New Sensitizers for Photodynamic Therapy: Controlled Synthesis of Purpurins and Their Effect on Normal Tissue

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Purpurins are a class of porphyrin derivative that have been shown to have good in vivo cytotoxicity to *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide (FANFT) induced rat bladder tumors (AY-27) implanted into Fisher 344 rats. The synthesis of purpurins from etioporphyrin I and coproporphyrin I proceeds in high yield and with a high degree of regioselectivity. Product formation can be rationalized in terms of relief of steric strain about the periphery of the purpurin macrocycle. The effect of therapeutic light doses using the rat footpad model suggests that, at therapeutic sensitizer doses, normal tissue damage is within acceptable limits, particularly for metalated purpurins.

Photodynamic therapy (PDT) has drawn some attention as a new approach to the treatment of selected human neoplasms. The technique uses an exogenously administered photosensitizer that "localizes" in the neoplasm. In combination with visible (red) light from a laser, a photodynamic effect is generated that leads to tumor destruction. Currently, the most widely used photosensitizer for PDT is hematoporphyrin derivative (HpD) or its putative active component Photofrin II.[1] Although somewhat effective in uncontrolled clinical trials, both HpD and Photofrin II are mixtures of various porphyrin species, each of whose contribution to the total biological effect remains unclear. In addition, the absorption maxima of these species are at 630 nm, a region in which light penetration of tissue is relatively poor. Finally, these absorptions (of HpD) are weak (extinction < 5000 L mol^{-1} cm^{-1}), which is a disadvantage for photodynamic reactions where efficient capture of photons is needed.

The only significant side effect noted to date during HpD-based PDT is related to concentrations of the drug that reside in skin and are cleared only very slowly. Patients are therefore advised to avoid strong sunlight for periods of 2–4 weeks. Failure to do so can lead to quite dramatic effects in the exposed tissue.[2]

During the past few years, therefore, a number of alternative sensitizers have been developed and proposed as potential candidates for PDT. These sensitizers, which absorb further into the red region of the visible spectrum (where light penetration of tissue is optimum[3]) include the phthalocyanines,[4-6] naphthalocyanines,[7] chlorins (in particular monoaspartylchlorin e_6[8] and the pheophorbide series[9]), tetraphenylporphyrin derivatives (e.g. tetrakis(hydroxyphenyl)porphyrins[10]), hematoporphyrin diethers,[11] and Diels–Alder adducts of protoporphyrin IX.[12]

We ourselves have developed an alternative series of sensitizers called purpurins, and have described the in vivo cytotoxicity of these compounds to the FANFT-induced urothelial cell carcinoma transplanted onto Fischer CDF (F344/CrlBR) rats.[13] We found that histological examination of tumors treated with purpurin-based photodynamic therapy, 24 h after treatment, showed extensive hemorrhagic necrosis, suggesting that the mechanism of action of these sensitizers included disruption of the tumor vasculature.[13a]

More extensive studies, including a dose–response analysis both 12 and 30 days after treatment indicated that, at appropriate doses, all purpurins caused extensive tumor regression and in many cases complete cure (based on 30-day studies).[13b,d]

Subsequent studies on metallopurpurins suggested that they were also suitable candidates for use in photodynamic therapy, with histological studies and preliminary data on dose–response analyses suggesting even greater cytotoxicity than found for the metal-free analogues.[13c,d]

Some of the advantages of using purpurins as sensitizers for photodynamic therapy include the ease of synthesis of these compounds from the corresponding porphyrins and the high degree of purity with which products are obtained. As stated above, however, one of the limitations of PDT in general is related to the clearance of the sensitizer used, from normal tissue. We report here the synthesis of purpurins from porphyrins with "type 1 symmetry" and the effect of purpurins on normal tissue in the Fisher 344 rat.

Synthesis

We have previously reported on the synthesis of the

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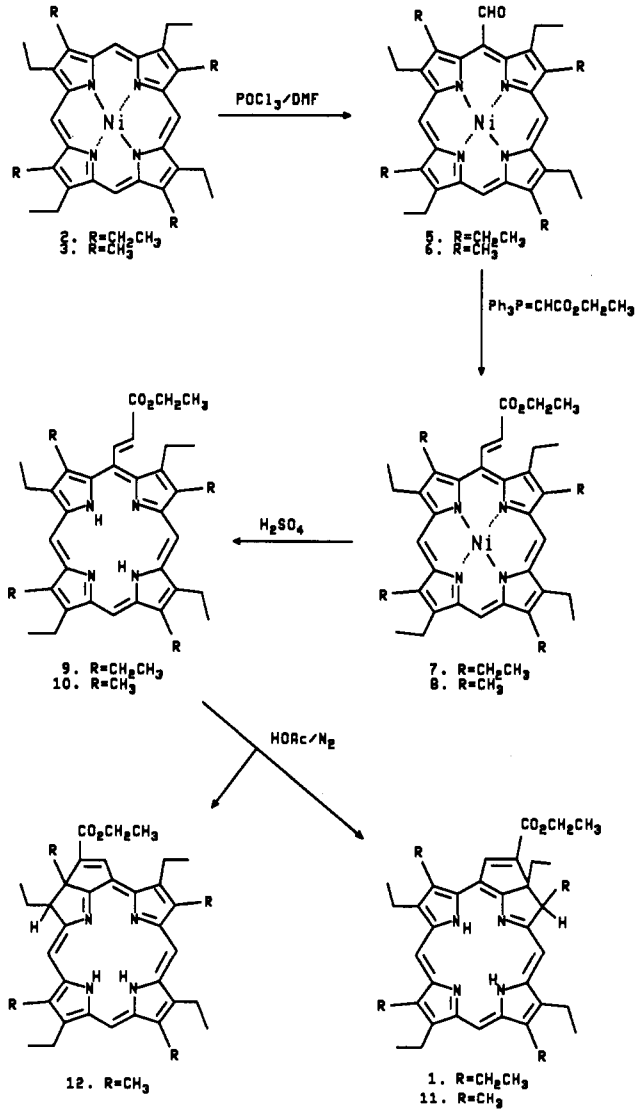

Figure 1.

octaethylpurpurin 1 from nickel octaethylporphyrin 2 (Figure 1).[14] Since the symmetry of 2 is not present in most porphyrins, the stereoselectivity and specificity of purpurin formation from porphyrins capable of forming more than one product (e.g. nickel etioporphyrin, 3, or nickel coproporphyrin I tetramethyl ester, 4) becomes critical.

We have recently established that although cyclization of the meso acrylic intermediate 9 to octaethylpurpurin 1 results in initial formation of both cis and trans isomers, rapid equilibration of the kinetically favored cis isomer to the thermodynamically favored trans isomer results in a high yield of this species.[14] With respect to regioselectivity during the cyclization process, previous observations have shown that, in the case of the acrylate 13, cyclization occurs to give purpurin 14 as the major product (Figure 2).[15] In addition, Fuhrhop et al. noted no purpurin formation from the unsubstituted reactant porphin, indicating that the driving force for cyclization that occurred in the octaethyl case (described above) was coupled to relief of steric strain.[16]

While relaxation of steric stress may be important in the latter case, it can be argued that the selectivity noted in the transformation of 13 to 14 is a consequence of electronic factors in which the unfavorable disruption of the conjugated peripheral carboxylate drives cyclization to the adjacent ring. The question therefore remains, can cyclization be controlled in unsymmetric porphyrins in which electronic factors can play little or no part? We now present evidence that the specificity of these cyclizations can also be controlled.

Thus, transformation of nickel etioporphyrin I, 3, into *meso*-[β-(ethoxycarbonyl)vinyl]etioporphyrin I, 10, by methods similar to those previously described for the octaethyl series[14] results in a 90% yield of 10 (Figure 1). Cyclization (of 10) by refluxing in glacial acetic acid, under a nitrogen atmosphere, could occur to give either purpurin 11, 12, or a mixture of both. In practice, workup of the resulting reaction mixture resulted in isolation of only one product in a yield of 92%. Ring closure to the purpurin form was established by mass spectrometry (product isomeric with reactant) and by visible spectroscopy (λ max 695 nm, similar to reported values for other purpurins).[14,15,17]

NMR spectroscopy was used to distinguish between the possible products 11 and 12. In particular, ring closure of acrylate 10 to 11 would yield a product in which the methyl group of the reduced pyrrolic ring would show coupling to the ring proton in the proton NMR spectrum, while production of 12 would produce a noncoupled methyl group. The proton NMR spectrum of the product was therefore inspected for the occurrence of an upfield doublet (attributable to the methyl group of 11) or of an upfield singlet (assignable to the methyl group of 12). A three-proton doublet was observed (δ = 2.50 ppm), confirming the structure of the purpurin as 11, and in good agreement with the reported chemical shift (δ = 2.10 ppm) of a methyl group in a similar environment in methyloctaethylchlorin 15 (Figure 3).[18]

The preferential formation of 11 over 12 confirms the role of relief of steric stress as the driving force of the reaction. Clearly, the sp^3 hybridization of the ethyl-bearing carbon results in a deviation of this group from planarity with greater relief of steric stress than would be gained from similar hybridization of the methyl-bearing carbon.

Studies on coproporphyrin I tetramethyl ester, 4, substantiate this observation. Thus, cyclization of the *meso*-acrylate intermediate 18 results in formation of a purpurin (characterized by mass spectrometry and visible spectroscopy) formulated as 19 on the basis of the presence in the NMR spectrum (of 19) of a three-proton doublet (δ = 2.54 ppm) assignable to the methyl group of the reduced pyrrolic ring (Figure 3).

The insertion of metals (zinc and tin) into the two purpurins 1 and 11 was performed as previously described.[13c]

Purpurin Tautomerization

It has previously been reported that the preparation of purpurins results in an equilibrium mixture, with $K_{purpurin}/K_{porphyrin}$ = 5:3 (for 13 → 14).[15] The purpurin can, however, be separated from the porphyrin tautomer in a

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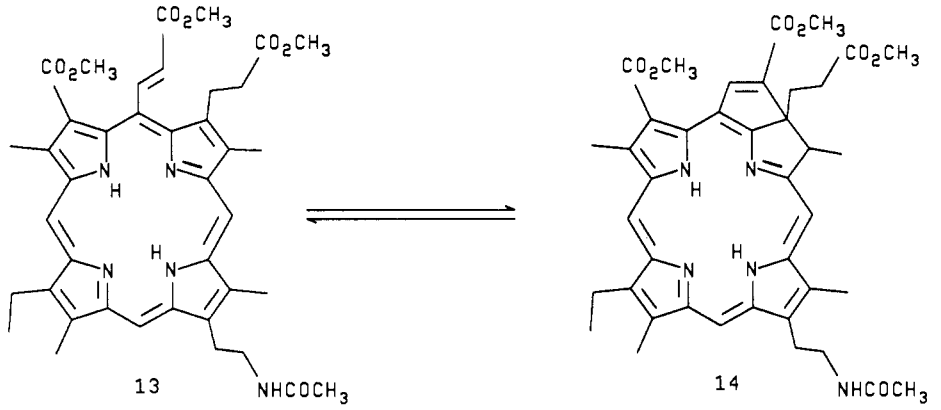

Figure 2.

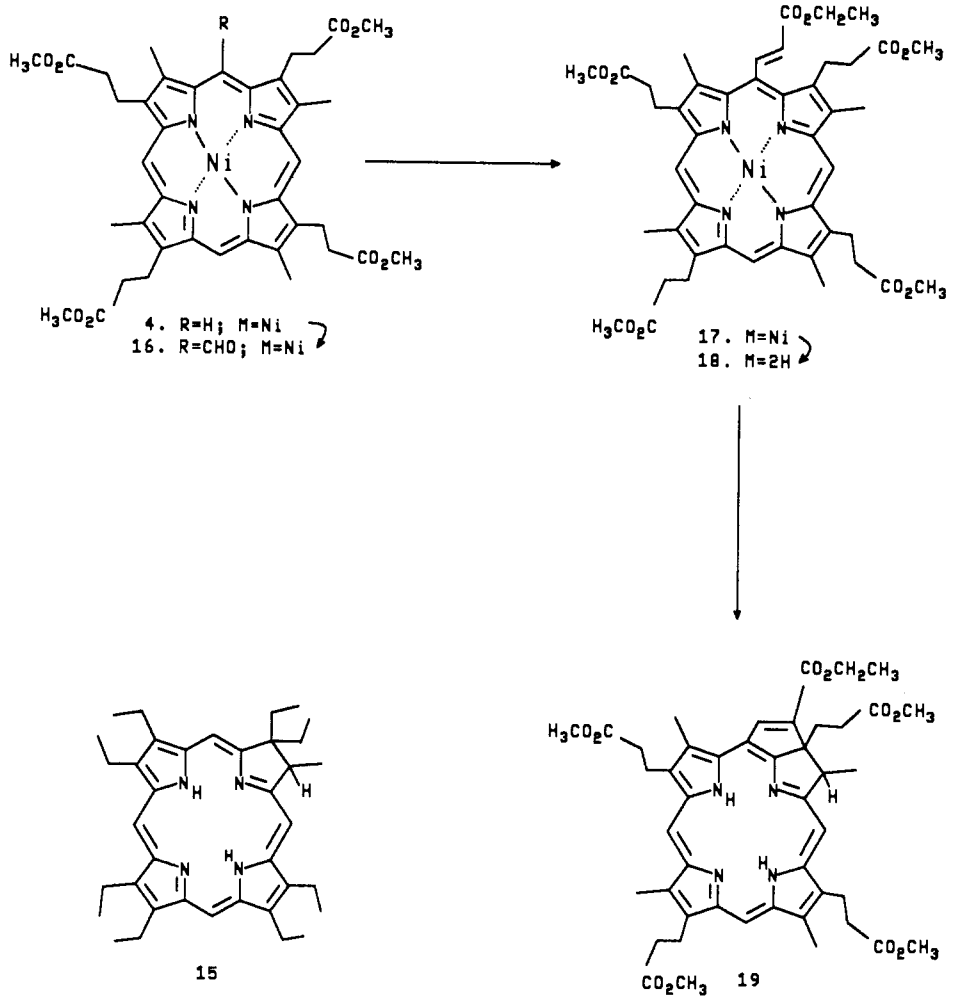

Figure 3.

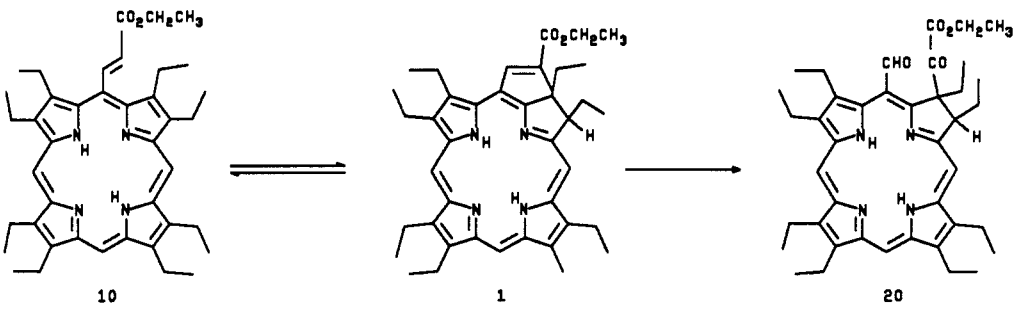

Figure 4.

high degree of purity. We have recently reported the chromatographic purification of purpurin 1 using silica gel and 2% methanol in dichloromethane as eluent.[14] With this system, however, care must be taken since prolonged exposure to the column and the presence of light result in two transformations of the purpurin: to the porphyrin tautomer **10** and to the oxidized form **20**, respectively (Figure 4).

This problem can be overcome by simple recrystallization of the crude product (2% hexane in dichloromethane), which results in a high yield of the purpurin **1**, at least 95% pure by ^{1}H NMR spectroscopy. The remaining 5% appears to be the porphyrin form **10**, with no oxidation product **20** detectable, as determined by ^{1}H NMR spectroscopy.

A similar observation was made with the purpurin **11** and with the metallo derivatives. The use of heat to insert metals (e.g. Zn, Sn) into the purpurin results in a change in K_{eq} such that the crude products, by ^{1}H NMR spectroscopy, are mixtures of the purpurin/porphyrin tautomers (80:20). In the case of the zinc derivatives, chromatography results in a lowering of the yield of metallopurpurin due to the same processes of tautomerization and oxidation observed for the metal-free systems. However, recrystallization of the crude product from hexane/dichloromethane results in a product free of these byproducts (as determined by NMR). In the case of the tin derivatives, successful chromatographic conditions have not been found; however, pure metallopurpurin can be prepared by recrystallization as described for the zinc derivatives above.

Normal Skin Response

We have previously determined that octaethylpurpurin **1** and etiopurpurin **11** cause significant tumor regression and in some cases complete cures when used in PDT to treat the transplantable FANFT-induced rat bladder tumor implanted into the flanks of Fisher 344 rats.[13a,b,d] We have also shown that incorporation of tin or zinc into the purpurin cavity results in an increase in tumoricidal response in the same tumor model.[13c] Conversely, copropurpurin I tetramethyl ester, **19**, had very little cytotoxic effect.[13d]

To study the effect of the more active purpurins on normal tissue, we therefore chose to use octaethylpurpurin **1** and its tin derivative and etiopurpurin **11** and its tin and zinc derivatives.

Fischer CDF (F344/CrPBR) rats (nine groups, five animals per group) were injected intravenously, via the tail vein, with either 2.5 or 1 mg/kg body weight of sensitizer, solubilized by emulsification as previously described.[13a] Twenty four hours after injection, one footpad of each animal was irradiated with red light from a 500-W G.E. Quartzline lamp (GECBA, Cleveland, OH) in a Kodak slide projector equipped with a Kodak Ektanar lens. The output lens of the projector was fitted with a filter (no. 2418, Dow Corning, Corning, NY) to allow only light with a wavelength greater than 590 nm to pass. The light was reflected 90° by placing a 5 × 5 cm silver mirror 45° to the axis of the light beam, 24 cm from the output lens of the projector. The beam was then passed through a 6 cm diameter double convex lens with a focal length of 12 cm and focussed to give a 1 cm diameter light beam at the surface of the tumor. The irradiation spectrum of this light has previously been described[13c] and was measured with a radiometer (UDT no. 351S, Culver City, CA). Total light dose was 200 mW/cm^2 for 30 min (360 J/cm^2).

The thickness of the treated footpad was measured as a function of time after phototherapy, and the data compared to the unirradiated footpad of the same animal, this acting therefore as an internal control.

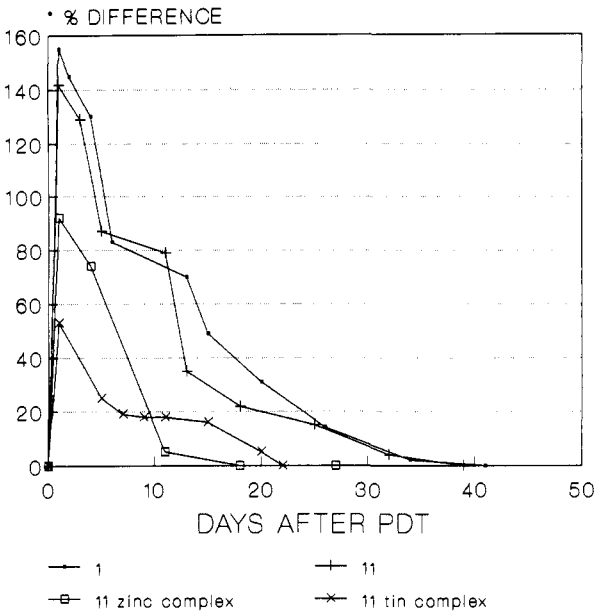

Figure 5. Footpad response: 2.5 mg/kg body weight of sensitizer; (*) (treated − control)/control × 100; footpad thickness in millimeters.

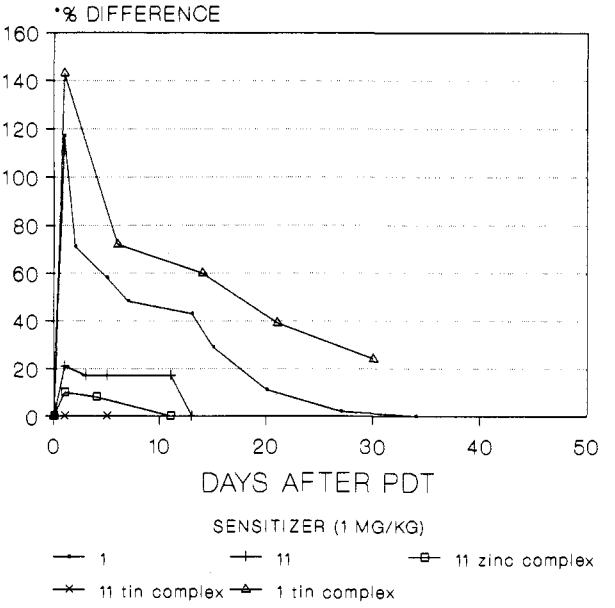

Figure 6. Footpad response: 1.0 mg/kg body weight of sensitizer; (*) (treated − control)/control × 100; footpad thickness in millimeters.

The results are shown below in Figures 5 and 6. As expected, significantly greater response was seen at the higher doses; however, the relative reactivities of sensitizers at the high dose appears to be consistent with the relative reactivities at the lower dose. In particular, it can be seen that etiopurpurin **11** and its metallo derivatives cause less damage to the footpad than octaethylpurpurins under this protocol.

We have previously reported that in terms of tumoricidal activity, **11** is more active than **1** (therapeutic doses = 2.5 and 5.0 mg/kg, respectively).[13b,d] It is interesting to postulate that the higher tumoricidal activity and lower footpad response of **11** compared to **1** suggests that the concentration of **11** in tumors is higher and in skin lower than corresponding concentrations of **1**. This explanation assumes, of course, that both **1** and **11** have similar pho-

tophysical properties. Since both have exactly the same visible spectrum and extinction coefficients, this assumption would appear to be valid; however, clearly the photophysical properties and tissue distribution patterns of these sensitizers are questions that need to be addressed.

Experimental Section

Visible spectra were recorded on a Bausch and Lomb Spectronic 2000; absorptions are given in nanometers (solutions in dichloromethane). Proton nuclear magnetic resonance spectra (^{1}H NMR) were obtained on a JEOL FX-90Q or a Varian VXR400 spectrometer, and chemical shifts are expressed in parts per million downfield from internal tetramethylsilane. Low-resolution mass spectra were measured (direct insertion probe) on a Hewlett-Packard 5987 mass spectrometer. Analytical TLC was performed by using Merck silica gel 90F 254 precoated sheets (0.2 mm); preparative chromatography was performed on a Chromatotron,[19] using Merck silica gel 60F 254 with $CaSO_4 \cdot {}^1/_2H_2O$ (catalog no. 7749). Elemental analyses were performed by MicAnal, Tucson, AZ 85717. Nickel etioporphyrin 3 was prepared as previously described.[20]

Nickel Coproporphyrin I Tetramethyl Ester (4). Coproporphyrin I tetramethyl ester (100 mg) was dissolved in glacial acetic acid (100 mL), and nickel acetate (50 mg) was added. The resulting solution was refluxed for 12 h and allowed to cool. Filtration afforded the desired product in a quantitative yield: vis λ_{max} 548, 511, 388 (ϵ 17 832, 6923, 114 335); ^{1}H NMR ($CDCl_3$) δ 9.69 (s, 4 H, meso-H), 4.21 (t, 8 H, CH_2-Ar), 3.71 (s, 6 H, CH_3 ester), 3.70 (s, 12 H, CH_3), 3.45 (s, 6 H, CH_3), 3.14 (t, 8 H, CH_2CH_2).

General Method for Formylation of Nickel Porphyrins. The method of Johnson et al.[20] was used without revision.

Nickel *meso*-formyletioporphyrin I (6): prepared in 95% yield; spectroscopic properties identical with those previously reported.[20]

Nickel *meso*-formylcoproporphyrin I, tetramethyl ester (16): prepared in 83% yield; vis λ_{max} 639, 554, 522, 420, 397 (ϵ 7294, 8425, 5548, 71 918, 92 123); ^{1}H NMR ($CDCl_3$) δ 11.91 (s, 1 H, CHO), 9.39 (s, 2 H, meso-H), 9.36 (s, 2 H, meso-H), 4.06 (t, 8 H, CH_2-Ar), 3.76, 3.71, 3.69, 3.40, 3.32, 3.30 (all s, 24 H, CH_3 of ester and ring methyl), 3.05 (t, 8 H, CH_2CH_2). Anal. ($C_{41}H_{44}N_4O_9Ni$) C, H, N.

General Method for Reaction of Nickel *meso*-Formylporphyrins with (Carbethoxymethylene)triphenylphosphorane. A solution of the nickel *meso*-formylporphyrin (500 mg) and (carbethoxymethylene)triphenylphosphorane (1.00 g) in xylene (50 mL) was heated under reflux for 24 h. The solution was cooled, the solvent removed in vacuo, and the crude product crystallized from methylene chloride–methanol (10:1). The product was chromatographed on silica with dichloromethane for elution. The major brown band was collected and recrystallized from dichloromethane–methanol (10:1) to give the product.

Nickel *meso*-[β-(ethoxycarbonyl)vinyl]etioporphyrin I (8): prepared in 96% yield; vis λ_{max} 557, 523, 399 (ϵ 12 890, 8750, 121 000); ^{1}H NMR ($CDCl_3$) δ 10.08 (d, J = 17 Hz, 1 H, β-H of acrylic ester), 9.48 (s, 3 H, meso H), 5.34 (d, J = 17 Hz, 1 H, α-H of acrylic ester), 4.34 (q, 2 H, CH_2 of ester), 3.83 (q, 16 H, CH_2 of peripheral ethyls), 3.38 (s, 9 H, CH_3), 3.31 (s, 3 H, CH_3), 1.70 (m, 12 H, CH_3 of peripheral ethyl), 1.36 (t, 3 H, CH_3 of ester).

Nickel *meso*-[β-(ethoxycarbonyl)vinyl]coproporphyrin I tetramethyl ester (17): prepared in 61% yield; λ_{max} 559, 524, 401 (6593, 4450, 70 000); ^{1}H NMR ($CDCl_3$) δ 9.99 (d, J = 18 Hz, β-H of acrylate), 9.46 (s, 3 H, meso-H), 5.28 (d, J = 18 Hz, α-H of acrylate), 4.13 (m, 10 H, CH_2-Ar and CH_2 of acrylate ester), 3.74, 3.68, 3.37, 3.35, 3.28 (all s, 24 H, CH_3 of ester and ring methyl), 1.32 (t, 3 H, CH_3 of acrylate ester). Anal. ($C_{45}H_{51}N_4O_{10}Ni \cdot H_2O$) C, H.

General Method for Demetalation of Nickel *meso*-Acrylic Porphyrins. A solution of the nickel complex (600 mg) was dissolved in concentrated sulfuric acid (10 mL) and kept at room temperature for 2 h. Dichloromethane (100 mL) was added, followed by saturated aqueous sodium bicarbonate. After neutralization was complete, the organic layer was collected, washed, and dried, and the solvent was removed. Crystallization of the crude product from methylene chloride–methanol (10:1) gave the product, pure by TLC.

***meso*-[β-(Ethoxycarbonyl)vinyl]etioporphyrin I (10):** prepared in 92% yield; vis λ_{max} 622, 570, 532, 501, 404 (ϵ 2660, 6300, 7000, 1346, 175 400); ^{1}H NMR ($CDCl_3$) δ 10.31 (d, J = 17 Hz, 1 H, β-H of acrylate ester), 10.07 (s, 2 H, meso H), 9.92 (s, 1 H, meso H), 6.21 (d, J = 17 Hz, α-H of acrylate ester), 4.48 (q, 2 H, CH_2 of ester), 4.04 (m, 16 H, CH_2 of peripheral ethyls), 3.60 (s, 9 H, CH_3), 3.38 (s, 3 H, CH_3), 1.85 (m, 12 H, CH_3 of peripheral ethyls), 1.46 (s, 3 H, CH_3 of ester); mass spectrum, m/e 576 (M^+); calcd for $C_{37}H_{44}N_4O_2$, MW = 576.

***meso*-[β-(Ethoxycarbonyl)vinyl]coproporphyrin I tetramethyl ester (18):** prepared in 90% yield; vis λ_{max} 626, 571, 534, 501, 400 (ϵ 2927, 5122, 5487, 9024, 120 444); ^{1}H NMR ($CDCl_3$) δ 10.24 (d, J = 18 Hz, β-H of acrylate), 10.09 (s, 2 H, meso-H), 9.93 (s, 1 H, meso-H), 6.20 (d, J = 18 Hz, α-H of acrylate ester), 4.37 (m, 10 H, CH_2-Ar and CH_2 of acrylate ester), 3.73, 3.68, 3.63, 3.60, 3.38 (all s, CH_3 of ring and CH_3 of ester), 3.25 (m, 8 H, CH_2CH_2), 1.46 (t, 3 H, CH_3 of acrylate ester).

General Method for Purpurin Formation. A solution of the *meso*-acrylic porphyrin (100 mg) in glacial acetic acid was heated under reflux in a nitrogen atmosphere for 24 h. After the mixture was cooled, the solvent was removed in vacuo and the crude product either (a) chromatographed on silica with dichloromethane–hexane (60:40) (for the etio series) or dichloromethane (for the copro series) as eluent, the major purple band collected, the solvent removed, and the residue recrystallized from dichloromethane–hexane (10:1) or (b) recrystallized directly from dichloromethane–hexane.

Etiopurpurin I (11): prepared in 92% yield; vis λ_{max} 695, 633, 563, 526, 500, 423 (ϵ 46 080, 8362, 19 710, 9407, 7466, 148 453); ^{1}H NMR ($CDCl_3$) δ 9.45 (s, 2 H, meso H), 9.40, (s, 1 H, H of isocyclic ring), 8.52 (s, 1 H, meso H), 4.53 (q, 2 H, CH_2 of ethyl ester), 4.33 (q, 1 H, C-2 H), 3.75 (m, 6 H, CH_2 of ethyl), 3.49 (s, 1 H, CH_3), 3.39 (s, 3 H, CH_3), 3.28 (s, 3 H, CH_3), 2.70, 1.65 (both m, both 1 H, CH_2 of sp^3 ethyl), 2.50 (d, 3 H, CH_3 of reduced ring), 1.80 (m, 9 H, CH_3 of ethyl), 1.70 (t, 3 H, CH_3 of ethyl ester), –0.23 (t, 3 H, CH_3 of sp^3 ethyl), –3.2 (br s, 2 H, 2NH); mass spectrum, m/e 576 (M^+); calcd for $C_{37}H_{44}N_4O_4$, MW = 576.

Copropurpurin I tetramethyl ester (19): prepared in 68% yield; vis λ_{max} 695, 635, 562, 525, 499, 407 (ϵ 32 727, 10 128, 16 362, 13 245, 16 362, 198 000); ^{1}H NMR ($CDCl_3$) δ 9.46 (s, 1 H, meso-H), 9.41 (s, 2 H, meso-H and acrylate-H), 8.55 (s, 1 H, meso-H), 4.52 (q, 2 H, CH_2 of ethyl ester), 4.19 (m, 1 H, H of reduced ring), 4.06 (m, 6 H, CH_2 of propionates), 3.67 (s, 12 H, CH_3 of methyl esters), 3.49, 3.42, 3.30 (all s, 3 H, 3 CH_3), 3.07 (m, 8 H, CH_2 of propionates), 2.54 (d, 3 H, CH_3 of reduced ring), 2.15 (m, 2 H, CH_2 of propionate on reduced ring), 1.54 (t, 3 H, CH_3 of ethyl ester). Anal. ($C_{45}H_{51}N_4O_{10}$) C, H.

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