Synthesis and Assignment of the Four Possible *meso*-Methylmesoporphyrin IX Regioisomers

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Meso-alkylated porphyrins are formed in the reactions of hemoproteins with alkylhydrazines and are of interest as probes of heme-dependent enzymes. Their biological utility has been limited, however, by the unavailability of a set of the four individual *meso*-alkyl regioisomers of a biologically relevant porphyrin. We report here synthesis of the dimethyl esters of the regioisomers of *meso*-(hydroxymethyl)mesoporphyrin IX from the dimethyl ester of mesoporphyrin IX, separation of the four isomers by column chromatography, identification by NMR methods of the *meso* position occupied by the hydroxymethyl group in each regioisomer, and reduction of the *meso*-(hydroxymethyl) regioisomers to the corresponding *meso*-methylporphyrins. Ester hydrolysis and iron insertion provide the four individual regioisomers of iron *meso*-methylmesoporphyrin IX (*meso*-methylmesoheme). Since mesoheme is accepted by most heme-dependent systems as a substitute for iron protoporphyrin IX, its *meso*-methyl derivatives should prove to be useful probes of hemoprotein function.

Meso-alkylated protoporphyrin IX derivatives are formed in the reactions of horseradish peroxidase with nitromethane, cyclopropanone hydrate, and several alkylhydrazines.[1-4] In each instance, only the δ-*meso*-substituted regioisomer is obtained. The site of substitution in these adducts has been established by NMR analysis of the single regioisomer that is isolated from each reaction.[3,4] The reaction of myoglobin with alkylhydrazines also yields a single *meso*-alkylated protoporphyrin IX derivative, but in this instance NMR studies indicate that the substituent is located at the γ-*meso* position.[5] Using the γ- and δ-*meso*-alkyl porphyrins extracted and purified from the reactions of horseradish peroxidase and myoglobin with alkylhydrazines, it has been possible to reconstitute apohorseradish, cytochrome c, and manganese peroxidases and to determine the effects of a δ-*meso*-alkyl substituent on their catalytic activities .[4,6-8] It has not been possible to do this, however, with hemes substituted at the α or β positions. Although methods have been reported for the synthesis of mixtures of the four possible *meso*-alkyl regioisomers,[9,10] the individual isomers of a biologically useful porphyrin have never been separated or individually characterized, nor has the specific *meso* position substituted in each of the isomers been identified. Assignment of the γ- and δ-substituted porphyrins is expected to be relatively straightforward due to the unique pattern of substituents about those positions, but assignment of the α- and β-substituted analogs is likely to be more difficult because both of these *meso* positions are flanked by the same substituents. In spite of their potential utility, the unavailability of a well defined set of *meso*-alkylporphyrin regioisomers has hindered the use of such molecules as probes of hemoprotein structure and function. We report here preparation of the four individual regioisomers of *meso*-methylmesoporphyrin IX, determination of the *meso* position substituted in each by NMR methods, and conversion of the individual isomers to the corresponding iron complexes. Mesoporphyrin IX was used in these studies to avoid the chemical reactivity associated with the two vinyl groups of protoporphyrin IX, the natural heme ligand, as this reactivity is not compatible with the chemistry used to introduce the *meso*-methyl group. Mesoheme (iron mesoporphyrin IX), however, is accepted by most hemoproteins as a replacement for the normal heme moiety.

Results and Discussion

Synthesis of the *meso*-Methylmesoporphyrin IX Regioisomers. The four isomers of *meso*-methylmesoporphyrin IX (**6a–d**) have been synthesized as shown in Scheme 1. The copper complex of mesoporphyrin IX was monoformylated at the *meso* positions by a Vilsmeier reaction to give **3a–d**. The formyl groups of these porphyrins were directly reduced to give the *meso*-hydroxymethyl derivatives **4a–d** by treatment with tetra-*n*-butylammonium borohydride in CH_2Cl_2. Demetalation of the copper–(hydroxymethyl)porphyrin complexes yielded a mixture of **5a–d** as well as some starting material **1**. Exploratory studies demonstrated that the *meso*-(hydroxymethyl)porphyrin isomers are more readily separated by chromatography than the *meso*-methylporphyrin isomers. Thus, the R_f values of isomers **5a–d** on silica gel thin layer chromatography with diethyl ether as the solvent are γ (0.72), α (0.66), β (0.55), and δ (0.46). Porphyrins **5a–d** were therefore separated and purified

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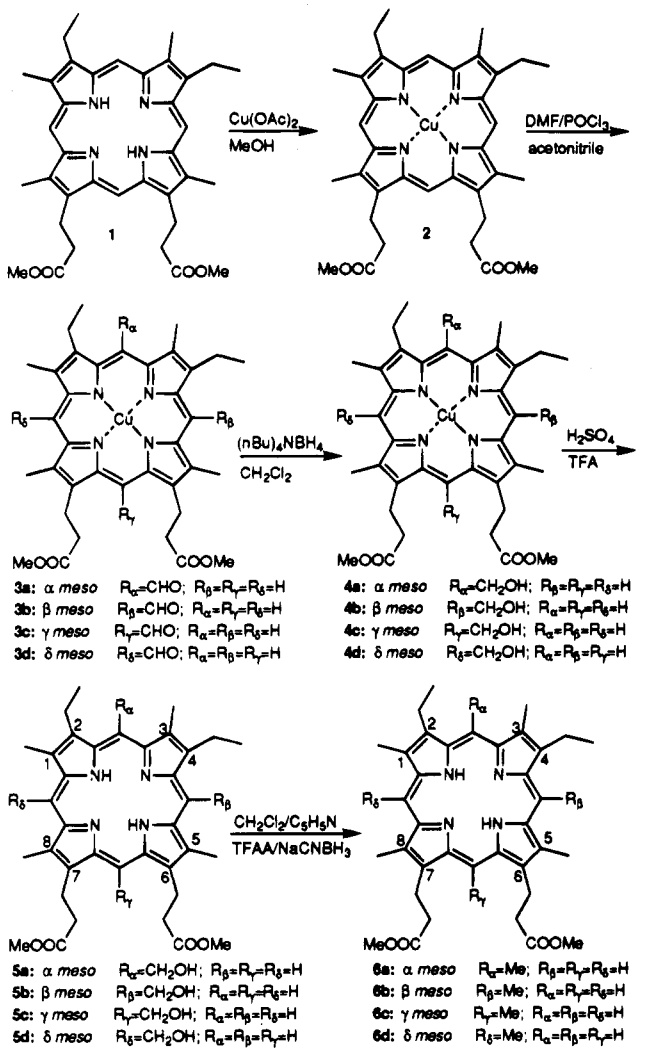
Scheme 1. Synthesis of the *meso*-Methylmesoporphyrin IX Regioisomers



by flash column chromatography, and each isomer was subsequently rechromatographed for optimum purity. The individual *meso*-(hydroxymethyl)porphyrins **5a–d** were then reduced to the corresponding *meso*-methylporphyrins **6a–d** by treatment with trifluoroacetic anhydride/pyridine and sodium cyanoborohydride in CH_2Cl_2. The overall yield of each of the *meso*-methylporphyrins correlates with the degree of steric hindrance surrounding the *meso* position substituted in each. Thus, the yield of the δ-isomer, in which the *meso* position is flanked by two methyls, was the highest (20%) and that of the γ-isomer, in which the *meso* position is flanked by the two propionic acid side chains, the lowest (3.5%).

Identification of the *meso*-(Hydroxymethyl)mesoporphyrin IX Regiosomers. The mixture of porphyrin isomers was separated at the *meso*-(hydroxymethyl) stage because advantage could be taken of the relatively high differences in the polarities of the *meso*-(hydroxymethyl) regioisomers. The regiochemistry of the *meso*-substitution was established at the same stage. As shown in Figure 1, the ^1H NMR spectra of porphyrins **5a–d** are very similar. These pseudosymmetric molecules exhibit many overlapping resonances, including a highly congested region of methyl signals. As expected, however, porphyrins **5c** and **5d** are readily identified as the γ- and δ-isomers, respectively, by NOE experiments. Thus, irradiation of the propionate resonance of **5d** at 4.39 ppm (Figure 2, lower trace) enhances and identifies the γ-*meso*

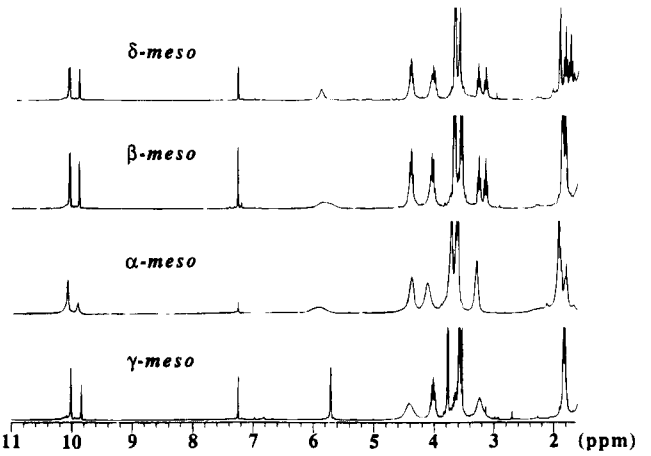


Figure 1. Comparison of the ^1H NMR spectra of the four regioisomers of dimethyl-esterified *meso*-(hydroxymethyl)-mesoporphyrin IX. The position of the hydroxymethyl group is indicated on each spectrum.

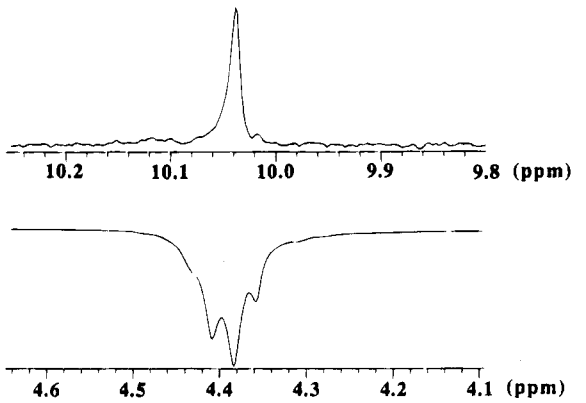


Figure 2. NOE of the γ-meso proton at 10.04 ppm (upper tracing) of isomer **5d** caused by irradiation of the propionate resonances at 4.39 ppm (lower tracing).

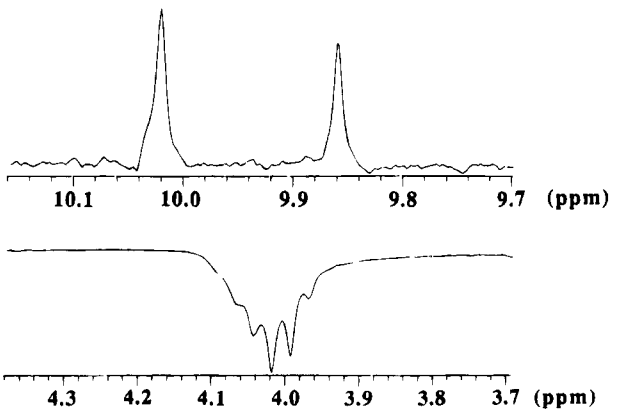


Figure 3. NOE enhancement of the α- and β-*meso* protons at 10.02 and 9.86 ppm (upper tracing) of isomer **5d** caused by irradiation of the ethyl methylene resonances at 4.02 ppm (lower tracing).

proton signal (upper trace), while irradiation of the ethyl methylene resonance at 4.02 ppm (Figure 3, lower trace) enhances and identifies both the α- and β-*meso* proton resonances (upper trace). By exclusion, porphyrin **5d** is the δ-*meso*-(hydroxymethyl) isomer. The γ-isomer is unambiguously identified by two independent experiments. The α- and β-*meso* protons are identified in NOESY plots by their NOE interactions with the protons of both a methyl and an ethyl methylene, and the δ-*meso*

Table 1. ^{1}H NMR Assignments for the *γ-meso*-(Hydroxymethyl) Isomer 5c

proton	chemical shift (ppm)	NOE[a]
α-*meso*-*H*	9.84	2-Eth; 3-Me
β-*meso*-*H*	10.01	4-Eth; 5-Me
γ-*meso*-CH_2OH	1.84	γ-CH_2OH; 6-Pr; 7-Pr
γ−*meso*-CH_2OH	5.69	γ-CH_2OH; 6-Pr; 7-Pr
δ-*meso*-*H*	10.01	1-Me; 8-Me
1-CH_3	3.56	δ-H; 2-Eth
2-CH_2CH_3	3.99	1-Me; α-H
2-CH_2CH_3	1.80	
3-CH_3	3.54	α-H; 4-Et
4-CH_2CH_3	4.03	3-Me; β-H
4-CH_2CH_3	1.81	
5-CH_3	3.59	β-H; 6-Pr
6,7-$CH_2CH_2CO_2Me$	4.47/4.32	5,8-Me; γ-H
6,7-$CH_2CH_2CO_2Me$	3.24	
8-CH_3	3.59	7-Pr; δ-H
diester CH_3	3.77/3.78	

[a] NOE's in the NOESY spectrum of **5c** used to "walk" around the porphyrin periphery.

Table 2. ^{1}H NMR Assignments for the *β-meso*-(Hydroxymethyl) Isomer 5b

proton	chemical shift (ppm)	NOE[a]
α-*meso*-*H*	10.01	2-Eth; 3-Me
β-*meso*-CH_2OH	5.90 (broad)	
β-*meso*-CH_2OH	1.88	4-Eth; 5-Me
γ-*meso*-*H*	10.03	6-Pr; 7-Pr
δ-*meso*-*H*	9.85	1-Me; 8-Me
1-CH_3	3.53	δ-H; 2-Eth
2-CH_2CH_3	4.02	1-Me; α-H
2-CH_2CH_3	1.83	
3-CH_3	3.56	α-H; 4-Et
4-CH_2CH_3	4.07	3-Me; β-CH_2OH
4-CH_2CH_3	1.83	
5-CH_3	3.68	β-CH_2OH; 6-Pr
6-$CH_2CH_2CO_2Me$	4.41	5-Me; γ-H
6-$CH_2CH_2CO_2Me$	3.15	
7-$CH_2CH_2CO_2Me$	4.39	γ-H; 8-Me
7-$CH_2CH_2CO_2Me$	3.25	
8-CH_3	3.56	7-Pr; δ-H
diester CH_3	3.67/3.65	

[a] NOE's in the NOESY spectrum of **5b** used to "walk" around the porphyrin periphery.

proton by its NOE interactions with two different methyl groups (not shown). By exclusion, the *γ-meso* position in isomer **5c** must bear the hydroxymethyl substituent. This assignment is confirmed by irradiation of the inner methylenes of the propionic acid substituents, which results in enhancement of both the *γ-meso*-(hydroxymethyl) OH proton at 1.84 ppm and methylene protons at 5.69 ppm (not shown). The complete resonance assignments for the γ isomer (**5c**) are given in Table 1.

Differentiating the α- and β-isomers, however, requires a NOESY spectrum that contains the NOE's for the entire molecule. The complete proton assignments for the β-isomer **5b** are shown in Table 2. These proton assignments make possible a "walk" around the porphyrin periphery that identifies the *β-meso*-(hydroxymethyl) group by its NOE cross-peaks to the surrounding substituents. As shown in Figure 4, the *δ-meso* proton at 9.85 ppm exhibits cross-peaks to the flanking 1- and 8-methyl groups at 3.53 ppm and 3.56 ppm, respectively. The methyl group at 3.56 ppm has an NOE to the 7-propionate inner methylene protons at 4.39 ppm (Figure 5) and can therefore be identified as the 8-methyl. The other propionate at 4.41 ppm, which must be the 6-propionate, has an NOE to the methyl at 3.68 ppm, which is therefore identified as the 5-methyl (Figure 5). The 5-methyl at 3.68 ppm exhibits a cross-peak to the *meso*-(hydroxymethyl) OH proton at 1.88 ppm rather

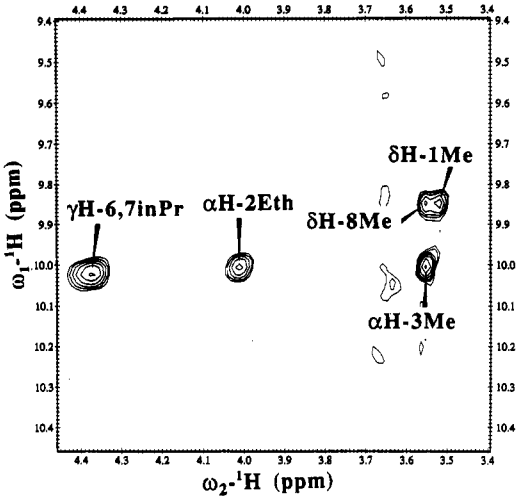


Figure 4. Region of the NOESY spectrum of isomer **5b** showing the cross-peaks between the *δ-meso* proton at 9.85 ppm and the flanking 1- and 8-methyl groups at 3.53 and 3.56 ppm.

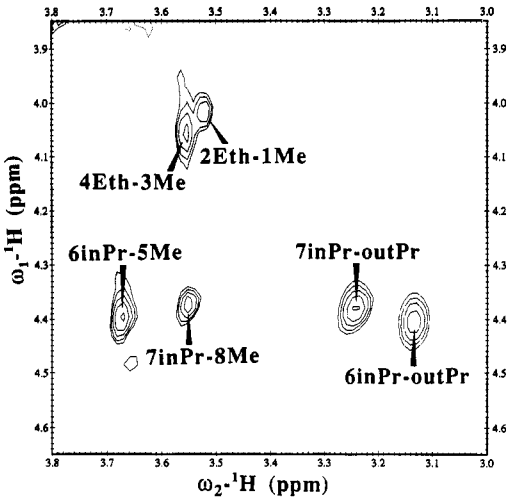


Figure 5. Region of the NOESY spectrum of isomer **5b** showing the NOE between the methyl group at 3.56 ppm and the 7-propionate inner methylene protons at 4.39 ppm and between the propionate inner methylene protons at 4.41 ppm and the 5-methyl at 3.68 ppm.

than to a *meso* proton. The *meso*-(hydroxymethyl) group with the OH signal at 1.88 ppm therefore must be at the *β-meso* position vicinal to the 5-methyl (Figure 6). The location of the *meso*-(hydroxymethyl) group is independently confirmed by the observation of an NOE between the *meso*-(hydroxymethyl) OH proton at 1.88 ppm and the 4-ethyl methylene protons at 4.07 ppm (Figure 6). The 4-ethyl methylene protons at 4.07 ppm are distinguished from the 2-ethyl methylene protons at 4.02 ppm by the presence of an NOE correlating the resonance at 4.02 ppm with that of the 1-methyl at 3.53 ppm (Figure 5). Both results positively identify **5b** as the β-isomer. By exclusion, isomer **5a** must be the α-*meso*-(hydroxymethyl) isomer.

Separation of the four regioisomers of *meso*-(hydroxymethyl)mesoporphyrin IX, identification of the *meso* position substituted in each, and subsequent reduction to the corresponding *meso*-methylporphyrins makes available for the first time a set of well-defined, *meso*-alkyl-substituted porphyrins. The individual dimethyl-esterified *meso*-methylmesoporphyrin IX regioisomers were hydrolyzed to the free acids, and iron was inserted by

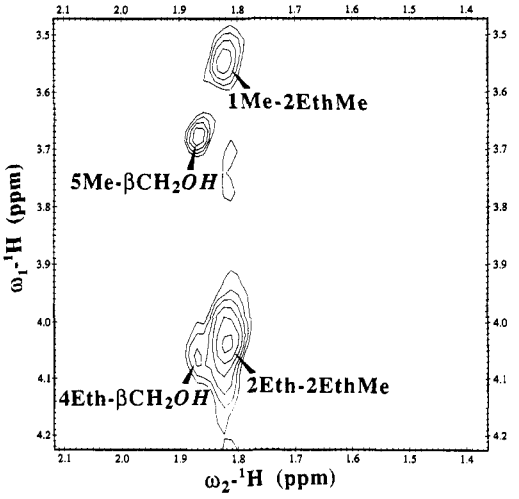


Figure 6. Region of the NOESY spectrum of isomer **5b** showing the NOE between the 5-methyl at 3.68 ppm and the *β-meso*-(hydroxymethyl) OH proton at 1.88 ppm.

conventional procedures to provide the individual ferric *meso*-methylmesohemes. Although the normal prosthetic group of most hemoproteins is iron protoporphyrin IX, iron mesoporphyrin IX (mesoheme) is acceptable as a replacement for the normal heme in most biological systems. Experiments are under way to examine the effect of the *meso*-methyl group on the cleavage of ferric *meso*-methylmesoheme by heme oxygenase, an enzyme that cleaves the porphyrin at the α-*meso* position. The *meso*-methylmesoheme regioisomers should also be highly useful as probes of the structure and mechanism of other hemoproteins.

Experimental Section

General. ^{1}H NMR spectra were measured in deuterated chloroform (porphyrin concentration 3-4 mg/mL) on either a 300 or 500 MHz instrument. One-dimensional ^{1}H NOE experiments were carried out using a 4 s decoupler pulse and a delay time of 1 s. NOE difference spectra were obtained by subtracting the off resonance spectrum from the on resonance spectrum. ^{1}H NOESY data were obtained using a delay time of 2s and a mixing time of 300 ms. The NOESY spectra were obtained with 8K data points in the t_2 dimension and 400 blocks of 16 scans each in the t_1 dimension. The free induction decays were zero filled once in both dimensions, and a 70° shifted sine-squared apodization window was applied in both dimensions. ^{13}C NMR spectra were acquired on a 300 MHz instrument and are completely decoupled. Mesoporphyrin IX dimethyl ester was purchased from Porphyrin Products (Logan, UT). Thin layer chromatography was carried out on silica gel GF (250 micron) plates (Analtech, Newark, DE).

Cu Mesoporphyrin IX Dimethyl Ester (2). As described by Fuhrhop and Smith,[11] 20 mL of a saturated solution of cupric acetate in methanol was added to a solution of the dimethyl ester of mesoporphyrin IX (100 mg, 168 μmol) in 10 mL of CH_2Cl_2. After being refluxed for 30 min, the reaction was allowed to cool and was then washed with water and extracted with CH_2Cl_2. The organic layer was again washed with water, dried over Na_2SO_4, and concentrated *in vacuo* to give **2** in essentially quantitative yield. TLC in chloroform shows a single product (**2**) with R_f = 0.41 and no residual mesoporphyrin IX dimethyl ester (R_f = 0.20). Compound **2** was used without further purification.

Dimethyl Esters of the Regioisomers of Cu *meso*-Formylmesoporphyrin IX 3a–d. Porphyrin **2** was formylated by a Vilsmeier reaction as described by Fuhrhop and Smith[9] with the following modifications. A solution of **2** (110 mg, 168 μmol) in 10 mL of CH_2Cl_2 and 20 mL of acetonitrile in a two-necked round bottom flask equipped with stir bar and condenser was purged with argon while freshly distilled phosphorus oxychloride (2.0 mL, 21.3 mmol) was added to dry dimethylformamide (2.0 mL, 25.8 mmol). The Vilsmeier reagent was stirred at room temperature for 10 min before a 3 mL aliquot was transferred to the reaction flask and the mixture was refluxed under argon for 1 h. To the reaction was then added 50 mL of saturated sodium acetate, and the reaction was stirred for another hour before it was allowed to cool. The reaction was worked up by diluting it with water (50 mL), extracting it with CH_2Cl_2 (2 × 200 mL), washing the combined organic phase with water, drying it over Na_2SO_4, and finally concentrating it *in vacuo*. The residue was used without further purification.

Dimethyl Esters of the Regioisomers of Cu *meso*-(Hydroxymethyl)mesoporphyrin IX (4a–d). *meso*-Formylporphyrins **3a–d** were reduced to the corresponding *meso*-(hydroxymethyl)porphyrins **4a–d** by treatment with tetra-*n*-butylammonium borohydride (50 mg, 196 μmol) in CH_2Cl_2 (30 mL).[10] The reaction mixture was stirred at room temperature for 5 min. Workup of the reaction involved extraction with 100 mL of 3% H_2O_2, thorough washing with water, drying over Na_2SO_4, and concentration in *vacuo*. TLC of the residue with diethyl ether as solvent indicates the formation of all four *meso*-(hydroxymethyl) isomers (R_f = 0.74, 0.68, 0.56, 0.48) as well as detectable amounts of starting material **2** (R_f = 0.84) due to incomplete Vilsmeier reaction.

Dimethyl Esters of *meso*-(Hydroxymethyl)mesoporphyrin IX Isomers 5a–d. The copper ion was removed from the mixture **4a–d** by treatment with 1:1 H_2SO_4/trifluoroacetic acid as reported by Fuhrhop and Smith.[11] Prolonged exposure of these metalloporphyrins to acidic conditions causes retroelimination of the *meso*-(hydroxymethyl) group to give mesoporphyrin IX and formaldehyde, but removal of the copper ion occurs almost instantaneously under the indicated conditions. Therefore, the product mixture **4a–d** was dissolved in 10 mL of the acid solution, and the solution was immediately diluted with water (100 mL) and extracted with CH_2Cl_2 (2 × 200 mL). The organic layer was washed with water until the pH was neutral and was then dried and concentrated *in vacuo*. Thin layer chromatography with long wave UV light detection shows the presence of five fluorescent spots, indicating that the metal was successfully removed from the four product regioisomers as well as the residual starting material. The product mixture was purified by flash column chromatography in 30% hexanes/70% diethyl ether, and each isomer was subsequently rechromatographed using diethyl ether as solvent: TLC (diethyl ether) R_f = 0.87 (**2**), 0.72 (**5c**), 0.66 (**5a**), 0.55 (**5b**), 0.46 (**5d**). **5a**: λ_{max} (CH_2Cl_2) 406, 508, 542, 578, 628 nm; ^{1}H NMR ($CDCl_3$) δ 1.79 (brd t, 3H), 1.89 (brd t, 3H), 1.93 (s, OH), 3.28 (brd m, 4H), 3.60 (s, 6H), 3.63 (s, 3H), 3.70 (s, 9H), 4.10 (brd m, 4H), 4.37 (brd m, 4H), 5.90 (brd s, 2H), 9.88 (s, 1H), 10.05 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 11.6, 11.9, 16.6, 17.1, 17.5, 20.0, 21.8, 22.9, 36.9, 45.4, 51.7, 59.6, 95.8, 96.7, 96.9, 135.1, 136.5, 136.7, 137.6, 137.7, 138.1, 141.6, 142.0, 142.4, 143.1, 143.6, 144.2, 144.8, 146.5, 147.9, 173.6; LSIMS *m/z* 607 (MH^+ - H_2O). **5b**: λ_{max} (CH_2Cl_2) 406, 506, 542, 578, 628 nm; ^{1}H NMR ($CDCl_3$) δ 1.83 (m, 6H), 1.88 (s, O-H), 3.15 (t, 2H, *J* = 7.8 Hz), 3.25 (t, 2H, *J* = 7.5 Hz), 3.53 (s, 3H), 3.56 (s, 6H), 3.65 (s, 3H), 3.67 (s, 3H), 3.68 (s, 3H), 4.02–4.07 (m, 4H), 4.39–4.41 (m , 4H), 5.80 (brd s, 2H), 9.85 (s, 1H), 10.01 (s, 1H) 10.03 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 11.3, 11.5, 12.0, 16.7, 17.1, 17.6, 19.8, 21.9, 22.2, 23.1, 37.0, 45.3, 51.7, 59.5, 96.3, 96.6, 96.8, 134.8, 135.8, 136.0, 138.2, 138.6, 140.7, 141.3, 141.6, 142.3, 142.7, 142.7, 143.3, 145.5, 146.1, 146.5, 147.2, 173.6; LSIMS *m/z* 607 ($MH^+ - H_2O$). **5c**: λ_{max} (CH_2Cl_2) 408, 510, 546, 580, 636 nm; ^{1}H NMR ($CDCl_3$) δ 1.81(m, 6H), 1.83 (s, O-H), 3.24 (m, 4H), 3.53 (s, 3H), 3.56 (s, 3H), 3.58 (s, 3H), 3.59 (s, 3H), 3.76 (s, 3H), 3.77 (s, 3H), 4.01 (m, 4H), 4.40 (brd m, 4H), 5.70 (s, 2H), 9.83 (s, 1H), 10.01 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 11.4, 11.5, 12.2, 17.5, 17.6, 19.7, 19.8, 25.4, 36.4, 45.5, 51.8, 60.2, 96.2, 96.9, 97.0, 134.7, 135.6, 137.6, 138.9, 141.8, 142.3, 142.7, 143.0, 143.8, 143.8, 145.2, 145.6, 145.8, 146.3, 173.6; LSIMS *m/z* 607 ($MH^+ - H_2O$). **5d**: λ_{max} (CH_2Cl_2) 406, 506, 542, 580, 628 nm; ^{1}H NMR ($CDCl_3$) δ 1.74 (t, 3H, *J* = 7.5 Hz), 1.82 (t, 3H, *J* = 7.5 Hz), 1.92 (s, O-H), 3.14 (t, 2H, *J* = 7.5 Hz), 3.26 (t,

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2H, J = 7.5 Hz), 3.57 (s, 6H), 3.64 (s, 3H), 3.66 (s, 6H), 3.67 (s, 3H), 4.01–4.03 (m, 4H), 4.38–4.40 (m, 4H), 5.75 (s, 2H), 9.86 (s, 1H), 10.02 (s, 1H), 10.04 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 11.5, 11.6, 16.9, 17.5, 17.6, 19.7, 20.0, 21.9, 22.1, 37.0, 45.4, 51.7, 59.5, 96.0, 96.5, 96.6, 134.8, 135.5, 135.7, 135.8, 137.1, 138.7, 140.6, 141.0, 141.9, 141.9, 142.4, 143.9, 144.8, 145.0, 145.7, 146.9; LSIMS m/z 607 ($MH^+ - H_2O$).

The Dimethyl Esters of the Regioisomers of *meso*-Methylmesoporphyrin IX 6a–d. The *meso*-(hydroxymethyl) substituent of **5a–d** was reduced to the *meso*-methyl of **6a–d** by dissolving the porphyrin (16.0 mg, 20.4 μmol) in CH_2Cl_2 (10 mL), adding pyridine (100 μL), trifluoroacetic anhydride (100 μL), and sodium cyanoborohydride (8.0 mg, 127 μmol), and stirring at room temperature for 10 min. The reaction was then sequentially extracted with aqueous $NaHCO_3$, 1.0 N HCl, and brine. The organic layer was dried and concentrated under vacuum. Each isomer was individually reduced using a similar procedure with yields varying between 60 and 80%. After workup the products **6a–d** were purified by flash column chromatography with ether as the solvent: **6a**: λ_{max} (CH_2Cl_2) 406, 504, 540, 580 nm; ^{1}H NMR ($CDCl_3$) δ 1.79 (m, 6H), 3.26 (m, 4H), 3.51 (s, 3H), 3.56 (s, 3H), 3.58 (s, 3H), 3.61 (s, 3H), 3.67 (s, 6H), 4.02 (m, 4H), 4.35 (m, 4H), 4.48 (s, 3H), 9.8 (s, 1H), 10.0 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 11.7, 16.0, 17.3, 17.6, 19.9, 21.8, 22.8, 22.9, 36.9, 51.7, 94.6, 96.4, 96.5, 114.4, 135.1, 136.5, 137.1, 137.5, 137.7, 141.2, 141.4, 142.1, 142.2, 144.1, 144.5, 146.0, 173.7; HRMS m/z 608.3371, calcd for $C_{37}H_{44}O_4N_4$ 608.3363. **6b**: λ_{max} (CH_2Cl_2) 406, 506, 540, 576 nm; ^{1}H NMR ($CDCl_3$) δ 1.78 (t, 3H, J = 9.0 Hz), 1.83 (t, 3H, J = 9.0 Hz), 3.20 (t, 2H, J = 7.5 Hz), 3.26 (t, 2H, J = 7.5 Hz), 3.52 (s, 3H), 3.54 (s, 3H), 3.56 (s, 3H), 3.58 (s, 3H), 3.65 (s, 3H), 3.69 (s, 3H), 4.03 (brd q, 4H), 4.38 (t, 4H, J = 7.5 Hz), 4.49 (s, 3H), 9.81 (s, 1H), 10.00 (s, 1H), 10.01 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 11.4, 11.5, 11.8, 16.0, 17.4, 17.6, 19.8, 21.9, 22.0, 22.8, 22.9, 37.0, 51.7, 95.1, 96.1, 96.4, 114.4, 134.8, 135.3, 137.5, 138.5, 140.4, 141.6, 143.0, 143.8, 144.9, 145.3, 146.8, 147.4, 173.6; HRMS m/z 608.3378, calcd for $C_{37}H_{44}O_4N_4$ 608.3363. **6c**: λ_{max} (CH_2Cl_2) 406, 506, 540, 576 nm; ^{1}H NMR ($CDCl_3$) δ 1.82 (m, 6H), 3.14 (brd t, 4H), 3.53 (s, 3H), 3.56 (s, 3H), 3.59 (s, 3H), 3.60 (s, 3H), 3.77 (s, 3H), 3.78 (s, 3H), 4.01 (m, 4H), 4.36 (brd t, 4H), 4.53 (s, 3H), 9.79 (s, 1H), 9.99 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 11.4, 11.5, 12.0, 17.5, 17.6, 19.7, 19.8, 25.4, 29.7, 36.0, 51.9, 96.5, 96.6, 98.1, 134.8, 135.2, 135.8, 137.4, 137.5, 138.6; HRMS m/z 608.3345, calcd for $C_{37}H_{44}O_4N_4$ 608.3363. **6d**: λ_{max} (CH_2Cl_2) 404, 504, 538, 576 nm; ^{1}H NMR ($CDCl_3$) δ 1.79 (t, 3H, J = 9.0 Hz), 1.85 (t, 3H, J = 9.0 Hz), 3.19 (t, 2H, J = 7.5 Hz), 3.26 (t, 3H, J = 7.5 Hz), 3.55 (s, 3H), 3.57 (s, 3H), 3.58 (s, 6H), 3.64 (s, 3H), 3.67 (s, 3H), 4.04 (m, 4H), 4.38 (m, 4H), 4.47 (s, 3H), 9.82 (s, 1H), 10.01 (s, 1H), 10.02 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 11.5, 11.6, 17.1, 17.2, 17.5, 19.7, 19.8, 21.9, 23.6, 37.0, 51.7, 94.9, 96.0, 96.3, 114.7, 134.8, 135.6, 138.7, 140.0, 140.6, 141.5, 142.2, 143.8, 144.1, 145.5, 145.7, 146.3; HRMS m/z 608.3353, calcd for $C_{37}H_{44}O_4N_4$ 608.3363.

Ferric *meso*-Methylmesoheme Regioisomers. Conversion of the dimethyl esters of the *meso*-methylmesoporphyrin IX regioisomers to the corresponding hemes was done by hydrolyzing the dimethyl esters in 25% (v/v) HCl at 25 °C in the dark for 8 h.[12] The hydrolysis reaction was worked up by neutralizing to pH 4, extracting with CH_2Cl_2, washing the organic phase with water before drying it with anhydrous Na_2SO_4, and removing the solvent under vacuum. The porphyrins were used without further purification for the subsequent reaction in which the iron was inserted by the ferrous sulfate method.[13] An argon-purged, saturated aqueous Fe_2SO_4 solution (1 mL) was added to a stirred, argon-purged solution of the porphyrins in 1 mL of pyridine and 20 mL of acetic acid. The mixture was warmed for 10 min under a stream of argon. The stirred reaction mixture was then exposed to the air while it cooled to promote autooxidative formation of the ferric state before it was combined with 25 mL of brine and extracted with diethyl ether. The organic layer was washed with 25% HCl to remove unreacted porphyrin, washed with water, and dried over Na_2SO_4. Removal of the solvent under vacuum provided the *meso*-methylmesoheme regioisomers, which were purified by reversed-phase HPLC on a Whatman Partisil 10 semi-preparative ODS-3 column at 5 mL/min using 100% solvent A (55:40:10 acetonitrile:water:acetic acid). The *meso*-methylmesohemes had poorly resolved retention times between 9.9 and 10.8 min and by HPLC were >95% pure.

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Supplementary Material Available: ^{13}C NMR spectra of **5a–d** and **6a–d** (8 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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