SYNTHESIS AND PHOTOCYTOTOXICITY OF SOME NEW SUBSTITUTED PHTHALOCYANINES

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Abstract—Some new, ring-substituted phthalocyanines have been synthesized and underwent preliminary testing for photodynamic activity using the colony forming ability of Chinese hamster cells in culture as an endpoint. Using 4-(3-N,N-diethylaminopropyl)phthalonitrile as a precursor, the previously unknown metal-free 2,9,16,23-tetra-(3-N,N-diethylaminopropyl)phthalocyanine was prepared and converted to its zinc (II) and its cationic water-soluble 2,9,16,23-tetramethylammonium zinc (II) iodide derivatives. Other new phthalocyanine derivatives tested, include a 2,9,16,23-tetra(2-hydroxymethyl-2-methylbutoxy)phthalocyaninato zinc (II) and 2,3,9,10,16,17,23,24-octahydroxyphthalocyaninato zinc (II) derivatives. Boron tribromide cleavage of the newly prepared and fully characterized 2,3,9,10,16,17,23,24-octamethoxyphthalocyanine gave the unstable metal-free octahydroxyphthalocyanine, which could only be characterized by ultraviolet-visible, NMR and IR spectroscopy.

INTRODUCTION

The phthalocyanine dyes have been recently suggested as sensitizers in the photodynamic therapy of cancer (Ben-Hur and Rosenthal, 1985). The relationship between chemical structure and ability to act as photodynamic sensitizers has not yet been entirely defined. Two photobiologically critical parameters are affected by the structural diversity: (a) the lifetime of the excited triplet state and (b) the cellular uptake of the dye. Both these parameters depend on the nature of the central metal atom. Since a long life triplet state is a prerequisite for photosensitization, a phthalocyanine dye containing a diamagnetic metal is better suited for this function than an analog compound containing a paramagnetic metal (Rosenthal et al., 1986). Furthermore, preliminary screening indicates that metals higher than trivalent, like Si, which require out-of-plane counterions on both sides of the phthalocyanine ring, are not taken up by cells, most probably due to the physical hindrance induced by the axial substituents (Ben-Hur et al., 1987). A similar explanation is provided for the absence of photodynamic activity of phthalocyanine substituents with bulky peripheral substituents such as neopentoxy groups. In addition, the ring substituents, which define the solubility features of the dye, will affect the rate of uptake, as exemplified by the comparison between unsubstituted, tetrahydroxy, and tetrasulfonated derivatives (Rosenthal et al., 1987; Brasseur et al., 1988).

We have now prepared metal-free 2,9,16,23-tetra-(3-N,N-diethylaminopropyl) phthalocyanine (1), its zinc (II) (2), and its quaternized tetramethyl zinc (II) iodide (3) derivatives. Other unusual phthalocyanines tested in this study are 2,9,16,23-tetra(2-hydroxymethyl-2-methylbutoxy) phthalocyaninato zinc (II) (4) (Leznoff et al., unpublished data) and 2,3,9,10,16,17,23,24-octahydroxyphthalocyaninato zinc (II) (5) prepared from 2,3,9,10,16,17,23,24-octamethoxyphthalocyaninato zinc (II) (6). It was expected that the cationic water-soluble phthalocyanine 3, the partially hydrophilic-lipophilic phthalocyanines 2 and 4, and the catechol-like phthalocyanine 5 would provide new and interesting candidates for photodynamic sensitization.

MATERIALS AND METHODS

Procedures for synthesis

Instrumental methods and equipment used in the characterization of new phthalocyanines have been previously outlined (Marcuccio et al., 1985).

Preparation of 4-(3-N,N-diethylaminoprop-1-ynyl) phthalonitrile (9). To a solution of 1.0 g (3.94 mmol) of 4-iodophthalonitrile (7) (Marcuccio et al., 1985; Keller and Griffith, 1980) in 40 mℓ of dry, freshly distilled diethylamine were added 0·6 g (5.40 mmol) of 3-N,N-diethylamino prop-1-yne (8) (Wolf, 1952), 5.0 mg (0.026 mmol) of copper (I) iodide, and 40 mg (0.06 mmol) of bis(triphenylphosphine)palladium dichloride under an Ar atmosphere, using a recent procedure (Takahashi et al., 1980). The reaction mixture was stirred at room temperature for 24 h, during which a precipitate of diethylamine hydroiodide appeared. Thin layer chromatography showed traces of 7, so small amounts of copper(I) iodide, bis(triphenylphosphine) palladium dichloride and 3-N,N-diethylaminoprop-1-yne were added, and the mixture was stirred for another 3 h. The mixture was filtered to separate the precipitated salt. The salt was washed with diethyl ether and the filtrate concentrated on the rotary evaporator. The residue was extracted with diethyl ether then

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washed with water (2 × 100 mℓ), 5% NaHCO$_3$ (2 × 100 mℓ) and water (2 × 100 mℓ). The ether fraction was dried over anhydrous magnesium sulfate and then concentrated on the rotary evaporator. The residue was purified by column chromatography using basic alumina (Act 2-3) and hexane/ether (3:7) as the eluting solvents. Evaporation of the solvent left a yellow oil which was stored under Ar in the refrigerator overnight to give 0.83 g (89% yield) of a yellow solid (9). Recrystallization from ethyl acetate/hexane produced long, yellow, needle-like crystals, mp 43–45°C, IR spectrum (KBr pellet): 3120–2820, 2240, 1600, 1490, 1430, 1380, 1360, 1296, 1260, 1190, 1160, 1120, 910, 850, 770 cm^{-1}. ^{1}H NMR spectrum (300 MHz, CDCl$_3$): δ 7.81–7.73 3H, 3.68 2H, 2.65–2.57 4H, 1.14–1.09 6H, ^{13}C NMR spectrum (300 MHz, CDCl$_3$): δ 136.16, 135.78, 133.36, 129.28, 116.11, 115.08, 114.69, 114.10, 93.23, 81.70, 47.39, 41.53, 12.52. Mass spectrum m/z 237 (M$^+$), 222, 138. Analysis: C$_{15}$H$_{15}$N$_3$. Calc. (%): C(75.90), H(6.37), N(17.70). Found (%): C(75.95), H(5.97), N(17.42).

Preparation of 4-(3-N,N-diethylaminopropyl)phthalonitrile (10). A solution of 2.4 g (10.13 mmol) of 9 in 100 mℓ of dry ethyl acetate was hydrogenated over 10% palladium on charcoal, at 60 psi, in a Parr hydrogenation bottle for 1 h at room temperature. The solution was filtered from the catalyst and concentrated. Column chromatography of the product on 80 g of basic alumina (Act 3) using hexane/ether (3:7) as the eluant, gave upon evaporation of the solvent, 1.9 g (78% yield) of a yellow oil, bp 173–175°C/0.05 torr. IR spectrum (neat, NaCl disc): 2980–2700, 2220, 1600, 1480, 1440, 1380, 1290, 1200, 1070, 910, 840, 760 cm^{-1}. ^{1}H NMR spectrum (300 MHz, CDCl$_3$): δ 7.73 1H, 7.66 1H, 7.59 1H, 2.77 2H, 2.53 4H, 2.45 2H, 1.84 2H, 1.04 6H. ^{13}C NMR spectrum (300 MHz, CDCl$_3$): δ 149.24, 133.48, 133.32, 133.26, 115.59, 115.48, 115.43, 112.86, 51.59, 46.56, 33.32, 28.05, 11.42. Mass spectrum: m/z 241 (M$^+$), 226, 141. Analysis: C$_{15}$H$_{19}$N$_3$. Calc(%): C(74.75), H(7.95), N(17.43). Found(%): C(75.04), H(8.39), N(17.75).

Preparation of 5-(3-N,N-diethylaminopropyl)-1,3-diiminoisoindoline (11). To a solution of 200 mg of sodium in 100 mℓ of dry methanol, was added 1.8 g (7.47 mmol) of 10. The mixture was stirred for 1 h at room temperature, and for 4 h under reflux conditions, during which ammonia gas was bubbled into the solution. The reaction mixture gradually turned a dark green color. The reaction was cooled to room temperature and the addition of ammonia was discontinued. Evaporation of the solvent gave crude 11, which was used directly in a condensation reaction without further purification. Compound 11 did not exhibit a nitrile absorption in its IR spectrum.

Preparation of 2,9,16,23-tetra(3-N,N-diethylaminopropyl) phthalocyanine (1). The crude 5-(3-N,N-diethylaminopropyl)-1,3-diiminoisoindoline (11), made from 1.8 g (7.47 mmol) of 10, underwent self-condensation as previously described (Leznoff et al., 1985; Brach et al., 1970). Compound 11 was heated in 30 mℓ of 2-N,N-dimethylaminoethanol at 150–160°C (oil bath) for 48 h under an Ar atmosphere. The mixture gradually changed from dark green to dark blue. After cooling to room temperature, the mixture was evaporated under pump-vacuum to remove the 2-N,N-dimethylaminoethanol. The residue was extracted into ether and purification was performed by column chromatography on basic alumina (Act 2-3). The ether solution containing the phthalocyanine (1) was preadsorbed onto a small amount of basic alumina (Act 2-3). Elution with 200 mℓ of ether followed by increasing amounts of methanol/ether (1:100) to (3:100) mixtures gave two fractions. The first fraction contained pure tetra-substituted phthalocyanine 1, and the second contained a mixture of yellow-green impurity plus compound 1. All of 1 was eventually recovered by rechromatography of all mixed fractions. The total yield of mononuclear phthalocy-

anine 1 was 0.5 g (28% yield) as a dark blue solid. Absorption spectrum: λ$_{max}$ (tetrahydrofuran) 696, 660, 602, 342, 290, 232, (log ε: 5.22, 5.17, 4.51, 4.90, 4.56, 5.02). ^{1}H NMR spectrum (300 MHz, CDCl$_3$): δ 8.40, 8.00, 7.53, (broad signals) 12H, 3.02–2.66 (broad signals) 8H, 2.09 (broad signal) 2H, 1.10 (broad signal) 6H, 3.1 (broad signal) NH. IR spectrum (KBr pellet) 3450, 3290, 2980, 2800, 1610, 1500, 1460, 1380, 1320, 1200, 1100, 1015, 750 cm^{-1}. Mass spectrum: m/z 967 (M$^+$). Analysis: C$_{60}$H$_{78}$N$_{12}$. Calc.(%): C(74.49), H(8.12), N(17.37). Found(%): C(75.15), H(8.35), N(17.71).

Preparation of 2,9,16,23-tetra(3-N,N-diethylaminopropyl) phthalocyaninato zinc (II) (2). To a solution of 130 mg (0.13 mmol) of 1 in 10 mℓ toluene/2-methoxyethanol (1:1), was added 300 mg (1.63 mmol) of anhydrous zinc acetate. The solution was stirred at 120°C (reflux), under an argon atmosphere for 20 h. The crude reaction mixture was applied directly to a short (10 cm × 1 cm), basic alumina column (Act 3) and eluted with toluene/2-methoxoethanol (3:1). Evaporation of the solvent gave 135 mg of 2 as dark blue shining solid in 97% yield. This metal insertion reaction is similar to other reactions previously described (Leznoff et al., 1985). IR spectrum (KBr pellet): 3450, 2940, 1610, 1490, 1340, 1090, 1050, 830, 750 cm^{-1}. Mass spectrum: m/z 1030 (M$^+$). Absorption spectrum: λ$_{max}$ (chloroform) 686, 672, 616, 352, 246 (log ε: 5.04, 4.93, 4.41, 4.74, 4.45).

Preparation of 2,9,16,23-tetra(3-N,N-diethyl-N-methyl-aminopropyl) phthalocyaninato zinc (II) tetraiodide (3). To a solution of 84 mg (0.081 mmol) of 2 in 10 mℓ toluene was added 0.5 mℓ (8 mmol) of methyl iodide. This solution was stirred for 20 h at room temperature. The observed precipitate was filtered and washed with ether. The yield of the green powdery solid 3 was 96.5 mg (74.2%). Absorption spectrum: λ$_{max}$ (water) 634, 338, 290, 226, (log ε: 4.89, 4.84, 4.50, 5.00). IR spectrum (KBr pellet): 3440, 2960, 1610, 1490, 1400, 1330, 1090, 1050, 750 cm^{-1}. Analysis: C$_{46}$H$_{88}$N$_{12}$Zn$_4$I$_4$. Calc (%): C(48.09), H(5.55), N(10.51), Zn(4.09). Found (%): C(48.10), H(5.48), N(10.54), Zn(3.97).

Preparation of 2,3,9,10,16,17,23,24-octamethoxyphthalocyaninato zinc (II) (6). To 1.01 g (5.39 mmol) of 1,2-dicyano-4,5-dimethoxybenzene (Metz et al., 1984) in 10 mℓ of dry gold label dimethylformamide (DMF), was added 1.01 g (5.53 mmol) of anhydrous zinc acetate (Pawlowski and Hanack, 1980). After 120 h heating at 160°C (oil bath) under Ar, the mixture was cooled to room temperature and the DMF was removed under reduced pressure (40°C, 0.1 torr). The crude product was filtered and washed with methanol to remove brown impurities. The residue was then extracted with methanol in a Soxhlet apparatus until the extract was clear (26 h) followed by extraction with acetone. It was then dried at 80°C overnight to give 420 mg (38%) of 6 (Meier et al., 1986) as a dark blue solid. Absorption spectrum: λ$_{max}$ (pyridine) 680, 672, 610, 364 (log ε: 5.20, 5.24, 4.55, 4.97). IR spectrum (KBr pellet): 2930, 1600, 1440, 1395, 1275, 1095, 1050, 875, 740 cm^{-1}. Mass spectrum: m/z 816, 817, 818, 819, 820, 821.

Preparation of 2,3,9,10,16,17,23,24-octahydroxyphthalocyaninato zinc (II) (5). To 206.5 mg (0.25 mmol) of 6 in 50 mℓ of dry benzene, was added 5 mℓ (52.6 mmol) of boron tribromide at 10°C, under an Ar atmosphere. The mixture was warmed to room temperature and was then refluxed under argon for 20 h. After cooling to room temperature, ice cold water was added and the water–benzene mixture was evaporated under reduced pressure. The crude product was filtered, washed with water and air dried to give 1.87 g of a black-grey powder. Further purification of the product was achieved by extraction with acetonitrile in a Soxhlet apparatus for 24 h. This process removed 880 mg of white material. The acetonitrile extraction was repeated until all of the impurities were

removed (an additional 560 mg of off-white powder). The amount of **5** recovered after Soxhlet extractions was 146 mg (82%). A sample of 25.8 mg was further purified by recrystallization from $DMSO/H_2O$ to yield 16.0 mg of **5** (62% recovery). Absorption spectrum: λ_{max} (10% DMF in ethanol) 682, 674, 622, 354, 296 (log ϵ: 4.78, 4.70, 4.12, 4.60, 4.49). ^{1}H NMR spectrum (300 MHz, DMSO d_6): δ 10.1 (broad signal) 8H, OH, 8.6 8H. IR spectrum (KBr pellet): broad band from 3100 to 3500 cm^{-1} OH, 1615, 1470, 1380, 1300, 1060, 1030, 880 cm^{-1}. Mass spectrum: m/z 704, 705, 706, 707, 708, 709.

Preparation of 2,3,9,10,16,17,23,24-octamethoxyphthalocyanine (**12**). Ammonia was bubbled into a solution of 1.88 g (10.0 mmol) of 1,2-dicyano-4,5-dimethoxybenzene (Metz *et al.*, 1984) in dry methanol containing sodium methoxide, for 1 h at 85°C and for 8 h under reflux conditions, similar to, but not identical with conditions previously described (Leznoff *et al.*, 1985). Evaporation of the solvent gave the crude 5,6-dimethoxy-1,3-diiminoisoindoline (which did not exhibit any CN absorption peaks in its infrared spectrum), which was dissolved in 20 mℓ of 2-N,N-dimethylaminoethanol and heated to 165°C (oil bath) under an argon atmosphere. After 48 h reflux, the mixture was cooled to room temperature and the solvent was evaporated under reduced pressure. The crude product was digested with methanol containing large amounts of ether, filtered and washed with ether followed by increasing amounts of methanol in ether (1:9) to (1:1), and finally it was washed with methanol and air dried overnight to give 924 mg of a very fine purple powder. The product was then extracted with methanol in a Soxhlet apparatus for 20 h followed by acetone to give 790 mg (42%) of **12** as a dark blue solid. Absorption spectrum: λ_{max} (CF_3CO_2H) 734, 702, 406, 292 (log ϵ: 5.14, 4.84, 4.64, 4.95). IR spectrum (KBr pellet): 3285, 2920, 1600, 1475, 1270, 1195, 1095, 1015, 845, 740 cm^{-1}. Mass spectrum: m/z 754, 739, 724, 709, 694, 679. Analysis: $C_{40}H_{34}N_8O_8$. Calc. (%): C(63.66), H(4.54), N(14.84). Found (%): C(63.28), H(4.84), N(14.77).

Preparation of 2,3,9,10,16,17,23,24-octahydroxyphthalocyanine (**13**). To 40 mg (0.053 mmol) of **12** in 20 mℓ of dry benzene, was added 0.3 mℓ (3.17 mmol) of boron tribromide at 10°C under an argon atmosphere. The mixture was warmed to room temperature and then refluxed under Ar for 22 h. After cooling to room temperature, 10 mℓ of distilled water was added and the suspension was stirred for 10 min. The crude product was filtered under suction and washed with water, ether and air dried. Exhaustive Soxhlet extractions with methanol and then ether gave 23.0 mg (67%) of **13** as a dark blue solid. Absorption spectrum: λ_{max} (pyridine) 708, 678, 672, 652, 612, 428, 350 (log ϵ: 4.72, 4.61, 4.65, 4.27, 4.08, 4.22, 4.57). ^{1}H NMR spectrum (300 MHz, DMSO d_6): δ 8.6, 10.1 (broad signal) OH. ^{1}H NMR spectrum (300 MHz, pyridine d_5): δ-3.7 (broad signal) NH. IR spectrum (KBr pellet): 3420 (broad), OH, 1605, 1475, 1380, 1260, 1085, 1015, 850, 740 cm^{-1}.

Preparation of 2,9,16,23-tetra(3-N,N-diethyl-N-methylaminopropyl) phthalocyanine tetraiodide (**14**). Methyl iodide (0.5 mℓ, 8 mmol) was added to a solution of 34 mg (0.035 mmol) of **1** in 25 mℓ of diethyl ether. This mixture was stirred for 48 h at room temperature. The above observed precipitate was filtered and dried to give 48 mg (89% yield) of **14** as a dark blue powder. Absorption spectrum λ_{max} (H_2O) 639, 332, 292, 226 (log ϵ: 4.76, 4.75, 4.60, 4.92). IR spectrum (KBr pellet): 3440 NH, 2980, 1610, 1390, 1320, 1100, 1010, 810, 750 cm^{-1}. Analysis: $C_{64}H_{90}N_{12}I_4$. Calc.(%): C(50.07), H(5.91), N(10.95). Found (%): C(50.19), H(5.82), N(11.07).

Preparation of 2,9,16,23-tetra(3-N,N-diethylaminopropyl) phthalocyanine tetrahydrochloride (**15**). Hydrogen chloride gas was bubbled into a solution of 45.5 mg (0.47 mmol) of metal free phthalocyanine **12** in 25 mℓ of diethyl

ether. This mixture was stirred for 15 min at room temperature. The observed dark blue solid precipitate was filtered and dried to yield 47.1 mg (90% yield) of **15**. Absorption spectrum: λ_{max} (H_2O) 636, 332, 292, 212 (log ϵ: 4.69, 4.68, 4.55, 4.60). IR spectrum (KBr pellet): 3450 NH, 2980, 1610, 1470, 1100, 1010, 750 cm^{-1}. Mass spectrum m/z 968 (M^+), 937. Analysis: $C_{64}H_{82}N_{12}Cl_4$. Calc. (%): C(64.74), H(7.42), N(15.10). Found (%): C(64.39), H(7.19), N(15.27).

Chinese hamster cells and light exposure

Chinese hamster fibroblasts, line V79-B310H, were grown as a monolayer in 10 cm plastic Petri dishes. Growth was in Dulbecco's modified Eagle medium containing 10% fetal calf serum, at 37°C in a humidified atmosphere adjusted to 5% CO_2. The detailed experimental procedure for determination of phototoxicity was described by Rosenthal *et al.* (1987).

RESULTS AND DISCUSSION

Syntheses

The preparation of the aminophthalocyanines **1** and **2** and the tetramethyl derivative **3** have not been previously described. Thus, as shown in Fig. 1, treatment of 4-iodophthalonitrile (**7**) with 3-N,N-diethylaminoprop-1-yne (**8**), bis(triphenylphosphine palladium dichloride and copper (I) iodide (Takahashi *et al.*, 1980) gave 4-(3-N,N-diethylaminoprop-1-ynyl)phthalonitrile (**9**) in 89% yield. Catalytic hydrogenation of **9** gave 4-(3-N,N-diethylaminopropyl) phthalonitrile (**10**) in 78% yield. Conversion of **10** into 5-(3-N,N-diethylaminopropyl)-1,3-diiminoisoindoline (**11**) was achieved by bubbling ammonia into a solution of **10** and sodium methoxide, in methanol. Compound **11** underwent self-condensation when heated in 2-N,N-dimethylaminoethanol to give the dark blue 2,9,16,23-tetra(3-N,N-diethylaminopropyl) phthalocyanine (**1**) in 28% yield after purification by chromatography. Insertion of zinc into metal-free **1** was readily attained by refluxing **1** with anhydrous zinc acetate in 1:1 toluene/2-methoxyethanol to give 2,9,16,23-tetra(3-N,N-diethylaminopropyl)phthalocyaninato zinc (II) (**2**). Quaternization of the four amino groups of **2** occurred on treatment of **2** with excess iodomethane in toluene at room temperature to give 2,9,16,23-tetra(3-N,N-diethyl-N-methylaminopropyl)phthalocyaninato zinc (II) tetraiodide (**3**) in 97% yield. The synthesis of 2,9,16,23-tetra(2-hydroxymethyl-2-methylbutoxy)phthalocyaninato zinc (II) (**4**) and related compounds will be reported elsewhere (Leznoff *et al.*, unpublished data). It should be noted that **1–4**, each exist as a closely related mixture of isomers containing the depicted structures (Fig. 1) and their 2,9,16,24-, 2,9,17,24- and 2,10,16,24- derivatives.

Cleavage of the known 2,3,9,10,16,17,23,24-octamethoxyphthalocyaninato zinc (II) (**6**) (Meier *et al.*, 1986) with boron tribromide (BBr_3) (McOmie and Watts, 1963; McOmie *et al.*, 1968) according to our

CN
CN
I
7
+ (Et)$_2$NCH$_2$C≡CH   **8** $\xrightarrow{\text{[(Ph)}_3\text{P]}_2\text{PdCl}_2}{\text{Cu I, Et}_2\text{NH}}$

(Et)$_2$NCH$_2$C≡C
CN
CN
9
$\xrightarrow{\text{H}_2}{\text{Pd/C}}$

(Et)$_2$N(CH$_2$)$_3$
CN
CN
10
$\xrightarrow{\text{NH}_3}{\text{NaOMe}}$

(Et)$_2$N(CH$_2$)$_3$
NH
NH
NH
11
$\xrightarrow{\text{Me}_2\text{NCH}_2\text{CH}_2\text{OH}}{\Delta}$

R' R
R
R'
R'
R
R R'
M

1 R=(Et)$_2$N(CH$_2$)$_3$, R'=H, M=H$_2$
2 R=(Et)$_2$N(CH$_2$)$_3$, R'=H, M=Zn
3 R=Me(Et)$_2$N$^{\oplus}$(CH$_2$)$_3$I$^{\ominus}$, R'=H, M=Zn
4 R=HOCH$_2$C(Me)(Et)CH$_2$O, R'=H, M=Zn
5 R=R'=OH, M=Zn
6 R=R'=OCH$_3$, M=Zn
12 R=R'=OCH$_3$, M=H$_2$
13 R=R'=OH, M=H$_2$
14 R=Me(Et)$_2$N$^{\oplus}$(CH$_2$)$_3$I$^{\ominus}$, R'=H, M=H$_2$
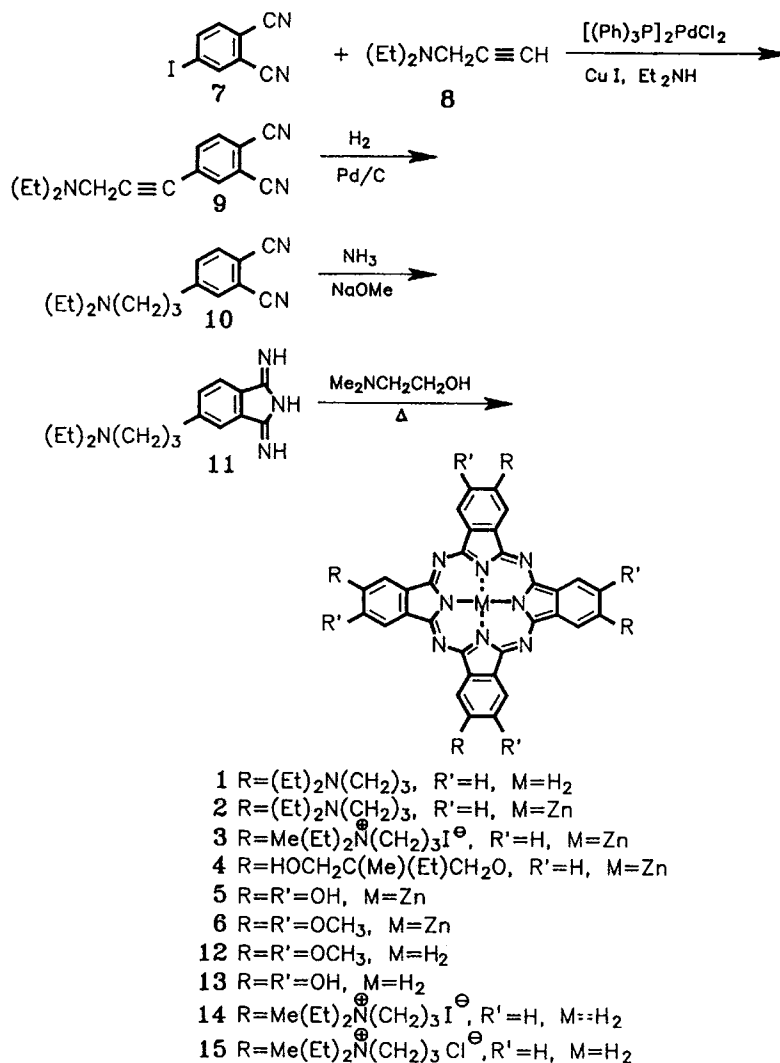15 R=Me(Et)$_2$N$^{\oplus}$(CH$_2$)$_3$Cl$^{\ominus}$,R'=H, M=H$_2$

Figure 1. A synthetic scheme for some substituted phthalocyanines.

earlier preparation of 2,9,16,23-tetrahydroxyphthal-ocyaninato zinc (II) (Rosenthal et al., 1987) gave the previously unknown 2,3,9,10,16,17,23,24-octa-hydroxyphthalocyaninato zinc (II) (5) as a fairly insoluble dark blue compound. Although metal-free 2,3,9,10,16,17,23,24-octamethoxyphthalocyanine (12) has now been prepared for the first time (see Materials and Methods), boron tribromide cleavage of 12 produced a highly insoluble material (slightly soluble in dimethyl sulfoxide or pyridine) which exhibited ultraviolet-visible, infrared, and NMR spectra characteristic of the metal-free, 2,3,9,10,16,17,23,24-octahydroxyphthalocyanine (13), but mass spectral and elemental analysis were unsatisfactory. Thus attempts at making 5 by zinc

insertion into 13 could not be achieved. In addition, zinc insertion into 12 in order to make 5 was incomplete due to the insolubility of 12.

Photocytotoxicity

The photodynamic activity of these previously unknown phthalocyanines have been estimated using the colony forming ability of Chinese hamster cells as an endpoint. Since other metal-free phthalocyanines showed minimal photodynamic activity (Chan et al., 1987; Abernathey et al., 1987; Sonoda et al., 1987), at the present only zinc-containing derivatives were assayed. The results are displayed in Fig. 2.
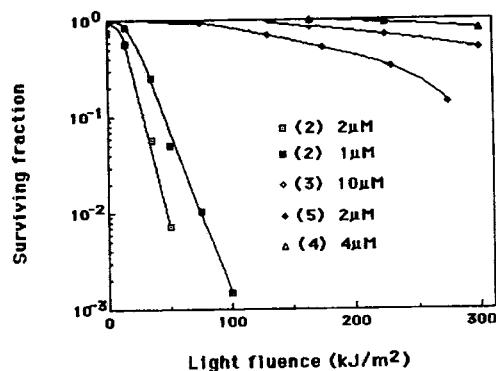
Figure 2. Survival curves of Chinese hamster cells photo-sensitized by phthalocyanines. Cells were incubated for 18 h with the indicated compounds, rinsed and exposed to graduated light fluence. Cell survival was determined using colony-forming ability as an endpoint.

It was suggested that the photodynamic activity of phthalocyanines is expressed in membrane deterioration (Ben-Hur and Rosenthal, 1986). Since charged groups impart a net surface charge to most biological membranes, this surface charge might be expected to affect the association of the sensitizer with the membranes, either at a specific site, or globally. Thus, mitochondria accumulate positively charged lipophilic dyes, apparently due to the trans-membrane potential which is 100–300 mV negative inside the organelle; up to 10^4 fold concentration gradient is theoretically possible. Mitochondria of carcinoma cells take up higher concentration of cat-ionic molecules, and retain them longer than most normal cells. Thus photodynamically active cationic dyes may serve as carcinoma cell-specific phototoxic agents (Oseroff et al., 1986).

Up to now, only neutral or negatively charged. phthalocyanines have been tested. Compound 3 is a cationic, water-soluble, phthalocyanine, contain-ing a tetraalkylammonium group at the remote end of the lipophilic side chains. However, this cationic phthalocyanine was less toxic to mammalian cells in culture than its neutral counterpart. The present results point to 2 as the most active compound in this group of new dyes. Compounds 4 and 5 dis-played very little phototoxicity. The octahydroxy derivative 5 is therefore inferior to its tetrahydroxy analog which possesses high photobiological activity (Rosenthal et al., 1987). This is presumably due to the decreased uptake of 5 into the cells, either because of the greater substitution and consequent steric hindrance, or lower solubility which is charac-teristic of highly symmetrical derivatives.

While these results are indicative of general pho-totoxicity, it remains to be seen if the efficiency of the cationic compound 3 is enhanced toward carcinoma cells in vivo.

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