New Efficient Total Syntheses of Derivatives of Protoporphyrin-IX Bearing Deuteriated Methyl Groups

Kevin M. Smith* and Ravindra K. Pandey

Department of Chemistry, University of California,
Davis, California 95616, USA

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New total syntheses of hemins which are regioselectively deuteriated in the 1,5 (**2**), 5 (**3**) or 8 (**4**) methyl groups are described. Syntheses of hemins **2** and **3** follow the progression from pyrromethane **12** to *t*-butyl tripyrrene-carboxylate hydrobromide (**17** and **30**) and then to *a,c*-biladiene dihydrobromide (**19** and **31**), but for reasons of economy in use of labeled monopyrroles, the *a,c*-biladiene dihydrobromide **40** for hemin **4** is approached in an initially "clockwise" manner by synthesis of a benzyl tripyrrene-carboxylate hydrobromide **37** from the pyrromethane **5**. Cyclization of the *a,c*-biladienes (**19**, **31**, and **40**) was accomplished by brief heating in dimethylformamide in the presence of copper(II) chloride.

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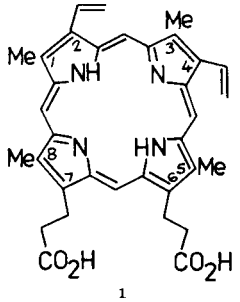
Synthetic derivatives of protoporphyrin-IX (**1**) in which the methyl groups at positions 1, 3, 5 and 8 have been substituted with trideuteriomethyl functions [1-3] have been instrumental in obtaining definitive proton nmr assignments of the heme methyl peaks in a variety of hemoproteins [4-16]. In particular, such compounds were vitally important in the characterization of "heme rotational disorder" in several natural [8,9,11] and reconstituted [7,9,10] hemoproteins. However, the major importance of resonance assignment in hemoprotein nmr spectroscopy is that such information can be used to probe the electronic structure of various hemes embedded in apoproteins, and to investigate the phenomena which, for the same heme prosthetic group, cause the apoprotein to impart a unique function to the heme/apoprotein adduct.

Gradual extension of our studies to a larger assortment of hemoproteins has caused additional syntheses of methyl-labeled hemes to be required; in this paper we describe new improved total syntheses of 1,5-di-(trideuteriomethyl)- (**2**), 5-trideuteriomethyl- (**3**), and 8-trideuteriomethyl-protohemin-IX (**4**).



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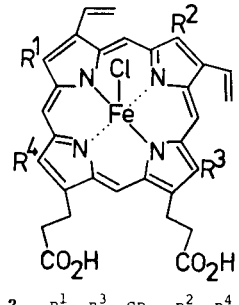
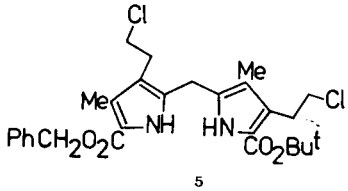


2, $R^1 = R^3 = CD_3$; $R^2 = R^4 = Me$
3, $R^3 = CD_3$; $R^1 = R^2 = R^4 = Me$
4, $R^4 = CD_3$; $R^1 = R^2 = R^3 = Me$

Synthesis of Hemins **2** and **3**.

In our earlier synthesis of **2** [3] the ring AB pyrromethane **5** was elaborated to give an *a,c*-biladiene which was subsequently cyclized to give porphyrin. This approach required a great deal of complex manipulation of monopyrroles in order to obtain the deuterium labeled, unsymmetrically substituted pyrromethane **5**. In our new approach, this problem was overcome by use of the pyrromethane **6** in which the beta-pyrrole substituents are symmetrically arranged. Thus, the deuteriated pyrrole **7** [1] was transformed into its acetoxymethyl derivative **8** by treatment with lead tetraacetate. The original synthesis of such labeled pyrroles was improved by more efficient preparation of the dione **9** using deuteriated acetylacetone **10** and methyl acrylate in a sealed tube at 160° in presence of a catalytic quantity of nickel(II) acetylacetonate; [17] condensation of **9** with *t*-butyl oximinoacetoacetate under standard Johnson-Kleinspehn conditions [18] gave pyrrole **7** in 50% yield.



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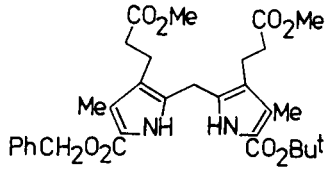

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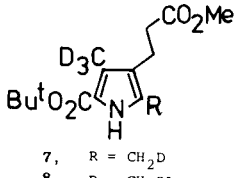
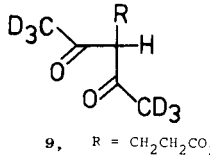
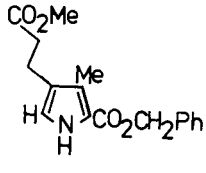


7, R = CH_2D
8, R = CH_2OAc



9, R = $CH_2CH_2CO_2Me$
10, R = H



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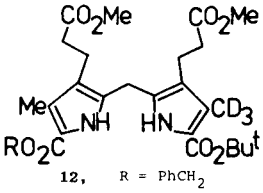
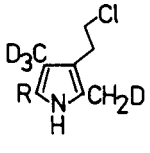


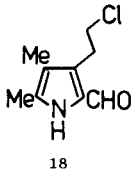
12, R = $PhCH_2$
13, R = H

Treatment of the deuteriated acetoxymethylpyrrole **8** with the 2-unsubstituted pyrrole **11** gave the pyrromethane **12** in 70% yield. Catalytic debenzylation gave the pyrromethane carboxylic acid **13** which was condensed, in the presence of *p*-toluenesulfonic acid, with the methyl-labeled 2-formylpyrrole **14** (obtained from the corresponding known [3] benzyl pyrrole-2-carboxylate **15** by catalytic hydrogenation to give **16**, followed by Vilsmeier formylation) to give the tripyrrene hydrobromide, **17**, in 70% yield after brief treatment with hydrogen bromide gas to accomplish anion exchange. Upon treatment with trifluoroacetic/hydrobromic acids, the tripyrrene hydrobromide **17** condensed with the formylpyrrole **18** to give the *a,c*-biladiene dihydrobromide **19**, in 85% yield. Formylpyrrole **18** was prepared from pyrrole **20** by transesterification (using benzyl alcohol and sodium) to give **21**, followed by formylation with trimethyl orthoformate and trifluoroacetic acid (to afford **22**) and diborane reduction, which accomplished both reduction of the formyl group to methyl and of the acetate group to give the 2-hydroxyethylpyrrole **23** in good overall yield. Finally, treatment with thionyl chloride and pyridine gave the 2-chloroethylpyrrole **24** which was debenzylated by catalytic hydrogenation and then formylated *via* the Vilsmeier procedure to give **18**.

Brief treatment of the *a,c*-biladiene dihydrobromide **19** with copper(II) chloride in hot dimethylformamide gave the copper(II) porphyrin **25**, and this was demetallated by treatment with 10% sulfuric acid in trifluoroacetic acid (to give **26**) before dehydrochlorination using sodium hydroxide in pyridine/methanol. The resulting 1,5-labeled protoporphyrin-IX dimethyl ester, **27**, was treated with ferrous chloride in methanol, to give the hemin **28** after a chloride wash. Finally, the propionic esters were hydrolyzed by treatment of **28** with potassium hydroxide/methanol/water, and gave a good yield of the required hemin, **2**.



14, R = CHO
15, R = $PhCH_2O_2C$
16, R = HO_2C



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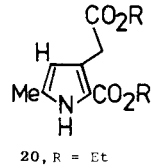
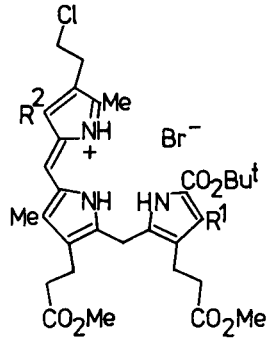
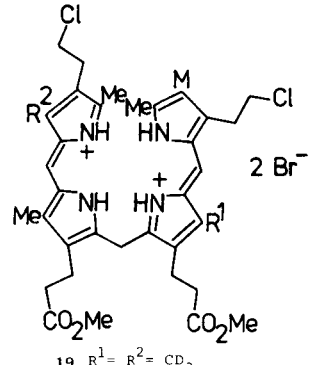


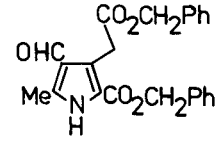
20, R = Et
21, R = CH_2Ph



17, $R^1 = R^2 = CD_3$
30, $R^1 = CD_3$; R^2 = Me



19, $R^1 = R^2 = CD_3$
31, $R^1 = CD_3$; R^2 = Me



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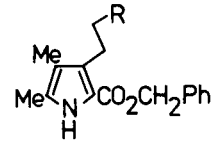
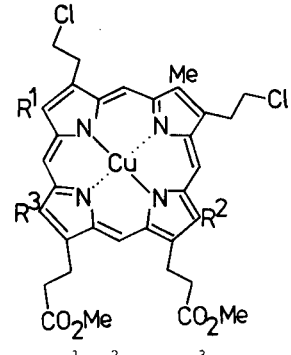
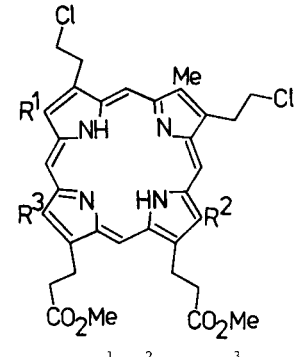


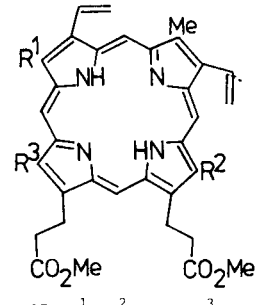
23, R = OH
24, R = Cl



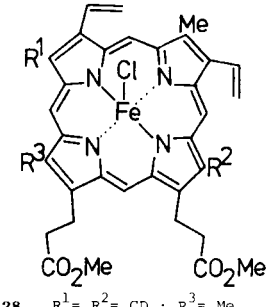
25, $R^1 = R^2 = CD_3$; R^3 = Me
32, $R^1 = R^3$ = Me; $R^2 = CD_3$
41, $R^3 = CD_3$; $R^1 = R^2$ = Me



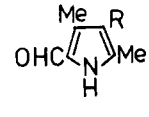
26, $R^1 = R^2 = CD_3$; R^3 = Me
33, $R^1 = R^3$ = Me; $R^3 = CD_3$
42, $R^3 = CD_3$; $R^1 = R^2$ = Me



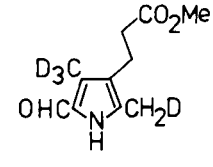
27, $R^1 = R^2 = CD_3$; R^3 = Me
34, $R^1 = R^3$ = Me; $R^2 = CD_3$
43, $R^1 = R^2$ = Me; $R^3 = CD_3$



28, $R^1 = R^2 = CD_3$; R^3 = Me
35, $R^1 = R^3$ = Me; $R^2 = CD_3$
44, $R^1 = R^2$ = Me; $R^3 = CD_3$



29, R = CH_2CH_2Cl
36, R = $CH_2CH_2CO_2Me$



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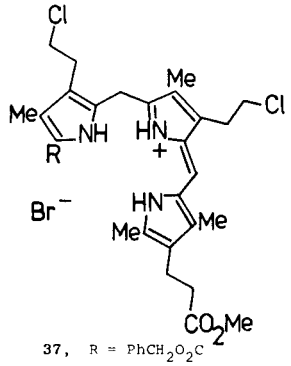
The mono-methyl labeled hemin **3** was prepared using a slight variation of the method above. In this case, the pyrromethane **12** was condensed in a stepwise manner with the formylpyrroles **29** and **18**, and gave a high yield of the *a,c*- biladiene dihydrobromide **31**, by way of the tripyrene hydrobromide **30**. The *a,c*-biladiene was then transformed as described above for the analogous dimethyl labeled hemin **2** into the 5-deuteriomethylhemin **3**, by way of the copper(II) porphyrin **32**, metal-free bis-(2-chloroethyl)porphoryn **33**, protoporphyrin-IX dimethyl ester **34**, and hemin dimethyl ester **35**. Yields in all cases were comparable with those described above for compound **2**.

Synthesis of Hemin **4**.

By far the most laborious part of the syntheses described above involves the synthesis of the methyl-labeled pyrroles **7** and **14**. Synthesis of the required 8-trideuteriomethylhemin **4** using the same initially "anticlockwise" elongation of the dipyrrole **12** would require uneconomical use of deuteriated pyrroles; in order to avoid this problem, the dipyrrole was extended to tripyrrole in a "clockwise" manner [19]. Thus, the unlabeled pyrromethane **5** [1] was treated with formylpyrrole **36** in trifluoroacetic and hydrobromic acids, to give an 80% yield of the benzyl tripyrrenecarboxylate hydrobromide **37**. After stirring in hydrobromic/acetic acids for 6 hours, the resulting (but unisolated) debenzylated tripyrrene **38** was treated with the labeled formylpyrrole [3] **39** and gave a 75% yield of the *a,c*-biladiene dihydrobromide, **40**. This compound, (by way of the copper(II) porphyrin **41**, metal-free porphyrin **42**, protoporphyrin-IX dimethyl ester **43**, and hemin dimethyl ester **44**), was transformed into the required hemin, **4**, in yields comparable with those described for hemin **2**.



37, R = $PhCH_2O_2C$
38, R = H

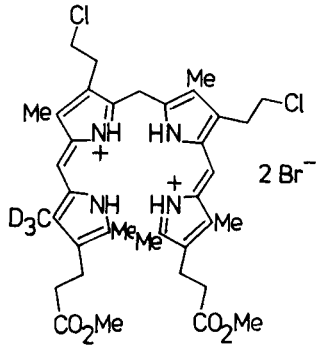


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Figure 1 shows the hyperfine-shifted methyl region in the proton spectra of the corresponding low-spin dicyanoferrihemins **2**, **3**, and **4**, and a comparison with the corresponding, unlabeled, natural hemin.

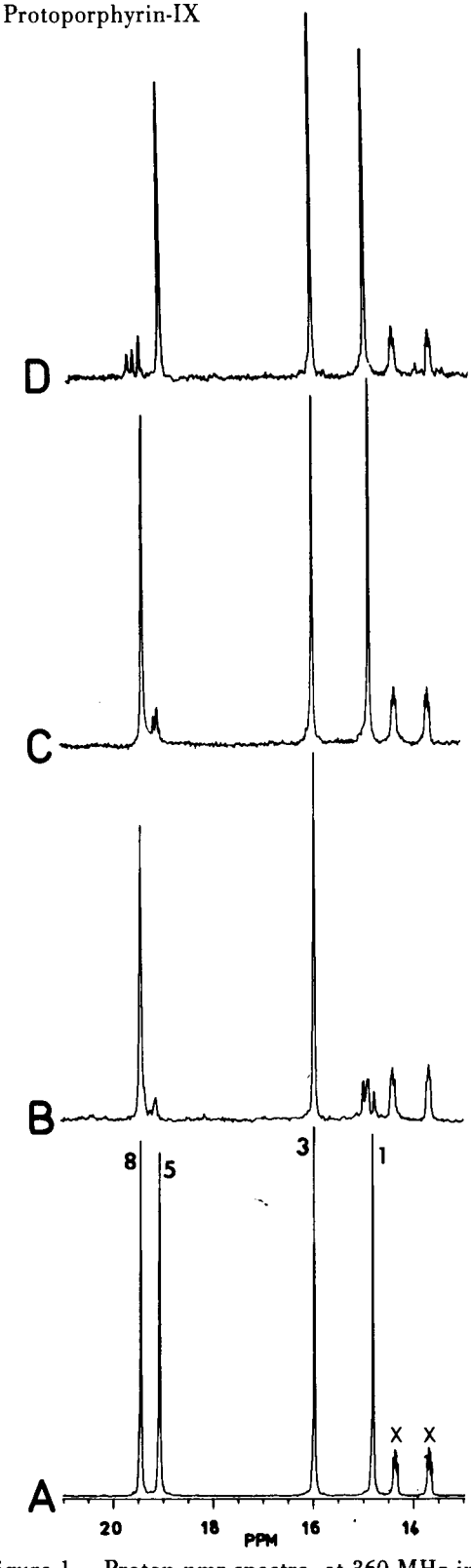

Figure 1. Proton nmr spectra, at 360 MHz in deuterium oxide solution, of the methyl region in the dicyanoferrihemins: A, unlabeled natural hemin; B, 1,5-labeled hemin, **2**; C, 5-labeled hemin, **3**; D, 8-labeled hemin, **4** Small peaks to low field of the deuteriated resonances in B, C and D are CH_2D and CD_2H lines. Peaks marked with an X (in A) are the vinyl alpha-CH resonances.

EXPERIMENTAL

Melting points were measured on a hot-stage apparatus, and are uncorrected. Silica gel 60 (70-230 mesh; Merck) or neutral alumina (Merck) was used for column chromatography, and preparative tlc was carried out on 20 × 20 cm glass plates coated wtih Merck GF 254 silica gel (1 mm thick). Analytical tlc 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) and proton nmr spectra were measured either at 90 MHz (Varian EM-390) or at 360 MHz (Nicolet NT-360) in deuteriochloroform solution. Elemental analyses were performed at the Berkeley Microanalytical Laboratory, Department of Chemistry, UC Berkeley.

4-(2-Chloroethyl)-2-formyl-3,5-dimethylpyrrole (**29**).

Benzyl 4-(2-chloroethyl)-3,5-dimethylpyrrole-2-carboxylate [20] (3.0 g) in tetrahydrofuran (100 ml) and triethylamine (0.5 ml) containing 10% palladized charcoal (300 mg) was hydrogenated at room temperature and atmospheric pressure until uptake of hydrogen ceased. The catalyst was removed by filtration through Celite and the filtrate was evaporated to give a solid residue of the pyrrole-2-carboxylic acid **16**, which was immediately treated with trifluoroacetic acid (15 ml) and allowed to stir for 30 minutes. Methylene chloride (250 ml) was then added, followed by water (250 ml), and the organic layer was separated and washed wth saturated aqueous sodium bicarbonate (250 ml) and then with water (250 ml). The organic phase was evaporated to dryness and the residue was dissolved in dry methylene chloride (20 ml) and added dropwise to the Vismeier complex prepared by addition of phosphoryl chloride (4.95 ml) to *N,N*-dimethylformamide (4.13 ml) in methylene chloride (30 ml). After addition was complete, the mixture was stirred at room temperature for 30 minutes, and then at 40° for 1 hour. After cooling of the mixture, saturated aqueous sodium acetate was added (to achieve *p*H 7) and the mixture was stirred for 20 hours at room temperature. To this solution was added saturated aqueous sodium bicarbonate (to *p*H 8) and the mixture was stirred, at this *p*H, for a further 4 hours. The organic layer was separated, washed with water (100 ml), chromatographed on a short silica column (elution with methylene chloride), and the appropriate eluates were evaporated to dryness to give a residue which was crystalliazed from methylene chloride/hexane, to give 1.30 g (68%) of the formylpyrrole, mp 143-144°; nmr: δ, ppm, 9.50 (s, 1H, CHO), 3.55, 2.82 (each t, 2H, CH_2CH_2Cl), 2.25 (s, 6H, Me), 10.10 (br, s, 1H, NH). When the synthesis was carried out using deuteriated pyrrole **15**, the resulting formylpyrrole **14** had mp 143-144°, and showed reduced intensity of the methyl signal at 2.25 ppm (2H) in its proton nmr spectrum.

Anal. Calcd. for $C_9H_{12}ClNO$: C, 58.22; H, 6.46; N, 7.54. Found: C, 58.12; H, 6.50; N, 7.46.

3-(2-Chloroethyl)-2-formyl-4,5-dimethylpyrrole (**18**).

This pyrrole was likewise obtained from benzyl 3-(2-chloroethyl)-4,5-dimethylpyrrole-2-carboxylate [21] (**24**) (2.5 g), and was obtained in 53% yield (840 mg), with mp 101-102°; nmr: δ, ppm, 9.50 (s, 1H, CHO), 3.60, 3.22 (each t, 2H, CH_2CH_2Cl), 2.25, 1.98 (each 3H, s, Me), 10.0 (br s, 1H, NH).

Anal. Calcd. for $C_9H_{12}ClNO$: C, 58.22; H, 6.46; N, 7.54. Found: C, 58.40; H, 6.62; N, 7.74.

t-Butyl 4-(2-Methoxycarbonylethyl)-3,5-dimethylpyrrole-2-carboxylate (**7**, Undeuteriated).

A well-stirred mixture of *t*-butyl acetoacetate (Aldrich) (5.5 g) in acetic acid (8 ml) was treated with a solution of sodium nitrite (2.8 g) in water (5 ml) at a rate which allowed the reaction temperature to be maintained below 10° (ice bath). After 3 hours of further stirring, the solution was stored overnight in a refrigerator and then added, during 2 hours, to a solution of 4-acetyl-5-oxohexanoate in acetic acid (25 ml) kept at 65°, simultaneously adding an intimate mixture of zinc dust (6.3 g) and sodium acetate (6.3 g) in portions such that the zinc dust was in excess of the added dione. The mixture, after complete addition, was maintained at 65° for a further 2 hours, and was poured, hot, into ice-water mixture (500 ml). After stirring overnight, the precipitated product was filtered off and recrystallized from methylene chloride/hexane, to give 4.9 g (50%) of pyrrole-2-carboxylate, with mp 100°; nmr: δ, ppm, 9.20 (br s, 1H, NH), 3.70 (s, 3H, OMe), 2.20, 2.25 (each 3H, s, Me), 2.5-2.8 (m, 4H, CH_2CH_2CO), 1.68 (s, 9H, *t*-Bu). When the reaction was repeated using d_6-hexanoate **9** (acetyl methyls labeled, prepared [17] from d_6-acetylacetone [1], **10**, and methyl acrylate in presence of Ni(II) at 160° in a sealed tube), on the same scale, a similar yield of the labeled pyrrole **7** was obtained. Its mp was 100-101°, and the nmr spectrum was identical with unlabeled material except that the resonances at 2.20 and 2.25 ppm were reduced in intensity.

Anal. Calcd. for $C_{15}H_{23}NO_4$: C, 64.04; H, 8.24; N, 4.98. Found: C, 64.16; H, 8.19; N, 4.94.

t-Butyl 5-Acetoxymethyl-4-(2-methoxycarbonylethyl)-3-methylpyrrole-2-carboxylate (**8**, Undeuteriated).

The foregoing pyrrole (3 g) in acetic acid (112 ml) and acetic anhydride (45 ml) was stirred and treated with aliquots of lead(IV) tetraacetate (4.95 g total) over a period of 1 hour at room temperature. The mixture was then stirred for 16 hours before being poured into water (500 ml) with vigorous stirring. The precipitated solid was filtered off and recrystallized from methylene chloride/hexane to give 3.40 g (95%) of white crystals, mp 80°; nmr: δ, ppm, 9.25 (br s, 1H, NH), 5.10 (s, 2H, CH_2), 3.65 (s, 3H OMe), 2.82, 2.50 (each t, 2H, CH_2CH_2CO), 2.25 (s, 3H, Me), 2.10 (s, 3H, COMe), 1.60 (s, 9H, *t*-Bu). When the reaction was repeated on the same scale with labeled pyrrole **7**, a similar yield of acetoxymethylpyrrole **8** was obtained, mp 79-80°. In the proton nmr spectrum of **8**, the methyl singlet at 2.25 and the methylene at 5.10 ppm were reduced by 95 and 50% respectively.

Anal. Calcd. for $C_{17}H_{25}NO_6$: C, 60.16; H, 7.43; N, 4.13. Found: C, 60.07; H, 7.36; N, 4.15.

Benzyl 5′-*t*-Butoxycarbonyl-3,3′-di(2-methoxcyarbonylethyl)-4-methyl-4′-trideuteriomethylpyrromethane-5-carboxylate (**12**).

Benzyl 4-(2-methoycarbonylethyl)3-3-methylpyrrole-2-carboxylate (990 mg) in acetic acid (20 ml) was treated with the foregoing labeled acetoxymethyl pyrrole **8** (1.026 g) and *p*-toluenesulfonic acid (27 mg), and then stirred under nitrogen at 45° for 4.5 hours. The mixture was then poured into water (100 ml), extracted with methylene chloride (2 × 100 ml), which was dried (sodium sulfate) and evaporated to dryness to give a residue. This was chromatographed on silica (elution with 20% ethyl acetate in cyclohexane); evaporation of the appropriate eluates and crystallization from methylene chloride/hexane gave the labeled pyrromethane (1.28 g, 70%), mp 113-115° (lit mp [20] 114-115°); nmr δ, ppm, 8.98, 8.68 (each br s, 1H, NH), 7.35 (s, 5H, Ph), 5.25 (s, 2H, CH_2Ph), 3.95 (s, 1H, -CHD-), 3.65, 3.55 (each s, 3H, OMe), 2.5-2.8 (m, 8H, CH_2CH_2CO), 2.28 (s, 3H, Me), 1.55 (s, 9H, *t*-Bu).

t-Butyl 1-(2-Chloroethyl)-4,5-di(2-methoxycarbonylethyl)-1′,2,3,6-tetramethyltripyrrene-a-6′-carboxylate Hydrobromide (**17** or **30** Undeuteriated).

A stirred solution of the pyrromethane-5-carboxylic acid obtained by catalytic debenzylation of the pyrromethane **6** (530 mg) in tetrahydrofuran using 10% palladized charcoal (53 mg) in methylene chloride (50 ml), was treated with formylpyrrole **29** under nitrogen. To this solution was added *p*-toluene sulfonic acid hydrate (514 mg) in methanol (5 ml), and the mixture was stirred at room temperature for 40 minutes. The solution was diluted with more methylene chloride (100 ml), washed successively with water (100 ml), aqueous sodium bicarbonate (100 ml), and water (100 ml) again, before evaporation to dryness. The residue was quickly taken up in dry methylene chloride (50 ml), treated for 5 seconds with hydrogen bromide gas, then rapidly evaporated to dryness. Dry toluene (25 ml) was then added and flash evaporated to azeotrope any water or hydrobromic acid. This procedure was repeated before addition of ether (50 ml) and evaporation. The resulting residue was taken up in a

minimum volume of ether, and upon standing, crystallization took place. Filtration afforded 444 mg (70%) of the required tripyrrene hydrobromide, mp >150° dec; nmr: δ, ppm, 13.25 (br s, 2H, NH), 10.22 (br s, 1H, NH), 7.10 (s, 1H, =CH-), 4.41 (s, 2H, CH_2), 3.65, 3.67 (each s, 3H, OMe), 2.6-2.95 (m, 12H, CH_2CH_2O and CH_2CH_2Cl), 2.35 (s, 3H, Me), 2.25 (s, 9H, Me), 1.55 (s, 9H, *t*-Bu); vis: λ max 486 nm (ε 79,000). When this procedure was repeated with the deuteriated pyrromethane **12** and the deuteriated pyrrole **14**, a 70% yield of the doubly labeled tripyrrene hydrobromide, **17**, was obtained, mp >150° dec. In its nmr spectrum, the methyl resonance at 2.35 was absent, and that at 2.25 integrated for only 6 protons. Repetition of the synthesis with the above deuteriated pyrromethane **12** and the unlabeled formylpyrrole **29** gave 71% of the monolabeled tripyrrene hydrobromide, **30**, mp >150° dec; the proton nmr spectrum showed the methyl singlet at 2.35 ppm to be absent.

Anal. Calcd. for $C_{33}H_{45}BrClN_3O_6$: C, 57.01; H, 6.47; N, 6.04. Found: C, 56.95; H, 6.48; N, 5.97.

Benzyl 3,5-Di(2-chloroethyl)-1-(2-methoxycarbonylethyl)-1',2,4,6-tetramethyltripyrrene-a-6'-carboxylate Hydrobromide (**37**, Undeuteriated).

The pyrromethane **5** (513 mg) in trifluoroacetic acid (3 ml) was stirred for 5 minutes before being treated with the formylpyrrole **36** (207 mg) in dry methanol (25 ml) under an atmosphere of nitrogen. After stirring for 90 minutes, 31% hydrogen bromide in acetic acid (1 ml) was added at ice bath temperature. Slow dropwise addition of ether caused the tripyrrene hydrobromide (475 mg, 70%) to crystallize as orange prisms, mp >150° dec. The sample was shown, by nmr analysis to be identical with an authentic sample [19].

4,6-Di(2-chloroethyl)-1,8-di(2-methoxycarbonylethyl)-1',2,3,5,7,8'-hexamethyl-*a,c*-biladiene Dihydrobromide (**19** or **31**, Undeuteriated).

The tripyrrene hydrobromide (**17** or **30**, undeuteriated) (464 mg) was stirred in trifluoroacetic acid (5 ml) for 5 minutes before addition of the formylpyrrole **18** (124 mg) in methanol (5 ml). A solution of 31% hydrogen bromide in acetic acid (5 ml) was immediately added and the mixture was stirred for 30 minutes during precipitation of a bright red solid. Dry ether (100 ml) was then added dropwise over 30 minutes and the *a,c*-biladiene dihydrobromide was filtered off to give 455 mg (80%) of bright red crystals, mp >300°; nmr: δ, ppm, 13.6, 13.4 (each s, 2H, NH), 7.30 (s, 2H, =CH-), 5.30 (s, 2H, $-CH_2$), 3.70, 2.90, 2.80, 2.00 (each t, 4H, CH_2CH_2CO and CH_2CH_2Cl), 3.40 (s, 6H, OMe), 2.45, 2.35, 2.25 (each s, 6H, Me); vis: λ max 455 nm (ε 47,000) and 518 (87,000). When the reaction was repeated on the same scale, but using the doubly labeled tripyrrene hydrobromide **17**, an 81% yield of *a,c*-biladiene dihydrobromide **19** was obtaind, with mp >300°. Repetition of the reaction using the monomethyl-labeled tripyrrene hydrobromide **30** and formylpyrrole **18** gave an 80% yield of the *a,c*-biladiene dihydrobromide **31**, mp >300°.

Anal. Calcd. for $C_{37}H_{48}Br_2Cl_2N_4O_4$: C, 51.55; H, 5.44; N, 6.87. Found: C, 52.29; H, 5.75; N, 6.45.

4,6-Di(2-chloroethyl)-1,8-di(2-methoxycarbonylethyl)-1',2,3,5,7,8'-hexamethyl-*a,c*-biladiene Dihydrobromide (**39**, Undeuteriated).

The benzyl tripyrrene-carboxylate **37** (undeuteriated) (395 mg) in 31% hydrogen bromide in acetic acid (2.6 ml) and acetic acid (10 ml) was stirred at room temperature for 6 hours. The formyl pyrrole **36** (120 mg) in methanol (15 ml) was then added to the solution at a temperature <10° (ice bath). The mixture was then stirred and allowed to warm to room temperature over a period of 30 minutes before ether (100 ml) was allowed to drop slowly into the mixture. The precipitated *a,c*-biladiene dihydrobromide (358 mg, 75%) had mp >300°, and was identical with a previously prepared sample [22]. When the reaction was repeated, but using the monomethyl-labeled tripyrrene **37**, a similar yield of the required labeled *a,c*-biladiene dihydrobromide **39** was obtained, mp >300°. In its proton nmr spectrum the singlet methyl resonance at 2.38 ppm was absent.

2,4-Di(2-chloroethyl)-6,7-di(2-methoxycarbonylethyl)-1,3,5,8-tetramethylporphyrin **26, 33** or **42**, Undeuteriated).

To a solution of copper(II) chloride dihydrate (1.7 g) in *N,N*-dimethylformimide (30 ml) kept at 150° was added the *a,c*-biladiene dihydrobromide **19** or **31** (undeuteriated) (342 mg). After 4 minutes stirring at this temperature, the mixture was poured into water (100 ml), extracted with methylene chloride (2 × 100 ml), washed with water (100 ml), dried (sodium sulfate), and then evaporated to dryness. The residue was dissolved in 10% sulfuric acid in trifluoroacetic acid (25 ml) and stirred vigorously for 30 minutes (spectrophotometry at that time indicated complete removal of copper from the porphyrin), and then poured into water (100 ml), extracted with chloroform (2 × 75 ml), and washed successively with water (100 ml), aqueous sodium bicarbonate solution (100 ml), and finally water (100 ml) again. The solvent was evaporated and the residue was treated with 5% (w/v) sulfuric acid in methanol (25 ml) overnight at room temperature. After pouring into aqueous sodium acetate (100 ml) the mixture was extracted with methylene chloride (3 × 100 ml) then washed with aqueous sodium bicarbonate solution (100 ml) and water (100 ml). The organic layer was dried (sodium sulfate), evaporated to dryness, and the residue was chromatographed on alumina (Brockmann Grade III, elution with methylene chloride). Evaporation of the red eluates gave a red residue which was crystallized from methylene chloride/methanol to give the 2,4-di(2-chloroethyl)porphyrin, **26, 33** or **42** (unlabeled), 133 mg (50%), mp 217-218°, (lit [22] mp 216-218°). When the reaction was repeated wtih the *a,c*-biladienes **19, 31**, and **40**, similar yields of chloroethylporphyrin were obtained, and in each case the mp was in the 215-218° region. The proton nmr spectra of the products, **26, 33**, and **42** indicated absence of the appropriate methyl singlet resonances.

6,7-Di(2-methoxycarbonylethyl)-1,3,5,8-tetramethyl-2,4-divinylporphyrin (**27, 34** or **43**, Undeuteriated). "Protoporphyrin IX Dimethyl Ester".

The foregoing 2,4-di(2-chloroethyl)porphyrin, **26, 33** or **42** (undeuteriated), (130 mg) in pyridine (60 ml) was heated under reflux under a nitrogen atmosphere for 10 minutes before water (15 ml) and 3% aqueous sodium hydroxide (15 ml) were added. The mixture was refluxed for a further 2.5 hours before being cooled, treated with 25% acetic acid in water (15 ml) and evaporated to dryness using toluene as a chaser. Water (70ml) was added and this caused a solid to precipitate, which was filtered off, washed with water, and dried under vacuum. The porphyrin was dissolved in 5% sulfuric acid in methanol (30 ml) and set aside overnight at room temperature. Chloroform (100 ml) and water (100 ml) were then added and the organic phase was separated, washed with aqueous sodium bicarbonate (100 ml), water (100 ml), and then evaporated to dryness to give a residue which was chromatographed on alumina (Brockmann Grade III, elution with methylene chloride). The red eluates were collected and evaporated to dryness to give a residue which was crystallized from methylene chloride/methanol to give 81 mg (70%), mp 225-226°, (lit [23] mp 228-229°). The product was in all respects identical with an authentic sample of protoporphyrin IX dimethyl ester. When the reaction was repeated using the methyl labeled 2-chloroethyl porphyrins **26, 33** and **42**, the proton nmr spectra exhibited the expected [1,24] methyl absences. These signals, in protoporphyrin IX dimethyl ester are concentration dependent, so Figure 1 shows the spectra in the methyl region of the corresponding iron(III) complexes (**2, 3** or **4**), measured in deuterium oxide as the corresponding low-spin dicyanoferrihemins. The hemins were synthesized in good yields from the corresponding porphyrin dimethyl esters using the ferrous chloride in acetonitrile method [25]. Finally, the hemin dimethyl esters were hydrolyzed using 1% potassium hydroxide in 94% methanol and 5% water, as described elsewhere [25].

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