

Photoacoustic Measurements of Porphyrin Triplet-State Quantum Yields and Singlet-Oxygen Efficiencies

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Abstract: Photoacoustic calorimetry was used to measure the quantum yields of singlet molecular oxygen production by the triplet states of tetraphenylporphyrin (TPP), Zn–TPP and Cu–TPP in toluene, yielding values of 0.67 ± 0.14 , 0.68 ± 0.19 and 0.03 ± 0.01 , respectively. We show that a novel dichlorophenyl derivative of Zn–TPP is capable of singlet-oxygen production with a 0.90 ± 0.07 quantum yield. The synthesis and characterisation of a new photostable chlorin with high absorptivity in the red that is capable of singlet-oxygen production with 0.54 ± 0.06 quantum yield is described. Our results suggest that chlorinated chlorins may be interesting new sensitizers for photodynamic therapy.

Keywords: metalloporphyrins • photoacoustic calorimetry • porphyrinoids • singlet oxygen • sensitizers

Introduction

The ubiquitous tetrapyrrolic macrocycles play highly diverse roles in biological systems.^[1–5] The natural roles of these structures stimulated the search for new applications, exploiting in particular the use of new synthetic porphyrins.^[6] One of the more recent and promising applications of porphyrin chemistry in medicine is in the detection and cure of tumours,^[7,8] referred to as photodynamic therapy (PDT). The first reports of clinical trials of haematoporphyrin derivatives (HPD) in PDT were followed by systematic research for improved sensitizers over the last 20 years.^[9]

A good photosensitizer must be able to selectively photo-damage the tumour tissue, while being irradiated with visible or, preferably, near-infrared light. Two mechanisms are possible. In one mechanism the excited photosensitizer reacts directly with substrate molecules in the tissue by electron- or hydrogen-transfer reactions (Type I process). In the other, it transfers energy to the ground state of molecular oxygen, generating singlet oxygen ($^1\Delta_g$), which is the tissue-damaging species (Type II process). Evidence favours the role of the

Type II photooxygenation process in cells.^[7,8] Adequate sensitizers have specific biological and photochemical properties. The desired biological features of the sensitizer are:

- 1) little or no dark toxicity
- 2) selective accumulation and prolonged retention in tumour tissues
- 3) controlled photofading to reduce the unwanted skin photosensitivity side effects and increase light penetration during therapy.

The chemical and photochemical requisites are:

- 1) stability, purity and long shelf-life
- 2) high absorption coefficient in the phototherapeutic window (600–1000 nm)
- 3) high quantum yield for singlet molecular O_2 ($^1\Delta_g$) sensitisation.

The most important precursor of singlet oxygen is the triplet state of the sensitizer, and a high singlet-oxygen quantum yield requires at least three sensitizer triplet-state properties: a near-unity quantum yield ($\Phi_T \approx 1$), an electronic energy at least 20 kJ mol^{-1} above that of singlet oxygen ($E_\Delta = 94 \text{ kJ mol}^{-1}$), and a long lifetime ($\tau_T > 5 \mu\text{s}$).

The quantum yield of the triplet state of porphyrins and related macrocycles is a critical quantity in determining their efficiency in PDT. However, uncertainties persist concerning the triplet quantum yields of basic members of this family of compounds. For example, studies on the triplet quantum yield of 5,10,15,20-tetraphenylporphyrin (TPP) reported values ranging from as low as $\Phi_T = 0.67 \pm 0.07$ ^[10] to as high as $\Phi_T = 0.88 \pm 0.03$.^[11] The triplet quantum yield of zinc 5,10,15,20-tetraphenylporphyrinate (Zn–TPP) is also subject to some scatter; values of $\Phi_T = 0.86$ ^[12] and $\Phi_T = 0.97$ ^[13] can be found in

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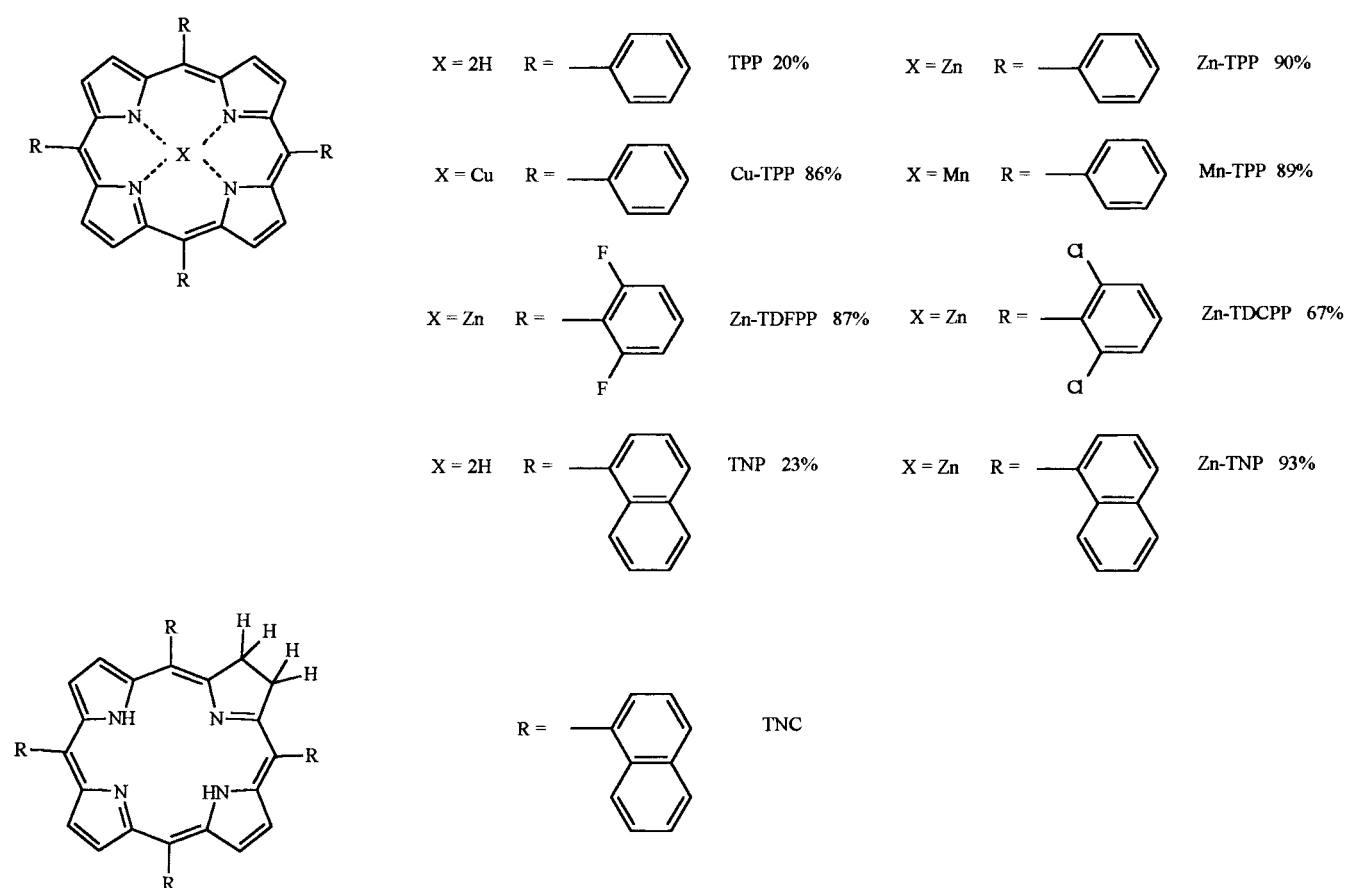


Figure 1. Structural diagram of porphyrin and chlorin macrocycles. Abbreviations: TPP, 5,10,15,20-tetraphenylporphyrin; Zn-TPP, Zn^{II} 5,10,15,20-tetraphenylporphyrinate; Cu-TPP, Cu^{II} 5,10,15,20-tetraphenylporphyrinate; Mn-TPP, Mn^{III} 5,10,15,20-tetraphenylporphyrinate; Zn-TDFPP, Zn^{II} 5,10,15,20-tetrakis(2,6-difluorophenyl)porphyrinate; Zn-TDCPP, Zn^{II} 5,10,15,20-tetrakis(2,6-dichlorophenyl)porphyrinate; TNP, 5,10,15,20-tetranaphthylporphyrin; Zn-TNP, Zn^{II} 5,10,15,20-tetranaphthylporphyrinate; TNC, 5,10,15,20-tetranaphthylchlorin.

the literature. The various methods available for measuring the quantum yield of singlet-oxygen production have been recently reviewed,^[14] and the value of 0.62 was selected as the standard value for Φ_{Δ} of TPP in aerated CCl₄. Other Φ_{Δ} values relevant to this work are 0.68–0.93 for Zn-TPP in aerated benzene or toluene and <0.01 for Cu-TPP in aerated CCl₄. The use of photothermal methods to measure Φ_{Δ} , pioneered by Braslavsky and co-workers,^[15, 16] is particularly relevant to this work.

This work reports the use of photoacoustic calorimetry (PAC),^[17, 18] to measure energy-transfer rates and singlet-oxygen sensitisation quantum yields for a selected range of porphyrins and a chlorin (Figure 1). Some of these species are new and include basic structures that can be derivatized with more polar groups, notably hydroxyl and sulfonamide,^[19] which modulate the solubility of the photosensitisers and their selective accumulation in specific tissues.^[20]

Abstract in Portuguese: *A calorimetria fotoacústica foi utilizada para determinar os rendimentos quânticos de formação de oxigênio singuleto molecular pelos estados tripleto de TPP, Zn-TPP e Cu-TPP em tolueno, onde TPP representa a tetrafenilporfirina na sua forma protonada ou desprotonada, tendo-se obtido 0.67 ± 0.14 , 0.68 ± 0.19 e 0.031 ± 0.01 , respectivamente. Um novo derivado da Zn-TPP, com átomos de cloro nas posições orto do grupo fenilo, produz oxigênio singuleto com um rendimento quântico de 0.90 ± 0.07 . É descrita a síntese e feita a caracterização de uma nova clorina foto-estável, capaz de produzir oxigênio singuleto com um rendimento quântico de 0.54 ± 0.06 . Estes resultados sugerem que clorinas cloradas podem ser sensibilizadores apropriados para a terapia fotodinâmica.*

Results

Porphyrin synthesis: 5,10,15,20-Tetrakisarylporphyrins have a simple basic structure, but their characteristics can be modified by peripheral structural changes. The methodology first described by Rothmund in 1935^[21] and modified by Adler in 1967^[22] was recently improved by Rocha Gonsalves et al.^[23] This methodology is now useful for the preparation of large amounts of a wide range of pure porphyrins, which were previously very difficult or impossible to prepare. A source of potentially useful new porphyrins for PDT applications became available.

In our approach, the aldehyde and pyrrole are made to react in acetic acid or propionic acid in the presence of nitrobenzene at 120 °C to give, very often by direct crystal-

lisation, the corresponding porphyrin free of any chlorin contamination. Yields of porphyrins are presented in Figure 1, and their full characterisation is described in the Experimental Section. The effect of nitrobenzene as oxidant and aromatizing agent was described in the synthesis of other aromatic compounds^[24, 25] and also in the oxidation of porphyrinogen to porphyrins or chlorins.^[26]

The Zn and Cu complexes were easily prepared by refluxing the porphyrins in the presence of the desired acetate salt with dimethylformamide as solvent.^[27] Yields are presented in the Experimental Section. Our interest in obtaining compounds with high absorption coefficients prompted us to synthesise 5,10,15,20-tetranaphthylchlorin (TNC) by refluxing the corresponding porphyrin in γ -picoline in the presence of *p*-toluenesulfonylhydrazine and sodium carbonate for about 6 hours.^[28] After preparative TLC purification, the new 5,10,15,20-tetranaphthylchlorin was isolated; its full characterisation is presented in the Experimental Section.

Porphyrin photophysics and photochemistry:

The relevant data from the absorption and luminescence spectra of the porphyrins and chlorin are summarised in Table 1. The concentrations used in our spectroscopic and PAC studies were in the 10^{-7} – 10^{-5} M range. The Beer–Lambert Law was always obeyed in this concentration range, and we found no evidence for aggregation. The present results are

in good agreement with the available literature data for TPP and the corresponding porphyrinates.^[29–31] The spectroscopic singlet-state energies (E_S) were obtained from the intersection of the normalised absorption and fluorescence spectra. In Figure 2 we show the absorption, fluorescence excitation and fluorescence emission spectra of TNC. The Stokes shifts of the free bases are very small, and the spectroscopic energies are nearly identical to the relaxed energies of the singlet states. The same is probably true for the triplet states^[32], and the

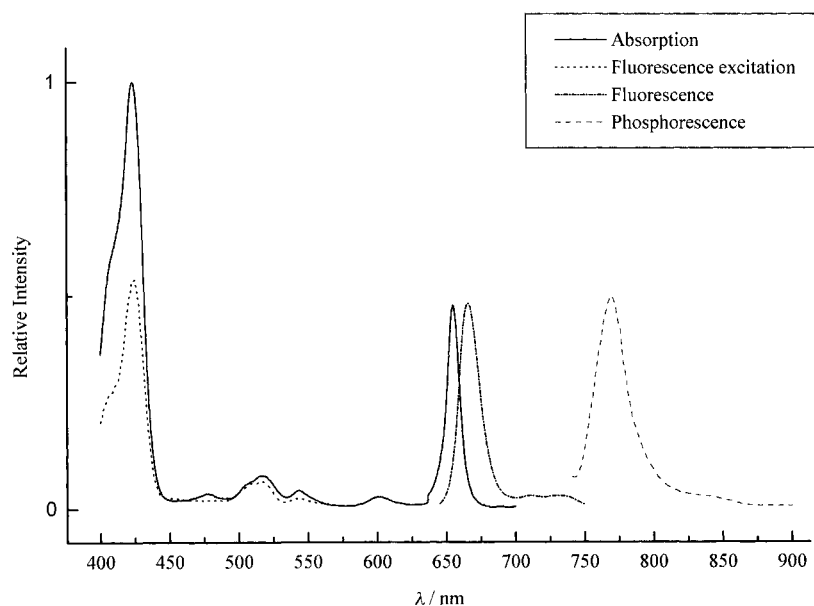


Figure 2. Absorption, fluorescence excitation and emission (normalised) spectra of TNC in toluene at room temperature. The phosphorescence spectrum of TNC was obtained in deaerated toluene at 77 K.

Table 1a. Absorption and luminescence data of the free bases in deaerated toluene solutions.

	Absorption λ_{\max} [nm] (ϵ [$M^{-1} \text{cm}^{-1}$])					Fluorescence λ_{\max} [nm] (RT)		Phosphorescence λ_{\max} [nm] (77 K)		E_S [kJ mol^{-1}]	Φ_F	E_T [kJ mol^{-1}]
	$Q_x(0-0)$	$Q_x(1-0)$	$Q_y(0-0)$	$Q_y(1-0)$	$B(0-0)$	$Q(0-0)$	$Q(0-1)$	$T(0-0)$	$T(0-1)$			
TPP	649.8 (9.6×10^3)	592.0 (1.0×10^4)	548.0 (1.16×10^4)	514.6 (1.8×10^4)	418.8 (2.67×10^5)	652	719			183.9 ± 0.3	0.10 ± 0.01	$138.0^{[a]}$
TNP	654.0 (5.16×10^3)	589.5 (8.61×10^3)	548.5 (7.67×10^3)	514.0 (2.4×10^4)	423.0 (3.81×10^5)	654	715	766	778	183.8 ± 0.3	0.16 ± 0.02	153.9 ± 1.9
TNC	652.0 (3.7×10^4)	601.8 (1.9×10^3)	542.8 (4.5×10^3)	517.0 (1.0×10^4)	423.4 (1.57×10^5)	657	721	775		182.9 ± 0.7	0.36 ± 0.02	154.5 ± 0.7

[a] Ref [27].

Table 1b. Absorption and luminescence data of the free bases in deaerated toluene solutions.

	Absorption λ_{\max} [nm] (ϵ [$M^{-1} \text{cm}^{-1}$])			Fluorescence λ_{\max} [nm] (RT)			Phosphorescence λ_{\max} [nm] (77 K)		E_S [kJ mol^{-1}]	Φ_F	E_T [kJ mol^{-1}]
	$Q(0-0)$	$Q(1-0)$	$B(0-0)$	$Q(0-0)$	$Q(0-1)$	$Q(0-2)$	$T(0-0)$	$T(0-1)$			
Zn-TPP	588.4 (2.8×10^3)	549.6 (1.21×10^4)	423.4 (4.52×10^5)	600	648	715	757	–	201.5 ± 2.0	0.033 ± 0.005	160.0 ± 1.0
Zn-TNP	583 (8.28×10^2)	549.2 (1.56×10^4)	426.2 (2.73×10^5)	595	644		770	–	203.5 ± 2.1	0.077 ± 0.005	157.0 ± 1.0
Zn-TDFPP		546.0 (1.45×10^4)	420.8 (3.15×10^5)	588	644	720	737	–	211.5 ± 7.8	$< 10^{-4}$	160.3 ± 1.0
Zn-TDCPP	586.6 (3.6×10^3)	550.4 (1.22×10^4)	423.8 (2.42×10^5)	595	652	725	754	–	202.7 ± 1.4	$< 10^{-4}$	163.9 ± 1.9
Cu-TPP		539.8 (1.65×10^4)	418.4 (2.4×10^5)	[a]	[a]		738	–	$205.3^{[b]}$	[a]	163.7 ± 1.2

[a] We observe a residual fluorescence attributable to TPP, suggesting that our sample of Cu-TPP is only 98 % pure. [b] Ref. [29].

maximum of the highest energy phosphorescence band was used to obtain the triplet energies (E_T) presented in Table 1. The Stokes shifts of the zinc porphyrins correspond to 3–4 kJ mol⁻¹. Thus, we add 1.5 kJ mol⁻¹ to the energy corresponding to the maximum of the first vibrational band in the phosphorescence spectrum of Zn-porphyrins to obtain the E_T energies presented in Table 1. The same procedure was followed to obtain the triplet energy of Cu-TPP from the phosphorescence maximum obtained in this work ($\lambda_{\text{max}} = 738$ nm), which is in good agreement with that reported by Harriman.^[31]

Time-resolved PAC is based on the measurement of the acoustic wave generated by the heat released in the non-radiative processes following electronic excitation. The experimental wave (E-wave) of the sample studied is compared with that of the pressure transducer (T-wave). The T-wave is obtained with a calorimetric reference absorbing the same fraction of light as the sample and releasing it as thermal energy in a time much shorter than the transducer oscillation frequency. The phase and amplitude differences between the T- and E-wave allow for the simultaneous determination of the thermal energy released by the transients and their lifetimes. Typical background-corrected reference and sample signals are shown in Figure 3.

Co-TPP and Zn-TPP have been used as PAC references.^[32] Whereas the first of these compounds is radiationless, the use of the second one in PAC requires a correction for its fluorescence. The use of 5,10,15,20-tetrakis(*p*-sulfonylphenyl)porphyrin as calorimetric reference in aqueous solutions^[33] has been shown to be inadvisable.^[34] The same is probably true for any compounds yielding long-lived tran-

sients. For this reason, we selected Mn-TPP as the calorimetric reference for our PAC studies in the visible and tested it against reliable calorimetric references in the UV and visible. It is known that Mn-TPP is very weakly luminescent,^[35, 36] is soluble in a wide range of solvents and absorbs strongly in the 350–550 nm region. The absorption spectrum of Mn-TPP did not change as a result of prolonged irradiation with the N₂ laser. We compared the acoustic waves of Mn-TPP and 2-hydroxybenzophenone (HBP), an established PAC reference for UV irradiation,^[17] using the N₂ laser. The linearity of the photoacoustic response of Mn-TPP with the fraction of laser energy absorbed is not distinguishable from that of HBP in toluene solutions. We also tested the photoacoustic response of Mn-TPP with irradiation at 337 nm in ethanol/water (1:1 by volume) against that of K₂CrO₄. When the fraction of energy absorbed is less than 50% of the N₂-laser energy, the photoacoustic responses of Mn-TPP and K₂CrO₄ solutions are linear (correlation coefficient better than 0.990) and have indistinguishable slopes. Mn-TPP and K₂CrO₄ give slightly larger waves than HBP in this solvent mixture. It is known that the ground-state repopulation of HPB in non-hydrogen-bonding solvents is 35 ± 5 ps, but in ethanol a fraction of the molecules populate the triplet state, which has a 1.5 ns lifetime.^[37] This advises against the use of HPB as a PAC reference in hydrogen-bonding solvents. Finally, we tested Mn-TPP against *trans*- β -carotene (Aldrich) in toluene at 421 nm, because *trans*- β -carotene has a singlet-state lifetime of 8.4 ± 0.6 ps^[38] and a fluorescence quantum yield of 6 × 10⁻⁵.^[39] The photoacoustic responses of Mn-TPP and *trans*- β -carotene are linear with the fraction of laser energy absorbed and have identical slopes.

We interpret the waves of N₂-saturated samples with two sequential exponentials, the first one for the formation of the triplet state of the sensitizer and the second one for its decay (Figure 4). The formation of the triplet state is faster than the time resolution of our experiments, and we arbitrarily set the lifetime of the first exponential decay to $\tau_1 = 1$ ns; smaller values of τ_1 do not change the other parameters in the deconvolution. This is not strictly true for Cu-TPP, because τ_2 is small. For this system we set τ_1 to 0.1 ns. We interpreted the waves of air-saturated samples with three sequential exponentials, the second one representing the two decay channels now available for the triplet (energy transfer to oxygen or nonradiative decay to the ground state), and the last one associated with the decay of singlet oxygen. Each decay step is described by two parameters: the lifetime of the transient and the fraction of thermal energy released in that lifetime (Figure 4). The convolution of the reference wave with parameters of the kinetic model for the decay of transient species gives a calculated E-wave. The appropriateness of the kinetic model and its parameters to describe the observed E-wave can be evaluated by the difference between the amplitudes of observed

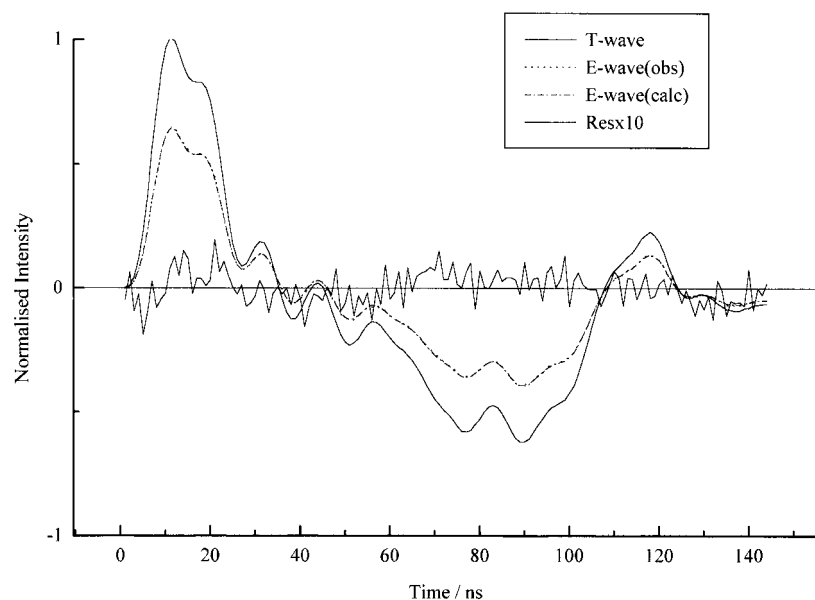


Figure 3. Typical sample photoacoustic wave, E-wave (obs) and reference wave (T-wave), obtained in a PAC experiment. The E- and T-waves depicted were corrected for the background signal and normalised. The normalisation factor is the reciprocal of the largest absolute value of the T-wave. The sample (TNP), reference (Mn-TPP) and solvent (toluene) data were obtained under the following experimental conditions: irradiation at 517 nm of N₂-saturated solutions with a filter with 93% transmittance; absorbance of 1.50 at 517 nm; solution flow of 1 mL min⁻¹. The calculated wave, E-wave (calcd), was obtained with two sequential exponential decays with lifetimes $\tau_1 = 1$ ns and $\tau_2 = 5.5$ μ s and fractions of heat released $\phi_1 = 0.2995$ and $\phi_2 = 0.3052$. Res = E-wave(calcd) – E-wave(obs).

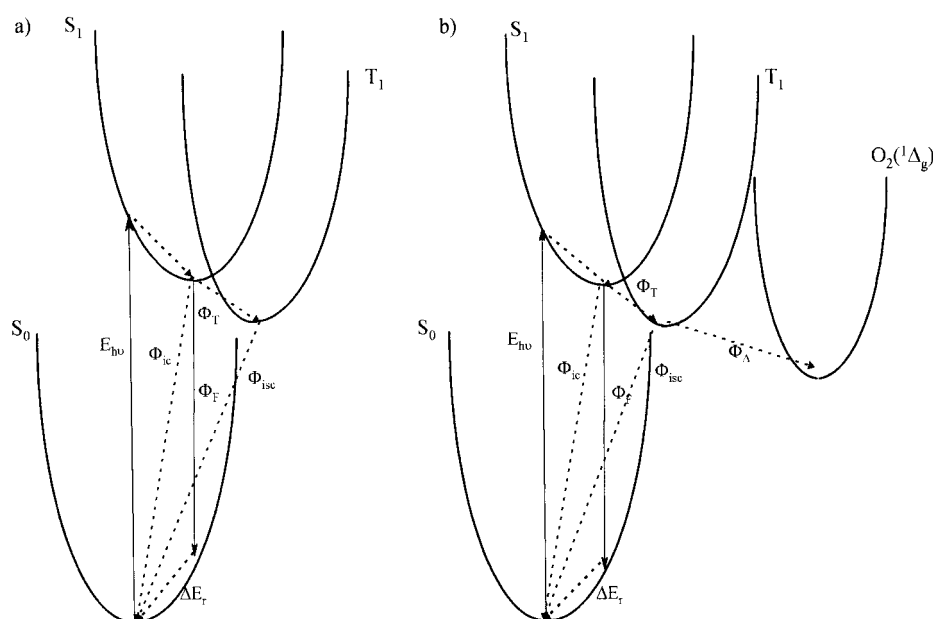


Figure 4. Photoinduced processes: a) in the absence and b) in the presence of molecular oxygen. Full lines: radiative processes; dashed lines: radiationless processes. ΔE_r represents the relaxation energy of the ground-state species formed radiatively from the lowest excited singlet state. In our PAC measurements the radiationless processes in a) are described by two sequential exponentials: formation of the triplet state and internal conversion to the ground state, followed by decay of the triplet. In b) there are three sequential exponentials: formation of the triplet state and internal conversion to the ground state, followed by decay of the triplet by intersystem crossing or energy transfer to oxygen and, finally, the decay of singlet oxygen.

and calculated E-waves at each decay time. As shown in Figure 3, that difference is typically 1/100 of the amplitude of the sample wave. The decay parameters were obtained by deconvolution of the background-corrected and normalised E- and T-waves with the algorithm described by Melton and co-workers.^[40]

The fractions of laser energy released by each system were measured at four different laser intensities. In some systems, the first fraction of energy released varied with the laser intensity. We used the Student's *t* test, at the 95% confidence level, to decide whether the values obtained at the different laser energies were significantly different. When the differences were significant, we plotted the first fraction of energy released as a function of the laser energy and obtained linear correlation coefficients greater than 0.96. The difference was assigned to transient–transient absorption and was corrected by extrapolating the fraction of energy released to zero laser intensity.

Discussion

The absorption and emission spectra of porphyrins and chlorin exhibit the typical features of this class of compounds. It is worth mentioning the large molar absorption coefficient of the $Q_x(0,0)$ band of TNC ($\epsilon_{652} = 37000 \text{ M}^{-1} \text{ cm}^{-1}$).

The series of halogenated Zn^{II} complexes show fluorescence quenching due to the heavy-atom effect in the intersystem-crossing rate. This effect was also described by Quimby and Longo^[30] for halogen substituents positioned on phenyl rings of the free base and Zn^{II} 5,10,15,20-tetrakis-(2-chlorophenyl)porphyrinates in benzene. However, these

authors reported that the fluorescence yield of Zn^{II} 5,10,15,20-tetrakis(2-chlorophenyl)porphyrinate was larger than that of Zn -TPP. This unexplained result has no parallel in Zn -TDCPP. Actually we find that introducing two halogens in the *ortho* positions of the phenyl ring is as effective in quenching fluorescence as halogenation in the *meso* positions of the porphyrin.^[41]

Energy conservation in N_2 -saturated samples requires that the energy of the laser light absorbed (E_{hv}) be given by Equation (1), where E_{F} is the integrated radiative

$$E_{\text{hv}}(\text{N}_2) = E_{\text{F}} + E_{\text{hv}}\phi_1 + E_{\text{hv}}\phi_2 \quad (1)$$

energy of the singlet state, ϕ_1 and ϕ_2 are the fractions of laser energy released as thermal energy in the lifetimes τ_1 and τ_2 , respectively. The spectroscopic energy of the singlet (E_{S}) is an upper limit of E_{F} . This is given by Equation (2),

where I_{F} and I_0 are the intensities of emitted and absorbed light, and accounts for the energy emitted by the singlet at each frequency. For a Gaussian emission band, Equation (2) can be approximated by Equation (3), where $E_{\nu_{\text{max}}}$ is the energy at the maximum fluorescence intensity.

$$E_{\text{F}} = \int E_{\text{F}}(\nu) I_{\text{F}}(\nu) / I_0 \, d\nu \quad (2)$$

$$E_{\text{F}} = E_{\nu_{\text{max}}} \Phi_{\text{F}} \quad (3)$$

When two or more emission bands are observed, they can be broken into a series of Gaussians, each centred around a maximum. The thermal energy released in a time shorter than the resolution of the 2.25 MHz transducer ($\tau_1 < 10 \text{ ns}$) is due to the formation of the relaxed singlet followed by the formation of the triplet and by the internal conversion to the ground state (internal conversion quantum yield Φ_{IC}). The ground-state species formed by fluorescence also relax in this time window and contribute ($\Delta E_{\text{r}} \Phi_{\text{F}}$) to the thermal energy dissipated in lifetime τ_1 [Eq. (4)].

$$E_{\text{hv}}\phi_1 = (E_{\text{hv}} - E_{\text{S}}) + (E_{\text{S}} - E_{\text{T}})\Phi_{\text{T}} + E_{\text{S}}\Phi_{\text{IC}} + \Delta E_{\text{r}}\Phi_{\text{F}} \quad (4)$$

The thermal energy released in the longer decay is associated with the triplet-state energy [Eq. (5)], but triplets with lifetimes longer than $\tau_2 > 10 \mu\text{s}$ are difficult to follow with

$$E_{\text{hv}}\phi_2 = E_{\text{T}}\Phi_{\text{T}} \quad (5)$$

the 2.25 MHz transducer. In such cases, Φ_{T} can be obtained from Equation (4) from the value of ϕ_1 determined by PAC, E_{T} from phosphorescence measurements (Table 1) and Equa-

tion (6), where Φ_F is given by fluorescence measurements (Table 1). From Equations (4) and (6) we obtain Equation (7), because $E_S = E_{v_{\max}} + \Delta E_r$. The values of Φ_T determined by this procedure are presented in Table 2.

$$\Phi_{IC} = 1 - \Phi_T - \Phi_F \quad (6)$$

$$\Phi_T = [(1 - \phi_1)E_{hv} - \Phi_F E_{v_{\max}}]E_T \quad (7)$$

Energy conservation in air-saturated samples yields Equation (8), where $E_{hv}\phi_1$, $E_{hv}\phi_2$ and $E_{hv}\phi_3$ are given by Equations (9), (10) and (11), respectively, and Φ_{isc} represents the intersystem-crossing quantum yield from the triplet to the ground state in the presence of oxygen ($\Phi_T f_{\Delta}^T + \Phi_{isc} = \Phi_T$).

$$E_{hv}(\text{air}) = E_{v_{\max}}\Phi_F + E_{hv}\phi_1 + E_{hv}\phi_2 + E_{hv}\phi_3 \quad (8)$$

$$E_{hv}\phi_1 = (E_{hv} - E_S) + (E_S - E_T)\Phi_T + E_S\Phi_{IC} + \Delta E_r\Phi_F \quad (9)$$

$$E_{hv}\phi_2 = (E_T - E_{\Delta})\Phi_T f_{\Delta}^T + E_T\Phi_{isc} \quad (10)$$

$$E_{hv}\phi_3 = E_{\Delta}\Phi_T f_{\Delta}^T \quad (11)$$

The decay of singlet oxygen in toluene ($\tau_{\Delta} = 17.4 \mu\text{s}$ ^[42]) is difficult to follow with the 2.25 MHz transducer. However, we can obtain the fraction of triplet states quenched by oxygen, which gives singlet oxygen (f_{Δ}^T) either by rearranging Equation (10) to Equation (12) or by adding Equations (9) and (10) together to give Equation (13). The values of f_{Δ}^T and Φ_{Δ} ($=\Phi_T f_{\Delta}^T$) presented in Table 2 were obtained with Equation (12) because they are not sensitive to cumulative errors in the measurement of ϕ_1 and ϕ_2 .

$$\Phi_T f_{\Delta}^T = \frac{(\Phi_T E_T - E_{hv}\phi_2)}{E_{\Delta}} \quad (12)$$

$$\Phi_T f_{\Delta}^T = \frac{(1 - \phi_1 - \phi_2)E_{hv} + (1 - \Phi_T)E_T - E_S\Phi_F}{E_{\Delta}} \quad (13)$$

It has been argued that structural volume changes accompany thermal expansion in the triplet state formation of some porphyrins, and that both contribute to PAC signals.^[34, 43] The relevance of such structural volume changes to our studies can be assessed by use of Zn-TDCPP, because its triplet decays in the time window of our experiments, and because the energy

lost by room-temperature luminescence can be neglected. Equation (7) with the experimental value of $\phi_1 = 0.4121 \pm 0.058$, $\Phi_T \approx 1$ and $\Phi_F \approx 0$, gives $E_T = 167.2 \pm 0.5 \text{ kJ mol}^{-1}$ in good agreement with the energy obtained from phosphorescence ($E_T = 163.9 \pm 1.9 \text{ kJ mol}^{-1}$). On the other hand, Equation (5) with the experimental value of $\phi_2 = 0.487 \pm 0.197$, gives $E_T = 139 \pm 56 \text{ kJ mol}^{-1}$. In this system we obtain $\phi_1 + \phi_2 = 0.90 \pm 0.25$ in the absence of oxygen. The large uncertainty in the value of ϕ_2 reflects the difficulty of measuring microsecond lifetimes with the 2.25 MHz transducer.

According to the mechanism of triplet-state quenching discussed in detail by Wilkinson [Eq. (14)–(16)],^[44] the rate constant of the quenching by oxygen ($k_q = k_{T\Delta} + k_{T\Sigma}$) can be obtained from the lifetimes of the triplet in N_2 and air-saturated solutions from Equation (17), taking $[O_2] = 1.81 \times 10^{-3} \text{ M}$ in toluene at room temperature.^[42]



$$k_q[O_2] = \frac{1}{\tau_2(N_2)} - \frac{1}{\tau_2(\text{air})} \quad (17)$$

When $\tau_2(N_2) > 10 \mu\text{s}$ we simply take $k_q[O_2] = 1/\tau_2(\text{air})$. Some properties of the triplet states of the porphyrins and chlorin are presented in Table 2. The present results are in good agreement with the literature data for TPP, Zn-TPP and Cu-TPP. The fractions of O_2 (${}^1\Delta_g$) formed from the triplet state of the sensitizers are all close to unity ($f_{\Delta}^T \approx 1$), except for Cu-TPP, which will be discussed below. This indicates that $k_{T\Delta} \geq 10 k_{T\Sigma}$.

Following the pioneering work of Porter,^[45] it is believed that when $k_q \approx {}^1/9 k_{diff}$ the quenching of triplet states by molecular oxygen follows an energy-transfer mechanism. In toluene at room temperature ${}^1/9 k_{diff} = 3.4 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$.^[42] The rates measured in our study are slightly below ${}^1/9 k_{diff}$ and, with the exception of Cu-TPP, tend to decrease as E_T increases (Figure 5). A similar trend for energy gaps ($\Delta E = E_T - E_{\Delta}$) between 40 and 200 kJ mol^{-1} was also apparent in the work of Porter and was investigated in detail by Schmidt and co-workers.^[46] The energy-gap dependence of long-distance

Table 2. Quantum yields and triplet lifetimes in toluene measured by time-resolved photoacoustic calorimetry.^[a]

	Φ_T	Φ_{Δ} ^[b]	Φ_{Δ} ^[c]	f_{Δ}^T	$\tau_T(N_2)$ [ns]	$\tau_T(O_2)$ [ns]	$k_q \times 10^{-9}$ [M ⁻¹ s ⁻¹]
TPP	0.73 ± 0.10	0.67 ± 0.14	0.55–0.72	0.92 ± 0.22	> 10000	196 ± 6	2.82 ± 0.02
TNP	0.86 ± 0.13	0.97 ± 0.10		1.13 ± 0.20	> 10000	264 ± 7	2.09 ± 0.01
TNC	0.55 ± 0.10	0.54 ± 0.06		0.98 ± 0.17	1390 ± 99	545 ± 130	0.62 ± 0.12
Zn-TPP	0.86 ± 0.17	0.68 ± 0.19	0.68–0.92	0.79 ± 0.26	> 10000	309 ± 31	1.79 ± 0.07
Zn-TNP	0.90 ± 0.10	0.76 ± 0.11		0.84 ± 0.15	> 10000	424 ± 34	1.30 ± 0.08
Zn-TDFPP	0.99 ± 0.04	0.99 ± 0.05		1.00 ± 0.06	5921 ± 597	604 ± 5	0.82 ± 0.06
Zn-TDCPP	1.02 ± 0.01	0.90 ± 0.07		0.88 ± 0.07	4185 ± 890	738 ± 59	0.62 ± 0.02
Cu-TPP	0.88 ± 0.02	0.03 ± 0.01	< 0.01 ^[d]	0.04 ± 0.01	17.8 ± 1.5	15.9 ± 0.7	11.6 ± 0.09

[a] The uncertainties are calculated for a 95% confidence level. [b] Values of $\Phi_{\Delta}(\text{TNP}) = 0.66$, $\Phi_{\Delta}(\text{TNC}) = 0.68$ and $\Phi_{\Delta}(\text{Zn-TNP}) = 0.77$ relative to $\Phi_{\Delta}(\text{TPP}) = 0.66$ were measured, with the same samples, at the Free Radical Research Facility of the Paterson Institute for Cancer Research (Manchester, U.K.) by means of the method described by Bonnett et al in ref. [10]. [c] Range of values in benzene or toluene solutions reported in ref. [14]. [d] Limit in CCl_4 reported in ref. [14].

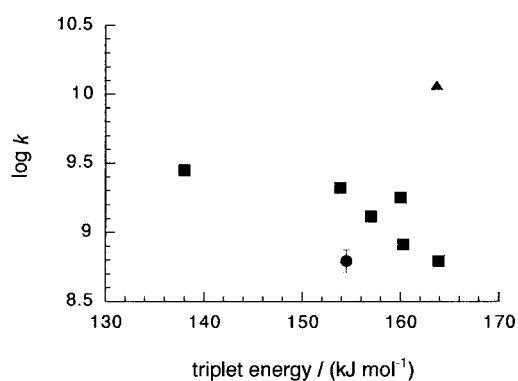


Figure 5. Energy-gap dependence of triplet-quenching rate constants by molecular oxygen ($E_{\Delta} = 94 \text{ kJ mol}^{-1}$). The circle represents the quenching of the triplet state of TNC, and suggests that chlorins and porphyrins have different Franck–Condon factors. The triangle represents the quenching of Cu–TPP, which follows a different mechanism.

triplet energy-transfer rates was interpreted as the simultaneous transfer of two electrons,^[47, 48] with the electron-exchange mechanism originally proposed by Dexter.^[49] A quantitative interpretation of long-distance triplet energy-transfer reaction rates was offered by the intersecting-state model (ISM).^[50] The rates of energy transfers taking place between reactants in close contact, such as those measured in this study, will depend on the details of the orbital interactions that allow electron exchange.^[51] Such electron exchanges may have some inner-sphere character, and the energy-transfer rates calculated with ISM assuming an outer-sphere mechanism tend to underestimate the experimental rates, especially for near-resonance transfers.

The luminescence of Cu–TPP originates from the triplet manifold of the porphyrin.^[52, 53] Adding the 1.5 kJ mol^{-1} correction to the energy obtained from the maximum of the emission band we obtain $E_{\text{T}} = 163.7 \text{ kJ mol}^{-1}$. Equation (7), with $\phi_1 = 0.4967 \pm 0.0133$ and $\Phi_{\text{F}} = 0$, gives $\Phi_{\text{T}} = 0.88 \pm 0.02$. This result may be unexpected in view of the fast intersystem-crossing rates. However, the transient absorption of Cu–TPP in toluene relaxes by 10–15% between 40 ps and 2.4 ns,^[54] and Φ_{T} should be in the range of 0.85–0.90. Our results suggest that most of the initially excited singdoublet (^2Q) relaxes very rapidly to the emissive tripdoublet (^2T) and tripquartet (^4T) states, while a smaller fraction returns to the ground state ($^2\text{S}_0$) probably via a charge-transfer (CT) state.

The relevance of a low-energy CT state in Cu–TPP is supported by the large triplet-quenching rate in the presence of molecular oxygen. Wilkinson proposed the involvement of CT complexes in oxygen quenching to account for rate constants larger than $1/9 k_{\text{diff}}$.^[55, 56] When triplet quenching via CT complexes is diffusion-limited, the quenching rate constant expected is $4/9 k_{\text{diff}}$ ($= 1.4 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ in toluene), and the limiting value of f_{Δ}^{T} is 0.25. We calculated $f_{\Delta}^{\text{T}} = 0.038$ using Equation (12), which presumes a phosphorescence quantum yield $\Phi_{\text{p}} \approx 0$ in room-temperature air-saturated toluene solutions of Cu–TPP. It seems that in the encounter complex between excited Cu–TPP and molecular oxygen the energy of the CT state is lowered and becomes a very effective dissipative channel.

Conclusions

This work describes a reliable methodology for the measurement of singlet-oxygen quantum yields in solution by means of time-resolved photoacoustic calorimetry and luminescence techniques. We show that the presence of two chlorine atoms in the *ortho* positions of the phenyl ring in TPP is sufficient to increase the intersystem crossing rate to yield the triplet state quantitatively, and yet this state lives long enough to sensitise singlet oxygen with unit quantum yield. The presence of low-energy charge-transfer states may accelerate the sensitisation of singlet oxygen, but it also opens other radiationless channels that waste a significant part of the energy absorbed.

The molecules synthesised in this study have the photochemical properties required for a good PDT sensitiser. However, none gather all the necessary biological and photochemical properties simultaneously. The synthesis of chlorins with hydroxyl and sulfonamide substituents, in addition to chlorine substituents, is in progress in our laboratory and is expected to more closely approach the desired properties of a PDT sensitiser.

Experimental Section

Instrumentation: ^1H NMR spectra were recorded on 300 Mhz Bruker-AMX spectrometer. Mass spectra were obtained on a VG autospec and elemental analysis on EA1108-CHNS-0 Fisons Instruments. Absorption and luminescence spectra were recorded with Shimadzu UV-2100 and SPEX Fluorog 111 spectrophotometers, respectively.

Luminescence measurements: Fluorescence quantum yields (Φ_{F}) were measured by the method described by Parker and Rees.^[57] Carefully diluted solutions of each porphyrin in toluene with absorbance 0.02 at the excitation wavelength (417 nm) were deaerated with N_2 saturated in toluene and kept in the dark before the measurements. Using 1,4-dihydroxyanthraquinone as reference,^[58] we obtained $\Phi_{\text{F}} = 0.10$ for TPP, in agreement with literature data.^[50] The other Φ_{F} values were obtained with $\Phi_{\text{F}} = 0.10$ for TPP. The phosphorescence studies were carried out in toluene at liquid nitrogen temperature, with excitation at the Soret band. Second-order harmonics were eliminated with appropriate filters. Fluorescence and phosphorescence excitation spectra were obtained for all the porphyrins and agreed well with the corresponding absorption spectra.

Photoacoustic measurements: The PAC apparatus follows the front-face irradiation design described by Arnaut et al. (Figure 6).^[18] The solutions were pumped through a 0.11 mm thick cell at a 1 mL min^{-1} flow rate with an SSI chromatography pump, and irradiated with an unfocused PTI dye laser (model PL2300), pumped by a N_2 laser working at a frequency of 2 Hz. TPP, Zn–TPP, Zn–TNP, Zn–TDFPP, Zn–TDCPP and Cu–TPP were irradiated at 421 nm, whereas TNP and TNC were irradiated at 517 nm. More than 99% of the light impinging on the front-face dielectric mirror is reflected back into the solution; the rest is transmitted, minimising the background signal. We measured the N_2 laser energy using Aberchrome (Aberchromics Ltd.) as actinometer and obtained 1.0 mJ/pulse in the PAC cell; the energy of the dye lasers is about 10% that of the N_2 laser. A small fraction of the laser beam was deflected to a photodiode in order to trigger the digital storage oscilloscope (Tektronix DSA 601, 1 Gs^{-1} , two channels). The acoustic waves generated by nonradiative processes following light absorption in the cell were detected with a 2.25 MHz Panametrics transducer (model A106S), pre-amplified with a Panametrics ultrasonic preamplifier (model 5676), captured by the transient recorder and transferred to a PC for data analysis. The decay of the triplet state of Cu–TPP was measured with a Panametrics 15 MHz transducer (model A113S) given the short lifetime expected: 29 ns in toluene.^[59] Prior to each PAC experiment, we matched to better than 1% the absorbances of sample and reference at the irradiation wavelength. We collected and averaged

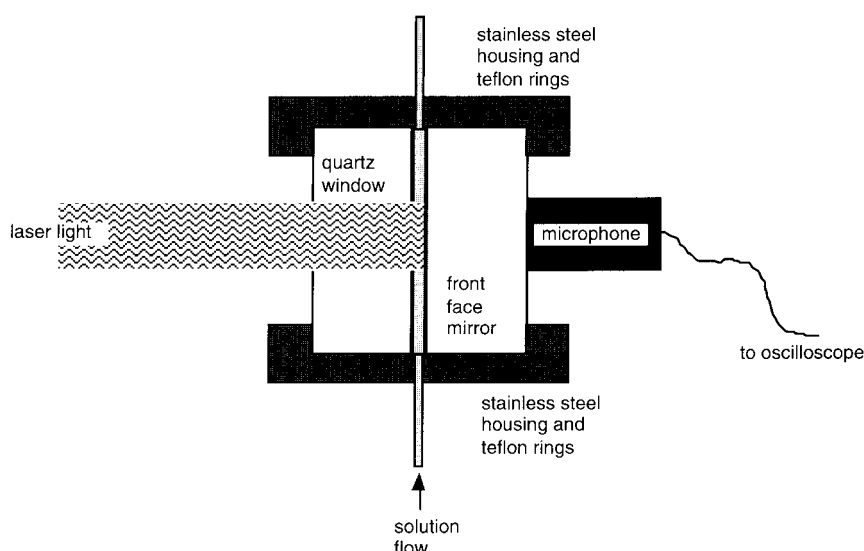


Figure 6. Front-face cell used in PAC. The dielectric mirrors are specific for each dye laser and reflect more than 99.5% of the incident light. The remaining light is transmitted. The laser beam is defocused to match the diameter of the transducer.

100 acoustic waves of the sample, reference and pure solvent in the same conditions. Four sets of averaged sample, reference and solvent waves were used for data analysis at a given laser intensity, and four laser intensities were employed in each experiment. These laser intensities were obtained by interposing filters with transmissions in the 30 to 100% range. The sample solutions used in the oxygen photosensitisation measurements were saturated with air; in the other measurements they were deaerated by purging with solvent-saturated N_2 . The pure solvent signal was scaled by the fraction of light absorbed by the sample in the PAC cell (typically 5%) and subtracted from the sample and reference signals. All the measurements were made in toluene solutions with Mn-TPP as photoacoustic reference.

Materials: All solvents and reagents (Aldrich) were purified by standard methods before use. The toluene employed in PAC studies was first washed with sulfuric and nitric acids and then with distilled water until neutralisation. Most of the water was removed by adding anhydrous $CaCl_2$; the toluene was then filtered and distilled over P_2O_5 . We added sodium and benzophenone to the distilled toluene, and refluxed the mixture until it turned blue, indicating that all traces of water were eliminated. The toluene was then re-distilled.

General porphyrin syntheses: Porphyrins were synthesised by a method developed by Rocha Gonsalves et al.^[23] The general procedure is as follows. In a typical reaction, arylaldehyde (0.4×10^{-3} mol) were dissolved in a mixture of acetic acid (140 mL, 2.45 mol) and nitrobenzene (70 mL, 0.68 mol), and the temperature was raised to 120 °C. Pyrrole (2.8 mL, 0.4×10^{-3} mol) was then added. The reaction was followed by UV/Vis spectroscopy, and the temperature was maintained until the Soret-band intensity reaches a maximum. The solution was cooled to room temperature to give porphyrin crystals, which were filtered off, washed with methanol and dried. Simple recrystallisation from dichloromethane/methanol (10:1) gave the compounds with yields and characterisation presented below.

5,10,15,20-Tetraphenylporphyrin: Yield: 20%; 1H NMR (300 MHz, $CDCl_3$): δ = 8.89 (s, 8H), 8.19–8.31 (m, 12H), 7.75 (m, 8H), –2.77 (brs, 2H); MS (FAB): m/z = 615 (molecular ion); $C_{44}H_{30}N_4$: calcd C 85.97, H 4.92, N 9.12; found C 85.71, H 4.78, N 9.02.

5,10,15,20-Tetrakis(2,6-difluorophenyl)porphyrin: Yield: 11%; 1H NMR (300 MHz, $CDCl_3$): δ = 8.88 (s, 8H), 7.73–7.89 (m, 4H), 7.32–7.44 (m, 8H), –2.76 (brs, 2H); MS (FAB): m/z = 759 (molecular ion); $C_{44}H_{22}N_4F_8$: calcd C 69.66, H 2.92, N 7.38; found C 69.72, H 2.91, N 6.91.

5,10,15,20-Tetrakis(2,6-dichlorophenyl)porphyrin: Yield: 5%; 1H NMR (300 MHz, $CDCl_3$): δ = 8.62 (s, 8H), 7.65–7.85 (m, 12H), –2.59 (brs, 2H); MS (FAB): m/z = 887–905 (Cl isotopes for molecular ion); $C_{44}H_{22}N_4Cl_8$: calcd C 59.36, H 2.49, N 6.29; found C 59.30, H 2.47, N 6.25.

5,10,15,20-Tetranaphthylporphyrin: Yield: 23%; 1H NMR (300 MHz, $CDCl_3$): δ = 8.47 (s, 8H), 8.21–8.30 (m, 8H), 8.09–8.12 (d, 4H), 7.79–7.85 (m, 4H), 7.44–7.49 (m, 4H), 7.08–7.21 (m, 8H), –2.25 (brs, 2H); MS (FAB): m/z = 815 (molecular ion); $C_{60}H_{38}N_4$: calcd C 88.42, H 4.70, N 6.88; found C 88.44, H 4.71, N 6.86.

5,10,15,20-Tetranaphthylchlorin synthesis: We used the method of Whitlock^[28] to synthesise this chlorin. 5,10,15,20-Tetranaphthylporphyrin (0.05 g, 0.06 mmol) and sodium carbonate (3 g, 0.02 mol) were dissolved in freshly distilled γ -picoline (50 mL), and the mixture was heated to 140 °C. A solution of *p*-toluenesulfonylhydrazine in γ -picoline (10 mL, 0.4M) was added to the reaction mixture. The reaction, monitored by UV/Vis spectroscopy, was complete in 6 hours. After workup, the product was chromatographed with dichloromethane/hexane (1:3) in silica preparative thin-layer, and the second fraction was separated. The silica was washed with dichloromethane, and the chlorin was recrystallised from dichloromethane/hexane (10:1). Yield: 51%; 1H NMR (300 MHz, $CDCl_3$): δ = 8.29–8.31 (m, 6H), 8.14–8.22 (m, 8H), 8.08–8.09 (m, 8H), 7.94–8.03 (m, 8H), 7.78–7.83 (m, 4H), 7.40–7.54 (m, 4H), 3.80 (s, 4H), –0.99 (s, 2H); MS (FAB): m/z = 817 (molecular ion); $C_{60}H_{40}N_4$: calcd C 88.20, H 4.94, N 6.86; found C 88.18, H 4.91, N 6.84.

General metalloporphyrin syntheses: Porphyrin metal complexes were synthesised by the method of Adler.^[27] Porphyrin (0.07 mmol) was dissolved in dimethylformamide (50 mL) and heated to 150 °C. The desired metal acetate salt (0.4 mol) was added. The reaction was monitored by UV/Vis spectroscopy. After workup, the metal complex was purified by silica-gel column chromatography with the appropriate dichloromethane/hexane ratio. Recrystallisation from dichloromethane/hexane gave the isolated products with yields described below.

Mn^{III} 5,10,15,20-tetraphenylporphyrinate acetate: Yield: 89%; MS (FAB): m/z = 667 (molecular ion – acetate); UV/Vis (toluene): λ (ϵ) = 477.40 (1.1×10^5), 534.0 (5.4×10^3), 585.4 (8.4×10^3), 621.0 nm (9.9×10^3). Although this is a Mn^{III} complex involving acetate ion as ligand, we abbreviate it as Mn-TPP. $C_{46}H_{31}N_4O_2Mn$: calcd C 77.74, H 4.40, N 7.88; found C 77.64, H 4.51, N 7.84.

Zn^{II} 5,10,15,20-tetraphenylporphyrinate: Yield: 90%; 1H NMR (300 MHz, $CDCl_3$): δ = 8.85 (s, 8H), 8.20 (m, 8H), 7.75 (m, 12H); MS (FAB): m/z = 676 (molecular ion). $C_{44}H_{28}N_4Zn$: calcd C 77.93, H 4.16, N 8.26; found C 76.94, H 4.12, N 7.80.

Cu^I 5,10,15,20-tetraphenylporphyrinate: Yield: 86%; $C_{44}H_{28}N_4Cu$: calcd C 78.15, H 4.17, N 8.28; found C 78.12, H 4.48, N 8.28.

Zn^{II} 5,10,15,20-tetranaphthylporphyrinate: Yield: 93%; 1H NMR (300 MHz, $CDCl_3$): δ = 8.56 (s, 8H), 8.20–8.30 (m, 8H), 8.09–8.12 (d, 4H), 7.80–7.85 (m, 4H), 7.44–7.49 (m, 4H), 7.07–7.23 (m, 8H); $C_{60}H_{36}N_4Zn$: calcd C 82.05, H 4.13, N 6.38; found C 81.97, H 4.71, N 6.41.

Zn^{II} 5,10,15,20-tetrakis(2,6-difluorophenyl)porphyrinate: Yield: 87%; 1H NMR (300 MHz, $CDCl_3$): δ = 8.81 (s, 8H), 7.60–7.74 (m, 4H), 7.20–7.36 (m, 8H); MS (FAB): m/z = 822 (molecular ion); $C_{44}H_{20}N_4F_8Zn$: calcd C 64.29, H 2.45, N 6.82; found C 63.71, H 2.96, N 6.35.

Zn^{II} 5,10,15,20-tetrakis(2,6-chlorophenyl)porphyrinate: Yield: 67%; 1H NMR (300 MHz, $CDCl_3$): δ = 8.70 (s, 8H), 7.60–7.80 (m, 12H); MS (FAB): m/z = 954 (peak centred); $C_{44}H_{20}N_4Cl_8Zn$: calcd C 55.42, H 2.11, N 5.87; found C 55.64, H 2.42, N 5.64.

Acknowledgments: The authors are grateful to Dr. Hugh Burrows for the measurements at the Paterson Institute for Cancer Research and to Dr. C. Claver (University of Rovira Virgili, Tarragona, Spain) for mass spectral measurements. We thank the Fundação para a Ciência e a Tecnologia (PRAXIS XXI contract no. 2/2.1/QUI/390/94) for financial support. M.P. received a scholarship from PRAXIS XXI/BD/11398/97.

Received: March 12, 1998 [F1049]

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