Progress with Heterocyclic Photosensitizers for the Photodynamic Therapy (PDT) of Tumours

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J. Heterocyclic Chem., **39**, 455 (2002).

Introduction.

The aim of this review is to highlight aspects of heterocyclic chemistry which have played a role in the development of photosensitizers for the photodynamic therapy (PDT) of tumours. In the general field of pharmaceutical compounds, heterocyclic systems have been of considerable importance, and it is not surprising therefore that amongst photosensitizers for PDT they also have a dominant position.

Photodynamic therapy is based upon the photodynamic effect, which may be defined as the destruction or damage of living things by the combined action of a photosensitizer, visible or near visible light, and oxygen. An important part of this is that the radiation employed is not of high energy, and that by itself (unlike X-radiation) it is virtually harmless to living things. The expression of the photodynamic effect is sometimes referred to as "photodynamic action", and this is the term most often used historically, being a translation of the German "photodynamische wirkung". The historical background of the subject has been reviewed [1-3].

The basic process in tumour PDT is presented in schematic form in Figure 1. The photosensitizer is applied, usually by intravenous injection, although oral and topical administration have also been used. After an interval (the drug - light interval) to allow the substance to become suitably distributed (to give maximum therapeutic advantage of tumour damage with respect to damage to normal tissues), the tumour is irradiated with a measured dose of red light, the wavelength of which matches an appropriate absorption band of the photosensitizer. The tumour tissue then becomes necrotic. The selectivity of damage depends on two main factors: the selectivity of the sensitizer for localization in the tumour with respect to

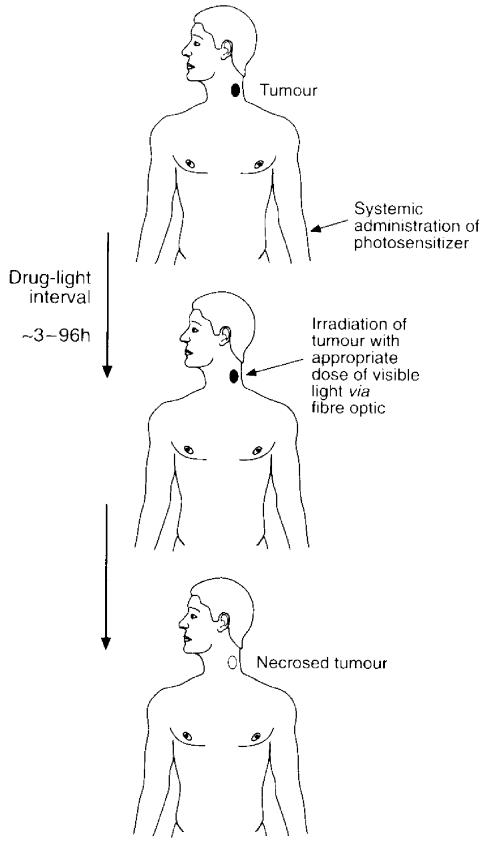
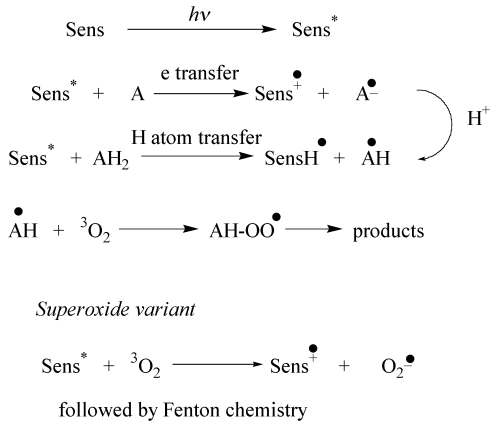


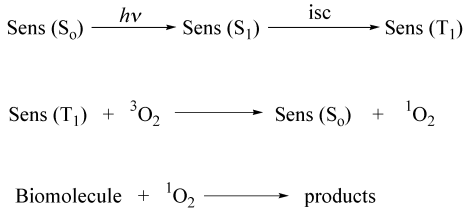
Figure 1. Schematic to show stages in the photodynamic therapy (PDT) of tumours using a photosensitizing drug. (From reference 3, with permission, Royal Society of Chemistry and Marius Press).

Scheme 1

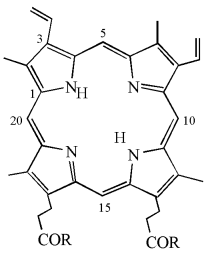
(a) Type I



(a) Type II



Type I and Type II photooxygenations [6]. (Sens = photosensitizer, Sens* = photosensitizer excited state, A = acceptor (e.g. biomolecule, $^3O_2$), S_0 = ground singlet state, S_1 = first excited singlet state, T_1 = first excited triplet state, $^3O_2$ = ground state triplet oxygen, $^1O_2 = {}^1\Delta_g$ singlet oxygen).

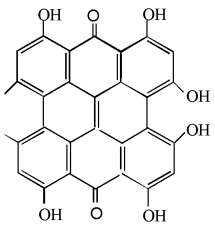


1 **a,** R = $NH(CH_2)_3NMe_2$

b, R = $NH(CH_2)_3\overset{+}{N}Me_3\ I^-$

c, R = $NH(CH_2)_2\overset{+}{N}Me_3\ I^-$

d, R = OH, Protoporphyrin



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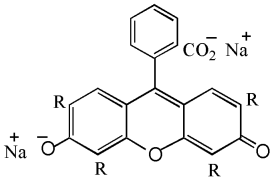
Block 1

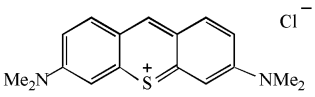
localization in normal tissue; and the directional properties of the light beam (usually a laser). Visible light in the red region is preferred because tissue absorbs and scatters in the red much less than at shorter wavelengths, so that tissue penetration is greater [5].

In mechanistic terms, the destruction of living tissue is ascribed to reactive oxygen species. Two main photooxidation pathways are possible, the well-known Type I (radical intermediates, Scheme 1a) and Type II (with singlet oxygen as an intermediate, Scheme 1b) processes. The relative importance of these continues to be a matter for experiment and discussion, but the general view is emerging that singlet oxygen is a major pathway. For example [7], studies on the singlet oxygen quantum yields (ϕ_Δ) and superoxide quantum yields (ϕ_{sup}) for a series of porphyrin amide derivatives **1a-c** in



3 R = Br

6 R = H



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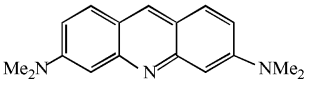

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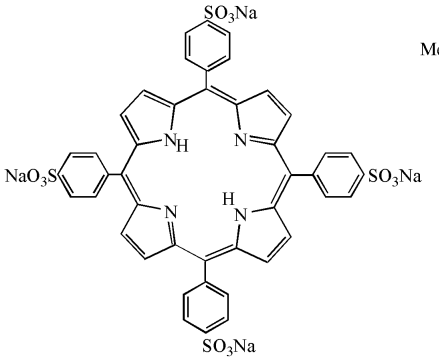
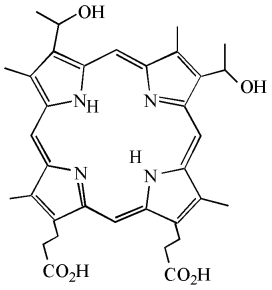


5, tetrasodium salt



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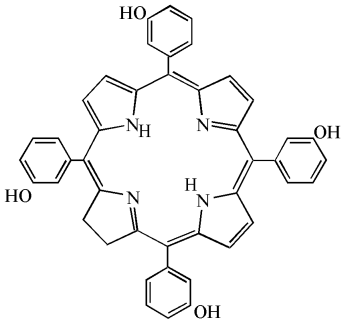

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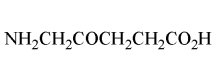

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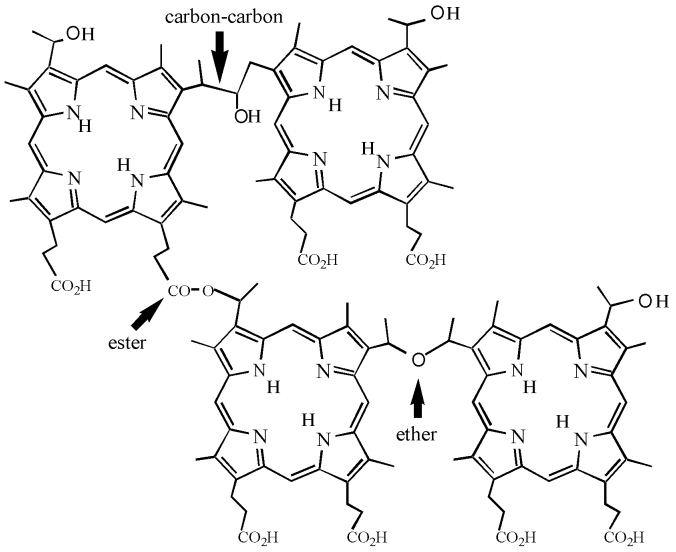


Figure 2. Schematic structure for an HpD oligomer illustrating ether, carbon-carbon and ester internuclear linkages. Ether linkages are thought to predominate[21]. Adapted from Figure 6.4 of reference [1] with permission of Gordon and Breach Science Publishers.

aqueous medium showed $\phi_\Delta >> \phi_{sup}$, and a broad correlation between ϕ_Δ and cell kill of a melanoma culture *in vitro*.

Although some non-heterocyclic compounds, such as the extended quinones (*e.g.* the natural product hypericin **2** [8]) have attracted attention as PDT photosensitizers, the bulk of published work in this field has been on heterocyclics, and especially on porphyrins and their relatives. In my view this situation has arisen because: (a) haematoporphyrin derivative (HpD), a "first generation" sensitizer, was the first sensitizer to show substantial clinical promise [9,10] and was later developed into the first product (Photofrin) which was accorded regulatory approval (Canada, 1993); (b) the porphyrins show absorption in the red (the lowest energy absorption, referred to as Band I, being at about λ max 630 nm), and this absorption shifts further into the red, and increases in intensity, on going to the hydro compounds (that is, the chlorins and the bacteriochlorins) and to the related phthalocyanines; (c) the porphyrins are aromatic, and so the chromophore is rugged and survives biological manipulation; (d) many porphyrins appear to have a low toxicity in the dark.

First Generation Photosensitizers

Although HpD was the first photosensitizer to show substantial and reproducible clinical PDT activity, several other heterocycles had shown promise. Eosin **3** was the earliest example and was applied, for example, to the therapy of rodent ulcers of the lip [11]. Methylene blue **4** [12], tetraphenylporphyrin tetrasulphonic acid **5** [13], fluorescein **6** [14], and acridine orange **7** [15] have all been studied with respect to tumour PDT.

However, the clinical papers on HpD focussed attention upon this substance, and it is commonly referred to as representing the first-generation photosensitizers [16]. Chemically, it is exceedingly complex, although the preparation is disarmingly simple [17]. Haematoporphyrin **8** is treated at room temperature with 5% sulphuric acid in acetic acid for 15 minutes. The product is precipitated by adding water, and the red-brown precipitate (HpD Stage I) is made alkaline, and brought to neutrality to give HpD (Stage II) for injection.

The first stage leads to a mixture of acetylation and elimination products: haematoporphyrin *O,O'*-diacetate is the main component [18]. The second stage involves some hydrolysis of acetate functions to give porphyrin monomers (*e.g.* regenerated haematoporphyrin), but the biological activity resides mainly in a polymeric fraction (recognised as a covalent polymer, and called Fraction D by its discoverers [19]) in which the internuclear linkages comprise ether, ester, and carbon-carbon bonds [19-21] in various combinations, as illustrated schematically in Figure 2.

Removal, or partial removal, of the monomeric fraction from HpD Stage II, using a commercial gel filtration procedure, eventually led to the product with the trade name "Photofrin" [22]. Originally developed as a drug by QLT PhotoTherapeutics Inc., Vancouver, the rights to this photosensitizer have recently been acquired by Axcan Pharma Inc. (Mont-Saint-Hilaire, Quebec) (www.axcan.com).

Capillary electrophoresis of Photofrin has revealed about 60 peaks [23], but there must be far more individual components. Our extensive early attempts to separate a species of high *in vivo* biological activity from this sort of

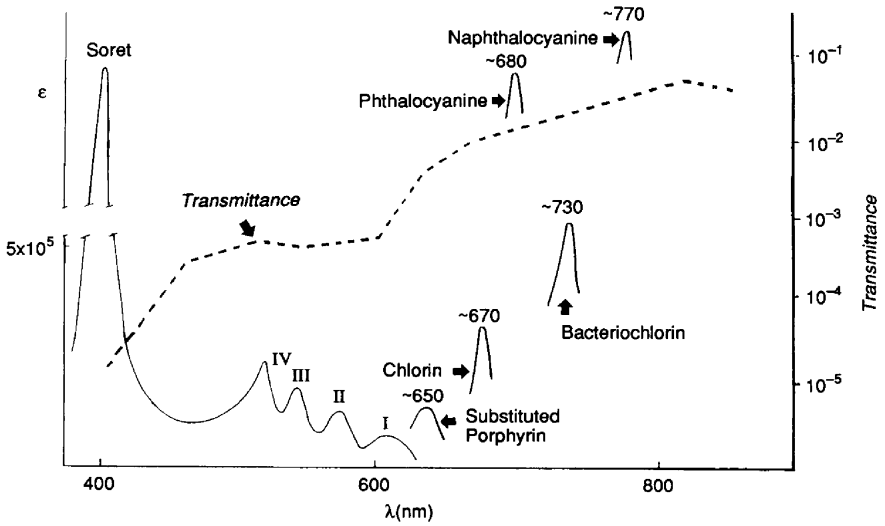


Figure 3. Absorbance of some common PDT photosynsitzers in relation to tissue transmittance. The absorption spectra are schematic, and only Band I is shown, apart from the porphyrin spectrum on the left-hand side. The transmittance curve, based on reference [5], refers to 0.7 cm thickness of human scrotal sac. The broad feature in the transmittance curve at 500-600 nm is ascribed to absorption by haemoglobin. (From reference [4], with permission of the Royal Society of Chemistry).

mixture were without success. It was principally for this reason that attention turned to photosensitizers that were single substances, more active and more selective, the "second generation" of photosensitisers.

The rest of this review concentrates on the design and synthesis of selected second generation heterocyclic sensitizers suitable for PDT.

Second Generation Photosensitizers

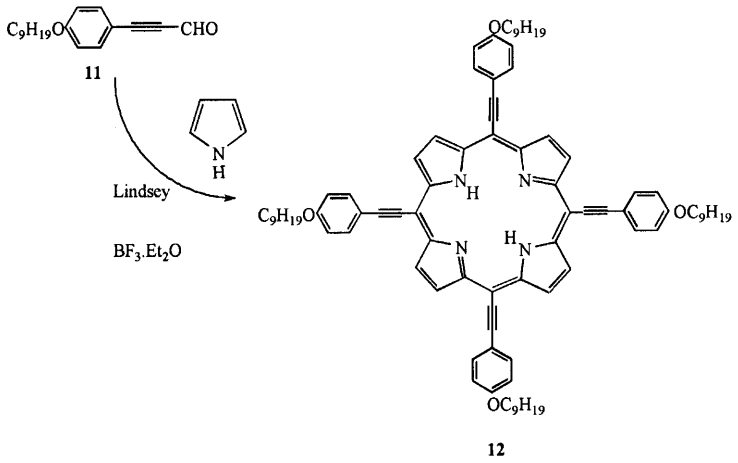
In considering the design of a new drug for photo-therapeutic use it is necessary to take into account all the conventional criteria in drug design, plus additional ones which arise because of the use of light [24]. The light source is often a laser, although this is not strictly necessary and sometimes much less costly filament or arc sources are employed with appropriate filters. The important light-based variables are the wavelength, the fluence (*i.e.* the total energy per unit area), the fluence rate, and the drug-light interval (Figure 1).

The dose of light may be given continuously, or as a series of short exposures. For example, in the use of 5,10,15,20-tetrakis(*m*-hydroxyphenyl)chlorin (m-THPC, **9**, "Foscan") in the treatment of head and neck cancer, the drug - light interval is 96 hours, and a laser source at 652 nm is used with continuous irradiation to give a fluence of 20 J cm^{-1} at a fluence rate of 100 mW cm^{-1}. From an operational point of view, this means that the irradiation session lasts just over 3 minutes in this case.

As far as the photosensitizer is concerned, the design criteria can be summarised as follows: (a) a single substance, not chiral, which can be conveniently synthesized in good yield (b) little or no toxicity in the dark (c) effective tumour photonecrosis and selectivity, but behaviour such that the drug is cleared quickly, and any generalized photosensitivity remaining after the treatment is minimal. As with conventional drugs, these pharmacokinetic and localization properties depend largely on the solution physical chemistry of the substance, and amphiphilic character appears to be important here. Since the heterocyclic nuclei under consideration are generally hydrophobic, it is often hydrophilic substitution that is looked for; (d) if it is the case that the singlet oxygen mechanism is important (above), the energy of the first excited triplet (E_T) of the sensitizer needs to be greater than 94 kJ mol^{-1} (the energy of the $^1\Delta_g$ singlet state of dioxygen). The triplet of the sensitizer must be generated with a high quantum yield, and have a lifetime sufficient that the overall quantum yield of singlet oxygen (ϕ_Δ) is appreciable (say, > 0.3); (e) as mentioned earlier, strong absorption in the red region is advantageous, because mammalian tissue absorbs and scatters much less in the red than at shorter wavelengths. Figure 3 shows in a schematic form a plot of transmittance of human tissue in relation to the major red absorption bands of a number of relevant heterocyclic nuclei. The comparisons in the Figure explain why it is that the new photosensitizers that are currently being developed are usually not porphyrins, but those relatives (*e.g.* chlorins, bacteriochlorins, phthalocyanines, naphthalocyanines) which have strong absorption bands at longer wavelength.

It is now appropriate to examine some specific examples of heterocyclic systems. Because criterion (e) above has been of considerable significance in guiding

Scheme 2



recent synthetic work, specific mention will be made of the longest wavelength absorption (Band I) for the various systems.

Porphyrins.

Only one example of a simple porphyrin is currently showing clinical promise, and that is protoporphyrin **1d** itself. When injected as such, this does not behave as an effective tumour sensitizer [19], but when generated *in situ* from its biochemical precursor, δ-aminolaevulinic acid (δ-ALA) **10**, it is found to be so [25,26,27]. Thus, δ-ALA is a pro-drug. Typically, **10** is formulated for topical or intravenous administration, and the localization of the resulting porphyrin in tumour tissue is demonstated (as with several other photosensitizers of this series *e.g.* HpD [28]) by a red fluorescence of the tumour in ultraviolet light (366 nm). Hence δ-ALA has applications as a photodiagnostic agent. For photodynamic therapy δ-ALA appears to be most successful with superficial lesions. It is being developed (trade name: Levulan) by Dusa Pharmaceuticals (www.dusapharma.com) and in December 1999 received FDA approval for the treatment of actinic keratosis. Little chemical manipulation of this pro-drug seems to be possible because of the nature of enzymatic specificity. However, some improvement in bioactivity is recorded when esters of δ-ALA are used [29]. The increase in lipophilicity conferred by the esterification presumably increases membrane penetration, and the ester is subsequently cleaved by adventitious esterase activity.

It has recently been shown that conjugation of a porphyrin at the *meso* positions using the sterically undemanding ethynyl function gives products with strong absorption in the red region [30,31]. For example (Scheme 2), treatment of the aldehyde **11** with pyrrole using the Lindsey modification of the Rothemund-Adler porphyrin synthesis gives 5,10,15,20-tetrakis(*p*-nonyloxyphenyl)porphyrin **12**.

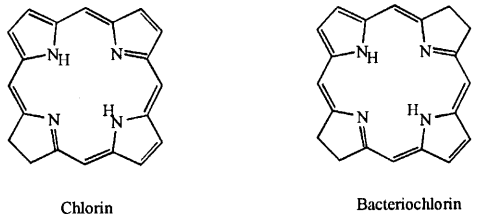


Figure 4. Structures of the chlorin and bacteriochlorin macrocycles.

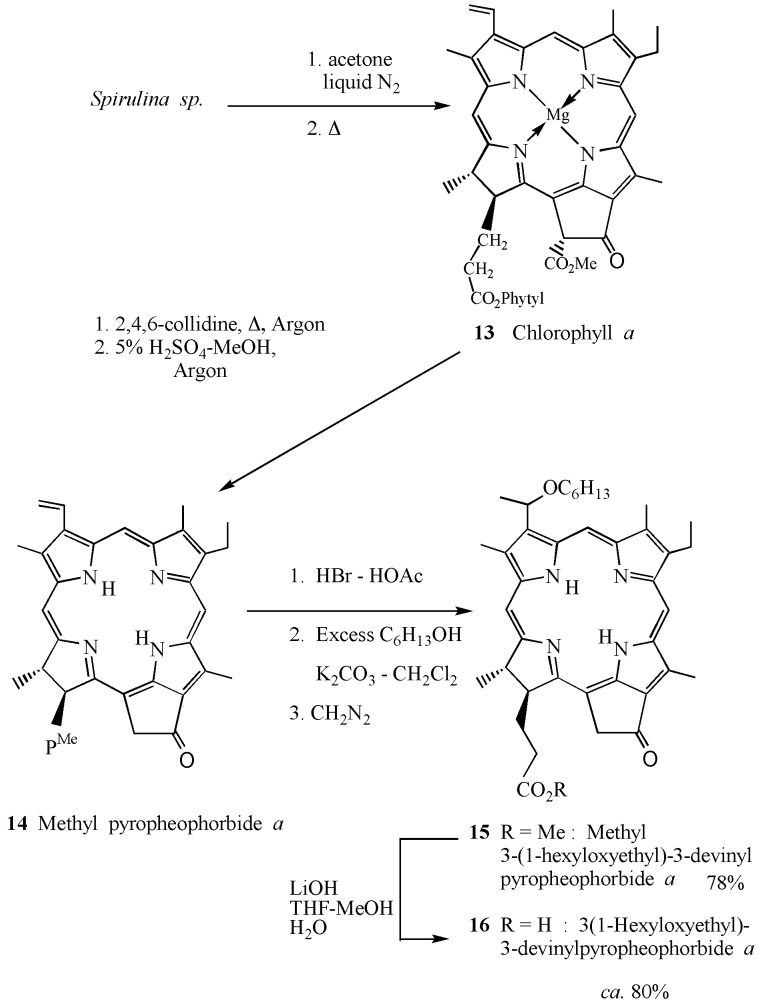
Although the yield is very low (3%), the striking result is the electronic spectrum of **12,** which in dichloromethane (*green* solution) has λ max 472 (297000), 653 (89100) and 744 nm (ε 55900) [30]. The zinc(II) complex of the analogous *p*-octylphenyl compound has $\phi_\Delta = 0.97$ [31].

Chlorins and Bacteriochlorins

The parent systems are shown in Figure 4**.** The reduced ring of the chlorin is shown here at ring D, as drawn in the conventional representation of chlorophyll *a*. In my view, this is currently the most important group of compounds under development, and four compounds will be highlighted which are in an advanced stage of assessment.

Chlorophyll *a*, available uncontaminated by chlorophyll *b* from the alga *Spirulina maxima*, is a feasible commercial source of the chlorin nucleus. An example is provided by the preparation of a series of ether derivatives, based on 3(1-alkoxyethyl)-3-devinyl-pyropheophorbide *a*, in which lipophilicity increases as the alkyl chain length increases. The preparative route (Scheme 3) shows the hexyl ether, at which point along the homologous series the PDT activity has been found to

Scheme 3



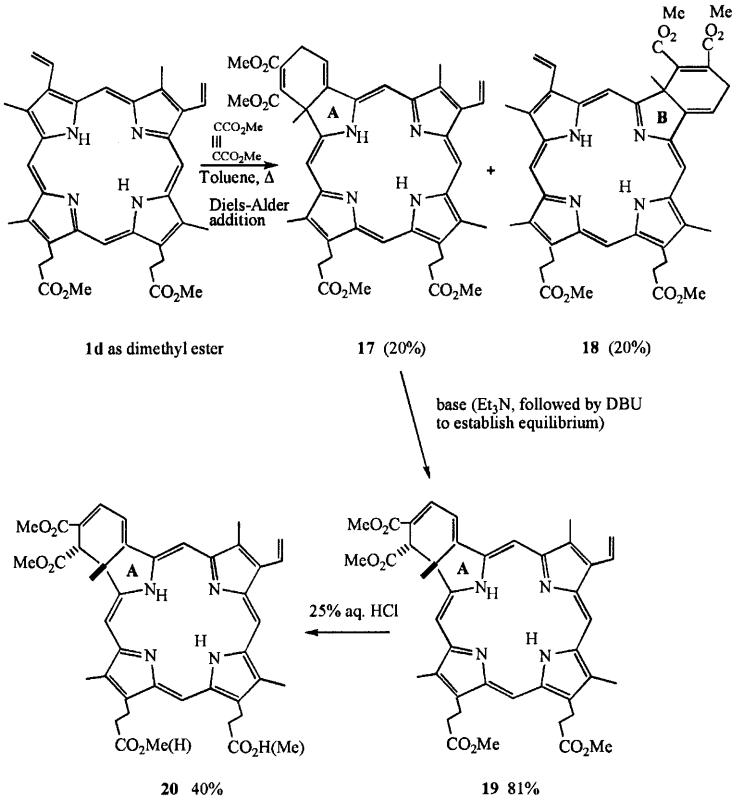
be greatest [32,33]. Chlorophyll *a* **13** suffers thermal decarboxymethylation in hot collidine to give pyropheophorbide *a*, isolated as the methyl ester **14**. (This removal of the β-ketoester system of chlorophyll is highly desirable, since the C-13[2] position of **13** is highly reactive, *e.g.* as a centre for autoxidation, leading to undesirable side products). Markovnikov addition of hydrogen bromide to the 3-vinyl function, followed by ether synthesis (1-hexanol, potassium carbonate) and treatment with diazomethane gives the hexyl ether as its methyl ester **15.** The latter is a sticky solid, but hydrolysis (LiOH) gives the corresponding propionic acid **16** which is obtained from dichloromethane/ hexane as a fine powder [34]. The hexyl ether **16** in dichloromethane has a strong absorption band at 663 nm (ε *ca.*47,500) and a high ϕ_Δ value (0.48) [34]. Associated with much less residual skin photosensitivity than Photofrin, this substance (under the trade name "Photochlor") is now in clinical trial [35].

The second chlorin originates from another naturally derived heterocycle, protoporphyrin **1d**. Although protoporphyrin has been synthesized, it is available much more cheaply from slaughterhouse blood. The vinyl groups on rings A and B each form a diene system with the conjugated ββ'-double bond, and a number of Diels-Alder reactions are known. Thus, addition of dimethyl acetylenedicarboxylate to protoporphyrin dimethyl ester gives a mixture of two mono-adducts **17** and **18**, each in about 20% yield (Scheme 4).

The two regioisomers are separated and the ring A adduct **17** is isomerized by base to produce the conjugated system **19**. This has two chiral centres, but the thermodynamically more stable transoid diastereoisomer is the only one that is isolated after DBU treatment.

The next step is a partial hydrolysis, which occurs preferentially at the propionic esters for steric reasons, and which is conducted so as to produce a mixture of the two

Scheme 4



monocarboxylic acids represented at **20** [36]. This mixture is called benzoporphyrin derivative (mono acid, ring A) with the acronym BPDMA, sometimes shortened to BPD, although it is a chlorin, not a porphyrin, and it does not contain a benzenoid ring. Purists may prefer to use its trade names. It has two, "Verteporfin" and "Visudyne".

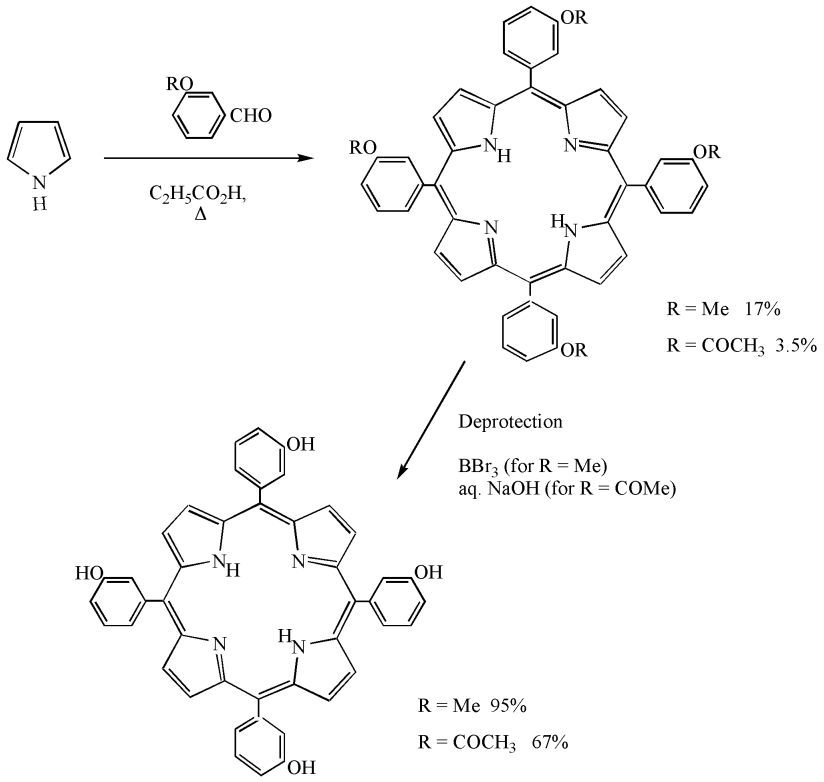
This compound **20** has a strong absorption in the red (λ max 686 nm, ε 34,000 in methanol) and a high singlet oxygen quantum yield (ϕ_Δ = 0.7). In PDT assays it is an effective sensitizer, and is characterised by rapid clearance, and a low residual generalized photosensitivity. Although a monocarboxylic acid, it is a hydrophobic compound, and needs assistance in delivery, for example in a liposomal system. It has been in clinical trial against basal cell carcinoma and psoriasis. Recently, it has received regulatory approval in Switzerland and the USA for the treatment of wet age-related macular degeneration (AMD) under the trade name "Visudyne" (CibaVision / QLT Phototherapeutics. www.visudyne.com and www.qltinc.com). AMD is an ophthalmic disease of the elderly for which there appears to have previously been no effective remedy [37]. The condition is associated with pathological development of blood vessels in parts of the eye, and leakage from them. One of the biological characteristics of PDT is the sealing of blood vessels, and this is thought to be a basis of the improvement observed. These developments serve to illustrate a general point, that it is increasingly being realized that there are clinical applications for PDT well outside the oncology field.

The third and fourth examples are chlorins which are made by total synthesis. Scheme 6 shows the synthesis of 5,10,15,20-tetrakis(*m*-hydroxyphenyl)chlorin (m-THPC, **9**), which has the trade name "Foscan" (Scotia Pharmaceuticals, Stirling). The corresponding porphyrin **21** is the starting material and is made by the Rothemund-Adler synthesis (Scheme 5) [38]. The phenolic hydroxy group is protected either as acetate (hydrolytic deprotection) or as the methyl ether (removal with boron tribromide). In either event the yields are modest, as is often the case with this synthesis, but are tolerated since a complex macrocycle is produced in a one-pot reaction from simple (and inexpensive) starting reagents.

Reduction of the porphyrin **21** (Scheme 6) with diimide gives a mixture of the chlorin **9** and the corresponding bacteriochlorin (*m*-THPBC) **22**: if it is the chlorin that is required, the bacteriochlorin is converted into it by dehydrogenation with *o*-chloranil [39].

In methanol, *m*-THPC **9** has λ max 650 nm (ε 29,600) and a ϕ_Δ value of 0.43 [39]. Both the chlorin and the bacteriochlorin are very effective PDT sensitizers [39],

Scheme 5



21 5,10,15,20-Tetrakis (*m*-hydroxyphenyl)porphyrin

and the chlorin has regulatory approval in Europe for treatment of cancer of the head and neck [40] (www.quantanova.com).

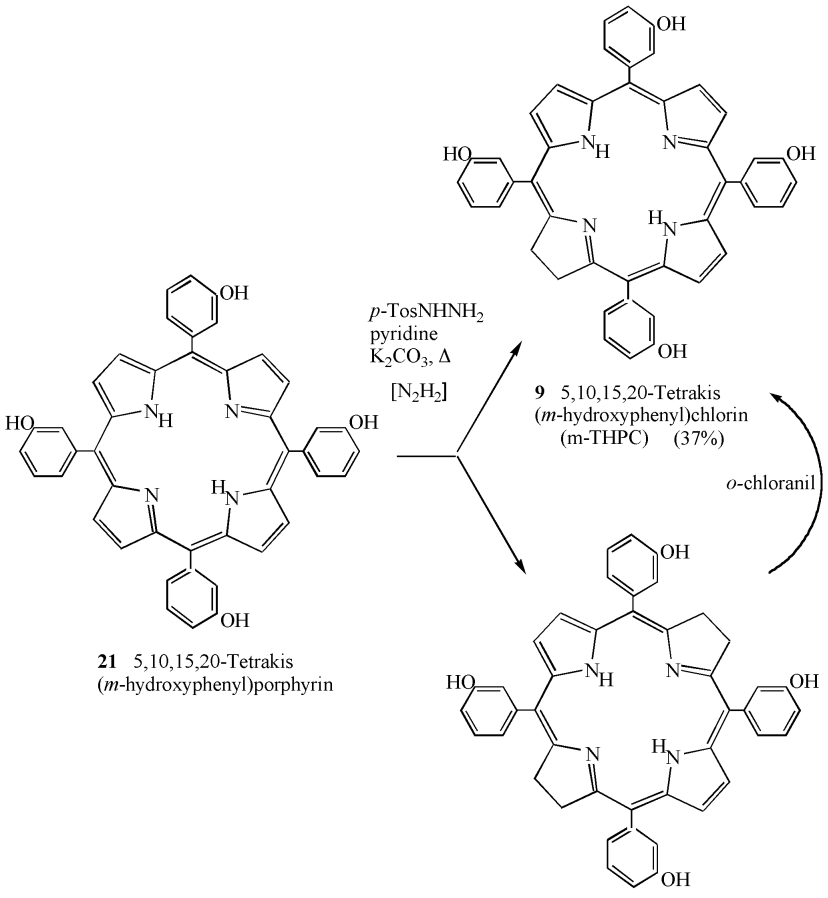
The high activity of *m*-THPC may be judged from the clinical dose, which is only 0.15 mg kg^{-1} for a light dose of only 20 J cm^{-2}. From a chemical composition point of view, it is a single substance with no chiral centres, the synthesis is straightforward and the yields are acceptable.

Another chlorin, tin etiopurpurin **29**, now in clinical trial, is being developed by Miravant Medical Technologies (Santa Barbara, CA) under the trade name "Purlytin". It is derived (Scheme 7) from etioporphyrin I **23**, which in turn is available from kryptopyrrole **24** via the pyrromethene perbromide **25** in a well-established porphyrin synthesis of the 2x2 type [41]. Etioporpyrin I as its nickel(II) complex **26** is *meso*-formylated under Vilsmeier-Haack conditions, and then subjected to a Wittig reaction followed by demetallation to give the *meso*-acrylate **27** [42,43]. In an example of a cycloisomerization reaction discovered by Woodward during the synthesis of chlorophyll *a* [44], the *meso*-acrylate **27** is equilibrated in hot acetic acid under nitrogen with the cyclized derivative **28.** This has a reduced pyrrole ring, and so belongs to the chlorin series: but having a conjugating substituent at the adjacent *meso* position, it falls into the subset of chlorins known as purpurins (which are generally characterized by a puce-kharki rather than a bright green colour). The predominant regioisomer **28** is rationalized on the basis that its formation involves displacement of the larger group (here, ethyl rather than methyl) from peripheral overcrowding. Insertion of tin with $SnCl_2$-HOAc (a step which presumably is accompanied by aerial oxidation) gives the dichlorotin(IV) complex **29**, which is referred to in the literature as tin purpurin or SnEt2.

Tin etiopurpurin has its Band I at λ max 659 nm (ε 30,300) in dichloromethane, and a substantial ϕ_Δ value (0.6). It is a lipophilic substance, and has to be administered in a lipid emulsion (Cremophor). Earlier clinical work has been summarised [45]. **29** is currently in Phase III clinical trials for wet age-related macular degeneration and in Phase I trials for cancer of the prostate (www.miravant.com).

A number of other chlorin compounds are under study [46]. As indicated in Figure 3, Band I in the bacteriochlorins is further into the red, and of greater intensity, than the analogous band in the chlorins. Typically, bacteriochlorins have λ max ~740 nm (ε 80,000). The bacteriochlorins prepared for PDT studies include

Scheme 6



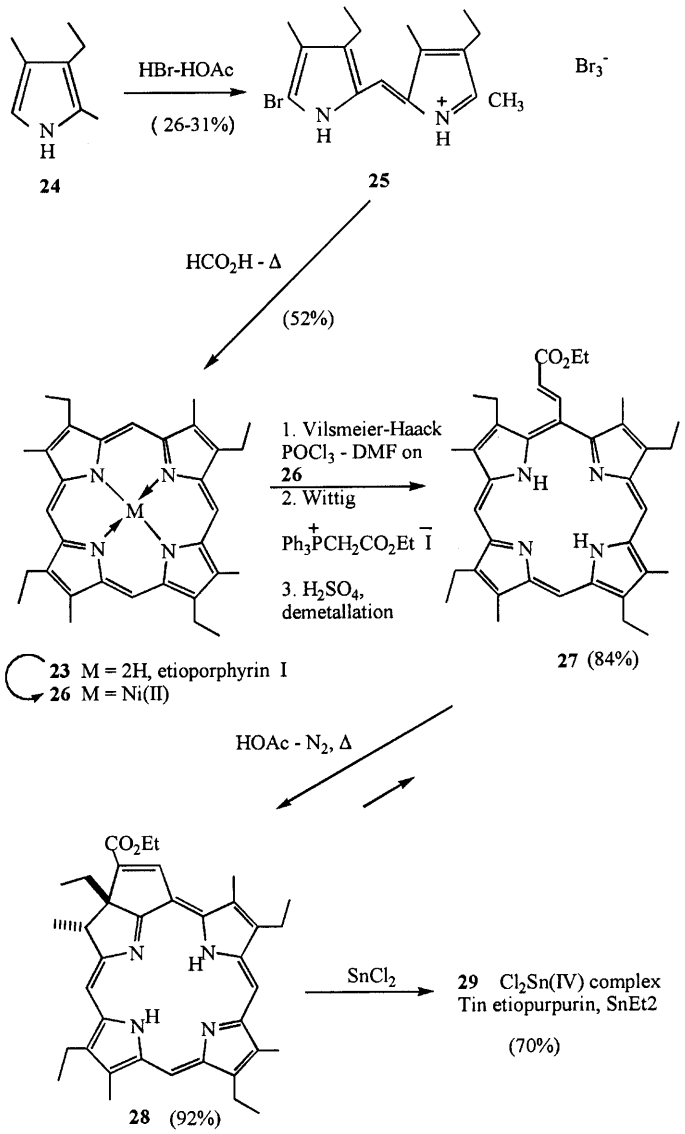
m-THPBC **22** [39,47], which has λ max 735 nm (ε 91,000) in methanol; the oxobacteriochlorin **30**, which has λ max 786 nm (ε 64,000) in dichloromethane [48]; and bacteriochlorin *a* (bacteriochlorin e_6) **31** which has λ max 760 nm (ε 32,000) in phosphate buffered saline[49].

Porphycenes

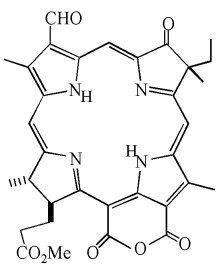
Porphycene **32** is an isomer of porphyrin. Like porphyrin, it has an 18πe aromatic system. The corresponding 2,3-dihydroporphycene has also been characterized, but rather surprisingly does not have the enhanced Band I features enjoyed by chlorin [50]. Porphycene was first encountered in 1986, and is a synthetic system, not found in Nature. The parent **32** is known, and was prepared by the McMurry type coupling of α,α'-diformylbipyrrole [51]. The 2,7,12,17-tetrapropyl derivative **33** is synthesised analogously (Scheme 8) [52]. The α-iodopyrrole **36** is prepared following standard pyrrole chemistry. Ullmann type coupling gives the bipyrrole system **37**, which is hydrolyzed, decarboxylated, and *bis*-formylated to give **40.** McMurry coupling then gives the tetrapropylporphycene **33** in modest yield. The importance of **33** is that it has satisfactory physical properties (*e.g.* solubility) which allows the chemistry of the system and its metal complexes to be explored. **33** is a single substance with a purity of >99%. In methanol it has λ max 631 nm (ε 53,400), and, administered in a liposomal preparation, it shows PDT activity *in vivo* (tumour implants in mice) [53]. A series of compounds **41** in which amphiphilicity may be varied by changing the substituent at C-9 (**41**, R = *e.g.* -$NHCO(CH_2)_3CO_2H$,-$NHCO(CH_2)_3$-$CONHCH(CO_2H)(CH_2)_2CO_2H$, -$NHCO(CH_2)_{16}CH_3$, OC_6H_{13}) has been prepared for pharmacokinetic studies [54]. The 9-acetoxy derivative (**41**, R = OAc) has also attracted attention [55].

This series of compounds has been studied by Cytopharm (Menlo Park, CA) in association with Glaxo-Wellcome. The compounds have strong intellectual property status, but from a purely synthetic viewpoint the preparations require several steps, some of which give low

Scheme 7



yields. Nevertheless, the compounds are well characterized single substances, achiral (unless the C-9 substituent has a chiral centre), with satisfactory ϕ_Δ values (*ca.* 0.3) [56], and can be formulated for topical application [57] which has obvious benefits as far as selectivity is concerned.



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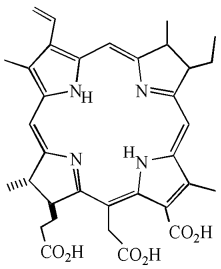

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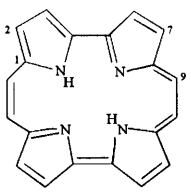
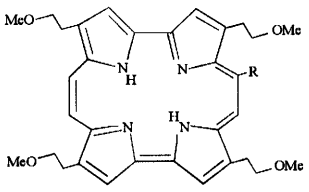
Block 3



32 Porphycene



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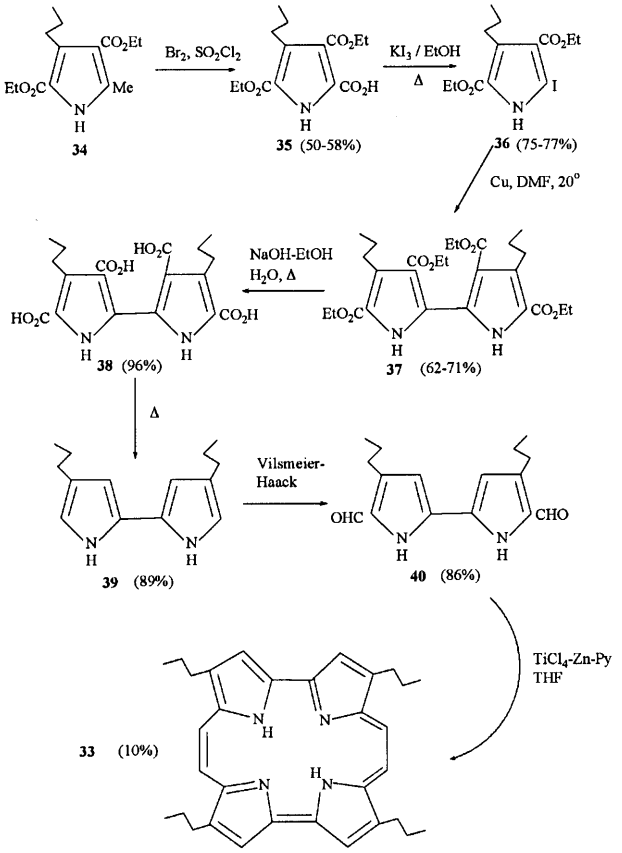
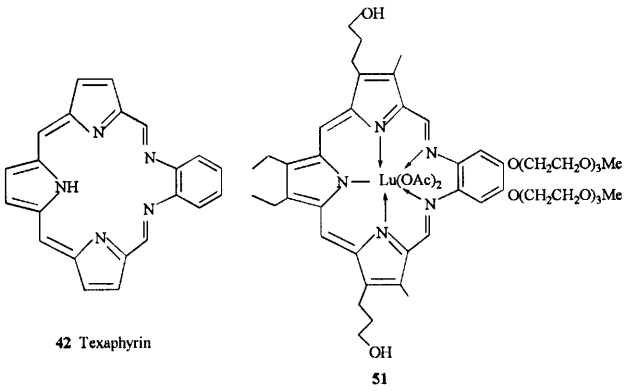
Block 4

Scheme 8



Texaphyrins

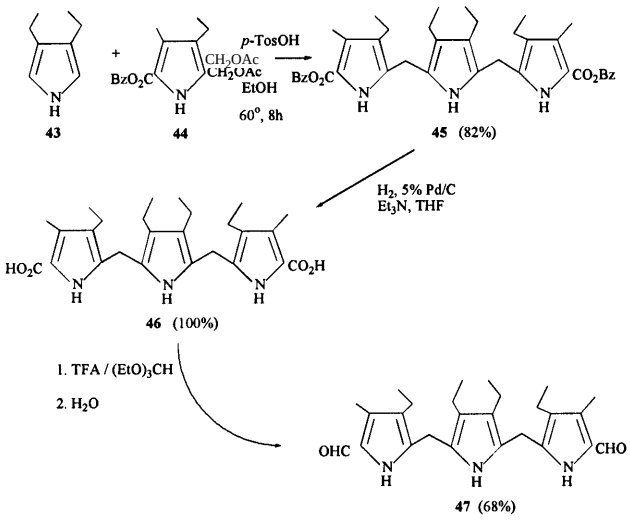
Texaphyrin (parent ring system **42**) is another relative newcomer amongst polypyrrolic macrocycles. Again it has the 18πe aromaticity, but the coordination hole is now expanded from four nitrogen atoms to five, and can consequently accommodate larger ions. For example, whereas cadmium(II) and gadolinium(III) porphyrins are rather unstable because the metal ions are too large, the corresponding metallotexaphyrins are relatively stable.



Block 5

The generation of the tripyrrane system (Scheme 9) is an important and striking feature of the synthetic route. For example [58], condensation of 3,4-diethylpyrrole **43** with the acetoxymethylpyrrole **44** in the presence of *p*-tosic acid in ethanol gives the tripyrrane **45**. Hydrogenolysis of the two benzyl ester groups leads to the free dicarboxylic acid **46**, which can be *bis*-formylated using trifluoroacetic acid and triethylorthoformate to give the diformyl-tripyrrane **47**. Acid catalysed redistribution of pyrrole moieties in the dipyrrylmethane units does not appear to occur. Although lower yields are reported in the original accounts [58], the normal development operation has resulted in overall yields of about 80% for the diformyl-tripyrranes [59].

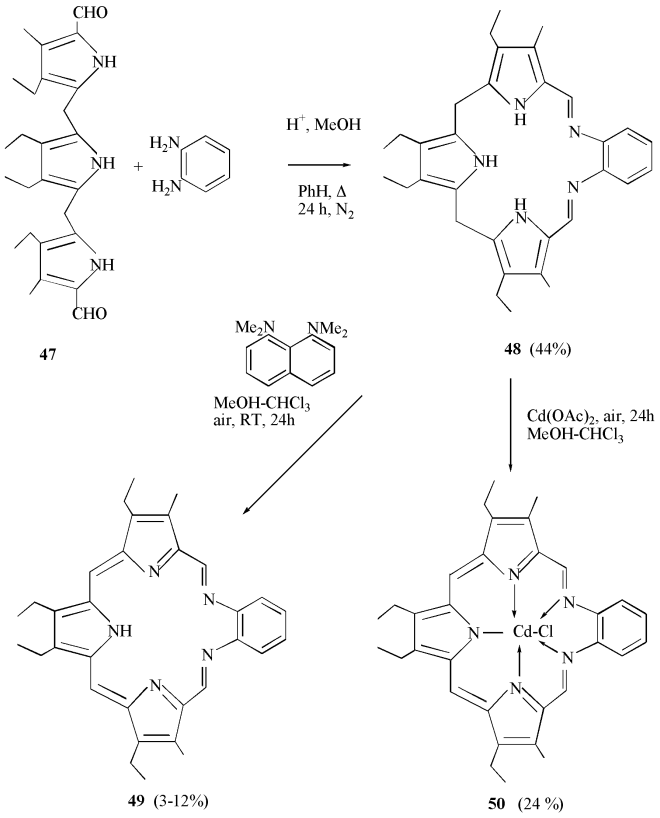
Scheme 9



Condensation of **47** with *o*-phenylenediamine (Scheme 10) generates the macrocycle **48**, which is colourless when pure (like a porphyrinogen) [58]. Aerial oxidation of this in the presence of base (proton sponge) gives the aromatic texaphyrin **49**, albeit in low yield [58]. Better still, metallation and aromatization can be carried out in one step *e.g.* to give the cadmium(II) complex **50** [Scheme 10]. Complexes with a variety of lanthanides [e.g. Gd(III), Lu(III)] can be made in this way [60]. Usually the reaction is carried out on the hydrochloride of the free base (*ie* **48** or analogous structure) in the presence of a quaternary ammonium salt and triethylamine as a buffering system [60]. It is possible to vary the substitution pattern of the pyrrole rings and of the *o*-phenylenediamine unit to modulate solution characteristics.

Pharmacokinetic studies (on **51**) in hamsters show that fluorescence intensity reaches a maximum at about 3 hours after injection, and is greater by a factor of up to 1.5 in

Scheme 10



tumour tissue compared with normal tissue. After 24 hours, fluorescence from **51** is no longer detectable, *ie* long term sensitivity is not observed [62, but see also 63].

Two types of medical application of these substances have emerged. The gadolinium(III) complexes have potential applications as contrast-enhancing agents for magnetic resonance imaging [59], while diverse photodynamic therapeutic activities have been described for lutetium(III) complexes. For example, the lutetium(III) complex **51** with triethylene ether substituents to modulate solution properties (conferring water solubility) has the trade name "Lutrin" (it has also been referred to as PCI-0123 , Lut-ex and motexafin lutetium). It has λ max 732 nm (ε 42,000) in methanol [61]. It is being developed by Pharmacyclics (Sunnyvale, CA) as a PDT drug, and is reported to have completed Phase II trials for recurrent breast cancer of the chest wall in patients where radiation therapy has failed. Other cancers are in clinical study, as well as quite different applications of PDT (*e.g.* AMD, **51** is then referred to as "Optrin"; photoangioplasty of atherosclerosis, **51** is then referred to as "Antrin"). The gadolinium(III) analogue of **51** (called "Xcytrin") is being assessed as a combined MRI contrast agent and X-radiation enhancer [59] (www.pcyc.com).

Phthalocyanines and Naphthalocyanines

The structures of the parent systems are shown in Figure 5.

These are attractive compounds for PDT applications from the point of view of photophysical properties, having strong absorption bands at about 680 nm (phthalocyanines) and about 780 nm (naphthalocyanines) and appreciable ϕ_Δ values. However, solution properties are poor: for example, phthalocyanine and copper(II) phthalocyanine are almost insoluble in common solvents (except concentrated sulphuric acid). Solubility in organic solvents

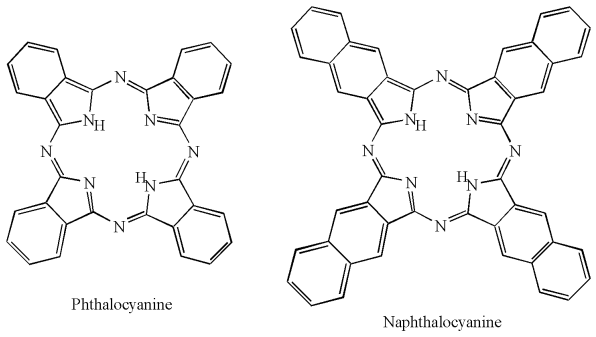
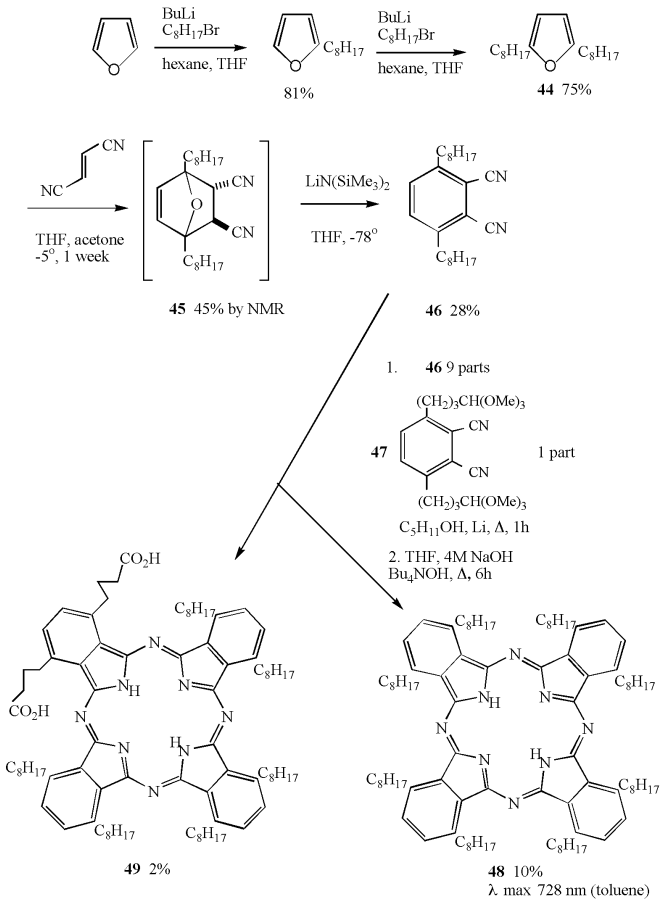


Figure 5. Structures of phthalocyanine and naphthalocyanine.

Scheme 11



can be improved by alkyl substitution - thus, zinc(II) octapentylphthalocyanine (synthesis analogous to Scheme 11) is more soluble in organic solvents, and more effective as a PDT agent, than is zinc(II) phthalocyanine itself [64]. It still has to be administered *in vivo* as an emulsion, however.

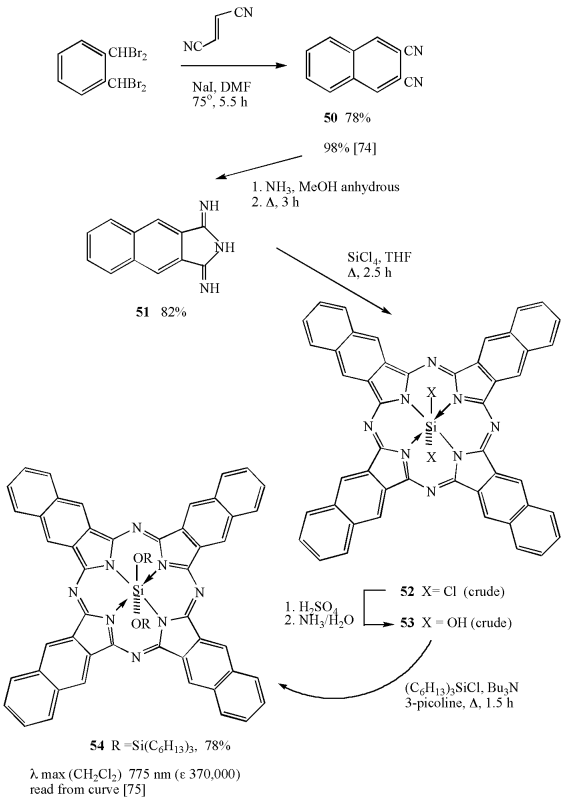
Solubility in aqueous media can be conferred by sulphonation. Thus, sulphonation of chloroaluminium(III) phthalocyanine gives a mixture of mono to tetra sulphonic acids. The mixture is complex, but components can be separated by HPLC [65]. Under the trade name "Photosens" this mixture, thought to be mainly a mixture of the di and tri sulphonic acids **43**, has been in clinical use in Russia for the treatment of cancer by PDT for some years [66].

Another way to change solubility characteristics in a metal complex is to alter axial ligands. Thus, a series of silicon(IV) phthalocyanines with substituted alkoxyl groups as axial ligands have been synthesised (see Scheme 12, for an analogous example) and have shown PDT activity in experimental animals *in vitro* [67] and *in vivo* [68]. Naphthalocyanines have been addressed in the same way. The interest in these long wave absorbers, and especially the naphthalocyanines, is that they offer an approach to the pigmented melanomas, which absorb strongly in the visible [69], and effectively block out the activity of photosensitizers with long wavelength absorption bands at < 700 nm.

Synthetic work in these two series still relies heavily on the 4 x 1 type synthesis devised by Linstead [70,71]. These reactions are reductive cyclotetramerisations [72] where the monomer is typically a phthalonitrile or the derived 1,3-diiminoisoindoline in the phthalocyanine series (Scheme 11); and the corresponding naphthalene compounds in the naphthalocyanine series (Scheme 12). The corresponding imides, anhydrides and carboxylic acids can also serve as starting materials, but in the research laboratory the dinitriles and diiminoisoindolines usually give better yields and purer products.

Thus, in Scheme 11, furan is doubly α-alkylated in two stages to give 2,5-dioctylfuran **44**. Diels-Alder addition of fumaronitrile occurs slowly to give the adduct **45** which is not isolated but treated with lithium di(trimethylsilyl)amide to generate the phhthalonitrile **46** [73].

Scheme 12



When **46** (9 mol.) is heated under basic/reducing conditions ($C_5H_{11}OH$, Li) in the presence of the specially constructed phthalonitrile **47** (1 mol.), (followed by a hydrolytic step), the symmetrically-substituted octaoctylphthalocyanine **48** is isolated in 10% yield, and the prized unsymmetrically-substituted product **49** of the crossed condensation is obtained in 2% yield. **49** can readily be separated because it is a dicarboxylic acid. This mixed synthesis is an example of one approach to non-centrosymmetric systems (see below) which are needed, for example, in the study of molecules possessing non-linear optical properties.

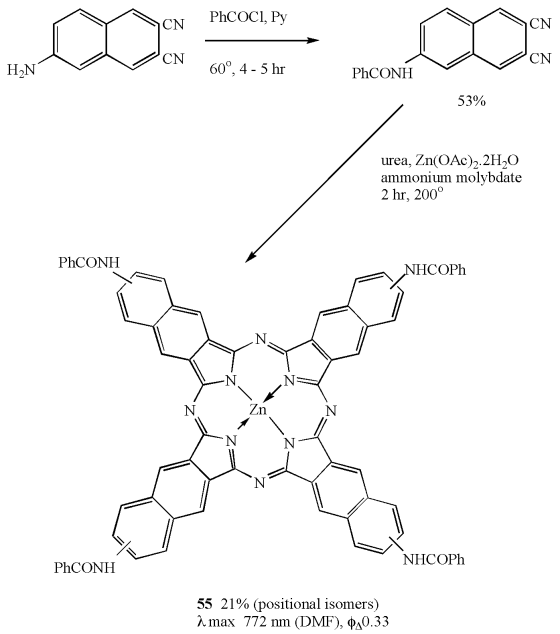
Scheme 12 provides an example of an approach to naphthalocyanines, this time *via* diiminobenzoisoindolines. Reaction of α,α,α′,α′-tetrabromo-*o*-xylene with fumaronitrile in DMF in the presence of sodium iodide gives the 2,3-dicyanonaphthalene **50** [74] (elimination, Diels-Alder, elimination). Cyclisation with anhydrous ammonia gives the 1,3-diiminobenzo[*f*]isoindoline **51** [75]. When this is heated under relatively mild conditions with silicon tetrachloride, the dichlorosilicon(IV) naphthalocyanine **52** is produced. Hydrolysis gives the corresponding dihydroxysilicon(IV) complex **53**, which is pure enough for reaction to give soluble complexes with bis-trialkylsilyloxy axial ligands, such as **54** [75], which can be manipulated much more readily than can naphthalocyanine itself.

Scheme 13 shows another example: in this case the product **55** has been shown, out of a series of closely related structures, to have the highest PDT activity in experimental animals [76].

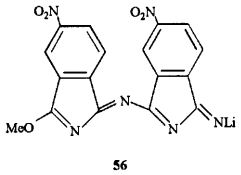
Although many of these syntheses (and especially those starting with diacids or anhydrides and metal salts in the presence of urea as an ammonia source) require rather forcing conditions, it has been shown recently that metal-free phthalocyanines can be obtained in acceptable yields, albeit slowly, from phthalonitriles using lithium metalin 1-octanol at room temperature [77].

The phthalocyanines and naphthalocyanines which can be synthesised by currently available methods have been sufficient to show the potential of these systems as PDT agents, not only in oncological applications [66] but also as photovirucides in the sterilisation of blood products [78]. Nevertheless it would be useful, both here and in electroactive materials applications, to have more flexible stepwise syntheses to hand, such as exist in the related porphyrin chemistry. The synthesis of **49** in Scheme 10 shows one example of the use of a mixed synthesis to produce a non-centrosymmetric system, but yields in such a mixed synthesis are inevitably low, and product separation is needed (although relatively easy in the **48**/**49** case). What is really missing is a true step-by-step synthesis. Some attention has been paid to stepwise synthesis [71,79-81]. However, the *meso*-aza analogues of the pyrromethene system (*e.g.* **25** in Scheme 7), which

Scheme 13



might comprise a suitable intermediate stage, are not well known, and the few examples (*e.g.* **56** [82]) that are recorded appear to be rather sensitive substances [83]. This is an area that deserves further exploration.



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Block 6

Outlook.

As discussed above, several groups of heterocyclic PDT photosensitisers have been identified for use in tumour treatment (porphyrins, chlorins, bacteriochlorins, porphycenes, texaphyrins, phthalocyanines, naphthalocyanines). Amongst these, the chlorins appear *at present* to possess the greatest potential. It can be expected that over the next few years a small number of second generation compounds will receive regulatory approval for tumour PDT, while the synthesis of novel heterocyclic systems for this purpose will continue to be explored.

Moreover, significant new clinical applications for PDT are likely to be identified. Indeed, with **20** ("Visudyne") being granted regulatory approval (2000) in Switzerland and the USA for the photodynamic therapy of wet age-related macular degeneration, they may be said to be already appearing.

REFERENCES AND NOTES

[1] R. Bonnett, Chemical Aspects of Photodynamic Therapy, Gordon and Breach Science Publishers, Amsterdam, 2000.

[2] T. J. Dougherty, B. W. Henderson, S. Schwartz, J. W. Winkelman and R. L. Lipson, in Photodynamic Therapy, B. W. Henderson and T. J. Dougherty, eds, Marcel Dekker, New York, NY, 1992, pp 1-15.

[3] R. Bonnett, *Rev.Contemp. Pharmacother.*,**10**, 1 (1999).

[4] R. Bonnett, *Chem. Soc. Rev*., **24**, 19 (1995).

[5] S. Wan, J. A. Parrish, R. R. Anderson and M. Madden, *Photochem. Photobiol.*, **34**, 679 (1981).

[6] C. S. Foote, J. S. Valentine, A. Greenberg and J. F. Liebman (eds), Active Oxygen in Chemistry, Blackie Academic and Professional, London, 1995.

[7] A. K. Haylett, F. I. McNair, D. McGarvey, N. J. F. Dodd, E. Forbes, T. G. Truscott and J. V. Moore, *Cancer Letters*, **112**, 233 (1997).

[8] A. Colasanti, A. Kisslinger, R. Liuzzi, M. Quarto, R. Riccio, G. Roberti, D. Tramontano and F. Villani, *J. Photochem. Photobiol., B: Biol.*, **54**, 103 (2000).

[9] J. F. Kelly and M. E. Snell, *J. Urol.*, **115**, 150 (1976).

[10] T. J. Dougherty, J. E. Kaufman, A. Goldfarb, K. R. Weishaupt, D. Boyle and A. Mittelman, *Cancer Res.*, **38**, 2628 (1978).

[11] A. Jesionek and H. von Tappeiner, *Dtsch. Arch. Klin. Med.*, **82**, 223 (1905).

[12] H. Berg and W. Jungstand, *Naturwiss.*, **53**, 481 (1966).

[13] J. W. Winkelman, *Cancer Res.*, **22**, 589 (1962).

[14] T. J. Dougherty, *J. Nat. Cancer Inst.*, **52**, 1333 (1974).

[15] S. H. Thomson, E. A. Emmett and S. H. Fox, *Cancer Res.*, **34**, 3124 (1974).

[16] M. Kreimer-Birnbaum, *Seminars in Hematology*, **26**, 157 (1989).

[17] R. L. Lipson and E. J. Baldes, *Arch. Dermatol.*, **82**, 508 (1960).

[18] R. Bonnett, R. J. Ridge, P. A. Scourides and M. C. Berenbaum, *J. Chem. Soc., Perkin Trans. 1*, 3135 (1981).

[19] M. C. Berenbaum, R. Bonnett and P. A. Scourides, *Br. J. Cancer*, **45**, 571 (1982).

[20] C. J. Byrne, L. V. Marshallsay and A. D. Ward, *J. Photochem. Photobiol., B: Biol.*, **6**, 13 (1990).

[21] M. M. Siegel, K. Tabei, R. Tsao. M. J. Pastel, R. K. Pandey, S. Berkenkamp, F. Hillenkamp and M. S. de Vries, *J. Mass Spectrom.*, **34**, 661 (1999).

[22] K. R. Weishaupt, T. J. Dougherty and W. R. Potter, Int. Patent WO 84/01382, (1984). [*Chem. Abstr*., **101**, 116725 (1984)]. Y.-K. Ho, J. R. Missert and T. J. Dougherty, *Photochem. Photobiol.*, **54**, 83 (1991) and references therein.

[23] M. T. Bowser, E. D. Sternberg and D. D. Y. Chen, *Analyt. Biochem.*, **241**, 143 (1996).

[24] Reference 1, pp 19-23, 131, 281.

[25] J. C. Kennedy and R. H. Pottier, *J. Photochem. Photobiol., B: Biol.*, **14**, 275 (1992).

[26] K. Tabata, S. Ogura and I. Okura, *Photochem. Photobiol.*, **66**, 842 (1997).

[27] Review: Q. Peng, T. Warloe, K. Berg, J. Moan, M. Kongshaug, K.-E. Giercksky and J. M. Nesland, *Cancer*, **79**, 2282 (1997).

[28] R. L. Lipson, E. J. Baldes and A. M. Olsen, *J. Nat. Cancer Inst.*, **26**, 1 (1961).

[29] J.-M. Gaullier, K. Berg. Q. Peng, H. Anholt, P. K. Selbo, L.-W. Ma and J. Moan, *Cancer Res.*, **57**, 1481 (1997).

[30] L. R. Milgrom and G. Yahioglu, *Tetrahedron Letters*, **36**, 9061 (1995).

[31] J. A. Lacey, D. Phillips, L. R. Milgrom, G. Yahioglu and R. D. Rees, *Photochem. Photobiol.*, **67**, 97 (1998).

[32] D. A. Bellnier, B. W. Henderson, R. K. Pandey, W. R. Potter and T. J. Dougherty, *J. Photochem. Photobiol., B: Biol.*, **20**, 55 (1993).

[33] W. R. Potter, B. W. Henderson, D. A. Bellnier, R. K. Pandey, L. A. Vaughan, K. R. Weishaupt and T. J. Dougherty, *Photochem. Photobiol.*, **70**, 781 (1999).

[34] R. K. Pandey, A. B. Sumlin, S. Constantine, M. Aoudia, W. R. Potter, D. A. Bellnier, B. W. Henderson. M. A. Rodgers, K. M. Smith and T. J. Dougherty, *Photochem. Photobiol.*, **64**, 194 (1996).

[35] R. K. Pandey and C. K. Herman, *Chem. Ind.*, 739 (1998).

[36] V. S. Pangka, A. R. Morgan and D. Dolphin, *J. Org. Chem.*, **51**, 1094 (1986). E. D. Sternberg, D. Dolphin and C. Brückner, *Tetrahedron*, **54**, 4151 (1998).

[37] G. Donati, A. D. Kapetanios and C. J. Pournaras, *Seminars Opthalmol.*, **14**, 2 (1999).

[38] R. Bonnett, S. Ioannou, R. D. White, U.-J. Winfield and M. C. Berenbaum, *Photobiochem. Photobiophys.*, Suppl.., 45 (1987).

[39] R. Bonnett, S. Ioannou, R. D. White, U.-J. Winfield and M. C. Berenbaum, *Biochem. J.*, **261**, 277 (1989). R. Bonnett, P. Charlesworth, B. D. Djelal, S. Foley, D. J. McGarvey and T. G. Truscott, *J. Chem. Soc., Perkin Trans. 2*, 325 (1999).

[40] M. G. Dilkes, M. L. De Jode, A. Rowntree-Taylor, J. A. McGilligan, G. S. Kenyon and P. McKelvie, *Lasers Med. Sci.*, **11**, 23 (1996).

[41] H. Fischer, E. Baumann and H. J. Riedl, *Liebig's Ann. Chem.*, **475**, 205 (1929). K. M. Smith, *J. Chem. Soc., Perkin Trans 1*, 1471 (1972).

[42] A. R. Morgan and N. C. Tertel, *J. Org. Chem.*, **51**, 1347 (1986). A. R. Morgan, A. Rampersaud, G. M. Garbo, R. W. Keck and S. H. Selman, *J. Med. Chem.*,**32**, 904 (1989).

[43] A. R. Morgan, G. M. Garbo, R. W. Keck and S. H. Selman, *Cancer Res.*, **48**, 194 (1988).

[44] R. B. Woodward, W. A. Ayer, J. M. Beaton, F. Bickelhaupt, R. Bonnett, P. Buchschacher, G. L. Closs, H. Dutler, J. Hannah, F. P. Hauck, S. Ito, A. Langemann, E. Le Goff, W. Leimgruber, W. Lwowski, J. Sauer, Z. Valenta and H. Volz, *J. Am. Chem. Soc.*, **46**, 7599 (1990).

[45] N. Razum, A. Snyder and D. Dorion, *Proc. SPIE*, **2675**, 43 (1996).

[46] Reference 1, pp 183-198.

[47] M. F. Grahn, A. McGuinness, R. Benzie, R. Boyle, M. L. de Jode, M. G. Dilkes, B. Abbas and N. S. Williams, *J. Photochem. Photobiol., B: Biol.*, **37**, 261 (1997).

[48] A. N. Kozyrev, R. K. Pandey, C. J. Medforth, G. Zheng, T. J. Dougherty and K. M. Smith, *Tetrahedron Letters*, **37**, 747 (1996).

[49] M. Hoebeke, H. J. Shuitmaker, L. E. Jannick, T. M. A. R. Dubbelman, A. Jakobs and A. Van der Vorst, *Photochem. Photobiol.*, **66**, 502 (1997).

[50] E. Vogel, M. Köcher, M. Balci, I. Teichler, J. Lex, H. Schmickler and O. Ermer, *Angew. Chem. Int. Ed. Engl.*, **26**, 931 (1987).

[51] E. Vogel, M. Köcher, H. Schmickler and J. Lex, *Angew. Chem. Int. Ed. Engl.*, **25**, 257 (1986).

[52] E. Vogel, M. Balci, K. Pramod, P. Koch, J. Lex and O. Ermer, *Angew. Chem. Int. Ed. Engl.*, **26**, 928 (1987).

[53] M. Guardiano, R. Biolo, G. Jori and K. Schaffner, *Cancer Letters*, **44**, 1 (1989).

[54] A. Segalla, F. Fedeli, E. Reddi, G. Jori and A. Cross, *Int. J. Cancer*, **72**, 329 (1997).

[55] R.-M. Szeimies, S. Karrer, C. Abels, P. Steinbach, S. Fickweiler, H. Messmann, W. Bäumler and M. Landthaler, *J. Photochem. Photobiol., B: Biol.*, **34**, 67 (1996).
S. Fickweiler, C. Abels, S. Karrer, W. Bäumler, M. Landthaler, F. Hofstädter and R.-M. Szeimies, *J. Photochem. Photobiol., B: Biol.*, **48**, 27 (1999).

[56] P. F. Aramendia, R. W. Redmond, S. Nonell, W. Schuster, S. E. Braslavsky, K. Schaffner and E. Vogel, *Photochem. Photobiol.*, **44**, 555 (1986).

[57] S. Karrer, C. Abels, R.-M. Szeimies, W. Bäumler, M. Dellian, U. Hohenleutner, A. E. Goetz and M. Landthaler, *Arch. Dermatol. Res.*, **289**, 132 (1997).

[58] J. L. Sessler, M. R. Johnson and V. Lynch, *J. Org. Chem.*, **52**, 4394 (1987). J. L. Sessler, T. Murai and V. Lynch, *Inorg. Chem.*, **28**, 1333 (1989). J. L. Sessler, T. Murai, V. Lynch and M. Cyr, *J. Am. Chem. Soc.*, **110**, 5586 (1988).

[59] Reviews: J. L. Sessler, G. Hemmi, T. D. Mody, T. Murai, A. Burrell and S. W. Young, *Acc. Chem. Res.*, **27**, 43 (1994). J. L. Sessler and R. A. Miller, *Biochem. Pharmacol.*, **59**, 733 (2000).

[60] J. L. Sessler, T. D. Mody, G. W. Hemmi and V. Lynch, *Inorg. Chem.*, **32**, 3175 (1993).

[61] S. W. Young, K. W. Woodburn, M. Wright, T. D. Mody, Q. Fan, J. L. Sessler, W. C. Dow and R. A. Miller, *Photochem. Photobiol.*, **63**, 892 (1996).

[62] M. Zellweger, A. Radu, P. Monnier, H. van den Bergh and G. Wagnières, *J. Photochem. Photobiol.,B: Biol.*, **55**, 56 (2000).

[63] G. Kostenich, A. Orenstein, L. Roitman, Z. Malik and B. Ehrenberg, *J. Photochem. Photobiol., B: Biol.*, **39**, 36 (1997).

[64] C. Fabris, C. Ometto, C. Milanesi, G. Jori, M. J. Cook and D. A. Russell, *J. Photochem. Photobiol., B: Biol.*, **39**, 279 (1997).

[65] M. Ambroz, A. Beeby, A. J. MacRobert, M. S. C. Simpson, R. K. Svensen and D. Phillips, *J. Photochem. Photobiol., B: Biol.*, **9**, 87 (1991).

[66] E. F. Stradnadko, O. K. Skobelkin, G. N. Vorozhtsov, A. F. Mironov, S. E. Besheul, N. A. Markitchev and M. V. Riabov, *Proc. SPIE*, **3191**, 253 (1997).

[67] J. He, H. E. Larkin, Y.-S. Li, B. D. Rihter, S. I. A. Zaidi, M. A. J. Rodgers, H. Mukhtar, M. E. Kenney and N. L. Oleinick, *Photochem. Photobiol.*, **65**, 581 (1997).

[68] D. Wöhrle, S. Muller, M. Shopova, V. Mantareva, G. Spassova, F. Vietri, F. Ricchelli and G. Jori, *J. Photochem. Photobiol., B: Biol.*, **50**, 124 (1999).

[69] M. Soncin, A. Busetti, R. Biolo, G. Jori, G. Kwag, Y.-S. Li, M. E. Kenney and M. A. J. Rodgers, *J. Photochem. Photobiol., B: Biol.*, **42**, 202 (1998).

[70] G. T. Byrne, R. P. Linstead and A. R. Lowe, *J. Chem. Soc.*, 1017 (1934). R. P. Linstead and A. R. Lowe, *J. Chem. Soc.*, 1022 (1934).

[71] For a review of synthetic work on metal-free phthalocyanines see C. C. Leznoff, in Phthalocyanines: Properties and Applications, Vol. **1**, C. C. Leznoff and A. P. B. Lever, eds, VCH Publishers, New York, NY, 1989, pp 1-54.

[72] Reference 1, p 219.

[73] N. B. McKeown, I. Chambrier and M. J. Cook, *J. Chem. Soc., Perkin Trans. 1*, 1169, (1990).

[74] E. I. Koshev, V. A. Puchova and E. A. Luk'yanets, *Zh. Org. Khim.*, **7**, 369 (1971). [English translation: *J. Org. Chem. USSR*, **7**, 364 (1971)].

[75] B. L. Wheeler, G. Nagasubramanian, A. L. Bard, L. A. Schectman, D. R. Dininny and M. E. Kenney, *J. Am. Chem. Soc.*, **106**, 7404 (1984).

[76] S. Müller, V. Mantareva, N. Stoichkova, H. Kliesch, A. Sobbi, D. Wöhrle and M. Shopova, *J. Photochem. Photobiol., B: Biol.*, **35**, 167 (1996).

[77] C. C. Leznoff, A. M. D'Ascanio and S. Z. Yildiz, *J. Porphyrins Phthalocyanines*, **4**, 103 (2000).

[78] A. C. E. Moor, A. E. Wagenaars-van Gompel, R. C. A. Hermanns, J. van der Meulen, J. Smit, J. Wilschut, A. Brand, T. M. A. R. Dubbelmen and J. VanSteveninck, *Photochem. Photobiol.*, **69**, 353 (1999). For a Symposium-in Print (E. Ben-Hur and B. Horowitz, eds) on this topic see: *Photochem. Photobiol.*, **65**, 427-470 (1997).

[79] R. Bonnett and K. A. McManus, *J. Chem. Soc., Perkin Trans. 1*, 2461 (1996). R. Bonnett and K. Okolo, *J. Porphyrins Phthalocyanines*, **3**, 530 (1999).

[80] S. Dabak and Ö. Bekaroglu, *J. Chem. Res. (S)*, 8 (1997); *J. Chem. Res. (M)*, 0152 (1997).

[81] T. Torres, *J. Porphyrins Phthalocyanines*, **4**, 325 (2000).

[82] S. W. Oliver and T. D. Smith, *J. Chem. Soc., Perkin Trans. 2*, 1579 (1987).

[83] M. Hu, N. Brasseur, S. Z. Yildiz, J. E. van Lier and C. C. Leznoff, *J. Med. Chem.*, **41**, 1789 (1998).