of benzene followed by column chromatography gave **6b** (92%) and **8** (95%).

Thermolysis of 1b in Acetonitrile (4–5 h). **1b** (0.92 mmol, 0.25 g) was refluxed in 15 mL of acetonitrile. TLC analysis revealed that the product was **6a** contaminated with **1b**. Recrystallization (3:7 methanol/water) gave **6a** (72%).

Reaction of 1a in Toluene at Room Temperature. After 5 weeks **1a** was totally converted as was measured by TLC. GPLC and mass spectroscopy showed the presence of benzyl bromide (50%). No benzyltoluene isomers were detected, but traces of benzoyl bromide were found. Collected fractions after column chromatography contained according to IR spectroscopy 3,4,5-tribromo-1*H*-pyrazole (**3a**, 8%) and 3,4-dibromo-5-nitro-1*H*-pyrazole (**6a**, 66%) contaminated with some 3,5-dibromo-4-nitro-1*H*-pyrazole (**2**). The test on the presence of NO_2 was negative, and the test on Br_2 was inconclusive. The yield of the residue was not determined.

Acknowledgment. We express our sincere gratitude to Dr. Pauline Cohen-Fernandes who obtained for us the results of the thermolysis reactions of **1b** and for helpful advice and stimulating discussions. We are also indebted to Ellen van den Berg for synthesizing **4**. We are grateful to B. van Vliet for the IR spectra and for help with the GLC analyses, to Dr. J. van Thuijl and J. J. van Houte for the mass spectral analyses, and to C. Erkelens for the NMR spectra. We thank M. Kloosterman for the preparation of **5**[15] and 1-benzyl-3,4,5-tribromo-1*H*-pyrazole.[15]

Appendix

Thermolysis of 4-Bromo-1-nitro-1*H*-pyrazole.[23] No decomposition was observed on refluxing in acetonitrile for 1 day. After refluxing for 5 days in acetonitrile 95% of 4-bromo-1-nitro-1*H*-pyrazole was recovered. Heating a solution of 4-bromo-1-nitro-1*H*-pyrazole mixed with an equimolar amount of **7** for 3 days followed by column chromatography also gave recovered **7** (100%) and 4-bromo-1-nitro-1*H*-pyrazole (75%) and 4-bromopyrazole (18%).

Registry No. **1a**, 104599-40-8; **1b**, 104599-41-9; **2**, 104599-36-2; **3a**, 17635-44-8; **3b**, 67460-86-0; **4**, 104599-39-5; **5**, 51039-46-4; **6a**, 104599-37-3; **6b**, 104599-38-4; **7**, 67-51-6; **8**, 3398-16-1; 3,5-dibromo-1-phenyl-1*H*-pyrazole, 51039-44-2; 4-bromo-1-nitro-1*H*-pyrazole, 7185-93-5; anisole, 100-66-3; pyrazole, 288-13-1.

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Syntheses of Derivatives of Protoporphyrin IX Bearing Deuteriated Methyls on the Propionate (C and D) Rings

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New syntheses of hemins that are regioselectively deuteriated in the 5- and 8-methyls (**14**) and the 8-methyl (**31**) are described. The 5,8-dilabeled porphyrin **3** was obtained via an acrylate porphyrin by conversion of deuteroporphyrin IX dimethyl ester (**2**) into the corresponding bis(acrylate) **5** using LDA, benzeneselenenyl bromide, and oxidative elimination. After base-catalyzed deuterium exchange, reduction of the acrylate to propionate, and vinylation, the required 5,8-dilabeled porphyrin was obtained. The 8-methyl-deuteriated compound **15** was obtained by total synthesis through a porphyrin **16** bearing an unsubstituted 7-position. By a mercuration/palladium–olefin reaction, the vacant position was substituted with an acrylate, and following base-catalyzed exchange, hydrogenation, and construction of the 2- and 4-vinyls, the required product was obtained. These compounds, as the corresponding iron complexes (hemins), are of interest in connection with heme/apoprotein reconstitution studies and for characterization of structure/function relationships in heme proteins.

Regioselectively deuteriated hemes have been critically important to recent advances in the characterization of heme protein structure/function relationships using proton NMR[1] and resonance Raman spectroscopy.[2] In our laboratory, methods have been developed for regioselective deuterium labeling of vinyls[3,4] and the 1-, 3-, 5-, and 8-methyls[5–7] in protoporphyrin IX dimethyl ester (**1**). Initial methyl deuteriation studies centered on total synthesis from deuteriated acetylacetone[5] and more recently via simple exchange processes using intact porphyrins.[7] Thus,

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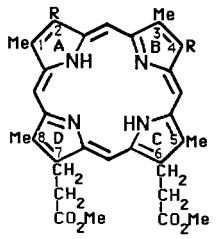


1 R = $CH=CH_2$

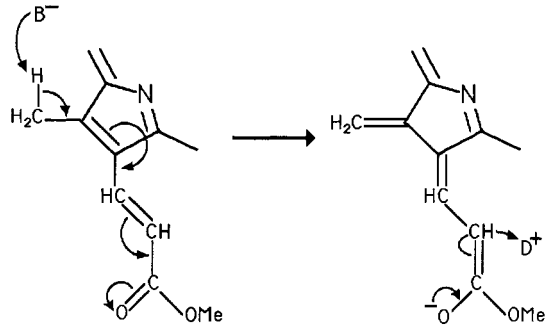
2 R = H

protoporphyrin IX dimethyl ester (1) can be selectively deuterated in the 1- and 3-methyls,[7] while use of more strongly electron-withdrawing substituents such as acetyl[4,6] and acrylate[8,9] cause more efficient base-catalyzed deuterium exchange at adjacent methyl groups on the same pyrrole subunit. Use of the vinyl/acetyl activation process effectively provided routes to 1- and/or 3-methyl-labeled derivatives of protoporphyrin IX, but a significant methodology gap still existed for the preparation of reasonable quantities of 5- and/or 8-labeled derivatives of 1. In this paper we describe two approaches to 5- and/or 8-methyl-deuteriated derivatives of protoporphyrin IX. In the first, the propionic side chains in deuteroporphyrin IX dimethyl ester (2) are transformed into acrylates before base-catalyzed deuterium exchange, while in the second a β-unsubstituted porphyrin is prepared by total synthesis and this is modified via a mercury/palladium–olefin reaction[10] to give an acrylate; subsequent exchange[8] and vinyl fabrication provides the individually methyl-labeled porphyrin. In both approaches, the deuterium labels are introduced at a late stage in the synthesis and thereby provide significant advantages over total syntheses via deuterium-labeled pyrroles.

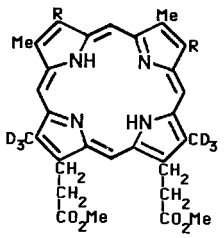
Synthesis of the 5,8-Bis(trideuteriomethyl)-porphyrin (3). Since the base-catalyzed exchange process for incorporation of deuterium into porphyrin methyls relies upon activation by an adjacent electron-withdrawing group, it was decided to attempt the transformation of the 6- and 7-propionate side chains into acrylates prior to the exchange reaction. Such reactions had been reported, using selenium chemistry,[11] in standard chemical transformations and, subsequent to the completion of our own investigations, in the porphyrin series.[9]

Because one of the steps after deuterium exchange is catalytic hydrogenation (acrylate to propionate), vinyl groups could not be carried through the transformation intact; moreover, presence of vinyls might also promote some exchange of the 1- and 3-methyls and thereby significantly reduce the regioselectivity in the reaction. Thus, deuteroporphyrin IX dimethyl ester (2) was chosen as the substrate. Treatment of 2 with LDA gave the dianion, which with benzeneselenenyl bromide gave the intermediate porphyrin 4. With hyrogen peroxide, a 28% yield of the bis(acrylate) 5 was obtained, and this was separated by chromatography from a small amount of isomeric monoacrylates 6 and 7 and deuteroporphyrin IX dimethyl ester (2). The NMR spectrum of 5 clearly showed the presence of trans acrylates at δ 9.08, 9.06, 7.05, and 6.98, with J = 16 Hz. The bis(acrylate) 5 was treated for 30 h

Scheme I



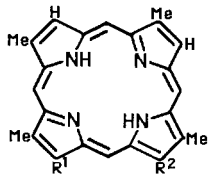
at 60 °C in methoxide/methanol-d_1 and, after workup with D_2O and reesterification, gave a 51% recovery of the 5,8-bis(trideuteriomethyl)porphyrin (8), in which mass spectrometry and NMR spectroscopy showed the methyls to be labeled to an extent greater than 80%. Somewhat unexpectedly, the β-acrylate protons in 8 were also almost completely exchanged. The NMR spectrum of 8 showed that the doublets of the 7′-vinyl protons (ca. 9.0 ppm) had collapsed to singlets. No signals were apparent for the 7″-vinyl protons that previously appeared around 7.0 ppm, thus indicating deuteriation at this position.



3 R = $CH=CH_2$

11 R = COMe

12 R = CH(OH)Me

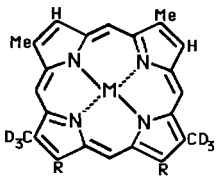


4 $R^1 = R^2 = CH_2CHCO_2Me$ (SePh)

5 $R^1 = R^2 = CH=CHCO_2Me$

6 $R^1 = CH_2CH_2CO_2Me$; $R^2 = CH=CHCO_2Me$

7 $R^1 = CH=CHCO_2Me$; $R^2 = CH_2CH_2CO_2Me$



8 R = $CH=CDCO_2Me$; M = 2H

9 R = $CH=CDCO_2Me$; M = Cu

10 R = $CH_2CH_2CO_2Me$; M = Cu

We have already discussed the mechanism for vinyl- or acetyl-promoted methyl exchange.[6,7] A large variety of structures can similarly be drawn to demonstrate acrylate proton exchange, some involving six-membered transition states. However, we believe that Scheme I rationalizes the basic mechanism upon which further enhancements can be built.

The copper(II) complex 9 of compound 8 underwent catalytic hydrogenation (acrylate to propionate) with great reluctance, so it was reduced with Raney nickel to give an 84% yield of the copper(II) deuteroporphyrin IX (10). This was in all respects identical with an authentic sample,[4] except that the 5- and 8-methyls were deuteriated. Fol-

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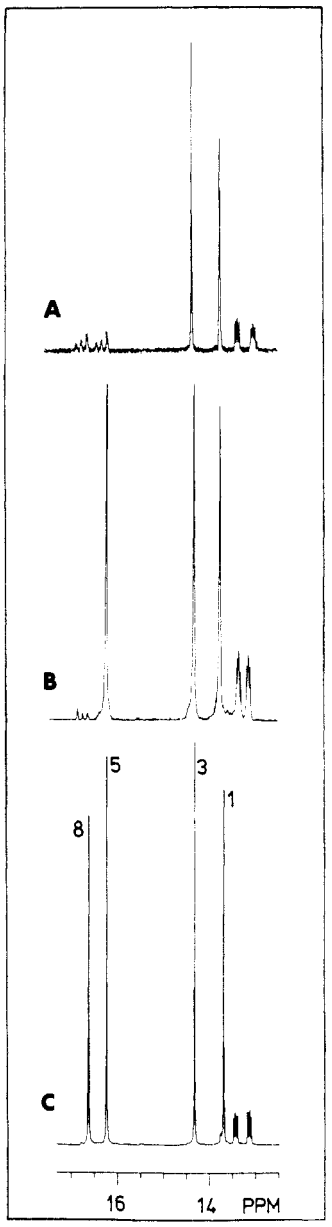
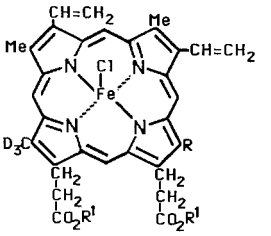


Figure 1. Proton NMR spectra (360 MHz, in methanol-d_4 containing KCN): (A) 5,8-bis(trideuteriomethyl)hemin (**13**); (B) 8-(trideuteriomethyl)hemin (**30**); (C) unlabeled protohemin IX dimethyl ester. Numbers in (C) refer to the methyl assignments;[1] also shown (ca. 13–13.5 ppm) are the vinyl α-CH resonances. Small peaks to low field of exchanged methyls are from the partially deuteriated CH_2D and CHD_2 methyls.

lowing standard methodology,[4,12] the copper(II) porphyrin **10** was acetylated under Friedel–Crafts conditions (acetic anhydride/stannic chloride), demetalated to give **11**, reduced ($NaBH_4$) to give **12**, and finally dehydrated (in *o*-dichlorobenzene containing *p*-toluenesulfonic acid) to give the labeled protoporphyrin IX dimethyl ester **3**. Insertion of iron (to give **13**) was accomplished by using the ferrous sulfate method,[4] and hydrolysis gave the hemin **14**. Proton NMR spectroscopy (Figure 1A) showed that the 5- and 8-methyls had been deuteriated greater than 80% (cf. Figure 1C).

Because of the erratic yields obtained in the mono(acrylate) (**6**, **7**) syntheses (vide supra) and concomitant chromatographic problems, it was decided to approach 5- or 8-monomethyl deuteriated porphyrins by total synthesis. This work is described in the following section.

Synthesis of the 8-(Trideuteriomethyl)porphyrin (15). Since the deuteriation was to be accomplished by way of acrylate activation on a porphyrin followed by catalytic reduction of the acrylate to propionate, the vinyl groups in the final product **15** needed to be protected. The usual group used is the 2-chloroethyl,[13] but this was determined to be unsuitable because under the basic exchange conditions it can dehydrochlorinate to afford vinyl. The newly created vinyls could then activate the 1- and 3-methyls toward exchange, which would reduce the regiospecificity of the exchange process and would also be labile toward catalytic reduction to ethyl. The target porphyins, **16** and **17**, therefore, were selected with acetoxyethyl groups for vinyl protection; though the acetoxyethyl groups would almost certainly hydrolyze to hyroxyethyl under basic conditions, they will not subsequently dehydrate to vinyls.

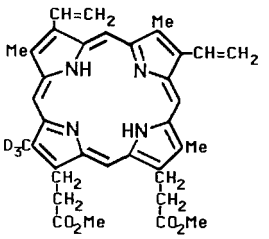


13 R = CD_3; R^1 = Me

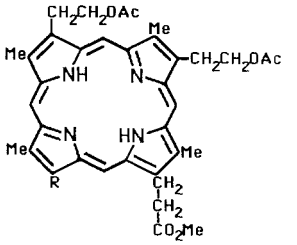
14 R = CD_3; R^1 = H

30 R = Me; R^1 = Me

31 R = Me; R^1 = H



15



16 R = H

17 R = CH=CHCO$_2$Me

23 R = I

The synthetic route is shown in Scheme II. The *a,c*-biladiene route[14] was chosen, incorporating the A- and B-ring pyrroles in the starting pyrromethane **18**; this compound has been synthesized previously.[15] Condensation with formylpyrrole **19** gave the tripyrrene hydrobromide **20** in 70% yield. Final condensation of the tripyrrene with formylpyrrole **21** gave the *a,c*-biladiene dihydrobromide **22** in 96% yield.

Cyclization of the *a,c*-biladiene in refluxing *o*-dichlorobenzene in the presence of iodine gave the desired porphyrin **16** in 38% yield. Two minor byproducts were also isolated. One was identified as the iodoporphyrin **23** by mass and NMR spectroscopies; the electronic absorption

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(13) Cavaleiro, J. A. S.; Rocha Gonsalves, A. M. d'A.; Kenner, G. W.; Smith, K. M. *J. Chem. Soc., Perkin Trans. 1* **1973**, 2471–2478.

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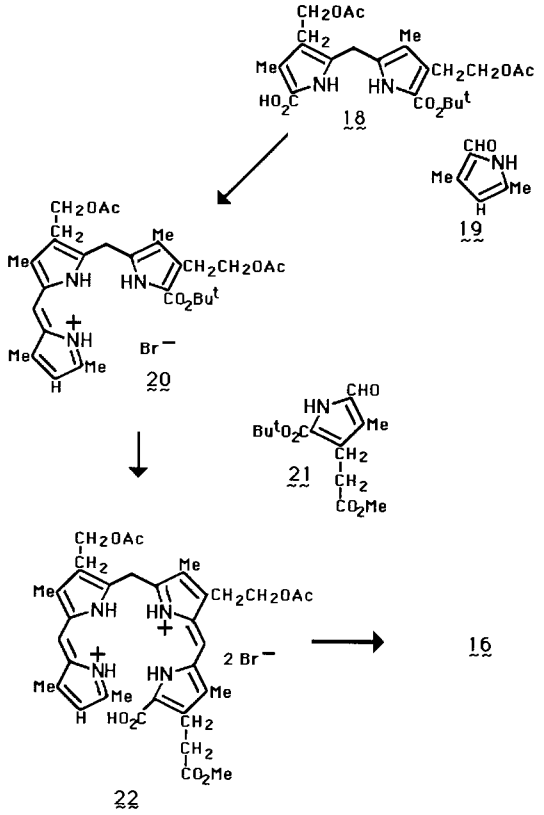
(15) Jackson, A. H.; Kenner, G. W.; Smith, K. M. *J. Chem. Soc. C* 1971, 502–509.

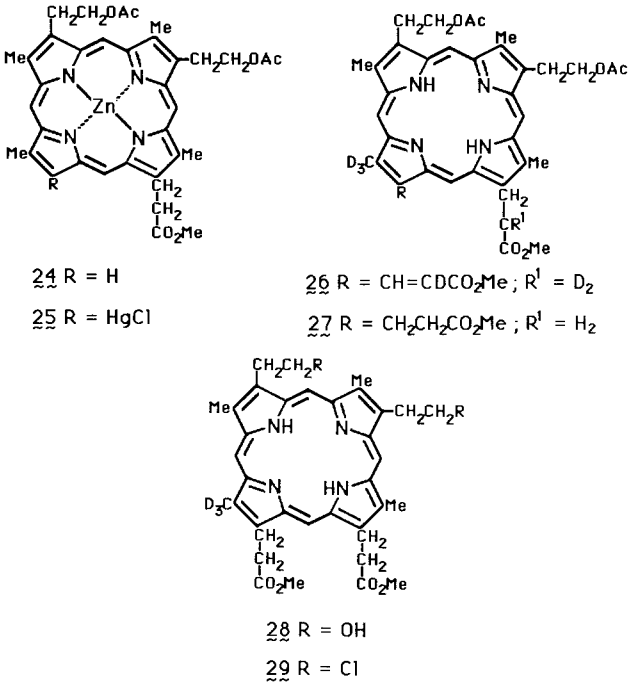
Scheme II



spectrum is of the rhodo type,[16] and on TLC it ran slightly faster than porphyrin **16**. Russian workers also isolated an iodoporphyrin[17] from cyclization of an *a,c*-biladiene containing two iodo groups (at the terminus and 7-position) in the presence of 20 equiv of iodine in refluxing *o*-dichlorobenzene. The second byproduct, which possessed an etio type absorption spectrum, remains uncharacterized.

Zinc was inserted into the porphyrin **16** to give **24**, and this was accomplished in 88% yield. The mercuration reaction[10] was carried out by adding 2.2 equiv of mercuric acetate in methanol to a solution of the zinc porphyrin in tetrahydrofuran and heating at reflux for 4 h, and the mercurated porphyrin **25** was isolated in 87% yield. This material was dissolved in dimethyl sulfoxide and tetrahydrofuran and then treated with methyl acrylate and $LiPdCl_3$ in acetonitrile at 50 °C for 1 h. After workup and zinc removal with trifluoroacetic acid, the mixture was purified by flash chromatography on silica gel and the desired acrylate **17** was isolated in 63% yield.

The base-exchange reaction was carried out with sodium methoxide in methanol-d_1 at 65 °C for 22 h. The product was difficult to extract with an organic solvent due to insolubility introduced by multiple ester hydrolysis. Therefore, after neutralization with acetic acid, water was added, the solvents were reduced in volume, and the product was collected by filtration. This was dried and reesterified with 5% methanol in sulfuric acid before the hydroxyl groups were reacetylated by treatment with acetic anhydride and pyridine to give a 44% yield of porphyrin **26**. As described for the exchange process on the bis(acrylate) the β-vinyl proton had again been exchanged, but in the case of the present compound, the methylenes at the 6″-position of the propionate ester had also exchanged (no signal at 3.28 ppm). The methyl region integrated to approximately 15 protons (which includes 3 ring methyls and 2 acetyl methyls), indicating absence, through deuteriation, of a methyl.



In the hydrogenation procedure, the above acrylate porphyrin was dissolved in formic acid and allowed to stir under a hydrogen atmosphere with palladium/carbon as catalyst for 3 h. The electronic absorption spectrum at this time (after neutralization) showed an etio-type spectrum, as expected, because the electron-withdrawing acrylate group had been reduced to an aliphatic ester. The product was worked up, and a 30% yield of the porphyrin **27** was obtained. The 2-acetoxyethyl groups were then hydrolyzed with 5% sulfuric acid in methanol, and the crude diol **28** was chlorinated with thionyl chloride in dry dimethylformamide and chloroform, to give a quantitative yield of **29**. Dehydrochlorination, using potassium hydroxide in pyridine/methanol, gave an 88% yield of the protoporphyrin IX dimethyl ester **15**, which was chelated with iron (to give **30**) and hydrolyzed to afford **31**. The NMR spectrum (Figure 1B) showed clean deuteriation of the 8-methyl group when compared to a spectrum of an undeuteriated protohemin (Figure 1C) with assigned methyl peaks.

Experimental Section

Melting points were measured on a hot-stage apparatus and are uncorrected. Silica gel 60 (Merck, 70–230 mesh) or alumina (Merck) were used for column chromatography, and preparative TLC was carried out on 20 × 20 cm glass plates coated with Merck GF 254 silica gel (1 mm thick). Analytical TLC was performed using Merck silica gel 60 F 254 precoated sheets (0.2 mm). Whenever possible, reactions were monitored by TLC and/or spectrophotometry and were carried out under nitrogen in the dark (aluminum foil). Proton NMR spectra were measured in deuteriochloroform solution at 360 MHz on a Nicolet NT-360 spectrometer or at 90 MHz with a Varian EM-390 spectrometer with tetramethylsilane as internal standard. Electronic absorption spectra were measured, in dichloromethane solution, on a Hewlett-Packard 8450A spectrophotometer. Elemental analyses were performed at the Berkeley Microchemical Analysis Laboratory, University of California—Berkeley.

Synthesis of the 5,8-Dilabeled Porphyrin 3. 6,7-Bis[2-(methoxycarbonyl)vinyl]-1,3,5,8-tetramethylporphyrin (5). To a solution of deuteroporphyrin IX dimethyl ester **2** (500 mg) in dry tetrahydrofuran (75 mL) under a nitrogen atmosphere at

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−78 °C was added dropwise a 0.5 M solution of LDA in tetrahydrofuran (8.90 mL). The mixture was stirred for 15 min before rapid addition of benzeneselenenyl bromide (701 mg) in tetrahydrofuran. After a further 5 min a solution of 30% hydrogen peroxide (2.5 mL), glacial acetic acid (2 mL), and water (0.75 mL) was added to the cold mixture that was then allowed to warm slowly to room temperature and then left to stir for a further 30 min. The mixture was diluted with dichloromethane (150 mL), washed with water (3 × 100 mL), dried (Na_2SO_4), and evaporated to dryness with a toluene chaser. The residue was dissolved in dichloromethane, passed through a short column of neutral alumina (Brockmann grade III), eluted with dichloromethane, and then chromatographed on silica gel (elution with 1% methanol in dichloromethane). This accomplished separation of the slow-running bis(acrylate) from a small quantity of the corresponding mono(acrylates) **6** and **7** [NMR (ppm) 10.13 (s, 3 H, meso H); 10.07, 10.05, 10.03, 10.00, 9.93 (each s, 1 H, meso H); 9.34 (d, J = 16 Hz, 2 H, C*H*=CHCO); 9.14, 9.13, 9.04, 9.02 (each s, 1 H, 2,4-H); 7.12 (d, J = 16 Hz, 2 H, CH=C*H*CO); 4.44 (t, 4 H, CH_2CH_2CO); 4.08 (s, 6 H, OMe); 3.77, 3.75, 3.74, 3.70 (each s, 3 H, Me), 3.68 (s, 6 H, Me), 3.66, 3.64 (each s, 6 H, Me); 3.28 (t, 4 H, CH_2CH_2CO); −3.88, −3.89 (each s, 2 H, NH)] and deuteroporphyrin IX dimethyl ester (**2**). The appropriate eluates were collected, and the solvent was evaporated to give a residue that was crystallized from dichloromethane/methanol to give 140 mg (28%): Mp >300 °C dec (lit.[9] mp 301 °C); NMR (ppm) 9.85, 9.76, 9.73, 9.72 (each s, 1 H, meso H); 9.08, 9.06 (each d J = 16 Hz, 1 H, C*H*=CHCO); 8.95, 8.94 (each s, 1 H, 2,4-H); 7.05, 6.98 (each d, J = 16 Hz, 1 H, CH=C*H*CO); 4.12 (s, 6 H, OMe); 3.67, 3.63, 3.56, 3.52 (each s, 3 H, Me); −4.58 (s, 2 H, NH).

6,7-Bis[2-(methoxycarbonyl)vinyl]-1,3-dimethyl-5,8-bis(trideuteriomethyl)porphyrin (8). Sodium metal (25 mg) was dissolved under a nitrogen atmosphere in methanol-d_1 (12 mL), and to this solution was added the foregoing bis(acrylate) **5** (50 mg) in dry tetrahydrofuran (8 mL) and dry pyridine (8 mL). This mixture was stirred at 60 °C for 30 h before being cooled and the reaction quenched with D_2O (10 mL) and acetic acid (1 mL). The mixture was evaporated (vacuum pump) to a volume of ca. 10 mL before the product was collected by filtration, washed with water, dried overnight under vacuum, and then taken up in 5% sulfuric acid in methanol (10 mL). After standing overnight, the mixture was diluted with dichloromethane (50 mL), washed with water (50 mL), aqueous sodium bicarbonate (50 mL), and water again (50 mL) before being dried (Na_2SO_4) and evaporated to dryness. The residue was crystallized from dichloromethane/methanol to give 26 mg (51%) of the title compound, identical with the sample above except in the NMR and mass spectra: NMR (ppm) 10.05, 9.99, 9.95, 9.92 (each s, 1 H, meso H); 9.22, 9.03 (each s, 1 H, C*H*=CDCO); 4.10 (s, 6 H, OMe); 3.71, 3.68 (each s, 3 H, 1,3-Me); −4.0 (s, 2 H, NH); integration indicated >80% deuteriation at the 5- and 8-methyls. MS, m/e (%) 543 (49, d_9), 542 (85, d_8), 541 (99, d_7), 540 (100, d_6), 539 (100, d_5), 538 (88, d_4), 537 (71, d_3). MW for $C_{32}H_{24}D_6N_4O_4$, calcd 540.2645, found 540.2635.

Copper(II) 6,7-Bis[2-(methoxycarbonyl)ethyl]-1,3-dimethyl-5,8-bis(trideuteriomethyl)porphyrin (10). Copper was inserted into the foregoing compound, using standard methodology (porphyrin in dichloromethane, copper(II) acetate in methanol),[18] in quantitative yield. Neutral Raney nickel (W2, Fluka) (1.6 g) which had previously been washed with D_2O in tetrahydrofuran was added to a solution of the copper(II) porphyrin complex (80 mg) in tetrahydrofuran (150 mL). The mixture was placed beneath a hydrogen atmosphere and stirred vigorously at room temperature with careful monitoring by TLC (product R_f 0.7; starting material R_f 0.25, developed with 2% methanol/dichloromethane) and spectrophotometry (λ_{max} from 544, 586 nm to 522, 560 nm). Typically the reaction took 30 min to accomplish complete hydrogenation, and the Raney nickel was then removed by filtration through Celite. The filtrate was titrated dropwise (spectrophotometric monitoring) with 2,3-dichloro-5,6-dicyanobenzoquinone in tetrahydrofuran to convert any chlorin or isobacteriochlorins back to porphyrin. Evaporation gave a residue that was chromatographed on a silica gel column (elution with 1% methanol in dichloromethane); evaporation of the appropriate eluates gave a residue that was crystallized from dichloromethane/methanol to give 68 mg (84%) of the title compound. TLC and spectrophotometry of this material showed it to be identical with an authentic sample of copper(II) deuteroporphyrin IX dimethyl ester, so this labeled material was used without further purification.

2,4-Diacetyl-6,7-bis[2-(methoxycarbonyl)ethyl]-1,3-dimethyl-5,8-bis(trideuteriomethyl)porphyrin (11). The foregoing porphyrin (55 mg) in dichloromethane (12 mL) was treated with acetic anhydride (2 mL) and then cooled to 0 °C (some porphyrin precipitated out). Freshly distilled stannic chloride (0.4 mL), was added and the homogeneous green solution was stirred for 2.5 min before being poured into iced water (100 mL). The organic products were extracted with dichloromethane (50 mL), dried (Na_2SO_4), and evaporated to dryness to give a residue that was chromatographed on neutral alumina (Brockmann grade III, elution with dichloromethane initially and then with 0.5% methanol in dichloromethane). The appropriate eluates were collected and after evaporation afforded the copper(II) complex of the title porphyrin. This was dissolved in a mixture of trifluoroacetic acid (10 mL) and sulfuric acid (1 mL) and left for 1 h at room temperature under nitrogen. The mixture was then diluted with dichloromethane (100 mL), washed with water (3 × 100 mL), dried (Na_2SO_4), and evaporated to dryness. The residue was dissolved in 10% sulfuric acid in methanol (10 mL) and stirred at room temperature under nitrogen overnight. (Spectrophotometry showed that the acetyl groups had been transformed into dimethyl ketals at this stage.) The mixture was poured into dichloromethane (50 mL) and washed with water (50 mL), aqueous sodium bicarbonate (50 mL), and then water (50 mL) again, before being dried (Na_2SO_4) and evaporated to dryness. The residue was chromatographed on a silica gel column (elution with 0.5% methanol in dichloromethane), and the appropriate eluates were collected. Evaporation and crystallization from dichloromethane/heptane gave 36 mg (63%) of the title compound: Mp 238–241 °C (lit.[19] mp 242.5 °C). NMR (ppm) 10.91, 10.68, 10.04, 9.92 (each s, 1 H, meso H); 4.34, (t, 4 H, CH_2CH_2CO); 3.93, 3.90 (each s, 3 H, Me); 3.65 (s, 6 H, OMe); 3.34 (s, 6 H, COMe); 3.25, (t, 4 H, CH_2CO); −3.31 (s, 2 H, NH); MS, m/e (%) 631 (59, d_9), 630 (100, d_8), 629 (66, d_7), 628 (48, d_6), 627 (28, d_5). MW for $C_{36}H_{32}D_6N_4O_6$, calcd 628.3166, found 628.3197.

6,7-Bis[2-(methoxycarbonyl)ethyl]-1,3-dimethyl-5,8-bis(trideuteriomethyl)-2,4-divinylporphyrin (3). The foregoing labeled diacetylporphyrin **11** (21 mg) in dichloromethane (12 mL) was treated with an ice-cold solution of sodium borohydride (30 mg) in methanol (2.5 mL). The mixture was stirred for 20 min at room temperature, after which it was determined by TLC that reaction was complete. Acetic acid (0.5 mL) was added to quench the reaction, and the solution was diluted with dichloromethane (50 mL) and then washed with water (2 × 100 mL). The organic fraction was collected, and the solvent was evaporated to give a residue [labeled hematoporphyrin IX dimethyl ester (**12**)] that was taken up in o-dichlorobenzene (5 mL) containing p-toluenesulfonic acid (75 mg) and heated at 145 °C for 40 min as nitrogen gas was bubbled through the solution. The cooled reaction mixture was diluted with dichloromethane (100 mL) that was washed with water (3 × 100 mL), dried (Na_2SO_4), and evaporated to dryness. The residue was dissolved in methanol (10 mL) containing sulfuric acid (1 mL), and the solution was stirred overnight under nitrogen. It was then diluted with dichloromethane (50 mL) and washed with water (50 mL), aqueous sodium bicarbonate (50 mL), and then water again (50 mL) before being dried (Na_2SO_4) and evaporated to dryness. The red residue was chromatographed on a silica gel column (elution with 0.5% sulfuric acid in methanol), and the appropriate eluates were collected and evaporated to dryness to give a residue that was crystallized from dichloromethane/heptane to give the title compound: Yield 12.5 mg (62%). Mp 219–222 °C (lit.[20] mp 224–225 °C). NMR (ppm) 10.27, 10.21, 10.13, 10.07 (each s, 1

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H, meso H); 8.36–8.27 (dd, 2 H, $=CH_2$); 6.42–6.16 (dd, 2 H, CH=); 4.42 (t, 4 H, CH_2CH_2CO); 3.75, 3.74 (each s, 3 H, 1,3-Me); 3.66 (s, 6 H, OMe); 3.29 (t, 4 H, CH_2CO); −3.59 (s, 2 H, NH).

Synthesis of the 8-Methyl-Deuteriated Porphyrin 15. *tert*-Butyl 2-Formyl-4-[2-(methoxycarbonyl)ethyl]-3-methylpyrrole-5-carboxylate (21). The Vilsmeier complex from dry phosphoryl chloride (4.3 mL) and dimethylformamide (4.7 mL) was stirred at 0 °C before dropwise addition of *tert*-butyl 4-[2-(methoxycarbonyl)ethyl]-3-methylpyrrole-5-carboxylate[21] (3.69 g) in dry dichloromethane (25 mL). The mixture was stirred at room temperature for 1 h, after which time TLC monitoring indicated the reaction to be complete. The mixture was first neutralized with aqueous saturated sodium acetate (30 mL) and stirred overnight. Then, 1 N sodium hydroxide solution (100 mL) was added to bring to pH 7, and the mixture was extracted with dichloromethane (100 mL). The organic phase was washed twice with water, dried (Na_2SO_4), and evaporated to give an oil that was subjected to an 11-cm flash column (silica gel, elution with 30% ethyl acetate/*n*-hexane). The appropriate fractions (TLC analysis) were collected and evaporated to give a pale pink oil that was triturated with cyclohexane. The white fluffy solid was collected by filtration, washed with cold cyclohexane, and then dried in a vacuum oven at 50 °C to give 2.947 g (72%): Mp 74–75 °C. NMR (ppm) 9.83 (s, 1 H, CHO); 9.46 (s, 1 H, NH); 3.68 (s, 3 H, OMe); 3.01 (m, 2 H, CH_2CO); 2.53 (m, 2 H, CH_2CH_2CO); 2.32 (s, 3 H, Me); 1.62 (s, 9 H, *t*-Bu). Anal. Calcd for $C_{15}H_{21}NO_5$: C, 61,00; H, 7.17; N, 4.74. Found: C, 61.22; H, 6.86; N, 4.81.

***tert*-Butyl 1,3-Bis(2-acetoxyethyl)-2,4,5,6′-tetramethyltripyrrene-*b*-1′-carboxylate Hydrobromide (20).** Into a dry 300-mL round-bottom flask equipped with a stir bar were dissolved 3,4′-bis(2-acetoxyethyl)-5′-(*tert*-butoxycarbony')-3′,4-dimethylpyrromethane-5-carboxylic acid (**18**;[15] 3.342 g) and 2-formyl-3,5-dimethylpyrrole[22] (826 mg, 1.05 equiv) in dry dichloromethane (180 mL) and dry methanol (23 mL). *p*-Toluenesulfonic acid hydrate (2.567 g, 2.1 equiv.) was added, and this mixture was stirred at room temperature under nitrogen for 45 min (TLC monitoring). Water (ca. 200 mL) was then added, and the organic layer was extracted. Another 100 mL of dichloromethane was added, and the combined organic extracts were washed with water, saturated sodium bicarbonate solution, and then water again. The organic phase was dried (Na_2SO_4) and evaporated to dryness (under vacuum, no heating), and the residue was dissolved in a minimum volume of dichloromethane before being treated, at 0 °C, with HBr gas for 5 s. Dry ether (ca. 10 mL) was then added, and the mixture was left overnight at 0 °C. A yellow-gold solid was collected in two crops by filtration, washed with ether, and then dried under vacuum to give 2.818 g (70%): Mp 173–176 °C. NMR (ppm) 13.356, 13.207, 10.362 (each s, 1 H, NH); 7.122 (s, 1 H, bridge CH); 6.219 (s, 1 H, ring H); 4.366 (s, 2 H, bridge CH_2); 4.135, 3.995 (each t, 2 H, CH_2O); 3.021, 2.762 (each t, 2 H, CH_2CH_2O); 2.713, 2.374, 2.297, 2.078 (each s, 3 H, Me); 2.040, 2.019 (each s, 3 H, OCOMe); 1.562 (s, 9 H, *t*-Bu). Vis (2% trifluoroacetic acid/CH_2Cl_2) λ_{max} 484 nm (ϵ 87.7 × 10^3). Anal. Calcd for $C_{31}H_{39}BrN_3O_6$: C, 59.14; H, 6.24; N, 6.67. Found: C, 58.87; H, 6.00; N, 6.64.

2,4-Bis(acetoxyethyl)-8-[2-(methoxycarbonyl)ethyl]-1,2,5,8-tetramethyl-*a*,*c*-biladiene-8′-carboxylic Acid Dihydrobromide (22). The foregoing tripyrrene hydrobromide **20** (2.818 g) in trifluoroacetic acid (18 mL) was stirred under nitrogen for 10 min before addition of formylpyrrole **21** (1.392 g, 1.05 equiv) in dry dichloromethane (45 mL) and dry methanol (45 mL) in one aliquot at 0 °C. After the mixture was stirred for 45 min, TLC analysis showed no formylpyrrole to be left so the solution was diluted with dichloromethane (200 mL) and washed twice with water, then with saturated sodium bicarbonate solution (color from red to green), and finally with water again. The organic phase was dried (Na_2SO_4), then evaporated to dryness, and dissolved in a minimum volume of dry dichloromethane. HBr gas was bubbled through the mixture for 10 s and ether was added. After 1 h at 0 °C the red powder was collected by filtration, washed with ether, and then dried under vacuum to give 3.298 g (91.7%): Mp 171–172 °C. NMR (ppm) 15.144, 13.522, 13.324, 12.502 (each s, 1 H, NH); 7.496 (s, 1 H, CO_2H); 7.175, 6.273 (each s, 1 H, bridge CH); 5.409 (s, 2 H, bridge CH_2); 4.121 (t, J = 7.5 Hz, 4 H, 2 × CH_2O); 3.672 (s, 6 H, 2 × OMe); 2.964 (t, J = 7.2 Hz, 2 H, CH_2CH_2CO); 2.598 (t, J = 7.6 Hz, 2 × CH_2CH_2O); 2.409, 2.331, 2.041, 2.016 (each s, 3 H, Me); 2.000 (s, 6 H, 2 × COMe). Vis (2% trifluoroacetic acid/CH_2Cl_2) λ_{max} 444 nm (ϵ 80.4 × 10^3), 516 (22.2 × 10^3). Anal. Calcd for $C_{37}H_{46}Br_2N_4O_8$: C, 55.37; H, 5.78; N, 6.98. Found: C, 55.02; H, 5.89; N, 6.81.

2,4-Bis(2-acetoxyethyl)-6-[2-(methoxycarbonyl)ethyl]-1,3,5,8-tetramethylporphyrin (16). The foregoing *a*,*c*-biladiene **22** (539 mg) in 1,2-dichlorobenzene (130 mL) was treated with iodine (1.11 g) and then stirred at reflux for 20 min. After the mixture was cooled to room temperature, triethylamine (0.5 mL) was added dropwise and the mixture was applied to a 12-cm flash chromatography column (neutral alumina, Brockmann grade V, elution with three column volumes of *n*-hexane and then with 5% tetrahydrofuran in dichloromethane). A second flash chromatography was then carried out (10 cm, silica gel, elution with 5% tetrahydrofuran in dichloromethane). The red eluates were collected and evaporated to dryness, and the residue was recrystallized from dichloromethane/cyclohexane to give 150 mg (38%): Mp 211–214 °C. NMR (ppm) 10.102, 10.093, 10.085, 9.985 (each s, 1 H, meso H); 9.086 (2, 1 H, 7-H); 4.8–4.9 (m, 4 H, 2 × CH_2O); 4.3–4.4 (m, 6 H, 2 × CH_2CH_2O, CH_2CH_2CO); 3.745, 3.732, 3.669, 3.661, 3.636 (each s, 3 H, Me or OMe); 3.276 (t, 2 H, CH_2CO); 2.089, 2.082 (each s, 3 H, COMe); −3.908 (s, 2 H, 2 × NH); vis (CH_2Cl_2) λ_{max} 400 nm (ϵ 181 × 10^3), 496 (13.0 × 10^3), 530 (8.55 × 10^3), 566 (3.36 × 10^3), 620 (8.18 × 10^2). Anal. Calcd for $C_{36}H_{40}N_4O_6$: C, 67.28; H, 6.59; N, 8.72. Found: C, 67.28; H, 6.27; N, 8.65. A minor amount of a slightly less polar byproduct was also isolated from this reaction (see text), and this was characterized as 2,4-bis(2-acetoxyethyl)-7-iodo-6-[2-(methoxycarbonyl)ethyl]-1,3,5,8-tetramethylporphyrin (**23**): NMR (ppm) 10.134, 10.057, 10.054, 10.029 (each s, 1 H, meso H); 4.868 (t, J = 7.4 Hz, 2 H, CH_2OAc); 4.845 (t, J = 7.7 Hz, 2 H, CH_2OAc); 4.506 (t, J = 7.7 Hz, 2 H, CH_2CH_2OAc); 4.438 (t, J = 7.3 Hz, 2 H, CH_2CH_2OAc); 4.308 (t, J = 7.3 Hz, 2 H, $CH_2CH_2CO_2Me$); 3.723 (s, 3 H, OMe); 3.694, 3.675, 3.654, 3.594 (each s, 3 H, Me); 3.335 (t, J = 7.7 Hz, 2 H, CH_2OMe); 2.100, 2.052 (each s, 3 H, COMe); −4.120 (s, 2 H, 2 × NH).

2,4-Bis(2-acetoxyethyl)-6-[2-(methoxycarbonyl)ethyl]-7-[2-(methoxycarbonyl)vinyl]-1,3,5,8-tetramethylporphyrin (17). The foregoing 7-unsubstituted porphyrin **16** (204.8 mg) in dichloromethane (35 mL) was treated with a saturated solution of zinc(II) acetate in methanol (15 mL). After the mixture was stirred at room temperature for 30 min, spectrophotometry showed the reaction to be complete (λ_{max} 400, 530, 568 nm). The mixture was diluted with dichloromethane (75 mL), washed twice with water, dried (Na_2SO_4), and then evaporated to dryness. The magenta zinc(II) complex was recrystallized to give 195 mg (88%). Without further purification, this porphyrin (195 mg) in freshly distilled tetrahydrofuran (30 mL) was heated under nitrogen at 60 °C while mercury(II) acetate (202 mg) in dry methanol (5 mL) was added. After 4 h, TLC analysis showed the reaction to be virtually complete, so saturated sodium chloride solution (10 mL) was added and the solution was diluted with dichloromethane (75 mL) and washed twice with water. After drying (Na_2SO_4), the solvent was evaporated and the pink-red residue was suspended in ethanol, collected by filtration, and then dried under vacuum to give 226.6 mg (87%) of the mercurated zinc(II) complex **25**. $LiPdCl_3$ was prepared by stirring lithium chloride (8 mg) with palladium(II) chloride (52 mg) in refluxing dry acetonitrile (5 mL) under nitrogen for 30 min. To the mercurated porphyrin **25** (226.6 mg) in dry dimethyl sulfoxide (7 mL) and dry tetrahydrofuran (15 mL) at 50 °C under nitrogen was added distilled methyl acrylate (3.0 mL) in one aliquot, followed by the (cooled) $LiPdCl_3$ solution. After 1 h at 50 °C TLC analysis showed no base-line material, so the mixture was filtered through Celite (to remove precipitated palladium), then diluted with dichloromethane (100 mL), and washed three times with water. The organic phase was dried (Na_2SO_4) and evaporated, and the residue was dissolved in trifluoroacetic acid (5 mL) to remove the chelated zinc. After stirring at room temperature under nitrogen for 10 min, the solution was poured into water (100 mL) and then extracted with dichloromethane (2 × 50 mL), and the organic layer was washed

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twice with water, dried (Na_2SO_4), and evaporated to dryness. The residue was flash chromatographed on an 8-cm column (silica gel, elution with 3% tetrahydrofuran in dichloromethane); five fractions were collected containing the pure product, these were evaporated, and the residue was recrystallized from dichloromethane/cyclohexane to give 110 mg (63%): Mp 205–207 °C. NMR (ppm) 10.130 (s, 1 H, meso H); 10.063 (s, 3 H, meso H); 9.335 (d, J = 16.1 Hz, 1 H, C*H*=CHCO); 7.116 (d, J = 16.1 Hz, 1 H, CH=C*H*CO); 4.867 (t, J = 7.2 Hz, 4 H, 2 × CH_2O); 4.440 (t, J = 7.7 Hz, 2 H, C$H_2$$CH_2CO$); 4.389 (t, J = 7.3 Hz, 2 H, C$H_2$$CH_2O$); 4.329 (t, J = 7.3 Hz, 2 H, C$H_2$$CH_2O$); 4.085, 3.731 (each s, 3 H, OMe); 3.687 (s, 6 H, 2 × Me); 3.645, 3.614 (each s, 3 H, Me); 3.282 (t, J = 7.7 Hz, 2 H, CH_2CO); 2.099, 2.084 (each s, 3 H, COMe); −4.167 (s, 2 H, 2 × NH); vis (CH_2Cl_2) λ_{max} 412 nm (ϵ 165 × 10^3), 508 (20.1 × 10^3), 548 (24.3 × 10^3), 576 (18.1 × 10^3). Anal. Calcd for $C_{40}H_{44}N_4O_8$: C, 67.78; H, 6.26; N, 7.90. Found: C, 67.55; H, 6.34; N, 7.99.

2,4-Bis(2-acetoxyethyl)-6-[2,2-dideuterio-2-(methoxycarbonyl)ethyl]-7-[2-deuterio-2-(methoxycarbonyl)vinyl]-8-(trideuteriomethyl)-1,3,5-trimethylporphyrin (26). Sodium metal (96 mg) was dissolved under nitrogen in methanol-d_1 (10 mL) in a 100-mL round-bottom flask equipped with a stir bar and a condenser. The foregoing acrylate porphyrin **17** (91 mg) was dissolved in dry tetrahydrofuran (20 mL) and added to the methoxide solution in one aliquot. The mixture was stirred at 65 °C for 22 h before being cooled and neutralized with acetic acid (6 mL). Water (50 mL) was added, and the mixture was evaporated to a volume of 10 mL before the product was collected by filtration, washed well with water, and then dried under vacuum to give 49 mg of the bis(2-hydroxyethyl)porphyrincarboxylic acid. The material was esterified using 5% sulfuric acid in methanol (10 mL) overnight and was then diluted with dichloromethane (75 mL) and washed three times with water. The organic phase was dried (Na_2SO_4) and evaporated to dryness, and the residue was taken up in pyridine (5 mL) and acetic anhydride (4 mL) and then stirred at room temperature overnight. The solution was diluted with dichloromethane (75 mL) and then washed three times with water and dried (Na_2SO_4). Evaporation gave a residue, which, by TLC and UV/vis, was identical with the starting material. It was collected by filtration and then dried to give 42.4 mg (44% recovery): Mp 210–212 °C. NMR (ppm) 10.243, 10.214, 10.150, 10.142 (each s, 1 H, meso H); 9.388 (s, 1 H, vinyl H); 4.912 (t, J = 7.1 Hz, 2 H, CH_2OAc); 4.889 (t, J = 7.2 Hz, 2 H, CH_2OAc); 4.469 (m, 4 H, 2 × C$H_2$$CH_2$OAc); 4.364 (t, J = 7.1 Hz, 2 H, C$H_2$$CH_2CO$); 4.079, 3.729, 3.723, 3.693, 3.648 (each s, 3 H, ring methyl or OCH_3); 2.101, 2.074 (each s, 3 H, OCOMe); −3.599 (s, 2 H, 2 × NH).

2,4-Bis(2-acetoxyethyl)-6-[2-(methoxycarbonyl)ethyl]-7-[2-(methoxycarbonyl)ethyl]-8-(trideuteriomethyl)-1,3,5-trimethylporphyrin (27). The foregoing deuteriated porphyrin acrylate **26** (16 mg) in 97% formic acid (15 mL) containing 10% palladized charcoal (*Caution*! Sparks.) (16 mg) and 70% perchloric acid (2 drops) was stirred at room temperature and atmospheric pressure under hydrogen until spectrophotometry showed a change from rhodo to etio spectral type (3 h). The solution was filtered through Celite and then diluted with dichloromethane (100 mL). It was washed three times with water, then dried (Na_2SO_4), and evaporated to dryness to give a residue that was chromatographed on preparative TLC plates (silica gel, elution with 3.5% tetrahydrofuran in dichloromethane). The major band, red, was extracted from the silica gel, the solvent was evaporated, and the residue was recrystallized from dichloromethane/cyclohexane, washed with excess hexane, and then dried under vacuum to give 4.7 mg (30%): Mp 179–181 °C (lit.[20] mp 167–168 °C). NMR (ppm) 10.179, 10.165 (each s, 1 H, meso H); 10.122 (s, 2 H, 2 × meso H); 4.903 (t, J = 7.1 Hz, 4 H, 2 × CH_2OAc); 4.428 (m, 8 H, 2 × C$H_2$$CH_2$OAc, 2 × C$H_2$$CH_2CO$); 3.705, 3.686 (each s, 6 H, 2 × Me); 3.651 (s, 3 H, Me or OMe); 3.27 (t, 4 H, CH_2CH_2CO); 2.081 (s, 6 H, 2 × COMe); −3.726 (s, 2 H, 2 × NH).

2,4-Bis(2-chloroethyl)-6,7-bis[2-(methoxycarbonyl)ethyl]-8-(trideuteriomethyl)-1,3,5-trimethylporphyrin (29). The foregoing diacetoxyporphyrin **27** (4.8 mg) was dissolved in 5% sulfuric acid in methanol (5 mL) and was allowed to stir at room temperature under nitrogen for 3 h. It was worked up by pouring the reaction mixture into water (30 mL) and was extracted with dichloromethane (30 mL). The organic layer was washed with saturated sodium bicarbonate solution followed by water. It was dried (Na_2SO_4), and the solvent was evaporated. The residue (**28**) was left under high vacuum overnight. The chlorination procedure involved dissolving the residue in distilled dimethylformamide (2 mL) and distilled chloroform (4 mL), and potassium carbonate (anhydrous, 426 mg) was added, forming a heterogeneous solution. Freshly distilled thionyl chloride (0.106 mL) was added dropwise, and the solution was allowed to stir under nitrogen for 1 h. The reaction was quenched with 2 N ammonium hydroxide (1 mL) at 0 °C. Water (40 mL) was added, and the porphyrin was extracted with dichloromethane (30 mL). The organic layer was washed with water twice and dried (Na_2SO_4), and the solvent was evaporated to give 4.9 mg (100%): Mp 204–205 °C (lit.[20] mp 215–217 °C). NMR (ppm) 10.089, 10.073, 9.993, 9.987 (each s, 1 H, meso H); 4.503 (m, 4 H, 2 × CH_2Cl); 4.401 (m, 2 H, C$H_2$$CH_2CO_2$Me); 4.320 (m, 4 H, 2 × C$H_2$$CH_2$Cl); 3.662 (s, 3 H, Me); 3.652 (s, 6 H, Me); 3.646, 3.636 (each s, 3 H, OMe); 3.282 (q, 4 H, CH_2CO); 3.798 (s, 2 H, 2 × NH).

6,7-Bis[2-(methoxycarbonyl)ethyl]-8-(trideuteriomethyl)-1,3,5-trimethyl-2,4-divinylporphyrin (15). The foregoing bis(chloroethyl)porphyrin **29** (4.9 mg) was dissolved in distilled pyridine (2.3 mL), and the resultant mixture was allowed to stir at reflux for 5 min. Water (0.44 mL) was added followed by a 3% aqueous sodium hydroxide solution (0.52 mL), and this was left at reflux for 4 h. The reaction was then cooled, and over an ice bath 25% acetic acid (0.52 mL) was added. The solvent was evaporated, leaving a red residue. This was treated with 5% H_2SO_4/methanol (4 mL) overnight. The workup involved diluting with dichloromethane (40 mL) and washing with water followed by saturated sodium bicarbonate and then water again. After drying over Na_2SO_4 and removal of solvent, the red residue was purified with preparative TLC (silica gel, elution with 5% tetrahydrofuran/dichloromethane), which gave 3.9 mg (88%): Mp 218–219 °C (lit.[20] mp 224–225 °C). NMR (ppm) 10.179, 10.129, 10.029, 9.983 (each s, 1 H, meso H); 8.277 (dd, 2 H, 2 × C*H*=CH_2); 6.377 (d, J = 17.7 Hz, 2 H, 2 × CH=C*H*H); 6.192 (d, J = 11.1 Hz, 2 H, 2 × CH=CH*H*); 4.381 (m, 4 H, 2 × C$H_2$$CH_2CO$); 3.709, 3.701, 3.613 (each s, 3 H, Me or OMe); 3.655 (s, 6 H, Me or OMe); 3.270 (q, 4 H, CH_2CO); −3.832 (s, 2 H, 2 × NH).

8-(Trideuteriomethyl)hemin Chloride (30). The foregoing protoporphyrin IX **15** (3.87 mg) was dissolved in chloroform (1.2 mL; which was vigorously purged with nitrogen for 20 min). Acetonitrile (2.0 mL) was stirred at reflux under nitrogen for deoxygenation. Iron(II) chloride hydrate (8 mg) was added to the hot solution, which was allowed to cool. The porphyrin solution was added and allowed to stir at room temperature under nitrogen for 10 min. TLC indicated very little free-base porphyrin to be present as the iron complex sticks to the base line. The now brown solution was diluted with dichloromethane (30 mL) and washed with water followed by 0.12 M HCl solution followed by water again. This was dried (Na_2SO_4) and the solvent removed. This was left under high vacuum in the dark. For analysis by NMR, the low-spin cyano complex was made by adding crystals of potassium cyanide to a methanolic solution of the hemin: NMR (ppm) (CD_3OD + KCN) 16.031 (s, 3 H, 5-Me); 14.243 (s, 3 H, 3-Me); 13.681 (s, 3 H, 1-Me); 13.279 (t, 1 H, CH=); 13.058 (t, 1 H, CH=); 7.374 (s, 2 H, 7-C$H_2$$CH_2CO$); 7.005 (br s, 2 H, 6-C$H_2$$CH_2CO$); 1.18, 1.16 (each br s, 2 H, CH_2CO); −2.250 (t, 2 H, =CH_2); −2.880 (q, 2 H, =CH_2).

5,8-Bis(trideuteriomethyl)hemin Chloride (13). This hemin was similarly prepared from the 5,8-dilabeled protoporphyrin IX dimethyl ester **3** (12 mg) and afforded 12 mg of the corresponding hemin chloride: NMR (ppm) (CD_3OD + KCN) 14.435 (s, 3 H, 3-Me); 13.762 (s, 3 H, 1-Me); 13.510 (t, 1 H, CH=); 13.261 (t, 1 H, CH=); 7.488 (t, ca. 2 H, 7-C$H_2$$CH_2CO$); 7.112 (t, ca. 2 H, 6-C$H_2$$CH_2CO$); 1.02 1.01 (each br s, 2 H, CH_2CO); −2.226 (t, 2 H, =CH_2); −2.885 (q, 2 H, =CH_2).

Typical Ester Hydrolysis. The hemin dimethyl ester (50 mg) was dissolved in 15 mL of a solution made by mixing methanol (95 mL), water (5 mL), and potassium hydroxide (1 g). The solution was refluxed for 5 h at 60 °C under an atmosphere of dry nitrogen. The warm solution was then diluted with dichloromethane (20 mL) and washed with 2 M hydrochloric acid (2 × 50 mL). The organic phase was collected, dried (Na_2SO_4), and evaporated to dryness to give a residue that was recrystallized from tetrahydrofuran to give 80–85% yield of the hemin, identical

with an authentic sample by TLC, spectrophotometry, and proton ^1H NMR spectroscopy (in D_2O, as the dicyanoferrihemin).

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Total Syntheses of Derivatives of Protoporphyrin IX Regioselectively Labeled with Carbon-13 in the Methyls

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Total syntheses, from monopyrroles via tripyrrenes and *a,c*-biladienes, of the four isomers **2**–**5** of protoporphyrin IX dimethyl ester in which the 1-, 3-, 5-, and 8-methyl groups are individually and regioselectively enriched with carbon-13 are described. The source of labeled carbon was 90% carbon-13-enriched paraformaldehyde, and methyls were inserted at the monopyrrole stage by reductive C-alkylation. The carbon-13-labeled porphyrins, as the corresponding hemes, are of interest as probes in carbon-13 NMR spectroscopic studies of reconstituted heme proteins.

Proton NMR spectroscopy has been extremely productive for studying the electronic structure of paramagnetic porphyrins and heme proteins.[2,3] Significant advances in interpretation of the large isotropic NMR shifts[4–25] in hemes and heme proteins have been made possible by the availability of regioselectively deuterium-labeled hemes.[26–31] Recently, interpretation of resonance Raman spectra of nickel porphyrins,[32] hemes,[33] and heme proteins[33–35] has also been aided by the use of hemes from vinyl-labeled protoporphyrin IX dimethyl ester (**1**). It is to be expected

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