Photosensitizers of the Porphyrin and Phthalocyanine Series for Photodynamic Therapy

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1 Introduction

Phototherapy is the use of visible or near-visible light as a therapeutic (hopefully, curative) agent in clinical medicine. It falls into two categories: *direct*, without an administered photosensitizer (the treatment of neonatal jaundice with blue/white light being an important example here); and *indirect*, where the effect is achieved *via* an administered photosensitizer which is the effective light absorber. Of course, light must be absorbed in either case, but it is useful nonetheless to make the distinction because the administration of both sensitizing drug and a dose of light in the indirect category means that consideration must be given to additional parameters (*e.g.* chemical structure of the photosensitizer, length of drug–light interval). This essay concerns the indirect category, and in particular the design of sensitizing molecules which can photoinitiate the destruction of tumours.

Phototherapy is generally considered to have originated with Finsen, who in the 1890's treated *lupus vulgaris*, a tubercular condition of the skin, in the direct mode with heat-filtered light from a carbon arc lamp.[1] Finsen was a Dane, a countryman of Queen Alexandra, and it was she who was instrumental in establishing the treatment in Great Britain at the London Hospital, Whitechapel (which, indeed, had a Light Department in the first decade of this century. The original light source is now in the Science Museum in South Kensington). In spite of this royal patronage, and the award of the Nobel prize to Finsen in 1903, in subsequent years phototherapy seems to have made rather halting progress, partly, I suspect, because light, as a feature of everyday experience, seems unlikely as a curative agent, and partly because exaggerated claims were made along the way. However, phototherapy has now established a significant niche position in medicine, important for example in the treatments of vitamin D deficiency, of psoriasis, and of neonatal hyperbilirubinemia.[2]

One of the most active research areas in this field at present is tumour phototherapy. The subject has become known as photodynamic therapy (PDT),[3] which arises from the term *photodynamische Wirkung* (photodynamic effect) coined by German physiologists to describe the damage of living tissue by a combination of photosensitizer, visible light, and oxygen.

The basic idea of tumour phototherapy is this: (a) find a good photosensitizer which shows some selectivity for photodamage to tumour tissue; (b) inject the photosensitizer and wait for a certain time (which will depend on the photosensitizer and the tumour type) for equilibration between biological compartments to give the maximum differentiation between normal and tumour tissue—at this stage the tumour will fluoresce in ultraviolet light if the photosensitizer is a porphyrin (diagnostic mode); and (c) irradiate the tumour with visible light. For Type II photosensitizers singlet oxygen will then be generated within the tumour, which will be destroyed preferentially (therapeutic mode). The three steps are illustrated schematically in Figure 1.

Since the photosensitizer is chosen to have some selectivity for the tumour, and since light is highly directional, it is possible to target the tumour with some precision. In principle any visible light source can be used: Kennedy in Ontario has for several years used a slide projector lamp with considerable success. However laser sources have certain advantages. Using fibre optics and a laser source it is possible to irradiate internal tumours, so that the method is not restricted to tumours at or near the surface. It is not the power of the laser that is of chief importance, since in this application the laser is *not* being used as a cutting tool. Rather the advantage of the laser beam resides in its coherence, and the resulting efficient address of, and transmission by, optical fibres.

2 Mechanisms

Photobiochemical processes fall broadly into two classes. One group, which includes photosynthesis and vision, has had the benefit of Darwinian evolution, with molecular and macroscopic structures, assemblies and compartments so contrived that the reaction process follows essentially the biologically desirable pathway. The second group, which includes the photodynamic effect, is adventitious. The photosensitizer is a foreign molecule, a xenobiotic, and as with any other drug its metabolism is likely to follow a multiplicity of pathways. Moreover photochemical processes are involved here, and the excited state, and activated species derived from it, will inevitably find a variety of target biomolecules in the cell. Hence it does not make much sense to think in terms of a single mechanism for the photodynamic effect.

It is also important to regard the matter of mechanism in a broader way, which relates to the obviously interdisciplinary nature of the topic. Besides the molecular reactions which the organic chemist is used to rationalize in terms of electronic mechanism (items 3 & 4 of Scheme 1), there are the transport and localization processes (item 1) and the photophysical processes (item 2) which are an important part of the overall mechanism.

Raymond Bonnett was born in London in 1931. After a spell in the Royal Air Force, he continued his education at Imperial College. He moved to Cambridge for his Ph.D. (1957) under Sir Alexander Todd and A. W. Johnson on the chemistry of vitamin B_{12} and then joined R. B. Woodward's group working on the synthesis of chlorophyll. After two years at the University of British Columbia (1959–61) Bonnett returned to London, to Queen Mary College, where he has been ever since. (Chair in organic chemistry in 1976; head of the Chemistry Department 1982–1987.) He is now Scotia Research Professor. His research interests concern the chemistry of porphyrins and related compounds, on which he has published over 200 papers.

Abbreviations: δ-ALA = δ-aminolaevulinic acid; GPC = gel permeation chromatography; HpD = haematoporphyrin derivative; $MPcS_n$ = metallophthalocyanine with *n* sulfonic acid groups; Pc = phthalocyanine; PDT = photodynamic therapy; o,(m,p)-THPP = 5,10,15,20-tetrakis(*o,m,* or *p*-hydroxyphenyl)porphyrin; o,(m,p)-THPC = the corresponding chlorin; m-THPBC = the *meta* isomer of the corresponding bacteriochlorin; TPP = 5,10,15,20-tetraphenylporphyrin; $TPPS_x$ = TPP with *p*-sulfonic acid groups on *x* phenyl rings; $TPPS_{2o}$, $TPPS_{2a}$ = disulfonic acids of TPP with the *p*-sulfonic acid functions on opposite and adjacent phenyl rings, respectively.

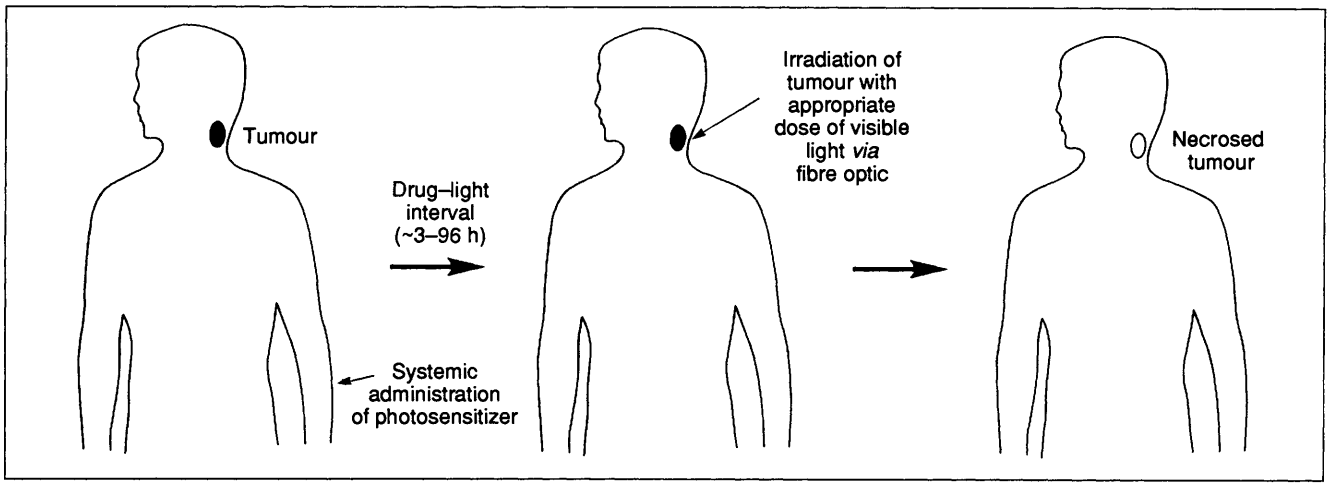


Figure 1 Schematic of tumour phototherapy using a sensitizing drug (photodynamic therapy).

(1) *In vivo* Interactions
Pharmacokinetics: distribution of photosensitizer in tissues and cells; localization, clearance rate
Structure–activity: chemical structure, partition coefficient
Relation of photonecrosis to photosensitizer structure, location, concentration

(2) Excitation
Absorption characteristics
Energies, quantum yields, lifetimes of excited states
Quantum yield of $^1O_2$ formation

(3) Reaction of Excited Species
(a) Electron transfer → radical processes (*e.g.* crosslinking of proteins) (TYPE I)
(b) Energy transfer → singlet oxygen (*e.g.* 5α-hydroperoxide from cholesterol) (TYPE II)
(c) Photobleaching

(4) Reactions of Reactive Secondary Products *in vivo*
e.g. singlet oxygen, superoxide, cyclic peroxides

Scheme 1 The photodynamic effect – four areas of mechanistic consideration.

Having said that, it is not unreasonable to look for a major pathway of photonecrosis, and at present it appears to be generally agreed that singlet oxygen is the key agent of cellular damage. In other words the Type II photooxygenation process predominates over the Type I. The Type II (singlet oxygen) reaction is well documented in the chemical literature, where porphyrin photosensitizers, *e.g.* zinc(II) tetraphenylporphyrin, are quite commonly employed in photooxygenation processes. Thus, the principal cause of photodamage in PDT is regarded as involving the following processes [where P = photosensitizer, S_0,S_1... ground, first excited, singlet states, T_0,T_1... ground, first excited, triplet states, isc = intersystem crossing, $^3O_2$ = ground state triplet dioxygen, $^1O_2$ = first excited singlet state of dioxygen ($^1\Delta_g$)].

$$P(S_0) \xrightarrow{h\nu} P(S_1) \xrightarrow{\text{isc}} P(T_1)$$
$$P(T_1) + {}^3O_2 \longrightarrow P(S_0) + {}^1O_2$$
$$\text{Biomolecules} + {}^1O_2 \longrightarrow \text{products}$$

The processes involved are summarized in the extended Jablonski diagram shown in Figure 2. The biomolecules which react

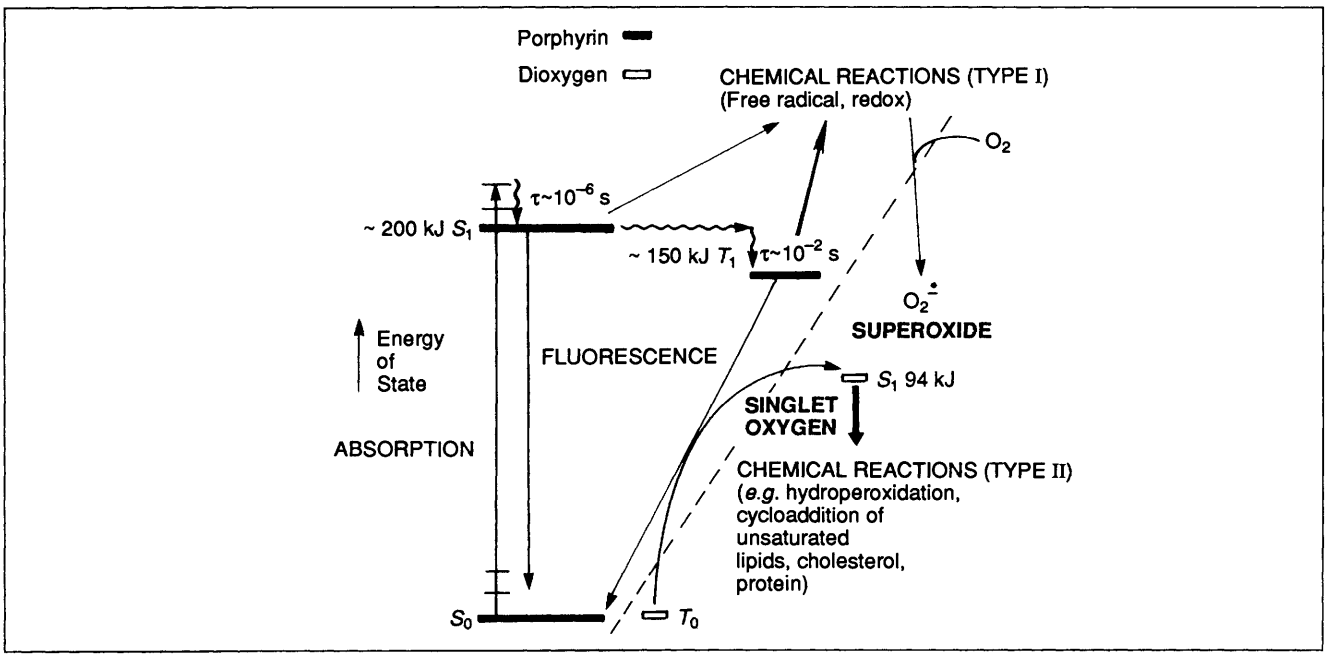


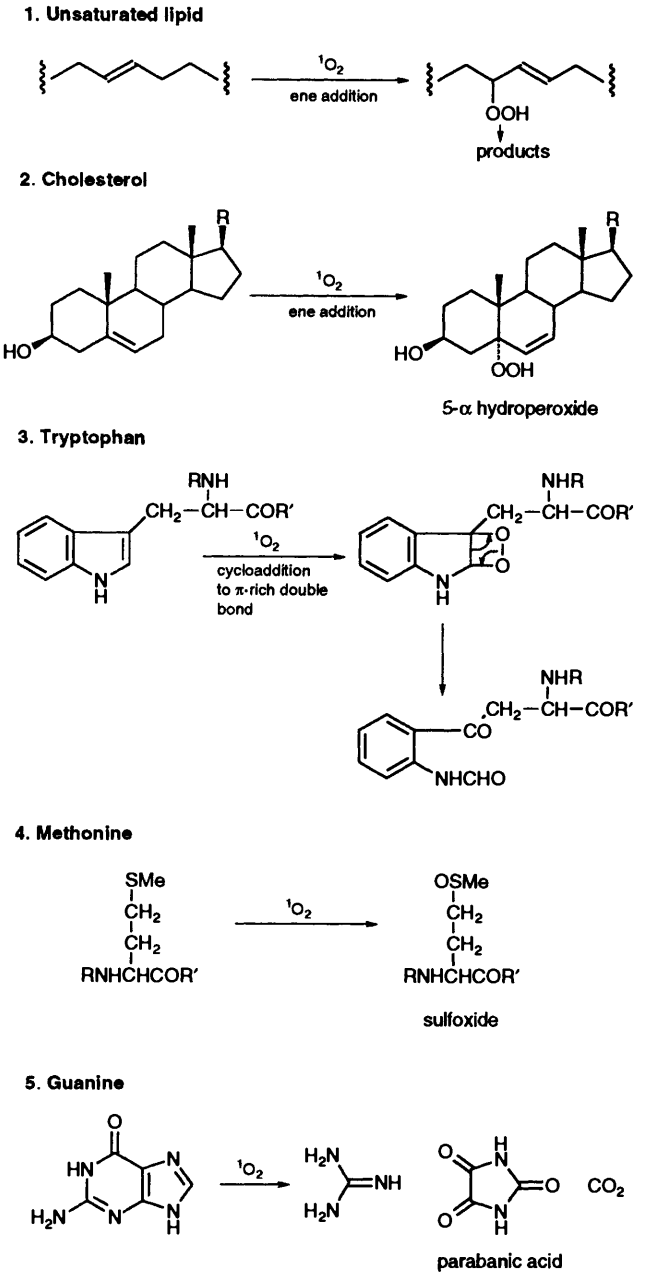
Figure 2 Generation of excited porphyrin states and reactive dioxygen species. State energies are represented by thick lines: ▬ porphyrin sensitizer, ▭ dioxygen. Reactive dioxygen intermediates are in bold type.

with singlet oxygen include unsaturated lipids (including cholesterol), and α-amino-acid residues. Tryptophan is the most reactive, but histidine and methionine also react (see Scheme 2). Since unsaturated lipids and proteins are essential constituents of biological membranes, which have important roles in containing and organizing biological systems, it is not unreasonable to suppose that membrane damage, leading, for example to vascular shutdown, could be one important cause of photonecrosis. There is now a considerable body of evidence which supports this idea.

It has been difficult to obtain direct spectroscopic evidence for the involvement of singlet oxygen in photodynamic therapy: recent experiments indicate that singlet oxygen emission can be detected from porphyrins adsorbed on the cell surface,[4] but such emission is difficult to detect from internally localized porphyrin generators, presumably because of the rapid reaction of the singlet oxygen (Scheme 2). However, cell cultures *in vitro* are to some extent protected against the photodynamic effect in the presence of 1,3-diphenylisobenzofuran, which is interpreted in terms of the scavenging of singlet oxygen by the additive.[5]

Recently a copper complex (of a benzochlorin iminium salt) has been found to be a tumour photosensitizer.[6] The result is surprising because the excited states of transition metal complexes are so short-lived that such compounds are not effective photosensitizers in Type II reactions (for example, the haem in haemoglobin). The process requires oxygen, and the rate is not increased in D_2O. Possibly an electron-transfer process to give superoxide (Figure 2) is occurring since photosensitization in an erythrocyte ghost model was decreased in the presence of superoxide dismutase and of catalase.

Transport mechanisms for systemically administered sensitizers involve various plasma components, proteins and lipoproteins, depending on the hydrophobicity of the compound. The processes by which tumour localization occurs are not understood, but six hypotheses have been considered.[7] Macroscopic damage appears to occur by three pathways: (i) direct damage to tumour cells, (ii) damage to endothelial cells of the vascular system of the tumour, and (iii) macrophage-mediated infiltration of the tumour.[7]



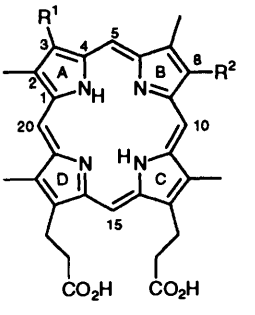
Scheme 2 Reactions of singlet oxygen with some biomolecules.

3 Haematoporphyrin Derivative

Haematoporphyrin derivative, together with its commercial variants Photofrin, Photosan, Photogem, and Photocarcinorin, holds an important place in the story of tumour phototherapy. These are the first generation photosensitizers. The first clearcut observations of activity were made with this material, and the first regulatory authorizations for clinical use were obtained on its behalf (for Photofrin).

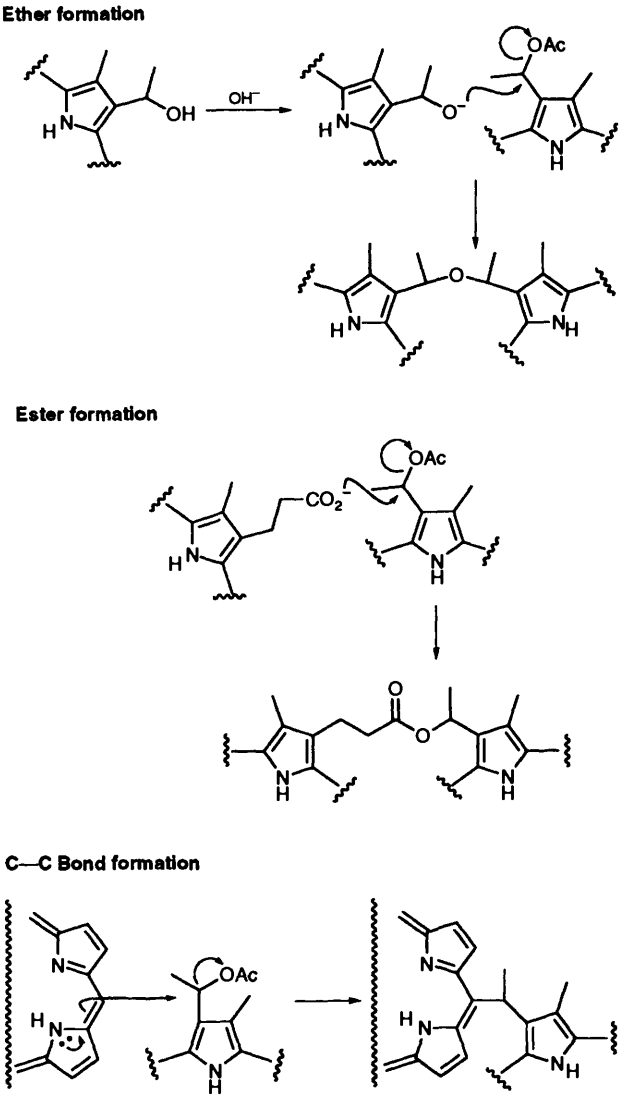
Haematoporphyrin derivative (HpD) was described by Lipson and his colleagues[8] in 1961 as a diagnostic tool. After systemic administration of HpD, tumour tissue could be visualized in ultraviolet light because of the red emission that it showed due to porphyrin fluorescence. It was some 11 years later that the tumour-killing potential was clearly demonstrated (actually with haematoporphyrin),[3] and shortly afterwards there were several reports of the efficacy of HpD in PDT.[9] Haematoporphyrin derivative seems originally to have been produced as a way of solubilizing haematoporphyrin in aqueous media at neutral pH. It is prepared by treating haematoporphyrin (1) or its hydrochloride with 5% sulfuric acid in acetic acid at room temperature for 30 minutes. This gives a purple solid (HpD Stage I) which contains about ten principal components, the major one being haematoporphyrin diacetate (2).[10]

In order to produce a solution for injection, this solid is treated with aqueous base and then brought back to neutrality (HpD Stage 2). This causes hydrolysis and elimination of the 2-acetoxyethyl functions, to give back haematoporphyrin and to generate 3(8)-hydroxylethyl-8(3)-vinyldeuteroporphyrin (3) and protoporphyrin (4). However *in vivo* bioassay shows that the photonecrotic activity of HpD stage 2 resides not with these components, but with higher molecular weight material ('frac-



(1) $R^1 = R^2 = CH(OH)Me$
(2) $R^1 = R^2 = CH(OAc)Me$
(3) $R^1(R^2) = CH(OH)Me$, $R^2(R^1) = CH{=}CH_2$
(4) $R^1 = R^2 = CH{=}CH_2$

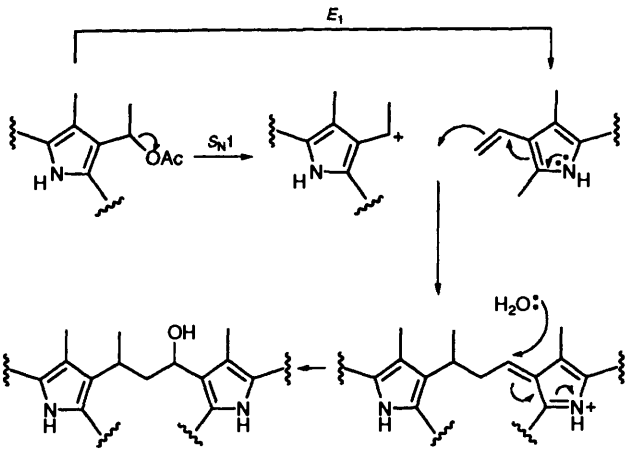
tion D') which we originally postulated to be a mixture of porphyrin dimers and oligomers involving ether, ester, and carbon–carbon interporphyrin linkages.[11] The formation of these linkages is rationalized in Scheme 3. The commercially produced purified photosensitizers (Photofrin, Photocarcinorin, Photosan, Photogem*) are prepared from HpD Stage II by removing much of the monomer fraction using HPLC or gel permeation chromatography.



Scheme 3 Rationalization of oligomer formation during the alkaline treatment of HpD Stage I [or of haematoporphyrin diacetate, (2)].

A considerable amount of work has been published on these polymers. Generally the ester and (especially) the ether interporphyrin linkages have been supported. Indeed, in one ill-considered development the active component was formulated as 'dihaematoporphyrin ether', but it is now recognized that this is erroneous. A GPC analysis by Russian workers[12] gives the composition as monomer: dimer: oligomer = 22:23:55 for HpD and 14:19:67 for Photofrin. The carbon–carbon bridged system (meso-βC^1) shown in Scheme 3 has not yet been found, but a carbon–carbon bridge (βC^1–βC^2) involving the 2-hydroxyethyl functions has been identified by Australian and U.S. chemists.[13] The formation of this internuclear linkage is rationalized in Scheme 4. As a result of this interest, a number of synthetic approaches to porphyrin dimers and trimers with ether, ester,

* Since Russian lacks an h, this should presumably be transliterated as Photohem: but I prefer it the way it is.



Scheme 4 Formation of C_3-bridged porphyrin oligomer.

and carbon–carbon linkages have been described. Interestingly, it appears that vinyl substituents confer bioactivity.

Although HpD and its commercial variants have been used extensively in experimental clinical work, these first generation photosensitizers have three important disadvantages. Firstly they are not very selective, and cause skin sensitivity for some weeks. Secondly the absorption band in the red (Band I at *ca.* 630 nm) is weak, *i.e.* the material is not a good absorber of the light available (Section 4.5). Thirdly they are complex and variable mixtures from which it has not proved possible to isolate a single highly active constituent, and which probably does not contain one. The complexity of the mixture arises from both positional isomerism and stereoisomerism as shown in Figure 3 for the case of 'dihaematoporphyrin ether'.

While the gradually mounting appreciation of the benefits of this form of cancer treatment has been such as to outweigh these disadvantages, so that regulatory bodies (for example in

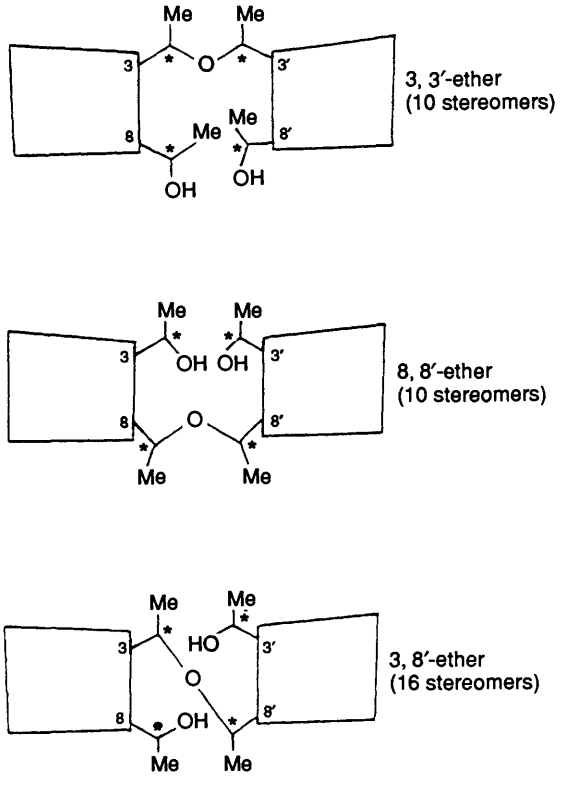


Figure 3 Positional and stereo-isomerism of 'dihaematoporphyrin ether'. Each irregular quadrilateral represents the rest of the haematoporphyrin structure shown in (1).

Canada, Japan, and Holland) have at last approved the clinical use of Photofrin, it has been evident from the early 1980's that the development of one or more second generation PDT photosensitizers with enhanced activity and selectivity would be an important step forward The rest of this review is devoted to this development

4 Design Criteria for Second Generation Photosensitizers

In the early 1980's we began a systematic search for an improved tumour photosensitizer The plan we formulated was to design likely candidate molecules, to synthesize them, and then to screen them using an *in vivo* biological assay The chemistry was to be done at Queen Mary and the biology at St Mary's in doing this Morris Berenbaum and I built up a productive working partnership which lasted from 1976 until he died in 1991 We had considered an *in vitro* bioassay (tissue or cell culture) for the screening process It was concluded that this was too far removed from the clinical situation to be a useful screen, although it was useful as a preliminary test of dark toxicity

Berenbaum devised an *in vivo* biological assay for tumour photonecrosis based on the depth of vascular destruction in a tumour implant (a PC6 myeloma) growing on the flank of a mouse In order to screen a reasonable number of substances it was necessary to standardize the light dose (at 10 J cm^{-2}) and the drug-light interval (at 24 hours), but photosensitizer doses were varied

The light was delivered at the wavelength corresponding to the lowest energy wavelength maximum (Band I) of the photosensitizer when dissolved in a biological fluid (foetal calf serum) It is worth noting that this was at slightly longer wavelength (by a few nanometers) than λ_{max} in an organic solvent

Initial experiments with HpD prepared from various haematoporphyrin samples on different scales showed that the biological activity was difficult to reproduce (It is well known that, because it is a bisbenzylic alcohol, haematoporphyrin (1) is obtained pure only with much difficulty Commercial samples are usually very impure) At first there was little to guide us, but as the number of new photosensitizers built up, and as literature reports appeared from other laboratories, we began to get a feel for the design features of a good photosensitizer for tumours, which appear to be as follows

4.1 Single Substances

The preference must be for single substances, because this simplifies the interpretation of dose–response relationships in a situation which is already much more complex than usual because of the extra variables to do with the light treatment (drug–light interval, wavelength, total energy, fluence rate, continuous or intermittent) With a multi-component photosensitizer the rational interpretation of the causes of the overall effect becomes very difficult Hence the clear preference for a pure photosensitizer

Since the photosensitizers are solids, a liquid medium is needed for injection purposes Amphiphilic and polar molecules may be dissolved in polar solvents (*e g* buffer, EtOH–H_2O–polyethyleneglycol) while hydrophobic sensitizers have been administered in phospholipid liposomes, with the aid of plasma lipoproteins, or with an oil-based emulsion

Presumably because the porphyrin system is a good singlet oxygen generator, most of the second generation photosensitizers which have been described belong to the porphyrin series, broadly defined But there seems no inherent reason for this restriction Other singlet oxygen photosensitizers (*e g* perylenequinones, triarylmethane dyes, phenothiazines) have been considered and, indeed, the first experiments on cancer treatment in man by topical application (von Tappeiner and Jesoniak, 1903) used eosin as the photosensitizer

4.2 Toxicity and Stability

The substance should have little or no dark toxicity (The majority of the tetrapyrrole photosensitizers we have tested meet this criterion but many fail on the basis of inadequate, or too generalized, phototoxicity) It is desirable that the molecule should be sufficiently robust (kinetically and thermodynamically stable) to confer an adequate shelf-life, and that the synthetic route is short, convenient, and high-yielding

4.3 Photophysical Parameters

For use in the diagnostic mode, the fluorescence quantum yield needs to be appreciable For the therapeutic application it is the triplet yield, lifetime, and energy that are most important The energy, E_T, of the triplet needs to be $\geq$94kJ mol^{-1} for efficient energy transfer to ground state dioxygen For singlet oxygen reactions, the key parameter overall is the quantum yield (Φ_Δ) of singlet oxygen

It emerges that, in the absence of heavy atom substitution and coordination of transition metal ions, porphyrin and its relatives (Section 5) generally satisfy the photophysical criteria Φ_T and Φ_Δ values are in many cases greater than 0 5, which appears to be adequate for the purpose in hand Thus *meso*-tetraphenylporphyrin (10), which as such or as its zinc complex is frequently used as a sensitizer in photooxygenation reactions, has $\Phi_T = 0\ 67$ and $\Phi_\Delta = 0\ 63$ (benzene, air) [14] Haematoporphyrin (1) in aqueous methanol has $\Phi_T = 0\ 83$ and $\Phi_\Delta = 0\ 65$ in aqueous quaternary detergent the Φ_Δ value falls to 0 35 Some illustrative values for other sensitizers are given in Table 1

4.4 Hydrophobicity and Hydrophilicity

Some of the photosensitizers which are being studied are sufficiently hydrophobic that they have to be injected in micellar suspensions (Section 4 1) The porphyrin skeleton is essentially hydrophobic, and the working hypothesis has emerged that tumour localization can be improved by introducing polar substituents to confer amphiphilicity and improve selectivity Sulfonic acid, carboxylic acid, hydroxyl, and quaternary ammo-

Table 1 Quantum yields of photosensitizer triplet (Φ_T) and overall singlet oxygen generation (Φ_Δ) with photosensitizers for PDT applications Φ_Δ values refer to air-saturated solutions Illustrative values from the compilation by McGarvey and Truscott [14]

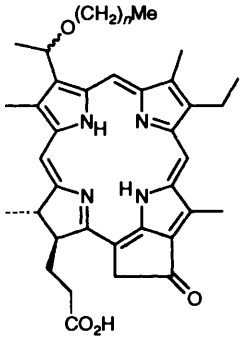
Photosensitizer		Solvent	Φ_T	Φ_Δ
Class	Example			
Porphyrin	Etioporphyrin	Benzene	0 83	0 72
	Protoporphyrin dimethylester [(4) as dimethyl ester)]	Benzene	0 80	0 57
	m-THPP (12)	Methanol	0 69	0 61
Chlorin	Octaethylpurpurin	Benzene	0 82	0 67
	Zn^{II} etiopurpurin [(27) as Zn complex]	Benzene	0 83	0 57
Bacteriochlorin	Bacteriochlorophyll *a*	Benzene	0 32	0 32
	Bacteriopheophytin *a*	Benzene	0 73	0 46
Phthalocyanine	Aluminium(III) phthalocyanine tetrasulfonic acid [(30) M = Al, R = SO_3Na]	Water	0 38	0 36 (MeOD)
	Zinc(II) PcS_4	Methanol	0 47	0 43
	Silicon naphthalocyanine	Benzene	0 39	0 35
Porphycene	Porphycene (32)	Toluene	0 42	0 30

nium salt have been the substituents most studied and differential interactions have been observed at the molecular (*e.g.* lipoprotein), cellular, and tumour tissue levels.

In experiments on tumour damage *in vivo* the effect of varying hydrophobicity with the length of the alkyl chain in alkyl ethers has been observed with both porphyrins [*e.g.* ethers derived from haematoporphyrin (1)] and chlorins, and with some methylene-linked diporphyrin systems. For example in the 3-[1-alkyloxyethyl]-3-devinyl pyropheophorbide *a* series (5), activity appears to be greatest for the C_6 ether.[15] In the sulfonated aluminium phthalocyanine series, tumour inactivation increases by an order of magnitude in the series $AlPcS_4 \rightarrow AlPcS_3 \rightarrow AlPcS_2$ (where S_n refers to the number of sulfonic acid groups on peripheral benzenoid positions):[16] evidently $AlPcS_4$ is too hydrophilic in the present context. In the *meso*-tetraphenylporphyrin polysulfonic acids (6), a similar observation has been made: cellular uptake of $TPPS_1$ and TPP_{2a} is more efficient than it is of $TPPS_{2o}$ and $TPPS_4$.

We have concentrated on the subtle amelioration of hydrophobicity with can be achieved with hydroxyl substitution. For example, in a series of chlorins with geminal substitution (to prevent dehydrogenation) we have introduced a single hydroxylated side-chain, and increased the number of hydroxyl groups from one (7a, 7b) to five (7e). The mono alcohols gave erratic results in the *in vivo* assay, probably because of solubility problems: for the other compounds in this series, the biological activity increased in the series (7c)→(7d)→(7e) *i.e.* with the number of hydroxyl substituents on the side chain.[17]

The mammalian system is an intricate assembly of compartment, phase, and flow. Our understanding of interaction with xenobiotics is limited, and it is not surprising that, in the PDT field, it is the solution-properties physical chemistry, rather than the photophysical chemistry, which is the most demanding criterion.



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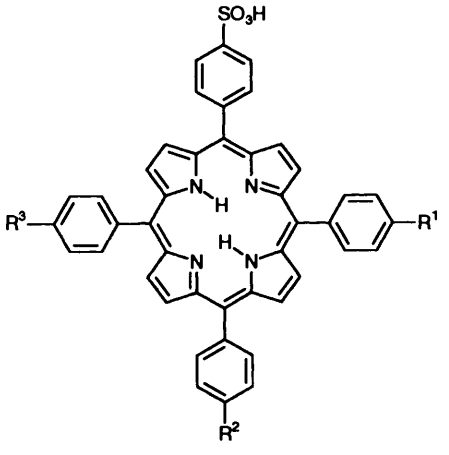
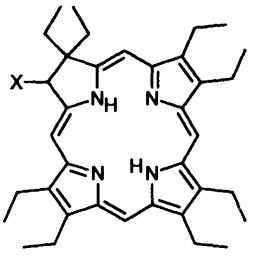


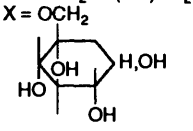
(6)

(6a) $TPPS_1$, $R^1 = R^2 = R^3 = H$
(6b) $TPPS_{2a}$, $R^1 = SO_3H$, $R^2 = R^3 = H$
(6c) $TPPS_{2o}$, $R^2 = SO_3H$, $R^1 = R^3 = H$
(6d) $TPPS_4$, $R^1 = R^2 = R^3 = SO_3H$

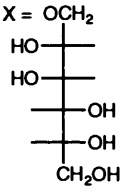


(7)

(7a) X = OH
(7b) X = OCH_2CH_2OH
(7c) X = $OCH_2CH(OH)CH_2OH$
(7d) X = OCH_2



(7e) X = OCH_2



4.5 Red Absorption

Because both absorption and scattering of light by tissue increase as the wavelength decreases, the most efficiently excited sensitizers are those which have strong absorption bands at the red end of the visible spectrum. The search for red absorbers has been a major activity in recent years (Section 5).

The situation is illustrated in Figure 4, where the dotted line represents in a generalized way the transmittance of human tissue in relation to the molar extinction coefficients of Band I of a number of second-generation photosensitizers.

The extension of the λ_{max} value into the red cannot be carried too far for two reasons. Firstly, if the red shift is related (as it often is) to an extension of the π-system of the heteroaromatic system, then the oxidation potential decreases, and the photosensitizer becomes less stable kinetically, and subject to photobleaching. The second reason has to do with singlet oxygen generation. Band I in Figure 4 represents the energy of the first excited singlet state: the triplet energy will be lower than this (Figure 2), but must not fall appreciably below 94kJ mol^{-1} (1270 nm) otherwise efficient energy transfer from triplet sensitizer to ground state dioxygen will not be possible.

An example of this is to be found in the remarkable 'expanded' porphyrin series.[18] The [26]porphyrin dication (8) has λ_{max} 783 nm (ϵ28 000) and a low but appreciable singlet oxygen quantum yield ($\Phi_\Delta = 0.19$). When the conjugated macrocycle is expanded to the [34]porphyrin dication (9), a blue salt results in which the lowest energy electronic transition (*i.e.* Band I) now falls at λ_{max} 997 nm (ϵ24 000). Compound (9) is not a photosensitizer for singlet oxygen formation ($\Phi_\Delta = 0$): presumably its triplet energy lies below 94 kJ mol^{-1}.

5 The New Photosensitizers

There has been an explosion of activity in this area and, indeed, a problem arises as to what to do with many of the potential photosensitizers which have been advocated. Reference is made in the bibliography to reviews which cover the topic in detail. Within the style and space criteria stipulated by *Chemical Society Reviews* I propose to mention each of the main structural types of photosensitizer with one or two recent references and a commentary.

5.1 Porphyrins

One obvious approach is to modify porphyrins [(haematoporphyrin (1), protoporphyrin (4)] which are readily available from bovine blood. Although haematoporphyrin (1) is difficult to purify, a range of analogues substituted at the reactive pseudobenzylic positions ($C3^1$ and $C8^1$) with ether, thioether, ester, and amino functions have been prepared: many of them show biological activity. In an immunological approach to increased

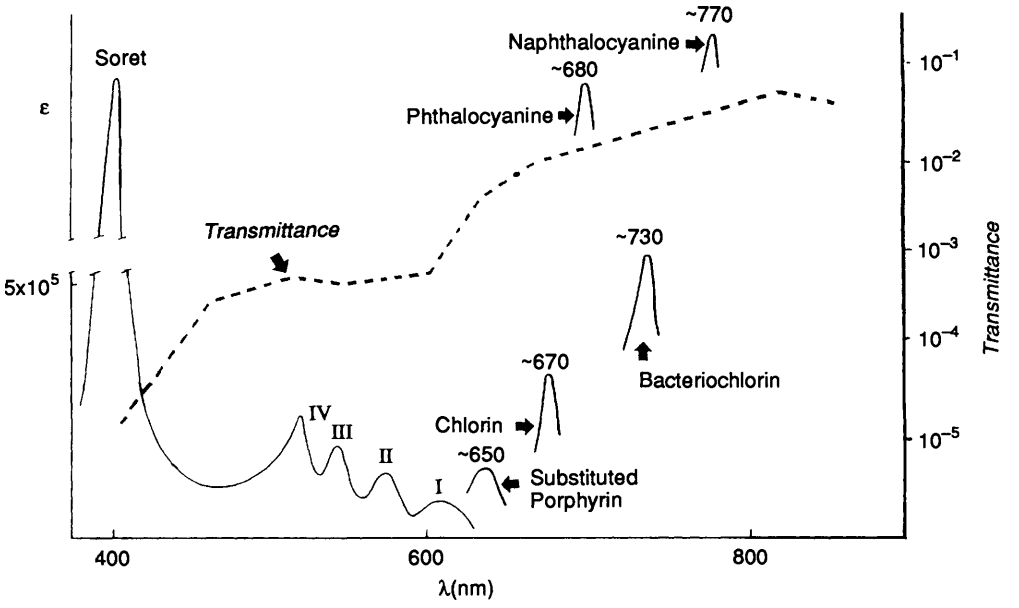
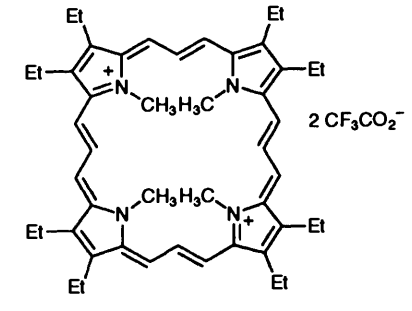
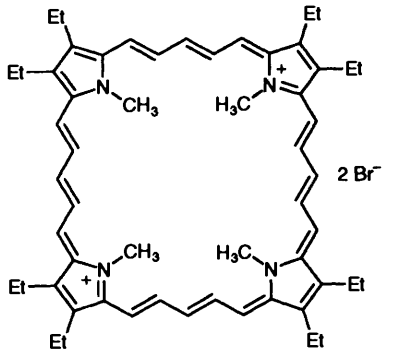


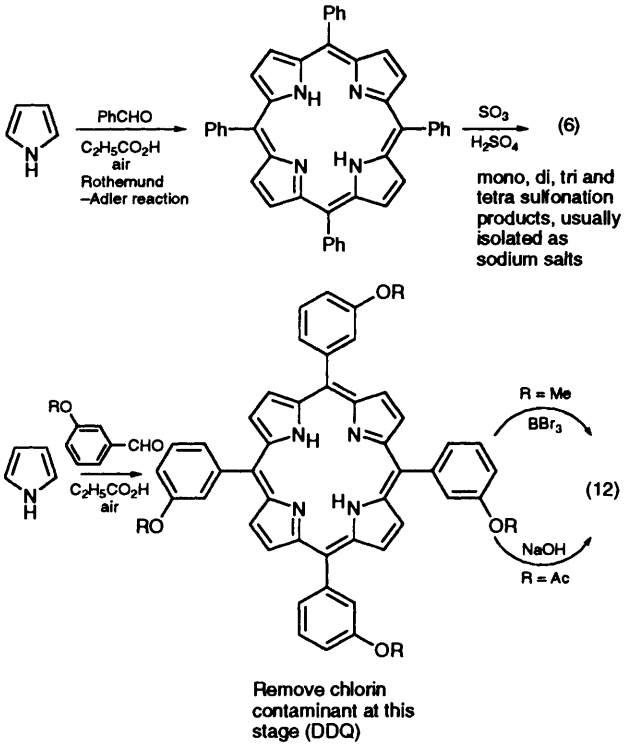
Figure 4 Photosensitizer absorbance in relation to tissue transmittance. The absorption spectra are schematic: only Band I is shown, except for the porphyrin absorption spectrum on the left. The transmittance curve refers to a sample of human scrotal sac, 7 mm thick (Wan, Parrish, Anderson, and Madden, *Photochem. Photobiol.*, 1981, **34**, 679). The broad feature at 500—600 nm is attributed to absorption by haemoglobin.



(8) [26]Porphyrin system



(9) [34]Porphyrin system



Scheme 5 Routes to tetraphenylporphyrins with hydrophilic substituents.

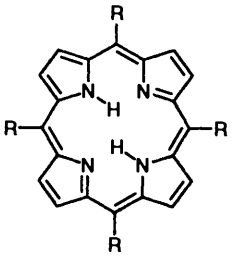
selectivity the conjugates of haematoporphyrin with antibodies (*e.g.* anti-colonic cancer monoclonal antibody) indeed show enhanced photocytotoxicity.[19]

meso-Tetraphenylporphyrins [parent, (10)] are the most readily available of the synthetic compounds. Although the Rothemund synthesis, even in its Adler ($C_2H_5CO_2H$,Δ) or Lindsey (BF_3/CH_2Cl_2/20°C; then DDQ) modifications, gives only modest yields, it is still acceptable, as a one-pot synthesis from simple starting materials (Scheme 5). Substitution in the phenyl groups confers some degree of hydrophilicity: sulfonic acids are generally prepared by direct sulfonation of *meso*-tetraphenylporphyrin (which usually gives a mixture of different levels of sulfonation), while hydroxyphenyl derivatives are made *via* the Adler synthesis, protecting the phenolic hydroxyl as a methyl ether or as an acetate (Scheme 5). In our screening of potential photosensitizers (Section 4) the first attractive group of compounds were the *meso*(hydroxyphenyl)porphyrins.[20] Of the three isomers (11), (12), (13) the *ortho* isomer (a mixture of atropisomers) was discarded because it caused skin photosensitization, but the remaining two isomers [(12), m-THPP and (13), p-THPP] both showed increased tumour photonecrosis and favourable selectivity with respect to HpD. m-THPP (12) was about 25—30 times as potent as HpD in sensitizing tumours. Recently these compounds, and a series of dihydroxyphenyl

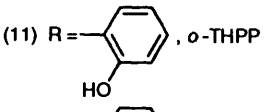
analogues, have been tested for toxicity and phototoxicity in *in vitro* experiments (human melanoma cell line); the 3,4-dihydroxy compound [(14) a tetracatechol!] was the most active.[21]

Hydrophilicity has also been increased using cationic substituents. For example, the methylpyridinium salt (15), prepared by the Rothemund route from pyrrole and 4-formylpyridine, followed by methylation, causes significant tumour photonecrosis characterized by gradually developing damage to tumour cells and the vascular endothelium.[22]

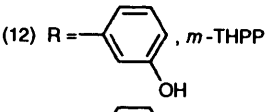
Although it is possible by substitution of the porphyrin system to cause a bathochromic shift and so move Band I to the red, the effect is generally small, and often the molar extinction is only modestly increased. For this reason work has concentrated on compounds with relatively intense bands in the 650—800 nm region (Figure 4).



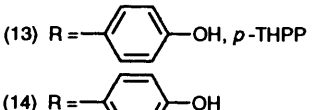
(10) R = Ph



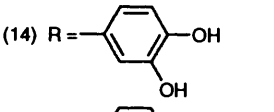
(11) R = , *o*-THPP



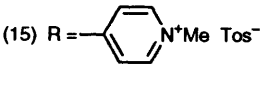
(12) R = , *m*-THPP



(13) R = OH, *p*-THPP



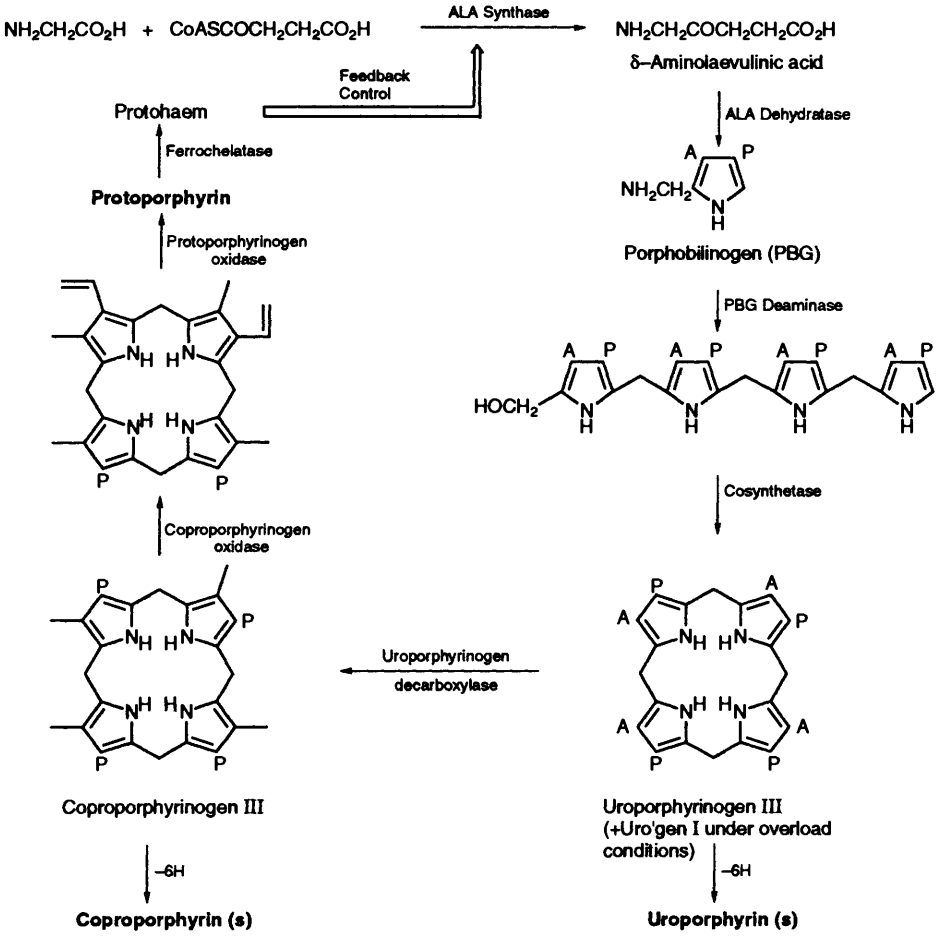
(14) R =



(15) R = $N^+Me\ Tos^-$

5.2 Endogenous Porphyrins

There is one exception to the foregoing statement, and this relates to a remarkable development which uses the biochemical machinery of the body to generate a natural photosensitizer. In the biosynthesis of tetrapyrroles in mammalian systems the production of haem is controlled by a feedback mechanism in which the concentration of haem regulates the production of δ-aminolaevulinic acid (δ-ALA). However, if δ-aminolaevulinic acid is artificially added to the system, the enzymes responsible for successive stages take over, and along the way to haem, protoporphyrin is generated. (Possibly other porphyrins, resulting from adventitious oxidation of intermediate porphyrinogens, are also formed under these overload conditions as shown in Scheme 6.) At any event a photosensitizer is generated within



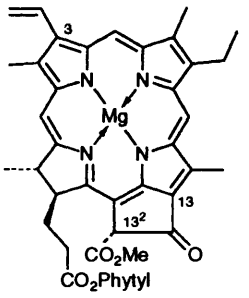
Scheme 6 Haem biosynthesis: abbreviated version to show porphyrin photosensitizer formation (bold) under conditions of δ-ALA overload. Protoporphyrin appears to be the main photosensitizer in the present context. (A = $-CH_2CO_2H$, P = $-CH_2CH_2CO_2H$.)

the tissue, and it has a photonecrotic effect. (A similar effect arises in certain of the porphyria diseases, such as erythropoietic protoporphyria, where excess porphyrin is generated because of the inadequate performance of one or more of the enzymes along the haem biosynthetic pathway.)

Kennedy and his co-workers[23] have shown that δ-ALA applied as a cream topically is able to penetrate the abnormal keratin of the epidermis of superficial basal cell or squamous cell carcinomas. Normal skin is less permeable, but wound tissue is susceptible. During a period of three years, over 300 basal cell carcinomas have been treated with δ-ALA topically, using a projector lamp as the light source, with a complete response rate at three months of 79% after a single exposure. Clearly this is a very promising development, and it is now being actively pursued in a number of laboratories. Interestingly, the protoporphyrin induced in this way *in situ* seems remarkably sensitive to photobleaching (as it does in protoporphyric erythrocytes). From a treatment point-of-view it appears that this approach can be generalized from cutaneous tumours to those at internal sites since the δ-ALA can also be given systemically and orally.[24]

5.3 Chlorins and Bacteriochlorins

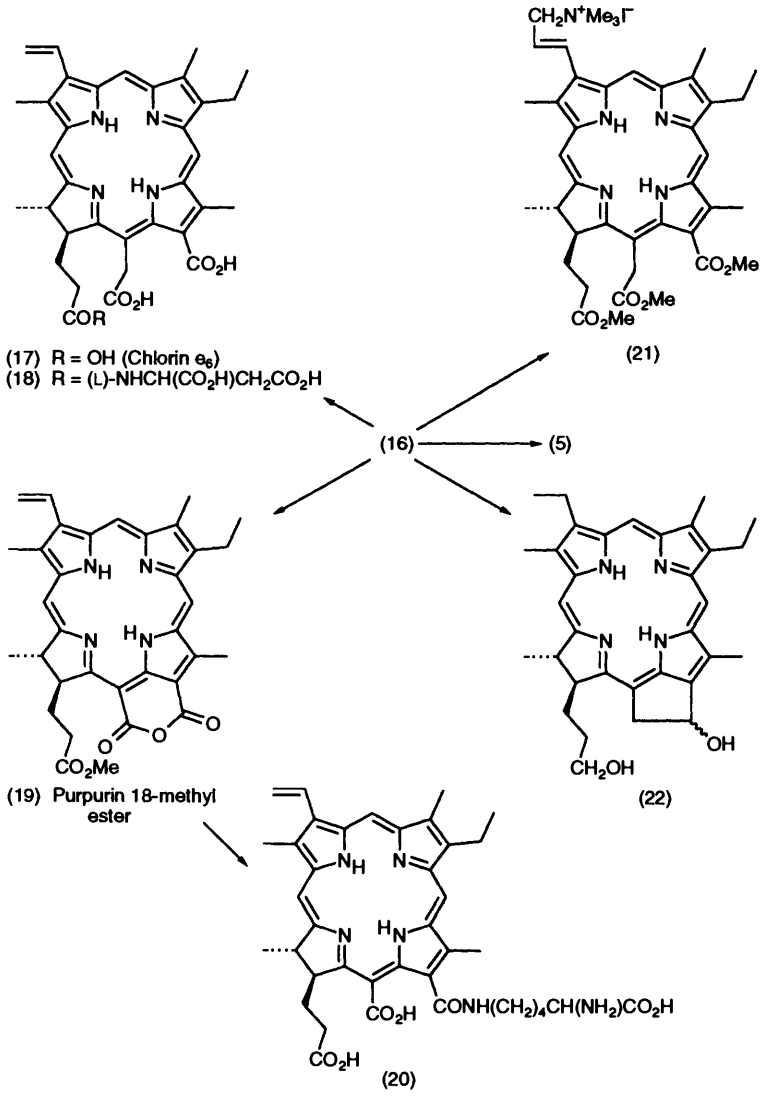
The natural compounds, chlorophyll *a* and bacteriochlorophyll *a*, have both been used as sources of PDT photosensitizers. However, both of these starting materials are sensitive substances (*e.g.* to autoxidation) and modifications are required if drugs with a reasonable shelf-life are to be produced.



(16)

Chlorophyll *a* (16) is available from certain *Spirulina* species, which, conveniently contain little or no chlorophyll *b*, thus avoiding a tedious separation. Most approaches have sought to remove the major points of fragility in the molecule – the metal, the isocyclic ring, the very reactive C-13² position (a benzylic position activated by two carbonyl groups), and the vinyl group at C-3. The phytyl group makes the systems very hydrophobic and is dispensed with.

Scheme 7 summarizes some of the major routes which have been used to generate amphiphilic chlorins with PDT activity. Chlorin e_6 (17) is available directly from chlorophyll *a* under vigorous basic conditions, and appears to have moderate *in vivo* activity.[25] Various derivatives of it have been studied, including the mono amide with L-aspartic acid [(18), 'MACE'], and the



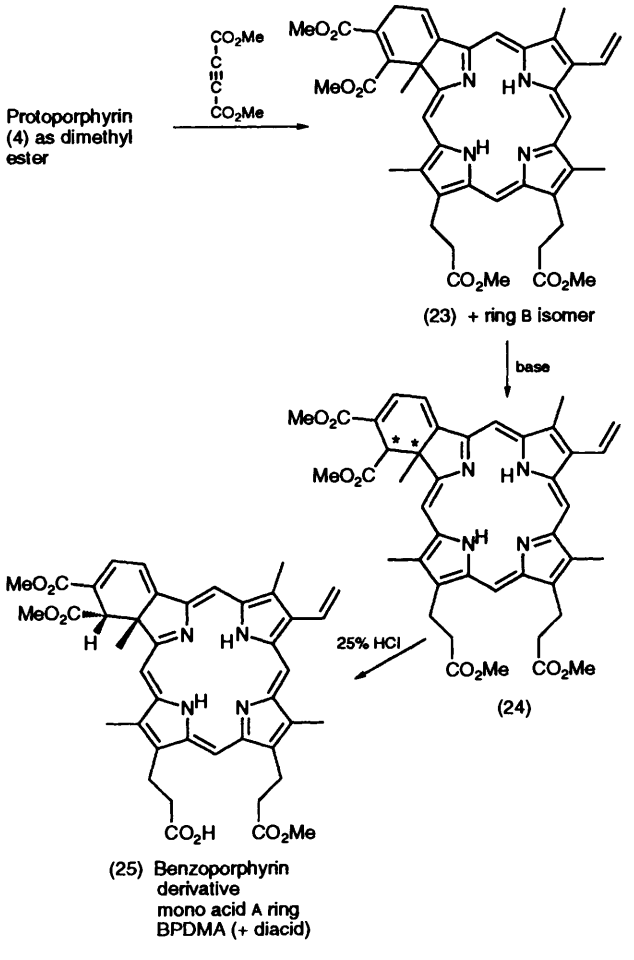
Scheme 7 Photosensitizers derived from chlorophyll *a* (16).

quaternary ammonium salt (21). Chlorin p_6 derivatives, such as the lysyl derivative (20) which is prepared from purpurin 18-methyl ester (19) by opening the anhydride ring with L-lysine in aqueous pyridine, are also attracting attention.[26]

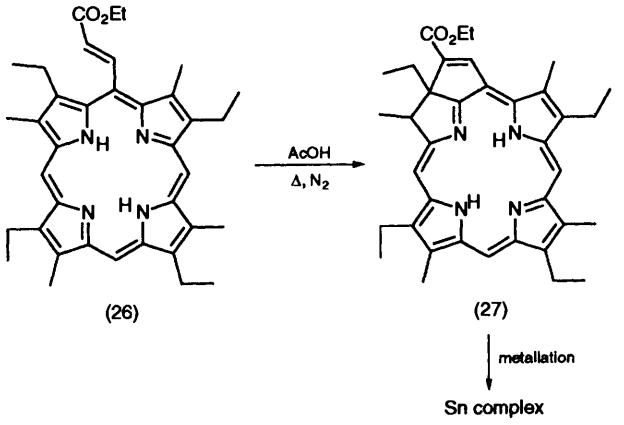
Systems in which the vinyl group at C-3 has been removed include adducts such as the hexyl ether (5),[15] and polyols [e.g. (22)] in which both the C-3 vinyl group and the carbonyl groups on the southern edge of the structure have been reduced.[27] Analogous routes are available in the bacteriochlorophyll series.

Some synthetic chlorins have also shown promising biological activity. *meso*-Tetrakis(*m*-hydroxyphenyl)chlorin [(34), m-THPC] has emerged from our own work and is discussed in Section 6. Other chlorins are also under commercial development. For example, benzoporphyrin derivative (a chlorin in spite of its name) is prepared by the Diels–Alder reaction of protoporphyrin dimethyl ester with dimethyl acetylenedicarboxylate. As shown in Scheme 8, base-catalysed rearrangement of the 1:1 adduct (23) gives the conjugated system (24). Chromatographic separation and partial hydrolysis then gives the mono acid, in which addition has occurred on ring A [(25), BPDMA]. In *in vivo* experiments, this sensitizer is administered in a liposomal system, and the drug–light interval is short (about 3 hours).[28]

A second example is furnished by the purpurin series. Substitution of a conjugating substituent at a *meso*-position of a chlorin changes the colour of the pigment from green to puce; the strong chlorin absorption is retained in the red, but extra absorption occurs in the 550 nm region, and Conant termed the resulting system a purpurin. Thus cyclization of the *meso*-acrylic acid derivative (26) under mild conditions (Δ,AcOH,N_2) occurs regiospecifically (maximizing relief of steric strain) to give the purpurin (27) in 92% yield (Scheme 9). These compounds are



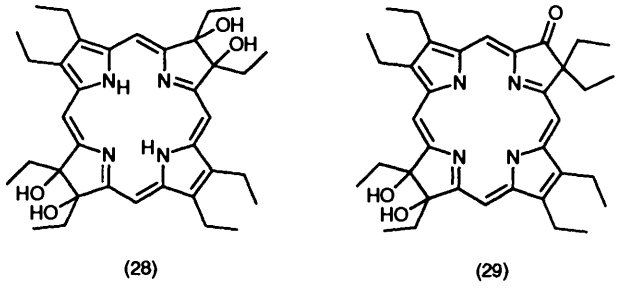
Scheme 8 Synthesis of the benzoporphyrin derivative monoacid, BPDMA.



Scheme 9 Synthesis of tin etiopurpurin.

hydrophobic, and need to be administered in an emulsifying agent. The tin complex of etiopurpurin ('SnEt2') appears to be the most active of the compounds studied.[29]

Synthetic bacteriochlorins have also been examined. Here attempts have been made to stabilize the tetrahydroporphyrin reduction level by appropriate peripheral substitution, as shown in structures (28) and (29), both of which show tumour photonecrotic activities *in vivo*.[17] The β-oxochlorin chemistry involved here has been extended to more complex systems.[30]

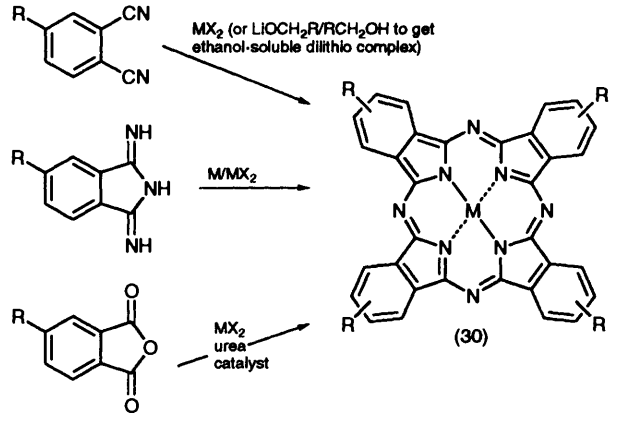


5.4 Phthalocyanines and Naphthalocyanines

The phthalocyanines and naphthalocyanines are readily prepared by the reductive tetramerization of phthalonitriles and naphthalene-2,3-dinitriles (or equivalent monomers), respectively (Scheme 10). A metal or metal salt is included in the reaction mixture to serve both as a template and an electron source, and a metal complex results. The metallophthalocyanines, especially the copper complex, have been important as commercial pigments since the 1930's: more recently metallophthalocyanines and related compounds have found multiple applications in electroreprographic (*e.g.* photocopiers) and electrooptical (*e.g.* CD discs) devices. However the synthesis is not very flexible. No stepwise synthesis is known (contrast porphyrin synthesis), and, except in special cases, substituted phthalocyanines are obtained as mixtures.

Various complexes of phthalocyanine with non-transition elements (*e.g.* Zn, Al, Si) show photobiological activity against tumours. Such compounds are hydrophobic and require administration in a transport agent such as a liposomal preparation. The zinc(II) complex [(30), R = H, M = Zn^{II}] and the germanium(IV) complex with cholesterolate axial ligands appear to be especially active,[31] and are now attracting commercial interest.

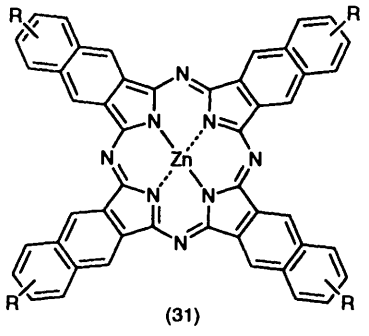
Amphiphilic phthalocyanines have been obtained either by direct sulfonation of the parent system, or by mixed phthalic acid–sulfophthalic acid reactions. In both cases mixtures ($MPcS_n$, where n = 1–4) are obtained. Activity is related to the degree of sulfonation: thus, the disulfonic acid of aluminium(III) phthalocyanine is more effective in tumour photonecrosis than is the tetrasulfonic acid (Section 4.4).[16] Characterization of a



Scheme 10 Principal routes to phthalocyanines by reductive tetramerization.

disulfonated aluminium phthalocyanine preparation by HPLC revealed eight components,[32] the major components having the sulfonic acid groups on adjacent rings. At a meeting in Lille in September 1994, Stranadko (Moscow) described the first clinical use of a sulfonated aluminium phthalocyanine (Photosens). Zinc(II) tetrahydroxyphthalocyanine [(30), R = OH, M = Zn^{II}] is inactive both *in vivo* and *in vitro*: however the corresponding tetraalkoxy derivative does show activity, which depends on the chain length of the alkyl group ($C_3 > C_6$).[33] This remarkable result indicates just how far we still have to go before we understand the complex physicochemical interactions involved here.

The metallonaphthalocyanines must surely be even more hydrophobic than are the phthalocyanine complexes, and some experiments have shown unpromising results. Nevertheless recent work with substituted zinc(II) naphthalocyanine delivered intraperitoneally in liposomes has shown significant *in vivo* activity which depends on the substituent [(31), activity R = $NHCOCH_3$ or OMe ≫ R = H ≫ R = NH_2].[34]

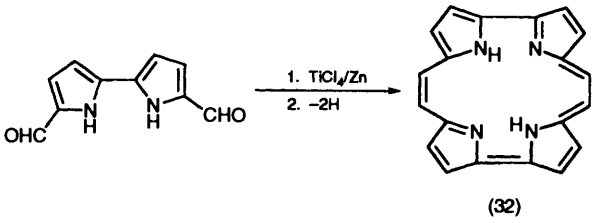


5.5 Related Macrocyclic Systems

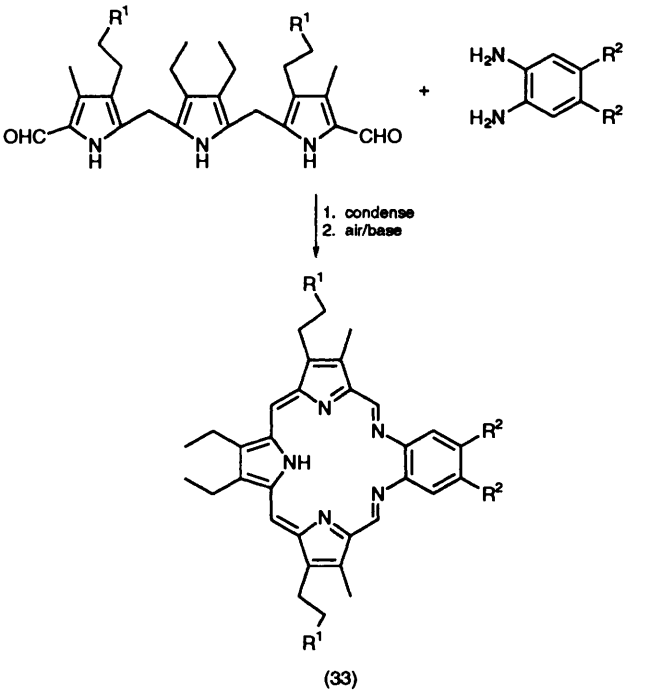
Other compounds analogous to phthalocyanines, including *meso*-azaporphyrins and chlorins, and di- and tetra-benzoporphyrins, show strong absorption in the red region, and are attracting attention. Many variant macrocyclic systems are now known, and two striking examples are provided here by way of illustration.

Porphycene (32) is an aromatic isomer of porphyrin: Band I occurs at λ_{max} 630 nm (ϵ 52000). The parent compound is prepared in low yield by coupling 5,5¹-diformyl-2,2¹-bipyrrole with itself in the presence of low-valent titanium (Scheme 11).[35] Tumour localization studies have been reported for substituted porphycenes: in some cases favourable tumour/normal skin accumulation is observed.[36]

The texaphyrins [*e.g.* (33)] are obtained by the condensation of diformyltripyrranes with diamino compounds.[37] When the diamine is an *o*-phenylenediamine (or an analogous system) the



Scheme 11 Synthesis of porphycene (32).



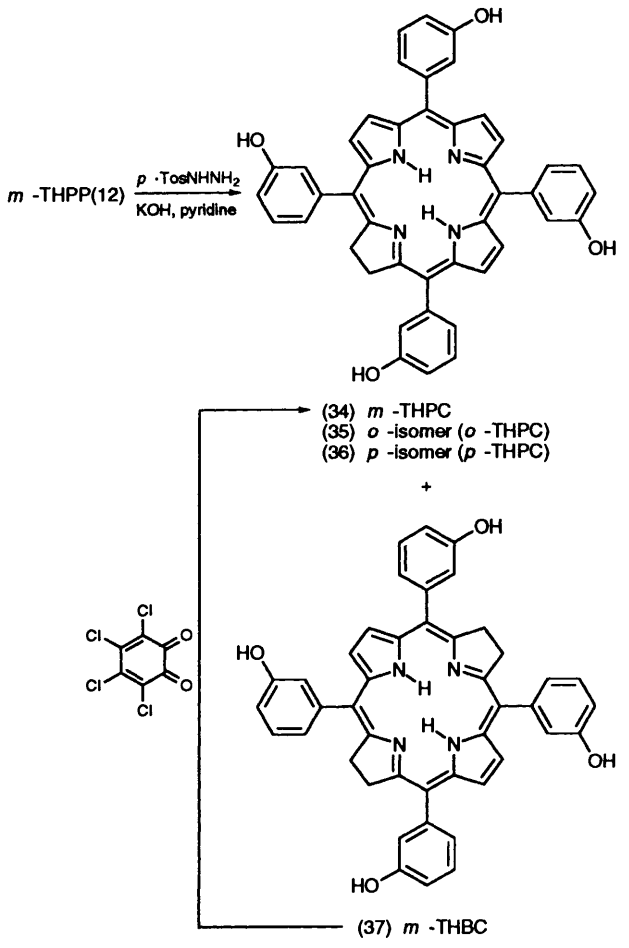
Scheme 12 Construction of the texaphyrin system.

intermediate bisazomethine is readily oxidized to the aromatic texaphyrin (Scheme 12). Such compounds readily form complexes with diamagnetic metal ions. The complexes have absorption in the 600—900 nm region which, as expected, can be tuned by variation of the substitution pattern. Singlet oxygen quantum yields are high. Complexes with lanthanum and lutecium [of (33), $R^1 = CH_2OH$, $R^2 = O(CH_2)_3OH$] show phototumoricidal activity *in vivo*; and experiments with the gadolinium complex as a contrast agent in magnetic resonance imaging are promising.[37]

6 The Development of m-THPC

In our search for a tumour photosensitizer which was more active than HpD, the first real success came with the tetrakis (hydroxyphenyl)porphyrins [(11),(12),(13), Section 5.2]. In order to increase absorption in the red region (Section 4.5) the corresponding chlorins [5,10,15,20-tetrakis(*m*-hydroxyphenyl) chlorin, m-THPC, (34); 5,10,15,20-tetrakis(*o*-hydroxyphenyl) chlorin, o-THPC, (35); and 5,10,15,20-tetrakis(*p*-hydroxyphenyl)porphyrin, p-THPC, (36)] were prepared: in the *m*-series the bacteriochlorin [(37), m-THPBC] was also isolated. It was found that the chlorins and bacteriochlorins could be prepared in satisfactory yield and purity by the diimide reduction of the tetrakis(hydroxyphenyl)porphyrins without the need for protection of the phenolic functions (Scheme 13). Thus, these compounds were readily available in three steps from pyrrole and substituted benzaldehyde (Schemes 5 and 13).

As expected, the successive levels of reduction caused increased absorption in the red region.[38] The absorption spectra



Scheme 13 Chlorins and bacteriochlorins of the tetrakis(hydroxyphenyl)porphyrin series by diimide reduction. Where the bacteriochlorin (37) was required an excess of the diimide reagent was used: where the chlorin, *e.g.* (34), was required the bacteriochlorin, inevitably formed as a by-product, was selectively dehydrogenated with *o*-chloranil.[38]

(methanol) in the Band I region for m-THPP (12), m-THPC (34), and m-THPBC (37) are shown in Figure 5. A combination of ^{1}H, ^{13}C, and natural abundance ^{15}N NMR spectra demonstrated that the predominant imino tautomer of m-THPC was that with imino hydrogens on opposite non-reduced rings, as shown in (34).[39] Presumably the other chlorins and the bacteriochlorin have analogous fine structure.

In vivo biological assay of tumour photonecrosis showed that activity increased in the sequence porphyrin < chlorin < bacteriochlorin. This is shown in Table 2, where the doses of photosensitizer, under standard conditions, required to cause necrosis of the tumour to a depth of 5 mm are compared. This remains the only example where this comparison has been made between these three reduction levels in molecules possessing the same substitution pattern. Table 2 also illustrates the small but significant bathochromic shift mentioned earlier (Section 4) which photosensitizers commonly show in going from an organic solvent (here, methanol) to a biological medium (here, foetal calf serum).

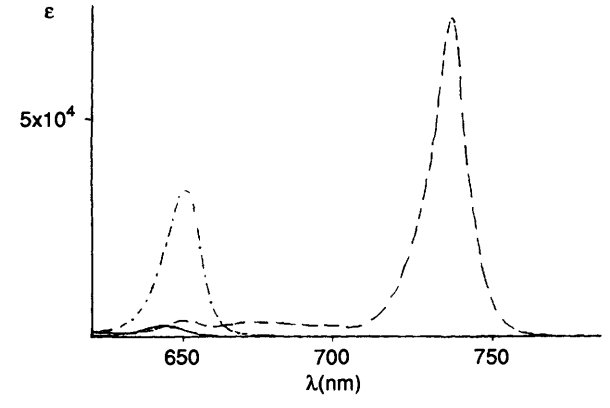


Figure 5 Band I for m-THPP (12), ———; m-THPC (34), —.—.; and m-THPBC (37), – – –; in methanol (B. D. Djelal, 1994).

Table 2 Comparison of tumour photonecrosis for a porphyrin, chlorin, and bacteriochlorin possessing the same substitution pattern[38]

Photosensitizer	Band I λ_{max} in MeOH nm	Band I* λ_{max} in foetal calf serum nm	Dose of photosensitizer μmol kg^{-1}	Depth of tumour necrosis mm
m-THPP (12)	646	648	6.25	4.6
m-THPC (34)	650	652	0.75	5.4
m-THPBC (37)	735	741	0.39	5.1

* Wavelength of tumour irradiation, 10J cm^{-2} 24 hours after drug administration.

A tumour photosensitizer is not going to be a practical proposition unless it shows a good selectivity for tumour over normal tissue. Having discovered a series of photosensitizers of low dark toxicity and high activity in tumour necrosis when irradiated, it was now necessary to examine selectivity with respect to normal tissue. To do this the damage to tumour was compared with damage to normal skin, muscle, and bladder under the same conditions: the photosensitizer dose was varied, but the other parameters (light dose, drug light interval) were kept constant as before (10J cm^{-2}, 24 hours).[40] Damage to skin and bladder was assessed by the extent of oedema after four hours; damage to muscle was assessed by oedema after 4 hours and muscle necrosis after 24 hours. Damage indices were defined as follows: tumour index – depth of necrosis in mm; muscle necrosis index – fraction of necrotic tissue expressed as a decimal; oedema (muscle, skin, bladder) – fractional increase in weight or Evans blue accumulation.

Out of the considerable number of sensitizers which we had assayed for tumour photonecrosis, the best four [(12),(13),(34),(36)] were selected for comparison with Photofrin II. (The *ortho* isomers [(11),(35)] were omitted because the porphyrin (11) had caused marked skin photosensitization;[20] and the bacteriochlorin (37), while a very active tumour photosensitizer, was relatively unstable). The results for the five compounds were compared by cost–benefit analysis.[40] This is a useful way of presenting a large amount of such comparative data and relates to tissue selectivity and therapeutic index. In the present case the benefit refers to damage to tumour while the cost refers to damage to normal tissue *under the same conditions of drug and light administration*. An example is given in Figure 6. Each line refers to a different photosensitizer; each point is the mean of several observations (tumour damage, normal tissue damage) made at the same dose of photosensitizer under the same conditions. Promising drugs (*i.e.* high benefit at acceptable cost) appear in the bottom right hand part of the field as drawn here.[40]

The upshot of this extensive series of experiments was that m-THPC (34) appeared to be the most promising of the four new sensitizers studied; and that it was more effective as a tumour photosensitizer, and caused less photosensitization of normal tissue (*e.g.* skin, muscle) than did the well-established Photofrin preparation.[40] Although the results with animal models must be treated with due reserve, m-THPC appeared to be a good candidate for clinical work.

Other experiments – physical, analytical, and biological – have supported this view. Thus (i) m-THPC has satisfactory

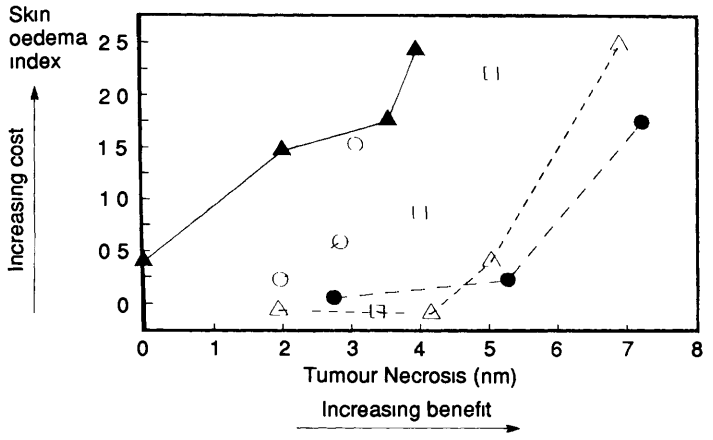


Figure 6 Cost–benefit analysis tumour necrosis (benefit) against skin oedema index (cost) for Photofrin II, ▲—▲, p-THPP (13), ○----- ○, m-THPP (12), □----- □, p-THPC (36), △– △, and m-THPC (34), ●— —●

photophysical parameters (Φ_T = 0 9 in methanol, Φ_Δ = 0 43 in air-saturated methanol *cf* Table 1),[41] (ii) purity is readily determined by ^{1}H-NMR and HPLC methods sensitive HPLC systems have been described for the detection and estimation of m-THPC and its metabolities in biological samples,[42] (iii) toxicity studies have shown that m-THPC is not mutagenic and of low toxicity the LD_{50} value is > 3 mg kg^{-1}, which is about 20—30 times the therapeutic dose, and (iv) protocol-development studies with mesothelioma implants in rodents have shown that, in that system, m-THPC is more effective if a longer drug–light interval is used (48—72) hours rather than the 24 hours used in the original screening, (Section 4), and with a different illumination regime (0 1 mg kg $^{-1}$, 20 J cm $^{-2}$ at 200 mW cm^{-2} rather than 0 3 mg kg^{-1}, 10 J cm^{-2} at 100 mW cm $^{-2}$) [43]

In vitro studies in hamster lung fibroblasts show diffuse distribution of m-THPC in the cytoplasm, and the presence of monomeric and aggregated species, of which only the former is photodynamically active Cell inactivation on irradiation is diminished in the presence of 1,3-diphenylisobenzofuran, a result in accord with the view (Section 2) that singlet oxygen is a significant reactive intermediate [5]

About 60 patients have now been treated with photodynamic therapy using m-THPC as the photosensitizer Ris and his colleagues in Berne reported the first clinical cases in 1991 where PDT with m-THPC was used after surgery ('intraoperatively') in four cases of malignant mesothelioma [44] The photosensitizer was delivered intravenously as a solution in ethanol–polyethylene glycol 400 – water (2 3 5) at a dose of 0 3 mg kg^{-1}, with a drug–light interval of 48 hours and a light dose of 10J cm^{-2} (650 nm) Normal structures (oesophagus, nerve ganglion cells, aorta) were retained even when full thickness tumour necrosis occurred Some negative features were noted, including a mild skin photosensitivity which lasted about 14 days, loss of appetite, fluid retention, and severe chest pain

Monnier and van den Bergh and their colleagues[45] in Lausanne have described the PDT of twelve patients with early carcinomas in the upper aerodigestive tract, the oesophagus, and the bronchus m-THPC was found to be a much more effective photosensitizer than HpD or Photofrin Of nine patients evaluated at 3 months, seven showed no residual tumour, but tumour persisted in the irradiated region in two cases Further progress was reported by this group at a recent conference in Florida (Fifth Biennial Meeting of the International Photodynamic Association, September 1994) Thirty two treatments have now been carried out, with one exception on early cancer With a mean follow-up of 9 months (and counting patients with a follow-up ≥ 3 months) a 90% tumour sterilization was seen, with a 10% recurrence Complications included photosensitivity of normal tissue, and risk (in oesophagal cancer) of perforation of oesophagus wall Irradiation in the green (514 5 nm, 75—100 J cm $^{-2}$, drug–light interval 96 hours, 0 15 mg kg^{-1}) appears to reduce the risk of such perforation 'All in all, excellent results for phase 1,2' [46]

At the Florida meeting Dilkes (Royal London Hospital) also gave a summary of seventeen treatments of carcinoma of the upper aerodigestive tract His protocol illustrates the sort of procedures now being developed The light source was a copper vapour dye laser set for 652 nm, the light being delivered with a bare fibre, a microlens, or a cylinder diffuser m-THPC was given intravenously at a dose of 0 1 mg kg^{-1}, with a drug–light interval of 96 hours The total light dose was 20 J cm^{-2}, at a fluence rate of 100 mW cm^{-2} (*i e* an exposure time of just over 3 minutes) The results were reported to be promising [47] Studies with m-THPC and laryngeal cancer are also in progress, and a multi-centre study coordinated by Dr J C M Stewart (Scotia Pharmaceuticals) on the effect of m-THPC PDT on primary squamous cell carcinomas of the head and neck is now underway

7 Conclusions

(a) As usual, the chemists have been very productive, and there are now more suggested photosensitizers than we know what to do with It is noticeable that the large pharmaceutical companies have not revealed much interest in this area maybe the unfamiliar requirement for laser technology has been a deterrent But the possibility that other diseases, including, for example, psoriasis and viral and fungal conditions, may be amenable to photodynamic therapy, may change that perception At any event, there is a need for organized biological testing, at which pharmaceutical companies have a wealth of experience, to compare the various photosensitizers with one another in a number of *in vivo* assays

(b) m-THPC (34) is a very powerful PDT photosensitizer It has the disadvantage that it causes some skin photosensitivity in Man (but less than that caused by Photofrin) but it has the following advantages (i) it was selected on the basis of tumour necrotic activity *in vivo* after examining a considerable number (about 140) of photosensitizer preparations, and on the basis of selectivity for tumour over normal tissue from the best four compounds, (ii) it has strong absorption at 652 nm, and a Φ_Δ value of 0 43, (iii) the synthetic route is convenient and short (three steps from pyrrole, Schemes 5 and 13), (iv) it is a single substance (It is now prepared commercially by Scotia Pharmaceuticals Ltd , Guildford, in a purity of > 99% and has a British Approved Name – Temoporfin), (v) it is sufficiently soluble in hydroxylic solvent mixtures (but not in water at pH7) to be injected in solution without a carrier, (iv) dark toxicity is low, and it is not mutagenic, (vii) the clinical experiments carried out so far show that it is a very powerful photosensitizer of tumour necrosis, and the results are generally encouraging

(c) It is not, of course, the only second generation photosensit-

izer (Section 5) Several others are being developed worldwide, and it may emerge that different photosensitizers will be found to be appropriate for the various conditions covered by the umbrella word cancer New photosensitizers under development include the following

Company	Head Office	Photosensitizer
Ciba Geigy/QLT	Basel/Vancouver	Zn^{II} phthalocyanine
Cytopharm/partner	Manlo Park, California	Porphycenes
DUSA (Deprenyl, USA)	Toronto	δ-ALA
Nippon Petrochemical	Tokyo	Monoaspartyl chlorin e_6('MACE')
PDT Inc	Santa Barbara, California	Purpurins (*e g* Sn etiopurpurin)
Pharmacyclics	Palo Alto, California	Texaphyrins
Quadra Logic Technologies	Vancouver	Benzoporphyrin Derivative
Scotia Pharmaceuticals	Guildford, England	m-THPC

(d) Much remains to be done Sensitizers will undoubtedly be modified and improved, and more work is needed to reveal the mechanisms which make them effective More, too, is needed on the chemical mechanisms which cause the photobleaching of photosensitizers since this could, in itself, be an important process by which generalized photosensitivity could be diminished

(e) "In conclusion, the treatment which I have described seems to have proved its value, and there is every reason to give it the place that it deserves in therapeutics, a place which it is at present still far from having obtained, doubtless owing to its strangeness and unintelligibility In reality, its scientific basis is much better and more solid than that of many other methods of medical treatment "[1] This cry from the heart from the father of phototherapy in 1901, which referred to direct phototherapy, applied until very recently to photodynamic therapy of cancer Now with approvals for Photofrin treatment from regulatory bodies in three countries (1993, 1994), tumour PDT is at last becoming accepted It seems to me that it will emerge as part of our armoury in the battle against cancer, especially in the diagnosis and treatment of early primary cancer, and also in intraoperative and palliative procedures

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