Synthesis of the Heme *d* Prosthetic Group of Bacterial Terminal Oxidase

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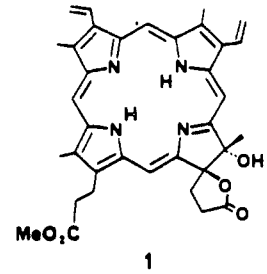
Abstract: The heme *d* prosthetic group of the *Escherichia coli* terminal oxidase has been proposed to be a hydroxychlorin γ-spirolactone. This "lactochlorin" structure has now been verified by total synthesis. Vicinal dihydroxylation of pyrrole ring C of protoporphyrin was accomplished by OsO_4 oxidation of 2,4-bis(2-chloroethyl)deuteroporphyrin followed by dehydrochlorination. Hydroxychlorins substituted with a geminal propionic ester group have a propensity to lactonize under general-base catalysis. Mild bases such as NaOAc cyclize the geminal groups without inversion of configuration while prolonged contact with silica gel invariably gives the trans diastereomer. ^1H NMR spectra have provided important information on the conformation of the cis and trans spirolactones. This work strongly supports the argument that the lactone structure of the isolated chromophore is an artifact. The true ligand structure of heme *d* in the enzyme is most likely 5,6-dihydroxyprotochlorin IX, the cis and trans isomers of which have also been synthesized.

As typical in many bacteria, the respiration chain of *Escherichia coli* contains two terminal oxidases, cytochrome *o* and cytochrome *d*. Cytochrome *o* prevails in the early exponential phase during aerobic growth of culture while cytochrome *d* becomes important in the late exponential phase, or when cells are grown under limiting oxygen supply.[1,2] The K_m value for oxygen of cytochrome *d* is about 8 times lower than that of cytochrome *o*.[3] The more efficient utilization of oxygen by cytochrome *d* presumably allows the microbes to maintain efficient oxidative energy conservation over a wide range of oxygen pressures by changing the relative ratio of the two oxidases. The oxygen binding site in the membrane-bound cytochrome *d* complex is a green heme prosthetic group displaying a prominent α-band near 630 nm. Keilin[4] originally designated the name "a_2" for this cytochrome absorbing in the red region; a_2 was later changed to *d* to avoid confusion with the aa_3 hemes of mammalian cytochrome oxidase. It should be made clear that cytochrome *d* does not reduce nitrite; it is different from the soluble cd_1-type oxidases[5,6] from *Pseudomonas aeruginosa* and *Paracoccus denitrificans*, which primarily function as nitrite reductase and are not components of the aerobic respiratory chain.

The green heme moiety of cytochrome *d* was first studied by Barrett (1956) who extracted cells of *Aerobacter aerogenes* and identified an iron chlorin core structure.[7] The site of saturation and the nature of the side chains on the chlorin macrocycle could not be determined at that time. Barrett suggested the possible presence of vinyl, hydroxyethyl, and propanoic acid substituents in an arrangement similar to a hydrated protoporphyrin IX. The tentatively formulated structure of Barrett remained unchallenged in the literature for almost 30 years and has served as the de facto model for many other green hemes[8] subsequently found in various bacterial cytochromes. Recently, Timkovich and collaborators[9] isolated sufficient amounts of the heme *d* prosthetic group from purified *E. coli* oxidase and characterized the structure of the metal-free, esterified chromophore by means of ^1H NMR, IR, UV-vis, and mass spectroscopy. The proposed structure comprises an unusual chlorin core with a γ-spirolactone group at the saturated pyrrole ring C (**1**). This "lactochlorin" structure is certainly



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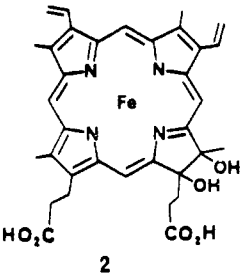

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unique, but as the authors noted, it is not clear whether the lactone ring found in the metal-free macrocycle is an authentic feature of the heme or whether it is an artifact formed during isolation. The evidence of the γ-lactone in the metal-free heme *d* was largely based on an intense IR absorption at ~1782 cm^{-1}. In a recent paper, however, the Timkovich group reported that this IR peak was not present in the extracted heme *d*,[10] thus supporting structure **2**. Considering the limitation imposed by the scarcity of natural material, we believe that a full-scale study of heme *d* as well as the confirmation of the proposed structure must rely upon organic syntheses. In this paper, we report the total synthesis of Timkovich's lactochlorin, its diastereomer, and the nonlactonized forms. The chemical reactivities inherent in these structures are also addressed.

Results and Discussion

A. Model Studies. During the past 2 years, we have been studying *vic*-dihydroxychlorins, and the principal results can be summarized here.[11-13] Dihydroxylation of the porphyrin β-β double bond can be routinely accomplished with osmium tetroxide. If the chlorin diol bears a geminal propionate ester side chain, it is unstable during chromatography, especially on a TLC plate. Given enough time, the initially slow-moving diol can completely change into a fast-moving green pigment. This new chlorin is the cyclized γ-spirolactone as evidenced by the characteristic strong IR band near 1783 cm^{-1}. It turns out that hydroxychlorins with

(1) (a) Lemberg, R.; Barrett, J. *Cytochromes*; Academic: New York, 1973; pp 8-14, 233-240. (b) Miller, M. J.; Gennis, R. B. *J. Biol. Chem.* **1983**, *258*, 9159-9165.
(2) Haddock, B. A.; Jones, C. W. *Bacteriol. Rev.* **1977**, *41*, 47-99.
(3) Kita, K.; Konishi, K.; Anraku, Y. *J. Biol. Chem.* **1984**, *259*, 3375-3381.
(4) Keilin, D. *Nature (London)* **1933**, *132*, 783.
(5) (a) Horio, T.; Higashi, T.; Yamanaka, T.; Matsubara, H.; Okunuki, K. *J. Biol. Chem.* **1961**, *236*, 944-949. (b) Timkovich, R.; Dhesi, R.; Martinkus, K. J.; Robinson, M. K.; Rea, T. M. *Arch. Biochem. Biophys.* **1982**, *215*, 47-58.
(6) (a) Chang, C. K. *J. Biol. Chem.* **1985**, *260*, 9520-9522. (b) Chang, C. K.; Wu, W. *J. Biol. Chem.* **1986**, *261*, 8593-8597. (c) Wu, W.; Chang, C. K. *J. Am. Chem. Soc.* **1987**, *109*, 3149-3150.
(7) Barrett, J. *Biochem. J.* **1956**, *64*, 626-639.
(8) For example, heme a_2 from *Pseudomonas* cyctochrome oxidase (now characterized as *dioneheme*): References 5 and 6. Catalase of *Neurospora crassa*: Jacob, G. S.; Orme-Johnson, W. H. *Biochemistry* **1979**, *18*, 2975-2980. Green heme from *Aspergillus niger*: Horie, S.; Watanabe, T.; Nakamura, S. *J. Biochem. (Tokyo)* **1976**, *80*, 579-593.
(9) Timkovich, R.; Cork, M. S.; Gennis, R. B.; Johnson, P. Y. *J. Am. Chem. Soc.* **1985**, *107*, 6069-6075.
(10) Vavra, M. R.; Timkovich, R.; Yap, F.; Gennis, R. B. *Arch. Biochem. Biophys.* **1986**, *250*, 461-468.
(11) (a) Chang, C. K.; Sotiriou, C. *J. Org. Chem.* **1985**, *50*, 4989-4991. (b) Chang, C. K.; Sotiriou, C. *J. Heterocycl. Chem.* **1985**, *22*, 1739-1742.
(12) Chang, C. K.; Sotiriou, C. *J. Org. Chem.* **1987**, *52*, 926-929.
(13) Andersson, L. A.; Sotiriou, C.; Chang, C.; Loehr, T. M. *J. Am. Chem. Soc.* **1987**, *109*, 258-264.

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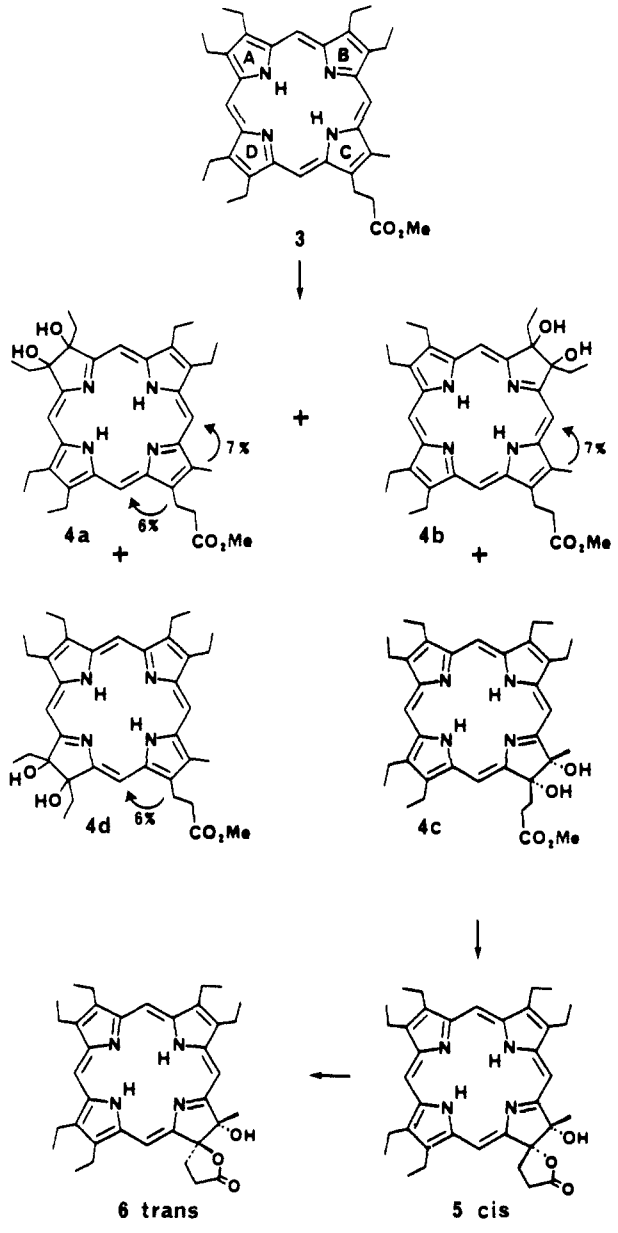
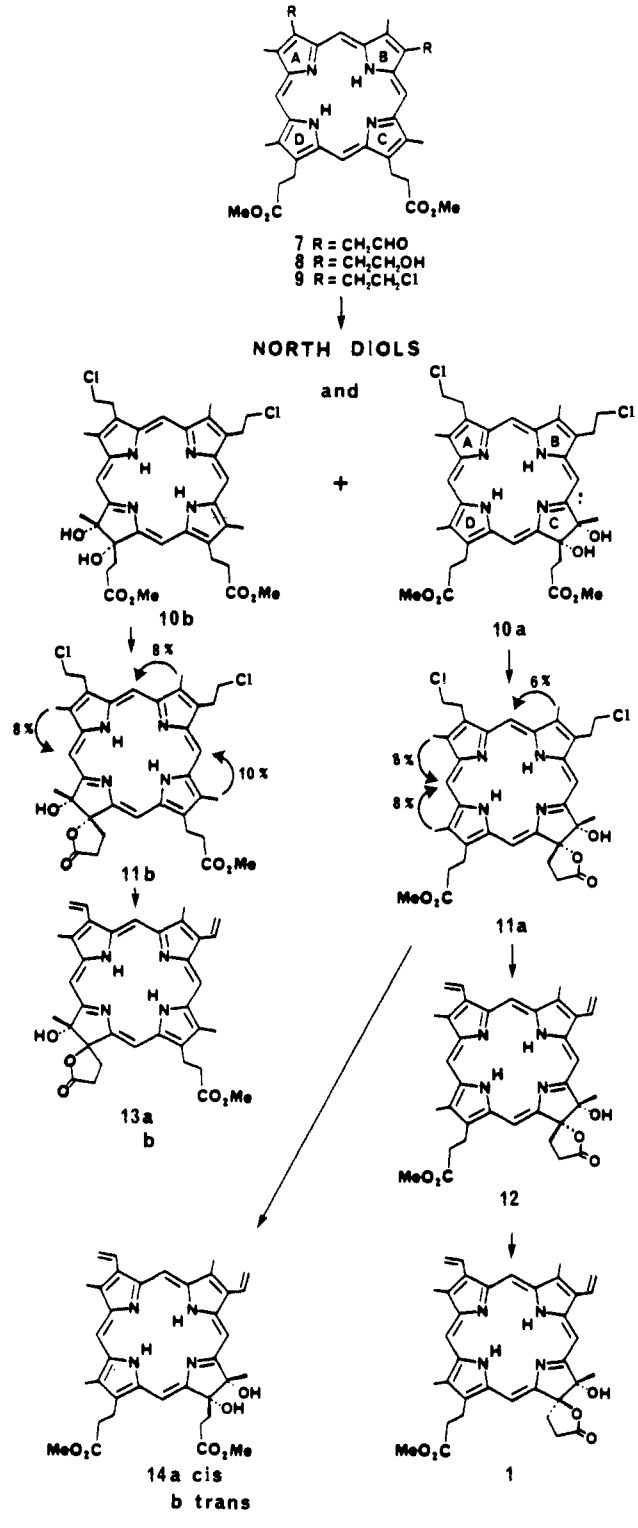
Scheme I



Scheme II



an angular propionate ester have a tendency to lactonize into the 5-membered ring also under the influence of general-base catalysis.[13] A variety of bases including pyridine and sodium acetate are effective to bring about this cyclization. However, preliminary results indicate that lactones resulting from bases are not identical with those cyclized by silica gel, a difference perhaps attributable to diastereomers.[13]

The above description can be examplified by the reaction of a model compound **3** in Scheme I; the reason for choosing this particular porphyrin will become evident in later discussion. Treatment of **3** with OsO_4–H_2S yielded a mixture of four isomeric chlorins, **4a–4d**, which can be separated by preparative TLC into three green bands. The two faster moving chlorins were characterized by ^1H NMR to be the two north diols, **4a** and **4b**; the site of saturation was unambiguously identified by nuclear Overhauser enhancements (NOE's), as indicated in Scheme I. The two south diols, contained in the slow-moving TLC band, were difficult to separate directly. Consequently, we recovered the mixture and heated it in CH_2Cl_2–MeOH with sodium acetate, and the cyclized **5** was then separated easily from **4d**. Lactone **5**, which has a strong IR peak at 1783 cm^{-1}, was found to undergo further changes during development on TLC plates; it slowly converted to another green compound possessing unchanged mass spectral and IR peaks. However, ^1H NMR of the two (**5** and **6**) were different, which eventually allowed us to deduce the configuration of the two lactone forms (vide infra). The more stable **6** could also be obtained directly by lactonizing diol **4c** under prolonged contact with silica gel.

B. Synthesis of Lactochlorin. The synthetic strategy, which was guided by the results of model compounds, is shown in Scheme II. We began with protoporphyrin, a probable precursor also in the biosynthesis of heme *d*. The reactive vinyl groups were protected as the chloroethyl side chains by oxidation with $Tl(NO_3)_3$ to the aldehyde **7**,[14] followed by reduction to **8** with $NaBH_4$ and

(14) Kenner, G. W.; McCombie, S. W.; Smith, K. M. *Justus Liebigs Ann. Chem.* **1973**, 1329–1338.

then by chlorination with PhCOCl–DMF[15] to **9**, each step giving essentially quantitative yield. Porphyrin **9** was treated with 1.3 equiv of OsO_4 for 1 day before being quenched with H_2S to effect dihydroxylation at the four possible sites: pyrrole ring A (6.8%), ring B (6.8%), ring C (22%), and ring D (26%). The separation of the north *vic*-diols from the south was easily accomplished by chromatography. The two south regioisomers were separated by TLC via the lactones cyclized with NaOAc. Structural assignments of **11a** and **11b** were based on NOE connectivities (key measurements are indicated by the structures in Scheme II). Regeneration of the vinyl side chains was brought about by heating **11a** in pyridine–30% KOH,[16] during which most of the lactone chlorin was hydrolyzed but some survived. The hydrolyzed chlorin was methylated with diazomethane to give a diol form **14a**. The dehydrated lactone **12** has all the structural elements of Timkovich's lactochlorin, yet it exhibited an ^1H NMR spectrum showing discrepancies in the lactone proton region as compared with that of the natural compound. An obvious explanation is that this compound does not have the correct configuration. Indeed, when this lactone was chromatographed on silica gel repeatedly, the expected isomeric chlorin emerged, which was shown by IR and mass spectra to be a γ-lactone; it exhibited ^1H NMR features basically indistinguishable from that of lactochlorin and showed a retention time in HPLC analyses identical with that of the natural compound. On the basis of their NMR spectra discussed below, the lactones **11a** and **12** were assigned a cis configuration (with respect to the two oxygen position) while the lactochlorin should have a trans configuration, as suggested by Timkovich.

1 could also be obtained by two other routes. If **11a** was first epimerized on silica gel and then dehydrochlorinated in base, **1** was obtained directly. Also during the base treatment, the majority of lactone was hydrolyzed to a diol **14b** (after methylation), which is different from **14a**. The two diols, shown by ^1H NMR to be cis and trans isomers, could then be lactonized to **12** or **1** under appropriate conditions.

When the regioisomer **10b** was subjected to the same reaction conditions described above, the cis and trans lactones, **13a** and **13b**, were obtained. These unnatural compounds provided us more variety of this class of chromophores and may be useful for probing the structure–function relationship in the heme protein.

C. ^1H NMR Spectra and Structure of the Chlorins. The two synthetic lactones **12** and **1** have distinctive ^1H NMR signatures, particularly in the 2.4–3.4 ppm region where the methylene protons of the γ-lactone appear. As shown in Figure 1A, at 250 MHz **1** has a spectrum almost identical with the published spectrum of lactochlorin.[9] Timkovich concluded the trans configuration based on the assumption that the single proton peaks around 2.4 ppm are from H4 (refer to Figure 2), which points away from the OH group and should have a chemical shift similar to those of α-methylene protons of normal pyrroline alkyl substituents. The peaks of H1, because of the large deshielding imparted by the nearby OH oxygen, should occur relatively downfield. The four protons were thus labeled as the following (ppm): H1, 3.169; H2, 3.032; H3, 3.231; H4, 2.431. However, these peak assignments and the reported simulation had a large degree of uncertainty due to the presence of overlapping peaks in this region. In fact, on close examination of Figure 1A and the spectra of the natural molecule, as well as several other synthetic analogues,[13] it becomes evident that there are peaks near 3.5 ppm (at the shoulder of the dominant 8-methyl singlet) that can be nothing but part of the lactone methylenes. This is illustrated by the spectrum of the model lactone **6** whose 4-spin system can be readily assigned and simulated (Figure 1B). Going back to the lactochlorin spectrum, we now assign H1 at 3.490 ppm and other parameters as tabulated in Table I, which fit the natural spectrum more closely in terms of relative heights of some of the resolved transitions and in explaining the weak signals overlapping with the 8-methyl.

(15) (a) Carr, R. P.; Jackson, A. H.; Kenner, G. W.; Sach, G. S. *J. Chem. Soc. C* **1971**, 487–502. (b) Clezy, P. S.; Fookes, C. J. R.; Sternhell, S. *Aust. J. Chem.* **1978**, *31*, 639–648. (c) Smith, K. M.; Fujinari, E. M.; Langry, K. C.; Parish, D. W.; Tabba, H. D. *J. Am. Chem. Soc.* **1983**, *105*, 6638–6646.
(16) Clezy, P. S.; Fookes, C. J. R. *Aust. J. Chem.* **1977**, *30*, 217–220.

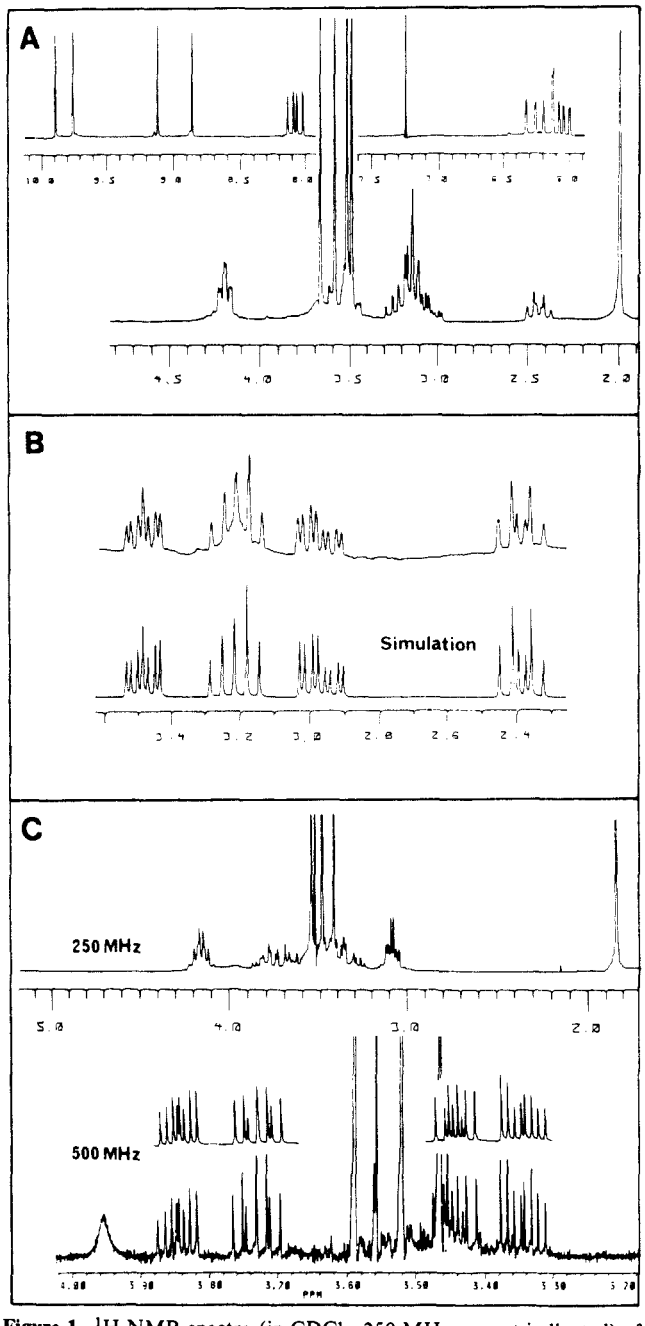


Figure 1. ^1H NMR spectra (in $CDCl_3$, 250 MHz, except indicated) of trans lactone **1** (A), hexaethyl trans lactone **6** (B), and cis lactone **12** (C).

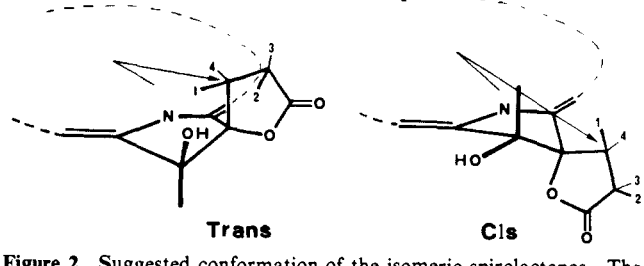


Figure 2. Suggested conformation of the isomeric spirolactones. The slope estimated for the trans isomer is 35° while that for the cis isomer is less than 10°.

The spectrum of the lactone isomer cyclized by NaOAc has very different features in the spectral region discussed above (Figure 1C). The most evident difference is that there is no lactone peaks lower than 3.3 ppm. The complex splitting pattern of the methylene protons is not discernible at 250 MHz but is reduced to first order at 500 MHz. The chemical shifts and parameters are listed in Table I. The lack of a large differentiation between

Table I. ^{1}H NMR Assignments for the Pyrroline Substituents of the Four Forms of 5,6-Dihydroxyprotochlorin

form	H	shift, ppm	coupling const, Hz
lactone			
trans	1	3.490	$J_{1,2}$ = 4.1, $J_{2,4}$ = 9.6
	3	3.213	$J_{1,3}$ = 9.6, $J_{3,4}$ = 9.6
	2	3.032	$J_{1,4}$ = −13.3
	4	2.430	$J_{2,3}$ = −17.9
	5-Me	1.992	
cis	4	3.846	$J_{1,2}$ = 7.4, $J_{2,4}$ = 9.8
	2	3.731	$J_{1,3}$ = 9.8, $J_{3,4}$ = 5.2
	1	3.443	$J_{1,4}$ = −13.3
	3	3.345	$J_{2,3}$ = −17.8
	5-Me	1.823	
diol			
cis	1	2.816	$J_{1,2}$ = 5.0, $J_{2,4}$ = 9.4
	4	2.614	$J_{1,3}$ = 9.2, $J_{3,4}$ = 5.0
	2	2.562	$J_{1,4}$ = −14.4
	3	2.508	$J_{2,3}$ = −16.2
	5-Me	2.110	
trans	1 (4)	2.660	$J_{1,2}$ = 7.3, $J_{2,4}$ = 7.3
	4 (1)	2.417	$J_{1,3}$ = 7.3, $J_{3,4}$ = 7.3
	2 (3)	2.116	$J_{1,4}$ = −14.6
	3 (2)	1.882	$J_{2,3}$ = −14.6
	5-Me	1.847	

H1 and H4 or, for that matter, any two protons in this group suggests that the shielding and deshielding effects of the OH group are absent or diminished. The fact that the chemical shifts are all located at a much downfield region indicates that the four methylene protons must be experiencing uniformly a greater deshielding than in the trans isomer. From the known pattern of isoshielding lines of porphyrin ring current,[17] our NMR data can best be fit into a *cis*-lactone in which the methylene protons are near the horizontal plane of the macrocycle (Figure 2). In the trans isomer, the relatively small deshielding effect experienced by the methylene protons, particularly H4, is an indication that they are located higher above the plane, near the "blank region" interfacing the opposite isotropic and anisotropic ring current effects. The presence of the adjacent OH oxygen to H1 could add up to 0.8 ppm deshielding to H1, but the shielding effect on H4 should not exceed 0.2 ppm. These arguments can be applied to the 5-methyl equally well. In the cis isomer (1.823 ppm), it is axial and has no nearby deshielding oxygen whereas in the trans form (1.992 ppm) it is more equatorial and also closer to the ester oxygen.

The broad peak at 3.924 ppm in Figure 1C is the 5-OH proton. NOE experiments revealed that irradiation of the 5-Me singlet enhances the lactone H1 peaks as well as the β-meso proton (9.272 ppm), and irradiation of the 5-OH peak enhances the same meso proton. This observation ruled out the possibility that the Na-OAC-cyclized product may be a 6-membered lactone fused across the 5,6-position.

The NMR spectra of the nonlactonized diols **14a** and **14b** are shown in Figure 3. The methylene protons of the angular propionate side chain were analyzed at 250 and 500 MHz. The 4-spin systems were simulated to give the chemical shifts and *J* values tabulated in Table I. Their configuration was deduced from two considerations: First, *cis*-diols were frequently obtained in model compounds following the osmate cleavage. In these *cis*-diols, the NMR peaks of the methylene protons of the pyrroline propionic acid side chain usually gave a densely packed pattern resembling the ABCM pattern of **14a**. Second, when either the *cis*- or *trans*-diol was lactonized in the presence of NaOAc, which should not epimerize the lactone, each gave only the corresponding cis or trans lactone, i.e., **14a** gave **12** and **14b** gave **1**. The symmetric pattern of diol **14b** suggests there is a greater rotational freedom of the pyrroline methylene protons in the trans configuration. The lack of a large differentiation between H1 and H4, in contrast

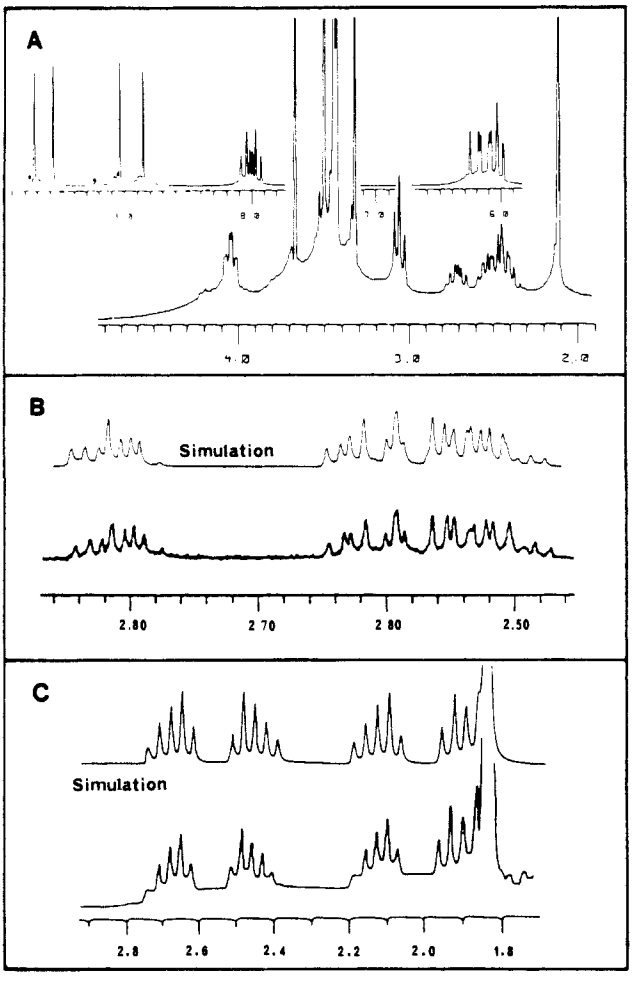
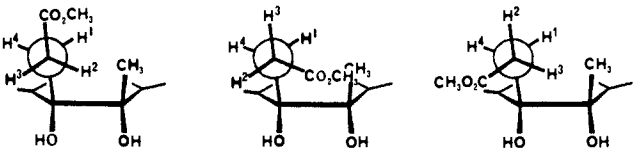


Figure 3. ^{1}H NMR spectra (in $CDCl_3$) of chlorin diols: **14a** at 250 MHz (A); partial spectrum of the methylene region at 500 MHz with simulation (B); partial spectrum of **14b** at 250 MHz with simulation.

Table II. Couplings (Hz)[a] and Rotamer Populations of Diol **14a**



I II III

	I	II	III
$J_{1,2}$	4.4	13.2	2.8
$J_{1,3}$	13.2	3.6	3.6
$J_{2,4}$	13.2	3.6	3.6
$J_{3,4}$	4.4	2.8	13.2
population	0.60	0.12	0.28

[a] From ref 19.

to the case of the trans lactone **1**, is again a consequence of this freedom of rotation. In both diols, no NOE could be detected between the 5-Me and any of the methylene protons.

The vicinal couplings of **14a** can be used to provide information about the conformation of the pyrroline propionic group. These measured numbers are average values of the component coupling constants in rotamers I–III weighted by their fractional populations.[18] The component coupling constants of I–III have previously been estimated for chlorophyll derivatives[19] and can be applied directly in this case. These *J* values and our calculated

(17) Scheer, H.; Katz, J. J. In *Porphyrins and Metalloporphyrins*; Smith, K. M.; Ed.; Elsevier: Amsterdam, 1975; p 402.

(18) Feeney, J. *J. Magn. Reson.* **1976**, *21*, 473–478.

(19) Smith, K. M.; Goff, D. A.; Abraham, R. J. *Tetrahedron Let.* **1981**, *22*, 4873–4876.

population for **14a** are given in Table II. While this analysis may have substantial error margins (±10%), the results are entirely consistent with what a molecular model would qualitatively predict: the anti conformer I is most favorable while the sterically congested rotamer II may be neglected. In the *trans*-diol **14b**, because of the greater rotational freedom, there should be no significant difference in the population of the rotamers.

D. Structure of Heme *d*: Lactone versus Diol. Before commenting on this question, we recapitulate the experimental observation. *cis,vic*-Dihydroxychlorins carrying a pyrroline propionate ester chain would readily cyclize into either a cis or trans lactone by reagents encountered in common heme extraction protocols. Mild bases such as sodium acetate, sodium bicarbonate, and pyridine cleanly lactonize the ester without inversion of configuration. Silica gel acts upon the diol in two steps: it first lactonizes the geminal groups and then promotes the inversion of lactone to give the more stable trans diastereomer. Presumably silica gel is able to effect the fission of the C–O bond in the spiro carbon. In an ideal case, all three compounds can be seen on TLC plate during developing, arising from a pure diol. Under alkaline hydrolytic conditions, the cis or trans lactone, if not hydrolyzed, apparently does not epimerize, and its hydrolysis yields the diol with complete retention of configuration, undoubtedly the result of a common $B_{AC}2$ mechanism.[20] It should be mentioned that chlorin diols without a pyrroline propionate ester side chain do not epimerize by silica gel nor by heating with pyridine–NaOH.[21]

Given these intrinsic labilities, we quite safely conjecture that the lactone ring found in lactochlorin is produced during the chromatographic purification of the demetalated and esterified chromophore. The observed trans configuration is also irrelevant as far as the true structure of the in vivo heme *d* is concerned. That issue, unfortunately, remains unanswered. From a biosynthetic point of view, saturation of the protoporphyrin ring is most likely brought about by an epoxidation. If the epoxy ring is opened in aqueous medium, most likely a *trans*-diol will result. However, one cannot be certain about the configuration as the propionate group may participate in epoxide opening even though the resulting lactone may not be the final form of heme *d*. To date, the strongest argument against the lactone being present in the in vivo heme *d* is the absence of IR peak in the extracted—but unesterified—heme *d*.[10] We noticed that the lactones are uniformly resistant to acid or base hydrolysis, and the extraction procedure employed by Timkovich et al. is too mild to open the lactone ring. In any case, the true structure of heme *d* awaits further confirmation. Our finding that the various forms of dihydroxychlorin have slightly different absorption and resonance Raman (RR) spectra[13] suggests that this is a problem solvable by RR studies of the heme enzyme.

In closing, we prefer the name "dihydroxyprotochlorin"[22] to be used to describe the basic structure of the iron-free heme *d* which, of course, may have two lactone forms. *Lacto*chlorin could be associated wrongly with milk (as in *lacto*peroxidase) and should be avoided.

Experimental Section

NMR spectra were routinely obtained at 250 MHz on a Bruker WM-250 instrument. Occasionally we managed to obtain spectra recorded at 360, 400, or 500 MHz on spectrometers (all Bruker make) located at other institutions. Spectra were recorded in $CDCl_3$; the residue $CHCl_3$ was used as the internal standard set at 7.240 ppm. The concentrations of chlorin samples were maintained at 2–3 mM to avoid concentration effects. All NOE's were positive and are expressed as the area of the enhanced resonance in difference spectra divided by the area in the control spectrum. Simulations were carried out first on an IBM PC with PMR (SERENA software) and then on Aspect 2000, which outputs to the NMR plotter. High-resolution mass spectra were obtained on a JEOL HX110-HF spectrometer equipped with a fast atom bombardment gun. Visible spectra (in CH_2Cl_2) were measured on a Cary 219 spectrophotometer. IR spectra were obtained from KBr pellets on a Perkin-Elmer 283B spectrophotometer. Preparative TLC plates were from Analtech (silica gel G, 1000 or 1500 μm).

Methyl 1,2,3,4,7,8-Hexaethyl-5-methylporphine-6-propionate (3). To a mixture of 4-[(ethoxycarbonyl)methyl]-3,5-dimethylpyrrole-2-carboxylic acid obtained by hydrogenolysis of the benzyl ester[23] (11.3 g, 0.05 mol) and 3,4-diethyl-2-formyl-5-methylpyrrole[24] (8.25 g, 0.05 mol) in methanol (80 mL) was added 40% HBr in acetic acid (20 mL). The mixture was heated on a steam bath for 15 min before being cooled to room temperature. The solid product was filtered, washed with ether (100 mL), and dried to give 16.5 g (83%) of 4′-(2-carboxyethyl)-3,4-diethyl-3′,5,5′-trimethyl-2,2′-dipyrrylmethene hydrobromide: mp 183–185 °C; NMR 1.06, 1.20 (3 H each, t, Et), 2.23 (3 H, s, Me), 2.63 (6 H, s, Me), 2.4–3.0 (8 H, m, Et and propionate), 6.90 (1 H, s, methine), 12.8 ppm (2 H, br s, NH). This salt (7.9 g, 0.02 mol) and 3,4,3′,4′-tetraethyl-5,5′-dibromo-2,2′-dipyrrylmethene hydrobromide[25] (15 g, 0.02 mol) were heated in anhydrous formic acid (100 mL) containing bromine (3.2 g, 0.02 mol). The mixture was heated under reflux for 2 h, and the solution was then boiled off. The residue was dissolved in methanol (200 mL) and trimethyl orthoformate (20 mL) for esterification. After standing in the dark for 1 day, the mixture was evaporated to dryness and the porphyrin was isolated by column chromatography on silica gel, eluting with CH_2Cl_2: yield 3.65 g (32%); mp 193–195 °C; ^1H NMR 1.93, 1.94 (9 H each, t, Et), 3.29 (2 H, t, $CH_2CH_2CO_2$), 3.65, 3.68 (3 H each, s, ring Me and OMe), 4.09, 4.12 (6 H each, q, Et), 4.42 (2 H, t, $CH_2CH_2CO_2$), 10.09, 10.10 (1 H each, s, meso β, γ), 10.12 (2 H, s, meso α, δ), −3.73 ppm (2 H, br s, NH); UV-vis λ_{max} (ϵ_M) 619 nm (8800), 566.5 (10 400), 533 (13 100), 499 (15 900), 399 (142 000). Anal. Calcd for $C_{37}H_{46}N_4O_2$: C, 76.78; H, 8.01; N, 9.67. Found: C, 76.68; H, 8.10; N, 9.55.

Dihydroxychlorins 4a, 4b, and 4d. To a solution of the above porphyrin (200 mg, 0.35 mmol) in CH_2Cl_2 (60 mL) and pyridine (0.25 mL) was added osmium tetroxide (114 mg, 0.45 mmol). The mixture was stirred at room temperature in the dark for 36 h; it was then diluted with methanol (20 mL) and bubbled with H_2S for 10 min. The precipitated osmium sulfide was removed by filtration, and the filtrate was concentrated, chromatographed on preparative TLC plates, and developed with CH_2Cl_2–5% EtOAc. There were four distinct bands: the red porphyrin moving at the front followed by **4a**, **4b**, and another green band containing **4c** and **4d**. Unambiguous structure assignments were achieved by NOE (see Scheme I). The two south diols were dissolved in a mixture of methanol (20 mL) and CH_2Cl_2 (5 mL), and the resultant solution was brought to reflux in the presence of anhydrous NaOAc (1 g). The reflux was continued for 20 min before the mixture was washed with water and evaporated. The residue was chromatographed on TLC to separate the lactonized **5** (higher R_f) and **4**. Overall yields: unchanged **3**, 22 mg (11%); **4a**, 34 mg (16%); **4b**, 29 mg (13.7%); **4d**, 32 mg (15%); **5**, 28 mg (14%).

1,2-Dihydroxy-1,2,3,4,7,8-hexaethyl-6-[2-(methoxycarbonyl)ethyl]-5-methylchlorin (4a): mp 138 °C dec; NMR 1.00 (6 H, t, pyrroline Et), 1.79, 1.81, 1.82, 1.85 (3 H each, t, Et), 2.59, 2.61 (2 H each, q, pyrroline Et), 3.11 (2 H, t, $CH_2CH_2CO_2$), 3.40 (3 H, s, Me), 3.68 (3 h, s, OMe), 3.91 (8 H, q, Et), 4.14 (2 H, t, $CH_2CH_2CO_2$), 9.01, 9.02 (1 H each, s, meso α, δ), 9.66 (1 H, s, meso γ), 9.69 (1 H, s, meso β), −2.53 ppm (2 H, br s, NH); UV-vis λ_{max} 642 nm, 590.5, 525, 496, 393; MS for $C_{37}H_{49}N_4O_4$, found *m/e* 613.4152 for $(M + H)^+$, calcd *m/e* 613.4189.

3,4-Dihydroxy-1,2,3,4,7,8-hexaethyl-6-[2-(methoxycarbonyl)ethyl]-5-methylchlorin (4b): mp 180–182 °C dec; NMR 0.90, 1.00 (3 H each, t, pyrroline Et), 1.80, 1.83 (6 H each, t, Et), 2.54, 2.64 (2 H each, q, pyrroline Et), 3.08 (2 H, t, $CH_2CH_2CO_2$), 3.36 (3 H, s, Me), 3.64 (3 H, s, OMe), 3.88, 3.89 (4 H each, q, Et), 4.15 (2 H, t, $CH_2CH_2CO_2$), 8.96 (1 H, s, meso β), 9.02 (1 H, s, meso α), 9.66, 9.73 (1 H each, s, meso γ, δ), −2.57 ppm (2 H, br s, NH); UV-vis λ_{max} 644 nm, 590.5, 523, 494, 392.

7,8-Dihydroxy-1,2,3,4,7,8-hexaethyl-6-[2-(methoxycarbonyl)ethyl]-5-methylchlorin (4d): NMR 0.92, 1.02 (3 H each, t, pyrroline Et), 1.80, 1.83 (6 H each, t, Et), 2.58, 2.64 (2 H each, q, pyrroline Et), 3.10 (2 H, t, $CH_2CH_2CO_2$), 3.41 (3 H, s, Me), 3.65 (3 H, s, OMe), 3.86, 3.92, (4 H each, q, Et), 4.11 (2 H, t, $CH_2CH_2CO_2$), 8.94 (1 H, s, meso γ), 9.04 (1 H, s, meso δ), 9.65, 9.72 ppm (1 H each, s, meso α, β); UV-vis λ_{max} 643.5 nm, 591, 523, 497, 391.5.

***cis*-1,2,3,4,7,8-Hexaethyl-5α-hydroxy-5β-methylchlorin 6,6-γ-spirolactone (5)**: mp 239–240 °C; NMR 1.82, 1.85 (9 H each, t, Et), 1.88

(20) Ingold, C. K. *Structure and Mechanism in Organic Chemistry*, 2nd ed.; Cornell University: Ithaca, NY, 1969; pp 1129–1131.

(21) In acids, most chlorin diols would undergo pinacolic rearrangement or elimination,[12] and these rearrangements appear to be more facile than the acid-catalyzed diol epimerization.

(22) According to the *Chemical Abstracts* convention: 17,18-dihydroxy-8,13-diethenyl-3,7,12,17-tetramethylporphine-2,18-dipropanoic acid.

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(24) Dolphin, D.; Harris, R. L. N.; Huppat, J. L.; Johnson, A. W.; Kay, I. T. *J. Chem. Soc. C* **1966**, 30–40.

(25) Fischer, H.; Baumler, R. *Justus Liebigs Ann. Chem.* **1929**, *468*, 94.

(3 H, s, Me), 3.29–3.54 (2 H, m, 13a2 and 13b4), 3.75–4.14 (14 H, m, 6 Et, 13a1, 13b3), 9.02, 9.20 (1 H each, s, meso β, γ), 9.78, 9.85 (1 H each, s, meso α, δ), −2.65, −2.57 ppm (1 H each, br s, NH); UV–vis λ_{max} (ϵ_M) 640.5 nm (42 000), 588 (5900), 522 (5000), 492 (14 000), 390 (161 000); MS for $C_{36}H_{45}N_4O_3$, found *m/e* 581.3480 for $(M + H)^+$, calcd *m/e* 581.3494.

***trans*-1,2,3,4,7,8-Hexaethyl-5α-hydroxy-5β-methylchlorin 6,6-γ-Spirolactone (6).** The cis lactone **5** (20 mg, 0.034 mmol) was loaded on a 1500-μm TLC plate and left in the dark overnight. The plate was developed with CH_2Cl_2–3% EtOAc to give the faster moving **6** in about 20% yield and the unchanged **5** (75%). If the plate was developed repetitively, **5** was completely converted to **6**; however, some minor degradation was also observed: mp 253–255 °C; NMR 1.79, 1.83 (9 H each, t, Et), 1.97 (3 H, s, Me), 2.38 (1 H, sext, 13a4), 2.97 (1 H, oct, 13b2), 3.21 (1 H, quint, 13b3), 3.48 (1 H, sept, 13a1) [J(13a1, 13b2) = 3.7 Hz, J(13a1, 13b3) = 9.6, J(13a1, 13a4) = −13.3, J(13b2, 13b3) = −17.9, J (13b2, 13a4) = 9.6, J(13b3, 13a4) = 9.6], 3.87, 3.90, 4.00 (4 H each, q, Et), 8.87, 9.00 (1 H each, s, meso β, γ), 9.76, 9.77 (1 H each, s, meso α, δ), −2.51 ppm (2 H, br s, NH); UV–vis λ_{max} (ϵ_M) 643 nm (31 500), 590 (2600), 522 (4800), 493 (11 000), 390 (153 000); MS for $C_{36}H_{45}N_4O_3$, found *m/e* 581.3473 for $(M + H)^+$, calcd *m/e* 581.3494.

Spirolactones 11a and 11b. To a solution of 2,4-bis(2-chloroethyl)-deuteroporphyrin IX dimethyl ester[15] (850 mg, 1.28 mmol) in CH_2Cl_2 was added osmium tetroxide (423 mg, 1.66 mmol), followed by addition of pyridine (0.2 mL). The reaction was stirred in the dark for 26 h before being diluted with methanol (50 mL) and quenched by H_2S. The chlorin products were chromatographed on a silica gel column. Porphyrin **9** (180 mg, 21% recovered) was eluted first with CH_2Cl_2 while the mixture of the four dihydroxychlorins was washed out with CH_2Cl_2–2% MeOH. This mixture was then chromatographed on TLC plates (CH_2Cl_2–10% EtOAc) to separate the two faster moving north diols (120 mg, 1:1 ratio) from the two south diols **10a** and **10b** (430 mg, 1.2:1 ratio). The distinction of the north and south diols was based on NMR of the methyl ester singlets: 3.64, 3.63, 3.60, 3.59 ppm (north diols) versus 3.67, 3.65, 3.55, 3.52 ppm (south diols).

The mixture of **10a** and **10b** in MeOH (100 mL) was refluxed with anhydrous NaOAc (5 g) for 30 min. The solution was evaporated, and the residue was dissolved in CH_2Cl_2 and chromatographed on TLC (CH_2Cl_2–10% EtOAc) without interruption, in a single path, to yield the faster moving **11a** (160 mg) and the slower moving **11b** (180 mg), the structure assignment of which had been based on NOE (see Scheme II).

***cis*-2,4-Bis(2-chloroethyl)-5α-hydroxydeuterochlorin IX dimethyl ester 6,6′-γ-spirolactone (11a)**: mp 226–228 °C; NMR 1.84 (3 H, s, 5-Me), 3.06 (2 H, t, 7-$CH_2CH_2CO_2$), 3.38, 3.41 (3 H each, s, 1.8-Me), 3.51 (3 H, s, 3-Me), 3.52 (3 H, s, OMe), 3.26–3.90 (4 H, m, 6-CH_2CH_2), 4.06 (1 H, br s, OH), 4.12, 4.20, 4.28 (10 H, t, 2,4-CH_2CH_2Cl and 7-$CH_2CH_2CO_2$), 9.08, 9.12 (1 H each, s, meso β, γ), 9.64 (1 H, s, meso δ), 9.71 (1 H, s, meso α), −2.67 ppm (2 H, br s, NH); UV–vis λ_{max} (ϵ_M) 641 nm (57 000), 588.5 (10 000), 522.5 (9000), 497 (21 900), 392 (226 000); MS for $C_{35}H_{39}N_4O_5Cl_2$, found *m/e* 665.2345 for $(M + H)^+$, calcd *m/e* 665.2300.

***cis*-2,4-Bis(2-chloroethyl)-8α-hydroxydeuterochlorin IX dimethyl ester 7,7′-γ-spirolactone (11b)**: mp 239–240 °C; NMR 1.85 (3 H, s, 8-Me), 3.12 (2 H, t, 6-CH_2CH2CO_2), 3.41, 3.49 (3 H each, s, 3,5-Me), 3.44 (3 H, s, 1-Me), 3.52 (3 H, s, OMe), 3.27–3.86 (4 H, m, 7-CH_2CH_2), 3.98 (1 H, br s, OH), 4.19, 4.22, 4.30 (10 H, t, 2,4-CH_2CH_2Cl and 6-$CH_2CH_2CO_2$), 9.14 (1 H, s, meso γ), 9.19 (1 H, s, meso δ), 9.66, 9.70 (1 H each, s, meso α, β), −2.70 ppm (2 H, br s, NH); UV–vis λ_{max} (ϵ_M) 641 nm (55 000), 588 (11 900), 524.5 (12 800), 494 (22 000), 395.5 (255 000).

***cis*-5α-Hydroxyprotochlorin IX Methyl Ester 6,6-γ-Spirolactone (12).** To a refluxing solution of cis lactone **11a** (100 mg, 0.15 mmol) in pyridine (50 mL) under argon was added KOH (1.2 g) in water (4 mL), and the heating was continued for 6 h before the mixture was evaporated to dryness under reduced pressure. The residue dissolved in ice water was treated with 10% HCl whereupon the product precipitated. The solid was filtered, washed with water, and esterified in MeOH with diazomethane. The product was chromatographed rapidly on TLC to separate the faster moving lactone **12** (8 mg) and the slower moving diol **14** (12 mg). The nonmobile material after elution with CH_2Cl_2–2% MeOH was believed to be degradation products due to the prolonged heating with strong base. However, if more dilute solution of KOH or less time (<3 h) was allowed for the reaction, the monovinyl compound 5,6-dihydroxy-2-(2-chloroethyl)-4-vinyldeuterochlorin IX dimethyl ester mainly resulted (structure determined by NOE). The condition for this elimination has not been optimized. ^{1}H NMR of **12** ring methyl (3 H each, s), 3.433 (8-Me), 3.490 (1), 3.527 (3) ppm; NMR of propionate, 4.263 (2 H, t, 7a), 3.087 (2 H, t, 7b) [J(7a,7b) = 7.4 Hz], 3.558 (3 H, s, OMe) ppm; NMR of vinyl (1 H each), 8.073 (X_i), 8.073 (X_j), 6.281 (A_i), 6.101 (B_i), 6.165 (A_j), 6.022 (B_j) [$J(X_i, A_i)$ = 17.8, $J(X_i, B_i)$ = 11.5, $J(A_i, B_i)$ = 1.3, $J(A_j, B_j)$ = 1.7] ppm; NMR of meso (1 H each, s), 9.856 (α), 9.272 (β), 9.048 (γ), 9.737 (δ) ppm; NMR of NH, −2.618 (2 H, br s) ppm; ^{1}H NMR of pyrroline substituents, see Table I; IR 1780, 1735 cm^{-1}; UV–vis λ_{max} (ϵ_M) 650 nm (37 200) 594 (7500), 530 (8000), 500 (14 200), 401 (147 000). MS for $C_{35}H_{37}N_4O_5$, found *m/e* 593.2742 for $(M + H)^+$, calcd *m/e* 593.2766.

***cis*-5,6-Dihydroxyprotochlorin IX dimethyl ester (14a)**: NMR 2.11 (3 H, s, 5-Me), 3.06 (2 H, t, 7b), 4.04 (2 H, t, 7a) [J(7a,7b) = 7.4], 3.32, 3.34, 3.44 (3 H each, s, ring Me), 3.49 (3 H, s, 6-$CCCO_2Me$), 3.66 (3 H, s, 7-$CCCO_2Me$), 5.99, 6.05, 6.13, 6.22 (1 H each, dd, vinyl), 8.01 (2 H, m, vinyl), 8.84 (1 H, s, meso γ), 9.03 (1 H, s, meso β), 9.57, 9.72 (1 H each, s, meso α, δ), −2.41 ppm (2 H, br s, NH); NMR for 6-$CH_2CH_2CO_2$, see Table I; UV–vis λ_{max} (ϵ_M) 650 nm (45 000), 594 (4600), 532 (4400), 498 (13 800), 401 (179 000); MS for $C_{36}H_{41}N_4O_6$, found *m/e* 625.3039 for $(M + H)^+$, calcd *m/e* 625.3028.

***trans*-5,6-Dihydroxyprotochlorin IX Dimethyl Ester (14b).** The lactone **11a** (50 mg) loaded on a 1500-μm silica gel plate was left in a developing tank overnight with 2% methanol in CH_2Cl_2 as solvent. When the pigment traveled to the top of the plate, it was scraped, extracted, and rechromatographed on a silica gel plate with CH_2Cl_2–10% EtOAc to separate the two isomers; the ratio was usually better than 50%.

***trans*-2,4-Bis(2-chloroethyl)-5α-hydroxydeuterochlorin IX dimethyl ester 6,6-γ-spirolactone**: NMR 1.99 (3 H, s, 5-Me), 2.41 (1 H, sext, 6a4), 3.03 (1 H, oct, 6b2), 3.23 (1 H, quint, 6b3), 3.45 (1 H, sept, 6a1) [J(6a1,6b2) = 4.1 Hz, J(6a1,6b3) = 9.6, J(6a1,6a4) = −13.3, J(6b2,6b3) = −17.9, J(6b2,6a4) = 9.6, J(6b3,6a4) = 9.6], 3.14 (2 H, t, 6-$CH_2CH_2CO_2$), 3.42, 3.52, 3.54 (3 H each, s, ring, Me), 3.66 (3 H, s, OMe), 4.16, 4.20, 4.21, 4.24, 4.30 (2 H each, t, 2,4-CH_2CH_2Cl and 6-$CH_2CH_2CO_2$), 8.90, 8.98 (1 H each, s, meso γ, δ), 9.66, 9.75 (1 H each, s, meso α, β), −2.46 ppm (2 H, br s, NH); UV–vis λ_{max} (ϵ_M) 641 nm (42 200), 588 (6300), 520 (4600), 495.5 (18 700), 392 (184 000); MS for $C_{35}C_{39}N_4O_5Cl_2$, found *m/e* 665.2335 for $(M + H)^+$, calcd *m/e* 665.2300.

trans-5,6-Dihydroxyprotochlorin IX dimethyl ester (**14b**) was obtained by heating the above lactone in KOH–pyridine following the procedure described for **12**: NMR 1.85 (3 H, s, 5-Me), 3.15 (2 H, t, 6b), 3.39, 3.49, 3.52 (3 H each, s, ring Me), 3.59 (3 H, s, 6-$CCCO_2Me$), 3.66 (3 H, s, 7-$CCCO_2Me$), 4.19 (2 H, t, 7a), 6.00, 6.07, 6.15, 6.29 (1 H each, dd, vinyl), 8.08 (2 H, m, vinyl), 8.93, 9.11 (1 H each, s, meso γ, δ), 9.72, 9.86 (1 H each, s, meso α, β), −2.08 ppm (2 H, br s, NH); NMR for 6-$CH_2CH_2CO_2$, see Table I; UV–vis λ_{max} (ϵ_M) 653 nm (37 200), 598 (4600), 533 (4800), 499.5 (12 800), 401 (154 000); MS for $C_{36}H_{41}N_4O_6$, found *m/e* 625.3014 for $(M + H)^+$, calcd *m/e* 625.3028.

***trans*-5α-Hydroxyprotochlorin IX Methyl Ester 6,6-γ-Spirolactone (1).** The cis lactone **12** (20 mg) was loaded on a TLC plates and was developed with CH_2Cl_2–3% EtOAc in the dark at least four times to convert **12** into the slightly faster moving **1**. NMR for ring methyl (3 H each, s), 3.484 (8-Me), 3.514 (1), 3.584 (3) ppm; NMR for propionate, 3.132 (2 H, t, 7b), 4.189 (2 H, t, 7b) [J(7a,7b) = 7.4 Hz], 3.662 (3 H, s, OMe) ppm; NMR for vinyl (1 H each), 8.069 (X_i), 8.069 (X_j), 6.286 (A_i), 6.091 (B_i), 6.157 (A_j), 6.010 ppm (B_j) [$J(X_i,A_i)$ = 17.8, $J(X_i,B_i)$ = 11.5, $J(A_i,B_i)$ = 1.3, $J(A_j,B_j)$ = 1.7]; NMR for meso (1 H each, s), 9.889 (α), 9.113 (β), 8.856 (γ), 9.752 (δ) ppm; NMR for NH, −2.254 (2 H, br s) ppm; NMR for pyrroline substituents, see Table I; IR 1780, 1738, 1718 cm^{-1}; UV–vis λ_{max} (ϵ_M) 653 nm (40 500), 596 (6300), 532 (6600), 501 (14 500), 401 (156 000); MS for $C_{33}H_{37}N_4O_5$, found *m/e* 593.2736 for $(M + H)^+$, calcd *m/e* 593.2766.

***cis*-8-Hydroxyprotochlorin IX Methyl Ester 7,7-γ-Spirolactone (13a).** The chlorine-bearing lactone **11b** was treated with KOH in refluxing pyridine and worked up in the same manner as described for **12**: NMR 1.84 (3 H, s, 8-Me), 3.06 (2 H, t, 6b), 3.27–3.88 (4 H, ABMN, lactone), 3.32, 3.39, 3.43 (3 H each, s, ring Me), 3.54 (3 H, s, OMe), 4.05 (1 H, br s, OH), 4.10 (2 H, t, 6a), 5.99, 6.07, 6.13, 6.25 (1 H each, dd, vinyl), 8.00 (2 H, m, vinyl), 9.04, 9.15 (1 H each, s, meso γ, δ), 9.71, 9.72 (1 H each, s, meso α, β), −2.70 ppm (2 H, br s, NH); UV–vis λ_{max} (ϵ_M) 650 nm (40 000), 596 (7300), 533 (7400), 501 (15 800), 402 (164 000).

***trans*-8-Hydroxyprotochlorin IX Methyl Ester 7,7-γ-Spirolactone (13b).** **13b** was obtained from the cis lactone **13a** by repetitive chromatography: NMR δ 2.01 (2 H, s, 8-Me), 2.43 (1 H, sext, 7a4), 3.04 (1 H, oct, 7b2), 3.22 (1 H, quint, 7b3), 3.48 (1 H, sept, 7a1) [J(7a1,7b2) = 3.7 Hz, J(7a1,7b3) = 9.6, J(7a1,7a4) = −13.3, J(7b2,7b3) = −17.9, J(7b2,7a4) = 9.6, J(7b3,7a4) = 9.6], 3.13 (2 H, t, 6b), 3.66 (3 H, s, OMe), 4.19 (2, H, t, 6a), 6.02, 6.13, 6.19, 6.35 (1 H each, dd, vinyl), 8.11 (2 H, m, vinyl), 8.88, 9.05 (1 H each, s, meso γ, δ), 9.86, 9.93 (1 H each, s, meso α, β), −2.34 ppm (2 H, br s, NH); UV–vis λ_{max} (ϵ_M) 652 nm (41 800), 597 (8200), 533 (9000), 500 (16 800), 401 (166 000); MS for $C_{35}H_{37}N_4O_5$, found *m/e* 593.2730 for $(M + H)^+$, calcd *m/e* 593.2766.

***cis*-7,8-Dihydroxyprotochlorin IX Dimethyl Ester.** This was obtained by pyridine–KOH treatment of **10b**: NMR δ 2.09 (3 H, s, 5-Me),

2.32-2.78 (4 H, ABCM, 7-$CH_2CH_2CO_2$), 3.01 (2 H, t, 6b), 3.24, 3.40, 3.41 (3 H each, s, ring Me), 3.50 (3 H, s, 7-$CCCO_2Me$), 3.65 (3 H, s, 6-$CCCO_2Me$), 3.97 (2 H, t, 6a), 5.99, 6.07, 6.11, 6.24 (1 H, each, dd, vinyl), 7.98 (2 H, m, vinyl), 8.82, 8.91 (1 H each, s, meso γ, δ), 9.62, 9.71 (1 H each, s, meso α, β), -2.45 ppm (2 H, br s, NH); UV-vis λ_{max} (ϵ_M) 651 nm (41 000), 597 (8000), 533 (8600), 501 (15 800), 402 (159 000).

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Multicomponent Redox Catalysts for Reduction of Large Biological Molecules Using Molecular Hydrogen as the Reductant

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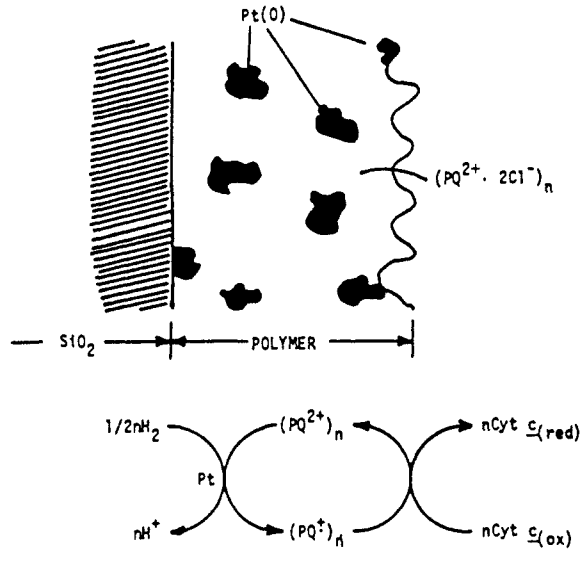
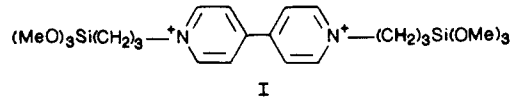
Abstract: One-electron reduction of the large biological molecules horse heart cytochrome *c*, sperm whale myoglobin, and horseradish peroxidase using H_2 as the reductant can be catalyzed by two-component, high surface area heterogeneous catalysts. The catalysts can be prepared by first functionalizing high surface area SiO_2 with a polycationic polymer into which is dispersed MCl_4^{2-} (M = Pd, Pt). Reduction with H_2 yields elemental Pd or Pt dispersed in the polymer. The particles are finally functionalized with a redox polymer derived from hydrolysis of $Si(OR)_3$ groups of an *N,N'*-dialkyl-4,4'-bipyridinium- or from a cobalticenium-based monomer. The two components of the heterogeneous catalysts are the buried noble metal capable of activating the H_2 and the redox polymer, which can equilibrate both with the noble metal and with the large biological molecule. Reduction of the large biological molecules in aqueous solution can be effected at room temperature and 1 atm H_2 using the catalysts under conditions where the biological materials would not be reducible with H_2 alone or when the noble metal alone would be used as the catalyst.

In this article we report the synthesis and characterization of two-component, high surface area catalysts for the reduction of certain biological redox molecules using H_2 as the reductant (Scheme I). The catalysts that have been synthesized have a component, Pd or Pt, for the activation of the H_2 that is interfaced with a second component, a redox mediator, that is a one-electron, outer-sphere type redox reagent that is reducible with the H_2 and can in turn reduce the biological redox reagent. The basic idea involved is to convert the inert, two-electron, reductant H_2 to two, reactive, one-electron, surface-confined, outer-sphere reducing equivalents. The need to use the one-electron, outer-sphere reducing reagents stems from the general finding that large biological molecules do not interface well with the surfaces of conductors,[1-3] particularly when contaminated with biological impurities.

Scheme I. Catalyst Particle for Reduction of Biological Redox Molecule Using H_2 as the Reductant



Previous work in this laboratory demonstrated the feasibility of effecting the H_2 reduction chemistry represented by Scheme I with a redox polymer/noble metal assembly as the catalyst deposited on the inside of ordinary Pyrex test tubes. The redox polymer used, $(PQ^{2+/+})_n$, is derived from an *N,N'*-dialkyl-4,4'-bipyridinium monomer I, and the noble metal M can be dispersed



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