

Syntheses of protoporphyrin-IX derivatives bearing extended propionate side-chains

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Dedicated to Professor António M. d'A. Rocha Gonçalves

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Abstract

In order to investigate the relationship between depth within membranes of singlet oxygen generation and effectiveness of photodynamic therapy of tumors, analogs of protoporphyrin-IX **1** bearing five **4** and seven **5** carbon atoms (in place of the 3-carbon atom chain in **1**) were synthesized from monopyrrole precursors.

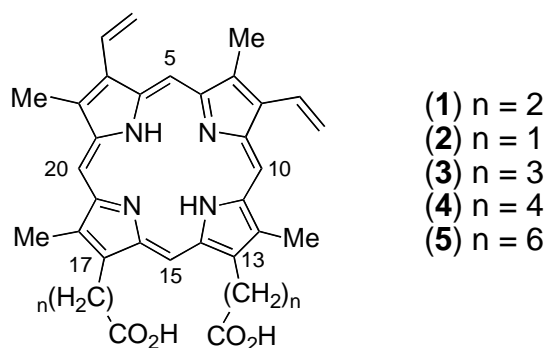
Keywords: Lipid bilayer, photodynamic therapy, protoporphyrin analogs, singlet oxygen

Introduction

Protoporphyrin-IX (PP-IX, **1**) is justifiably known as the 'first' porphyrin.¹ As its iron(II) complex, heme, it is the prosthetic group for a number of critically important heme proteins such as hemoglobins, myoglobins, cytochromes, catalases and peroxidases. In addition, PP-IX is also a biosynthetic precursor of chlorophylls and bacteriochlorophylls.² X-Ray studies have shown that the vinyl-bearing rings of PP-IX are the most deeply embedded in the protein pockets of heme proteins and that the acid groups are consequently pointing to the polar outside of the protein cleft.³ With the notion that varying the length of the propionate side-chains in **1** might affect physiological activity of a heme protein by altering the position of the heme iron atom within the protein cleft, we had earlier synthesized the bis-acetic **2** and bis-butyric porphyrin **3**;⁴ the bis-butyric porphyrin **3** was obtained by transformation of PP-IX. These porphyrins were also used in a study of the specificity of heme oxidation by microsomal heme oxygenase.⁵

We were prompted to expand our syntheses to include other chain-lengthened derivatives of PP-IX [e.g. the bis-pentanoate **4** and bis-heptanoate **5** porphyrins] by the needs of ongoing collaborative studies with Dr. Benjamin Ehrenberg. These studies aimed to investigate the relationship between effectiveness of photodynamic therapy (PDT) and the distance within a

membrane that singlet oxygen can be efficiently generated and used without diffusion out of the membrane.



Singlet oxygen is the toxic species most often generated during the PDT process.⁶ It is a powerful oxidant and initiates critical damage to the tissues *via* apoptosis or necrosis of cancer cells when localized there. Evidence suggests^{7,8} that lipophilic sensitizers act, at least partially, in the bilayer membrane, increasing cell permeability, membrane rupture, and cell lysis in the plasma and organelle membranes.⁹ A disadvantage of PDT is that singlet oxygen can rapidly diffuse out of membranes because of its relatively long intrinsic lifetime (13-35 ms).¹⁰ Thus, an additional new criterion has been proposed by Ehrenberg for the design and choice of new PDT sensitizers; along with long-wavelength absorption (the obvious and most-often quoted advantage), vertical depth of penetration into cell membranes (see 'X' in Figure 1) should also be a criterion.

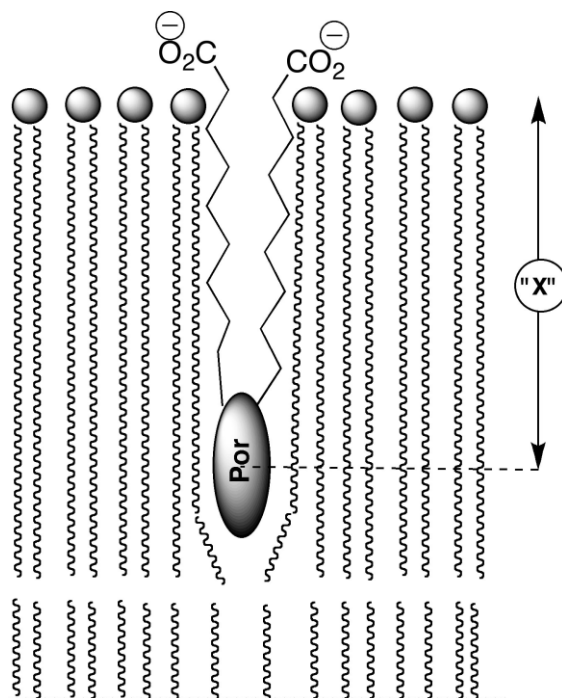
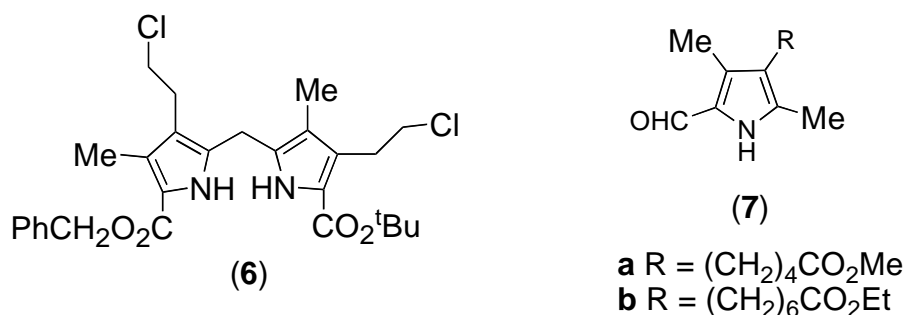


Figure 1. Cartoon showing vertical depth ('X') of a porphyrin (Por) in a lipid bilayer.

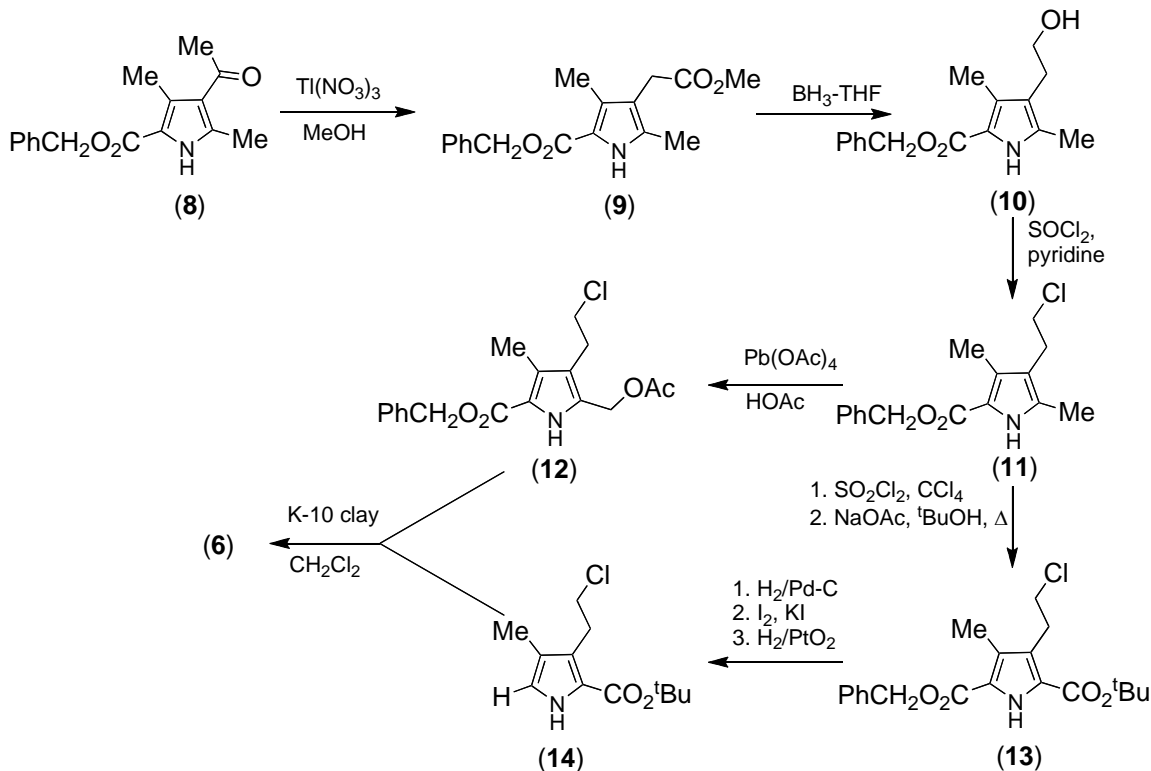
To date five articles¹¹⁻¹⁵ have reported our photochemical, photophysical and photobiological preliminary studies using the PP-IX analogs that we report herein, along with compounds **2** and **3** that were re-synthesized according to our literature procedures.⁴ In the first paper¹¹ we definitively showed that the photosensitization process is enhanced when the sensitizing chromophore is deeper into the lipid bilayer of liposomes. We next showed¹² that lengthening of the 13- and 17-side-chains caused an increase in the lipophilicity and the liposome-binding constant. In another study¹³ it was shown, using two fluorescence quenching techniques (namely iodide ions from the aqueous phase and using spin-probe-labeled phospholipids), that vertical location of the photosensitizer chromophore in a membrane has an effect on its activity; the extent of membrane damage can be modulated by adjustment of the depth in the membrane, this being accomplished by synthetic variability of the 13- and 17-side-chain length. In the fourth paper¹⁴ we showed that increased chain length in the corresponding hematoporphyrin-IX analogs of the synthetic PP-IX compounds imparts increased hydrophobicity, and that there is a strong pH dependence on the efficiency of fluorescence quenching by iodide; porphyrins in the neutral form penetrate deeper into the lipid bilayer and are less exposed to external quenching than when negatively charged at the carboxylic groups. Increasing the pH causes a significant decrease in the photosensitization efficiency in liposomes because protonation of the carboxylate groups allows the chromophore to sink deeper into the membrane, thus increasing the 'dwell-time' and effectiveness of the singlet oxygen produced upon irradiation. The fifth paper¹⁵ described an *in vitro* and *in vivo* comparison between PP-IX **1** and the synthetic PP analog **5** bearing a seven-membered carboxylate chain at positions 13 and 17. The inactivation (by singlet oxygen) of WiDr and CT26 cells increased linearly with length of the 13- and 17-alkylcarboxylic chains, and in mice, tumors treated with **5** and light showed significant delay in their growth (compared with PP-IX, **1**).¹⁵

Results and Discussion

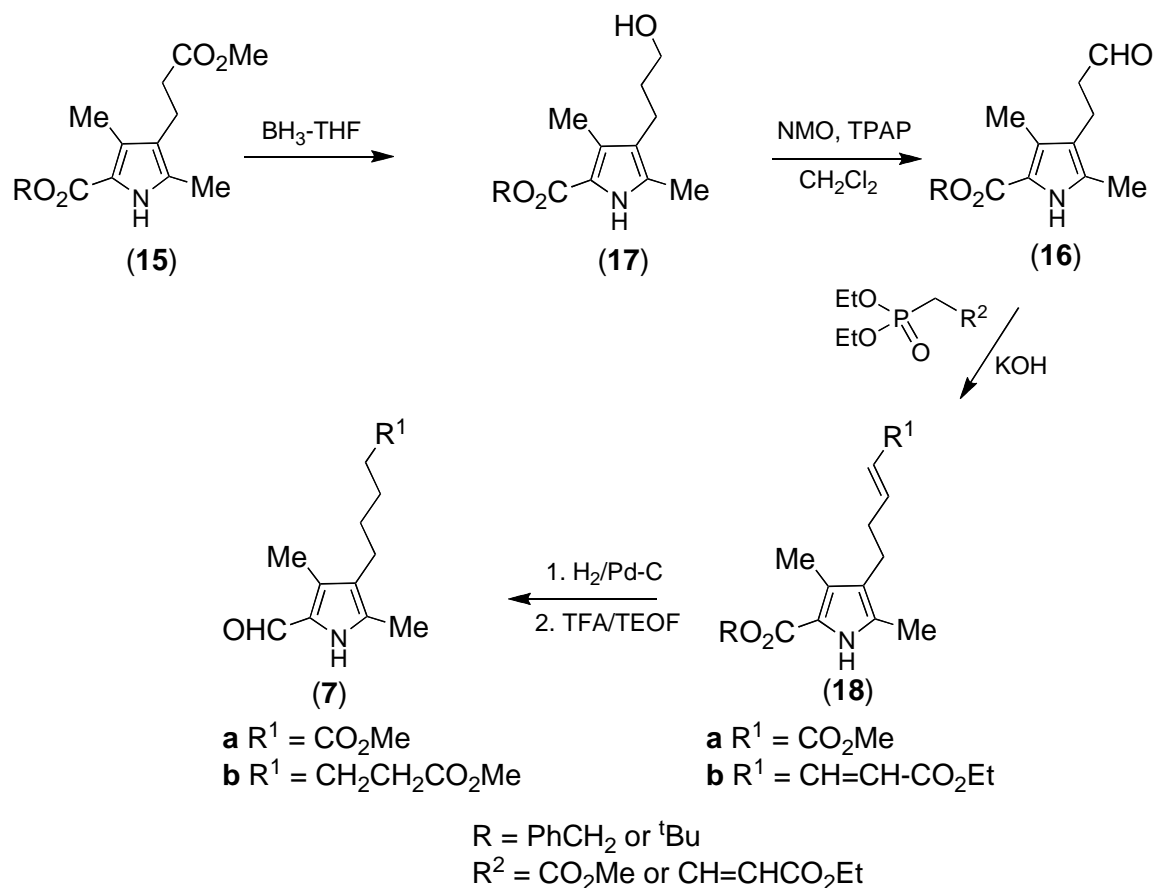
Our synthetic targets were the bis-pentanoic **4** and bis-heptanoic **5** analogs of PP-IX (**1**). Using IUPAC nomenclature, the side-chain modifications were designed at the 13- and 17-positions. For economy in synthesis, the approach was designed such that a common dipyrromethane **6** was prepared. The lower halves of the derivatives, however, were more challenging and required the synthesis of novel pyrroles **7a,b**.



Beginning with the top half of the molecule, known dipyrromethane **6** was prepared as outlined in Scheme 1.¹⁶ The desired unsymmetrical starting material was prepared from just one pyrrole **8**, which was in turn prepared *via* a standard Knorr-type synthesis. Thallium(III) nitrate rearrangement of **8** in methanol gave the (methoxycarbonylmethyl)pyrrole (**9**),^{17,18} which was reduced with borane-THF to give the (2-hydroxyethyl)pyrrole **10**. S_N2 displacement with a chloride ion led to the key chloroethylpyrrole **11**.¹⁷ This pyrrole was then either subjected to Pb(OAc)₄ oxidation in acetic acid to yield the acetoxymethylpyrrole **12** or further manipulated to form a *t*-butyl pyrrole-carboxylate **13** by sulfonyl chloride oxidation followed by *t*-butanolysis, (Scheme 1);¹⁹ hydrogenation, iodination, and a second hydrogenation using Adams' catalyst then afforded the desired α -free pyrrole **14**. Coupling of these two pyrroles with K10 clay as catalyst in CH₂Cl₂ completed the synthesis of **6**.

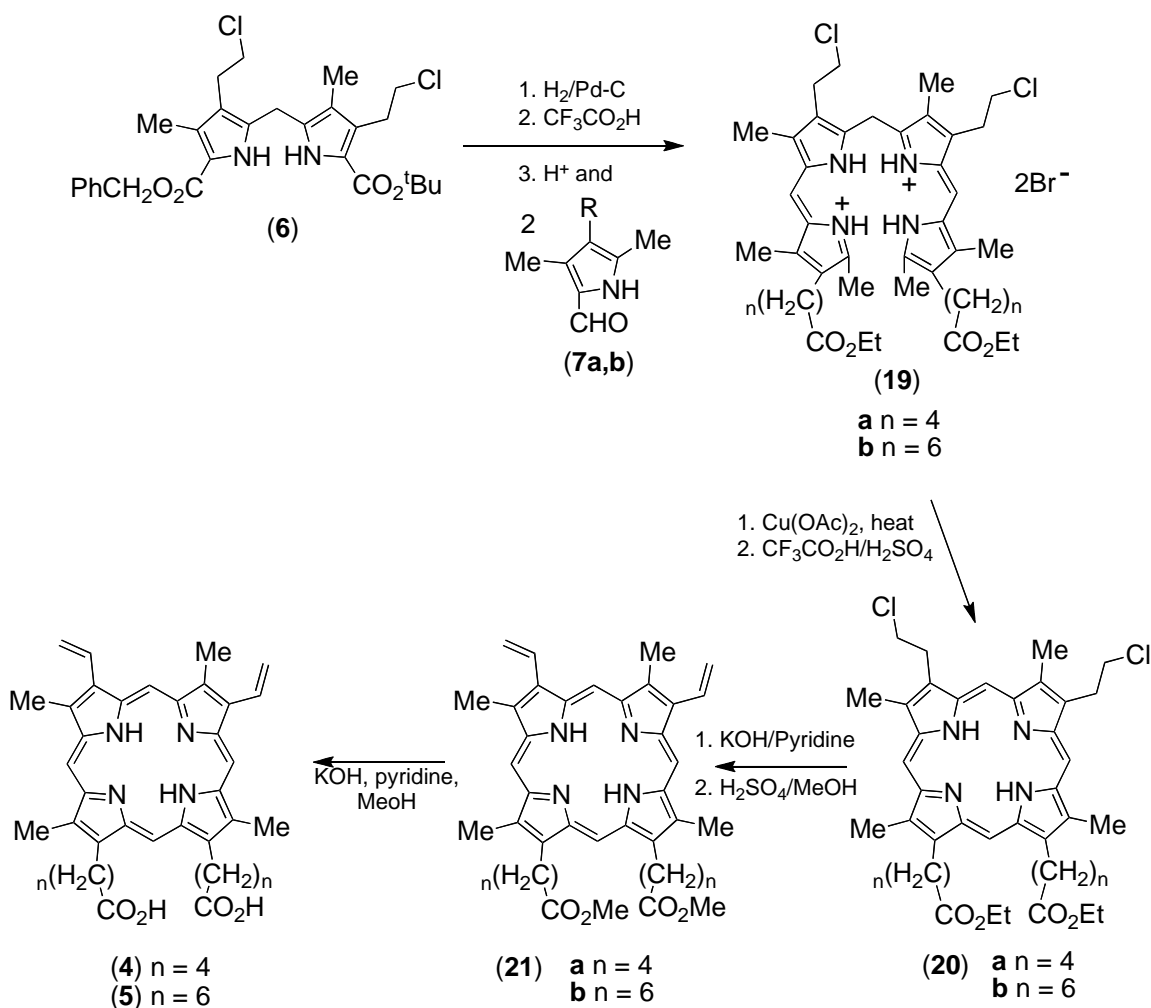


Scheme 1. Synthesis of the common dipyrromethane **6**.



Scheme 2. Syntheses of the formylpyrroles **7a,b**.

Turning to the lower portion of the targeted derivatives, pyrrole **7a** and **7b** required starting from known pyrrole **15**;²⁰ pyrrole aldehyde **16** was prepared by reduction of **15** with $\text{BH}_3\text{-THF}$ to give **17** which was then oxidized using NMO and TPAP²¹ to give **16**. The chain was then lengthened using the appropriate Horner-Wittig-Emmons reagent and base to give high yields of both acrylate pyrroles **18a** and **18b**. Catalytic hydrogenation, followed by formylation using TFA/TEOF led to the desired 2-formylpyrrole starting materials **7a** and **7b** (Scheme 2).



Scheme 3. Syntheses of porphyrins (4), and (5) via a,c-biladiene salts (19).

Removal of both ester protecting groups followed by condensation with the appropriate monoformyl pyrrole led to the corresponding a,c-biladienes **19a,b**. These were difficult to crystallize and to purify, so were used directly in a copper-induced cyclization reaction²² to yield the porphyrins **20a,b** in yields ranging from 30 to 42% (Scheme 3). Dehydrohalogenation of the porphyrins **20a,b** in the presence of base led to the required dimethyl esters of the divinyl products **21a,b**. During this step, partial hydrolysis of the esters was detected by TLC, so re-esterification (using 5% H₂SO₄ in MeOH) was carried out, and the products **21a,b** were purified using silica gel column chromatography. Once isolated, the esters were carefully hydrolyzed at room temperature in the presence of pyridine, KOH, and MeOH to yield the desired PP-IX analogs **4** and **5**.

Experimental Section

General Procedures. Melting points were measured on a Thomas/Bristoline microscopic hot stage apparatus and were uncorrected. Silica gel 60 (70-230 mesh, Merck) was used for column chromatography. $^1\text{H-NMR}$ spectra were obtained in CDCl_3 at 300 MHz using a General Electric QE300 spectrometer; chemical shifts are expressed in ppm relative to chloroform (7.26 ppm). Elemental analyses were performed at the Midwest Microlab. Inc., Indianapolis, Indiana, USA. Electronic absorption spectra were measured in dichloromethane solution using a Hewlett-Packard 8450A spectrophotometer. Mass spectra were obtained at the Mass Spectrometry Facility, University of California, San Francisco, California, USA and at the Mass Spectrometry Facility in the Department of Chemistry, Louisiana State University.

Benzyl 4-(3-hydroxypropyl)-3,5-dimethylpyrrole-2-carboxylate, (17, R = PhCH₂). Pyrrole **15**²² (10.00 g, 0.0317 mol) was placed in a 1000 mL round-bottomed flask and dissolved in THF (300 mL). An addition funnel, affixed with a nitrogen inlet was attached and $\text{BH}_3\text{-THF}$ (1M, 70 mL) was dripped in slowly while the solution was stirred over ice. Once the addition was complete the reaction was allowed to continue at room temperature overnight. MeOH was slowly added to quench the reaction and the solution was evaporated to dryness and re-suspended in CH_2Cl_2 before being passed through a pad of silica gel. The resulting crude material was dissolved in a minimum amount of CH_2Cl_2 and petroleum ether was added slowly to give the title compound **17**, R = PhCH₂ as an oil (7.2 g, 80% yield). $^1\text{H-NMR}$: (CDCl_3) δ 8.48 (bs, 1H, N-H), 7.36 (m, 5H, Ar-H), 5.28 (s, 2H, Ar-CH₂-), 3.64 (t, 2H, HO-CH₂-CH₂-CH₂-), 2.46 (t, 2H, HO-CH₂-CH₂-CH₂-), 2.29 (s, 3H, -CH₃), 2.20 (s, 3H, -CH₃), 1.72 (m, 2H, HO-CH₂-CH₂-CH₂-). HRMS: C₁₇H₂₁NO₃ requires m/z 287.15213. Found 287.1521.

tert-Butyl 4-(3-hydroxypropyl)-3,5-dimethylpyrrole-2-carboxylate (17, R = 'Bu). Using the same procedure as reported above, the title pyrrole (2.5 g, 91% yield) was obtained as an oil. $^1\text{H-NMR}$: (CDCl_3) δ 8.45 (bs, 1H, N-H), 3.64 (bt, 2H, HO-CH₂-CH₂-CH₂-), 2.46 (t, 2H, HO-CH₂-CH₂-CH₂-), 2.25 (s, 3H, -CH₃), 2.21 (s, 3H, -CH₃), δ 1.70 (m, 2H, HO-CH₂-CH₂-CH₂-), 1.56 (s, 9H, -C(CH₃)₃). HRMS: C₁₄H₂₃NO₃ requires m/z 252.16778. Found 253.1672.

Benzyl 4-(2-formylethyl)-3,5-dimethylpyrrole-2-carboxylate, (16, R = PhCH₂). To a 500 mL round-bottomed flask was added 3-hydroxyethylpyrrole **16**, R = PhCH₂ (5 g, 0.0174 mol), distilled CH_2Cl_2 (200 mL), and molecular drying sieves. N-Methylmorpholine-N-oxide (NMO, 2.1 g, 0.0174 mol) was then added followed by the addition of tetrapropylammonium perruthenate (TPAP, 0.5 g). The mixture was stirred under a stream of nitrogen for 45 min, after which time the solution was partitioned between H₂O and CH_2Cl_2 . The organic layer was evaporated and eluted through a silica gel column prepared in 1% MeOH/ CH_2Cl_2 . A clear colorless compound was collected which was crystallized from a minimum of CH_2Cl_2 and petroleum ether. The white solid obtained proved to be the title compound **16**, R = PhCH₂, (3.1 g, 63% yield), mp 80-82 °C. $^1\text{H-NMR}$: (CDCl_3) δ 9.78 (s, 1H, -CH₂-CH₂-CHO), 8.50 (bs, 1H, N-H), 7.39 (m, 5H, Ar-H), 5.28 (s, 2H, Ar-CH₂-), 2.70 (t, 2H, -CH₂-CH₂-CHO), 2.57 (t, 2H, -

CH₂-CH₂-CHO), 2.28 (s, 3H, **-CH₃**), 2.21 (s, 3H, **-CH₃**). HRMS: C₁₇H₁₉NO₃ requires *m/z* 251.15213. Found 251.1525.

tert-Butyl 4-(2-formylethyl)-3,5-dimethylpyrrole-2-carboxylate, (16, R = ^tBu). Using the same procedure as reported above, the title pyrrole was obtained (3.8 g, 75% yield), mp 105-107 °C. ¹H-NMR: (CDCl₃) δ 9.78 (s, 1H, **-CH₂-CH₂-CHO**), 8.42 (bs, 1H, **N-H**), 2.69 (t, 2H, **-CH₂-CH₂-CHO**), 2.57 (tr, 2H, **-CH₂-CH₂-CHO**), 2.24 (s, 3H, **-CH₃**), 2.20 (s, 3H, **-CH₃**), 1.55 (s, 9H, **-C(CH₃)₃**). HRMS: C₁₄H₂₁NO₃ requires *m/z* 251.15213. Found 251.1525.

Benzyl 4-(4-methoxycarbonyl-3-butenyl)-3,5-dimethylpyrrole-2-carboxylate (18a). (Formylethyl)pyrrole **16**, R = PhCH₂ (3.1 g, 0.0109 mol) was placed in a 250 mL round-bottomed flask. THF (150 mL), KOH (800 mg), and methyl diethylphosphonoacetate (3.4g, 0.0163 mol) were then added. The reaction was allowed to stir under nitrogen for 3 h after which time it was extracted 3x with CH₂Cl₂ and H₂O. The organic layers were combined, washed with brine and dried over Na₂SO₄. Upon evaporation, the crude oil was eluted through a silica gel column previously prepared in 1% MeOH/CH₂Cl₂. The title compound as a clear oil (3.7 g, 100% yield), was obtained which slowly crystallized, mp 75-76 °C. ¹H-NMR: (CDCl₃) δ 8.50 (bs, 1H, **N-H**), 7.43 (m, 5H, **Ar-H**), 6.96 (d of t, 1H, **-CH₂-CH₂-CH=CH-CO₂CH₃**), 5.81 (d, 1H, **-CH₂-CH₂-CH=CH-CO₂CH₃**), 5.28 (s, 2H, **Ar-CH₂-**), 3.72 (s, 3H, **-CH₂-CH₂-CH=CH-CO₂CH₃**), 2.51 (t, 2H, **-CH₂-CH₂-CH=CH-CO₂CH₃**), 2.28 (m, 2H, **-CH₂-CH₂-CH=CH-CO₂CH₃**), 2.27 (s, 3H, **-CH₃**), 2.17 (s, 3H, **-CH₃**). HRMS: C₂₀H₂₃NO₄ requires *m/z* 341.16270. Found 341.1624.

tert-Butyl 4-(6-ethoxycarbonyl-3,5-hexadienyl)-3,5-dimethylpyrrole-2-carboxylate (18b). (Formylethyl)pyrrole **16**, R = ^tBu (5.0 g, 0.019 mol) was placed in a 250 mL round-bottomed flask. THF (150 mL), KOH (800 mg), and triethyl 4-phosphonocrotonate (7.5g, 0.03 mol) were then added. The reaction mixture was allowed to stir under nitrogen for 3 h after which time it was extracted 3x with CH₂Cl₂ and H₂O. The organic layers were combined, washed with brine and dried over Na₂SO₄. After evaporation, the crude oil was eluted through a silica gel column previously prepared in 1% MeOH/CH₂Cl₂. A clear oil of the title compound (5.5 g, 80% yield) resulted which could not be crystallized or precipitated. ¹H-NMR: (CDCl₃) δ 8.96 (bs, 1H, **N-H**), 7.21 (m, 1H, **-CH₂-CH₂-CH=CH-CH=CH-CO₂CH₂CH₃**), 6.14 (m, 2H, **-CH₂-CH₂-CH=CH-CH=CH-CO₂CH₂CH₃**), 5.78 (d, 1H, **-CH₂-CH₂-CH=CH-CH=CH-CO₂CH₂CH₃**), 4.20 (q, 2H, **-CO₂-CH₂-CH₃**), 2.50 (t, 2H, **-CH₂-CH₂-CH=CH-CH=CH-CO₂CH₂CH₃**), 2.28 (m, 2H, **-CH₂-CH₂-CH=CH-CH=CH-CO₂CH₂CH₃**), 2.25 (s, 3H, **-CH₃**), 2.19 (s, 3H, **-CH₃**), 1.57 (s, 9H, **-(CH₃)₃**), 1.30 (t, 3H, **-CO₂-CH₂-CH₃**). HRMS: C₂₀H₂₉NO₄ requires *m/z* 347.20964. Found 347.2097.

2-Formyl-4-(4-methoxycarbonylbutyl)-3,5-dimethylpyrrole (7a). Acrylate pyrrole **18a**, R = PhCH₂ (3.3 g, 9.67x10⁻³ mol) was hydrogenated under H₂ gas at room temperature and atmospheric pressure using Pd/C (0.30 g) as a catalyst and THF (150 mL) as solvent. Upon completion of reaction, the catalyst was filtered off and the solvent evaporated. This product was not isolated, but was instead directly subjected to treatment with TFA (50 mL) while stirring under nitrogen in an ice bath. After 15 min had passed, triethyl orthoformate (25 mL) was added and the reaction mixture was allowed to stir an additional 3 h as it reached room temperature. The solution was then partitioned between CH₂Cl₂ and water. The organic layer was neutralized

with a saturated solution of NaHCO₃. The resulting organic layer was dried over Na₂SO₄ and evaporated to give a dark solid. This solid was re-suspended in a minimum of CH₂Cl₂ and eluted through a silica gel column prepared in CH₂Cl₂. A clear oil which proved to be the title compound was collected (2.2 g, 96%) and slowly crystallized (mp 70-72 °C). ¹H-NMR: (CDCl₃) δ 9.45 (s, 1H, -CHO), 9.20 (bs, 1H, -NH), 3.66 (s, 3H, -CO₂-CH₃), 2.37 (m, 4H, -CH₂-CH₂-CH₂-CH₂-CO₂CH₃), 2.16 (s, 3H, -CH₃), 2.13 (s, 3H, -CH₃), 1.62 (m, 2H, -CH₂-CH₂-CH₂-CH₂-CO₂CH₃), 1.48 (m, 2H, -CH₂-CH₂-CH₂-CH₂-CO₂CH₃). HRMS: C₁₃H₁₉NO₃ requires *m/z* 237.13648. Found 237.1360.

2-Formyl-4-(6-ethoxycarbonylhexyl)-3,5-dimethylpyrrole (7b). Using the same procedure as reported above, the title pyrrole was obtained (1.7 g, 38% yield), mp 52-55 °C. ¹H-NMR: (CDCl₃) δ 9.45 (s, 1H, -CHO), 9.35 (bs, 1H, -NH), 4.13 (q, 2H, -CO-CH₂-CH₃), 2.34 (m, 4H, -CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-CO₂-CH₂-CH₃), 2.23 (s, 3H, -CH₃), 2.19 (s, 3H, -CH₃), 1.48-1.31 (m, 8H, -CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-CO₂-CH₂-CH₃), 1.24 (t, 3H, -CO₂-CH₂-CH₃). HRMS: C₁₅H₂₃NO₃ requires *m/z* 279.18343. Found 279.1836.

2,4-Bis(2-chloroethyl)-6,7-bis(4-methoxycarbonylbutyl)-1,3,5,8-tetramethyl-porphyrin (20a). The dipyrromethane **6** (414 mg, 7.78x10⁻⁴ mol) in THF (25mL) containing 10% Pd-C (40 mg) was hydrogenated at atmospheric pressure and room temperature until uptake of hydrogen was complete. The solution was filtered through a bed of Celite and the solvent was evaporated to dryness to give a white solid. In a 100 mL round-bottomed flask over ice was placed the dipyrromethane carboxylic acid (0.344g, 7.78x10⁻⁴ mol) and TFA (25 mL) was added; the mixture was stirred under nitrogen for 15 min. Formylpyrrole **7a** (0.360 g, 1.52 x 10⁻³ mol) dissolved in 15 mL of MeOH was then added directly. A color change from yellow to dark red was observed over time and the reaction was monitored using visible light. After 2 h, HBr gas was bubbled into the solution and then ether (50 mL) was added to precipitate the a,c-biladiene salt **19a**. This compound was collected and used directly in the next step. This crude a,c-biladiene dihydrobromide **19a** was placed in a 100 mL round-bottomed flask and dissolved in DMF (40 mL). Cu(OAc)₂ (1.2 g) was then added and the mixture was heated at 100 °C until reaction was complete as indicated by spectrophotometry (appearance of a Soret band, ~30 min). The mixture was cooled and partitioned between CH₂Cl₂ and H₂O. The organic layer was extracted once with base and then dried over Na₂SO₄ and used directly in the next step. The crude copper(II) porphyrin was demetalated in the presence of ice cold H₂SO₄/TFA (20/80, 20 mL) during 6 h. This acidic solution was washed successively with water (100 mL), aqueous NaHCO₃ (3 x 50 mL) and then water again (100 mL) before being separated and evaporated to dryness. The crude porphyrin was placed on a silica gel column and eluted using 1% MeOH/CH₂Cl₂. The red band was collected and crystallized from a minimum of CH₂Cl₂/MeOH to give red crystals of the title compound **20a** (0.20 g, 42% yield), mp 195-200 °C. ¹H-NMR: (CDCl₃) δ 10.13 (s, 1H, meso-H), 10.05 (s, 1H, meso-H), 10.04 (s, 1H, meso-H), 10.03 (s, 1H, meso-H), 4.55 (t, 4H, -CH₂-CH₂-Cl), 4.33 (t, 4H, -CH₂-CH₂-Cl), 4.10 (t, 4H, -CH₂-CH₂-CH₂-CH₂-CO₂CH₃), 3.68 (s, 6H, -CO₂CH₃), 3.63 (s, 12H, -CH₃), 2.49 (t, 4H, -CH₂-CH₂-CH₂-CH₂-CO₂CH₃), 2.31 (m, 4H, -CH₂-CH₂-CH₂-CH₂-CO₂CH₃), 2.08 (m, 4H, -CH₂-CH₂-CH₂-CH₂-

CO₂CH₂). Anal. Calcd for C₄₀H₄₈Cl₂N₄O₄: C, 66.82; H, 6.73; N, 7.80% Found: C, 66.94; H, 6.75; N, 7.55% HRMS: C₄₀H₄₈Cl₂N₄O₄ requires *m/z* 718.30524. Found 718.3048.

2,4-Bis(2-chloroethyl)-6,7-bis(6-ethoxycarbonylhexyl)-1,3,5,8-tetramethylporphyrin (20b).

Using the same procedure as reported above, the title compound **20b** was obtained (0.30 g scale, 30% yield), mp 151-153 °C. ¹H-NMR: (CDCl₃) δ 10.12 (s, 1H, meso-**H**), 10.05 (s, 1H, meso-**H**), 10.04 (s, 1H, meso-**H**), 10.02 (s, 1H, meso-**H**), 4.54 (t, 4H, -CH₂-CH₂-Cl), 4.33 (t, 4H, -CH₂-CH₂-Cl), 4.06 (mq, 8H, -CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-CO₂CH₂CH₃), 3.69 (s, 3H, -CH₃), 3.68 (s, 3H, -CH₃), 3.67 (s, 3H, -CH₃), 3.66 (s, 3H, -CH₃), 2.29 (t, 4H, -CH₂-CH₂-CH₂-CH₂-CH₂-CO₂CH₂CH₃), 1.70-1.65 (m, 8H, -CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-CO₂CH₂CH₃), 1.19 (t, 3H, -CO₂-CH₂-CH₃). Anal. Calcd for C₄₆H₆₀Cl₂N₄O₄: C, 68.79; H, 7.54; N, 6.98% Found: C, 68.66; H, 7.02; N, 6.53% HRMS: C₄₆H₆₀Cl₂N₄O₄ requires *m/z* 802.39913. Found 802.3992.

6,7-Bis(4-methoxycarbonylbutyl)-1,3,5,8-tetramethyl-2,4-divinylporphyrin (21a).

To bischloroethylporphyrin **20a** (0.30 g) in a 100 mL round-bottomed flask was added 40 mL pyridine and aqueous 3% KOH solution (20 mL). This mixture was refluxed for 2.5 h in the dark and then cooled. Spectrophotometric analysis and TLC indicated that the vinyl groups had formed and that the esters were hydrolyzed to carboxylic acids. The crude mixture was then partitioned between CH₂Cl₂ (100 mL) and an aqueous phosphate buffer solution (100 mL, pH = 4). The organic layer was washed once with water (100 mL). Crystallization attempts were unsuccessful, so the crude mixture was re-esterified by stirring the material overnight in 5% H₂SO₄/MeOH (50 mL). The mixture was worked up by dilution with CH₂Cl₂ (100 mL) and washing with water and then saturated NaHCO₃ solution. After washing again with water (100 mL) the organic phase was evaporated. Purification using a silica gel column prepared with 1% MeOH/CH₂Cl₂ yielded compound **21a** (0.081 g, 30%), mp 210-212 °C. ¹H-NMR: (CDCl₃) δ 10.25 (s, 1H, meso-**H**), 10.18 (s, 1H, meso-**H**), 10.10 (s, 1H, meso-**H**), 9.97 (s, 1H, meso-**H**), 8.32 (dd, 2H, -HC=CH₂), 6.40 (dd, 4H, -HC=CH₂), 4.08 (t, 4H, -CH₂-CH₂-CH₂-CH₂-CO₂CH₃), 3.72 (s, 6H, -CO₂CH₃), 3.62 (s, 12H, -CH₃), 2.48 (t, 4H, -CH₂-CH₂-CH₂-CH₂-CO₂CH₃), 2.32 (m, 4H, -CH₂-CH₂-CH₂-CH₂-CO₂CH₃), 2.07 (m, 4H, -CH₂-CH₂-CH₂-CH₂-CO₂CH₃). Anal. Calcd for C₄₀H₄₆N₄O₄: C, 74.26; H, 7.17; N, 8.67. Found: C, 74.29; H, 7.34; N, 8.83. LRMS: C₄₀H₄₆N₄O₄ requires *m/z* 646.4. Found 646.4. To obtain the dicarboxylic acid **4** the pure porphyrin was dissolved in MeOH/H₂O/KOH (90 mL/10 mL/1 g) and stirred under argon in the dark at room temperature overnight. Acidic work-up, followed by extraction into CH₂Cl₂ using a pH 4 phosphate buffer as the aqueous phase led to the isolation of compound **4** (95% yield) by crystallization from minimum CH₂Cl₂ and petroleum ether; mp >250 °C. As is usually the case, a satisfactory elemental analysis of this porphyrin dicarboxylic acid could not be obtained (as follows); Anal. Calcd for C₃₈H₄₂N₄O₄: C, 69.69; H, 6.62; N, 8.56% Found: C, 66.06; H, 6.42; N, 8.11% LRMS: C₃₈H₄₂N₄O₄ requires *m/z* 618.3. Found 619.3.

6,7-Bis(6-methoxycarbonylhexyl)-1,3,5,8-tetramethyl-2,4-divinylporphyrin (21b). Using the same procedure as reported above, the title compound **21b** was obtained (0.30 g scale, 30% yield), mp 190-192 °C. ¹H-NMR: (CDCl₃) δ 10.26 (s, 1H, meso-**H**), 10.19 (s, 1H, meso-**H**), 10.11 (s, 1H, meso-**H**), 9.99 (s, 1H, meso-**H**), 8.32 (dd, 2H, -HC=CH₂), 6.20 (dd, 4H, -

HC=CH₂), 4.09 (t, 4H, -CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-CO₂CH₃), 3.74 (s, 6H, -CO₂CH₃), 3.61 (s, 12H, -CH₃), 2.30 (t, 4H, -CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-CO₂CH₃), 1.67 (m, 8H, -CH₂-CH₂-CH₂-CH₂-CH₂-CO₂CH₃). Anal. Calcd for C₄₄H₅₄N₄O₄: C, 75.17; H, 7.28; N, 7.10. Found: C, 75.17; H, 7.75; N, 6.97. HRMS: C₄₄H₅₄N₄O₄ requires *m/z* 702.41448. Found 702.4153. To obtain the dicarboxylic acid **5**, the pure porphyrin was dissolved in MeOH/H₂O/KOH (90 mL/10 mL/1 g) and stirred under argon in the dark at room temperature overnight. Acidic work-up, followed by extraction into CH₂Cl₂ using a pH 4 phosphate buffer as the aqueous phase led to the isolation of compound **5** (95% yield), mp >250 °C, after crystallization from a minimum amount CH₂Cl₂ and petroleum ether. A satisfactory elemental analysis of this porphyrin dicarboxylic acid could not be obtained. LRMS: C₄₂H₅₀N₄O₄ requires *m/z* 674.4. Found 675.4.

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