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Some Observations on the Synthesis of Polysubstituted Zinc Phthalocyanine Sensitisers for Photodynamic Therapy

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ABSTRACT

The syntheses and properties of a number of polysubstituted zinc phthalocyanines are described which are suitable as sensitisers for photodynamic therapy. Zinc phthalocyanine tetrasulphonic acid, itself a useful photosensitiser, has been used to prepare tetrasulphonamides by converting it to the tetrasulphonyl chloride and reacting this with various amino compounds. This has afforded lipophilic and water-soluble anionic and neutral tetra-sulphonamido derivatives. Chlormethylation of zinc phthalocyanine under carefully defined conditions followed by reaction with pyridine affords a water-soluble cationic dye with an average of two methylenepyridinium groups per molecule. Hydrolysis of zinc phthalocyanine tetracarboxyamide could not be carried out to completion to afford the tetracarboxylic acid, and the resultant product had an average composition corresponding to a dicarboxylic acid–dicarboxyamide structure. Visible absorption spectroscopic properties and singlet oxygen sensitising efficiencies of the dyes (measured relative to methylene blue) are described. All the dyes are photocytotoxic in vitro *and show anti-tumour photoactivity* in vivo. *Copyright © 1997 Elsevier Science Ltd*

Keywords: Zinc phthalocyanines, photodynamic therapy, singlet oxygen, photosensitisers, aggregation, visible spectra.

INTRODUCTION

The clinical use of photodynamic therapy (PDT) in the treatment of cancer is now well established, and although the original sensitiser (Photofrin II or

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PHP) is still the most frequently used, second generation photosensitisers are finding increasing use because of their improved selectivity and light absorption characteristics. Perhaps the most studied synthetic sensitisers are the phthalocyanines,[1] particularly the aluminium and zinc derivatives, and although some of these materials show excellent PDT behaviour and low general toxicity in animal models, they still have yet to find widespread clinical acceptance. For example, one of the problems recognised with water-soluble phthalocyanines is that of unwanted skin coloration, which may or may not be accompanied by skin sensitisation to light. Some derivatives can show spectral shifts in cells which makes the correct selection of the light source critical. There is clearly still a need to explore in greater depth relationships between the structure of phthalocyanine sensitisers and their PDT behaviour.

Dyes effective in PDT are recognised as efficient sensitisers of singlet oxygen, and the involvement of singlet oxygen in the PDT process is now widely accepted, although not yet unequivocally proven. Good sensitisers must form triplet states efficiently and the triplet state energy should be greater than about 92 kJ mol^{-1}. A PDT dye should also absorb light at wavelengths longer than about 600 nm to avoid competitive absorption from blood, should show a good selectivity towards tumour cell uptake, and should be non-toxic. Certain phthalocyanine derivatives fulfil these general requirements very adequately, and this is particularly true of the zinc and aluminium complexes. The zinc derivatives have advantages over the aluminium complexes in that the metal ion is divalent and thus the structure of the central ion is unambiguous. On the other hand it is claimed that in the aluminium complexes the out-of-plane substituent makes aggregation of the dye molecules in solution much less likely than with the planar zinc analogues, and aggregation is known to reduce singlet oxygen sensitising efficiency. However, there is no *in vitro* or *in vivo* evidence that aluminium phthalocyanines are any more effective as PDT sensitisers than corresponding zinc derivatives.

Within the zinc phthalocyanine series, relatively few structures have been investigated as PDT sensitisers. Zinc phthalocyanine itself, because of its water insolubility, is utilised in liposomal form and has shown sufficient PDT activity to justify its commercialisation. Zinc phthalocyanine sulphonic acids have perhaps received the closest attention, and their water solubility is a distinct advantage. The tetrasulphonic acid has been regarded as inferior in its PDT characteristics to the lower sulphonated derivatives,[2,3] and it has been suggested that its strongly hydrophilic character is detrimental to cell uptake and localisation. However, the dye was found to be a good PDT photosensitiser *in vivo* provided the light source is chosen to take into account the red shift of the absorption band of the dye when absorbed within

cells.[4] Amino-substituted derivatives have received attention, as these offer the possibility of quaternisation and formation of cationic water soluble derivatives. Thus Leznoff *et al.* have prepared tetra-*NN*-diethylaminopropyl zinc phthalocyanine and its *N*-methylated tetra-cation,[5] and Wöhrle *et al.* have prepared tetra(3-pyridyloxy)- and tetra(2-dimethylaminoethoxy)-zinc phthalocyanines and have quaternised these with various alkyl groups to give a range of cationic dyes.[6] Other polar derivatives that have received attention include tetra(2-hydroxymethyl-2-methylbutoxy)- and octahydroxy-zinc phthalocyanine,[5] whereas non-polar derivatives of PDT interest have included tetraiodo-,[7] octa-*n*-butoxy-[8] and tetra(dibenzobarrelene)-octabutoxy[9] derivatives of zinc phthalocyanine.

We now describe routes to poly-substituted zinc phthalocyanines which offer the potential for varying the hydrophilic/hydrophobic character and the ionic charge of the dyes within wide limits. Initial results show that all the products synthesised are photocytotoxic *in vitro* and show PDT activity *in vivo* and details will be reported elsewhere. The spectroscopic properties and relative singlet oxygen sensitising abilities of the dyes have been examined, and the limitations of the synthetic methods are discussed.

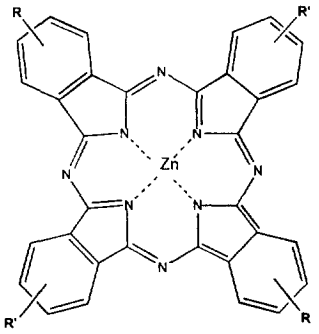
RESULTS AND DISCUSSION

Synthesis of dyes

Synthetic routes to zinc phthalocyanines may involve either the initial synthesis of the metal-free phthalocyanine derivative, followed by metallisation with a zinc salt, or may incorporate the zinc ion concurrently with the synthesis of the phthalocyanine ring system from a simple benzenoid precursor. Substituents can be introduced before or after the formation of the phthalocyanine ring system, and in the former case this normally leads to derivatives with substituents in all four rings, whereas in the latter case the degree of substitution will be variable, determined by reaction conditions. The situation is complicated by the numerous possibilities of positional isomerism. A single substituent in one ring may occupy position 3 (=6) (*ortho* to the imino bridge) or position 4 (=5) (*meta* to the bridge). For a tetra-substituted dye with a single substituent in each of the four rings, differences between positions 3 and 4 (or 5 and 6) are recognised, but differences between 3 and 6, and between 4 and 5, are generally ignored. Thus a mixed tetra-substituted phthalocyanine in which the substituents randomly occupy only the 4 and 5 positions would normally be regarded as a symmetrically substituted derivative, as would the corresponding family of compounds with substituents in the 3 and 6 positions only. Preparative separation of

positional isomers within these families has yet to be achieved, and it is reasonable to assume that chemical and physical differences between the isomers will be negligible.

The 'symmetrical' zinc phthalocyanine tetrasulphonic acid (**1**) (Scheme 1) with one sulphonic acid group occupying the 4 (= 5) position of each ring is well known in the literature, but details of its synthesis and purification do not appear to have been documented. Most researchers refer to the general synthetic procedure for metal phthalocyanines of Weber and Bush,[10] although these authors did not specifically describe synthesis of the zinc compound. By analogy with their general procedure, the zinc phthalocyanine tetrasulphonic acid can be made by reaction of 4-sulphophthalic anhydride with urea and a zinc salt in nitrobenzene as solvent. This procedure calls for the monosodium salt of 4-sulphophthalic acid as starting material, but as this was not commercially available, and as attempts to use the available trisodium salt in this reaction were unsuccessful, we sought an alternative starting material. 4-Sulphophthalic acid itself can be obtained commercially as a 50% aqueous solution, and careful neutralisation of this with ammonia



1: R = R' = $-SO_3H$

2: R = R' = $-SO_2Cl$

3: R = R' = $-SO_2N(C_8H_{17})_2$

4: R = R' = $-SO_2N(CH_2CH_2OH)_2$

5: R = R' = $-CONH_2$

6: R = $-CO_2H$; R' = $-CONH_2$

7: R = R' = $-SO_2NHCH_2CO_2H$

8: R = H, R' = $-CH_2-N^{\oplus}$ $Cl^{\ominus}$

Scheme 1.

to pH 4 and evaporation of the water gave the mono-ammonium salt which readily afforded the phthalocyanine under the reaction conditions of Weber and Bush.[10] However, we found that using nitrobenzene as solvent was unsatisfactory, and poor yields were obtained with a high level of water-soluble brown impurities. The situation could be improved by using excess urea as solvent rather than nitrobenzene, but optimum results were obtained with sulpholane as solvent, and from this reaction the crude dye could be obtained in 67% yield.

Although purification of the dye to a reasonable degree could be achieved by its conversion to the tetra sodium salt and chromatography of this over cellulose powder, high purity could only be achieved by converting the acid to its tetrasulphonyl chloride (**2**) with thionyl chloride followed by isolation and hydrolysis back to the tetrasulphonic acid. This gave analytically pure material, as the decahydrate of the tetra sodium salt (22% recovery from crude dye), with $\lambda_{max} = 672$ nm and $\varepsilon_{max} = 142{,}600$ litre mol^{-1} cm^{-1} in DMF.

The ready availability of pure **1** by this procedure prompted an examination of its possible use as a precursor for other tetra-substituted derivatives. As noted previously, reaction of **1** with thionyl chloride in chlorosulphonic acid affords the highly reactive tetrasulphonyl chloride **2**, and reaction of this with various amino compounds permitted the synthesis of a range of *N*-substituted tetra-sulphonamides. Sulphonamido zinc phthalocyanines do not appear to have been examined previously. Reaction of the tetra-sulphonyl chloride with excess di-*n*-octylamine in DMF gave the tetra-(*NN*-di-*n*-octyl-sulphonamide) derivative **3** as a deep blue solid, slightly soluble in DMF and readily soluble in toluene and dichloromethane.

In order to produce a non-ionic water-soluble tetrasulphonamide, reaction of the acid chloride with diethanolamine was considered. The fully substituted product would then have eight hydroxyethyl groups per molecule, and these were anticipated to confer sufficient hydrophilicity on the molecule to provide water solubility. The reaction was most conveniently carried out in a 1:1 (by volume) mixture of diethanolamine and water, and the resultant dye **4** was found to be very soluble in water. It was purified by dialysis in water to remove inorganic and low molecular weight impurities and isolated by evaporation of the water *in vacuo*. The product gave microanalytical data consistent with an octahydrate, and FAB mass spectrometry confirmed the molecular formula of the dye. The sulphonamide showed $\lambda_{max} = 673$ nm ($\varepsilon_{max} = 146{,}000$ litre mol^{-1} cm^{-1}) in DMF.

The sulphonamide route also offers many possibilities for synthesising tetra-carboxylic acid derivatives of zinc phthalocyanine by using amino acids as the reacting species. Carboxylic acid groups are interesting from a PDT point of view, because not only do they confer water solubility, but their lower hydrophilicity and higher pK_a in comparison with sulphonic

acid groups could have an important influence on cell uptake and localisation characteristics. Although the zinc phthalocyanine tetracarboxylic acid itself would be the simplest example, synthesis of this has so far proved surprisingly elusive. The most obvious route involves condensation of trimellitic anhydride with urea and a zinc salt, but we found that this afforded the tetracarboxyamide (**5**), which could be isolated in high yield and purity. Microanalytical data indicated that the product forms a pentahydrate.

Attempts to hydrolyse **5** to the tetracarboxylic acid under a variety of conditions (e.g. dilute and concentrated aqueous alkali, dilute and concentrated acids) were unsuccessful, however. Any attempt to force the reaction to completion invariably caused degradation of the chromophore, and the product isolated after the optimum hydrolysis time corresponded to **6**, with an average of two carboxylic acid groups and two carboxyamide groups per molecule. These observations are in agreement with the findings of Negri *et al.*, who also examined this route to the tetracarboxylic acid.[11]

Capillary electrophoresis showed that the product contained one main component, presumably **6**, and minor components which are most likely to be the mono-, tri- and tetra-hydrolysed products. Thus the dicarboxylic acid structure (**6**) assigned to the product represents a statistical average. The product proved to be hygroscopic, and differential scanning calorimetry indicated a water content corresponding to a pentahydrate. On heating up to 100°C, about 20% of the water was lost, and this was slowly regained on exposure to the atmosphere at room temperature. Negri *et al.* reported a lower degree of hydration for the product stored over phosphorus pentoxide under vacuum. The dye was readily soluble in water, but showed extensive aggregation in this solvent.

The difficulty found in making the zinc phthalocyanine tetracarboxylic acid thus prompted examination of the reaction between the tetrasulphonyl chloride and an amino acid. For this purpose glycine was used, and the acid chloride was treated with an excess of glycine in water at room temperature, maintaining an alkaline pH throughout. The highly water-soluble product was purified by dialysis, and the tetrasulphonamide structure (**7**) was confirmed by microanalysis, which also indicated that the dye existed as a decahydrate. The dye showed $\lambda_{max} = 672$ nm in DMF ($\varepsilon_{max} = 140{,}000$ litre mol^{-1} cm^{-1}).

Cationic groups (e.g. quaternised ammonium) offer an alternative way of producing water-soluble zinc phthalocyanine derivatives, and known tetrasubstituted examples have been prepared by using amino-substituted benzenoid precursors in the phthalocyanine synthesis, followed by quaternisation of the amino groups with suitable alkylating agents. We have examined an alternative approach where groups capable of alkylating tertiary amines are introduced directly into the zinc phthalocyanine molecule. It has long been known in copper phthalocyanine chemistry that the ring system can be

chloromethylated, and that the chloromethyl groups react readily with trialkylamines or pyridine to form water-soluble alkylammonium cationic derivatives. The degree of chlormethylation achievable has never been determined unequivocally, but under moderate conditions (e.g. HCl/HCHO in concentrated sulphuric acid at 80°C for 10 h) the mixed product approximates to a Tris–chloromethyl derivative. It has been claimed that a higher substituted product approximating to a tetra-chloromethyl derivative can be obtained by repeated additions of formaldehyde and chlorosulphonic acid with admission of HCl gas.[12]

Chlormethylation of zinc phthalocyanine was investigated, and a typical procedure involved dissolving the zinc phthalocyanine in a sulphuric acid–chlorosulphonic acid mixture and generating the chloroalkylating agent by addition of paraformaldehyde and sodium chloride. A 12-h reaction time at 80°C gave a product whose mass spectrum showed predominantly a bis-chloromethylated structure (found: m/z cluster at 676; $C_{34}H_{18}Cl_2N_8Zn$ requires $m/z = 673$–677). However, weak clusters at $m/z = 627$, 726 and 775 suggested the presence of mono-, tri- and tetra-substituted products in addition. The product was reacted with dibutylamine and the resultant amine isolated and examined by mass spectrometry. This also showed a dominant molecular ion ($m/z = 859$) corresponding to the bis-*NN*-dibutylaminomethylene derivative, with lesser peaks corresponding to the mono, tri and tetra derivatives.

The statistical composition of the product was determined by reacting a sample exhaustively with 4-aminoazobenzene and isolating and purifying the resultant azobenzene-phthalocyanine bichromophoric dye. The absorption spectrum of the product in DMF showed two visible absorption bands at *ca.* 400 and 665 nm corresponding, respectively, to the independent aminoazobenzene and zinc phthalocyanine chromophores, and the absorbance ratios, assuming the ε_{max} values are unchanged from those of the parent single chromophoric dyes, indicated an average value of *ca.* 2.3 chloromethyl groups in the original product.

To produce a cationic water-soluble dye, the chloromethyl derivative was condensed with pyridine, and the resultant *N*-methylenepyridinium chloride salt (**8**) was purified by repeated dissolution in water and precipitation with acetone, followed by dialysis.

Because of the high affinity of the dye for various substrates, attempts to analyse it by HPLC and capillary electrophoresis were unsuccessful. Thin-layer chromatography (cellulose/*n*-butanol: water; acetic acid 2:2:1 by volume) showed the presence of at least four blue poorly resolved components, one of which was present in much larger amounts than the others. Various methods of purification and modified methods of isolation gave essentially the same distribution pattern of components, which were

examined by mass spectrometry (FAB/nitrobenzyl alcohol matrix). The parent pyridinium ions could not be detected by this technique because of the facile loss of the pyridine groups, but the expected fragment ions were readily observable as a major cluster at *ca.* 606 (the disubstituted product) and at *ca.* 632 and 619, corresponding to the tetra- and tri-substituted products, respectively.

The presence of the methylenepyridinium residues was confirmed by 220 MHz p.m.r. spectroscopy, using solutions of **8** in deuteriosulphuric acid. The dye is readily soluble in this solvent and the phthalocyanine ring protons are well resolved from the pyridine ring and methylene protons. The average number of methylenepyridinium groups per molecule could be determined as the methylene protons show as a singlet at δ 7.17, and the phthalocyanine ring protons show as two broad singlets centred at *ca.* δ 10.6 and 9.5 ppm. The former can be assigned to the outermost (4- and 5-) protons on each benzene ring, and the latter to the 3- and 6-protons. [Progressive substitution of the 4 (or 5) phthalocyanine ring protons (e.g. comparison of unsubstituted zinc phthalocyanine with zinc phthalocyanine tetrasulphonic acid) results in a decrease in the δ 10.6 signal relative to the δ 9.5 signal.] In the case of **8** integration values showed that the methylenepyridinium groups had entered the 4(5) positions preferentially. The pyridinium protons showed two singlets at δ 9.1 and 9.8 ppm (identified by adding a small amount of pyridine to the solution), when both peaks (allowing for a small chemical shift relative to the original peaks) increased in intensity, whereas the phthalocyanine ring protons remained unaffected. By comparing the integration values for the phthalocyanine δ 10.6 peak and the methylene (δ 7.17) peak the degree of substitution could be estimated. For eight different samples this gave an average degree of substitution of 2.0 pyridinium groups per molecule, with a variation of *ca.* ±15%. Thus the product can be assigned the nominal

TABLE 1
Visible Absorption Spectroscopic Data for Water-soluble Dyes

Dye	*Solvent* λ_{max} *(nm)*	*DMF* ε_{max} *(litre mol^{-1} cm^{-1})*	*Solvent* λ_{max} *(nm)*	*MEM*[a] ε_{max} *(litre mol^{-1} cm^{-1})*
1	672	1.42×10^5	632	2.22×10^4
			672	2.22×10^4
4	673	1.46×10^5	626	4.28×10^4
6	680	5.3×10^4	636	2.35×10^4
7	672	1.40×10^5	631	4.38×10^4
			673	3.82×10^4
8	670	1.12×10^5	629	3.90×10^4

[a]Minimum essential medium (aqueous cell culture medium RPMI 1640 containing 10% calf serum) at pH 7.4.

structure **8**, and this is in reasonable agreement with the degree of substitution found for the chloromethyl precursor.

The degree of hydration of **8** could not be determined reliably as DSC analysis gave variable results. Microanalytical data were most closely in agreement with a decahydrate structure. The molar absorption coefficient (based on a decahydrate) was 112,000 litre mol^{-1} cm^{-1} in DMF ($\lambda_{max} = 670$ nm).

Visible absorption spectra

The dyes of specific PDT interest are those soluble in water, i.e. **1**, **4**, and **6–8**, and their visible absorption spectral properties, measured in DMF and in water (cell culture medium RPMI 1640) are summarised in Table 1. In water the dyes show a high tendency to aggregate, whereas in dimethylformamide they exist predominantly in monomeric form. Thus in the latter solvent they show a major intense peak near 670 nm (ε_{max} *ca.* 10^5 litre mol^{-1} cm^{-1}) with a secondary peak of about one-fifth the intensity near 605 nm, whereas in water a broad aggregate band at about 630 nm is observed in addition to the monomer band. The tetrasulphonate anion of **1** showed a lower tendency to aggregate in water than the non-ionic and cationic derivatives **4** and **8**. Thus the monomer band (672 nm) and aggregate band (632 nm) of **1** are almost equal in intensity, whereas for **4** and **8** the aggregate band is dominant and the monomer band is only detectable as a shoulder at longer wavelengths. The dicarboxylic acid-diamide **6** showed the greatest tendency to aggregate, and even in DMF the aggregate peak was significant. The principal effect of aggregation is to lower the apparent molar absorption coefficient of the dye, and thus the low ε_{max} of **6** in DMF can be attributed to this. The sulphonamide dye **7** contains carboxylic acid functionality and shows a somewhat greater tendency to aggregate than **1** but does not aggregate to the same extent as **6**.

TABLE 2
Relative Singlet Oxygen Sensitising Efficiencies of Water-soluble Zinc Phthalocyanines[a]

Dye	*% Photo-oxidation of DPBF*[b]	*Relative rate*[c]
1	45.3	1.00
4	53.5	1.18
6	11.5	0.25
7	50.3	1.10
8	41.8	0.92

[a]Solvent DMF/H_2O (9:1).
[b]1,3-Diphenylisobenzofuran.
[c]Relative to Methylene Blue.

Relative singlet oxygen sensitising efficiencies

An approximate indication of the relative singlet oxygen sensitising efficiencies of the water-soluble zinc phthalocyanine dyes was obtained by comparison experiments using Methylene Blue as a reference sensitiser. Thus the extents of photooxidation of 1,3-diphenylisobenzofuran caused by each sensitiser and by Methylene Blue under conditions of equal light absorption and in the same solvent mixture (DMF/H_2O, 9:1 by volume) were measured. The results are summarised in Table 2.

It can be seen that, with the exception of the dicarboxylic acid-diamide dye **6**, the dyes have very similar sensitising efficiencies to, or are slightly better than, Methylene Blue and are therefore potentially good PDT sensitisers. The reasons for the low efficiency of dye **6** are unclear, although it may be due to its propensity to aggregate in solution. This dye would probably be the least effective of the series for PDT purposes.

The PDT properties of the tetrasulphonic acid **1** have already been investigated in some detail, and contrary to some suggestions it is a very effective PDT sensitiser *in vivo*.[4] This dye shows an interesting red shift of the absorption band in tumour cells and thus the wavelength of the excitation source has to be adjusted to allow for this.[4] The methylenepyridinium derivative **8** has also been found to be more effective than PHP in LMC_1 mammary carcinoma.[13] All the water-soluble derivatives (i.e. **1**, **4**, **6–8**) are currently under investigation as PDT sensitisers and detailed results will be presented elsewhere. Preliminary results confirm that all are PDT active, with the dicarboxylic acid-diamide **6** being the least effective of the series.

EXPERIMENTAL

Synthesis of dyes

General procedure for purification by dialysis

The solution of crude dye in water was sealed in a length of Visking dialysis tubing (regenerated cellulose, pore size 2.4 nm) and immersed in a beaker of distilled water at room temperature. The external water was stirred and replaced at intervals by more distilled water. After the appropriate period of time the contents of the tubing were removed and evaporated to dryness under vacuum.

*Zinc phthalocyanine tetrasulphonic acid tetra-sodium salt (**1**)*

Commercial 50% 4-sulphophthalic acid solution (Aldrich) (56 g) was neutralised to pH 4 with aqueous ammonia and then evaporated to near

dryness. The paste was mixed with urea (41 g), zinc acetate (7.5 g), ammonium chloride (3.4 g), ammonium molybdate (0.5 g), boric acid (0.5 g), and the mixture added to sulpholane (50 cm^3), preheated to 80–90°C. The resultant slurry was then heated with stirring to 210–220°C, and held at that temperature for 3 h. After cooling the sulpholane was decanted off, and the residue washed with methanol to remove residual solvent. The solid was dissolved in water (200 cm^3) containing a small amount of sodium hydroxide, and activated carbon (1 g) was added and the solution filtered. The solution was applied to a column of cellulose powder and eluted with methanol to remove a brown impurity. The product was eluted from the column with water, and after evaporation of the solvent gave the tetrasodium salt as a dark blue solid, homogeneous by thin layer chromatography. Purification from inorganic salts was effected by dialysing an aqueous solution and then evaporating the solution to dryness under reduced pressure. An alternative method of purification involved conversion to the tetrasulphonyl chloride by the action of chlorosulphonic acid, followed by hydrolysis back to the sulphonic acid. (Found: C, 32.2; H, 3.2; N, 9.9%. $C_{32}H_{12}N_8O_{12}S_4Na_4Zn{\cdot}10H_2O$ requires C, 32.9; H, 2.8; N, 9.6%.)

*Zinc phthalocyanine tetrasulphonyl chloride (**2**)*

The sodium salt of zinc tetrasulphonic acid (**1**) (5.0 g) was added with stirring to chlorosulphonic acid (58 cm^3) at room temperature, and then heated at 70°C for 2 h. Thionyl chloride (7.5 cm^3) was added and stirring continued for 20 h at 70°C. The solution was cooled and added to ice with vigorous stirring, giving a final volume of *ca.* 500 cm^3. The solid was filtered off, washed with a little ice-water and dried as much as possible with the vacuum pump. The filter cake was then used immediately for subsequent reactions with amines.

Zinc phthalocyanine tetra-[NN-*bis(hydroxyethyl)sulphonamide*] (***4***)

The filter cake of the tetra-sulphonyl chloride (**2**) made from 5.0 g of the tetra-sulphonic acid was added to a mixture of water (15 cm^3) and diethanolamine (15 cm^3), and the suspension stirred vigorously for 24 h at 20°C. The solution was diluted with cold water (200 cm^3) and dialysed for 1 week, with frequent changes of water. The residual deep blue–green solution was evaporated to dryness under vacuum to give the tetra-sulphonamide (**4**) as a dark blue solid (1.32 g), which showed a single blue spot on thin-layer chromatography on silica. [Found: C, 39.5; H, 3.7; N, 12.0%. $C_{48}H_{44}N_{12}O_8S_4Zn{\cdot}10H_2O$ requires C, 40.4; H, 3.2; N, 11.8%. Mass spectrum (FAB) $m/z = 1245$ (expected M-$10H_2O$ = 1245).]

*Zinc phthalocyanine tetra(carboxyamide) (**5**)*
Benzene-1,2,4-tricarboxylic anhydride (44 g, 0.23 mol), urea (82.1 g, 1.37 mol), zinc acetate (15.0 g, 0.07 mol), ammonium molybdate (0.99 g, 0.8 mol) and ammonium chloride (6.69g, 0.13 mol) were ground together and then added with stirring to nitrobenzene (100 cm^3). The mixture was heated with stirring at 200°C for 3 h and after cooling, the blue–black solid was filtered off and washed thoroughly with methanol to remove all traces of nitrobenzene. A small portion of the product was further washed with water, then methanol and dried and confirmed to be the tetracarboxyamide by microanalysis. (Found: C, 52.0; H, 3.1; N, 20.6%. $C_{36}H_{20}N_{12}O_4Zn \cdot 4H_2O$ requires C, 52.5; H, 3.4; N, 20.4%.)

*Zinc phthalocyanine dicarboxylic acid dicarboxyamide (**6**)*
The product from the previous experiment was finely ground and stirred with sodium hydroxide solution (4 M) for 5 days. The solution was filtered and concentrated under reduced pressure, and then dialysed for 4 days in distilled water. The dialysed solution was evaporated to dryness under reduced pressure to give **6** as a dark blue solid (5.27 g). (Found: %C/%N = 3.2; $C_{36}H_{20}N_{12}O_4Zn$ requires %C/%N = 3.1).

*Zinc phthalocyanine tetra-(N-carboxymethyl)sulphonamide (**7**)*
Glycine (10.0 g) was dissolved in water (25 cm^3), and to the stirred solution at room temperature was added in portions the freshly prepared filter cake of zinc phthalocyanine tetrasulphonyl chloride made from 5 g **1**, whilst maintaining a slightly alkaline pH by simultaneous addition of dilute sodium hydroxide solution. The solution was stirred at room temperature for 12 h, and was then dialysed for 24 h. The deep blue solution was evaporated to dryness under vacuum and the residue washed thoroughly with acetone and dried to give **7** (1.95 g). (Found: C, 34.5; H, 3.25; N, 11.5%; $C_{40}H_{24}N_{12}O_{16}S_4ZnNa_4 \cdot 10H_2O$ requires C, 34.5; H, 3.2; N, 12.1%.)

*Bis-methylenepyridinium zinc phthalocyanine (**8**)*
Finely powdered paraformaldehyde (120 g) was added with stirring to a mixture of concentrated sulphuric acid (160 g) and chlorosulphonic acid (535 g) at 0°C, and stirring was continued for 20 min. A ground mixture of sodium chloride (20.5 g) and zinc phthalocyanine (20.5 g) was added slowly with stirring and strong cooling. After addition, the mixture was warmed carefully to 80°C over 1 h and maintained at this temperature for 12 h. The mixture was then cooled and added slowly to crushed ice (1500 g) to hydrolyse excess chlorosulphonic acid. The deposited zinc phthalocyanine chloromethyl derivative was filtered off, washed thoroughly with water until acid free, and dried *in vacuo* at 30°C for 12 h. (Yield: 41.0 g.) The solid was then

added to pyridine (200 cm^3) and heated under reflux for 20 min. Water (400 cm^3) was added and refluxing continued for 15 min. The deep blue solution was cooled and poured into acetone (1500 cm^3) to precipitate the methylenepyridinium chloride as a dark blue solid. This was filtered off and purified by repeated dissolution in distilled water/precipitation in acetone. Final purification was effected by dialysis. The product was obtained as a deep blue crystalline solid. (Found: %C/%N = 3.9. $C_{44}H_{29}N_{10}Cl_2Zn \cdot 10H_2O$ requires %C/%N = 3.8.)

Singlet oxygen sensitisation efficiencies

Test solutions were prepared containing 1,3-diphenylisobenzofuran (0.020 g $litre^{-1}$) and the phthalocyanine sensitiser or Methylene Blue (0.100 g $litre^{-1}$) in dimethylformamide containing 10% water. Aliquots were placed in 1 cm glass cuvettes in a merry-go-round apparatus, and were exposed at 0°C under air to filtered light from a focused 500 W quartz-halogen projector lamp. The light beam was passed through a combination of three filter solutions providing a transmission window between 600 and 700 nm. The concentration of the sensitiser solutions was sufficient for a 1 cm pathlength to absorb all the radiation within this range, and none of the radiation was absorbed by the diphenylisobenzofuran. The disappearance of the diphenylisobenzofuran was monitored by observing the decrease in absorbance at 410 nm, and the percent photo-oxidation after 10.0 min exposure was measured. For each trial, Methylene Blue was used as control. The results averaged over four runs for each sensitiser are summarised in Table 2.

CONCLUSIONS

Zinc phthalocyanine tetrasulphonyl chloride can be made readily from the tetrasulphonic acid and is a useful intermediate for the synthesis of a wide range of sulphonamides which have potential value as PDT sensitisers. Thus condensation with *NN*-dialkylamines, hydroxyalkylamines and amino acids affords lipophilic, water-soluble non-ionic, and water-soluble anionic derivatives, respectively. The reaction with amino acids is a particularly useful route to tetracarboxy-substituted zinc phthalocyanines, as the attempted direct synthesis of zinc phthalocyanine tetracarboxylic acid from trimellitic anhydride affords a tetracarboxyamide which cannot be fully hydrolysed. Synthesis of a poly-cationic zinc phthalocyanine could be achieved by chlormethylation of zinc phthalocyanine followed by quaternisation with pyridine. However, this gave a mixed product with an average degree of substitution corresponding to the bis-(methylenpyridinium) dication.

Comparative photo-oxidation studies showed all the derivatives, with the exception of the dicarboxylic acid diamide **6**, to be at least as effective as Methylene Blue as singlet oxygen sensitisers. Preliminary results indicate that the various compounds have good PDT activity, although the dicarboxylic acid diamide **6** is the least effective, in agreement with its poorer singlet oxygen sensitising properties.

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