Syntheses of Methyl-devinylporphyrins Related to Protoporphyrin-IX. Initial Studies on the Mechanism of the Copper(II) Catalysed Cyclization of 1′,8′-Dimethyl-*a,c*-biladiene Salts

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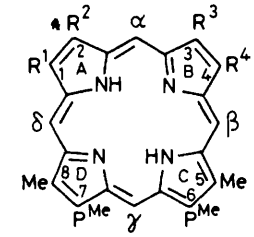
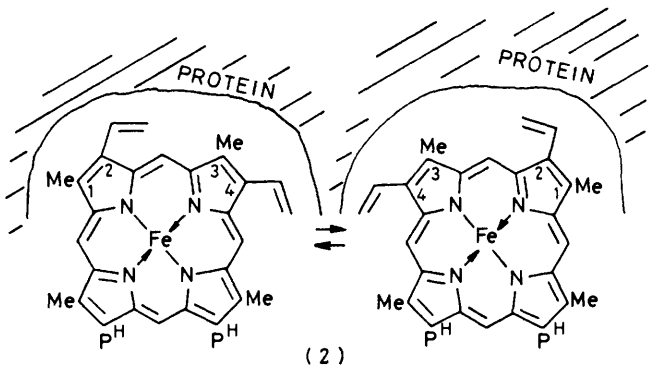
Using copper(II) catalysed cyclization of *a,c*-biladiene dihydrobromide salts, porphyrins [(3)—(5)] related to protoporphyrin-IX dimethyl ester (8), but in which both vinyls are replaced with methyls, or where either the 2- or 4-vinyls are individually replaced with methyls, are synthesized. These compounds are required for reconstitution of the corresponding hemes into hemoproteins, to enable the study of the rotational disorder of prosthetic heme groups in reconstituted hemoproteins. By way of ^{13}C n.m.r. spectroscopy of enriched *a,c*-biladienes and porphyrins, the copper(II) catalysed cyclization of 1′,8′-dimethyl-*a,c*-biladiene dihydrobromides is shown to afford porphyrins in which one of the 1′- and 8′-methyl groups becomes the new linking *meso* carbon atom.

In order to assist in the development of a useful model for the interpretation of the hyperfine-shifted resonances in hemoprotein n.m.r. spectra,[1] it became necessary to have available certain novel derivatives of deuteroporphyrin-IX dimethyl ester (1). These models needed to be related as closely as possible to protoporphyrin-IX but, upon 180° rotation about the α-γ *meso* axis, should interchange only one of the two possible pairs of substituents at positions 1,4 and 2,3. Rotation of protohemin (2) about this axis suffers the complication that two pairs of substituents are simultaneously interchanged. Attempts, therefore, to delineate which of the four substituents on rings A and B are responsible for the absolute configuration of the heme within the apoprotein have been complicated by the large number of groups which need to be considered. Compounds which would ideally fit the requirements of the proposed model are the deuteroporphyrin-IX dimethyl ester derivatives (3) and (4). In the case of compound (3), the vinyl group at position 2 moves to position 3 upon 180° rotation about the α-γ *meso* axis, but the methyl at position 1 merely exchanges its position with the identical substituent at the 4 position. Likewise, for compound (4), only the vinyl at position 4 changes its position (the 1-methyl notwithstanding) upon the 180° rotation. The ' parent ' molecule, 2,4-dimethyldeuteroporphyrin-IX dimethyl ester (5), was also a desirable synthetic target for two reasons. The structure of compound (5) has the built-in property that rotation by 180° about the α-γ *meso* axis produces no observable change at all. In this respect, compound (5) resembles protoporphyrin-III dimethyl ester (6) and protoporphyrin-XIII dimethyl ester (7). Compound (6), as its iron complex, has already been used with advantage in certain n.m.r. studies of hemoglobins from the larva of the aphid, *Chironomus thummi thummi*.[2,3] The second use for compound (5) was that it was structurally suitable to be used in projected studies on the mechanism of the copper(II) catalysed cyclization of 1′,8′-dimethyl-*a,c*-biladienes to give porphyrins. Although a multitude of synthetic approaches to porphyrins bearing the types of substituents and symmetry characteristics to be found in compounds (3)—(5) are available,[4,5] the most generally useful approach seems to be that through copper(II) catalysed cyclizations of 1′,8′-dimethyl-*a,c*-biladiene dihydrobromides.



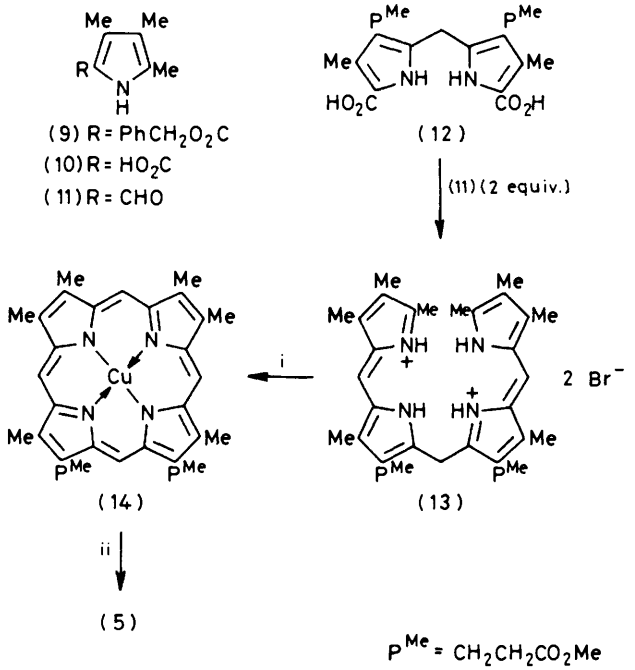
(1) $R^1 = R^3 = Me$, $R^2 = R^4 = H$
(3) $R^1 = R^3 = R^4 = Me$, $R^2 = CH{=}CH_2$
(4) $R^1 = R^2 = R^4 = Me$, $R^3 = CH{=}CH_2$
(5) $R^1 = R^2 = R^3 = R^4 = Me$
(6) $R^1 = R^4 = Me$, $R^2 = R^3 = CH{=}CH_2$
(7) $R^2 = R^3 = Me$, $R^1 = R^4 = CH{=}CH_2$
(8) $R^1 = R^3 = Me$, $R^2 = R^4 = CH{=}CH_2$



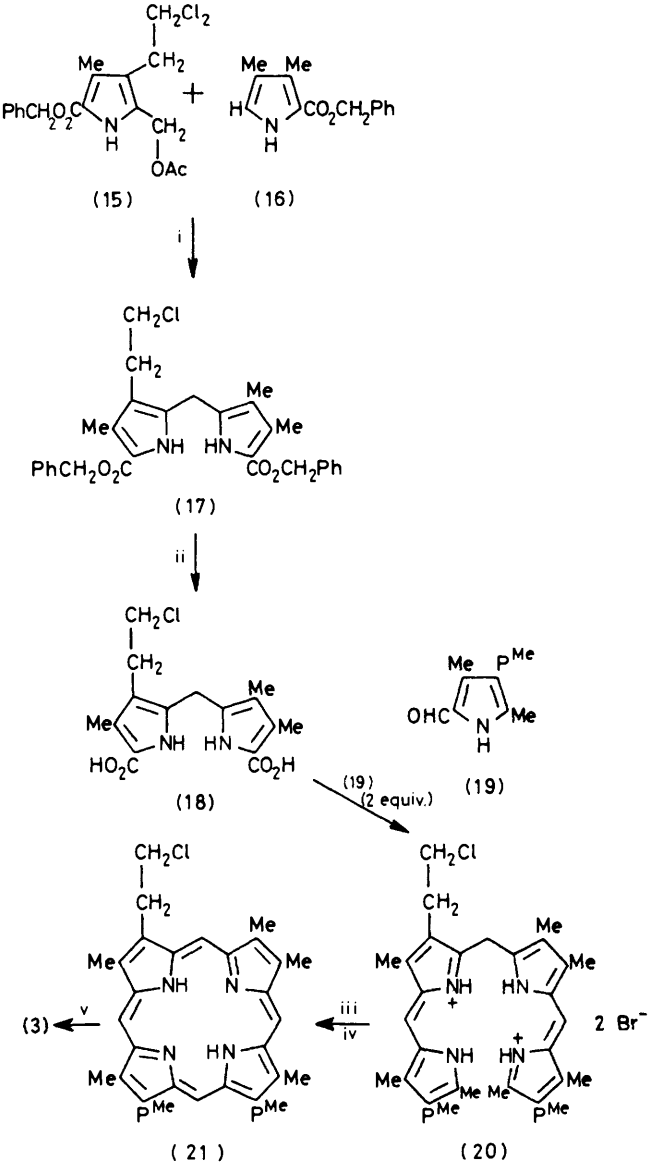
Results and Discussion

Synthesis of 2,4-*Dimethyldeuteroporphyrin*-IX *Dimethyl Ester* (5).—Since this particular compound lacked the complicating vinyl substituents in compounds (3) and (4), the synthesis of (5) was completed first. This compound has previously been synthesized [6] in low yield by fusion of pyrromethanes, and also by reduction of 2,4-diformyldeuteroporphyrin-IX; [7] the latter approach, involving modification of commercially available protoporphyrin-IX dimethyl ester (8), would not be appropriate for the related syntheses of the monovinyl compounds (3) and (4).

The synthesis of porphyrin (5) is shown in Scheme 1; the trimethylpyrrole (9) was hydrogenated to give the carboxylic acid (10), which was formylated directly using Vilsmeier conditions ($POCl_3$–DMF) to afford a good yield of the formyl-



Scheme 1. *Reagents:* i, $CuCl_2$, DMF; ii, H_2SO_4, TFA



Scheme 2. *Reagents:* i, TosOH, HOAc; ii, H_2/Pd–C; iii, $CuCl_2$, DMF; iv, H_2SO_4, TFA; v, KOH, H_2O–pyridine

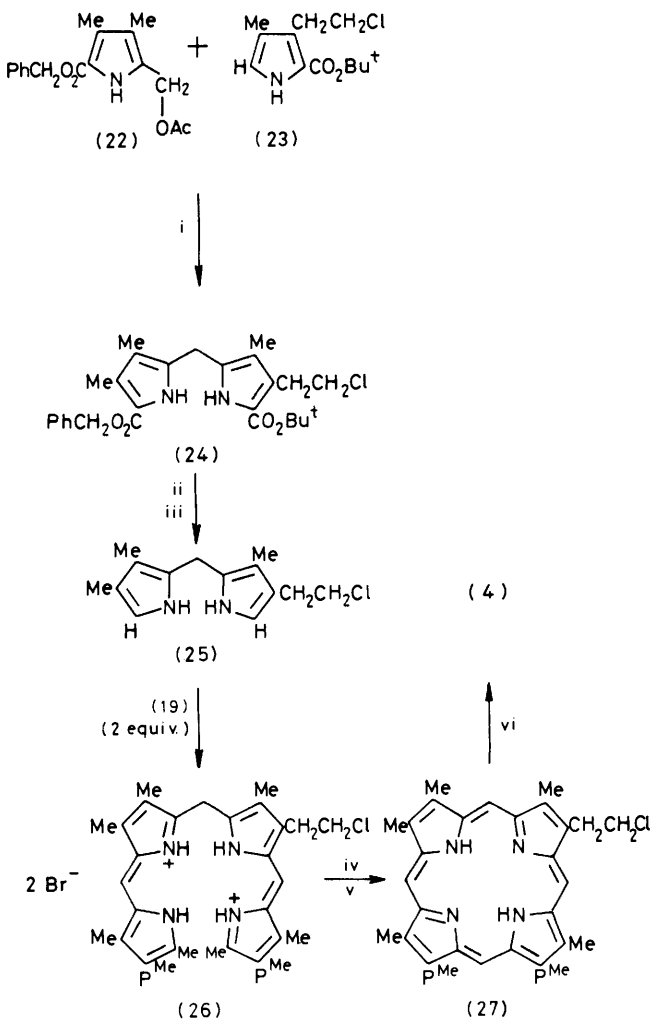
pyrrole (II). The symmetrically substituted pyrromethane (12) was then treated with 2 mol equiv. of the formylpyrrole (11) in the presence of methanol and trifluoroacetic acid (TFA) to give the 1′,8′-dimethyl-*a*,*c*-biladiene, which, after treatment with 40% HBr in acetic acid, gave the crystalline *a*,*c*-biladiene dihydrobromide (13) in 89% yield. The *a*,*c*-biladiene (13) was cyclized using copper(II) chloride in dimethylformamide (DMF) at 150 °C for 4 min, and afforded the copper(II) porphyrin (I4); this was directly demetallated using 20% sulphuric acid in TFA, to give the required 2,4-dimethyldeuteroporphyrin-IX dimethyl ester (5) [36% from (13)].

Synthesis of 4-*Methyl*-2-*vinyldeuteroporphyrin*-IX *Dimethyl Ester* (3).—The general approach to this compound was similar to that employed for compound (5), and is shown in Scheme 2.

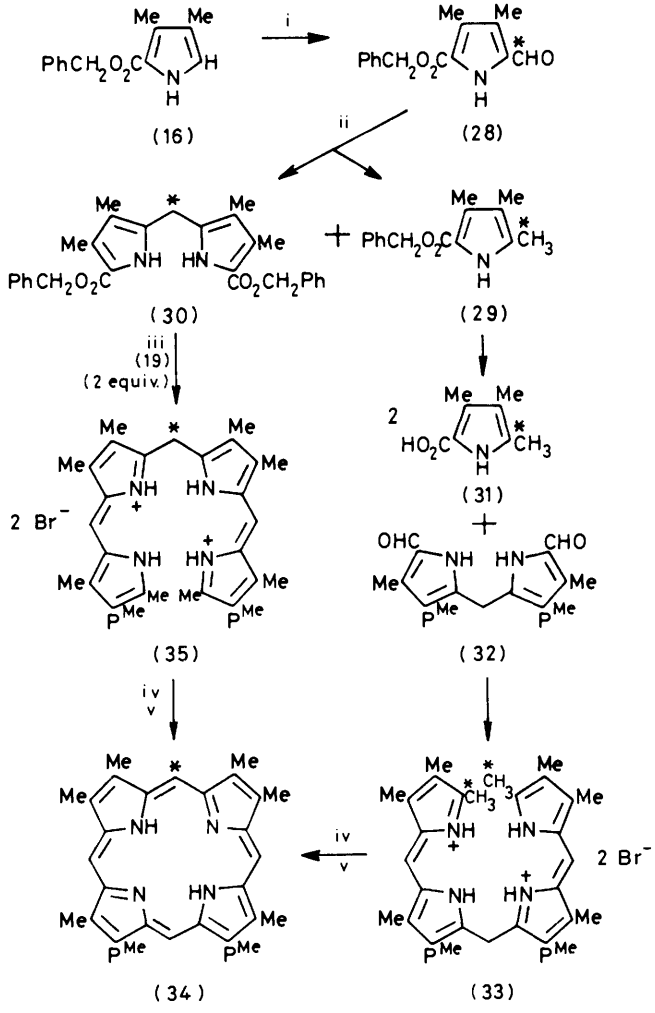
Synthesis of 2-*Methyl*-4-*vinyldeuteroporphyrin-IX Dimethyl Ester* (4).—Synthesis of this porphyrin again proceeded through an *a*,*c*-biladiene (26), and the route is presented in Scheme 3.

In preparation for reconstitution into various hemoproteins, iron was chelated with all three of the synthetic porphyrins (3)—(5) using the ferrous chloride method.[8] Finally, the methyl esters on the 7- and 8-propionates were hydrolysed in alkali. Studies of heme orientation using the reconstituted hemes are currently in progress.

Preliminary Studies on the Origin of the meso *Carbon in the Copper*(II) *Catalysed Cyclization of* 1′,8′-*Dimethyl*-a,c-*biladienes to give Porphyrins.*—One of the most remarkable reactions in contemporary porphyrin chemistry is the copper(II) catalysed cyclization of 1′,8′-dimethyl-*a*,*c*-biladiene salts to give copper complexes of porphyrins. This reaction was discovered by Johnson and Kay [9] during studies on the metal-catalysed cyclization of various *a*,*c*-biladiene salts, and Grigg *et al.*[10] subsequently studied a range of oxidants and introduced DMF as the solvent of choice for porphyrin syntheses. In this sequence, one of the methyl groups in the 1′,8′-dimethyl-*a*,*c*-biladiene [*e.g.* (13)] is extruded during its transformation into the copper(II) porphyrin [*e.g.* (14)]. Grigg *et al.*[10] also suggested a mechanism for the cyclization which utilized a terminal *a*,*c*-biladiene methyl group as the newly introduced porphyrin *meso* carbon. The work of Kulish *et al.*[11] in 1971 revealed certain facts pertinent to the mechanistic route of the oxidative cyclization of *a*,*c*-biladienes to give porphyrins. In this study, a 1,8-di-unsubstituted *a*,*c*-biladiene salt was cyclized with copper salts to give a formylporphyrin as the major product. A logical interpretation of the formation of the formylporphyrin is that the 1′- and 8′-methyls are modified during the cyclization to produce the *meso* carbon at the point of macrocycle formation and the anomalous formyl carbon (through some kind of oxidation reaction followed by migration to the unsubstituted peripheral position). Even though the oxidative cyclization of *a*,*c*-biladienes is one of the most commonly used reactions in porphyrin chemistry, and the novel results obtained by the Russian workers [11] suggest that a mechanistic study could be fruitful, no definitive study of this reaction sequence has so far



Scheme 3. *Reagents:* i, TosOH, HOAc; ii, H_2/Pd–C; iii, TFA; iv, $CuCl_2$, DMF; v, H_2SO_4, TFA; vi, KOH, H_2O–pyridine



Scheme 4. (* = ^{13}C label) *Reagents:* i, $POCl_3$, Me_2NCHO; ii, B_2H_6; iii, H_2/Pd–C; iv, $CuCl_2$, DMF; v, H_2SO_4, TFA

been described. In this initial study we report our results on the origin of the newly introduced *meso* carbon in the cyclization of *a,c*-biladiene salts. The overall reaction (13) ⟶ (14) closely resembles the transformation which occurs when 2-bromomethylpyrroles (or 2-acetoxymethylpyrroles) are self-condensed to give symmetrically substituted pyrromethanes.[12,13] Before any firm mechanism can be proposed for the *a,c*-biladiene cyclization, studies were required to ascertain the fate or origin of the various carbon atoms involved in the reaction; the mechanistic probe in the studies which we describe here was ^{13}C n.m.r. spectroscopy.

The origin of the new *meso* carbon in the porphyrin (5) was established by synthesizing the ^{13}C enriched pyrrole (31) from compound (16) as shown in Scheme 4. For reasons of economy in the use of expensive ^{13}C enriched precursors, it was decided to carry out the *a,c*-biladiene synthesis using 2 mol equiv. of the pyrrole (31) with a 5,5′-diformylpyrromethane (32), and this is also shown in Scheme 4. The diborane reduction of the formylpyrrole (28) gave both the required methylpyrrole (29) and the symmetrical pyrromethane (30), the latter presumably by self-condensation of the intermediate pyrrole carbinol during work-up. Both the pyrrole (29) and the pyrromethane (30) were used in complementary syntheses of the labelled porphyrin (34), and produced identical products. The *meso* proton region of the ^1H n.m.r. spectrum of the product (zinc complex) is shown in the Figure, and this confirms that the new *meso* carbon is derived from the 1′- and 8′-methyl groups in the *a,c*-biladiene (33).

Experimental

M.p.s were measured on a hot-stage apparatus, and are uncorrected. Neutral alumina (Merck 90, 70–230 mesh) was used for column chromatography, and preparative t.l.c. was carried out on 20 × 20-cm glass plates coated with Merck GF 254 silica gel (1 mm thick). Analytical t.l.c. was performed using Merck silica gel 60 F 254 precoated sheets (0.2 mm). Electronic absorption spectra were measured on a Hewlett-Packard 8450A spectrophotometer (solutions in dichloromethane), ^1H n.m.r. spectra were measured at 360 MHz using a Nicolet NT-360 spectrometer, and ^{13}C n.m.r. spectra were measured using a Nicolet NT-200 instrument (solutions in $CDCl_3$). Mass spectra were measured (direct insertion probe, 70 eV, 50 μA, source temp. *ca.* 200 °C) using a Finnegan 3 200 mass spectrometer. Elemental analyses were performed at the Berkeley Microanalytical Laboratory, Department of Chemistry, UC Berkeley.

Benzyl 3,4-Dimethylpyrrole-2-carboxylate (16).—Benzyl 5-iodo-3,4-dimethylpyrrole-2-carboxylate [14] (16.71 g) dissolved in methanol (440 ml) containing sodium acetate trihydrate

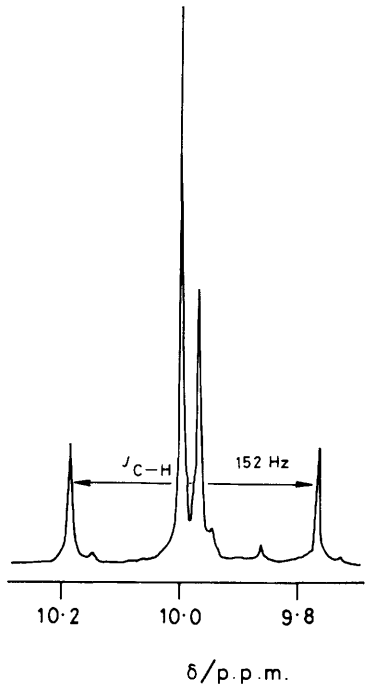


Figure. ^{1}H N.m.r. spectrum (360 MHz) of the *meso* proton region of the zinc(II) complex of the α *meso* ^{13}C labelled porphyrin (34). The solvent is $CDCl_3$ with a small amount of pyrrolidine added to ensure absence of aggregation

(17.6 g) and Adams catalyst (20 mg) was hydrogenated at room temperature and atmospheric pressure until uptake of hydrogen ceased (3 h). T.l.c. monitoring showed the reaction to be complete after this time, so the catalyst was filtered off on Celite, which was washed with methanol, and the combined filtrates were evaporated to give a brown oil. The residue was chromatographed, first on alumina [Brockmann Grade III, elution with hexane–dichloromethane (1 : 1), with unsatisfactory results], and then on silica gel (elution with dichloromethane), which gave an easy separation from baseline impurities. The combined eluates were evaporated to give the *product* (8.38 g, 78%) as an off-white solid. A sample was recrystallized for elemental analysis from dichloromethane–hexane, and had m.p. 73—75 °C (Found: C, 73.4; H, 6.6; N, 6.1. $C_{14}H_{15}NO_2$ requires C, 73.34; H, 6.59; N, 6.11%); δ 8.98 (1 H, br, NH), 7.31, 5.27 (5 H, 2 H, $PhCH_2$), 6.50 (1 H, d, *J* 3 Hz, 5-H), and 2.27, 1.97 (each 3 H, Me); *m/z* (%) 229 (80), 185 (83), 170 (80), 138 (66), 122 (75), 108 (86), and 91 (100).

Benzyl 5-Formyl-3,4-dimethylpyrrole-2-carboxylate (28; *unlabelled*).—Benzyl 3,4-dimethylpyrrole-2-carboxylate (16) (1.07 g) dissolved in dichloromethane (25 ml) was added dropwise during 20 min to the Vilsmeier complex prepared from phosphoryl chloride (0.87 ml) and DMF (0.72 ml). The mixture was heated under reflux for 30 min, and t.l.c. monitoring showed complete transformation into the Vilsmeier imine salt. Saturated aqueous sodium acetate (100 ml) was added cautiously, and then aqueous sodium hydrogen carbonate was added until the mixture reached pH 8. The mixture was extracted with dichloromethane (3 × 75 ml) and then washed with water (3 × 75 ml), dried (Na_2SO_4), and evaporated to give the required pyrrole (1 g, 83%) as an off-white solid; δ 9.71 (1 H, CHO), 9.65 (1 H, br, NH), 7.37, 5.29 (5 H, 2 H, $PhCH_2$), and 2.25 (3 H, Me). When the reaction was repeated using 90% enriched [^{13}C]dimethylformamide [on a scale of 1.48 g of pyrrole (16)] a 96% yield of the formylpyrrole (28) was obtained. This labelled material was identical with the unlabelled compound described above, except that the ^{1}H n.m.r. spectrum showed a ^{13}C-^{1}H coupling constant for the CHO group of 179 Hz.

Diborane Reduction of Pyrrole (28) *to give the Labelled 5-Methylpyrrole* (29) *and the Symmetrically Substituted Labelled Pyrromethane* (30).—Labelled formylpyrrole (28) (1.63 g) in 1,2-dimethoxyethane (20 ml) was cooled (iced water) under nitrogen during addition of 1.0M-BH_3·THF solution in tetrahydrofuran (THF) (19 ml). The stirred reaction mixture was allowed to warm to room temperature and then stirred for 20 h. T.l.c. analysis indicated incomplete reduction so more BH_3·THF (5.0 ml) was added. After a further 48 h the mixture was treated with methanol (20 ml), extracted with dichloromethane (3 × 50 ml), and then washed with water (3 × 50 ml). T.l.c. analysis and development with bromine vapour showed the major product to be a pyrromethane. The reaction product was chromatographed on alumina (Brockmann Grade III, elution with dichloromethane); the faster running band was shown, by comparison with an authentic sample, to be the 3,4,5-trimethylpyrrole (29) (60 mg), labelled in the 5-methyl with ^{13}C; δ 8.79 (1 H, br, NH), 7.30, 5.22 (5 H, 2 H, $PhCH_2$), 2.20, 1.84 (each 3 H, Me), and 2.08 (3 H, d, *J* 129 Hz, Me). The slower running chromatographic band was shown, by comparison with an authentic sample, to be labelled dibenzyl 3,3′,4,4′-tetramethylpyrromethane-5,5′-dicarboxylate (30) (700 mg); δ 8.98 (2 H, br, NH), 7.28, 5.24 (10 H, 4 H, $PhCH_2$), 3.77 (2 H, d, *J* 147 Hz, CH_2), and 2.23, 1.93 (each 6 H, Me).

Benzyl 5-Acetoxymethyl-3,4-dimethylpyrrole-2-carboxylate (22).—The pyrrole (9) (7.4 g) in acetic acid (156 ml) and acetic anhydride (3.9 ml) was stirred for 3 h with addition of lead tetra-acetate (14.25 g) in portions. It was then stirred at room temperature overnight. The reddish-brown solution was treated, dropwise, with water (150 ml) and a flaky precipitate formed. After filtration and washing with water, the crude precipitate was dissolved in dichloromethane, dried (Na_2SO_4) and evaporated to dryness. Recrystallization from hexane–dichloromethane gave the required *pyrrole* (4.71 g, 51%) as a fluffy white solid, m.p. 105.5—107 °C (Found: C, 67.9; H, 6.5; N, 4.6. $C_{17}H_{19}NO_4$ requires C, 67.76; H, 6.36; N, 4.65%); δ 9.00 (1 H, br, NH), 7.46, 5.35 (5 H, 2 H, $PhCH_2$), 5.05 (2 H, CH_2O), 2.26 (3 H, COMe), and 2.05, 2.00 (each 3 H, Me); *m/z* (%) 301 (68), 256 (30), 242 (58), 229 (80), 210 (12), 185 (27), 168 (50), and 91 (100).

Dibenzyl 3-(2-Chloroethyl)-3′,4,4′-trimethylpyrromethane-5,5′-dicarboxylate (17).—Benzyl 3,4-dimethylpyrrole-2-carboxylate (16) (2.04 g) was dissolved in acetic acid (125 ml) containing toluene-*p*-sulphonic acid (89 mg) and then treated under nitrogen with a solution of benzyl 5-acetoxymethyl-4-(2-chloroethyl)-3-methylpyrrole-2-carboxylate (15) (3.07 g) in acetic acid (105 ml), added dropwise during 45 min. The pink reaction mixture was stirred at 45 °C for 4 h, after which time it was diluted with dichloromethane (100 ml), washed with aqueous sodium acetate, then aqueous sodium hydrogen-carbonate, and finally water. The organic layer was dried (Na_2SO_4), filtered, and evaporated to give a residue which was chromatographed on alumina (Brockmann Grade II, elution with a dichloromethane–cyclohexane gradient from 1 : 1 to pure dichloromethane). The product was obtained from the appropriate eluates by evaporation and was recrystallized from dichloromethane–hexane to give the *pyrromethane* (4.28 g, 94%), with m.p. 138.5—140.5 °C (Found: C, 69.6; H, 6.2; N, 5.4. $C_{30}H_{31}ClN_2O_4$ requires C, 69.42; H, 6.02; N, 5.40%);

δ 10.10, 9.90 (each 1 H, br, NH), 7.20, 5.14 (10 H, 4 H, $PhCH_2$), 3.73 (2 H, CH_2), 3.37, 2.85 (each 2 H, t, CH_2CH_2), 2.24 (6 H, 2 × Me), and 1.95 (3 H, Me); *m/z* (%) 518 (100), 470 (36), 410 (18), 319 (23), 289 (15), 273 (29), 254 (45), 241 (30), 229 (26), 198 (17), and 184 (24).

Benzyl 5′-t-Butoxycarbonyl-4′-(2-chloroethyl)-3,3′,4-trimethylpyrromethane-5-carboxylate (24).—This pyrromethane was likewise prepared from t-butyl 4-(2-chloroethyl)-3-methylpyrrole-5-carboxylate (23) (200 mg) and benzyl 5-acetoxymethyl-3,4-dimethylpyrrole-2-carboxylate (22) (240 mg) in acetic acid (16 ml) containing toluene-*p*-sulphonic acid (8 mg). The yield of the required pyrromethane was 187 mg (48%), and the *product*, after recrystallization from dichloromethane–hexane, had m.p. 139—141 °C (Found: C, 67.0; H, 6.8; N, 5.6. $C_{27}H_{33}ClN_2O_4$ requires C, 66.84; H, 6.86; N, 5.78%); δ 9.04, 8.88 (each 1 H, br, NH), 7.36, 5.30 (5 H, 2 H, $PhCH_2$), 3.83 (2 H, CH_2), 3.63, 3.10 (each 2 H, CH_2CH_2), 2.27 2.00, 1.98 (each 3 H, Me), and 1.57 (9 H, Bu^t); *m/z* (%) 484 (88), 428 (100), 368 (19), 337 (10), 265 (53), 256 (15), 247 (45), 241 (29), 229 (15), 199 (39), and 128 (62).

4,5-*Bis*(2-*methoxycarbonylethyl*)-1′,1,2,3,6,7,8,8′-*octamethyl*-a,c-*biladiene Dihydrobromide* (13).—3,3′-Bis(2-methoxycarbonylethyl)-4,4′-dimethylpyrromethane-5,5′-dicarboxylic acid (12) (244 mg) was dissolved in TFA (1.5 ml) and stirred until evolution of carbon dioxide ceased. To the resulting dark red solution was added 2-formyl-3,4,5-trimethylpyrrole (11) (154 mg) in methanol (7 ml), and after 3 min, 40% HBr–acetic acid (1 ml) was added and the mixture was stirred for 30 min. At the end of that time, diethyl ether (15 ml) was added dropwise to the stirred solution, which was further stirred for 2 h. The precipitated *a,c*-biladiene was filtered through a Willstatter nail and washed with cold diethyl ether, giving the desired *product* (14) (374 mg; 89%) as dark red microcrystals, m.p. above 300 °C (Found: C, 55.4; H, 6.3; N, 7.2. $C_{35}H_{46}Br_2N_4O_4 \cdot H_2O$ requires C, 55.01; H, 6.33; N, 7.33%); δ 13.39, 13.34 (each 2 H, br, NH), 7.11 (2 H, methine-CH), 5.23 (2 H, methine-CH_2), 3.45, 2.69, 2.27, 2.24, 2.00 (each 6 H, Me and OMe), and 2.82, 2.06 (each 4 H, t, CH_2CH_2); $\lambda_{max.}$ 372 (ε 14 700), 454 (38 000), and 522 nm (216 000).

4-(2-*Chloroethyl*)-1,8-*bis*(2-*methoxycarbonylethyl*)-1′,2,3,5,-6,7,8′-*heptamethyl*-a,c-*biladiene Dihydrobromide* (20).—This compound was likewise prepared from dibenzyl 3-(2-chloroethyl)-3′,4,4′-trimethylpyrromethane-5,5′-dicarboxylate (17) (2.17 g), which was hydrogenated using 10% Pd–C (200 mg) and triethylamine (3 drops) in THF (150 ml). The catalyst was removed by filtration through Celite and gave the corresponding pyrromethane-5,5′-dicarboxylic (18) as a white solid. This material was used directly; it was dissolved in TFA (10 ml), stirring for 20 min, and then 2-formyl-4-(2-methoxycarbonylethyl)-3,5-dimethylpyrrole (19) (2.23 g) in methanol (40 ml) was added. The mixture was stirred for 30 min, 33% HBr–acetic acid (9 ml) was added, and the mixture stirred for a further 30 min; anhydrous diethyl ether (200 ml) was then added dropwise to the stirred reaction mixture. After 2.5 h the *precipitate* was collected and washed with dry diethyl ether to give orange-red microcrystals (2.81 g, 85%), m.p. >300 °C (Found: C, 54.1; H, 5.8; N, 6.9. $C_{36}H_{47}Br_2ClN_4O_4$ requires C, 54.38; H, 5.96; N, 7.05%); δ 13.44, 13.36, 13.31, 13.15, (each 1 H, br, NH), 7.16, 7.13 (each 1 H, methine-H), 5.22 (2 H, methine CH_2), 3.67 (6 H, 2 × OMe), 3.16, 2.98 (each 2 H, t, CH_2CH_2Cl), 2.78, 2.49 (each 4 H, t, CH_2CH_2CO), and 2.75, 2.70, 2.33, 2.32, 2.28, 2.22, 1.94 (each 3 H, Me); $\lambda_{max.}$ 372 (ε 14 800), 456 (24 300), and 524 nm (179 000).

3-(2-*Chloroethyl*)-1,8-*bis*(2-*methoxycarbonylethyl*-1′,2,4,5,-6,7,8′-*heptamethyl*-a,c,-*biladiene Dihydrobromide* (26).—This *a,c*-biladiene salt was likewise prepared by catalytic hydrogenation of benzyl 5′-t-butoxycarbonyl-4′-(2-chloroethyl)-3,3′,4-trimethylpyrromethane-5-carboxylate (24) (147 mg) followed by treatment with TFA [to give (25)] and addition of 2-formyl-4-(2-methoxycarbonylethyl)-3,5-dimethylpyrrole (19) (130 mg), as described above. The a,c,-*biladiene* (208 mg, 86%) was isolated as dark-red microcrystals, m.p. >300 °C (Found: C, 54.1; H, 5.8; N, 7.0. $C_{36}H_{47}Br_2ClN_4O_4$ requires C, 54.38; H, 5.96; N, 7.05%); δ 13.51, 13.37, 13.17, (1 H, 2 H, 1 H, each br, NH), 7.13, 7.10 (each 1 H, methine-H), 5.21 (2 H, methine-CH_2), 3.68 (6 H, OMe), 3.60, 3.08 (each 2 H, t, CH_2CH_2Cl), 2.79, 2.48 (each 4 H, t, CH_2CH_2CO), and 2.74, 2.71, 2.33, 2.31, 2.21, 1.93, 1.88 (each 3 H, Me); $\lambda_{max.}$ 376 (ε 12 400), 452 (31 400), and 526 nm (138 000).

6,7-*Bis*(2-*methoxycarbonylethyl*)-1,2,3,4,5,8-*hexamethylporphyrin* (5).—The *a,c*-biladiene dihydrobromide (13) (264 mg) was added to a stirred solution of copper(II) chloride dihydrate (1.21 g) in refluxing DMF (15 ml) and then heated at reflux temperature for 4 min. The mixture was poured immediately into a solution of pyridine (6.7 ml) in water (55 ml), then extracted with dichloromethane (3 × 100 ml), and the organic phase was washed with saturated sodium acetate solution (2 × 75 ml), water (2 × 75 ml), and then dried (Na_2SO_4) and evaporated to dryness, the last traces of DMF being removed with a high vacuum pump. The red residue [of (14)] was immediately column chromatographed on alumina (Brockmann Grade III, elution with dichloromethane), and the red eluates were evaporated to give a residue which was taken up in 20% sulphuric acid in TFA (15 ml) and stirred at room temperature for 30 min. The solution was diluted with dichloromethane (100 ml), washed with water (3 × 100 ml), aqueous sodium hydrogen carbonate solution (3 × 100 ml), and finally water (3 × 50 ml) again. The organic phase was evaporated to dryness to give a red residue which was chromatographed on alumina (Brockmann Grade III, elution with dichloromethane); evaporation of the red eluates gave the required porphyrin (72 mg, 36%), as red crystals from dichloromethane–hexane. (Note, a higher yield might have been obtained if the product had been re-esterified with 5% sulphuric acid in methanol after removal of the copper.) The product had m.p. >300 °C (lit.,[6] m.p. 318—320 °C); δ 10.06, 10.02, 9.96, (1 H, 2 H, 1 H, *meso*-H), 4.42, 3.28 (each 4 H, CH_2CH_2-CO), 3.66, 3.64, 3.56, 3.54 (each 6 H, Me and OMe), and −3.84 (2 H, br, NH). When the reaction was carried out using *a,c*-biladiene (33) in which the terminal 1′- and 8′-methyl groups were 90% enriched with ^{13}C, the resulting porphyrin was labelled at the α *meso* carbon, and was otherwise identical with the unlabelled material. The n.m.r. spectrum of the carbon-labelled porphyrin (34) was as follows: δ($CDCl_3$) 10.03, 9.97 (1 H, 2 H, *meso*-H), 10.89 (1 H, d, *J* 154 Hz, *meso*-H), 4.41, 3.29 (each 4 H, t, CH_2CH_2CO), 3.68, 3.61, 3.53, 3.52 (each 6 H, Me, OMe), and −3.85 (2 H, br, NH). The n.m.r. spectrum of the zinc(II) complex was as follows: δ($CDCl_3$), 9.13, 8.95 (1 H, 2 H, *meso*-H), 8.52 (1 H, d, *J* 152 Hz, *meso*-H), 4.14, 3.08 (each 4 H, t, CH_2CH_2), 3.70 (6 H, OMe), and 3.35, 3.12, 2.96 (each 6 H, Me). The ^1H n.m.r. spectrum of the zinc(II) complex, disaggregated by addition of excess of pyrrolidine (Figure), was as follows: δ 10.00, 9.97, (1 H, 2 H, *meso*-H), 9.98 (1 H, d, *J* 152 Hz, *meso*-H), 4.44, 3.27 (each 4 H, t, CH_2CH_2CO), and 3.66, 3.64, 3.58 (6 H, 6 H, 12 H, OMe and Me). When the ^{13}C enriched porphyrin was synthesized by the independent route involving the pyrromethane (30) and the *a,c*-biladiene dihydrobromide (35) [δ 13.38, 13.19 (each 2 H, br, NH), 7.11 (2 H, methine-H), 5.22 (2 H, d, *J* 150 Hz, CH_2), 3.68 (6 H, OMe), 2.80, 2.48 (each 4 H, t, CH_2CH_2),

2.77, 2.32, 2.21, 1.91 (each 6 H, Me)], the n.m.r. spectra of the materials were identical with those obtained by the route through the *a,c*-biladiene dihydrobromide (33).

2-(2-*Chloroethyl*)-6,7-*bis*(2-*methoxycarbonylethyl*)-1,3,4,5,8-*pentamethylporphyrin* (21).—This porphyrin was prepared, as described above, from the *a,c*-biladiene dihydrobromide (20) (160 mg). The *porphyrin* (21) (75%) was obtained by way of the copper(II) porphyrin; m.p. 229—230 °C, after recrystallization from dichloromethane–hexane (Found: C, 68.1; H, 6.4; N, 9.0. $C_{35}H_{39}ClN_4O_4$ requires C, 68.32; H, 6.39; N, 9.11%); δ 10.05, 9.98, 9.80 (2 H, 1 H, 1 H, *meso*-H), 4.41 (6 H, m, CH_2 next to porphyrin in propionates and chloroethyl), 4.27 (2 H, t, CH_2Cl), 3.72, 3.70, 3.66, 3.60, 3.55 (3 H, 6 H, 3 H, 3 H, 6 H, OMe and Me), 3.30 (4 H, t, CH_2CO), and −3.86 (2 H, br, NH); $\lambda_{max.}$ 400 (ε 165 000), 498 (11 800), 532 (8 000), 566 (5 200), and 622 nm (3 000); *m/z* (%) 616 (40), 614 (100), and 541 (9).

4-(2-*Chloroethyl*)-6,7-*bis*(2-*methoxycarbonylethyl*)-1,2,3,5,8-*pentamethylporphyrin* (27).—This porphyrin was similarly prepared, by way of the copper(II) complex, from the *a,c*-biladiene dihydrobromide (26) (2.14 g). The *product* (991 mg, 60%) was recrystallized from dichloromethane–hexane, m.p. 244—245 °C (Found: C, 67.8; H, 6.3; N, 8.9. $C_{35}H_{39}ClN_4O_4$ requires C, 68.32; H, 6.39; N, 9.11%); δ 10.10, 10.08, 10.07, 10.01 (each 1 H, *meso*-H), 4.5 (6 H, m, CH_2 next to porphyrin in chloroethyl and propionates), 4.40 (2 H, t, CH_2Cl), 3.68, 3.66, 3.65, 3.63, 3.62 (3 H, 6 H, 3 H, 3 H, 6 H, Me and OMe), 3.32 (4 H, t, CH_2CO), and −3.75 (2 H, br, NH); $\lambda_{max.}$ 400 (ε 164 600), 498 (12 100), 532 (8 300), 566 (5 700), and 622 nm (3 300).

6,7-*Bis*(2-*methoxycarbonylethyl*)-1,3,4,5,8-*pentamethyl*-2-*vinylporphyrin* (3).—The 2-(2-chloroethyl)porphyrin (21) (250 mg) was dissolved in pyridine (90 ml) and heated under reflux for 5 min; this was followed by addition of 3% sodium hydroxide in water (20 ml). The reaction mixture was then refluxed for a further 2.5 h, before being treated with 25% aqueous acetic acid (20 ml) and water (120 ml). The mixture was concentrated to *ca.* 60 ml, the precipitate was collected on Celite, and then washed with water before being left overnight in the vacuum oven. Next day the residue was dissolved in 5% sulphuric acid in dry methanol (350 ml) and then stirred in the dark for 9 h. The mixture was diluted with water (400 ml) and then extracted with chloroform (3 × 100 ml), dried (Na_2SO_4), and evaporated to dryness. The residue was chromatographed on alumina (Brockmann Grade III, elution with dichloromethane), and evaporation of the red eluates gave a residue which was recrystallized from dichloromethane–hexane to give the *vinylporphyrin* (230 mg, 98%), m.p. >330 °C (Found: C, 72.5; H, 6.6; N, 9.5. $C_{35}H_{38}N_4O_4$ requires C, 72.64; H, 6.62; N, 9.68%). δ 10.16, 10.11, 10.06, 10.02 (each 1 H, *meso*-H), 8.29 (1 H, q, vinyl-CH), 6.35, 6.17 (each 1 H, d, vinyl-CH_2), 4.45, 4.39 (each 2 H, t, CH_2 next to porphyrin ring), 3.71, 3.67, 3.66, 3.61, 3.60 (3 H, 6 H, 3 H, 3 H, 6 H, Me and OMe), 3.28, 3.27 (each 2 H, t, CH_2CO), and −3.78 (2 H, br, NH); $\lambda_{max.}$ 400 (ε 159 600), 502 (13 500), 538 (11 900), 570 (7 200), and 626 nm (4 000).

6,7-*Bis*(2-*methoxycarbonylethyl*)-1,2,3,5,8-*pentamethyl*-4-*vinylporphyrin* (4).—This *compound* was similarly prepared, in quantitative yield, from the 4-(2-chloroethyl)porphyrin (27). It was recrystallized from dichloromethane–hexane, m.p. 259—261.5 °C (Found: C, 72.8; H, 6.6; N, 10.0. $C_{35}H_{38}N_4O_4$ requires C, 72.64; H, 6.62; N, 9.68%); δ 10.13, 10.00, 9.94, 9.93 (each 1 H, *meso*-H), 8.25 (1 H, q, vinyl-CH), 6.33, 6.13 (each 1 H, d, vinyl-CH_2), 4.42, 4.36 (each 2 H, t, CH_2 next to porphyrin ring), 3.68, 3.66, 3.64, 3.63, 3.58, 3.53, 3.52 (each 3 H, Me and OMe), 3.28, 3.27 (each 2 H, t, CH_2CO), and −3.75 (2 H, br, NH); $\lambda_{max.}$ 400 (ε 149 700), 502 (12 200), 538 (11 000), 570 (6 400), and 626 nm (3 500).

Typical Iron Insertion Procedure.—Acetontrile (25 ml) was refluxed under nitrogen for 1 h, after which time ferrous chloride (1.5 g) was added, and stirred until it dissolved. After cooling to room temperature, a solution of the 2-vinylporphyrin (3) (111 mg) in chloroform (19 ml) was added *via* a septum with a syringe, at the rate of 0.5 ml per min. The mixture was then stirred for 40 min before being exposed to air for 10 min. The reaction mixture was diluted with dichloromethane (100 ml), then washed with 0.2M-HCl (3 × 150 ml). The organic layer was evaporated and dried (Na_2SO_4), and then chromatographed on preparative silica gel plates (elution with 5% methanol in dichloromethane). Some of the faster travelling metal-free band was retrieved (to be re-used). The yield of iron complex was typically 106 mg (87%) and this material was usually used directly for hydrolysis and protein reconstitutions.

Typical Hydrolysis to Produce Hemin Carboxylic Acid.—The foregoing hemin diester (239 mg) was treated with 1% KOH in water and methanol (50 ml) [prepared by dissolving KOH (1 g) in water (4 ml) and diluting with methanol to 100 ml], and then refluxed at 60 °C under nitrogen, overnight. The mixture was then diluted with water (100 ml), and treated with 1% NaOH in water (100 ml) to dissolve the precipitated solid. After being washed with chloroform (2 × 50 ml) the aqueous layer was filtered, and this was then re-acidified by dropwise addition of conc. HCl until the required hemin precipitated as red-brown flakes. It was filtered off on a sinter, washed with water (50 ml), and then dried overnight under vacuum. Typically a quantitative yield of material was obtained, and this could be recrystallized from THF–hexane.

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