yield) as a green amorphous powder: IR (KBr) 3290, 3058, 2960, 2928, 2860, 1394, 1044 cm^{-1}; UV (THF) 778 (ϵ 132000), 746 (ϵ 98300), 674 (ϵ 31900), 430 (ϵ 40700), 374 (ϵ 60800), 346 (ϵ 62800); ^1H NMR (300 MHz, benzene-d_6) -4.30 to -2.40 (2 H, m), 0.89 (3 H, t), 1.18-1.85 (60 H, m), 2.00 (2 H, br s), 3.55 (2 H, br s), 7.35-8.62 (20 H, m); MS m/z 947 (M^+, 100), 950 (25). Anal. Calcd for $C_{80}H_{89}N_7$: C, 83.13; H, 8.32; N, 8.49. Found: C, 82.91; H, 8.04; N, 8.63.

Acknowledgment. Financial support by the Natural Sciences and Engineering Research Council of Canada is gratefully acknowledged. The work was also funded by the Midwest Center for Mass Specrometry, a National Science Foundation Regional Instrumentation Facility (Grant No. CHE-8620177).

Registry No. **2**, 574-93-6; **3**, 93673-00-8; **4**, 35984-93-1; **5**, 93672-98-1; **6**, 32703-80-3; **7**, 91-15-6; **8**, 693-03-8; **9**, 125440-57-5; **10**, 6921-34-2; **11**, 125451-93-6; **12**, 125440-58-6; **13**, 125451-90-3; **14**, 125440-61-1; **15**, 125440-59-7; **16**, 125440-62-2; **17**, 125451-91-4; **18**, 125451-92-5; **19**, 32703-82-5; **20**, 58687-99-3; **21**, 125440-60-0; magnesium phthalocyanine, 1661-03-6.

Isomeric Monoacetylmono(1-hydroxyethyl)deuteroporphyrins: Syntheses, Characterization, and Use for the Syntheses of Regioselectively Methyl- and Vinyl-Deuterated Hemins

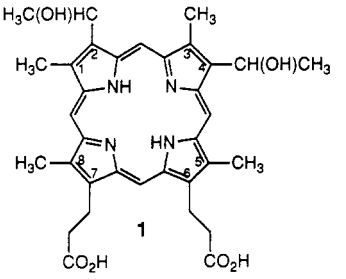
Fuu-Yau Shiau,[1] Ravindra K. Pandey,*[,2] S. Ramaprasad,[3] Thomas J. Dougherty,[4] and Kevin M. Smith*[,1]

Department of Chemistry, University of California, Davis, California 95616, Oncologic Foundation of Buffalo, 225 Oak Street, Suite 200, Buffalo, New York 14203, Department of Radiation Medicine, Roswell Park Memorial Institute, Buffalo, New York 14263, and Departments of Radiology and Pathology, 4301, West Markham Slot 582, University of Arkansas for Medical Sciences, Little Rock, Arkansas 72205

Received September 28, 1989

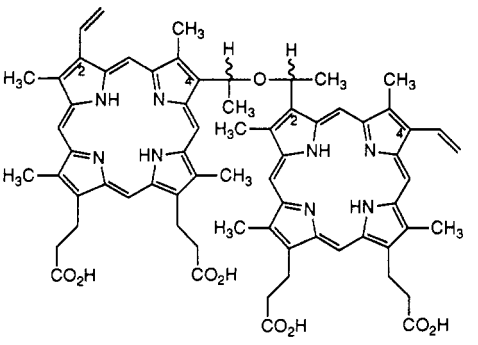
Treatment of hematoporphyrin IX dimethyl ester (**7**) with tetrapropylammonium perruthenate ($Pr^n_4N)(RuO_4$) and *N*-methylmorpholine *N*-oxide affords a high yield of the separable monoacetylmono(1-hydroxyethyl)-deuteroporphyrin isomers **5** and **6**. Proton NMR NOE experiments and chemical transformations involving specific individual deuteration at the 1- and 3-methyls and 2- and 4-vinyls are used to characterize the isomers.

Photodynamic therapy (PDT) is an experimental cancer treatment modality which selectively destroys cancer cells by interaction of light with a photosensitizing dye, presumably to form singlet oxygen.[5] Some porphyrins have been shown to be particularly effective sensitizers in this regard, and Photofrin II, a purified version of hematoporphyrin derivative which localizes in tumors, is currently in phase III clinical trials. The active constituent in Photofrin II appears to be an ether-ester linked oligmer containing between two and six hematoporphyrin (**1**) units.[6-8]



1

In our continuing efforts to characterize Photofrin II, and in the hope of preparing unique pure compounds contained in the active fraction of this drug, we have synthesized a number of dimers and trimers with both ether[9] and ester linkages.[10] In our animal tumor models, dimers with ester linkages were found to be biologically inactive,[10] while dimers (e.g. **2**) and trimers with ether linkages between positions 2 and 4 in **1** showed significant tumorcidal activity.[11] Our preliminary synthetic studies (Scheme I) utilized partial reduction of the acetyls in 2,4-diacetyldeuteroporphyrin IX dimethyl ester (**3**) and resulted in isolation of dimers (e.g. **4**) and trimers which were mixtures of regio- and stereoisomers at the 2- and 4-positions;[9-11] synthesis of a pure dimer or trimer, on the other hand, requires ready availability of large quantities of isomerically pure porphyrin monoacetylmono(1-hydroxyethyl)porphyrin isomers **5** and **6**.



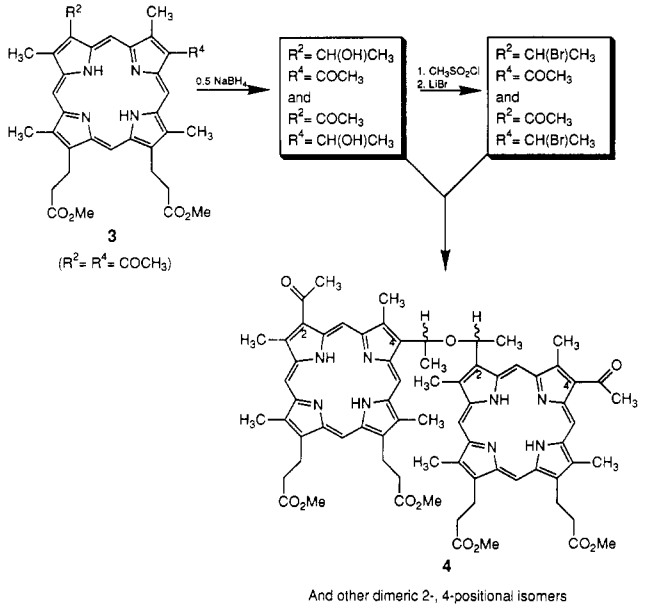
2

And other 2-, 4-connected ether positional isomers

(1) University of California.
(2) Oncologic Foundation of Buffalo.
(3) University of Arkansas for Medical Sciences.
(4) Roswell Park Memorial Institute.
(5) Weishaupt, K. R.; Gomer, C. J.; Dougherty, T. J. *Cancer Res.* **1976**, *36*, 2326.
(6) Dougherty, T. J.; Potter, W. R.; Weishaupt, K. R. *Adv. Med. Biol.* **1984**, 301.
(7) Kessel, D.; Thompson, P.; Musselman, B.; Chang, C. K. *Cancer Res.* **1987**, *47*, 4642.
(8) Kessel, D. *Photochem. Photobiol.* **1986**, *44*, 193.
(9) Pandey, R. K.; Dougherty, T. J.; Smith, K. M. *Tetrahedron Lett.* **1988**, *29*, 4657.
(10) Pandey, R. K.; Dougherty, T. J. *Cancer Res.* **1989**, *49*, 2042.
(11) Pandey, R. K.; Majchrzycki, D. A.; Smith, K. M.; Dougherty, T. J. *SPIE Proc.* **1989**, *1065*, 164.

0022-3263/90/1955-2190$02.50/0

Scheme I. Method for Preparation[9] of a Mixture of Ether-Linked Porphyrin Dimers Related to Photofrin-II

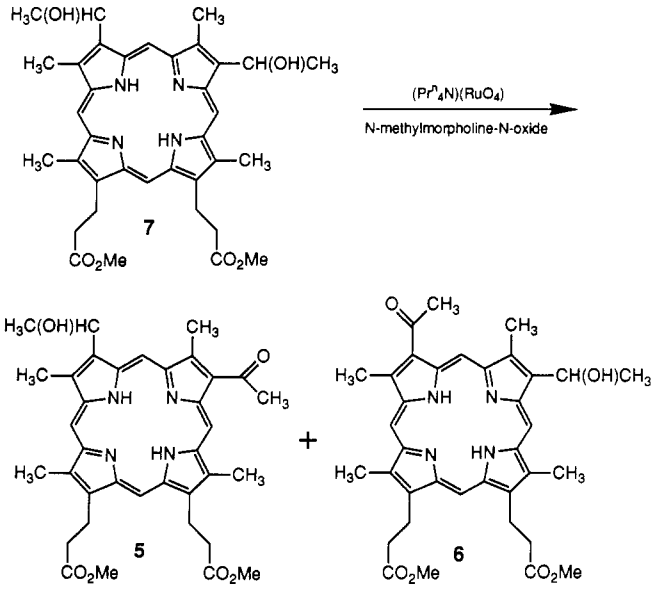


The compounds **5** and **6** have previously been prepared by Clezy and co-workers.[12] Our own earlier syntheses of **5** and **6** made use of partial reduction of 2,4-diacetyldeuteroporphyrin IX dimethyl ester (**3**) (Scheme I)[9] and separation of the mixture of **5** and **6** from (over-reduced) hematoporphyrin IX dimethyl ester (**7**) and unconsumed starting material **3**; the mixed monoacetyldeuteroporphyrins were used for dimer and trimer synthesis without separation.[9] Synthesis of pure dimers and trimeric photosensitizers related to Photofrin II requires, as a next step, the separation and characterization of the key building blocks **5** and **6**, and the present paper addresses that problem.

Synthesis of 5 and 6 from Hematoporphyrin IX Dimethyl Ester (7). As a result of Ley and co-worker's recent description of the use of tetrapropylammonium perruthenate ($Pr^n_4N)(RuO_4$) and *N*-methylmorpholine *N*-oxide for oxidation of alcohols to aldehydes and ketones,[13] we decided to apply the same methodology to controlled partial oxidation of hematoporphyrin IX dimethyl ester (**7**) to give **5** and **6** (Scheme II). A 47% yield of the required mixture of **5** and **6** was readily obtained by use of 0.1, and 3.2 equiv of perruthenate and *N*-methylmorpholine *N*-oxide, respectively, on the 100-mg scale. Access to these larger quantities led us to attempt separation of the two monoacetylmono(1-hydroxyethyl) isomers, and this was accomplished using silica gel thick-layer chromatography. Figure 1 shows the normal-phase HPLC separation of the two isomers, and assuming equal absorptivity at the wavelength of the detector (405 nm) indicates slightly unequal formation of the two isomers (45:55). Differential reactivity at the 2- and 4-positions in deuteroporphyrin IX derivatives has previously been noted.[14] The total yield of the two separated isomers **5** and **6** was 42%.

Traditional attempts to establish the unique isomeric identities of the two chromatographically separated fractions using melting point comparisons were confusing. Clezy and co-workers reported[12] melting points of 229–230 and 266–268 °C for **5** and **6**, respectively. By contrast, the melting points of our two pure separated fractions were 230–232 and 247–249 °C, and it was not completely clear which was which. We therefore resorted to proton NMR nuclear Overhauser enhancement (NOE) techniques as well as methyl and vinyl deuteration experiments to solve the problem.

Scheme II. Controlled Tetrapropylammonium Perruthenate/*N*-Methylmorpholine *N*-Oxide Oxidation of Hematoporphyrin IX Dimethyl Ester (7) To Give the Monoacetyl Isomers 5 and 6



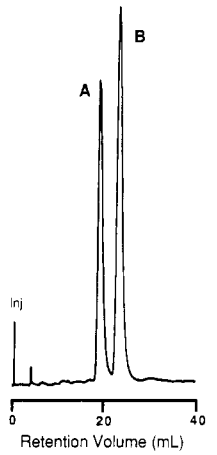


Figure 1. HPLC trace of mixture of **5** and **6** isolated from tetrapropylammonium perruthenate and *N*-methylmorpholine *N*-oxide oxidation of hematoporphyrin IX dimethyl ester (**7**). HPLC conditions: Waters Associates μPorasil 10-μm normal-phase stainless steel column (30 cm × 3.9 mm i.d.), 3% tetrahydrofuran in dichloromethane, 1.5 mL/min, Waters Associates 510 pump, 600E multisolvent delivery system, and 490E programmable multiwavelength detector set at 405 nm.

Nuclear Overhauser Enhancement Studies. There has been a continued interest in the application of NOE techniques for structure determination in porphyrin systems.[15] An unambiguous assignment of all meso protons is crucial for the structure determination studies. The two isomers are referred to, initially, on the basis of their

(12) Clezy, P. S.; Hai, T. T.; Henderson, R. W.; Thuc, L. *Aust. J. Chem.* **1980**, *33*, 585.
(13) Griffith, W. P.; Ley, S. V.; Whitcombe, G. P.; White, A. D. *J. Chem. Soc., Chem. Commun.* **1988**, 1625. See also: *Aldrichimica Acta* **1988**, *21*, 16.
(14) Smith, K. M.; Fujinari, E. M.; Langry, K. C.; Parish, D. W.; Tabba, H. D. *J. Am. Chem. Soc.* **1983**, *105*, 6638.
(15) Noggle, J. H.; Schirmer, R. E. *The Nuclear Overhauser Effect*; Academic Press: New York, 1971.

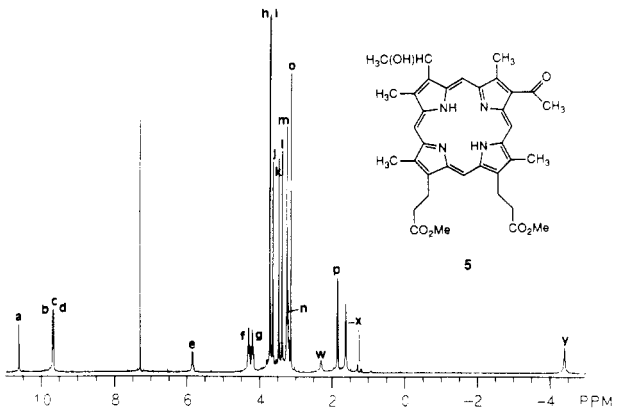


Figure 2. Proton NMR spectrum, at 300 MHz in $CDCl_3$, of monoacetylporphyrin **5**. Annotations a–p correspond with those discussed in text; w = $CH(OH)CH_3$, x = impurity, y = NH.

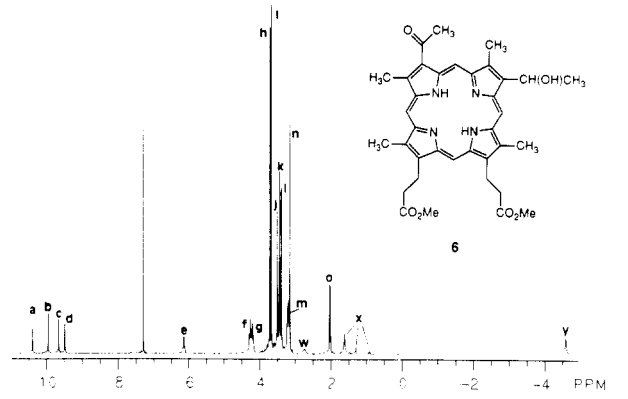


Figure 3. Proton NMR spectrum, at 300 MHz in $CDCl_3$, of monoacetylporphyrin **6**. Annotations a–o correspond with those discussed in text; w = $CH(OH)CH_3$, x = impurity, y = NH.

chromatographic behavior, with band A (Figure 1) being the faster running. Proton NMR spectra of band A and band B are shown in Figures 2 and 3, respectively. For both chromatographic bands, only the γ-meso resonance can be assigned with certainty, and this was peak c in both cases. The doublet methyl peak in the 1.8–2.0 ppm region was easily assigned to $CH(OH)CH_3$; peak e with single proton intensity and a quartet splitting pattern is likewise assigned to the $CH(OH)CH_3$. From Clezy's work, moreover, the peak at 3.1 ppm can be assigned to the methyl of the acetyl group. Peaks h, i (band A) and h, i (band B), which showed no NOEs, are assigned to the 6- and 7-methoxyls in each isomer (in no particular order).

With the above assignments as a basis, the remaining peaks in band A were assigned. The peaks j, k, l, and m are ring methyls (integrated intensities). Since both k and l showed NOEs to the same meso proton d, these must be the 1- and 8-methyls. Peak l also showed a NOE to e, from the $CH(OH)CH_3$ moiety. Thus, the (1-hydroxyethyl) group must be located at position 2, and peak l is the 1-CH_3. NOE connectivities between between g and k lead to assignment of g as the CH_2CH_2CO of the 7-propionate; the NOE between f (6-propionate) and j enable the latter to be assigned as the 5-CH_3. The complete set of NOE connectivities for band A (chromatographically faster-moving), 4-acetyl-2-(1-hydroxyethyl)deuteroporphyrin IX dimethyl ester, **5**, are provided in Figure 4.

Assignments for band B were not trivial, nor were they unambiguous because only a limited number of NOE connectivities were observed. Peak o was assigned to the methyl of the $CH(OH)CH_3$, and peak n at 3.13 ppm is the resonance from the acetyl CH_3. With the assignment of

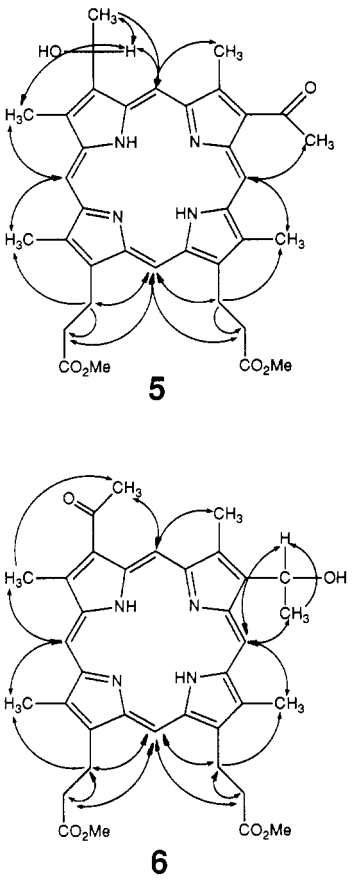


Figure 4. Networks of NOE connectivities for porphyrins **5** and **6**.

h and i as the methoxyls, peaks j, k, and l must be the ring methyls. Both j and k showed a NOE to peak d, the δ-meso proton, but j also showed an enhancement to peak n, the acetyl methyl. Thus, j must be the 1-CH_3 and the acetyl must be at the 2-position. The complete set of NOE connectivities for band B (chromatographically slower moving), 2-acetyl-4-(1-hydroxyethyl)deuteroporphyrin IX dimethyl ester, **6**, are provided in Figure 4.

Structural Assignments of 5 and 6 by Regioselective Deuteration: New Syntheses of 1- and 3-CD_3-Protohemins and Selectively 2- and 4-Vinyl-Deuterated Hemins. Though we regarded the NOE assignments to be definitive, at least for band A, we recognized the opportunity to use compounds **5** and **6** for synthesis of specifically methyl-deuterated and vinyl-deuterated protohemins, while at the same time using the known assignments of the 1- and 3-methyls in protohemin to confirm the results from the NOE study. Because of the paramagnetic nature of the hemins, proton NMR spectroscopy can be used as a diagnostic tool to study structure–function relationships in heme proteins, provided that unambiguous resonance assignments can be made.[16] Definitive assignments of proton NMR peaks in a number of heme proteins have been made in recent years by use of deuterated derivatives of protohemin, and reconstitution of these compounds with apoproteins.[17] A number of interesting phenomena, including that of heme orientational heterogeneity, for example, in myoglobins[18] and

(16) La Mar, G. N. In *Biological Applications of Magnetic Resonance*; Shulman, R. G., Ed.; Academic Press: New York, 1979; pp 305–343.
(17) E.g.: Smith, K. M. *Acc. Chem. Res.* **1979**, *12*, 374. Smith, K. M. *Rev. Port. Chem.* **1983**, *25*, 138.
(18) La Mar, G. N.; Budd, D. L.; Viscio, D. B.; Smith, K. M.; Langry, K. C. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 5755.

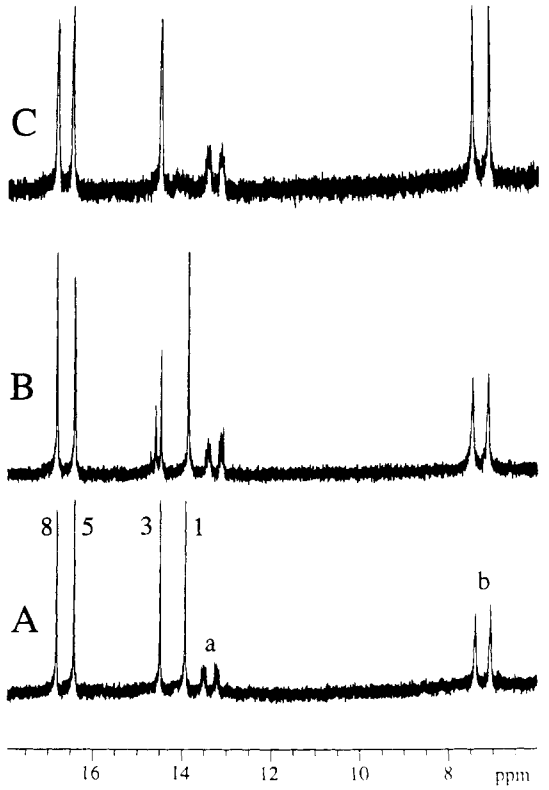
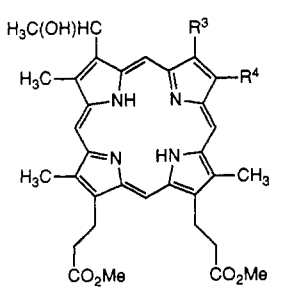


Figure 5. Proton NMR spectra, at 300 MHz in methanol-d_4, of low-field region of A, dicyanoferriprotoporphyrin IX dimethyl ester; B, [3-CD_3]dicyanoferriprotoporphyrin IX dimethyl ester, **12**; C, [1-CD_3]dicyanoferriprotoporphyrin IX dimethyl ester, **13**. Assignments: 8-, 5-, 3-, 1-methyls; a, α-vinyl CH; b, propionate CH_2CH_2CO. Peaks to low field in the 3-methyl region of B are due to presence of CHD_2 and CD_2H components. Differences in degree of deuteration between B (3-methyl) and C (1-methyl) are associated with time of reaction and speed of workup, both of which are not optimized.

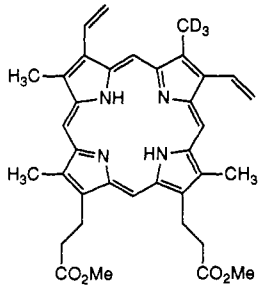
hemoglobins[19] have been investigated.

Methyl-deuterated hemes were originally obtained by total synthesis,[20] but subsequent discovery of a number of novel proton–deuteron exchange processes[14,21] have enabled methyls and vinyls to be regioselectively labeled without resort to time-consuming total synthesis. In particular, it has been shown that acetylporphyrins can be used for both methyl and vinyl isotope exchange,[14,21] and availability of monoacetylporphyrins (e.g. **5** and/or **6**), which can potentially be converted into protoporphyrin IX with ease,[22,23] should provide a very convenient access to a number of valuable labeled hemins.

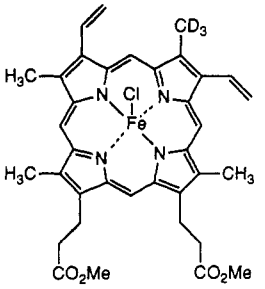
Thus, treatment of porphyrin **5** with methoxide in methanol-*O*-*d* gave the labeled porphyrin **8**, which was treated with sulfuric acid in methanol (to replace any cleaved esters and to back-exchange the acetyl CD_3 to CH_3, affording **9**) and then reduced with sodium borohydride to give the monolabeled hematoporphyrin IX dimethyl ester **10** in 70% yield. Dehydration using *p*-toluenesulfonic acid in *o*-dichlorobenzene gave a 75% yield of the protoporphyrin IX dimethyl ester **11**. Iron insertion using the ferrous chloride method[14] provided the hemin dimethyl ester **12**. Similarly, the hemin ester **13** was prepared from porphyrin **6** via the porphyrins **14**–**17**. Figure 5 shows the proton NMR spectra of the low spin cyanoferrihemin esters from **12** and **13**, as well as the corresponding spectrum of the unlabeled hemin (Figure 5A). Regioselective labeling of the previously assigned[24] 3-methyl starting from compound **5** (identified by NOEs, as above) clearly confirms the NMR assignment of this isomer as the 4-acetyl-2-(1-hydroxyethyl) derivative.



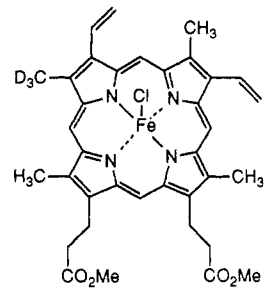
5 $R^3 = CH_3$; $R^4 = COCH_3$
8 $R^3 = CD_3$; $R^4 = COCD_3$
9 $R^3 = CD_3$; $R^4 = COCH_3$
10 $R^3 = CD_3$; $R^4 = CH(OH)CH_3$



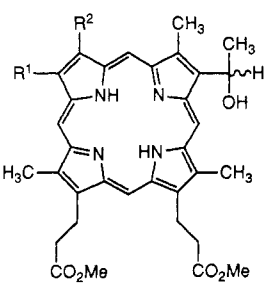
11



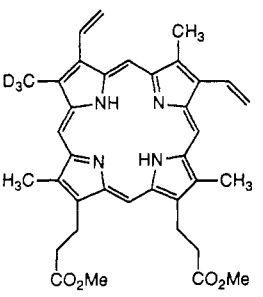
12



13



6 $R^1 = CH_3$; $R^2 = COCH_3$
14 $R^1 = CD_3$; $R^2 = COCD_3$
15 $R^1 = CD_3$; $R^2 = COCH_3$
16 $R^1 = CD_3$; $R^2 = CH(OH)CH_3$



17

Treatment of compound **5** with D_2SO_4 in methanol-d_1 and subsequently with DCl in D_2O gave the 4-acetyl-labeled material **18** in good yield. Sodium borohydride reduction gave **19**, and dehydration, as described above, gave the 4-vinyl-deuterated derivative **20** of protoporphyrin IX dimethyl ester, which was chelated with iron in the normal way to give the hemin **21**; in a similar fashion, compound **6** was transformed into hemin **22** via **23**, hematoporphyrin **24**, and the protoporphyrin dimethyl ester **25**. Figure 6 shows the high-field (vinyl CH_2) and low-field (vinyl CH) regions in the proton NMR spectra of the two labeled

(19) La Mar, G. N.; Yamamoto, Y.; Jue, T.; Smith, K. M.; Pandey, R. K. *Biochemistry* **1985**, *24*, 3826.
(20) Cavaleiro, J. A. S.; Rocha Gonsalves, A. M. d'A.; Kenner, G. W.; Smith, K. M. *J. Chem. Soc., Perkin Trans. 1* **1974**, 1771.
(21) Evans, B.; Smith, K. M.; La Mar, G. N.; Viscio, D. B. *J. Am. Chem. Soc.* **1977**, *99*, 7070. Smith, K. M.; Parish, D. W.; Inouye, W. S. *J. Org. Chem.* **1986**, *51*, 666. Smith, K. M.; Miura, M.; Morris, I. K. *J. Org. Chem.* **1986**, *51*, 4660. Smith, K. M.; Leung, H.-K.; Parish, D. W. *J. Chem. Res. (S)* **1986**, 324.
(22) Fuhrhop, J.-H.; Smith, K. M. In *Porphyrins and Metalloporphyrins*; Smith, K. M., Ed.; Elsevier: Amsterdam, 1975; Chapter 19.
(23) Smith, K. M.; Cavaleiro, J. A. S. *Heterocycles* **1987**, *26*, 1947.
(24) Cavaleiro, J. A. S.; Rocha Gonsalves, A. M. d'A.; Kenner, G. W.; Smith, K. M.; Shulman, R. G.; Mayer, A.; Yamane, T. *J. Chem. Soc., Chem. Commun.* **1974**, 392.

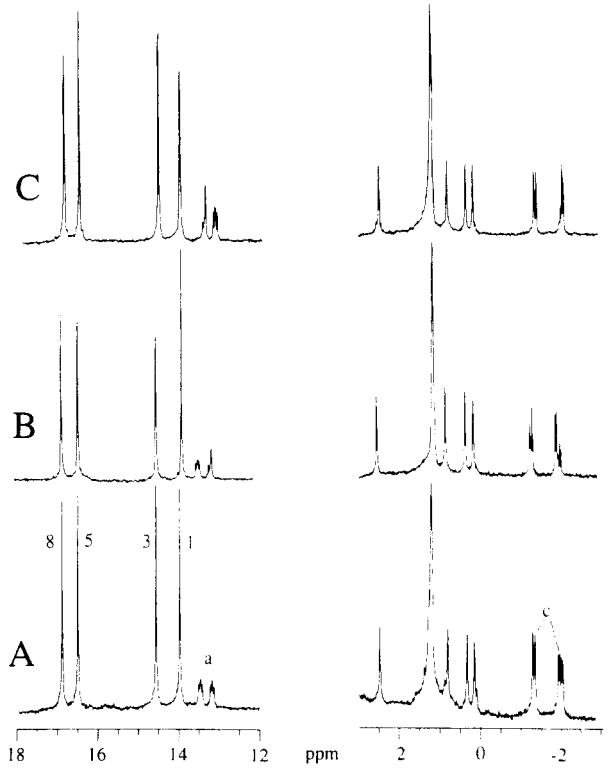


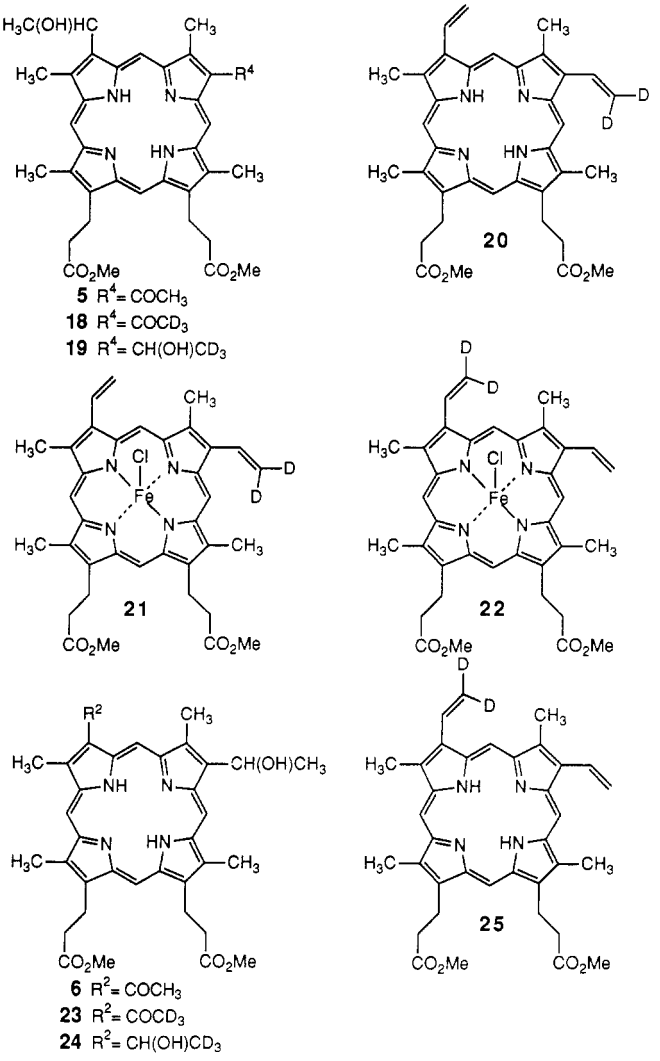
Figure 6. Proton NMR spectra, at 300 MHz in methanol-d_4, of methyl and vinyl CH_2 regions of A, dicyanoferriprotoporphyrin IX dimethyl ester; B, [4-β-CD_2]dicyanoferriprotoporphyrin IX dimethyl ester, **21**; C, [2-β-CD_2]dicyanoferriprotoporphyrin IX dimethyl ester, **22**. Assignments: 8-, 5-, 3-, 1-methyls; a, α-vinyl CH; c, β-vinyl CH_2. Differences in degree of deuteration between B (4-vinyl) and C (2-vinyl) are associated with time of reaction and speed of workup, both of which are not optimized.

cyanoferrihemins **21** and **22**, as well as the corresponding regions for unlabeled hemin as Figure 6A. The regioselective vinyl-labeling procedures described above represent a considerable improvement over a previous multistep synthesis of **21**.[14]

Experimental Section

General. Melting points are uncorrected and were measured on a Thomas/Bristoline hot stage. Electronic absorption spectra were measured on a Hewlett-Packard 8450A spectrophotometer using solutions in dichloromethane. Routine proton NMR spectra (^{1}H NMR) were obtained in $CDCl_3$ either at 90 MHz (Varian EM390) or 300 MHz (GE QE300) with chemical shifts reported in ppm relative to internal standards of tetramethylsilane (0 ppm, 90-MHz spectra) or chloroform (7.258 ppm, 300 MHz). Reactions were usually carried out in the dark (aluminum foil) under nitrogen and were monitored using thin-layer chromatography (TLC) on commercially available Eastman-Kodak 13181 (100 μm thick) silica sheets. Gravity and flash column chromatography employed either Merck neutral alumina (70–230 mesh) or Merck silica gel 60. The alumina was usually deactivated with 6% water (Brockmann Grade III) before use.

The 1D NOE measurements were performed on a GE GN300 (5-mm probe) instrument using 16K data points over a 5–6-kHz bandwidth. Samples of approximately 5 mg were dissolved in 0.5 mL of $CDCl_3$, and the residual $CHCl_3$ peak at 7.258 ppm was used as a chemical shift reference. A low power decoupler saturation pulse in the range of 0.2–0.3 W was used for a duration of 2 s to obtain steady-state NOEs. The decoupler was systematically stepped through all the required frequencies including a reference frequency shifted away from the spectral region of interest. Sixteen scans were collected for each saturation frequency before stepping to the next frequency, and the whole process was repeated over the required number of cycles to average out instrumental artifacts for all the spectra. The data were analyzed after obtaining difference spectra and were checked for reproducibility. The 1D spectra for compounds **5** and **6** are shown in Figure 2 and 3. The complete NOE networks for both compounds are given in Figure 4.



2-Acetyl-4-(1-hydroxyethyl)deuteroporphyrin IX Dimethyl Ester (6) and 4-Acetyl-2-(1-hydroxyethyl)deuteroporphyrin IX Dimethyl Ester (5). Hematoporphyrin IX dimethyl ester (**7**, 100 mg) [obtained by methanol/HCl(g) esterification of commercially available hematoporphyrin IX (**1**)] was mixed with *N*-methylmorpholine N-oxide (60 mg) dissolved in dichloromethane (25 mL) and stirred at room temperature under nitrogen for 10 min. Tetrapropylammonium perruthenate (10 mg) was added, and the mixture was stirred for 15 min, after which time TLC analysis indicated formation of a small amount of 2,4-diacetyldeuteroporphyrin IX dimethyl ester (**3**). The mixture was washed immediately with water (2 × 100 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was chromatographed on silica gel, eluting initially with dichloromethane (to remove excess *N*-methylmorpholine *N*-oxide) and then with 1% methanol in dichloromethane. The major fraction was collected, the solvent was evaporated to dryness [to give 47 mg (47% yield) of admixed **5** and **6**], and the residue was separated on a silica gel thick-layer plate (20 × 20 cm) eluting with 4% tetrahydrofuran in dichloromethane by repeated development until two clearly separated bands were apparent. Both isomers were crystallized from dichloromethane/*n*-hexane. (A) Fast running band, band A, 4-acetyl-2-(1-hydroxyethyl)deuteroporphyrin IX dimethyl ester (**5**): 18 mg (18% yield from **7**); mp 230–232 °C (lit.[12] mp 229–230 °C); ^{1}H NMR spectrum, Figure 2 δ, ppm, 10.59 (a), 9.64 (b, c) (each s, 1 H, meso-H), 9.62 (d) (s, 2 H, meso-H), 5.80 (q, 1 H, C*H*(OH)Me, e), 4.26 (f), 4.17 (g) (each t, 2 H, CH_2CH_2CO), 3.69 (h), 3.68 (i) (each s, 3 H, OMe), 3.61 (j), 3.44 (k), 3.34 (l), 3.20 (m) (each s, 3 H, ring Me), 3.19 (m, 4 H, CH_2CH_2CO, n), 3.10 (s, 3 H, COMe, o), 2.27 (s, 1 H, CH(O*H*)Me, w), 1.80 (d, 3 H, CH(OH)*Me*, p), −4.44

(s, 2 H, NH, y); λ_{max} 406 nm (ε 165 700), 508 (9100), 548 (10 400), 576 (7600), 636 (1500). (B) Slow running band, band B, 2-acetyl-4-(1-hydroxyethyl)deuteroporphyrin IX dimethyl ester (**6**): 24 mg (24% yield from **7**); mp 247–249 °C (lit.[12] mp 266–268 °C); ^{1}H NMR spectrum, Figure 3 δ, ppm, 10.37 (a), 9.92 (b), 9.63 (c), 9.45 (d) (each s, 1 H, meso-H), 6.10 (q, 1 H, C*H*(OH)Me, e), 4.18 (f), 4.25 (g) (each t, 2 H, CH_2CH_2CO), 3.70 (h), 3.66 (i) (each s, 3 H, OMe), 3.47 (j), 3.36 (l) (each s, 3 H, ring Me), 3.40 (s, 6 H, ring Me, k), 3.19 (m, 4 H, CH_2CH_2CO, m), 3.13 (s, 3 H, COMe, n), 2.72 (s, 1 H, CH(O*H*)Me, w), 2.00 (d, 3 H, CH(OH)*Me*, o), −4.54 (s, 2 H, NH, y); λ_{max} 408 nm (ε 164 200), 508 (9200), 546 (10 500), 576 (7500), 630 (1900).

3-(Trideuteriomethyl)protoporphyrin IX Dimethyl Ester (11). 4-Acetyl-2-(1-hydroxyethyl)deuteroporphyin IX dimethyl ester (band A, **5**, 25 mg) was dissolved in tetrahydrofuran (15 mL) and dichloromethane (5 mL). This solution was heated to reflux, and then sodium methoxide (20 mg) in methanol-d_1 (3 mL) was added dropwise before further heating of the mixture under dry nitrogen in the dark for 20 h. The mixture was then cooled, diluted with 90:10 dichloromethane/tetrahydrofuran (50 mL), and then washed with water (3 × 100 mL). The organic phase was dried over anhydrous Na_2SO_4 and evaporated to dryness to give **8**, which was dissolved in 10% sulfuric acid in methanol (8 mL) and allowed to stand overnight. The mixture was diluted with dichloromethane (150 mL), washed with water (3 × 300 mL), dried over anhydrous Na_2SO_4, and evaporated to dryness to give **9**. The residue was purified by thick-layer chromatography on silica gel, eluting with 3% methanol in dichloromethane, and the resulting porphyrin was crystallized from dichloromethane/*n*-hexane to give 17 mg of **9**, which was dissolved in dichloromethane (10 mL) and treated with ice-cold methanol (4 mL) containing sodium borohydride (50 mg). The mixture was stirred at room temperature for 10 min, diluted with dichloromethane, and then washed with 0.05 N HCl (20 mL). It was then washed with water (3 × 50 mL) and, after drying over anhydrous Na_2SO_4, evaporated to dryness to give **10**. The residue was dissolved in *o*-dichlorobenzene (5 mL) containing *p*-toluenesulfonic acid hydrate (70 mg) and heated at 145 °C for 45 min under nitrogen in the dark. The mixture was cooled to room temperature, diluted with dichloromethane (50 mL), and washed with water (3 × 100 mL). The organic phase was evaporated, and the residue, in dichloromethane (10 mL), was treated with excess ethereal diazomethane. After evaporation to dryness, the residue was chromatographed on silica gel, eluting with dichloromethane initially (to remove residual *o*-dichlorobenzene) and then with 5% methanol in dichloromethane. The major fraction was collected, the solvent was evaporated, and the residue was further purified on a thick-layer silica gel plate, eluting with 2% tetrahydrofuran in dichloromethane, and then crystallized from tetrahydrofuran/*n*-hexane to give 12 mg of the title compound, mp 193–195 °C (lit.[25] mp 228–229 °C, unlabeled). For Figure 5, iron was inserted to give **12** using the ferrous chloride method.[14]

1-(Trideuteriomethyl)protoporphyrin IX Dimethyl Ester (17). This compound (13 mg) was similarly prepared from 2-acetyl-4-(1-hydroxyethyl)deuteroporphyin IX dimethyl ester (band B, **5**, 20 mg) via **14**–**16**: mp 192–194 °C (lit.[25] mp 228–229 °C, unlabeled). For Figure 5, iron was inserted to give **13** using the ferrous chloride method.[14]

4-(2,2-Dideuteriovinyl)protoporphyrin IX Dimethyl Ester (20). 4-Acetyl-2-(1-hydroxyethyl)deuteroporphyin IX dimethyl ester (band A, **5**, 50 mg) was dissolved in methanol-d_1 (4 mL) containing concentrated D_2SO_4 (5 drops) and stirred overnight at room temperature. The mixture was diluted with dichloromethane (50 mL) and then washed quickly with water (2 × 100 mL). After drying over anhydrous Na_2SO_4 and evaporation, TLC indicated the presence of some dimethyl ketal, so the material was dissolved in tetrahydrofuran (10 mL) containing D_2O (1 mL) and 20% DCl in D_2O (5 drops) and allowed to stand at room temperature for 30 min. This mixture was then diluted with dichloromethane (50 mL), washed with water (3 × 100 mL), dried over anhydrous Na_2SO_4, and evaporated to give a residue (**18**), which was dissolved in dichloromethane (5 mL) and treated with excess ethereal diazomethane. After evaporation, the residue was dissolved in dichloromethane (10 mL) and treated with ice cold methanol (4 mL) containing sodium borohydride (50 mg). The mixture was stirred at room temperature for 1 h, diluted with dichloromethane (50 mL), and washed with 0.05 N HCl (10 mL) and then with water (3 × 50 mL). After being dried over anhydrous Na_2SO_4 and evaporated to dryness (to give **19**), the residue was dissolved in *o*-dichlorobenzene (10 mL) containing *p*-toluenesulfonic acid hydrate (150 mg) and heated at 145 °C for 45 min under nitrogen in the dark. The cooled mixture was diluted with dichloromethane (50 mL), washed with water (3 × 100 mL), and evaporated to dryness, and the residue in dichloromethane (10 mL) was treated with excess ethereal diazomethane and the immediately evaporated to dryness. The residue was chromatographed on silica gel, initially eluting with dichloromethane and then with 5% methanol in dichloromethane. The major red fraction was collected, the solvent was evaporated, and the residue was further purified on a thick-layer silica gel plate, eluting with 2% tetrahydrofuran in dichloromethane. The product was crystallized from tetrahydrofuran/*n*-hexane to give 25 mg of **20**, mp 199–201 °C (lit.[25] mp 228–229 °C, unlabeled). For Figure 6, iron was inserted to give **21** using the ferrous chloride method.[14]

2-(2,2-Dideuteriovinyl)protoporphyrin IX Dimethyl Ester (25). This compound (20 mg) was similarly prepared from 2-acetyl-4-(1-hydroxyethyl)deuteroporphyin IX dimethyl ester (band B, **6**, 35 mg) via **23** and **24**: mp 193–195 °C (lit.[25] mp 228–229 °C, unlabeled). For Figure 6, iron was inserted to give **22** using the ferrous chloride method.[14]

Acknowledgment. We thank the National Institutes of Health (HL-22252, K.M.S.) for support of this research. S.R. wishes to thank Dr. R. A. Komoroski (UAMS) for access to the GN-300 instrument and for helpful discussions.

Registry No. **5**, 66154-86-7; **6**, 66154-87-8; **7**, 32562-61-1; **8**, 125453-67-0; **9**, 125453-68-1; **10**, 125453-69-2; **11**, 105893-84-3; **12**, 125453-74-9; **13**, 125453-75-0; **17**, 105893-83-2; **18**, 125453-70-5; **19**, 125453-71-6; **20**, 125453-72-7; **21**, 125453-76-1; **22**, 125453-77-2; **25**, 125453-73-8; photofrin II, 87806-31-3.

(25) Carr, R. P.; Jackson, A. H.; Kenner, G. W.; Sach, G. S. *J. Chem. Soc. (C)* **1971**, 487.