Porphocyanines: Expanded Aromatic Tetrapyrrolic Macrocycles

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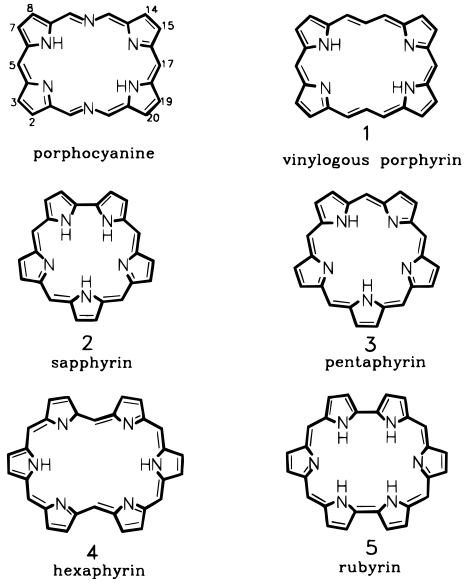
Abstract: Porphocyanines are tetrapyrrolic macrocycles containing two dipyrromethene units linked together by two C═N−C bridges. The syntheses of 2,3,7,8,14,15,19,20-octaethylporphocyanine, 5,17-diphenylporphocyanine, 2,3,7,8-tetraethyl-17-phenylporphocyanine, and other *meso*-aryl and *β*-alkyl, including a tetra-*β*-propionate, derivatives and metal complexes are described. The physical and chemical properties of porphocyanines show them to be aromatic. The aromaticity is reflected in the large deshielding of the outer *β*-protons and shielding of the inner pyrrolic protons in their NMR spectra. The optical spectra of these compounds exhibit Soret and Q bands similar to porphyrins but all significantly bathochromically shifted. The ability of porphocyanines to act as catalysts for the generation of singlet molecular oxygen was investigated using a cholesterol oxidation assay.

Introduction

Aromatic macrocyclic compounds capable of absorbing in the far red or near infrared region are now finding applications in biomedical research, specifically as photosensitizers for use in photodynamic therapy (PDT) of hyperprolipherative diseases,[1,2] e.g. cancer and psoriasis. PHOTOFRIN®, which is currently the only PDT agent approved for clinical use, consists of a mixture of oligomeric porphyrins derived from acetylation and subsequent base treatment of hematoporphyrin.[3] It is important to realize that this "first generation" photosensitizer can be improved upon. PHOTOFRIN® absorbs weakly above 630 nm and the depth of light penetration through tissue is attenuated due to the absorption by heme chromophores. The "second generation" of PDT sensitizers should, therefore, be compounds which absorb light at wavelengths significantly longer than 630 nm. Prior to the synthesis of porphocyanine, we had success in achieving strong absorptions in the far red region of the spectrum through modification of the porphyrin periphery.[4] As a result a well-characterized chlorin, called benzoporphyrin derivative (BPD),[5] is currently in phase II clinical trials. Another strategy for achieving strong absorption in the red region is to extend the conjugate bridges between the pyrrole groups in the porphyrin to form so-called "expanded porphyrins". Examples of such compounds[6] are the tetrapyrrolic vinylogous porphyrins (**1**), pentapyrrolic sapphyrins (**2**), pentaphyrins (**3**), hexapyrrolic hexaphyrins (**4**), and rubyrins (**5**), some of which have been shown to be good singlet oxygen producers[7] and promising photosensitizers for PDT and the photoinactivation of viruses in blood (PDI).[8] We adopted a similar approach in this study; extending the bridges between the dipyrromethene units in an α,*γ*-diazaporphyrin, we were able to synthesize a new class of aromatic expanded tetrapyrrolic macrocycle, to which we have given the generic name "porphocyanine".[9] The ultimate goal of this research is to synthesize a series of well-characterized compounds having the same "core" chromophore, but varying in peripheral substituents, to allow structure−activity relationships to be defined. Many of the new PDT photosensitizers have shown limited versatility with regard to altering the pattern of substitution, which has been shown to affect the biological activity.[10,11] We have, therefore, developed a synthetic scheme that promises a simple preparation and facile interchange of functional groups on a porphocyanine framework. Syntheses of 5-aryl- and 5,17-diarylporphocyanines are central to this strategy. We report here the facile syntheses of a range of these compounds having different substituents at both meso and *β* positions, all of which are shown to be capable of acting as catalysts for the generation of singlet molecular oxygen.

Chart 1



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Experimental Section

General. All reagents and solvents were used without purification unless otherwise stated. Ethanol used in the ammonia reaction was distilled over magnesium turnings under nitrogen prior to use. THF used in $LiAlH_4$ reductions was distilled over a sodium benzophenone complex under nitrogen. The purity of isolated compounds and the progress of all reactions were monitored by thin layer chromatography on silica gel plates (Merck kieselgel 60; 0.2 mm) or by observing the change in the optical absorption spectrum. Purification of porphocyanines was carried out on neutral deactivated Alumina (Grade II). In the case of 2,3,7,8-tetraethyl-17-phenylporphocyanine, reversed-phase HPLC (Waters μBondapak C_{18} column) on a Waters 994 instrument was performed for the separation. Molecular modeling was performed on a Silicon Graphics IRIS system using the program INSIGHT II by Biosym (San Diego). The lowest energy van der Waals configurations were determined using the INSIGHT interactive energy minimization routine. Microanalyses were performed at the Microanalytic Laboratory of the University of British Columbia.

Electronic absorption spectra were recorded on a Hewlett-Packard 8452A Diode Array spectrophotometer at room temperature. Fluorescence emission spectra were measured on an Aminco Bowman Series 2 Luminescence Spectrometer using a flash lamp excitation source. Values of pH were recorded on a Fisher Acumet pH meter model 210 with a glass combination electrode. The pH meter was calibrated at 25 °C with standard buffer solutions for pH 4.00 and 7.00 prior to pH measurements. Nuclear magnetic resonance spectra were recorded on a Bruker WH-400 spectrometer for ^{1}H and a Varian XL-300 spectrometer for ^{19}F. The chemical shift of ^{19}F was referenced to the chemical shift of CF_3CO_2H. Mass spectra were recorded on a Kratos/AEI MS-902 for EI and a Kratos Concept II HQ for both LSIMS and DCI.

Preparation of Compounds. 2,3,7,8-Tetraethyl-1,9-diformyldipyrromethane and dimethyl 2,8-dimethyl-1,9-diformyldipyrromethane-3,7-dipropionate were prepared according to literature procedures.[12] The 5-phenyl-dipyrromethanes were prepared from pyrrole and aromatic aldehydes in the presence of trifluoroacetic acid according to a reported procedure.[13] Preparation of 1,9-dicyanodipyrromethane derivatives (**6**, **8**−**10**) except 1,9-dicyano-5-phenyldipyrromethane (**7**) has been reported elsewhere.[14]

1,9-Dicyano-5-phenyldipyrromethane (7). The 5-phenyldipyrromethane (70 mg, 0.32 mmol) was dissolved in *N*,*N*-dimethylformamide (DMF) (5 mL) and the stirred mixture was cooled to −78 °C. To the cooled mixture was added dropwise a solution of chlorosulfonyl isocyanate (200 mg, 1.42 mmol) in acetonitrile (1 mL) under 1 atm of N_2. The mixture was stirred at −78 °C for 1 h and then at −40 °C for 1 h before being allowed to rise to room temperature. The reaction mixture was poured into aqueous KOH (3 N) and ice, then diluted with brine (200 mL) and extracted with CH_2Cl_2 (3 × 50 mL). The organic phase was dried over anhydrous K_2CO_3, the solvent evaporated *in vacuo*, and the residue chromatographed on silica gel to give the 1,9-dicyano-5-phenyldipyrromethane. Yield: 29 mg, 32%. ^{1}H NMR ($CDCl_3$): δ 5.5 (s, 1H), 5.95 (d, J = 1.5 Hz, 2H), 6.7 (d, J = 1.5 Hz, 2H), 7.05 (m, 2H), 7.4 (m, 3H), 9.65 (br s, 2H). FTIR ($CHCl_3$) ν_{CN} 2220 cm^{-1}. HRMS (EI) for $C_{17}H_{12}N_4$ calcd 272.1062, found 272.1060.

1,9-Di(aminomethyl)-2,8-diethyl-3,7-dimethyl-5-phenyldipyrromethane (18). A solution of 1,9-dicyano-2,8-diethyl-3,7-dimethyl-5-phenyldipyrromethane (**10**)[14] (44 mg, 0.12 mmol) in THF (5 mL) was added dropwise to a stirred suspension of $LiAlH_4$ (44 mg, 1.3 mmol) in THF (10 mL) under an atmosphere of N_2 at 0 °C. The reduction was followed by thin layer chromatography (silica gel), and when the reaction was complete, excess $LiAlH_4$ was quenched with water and the slurry was filtered. The filtrate was dried over anhydrous Na_2SO_4. The golden colored solution was concentrated and solid **18** was precipitated with hexane and collected by filtration. Yield: 11 mg (24%). ^{1}H NMR ($CDCl_3$): δ 1.0 (t, J = 7.4 Hz, 6H), 1.85 (s, 6H), 2.35 (q, J = 7.4 Hz, 4H), 2.7 (br s, 4H), 3.6 (m, 4H), 5.5 (s, 1H), 7.05 (m, 3H), 7.2 (m, 3H), 8.35 (br s, 2H). HRMS (DCI) for $C_{23}H_{32}N_4$ calcd 364.2627, found 364.2626.

2,3,7,8,14,15,19,20-Octaethylporphocyanine (11). Method 1. A dry THF solution of 1,9-dicyano-2,3,7,8-tetraethyldipyrromethane (**6**) (102 mg, 0.33 mmol) was added dropwise to a suspension of $LiAlH_4$ (102 mg, 3.0 mmol) in THF under N_2 at 0 °C. The resulting compound was not isolated. Instead, after quenching the excess reducing agent with water, filtering, and drying the solution over anhydrous Na_2SO_4, a 5-fold molar excess of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, Aldrich) was added to the stirred solution. The color of the solution turned from gold to dark green immediately. The resulting solution was concentrated and then chromatographed on a neutral alumina column (Grade II) eluting with 10% ethyl acetate in CH_2Cl_2. The bright green eluent containing the macrocycle was collected and evaporated to dryness. Yield: 46 mg, 48%. ^{1}H NMR (1% TFA in $CDCl_3$): δ −5.75 (br s, 4H), 2.30 (2t, J = 7.8 Hz, 24H), 4.78 (2q, J = 7.8 Hz, 16H), 11.97 (s, 2H), 13.75 (s, 4H). UV/vis $\lambda(\epsilon)$: 458 (2.4 × 10^5), 592 (1.7 × 10^4), 634 (5.8 × 10^3), 728 (3.2 × 10^3), 798 (2.7 × 10^4) nm in CH_2Cl_2; 454 (7.6 × 10^5), 600 (1.0 × 10^4), 608 (1.2 × 10^4), 624 (1.1 × 10^4), 668 (8.4 × 10^3), 682 (5.0 × 10^3), 744 (4.4 × 10^4) nm in TFA/CH_2Cl_2. HRMS (EI) for $C_{38}H_{48}N_6$ (M^+) calcd 588.3941, found 588.3933. Anal. Calcd for $C_{38}H_{48}N_6$·HCl: C, 73.00; H, 7.90; N, 13.43; Cl, 5.66. Found: C, 73.48; H, 7.89; N, 12.97; Cl, 5.67.

Method 2. 2,3,7,8-Tetraethyl-1,9-diformyldipyrromethane[15] (25 mg, 0.08 mmol) was suspended in dry ethanol (30 mL). The resulting ethanol solution was chilled to 0 °C and ammonia gas was bubbled through it for 30 min. The gas inlet was then removed and the flask, which was sealed with a septum (suba-seal; Aldrich), was placed in an oil bath and heated at 60 °C. The reaction was stopped after 80 h and cooled to 0 °C. Ethanol was removed by rotary evaporation and the residue was chromatographed on Neutral Alumina (Grade II) with 10% ethyl acetate in CH_2Cl_2. Yield: 6 mg (26%).

Spectroscopic data for this compound were found to be identical with those for compound **11** prepared by Method 1.

Tetraethyl 2,8,14,20-Tetramethylporphocyanine-3,7,15,19-tetrapropionate (16). This compound was prepared and purified according to Method 2 above using dimethyl 1,9-diformyl-2,8-dimethyldipyrromethane-3,7-dipropionate. Yield: 7%. ^{1}H NMR (1% TFA in $CDCl_3$): δ −5.80 (br s, 4H); 1.05 (t, J = 7.5Hz, 12H), 3.60 (t, J = 7.5 Hz, 8H), 4.03 (q, J = 7.5 Hz, 8H), 4.22 (s, 12H), 5.06 (t, J = Hz, 8H), 12.28 (s, 2H), 13.67 (s, 4H). UV/vis λ: 458, 592, 632, 724, 800 nm in CH_2Cl_2 and 454, 602, 612, 624, 672, 684, 746 nm in TFA/CH_2Cl_2. HRMS (EI) for $C_{46}H_{56}N_6O_8$ (M^+) calcd 820.4159, found 820.4153. Anal. Calcd for $C_{46}H_{56}N_6O_8$·2H_2O: C, 64.47; H, 7.08; N, 9.80; Found: C, 63.73; H, 7.18; N, 9.64.

5,17-Diphenylporphocyanine (12). A solution of 1,9-dicyano-5-phenyldipyrromethane (**7**) (29 mg, 0.1 mmol) in THF (5 mL) was added dropwise to a stirred suspension of $LiAlH_4$ (44 mg, 1.3 mmol) in THF (10 mL) under an atmosphere of N_2 at 0 °C. The reduction was followed by thin layer chromatography (silica gel). Excess $LiAlH_4$ was quenched with a few drops of water and the resulting slurry was filtered. The filtrate was dried over anhydrous Na_2SO_4 and filtered and the solvent was evaporated *in vacuo*. The residue was redissolved in dry CH_2Cl_2 (100 mL) and to the stirred solution was added DDQ in 5-fold molar excess. The oxidation was followed by UV/vis spectroscopy. When no further porphocyanine formation was detected, the reaction mixture was filtered through a plug of neutral alumina to remove excess DDQ. The crude 5,17-diphenylporphocyanine was purified by chromatography on alumina as described for octaethylporphocyanine. Yield: 15 mg (29%). ^{1}H NMR ($CDCl_3$): δ 7.92 (m, 6H), 8.44 (m, 4H), 9.38 (d, J = 5.7 Hz, 4H), 9.8 (d, J = 5.7 Hz, 4H), 12.95 (s, 4H). UV/vis λ: 452, 598, 640, 814 in CH_2Cl_2. HRMS (EI) for $C_{34}H_{24}N_6$ (M^+) calcd 516.2062, found 516.2058. Anal. Calcd for $C_{34}H_{24}N_6$: C, 79.07; H, 4.65; N, 16.28. Found: C, 78.94; H, 4.84; N, 15.98.

5,17-Bis(3′,4′,5′-trimethoxyphenyl)porphocyanine (13). This compound was prepared and purified according to the procedure outlined in the preparation of 5,17-diphenylporphocyanine using 1,9-dicyano-5-(3',4',5'-trimethoxyphenyl)dipyrromethane (**8**).[14] Yield: 32%. ^{1}H

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NMR ($CDCl_3$): δ 4.1 (s, 12H), 4.25 (s, 6H), 7.67 (s, 4H), 9.44 (d, J = 4.8 Hz, 4H), 9.8 (d, J = 4.8 Hz, 4H), 12.95 (s, 4H). UV/vis λ: 460, 602, 644, 818 nm in CH_2Cl_2. HRMS (EI) for $C_{40}H_{36}N_6O_6$ (M^+) calcd 696.2696, found 696.2690. Anal. Calcd for $C_{40}H_{36}N_6O_6 \cdot H_2O$: C, 67.22; H, 5.36; N, 11.75. Found: C, 67.12; H, 5.47; N, 11.67.

5,17-Bis(pentafluorophenyl)porphocyanine (14). This compound was prepared according to the procedure outlined in the preparation of 5,17-diphenylporphocyanine using 1,9-dicyano-5-(pentafluorophenyl)-dipyrromethane (**9**)[14] and purified by chromatography on alumina (Grade II) eluting with acetone. Solvent was evaporated from the eluent *in vacuo* and the residue was triturated with hexane and filtered to give **14** as lustrous green crystals. Yield: 28%. ^{1}H NMR (acetone-d_6): δ 9.55 (dd, J_1 = 15.22 Hz, J_2 = 4.15 Hz, 4H), 10.1 (d, J = 4.15 Hz, 4H), 13.24 (s, 4H). ^{19}F NMR (acetone-d_6): δ 60.53, 60.92, 61.45. UV/vis: λ 442, 582, 624, 814 nm in acetone. HRMS (EI) for $C_{34}H_{14}F_{10}N_6$ (M^+) calcd: 696.1120, found: 696.1124. Anal. Calcd for $C_{34}H_{14}F_{10}N_6 \cdot {}^1/_2H_2O$: C, 57.87; H, 2.10; N, 11.78. Found: C, 57.96; H, 2.44; N, 11.57.

2,3,7,8-Tetraethyl-17-phenylporphocyanine (17). 1,9-Dicyano-2,3,7,8-tetraethyldipyrromethane (**6**)[14] (62 mg, 0.2 mmol) and 1,9-dicyano-5-phenyldipyrromethane (**7**) (55 mg, 0.2 mmol) were dissolved in dry THF (5 mL) separately and the two solutions were mixed. To a suspension of $LiAlH_4$ (140 mg) in dry THF (10 mL) was added dropwise the mixed solution under 1 atm of N_2 at 0 °C. The reduction was followed by TLC and, when complete, excess $LiAlH_4$ was quenched with a few drops of H_2O. The resulting slurry was filtered and the filtrate dried over anhydrous Na_2SO_4. Evaporation of solvent *in vacuo* followed by redissolution in CH_2Cl_2 (20 mL) gave a golden solution. To this solution was added, dropwise, a solution of DDQ (0.45 g, 2.2 mmol) in toluene (7 mL). The oxidation was followed by UV/vis spectroscopy, and when no further porphocyanine formation was observed the solution was applied to an alumina (Grade II) column and eluted with 10% ethyl acetate in CH_2Cl_2. The bright green fractions containing all three porphocyanines were collected, yielding 44 mg of a dark green solid. 2,3,7,8-Tetraethyl-17-phenylporphocyanine (**17**) was separated from octaethylporphocyanine (**11**) and diphenylporphocyanine (**12**) on HPLC (reversed phase) with isocratic elution [0.1% trifluoroacetic acid in water/0.1% trifluoroacetic acid in acetonitrile (1:4)] yielding 23 mg (21%) of the isolated 2,3,7,8-tetraethyl-17-phenylporphocyanine (**17**). ^{1}H NMR ($CDCl_3$): δ 2.05 (t, J = 7.5 Hz, 3H), 2.07 (t, J = 7.5 Hz, 3H), 4.21 (q, J = 7.5 Hz, 2H), 4.32 (q, J = 7.5 Hz, 2H), 7.88 (m, 3H), 8.41 (m, 2H), 9.31 (d, J = 4.5 Hz, 2H), 9.7 (d, J = 4.5 Hz, 2H), 10.3 (s, 1H), 12.72 (s, 2H), 12.96 (s, 2H). UV/vis: λ 456, 592, 632, 806 nm in CH_2Cl_2 and 454, 602, 608, 670, 748 nm in TFA/CH_2Cl_2. HRMS (EI) for $C_{36}H_{36}N_6$ (M^+) calcd 552.3001, found 552.2996. Anal. Calcd for $C_{36}H_{36}N_6 \cdot {}^1/_2CF_3COOH$: C, 72.80; H, 6.11; N, 13.80. Found: C, 72.48; H, 6.39; N, 13.77.

Preparation of a Zn(II) Derivative of 2,3,7,8,14,15,19,20-Octaethylporphocyanine (24). Octaethylporphocyanine (**11**) (8 mg, 0.014 mmol) was dissolved in CH_2Cl_2 (5 mL), to which $ZnCl_2$ (20 mg, 0.15 mmol) in methanol (5 mL) was added. The resulting solution was heated at reflux for 20 min. The metalation was monitored spectroscopically and, when complete, the mixture was evaporated to dryness. The crude product was redissolved in CH_2Cl_2, washed three times with water, and dried over anhydrous $MgSO_4$. Filtration of the solution and evaporation to dryness gave a brown solid which was in turn dissolved in chloroform and transferred to a solvent diffusion apparatus. Diethyl ether was then slowly diffused into the chloroform solution. Several days later, the dark green crystals were collected. Yield: 46%. ^{1}H NMR ($CDCl_3$): δ −5.50 (s, 2H), 2.14, 2.20, 2.24, 2.27 (4t, J = 7.8 Hz, 24H), 4.48, 4.53, 4.60, 4.62 (4q, J = 7.8 Hz, 16H), 11.2, 11.38 (2s, 2H), 13.30, 13.55 (2s, 4H). UV/vis: λ 464, 476, 656, 666, 736 nm in CH_2Cl_2; 480, 658, 762 nm in Et_3N/CH_2Cl_2. HRMS (LSIMS) for $C_{38}H_{48}Cl_2N_6Zn$ (M^+) calcd 722.2603, found 722.2608.

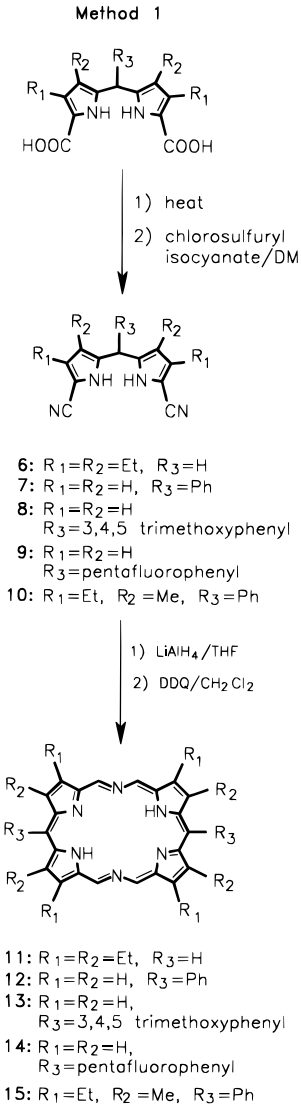
Cholesterol Oxidation Assay for Singlet Molecular Oxygen. Cholesterol (Sigma) freshly crystallized from methanol was dissolved in chloroform (Fisher HPLC grade) to a concentration of 5 mM. The porphocyanine was then dissolved in 10 mL of this solution to attain a photosensitizer concentration of 5 μM. The mixed solution was transferred to a stoppered glass tube fitted with gas inlet and outlet, and this was placed inside a dry-ice condenser with water flowing through the outer jacket. Air was bubbled through the solution and a filtered (Corion #2424) tungsten halogen lamp (250 W) was positioned at a distance of 1 cm so that the beam passed through the condenser and tube. The solution was irradiated with red light (>600 nm). The filter was cooled with a stream of air to prevent cracking. After 60 min the light was turned off and the tube removed. The solution was reduced in volume by 75% using a stream of nitrogen, and methanol (Fisher HPLC grade) was added to give a final volume of 5 mL. Sodium borohydride (Aldrich) (5 mg) was added and agitated to convert the unstable hydroperoxides to the corresponding alcohols. Samples (5 μL) were extracted by micropipet and spotted onto thin layer chromatograpy plates (Merck kieselgel 60; 0.2 mm) along with genuine samples containing 7α/β-hydroxycholesterol and 5α-hydroxycholesterol obtained by 2-methyl-1,4-naphthoquinone (Aldrich) and rose bengal (Aldrich) photosensitized oxidation of cholesterol. Plates were developed, twice, by elution with ethyl acetate/hexane (1:1) and products were visualized by immersing the plates in a 5% solution of concentrated sulfuric acid in ethanol and heating with a heat gun. Products were then compared against standards by R_f value relative to cholesterol: 5α-hydroxycholesterol, 0.39; 7β-hydroxycholesterol, 0.29; 7α-hydroxycholesterol, 0.22.

Results and Discussion

Preparation of the Compounds. Originally, 2,3,7,8,14,15,-19,20-octaethylporphocyanine (**11**) was isolated in very low yield by condensing 2,3,7,8-tetraethyl-1,9-diformyldipyrromethane and 1,9-di(aminomethyl)-2,3,7,8-tetraethyldipyrromethane in refluxing MeOH in the presence of molecular sieves (3 Å) followed by air oxidation. Considerable improvements in the synthesis of porphocyanines have been made in our laboratory since that time. The number of reaction steps have been reduced and the yield of **11** has increased significantly, from 6% to 48%, based on the dicyano derivative via Method 1 (Scheme 1). The key intermediate (**6**) was originally prepared by dehydration of the corresponding oxime in acetic anhydride. This procedure gave poor yields and involved a difficult separation of product from impurities.[15] A more efficient method for direct cyanation of dipyrromethanes was developed in our laboratory. The original lengthy reaction steps (formylation, oxime formation, and dehydration) were cut to a single step by employing chlorosulfonyl isocyanate in DMF/acetonitrile at low temperature. This cyanation reaction proceeds under mild conditions for all the 1,9-unsubstituted dipyrromethanes used in yields ranging from 30 to 60%.[14] The facile preparation of 1,9-dicyanodipyrromethanes (**6**−**10**) from the readily available dipyrromethanes gave us potential access to a wide range of differently substituted porphocyanines. Surprisingly, at this stage it was found that the THF solution of the supposed 1,9-bis(aminomethyl)-2,3,7,8-tetraethyldipyrromethane from $LiAlH_4$ reduction of **6** turned green after being left standing at room temperature in air for a few days. The optical spectrum of the resulting solution had bands characteristic of the previously isolated porphocyanine. This observation led to a 4-fold increase in the yield of **11** when the reduction product of **6** was simply condensed in refluxing MeOH/THF and subsequently oxidized with air at room temperature.[9] The 1,9-diformyldipyrromethane component was thus found to be superfluous! The yield of octaethylporphocyanine was further improved, to 48%, when the reduction product of **6** was oxidized *in situ* by DDQ immediately after quenching the excess $LiAlH_4$.

Diphenylporphocyanine derivatives (**12**−**14**) were readily prepared by the 1,9-dicyano route (Method 1) in moderate yields. Oxidation with DDQ during the formation of **11** was instantaneous; however, oxidations during the formation of **12**−**14** were slower and an excess of oxidant caused decomposition of the 5,17-diphenylporphocyanines over a prolonged period of time. Monitoring of porphocyanine formation by UV/vis was thus essential for compounds **12**−**14**. Excess DDQ must,

Scheme 1

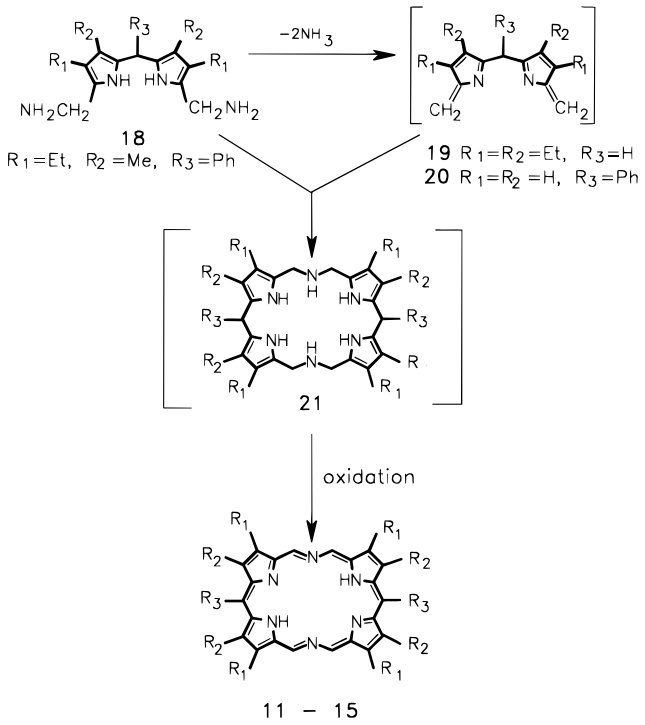


therefore, be removed immediately after porphocyanine formation is complete to limit degradation and ensure a good yield.

The uniqueness of this self-condensation reaction had attracted our attention from the outset. Spectroscopic analysis of the crude reduction product indicated a mixture of several species. Attempts to trap this supposed 1,9-bis(aminomethyl)-2,3,7,8-tetraethyldipyrromethane via acetylation resulted in decomposition. Purification via column chromatography also failed to provide cleaner compounds. It is known in the literature that α-(aminomethyl)pyrrole is unstable and can eliminate ammonia to form a resonance-stabilized azafulvene which is extremely electrophilic.[16] Nucleophilic attack of a primary amine on such an azafulvene could lead to the formation of a secondary amine. In the present case, attack of a 1,9-bis(aminomethyl)dipyrromethane molecule (e.g. **18**) on an azafulvene intermediate (e.g. **19**) could result in a cyclic, porphyrinogen-like structure (**21**, Scheme 2), which upon oxidation gives porphocyanine **11**. The differences in yields for octaethyl- and diphenylporphocyanines may be rationalized on the basis of the azafulvene intermediate since azafulvene **19**, with ethyl groups at all four β-pyrrolic positions, will be both electronically stabilized by induction and sterically protected from nucleophilic attack at these positions; conversely, **20** has no electronically stabilizing groups on the pyrrolic rings and is open to attack at both the exocyclic

(16) Paine, J. B., III *The Porphyrins*; Dolphin, D., Ed.; Academic Press: New York, 1978; Vol. 1, pp 101−234.

Scheme 2



methylene group and the β-pyrrolic positions. Formation of **11** should thus be more facile than the formation of **12**−**14**. The isolation and characterization of 1,9-bis(aminomethyl)-2,8-diethyl-3,7-dimethyl-5-phenyldipyrrromethane (**18**) might, at first, seem inconsistent with the instability of α-(aminomethyl)-pyrrole. However, the extremely low yield (<1%) of **15**[17] after DDQ oxidation of the 1,9-dicyano-2,8-diethyl-3,7-dimethyl-dipyrromethane reduction product mixture implied to us that both the 1,9-bis(aminomethyl) derivative **18** and the corresponding porphyinogen-like intermediate **21** were present in solution (Scheme 2), with **18** as the major product. Molecular modeling showed that steric interaction of the phenyl and methyl substituents in **18** limited the flexibility of the dipyrromethane, thus making the attainment of the correct transition state geometry for macrocycle formation more difficult. Isolation of **18** may, therefore, simply be the result of increased difficulty in forming **21**. Molecular modeling also indicated that porphocyanine **15** was sterically strained by interaction of the 5,-17-phenyl and 3,7,15,19-methyl groups causing the porphocyanine framework to ruffle. A slight twisting in the bis(*p*-nitrophenyl)amethyrin framework (a structurally similar non-aromatic hexapyrrolic version of porphocyanine) was recently reported by Sessler et al.[18] Porphocyanines bearing β-pyrrolic alkyl groups (**11** and **16**) are generally more acid resistant than those which are β-unsubstituted (**12**−**14**). Porphocyanines substituted on both 5,17 and pyrrolic β positions should have similar stability to β-alkylporphocyanines while retaining the possibility of placing a wide range of substituents on the phenyl groups. Knowing that even more strained porphyrins (perhalogenated porphyrins[19] and dodecasubstituted porphyrins[20])

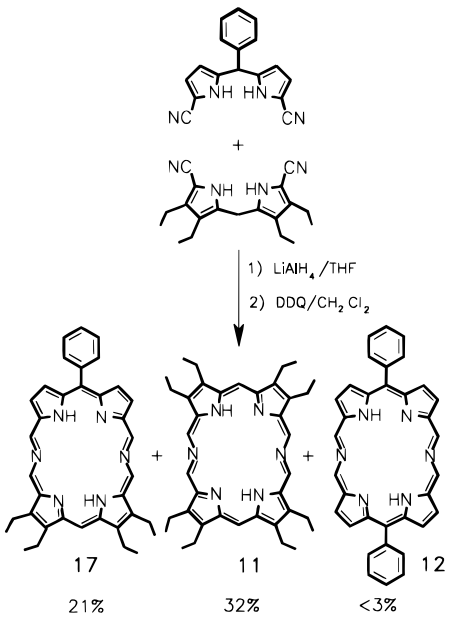
(17) This green compound was partially characterized by UV/vis and mass spectroscopy. UV/vis: λ 462, 604, 642, 802 nm in CH_2Cl_2. HRMS-(EI) for $C_{46}H_{48}N_6$ (M^+) calcd 684.3940, found 684.3932.

(18) Sessler, J. L.; Weghorn, S. J.; Hiseada, Y.; Lynch, V. *Chem. Eur. J.* **1995**, *1*, 56−67.

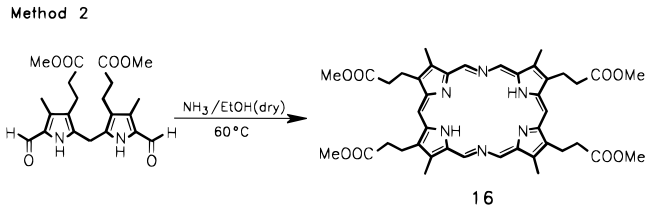
(19) Mandon, D.; Fischer, J.; Weiss, R.; Jayaraj, K.; Austin, R. N.; Gold, A.; White, P. S.; Brigaud, O.; Battioni, P.; Mansuy, D. *Inorg. Chem.* **1992**, *31*, 2044−2049.

(20) Shelnutt, J. A.; Medford, C. J.; Berber, M. O.; Barkigia, K. M.; Smith, K. M. *J. Am. Chem. Soc.* **1991**, *113*, 4077−4087.

Scheme 3



Scheme 4



have been synthesized and are stable, new routes for synthesizing porphocyanines of this type are being explored.

By analogy with porphyrinogen formation, it is reasonable to assume that an equilibrium exists between the 1,9-bis-(aminomethyl)dipyrromethanes and the corresponding non-aromatic tetrapyrrolic macrocycle. The presence of such an equilibrium offers the possibility of an efficient mixed condensation to obtain an asymmetric porphocyanine. When the 1,9-dicyano-5-phenyldipyrromethane (**7**) and 1,9-dicyano-2,3,7,8-tetraethyldipyrromethane (**6**) were reduced together and the products subsequently oxidized with DDQ (Scheme 3), three porphocyanines (**11**, **12**, **17**) were formed with **11** and **17** being detected by ^{1}H NMR spectroscopy in a 1.3:1 ratio and **12** only being detected by reversed-phase HPLC (<3%). The low yield of 5,17-diphenylporphocyanine in this case would seem to suggest an order of thermodynamic stability for the three products of **11** > **17** >> **12**.

We have also found that 2,3,7,8-tetraethyl-1,9-diformyldipyrromethane reacts with ammonia in dry ethanol (Scheme 4) (Method 2) leading to the formation of **11** in 26% yield.[15] To our knowledge, there has been no literature precedent where two aldehydes are directly condensed by the reaction with ammonia to form an aromatic macrocyclic structure. An obvious advantage of this method over the cyano route is that no reduction is involved in the synthesis. Consequently, porphocyanines with reduction-sensitive functional groups, such as **16**, can be readily prepared by this method. Under these conditions, transesterification takes place and small amounts of amide are also formed during the reaction. The amides thus formed were insoluble and easily removed. Attempts to condense 1,9-diformyl-5-phenyldipyrromethane with ammonia in dry ethanol were unsuccessful. The 1,9-diformyl-5-phenyldipyrromethane appeared to be unstable in the ethanolic ammonia solution, decomposing over the course of a few hours without the formation of porphocyanine.

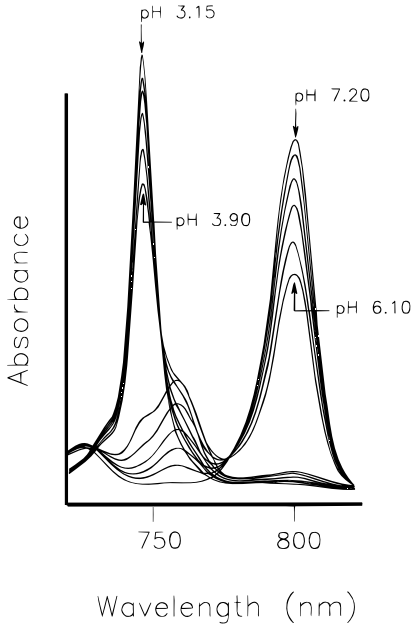


Figure 1. Spectral changes of octaethylporphocyanine (**11**) in CH_2Cl_2 equilibrated with 0.1 M NaCl solutions from pH 3.15 to 7.20 at 22 °C.

Chemical Properties. The porphocyanine nucleus contains six nitrogens. The relative basicity of the pyrroleneic nitrogens verses the bridging imino nitrogens and the pK_a values of these nitrogens are of particular interest to us, as we know that protonation of porphocyanine not only affects the light absorption properties but also plays an important role in the biodistribution of photosensitizers *in vivo*.[21] We have found in our study that when the pyrrolic β-positions are free of substituents porphocyanines become sensitive toward acid, particularly strong acids such as TFA, while β-alkylated porphocyanines are stable under such conditions. Octaethylporphocyanine (**11**) was therefore chosen, and titrated spectrophotometrically in a biphasic system (CH_2Cl_2/aqueous NaCl 1:1). Three species (free base, monocation and dication) were present in solution throughout the titration (eqs 1 and 2).[22] Two equivalents of acid were added to **11** to give the spectrum of the dication, which remained unchanged upon addition of more acid. The bridging nitrogen atom in an etioazaporphyrin is about 7 pK_a units less basic than the pyrroleneic nitrogen.[23] However , the protonation of more than two nitrogen atoms was never observed with porphocyanine even in concentrated H_2SO_4.

$$H_2P + H^+ \overset{K_3}{\leftrightarrow} H_3P^+ \quad (1)$$

$$H_3P^+ + H^+ \overset{K_4}{\leftrightarrow} H_4P^{2+} \quad (2)$$

The pK_a values of octaethylporphocyanine were estimated to be 6.0 and 4.4 from the spectral changes recorded at specific wavelengths for the monocation and dication, respectively[22] (Figure 1). Compared to that of sapphyrin (**2**) whose pK_a's were also estimated by the same method to be 9.5 and 3.5,

(21) (a) Pottier, R. H.; Kennedy, J. C.; Chow, Y. F. A.; Cheung, F. *Can. J. Spectrosc.* **1988**, *33*, 57−63. (b) Pottier, R. H.; Laplante, J. P.; Chow, Y. F. A.; Kennedy, J. C. *Can. J. Chem.* **1985**, *63*, 1463−1467.

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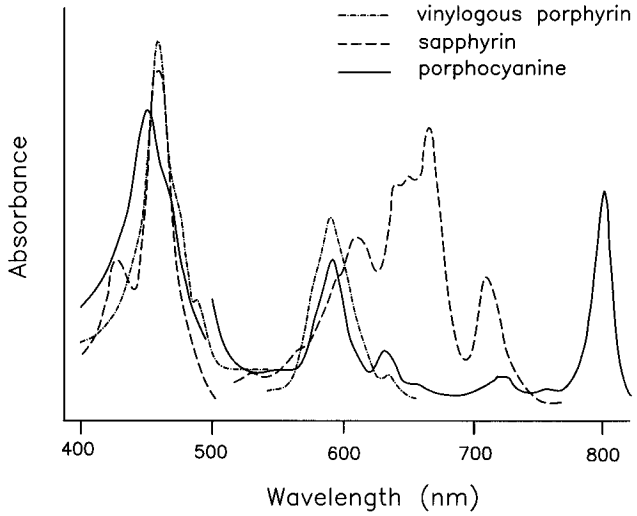


Figure 2. Electronic absorption spectra of porphocyanine (**11**), sapphyrin (**2**), and vinylogous porphyrin (**1**).

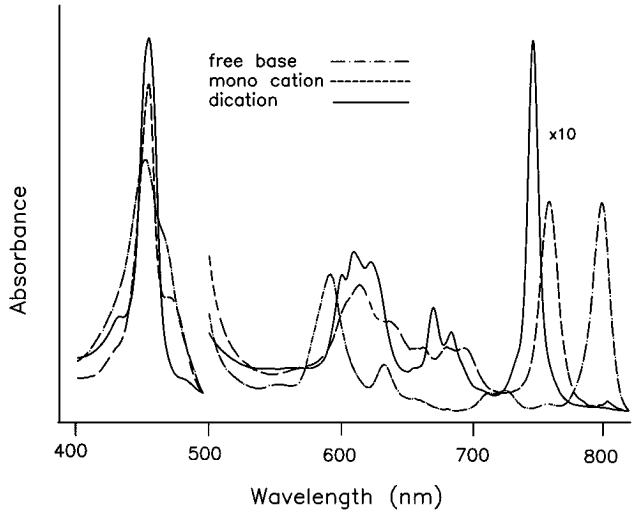
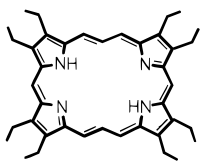


Figure 3. Electronic absorption spectra of octaethylporphocyanine (**11**) in methanol.

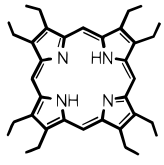
Table 1. ^{1}H NMR Data for Octaethyl Derivatives of Porphyrin, [22]Porphyrin, and Porphocyanine in $CDCl_3$/1% TFA

octaethylporphyrin (**23**)	[22]octaethylporphyrin (**22**)	octaethylporphocyanine (**11**)
	12.78 (d)	13.75 (s)
10.66 (s)	11.96 (s)	11.97 (s)
−3.85 (s)	−6.61 (s)	−5.75 (s)
	−9.54 (t)	

porphocyanine **11** appeared to be less basic than sapphyrin. The electronic absorption spectra of all the porphocyanines **11**−**17** exhibit an intense Soret-like band at 440−460 nm and a weaker band at 798−818 nm, consistent with a fully conjugated aromatic porphyrin-like structure. The position of the Soret-like band seems quite constant within the 22π-electron systems, porphocyanine, vinylogous porphyrin (**1**) sapphyrin (**2**), and pentaphyrin (**3**) (Figure 2). However, the Q-type band of porphocyanine **11** exhibited the largest bathochromic shift among the 22π-electron "expanded porphyrins". Most striking is the observation that replacement of the two C−C═C bridges of vinylogous octaethylporphyrin (**22**) with two C−N═C bridges in **11** shifts the longest wavelength absorption band by more than 200 nm in the free base form! Substituents on the porphocyanine perimeter cause shifts in the wavelength of the absorption maxima; however, the basic characteristics of the porphocyanine are retained. Addition of acid caused a normal hypsochromic shift of the Q-type absorption, whereas in **22** Q bands shift to longer wavelength upon protonation. The absorption spectra of the three forms of **11** in methanol are presented in Figure 3.



22



23

The ^{1}H NMR spectrum of **11** in 1% TFA/$CDCl_3$ showed well-resolved peaks at 13.75 (s, 4H) and 11.95 (s, 2H) ppm. The *meso* proton signals observed for this compound are more than 1 ppm downfield compared to the corresponding signals for the dihydrotrifluoroacetate salt of octaethylporphyrin (Table 1), but are strikingly similar to that of **22**.[24] The methine protons of the C−N═C bridge are shifted about 3 ppm downfield from the *meso* protons of octaethylporphyrin (**23**) and 1 ppm from that of **22**, presumably due to the deshielding effect of nitrogen. Attempts to measure the ^{1}H NMR spectra in 1% TFA/$CDCl_3$ led invariably to the decomposition of the β-unsubstituted porphocyanines **12**−**14**. The ^{1}H NMR of **12** in $CDCl_3$, however, also showed well-resolved peaks at 12.95 (s, 4H), 9.8 (d, 4H), and 9.38 (d, 4H) ppm. For comparison, the ^{1}H NMR spectrum of **11** in $CDCl_3$ was also recorded. Resonance of the C−N═C methine protons appeared at 12.92 (s, 4H) ppm, while the *meso* protons were observed at 10.50 (s, 2H) ppm. The chemical shift of the four protons on the C−N═C bridges for **12** appears at 12.95 ppm, very similar to that of **11**. This signal stayed relatively constant in **12**−**14**, the substituents on the phenyl groups do not seem to influence the chemical shifts of these protons. The β-pyrrolic hydrogen resonances observed in **12**−**14**, however, are about 1 and 0.5 ppm downfield compared to the corresponding signals for the tetraphenylporphyrin in $CDCl_3$.[25] The shifts reflect the larger ring current in 22π-electron porphocyanines relative to the 18π-electron porphyrins. The spectrum recorded for **11** in 1% TFA/$CDCl_3$ also shows a singlet upfield of TMS, located at −5.75 ppm, comparable to the signal of NH protons in **22**.[24]

Electrophilic substitution of the *meso* protons by deuterium is common in polypyrrolic aromatic macrocycles. The exchange is rapid for chlorins,[26] corroles,[27] and sapphyrins,[28] but slow for porphyrins, even at elevated temperature.[29] The outer *meso* protons of vinylogous octaethylporphyrin (**22**) readily undergo electrophilic substitution while the inner (CH) protons are completely inert. The ease of electrophilic attack on the porphocyanine nucleus was also demonstrated: when the CF_3-COOD solution of octaethylporphocyanine **11** was left standing at room temperature overnight complete exchange of all six protons attached directly to the macrocycle for deuterium resulted, as shown by mass spectral analysis.

(24) Konig, H.; Eickmeier, C.; Moller, M.; Rodewald, U.; Franck, B. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1393−1395.
(25) Storm, C. B.; Teklu, Y.; Sokolshi, E. A. *Ann. N.Y. Acad. Sci*. **1973**, *206*, 631−638.
(26) Woodward, R. B.; Skaric, V. *J. Am. Chem. Soc.* **1961**, *83*, 4676−4678.
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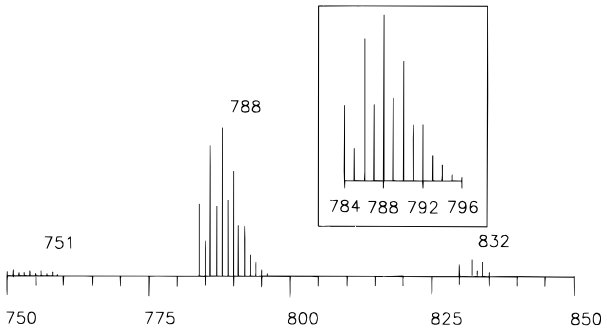

Figure 4. Observed mass spectrum of Zn(octaethylporphocyanine)Cl_2 by electron impact ionization (insert: theoretical mass spectrum of Zn_2(octaethylporphocyanine)Cl_2 [$C_{38}H_{46}N_6Zn_2Cl_2$]).

We had originially expected that the large cavity in the center of the porphocyanine ring and the planar multidentate array of nitrogens might make this macrocycle a good ligand for lanthanides[30] and actinides.[31−33] However, we have been unable to form a complex between UO_2^+ and octaethylporphocyanine. Conversely, when methanolic solutions of the acetate salts of Cd^{2+}, Mn^{2+}, Co^{2+}, and Zn^{2+} were added to CH_2Cl_2 or $CHCl_3$ solutions of octaethylporphocyanine at elevated temperatures, spectral changes indicated that metal complexes were formed and the Zn^{2+} complex (**24**) was isolated as a crystalline solid in low yield. The visible spectrum of the complex which exhibits a split Soret (464, 476 nm) and a weaker Q band (736 nm) changes to a single Soret (480 nm) and a red-shifted Q band (762 nm) upon addition of base, e.g. Et_3N, and can be restored by neutralization with acetic acid. This suggested the presence of acidic pyrrolic protons (NH), which was consistent with the observation of a sharp singlet at δ −5.50 (2H) in the ^{1}H NMR spectrum. The ^{1}H NMR spectrum of **24** displays two singlets for the protons of the C−N═C bridges (13.3 and 13.55 ppm), and also shows a spliting of the *meso* signal into two singlets. Coordination of zinc, therefore, causes the chemical environments of the two "dipyrromethene" halves of the porphocyanine to become nonequivalent. These spectroscopic data suggested the formation of a mono zinc complex. The mass spectrum, however, displayed an intense cluster of peaks centered at *m/e* 788.1717 (relative intensity 100%), indicative of the presence of two zinc and two chlorine atoms by isotope analysis (Figure 4). The mass spectrum finding was surprising in that chloride, which was present only in trace amount as impurity in the CH_2Cl_2 solvent, was strongly prefered over acetate anion which was present in large excess, when forming the zinc complex. The yield of zinc complex was dramatically increased by replacing zinc acetate with zinc chloride at the chelation step. The X-ray structure of the zinc complex (**24**) (Figure 5)[9] revealed that the metal was tetrahedrally coordinated to two pyrrolic nitrogens and two chlorine atoms, leaving the bridging nitrogens uncoordinated and the other two pyrrolic nitrogens protonated with hydrogen-bonding interactions to the chlorines. The crystal structure of the zinc complex compliments the ^{1}H NMR and electronic absorption spectral findings. The molecular mass, as revealed by liquid secondary ion mass spectrometry, indeed confirmed the structure revealed by X-ray. Therefore, it is likely that a disproportionation reaction occurs on the probe prior to evaporation. A dizinc unit can indeed be accommodated in the central cavity of amethyrin which has essentially the same core size as porphocyanine.[18]

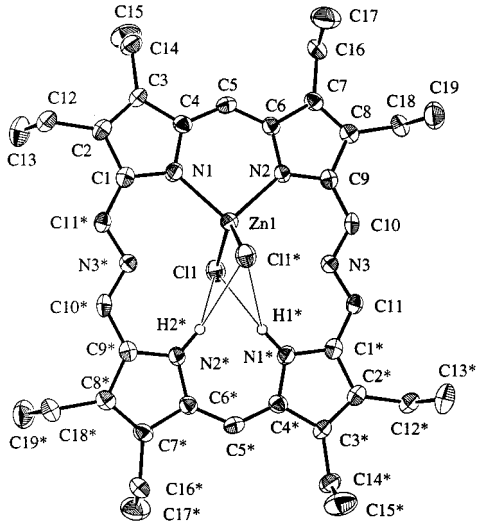

Figure 5. ORTEP presentation of molecular structure of Zn(octaethylporphocyanine)Cl_2 (**24**). View of the Zn complex showing the tetrahedral coordination around zinc and hydrogen-bonding interactions of NH and chlorine ligands. Mean derivation from the plane is 0.0095 Å. Some relevant bond lengths (Å) are Zn−N1 2.050, Zn−N2 2.063, Zn−Cl1 2.247, and Zn−Cl2 2.240. The molecule is situated at a crystallographic center of symmetry, and an asterisk implies 50% occupancy of the atom at these sites and 50% occupancy at the corresponding nonasterisked site.

As we were particularly interested in the porphocyanines as possible new, long-wavelength-absorbing photosensitizers for photodynamic therapy, we believed it would be prudent to assay these compounds for their ability to catalyze the singlet oxygen mediated oxidation of a biological substrate. The photosensitized oxidation of cholesterol can proceed by two different pathways: type I (radical mediated) and type II (singlet oxygen mediated).[34] Each pathway leads to discrete products: 7-α/β-hydroperoxycholesterol for type I and 5α-hydroperoxycholesterol for type II. Thin layer chromatographic analysis of the products of these photosensitized reactions against genuine samples of 7-α/β- and 5α-hydroxycholesterol indicated that compounds **11**−**15** all catalyzed the type II photooxidation of cholesterol under these experimental conditions.

Concluding Remarks

The ease of preparation of the porphocyanines, their long-wavelength absorptions in the visible−near infrared region, and their ability to generate singlet oxygen makes them potential candidates for photodynamic therapy.

Acknowledgment. We thank the Natural Sciences and Engineering Research Council of Canada for financial support.

JA953721K

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