Université de Sherbrooke

Synthesis of Novel Asymmetrically Substituted Phthalocyanines

by

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Great works are performed, not by strength,
but by perseverance.

Samuel Johnson

To Cynthia, the love of my life and to Ryan, my inspiration.

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Abbreviations and Symbols

A, absorption; Ad, adenovirus; Ad2, adenovirus serotype 2; AIBN, azoisobutyronitrile; ALA, 5-aminolaevulinic acid; AlPc, aluminum phthalocyanine; $AlPcS_2$, disulphonated aluminum phthalocyanine; $AlPcS_{2adj}$, adjacently disulphonated aluminum phthalocyanine; $AlPcS_4$, aluminum tetrasulphonated phthalocyanine; $AlPcS_4A_1$, aluminum mono-(6-carboxypentylaminosulfonyl)-tetrasulfophthalocyanine; $AlPcS_4A_2$, aluminum di-(6-carboxypentylaminosulfonyl)tetrasulfophthalocyanine; $AlPcS_4$(C16), aluminum (hexadecylaminosulfonyl)tetrasulfophthalocyanine; $AlPcS_4C_{12}$, $AlPcS_4$(C12), aluminum (dodecylaminosulfonyl)tetrasulfophthalocyanine; $AlPcS_4$(C8), aluminum (octylaminosulfonyl)tetrasulfophthalocyanine; $AlPcS_4$(C4), aluminum (butylaminosulfonyl)tetrasulfophthalocyanine; AMD, age-related macular degeneration; AOT, sodium bis(2-ethylhexyl)sulfosuccinate; ATMPn, [9-acetoxy-2,7,12,17-tetrakis-(β-methoxyethyl)]porphycene; BPD, benzoporphyrin derivative; BPD-MA, benzoporphyrin derivative monoacid ring A (verteporfin); bpy, dipyridine; BSA, bovine serum albumin; (br), broad; CAR, Coxsackie B and Adenovirus receptor; Ce6, chlorin e6; CoPc, cobalt phthalocyanine; CRM Cremophor™ EL; DABCO, 1,4-diazabicyclo[2,2,2]octane; DAMN, diaminomaleonitrile; dba, Tris(dibenzylidene acetone); DBI, N, N-dibromoisocyanuric acid; DBU, 1,8-diazabicyclo[5,4,0]undec-7-ene; DCC, dicyclohexylcarbodiimide; DDQ, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; Dex, dextran; DMA, dimethylacetamide; DMAP, p-N,N-dimethylaminopyridine; DMF, N,N-dimethylformamide; DMPO, 5,5-dimethyl-1-pyrrolidine-1-oxide; DMSO, dimethylsulfoxide; dpa, dibenzylideneacetone; DPPC, dipalmitoylphosphatidylcholine;

dppf, 1,1'-bis(diphenylphosphino)ferrocene; dsDNA, double stranded DNA; DTox, diphtheria toxin; DVD-R, recordable digital video disk; EC_{50}, effective concentration 50%; EDTA, ethylenediaminetetraacetic acid; EGF, epidermal growth factor; EMEA, European Medicines Evaluation Agency; EMT-6, a murine mammary adenocarcinoma cell line; EPR, electron paramagnetic resonance; F, fluorescence; FAB-MS, fast atom bombardment mass spectroscopy; FBS, fetal bovine serum; FDA, Food and Drug Administration; F-MRI, fluorine magnetic resonance imaging; GB_3, globotriaosylceramide; GePc, germanium phthalocyanine; HDL, high density lipoprotein; HIV, human immunodeficiency virus; HMP, Escherichia coli hemoglobin-like protein; HMPA, hexamethylphosphoramide; HMPT, hexamethylphosphorus triamide; HOMO, highest occupied molecular orbital; HP, haematoporphyrin; HPD, haematoporphyrin derivative; HPLC, high performance liquid chromatography; HPPI, 3α-hydroperoxy-1,2,3,3α,8,8α-hexahydropyrrolo[2,3β]indole-2-carboxylic acid; HSA, human serum albumin; IC, internal conversion; IR, infrared; ISA, intersystem crossing; LD50, light dose required for 50% cell inactivation; LD90, light dose required for 90% cell inactivation; LDL, low density lipoprotein; LUMO, lowest unoccupied molecular orbital; Lu-tex, lutetium texaphyrin; malBSA, maleylated bovine serum albumin; MCBPA, m-chloroperbenzoic acid; MgPc, magnesium phthalocyanine; MnSOD, manganese superoxide dismutase; MRT, modular recombinant transporters; MSH, α-melanocyte stimulating hormone; mTHPC, m-tetrahydroxyphenylchlorin (Temoporfin); MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-(diphenyltetrazolium) bromide; m/z, mass/charge; Na_2mnt, disodium maleonitriledithiolate; NBS, N-bromosuccinimide; NDA, New Drug Application; NLO, non-linear optics; NLS, nuclear localization signal; NMP, 1-methyl-2-

pyrrolidinone; NMR, nuclear magnetic resonance spectroscopy; NPC, nuclear pore complex; Npe6, mono-L-aspartyl chlorin e6; oxLDL, oxidized low density lipoprotein; P, phosphorescence; PBS, phosphate buffered saline; PEG, polyethylene glycol; Pc, phthalocyanine, PDP, patented Photodynamic cell therapy process; PDT, photodynamic therapy; PEG, polyethylene glycol; PpIX, protoporphyrin IX; PS, photosensitizer; PVA, polyvinyl alcohol; R, vibrational and rotational relaxation; RGD, Arg-Gly-Asp tripeptide; ROS, reactive oxygen species; rt, room temperature, Sens, sensitizer; Sens*, sensitizer in an excited electronic state; (sh), shoulder; SLTB, Shiga-like toxins; SOD, superoxide dismutase; S_0, ground state; S_1, first excited singlet state; Sn(IV)Ce6, tin(IV) chlorin e6; SnET2, tin etiopurpurin; subPc, boron subphthalocyanine, TBAB, tetrabutylammonium bromide; TBAF tetrabutylammonium fluoride; THF, tetrahydrofuran; T_1, first excited triplet state; TEMP, 2,2,6,6-tetramethyl-4-piperidone; TEMPO, 2,2,6,6-tetramethyl-4-piperidone-N-oxyl radical; TFA, trifluoroacetic acid; TPPS-2A, $TPPS_{2adj}$, adjacently disulphonated tetraphenylporphine; $TPPS_{2opp}$, oppositely disulphonated tetraphenylporphine; UV, ultraviolet; VLDL, very low density lipoprotein; ZnDPP, (5,15-diphenylporphinato)zinc; ZnPc, zinc phthalocyanine; $ZnPcF_{16}$, zinc hexadecafluorinated phthalocyanine; $ZnPcF_{12}S_1$, zinc dodecafluoro-4-sulfophthalocyanine.

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Abstract:

Phthalocyanines are among the more promising second generation photosensitizers for photodynamic therapy. Our research group has consistently shown that the more amphiphilic of these compounds display improved biological properties as photosensitizers for photodynamic therapy. However, synthetic approaches towards such asymmetrically substituted amphiphilic phthalocyanines are quite limited. As such, we have examined different methodologies for imparting amphiphilicity to phthalocyanine-based photosensitizers. Boron subphthalocyanines are the lower homologs of phthalocyanines and the reactivity of boron subphthalocyanines allows them to react with 1,3-diiminoisoindolines in a Kobayashi ring expansion reaction to give 3:1 asymmetrically substituted phthalocyanines. While several literature examples demonstrate that this protocol can lead to a mixture of substituted phthalocyanine products, the ring expansion reaction of halogenated boron subphthalocyanines in the current study has proven to proceed smoothly to selectively produce the desired 3:1 asymmetrically substituted products. Fluorinated photosensitizers have been previously demonstrated to have interesting properties for PDT and a series of 3:1 asymmetrically substituted dodecafluorinated phthalocyanines have been synthesized by the Kobayashi ring expansion reaction of (dodecafluorosubphthalocyaninato)boron(III) bromide. The asymmetry in these lipophilic compounds improves the photodynamic efficiency of these photosensitizers compared to previously examined symmetrically substituted fluorinated phthalocyanine derivatives. The chemical versatility of aryl iodides, in particular towards palladium-catalyzed reactions, allows for the controlled addition of novel functionality to 3:1 asymmetrically substituted iodinated phthalocyanines prepared by the Kobayashi ring

expansion reaction of iodinated boron subphthalocyanines. Palladium-catalyzed reactions have thus been employed in the preparation of new amphiphilic anionic and cationic water-soluble photosensitizers. These compounds should have interesting properties for photodynamic therapy. Lastly, boron subnaphthalocyanines absorb light at a wavelength around 660-680 nm. Their cone-shaped structure prevents aggregation and may impart amphiphilicity to the molecule depending on the nature of the substituents on the subnaphthalocyanine macrocycle and the axial ligand on the central boron. A series of boron subnaphthalocyanines has been synthesized and this class of photosensitizers has been shown to effectively generate singlet oxygen in an aqueous, biologically relevant environment while undergoing rapid photobleaching.

Chapter 1.

The Introduction

1. Introduction

1.1 The Beginning

The term phthalocyanine finds its origin in the Greek terms "naphtha" , which means rock oil and "cyanine", which means dark blue. This term was first used to describe these intensely blue-green compounds by Sir Reginald Linstead in the early 1930's during his pioneering work on the subject (Linstead, 1934). He coined this term for this new class of coloured compounds due to their origin and starting materials (phthalic acid derivatives) and due to the intensity and beauty of their blue-green colour.

As with most important discoveries made in science, serendipity played an important role in the discovery of phthalocyanines. Serendipity can be defined as the ability to make fortunate discoveries by accident or by a chance observation. Numerous important discoveries, including X-rays, the smallpox vaccine and the pharmaceutical utility of compounds such as cyclophosphamide, valproic acid, viagra, tamoxifen and chlorpromazine have all been due to chance observations. Among the most important serendipitous discoveries was made by Sir Alexander Flemming when he observed the destruction of staph bacteria in Petri dishes contaminated with penicillium mold. Along the same lines, phthalocyanines were discovered quite by accident. One day at Scottish Dyes Ltd., during the routine manufacture of phthalimide by passing ammonia gas through molten phthalic anhydride, blue impurities were observed around the charge hole of the iron reaction vessel (Gregory, 1999; Gregory, 2000). Upon noticing this blue colour and in the interest of good plant discipline, the plant chemist raised hell and roasted the foreman for allowing such sloppy work and contamination of the phthalimide

as he believed that the blue compound must be dibromoindanthrone, a blue dye produced in a neighboring reaction vessel. However, luckily for the foreman and ultimately for Scottish Dyes Ltd., the blue intermediate was not dibromoindanthrone. What was originally thought to be an impurity turn out to be iron phthalocyanine, ultimately leading to the birth of one of the most important classes of dyes and pigments in the colouring industry. A crack in the enamel lining of the iron reaction vessel allowed the reaction mixture access to a source of metal ions, serendipitously leading to the formation of iron phthalocyanine (Figure 1.1).

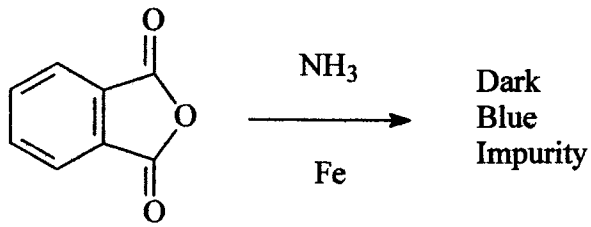

Figure 1.1. The original synthesis of phthalocyanine by Scottish Dyes Ltd.

While preliminary investigations by the chemists at Scottish Dyes Ltd. were only able to identify the blue intermediate as an iron-containing compound that was highly stable to various harsh reagents and conditions, they immediately recognized the potential utility of these intensely coloured compounds as highly stable dyes and colouring agents (Gregory, 2000). The first patent for compounds that are now known as phthalocyanines was granted to Scottish Dyes, Ltd. in 1929 (Dandridge et al., 1929). In this patent, a method for preparing colouring matters which may be used as vat dyes or pigments by reacting with ammonia or a primary amine of the aliphatic or of the benzene or naphthalene series on phthalic anhydride, phthalimide, or the mono- or di-amide of

phthalic acid in the presence of iron, nickel, or copper is disclosed. In addition, a method for preparing the first phthalocyanine dyes, polysulphonated phthalocyanines, was also disclosed by reacting phthalocyanines with sulphuric acid or oleum.

The structure of phthalocyanines was not elucidated until the mid 1930s. Supported by grants from the Research Committee of the Dyestuffs Group of Imperial Chemical Industries, Ltd., Sir Reginald Linstead and his research group identified phthalocyanines as tetrabenzotetraazaporphyrin macrocycles through molecular weight determination, oxidative degradation and synthetic analysis among other techniques (Elvidge, 1999). In a classic series of papers, Linstead and his research associates were able to elucidate the structure of phthalocyanines (Linstead, 1934; Linstead and Lowe, 1934b; Dent et al., 1934; Linstead and Robertson, 1936) while further determining methods for the synthesis of phthalocyanines and related macrocycles (Byrne et al., 1934; Linstead and Lowe, 1934a; Linstead et al., 1937; Elvidge and Linstead, 1955) and studying their relationship to porphyrins (Dent, 1939), their complexes with various metal ions (Dent and Linstead, 1934; Barrett et al., 1936; Barrett et al., 1938), their unusual degree of stability (Linstead, 1934; Dent and Linstead, 1934) and the mechanism of their formation (Elvidge and Golden, 1957; Baguley and Elvidge, 1957). In addition, the structure established by Linstead was later confirmed by J. Monteath Robertson via X-ray crystallography in a classic series of papers (Robertson, 1935; Robertson, 1936; Robertson and Woodward, 1937; Robertson and Woodward, 1940). In fact, phthalocyanines were the first organic compounds to have their structures confirmed by X-ray diffraction studies, validating this technique in structure determination. Interestingly, the first molecular and submolecular resolution images of an organic

compound by high resolution electron microscopy were also obtained using phthalocyanines (Uyeda et al., 1972). The exceptional stability of phthalocyanines allows them to survive the high electron flux needed for high resolution microscopy.

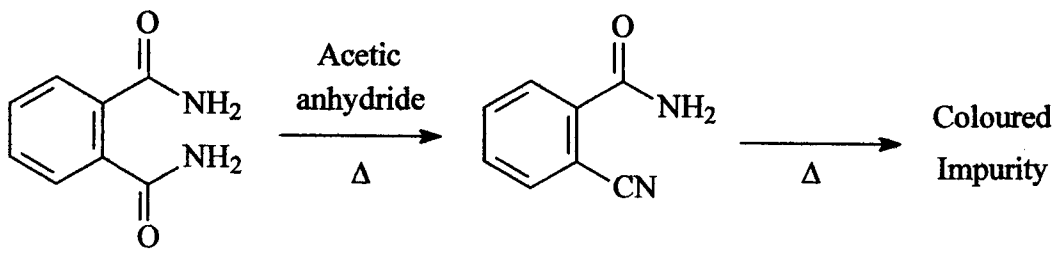

Figure 1.2. In hindsight, the first reported synthesis of phthalocyanine.

In hindsight, the first reported synthesis of phthalocyanines actually occurred in 1907, when Braun and Tscherniac observed the production of a coloured impurity of unknown origin during the synthesis of o-cyanobenzamide from phthalimide and acetic anhydride (Figure 1.2) (Braun and Tscherniac, 1907). Unfortunately, no further investigation of this coloured impurity was undertaken. The second recorded observation of a blue-green compound that was later determined to be phthalocyanine was in 1927 (de Diesbach and von der Weid, 1927). During attempts to synthesize of dinitriles, o-dibromobenzene was reacted with copper cyanide in pyridine at elevated temperature.

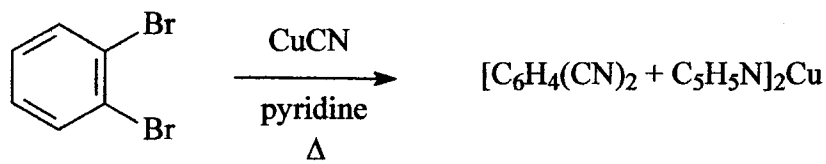

Figure 1.3. Complex copper salt proposed by de Diesbach and von der Weid

However, instead of obtaining the desired o-dicyanobenzene, a deeply coloured blue compound was obtained, which are tentatively believed to be a complex copper salt of o-dicyanobenzene and pyridine (Figure 1.3). Similar deeply coloured blue compounds were obtained when the reaction was carried out using either 1,2-dimethyl-4,5-dibromobenzene or 1,2-dibromonaphthalene. However, it was not until 1931 that it was suggested that these complex copper salts were in reality copper phthalocyanines. This was later verified by Linstead (Linstead and Lowe, 1934a).

1.2 Structure and Properties of Phthalocyanines

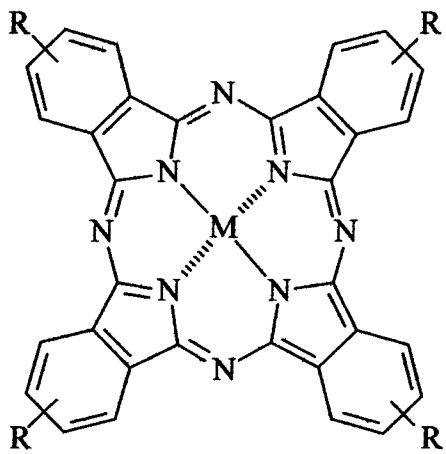

Figure 1.4. The classic general chemical structure of a tetrasubstituted phthalocyanine

Phthalocyanines are essentially tetrabenzotetraazaporphyrin macrocycles and are chemically and physically very closely related to the naturally occurring and biologically essential porphyrins. Similar to porphyrins, the central core of phthalocyanines consist of an 18-π electron aromatic system in the form of a cyclic tetrapyrrolic macrocycle. However, in contrast to porphyrins, the individual pyrrole rings are linked by nitrogen atoms and not by methine bridges. In addition, the chromophore is extended by the presence of the benzo rings on the periphery of the macrocycle. The additional π-orbital conjugation afforded by the benzo rings and the orbital perturbation caused by the nitrogen atoms at the four meso-positions of the tetrapyrrolic core macrocycle have profound effects on the properties of phthalocyanines compared to their porphyrin

cousins. Most evident is the entirely different electronic spectra exhibited by phthalocyanines, with an important red-shift in the Q band and a corresponding strong enhancement in the intensity of this absorption. In addition, the presence of lone pairs on the nitrogen atoms in the meso-positions lends a more pronounced aromaticity to phthalocyanines while also resulting in stronger, more stable metal complexes compared to metalloporphyrins. The inner nitrogens of phthalocyanines are also significantly weaker Lewis bases. However, it has been well established that the substitution of nitrogen atoms for methine bridges in aromatic heterocycles does very little to alter the actual physical structure of the heterocycle. Along these lines, the tetrapyrrolic macrocyclic core of phthalocyanines is physically very similar to the one found in porphyrins in terms of both size and shape (Berezin, 1981) with only slight differences in the size of the central core of the macrocycle. The nitrogen-nitrogen diagonal distance is only slightly smaller for phthalocyanines (396 pm) as compared to porphyrins (402 pm) (Stillman and Nyokong, 1989).

Based on molecular weight determination, oxidative degradation, synthetic analysis and other laboratory techniques, Sir Reginald Linstead proposed the classic chemical structure of phthalocyanines as depicted in Figure 4 (Dent et al., 1934). This general physical orientation of atoms was confirmed by X-ray diffraction studies by J. Monteath Robertson (Robertson, 1935; Robertson, 1936; Robertson and Woodward, 1937; Robertson and Woodward, 1940). The phthalocyanine molecule itself is planar, which along with the unusual degree of aromaticity of its tetrapyrrolic core explains the tendency of phthalocyanines to aggregate in solution via π-π orbital stacking. Unsubstituted phthalocyanines chelating metal ions are highly symmetrical, displaying

D_{4h} symmetry. Taken these factors into consideration, the structural formula proposed by Linstead and universally employed to describe phthalocyanines is adequate. However, there are a number of structural anomalies observed by Linstead and by Robertson that cannot be explained by the classical structural formula. For instance, Robertson observed that the carbon-nitrogen bond length in the tetrapyrrolic macrocycle is 1.34 Å and is the same throughout the molecule (Robertson, 1936). Such bond lengths are indicative of a single bond/double bond resonance for all C-N bonds within the macrocycle, which is not indicated by the classic structure. Furthermore, Robertson measured the carbon-carbon bonds that link the benzo rings to the inner tetrapyrrolic macrocycle at a constant length of 1.49Å. According to Pauling's empirical formula for determining the double bond character of a bond using bond lengths, these bonds would appear to have from 12% to 15% double bond character (Robertson, 1936). This is significantly less than would be expected from the classical structure. Finally, the homogeneity of the oxidation products of phthalocyanines when treated with hot acidic permanganate is at odds with the quinoid form of one of the benzene rings in the classical structure (Dent et al., 1934; Robertson, 1935). As such, while the classical structure first proposed by Linstead is adequate in representing phthalocyanines, it fails to completely describe the precise structure of phthalocyanines.

In order to explain the above mentioned observations, the structure depicted in Figure 1.5 was proposed as a more adequate representation of the structure of a metal-free phthalocyanine (Berezin, 1981). In this structure, the * represent the 24 π-electrons of the four benzo rings, the • represent the 16 π-electrons inherent to the tetrapyrrolic

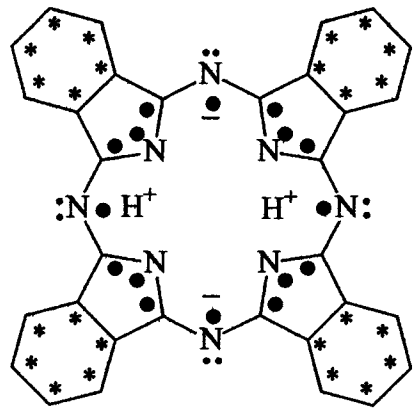

Figure 1.5. The structure of metal-free phthalocyanine as proposed by Berezin (Berezin, 1981)

macrocyclic core and the – represent the two ionization electrons resulting from the internal ionization of the two imino-hydrogen bonds. The four sextets of π-electrons in the benzo rings intrinsically form stable aromatic shells that only weakly interact with the π-electrons of the tetrapyrrolic macrocycle. This weak interaction is evident by the minor double bond character of the carbon-carbon bonds joining the benzo rings to the tetrapyrrolic macrocycle. The remaining 18 π-electrons occupy molecular orbitals of the tetrapyrrolic macrocycle and independently form a π-electron system exhibiting aromatic stabilization. This aromaticity explains the uniformity in the length of all the C-N bonds as well as the homogeneity of the oxidation products.

In the above proposed representation of metal-free phthalocyanines, the inner protons are in the electrostatic field of three nitrogen atoms. It is energetically favourable to have these protons in the electrostatic field of multiple nuclei. Furthermore, such bonding liberates the two ionization electrons and allows them to augment the

conjugation in the tetrapyrrolic macrocycle, resulting in aromatic stabilization. In terms of experimental evidence supporting this type of bonding, Robertson observed a significant distortion from true tetragonal symmetry in metal-free phthalocyanines that was not observed for the corresponding phthalocyanines that were chelating metal ions (Robertson, 1936). While Robertson explained this distortion in terms of hydrogen bonding, it seems unlikely that weak hydrogen bonding would be enough to cause any significant distortion of the highly aromatic system present in phthalocyanines. However, the three-center bonding of the inner protons should cause some distortion in the structure of metal-free phthalocyanines, which would disappear upon chelation of a metal ion. In addition, in the NMR spectra of metal-free phthalocyanines, the signal due to the two strongly shielded inner protons ($\delta \approx$ -5.00 ppm) disappears upon deuterium exchange (Dabak et al., 1994). While covalently bound protons should not be susceptible to deuterium exchange, protons involved in three-center bonding would undergo deuterium exchange. As a result, the structure proposed by Berezin may represent a more accurate representation of the true physical structure of phthalocyanines.

When coordinating a metal ion, the phthalocyanine molecule acts as a tetradentate ligand capable of forming four dative σ bonds with the metal ion. Typically, metallophthalocyanine complexes exhibit octahedral geometry with the phthalocyanine tetradentate ligand occupying the equatorial plane and axial ligands coordinated to the metal both above and below the phthalocyanine plane. Stable phthalocyanine complexes involve the formation of four equivalent dative σ bonds between the pyrrolic nitrogen atoms of the phthalocyanine and the metal ion. These dative σ bonds involve the filling of vacant s, p_x, p_y and $d_{x^2-y^2}$ orbitals of the metal ion (those orbitals orientated toward the

pyrrolic nitrogen atoms) with the electrons of the lone pairs of the nitrogen atoms. This filling of vacant orbitals of the metal ion results in the formation of stable dative bonds wherein both electrons involved in bonding originate from the same atom. The most stable σ bonds require the best possible overlap of orbitals, which in the case of phthalocyanines occurs with metal ions having a radius of approximately 1.35Å (the radius of the inner core of phthalocyanines) (Berezin, 1981). Metal ions such as Cu^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+}, Pt^{2+}, Pd^{2+}, Al^{3+}, Ga^{3+} and VO^{+2} have covalent radii of this size and are know to form extremely stable metallophthalocyanine complexes. More labile phthalocyanine complexes are formed by metal ions that do not have the appropriate radius. For instance, several 3d and 4d metal ions and all of the lanthanides form out-of-plane and sandwich phthalocyanine complexes wherein the metal ion sits above the phthalocyanine plane (1.11 Å above the phthalocyanine plane for dichlorotin(IV)phthalocyanine (Kroenke et al., 1964)) with incomplete orbital overlaps and distortion of the phthalocyanine geometry. Labile phthalocyanine complexes are also obtained with metal ions that are not capable of forming strong σ bonds, either due to weak electron affinities or an inability to adopt the planar geometry of orbitals necessary for forming σ-bonds with phthalocyanines. Metal ions such as Li^{+}, Na^{+}, Mg^{2+}, Ca^{2+}, Ag^{+}, Mn^{2+}, Sn^{+2} and Pb^{2+} form labile metallophthalocyanine complexes involving primarily ionic bonding and these metal ions are easily removed by treatment with acid. This method is in fact often utilized to prepare metal-free phthalocyanines.

In addition to σ-bonds, metal ions with filled d-orbitals with π symmetry (d_{xy}, d_{xz} or d_{yz}) can also for dative π backbonding, with the metal ion donating electrons into antibonding π* molecular orbitals of the phthalocyanine. Phthalocyanines have an

unusually high capacity to form dative π backbonds with metal ions, significantly increasing the coordinative strength of the interactions between the metal ion and the phthalocyanine ligand and thus, increasing the stability of the complex.

Depending on the strength of the coordinative metal-phthalocyanine ligand interactions (which itself is determined by the strength of the dative σ-bonds between the metal ion and the pyrrolic nitrogen atoms and the formal charge of the metal ion) metal ions may be deemed as being either coordinatively saturated or unsaturated. Coordinatively saturated metal ions such as Ni^{2+}, Pt^{+2} and Pd^{+2} have a formal charge of +2 and form extremely stable complexes with both strong σ forward- and π backbonding. Phthalocyanine complexes with such metal ions lack low lying antibonding orbitals at the metal and show no tendency towards coordinating axial ligands. It is well-known that d^7 ions such as Ni^{2+}, Pt^{+2} and Pd^{+2} form extremely stable square planar complexes (Butler and Herrod, 1989) and metallophthalocyanines prepared using these metal ions exhibit a square planar geometry with the phthalocyanine ligand and the metal ion occupying the same equatorial plane.

Coordinatively unsaturated metal ions include those that have no tendency towards dsp^2 hybridization at a formal charge of +2 (Zn^{2+}, Fe^{2+}, Cr^{2+} and Mn^{2+}), involve unstable oxidation states of the metal (Co^{2+}, Re^{2+} and Cu^{0}) or have inappropriate ionic radii, resulting primarily in ionic complexes (Be^{2+}, Mg^{2+}, Cd^{2+}, Hg^{+2} and Ca^{+2}). Phthalocyanines complexes with these metal ions tend to adopt octahedral geometries, typically with solvent molecules acting as axial ligands. Other anions such as Cl^- or HO^- can also act as the axial ligand when present in solution. In solution, phthalocyanines tend to aggregate via stacking. In this instance, the metal ion from an adjacent

phthalocyanine complex is actually acting as the axial ligand. Phthalocyanines with a tendency to aggregate in solutions of water or alcohol become monomeric in DMF or pyridine. Such solvents are more strongly coordinating (more readily donate electron density to the metal ion) and thus remain tightly bound to the metal in solution. This prevents the phthalocyanines from stacking. Overall, it should be noted that phthalocyanines chelating coordinatively unsaturated metal ions lack axial ligands as solids and usually form crystals via some type of stacking, usually either as a α or β polymorph.

Coordinatively unsaturated metal ions also include any metal with a formal charge exceeding +2, irrespective of the strength of the dative σ and π interactions. Phthalocyanine complexes with such metal ions readily coordinate axial ligands in order to neutralize the excess charge of the metal ion. As a result, the axial ligands are more tightly coordinated and are present in the crystal forms of these complexes. Axial ligand binding involves the s, p_z and d_z^2 orbitals of the metal ion, along with any empty d_{sp}^2-hybridized orbitals. In solution, the presence of these more tightly bound axial ligands tends to prevent aggregation to varying degrees.

The geometry, energy and occupancy of the d-orbitals of the metal ion influences the magnetic properties of the metallophthalocyanine complex. Phthalocyanine complexes of Cu^{2+}, Co^{2+} and Fe^{+2} are paramagnetic since they contain unpaired d-orbital electrons. In contrast, Zn^{2+}, Ga^{3+} and Al^{3+} are d^{10} ions with a filled outer d-shell and phthalocyanine complexes with these metal ions are thus diamagnetic. Metal ions such as Ru^{2+} also form diamagnetic phthalocyanine complexes, even though it is a d^6 ion. Since it requires energy to pair electrons in any given orbital, atoms and molecules tend

to fill degenerate orbitals (such as d-orbitals) before pairing up electrons. However, in the case of coordination complexes, crystal field theory dictates that the normally degenerate d-orbitals are split into different energy levels as a result of electrostatic interactions between the individual d-orbitals of the metal ion and the ligand. In the case of metallophthalocyanine complexes, d-orbitals orientated towards the ligand are destabilized ($d_{x^2-y^2}$ and d_{z^2}) while the remaining d-orbitals (d_{xy}, d_{xz} or d_{yz}) are stabilized as compared to an uncomplexed metal ion due to the presence of the ligand. In the case of ruthenium (II) phthalocyanine, it is energetically favourable to pair electrons in the lower energy d-orbitals rather than having the electrons remain unpaired and filling the higher energy d-orbitals. Thus, the six d-electrons of ruthenium fill the d_{xy}, d_{xz} or d_{yz} orbitals, resulting in diamagnetic complexes with phthalocyanines. The magnetism of phthalocyanine complexes significantly influences a number of properties. For instance, upon illumination with light of an appropriate wavelength, diamagnetic phthalocyanine complexes exhibit high triplet state yields and long triplet state lifetimes. In the meanwhile, the first excited singlet electronic state of paramagnetic phthalocyanine complexes is rapidly deactivated, leading to poor triplet state yields. Such alterations of the electronic characteristics of phthalocyanine complexes are particular important in applications such as photodynamic therapy, which requires high triplet state yields and long triplet state lifetimes in order to effectively produce the cytotoxic species responsible for the biological effects. On the other hand, the dye industry prefers to use paramagnetic phthalocyanine complexes in order to avoid oxidative damage caused by the generation of these same reactive species.

1.3 Electronic Spectra of Phthalocyanines

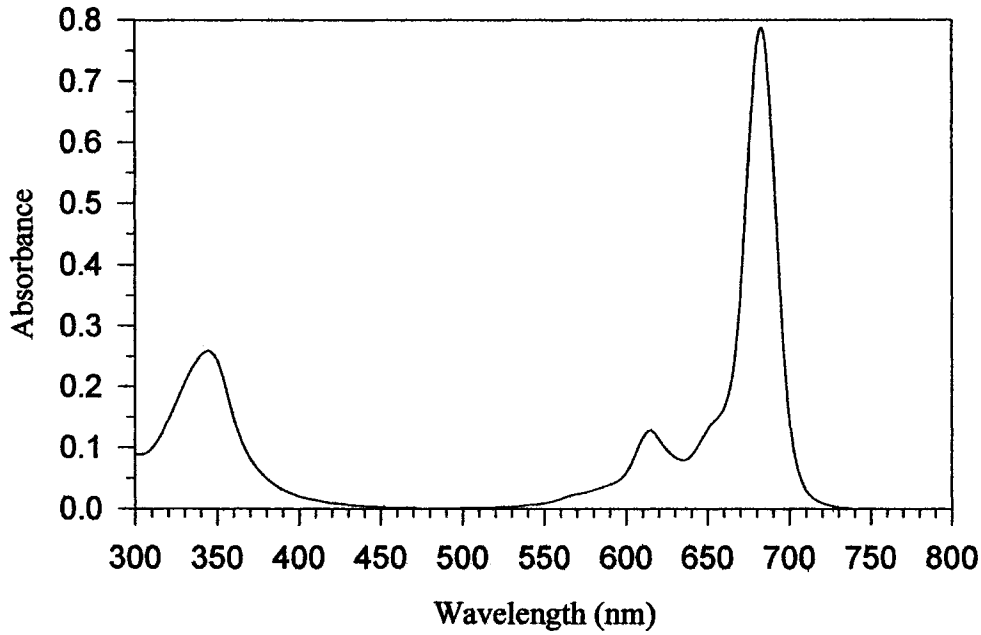

Figure 1.6. The typical UV-visible electronic spectra of a metallophthalocyanine complex

The UV-visible spectra of naturally occurring porphyrins such as heme and chlorophyll is extremely complex, characterized by low extinction coefficients for the visible absorptions and an intensity B band around 400 nm. There is a great variability in the position and intensity of the absorptions in the UV-visible spectra as a function of the structure of the porphyrins, in particular with respect to substitution at either the meso or β positions. The position and intensity of the absorptions of porphyrins are however relatively insensitive to the nature of solvents while being extremely sensitivity to

protonation, complexing agents and strong bases, indicative of the relatively strong Lewis basicity of porphyrins.

The electronic spectra of phthalocyanines are radically different from the spectra observed for porphyrins. The extended π-orbital conjugations and the orbital perturbation resulting from the nitrogen atoms at the meso positions of the tetrapyrrolic macrocycle lowers the energy of the Q band, with a corresponding important increase in the intensity of this absorption. In addition, the greatly reduced coupling between the Q and B bands means that the Q band in phthalocyanines is unaffected by charge transfer effects. Overall, the most important difference between the electronic spectra of porphyrins and phthalocyanines is the well-resolved, isolated and intense Q band near 670 nm of the phthalocyanine compared to the intense B band located around 400 nm of the porphyrins.

A typical UV-visible spectrum for a monomeric metallophthalocyanine complex is depicted in Figure 1.6. The Q band is observed around 670-680 nm, with an extinction coefficient in the range of 2.5×10^5 $M^{-1}cm^{-1}$. A much less intense B band appears around 340 nm and has an extinction coefficient of approximately 10^4 $M^{-1}cm^{-1}$. A weak absorption around 600-620 nm, due to an $n \rightarrow \pi^*$ transition out of the aza-nitrogen lone pair orbitals (Mack and Stillman, 2001), is often observed and does not interact with the Q band absorption. The isolation and intensity of the Q band absorption in the visible region of the spectrum results in the strength and purity of the blue/green colours of these compounds, leading to their industrial importance as dyes and pigments.

The Q band absorption is relatively insensitive to changes in the complexed metal ion or any bound axial ligands. In light of these observations, it is evident that the Q band in the electronic spectra of phthalocyanines is due to electronic transitions centered

on the phthalocyanine ligand, thus making changes to the metal ion or to any axial ligands irrelevant. Similarly, changes to the functional groups substituted onto the benzo rings on the periphery of the phthalocyanine macrocycle usually has only a minor effect on the position and intensity of the Q band. This is in sharp contrast to porphyrins, where substitution at either the meso or β positions of the macrocycle results in significant alterations to the UV-visible spectra. This is due to the fact that, unlikely in porphyrins, the electronic transition leading to the Q band absorption is essentially symmetry allowed for phthalocyanines and is therefore less dependent upon any gains in intensity resulting from differences in substituents. In addition, the benzo rings on the periphery of phthalocyanines and the relative electronic independence of the tetrapyrrolic macrocyclic core from these benzo rings (as observed by the weak double bond character of the carbon-carbon bonds attached the benzo rings to the tetrapyrrolic core) isolates the substituents from the tetrapyrrolic core and inhibits their ability to affect the energy of the molecular orbitals involved in the Q band transition. On the other hand, substituents at the meso and β positions of porphyrins are directly bound to the tetrapyrrolic macrocycle and thus, readily stabilize or destabilize molecular orbitals involved in the corresponding transitions in porphyrins.

Unlike the Q band absorption of phthalocyanines, the B band in the absorption spectra of phthalocyanines is extremely sensitive to changes in the structure of the chromophore. This is presumably due to the symmetry and orientation of the molecular orbitals from which the underlying electronic transitions arise. These orbitals must be oriented in such a way that they are more susceptible to changes in electron density caused by alterations in the structure of the phthalocyanine macrocycle, ultimately

leading to changes in the energy of the electronic transition (and thus, changes in the wavelength of the absorption) with accompanying changes in the overall intensity of the absorption.

Several detailed theoretical calculations on the molecular orbitals of phthalocyanines have been considered and used to account for empirical observations (Gurinovich et al., 1968; Lee et al., 1982; Hale et al., 1987; Stillman and Nyokong, 1989; Gantchev et al., 1993; Owens et al., 1998; Mack and Stillman, 2001; Stillman et al., 2002; Mack and Stillman, 2003). While lacking in terms of explaining some of the more detailed features of the absorption spectra of phthalocyanines, the use of a four orbital model which considers the top two highest occupied molecular orbitals (HOMO) and the degenerate lowest unoccupied molecular orbital (LUMO) readily explains the first few electronic transitions in the UV-visible region of the phthalocyanine spectrum and the differences between the spectra of porphyrins and phthalocyanines. Diagrammatically, this basic theory is described in Figure 1.7 (Stillman and Nyokong, 1989).

In the case of porphyrins, the a_{1u} and a_{2u} HOMO orbitals are accidentally degenerate (i.e. of the same energy), resulting in extensive interactions between the Q and B band absorption. The addition of aza linkages and, to a lesser extent, the increased π-orbital conjugated caused by the fused benzo rings on the tetrapyrrolic core of phthalocyanine breaks this accidental degeneracy and stabilizes the a_{1u} HOMO orbital. This results in reduced mixing between the Q and B excited states so that the previously forbidden Q transition gains significant intensity and shifts to a longer wavelength. Interestingly, this model predicts that the molecular orbitals responsible for the B band

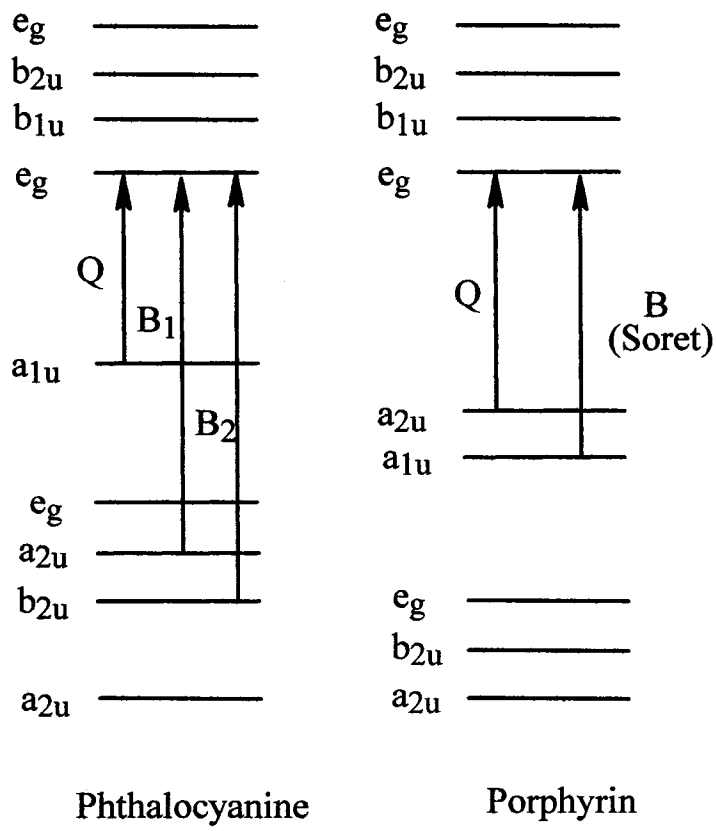

Figure 1.7. A molecular orbital explanation for the origin of the UV-visible spectra of phthalocyanines (adapted from Stillman et al., 1989)

in phthalocyanines are now accidentally degenerate, resulting in a two component absorption (B_1 and B_2). These two absorptions occur at roughly the same energy, resulting in the broad B band present in the spectra of phthalocyanines. Such a split B band has been observed in the spectra of ZnPc, MgPc and Li_2Pc (Stillman and Nyokung, 1989). It should be noted that while the weak double bond character of the carbon-carbon bonds linking the benzo rings to the tetrapyrrolic core indicates only a weak conjugation between π-orbitals of the benzo rings and those of the tetrapyrrolic core, the presence of the benzo rings in phthalocyanines does result in a significant stabilization of the HOMO orbital. This is clear from the UV-visible spectra of phthalocyanines

derivatives having increased conjugation such as naphthalocyanines and anthralocyanines whose Q band absorptions are at 780 nm and 980 nm respectively (Brasseur et al., 1994; Bedworth et al., 1997).

This model can also be employed to explain the effect of aggregation on the electronic spectra of phthalocyanines. It is well-known that phthalocyanines tend to aggregate via stacking in solution, the result of the highly hydrophobic and aromatic tetrapyrrolic core of these macrocycles and the corresponding stabilization achieved by overlapping the π-electron cloud of adjacent phthalocyanine molecules. In solution, aggregation by this type of stacking causes a significant broadening of both the Q band and B absorptions with a blue shift in the wavelength of the absorption. The Q band, for instance, is frequently shifted to approximately 620 nm as a broad, less intense peak.

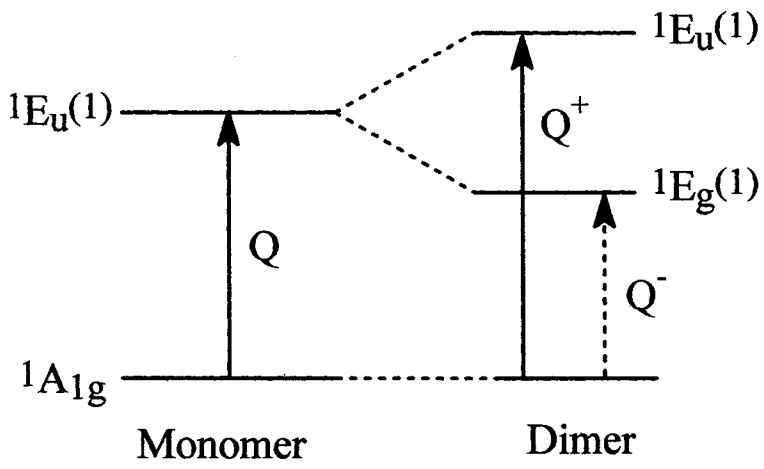

Figure 1.8: The effect of aggregation on the LUMO orbitals of phthalocyanines

Upon aggregation and the corresponding overlap of the π-orbitals in adjacent phthalocyanine molecules, there is a loss of degeneracy in the LUMO orbital, resulting in

two LUMO orbitals with $^1E_u^{(1)}$ and $^1E_g^{(1)}$ symmetries (Figure 1.8) (Stillman and Nyokong, 1989). Since, according to quantum mechanics, electronic transitions must transform with either E_u or A_{2u} symmetry, only the electronic transition to the higher LUMO orbital (Q^+) is allowed, thus resulting in the shift in the wavelength of the Q band to higher energy. The broadening of the absorption is the result of two factors. While the transition to the lower LUMO orbital is forbidden according the quantum mechanics, it does occur to a small extent, resulting in a wider overall absorption. In addition, the magnitude of π-orbital overlap depended directly on the extent of aggregation, which depends on the physical proximity of the macrocycles, their physical overlap, and the tilt angle. All these factors dictate the effectiveness of the overlap of the π-electron clouds of the overlapping phthalocyanines and surely differ for each aggregate in solution. As a result, the extent of the splitting between the non-degenerate LUMO differs for each aggregate, depending on the effectiveness of the π-orbital overlap. Hence, each aggregate will absorb at a slightly different wavelength, leading to a broad absorption spectra.

Shown in Figure 1.9 is the typical UV-visible spectrum of a metal-free phthalocyanine, characterized by a split Q band centered at slightly lower energy than the Q band of a metallophthalocyanine complex. The presence of two protons bound in the inner core of the phthalocyanine tetrapyrrolic macrocycle drops the symmetry of the molecule from D_{4h} to D_{2h}. Similar to aggregation, the decrease in symmetry results in a break in the degeneracy of the LUMO orbital. However, in metal-free phthalocyanines, the electronic transition to both the $^1E_u^{(1)}$ and $^1E_g^{(1)}$ LUMO orbitals is allowed due to

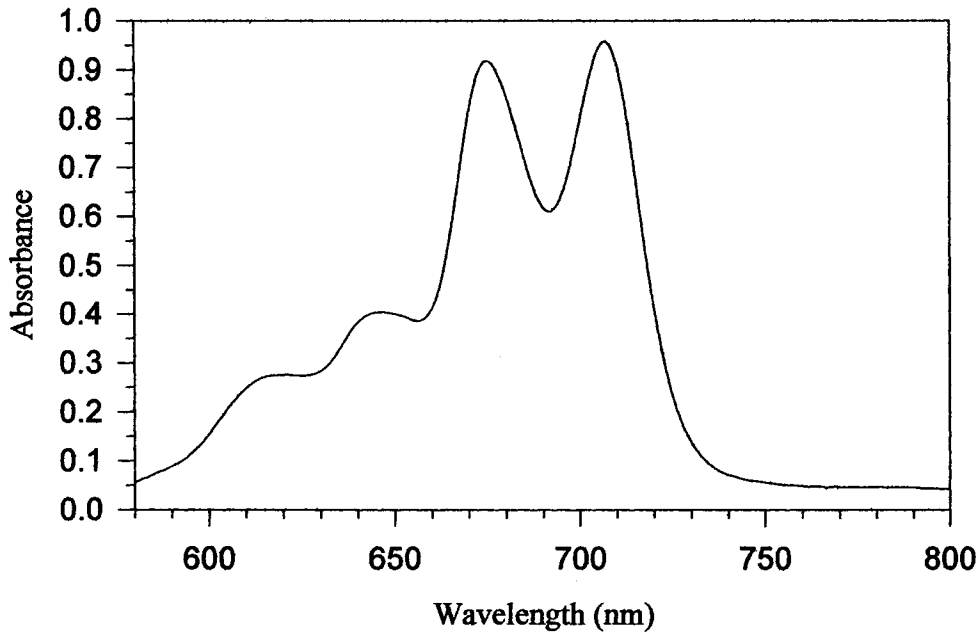

Figure 1.9. The typical UV-visible spectra of a metal-free phthalocyanine

polarization in either the x or y direction. As such, the Q band of metal-free phthalocyanines is split, with electronic transitions to either of the LUMO orbitals. Similar splitting of the Q band may be observed in asymmetrically substituted metallophthalocyanines. While the substituents on the benzo rings of phthalocyanines generally has only a minor effect on the position and intensity of the Q band, asymmetrically substituted phthalocyanines bearing both electron-donating and electron-withdrawing substituents can significantly alter the geometry of the π-electron cloud of phthalocyanines. This disruption of the π-electron cloud will also cause a break in the degeneracy of the LUMO orbital and lead to a split Q band. Such splitting is observed in novel push-pull asymmetrically substituted phthalocyanine bearing alkynyl functional

groups (Maya et al., 2000) and in asymmetrically substituted benzonaphthaloporphyrazines wherein the π-electron cloud is disturbed by the extended conjugation introduced by the napthalo rings asymmetrically present in the chromophore (Margaron et al., 1992; Michelsen et al., 1996). Indicative of the effect of symmetry on the UV-visible spectra of these asymmetrically substituted phthalocyanine derivatives is the observation that the dinapthalodibenzoporphyrazine derivative wherein the two napthalo groups are on adjacent pyrrolic rings exhibits a split Q band. On the other hand, UV-visible spectra of the dinapthalodibenzoporphyrazine derivative wherein the two napthalo groups are on opposite pyrrolic rings has a single Q band, the direct result of the symmetry in this molecule compared to the chromophore with the naphthalo rings on adjacent pyrrolic rings.

1.4 Utility of Phthalocyanines

The unique structure along with the distinct physical and chemical characteristics and remarkable stability of the phthalocyanine molecule has been exploited by researchers in widely divergent high-tech fields. In addition, phthalocyanines have an important position in the annals of a number of these high-tech applications, with landmark experiments involving phthalocyanine serving to propel the technology forward towards actual utility. As has been previously mentioned, the fact that phthalocyanine tend to sublime at high temperatures to form large crystals allowed Robertson to confirm the structure of phthalocyanines via X-ray diffraction, validating this important method of structure determination (Robertson, 1935; Robertson, 1936; Robertson and Woodward, 1937; Robertson and Woodward, 1940). The unusual stability of the phthalocyanine ligand under intense electron flux permitted Uyeda and Kobayashi to use phthalocyanines to obtain the first molecular and sub-molecular resolution images of an organic molecule (Uyeda et al., 1972). Copper phthalocyanine was used in some of the first experiments to demonstrate that organic solid could act as electronic semiconductors (Eley, 1948), thus exploiting the electronic properties of the phthalocyanine molecule to validate the use of organic molecules in semiconductors. Phthalocyanines also form charge-transfer complexes with iodine, with these charge-transfer complexes exhibiting metal-like conductivity (Schramm et al., 1978; Marks, 1985; Marks, 1990). With the large increases in conductivity upon forming such charge-transfer complexes, phthalocyanines have found utility as chemical sensors that are able to detect minute amounts (down to as low as a few parts per billion) of toxic, oxidizing gas such as nitrogen dioxide (Bott et al., 1984; Wright, 1989; Ishii et al., 2000; Nguyen Van et al., 2001; Slota et al., 2002).

Chemical sensors based on resistance or capacitance along with field-effect transistor sensors, solid state ionic sensors, quartz crystal microbalance sensors, surface acoustic wave sensors and optical sensors have all been prepared using phthalocyanines as the means of sensing (Snow et al., 1989; Guillard et al., 1998; Ding et al., 1999; Kudo et al., 1999).

Ever since their serendipitous discovery, phthalocyanines have been used as dyes and pigments by the colouring industry. In fact, chemists at Scottish Dyes Ltd. attempted to use the novel blue compound later identified as aphthalocyanine to colour cotton using a vat dyeing technique long before the structure and nature of phthalocyanines was fully elucidated (Gregory, 1999; Gregory, 2000). This led to the first issued patent on the use of phthalocyanines as dyes and pigments in 1929 (Dandridge et al., 1929). In this patent, a method for preparing colouring matters which may be used as vat dyes or pigments by reacting with ammonia or a primary amine of the aliphatic or of the benzene or naphthalene series on phthalic anhydride, phthalimide, or the mono- or di-amide of phthalic acid in the presence of iron, nickel, or copper is disclosed. In addition, a method for preparing the first phthalocyanine dyes, polysulphonated phthalocyanines, was also disclosed by reacting phthalocyanines with sulphuric acid or oleum.

The intensity of the blue/green colour of phthalocyanines along with their important fastness to light and heat, chemical inertness and high dyeing power and tinctorial strength has ensured the reputation of phthalocyanines in the painting, dyeing, textile and paper industries as superior quality blue, blue-green and green dyes and pigments. Additionally, phthalocyanines are extremely cost effective, being relatively inexpensive to prepare on an industrial scale with Pigment blue 15 (the alpha form of

copper phthalocyanine) cost $5.73 per lbs (http://www.horsta-pigment.com/Products_And_Pricelist.htm). The synthetic flexibility in adding and altering the substituents bound to the phthalocyanine chromophore also allows for the control of their solubility and the colour of the individual dye or pigment. Finally, the extreme insolubility of certain phthalocyanines makes them valuable blue/green pigments (dyes being soluble organic colourants while pigments are insoluble organic or inorganic colourants).

The amount of phthalocyanine produced for use as dyes and pigments is around 80 000 tons per year with copper phthalocyanine being the highest volume colourant produced worldwide (Wöhrle, 2001). Phthalocyanine dyes and pigments are used to colour everything from paints and ink through to plastics, rubbers, leather, fabrics and paper. Phthalocyanines are also used to colour detergents and cleaning solutions, contact lenses, sutures and tattoos while also serving as the major coloured pigment employed by the packaging industry. In addition, phthalocyanines are used as biological stains with Alcain Blue staining bacteria acid mucopolysaccharides, histocytes and fibroblast and Luxol fast blue staining myelin. In terms of the colourant industry, phthalocyanine pigments are more important as the molecular size and rigidity of phthalocyanines make them of limited usefulness in dyeing synthetic fibers such as polyester, polyacrylonitrile and nylon. As such, phthalocyanine dyes are nearly exclusively used to colour cellulosic substrates such as cotton and paper.

While unsubstituted phthalocyanines are important blue/green pigments, they cannot be used directly as dyes because of their extreme insolubility in most solvents. However, some metallophthalocyanines can be reduced by dithionite to give more

soluble vat dyes (Struve, 1955; Jackson, 1978). When steeped onto textile material and exposed to air, these dyes reoxidize to the insoluble form and precipitate onto the fibers of the textile, thus colouring the textile. Indanthrene brilliant blue 4G (cobalt phthalocyanine) is an important vat dye of this type. In the meanwhile, a number of more soluble phthalocyanine derivatives have been synthesized and investigated as potential dyestuffs. Water-soluble polysulphonated phthalocyanine was described in the first patent issued concerning phthalocyanines (Dandridge et al., 1929) and since then, functional groups such as sulphonic acids, sulphonyl chlorides, amides, thiols and tertiary and quaternary ammonium groups have been added to phthalocyanines to impart solubility and to improve the properties of the macrocycle as a dyestuff (Bigelow et al., 1955; Struve, 1955; Booth, 1971; Vollman, 1971). Reactive phthalocyanine dyes containing functional groups that will react with free functional groups of the substrate and phthalogen dyes wherein phthalocyanines are formed in situ from phthalocyanine precursors (such as 1,3-diiminoisonidolines) during the formation of the fabric are important methods of colouring using phthalocyanine chromophores (Sturve, 1955; Vollman, 1971; Jackson, 1978; Gregory, 1999). The patent literature is replete with novel phthalocyanine derivatives prepared for use as dyestuffs and pigments (see, for instance, James et al., 1980; Lacroix et al., 1980; Marraccini et al., 1987; Saitmacher et al., 1994) and this vast repository of phthalocyanine synthetic knowlege remains under-utilized by synthetic phthalocyanine chemist and scientist interested in employing phthalocyanines in high-tech applications. Phthalocyanine dyes have found high-tech utility in ink jet printing, where dyes that are soluble at alkaline pH and insoluble at the more acidic pH of the paper surface (pH 4.5-7.0) are preferred (Gregory, 1999; Gregory,

2000; Shawcross et al., 2003). Currently, research is underway to prepare phthalocyanine zwitterions containing amine and sulphonic acid groups wherein at the pH of paper, the amine is protonated and the dye becomes insoluble.

Phthalocyanines exhibit an increase in conductivity upon illumination with light. This photoconductivity was first observed in 1948 (Vartanyan, 1948) and has been exploited with phthalocyanines being employed as photoconducting agents in photocopying devices and laser printers (Law, 1993; Nguyen, 1994; Haisch, 1997; Gregory, 2000). A number of crystallographic forms of metal-free phthalocyanines, titaniumoxo phthalocyanines and vanadiumoxo phthalocyanines absorb in the near-infrared region of the spectrum and offer excellent photoconductivity when used in conjunction with cheaper semiconducting diode lasers (Law, 1993).

Uniform films composed of a suitable phthalocyanine derivative deposited on a smooth reflective metal have been shown to absorb highly focused laser light to form well-defined microscopic deformations. Such deformation or pits are used to record data on recordable compact disk (Seto et al., 1996; Berneth et al., 2004; Stawitz et al., 2004). Phthalocyanine dyes are more stable and have preferable properties for such data storage as compared to cyanine and azo dyes and are thus becoming the dye of choice for recordable compact disk (see http://mitsuicdr.com/). Phthalocyanine dyes are more responsive to the writing laser, thus giving cleaner, better defined pits and are more transparent, contributing to the high reflectivity of phthalocyanine recordable compact disk. It has also been estimated that recordable compact disk employing phthalocyanine dyes should reliably store data for over 100-150 years compared to the 20 years estimated for disk utilizing azo or cyanine dyes (http://mitsuicdr.com/). In addition, the

photophysical, photochemical and structural properties of phthalocyanines may allow for superresolution read-out and photochemical hole burning, which would significantly improve recording density (by up to two or three orders of magnitude) in order to prepare recordable compact disk capable of recording significantly more data (Seto et al., 1996).

The large, delocalized π-electron cloud, planar structure and metal-chelating capability of phthalocyanines have been employed to prepare catalyst for numerous and varied chemical reactions. Phthalocyanines chelating transition metal ions are known to efficiently catalyze numerous chemical reactions, in particular redox reactions and have found important roles in a number of important industrial processes. In fact, phthalocyanines are the only tetrapyrrolic compounds used as an industrial catalyst with cobalt phthalocyanine derivatives employed as the catalyst in the Merox process (Douglas, 1978; Salazar, 1986; Kaliya et al., 1999; Navid et al., 1999). The Merox process has important environmental implications as it involves the oxidation and removal of sulfur compounds from gasoline and petroleum products (see Figure 1.10).

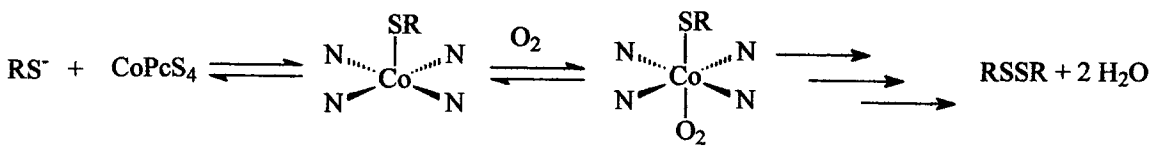

Figure 1.10. Schematic representation of the Merox process

Phthalocyanine-based catalysis are also in development for a new type of heterogeneous catalyst for removal of sulfide ion from waste water and in a new variant of Altax vulcanization accelerator process which allows for the use of O_2 instead of the

environmental unfriendly NO_2 as the oxidant (Kaliya et al., 1999). Investigations are also underway for the photooxidative degradation of organic pollutants from water using water-soluble phthalocyanines (Schneider et al., 1994; Tao et al., 2002).

Phthalocyanines are particularly useful in the electroreduction of oxygen by hydrogen and thus have been extensively investigated as catalyst in fuel cells (Jasinski, 1968; Randin, 1974; Moser et al., 1983; Hempstead et al., 1987; Janda et al., 1989; Ouyang et al., 1991; McKeown, 1999; Wöhrle, 2001). While the electroreduction of oxygen would ideally lead to the production of water via a four-electron reduction mechanism, the electroreduction of oxygen typically proceeds via a two-electron reduction to yield peroxide ion. This process can be used as the basis of cheaper fuel cells using metallophthalocyanines adsorbed onto suitable electrodes (such as highly oriented pyrolytic graphite electrodes) instead of the more expensive conventional used platinum electrodes. It has been suggested that the electroreduction proceeds via the adsorption of molecular oxygen onto the phthalocyanine as an axial ligand of the central metal ion (Beck, 1973, Moser et al., 1983). The phthalocyanine is then oxidized, leading

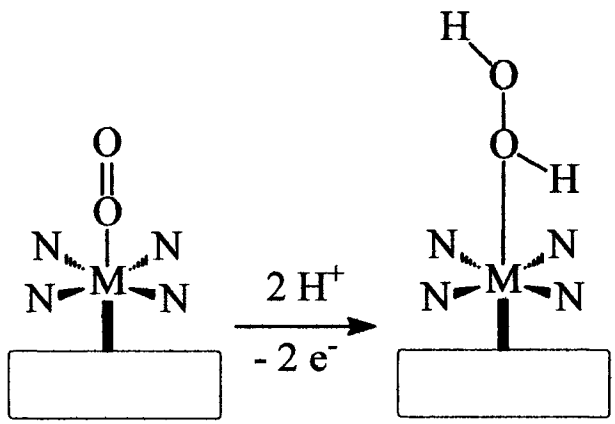

Figure 1.11. Mechanism of phthalocyanine catalysis in fuel cells.

to the production of the reduced oxygen species. The phthalocyanine is then reduced in an electrochemical follow-up step (see Figure 1.11). This view of the catalytic activity of phthalocyanines is based on the observation that strong ligands such as CN^-, PO_4^{-2} and ethylenediaminetetraacetic acid (EDTA) effectively poison the catalyst by displacing oxygen from the axial ligand position and preventing oxygen binding to this reactive site (Kozawa et al., 1971; Beyer et al., 1972). It has also been observed that catalytic activity greatly depends on the crystal structure of the phthalocyanine (Moser et al., 1983) with the planar oriented α crystal form having higher catalytic activity as compared to the more diagonally aligned β form. The molecular orientation of the phthalocyanine also is important in the overall efficiency of the electroreduction with phthalocyanines bearing four peripheral crown ether substituents exhibiting the desirable four-electron reduction because of their tendency to adsorb uniformly onto graphite (Kobayashi et al., 1992).

Phthalocyanines are also known to catalyze other reactions (Moser et al., 1983; Chen et al., 1996; Kasuga, 1996), including the oxidation of alkanes, alkenes and alkynes (Middleton et al., 1986; Ellis et al., 1992; Pérollier et al., 2002), the hydroformylation of olefinic compounds (Homeier, 1979) and even as a catalyst in a method for the prevention of dye transfer in washing or bleaching detergents (Johnston et al., 1979; Fredj et al., 1994). Unfortunately, the synthetic utility of these catalysts is somewhat limited due to self-oxidation of the phthalocyanine chromophore which significantly reduces their catalytic efficiency. This problem has been overcome by incorporating the phthalocyanine into the cavity of zeolite by undertaking the cyclotetramerization reaction of phthalocyanine precursors within the cavity. The resulting phthalocyanine is too large

to escape the cavity and is isolate from other phthalocyanines, significantly decreasing phthalocyanine degradation by oxidation. However, small organic molecules are able to enter the porous lattice of the zeolite and can be efficiently oxidized by the phthalocyanine catalyst.

The redox properties of the phthalocyanine molecule can also be altered by adding suitable substituents to the phthalocyanine periphery. Alkoxy groups (Sakamoto et al., 1999), ferrocene (Jin et al., 1994; Poon et al., 2001), tetrathiafulvalenes (Blower et al., 1996; Wang et al., 1997), buckminsterfullerene derivatives (Sastre et al., 1999; Goulomis et al., 2000), dendritic groups (Kimura et al., 1999), chelating groups (Altuntaş Bayir et al., 1997; Kandaz et al., 1997) and crown ethers (Gümüs et al., 1992) have been added to phthalocyanines and have been used to alter the redox properties of the molecule. With the physical similarities with naturally occurring porphyrins, phthalocyanines have been investigated as synthetic analogs of naturally occurring enzymes. Zeolite-entrapped phthalocyanine incorporated into a polymer membrane mimics the behavior of cytochrome P-450, an enzyme that plays a vital role in a number of important biological functions (Parton et al., 1994). In addition, phthalocyanines have been studies as possible dual function mimic enzymes of superoxide dismutase and catalase, both vital biological antioxidants (Feng et al., 2001) and phthalocyanines have been reconstituted with hemoproteins (Neya, 1996). These raise the possibility of preparing synthetic analogs to naturally occurring and biological vital enzymes and macromolecules using phthalocyanines.

Even with the realized applications mentioned above, the full potential of phthalocyanines remains relatively untapped. Due to their unique physical, chemical and

electronic properties, phthalocyanines have been extensively investigated in such wide-ranging and diverse fields as electrochromism (Nicholson, 1980; Bardin et al., 1989), photovoltaic junctions (Wöhrle et al., 1996; Forrest et al., 2004), solar cells (Tang, 1986; Flatz et al., 1994; Nazeeruddin et al., 1998; Lane et al., 2000; Tsuzuki et al., 2000; Yanagisawa et al., 2002), molecular metals (Martinsen et al., 1984; Achar et al. 1999), liquid crystals (van Nostrum et al., 1995; McKeown, 1999; McKeown et al., 1999; Gürek et al, 2000), Landmuir-Blodgett films (Cho et al., 1988; Burghard et al., 1994a; Burghard et al., 1994b; Kobayashi et al., 1994; Kenney et al., 1995; Fouriaux et al., 1996; Davidson et al., 2001), ionoelectronics (Piechocki et al., 1982; Sielcken et al., 1987; Toupance et al., 1994; van Nostrum et al., 1995), functional polymers (Gotoh et al., 1989; McKeown, 2000; Wöhrle, 2000; Wöhrle, 2001), semiconductors (Clarisse et al., 1991; Bao, 1999), photodynamic therapy (van Lier et al., 1989; van Lier, 1990; Rosenthal, 1996; Kenney et al., 1996; Ali et al., 1999; Allen et al., 2001) and non-linear optical applications (Nalwa et al., 1996; de la Torre et al., 1997; de la Torre et al., 1998; Claessens et al., 2001; Dini et al., 2001; Hanack et al., 2001; de la Torre et al., 2004). While phthalocyanines have shown potential in all of these fields, the utility and importance of phthalocyanines in non-linear optical applications is particularly impressive. Following the discovery of the laser, considerable research has been undertaken in photonics, wherein light photons are used to acquire, store, transmit and process information instead of electrons (electronics). In order to manipulate the optical signals used in photonics, materials exhibiting non-linear responses to light are needed as high speed electro-optical and all optical switches and modulators. In addition, materials with non-linear optical properties have applicability in high density data storage, phase conjugation, holography, spatial light

modulators and in laser frequency conversion devices such as optical parametric oscillators and second and third harmonic generators (Nalwa et al., 1996). While early stages of research employed inorganic materials, organic materials exhibiting non-linear optical properties have markedly increased and exhibit a number of advantages including sub-picosecond response times, large non-linearities, low losses, small dielectric constants and greater synthetic versatility. Phthalocyanines, with their highly polarizable and conjugated π-electron system, have ideal properties for the development of non-linear optical materials (Nalwa et al., 1996). In addition to their incredible stability, phthalocyanines provide tremendous architectural flexibility, allowing for engineering of the non-linear optical response, which may be based on several varied mechanisms. Phthalocyanines are known to provide large nonlinearities with sub-picosecond response times and the absorption losses are small over wide regions of the near IR spectral range, reducing the power requirements and heat load for non-linear optical devices comprising phthalocyanines. The centrosymmetry of the phthalocyanine molecule is ideal for third harmonic generation and the third order non-linear response of phthalocyanines has been extensively investigated (Shirk, 1996; de la Torre et al., 1998). However, second harmonic generation requires non-centrosymmetry and a strong molecular dipole (Shirk, 1996; de la Torre et al., 1997; de la Torre et al., 1998). As such, phthalocyanines would seem to lack the necessary properties for second order non-linearity. However, theoretical calculations have suggested that asymmetrically substituted push-pull phthalocyanines bearing both electron-donating and electron-withdrawing functionality may provide efficient intramolecular charge transfer and should yield compounds with interesting second order non-linear optical properties. However, to date, the second order

non-linear optical properties of such phthalocyanines have only been studied in a limited number of cases (Shirk, 1996; de la Torre et al., 1997; de la Torre et al., 1998), the result of the difficulty in the synthesis and purification of asymmetrically substituted phthalocyanines. In terms of non-linear optical properties, phthalocyanines have found utility in optical limiting, wherein materials increase their absorbance when illuminated with high intensity light (Dini et al., 2001; Hanack et al., 2001). The ability to fine-tune the absorbance of phthalocyanines over the range 650-850 nm should allow for optical limiting devices specifically designed for particular lasers. Overall, the size of the non-linear response of organic materials has been too modest for commercial utility. However, phthalocyanines clearly have utility as third order non-linear optical materials and as optical limiters. In addition, new synthetic pathways towards asymmetrically substituted phthalocyanines should lead to organic materials with utility as second order non-linear optics.

1.5 Photodynamic Therapy

Cancer is one of the leading causes of disease and death in Canada. It is estimated that 149 000 new cases of cancer will be diagnosed and 69 500 deaths as a result of cancer will occur in Canada in 2005 (www.cancer.ca). This makes cancer the leading cause of premature death in Canada. Based on current incidence rates, 38% of Canadian women and 44% of Canadian men will develop some form of cancer during their lifetimes. Among the leading causes of cancer-related deaths are lung cancer, colorectal cancer, breast cancer, prostate cancer, non-Hodgkin's lymphoma, leukemia, bladder cancer, esophagus cancer and stomach cancer.

Traditional cancer therapies such as surgery, chemotherapy and radiation therapy involve a delicate balance between removing or destroying diseased tissue and sparing surrounding healthy cells. These conventional treatments result in serious side effects caused by the loss of normal cell function since traditional cancer therapies exhibit relatively indiscriminate cytotoxic properties. Surgery, for instance, involves a delicate balance between removing enough tissue to completely eliminate the malignant cells while preserving healthy tissue, thus maintaining tissue function while avoiding possible foci for future tumor development. Chemotherapy and radiation therapy, on the other hand, do not exhibit appreciable selectivity towards malignant cells, resulting in the destruction of normal, healthy cells. Consequently, the development of new treatment protocols that display improved selectivity for diseased tissue is required.

Among the more promising new therapies for cancer are binary therapies such as binary gene therapy (Pirocanac et al., 2002; Fretya et al., 2004; Gridley et al., 2004; Isayeva et al., 2004; Saukkonen et al., 2004; Buchsbaum et al., 2005), radiosensitizers

(Skov et al., 1994; Mehta et al., 2000; Schaffer et al., 2003; Weinmann et al., 2003), neutron capture therapy (Gahbauer et al., 1998; Barth et al., 1999; Diaz et al., 2000; Barth, 2003) and photodynamic therapy (Moore et al., 1997; Oschner, 1997; Dougherty et al., 1998; Bonnett, 1999; Gudgin Dickson et al., 2002; Lukšienė, 2003; Brown et al., 2004; Detty et al., 2004). The primary advantage of binary therapies such as these is that each component of these two component systems is innocuous by themselves and must be combined in order to produce cytotoxic effects. Greater selectivity is thus achieved since each component can be manipulated independently and only cells simultaneously exposed to all the necessary components are exposed to the cytotoxic effects.

Photodynamic therapy is based on the dye-sensitized photooxidation of biological matter in a target tissue and by definition requires three components: a photosensitizer (a compound capable of absorbing light of a particular wavelength and transforming the light energy into cytotoxic agents), light of the appropriate wavelength and molecular oxygen. The elegance of photodynamic therapy is the same as for other binary therapies. Both the photosensitizer and the light are harmless by themselves. However, when combined, in the presence of oxygen, they can produce lethal cytotoxic species that can inactivate tumour cells. As such, photodynamic therapy provides for a dual selectivity, with preferential tumour uptake of the photosensitizer and the ability to illuminate the target tissue precisely allowing for the activation of photosensitizer only in the tumour volume.

The photochemical and photophysical mechanism involved in photodynamic therapy have been extensively investigated (Oschner, 1997; Philips, 1997). Briefly, upon

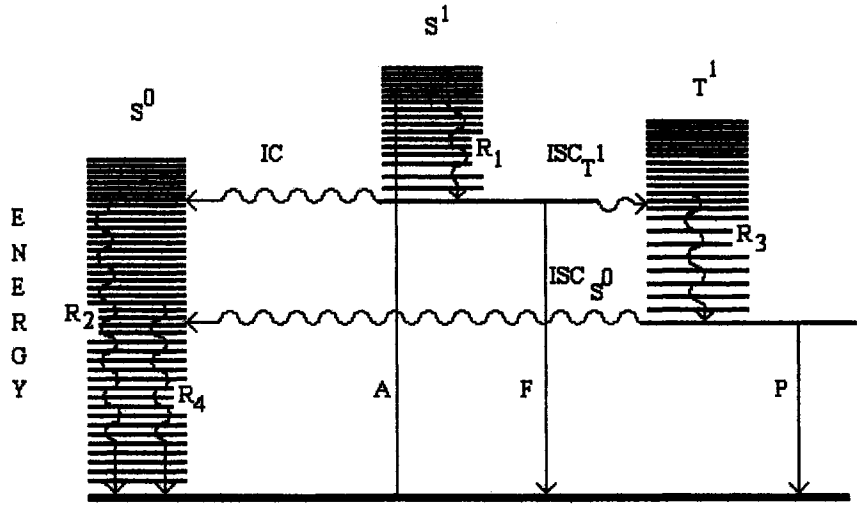

Figure 1.12. Jablonski Diagram illustrating some of the physical processes that can occur after a molecule absorbs a photon, excited state levels and transitions. S^0 is the ground electronic state of the molecule. S^1 and T^1 are the lowest excited singlet and triplet states, respectively. Straight arrows represent processes involving photons and wavy arrows represent radiationless transitions. (A), absorption; (F), fluorescence; (P), phosphorescence; (IC), internal conversion; (ISC), intersystem crossing; (R), vibrational and rotational relaxation (adapted from Philips, 1997).

illumination with light of the appropriate wavelength, the photosensitizer is electronically excited from the ground state S_0 to its first excited singlet state (S_1) (see Figure 1.12). This short lived excited state can dissipate its energy by radiative decay (fluorescence (F)), which may be used to monitor sensitizer distribution both *in vitro* and *in vivo*. The excitation energy of the S_1 states can also be lost by non-radiative internal conversion (IC), which entails the loss of energy via collusions with surrounding molecules, resulting in the generation of heat. It has been suggested that photothermal effect such as those

involved in internal conversion may be one of the more important mechanisms for photosensitized cell killing. For instance, illumination of cells stained with merocyanine 540 may increase the internal cell temperature as much as 12°C/minute provided the cell membrane acts as an adiabatic sink (Davila et al., 1991). However, the most important transition in terms of photodynamic therapy is intersystem crossing (ISC), resulting in the population of the much longer lived first excited triplet electronic state (T_1) of the photosensitizer. Lifetimes of the first excited triplet electronic state of photosensitizers are typically in the micro- to millisecond range as the $T_1 \rightarrow S_0$ transition is spin-forbidden (Oschner, 1997). This longer lifetime allows for efficient interaction between the electronically excited photosensitizer and surrounding molecules and it is accepted that the excited triplet state of photosensitizers is responsible for the generation of the cytotoxic species produced during PDT.

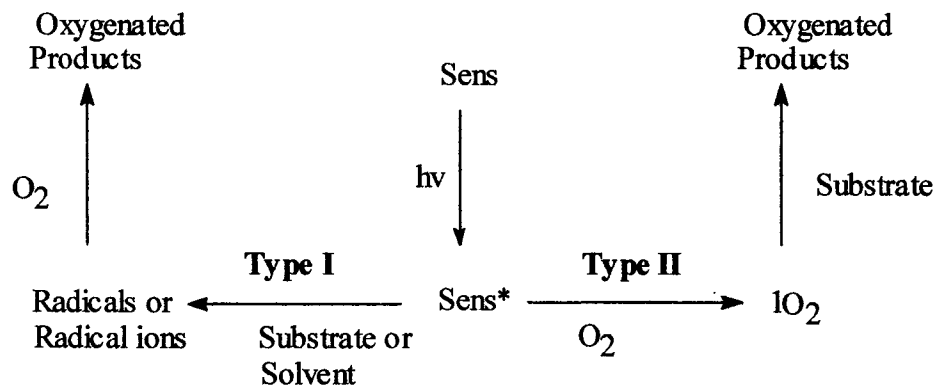

Figure 1.13. Diagrammatic presentation of Type I and Type II photosensitized oxidation reactions (Foote, 1991).

The excited triplet electronic state of the photosensitizer may react with its surroundings in two ways, defined as Type I and Type II mechanism (Figure 1.13) (Foote, 1991). A Type I mechanism involves hydrogen atom extraction or electron transfer reactions between the excited triplet state of the photosensitizer and a surrounding substrate (a biological molecule, solvent or another photosensitizer), yielding free radicals and radical ions. These radicals are highly reactive and efficiently react with molecular oxygen to give reactive oxygen species such as superoxide anion or hydroxyl radicals or to fix the damage, thus making it irreparable. These reaction results in oxidative damage that ultimately lead to cellular inactivation.

By contrast, a Type II mechanism results in an energy transfer from the first excited triplet electronic state of the photosensitizer and ground-state molecular oxygen, generating singlet oxygen. This highly reactive oxygen species can react with a large number of biologically important substrates, causing oxidative damage and ultimately, cell death. While it is generally accepted that Type II mechanisms predominate during photodynamic therapy and that singlet oxygen is the most important cytotoxic agent involved, Type I reactions become important at low oxygen concentrations and in more polar environments (Oschner, 1997). However, both Type I and Type II reactions lead to similar oxidative damage and comparable free radical chain reactions. For a more in-depth review of the mechanisms involved in photodynamic therapy and the role of reactive oxygen species in the cytotoxic effects observed during photodynamic therapy, please see Chapter 2 (Sharman W. M., C. M. Allen and J. E. van Lier (2000) Role of activated oxygen species in photodynamic therapy, *Methods Enzymol.*, **319**, 376-400).

While it is evident that photodynamic therapy can induce the production of cytotoxic species that can readily destroy neoplastic cells, the *in vivo* response is affected by the complexity of biological systems (Mason, 1999). Any number of subcellular targets, including the mitochondria, lysosomes, plasma membrane and nuclei, can be targeted (Moore et al., 1997; Dougherty et al., 1998). Cell death may occur by necrosis or apoptosis (Jori et al., 1998; Olenick et al., 2002). In addition, the cytotoxic effects of photodynamic therapy induce numerous signaling pathways which dictate the cellular response to this cytotoxic treatment (Moor, 2000). In addition, while it has been shown that the action of some amphiphilic photosensitizers proceeds via direct tumor cell death, most photosensitizers that have been investigated induce tumour necrosis via vascular shutdown (Henderson et al., 1989; Margaron et al., 1996a; Moore et al., 1997; Oschner, 1997). In addition, photodynamic therapy can induce inflammation and other tumour-specific immune reactions (Oschner, 1996; Moore et al., 1997; Dougherty et al., 1998) and has been investigated as a potential method of inducing tumour immunity (van Duijnhoven et al., 2003). The exact method of photodynamic therapy-induced tumour destruction depends greatly on the photosensitizer used as well as the light dose and the conditions being treated. However, understanding the biological mechanisms involved will enable the design and synthesis of ideal photosensitizers for a given condition or disease.

The first generation photosensitizer is Photofrin®, a haematoporphyrin derivative originally synthesized by combining haematoporphryin with 5% sulphuric acid in acetic acid at room temperature, followed by treatment with aqueous base and neutralization. This gives a complex mixture of haematoporphyrin dimers and oligomers, primarily

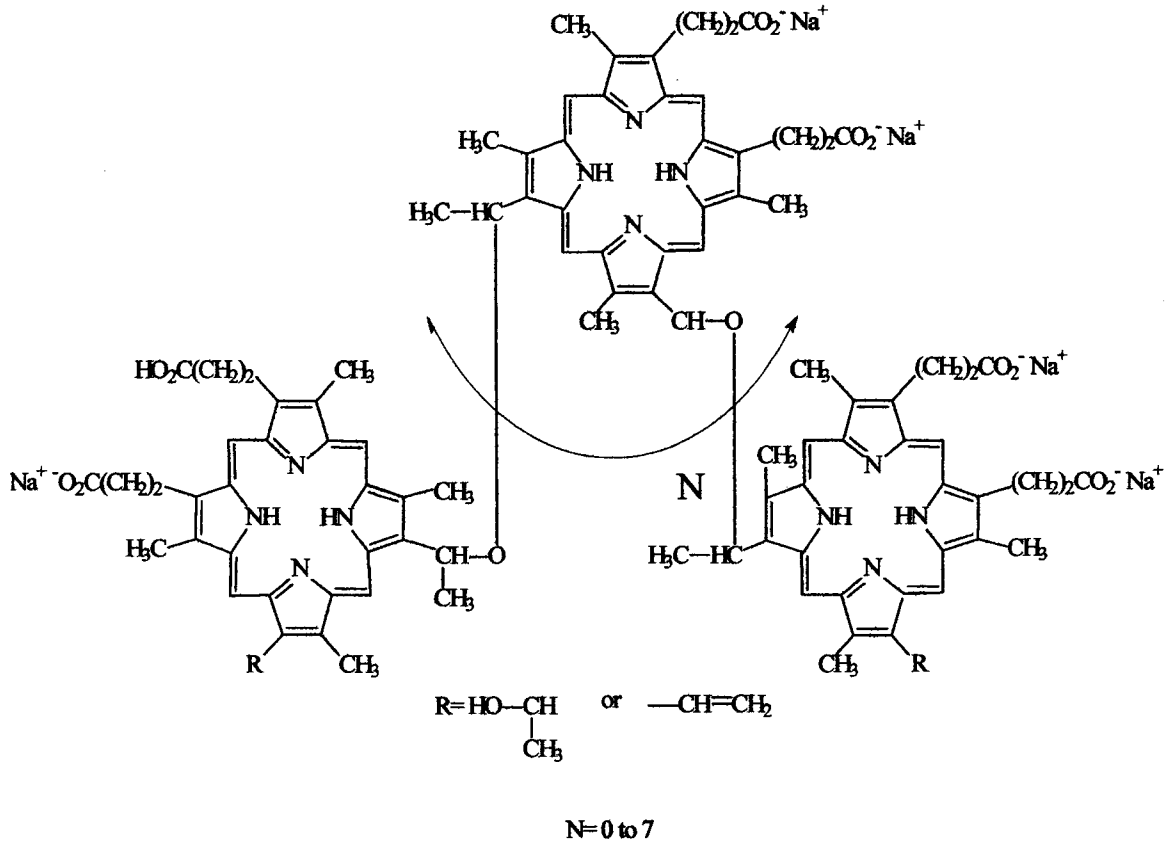

Figure 1.14. Photofrin® structure consisting of a complex mixture of dimers and oligomers ranging from two to nine porphyrin units linked via ether or ester bonds.

attached by ester and ether linkages (see Figure 1.14) (Bonnett, 1995). Partial purification of the more active oligomers by HPLC or size exclusion gel chromatography leads to Photofrin®, which is 90-95% active component (Dougherty et al., 1992). Photodynamic therapy using Photofrin® has been accepted in clinic in several countries for the treatment of early and late stage lung cancer, superficial and advanced oesophageal cancer, bladder cancer, superficial and early stage gastric cancer, early stage cervical cancer and cervical dysplasia. It has also recently been approved for the ablation of high-grade dysplasia in Barrett's esophagus patients (www.photofrin.com). However,

despite the apparent success of Photofrin®, haematoporphyrin derivatives have a number of serious disadvantages in terms of their use as photosensitizers for photodynamic therapy (Philips, 1997; Kessel et al., 1999). Photofrin® is a complex chemical mixture of oligomers that can vary with different preparations and storage times and that makes structure-activity relationships impossible to determine. Haematoporphyrin derivatives are readily taken up and retained by cutaneous tissue for up to ten weeks after administration, causing a marked skin photosensitivity that requires the patient to avoid bright sunlight. This is an obvious disadvantage, particularly for patients with late-stage malignancies. In addition, haematoporphyrin derivatives have only a weak absorption at the therapeutic wavelength of 630 nm, limiting treatment to tumour depths of no more than 5 mm. With these disadvantages in mind, a number of second generation photosensitizers have been developed and investigated from their potential as photosensitizers for photodynamic therapy. An ideal photosensitizer for photodynamic therapy should (MacRoberts et al., 1989; Bonnett, 1996):

1) be chemically pure and of known and constant composition.

2) have a minimal dark toxicity and only be cytotoxic in the presence of light of the appropriate wavelength.

3) be preferentially retained by the target tissue.

4) be rapidly excreted from the body, thus inducing a low systemic toxicity.

5) have high photochemical reactivity, with high triplet state yields and long triplet state lifetimes and be able to effectively produce singlet oxygen and other reactive oxygen species upon illumination.

6) have a strong absorbance with a high extinction coefficient at a longer wavelength (600-800 nm) where tissue penetration of light is at a maximum while still being energetic enough to produce singlet oxygen and where cheaper diode laser can be employed.

While no photosensitizer can be deemed to be ideal for every possible application of photodynamic therapy, a number of second generation photosensitizer have been investigated with the hopes of overcoming the shortcomings of Photofrin® while taking advantages of their more ideal properties. These second generation photosensitizers include methylene blue, verteporfin, tin etiopurpurin, temoporfin, texaphryins, phthalocyanines, n-aspartyl chlorin e6, rhodamines, hypericin and 5-aminolaevulinic acid, a natural precursor in the endogenous production of protoporphyrin IX. For a complete review of the various compounds that have been investigated as photosensitizers for photodynamic therapy and the conditions possibly treated by each of these photosensitizers, please see Chapter 3 (Sharman W. S., C. M. Allen and J. E. van Lier (1999) Photodynamic therapeutics: Basic principles and clinical applications, *Drug Discovery Today*, **4**, 507-517).

While photodynamic therapy has been primarily considered as a treatment for cancer, preclinical and clinical investigations have been undertake for the treatment of a number of other diverse conditions. Photodynamic therapy using 5-aminolaevulinic acid, (a precursor to the nature production of protoporphyrin IX, which is itself a precursor in the biosynthesis of haem) has been accepted in clinic for the treatment of actinic keratoses, a sun-induced precancerous skin lesion (www.dusapharma.com). Methylene

blue is currently used in clinic by the Swiss and German Red Cross for the photodynamic decontamination of freshly frozen plasma units (Mohr et al., 1993; Mohr et al., 1995). Most interesting however is the clinical utility of verteporfin in the treatment of wet age-related macular degeneration (AMD) (www.qlt-pdt.com). Wet age-related macular degeneration involves the rapid growth of abnormal blood vessels under the central retina, with leakage from these underdeveloped vessels causing swelling and scarring. This ultimately leads to vision loss. Verteporfin, like many other photosensitizers, is capable of inducing vascular stasis upon illumination and treatment of AMD with verteporfin and light of the appropriate wavelength (690 nm) effectively closes off the abnormal blood vessels and stops the progression of the disease (Miller et al., 1999; Schmidt-Erfurth et al., 1999; Bressler et al., 1999; Fine, 1999; Azab et al., 2005). While the photodynamic treatment of AMD does not repair destroyed photoreceptors, it does halt the progression of the disease by easing swelling and maintaining vision. With a common mechanism of action, other photosensitizers have also been under investigation as photosensitizers in the treatment of AMD (Gohto et al., 2000; Obana et al., 2000; Sessler et al., 2000). Verteporfin and other photosensitizers have also been examined in the treatment of other conditions caused by abnormal choroidal neovascularization as well as other conditions of the eye (Donati et al., 1999; Okunaka et al., 1999; Rivellese et al., 1999; Gohto et al., 2000; Sickenberg et al., 2000). Photodynamic therapy has also been investigated in the treatment of a number of dermatological conditions including psoriasis, acne, viral warts, alopecia areata, port-wine stains, hair removal and hair loss (Fritsch et al., 1998; Boehncke et al., 2000; Simkin et al., 2003; Touma et al., 2003; Kimura et al., 2004; Evans et al., 2005; Schroeter et al., 2005). Among other conditions

that have been treated with photodynamic therapy are cardiovascular disease (photoangioplasty of vascular atherosclerotic and restenotic lesions) (Rockson et al., 2000; Sessler et al., 2000), rheumatoid and inflammatory arthritis (Trauner et al., 1996; Okunaka et al., 1999; Hendrich et al., 2000), autoimmune diseases (Leong et al., 1996a; Roy et al., 2001), menorrhagia (endometrial ablation) (Brown, 1998; Mhawech et al., 2003; Degen et al., 2004), microbial (Bertoloni et al., 1992; Ichinohe et al., 1998; Roncucci et al., 2001; Habi et al., 2002a; Soncin et al, 2002; Dupouy et al., 2004; Jori et al., 2004) and viral infections (Diwu et al., 1994; Kempf et al., 1997; Okunaka et al, 1999; Habi et al., 2002a; Wainwright, 2003). Photodynamic therapy has also been shown to be useful in bone marrow purging (Gaboury et al., 1996; Gaboury et al., 1998; Okunaka et al., 1999; Habi et al., 2002a; Habi et al., 2002b; Huang et al., 2005), the sterilization of blood components (Allen et al., 1995; Wainwright et al., 2002; Horowitz et al., 2003; Wagner et al., 2003; Trannoy et al., 2004), the prevention of transplant rejection (LaMuraglia et al., 1995; Honey et al., 2000; Roy et al., 2001) and the treatment of multiple sclerosis (Leong et al., 1996b). Essentially, conditions to which there is easy assess to light can be treated using photodynamic therapy. For a more complete review of the diseases and conditions that can be treated with photodynamic therapy, please see Chapter 3 (Sharman W. S., C. M. Allen and J. E. van Lier (1999) Photodynamic therapeutics: Basic principles and clinical applications, *Drug Discovery Today*, **4**, 507-517).

As has been previously mentioned, part of the selectivity demonstrated during photodynamic therapy is due to a preferential uptake of the photosensitizer in the target tissue. In terms of the treatment of cancer, the preferential uptake of the photosensitizer

by the tumor most likely involves a combination of factors specific for malignancies. These include increased malignant cell metabolism, leaky tumour vasculature, poor lymphatic drainage, lower intratumoural pH, cellular heterogeneity within the tumor and increased LDL receptors on malignant cells (Henderson et al., 1992; Pass, 1993; Hamblin et al., 1994a; Stables et al., 1995; Dougherty et al., 1998). Despite these factor, however, most first and second generation photosensitizer exhibit only marginally higher tumour retention as compared to surrounding healthy tissues. For instance, only 0.1-3% of the injected dose of haematoporphyrin derivative accumulates in the tumour tissue (Wöhrle et al., 1998) while tumour-to-normal tissue ratios for most photosensitizers range from 2:1 to 5:1 (Pass, 1993). In order to improve photosensitizer delivery to target tissues, a number of different photosensitizer delivery vehicles and photosensitizers conjugated to targeting molecules have been developed (Klyashchitsky et al., 1994; Niamien Konan et al., 2002; Allen et al., 2002). Polymeric micelles (Taillefer et al., 2000; van Nostrum, 2004), liposomes (Morgan et al., 1989; Richter et al., 1993; van Leengoed et al., 1994; Love et al., 1996; Renno et al., 2001; Derkycke et al., 2004), nanoparticles (Allémann et al., 1995; Allémann et al., 1996; Russell et al., 2003), microsphere (Bachor et al., 1991) and cyclodextrins (Ruebner et al., 1997; Ruebner et al., 1999) have all been used as vehicles for the delivery of photosensitizers. Photosensitizers conjugated to polymers (Soukos et al., 1997; Hamblin et al., 1999; Lu et al., 1999), antibodies (Morgan et al., 1989; Carcenac et al., 1999; Del Governature et al., 2000; Vrouenraets et al., 2000; van Dongen et al., 2004), viral proteins (Allen et al., 1999), serum proteins (Hamblin et al., 1994b; Larroque et al., 1996; Nagae et al., 1998; Hamblin et al., 2000; Urizzi et al., 2001; Cavanaugh, 2002a; Cavanaugh, 2002b), growth factors (Gijsens et al., 1998; Lutsenko et

al., 1999; Gijsens et al., 2000), hormones (Mohr et al., 1997; James et al., 1999; Ray et al., 2001; Swamy et al., 2002) and nuclear localization signals (Bisland et al., 1999; Rosenkranz et al., 2000; Sobolev et al., 2000; Sobolev et al., 2002) have been prepared in order to improve the targeting of photosensitizers to the target tissue and also to specific intracellular sites. For a review of targeted photosensitizers and their use in photodynamic therapy, please see Chapter 4 (Sharman W. M., J. E van Lier and C. M. Allen (2004) Targeted photodynamic therapy via receptor mediated delivery systems, *Adv. Drug Delivery Rev.*, **56**, 53-76.)

1.6 Structure-Activity Relationships in Photodynamic Therapy

In addition to its photophysical and photochemical properties, the overall photodynamic efficiency of a given photosensitizer is dictated by the interaction of the photosensitizer with serum proteins, the degree of aggregation, the total cellular uptake and the subcellular localization. These factors are influenced by the chemical structure and properties of the photosensitizer, in particular by the overall charge and the lipophilicity/hydrophilicity of the molecule. It has been found that amphiphilic photosensitizers are generally more photodynamically active than the corresponding hydrophilic or lipophilic derivatives (Boyle et al., 1996a). Amphiphilicity describes photosensitizers that have both hydrophilic and lipophilic characteristics in distinct parts of the molecule. Such structural features permit these distinct portions to interact differently with their biological environment while bestowing enhanced solubility, modulating molecular aggregation, improving cellular uptake and directing intracellular localization to more photosensitive subcellular sites. It has been suggested that the favourable pharmacokinetics necessary to ensure selective tumour uptake and rapid systemic clearance is directly related to the degree of amphiphilicity of the photosensitizer (Bonnett, 1999). Both sulphonated tetraphenylporphines and sulphonated phthalocyanines exhibit similar trends in their photocytotoxicity with the more amphiphilic adjacently substituted disulphonated photosensitizers being the most photocytotoxic (Kessel et al., 1987; Brasseur et al., 1988; Brasseur et al., 1988; Berg et al., 1989; Margaron et al., 1996). While the oppositely and adjacently substituted disulphonated tetraphenylporphines ($TPPS_{2adj}$ and $TPPS_{2opp}$) (Figure 1.15) have identical

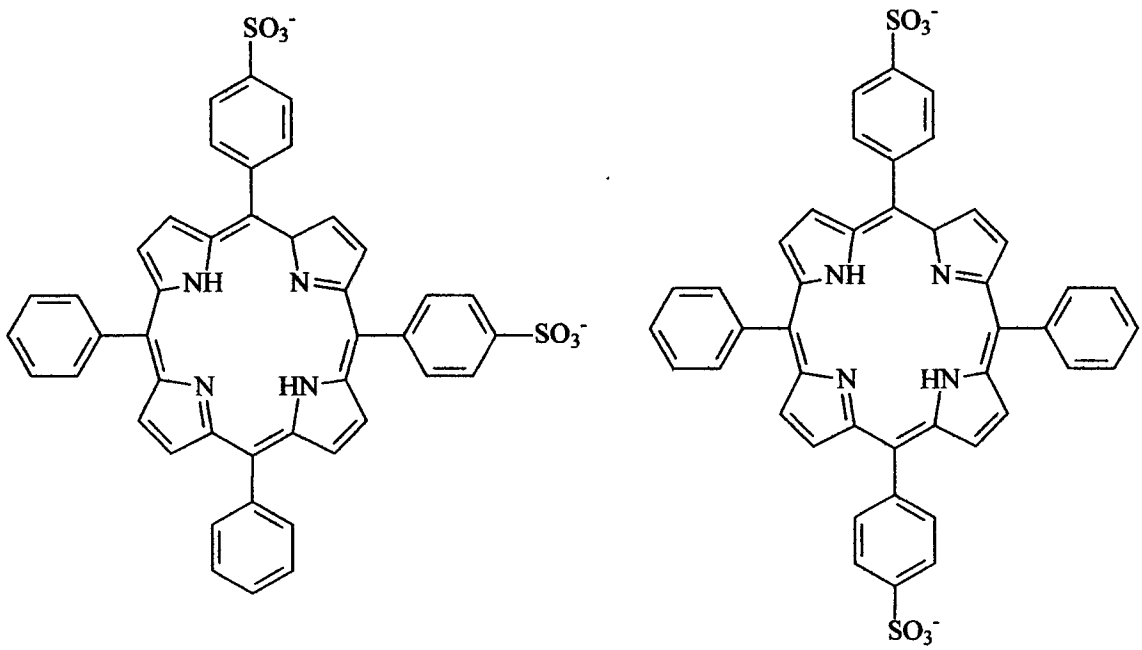

Figure 1.15. Adjacently and oppositely disulphonated 5,10,15,20-tetraphenylporphines (Kessel et al., 1987)

hydrophobicities as measured by their partition coefficients, these photosensitizers feature significantly different tumour localization and photocytotoxicities with $TPPS_{2adj}$ being significantly more photocytotoxic (Kessel et al., 1987). This clearly indicates the importance of amphiphilicity in the overall photodynamic efficiency of photosensitizers. Furthermore, while the tetrasulphonated tetraphenylporphine has the highest levels of tumor cell uptake and the most significant tumor selectivity, this compound remains the least photodynamically active member of this family of photosensitizers (Kongshaug et al., 1989). This suggests that the amphiphilicity of a photosensitizer also plays an

important role in the subcellular targeting of photosensitizer to more photosensitive organelles and subcellular sites.

The improved photodynamic activity of amphiphilic photosensitizers is not limited to anionic photosensitizers. In a study involving novel cationic photosensitizers based on either protoporphyrin or mesotetra(4-carboxyphenyl)porphine, the asymmetric cationic photosensitizers were more efficient in destroying mouse and human melanoma cells than anionic haematoporphyrin derivative (HpD) (Haylett et al., 1995). HpD was in turn more effective than the symmetrically substituted cationic photosensitizers examined. Photosensitizer cell uptake was relatively high for the cationic photosensitizers when compared to HpD. Photosensitizer cell uptake correlated with the partition coefficient of the asymmetrically substituted protoporphyrin derivatives (Haylett et al., 1996). However, cell-associated uptake did not correlate with clonogenic cell survival. Interestingly, while the photophysical properties (including singlet oxygen quantum yields) did not seem to be responsible for the improved photodynamic efficiency of amphiphilic sulphonated phthalocyanines (Allen et al., 2002; Cauchon et al., 2005), a broad association was found between singlet oxygen quantum yield and clonogenic cell killing for the series of asymmetrically substituted protoporphyrin derivatives (Haylett et al., 1997). This is in contrast with the knowledge that monomeric phthalocyanine molecules with identical central metal atoms and varying degrees of sulfonation retaining the same photochemical activity (Wagner et al., 1987). However, this apparent link between singlet oxygen quantum yield and clonogenic cell killing may be due to aggregation of the more lipophilic photosensitizers, with aggregation known to

decrease singlet oxygen quantum yields and to decrease cellular uptake (Haylett et al. , 1997).

In terms of phthalocyanines, it has also been found that asymmetrically substituted amphiphilic phthalocyanines are more photodynamically active than the corresponding symmetrically substituted phthalocyanines (Paquette et al., 1991a; Allen et al., 1995; Margaron et al., 1996b; Kudrevich et al., 1997; Edrei et al., 1998; Allen et al., 2002; Cauchon et al., 2005). A quantitative structure-activity relationship comparing the phototoxicity and the log of the partition coefficients (PBS and n-octanol) of sulphonated zinc phthalocyanines gave a parabola with optimal partition values corresponding to the amphiphilic adjacently substituted disulphonated zinc phthalocyanine (Margaron et al., 1996b). It should however be noted that the oppositely substituted disulphonated zinc phthalocyanines was not included in this study and is known to be significantly less photocytotoxic as compared to the adjacently substituted disulphonated zinc phthalocyanine while having a similar partition coefficient.

While more amphiphilic phthalocyanines are more photodynamically active, cellular uptake of phthalocyanines has been shown to increase with increasing lipophilicity of the molecule (Brasseur et al., 1988; Berg et al., 1989; Paquette et al., 1991a; Margaron et al., 1996b). For instance, in EMT-6 cells, the more lipophilic tetraiodinated zinc phthalocyanine displayed the most important cellular uptake followed by the amphiphilic adjacently disulphonated zinc phthalocyanine (Margaron et al., 1996b). However, despite having the highest cellular uptake, the tetraiodinated zinc phthalocyanine were only slightly phototoxic while the amphiphilic photosensitizer exhibiting the most important photocytotoxicity. Increasing the lipophilic character of

amphiphilic phthalocyanines has been shown to increase the photocytotoxicity of these photosensitizers, with addition of two t-butyl groups to adjacently substituted sulphonated phthalocyanine improving the photocytotoxicity of the photosensitizer four-fold as a result of the additional lipophilic character added by the t-butyl groups (Paquette et al., 1991b). The increase in the amphiphilic character of the gallium disulphonated phthalocyanine with the t-butyl groups not only promoted cell uptake but also resulted in improved targeting of the dye to photosensitive intracellular sites.

Similarly, cell uptake in Ehrlich ascites mouse tumour cells has been shown to correlate well with the overall hydrophobicity of the sulphonated phthalocyanine preparation and inversely with the degree of aggregation in the extracellular environment (Edrei et al., 1998). While the amphiphilic adjacently substituted disulphonated aluminum phthalocyanine ($AlPcS_{2adj}$) exhibited the highest membrane-penetrating properties, even higher cell uptake was observed for a mixture of $AlPcS_2$ comprising both oppositely and adjacently substituted disulphonated phthalocyanines. It has been suggested that this is due to a combination of optimal amphiphilicity and a lower degree of aggregation. Similar observations of the relationship between cellular uptake and aggregation of the photosensitizer have been reported (Margaron et al., 1996b). In light of this, the decreased photocytotoxicity of the more lipophilic phthalocyanines may be the result of increased aggregation of the phthalocyanine chromophore, which would lead to decreased singlet oxygen quantum yields and to decreased cellular uptake. However, the decreased photocytotoxicity may also be the result of partitioning to less photosensitive subcellular sites.

Increasing the amphiphilicity of trisulphonated phthalocyanines by the addition of a t-butyl group to the unsubstituted benzo ring has also been shown to result in a 5-40 fold increase in anti-viral potency versus vaccinia virus in red blood suspension (Allen et al., 1995). Importantly, the heightened anti-viral potency did not correlate with photohemolytic activity since these t-butyl substituted trisulphonated phthalocyanines also exhibit favourable toxicity indices, a measure of the anti-viral activity over the photohemolytic activity. These results suggest that the increased amphiphilicity of these phthalocyanines permits effective photosensitizer/viral particle interactions, negatively affecting the cell fusion function required for infectivity of the viral particle. In the meanwhile, possible photodynamically-induced structural modifications of the red blood cell membranes were only negligible.

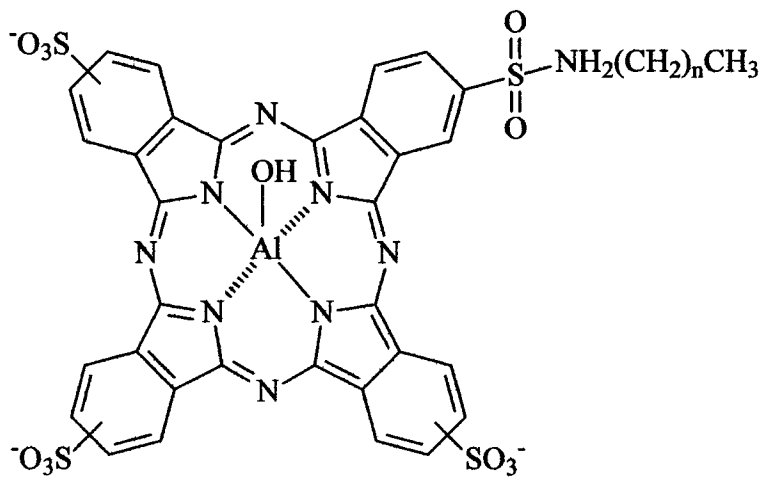

Figure 1.16. Amphiphilic derivatives of aluminum tetrasulphonated phthalocyanines substituted with long straight chain aliphatic groups via a sulfonamide bond (n = 3, 7, 11 and 15) (Allen et al., 2002)

A series of tetrasulphonated aluminum phthalocyanines or trisulphonated zinc phthalocyanines with varying degrees of hydrophilicity have been prepared by adding long straight chain aliphatic groups either via a sulfonamide bond (Urizzi et al., 2001; Allen et al., 2002) or via an alkynyl bond using palladium-catalzyed reactions (Tian et al.,

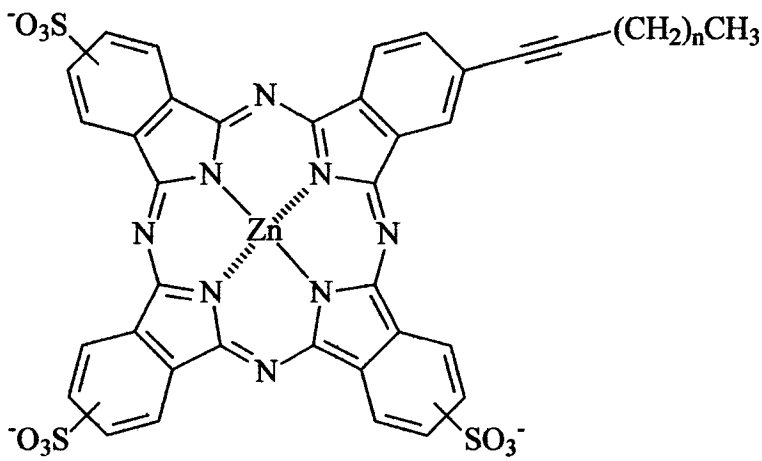

Figure 1.17. Amphiphilic trisulphonated zinc phthalocyanines substituted with long straight chain aliphatic groups via alkynyl bonds (n = 3, 6, 9 and 13) (Tian et al., 2000)

2000; Cauchon et al., 2005) (Figures 1.16 and 1.17). In both studies, the various photosensitizers gave similar singlet oxygen yields when monomerized using Cremophor™ EL (Allen et al., 2002; Cauchon et al., 2005), suggesting that aggregation is not the determining factor in the differences in photodynamic potency since phthalocyanines are known to monomerize in the presence of cellular components (Paquette et al., 1991a). Trisulphonated zinc phthalocyanines bearing hexynyl and nonynyl substituents (Figure 1.17, n = 3 or 6) exhibited high cellular uptake with

important localization at the mitochondrial membranes, which coincided with effective photocytotoxicity toward EMT-6 tumour cells (Cauchon et al., 2005). Further increasing the lipophilicity of these trisulphonated zinc phthalocyanines by increasing the length of the alkynyl chain to dodecynyl or hexadecynyl (Figure 1.17, n = 9 or 13) did not further improve the phototoxicity of the photosensitizers. This may be the result of extensive aggregation of the dye in aqueous medium, resulting in reduced cell uptake. On the other hand, when long alkyl chains are bonded to tetrasulphonated aluminum phthalocyanine via a sulfonamide bond, it was observed that both cell uptake and photocytotoxicity varied directly with the length of the alkyl chain (ie. with the lipophilicity of the dye) (Allen et al., 2002). This difference is most likely a result of the nature of the central metal atom and the bonding used to attach the long aliphatic chains to the phthalocyanines, with the axial ligand of the aluminum central metal atom and the sulfonamide bond helping to prevent aggregation in a cellular environment and allowing efficient interaction of the long aliphatic chains with the lipophilic cellular membranes. Interestingly, addition of human LDL in appropriate amounts during incubation decreased cell uptake of the more lipophilic phthalocyanines while the cytotoxic potency increased or remained unaffected (Allen et al., 2002). This seemingly indicates that the improved cytotoxicity of these amphiphilic derivatives of zinc tetrasulphonated phthalocyanine is at least in part due to improved intracellular targeting. Both of these studies indicate that amphiphilic in phthalocyanines improves their photodynamic potency by increasing cell uptake and improving subcellular trafficking to more photosensitive sites such as the mitochondria.

Increased amphiphilicity in aluminum tetrasulphonated phthalocyanines also increased the *in vivo* potential of the photosensitizer (Allen et al., 2002). Complete tumour regression was observed for aluminum tetrasulphonated phthalocyanines substituted with octyl, dodecyl and hexadecyl chains (Figure 1.15, n = 7, 11 or 15) at concentrations that failed to give a tumour response for the parent compound $AlPcS_4$. This is at least in part due to interactions of the phthalocyanines with low density lipoproteins. It is well established that many tumour cell types have increased LDL receptor expression (Ho et al., 1978; Gal et al., 1981; Lombardi et al., 1989; Gueddari et al., 1993) and that lipophilic and amphiphilic phthalocyanines associate with LDL upon administration *in vivo* (Reddi et al., 1990; Versluis et al., 1994; Reddi, 1997). It has been observed that aluminum tetrasulphonated phthalocyanines bearing a dodecyl chain via a sulfonamide bond exhibits improved photodynamic activity both *in vitro* and *in vivo* compared to the unsubstituted aluminum tetrasulphonated phthalocyanine (Urizzi et al., 2001). While incorporation of the long alkyl chains into LDL particles prior to *in vitro* administration significantly increased the *in vitro* phototoxicity, this incorporation did not affect *in vivo* results. This suggest that the long alkyl chains naturally redistribute to LDL particles upon *in vivo* administration and that this association with LDL particles is at least partially responsible for the improved photodynamic efficacy of these amphiphilic photosensitizers.

Asymmetry and the resulting amphiphilicity have also been shown to increase the photodynamic efficacy of both anionic and cationic naphthobenzoporphyrazines. These phthalocyanine derivatives have the advantage of shifting the wavelength of absorption to longer wavelengths, where tissue penetration of light is optimized. A comparison of

photodynamically-induced tumour regression of zinc (4-t-butyl)tri(4-sulfo)phthalocyanine and zinc 6-t-butylnaphthotris(4-sulfobenzo)porphyrazine indicated that the asymmetrical anionically charged napthotribenzoporphyrazine induced the best tumour response (Kudrevich et al., 1997). The asymmetrically substituted amphiphilic zinc (4-t-butyl)tri(4-sulfo)phthalocyanine caused severe damage to surrounding healthy muscle tissue under similar PDT conditions. Both of these asymmetrically substituted phthalocyanine derivatives however gave similar results in photo-inactivating cells *in vitro*, demonstrating the difficulties in extending *in vitro* results to *in vivo* tumour environments. Metallo naphthosulfobenzoporphyrazines sulphonated to different degrees have also been synthesized and their potential as photosensitizers for PDT of cancer has been evaluated (Margaron et al., 1992). *In vitro*, the disulphonated dinaphthodisulfobenzoporphyrazine proved to be slightly more photoactive than the trisulphonated naphthalotrisulfobenzoporphyrazine. In the meanwhile, the monosulphonated trinapthalosulfobenzoporphyrazine was inactive in spite of a six-fold higher cell uptake. Intriguingly, difference in the in vitro phototoxicity of the disulphonated and trisulphonated derivatives correlated well with their relative cell uptake, seeming to indicate the di- and trisulphonated derivatives experiences similar subcellular trafficking and that this trafficking differed from that the monosulphonated derivative. *Ex vivo* and *in vivo* PDT using these derivatives followed the same trend. It is important to note that while the corresponding monosulphonated phthalocyanine is less active than the disulphonated phthalocyanines, it still exhibits substantial photocytotoxicity. The lack of activity of the monosulphonated trinapthalo-sulfobenzoporphyrazine cannot be explained by a decreased cell uptake as this derivative

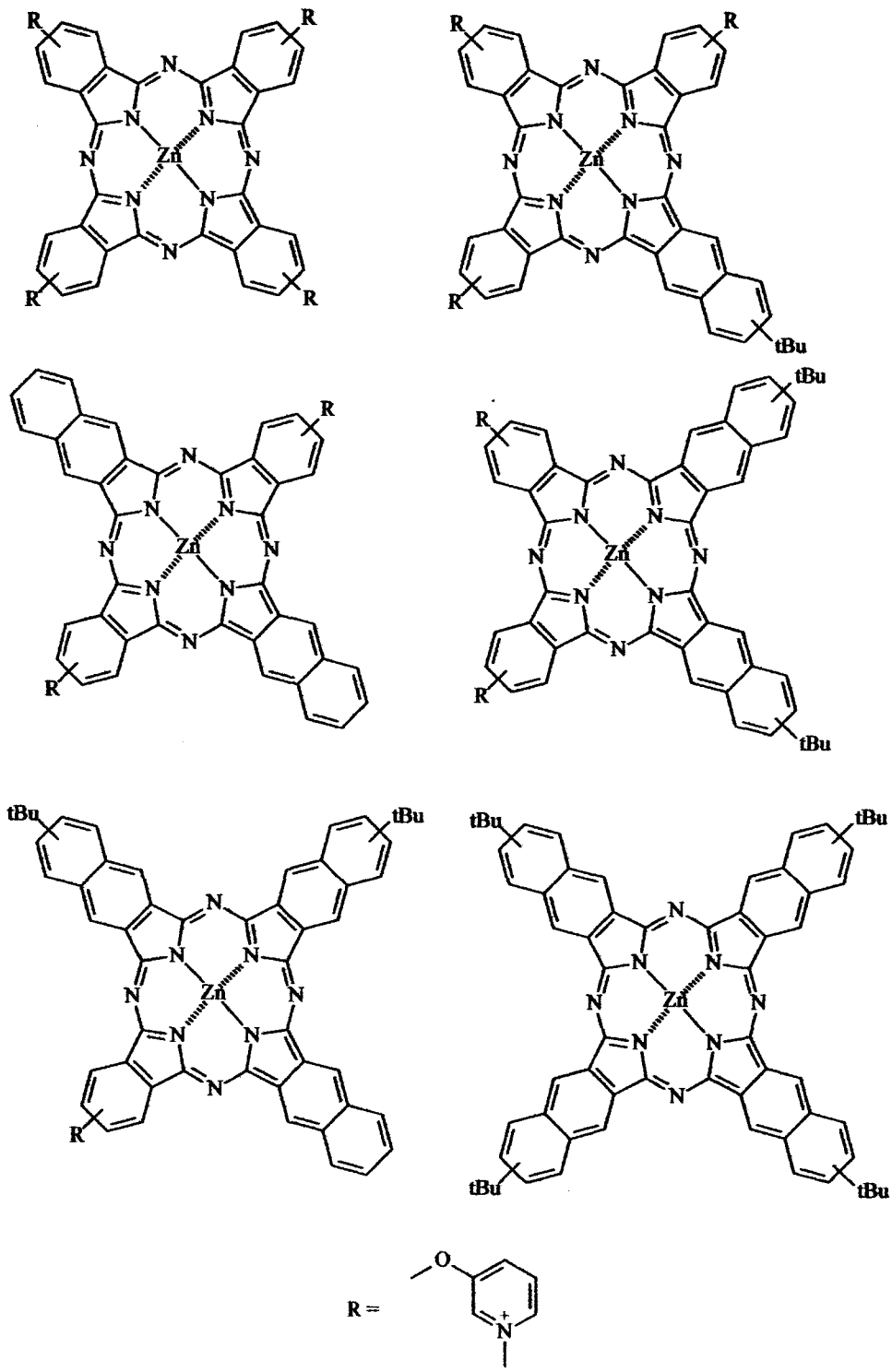

Figure 1.18. Asymmetrically substituted cationic naphthobenzoporphyrazines

actually has a six fold higher cell uptake compared to the highly phototoxic disulphonated derivative. While differences in subcellular trafficking may explain some of this inconsistency, the bulky hydrophobic naphthalo groups probably also increase aggregation, even in biological environments, with aggregation leading to depression of the singlet oxygen yields.

Along the same lines, a series of naphthobenzoporphyrazines substituted on the benzo rings with 3-pyridyloxy and 3-(N-methyl)pyridyloxy groups and with t-butyl groups on the napthalo rings (Michelsen et al., 1996) (Figure 1.18) and were studied as potential photosensitizers for photodynamic therapy (Peeva et al., 2001). Surprisingly, the non-methylated naphthotribenzoporphyrazine exhibited a higher singlet oxygen quantum yield than unsubstituted zinc phthalocyanine or the corresponding symmetrically substituted phthalocyanine bearing four 3-pyridyloxy groups (Michelsen et al., 1996). It is suggested that this increase in the singlet oxygen quantum yield may be the result of the significantly lower symmetry in this molecule, which may result in additional electronic transitions and different excited state properties. The corresponding cationic charged methylated compounds had decreased singlet oxygen quantum yields compared to their non-methylated derivatives, most likely a result of quenching by the counter ion. As expected, the photostability decreased with increasing number of naphthalene rings (Michelsen et al., 1996) as it is well-known that naphthalocyanines are less photostable than phthalocyanines (Yates et al., 1990; Brasseur et al., 1994; Brasseur et al., 1995; Spikes et al., 1995).

In terms of photodynamic activity, the cationic methylated naphthotribenzoporphyrazine was the most phototoxic against EJ human bladder

carcinoma cells *in vitro* followed by the symmetrically substituted cationic phthalocyanine compound. The monocationic and dicationic porphyrazines did not exhibit any phototoxicity, perhaps a result of important aggregation of the macromolecules and decreased cell uptake. The best *in vivo* phototherapeutic effect was also observed for the cationic methylated naphthotribenzoporphyrazine. In both cases, this compound was accumulated to a higher degree in the cells *in vitro* and the tumour tissue *in vivo*. In addition, this photosensitizer showed higher singlet oxygen quantum yields compared to the symmetrically substituted cationic phthalocyanine. Finally, it is suggested that the unsymmetrically structure of this photosensitizer may be responsible for a more suitable orientation towards cellular and subcellular membranes, resulting in more effective damage to membranous cellular organelles. This assumption is supported by electron microscopy observations demonstrating typical features of random tumour necrosis, which includes heavy dystrophic changes in the membranous subcellular organelles.

In general, the results discussed above indicate that amphiphilic in phthalocyanines leads to favourable properties for photodynamic therapy. Depending on the specific phthalocyanine derivatives and conditions employed, these properties may include decreased aggregation, increased singlet oxygen quantum yields, increased association with serum lipoproteins, improved tumour cell uptakes and increased trafficking and improved interactions with photosensitive subcellular organelles. Overall, there remains a need for new amphiphilic phthalocyanines that take advantage of any or all of these factors, resulting in new photosensitizers with increased efficiency as photodynamic therapy agents.

1.7 General Synthesis of Phthalocyanines

Unlike metalloporphyrins derivatives, metallophthalocyanines are seldom obtained from an available metal-free phthalocyanine ligand. Typically, metallophthalocyanine complexes are synthesized by a metal-templated cyclotetramerization reaction involving the heating of an appropriate phthalocyanine precursor in the presence of a metal salt. Appropriate phthalocyanine precursors are aromatic *ortho*-carboxylic acid derivatives and include phthalic acids, phthalonitriles, phthalic anhydrides, phthalimides, diiminoisoindolines and phthalimides (see Chapter 5, Figure 5). *Ortho*-substitution is absolutely required as compounds having the carboxylic acid or related functional group separated from the aromatic system by a saturated bond or by extended unsaturation fail to undergo the cyclotetramerization reaction. Such compounds include isophthalic acid, terephthalonitrile, 1,2-bis(cyanomethyl)benzene, 2-carboxyphenylacetonitrile and 1,2-dicyanohexane (see Figure 5.6). Interestingly, 1-cyclohexene-1,2-dicarboxylic acid does yield tetracyclohexenetetraazaporphyrin via a cyclotetramerization reaction, with the corresponding phthalocyanine being obtained by dehydration of the tetraazaporphyrin derivative via sublimation at 300-320°C, heating in sulfur, boiling in chloronaphthalene in the presence of palladium or treating with DDQ (Ficken et al., 1952, Ficken et al., 1958). In addition, *o*-halobenzonitriles and *o*-dihalobenzenes can be used as precursors for phthalocyanines if the cyclotetramerization reaction is done in the presence of cuprous cyanide. These reactions most probably occur via the *in situ* generation of the corresponding phthalonitriles. Naturally, phthalocyanine derivatives with extended conjugation can also be prepared with 2,3-napthalenedicarbonitrile and 1,2-napthalenedicarbonitrile leading to naphthalocyanines.

For a complete review of phthalocyanine derivatives and their preparation, please see Chapter 5 (Sharman W. S. and J. E. van Lier (2002) "Synthesis of Phthalocyanine Precursors" in *The Porphyrin Handbook. Vol. 15 Phthalocyanine: Synthesis* (eds. K. M. Kadish, K. M. Smith and R. Guilard), London & Amsterdam: Elsevier Publishers, pp. 1-60).

Mechanistically, the cyclotetramerization reaction involved in the formation of the phthalocyanine macrocycle probably involves a stepwise polymerization of phthalocyanine precursors or reactive intermediates followed by coordination of the metal ion and ring closure (Dent, 1938; Owen et al., 1962; Hurley et al., 1967; Berezin, 1981; Gaspard et al., 1987; Leznoff, 1989). Ring closure is driven not only by the template effect of the metal ion and the inherent stabilization achieved by the resulting coordination but also by the thermodynamic stabilization and increased aromaticity involved in the formation of the phthalocyanine macrocycle. This increased aromaticity is clearly demonstrated by the magnetic anisotropy of phthalocyanines that is 15 times larger than that of benzene (Lonsdale, 1937).

This basic mechanism of phthalocyanine formation is supported by experimental observation, in particular in the nature of reaction intermediates isolated during phthalocyanine formation. For instance, in the preparation of metal-free phthalocyanine using sodium methoxide, a sodium salt of methoxyiminoisoindoline (Figure 1.19, Structure I) has been isolated (Borodkin, 1958). Such an intermediate suggest that formation of the iminophthalimidine is implicated in the cyclotetramerization reaction. In the meanwhile, nickel complexes II and III (Figure 1.19) have been isolated during the

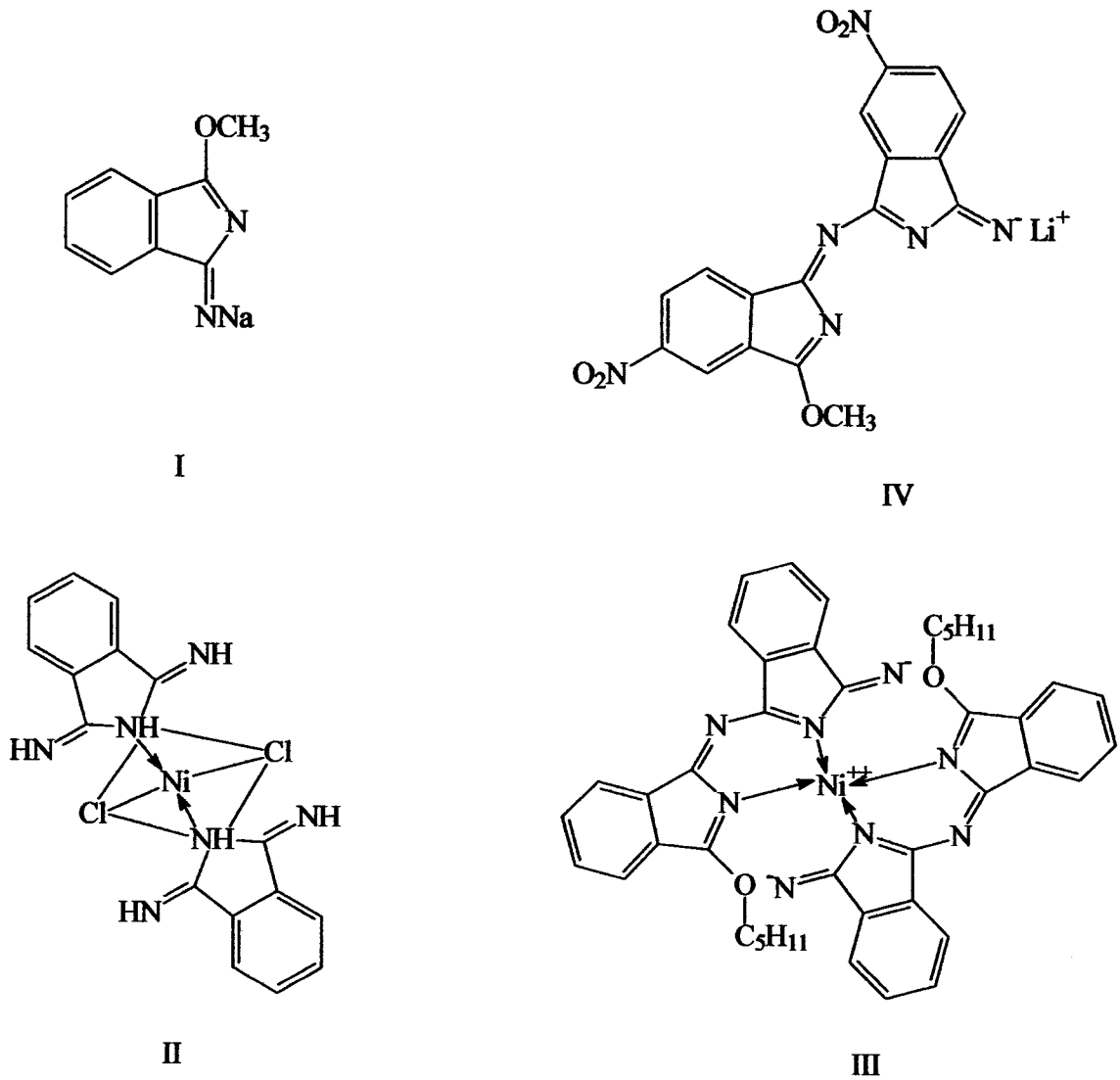

Figure 1.19. Intermediates isolated during the synthesis of phthalocyanines.

synthesis of nickel phthalocyanine (Hurley et al., 1967), pointing to the role of the metal ion as a template for the cyclization. During the synthesis of lithium tetranitrophthalocyanine, lithium salts such as IV (Figure 1.19) were observed (Oliver et al., 1987). This indicates that the reaction may proceed by a stepwise condensation of phthalocyanine precursors. Overall, however, the exact mechanism involved in

phthalocyanine macrocycle formation remains unclear. As observed by Elvidge and Linstead as early as 1955, the cyclotetramerization reaction of diiminoisoindolines should lead to a hydrophthalocyanine (Elvidge et al., 1955). Thus, a reductant of some type must be involved in the synthesis in order to get the corresponding phthalocyanine. While it has been theorized that the reduction occurs on the phthalocyanine precursor prior to complexation of the metal (Gaspard et al., 1987), the nature and the role of the reductant remains unclear.

As has been previously mentioned, unsubstituted phthalocyanines are extremely insoluble in most common solvents. In order to increase the solubility and to improve the physical, chemical and electronic properties of the phthalocyanine macrocycle, a seemingly endless number of functional groups and substitutions have been added to the phthalocyanine framework via covalent attachment to the benzene rings on the periphery of the macrocycle. Careful consideration of the functional groups added to the phthalocyanine can be used to fine-tune the properties of the macrocycle, leading to compounds with heightened characteristics for a given application. Simple functional groups such as alkyl chains, higher order aromatics, ethers, amines, thiols, halides and various acidic groups have been used to improve the solubility and the characteristics of phthalocyanines. More exotic substituents including crown ethers, dendrimers, ferrocenes and tetrathiafulvalenes lend other properties to the macrocycle that may enhance their activity and utility in various applications. Polynuclear phthalocyanine systems have also been prepared in order to synthesize novel organic materials, new chemical catalysts and high temperature polymers. Reactions involving the preparation of ether, amine, thiol and carbon-carbon bonds have been employed along with countless

other possibilities in order to add a variety of substituents and functional groups to the phthalocyanine framework. Overall, this rich chemistry is the driving force behind the tremendous versatility of phthalocyanines and their value in such a wide array of technological fields.

Substitution onto a phthalocyanine can basically be accomplished by one of two methods. The first involves direct substitution onto a pre-existing phthalocyanine macrocycle. An excellent example of this is the sulphonation of phthalocyanines, which can be accomplished by heating a phthalocyanine in oleum (Ali et al., 1988). Such harsh reaction conditions can result in substitution at any or all of the available positions (see Figure 5.2), leading to a complex isomeric mixture and varying degrees of substitution. Though direct substitution is the preferred method for adding functionality to phthalocyanines in the colourant industry (Gregory, 1999; Gregory 2000), the resulting phthalocyanine mixture lacks a distinct structure and isolation and purification of the desired phthalocyanine product is extremely difficult and time-consuming. This greatly limits the utility of this methodology in applications calling for well-defined phthalocyanine structures. It should be noted however that pre-existing substituted phthalocyanines have been extensively used in the preparation of novel substituted phthalocyanines by chemically modifying the existing functionality. An excellent example of such chemistry is the use of palladium-catalyzed reactions to prepare novel phthalocyanine derivatives. For a complete review of the use of palladium-catalyzed reactions in the preparation of novel phthalocyanine and porphyrin derivatives, please see Chapter 6 (Sharman W. S. and J. E. van Lier (2000) Use of palladium catalysis in the

synthesis of novel porphyrins and phthalocyanines, *J. Porphyrins Phthalocyanines,* **4,** 441-453).

The second methodology involves the condensation of a substituted phthalocyanine precursor. This obviously leads to a far cleaner reaction, with the degree of substitution and the relative position of the substituents readily known from the nature of the substituted starting material. For instance, a monosubstituted precursor will yield a tetrasubstituted phthalocyanine while a 4,5-disubstituted phthalonitrile will yield a 2,3,9,10,16,17,23,24-octasubstituted phthalocyanine (see Chapter 5, Figure 2 for numbering scheme used in phthalocyanine nomenclature). However, this method still leads to constitutional isomers when it involves asymmetrically substituted phthalocyanine precursors (see Figure 5.3 for the constitutional isomers obtained in the synthesis of a tetrasubstituted phthalocyanine from a monosubstituted precursor). While it is theoretically possible to separate these isomers due to their differing geometries, it has only been accomplished used specialized HPLC columns and the best results often only lead to enriched isomeric fractions (Hanack et al., 1993a, Hanack et al., 1993b; Hanack et al., 1994; Sommerauer et al., 1996; Schmid et al., 1996). While isomeric mixtures are suitable for most applications, high tech fields require phthalocyanines with distinct structural features and thus require synthetic methods that can lead to the preparation of single phthalocyanine isomers. Nonetheless, the methodology has been used extensively for the preparation of phthalocyanines bearing novel substituents and extensive chemical modification of phthalocyanine precursors has been undertake to ultimately prepare novel phthalocyanines and phthalocyanine derivatives. For a complete review of the preparation of substituted phthalocyanine precursors and their use in the

preparation of phthalocyanines, please see Chapter 5 (Sharman W. S. and J. E. van Lier (2002) "Synthesis of Phthalocyanine Precursors" in *The Porphyrin Handbook. Vol. 15 Phthalocyanine: Synthesis* (eds. K. M. Kadish, K. M. Smith and R. Guilard), London & Amsterdam: Elsevier Publishers, pp. 1-60).

1.8 Synthesis of Asymmetrically Substituted Phthalocyanines

Further complicating matters is the preparation of asymmetrically substituted phthalocyanines, especially since these phthalocyanine derivatives have important utility in fields such as photodynamic therapy and non-linear optics. Such asymmetrically substituted phthalocyanines are generally prepared by a statistical mixed condensation using two differently substituted phthalocyanine precursors (Schmid et al., 1996). This method can result in reaction mixtures enriched with the desired substitution pattern due to the different reactivities of the differently substituted precursors. In most cases, by experimentation, the necessary proportions of the individual precursors and the optimal reaction conditions can be determined in order to obtain predominately the desired asymmetrically substituted phthalocyanine. However, a mixed condensation still leads to six differently substituted phthalocyanine products (see Figure 5.4 for the six differently substituted phthalocyanines obtained by a mixed condensation using two differently substituted precursors). Isolation of the desired product from such a mixture can be accomplished by extensive column and HPLC chromatography. Nonetheless, such isolation and purification is very tedious and the resulting product can still ultimately be contamination with the other substituted phthalocyanines. Clearly, the synthesis of asymmetrically substituted phthalocyanines via a mixed condensation lacks sophistication and new methods are needed to prepare phthalocyanines with exact compositions and pure isomeric distributions.

Due to the need for isomerically pure phthalocyanines and for asymmetrically substituted phthalocyanines with precise degrees of substitution, a number of novel synthetic approaches have been investigated. One of these methods actually involves

avoiding the problems inherent in preparing substituted phthalocyanines and uses the axial ligand on the central metal ion of the phthalocyanine complex to impart the desired physical and chemical properties to the molecule. In doing this, the phthalocyanine

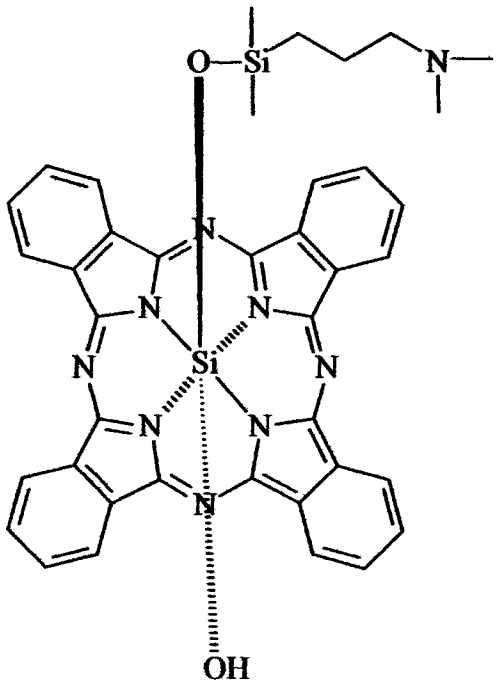

Figure 1.20. Pc4

macrocycle itself can be left unsubstituted. Furthermore, covalently attaching a bulky axial ligand to the phthalocyanine will help prevent aggregation by sterically inhibiting aromatic stacking. Pc4, an unsubstituted silicon phthalocyanine with an alkylsilyl axial ligand bearing a terminal amine group (see Figure 1.20) (Oleinick et al., 1993; Rywkin et al., 1994) is one of the more promising phthalocyanines for photodynamic therapy and this dye entered clinical trials in 2001 (Allen et al., 2001). A number of other phthalocyanines with novel axial ligands have also been prepared in view of obtaining

molecules with useful properties for various diverse applications (Charlesworth et al., 1994; He et al., 1997; Vollano et al., 1997; Brewis et al., 1998; Decréau et al., 2000; Kobayashi et al., 2000; O'Flaherty et al., 2003; Dudnik et al., 2004). However, while preventing aggregation and the inherent molecular interactions is important in some cases, the ability of phthalocyanines to stack and interact is vital for other applications. In light of this, while the use of specifically designed axial ligands enhances the utility of phthalocyanines for some uses, it greatly decreases or eliminates the utility in others. As a result, the use of an axial ligand to increase solubility and to enhance certain properties of phthalocyanines has only limited potential.

The overall synthetic mechanism of phthalocyanine formation is essentially extremely symmetric, with the cyclotetramerization reaction occurring from any number of possible orientations. This symmetry results in the constitutional isomers observed during the preparation of tetrasubstituted phthalocyanines as the orientation of the substituents during phthalocyanine formation is not affected significantly by steric or electronic factors. Attempts have been undertaken to prepare novel phthalocyanine precursors that will break this symmetry and force the condensation reaction to proceed in only one possible orientation, thus leading to the exclusive preparation of a single isomer or substitution pattern.

It has been demonstrated that dithiophthalimides undergo the cyclotetramerization reaction with 1,3-diiminoisoindolines under relatively mild conditions (temperatures around 80-90°C) (Leznoff et al., 1987). At these reaction temperatures, neither the 1,3-diiminoisoindolines nor the dithiophthalimides should self-condense. Thus, due to the lower reaction temperature employed, this reaction should

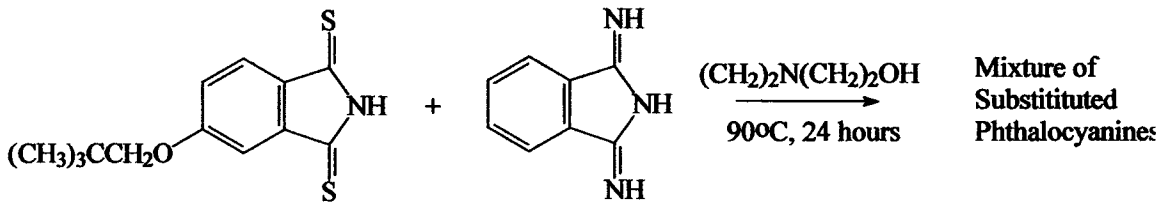

Figure 1.21. Attempted synthesis of pure oppositely disubstituted phthalocyanines using dithiophthalimides

proceed by the selective displacement of a thiol group of the dithiophthalimide by an imino group of the 1,3-diiminoisoindoline. Attempts have been undertake to use this methodology to prepare pure oppositely disubstituted phthalocyanines wherein one of the two precursors bearing a substituents. The dithiophthalimide (such as 1-H-isoindole-1,3(2H)-dithione or 5-neopentoxy-1-H-isoindole-1,3(2H)-dithione) were readily prepared from the corresponding phthalimides by reaction with Lawesson's reagent (see Chapter 5, Figure 70). However, all possible substitution patterns were obtained when 5-neopentoxy-1-H-isoindole-1,3(2H)-dithione was reacted with unsubstituted 1,3-diiminoisoindoline (Figure 1.21). From this, it was proposed that the first step in this synthesis of phthalocyanines would lead to the formation of an intermediate trimeric species (see Figure 1.22). Ideally this intermediate would reaction with a second dithiophthalimide to give the desired oppositely disubstituted phthalocyanine. However, while the initiation step of phthalocyanine formation has been lowered by using the dithiophthalimide precursor, the propagation step remains rapid at these modest temperatures and this trimeric species may react with another molecule of the unsubstituted 1,3-diiminoisoindoline to give the corresponding monosubstituted

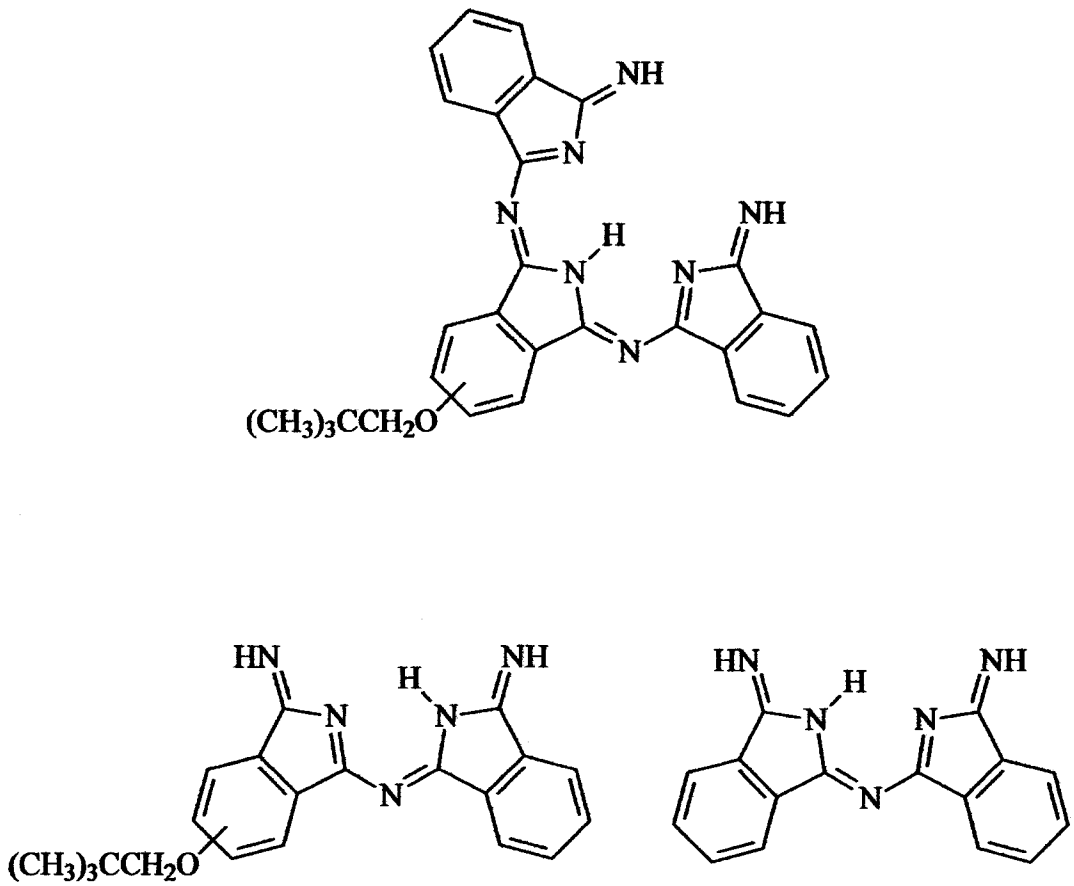

Figure 1.22. Intermediate trimeric and dimeric species proposed for reaction of 1,3-diiminoisoindolines and dithiophthalimides

macrocycle. Furthermore, it is theorized that this trimeric species may undergo N-H tautomeric shifts with the resulting trimeric intermediate reacting with the 1,3-diiminoisoindoline to give two dimeric species (see Figure 1.22). These dimeric species may react with other dimeric species to give the corresponding unsubstituted or disubstituted phthalocyanines or undergo further reaction with 1,3-diminoisoindoline to give the corresponding monomeric substituted 1-3-diiminoisoindolines. The presence of

these dimeric and substituted 1,3-diiminoisoindolines in the reaction mixture are the reason that the reaction proceeds to give all possible substitution patterns.

Along the same line of reason as the above technique in phthalocyanine synthesis, the condensation of 1,3,3-trichloroisoindolines with 1,3-diiminoisoindolines should lead to the preparation of pure oppositely disubstituted phthalocyanines. In fact, reacting 1,3,3-trichloroisoindoline with 5-phenyl-1,3-diiminoisoindoline at room temperature in the presence of triethylamine (an organic base) and hydroquinone (a reducing agent) lead to exclusive formation of the desired 2,16(17)-diphenylphthalocyanine in a 7% yield (Idelson, 1977). When the reaction temperature is raised, however, the reaction leads to a mixture of substituted products (Wimmer, 1969). The basis behind the controlled reaction of 1,3,3-trichloroisoindolines with 1,3-diiminoisoindolines lies with the lower reaction temperatures employed along with the steric hindrance in the 1,3,3-trichloroisoindolines (Young et al., 1990; Stihler et al., 1997; Hanack et al., 2000). At the lower reaction temperatures employed, the diiminoisoindolines do not self-condense while the steric hindrance in the 1,3,3-trichloroisoindolines prevent self-condensation despite the increased reactivity of these precursors. Thus, only condensation between the 1,3-diiminoisoindolines and the 1,3,3-trichloroisoindolines is possible, leading to pure trans disubstituted products. Note that in some cases, trisubstituted products have been obtained (Wimmer, 1969; Stihler et al., 1997; Hanack et al., 2000), most likely the result of the presence of small traces of water in the reaction system, which would hydrolyze the 1,3,3-trichloroisoindoline and upset the stoichiometric balance of the two precursors. It should however be noted that 1,3,3-trichloroisoindolines are generally extremely reactive and highly unstable, thus greatly limiting their overall synthetic utility.

Furthermore, the harsh reaction conditions used in their synthesis (see Chapter 5, Figure 68) restricts the functional groups that can be included in these precursors.

The condensation reaction of substituted iminothioamides may lead to the production of a single isomer by controlled reaction of the thiol group of one precursor with the imino group of an adjacent precursor. Such iminothioamides (1-imino-3-alkylthioisoindolines) are prepared by reacting the corresponding substituted phthalonitrile with hydrogen sulfide followed by methylation with methyl iodide (Greenberg et al., 1988) (see Figure 5.69). S-methylation is necessary in order for these substituted phthalocyanine precursor to undergo the cyclotetramerization reaction to give the desired phthalocyanines. The unsubstituted 1-imino-3-methylthioisoindoline readily condensed to the corresponding phthalocyanines at room temperature. Unfortunately, while the resulting 1-imino-3-methylthio-6-neopentoxyisoindoline and 1-imino-3-methylthio-5-neopentoxyisoindoline were readily isolated and separated by column chromatography, these unique phthalocyanine precursors self-condensed at room temperature to give a mixture of constitutional isomers. However, when small-scale condensation reaction was carried out at -20°C in DMF using zinc acetate as a metal ion source, a single isomer was obtained (Figure 1.23) (Greenberg et al., 1988). Interestingly, increasing the scale of this reaction again gave a mixture of structural isomers. Furthermore, poor yields are obtained due to extensive by-product formation. It is unclear why condensation of 1-imino-3-methylthio-6-neopentoxyisoindoline or 1-imino-3-methylthio-5-neopentoxyisoindoline resulted in a mixture of isomers. However, it should be noted that the mixture of isomers obtained was not identical to the statistical distribution of isomers previously observed for the same phthalocyanine product

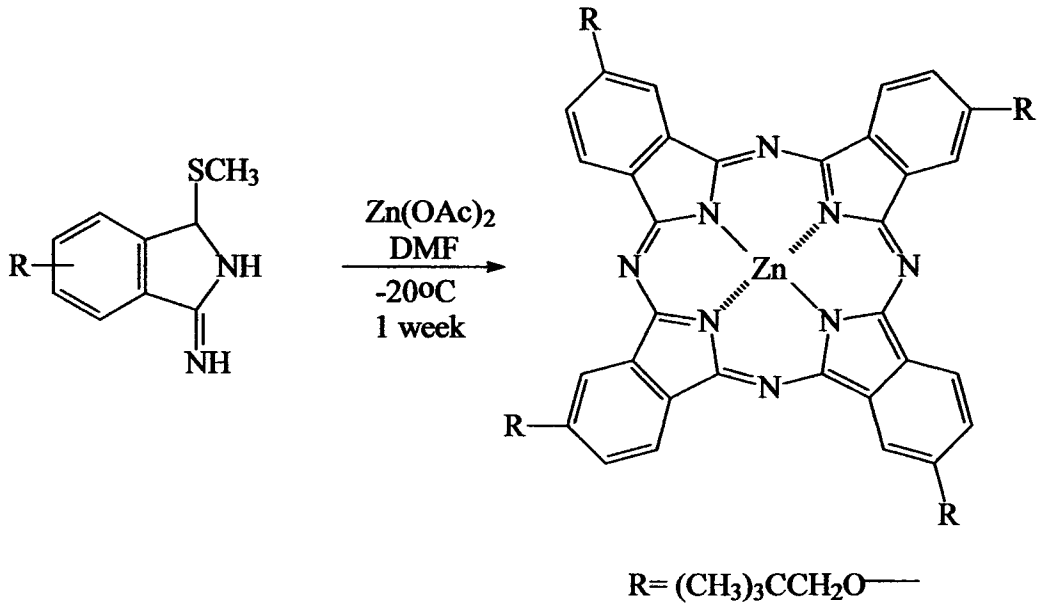

Figure 1.23. Synthesis of pure 2,9,16,23-tetrasubstituted phthalocyanines using 1-imino-3-methylthioisoindolines

synthesized from the corresponding substituted 1,3-diiminoisoindoline (Marcuccio et al., 1985). This suggests that a different reaction pathway is involved for these two structurally similar phthalocyanine precursors.

For a thorough review of the synthesis and use of these novel designed phthalocyanine precursors, please see Chapter 5 (Sharman W. S. and J. E. van Lier (2002) "Synthesis of Phthalocyanine Precursors" in *The Porphyrin Handbook. Vol. 15 Phthalocyanine: Synthesis* (eds. K. M. Kadish, K. M. Smith and R. Guilard), London & Amsterdam: Elsevier Publishers, pp. 1-60).

In order to prepare 3:1 asymmetrically substituted phthalocyanines, the use of polymer supports has been investigated. As would be expected, this methodology

involves the covalent attachment of a phthalocyanine precursor (typically a 1,3-diiminoisoindoline or a phthalonitrile) to a solid phase polymer support. This precursor is then reacted with a large excess of a differently substituted precursor in solution. The desired 3:1 asymmetrically substituted phthalocyanine is then readily separated from the symmetrically substituted phthalocyanine that is produced due to the self-condensation of the unbound precursor by filtration as the polymer-bound phthalocyanine product remains suspended in the solution. The desired phthalocyanine can then be isolated by cleaving off the solid support. This methodology has been successively employed to prepare a number of 3:1 asymmetrically substituted phthalocyanines either using polymer-bound trityl chloride derived from a 1% divinylbenzene-co-styrene copolymer (Hall et al., 1982; Leznoff et al., 1982; Leznoff et al., 1991) or silica modified by aminopropyl groups (Hirth et al., 1997). While this methodology produces the desired phthalocyanine product exclusively and in good yields, there are several limitations. It can only be used to prepare 3:1 asymmetrically substituted phthalocyanines. Furthermore, the functional group on the single phthalocyanine precursor must be capable of being chemically attached to the polymer support. The reaction is achieved using a large excess of the unbound precursor, which is essentially lost because it self-condenses under the reaction conditions employed to give the corresponding symmetrical tetrasubstituted macrocycle. Finally, while yields are often extremely good, only small amounts of phthalocyanine can be prepared due to the low binding capacity of the polymeric carrier and the inherent limitations of solid phase chemistry. Thus, while small scale reactions work very well, the polymer support method is not appropriate for larger scale synthesis.

For a thorough review of the synthesis and use of these polymer-bound phthalocyanine precursors, please see Chapter 5 (Sharman W. S. and J. E. van Lier (2002) "Synthesis of Phthalocyanine Precursors" in *The Porphyrin Handbook. Vol. 15 Phthalocyanine: Synthesis* (eds. K. M. Kadish, K. M. Smith and R. Guilard), London & Amsterdam: Elsevier Publishers, pp. 1-60).

1.9 Boron Subphthalocyanines

One final method of preparing 3:1 asymmetrically substituted phthalocyanines is the use of boron subphthalocyanines as a template. The preorganization of three isoindoline units is boron subphthalocyanines make them extremely attractive reagents for the synthesis of asymmetrically substituted phthalocyanines. A simple ring enlargement reaction with various 1,3-diiminoisoindolines should readily yield the desired 3:1 asymmetrically substituted phthalocyanine.

Boron subphthalocyanines themselves are the lower homologs of phthalocyanines with the smaller ionic radius of the boron central metal ion allowing formation of a tripyrrolic macrocyclic system (see Figure 1.24). The loss of an isoindoline unit as compared to phthalocyanines results in a hypsochromic shift in the Q band absorption to

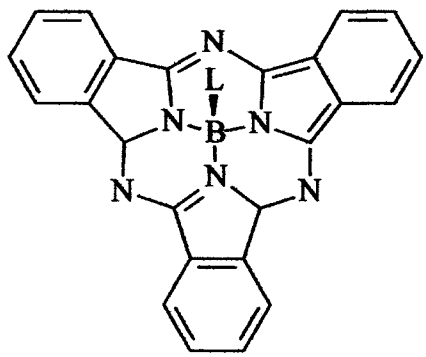

Figure 1.24. General structure of a boron subphthalocyanine

around 560 nm, giving solutions of boron subphthalocyanines a distinct red/purple colour. However, despite the loss of the isoindoline unit, the inner macrocyclic system of boron subphthalocyanines is still aromatic in nature, obeying Huckel's 4N+2 rule for

aromaticity as there are 14 π electrons delocalized within the tripyrrolic core. Importantly, while Pcs are extremely stable molecules exhibiting a high degree of planarity in their central aromatic core, subPcs have a cone-shaped structure, with the boron coordinated in a tetrahedral geometry with a single axial ligand, typically a halide that is present during their synthesis. This cone-shaped structure is clearly evident in the X-ray crystal structure (Rauschnabel et al., 1995; Geyer et al., 1996; Sastre et al., 1996; Kobayashi, 1999; Claessens et al., 2002) and has been confirmed by scanning tunneling microscopy using chloro[tri-tert-butyl-subphthalocyaninato]boron(III) on a Au(111) surface (Suzuki et al., 2003) and quantum mechanical calculations at semiempirical and ab initio levels (Ferro et al., 2000).

The boron in these complexes is in the +3 oxidation state and therefore is coordinatively unsaturated. As a result, boron subphthalocyanines have an σ-bonded axial ligand. In general, this axial ligand is a halide ion released during subphthalocyanine formation. However, when the boron reagent employed contains a phenyl group, such as $PhBCl_2$ or Ph_3B, the phenyl group becomes axially coordinated to the boron atom. The ability of the π orbitals of the phenyl group to overlap the π-oriented d-orbitals of the boron allows for stabilizing dative backbonding, leading to stronger coordination of the phenyl group. Boron subphthalocyanines readily undergo axial ligand exchange reactions with the halogen atom easily displaced by nucleophiles such as alcohols and silanols (Geyer et al., 1996; del Rey et al., 2000; Zyskowski et al., 2000; Claessens et al., 2002). Peripheral donors groups increase the rate and the yield of axial ligand exchange, presumably due to the resulting stabilization of the positive charge that temporarily forms on the boron atom (Claessens et al., 2002).

Due to their unique chemical and physical properties, the lack of a center of symmetry in these macrocyclic molecules along with the delocalized nature of this 14 π-electron aromatic system make these compounds extremely attractive chemical moieties. In particular, their extensively delocalized 14-π electron system and their cone-shaped structure have led to investigations in the utility of subPcs in non-linear optical applications (Diaz-Garcia et al., 1995; Sastre et al., 1996; Rojo et al., 1997; del Rey et al., 1998; Kang et al., 1999; de la Torre et al., 2004; Claessens et al., 2005). The cone-shape of boron subphthalocyanines results in non-centrosymmetry and a strong molecular dipole, ideal properties for second harmonic generation. Boron subphthalocyanines with long thioalkyl chains have been prepared and exhibit hexagonal columnar mesophases, potentially adding value to these compounds in molecular devices (Kang et al., 1999). In addition to utility in non-linear optical applications, the intense reddish colour of boron subphthalocyanines has lead to investigations in their use as dyes and pigments (Nohr et al., 2002) while their similarities with phthalocyanines has resulted in the use of subphthalocyanines in organic photoconductors (Reynolds et al., 1994) and as an optical recording medium for an optical data memory of the DVD-R type (Zafirov et al., 2002). Significant research has also been undertaken in the preparation, properties and utility of subphthalocyanine-fullerene dyads which have unique photophysical properties and photoinduced energy- and electron-transfer events (Gonzalez-Rodriguez et al., 2002; Claessens et al., 2004; Gonzalez-Rodriguez et al., 2004; Gonzalez-Rodriguez et al., 2005; Iglesias et al., 2005).

Boron subphthalocyanines were first prepared serendipitously during attempts to prepare boron phthalocyanine by the reaction of phthalonitrile with condensed gaseous

boron trichloride at 250°C (Meller et al., 1972). More recently, boron subphthalocyanines are typically prepared by a cyclotrimerization reaction using commercially available solutions of boron trihalides. The use of boron trihalides however may lead to halogenation of the periphery of the subphthalocyanine macrocycle (Dabak et al., 1994; Hanack et al., 1994; Weitemeyer et al., 1996; Kobayashi, 1999; Claessens et al., 2002) since halogen is released during subphthalocyanine formation and electronic aromatic substitution reactions with halogen are known to be catalyzed by boron trihalides (March, 1992). In addition, a significant number of functional groups are not compatible with the strong Lewis acidity of boron trihalides, limiting the possible substituents on subphthalocyanines formed using these boron sources. Other boron sources such as trialkylboron and triphenylboron avoid these disadvantages (Hanack et al., 1994; Rauschnabel et al., 1995; Geyer et al., 1996; Claessens et al., 2002). However, such boron sources are much lesser reaction, require an organic base such as DBU or a super base and give only low yields of the desired product. Overall, the reactivity of trisubstituted boron compounds towards phthalonitriles follows the order $B(alkyl)_3 < BPh_3 < BF_3 < BCl_3 < BBr_3$, which mimics the Lewis acidity of these compounds (Claessens et al., 2002). Despite the increased reaction of BBr_3 and its commercial availability as solutions in dichloromethane, heptane or hexanes, BCl_3 is by far the most commonly reported boron reagent employed for subphthalocyanine synthesis. This is primarily due the instability of bromosubphthalocyanines with respect to the bromine axial ligand which makes purification and characterization somewhat tedious. It has been found that dimethyl sulfide complexes with boron trichloride may be employed as a boron source for subphthalocyanine synthesis, with smoother reaction conditions and

greater compatibility with various functional groups (Claessens et al., 2002). Unfortunately, yields are lower using this methodology.

When boron subphthalocyanines are prepared from asymmetrically 4-substituted phthalonitriles, a mixture of C_3 and C_1 constitutional isomers is obtained, generally following a statistical distribution (1:3 respectively) without any steric effects from the substituents. This statistical distribution is not observed for 3-substituted phthalonitriles, where the C_3 is predominately obtained due to steric effects in the C_1 isomer (Claessens et al., 2000). Overall, the C_3 and C_1 structural isomers have been separated using preparative HPLC (Hanack et al., 1994) and column chromatography on silica gel (Claessens et al., 2000a). Each of these constitutional isomers is in fact a racemic mixture of enantiomers, which have also been resolved (Claessens et al., 2000c; Kobayashi, 2001). Very few organic compounds with C_3 symmetry have been obtained in optically active forms and the resolution of an aromatic chiral C_3 molecule has only been described for boron subphthalocyanines (Claessens et al., 2002). Such high-symmetry chiral molecules are of special interest in the investigation of the molecular origin of optical activity.

With the unique physical and chemical properties of boron subphthalocyanines and their potential utility in fields such as non-linear optics, numerous subphthalocyanines bearing different substituents and axial ligands have been prepared (Dabak et al., 1994; Geyer et al., 1996; del Rey et al., 1997; Kudrevich et al., 1997; del Rey et al., 1998; Kipp et al., 1998; Kobayshi, 1999; Claessens et al., 2000; del Rey et al., 2000; Zyskowski et al., 2000; Cao et al., 2002; Claessens et al., 2002; Ohno-Okumura et al., 2002; Claessens et al., 2005). The periphery of subphthalocyanines has also been

modified following subphthalocyanine formation in order to alter the properties of the macrocycle (del Rey et al., 1997; Kudrevich et al., 1997). In addition, asymmetrically substituted boron subphthalocyanines have been prepared by a mixed condensation of two differently substituted phthalonitrile derivatives (Ali et al., 1999; Stork et al., 1999; Claessens et al., 2000; Zyskowski et al., 2000). Boron subazaporphyrins and boron subnaphthalocyanines have also been prepared in low yields (Kobayashi, 1999; Kobayashi et al., 1999; Zyskowski et al., 2000; Nonell et al., 2000) and valuable properties have been identified in such compounds. For instance, unsubstituted boron subnaphthalocyanine, which absorbs at 663 nm, has a high triplet state yield and a long triplet state lifetime and can effectively generate singlet oxygen as a quantum yield significantly higher than phthalocyanines (Nonell et al., 2000), resulting in potential as a photosensitizer for photodynamic therapy. Various binuclear subphthalocyanines have also been prepared (Kobayashi, 1991; Geyer et al., 1996; Kobayashi, 1999; Kobayashi et al., 1999).

It has been demonstrated that the less stable subPcs readily react with 1,3-diiminoisoindolines in a ring enlargement reaction to yield 3:1 asymmetrically substituted Pcs (Kobayashi et al., 1990; Kobayashi, 1999; Kobayashi et al., 1999). Such a reaction would appear to be ideal for the preparation of 3:1 asymmetrically substituted phthalocyanines with the preorganization of three isoindoline units in the subphthalocyanines hopefully leading exclusively to the desired substituted product. Initial reactions of (tri-t-butyl)subphthalocyanatoboron(III) bromide with a series of diiminoisodoline derivatives of increasing aromaticity in a mixture of

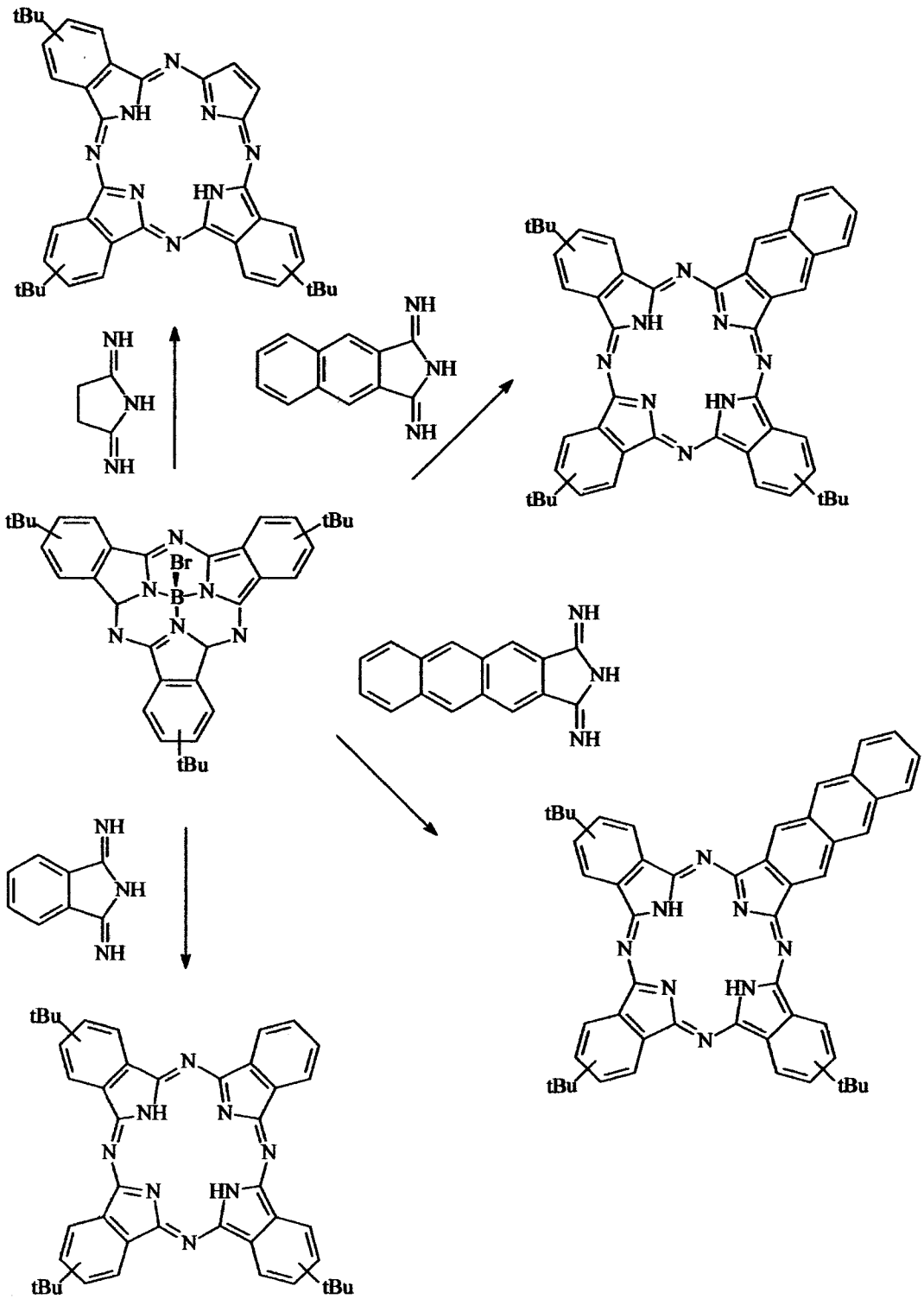

Figure 1.25. Initial Kobayashi ring expansion reaction of boron subphthalocyanines

(Kobayashi et al., 1990)

N,N-dimethylsulfoxide and either of increasing aromaticity in a mixture of N,N-dimethylsulfoxide and either chlorobenzene, o-dichlorobenzene, 1-chloronaphthalene or 2-chloronaphthalene at 80-90°C for 5-27 hours yielded the predicted 3:1 asymmetrically substituted phthalocyanine derivatives in yields of 8-20% (Figure 1.25) (Kobayashi et al., 1990). The yield of the desired phthalocyanine is good, even at 8-20%, compared to other methodologies, especially since only the desired 3:1 asymmetrically substituted phthalocyanine was obtained. Furthermore, isolation and purification of the desired phthalocyanine was easy, with a blue fraction for the 3:1 asymmetrically substituted phthalocyanine and a reddish purple fraction for unreacted subphthalocyanine.

Intuitively, it seems logical that the reactivity of subPcs towards a ring expansion reaction would be based on the steric hindrance present in the distorted molecular structure, with the cone shaped structure of the molecule leading to ineffective p-orbital overlap and a loss of aromatic stabilization. However, molecular orbital calculations comparing the bond energies of subPc with MgPc showed little deviation in the calculated C-N bond energies between these two macrocycles. This implies that distortion energy is not a major cause of the ring expansion reactivity (Kobayashi et al., 1999). Similar calculations suggest that the lack of electron-accepting orbitals in boron results in a lack of donor-acceptor stabilization in the B-N bonds, at least explaining in part the instability of subPcs. More importantly, however, these calculations indicates that the loss of the axial ligand alters the shape of the main skeleton of subPcs from a shuttlecock shaped to a more planar form, with a corresponding stabilization energy of approximately 100 kJ/mol. Furthermore, the resulting cationic charge on the central boron atom would be delocalized over the entire macrocycle. In light of this, it seems

likely that the initial step in the ring expansion reaction consist of a dehalogenation reaction, with the corresponding loss of the axial ligand.

Unfortunately, despite initial success in the preparation of novel 3:1 asymmetrically substituted phthalocyanines (Kobayashi et al., 1990; Kasuga et al., 1992: Musluoglu et al., 1992; Dabak et al., 1994), the Kobayashi ring expansion reaction of boron subphthalocyanine may lead to a mixture of substituted phthalocyanines (Sastre et al., 1995; Weitemeyer et al., 1995; Geyer et al., 1996; Sastre et al., 1996). Due to these results, it has been suggested that the Kobayashi ring expansion reaction is not a concerted process and that it must be a multistep process which greatly depends on the

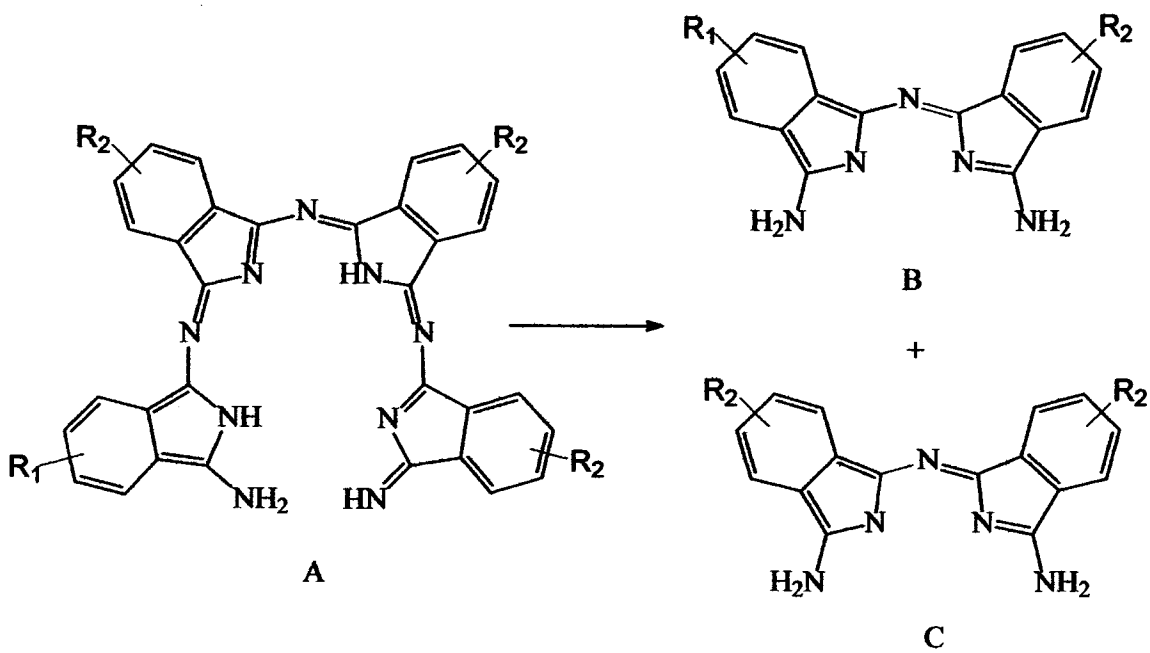

Figure 1.26. Proposed mechanism for the Kobayashi ring expansion reaction (R_1 is the substituents on the 1,3-diiminoisoindoline and R_2 are the substituents on the subphthalocyanine) (Sastre et al., 1995; Sastre et al., 1996)

nature of the substituents on the subphthalocyanine, the reactivity of the 1,3-diiminoisoindoline, the solvent and the reaction conditions employed. The following mechanism has been proposed wherein the initial step involves reaction of the subphthalocyanine with the 1,3-diiminoisoindoline to form an open four-membered intermediate A (Figure 1.26) (Sastre et al., 1995; Sastre et al., 1996). This intermediate can cyclize to the desired 3:1 asymmetrically substituted phthalocyanine. However, intermediate A can also undergo cleavage, promoted either thermally or by interactions with another 1,3-diiminoisoindoline molecule or a solvent molecule to yield dimers B and C. While condensation of B and C also gives the desired 3:1 asymmetrically substituted phthalocyanine, self-condensation of intermediates B or C lead to phthalocyanines with undesirable substitution patterns. These dimers can also undergo cleavage to give reactive monomers which may condense with dimers B and C, with unreacted 1,3-diiminoisoindoline (which is present in a large excess) or with other monomers to give other undesirably substituted phthalocyanines. Finally, depending the reaction conditions employed and the reactivity of the 1,3-diiminoisoindoline, the 1,3-diiminoisoindoline may self-condense to varying degrees, adding additional reactive monomer, dimers and trimers to the reaction mixture while also giving small amounts of its symmetrically substituted phthalocyanine derivative.

In a series of experiments involving the reaction of unsubstituted boron subphthalocyanine bearing a chlorine axial ligand with 5-(4-t-buylphenoxy)-1,3-diiminoisoindoline and 5,6-dimethyl-1,3-diiminoisoindoline, it was observed that the subphthalocyanines was stable under the reaction conditions employed for the ring expansion reaction (2:1 mixture of DMSO and chloronaphthalene, 80-90°C, 24 hours)

(Weitemeyer et al., 1995). The subphthalocyanine did not react with the corresponding phthalonitrile to give any phthalocyanine product under these conditions. The 1,3-diiminoisoindolines only self-condensed in trace amounts to give the corresponding symmetrically substituted phthalocyanine. Finally, addition of weakly basic zinc acetate dihydrate to a solution of the subphthalocyanine lead to the formation of unsubstituted zinc phthalocyanine while a similar addition of neutral zinc chloride gave no such reaction. Based on these results, it was assumed that the subphthalocyanine does not undergo a concerted ring expansion reaction. The reaction involves a multistep process wherein the first step is theorized to be a base-catalyzed decomposition of the subphthalocyanine. This would be followed by condensation of the reactive fragments produced by this decomposition with each other and with the 1,3-diiminoisoindolines. Such condensation and ring closure would result in the formation of the differently substituted phthalocyanines observed.

In a novel approach, the ring expansion reaction has been carried out in the presence of a metal ion (zinc acetate dihydrate) as a template in order to provide selectivity for the formation of the desired 3:1 asymmetrically substituted phthalocyanine and to prepare the metallophthalocyanine in one step (Weitemeyer et al., 1995). In the presence of Zn^{+2}, the reaction of the subphthalocyanine with substituted 1,3-diiminoisoindolines resulted in higher percentages of the symmetrically tetrasubstituted phthalocyanine, a result of the condensation of the 1,3-diiminoisoindolines in the presence of the metal ion. Using the corresponding substituted phthalonitrile, this reaction gave higher yields in terms of total phthalocyanine production but also relatively

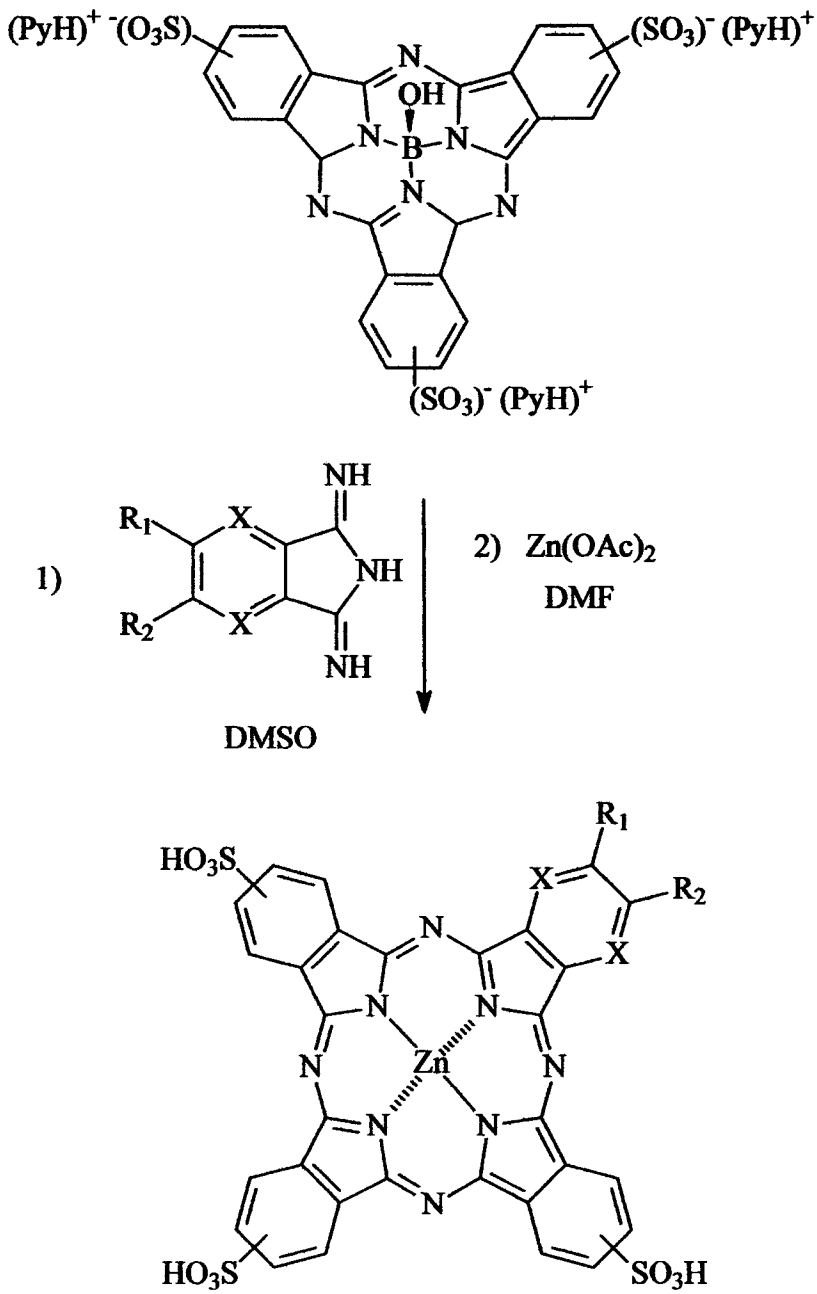

Figure 1.27. Synthesis of novel asymmetrically substituted trisulphonated phthalocyanines using the Kobayashi ring expansion reaction (a: X = CH, R_1 = t-butyl, R_2 = H; b: X = CH, R_1 = R_2 = -CH=CH-CH=CH-; c: X = N, R_1 = R_2 = phenyl) (Kudrevich et al., 1996, Kudrevich et al, 1997; van Lier et al., 1999).

high amounts of the di-, tri- and tetrasubstituted phthalocyanines. Formation of these undesirable substituted phthalocyanines could be reduced by the use of a catalytic amount of DBU and pentanol and by employing higher reaction temperatures, all of which promotes ring-opening of the subphthalocyanine.

Despite the lack of general synthetic utility and the above proposed mechanisms, the Kobayashi ring expansion reaction has been successfully employed to prepare certain 3:1 asymmetrically substituted phthalocyanines (Kobayashi et al., 1990; Kasuga et al., 1992; Musluoglu et al., 1992: Dabak et al., 1994; Kudrevich et al., 1996; Kudrevich et al., 1997; Kobayashi, 1999; Kobayashi et al., 1999; van Lier et al., 1999). For instance, the Kobayashi ring expansion reaction involving trisulphonated boron subphthalocyanines has been used to prepare novel asymmetrically substituted trisulphonated zinc phthalocyanines with utility as photosensitizer for photodynamic therapy (Kudrevich et al., 1996, Kudrevich et al., 1997, van Lier et al., 1999) (Figure 1.27). In a number of these examples, lower reaction temperatures and shorter reaction times along with electron-withdrawing groups on the subphthalocyanine and electron-donating groups on the 1,3-diiminoisoindoline may allow the reaction to proceed exclusively to give the desired 3:1 asymmetrically substituted phthalocyanines. Thus, while the Kobayashi ring expansion reaction of boron subphthalocyanines may not be a universal methodology for the preparation of 3:1 asymmetrically substituted phthalocyanines, it has proven to be still extremely useful and successful in specific cases.

1.10 Research Objectives

In view of their known utility and improved physical, chemical and spectral properties, the preparation of asymmetrically substituted phthalocyanines remains a vital objective in phthalocyanine synthesis. While a number of synthetic strategies (such as statistical mixed condensations, the use of polymer supports, the use of specially designed phthalocyanines precursors and the use of boron subphthalocyanines as templates) have been employed, each of these methodologies encounters significant drawbacks that limit their universal applicability. Nonetheless, with the importance of asymmetrically substituted phthalocyanines in such technical fields as non-linear optics and photodynamic therapy, methodologies such as these continue to be investigated and modified in order to prepare these crucial phthalocyanine derivatives.

The current research represents an extension of knowledge obtained in our lab concerning the use of certain boron subphthalocyanines in Kobayashi ring expansion reactions in order to prepare novel asymmetrically substituted phthalocyanines (Kudrevich et al., 1996, Kudrevich et al., 1997, van Lier et al., 1999) and the use of palladium-catalyzed reactions to modify the substituents on phthalocyanines and other photosensitizers in order to incorporate novel functional groups (Ali et al., 1994; Sharman et al., 1996; Ali et al., 1997; Tian et al., 2000; Khan et al., 2001; Khan et al., 2003; Cauchon et al., 2005) (also see Chapter 6). Halogenated boron subphthalocyanines were targeted as precursors for the synthesis of novel phthalocyanines. The corresponding Kobayashi ring expansion reaction of fluorinated boron subphthalocyanines would result in asymmetrically substituted phthalocyanines with important utility in photodynamic therapy in light of the heavy atom effect and the

established photodynamic efficiency of fluorinated photosensitizers as determined by our group (Allémann et al., 1995; Allémann et al., 1996; Boyle et al., 1996b; Allémann et al., 1997; Bench et al., 2002). In the meanwhile, the well-established reactivity of aryl iodides and in particular iodinated phthalocyanines towards palladium-catalyzed cross-coupling reactions (Ali et al., 1997) can be employed advantageously with iodinated phthalocyanines prepared by the Kobayashi ring expansion reaction of iodinated boron subphthalocyanines. Such reactions could be used in order to achieve the addition of novel functionality to asymmetrically substituted phthalocyanines. Of particular interest are novel asymmetrically substituted anionic and novel cationic water-soluble asymmetric phthalocyanines as these phthalocyanines would exhibit the amphiphilic character that has been shown to be advantageous for photosensitizers for photodynamic therapy (Paquette et al., 1991a; Allen et al., 1995; Margaron et al., 1996b; Kudrevich et al., 1997; Edrei et al., 1998; Allen et al., 2002; Cauchon et al., 2005).

Chapter 2.

Role of Activated Oxygen Species in Photodynamic Therapy

W. M. Sharman, C. M. Allen and J. E. van Lier (2000) *Methods in Enzymology,* **319**, 376-400.

THE ROLE OF ACTIVATED OXYGEN SPECIES IN PHOTODYNAMIC THERAPY

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Running title: Photodynamic therapy

Abbreviations: ALA, 5-aminolevulinic acid; BPD-MA, benzoporphyrin derivative monoacid ring A; DABCO, 1,4-diazabicyclo[2,2,2]octane; DMPO, 5,5-dimethyl-1-pyrolidine-1-oxide; EPR, electron paramagnetic resonance; HpD haematoporphyrin derivative; Lu-tex, lutetium texaphyrin; Npe6, mono-L-aspartyl chlorin e6; Pc, phthalocyanine; PDT, photodynamic therapy; PpIX, protoporphyrin IX; SOD, superoxide dismutase; S_0, ground state; S_1, first excited singlet state; SnET2, tin etiopurpin; T_1, first triplet state, mTHPC, tetra(m-hydroxyphenyl)chlorin; TEMP, 2,2,6,6-tetramethyl-4-piperidone; TEMPO, 2,2,6,6-tetramethyl-4-piperidone-*N*-oxyl radical

INTRODUCTION

Photodynamic therapy (PDT) finds its roots at the turn of the century when a young medical student found acridine orange killed paramecia upon exposure to sunlight.[1] Subsequently a plethora of information concerning the lethal effects of the combination of photosensitizers and light, both *in vitro* and *in vivo*, has been documented. Several reviews have recently been published outlining the clinical aspects of PDT and the preclinical and clinical studies predominantly accomplished within the past 25 year period.[2-7] This chapter outlined the basic principles of PDT using first and second generation photosensitizers. Attention will be given to their mode of action, either via singlet oxygen or other reactive oxygen species.

Conventional cancer therapies include radiation and chemotherapies, surgery and a combination of any or all of the above. The treatments themselves have important side effects, even life threatening. Consequently the development of an effective treatment that is more selective for diseased tissue is of utmost importance.

PDT offers an alternative, less invasive treatment for such illnesses as psoriasis and several types of cancers. It involves the use of three basic components. First, a photosensitizer, a light absorbing molecule which is activated by the second element, light of a corresponding wavelength. Third, by definition, molecular oxygen is consumed during the

photochemical reaction to produce cytotoxic agents thus destroying neoplastic tissue.

The traditional treatment protocol used in photodynamic therapy involves the intravenous injection of a photosensitizer. The photosensitizer is rapidly distributed throughout the body and experiences a differential uptake and/or retention time in tumor tissue such that, typically 48-72 hours post-injection, there is a marked increase in the photosensitizer concentration in the tumor as compared to surrounding normal tissue.[2] Several explanations have been proposed as to why there is more or less selective uptake by the tumor of the photosensitizer. They include, lower intratumoral pH, increased phagocytosis, increased permeability of the tumor vasculature as well as reduced lymphatic drainage and an increased number of receptors on the cell membrane for cellular proteins (i.e. lipoproteins) which are able to target the photosensitizer. [3,8,9]

Unlike laser surgery or psoralen UV-A treatment, PDT employs light with wavelengths typically 600-800 nm therefore not toxic as such. The photosensitizer alone is not able to generate cytotoxic agents hence following administration of the photosensitizer and localization in the malignant tumor, the beam of light can be directed to the tumor increasing the selectivity of the treatment. This is advantageous as it spares normal tissue which is not the case with conventional therapies. PDT offers

several advantages. It can be used in conjunction with other therapies, there is regeneration of healthy tissue following treatment and due to photosensitizer fluorescence, *in vivo* detection is possible providing a means of monitoring photosensitizer concentration and location.

PHOTOSENSITIZERS

There are hundreds of naturally occurring and synthetic dyes which can function as photosensitizers The most commonly used are first generation hematoporphyrin derivatives, most notably Photofrin II™ (PII) (Figure 1). Photofrin II™ is obtained by treating hematoporphyrin (Hp) with 5% sulfuric acid and acetic acid at room temperature.[10] Subsequently the mixture is treated with aqueous base then neutralized yielding a complex mixture of dimers and oligomers. The active compound being either an ether or an ester derivative. Purification of the most active compounds via HPLC leads to Photofrin II™. PII has been accepted in several countries including Canada, France, Germany, Japan, the Netherlands and the United States.[4] It is used for the treatment of advanced stage eosophageal, both early and advanced stage lung cancer and gastric cancers as well as cervical cancer and dysplasia. In addition it is used for the prophylactic treatment of bladder cancer. (See Dougherty et. al. 1998 for a complete review of clinical trials). Treatment of various other cancers are being thoroughly investigated using PII in the hopes of

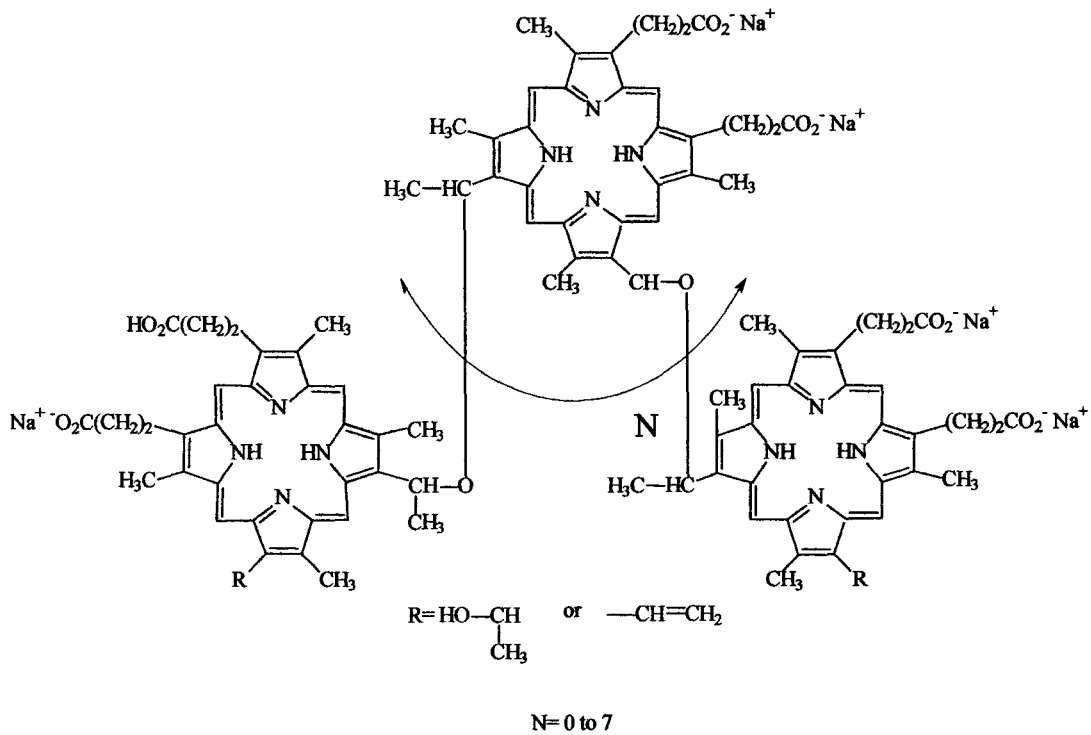


Figure 1. Photofrin II structure where N is from 0 to 7 repeating units.

licensing. Among them are early stage eosophageal cancer, head and neck cancers and superficial bladder cancer.[4]

Regardless of the success of PII, it has many important drawbacks.[2,11] The first being it is not a pure substance but a poorly reproducible mixture which varies with different preparations and storage times. It has poor absorption at 630 nm (molar extinction coefficient $\approx 10^3$ M^{-1} cm^{-1}). Tissue transmittance of light at this wavelength is minimal thus limiting the treatment to tumors at a depth of 5 mm or less. There is nonspecific accumulation of PII in various organs with only 0.1-3% of the injected dose retained by the malignant tissue. Lastly, PII is retained by cutaneous tissue for up to 2 months post PDT causing skin photosensitivity therefore the patient must avoid bright sunlight.[6,10] With that said, the search for the ideal photosensitizer has led to second generation photosensitizers in clinical trials.

Most importantly, the photosensitizer should be non-toxic with minimal systemic photosensitivity especially cutaneous. Ideally, the photosensitizer should absorb in the red or near infra-red (600-1000 nm) spectrum thus having enough energy to produce long lived triplet states so as to generate cytotoxic species usually believed to be singlet oxygen (see below). Light at a longer wavelength also allows for almost double the tissue penetration depth as compared to PII. There should be selective retention in tumor tissue to neighbouring healthy tissue. For convenience,

the photosensitizer should fluoresce to allow for visualization. Using fluorescent microscopy, the dye can be followed to determine sub-cellular targets. The mitochondria, lysosomes, plasma membrane, tumor cell nuclei and tumor vasculature have all been identified as important PDT targets depending on which photosensitizer is employed. Thus the fluorescence is a useful too in drug development. Lastly, a defined chemical composition is beneficial, preferably water soluble to avoid the need for emulsifiers and organic solvents for solubilization and delivery.[10,12]

Various second generation photosensitizers (Figure 2) have entered into phase I/II and II/III clinical trials. One such sensitizers is the very active chlorin, tetra (m- hydroxyphenyl) chlorin, mTHPC, having the greatest potential. It is now in clinical trials for head and neck cancers under the commercial name Temoporfin. It is activated at a number of wavelengths, 514 nm and 652 nm. It is very potent at a dose of 0.1 mg per kg of body weight and light doses as low as 10 J cm^{-2} for superficial esophagus cancers and Barrett's esophagus. As it is so potent at 652 nm, there is the option of illuminating at 514 nm where the product has a smaller extinction coefficient yet efficacy is not sacrificed. A potential drawback is that mTHPC has prolonged skin photosensitivity of about 6 weeks.[10,13]

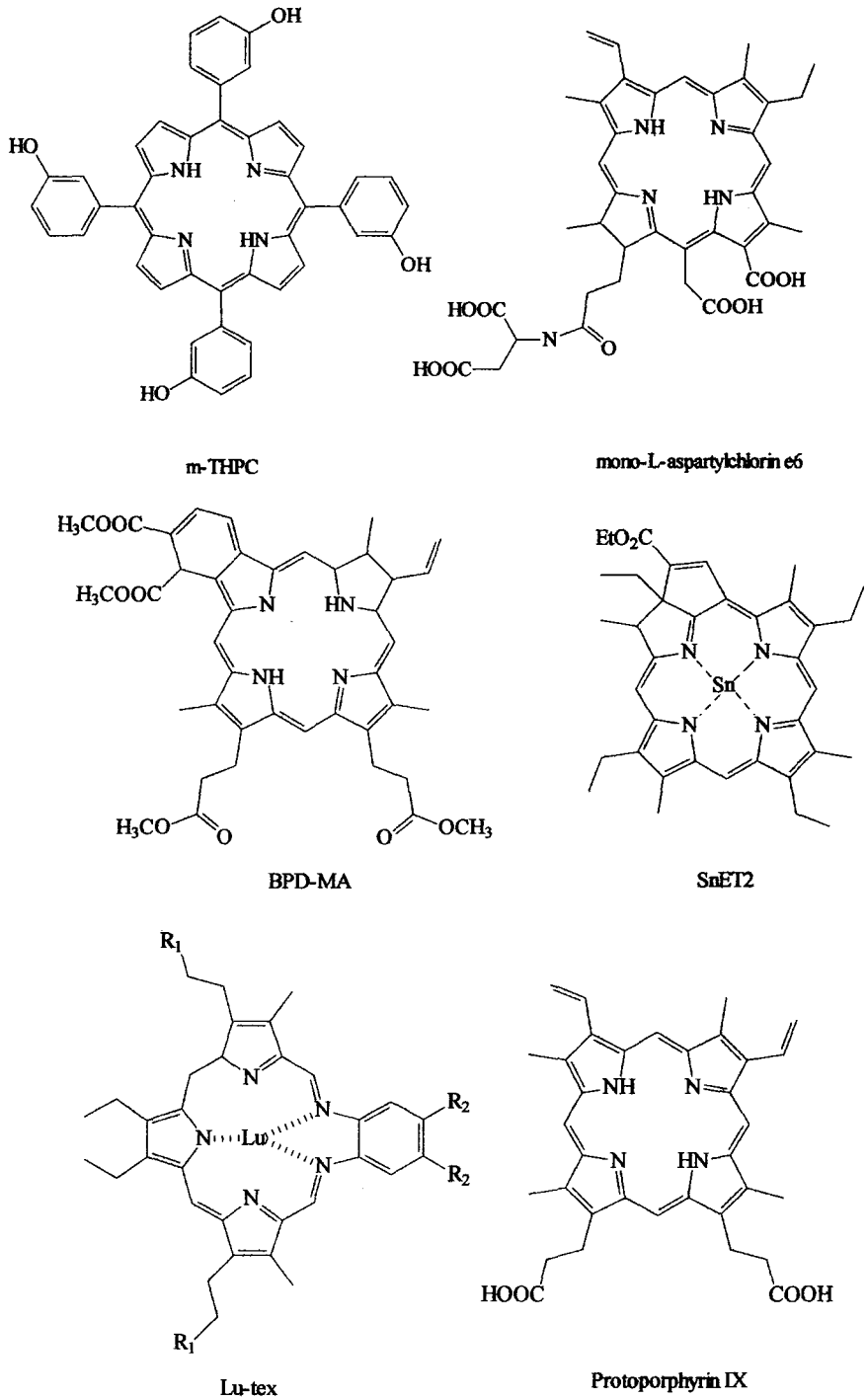


Figure 2. Second generation photosensitizers.

Subsequently NPe6, mono-L-aspartyl chlorin e6 is udergoing phase I/II trials for endobronchial lung cancer in Japan and the U.S. Npe6 is very hydrophilic and is therefore rapidly cleared from the blood. It is only effective as a photosensitizer if it is irradiated within 2 hours of injection when serum levels are at their peak. Limited skin photosensitivity is experiencd.[2,4]

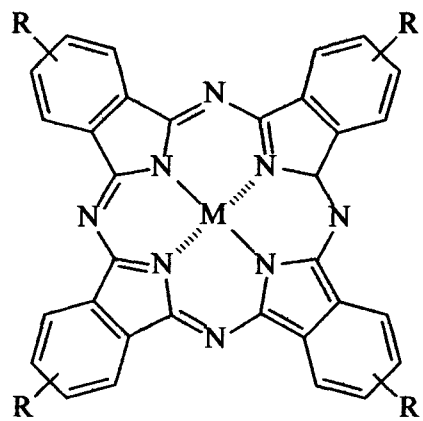
Benzoporphyrin derivative mono acid ring A (BPD-MA) has been in phase I/II trials for cutaneous tumors such as metastatic breast cancer and basal cell carcinoma. It is a lipophilic photosensitizer with a strong absorption maximum at 690 nm. BPD-MA rapidly localizes in neoplastic tissue therefore 3 hours post injection the light is administered on the tumor as opposed to 48-72 hours post injection as is the case for PII. With its rapid body clearance, BPD-MA causes mild cutaneous photosensitivity for only 3-5 days.[4,14,15]

Tin etiopurpin (SnET2) is under going phase II trials in the United States for cutaneous breast cancer and Kaposi's sarcoma for HIV patients.[16] Whereas lutetium texaphrin (Lu-tex) is in phase II/III trials for skin cancers. Lu-tex is one of the sensitizers with higher tumor to tissue ratios.[4]

A novel PDT method is the employment of endogenous photosensitization. 5-aminolevulinic acid (ALA) is a precusor in the heme pathway.[17,18] ALA formation is the rate limiting step in the formation of

protoporphyrin IX (PpIX) and is formed from glycine and succinyl CoA. Excess exogenous ALA can cause an accumulation of PpIX in the tissue where ALA was applied. It has been suggested that cells with higher turnover rates produce more PpIX possibly due to decreased ferrochelatase activity. In addition, it has been postulated that tumor cells require more iron thus limiting the amount available to proceed with the pathway to heme. The topical application of ALA to acinic keratoses, squamous cell carcinoma and superficial basal cell carcinoma induces a favourable biological response post illumination. ALA or its methyester give excellent results when applied topically yet show systemic toxicity when administered orally or intravenous injection. It is a noninvasive and convenient treatment and merits further investigation for any diseases dealing with epithelial surfaces, oral, vaginal, rectal, gastric, respiratory mucosa etc...

Phthalocyanines (Pc) (Figure 3)are another second generation class of photosensitizers.[19-21] They are azoporphyrin derivatives with four pyrrole subunits fused together with nitrogen atoms. The macrocycle is extended by four benzo rings on the pyrrole units. These modifications lead to enhanced absorption in the far red region of the spectrum as compared to PII. In addition, metallo-Pcs can be prepared by chelating one of several possible metal cations with the four central benzisoindole nitrogens to form stable complexes easily purified. Pcs are available for



Phthalocyanine

Figure 3. Phthalocyanine structure.

several modifications either by substitution on the benzene rings, for example the addition of sulphono and phosphono groups or by modifying the axial ligand. Pcs have attractive photophysical properties. They absorb strongly ($\varepsilon = 10^5\ M^{-1}\ cm^{-1}$) in the far red (680 nm) where tissue penetration is optimal. These photochemically stable compounds have been used for a number of *in vitro* and *in vivo* studies to evaluate their potential usefulness as anti-cancer agents. Both an aluminum and a zinc sulfonated phthalocyanine are in phase I/II clinical trials for early tracheobronchial, oesophagus and digestive tract cancers.[22]

MODE OF ACTION

Typically the photosensitizer is systemically administered and allowed to localized in the tumor. At this time, the photosensitizer is illuminated with light of the appropriate wavelength, exciting the photosensitizer to a higher energy state and ultimately leading to the production of cytotoxic species, resulting in cell death and tumor necrosis. The underlying mechanism behind the cytotoxic effects displayed during photodynamic therapy on the cellular level are described schematically below:

Sen → Sen* → Cytotoxic Agents → Biological Damage → Cell Death

While the first and last steps are indeed well known as it is clear that excitation of the photosensitizer will ultimately lead to cell death, the intervening steps are not so clearly understood and are most often assumed

relying on conjecture and indirect evidence. However, the overall mechanism involved in photodynamic therapy can easily be divided into two distinct and well-defined steps. The photophysical and photochemical properties of the photosensitizer and its ability to generate cytotoxic agents govern the first. The second results from the biological response of the cell towards the cytotoxic agents produced.

The initial photophysical processes experienced by the photosensitizer upon illumination have been extensively examined for a wide range of potential compounds. Upon illumination with light of the appropriate energy, ground state sensitizer (S_0) is promoted to its short-lived excited singlet state (S_1) (Figure 4). The lifetime of this excited singlet state (τ_S) is generally in the nanosecond range,[2] which is far too short to allow for significant interaction with the surrounding molecules. As such, it is generally accepted that photodynamic damage induced by the excited singlet state of the sensitizer is negligible. The excited singlet state can dissipate its energy via radiative emission of its excitation energy (fluorescence) or non-radiative decay (internal conversion). Internal conversion entails the lose of energy via collisions with solvent molecules, resulting in the generation of heat. It has been suggested that photothermal effects caused by internal conversion are one of the most important mechanisms for photosensitized cell killing.[23] For instance, Davila et al. Estimated that illumination of a cell stained with

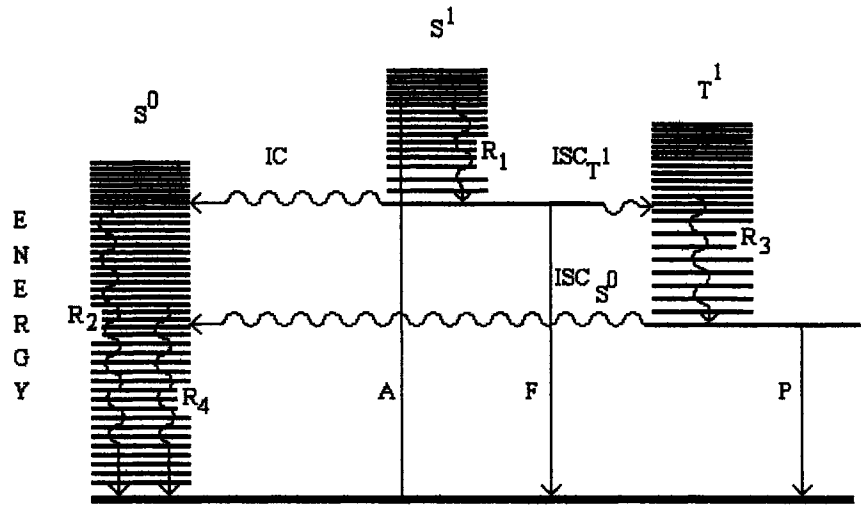


Figure 4. Jablonski Diagram illustrating some of the physical processes that can occur after a molecule absorbs a photon, excited state levels and transitions. S^0 is the ground electronic state of the molecule. S^1 and T^1 are the lowest excited singlet and triplet states, respectively. Straight arrows represent processes involving photons and wavy arrows represent radiationless transitions. (A), absorption; (F), fluorescence; (P), phosphorescence; (IC), internal conversion; (ISC), intersystem crossing; (R), vibrational and rotational relaxation.

D. Phillips, Progress Reaction Kinetics 22, 175 (1997).

Merocyanine 540, a polymethine dye, could increase the internal temperature of the cell by about 12°C/min. provided the cell membrane functioned as an adiabatic sink.[24] On the other hand, photosensitizer fluorescence can be used to monitor compound distribution in tissues both in vitro and in vivo.

Despite these important factors, the most important method of dissipating excited photosensitizer singlet state energy in terms of photodynamic therapy is non-radiative intersystem crossing to populate the much longer-lived triplet state (T_1). Lifetimes for longer-lived triplet species are typically in the microsecond to millisecond range since the $T_1 \rightarrow S_0$ transition is spin-forbidden.[25] This allows for sufficient time for interaction between the excited photosensitizer and surrounding molecules. Accordingly, it is believed that excited triplet states of the photosensitizer are responsible for the generation of the cytotoxic species produced during PDT. In fact, Takemura et al. have shown that the phototoxic effects of a given porphyrin are significantly enhanced as its triplet state quantum yield and lifetime is increased.[26] In addition, phthalocyanines containing paramagnetic central metal ions (Cu^{2+}, Fe^{2+}, Ni^{2+}), which greatly shorten the lifetime of the triplet state, are far less effective photosensitizers as compared to phthalocyanines chelating diamagnetic metal ions (Al^{3+}, Ga^{3+}, Zn^{2+}), whose triplet lifetimes are much longer.[19] Finally, it has been well established that addition of heavy atoms

such as bromine or chlorine to a photosensitizer improves it photosensitizing activity by improving triplet state quantum yields.[27] For instance, the triplet yield of rhodamine dyes has been greatly enhanced by the addition of bromine to the chromophore.[28] This is due to the internal heavy atom effect which increases spin-orbital coupling and facilitates intersystem crossing, thus allowing otherwise forbidden changes in the spin state ($S_1 \rightarrow T_1$). Typical photosensitizers being examined for use in photodynamic therapy have triplet state quantum yields (ϕ_T) of 0.2 to 0.7 [9] while triplet state lifetimes (τ_T) greater than 500 ns[25] are generally considered a prerequisite for efficient photosensitization.

Once produced, the excited triplet state can lose its excitation energy via radiative triplet-singlet emission known as phosphorescence. Of more importance for PDT however is the quenching of the excited triplet state which turns out to be the process which generates the majority of the cytotoxic agents needed to induce a biological effect. The quenching mechanism of the T_1 state of the photosensitizer can be distinguished as occurring via a Type I or a Type II mechanism (Figure 5).[29] A Type I mechanism involves hydrogen atom extraction or electron transfer reactions between the excited state of the sensitizer and some substrate, either biological, solvent or another photosensitizer, to yield radicals and radical ions. These radical species are highly reactive and can readily interact with molecular oxygen to either generate reactive oxygen

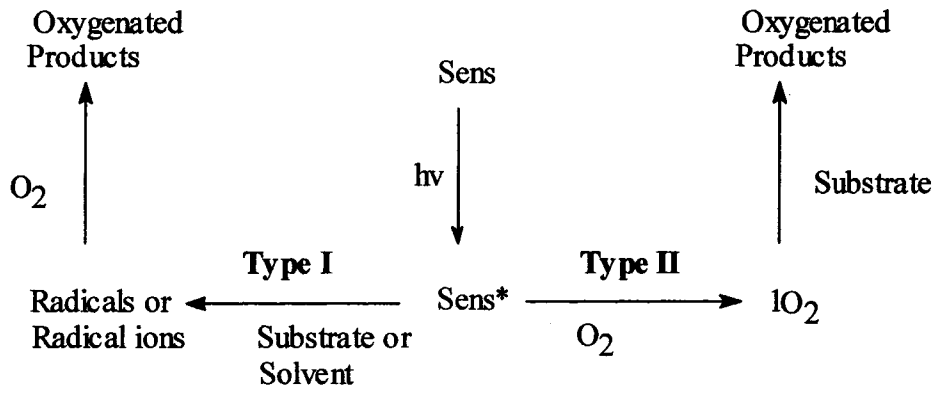


Figure 5. Diagramatic presentation of Type I and Type II photosensitized oxidation reactions.

C. S. Foote, Photochem. Photobiol. 54, 659 (1991)

species such as superoxide anion or fix the damage so it is unrepairable. These reactions cause the formation of oxidative damage and eventually lead to the cytotoxic effects seen during photodynamic therapy. The Type II mechanism, on the other hand, results from an energy transfer from the triplet state of the photosensitizer to ground state molecular oxygen, leading to the generation of an excited state of oxygen known as singlet oxygen. Due to its high reactivity, singlet oxygen can react with a large number of biological substrates, causing oxidative damage and cell death.

ROLE OF OXYGEN

Ever present in either Type I or Type II photodestruction is the role of molecular oxygen. It has been well established that the presence of oxygen is an absolute requirement for the photoinactivation of cells via photodynamic therapy as anoxic conditions totally abolish PDT-mediated cellular inactivation. For PhotofrinII™ photosensitization of cells in vitro, full effects were observed at around 40 torr oxygen tension with half-values at about 8 torr.[30,31] In addition, PDT-dependent oxygen consumption rates for PhotofrinII™ have been estimated to be as high as 6-9 μM/s using an incident light intensity of 50 mW/cm^2.[32] As such, it is clear that oxygen plays a vital and absolute role in photodynamic therapy and is in fact one of its key limiting factors. For instance, tumor cells are poorly supplied with blood, leading to local areas of hypoxia. In addition, during irradiation, oxygen levels within a tumor will be affected by PDT-

induced vascular damage and even by the production of reactive oxygen species by PDT. These effects can greatly limit the potential of PDT against solid tumors where regions of hypoxia become important.[4,33]

TYPE II: SINGLET OXYGEN

Until recently, it has been universally accepted that the Type II mechanism predominates in photodynamic therapy. As such, the single most important cytotoxic agent generated during PDT is viewed as singlet oxygen. Singlet oxygen is produced during PDT via a triplet-triplet annihilation reaction between ground state molecular oxygen (which is in a triplet state) and the excited triplet state of the photosensitizer. Such an energy transfer reaction requires a collision between the two species involved thus the need for a long triplet state lifetime for the photosensitizer and a minimum oxygen concentration in the tissue. It should be noted that, with certain sensitizers, singlet oxygen can be produced via an energy transfer reaction between the S_1 state of the sensitizer and molecular oxygen.[25] This is possible when the S_1-T_1 energy gap is large enough and the sensitizer has a singlet state lifetime that is sufficiently long to allow ample bimolecular collisions with molecular oxygen.

There exist two excited singlet S_1 states of molecular oxygen.[34] The $^1\Delta_g$ state has an energy level of 94 kJ/mol. above the ground state

while the $^1\Sigma_g^+$ state is excited by 156.7 kJ/mol. The higher energy $^1\Sigma_g^+$ state of singlet oxygen has a very short lifetime (20 ps in methanol[35]) and is rapidly quenched to yield the $^1\Delta_g$ singlet state in a spin-allowed process. Considering this, the $^1\Delta_g$ singlet state of molecular oxygen is assumed to be the only singlet oxygen species involved in the photodynamic effect and any further reference to singlet oxygen refers to the $^1\Delta_g$ state.

The two highest energy electrons of the $^1\Delta_g$ state are paired and in the same orbital, leading to zwitterionic reactivity.[34] Such an electronic configuration lends a relatively high reactivity to singlet oxygen towards a number of biological substrates. The major chemical entities that constitute biological matter are water, amino acids, pyrimidine and purine bases and phospholipids. Except for water, all these constituents are sensitive to being oxidatively damaged by singlet oxygen. Presumably such chemical modifications can lead to biological lesions and possibly cell death. Singlet oxygen readily adds to unsaturated carbon-carbon bonds of biomolecules via a 1:4 addition to eventually yield hydroperoxides among primary oxidation products.[36] Reaction of singlet oxygen with membrane lipids, proteins or nucleic acids can lead to disruption of the cell membrane, the lose of functionality of vital proteins, and unrepairable DNA damage. Any or all of these biological lesions can lead to cell death.

Singlet oxygen was first proposed as the cytotoxic agent responsible for photoinactivation of tumor cells in 1976 by Weishaupt et al..[37] Despite this, direct evidence of the involvement of singlet oxygen in PDT has been illusive. However, extensive indirect evidence is available that backs up the hypothesis of the involvement of singlet oxygen in PDT-induced biological damage.

One of the main problems associated with the detection of singlet oxygen in biological systems is its high reactivity, which results in extremely short singlet oxygen lifetimes. Singlet oxygen is known to have a lifetime of about 3 μs in water.[38,39] In the meantime, due to its high reactivity with biological substrates, the lifetime of singlet oxygen in cells has been estimated to be in the region of 200 ns.[40] In cellular systems, saturated with oxygen, the rate of singlet oxygen generation in the cell interior has been assessed to be in the range of $2x10^5$ to $4x10^5$ s^{-1}.[41] As such, in the best possible conditions (oxygen saturation), the decay of singlet oxygen will occur at a rate that is 5-10 times more rapid than is rate of formation. The situation is even more precarious in biological systems equilibrated with air only. The resulting five-fold decrease in oxygen concentration reduces the rate of generation of singlet oxygen to about $1x10^4$ to $4x10^4$ s^{-1}. Clearly, this results in an infinitesimally small amount of singlet oxygen being available for detection.

The other consequence of the extremely short lifetime of singlet oxygen in cells is its short diffusion range, which has been predicted to be limited to approximately 45 nm.[42] This is in excellent agreement with experimental results where singlet oxygen generated by haematoporphyrin outside the cell wall of E. coli could not induce DNA strand breaks within the bacteria.[43] Thus, since the diameter of human cells range from 10-100 μm, the primary site of singlet oxygen generation will determine what subcellular target will be attacked. Hence, the importance of the subcellular distribution of the photosensitizer is obvious in determining the efficiency of the compound as a therapeutic agent.

The only true method of detecting singlet oxygen directly is by means of its monomol luminescence at approximately 1270 nm.[44,45] Singlet oxygen also has a dimol luminescence emission at 610 nm[46] and while this emission has proven an effective tool in the identification of the $^1\Delta_g$ state, its production requires a bimolecular reaction between a pair of singlet oxygen species. Obviously, this is extremely disadvantageous at the micromolar levels seen in PDT. Furthermore, the emission at 610 nm is clearly far from ideal as most photosensitizers absorb in that range of the electromagnetic spectrum.

While the demonstration of the intermediacy of singlet oxygen in reactions carried out in homogeneous environments using the monomol luminescence is a relatively easy procedure, the molecular complexity and

heterogeneity of biological systems has made such demonstrations both difficult and ambiguous. The ability of a given photosensitizer to generate singlet oxygen can be easily determined and is in fact often considered to be one of the first parameters to be determined in order to evaluate the potential of the sensitizer in PDT. Photofrin™ has a singlet oxygen quantum yield of 0.87 [47] while second generation photosensitizers have similar or better quantum yields.

While the triplet excited state of the photosensitizer can easily be detected by laser flash photolysis techniques for a sensitizer localized intracellularly, there is no such detection of singlet oxygen luminescence in intact cells.[48] However, recent improvement in techniques has allowed for the detection of singlet oxygen luminescence in a variety of cell suspensions. For instance, Girotti and associates have detected singlet oxygen luminescence from porphyrins bound to erythrocyte ghosts and other membranes.[49-51] Singlet oxygen luminescence in the case of porphyrin sensitization was also reported by Bohm et al. using Fourier transform techniques for porphyrins bound to membrane surfaces.[52] Yeast cells sensitized with tetra-(p-phenosulphide)porphyrin also displayed singlet oxygen luminescence using flash photolysis techniques.[38,53] Baker and Kanofsky have detected singlet oxygen in sensitized suspensions of red blood cell ghosts and L1210 leukemia cell using 5-(N-hexadecanoyl)amino eosin via its emission at 1270 nm.[54-56] Lifetimes

determined in all cases suggested that the vast majority of the detected luminescence was due to singlet oxygen that had diffused into the buffer.[54-57]

Considerations such as these, along with a number of other theoretical considerations of the *in vivo* singlet oxygen detection problem (including the short lifetime in singlet oxygen in cells, the rapid rate of singlet oxygen consumption and low luminescent quantum yields of singlet oxygen (only 200 singlet oxygen molecules per billion undergo radiative decay[41])) have suggested that it is unlikely that luminescence emission will be detected emitting from within cells. However, extensive work using indirect means of evaluating the presence of singlet oxygen in biological systems has been used to verify the hypothesis of the involvement of singlet oxygen in PDT. For instance, it has been shown that photo-oxidation of cholesterol by haematoporphyrin or tetrasulphonated chloro-aluminium(III) phthalocyanine yields characteristic reaction products due to singlet oxygen oxidation, 3β-hydroxy-5α-hydroperoxy-cholest-6-ene, 3β-hydroxy-6α-hydroperoxy-cholest-4-ene and 3β-hydroxy-6β-hydroperoxy-cholest-4-ene.[58-61] In addition, typical singlet oxygen products are acquired upon sensitization of guanosine (4-hydroxy-8-oxo derivatives).[62] Cholesterol and guanosine both give complex mixtures of species when oxidized by radicals (Type I mechanism), mainly leading to epimeric 7-hydroperoxyl derivatives from

cholesterol and imidazole ring opening products from guanosine. In the case of cholesterol, reduction of the products using sodium borohydride yields the corresponding stable hydroxyl analogues, which can be readily identified using chromatographic procedures. This leads to a simple diagnostic test to distinguish between the two reaction pathways in homogeneous systems.[34] However, in complex biological milieu, the ensuing lipid peroxidation of cholesterol results in the formation of complex mixtures of oxysterols which obscure the initial products.[63] In addition, the 5-α-hydroperoxyl derivative is slowly converted via an intramolecular rearrangement to the 7-α-hydroperoxide, which subsequently epimerizes to the 7-β-hydroperoxyl derivative via a dissociative radical mechanism.[64] Accordingly, although the presence of the 5-α-hydroperoxyl derivative of cholesterol is unambiguous evidence for a Type I mechanism, its absence or the presence of 7-hydroperoxycholesterols alone does not rule out the involvement of singlet oxygen in the photochemical process. Despite these problems involving biological systems, studies using [^{14}C]cholesterol clearly showed the intermediacy of singlet oxygen in unilamellar phospholipid vesicles, ghost erythrocytes and in L1210 leukemia cells.[59]

Routinely, the responsibility of singlet oxygen in the photodamage caused during PDT has been indicated by the inhibition of the biological effect using competitive quenchers of singlet oxygen. Numerous

compounds exist that can competitively react with singlet oxygen, thus providing protection against its cytotoxic effects. Among these are sodium azide, histidine, 2,5-dimethylfuran, β-carotene and 1,4-diazabicyclo[2,2,2]octane (DABCO).[36,41,65-67] In addition, compounds like 1,3-diphenylisofuran and 9,10-diphenylanthracene has been used to determine singlet oxygen quantum yields in heterogeneous environments by following their photo-oxidation via fluorescence.[68,69] These quenchers work via different mechanisms. β-carotene involves an electronic energy transfer[70] while amines like DABCO and sodium azide react by charge transfer quenching.[71] Others like 1,3-diphenylisobenzofuran and 9,10-diphenylanthracene undergo chemical reactions with singlet oxygen to form peroxy-derivatives.[41]

Quenchers have routinely been used to show not only the intermediacy of singlet oxygen in PDT but to also distinguish between the reaction mechanism involved in a given system. For instance, PDT-induced damage to cytochrome P-450 and associated monooxygenases (aryl hydrocarbon hydroxylase, 7-ethoxycoumarin-O-deethylase and 7-ethoxyresorufin-O-deethylase) along with lipid peroxidation by chloroaluminium phthalocyanine tetrasulphonate was studied by Agarwal et al. in hepatic microsomes.[66,67] It was determined that among quenchers of reactive oxygen species, only those of singlet oxygen, such as sodium azide, histidine and 2,5-dimethylfuran, afforded substantial protection

towards photodestruction of cytochrome P450 and associated monooxygenase activities along with photo-oxidation of lipids. As such, it was suggested that PDT-induced damage was due to the production of singlet oxygen. Similar results were obtained for mammalian cells using phthalocyanines as the photosensitizer.[72]

Unfortunately, the use of quenchers is not entirely specific for singlet oxygen. The quenchers used are typically systems of low oxidation potential and are almost certainly capable on interacting with other reactive oxygen species produced during irradiation of the photosensitizer. For instance, sodium azide, an effective quencher of singlet oxygen, is also known to react with hydroxyl radicals but at a much slower rate and inhibition of lipid peroxidation by sodium azide has been found to occur at a rate constant that is 50 times lower than expected.[59] This could be explained by the limited access of the quencher to the singlet oxygen generated in the membrane. However, it could also indicate the intermediacy of hydroxyl radical. Tryptophan is also a popular quencher of singlet oxygen but has recently been shown to react under certain circumstances via a mixed Type I/Type II mechanism.[73] Only careful identification of the reaction products can clearly identify the reaction mechanism involved in a given case.[61]

A second diagnostic test for the involvement of singlet oxygen depends on the truly amazing and totally characteristic lifetime variation

in different solvents exhibited by singlet oxygen. As was stated previously, singlet oxygen has a lifetime in water of about 3 μs. This increases to 65 μs in heavy water (D_2O).[41,74] The result of changing from water to heavy water leads to an increased lifetime for singlet oxygen and presumably an increase in the biological effect and this has been used to verify the presence on singlet oxygen during PDT. However, while large increases in the rate of photo-oxidation have been seen, the results are not unequivocal. For instance, the quantum yield for triplet state formation of the photosensitizer is greater in D_2O which will favor both Type I and Type II reaction pathways.[2,75] In addition, the structural conformation of proteins may not be comparable in H_2O and D_2O environments, thus making the protein more susceptible to oxidative damage, be it via a Type I or Type II mechanism.[2] The replacement of water with heavy water may also hamper the biological processes involved in recovery from sublethal damage and would therefore potentiate cell killing.[76] Finally, D_2O may be expected to have little or no effect on the lifetime of singlet oxygen that is generated with a lipid membrane or another hydrophobic environment where water does not have access. Thus, while the use of D_2O can be used to show the involvement of singlet oxygen, it is not unequivocal proof of which mechanism is involved.

One final indirect method for the detection of singlet oxygen in a biological system is the use of electron paramagnetic resonance

(EPR).[69,77-83] While singlet oxygen is not radical in nature and will not give a signal in the EPR spectrum, it has been shown to readily react with the spin trap 2,2,6,6-tetramethyl-4-piperidone (TEMP).[77] Such a reaction leads to the formation of 2,2,6,6-tetramethyl-4-piperidone-*N*-oxyl radical (TEMPO) (Figure 6) that gives a clear and easily identifiable signal in the EPR spectrum. To ensure that the production of TEMPO was due to the reaction of TEMP with singlet oxygen, various quenchers and the effects of D_2O were used. EPR has been used to identify singlet oxygen production in liposomes,[79] in human erythrocytes[80] and in bronchial epithelial cells[83] and has proven to be a useful technique. Of particular interest is the reported feasibility of *in vivo* skin EPR spectroscopy and imaging which might provide for detection of singlet oxygen production *in vivo*.[84,85]

TYPE I: RADICAL AND OTHER REACTIVE OXYGEN SPECIES

While the techniques described above have surely identified singlet oxygen as an important reactive oxygen species implicated in photodynamic therapy, it is not the only reactive oxygen species formed during PDT. Reactive oxygen species such as superoxide anion, hydrogen

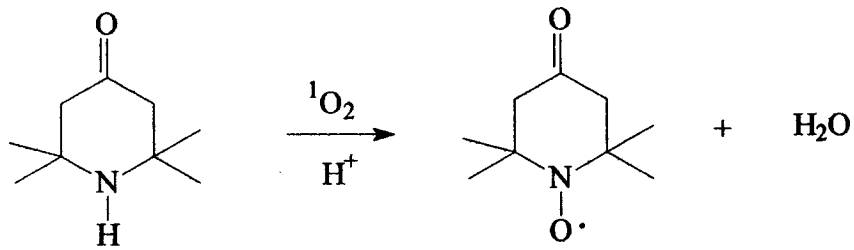


Figure 6. The reaction of TEMP with singlet oxygen.

A. Viola, A. Jeunet, R. Decreau, M. Chanon, and M. Julliard, Free Rad. Res. 28, 517 (1998).

peroxide and hydroxyl radicals can be easily formed via Type I processes. In general, the excitation of the photosensitizer to its excited triplet state and the resulting promotion of an electron from an occupied to an unoccupied orbital leads to the formation of a reducing electron and an oxidizing hole. As such, the triplet state of the photosensitizer is more easily oxidized and reduced as compared to the ground state molecule. The excited triplet state can react with another photosensitizer in the ground state to form a radical anion and radical cation pair. Furthermore, the excited triplet state can easily react with electron donating molecules to form the sensitizer anion radical. In fact, there exist numerous molecules capable of donating electrons to the triplet state of the sensitizer in biological matter. They include NADH, vitamin C, cysteine, methionine, tyrosine, uracil and guanine among many others.[86] The sensitizer anion radical can react with molecular oxygen in an electron-exchange reaction, leading to the formation of superoxide anion ($\cdot O_2^-$). It should be noted that superoxide anion can also theoretically be formed via an electron transfer reaction between molecular oxygen and the triplet state of the photosensitizer. However, this process has been shown to be thermodynamically unfavorable as compared to the energy transfer reaction that forms singlet oxygen.[87]

Superoxide anion can react with biological substrates either by electron transfer or oxidation reactions and can interact directly with a

number of cellular structures such as polyunsaturated fatty acids, alcohols, amino acids and proteins. It has been shown that superoxide anion can inactivate several enzymes as well. In addition, superoxide anion reacts rapidly with ascorbic acid and α-tocopherol while one of the most important biologically relevant reactions is that of superoxide anion with sulfhydryl compounds, leading to the formation of RS· radicals and hydrogen peroxide.[36] Overall, however, the reactivity of superoxide anion is rather limited. Its most important role in the induction of biological damage induced by PDT is in the generation of the highly reactive hydroxyl radical via the Fenton reaction (Figure 7). The hydroxyl radical can readily add to double bonds or abstract hydrogen atoms, resulting in the formation of secondary radicals ultimately leading to chain reactions such as those implicated in lipid peroxidation.[88]

Reactive oxygen species such as superoxide anion, hydrogen peroxide and hydroxyl radical have been identified in photodynamic therapy using a number of indirect methods. Superoxide anion, for instance, has been implicated in photodynamic induced damage using the quenching effects of superoxide dismutase (SOD). However, superoxide dismutase is too big to enter intact cells and therefore, SOD is less useful in cellular systems.[89] In addition, the dismutation of superoxide anion by SOD leads to the formation of hydrogen peroxide, which can also induce a biological effect.[36,41,65-67] Another quencher of superoxide anion, p-

$$2\ O_2^{\cdot-} + 2\ H^+ \rightarrow O_2 + H_2O_2$$

$$Fe^{3+} + O_2^{\cdot-} \rightarrow Fe^{2+} + O_2$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\cdot}$$

Figure 7. Fenton reaction

benzoquinone, has also been used, primarily to distinguish between effects due to superoxide anion and the other reactive oxygen species.[90] The reduction of ferri-cytochrome c to ferro-cytochrome c has been used to quantify the amount of superoxide anion formed during PDT.[77] Quenching has also been used to examine the presence of hydrogen peroxide (using catalase) and hydroxyl radical (using sodium benzoate, mannitol and ethanol) with varying results.[30,36,66,67] As was mentioned above, quenching experiences seemed to identify singlet oxygen as the only reactive species involved in the photodestruction of cytochrome P450 activity. However, the use of quenchers in studying photodynamic cell killing of EMT6 and CHO cells by Photofrin™ reaffirmed the predominant role of singlet oxygen in PDT but also indicated some involvement of free radical species such as hydroxyl radical.[65] In addition, using quenching experiments and studying the effects of D_2O, it was determined that inactivation of catalase in erythrocytes and K562 leukemia cells using tetrasulphonated metallophthalocyanines involved a mixed Type I/Type II mechanism.[91] Similar studies indicated a mixed Type I/Type II mechanism in the photoinactivation of Chinese hamster lung fibroblasts.[92] Clearly, quenching experiments such as these seem to show that the mechanism behind photodynamic therapy is not quite so straightforward and most likely involves a combination of Type I and Type II mechanisms.

The presence of reactive oxygen species such as superoxide anion and hydrogen peroxide has also been examined using flow cytometry.[93] Using fluorescence probes like hydroethidine (which reacts with superoxide anion to give ethidium bromide which emits a red fluorescence) and dihydrorhodamine 123 (which reacts with hydrogen peroxide to give rhodamine 123 which emits a green fluorescence), it was determined that ALA-induced photodestruction of primary human skin fibroblast correlates with intracellular superoxide anion production but does not correlate with intracellular hydrogen peroxide production. This apparent discrepancy was explained by the fact that the flow cytometric assay used focused on early stages of cell death and that hydrogen peroxide can readily diffuse out of the cell and might not be seen by the intracellular method used.

One of the most important methods for detecting radicals in biological systems is electron paramagnetic resonance and it has been used extensively in the case of photodynamic therapy. Superoxide anion, for instance, can react with the spin trap 5,5-dimethyl-1-pyrolidine-1-oxide (DMPO) (Figure 8) to give the superoxide spin trap adduct DMPO-OOH.[77] However, DMPO-OOH is relatively unstable, especially in the presence of transition metals, and rapidly decomposes into various species including DMPO-OH, the spin trap adduct of the hydroxyl radical. Despite this, the EPR signal due to DMPO-OOH can be seen when

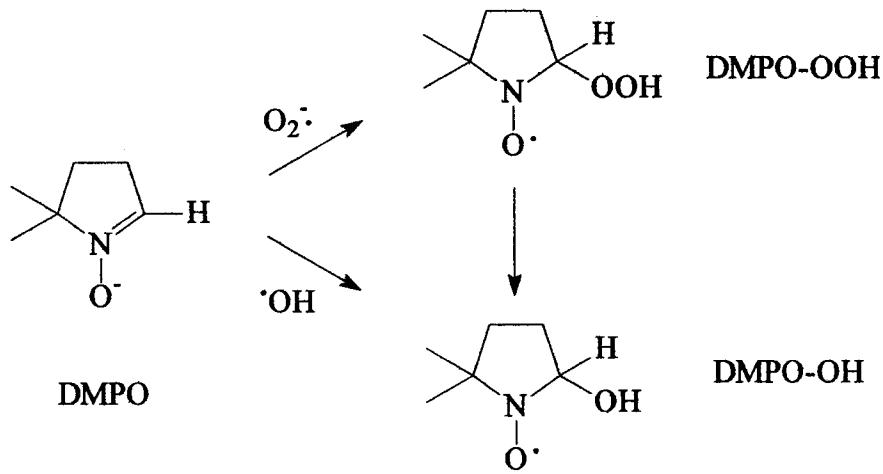


Figure 8. Formation of the DMPO spin adducts of superoxide anion and hydroxyl radical.

A. Viola, A. Jeunet, R. Decreau, M. Chanon, and M. Julliard, Free Rad. Res. 28, 517 (1998).

desferrioxamine is used to chelate the iron ions present in solution and this technique has been used to show the presence of superoxide anion during photosensitization of numerous photosensitizers.[69] The same goes for hydroxyl radical, which has been shown to be present in a number of photodynamic systems. In one very interesting EPR study, Viola et al. demonstrated that a series of phthalocyanines readily generated both Type I and Type II reactive oxygen species in a homogenous solution of DMF with Type II products predominating.[77] In a membrane model, those phthalocyanines tested have fixed axial ligands were unchanged, with Type II products predominating. However, in the same membrane model, Type I products became important for those phthalocyanines without axial ligands while the Type II pathway was shown to be negligible. Results such as these clearly show the importance of environment on the mechanism involved in PDT.

In addition to the spin trapping of superoxide anion and hydroxyl radical, spin adducts of the sensitizer anion radical have been detected in certain systems,[69,81,82] clearly identifying this species as the precursor to the formation of the other reactive oxygen species. Furthermore, carbon-centered radicals have been seen, most likely being due to the formation of radicals on biological targets.[94,95] Finally, recent studies have shown that the radical cation of the photosensitizer may be involved in

photosensitized decomposition of biological peroxides resulting in the generation of peroxyl radicals.[96]

TYPE I VERSUS TYPE II

Although the factors that govern the competition between Type I and Type II processes are reasonably well understood, the complexity of the biological environment, as well as uncertainty concerning the localization and binding of the sensitizer to tissue and cell constituents, combined with fluctuations in oxygen concentrations in tissues and even in cellular compartments make it impossible to predict which type of reaction mechanism will prevail during PDT. It has been well established, for instance, that Type II processes predominate in oxygenated systems while Type I reactions prevail under hypoxic conditions.[33] At low oxygen concentrations, Type I processes have been demonstrated to contribute significantly to the photo-oxidation of membrane components and amino acids using flash photolysis studies with tetrasulphonated chloro-gallium (III) phthalocyanine.[97] In fact, the consumption of oxygen during PDT has been shown to change the mechanism of action from Type II to Type I as the oxygen concentration decreases below a critical level and tumor vascularitity is damaged.[33,81,98] For instance, electrochemical measurements have demonstrated that the number of cells with a regular intracellular oxygen concentration is obviously reduced following irradiation of photosensitizer-loaded cells.[99] Moreover, it has been clearly

demonstrated that Type I processes are favoured in more polar environments where the high dielectric constant of the medium should stabilize the radical pair that is generated. In the meantime, Type II reactions should predominate in more lipophilic environments where the lifetime of singlet oxygen is much longer.[25] However, despite this, it has been shown that even in relatively polar media, singlet oxygen mechanisms are often more efficient. Results by Rossi et al. indicated that indole derivatives appear to be photo-oxidized largely by a Type I mechanism involving electron transfer from the triple state of haematoporphyrin to the indole moiety in water.[100] Type II processes only become important as the environment becomes more lipophilic. Despite this, the situation is not quite so clear as at low trytophan concentrations, singlet oxygen has been shown to be able to compete with radical-type reactions even in highly polar media.[73] Finally, binding of the sensitizer to cellular components should favor Type I hydrogen abstraction or electron transfer reactions, which will not be evident under *in vitro* conditions. An interesting example of the importance of Type I mechanisms involves a recently studied photosensitizer copper(II)-α-meso-N,N-dimethyloctaethylbenzochlorin iminium chloride (Figure 9).[101] This sensitizer has a triplet lifetime of less than 20 ns, which is far too short to allow efficient energy transfer to oxygen in order to form singlet oxygen. Despite this, this compound has been demonstrated to be an effective

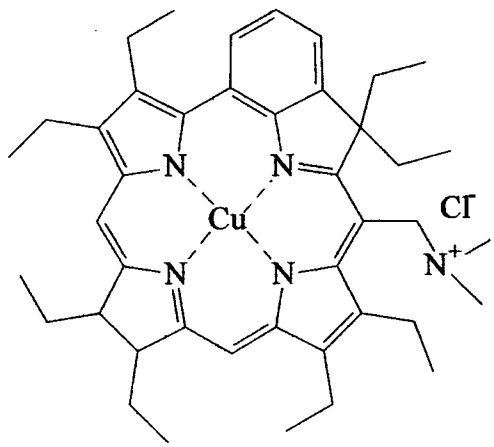


Figure 9. Copper(II)-α-meso-N,N-dimethyloctaethylbenzochlorin imminium chloride

D. Skalkos, J. A. Hampton, R. W. Keck, M. Wagoner, and S. H. Selman, Photochem. Photobiol. 59, 175 (1994).

photosensitizer against urothelial tumors in rats. Using superoxide dismutase and catalase, it was shown that this sensitizer induces its damage via reactive oxygen species other than singlet oxygen. It was determined that the close proximity and/or binding of this compound to important biological molecules and the rapid timescale of electron transfer reactions (less than 10 ps[102]) most likely promoted a Type I mechanism and helped explain the usefulness of this compound as a PDT agent.

Further complicating the identification of the processes responsible in a given system is the finding that the mechanism involved may depend on the cell type.[2] It has been demonstrate that killing of bacteria depended on whether the bacteria was gram-positive or gram-negative. By adding toluidine blue (Figure 10) to sepharose beads, the effects of Type I cytotoxic agents could be ignored as cells could not penetrate the bead Type I cytotoxic agents could not escape it. Using this, it was determined that singlet oxygen was responsible for the cell killing of S. mutans, a gram-positive bacteria. On the other hand, gram-negative bacteria such as P. gingivalis and E. coli along with a yeast, C. albicans were not killed using this beads while toludine blue is capable of inactivating these bacteria by itself. From this, it was concluded that the action of Type I free radicals was vital for the cell killing ability of toludine blue towards these cells. In addition, it was determined that the kinetics of cell killing of these cells by toludine blue was binomial, suggesting a two step

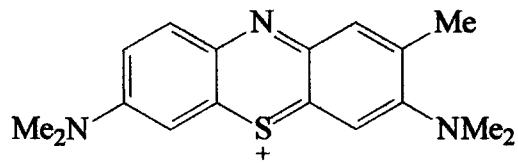


Figure 10. Toluidine Blue

mechanism whereby free radicals caused disruption of the cell wall which allowed access to the cell interior to toludine blue and the cytotoxic effects of singlet oxygen. Such cell specificity is backed up by finding by Rywkin et al. who, using various quenchers, established that virus kill in red blood cell concentrates operated through a Type II mechanism whereas both Type I and Type II mechanisms contributed to red blood cell damage.[103]

CONCLUSION

Our knowledge of photodynamic therapy has increased substantially. So much so that it is now an approved treatment for various cancers and subsequent uses for PDT are being determined regularly. Although it is well known that PDT is very toxic under specific circumstances and conditions, there is much to learn about its exact mode of action. For the overall photodamage, the initial reaction is of less importance since both Type I and Type II reactions lead to similar oxidative damage and can lead to comparable radical chain reactions in the presence of oxygen. Overall, the effect of either Type I or Type II reactions is the production of oxidative damage within the cell which will ultimately lead to cell death. Where distinguishing between these two reaction pathways becomes important is in order to thoroughly understand how PDT actually works so that modulation of its effect can be achieved to maximize the biological effect. It is highly unlikely that a given

photosensitizer or a given PDT mechanism is ideal for every application. It is only in completely understanding the processes involved, that ideal compounds and conditions can be determined for each use.

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Chapter 3.

Photodynamic Therapeutics: Basic Principles and Clinical Applications

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Photodynamic therapeutics:

Basic principles and clinical applications

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Keywords: Photodynamic therapy, Photosensitizer, Clinical trials, Photofrin®, Cancer, Macular Degeneration, Psoriasis

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Preface Paragraph

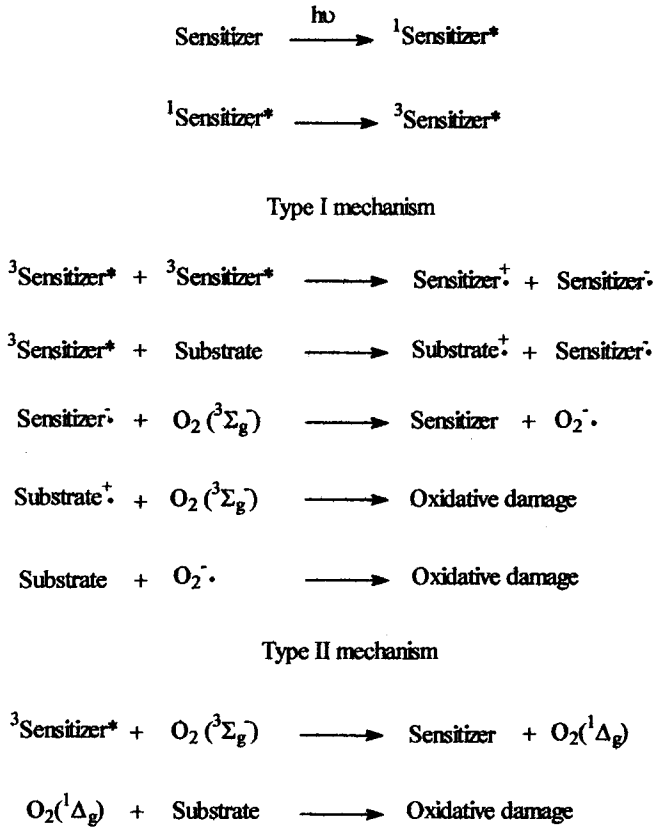
Photodynamic therapy (PDT) is a promising new cancer treatment recently accepted in clinic. PDT involves the localization of a light-sensitive drug (photosensitizer) in the target tissue prior to illumination with light of an appropriate wavelength. Cytotoxic agents generated upon illumination trigger a cascade of biochemical responses, effectively inactivate cancer cells either directly or via the induction of vascular stasis. Such a treatment is better tolerated as it destroys diseased tissue while leaving normal tissue intact. Photofrin®, an haematoporphyrin derivative, has been approved in a number of European and Asian countries, as well as in North America. To further enhance the potential of PDT and explore it application for various other conditions, second generation photosensitizers are being rigorously investigated.

Traditional cancer therapies such as surgery, radiation therapy and chemotherapy involve a delicate balance between removing or destroying diseased tissue while sparing surrounding normal, healthy cells. All of these conventional treatments result in very important side effects due to undesirable loss of normal cell function as a result of the rather indiscriminate nature of their cytotoxic properties. Consequently, the development of new treatment protocols that display more selectivity for diseased tissue is extremely important.

Photodynamic therapy (PDT) is a promising new cancer treatment modality that has recently been accepted into clinic in a number of countries. PDT involves the combination of visible light and a photosensitizer, both harmless by themselves but together with oxygen, they are capable of producing lethal cytotoxic agents that can inactivate tumour cells. This allows for greater selectivity towards diseased tissue as only those cells that are in the presence of the photosensitizer, light and oxygen simultaneously are exposed to a cytotoxic effect. This dual selectivity is due not only to a preferential uptake of the photosensitizer by the diseased tissue but also because light delivery can be restricted to specific regions, therefore confining activation of the photosensitizer to these regions only. Such a therapy allows for the destruction of diseased tissue while leaving normal tissue intact.

Mechanism of action

The photochemical and photophysical principles behind the mechanisms involved during PDT have been extensively studied.[1,2] (Box 1) Briefly, upon illumination, the photosensitizer is excited from its ground state (S_0) to its first excited single state (S_1). This short-lived excited singlet state rapidly converts to the much longer lived triplet state



Box 1. Photochemical and photophysical principles of photodynamic therapy

The underlying mechanism involved in photodynamic therapy is governed by the ability of the photosensitizer to absorb light of a specific wavelength and jump to its first excited singlet state. From there, it can readily transform to the much longer lived triplet state via intersystem crossing. This excited state of the photosensitizer can effectively interact with its surroundings, be it via a Type I hydrogen atom abstraction or electron transfer reaction or a Type II energy transfer to ground state molecular oxygen ($^3\Sigma_g^-$) to form singlet oxygen ($^1\Delta_g$). The reactive species generated (radicals and reactive oxygen species) will ultimately lead to oxidative damage and cell death. (Note: hν = light energy, * = excited state, • = radical)

(T_1) via intersystem crossing. The longer lifetime of the triplet state of the photosensitizer allows for sufficient time for interaction between the excited photosensitizer and surrounding molecules. It is generally accepted that the triplet state of the photosensitizer is responsible for the generation of the cytotoxic species produced during PDT.

The excited triplet state of the photosensitizer can react in two ways, defined as Type I and Type II.[3] A Type I mechanism involves hydrogen atom abstraction or electron transfer reactions between the excited state of the sensitizer and some substrate, either biological, solvent or another sensitizer, to yield radicals and radical ions. These radical species are generally highly reactive and can readily react with molecular oxygen to either generate reactive oxygen species such as superoxide anion or hydroxyl radical or can fix the biological damage so it is not repairable. Such reactions cause the formation of oxidative damage and eventually lead to the biological lesions expressed during PDT.

A Type II mechanism, on the other hand, results from an energy transfer between the excited triplet state of the sensitizer and ground state molecular oxygen, leading to the generation of the first excited state of oxygen, singlet oxygen. This zwitterionic species is extremely reactive and can react with a large number of biological substrates, inducing oxidative damage and ultimately cell death. While it is generally accepted that Type II processes predominate during PDT and that singlet oxygen is the primary cytotoxic agent responsible for the biological effects displayed,[4-7] it has been shown that Type I reactions become important at low oxygen concentrations or in more polar environments.[1,8] Overall, however, the initial reaction is of less importance since both Type I and Type II reactions lead to similar oxidative damage and can lead to comparable radical chain

reactions in the presence of oxygen. Overall, the effect of either a Type I or Type II reaction pathway is the production of oxidative damage within the target cell which will ultimately lead to tumour destruction.

Biological response

While it is clear that PDT can induce the production of cytotoxic agents that can readily destroy neoplastic cells, the complexity of biological systems greatly complicates the actual effects involved in the overall PDT response in vivo. Any number of subcellular targets can be attacked during PDT. These would include mitochondria, lysosomes, plasma membranes and nuclei and the exact target can greatly affect the mechanism of cell death, be it via necrosis or apoptosis.[9,10] In addition, while it has been shown that the action of some amphiphilic sensitizers involve direct tumour cell kill, most photosensitizers induce tumour necrosis via vascular shutdown.[1,10-14] Finally, it has been shown that PDT can induce immunological effects such as inflammation and other important tumour-specific immune reactions.[1,9,10] The exact nature behind PDT-induced tumour destruction depends on the photosensitizers used and will vary greatly depending on the condition being treated along with the photosensitizer and light dose used. However, knowing the biological effects behind cell death can lead to the selection of an ideal photosensitizer to treat a given disease.

Photosensitizers

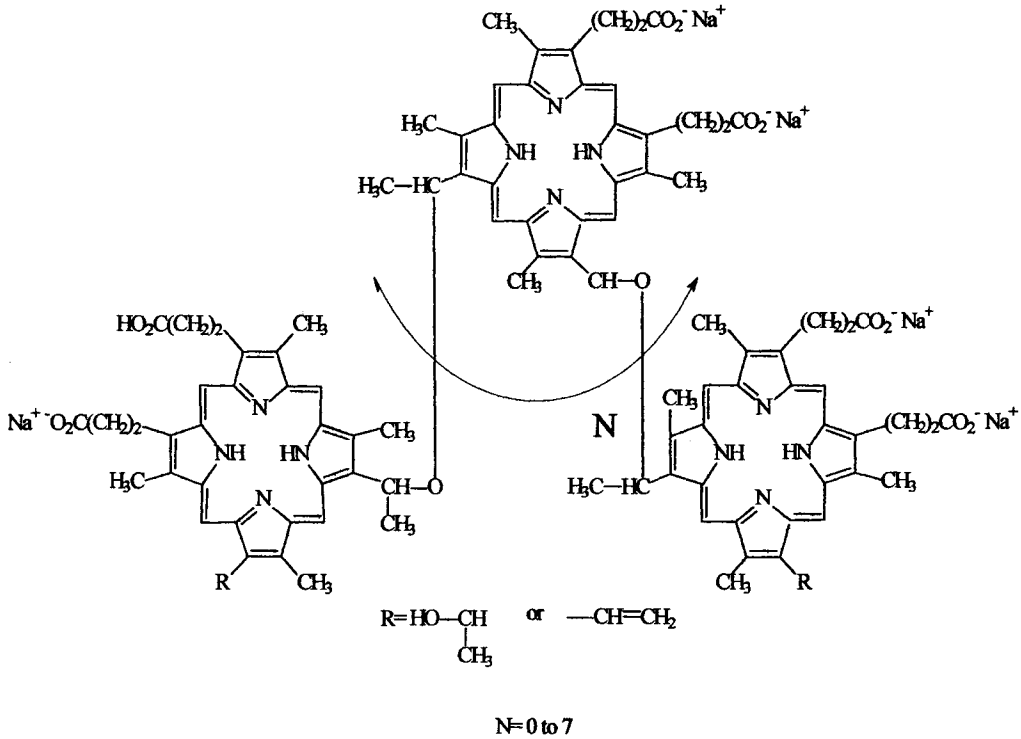
Photosensitizers are compounds that are capable of absorbing light of a specific wavelength and transforming it into useful energy. In the case of PDT, this would involve the production of lethal cytotoxic agents. There are hundreds of naturally occurring and synthetic dyes that can function as photosensitizers for PDT. These range

from naturally occurring plant abstracts to complex synthetic macrocycles. The key characteristic of any photosensitizers will be its ability to accumulate preferentially in diseased tissue and once there, its ability to generate cytotoxic agents so as to induce the desired biological effect. Table 1 provides an overview of the fundamental clinical characteristics of various photosensitizers currently in clinical or preclinical trials.

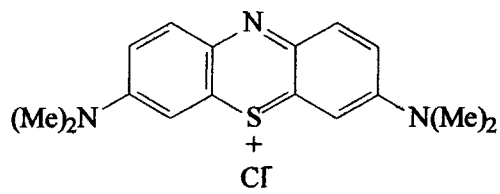
Photofrin®

The first generation photosensitizers are haematoporphyrin derivatives[15] such as Photofrin® (Figure 1) and are the most commonly used photosensitizers, having been accepted in clinic in a number of countries. Haematoporphyrin derivative was originally synthesized[16] by treating haematoporphyrin with 5 % sulphuric acid and acetic acid at room temperature. Subsequently, the mixture was treated with aqueous base and then neutralized. This lead to the formation of a complex mixture of dimers and oligomers involving primarily ester and ether linkages.[17] Partial purification of the most active of these oligomers via high performance liquid chromatography (HPLC) or size exclusion gel chromatography lead to Photofrin®, which is about 90-95 % active component.[18]

Photofrin® is marketed by QLT PhotoTherapeutics (Vancouver, British Columbia, Canada) and has been accepted in clinic in a number of countries (see QLT PhotoTherapeutics 1997-1999 Company reports and press releases). It was approved in the United Kingdom in 1999 for the palliative treatment of late-stage lung cancer and advanced esophageal cancer. The Food and Drug Administration (FDA) in the United States has accepted Photofrin® for the treatment of advanced esophageal cancer as well as for early and late stage lung cancer. France and the Netherlands have accepted Photofrin® as a therapy for lung and esophageal cancer[19] while it has been accepted in



Photofrin®



Methylene Blue

Figure 1. Photosensitizers presently accepted in clinic

The two photosensitizers accepted in clinic are Photofrin®, which is used to treat a number of cancers in several countries and methylene blue, which is used by the Swiss and German Red Cross for the sterilization of freshly frozen plasma units.

Germany for early stage lung cancer, in Canada for esophageal and bladder cancer and in Finland for esophageal and lung cancer. In Japan, Photofrin® is used against early stage lung cancer, superficial esophageal cancer, superficial and early stage gastric cancer, early stage cervical cancer and cervical dysplasia, a precancerous condition.[19] Furthermore, Photofrin® is awaiting approval in a number of European countries such as Italy and Spain for esophageal cancer and in Canada and European countries for lung cancer. In addition, Photofrin® is being investigated as a possible therapy against head and neck cancer, intestinal cancer, lung cancer, skin cancer (both primary and metastatic breast cancers), urinary bladder cancer, abdominal cancer, thoracic cancer, brain cancer, and Kaposi's sarcoma. Other conditions include Barrett's esophagus, psoriasis and arterial restenosis.[9,20] All of these conditions are being investigated and are currently in various clinical trials with promising results being acquired in most cases.

Variations on Photofrin® are being used in other countries as well. Photoheme is produced in Russia for instance and has been accepted by the Pharmacological Committee of Russia (Moscow, Russia) for a wide range of clinical uses including skin, breast, oropharingeal, lung, larynx and gastrointestinal cancers. Non-oncological uses include psoriasis and prophylaxis for corneal transplant opacity and recurrent blindness.[21-23]

Second generation photosensitizers

Despite its apparent successes, haematoporphyrin derivatives such as Photofrin® have two very important drawbacks.[2,24] First of all, these compounds are readily taken up and retained by cutaneous tissue for up to eight to ten weeks post-injection. This causes a marked skin photosensitivity that requires the patient to avoid bright sunlight, which is

obviously a disadvantage especially for patients with late stage malignancies. Secondly, while Photofrin® has a number of absorption peaks between 400 and 650 nm, its weakest absorption band at 630 nm is most often used to excite the photosensitizer as the tissue penetration of light increases with increasing wavelength. While such disadvantages have not stopped Photofrin® from becoming a useful tool against cancer and other conditions, the search for new photosensitizers remains an important goal.

An ideal photosensitizer for PDT should have the following characteristics:[16,24-26]

1) It should be chemically pure and of known and constant composition.
2) It should have a minimal dark toxicity and only be cytotoxic in the presence of light.
3) It should be preferentially retained by the target tissue.
4) It should be rapidly excreted from the body, thus inducing a low systemic toxicity.
5) It should have a high photochemical reactivity, with high triplet state yields (ϕ_T) and long triplet state lifetimes (τ_T) and should be able to effectively produce singlet oxygen and other reactive oxygen species.
6) Finally, it should have a strong absorbance, with a high extinction coefficient (ε), at a longer wavelength, between 600-800 nm, where tissue penetration of light is at a maximum while still being energetic enough to produce singlet oxygen. Furthermore, cheaper diode lasers can be used in this range, thus increasing the potential utility of PDT in a clinical setting.

While no photosensitizer can be deemed ideal for every possible application, a number of second generation photosensitizers have been developed in order to overcome the shortcomings of Photofrin® and to take advantage of their more ideal properties.

Methylene Blue

The only photosensitizer other than haematoporphyrin derivative that is presently used in clinic is methylene blue (Figure 1), which is used by the Swiss and German Red Cross for the decontamination of freshly frozen plasma units.[27,28] This photosensitizer has been shown to effectively inactivate extracellular enveloped viruses[28] and is used in clinic as a treatment for methemoglobinemia, thus showing its lack of toxicity in humans.[29] This phenothiazinium dye has been used extensively for over a century as a vital stain in biological assays and can be used in the clinical diagnosis of a variety of diseases and as a tumour marker in surgery. However, its use as an in vivo photosensitizer is limited by its facile reduction in biological milieu by ubiquitous cellular enzymes to the colourless leuco methylene blue, which is photodynamically inactive.[30]

5-Aminolaevulinic acid (ALA)

The use of ALA-induced endogenous photosensitizers is a novel method currently being investigated for PDT.[31] The natural porphyrin haem is synthesized in every energy-producing cell and is the prosthetic group for haemoglobin, myoglobin and other haematoproteins. The rate-limiting step in the synthetic pathway for haem is the conversion of glycine and succinyl coenzyme A to 5-aminolaevulinic acid (ALA), this step being under a negative feedback control by haem. However, the addition of excess exogenous ALA can bypass this negative feedback and overload the system, leading to a build-up of protoporphyrin IX (PpIX), an effective photosensitizer for PDT.[32] As such ALA has been extensively studies as a prodrug for the endogenous production and accumulation of the photosensitizer protoporphyrin IX in diseased tissue, in particular

malignancies (see Figure 2). In the case of ALA, tumor selectivity is influenced by a number of factors. Increased permeability of abnormal keratin, increased levels of porphobilinogen deaminase and decreased levels of iron and decreased activity of ferrochelatase in tumor cells all result in an accumulation of protoporphyrin IX in these diseased cells, thus resulting in the preferential accumulation of the photosensitizer in the target tissue.[31,33]

Marketed under the name Levulan® by DUSA Pharmaceuticals Inc. (Toronto, Ontario, Canada), ALA is the photosensitizer that is closest to being the next compound accepted into clinic, with its New Drug Application (NDA) having been accepted for submission by the FDA for the treatment of actinic keratoses, a common sun-induced precancerous skin lesion. DUSA Pharmaceuticals Inc. has also announced Phase I/II clinical trials involving Levulan® as a treatment for acne, hair removal and the photodetection of bladder cancer (DUSA Pharmaceuticals Inc., 1998 Annual Company Report). Other clinical trials are underway using ALA as a therapy for non-melanoma skin cancer[34], endometrial ablation[35], late stage esophageal cancer[36], gastrointestinal cancer[20], Barrett's esophagus[37] and psoriasis.[35] Because of the low molecular weight and polar properties of ALA, it can be used as a topical PDT agent against a number of dermatological conditions and has been shown to be effective against superficial basal cell carcinomas, Bowen's disease, erythroplasia of Queyrat, cutaneous T-cell lymphoma and Hirsutism.[35]

One of the problems associated with ALA is that it does not penetrate very deeply into the skin when used as a topical agent. Because of this, attention has been given to ALA esters, which possess slightly different properties. PhotoCure AS, a Norwegian

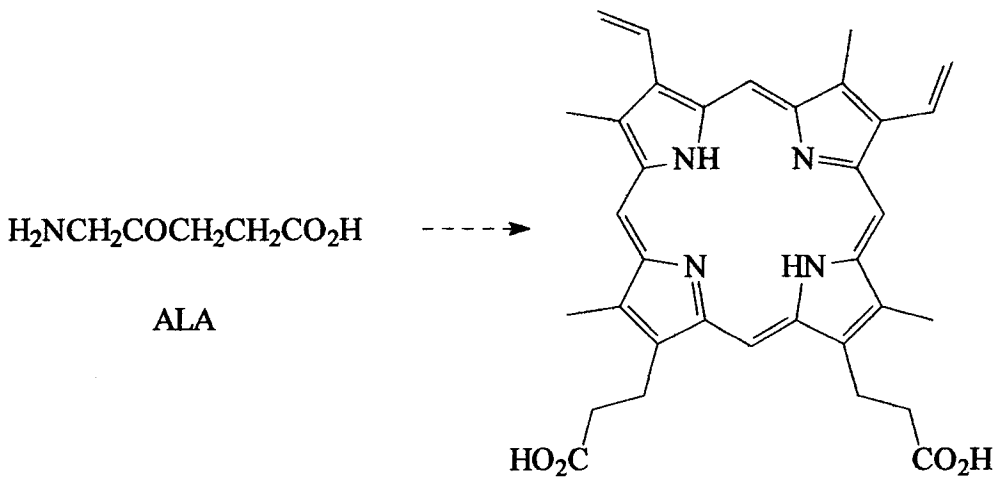


Figure 2. 5-Aminolaevulinic acid and protoporphyrin IX

The stimulation of the production of endogenous photosensitizers such as protoporphyrin IX by ALA is a novel method being examined for PDT with an NDA submitted for the treatment of actinic keratoses.

company (Oslo, Norway), is marketing one such ALA ester, P-1202, a methyl ester, and is studying its potential against basal cell carcinomas and other skin lesions along with several of the conditions that have been shown to be effectively treated using ALA.[9]

Verteporfin

QLT PhotoTherapeutics Inc. has done extensive work on the second generation photosensitizer verteporfin or benzoporphyrin derivative monoacid ring A (BPD-MA) (Figure 3).[38] In collaboration with CIBA Vision Corporation (Duluth, Georgia, USA), verteporfin, under the tradename Visudyne™, is presently undergoing Phase III clinical trials for the treatment of wet age-related macular degeneration (AMD) (see QLT PhotoTherapeutics and CIBA Vision Joint 1998-1999 Press Releases). AMD is the leading causes of blindness for people over the age of 50 and involves the rapid growth of abnormal blood vessels under the central retina. Leaking from these abnormal vessels causes scarring and an accelerated loss of visual acuity. There is no adequate treatment protocol for 80-90 % of patients suffering from AMD.[39] Since PDT is known to induce vascular shutdown, compounds such as verteporfin are ideal for treating this condition. Initial results are excellent and show a significant preservation of vision in a number of patients.

Verteporfin is also in Phase III clinical trials against cutaneous non-melanoma skin cancer and I/II clinical trials against other non-melanoma skin cancers (such as multiple non-melanoma skin cancer)[40], psoriasis[41] and psoriatic arthritis along with rheumatoid arthritis (also see the 1997 QLT PhotoTherapeutics Annual Company Report). Extensive preclinical work has been done using verteporfin as a therapy for multiple sclerosis and Barrett's esophagus and as an agent to achieve endometrial

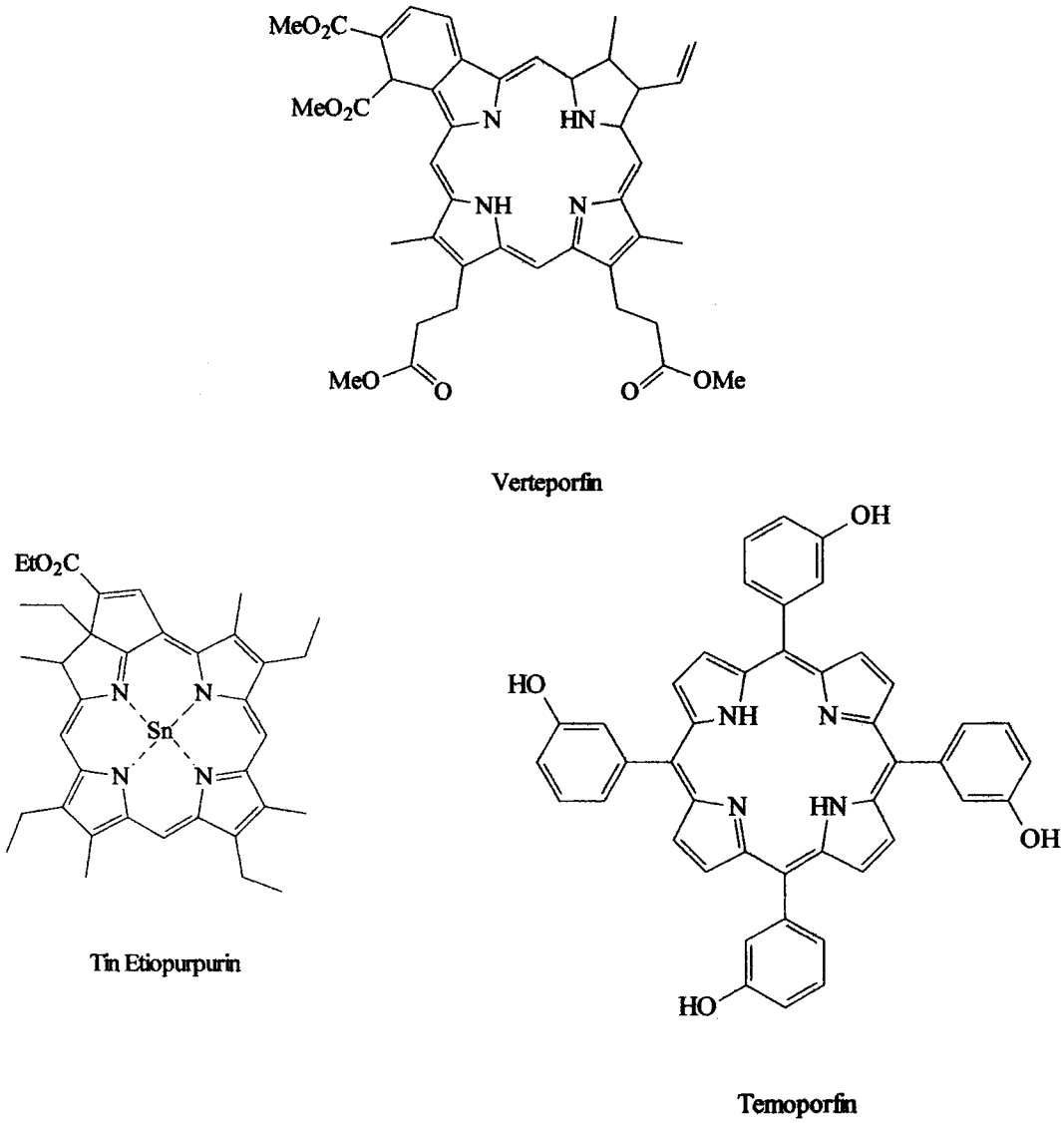


Figure 3. Photosensitizers currently in Phase III clinical trials

Verteporfin and tin etiopurpurin are both involved in Phase III clinical trials for the treatment of macular degeneration while Phase III trials are underway using temoporfin against head and neck cancers. Early stage clinical trials and preclinical work using these photosensitizers are also underway (see Table 2).

ablation and bone marrow purging.[40] Verteporfin has a much stronger absorbance at a longer wavelength (690 nm) where tissue penetration of light is 50% greater as that of Photofrin® at 630 nm. In addition, verteporfin is rapidly taken up by the tumor, reaching optimal tumor/normal tissue ratios between 30-150 minutes post intravenous injection and is rapidly cleared so that skin photosensitivity last only a few days.[32]

Tin Etiopurpurin

Miravant Medical Technologies (Santa Barbara, California, USA) markets tin etiopurpurin (SnET2)[42] (Figure 3) under the tradename Puryltin™ as part of their PhotoPoint™ procedure. The PhotoPoint™ procedure involves three components, a light-activated photosensitizer, a light-producing device and a light-delivery system and is described at the Miravant Medical Technologies Internet site (www.miravant.com). Among the light-activated compounds under development by Miravant, the furthest along is definitely Puryltin™, which is presently in Phase III clinical trial for the treatment of wet age-related macular degeneration (in cooperation with Pharmacia & Upjohn (Bridgewater, New Jersey, USA)). It is also in Phase I clinical trials against prostatic cancer (cancer that has not spread to the prostate itself)[43] and Phase II for cutaneous metastatic breast cancer and Kaposi's sarcoma in patients with acquired immunodeficiency syndrome.[9] Preclinical work done using SnET2 include extensive work on other malignancies such as brain, lung, skin, and head and neck cancer. Non-malignant conditions such as psoriasis and restenosis have also been shown to be effectively treated using SnET2.

Temoporfin

Temoporfin (Figure 3) or tetra(m-hydroxyphenyl)chlorin (mTHPC),[16,44] under the tradename Foscan®, is being marketed by Scotia Pharmaceuticals (Guildford, Surrey, UK) as a new second generation photosensitizer for PDT (see the company website at www.quantanova.com). Phase III clinical trials have begun in Europe and in the USA using Foscan® against head and neck cancers.[45] Trial work has concentrated on this area since conventional treatments are difficult, ineffective and disfiguring. Recent press releases from the company state that Foscan® has been given fast track designation by the FDA for the palliative treatment of recurrent, refractory or second primary squamous cell carcinomas of the head and neck in patient considered to be incurable with surgery or radiotherapy with NDA file submission expected by the end of September 1999. Late stage esophageal cancer and dysplasia in Barrett's esophagus are also being treated using Foscan® in clinical trials.[36] Future trials using this photosensitizer in Europe, USA and the Far East against malignant and non-malignant diseases are anticipated and will include trials against gastric cancer, prostate cancer and hyperplasia and for field sterilization after cancer surgery and control of antibiotic-resistant bacteria.[45] In addition, topical formulations of temoporfin are being developed in order to compete with ALA against skin cancers and other dermatological conditions.[45]

Temoporfin appears to be one of the most phototoxic of all the second generation photosensitizers presently being investigated. It requires very low drug doses (as little as 0.1 mg/kg) as well as an unusually low light dose (as low as 10 J/cm^2), making it 100 times more photoactive than Photofrin®, where drug doses range from 2-5 mg/kg and light doses between 100-200 J/cm^2 are generally used.[9] The reasons behind this exceptionally high activity are not fully known. While improved optical properties and

singlet oxygen quantum yields can partially explain this increased phototoxicity, it appears the explanation resides in subtumoral and subcellular localization of the compound. While lipophilic sensitizers have been shown to bind with lipoproteins and hydrophilic compounds with serum albumin upon intravenous administration,[46] temoporfin has been shown to bind to an unknown plasma protein presumably involving the PEG vehicle, possibly leading to differences in subcellular localization.[47] Furthermore, the interaction with a plasma protein other than albumin or lipoproteins could explain the novel pharmacokinetics. The immediate peak in plasma drug levels following intravenous administration is followed by a second plasma peak some hours later[48,49] may be a factor in the high phototoxicity of this compound.

Texaphyrins

Texaphyrins (Figure 4) are "Texas-sized" porphyrins[50,51] and are marketed by Pharmacyclics, Inc. (Sunnyvale, California, USA) as a photosensitizer (see Pharmacyclics, Inc. internet site (www.pcyc.com). Under the trade name Lutrin™, lutetium texaphyrin is undergoing Phase II clinical trials as a possible therapy for breast cancer. The main advantage of using texaphyrins as a PDT agent is its strong absorbance at a much longer wavelength (732 nm) so that treatment can be effectively done on a much larger tumour or at a much greater depth. Lutetium texaphyrin derivatives are also being investigated in Phase I clinical trials for angioplasty of atherosclerotic cardiovascular disease and the treatment and prevention of restenosis under the tradename Antrin™ and Optrin™ is under Phase I trials for age-related macular degeneration. In addition, both radiosensitizers and chemosensitizers based on the texaphyrin framework are also being developed by this company, with Xcytrin™, a

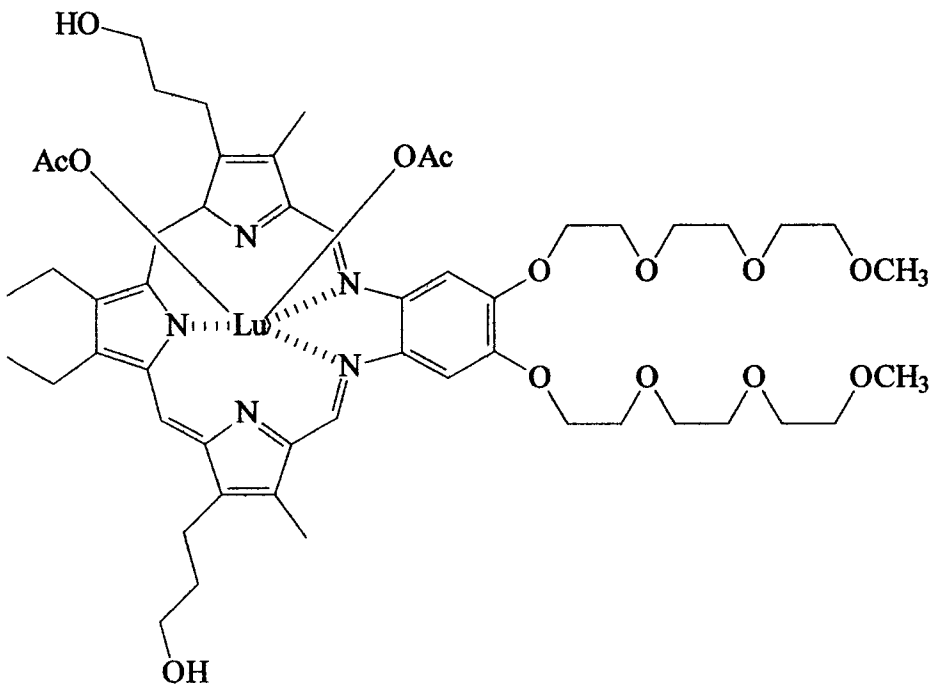


Figure 4. Lutetium texaphyrin (Lutrin™)

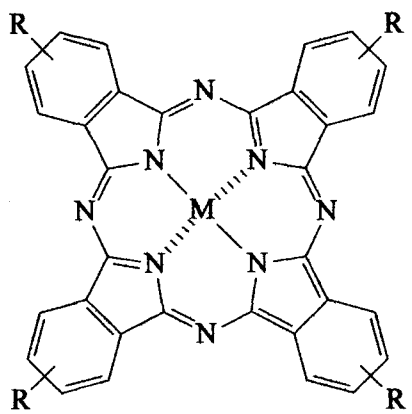
radiation sensitizer, presently involved in Phase III clinical trials for the treatment of brain metastases and Phase I trials for newly diagnosed primary brain tumors.

Phthalocyanines

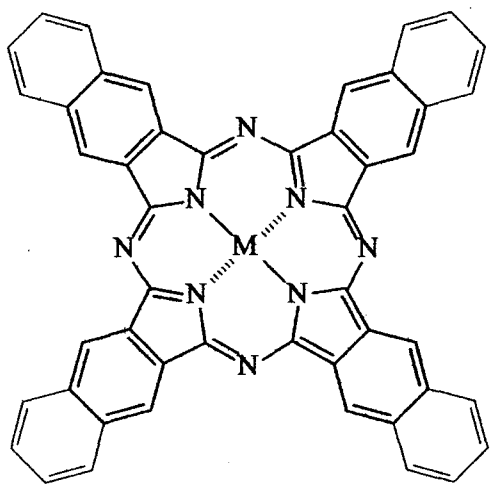
Phthalocyanines are tetrapyrrolic macrocycles where, unlike porphyrins, the individual pyrrole units are linked by nitrogen atoms and not methine bridges (Figure 5). The periphery of the macrocycle is extended by benzene rings, which leads to stronger absorptions at longer wavelengths than porphyrins such as Photofrin®. Phthalocyanines have long been used as dyes and colouring agents in industry and have recently found use as photoconducting agents in photocopying machines. They have also been extensively studied at PDT agents especially due to their favourable photophysical properties and the ability to change its properties, such as solubility, through the addition of substituents to the periphery of the macrocycle.[2,52]

Ciba-Geigy Ltd. (Basle, Switzerland), in partnership with QLT PhotoTherapeutics, has developed a liposomal preparation of zinc phthalocyanine (CGP 55847) that was involved in Type I/II clinical trials in Switzerland in patients suffering from squamous cell carcinomas of the upper aerodigestive trace.[53] Attempts to develop a topical application for this photosensitizer in the hopes of treating psoriasis were also made.[20]

Sulphonated aluminium phthalocyanine, under the name Photosense, is currently undergoing clinical trials in Russia.[21,54,55] The Oncological Centre of the Russian Academy of Medical Sciences (Moscow, Russia) and the Surgical Clinic of Moscow Medical Academy (Moscow, Russia) are carrying out trials using this mixture of sulphonated derivatives against a number of malignancies including skin, breast, lung and



Phthalocyanines



Naphthalocyanines

Figure 5. Phthalocyanines and napthalocyanines

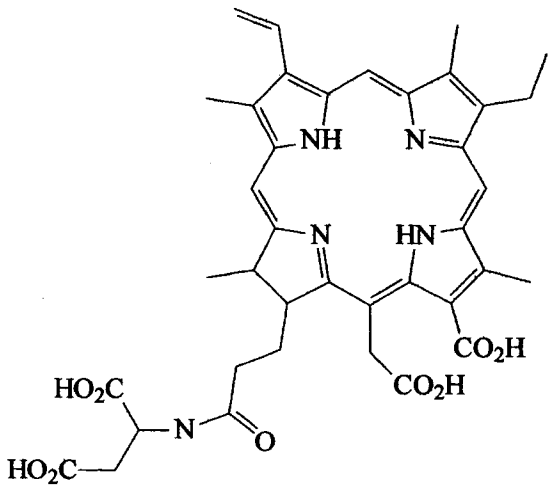
gastrointestinal cancers. The addition of the sulphonate groups to the periphery of the phthalocyanine greatly increases the solubility of these compounds, removing the need for liposomal delivery vehicles. Success using Photosense has been relatively good.

V. I. Technologies Inc. (Vitex) (Melville, New York, USA), a company based at the New York Blood Center, has been studying a silicon-based phthalocyanine[56], Pc4, for the sterilization of blood components (1999 Press Release from V. I. Technologies, Inc.). Preclinical results have been extremely promising and it is hoped that the procedure used will enter clinical trials in late 1999.

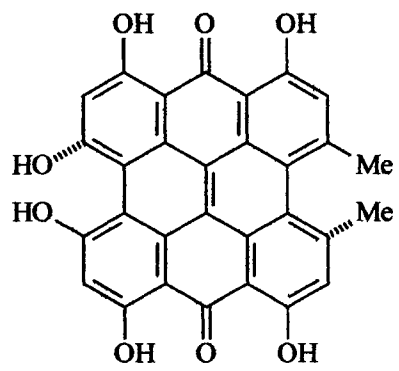
Addition of a second benzene ring to the periphery of the phthalocyanine leads to napthalocyanines.[57] (Figure 5) These compounds absorb at a high wavelength than do phthalocyanines (770 nm versus 680 nm), thus increases the therapeutic depth that can be achieved and rendering them potential photosensitizers for highly pigmented tumours such as melanomas.[58] Significant work has been done evaluating these compounds as photosensitizer for PDT[58-60] and they are being pushed towards clinical trials in Bulgaria by the Bulgarian Academy of Sciences (Sofia, Bulgaria).[24]

N-Aspartyl chlorin e6

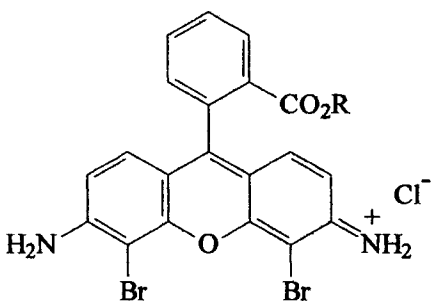
Under the supervision of Nippon Petrochemical (Osaka, Japan), N-aspartyl chlorin e6 (Npe6)[61] (Figure 6) is being studied as a possible photosensitizer for PDT.[62,63] Phase I clinical trials are underway for the treatment of cutaneous malignancies[20] and it is also being investigated in Japan as a possible therapy for endobronchial lung cancer.[9] It has been shown to be an effective photosensitizer against skin cancers with little or no long term cutaneous photosensitivity.[9] The photodynamic activity of Npe6 has also been



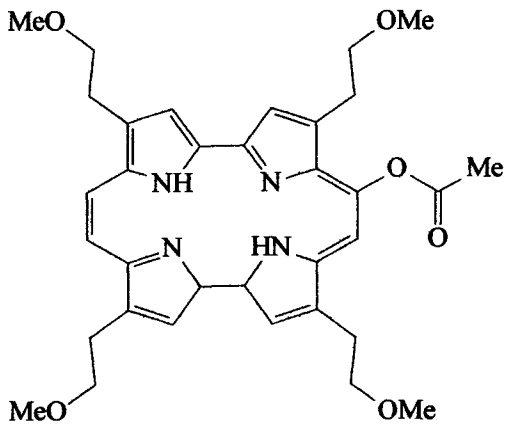
N-aspartyl chlorin e6



Hypericin



Brominated rhodamines



Porphycene (ATMPn)

Figure 6. Some photosensitizers in early clinical or preclinical trials

Phase I/II clinical trials and preclinical work are being done using photosensitizers such as these for the treatment of a number of conditions (see Table 2)

demonstrated to involve a combination of vascular and direct anti-tumor photodamage (both direct and indirect effect), another potential advantage of this photosensitizer.[64]

Rhodamines

Due to their specific uptake by mitochondria and their known use as a fluorescent probe, rhodamines have been used extensively.[30] This has been naturally extended to using these sensitizers in the treatment of malignant tumours. However, rhodamine 123, a readily available commercial dye, is a poor phototoxin due to the high fluorescence quantum yield of the compound.[65] This problem can be remedied by adding heavy atoms such as bromine or chlorine to the macrocycle (Figure 6). Known as the heavy atom effect, the addition of heavy atoms to the chromophore increases intersystem crossing from the singlet to the triplet state by increases spin-orbital coupling, thus allowing an otherwise forbidden changes in the spin state ($S_1 \rightarrow T_1$). The addition of halogens to the chromophore also red-shifts the absorption, an important feature in the case of rhodamines, which absorb around 500 nm, a wavelength where tissue penetration of light is minimal.[30] Despite this, rhodamines have been shown to be very effective photosensitizers against malignant cells in vitro and a Quebec-based company, Theratechnologies, Inc., has undertaking extensive preclinical studies in the use of brominated rhodamine derivatives in the eradication of leukemia cells from bone marrow extracts in preparation for transplantation.[66] Phase I clinical trials have begun using TH 9402, a brominated rhodamine analog, for the treatment of chronic myeloid leukemia using the patented PhotoDynamic cell therapy Process (PDP) as described at www.theratech.com, the company's internet site. This ex vivo photodynamic therapy, used for purging autologous bone marrow, has been shown to destroy diseased cells

while sparing normal healthy cells, an important prerequisite for such a treatment protocol.

Porphycenes

Glaxo Dermatology, a division of Glaxo-Wellcome Inc. (Research Triangle Park, North Carolina, USA) along with Cytopharm (Menlo Park, California, USA), has done extensive preclinical work using the porphycene ATMPn (9-acetoxy-2,7,12,17-tetrakis-(β-methoxyethyl)-porphycene) (Figure 6).[67-69] Its four β-methoxyethyl side chains leads to accelerated cellular uptake and the acetoxy function increases the solubility and hydrophilicity of the molecule.[67] It has been shown that this compound can be applied topically, which would make it useful in dermal applications.[20] *In vitro* studies has shown that ATMPn has an unusually fast uptake into skin cells not seen for other second generation photosensitizers. ATMPn is undergoing preclinical testing as a possible agent against psoriasis vulgaris and superficial non-melanoma skin cancer.[67]

Other photosensitizers

The success exhibited by Photofrin® and the potential shown by a number of the second generation photosensitizers has caused an explosion in photodynamic therapy, resulting in the unveiling of new photosensitizers along with an investigation into well-known naturally occurring chromophores. Hyericin (Figure 6), for example, is well-documented as having photodynamic activity as it causes hyericism or photopoisoning in grazing animals that consume large quantities of plants containing this compound, often leading to skin irritation, fever and even death.[70] This multicyclic quinione, which absorbs around 590 nm,[65] is being investigated as a photosensitizer for PDT and is presently in Phase I clinical trials for the treatment of psoriasis, warts and skin cancer

(see www.sante.univ-nantes.fr/med.laser/sensitizer.html). The naturally occurring perylenequinones such as hypocrellins, which are produced by fungi and insects[65], are also under evaluation for PDT. Several pharmaceutical companies are also actively developing new synthetic photosensitizers. Scotia Pharmaceuticals are interested in bacteriochlorins for photodynamic therapy (see the company website mentioned above) while Hamamatsu Phototonics are investigating ATX-S10, a chlorin derivative.[71] In reality, any chromophore able to effectively produce photocytotoxicity upon illumination has the potential to be used in photodynamic therapy, leading to endless possibilities.

Conclusion

As the new millenium nears, the need for new protocols for the treatment of cancer and other diseases is becoming acute. With the population aging and established therapies operating close to optimal levels, new therapies that can effectively treat cancer and other conditions while being cost effective are at a premium. Photodynamic therapy is essentially a very simple concept that still offers the possibility of an effective and specific method of destroying malignant, premalignant and benign tissues while sparing surrounding normal, healthy cells. Initial clinical studies have shown that PDT is effective against cancer and a variety of other diseases (see Table 2) and offers a promising treatment option for patients with conditions that have no established or effective cure or whose condition has become refractory to existing therapies.

Since cancer is a large family of diseases with widely different clinical patterns, it is highly unlikely that a single photosensitizer will ever serve all purposes in oncology. Add to this the desire to extend PDT into the treatment of other conditions and the need to develop new photosensitizers with optimal properties for treating a given condition

becomes obvious. With the acceptance of the first generation photosensitizer Photofrin® in clinic around the world, second generation photosensitizers are being tested against numerous pathogenic states (Table 2). Photodynamic therapy is the treatment of the future.

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Table 1. Fundamental clinical characteristics of the photosensitizers currently in clinical or preclinical trials

Photosensitizer	λ_{max} (nm)	Extinction coefficient (M^{-1} cm^{-1})	Mode of delivery	Delivery vehicle	Typical dose (mg/kg)	Light dose (J/cm^2)	Time post-injection	Duration of skin photosensitivity
Haematoporphyrin derivative	630	$3x10^3$	i.v. or topical	5% dextrose	2-5	100-200	24-48h	2-3 months
Methylene blue	668	$9.5x10^4$	Ex vivo	water-soluble	1μM	50 000 lux	n.a.	n.a.
5-aminolaevulinic acid (protoporphyrin IX)	635	$<5x10^3$	topical, oral or i.v.	water-soluble	<60 (orally) <30 (i.v.)	100-200	-	1-2 days
Verteporfin	690	$3.5x10^4$	i.v.	liposomal	0.1-2	100-200	30-150 min	3-5 days
Tin Etiopurpurin	660	$2.8x10^4$	i.v.	lipid emulsion	1-2	100-200	24h	Up to 1 month
Temoporfin	652	$3x10^4$	i.v.	PEG/EtOH/ H_2O	0.1-0.3	8-12	24-48h	Up to 6 weeks
Texaphyrins	732	$4.2x10^4$	i.v.	water-soluble	0.6-7.2	150	3-5h	minimal
Phthalocyanines	670-680	$2.5x10^5$	i.v.	liposomal or water-soluble	0.5-2	100	24-72h	8-10 days
Napthalocyanines	750-780	$>10^5$	i.v.	liposomal	-	-	-	-
N-aspartyl chlorin e6	664	$4.0x10^4$	i.v.	water-soluble	0.5-3.5	25-100	4h	3-7 days
Rhodamines	511	$2.0x10^4$	ex vivo	water-soluble	25μM	1-10	n.a.	n.a.

Porphycenes	630	$5.2x10^4$	topical	liposomal	1-3	-	n.a.	-
Hypericin	590	$4.4x10^4$	topical	liposomal	-	-	-	-

Table 2. Photosensitizers currently in clinical trials or late preclinical development

Company	Photosensitizer	Tradename	Clinical Application	Clinical Status
QLT PhotoTherapeutics	Haematoporphyrin derivative	Photofrin®	Esophageal, Lung, Bladder, Gastric and Cervical Cancer, Cervical Dysplasia	Approved
QLT PhotoTherapeutics	Haematoporphyrin derivative	Photofrin®	Head and Neck, Intestinal, Lung, Skin, Bladder and Metastatic Breast cancer, Kaposi's Sarcoma, Barrett's Esophagus, Psoriasis, Arterial Restenosis	Phase I/II through Phase III
State Research Centre for Laser Medicine (Russia)	Haematoporphyrin derivative	Photoheme	Skin, Breast, Oropharingeal, Lung, Larynx and Gastrointestinal Cancer, Psoriasis, Prophylaxis for Corneal Transplant Opacity	Approved
German and Swiss Red Cross	Methylene Blue	-	Sterilization of Freshly Frozen Plasma	Approved
DUSA Pharmaceuticals Inc.	5-aminolaevulinic acid (ALA)	Levulan®	Actinic Keratoses Hair removal, Acne, Non- Melanoma Skin, Esophageal and Gastrointestinal Cancer, Endometrial Ablation, Psoriasis, Barrett's Esophagus	NDA submitted Phase I/II Preclinical
PhotoCure AS	5-aminolaevulinic acid (ALA)	P-1202	Basal Cell Carcinoma and Other Skin Lesions	Preclinical
QLT PhotoTherapeutics	Verteporfin	Visudyne™ Verteporfin	Macular Degeneration Non-Melanoma Skin Cancer, Psoriasis, Psoriatic and Rheumatoid Arthritis Multiple Sclerosis, Barrett's Esophagus, Endometrial Ablation, Bone Marrow Purging	Phase III Phase I/II Preclinical
Miravant Medical Technologies	Tin Etiopurpurin	Purlytin™	Macular Degeneration Metastatic Breast Cancer, Kaposi's Sarcoma Prostatic Cancer Brain, Lung, Skin and Head and Neck Cancer,	Phase III Phase II Phase I Preclinical

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			Psoriasis, Restenois	
Scotia Pharmaceuticals	Temoporfin	Foscan®	Head and Neck Cancer Esophageal Cancer, Barrett's Esophagus Gastric and Prostate Cancer, Hyerplasia, Sterilization, Antibiotic	Phase III Phase I/II Preclinical
Pharmacylics Inc.	Texaphyrins	Lutrin™ Antrin™ Optrin™	Breast cancer Angioplasty Macular Degeneration	Phase II Phase I Phase I
Ciba-Geigy	Phthalocyanine	CGP 55847	Squamous Cell Carcinoma of Upper Aerodigestive Tract, Psoriasis	Phase I/II
State Research Centre (Russia)	Phthalocyanine	Photosense	Skin, Breast, Oropharingeal, Lung, Larynx and Gastrointestinal Cancer, Psoriasis	Phase III
V. I. Technologies Inc.	Phthalocyanine	Pc4	Sterilization of Blood Products	Phase I/II (late 1999)
Nippon Petrochemical	N-aspartyl chlorin e6	NPe6	Endobronchial Lung Cancer and Cutaneous Malignancies	Phase I
Theratechnologies	Rhodamines	TH 9402	Bone Marrow Purging	Phase I
Cytopharm/Glaxo	Porphycenes	ATMPn	Dermal Applications (Psoriasis, Non-Melanoma Skin Cancer)	Preclinical
Vim Rx Pharmaceuticals	Hypercin	-	Psoriasis, Warts and Skin Cancer	Phase I

Chapter 4.

Targeted Photodynamic Therapy via Receptor Mediated Delivery Systems

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Targeted Photodynamic Therapy via Receptor Mediated Delivery Systems

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Keywords: chlorin e6, photosensitizers, albumin, lipoproteins, transferrin, nuclear localizing signal, epidermal growth factor, steroids, adenovirus, bisphosphonates, annexins, phthalocyanines

Abbreviations: Ad, adenovirus; Ad2, adenovirus serotype 2; AlPc; aluminium phthalocyanine; $AlPcS_{2adj}$, adjacently disulphonated aluminium phthalocyanine; $AlPcS_4$, aluminium tetrasulphonated phthalocyanine; $AlPcS_4A_1$, aluminium mono-(6-carboxypentylaminosulphonyl)-tetrasulphophthalocyanine; $AlPcS_4A_2$; aluminium di-(6-carboxypentylaminosulphonyl)-tetrasulphophthalocyanine; $AlPcS_4C_{12}$, aluminium (dodecylaminosulphonyl) tetrasulphophthalocyanine; BPD, benzoporphyrin derivative; BPD-MA, benzoporphyrin derivative monoacid ring A; BSA, bovine serum albumin; CAR, Coxsackie B and Adenovirus receptor; Ce6, chlorin e6; CoPc, cobalt phthalocyanine; CRM, Chremophor EL™; Dex, dextran; dsDNA, double stranded DNA; DTox, diphtheria toxin; DPPC, dipalmitoylphosphatidylcholine; EC_{50}, effective concentration 50%; EGF, epidermal growth factor; Gb_3, globotriaosylceramide; GePc, germanium phthalocyanine; HDL, high density lipoprotein; HMP, *Escherichia coli* hemoglobin-like protein; HP, hematoporphyrin; HAS, human serum albumin; LD_{50}, lethal dose 50%; LDL, low density lipoprotein; malBSA, maleylated bovine serum albumin; MnSOD, manganese superoxide dismutase; MRT, modular recombinant transporters; MSH, α-melanocyte stimulating hormone; mTHPC, m-tetrahydroxyphenylchlorin; NLS, nuclear localization signal; NPC, nuclear pore complex; oxLDL, oxidized low density lipoprotein; Pc, phthalocyanine; PDT, photodynamic therapy; PS, photosensitizer; PVA, polyvinyl alcohol; RGD, Arg-Gly-Asp tripeptide; ROS, reactive oxygen species; SLTB, Shiga-like toxins; Sn(IV)Ce6, tin(IV) chlorin e6; $SnET_2$, tin etiopurpurin; TPPS-2A, adjacently disulphonated tetraphenylporphine; VLDL, very low density lipoprotein; ZnPc, zinc phthalocyanine

Table of Contents

1. Abstract

Targeted photodynamic therapy offers the opportunity of enhancing photodynamic efficiency by directly targeting diseased cells and tissues. While antibody-conjugates have received the most attention, cellular transformations offer numerous other potent targets to exploit during the delivery of photosensitizers for PDT. Alterations in receptor expression, increased levels of specific cell surface membrane lipids and proteins as well as changes in the cellular microenvironment all occur in diseased cells. Along with other biochemical and physiological changes that occur during diseased and malignant cell transformation, these factors have been utilized in order to improve the efficacy of PDT. Attempts have been made to either increase the uptake of the dye by the target cells and tissue or to improve subcellular localization so as to deliver the dye to photosensitive sites within the cells. This review discusses various photosensitizer bioconjugates that utilize these factors and summarizes the results obtained to date.

2. Introduction

Traditional cancer treatments including surgery, radiation therapy and chemotherapy all result in serious side effects caused by the loss of normal cell function. This is a result of the relative indiscriminate cytotoxic properties of modern treatment modalities. Researchers have thus invoked the search for the "magic bullet", that single underlying process that will allow for selectively targeting and destroying diseased cells while sparing their healthy functional neighbors. Despite decades of experimentation, success has been fleeting. Complicating the search is the fact that cancer is not a single entity but is a family of diseases characterized by uncontrolled proliferative growth and the unwanted spread of aberrant cells from their site of origin [1]. Each malignancy exhibits their own characteristics and each expresses their own possible target antigens. Furthermore, individual tumours are incredibly heterogeneous, where therapy that causes cell death in one subset of cells might in fact strengthen another subset.

Despite much early promise, antibody targeting has had little real success in cancer therapy [2]. There are a number of problems associated with antibody-based therapies that preclude them from being the "magic bullet" so long sought after. Among these problems are the following:

1) It is remarkably difficult to achieve tumour-specific antibodies that also display high affinity.
2) Clinical tumours are highly heterogeneous and do not have consistent expression of target antigen throughout their mass.
3) Antibodies are large proteins and do not penetrate well into the tumour mass.

4) Only a very small amount of the antibody dose (much less than 1%) actually reaches the tumour and most of that is localized to the tumour vasculature.
5) Antibodies are often not internalized by the cell, leaving the cytotoxic agent to do its damage on the cell surface, away from the most sensitive sites within the cell.
6) Antibody-drug conjugates will only be active against those tumour cells that express the corresponding antigen and any chemical instability in the chemical bond between the antibody and the drug could result in undesirable systemic effects.

These important disadvantages have led research towards new areas.

Photodynamic therapy is one step towards the "magic bullet" as only those cells that are simultaneously exposed to the photosensitizing dye, molecular oxygen and light receive the cytotoxic insult [3-5]. The ability to confine activation of the photosensitizer by restricting illumination to the diseased tissue allows for a certain degree of selectivity towards these cells. Ideally, photodynamic therapy holds the promise of dual selectivity with preferential tumour uptake of the photosensitizer leading to improved efficiency. To date, most first and second-generation photosensitizers studied for photodynamic therapy display only a slight preference for malignant cells, often leading to significant skin photosensitivity and high uptake by healthy cells and tissues. In order to overcome this, third generation photosensitizers that are actively targeted towards diseased tissue are being designed and synthesized [6]. These can be said to include targeted vehicles used to improve photosensitizer delivery along with photosensitizer-antibody conjugates. This chapter deals with other methods of targeting photosensitizers to cancer cells, paying particular attention to non-antibody based protein carriers and protein/receptor systems. Several of these targeting methodologies offer the added advantage of trafficking the

photosensitizer across the cellular plasma membrane, resulting in intracellular accumulation of the dye. Such intracellular accumulation may allow for targeting of photosensitive intracellular sites, thus improve photodynamic efficiency.

3. Serum Proteins

Upon administration into the blood stream, most drugs associate with various serum proteins including both high and low density lipoproteins and albumin. The nature of this interaction depends on the physical characteristics of the drug and the serum protein involved. Presumably, hydrogen bonding, van der Waal forces, π bond stacking, hydrophobic interactions, physical entrapment and ionic pairings all play a role in the attachment of the drug to the carrier serum protein. Along these lines, it is well known that serum proteins are also predominantly responsible for the transportation of photosensitizers throughout the body [7-10]. More hydrophilic photosensitizers such as tetrasulphonated aluminium phthalocyanine tend to associate with serum albumin while low density lipoproteins (LDL) carry zinc phthalocyanine and other more hydrophobic photosensitizers in the blood stream. The *in situ* generation of these carrier systems can lead to improved photodynamic action as they may lead to enhanced intracellular accumulation of the dye via receptor-mediated endocytosis along with improved targeting. In order to further profit from this improved PDT efficiency, means of strengthening the association of the photosensitizer with the serum protein have been investigated in the hopes of increasing the target specificity of the dye (Table 1).

Table 1) Summary of serum-based protein conjugated to photosensitizers.

Serum Protein	Photosensitizer	Target	**Reference**
BSA	Hematoporphyrin	Macrophages	17
BSA	ZnPc	Murine mammary and human colon carcinoma	14
BSA, Fibrinogen, Gelatin	Chlorin e6	Tissue solder	22
Maleylated BSA	Chlorin e6	Scavenger receptor, Intimal hyperplasia	21
Maleylated BSA	$AlPcS_4A_1$ and A_2	Scavenger receptor	18
BSA and Maleylated BSA	Chlorin e6	Scavenger receptor	19,20
LDL	ZnPc	MS-2 fibrosarcoma	15
LDL	Hp	HT1080 fibroblast	34
LDL	BPD-MA	GM3348B fibroblast	41
LDL	$TPPS_n$	LDL receptor on human hepatocyte tumour	35
LDL (human)	BPD-MA	Choroidal melanomas, choroidal neovasculature	42-44, 48,
LDL, HDL	Hematoporphyrin	LDL receptor on fibroblast, Scavenger receptor	10, 17, 47
LDL	BPD-MA	LDL receptor on rhabdomyosarcoma	41
LDL (human)	BPD-MA	Greene melanoma	44
LDL (human)	Chlorin e6	Retinoblastoma	48
LDL (oxidized)	AlPc	Scavenger receptor	45
LDL	$AlPcS_4A_2$ and $AlPcS_4(C_{12})$	LDL receptor of lung adenocarcinoma	40
Transferrin	Hematoporphyrin	Transferrin receptor	17
Transferrin	Chlorin e6	Adenocarcinoma	53, 54

3.1 Albumin

Albumin is by far the most abundant serum protein in humans, with concentrations in the range of 0.6 mM [11]. As such, it is at least ten times more concentrated than the total concentration of all lipoproteins. Human serum albumin consists of 585 amino acids forming a single polypeptide [12]. It has a molecular weight of approximately 66 000 Daltons and is 50% α helical, giving the protein an overall ellipsoidal shape. Albumin plays several important biological roles including involvement in regulating osmotic blood pressure and transporting fatty acids from the liver to tissues. Serum albumin possesses a unique capability to bind, covalently or reversibly, a great number of various endogenous and exogenous compounds [13]. Several different transport proteins exist in blood plasma but albumin alone is able to bind a wide diversity of ligands reversibly with high affinity. This broad specificity of human serum albumin towards structurally diverse ligands is related to its flexible structure, enabling multiple three dimensional rearrangements of the protein depending upon drug binding conditions. This blood serum protein also serves as an important source of amino acids for cells [12]. In fact, over 60% of albumin can be found in interstitial fluid. With the increased metabolism and proliferation of cancer cells, this would explain the high rate of serum albumin turnover in tumours.

Initial studies utilizing serum albumin as a targeting vehicle involved noncovalent binding of unsubstituted zinc phthalocyanine (Figure 1) into BSA prior to iv administration [14]. EMT-6 mouse mammary tumours on Balb/c mice and T380 human colon carcinomas on nude mice displayed tumour regression at doses of 0.5 and 2.0 μmol per kg, 24 hours post PDT. Importantly, no hepatic toxicity was observed using the

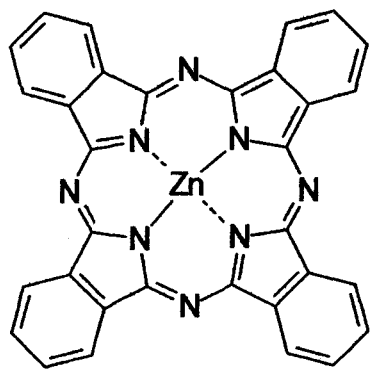


Figure 1. Unsubstituted zinc phthalocyanine

ZnPc-BSA conjugate, thus avoiding an obstacle when administering ZnPc in Chremophor EL™ (CRM). Interestingly, analysis of serum fractions from treated animals showed that, following injection, the ZnPc redistributed towards the high density lipoprotein fraction of the serum. This is similar to observations made using similar dyes delivered in liposomal formulations [15].

In order to avoid this redistribution, studies have been undertaken to covalently bind various photosensitizers to albumin, in particular BSA. It is known that physically altered albumin is targeted by scavenger receptors, which are expressed in high numbers on macrophages. These scavenger receptors are able to bind a wide range of different ligands and shuttle them to endosomes and lysosomal compartments within the cell. Both oxidized LDL and maleylated BSA readily bind to the scavenger receptor while the native proteins do not [16]. As it is estimated that over 50% of the tumour mass is of macrophage lineage in several cancers, this provides an opportunity to target photosensitizers to the tumour volume [6]. For instance, it has been observed that tumour-associated macrophages accumulate higher levels of photosensitizer than do neighboring tumour cells, with a nine-fold increase being observed in the case of porphyrins [17].

Among initial studies, Hamblin and Newman covalently coupled hematoporphyrin (Figure 2) to BSA via a simple peptide bond to give monomeric and cross-linked conjugates [17]. While the fluorescence of these conjugates was quenched to a certain degree, single oxygen quantum yields were comparable to those of the free porphyrins. In NIH 3T3 fibroblast cells and HT29 tumour cells, it was observed that native albumin did not compete with the uptake of the HP-BSA conjugate while the

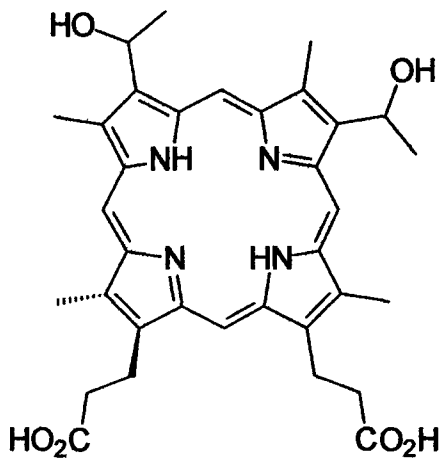
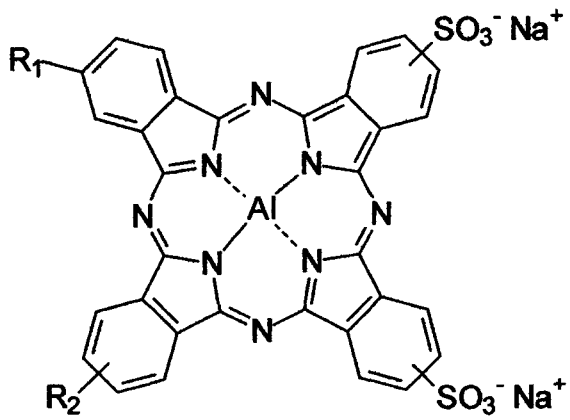


Figure 2. Hematoporphyrin

uptake of the HP-BSA was greatly enhanced in the presence of poly-L-lysine and in the absence of serum. These observations led to the conclusion that the HP-BSA was most likely associated with the plasma membrane of these cells. On the other hand, J774 macrophage-like cells accumulated large amounts of the conjugate as observed by increased fluorescence and this uptake could be drastically impeded by naturally binding ligands. Of some importance, the J774 cells degraded the conjugate, leading to free photosensitizer within the cell and allowing the possibility of redistribution to more photosensitive sites [17].

Altered albumin such as maleylated BSA has also been investigated as a possible vehicle for targeting photosensitizers. $AlPcS_4$ was covalently bound to BSA in a 9:1 molar ratio via one or two sulphonamide hexanoic amide spacer chains (Figure 3) [18]. The resulting conjugate was then treated with maleic anhydride to yield the corresponding maleylated BSA-phthalocyanine complex. The mal-BSA-Pc conjugate showed greater affinity for the scavenger receptor as compare to its BSA-Pc counterpart. These photosensitizer conjugates exhibited higher uptake and improved photodynamic efficiency in the macrophage-like J774 cells as compared to the non-phagocytic EMT-6 murine mammary tumour cells. Competitive binding studies showed that this difference was due to recognition of the mal-BSA conjugate by the scavenger receptors expressed on the J774 cell line. Unfortunately, these protein-photosensitizers were less active than free $AlPcS_{2adj}$, most likely a result of aggregation of the photosensitizer within the conjugate.

Similarly, chlorin e6 (Ce6) (Figure 4) was covalently attached to BSA and this conjugated photosensitizer was further modified by maleylation [19,20]. Dye to protein



$AlPcS_{2(adj)}$	$R_1 = R_2 = H$
$AlPcS_4$	$R_1 = R_2 = SO_3^- Na^+$
$AlPcS_4A_1$	$R_1 = SO_3^- Na^+ R_2 = SO_2(CH_2)_5CO_2H$
$AlPcS_4A_2$	$R_1 = R_2 = SO_2NH(CH_2)_5CO_2H$
$AlPcS_4(C_{12})$	$R_1 = SO_3^- Na^+ R_2 = SO_2NH(CH_2)_{11}CH_3$

Figure 3. Modified sulphonated aluminium phthalocyanines

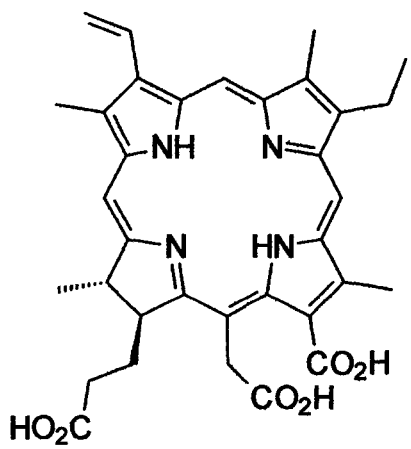


Figure 4. Chlorin e6

ratios of 1:1 to 3:1 were obtained. As in the study above, these photosensitizers displayed increased uptake and higher photodynamic activity in J774 cells as compared to the non-phagocytic OVCAR-5 human ovarian cancer cells. The uptake and phototoxicity in J774 cells was greatly diminished after incubation at 4°C, seemingly indicating an endocytotic route of entry. Interestingly, during *in vivo* studies, mal-BSA-Ce6 had a significant effect on tumour growth delay and reduction in tumour growth in scavenger-receptor negative EMT-6 tumours as compared to free chlorin e6. Naturally, J774 tumours were effectively treated *in vivo* using this conjugate.

Albumin-conjugated photosensitizers have been examined as possible therapies for conditions other than cancer, in particular other conditions involving macrophages and macrophage recruitment. Mal-BSA-Ce6 has been shown to be readily taken up by intimal macrophages and smooth muscle cells that are recruited during the formation of hyperplastic lesions [21]. PDT using this agent effectively inhibited intimal hyperplasia and decreased restenosis following therapy for arterial occlusion. On the other hand, BSA-Ce6 has been investigated as an agent to induce photodynamic tissue adhesion via tissue soldering. Scleral incisions in human cadaveric eyes were welded using conjugates and mixtures of chlorin e6 with various proteins including albumin, fibrinogen and gelatin [22]. The BSA-Ce6 conjugate formulated with additional free albumin showed significantly higher weld strength than the other protein conjugates and mixtures. One possible reason for this observation is increased intermolecular cross-linking between the BSA-Ce6 conjugate and the free albumin, which would result in improved weld strength.

3.2 Lipoproteins

Cholesterols, triacylglycerols and other lipids are transported in the serum by lipoproteins classified according to increasing density: chylomicrons, chylomicron remnants, very low density lipoproteins, intermediate density lipoproteins, low density lipoproteins and high density lipoproteins [23]. Basically, a lipoprotein is a particle consisting of a central core of hydrophobic lipids surrounded by a shell of hydrophilic polar lipids and apoproteins, with seven principle apoproteins having been isolated and characterized. These serum particles have two important biological functions. They solubilize highly hydrophobic lipids and allow the transportation of these important molecules throughout the body while also containing signals that regulate the movement of the particular lipid into and out of specific cells and tissues.

Of these lipoproteins, the most important in terms of drug delivery are the low density lipoproteins. LDLs are the major carrier of cholesterol in the blood [23]. Having a diameter of 22 nm and a mass of approximately three million Daltons, these particles contain a core of about 1500 esterified cholesterol molecules (mostly linoleate esters) surrounded by a shell of phospholipids and unesterified cholesterols. This outer shell also contains a single copy of the very large (514 kd) B-100 apolipoprotein. It is this apolipoprotein that is responsible for recognition and binding by the LDL receptor and leads to receptor-mediated endocytosis of the LDL particle. As cholesterol is a key component of all eukaryotic plasma membranes and is thus essential for the growth and viability of cells in higher organisms, it is natural that tumour cells and tumour vascular endothelial cells express the LDL receptor in higher numbers due to either their increased proliferation or increased membrane turnover [24]. This makes LDL particles extremely attractive vehicles for drug delivery and targeting. An additional advantage of using

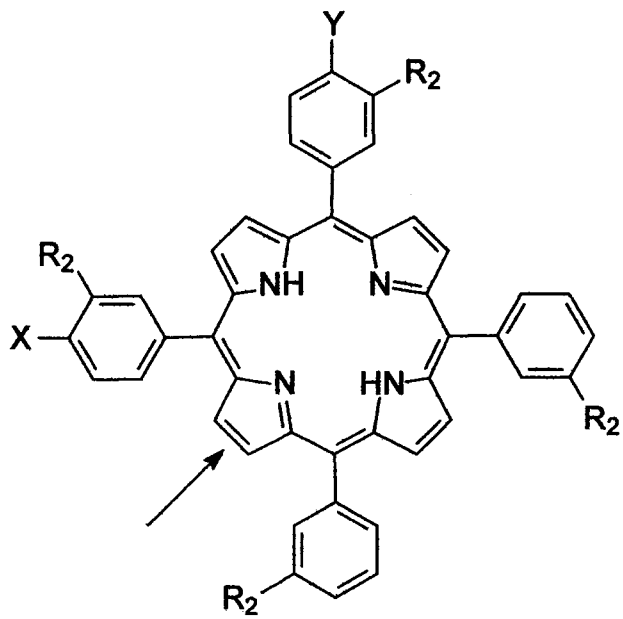
LDLs during PDT is that following irradiation, the LDLs will become highly oxidized and the resulting oxidized species are cytotoxic towards endothelial cells, thus further extending the photodynamic action [25]. In fact, it has been observed that Pc-loaded LDLs are increasingly susceptible to oxidation even without illumination and that this causes a local oxidative stress that can further induce cytotoxic effects in neighboring cells [26].

As was mentioned previously, LDLs are believed to be important in the transportation of the more hydrophobic photosensitizers. The interaction between the PS and the LDL is believed to be a result of two classes of binding sites on the lipoprotein, most likely located in either the matrix of the apolipoprotein or within the lipid core [27,28]. This dual binding would seem to dictate where the LDL-PS particles are targeted, either to the cellular or vascular components of the tumour, depending on the site of binding.

The more hydrophobic photosensitizers must be formulated in some sort of lipid material prior to administration in order to solubilize the dyes [9,25]. Several studies were initiated wherein the highly hydrophobic unsubstituted zinc phthalocyanine (Figure 1) was enclosed into various liposomes and the interaction of these liposomes and serum proteins, in particular LDLs, was examined in order to determine the ultimate fate of the ZnPc in the blood [29-31]. Depending on the liposomal formulation used, it was found that the ZnPc readily redistributed to the LDLs. Incubation of LDLs with liposomes containing ZnPc resulted in a progressive increase in the net negative charge of the lipoprotein as determined by agarose gel electrophoresis and both ZnPc and liposomal phospholipid were found to be incorporated into the LDL particles [30]. Immunoaffinity

experiments indicated that upon incubation, a heterogeneous population of apolipoprotein B-100 was obtained. This would seem to confirm that the LDL particles have two distinct binding sites, one of which involving the apolipoprotein. Importantly, the loss of antibody affinity in this subpopulation may indicate a potential loss of affinity for the LDL receptor. In another study, it was observed that the ZnPc was incorporated into both LDL and HDL following incubation with pooled lipoproteins [32,33]. In pooled plasma, HDL and LDL took up most of the photosensitizer with some of the dye found in association with VLDL. Overall, the density of the ZnPc liposomes increased when the liposomes were incubated with plasma. This suggests that the liposomes are at least partially opsonized by plasma proteins [31]. It seems likely that lipid-type delivery systems partially fuse with the lipid core of lipoproteins. This is supported by the observation that CRM alters the density of HDL and LDL particles [32,33].

The role of the LDL receptor was investigated using both the amphiphilic hematoporphyrin IX (Figure 2) and the hydrophobic ZnPc (Figure 1) bound to human LDL in molar ratios of 5-6:1 and 10-12:1 respectively [34]. In human HT1080 fibroblasts, accumulation of the HP-LDL complex was due to high affinity LDL receptors while the ZnPc-LDL complex was internalized through non-specific endocytosis. The lack of LDL receptor affinity for the ZnPc-LDL was due to changes in the apolipoprotein B structure induced by complexation of the LDL with the phthalocyanine. This structural modification was suggested by spectroscopic studies. On the other hand, in studies using tetraphenylporphinesulphonates, it was found that the monosulphonated (Figure 5 A) and adjacently disulphonated derivatives (Figure 5B) associate strongly with LDLs [35]. Furthermore, despite the photosensitizer strongly



Fiugure 5. 5, 10, 15, 20-Tetraphenylporphyrins (5A, X = SO_4^-, Y = R_1 = H; 5B, X = Y = SO_4^-, R_1 = H; 5C, X = Y = H, R_1 = OH (with the double bond indicated being reduced).

influencing the charge of the lipoproteins, Hep G2 cells showed that up to 250 molecules of TPPS-2A per LDL resulted in unchanged LDL receptor recognition. Furthermore, *in vivo* studies in rats indicated that LDLs incorporating up to 1000 photosensitizer molecules were still processed like native LDLs. As such, these two studies put into question how photosensitizer structure and loading truly influences LDL receptor recognition.

In vivo studies confirmed that LDLs were indeed involved in Pc transport within the body. The relative amount of photosensitizer bound to LDL following iv injection depends on the physical characteristics of the compound. The nature of the delivery system may also affect the amount of photosensitizer bound to LDL. For instance, it is known that the relative amount of $SnET_2$ bound to LDL increases with the vehicle used in the order CRM > cyclodextrin > liposomes following *in vitro* incubation with dog serum [36]. Similar results were obtained *in vivo* using a germanium(IV) octabutoxyphthalocyanine, where CRM-administered GePc let to prolonged serum retention and stronger association with LDL as compared to the corresponding liposome-delivered Pc [37].

Improved incorporation into LDLs enhances the PDT efficiency of most photosensitizers. In the case of ZnPc, non-covalent complexation of the photosensitizer with LDLs prior to injection enhanced both tumour uptake and photodynamic activity of the photosensitizer as compared to ZnPc incorporated into DPPC liposomes [38]. Accordingly, the LDL receptor pathway should be an effective method of enhancing the selectivity of PDT. Interestingly, while albumin is known to mediate the accumulation of photosensitizing dye into the vascular stroma, LDL-transported dye is mostly delivered to

intracellular sites such as the mitochondria [32,38]. As such, these two vehicles would result in different modes of tumour control, with albumin causing photodamage to the extracellular matrix and LDLs resulting in more direct cell death [39]. This difference was observed in the case of hematoporphyrin. Electron microscopy demonstrated that Hp-LDL complexes induced direct cell kill while free Hp is known to induce tumour regression via vascular shutdown [9,32,38].

In order to enhance the incorporation of phthalocyanines into LDLs, $AlPcS_4$ has been modified to include a twelve-carbon long alkyl chain by a sulphonamide bond (Figure 3). The long alkyl chain readily inserts into the lipid core of the LDL [40]. *In vitro* PDT studies against A549 adenocarcinoma lung cancer cells showed the effectiveness of the $AlPcS_4C_{12}$-LDL conjugate. The LDL conjugate was found to be twice as phototoxic as compared to the unconjugated $AlPcS_4C_{12}$ Under the same conditions, the parent tetrasulphonated aluminium phthalocyanine was inactive. No difference was observed between the conjugated and unconjugated $AlPcS_4C_{12}$ during *in vivo* studies. However, this would be as expected since, upon iv injection, unconjugated $AlPcS_4C_{12}$ would naturally distribute to LDLs in the blood stream. Both the conjugated and unconjugated dye exhibited EMT-6 tumour regression at doses as low as 0.2 μmol/kg.

Benzoporphyrin derivative (Figure 6) has been non-covalently complexed with LDLs and these conjugates have been investigated in the treatment of ocular conditions. The importance of the LDL receptor was obvious when it was observed that BPD-LDL intracellular accumulation was insignificant when the LDL was chemically modified by acetylation or when incubation with LDL receptor negative GM2000E fibroblast

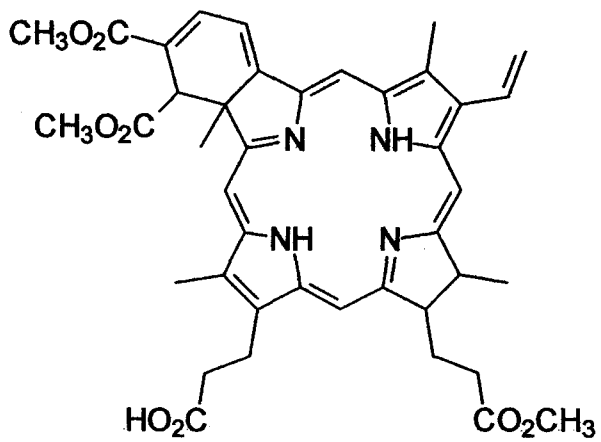


Figure 6. Benzoporphyrin derivative monoacid Ring A (verteporfin)

cells [41]. In the meanwhile, normal GM3348B fibroblast accumulated the BPD-LDL by a specific binding and internalization via the LDL receptor. *In vivo* studies using M1 tumour bearing DBA/2J mice showed a pronounced enhancement in the uptake of the BPD-native LDL conjugate as compared to the acetyl-LDL associated BPD. This result shows the importance of the LDL receptor both *in vitro* and *in vivo*. Subsequently, BPD-LDL photodynamic therapy was tested on experimental models of choroidal melanoma [42], choroidal neovascularization [43] and Green melanoma [44]. Results were favorable in all three of these models. However, despite evidence suggesting direct tumour cell damage, evidence for the role of direct PDT-mediated cell death is lacking and the possibility that the BPD-LDL conjugate targets the neovascular endothelial cells remains [44].

Unsubstituted AlPc has been non-covalently inserted into oxidized LDL [45]. Like albumin, altered LDLs are targeted by the scavenger receptor of macrophages. In this case, the oxLDL-Pc complex was stable upon incubation with serum, indicating that there was little redistribution of the dye to other serum components. Studies against RAW 264.7 macrophage cells showed that these conjugates were highly photoactive. In the presence of a specific ligand against the scavenger receptor, no photocytotoxicity was observed, indicating the importance of scavenger receptor in the targeting of the dye to these cells.

In a similar fashion to albumin, LDLs have been covalently attached to photosensitizers in an attempt to take advantage of the increased LDL receptor expression by malignant cells. Unfortunately, results to date have been disappointing. LDLs were covalently bound to $AlPcS_4$ bearing two sulphonamide hexanoic acid spacer chains

($AlPcS_4A_2$) in the same way as was described above for $AlPcS_4$-albumin conjugates (Figure 3) [40]. The LDL conjugate was not photodynamically active at the highest drug and light doses studied. It seems reasonable to hypothesize that covalent coupling of the Pc to the apolipoprotein of the LDL led to reduced receptor recognition and as such, decreased cell/LDL-Pc interactions. A different explanation involves altered trafficking of the LDL-Pc upon internalization with no redistribution of the LDL-Pc to sites more susceptible to photodynamic damage. This hypothesis is supported by observations made during an investigation on the effects of human serum components on the *in vitro* uptake and photodynamic activity of ZnPc (Figure 1) [46]. In this study, high density lipoproteins increased ZnPc uptake in V-79 cells by 23% but the corresponding photodynamic efficiency was basically unaffected after correcting for the cellular ZnPc concentration. In the meanwhile, both LDL and albumin inhibited the uptake of ZnPc yet increased its cellular photocytotoxicity, seeming to indicate that these serum proteins facilitate the localization of the dye to photosensitive subcellular sites.

Conjugates of hematoporphyrin (Figure 2) and LDL exhibited increased uptake in NIH 3T3 cells, presumably due to receptor-mediated endocytosis as judged by increased uptake when LDL receptors were artificially upregulated [47]. Both HP-LDL and HP-HDL conjugates faced competition for binding sites with unlabelled LDL, suggesting that both lipoprotein conjugates may have other cell surface binding sites along with the specific LDL receptor. Importantly, both the HP-LDL and HP-HDL conjugates were avidly taken up by J774.2 macrophages, although the HP-HDL required aggregation prior to endocytosis. It is interesting to note that the method used to prepare these covalently bonded complexes caused important aggregation of the HP-LDL, possibly due to

apolipoprotein B-100 cross-linking. This aggregation would help explain the increased uptake of the HP-LDL by phagocytic cells.

Chlorin e6 (Figure 4) has also been covalently bound to LDLs via a carbodiimide method. The LDL-Ce6 conjugates were compared to both free Ce6 and Ce6 non-covalently complexed with LDL against a fibroblast cell line (GM 03348 C) and a retinoblastoma cell line (Y79) [48]. Covalent bonding to LDLs significantly increased the uptake of Ce6 in both cell lines. Saturability and competitive inhibition studies indicated a receptor-mediated uptake. However, binding at 2°C also occurred, indicating a degree of non-specific associations. These conjugates had improved photocytotoxic activity, with the LDL-Ce6 reducing cell survival by 80% under conditions where both the free and mixed Ce6 induced a maximum of 10% cell kill.

3.3 Transferrin

All rapidly dividing cells require a continuous influx of iron in order to divide. Free iron or iron ions are absent from biological systems as they catalyze a number of biologically unfavorable reactions including Fenton reactions [49]. As such, all iron is delivered, stored and transported as chelation complexes with various proteins. Transferrin is the major circulating iron transport protein. It is present in the blood at levels of around 200-400 mg/100ml and each transferrin molecule can bind two iron ions [50]. Cells express specific transferrin receptors, which allow for binding and internalization of the two iron saturated transferrin [51]. Following internalization, the iron is delivered to the necessary sites. Several types of cancer cells exhibit increased expression of the transferrin receptor. Furthermore, the expression of the transferrin receptor correlates with tumour grade, stage, progression and metastasis [50]. Hence,

transferrin is an interesting potential vehicle for transporting drugs and photosensitizers to cancerous cells [52].

Initial studies showed that transferrin-photosensitizer conjugates had potential as delivery agents in targeted PDT. Transferrin was covalently coupled to hematoporphyrin (Figure 2) using the N-hydroxysuccinimide ester of HP [17]. While the fluorescence of the dye was somewhat quenched following bonding to transferrin, the conjugate had similar singlet oxygen quantum yields. The uptake of the HP-transferrin in NIH 3T3 and HT29 cells was shown to be receptor-mediated as it was partially inhibited by native protein. In addition, the uptake was greatly enhanced when the transferrin receptor was upregulated by incubation with desferrioxamine.

Transferrin has also been covalently bonded to chlorin e6 (Figure 4). In this case, a novel method was developed wherein the conjugation was accomplished in the presence of a zwitterionic detergent (3-[3-cholidamidopropyl)-dimethylammonio]-1-propanesulphonate (CHAPS)) while the protein was immobilized on QAE-Sephadex®. [53,54] This new methodology allowed the conjugated transferrin to retain its biological activity, the loss of which is a common problem encountered when attaching drug moieties to this protein. The transferrin-Ce6 conjugate has a singlet oxygen quantum yield of approximately 70% of that of the free Ce6. However, during *in vitro* studies against MTLn3 rat mammary adenocarcinoma cells , it was found that the transferrin-Ce6 conjugate was 10-40 times more photocytotoxic than the free Ce6. Similar results were obtained using human MCF-7 mammary adenocarcinoma cells. It was theorized that this treatment might be particularly useful against primary or intraductal breast neoplasms as

the more aggressive of these types of cancers are known to express high numbers of transferrin receptors.

3.4 Other serum proteins

A number of other serum proteins may be involved in photosensitizer uptake and have potential as vehicles for delivering PDT to target molecules. As was previously mentioned, HDL display important associations with various photosensitizers. In fact, it has been generally reported for a number of porphyrins, phthalocyanines and purpurins that HDL bind the highest amount of the photosensitizer as compared to LDL and VLDL [33,55]. In the case of Photofrin®, there is initially binding to both albumin and lipoproteins, with an equal binding to LDL and HDL [28]. However, over a longer time period, the binding is shifted and occurs almost exclusively to HDL. Despite this, it is believed that it is the LDL bound fraction that is delivered to the tumour. Furthermore, findings such as those mentioned earlier, where association of ZnPc with HDL leads to increased cell uptake but no change in PDT efficiency, seem to indicate that this serum protein may be less useful as a targeting moiety [46]. Still, the study by Hamblin and Newman did show that HP-HDL was accumulated by NIH 3T3 and J774 cells [47]. However, the mode of uptake was clearly different from that observed for the HP-LDL conjugate, suggesting that the HP-LDL was not accumulated by an HDL-specific, receptor-mediated mechanism.

There is evidence of the involvement of other serum proteins in the delivery of photosensitizers. An important example is in the case of tetra(*m*-hydroxyphenyl)chlorin (mTHPC) (Figure 5C). This compound is one of the most phototoxic of all second generation photosensitizers [3]. It is 200-fold more photoactive than Photofrin® in some

cases [56]. While improved photophysical properties and singlet oxygen generation can partially explain this increased activity, it is believed that superior subtumoural and subcellular localization is responsible [57]. It appears that mTHPC can bind to an unknown plasma protein, presumably at least partially due to the polyethylene glycol vehicle used to administer this dye, which results in these improved biological properties. Identification of this protein may lead to a new targeting element for improved photosensitizer conjugates.

A final interesting possibility and one that remains unexplored to date is hemoglobin, which has been proposed as an intravascular drug delivery agent [58]. Hemoglobin is readily abundant in the blood as a result of release from aging erthyrocytes. As such, it is felt that the half-life in the blood should be longer for hemoglobin conjugates as compared to albumin and lipoprotein counterparts. In addition, hemoglobin has only one free cysteine available for reaction with reagents, thus helping to avoid cross-linking, an important problem when using albumin. Hemoglobin also provides the fascinating chance to simultaneously deliver both the photosensitizer (or another biologically active compound) and oxygen. This would be clearly advantageous for PDT, which by definition requires oxygen in order to form the necessary cytotoxic species that ultimately lead to cell death and/or tumour shutdown. In addition to the above-mentioned possibility, hemoglobin-like proteins have been used in more sophisticated targeting agents wherein the hemoglobin-like protein acts as the backbone on which is attached a photosensitizer, a cellular targeting agent and a subcellular localizing agent. Similar sophisticated targeting agents have also used BSA and polypeptides as the backbone. These will be discussed later (see Section 9).

4. Annexins

Annexins are normally found in high levels in the cytoplasm of a number of normal healthy cells including lymphocytes, monocytes, biliary and renal tubular epithelium and placenta [59]. Its physiological function has not been fully elucidated although it may involve phospholipid membrane associated processes and calcium binding [60]. However, annexins, in particular annexin V, have numerous properties that make them useful in preparing diagnostic and therapeutic agents. In particular, annexins possess very high affinity for anionic phospholipids such as membrane leaflets having an exposed surface of phosphatidylserine [60,61].

In general, biological membranes are asymmetrical with respect to specific membrane phospholipids [62]. In eukaryotic cells, the outer leaflet of the plasma membrane is formed predominantly with cholinephospholipids such as sphingomyelin and phosphatidylserine while the inner leaflet is composed of aminophospholipids including phosphatidylserine and phosphatidylethanolamine. The asymmetry of the membrane is maintained by a number of enzymes including ATP-dependent floppase and lipid scramblase.

While membrane asymmetry is usual for healthy cells, loss of this asymmetry is associated with various physiological and pathogenic processes. Among the most important is apoptosis, where the appearance of phosphatidylserine on the outer leaflet of the plasma membrane is one of the earliest manifestations of this programmed cell death [61]. As such, annexin V would be ideal in targeting agents to prominent sites of apoptosis. However, it is important to note that phosphatidylserine exposure is also a

component of necrosis, somewhat limiting the utility of annexin V to selectively target sites of apoptosis.

WO patent application publication number 02/080754 teaches the use of annexin V coupled to optically active molecules such as photosensitizers and proposes their use in the diagnosis and treatment of conditions characterized by inappropriate apoptosis and rapid cell turnover [63]. Among photosensitizers that are mentioned as being useful in compositions comprising annexin coupled to a biologically compatible and optically active molecule are fluorescein dyes, Photofrin®, lutetium texaphyrin, hypericin and aluminium phthalocyanines. The ability of annexin to localize at sites of tumour cell apoptosis also makes a photosensitizer-annexin conjugate an ideal drug to use in combination with anti-cancer treatments that cause either apoptosis or necrosis of tumour cells.

Annexin is also mentioned in US patent 6,217,869 as a possible targeting agent in a novel targeting methodology [64]. In this case, targeting agents such as annexin, LDLs, transferrin and insulin along with antibodies, antibody fragments, peptides and hormones are attached to and used to deliver an anti-ligand such as avidin to the target cell. Following this, a photosensitizer bound to the ligand of the ligand/anti-ligand pair (most predominantly biotin) is administered, with ligand/anti-ligand bonding resulting in delivery of the photosensitizer to the target cells. Among possible ligand/anti-ligand pairs are zinc finger protein/dsDNA fragment, lectin/carbohydrate, ligand/receptor and enzyme/inhibitor. Possible photosensitizers include chlorin e6, benzoporphyrin derivatives and sulphonated derivatives of tetraphenylporphine. Preferably the photosensitizing agent has a carboxylic acid group available for conjugation.

5. Bisphosphonates

Bones are constantly being built and destroyed, with the human skeleton being rebuilt every 8 to 10 years [65]. This physiological balance is maintained by osteoclasts, which mediate bone resorption and osteoblasts, which mediate new bone formation [66,67]. Enhanced bone resorption is typical of a number of metabolic bone disorders including Paget's disease, malignant hypercalcemia, osteoporosis and bone metastases [68]. It has been proposed that PDT might be useful in treating these conditions by selectively destroying osteoclasts or other cells involved in metabolic bone disorders. In this case, bisphosphonates (Figure 7) have been proposed as targeting agents [69]. Like inorganic pyrophosphates, bisphosphonates bind to hydroxyapatite crystals in mineralized bone matrix, thus inhibiting osteoclast recruitment and function [68,70]. Furthermore, bisphosphonates stimulate osteoblasts to produce inhibitors of osteoclast function while avoiding degradation by enzymes within the cell. Indocyanine green, methylene blue, chlorins, phthalocyanines, porphyrins, purpurins and texaphyrins are all proposed as potential useful photosensitizers for this targeted PDT [69]. These targeted moieties could be further conjugated to other molecules such as antibodies, peptides and polymers to improve the specificity of the photosensitizer to bone targets. The possibility of using photosensitizer conjugates of this type against bone metastases, Paget's disease, hypercalcemia and Type 1 osteoporosis is mentioned.

6. Steroids

Steroids form an interesting and potential useful method of targeting photosensitizers to diseased tissue. As was previously mentioned, cholesterol is a vital component of eukaryotic cell membranes and as such, is rapidly taken up by proliferating

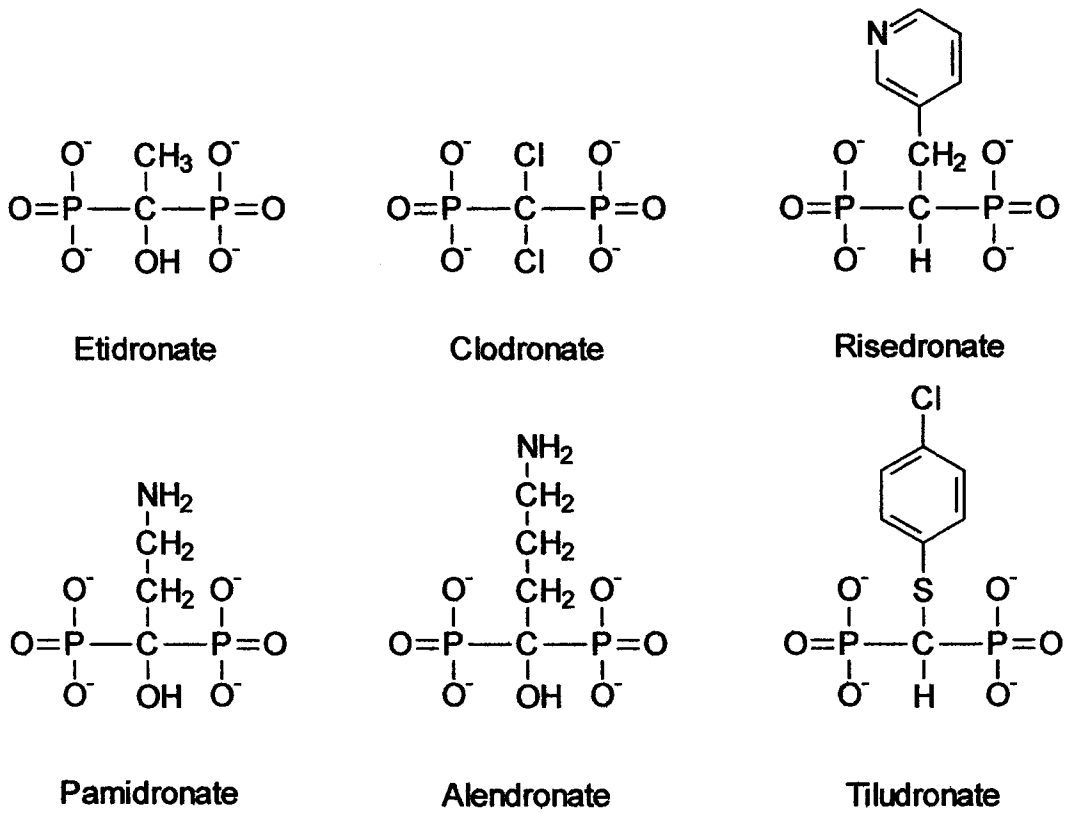


Figure 7. Bisphosphonates

cells [23]. As has been previously stated, LDLs are the primary source of cholesterol for cells as they are made up of a cholesterol ester core surrounded by a shell of phospholipids and unesterified cholesterol. In order to improve non-covalent LDL-photosensitizer interactions, pyropheophoride cholesterol oleate conjugates have been synthesized [71]. These steroid-photosensitizer conjugates were successfully reconstituted into the LDL lipid core. Internalization of the reconstituted LDL via the LDL receptor in human hepatoblastoma G(2) tumour cells was demonstrated using laser scanning confocal microscopy. With the same motivation, dicholesteryl-substituted germanium phthalocyanine has been synthesized where the cholesterol moieties act as axial ligands, bound to the germanium central metal ion via a diphenylsilanediol [72]. The length of the spacer chain between the cholesterol and the silane was varied in order to alter the overall amphiphilicity of the molecule. Singlet oxygen quantum yields for these conjugates were quite good in organic solvents. Silicon phthalocyanines bearing two cholesterol axial ligands have also been prepared by reacting dihydroxysilicon phthalocyanine with chlorocholesteryloxydiphenylsilane, bis(triflate)-silicon phthalocyanine with cholesterol or dichlorosilicon phthalocyanine with cholesterol alcoholate [73]. The synthesis of galactopyranosyl-cholesteryloxy substituted porphyrin has been reported as well [74]. The amphiphilicity of these new carbohydrate cholesterol substituted porphyrins led to easy incorporation into model membranes. Furthermore, the vesicle forming properties of these compounds have been investigated by light scattering experiments and electron microscopy and have been found to be adequate for forming vesicles with potential for PDT.

Steroids are also useful as an adjunct therapy to PDT. For instance, the anti-tumour effects of photodynamic therapy are potentiated by 2-methoxyestradiol [75]. Pre-incubation of 3 murine and 5 human tumour cell lines with 2-methoxyestradiol prior to PDT gave a synergistic anti-tumour effect. Retardation of tumour growth and prolonged survival of tumour-bearing mice was also observed when this combination therapy was used *in vivo*. PDT in these series of experiments was shown to induce the expression of MnSOD in cancer cells. With 2-methoxyestradiol known as a superoxide dismutase inhibitor, the suggestion was made that the synergistic effect of this combined therapy is due to inhibition of this important oxidative stress defense mechanism. In another study, the photodynamic efficiency of hematoporphyrin derivatives was potentiated by glucocorticoids when administered after irradiation [76]. Both Lewis lung carcinoma and B16 melanoma were examined in a transplantable mouse tumour model. Interestingly, concurrent administration of the glucocorticoid with the photosensitizer either inhibited the PDT response or had no effect. Finally, it was observed that administration of lovastatin, a sterol synthesis inhibitor, increases the phototoxic effect of Photofrin® when the Photofrin® is delivered by LDLs [77]. In this case, the increased efficiency is due to improved photosensitizer uptake via some sort of LDL receptor-mediated process.

In terms of targeting, steroids and other hormones provide the opportunity to deliver the photosensitizer to one of the most photosensitive sites within the cell, namely the nucleus. Hormones such as estrogens, androgens, progesterone, mineralocorticosteroids, glucocorticosteroids, thyroid hormones, retinoic acid, vitamin D and ecdysone all bind with high affinity to a specific member of the nuclear hormone receptor superfamily [78]. Each member of this family of receptors has a ligand binding

domain and a DNA binding domain [79]. While the ligand binding domain is highly conserved between the various family members, the DNA binding domain varies, leading the family to be divided into at least two subgroups [80]. Most of the steroid receptor family, with the exception of the estrogen receptor, are translocating receptors, meaning that they have principally a cytoplasmic distribution in the absence of their ligand [81]. Upon diffusion of their ligand through the plasma membrane and binding to the ligand binding site, these receptors undergo a conformational change and are trafficked to the nucleus where they target sequences of DNA. This ultimately leads to gene expression. The estrogen receptor, on the other hand, is found exclusively in the nucleus. However, binding with its ligand again leads to DNA sequence targeting and gene expression. Furthermore, due to the high affinity binding involved, nuclear receptors act like beacons to attract and selectively localize their ligands into cells where these receptors are expressed. This makes such ligands important in attempts to target these cells with photosensitizer and other drugs.

Obviously, photosensitizers conjugated with ligands for nuclear hormone receptors would be useful tools for targeted PDT. This is especially the case for the estrogen receptor, which binds estradiol, estriol, estrone and synthetic estrogen agonists/antagonists. Breast tumour cells are known to over-express estrogen receptors in high levels, particularly in their earlier stages and under hormone treatment [82-84]. As such, estrogen receptors represent a potential site for directing photosensitizers, in order to both increase cellular uptake and to deliver the dye to the nucleus of these cancer cells. A number of attempts have been made to covalently attach estrogen and other hormone receptor ligands to photosensitizers. For instance, both estrogen and progesterone

derivatives have been conjugated to the zinc(II) and nickel (II) complexes of 5-15-diphenylporphyrin (Figure 5) and deuteroporphyrin IX dimethyl ester [85,86]. In addition, palladium catalyzed cross coupling methodologies have been used to covalently bind the 17α-ethynyl derivatives of estradiol, testosterone and 19-nortestosterone to zinc (II) 5, 10, 15, 20-tetraphenylporphyrin (Figure 5) either on a phenyl ring or in the 2 and/or 7-beta positions [86].

A major problem with steroid-based conjugates in general and photosensitizer-steroid conjugates in particular is a decrease or loss of receptor recognition. The nuclear hormone receptor superfamily is very susceptible to minor variations in the structure of its ligand, leading to rapid decreases in receptor binding and receptor recognition, even with only slight structural modifications. As such, it is essential to identify positions on the parent steroid where an appendage such as a photosensitizer can be attached without seriously compromising receptor binding. In the case of estradiol, both structure/activity studies and crystal structures of hormone binding domains have demonstrated that substitution at the 7α, 11β and 17α positions is well tolerated [87-90]. In particular, modifications at the 11β and 17α positions with hydrophobic moieties will still lead to significant binding with the estrogen receptor. Even with this however, the covalent attachment of a large photosensitizer to a steroid molecule most likely will affect steroid/nuclear hormone receptor interactions to some extent. For instance, chlorin e6 dimethyl ester bonded to estradiol via a C17-amino or a C17-ether group at the C17-hydroxy position do not bind to any significant extend to the estrogen receptor [91,92]. Cell uptake and photocytotoxicity studies using receptor positive and receptor negative

cells varied greatly and seem to depend more on the inherent photosensitivity of the cell line instead of estrogen receptor status.

With these positions in mind, estrogen-photosensitizer conjugates have been prepared and their receptor binding and PDT photocytotoxicity have been studied. 17α-Ethynylestradiol, 17α-(2-buta-1,7-diynyl)estradiol and 17α-(phenyl-1,3-diynyl)estradiol have been covalently bound to a series of lipophilic and hydrophilic phthalocyanines using catalytic palladium chemistry [93]. The more lipophilic conjugates displayed higher binding affinity for the estrogen receptor during *in vitro* testing. At its best, this binding was only 12% of that found for native estradiol. Surprisingly, the more hydrophilic trisulphonated phthalocyanine conjugates were more phototoxic against the EMT-6 mouse mammary tumour model. In fact, the lipophilic conjugates were photodynamically inactive at 1 μM while exhibiting a dark toxicity at 5 μM. It is possible that this lack of photodynamic activity was due to the vehicle used to solubilize these conjugates.

Estradiol has also been attached to tetraphenylporphyrin via the 11β-position (Figure 8) [91,92,94,95]. Competitive radioligand binding assays demonstrated that the estradiol-porphyrin conjugate could displace [^{3}H]-estradiol in a dose-dependent manner, indicating that estradiol receptor recognition remained even upon substitution with a large moiety such as a porphyrin [94]. Still, receptor binding affinity was significantly decreased compared to estradiol, with the EC_{50} for estradiol and the conjugate being 1 nM and 274 nM respectively. Studies with estrogen receptor-positive MCF-7 breast cancer cells revealed that the conjugate was selectively taken up by the receptor-positive cells in a dose-dependent manner [95]. This uptake was obliterated when the conjugate

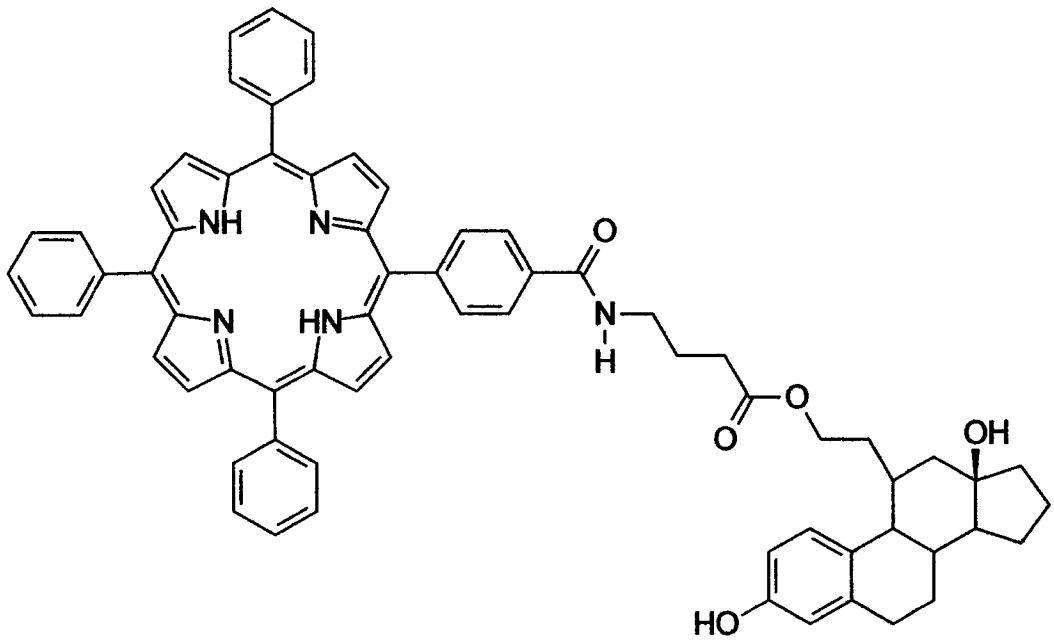


Figure 8. C11-β-estradiol-tetraphenylporphyrin conjugate

was co-incubated with estradiol, an obvious result with the large variation in binding affinities. Little uptake was observed using the non-conjugated parent porphyrin or in receptor-negative Hs578t breast cancer cells. These results would seem to indicate that specific interaction between the estradiol-porphyrin conjugate and the receptor enables the cells to selectively internalize the conjugate over its unconjugated parent. Unfortunately, neither the conjugate nor the unconjugated dye were very effective in killing either the MCF-7 or the Hs578t breast cancer cells [92,95]. It was believed that this lack of photocytotoxicity was due to inadequacies in the photophysical and photobiological properties of the dye used.

In order to improve on the photocytotoxicity of the conjugates, Ce6 (Figure 4) was attached to estradiol via 17α-position using tethers of varying length [92]. While binding to the receptor was poor (300 times less than estradiol), these conjugates proved to be photoactive against MCF-7 cells. Conjugates were also prepared using 4-hydroxytamoxifen, an anti-estrogen that binds strongly to the estrogen receptor. When covalently bound to chlorin e6, estrogen receptor binding by the tamoxifen was still observed, though in a very small amount. Light-induced cell killing experiments against MCF-7 breast cancer cells did demonstrate that these tamoxifen conjugates were photoactive. It has been proposed that the methodology described here could be extended to other nuclear hormone receptor ligands including testosterone and vitamin D [91].

7. Toxins and Lectins

In order to enhance the specificity of cancer therapies, studies have been undertaken in order to determine biochemical and physiological changes that occur during malignant cell transformation. Among these changes is the expression of cell

surface molecules, which are not expressed in the non-transformed cells. The differential expression of many cell surface molecules in human cancers has been well studied and provide yet another opportunity to target these cells specifically.

One molecule that has been found to be overexpressed in malignancies such as Burkitt's lymphoma and breast, brain, gastric and testicular cancers is globotriaosylceramide (also known as Gb_3, CD77 and P^k antigen) [96]. The Gb_3 glycosphingolipid is normally expressed in several tissues including intestinal and kidney epithelium. This molecule is specifically targeted by bacterial toxin proteins belonging to the verotoxin family and include Shiga toxins and Shiga-like toxins. These bacteriotoxins are produced by *S. dysenteria* and *E. coli* and are responsible for the disease symptoms associated with these bacteria. Verotoxins comprise two protein components. The catalytic A subunit inhibits protein synthesis, inducing disease while the pentameric B subunit is responsible for targeting specific cells expressing Gb_3 [97]. In addition, binding to Gb_3 leads to internalization of the ligand/receptor complex. A number of studies have been undertaken to utilize the specific binding of the B subunit of Shiga toxins to specifically target malignant tissues with promising results [98-101].

In terms of PDT, the B subunit of Shiga-like toxins (SLTB) has been covalently conjugated to chlorin e6 (Figure 4)and the efficiency of the conjugate has been examined [102]. *In vitro* uptake experiments showed that the cell associated chlorin e6-specific fluorescence was readily detected following incubation of Gb_3 positive Vero monkey kidney fibroblasts with the conjugate. No cell-associated fluorescence was observed under identical conditions using free chlorin e6. The conjugate accumulated in the Golgi apparatus and endoplasmic reticulum as indicated by MitoTracker® Green FM

experiments The chlorin e6-SLTB were also significantly more efficient at inducing photodynamic cell death than free chlorin e6, with an LD_{50} of 0.1 nmol/ml compared to 1.2 nmol/ml for free Ce6. Interestingly, Ce6 simply absorbed into the SLTB was found to localize in mitochondria and had an LD_{50} of 0.6 nmol/ml. This would seem to indicate that the ultimate intracellular site of accumulation greatly depends on the method of association between the dye and the toxin.

Based on these results, it was hypothesized that the targeting portion of other toxin or lectin molecules may be used to target photosensitizers. Among possible toxins and lectins are abrin, heat labile toxins, botulinum toxin, cholera toxin, helix pomatia, jacalin, peanut agglutinin, ricin toxin, sambucus nigra, tetanus toxinulex europeaus and viscumin. The targeting fragment of these toxins and lectins would allow for targeting of a selection of cell surface receptors and help deliver the dye to such potential targets as sarcomas, breast cancer, colon cancer and vasoformative tumours (which include angiosarcomas and hemangiopericytoma) among many others. Jacalin, which specifically recognizes the tumour-associated T-antigenic disaccharide structure Galβ13GalNAc, has been interacted with various porphyrins to form inclusion complexes [103]. Each lectin subunit was found to bind one porphyrin molecule with an association constant in the range of 2×10^3 to 1.3×10^5 M^{-1} at room temperature. Such binding suggests that jacalin may potentially be useful as a targeting vehicle for porphyrins, possibly leading to accumulation of the dye in gastric, pancreatic and mammary cancers.

Attempts are also underway to combine the B subunit of Shiga toxin with a new family of photosensitizers [104]. These tri-metallic supramolecular organometallic compounds consist of three subunits, two light absorbing chromophores and a bioactive

site that induces cell death following excitation. Upon illumination, osmium and ruthenium based chromophores become excited and pass an electron to the bioactive site, which contains rhodium. Utilizing two chromophores increases the range of light that can be captured. The passed electron excites the rhodium, creating a charged radical that will wreak havoc in the cell, inducing cell death. Using such a system removes the need for oxygen, alleviating an important problem with traditional photosensitizers.

8. Epidermal growth factor

Epidermal growth factor (EGF) is a small 6 kDa polypeptide that binds specifically to a cell surface receptor, stimulating the growth of epidermal and epithelial cells [105]. Like the insulin receptor, the EGF receptor has tyrosine kinase activity and is activated upon binding of EGF to the extracellular portion of this transmembrane 175 kDa protein. EGF is a potent mitogen found throughout the body and is an angiogenesis-stimulating factor. EGF receptors are overexpressed in a number of cancer cell lines including ovarian cancer. In fact, over 30% of ovarian cancers have increased EGF receptors [6], making EGF a potential drug carrier and EGF receptors important targets in treating these types of cancers [106]. This is especially so in view of the fact that over-expression of the EGF receptor is frequently correlated with poor prognosis and cure rates. It has been demonstrated that if EGF is added following PDT with hematoporphyrin derivative, photocytotoxicity is greatly decreased in three glioma cell lines [107]. Pre-incubation with EGF, on the other hand, did not affect PDT efficiency.

EGF has been coupled with various photosensitizers in the hopes of specifically targeting cells over-expressing the EGF receptor. The cytotoxic activities of aluminium and cobalt disulphonated phthalocyanines bound to EGF were determined against a

human breast carcinoma cell line (MCF-7) [108]. The AlPc-EGF conjugate was 7 times more photoactive than the corresponding free disulphonated phthalocyanine. Binding EGF to the CoPc, which required ascorbic acid to induce activation instead of light, greatly increases the photoactivity of this photosensitizer, improving cytotoxicity over 100 times in MCF-7 cells. Subsequent *in vivo* studies using B16 melanoma were carried out with the conjugates delaying tumour growth while free dye had little effect.

More elaborate EGF-photosensitizer conjugates have been prepared where Sn(IV)chlorin e6 and EGF are combined via a carrier such as dextran, polyvinyl alcohol and human serum albumin [109,110]. The EGF-HSA-Sn(IV)Ce6 conjugate had the best receptor affinity [110]. Binding of the EGF-Dex-Sn(IV)Ce6 was substantially impaired, with approximately 100 times more conjugate needed to obtain equal displacement of ^{125}I labeled EGF as compared to unconjugated EGF [109]. The PVA conjugate, on the other hand, displayed no affinity for the receptor at all. Photocytotoxicity against MDA-MB-468 and A431 cells, both of which over-express the EGF receptor, also varied depending on the carrier used. The EGF-HSA-Sn(IV)Ce6 conjugate displayed a high phototoxicity with an IC_{50} of 63 nM at a light dose of 27kJ/m^2 [110]. Despite the receptor binding results, it was found that the PVA conjugate exhibited a higher photocytotoxicity than the dextran conjugate [109]. However, it was noted that both the Dex-Ce6 and PVA-Ce6 displayed similar results as compared to their EGF conjugated counterparts. Thus, it would appear that the difference in photocytotoxicity is a result of the carrier and not the EGF ligand. In the case of the EGF-HSA-Sn(IV)Ce6, the results were clearly due to receptor mediated processes as native EGF could compete for binding sites and decrease the photoactivity of the conjugate [110]. Increased production of intracellular ROS upon

irradiation was observed using the HSA as compared to the dextran derivative, seeming to show increased intracellular accumulation of the dye in the active form.

Preliminary studies are also underway wherein the EGF receptor is targeted using an antibody against the receptor [111]. BPD was conjugated with the C225 antibody, which targeted the EGF receptor on ovarian cancer cells. Such photoimmunotherapy remains promising.

9. Insulin and nuclear localizing signals

Drug targeting is an integral part in the planning of novel medications. The vast majority of disease treatments are delivered systemically, thus the importance of cell specificity is apparent. Initial attempts to improve photosensitizer delivery focused on improving target to non-targeted tissue ratios. However, it was shown that elevated tumour to normal tissue ratios did not ensure improved tumour eradication *in vivo* [112]. Photodynamic therapy acts through the production of free radicals and singlet oxygen ($^1O_2$) to induce cell death. It has been postulated that singlet oxygen is the more important of these reactive species. Singlet oxygen has a life span of 200 ns and a migrating circumference of 45 nm. Mammalian cells are 10^4 to 10^5 nm in diameter, making the importance of PS distribution within the target tissue readily evident [4]. The site of $^1O_2$ production is the site of photosensitization. Research efforts have concentrated on enhanced cellular targeting as a valid approach to improve the photodynamic response.

Initial studies using chlorin e_6 (Figure 4) conjugated to either insulin or concanavalin A (Con A) demonstrated increased photosensitization due to receptor mediated endocytosis [113]. A human hepatoma cell line was incubated with either free

chlorin e_6 or chlorin e_6 conjugated with one of the two ligands followed by illumination. The bioconjugates were 4.2 fold more efficient in cell killing than the free photosensitizer. It was shown that this effect could be abrogated if an excess of unconjugated ligand was present. In addition, photosensitization was greatly reduced if incubated at a lowered temperature (4°C), suggesting that internalization of the photosensitizer led to increased phototoxicity. Akhlynina and associates have expanded this work to include subcellular targeting. Nucleic acids are very photosensitive, therefore efforts have been made to enhance delivery of the PS to the cell nucleus. Numerous chlorin e6 were bound to bovine serum albumin (BSA) which acts as a linker to allow the attachment of more PS molecules on an internalizable ligand such as insulin. It was shown that once endocytosed, the BSA-insulin-Ce6 conjugate localized in the cell nucleus [114]. Singlet oxygen production was monitored using 2'7'-dichlorofluorescein diacetate, which yields a fluorescent derivative, 2',7'-dichlorofluorescein when reacted with active oxygen species. Following a 4 hour incubation at physiological temperature with the BSA-insulin-Ce6 and subsequent irradiation to produce ample amounts of $^1O_2$, there was increased fluorescence around and within the cell nucleus.

All transport across the nuclear membrane occurs through the nuclear pore complex (NPC). This acts as a sieve to allow smaller proteins of less than 45 kDa to gain access to the nucleus via passive diffusion whereas larger molecules, such as would be the case with these bioconjugated Ce6 molecules, require specific targeting signals in order to penetrate the NPC. Elaborate constructs were studied to exploit the photosensitivity of the cell nucleus by directing intracellular transport of the PS with a variant of the simian virus SV40 large tumour antigen nuclear localization signal (NLS).

The insulin-BSA-Ce6 was either tagged with a NLS peptide cross-linked to the carrier, BSA, or the NLS peptide sequence was encoded within a β-galactosidase fusion protein carrier [115]. The use of nuclear localizing signals increases the photosensitizing activity of the Ce6 bioconjugates such that the EC_{50} is reduced by over 2400 times as compared to free Ce6. Sobolev and co-workers constructed different Ce6 bioconjugates by altering the NLS, carriers and internalizable ligands and found some to be more photodynamically efficient than others. However in all cases, the bioconstructs exhibited greater efficacy than the parental photosensitizer, chlorin e6 [116].

Tumour cells typically have increased expression of cell surface receptors for various growth factors. For example, melanomas have upregulated α-melanocyte-stimulating hormone (MSH) receptors. Rosenkranz *et al.* have produced a bacterial expressed recombinant polypeptide vehicle for photosensitizers, which comprises the MSH internalizable ligand, the NLS of SV40 T-ag, the *Escherichia coli* hemoglobin-like protein HMP as a carrier and an endosomolytic peptide that is required for disruption of the endosome once the conjugate is internalized in the cell [117-119]. Using the M3 murine melanoma cell line, the PS coupled to the MSH-NLS-HMP-peptide required a light dose of 67 kJ/m^2 to eradicate the melanoma cells as opposed to 620 kJ/m^2 for the free photosensitizer [112]. This technology can be applied to several internalizable peptide ligands, i.e. EGF, somatostatin, acidic and basic fibroblast growth factor, platelet-derived growth factor, interleukin-1, -2, -5 as well as various tumour cell specific antibodies. These ligands have a dual advantage for PS trafficking as they have cell specific delivery as well as being nuclear localizing in nature.

Recent efforts have focused on the development of chimeric modular recombinant transporters (MRT) expressed in E. coli and used for PS targeting [117]. The bacteriochlorin p6 was conjugated to a MRT which consisted of 4 components: (a) the same localizing and internalizing ligand, α-MSH, (b) the SV40 large T antigen as the NLS, (c) the HMP carrier and (d) the translocation domain of the diphtheria toxin DTox as the endosomolytic module. The MRT (DTox-HMP-NLS-MSH-Ce6) enters the target cells, either the murine melanoma B16-F1 or M3 cells, both with abundant expression of the MSH receptor, via receptor mediated endocytosis. The endosome is a closed membrane structure which is considered to be extracellular. In order to deliver the PS to the photosensitive cell nucleus, the endosome must be ruptured and its contents emptied into the cytoplasm where the NLS directs the construct to interact with α/β-importins, a class of proteins which mediates cytoplasm to nucleus transport. The DTox-HMP-NLS-MSH was detected in the nuclei of 87.5% of the cell population in comparison to only 12.2% of the cells' nuclei when using only the HMP-NLS-MSH without the DTox polypeptide for endosomolysis. This resulted in a 250-fold increase in photodynamic efficacy when administered to the B16-F1 melanoma cells. These MRT constructs are advantageous in that they can be used to target any number of cell types by exchanging the internalizable ligand. A similar MRT conjugated with bacteriochlorin p6 has been developed where the α-MSH has been replaced with epidermal growth factor, EGF. This proved to be 960 times more cytotoxic against human epidermoid carcinoma A431 cells than the free PS [117].

Constructs have also been prepared whereby chlorin e6 (Figure 4) has been conjugated to a targeting moiety and a subcellular localizing motif via an α-helical

polypeptide [120]. Preferred target moieties were antibodies and antibody fragments. Interestingly, α-helical polypeptides in the form of a fusion polypeptide containing antibody fragments such as single chain Fvs showed promise in targeting chlorin e6 to cells. Subcellular targeting peptides proposed include NLS, mitochondrial localization sequences, lysosomal targeting peptides, endoplasmic reticulum retrieval signals and Golgi targeting sequences.

Alternatively, vectorial delivery of nucleus-directed complexes into cells has been accomplished using synthetic peptides composed of a branched polylysine core with 8 identical arms [121,122]. These molecules, known as loligomers, have 8 pentalysine import signals, which are cytoplasmic translocation signaling peptides, and a NLS from SV40 T antigen for localization within the cell (Figure 9). The photosensitizer, Ce6 was coupled to a nucleus-directed linear peptide or the branched loligomer via solid-phase synthesis. The use of eight Ce6 molecules in a single loligomer resulted in a 400-fold increase in CHO photocytotoxicity and a 40-fold increase in RIF-1 cell death over Ce6 alone. Following a six hour incubation of RIF-1 cells, there was 3 times more Ce6-loligomer located in the cell nuclei than Ce6-peptide. This accumulation in the more photosensitive cell nuclei most probably accounts for the increased toxicity of these constructs.

10. Adenoviruses and adenoviral proteins

As previously discussed, endosomal disruption represents a serious limitation in photodynamic efficiency. If the PS remains trapped within this membrane bound vesicle, upon illumination, the endosome will quench the PDT reaction. To circumvent this problem, attenuated Adenovirus (Ad) type 5 had been used. Adenoviruses efficiently

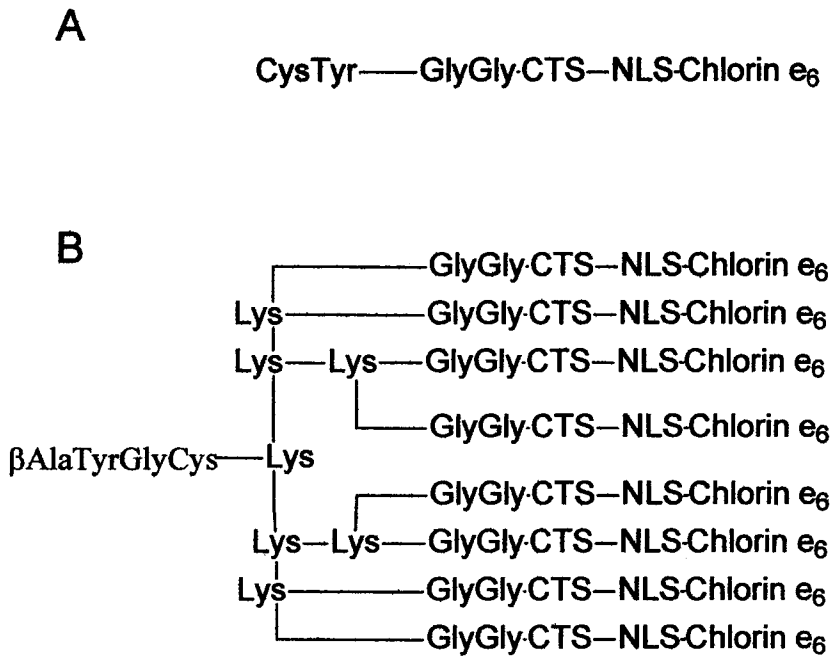


Figure 9. Nuclear directed chlorin e6-peptide (A) and loligomer (B) conjugates

break open the endosomes upon infection and therefore it was hypothesized that the bioconstructs would target the cell nucleus more quickly when delivered in conjunction with Ad. This resulted in a 2.5 fold increase in nuclear photosensitizer targeting as opposed to that photosensitizing activity when localized in the cytoplasm [123].

Using similar principles as described previously, adenoviral proteins were investigated as potential targeting agents [124]. Adenoviral particles gain entrance into the cells via receptor mediated endocytosis. In place of insulin as an internalizable ligand, studies using adenoviral proteins as targeting agents have been carried out to improve PDT efficiency against human lung adenocarcinoma cells, A549. This family of viruses requires two separate receptors, the first for attachment and the other mediating internalization. The Ad fiber capsid protein attaches to the cells via the Coxsackie B and Adenovirus receptor (CAR) [125]. Internalization of the virus particle is via the α_v integrin receptors which are able to bind with high affinity to the RGD (Arg-Gly-Asp) binding motif found in the penton base protein of adenovirus [126-128]. This class of integrin receptors is up-regulated in several cancer cell lines as well as having a robust expression in epithelial cells lining the blood vessels that feed tumours [129,130]. Studies using purified adenovirus capsid proteins covalently labeled with $AlPcS_4$ derivatives have shown this to be a valid approach to tumour cell eradication [124]. The hexon, the penton base and the fiber antigen of adenovirus type 2 were purified using anion exchange chromatography and SDS-PAGE verification of protein content. This was followed by ammonium sulfate precipitation and dialysis. The Ad proteins were covalently coupled to $AlPcS_4A_1$ or A_2 via a one or two six carbon spacer chains containing a terminal free carboxy group using a diimide active ester (Figure 3). The Ad-

protein-$AlPcS_4$ derivatives were tested both *in vitro* and *in vivo* for their PDT efficacy. The penton base-$AlPcS_4A_2$ derivative was the most efficient *in vitro* with LD_{50} values half as much as the free $AlPcS_4A_2$ as measured in two different cell lines both expressing the cell surface integrin receptors. This LD_{50} value was still twice as high as that measured for a reference photosensitizer, $AlPcS_{2adj}$.

Endosomal entrapment of the photosensitizer has been proposed as an explanation for the decreased phototoxicity of the penton base-$AlPcS_4A_2$. It is plausible that the penton base-Pc/receptor complex is trapped within the endosome and is unable to redistribute to cellular targets. Adenovirus cell infection is dependent not only on cell recognition and internalization but also on endosome disruption. This is a two step process with an initial cleavage of viral capsid proteins followed by a pH-dependent endosome disruption. The Ad protease is required to cleave various cellular proteins to facilitate passage from within the endosome into the cytoplasm [131]. Therefore tumour response studies using Balb/c mice with EMT-6 tumour implants were carried out using a mixture of adenovirus type 2 soluble proteins covalently bound to $AlPcS_4A_2$. The free $AlPcS_4A_2$ induced tumour regression at a dose of 1μmol/kg and 400J/cm^2 which is comparable to $AlPcS_{2adj}$. The mixture of Ad2 soluble proteins coupled to $AlPcS_4A_2$ required a dye dose of 0.5 μmol/kg to cause tumour ablation.

Tumour targeting using protein vehicles may have serious limitations invoking adverse immune responses. The search for small peptidic vectors has led to the investigation of the RGD sequence as a possible PS carrier [132]. The RGD motif is currently being investigated as a targeting vehicle for conventional chemotherapeutics and may be useful for PDT.

11. Conclusion

The diversity of cellular characteristics will eventually lead to the discovery of appropriate drug targets and targeting mechanisms. Research is ongoing to find the infamous "magic bullet". However, a less general approach is probably more realistic. Each disease type must be targeted on an individual basis. In order for photodynamic therapy to reach its full potential, there will be a need for varied photosensitizers and numerous targeting motifs so that all cell and tissue types can be selectively destroyed.

In addition to finding the ideal photosensitizer and targeting moiety for each individual condition, there are a number of other factors specific to PDT that need to be addressed. The primary photosensitizers used for PDT have an important tendency to aggregate by virtue of their large planar aromatic ring systems, leading to strong photosensitizer-photosensitizer interactions and non-covalent complexes with proteins and other potential targeting compounds [19, 111]. Despite recent advances, this aggregation frequently results in difficulties in purifying the covalently bound conjugates from unbound photosensitizers. The presence of non-covalently bound photosensitizer within the conjugates will affect the biological results obtained using these targeted dyes, making the comparison of results difficult and evaluation of the targeting moiety questionable.

Aggregation of photosensitizers within the conjugations along with hydrophobic interactions between the aromatic core of the photosensitizers and the targeting protein can also greatly affect the photophysical and photochemical properties of the PS [4]. Decreases in the absorption coefficient, singlet state lifetimes and triplet state yields and lifetimes caused by conjugation will all negatively affect the production of ROS during

illumination, thus decreasing the photocytotoxicity of the dye conjugates. Furthermore, as has been mentioned above, it is necessary not only to consider delivery of the photosensitizer to the target cell but also to get efficient accumulation of the PS at susceptible subcellular locations. Therefore, PS conjugates that accumulate in cells within endosomes and lysosomes may be less effective than the corresponding non-conjugated PS despite increased intracellular uptake.

While the majority of targeted PDT has involved the treatment of cancer, the use of receptor mediated delivery systems also has immense promise for the treatment of other conditions including atherosclerosis, age-related macular degeneration, bacterial and parasitic infections and autoimmune diseases. It remains only to find the ideal photosensitizer/receptor mediated delivery system for each pathological condition for targeted PDT to a useful treatment modality and for PDT itself to fulfill the potential it has exhibited since its discovery a little over a century ago.

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Chapter 5.

Synthesis of Phthalocyanine Precursors

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SYNTHESIS OF PHTHALOCYANINE PRECURSORS

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Key words: phthalocyanines, phthalonitriles, diiminoisoindolines, crown ethers, dendrimers, precursors

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Abbreviations:

Abbreviation	Term
AIBN	Azoisobutyronitrile
DAMN	Diaminomaleonitrile
dba	Tris(dibenzylideneacetone)
DBI	N,N-dibromoisocyanuric acid
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	Dicyclohexylcarbodiimide
DDQ	2,3-Dichloro-5,6-dicyanobenzoquinone
DMAP	*p*-N,N-dimethylaminopyridine
DMF	N,N-dimethylformamide
DMSO	Dimethylsulphoxide
dppf	Bis(diphenylphosphino)ferrocene
HMPA	Hexamethylphosphoramide
HMPT	Hexamethylphosphorous triamide
HPLC	High performance liquid chromatography
MCBPA	*m*-chloroperbenzoic acid
NBS	N-bromosuccinimide
Na_2mnt	Disodium maleonitriledithiolate
NMP	1-methyl-2-pyrrolidinone
Pc	Phthalocyanine
rt	Room temperature
TBAB	Tetrabutyl ammonium bromide
TBAF	Tetrabutylammonium fluoride
THF	Tetrahydrofuran

I. Introduction

The importance of tetrapyrrolic macrocycles in nature is obvious. Compounds such as haem and chlorophyll play such vital roles in the biological systems responsible for the transportation of oxygen to cells in the body and the transformation of light into useful energy in plants. Others are involved in the electron transport chain in the mitochondria and the protection of cells from oxidative damage. The unique physical, chemical and spectral properties of this class of compounds along with the diversity found in their structural features are significant factors governing their importance and wide distribution throughout nature and their utility in an impressive list of potential applications. Synthetic tetrapyrrolic compounds such as phthalocyanines **(1)** (Figure 1) have been proposed as convenient molecular models for the study of the physicochemical properties of naturally occurring tetrapyrrolic macrocycles including porphyrins due to their structural similarities. However, owing to their increased stability, improved spectroscopic characteristics, diverse coordination properties and architectural flexibility, phthalocyanines have surpassed porphyrins in a number of applications and their immense potential in diverse fields makes them one of the most highly studied macrocyclic and coordination compounds.[1-3]

Ever since their serendipitous discovery[4-6] and identification[7-13] in the early 1900s, phthalocyanines have been extensively used as dyes and pigments in the paint, printing, textile and paper industries due to their extremely intense blue-green color, high dyeing power, photostability, insolubility in most solvents and chemical inertness.[11-16] Copper phthalocyanine is the single largest synthetic colorant produced today.[15] In addition, phthalocyanines are known catalysts for numerous chemical reactions.[2,20,21] In

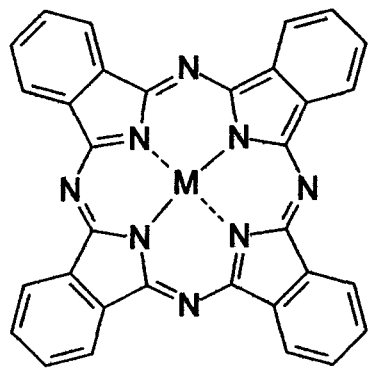


Figure 1. General phthalocyanine macrocycle.

fact, Pc are the only tetrapyrrolic compound used as an industrial catalyst with cobalt phthalocyanine derivatives used in the Merox process for oxidation of sulphur compounds in gasoline fractions.[20,22-24] More recently, phthalocyanines have found high tech applications in electrophotography and ink jet printing[18,19] and as photoconducting agents in photocopying devices.[3,25] In addition, the importance and potential of phthalocyanines is rapidly growing in many other fields. These include chemical sensors[26-29], electrochromism[30,31], molecular metals[32,33], liquid crystals[21,29,34], Langmuir-Blodgett films[21,35], functional polymers[36,37], semiconductors[27,28], photosensitizers for photodynamic therapy[38-42] and non-linear optical applications[43-45]. The potential to adapt to such a wide range of applications originates with their singular chemical structure, high degree of aromaticity, unique electronic spectra and the flexibility involved in the synthesis of phthalocyanines. Diverse applications such as those proposed for phthalocyanines require compounds with distinct and well-defined physical, chemical and electronic properties in some cases. This necessitates synthetic methods with control of regioselectivity and with access to assorted types of substituents. Current synthetic approaches often come up lacking and as such new methods are needed in order for phthalocyanines to fulfill their promise.

A notorious disadvantage of phthalocyanines is the extreme insolubility of their unsubstituted derivatives. This can be primarily traced to the extreme hydrophobicity of the aromatic core and planarity of the phthalocyanine, which leads to a tendency to stack upon themselves and results in highly stable crystal structures with high molecular lattice energies. The solubility of unsubstituted phthalocyanines in the more universal organic solvents like sulpholane, dimethyl sulphoxide (DMSO), tetrahydrofuran (THF) and N,N-

dimethylformamide (DMF) is negligible[1]. Even highly aromatic solvents such as quinoline and 1-chloronaphthalene rarely give solutions of concentrations exceeding 10^{-5} M. The only effective solvent is sulphuric acid at concentrations greater than 8 M. However, this induces solubility via protonation of the aza nitrogens, thus modifying the properties of the macrocycle and severely limiting the usefulness of these solutions. For instance, protonation of the aza nitrogens causes a strong bathochromic shift of up to 80-120 nm of the Q band.[1]

While the use of phthalocyanines as pigments relies on their insolubility to ensure fixation to material and durability against light, heat and chemicals to avoid fading, most Pc applications require solubility in water or a common organic solvent. In order to induce solubility, a number of functional groups have been added to the Pc framework via attachment to the benzene rings on the periphery of these macrocycles. The physical, chemical and electronic properties of phthalocyanines can also be fine-tuned via the addition of appropriate substituents and functional groups to the molecule. Simple functional groups such as alkyl chains and higher order aromatics to ethers, amines, thiols, halides and various acid groups have been used to improve the overall properties of phthalocyanines. More exotic substituents including crown ethers, dendrimers, ferrocenes and tetrathiafulvalenes lend other characteristics to the macrocycle that increase their utility. Polynuclear phthalocyanine systems have also been prepared with novel organic materials, new chemical catalysts and high temperature polymers in mind. Ether, amine, thiol and new carbon-carbon bonds have been used among countless other possibilities in order to add a variety of substituents to the Pc framework. Overall, this

rich chemistry is the driving force behind the tremendous versatility of phthalocyanines and their value in such a wide array of fields.

Substitution onto a phthalocyanine can essentially be accomplished by one of two basic methods. The first involves the direct substitution onto a pre-existing phthalocyanine. An example of this is the sulphonation of phthalocyanines,[46] which can be accomplished by heating a phthalocyanine macrocycle in oleum (concentrated sulphuric acid containing 20-30% free SO_3). While direction substition is the preferred method for adding functionality to Pc for the colorant industry,[18,19] the harsh reaction conditionsgenerally employed result in complex isomeric mixtures and varying degrees of substitution, with substituents added at any or all of the sixteen available positions on the phthalocyanine (Figure 2). Obviously, the resulting phthalocyanine mixture lacks a distinct structure and isolation and purification of the desired product is extremely difficult. This greatly limits the utility of this methodology in applications calling for well-defined phthalocyanines such as those needed in more high-tech fields.

The second basic method involves condensation of substituted precursors. This leads to far cleaner reactions in terms of the degree of substitution, with, for example, monosubstituted precursors leading to tetrasubstituted phthalocyanines. Furthermore, the basic position of these substituents is known as, for instance, 4,5-disubstititued phthalonitriles condense to form 2,3,9,10,16,17,23,24-octasubstituted phthalocyanines (see Figure 2 for the numbering scheme used in phthalocyanine nomenclature). However, while the number of substituents and their relative position is known, this method still leads to constitutional isomers for unsymmetrically substituted precursors (Figure 3). This is primarily due to the symmetry involved in the condensation reaction

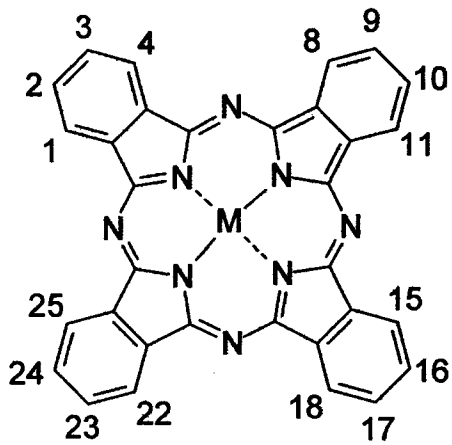


Figure 2. Potential sites for phthalocyanine substitution. Note that the numbering scheme used is that traditionally used for phthalocyanine nomenclature.

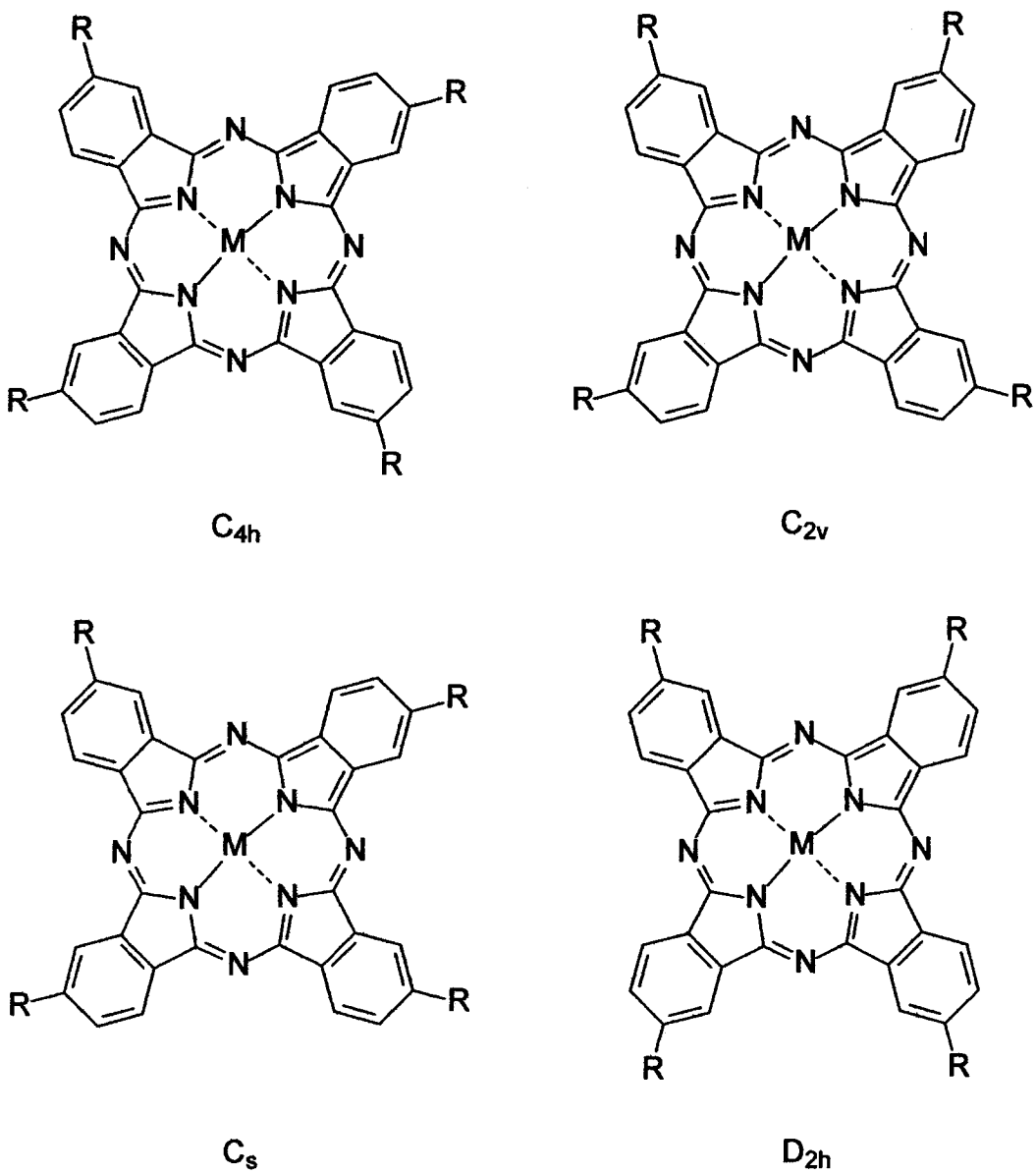


Figure 3. The four constitutional isomers possible for a tetrasubstituted phthalocyanine. The geometry indicated is for the phthalocyanine macrocycle itself with overall molecular geometry depending on the nature of the substituent.

used in phthalocyanine synthesis. While it is theoretically possible to separate these isomers due to their differing geometries, it has only been accomplished for very specific phthalocyanines using specially designed HPLC columns.[47-50] Often, even in these cases, the best results possible give only enriched isomeric fractions. While an isomeric mixture is usually suitable for most applications, high tech fields such as non-linear optics require distinct molecular geometries. This has led to research on new synthetic protocols and specially designed phthalocyanine precursors for the preparation of single isomers. Nevertheless, while this method has clear drawbacks, it is still the highly preferred method for adding substituents to phthalocyanines and as such, the synthesis of substituted precursors is vital in the preparation of new phthalocyanine derivatives with improved properties and designed chemical structures.

Further complicating the syntheses of phthalocyanines are mixed condensations where phthalocyanines substituted with different functional groups are desired. This is the case for several applications. Langmuir-Blodgett films require different substituents in order to achieve the molecular orientations necessary to ensure that transferred films have similar orientations.[26,51] In addition, more amphiphilic phthalocyanines bearing both hydrophobic and hydrophilic moieties have been shown to be more potent as photosensitizers for photodynamic therapy.[42] Generally, unsymmetrically substituted phthalocyanines are synthesized by a statistical condensation of appropriately substituted precursors followed by chromatographic isolation of the desired products. Although this method, via trial and error, can lead to reaction mixtures enriched with the product with the desired substitution pattern, it still leads to six differently substituted phthalocyanines (Figure 4), not including constitutional isomers when they are possible. Column and

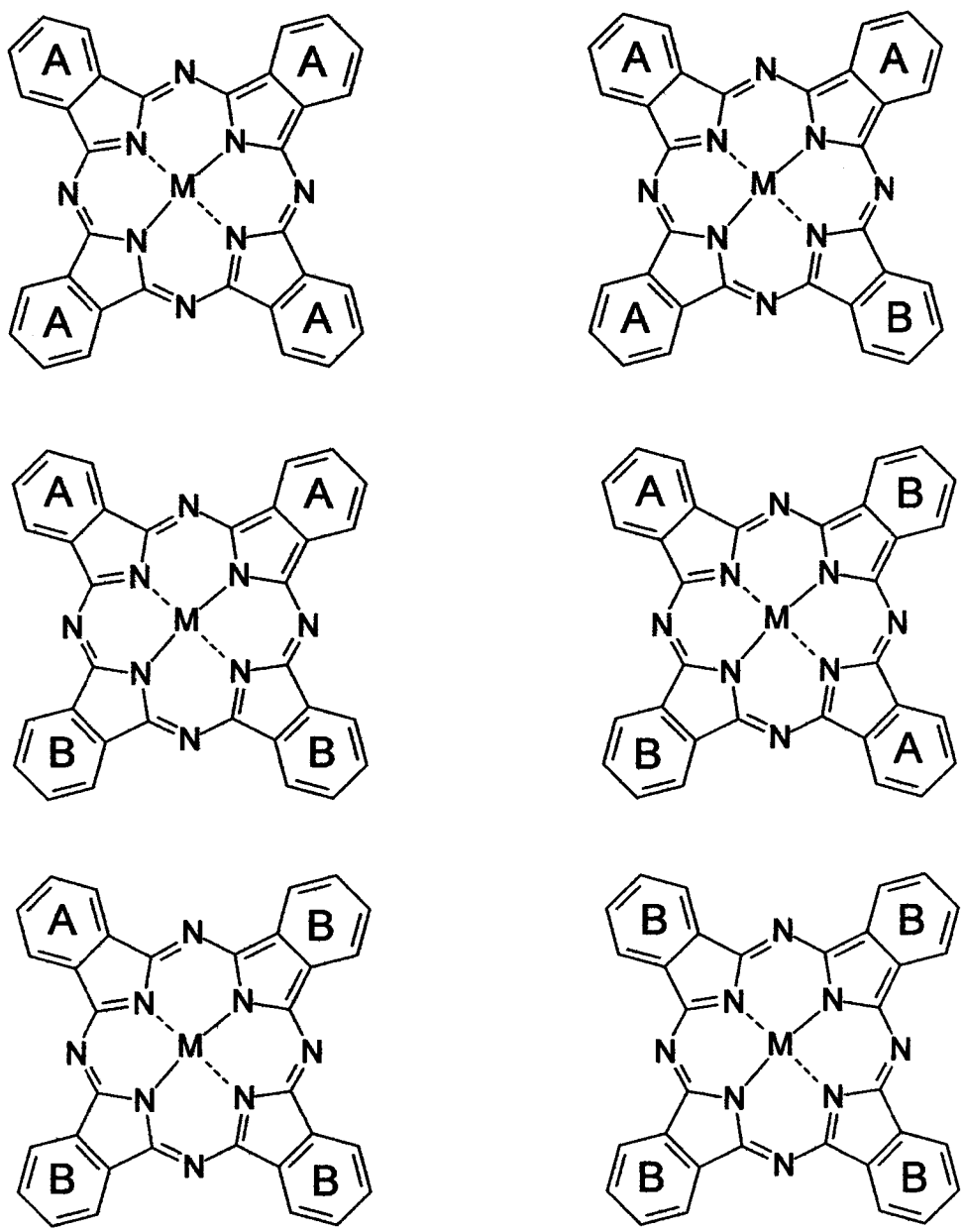


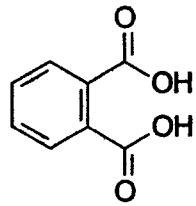
Figure 4. The six differently substituted phthalocyanine possible from a mixed condensation reaction of two differently substituted precursors. A and B represent two differently substituted isoindoline units.

HPLC chromatography can be used to isolate the desired substituted product in most cases. However, this is often very tedious and difficult and can lead to contamination with differently substituted phthalocyanines. As such, new synthetic procedures would be advantageous in order to synthesize phthalocyanines with exact composition and of pure isomeric distribution and a number of novel synthetic approaches have been designed to accomplish this. Among these is the use of axial ligands on the central metal ion of the phthalocyanine complex to impart the desired physical and chemical properties to the molecule while leaving the periphery unsubstituted, thus eliminating the problem of isomers. Among the more promising phthalocyanines for photodynamic therapy is Pc4, an unsubstituted silicon phthalocyanine with an alkylsilyl axial ligand bearing a terminal amine and this compound entered clinical trials in 2001.[42] Attempts have also been made to break the symmetry inherently found in the synthesis of phthalocyanines by using designed phthalocyanine precursors.[52-56] Such precursors seek to force the condensation reaction to occur in one direction, thus producing exclusively a single isomer or substitution pattern. In a similar fashion, polymer-supported precursors[57-60] and boron subphthalocyanines[61-65] have been investigated as starting materials for the synthesis of 3:1 unsymmetrically substituted phthalocyanines with varying success. While these methodologies have displayed some promise in specific cases, further research remains to be done in this area of phthalocyanine synthesis. Clearly, however, the preparation of new phthalocyanine precursors and the addition of novel functional groups to these molecules remains an important goal.

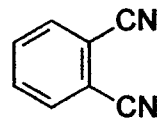
II. Phthalocyanine Precursors

Unlike metalloporphyrins, phthalocyanine complexes are seldom obtained from an available phthalocyanine ligand. More often, the complex is formed from precursors via a metal-templated cyclotetramerization reaction. As was mentioned previously, the addition of substituents to phthalocyanines in order to improve their properties is also far easier to control using appropriately substituted starting materials rather than adding them to a pre-existing macrocycle. Moreover, free phthalocyanine ligands (or metal-free phthalocyanines) tend to be prepared via demetalation of labile alkali and alkali earth metal complexes, which themselves are formed by the reaction of phthalonitriles and other phthalocyanine starting materials with metal alcoholates.[1,66] The mechanism of this condensation reaction has been extensively examined and probably involves a stepwise polymerization of phthalocyanine precursors or reactive intermediates followed by coordination of the central metal ion and ring closure to the macrocycle.[1,66-70] Ring closure is driven not only by the template effect of the metal ion and the inherent stabilization this coordination implies but also by the thermodynamic stabilization and added aromaticity involved in formation of the phthalocyanine macrocycle. The aromatic character of the phthalocyanine system is clearly demonstrated by its magnetic anisotropy, which is 15 times larger than that for benzene.[71]

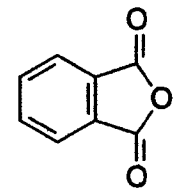
Phthalocyanines can be prepared from aromatic ortho-dicarboxylic acid derivatives and these include phthalic acids **(2)**, phthalonitriles **(3)**, phthalic anhydrides **(4)**, phthalimides **(5)**, diiminoisoindolines **(6)** and *o*-cyanobenzamides **(7)** (Figure 5). Ortho-substitution is a definite prerequisite. The carboxylic acid or related functional group may not be separated from the aromatic system by a saturated atom or by extended



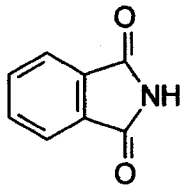
Phthalic acid (2)



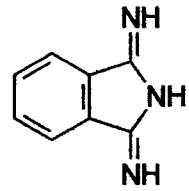
Phthalonitrile (3)



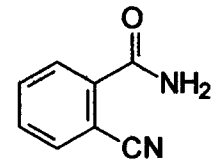
Phthalic anhydride (4)



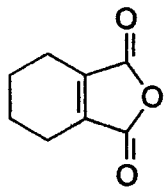
Phthalimide (5)



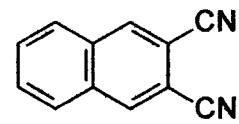
Diiminoisoindoline (6)



o-Cyanobenzamide (7)

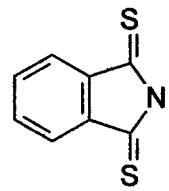


Cyclohex-1-ene-
1,2-dicarboxylic anhydride (8)

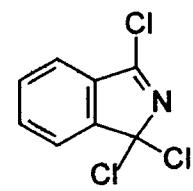


2,3-Naphthalenedicarbonitrile (9)

Iminothioamide (10)



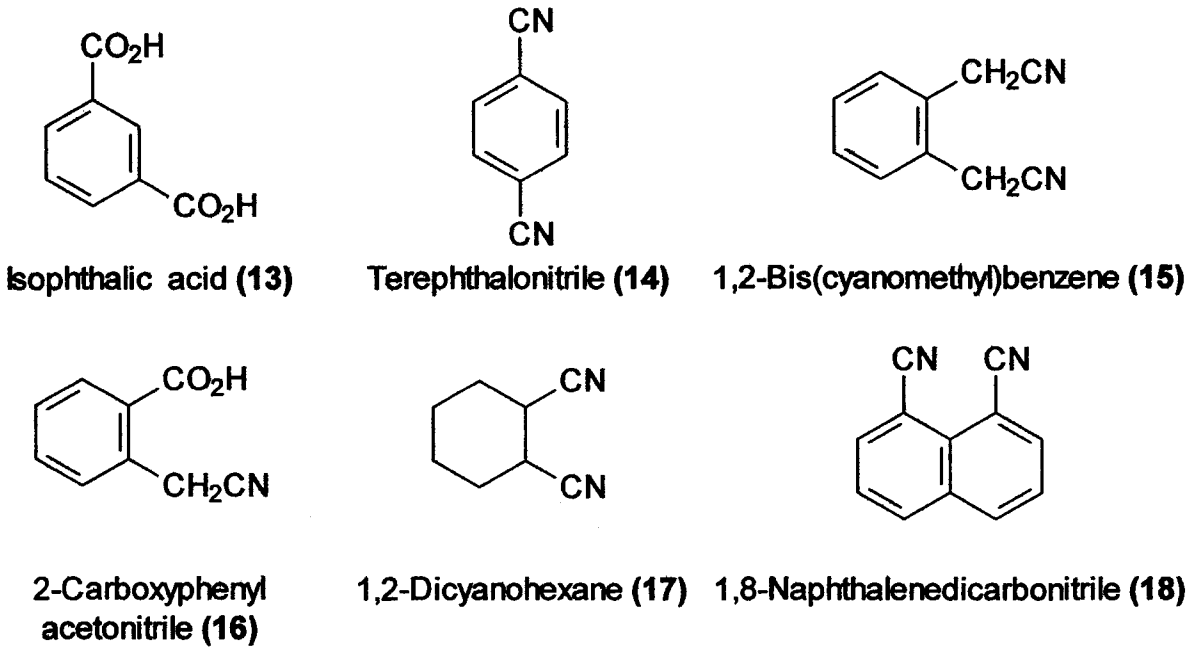
Dithioimide (11)



1,3,3-Trichloroisoindoline (12)

Figure 5. Basic phthalocyanine precursors.

unsaturation. There also must be a double bond between the atoms carrying these functional groups or there must be the possibility for a rearrangement to form such a double bond during the condensation reaction. As such, compounds such as isophthalic acid **(13)**, terephthalonitrile **(14)**, 1,2-bis(cyanomethyl)benzene **(15)**, 2-carboxyphenylacetonitrile **(16)** and 1,2-dicyanohexane**(17)** fail to give complex formation (Figure 6). On the other hand, compounds like 1-cyclohexene-1,2-dicarboxylic anhydride **(8)** do yield tetracyclohexenetetraazaporphyrins, which can be dehydrated by sublimation at 300-320 °C, heating with sulphur, boiling with palladium in chloronapthalene or treating with DDQ to yield the phthalocyanine.[72,73] In addition to the phthalic acid derivatives already mentioned, *o*-halobenzonitrile and *o*-dihalobenzenes also give phthalocyanines when heated in the presence of cuprous cyanide, probably via the *in situ* generation of phthalonitrile. Phthalocyanine-like complexes can be synthesized with extended aromatic systems as well and starting materials for such macrocycles including 2,3-naphthalenedicarbonitriles **(9)**. However, the same rules apply as 1,8-naphthalenedicarbonitriles **(18)** fail to give a Pc macrocycle under condensation reaction conditions.[74] Finally, a number of specially designed precursors, based on phthalimide, have been prepared. Iminothioamides **(10)**, dithioamides **(11)** and 1,3,3-trichloroisoindolines **(12)** attempt to control the cyclotetramerization reaction by altering the geometry of the cyclotetramerization reaction.[52-56] Nevertheless, these more sophisticated starting materials are still based on *o*-dicarboxylic acid derivatives. Clearly, the nature and structure of phthalocyanine precursors is quite restricted, substantially limiting modification of the core phthalocyanine structure although aza derivatives and a few non-Hückel systems have been prepared. Notwithstanding this, the comparative ease



Isophthalic acid **(13)**

Terephthalonitrile **(14)**

1,2-Bis(cyanomethyl)benzene **(15)**

2-Carboxyphenyl acetonitrile **(16)**

1,2-Dicyanohexane **(17)**

1,8-Naphthalenedicarbonitrile **(18)**

Figure 6. Examples of compounds with molecular structures incompatible with the phthalocyanine cyclotetramerization reaction.

with which phthalocyanines undergo substitution on their four benzene rings and the synthetic possibilities involved in adding substituents to phthalonitriles and other phthalocyanine precursors lend incredible structural flexibility to phthalocyanines. Despite the restrictive prerequisites implicated in phthalocyanine synthesis, a rich and varied chemistry for the preparation of appropriate precursors and addition of interesting and beneficial functional groups and chemical moieties has been developed.

III. Phthalonitriles

The most useful of the phthalocyanine precursors are phthalonitriles **(3)**.These compounds readily yield phthalocyanine complexes in good yields with most metals except silver and mercury.[1] Reactions often involve simply heating the phthalonitrile in the presence of a metal ion source as either a melt of reagents or in a suitable high boiling solvent. Conversely, syntheses using precursors like phthalic anhydrides **(4)**and phthalimides **(5)** require the presence of a nitrogen source such as urea and a catalyst such as ammonium molybdate or boric acid in order to get phthalocyanine macrocycle formation. Moreover, phthalimides **(5)** and some of the other phthalic acid derivatives give far more erratic results. These factors greatly limit the usefulness of these compounds as precursors, especially by restricting the nature of the functional groups that can be present during the condensation reaction. However, phthalic anhydrides **(4)** are used extensively for the large scale production of phthalocyanine by industry mainly due to the inexpensive cost of such starting materials.[11-16] Phthalonitriles **(3)**, on the other hand, lead to higher purity and since phthalonitriles are generally more expensive, their use tends to be restricted to high-technological applications and small scale syntheses

where quality and not cost are the main considerations. Beyond this, phthalonitriles are generally the precursor of choice for most phthalocyanine syntheses as these compounds can be readily prepared via a number of synthetic pathways and their condensation reaction generally proceeds more smoothly and with improved yields as compared to other *o*-phthalic acid derivatives.

As previously described, phthalonitriles are transformed into phthalocyanines via a metal template assisted cyclotetramerization reaction. In general, these reactions are carried out at elevated temperatures in either a melt of reagents or in a high boiling solvent such as chlorobenzene, quinoline, nitrobenzene or 1-chloronaphthalene. Phthalocyanine macrocycle formation can also be accomplished in refluxing 1-pentanol or another similar alcohol in the presence of an organic base such as DBU, piperidine or cyclohexylamine.[66,75,76] The presence of the strong organic base permits the reaction to proceed under more mild reaction conditions by acting as an electron acceptor, thus promoting formation of the alcoholate. This in turn adds to the cyano groups of the dinitrile with formation of an alkoxyisoindoline, which rapidly cyclizes to the Pc. While not strictly necessary, urea and ammonium molybdate are added on occasion to the reaction to help promote cyclization.[14-16] Metal-free phthalocyanine can also be prepared from phthalonitriles via the reaction with hydrogen in dioxane or with ammonia in 2-N,N-dimethylaminoethanol.[66,76] Compounds of molybdenum, titanium and iron are known to catalyze the cyclotetramerization reaction, greatly decreasing reaction times and reaction temperatures in certain cases.[16]

Phthalonitriles readily react with various metal sources including metals themselves along with their salts, oxides, sulphates and halides to form

metallophthalocyanine complexes. Such metal complexes have been reported for most metals with the most obvious exceptions being silver and mercury. Note that metal halides can give halogenated phthalocyanine contaminants via an *in situ* halogenation of the aromatic system and are thus generally avoided. Reactions involving phthalonitriles are generally much cleaner than those using other phthalocyanine precursors and give the best yields, typically in the range of 30-50% and occasionally as high as 90%. However, cyclotetramerization reactions involving phthalonitriles have been shown to be somewhat sensitive to solvent, temperature and metal ion source.[1] Still, they tend to be the preferred starting material for phthalocyanine synthesis and as such, most research encompassing phthalocyanines and their preparation has involved phthalonitrile starting materials. Numerous methods have been developed for their preparation and functionalization. Below is a brief resume of each of the main synthetic methods used to synthesize phthalonitriles along with an extensive list of substituents that have been added to these molecules and the protocols used for their addition. While far from complete, these dicussions clearly demonstrate the wide array of potential functional groups that have been added to phthalocyanines and hopefully will act as a guide for phthalocyanine chemist.

A) Ammonolysis/Dehydration of Phthalic Acid Derivatives

Phthalonitriles can be synthesized from the other phthalic acid derivatives by a stepwise progression from the dicarboxylic acid through to the anhydride, imide and diamide and finally to the desired phthalonitrile. Overall, the reaction pathway is a reductive ammonolysis and involves ammonolysis of the dicarboxylic acid followed by

dehydration of the resulting diamide to give the corresponding nitrile. An excellent example of this was employed in the synthesis of 4,5-dichlorophthalonitrile **(23)** from the inexpensive commercially available 1,2-dichloro-3,4-benzenedicarboxylic acid **(19)** (Figure 7).[77]

Phthalic acids **(2)** readily form the corresponding anhydrides **(4)** by refluxing in acetic anhydride with yields usually being nearly quantitative.[77-79] This dehydration reaction to form anhydrides can be accomplished using a number of other dehydrating agents such as methoxyacetylene, dicyclohexylcarbodiimide (DCC) or diphosphorus pentoxide.[80,81] However, reaction rates and yields are sufficient using acetic anhydride in the case of phthalic acids. The conditions used are also very mild and the resulting phthalimide precipitates from solution upon cooling, leading to a simple workup. Of course, ring closure to the cyclic anhydride drives the reaction towards anhydride formation[81] and leads to the excellent yields and high purity obtained in these reactions.

Transformation of the phthalic anhydride **(4)** to the phthalimide **(5)** is usually accomplished by refluxing in formamide. Formamide partially decomposes into carbon monoxide and ammonia beginning at 180°C[82] and therefore acts as both the solvent and the source of ammonia for this reaction. Urea acts in a very similar fashion, decomposing to ammonia, biuret and cyanuric acid above its melting point of 132.7°C[82] and as such, has been used in the conversion of phthalic anhydrides to phthalimides as well. However, the byproducts of this reaction are somewhat harder to eliminate. An example of a reaction where urea is used is in the synthesis of 4-t-butyl and 4-trimethylsilylphthalimide **(30)** (Figure 8).[78-79] Other nitrogen sources that have been employed include ammonia gas and ammonium hydroxide, carbonate or cyanate,

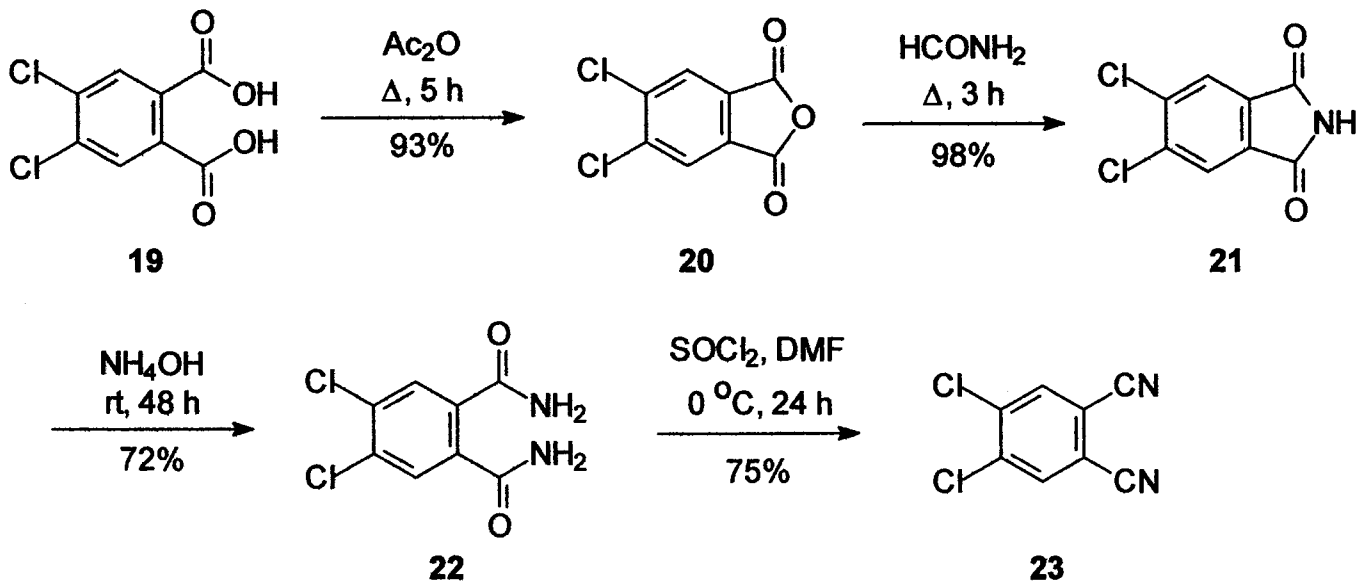


Figure 7. Synthesis of 4,5-dichlorophthalonitrile from its phthalic acid derivative[77]

although most of these utilize extremely high reaction temperatures and harsh reaction conditions and often lead to unsatisfactory yields.[81,83]

The basic reaction mechanism here entails a two step procedure, the first being a nucleophilic addition of the ammonia to one of the carbonyl carbons with subsequent opening of the cyclic anhydride to give an *o*-carboxybenzamide or phthalamic acid **(32)**. The second slower step is a second nucleophilic attack by the amide nitrogen on the carboxylic acid carbon, with loss of water and resulting in the ring closure to the phthalimide.[84,85] In the case of unsubstituted phthalic anhydride, at lower temperatures, the reaction is known to proceed via ammonium phthalamate **(31)**, which upon acidification gives phthalamic acid **(32)** (Figure 9).[85,86] However, these compounds readily transform into the analogous phthalimide upon heating and under the elevated reaction temperatures used with refluxing formamide, the phthalimide is formed directly.

As is the case for the phthalic anhydrides, the resulting phthalimide usually precipitates from solution upon cooling and after washing to remove the formamide solvent, is generally sufficiently pure to be used in the next step. It should be noted that it is possible to obtain the imide directly from the dicarboxylic acid. One method of accomplishing this is via the monoammonium salt formed by neutralizing the phthalic acid with ammonium hydroxide. Following concentration to give the crystalline salt, pyrolysis is induced by heating, giving the final phthalimide product. Such a methodology has been used to prepare 3- and 4-nitrophthalimide **(40)**[87] from the corresponding phthalic acids with yields of 68-82%, which is highly satisfactory considering the number of steps that are avoided.

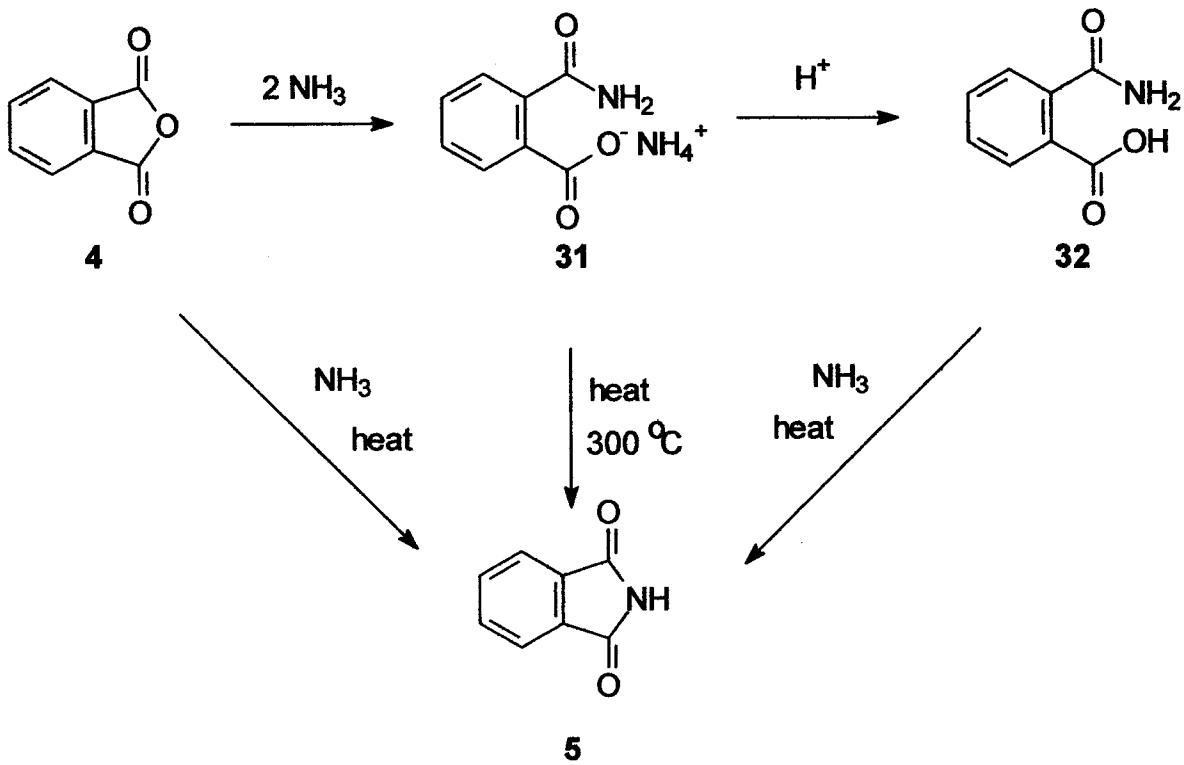


Figure 9. Synthesis of phthalimide from phthalic anhydride passing via ammonium phthalamate and phthalamic acid.[86]

Transformation of the phthalimide to the corresponding phthalamide can be achieved by reacting in concentrated aqueous ammonium hydroxide or ammonium carbonate. The reaction temperature depends on the phthalimide involved and these reactions are at times prolonged. In the case of 4,5-dichlorophthalimide **(21)**, the reaction is carried out over 48 hours at room temperature with fresh addition of ammonium hydroxide after 24 hours (Figure 7).[77] 4-t-Butylphthalimide requires 24 hours at room temperature.[78] On the other hand, 4,5-diiodophthalimide **(33)** reacts at 50-60°C with concentrated aqueous ammonia with a 81% yield of the resulting phthalamide **(36)** being acquired after only 1.5 hours (Figure 10).[88,89] Interestingly, despite ammonium hydroxide being a potential reagent for both the preparation of phthalimides from phthalic anhydrides and phthalamides from phthalimides, the two reactions involved are completely different. The synthesis of phthalamide requires aqueous ammonium hydroxide and is accomplished at temperatures ranging from 20-60 °C. Phthalimide, on the other hand, encompasses the removal of aqueous solvent and extremely elevated temperatures, occasionally as high as 290 °C.

An extremely interesting variation of the classic protocol has been used during the preparation of 4-nitrophthalamide **(41)** from 4-nitrophthalimide **(40)** (Figure 11). It utilizes THF as the solvent and induces the reaction by adding excess concentrated ammonium hydroxide and bubbling ammonia gas through the reaction at only 40 °C for 2 hours.[54] Even with these much milder conditions, yields are comparable to the tradition conditions employing simple aqueous ammonia. Other potential methods for synthesizing these *ortho*-diamides often give disappointing results, with, for instance, the reaction of phthaloyl chloride with ammonia leading to only the monoamide.[87] There

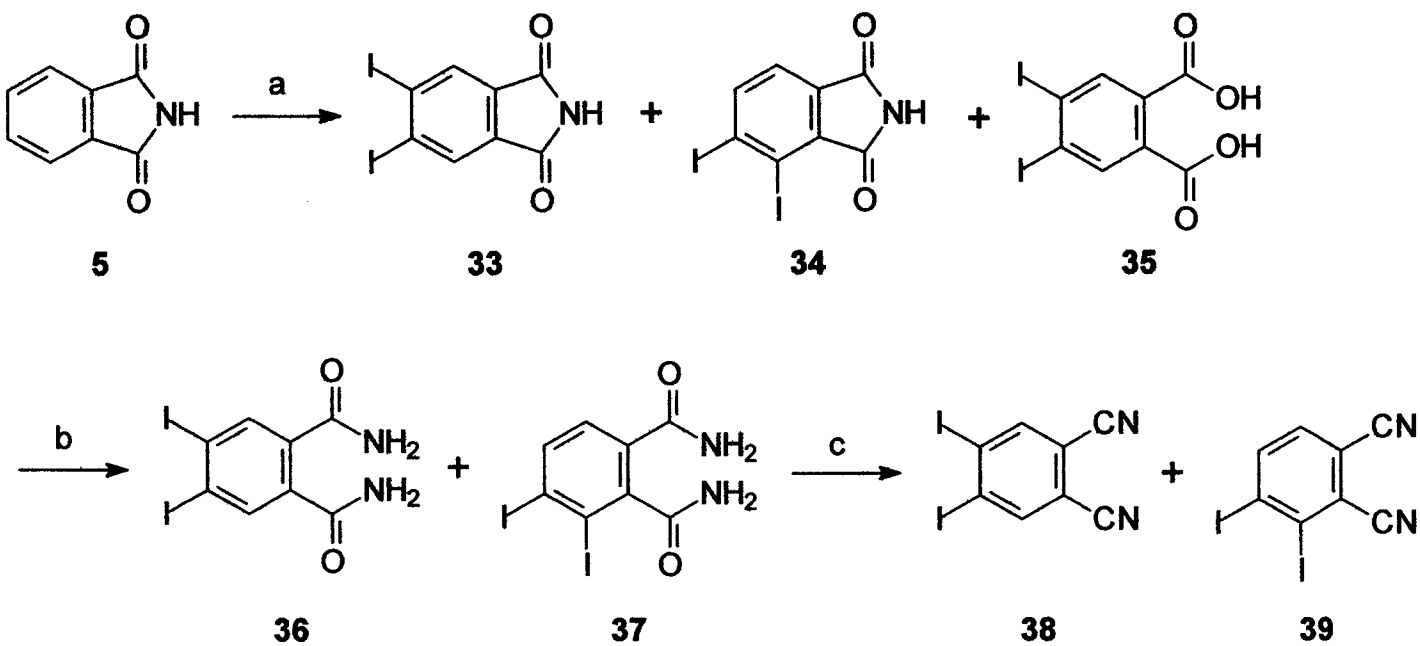


Figure 10. Synthesis of 4,5-diiodophthalonitrile. a) I_2, 30% fuming H_2SO_4, 75-80 °C, 24 h; b) NH_4OH, 50-60 °C, 1,5 h; c) $(CF_3CO)_2O$, pyridine, dioxane, 0-5 °C, 24 h.[88]

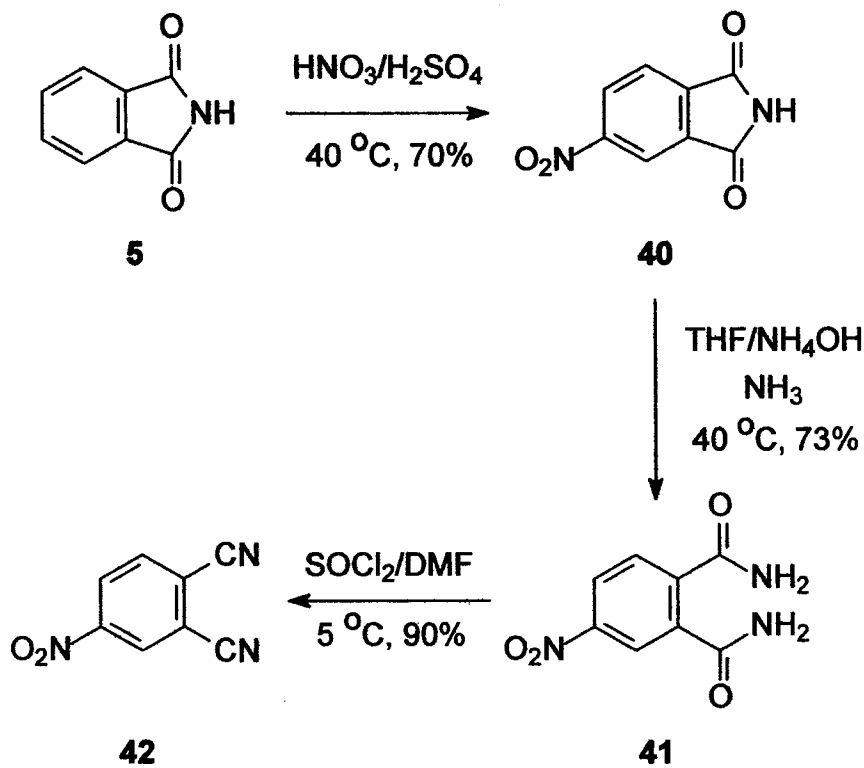


Figure 11. Synthesis of 4-nitrophthalonitrile from phthalimide.[54]

also appears to be steric effects involved as tetrabromo- and tetraiodophthalimide fail to give the corresponding phthalamide under traditional conditions while the tetrachlorinated compounds gives only small yields of the desired product (personal observation). Altogether, while the earlier steps in the overall reaction scheme towards phthalonitrile synthesis from phthalic acids often give near quantitative yields, the synthesis of phthalamides tends to produce roughly 80% yields although higher yields are obtained in some cases.[78,90] Note that after initially dissolving, these reactions tend to become very thick and effective and vigorous stirring is often needed to ensure satisfactory results.

The most delicate of the reactions involved in this method of synthesizing phthalonitriles is the final dehydration. While the dehydration of amides to the corresponding nitriles are most commonly carried out with phosphorus pentoxide, a wide range of dehydrating agents can been employed.[81,91] In terms of phthalonitrile synthesis, a number of different protocols have been used in order to accomplish this reaction. Among the more popular are trifluoroacetic anhydride in dry dioxane/pyridine (Figure 10)[88,89,92,93] and thionyl chloride in DMF (Figure 7).[54,77,94] For thionyl chloride in DMF, it is important to first prepare the highly reactive dimethylformiminium chloride (Vilsmeier reagent)[95,96] via the dropwise addition of the thionyl chloride in either DMF or a solution of DMF in another appropriate solvent at 0°C prior to adding the phthalamide (Figure 12).[81,97] Oxalyl chloride can be used to form the reactive Vilsmeier reagent as well. As an example, oxalyl chloride and DMF were reacted in acetonitrile at 0°C prior to addition of 4,5-bis(benzyloxyethoxy)phthalamide and gave excellent yields of the corresponding phthalonitrile.[90] It has been observed that the Vilsmeier reagent

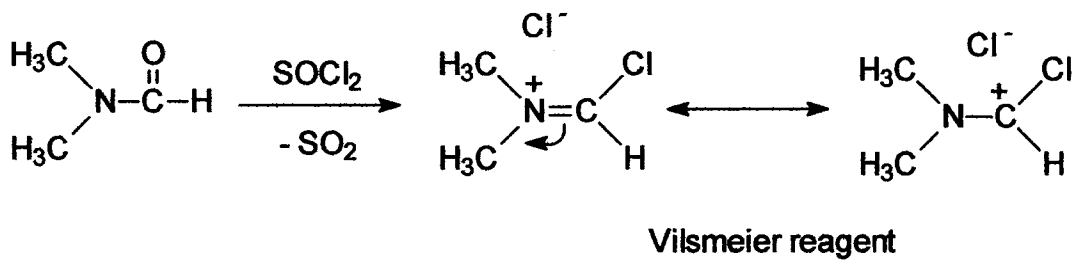


Figure 12. Preparation of Vilsmeier reagent (Dimethylformiminium chloride).[81,95-97]

tends to give the purest dinitriles in the highest yields.[54] Other dehydrating conditions that have been used include phosphorus oxychloride in pyridine (Figure 8)[78-79] and acetic anhydride either neat or in chlorobenzene.[87,93,98,99] In the case of the synthesis of pyromellitonitrile (1,2,4,5-tetracyanobenzene), harsher reaction conditions were necessary in order to get complete dehydration, calling for the addition of thionyl chloride to a stirred solution of pyromellitamide in DMF at 60°C and a six hour reaction.[100] Note that when this reaction is carried out at slightly higher temperatures in an excess of thionyl chloride, 4,5-dicyanophthalimide was obtained. Using phosphorus pentachloride, phosphorus pentoxide and benzenesulphonyl chloride as the dehydrating agent yielded only starting material in this case. In general, however, yields for dehydration reactions of phthalamides tend to be very good, often in the 70-90% range although earlier reactions using acetic anhydride gave much inferior results. During the synthesis of 3-nitrophthalonitrile, the use of Vilsmeier reagent as the dehydrating agent leads to a 89% yield[94] while acetic anhydride in chlorobenzene only resulted in a 26% yield, although this yield was not optimized.[87] Similarly, phosphorus oxychloride proved to involve a laborious protocol with marginal yields for this example.[94,101] This clearly demonstrates that while this reaction can lead to excellent yields of the desired phthalonitrile, it depends greatly on the conditions used and it would appear that the Vilsmeier reagent is the better dehydrating agents to use for this reaction.

Mechanistically, this reaction may be formally looked at as a β-elimination from the enol form of the amide (RC(OH)=NH).[81,91] In some cases, the dehydrating agent forms an ester with the hydroxyl group, forcing the formation of the enol and inducing elimination by a E1 or E2 mechanism.[81,102] Whether this elimination proceeds via a

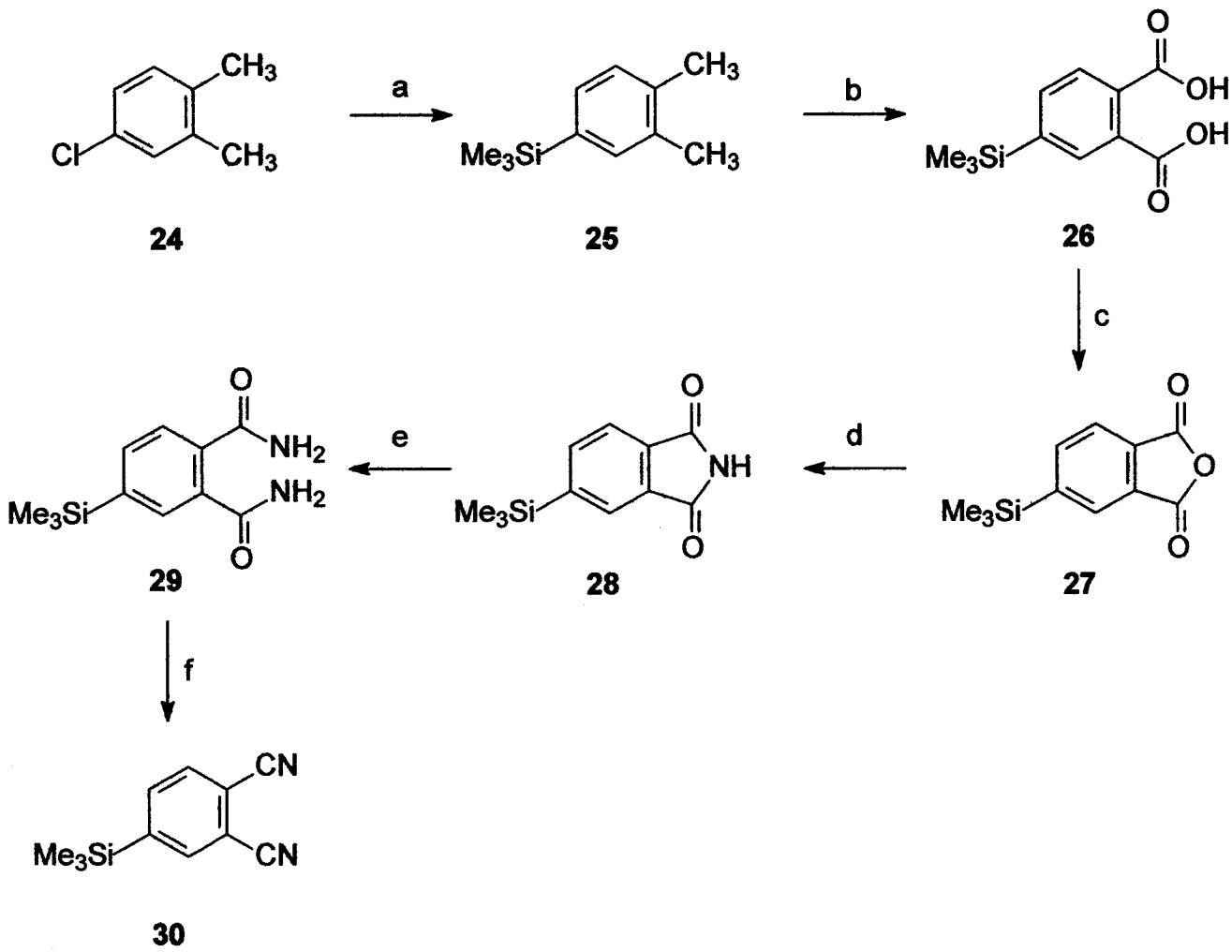


Figure 8. Synthesis of 4-trimethylsilylphthalonitrile. a) CH_3SiCl, sodium, benzene, EtOAc, 75%; b) $KMnO_4$, pyridine, 77%; c) Ac_2O, reflux, 99%; d) urea, 170 °C, 95%; e) NH_4OH, rt, 96%; f) $POCl_3$, pyridine, 5 °C, 86%.[78] Note that the corresponding 3-trimethylsilylphthalonitrile was prepared via the same pathway with similar yields at each step.

bimolecular E2 mechanism or via a carbocation (E1 mechanism) depends on the overall reaction conditions and has not been fully determined. However, evidence exists that would seem to point towards the mechanism depending on the dehydrating agent used.[102]

The transformation of phthalic acid derivatives into phthalonitriles is an extremely useful method for forming these valuable phthalocyanine precursors and has several important advantages. It involves reactions with readily available and inexpensive starting materials, be it the phthalic acid **(2)**, phthalic anhydride **(4)**, phthalimide **(5)** or phthalamide. In fact, this reaction pathway can be initiated from the corresponding *o*-xylenes as well since these compounds can be oxidized to the corresponding *o*-benzodicarboxylic acids (Figure 8). Trimethylsilyl- **(30)** and 3- and 4-t-butylphthalonitrile have been prepared from the corresponding *o*-xylenes in a multistep synthetic pathway where sodium permanganate-induced oxidation of the *o*-xylenes **(25)** giving rise to the phthalic acids **(26)**.[78,79] *o*-Phthalaldehydes can also be transformed into phthalonitriles by treating with hydroxylamine salts in the presence of base followed by reaction with acidic dehydrating agents.[103] Overall, such multi-step reaction pathways are not limited to phthalonitrile synthesis as aza derivatives including 4,5-dicyanopyridazine can be prepared following a similar ammonolysis/dehydration pathway.[104]

Several vitally important phthalonitriles are formed by this methodology. These include both 3- and 4-nitrophthalonitrile **(42)**, which are synthesized either following nitration of phthalimide with nitric acid in concentrated sulphuric acid (Figure 11)[54,93] or from commercially 3-nitrophthalic anhydride.[94] These two phthalonitriles are extremely valuable due to the reactivity of aromatic nitro groups towards nucleophilic displacement

and the potential for transformation into a diazonium salt. This truly makes these two compounds the parent for a large majority of monosubstituted phthalonitriles and as such, for tetrasubstituted phthalocyanines (see below). In addition, 4,5-dihalophthalonitriles are also prepared in this manner, with starting material derived from the iodination[88,89] or bromination[92] of phthalimide in 30% fuming sulphuric acid (Figure 10) or from commercially available 1,2-dichloro-4,5-benzenedicarboxylic acid **(19)** (Figure 7).[77] These starting materials are highly utilized in the preparation of 4,5-disubstituted phthalonitriles and are thus important in the synthesis of octasubstituted phthalocyanines. Another intriguing 4,5-disubstituted compound recently synthesized by this methodology is 4-bromo-5-nitrophthalonitrile **(47)** whose synthesis entails successive bromination of phthalic anhydride **(4)**, imidization of 4-bromophthalic acid **(43)**, nitration of 4-bromophthalimide **(44)**, amidization of 4-bromo-5-nitrophthalimide **(45)** and dehydration of 4-bromo-5-nitrophthalamide **(46)** (Figure 13).[105]

Despite the advantages of this overall synthetic scheme, it does have drawbacks as well. It is often a multi-step process and while each step gives adequate to excellent yields, overall yields are decreased due to the number of steps involved. For instance, despite excellent yields for each step in the pathway, the overall yield for the synthesis of 4,5-dichlorophthalonitrile **(23)** from 1,2-dichloro-4,5-benzenedicarboxylic acid **(19)** is only 49%.[77] Furthermore, the dehydration reaction to yield the final phthalonitrile involves rather harsh reaction conditions, limiting the functional groups that can be present during this step. As such, this restricts the nature of the phthalonitriles that can be formed using this procedure, although it is used to prepare phthalonitriles bearing some extremely versatile functional groups. Nevertheless, in general, this stepwise synthetic

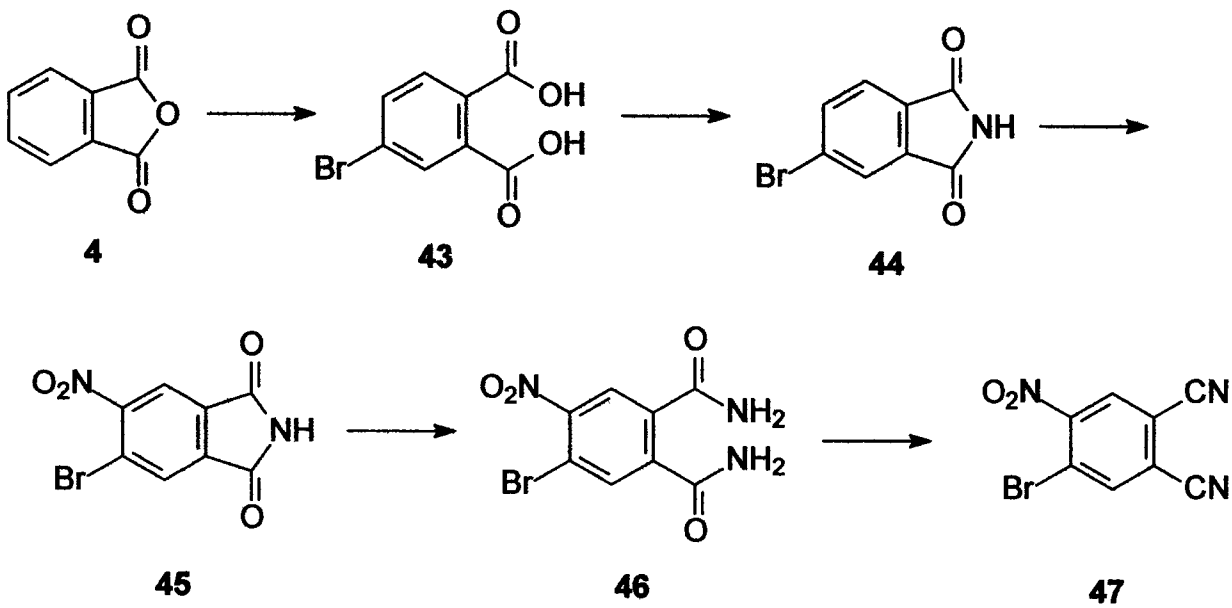


Figure 13. Synthesis of 4-bromo-5-nitrophthalonitrile.[105]

pathway for the preparation of phthalonitriles is very important for its ability to transform less effective phthalic acid derived starting materials into the preferred phthalonitrile precursor.

With phthalonitrile being a principal intermediate for phthalocyanine production by industry, a number of processes for the continuous catalytic production of phthalonitrile from phthalic acid derivatives have been developed.[16,17,106,107] While of little synthetic use as they tend to use extremely elevated temperature and harsh reaction conditions, they are of some interest. Examples include passing the vapor of one of the phthalic acid derivatives, together with ammonia, over a selected catalyst such as aluminum phosphate, silicate, arsenate or borate. Phthalonitrile has also been prepared industrially by catalytic dehydration of phthalimide or ammonium phthalate using a basic aluminum phosphate catalyst at 300-550°C. Phthalamides have also be dehydrated under pressure with an acid halide, an acylated secondary amide and if necessary, a tertiary base and a solvent. Thus, a sample protocol that has been used employs phosgene, thionyl chloride or phosphorus trichloride and N-ethyl-formanilide. A final example passes ammonia at 340°C through molten phthalic anhydride. The resulting vapor is then heated to 400-430°C and led over a bauxite catalyst. Following rapid cooling, phthalonitrile is obtained in 91-92% yields with a overall purity of roughly 90%. Due to the exceptional amount of phthalocyanine product by industry, with the production of phthalocyanine-based dyes and pigments exceeding 80 000 tons per year,[37] attempts to upgrade these reactions, especially in terms of improved catalytic activity and decreased cost, are constantly underway.[108] Furthermore, in addition to the traditional phthalic acid derivatives, methods for the ammoxidation of *o*-xylene have been and continue to be

developed.[109-112] In one such method, tetrachlorophthalonitrile, an important precursor for other tetrasubstituted phthalonitriles, has been prepared by oxidative ammonolysis of tetrachloro-*o*-xylene.[113] The synthetic transformation of phthalic acid derivatives is thus not only a valuable methodology for the detailed synthesis of important phthalonitriles but also for the industrial preparation of important dye and pigment precursors as well.

As an interesting aside, it has been observed that phthalonitrile can also be synthesized from 1,4-dichlorophthalazine and 1-chloro-4-alkoxyphthalazine **(48)** by a two electron reduction (Figure 14).[114] This two electron reduction involves the transfer of two electrons, the cleavage of three σ bonds (two C-Cl bonds and an N-N bond) and the formation of two new π bonds. All stages of this reaction are rapid. Polarography indicates that the resulting phthalonitrile can then undergo a one electron reduction to the phthalonitrile radical anion **(49)**, which can then itself undergo a two electron reduction in the presence of a hydrogen donor to the benzonitrile radical anion **(50)**. Interestingly, while 1,4-dichlorophthalazine gives a 5-electron reduction wave, the 1-chloro-4-alkoxyphthalazine **(48)** only gives a 3-electron wave. This is because these compounds are reduced at the same potential range as phthalonitrile.

B) Rosenmund-von Braun reaction

A highly popular method of synthesizing phthalonitriles is by a cyano-dehalogenation reaction known as the Rosenmund-von Braun reaction.[115] In this method, aryl halides are converted into the corresponding aryl nitriles using cuprous cyanide. Other cyanides such as KCN or NaCN do not react with aryl halides, even activated ones, in the same manner. However, alkali cyanides do covert aryl halides into nitriles in the

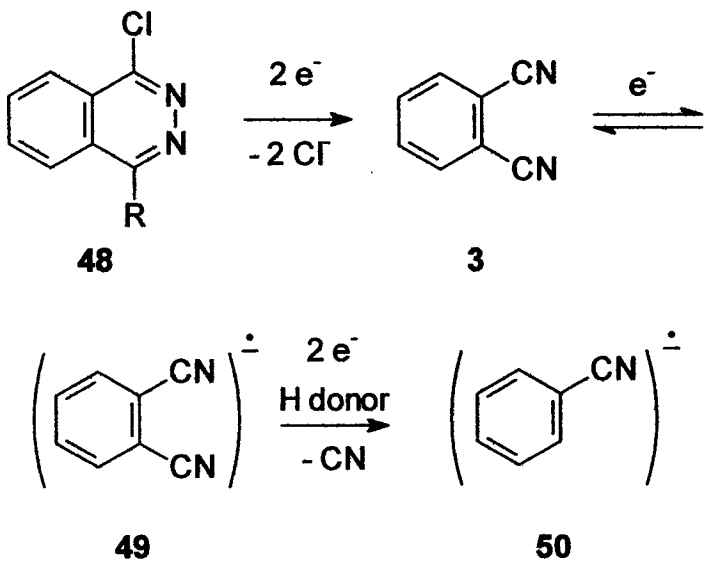


Figure 14. Reduction of 1,4-dichloro- and 1-chloro-4-alkoxyphthalazine. R = OMe, OPh.[114]

presence of certain nickel, cobalt and palladium complexes.[81,115-119] To date, this transition metal-catalyzed synthesis of aryl nitriles has not been fully exploited in the synthesis of phthalonitriles, with only rare examples present in the literature. One potential drawback is that an *o*-cyano or *o*-halogen group greatly reduces the reactivity of aryl halides towards this transition metal catalyzed reaction.[115] One literature example of this transition metal-catalyzed procedure utilizes NaCN and tris(triphenylphosphine)nickel as reagents for preparing phthalonitrile from 1,2-dichlorobenzene. While the identical conditions gave the 1,3 and 1,4-dicyanobenzene in excellent yields, minimal amounts of phthalonitrile were obtained by this method.[120] Likewise, while 5,6-diphenylpyrazine-2,3-dicarbonitrile can be synthesized using $Pd(Ph_3P)$ and KCN in a 16% yield, the 2,6-derivative is obtained in a 68% yield.[121] As such, the traditionally used cuprous cyanide and Rosenmund-von Braun conditions have been employed almost exclusively in preparation of *o*-dinitriles from aryl halides. However, cases do exist of the successful use of other cyanodehalogenation reactions. One example is the synthesis of 2,3,6,7,10,11-hexacyanotriphenylene.[122] In this case, the hexanitrile was prepared using KCN and $(Ph_3P)_4Pd$ in the presence of the dibenzo-18-crown-6. More traditional Rosenmund-von Braun conditions gave only partial exchange of the halogens by cyanide anions. With results such as these, transition-metal assisted cyanodehalogenation reactions may still become important in the synthesis of phthalonitriles as new catalytic systems are developed.[123]

While the Rosenmund-von Braun reaction has been extensively utilized and studied ever since it was first described in 1927,[115,124] the mechanism behind this cyanodehalogenation using CuCN is not fully understood. It is known that reactivity

varies in the order I > Br > Cl > F, which would seem to rule out a SNAr mechanism.[81] The reaction also has unusual kinetics, with a second order rate constant that increases as the reaction progresses and tends to become constant later on.[125,126] It would appear that the reaction with CuCN involves either an electron-transfer process or a π-complex, which is formed between the copper (I) cyanide and the aryl halide.[115,126] In the electron-transfer process, there is formation of a complex such as ArCu(X)CN that leads to the momentary formation of an arene radical or arene radical ion. These radical species would not be able to escape the immediate vicinity of the reacting species as radical traps do not affect this reaction.[126] The second possibility requires the formation of an 18-electron π-complexed organocuprate **(52)** (Figure 15). Intermolecular attack of the cyanide ion on the aryl halide carbon would give a tetrahedral intermediate **(53)** and is followed by the rate-determining elimination of the halide and rapid formation of the final product **(55)**.[126] Whether the cyanation of aryl halides occurs via electron-transfer, π-electron complexation or a mixture of the two in the synthesis of phthalonitriles has still not been resolved in spite of significant research.

While the Rosenmund-von Braun reaction has previously been achieved in the absence of solvent or in a basic solvent such as quinoline or pyridine, studies have indicated that DMF is superior as the reaction medium.[127] Clearly, this is the case for the synthesis of phthalonitriles as it is used also exclusively as the reaction solvent. Cases do exist however where pyridine is added as a catalyst.[128-130] This catalytic activity is a result of complex formation between the cuprous cyanide and pyridine, which facilitates the interaction of the CuCN with the aryl halide.[131] Reaction conditions used for phthalonitrile synthesis using Rosenmund-von Braun conditions are extremely uniform,

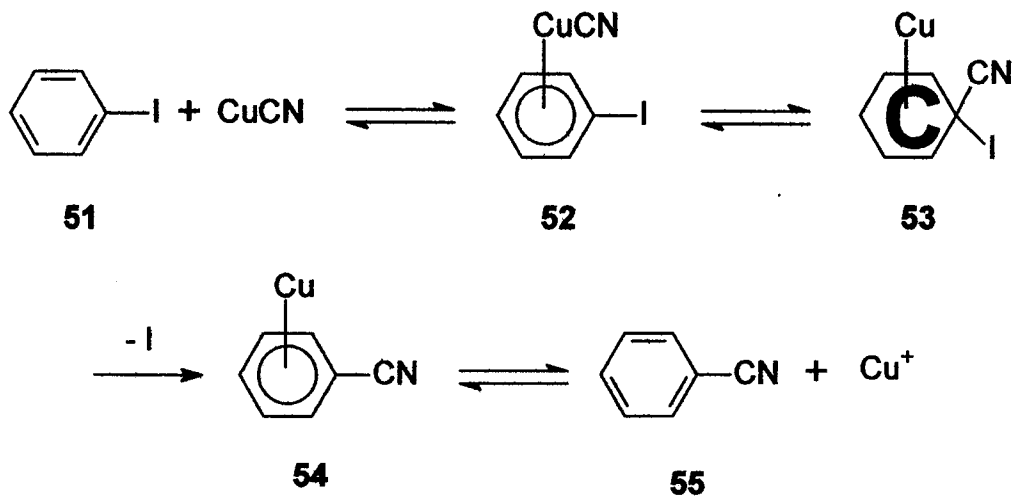


Figure 15. The proposed π complex mechanism for the Rosenmund-von Braun reaction.[115,126]

with the reaction most commonly being carried out at reflux or near reflux, though slightly lower temperatures have been used on occasion. One inconvenience experienced during the experimental protocol of Rosenmund-von Braun reactions is that the resulting nitriles are known to form complexes with the cuprous halide byproducts. One of the main advantages of using DMF as a solvent is that such complexes remain in solution.[127] These complexes must be decomposed in order to obtain the desired nitrile and a number of methods have been developed in order to accomplish this.[115] In most cases, phthalonitriles are obtained from the cuprous halide complexes by treating with concentrated ammonium hydroxide, often concurrent with bubbling of air or oxygen.[115] A second method that has been shown to be very effective involves treating with ferric chloride and hydrochloric acid, which oxidizes the cuprous ion and frees the nitrile.[127] This methodology has been used successively in order to obtain certain phthalonitriles from their cuprous halide complexes.[132-134] In some cases, the complexes appear to be slightly unstable and the desired phthalonitriles can be obtained by extraction with dichloromethane.[135-136] Overall, aryl dihalides have been converted into phthalonitriles in the presence of numerous functional groups including alkyl, alkoxy, alkylthio, hydroxy, acyl, formyl, carboxy, carboxy ester, nitro and amines. However, the reactivity of the aryl halide and the resulting yield of the Rosenmund-von Braun reaction are pronouncedly affected by the substituents present during the reaction.[137] For instance, 5,6-dibromobenzimidazole **(60)** cannot be converted to the 5,6-dicyanobenzimidazole using the Rosenmund-von Braun reaction conditions unless the imidazole nitrogen is alkylated **(62→63)**.[138] The unalkylated form **(60)** leads to the mono exchange product exclusively **(61)** (Figure 16). Attempts using solvents such as DMSO or HMPA only

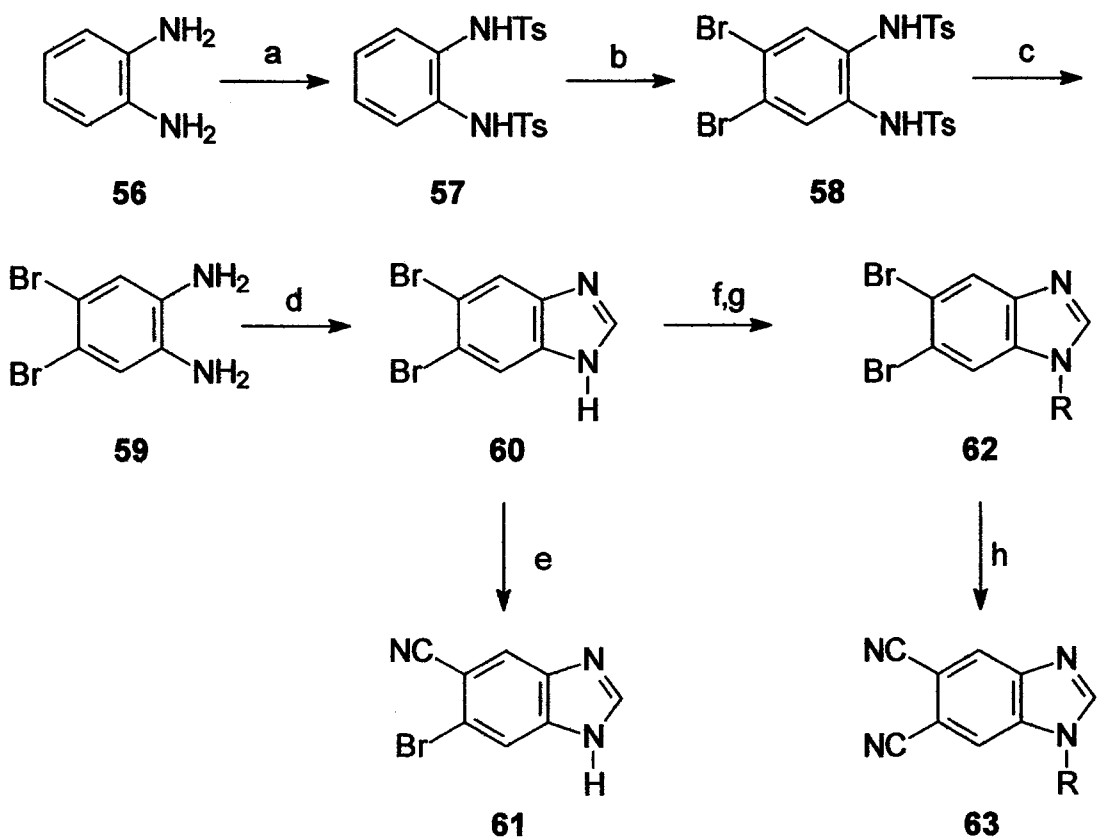


Figure 16. Synthesis of 1-alkyl-5,6-dicyanobenzimidazoles. a) TsCl, pyridine, 95%; b) Br_2, NaOAc, 95%; c) H_2SO_4, 90%; d) HCO_2H, 80%; e) CuCN, DMF, Δ, 70%; f) KOH, TBAB; g) RX, 68-98%; h) CuCN, DMF, Δ, 57-59%.[138]

yielded starting material. Note however that unalkylated 5,6-dicyanobenzimidazole has been synthesized starting from 4,5-diaminophthalonitrile.[139]

It is readily apparent that the synthesis of phthalonitriles necessitates the corresponding 1,2-dihalo compounds. While it is known that aryl iodides react 40-100 times more readily than aryl bromides,[115,126] phthalonitrile synthesis is generally accomplished using the corresponding aryl bromides and the general ease of their synthesis. This is primarily due to the ready availability of brominated precursors. Nevertheless, it was observed during the synthesis of 4-triphenylmethylphthalonitrile that the diiodinated precursor reacted much more effectively under Rosenmund-von Braun conditions than the corresponding 1-bromo-2-iodo-4-triphenylmethylbenzene.[140] 3,4,5-Trimethylphthalonitrile has also been prepared from the aryl iodide using CuCN in hexamethylphosphorous triamide (HMPT).[141] Other aryl dihalides other than bromides and iodides have also been used in order to add the necessary functionality to the molecule. A case in point being 4,5-didodecylphthalonitrile, which has been synthesized by palladium-catalyzed addition of dodecylmagnesium bromide to 1,2-dichlorobenzene followed by bromination to the dibromide and Rosenmund-von Braun cyanodehalogenation to the dinitrile.[142]

While a large number of 1,2-dibromobenzene derivatives have been transformed into phthalonitriles using Rosenmund-von Braun conditions, a few take on special significance due to the large number of substituted phthalonitriles derived from them. 1,2-Dibromo-4,5-di(bromomethyl)benzene **(65)** is prepared from *o*-xylene by a two step reaction (Figure 15). Bromination of the aromatic core in the 4,5 position is accomplished using bromine in the presence of iron and iodine.[143-146] This is followed by

free radical bromination of the methyl groups using NBS and a radical chain initiator to give the desired brominated product.[135,146] 1,2-Dibromo-4,5-di(bromomethyl)benzene **(65)** undergoes various reactions on the benzylic bromomethyl groups with ether[135,146-148] and amine bond formation[129,149-151] predominating. Following cyanodehalogenation by the Rosenmund-von Braun reaction, 4,5-disubstituted phthalonitriles are obtained **(67)** (Figure 17). Furthermore, 1,2-dibromo-4,5-dimethylbenzene **(64)** also undergoes the Rosenmund-von Braun reaction effectively[135,152-154] and the methyl groups can be brominated using NBS to yield 4,5-di(bromomethyl)phthalonitrile **(69)**.[153-155] This compound is a valuable starting material for other 4,5-disubstituted phthalonitriles including phthalonitriles appended to tetrathiafulvene **(71)**, glycoluril **(73)** and phosphonates **(74)** (Figure 18).[153,154,156] The particular advantage of performing the Rosenmund-von Braun reagent before the addition of the desired functional group is that it allows addition of more labile functional groups to the phthalonitrile molecule by avoiding their presence during the harsh cyanodehalogenation reaction.

A second valuable starting material is 4,5-dibromocatechol **(77)**, which is involved in the synthesis of 4,5-disubstituted phthalonitriles bearing ether and crown ether substituents. Functional group addition via ether bond formation has been accomplished both before and after bromination of the catechol aromatic ring (Figure 19). However, these 4,5-disubstituted 1,2-dibromobenzenes **(78)** are more often than not prepared from 4,5-dibromocatechol **(77)** in order to avoid bromination of the ether side chains.[157] This is despite potential contamination by tribrominated products that can be formed during the bromination of catechol using bromine in acetic acid.[157,158] Either way, the resulting compounds undergo the Rosenmund-von Braun reaction to give a large

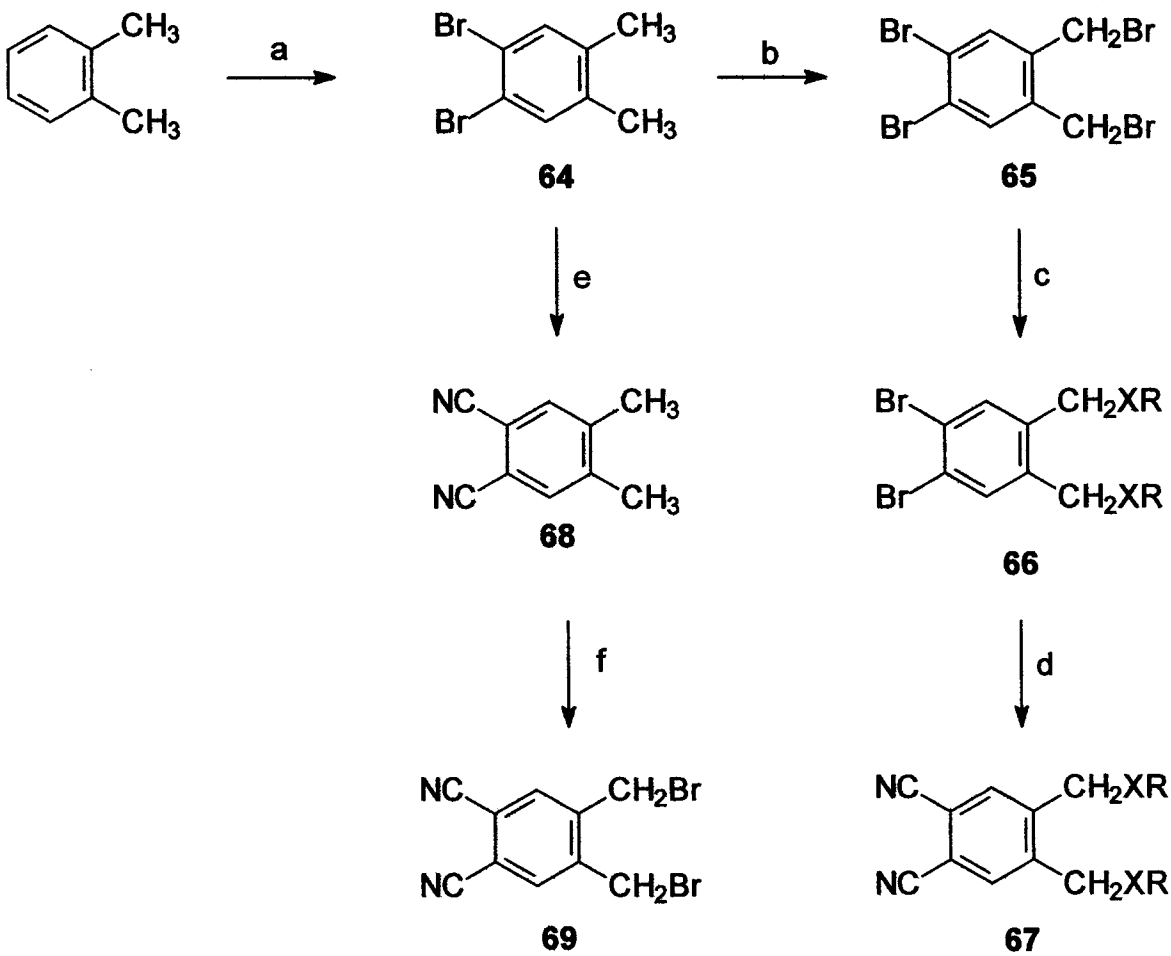


Figure 17. General synthesis of 4,5-disubstituted phthalonitriles from 1,2-dibromo-4,5-bis(bromomethyl)benzene (X = O or N). a) Br_2, I_2, Fe; b) NBS, CCl_4, radical chain initiator; c) RONa or RR'NH and K_2CO_2; d) CuCN, DMF; e) CuCN, DMF; f) NBS, CCl_4, radical chain initiator.

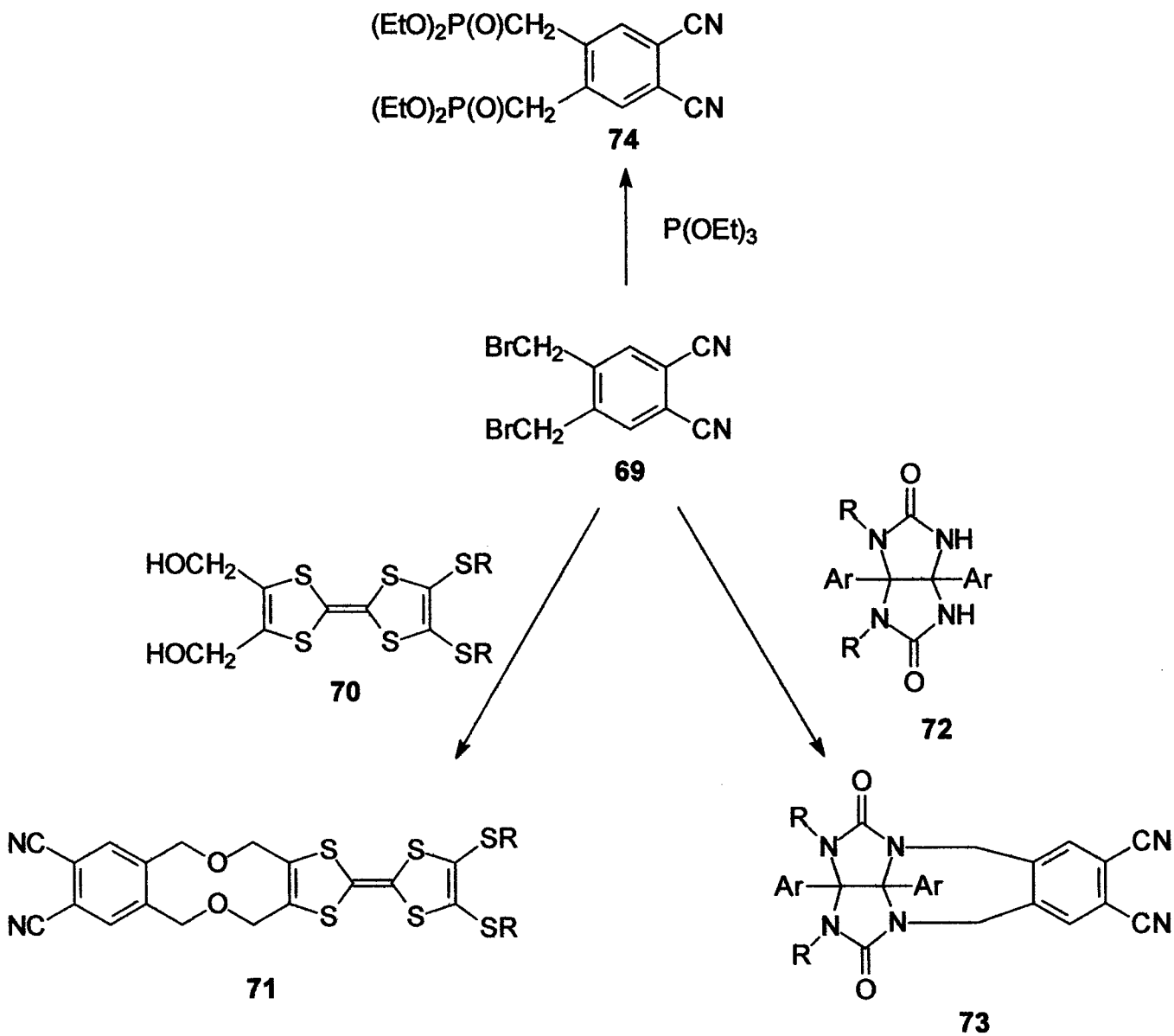


Figure 18. Examples of 4,5-disubstituted phthalonitriles prepared from 4,5-bis(bromomethyl)phthalonitrile.[153-156,]

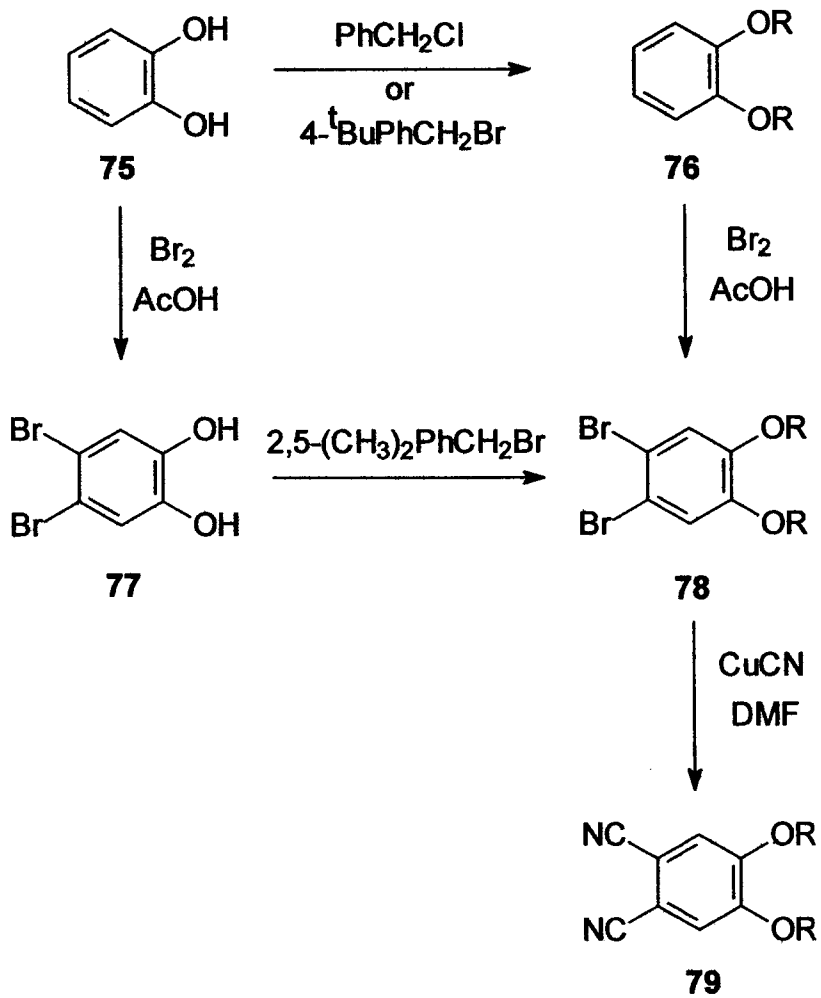


Figure 19. Synthesis of various benzyl protected 4,5-dihydroxyphthalonitriles.[157]

number of 4,5-diether-substituted phthalonitriles and phthalonitriles extended by crown ether substituents (Figure 19-21). Examples include protecting benzyl groups **(79)**,[157] simple alkyl and long chain ethers **(81)**[142,159,160] and polyethers,[161,162] alkyl ethers with terminal ester[134,163] and amide **(84)**[164-168] and various crown **(90,91)** and aza crown ethers.[128,130,169,170] 4,5-Dihydroxyphthalonitrile can also be synthesized via the Rosenmund-von Braun reaction using a suitably protected 4,5-dibromocatechol followed by deprotection.[169,171,172] The corresponding hydroxylated phthalocyanines can be prepared from protected 4,5-dicyanocatechols.[157] However, the electron withdrawing cyano groups greatly hinder alkylation of the phenolic hydroxy groups, limiting the potential of 4,5-dihydroxyphthalonitriles towards the synthesis of novelly substituted phthalonitriles.[169]

In addition to phthalonitriles, a number of substituted 2,3-naphthalenedicarbonitriles and other dinitriles with extended conjugation have been prepared using the Rosenmund-von Braun reaction (Figure 22). The conditions used are identical to those used for phthalonitriles in most cases. Naphthalonitriles substituted with 1,4-dialkyl **(99)**,[173] 5,8-dialkyl[174,175] and 6,7-diether groups[176] have been prepared in this manner along with others. In addition, synthetic procedures towards various 6,7-dicyano-1,4-diepoxynaphthalenes utilize the Rosenmund-von Braun reaction to introduce the nitrile functionlity into the molecule as well.[177,178] 1,2-Naphthalenedicarbonitrile **(102)** has been prepared from 2-amino-1-naphthalenesulphonic acid **(100)** using a modified procedure, with cyanation by a Sandmeyer reaction at the 2 position followed by cyanodesulphonation using potassium ferrocyanide (Figure 23).[179,180] A similar stepwise procedure, with a Sandmeyer reaction followed by a Rosenmund-von Braun

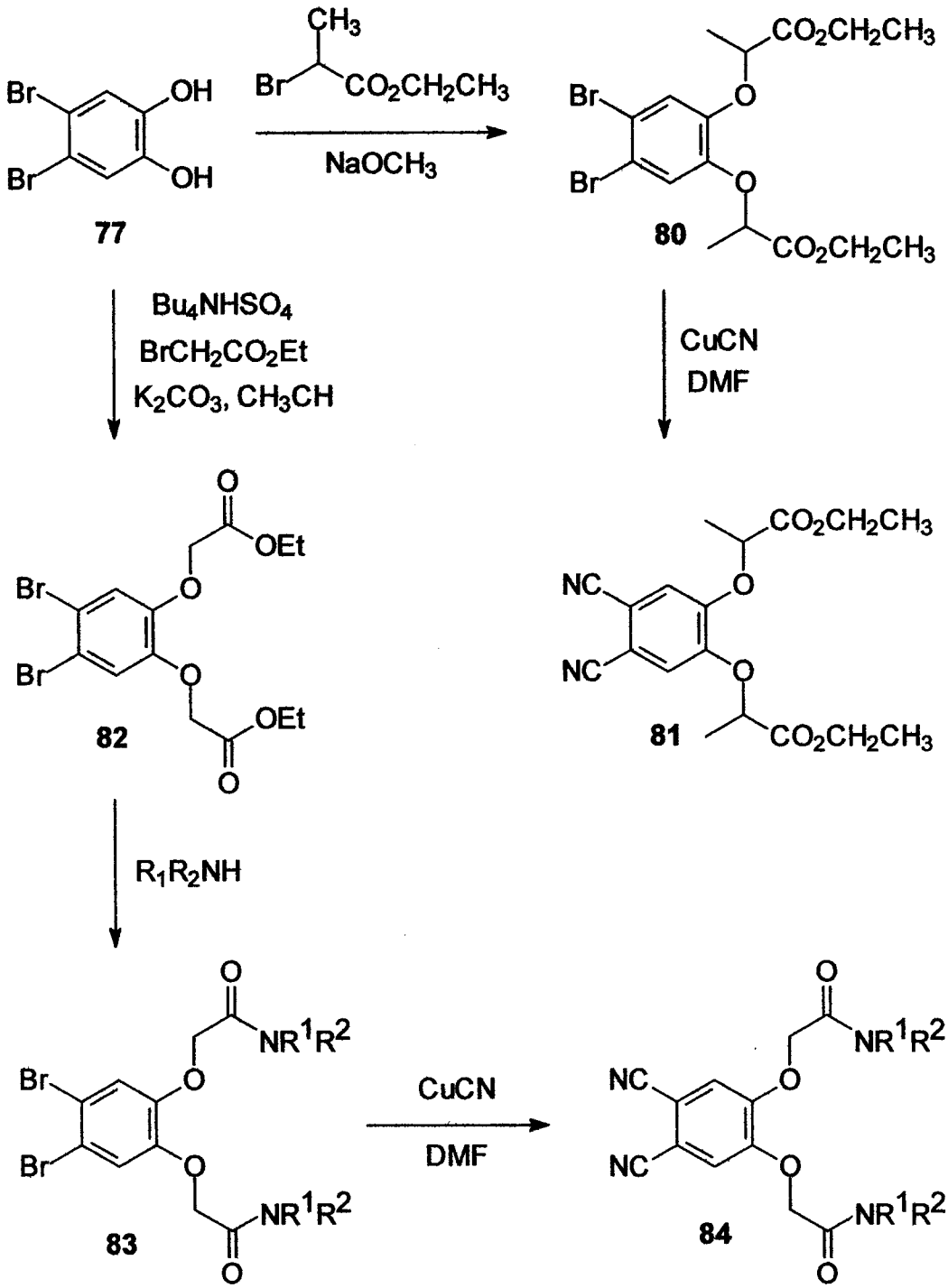


Figure 20. Examples of 4,5-disubstituted phthalonitriles synthesized from 4,5-dibromocatechol.[134,168]

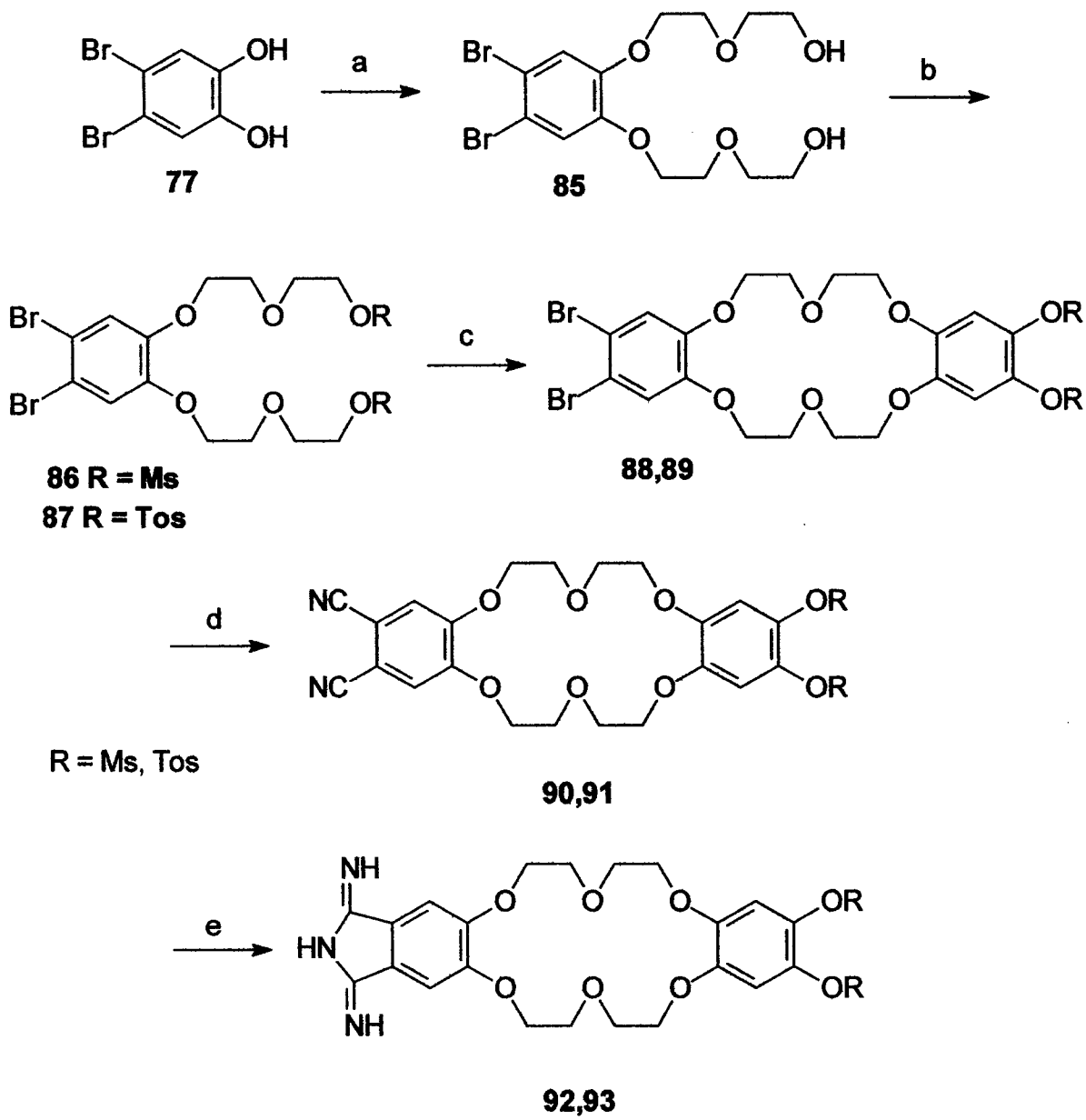


Figure 21. Synthesis of a crown-ether substituted phthalonitrile from 4,5-dibromocatechol. a) $THPO(CH_2)_2O(CH_2)_2Cl$, Δ, 16 h, 49%; b) TsCl, pyridine, -10 °C, 24 h, 93%; c) 1,2-bis(decoxy)-4,5-bis(acetoxy)benzene, NaOH, 1-butanol, 16 h, 58%; d) CuCN, DMF, Δ, 40 h, 75%; e) NH_3, NaOMe, MeOH, Δ, 30%.[130]

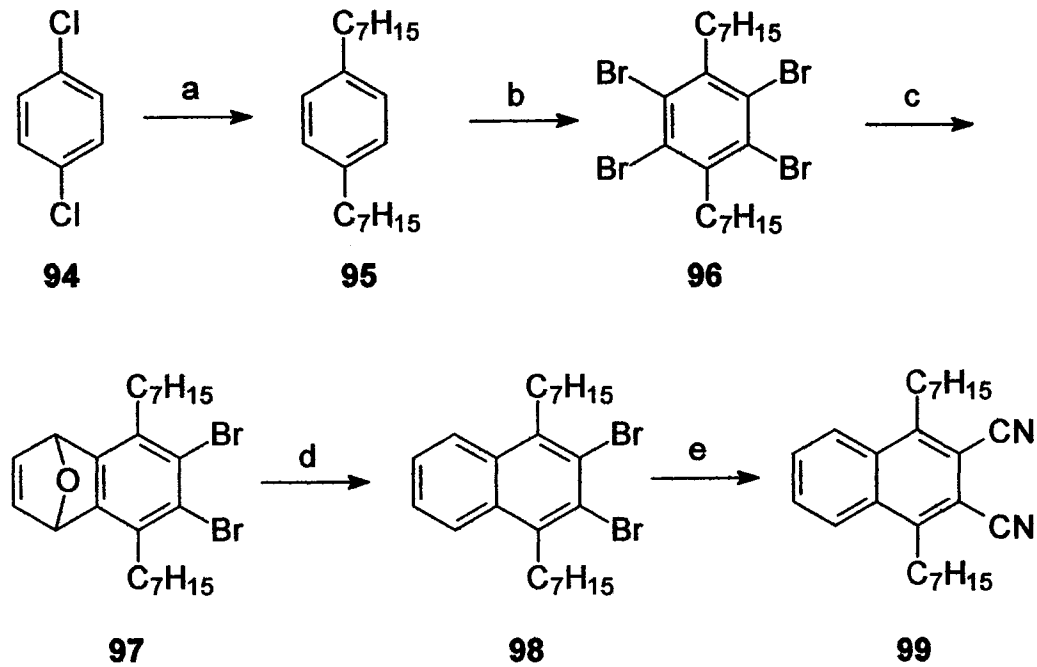


Figure 22. Synthesis of 1,4-diheptyl-2,3-naphthalenedicarbonitrile. a) $C_7H_{15}MgBr$; b) Br_2, I_2; c) furan, n-BuLi, toluene; d) Zn, $TiCl_4$, THF; e) CuCN, DMF.[173]

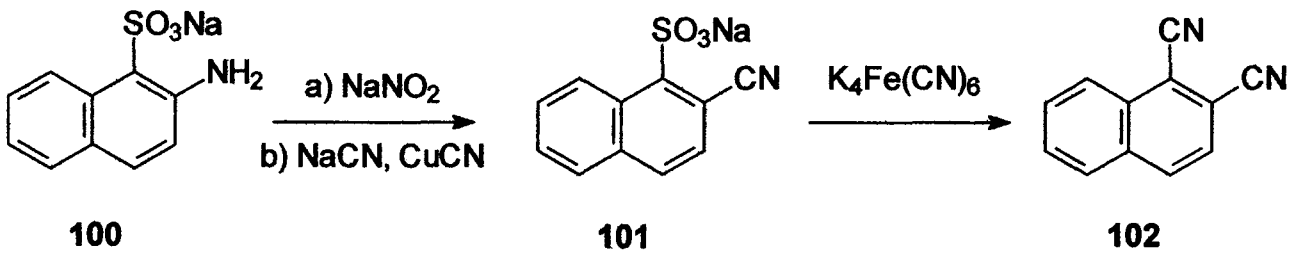


Figure 23. Synthesis of 1,2-naphthalenedicarbonitrile.[179,180]

cyanation, has been used to synthesize 3,5-di-t-butylphthalonitrile from 2-bromo-1-cyano-3,5-di-t-butylbenzene.[181] The synthesis of 2,3-naphthalenedicarbonitrile can proceed via the Sandmeyer reaction with transformation of commercially available 2-amino-3-naphthaloic acid into the corresponding imide.[179] This can then be turned into the dinitrile via amidization and dehydration. Somewhat interestingly, 2,3-dicyano-1,4-dihydroxyanthracene can be prepared in a moderate yield by treating 2,3-dichloroanthracene-1,4-dione with an excess of KCN in hot ethanol.[182,183] The more mild reaction conditions are the result of the adjacent carbonyl groups of this quinone derivative. 1,4-Dihydroxy-2,3-dicyanonaphthalene is synthesized taking advantage of this increased reactivity as well and this compound is an important starting material for 1,4-dialkoxynaphthalonitriles.[173]

The Rosenmund-von Braun reaction is a well-established method for preparing nitriles such as phthalonitriles from readily available and easily synthesized halogenated starting material. As can be seen in the examples mentioned above, it has been used successfully in the preparation of a rather wide-range of substituted phthalonitriles. However, this reaction is often less than satisfactory. The harsh reaction conditions and the oxidative workup prohibit the presence of a large number of functional groups. Furthermore, the elevated reaction temperatures and the use of cuprous cyanide, an obvious source of cuprous ions, often result in the production of the corresponding copper phthalocyanine as a byproduct.[90,93,] In fact, copper phthalocyanines can be directly synthesized from the 1,2-dibromo compounds by increasing the temperature of the reaction and changing solvents to tetramethylurea (TMU).[149-151,170] On occasion, product mixtures obtained using the Rosenmund von-Braun reaction are extremely

complex and are very difficult to purify.[90,131] This is the case for cyanodehalogenation of 4-nitro-1,2-dibromobenzene using traditional conditions.[93] Overall, yields for the Rosenmund-von Braun reaction, though often acceptable, can be very low and often depend greatly on the substituents on the starting material and can fail to give the desired product in some cases.[140] Finally, it is known that substrates containing more than one halogen usually react to give the polycarbonitrile, as is desired in the synthesis of phthalonitriles.[115,184] However, one halogen may remain unaffected or may even be lost.[115,185,186] This inability to drive the reaction to completion has been observed in the synthesis of several phthalonitriles.[122,138,154,185,187,188] Cyanodehalogenation of 2,3-dichloronitrobenzene using CuCN in refluxing DMF only gave 2-chloro-6 nitrobenzonitrile[187] while 1,2-dibromo-4,5-bis[4',5'-bis(hexylthio)tetrathiafulvalen-4-yl-methoxymethyl]benzene **(104)** gave a roughly 50:50 mixture of the mono- and dinitrile compounds **(105,106)** (Figure 24).[154] Such incomplete reactions are highly undesirable and isolation of the dinitrile from the mononitrile is very difficult. Still, despite these important problems, the Rosenmund-von Braun reaction is a highly successful and eminently used methodology for the preparation of phthalonitriles.

C) Palladium-catalyzed cyanation of aryl triflates

While the Rosenmund-von Braun reaction has been used successfully in the synthesis of a large number of phthalonitriles, in particular 4,5-disubstituted derivatives, its drawbacks have lead to the development of other procedures for procuring the ortho dinitrile functionality. A recently developed technique involves the transition metal-catalyzed cyanation of aryl triflates and aryl nonaflates.[122,134,189] Although halides are

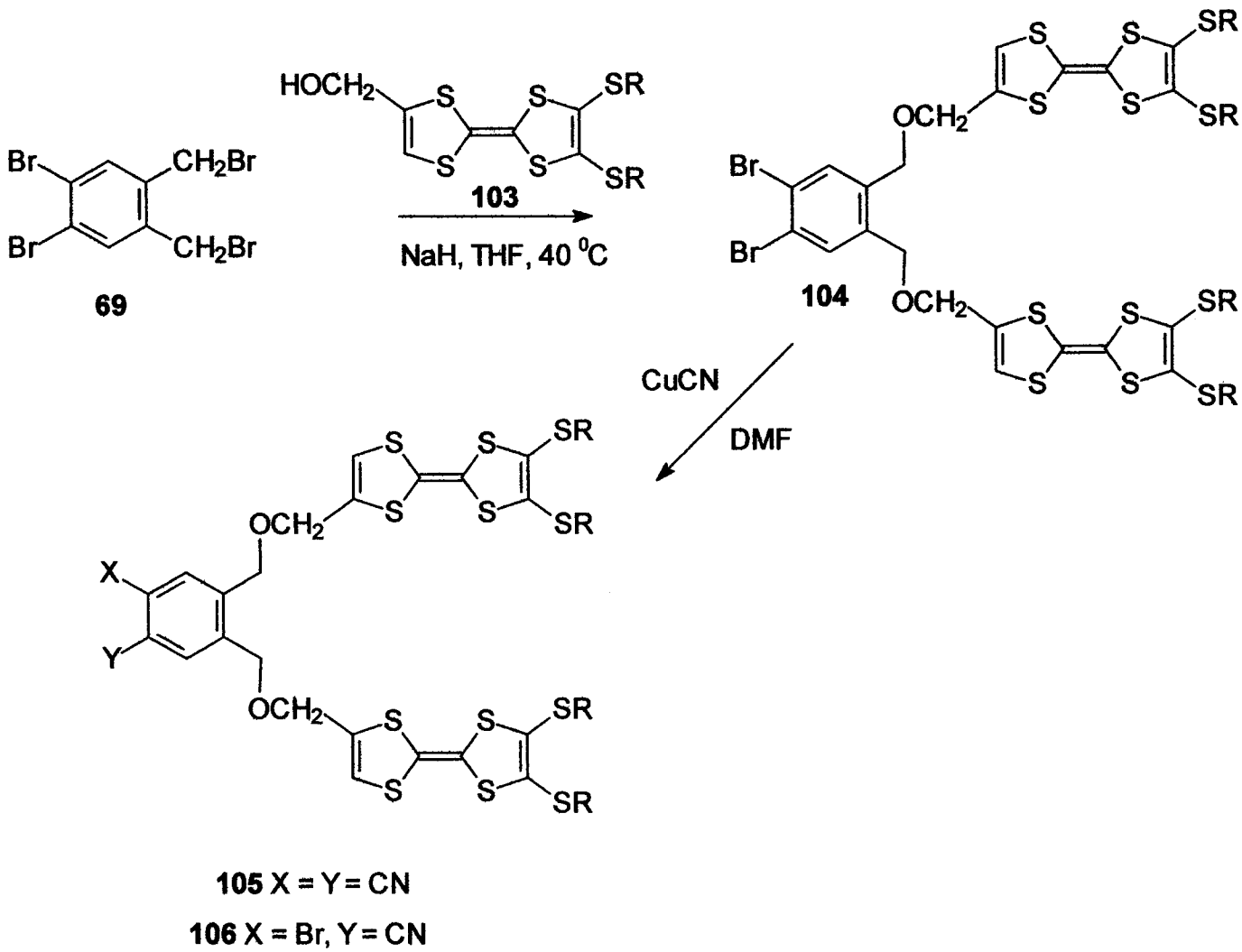


Figure 24. Synthesis of 4,5-bis[4',5'-bis(hexylthio)tetrathiafulvalen-4-yl-methoxymethyl]phthalonitrile.[154] Note that the conversion of the dibromo compound to the corresponding phthalonitrile leads to a roughly 50:50 mixture of the mono- and dinitrile.

common leaving groups in nucleophilic substitution reactions, for instance in the Rosenmund-von Braun reaction, it is often more convenient to use alcohols. Since the hydroxyl group is a poor-leaving group, a number of methods have been used to convert them into a more labile substituent. The most popular of these is conversion to a reactive ester, most commonly a sulphonic ester. While a tosylate sulphonic ester is commonly used, it is known that perfluoroalkanesulphonic esters are much better leaving groups and therefore, are starting to become extensively used in organic chemistry.[190] For instance, triflates are 2×10^4 to 2×10^5 times more reactive towards nucleophilic substitution than comparable tosylates while nonaflates are slightly more reactive still. Hence, the superior leaving ability and low nucleophilicity of these perfluoroalkanesulphonates makes them important functional groups and synthetic tools in organic chemistry and they have been widely used in both preparative and mechanistic investigations.

Aryl triflates and nonaflates are readily synthesized from the appropriate phenols or their metal phenoxides using the anhydride, halide or another derivative of the perfluorosulphonic acid.[190] Like halogens, these perfluoroalkanesulphonic ester groups are deactivating (electron withdrawing) and yet are ortho-para directing towards electrophilic aromatic substitution. In addition, they are highly stable, resulting in a relatively low reactivity. Nonetheless, they have been shown to undergo nucleophilic displacement, for instance, using dimethyl malonate anion.[191] Reaction with $R_2Cu(CN)Li_2$,[192] $RZnX$[193] and R_3Al[194] all lead to alkylation of the aromatic ring. Using certain palladium catalysts, carboxylic acid derivatives can also be synthesized from aryl triflates in a reaction with carbon monoxide and either water, alcohols or amines.[195]

Palladium and nickel catalysts have also been used in the cyanation of aryl triflates.[196,197] The premise behind this method is based on the fact that aryl triflates are easily synthesized from phenols and their carbon-oxygen bonds are readily cleaved by traditional metals to form oxidative adducts.[190] The basic synthetic procedure for this reaction involves a reagent system composed of a source of cyanide anion, usually either KCN or $Zn(CN)_2$, and a palladium(0)[197,198] or nickel (0) catalyst.[196,199] The nickel catalyst is customarily $Ni(Ph_3P)_4$ and is generated *in situ* from nickel(II) complexes and metallic zinc in the presence of excess phosphine.[196] In the case of palladium(0) catalyst, 1,1'-bis(diphenylphosphino)ferrocene (dppf) is often used as the ligand as it improves the catalytic efficiency to a great extent.[197] This is because cyanide anion forms highly stable tetracyano-metal complexes with palladium and these complexes do not participate in the catalytic cycle.[119,189,197] Hence, the catalyst must be shielded by a strongly chelating ligand while the concentration of free cyanide anion must be diminished as much as possible. Dppf is a suitable ligand as it can effectively protect the palladium catalyst while stabilizing the intermediate cationic species formed during the reaction.[189] Concentration of free cyanide anion are minimized in the reaction mixture by both adding the cyanide salt portionwise to the reaction mixture over a prolonged period and by using solvents such as DMF, acetonitrile and 1-methyl-2-pyrrolidinone (NMP) in which cyanide salts have only a slight solubility.[122,189] Yields for this reaction are often exceedingly good.

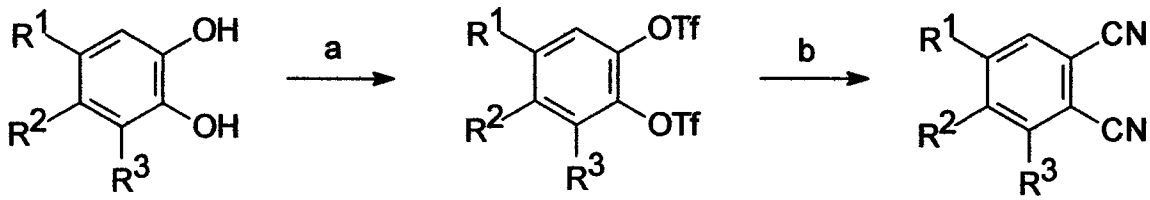
The mechanism of this reaction can be described best as a series of individual reactions.[189,200-202] The initial step is the oxidative addition of the transition metal into the aryl triflate bond to form an arylmetal triflate. In polar solvents, a subsequent

dissociation of the triflate leaving group gives a positively charged, coordinatively unsaturated metal complex.[202] The free coordination site is then filled with a cyanide anion and this is followed by reductive elimination of the transition metal to give the desired aryl cyanide with regeneration of the zero-valent metal species. The palladium catalytic system has been used successfully with functional groups such as alkyl, aryl, chloro, acyl, alkoxycarbonyl, cyano and nitro substituents present in the starting material.[197] However, electron-donating groups including alkoxyl and acylamino tend to retard the reaction, requiring more severe conditions and lead to incomplete conversion of the starting material. The nickel catalyst has the disadvantage that halogen substituents and certain other functional groups cannot be present due to side reactions involving the catalyst.[199]

The availability of catechols and the ease of converting them into the corresponding triflates[190] makes this procedure an attractive pathway for the synthesis of phthalonitriles. A number of phthalonitriles have been prepared from the corresponding aryl triflates[134,189] and the more reactive aryl nonaflates.[122] Tris(dibenzylideneacetone)dipalladium [$Pd_2(dba)_3$] and dppf is the preferred catalytic system as attempts to use nickel(0) complexes have given unsatisfactory results.[189] In all cases, the reaction is carried out in DMF and the $Zn(CN)_2$ is added portionwise in order to keep the concentration of free cyanide to a minimum. When the $Zn(CN)_2$ was added in one portion, no phthalonitrile is formed.[189] Note that the more reactive nonaflates gave the desired product in good yields despite not adding the protective dppf ligand to the reaction mixture.[122] Phthalonitriles bearing alkyl **(126,127)**, alkoxycarbonyl **(128)**, 1-carboxyalkyl **(129,131,132)** and amino acid **(130)** functional groups have been prepared

by this method (Figure 25)[134,189]. Furthermore, 2,3-naphthalenedicarbonitrile **(133)**[189] and 2,3,7,8,12,13-hexacyanotribenzylene[122] have also been synthesized from the corresponding perfluoroalkanesulphonates. Yields for these cyanation reaction are in the 80-90% range although, as expected, electron-donating substituents decrease the yields somewhat. While reports indicate that the reaction is not affected by ortho substituents,[196] steric hindrance in the case of catechols requires higher reaction temperatures for compounds bearing functionality in the 3-position.[189] Interesting, the synthesis of 4,5-bis[2-ethyloxycarbonyl)ethyl]phthalonitrile **(131)** and 4,5-bis[2-ethyloxycarbonyl)propyl]phthalonitrile **(132)** commenced with 4,5-dibromocatechol **(77)** (Figure 26),[134] an important starting material in the preparation of phthalonitriles using the Rosenmund-von Braun reaction. However, in these cases, the role of the halide and hydroxy functional groups are reversed, with the bromides used to add the desired substituents and the catechol employed to add the ortho dinitriles. An advantage of this inversion of roles is the altered reactivity of the starting material. For instance, the strong electron-donating effect of phenolic hydroxyl groups makes it possible to metallate the 3-position of a protected catechol with n-butyl lithium.[134] This can then be alkylated with alkyl halides, allowing the addition of new functionality to the compound, even in the harder to access 3 position. 3-(4-Methoxycarbonyl)butyl-1,2-benzenedicarbonitrile **(146)** was synthesized taking advantage of this point (Figure 27).

While only being recently exploited in the synthesis of phthalonitriles, the palladium-catalyzed cyanation of aryl triflates is a highly advantageous method for preparing this class of compounds. The ready availability of catechols, including naturally occurring and biologically important compounds, greatly increases the potential



107-115 **116-124** **125-133**

	R^1	R^2	R^3
107,116,125	H	H	H
108,117,126	H	CH_3	H
109,118,127	H	H	CH_3
110,119,128	H	CO_2Et	H
111,120,129	H	H	$(CH_2)_4CO_2CH_3$
112,121,130	H	$CH_2CH(NHBoc)CO_2CH_3$	H
113,122,131	$(CH_2)_2CO_2Et$	$(CH_2)_2CO_2Et$	H
114,123,132	$CH_2CH(CH_3)CO_2Et$	$CH_2CH(CH_3)CO_2Et$	H
115,124,133	-C=CH-CH=CH-		H

Figure 25. Phthalonitriles prepared from aryl triflates. a) Tf_2O, Et_3N, CH_2Cl_2, -20 °C, 72-89%; b) $Zn(CN)_2$, Pd_2dba_3, dppf, DMF, RT, 75-98%.[122,134,189]

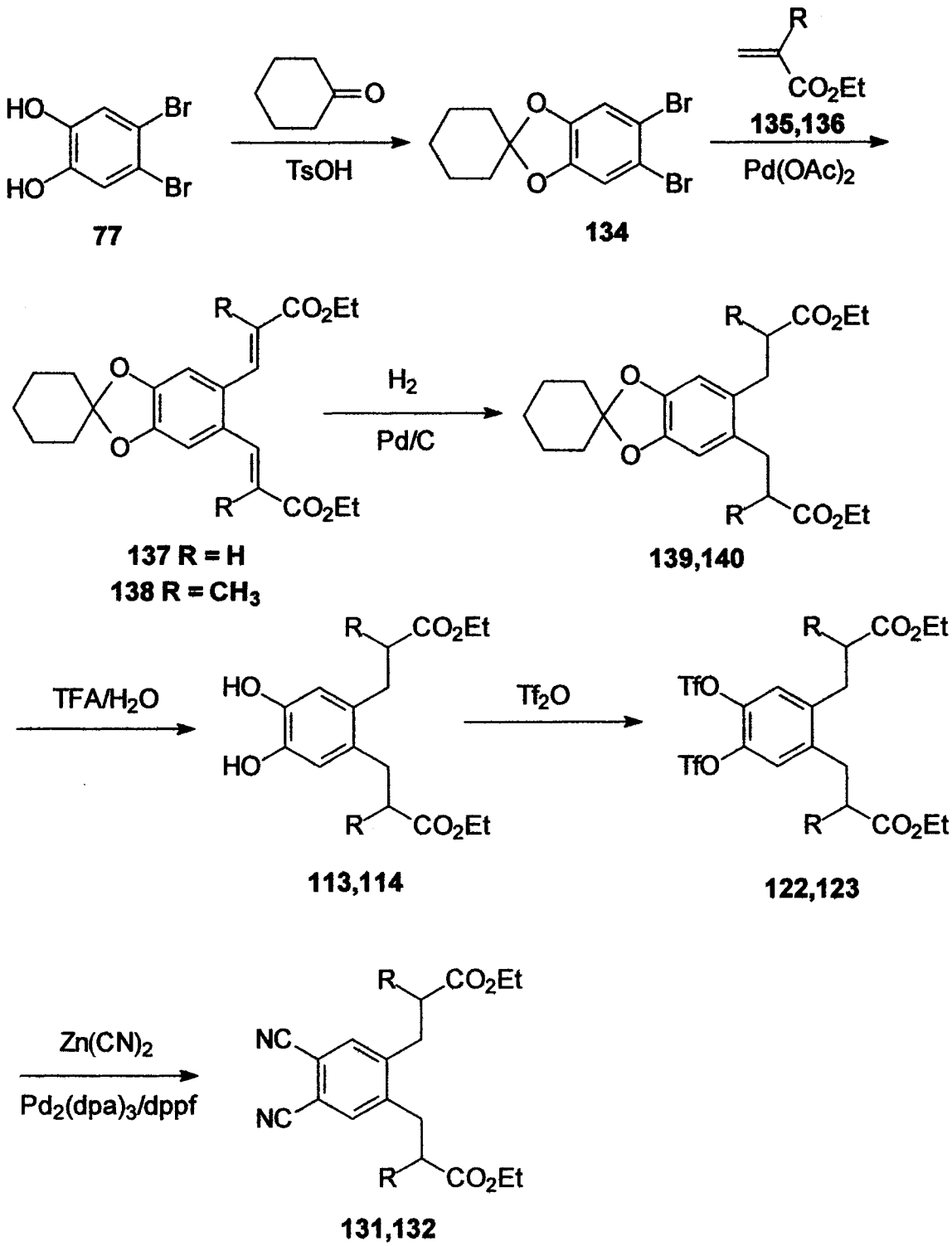


Figure 26. Synthesis of 4,5-bis[2-ethyloxycarbonyl)ethyl]phthalonitrile and 4,5-bis[2-ethyloxycarbonyl)propyl]phthalonitrile (R = H or CH_3).[134]

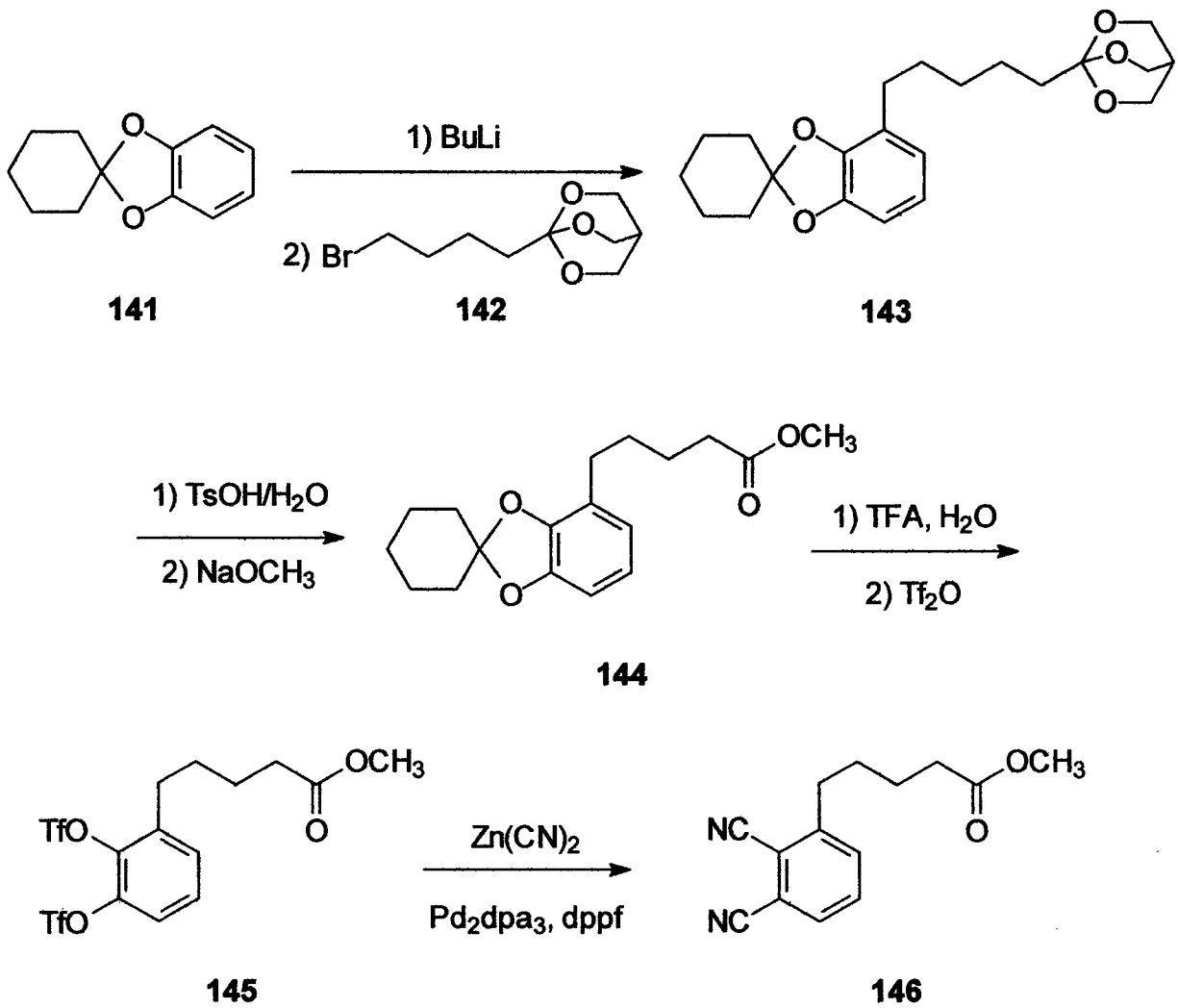


Figure 27. Synthesis of 3-(4-methyloxycarbonyl)butylphthalonitrile.[134]

of this methodology. Dopa or 3-hydroxytyrosine, whose *L*-isomer is the biological precursor of catecholamines including dopamine and is used as an anti-Parkinsonian,[82] has been protected and transformed into the corresponding dinitrile **(130)**.[134] In addition to their availability, catechols are easily converted to the corresponding triflates.[190] Despite the difficulties with the catalytic system, this reaction would appear to have an important future in phthalonitrile synthesis. In addition, other reactions involving aryl triflates, such as those mentioned above, may provide a method for adding other new and important functional groups to the molecule through the catechol moiety of 4,5-dibromocatechol **(77)** while using the Rosenmund-von Braun reaction to add the dinitrile via the bromides. Overall, this synthetic route represents an important alternative to the Rosenmund-von Braun reaction. The mild reaction conditions involved in forming the aryl triflate and the subsequent transformation to the dinitrile tolerates numerous functional groups, which makes this method extremely useful for preparing substituted phthalonitriles. Several of the disadvantages of the Rosenmund-von Braun reaction, in particular the product of copper phthalocyanine as a byproduct is avoided while yields for this method are generally significantly better.

D) Diels Alder and other cycloaddition reactions

A seemingly obvious method of forming phthalonitriles, naphthalonitriles and other higher order aromatic *ortho* dinitriles is via Diels Alder and other cycloaddition reactions. While not extensively used for this goal, Diels Alder reactions have numerous advantages that would be beneficial for the synthesis of phthalonitriles. They are well suited for the synthesis of multiply substituted six-membered rings such as phthalonitriles

and the versatility and generality of these reactions make it possible to place a variety of substituents onto the molecule in a controlled and predictable fashion. Whether induced thermally or photochemically, the Woodard-Hoffman rules can be used to predict the nature and stereochemistry of the relevant products.[203] These 4+2 cycloadditions involving a diene and a dienophile have been shown to proceed almost exclusively via a concerted mechanistic pathway with the simultaneous formation of both new σ bonds. However, both a diradical and diionic mechanism can not be ruled out in some very specific cases.[82,204,205] Overall, the reaction can be understood by orbital symmetry principles, the three most popular theories being the Frontier Orbital Method,[206] the Möbius-Hückel method[81] and the correlation diagram method.[203]

While nearly all conjugated dienes undergo cycloaddition reactions, ethylene and simple olefins make poor dienophiles. This is overcome by adding electron-withdrawing functional groups to the molecule, which activate the dienophile by drawing electron density away from the reacting carbon centers. The most important of these in terms of phthalonitriles is clearly CN. By far the most popular dienophile used in the synthesis of phthalonitriles is fumaronitrile (NCCH=CHCN) **(151)**. Following cycloaddition, the resulting six membered rings bear the desired 1,2-dinitrile functionality needed for phthalocyanine synthesis.

Cycloaddition reactions have not been extensively utilized in the synthesis of phthalonitriles. However, Diels Alder reactions have been used to prepare phthalonitriles with important functionality in novel positions on the molecule. For instance, 3,6-dialkylated phthalonitriles **(153)** have been successfully prepared using such cycloaddition reactions.[207-209] Fumaronitrile **(151)** was used as the dienophile while the

dienes were 2,5-dialkylthiophene-1,1-dioxides **(150)**. These starting materials were prepared by dialkylation of thiophene **(147)** via its dilithiated derivative **(148)** and subsequent oxidation (Figure 28). While the oxidation of the dialkylated thiophene **(149)** to the sulphones **(150)** proved problematic, this was not insurmountable with conditions varying depending on the length of the alkyl chains and thus on the steric hindrance in the molecule.[209] Cycloaddition using fumaronitrile **(151)** was followed by *in situ* extrusion of sulphur dioxide and dehydrogenation to give the corresponding 3,6-dialkylphthalonitrile **(153)**. Alkyl chain lengths ranging from methyl to octadecyl have been affixed to phthalonitriles in the 3,6-positions using this procedure.[207-209] In addition, terminal alkenes, phenyl, bis orthoesters and alkoxycarbonyl groups are permitted.[207] Attempts to carry out the reaction using dialkylated furans instead of the sulphones proved more problematic, with difficulties encountered in both the cycloaddition and in the dehydration of the isolated oxygenated adduct.[207] However, both 3,6-bis(6-hydroxyhexyl)- and 3-hydroxyalkyl-6-methylphthalonitriles **(157)** have been synthesized starting from furan and 2-methylfuran **(154)** respectively (Figure 29).[210] The alkylation reactions in these cases were very slow, with conversion to the desired product being only 50% after several days. Dehydration of the isolated oxygenated adduct was done using lithium bis(trimethylsilyl)amide **(175)** in both cases. Yields starting from furans were low, being around 20-30%.

Despite the difficulties encountered above, substituted furans have been used successfully as dienes in the synthesis of phthalonitriles. For instance, both 3-heptyl- and 4-pentylphthalonitrile were prepared via the Diels Alder reaction of fumaronitrile **(151)** and the appropriately substituted furan.[211] Dehydration was again accomplished using

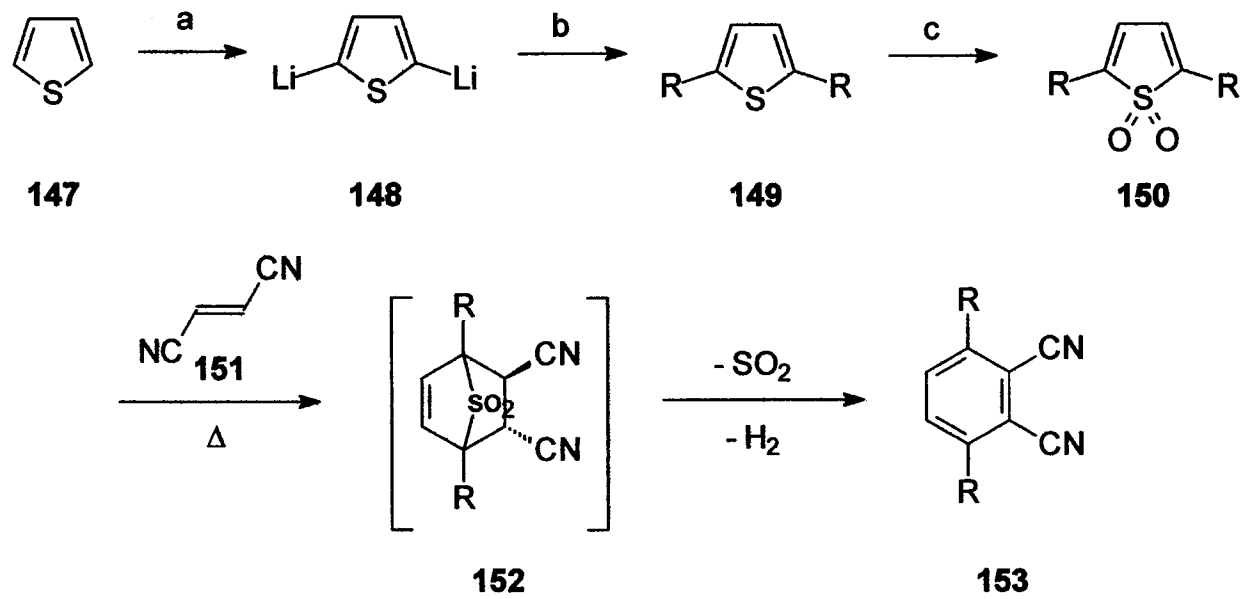


Figure 28. Synthesis of 3,6-dialkylphthalonitriles via Diels Alder reactions with thiophenes. a) nBuLi, THF; b) RX, THF; c) $NaBO_3 \cdot 4H_2O$, AcOH or MCPA, $NaHCO_3$, CH_2Cl_2 or Oxone, $NaHCO_3$, H_2O, acetone.[207-209]

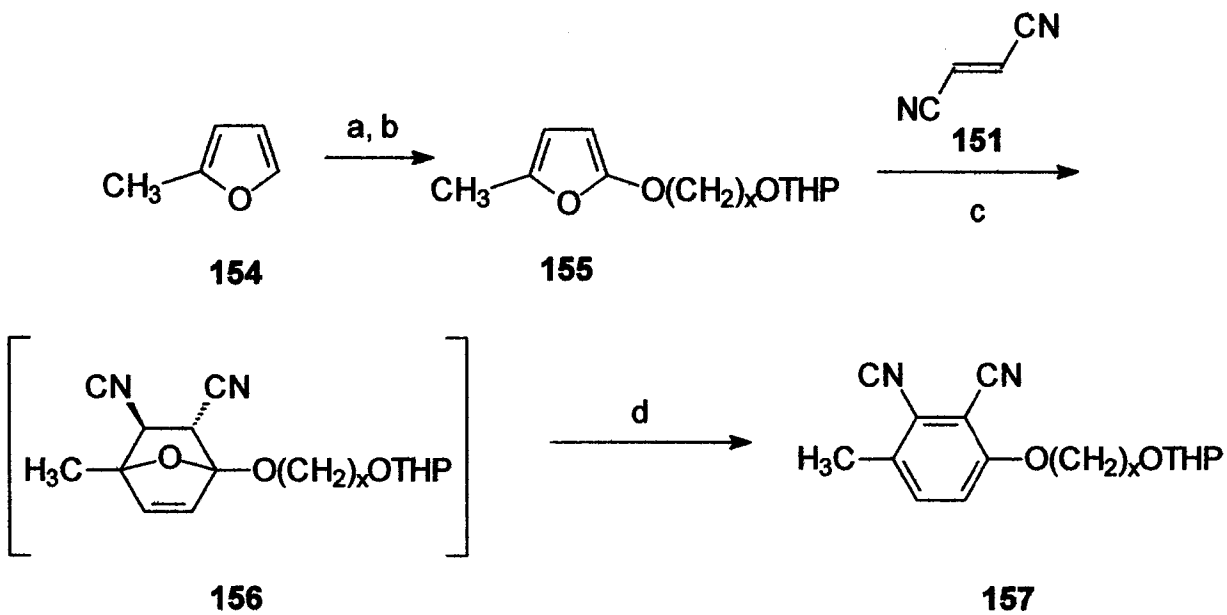


Figure 29: Preparation of 3-hydroxyalkyl-6-methylphthalonitrile.[210] a) nBuLi, THF; b) $THPO(CH_2)_xBr$, THF; c) THF, -5 °C; d)$LiN(Si(CH_3)_3)_2$, THF, -78 °C.

lithium bis(trimethylsilyl)amide **(175)** at -40°C. The reaction of furfural dimethylhydrazone and fumaronitrile leads to 2,3-dicyanobenzaldehyde with dehydration induced using diphosphorus pentoxide in this case.[212] Using a somewhat similar protocol, a series of tetrasubstituted phthalonitriles have also been synthesized in a cycloaddition reaction (Figure 30). In this case, tetrasubstituted cyclopentadienones **(158)** were reacted with chloromaleonitrile **(159)**.[213] This reaction involves the extrusion of carbon monoxide and the loss of hydrogen chloride to give the final product. 3,5-Dialkyl-4,5-diphenyl substituted phthalonitriles **(161)** were obtained along with tetraphenylphthalonitrile and 7,10-diphenyl- and 7,10-dimethyl-8,9-fluoranthenedicarbonitrile. Interestingly, tetrachlorocyclopentdienone acetal **(162)** was quite unreactive under the same conditions and unexpectedly gave 3,4,5-trichloro-6-alkoxycarbonylphthalonitrile **(165)** via the loss of HCl and CH_3Cl (Figure 30).

With its ability to form six membered rings and to extend conjugation, Diels Alder reactions have been extensively used in preparing dinitriles of higher order aromatics, in particular 2,3-naphthalenedicarbonitriles (naphthalonitriles) **(9)**. While isobenzofurans have been used, for instance, in the synthesis of 1,4-diphenyl-2,3-naphthalenedicarbonitrile,[214] such furans are not the most popular dienes in the synthesis of naphthalonitriles directly. Furan and substituted furans are more highly used in the preparation of substituted 2,3-dibromonaphthalenes **(98)** via the 1,4-epoxy derivative **(97)** (Figure 22).[173-175,215] Zinc and titanium tetrachloride is used to invoke dehydration. Subsequently, the dinitrile is formed by the Rosenmund-von Braun reaction. Note that in some cases, the 1,4-epoxy derivative is not dehydrated, allowing for the synthesis of dienophilic phthalocyanine precursors such as 6,7-dicyano-1,4-dihydro-5,8-dialkoxy-1,4-

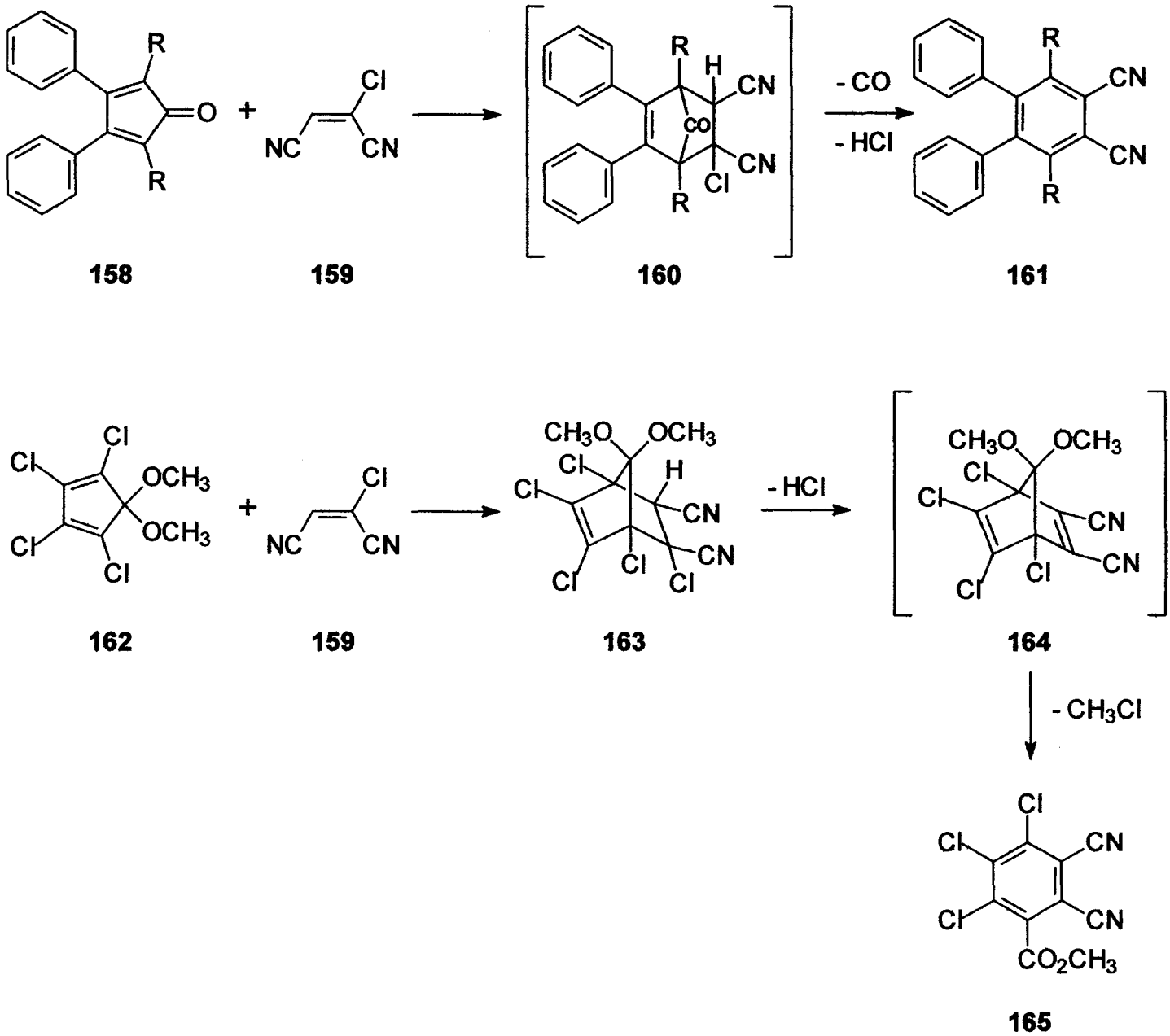


Figure 30. Synthesis of tetrasubstituted phthalonitriles using Diels Alder chemistry.[213] R = CH_3, CH_2CH_3, $CH_2CH_2CH_3$, C_6H_5.

diepoxynaphthalenes.[178] These compounds were introduced into phthalocyanines, hemiporphyrazines and other phthalocyanine derivatives and the resulting macrocycles were used to synthesize oligomeric ladders by repetitive Diels-Alder reactions.[55,56,177,178,] In Diels Alder reactions involving 1,2,4,5-tetrabromobenzene, the dienophile is formed *in situ* using n-butyllithium. This results in the formation of a dehydrobenzene or benzyne, a powerful dienophile that is trapped by the furan derivative in a Diels Alder reaction. A number of highly substituted naphthalonitriles have been prepared using this reaction pathway. These include 1,4- and 5,8-dialkylated 2,3-naphthalenedicarbonitriles **(99)**,[173-175,215-217] synthesized using substituted 1,2,4,5-tetrabromobenzenes and substituted furans respectively. Additionally, 6,7-dialkoxynaphthalonitriles have been formed from 1,2-dibromo-4,5-dialkoxybenzene[174] 6,7-Dialkoxynaphthalonitriles **(176)** have also been synthesized using an elaborate scheme starting from catechol (Figure 31). The *in situ* generation of the appropriate dialkoxy-substituted isobenzofuran **(173)**, followed by reaction with fumaronitrile **(151)** and subsequent dehydration gives the coveted dialkoxylated product.[174]

An important source of dienes for the synthesis of naphthalonitriles is 1,2-bis(dibromomethyl)benzenes **(177b-192b)**. Treatment of these compounds with sodium iodide in DMF yields 1,2-bis(bromomethylene)cyclohexa-3,5-dienes **(177c-192c)** *in situ*, which then reacts in the presence of a dienophile in the manner of a Diels Alder cycloaddition reaction.[218] As such, generation of 1,2-bis(bromomethylene)cyclohexa-3,5-dienes in the presence of fumaronitrile **(151)** leads to the corresponding substituted 2,3-naphthalenedicarbonitriles **(177e-192e)** in one step (Figure 32). In addition to the synthesis of unsubstituted 2,3-naphthalenedicarbonitrile,[74,216,219-222] a number of

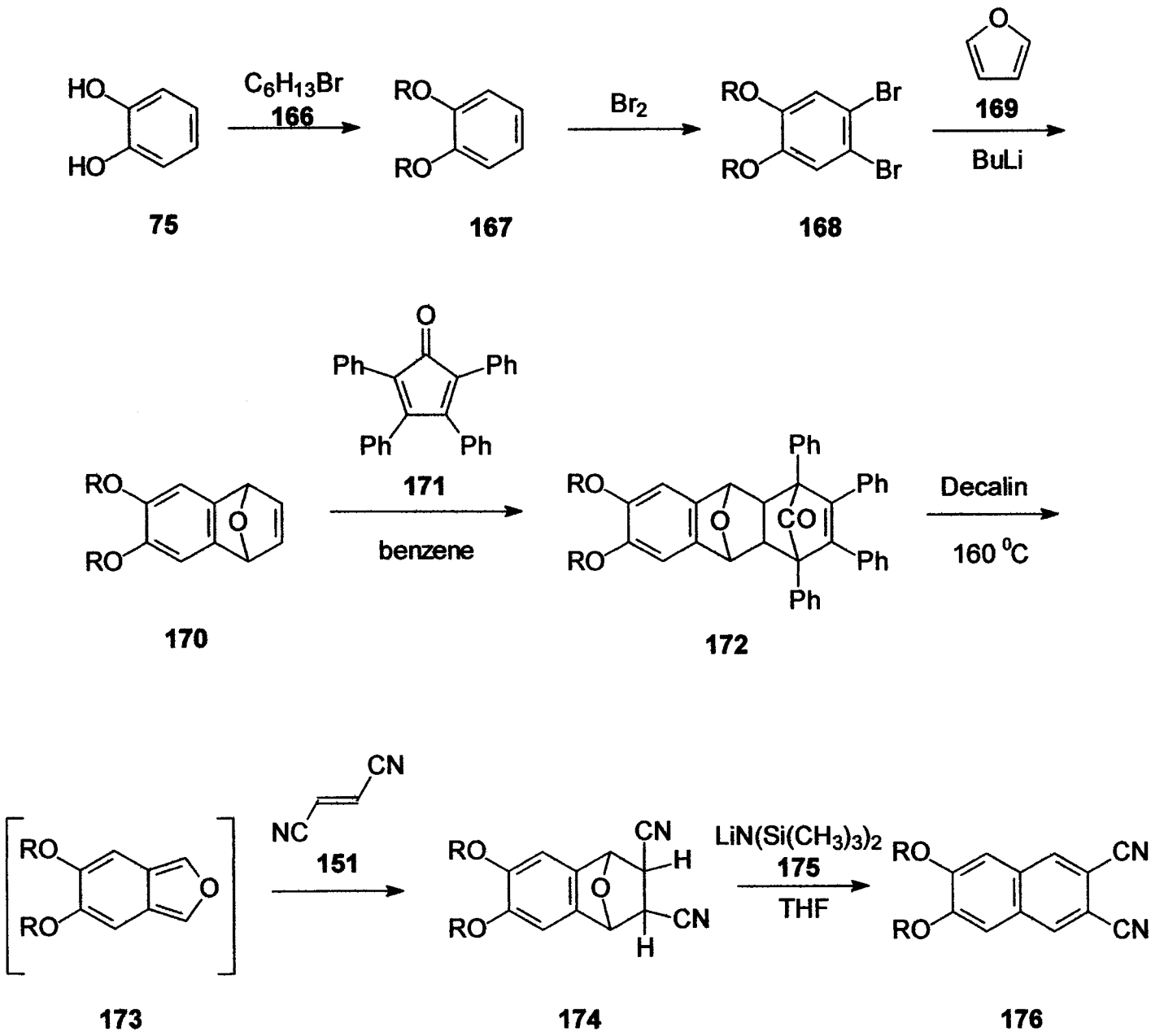
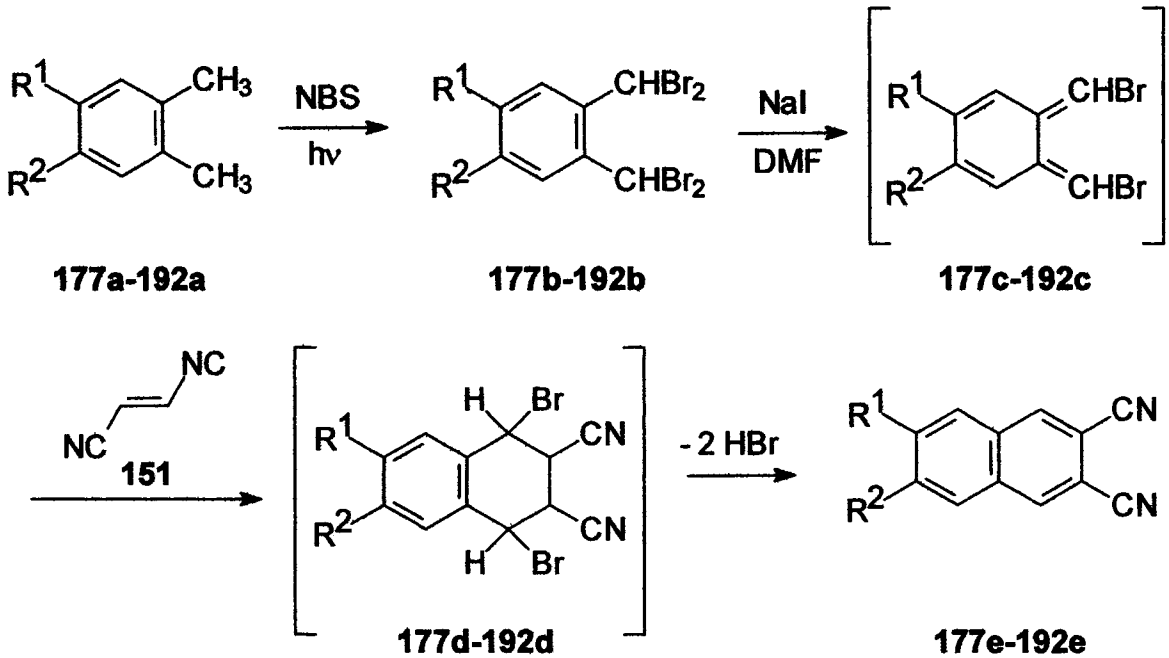


Figure 31. *In situ* generation of dialkoxylated isobenzofuran during the preparation of 6,7-dialkoxy-2,3-naphthalenedicarbonitrile.[174] R=$(CH_2)_5CH_3$



	R^1	R^2		R^1	R^2
177	H	H	**185**	CN	H
178	t-butyl	H	**186**	$NHC(O)CH_3$	H
179	OH	H	**187**	Cl	H
180	OCH_3	H	**188**	Br	H
181	OC_6H_{13}	H	**189**	$C_{10}H_{21}$	$C_{10}H_{21}$
182	$OC(O)C_6H_5$	H	**190**	$C_{11}H_{23}$	$C_{11}H_{23}$
183	COOH	H	**191**	Cl	Cl
184	NO_2	H	**192**	Br	Br

Figure 32. Synthesis of 2,3-naphthalenedicarbonitriles from 1,2-bis(dibromomethyl)benzenes.[174,176,218,221,223]

substituted naphthalonitriles have been prepared using this methodology. Bis(dibromomethyl)benzenes have been primarily synthesized by free radical bromination of substituted 1,2-dimethylbenzenes. Functional groups such as alkyl,[176,218] carboxylic acids,[223] halogen,[216,218] nitro, cyano,[218] amides and ethers[216,221] can be present during this free radical reaction and the ensuing cycloaddition. Amino and hydroxyl groups, on the other hand, must be protected as the corresponding acetylamine[221] or benzoyl ester.[174] 2,3-Dicyanoanthracene has also been prepared via the reaction of fumaronitrile with the corresponding bis(dibromomethyl)naphthalene.[74] Note that tetrabromination of 3-substituted 1,2-dimethylbenzenes is sometimes problematic. However, the corresponding 2-(bromomethyl)-3-(dibromomethyl)benzenes can be used as diene sources using chlorofumaronitrile **(159)** as the dienophile.[174] Finally, 1,4-dialkyl-2,3-naphthalenedicarbonitriles **(198)** are obtainable using a slight modification of this general protocol (Figure 33). It is possible to brominate 1,2-dialkylbenzenes **(194)** using free radical conditions at the benzyl position. Treatment of these α,α'-dibrominated compounds **(195)** with Zn in THF led to *in situ* generation of the diene **(196)**, which reacted with fumaronitrile **(151)** to give 1,4-dialkyl-2,3-dicyanotetralins **(197)**. Aromatization to the desired 1,4-dialkylnaphthalonitrile **(198)** is then accomplished by a bromination/elimination reaction sequence.[176]

Hetero Diels Alder reactions involving 4,5-dicyanopyridazine[104] **(199)** provide a straightforward complementary route to substituted phthalonitriles. The pyridazine system behaves as an excellent azadiene and displays a remarkable reactivity with dienophiles, even unactivated ones.[224-226] The 4+2 cycloaddition reactions of 4,5-dicyanopyridazine with alkynes and enamines **(200)** are followed by nitrogen extrusion

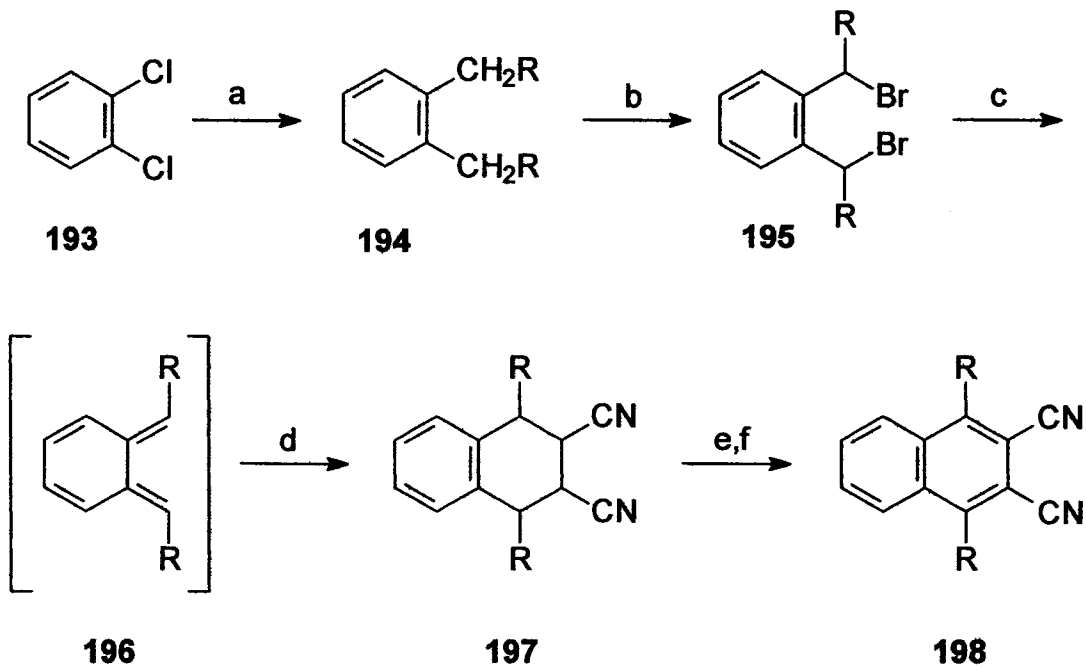


Figure 33. Synthesis of 1,4-dialkyl-2,3-naphthalenedicarbonitriles. a) RCH_2MgBr, $Ni(dppp)Cl_2$, Et_2O; b) NBS, AIBN, $HCOOCH_3$; c) Zn, THF; d) Fumaronitrile, THF; e) NBS, AIBN, $HCOOCH_3$; f) ^tBuOK, CCl_4.[176]

or sequential loss of nitrogen and amine to give the desired dinitriles **(202)** (Figure 34).[227] Alkyl, amine, silyl, phenyl and carboxy substituents have been successfully incorporated into the final phthalonitriles. Increasingly forcing conditions are required on going from the more reactive enamines to the less reactive acetylenes. Cyclic enamines give phthalonitriles bearing saturated ring systems including 5,6-dicyano-2,3-dihydroindene, 2,3-dicyano-5,6,7,8-tetrahydronaphthalene and 2,3-dicyano-6,7,8,9-tetrahydrobenzocycloheptane.[226] Using 1,2-diphenyl- or 1,2,3-triphenylcyclopropene **(203,204)** as the diene affords the corresponding 1,6-diphenyl- and 1,6,7-triphenyl-3,4-dicyanocycloheptatriene **(207,208)** (Figure 35)[226,229] and these compounds have recently been used to prepare novel seven-membered carbon ring-fused phthalocyanine analogs.[229] Cyclopropenone, on the other hand, affords a mixture of bicyclic products.[226]

A potentially interesting method of preparing naphthalonitriles and other acene dinitriles is the Bergman cycloaromatization reaction, especially as described by Bowles and Anthony as it provides 2,3-dibrominated products.[230] However, this interesting possibility for the synthesis of dinitriles has not been investigated to date. Another interesting series of higher order aromatic compound, the iptycenes,[231] have been prepared bearing dinitriles using Diels Alder chemistry.[220,232-234] Initial preparation of the iptycene involves cycloaddition of a diene such as quinone **(210)** to an appropriate aromatic dienophile. The resulting quinone can then be halogenated and transformed into the corresponding dinitrile using conditions similar to those reported above for 2,3-dicyano-1,4-dihydroxyanthracene (Figure 36).[182,183,234] In addition to the few literature examples, a large number of other iptycenes have been prepared that could be transformed into dinitriles as useful synthons for triptycene synthesis generally involve

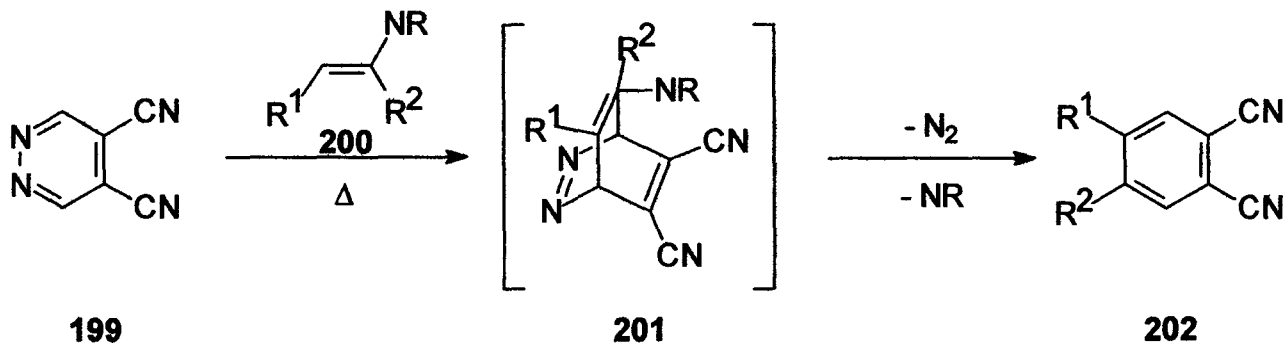
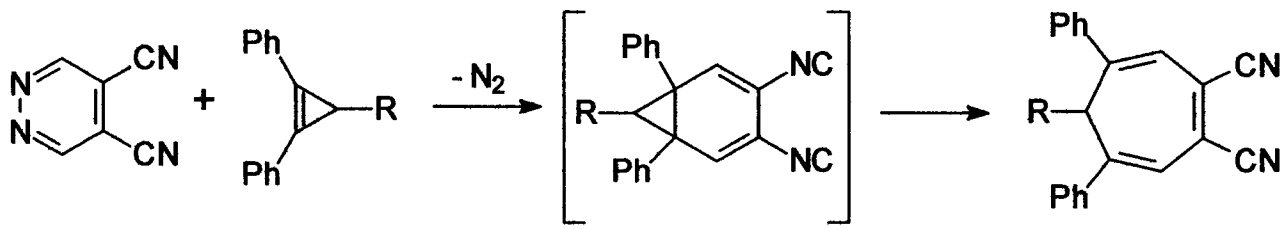


Figure 34. Reaction of 2,3-dicyanopyridazine with cyclic enamines.[226-228]



199 **203 R = H** **205 R = H** **207 R = H**

204 R = Ph **206 R = Ph** **208 R = H**

Figure 35. Synthesis of corresponding 1,6-diphenyl- and 1,6,7-triphenyl-3,4-dicyanocycloheptatriene (R = H or phenyl).[226,229]

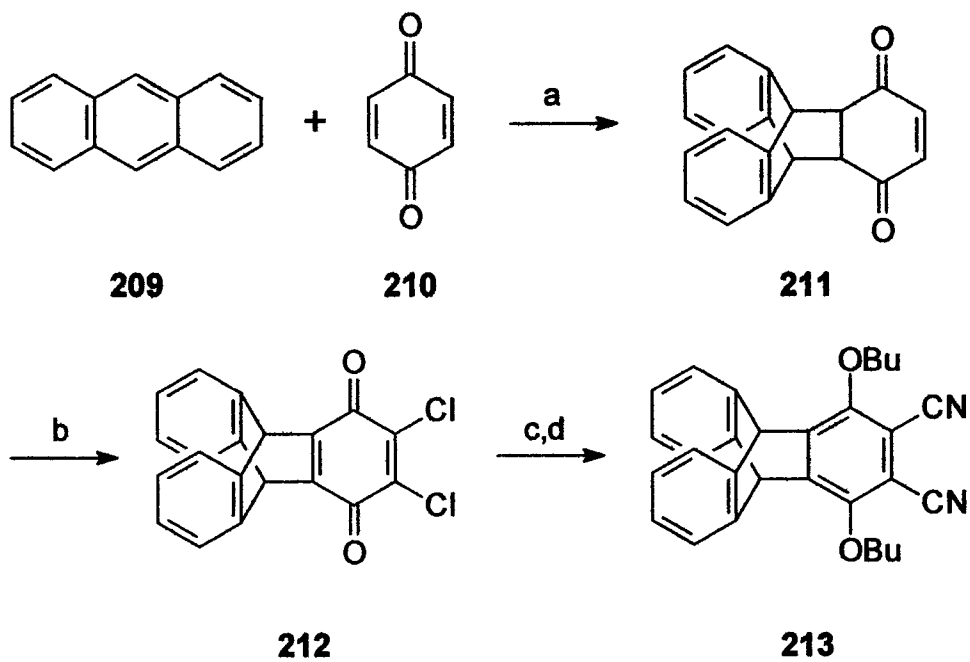


Figure 36. Synthesis of iptycene dinitriles. a) *p*-xylene, Δ, 88%; b) Cl_2, AcOH, 63%; c) i) KCN, NaOH, ii) HCl (82%); d) n-BuI, K_2CO_3, acetone, 86%.[234]

aryl dibromides.[231] Overall, while naphthalonitriles have been extensively examined and their synthesis using Diels Alder cycloaddition reactions have been exploited, higher order aromatic systems have not been so highly examined. This is most likely due to the greatly decreased stability of the resulting phthalocyanine-based complexes.

The usefulness of Diels Alder cycloaddition reactions in the synthesis of phthalonitriles and phthalonitrile derivatives is self-evident. In addition to the reactions examined above, Diels Alder chemistry has been implicated in the synthesis of acetylenic phthalonitriles **(217)** using dicyanoacetylene **(215)** as the dienophile and cross-conjugated dimethylenehexadiynes **(214)** as dienes (Figure 37).[235,236] Diels Alder approaches have likewise been turned to in order to prepare 4,5-disubstituted phthalic acid derivatives in an attempt to avoid the use of the Rosenmund-von Braun reaction during the synthesis of 4,5-dialkoxyphthalonitriles.[90] A series of 4-carboxy-5-aryl-substituted phthalic acids **(222)** have likewise utilized the Diels Alder reactions of 2,3-dimethyl-1,3-butadiene **(219)** and various cinnamic acid derivatives **(218)** for their synthesis (Figure 38).[15] These phthalic acids have been used in the fabrication of phthalocyanine dyes by fusion in excess urea in the presence of ammonium molybdate. In a novel synthetic approach, Diels Alder reactions have been used to prepare 3,4-dialkyl-1,2-dimethylphthalates, which are subsequently transformed to the *o*-phthalic aldehyde.[176] The *o*-phthalic aldehydes can be brominated to the bis(dibromomethyl) compounds and then reacted with fumaronitrile or directly converted into the naphthalonitriles by reacting with succinonitrile. Note that the same phthalaldehydes can be synthesized from 1,2-dialkylbenzene in a reaction scheme involving chloromethylation and oxidation using potassium benzeneselenite.[176] In a different pericyclic reaction, photocyclization of cis-

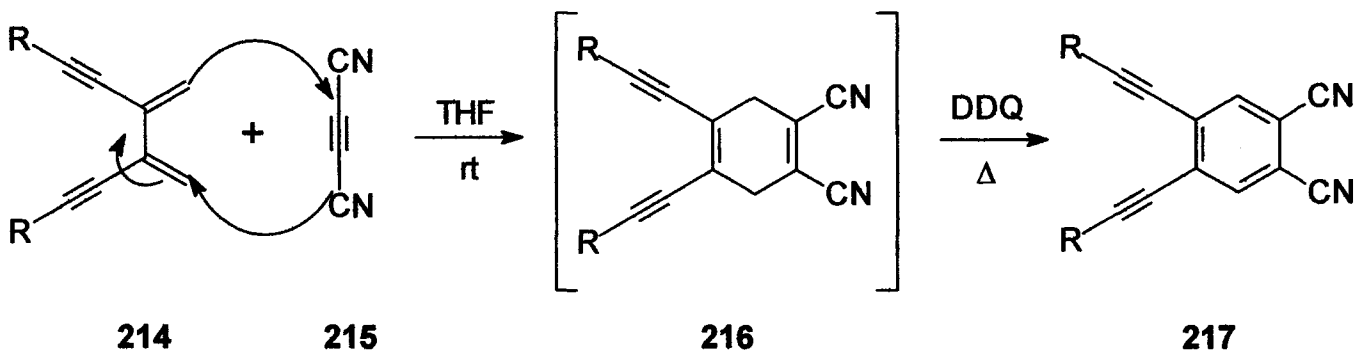


Figure 37. Preparation of acetylenic phthalonitriles via Diels Alder chemistry.[235,236]

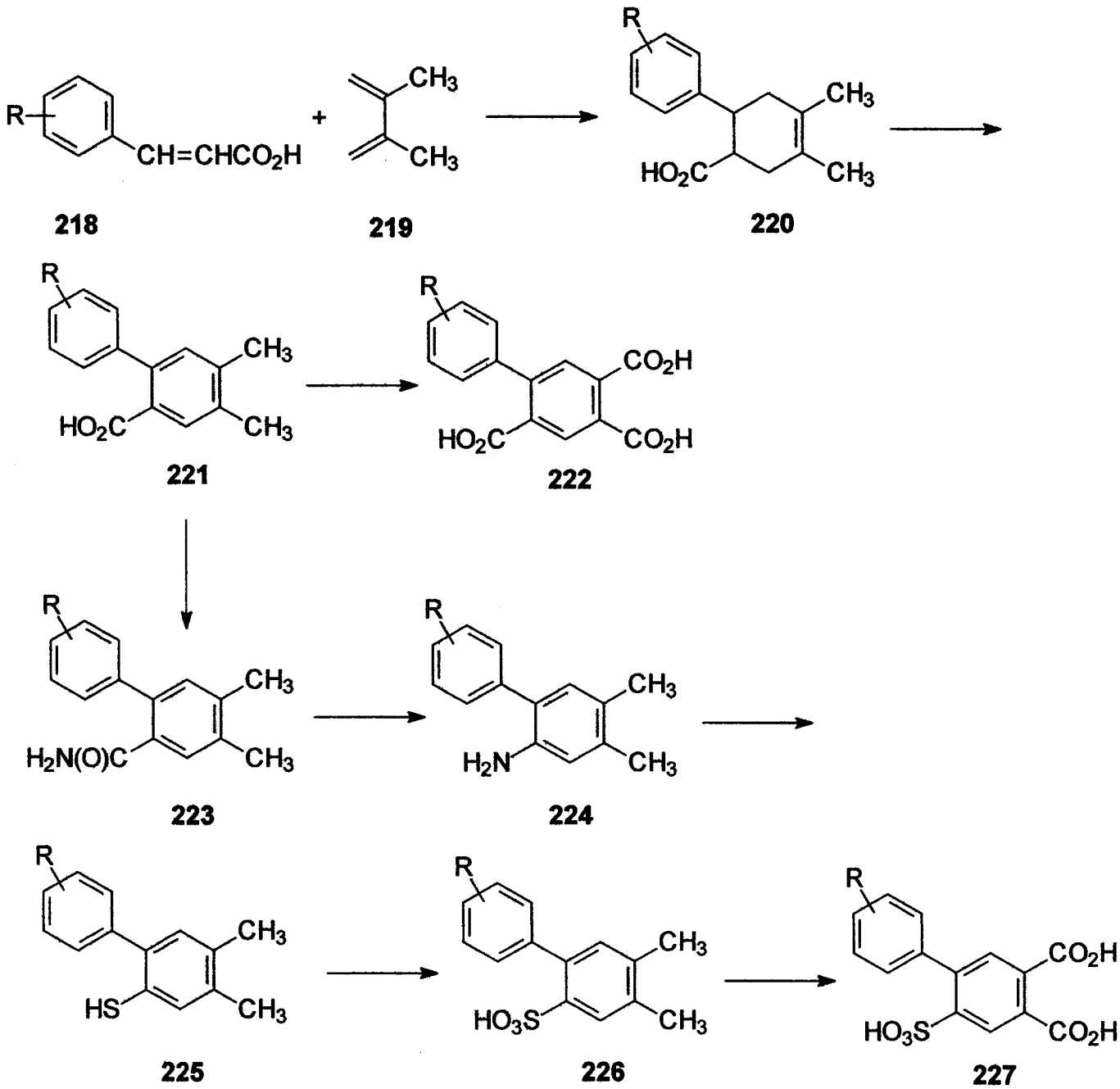


Figure 38. Reaction of 2,3-dimethyl-1,3-butadiene and various cinnamic acid derivatives and the conversion of the resulting product to the corresponding mixed substituted phthalic acid derivatives.[15] R = CH_3, OCH_3, COOH, Cl, NO_2 among others.

and trans-1,2-bis(3,4-dicyanophenyl)ethene produced the corresponding tetracyanophenanthrenes.[237] Finally, substituents have even been added to a pre-existing phthalonitrile using Diels Alder reactions. An excellent example is the synthesis of polyphenylated phthalonitriles, which have been prepared via the cycloaddition reaction of 2,3,4,5-tetraphenylenecyclopentadiene-1-one **(230)** and 4-phenylethynylphthalonitrile **(229)** (Figure 39).[238] With all these examples, it is clear that the versatility of the Diels Alder reaction has been extensively profited in order to synthesize phthalonitriles and other dinitriles useful in the preparation of phthalocyanines, naphthalocyanines and other phthalocyanine derivatives.

E) Modification of substituted phthalonitriles

The protocols discussed above describe the major methods used in the addition of the ortho dinitrile functionality into phthalonitriles and have been invaluable in attaching important functional groups onto these phthalocyanine precursors in distinct locations. However, these methods often involve multi-step and sophisticated reaction pathways and give poor overall yields. A more simplistic approach to preparing substituted phthalonitriles encompasses the modification of pre-existing phthalonitrile molecules. A number of substituted phthalonitriles bearing chemically versatile functional groups are commercially available. Others can be easily synthesized from inexpensive commercially available starting materials using one of the classic methods discussed above in good yields and in large quantities. It is truly beyond the scope of this review to probe every reaction used to modify phthalonitriles and every substituent that has been

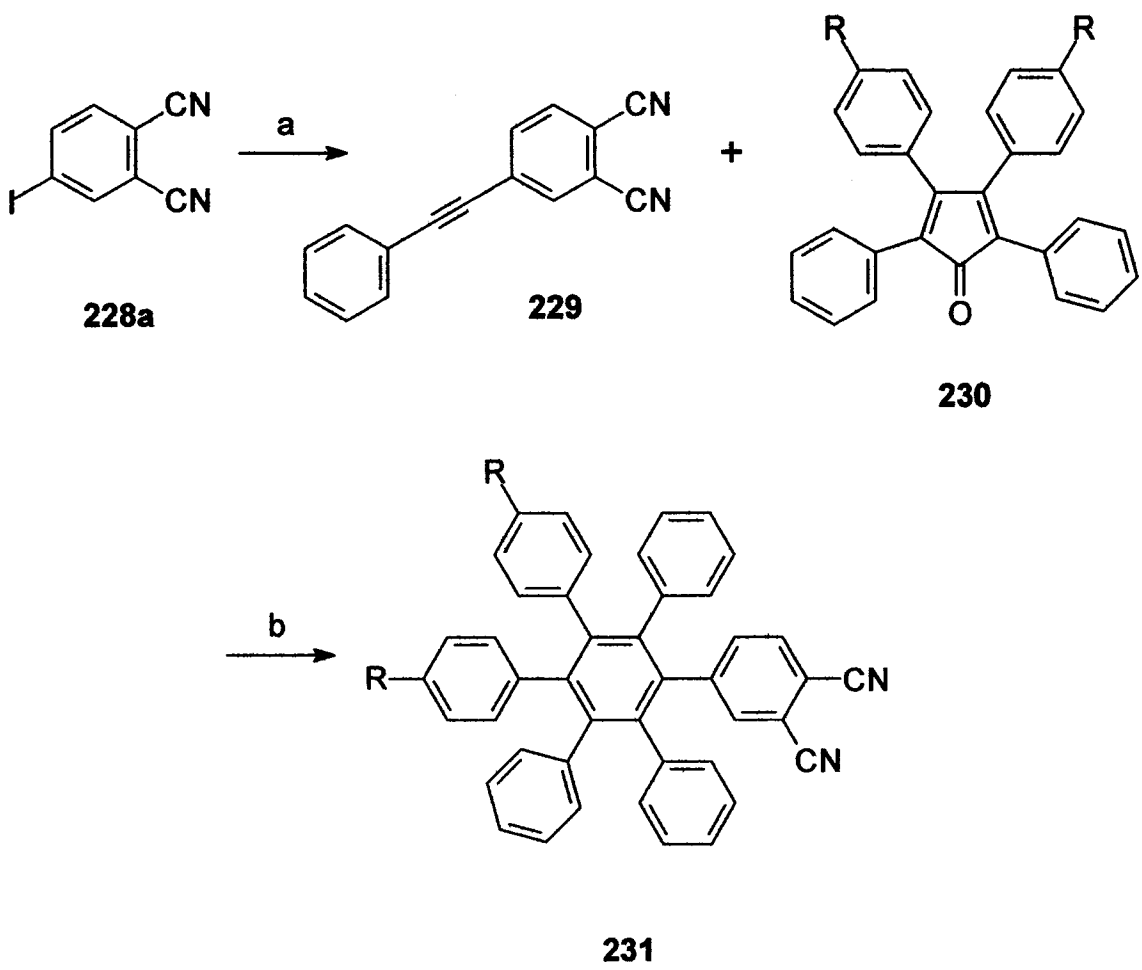


Figure 39. Synthesis of polyphenylated phthalonitriles (R = H, OMe, OC_4H_9, $OC_{12}H_{25}$).

a) $C_6H_5C{\equiv}CH$, $(Ph_3P)_4Pd$, CuI, piperidine, 80%; b) cyclohexylbenzene, Δ, 65-80%.[238]

added to these important compounds. However, a brief overview of the more important and interesting of these reactions is in order and could be extremely useful.

i) Nucleophilic Aromatic Substitution

Far and away the most highly used reactions employed in the modification of substituted phthalonitriles are nucleophilic aromatic substitution reactions. While the high electron density of the aromatic system attracts positive species and tends to favor electrophilic substitution reactions, nucleophilic aromatic substitution reactions are successful when appropriate leaving groups are present in the molecule. In the case of phthalonitriles, the molecule is particularly susceptible to nucleophilic attack due to the electron-withdrawing capability of the dinitrile functionality. These reactions are known to proceed almost exclusively via three basic mechanism:[81,239,240] 1) an SNAr mechanism, which passes via a tetrahedral intermediate containing a delocalized negative charge that is stabilized by electron withdrawing groups such as nitriles, 2) an SN1 mechanism where, in a two step reaction, the leaving group (almost exclusively N_2 from a diazonium salt) departs, giving an aryl cation as an intermediate, which then reacts with the nucleophile, 3) a benzyne mechanism that involves a strong base that induces the loss of the leaving group and production of the highly reactive benzyne intermediate. All three of these mechanisms have been employed in the production of substituted and modified phthalonitriles. Of course, a benzyne intermediate as a diene was described above in the preparation of naphthalonitriles from 1,2,4,5-tetrabromobenzenes.[173-175,215] Furthermore, the Rosenmund-von Braun reaction can be seen as nothing more than a transition metal-assisted nucleophilic substition of halide by cyanide and it has been proposed that this

reaction may proceed by a tetrahedral intermediate (see Figure 15). Numerous nucleophiles and leaving groups have been utilized in the general reaction pathway towards substituted phthalonitrile synthesis. Below is a brief description of the use of nucleophilic aromatic substitution reactions for the synthesis of substituted phthalonitriles in terms of the leaving group and nucleophile used.

a) Nitro group as leaving group

NO_2 is a surprising good leaving group for nucleophilic aromatic substitution reactions. This is despite the fact that NO_2 is generally not lost in aliphatic substitution reactions and that halogens has been shown to act preferentially as a leaving group in certain nucleophilic aromatic substitution reactions involving both NO_2 and Cl such as in the synthesis of picric acid. Overall, the approximate order of leaving group ability is: F > NO_2 > OTs > SOPh > Cl, Br, I > N_3 > NR_3^+ > OAr, OR, SR, NH_2.[81,241] However, this order greatly depends on the nature of the nucleophile employed during the reaction. The explanation for fluoro and nitro being such good leaving groups in nucleophilic aromatic substitution reactions lies in the mechanism involved. Under the SNAr mechanism, the rate determining initial step involves formation of a tetrahedral intermediate and its formation is promoted by leaving groups having strong *–I* effects such as F and NO_2.[81] The popularity of the NO_2 leaving group in terms of the preparation of monosubstituted phthalonitriles is directly linked to the commercial availability of both 4- and 3-nitrophthalonitrile **(42,235)** and/or their facile synthesis via nitration of phthalimide and subsequent conversion to the phthalonitrile (Figure 11).[54,93] Furthermore, other potential

leaving groups such as halogens are generally prepared from the nitro compound via a diazonium salt **(253)**.

a-1) Alcohols as nucleophiles

An exceptional amount of work has been carried out using the NO_2 group of both 4- and 3-nitrophthalonitrile **(42,235)** as the leaving group and various alcohols as the nucleophile. The resulting aryl ethers are readily synthesized, with the reaction generally being carried out in a dry polar aprotic solvent such as DMSO or DMF using sodium or potassium carbonate as a base. Frequently, the dry potassium carbonate is added portionwise over time. Other solvents that have been used include dioxane, N-methyl-2-pyrrolidone and N,N-dimethylacetamide. The use of aqueous DMF has been reported as well, with these conditions allowing the reaction to be carried out under homogeneous conditions.[105] It has been observed that the kinetics of the nucleophilic substitution of the nitro group of 3- and 4-nitrophthalonitriles with arylhydroxy groups are dependent on the water content when aqueous DMF is employed as the solvent.[242,243] However, more often than not, such protogenic conditions fail to give any reaction except for highly activated cases. In the meanwhile, it has been observed that using lithium hydroxide as the base instead of potassium carbonate can be a useful modification to reported procedures in some instances.[54] Alcohols of weaker nucleophilicity sometimes call for slightly modified conditions including higher reaction temperatures, more polar solvents and stronger bases. For instance, sodium 2,2,2-trifluoroethoxide is added to 4-nitrophthalonitrile in anhydrous hexamethylphosphortriamide (HMPA) while other fluorinated alcohols were added using NaOH, KOH or

tris(diethylamino)phosphazomethane as the base.[244] A modified procedure has also been used to attach phthalocyanine precursors to a polymer support (Figure 40).[57-59] The nucleophilic aromatic substitution reaction was induced using 25% KOH and Adogen 464 in nitrobenzene in a modification of Fréchet's three-phase reaction and resulted in attachment of the phthalonitrile to the polymer support via a long alkyl spacer chain **(233)**.[57,58] These polymer bound precursors can also be prepared from polymer-bound trityl chloride and (6'-hydroxyalkoxy)phthalonitrile **(232)** and other terminal alcohols (Figure 40), which are themselves synthesized by the reaction of 4-nitrophthalonitrile **(42)** with monoprotected dialcohols such as the monotetrahydropyranyl ether of 1,6-hexanediol.[57-59] Interestingly, the first method with the nucleophilic displacement of NO_2 using phase transfer conditions gave 0.26 mmol of phthalonitrile per gram of polymer while the second method gave 0.53 mmol per gram.[58] Such polymer bound phthalocyanine precursors have been used in the preparation of 3:1 unsymmetrically substituted macrocycles.

Both aliphatic[48,49,54,245-250] and phenolic alcohols[51,54,94,223,245,248,250-261] **(237)** have added to phthalonitriles successfully via nucleophilic displacement of the nitro groups of both 3- and 4-nitrophthalonitrile. In addition, poly(oxyethylene)ethers,[261-263] poly(aryl ether) dendrimers **(234a-c)** (Figure 41)[264-269] and crown ethers[270,271] have been introduced into phthalonitriles via this method. Other interesting functional groups attached to phthalonitriles via an ether bond include cyclic phosphazenes,[135] pentose and hexose rings,[272] alkylsilyloxy groups[273] and 1,3-bis-(dimethylamino-2-propyloxy) substituents.[274] The free 2,3- and 3,4-dicyanophenols are available as well, by treating the corresponding

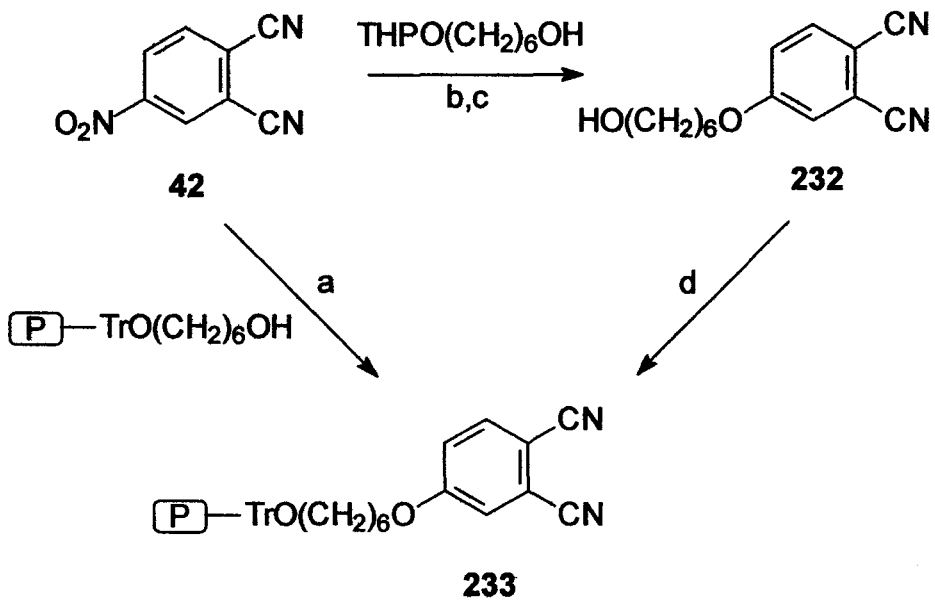


Figure 40. Synthesis of polymer-bound phthalonitrile. a) 25% KOH, Adogen 464, nitrobenzene, 60 °C, 22 hr; b) KOH, DMF, 100 °C, 15 hr; c) 4 N HCl; d) polymer bound-TrCl, pyridine, CH_2Cl_2, DMAP.[58]

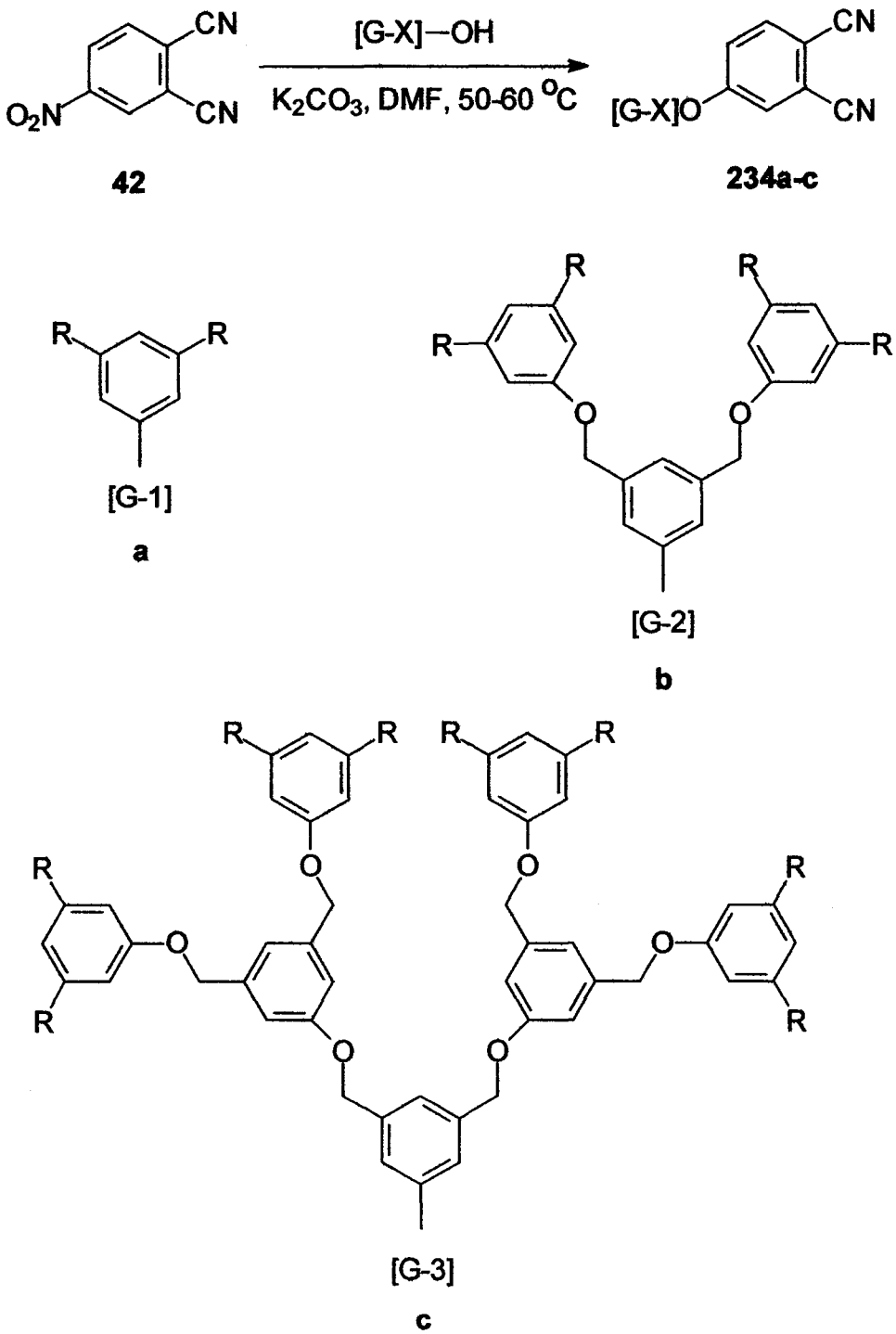


Figure 41. Preparation of dendritic phthalonitriles (R = H, CO_2CH_3, $O(CH_2CH_2O)_3CH_3$, [G-1] (R = H)).[264-269]

nitrophthalonitrile with sodium nitrite in DMSO or NMP in the presence of base in a reaction that proceeds via the nitrous acid ester.[275-277]

The nitro group of 6-nitro-2,3-dicyanonaphthalenedicarbonitrile **(240)** has also been substituted with sterically hindered aryl alcohols using K_2CO_3 and DMF.[258] Using a halogen-leaving group, 6-fluoro-2,3-dicyanonaphthalenedicarbonitrile gave better yields of the desired product in some cases although more sterically hindered phenols gave no reaction.[258] Note that the more steric hindered 2,6-di-t-butylphenol **(238)** did not react via nucleophilic addition with 6-nitro-2,3-dicyanonaphthalenedicarbonitrile **(240)** either. Instead, an interesting oxidative coupling was observed to give 5-(3',5'-di-t-butyl-4'-hydroxyphenyl)-6-nitro-2,3-naphthalenedicarbonitrile **(242)** (Figure 42). In a somewhat similar reaction with 2,6-di-t-butylphenol **(238)**, the nitro groups of both 3- and 4-nitrophthalonitrile **(42,235)** were replaced by a 3',5'-di-t-butyl-4'-hydroxyphenyl group **(239)** with the anion of 2,6-di-t-butylphenol acting as an effective carbon nucleophile. The ability of the anion of 2,6-di-t-butylphenol to act as a carbon nucleophile has precedents, with tetrachlorophthalonitrile reacting to give a similar monoaddition product.[278-279]

Modification of the alkyl and aryl ether substituents added to the phthalonitriles via the nucleophilic displacement of the nitro group remains a possible strategy for adding novel functional groups to the molecule as well. A previous mentioned example is with polymer-bound phthalonitrile, where (6'-hydroxyalkoxy)phthalonitriles **(232)** are used to attached the phthalocyanine precursor to a polymer support (Figure 40).[57-59] In a like manner, 4-(3,4-dicyanophenoxy)benzoic acid has been prepared and attached to modified silica by the reaction of its acid chloride with free amine groups on the modified

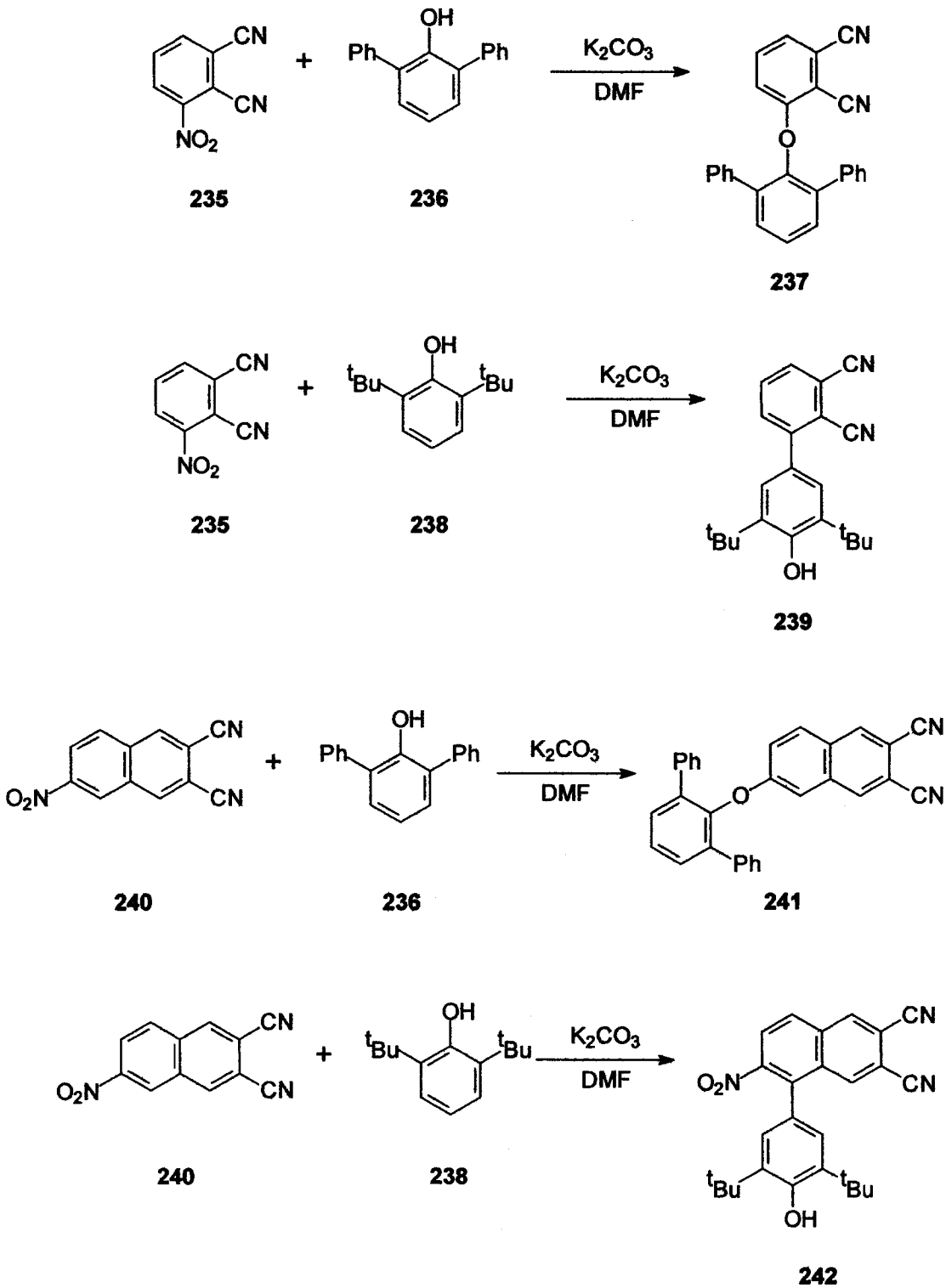


Figure 42. Nucleophilic aromatic substitution reactions of sterically hindered phenols and 3- and 4-nitrophthalonitrile and 6-nitro-2,3-naphthalenedicarbonitrile.[258]

silica.[60] In another instance, 2,3-dicyanophenol was synthesized from 3-nitrophthalonitrile and this was in turn reacted with (chloromethyl)trimethylsilane in DMF in the presence of K_2CO_3 to give 3-(trimethylsilylmethoxy)phthalonitrile.[277] The vinyl group in 4-(*o*-allylphenoxy)phthalonitrile has been applied to the addition of polymethylsiloxane to the phthalonitrile molecule.[280] And finally, 3-propargyloxyphthalonitrile derivatives **(243)** prepared by this methodology have been shown to undergo cyclization reactions to the corresponding dicyanobenzopyran **(244)** along with some dicyanobenzofuran **(245)** (Figure 43).[281,282]

In addition to the synthesis of phthalonitriles substituted through ether bonds, these nucleophilic aromatic substitution reactions have been instrumental in the preparation of polynuclear phthalonitriles. Simply by using polyalcohols, this reaction readily leads to bis- and tetranuclear phthalonitriles that are extremely useful in preparing both polynuclear phthalocyanines and phthalocyanines with controlled geometries (Figure 44). Binuclear phthalonitriles have been prepared using catechols[283] and simple alkyl diols,[283-287] while a novel optically active bisphthalonitrile has been synthesized using (S)-(-)- or (R)-(+)-2,2'-dihydroxy-1,1'-binaphthyl as the alcohol.[288,289] Alkyl-, fluoroalkyl-, oxy-, alkylenedioxy- and arylenedioxy-bridged bisphthalonitriles[59,251,286,290-292] **(246a-h)** along with phthalonitrile end-capped poly(ether sulphone)s[293] and multiple aromatic ethers[294] **(246i-j)** have implemented similar reactions in their formation. Similarly, tetranuclear phthalonitriles based on pentaerythritol nuclei can be synthesized and provide access to capped[295] and tetranuclear phthalocyanines.[296] 4-Aminophenoxyphthalonitriles, formed by a nucleophilic aromatic substitution reaction, has been reacted with 4,4'-bismaleimidodiphenylmethane to give a dimeric species.[297]

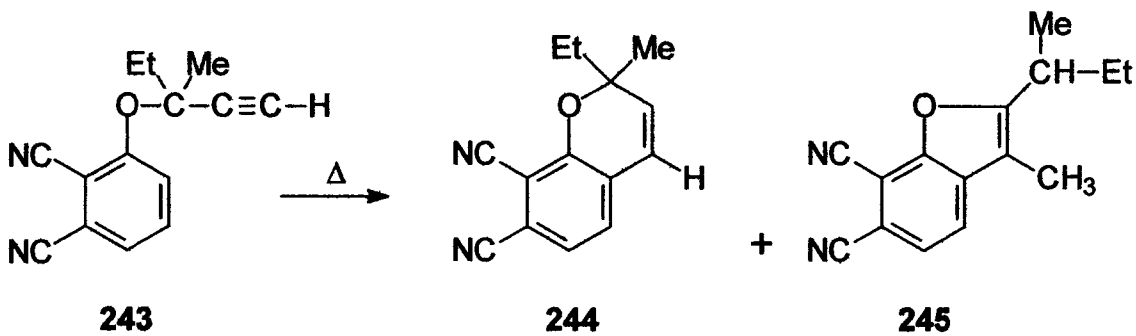
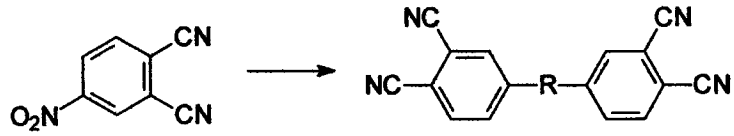


Figure 43. Cyclization of 3-propargyloxyphthalonitrile derivatives to the corresponding dicyanobenzopyran and dicyanobenzofuran.[281,282]



42 246a-j

246a —O—

246b —O—$(CH_2)_x$—O—

246c —O—$(CF_2)_3$—O—

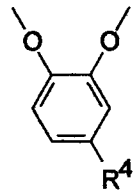
246d —O—CH_2—C(R^1)(R^2)—CH_2—O—

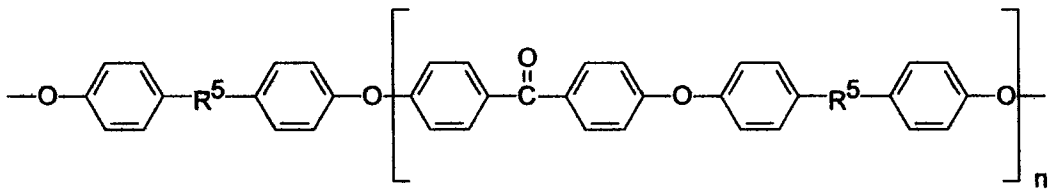
246e —O—C$_6$H$_4$—O—

246f —O—C$_6$H$_4$—C$_6$H$_4$—O—

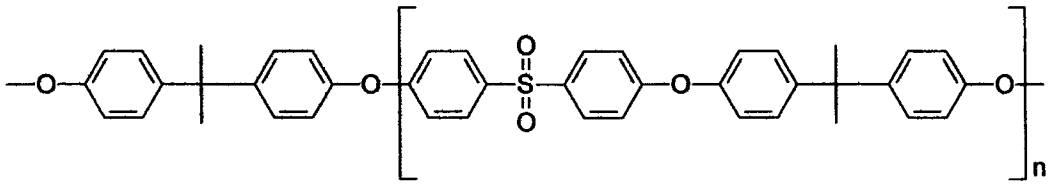
246g —O—C$_6$H$_4$—C(R^3)(R^3)—C$_6$H$_4$—O—

246h





246i



246j

Figure 44. Various binuclear phthalonitrile synthesized from 4-nitrophthalonitrile via nucleophilic aromatic substitution (x = 3, 6, 10 or 12; $R^1 = R^2 = CH_3$; $R^1 = CH_3$, $R^2 = CH_2CH_3$; R^3 = H or CH_3; R^4 = H or t-butyl).[251,283,284,287,290-292]

Numerous other bridged and polymer linked phthalonitriles have been prepared and their use in the preparation of phthalocyanine-containing polymers has been recently reviewed.[36,37]

a-2) Thiol as nucleophiles

Thiols are also effective nucleophiles in nucleophilic aromatic substitution reactions and numerous monosubstituted phthalonitriles have been prepared via the nucleophilic substitution reaction of nitrophtalonitriles with thiols (Figure 45). Simple and long chain thiols[298-300] and thiophenols[301] **(247)** have been used as nucleophiles. Among more interesting functional groups added via a thiol bond is cysteamine,[302] N,N-dialkyldithiocarbamates[303] and triethyleneoxysulphanyl groups.[304] Selenophenols yield the corresponding selenium-substituted phenyl phthalonitrile ethers in good yields.[251] Importantly, mercaptophthalonitrile can be prepared by the decomposition of N,N-dialkyldithiocarbamates and this free thiol functional group can be alkylated or alkoxylated with alkyl or alkoxy halides.[303] Phthalonitriles equipped with alkyl- and arylsulphonyl groups have been prepared by oxidation of the thioethers **(249)** using *m*-chloroperbenzoic acid (MCPBA) (Figure 45).[299-301] Note that carrying this oxidation out at lower temperatures gave access to the arylsulphinyl compounds **(250)** as well.[301] Finally, thioether-linked bisphthalonitriles can be synthesized by nucleophilic substitution of NO_2.[251] The basic reaction proceeds via the nitrous acid ester[275] and involves treating the nitrophthalonitrile with sodium nitrite in DMSO followed by reaction with sodium monosulphide. It was very important to adhere to completely anhydrous conditions in this reaction to avoid the bisphthalonitrile ether from becoming

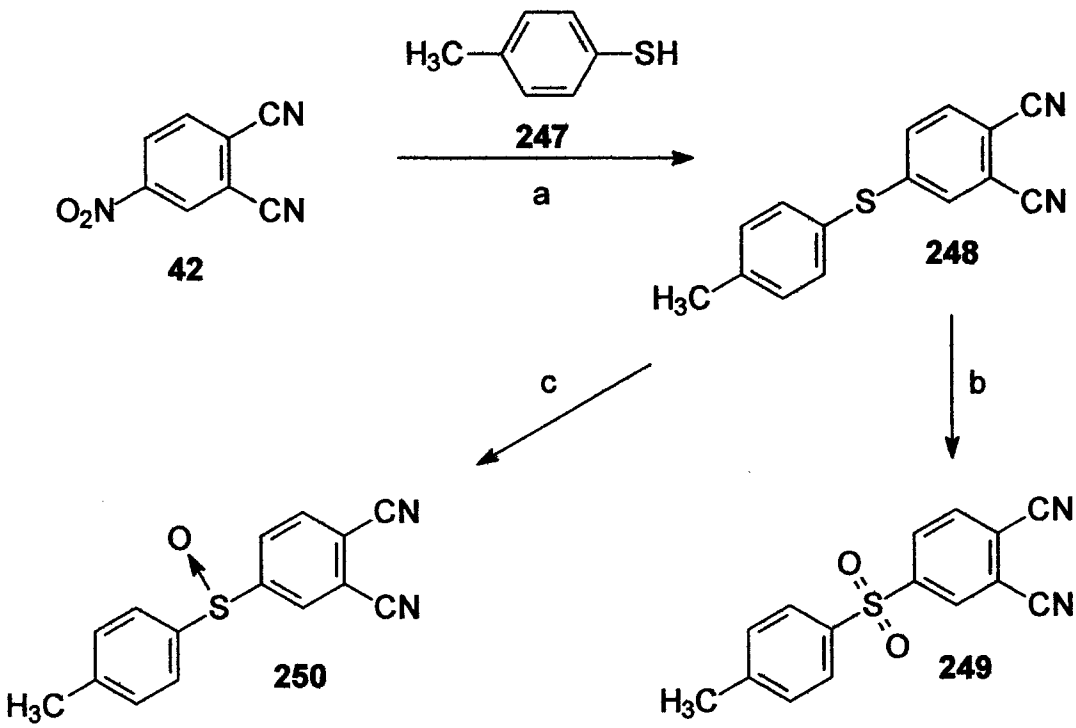


Figure 45. Synthesis of phthalonitriles bearing *p*-tolyl , *p*-tolylsulphinyl, and *p*-tolylsulphonyl groups. a) K_2CO_3, DMSO, rt, 12 h, 90%; b) MCPBA, CH_2Cl_2, 0 °C to rt, 30 min, 96%; c) MCPBA, CH_2Cl_2, -78 °C, 94%.[301]

the major product. Using the same basic protocol and sodium selenide gives the bisphthalonitrile selenoether.

a-3) Amine as nucleophiles

Despite the fact that activated nitro aryl compounds such as 3- and 4-nitrophthalonitrile are known to react quite well with ammonia and primary and secondary amines to give the corresponding aryl amines, little attention has been given to amine nucleophiles for the synthesis of monosubstituted phthalonitriles from nitrophthalonitriles. Perhaps this is because 3- and 4-aminophthalonitriles are easily synthesized from the nitro compounds and can readily be transformed into phthalonitriles bearing amine and amide substituents. Among compounds mentioned in the literature that have been prepared using amine nucleophile are 4-(phenothiazin-10-yl)phthalonitrile[305] **(251c)** and a series of heterylphthalonitriles where heterocyclic N-nucleophiles were used (Figure 46).[305,306] These include benzotriazole **(251a)**, 3,5-diphenyl-1,2,4-triazole **(251b)**, phthalazine **(251d)** and 4-quinazolinone **(251e)**. As with both alcohol and thio nucleophiles, amine-linked bisphthalonitriles have been formed using amine nucleophiles. One particular example is butane-1,4,7-tri-*p*-tolylsulphonyl-1,4,7-triamine.[307]

b) N_2^+ as the Leaving Group

Both 3- and 4-nitrophthalonitrile **(42,235)** are readily hydrogenated to the corresponding aminophthalonitrile **(252a,b)** using various catalysts, primarily 10% palladium/carbon.[89,93,237,283] This reduction can also be accomplished using iron metal or

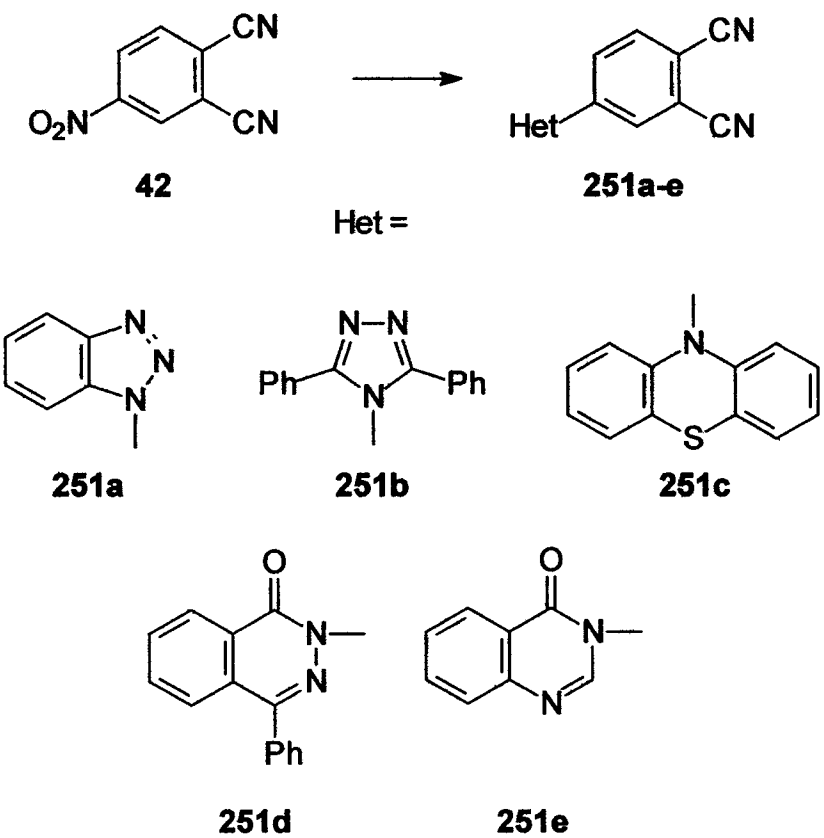
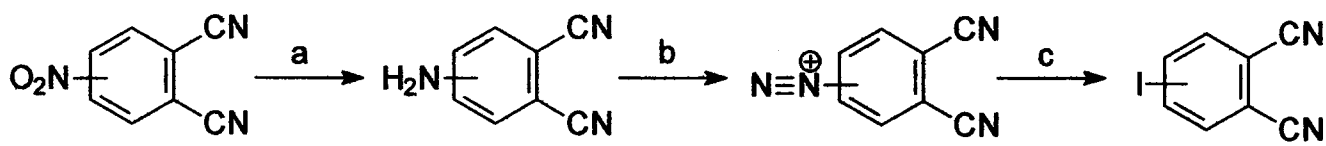


Figure 46. Examples of phthalonitriles substituted with heterocycle substituents synthesized using amine nucleophiles.[305,306]

stannous chloride in concentrated HCl/MeOH.[214,238] The resulting amino group can be protected and used to synthesize amino-substituted phthalocyanines.[214] It can also be used to add other functional groups to the molecule such as in 4-(octanoylamino)- and 4-[N-(methyloctanoyl)amino]phthalonitrile.[308] Nevertheless, the primary application of 3- and 4-aminophthalonitrile is to form substituted derivatives via diazonium salts **(253a,b)**. Diazotization of these compounds is accomplished using traditional conditions by treating the aminophthalonitrile **(252a,b)** with sodium nitrite in acidic media.[283] Note that 3-aminophthalonitrile requires slightly more forcing conditions, probably a result of increased steric hindrance.

The diazonium group is an excellent leaving group and can be replaced by a large number of possible substituents. Some of these reactions are indeed nucleophilic aromatic substitution reactions and proceed via the dissociative SN1 mechanism. In fact, the diazonium group is almost the only leaving group for aryl SN1 reactions. The only other example is aryl triflates where bulky groups occupy both ortho positions.[309] Reaction via the diazonium salt have been used to add interesting groups to the phthalonitrile molecule, one of which is ferrocene.[310] A primary reaction of diazonium salts in preparing substituted phthalonitriles is in the addition of iodide to the molecule **(228a,b)** (Figure 47). This is simply accomplished by reacting the diazonium salt with potassium iodide in water.[237,283] Similarly, 5-iodonaphthalonitrile has been prepared via the diazonium salt of 5-aminonaphthalonitrile.[237] Aryl iodides are versatile and are useful in reactions that add a number of different functional groups to phthalonitriles, especially via palladium-catalyzed reaction. Also of importance is the preparation of 3-



42, 235 **252a,b** **253a,b** **228a,b**

Figure 47. Synthesis of 3- and 4-iodophthalonitrile. a) H_2, 10% Pd/C, 95% EtOH; b) $NaNO_2$, 2.5M H_2SO_4; c) KI.[283]

and 4-chlorosulphonylphthalonitriles **(317)**,[311] useful precursors in the synthesis of sulphonated phthalocyanines (see below).[65,312]

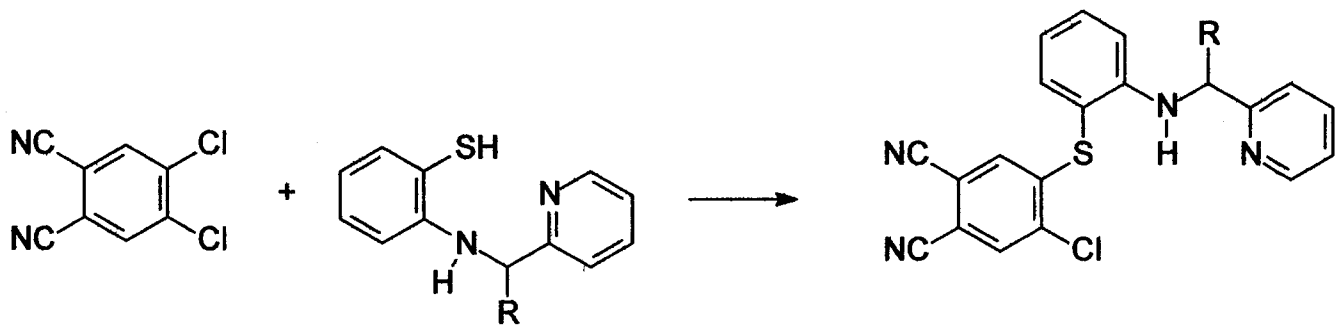
The analogous monochlorinated and monobrominated phthalonitriles can also prepared from the diazonium salt using cuprous chloride or cuprous bromide via the Sandmeyer reaction.[92] However, the Sandmeyer reaction does not proceed via nucleophilic aromatic substitution but via a free radical mechanism that is initiated by reduction of diazonium salt by the cuprous ion.[81] This is followed by halogen abstraction from the resulting cupric halide, resulting in the desired aryl halide and regeneration of the cuprous ion. Overall, however, the reaction conditions are very similar to the preparation of 3- and 4-iodophthalonitrile **(228a,b)**. For instance, 3-bromophthalonitrile is prepared via mixing 3-aminophthalonitrile with 48% hydrobromic acid and reacting it with a solution of sodium nitrite. The resulting diazonium salt is then treated with freshly prepared anhydrous cupric bromide.[92] Using a similar protocol, 5-chloro- and 5-bromo-2,3-naphthalenedicarbonitriles **(102)** have been synthesized from corresponding amino compound.[214] The Sandmeyer reaction is also useful in adding cyanide to aromatic compounds and its use has been described above in the preparation of 1,2-naphthalenedicarbonitrile and 3,5-di-t-butylphthalonitrile.[179-181] Another example is the synthesis of 4-methylphthalonitrile, which can be prepared from *p*-amino-*m*-toluonitrile using the Sandmeyer reaction.[313]

c) Halogen as the Leaving Group

While the commercial availability of 3- and 4-nitrophthalonitrile **(42,235)** make them the precursor of choice for the synthesis of monosubstituted phthalonitriles, similar

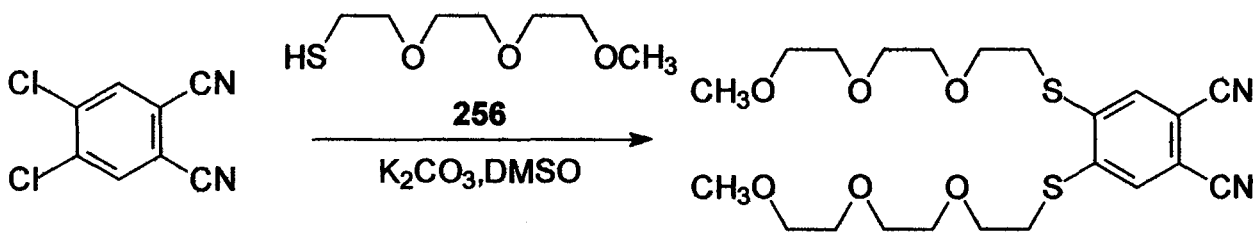
dinitro compounds are not so readily available. In this case, dihalogenated precursors such as 4,5-dichlorophthalonitrile **(23)** are easily prepared (Figure 7) and as such, halogen-leaving groups have been extensively used in the preparation of disubstituted phthalonitriles. In addition, halogenated precursors have been used in some cases for the synthesis of other substituted phthalonitriles and phthalonitrile derivatives. Reaction conditions are quite similar to those used with nitro leaving groups, though solvents such as THF and dimethylacetamide are also common. Both alcohols[314-316] and amines[317-319] have been used as nucleophiles in these halogen substitution reactions. However, unlike in the case of nitro leaving groups, alcohols are not as extensively used as nucleophiles. The majority of the work has been accomplished using thiols, in part because they are superior nucleophiles compared to alcohols and amines and give higher yields of the desired products.

Simple mercaptophthalonitrile, available from both nitrophthalonitrile[303] and its diazonium salt,[311] can also be prepared from the 3- and 4-iodophthalonitrile **(228a,b)** using thiourea as a nucleophile in the presence of a nickel(0) catalyst.[320] Simple thiols[299,314,315,321-323] and thiophenols[299,316] have been added to 4,5-dichloro- **(23)** and 4,5-diiodophthalonitrile **(38)**. Steric effects do influence this reaction to some extent as the bulky 2-(2-pyridylmethylamino)benzenethiol and 2-(2-pyridylethylamino)benzenethiol lead only to substitution of one of the two chloro groups of 4,5-dichlorophthalonitrile (Figure 48).[324] Thioethers synthesized in this manner can be oxidized to the corresponding alkyl- and arylsulphonyl groups using MCPBA- or hydrogen peroxide.[299,323] Disubstituted phthalonitriles containing polyethers such as 1-mercapto-4,7,10-trioxaundecane[304] **(257)** (Figure 49) and 3,6-dioxa-1-decylthiol[321] have also been



23 **254** **255**

Figure 48. Steric effects in the nucleophilic displacement of chloro groups in 4,5-dichlorophthalonitrile.[324]



38 **257**

Figure 49. Synthesis of 1-mercapto-4,7,10-trioxaundecane.[304]

synthesized. In the second example, the thiol was transformed to the thiolate ion, with the nucleophilic substitution promoted by copper(I) oxide. A similar procedure was used with butylmercaptan.[321] Among novel monosubstituted phthalonitriles prepared using nucleophilic displacement of halogen are phthalonitriles bound to adamantane via ether and thioether bonds.[314,315] In addition to phthalonitriles, halogenated naphthalonitriles can also undergo nucleophilic displacement of their halogen group by various nucleophiles. For instance, alkyl thiols and polyether thiols have been added to 6,7-dibromo-2,3-naphthalenedicarbonitrile using Cu_2O to promote the addition of the corresponding thiolate ion.[175,215,321] In other cases, the bromides of 6,7-dibromo-2,3-naphthalenedicarbonitrile were substituted with 1-dodecanethiol using DBU as the base[217] and by benzenethiolate, benzeneselenolate and benzenetellurolate ions by simply heating in DMF.[325]

Phthalonitriles fitted with oxygen-, nitrogen- and sulphur-containing crown ethers[317,326,327] and dioxa-dithia macrocycle-bridged dimeric phthalonitriles[328,329] have been prepared by nucleophilic displacement of the chloro groups of 4,5-dichlorophthalonitrile **(23)**. In addition, metal chelators have been added to phthalonitriles and ultimately phthalocyanines using such nucleophilic substitution reactions as well. Examples are based on 2-mercaptoethanol[330] (Figure 50) and 2-aminothiophenol.[316] It is definitely worth noting that when the reaction of 2-mercaptoethanol with 4,5-dichlorophthalonitrile **(23)** was carried out using sodium carbonate, both chloro groups were replaced.[328,329] However, when potassium or cesium carbonate was used as a base, only one of the chloro groups was replaced by 2-mercaptoethanol while the second was replaced by a hydroxyl group.[328] The differing

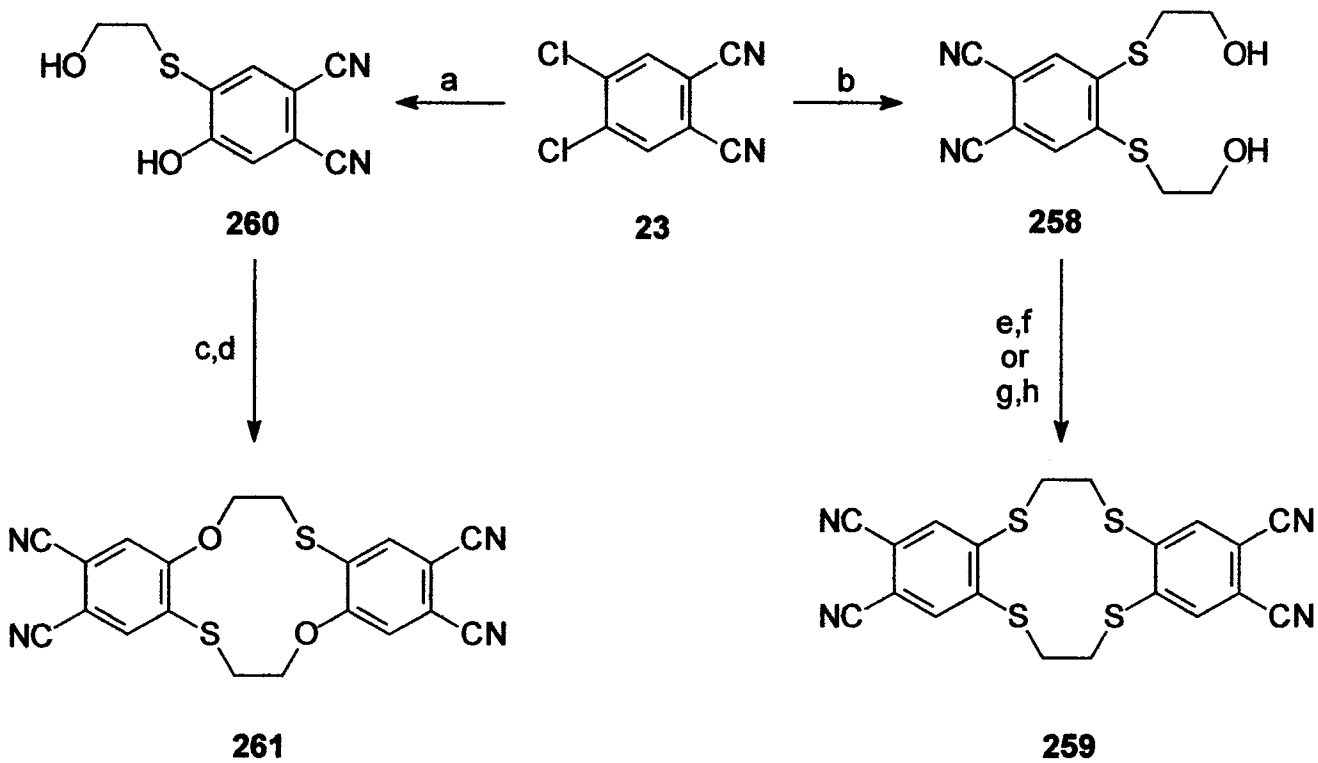


Figure 50. Synthesis of dioxa-dithia- and tetrathia macrocycle-bridged bisphthalonitriles. a) 2-mercaptoethanol, K_2CO_3, DMF; b) 2-mercaptoethanol, Na_2CO_3, DMF c) TsCl, pyridine; d) K_2CO_3, DMF; e) TsCl, DMF; e) 1,3-dimercaptoacetone, DMF; f) $SOCl_2$, DMF; g) 1,3-dimercaptoacetone, DMF or 1,2-dimercaptoethane, Na_2CO_3, DMF.[170,330]

template effects of Na^+, K^+ and Cs^+ ions was used to explained this outcome. The resulting 2-hydroxy-1-mercaptoethanol-phthalonitrile **(260)** was transformed to its tosylate and then readily underwent dimerization to give tetracyanodibenzo-[1,7-dithia(12 crown-4)] **(261)**.[328] The completely substituted 1,2-bis(hydroxyethylmercapto)-4,5-dicyanobenzene **(258)** could also be made into its tosylate or its chloride.[329] Attempts to close the ring to the crown ether with 1,3-dimercaptoacetone or 1,2-dimercaptoethane failed, leading exclusively to the 1,4,7,10-tetrathia-(12-crown-4)-bridged bisphthalonitrile **(259)**.

In addition to phthalonitriles and naphthalonitriles, substituted pyrazine dicarbonitriles have been prepared via nucleophilic aromatic substitution reactions using chloro leaving groups. In particular, 2,3-dichloro-5,6-dicyanopyrazine **(264)** is easily synthesized in high yields using diaminomaleonitrile **(262)** as a starting material (Figure 51)[331,332] and is in fact commercially available. This compound readily reacts with alcohol,[333] thiol,[332] and amino nucleophiles[331,334,335] to give the corresponding disubstituted 5,6-dicyanopyrazine. Reaction conditions vary, with alcohols added using triethylamine, thiols using pyridine in acetone and amines using sodium hydride in dioxane. Note that at least in the case of alcohols, the mono-substitution is clearly evident in the reaction mixture by TLC and the monosubstituted product can be obtained by carrying the reaction out at lower temperature or for shorter times.[333,336] Among the more interesting groups added are morpholine **(265)**, thiomorpholine, piperidine and pyrrolidine through amine linkages[334,335] and ethyl-, benzyl-, *p*-tolyl- and (5-methyl-1,3,4-thiadiazol-2-yl)sulphonyl groups by thiol linkages.[332] Unfortunately, the aromatic S-substituents are less useful as they are less stable and tend to decompose during the

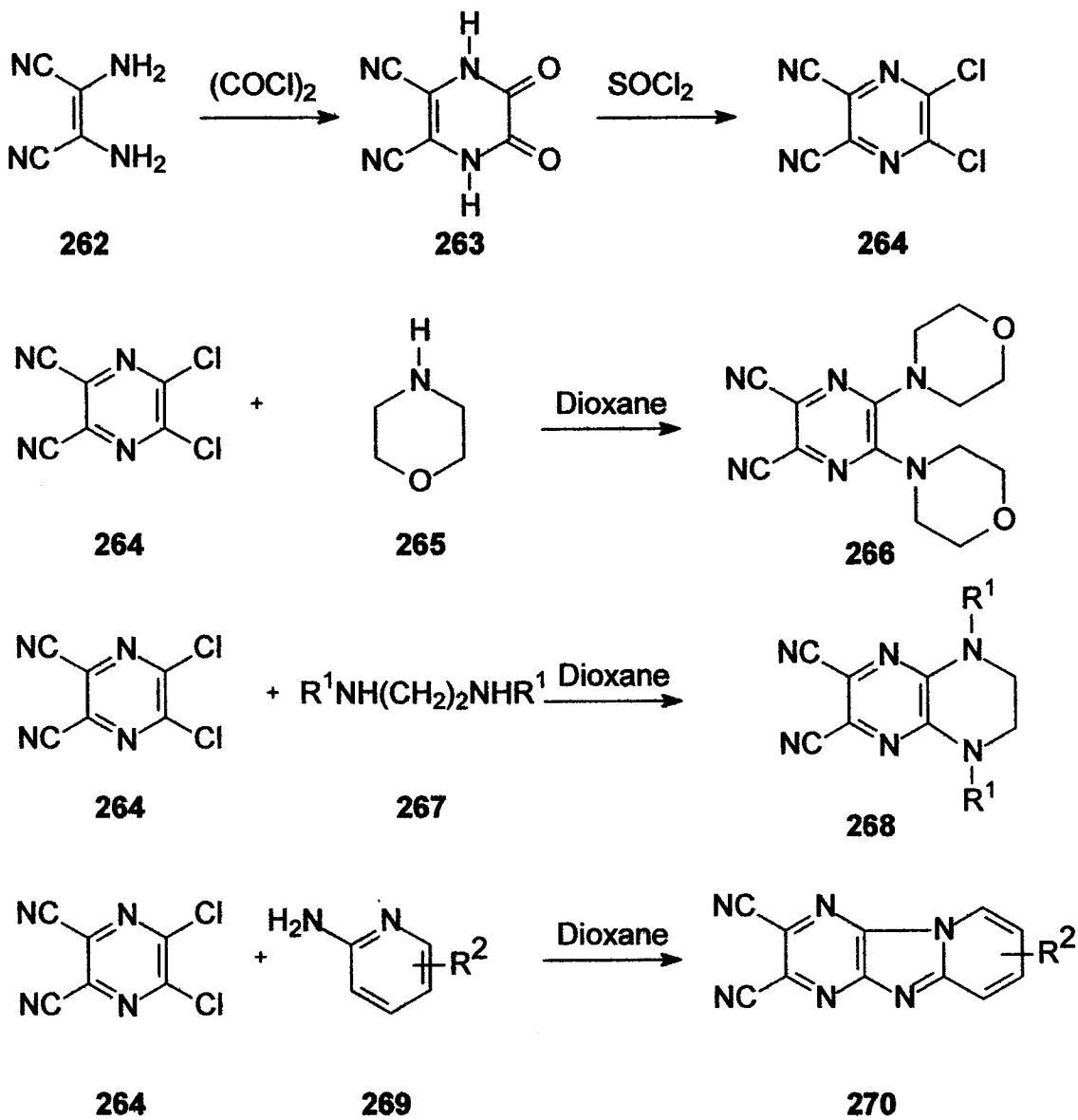


Figure 51. Synthesis of 2,3-dichloro-4,5-dicyanopyrazine and its reaction with various nucleophiles.[331,334] (R^1 = CH_2CH_3, Ph, CH_2Ph; R^2 = CH_3, OCH_2Ph, Cl, Br, NO_2).[331]

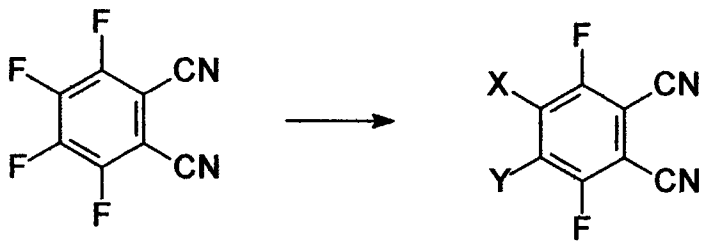
condensation reaction. One final interesting synthesis involves the reaction of 2,3-dichloro-5,6-dicyanopyrazine **(264)** with 2-aminopyridines **(269)**.[331] This simple reaction generates 2,3-dicyanopyrido[1′,2′:1,2]imidazo[4,5-b]pyrazine triheterocyclic ring systems **(270)** (Figure 51). This heterocyclic compounds are formed under mild conditions (dioxane at 20-80 °C). However, these ortho dinitriles have not been investigated as precursors to phthalocyanines. Similarly, reactions with amines, ethylenediamines **(267)**, propylenediamines and *o*-phenylenediamines give amine-substituted dicyanopyrazine along with six **(268)** and seven membered heterocyclic substituents.[334] These disubstituted 2,3-dicyanopyrazines were reacted with hydrazine hydrate to form nitrogen-rich heterocycles and were not examined as precursors for Pcs.

While the 4,5-dichlorophthalonitrile **(23)** is an extremely useful precursor for the synthesis of disubstituted phthalonitriles, tetrafluorophthalonitrile **(271)** is vital in the preparation of tetrasubstituted derivatives. Fluoride is far and away the best of the halogen leaving groups and is in fact, depending upon the nucleophile, superior to even NO_2 in terms of being a leaving groups for nucleophilic aromatic substitution reactions. Moreover, the addition of four strong electron-withdrawing fluorine groups further activates the phthalonitrile aromatic system towards nucleophilic attack. As such, nitrogen, oxygen, sulphur and phosphorus nucleophiles readily effect nucleophilic displacement of fluoride ion from tetrafluorophthalonitrile to give mono-, di- and tetrasubstitution products.

Tetrafluorophthalonitrile is commercially available and can be prepared by the reaction of tetrachlorophthalonitrile[337] with excess anhydrous potassium fluoride at 200-250 °C or via the Rosenmund-von Braun cyanodehalogenation of 1,2-

dibromotetrafluorobenzene.[132] This tetrasubstituted compound is particularly susceptible to nucleophilic attack at the four position. Dimethylformamide (dimethylamino), sodium 1-naphthaloate, aniline, N-methylaniline and ammonia all replace fluoride at the 4-position to give the corresponding trifluoro-monosubstituted phthalonitriles **(272a-e)** (Figure 52).[338] This chemistry has been extensively used in a number of Japanese patents to form trifluorinated phthalonitriles with amino, alkoxy and thioalkoxy bonded substituents at the four position.[339-345] Even diethyl malonate has been successfully added to the four position simply by using K_2CO_3 in DMF.[346] This functional group can be hydrolyzed to 2-(3,4-dicyano-2,5,6-trifluorophenyl)acetic acid **(274)** using hydrochloric acid in acetic acid (Figure 53). Interestingly, while the reaction of tetrafluorophthalonitrile **(271)** with lithium chloride in refluxing NMP gives tetrachlorophthalonitrile in good yields, the corresponding reaction in refluxing DMF yields 3,5,6-trichloro-4-dimethylaminophthalonitrile.[338] The same product can be obtained by refluxing tetrachlorophthalonitrile in DMF although the reaction is much slower, indicating that the overall reaction of tetrafluorophthalonitrile with LiCl in DMF proceeds via an initial displacement of fluorine by the dimethylamino group prior to chlorine substitution.

Despite the 4-position being the most susceptible to nucleophilic attack, the other fluoride ions can be replaced, with the extent depending on the nature of the nucleophile and the molar ratios used. For instance, as was stated above, tetrafluorophthalonitrile reacts with an excess of lithium chloride in refluxing NMP to give tetrachlorophthalonitrile.[338] However, using a 1:1 ratio of reagents leads to a mixture of 4-chlorotrifluoro and 4,5-dichloro-3,6-difluorophthalonitrile **(272f)** (Figure 52). Similar



271 **272a-h**

272a X = NH_2, Y = F

272b X = $N(CH_3)_2$, Y = F

272c X = 1-$C_{10}H_7O$, Y= F

272d X = NHC_6H_5, Y = F

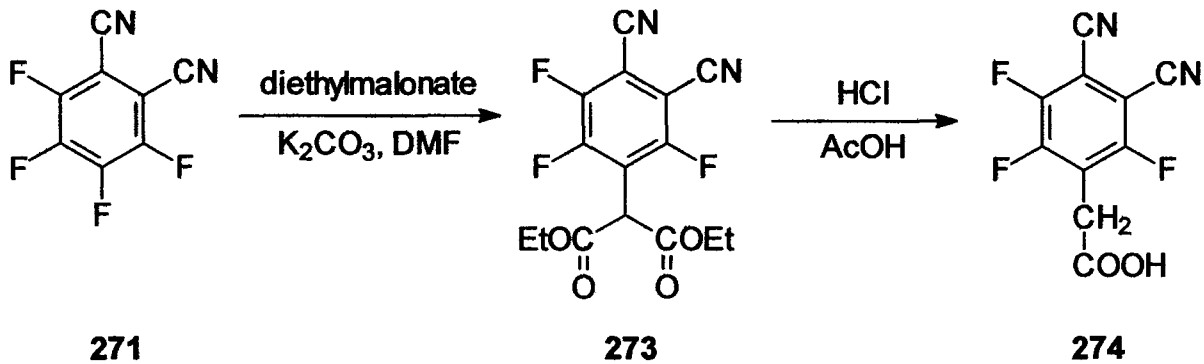
272e X = $N(CH_3)C_6H_5$, Y = F

272f X = Y = Cl

272g X = Y = PCH_2CH_3

272h X = Y = SC_6H_5

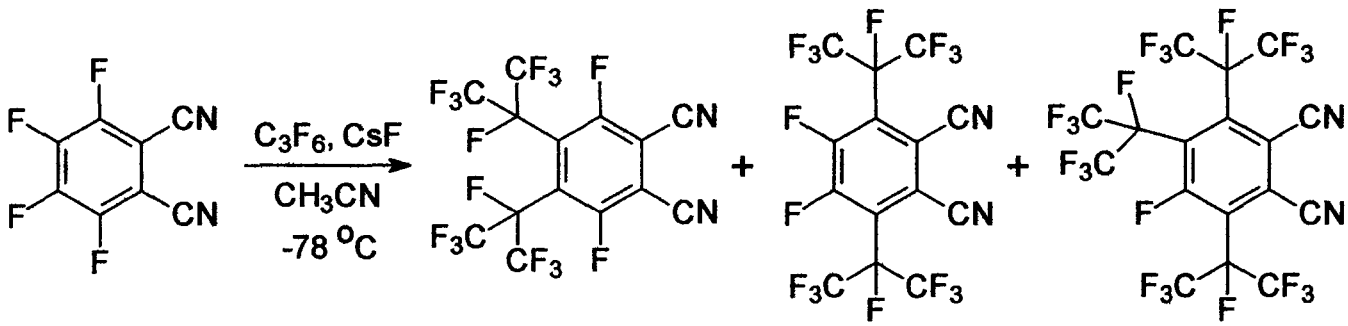
Figure 52. Nucleophilic displacement of fluorine atoms in tetrafluorophthalonitrile by various nucleophiles.[338]



271 **273** **274**

Figure 53. Synthesis of 2-(3,4-dicyano-2,5,6-trifluorophenyl)acetic acid.[346]

observations have also been observed in the reaction of tetrafluorophthalonitrile and lithium bromide. In the same fashion, equimolar quantities of sodium benzenethiolate and tetrafluorophthalonitrile in methanol give 62% 3,6-difluoro-4,5-bisphenylthiophthalonitrile **(272h)** and 8% tetrakisphenylthiophthalonitrile with the rest being unreacted starting material (Figure 52). Higher molar quantities of the thiolate results in the tetrasubstituted product exclusively. As such, a wide array of mixed tetrasubstituted phthalonitriles is possible. Perfluoro-(4,5-diisopropyl)- **(275)**, perfluoro-(3,6-diisopropyl)- **(276)** and perfluoro(3,4,6-triisopropyl)phthalonitriles **(277)** have been prepared by the reaction of tetrafluorophthalonitrile **(271)** with perfluoropropene in the presence of cesium fluoride in acetonitrile at –78 °C (Figure 54). The 4,5-disubstituted derivative is obtained in the highest yield.[347] 4-Monophenoxy- and 4,5-dithiophenoxy-substituted fluorophthalonitriles bearing sulphonate groups on the phenoxy and thiophenoxy benzene rings have presumably been prepared by nucleophilic displacement of fluoride ions as well.[348,349] Similar compounds substituted with benzene rings containing alkyl, alkoxy and ester groups have also been reported.[350] Other multiple substitution of fluorinated phthalonitriles has been accomplished, with the extent of substitution depending on the nucleophile involved.[318,319] Finally, a number of tetraalkoxyphthalonitriles **(278a-e)** have been synthesized from tetrafluorophthalonitrile **(271)** by reaction with the corresponding alcohol in DMF using potassium carbonate as the base.[351,352] Among the alcohols added were 1-hexanol, 2,2,2-trifluoroethanol, 2,2,3,3,3-pentafluoropropanol and 3,5-di-t-butylphenol (Figure 55).



271 275 276 277

Figure 54. Reaction of tetrafluorophthalonitrile with perfluoropropene in the presence of CsF.[347]

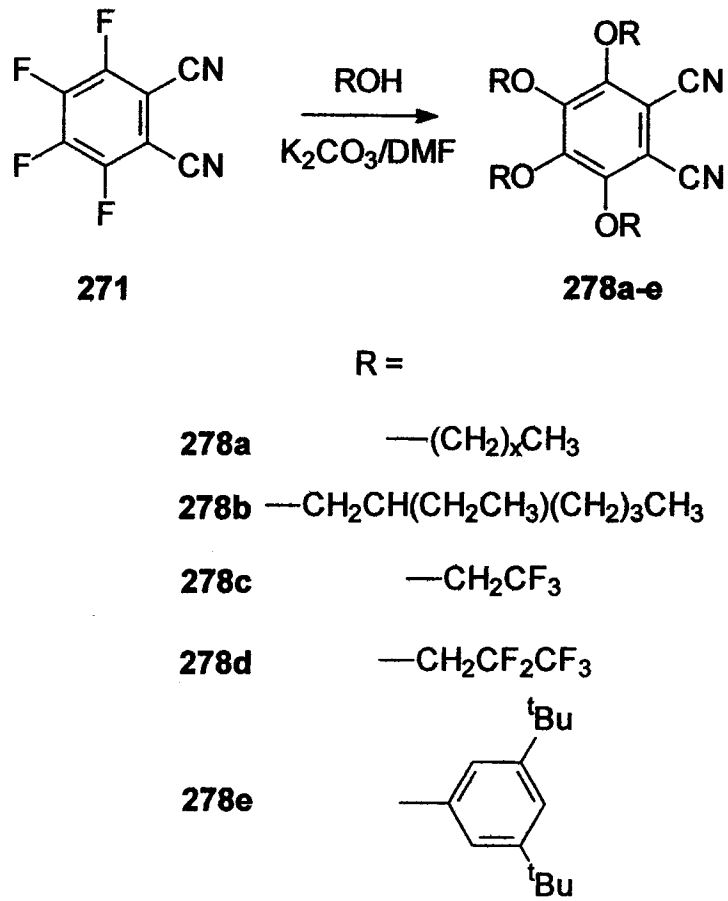


Figure 55. Synthesis of tetraalkoxyphthalonitriles by the nucleophilic aromatic substitution of the fluorine atoms of tetrafluorophthalonitrile.[351,352]

d) NO_2 and Br as the Leaving Groups

4-Bromo-5-nitrophthalonitrile **(47)** has been recently synthesized[105] and provides an excellent starting material for the preparation of 4,5-disubstituted phthalonitriles (Figure 13). Both the bromo and the nitro groups are extremely mobile leaving groups in SNAr reactions and thus, can be substituted with various nucleophiles. Importantly, these leaving groups exhibit different mobilities with the bromo group easier to eliminated. This is due to the fact that the nitro group, due to its electron-withdrawing effect, acts as an activator of nucleophilic attack on its ortho positions, thus activating the bromine carbon atom. Bromine, on the other hand, does not induce this effect. This difference in mobility enables the synthesis of asymmetric disubstituted phthalonitriles depending on the nucleophile and the conditions used. For instance, 4-bromo-5-nitrophthalonitrile **(47)** readily reacts with various phenols using potassium carbonate as the base under homogeneous (aqueous DMF) or heterogeneous (anhydrous DMF) conditions at 90 °C to give the 4,5-diphenoxyphthalonitriles **(279)** (Figure 56).[105] However, when the reaction temperature is lowered to 30 °C, only the bromide ion is replaced, giving rise to the 4-phenoxy-5-nitrophthalonitrile derivatives **(280)**. This can then be reacted with a different phenol at 90 °C to give novel asymmetrically substituted phthalonitriles **(281)**.

The mobility of the bromide ion is so high that it allows the nucleophilic substitution reactions with primary and secondary amines, substituted anilines and diamines to be carried out using protogenic solvents such as isopropanol and triethylamine as a base.[105] Under similar conditions, both 4-nitrophthalonitrile and 4-bromophthalonitrile give no reaction although reactions do proceed in aprotic solvents

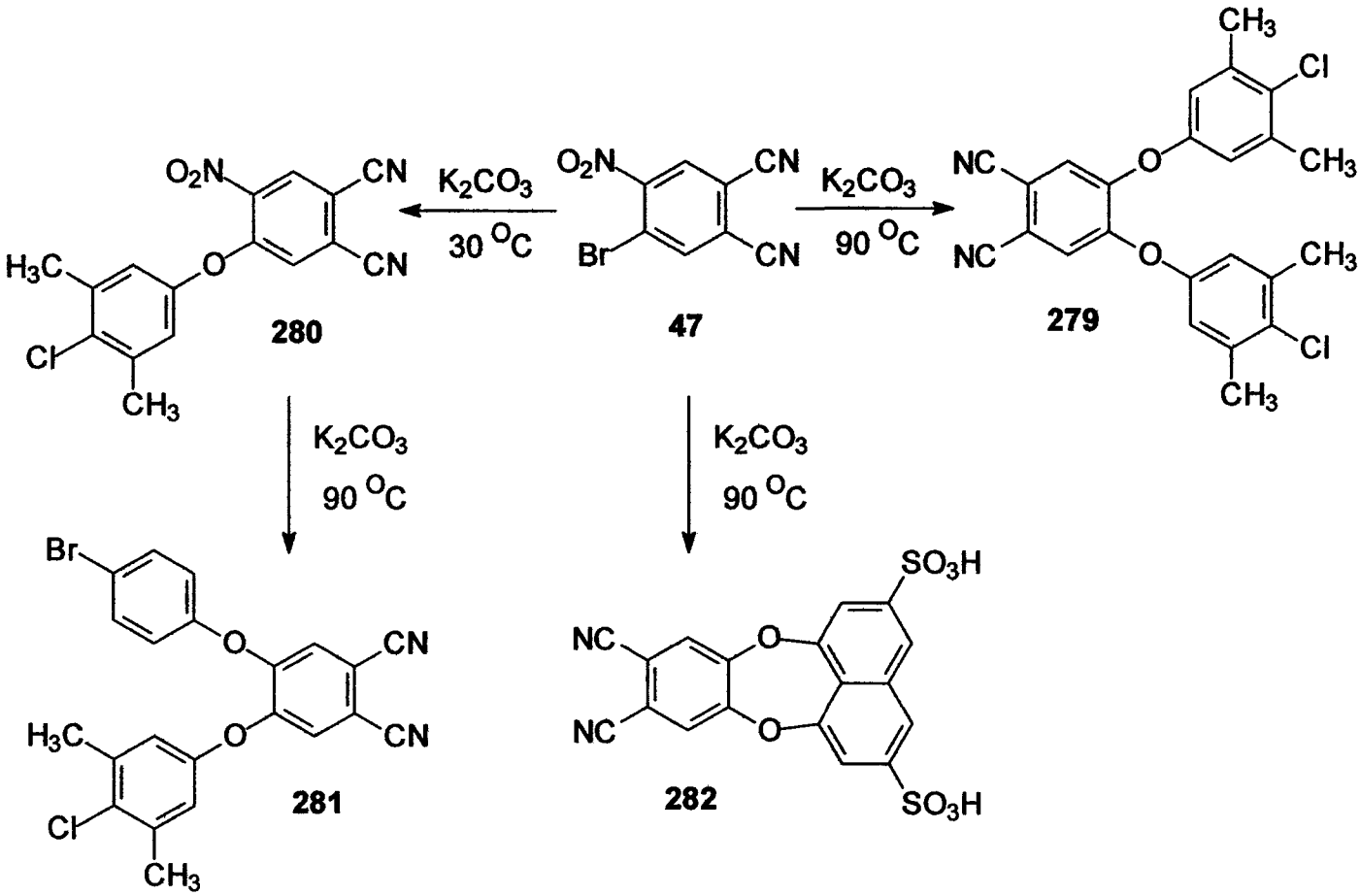


Figure 56. Synthesis of novel phthalonitriles from 4-bromo-5-nitrophthalonitrile.[105,354]

like DMF. Amines such as 3-aminomethyltetrahydrofuran, 4-chloroaniline and morpholine have replaced the bromide ion using protogenic solvents while leaving the nitro group unchanged. Furthermore, some bisphthalonitriles linked by 4,4'-diamino-biphenyl and 2,2'-diaminodiphenylether have also been prepared. On the other hand, reactions with 2-(2'-hydroxyphenyl)phenol, 1-(2-hydroxynaphth-1-yl)naphth-2-ol, 4,5-dihydroxynaphthalene-2,7-disulphonic acid (Figure 56) and quinoxaline-2,3-diol under both homogeneous and heterogeneous conditions at 90 °C gave the corresponding oxygen-containing heterocyclic ortho dinitriles.[354] Interestingly, no reports exist using the phthalonitriles synthesized using 4-bromo-5-nitrophthalonitrile to prepare phthalocyanines.

ii) Palladium-catalyzed reactions

Palladium-catalyzed reactions such as the Heck, Stille and Suzuki reactions have several features that make them extremely useful and versatile synthetic tools in organic chemistry. Palladium offers numerous possibilities for carbon-carbon bond formation and the addition of novel functional groups to the molecule. The mild conditions generally required for palladium-catalyzed organic synthesis make these methodologies extremely valuable in the addition of new functional groups to the molecule as palladium-based reactions can tolerate different and diverse functionalities. High chemical yields, facile reaction procedures and short synthetic sequences are among the many advantages of these important reactions. The accessibility of appropriate starting materials and the extreme versatility of these transition metal-assisted reactions also make them highly useful in the preparation of both substituted phthalonitriles and

phthalocyanines and the use of palladium catalysis to this end has been recently reviewed.[354]

a) Heck reaction

Palladium-catalyzed coupling of haloarenes and alkenes was first observed in the late 1960's and has come to be known as the Heck reaction.[355] This well established reaction and other mechanistically related palladium-catalyzed transformations between haloarenes and alkene or alkynes are indispensable reactions in the arsenal of synthetic organic chemists and have been used in vital steps towards the synthesis of compounds such as crinan and morphine.[356] The basic protocol tends to involve the reaction of terminal alkene or alkyne with an aryl halide (I > Br >> Cl)[356] in the presence of a palladium catalyst such as $(Ph_3P)_4Pd$, $(Ph_3P)_2PdCl_2$ and $Pd(OAc)_2$. More often than not, CuI is used as a co-catalyst and an organic base such as diethyl- or triethylamine is added. Mechanistically, this reaction, much like most transition metal-catalyzed reactions, involves oxidative addition of the palladium to the aryl halide bond followed by transmetalation and a reductive elimination of the palladium to give the desired product.[356,357]

In terms of phthalonitrile synthesis, extensive studies have been undertaken in order to add terminal alkynes to the molecule. Appropriate halogenated starting materials include 3- and 4-iodophthalonitrile **(228a,b)**, 4,5-dichloro- **(23)** and 4,5-diiodophthalonitrile **(38)** and 3,4-dibromophthalonitrile. For an example of their use in Heck reactions, a series of alkynyl-substituted phthalonitriles **(283a-e)** have been prepared using copper-free palladium(II) catalysis starting from 4,5-diiodophthalonitrile

(38) (Figure 57).[88,92] More sterically strained terminal alkynes such as 3,3-dimethyl-1-butyne and t-butyldimethylsilylethyne required more forcing conditions using CuI as a co-catalyst. In a similar fashion, 3,4-dibromophthalonitrile has been successfully coupled with 3,3-dimethyl-1-butyne using $(Ph_3P)_2PdCl_2$ and CuI in triethylamine/DMF.[92] While this reaction does not go to completion due to steric effects, leaving some monobrominated product, good yields of the desired 3,4-bis(3,3-dimethyl-1-butynyl)phthalonitrile were obtained. Reactions of terminal alkynes with 4,5-diiodophthalonitrile **(38)** can also lead to incomplete coupling when a 1:1 ratio of phthalonitrile and terminal alkyne is used.[88] Monoprotected acetylenes can be added to halogenated phthalonitriles using Heck chemistry and can be used in the synthesis of ethynyl-substituted phthalocyanines.[92,358] The protecting group can also be removed from the phthalonitrile, with, for example, the silyl groups of 4,5-bis(t-butyldimethylsilylethynyl)phthalonitrile **(284f)** being removed using tetrabutylammonium fluoride (TBAF) to give the unprotected terminal alkynes **(285)** (Figure 57).[92]

Long alkyl chains[88] have been added to phthalonitriles using this method as the alkyne group can be reduced using hydrogen and palladium on carbon (Figure 57). This is a very much desirable alternative to other multistep preparations of 4,5-dialkylphthalonitriles, which utilize the Rosenmund-von Braun reaction.[133,359-361] Other interesting disubstituted phthalonitriles synthesized using this coupling procedure include 4,5-bis(ferrocenylethynyl)phthalonitrile[362] and 4,5-bis[2-(4-nitrophenyl)ethynyl]phthalonitrile.[323] In the meanwhile, some novel alkyl-substituted phthalonitriles have been prepared via selective coupling of 1,2-dibromo-3,6-diiodo-4,5-

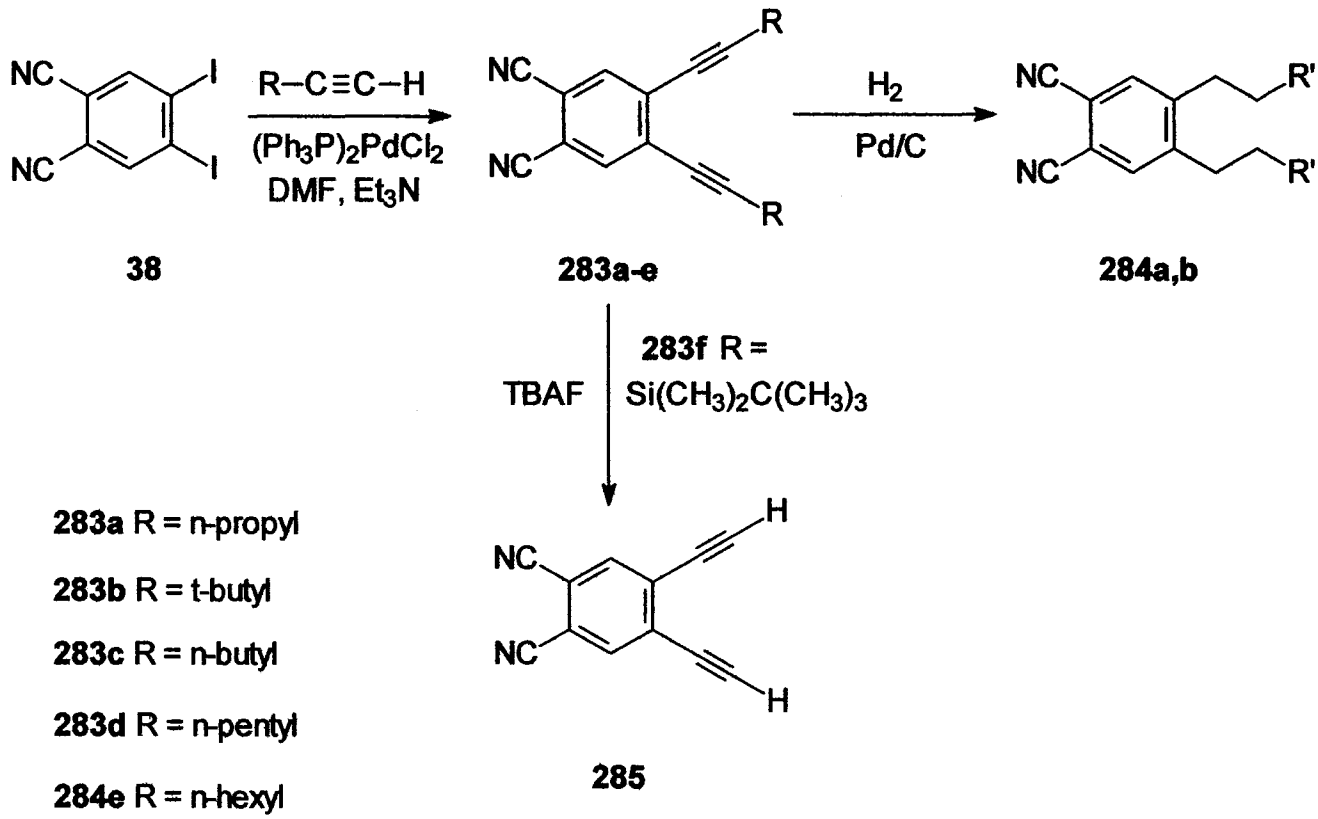


Figure 57. The use of the Heck reaction in the synthesis of substituted phthalonitriles (R = n-hexyl, n-pentyl, n-butyl, n-propyl, t-butyl; R' = n-propyl, t-butyl).[88] Note that for R = $Si(CH_3)_2C(CH_3)_3$, the Heck reaction utilizes CuI as a co-catalyst.[92]

dimethylbenzene **(286)** and 1,2-dibromo-3,6-diiodo-4,5-dihexylbenzene **(287)** with 1-hexyne and 1-heptyne using $(Ph_3P)PdCl_2$ and CuI as catalysts in triethylamine (Figure 58).[363] This coupling gave dibromobenzenes substituted with two alkyl chains and two alkynyl chains. Following conversion to the phthalonitrile using the Rosenmund von Braun reaction, catalytic hydrogenation gave novel mixed tetraalkylphthalonitriles **(290,291)**.

A number of monosubstituted phthalonitriles have been prepared using the same methodology, primarily starting from 4-iodophthalonitrile **(228a)** (Figure 39). Simple alkynes such as phenylacetylene,[238] 4-nitrophenylethyne,[323] 2-methylbut-3-yn-2-ol,[358] *tert*-butylacetylene and trimethylsilylacetylene[283] have been coupled successfully to 4-iodophthalonitrile using Heck conditions. Note that again, the silyl group can be removed to give ethynyl-substituted phthalonitriles.[283] In addition, novel functional groups have been added to the molecule using palladium-based chemistry. For instance, alkyl carboxylic acids have been added using $Pd(OAc)_2$ catalyzed coupling of benzyl pent-4-enoate to a halogenated phthalonitrile. Following hydrogenation of the double bond and hydrogenolysis of the benzyl-protecting group, the free alkyl terminal carboxylic acid is obtained.[212] Palladium-catalyzed alkynation of 4-iodophthalonitrile **(228a)** with propyn-1-ol and 5-hexyn-1-ol followed by catalytic hydrogenation gave 4-(propylhydroxy)phthalonitrile and 4-(hexylhydroxy)phthalonitrile, both of which can easily be transformed into the corresponding hydroxylated phthalocyanines.[364,365] The terminal hydroxy group has also been used in a Michaelis-Arbuzov reaction to prepare phosphonated phthalonitriles **(297)** (Figure 59).[365] Interestingly, aryl phosphonates can

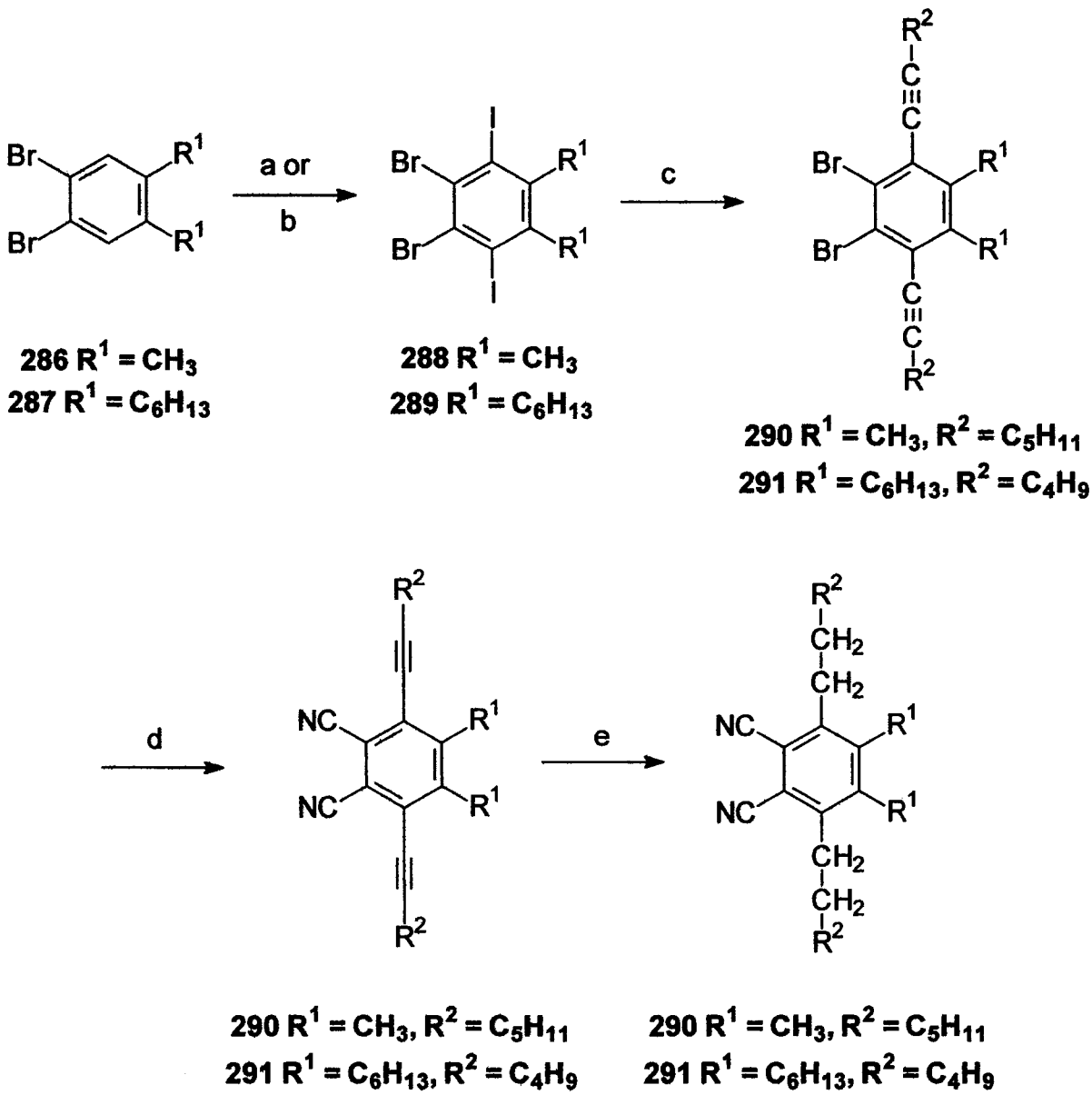


Figure 58. Mixed alkylated phthalonitriles prepared via palladium-catalyzed coupling of alkynes to the iodo group of 1,2-dibromo-3,6-diiodo-4,5-dialkylbenzene followed by a hydrogenation and a Rosenmund-von Braun reaction.[363] a) H_5IO_6, I_2, H_2SO_4; b) i) HgO, CF_3CO_2H, ii) NaI, I_2; c) alkyne, $(Ph_3P)_2PdCl_2$, CuI, Et_3N, DMF; d) CuCN, DMF; e) H_2, Pd. $R^1 = CH_3$ or $(CH_2)_5CH_3$, $R^2 = (CH_2)_3CH_3$ or $(CH_2)_4CH_3$

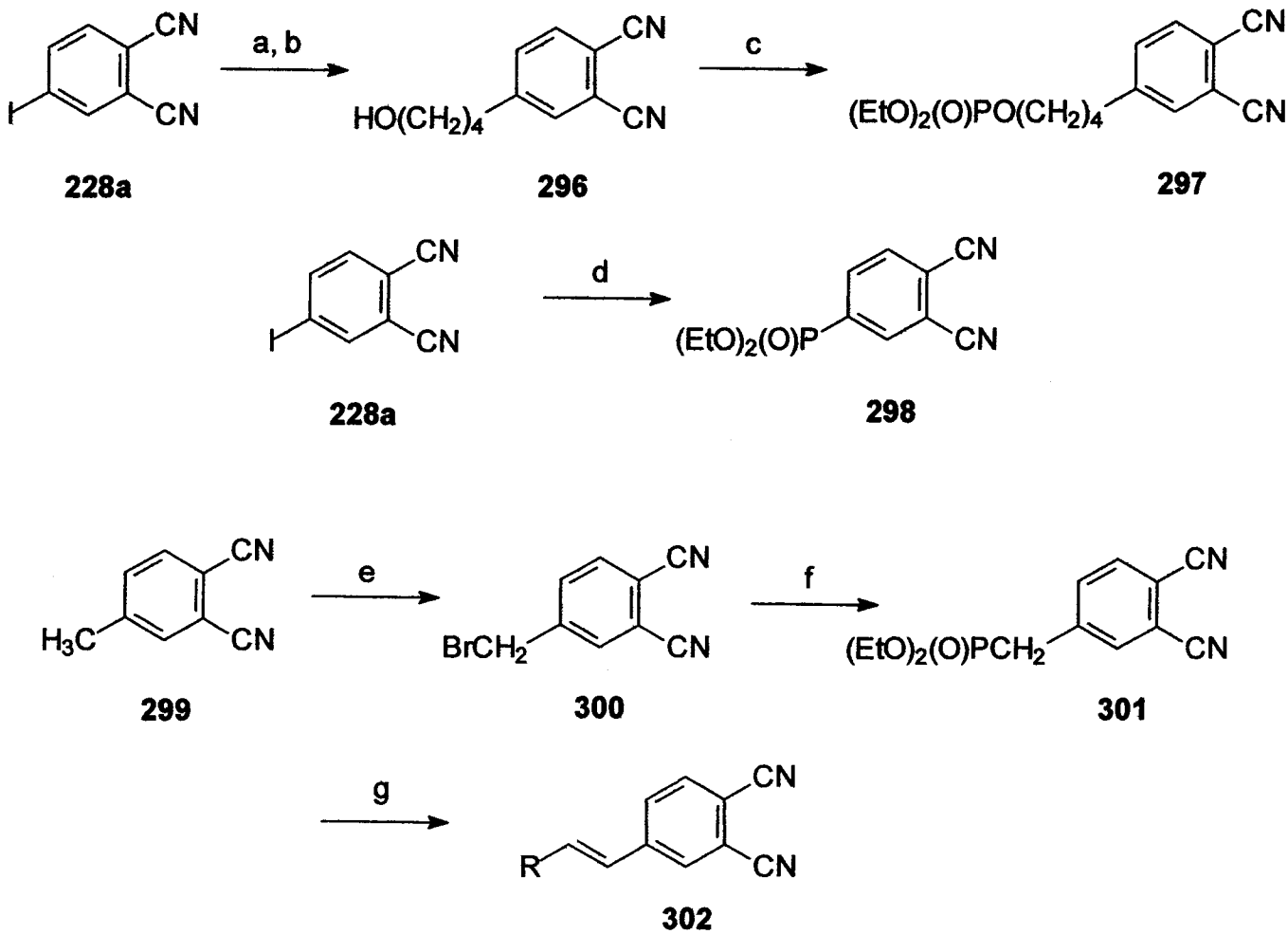


Figure 59. Synthesis of phosphonated phthalonitriles[153,365-369] and their conversion of vinyl groups by the Wittig-Horner reaction.[369] a) $HCCCH_2CH_2OH$, $(Ph_3P)_2PdCl_2$, CuI, Et_3N, DMF; b) H_2, 10% Pd/C, THF; c) $(EtO)_2P(O)Cl$, pyridine; d) $(EtO)_2P(OH)$, $(Ph_3P)_4Pd$, Et_3N, toluene; e) NBS, CCl_4; f) $P(OEt)_3$; g) RCHO, H_2O.

also be prepared using a palladium(0) catalyst and 4-diethyoxyphophinylphthalonitrile **(298)** has been synthesized by means of this methodology (Figure 59).[366] Other phthalonitrile phosphonate esters have been prepared via the Arbuzov rearrangement starting from 4-bromomethyl- **(300)** and 4,5-dibromomethylphthalonitrile **(69)** (Figure 18,59).[153,367,368] The Wittig-Horner reaction between aryl phosphonates and carbonyl compounds has been exploited to add substituted alkenes to such phthalonitriles (Figure 59), indicating the utility of phosphonated phthalonitriles.[369]

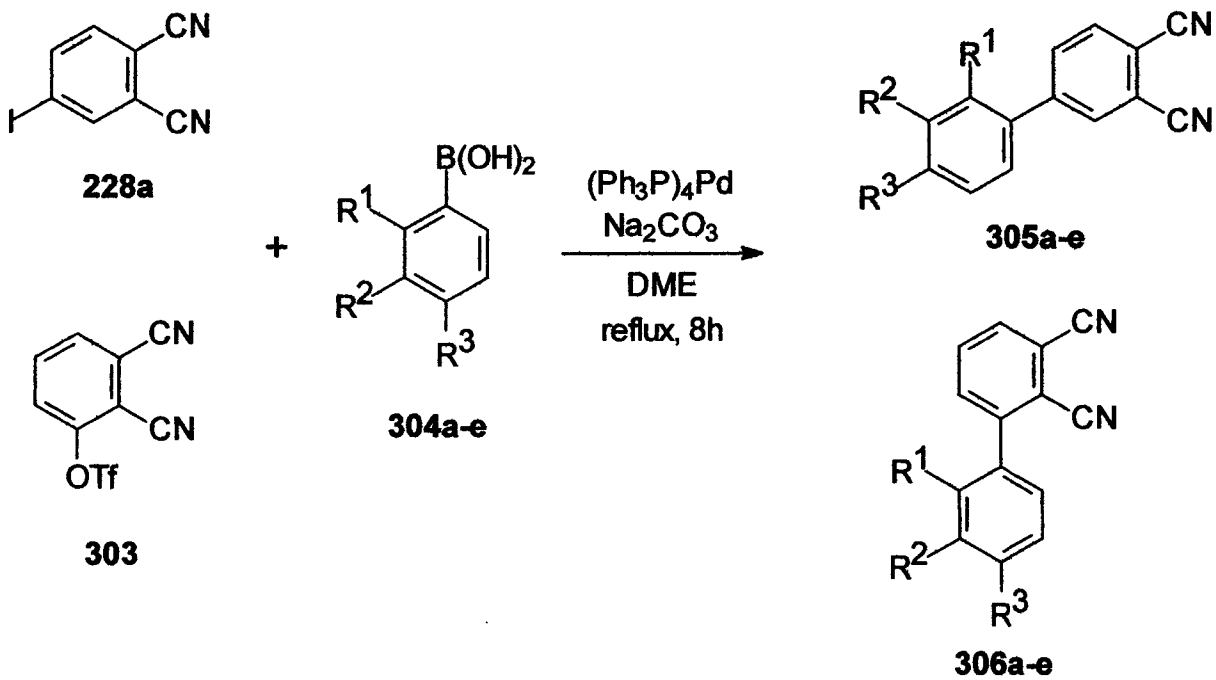
Coupling of 4-iodophthalonitrile **(228a)** with acetylene by way of identical Heck conditions gives phthalonitrile dimers linked by an alkyne bridge.[78,283] Partial[370] or complete[78,283] hydrogenation of the triple bond gives interesting linked phthalonitriles. Similar linked naphthalonitriles have also been prepared from 5-iodo-2,3-naphthalenedicarbonitrile.[237] In addition, butadiyne-linked phthalonitrile dimers have also been prepared from 4-(2-trimethylsilylethynyl)phthalonitrile (following removal of the trimethylsilyl group) by the copper-assisted coupling of terminal alkynes.[283] Hydrogenation ultimately led to butane-bridged phthalonitriles.

b) Suzuki reaction

The Suzuki reaction or Suzuki-Miyaura coupling involves the palladium-catalyzed cross coupling of organoboron compounds and organic halides or triflates and has been used extensively since its development in the mid 1990's in synthetic schemes towards important organic molecules.[371-373] In general, the reaction employs a palladium (0) catalyst such as $(Ph_3P)_4Pd$ and a base, typically potassium or sodium carbonate. The availability of the necessary reagents and the mild reaction conditions all contribute to the

versatility of this reaction. The Suzuki coupling reaction offers several important advantages including toleration of a broad range of functional groups, lenience for water in the reaction mixture and the ability to control regio- and stereoselectivity.[371-373]

While the Suzuki-Miyaura coupling has not been highly investigated in phthalonitrile synthesis, it has recently started to gain attention as a valuable method for adding functionality to these molecules. Starting with 4-iodophthalonitrile **(228a)** and 3-trimethylfluoromethanesulphonyloxyphthalonitrile **(303)**, phenyl-substituted phthalonitriles **(305,306)** were synthesized in high yields based on the Suzuki-Miyaura cross coupling reaction (Figure 60).[374] The necessary phenyl boronic acids **(304)** were readily prepared from bromobenzene derivatives via a Grignard reagent. The coupling reaction with the appropriate phthalonitrile precursor was carried out at 90 °C in 1,2-dimethoxyethane using tetrakis(triphenylphosphine)palladium(0) and potassium hydroxide. Phenyl groups both unsubstituted and substituted with methyl and methoxy groups were successfully introduced. Yields were roughly 80%. Disubstitution with 2-thienyl substituents has also been accomplished via a Suzuki coupling (Figure 61).[375] In this case, thiophene-2-boronic acid **(308)** was coupled to 4,5-dibromophthalonitrile **(307)** using $(Ph_3P)_4Pd$ as the palladium catalysis and gave the desired product **(309)** in a 62% yield. As an indication of the versatility of the Suzuki coupling and the range of reaction conditions available, this reaction was carried out using toluene/THF/EtOH as the solvent mixture and employed 2 M aqueous Na_2CO_3 as the base. Work is also be undertaken towards the synthesis of phthalonitrile boronic acid derivatives in the hope of using them to prepare a large range of substituted phthalonitriles (unpublished results).



a $R_1 = R_2 = R_3 = H$
b $R_1 = CH_3$, $R_2 = R_3 = H$
c $R_1 = H$, $R_2 = CH_3$, $R_3 = H$
d $R_1 = R_2 = H$, $R_3 = CH_3$
e $R_1 = OCH_3$, $R_2 = R_3 = H$

Figure 60. Synthesis of phthalonitriles substituted with phenyl substituents using the Suzuki coupling reaction.[374]

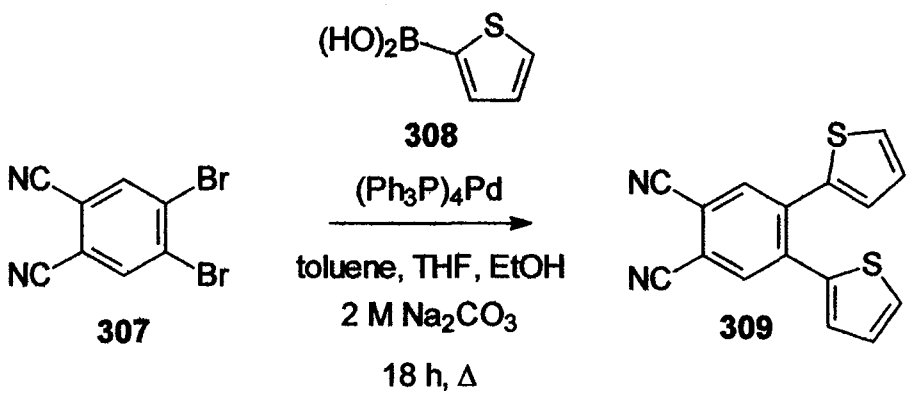


Figure 61. Use of the Suzuki coupling reaction to prepare 4,5-di-2-thienylphthalonitrile.[375]

c) Stille coupling

The Stille coupling of organotin reagents with a variety of organic electrophiles using palladium catalysis provides a novel method for generating carbon-carbon bonds and is becoming an important reaction in organic synthesis.[376,377] Like most palladium-catalyzed reactions, Stille coupling reactions are mild and extremely versatile and tolerate a wide variety of functional groups in either coupling partner. They also exhibit stereo- and regioselectivity and give high yields of the desired product. Compared to other cross-coupling reactions including those of organomagnesium, organozinc, organoboron and organosilicon reagents, the Stille reaction has a wide scope mainly due to the stability and low cross reactivity of organotin compounds. Like in Heck reactions, the mechanism employs a palladium (0) catalyst and involves an oxidative addition/transmetalation/reductive elimination pathway.[378] Unfortunately, the low reactivity of organostannanes also has drawbacks, with high reaction temperatures and resulting side reactions limiting its usefulness. However, recent attempts to improve the reaction by increasing the rate of the transmetalation step (the rate-determining step) using various different ligands for the palladium catalyst and employing copper co-catalyst have refined the situation immensely.[378]

It has only been recently that the versatility of the Stille coupling reaction has been taken advantage of in the preparation of novel phthalocyanines. One such example employed the Stille coupling in the preparation of arylated phthalonitriles.[93] 4-Phenylphthalonitrile, 4-(2,5-dimethoxyphenyl)phthalonitrile and 2-(3,4-dicyanophenyl-4-methylpyridine **(312)** were synthesized in good yields from 4-iodophthalonitrile and

appropriate organotin reagents (Figure 62). The organotin compounds were simply acquired from aryl bromides by treating with n-butyl lithium to form the anion followed by treatment with trimethyltin chloride. It was found that $(Ph_3P)_4Pd$ was not an ideal catalyst for this reaction with coupling occurring only in certain cases. Dibenzylideneacetone (dba) proved to be a superior ligand for the Pd(0) and gave satisfactory results. Stille coupling was also employed in the preparation of bisphthalonitriles and bisnaphthalonitriles coupled by a alkene bridge.[237] Trans-1,2-bis(tri-n-butylstannyl)ethene was coupled with 3-iodophthalonitrile **(228b)** and 5-iodonaphthalonitrile to give the corresponding trans bridged compounds using $(Ph_3P)_4Pd$ as the catalyst. Coupling of 4-iodophthalonitrile **(228a)** with both the cis and trans-1,2-bis(tri-n-butylstannyl)ethene[214,237] proceeds smoothly as well and these compounds were photocyclized to tetracyanophenanthrene derivatives. Finally, in a interesting series of reactions, 4-vinylphthalonitrile **(313)** was synthesized via the Stille coupling of 4-iodophthalonitrile **(228a)** with tributyl(vinyl)tin induced by $(Ph_3P)_4Pd$ at 100 °C in toluene (Figure 62).[379] The same compound was also prepared by the reaction of vinylzinc in the presence of the same Pd(0) catalyst though yields were significantly less in this case.[379] Dehydration of 3,4-dibromo-α-phenyl ethyl alcohol followed by the Rosenmund-von Braun cyanodehalogenation also gives the same product.[380] Oxidative cleavage of the resulting 4-vinylphthalonitrile **(313)** by ozonolysis gave rise to 4-formylphthalonitrile **(314)**.[379] This precursor reacted with fullerene (C_{60}) and sarcosine to yield the phthalonitrile fulleropyrrolidine derivative.

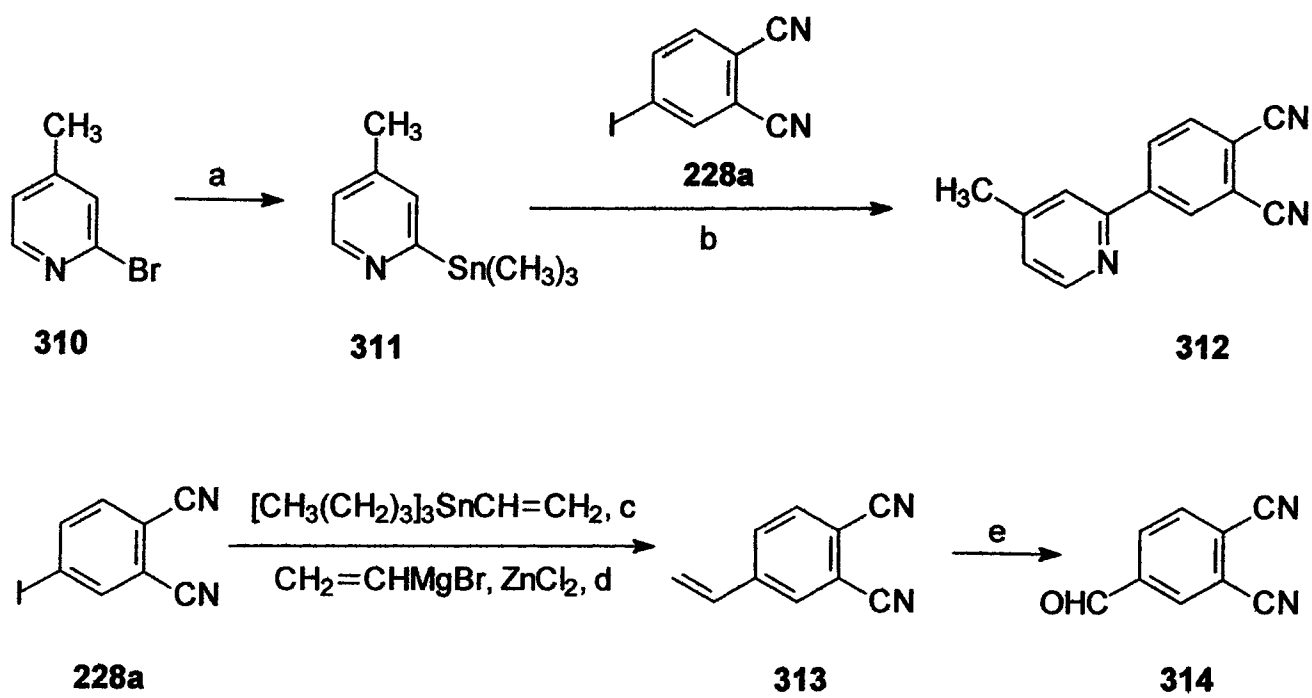


Figure 62. Examples of the use of the Stille coupling reaction in the synthesis of substituted phthalonitriles. a) i) nBuLi, THF, -78 °C, ii) CH_3SnCl, THF, -78 °C to RT, 85%; b) Pd_2dba_3, DMF, 80-85 °C, 66%; c) $(Ph_4P)_4Pd$, toluene, 100 °C, 97%; d) i) $(Ph_4P)_4Pd$, THF, 45 °C, 70%; e) O_3, CH_2Cl_2, -78 °C, 90%.[93,379]

d) Other transition metal-catalyzed coupling reactions

Other phthalonitrile dimers have also been formed using transition metal catalysis. The synthesis of directly linked phthalonitriles and ones linked by a naphthalene bridge utilize nickel(0) catalyzed coupling of aryl iodides.[381] Anthracene-bridged phthalonitrile dimers require transformation of 1,8-dichloroanthracene to its arylzinc derivative prior to palladium-catalyzed coupling with 4-iodophthalonitrile. This is primarily due to the lack of reactivity of chloro groups towards the nickel(0) coupling reaction and the fact that 1,8-diiodoanthracene is unknown.

iii) 2,3-Dicyanohydroquinone derivatives

An important series of precursors for the preparation of 3,6-dialkoxyphthalonitriles are 2,3-dicyanohydroquinone derivatives. These include 2,3-dicyanohydroquinone (or 1,4-dihydroxyphthalonitrile) **(315)** itself along with 2,3-dichloro-4,5-dicyanohydroquinone and 2,3-dicyanonaphthalene-1,4-diol. 2,3-Dicyanohydroquinone **(315)** is commercially available while 2,3-dichloro-4,5-dicyanohydroquinone can be easily prepared by sodium metabisulphite reduction of the corresponding quinone.[382] The synthesis of 2,3-dicyanonaphthalene-1,4-diol has already been mentioned and involves the reaction of 2,3-dichloronaphthoquinone with potassium cyanide.[173,182,383] These compounds provide access to important 1,4-dialkoxyphthalonitriles and 1,4-dialkoxy-2,3-naphthalenedicarbonitriles. The necessary ether bond is simply formed by the reaction of these diols with alkyl halides under basic

conditions (usually potassium carbonate). While reactions tend to be prolonged, good yields of the desired alkoxy compounds are obtained.

A whole series of 1,4-dialkoxyphthalonitriles has been prepared with alkoxy chains ranging from methyl through to dodecyl **(316a-k)** (Figure 63).[276] In addition, alkene (pent-4-enyl) **(316l)** and phenyl (3-phenylpropyl) **(316m)** functional groups have been included as well. Other 3,6-dialkoxyphthalonitriles that have been synthesized in this manner include 3,6-bis(isopropyloxy)phthalonitrile,[384] 3,6-bis(2-hydroxyethoxy)phthalonitrile,[385] 3,6-bis[(trimethylsilyl)methoxy]phthalonitrile[277] and 3,6-bis[methoxy(oligoethyleneoxy)]phthalonitrile.[263] Alkylation of 2,3-dicyanonaphthalene-1,4-diol has led to simple 1,4-dialkoxy-2,3-naphthalenedicarbonitriles as well.[173,276] Of particular interest however are 4,5-dichloro-3,6-dialkoxyphthalonitriles[276] as such chlorinated phthalonitriles are susceptible to nucleophilic attack. Numerous nucleophiles including thiols[386-389] and amines[386] have been used in preparing novel tetrasubstituted phthalonitriles with mixed substitution. In some cases, this nucleophilic attack can be accomplished stepwise, allowing the addition of two different substituents to the molecule.[386,387] Furthermore, heterocyclic phthalonitrile derivatives can be synthesized using nucleophiles such as 2-aminothiophenol.[286] Note that in addition to the simple alkoxy groups mentioned above, other alkoxyalkyl, dialkylaminoalkyl, and alkylene heterocyclic substituents are also possible functional groups that can be added via an ether bond to these phthalonitrile derivatives.[386,387,389]

4,5-Dialkoxyphthalonitriles, though generally prepared from catechols using the Rosenmund-von Braun reaction , can also be synthesized from 4,5-

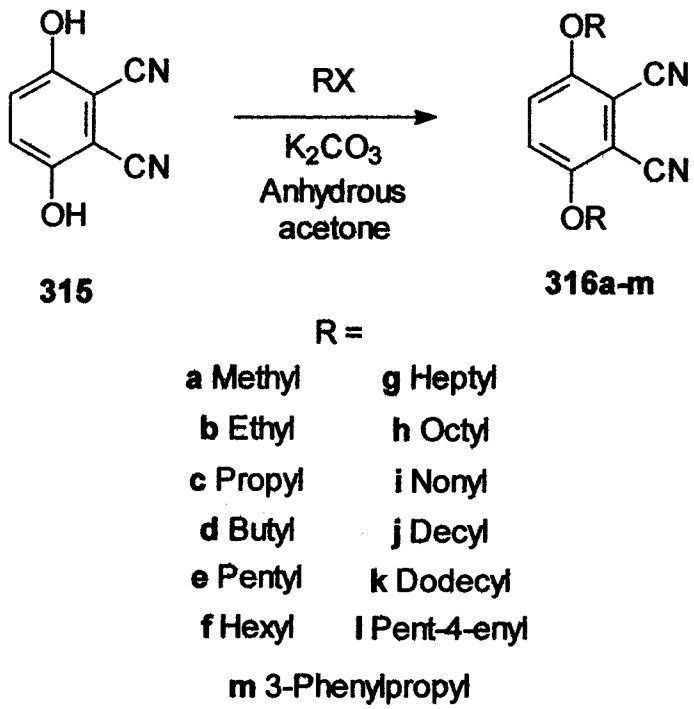


Figure 63. Series of 1,4-dialkoxyphthalonitrile synthesized by the alkylation of 2,3-dicyanohydroquinone.[276]

dihydroxyphthalonitrile via ether bond formation. Dithioalkoxyheterocycle-substituted phthalonitriles are examples of phthalonitriles synthesized in this fashion.[390]

iv) Halogenation

Halogenated phthalonitriles are clearly extremely important starting materials in the preparation of novel substituted phthalonitriles, especially via nucleophilic substitution and palladium-catalyzed reactions. Simple monohalogenated phthalonitriles are prepared from the corresponding amino compound via the diazonium salt as has been previously mentioned (Figure 47). However, polyhalogenated phthalonitriles are not so easily accessible and require more forcing conditions in their synthesis. Generally, such reactions proceed by an electrophilic aromatic substitution pathway. The synthetic pathway towards 4,5-diiodophthalonitrile **(38)**, for instance, begins with the iodination of phthalimide **(5)** with iodine in fuming sulphuric acid (Figure 12).[88,89] This results in the formation of primarily 4,5-diiodophthalimide **(33)** with both 3,4-diiodophthalimide **(34)** and 4,5-diiodophthalic acid **(35)** as byproducts. Increased amounts of 3,4-diiodophthalimide **(33)** can be obtained when the iodination is carried out at higher reaction temperatures. The desired products can then be isolated, with the phthalic acid removed by Soxhlet extraction and the 3,4- and 4,5-diiodo derivatives separated following ammonolysis and dehydration to the corresponding phthalonitriles. Note that the 4,5-diiodophthalimide **(33)**, even at the higher reaction temperatures, is obtained in an 80% yield compared to only 10% of the 3,4-diiodo derivative. The corresponding tetraiodophthalimide can be obtained by iodination of phthalimide or phthalonitrile with iodine and periodic acid in concentrated sulphuric acid.[391] However, attempts to

transform the resulting tetraiodophthalimide into tetraiodophthalonitrile have failed, possibly due to steric hindrance. A number of other iodinated phthalic acids and oxidized iodinated phthalic acids have also been prepared.[392] Attempts to transform these into phthalonitriles have not been undertaken.

Bromination of phthalimide can be accomplished in the manner akin to the preparation of 4,5-diiodophthalimide **(33)** using bromine in fuming sulphuric acid using iron as a catalyst.[92] 4,5-Dibromophthalimide is obtained with 4,5-dibromophthalic acid as an important byproduct that can be relatively easily removed. The corresponding 4,5-dibromophthalonitrile **(307)** is then obtained following ammonolysis and dehydration as seen above. In addition, phthalonitrile can be brominated using N,N-dibromoisocyanuric acid (DBI) in 8% fuming sulphuric acid.[89,92] These rather harsh conditions lead to a mixture of mono- and dibrominated phthalonitrile along with traces of tri- and tetrabrominated compounds as well. The primary product is 4-bromophthalonitrile (45.2%) with significant amounts of 3,6-dibromophthalonitrile (7%), 4,5-dibromophthalonitrile (6.7%) and 3,4-dibromophthalonitrile (5.9%). Each of these could be isolated using column chromatography. 3-Bromophthalonitrile could not be isolated from unreacted starting material however. As has been mentioned before, the tetrabrominated phthalonitrile can also be obtained from tetrafluorophthalonitrile by nucleophilic exchange[338]

The bromination of 2,3-dicyanohydroquinone has also been accomplished using N-bromosuccinimide (NBS) in t-butyl alcohol, giving rise to 2,3-dibromo-4,5-dicyanohydroquinone.[393] Unlike above, alkoxylation with 1-butanol was accomplished using triphenylphosphine and diisopropylazodicarboxylate in dry THF. Using potassium

carbonate and N,N,N-tributyl-1-butaninium bromide in 2-butanone with 1-iodobutane led to loss of one of the bromine atoms and gave 4-bromo-3,6-dibutoxyphthalonitrile. These compounds have both been used to prepare phthalocyaninodehydroannulenes.

Much less work has been done on the synthesis of chlorinated phthalonitriles. However, with tetrachlorophthalonitrile being an essential precursor to tetrafluorophthalonitrile **(271)**, much work has been done on the preparation of this compound. One method that has been developed utilizes vaporized phthalonitrile and treats it with chlorine gas in the vapour phase over various catalysts.[394] A second possibility involves chlorination of *o*-xylene and inducing phthalonitrile formation via reductive ammonolysis.[113] Note that the corresponding trichlorinated and tribrominated phthalonitriles can be obtained by reaction of the tetrahalogenated compound with solid metals in water and solvents incompatible with water.[395] The trifluorophthalonitrile can also be obtained by heating these trihalogenated compounds with a fluorinating agent.

Other halogenation reactions of interest include the bromination of catechol and its alkoxy derivatives, which is generally done using bromine in acetic acid. The free radical benzylic bromination of 4-methyl- **(299)** and 4,5-dimethylphthalonitrile **(68)** and 1,2-dibromo-*o*-xylene **(64)** is accomplished using NBS and a free radical initiator (Figure 17, 59).[157,158] A somewhat similar reaction that proceeds via an electrophilic aromatic substitution mechanism is nitration of phthalimide and 2,3-naphthalenedicarbonitrile, which utilizes nitric acid in sulphuric acid (Figure 11).[54,93,214] This reaction occurs in nearly identical fashion as the halogenation of the aromatic compounds.

v) Reactions of sulphonated phthalonitriles

Sulphonated phthalocyanines are extremely important compounds in a number of applications, in particular in the photodynamic therapy of cancer.[38-42] While these compounds are often synthesized from 4-sulphophthalic acid[46] and sulphonated phthalocyanines are often modified following macrocycle formation,[396] a number of sulphonated phthalonitriles have been prepared and are used in the synthesis of phthalocyanines.[311] Among appropriate precursors for the synthesis of sulphonated phthalocyanines are 3- and 4-chlorosulphonylphthalonitrile **(317a,b)** (Figure 64) , 3- and 4-mercaptophthalonitrile **(320a,b)** and bis(2,3-dicyanophenyl) disulphide **(321)** (Figure 65).[311] 3- and 4-Chlorosulphonylphthalonitriles **(317a,b)** can simply be prepared by the reaction of the diazonium salt of the corresponding aminophthalonitrile **(252a,b)** with sulphur dioxide in acetic acid in the presence of cuprous chloride (Figure 64).[311] Reaction of the same diazonium salts with thiourea followed by reflux at pH 10-12 gives the mercaptophthalonitriles **(320a,b)**, which are simply transformed to the sulphonic acid **(322a,b)**by oxidation using hydrogen peroxide in formic acid.[311] The sulphonic acid **(322a)** can be prepared from the aminophthalonitrile **(252a,b)** via an intermediate dithiocarbonate **(319)** as well. Note that attempts to synthesize the sulphophthalonitriles **(322a,b)** from halogenated 1,2-dicyanobenzenes with sodium sulphite in 50% aqueous dioxane in the presence of copper sulphate led to low yields of the desired products. Protected N,N-dialkyl-3,4-dibromobenzenesulphonamides provide another starting material for sulphonated phthalonitriles. The phthalonitrile is prepared using the Rosenmund-von Braun reaction as previously discussed with phenyl, pyrrole and indole protecting groups proving adequate for the protection of the sulphonic acid functional group while being cleavable to free the desired sulphonic acid after Pc formation.[136] A

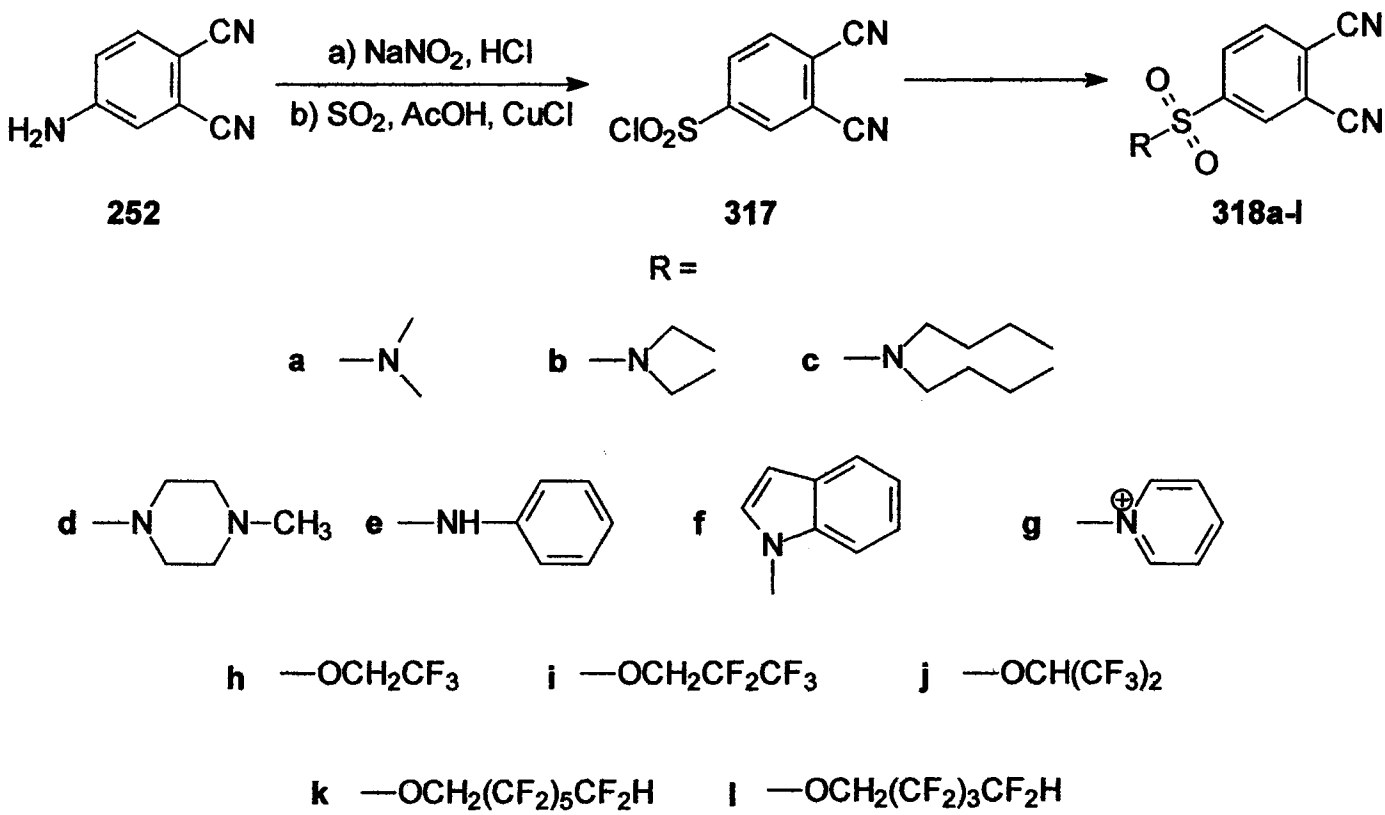


Figure 64. Synthesis of 4-chlorosulphonylphthalonitrile and its reactions to form sulphonyl-, sulphonylamido- and polyfluoroalkoxysulphonyl-substituted phthalonitriles.[311,312,398] Note that **317b** refers to the corresponding 3-chlorosulphonylphthalonitrile.

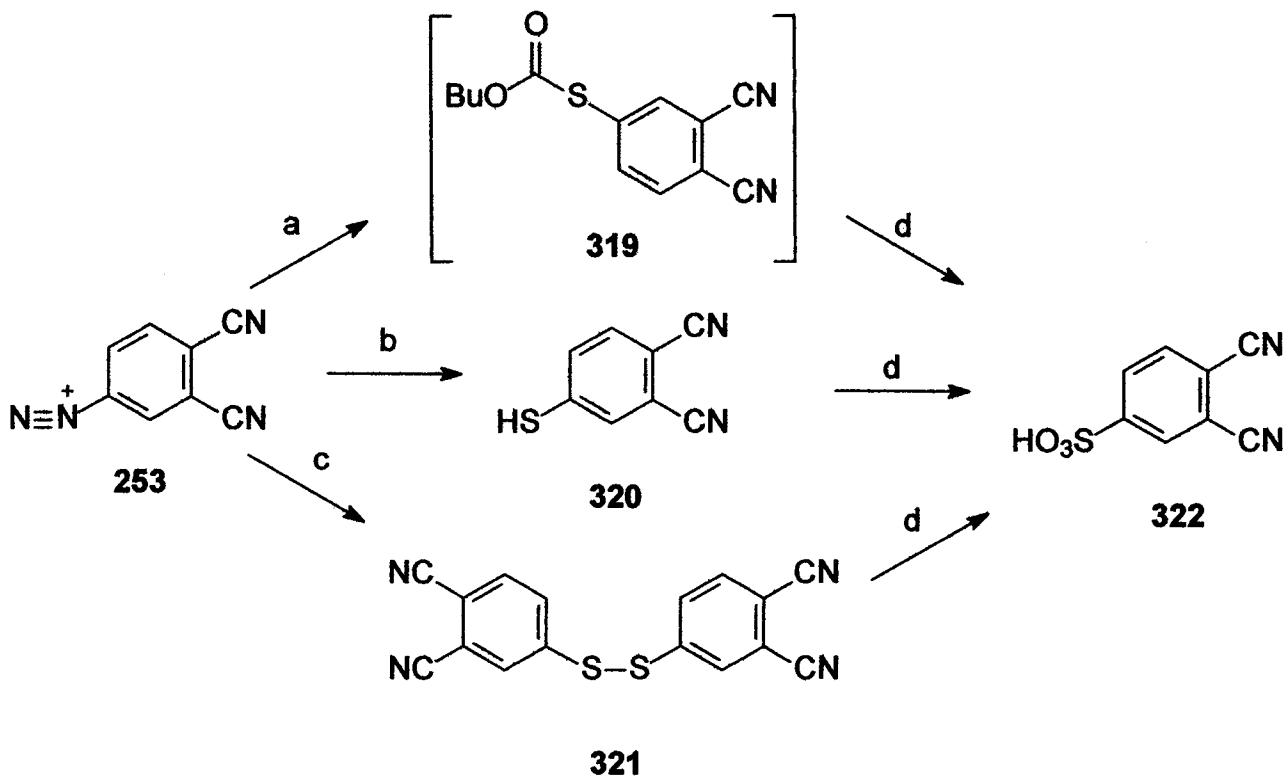


Figure 65. Reaction of the diazonium salt of 4-aminophthalonitrile with various sulfur nucleophiles and the conversion of the reaction products to the 4-sulfophthalonitrile.[311] a) $BuOCS_2K$, H_2O; b) i) $(NH_2)_2CS$, ii) NaOH; c) Na_2S_2, S, H_2O; d) H_2O_2, HCOOH. Note that **320b** refers to the corresponding 3-mercaptophthalonitrile and **332b** to the corresponding 3-sulphophthalonitrile.

number of other N,N-dialkyl-3,4-dibromobenzenesulphonamides have been prepared for use in phthalonitrile and phthalocyanine synthesis in which the sulphonamide groups are not readily cleaved to the free acid.[397] Forming the protected sulphonated phthalonitrile (example being **318f**) from 4-chlorosulphonylphthalonitrile (**317a**) gives decidedly better results by avoiding the Rosenmund-von Braun reaction (Figure 64) and has been used to prepare a number of novel trisulphonated phthalocyanines.[312] These protected sulphonic acids greatly ease the purification of the phthalocyanines, especially those formed via mixed condensations by imparting solubility in organic solvents. Furthermore, they allow for modification of the substituents on unsymmetrically substituted phthalocyanines, for instance via palladium-catalyzed reactions.

Of these sulphonated precursors, it is clearly the 3- and 4-chlorosulphonylphthlonitriles (**317a,b**) are the most interest due to their reactivity. A number of important sulphonated phthalocyanines have been prepared using these starting materials. For instance, sulphonated boron subphthalocyanine has been synthesized from 4-chlorosulphonylphthalonitrile (**317a**) in a manner that cannot be accomplished using the traditional 4-sulphophthalic acid due to cross reactivity between the Lewis acid boron source and the carboxylic acids of the starting material. From this subphthalocyanine, several trisulphonated Pcs have been synthesized via a ring expansion reaction.[65] In addition, these chlorosulphonyl groups readily react with amines to form sulphonylamido substituted phthalonitriles (**318a-g**).[311] Amines such as diethylamine, aniline, N-methylpiperazine and pyridine have been attached to the sulphonate group in this fashion (Figure 64). Furthermore, various polyfluorinated sulphonyl esters (**318h-l**)

have been reported, prepared by the reaction of 4-chlorosulphonylphthalonitrile with various polyfluorinated terminal alcohols in triethylamine and dichloromethane.[398]

While these few examples only give a brief glimpse into the enormous number of substituted phthalonitriles that have been prepared, it clearly shows the rich chemistry employed to prepare novel substituted phthalonitriles and from them, novel substituted phthalocyanines. Overall, it is this ability to add and control the substituents on phthalocyanine precursors that will be extremely important in the preparation of important phthalocyanines and will be of vital importance if phthalocyanines are to reach their full potential as important organic materials.

IV) Diiminoisoindolines

As can be clearly seen in the discussion above, the vast majority of phthalocyanine synthesis involves phthalonitriles as starting materials and their synthesis and modification has been vital in the preparation of novel phthalocyanines. However, a number of other starting materials can be used in the synthesis of the phthalocyanine macrocycle. Among these, the most highly used are diiminoisoindolines **(6)** (Figure 5). This reactive phthalocyanine precursor has been in fact proposed as key intermediates formed during the synthesis of phthalocyanines from other precursors including phthalonitriles.[1,3,16,399] Evidence showing the plausibility of diiminoisoindolines intermediates comes from the reaction of diiminoisoindolines with diamines.[400] The stable 2:1 adduct formed. would seem to indicate the pathway for imidine-imidine condensation and the relevance of such diiminoisoindoline intermediates in the synthesis of phthalocyanines. Others have concluded that during the condensation of *o*-

cyanobenzamide, iminophthalimidine could be an intermediate and it should have to pass via the diiminoisoindoline on the way towards phthalocyanine formation.[399] Proof of this iminophthalimidine intermediate in the condensation of various phthalic acid precursors comes from the synthesis of Pcs using phthalic anhydride as the precursor and urea as an amminating agent (Figure 66). In this case, ^{14}C-labeling experiments indicate that the urea provides the nitrogen for the macrocycle but none of the carbon.[401] As such, the phthalic anhydride must have been converted to the iminophthalimidine and then to the diiminoisoindoline prior to Pc formation. In fact, both of these intermediates have been isolated from the reaction mixtures and their conversion to Pcs has been demonstrated.[402] Other isolated intermediates such as the sodium salt of methoxyiminoisoindoline would also seem to show that the cyclotetramerization reaction passes through a diiminoisoindoline intermediate.[403-405]

Diiminoisoindolines **(6)** are almost exclusively formed from the corresponding phthalonitrile **(3)** by reacting it with ammonia gas in the presence of sodium methoxide in methanol (Figure 67). Another method that has been developed undertakes the reaction in an amide solvent such as formamide or acetamide in the presence of a base other than an alcoholate (such as sodium hydroxide).[406] Overall, the mild reaction allows the presence of halo, nitro, and linear and branched alkyl, alkoxy and aryl groups on the reacting phthalonitrile. On the other hand, aza derivatives of diiminoisoindolines have been prepared in a stepwise fashion.[335] The initial reaction is carried out in an alcohol at room temperature and involves ammonia gas and sodium hydride and leads to a monocarboximidate. Conversion of this carboximidate to the diiminoisoindoline is then accomplished by a second reaction with ammonia and sodium hydride at reflux. Similar

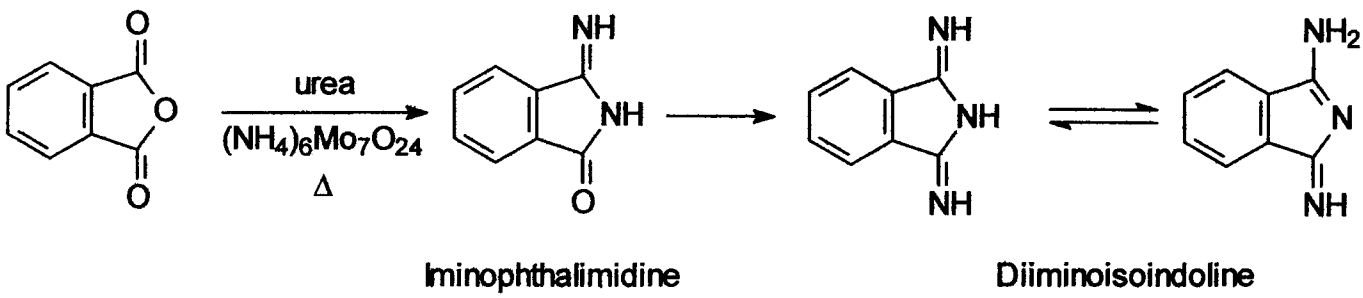
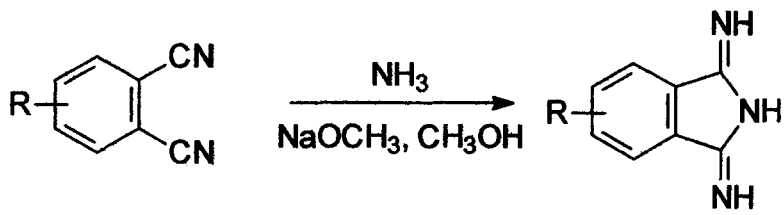


Figure 66. Iminophthalimidine and diiminoisoindoline as intermediates in the synthesis of phthalocyanines.[399,401]



3 6

Figure 67. Synthesis of diiminoisoindoline.

stepwise reactions were observed using ammonia in methanol with a catalytic amount of sodium methoxide.

Being a key intermediate in the cyclotetramerization reaction, diiminoisoindolines naturally display an increased tendency towards cyclization and readily react to form phthalocyanine even at room temperature. They are particularly useful in the synthesis of metal-free phthalocyanines as they readily cyclize under the milder conditions employed. In fact, cases exist where phthalonitriles that are resistive towards condensation or give low yields of Pc have been transformed into the corresponding diiminoisoindoline in order to induce Pc formation and improve yields. Others have invoked the increased yields of Pc formation using diiminoisoindolines to increase the efficiency of the cyclotetramerization reaction in order to avoid unnecessary loss of precursors that were prepared via a multistep pathway. Crown ether substituted phthalonitriles (Figure 21),[130,169,326,327] crown ether bridged bisphthalonitriles[328,329] and other phthalonitrile dimers[283,287,288,296,370,381] have all used this reasoning in order to increase the overall efficiency of their total synthesis.

Overall, abundant monosubstituted and disubstituted diiminoisoindolines have been prepared from the analogous phthalonitrile. Substituents such as simple alkyl,[56,283,301,407,408] alkoxy,[49,52-54,306,409,410] thioalkoxy,[301,302,315] aryl,[406] nitro,[408] silyl,[78,283,277] silyloxy[273] and iodo groups[358]can be present during the synthesis of diiminoisoindolines as they can endure the basic conditions used. Phthalonitriles bearing other functional groups such as vinyl and alkene groups,[369,380] heterocycles,[314] long chain amide groups[166,168] and polymer-bound phthalonitriles[57-59] have also been transformed into the corresponding diiminoisoindolines. Even tetracyanobenzene can be transformed

to dicyanodiiminoisoindoline under the right conditions.[411] The analogous benzene and naphthalene bis-diiminoisoindolines can also be prepared with slightly more forcing conditions.[100,412,413] In addition, naphthalene-based diiminoisoindolines[214,216,217,220,222,] and various 6,7-dicyano-1,4-diepoxynaphthalene-based diiminoisoindolines[55,177.178] are obtainable using the same protocol. Finally, aza derived diiminoisoindolines have been prepared, not only stepwise as previously mentioned but also by using the traditional conditions.[414-416] Note, however, that few examples exist of tetrasubstituted diiminoisoindolines. Tetrafluorophthalonitrile **(271)** is one of the primary source of tetrasubstituted phthalonitriles and this compound is extremely reactive towards alcoholates. Clearly the key drawback of using diiminoisoindolines as phthalocyanine precursors is the reaction conditions involved in their preparation, which limits the functionality that can be present in the molecule. Furthermore, their parent phthalonitriles generally tetramerize to the corresponding phthalocyanine in good yields and are definitely more stable. As such, phthalonitriles are the precursor of choice. However, diiminoisoindolines play important roles in Pc synthesis due to their higher reactivity and have been eminently used as Pc precursors as well.

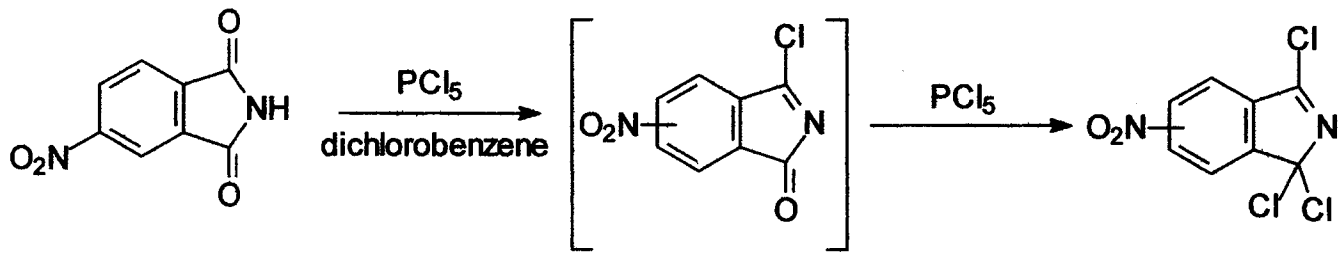
V) Novel designed phthalocyanine precursors

The general synthesis of phthalocyanines is a very highly symmetrical one, with the condensation reaction occurring in any number of possible orientations. It is this symmetry that leads to the production of the constitutional isomers seen for tetrasubstituted phthalocyanines (Figure 3) and the difficulties encountered in the synthesis of unsymmetrically substituted phthalocyanines (Figure 4). As such, novel

phthalocyanine precursors have been designed that will eliminate this symmetry and force the condensation to occur in only one direction, thus producing only one isomer or one unsymmetrically substituted product.

One such method of breaking the symmetry of cross condensation reactions led to a rare bonafide synthesis of pure disubstituted phthalocyanine. When 5-phenyl-1,3-diiminoisoindoline is reacted with 1,3,3-trichloroisoindoline **(12)** (Figure 68)[417] at room temperature in the presence of an organic base like triethylamine and reducing agent like hydroquinone, pure 2,16/17-diphenylphthalocyanine is produced in a 7% yield.[418] When the temperature is raised however, the reaction leads to a mixture of substituted products.[419] Such 1,3,3-trichloroisoindolines are well-known phthalocyanine precursors, having been used in the preparation of phthalogen dyestuffs.[17] In fact, they have been extensively used as intermediates in the preparation of diiminoisoindolines from phthalimides by their reaction with ammonia. They are prepared by the reaction of phthalimides with phosphorus pentachloride in a solvent such as 1,2-dichlorobenzene (Figure 68).[17,54-56] A number of substituted derivatives have been synthesized, including 6/7-nitro-1,3,3-trichloroisoindoline **(325)**,[54] 6/7-t-butyl-1,3,3-trichloroisoindoline, 6,7-dihexyl-1,3,3-trichloroisoindoline[56] and tetrachloro-1,3,3-trichloroisoindoline[17] among others.

The basis behind their controlled reaction lies with lower reaction temperatures used along with the steric hindrance in the 1,3,3-trichloroisoindoline **(12)**.[54-56] At the lower reaction temperatures, the diiminoisoindolines used for this condensation reaction do not self-condense while the steric hindrance of the chlorine atoms of the 1,3,3,-trichloroisoindoline prevents self-condensation of these more reactive precursors. Thus,



323 **324** **325**

Figure 68. Synthesis of 6/7-nitro-1,3,3-trichloroisoindoline.[54]

only condensation between the diiminoisoindoline and the 1,3,3-trichloroisoindoline **(12)** is possible, leading to pure trans disubstituted products. Note that in some cases, trisubstituted products are also obtained.[55,56,419] It has been proposed that this is due to traces of water in the reaction mixture, which results in partial hydrolysis of some of the 1,3,3-trichloroisoindolines.[55] This slight loss of the reactive precursor would upset the stoichiometric balance of the two reagents and under conditions where the diiminoisoindoline is in excess, trisubstituted products would be expected to be formed. Also note that some of these 1,3,3-trichloroisoindolines are extremely reactive and unstable. For example, 6,7-dihexyl-1,3,3-trichloroisoindoline cannot be fully purified, even under an inert atmosphere, due to its high reactivity.[55] This results in poor yields in its condensation reactions. While this controlled condensation reaction generally leads to metal-free phthalocyanines, it has also been extended to the synthesis of metallophthalocyanines by carrying out the condensation in the presence of $NiCl_2(py)_4$ or $ZnCl_2$ and tetrabutylammonium bromide as a phase transfer catalyst.[55,56] However, in reality, this procedure involves condensation of the two reagents to the macrocycle, followed by metal insertion *in situ*. Overall, while these reactive precursors have been successfully in preparing pure trans disubstituted phthalocyanines, their difficult synthesis and instability limits their usefulness.

An symmetry-based approach towards the synthesis of a pure tetrasubstituted phthalocyanine isomer has also been examined. Iminothioamides **(10)** have been envisioned as precursors that could be used in the synthesis of isomerically pure phthalocyanines.[420] It has been shown that dithioamides **(11)** readily form phthalocyanines at relatively low temperatures (around 80-90 °C).[52] As such, it has been

proposed that using substituted iminothioamides **(328)** would induce the tetramerization to occur in only one direction, with the alkylthiol functionality of one molecule being selectively displaced by the imino group of another molecule, especially at the reaction temperatures employed. Iminothioamides **(328)** are synthesized by the reaction of phthalonitriles with hydrogen sulphide (Figure 69).[53,420] The resulting 1-imino-3-thioisoindolines **(327)** are then methylated with dimethyl sulphate or iodomethane as the non-alkylated derivative failed to condense to the desired phthalocyanine. Note that while monosubstituted phthalonitriles give rise to two isomers, they are separable by flash chromatography. Importantly, these S-methylated derivatives readily condense to the desired phthalocyanine at room temperature. Unfortunately, the 5-or 6-substituted 1-imino-3-thioisoindoline **(328)** self-condense at room temperature not as a single isomer as expected but as a mixture of isomers.[53] However, if the condensation is carried out at –20 °C in DMF using zinc acetate as a template, small-scale reactions over a prolonged time do lead to a single isomer. It should be noted that the statistical condensation of a substituted diiminoisoindoline leads to a different isomeric distribution as that obtained using these iminothioamides **(328)**. As such, a different reaction pathway must be at play. These precursors also result in a series of isoindigo byproducts at room temperature, perhaps indicating that a metal ion template may be useful.

Attempts to use dithioimides **(11)** in the synthesis of pure unsymmetrically substituted phthalocyanines have also been undertaken.[52] Substituted dithioimides **(330)** are prepared from the corresponding phthalimide **(329)** using Lawesson's reagent (Figure 70). This gives rise to the desired dithioimide **(330)** along with thiophthalimides **(331)**. These reagents also condense at low reaction temperature and it was proposed that

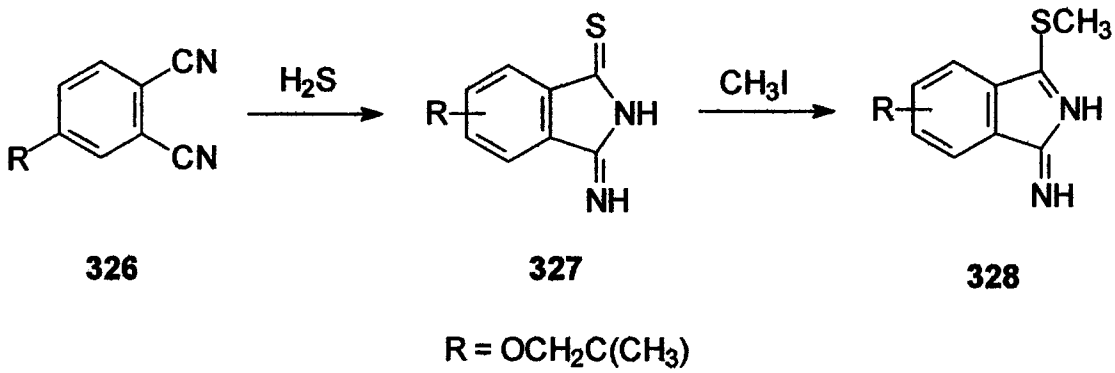
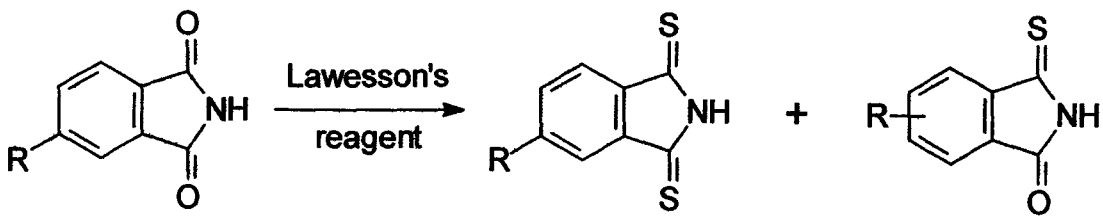


Figure 69. Synthesis of 1-imino-3-methylthio-5/6-neopentoxyisoindoline.[53]



329 **330** **331**

R = $OCH_2C(CH_3)$

Figure 70. Synthesis of thiophthalimides.[52]

carrying out a mixed condensation involving dithioimides **(330)** and diiminoisoindolines at low temperature would result in condensation only between the thio groups and the imino groups, much as is the case with 1,3,3-trichloroisoindolines **(325)**. Unfortunately, a mixture of substituted products was obtained, most likely due to self-condensation of the dithioimides at the reaction temperatures used.

VI) Other phthalic acid derivatives

As has been previously indicated, any number of phthalic acid derivatives can be used as starting materials in the synthesis of phthalocyanines (Figure 5). While phthalonitriles and diiminoisoindolines are clearly the most popular and most highly used of this class of compounds, others such as *o*-dibromobenzenes, phthalic acids, phthalic anhydrides and phthalimides are also appropriate precursors for phthalocyanines. Of course, reactions of *o*-dibromobenzenes require a source of cyanide ion, typically cuprous cyanide and most likely proceeds via an *in situ* Rosenmund-von Braun reaction to generate the phthalonitrile. The other phthalic acid derivatives necessitate the use of amminating agents and catalyst in order to invoke cyclotetramerization to the phthalocyanine. While not extensively utilized in the delicate synthesis of phthalocyanines for technological purposes, the economical cost of these reagents and the ease in their preparation make them the most used precursors in the dye and pigment industry.[14-19]

By virtue of this, most phthalic acid derivatives other than phthalonitriles and diiminoisoindolines that have been used in the synthesis of phthalocyanines are readily available, either commercially or via simple organic transformations. A number of

simply substituted phthalocyanines such as tetrasulphonated[46] and tetracarboxyphthalocyanine[421] are synthesized for the readily available 4-sulphophthalic acid and trimellitic anhydride (4-carboxyphthalic anhydride) respectively. Other important precursors like 4-nitrophthalimide **(40)** can be easily prepared by nitration of the proper starting material (Figure 11).[93] Additional substituted phthalic acids can be synthesized by Diels Alder reactions using dimethyl-1,3-butadiene **(219)** as the diene followed by potassium permanganate oxidation of the resulting substituted *o*-xylenes **(221)** as described in the Diels Alder section (Figure 38).[15] For instance, the Diels Alder reaction between cinnamic acid **(218)** and dimethyl-1,3-butadiene **(219)**, followed by dehydration and oxidation with $KMnO_4$ gives 4-carboxy-5-phenylphthalic acid **(220)** and this precursor is used in the synthesis of the copper Pc dye Sirius Light Green FFGL. 4-Sulpho-5-acylaminophthalic acid can be procured from 4-chloro-5-sulpho-*o*-xylene following a series of steps including $KMnO_4$ oxidation, amination and acylation. Other interesting disubstituted phthalic acids can also be achieved via modification of substituted *o*-xylenes or by using other dienophiles in the Diels Alder reaction with dimethyl-1,3-butadiene **(219)**. An additional example is 4-sulpho-5-phenylphthalic acid **(227)** whose starting material is 4-carboxy-5-phenyl-*o*-xylene **(221)** (Figure 38). *o*-Dicarboxyphenylphosphonous and phosphonic acids along with diphenylsulphone-3,4-dicarboxylic acid have also been described and used in the preparation of phthalocyanines.[16]

Other important starting materials for industry are phthalic anhydrides, which are extensively used in the production of dyes. Industrially important halogenated phthalocyanines, for instance, are sometimes prepared from the appropriately substituted

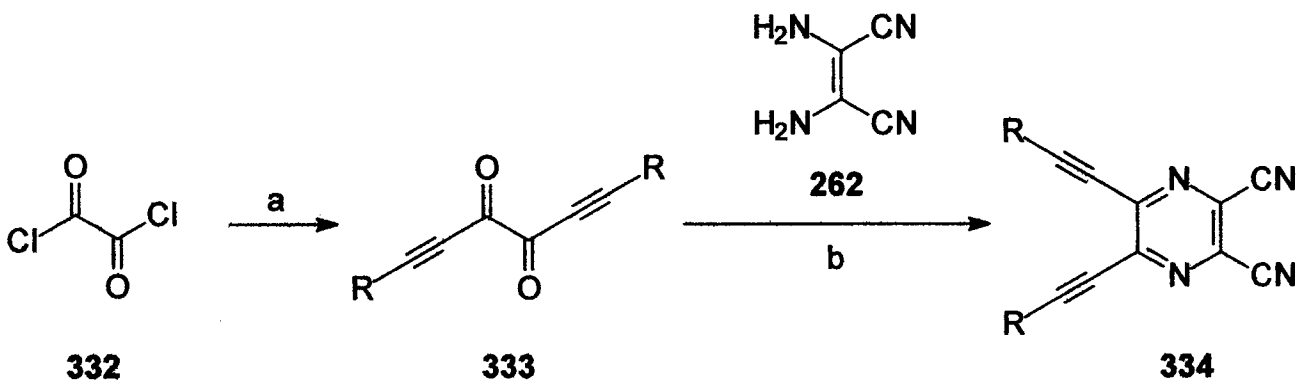
phthalic anhydride[16] although hexadecachlorophthalocyanine is also available by chlorination of a pre-existing phthalocyanine molecule.[18,19] In addition, substituted phthalocyanine is usually synthesized by the dye and pigment industry using substituted phthalic anhydrides as starting materials.[16,18,19] Overall, there is an endless list of substituents that have been added to various phthalic acid derivatives in attempts to develop better dyes and pigments. On the whole, while the use of phthalic acid derivative other than phthalonitriles and diiminoisoindolines is not prominent in the detailed synthesis of phthalocyanines in research, they are used extensively by industry. Precursors such as those mentioned above should not be ignored as they provide access to a multitude of rediscovered and important functional groups that can be added to phthalocyanines in order to improve their physical and chemical traits for more high tech applications.

VII) Aza derivatives

Numerous phthalocyanine analogs have been developed in order to improve the characteristics of these macrocycles for various applications. Among these are the aza analogs where the peripheral benzene rings are replaced by nitrogen-containing aromatic heterocycle. Such aza phthalocyanine analogs have several interesting properties and their synthesis along with their chemical and physical characteristics have been extensively reviewed.[414,422]

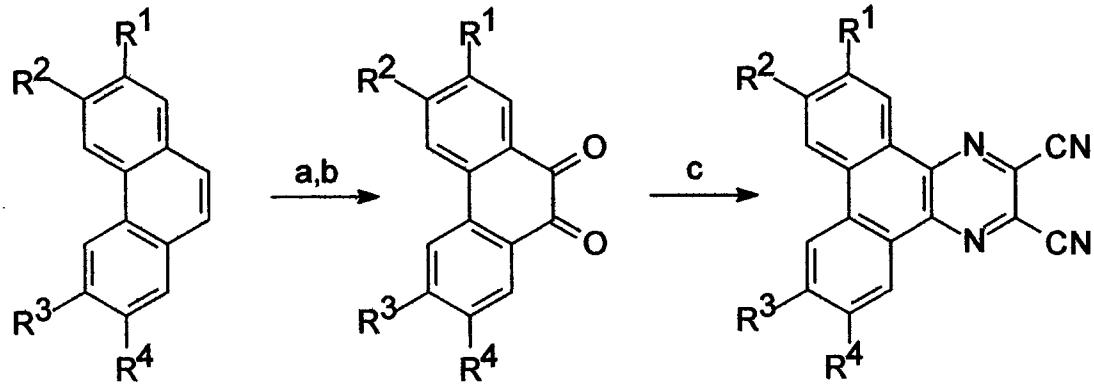
The most predominate members of this family of phthalocyanine analogs are tetrapyrazinoporphyrazines and are based on 2,3-dicyanopyrazines. For the most part, substituted 2,3-dicyanopyrazines are synthesized by the reaction of 1,2-diketones and

diaminomaleonitrile (DAMN) **(262)** (Figure 71). DAMN itself has a very rich and high studied chemistry[423-425] and can be used to create various 1,2-dinitriles that can be used to prepare phthalocyanine-like macrocycles. A large number of diketones have been investigated and the synthesis of such diketones has been accomplished with a wide array of methods.[236] While their preparation is beyond the scope of this review, their importance in the synthesis of azaphthalocyanine derivatives cannot be underestimate. Among some of the diketones used are oxalyl chloride **(332)**,[331] diethyl dioxosuccinate[426] and various alkyl,[415,427,428] alkyne **(333)** (Figure 71),[236,429] aryl[414,430] and heterocyclic[431,432] substituted diketones. Note that the use of oxalyl chloride **(332)** leads to the formation of a cyclic diketodicyanodiamine **(263)** and this must be reacted with thionyl chloride to yield the desired 1,2-dichloro-5,6-dicyanopyrazine **(264)** (Figure 51).[331,332] Symmetrically disubstituted 2,3-dicyanopyrazines bearing chlorine, carboxylic ester, alkanes, alkynes and furan, thiophene and pyridine heterocycles including polythiophene substituents have been prepared.[431] Unsymmetrically substituted 2,3-dicyanopyrazines are conceivable as well.[236,414] As mentioned before, the 1,2-dichloro-5,6-dicyanopyrazine **(264)** offers access to other substituted pyrazine derivatives by nucleophilic displacement of the chlorine atoms (Figure 51). In addition, numerous higher order aromatics **(335)**have been transformed into *o*-quinones **(336)** and reacted with DAMN **(262)** to give dinitriles **(337)** (Figure 72).[433-438] A particular interesting example of these higher order aromatic aza analogs include those fused with [7]helicenes.[437,438] Other possible reactions for the preparation of 2,3-dicyanopyrazine derivative include the reaction of DAMN **(262)** with α-hydroxyiminoketones and diamines.[414] Note that the reaction of diketones with 4,5-diaminophthalonitrile gives 2,3-



332 333 334

Figure 71. Synthesis of acetylenic 2,3-dicyanopyrazines. a) $(CH_3)_2CHSi{\equiv}CH$, BuLi, CuBr, LiBr, THF, O °C, 85%; b) AcOH, rt, 81%.[429]



335a-c **336a-c** **337a-c**

a $R^1 = R^4$ = t-butyl, $R^2 = R^3 = H$
b $R^1 = R^3$ = t-butyl, $R^2 = R^4 = H$
c $R^2 = R^3$ = t-butyl, $R^1 = R^4 = H$

Figure 72. Synthesis of di-t-butyl-substituted 9,10-phenanthro-2,3-dicyanopyrazines. a) CH_3COCl, $AlCl_3$, toluene; b) CrO_3, AcOH; c) DAMN, EtOH, AcOH, Δ (Azamacro-3 + ref).[433-438]

disubstituted 6,7-quinoxalinedinitriles, which are slightly different aza analogs as the nitrogen containing aromatic ring is seperated from the dintrile functionality by a benzene ring.[236] This should greatly alter the properties of the resulting macrocycle as compared to other aza compounds.

Work has also been done on pyridine-based aza analogs using both 2,3-dicyano- and 3,4-dicyanopyridine molecules.[416,422,434,439,440] In addition, quinolinedicarbonitriles and other mono- and polynitrogen-containing aromatic dinitriles have been prepared.[414,441] In the case of 2,3-dicyanoquinoline **(342)**, the synthesis commences with the reaction of 2-aminobenzaldehyde **(338)** with dimethyloxaloacetate **(339)** ensued by ammonolysis and dehydration (Figure 73).[441] The seven membered 5,7-diphenyl-2,3-dicyano-6*H*-1,4-diazepine ring system can be made as well, in this case from dibenzoylmethane and DAMN **(262)**.[430] In reality, vast arrays of aza phthalonitrile and phthalocyanine analogs have been prepared, with both varying nitrogen-containing heterocycles and differing substituents. Moreover, numerous bridged analogs have also been prepared.[414] Aza substitution is a promising method for structural modification of phthalocyanines along with modulation of their electronic properties. As such, work continues in this area in efforts to design new phthalocyanine-based materials with fine-tuned absorption maxima and the ability to modify their characteristics to enhance the macrocycles performance in a given application.

VIII) Other phthalocyanine precursors

In addition to aza analogs, a number of other phthalocyanine-like macrocycles have been designed. Linstead originally investigated several in order to fully study

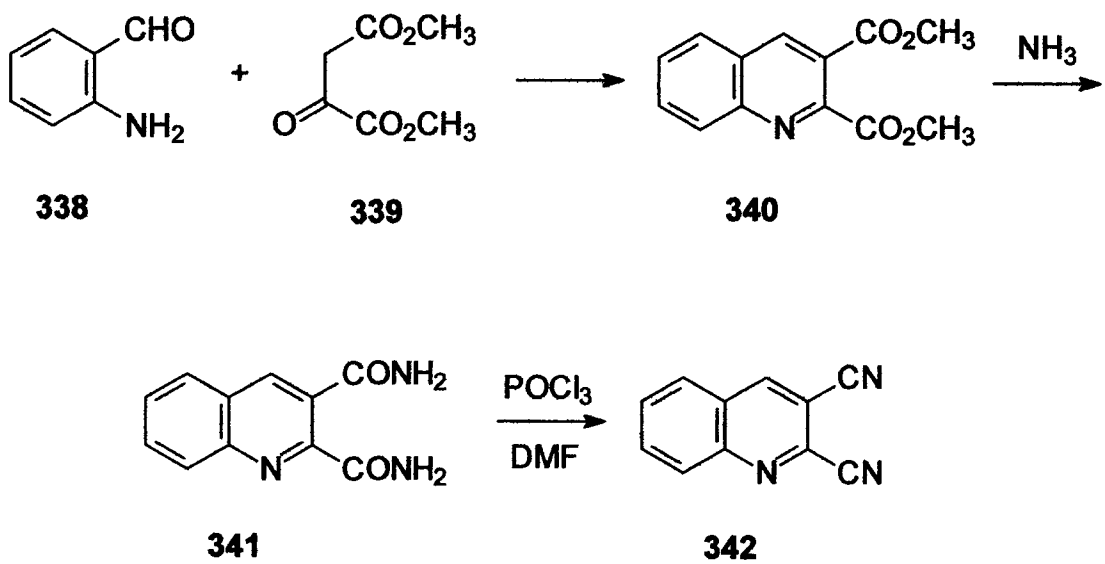
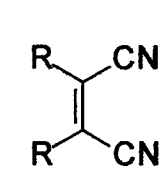


Figure 73. Synthesis of 2,3-dicyanoquinoline.[441]

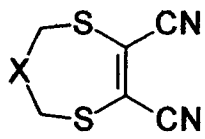
phthalocyanines and phthalocyanine-like macrocycles. Others were developed by industry in attempts to design and fabricate new and improved dyes with improved colors. More sophisticated examples have been recently prepared in order to control macrocycle formation and to adjust the characteristics of the molecule. These all require the synthesis of novel 1,2-dinitriles and it is outside the intent of this review to go into each in detail. Suffice it to give a few important examples (Figure 74). A variety of nitrogen, oxygen and sulphur containing 1,2-dinitriles have been prepared, a large number of these being maleonitrile derivatives **(343)**. Compounds such as dialkylmaleonitrile,[384,442,443] bis(dimethylamino)maleonitrile,[384] bis(benzylthio)maleonitrile[384,444] and dispiromaleonitrile[384,443,445] **(356)** (Figure 75) can be included in these. Other sulphur-containing maleonitrile derivatives are obtained from disodium maleonitriledithiolate (Na_2mnt), which itself is synthesized in two steps from sodium cyanide and carbon disulphide.[446-448] Reaction of Na_2mnt with various alkyl halides results in bis(alkylthio)maleonitriles.[17,444,448-450] Furthermore, chalogen atom substituted dinitriles such as 3,7-dithiocycloheptamaleonitrile **(344** X = CH_2) and 3,7-dithia-5-oxocycloheptamaleonitrile **(344** X = O) can also be prepared using the necessary alkyl dihalides (Figure 74).[451] The corresponding 3,5,7-trithiacycloheptamaleonitrile **(344** X = S) however requires the more labile $(Bu_4N)_2[Zn(mnt)_2]$ as the starting material (Figure 74). Condensation of any of these maleonitrile derivatives gives porphyrazines or tetraazaporphyrins while examples also exist where these starting materials were employed in mixed condensations to give porphyrazine-phthalocyanine derivatives.[384]

Sulphur containing phthalocyanine derivatives have also been realized using different dicyanothiophene derivatives.[430,440,452-455] Amid these are 2,3- and 3,4-



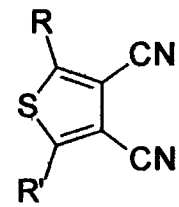
Maleonitriles

343



Chalcogen Atom Substituted Maleonitriles

344



3,4-Dicyanothiophenes

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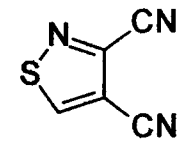

3,4-Dicyanothiadiazole

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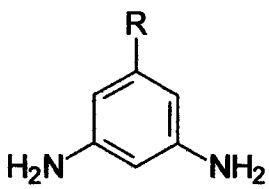
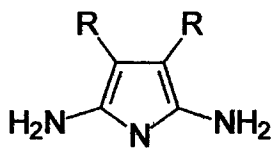


2,6-Diaminopyridines

347



2,5-Diaminopyrroles

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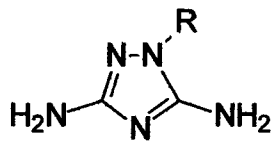
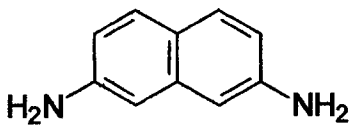


3,5-Diamino-1,2,4-triazoles

349



2,7-Diaminonaphthalene

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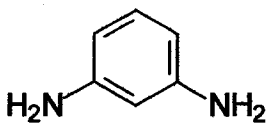
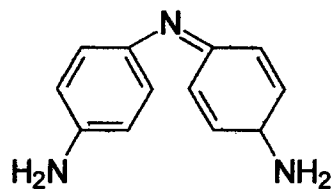


m-Phenylenediamine

351



4,4'-Diaminodiphenylamine

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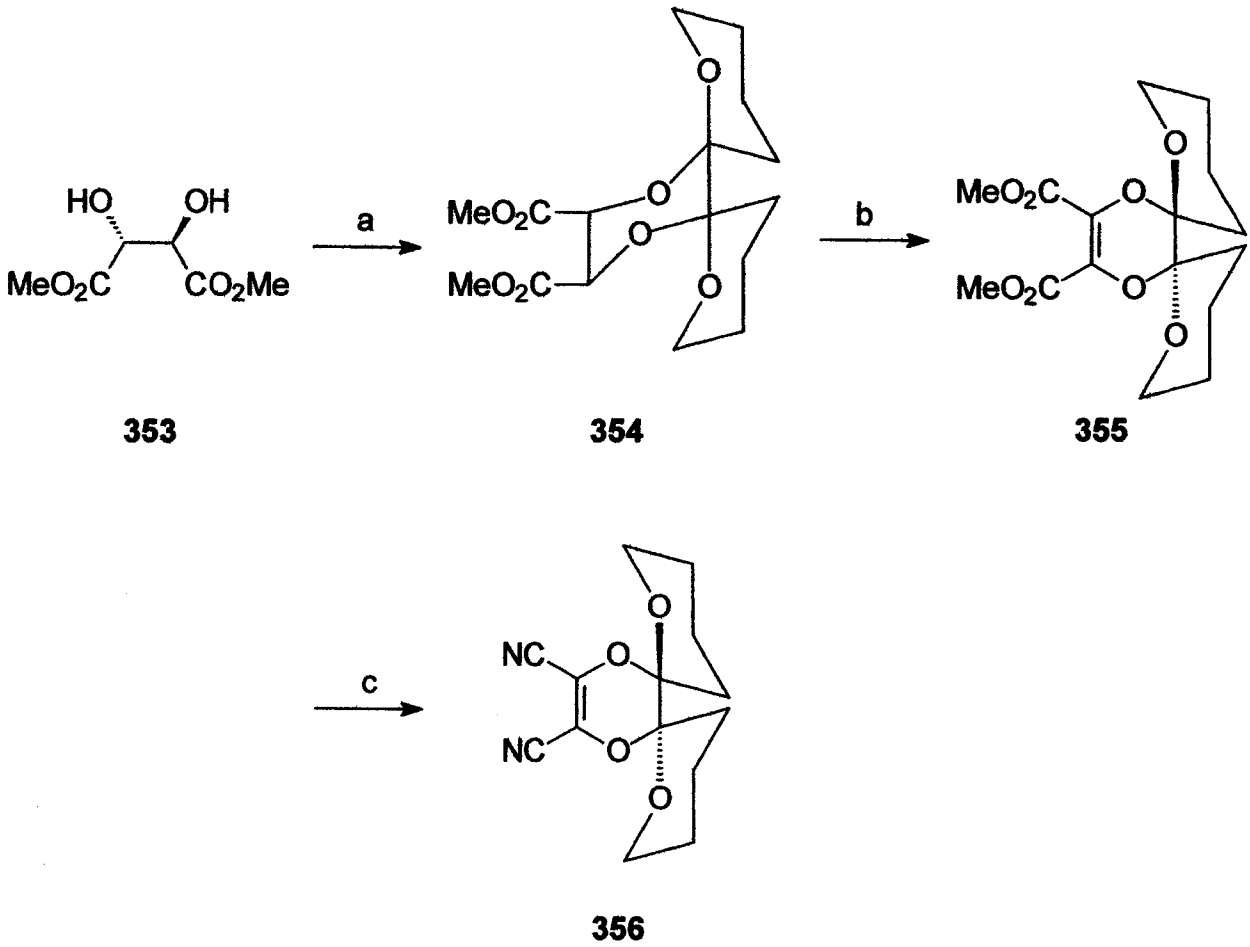
Figure 74. Examples of some precursors for phthalocyanine-like macrocycles.



353 354 355

356

Figure 75. Synthesis of dispiromaleonitrile. a) bis-DHP, HCl, Et_2O, 0 °C, 60%; b) i) lithium 2,2,6,6-tetramethylpyperidine, THF, -78 °C; ii) I_2, THF, -78 °C, 53%; c) i) NH_3, MeOH, rt; ii) $(CF_3CO)_2O$, pyridine, -30 °C to rt, 77%.[443,445]

dicyanothiophenes[440,452,455] **(345)** and 3,4-dicyanothiadiazole[430] **(346)** (Figure 74). Substituents such as 2,5-amino[453,454] and alkyl groups[455] have been added onto the 3,4-dicyanothiophene. Note that the corresponding selenophenes,[452] selenodiazole[430] and 2,3-dicyanothionaphthalene[440] are also available as is N-alkyl-4,5-dicyanoimidazoles.[456] In most cases, these compounds are prepared via the Rosenmund-von Braun reaction of the parallel dibromide.[452] However, synthetic pathways from the dicarboxylic acid also are possible.[440] In the case of the thiadiazole and selenodiazole derivatives, their synthesis commences with DAMN **(262)** and inexpensive thionyl chloride and selenium oxide.[430] The cyclotetramerization of these compounds tend to involve mixed condensations to the tribenzoporphyrazines. Along with tribenzoporphyrazines formed from the precursors mentioned above are thiophenotribenzoporphyrazines and pyridino[3,4]tribenzoporphyrazines.[457,458]

Other interesting precursors that lead to pigments related to phthalocyanines are fairly simple organic molecules (Figure 74). For example, 2,6-diaminopyridines **(347)** can be used as precursors in mixed condensation reactions to form hemiporphyrazines[107,178,459] and similar compounds can be obtained when replacing the diaminopyridine with 2,5-diaminopyrrole **(348)**,[402] 3,5-diamino-1,2,4-triazole **(349** R = H) and 3,5-diamino-1-phenyl-1,2,4-triazole **(349** R = Ph).[107] In addition, reacting diiminoisoindoline with 2,7-diaminonaphthalene **(350)** or *m*-phenylenediamine **(351)** give hemiporphyrazines lacking two of the donating nitrogen atoms.[107,460] Comparable macrocyclic compounds can be obtained by reacting phthalonitriles with diamines such as 4,4'-diaminodiphenylamine **(352)**.[107] Note that many of these compounds are non-Hückel systems and therefore have significantly different physical, chemical and spectral

characteristics. Of particular interest is the previously mentioned 3,5-diamino-1,2,4-triazoles **(349)**, which has been used quite extensively in the synthesis of triazolephthalocyanines.[140,169] It turns out that a three unit phthalocyanine-like precursor can be prepared from these molecules and a subsequent condensation with diiminoisoindolines gives a controlled synthesis of mixed phthalocyanine systems.[461] An identical procedure has been applied to the synthesis of thiadiazolephthalocyanines as well.[462]

While a number of interesting and singular phthalocyanine-like macrocycles and their prerequisite precursors have been mentioned above, various other examples do exist. Clearly, this indicates the rich and important chemistry of phthalocyanine macrocycles and their value in several important applications. Much more attention needs to be paid to these unusual phthalocyanine-like macrocycles as they could provide the key to unlocking the potential of phthalocyanines.

IX) Conclusion

Phthalocyanines have a rich and varied chemistry and an immense potential in a vast array of widely diverging fields. They owe this not only to their unique physical, chemical and spectral properties but also to the high degree of versatility displayed in their synthesis. The variety of possible starting materials for phthalocyanine synthesis and the diversified methods for their preparation essentially opens the door for phthalocyanine to fulfill some of their promise. Not only do countless possibilities exist for adding significant and original substituents to the molecule, novel phthalocyanine precursors exist that change the very nature of the macrocyclic system. The key to

phthalocyanine reaching their potential lies not in developing specific systems where phthalocyanines will work but in the design, preparation and modification of their precursors so that phthalocyanines ideal for a specific application can be synthesized.

X) Acknowledgements

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Chapter 6.

The Use of Palladium Catalysis in the Synthesis of Novel Porphryins and Phthalocyanines

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The Use of Palladium Catalysis in the Synthesis of Novel Porphyrins and Phthalocyanines

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Abstract:

Palladium catalysts offer a rich and highly versatile chemistry for the synthesis of novel porphyrins and phthalocyanines. These mild and flexible reactions have been used extensively in the preparation of interesting porphyrins and phthalocyanines, either in the synthesis of substituted precursors or the modification of pre-existing macrocycles. For these tetrapyrrolic compounds, metal-mediated reactions such as these offer extensive advantages, which have been taken advantage of in order to add novel substituents, synthesize naturally occurring molecules and prepare multi-macrocyclic arrays. This review gives an overview of the use of palladium catalysts in the synthesis of porphyrins and phthalocyanines along with the applications of some of the compounds prepared.

Keywords: porphyrins, phthalocyanines, palladium catalysts, Heck reaction, Suzuki reaction, Stille coupling, multiporphyrin arrays

Introduction

Tetrapyrrolic macrocycles are found throughout nature and serve a number of essential biological functions. Porphyrins such as haem and chlorins including the macrocycles found in chlorophylls not only serve to transport oxygen through our bodies and transform light into useful energy but have also been extensively studied due to their unique physical, chemical and spectral properties. Synthetic tetrapyrrolic analogs such as phthalocyanines have also been examined in great detail due to their increased stability and improved spectroscopic features.

In addition to their traditional applications as dyes, photoconducting agents in photocopying devices and catalysis for numerous chemical reactions [1,2], the importance of phthalocyanines is rapidly growing in many other fields. These include chemical sensors [3], electrochromism [4], molecular metals [5], liquid crystals [6], photosensitizers for photodynamic therapy [7-10] and non-linear optical applications [11]. Wide-ranging applications such as these call for new synthetic methods with greater control of regioselectivity and access to diverse types of substituents.

The unique properties of porphyrins lead to their importance in the development of molecular optoelectronic gates and switches [12], molecular wires [13], photoinducable energy [14] or electron–transfer systems [15,16], light-harvesting arrays [17], unidimensional conductors [18] and semiconductors [19], enzyme models [20,21] and photosensitizers for photodynamic therapy [10,22]. Of particular importance is the preparation of multiporphyrin arrays as they may provide insight into the mechanisms of photosynthesis and energy transfer processes. In addition, the synthesis of substituted porphyrins is of considerable chemical interest as slight changes in the substituents can

significantly alter the fundamental properties of the porphyrin macrocycle. Attachment of unusual organic moieties and novel functional groups to the porphyrin periphery often involves elaborate and multistep synthetic strategies and tedious purification procedures. Furthermore, potential incompatibility between a component in the synthetic strategy and the conditions used for cyclization greatly limits the functional groups that can be present during synthesis.

Transition metal-catalyzed reactions are powerful tools in organic synthesis. Palladium-catalyzed reactions [23] such as the Heck [24,25], Stille [26,27] and Suzuki reactions [28-30] in particular have several features that make them extremely useful and versatile. Palladium offers a number of possibilities for carbon-carbon bond formation and can tolerate many different functional groups such as carbonyl and hydroxyl groups. The mild reaction conditions required for these reactions make them ideal for the synthesis of substituted porphyrins and phthalocyanines. Other advantages include high chemical yields, facile reaction procedures, readily accessible starting materials and short synthetic sequences.

Palladium in Porphyrin Synthesis

Palladium-catalyzed reactions have been used extensively in the preparation of novel porphyrins. In addition to the above-mentioned advantages, these reactions provide for the special needs in porphyrin synthesis. The mild, non-forcing reaction conditions prevent metalation or transmetalation from occurring. Furthermore, palladium does not insert into porphyrins unless high temperatures are employed [31]. Finally, palladium-catalyzed reactions can be carried out at low concentrations, an important requirement with the low intrinsic solubilities of porphyrins. These reactions have been

used to synthesize novel precursors for traditional porphyrin synthesis along with the preparation of naturally occurring porphyrins, porphyrins bearing novel substituents and oligomeric conjugated porphyrin arrays.

a) Synthesis of Porphyrin Precursors

Traditional synthesis of porphyrins usually involves the acid-catalyzed condensation of aldehydes with pyrroles [32]. While most of the work done with palladium catalysis involves the modification of pre-existing macrocycles, some attention has been given to the use of this chemistry to form novel aldehydes and pyrroles for porphyrin synthesis. In a study concerning the stepwise synthesis of porphyrin bioorganic model systems [33,34], 2,6-dimethyl-4-bromobenzaldehyde was treated with trimethylsilylacetylene in triethylamine using tetrakis(triphenylphosphine)palladium(0) ($Pd(Ph_3P)_4$) as a catalyst to afford the ethynyl-substituted benzaldehyde (Figure 1). Condensation of the protected alkynyl aldehyde with pyrrole lead to symmetric *meso*-tetraarylporphyrins while asymmetric porphyrins were formed via a mixed aldehyde condensation. The resulting alkynyl-substituted porphyrins can be readily deprotected to give terminal alkynes, which can be coupled to form oligomeric porphyrin arrays as is described below. In addition, novel furyl-, thienyl- and dipyrromethane precursors have been prepared in order to synthesize porphyrin building blocks containing one sulfur or oxygen atom in place of nitrogen in a designated site in the porphyrin core [35]. This allows fine-tuning of the properties of the porphyrin macrocycle, possibly optimizing their usefulness in preparing biomimetic energy transduction systems.

Palladium-catalyzed carbonylation was used to form novel pyrrole diesters in a two step protocol in fair yields (Figure 1) [36]. Condensation of the resulting compound

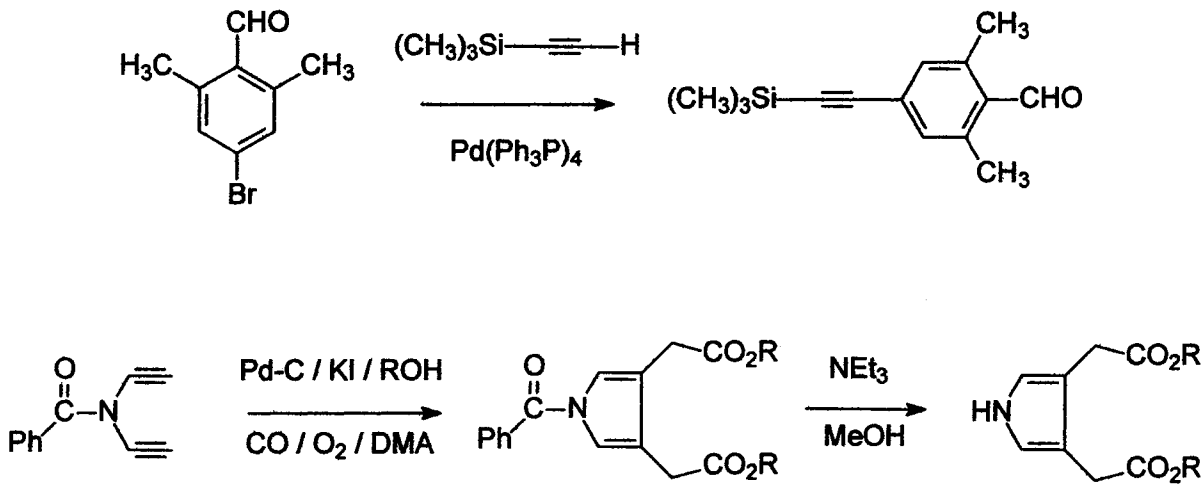


Figure 1. Examples of the use of palladium-mediated reactions in the synthesis of porphyrin precursors [33,36]

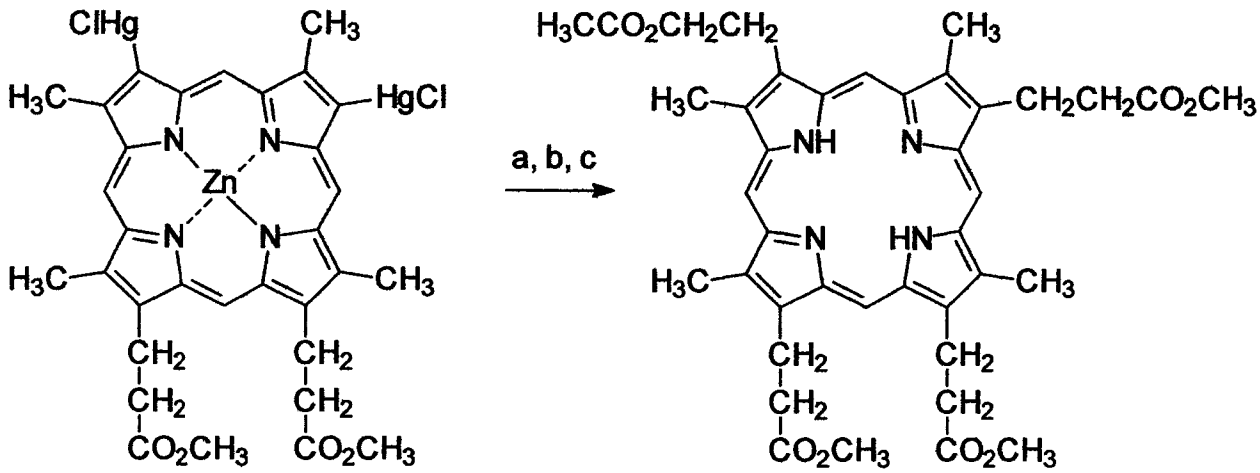
with paraformaldehyde in the presence of boron trifluoride etherate gave octasubstituted porphyrins. Novel dodecasubstituted porphyrins were also prepared using appropriately substituted aldehydes during the condensation. The yields of these porphyrins were low due to their inherent instability caused by the steric strain and resulting non-planarity of the porphyrin core. These porphyrins were investigated for their mesogenic properties and their ability to self-organize in well-ordered fluid structures.

b) Modification of Porphyrin Macrocycles

Even with these examples, the majority of the studies done encompassing the synthesis of porphyrins have involved the modification of the porphyrin periphery using palladium-catalyzed reactions to either prepare naturally occurring porphyrins or add novel functionality to the macrocycle. The readily availability and controlled synthesis of halogenated and tritolylated porphyrins [37-42] along with the stability of porphyrins towards mercuration [37,43] give the porphyrin the necessary functionality for palladium-catalyzed reactions. As was mentioned previously, the controlled reactivity along with the neutral, mild conditions involved prevents metalation and maintains the porphyrin macrocycle intact. Both β- and *meso*-positions along with *meso*-aryl substituents can be modified by this procedure in good yields, arriving at novel functional groups on the porphyrin that can greatly influence the properties of this macrocycle.

Naturally occurring porphyrins are usually synthesized using synthetic routes starting from pyrroles. Such schemes, while avoiding the necessity of regioselectively adding substituents to cyclized macrocycles, preclude the preparation of a large number of porphyrins due to the length and complexity of the synthetic procedure. Modification of pre-existing porphyrins to prepare naturally occurring porphyrins avoids these

problems and allows ready access to several porphyrin analogs that would otherwise be difficult to prepare. The controlled mercuration of deuteroporphyrin IX was shown and the resulting mercurated compounds were used in the synthesis of coproporphyrin III, harderoporphyrin, isoharderoporphyrin and S-411 porphyrin [44] using the chemistry developed by Heck [45]. For instance, treatment of 2,4-dimercurated zinc(II) deuteroporphyrin IX (formed by reacting zinc(II) deuteroporphyrin IX dimethyl ester with mercuric acetate in methanol, followed by addition of aqueous sodium chloride) with methyl acrylate in the presence of triethylamine and $LiPdCl_3$ (generated *in situ* from $PdCl_2$ and LiCl) followed by catalytic hydrogenation and demetalation lead to coproporphyrin III as its tetramethyl ester in a 37% overall yield (Figure 2) [44]. Similar chemistry has been exploited in the syntheses of both novel substituted porphyrins and chlorins [43]. β-Substitution was accomplished by either mercurating the macrocycle or by adding arylmercurials to unsaturated substituents on the porphyrin. Substituents added include methoxystyryl, nitrostyryl, carbomethoxystyryl and sulphonylstyryl groups along with ferrocene. Finally, after numerous attempts using various conditions and some difficulty in preparing the necessary precursors, zinc complexes of various vinylporphyrins and chlorins were successfully reacted with an acetyl-protected 5-chloromercuriuridine catalyzed by $LiPdCl_3$ to give fair yields of the corresponding nucleoside adducts [46]. Note that the desired *trans*-vinyl isomer was obtained surprising with equal amounts of a *gem*-isomer. Recently, porphyrins coupled with nucleosides have attracted attention owing to their strong tumouricidal activity and potential as inhibitors of HIV replication. While yields were not great in this study, the pharmaceutical potential of such compounds makes them of extreme importance.



a = methyl acrylate, $PdCl_2$, LiCl, DMSO, THF, Et_3N, b = TFA

c = 10% Pd/C, 97% formic acid, 35% perchloric acid

Figure 2. Synthesis of coproporphyrin III tetramethyl ester using palladium catalyst [44]

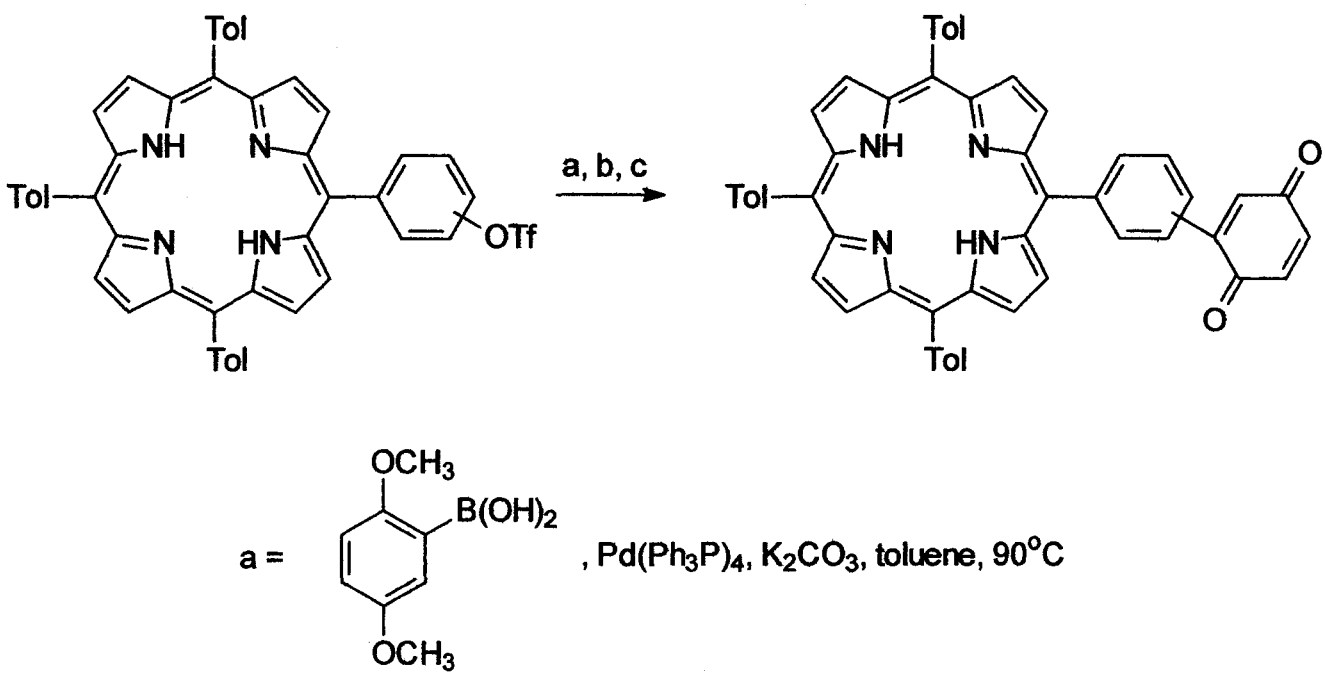


Figure 3. Synthesis of quinonylporphyrins via palladium-catalyzed cross-coupling reaction [49,50]

Numerous other functional groups have been added to the porphyrin macrocycle at both the β- and *meso*-positions using a wide variety of palladium-catalyzed reactions. The Suzuki reaction between organic halides and organoboranes was taken advantage of in order to add β-aryl substituents [47,48]. β-Monobromo-, β-tetrabromo, and β-octabromoporphyrins all underwent smooth Suzuki cross-coupling with various *p*-substituted aryl boronic acids to give the corresponding aryl substituted porphyrins in excellent yields. Electron-donated and withdrawing groups were introduced equally as well with the only prerequisite being completely anhydrous conditions. The β-brominated porphyrins are easily obtainable by controlled bromination, thus providing easy access to aryl-substituted porphyrins without the need for the tedious chromatographic separation of regioisomers needed when using a mixed condensation of porphyrin precursors. Analogously, palladium-catalyzed cross-coupling reactions between (2,5-dimethoxyphenyl)boronic acid and porphyrin *meso*-aryl meta and para triflates were carried out using catalytic amounts of $Pd(Ph_3P)_4$ and 2 equivalents of anhydrous potassium carbonate in toluene at 90°C for 2 hours under an inert atmosphere [49,50]. Following deprotection with boron tribromide and oxidation with DDQ, the corresponding quinonylporphyrins were obtained (Figure 3). Both mono- and tetraquinonylporphyrins were synthesized. These compounds may possibly be used as electrocatalysts where the reducible quinones can serve as electron reservoirs to facilitate multi-electron transfer reactions. In addition, chlorophyll and quinones are precisely positioned in photosynthetic reaction centers, making this new quinonylporphyrins models for the reactions involved in photosynthesis.

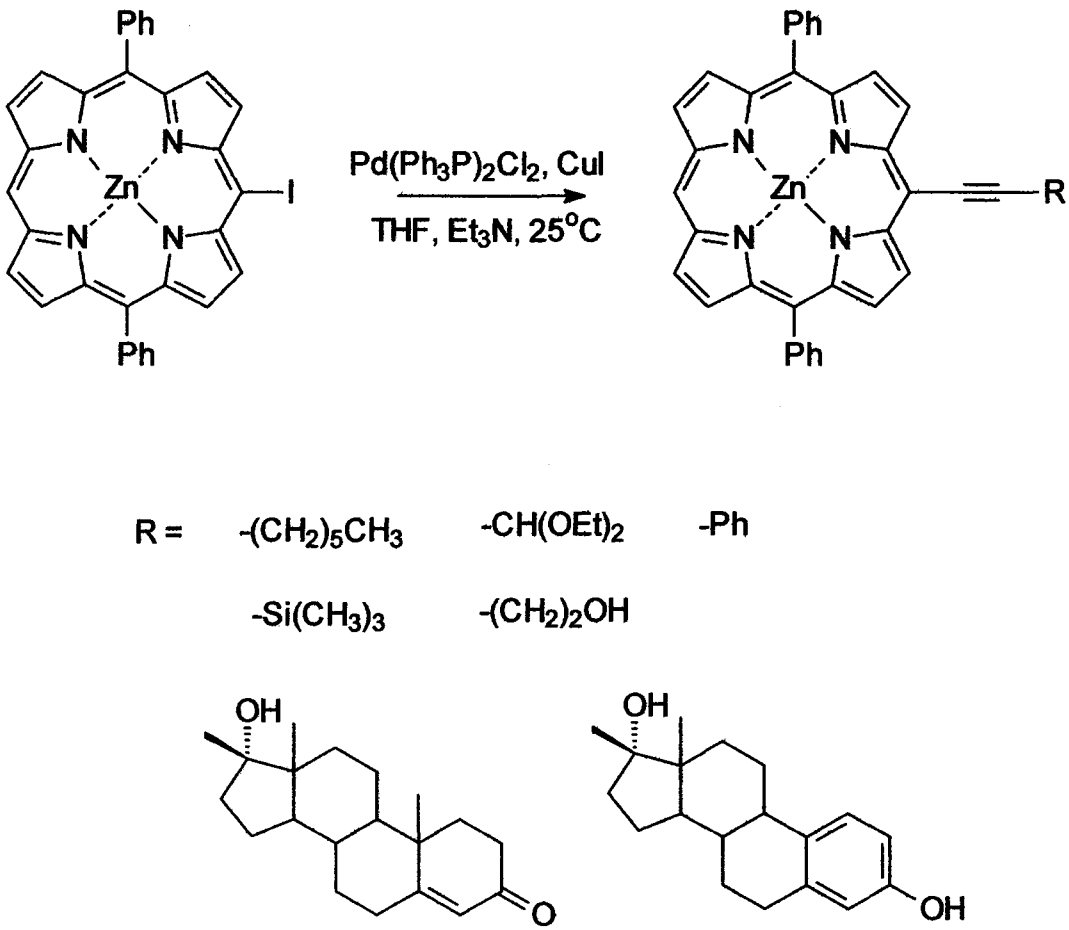


Figure 4. Example of Heck alkynylation in the synthesis of unsymmetrically *meso*-substituted porphyrins [41]

β- and *meso* substituents have also been added to the porphyrin core using the Heck alkynylation reaction between aryl halides and terminal alkynes. β-Substituents were added by reacting various brominated porphyrins with terminal alkynes [51]. Reaction rates and conversion ratios were dependent on steric effects, which can become prominent in β-substituted porphyrins. Typical reaction conditions involved catalytic quantities of bis(triphenylphosphine)palladium(II) chloride ($P(Ph_3P)_2Cl_2$) and copper(I) iodide (CuI) in a mixture of triethylamine and dimethylformamide (DMF). Nickel and zinc 3(8)-monoiododeuteroporphyrin IX dimethyl ester was also modified using this methodology. Similarly, this work was extended to *meso*-p-phenyl groups, giving high yields on the resulting *meso*-alkynyl-substituted products. *Meso*-p-phenyl groups were also modified with [4-(N,N-dimethylamino)phenyl]ethyne to give novel push-pull porphyrins with interesting nonlinear optical properties [52]. In the meanwhile, Stille and Heck reactions between *meso*-aryl substituents fitted with triflate leaving groups and either organostannes or terminal alkynes gave a secondary method towards such *meso-p*-alkynylaryl-substituted porphyrins [53].

Selective monoiodination with bis(trifluoroacetoxy)iodobenzene-iodine (1.2:1) followed by Heck alkynylation gave access to asymmetrically *meso*-substituted porphyrins [41]. Similar reaction conditions as those mentioned above were used, with $Pd(Ph_3P)_2Cl_2$ and CuI serving as catalysts and the reaction carried out in the presence of the organic base triethylamine, which scavenges the acid formed, thus protecting the palladium catalyst from degradation. Terminal alkynes such as 1-octyne, trimethylsilylacetylene, 3-butyn-1-ol, 17α-ethynyltestosterone and 17α-ethynylestradiol were used with yields ranging from 50-90% (Figure 4). Metalated porphyrins were

necessary to avoid potential metalation by the copper(I) catalyst and demetalation could be accomplished using traditional conditions.

Palladium-mediated cross coupling reactions can be used to add an endless list of substituents and functional groups to both the β- and *meso*-positions of porphyrins. DiMagno et al., for instance, used variations of the Stille coupling reaction to add alkyl, vinyl, aryl and pyridyl functional groups to both β- and *meso*-halogenated porphyrin macrocycles [54,55]. Using an organostannes or organozinc reagents and either $Pd(PPh_3)_4$ or Pd(dppf) as the catalyst, excellent yields (isolated yields are greater than 90%) of the substituted porphyrin were obtained (Figure 5 and 6), including high yields of perfluoroaryl substituents, which are absence elsewhere in the literature. It was noted that the reaction rate depended only on the catalyst used, with the Pd(dppf) reactions occurring under much more mild conditions with complete conversion of the starting material happening much more rapidly in most cases. Thus, this reaction would appear to be unimpeded by steric constraints with substrate electronic features playing the key role in determining the reactivity of the halogenated porphyrin template [55].

Palladium-catalyzed carbonylation reactions have also been shown to be useful in the modification of pre-existing porphyrins [56]. Similar to the reaction used to form pyrrole diester precursors, this reaction involves the use of a palladium(0) catalyst to mediate the alkoxycarbonylation of zinc *meso*-aryl brominated porphyrins. In this case, yields and conversion ratios depended greatly on the solvent and organic base used, most likely due to the limited solubility of the starting porphyrins. However, complete conversion of zinc-*meso*-(*p*-bromophenyl)porphyrin could be achieved using triethylamine as the base and n-butanol as both the nucleophile and the solvent. While

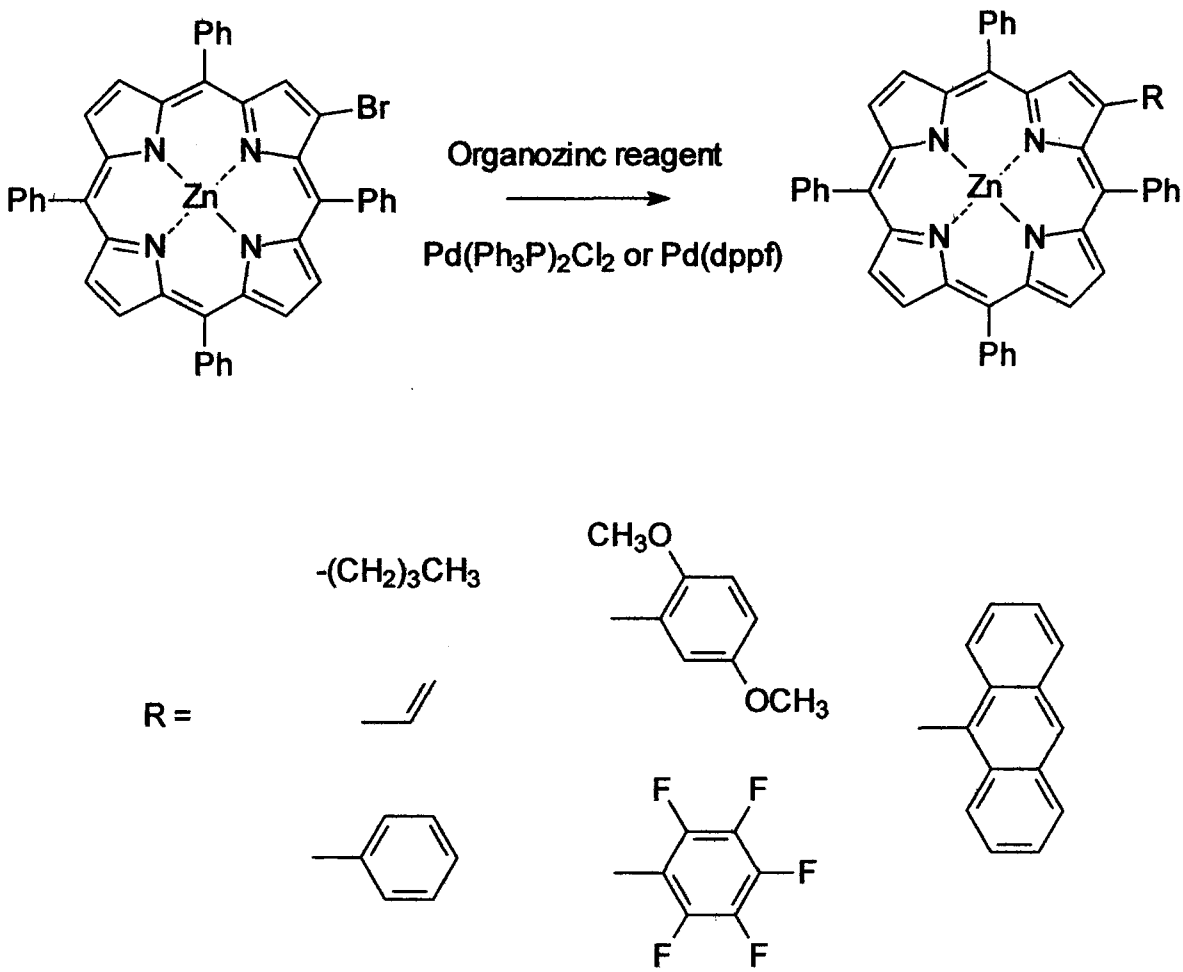
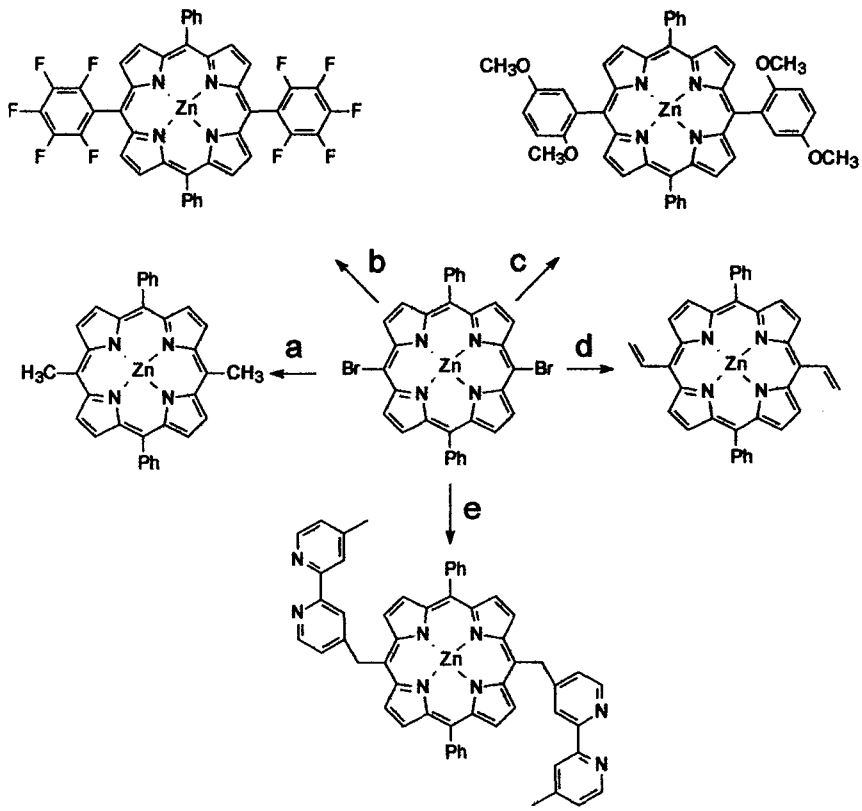


Figure 5. Palladium-catalyzed synthesis of β-substituted porphyrins [54,55]



a = MeZnCl, $Pd(PPh_3)_2Cl_2$, b = C_6F_5ZnCl, Pd(dppf)

c = $2,5\text{-}(CH_3O)_2C_6H_3ZnCl$, Pd(dppf), d = $Bu_3Sn(CH{=}CH_2)$, $Pd(PPh_3)_2Cl_2$

e = $Bu_3Sn[(4\text{-}CH_2)\text{-}4'\text{-}CH_3\text{-}bpy]$, $Pd(PPh_2)Cl_2$

Figure 6. Palladium-catalyzed synthesis of *meso*-substituted porphyrins [54,55]

longer reaction times were needed, catalytic carbonylation of the dibrominated porphyrin can the corresponding octaalkoxycarbonylporphyrin in good yield. In addition, zinc tetrabutoxytetrabenzoporphyrin was also prepared using the same chemistry and it was thought that after transmetalation with palladium, the complex could be quantitatively deesterified, giving the tetracarboxylated derivative, a novel water-soluble near-IR phosphorescent dye.

c) Synthesis of Multiporphyrin Arrays

Perhaps of more importance than the addition of novel substituents is the use of palladium-catalyzed reactions in the formation of oligomeric porphyrin arrays. The preparation of porphyrin arrays is an important area of research, not only for their possible application in the elucidation of natural photosynthetic mechanisms but also for probing the fundamental physical and chemical properties of the porphyrin chromophore. Furthermore, such arrays are vital in the development of molecular electronic devices for applications such as optical sensors and other optoelectronic materials. Porphyrin-based catalysts, magnetic materials and photosensitizers for photodynamic therapy also partly rely on incorporation of porphyrins into supramolecular systems and polymers. A common objective in the fabrication of many of these macromolecular arrays is the facile organization of individual porphyrin molecules into a multichromophoric assembly in order to achieve maximal electronic and excitonic interactions.

Palladium-catalyzed coupling reactions provide a number of desirable features for the synthesis of such arrays. They allow for the coupling reaction to be carried out in dilute solutions and form the desired product in extremely high yields and high purity. Using the appropriate catalyst, these reactions are compatible with both free base and

metalloporphyrins. This is particularly important in synthesizing artificial photosynthetic arrays where it is desirable to incorporate both free base and metalloporphyrins in distinct arrangements. The readily accessible ethynyl and oligoethynyl bridges are ideal linkages that enable unusually high excitonic and electronic coupling between the chromophores while providing the appropriate molecular rigidity. Finally, this synthetic protocol allows for the stepwise formation of multi-porphyrin arrays using molecular building blocks, greatly increasing the degree of control possible in their formation, thus allowing for the design of distinct chromophore arrangements.

With these important and highly desirable characteristics, palladium-catalyzed cross-coupling reactions have been extensively used in the synthesis of multi-porphyrin arrays and novel molecular geometries. Using the chemistry developed for the addition of alkynyl substituents to the porphyrin framework, Therien and collaborators synthesized an extended family of acetylenyl porphyrins (Figure 7) from brominated (5,15-diphenylporphinato)zinc (ZnDPP) [57]. Trimethylsilylacetylene was added to both the β- and *meso* positions with a di-*meso* ethynyl porphyrin was also prepared. These porphyrins displayed interesting electronic spectra, the di-meso-ethynyl porphyrin showing a distinct splitting of the Soret band due to an increase in conjugation along the C_2 molecular axis. Bis[(2,2',-5,10,15,20-tetraphenylporphinato)zinc(II)]butadiyne was prepared by the Eglinton reaction and this dimer revealed electronic and electrochemical properties characteristic of the porphyrin having essentially decoupled ground states. These suggest that the two porphyrin macrocycles lie approximately orthogonal to each other in this array. On the other hand, bis[5,5',-10,20-diphenylporphinato)zinc(II)]ethyne (the *meso*-linked dimer) and 5,15[bis{[(5',-10,20-diphenylporphinato)-

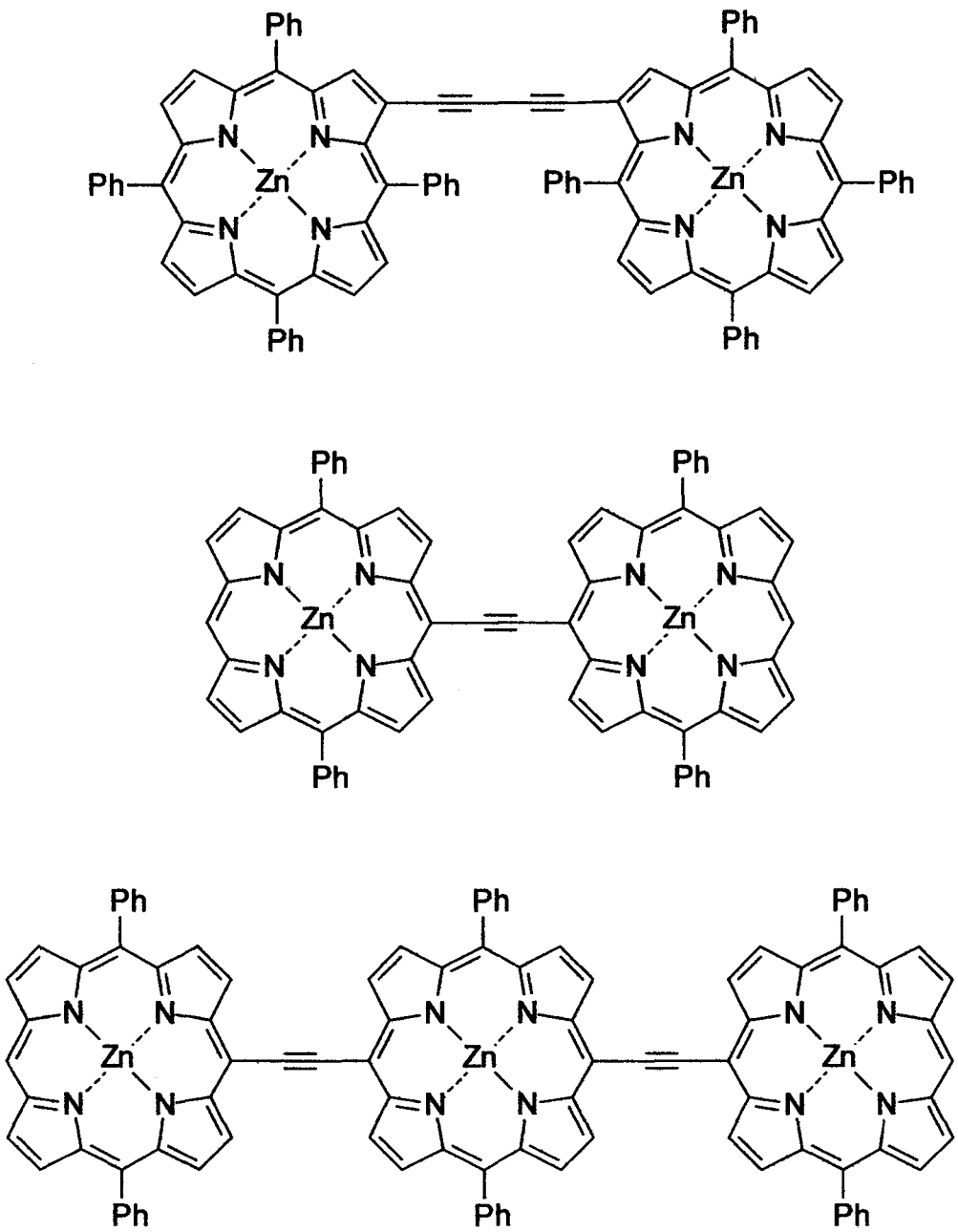


Figure 7. Examples of acetylenyl bridged porphyrins prepared using palladium-catalyzed coupling reaction [57]

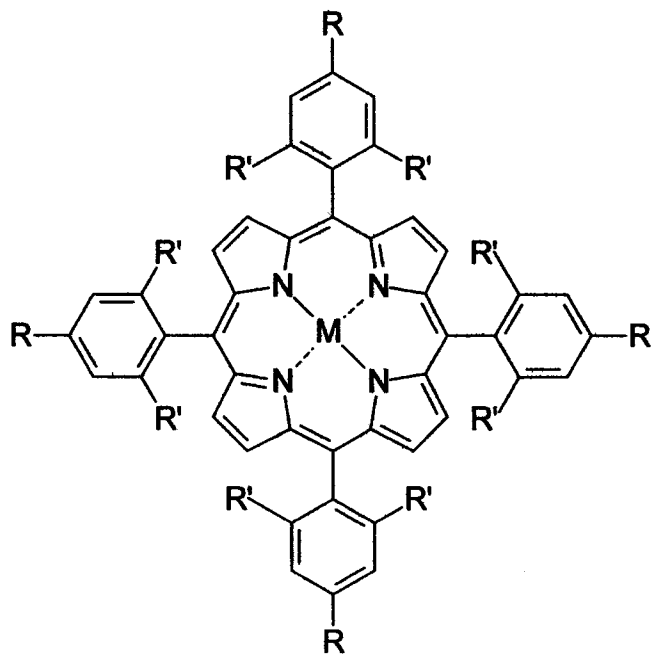
zinc(II)]ethynyl}[10,20-diphenylporphinato] zinc(II) (the *meso* linked trimer) was prepared by the palladium-catalyzed coupling of desilylated mono and di-*meso*-ethynyl porphyrins with (5-bromo-10,20-diphenylporphinato)zinc. The resulting dimer and trimer show significant broadening and usual splitting of the Soret band with a progressive and strong red-shift in the Q band with the trimer displaying a broad, strong Q-band absorbance centered at 802.2 nm. Such optical characteristics would be indicative of strong ground-state electronic interactions between the chromophores and suggest that the ethynyl-linked porphyrins are essentially co-planar. Electrochemical results also point towards strongly interacting ground-state redox centers and seem to indicate that the resulting charge accumulated during the reduction of the porphyrin rings is delocalized over the entire oligomer.

The suitability of these highly coupled porphyrins and thus the immense value of ethynyl-bridges and palladium-catalyzed cross-coupling reactions becomes ready apparent in the trimeric porphyrin array. This complex is the first synthetic system that accurately models the spectroscopic characteristics of specific subunits of a number of purple photosynthetic bacteria. Furthermore, the unusual photophysical and electrochemical properties of these acetylenyl-bridged porphyrins lend themselves to a number of other possible applications including the development of optical sensors, non-linear optic materials and new photosensitizers for photodynamic therapy.

Palladium-catalyzed cross-coupling reactions have also been used by Lindsey and his group to build ethyne- and butadiyne-linked porphyrins via *meso*-aryl substituents [13,17,31,33-35,58-62]. Such porphyrin oligomers have been extensively used to study energy and electron transfer process in such conjugated arrays. Molecular building block

strategies were employed to prepare the necessary ethynyl- and iodo-substituted porphyrins usually via a mixed condensation of appropriate precursors [33]. These porphyrin build blocks were then used to construct multiporphyrin arrays (Figure 8). *Meso*-iodoaryl-substituted porphyrins were used due to the increased reactivity of the aryl iodides towards the coupling reaction. Solvent mixtures varied depending on the solubility of the porphyrins involved and still required an organic base such as triethylamine. Initially, the synthesis of the ethyne-linked porphyrins were carried out using $Pd(Ph_3P)_4$ as the catalysis, which gave good yields of the desired product along with small amounts of the butadiyne-linked porphyrin and other higher molecular weight materials. Lower reaction temperatures and inert atmospheres lowered the amounts of these impurities. Finally, under these mild conditions, no metalation was observed, allowing this procedure to be used to form arrays containing both free base and metalloporphyrins, thus allowing the insertion of energy donors (metalloporphyrins) and energy acceptors (free base porphyrins) in the same oligomer.

Cross coupling reaction involving palladium are greatly enhanced by the presence of copper(I) halides [23,25,63]. Such co-catalysts are highly undesirable in forming multi-porphyrin arrays due to the potential for metalation of free base porphyrins within the array. However, it has been shown that reaction rates can be greatly accelerated using tri-2-furylphosphine and triphenylarsine as ligands for the palladium catalyst used [64]. It has been demonstrated that optimal ethyne-linked porphyrin trimer formation can be achieved using an *in situ* generated palladium catalyst from tris(dibenzylideneacetone)dipalladium(0) ($Pd_2(dpa)_3$) and triphenylarsine under an inert atmosphere [31,34]. Superior yields were obtained for the butadiyne-linked porphyrins



R = —CH_3 —OCH_3 —I —≡—H —≡—$Si(CH_3)_3$

—$CO_2(CH_2)_2Si(CH_3)_3$ —CO_2H

R' = —H —CH_3 —OCH_3

M = H_2 Zn Cu Ni

Figure 8. Porphyrin-based building blocks for the molecular construction of multiporphyrin arrays [24]

using tri-2-furylphosphine as the catalytic ligand under aerated conditions. This oxidative coupling could also be accomplished using stoichiometric amounts of a palladium(II) compound such as palladium diacetate or bis(triphenylphosphine) palladium(II) chloride. Enyne-linked porphyrins were obtained using $Pd(PPh_3)_4$ at more elevated temperatures and anaerobic conditions.

A number of multiporphyrin arrays have been prepared using this chemistry having various geometries and functional groups in order to examine factors influencing energy-transfer and electronic communication process in oligomeric porphyrins [34,35,58-62]. For instance, molecular squares containing four mutually coplanar porphyrins were synthesized in order to study the effect of porphyrin geometry on energy transfer process [62]. The molecular square was easily synthesized from the corresponding cis-substituted diethynyl and diiodo porphyrin building blocks using palladium-catalyzed coupling reactions as described above in acceptable yields. Free base or zinc porphyrins were placed on alternate corners of the square (Figure 9). The absorption spectrum of the molecular square was essentially the sum of the spectra of the individual components, indicating a relatively weak electronic interaction among the porphyrins. However, illumination of the square at 550 nm, a wavelength absorbed primarily by the zinc porphyrin afforded emission almost exclusively from the free base porphyrin, thus indicating an effective energy transfer between the energy donor and acceptor. In fact, the yield of energy transfer was 99.5% with the rate of energy transfer being identical to that of a free base-metalloporphyrin dimer where there is free rotation of the porphyrin rings [59,60]. As such, the molecular orientation of the porphyrin rings matters little in the energy transfer processes involved. Identical results were obtained

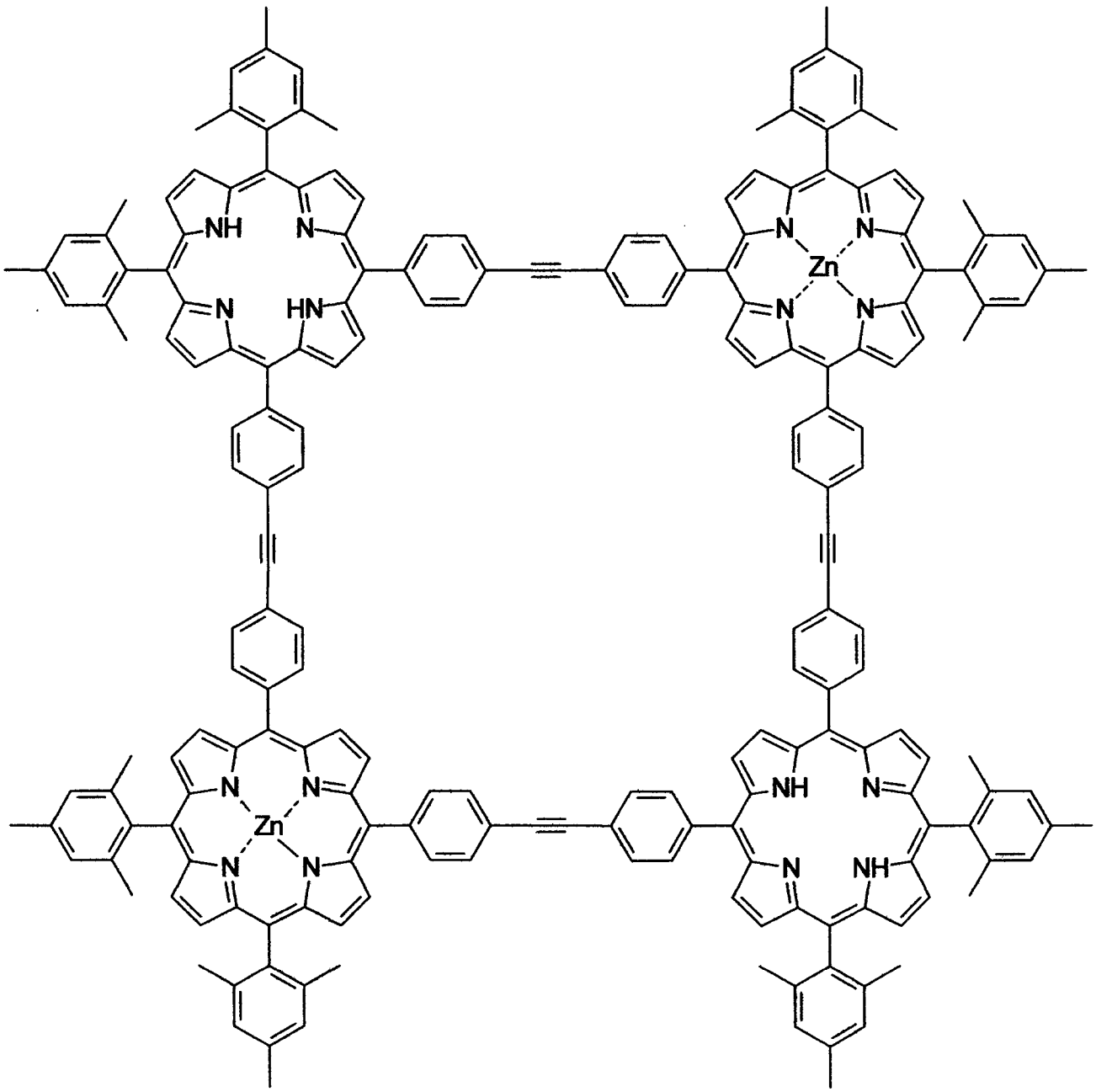


Figure 9. Porphyrin molecular square used to study the effect of porphyrin geometry on energy transfer processes in multiporphyrin array [62]

using a multiporphyrin array with a free base porphyrin surrounded by four zinc porphyrins linked by ethynyl groups [17]. Rapid and efficient energy transfer was observed and shown to involve through-bond processes. Conversely, it was determined that the nature and symmetry of the HOMO orbital greatly influenced the rate of energy transfer processes despite similar through-bond processes being involved [61]. Water-soluble amphiphilic porphyrin dimers and trimers have also been prepared to enable studies of transmembrane charge separation, electron transport and signal transduction through membrane bilayers (Figure 8) [58]. Identical palladium-catalyzed chemistry was employed with modification of the oligomer periphery leading to hydrophilic groups on the ends of the porphyrin arrays while the array itself is extremely hydrophobic. Thus, amphiphilic array was obtained and can be easily incorporated into vesicles.

Molecular photonic wires have also been prepared using identical palladium-catalyzed cross-coupling reactions [13,34]. In this case, linear arrays of zinc porphyrins are linked by ethyne bridges with terminal positions fitted with an optical input (a boron dipyrromethene dye) and an optical output (a free base porphyrin) (Figure 10). Illumination at 485 nm, the input dye absorbs the majority of the light while emission is 92% due to the free base porphyrin, indicating again a very effective energy transfer through the ethyne linkages.

A number of studies have involved the use of bis(phenylethynyl)arylene-linked porphyrins, either in the preparation of soluble conjugated metalloporphyrin polymers [65] or in the synthesis of porphyrin dimers for the study of intramolecular energy transfer [66,67]. In these cases, linkage involved the coupling of *meso*-alkynylaryl-substituted porphyrins with various iodobenzene derivatives. These reactions can either

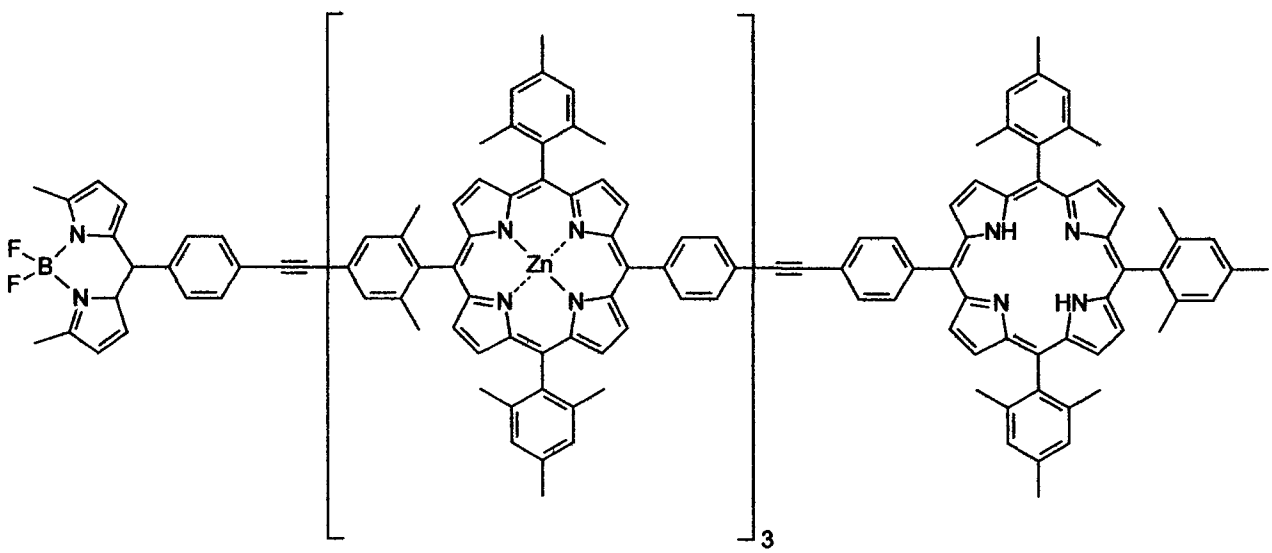


Figure 10. A porphyrin-based molecular wire [13,34]

be done stepwise, progressively adding each component [67] or in a one-step process to form a conjugated porphyrin-based polymer [65]. In addition, bis(phenylethynyl)phenylene linkers can be synthesized onto protected aldehydes, which following deprotection, can be used to synthesize the desired *ortho*, *meta* and *para*-bis(phenylethynyl)phenylene-bridged zinc-free base porphyrin dimers directly, using traditional porphyrin synthetic techniques [66]. Soluble conjugated metalloporphyrin polymers displayed high electron coupling along the conjugation of the polymer backbone and displayed strong red-shifted Q bands that were highly solvent dependent [65]. Such strong dependence opens the opportunity to use these polymers as optical sensors and molecular switches. Copper-free palladium-catalyzed cross-coupling of aryl iodides and terminal alkynes was used for the stepwise formation of porphyrin dimers linked diethynylphenylene bridges involving benzene, naphthalene and anthracene [67]. Triphenylarsine was used as a ligand for the palladium(0) catalyst to improve yields and rates of reaction. In the meanwhile, the preparation of *ortho*, *meta* and *para*-bis(phenylethynyl)phenylene-bridged porphyrin dimers allowed for the examination of the effect of molecular geometry on the energy transfer mechanism involved [66]. Using picosecond time-resolved fluorescence spectroscopy, the decay of the zinc porphyrin fluorescence was shown to correspond to an increase in the free base porphyrin fluorescence. Furthermore, it was shown that the 1,4-substituted bridge (*para*) has stronger electronic interactions than the 1,2- (*ortho*) and the 1,3-substituted ones (*meta*). Finally, similarities between calculated rates of energy transfer between the *ortho* and *meta* bridges clearly demonstrates that energy transfer entails through-bond interactions since the center-to-center distance is much shorter in the *meta*-bridge, and thus larger

rates would be expected if dipole-dipole interactions were involved. Similar bridged compounds were prepared using palladium(II) chloride and triphenylphosphine in triethylamine using nickel *meso*-ethynyloctaethylporphyrin and 1,2- and 1,4-diiodobenzene [68]. This procedure lead to important yields of the corresponding butadiyne bridges porphyrins, which could be removed using extensive chromatography. Reactions with 1,2-diiodobenzene were also complicated by much slower reaction rates, due to steric hindrance and the decomposition of the product into more polar compounds, potentially via a known *cis*-enediyne rearrangement. Finally, butenyne-linked porphyrins were also prepared via the palladium-catalyzed coupling of an alkynylporphyrin and nickel *meso*-(β-bromovinyl)octaethylporphyrin. This was the first example of such a linked porphyrin dimer.

Porphyrin dimers and trimers have also been synthesized using bridges linked to the β-position. Either linking β-alkynyl porphyrins with iodinated benzenes [69] or via oxidative coupling [70] or attaching of β-halogenated porphyrins with vinyl benzenes [71] or α,β-unsaturated carbonyl compounds [72] have been accomplished. Phase transfer conditions were used for palladium-catalyzed coupling of zinc(II)-monobromodeuteroporphyrin dimethylester with 1,4-divinyl- and 1,3,5-trivinylbenzene [71]. Coupling was accomplished in DMF using tetra-n-butyl-ammonium bromide, potassium carbonate, lithium chloride and palladium(II) acetate. Of interest is that the Soret band of the porphyrin dimer is further red-shifted than that of the trimer, indicating less conjugation in the *meta*-linked bridge. Similar conditions were used for the coupling reaction of zinc(II)-monobromodeuteroporphyrin dimethylester with various α,β-

unsaturated carbonyl compounds [72]. This reaction is plagued by low yields of the coupled product and increased yields of the corresponding monomer.

Oxidative coupling of β-ethynylmetalloporphyrins can be achieved using $Pd(Ph_3P)_4$ and copper iodide as catalysts in the presence of triethylamine [70]. While the electronic spectra of these dimers show some perturbation, it is far less than that observed above, seeming to indicate that meso-connection leads to greater electronic coupling between the individual porphyrin macrocycles. Such β-ethynylmetalloporphyrins can also be coupled to p-iodotoluene to form novel porphyrins, or to o-diiodobenzene and 1,3,5-triiodobenzene to form porphyrin dimers and trimers [69]. Traditional conditions ($Pd(Ph_3P)_4$, CuI, Et_3N, toluene) were used. Note that both of the β-ethynylmetalloporphyrins used were prepared via modification of formylated porphyrins.

A large number of other novel porphyrin oligomeric geometries have been prepared using palladium-catalyzed cross-coupling reactions. Gossauer and this group have successfully prepared porphyrin trimers and oligomers starting from 1,3,5-triiodobenzene [73]. Substitution of the iodo groups with trimethylsilylacetylene using traditional palladium-catalyzed Heck conditions gave poly(phenylacetylene)linkers capable of coupling with iodinated porphyrins following deprotection. Longer poly(phenylacetylene)linkers can be added either to the benzene core or to the iodinated porphyrins, providing the terminal phenyl groups contains an iodo leaving group. Furthermore, selective alkynylation of the starting 1,3,5-triiodobenzene can lead to unsymmetrical trimers where the individual porphyrin macrocycles are separated from the benzene core by different lengths. Note that the opposite approach in which the reactive terminal ethynyl groups are located on the porphyrin and the iodine leaving

groups in the core was discarded due to the formation of butadiyne-linked porphyrin dimers as byproducts. This group has also prepared dentritic porphyrin hexamers [73] along with tripodaphryins (Figure 11), which are tetrahedral assemblies in which a porphyrin macrocycle situated on the top of the molecule is supported by three legs consisting of linear arrays of covalently linked rigid constitutive elements usually with a terminal porphyrin molecule [74]. These compounds are based on tetrakis(4-iodophenyl)methane and are synthesized via the palladium-catalyzed coupling of tetrakis(4-ethynylphenyl)methane with 5-(4-iodophenyl)-10,15,20-triphenylporphine or the metalated equivalent. Elongation of the legs of the molecule can be accomplished by using phenylacetylene units as spaces, either attached to the methane core or to the porphyrins as describe above. All of these compounds were synthesized via the stepwise addition of phenylacetylene linkers using various palladium catalysts. Addition of phenylacetylene linkers to the tetrakis(4-iodophenyl)methane or 5-(4-iodophenyl)-10,15,20-triphenylporphine was accomplished using $Pd(Ph_3P)_2Cl_2$ and CuI. Addition of further linkers onto this core, along with to the 1,3,5-triiodobenzene core involved $Pd(Ph_3P)_4$ and CuI. Coupling of these cores to 5-(4-iodophenyl)-10,15,20-triphenylporphine also used $Pd(Ph_3P)_4$ and CuI. However, in cases where mixed metalation was desired in the final oligomer, CuI was not used and it was shown that triphenylarsine greatly increased the reaction rates and the overall yields of the reaction [73]. In fact, it was found that the use of triphenylarsine greatly increased the overall efficiency of the coupling reaction. Finally, note that owing to the dimensions of the molecules, no intramolecular interactions between the chromophores were observed [73,74]. Other unsymmetrically cyclized porphyrin oligomers have also been synthesized

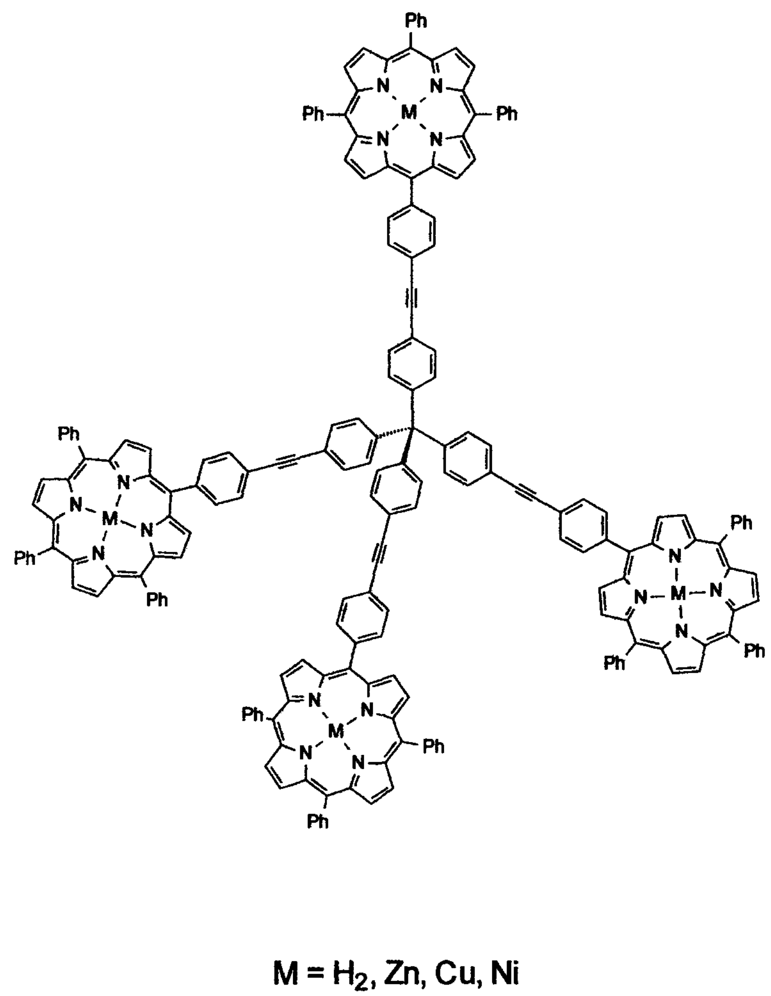


Figure 11. Example of a tripodaphyrins [74]

using palladium-catalyzed cross-coupling reactions and have been shown to be useful as cavities for molecular recognition [75].

Palladium in Phthalocyanine Synthesis

Unlike the case of porphyrins where palladium-catalyzed reaction were extensively utilized on pre-existing macrocycles, palladium-catalyzed reactions involving phthalocyanine synthesis have primarily involved the preparation of precursors. Only recently have attempts been made to take advantage of these mild and highly versatile reactions in modifying phthalocyanine macrocycles themselves. There are probably a number of reasons for this. Phthalocyanines are notoriously insoluble as compared to porphyrins, making the coupling reaction difficult in a number of potential cases. While the necessary porphyrin precursors, in particular the halogenated porphyrins, are readily available via controlled reactions and facile purification, such phthalocyanine-based precursors are not so easily obtained, generally leading to complex isomeric mixtures from which the desired product can be difficult to isolate and purify. Furthermore, most of the palladium-based chemistry involving porphyrins has been directed towards the preparation of multiporphyrin arrays, either in the synthesis of precursor, porphyrins or in the coupling of porphyrins. Such arrays, while of interest, are not nearly so important in the case of phthalocyanines, helping to explain the lag in the use of palladium-catalyzed reactions in modifying pre-existing phthalocyanine macrocycles.

a) Synthesis of Phthalocyanine Precursors

Leznoff and his group have done extensive work using palladium-catalyzed reactions in forming new precursors for the synthesis of phthalocyanines. For instance, multisubstituted phthalonitriles, naphthalenedicarbonitriles and phenanthrene-

tetracarbonitriles were synthesized using multistep reaction procedures involving palladium catalyzed coupling reactions [76]. Ethyne and ethene linked phthalonitriles were synthesized via the Heck and Stille coupling of 3-iodophthalonitrile with either acetylene with $Pd(Ph_3P)_2Cl_2$ and triethylamine or *trans*-1,2-bis(tri-*n*-butylstannyl)ethene with $Pd(Ph_3P)_4$. Similarly, 4-iodonapthalonitrile was coupled using identical procedures. Finally, the *cis* and *trans*-ethene-linked phthalonitriles were induced to cyclize photochemically to produce the corresponding 2,3,5,6- and 2,3,6,7-tetracyanophenanthrene.

4,5-Diiodophthalonitrile was synthesized and used to prepare a series of alkynyl-substituted phthalonitriles using copper-free palladium(II) catalysis [77,78]. Note that the more steric strained 3,3-dimethyl-1-butyne required more forcing conditions using CuI as a co-catalyst [77]. The copper co-catalyst was also used to form 4,5-bis(t-butyldimethylsilylethynyl)phthalonitriles [78]. Simple phthalonitriles were cyclized using lithium pentoxide to the corresponding octaalkynyl-substituted phthalocyanines [77]. The 4,5-bis(t-butyldimethylsilylethynyl)phthalonitrile was cyclized by heating in N,N-dimethylaminoethanol under continuous bubbling of ammonia gas [78]. In addition, 3,4-dibromophthalonitrile was synthesized and was successfully coupled with 3,3-dimethyl-1-butnyne using the same conditions are used for the 4,5-diiodophthalonitrile [78]. While the reaction did not go to completion, good yields of the desired 3,4-di(3,3-dimethyl-1-butynyl)phthalonitrile were obtained. This phthalonitrile readily cyclized when treated with lithium 1-pentoxide to give the first known 1,2,8,9,15,16,22,23-octasubstituted phthalocyanine. Metalation of the octaalkynyl-substituted phthalocyanines with zinc acetate in refluxing DMF was unsuccessful, with incomplete

metalation and some bleaching of the dye. Zinc was inserted by treating the dilithium phthalocyanines in situ with zinc acetate at 60°C [77]. The resulting phthalocyanines exhibited strong red-shifts in their Q-band by as much as 1 eV per alkynyl group as compared to unsubstituted phthalocyanines. NMR analysis of these phthalocyanines were undertaken and clearly demonstrated important dependences on the temperature and the concentration of phthalocyanine used [77]. Electrochemical and spectroelectrochemical analysis of [2,3,9,10,16,17,23,24-octa(3,3-dimethyl-1-butynyl)phthalocyaninato]cobalt(II) complex showed unusual electrochemical behavior, presumably due to the high solubility of these complexes and thus, their high degree of aggregation [79].

Coupling of 4-iodophthalonitrile with acetylene using the same Heck conditions as described above followed by either partial or complete hydrogenation of the triple bond gave phthalonitrile dimers. Following transformation into the corresponding diiminoisoindolines, binuclear phthalocyanines were synthesized using traditional procedures [80,81]. In addition, butadiyne-linked dimers were also prepared and hydrogenation ultimately gave butane-linked phthalocyanines [80]. The electronic spectra of the resulting ethynyl and ethene-linked phthalocyanine dimers were examined [81]. Interesting, the trans-ethene binuclear phthalocyanine had the spectra of a typical mononuclear phthalocyanine. On the other hand, the cis isomer has a blue-shifted Q-band, consistent with extensive intramolecular coupling. Cobalt complexes of these phthalocyanines were deposited as monolayer films on graphite electrodes and tested against the reduction of oxygen. Unfortunately, the expected four-electron reduction was not observed [81]. In the meanwhile, the spectroscopic properties of the ethane and

butane-link binuclear phthalocyanines were typical of phthalocyanine aggregates while their NMR spectra display a strong concentration dependence [80].

Palladium-catalyzed reactions have also be used to synthesize novel alkyl substituted phthalocyanines. Selective coupling of 1,2-dibromo-3,6-diiodo-4,5-dimethylbenzene and 1,2-dibromo-3,6-diiodo-4,5-dihexylbenzene with 1-heptyne and 1-hexyne using $Pd(Ph_3P)_2PdCl_2$, CuI, and triethylamine gave dibromobenzene substituted with two alkyl chains and two alkynyl chains. [82]. Following conversion to the phthalonitrile in a Rosenmund von Braun reaction with CuCN and catalytic hydrogenation gave tetraalkylphthalonitriles. Subsequent cyclization gave novel hexadecaalkyl-substituted ruthenium phthalocyanines. Other novel alkyl substituted phthalonitriles have been prepared bearing trimethylsilylethane, and 3,3-dimethylbutane substituents [80] while the 4,5-dialkynylphthalonitriles can be readily hydrogenated to give 4,5-dialkylphthalonitriles as well [77]. Furthermore, alkynyl-substituted phthalonitriles have been used to synthesize half-phthalocyanine intermediates for the synthesis of adjacent di-substituted phthalocyanines [83]. Stille coupling between 4-iodophthalonitrile and various aryl organostannanes has also been used to prepare phthalonitriles bearing novel aryl substituents [84], including phenyl, dimethoxybenzene and methylpyridine. In this case, $Pd_2(dpa)_3$ was found to be the most acceptable palladium(0) source.

Our own group has worked extensively on the synthesis of novel water-soluble phthalocyanines and has used palladium-catalyzed reactions to add functional groups that will enhance the solubility of these macrocycles in aqueous conditions. 4-Diethoxyphosphinyl phthalonitrile was prepared [85] using well-known palladium

chemistry [86] by reacting 4-iodophthalonitrile with diethyl phosphite in the presence of $Pd(Ph_3P)_4$ and triethylamine under an inert atmosphere. Complexation was accomplished by heating in the presence of a metal ion using quinoline or imidazole as a solvent. Hydrolysis of the diethylphosphinyl group was accomplished by heating the complex in 6 N HCl. The resulting phthalocyanines were highly water-soluble and display interesting aggregation properties. On the other hand, the Michaelis-Arbuzov reaction was used to synthesize 4-(diethylmethylphosphonato)phthalonitrile [87,88] while phosphonate derivatives with butyl spacer chains were prepared by treatment of 4-iodophthalonitrile with but-3-yn-1-ol under Heck conditions to yield 4-(1-hydroxybut-3-ynyl)phthalonitrile. Catalytic hydrogenation gave 4-(1-hydroxybutyl)phthalonitrile, which readily reacted with diethyl chlorophosphate to give the corresponding phosphonated phthalonitrile [89], which could be simply cyclized to the phthalocyanine. Similarly, palladium-catalyzed alkynation of 4-iodophthalonitrile with propyn-1-ol and hex-5-yn-1-ol, followed by catalytic hydrogenation gave 4-(propylhydroxy)phthalonitrile and 4-(hexylhydroxy)phthalonitrile, both of which could easily be transformed into the hydroxylated phthalocyanine [90].

b) Modification of Phthalocyanine Macrocycles

As was mentioned above, very few examples exist in the literature for the modification of pre-existing phthalocyanines using palladium-based chemistry. However, the immediate benefits of this methodology were readily apparent. In attempts to synthesize tetraethynylphthalocyanines, it was observed that cyclization of 4-ethynylphthalonitrile [80] in N,N-dimethylaminoethanol lead to a complex mixture of higher molecular weight compounds [91]. Protecting this terminal alkyne with a

trimethylsilyl protecting group still gave significant yields of oligomeric material, probably due to partial removal of the protecting group under the harsh conditions used for cyclization. Alkynation of metallotetraiodophthalocyanine with 2-methyl-but-3-ynol in the presence of $Pd(Ph_3P)_2Cl_2$ and CuI in diethylamine, on the other hand, gave high yields of the protected tetraalkynylphthalocyanines (Figure 12). Metal-free phthalocyanines were prepared using $Pd_2(dpa)_3$ and triphenylarsine as the catalyst for the reaction as it was observed that introduction of a copper ion occurred when CuI was present in the reaction mixture. Conversely, acceptable yields were obtained for the same phthalocyanines via the cyclotetramerization of 4-(3-hydroxy-3-methyl-1-butynyl)phthalonitrile [92]. Removal of the dimethylcarbinol-protecting group was achieved by treatment with sodium hydroxide. The resulting tetraethynylphthalocyanines were scarcely soluble in organic solvent, pointing out one problem when working with phthalocyanines. However, the utility of palladium-catalyzed techniques was obvious.

Our group has used palladium-catalyzed cross-coupling reactions to synthesize novel unsymmetrically substituted phthalocyanines [93]. Zinc(II) tri(*t*-butyl)-4-iodophthalocyanine was used as a starting material and was prepared via a mixed condensation. Treatment of this phthalocyanine with trimethylsilylacetylene in the presence of $Pd(Ph_3P)_2Cl_2$ and CuI in toluene containing triethylamine readily gave the trimethylsilylacetylene-substituted phthalocyanine. The acetylene derivative was obtained by removing the trimethylsilyl-protecting group with dilute aqueous sodium hydroxide in methanol. This terminal alkyne could be used as starting material for the preparation of dimeric phthalocyanines using the same reactions used for porphyrins. Furthermore, other terminal alkynes can be used to add phenyl, pyridinic, purinic and

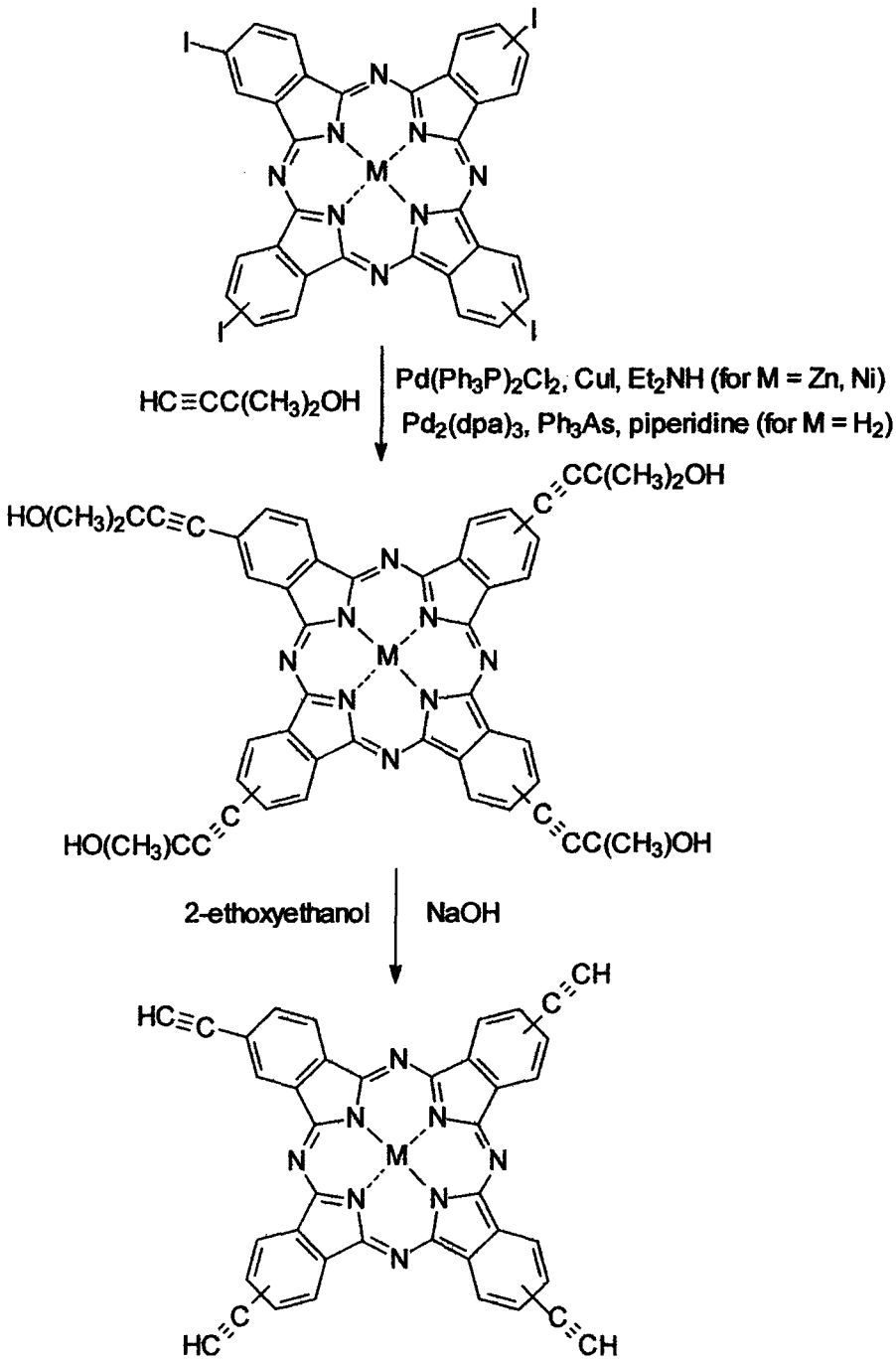


Figure 12. Synthesis of tetraethynylphthalocyanines using palladium-mediated coupling reactions [91]

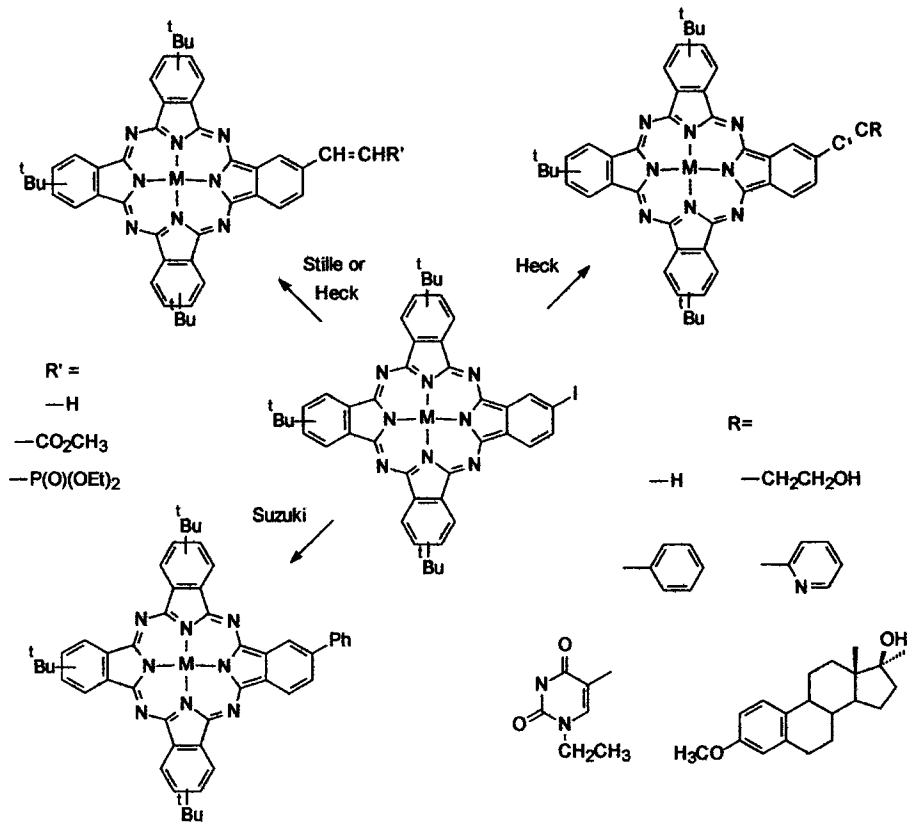


Figure 13. Synthesis of monofunctionalized phthalocyanines using Heck, Stille and Suzuki reactions [93]

estrogenic groups to the macrocycle (Figure 13). Similar products were obtained from the Heck reaction of a monoiodobenzonaphthaloporphyrazine with alkynes. Heck reaction conditions can also be used to add alkenes to the phthalocyanine framework and this has been used to add diethylvinylphosphonate to the macrocycle. Using palladium(II) acetate as a catalyst allowed for the coupling with methyl acrylate, thus adding carboxylic esters to the list of substituents that can be added. Moreover, Stille coupling reactions of organotin reagents such as vinyltributyltin offered another technique for preparing phthalocyanine-styrene derivatives (Figure 13). Finally, phthalocyanines bearing aryl substituents could readily be synthesized using Suzuki cross-coupling with arylboronic acids (Figure 13). All this reactions lead to novel unsymmetrically substituted phthalocyanines and demonstrate that palladium-catalyzed coupling methodologies greatly simplify the preparation of novel phthalocyanine derivatives.

Palladium-catalyzed reactions have also been used to prepare dimeric phthalocyanine arrays with extended conjugation [92], much like in the case of porphyrins. 4-(3-hydroxy-3-methyl-1-butynyl)phthalonitrile was synthesized by reacting 4-iodophthalonitrile with 2-methyl-but-3-yn-2-ol in the presence of $Pd(Ph_3P)_2Cl_2$ and CuI. The mixed condensation of this phthalonitrile with excess 4-tert-butylphthalonitrile in the presence of metal(II) chlorides followed by extensive purification gave the corresponding monoalkynylated zinc and nickel phthalocyanines in good yields. Deprotection with sodium hydroxide gave the desired terminal alkyne. Oxidative homocoupling to give butadiyne-linked phthalocyanine dimers was achieved in the same way as porphyrins using copper(II) acetate (Eglinton reaction) (Figure 14). Ethynyl-

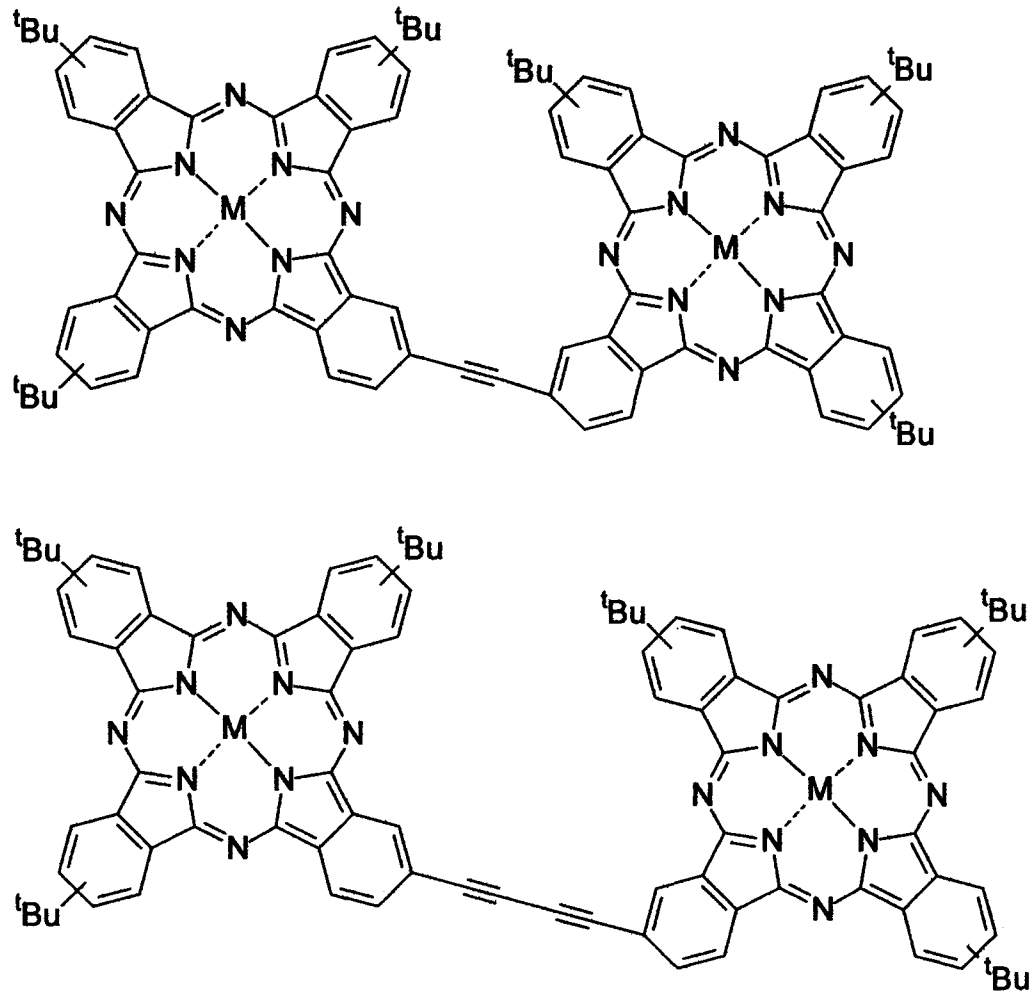


Figure 14. Homo- and hetero-dimetallic ethynyl- and butadiynl-bridged phthalocyanine complexes [92]

linked phthalocyanine dimers were formed by cross-coupling of the terminal alkyne with monoiodophthalocyanine (zinc tri(*t*-butyl)-4-iodophthalocyanine) (Figure 14). $Pd_2(dpa)_3$ and triphenylarsine were used for the in situ generation of the desired palladium(0) catalyst and gave superior yields in shorter reaction times as compared to $Pd(Ph_3P)_2Cl_2$ and CuI. All the resulting dimers, either the homometallic butadiyne-linked phthalocyanines or the homometallic and heterometallic ethynyl-bridged binuclear compounds, showed red-shifting and splitting of the Q-band in the electronic spectrum. This is presumably due to in increased conjugation and loss of symmetry in the dimers. As in the example of porphyrins, such phthalocyanine building blocks will allow for the stepwise synthesis of multiphthalocyanine arrays with different central metals and distinct substituent patterns and could be of interest in nonlinear optical applications.

Palladium-mediated cross-coupling of alkynes has also been applied subphthalocyanines as well. These lower homologs to phthalocyanines display a Hückel aromatic delocalized system and their non-planar cone-shaped geometry make these molecule attractive targets for nonlinear optical applications. Attempts to extend the conjugation of subphthalocyanines have centered on using palladium-catalyzed coupling reactions [94]. Boron triiodosubphthalocyanine readily reacted with terminal alkynes such as trimethylsilylacetylene, 1-pentyne, 3-methoxy-1-propyne and *p*-nitrophenylacetylene in the presence of $Pd(Ph_3P)Cl_2$ and CuI in triethylamine under an inert atmosphere, allowing for the synthesis of highly conjugated trialkynyl subphthalocyanines (Figure 15). Q-band absorptions for this highly conjugated macrocycles were strongly red-shifted as would be expected. Our lab is presently attempting to extend this work to hexaiodosubphthalocyanines. In addition, we have

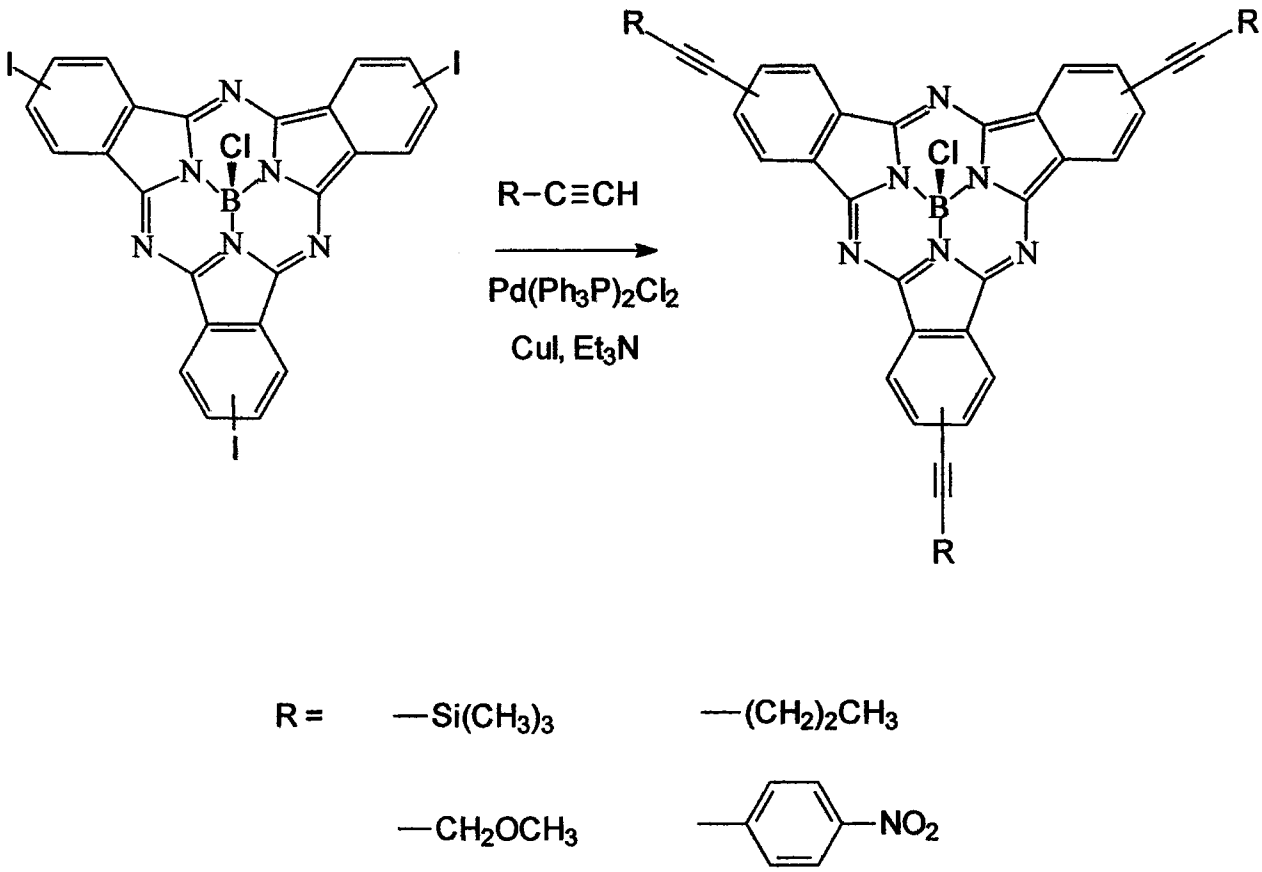


Figure 15. Synthesis of highly conjugated boron subphthalocyanines using palladium catalyst [94]

recently employed the well-known ring enlargement reaction on both the boron triiodo- and hexaiodosubphthalocyanines, successfully preparing the corresponding phthalocyanines (unpublished results) and are using these phthalocyanines to extend palladium-catalyzed reactions to these macrocycles as well.

Conclusion

In conclusion, the rich chemistry of palladium catalysts has lead to the preparation of vast array of novel porphyrins and phthalocyanines. The mild reaction conditions involved and the compatibility with most functional groups make palladium-mediated reactions extremely versatile procedures for the synthesis of novel porphyrins and phthalocyanines. Moreover, utilizing palladium-mediated methodologies on pre-existing macrocycles have several synthetic advantages. 1) A catalytic, quantitative conversion of reactants to products in high yields allows for highly functionalized macrocycles. 2) Facile reaction conditions permit the addition of sensitive organic functional groups. 3) Decoupling of the ring cyclization from the elaboration of the macrocyclic periphery facilitates syntheses of target macrocyclic molecules. It is evident that palladium-catalyzed reactions hold immense promise in preparing new porphyrins and phthalocyanines that may well allow them to fulfill a variety of roles in a wide-range of applications.

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Chapter 7.

Synthesis and Photodynamic Activity of Novel Asymmetrically Substituted Fluorinated Phthalocyanines

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Synthesis and Photodynamic Activity of Novel Asymmetrically Substituted Fluorinated Phthalocyanines

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TITLE RUNNING HEAD: Photodynamic properties of fluorinated phthalocyanines.

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[1]Abbreviations: $AlPcS_4$, aluminum tetrasulphonated phthalocyanine; CRM, Cremophor™ EL; DDQ, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; DMF, N,N-dimethylformamide; DMSO, dimethylsulfoxide; FBS, fetal bovine serum; HPLC, high performance liquid chromatography; HPPI, 3α-hydroperoxy-1,2,3,3α,8,8α-hexahydropyrrolo[2,3β]indole-2-carboxylic acid; LD50, light dose required for 50% cell inactivation; LD90, light dose required for 90% cell inactivation; LDL, low density lipoproteins; MgPc, magnesium phthalocyanine; MTT, 3(4,5-dimethylthiazol-2-yl)-2,5-(diphenyltetrazolium bromide); NLO, non-linear optics; NMP, 1-methyl-2-pyrrolidinone; PBS, phosphate buffered saline; PEG, polyethylene glycol; Pc, phthalocyanines; PS, photosensitizers; subPc, subphthalocyanines; THF, tetrahydrofuran.

ABSTRACT.

A series of asymmetrically substituted dodecafluorinated phthalocyanines has been synthesized via the Kobayashi ring expansion reaction of the corresponding dodecafluorinated boron subphthalocyanine with differently substituted diiminoisoindolines. The mild reaction conditions employed during this ring expansion reaction gave rise exclusively to 3:1 asymmetrically substituted dodecafluorinated phthalocyanines. Metal insertion into the metal-free phthalocyanines was accomplished by heating at 40°C in DMF in the presence of zinc bromide. The resulting zinc dodecafluorophthalocyanines were formulated as Cremophor™ EL (CRM) oil-water emulsions and evaluated as photosensitizers in vitro against EMT-6 mouse mammary tumor cells. As compared to the previously studied zinc hexadecafluorophthalocyanine, these new asymmetrical zinc dodecafluorophthalocyanines exhibited improved photodynamic activity.

KEYWORDS: Fluorinated phthalocyanines, SAR, photosensitizer, photodynamic therapy, cancer

INTRODUCTION

Tetrapyrrolic macrocycles such as porphyrins represent one of the most important and more interesting ligand systems, not only due to their ubiquitous presence in Nature but also because of their unique physical, chemical, biological and spectral properties. Phthalocyanines (Pc) are azoporphyrin derivatives and have been extensively studied in order to examine structure-active relationships and to improve upon some of the unique characteristics of the tetrapyrrolic chromophore *(1)*. Ever since their serendipitous discovery and identification *(2-4)*, phthalocyanines have been used extensively as dyes and pigments in the paint, printing, textile and paper industries due to their intense blue-green color, high dyeing power, photostability, insolubility in most solvents and chemical inertness *(4)*. In addition, phthalocyanines have found industrial applicability as photoconducting agents in photocopying devices and as catalyst for important industry reactions *(1,5)*. In fact, cobalt phthalocyanine derivatives are used in the Merox process for the oxidation of sulfur compounds in gasoline fractions *(6)*. More recently, phthalocyanines have found high-tech applications in electrophotography *(7)*, ink jet printing *(8)* and data storage *(9,10)*. Even with these important industrial and high-tech applications, the potential of phthalocyanines remains relatively untapped. Due to their unique chemical and physical properties, the importance of phthalocyanines is rapidly growing in a number of other fields. These fields include chemical sensors *(11)*, electrochromism *(12)*, molecular metals *(13)*, liquid crystals *(14)*, Langmuir-Blodgett films *(15)*, functional polymers *(16)*, semiconductors *(17)*, non-linear optical applications *(18)* and photosensitizers for photodynamic therapy *(19,20)*.

Diverse applications such as those proposed for Pcs require compounds with distinct and well-defined physical, chemical and electronic properties. These necessitates synthetic approaches for the preparation of single isomers or well-defined isomeric mixtures of substituted Pcs and for the preparation of Pcs bearing novel substituents. In addition, for a number of these applications, the physical and chemical properties of Pcs are enhanced when the Pcs are asymmetrically substituted. For instance, while symmetrically substituted Pcs exhibit large optical nonlinearities and third order harmonic generation due to their extensively delocalized 18-π electron system, second order nonlinear optical effects are only present in non-centrosymmetric molecules and are enhanced by electron-donating, electron withdrawing push-pull systems *(18)*. In light of this, the synthesis of unsymmetrically substituted Pcs bearing both electron-donating and electron-withdrawing functional groups has been investigated *(21,22)*. In addition to the potential utility in NLO applications, it has been established that asymmetrically substituted phthalocyanines often exhibit increased potential as photosensitizers for photodynamic therapy *(23,24)*.

A number of different strategies have been investigated in order to prepare asymmetrically substituted non-centrosymmetric Pcs. The most commonly used synthetic approach is the statistical mixed condensation of differently substituted phthalocyanine precursors *(25)*. While the yield of the desired substitution pattern have been enhanced by carefully selecting the substituents on the phthalocyanine precursors, the molecular proportions of the individual precursors and the reaction conditions used, the statistical mixed condensation method involving two differently substituted phthalocyanine precursors does lead to a mixture of six differently substituted

phthalocyanine products which must be separated, usually by chromatography. If the two Pc precursors used differ in their solubility significantly in a given solvent, separation of the differently substituted Pcs may be relatively simple due to the different solubility of the resulting Pcs. However, in most cases, this separation has proven troublesome.

Another method investigated is a polymer-support route where one of the phthalocyanine precursors is covalently attached to a polymer support prior to the synthesis of the Pc *(26)*. Following the synthesis of the macrocycle, the polymer bound Pc is obtained by filtration and the bond to the polymer is cleaved to yield pure 3:1 unsymmetrically substituted Pc. While this method has been used successfully to prepare pure unsymmetrically substituted Pcs, it is limited by the number of functional groups that can be covalently bonded to the polymer support. Furthermore, yields are less than satisfactory due to the low bonding capacity of the polymer support.

The Kobayashi ring expansion reaction of boron subphthalocyanines provides an efficient method for preparing 3:1 unsymmetrically substituted Pcs *(27)*. Boron subPc are the lower homologs of phthalocyanines, consisting of a tripyrrolic macrocycle *(28)*. The loss of an isoindoline unit causes a hypsochromic shift in the Q band of the electronic spectra to around 560-580 nm, lending a reddish purple color to solutions of these subPcs. While Pcs are extremely stable molecules exhibiting a high degree of planarity in their central aromatic core, subPcs have a cone-shaped structure, with the boron coordinated in a tetrahedral geometry with a single axial ligand. Their extensively delocalized 14-π electron system and their cone-shaped structure have led to investigations in the utility of subPcs in non-linear optical applications *(29)*. In light of this, a number of substituted subPcs have been prepared *(27-34)*. In addition, attempts

have been made to alter the properties of subPcs by varying the axial ligand coordinated to the boron *(32,35,36)*. It has been demonstrated that the less stable subPcs readily react with 1,3-diiminoisoindolines in a ring enlargement reaction to yield 3:1 unsymmetrically substituted Pcs *(27,37)*. First disclosed by Kobayashi in 1990, this ring enlargement reaction unfortunately may lead to a mixture of differently substituted Pcs and it has been proposed that the ring enlargement reaction of subPcs is a non-selective multistep reaction which depends greatly on the nature of the substituents on the subPcs, the reactivity of the 1,3-diiminoisodoline, the solvent and the reaction conditions used *(21,38,39)*. While this greatly limits the general synthetic utility of this reaction, this reaction protocol has been shown to be useful in a number of examples, giving the desired 3:1 unsymmetrically substituted Pc in good yields and as a pure single phthalocyanine product *(27,37,40-43)*.

In order to further investigations carried out by our group using zinc hexadecafluorophthalocyanine and zinc dodecafluorophthalo-4-sulphophthalocyanine, the Kobayashi ring expansion reaction of boron dodecafluorosubphthalocyanine (**2**) has been examined. We have found that subPc **2** readily reacts with 1,3-diiminoisoindolines under extremely mild conditions to give the corresponding 3:1 unsymmetrically substituted phthalocyanine in good yields and without contamination with phthalocyanines with differing substitution patterns. Furthermore, we have found that the starting boron subPc **2** can be prepared under much more mild conditions than those described in the literature *(34)*. The photodynamic activity, both in terms of singlet oxygen generation and in vitro photocytotoxicity, of some of the resulting unsymmetrically substituted

dodecafluorinated Pcs has been determined and has indicated that asymmetry, even in lipophilic photosensitizers, improves the photodynamic efficacy of these compounds.

EXPERIMENTAL PROCEDURES

All solvent were HPLC grade and were used without further purification unless otherwise stated. Tetrafluorophthalonitrile, 3-nitrophthalonitrile and 4-tert-butylphthalonitrile were purchased from TCI America (Portland, Oregon). 1-Dodecyne, 1,2-dicyanobenzene, 1,2,4,5-tetracyanobenzene, 4-nitrophthalonitrile, 1,3-diiminoisoindoline, dichlorobis(triphenyl-phosphine)palladium(II), 10% palladium on carbon, 1M boron tribromide in dichloromethane and zinc bromide were purchased from Aldrich (Oakville, Ontario, Canada). 4-Iodophthalonitrile *(44)*, 3-iodophthalonitrile *(45)* and 4,5-diiodophthalonitrile *(46)*, 5-*tert*-butyl-1,3-diiminoisoindoline, 5-nitro-1,3-diiminoisoindoline, 5-iodo-1,3-diiminoisoindoline, 4-iodo-1,3-diiminoisoindoline, 5,6-diiodo-1,3-diiminoisoindoline, 5,6-dicyano-1,3-diiminoisoindoline and 2,3-dihydro-1,3-diimino-1H-benz[f]isoindole *(47-50)*, $ZnPcF_{16}$ *(51)*, $ZnPcF_{12}S_1$ *(52)* and $AlPcS_4$ *(53,54)* were prepared by using modified literature procedures. UV-visible spectra were recorded with a Hitachi U-2000 spectrophotometer. ^1H and ^{19}F NMR were obtained on a Brucker AC-300 spectrometer. FAB-MS were obtained on an LG Autospec Q mass spectrometer.

4,5-(1-Dodecynl)phthalonitrile: 4,5-Diiodophthalonitrile (2.61 g; 6.87×10^{-3} mol) was dissolved in 50 mL of triethylamine and 5 mL of DMF. To this was added 300 mg of dichlorobis(triphenylphosphine)palladium(II). The reaction mixture was stirred at 100°C under an inert atmosphere and 6.4 mL of 1-dodecyne (2.99×10^{-2} mol; 4.35 equiv.). The reaction was monitored by TLC (10% diethyl ether in hexanes). After two

hours, the reaction was cooled, filtered and the filtrate was evaporated to dryness under reduced pressure. The resulting solid was purified by column chromatography on silica using 10% diethyl ether in hexanes as eluant. The desired product was obtained in a yield of 2.66 g (84.7%). $C_{32}H_{44}N_2$ MS (EI) *m/e* 456 (M^+), 385 (M^+-$CH_3(CH_2)_4$), 371 (M^+-$CH_3(CH_2)_5$), HR MS (EI) *m/z* calculated for $C_{32}H_{44}N_2$ 456.3504, found, 456.3522.

4,5-(Dodecyl)phthalonitrile: 4,5-(1-Dodecynl)phthalonitrile (2.5 g; 5.47 x 10^{-3} mol) was dissolved in 200 mL of THF. To this solution was added 500 mg of 10% palladium on carbon. The suspension was stirred at room temperature and hydrogen gas was bubbled through the reaction mixture. The reaction was monitored by TLC (10% diethyl ether in hexanes). After 6 hours, the reaction mixture was filtered to remove the catalyst and the filtrate was evaporated to dryness under reduced pressure. The resulting solid was purified by column chromatography on silica using 10% diethyl ether in hexanes as eluant. The desired product was obtained in a yield of 2.42 g (95.2%). $C_{32}H_{52}N_2$ MS (EI) *m/e* 464 (M^+), 449 (M^+-CH_3), 435 (M^+-2 CH_3), 421 (M^+- CH_3, CH_3CH_2), HR MS (EI) *m/z* calculated for $C_{32}H_{52}N_2$ 464.4130, found, 464.4122

5,6-Didodecyl-1,3-diiminoisoindoline (3g): Sodium metal (68.5 mg; 2.98 x 10^{-3} mol) was dissolved in 30 mL of methanol. Ammonia gas was bubbled through the reaction mixture for 5 minutes prior to the addition of 4,5-(dodecyl)phthalonitrile (919 mg; 1.98 x 10^{-3} moles). The mixture was stirred at 60°C with periodic bubbling of ammonia. The reaction was monitored by TLC (20% methanol in toluene). After 10 hours, the mixture was cooled to 0°C, saturated with ammonia gas, sealed and stirred at 4°C overnight. The reaction mixture was then warmed to room temperature and the solvent was removed under reduced pressure. The resulting solid was washed with

concentrated NH_4Cl, water and ice cold methanol and dried. The desired product was obtained in a yield of 938 mg (98.5%). $C_{32}H_{55}N_3$ MS (EI) *m/e* 482 (M^++H), HR MS (EI) *m/z* calculated for $C_{32}H_{55}N_3$ 481.4396, found, 481.4401

Dodecafluorosubphthalocyanato boron(III) bromide ($SubPcF_{12}$) (2). Tetrafluorophthalonitrile (**1**) (518 mg; 2.59 x 10^{-3} mol) was dissolved in a minimum amount of chlorobenzene (1.8 mL). The resulting solution was stirred at room temperature and 1.8 mL of 1M BBr_3 in dichloromethane (1.80 x 10^{-3} mol of BBr_3; 0.7 equiv.) was added. After 5 minutes, the reaction mixture was heated to 60°C and after 1 hour cooled to room temperature. The solvent was removed by rotary evaporation at reduced pressure and the resulting solid was dissolved in 30% THF in hexanes. This solution was filtered and purified by column chromatography over neutral alumina using 30% THF in hexanes as eluant. The desired product was obtained in a yield of 402 mg (67.4%). $C_{24}N_6BBrF_{12}$ MS (FAB) *m/e* 692 (M^++H), 612 (M^+-Br), ^{19}F NMR (dichloromethane) δ -59.76, -59.81, -70.18, -70.23 (δ = 0 for TFA), UV-vis λ (dichloromethane) (log ε) 577 nm (4.93), 532 nm (2.01).

Synthesis of dodecafluorinated phthalocyanines.

1,2,3,4,8,9,10,11,15,16,17,18-dodecafluorophthalocyaninate (H_2PcF_{12}) (4a): To a stirred solution of (dodecafluorosubphthalocyanato)boron(III) bromide (**2**) (400 mg; 5.78 x 10^{-4} mol) in 4 mL of DMSO was added a solution of 1,3-diiminoisoindoline (**3a**) (451 mg; 3.11 x 10^{-3} moles; 5.4 equiv.) in 6 mL of DMSO at room temperature. The reaction mixture instantly lost its intense purple color and gradually became blue. After 2-4 hours, the reaction mixture was added to a large excess of methanol (50 mL). The suspension was left at 4°C overnight and then the green solid was collected by

centrifuging. The desired product was obtained in a yield of 244 mg (59.5%). MS (FAB) *m/e* 731 (M^++H), HR MS (FAB) *m/z* calculated for $C_{32}H_6N_8F_{12}$ 730.0524, found, 730.0572, UV-vis λ (DMF) 671 nm, 601 nm.

The following dodecafluorinated phthalocyanine derivatives were prepared using the same synthetic procedure described above. Note that for 5,6-didodecyl-1,3-diiminoisoindoline (**3g**), the reaction was accomplished using a 1:1 solvent mixture of DMSO and chlorobenzene.

1,2,3,4,8,9,10,11,15,16,17,18-Dodecafluoro-23-t-butylphthalocyaninate (4b). Yield 40.5%. MS (FAB) *m/e* 786 (M^++H) UV-vis λ (DMF) 673nm, 606 nm.

1,2,3,4,8,9,10,11,15,16,17,18-Dodecafluoro-23-nitrophthalocyaninate (4c). Yield 20.7%. MS (FAB) *m/e* 776 (M^++H), UV-vis λ (DMF) 684 nm, 667 nm, 635 nm.

1,2,3,4,8,9,10,11,15,16,17,18-Dodecafluoro-23-iodophthalocyaninate (4d). Yield 25.9%. MS (FAB) *m/e* 857 (M^++H), UV-vis λ (DMF) 671 nm, 607 nm.

1,2,3,4,8,9,10,11,15,16,17,18-Dodecafluoro-22-iodophthalocyaninate (4e). Yield 18.7%. MS (FAB) *m/e* 857 (M^++H), UV-vis λ (DMF) 680 nm, 612 nm.

1,2,3,4,8,9,10,11,15,16,17,18-Dodecafluoro-23,24-diiodophthalocyaninate (4f). Yield 30.3%. MS (FAB) *m/e* 983 (M^++H), UV-vis λ (DMF) 669 nm, 612 nm.

1,2,3,4,8,9,10,11,15,16,17,18-Dodecafluoro-23,24-didodecylphthalocyaninate (4g). Yield 11.7%. MS (FAB) *m/e* 1067 (M^++H), UV-vis λ (DMF) 676 nm, 613 nm.

1,2,3,4,8,9,10,11,15,16,17,18-Dodecafluoro-23,24-dicyanophthalocyaninate (4h). Yield 28.8%. MS (FAB) *m/e* 780 (M^+). UV-vis λ (DMF) 689nm, 661 nm, 636 nm.

(1,2,3,4,8,9,10,11,15,16,17,18-Dodecafluoro)tribenzo[b,g,l]napthalo[2,3-q]-porphyrazine (4i). Yield 51.8%. MS (FAB) *m/e* 780 (M^+), UV-vis λ (DMF) 700nm, 675 nm, 620 nm, 604 nm.

Synthesis of dodecafluorinated zinc phthalocyanines.

1,2,3,4,8,9,10,11,15,16,17,18-Dodecafluorophthalocyaninato zinc (5a).

1,2,3,4,8,9,10,11,15,16,17,18-Dodecafluorophthalocyaninate (**4a**) (100mg; 1.37 x 10^{-4} mol) was suspended in 5 mL of DMF. To this was added zinc bromide (72 mg; 3.20 x 10^{-4}; 2.3 equiv.). The reaction mixture was heated to 40°C for 2 hours and was then cooled to room temperature. The solvent was removed by rotary evaporation under reduced pressure and the resulting solid was washed twice with 1.2 M hydrochloric acid (25 mL each) and twice with 95% ethanol (25 mL each). The solid was dried and then dissolved in tetrahydrofuran and purified by column chromatography on silica gel (40% THF in hexanes). The desired product was obtained in a yield of 105 mg (96%). MS (FAB) *m/e* 793 (M^++H), HR MS (FAB) *m/z* calculated for $C_{32}H_4N_8F_{12}Zn$ 791.9659, found, 791.9648, UV-vis λ (THF) (log ε) 674 nm (5.13), 604 nm (4.38), 356 nm (4.57).

The following dodecafluorinated zinc phthalocyanine derivatives were prepared using the same synthetic procedure described above.

1,2,3,4,8,9,10,11,15,16,17,18-Dodecafluoro-23-t-butylphthalocyaninato zinc ($ZnPcF_{12}$(t-butyl)) (5b). Yield 98%. MS (FAB) *m/e* 849 (M^++H, UV-vis λ (THF) (log ε) 663 nm (5.16), 629 nm (4.91), 355 nm (4.89).

1,2,3,4,8,9,10,11,15,16,17,18-Dodecafluoro-23-nitrophthalocyaninato zinc ($ZnPcF_{12}(NO_2)$) (5c). Yield 91%. $C_{32}H_3N_9O_2F_{12}Zn$ MS (FAB) *m/e* 838 (M^++H), UV-vis

λ (THF) (log ε) 678nm (4.92), 667 nm (4.88), 620 nm (4.55), 601nm (4.53), 340 nm (4.89).

1,2,3,4,8,9,10,11,15,16,17,18-Dodecafluoro-23-iodophthalocyaninato zinc (ZnPcF$_{12}$(4-I)) (5d). Yield 95%. $C_{32}H_3N_8F_{12}IZn$ MS (FAB) *m/e* 919 (M^++H), UV-vis λ (THF) (log ε) 671 nm (5.10), 640 nm (4.66), 608 nm (4.48) 357 nm (4.66).

1,2,3,4,8,9,10,11,15,16,17,18-Dodecafluoro-22-iodophthalocyaninato zinc (ZnPcF$_{12}$(3-I)) (5e). Yield 92%. $C_{32}H_3N_8F_{12}IZn$ MS (FAB) *m/e* 919 (M^++H)) UV-vis λ (THF) (log ε) 680 nm (5.19), 612 nm (4.54), 344 nm (4.66).

1,2,3,4,8,9,10,11,15,16,17,18-Dodecafluoro-23,24-diiodophthalocyaninato zinc (ZnPcF$_{12}$(I$_2$)) (5f). Yield 97%. $C_{32}H_2N_8F_{12}I_2Zn$ MS (FAB) *m/e* 1045 (M^++H), UV-vis λ (THF) (log ε) 676 nm (5.21), 643 nm (4.69), 355 nm (4.91).

1,2,3,4,8,9,10,11,15,16,17,18-Dodecafluoro-23,24-didodecylphthalocyaninato zinc (ZnPcF$_{12}$(C$_{12}$)$_2$) (5g). Yield 88%. $C_{56}H_{52}N_8F_{12}Zn$ MS (FAB) *m/e* 1129 (M^++H) UV-vis λ (THF) (log ε) 683 nm (4.90), 664 nm (4.86), 359 nm (4.61).

1,2,3,4,8,9,10,11,15,16,17,18-Dodecafluoro-23,24-dicyanophthalocyaninato zinc (ZnPcF$_{12}$(CN)$_2$) (5h). Yield 90%. $C_{34}H_2N_{10}F_{12}Zn$ MS (FAB) *m/e* 843 (M^++H), UV-vis λ (THF) (log ε) 675 nm (5.11), 620 nm (4.75), 359 nm (4.88).

(1,2,3,4,8,9,10,11,15,16,17,18-dodecafluoro)tribenzo[b,g,l]napthalo[2,3-q]-porphyrazine zinc (ZnPcF$_{12}$nap) (5i). Yield 98%. $C_{36}H_6N_8F_{12}Zn$ MS (FAB) *m/e* 843 (M^++H), UV-vis λ (THF) (log ε) 710 nm (4.96), 679 nm (4.92), 352 nm (4.55), 337 nm (4.39).

Drug formulation. In order to impart water-solubility, phthalocyanines **5a-5i** were formulated as Cremophor™ EL emulsions as previously published *(55)*. Briefly, the Pcs were dissolved in THF and 1 mL of Cremophor™ EL and 0.3 mL of 1,2-propanediol were added to the solutions. The solutions were then sonicated for thirty minutes, followed by removal of the THF solvent by rotary evaporation under reduced pressure. Phosphate buffered saline (PBS) (8.7 mL) was added to the viscous liquid and the mixture was sonicated for 30 minutes. The resulting emulsion was filtered (0.45 μm, Millipore). Concentrations were determined by serial dilution of the stock preparation with THF.

L-Tryptophan photooxidation *(56)*. A solution of 5 μM Pc and 5 mM L-tryptophan in PBS (1 mL total volume) was irradiated using a high intensity xenon light source (model IL 302) equipped with a CermaxR xenon short arc 300 W lamp (model LX300F) (ILC Technology, Sunnydayle, CA). A liquid guide fiber-optic (model 77556, Oriel Corp., Stratford, CT) was used to deliver the light. Two filters, a LS-700 and a LL-600 (Corion, Holliston, MA), were used to allow transmission of light in the 600-700 nm range (Note that the LS-700 filter was removed when (1,2,3,4,8,9,10,11,15,16,17,18-dodecafluoro)-tribenzo[b,g,l]napthalo[2,3-q]-porphyrazine zinc was used). The fluence rate was 150 mW cm^{-2}. After 2 and 4 minutes of irradiation, 80 μL samples were removed from the photooxidation reaction and were analyzed by HPLC to quantify the characteristic hydroperoxide products of singlet oxygen tryptophan peroxidation (3a-hydroperoxy-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indole-2-carboxylic acid; HPPI isomers) (t_R = 5.8 and 8.6 minutes, t_R for tryptophan = 15.7 minutes). The relative HPPI yield was measured from the HPPI chromatogram peak areas using a Shimadzu HPLC

system (Kyoto, Japan) composed of an LC-600 pump, SP-6AV UV-visible detector, a DGV-4A degasser, a LPM-600 low pressure mixing system, a rheodyne injector and EZChrom chromatography data acquisition and analysis system. During this analysis, a C18 Radial Pak cartridge (10 x 0.8 cm) filled with 4 μm Nova Pak C18 reversed phase packing was eluted at 1.5 mL min^{-1} with 0.1% trifluoroacetic acid (TFA) in water and a linear gradient over 25 minutes from 1 to 30% methanol (0.1% TFA). The wavelength of a UV-visible detector was set at 280 nm. The relative HPPI yield was evaluated by comparing the peak areas corresponding to the HPPI isomers to the total tryptophan peak area and then arbitrarily comparing this ratio to the ratio obtained using $AlPcS_4$ in PBS.

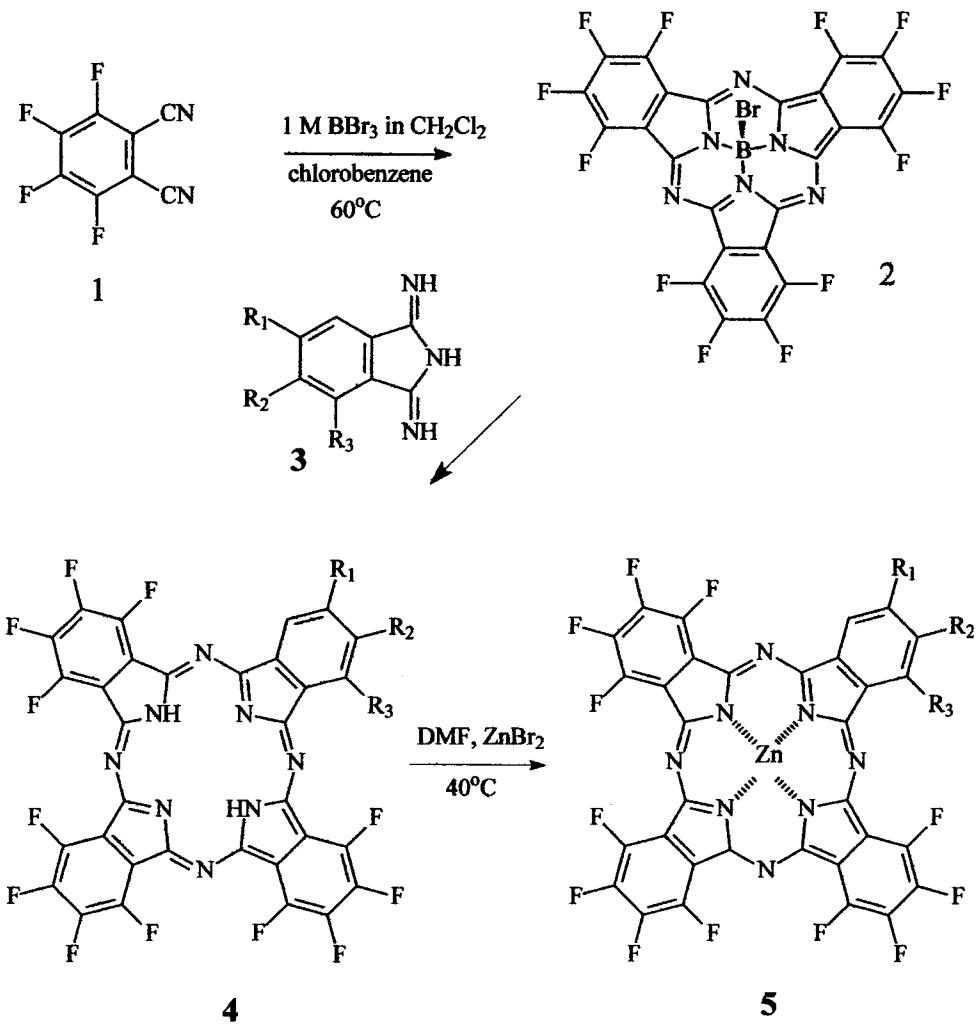
In vitro photocytotoxicity. A suspension of approximately 1.5 x 10^4 EMT-6 cells per well in 100 μL of Waymouth, 15% FBS were incubated overnight at 37°C, 5% CO_2 in 96 well microtitration plates (Falcon). The FBS solution was supplemented with 1% L-glutamine and 1% Penicillin-Streptomycin. The first column (8 wells) served as a blank control (no cells) while the second and third columns (16 wells) served as a control (no phthalocyanine added, 100% cell survival). The cells were rinsed twice with PBS and then 50 μL of Pcs **5a**, **5b** and **5g** were added at concentrations of 1 μM and 5 μM in Waymouth, 1% FBS. The plates were incubated at 37°C, 5% CO_2 for 1 or 24 hours. Before irradiation, the cells were rinsed twice with PBS and then re-fed with 100 μL of Waymouth, 15% FBS. Irradiation consisted of illuminating the microtitration plates with red light (660-700 nm) at graded fluences of 0 to 54 J cm^{-2} at a fluence rate of 100 mW cm^{-2}. The light source consisted of two 500 W tungsten/halogen lamps (GTE Sylvania, Drummondville, Quebec, Canada) fitted with a circulating refrigerated filter containing

aqueous rhodamine B (Sigma, Ontario, Canada) (OD_{580} = 1.25). Cell survival was then assessed by means of the colorimetric MTT assay *(23)*.

A MTT (Aldrich Canada Ltd., Oakville, Ontario, Canada) stock solution of 5 mg mL^{-1} was prepared and kept at 4°C in the dark. Upon a five-fold dilution in Waymouth, 15% FBS, 50 µL of the MTT solution was added to all the wells of the microtitration plate and the cells were incubated for four hours at 37°C, 5% CO_2. Acidic sodium dodecyl sulfate (SDS) solution (10% SDS in 0.01N HCl) (100 µL) was then added to each well and the cells were incubated for 24 hours at 37°C, 5% CO_2. Following this incubation, the microtitration plates were agitated at room temperature for 10 seconds and the absorbance at 595 nm was read on a microplate reader (BioRad, Ontario, Canada). The percent cell survival was determined as follows. The average absorbance of the blank cells containing only MTT solution was subtracted from the average absorbance obtained from the wells of identical treatment as well as subtracted from the average absorbance measured from the control wells containing untreated cells (which represent 100% cell viability). The percent cell survival was calculated by dividing the absorbance of the treated cells by the absorbance of the non-treated cells and multiplying by 100. Eight replicates were run and experiments were repeated three times.

RESULTS

Synthesis of boron dodecafluorinated subphthalocyanine (2). The tetrafluorophthalonitrile readily reacts with the stronger Lewis acid boron tribromide in chlorobenzene at temperatures of 40°C - 60°C, with the reaction mixture rapidly adopting



a: $R_1 = R_2 = R_3 = H$
b: $R_1 = R_3 = H$, R_2 = t-butyl
c: $R_1 = R_3 = H$, $R_2 = NO_2$,
d: $R_1 = R_3 = H$, $R_2 = I$
e: $R_1 = R_2 = H$, $R_3 = I$

f: $R_1 = R_2 = I$, $R_3 = H$
g: $R_1 = R_2 = CH_3(CH_2)_{11}$, $R_3 = H$
h: $R_1 = R_2 = CN$, $R_3 = H$
i: $R_1 = R_2 =$ -CH=CH-CH=CH-, $R_3 = H$

Figure 1. Synthesis of dodecafluorinated phthalocyanines **4a-i** via the Kobayashi ring expansion reaction of dodecafluorosubphthalocyanato boron(III) bromide (**2**).

the characteristic intense purple color of the corresponding subPc (Figure 1). In fact, the reaction proceeded relatively smoothly in dichloromethane at room temperature, though yields of the desired tripyrrolic macrocycle were significantly lower and reaction times were longer. The use of chlorobenzene instead of the higher boiling 1-chloronaphthalene greatly eased the purification of the dodecafluorosubphthalocyanine **2**. The latter product was purified by column chromatography on neutral alumina in the dark (70% yield).

At lower reaction temperatures (0 °C) in either dichloromethane or chlorobenzene a transient blue intermediate which absorbed around 660 nm was obtained, instead of the desired intensely purple subPc **2**. This transient blue intermediate transformed into the purple subphthalocyanine product upon heating. Interestingly, treatment of a solution of the transient blue species with DDQ lead to a short-lived absorption around 660 nm in the electronic spectra. Furthermore, treatment of a solution of the dodecafluorinated subPc with NaBH4 resulted in the loss of the characteristic Q band at 577 nm and the formation of a broad transient absorption around 660 nm.

Kobayashi ring expansion reaction of the boron dodecafluorinated subphthalocyanine (2). SubPc **2** readily reacted with various 1,3-diiminoisoindolines (**3a-3i**) in DMSO to give the corresponding 3:1 unsymmetrically substituted dodecafluorinated phthalocyanines (**4a-4i**) (Figure 1). The reaction proceeded exceptionally rapid, being complete after 1 hour, with an immediate loss of the intense purple color of the subPc solution. In the case of 5,6-didodecyl-1,3-diiminoisoindoline (**3g**), the 1,3-diiminoisoindoline was only marginally soluble in DMSO, necessitating the use of a 1:1 mixture of DMSO and chlorobenzene as reaction solvent. The metal-free Pc

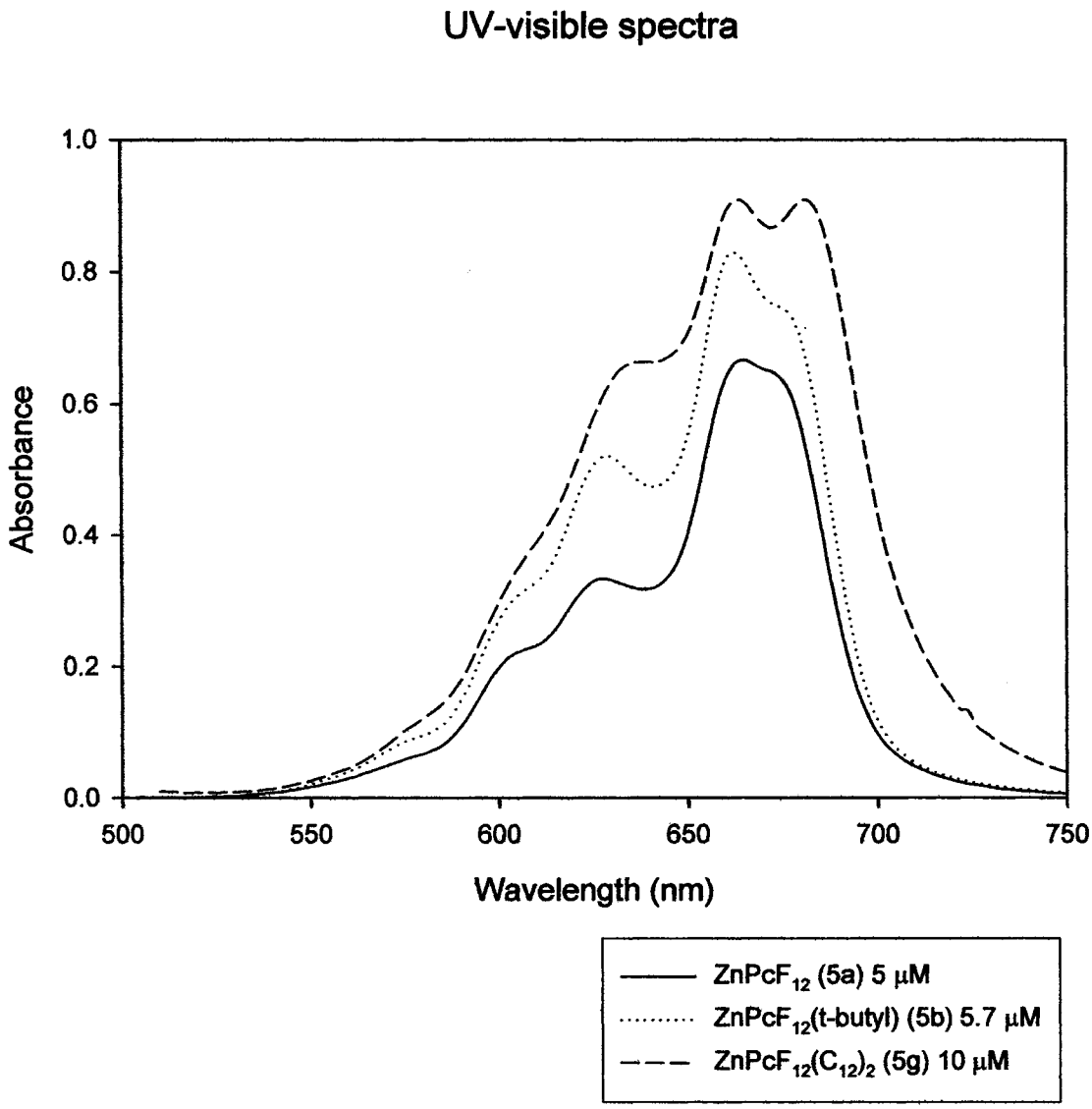


Figure 2. UV-visible spectra of **5a**, **5b** and **5g** in THF.

4a-4i prepared by this reaction were typically insoluble in the reaction solvent and precipitated from solution in yields ranging from 10-60%. In cases where the Pc product was slightly soluble in the reaction solvent, the product could readily be isolated by adding the reaction mixture to a large excess of methanol. The resulting green precipitate could be isolated by centrifugation or filtration. Mass spectra of all final Pc products exhibited intense peaks corresponding to the molecular ion (M^+) or M^++1. The metal-free dodecafluorinated phthalocyanines **4a-4i** were practically insoluble in most common organic solvents but slight solubility in DMF. Electronic absorption spectra in DMF displayed characteristic Q band absorptions around 670-680 nm with a weaker satellite in the range of 600-620 nm (Figure 2). The extended conjugation of (1,2,3,4,8,9,10,11,15,16,17,18-dodecafluoro)tribenzo [b,g,l]napthalo[2,3-q]-porphyrazine (**4i**) shifted the absorption to a longer wavelength (700 nm, 675 nm) as would be expected. Metal ion insertion was accomplished in nearly quantitative yield by suspending the metal-free dodecafluorinated Pcs **4a-i** in DMF at 40-60°C in the presence of zinc acetate (Figure 1). Other reaction solvents such as NMP failed to give the corresponding ZnPc and led to important macrocycle degradation. Attempts were undertaken to prepare the corresponding dodecafluorinated ZnPc directly by adding zinc ions to the Kobayashi ring expansion reaction. However, while the reaction appeared to give the desired 3:1 unsymmetrically substituted Pc, yields were greatly decreased. In addition, the ZnPcs exhibited higher solubility in the reaction solvent, leading to significant difficulties in isolating and purifying the final phthalocyanine product.

Tryptophan photooxidation. The asymmetrically substituted dodecafluorinated ZnPcs **5a-i** formulated as Cremophor™/PBS emulsions were exposed to red light in the

Table 1. Relative hydroperoxide (HPPI) yields as a result of L-tryptophan photooxidation

Photosensitizer[a]	λ_{max} (nm)	Relative peroxide yields
$AlPcS_4$ (in PBS)[b]	680 nm	1
$AlPcS_4$	678 nm	1.15
$ZnPcF_{16}$	678 nm	1.40
$ZnPcF_{12}S$	674 nm	1.82
$ZnPcF_{12}$ (**5a**)	674 nm	1.38
$ZnPcF_{12}$(t-butyl) (**5b**)	663 nm	1.49
$ZnPcF_{12}(NO_2)$ (**5c**)	678 nm, 667 nm	2.30
$ZnPcF_{12}$(4-I) (**5d**)	671 nm	2.43
$ZnPcF_{12}$(3-I) (**5e**)	680 nm	2.31
$ZnPcF_{12}(I_2)$ (**5f**)	676 nm	2.00
$ZnPcF_{12}(C_{12})_2$ (**5g**)	683 nm, 664 nm	1.17
$ZnPcF_{12}(CN)_2$ (**5h**)	675 nm	1.64
$ZnPcF_{12}$nap (**5i**)	710 nm, 679 nm	1.85

[a] Formulated as 0.5% Cremophor™ EL emulsions unless otherwise noted. [b] The singlet oxygen yield for $AlPcS_4$ in phosphate buffer (1% Triton X) has been reported to be 0.43 *(72)*.

presence of L-tryptophan. The relative yield of HPPI isomers was then determined by HPLC and compared to that of $AlPcS_4$ in PBS, whose yield was arbitrarily set at 1 (Table 1). The CRM emulsions of **5a-i** exhibited improved yields of the HPPI isomers as compared to $AlPcS_4$ in PBS. Formulation of $AlPcS_4$ in CRM also led to slightly improve L-tryptophan photooxidation, most likely as a result of decreased aggregation of the chromophore. The most significant photooxidation of L-tryptophan was obtained using the iodinated Pc derivatives, with relative HPPI yields of 2.43, 2.31 and 2.00 obtained for Pcs **5d**, **5e** and **5f**, respectively. The nitro-substituted derivative **5c** also provide L-tryptophan photooxidation that was over two times higher than that observed for $AlPcS_4$ in PBS. On the other hand, the dodecafluorinated ZnPc substituted with two long alkyl chains (**5g**) gave HPPI yields comparable to $AlPcS_4$ in 1% CRM.

In vitro photodynamic activity. Using a constant dye concentration of 1 μM or 5 μM, the photocytotoxicity of dodecafluorinated zinc phthalocyanines **5a**, **5b** and **5g** on EMT-6 murine mammary tumor cells was measured after illumination for various amounts of time at a fluence rate of 100 mW/cm^2 as calculated over the range of the Q band of phthalocyanines (660-700 nm). The light doses required to induce 50% and 90% cell death (LD50 and LD90) were extrapolated from the resulting cell survival curves. No significant dark toxicity was observed for any of the photosensitizers examined at either concentration. Figure 3 shows histograms reporting the LD50 and LD90 after 1 hour and 24 hour incubations with photosensitizers **5a**, **5b** and **5g** and for hexadecafluorinated ZnPc ($ZnPcF_{16}$), a photosensitizer that has already been extensively studied *(55,57-59)*. The asymmetrically substituted photosensitizers **5a**, **5b** and **5g** were more phototoxic than the symmetrically substituted $ZnPcF_{16}$ at both dye concentrations.

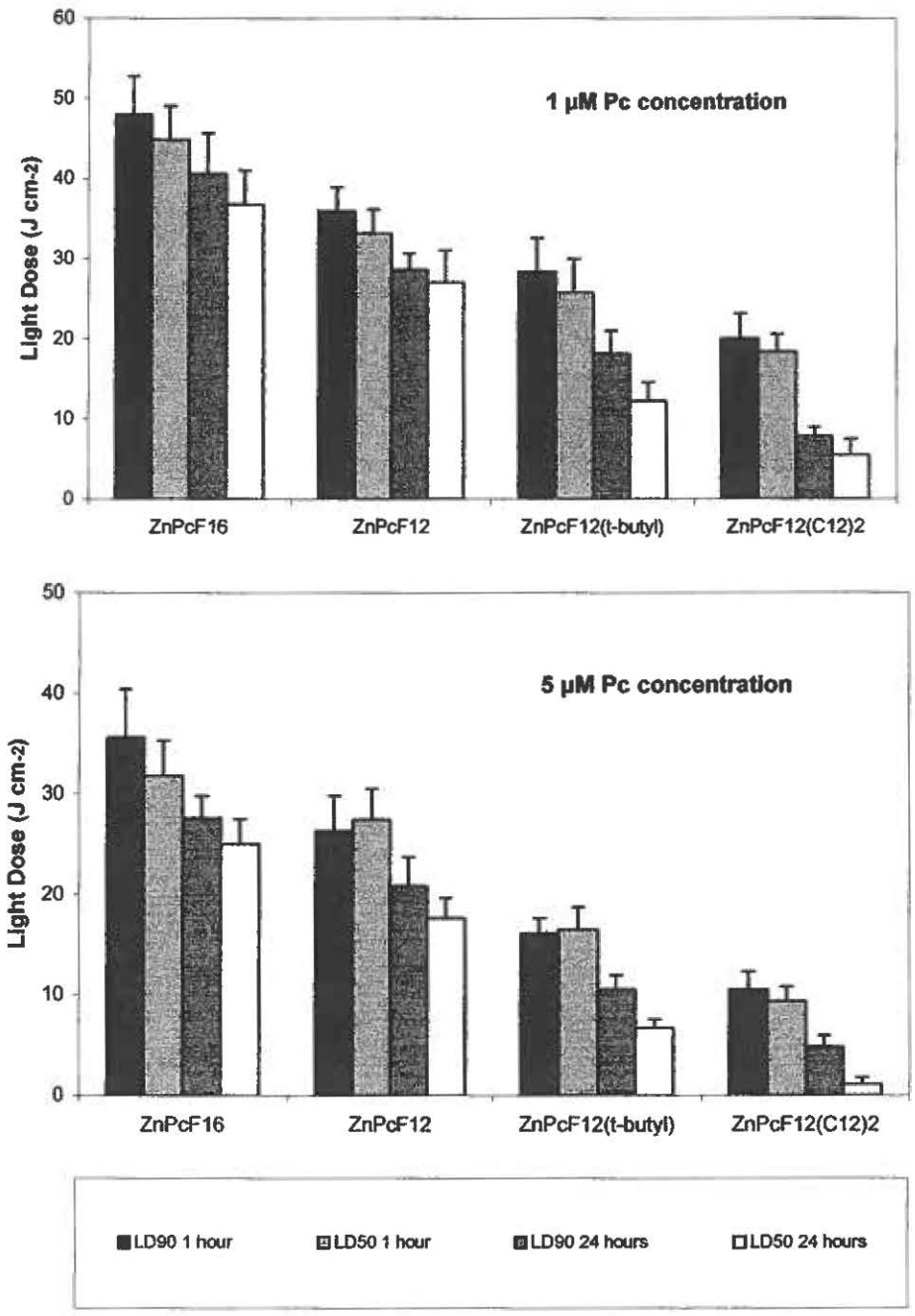


Figure 3. Photocytotoxicity of asymmetrically substituted fluorinated phthalocyanines. Phototoxic activity of a 1 µM (top) and 5 µM (bottom) solution of zinc dodecafluorinated phthalocyanines **5a**, **5b** and **5g** towards EMT-6 murine mammary tumor cells.

The improved photocytotoxicity was more pronounced for the photosensitizers substituted with a t-butyl group (**5b**) and was even more prominent for the dodecafluorinated phthalocyanine substituted with two dodecyl groups (**5g**). The difference in photocytotoxicity between these two photosensitizers and $ZnPcF_{16}$ is appreciably more important after incubating for 24 hours.

DISCUSSION

Dodecafluorosubphthalocyanato boron(III) bromide (**2**) was successfully synthesized by the cyclotrimerization reaction of tetrafluorophthalonitrile with BBr_3 in chlorobenzene at 40-60°C. Yields as high as 70% were obtained, which compare to 26% yield previously reported by the reaction of boron trichloride and tetrafluorophthalonitrile in benzene or 1-chloronaphthalene at elevated temperatures *(34)*. It has been established that the reactivity of trisubstituted boron compounds towards the cyclotrimerization reaction is $B(Alkyl)_3 < BPh_3 < BF_3 < BCl_3 < BBr_3$, an order that is closely related to the Lewis acidity of the boron compounds *(29)*. In addition, the presence of four strongly electron-withdrawing fluorine atoms on the tetrafluorophthalonitrile starting material will activate the nitrile groups towards the cyclotrimerization reaction. A combination of the increased reactivity of the boron source and the activation of the nitrile groups of tetrafluorophthalonitrile towards the cyclotrimerization reaction results in the possibility for the reaction being carried out under more mild reaction conditions, thus ensuring a higher yield of the desired boron dodecafluoro subPc. No exchange of fluorine for

bromine was observed. This is despite the strong Lewis acidity of the boron tribromide and the known reactivity of tetrafluorophthalonitrile towards exchange reactions *(60)*.

The resulting dodecafluorinated tripyrrolic macrocycle was purified by flash column chromatography on neutral alumina in the dark. It has been reported that subPcs bearing bromine axial ligands are fairly unstable with respect to axial ligand exchange and that purification of bromosubPcs via column chromatography resulted in an appreciably amount of replacement of the bromine axial ligand by OH *(29)*. To overcome this, it has been suggested that these labile subPcs can be purified by precipitation or Soxhlet extraction with an appropriate solvent *(33)*. However, in the current study, it was observed that addition of the chlorobenzene reaction mixture to excess hexanes lead to degradation of the macrocycle. This is despite reports that subPcs are stable in apolar solvents *(61)*. On the other hand, it has been reported that dodecafluorosubphthalocyanato boron(III) chloride undergoes thermal decomposition in hexanes *(62)*. Soxhlet extraction with dry dichloromethane of either the solid obtained by precipitation from excess hexanes or by evaporation of the dichlorobenzene from the reaction mixture also led to degradation of the subPc, significantly decreasing the yield of the desired product. Purification by flash column chromatography using 30% THF in hexanes or using dichloromethane proved to be the most efficient method of purifying dodecafluorosubphthalocyanato boron(III) bromide (**2**).

When attempts were made to prepare **2** at 0°C, a labile blue product was obtained. Interestingly, we observed similar labile blue products when the cyclotrimerization of other phthalonitriles substituted with electron-withdrawing groups (such as 4-nitrophthalonitrile, 4-iodophthalonitrile and 4,5-dicyanophthalonitrile) was carried out at

0°C (results not presented). While the identity of this labile species has not be fully elucidated, identification of this species may help determine the mechanism of the cyclotrimerization reaction, which in turn may help investigators in improving the synthesis and the utility of subPcs. The prior art describes that dodecafluorosubphthalocyanato boron(III) chloride undergoes thermal decomposition in hexanes to yield a light blue product whose structure was not determined *(62)*. We attempted to prepare Pc by reacting the transient blue complex with various 1,3-diiminoisoindolines. However, no green/blue product corresponding to phthalocyanines was obtained.

There are a number of possible identities for this transient blue species. It is well-known that boron trihalides readily form charge transfer complexes with nitrile functional groups *(63)* and one would expect that some type of charge transfer complex between the boron atom and dinitrile functionality of phthalonitriles would form at some point during the synthesis of subPcs. However, it was observed that this transient blue intermediate transformed into the desired $subPcF_{12}$ upon heating of the reaction mixture. Furthermore, treatment of $subPcF_{12}$ with $NaBH_4$ resulted in a loss of the characteristic Q band at 577 nm and the formation of a broad absorption around 660 nm while treatment of the blue intermediate with DDQ led to a broad transient absorption centered near 580 nm. Since the bromide **2** absorbs at 577 nm and the transient blue species exhibits a broad absorption centered approximately at 660 nm, these results imply that the transient blue species requires oxidation in order to form the subPc. The bathochromic shift of the absorption from the subPc to the incompletely oxidation blue intermediate would not be unexpected. A loss of conjugation in tetrapyrrolic macrocycles is known to led to red

shifts in the λ_{max}. For instance, while the completely conjugated protoporphyrin IX absorbs at 630 nm, the incompletely conjugated verteporfin absorbs at 690 nm *(64)*. It has been reported that the singlet excited state of dodecafluorosubphthalocyanato boron(III) chloride is a strong oxidant *(62)* with a one-electron reduction potential estimated to be approximately 1.7 V, suggesting that the relationship between the subPc and the blue species may pass via a photoexcited state of the subPc.

In the current study, the Kobayashi ring expansion reaction of the subPc **2** with various 1,3-diiminoisoindolines (**3a-i**) selectively yielded the desired 3:1 asymmetrically substituted dodecafluorinated Pcs (**4a-i**). Yields for the dodecafluorinated Pcs ranged from 18% to as high as 60%, although lower yields were obtained using 4-iodo-1,3-diiminoisoindoline (**3e**), probably a result in steric effects. Lower yields of Pc were also obtained using 5,6-didodecyl-1,3-diiminoisoindoline (**3g**). Both steric effect and solubility of **3g** may have played a part in the lower yields obtained for 2,3,4,8,9,10,11,15,16,17,18-dodecafluoro-23,24-didodecylphthalocyaninate (**4g**). While the other 1,3-diiminoisoindoline derivatives employed could be solubilized in DMSO upon sonication, **3g** required the use of a 1:1 mixture of DMSO and chlorobenzene in order to obtain a solution. Interestingly, prior studies have indicated that the Kobayashi ring expansion reaction of (2,9,16(17)-tri-t-butylsubphthalocyanato)boron(III) bromide fails when DMSO is used as the reaction solvent and requires a mixture of DMSO and an aromatic solvent for the reaction to proceed *(37)*. Conversely, the use of DMSO as a solvent allowed for the selective preparation of trisulphonated phthalocyanines via the Kobayashi ring expansion reaction *(42,43)*. With its mild oxidant properties, the use of DMSO as the reaction solvent in the current study may promote the selectivity and

improved yields observed. On the other hand, molecular orbital calculations suggest the the initial step in the Kobayashi ring expansion reaction consist of loss of the axial ligand *(37)*. In light of the decreased bond energy of a B-Br bond compared to a B-Cl bond, the Kobayashi ring expansion reaction involving subphthalocyanines with bromine axial ligands should initiate and proceed under milder reactions conditions, perhaps promoting selectivity. Along those lines, most literature examples detailing selective ring expansion reactions have utilized subPcs with bromine axial ligands *(27,37,42,43)*. However, the increased solubility of the subPcF_{12} along with the electron-withdrawing properties of the fluorine substituents almost certainly play a role as well.

Due to the known tendency of tetrafluorophthalonitrile to undergo an exchange reaction with DMF *(60)*, attempts to insert metal ions such as Zn^{+2} into the metal free dodecafluorinated phthalocyanines using solvents such as NMP were undertaken. Unfortunately, these metal ion insertion reactions were sluggish and gave unacceptable yields. In order to avoid any possible reaction between DMF and the perfluorinated macrocycle, reaction temperatures between 30 and 50°C were employed and led to the corresponding zinc dodecafluorinated phthalocyanines in nearly quantitative yields.

Singlet oxygen is believed to be the most important reactive species generated during PDT and is capable of inducing important characteristic oxidative damage to a number of biologically important molecules including amino acids *(65)*. L-Tryptophan photooxidation was used to evaluate the photodynamic potential of the asymmetrically substituted zinc dodecafluorinated phthalocyanines by comparing the relative HPPI yields obtained using these Pcs to the yield obtained using AlPcS_4 in PBS (which was arbitrarily set as 1) *(56)*. In light of the heavy atom effect, it is not unexpected that these

dodecafluorinated photosensitizers exhibit improved HPPI yields as compared to $AlPcS_4$ with the iodinated derivatives giving the highest singlet oxygen yields. The heavy atom effect dictates that the exchange of hydrogen atoms with heavier atoms (such as fluorine atoms) on a chromophore increases intersystem crossing from the singlet to the triplet excited state by increasing spin-orbital coupling, thus allowing otherwise forbidden changes in the spin state ($S_1 \rightarrow T_1$) *(66)*. Accordingly, the exchange of hydrogen atoms for fluorine atoms of the periphery of phthalocyanine photosensitizers improves the photodynamic potential of the photosensitizer by improving triplet state yields.

Interestingly, for a series of zinc tetrahalogenated Pcs ($ZnPcX_4$ where X is Cl, Br or I), it was observed that increases in triplet state formation and decreases in triplet state lifetimes increased in the order Cl < Br < I, as would be expected in terms of spin-orbital coupling theory *(67)*. However, the production of singlet oxygen was only slightly increased by the nature of the halogen atom. While these results were not fully explained, it was noted that the tendency of these macrocycles to aggregate was in the order Br > I > Cl > H. As such, the improved photodynamic potential that results from the heavy atom effect may be somewhat counteracted by the tendency of Pcs to aggregate in solution. It has been shown that amphiphilic unsymmetrically substituted phthalocyanines bearing long alkyl chains exhibit decreased HPPI yields in the L-tryptophan photooxidation assay *(24)*, even as CRM emulsion, due to important aggregation of the macrocycle, with the long alkyl chains increasing the lipophilicity of the Pcs. While the zinc dodecafluorinated phthalocyanines are all lipophilic photosensitizers, it is believed that the longer alkyl chains promote aggregation of the

phthalocyanine **5g**, even in CRM emulsions, resulting in decreased HPPI yields under these experimental conditions.

Most lipophilic Pcs, including unsubstituted ZnPc and AlOHPc, are highly insoluble in most common solvents. Thus, despite important photodynamic activity, the utility of such lipophilic Pcs is rather limited. Fluorine is very similar in atomic radius to hydrogen and can mimic hydrogen in biological environments. In addition, exchange of hydrogen atoms for fluorine atoms increases lipid solubility and thus may lead to enhanced interactions with biological membranes. Our group has extensively examined $ZnPcF_{16}$ and $ZnPcF_{12}S_1$ and have found that $ZnPcF_{16}$ is effective in inactivating EMT-6 tumour cells with selective tumour uptake and improved pharmacokinetics *(55,57-59)*. A number of water-soluble amphiphilic fluorinated zinc phthalocyanines have also been synthesized and shown to have enhanced properties for photodynamic therapy *(68,69)*. Additionally, a novel three-dimensional zinc perfluorinated phthalocyanine comprising 64 fluorine atoms has been synthesized from a novel non-planar perfluorinated phthalonitrile and has been shown to have improved PDT efficiency as compared to $ZnPcF_{16}$ *(70)*.

Preliminary in vitro PDT against EMT-6 tumor cells indicate that asymmetrically substituted dodecafluorinated phthalocyanines **5a**, **5b** and **5g** are more photodynamically active than the symmetrically substituted $ZnPcF_{16}$ in CRM emulsions after incubations of 1 or 24 hours (see Figure 3). Asymmetrically substituted $ZnPcF_{12}S_1$ is 50 times more photoactive than ZnPcF16 against EMT-6 tumour cells in vitro *(59)*.. Unfortunately, in vivo photodynamic therapy induced mortality, indicating a small therapeutic window. The current asymmetrically substituted fluorinated phthalocyanines exhibit increased

photoactivity and may have an increased therapeutic window. Intriguingly, despite lower HPPI yields in the L-tryptophan photooxidation assay, **5g** was the most photodynamically active Pc examined. While it was proposed that the lack of an appreciable activity of $ZnPcF_{16}$ and $ZnPcCl_{16}$ against M6 melanoma cells was due to aggregation of the PS even in the complicated environment of cells *(71)*, the current study indicates that aggregation becomes less important in such biological systems, possibly due to interaction of proteins and lipoproteins with the planar Pc reducing the extent of aggregation.

CONCLUSION

DodecafluorosubPc boron(III) bromide (**2**) was prepared under milder conditions and shown to react readily with numerous 1,3-diiminoisoindolines in DMSO (Kobayashi ring expansion reaction) leading to the selective preparation of 3:1 unsymmetrically substituted dodecafluorinated phthalocyanines. Upon chelation to Zn^{+2}, the resulting asymmetrically substituted dodecafluorinated ZnPc were shown to be effective photosensitizers for the production of singlet oxygen in an aqueous environment. Preliminary in vitro photodynamic therapy against EMT-6 murine mammary tumor cells indicate that these compounds have potential as photosensitizers for PDT.

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Chapter 8.

A New Procedure for the Synthesis of Water-soluble Tri-Cationic and –Anionic Phthalocyanines

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A new procedure for the synthesis of water-soluble tri-cationic and -anionic phthalocyanines

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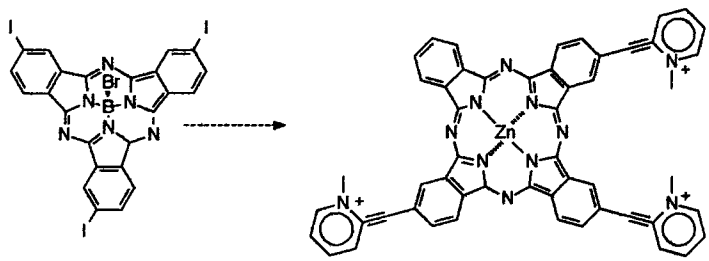
The Kobayashi ring expansion reaction of iodinated subphthalocyanines with 1,3-diiminoisoindolines lead exclusively to the corresponding 3:1 asymmetrically substituted iodinated phthalocyanines. These iodinated Pcs proved to be ideal building blocks for the synthesis of novel asymmetrically substituted water-soluble Pcs, with palladium-catalyzed cross-coupling reactions with appropriate terminal alkynes ultimately leading to both anionic and cationic Pcs.



A new procedure for the synthesis of water-soluble tri-cationic and -anionic phthalocyanines

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ABSTRACT: A series of water-soluble tri-anionic and -cationic substituted phthalocyanines has been synthesized via iodinated boron subphthalocyanines. The latter were opened with differently substituted diiminoisoindolines via the Kobayashi ring expansion reaction followed by metal insertion to exclusively yield the asymmetrically 3:1 substituted iodinated zinc phthalocyanines. These iodinated phthalocyanines readily underwent palladium-catalyzed coupling reactions with terminal alkynes such as 5-hexynoic acid and 10-undecynoic acid to give anionic phthalocyanines, or with 2-ethynylpyridine and 3-ethynylpyridine followed by N-methylation to give cationic phthalocyanines. These novel asymmetrically substituted charged phthalocyanines could have interesting properties as photosensitizers for photodynamic therapy.

KEYWORDS: phthalocyanines, photodynamic therapy, cationic, anionic, asymmetric, Kobayashi ring expansion, boron subphthalocyanines

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INTRODUCTION

Phthalocyanines and related tetrapyrrolic macrocycles have numerous properties that lend applicability in numerous important technological fields [1-3]. While the utility of unsubstituted Pcs is limited due to the extreme insolubility of the macrocycle, Pcs substituted with various functional groups have been prepared in order to improve their solubility and to increase their effectiveness in many of these potential applications. Asymmetrically substituted phthalocyanines exhibit improved properties compared to their unsubstituted and symmetrically substituted derivatives in a number of these applications. For instance, the extensively delocalized 18-π electron system of phthalocyanines imparts large optical nonlinearities and third order harmonic generation to symmetrically substituted Pcs [4,5]. However, second order nonlinear optical effects are only present in non-centrosymmetric molecules such as asymmetrically substituted Pcs and these effects are increased in systems characterized by both electron-donating and electron-withdrawing functionalities [4-7]. Furthermore, the amphiphilicity exhibited by Pcs bearing both hydrophilic and hydrophobic functionalities has been demonstrated to enhance their photodynamic potential [8-11]. In light of these factors, asymmetrically substituted Pcs remain important synthetic targets.

Despite attempts to use a polymer support [12,13] or novel phthalocyanine precursors [14-16] to control the cyclotetramerization reaction and thus develop superior methods for preparing asymmetrically substituted Pcs, the traditional employed method of preparing asymmetrically substituted Pcs by the condensation of two differently substituted phthalocyanine precursors remains the most widely used methodology [17]. However, even though careful selection of the substituents on the phthalocyanine precursors, the molecular proportions of each individual precursors and the reaction conditions used can help to control the relative amounts of each substituted pattern, these mixed condensations lead to a mixture of six differently substituted Pc products from which the isolation of the desired asymmetrically substituted Pc remains tedious and difficult. The prearrangement of three isoindoline units in boron subphthalocyanines make them extremely attractive reagents in the preparation of 3:1 asymmetrically substituted Pcs. Despite the early promise demonstrated by Kobayashi [18,19], the ring enlargement reaction of these lower homologs with 1,3-diiminoisoindolines has been shown to proceed by a non-selective multistep mechanism which depends to a great extent on the substituents on the subPc, the solubility and reactivity of the 1,3-diiimoisoindoline, the reaction solvent and the reaction conditions employed [6,20,21]. While this multistep mechanism may lead to a mixture of substituted Pcs, it has been established that the ring expansion reaction of subPcs does indeed give rise to the selective preparation of 3:1 asymmetrically substituted Pcs in certain cases [18,19,22]. Interestingly, even though intuitively the driving force behind the ring expansion reaction of subPcs would be expected to be based on the known distortion in the cone-shaped structure of subPcs, molecular orbital calculations suggest that this distortion is not a major reason for the ring expansion reactivity of subPcs and that the lack of donor-acceptor stabilization in the boron-nitrogen bonds destabilizes subPcs towards expansion [19]. These calculations also indicate that the first step in these ring expansion reactions is the loss of the halogen axial ligand.

We previously demonstrated methods for the synthesis of monofunctionalized Pc [23,24]. However to our knowledge no reports to prepare tri-cationic and -carboxylic substituted Pc have appeared. In the current study, the Kobayashi ring expansion reaction of iodinated boron subPc followed by palladium-catalyzed cross-coupling reactions provide a new method for the synthesis of novel asymmetrically substituted anionic and cationic phthalocyanines.

EXPERIMENTAL

Materials

All solvent were HPLC grade and were used without further purification unless otherwise stated. 3-Nitrophthalonitrile and 4-tert-butylphthalonitrile were purchased from TCI America (Portland, Oregon, USA). 1,2-Dicyanobenzene, 2,3-dicyanonaphthalene, 4-nitrophthalonitrile, 1,3-diiminoisoindoline, dichlorobis(triphenyl-phosphine)palladium(II), copper iodide, 1M boron tribromide in dichloromethane, zinc bromide, 5-hexynoic acid, 10-undecynoic acid, 2-ethynylpyridine and 3-ethynylpyridine were purchased from Aldrich (Oakville, Ontario, Canada). 4-Iodophthalonitrile [25], 3-iodophthalonitrile [26] and 4,5-diiodophthalonitrile [27] were prepared using modified literature procedures. 5-*Tert*-butyl-1,3-diiminoisoindoline, 5- and 2,3-dihydro-1,3-diimino-1H-benz[f]isoindole were prepared by reacting the corresponding phthalonitrile or 2,3-dicyanonaphthalene with sodium methoxide and ammonia gas in methanol at reflux as disclosed in the literature [28,29]. UV-visible spectra were recorded with a Hitachi U-2000 spectrophotometer. FAB-MS were obtained on an LG Autospec Q mass spectrometer.

Synthesis

Preparation of (2,9,16(17)-triiodosubphthalocyaninato)boron(III) bromide 2a

1.02 g of 4-iodophthalonitrile (**1a**) (4.02 x 10^{-3} moles) was dissolved in a minimum amount of chlorobenzene (1.1 ml). The resulting solution was stirred at room temperature and 3 ml of 1M BBr_3 in dichloromethane (3.00 x 10^{-3} moles of BBr_3) (0.75 equiv.) were added. After 5 minutes, 500 µl of THF were added and the reaction mixture was heated to 60°C. The reaction mixture was maintained at 60°C for 1 hour and was cooled to room temperature. The solvent was removed by rotary evaporation at reduced pressure and the resulting solid was dissolved in CH_2Cl_2. This solution was filtered and purified by column chromatography over neutral alumina using CH_2Cl_2 as eluant. The desired product was obtained in a yield of 706 mg (61.8%). $C_{24}H_9N_6BBrI_3$ MS (FAB): m/e 853 (M^++H), 773 (M^+-Br). UV-vis λ (dichloromethane) (log ε) 573 nm (4.67).

Preparation of (1,8,15(18)-triiodosubphthalocyaninato)boron(III) bromide 2b

The same procedure as disclosed above was employed using 568 mg of 3-iodophthalonitrile (**1b**) (2.24 x 10^{-3} moles) and 1.75 ml of 1M BBr_3 in dichloromethane (1.75 x 10^{-3} moles of BBr_3) (0.78 equiv.). Yield: 221 mg (35.0%). $C_{24}H_9N_6BBrI_3$ MS (FAB): m/e 853 (M^++H), 773 (M^+-Br).). UV-vis λ (dichloromethane) (log ε) 577 nm (4.63).

Preparation of (2,3,9,10,16,17)-hexaiodosubphthalocyaninato)boron(III) bromide 2c

The same procedure as disclosed above was employed using 785 mg of 4,5-diiodophthalonitrile (**1c**) (2.07 x 10^{-3} moles) and 2.00 ml of 1M BBr_3 in dichloromethane (1.75 x 10^{-3} moles of BBr_3) (0.88 equiv.). Yield: 514 mg (60.5%). $C_{24}H_6N_6BBrI_6$ MS (FAB): m/e 1230 (M^++H), 1150 (M^+-Br).). UV-vis λ (dichloromethane) (log ε) 583 nm (4.71).

Preparation of iodinated phthalocyanines 4a-4g

2,9,16(17)-triiodophthalocyaninate (4a). To a stirred solution of 506 mg of **2a** (5.93 x 10^{-4} moles) in 3 ml of DMSO was added a solution of 910 mg (6.27 x 10^{-3} moles, 10.6 equiv.) of 1,3-diiminoisoindoline (**3a**) in 6 ml of DMSO at room temperature. The reaction was heated at 60°C. After 24 hours, the reaction mixture was added to a large excess of methanol (50 ml). The suspension was left at 4°C overnight and then the green solid was collected by centrifuging. The desired product was obtained in a yield of 175 mg (43.1%). MS (FAB): m/e 892 (M^++H).

Iodinated phthalocyanine derivatives **4b-4g** were prepared using the same synthetic procedure described above.

Selective spectral data for compounds 4b-4g: **4b** Yield 36.5% MS (FAB): m/e 949 (M^++H); **4c** Yield 38.9% MS (FAB): m/e 943 (M^++H) **4d** Yield 21.3% MS(FAB): m/e 892 (M^++H) **4e** Yield 22.7% MS (FAB): m/e 949 (M^++H) **4f** Yield 19.6% MS (FAB): m/e 943 (M^++H) **4g** Yield 14.6% MS (FAB): m/e 1271 (M^++H).

Preparation of iodinated zinc phthalocyanines 5a-5g

(2,9,16(17)-triiodophthalocyaninato)zinc (5a). 100 mg of **4a** (1.12 x 10^{-4} moles) was suspended in DMF (5 ml).

To this was added 144 mg of zinc bromide (6.40×10^{-4}) (5.7 equiv.). The reaction mixture was heated to 60°C for 2 hours and was then cooled to room temperature. The solvent was removed by rotary evaporation under reduced pressure and the resulting solid was washed twice with 1.2M hydrochloric acid (25 ml each) and twice with 95% ethanol (25 ml each). The solid was dried and then dissolved in tetrahydrofuran and purified by column chromatography on silica gel (40% THF in hexanes). The desired product was obtained in a yield of 106 mg (99%). MS (FAB): m/e 955 (M^++H) UV-vis λ (THF) (log ε) 671 nm (5.37), 606 nm (4.59), 350 nm (4.88).

Iodinated zinc phthalocyanine **5b-5g** derivatives were prepared using the same synthetic procedure described above with near quantitative yields.

*Selective spectral data for compounds **5b-5g**:* **5b** MS (FAB): m/e 1011 (M^++H), UV-vis λ (THF) (log ε) 673 nm (5.31), 607 nm (4.56), 352 nm (4.89). **5c** MS (FAB): m/e 1004 (M^+), UV-vis λ (THF) (log ε) 708 nm (4.93), 680 nm (5.00), 650 nm (4.50), 616 nm (4.39), 349 nm (4.71). **5d** MS (FAB): m/e 955 (M^++H) UV-vis λ (THF) (log ε) 678 nm (5.21), 611 nm (4.45), 339 nm (4.63). **5e** MS (FAB): m/e 1011 (M^++H), UV-vis λ (THF) (log ε) 679 nm (5.26), 611 nm (4.52), 339 nm (4.72). **5f** MS (FAB): m/e 1004 (M^+), UV-vis λ (THF) (log ε) 708 nm (5.01), 680 nm (5.08), 650 nm (4.54), 616 nm (4.44), 349 nm (4.80). **5g** Yield 98.7% MS (FAB): m/e 1331 (M^+), UV-vis λ (THF) (log ε) 677 nm (5.31), 612 nm (4.61), 355 nm (4.90).

Preparation of asymmetrically substituted anionic zinc phthalocyanines 6a-6j

6a: 100 mg of **5a** (1.05×10^{-4}) was dissolved in a 1:1 mixture of DMF and triethylamine (6 mL). To this solution were added 50 mg of dichlorobis(triphenyl-phosphine)palladium(II) and 10 mg of copper iodide. The reaction mixture was stirred under nitrogen and a solution of 200 μL of 5-heyxnoic acid in 2 ml of DMF was added. The reaction was heated to 70°C and stirred overnight in the dark.. The reaction mixture was then filtered and the solvent removed under vacuum. The resulting solid was purified by column chromatography on silica gel (2:1 THF/hexanes). The desired product was obtained in a yield of 78 mg (81.2%) MS (FAB): m/e 907 (M^++H). UV-vis λ (THF) (log ε) 678 nm (5.34), 612 nm (4.62), (0.1 N NaOH) 623 nm (br). The corresponding water-soluble salt was readily obtained by dissolving the Pc in 1 N NaOH, neutralizing the resulting solution and precipitating the salt by adding the solution to a large excess of cold acetone.

Asymmetrically substituted anionic zinc phthalocyanine derivatives **6b-6j** were prepared using the same synthetic procedure described above.

*Selective spectral data for compounds **6b-6j**:* **6b** Yield 75.5% MS (FAB): m/e 1017 (M^++H) UV-vis λ (THF) (log ε) 679 nm (5.33), 612 nm (4.61), (0.1 N NaOH) 633 nm (br). **6c** Yield 83.5% MS (FAB): m/e 963 (M^++H) UV-vis λ (THF) (log ε) 680 nm (5.25), (0.1 N NaOH) 638 nm (br). **6d** Yield 61.5% MS (FAB): m/e 1173 (M^++H) UV-vis λ (THF) (log ε) 683 nm (5.33), 614 nm (4.60), (0.1 N NaOH) 623 nm (br). **6e** Yield 88.4% MS (FAB): m/e 957 (M^++H) UV-vis λ (THF) (log ε) 713 nm (5.00), 688 nm (5.07), (0.1 N NaOH) 643 nm (br). **6f** Yield 88.8% MS (FAB): m/e 1167 (M^++H) UV-vis λ (THF) (log ε) 716 nm (5.09), 689 nm (5.16), (0.1 N NaOH) 628 nm (br). **6g** Yield 81.5% MS (FAB): m/e 907 (M^++H) UV-vis λ (THF) (log ε) 679 nm (5.29), 612 nm (4.60), (0.1 N NaOH) 640 nm (br). **6h** Yield 89.2% MS (FAB): m/e 963 (M^++H) UV-vis λ (THF) (log ε) 681 nm (5.28), 614 nm (4.54), (0.1 N NaOH) 643 nm (br). **6i** Yield 88.5% MS (FAB): m/e 957 (M^++H) UV-vis λ (THF) (log ε) 717 nm (4.99), 689 nm (5.01), (0.1 N NaOH) 631 nm (br). **6j** Yield 78.7% MS (FAB): m/e 1237 (M^++H) UV-vis λ (THF) (log ε) 687 nm (5.33), (0.1 N NaOH) 632 nm (br).

Preparation of asymmetrically substituted zinc phthalocyanines 7a-7n and asymmetrically substituted cationic zinc phthalocyanines 8a-8n

7a, 8a: 100 mg of **5a** (1.05×10^{-4} moles) was dissolved in a 1:1 mixture of DMF/triethylamine (8 mL). To this solution were added 50 mg of dichlorobis(triphenyl-phosphine)palladium(II) and 10 mg of copper iodide. The reaction

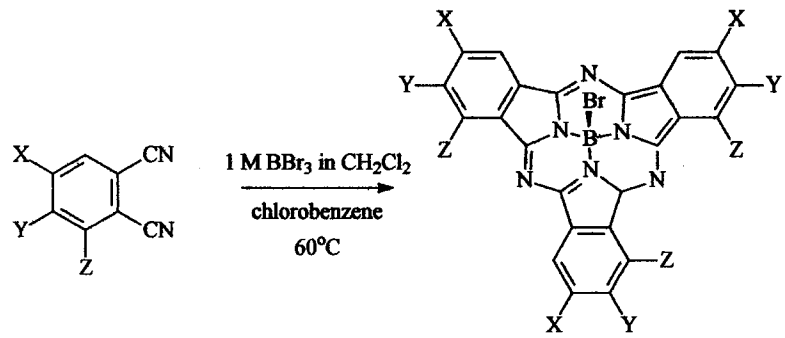
mixture was stirred under nitrogen and a solution of 165 mg of 2-ethynylpyridine in 4 mL of DMF was added. The reaction was heated to 60°C and stirred overnight in darkness. The reaction was then cooled and added to a large excess of water. The resulting solid was obtained by filtration and washed extensively with 0.1 N NaOH, diethyl ether and acetone. The desired product was obtained by dissolving the remaining solid in pyridine, filtering and removing the pyridine under vacuum. The resulting product **7a** was suspended in DMF (5 mL) and 1 mL of dimethyl sulfate was added. This reaction was stirred at 70°C in the dark overnight. The reaction mixture was then added to a large excess of cold acetone (1 L). After leaving to stand at 4°C, the resulting precipitate was collected (**8a**).

Asymmetrically substituted zinc phthalocyanine derivatives **7b-7n** and asymmetrically substituted cationic zinc phthalocyanines **8b-8n** were prepared using the same synthetic procedure as described above.

Selected spectral data: **7a** MS (FAB): m/e 880 (M^++H) UV-vis λ (DMF) (log ε) 681 nm (5.31), 617 nm (4.57). **8a** λ (DMF) 712 nm (sh), 695 nm, (H_20) 636 (br). **7b** MS (FAB): m/e 880 (M^++H) UV-vis λ (DMF) (log ε) 687 nm (5.28), 622 nm (4.57). **8b** λ (DMF) 696 nm, 682 nm, (H_20) 628 (br). **7c** MS (FAB): m/e 936 (M^++H) UV-vis λ (DMF) (log ε) 696 nm (5.32). **8c** λ (DMF) 714 nm, 689 nm, (H_20) 650 (br). **7d** MS (FAB): m/e 936 (M^++H) UV-vis λ (DMF) (log ε) 700 nm (5.34) **8d** λ (DMF) 700 nm, 685 nm (sh), (H_20) 648 (br). **7e** MS (FAB): m/e 930 (M^++H) UV-vis λ (DMF) (log ε) 727 nm (4.99), 693 nm (5.05), 627 nm (4.51). **8e** λ (DMF) 742 nm, 702 nm, (H_20) 636 (br). **7f** MS (FAB): m/e 930 (M^++H) UV-vis λ (DMF) (log ε) 732 nm (5.07), 699 nm (5.13), 632 nm (4.53). **8f** λ (DMF) 730 nm, 695 nm, (H_20) 633 (br). **7g** MS (FAB): m/e 880 (M^++H) UV-vis λ (DMF) (log ε) 686 nm (5.27). **8g** λ (DMF) 691 nm, 679 nm (sh), (H_20) 628 (br). **7h** MS (FAB): m/e 880 (M^++H) UV-vis λ (DMF) (log ε) 691 nm (5.33), 620 nm (4.59). **8h** λ (DMF) 698 nm, 684 nm (sh), (H_20) 636 (br). **7i** MS (FAB): m/e 936 (M^++H) UV-vis λ (DMF) (log ε) 685 nm (5.29), 615 nm (4.58). **8i** λ (DMF) 700 nm (sh), 688 nm, (H_20) 620 (br). **7j** MS (FAB): m/e 936 (M^++H) UV-vis λ (DMF) (log ε) 692 nm (5.24), 622 nm (4.54). **8j** λ (DMF) 698 nm (sh), 689 nm, (H_20) 627 (br). **7k** MS (FAB): m/e 930 (M^++H) UV-vis λ (DMF) (log ε) 717 nm (4.94), 689 nm (4.99). **8k** λ (DMF) 722 nm, 691 nm, (H_20) 636 (br). **7l** MS (FAB): m/e 930 (M^++H) UV-vis λ (DMF) (log ε) 724 nm (5.00), 695 nm (5.02). **8l** λ (DMF) 718 nm, 689 nm, (H_20) 623 (br). **7m** MS (FAB): m/e 1939 (M^++H) UV-vis λ (DMF) (log ε) 696 nm (5.31). **8m** λ (DMF) 736 nm (sh), 703 nm, (H_20) 644 (br). **7n** MS (FAB): m/e 1939 (M^++H) UV-vis λ (DMF) (log ε) 702 nm (5.30), **8n** λ (DMF) 726 nm (sh), 700 nm, (H_20) 648 (br).

RESULTS AND DISCUSSION

Boron subPcs are generally synthesized by the cyclotrimerization reaction of phthalonitriles with boron trichloride at elevated temperatures [21]. In the current study, the iodinated subPcs **2a-c** could in fact be prepared by reacting the appropriate iodinated phthalonitrile **1a-c** in chlorobenzene with 1M boron tribromide in dichloromethane at 60°C (Figure 1). This represents much milder reaction conditions than those traditionally employed, the milder conditions be at least in part due to the use of the stronger Lewis acid boron tribromide. These conditions allowed for improved yields of the macrocycles. While the corresponding triiodinated subPcs bearing chloride axial ligands have been prepared in yields of 45% and 11.0% respectively [21,30], subPcs **2a** and **2b** were prepared in yields of 61.8% and 35% following purification by column chromatography on neutral alumina in the dark using dichloromethane as eluant. The hexaiodinated subPc **2c** was obtained in a yield of 60.5%. Unexpectedly, the addition of a small amount of THF into the reaction mixture increased the reaction rate and the resulting yields. FAB-MS spectral data was



1 2

Fig. 1. The synthesis of (2,9,16(17)-triiodosubphthalocyaninato)boron (III) bromide (2a), (1,8,15(18)-triiodosubphthalocyaninato)boron(III) bromide (2b) and (2,3,9,10,16,17-hexaiodosubphthalocyaninato)boron(III) bromide (2c). **1a** and **2a** X = I, Y = H, Z = H; **1b** and **2b** X = H, Y = H, Z = I; **1c** and **2c** X = I, Y = I, Z = H. Note that both 2,9,16(17)-triiodosubphthalocyaninato)boron (III) bromide (2a) and (1,8,15(18)-triiodosubphthalocyaninato)boron(III) bromide (2b) are obtained as a mixture of two constitutional isomers having either C_1 or C_3 geometries (not depicted in the current figure).

consistent with the proposed structures of subPcs **2a-c** and did not reveal any evidence of bromination of the macrocycles, which is a common side reaction in the preparation of subPcs due to the strong Lewis acidity of the boron sources. The Q band in the UV-visible spectra of subPcs **2a-c** was typical for these lower homologs of Pcs, with absorptions at 573 nm, 577 nm and 583 nm respectively.

Iodinated subPcs **2a-c** readily underwent a ring expansion reaction with various 1,3-diiminoisoindolines using DMSO as the reaction solvent (Figure 2). FAB-MS spectral data confirm that these ring expansion reactions proceed selectively to yield the desired 3:1 asymmetrically substituted iodinated Pcs **4a-g**, with m/z values corresponding to the expected molecular ion (M^++1) and no m/z peaks corresponding to the other possible substitution patterns. Yields obtained ranged from 14.6% to 43.1%, which are exceeding good considering that only the 3:1 asymmetrically substituted iodinated Pcs were obtained. While the Kobayashi ring expansion reaction typically is accomplished in a mixture of DMSO and either chlorobenzene, o-dichlorobenzene, 1-chloronaphthalene or 2-chloronaphthalene [19], the ring expansion reaction of the iodinated subPcs proceeded smoothly in DMSO. It has been observed that the use of DMSO as the reaction solvent in similar ring expansion reactions allows the reaction to selectively yield the desired 3:1 asymmetrically substituted Pc [22]. It is possible that the mild oxidant DMSO helps control the reaction towards the necessary selectivity. On the other hand, if the loss of the axial ligand is the initial step in the ring expansion reaction, one would expect subPcs bearing bromide axial ligands to react under more mild reaction conditions, perhaps allowing the reaction to proceed selectively since the bond energy for a B-Br bond is 396 kJ/mol compared to 536 kJ/mol for a B-Cl bond [24]. Notably, a number of the literature examples detailing selective Kobayashi ring expansion reactions employed subPcs with bromine axial ligands [18,19,22].

Metal insertion into the resulting metal-free Pcs was readily accomplished by heating a suspension of the metal-free Pcs in DMF to 60°C in the presence of zinc acetate in near quantitative yields. Contrary to previous reports [20], the asymmetrically substituted iodinated zinc Pcs could also be obtained selectively by a templated Kobayashi ring expansion reaction wherein zinc ions added to the reaction act as a template for Pc ring closure. However, yields were significantly reduced in these templated reactions while isolation and purification of the Pc was complicated by the higher solubility of the zinc Pcs in the reaction solvent.

The asymmetrically substituted iodinated Pcs **4a-g** are ideal building blocks for the preparation of novel 3:1 asymmetrically substituted Pcs bearing novel functionality. Palladium-catalyzed cross-coupling reactions have been extensively used in the preparation of novel porphyrins and phthalocyanines [31], in particular in order to add new substituents to both iodinated subPcs [32] and Pcs [33], including asymmetrically substituted iodinated Pcs that were prepared by a mixed condensation [24,34]. It has been established that amphiphilic water-soluble photosensitizers exhibit enhanced photodynamic activity [8-11]. With that in mind, iodinated Pcs **4a-g** were used as building blocks for the preparation of novel water-soluble asymmetrically substituted Pcs. Iodinated Pcs **4a-g** readily reacted with terminal alkynes such as 5-hexynoic acid and 10-undecynoic acid in the presence of dichlorobis(triphenyl-phosphine)palladium(II) and copper iodide in DMF/Et_3N to give novel 3:1 asymmetrically substituted anionic phthalocyanines **6a-j** (Figure 3). The salts of these Pcs were readily soluble in water at physiological pH. However, they were highly aggregated in aqueous solution as exemplified by their visible spectra, with a broad Q band centered between 620 and 640 nm. When dissolved in DMF, the corresponding acids displayed the typical sharp Q band in the region around 680 nm. As expected, the lack of symmetry in **6e**, **6f** and **6i** lead to a splitting of the Q band of the phthalocyanines along with a shift of the absorbance to a longer wavelength.

While less extensively studied than their anionic counterparts, cationic Pcs have important properties that make them extremely attractive PS for PDT. Not only do cationic PS exhibit water-solubility, high singlet oxygen yields and excellent accumulation in target tissue [35-38], their cationic charge allows them to target important subcellular

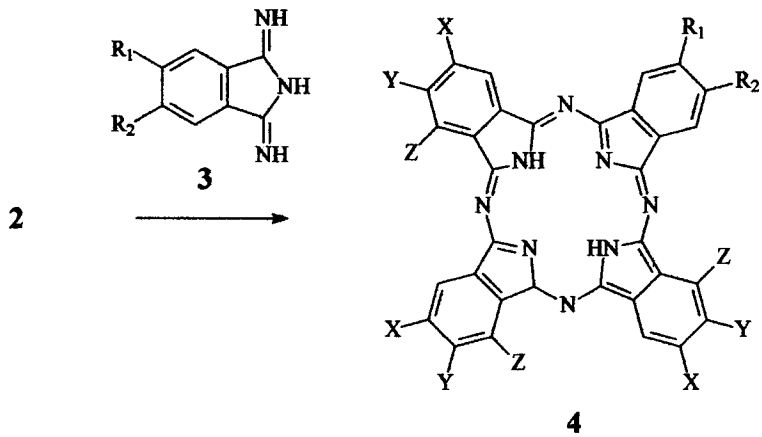


Fig. 2. The Kobayashi ring expansion reaction of iodinated boron subphthalocyanines with various 1,3-diiminoisoindolines. **4a** R_1 = H, R_2 = H, X = I, Y = H, Z = H; **4b** R_1 = H, R_2 = t-butyl, X = I, Y = H, Z = H; **4c** $R_1 = R_2$ = -CH=CH-CH=CH-, X = I, Y = H, Z = H; **4d** R_1 = H, R_2 = H, X = H, Y = H, Z = I; **4e** R_1 = H, R_2 = t-butyl, X =H, Y = H, Z = I; **4f** $R_1 = R_2$ = -CH=CH-CH=CH-, X = H, Y = H, Z = I; **4g** R_1 = H, R_2 = H, X = I, Y = I, Z = H. Note that the ring expansion reaction of both 2,9,16(17)-triiodosubphthalocyaninato)boron (III) bromide (2a) and (1,8,15(18)-triiodosubphthalocyaninato)boron(III) bromide (2b) leads to a mixture of constitutional isomers (not depicted in the current figure).

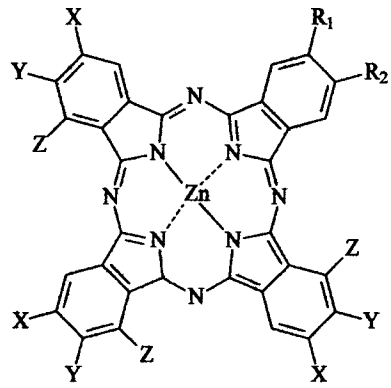


Fig. 3. Asymmetrically substituted phthalocyanines comprising carboxylic acid moieties. **6a** R_1 = H, R_2 = H, X = -C≡C-$(CH_2)_3$COOH, Y = H, Z = H; **6b** R_1 = H, R_2 = H, X = -C≡C-$(CH_2)_8$COOH, Y = H, Z = H; **6c** R_1 = H, R_2 = t-butyl, X = -C≡C-$(CH_2)_3$COOH, Y = H, Z = H; **6d** R_1 = H, R_2 = t-butyl, X = -C≡C-$(CH_2)_8$COOH, Y = H, Z = H; **6e** R_1 = R_2 = -CH=CH-CH=CH-, X = -C≡C-$(CH_2)_3$COOH, Y = H, Z = H; **6f** R_1 = R_2 = -CH=CH-CH=CH-, X = -C≡C-$(CH_2)_8$COOH, Y = H, Z = H; **6g** R_1 = H, R_2 = H, X = H, Y = H, Z = -C≡C-$(CH_2)_3$COOH; **6h** R_1 = H, R_2 = t-butyl, X = H, Y = H, Z = -C≡C-$(CH_2)_3$COOH; **6i** R_1 = R_2 = -CH=CH-CH=CH-, X = H, Y = H, Z = -C≡C-$(CH_2)_3$COOH; **6j** R_1 = H, R_2 = H, X = Y = -C≡C-$(CH_2)_3$COOH, Z = H.

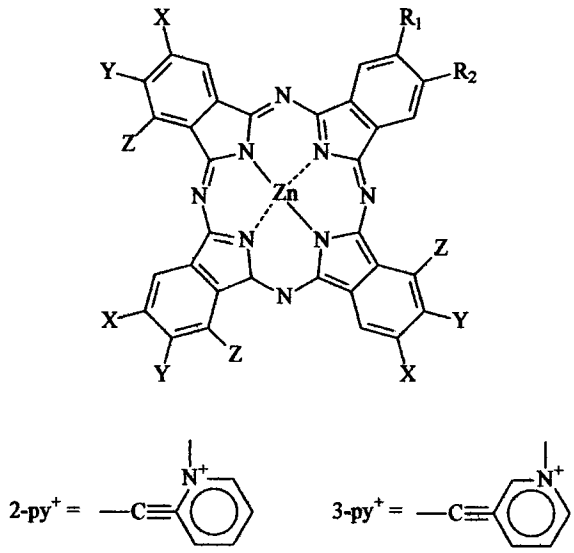


Fig. 4. Asymmetrically substituted cationic phthalocyanines comprising 2-ethynylpyridinyl and 3-ethynylpyridinyl moieties. **8a** R_1 = H, R_2 = H, X = -2-py$^+$, Y = H, Z = H; **8b** R_1 = H, R_2 = H, X = -3-py$^+$, Y = H, Z = H; **8c** R_1 = H, R_2 = t-butyl, X = -2-py$^+$, Y = H, Z = H; **8d** R_1 = H, R_2 = t-butyl, X = -3-py$^+$, Y = H, Z = H; **8e** R_1 = R_2 = -CH=CH-CH=CH-, X = -2-py$^+$, Y = H, Z = H; **8f** R_1 = R_2 = -CH=CH-CH=CH-, X = -3-py$^+$, Y = H, Z = H; **8g** R_1 = H, R_2 = H, X = H, Y = H, Z = -2-py$^+$; **8h** R_1 = H, R_2 = H, X = H, Y = H, Z = -3-py$^+$; **8i** R_1 = H, R_2 = t-butyl, X = H, Y = H, Z = -2-py$^+$; **8j** R_1 = H, R_2 = t-butyl, X = H, Y = H, Z = -3-py$^+$; **8k** R_1 = R_2 = -CH=CH-CH=CH-, X = H, Y = H, Z = -2-py$^+$; **8l** R_1 = R_2 = -CH=CH-CH=CH-, X = H, Y = H, Z = -3-py$^+$; **8m** R_1 = H, R_2 = H, X = Y = -2-py$^+$, Z = H; **8n** R_1 = H, R_2 = H, X = Y = -3-py$^+$, Z = H.

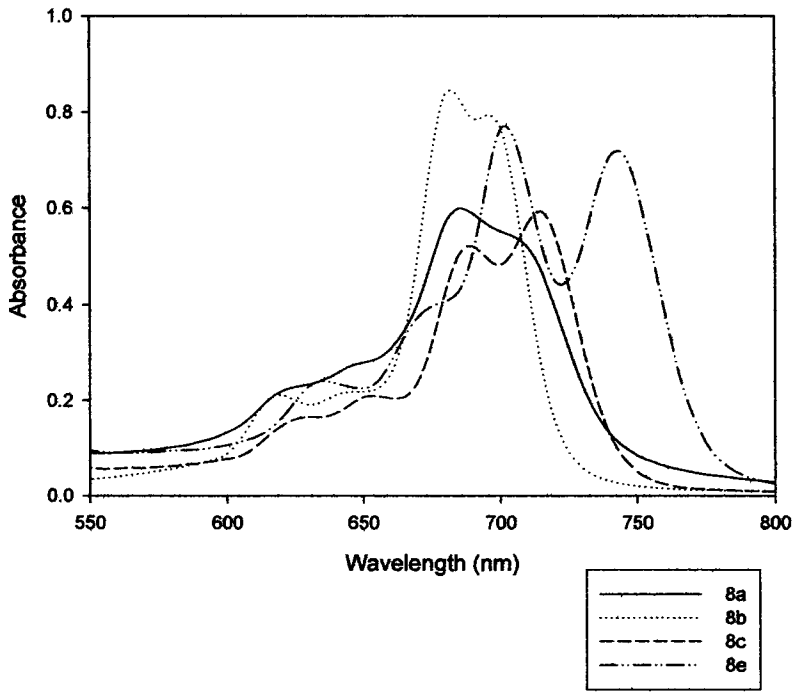


Fig. 5. UV-visible spectra of asymmetrically substituted cationic phthalocyanines **8a**, **8b**, **8c** and **8e** in DMF.

targets such as the mitochondria [39,40] and DNA [41] along with bacteria and other infectious agents [42-46]. Interestingly, comparative studies of three different charged (anionic, cationic and neutral) structurally similar zinc phthalocyanines have shown that the cationic phthalocyanine is the most effective photosensitizer [42,47-50]. In addition, asymmetrically substituted cationic photosensitizers are more efficient in destroying melanoma cells than the corresponding symmetrically substituted photosensitizers [51] while phthalocyanines substituted on only one benzo ring with cationic groups or protonable groups are particularly photodynamic active with impressive activities against fungi, Gram-positive and Gram-negative bacteria [52].

Asymmetrically substituted iodinated Pcs **4a-g** underwent palladium-catalyzed cross-coupling reactions with 2-ethynlpyridine and 3-ethnylpyridine (Figure 4). The FAB mass spectral data for the resulting Pcs **7a-n** was consistent with the proposed structures while the UV-visible spectra gave typical sharp Q bands around 680 nm. Pcs **7a-n** reacted readily with methyl iodide or dimethyl sulfate in DMF at 70°C to give the corresponding asymmetrically substituted cationic Pcs **8a-n**. In aqueous solution, cationic Pcs **8a-n** are highly aggregated, exhibited a broad Q band centered between 620-640 nm. However, N-methylation in Pcs **8a-n** lead to significant splitting of the Q band in DMF along with impressive shifts of the λmax to longer wavelengths (Figure 5). Such shifts are advantageous as light penetration of tissue increases with increasing wavelengths. In a studies involving a series of zinc complexes of benzonaphthaloporphyrazines bearing positively charged methylpyridinium substituents on the benzo rings, it was determined that the 3:1 asymmetrically substituted Pc containing one naphthalo ring and three benzo rings bearing positively charged methylpyridinium substituents (akin to Pcs **8f** and **8l** with the methylpyridinium group bound to the Pc by a ether bond instead of an ethynyl bond) are the most photodynamically active [53,54], thus making Pcs **8e, 8f, 8j** and **8l** of particular interest. In addition, shifting the quarternized nitrogen from the 3 to the 2 position in the pyridine moiety may also have interesting properties for PDT.

In conclusion, the Kobayashi ring expansion reaction of iodinated subPcs **2a-c** selectively yielded the corresponding 3:1 asymmetrically substituted iodinated Pcs **4a-g**. These 3:1 asymmetrically substituted iodinated Pcs are ideal building blocks for the preparation of novel 3:1 asymmetrically substituted Pcs bearing novel functionality and will be useful in the synthesis of Pcs with improve properties for numerous applications. In terms of PDT, palladium-catalyzed cross-coupling reactions have been successfully employed to prepare novel 3:1 asymmetrically substituted water-soluble anionic and cationic photosensitizers. Of particular interest are the novel asymmetrically substituted cationic Pcs since cationic photosensitizers have been shown to have a number of interesting properties in the photodynamic treatment of numerous conditions. In addition, the lipophilic/hydrophilic balance in these PS can be further modified by altering the number of carbon atoms in the side chains of the quarternized nitrogens.

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Chapter 9.

The Discussion

9. Discussion

As disclosed in the introduction, phthalocyanines have immense potential in an array of widely diverging fields. While these impressive macrocycles have found tangible utility as dyes and pigments, as photoconducting agents in photocopying devices and laser printers, as catalysts for important chemical reactions with environmental consequences and as the active element in optical data storage, the true potential of phthalocyanines can only be realized with macrocycles with distinct physical structures and well-defined chemical and electronic properties. With this in mind, new synthetic procedures and modification and adaptation of established synthetic protocols continue to be scrutinized in order to prepare phthalocyanines with the required physical, chemical and spectral properties for application in a given technical field.

Among pre-existing synthetic protocols, the Kobayashi ring expansion reaction of boron subphthalocyanines provides means for preparing novel 3:1 asymmetrically substituted phthalocyanines while retaining sufficient synthetic versatility to incorporate diverse substituents into the final phthalocyanine product. While it has been demonstrated that the ring expansion reaction does not proceed via a concerted, one-step mechanism and is thus not universally applicable (Figure 1.26) (Sastre et al., 1995; Weitemeyer et al., 1995; Geyer et al., 1996; Sastre et al., 1996), this synthetic procedure has been revealed to be valuable in a number of specific cases. In particular, our group has successfully used the Kobayashi ring expansion reaction of boron tri(4-sulfo)subphthalocyanine to synthesize novel trisulphonated zinc phthalocyanine derivatives (Figure 1.27) and has examined the photodynamic activity of these water-

soluble macrocycles (Kudrevich et al., 1996, Kudrevich et al, 1997; van Lier et al., 1999). Taking this successful use of the Kobayashi ring expansion reaction into consideration, halogenated boron subphthalocyanines were targeted as precursors for the synthesis of novel 3:1 asymmetrically substituted phthalocyanines. Our group has demonstrated that fluorinated phthalocyanines are efficient photosensitizers for photodynamic therapy (Allémann et al., 1995; Allémann et al., 1996; Boyle et al., 1996b; Allémann et al., 1997; Bench et al., 2002) and it is assumed that the asymmetrically substituted fluorinated phthalocyanines potentially obtained via the ring expansion reaction of the corresponding fluorinated subphthalocyanines will have important advantages as photosensitizers. In the meanwhile, iodinated subphthalocyanines provide useful precursors for the preparation of asymmetrically iodinated phthalocyanines. In turn, by employing palladium-catalyzed reactions, these iodinated macrocycles provide access to novel asymmetrically substituted phthalocyanines bearing interesting functionality. Such reactions have been extensively used to prepare novel porphyrins and phthalocyanines (Ali et al., 1994; Boyle et al., 1995; Sharman et al., 1996; Ali et al., 1997; Maya et al., 1998; Vinogradov et al., 1998; Aranyos et al., 1999; Leznoff et al., 1999; Maya et al., 2000; Sugimori et al., 2000; Tian et al., 2000; Khan et al., 2001; Khan et al., 2003; Cauchon et al., 2005) (also see Chapter 6).

Boron subphthalocyanines were first serendipitously synthesized in 1972 during attempts to prepare boron phthalocyanines by the condensation reaction of phthalonitrile with boron trichloride in chloronaphthalene at 200°C (Meller et al., 1972). It was not for another twenty years that subphthalocyanines started to receive attention as more than just the smaller analogues of phthalocyanines. Their unique physical, chemical and

structural properties have led investigators to propose their utility as building blocks for the synthesis of 3:1 asymmetrically substituted phthalocyanines (Kobayashi et al., 1990; Kasuga et al., 1992; Musluoglu et al., 1992; Dabak et al., 1994; Kudrevich et al., 1996; Kudrevich et al, 1997; Kobayashi et al., 1999; van Lier et al., 1999) and as materials for nonlinear optics (Diaz-Garcia et al., 1995; Sastre et al., 1996; Rojo et al., 1997; del Rey et al., 1998; Kang et al., 1999; de la Torre et al., 2004; Claessens et al., 2005). However, despite intense investigation into the synthesis, properties and reactivity of subphthalocyanines, the synthetic procedure employed during their first serendipitous synthesis remains the only basic method for obtaining their unique tripyrrolic architecture. The only method to date for preparing subphthalocyanines remains the cyclotrimerization of phthalonitriles in the presence of a boron source. While this boron source is typically a boron trihalide, other boron sources such as triphenylboron have been successfully employed though yields have been significantly decreased in most cases.

(Dodecafluorosubphthalocyaninato)boron(III) bromide, (2,9,16(17)-triiodo-subphthalocyaninato)boron(III) bromide, (1,8,15(18)-triiodosubphthalocyaninato)boron(III) bromide and (2,3,9,10,16,17-hexaiodosubphthalocyaninato)boron(III) bromide have all been successfully synthesized by the cyclotrimerization reaction of the corresponding halogenated phthalonitrile with 1M boron tribromide in dichloromethane using chlorobenzene as a solvent at a reaction temperature of 40-60°C (Figure 7.1 and 8.1). Yields ranged from 35% for the reaction with 3-iodophthalonitrile to 70% for tetrafluorophthalonitrile. These reaction conditions are much milder than those traditionally used in the preparation of subphthalocyanines

and even give improved yields. For instance, the corresponding (dodecafluorosubphthalocyaninato)boron(III) chloride has been previously synthesized via the reaction of tetrafluorophthalonitrile and boron trichloride (1M solution in hexane) in 1-chloronaphthalene at elevated temperatures (Kipp et al., 1998; Ohno-Okumura et al., 2002). Under these conditions, yields were decreased to 26%. (2,9,16(17)-Triiodosubphthalocyaninato)boron(III) chloride and (1,8,15(18)-triiodosubphthalocyaninato)boron(III) chloride have also prepared under harsh conditions (condensed boron trichloride, 1-chloronaphthalene, 120-240°C) in yields of 45% and 11% respectively (Geyer et al., 1996; del Rey et al., 1997; Claessens et al., 2000a). Interestingly, the addition of small amounts of tetrahydrofuran was found to increase reaction rates and the resulting overall yield. While the reason for this is unclear, it may involve improved solubilization of the reagents.

It is known that the reactivity of trisubstituted boron compounds towards the cyclotrimerization reaction is closely related to the Lewis acidity of the boron compound and follows the order $B(Alkyl)_3 < BPh_3 < BF_3 < BCl_3 < BBr_3$ (Claessens et al., 2002). Thus, the increased reactivity of boron tribromide compared to boron trichloride may lead to the possibility of employing milder reaction conditions in the formation of these halogenated boron subphthalocyanines, therefore ensuring enhanced yields. However, the presence of four strongly electron withdrawing fluorine atoms will also activate the nitrile groups of the tetrafluorophthalonitrile towards the subphthalocyanine formation. Furthermore, the relative stability of boron subphthalocyanines depends on the nature of the peripheral substituents with (2,9,16(17)-triiodosubphthalocyaninato)boron(III) chloride being the most stable subphthalocyanine studied (Claessens et al., 2002). Thus,

the halogen substituents increase the reactivity of the phthalonitrile and stabilize the final boron subphthalocyanine product, leading to improved yields while employing milder reaction conditions. As confirmed by mass spectroscopy, the use of milder reaction conditions also seems to prevent the bromination of the iodinated subphthalocyanines, a common side reaction due to the Lewis acidity of the boron source. Furthermore, no exchange of fluorine for bromine is observed in the preparation of dodecafluorinated subphthalocyanine, this despite the strong Lewis acidity of boron tribromide and the well-established reactivity of tetrafluorophthalonitrile towards exchange reactions (Birchall et al., 1970).

Boron subphthalocyanines bearing a bromine axial ligand are quite unstable with respect to axial ligand exchange with bromosubphthalocyanines readily undergoing nucleophilic substitution while chlorosubphthalocyanines require harsher reaction conditions (Claessens et al., 2002). This observation has been exploited for the preparation of subphthalocyanines bearing novel axial ligands (Kasuga et al., 1996; Engel et al., 1997; Yanagiba et al., 1997). However, this property of bromosubphthalocyanines also makes their purification and characterization difficult and only a small number of examples are described in the literature (see for instance Kobayashi et al., 1990; Kasuga et al., 1996; Kudrevich et al., 1997; Kobayashi et al., 1999; Wang et al., 2000; Cao et al., 2002). Use of column chromatography may lead to axial ligand exchange with free OH groups in the stationary phase (Claessens et al., 2002) and purification of these labile compounds is preferably accomplished by precipitation or Soxhlet extraction with appropriate solvents (Cao et al., 2002). However, it was found that the halogenated subphthalocyanines, in particular the dodecafluorinated subphthalocyanines, degraded

significantly when the chlorobenzene reaction mixture was added to excess hexanes. This is despite reports that subphthalocyanines are stable in aprotic solvents (del Rey et al., 1998). Furthermore, (2,9,16(17)-triiodosubphthalocyaninato)boron(III) chloride is the most stable subphthalocyanine that has been investigated (Claessens et al., 2002). On the other hand, (dodecafluorosubphthalocyaninato)boron(III) chloride is known to undergo thermal decomposition in hexanes (Kipp et al., 1998). Soxhlet extraction of the halogenated subphthalocyanines with dry dichloromethane also led to decreased yields of the macrocycles. Purification by flash column chromatography on neutral alumina in the dark using 30% THF in hexanes or using dichloromethane as eluant proved to be the most efficient method of obtaining the desired halogenated tripyrrolic macrocycles. Using neutral alumina decreased the amount of axial ligand substitution while avoiding light prevented photodegradation of the subphthalocyanines on the column. The resulting halogenated subphthalocyanines exhibited typical electronic spectra, with a sharp, strong Q band around 580 nm.

In view of the observation that lowering the reaction temperature and employing milder reaction conditions increased yields, attempts were undertaken to prepare (dodecafluorosubphthalocyaninato)boron(III) bromide at 0°C. Under these conditions, a labile blue product absorbing at 660 nm was obtained instead of the desired purple subphthalocyanine (with its characteristic absorption at 577 nm) (Figure 9.1). A similar labile blue product was observed when the cyclotrimerization reaction was undertaken at 0°C using other phthalonitriles substituted with electron-withdrawing substituents (including 4-iodophthalonitrile, 4,5-diiodophthalonitrile, 4-nitrophthalonitrile and

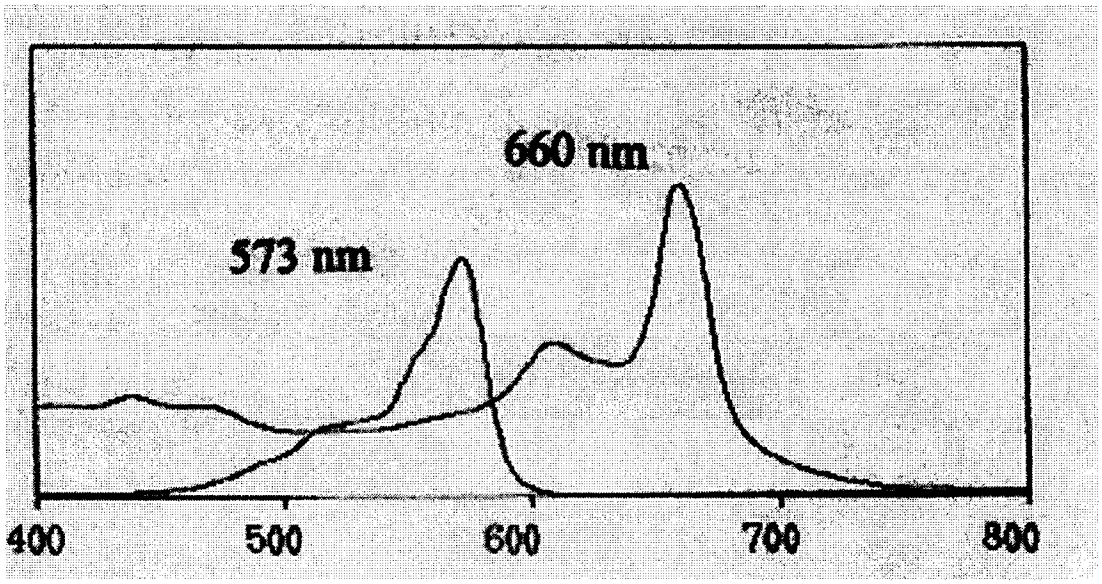


Figure 9.1. The absorption spectra of (dodecafluorosubphthalocyaninato) boron(III) bromide (573 nm) and of the blue intermediate species (660 nm) in THF.

1,2,4,5-tetracyanobenzene). (Dodecafluorosubphthalocyaninato)boron(III) chloride has been reported to undergo thermal decomposition to yield a light blue product whose structure was not determined (Kipp et al., 1998). While the identity of these labile blue species has not been fully elucidated, determination of the nature of these species would aid in the determination of the mechanism of the cyclotrimerization reaction. This would in turn allow investigators to improve the synthesis and ultimately the utility of subphthalocyanines.

Although the nature of the transient blue species has not been fully elucidated, speculation leads to a number of possibilities. Boron trihalides are known to form charge transfer complexes with nitrile functional groups ($RC{\equiv}N{:}BCl_3$) (Gerrard et al., 1958) and a charge transfer complex with the two nitrile groups of the phthalonitrile would be

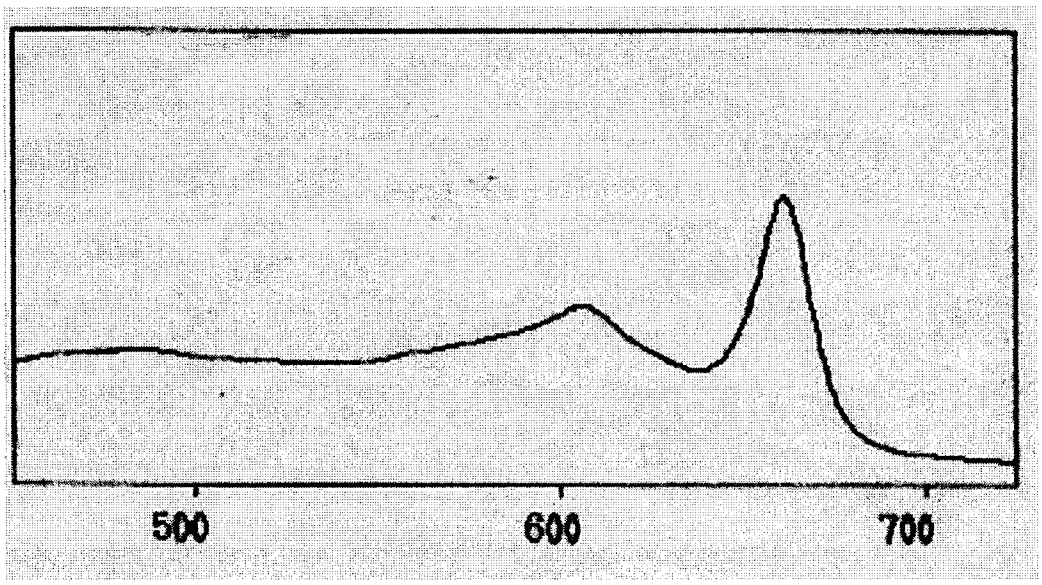
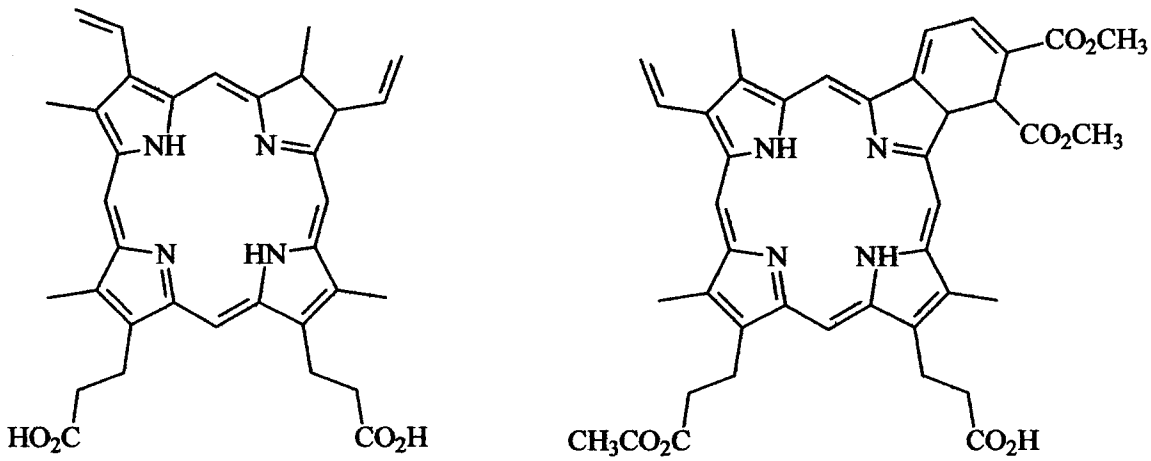


Figure 9.2. The absorption spectra of the reaction of (dodecafluorosubphthalocyanato) boron(III) bromide (2) with $NaBH_4$.

expected to form during the cyclotrimerization reaction. It has been observed that heating a solution of the labile blue species leads to formation of the desired subphthalocyanine in decreased overall yield. Furthermore, treatment of the blue species with the oxidant DDQ leads to a broad transient absorption in the UV-visible spectra centered around 580 nm. On the other hand, addition of reductant such as $NaBH_4$ to a solution of (dodecafluorosubphthalocyaninato)boron(III) bromide results in a loss of the absorption at 577 nm and the formation of a broad transient absorption at 660 nm (Figure 9.2). These results suggest that the transient blue species requires an oxidation step in order to form the subphthalocyanine and that this oxidation step occurs late in the cyclotrimerization reaction pathway. A bathochromic shift in the Q band as one goes from an incompletely oxidized species to the completely oxidized subphthalocyanines is



Protoporphyrin IX
λ_{max} = 635 nm

Verteporfin
λ_{max} = 690 nm

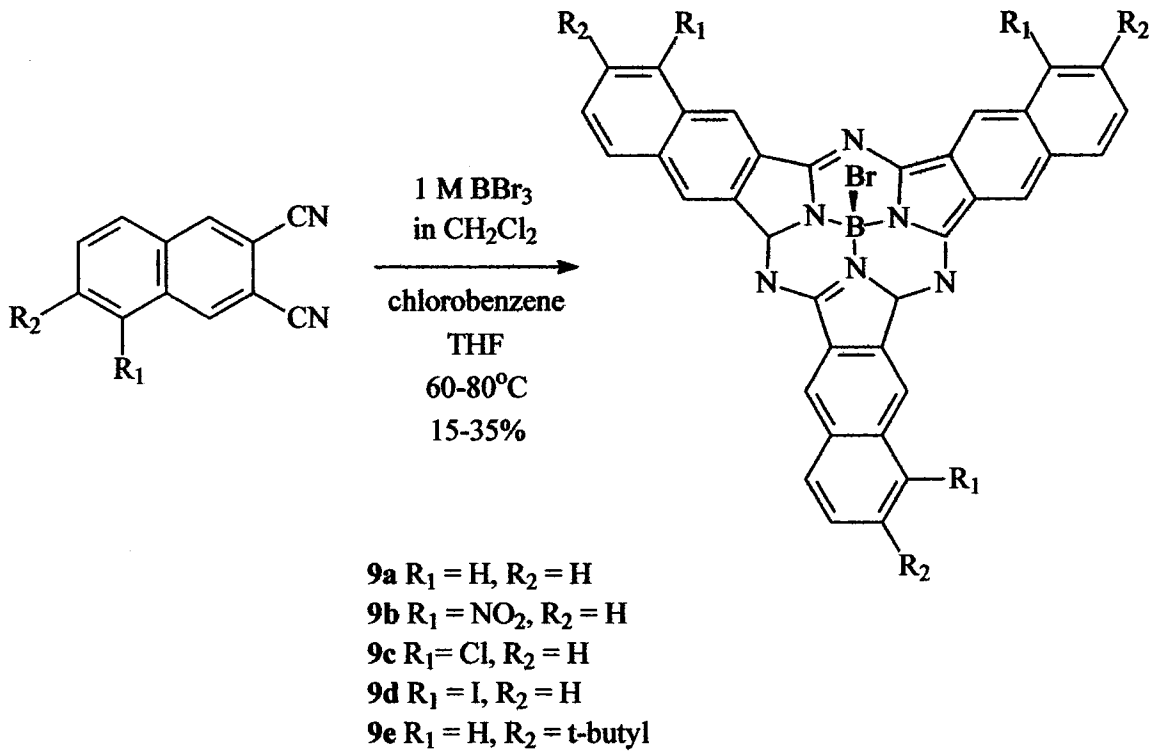
Figure 9.3. Protoporphyrin IX and Verteporfin and the bathchromic shift in the λ_{max} with incomplete conjugation.

not unexpected. A loss of conjugation in tetrapyrrolic macrocycles is known to lead to a red shift in the λ_{max}. For instance, while the completely conjugated protoporphyrin IX absorbs at 630 nm, the structurally similar incompletely conjugated verteporfin absorbs at 690 nm (Figure 9.3) (Mody, 2000). The singlet excited state of (dodecafluorosubphthalocyaninato)boron(III) chloride has also been shown to be a strong oxidant with a one electron potential estimated to be 1.7 V (Kipp et al., 1998), suggesting that the connection between the subphthalocyanines and the blue intermediary species pass via a photoexcited state of the subphthalocyanine.

Due to their cone-shaped structure, subphthalocyanines are less prone to aggregate in solution compared to planar phthalocyanines. Furthermore, subphthalocyanines fluoresce with quantum yields around 0.25 (which is lower than typical quantum yields for phthalocyanines) and have larger triplet state quantum yields

than phthalocyanines with triplet state lifetimes in the range of 100 μs (del Rey et al., 1998). As such, subphthalocyanines can effectively sensitize molecular oxygen to singlet oxygen upon illumination, with quantum yields ranging from 0.23 to 0.75. While these properties are exceptional for photosensitizers in terms of photodynamic therapy, subphthalocyanines absorb around 580 nm where tissue penetration of light is minimal. With this in mind, unsubstituted boron subnaphthalocyanine has been prepared and its optical absorption and photophysical properties have been investigated (Nonell et al., 2000). In a homogenous environment using toluene as a solvent, it was determined that this photosensitizer has excellent triplet state and singlet oxygen quantum yields (Φ_T = Φ_Δ = 0.68) which are substantially higher than those of phthalocyanines or naphthalocyanines. Since (subnaphthalocyaninato)boron(III) bromide absorbs at 663 nm with an absorption coefficient of 7.94 x 10^4 $M^{-1}cm^{-1}$, these photophysical properties, along with their synthetic availability, high solubility and low tendency to aggregate, has led to the suggestion that subnaphthalocyanines may represent a new class of photosensitizers for photodynamic therapy that warrant further investigation.

To ascertain the photodynamic potential of subnaphthalocyanines in a more biological relevant environment, a series of boron subnaphthalocyanines have been prepared from known 2,3-naphthalenedicarbonitrile derivatives and purified using the same methodologies as the one employed for the halogenated subphthalocyanines (Figure 9.4) (see Chapters 7 and 8 for the procedure employed in their synthesis). While these compounds have not yet been fully characterized, they provide an excellent opportunity to determine the relevance of boron subnaphthalocyanines as photosensitizers for photodynamic therapy.



9a $R_1 = H$, $R_2 = H$
9b $R_1 = NO_2$, $R_2 = H$
9c $R_1 = Cl$, $R_2 = H$
9d $R_1 = I$, $R_2 = H$
9e $R_1 = H$, $R_2 =$ t-butyl

Figure 9.4. Synthesis of boron subnaphthalocyanines (consititutional isomers not depicted)

In order to determine if these compounds could effectively generate singlet oxygen and induce oxidative damage in an aqueous, biologically relevant environment, thee (naphthalosubphthalocyaninato)boron(III) bromide derivatives were formulated as Cremophor™ EL (CRM) emulsions (see Chapter 7 for the procedure used for drug formulation). A solution of 5 μM boron subnaphthalocyanine (0.5% CRM) and 5 mM L-tryptophan in PBSwas irradiated with light between 600 and 700 nm at a fluence rate of 150 mW cm^{-2} using the L-tryptophan photooxidation protocol described in Chapter 7. Singlet oxygen is believe to be the single most important reactive species generated during photodynamic therapy and is ultimately responsible for most of the biological damage observed during PDT(see Chapter 2). Singlet oxygen induces characteristic

	λmax (nm)	Relative HPPI Yields	Photobleaching rate constant (sec^{-1})		λmax (nm)	Relative HPPI Yields	Photobleaching rate constant (sec^{-1})
$AlPcS_4$ (PBS)	680	1	-	9b (R_1=NO_2, R_2=H)	655	1.51	$1.01x10^{-2}$
$AlPcS_4$	678	1.15	-	9c (R_1=Cl, R_2=H)	657	1.55	$2.23x10^{-2}$
AlPc	671	0.25	-	9d (R_1=I, R_2=H)	659	1.68	$2.50x10^{-2}$
9a (R_1=R_2=H)	659	1.18	$2.14x10^{-2}$	9e (R_1=H, R_2=t-butyl)	660	1.38	$2.34x10^{-2}$

Table 9.1. Q band absorption, relative L-tryptophan photooxidation and photobleaching rate constant of boron subnaphthalocyanines (0.5% CRM solution unless otherwise noted)

oxidative damage to biologically important molecules including amino acids and DNA (Singh, 1982; Langlois et al., 1986; Ferraudi et al., 1988; Halliwell et al., 1991; Langlois et al., 1993; DeRosa et al., 2002). For instance, L-tryptophan oxidation by singlet oxygen yields characteristic hydroperoxide isomers (HPPI) which can be quantified by HPLC (Langlois et al., 1986; Langlois et al., 1993) and as a result, relative singlet oxygen yields can be inferred from the measurement of the yield of HPPI isomers relative to a known standard.

Table 9.1 discloses the relative tryptophan photooxidation after two minutes of irradiation for the series of boron subnaphthalocyanines examined. Each example is a

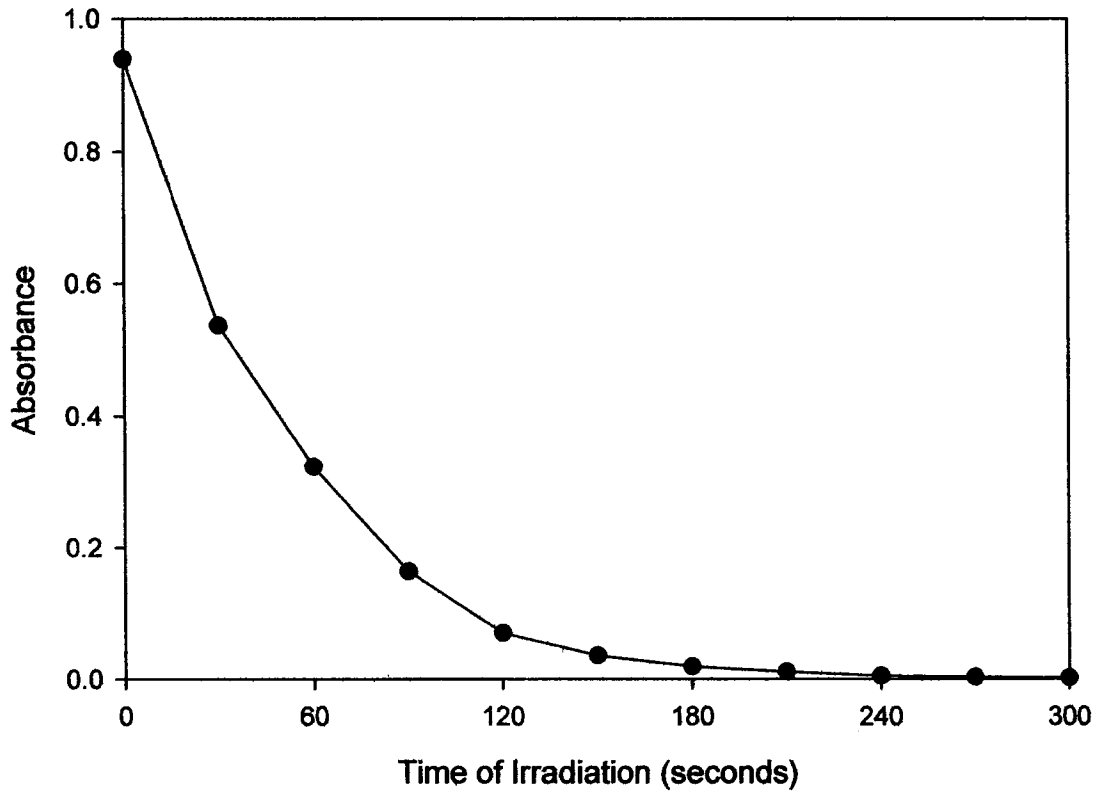


Figure 9.5. Photobleaching of **9a** ($R_1 = R_2 = H$) at a fluence rate of 150 $mWcm^{-2}$

0.5% CRM emulsion unless otherwise noted. The yield for $AlPcS_4$ in PBS is arbitrarily set as 1 (with the singlet oxygen quantum yield for $AlPcS_4$ in PBS (1% Triton) reported as 0.43 (Redmond et al., 1999)). Formulating $AlPcS_4$ in 0.5% CRM slightly increases hydroperoxide yields, most likely due to slightly decreased aggregation of the photosensitizer. Unsubstituted aluminum phthalocyanine, on the other hand, gives significantly less hydroperoxide, a result of important aggregation of the chromophore under these conditions. In contrast, unsubstituted boron subnaphthalocyanine yields similar hydroperoxide yields as $AlPcS_4$ in the same formulation. Obviously, the cone-shaped structure of these macrocycles prevents aggregation, leading to the important

difference between unsubstituted phthalocyanines and subnaphthalocyanines. As expected, the most effective of the boron subnaphthalocyanines are the halogenated derivatives, most likely a result of the heavy atom effect that increases spin-orbital coupling and facilitates intersystem crossing to the excited triplet state.

Subphthalocyanines and by extension subnaphthalocyanines are known to be considerably less photostable than phthalocyanines (del Rey et al., 1998; Kobayashi et al., 1999; Claessens et al., 2002). Figure 9.5 represents a graph of the absorbance of a 15μM solution of unsubstituted boron subnaphthalocyanine versus the time of irradiation with light between 600 and 700 nm at a fluence rate of 150 mW cm^{-2}. As can clearly be seen, these boron subnaphthalocyanines are rapidly photobleached, with most of the chromophore being destroyed after 2 minutes of irradiation. The improved tryptophan photooxidation by the trinitro-substituted derivative disclosed in Table 9.1 is most likely due to the slightly decreased rate of photobleaching observed for this photosensitizer. The strongly electron-withdrawing nitro functionality most likely draws electron density away from the macrocycle, protecting the trinitro-substituted derivative from photo-induced destruction. Table 9.1 discloses the photobleaching half-life of this series of boron subnaphthalocyanines at a fluence rate of 150 mW cm^{-2} with light of a wavelength between 600-700 nm. While photobleaching may decrease the photodynamic efficiency of the photosensitizer by decreasing the overall concentration in the irradiated target, photobleaching *in vivo* may be advantageous in terms of improved target selectivity (MacRoberts et al., 1989) and decreasing photosensitization (Bonnett, 1995; Bonnett, 1999). Furthermore, decreased photostability increases the therapeutic depth of laser light penetration into tissue because photosensitizer molecules in the upper cellular layers

photobleach more rapidly than those in deeper cellular layers (Wöhrle et al., 1998). Thus, the decreased shielding by photobleached photosensitizer in upper layers allows deeper light penetration during photodynamic therapy.

The ring expansion reaction of subphthalocyanines was first demonstrated by Kobayashi et al. in 1990 with the reaction of (2,9,16(17)-tri-t-butylsubphthalocyaninato)boron(III) bromide with 1,3-diiminoisoindolines of increasing aromaticity (Figure 1.25) (Kobayashi et al., 1990). The advantages of this ring expansion reaction were immediately recognized. Yields ranged from 8-20%, which are especially good in view of the fact that only the 3:1 asymmetrically substituted phthalocyanine derivatives were obtained. Furthermore, purification was deemed facile as only two easily separated bands existed, one for the reddish-purple unreacted subphthalocyanine and one for the green 3:1 asymmetrically substituted phthalocyanine. The initial promise of this reaction protocol for the synthesis of asymmetrically substituted phthalocyanines seemed to be verified by the synthesis of pure monosubstituted phthalocyanines fitted with crown ether substituents (Musluoglu et al., 1992) and with dipropoxy functionality (Kasuga et al., 1992) and by the synthesis of hexakis(alkylthio)-substituted phthalocyanines (Dabak et al., 1994).

In light of these promising initial studies and the important utility of 3:1 asymmetrically substituted phthalocyanines, extensive studies have been undertaken using this methodology in order to prepare novel phthalocyanine derivatives (Sastre et al., 1995; Weitemeyer et al., 1995; Geyer et al., 1996; Sastre et al., 1996; Kudrevich et al., 1996; Kudrevich et al., 1997; Ali et al., 1999; Kobayashi, 1999; Kobayashi et al., 1999; van Lier et al., 1999; Claessens et al., 2002). Unfortunately, success varies greatly,

depending on the substituents on the boron subphthalocyanine, the nature of the 1,3-diiminoisoindoline and the reaction conditions employed. In a number of reactions, the Kobayashi ring expansion reaction of boron subphthalocyanines led to a mixture of differently substituted phthalocyanines (Sastre et al., 1995; Weitemeyer et al., 1995; Geyer et al., 1996; Sastre et al., 1996; Claessens et al., 2002). In order to explain these results, it has been suggested that the ring expansion reaction must not be a concerted process (Sastre et al., 1995; Weitemeyer et al., 1995; Sastre et al., 1996). The reaction must proceed by a 1,3-diiminoisoindoline-catalyzed cleavage of the boron subphthalocyanine into different fragments that subsequently condense to give phthalocyanines with varying substitution patterns (Figure 1.26). Proceeding via such a mechanism greatly limits the universal applicability and the general synthetic utility of the Kobayashi ring expansion. However, in spite of these less than favorable results, this synthetic approach to the synthesis of 3:1 asymmetrically substituted phthalocyanines has proven valuable in specific cases where the substituents on the subphthalocyanine, the nature of the 1,3-diiminoisoindoline and/or the reaction conditions employed have allowed the reaction to proceed smoothly to yield only the desired 3:1 asymmetrically substituted product (Kobayashi et al., 1990; Musluoglu et al., 1992; Kasuga et al., 1992; Dabak et al., 1994; Kudrevich et al., 1996; Kudrevich et al., 1997; Ali et al., 1999; Kobayashi, 1999; Kobayashi et al., 1999; van Lier et al., 1999).

(Dodecafluorosubphthalocyaninato)boron(III) bromide, (2,9,16(17)-triiodo-subphthalocyaninato)boron(III) bromide, (1,8,15(18)-triiodosubphthalocyaninato)boron(III) bromide and (2,3,9,10,16,17-hexaiodosubphthalocyaninato)boron(III) bromide all readily underwent the Kobayashi

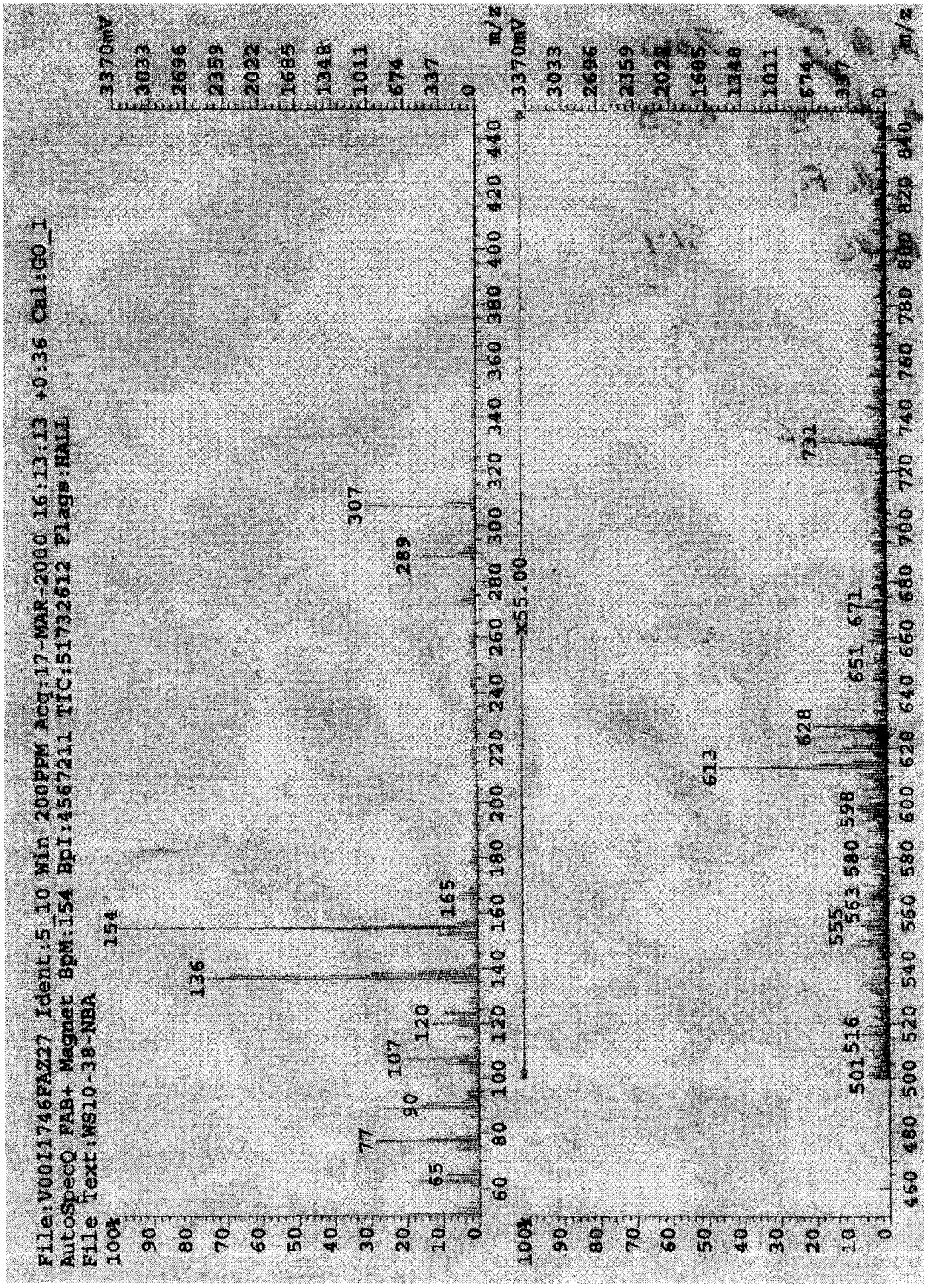


Figure 9.6. FAB-MS spectra of 1,2,3,4,8,9,10,11,15,16,17,18-dodecafluorophthalocyaninate (Compound **4a** in Figure 7.1).

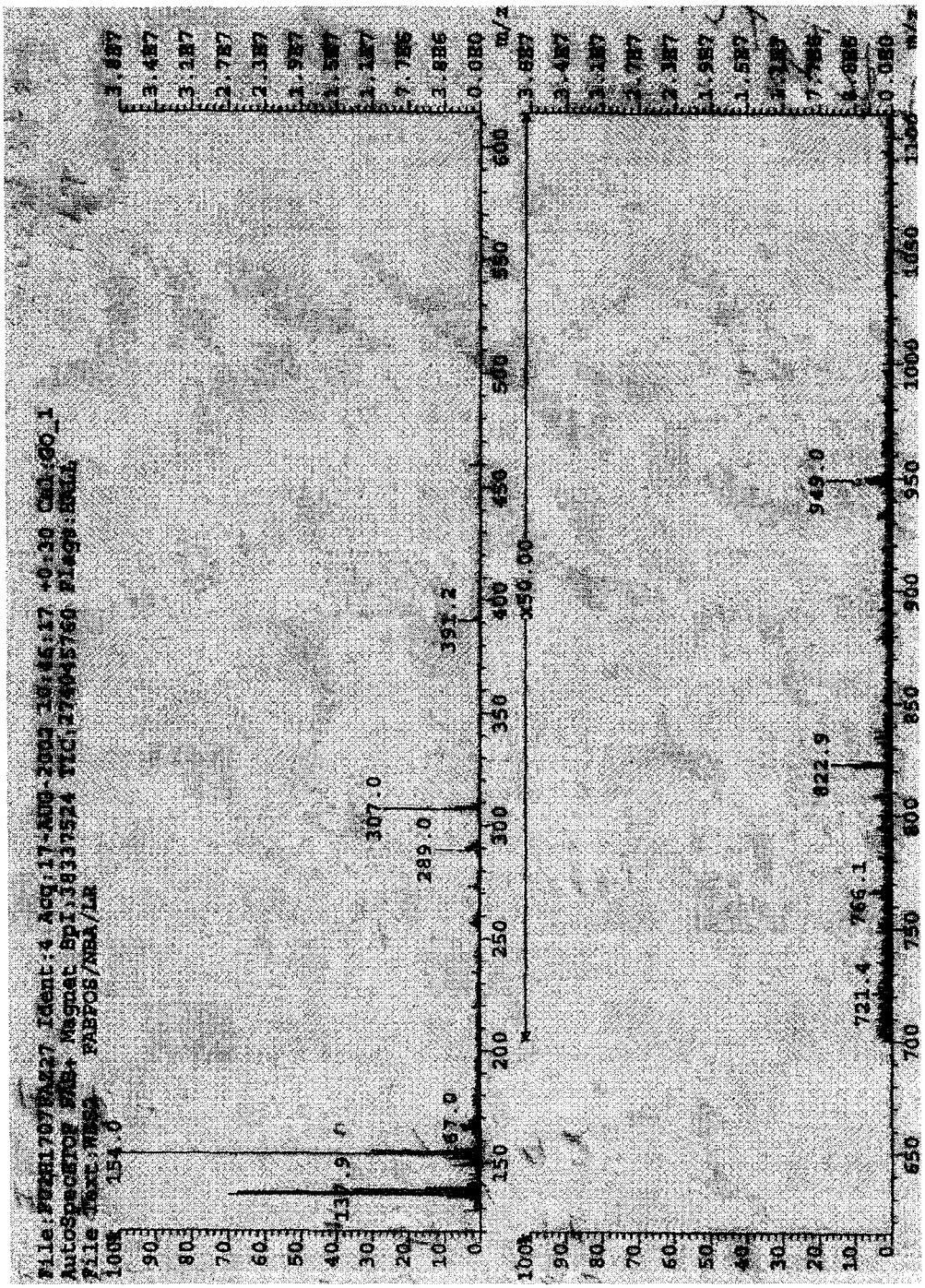


Figure 9.7. FAB-MS spectra of 2,9,16-triiodo-23(24)-t-butylphthalocyaninate

(Compound **4b** in Figure 8.2).

ring expansion reaction with various diiminoisoindolines using DMSO as a reaction solvent (Figures 7.1 and 8.2). FAB-MS data confirm that these ring expansion reactions proceeded selectively to yield only the desired 3:1 asymmetrically substituted halogenated phthalocyanines. While m/z values corresponding to the expected molecular ion (M^+ or $(M+1)^+$) were observed, no m/z peaks corresponding to the other possible substitution patterns were obtained. For instance, Figures 9.6 and 9.7 depict the FAB-MS spectra of 1,2,3,4,8,9,10,11,15,16,17,18-dodecafluorophthalocyaninate (Compound **4a** in Figure 7.1) and 2,9,16-triiodo-23(24)-t-butylphthalocyaninate (Compound **4b** in Figure 8.2). While the $(M+1)^+$ is present for the desired 3:1 asymmetrically substituted product, no ions represents in other substitution patterns are observed (514, 586, 658 and 802 for the unsubstituted, tetrafluorinated, octafluorinated and hexadecafluorinated phthalocyanines and 738, 808, 878, 1018 for tetra-t-butyl-substituted, tri-t-butyl-monoiodo-substituted, di-t-butyl-di-iodo-substituted and tetraiodo-substituted phthalocyanines respectively). Yields for the dodecafluorinated phthalocyanines ranged from 18-60% while those obtained for the iodinated phthalocyanines ranged from 15-43%. It should however be noted that these yields were obtained for small scale reactions with increases in the scale of the reaction often leading to critical decreases in the overall yield of the phthalocyanine product. Lower yields were obtained for the reaction of the dodecafluorinated subphthalocyanine with 4-iodo-1,3-diiminoisoindoline, most likely the result of steric effects. Decreased yields were similarly obtained for Kobayashi ring expansion reactions of the more sterically involved (1,8,15(18)-triiodosubphthalocyaninato)boron(III) bromide. Reactions of the dodecafluorinated subphthalocyanine with 5,6-didodecyl-1,3-diiminoisoindoline also led to lower yields.

While steric effects may play a role in the lower yields with the long alkyl chains interfering with the reaction of the subphthalocyanine with the 1,3-diiminoisoindoline, the solubility of 5,6-didodecyl-1,3-diiminoisoindoline undoubtedly is involved as well. While the other 1,3-diiminoisoindoline employed could be solubilized in DMSO upon sonication, 5,6-didodecyl-1,3-diiminoisoindoline require a 1:1 mixture of DMSO and chlorobenzene. The chlorobenzene in the reaction solvent may possibly interfere in some manner with the ring expansion reaction, resulting in lower phthalocyanine yields. Interestingly, the Kobayashi ring expansion reaction traditionally uses a mixture of DMSO and either chlorobenzene, o-dichlorobenzene, 1-chloronaphthalene or chloronaphthalene as the reaction solvent. In fact, it has been reported that the Kobayashi ring expansion reaction of (2,9,16(17)-tri-t-butylsubphthalocyaninato)boron(III) bromide with unsubstituted 1,3-diiminoisoindoline failed to give any phthalocyanine when pure DMSO was used as the reaction solvent (Kobayashi et al., 1999). Reaction of 2,9,16(17)-tri-t-butylsubphthalocyaninato)boron(III) bromide with 5-t-butyl-1,3-diiminoisoindoline in pure DMSO led to a small yield of the tetra-t-butylphthalocyanine. On the other hand, DMSO has been used successfully as the reaction solvent for the Kobayashi ring expansion reaction of (2,9,16(17)-tris(chlorosulfonyl)subphthalocyaninato)boron(III) bromide, allowing for the preparation of novel 3:1 asymmetrically substituted water-soluble phthalocyanines (Figure 1.27) (Kudrevich et al., 1996; Kudrevich et al., 1997; van Lier et al., 1999). As such, there is the possibility that the use of DMSO, a mild oxidant, as the solvent may be responsible for the selectivity and the enhanced yields observed in the Kobayashi ring expansion reaction of the halogenated subphthalocyanines studied. However, the increased solubility of the halogenated boron

subphthalocyanines and the more important polarity of the reaction solvent most certainly plays a role as well.

Intuitively, it seems logical that the reactivity of the subphthalocyanines towards a ring expansion reaction would be due to the steric strain present in the distorted molecular structure, with the cone-shaped geometry of boron subphthalocyanines leading to ineffective p-orbital overlap and loss of aromatic stabilization. However, molecular orbital calculations comparing the bond energies of unsubstituted boron subphthalocyanine with unsubstituted magnesium phthalocyanine indicated little deviation in the calculated C-N bond energies between the two macrocycles (Kobayashi, 1999; Kobayashi et al., 1999). This implies that the distortion is not a major cause of the ring expansion reactivity. Similar calculations suggest that the lack of electron-accepting orbitals in boron result in a lack of donor-acceptor stabilization in the B-N bonds, which in part explains the decreased stability of boron subphthalocyanines. More importantly, these calculations indicate that the loss of the axial ligand alters the shape of the main skeleton of subphthalocyanines from a shuttlecock to a more planar form, with a corresponding stabilization of approximately 100 kJ/mol. Furthermore, the resulting cationic charge on the central boron atom can be effectively delocalized over the entire macrocycle. In view of these calculations, it appears likely that the initial step in the Kobayashi ring expansion reaction consist of a dehalogenation reaction, causing the loss of the axial ligand.

In light of these molecular orbital calculations, the Kobayashi ring expansion reactions of (dodecafluorosubphthalocyaninato)boron(III) bromide, (2,9,16(17)-triiodo-subphthalocyaninato)boron(III) bromide, (1,8,15(18)-

triiodosubphthalocyaninato)boron(III) bromide and (2,3,9,10,16,17-hexaiodosubphthalocyaninato)boron(III) bromide may have proceeded with the desired selectivity and with important yields at least in part due to the use of boron subphthalocyanines bearing a bromine axial ligand in lieu of a chlorine axial ligand. The bond energy of a B-Br bond is 396 kJ/mol compared to 536 kJ/mol for a B-Cl bond (Lide, 1992). Therefore, if the loss of the axial ligand is the initial step in the ring expansion reaction, it would be expected that subphthalocyanines bearing bromine axial ligands would react under milder reaction conditions, perhaps allowing the reaction to proceed selectively. Notably, a number of literature examples detailing selective Kobayashi ring expansion reactions employ subphthalocyanines with bromine axial ligands (Kobayashi et al., 1990; Kudrevich et al., 1996; Kudrevich et al., 1997; Kobayashi et al., 1999; van Lier et al., 1999). It is also a possibility that the electron-withdrawing nature of the halogen functional groups on the boron subphthalocyanines examined and the lower overall reaction temperature play a role in the selectivity.

Metal insertion into the resulting metal-free phthalocyanines was readily accomplished by heating a suspension of the halogenated metal-free phthalocyanines in DMF to 30-60°C in the presence of zinc bromide. FAB-MS again indicate that the Kobayashi ring expansion reaction proceed with exclusive production of the desired 3:1 asymmetrically substituted phthalocyanine. For example, Figure 9.8 depicts the FAB-MS spectra of ((2,9,16(17)-triiodo)tribenzo[b,g,l]napthalo[2,3-q]-porphyrazine)zinc (Compound **4c** in Figure 8.2 after reaction with Zn^{+2}), with the M^{+} ion present for the desired 3:1 asymmetrically substituted zinc phthalocyanine and no ions observed for the other possible substitution patterns (774, 852, 928 and 1080 for the substituted zinc

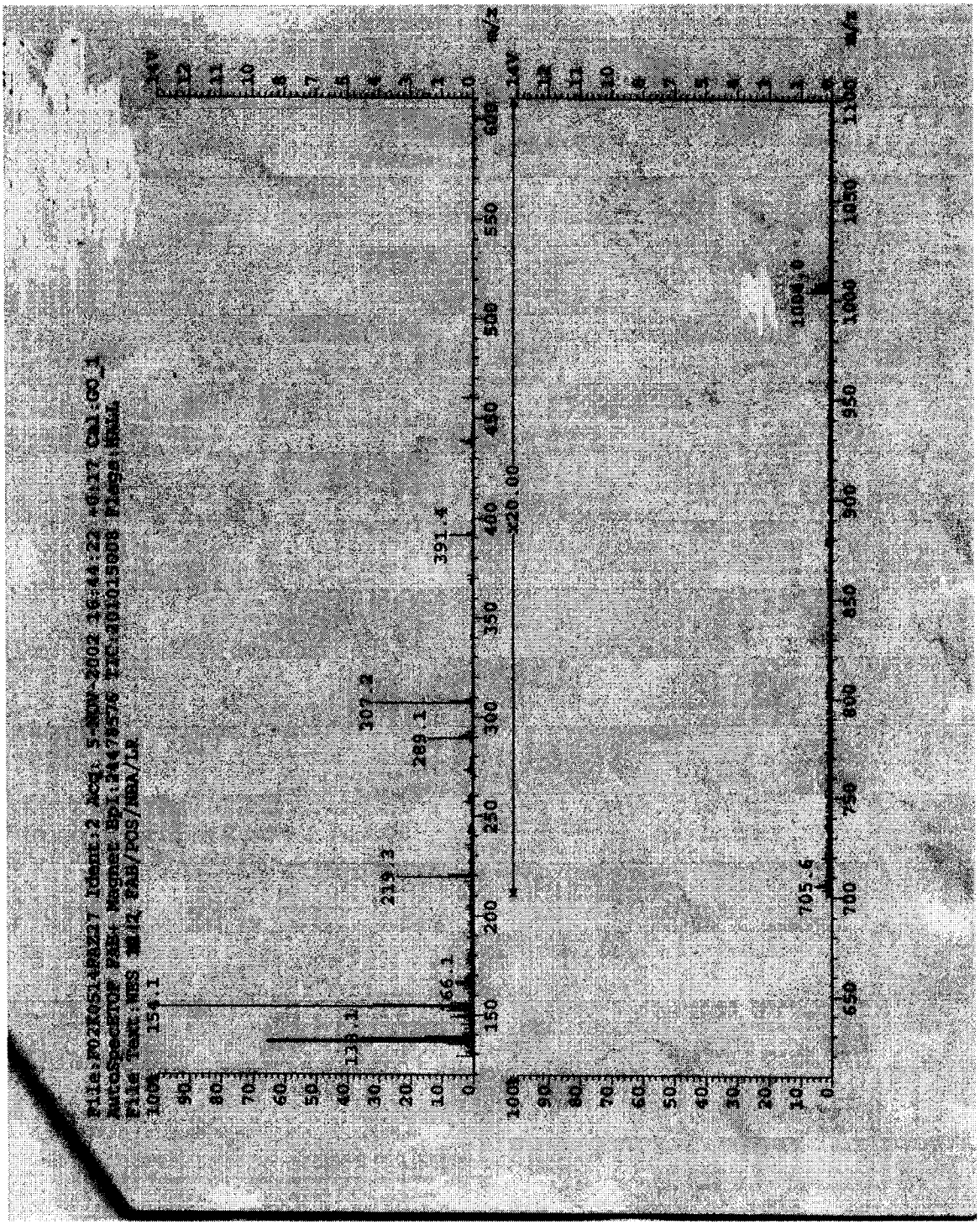


Figure 9.8. FAB-MS spectra of ((2,9,16(17)-triiodo)tribenzo[b,g,l]napthalo[2,3-q]-porphyrazine)zinc (Compound **5c** in Figure 8.2 after reaction with Zn^{+2})

naphthlaocyanine, monoiodo-trinaphthalo derivative, diiodo-dinaphthalo derivative and the tetraiodinated zinc phthalocyanine respectively). Yields were nearly quantitative for all phthalocyanines. The metal insertion reaction for the dodecafluorinated phthalocyanines was also attempted using solvents such as NMP due to the known reaction of tetrafluorophthalonitrile with DMF (Birchall et al., 1970). Unfortunately, metal insertion reactions using NMP were sluggish and gave unacceptable yields and incomplete reactions. Thus, in order to avoid any possible reaction with the DMF solvent, the reaction temperatures were maintained between 30-50°C. In the meanwhile, contrary to previous reports (Weitemeyer et al., 1995), the asymmetrically substituted iodinated zinc phthalocyanines could be obtained selectively by a templated Kobayashi ring expansion reaction wherein zinc ions are added to the reaction mixture to act as a template for phthalocyanine ring closure. In these reactions, yields were significantly reduced while isolation and purification of the desired iodinated zinc phthalocyanines was complicated by important increases in the solubility of the macrocycle in DMSO, the reaction solvent.

The zinc dodecafluorinated phthalocyanines (Figure 7.1) were significantly more soluble, exhibiting important solubilities in common organic solvents such as THF. This allowed purification by column chromatography on silica gel using solvent mixtures of THF or ethyl acetate in hexanes or toluene. Electronic spectra of these phthalocyanine derivatives were characteristic by a sharp Q band absorption between 663 nm and 683 nm (Table 7.1). The extended conjugation of the tribenzo[b,g,l]naphthalo[2,3-q]porphyrazine derivative **5i** (Figure 7.1) caused a red-shifted and split Q band (710 nm and 679 nm) (Figure 7.2). Similar splitting of the Q band was observed for

phthalocyanines **5c** and **5g** while t-butyl-substituted phthalocyanine **5b** exhibited a Q band with a prominent shoulder at longer wavelength (Figure 7.1). Those phthalocyanines that did not have a distinct shoulder on the Q band showed evidence of slight broadening. These results clearly demonstrate the decreased symmetry in these novel asymmetrically substituted zinc phthalocyanines with this decreased symmetry causing a loss in the degeneracy of the LUMO orbital (Figure 1.8). This effect was not nearly as prominent for the asymmetrically substituted iodinated zinc phthalocyanines, suggesting that the strong electron-withdrawing properties of the fluorine substituents promote this effect.

Palladium-catalyzed reactions have been extensively used in the preparation of novel porphyrins and phthalocyanines (see Chapter 6). In particular, palladium-catalyzed cross-coupling reactions have been employed to add novel substituents and functionalities to iodinated subphthalocyanines (del Rey et al., 1997) and phthalocyanine (Maya et al., 1998), including asymmetrically substituted iodinated phthalocyanines that had been prepared by a mixed condensation (Ali et al., 1997; Tian et al., 2000; Cauchon et al., 2005). Reactions such as the Heck, Stille and Suzuki reactions with asymmetrically substituted iodinated phthalocyanines allow for the preparation of novel monofunctionalized phthalocyanines (Ali et al., 1997). Amongst novel functionalities added to these phthalocyanines were phenyl, pyridyl, purinyl, phosphonates, carboxylic esters and estrogenic groups. In the meanwhile, novel water-soluble asymmetrically substituted trisulphonated zinc phthalocyanines with increased amphiphilicity and functionalized with novel substituents were synthesized by palladium-catalyzed cross coupling reactions such as the Heck reaction and Buchwald amination (Figure 1.17)

(Tian et al., 2000). In these reactions, the sulphonate groups of the starting phthalonitrile and the resulting trisulphonated zinc phthalocyanine were protected as an indole (Li et al., 1999), permitting the solubilization of the monoiodinated trisulphonated zinc phthalocyanine in most polar organic solvents. This allowed for easy purification by silica gel column chromatography and permitted the palladium-catalyzed reactions to be accomplished in organic solvent. Deprotection of the indolylsulphonate was readily accomplished by hydrolysis using lithium methoxide in methanol and THF to give novel asymmetrically substituted trisulphonated zinc phthalocyanines. A series of these photosensitizers have been examined in terms of their photodynamic efficiency and it has been found that trisulphonated zinc phthalocyanines bearing hexynyl and nonynyl substituents (Figure 1.17, n = 3 or 6) exhibited high cellular uptake with important localization at the mitochondrial membranes, with coinciding effective photocytotoxicity toward EMT-6 tumour cells (Cauchon et al., 2005).

The asymmetrically substituted iodinated zinc phthalocyanines prepared by the Kobayashi ring expansion reaction of iodinated boron subphthalocyanines are ideal building blocks for the preparation of novel 3:1 asymmetrically substituted phthalocyanines substituted with novel functionalities. As disclosed in the introduction, amphiphilic water-soluble phthalocyanines exhibit enhanced photodynamic activities (Paquette et al., 1988; Margaron et al., 1996; Allen et al., 2002; Cauchon et al., 2005). In light of this, the 3:1 asymmetrically substituted iodinated zinc phthalocyanines were used as building blocks for the preparation of novel asymmetrically substituted amphiphilic water-soluble photosensitizers. The iodinated zinc phthalocyanine readily reacted with terminal alkynes such as 5-hexynoic acid and 10-undecynoic acid in the presence of

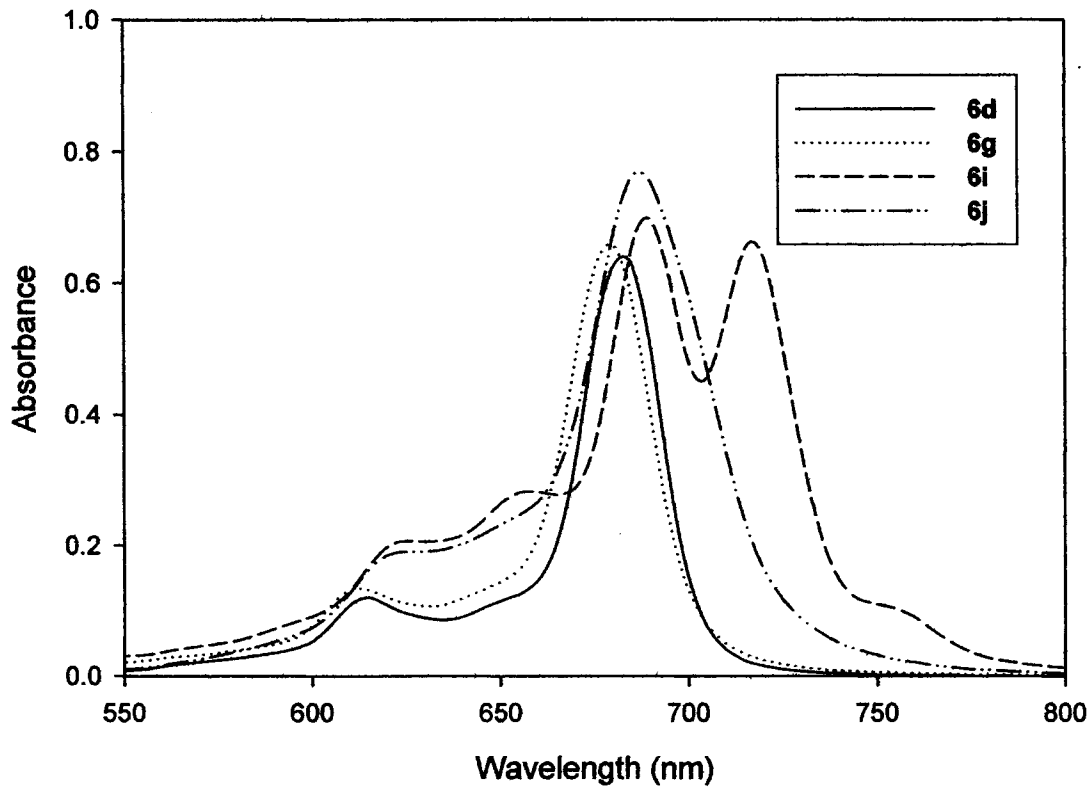


Figure 9.9. UV-visible spectra of asymmetrically substituted anionic phthalocyanines in THF (see Figure 8.3 for structures of **6d, 6g, 6i** and **6j**)

dichlorobis(triphenylphosphine)palladium(II) and copper iodide in DMF/Et_3N to give novel asymmetrically substituted anionic phthalocyanines (Figure 8.3). The sodium salts of these phthalocyanines were readily soluble in water at physiological pH. Unfortunately, these photosensitizers are highly aggregated in aqueous solution as exemplified by their UV-visible spectra with a broad Q band centered between 620 and 640 nm. When dissolved in organic solvent such as DMF, the corresponding acids display a typical sharp, strong Q band in the region around 680 nm (Figure 9.9). The

tribenzomononaphthalo derivatives exhibit a split Q band as expected due to the decreased symmetry in these molecules. The isolation of the functionality responsible for water solubility from the phthalocyanine macrocycle by long alkynyl chains may provide novel properties to these photosensitizer. Novel water-soluble tetra[1-(O-ethylphosphatobutyl)]zinc phthalocyanine where the phosphonate group, responsible for water solubility, is isolated from the phthalocyanine macrocycle by a butylene aliphatic chain, has been demonstrate to be an effective photosensitizer *in vivo* (Boyle et al., 1995). High biological activity has also been observed for zinc phthalocyanines substituted with short aliphatic chains terminated with hydroxyl groups (Boyle et al., 1993). Furthermore, intracellular cleavage of the alkynyl group by cellular enzymes would result in a loss in solubility, effectively trapping the photosensitizer within the cell. A comparable *in vivo* hydrolysis of the butoxy-phosphorus bonds in tetra[1-(O-ethylphosphatobutyl)]zinc phthalocyanine has been hypothesized as playing a role in the photodynamic activity of this photosensitizer (Boyle et al., 1995).

In spite of being far less extensively studied, cationic porphyrins and phthalocyanines exhibit a number of notable properties that make them attractive photosensitizers for photodynamic therapy. Cationic porphyrins and phthalocyanines are water-soluble, exhibit high singlet oxygen quantum yields, are less aggregated in solution and possess good tumour-localizing properties (Verlhac et al., 1984; Wöhrle et al., 1990; Villanueva, 1993; Villanueva et al., 1993; Fernandez et al., 1997; Spiller et al., 1998; De Filippis et al., 2000). Furthermore, cationic photosensitizers such as meso-tetra(4N-methyllpyridyl)porphine exhibit preferential tumor uptake compared to surrounding normal, healthy tissues (Villanueva et al., 1993). In addition, their cationic charge

permits these photosensitizers to target important subcellular sites including cellular membranes (Villanueva et al., 1994), the mitochondria (Leznoff et al., 1989; Moan et al., 1989; Dummin et al., 1997), lysosomes (Wood et al., 1997), the nucleus (Villanueva et al., 1994) and DNA (Gantchev et al., 1993; Villanueva et al., 1993). It has however been determined that meso-tetra(4N-methyl-pyridyl)porphine induces cell death by both direct tumour cell kill and vascular shutdown (Villanueva et al., 1994). Increased photodynamic efficiency of cationic photosensitizers may also be due to tighter binding and higher capability for intercalation with biological targets (Wöhrle et al., 1990). Interestingly, comparative studies of three different charged (anionic, cationic and neutral) structurally similar zinc phthalocyanines have shown that the cationic phthalocyanine is the most effective photosensitizer (Wood et al., 1997; Ball et al., 1998; Ball et al., 1999; Bremer et al., 1999). Furthermore, the cationic phthalocyanine was more effective than established photosensitizers mTHPC and polyhaematoporphyrin (Ball et al., 1998). This improved photodynamic activity of the cationic phthalocyanine may be due to increased cellular uptake (Ball et al., 1999), important relocalization to more important photosensitive subcellular sites upon irradiation (Wood et al., 1997; Ball et al., 1999), different plasma protein binding characterisitics (Ball et al., 1999), improved photophysical properties and decreased aggregation (Ball et al., 1998) and increased indirect vascular effects (Bremner et al., 1999). However, the cationic phthalocyanine has an average of two pyridinium charged groups per phthalocyanine (Wood et al., 1997). Thus, as well as being a function of charge, the improved photodynamic activity of this photosensitizer may also be the result of the number and distribution of the charge with the molecule. Cationic phthalocyanines with the two

pyridinium charged groups on adjacent benzo groups would be similar to phthalocyanines substituted on adjacent benzo groups with sulphonate groups, with the amphiphilicity of these molecules leading to improved photodynamic activity (see introduction).

It is well-established that certain bacterial cell walls have a high degree of negative charge and therefore, cationic photosensitizers may bind to and penetrate these cell wall barriers more readily (Chen et al., 2001). This makes cationic porphyrins and phthalocyanines valuable photosensitizers in the photodynamic treatment of microbial infections. In fact, photodynamic therapy using cationic porphyrins and phthalocyanines is effective in treating infections caused by both Gram-positive and Gram-negative bacteria (Merchat et al., 1996; Minnock et al., 1996; Marti et al., 2000; Minnock et al, 2000; Roncucci et al., 2001; Soncin et al., 2002; Roncucci et al., 2003; Dupouy et al., 2004) including biofilms (Wood et al., 1999). Importantly, careful selection of photodynamic therapy protocols allows for selective inactivation of the microbe while leaving potential host tissues, including fibroblast and keratinocytes, unaffected (Soncin et al., 2002).

Studies have indicated that while anionic phthalocyanines can efficiently photoinactivate Gram-positive bacteria, Gram-negative bacteria became photosensitive only after modification of the permeability of their outer membrane (Merchat et al., 1996). Interestingly, while cationic tetra(4N-methylpyridyl)porphine tetraiodide and tetra(4N,N,N-trimethylanilinium)porphine efficiently photoinactivated Gram-negative bacteria such as Vibrio anguillarum and Escherichia coli, structurally similar anionic tetra(4-sulphonatophenyl)porphine exhibit no appreciable photosensitizing activity. This

is despite similar subcellular distribution patterns and similar photodynamic activity against Gram-positive bacteria Entorecoccus seriolicida. In a comparative studies of three different charged (anionic, cationic and neutral) structurally similar zinc phthalocyanines, it was found that the cationic phthalocyanine effectively photoinactivated both Gram-negative (Escherichia coli and Pseudomonas aeruginosa) and Gram-positive bacterium (Enterococcus seriolicida) under conditions where neither the neutral or anionic phthalocyanine induced photoinactivation (Minnock et al., 1996). Although uptake studies indicate that the lack of activity of the anionic phthalocyanine was due to the fact that it has very low affinity for these bacteria, the neutral and cationic phthalocyanines had similar overall cellular uptakes. Thus, localization and subcellular distribution must be a critical factor in photoinactivation of bacterium. Interestingly, incubation of E. coli cells with the cationic phthalocyanine in the dark causes alterations in the outer membrane permeability barrier of the cells, rendering the bacteria much more sensitive to hydrophobic compounds (Minnock et al., 2000). Furthermore, the presence of Mg^{2+} in the medium prior to incubation prevents these alterations and prevents the photoinactivation of the bacteria. This suggest that the cationic phthalocyanine gains access across the outer membrane of the bacteria via a self-promoting uptake pathway as has been suggested for the uptake of other cationic compounds. Along these lines, a synergistic effect between cationic phthalocyanine photosensitizers and metal chelating agents has been observed, with the corresponding pharmaceutical compositions have enhanced photoinactivation properties against Gram negative bacteria (Roncucci et al., 2003).

Cationic porphyrins and phthalocyanines have also proven useful in the photodynamic sterilization of red blood cells (Ben-Hur et al., 1995; Roncucci et al., 2001; Roncucci et al., 2003; Trannoy et al., 2004). With red blood cells, it was found that cationically charged Zn(II) tetramethylpyridinoporphyrazinium salt and the corresponding neutrally charged Zn(II) tetrapyridinoporphyrazine both produced similar photohemolysis (Dupouy et al., 2004). However, the cationic photosensitizer produced significantly higher photoinactivation of the bacteria E. coli, resulting in the potential to use lower doses and achieving an enhanced therapeutic effect. Other cationic porphyrins give lower hemolysis under conditions that result in 5 log-kill of extracellular vesicular stomatitis virus (Trannoy et al., 2004). Certain cationic phthalocyanines also render the HIV virus non-infectious, with these compounds having potential as microbiocides that might provide protection against sexually transmitted HIV (Vzorov et al., 2003).

In view of the known and established photodynamic potential of cationic photosensitizers, the asymmetrically substituted iodinated zinc phthalocyanines were used as templates for the preparation of novel asymmetrically substituted cationic photosensitizers. These templates readily reacted with 2-ethynylpyridine and 3-ethynylpyridine in palladium-catalyzed cross-coupling reactions (Figure 8.4). The FAB mass spectra data for the resulting phthalocyanines was consistent with the proposed structure. UV-visible spectra of the macrocycles in DMF were characterized by sharp Q bands around 680-700 nm. As would have been expected, the tribenzomononaphthalo derivatives exhibited a split Q band due to the lack of symmetry in these molecules.

These pyridyl substituted phthalocyanines readily reacted with methyl iodide or dimethyl sulfate in DMF at 70°C with methylation of the pyridyl nitrogens, thus giving

the corresponding asymmetrically substituted cationic zinc phthalocyanines (Figure 8.4). While these compounds failed to give well resolved NMR spectra, complete methylation of the pyridyl nitrogen atoms was indicated by HPLC analysis of representative examples 8a, 8d, 8k and 8n, all of which exhibit a single peak. Furthermore, slab gell electrophoresis of tricationic examples 8a and 8d indicated that these compounds moved roughly the same distance towards the cathode as $AlPcS_3$ moved towards the anode. This demonstrates that these compounds have the identical but opposite charge as $AlPcS_3$ and are thus completely alkylated. It has been demonstrated that asymmetrically substituted cationic photosensitizers are more efficient in destroying melanoma cells than the corresponding symmetrically substituted photosensitizers (Haylett et al., 1995). In addition, unlike anionic phthalocyanines in which two sulphonate groups on adjacent benzo rings lead to the most important photocytotoxic properties, most like a result of optimal cellular uptake, phthalocyanines substituted on only one benzo ring with cationic groups or protonable groups are particularly photodynamic active with impressive activities against fungi, Gram-positive and Gram-negative bacteria (Roncucci et al., 2001). It is suggested by extension that such phthalocyanines would also be useful in the photodynamic treatment of tumours, pre-cancerous and proliferative pathologies and for blood and blood derivative sterilization. In light of these observations, asymmetrically substituted cationic phthalocyanines **8a-8n** (Figure 8.4) may have interesting photodynamic potentials. In aqueous solution, these cationic phthalocyanines are highly aggregated, exhibiting a broad Q band centered between 620-640 nm. However, in DMF, the macrocycle was monomerized, exhibiting a significant splitting of the Q band accompanied by an important red shift in the wavelength (Figure 8.5). Such shifts to

longer wavelength are advantageous as light penetration of tissue increases with increasing wavelength, thus increasing the therapeutic depth. As in the case of the fluorinated phthalocyanines, splitting of the Q band is indicative of decreased symmetry in the molecule with the cationic pyridine groups altering the electronic character of the molecule sufficiently to degenerate the symmetry of the aromatic electronic system. Monomerization of cationic phthalocyanines has been achieved using AOT reversed micelles (Chen et al., 2001) and in CRM emulsions (Peeva et al., 2001). Furthermore, in biological environments, the cationic charge of these photosensitizers should lead to tighter binding with biologically relevant molecules and serum proteins, effectively monomerizing the photosensitizer *in vivo*. Comparative studies involving a series of zinc complexes of benzonaphthaloporphyrazines substituted with positively charged methylpyridinium groups on the benzo rings (Figure 1.18) indicate that the 3:1 asymmetrically substituted mononaphthalotribenzo derivative having three methylpyridinium groups was the most photodynamically active (Michelsen et al., 1996; Peeva et al., 2001). The structurally similarities between this photosensitizer and phthalocyanines **8e**, **8f**, **8j** and **8l** (Figured 8.4) make these compounds of particular interest. In addition, the amphiphilicity of this mononaphthalotribenzo derivative suggest the asymmetrically substituted cationic phthalocyanines prepared in this case should have important properties for photodynamic therapy. The overall hydrophilic/lipophilic balance of these photosensitizers can also be fine-tuned by changing the nature and length of the alkyl chains on the pyridyl nitrogens. For instance, for zinc phthalocyanines tetrasubstituted with 3-pyridyloxy groups or (2-dimethylamino)ethoxy groups, alkylation of the nitrogen atoms by longer aliphatic chains (1-hexyl or 1-dodecyl) lead to

photosensitizers with increased photodynamic activity as compared to alkylation with only methyl groups (Wöhrle et al., 1990). This is most likely the result of increased cellular uptake due to the increasing lipophilicity of the compounds. Similar results showing increased photodynamic activity with increasing lipophilicity of the alkyl groups on the nitrogen atoms have been reported for other cationic phthalocyanines (Dummin et al., 1997).

Although they have been shown to have important photodynamic activities, the utility of most lipophilic phthalocyanines for photodynamic therapy is limited by the extreme insolubility of these compounds in most common solvents. Fluorine is very similar to hydrogen in terms of atomic radius and as such, compounds containing fluorine often mimic the corresponding compounds containing hydrogen in biological environments. Exchange of hydrogen atoms with fluorine atoms in molecules also significantly increases lipid solubility and may in fact lead to enhanced interactions with biological membranes and biologically relevant molecules. The increased atomic weight of the fluorine atoms will also improve the photophysical properties of photosensitizers due to the heavy atom effect. The heavy atom effect dictates that exchange of lighter atoms (such as hydrogen) with heavier atoms (such as fluorine) on a chromophore increases spin-orbital coupling. This in turn facilitates intersystem crossing from the first excited singlet state to the first excited triplet state (Figure 1.12) by allowing otherwise forbidden changes in the spin state of the molecule ($S_1 \rightarrow T_1$). In terms of photodynamic therapy, the exchange of hydrogen for fluorine on the periphery of phthalocyanines improves the photodynamic potential of the compound by increasing triplet state yields

and ultimately increasing singlet oxygen quantum yields. Finally, the presence of fluorine in the phthalocyanines offers the potential for F-MRI imaging.

The Kobayashi ring expansion reaction of (dodecafluorosubphthalocyaninato)-boron(III) bromide allowed for the synthesis of novel asymmetrically substituted dodecafluorinated phthalocyanines (Figure 7.1). This allows an extension of studies accomplished in our lab on the photodynamic activity of fluorinated phthalocyanines (Allémann et al., 1995; Boyle et al., 1996; Allémann et al., 1995; Allémann et al., 1997; Bench et al., 2002). Initial investigation of the photodynamic potential of these photosensitizers was achieved by the L-tryptophan photooxidation assay using Cremophor™ EL emulsions. In light of the heavy atom effect, it is not unexpected that these dodecafluorinated phthalocyanines exhibited increased HPPI yields compared to $AlPcS_4$ (Table 7.1), with the singlet oxygen quantum yield of $AlPcS_4$ in phosphate buffer (1% Triton X) known to be 0.43 (Redmond et al., 1999). Substitution with iodine on the fourth benzo ring led to further increases in the HPPI yields. In previously studies involving tetrahalogenated zinc phthalocyanines, singlet oxygen production was only slightly increased by the nature of the halogen atom (Zhang et al., 1993). This is despite the expected increases in triplet state formation with increasing atomic mass of the halogen (Cl < Br < I). It was also noted that the tendency of these phthalocyanines to aggregate was in the order Br > I > Cl > H. Therefore, it seems that any increases in the photodynamic potential that is caused the heavy atom effect and the resultant increases in triplet state formation may be negated to some extent by increased aggregation of the phthalocyanine in solution. Upon illumination, aggregated photosensitizers dissipate their energy through internal conversion rather than via the intersystem crossing to the

first excited triplet state with subsequent formation of singlet oxygen (see Chapter 2). The extent of aggregation is dictated in large part by the ring substituents and the axial ligands on the central metal ion. The extent of aggregation is an important factor in determining the overall photodynamic efficiency of a given photosensitizer (Wagner et al., 1987). In the current study, aggregation was avoided to a certain extent by formulation of the dodecafluorinated zinc phthalocyanines as CRM emulsion, with the emulsion somewhat preventing interactions between individual chromophores. This is evident from the slightly improved HPPI yield obtained using $AlPcS_4$ when formulated as a 0.5% CRM emulsion in comparison to PBS solution. However, despite formulation as CRM micelles, aggregation still led to significantly lower HPPI yields for the dodecafluorinated phthalocyanine substituted with two long dodecyl alkyl chains as compared to the other fluorinated phthalocyanines. Amphiphilic tetrasulphonated phthalocyanines substituted on one of the sulphonate groups by long chain alkyl groups via a sulfonamide bond (Figure 1.16) exhibit decreased HPPI yields in the L-tryptophan photooxidation assay, even when formulated as CRM emulsions, due to important aggregation of the macrocycle (Allen et al., 2002). The decrease in HPPI yields was directly related with the length of the alkyl chain with longer alkyl chains increasing the lipophilicity of the molecule and enhancing aggregation. While the zinc dodecafluorinated phthalocyanines are lipophilic, it is suggested that the longer alkyl chains of (1,2,3,4,8,9,10,11,15,16,17,18-dodecafluoro-23,24-didodecylphthalocyaninato) zinc promotes aggregation in the CRM emulsions, resulting in the decreased HPPI yields observed under the experimental conditions employed.

The results of the L-tryptophan photooxidation assay clearly indicate that the asymmetrically substituted dodecafluorinated zinc phthalocyanines are capable of generating singlet oxygen in an aqueous environment upon illumination. This, however, is not necessarily indicative of the photodynamic potential of these photosensitizers. Many factors play key roles in the determination of the utility of a given photosensitizer for photodynamic therapy. These include cellular uptake, subcellular localization, selective target tissue accumulation and the degree of *in vivo* aggregation (Bonnett, 1995; Oschner, 1997; Dougherty et al., 1998) (also see Chapters 2 and 3). Our group has extensively studied water-soluble phthalocyanines and has repeated found that their photodynamic efficiency is related to the amphiphilicity of the compound (Paquette et al., 1991a; Allen et al., 1995; Margaron et al., 1996b; Kudrevich et al., 1997; Edrei et al., 1998; Allen et al., 2002; Cauchon et al., 2005). It is believed that amphiphilicity upgrades cellular uptake due to better membrane penetrating properties of the lipophilic portion of the molecule. Overall, however, cellular uptake of phthalocyanines has been shown to increase with increasing lipophilicity of the molecule, a result of better affinity of lipophilic photosensitizers for cell membranes (Brasseur et al., 1988; Berg et al., 1989; Paquette et al., 1991a; Margaron et al., 1996b; Dougherty et al., 1998; Decreau et al., 2001). Furthermore, serum proteins are predominantly responsible for the transportation of photosensitizers throughout the body, with lipophilic photosensitizers partitioning to LDLs in the blood stream (Reddi et al., 1990; Jori et al., 1993; Versluis et al., 1994; Reddi, 1997) (also see Chapter 4). Many tumour types have increased LDL receptor expression as compared to normal healthy cells (Ho et al., 1978; Gal et al., 1981; Lombardi et al., 1989; Gueddari et al., 1993). Therefore, the interaction of lipophilic

phthalocyanines with LDL particles may lead to improved targeting and enhanced tumour-to-healthy tissue ratios (Urizzi et al., 2001; Allen et al., 2002b). Thus, despite the need for an appropriate vehicle in order to solubilize water-insoluble lipophilic photosensitizers in biologically relevant solvents, lipophilic photosensitizers may provide means for augmenting the efficiency of photodynamic therapy. In addition, selection of appropriate vehicles may further improve the properties of the pharmaceutical preparations used for photodynamic therapy (Allen et al., 2002b) (also see Chapter 4).

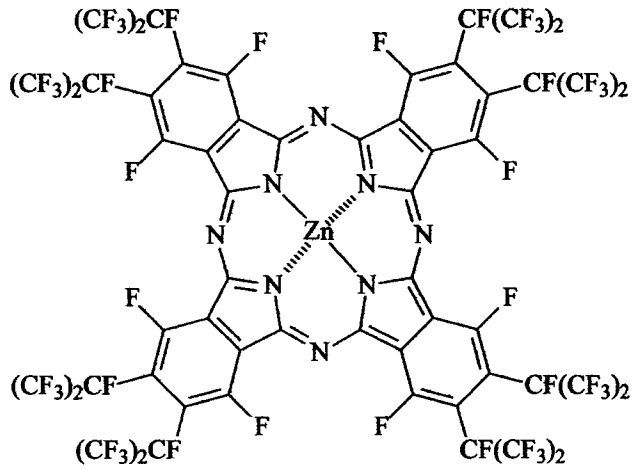


Figure 9.7. $ZnPcF_{64}$, a novel three-dimensional non-planar zinc perfluorinated phthalocyanine comprising 64 fluorine atoms

In light of the potential of lipophilic photosensitizer and in order to take advantage of the heavy atom effect and the improvement in solubility, hexadecafluorinated zinc phthalocyanine ($ZnPcF_{16}$) has been prepared (Haszeldine, 1966; Birchall et al., 1970; Boyle et al., 1996) and its effectiveness as a photosensitizer for photodynamic therapy

has been evaluated using various delivery vehicles (Allémann et al., 1995; Boyle et al., 1996; Allémann et al., 1995; Allémann et al., 1997; Decreau et al., 2001). Enhanced photodynamic activities have also been observed for a number of water-soluble fluorinated zinc phthalocyanines (Fukushima et al., 1998; Oda et al., 2000; Tabata et al., 2000). In addition, a novel three-dimensional non-planar zinc perfluorinated phthalocyanine comprising 64 fluorine atoms has been synthesized (Figure 9.7) (Bench et al., 2002; Gorun et al., 2003) from a novel non-planar perfluorinated phthalonitrile (Gorun et al., 1998). As a CRM emulsion, this perfluorinated phthalocyanine has improved photodynamic activity as compared to $ZnPcF_{16}$, at least partially due to the three-dimensional non-planar structure and the resulting lack of aggregation. A number of other fluorinated phthalocyanines are known and may have important potential for photodynamic therapy, particular in light of their absorption at near IR wavelengths (Birchall et al., 1970; Ito et al., 1996; Sato et al., 1996; Aoki et al., 1997; Kondratenko et al., 1997; Tian et al., 1997;; Aoki et al., 1998; Kaieda et al., 1998; Okumura et al., 1998; Kondratenko et al., 1999; Narizuka et al., 1999; Schlettwein et al., 2000; Gao et al., 2001). Of particular interest are polysubstituted zinc phthalocyanines prepared by the nucleophilic substitution of zinc hexadecafluorophthlaocyanine with a variety of oxygen, nitrogen, carbon and sulfur nucleophiles (Leznoff et al., 2004). The resulting narrowly defined mixture of polysubstituted zinc phthalocyanines are often completely inaccessible by classical phthalonitrile condensation reactions and may be useful as photosensitizers for photodynamic therapy.

Despite observations that $ZnPcF_{16}$ and $ZnPcCl_{16}$ are not phototoxic against M6 melanoma cells under standard experimental conditions (1 h incubation, 20 minutes

irradiation, 12 J/cm^2 light dose) (Decreau et al., 2001), CRM emulsions of $ZnPcF_{16}$ are effective in photoinactivating EMT-6 tumour cells (Allémann et al., 1995; Allémann et al., 1996; Boyle et al., 1996; Allémann et al., 1997). Biodistribution studies indicate that CRM emulsions of $ZnPcF_{16}$ exhibit a degree of selective tumour uptake while having improved pharmacokinetics. The action mechanism of photo-induced tumor necrosis mimics that of Photofrin®, with tumour regression primarily due to vascular stasis (Boyle et al., 1996). In light of the increased photodynamic activity of amphiphilic photosensitizers, zinc dodecafluoro-4-sulfophthalocyanine ($ZnPcF_{12}S_1$) was prepared by a modified Meerwein reaction (Kudrevich et al., 1994). This amphiphilic photosensitizer was shown to be almost 50 times more photoactive than $ZnPcF_{16}$ against EMT-6 tumour cells *in vitro* while having improved pharmacokinetics in mice with lower liver and spleen retention and higher tumour to non-target tissue ratios (Allémann et al., 1997). Unfortunately, *in vivo* photodynamic therapy of EMT-6 tumours on BALB/c mice with red light either 24 or 48 hours post-injection of 1 μmol/kg of $ZnPcF_{12}S_1$ caused mortality. This is likely a result of an acute phototoxic shock attributed to extensive cellular damage followed by a fatal biochemical response. Although similar conditions may result in tumour control in larger animals, this mortality is indicative of a small therapeutic window. Results suggest that a small therapeutic window may also limit the utility of $ZnPcF_{16}$ (Allémann et al., 1995; Allémann et al., 1996; Boyle et al., 1996; Allémann et al., 1997).

The Kobayashi ring expansion reaction of (dodecafluorophthalocyaninato)-boron(IIII) bromide provides means to prepare asymmetrically substituted dodecafluorinated phthalocyanines that may exhibit similar increases in photoactive as

$ZnPcF_{12}S_1$ while avoiding PDT-induced mortality and increasing the therapeutic window. Furthermore, while it has been clearly established that asymmetrically substituted amphiphilic photosensitizers have improved properties for photodynamic therapy, it is unclear whether asymmetry in lipophilic photosensitizers may also increase photodynamic potential. As representative examples, (1,2,3,4,8,9,10,11,15,16,17,18-dodecafluorophthalocyaninato)zinc, (1,2,3,4,8,9,10,11,15,16,17,18-dodecafluoro-23-t-butylphthalocyaninato)zinc and (1,2,3,4,8,9,10,11,15,16,17,18-dodecafluoro-23,24-didodecylphthalocyaninato)zinc (compounds **5a, 5b** and **5g** in Figure 7.1) were formulated as Cremophor™ EL emulsions and their *in vitro* photodynamic activity was evaluated against EMT-6 cells (Figure 7.3). These preliminary studies demonstrate that CRM emulsions of these dodecafluorinated phthalocyanines are more photodynamically active than symmetrically substituted $ZnPcF_{16}$ after either 1 or 24 hour incubations. Intriguingly, despite lower HPPI yields in the L-tryptophan photooxidation assay, the didodecyl derivative was the most photodynamically active phthalocyanine examined. While it was proposed that the lack of appreciable activity of $ZnPcF_{16}$ and $ZnPcCl_{16}$ against M6 melanoma cells was due to aggregation of the photosensitizer, the current study suggest that aggregation become less important in the more complicated cellular environment, with the multitude of cellular components possibly interacting with the planar phthalocyanine and thus reducing the extent of aggregation *in vitro*. It is also known that subcellular localization to more sensitive organelles is a vital factor in the overall photodynamic efficiency of a given photosensitizer (Dougherty et al., 1998). The presence of two long dodecyl alkyl chains in (1,2,3,4,8,9,10,11,15,16,17,18-dodecafluoro-23,24-didodecylphthalocyaninato)zinc (**5g** in Figure 7.1) may lead to

improved cellular uptake and trafficking to more photosensitive cellular organelles. In a study of the efficiency of a series of asymmetrically substituted sulphonated phthalocyanines for blood product sterilization, it was suggested that the addition of a t-butyl group may act as an anchor for the phthalocyanine to attach to the cellular membrane, thus leading to improved photodynamic activity (Allen et al., 1995). As previously mentioned, amphiphilic tetrasulphonated phthalocyanines substituted on one of the sulphonate groups by long chain alkyl groups via a sulfonamide bond (Figure 1.16) exhibit decreased HPPI yields in the L-tryptophan photooxidation assay with the decrease HPPI yield directed related to the length of the alkyl chain (Allen et al., 2002). However, biological activity decreased in the order $AlPcS_4(C16) > AlPcS_4(C12) > AlPcS_{2adj} > AlPcS_4(C8) > AlPcS_4(C4)$. Biological activity correlated with the cell uptake of the given phthalocyanine, suggesting that the longer alkyl chain promoted cell uptake and that aggregation in the cellular environment was negated. On the other hand, trisulphonated zinc phthalocyanines bearing long alkynyl chains gave contrary results (Figure 1.17) (Cauchon et al., 2005). While hexynyl- and nonynyl-substituted derivatives exhibited high cellular uptake with important localization at the mitochondrial membranes which coincided with effective photocytotoxicity toward EMT-6 tumour cells, further lengthening of the alkynyl chains to dodecynyl or hexadecynyl did not further increase photodynamic activity. It was suggested that the longer alkynyl chains promoted aggregation, with this causing reduced cell uptake. The effect of loading LDL with $AlPcS_4(C12)$ was evaluated in a separate study. While *in vitro* photodynamic activity was significantly improved by incubating the $AlPcS_4(C12)$ with LDL (leading to insertion of the long alkyl chain into the lipid portion of the LDL) prior to treatment, *in*

vivo results were equivalent for $AlPcS_4(C12)$ and $AlPcS_4(C12)$ inserted into LDL prior to administration. This is due to the $AlPcS_4(C12)$ naturally redistributing to LDL upon administration to the blood and thus, leading to in vivo formation of the $AlPcS_4(C12)$-LDL conjugate.

With the known increase in photodynamic potential of amphiphilic photosensitizers, it has been hypothesized that the results using the aluminum phthalocyanines bonded to long alkyl chains via a sulfonamide bond (Figure 1.16) were the result of increased amphiphilicity, with the longer alkyl chains increasing the importance of the lipophilic portion of the molecule. As a result, increasing the length of the alkyl chain will increase the amphiphilicity of the photosensitizer and will thus result in improved photodynamic activity. However, the current study seems to indicate that the presence of long alkyl chains may also increase the photodynamic efficiency of lipophilic phthalocyanines, thus suggesting that long alkyl chains act as an anchor for attachment of the photosensitizer to the cellular membrane. This promotes cellular uptake and perhaps subcellular trafficking to more susceptible subcellular sites. Along these lines, it is pointed out that in the present study, the improvement in photodynamic activity of (1,2,3,4,8,9,10,11,15,16,17,18-dodecafluoro-23-t-butylphthalocyaninato)zinc and (1,2,3,4,8,9,10,11,15,16,17,18-dodecafluoro-23,24-didodecylphthalocyaninato)zinc (compounds **5b** and **5g** in Figure 7.1) as compared to $ZnPcF_{16}$ was more prominent after long incubations (Figure 7.3). This implies that cell uptake probably plays a role in the increased photodynamic activity.

While CRM emulsions have been previously used to solubilize hydrophobic photosensitizers for intravenous administration and may promote tumour uptake, clinical

use of CRM has been associated with anaphylactic reactions (Dye et al., 1980), hyperlipidemia and abnormal electrophoretic lipoprotein patterns (Bagnarello et al., 1977). The improved solubility of fluorinated phthalocyanines fortunately allows for the incorporation of this type of photosensitizer into vehicles other than CRM emulsions. This may further improve the pharmaceutical characteristics of the lipophilic photosensitizer compositions. For instance, formulation of $ZnPcF_{16}$ in PEG-coated poly(lactic acid) nanoparticles result in compositions that display a delayed blood clearance, reduced liver uptake and an improved tumour response compared to CRM emulsions of the same photosensitizer (Allémann et al., 1995; Allémann et al., 1996). Furthermore, these nanoparticle preparations make it possible to prolong the delay between dye injection and light treatment while maintaining similar tumour responses. This increased time period helps to enlarge the therapeutic window of $ZnPcF_{16}$. The characteristics of fluorinated phthalocyanine-based pharmaceutical compositions may also be enhanced by using other vehicles such as liposomes (Morgan et al., 1989; Richter et al., 1993; van Leengoed et al., 1994; Love et al., 1996; Renno et al., 2001; Derkycke et al., 2004, microspheres (Bachor et al., 1991), polymeric micelles (Taillefer et al., 2000; van Nostrum, 2004) and cyclodextrins (Ruebner et al., 1997; Ruebner et al., 1999) among others. Chapter 4 reviews the use of receptor-mediated delivery systems for targeted photodynamic therapy and such methodology may be useful in targeting fluorinated phthalocyanines to target tissue and infections.

Asymmetrically substituted dodecafluorinated zinc phthalocyanines substituted with iodine on the fourth benzo ring provide the opportunity to alter the characteristics of these phthalocyanines via palladium-catalyzed reactions (see Chapter 6). Such reaction

will allow the addition of novel functionality to the molecule as was accomplished in the study described in Chapter 8. For instance, addition of 1-alkynes via palladium-catalyzed reaction will permit the control of the length of the chains attached to the phthalocyanine while the presence of the triple bond will increase conjugation in the macrocycle and will push the Q band absorbance further towards the near infrared. Coupling to 1-alkynes with terminal carboxy groups will add anionic charge to the molecule while also lending a function group to the molecule that can be used to couple the phthalocyanine to biologically important molecules such as proteins, peptides and antibodies by amide bond formation. Finally, 1-alkynes with pyridine groups permits addition of cationic charge to the molecule, with such molecules potentially have interesting properties in numerous fields including photodynamic therapy.

In conclusion, since their fortuitous beginnings a century ago, the field of phthalocyanines has grown exponentially. Beyond their use as dyes and pigments, phthalocyanines have found utility in fields such as chemical catalysis, electrophotography, photoreproduction and optical data storage. The potential of phthalocyanines still remains relatively unrealized however, with phthalocyanines being heralded as valuable and functional in an incredible list of widely divergent technological fields. The potential of phthalocyanines to be adapted to widely divergent applications originates with their singular chemical structure, high degree of aromaticity, unique electronic spectra and the flexibility involved in their synthesis. Diverse applications such as those proposed for phthalocyanines require compounds with distinct and well-defined physical, chemical and electronic properties. This necessitates synthetic methods with control of regioselectivity and with access to assorted types of substituents and to

well-controlled substitution patterns. Current synthetic methods often are lacking and new methods are thus needed for phthalocyanines to fulfill their immense promise.

The studies herein described indicate the potential of the Kobayashi ring expansion reaction in the preparation of 3:1 asymmetrically substituted phthalocyanines. Furthermore, the importance of combining synthetic approaches is readily apparent, with the combination of the Kobayshi ring expansion reaction and palladium-catalyzed cross-coupling reaction leading to the synthesis of novel amphiphilic water-soluble phthalocyanines with interesting potential as photosensitizers for photodynamic therapy. Overall, despite not being a universal approach for the synthesis of 3:1 asymmetrically substituted phthalocyanines, the Kobayashi ring expansion reaction of halogenated boron subphthalocyanines with various 1,3-diiminoisoindolines proceeded smoothly to exclusively give the desired 3:1 asymmetrically substituted phthalocyanines in good yields. The starting halogenated boron subphthalocyanines themselves were synthesized using much milder reaction conditions than those previously reported, allowing for improved yields and purities while permitting the observation of an unidentified intermediate in the synthesis of boron subphthalocyanines.

Unsubstituted boron subnaphthalocyanines has been shown to be effective in generating singlet oxygen in a homogeneous organic solvent. In the current study, a series of boron subnaphthalocyanines were prepared using the same protocol as the one employed for the preparation of the halogenated boron subphthalocyanines. Formulated as CRM emulsion, these photosensitizers effectively generated singlet oxygen in a biologically relevant media as determined by the L-tryptophan photooxidation assay. These compounds however underwent extremely rapid photobleaching under the

irradiation conditions used. While this photobleaching may limit their potential as photosensitizers for photodynamic therapy due to rapid destruction of chromophore, photobleaching may be desirable in certain situations. Thus, determination of methods to photostablize these compounds remains an important goal.

Changing the hydrogen atoms on the phthalocyanine macrocycle with fluorine atoms significantly increases the solubility of phthalocyanines, greatly improving their utility. In addition, the heavy atom effect leads to increased triplet state yields, an important consideration for applications such as photodynamic therapy. The ring expansion reaction of dodecafluorinated boron subphthalocyanine opens the door to a new series of fluorinated phthalocyanines with enhanced properties. Upon chelation with Zn^{+2} and formulation as CRM emulsions, illumination of the dodecafluorinated phthalocyanines with light of the appropriate wavelength readily led to an increased production of singlet oxygen as compared to $AlPcS_4$. Preliminary studies of *in vitro* photodynamic activity of selected asymmetrically substituted zinc dodecafluorophthalocyanines against EMT-6 tumours indicate that these compounds have potential as photosensitizers for photodynamic therapy. In particular, the addition of long alkyl chains to dodecafluorinated phthalocyanines improves the photodynamic efficiency as compared to symmetrically substituted $ZnPcF_{16}$. While it has been demonstrated that asymmetry in water-soluble photosensitizers with the resulting increase in amphiphilicity improves the photocytotoxicity, the present results suggest that asymmetry in lipophilic phthalocyanines, despite not altering the hydrophilic/lipophilic balance in the molecule, also enhances the efficiency of photosensitizers.

In the meanwhile, the ring expansion reaction of iodinated boron subphthalocyanines provides exceptional building blocks for the preparation of novel asymmetrically substituted phthalocyanines. The use of palladium-catalyzed cross-coupling reactions of the resulting iodinated phthalocyanines provide means for adding novel functionality to these phthalocyanines while altering their characteristics for applications such as photodynamic therapy. The use of palladium catalysis in the coupling of the 3:1 asymmetrically substituted iodinated phthalocyanines with 5-hexynoic acid and 10-undecynoic acid results in novel amphiphilic anionic water-soluble phthalocyanines wherein the functional group responsible for the water-solublity is separated from the chromophore by long alkynyl chains. Furthermore, the triple bond of the alkynyl group increases the conjugation of the molecule and pushes the Q band absorption to longer wavelength. Of particular interest however are asymmetrically substituted water-soluble cationic phthalocyanines prepared by the palladium-catalyzed coupling of the 3:1 asymmetrically substituted phthalocyanines with 2-ethynylpyridine or 3-ethynylpyridine. Following methylation with methyl iodide or dimethyl sulfate, solutions of the resulting cationic phthalocyanines in DMF exhibit important shifts in the λ_{max} to longer wavelengths along with significant splitting fo the Q band. Though less extensively studied, cationic phthalocyanines have been shown to have a number of interesting properties for the photodynamic treatment of numerous conditions. In addition, the lipophilic/hydrophilic balance of these phthalocyanines can be further modified and adjusted by altering the size and nature of the aliphatic group bound to the quarternized nitrogen atoms.

Chapter 10.

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Chapter 11.

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Note: Please see the reference in individual chapters 2-8 for further relevant references.