Pergamon

Tetrahedron 57 (2001) 9513–9547

TETRAHEDRON

Tetrahedron report number 591

Photobleaching of sensitisers used in photodynamic therapy

Raymond Bonnett* and Gabriel Martínez

Department of Chemistry, Queen Mary, University of London, Mile End Road, London E1 4NS, UK

Received 12 September 2001

Contents

1. Introduction: tumour phototherapy

The beneficial and therapeutic properties of light either alone or in combination with a chemical agent have long been recognised by mankind. The solarium, for example, was a feature of the domestic architecture of the ancient Greeks and Romans and, in India, plant extracts containing furocoumarins were administered orally followed by sunlight exposure to treat vitiligo.[1a,b] In modern times, Finsen's seminal work on phototherapy,[2] and especially

* Corresponding author. Fax: +44-20-7882-7794; e-mail: r.bonnett@qmul.ac.uk

Abbreviations: A=Absorbance; aq=Aqueous; Ar=Aryl; BPD-MA=Benzoporphyrin derivative monoacid ring A; Brij 35=Polyoxyethylene(23)lauryl ether; *c*=Concentration; CPC=Cetyl pyridinium chloride; CTAB=Cetyl trimethylammonium bromide; DABCO=Diazabicyclo[2.2.2]octane; δ-ALA=δ-Amino-laevulinic acid; DDQ=2,3-Dichloro-5,6-dicyano-1,4-benzoquinone; DMF=Dimethylformamide; DMSO=Dimethylsulfoxide; DPPC=Dipalmitoyl-L-α-phosphatidylcholine; DTAB=Dodecyl trimethylammonium bromide; EPR=Electron paramagnetic resonance; FCS=Foetal calf serum; *h*=Hour(s); Hp=Haematoporphyrin; HpD=Haematoporphyrin derivative; HPLC=High pressure liquid chromatography; HPMA=*N*-(2-Hydroxypropyl)methacrylamide; HSA=Human serum albumin; *k*=Rate constant; MACE=Mono-L-aspartylchlorin e_6; MALDI=Matrix assisted laser desorption ionization; min=Minute(s); *m*-THPBC=5,10,15,20-Tetrakis(3-hydroxyphenyl)bacteriochlorin; *m*-THPC=5,10,15,20-Tetrakis(3-hydroxyphenyl)chlorin; *m*-THPP=5,10,15,20-Tetrakis(3-hydroxyphenyl)porphyrin; *m*-TMeOPP=5,10,15,20-Tetrakis(3-methoxyphenyl)porphyrin; MS=Mass spectrometry; MV=Methyl viologen; PBS=Phosphate buffered saline; Photofrin®, Photosan®=Proprietary names for purified HpD; PDT=Photodynamic therapy; rel.=Relative; SDS=Sodium dodecyl sulfate; SOD=Superoxide dismutase; *t*=Time; *T*=Temperature; TPP=*meso*-Tetraphenylporphyrin; $TPPS_4$=5,10,15,20-Tetrakis(4-sulphonatophenyl)-porphyrin; UV=Ultra violet; UV–Vis=Absorption spectroscopy; vis=Visible.

PII: S0040-4020(01)00952-8

his successful treatment of lupus vulgaris (a tubercular condition of the skin), established the foundations for the use of light in contemporary medicine. Since then, phototherapy has been successfully used in the treatment of vitamin D deficiency, psoriasis, and neonatal hyperbilirubinemia.[1c]

Photodynamic therapy (PDT) is an emerging treatment for a variety of conditions, but particularly for cancer and for wet age-related macular degeneration. It requires a combination of oxygen, visible light and a drug (a photosensitiser), which causes damage to living tissue. The scientific basis for this treatment is the photodynamic effect, which was discovered a century ago by Raab.[3] By 1913, Meyer Betz[4] had demonstrated the photodynamic effect in the human body in a celebrated (if foolhardy) experiment in which he injected himself with a solution of 200 mg of haematoporphyrin **8**: subsequent exposure to sunlight caused severe damage to his hands and face. Several decades later, in 1942, Auler and Banzer[5] observed tumour fluorescence and necrosis under UV light after porphyrin administration in experimental animals. In 1961, Lipson and his colleages[6] reported clear evidence that a porphyrin preparation (haematoporphyrin derivative, HpD) was selectively taken up by tumours. Another decade had to pass before Diamond and his coworkers[7] proved that such sensitised tumours could be destroyed by visible irradiation. In 1976, Kelly and Snell[8] made the first record of a clinical example (a single bladder cancer case, briefly reported). Shortly afterwards, an extensive and detailed clinical study was described by Dougherty and his colleagues[9] which eventually led to the first regulatory approval of a PDT sensitiser (Photofrin®). In 1993, Photofrin® was authorised for use against certain types of bladder tumour in Canada, and was later approved for the treatment of various sorts of tumour in the USA, Japan, and some European countries. By this time, much effort was being devoted to discover more efficient photosensitisers (often referred to as 'second generation' sensitisers).

PDT essentially consists of the following steps: (i) a good photosensitiser (and preferably a single substance with a straightforward synthesis) is identified which shows some preferential accumulation in the tumour tissue; (ii) this compound is administered and a certain time (the drug-light interval) is allowed to elapse in order to obtain the maximum differentiation in photonecrosis between normal and malignant tissue; and (iii) the tumour is irradiated with visible light in order to destroy the abnormal cells. The photosensitiser is usually administered using an aqueous buffer solution or liposomes and the light source is often a laser since this can be efficiently connected to optical fibres to treat deep tumours with good precision. While the process of photosensitiser localisation, which favours tumour accumulation, is not really understood, the process of tumour photonecrosis has received mechanistic rationalisation.

Two oxidative mechanisms are considered to be principally involved in the photodestruction of tissue,[1c] namely Type I and Type II. In the Type I mechanism, the photosensitiser interacts with a biomolecule (or oxygen) with a resulting hydrogen atom (or electron) transfer to produce radicals. In the Type II mechanism, singlet oxygen is generated as a result of energy transfer from the triplet exited state of the photosensitiser to the triplet ground state of the dioxygen molecule (Fig. 1).

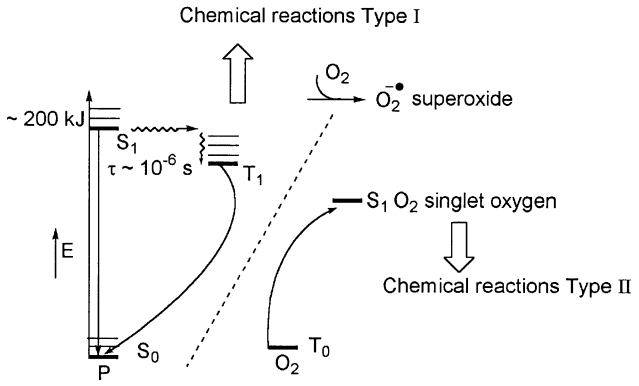


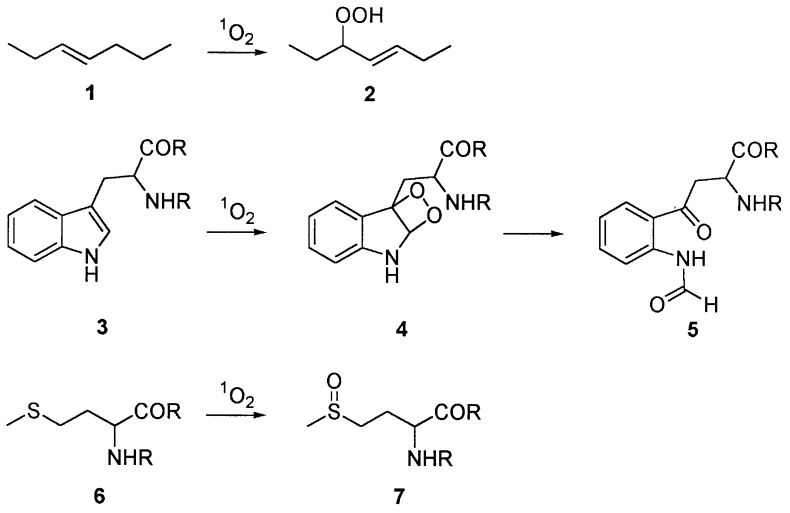
Figure 1. Modified Jablonski diagram to show Type I and Type II mechanisms (P=porphyrin photosensitiser).

Several sorts of biomolecules, such as unsaturated lipids (exemplified by **1**), cholesterol, and side chains of certain α-amino acids, such as tryptophanyl **3**, histidyl, and methionyl **6** react readily with singlet oxygen, as illustrated in Scheme 1. These components are essential constituents of various biological membranes, and it is therefore considered likely that membrane damage is an important process leading to both photonecrosis and vascular shutdown.

In general, the photosensitisers employed in cancer phototherapy have been porphyrin-type materials: there is no reason to suppose that the field is restricted to this series, although some advantages are discernible amongst these compounds (aromatic stability, high singlet oxygen quantum yields, apparent lack of dark toxicity, and absorption in the red region). HpD and its more purified versions, such as Photofrin®, Photosan® and Photohem®, were the first photosensitisers employed in PDT and they are referred to as first generation. HpD is a mixture of porphyrin monomers, dimers and oligomers which are formed from haematoporphyrin **8** as shown in Scheme 2.[1c,10] The antitumour activity basically resides in the covalently-linked oligomeric fraction, and therefore in the commercial preparations the less active monomeric fraction is removed using HPLC or gel permeation chromatography.

The first generation photosensitisers, however, possess three major drawbacks: their selectivity is low and, consequently, skin photosensitivity is a major side effect; absorption in the red region is weak, and so deep tumours are difficult to treat; and the photosensitisers are complex mixtures of active components.

In designing improved photosensitisers, a number of factors need to be considered in addition to their ease of synthesis.[11] A photosensitiser should have: little or no toxicity in the dark; good pharmacokinetic behaviour, i.e. high selectivity for tumour tissue and easy elimination from the body (amphiphilic character seems to be advantageous); a constant composition (preferably a single achiral substance); a high triplet quantum yield and a triplet energy $>94\ kJ\ mol^{-1}$ with efficient energy transfer to



Scheme 1. Reactions of singlet oxygen with some biomolecules.

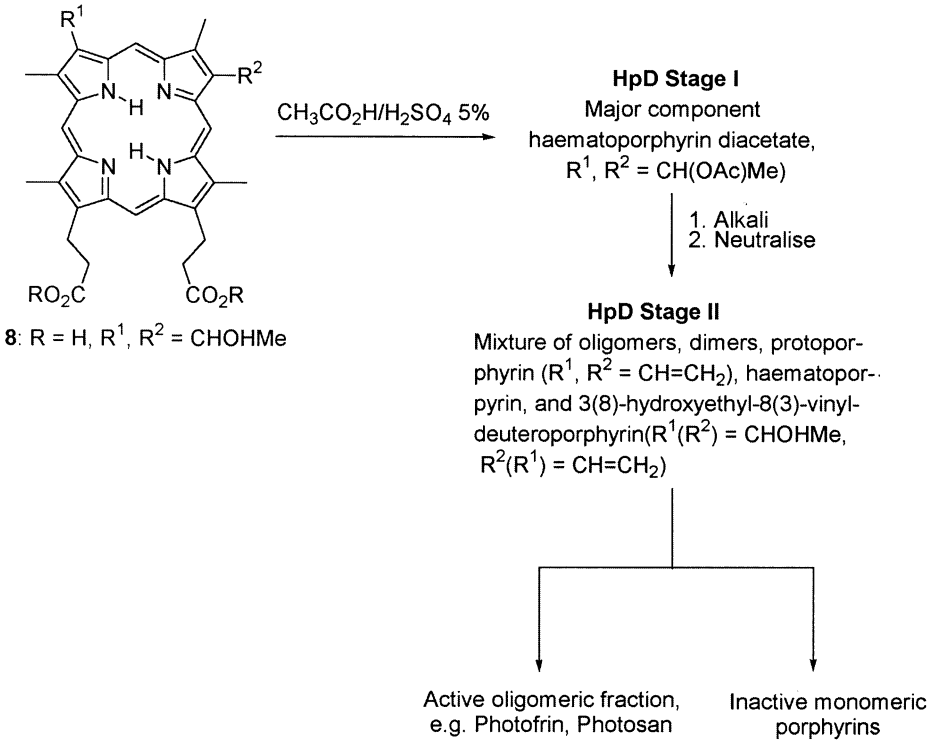
produce singlet oxygen; and red absorption to take advantage of deep light penetration.

A large number of novel photosensitisers have been synthesised with a variety of structures ranging from porphyrin-type compounds (chlorins, bacteriochlorins) to phthalocyanines, texaphyrins and porphycenes (Fig. 2). Detailed reviews have appeared elsewhere on this subject.[1b,c,13]

A significantly different approach which does not require the administration of a synthetic photosensitiser is the use of δ-aminolaevulinic acid (δ-ALA).[14] This procedure makes use of the biosynthesis of protohaem which is controlled by a feedback process in which the concentration of haem regulates the production of δ-ALA. When δ-ALA is administered, however, this regulatory mechanism is bypassed, and the biosynthesis sequence is triggered, producing mainly protoporphyrin **9** which acts as an endogenous photosensitiser. Patients suffering from basal cell carcinomas have been treated using a topical application of δ-ALA and a positive response was observed in 79% of the cases.[14] This treatment is being developed commercially by DUSA (Deprenyl USA, Toronto), and now has regulatory approval for use in actinic keratosis. Table 1 lists some other second generation sensitisers currently being developed.

2. Photobleaching and PDT

The resistance of dyes towards degradation by light has long



Scheme 2. Preparation of haematoporphyrin derivative (HpD) and its commercial variants.[1c]

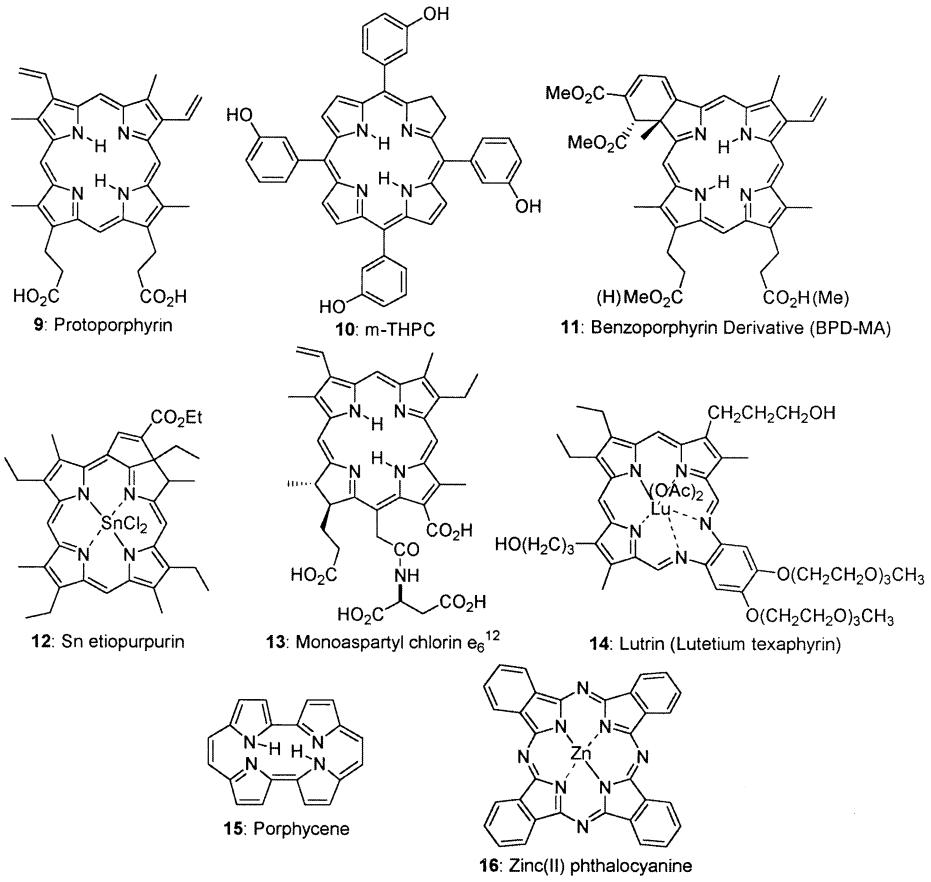


Figure 2. Structures of some second generation photosensitisers.

preoccupied scientists, especially in the textile industry.[15] The fading of dyed textiles upon exposure to daylight was already reported in Roman chronicles.[15a] In the first printed books about dyeing published in 1532 and 1548, sunlight was recognised as the cause of the fading of certain dyed textiles.[15a] At the beginning of the eighteenth century, Dufay carried out the first systematic study in order to classify dyes according to their resistance towards air and sunlight.[16] By the end of the eighteenth century, Senebier[17] and later Bancroft[18] began to discuss the chemical mechanisms of photofading and Bancroft suggested that the photobleaching process could be either oxidative or reductive.[18] In the 1830s, Chevreul[19] carried out detailed experiments in which dyes were exposed to sunlight under different conditions, for example, in oxygen, in a vacuum, and in the presence of moisture. He concluded that light, air and moisture had a great effect on the degradation of dyes in textiles. An early scientific investigation using more modern methods was carried out by Russell and Abney[20] who studied artists' pigments in 1886–88. A few years later, the issue of light fastness attracted the attention of the dye industry and after a communication presented by Hummel[21] in 1890 to the British Association, studies were made on the fastness of natural dyes in comparison with synthetic dyes, which were available since the discovery of mauve by Perkin in 1856. Since then, much progress has been made in the understanding of the mechanism of dye photodegradation. The mechanisms are, however, far from simple, and the nature of the photoproducts has often not been determined. In general, the accelerative effect of oxygen has been recognised and it has been suggested that singlet oxygen and related species are responsible for the photofading of certain

Table 1. Second generation photosensitisers under commercial development[1c]

Company	Headquarters	Photosensitiser
Cytopharm-Glaxo Wellcome	Menlo Park	Hydroxyalkylporphycene (cf **15**)
DUSA (Deprenyl USA)	Toronto	δ-Aminolaevulinic acid (ALA)
Miravant Medical Tech.	Santa Barbara	Tin etiopurpurin **12**
Negma	Strasbourg	Bacteriochlorophyll derivatives
Nippon Petrochemicals	Tokyo	MACE **13**
Novartis	Basel	Zinc phthalocyanine **16**
Pharmacyclics	Palo Alto	Lu texaphyrin derivative **14**
QLT	Vancouver	Benzoporphyrin derivative
CibaVision/QLT	Vancouver/Basle	(Visudyne®, for age-related macular degeneration) **11**
Russian group	Moscow	Chloroaluminium phthalocyanine sulphonic acids ('Photosens')
Scotia Pharmaceuticals	Stirling	*m*-THPC (Foscan®, **10**) *m*-THPBC, pegylated derivatives

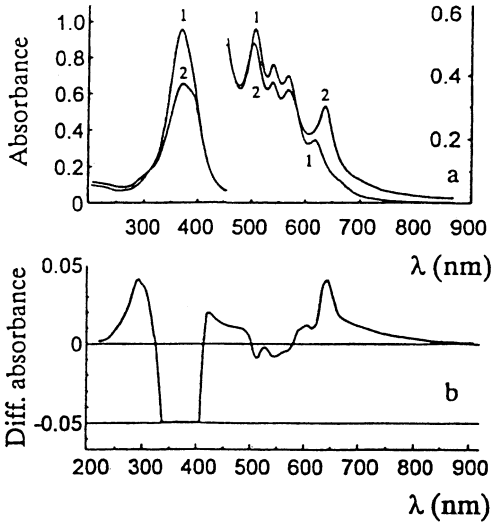


Figure 3. Top spectrum: absorption spectra of a solution of haematoporphyrin **8** (10^{-4} M in PBS) before (1) and after (2) irradiation at 514 nm. Light dose 1050 J. Bottom spectrum: difference spectra (irradiated minus non-irradiated) of a solution of haematoporphyrin (10^{-5} M in PBS). Light dose 380 J. (Reprinted from Ref. 36a with permission from Elsevier Science).

dyes.[22] The interest in the photodegradation of dyes and pigments has also been embraced by other fields which have appeared over the years with the advances of science and technology, so that nowadays, this property is relevant in a variety of fields from laser technology to photomedicine.

In the literature, there are several terms which are employed to refer to the degradation of dyes and pigments upon irradiation. Those most frequently used are photofading, lack of light fastness, and photobleaching. In photochemistry and photobiology, the term photobleaching (or bleaching) is widely used and is defined as the loss of absorption or emission intensity caused by light.[23]

There are two types of irreversible photobleaching which lead to chemical change in the chromophore:[24]

(i) *Photomodification*, where loss of absorbance or fluorescence occurs at some wavelengths, but the chromophore is retained in a *modified* form. An example—the photoreaction of haematoporphyrin **8** in aqueous buffer—is illustrated in Fig. 3. As the original absorption spectrum diminishes, a new absorption peak emerges at 640 nm and

(ii) *True photobleaching*, where chemical changes are deep-seated, and result in small fragments which no longer have appreciable absorption in the visible region. The sample becomes essentially colourless. Fig. 4 illustrates this effect when 5,10,15,20-tetrakis(*m*-hydroxyphenyl)chlorin **10** is irradiated in aqueous methanol with a 514 nm laser source, and all the visible peaks diminish.

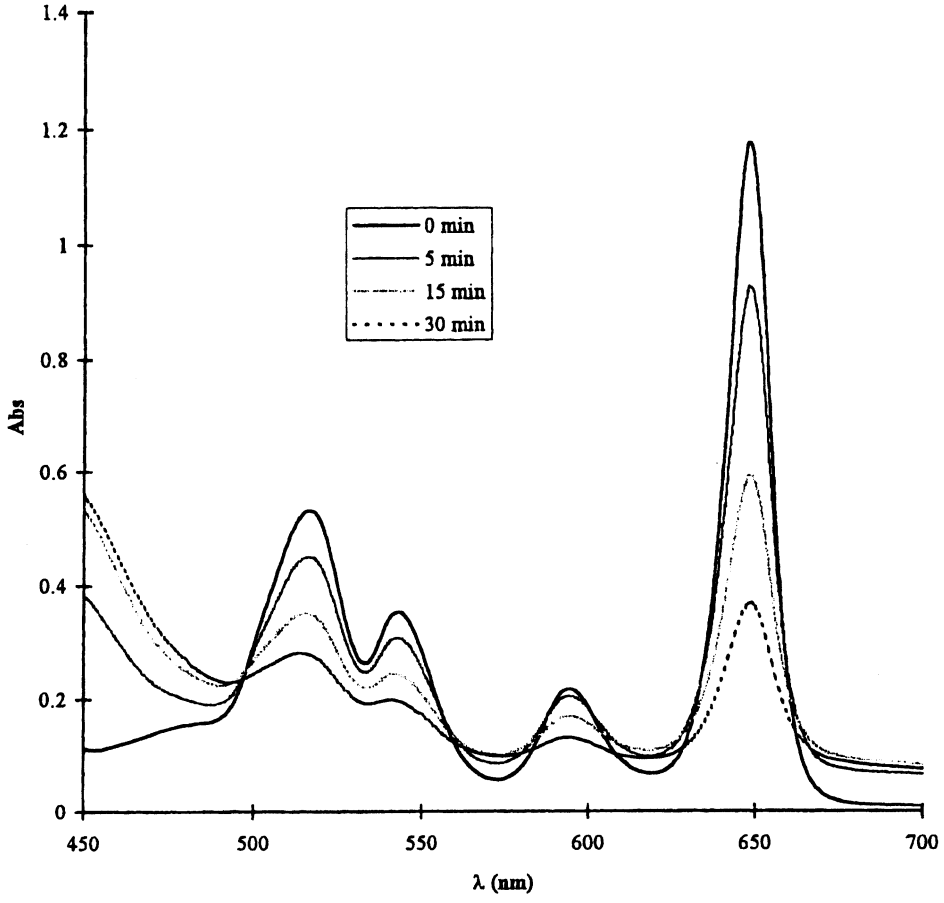


Figure 4. Changes in the absorption spectrum of a solution of *m*-THPC **10** 0.04 mM in methanol–water (3:2, v/v) in equilibrium with air on irradiation at 514 nm with an argon laser (P=0.78 W). (Reprinted from Ref. 24a with permission from Elsevier Science).

It appears that, where photomodification occurs, true photobleaching often occurs concomitantly.

In mechanistic terms, the pathways of the photobleaching or photomodification can be very varied. Photoaddition and photocyclisation may be accompanied by the loss of chromophore; and electron transfer processes can lead to the formation of broad bands showing red shifts, although these changes can often be reversed. Irreversible photoreductions may occur on irradiation in the presence of a reducing agent. Porphyrin photoreduction occurs by hydrogen addition at sites selected from *meso*-, β- and nitrogen, depending on the conditions.[25] In some circumstances, the photoreduction appears to be regiospecific, as in the photoreduction of zinc(II) β-vinylporphyrins in the presence of ascorbic acid–organic base mixtures, where the reduction appears to occur specifically at the ring bearing the vinyl substituent to give the *cis*-chlorin.[25e]

In the majority of examples of photobleaching in PDT, however, where oxygen is necessarily present, the mechanistic processes are oxidative, and usually involve singlet oxygen or radical reactive oxygen species (Fig. 1). In solution experiments, convincing evidence can be obtained for the involvement of singlet oxygen and, indeed, photochemically generated singlet oxygen is a valuable reagent in synthetic chemistry.[1c] In in vivo systems, however, it is much more difficult to establish the nature of the intermediate, although Forster and his colleagues[26] have reported evidence based on oxygen tension measurements in multicellular tumour spheroids which accords with the view that photobleaching of Photofrin® and protoporphyrin involves singlet oxygen. The photobleaching of Nile blue selenium, however, follows a non-singlet oxygen pathway.

In the PDT field, the first relevant observation of photobleaching was reported in 1986 by Moan et al.[27] using NHIK 25 cells incubated with Photofrin®. Shortly afterwards, photobleaching was observed by Mang et al.[28] in patients receiving treatment with Photofrin®. The potential benefit of photobleaching upon treatment was recognised at an early stage, although the first attempts were not promising. Boyle et al.[29] for example, proposed that skin photosensitivity (the major side effect of PDT treatment which can last up to five weeks when Photofrin® is used) could be reduced if the patient was pre-irradiated with increasing light doses. In animal experiments, Swiss mice treated in this way suffered only one week of skin photosensitivity when treated with Photofrin®. Subsequent studies, however, do not appear to have been successful.[30]

Another idea which also appeared at an early stage was to use a low dose of the photosensitiser in combination with a high dose of light.[27b,28] By taking this approach, Mang et al.[31a] and Ris et al.[31b] succeeded in limiting damage to normal tissue and achieving high tumour necrosis during PDT treatment using Photofrin® and *m*-THPC **10**, respectively. In recent years, as the potential benefits of controlling photobleaching have been recognised and the complexity of the process has been revealed, this property has been increasingly considered.

3. Photobleaching studies in solution

3.1. Haematoporphyrin and related photosensitisers (HpD, Photofrin®)

The photobleaching of haematoporphyrin and related compounds, which include Photofrin®, has been extensively studied in kinetic terms. Very little is known, however, about the structures of the photoproducts.

Starting in 1988, Rotomskis and coworkers[32] have reported the photoreactions of haematoporphyrin and various derivatives, irradiating with an argon ion laser (λ 514 nm). They observed the decrease of absorption throughout the spectrum and the simultaneous appearance of a band at about 640 nm, when an aerated solution of haematoporphyrin diacetate in phosphate buffer was irradiated [compare Fig. 3, which refers to the di-alcohol haematoporphyrin **8**]. While haematoporphyrin diacetate is a mixture of monomer and aggregates in aqueous solution, it exists as the monomer in ethanol. When haematoporphyrin diacetate in ethanol was exposed to light, only photobleaching was observed. The 640 nm photoproduct formed in aqueous media was also detected under a nitrogen atmosphere; but oxygen bubbling enhanced photobleaching. These observations, together with the fact that the ratio of aggregated to monomeric material decreased on irradiation, led these authors to conclude that the photoproduct may have arisen from the aggregated porphyrins without oxygen involvement.

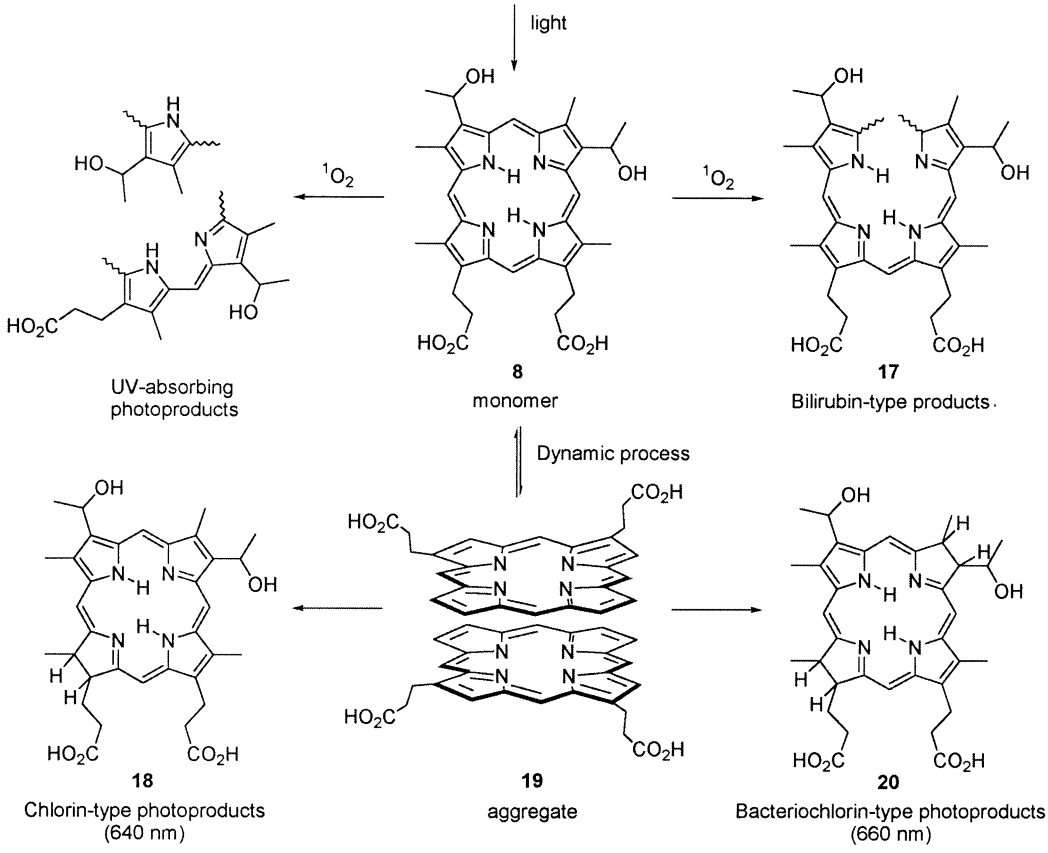
In order to support these ideas, Rotomskis et al.[33] carried out a set of experiments in which the effects of aggregation on photoproduct formation were evaluated. Haematoporphyrin dimethyl ether, haematoporphyrin **8** and Photosan® were irradiated in phosphate buffer solutions, and the same effects were observed as before (i.e. absorbance decay, disappearance of aggregated porphyrins, and the new band at 640 nm). While haematoporphyrin dimethyl ether yielded the largest amount of photoproduct, Photosan® appeared to be relatively photostable. In micellar media (Triton X-100), haematoporphyrin dimethyl ether led to a lower amount of photoproduct, whereas Photosan® and haematoporphyrin **8** each showed a new peak in the spectrum at 660 nm. These changes were attributed to different aggregation states of each photosensitiser under different reaction conditions. Haematoporphyrin dimethyl ether was thought to be aggregated, forming 'sandwich-type' dimers (H-type) according to its absorbance spectrum (Soret band blue-shifted, the width at half-height of the band ($\Delta\nu$) being increased, and Band I showing a small red shift); haematoporphyrin (**8**) was thought to exist as sandwich and linear dimers (linear dimers—J-type—are characterised by a slightly red-shifted Soret band, a larger $\Delta\nu$, and only a marginal change in λ_{max} of Band I, although some variation appears to be possible in these criteria); and Photosan® was regarded as existing as covalently-linked linear oligomers held together by non-covalent bonds and sandwich-type dimers or oligomers. In interpreting these results, Rotomskis[33] suggested that photoproduct formation with an absorption band at 640 nm was limited by the presence of sandwich-type dimers, while the product with a band at 660 nm depended on the existence of a linear-type covalently-linked structure.

Studies[34] on HpD using fluorescence and absorption spectroscopy, led to the suggestion that the photoproduct with an absorption band at 640 nm was a chlorin or a covalently-linked porphyrin–chlorin system, but no specific structure was proposed. Rotomskis et al.[35] reported the effect of pH on the photobleaching of, and photoproduct formation from, HpD and haematoporphyrin **8** in phosphate-buffered solutions. Photobleaching was slightly increased when the pH was increased, whereas the formation of the 640 nm photoproduct was strongly pH dependent: below pH 5 it was not detected; between pH 6–10 it was favoured; and above pH 10 its formation was again diminished.

More recently,[36] the same group isolated small amounts of several photoproducts from the photooxidation of haematoporphyrin **8** in aqueous buffer solution. Three types of photoproducts, characterised only by their absorption and fluorescence emission spectra, were isolated: UV absorbing photoproducts (λ_{max} 240–320 nm) regarded as mono- or di-pyrroles; supposed bilirubin-type molecules (λ_{max}~545 nm); and two red-absorbing photoproducts, which were regarded as compounds of the chlorin and bacteriochlorin type, with maxima at 640 and 660 nm, respectively (Scheme 3). It seems unlikely, however, that a bacteriochlorin of this substitution pattern would have λ_{max} for Band I as low as 660 nm. The efficiency of formation of these red-absorbing photoproducts with respect to structure was also evaluated and the following sequence was found: haematoporphyrin dimethyl ether>haematoporphyrin>HpD>Photosan®>Photofrin®. The authors concluded that the formation of the red-absorbing photoproducts from different precursors is favoured when sandwich-type aggregates are present. It was considered that in these aggregates, oxygen penetration would be limited and a photoreduction process would occur to yield the chlorin- and bacteriochlorin-type photoproducts (Scheme 3).

In a separate study, König et al.[37] observed a new fluorescent photoproduct (λ_{em} 642 nm) from HpD in phosphate-buffered solutions on irradiation with an argon ion laser (λ 514 nm). In order to demonstrate the involvement of singlet oxygen in photoproduct formation, several of the common tests[1c] were carried out, including using sodium azide (a singlet oxygen scavenger), and D_2O as solvent [depending on circumstances, the photooxidation efficiency may be improved in D_2O because singlet oxygen has a much longer lifetime in D_2O (τ=30–50 μs) than in H_2O (τ=2–3 μs)]. These results indicated a role for oxygen, in contrast to observations reported by Rotomskis et al.[32] It was also noted that the photoproducts appeared to have a lower photodynamic activity than HpD. Jones and Grosweiner[38] also detected the formation of a photoproduct (λ_{max}~663 nm) and photobleaching of the Soret band when solutions of Photofrin® in Triton X-100 were irradiated with a filtered 200 W high-pressure Hg–Xe arc (360–630 nm) or a tunable dye laser. In this example also, additives, such as sodium azide and imidazole, provided indirect evidence that singlet oxygen was responsible for the reaction.

Spikes[39] made a comprehensive study of reaction conditions



Scheme 3. Rotomskis' mechanism for the photooxidation of compounds of the haematoporphyrin series.[36]

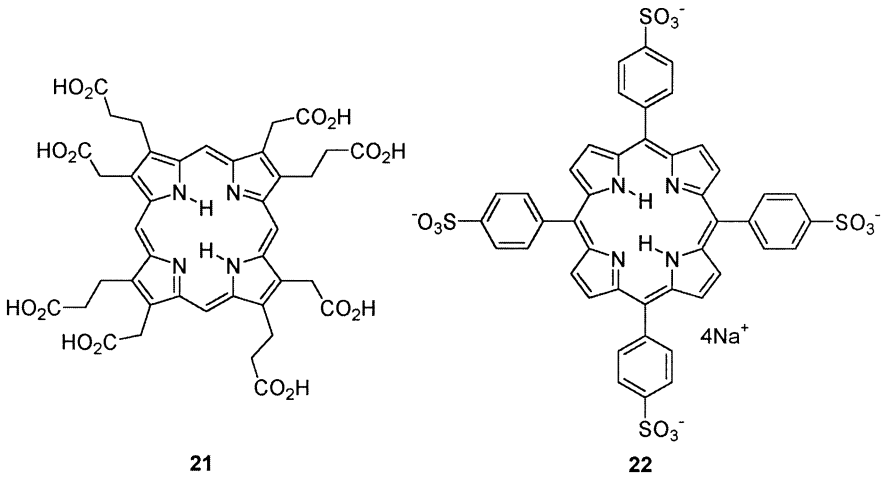


Figure 5. Uroporphyrin I **21** and $TPPS_4$ as the tetrasodium salt **22**.

and the effect of several additives on the quantum yields and kinetics of the photobleaching of haematoporphyrin **8**, Photofrin®, uroporphyrin I **21** and 5,10,15,20-tetrakis(4-sulphonatophenyl)porphyrin tetrasodium salt ($TPPS_4$, **22**) (Fig. 5) in phosphate buffer solution. The solutions were irradiated in the region of the Soret band with filtered light from a slide projector lamp. The photobleaching of haematoporphyrin **8**, uroporphyrin I **21** and $TPPS_4$ **22** followed first order kinetics, whereas the Photofrin® photobleaching rate was a mixed process. New products absorbing in the visible region were not observed on irradiation, in contrast to previous reports in which higher concentrations had been employed. The quantum yields for photobleaching in aqueous buffer reported by Spikes are listed in Table 2.

Table 2. Photobleaching quantum yields (Φ_{pb}): 100 mM $NaHPO_4$ buffer of pH 7 and 0.22 mM O_2[39]

Substrate	Photobleaching quantum yield (Φ_{pb})
Haematoporphyrin **8**	4.7×10^{-5}
Photofrin®	5.4×10^{-5}
Uroporphyrin I **21**	2.8×10^{-5}
$TPPS_4$ **22**	9.8×10^{-6}

Monomeric forms of Photofrin®, $TPPS_4$ **22** and haematoporphyrin **8** appeared to be more susceptible to photobleaching than aggregated porphyrins. Nitrogen bubbling (30 min, reducing oxygen concentration from 0.22 mM to 2 μM)[39] led to lower quantum yields (~90% decrease) for Photofrin®, haematoporphyrin **8** and $TPPS_4$ **22**, whereas the initial rate of uroporphyrin I **21** photobleaching was enhanced. Using D_2O as the solvent, and sodium azide addition, seemed to have little effect.

The dielectric constant of the solvent did appear to influence the quantum yield, photobleaching being increased when the dielectric constant of the solvent was raised. Since ionic species are more stable in solvents with high dielectric constants, ionic intermediates may be involved in the photobleaching process and, therefore, be associated with increased bleaching rates.

The effects of different photooxidisable organic compounds, namely cysteine, methionine, tryptophan, histidine, furfuryl alcohol, dithiomenthol and human serum albumin (HSA), on the quantum yields of photobleaching were also assessed. Apparently random effects on the rates of photobleaching of the four porphyrins were observed when the amino acids were added. On the other hand, quantum yields were increased 2- to 3-fold in the presence of HSA, in which monomeric forms are favoured due to protein binding. The presence of furfuryl alcohol also increased the photobleaching quantum yields of the four porphyrins, the largest effects occurring with uroporphyrin I **21** and haematoporphyrin **8** (>4-fold increase). The photooxidation products of furfuryl alcohol, such as hydrogen peroxide, may react with the porphyrin leading to bleaching (macrocycle cleavage). The effects of other additives (e.g. methyl viologen, metronidazole, catalase, superoxide dismutase, cationic detergents) were also observed, although a general rationalisation of the results of the additive experiments for the four porphyrins studied did not emerge.

Bezdetnaya et al.[40] reported the photobleaching of HpD and protoporphyrin **9** in solution containing foetal calf serum (FCS), irradiating with a krypton laser (λ 405, 413 nm). The photobleaching quantum yields were calculated from absorption decay, fluorescence decay and photocytotoxicity decay (using a cell line, HT 29, derived from human adenocarcinoma). The observed absorption decay was 10-fold smaller than the fluorescence decay. Since fluorescence mainly arises from monomers rather than from aggregates, this result was attributed to the greater photolability of the monomers compared to the aggregates. It was proposed that the difference between the fluorescence quantum yield and photocytotoxicity quantum yield (the former being 1.4- and 2.6-fold larger than the latter for HpD and protoporphyrin **9**, respectively) was caused either by the formation of photoproducts having emission bands which overlap those of the parent compound, or by a change in hydrophilicity of photobleached porphyrin, which could influence cell incorporation. A different test was also carried out. Pre-irradiated solutions, which had then been kept in the dark for several hours, recovered cytotoxicity and fluorescence, this recovery being 100% for protoporphyrin **9** and lower for HpD. The authors attributed this behaviour to

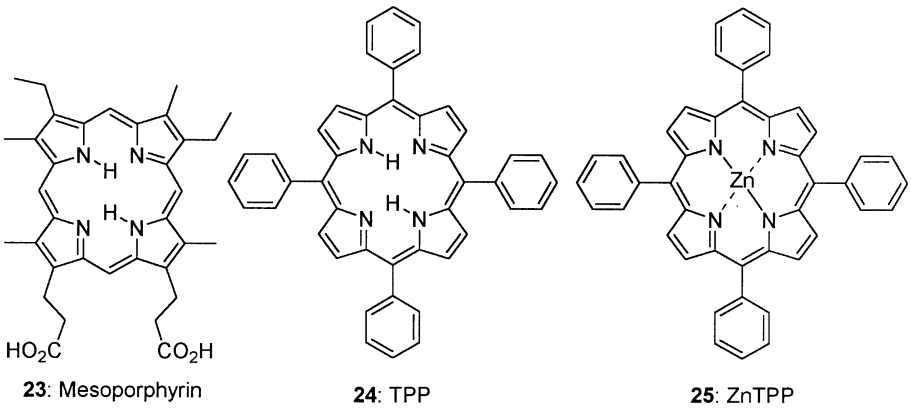


Figure 6. Mesoporphyrin **23**, tetraphenylporphyrin **24** (TPP) and zinc(II) tetraphenylporphyrin **25** (ZnTPP).

disaggregation of the pre-irradiated dye. Further studies are desirable to extend these findings.

3.2. Protoporphyrin (δ-ALA as a prodrug)

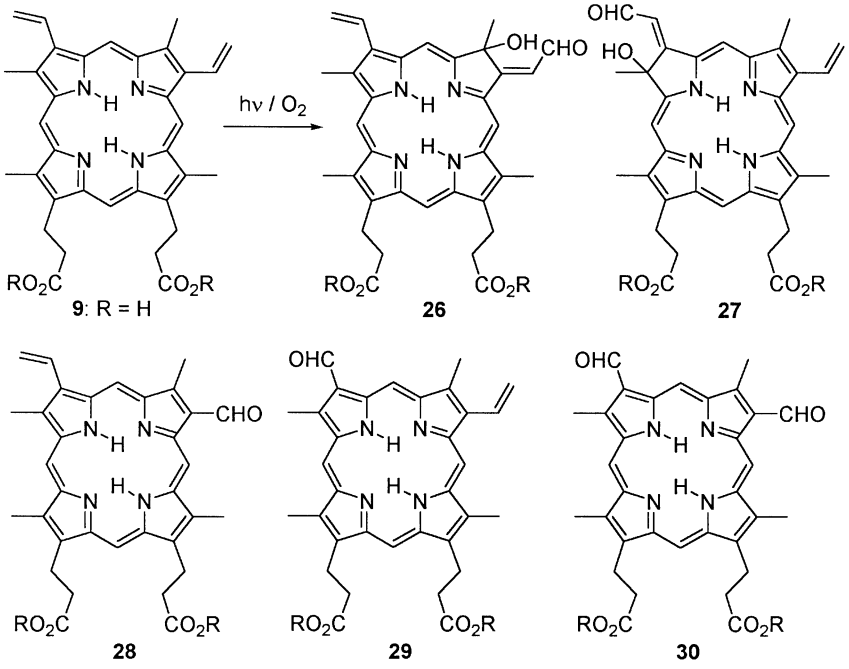
Administration of δ-ALA, usually in the form of a lotion applied directly to the tumour, leads to the formation of protoporphyrin **9** within the tumour (Section 1). Because of its relevance to the photosensitivity of patients suffering from erythropoietic protoporphyria, the photooxidation of protoporphyrin is one of the most thoroughly studied examples of photosensitiser degradation.[41–48]

The photooxygenation of protoporphyrin **9** was first studied by Fischer[41a] in 1938, although the products were identified much later.[42,43] Gurinovich et al.[41b] undertook spectroscopic studies which appeared to show that protoporphyrin, etioporphyrin and mesoporphyrin (**23**, Fig. 6) were photooxidised, giving chlorin-type products (absorption bands at λ_{max} 650–670 nm), whereas porphyrin, octaethylporphyrin and tetraazaporphyrin were photostable. TPP **24** was relatively photostable in aerated organic solvents, whereas ZnTPP **25** gave a photoproduct with λ_{max} 465 nm. No structures were proposed.

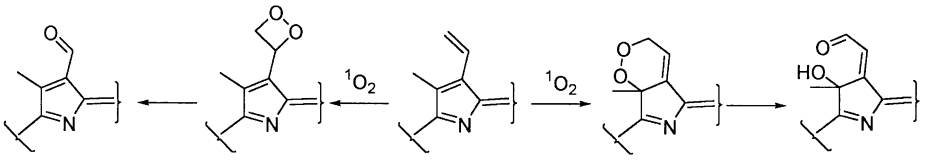
Inhoffen[42] and later Whitten[43] identified the photoproducts from protoporphyrin **9** as the isomeric hydroxyaldehydes **26** and **27** and the formylporphyrins **28**–**30** (Scheme 4).

The formation of **26** and **27** (also called photoprotoporphyrins A and B) is believed to involve a [4+2] cycloaddition of singlet oxygen to the diene unit formed by an endocyclic double bond and a vinyl group, whereas the formyl porphyrins **28**–**30** arise from a [2+2] addition to the vinyl group yielding a dioxetane (Scheme 5).

Whitten and his colleagues have carried out a detailed and very relevant study of this reaction, which in the authors'



Scheme 4. Photooxidation products from protoporphyrin **9**.[42–48] Experiments in organic solvents have sometimes been conducted with esters (e.g. R=Me, $C_{18}H_{37}$) to facilitate manipulation of the products.



Scheme 5. Reaction of singlet oxygen with protoporphyrin **9**.

view has been insufficiently regarded. Cox and Whitten[44] studied the photooxidation of protoporphyrin **9** derivatives in several organic solvents, irradiating with a 450 W medium pressure mercury lamp or a 1000 W mercury xenon arc lamp. In all cases, the formation of the chlorin-type products **26** and **27** was favoured over that of the formylporphyrins **28**–**30**, a typical ratio of about 10:1 being obtained. On the other hand, the total product yield was relatively low (25–51%), suggesting further decomposition of the primary photoproducts and **9**.

Although the singlet oxygen quantum yields of the hydroxyaldehydes **26** and **27** and the formylporphyrins **28**–**30** are high, these products are reported to be more stable towards photooxidation than protoporphyrin.[48]

Experiments with additives were carried out in order to try to distinguish between Type I and Type II mechanisms. With the addition of singlet oxygen quenchers such as cholesterol and diazabicyclo[2.2.2]octane (DABCO), the yields of all the products decreased, suggesting that singlet oxygen attack on the ground state of **9** was the most likely origin of these photoproducts. A slight decrease in the ratio of hydroxyaldehyde to formyl products was noticed, however, suggesting, perhaps, that a minor portion of the formylporphyrins could come from a non-singlet oxygen pathway. The role of superoxide was also assessed. The experiments using superoxide scavengers such as methanol or 2-propanol, which catalyse its dismutation, were rather inconclusive. On the other hand, the presence of methyl viologen led to more clear-cut results. The formation of the hydroxyaldehydes **26** and **27** was almost suppressed, whereas the yield of the formyl products **28**–**30** was little affected. It was concluded that, under the conditions studied, singlet oxygen (Type II mechanism) was the major pathway of photooxidation of **9** in solution to give the major products **26** and **27**, but that the formyl products **28**–**30** could also arise by a Type I pathway.

The same group investigated this process in more complex media. A preliminary study had been carried out in monolayers, organised monolayer assemblies and micelles,[43] which gave completely different ratios of photoproducts to those observed in organic solvents. The formylporphyrins **28**–**30** were now the major products rather than the hydroxyaldehydes **26** and **27**. A more detailed account on micelles (SDS, DTAB, and Brij 35), and on vesicles (DPPC), was published a few years later.[45] In these systems, the total yield of photoproducts **26**–**30** was lower (15–28%) than that observed in solution, indicating a more extensive true photobleaching of protoporphyrin **9**. The ratio of formyl to hydroxyaldehyde products also increased (typical ratio 43:57), but not as dramatically as seen with monolayers and assemblies. In order to investigate the mechanism involved in the aqueous surfactant solutions, the photooxidation was examined in the presence of different quenchers. DABCO and cholesterol did not provide any clear information on this occasion. Sodium azide, a singlet oxygen quencher which, being ionised, resides essentially in the aqueous phase, led to a reduction of the yields of the photoproducts **26** and **27**, and the formation of the formyl porphyrins **28**–**30** was slightly increased. When high concentrations of azide were employed, the quenching effect reached a plateau that was attributed to an intramicellar reaction unquenchable by the azide. The change from H_2O to D_2O was also examined. Both product yields were increased, but that of the hydroxyaldehydes **26** and **27** was particularly enhanced. The quenching of the hydroxyaldehyde formation by azide was much more pronounced in D_2O. From this evidence, it was proposed that there are at least two major pathways for the photooxidation of **9** in organised media.

Kinetic analysis (see Ref. 45 for more details) led to the conclusion that photooxidation by singlet oxygen in micellar systems can be split into two contributions: an intramicellar portion and an intermicellar portion. The intermicellar reaction would be limited by singlet oxygen decay in the aqueous phase and the intramicellar reaction would be independent of total porphyrin concentration since each micelle can only accommodate a limited amount of porphyrin. It was argued that the formation of the porphyrins **28**–**30** essentially did not depend on a singlet oxygen pathway, as indicated by the small effect of D_2O on **28**–**30** formation and the small yield increase when azide was used. An intramicellar path was therefore proposed, and an electron transfer leading to the generation of superoxide ion was favoured.

Krieg and Whitten[46,47] reported the behaviour of several porphyrins in the presence of molecular oxygen when studied in the presence of erythrocyte ghosts, and in oil-in-water microemulsions. The erythrocyte ghosts are the washed natural membrane systems from red blood corpuscles, and contain saturated lipids, unsaturated lipids, and a full complement of membrane proteins. The oil-in-water microemulsion provides a more limited model for just the lipid portion of a natural membrane. The porphyrins studied were protoporphyrin **9**, mesoporphyrin **23**, haematoporphyrin **8**, HpD, *meso*-tetraphenylporphyrin (TPP, **24**) and zinc(II) *meso*-tetraphenylporphyrin (ZnTPP, **25**).

In contrast to the results obtained in organic solutions or in micellar or vesicular systems, where protoporphyrin **9** gave the identified photoproducts **26**–**30**, this porphyrin *now underwent true photobleaching* when irradiated in aerated ghosts. Since mesoporphyrin **23** and haematoporphyrin **8**, which do not contain vinyl groups, also showed such

photobleaching, a possible photooxidation of the macrocycle, mediated by photooxygenated protein residues, was suggested. HpD photobleached at similar rates to those observed for protoporphyrin **9** and faster than in solution. On the contrary, TPP **24** was little affected and ZnTPP **25** gave a product absorbing strongly below 500 nm and in the 700–800 nm region. This behaviour is very similar to that of zinc, cadmium, and magnesium complexes of tetraphenylporphyrin in solution,[49,50] where the formation of a linear tetrapyrrole derivative occurs as a result of singlet oxygen addition to, and cleavage of, the macrocycle (Section 3.4).

In order to understand which pathway was involved in such a degradation in the presence of erythrocyte ghosts, systematic studies were carried out in oil-in-water emulsions comprising benzene, surfactant (anionic SDS or cationic DTAB), *n*-butanol and water. Several amino acids (arginine, cysteine, histidine, methionine and tryptophan) and other additives were introduced into the mixture and their effects evaluated.

When protoporphyrin **9** was irradiated in an aerated oil-in-water emulsion containing no further additive, it showed the same product distribution as in organic solvents. While arginine did not produce much change, the rate of photobleaching was enhanced when cysteine, histidine and methionine were added. HpD, haematoporphyrin **8** and mesoporphyrin **23** were photostable in emulsions, but addition of methionine induced photobleaching. No products were detected in any examples. In contrast, TPP **24** suffered little bleaching with or without added amino acids; ZnTPP **25** underwent its normal photooxidation in emulsion, but, surprisingly, this was quenched by methionine. On the other hand, diethyl sulfide did accelerate the photodegradation of protoporphyrin **9**. On the basis of these results, the thioether group might be considered to be the key factor in the photodegradation of **9** and the octaalkylporphyrins. Experiments in which the H_2O solvent was replaced by D_2O did not show any isotopic effect, i.e. the photobleaching rate was unaltered. It was shown that the photodegradation of protoporphyrin **9**, mesoporphyrin **23**, haematoporphyrin **8** and HpD did not occur in deaerated solutions, indicating that oxygen must have been involved in the process.

A new photodegradation mechanism was proposed to account for the observations on the photodegradation of protoporphyrin **9**, mesoporphyrin **23**, haematoporphyrin **8** and HpD in the presence of biomolecules. Singlet oxygen

$$P(S_0) \xrightarrow{h\nu} P(S_1) \longrightarrow P(T_1)$$

$$P(T_1) + O_2(T_0) \longrightarrow P(S_0) + O_2(S_1)$$

$$O_2(S_1) + BM \longrightarrow BM\text{-}O_2 \longrightarrow \text{Substrate oxidation products}$$

$$BM\text{-}O_2 + P \longrightarrow Pox + BM \text{ or } BMox$$

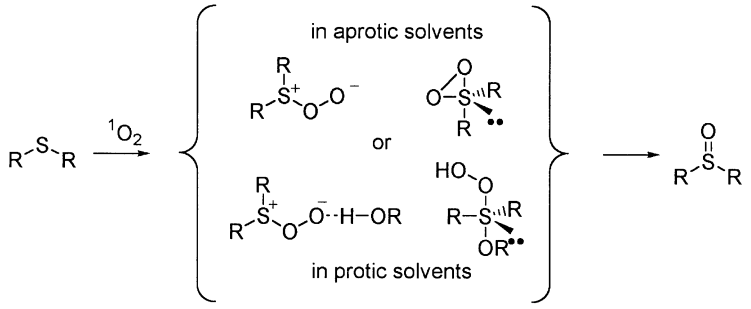
Scheme 6. Proposed photodegradation mechanism in microemulsions (BM=biomolecule).

was expected to be quenched very rapidly by an easily oxidised substrate, such as methionine. This would in turn provide a good oxidant which could oxidise the porphyrin (Scheme 6). According to the results described above, thioethers would be suitable candidates which could generate very reactive intermediates such as persulfoxides (Scheme 7). The effect of amino acids in microemulsions suggested that protein components would play a similar role in such a photobleaching in erythrocyte ghosts, where, in addition to persulfoxides, hydroperoxides (ROOH) and other organic peroxides (ROOR, RCO_3R', etc.) could also be involved as reactive residues. Although no photoproducts were identified in these studies, the cleavage of the porphyrin ring leading to linear tetrapyrrole intermediates, such as biliverdin and bilirubin analogues, was proposed as a possible explanation for the rapid photobleaching, since biliverdin and, especially, bilirubin are degraded very rapidly in the presence of singlet oxygen.[52]

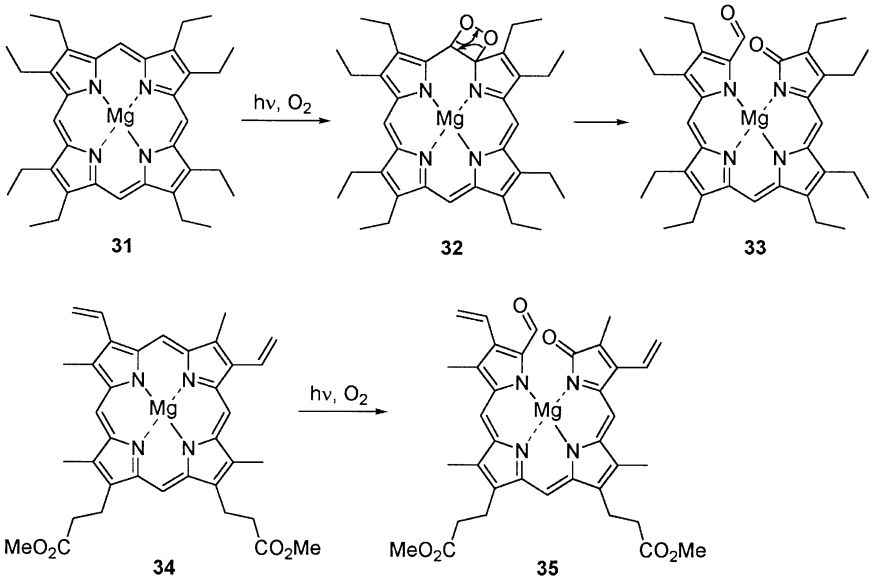
3.3. Other β-polyalkylporphyrins

The behaviour of some of these compounds has already been considered as it arose in Sections 3.1 and 3.2. The situation is confusing, since, whereas etioporphyrin is photooxidised, octaethylporphyrin under the same conditions is apparently not photooxidised.[41b] Certainly, the metal-free compounds are more difficult to photooxidise than those metal complexes [e.g. Mg(II) and Zn(II)] which have a lower redox potential.

Barrett[53] appears to have been the first to report the photoconversion of certain metalloporphyrins into linear tetrapyrrole pigments upon irradiation. Fuhrhop et al.[54] studied this reaction in greater detail and showed that the photooxidation of the magnesium(II) complex of octaethylporphyrin **31** yielded the formylbilinone **33**. Analogously, the magnesium complex of protoporphyrin dimethyl ester **34** led to **35**. The reaction was regarded as proceeding via



Scheme 7. Sulphide photooxidation.[51]



Scheme 8. Photooxidation of β-substituted metalloporphyrin complexes possessing low oxidation potentials.[54c]

singlet oxygen [2+2] addition, followed by ring opening (Scheme 8).

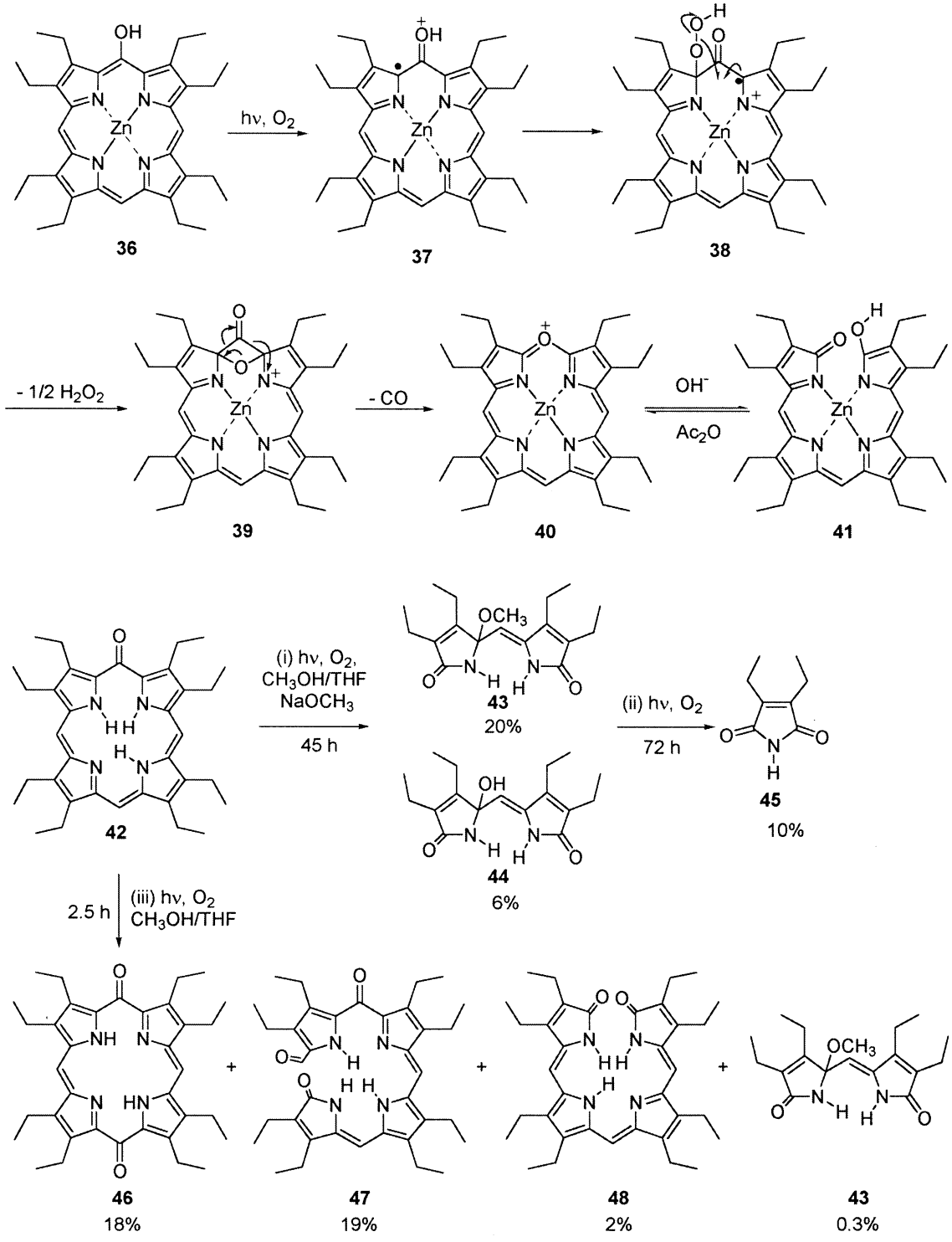
The photooxidation of the zinc(II) complex of octaethyl-α-hydroxyporphyrin **36** led to a biliverdin type product **41**, but on this occasion a methine carbon was lost.[55a] A mechanism was proposed which involved the formation of a radical cation of **36** and the addition of a molecule of oxygen (Scheme 9). Bonnett et al.[55b] studied the photooxidation of the free base of octaethyloxophlorin **42** (Scheme 9). The reaction of the dianion of **42** yielded small fragments **43**–**45** after long periods of irradiation. Under milder conditions, the neutral free base of **42** gave octaethyl-5,15-dioxo-5,15-dihydroporphyrin **46**, octaethyl-19-formyl-21*H*,24*H*-bilin-1,15-dione **47**, octaethyl-21*H*,24*H*-bilin-1,19-dione **48**, and tetraethyl(methanol)propentdyopent **43**. Both reactions were inhibited when air was replaced by a flow of nitrogen. The mechanism of formation of the pigments **46**–**48** was not established, although mediation of the cation radical of **42** was thought not to be involved. Reaction between the excited state of **42** with ground state oxygen to give a mixture of hydroperoxides could explain the formation of **46**–**48**. Pigments **47** and **48** could, however, also have arisen from a singlet oxygen [2+2] addition (cf Scheme 8).

3.4. *meso*-Tetraphenylporphyrin and its relatives

Some photoproperties of TPP **24** and its zinc(II) complex **25** have been mentioned as they arose in Whitten's work (Section 3.2). The photooxidation reactions of the metal complexes and of the dianion of TPP **24** have been studied extensively as models to understand oxidative haem metabolism, the formation of algae biliproteins, and chlorophyll degradation in senescent leaves.[49,50,56] Although none of these compounds has been important in PDT, their photooxygenation mechanism is relevant in the photobleaching context due to their structural relationship to biologically potent PDT photosensitisers such as *m*-THPC **10**.

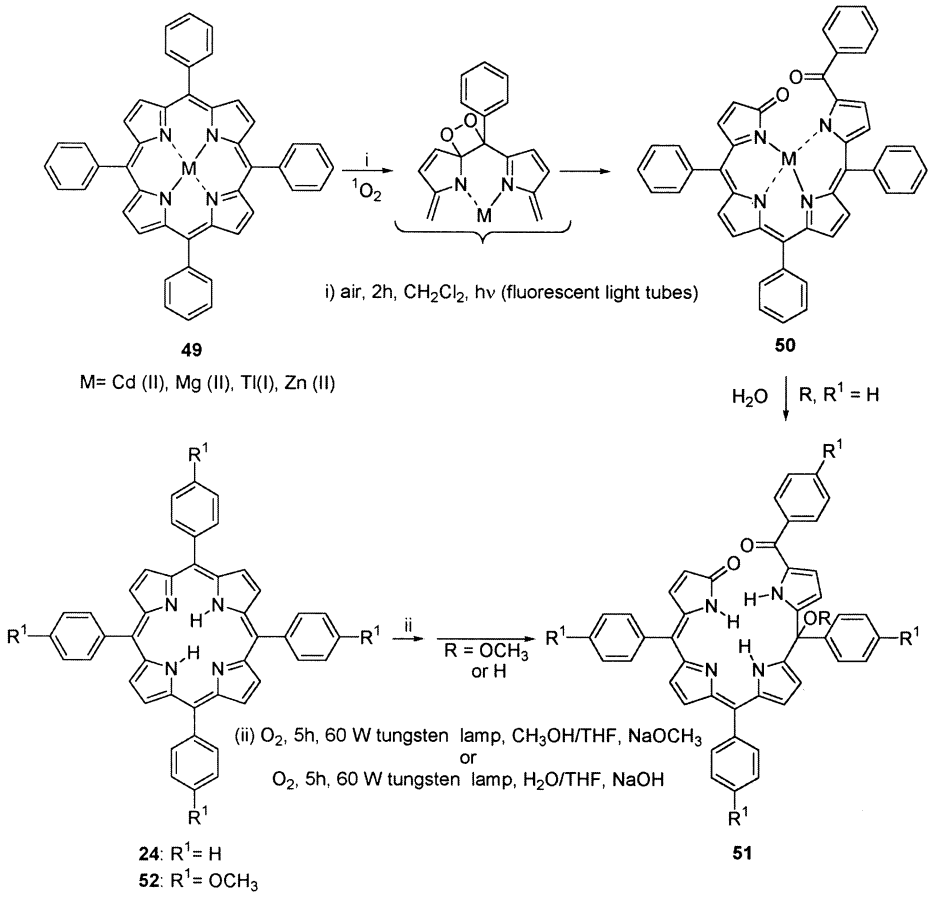
The major photoproduct isolated from the photooxidation of the cadmium(II) complex of **24** was a bilinone, reformulated[50b,56] as **51**, which was formed as a result of a singlet oxygen [2+2] addition.[50] Identical behaviour was observed when the magnesium(II), thallium(I) and zinc(II) complexes[50] and the dianion[56] of **24** were irradiated. The same type of ring-opened product was identified when the dianion of 5,10,15,20-tetrakis(*p*-methoxyphenyl)porphyrin **52** was photooxidised (Scheme 10).[56] When solutions of a series of zinc(II) and cadmium(II) complexes **53** of substituted *meso*-tetraphenylporphyrins were irradiated in air, the ease of the ring opening was dependent on steric rather than electronic factors.[57] If positions 2 and 6 of the phenyl rings were blocked, the reaction did not occur. When positions 2 and 6 were free, however, the reaction proceeded readily and was unaffected by the electronic nature of the substituents in the phenyl or pyrrole rings (Scheme 11).

Krasnovskii et al.[58] determined the rate constants of the interaction between singlet oxygen and a series of *meso*-tetraphenylporphyrin derivatives. The values of singlet oxygen physical quenching (k_p), which would not cause chemical change in the pigment, were 1- to 5-orders of magnitude higher than those of chemical quenching (k_c), which would lead to the irreversible degradation of the pigment (Table 3). Values for the metal-free porphyrin, chlorin and bacteriochlorin are included for comparison, which demonstrate the higher activity of the chlorin and the bacteriochlorin, most notably of the bacteriochlorin in chemical reaction. It was proposed that porphyrin complexes with iron, nickel, cobalt and manganese quenched singlet oxygen by an energy transfer process from the oxygen to the low-lying d electron levels, the other compounds quenching singlet oxygen via a charge transfer process, in which chemical reaction with singlet oxygen (chemical quenching) would occur much more readily. This interpretation was supported by the values of the rate constants, since in general, the diamagnetic metal complexes have high chemical quenching rates (Table 3).

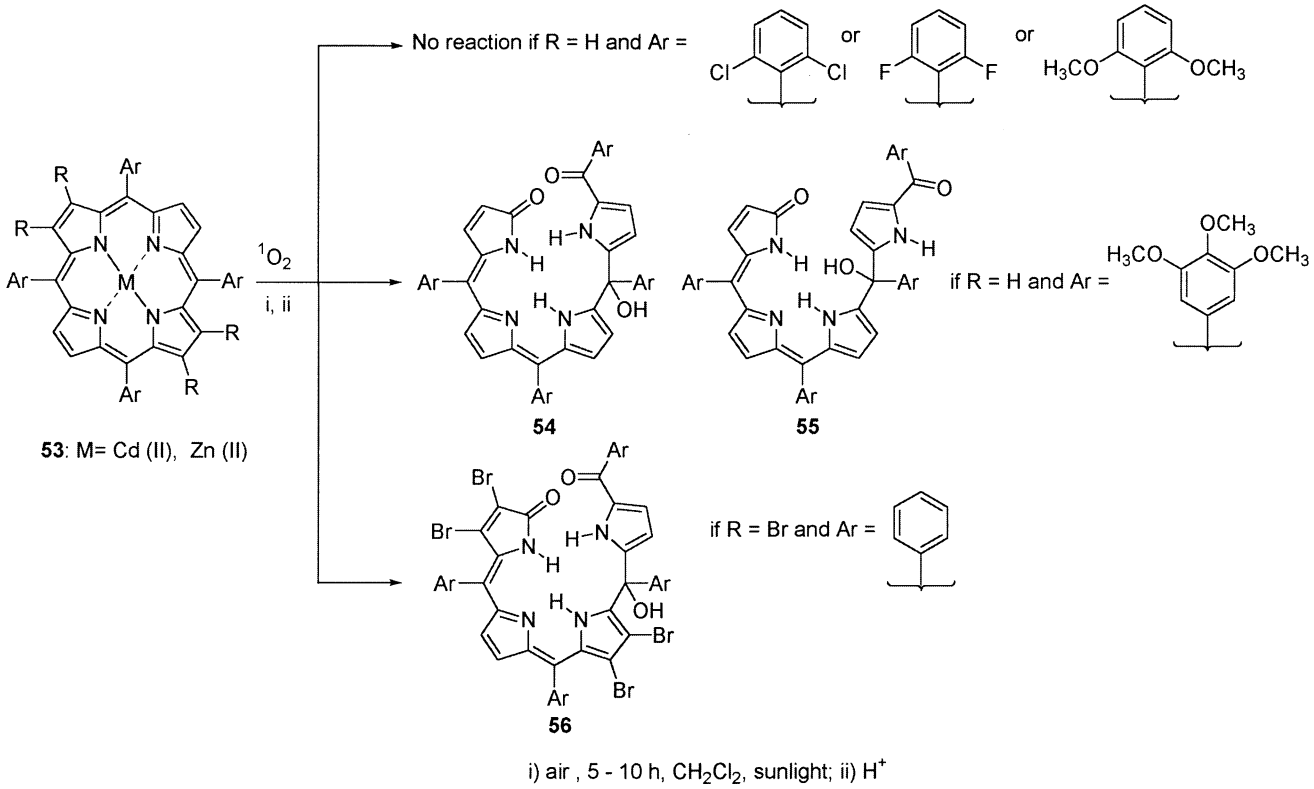


Scheme 9. Photooxidation of octaethyloxophlorin **42**[55b] and its zinc(II) complex **36**.[55a]

In the *meso*-tetrakis(*m*-hydroxypheny)porphyrin series, Bonnett et al. have made a comparative kinetic study across the hydrogenation levels [*m*-THPP **57**, *m*-THPC **10**, and *m*-THPBC **58**] (Fig. 7), irradiating in methanol or aqueous methanol solutions with an argon laser at 514 nm.[24] True photobleaching was observed for the chlorin and the bacteriochlorin (Section 3.5), but the porphyrin reacted more slowly, with photomodification. Evidence was found for a singlet oxygen involvement in all three experiments. In preparative studies[24b] using broad band UV–Vis radiation, a similar photomodification process was observed. In aqueous methanol the major photoproduct was formulated as the novel quinonoid porphyrin **59**, while from methanolic solution the fragments **60** and **61** of true photobleaching were also identified (Scheme 12). The formation of *p*-quinones from phenols mediated by singlet oxygen has been previously reported,[59] but this appears to be the first example in the porphyrin field. Linear tetrapyrrole products



Scheme 10. Photooxidation of metal complexes of tetraphenylporphyrin.[50,56]



Scheme 11. Photooxidation of metal complexes of TPP derivatives.[57]

Table 3. Rate constants of the physical and chemical quenching of singlet oxygen by TPP derivatives in CCl_4[58]

Substrate	Physical quenching k_p ($M^{-1}\,s^{-1}\pm15\%$)	Chemical quenching k_c ($M^{-1}\,s^{-1}\pm65\%$)
meso-Tetraphenylporphyrin (TPP, **24**)	0.09×10^7	30
meso-Tetraphenylchlorin (TPC)	0.2×10^7	120
meso-Tetraphenylbacteriochlorin (TPBC)	10×10^7	1.5×10^5
MgTPP	80×10^7	6×10^6
CdTPP	60×10^7	3×10^6
ZnTPP	4×10^7	8×10^3
CuTPP	0.4×10^7	<40
NiTPP	150×10^7	$<10^4$
CoTPP	200×10^7	$<10^4$
ZnTPC	400×10^7	2×10^8

were not identified: they may be present, but the attack on the phenyl rings can be understood in terms of activation by the electron donor effect of the hydroxy substituents. It may be noted that this effect is not transmissible mesomerically to the porphyrin ring, since the substituents are at the *meta* positions.

There has been some interest in the association of photosensitising molecules with larger molecular (usually polymeric) systems for the purposes of improved administration or targeting. Wöhrle and coworkers[60] have observed the photostability in water of *meso*-tetrakis(*p*-hydroxyphenyl)porphyrin covalently attached to methoxypoly(ethylene glycol) side chains **62** (Fig. 8), which appears to have suitable properties, such as good photoactivity and high tumour accumulation, for use as a photosensitiser.[60] Irradiation of **62** with near-UV light (λ 350–380 nm) using a halogen lamp, led to complete decomposition in 96 h, the first order rate constant being $2.3\times10^{-4}\,min^{-1}$. In another example, photooxidation of the zinc(II) complex

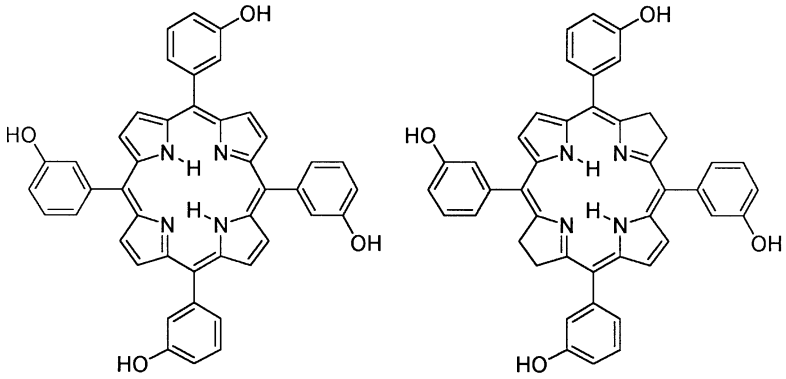
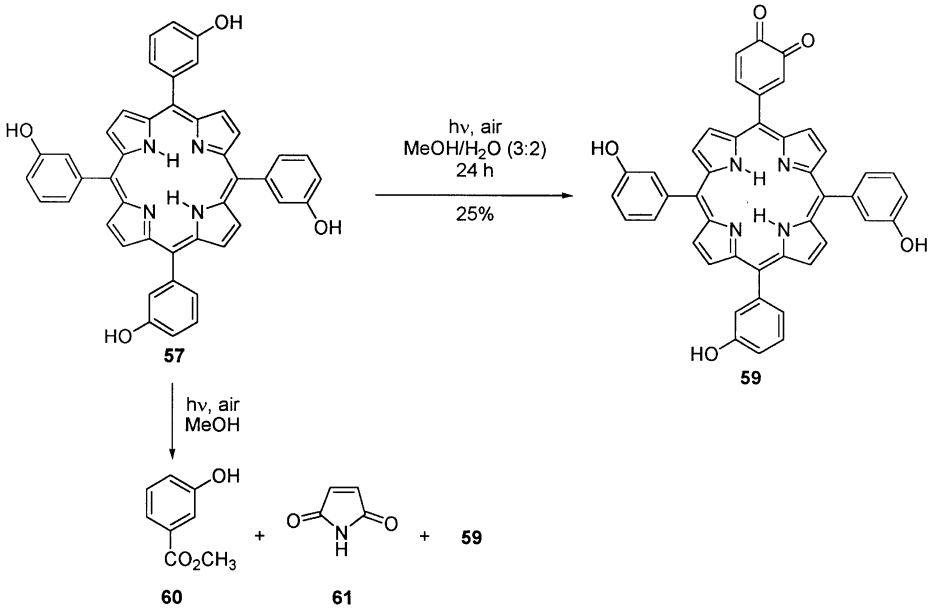
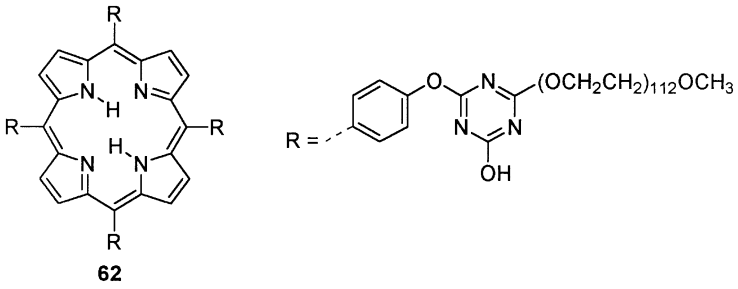


Figure 7. Structures of 5,10,15,20-tetrakis(*m*-hydroxyphenyl)porphyrin **57**, (*m*-THPP) and 5,10,15,20-tetrakis(*m*-hydroxyphenyl)bacteriochlorin **58**, (*m*-THPBC).



Scheme 12. Photooxidation of *m*-THPP **57** in MeOH and MeOH–H_2O (3:2) irradiating with 2×300 W MLU lamps (Philips).[24b]



62

Figure 8. *p*-THPP covalently bound to methoxypoly(ethylene glycol) **62**.

of 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrin **63** in cyclodextrin cavities was investigated by Mosseri et al.[61] When the cyclodextrin–porphyrin complex was irradiated at 422 nm at pH 12, the quantitative formation of a product formulated as the 2,3-dihydroxychlorin **64** was observed (Scheme 13). The product showed a strong Band I (λ_{max} 625 nm), but other support for the structural assignment appears to be lacking. The reaction required oxygen as degassed solutions did not show the presence of a photoproduct. The mechanism of the reaction was not elucidated, but the mediation of singlet oxygen seemed unlikely: it was proposed that superoxide might be involved. This interesting reaction deserves further study.

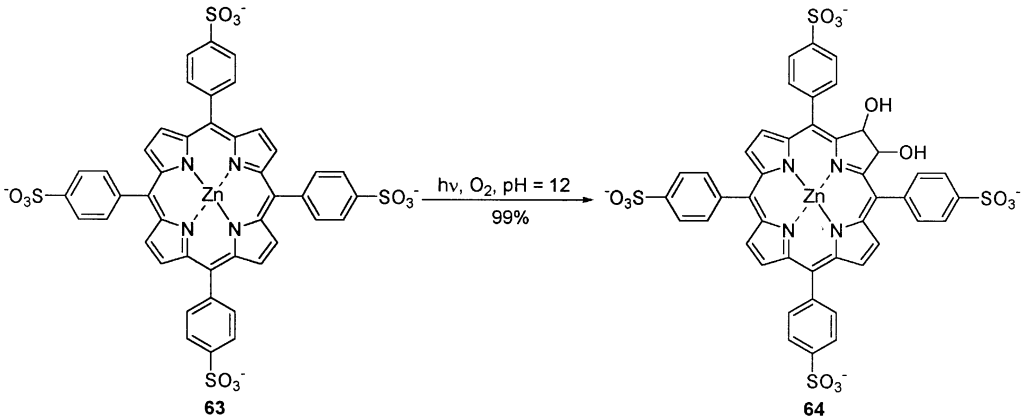
3.5. Chlorins and bacteriochlorins

Chlorins have been extensively studied as possible photosensitisers in PDT due to their strong absorption in the red region combined with their relatively rugged character. In general, chlorin photosensitisers are either obtained by chemical synthesis or by modification of a naturally-derived molecule (protohaemin or chlorophyll *a*).[1c] Two promising second generation photosensitisers, 5,10,15,20-tetrakis(*m*-hydroxyphenyl)chlorin (*m*-THPC, **10**) and benzoporphyrin derivative monoacid ring A (BPD-MA, **11**), are chlorins.

3.5.1. 5,10,15,20-Tetrakis(*m*-hydroxyphenyl)chlorin, *m*-THPC 10. 5,10,15,20-Tetrakis(*m*-hydroxyphenyl)chlorin (*m*-THPC, temoporfin, Foscan®, **10**) is a potent second generation photosensitiser which was discovered in 1989.[62] *m*-THPC **10** with a purity of ⊇99% has been commercially developed by Scotia Pharmaceuticals Ltd[1b,c,13] (and has recently received European regulatory approval for use in the treatment of head and neck cancer). The photobleaching of **10** has attracted interest in recent years, although, until recently, there has been little information on the nature of the photoproducts.

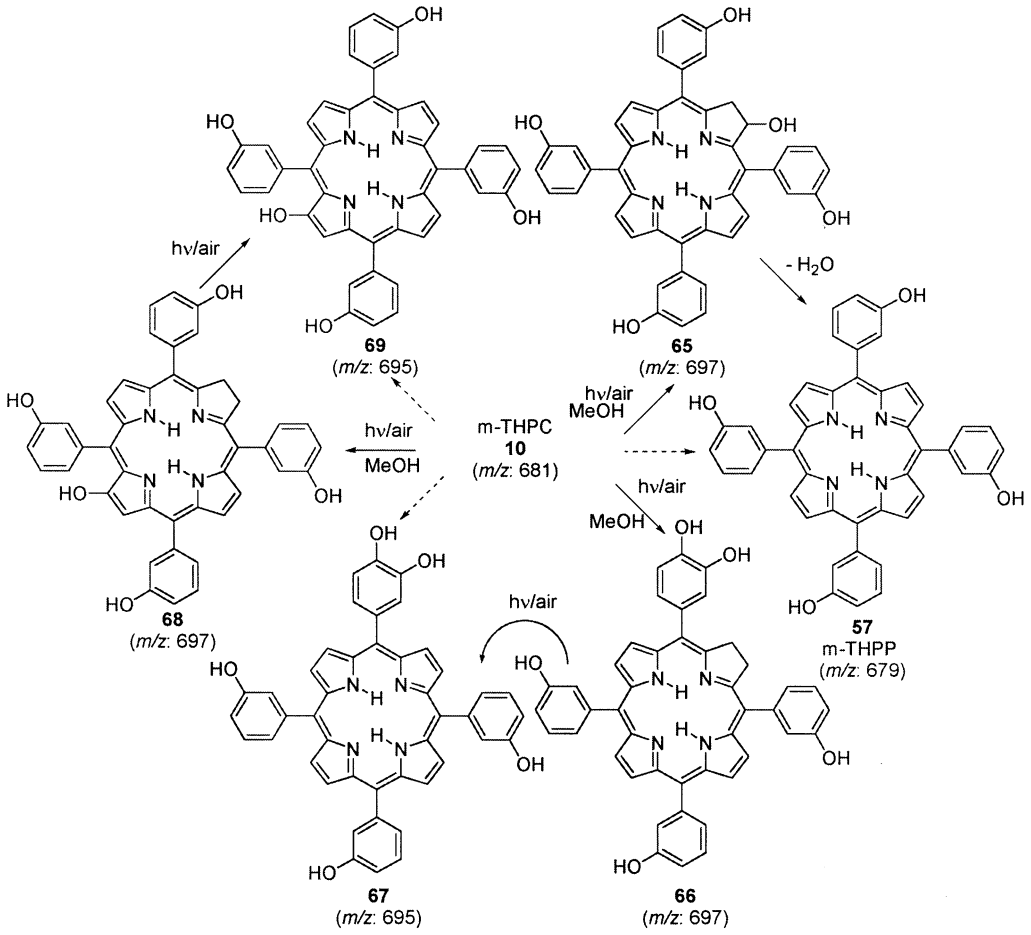
Jones et al.[63] have studied the photooxidation of *m*-THPC **10** by on-line HPLC-electrospray ionisation mass spectrometry. Air-saturated solutions of **10** (1 mg ml^{-1}) in methanol were exposed to ambient white laboratory light from double fluorescent tubes behind a diffuser, as well as incidental sunlight. After one week, five major porphyrin-type compounds were detected, with M+1 ions at *m/z* 697 (three compounds: $C_{44}H_{33}N_4O_5$), 695 ($C_{44}H_{31}N_4O_5$) and 679 ($C_{44}H_{31}N_4O_4$). The major of the three compounds having a molecular formula $C_{44}H_{33}N_4O_5$ was formulated as a β-hydroxy-*m*-THPC **65** with the hydroxyl group in the reduced pyrrole ring, since the product of water elimination, 5,10,15,20-tetrakis(*m*-hydroxyphenyl)porphyrin (*m*-THPP, **57**), was obtained when this fraction was heated at 60°C. The formation of **65** was possibly due to a radical chain autooxidation, since it was also detected in the absence of light. It was proposed that the other two isomers had the extra hydroxyl group either in a phenol ring **66** or in a pyrrole ring **68**. The other two photoproducts were formulated as dehydrogenated products from **66** (porphyrin, **67** or **69**) and **10** (*m*-THPP, **57**), respectively. Although the HPLC technique proved very useful, it did not provide enough sample for further structural analysis which could help to confirm these assignments (Scheme 14).

Hadjur et al.[64] studied the photobleaching kinetics of *m*-THPC **10** (2 μM) in 10% FCS, irradiating with an argon laser pumping a dye laser (λ 650 nm). The



63 64

Scheme 13. Proposed photooxidation of the $ZnTPPS_4$–cyclodextrin complex in aqueous solution.[61]



Scheme 14. Proposed photooxidation products of *m*-THPC in methanol. Light source: fluorescent lamps.[63]

photobleaching quantum yields under different conditions were calculated using the absorbance decay by monitoring Band I at 650 nm (Table 4). All major bands decayed during irradiation and a new band at 320 nm, which was attributed to a new photoproduct, was observed. (Rezzoug et al.[65] have noted that the 320 nm peak is formed in FCS but not in methanol, and suggested that this absorption may be related to the reaction of a protein constituent such as tryptophan). The fluorescence spectrum[64] also decreased and the photoproduct did not fluoresce. Several experiments were carried out in order to support the view that singlet oxygen was the reactive species responsible for *m*-THPC photobleaching. When the concentration of oxygen was reduced by bubbling nitrogen, the quantum yield was reduced by ~50%. Addition of singlet oxygen quenchers, namely histidine and DABCO, caused the quantum yield to decrease by 35 and 25%, respectively, whereas the addition of Type I quenchers (superoxide dismutase, catalase and deferoxamine) did not produce any appreciable change (Table 4). The quantum yield of singlet oxygen formation from **10** in 10% FCS and in ethanol was also calculated using two different techniques: 1,3-diphenylisobenzofuran photobleaching and EPR spectroscopy using 2,2,6,6-tetramethyl-4-piperidone-*N*-oxyl. A similar value of ~0.30 was obtained in both solvents (the value obtained from luminescence determination at 1270 nm is 0.43 in methanol).[66] It was therefore concluded that, under these experimental conditions, *m*-THPC **10** was able to generate singlet oxygen which then caused self-photobleaching.

Table 4. Quantum yields of photobleaching of *m*-THPC **10** in 10% foetal calf serum (FCS)[64]

Conditions	Photobleaching quantum yield (Φ_{pb})
Control	1.54×10^{-5}
Bubbled O_2	2.28×10^{-5}
Bubbled N_2	1.80×10^{-6}
Histidine	1.03×10^{-5}
Diazabicyclo[2.2.2]octane (DABCO)	1.16×10^{-5}
Superoxide dismutase (SOD)	1.53×10^{-5}
Catalase (CAT)	1.51×10^{-5}
Deferoxamine	1.55×10^{-5}

Bezdetnaya et al.[67a] also studied the photobleaching of *m*-THPC **10** (4.4 μM) in 2% FCS, irradiating with a krypton laser (λ 405 and 413 nm). Under these conditions and prior to irradiation, *m*-THPC **10** appeared to be a mixture of monomeric and aggregated material. A new band with an absorption maximum at about 500 nm appeared upon irradiation and was attributed to changes in the monomer–aggregate equilibrium. The quantum yield of photobleaching (Φ_{pb}) obtained using absorption spectroscopy was 15-fold smaller than that calculated using fluorescence spectroscopy, the latter technique mainly measuring the

monomers in a mixture. These authors concluded that this difference between the quantum yields shows that the monomers and the aggregated material have different reactivities towards photobleaching, the latter being much more resistant than the former.

The same group carried out MALDI mass spectrometric studies of the photoproducts of *m*-THPC **10**.[67b] After irradiating ethanol solutions of *m*-THPC **10** (10 μM) using laser irradiation (λ 650 nm) for 45 min, 30% absorbance decay for the Band I (650 nm) of **10** was observed, together with the formation of new bands at 620 and 418 nm. The irradiated solution was then stored in the dark for 9 h, whereupon a further increase of the absorbances at 620 and 418 nm occurred. After another irradiation period, the band at 620 nm decreased and two new bands were detected at 775 and 460 nm. The mass analysis of the irradiated solutions of **10** showed the appearance of two new peak clusters at *m*/*z* 713 and 744. After dark storage, the intensity of the cluster at *m*/*z* 744 increased. The second irradiation period led to an enhancement of the intensity of the *m*/*z* 713 cluster and to a decrease in that at *m*/*z* 744. More studies were required to elucidate the mechanism and the nature of the photoproducts.

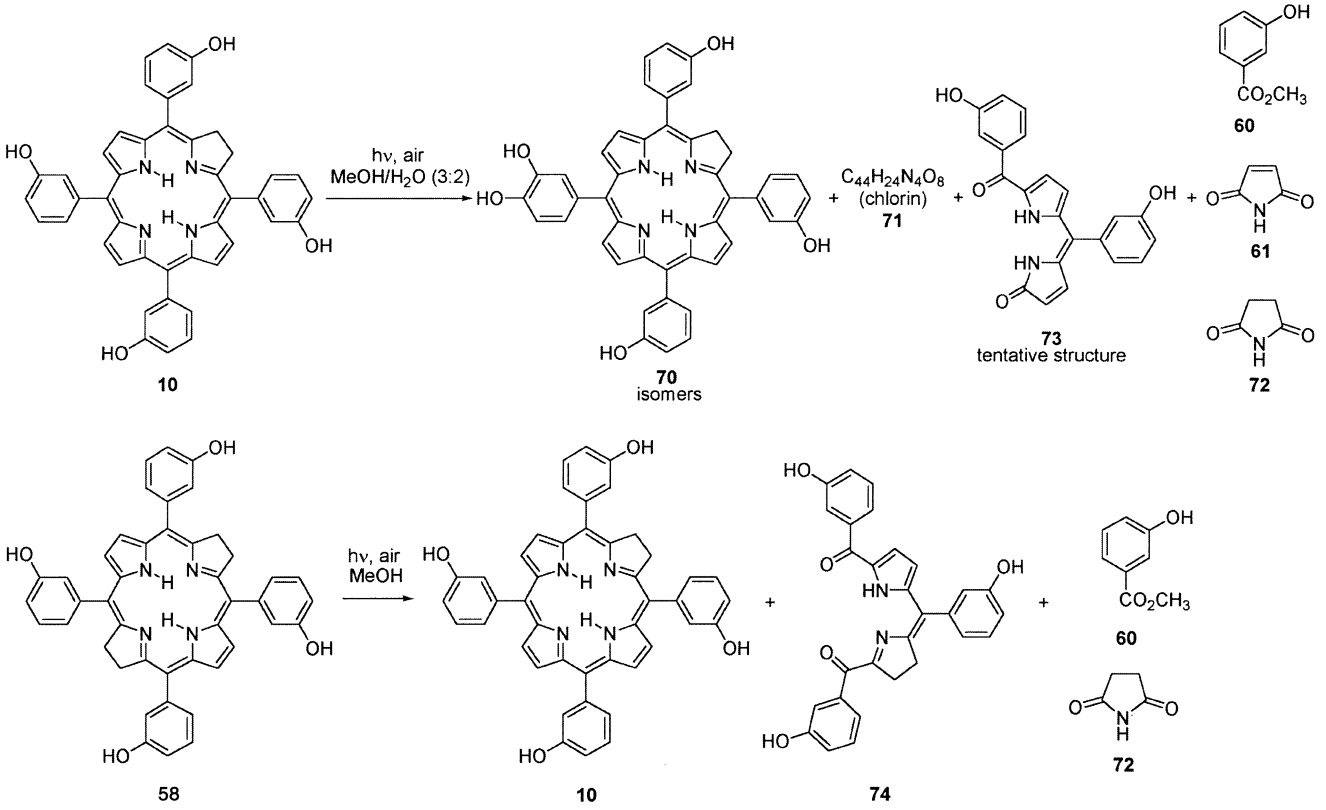
Recently, Bonnett et al.[24] reported the first comparative study across the *m*-THPP series, including *m*-THPC **10** and *m*-THPBC **58**, irradiating methanolic solutions with an argon laser at 514 nm. True photobleaching was observed for *m*-THPC (Fig. 4) and *m*-THPBC, the chlorin being more photostable than the bacteriochlorin. The photobleaching rates for the three substances increased as the polarity of the solvent increased. Singlet oxygen was found to be the key species responsible for the photobleaching of these compounds (Table 5).

Table 5. The photobleaching of *m*-THPC **10** and *m*-THPBC **58**[24]

Substrate	Medium	Initial[a] first order k (s^{-1})
m-THPC [a]	MeOH	1.7×10^{-4}
m-THPC [a]	MeOH–NaN_3 [b]	6.7×10^{-5}
m-THPC [a]	MeOH–H_2O (3:2)	6.3×10^{-4}
m-THPC [a]	MeOH–$HCONH_2$ (1:1)	1.1×10^{-3} [c] 2.2×10^{-4} [d]
m-THPC [a]	$HCONH_2$	2.8×10^{-3} [c] 1.2×10^{-4} [d]
m-THPBC [e]	MeOH	1.0×10^{-2}
m-THPBC [e]	MeOH–NaN_3 [a]	7.1×10^{-3}
m-THPBC [e]	MeOH–H_2O (3:2)	5.7×10^{-2}

[a] Except for [d]; [a] 0.04 mM; [b] 8 mM; [c] t=0–7.5 min; [d] t=10–30 min; [e] 0.02 mM.

The same research group has also studied the nature of the photoproducts formed during the photobleaching of *m*-THPC and *m*-THPBC under the similar conditions to those used with *m*-THPP (**57**, Section 3.4).[24b] Under the conditions used, *m*-THPC photobleaching yielded mainly the fragmentation products **60**, **61** and **72** and small amounts of a pentahydroxylated chlorin **70** (Scheme 15). The main photoproducts from *m*-THPBC photobleaching were also the fragmentation products **60** and **72**, although small amounts of *m*-THPC were also formed (Scheme 15). In



Scheme 15. The photobleaching products from *m*-THPC **10** and *m*-THPBC **58** in aqueous methanol and methanol, respectively. Light source: 2×300 W MLU lamps (Philips).[24b]

each case, small amounts of cleavage products formulated as dipyrrin derivatives (**73** from **10**; **74** from **58**) were detected.

3.5.2. Benzoporphyrin derivative monoacid A, BPDMA 11. The photobleaching quantum yields of benzoporphyrin derivative monoacid ring A (Verteporfin®, BPD-MA, **11**) have been measured by Aveline et al.[68] in different solvents, irradiating with an argon ion laser pumping a dye laser (λ 690 nm) (Table 6). No significant new bands were observed upon irradiation, and true photobleaching is therefore being observed. As with other photosensitisers, the presence of surfactants enhanced photobleaching, suggesting that the monomers were less resistant than the aggregates. The addition of 1% FCS also enhanced the photobleaching, illustrating that photooxidisable substrates in the proteins and/or monomerisation can accelerate photobleaching.[47,64,67] The quantum yields of photobleaching were not significantly affected by degassing the solution with nitrogen or by bubbling oxygen instead of air. The presence of oxygen does not therefore appear to affect the photobleaching of BPD-MA **11**.

Table 6. Photobleaching quantum yields for BPD-MA (10 μg ml^{-1})[68]

Solvent	Gas	Quantum yield of photobleaching (Φ_{Pb})
Methanol	Air	5.75×10^{-5}
Ethanol	Air	6.80×10^{-5}
$CHCl_3$	Air	3.70×10^{-5}
PBS+2% Triton 100	Air	5.35×10^{-5}
H_2O	Air	2.00×10^{-5}
PBS	Air	2.80×10^{-5}
PBS+1% FCS	Air	6.60×10^{-5}
PBS+10% FCS	Air	2.07×10^{-4}
Methanol	N_2	6.07×10^{-5}
PBS	N_2	2.00×10^{-5}
Methanol	O_2	6.00×10^{-5}
PBS	O_2	2.70×10^{-5}

Gillies et al.[69] have also studied the photobleaching of the photosensitiser **11** in phosphate buffer solutions containing 10% FCS and irradiating with an argon ion laser pumping a dye laser (λ 694 nm), new absorption bands being detected at 320 and 650 nm after irradiation. Fluorescence studies additionally showed photobleaching, and a photoproduct was detected. Although the structure of the photoproduct is unknown, it was suggested that it could be a compound of the hydroxyaldehyde type analogous to that observed for protoporphyrin **9** which also bears vinyl side chains. This would, of course, require the involvement of molecular oxygen, contradicting the conclusion of the previous paragraph.

3.5.3. Monoaspartylchlorin e_6, MACE 13. Spikes et al.[70a] studied the photobleaching of mono-L-aspartylchlorin e_6 (MACE) (**13**, revised structure[12]), a hydrophilic sensitiser, in phosphate buffer (pH 7.4) solution (5 μM), irradiating at 400 nm. The rate of photobleaching of **13** was first order until 70% of the sensitiser had bleached, and new fluorescence or new absorption peaks were not observed. The zinc(II) and tin(IV) complexes of MACE showed a similar behaviour, but the tin chlorin bleached more slowly (two orders of magnitude) and the zinc chlorin more quickly (two orders of magnitude) than the free base (Table 7). A similar behaviour was observed when chlorin e_6 and its zinc(II) and tin(IV) complexes were irradiated.[70b] The photobleaching rate of **13** was dramatically lowered when nitrogen was bubbled through the solution, indicating that oxygen was required. Sodium azide, however, had little effect on the quantum yield whilst D_2O increased the bleaching rate, but to a relatively small extent. In order to account for these intriguing results, Spikes[70a] suggested that singlet oxygen was being generated very close to the chlorin and would react very rapidly. Singlet oxygen lifetime would therefore not be a rate-limiting factor and essentially there is not enough time for azide quenching or a large solvent isotope effect.

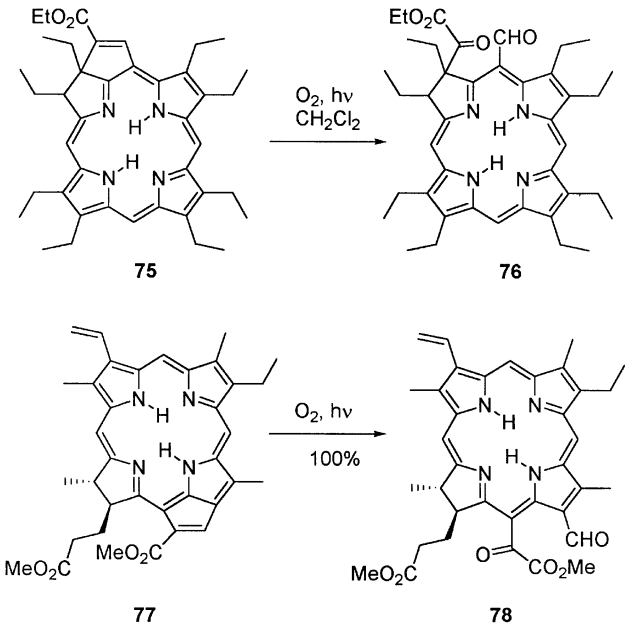
Table 7. Quantum yields of photobleaching (Φ_{pb}): 100 mM $NaHPO_4$ buffer of pH 7 and 0.22 mM O_2[70a]

Chlorin	Photobleaching quantum yield (Φ_{pb})
Mono-L-aspartylchlorin e_6 (MACE, **13**)	8.2×10^{-4}
Tin(IV) mono-L-aspartylchlorin e_6	5.7×10^{-6}
Zinc(II) mono-L-aspartylchlorin e_6	1.9×10^{-2}
Chlorin e_6	1.9×10^{-3}
Tin(IV) chlorin e_6	1.3×10^{-5}
Zinc(II) chlorin e_6	1.8×10^{-2}

Mono-L-aspartylchlorin e_6 (MACE, **13**) photobleaching was also observed by Roberts et al.[30] in phosphate buffer (PBS) and FCS solutions (c=5 μg ml^{-1}), irradiating with a krypton ion laser (λ 405–415 nm). The chlorin fluorescence decay was faster in FCS than in PBS solutions.

3.5.4. Purpurin derivatives. The photobleaching kinetics of tin(IV) etiopurpurin **12**, which is another second generation photosensitiser (currently undergoing Phase III clinical studies for cutaneous metastatic breast cancer and age-related macular degeneration), have been reported by Sekher and Garbo.[71] Rate constants were calculated for different solvents by following the decay of fluorescence emission, irradiating at 437 nm. In addition to true photobleaching, formation of a photoproduct with an emission maximum at 625 nm was detected in ethyl acetate solution. Tin(IV) etiopurpurin **12** was more photostable in organic solvents than in aqueous buffer. In DPPC liposomes, a plateau was reached after 25% of the pigment had photobleached. When cholesterol was incorporated into the liposomes, however, the photosensitiser could be completely photobleached.

Morgan et al.[72] have described the cleavage of the isocyclic double bond of purpurin **75** when exposed in dichloromethane to sunlight under air (Scheme 16). Ma and Dolphin[73] observed the analogous photocleavage of the isocyclic ring of a pheophorbide derivative **77**, the 3-vinyl group being unaffected. The yield of the latter reaction was reported as quantitative, in contrast to many photomodification processes which are often accompanied by side reactions, including photobleaching. The two cleavage reactions in Scheme 16 find precedent in step 39 of Woodward's synthesis of chlorophyll (yield: 74% spectroscopic; 59% recrystallised).[74]



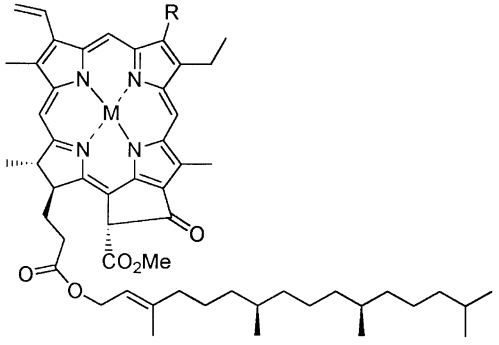
Scheme 16. Photooxidation of chlorins **75** and **77**.[72,73]

3.5.5. Chlorophyll and related compounds. The photooxidation of metal complexes and free bases of chlorins has been studied in some detail, due in part to the interest in chlorophyll catabolism. Chlorophyll **79a**, **79b** photooxidation has often been studied in solution. Small fragments have been identified from the photobleaching of chlorophyll adsorbed onto lipophilic particles surrounded by water.[75a] Glycerol, lactic, citric, succinic and malonic acids, and alanine have been detected (Scheme 17). It was proposed that glycerol and alanine came from the cleavage of the macrocyclic ring, since they were also isolated in the oxidation of pheophytin **79c** and pheophorbide **80** under the same conditions. Extraction of senescent green plant material has yielded methylethylmaleimide and methylvinylmaleimide,[75b] presumably derived from rings B and A, respectively, of chlorophyll *a*, although whether by enzymatic or photochemical agency is not yet clear.

Krasnovskii et al.[58] extending their work on tetraphenylporphyrins (Table 3), determined the rate constants of the interaction between singlet oxygen and a series of chlorophyll-type compounds (Table 8). Again, physical quenching occurs more quickly than chemical reaction.

Several PDT sensitisers (e.g. MACE **13** and bacteriochlorophyll derivatives) are structurally related to some of the model chlorins which have been studied in the difficult field of chlorophyll photodegradation. Analogously to the reactions of the metalloporphyrins (Scheme 8), the photooxidation of zinc(II) octaethylchlorin (**81**, Scheme 18) gives the isomeric formyldihydrobilinones **82** and **83**.[54] Smith et al.[76] showed that bilatrienes (**85**, **87** and **88**) were readily formed when *meso*-methyloctaethylchlorins (**84** and **86**) were exposed to sunlight (Scheme 19). The number of *meso*-methyl groups present when the free base chlorin was studied appeared to affect the reactivity. When one *meso*-methyl group was present, the free base was more photostable and the ring-opened product was not isolated. The zinc complex **86** was, however, very susceptible to photooxidation and yielded the bilatriene derivatives **87** and **88** in moderate yields.

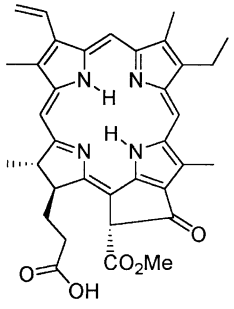
The ring-opening photooxidations of bacteriochlorophyll *c* and *e* derivatives have also been examined,[77] mainly in relation to the catabolism of naturally occurring chlorophylls (Scheme 20). Both bacteriochlorophyll *c* and *e*



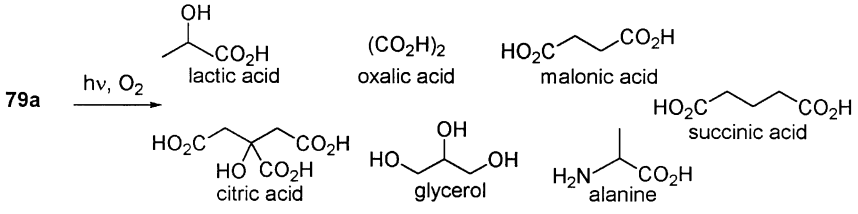
79a: M = Mg, R = CH_3 Chlorophyll *a*

79b: M = Mg, R = CHO Chlorophyll *b*

79c: M = 2H, R = CH_3 Pheophytin *a*



80: Pheophorbide *a*



Scheme 17. Photooxidation of chlorophyll.[75a]

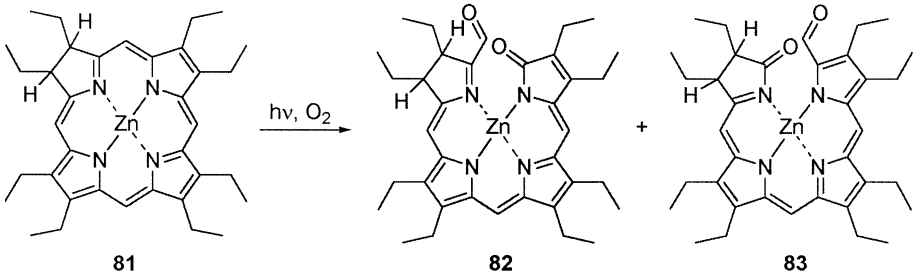
Table 8. Rate constants of the physical and chemical quenching of singlet oxygen by chlorophyll derivatives and naturally-derived porphyrins in CCl_4[58]

Substrate	Physical quenching k_p ($M^{-1}\,s^{-1}$ ±15%)	Chemical quenching k_c ($M^{-1}\,s^{-1}$ ±65%)
Bacteriochlorophyll *a*	100×10^7	2×10^7
Chlorophyll *a*	70×10^7	2×10^6
Chlorophyll *b*	30×10^7	6×10^5
4-Vinylprotochlorophyll	$<10\times10^7$	$<3\times10^5$
Pheophytin *a*	2×10^7	1.4×10^4
Pheophytin *b*	0.5×10^7	10^3
4-Vinylprotopheophytin	20×10^7	2×10^5
Protoporphyrin dimethyl ester	0.05×10^7	5×10^3
Mesoporphyrin dimethyl ester	0.2×10^7	400

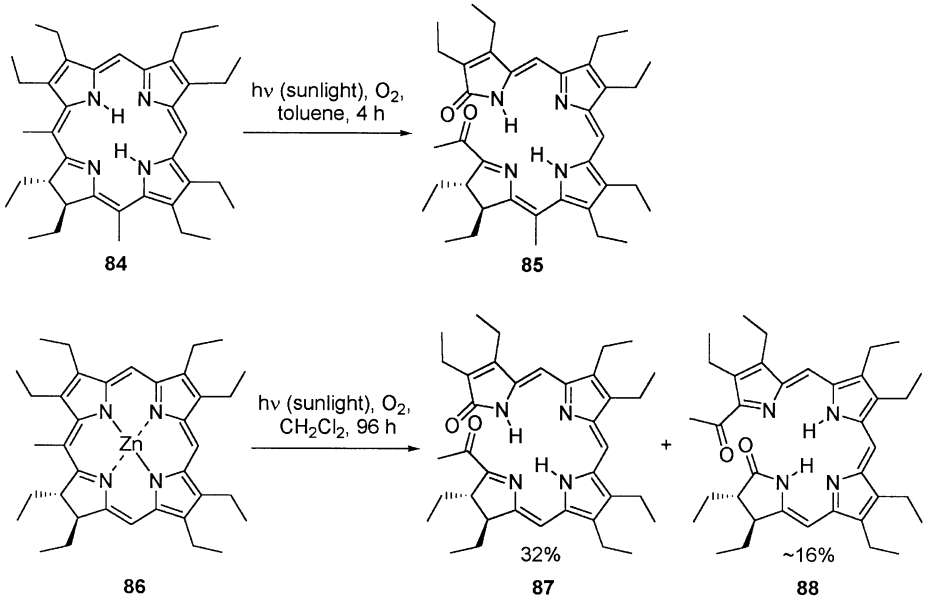
methyl pheophorbides (**89** and **92**) formed bilatriene derivatives (photobilins c, **91** and e, **93**) by cleavage at the C-20–C-1 bond when irradiated in the presence of air. The mechanism involves one molecule of oxygen, as reported by Brown et al.[77c] who showed that each one of the two oxygen atoms incorporated into the product is derived from a single molecule of oxygen. This result strongly supports a singlet oxygen [2+2] addition to produce a dioxetane which then collapses to yield two carbonyl groups, as in the case of metalloporphyrins. The same regioselective cleavage was shown by mesopyropheophorbide *a* methyl ester **94**.

Iturraspe and Gossauer[78,79] have also studied the photooxygenation of chlorophyll derivatives. Photooxidation of the zinc(II) complexes of 20-trifluoroacetyloxy- and 20-chloro-chlorophyll *a* derivatives (**96** and **97**) led to selective ring cleavage together with loss of the C-20 atom (Scheme 21). The primary photoproducts were identified as the oxoniachlorin **98**, the formation of which was proposed to be mediated by singlet oxygen as described earlier. After aqueous acidic or basic treatment of **98**, the dihydrobiliverdin **99** was isolated.

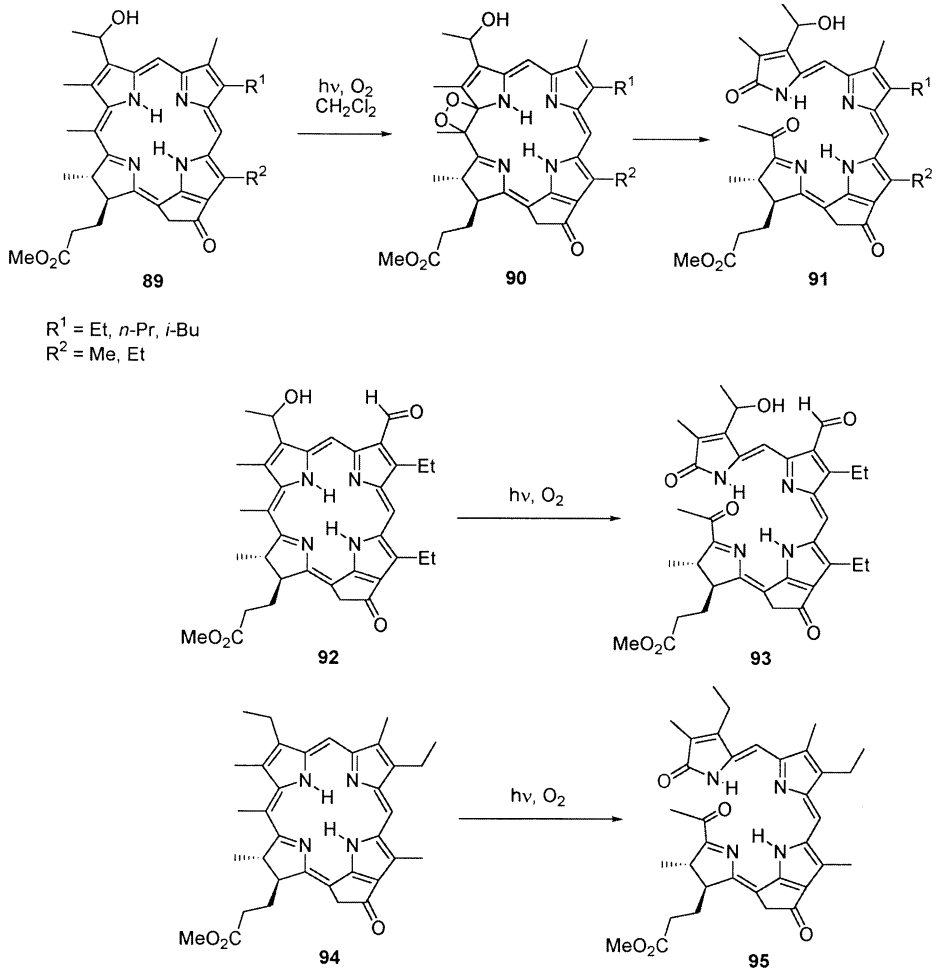
Analogously to the photooxidation studies on octaethylchlorin complexes (Scheme 18), solutions of the zinc(II) **101** and cadmium(II) **102** complexes of pyropheophorbide *a* methyl ester were irradiated in air. Ring cleavage to yield formylbilinones occurred, but the regioselectivity changed with the metal. While the zinc complex **101** led to the normal C-20-cleaved photoproduct **100**, the cadmium complex **102** provided the formylbilinone **104** in which cleavage had occurred at C-5. Further investigation into the mechanism of photooxidation of the cadmium complex **102**[80] provided evidence for a one oxygen molecule mechanism. Singlet oxygen [2+2] addition was therefore



Scheme 18. Photooxidation of zinc(II) octaethylchlorin.[54]



Scheme 19. Photooxidation of *meso*-methyloctaethylchlorins.[76]



Scheme 20. Photooxidation of bacteriochlorophyll *c* and *e* type compounds.[77]

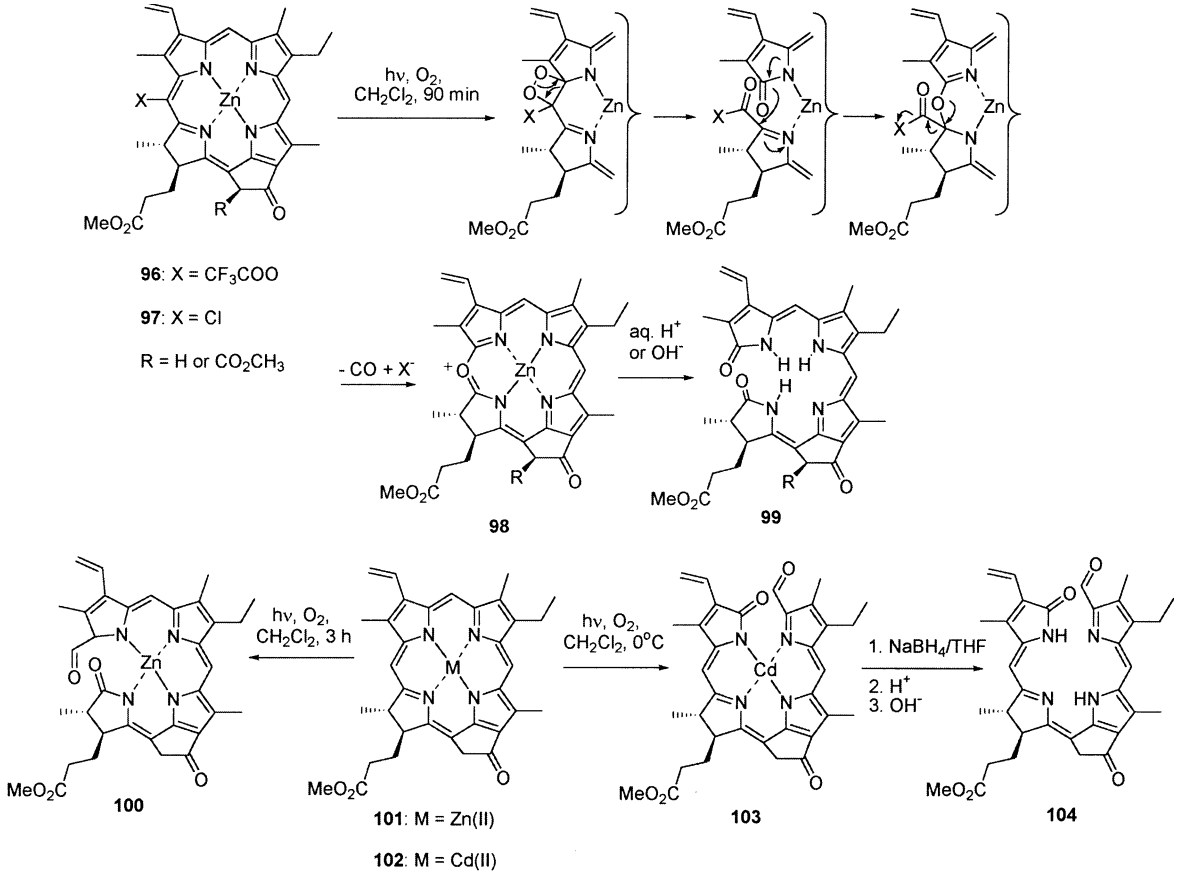
again envisaged as the key step in the formation of the formylbilinone complex **103** (Scheme 21). No explanation has been provided, however, to account for the different regioselectivities. This chemistry has been related to the products of chlorophyll catabolism.[81]

The number of bacteriochlorins currently being developed as photosensitisers for PDT (e.g. 5,10,15,20-tetrakis(*m*-hydroxypheny)bacteriochlorin and bacteriochlorophyll derivatives) is still relatively small. The photooxidation of bacteriochlorophyll *a* **105** was examined by Lindsay Smith and Calvin,[82a] but only the dehydrogenation product **106** was identified, although other components were detected (Scheme 22). The self-sensitised and rubrene-sensitised photooxidations of **105** were studied by Marsh et al.[82b] in oxygen-saturated organic solutions and it was concluded that the complex was generally more photostable when hexacoordinated than when pentacoordinated.

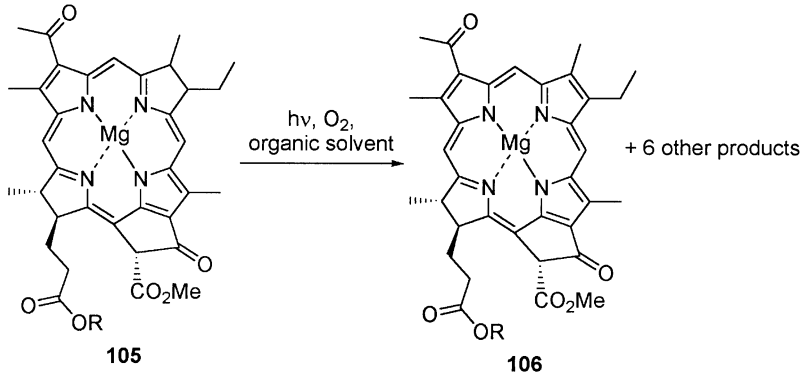
3.6. Phthalocyanine and related compounds

The phthalocyanines and naphthalocyanines, examples of which are shown in Fig. 9, are successive stages of benzenoid tetraannelation of the 5,10,15,20-tetraazaporphyrin system. The phthalocyanines have well established applications as blue/green pigments and as dyes and are finding increasing interest in the materials area (e.g. electrophotography and compact disc technology) because of their optoelectronic properties.[83] The naphthalocyanines are closely related compounds, but they are currently of much less commercial interest. Both series have strong absorption peaks in the red (phthalocyanines typically at 680 nm and naphthalocyanines at ~760 nm), which is an important property to consider when developing a new sensitiser for PDT (Section 1). Indeed, the zinc and aluminium complexes have been developed as PDT agents and the aluminium(III) complex of sulphonated phthalocyanine is in clinical use in Russia.[1b,c]

The photobleaching of several phthalocyanines has been studied in some detail in recent years. In an early report, McCubbin and Phillips[84] compared the photostability of aluminium(III) sulphonated phthalocyanine and aluminium(III) sulphonated naphthalocyanine (average of three sulphonic acid groups per molecule) in aerated solutions, irradiating with a 450 W xenon lamp (Table 9). The photobleaching quantum yields were measured in water and in aqueous methanol by monitoring both the Soret and the Q bands. The methanol component was needed to dissolve the naphthalocyanine derivative since, although soluble in water, it tended to aggregate in that solvent (even at 10^{-6} M) with broadening of the absorption bands. The naphthalocyanine was found to be >1000-fold more reactive to photobleaching than the phthalocyanine. The



Scheme 21. Photooxidation of chlorophyll derivatives.[78,79]



Scheme 22. Photooxidation of bacteriochlorophyll *a* **105**.[82a]

pigments were stable in deaerated solutions and singlet oxygen was suggested to be the main species responsible for the photodegradation. Thus, when the phthalocyanine was irradiated in D_2O, the quantum yield increased 8.8-fold (Q band). The electron transfer to oxygen process was, however, considered possible in the case of the napthalocyanine due to the greater conjugation (easier electron loss and lower oxidation potential) of this system.

Table 9. Photobleaching quantum yields of Al(III) sulphonated phthalocyanine and naphthalocyanine, based on the disappearance of the Q bands[84]

Substrate	Solvent	Quantum yield (Φ_{pb})
Al(III) sulphonated phthalocyanine (Q Band)	H_2O	1.7×10^{-6}
Al(III) sulphonated phthalocyanine (Q Band)	D_2O	1.5×10^{-5}
Al(III) sulphonated phthalocyanine (Q Band)	5%H_2O in MeOH	1.1×10^{-6}
Al(III) sulphonated naphthalocyanine (Q Band 1)	5%H_2O in MeOH	3.0×10^{-3}
Al(III) sulphonated naphthalocyanine (Q Band 2)	5%H_2O in MeOH	3.1×10^{-3}

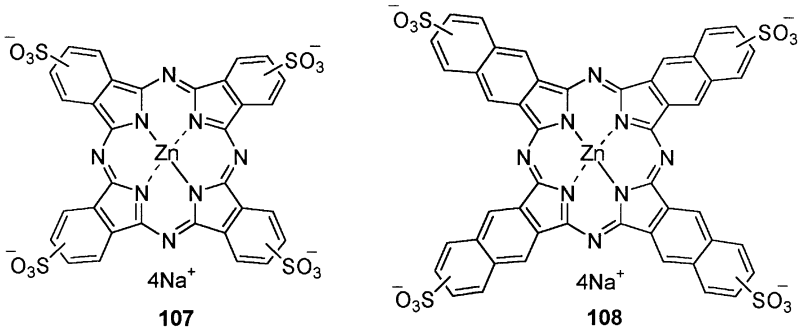


Figure 9. Zinc(II) phthalocyanine tetrasulphonic acid salt **107** and the corresponding naphthalocyanine compound **108**.

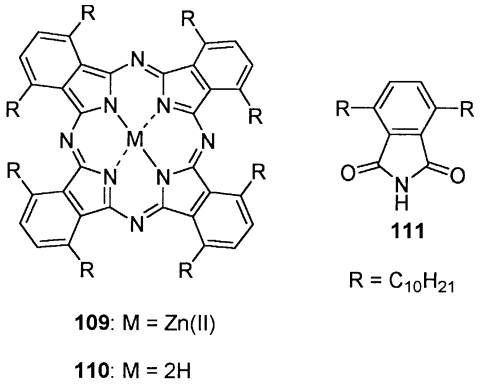


Figure 10. Zinc(II) and metal-free 1,4,8,11,15,18,22,25-octa(decyl)phthalocyanine **109**, **110** and 3,6-*bis*-decylphthalimide **111**.

Luk'yanets and coworkers[85] also studied the photobleaching of sulphonated hydroxyaluminium(III) phthalocyanines (from two to four sulphonated groups) in aerated aqueous solutions, irradiating with a pulsed He–Ne laser. The quantum yield for photobleaching was found to be about 5×10^{-6} and was independent of the dye concentration, the oxygen concentration and the activating irradiation power density. When HSA was added to the solution, the quantum yield increased 10-fold. Sulphonated chloroaluminium(III) phthalocyanine was also photobleached in FCS irradiated with an argon ion laser (λ 336–364 nm). The decrease of fluorescence was, however, only 33% after 10 min of irradiation, a much smaller decrease than that of MACE **13** and Photofrin®, which were photobleached to the extent of 98% in 6 min and 50% in 10 min, respectively.[30]

Spikes et al.[86] have measured the photobleaching quantum yields of the zinc(II) phthalocyanine **107** and zinc(II) naphthalocyanine **108** tetrasulphonic acid salts (Fig. 9) in aerated phosphate buffer solutions (pH 7.4), irradiating the Q band with filtered 500 W quartz–halogen light, and following the decay of the Q band. The quantum yields were 100-fold smaller in aqueous phosphate buffer than in buffer containing 10 mM cetylpyridinium chloride (a cationic detergent which causes disaggregation), again consistent with the view that the aggregated pigment is more resistant to photobleaching than the monomer. The photobleaching quantum yield was 250-fold larger for the naphthalocyanine **108** than for the phthalocyanine **107**.

The photobleaching of zinc(II) octadecylphthalocyanine **109** was shown by Cook et al.[87] to be much faster than that of the metal free octadecylphthalocyanine **110** in aerated cyclohexane solutions (irradiation with two 8 W lamps, λ 364–366 nm) (Fig. 10).The free base **110** needed 230 min to reach 50% reduction in the absorbance of the Q band, whereas the zinc(II) complex **123** needed only

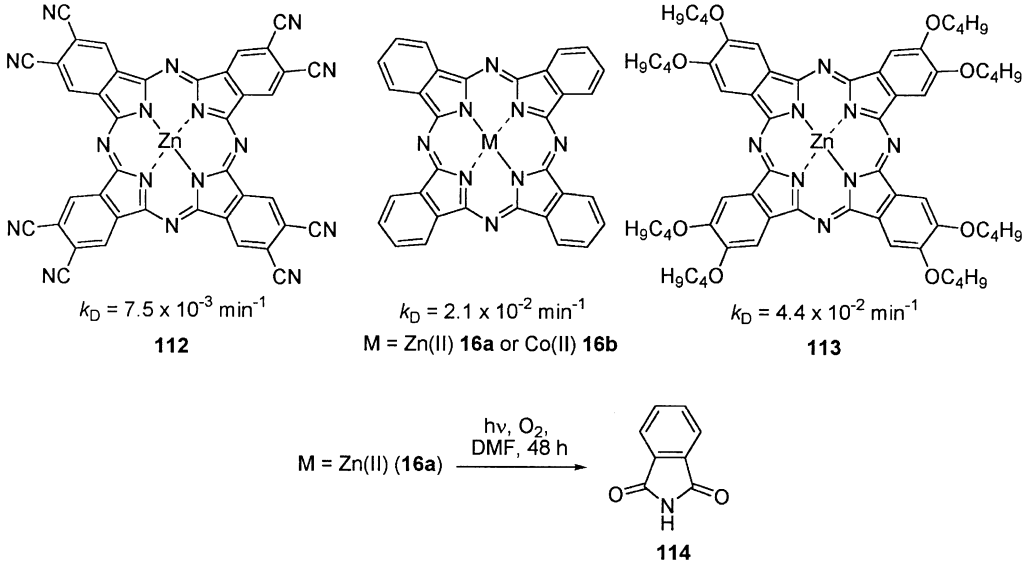


Figure 11. Zinc(II) phthalocyanines **16a**, **112** and **113**, and phthalimide **114**.

100 min. The rate of photobleaching was reduced by singlet oxygen quenchers such as β-carotene. The electron acceptor, metronidazole, also reduced the rate of photobleaching. In addition, degassed solutions showed <1% decomposition. A product of true photobleaching was isolated and identified as 3,6-*bis*-decylphthalimide **111**.

In an earlier study, Wöhrle and coworkers[60] examined the effect of substituents in the macrocyclic ring on the photobleaching of a series of zinc(II) phthalocyanines (**16a**, **112** and **113**) (Fig. 11) in aerated DMF solutions, irradiating with visible light (λ 350–850 nm) using a 24 V 250 W halogen lamp. The photobleaching, monitored by the loss of absorbance, increased in the sequence **112**<**16a**<**113** and, as expected, electron withdrawing groups appeared to stabilise the system, whereas electron donating groups enhanced the photobleaching. When zinc(II) was replaced by Co(II) **16b**, the rate of photobleaching slowed down. In all cases, photobleaching was halted in deaerated solutions of **16a**, **112** and **113**. The only identified photoproduct of zinc(II) phthalocyanine **16a** photooxidation was phthalimide **114**, although other products were detected. It was proposed that singlet oxygen was involved in the degradation of these pigments as found for porphyrin-type compounds. The photobleaching of phthalocyanine **113** was also investigated in different solvents, the rate of the process increasing in the order: toluene, pyridine <DMF, dichloromethane (Table 10).

The photobleaching rates of water soluble anionic zinc(II) tetrasulfonated phthalocyanine **107** and cationic tetraazaporphyrin tetra quaternary salt **115** (Fig. 12) were also measured in aqueous solution (pH 7).[60] The rates were smaller than those of **16a**, **112** and **113** in organic solvents, a result ascribable to the higher photostability of the aggregated material in aqueous media. In the studies in organic solvents, zinc(II) *tert*-butylnaphthalocyanine **117** (Fig. 12), with the lowest redox potential, showed the most rapid photobleaching. At the other extreme, zinc(II) octamethylthiotetraazaporphyrin **116** (Fig. 12), a porphyrazine without annelation, showed the slowest rate of all the systems studied in organic solvents (Table 10).

Table 10. Photobleaching rates of phthalocyanines in aerated organic solvents[60]

Substrate	Solvent	k (min^{-1})	Degradation time (min)		
			5%	10%	50%
112	DMF	7.5×10^{-3}	6.9	14	92
16a	DMF	2.1×10^{-2}	2.5	5	33
16b	DMF	6.5×10^{-3}	8	16	110
113	DMF	4.4×10^{-2}	1.2	2.4	16
113	Pyridine	7.8×10^{-3}	6.6	14	89
113	Toluene	8.6×10^{-3}	6	12	81
113	CH_2Cl_2	6.2×10^{-2}	0.8	1.7	11
107	H_2O	1.1×10^{-3}	47	96	630
115	H_2O	3.2×10^{-4}	160	330	2200
117	DMF	1.5×10^{-1}	0.3	0.7	5
116	DMF	1.0×10^{-3}	21	42	280

The photooxidation of tetra(*tert*-butyl)phthalocyanine has been observed in negatively charged vesicles (dihexadecylphosphate) upon irradiation with a filtered 300 W projector lamp.[88] It was found that the rate of photobleaching depended on the concentration of oxygen, and was reduced in argon-purged solutions. Monomers were also more susceptible to photobleaching than the aggregated material, and the formation of OH radicals was detected by spin trapping. Remarkably, the photobleaching was completely suppressed when the phthalocyanine was solubilised in positively charged vesicles (dioctadecyldimethylammonium bromide), the spectra showing that, in these vesicles, the phthalocyanine is extensively aggregated.

The photobleaching of zinc(II) 1,4,8,11,15,18,22,25-octakisbutyloxyphthalocyanine in ethanol solution, irradiating with a pulsed laser (λ 750 nm),[89a] appeared to be independent of the dye concentration. New absorption bands were not observed during the irradiation period, so that true photobleaching was being observed; a similar result was reported on irradiating a solution of zinc(II) 2(3),9(10),16(17),23(24)-tetrakis-(*N*-methylpiperidinyl-4-oxy)phthalocyanine in DMF.[89b]

The rate of photobleaching of zinc(II) mono-*N*-glycyl-2,9,16,23-tetraaminophthalocyanine (G-TAPC-Zn) was measured in DMSO and in aqueous micelles (CTAB-B), irradiating with a 500 W incandescent lamp.[90] The process was faster in aqueous media than in DMSO, and increased with the dielectric constant of the solvent. The photobleaching quantum yields were also calculated for zinc(II) mono-*N*-glycyl-2,9,16,23-tetraaminophthalocyanine (G-TAPC-Zn) bound to *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymer and were about 3-fold smaller than those for the unbound form. Similar results were also observed for mesochlorin e_6 when bound to a polymer.

Some systems closely related to the phthalocyanines have

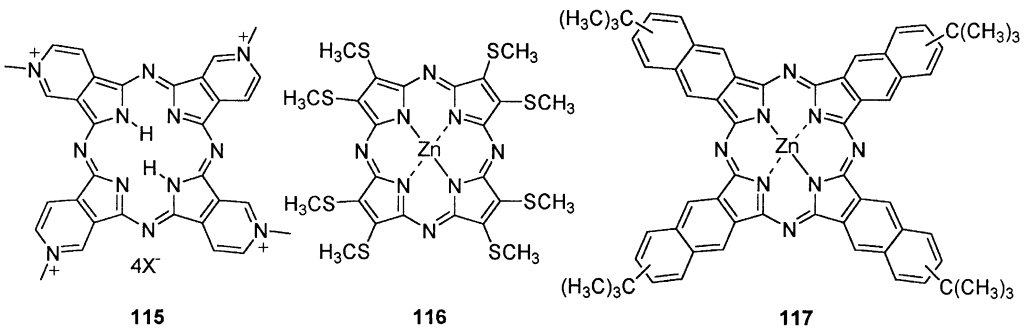
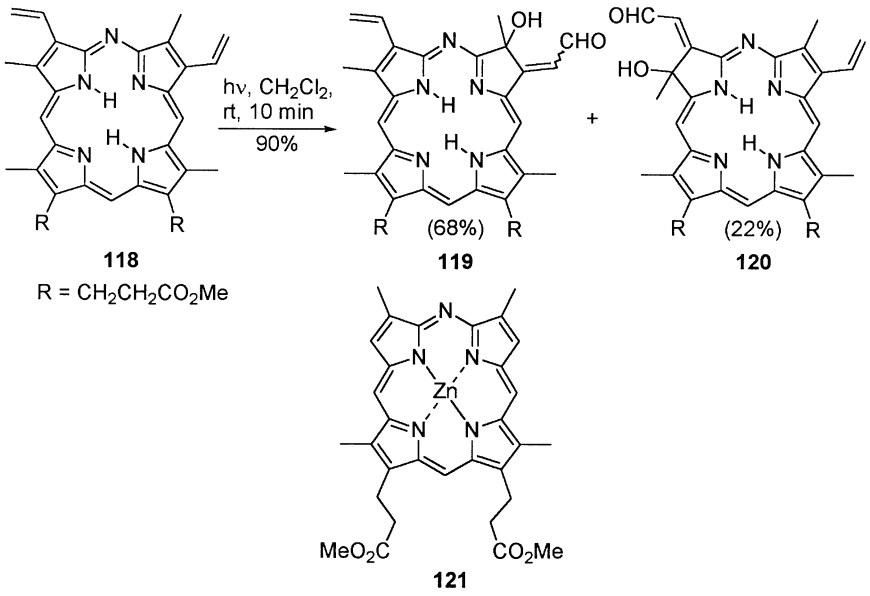
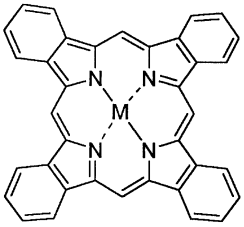


Figure 12. Structures of **115**, **116** and **117**.



Scheme 23. Photooxidation of monoazaporphyrin **118**. Structure of **121**.[91]

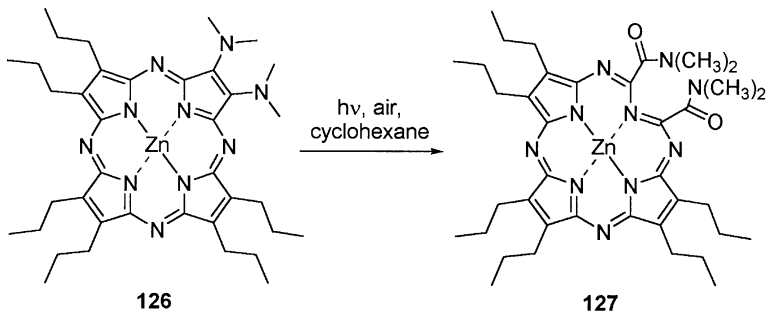


122: M = 2H
123: M = Zn(II)
124: M = Mg(II)
125: M = Cd(II)

Figure 13. Tetrabenzoporphyrin and its metal complexes.

been examined. Montforts et al.[91a] reported that the monoazaporphyrin **118** underwent oxygen addition when irradiated, yielding the photoproducts **119** and **120** (Scheme 23), analogous to those observed for protoporphyrin **9** (Scheme 4).[41–48] In this case, regioisomer **119** (*E* and *Z* isomers) was favoured in a 3:1 ratio over regioisomer **120** (*Z* stereoisomer only). The photoproducts **119** and **120** and the zinc complex of the monoazaporphyrin **121** were shown to be more photostable than **118**.[91b]

Berezin and coworkers[92] investigated the photooxidation of a series of metal complexes of tetrabenzoporphyrin in pyridine solution, irradiating with a projector lamp. The solutions photobleached upon irradiation and the rates increased as follows: tetrabenzoporphyrin (TBP, **122**)< zinc(II) TBP **123**<magnesium(II) TBP **124**<cadmium(II) TBP **125** (Fig. 13), i.e. with decreasing oxidation potential of the complexes. The photobleaching was suppressed when degassed solutions were irradiated, confirming oxygen involvement in the process. The mechanism of oxidation was proposed to involve both singlet oxygen and a charge transfer complex between the triplet excited state of the porphyrin and oxygen. Barrett et al.[93] reported that porphyrazine **126**, when irradiated in cyclohexane, underwent a [2+2] singlet oxygen addition, leading to the secoporphyrazine **127** (Scheme 24). Interestingly, the reaction is autocatalytic, since it is found that the photomodification product **127** has a substantial singlet oxygen quantum yield (0.54) whereas that of **126** appears to be low, and could not be measured accurately. Bonnett and Martínez[94] reported a comparative study of the photobleaching of structurally related aza and benzoporphyrin zinc complexes **128**–**130** (Fig. 14), irradiating at 605 nm with a light-emitting diode device. The solvents were varied, pyridine being employed as one solvent and, in other examples, the solvent (MeOH, HCO_2NMe_2 and MeCN)



Scheme 24. Formation of secoporphyrazine **127** via a singlet oxygen reaction.[93]

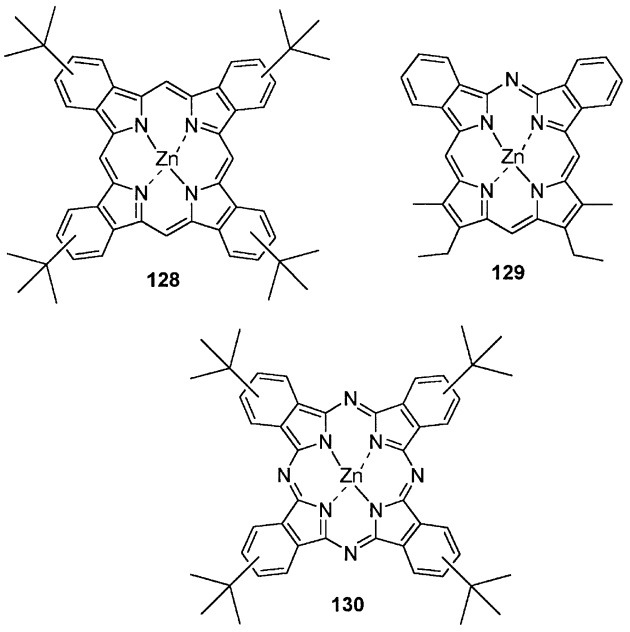


Figure 14. Zinc(II) complexes **128**–**130** of various benzo- and azabenzoporphyrins.

always contained ca. 0.1% pyridine to regulate the coordination sphere. The differences in reactivity were noteworthy: the zinc(II) *tert*-butylphthalocyanine **130** was the least photostable (true photobleaching) and small colourless fragments (the expected imide **131** and the unusual depolymerisation product **132**) were isolated in preparative studies (Scheme 25); the zinc(II) monoazadibenzoporphyrin **129** was relatively stable towards irradiation in air-saturated solutions; and the zinc(II) *tert*-butytetrabenzoporphyrin **128** in methanol and methanol-formamide underwent a broadening which was interpreted as a photoinduced aggregation process (examples of photoinduced aggregation are uncommon: Bezdetnaya et al.[40] have proposed it to account for the recovery of fluorescence in irradiated protoporphyrin solutions in 2% FCS kept in the dark). The reduction of the photobleaching rates when the solutions were degassed, and when sodium azide or DABCO were added, led the authors to suggest the involvement of singlet oxygen (Table 11).

4. Photobleaching studies in cell cultures (in vitro)

An initial caution is in order here. The experimental method generally used in photosensitiser assays in living systems, whether in vitro or in vivo, is the measurement of fluorescent emission. Many of the photosensitisers are strongly fluorescent, and the method is very sensitive and convenient. It has certain features, however, which need to be appreciated: normal tissue itself has a green fluorescence (autofluorescence) which may need to be allowed for; fluorescence is very sensitive to quenching by aggregation or by interaction with various biomolecules; and fluorescence can vary with the environment, e.g. the compartment where the sensitiser is located. Occasions have been reported where, on irradiation of the cell, the photosensitiser moves from one environment to another, with a change in fluorescence characteristics.[95a,b] A philosophy of perfection would have concentrations based on fluorescence results confirmed by an independent assay, such as extraction of the sensitiser, followed by HPLC. Sometimes this is undertaken, but it is not always feasible (e.g. with microspectrofluorimetry).

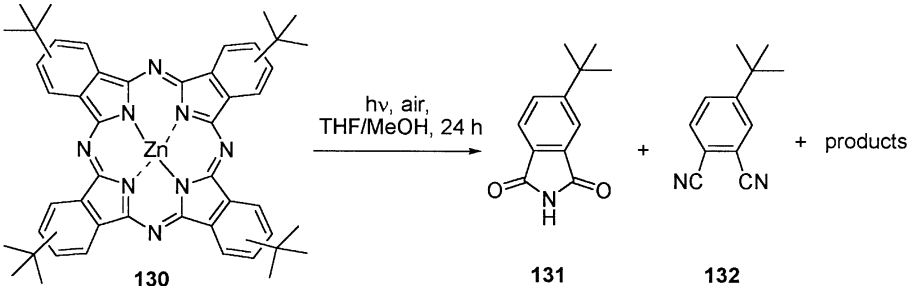
Table 11. Apparent first-order rate constants for diminution of the α-band in the photobleaching of various zinc complexes (605 nm, 9 mW cm^{-2})[94]

Substrate	Medium	k (s^{-1})
128	MeCN	4.5×10^{-5}
128	MeCN–DABCO[a]	1.7×10^{-5}
129	MeCN	3.3×10^{-6}
129	MeOH–$HCONH_2$ (1:1)	4.0×10^{-5}
129	MeOH–$HCONH_2$ (1:1)–NaN_3[a]	1.8×10^{-5}
130	MeCN	8.3×10^{-5}
130	MeOH	6.3×10^{-5}
130	MeOH–NaN_3[a]	3.3×10^{-5}

[a] 4 mM.

4.1. Haematoporphyrin derivative and related compounds

Moan et al.[27,95] studied the spectral changes during light exposure of cells of the line NHIK 25 (derived from human carcinoma in situ) incubated with Photofrin®. In addition to fluorescence loss (true photobleaching), at least two products were formed with emission peaks at 640–650 and 660–670 nm (similar absorption changes are seen in solution, Section 3.1). The first product was formed slowly and was stable during extended exposures, whereas the latter substance was formed faster and decayed more quickly. Their spectroscopic features suggested that both compounds were porphyrins.



Scheme 25. Photobleaching products of zinc(II) tetrakis(*t*-butyl)phthalocyanine **130**.[94]

The excitation spectrum of Photofrin® bound to NHIK 25 cells also showed a peak at 285–290 nm, which is regarded as corresponding to energy transfer from proteins to porphyrins located close (~1.5 nm) to aromatic (e.g. tryptophan) residues. When the Photofrin® concentration increased, the energy transfer peak decreased in intensity. This was interpreted in terms of high affinity binding sites which are increasingly occupied, whereupon the pigment starts to bind to more lipophilic environments. This energy transfer band was additionally followed during irradiation. At high Photofrin® concentrations, its decay was faster than that of the Soret band. The photobleaching rate of the Soret band itself was almost independent of the Photofrin® concentration, i.e. the process is mainly first order, but there appear to be higher order contributions at high concentrations. The decay of the energy transfer band was attributed mainly to a destruction of the binding sites, rather than a degradation of the porphyrin molecules which were bound there.

The appearance of the new bands at about 640 and 660 nm was explained in terms of photoinduced displacement of Photofrin® (or a porphyrin photoproduct) from binding sites on proteins to more hydrophobic environments. Changes in the fluorescence emission spectrum of protoporphyrin in red cells from patients suffering from erythropoietic protoporphyria have been explained in this way.[96] Cell suspensions bubbled with nitrogen showed a slower decay, suggesting oxygen involvement, but only a small isotope (D_2O) effect was observed. This latter finding, together with the fact that at high concentrations there are higher order contributions in the Soret decay rate, led Moan to propose that a small fraction of singlet oxygen was in the aqueous phase, probably escaping from the membrane to cause oxidation of some amino acids and proteins. These oxidised residues were then thought to attack the porphyrins bound in the membrane, causing the observed increase in the order of the decay rate. The main contribution to the Soret band decay was possibly due to the production of singlet oxygen. It was supposed that singlet oxygen oxidises the proteins and amino acids[45,46] in the near neighbourhood, and the oxidised biomolecules then react with the same porphyrin from which the singlet oxygen was generated. Moan et al.[95c] adduced an argument to show that singlet oxygen generated by a porphyrin would principally damage that porphyrin rather than others in the vicinity, as follows: 5,10,15,20-tetrakis(*m*-hydroxyphenyl)porphyrin (*m*-THPP, **57**) and Photofrin®, the monomeric fractions of which could be excited at different wavelengths (420 and 400 nm, respectively), were incubated together in NHIK 25 cells and the quantum yields of *m*-THPP **57** photobleaching due to light absorbed by Photofrin®, and vice versa, were measured. It was found that the quantum photobleaching quantum yields were 2- to 6-fold higher when the porphyrin was directly irradiated than when photobleaching was induced by indirect irradiation. The lifetime of singlet oxygen and the distance that singlet oxygen could diffuse under these conditions were estimated to be 0.01–0.04 μs and 0.01–0.02 μm, respectively. Such small values would account for the lack of an appreciable D_2O effect.

The absorption spectra of Photofrin® after irradiation in tissue culture also showed another product with a band about 310–450 nm. This band had also been observed when 5,10,15,20-tetrakis(4-sulfonatophenyl)-porphyrin ($TPPS_4$, **22**) or *m*-THPP **57** were irradiated in cells. The identity of this product is unknown, but it could arise from the oxidation of a cell component.[65]

Reyftmann et al.[97] have reported photobleaching of haematoporphyrin **8**, HpD and uroporphyrin I **21** in cells. Rück et al.[98] showed that porphyrins have different decay rates depending on their location in the cell, hydrophobic porphyrins such as protoporphyrin **9** and Photosan® photobleaching faster than hydrophilic porphyrins, such as uroporphyrin I **21** (Table 12). In 3T3 murine fibroblast cells, the photobleaching rates were calculated by monitoring fluorescence changes. The process appeared to be biexponential, i.e. two first order rates could be calculated (Table 12). Similar results were obtained with RR 1022 epithelial cells when incubated with protoporphyrin **9** and with Photosan®. It was found that photobleaching rates could be related to cell killing, since, when the rate of photobleaching was small, the cell destruction was low.

Table 12. Photobleaching rates in 3T3 murine fibroblasts[98]

Pigment	k (s^{-1})	
	0–5 s	5–15 s
Uroporphyrin I **21** (10 mg ml^{-1})	0.046	0.0085
Photosan® (2.5 mg ml^{-1})	0.10	0.014
Protoporphyrin **9** (1 mg ml^{-1})	0.29	0.053

Mang et al.[28] irradiated NHIK 25 cells containing Photofrin®. Loss of fluorescence (photobleaching) was also observed. Component 2 (haematoporphyrin, **8**) and components designated 4A and 4B showed less decay than did components designated 7D and 7F. The rate of photobleaching also appeared to be independent of Photofrin® concentration.

4.2. Second generation photosensitisers

Roberts et al.[30] compared the photobleaching rates of two new photosensitisers, namely mono-L-aspartylchlorin e_6 (MACE, **13**) and sulphonated chloroaluminium phthalocyanine, with that of Photofrin® in Chinese hamster ovary cells. Loss of fluorescence on irradiation was similar to that observed in FCS solutions. MACE **13** was the least photostable, followed by Photofrin® and sulphonated chloroaluminium phthalocyanine.

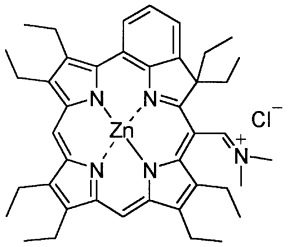
5,10,15,20-Tetrakis(*m*-hydroxyphenyl)chlorin (*m*-THPC, **10**) and 5,10,15,20-tetrakis(*m*-hydroxyphenyl)porphyrin (*m*-THPP, **57**) were incubated in V79 cells (Chinese hamster lung fibroblasts) and irradiated.[99] The fluorescence emission and excitation spectra showed loss of fluorescence, but there was no significant change in the shape of the emission spectrum, i.e. no new photoproduct was detected in this way. Photodestruction of the ring would therefore apparently be the only process involved. If *m*-THPC **10** bleaching is compared to those of *m*-THPP **57** and Photofrin®, the former process is much faster than those for the latter drugs. The *m*-THPC **10** rate was virtually independent of

concentration, i.e. it appeared to be a first order process (similar to Photofrin® behaviour).

Protoporphyrin **9** photobleaching in cells incubated with δ-ALA has also been observed.[100–102] Moan et al.[100] showed that, in cells of the human adenocarcinoma cell line Widr, the fluorescence of protoporphyrin **9** decayed upon irradiation. This process depended on oxygen and was faster in D_2O, and therefore singlet oxygen was thought to be involved. Photoprotoporphyrin **26**, **27** was identified as a minor photoproduct by HPLC comparison of the cell extracts with an authentic sample of photoprotoporphyrin. This photoproduct was also degraded upon irradiation, but, in contrast to observations in solution[48] (Section 3.2), it appeared to have a photostability similar to that of **9**. The decay rates of protoporphyrin **9** increased with increasing initial concentrations of **9**, so the process was not first order. Although the curves could be fitted to a second order kinetic analysis, the authors suggested that the degradation of **9** was possibly a mixture of several first order processes, with contributions from higher order processes. A more stable protoproduct, having a fluorescence peak at 652 nm, has also been detected, and attributed tentatively to a dimeric system. No products with rhodo-type spectra, expected for the monoformyl porphyrins **28** and **29**, were found.[100b]

In murine leukemia L1210 cells incubated with δ-ALA and irradiated at 630 nm, protoporphyrin **9** fluorescence decayed by 45% due to photobleaching.[101] The formation of a photoproduct with a fluorescence emission around 675 nm was observed in this case. Photobleaching of protoporphyrin (**9**, incubated as such) was studied by Strauss et al.[102] in endothelial cell cultures from normal calf aorta. Biexponential photobeaching was observed by following fluorescence decay, using total internal reflection (TIR) illumination. The fast portion of the curve was ascribed to the photodegradation of protoporphyrin **9** monomers and the slow portion to aggregated material in close proximity to the plasma membrane. Neither formation of photoproducts nor redistribution of **9** in the cells were observed and it was therefore concluded that the photobleaching was due to the formation of aggregates and/or destruction of the macrocycle. The decrease of PDT efficacy was proportional to photobleaching, suggesting that the monomers rather than the aggregates of **9** were responsible for inducing the cell killing.

Photoproduct formation was detected when the zinc benzochlorin iminium salt **133** (Fig. 15) was irradiated (740–760 nm, 540 mJ cm^{-2}) in murine colon carcinoma cells. The new species showed an emission band at 640 nm.[103]



133

Figure 15. Zinc(II) benzochlorin iminium salt **133**.

5. Photobleaching studies in vivo

Although the measurement of photobleaching in vivo poses great experimental difficulty, the number of studies has increased considerably in recent years.

5.1. First generation photosensitisers (Photofrin®)

Mang et al.[28] detected photobleaching of Photofrin® in mice (SMT-F tumour) and in metastatic skin tumours in patients undergoing PDT. In mice, the loss of fluorescence was accompanied by a reduction of the extractable amount of drug and, in humans, a faster decay (nearly an order of magnitude) was observed compared to that in mice. The decay in mice was independent of the amount of drug injected. In another in vivo experiment using rats and hamsters injected with Photofrin® and irradiated at 630 nm, it was observed that Photofrin® in the pancreas of the normal rat suffered negligible bleaching, whereas Photofrin® in hamsters and rats with implanted pancreatic tumours did undergo photobleaching.[104] Porphyrin levels were determined by fluorimetry and by extraction and absorption measurements. Boyle et al.[29] also observed photobleaching in Swiss mice which were injected with Photofrin®.

5.2. Second generation photosensitisers

Photobleaching has been detected in mouse tumour and human skin lesions using protoporphyrin **9** as a photosensitiser (δ-ALA treatment).[101] Furthermore, the formation of a photoproduct, which had an emission band around 670 nm, has also been observed. Such a product, which is thought to be photoprotoporphyrin **26**, **27** also appeared to be photobleached. Thus, in normal mouse skin, following topical application of δ-ALA, irradiation at 635 nm caused photobleaching of the fluorescence of protoporphyrin, with the appearance of a main fluorescent emission at 675 nm attributed to photoprotoporphyrin **26**, **27**. Other fluorescent bands at 588 and 623 nm were tentatively attributed to zinc(II) protoporphyrin and water-soluble porphyrins such as coproporphyrin, respectively.[100c] Several reports have shown recently that photobleaching of protoporphyrin **9** and formation of a photoproduct with an emission band at 676 nm occurred both in normal[105–107] and in tumour[105] mouse tissue. The photoproduct was found to be more resistant towards photobleaching than protoporphyrin **9** in normal tissue[105] (as found in solution, Section 3.2). When the photobleaching rates between normal tissue and tumour tissue under restricted blood flow were compared, the process was slower in the tumour tissue, which may reflect a lower availability of oxygen in tumour tissue.[106] In normal mouse tissue, the photobleaching rates of protoporphyrin **9** were not first order and were independent of the fluence rates (from 37.5 to 500 mW cm^{-2}). This was ascribed either to oxygen depletion or to the photodegradation of protoporphyrin **9** being independent of oxygen.[107] In another study with normal mouse tissue, the photobleaching rates were consistent with a second order process.[26,108] Under

hypoxic conditions, the photobleaching of protoporphyrin **9** and of its photoproducts appeared to cease.[108] The opposite result was, however, found by other authors.[107] The rate of photobleaching with respect to light dose increases significantly with decreasing fluence rate (from 150 to 5 mW cm^{-2}),[108] again in disagreement with another report.[106] The experiments are difficult, but these issues require further clarification.

Ma et al.[109a] showed that the photobleaching rates of 5,10,15,20-tetrakis(*m*-hydroxyphenyl)chlorin (*m*-THPC, **10**) and 5,10,15,20-tetrakis(*m*-hydroxyphenyl)-bacteriochlorin (*m*-THPBC, **58**) were similar when mice bearing WiDr human colon adenocarcinoma implants were irradiated at 652 and 740 nm, respectively, with a fluence of 10 J cm^{-2}. *m*-THPBC **58** usually contains a small amount of the chlorin (<5%), so the fluorescence spectra of *m*-THPBC **58** in mice also showed a small peak due to *m*-THPC **10**. By irradiating the tumour first at 740 nm and then at 652 nm, it was suggested that damage to normal tissue could be avoided, since *m*-THPBC **58** could be easily cleared, leaving *m*-THPC **10** unaffected. If enough *m*-THPC **10** was left, tumour necrosis could then be effected by irradiating at 652 nm. Forrer et al.[109b] also reported the photobleaching of *m*-THPC **10** in patients with early squamous cell carcinomas in the oesophagus, irradiating at 514 nm (fluence rate 100 mW cm^{-2}). They observed a 60% decay of the fluorescence of *m*-THPC **10** at 652 nm, when a light dose of 100 mJ cm^{-2} was administered.

Mono-L-aspartylchlorin e_6 (MACE, **13**) photobleaching was detected in mice bearing Meth-A fibrosarcoma implants.[110] The fluorescence intensities (λ_{ex} 405 nm) of the residual MACE **13** at 3 mm from the tumour surface were measured after different light exposures (Table 13). The fluorescence loss increased with light dose. The photobleaching appeared to be in proportion to the effectiveness of the treatment, high or low photobleaching corresponding to high or low tumour destruction.

Table 13. Relative fluorescence intensity of the residual amount of MACE **13** (c_o=5 mg kg^{-1}) after PDT at 664 nm[110]

Fluorescence intensity	Laser dose (J cm^{-2})
1	0
0.73	50
0.36	100
0.22	150
0.16	200

The photobleaching (irradiation at 672 nm) of aluminium(III) phthalocyanine tetrasulphonic acid salts and zinc(II) phthalocyanine **16a** in rats with induced bladder carcinoma was investigated by Rück et al.[111a] The fluorescence of the aluminium(III) tetrasulfonated phthalocyanine tetrasulphonic acid increased almost 2-fold after 30 s irradiation and then decreased after longer light exposure. The initial increase was attributed to dye relocalisation in the cells. Tumour necrosis was minimal. In contrast, the fluorescence of zinc(II) phthalocyanine **16a** decreased exponentially during light exposure. The significant tumour necrosis correlated with the photobleaching behaviour of zinc(II) phthalocyanine. Photobleaching of aluminium(III) phthalocyanine tetrasulphonic acid has also been detected in normal rat bladder when irradiated with red light (675 nm).[111b]

In a comparative study,[112a] the photobleaching of several photosensitisers was evaluated in normal mouse skin, irradiating with an argon pumped laser tuned to the appropriate therapeutic wavelength at a fluence rate of 100 mW cm^{-2}. The fluorescence decay decreased in the following order: chlorin e_6, 5,10,15,20-tetrakis(*m*-hydroxyphenyl)chlorin (*m*-THPC, **10**)>zinc(II) phthalocyanine **16a**, protoporphyrin **9**>Photofrin®, 5,10,15,20-tetrakis(*m*-hydroxyphenyl)porphyrin (*m*-THPP, **57**)>aluminium(III) tetrasulphonated and disulphonated phthalocyanine, and 5,10,15,20-tetrakis(4-sulphonatophenyl)porphyrin ($TPPS_4$, **22**). In all cases, there was deviation from first order kinetics. An increase in the fluorescence intensity was observed at initial irradiation times for aluminium(III) tetrasulphonated and disulphonated phthalocyanines, and for 5,10,15,20-tetrakis(4-sulphonatophenyl)porphyrin ($TPPS_4$, **22**). The explanation given for this effect is that these dyes are localised in lysosomes, which are permeabilised after short light exposures. The dye is released into the cytoplasm and disaggregates. With an increase in the monomer population, there is an increase in fluorescence. A comparison of fluence rate effects and photobleaching has been made in a rat bladder tumour model in vivo, comparing protoporphyrin (**9**, induced by δ-ALA) and benzoporphyrin derivative **11**.[112b] The results suggest that photobleaching pathways may be independent of the pathways involved in tissue detruction. Fluorescent photoproducts were detected for **9** but not for **11**.

5.3. Dosimetry

In some examples in solution, such as the photooxygenation **77**→**78**, the photoreactions considered here are essentially single pathway, high-yielding processes. More typically, however, they are complex, following several pathways, and this is especially so in biological systems. Nevertheless, in this field, it is to biological systems that the photochemistry has to be applied, and the question arises as to what effect should photobleaching have on dosimetry. The subject has yet to be fully explored: it may be that, to accommodate the destruction of the photosensitiser, a programmed increase of the fluence rate ('ramping') may be introduced with advantage.

Svaasand and Potter[113] have considered the implications of photobleaching for dosimetry, and concluded that optimisation of the protocol could significantly reduce damage to normal tissue located near to the tumour, although it would be a requirement that the tumour continued to retain a higher concentration of drug than the normal tissue. There appears to be a threshold for photodynamic activity,[108,114] and it would clearly be advantageous to have the concentration of the drug in the normal tissue below this level by whatever means. A mathematical treatment of photobleaching and its monitoring by fluorimetry has been presented by Jacques and his colleagues.[115]

Georgakoudi and Foster[26] have developed a mathematical model to describe photobleaching by singlet oxygen and

other pathways in relation to dosimetry during the irradiation of tumour multicellular spheroids. They conclude that the widely used fluence-dependent simple exponential description of sensitiser decay is not an appropriate model of photobleaching in PDT. Photobleaching for constant fluence, and the corresponding PDT damage, are both found to increase as the fluence rate is decreased.[108,116] Put another way, when equal amounts of energy are delivered to the tissue, more photosensitiser and more tissue are destroyed when the energy is given at a low dose rate. Clearly these results have an important bearing on dosimetry.

The absorption of photoproducts formed by the photomodification of sensitisers may well be of secondary importance, but ways of taking account of this aspect have been suggested.[117]

6. Summary and outlook

The main points of the foregoing can be summarised, and supported by illustrative references, as follows:

(i) There have been extensive studies on the photobleaching of PDT sensitisers, particularly kinetic studies which measure loss of absorbance or loss of fluorescence. Both photomodification (change of visible chromophore, Fig. 3) and true photobleaching (loss of visible absorbance, Fig. 4) have been encountered.
(ii) Initial rates of photobleaching are often observed to be first order in photosenstiser,[60,94,102] but second order dependence has also been observed experimentally[26,65] and mixed order dependence has also been found.[27,95] In situations where light intensity is high, tissues can become deprived of oxygen, and fragmentation of light delivery then becomes advantageous.[1c] In the singular case of the porphyrazine derivative **126**, the reaction is autocatalytic.[93]
(iii) Most of the photobleaching processes which have been reported are oxidative, and there is evidence that they involve singlet oxygen.[24,64,87] There are fewer examples where radical oxygen species appear to be implicated.[88] Many authors report that the reaction slows down when oxygen is replaced by nitrogen in the reaction vessel, but there are exceptions to this observation.[68] There is also evidence that oxygen is required in vivo.[108]
(iv) In many examples (e.g. haematoporphyrin[36]) the products of photomodification have not been fully characterised, but, where they have, they can be rationalised as the products of $2\pi+2\pi$ cycloadditions or of $2\pi+4\pi$ cycloadditions of singlet oxygen (e.g. protoporphyrin,[43] tetraphenylporphyrin,[50] zinc(II) octaethylchlorin,[54] chlorophyll analogues and derivatives[77,80]). Products of oxidative photomodification from phthalocyanines and naphthalocyanines do not appear to have been characterised, and are presumed to be fugitive.
(v) The products of true photobleaching appear to result from deep-seated oxidative and hydrolytic reactions: they have often not been isolated and identified. They include propentdyopent adducts (from oxophlorin derivatives[55b]), imides (from chlorins and bacteriochlorins,[24] phthalocyanines[60,94] and chlorophyll[75b]) and aliphatic acids (from chlorophyll[75a]).
(vi) As expected for oxidative processes, rates of photobleaching are enhanced for systems of lower oxidation potential (e.g. *m*-THPBC **58** is more reactive than is *m*-THPC **10**,[24] while naphthalocyanine **108** is more reactive than the analogous phthalocyanine **107**[86]). Electron-donating substituents increase the rate of reaction.[60] The effect of a coordinated metal ion on redox potential is significant: zinc(II) and magnesium(II) complexes with lower oxidation potentials are, in general, more readily photobleached than complexes of first row transition metal ions.[58,60]
(vii) Large aromatic photosensitiser molecules tend to form non-covalently bonded aggregates under appropriate conditions. Aggregates have lower singlet oxygen quantum yields and PDT activities, are more resistant to photobleaching[39,67a,86] and are less fluorescent than the corresponding monomers. Some apparent photobleaching of fluorescence may be due to aggregation,[102] since fluorescence is readily quenched by such interactions.
(viii) Surfactants generally enhance photobleaching, presumably because they promote disaggregation in aqueous media.[68,86]
(ix) The addition of protein (e.g. HSA[30,85] or FCS[30,68]) enhances photobleaching, possibly because the photosensitiser monomer/aggregate equilibrium is disturbed as the monomer associates loosely with the protein and/or the oxidative process is mediated by a reactive oxidised amino acid side chain (e.g. of methionine[46,47]). Further work is needed on this aspect, and to disentangle the effect of various solvent characteristics (e.g. dielectric constant[39]) and of additives on the rate of photobleaching.

This report appears to be the first detailed review of this topic, which is revealed to be complex in many ways. To paraphrase an observation[100b] made recently by two of the most prominent workers in the field, Moan and Rotomskis (specifically with reference to in vivo studies on the photobleaching of protoporphyrin, but there is some generality in the comment): the reactions leading to photoproducts are dependent on internal features (such as the level of oxygenation, lipophilicity, pH, compartmentalisation in cells and tissues) as well as on external factors (such as the wavelength and fluence rate of the light). As a result, the studies are particularly complicated, and the published results are frequently inconsistent.

Further studies are clearly needed in some areas, including studies on the basic chemical systems: in the past, it has been found all too easy to carry out kinetic studies on these beautiful coloured photosensitisers, and to forget about the chemical structures of the products that are being formed. This is a field that deserves further cultivation. The results would be of interest in, and might be of real benefit to, the emerging practice of photodynamic therapy.

Acknowledgements

G. M. is indebted to the University of London Postgraduate trust for the award of a Laura de Saliceto Cancer Research Studentship (1996–1998).

References

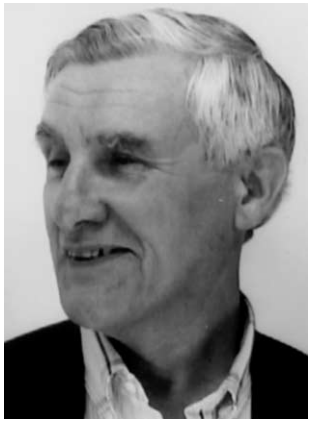
1. (a) Fitzpatrick, T. B.; Pathak, M. A. *J. Invest. Dermatol.* **1965**, *32*, 225–238. (b) Bonnett, R. *Rev. Contemp. Pharmacother.* **1999**, *10*, 1–17. Bonnett, R. *Chem. Soc. Rev.* **1995**, *24*, 19–33. (c) Bonnett, R. *Chemical Aspects of Photodynamic Therapy*; Gordon and Breach Science: Amsterdam, 2000.
2. Finsen, N. R. *Phototherapy*; Arnold: London, 1901 English translation: J. H. Sequeira.
3. Raab, O. *Z. Biol.* **1900**, *39*, 524–546.
4. Meyer Betz, F. *Dtsch. Arch. Klin. Med.* **1913**, *112*, 476–503.
5. Auler, H.; Banzer, G. *Z. Krebsforschung* **1942**, *53*, 65–68.
6. Lipson, R. L.; Baldes, E. J.; Olsen, A. M. *J. Natl Cancer Inst.* **1961**, *26*, 1–11.
7. Diamond, I.; Granelli, S.; McDonagh, A. F.; Nielsen, S.; Wilson, C. B.; Jaenicke, R. *Lancet* **1972**, *ii*, 1175–1177.
8. Kelly, J. F.; Snell, M. E. *J. Urol.* **1976**, *115*, 150–151.
9. (a) Dougherty, T. J. *Photochem. Photobiol.* **1993**, *58*, 890–895. (b) Dougherty, T. J.; Kaufman, J. E.; Goldfarb, A.; Weishaupt, K. R.; Boyle, D. G.; Mittelman, A. *Cancer Res.* **1978**, *38*, 2628–2635.
10. (a) Berenbaum, M. C.; Bonnett, R.; Scourides, P. A. *Br. J. Cancer* **1982**, *45*, 571–581. (b) Bonnett, R.; Berenbaum, M. C. *Adv. Exp. Med. Biol.* **1983**, *160*, 241–250.
11. Berenbaum, M. C.; Bonnett, R. In *Ciba Symposium 146. Photosensitising Compounds: Their Chemistry, Biology, and Clinical Use*; Bock, G., Harnett, S., Eds.; Wiley: Chichester, 1989; pp. 33–53.
12. Gomi, S.; Nishizuka, T.; Ushiroda, O.; Uchida, N.; Takahashi, H.; Sumi, S. *Heterocycles* **1998**, *48*, 2231–2243.
13. (a) Sternberg, E. D.; Dolphin, D.; Bruckner, C. *Tetrahedron* **1998**, *54*, 4154–4202. MacRobert, S.; Milgrom, L. *Chem. Br.* **1998**, *34*, 45–50. (b) Pandey, R. K.; Herman, C. K. *Chem. Ind.* **1998**, *18*, 739–743. (c) Boyle, R. W.; Dolphin, D. *Photochem. Photobiol.* **1996**, *64*, 469–485.
14. Kennedy, J. C.; Pottier, R. H. *J. Photochem. Photobiol. B. Biol.* **1992**, *24*, 275–292.
15. (a) Forrester, S. D. *J. Soc. Dyers Colour.* **1975**, *91*, 217–223 and references therein. (b) Giles, C. H. *J. Appl. Chem.* **1965**, *15*, 541–550 and references therein.
16. Hellot, J. *L'Art de la Teinture des Étoffes de la Laine, en Grand et Petit Teint*; Pissot, Herissant and Pissot: Paris, 1750. Wescher, H. *CIBA Rev.* **1939**, *18*, 626–641.
17. Senebier, J. *Memoires physico-chimiques sur l'influence de la lumière solaire pour modifier les êtres de trois règnes de nature, et surtout ceux du règne végétal*, Vol. 3; Barthelemi Chirol: Geneva, 1782.
18. Bancroft, E. *Experimental Researches Concerning the Philosophy of Permanent Colours*; 2nd ed.; Cadell and Davies: London, 1813.
19. Chevreul, M. E. *Mem. Acad. Sci.* **1838**, *16*, 53–116 see also pp. 181–228. Chevreul, M. E. *Mem. Acad. Sci.* **1845**, *19*, 491–551. *Chemical Reports and Memoires*, Graham, T., Ed.; Cavendish Society: London, 1848; pp. 165–238.
20. Russell, W. J.; Abney, W.deW. *Action of Light on Water Colours, Report to the Science and Art Department of the Committee of the Council on Education, C. 5453*; HMSO: London, 1888.
21. Hummel, J. J. *Text. Mfr.* **1890**, *16*, 506–507.
22. Sinclair, R. S. *Photochem. Photobiol.* **1980**, *31*, 627–629.
23. Verhoeven, J. W. *Pure Appl. Chem.* **1996**, *68*, 2223–2286.
24. (a) Bonnett, R.; Djelal, B. D.; Hamilton, P. A.; Martinez, G.; Wierrani, F. *J. Photochem. Photobiol. B. Biol.* **1999**, *53*, 136–143. (b) Martinez, G. PhD Thesis, University of London, 1999.
25. (a) Krasnovskii, A. A. *Dokl. Akad. Nauk SSSR* **1948**, *60*, 421–424. (b) Mauzerall, D. *J. Am. Chem. Soc.* **1960**, *82*, 1832–1833. (c) Seely, G. R.; Folkmanis, A. *J. Am. Chem. Soc.* **1964**, *86*, 2763–2770. (d) Scheer, H.; Katz, J. J. *Proc. Natl Acad. Sci. USA* **1974**, *71*, 1626–1629. (e) Iakovides, P.; Simpson, D. J.; Smith, K. M. *Photochem. Photobiol.* **1991**, *54*, 335–343. Hopf, F. R.; Whitten, D. G. *Porphyrins and Metalloporphyrins*; Dolphin, D., Ed.; Academic: New York, 1978; Vol. 2, pp. 178–187.
26. (a) Georgakoudi, I.; Nichols, M. G.; Foster, T. H. *Photochem. Photobiol.* **1997**, *65*, 135–144. (b) Georgakoudi, I.; Foster, T. H. *Photochem. Photobiol.* **1998**, *67*, 612–625.
27. (a) Moan, J.; Christensen, T.; Jacobsen, P. In *Porphyrin Localization and Treatment of Tumours*; Doiron, D. R., Gomer, C. J., Eds.; Alan Riss: New York, 1984; pp. 419–442. (b) Moan, J. *Cancer Lett.* **1986**, *33*, 45–53.
28. Mang, T. S.; Dougherty, T. J.; Potter, W. R.; Boyle, D. G.; Jones, S.; Moan, J. *Photochem. Photobiol.* **1987**, *45*, 501–506.
29. Boyle, D. G.; Potter, W. R. *Photochem. Photobiol.* **1987**, *46*, 997–1001.
30. Roberts, W. G.; Smith, K. M.; McCullogh, J. L.; Berns, M. W. *Photochem. Photobiol.* **1989**, *49*, 431–438.
31. (a) Mang, T. S.; Wilson, B. D.; Khan, S. *Photochem. Photobiol.* **1990**, *51s*, 72s–73s. (b) Ris, H.-B.; Alternatt, H. J.; Stewart, C. M.; Schaffner, T.; Wang, Q.; Lim, C. K.; Bonnett, R.; Althaus, U. *Int. J. Cancer* **1993**, *55*, 245–249.
32. Rotomskiene, J.; Kaspociute, R.; Rotomskis, R.; Jonusauskas, G.; Szito, T.; Nizhnik, A. *J. Photochem. Photobiol. B. Biol.* **1988**, *2*, 373–379.
33. Streckyte, G.; Rotomskis, R. *J. Photochem. Photobiol. B. Biol.* **1993**, *18*, 259–263.
34. Rotomskis, R.; Vaicaitis, V.; Piskarkas, A. *Chem. Phys. Lett.* **1993**, *202*, 233–236.
35. Rotomskis, R.; Bagdonas, S.; Streckyte, G. *J. Photochem. Photobiol. B. Biol.* **1996**, *33*, 61–67.
36. (a) Rotomskis, R.; Streckyte, G.; Bagdonas, S. *J. Photochem. Photobiol. B. Biol.* **1997**, *39*, 172–175. (b) Rotomskis, R.; Streckyte, G.; Bagdonas, S. *Proc. SPIE* **1995**, *2625*, 347–355.
37. König, K.; Wabnitz, H.; Dietel, W. *J. Photochem. Photobiol. B. Biol.* **1990**, *8*, 103–111.
38. Jones, L. R.; Grossweiner, L. I. *J. Photochem. Photobiol. B. Biol.* **1994**, *26*, 249–256.
39. Spikes, J. D. *Photochem. Photobiol.* **1992**, *55*, 797–808.
40. Bezdetnaya, L.; Zeghari, N.; Belitchenko, I.; Barberi-Heyob, M.; Merlin, J.-L.; Potapenko, A.; Guillemin, F. *Photochem. Photobiol.* **1996**, *64*, 382–386.
41. (a) Fischer, H.; Herrle, H. *Hoppe-Seyler's Z. Physiol. Chem.* **1938**, *251*, 85–96. (b) Gurinovich, I.; Gurinovich, G. P.; Sevchenko, A. N.; Tauger, S. M. *Dokl. Akad. Nauk SSSR* **1965**, *164*, 201–204.
42. Inhoffen, H. H.; Brockman, H.; Bliesener, K.-M. *Liebigs Ann. Chem.* **1969**, *730*, 173–185.
43. Horsey, B. E.; Whitten, D. G. *J. Am. Chem. Soc.* **1978**, *100*, 1293–1295.
44. Cox, G. S.; Whitten, D. G. *J. Am. Chem. Soc.* **1982**, *104*, 516–521

.

45. Cox, G. S.; Krieg, M.; Whitten, D. G. *J. Am. Chem. Soc.* **1982**, *104*, 6930–6937.
46. Krieg, M.; Whitten, D. G. *J. Am. Chem. Soc.* **1984**, *106*, 2477–2479.
47. Krieg, M.; Whitten, D. G. *J. Photochem.* **1984**, *25*, 235–252.
48. Cox, G. S.; Bobilier, C.; Whitten, D. G. *Photochem. Photobiol.* **1982**, *36*, 401–407.
49. Matsuura, T.; Inoue, K.; Ranade, A. C.; Saito, I. *Photochem. Photobiol.* **1980**, *31*, 23–26.
50. (a) Smith, K. M.; Brown, S. B.; Troxler, R. F.; Lai, J.-J. *Photochem. Photobiol.* **1982**, *36*, 147–152. (b) Cavaleiro, J. A. S.; Hewlins, M. J. E.; Jackson, A. H.; Neves, G. P. M. S. *J. Chem. Soc., Chem. Commun.* **1986**, 142–144.
51. Ando, W.; Takata, T. *Photooxidation of Sulfur Compounds*; Frimer, A., Ed.; CRC: Boca Raton, 1985; Vol. 3, pp. 1–117.
52. (a) Lightner, D. A.; Crandall, D. C. *Tetrahedron Lett.* **1973**, *12*, 953–956. (b) Bonnett, R.; Stewart, J. C. M. *J. Chem. Soc., Perkin Trans. 1* **1975**, 224–231. (c) Lightner, D. A. *Photochem. Photobiol.* **1977**, *26*, 427–436.
53. Barrett, J. *Nature* **1967**, *215*, 733–735.
54. (a) Fuhrhop, J.-H.; Mauzerall, D. *Photochem. Photobiol.* **1971**, *13*, 453–458. (b) Wasser, P. K. W.; Fuhrhop, J.-H. *Ann. NY Acad. Sci.* **1973**, *206*, 533–548. (c) Fuhrhop, J.-H.; Wasser, P. K. W.; Subramanian, J.; Schrader, U. *Liebigs Ann. Chem.* **1974**, 1450–1466.
55. (a) Besecke, S.; Fuhrhop, J.-H. *Angew. Chem.* **1974**, *86*, 125–126 *Angew. Chem., Int. Ed. Engl.* **1974**, *13*, 150–151. (b) Bonnett, R.; Chaney, B. D. *J. Chem. Soc., Perkin Trans. 1* **1987**, 1063–1067.
56. Cavaleiro, J. A. S.; Neves, M. G. P. S.; Hewlins, M. J. E.; Jackson, A. H. *J. Chem. Soc., Perkin Trans. 1* **1990**, 1937–1943.
57. Silva, A. M. S.; Neves, M. G. P. M. S.; Martins, R. R. L.; Cavaleiro, J. A. S.; Boschi, T.; Tagliatesta, P. *J. Porphyrins Phthalocyanines* **1998**, *2*, 45–51.
58. Krasnovskii, A. A.; Venediktov, Y. A.; Chernenko, O. M. *Biophysics* **1982**, *27*, 1009–1016 (*Biofizika* **1982**, *6*, 966–972).
59. Saito, I.; Matsuura, T. *Singlet Oxygen, Organic Chemistry*; Wasserman, H. H., Murray, R. W., Eds.; Academic: New York, 1979; Vol. 40, pp. 511–574.
60. Sobbi, A. K.; Wohrle, D.; Schlettwein, D. *J. Chem. Soc., Perkin Trans. 2* **1993**, 481–488 and references therein. Schnurpfiel, G.; Sobbi, A. K.; Michelsen, U.; Wöhrle, D. *Proc. SPIE* **1997**, *3191*, 299–308.
61. Mosseri, S. J. C.; Mialocq, J. C.; Perly, B.; Hambright, P. *J. Phys. Chem.* **1991**, *95*, 2196–2203.
62. (a) Bonnett, R.; White, R. D.; Winfield, U.-J.; Berenbaum, M. C. *Biochem. J.* **1989**, *261*, 277–280. (b) Bonnett, R.; Ioannou, S.; White, R. D.; Winfield, U.-J. *Photobiochem. Photobiophys. (Suppl.)* **1987**, 45–56. (c) Berenbaum, M. C.; Bonnett, R.; Chevretton, E. B.; Akande-Adebakin, S. L.; Ruston, M. *Lasers Med. Sci.* **1993**, *8*, 235–243. (d) Berenbaum, M. C.; Akande, S. L.; Bonnett, R.; Kaur, H.; Ioannou, S.; White, R. D.; Winfield, U.-J. *Br. J. Cancer* **1986**, *54*, 717–725.
63. Jones, R. M.; Wang, Q.; Lamb, J. H.; Djelal, B. D.; Bonnett, R.; Lim, C. K. *J. Chromatogr., A* **1996**, *722*, 257–265.
64. Hadjur, C.; Lange, N.; Rebstein, J.; Monnier, P.; van den Bergh, H.; Wagnieres, G. *J. Photochem. Photobiol. B. Biol.* **1998**, *45*, 170–178.
65. Rezzoug, H.; DeJode, M.; Rovers, J. P.; Grahn, M. F. personal communication.
66. Bonnett, R.; Charlesworth, P.; Djelal, B. D.; Foley, S.; McGarvey, D. J.; Truscott, T. G. *J. Chem. Soc., Perkin Trans. 2* **1999**, 325–328.
67. (a) Belitchenko, I.; Melnikova, V.; Bezdetnaya, L.; Rezzoug, H.; Merlin, J. L.; Potapenko, A.; Guillemin, F. *Photochem. Photobiol.* **1998**, *67*, 584–590. (b) Angotti, M.; Maunit, B.; Muller, J. F.; Bezdetnaya, L.; Guillemin, F. *Res. Commun. Mass Spectrom.* **1999**, *13*, 597–603.
68. Aveline, B.; Hasan, T.; Redmond, R. *Photochem. Photobiol.* **1994**, *59*, 328–335.
69. Gillies, R.; Kollias, N.; Hasan, T.; Diddens, H. *J. Photochem. Photobiol. B. Biol.* **1996**, *33*, 87–90.
70. (a) Spikes, J. D.; Bommer, J. C. *Photochem. Photobiol.* **1993**, *58*, 346–350. (b) Berezin, B.; Koifman, O. I.; Rashidova, S. T.; Askarov, K. A.; Golubchikova, N. L.; Enikolopov, N. S. *Russ. J. Phys. Chem.* **1985**, *59*, 560–562 (*Zh. Fiz. Khim.* **1985**, *59*, 971–973).
71. Sekher, P.; Garbo, G. M. *J. Photochem. Photobiol. B. Biol.* **1993**, *20*, 117–125.
72. (a) Morgan, A. R.; Tertel, N. C. *J. Org. Chem.* **1986**, *51*, 1347–1350. (b) Morgan, A. R.; Rumpersand, A.; Garbo, G. M.; Keck, R. W.; Selman, S. H. *J. Med. Chem.* **1989**, *32*, 904–908.
73. Ma, L.; Dolphin, D. *Tetrahedron Lett.* **1995**, *36*, 7791–7794.
74. Woodward, R. B.; Ayer, W. A.; Beaton, J. M.; Bickelhaupt, F.; Bonnett, R.; Buchschacher, P.; Closs, G. L.; Dutler, H.; Hannah, J.; Hauck, F. P.; Ito, S.; Langemann, A. E.; Le Goff, E.; Leimgruber, W.; Lwowski, W.; Sauer, J.; Valenta, Z.; Volz, H. *Tetrahedron* **1990**, *46*, 7599–7660.
75. (a) Llewellyn, C. A.; Mantoura, R. F. C.; Brereton, R. G. *Photochem. Photobiol.* **1990**, *52*, 1043–1047 and references therein. (b) Suzuki, Y.; Shioi, Y. *Plant Cell Physiol.* **1999**, *40*, 909–915.
76. Smith, K. M.; Bisset, G. M. F.; Bushell, M. J. *Bioorg. Chem.* **1980**, *9*, 1–26.
77. (a) Kenner, G. W.; Rimmer, J.; Smith, K. M.; Unsworth, J. F. *J. Chem. Soc., Perkin Trans. 1* **1978**, *9*, 845–852. (b) Risch, N.; Schormann, A.; Brockmann, H. *Tetrahedron Lett.* **1984**, *25*, 5993–5996 and references therein. (c) Brown, S. B.; Smith, K. M.; Bisset, G. M. F.; Troxler, R. F. *J. Biol. Chem.* **1980**, *255*, 8063–8068.
78. Iturraspe, J.; Gossauer, A. *Photochem. Photobiol.* **1991**, *54*, 43–49.
79. Iturraspe, J.; Gossauer, A. *Helv. Chim. Acta* **1991**, *74*, 1713–1717.
80. Curty, C.; Engel, N.; Iturraspe, J.; Gossauer, A. *Photochem. Photobiol.* **1995**, *61*, 552–556.
81. (a) Engel, N.; Jenny, T. A.; Moser, V.; Gossauer, A. *FEBS Lett.* **1991**, *292*, 131–133. (b) Kräutler, B.; Jaun, B.; Bortlik, K.; Schellenberg, M.; Matile, P. *Angew. Chem.* **1991**, *103*, 1354–1357 *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 1315–1318. Review: (c) Gossauer, A.; Engel, N. *J. Photochem. Photobiol. B. Biol.* **1996**, *32*, 141–151.
82. (a) Lindsay, J. R.; Calvin, M. *J. Am. Chem. Soc.* **1966**, *88*, 4500–4506. (b) Marsh, K. L.;
Connolly, J. S., J. S. *J. Photochem.* **1984**, *25*, 183–195.
83. McKeown, N. B. *Phthalocyanine Materials: Synthesis, Structure and Function*; University Press: Cambridge, 1998.
84. McCubbin, I.; Phillips, D. *J. Photochem.* **1986**, *34*, 187–195.
85. (a) Kogan, B. Y.; Butenin, A. V.; Kaliya, O. L. E. A.; Luk'yanets, E. A. *Proc. SPIE* **1996**, *2924*, 69–74.
86. Spikes, J. D.; van Lier, J. E.; Bommer, J. C. *J. Photochem. Photobiol. A. Chem.* **1995**, *91*, 193–198.

87. Cook, M. J.; Chambrier, I.; Cracknell, S. J.; Mayes, D. A.; Russell, D. A. *Photochem. Photobiol.* **1995**, *62*, 542–545.
88. Lukac, S.; Harbour, J. R. *J. Chem. Soc., Chem. Commun.* **1982**, 154–157.
89. (a) Giering, K.; Dressler, C.; Herter, R.; Senz, R.; Moser, J. G.; Berlien, H.-P. *Proc. SPIE* **1996**, *2625*, 77–83. (b) Kassab, K.; Fabris, C.; Defilippis, M. P.; Dei, D.; Fantetti, L.; Roncucci, G.; Reddi, E.; Jori, G. *J. Photochem. Photobiol. B. Biol.* **2000**, *55*, 128–137.
90. Gu, Z.; Spikes, J. D.; Kopeckova, P.; Kopecek, J. *Collect. Czech. Chem. Commun.* **1993**, *58*, 2321–2336.
91. (a) Montforts, F.-P.; Gerlach, B. *Tetrahedron Lett.* **1992**, *33*, 1985–1988. (b) Schiwon, K.; Brauer, H.-D.; Gerlach, B.; Müller, C. M.; Montforts, F.-P. *J. Photochem. Photobiol. B. Biol.* **1994**, *23*, 239–243.
92. Venediktov, E. A.; Kitaev, V. I.; Berezin, B. D. *Russ. J. Phys. Chem.* **1987**, *61*, 1278–1280 (*Zh. Fiz. Khim.* **1987**, *61*, 2445–2449).
93. Montalban, A. G.; Meunier, H. G.; Ostler, R. B.; Barrett, A. G. M.; Hoffman, B. M.; Rumbles, G. *J. Phys. Chem. A* **1999**, *103*, 4352–4358.
94. Bonnett, R.; Martinez, G. *J. Porphyrins Phthalocyanines* **2000**, *4*, 544–550.
95. (a) Moan, J.; Kessel, D. *J. Photochem. Photobiol. B. Biol.* **1988**, *1*, 429–436. (b) Moan, J.; Rimington, C.; Malik, Z. *Photochem. Photobiol.* **1988**, *47*, 363–367. (c) Moan, J.; Berg, K. *Photochem. Photobiol.* **1991**, *53*, 549–553.
96. Brun, A.; Sandberg, S. *J. Photochem. Photobiol. B. Biol.* **1988**, *2*, 33–41.
97. Reyftman, J. P.; Kohen, E.; Moliere, P.; Santus, R.; Kohen, C.; Mangel, W. F.; Dubertret, L.; Hirschberg, J. G. *Photochem. Photobiol.* **1986**, *44*, 461–469.
98. (a) Rück, A.; Hildebrandt, C.; Köllner, T.; Schneckenburger, H.; Steiner, R. *J. Photochem. Photobiol. B. Biol.* **1990**, *5*, 311–319. (b) Schneckenburger, H.; Rück, A.; Bartos, B.; Steiner, R. *J. Photochem. Photobiol. B. Biol.* **1988**, *355*, 363.
99. Ma, L. W.; Moan, J.; Berg, K. *Lasers Med. Sci.* **1994**, *9*, 127–132.
100. (a) Moan, J.; Streckyte, G.; Bagdonas, S.; Bech, O.; Berg, K. *Int. J. Cancer* **1997**, *70*, 90–97. (b) Bagdonas, S.; Ma, L.-W.; Iani, V.; Rotomskis, R.; Juzenas, P.; Moan, J. *Photochem. Photobiol.* **2000**, *72*, 186–192. (c) Juzenas, P.; Iani, V.; Bagdonas, S.; Rotomskis, R.; Moan, J. *J. Photochem. Photobiol. B. Biol.* **2001**, *61*, 78–86.
101. Ahram, M.; Cheong, W.-F.; Ward, K.; Kessel, D. *J. Photochem. Photobiol. B. Biol.* **1994**, *26*, 203–204.
102. Strauss, W. S. L.; Sailer, R.; Gschwend, M. H.; Emmert, H.; Steiner, R.; Schneckenburger, H. *Photochem. Photobiol.* **1998**, *67*, 363–369.
103. Amit, I.; Malik, Z.; Kessel, D. *Photochem. Photobiol.* **1999**, *69*, 700–702.
104. Mang, T. S.; Wieman, T. J. *Photochem. Photobiol.* **1987**, *46*, 853–858.
105. Dickson, E. F. G.; Pottier, R. H. *J. Photochem. Photobiol. B. Biol.* **1995**, *29*, 91–93 and references therein.
106. Dietel, W.; Fritsch, C.; Pottier, R. H.; Wendenburg, R. *Lasers Med. Sci.* **1997**, *12*, 226–236.
107. Sorensen, R.; Iani, V.; Moan, J. *Photochem. Photobiol.* **1998**, *68*, 835–840.
108. Robinson, D. J.; de Bruijn, H. S.; van der Veen, N.; Stringer, M. R.; Brown, S. B.; Star, W. M. *Photochem. Photobiol.* **1998**, *67*, 140–149.
109. (a) Ma, L. W.; Moan, J.; Grahn, M. F.; Iani, V. *Proc. SPIE* **1996**, *2924*, 219–224. (b) Forrer, M.; Glanzmann, T.; Braichotte, D.; Wagnieres, G.; van den Bergh, H.; Savary, J. F.; Monnier, P. *Proc. SPIE* **1995**, *2627*, 33–39.
110. Katsumi, T. A.; Aizawa, K.; Kuroiwa, Y.; Saito, K.; Kurata, Y.; Li, Y.; Okunata, T.; Konaka, C.; Kato, H. *Photochem. Photobiol.* **1996**, *64*, 671–675.
111. (a) Rück, A.; Beck, G.; Bachor, R.; Akgün, N.; Gschwend, M. H.; Steiner, R. *J. Photochem. Photobiol. B. Biol.* **1996**, *36*, 127–133. (b) Pope, A. J.; MacRobert, A. J.; Phillips, D.; Bown, S. G. *Br. J. Cancer* **1991**, *64*, 875–881.
112. (a) Moan, J.; Iani, V.; Ma, L. W.; Peng, Q. *Proc. SPIE* **1996**, *2625*, 187–193. (b) Iinuma, S.; Wagnieres, G.; Schomacker, K. T.; Bamberg, M.; Hasan, T. *Proc. SPIE* **1995**, *2391*, 225–231.
113. Svaasand, L. O.; Potter, W. R. In *Photodynamic Therapy: Basic Principles and Applications*; Henderson, B. W., Dougherty, T. J., Eds.; Marcel Dekker: New York, 1992; pp. 369–385.
114. Patterson, M. S.; Wilson, B. C.; Graff, R. *Photochem. Photobiol.* **1990**, *51*, 343–349. Iinuma, S.; Farshi, S. S.; Oetel, B.; Hasan, T. *Br. J. Cancer* **1994**, *70*, 21–28.
115. Jacques, S. L.; Joseph, R.; Gofstein, G. *Proc. SPIE* **1993**, *1881*, 168–179. Awazu, K.; Jacques, S. L. *Proc. SPIE* **1995**, *2392*, 77–84.
116. Robinson, D. *Photodynamic News* **2000**, *3*, 4–7.
117. Rotomskis, R.; Bagdonas, S.; Juzenas, P.; Frolov, D.; Streckyte, G. *Proc. SPIE* **1999**, *3863*, 489–495.

Biographical sketch



Professor Raymond Bonnett, Scotia Research Professor at Queen Mary College, University of London, 1994, received his BSc degree (1954) in Linstead's department at Imperial College, and then proceeded to Cambridge for his PhD (1957) studies on B_{12} with Lord Todd and Alan Johnson. Post-doctoral work was carried out with R. B. Woodward on the synthesis of chlorophyll (Harvard, 1959–1960). During his subsequent appointments (Assistant Professor, Chemistry, UBC Vancouver, 1959–1961; Lecturer, Reader, Professor of Organic Chemistry at Queen Mary College, University of London, 1961–1994) he developed his research interests on the chemistry and biochemistry of porphyrins and related systems. His current interests include chemical aspects of photodynamic therapy, on which he published a textbook in 2000.

Dr Gabriel Martínez (Barcelona, 1972) received his first degree from the University of Barcelona where he worked in the laboratory of Dr Felix Urpi. He earned his PhD degree working under the supervision of Professor Raymond Bonnett at Queen Mary College, University of London in 1999. While at Queen Mary, he was awarded a Laura de Saliceto Cancer Research Studentship to work on the photobleaching of PDT photosensitisers. He then moved to Göttingen, where he worked with Professor A. de Meijere for a year. He is presently carrying out post-doctoral research in natural product synthesis in the laboratory of Professor T. Ross Kelly at Boston College, USA.