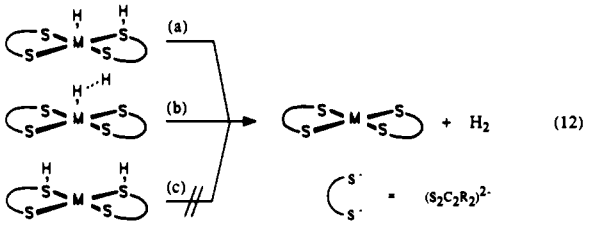
by the (higher) oxidation state compared to I or II. The resulting protons are then available for protonation of as yet unreacted $[Fe^{II}('S_2')_2]^{2-}$.

When 2 equiv or an excess of protons are added, the side reaction in which a second proton adds to I becomes more important. The resulting $[Fe('S_2')_2H_2]$ is labile and dissociates, yielding free $'S_2'-H_2$.

Theoretical calculations for the evolution of hydrogen at transition-metal sulfur centers were carried out for mononuclear bis(dithiolenes). They favor the heterolytic elimination of H_2 from metal hydride species according to either eq 12a or 12b rather than a concerted H_2 elimination from a dithiol species (eq 12c), which is thermally forbidden.[33]

The results described above and the mechanism of Scheme II show that such systems are only seemingly simple and that the evolution of H_2 at them can involve considerably more steps than



hitherto regarded. The results further show that it is possible to generate dihydrogen in protic media at iron sulfur centers whose formation does not require strongly reducing conditions.

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Proton NMR Study of High-Spin Iron(III) Isobacteriochlorins. The Effects of Five- vs Six-Coordination and Comparisons with Homologous Porphyrin and Chlorin Complexes

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Abstract: The two stereoisomers of free base 2,3,7,8-tetrahydro-2,3,7,8,12,13,17,18-octaethylporphyrin (octaethylisobacteriochlorin, H_2(OEiBC)), H_2(*ttt*-OEiBC) and H_2(*tct*-OEiBC), were separated to >90% purity with medium pressure liquid chromatography. These two macrocycles and *trans*-7,8-dihydro-2,3,7,8,12,13,17,18-octaethylporphyrin (*trans*-octaethylchlorin, H_2(*t*-OEC)) were used to prepare or generate in solution the following high-spin ($S = 5/2$) Fe(III) compounds: Fe(*ttt*-OEiBC)Cl, Fe(*tct*-OEiBC)Cl, $[Fe(ttt\text{-}OEiBC)(DMSO)_2]^+[CF_3SO_3]^-$, $[Fe(tct\text{-}OEiBC)(DMSO)_2]^+[CF_3SO_3]^-$, Fe(*t*-OEC)Cl, and $[Fe(t\text{-}OEC)(DMSO)_2]^+[CF_3SO_3]^-$. The isobacteriochlorin complexes are models for the siroheme active sites in the resting states of *E. coli* NADPH sulfite reductase and spinach ferredoxin nitrite reductase. Along with several specifically deuterated derivatives, the model complexes were studied by ^1H and ^2H NMR spectroscopy. (The six-coordinate complexes, reported here for the first time, were also characterized by UV-vis and EPR spectroscopy.) The OEiBC spectra are well enough resolved to show, for example, separate resonances for nearly all of the individual protons (not counting methyl protons) of the two C_s stereoisomers of Fe(*tct*-OEiBC)Cl. For both ligation states, the change from *ttt*-OEiBC to *tct*-OEiBC results in only minor changes in contact shift patterns. The range of contact shifts exhibited by pyrrole methylene protons, pyrroline protons, and meso protons is *far greater* for the OEiBC complexes than for the corresponding *t*-OEC complexes. Nevertheless, the estimated amount of unpaired spin density in pyrrole rings is essentially the same, at parity of axial ligation, across the series OEP, *t*-OEC, *ttt*-OEiBC. For both types of hydroporphyrin complexes, the change from five- to six-coordination induces diagnostic changes in meso and pyrroline proton isotropic shifts. Thus, pyrroline ^1H NMR resonances, which are relatively sharp, can be used to elucidate whether high-spin Fe(III) hydroporphyrin prosthetic groups in green heme and siroheme enzymes are five- or six-coordinate. T_1 relaxation times at 293 K for pyrrole methylene protons (~2 ms) and for meso protons (~0.3 ms) are qualitatively the same across the series OEP, *t*-OEC, *ttt*-OEiBC. For the hydroporphyrin complexes, the order of decreasing T_1 relaxation times is pyrrole methylene > pyrroline H ~ pyrroline methylene > meso. The pyrroline H and pyrroline methylene T_1's are measurably longer for the six-coordinate derivatives.

Introduction

Iron chlorins (e.g., heme-*d*,[3] sulfheme,[4] and the green heme in myeloperoxidase[5]) and isobacteriochlorins (e.g., heme-d_1[6] and siroheme[7]) are now widely recognized as important prosthetic groups in so-called green heme and siroheme proteins. Many of the physicochemical techniques that have been used to study the structure and dynamics of heme proteins (i.e., those containing iron porphyrins), such as X-ray crystallography and resonance Raman, EPR, MCD, Mössbauer, and NMR spectroscopy, have been recently applied to green heme and siroheme proteins and

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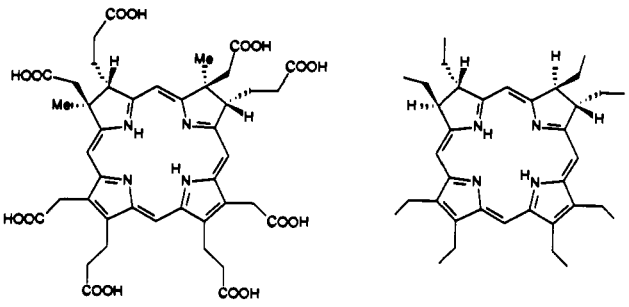

Figure 1. Drawings of sirohydrochlorin and H_2(*ttt*-OEiBC). Not counting the NH protons, these two macrocycles have 22 protons in common.

to synthetic iron chlorins and isobacteriochlorins (iBCs).[8] What has emerged so far is a better, if not yet complete, understanding of the structural and electronic differences between porpyrins and hydroporphyrins. It is now clear that synthetic iron porphyrins are not competent spectroscopic models for hydroporphyrin-containing proteins in many cases.

There is a growing body of data on the NMR properties of synthetic paramagnetic iron chlorins,[5,8b,m,o,9,10] but only two brief studies of iron iBCs.[9b,10] In this paper we report NMR spectra and T_1 relaxation times for the five- and six-coordinate high-spin Fe(III) ($S = 5/2$) complexes Fe(*ttt*-OEiBC)Cl, Fe(*tct*-OEiBC)Cl, $[Fe(ttt\text{-}OEiBC)(DMSO)_2]^+[CF_3SO_3]^-$, and $[Fe(tct\text{-}OEiBC)(DMSO)_2]^+[CF_3SO_3]^-$.[11] Factors that distinguish five- from six-coordination are identified, and comparisons with homologous chlorin and porphyrin complexes are made.

Our high-spin Fe(III) isobacteriochlorins are especially significant because they are models for the high-spin sirohemin active site in the resting state of both *E. coli* NADPH sulfite reductase and spinach ferredoxin nitrite reductase.[7d,e] Furthermore, while EPR results unambiguously indicate that these sirohemins are high-spin ($S = 5/2$),[7d,e] they are silent on the question of coordination number. The crystallographically defined structure of the active site of the *E. coli* enzyme currently lacks sufficient detail to determine whether a sixth ligand is present (the fifth ligand is probably a cysteine sulfur atom that bridges the siroheme iron atom and one of the iron atoms of the Fe_4S_4 cluster that is present at the active site).[8a] A ^{1}H ENDOR study of the *E. coli* enzyme conclusively ruled out the possibility that an exchangeable water molecule is the sixth ligand for resting state sirohemin.[7c] However, since other "sixth-ligand" possibilities remain, the ligation state of the resting state active sites is still very much an open issue.

Octaethylisobacteriochlorin and sirohydrochlorin, the macrocycle contained in siroheme enzymes,[7] share a large degree of structural homology, as shown in Figure 1. Previous work has shown that their electronic spectra are essentially congruent.[12] While the H_2(OEiBC) macrocycle does not possess methyl groups attached to pyrroline β-carbon atoms, unlike another synthetic isobacteriochlorin,[13] it does share 22 protons in common with sirohydrochlorin (not counting the NH protons: eight pyrrole methylene protons, eight pyrroline methylene protons, two pyrroline protons, and four meso protons). Therefore, paramagnetic complexes of OEiBC are reasonable ^{1}H NMR models for appropriate reaction states of siroheme enzymes. Furthermore, since the pyrroline protons in sirohydrochlorin have the same relative stereochemical relationship as do the pyrroline protons at the 3 and 8 positions in H_2(*ttt*-OEiBC), most of the detailed spectroscopic experiments reported herein (variable temperature studies, T_1 measurements) were performed with the *ttt* isomer of OEiBC.

Experimental Section

Preparation of Compounds. Reagents and solvents were the highest purity commercially available and were further purified and/or dried, where appropriate, by standard techniques. Celite (Fisher) and MgO (Mallinckrodt heavy powder) were used as received. The compounds H_2(OEP),[14] Fe(OEP)Cl,[15] H_2(*t*-OEC),[16] Fe(*t*-OEC)Cl,[16] $[Fe(t\text{-}OEC)]_2O$,[10] and $[Fe(OEP)]_2O$[9a] were prepared by literature procedures. Since metal complexes of hydroporphyrins are prone to air-oxidation (slow for chlorins and rapid (i.e., minutes) for iBCs—the OEiBC macrocycle undergoes facile oxidative dehydrogenation[12]), all preparations and manipulations were performed under a purified dinitrogen atmosphere, with Schlenk, glovebox, or high-vacuum techniques.

H_2(*t*-OEC-*5,10-d*$_2$).[18] Deuteration of the meso positions adjacent to the pyrroline ring was accomplished by heating H_2(*t*-OEC) (25 mg) to 60 °C in trifluoroacetic acid-*O-d* (5 mL) for 24 h. After removal of the acid under vacuum, the residue was extracted into dichloromethane, washed with water until neutral, and filtered through a bed of MgO. Integration of a ^{1}H NMR spectrum of the product showed that this procedure resulted in >99% deuterium incorporation at positions 5 and 10 and ~7% incorporation at positions 15 and 20.

H_2(*t*-OEC-*7,8,15,20-d*$_4$).[19] Deuteration of the pyrroline β-carbon atoms and the meso positions adjacent only to pyrrole rings was accomplished by treating H_2(*t*-OEC) (50 mg) dissolved in 1-butanol-*O-d* (20 mL) with sodium (2 g). After the reaction mixture had stirred for 1 h, 25 mL of a 50:50 (v/v) mixture of concentrated aqueous hydrochloric acid and methanol were added, and the resulting mixture stirred for 20 min. The mixture was washed once with 0.5 M aqueous ammonia and three times with water. The solvent was removed by rotary evaporation. The crude product was purified by preparatory thin-layer chromatography (alumina, $CHCl_3$). Integration of a ^{1}H NMR spectrum of the product showed that this procedure resulted in >95% deuterium incor-

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poration at positions 7 and 8 and 75% incorporation at positions 15 and 20.

H_2(*ttt*-OEiBC) and H_2(*tct*-OEiBC). The procedure of Stolzenberg et al. was used to prepare H_2(OEiBC), which is a 50:50 mixture of the *ttt* and *tct* isomers.[12] The amount of H_2(*t*-OEC) produced can be greatly reduced by using a longer reaction time (up to 6 h). An elegant but impractical separation of the two stereoisomers by 11 sequential fractional crystallizations has been reported.[20] An alternate separation procedure using medium-pressure liquid chromatography was developed. Crude H_2(OEiBC) (~50 mg) containing H_2(*t*-OEC) and H_2(OEBC)[11] was dissolved in toluene (10 mL) and filtered through a bed of MgO. The mixture was eluted from a 350 mm × 40 mm Michelson Miller (Ace) column of MgO with 10:1 (v/v) hexane/benzene at ~40 psi. A yellow-green band of H_2(OEBC) eluted first, followed by a broad red band containing H_2(*ttt*-OEiBC) (first) and H_2(*tct*-OEiBC). A green band of H_2(*t*-OEC) eluted very slowly. Up to 30 4-mL fractions were collected, several of which were analyzed by visible and ^{1}H NMR spectroscopy. Separate meso proton resonances for H_2(*ttt*-OEiBC) (δ 8.51 (1), 7.46 (2), and 6.68 (1)) and H_2(*tct*-OEiBC) (δ 8.49 (1), 7.44 (2), and 6.85 (1)) can be distinguished (chloroform-*d*, 22 °C).[10] Separation of these two stereoisomers in combined fractions was routinely >90%. The best separation that was achieved in a single fraction was >99%.

H_2(OEiBC-*5,10,20-d*$_3$) (Mixture of Isomers). This compound was prepared from H_2(OEiBC) (mixture of isomers) with the procedure that was used to prepare H_2(*t*-OEC-*5,10-d*$_2$). Integration of a ^{1}H NMR spectrum of the product showed that this resulted in >99% deuterium incorporation at positions 5, 10, and 20.

H_2(OEiBC-*2,3,7,8,10,15,20-d*$_7$) (Mixture of Isomers). This compound was prepared from H_2(*ttt*-OEiBC) with the procedure that was used to prepare H_2(*t*-OEC-*7,8,15,20-d*$_4$), except that preparatory thin-layer chromatography was replaced with a simple filtration through a bed of MgO. Besides effecting the deuterium exchange, the strongly basic conditions caused isomerization of the *ttt* isomer to a 50:50 mixture of *ttt* and *tct* isomers. Integration of a ^{1}H NMR spectrum of the product showed that this resulted in 94% deuterium incorporation at positions 2, 3, 7, and 8, 75% incorporation at position 15, and ~12% incorporation at positions 10 and 20.

Fe(*ttt*-OEiBC)Cl and Fe(*tct*-OEiBC)Cl. Either isomer of Fe(OEiBC) was generated in solution by metalating either isomer of H_2(OEiBC) with $FeCl_2 \cdot 4H_2O$ in THF containing several drops of 2,6-lutidine. The reaction mixture was heated to 60 °C for 3 h. All volatiles were removed under vacuum, and Fe(OEiBC) was extracted into hexane and filtered through Celite. Air (1–2 mL) from above a portion of concentrated hydrochloric acid was bubbled through the solution of Fe(OEiBC), producting Fe(OEiBC)Cl according to

$$4\text{Fe(OEiBC)} + O_2 + 4\text{HCl} \rightarrow 4\text{Fe(OEiBC)Cl} + 2H_2O$$

To avoid oxidation of the OEiBC macrocycle, all volatiles were immediately removed from the reaction mixture under vacuum, leaving a solid residue of either Fe(*ttt*-OEiBC)Cl or Fe(*tct*-OEiBC)Cl. The *ttt* compound is a mixture of enantiomers, while the *tct* compound is a mixture of two C_s stereoisomers, with the chlorine atom either cis or trans to the 2,7 protons (see Figure 1).

Fe(P)(DMSO-d_6)$_2$]$^+$[CF_3SO_3]$^-$ (P = OEP, *t*-OEC, and Either OEiBC Isomer). The six-coordinate complexes [Fe(P)(DMSO-d_6)$_2$]$^+$[CF_3SO_3]$^-$ were generated in solution by dissolving the respective Fe(P) complex in 50:50 (v/v) dichloromethane-d_2/dimethyl sulfoxide-d_6 and treating the solution with trifluoromethanesulfonic acid (2 μL) and a small portion of oxygen

$$2\text{Fe(P)} + \tfrac{1}{2}O_2 + 4\text{DMSO-}d_6 + 2CF_3SO_3H \rightarrow 2[\text{Fe(P)(DMSO-}d_6)_2]^+[CF_3SO_3]^- + H_2O$$

These solutions were used for subsequent NMR experiments. The water produced in the reaction ($^1/_2$ equiv per equiv iron) is small relative to the amount of DMSO-d_6 present. As has been found by others,[21] there was no evidence that water coordinates to Fe(III) in lieu of DMSO-d_6 under these conditions. The idealized symmetry of the [Fe(*t*-OEC)(DMSO-d_6)$_2$]$^+$ and [Fe(*ttt*-OEiBC)(DMSO-d_6)$_2$]$^+$ cations is C_2, while that of [Fe(*tct*-OEiBC)(DMSO-d_6)$_2$]$^+$ is C_s.

Spectroscopic Studies. UV–vis spectra were recorded with a Perkin-Elmer λ3B spectrophotometer. EPR spectra were recorded at 77 K with a Bruker ESP-300 spectrometer. NMR spectra were recorded at the indicated frequencies with a Bruker EM-300 spectrometer: ^{1}H (300.14 MHz) and ^{2}H (46.07 MHz). Proton NMR samples of the five- and six-coordinate complexes were prepared with toluene-d_8 or 50:50 (v/v) dichloromethane-d_2/dimethyl sulfoxide-d_6, respectively, as solvents. Natural abundance solvents were used to prepare ^{2}H NMR samples. Resonance positions were assigned relative to solvent signals ($CHDCl_2$ in the case of experiments with the six-coordinate complexes). The pulse sequence used for T_1 measurements was a standard 180–τ–90 sequence with a composite 180 pulse and explicit spectral subtraction. T_1 values were determined from a plot of ln (intensity) vs τ. Typically 15 values of τ were used. The probe temperature in the variable temperature experiments was measured by the method of Van Geet.[22]

Results and Discussion

Characterization of Six-Coordinate Complexes. Figure S1 (supplementary material) shows visible spectra of [Fe(OEP)(DMSO-d_6)$_2$]$^+$[CF_3SO_3]$^-$, [Fe(*t*-OEC)(DMSO-d_6)$_2$]$^+$[CF_3SO_3]$^-$, and [Fe(*ttt*-OEiBC)(DMSO-d_6)$_2$]$^+$[CF_3SO_3]$^-$. Extinction coefficients for these compounds were determined by comparison with reported values[10] for the respective Fe(P) complexes, from which the six-coordinate complexes were generated.[23] EPR spectra of all three compounds at 77 K show a single strong $g \sim 5.4$ signal and a weaker signal at $g \sim 2$.[24] This is characteristic of tetragonally distorted high-spin Fe(III) complexes.[25] The OEiBC compound is the first reported example of a six-coordinate high-spin ($S = 5/2$) Fe(III) iBC. The OEC complex is only the second example of a six-coordinate high-spin Fe(III) chlorin.[8m] It is now apparent that the lower symmetry of hydroporphyrins relative to porphyrins is not sufficient to cause resolvable rhombicity in EPR spectra of six-coordinate high-spin derivatives. This complements a similar observation about five-coordinate high-spin Fe(III) hydroporphyrins reported earlier.[10]

The disordered structure of [Fe(*t*-OEC)(DMSO)$_2$]$^+$[CF_3SO_3]$^-$ has been determined by X-ray diffraction methods.[26] The pyrroline ring could not be distinguished from the pyrrole rings. This type of disorder is apparently caused by rotational interconversion of pyrroline and pyrrole ring sites and has been observed in other iron chlorin structures.[8b] However, certain features of the structure that bear on the composition and spin-state of [Fe(*t*-OEC)(DMSO)$_2$]$^+$[CF_3SO_3]$^-$ were unequivocally discerned. The iron atom is six-coordinate with two axial DMSO ligands and is centered in the mean plane of the nearly planar macrocycle. The Fe–N distances range from 2.015 (4) to 2.060 (4) Å, indicative of high-spin Fe(III)[27] (cf. the Fe–N distance of 2.043 (3) Å in high-spin [Fe(TPP)(TMSO)$_2$]$^+$[ClO_4]$^-$ (TMSO = tetramethylene sulfoxide)[28]).

NMR Spectral Results. Proton NMR spectra of the following five-coordinate (toluene-d_8) and six-coordinate (50:50) (v/v) dichloromethane-d_2/dimethyl sulfoxide-d_6) complexes were recorded over the indicated temperature ranges: Fe(OEP)Cl (293–203 K), Fe(*t*-OEC)Cl (293 K only), Fe(*ttt*-OEiBC)Cl (327 to 227 K), Fe(*tct*-OEiBC)Cl (293 K only), [Fe(OEP)(DMSO-d_6)$_2$]$^+$[CF_3SO_3]$^-$ (327–227 K), [Fe(*t*-OEC)(DMSO-d_6)$_2$]$^+$[CF_3SO_3]$^-$ (327–227 K), [Fe(*ttt*-OEiBC)(DMSO-d_6)$_2$]$^+$[CF_3SO_3]$^-$ (293 K only), [Fe(*tct*-OEiBC)(DMSO-d_6)$_2$]$^+$[CF_3SO_3]$^-$ (325–225 K). In addition, ^{1}H and/or ^{2}H NMR spectra

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(24) The following *g* values were observed in frozen 50:50 (v/v) dimethyl sulfoxide/dichloromethane at ~90 K: [Fe(OEP)(DMSO)$_2$]$^+$[CF_3SO_3]$^-$, 5.31, ~2; [Fe(*t*-OEC)(DMSO)$_2$]$^+$[CF_3SO_3]$^-$, 5.40, ~2; [Fe(*ttt*-OEiBC)(DMSO)$_2$]$^+$[CF_3SO_3]$^-$, 5.55, ~2.

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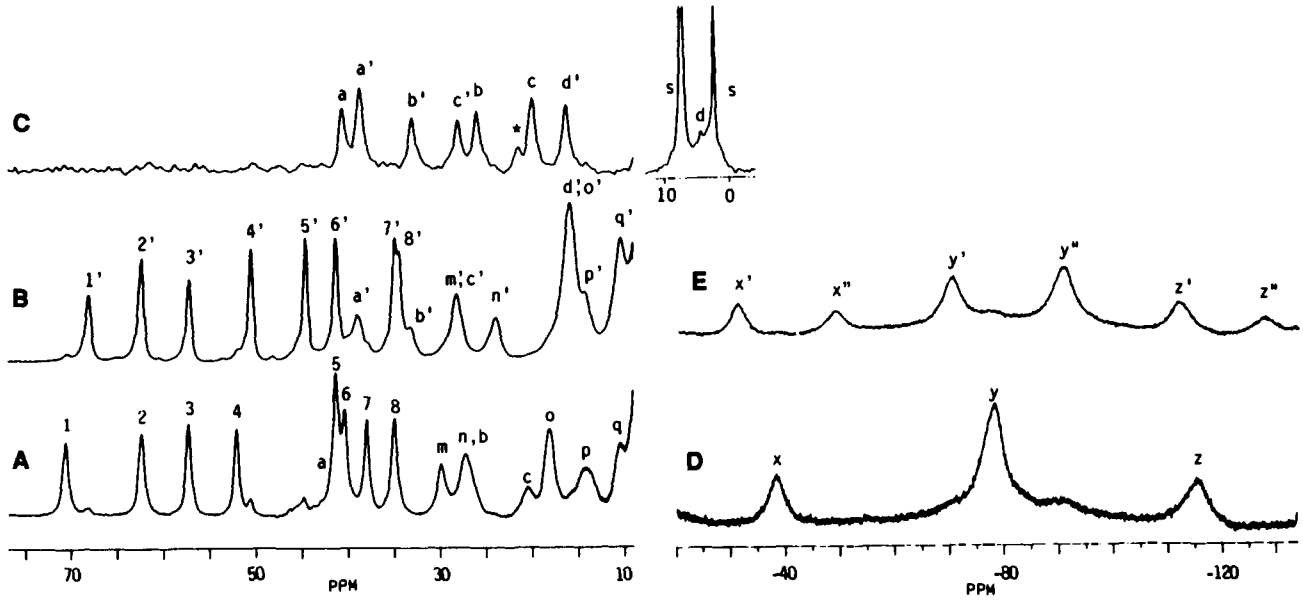

Figure 2. The 300-MHz ^{1}H NMR spectra of Fe(*ttt*-OEiBC)Cl (A and D) and Fe(*tct*-OEiBC)Cl (B and E) and 46-MHz ^{2}H NMR spectrum of a mixture of *ttt* and *tct* isomers of Fe(OEiBC-*2,3,7,8,10,15,20-d*$_7$)Cl (C). All of the spectra were recorded at 25 °C. Samples for ^{1}H NMR were dissolved in toluene-d_8, while samples for ^{2}H NMR were dissolved in natural abundance toluene.

were recorded for several selectively deuterated derivatives. T_1 values were determined at 293 K for the above compounds with the exception of Fe(*tct*-OEiBC)Cl and [Fe(*tct*-OEiBC)(DMSO-d_6)$_2$]$^+$[CF_3SO_3]$^-$. Table I lists chemical shifts at 293 K, T_1 values, and Curie plot slopes and $1/T = 0$ intercepts. Some peaks were obviously the superposition of two or more resonances—in these cases, the T_1 values in Table I are listed in parentheses.

Figure 2 shows 293 K ^{1}H NMR spectra of Fe(*ttt*-OEiBC)Cl and Fe(*tct*-OEiBC)Cl and the downfield portion of the ^{2}H NMR spectrum of a 50:50 mixture of *ttt* and *tct* isomers of Fe(OEiBC-*2,3,7,8,10,15,20-d*$_7$)Cl. The upfield portion of the latter spectrum (not shown) clearly shows that resonances labeled x, x′, and x″ are associated with the meso proton at position 15 (75% deuterium incorporation), while the resonances labeled y, y′, and y″ are associated with the meso protons at positions 10 and 20 (~12% deuterium incorporation). Figure 3 shows 293 K ^{1}H NMR spectra of [Fe(*ttt*-OEiBC)(DMSO-d_6)$_2$]$^+$[CF_3SO_3]$^-$ and [Fe(*tct*-OEiBC)(DMSO-d_6)$_2$]$^+$[CF_3SO_3] and a 50:50 mixture of *ttt* and *tct* isomers of [Fe(OEiBC-*2,3,7,8,10,15,20-d*$_7$)(DMSO-d_6)$_2$]$^+$[CF_3SO_3]$^-$. Note that in the latter spectrum, the resonance labeled 5′ only decreases slightly in intensity, while the resonance labeled 8′ has disappeared entirely. A 293 K ^{2}H NMR spectrum of a 50:50 mixture of *ttt* and *tct* isomers of [Fe(OEiBC-*5,10,20-d*$_3$)(DMSO-d_6)$_2$]$^+$[CF_3SO_3]$^-$ (not shown) shows, in addition to solvent resonances, three equal intensity broad resonances at δ 0.7, 29.8, and 34.2.

Figure 4 shows the 293 K ^{1}H NMR spectrum of Fe(*t*-OEC)Cl and 293 K ^{1}H and ^{2}H spectra of Fe(*t*-OEC-*7,8,15,20-d*$_4$)Cl. Figure 5 shows 293 K ^{1}H NMR spectra of [Fe(*t*-OEC)(DMSO-d_6)$_2$]$^+$[CF_3SO_3]$^-$, [Fe(*t*-OEC-*5,10-d*$_2$)(DMSO-d_6)$_2$]$^+$[CF_3SO_3]$^-$, and [Fe(*t*-OEC-*7,8,15,20-d*$_4$)(DMSO-d_6)$_2$]$^+$[CF_3SO_3]$^-$.

The ^{2}H resonances for all of the complexes have considerably smaller line widths than the corresponding ^{1}H resonances. In Figure 2, for example, $\Delta\nu_{1/2}$ for resonance y is 207 Hz in the ^{1}H NMR spectrum and 49 Hz in the ^{2}H NMR spectrum, while $\Delta\nu_{1/2}$ for resonance z is 218 Hz in the ^{1}H NMR spectrum and 46 Hz in the ^{2}H NMR spectrum. This is a well-known phenomenon[29] and is a consequence of less effective electron-nuclear dipolar relaxation for a deuterium nucleus relative to a proton.[30]

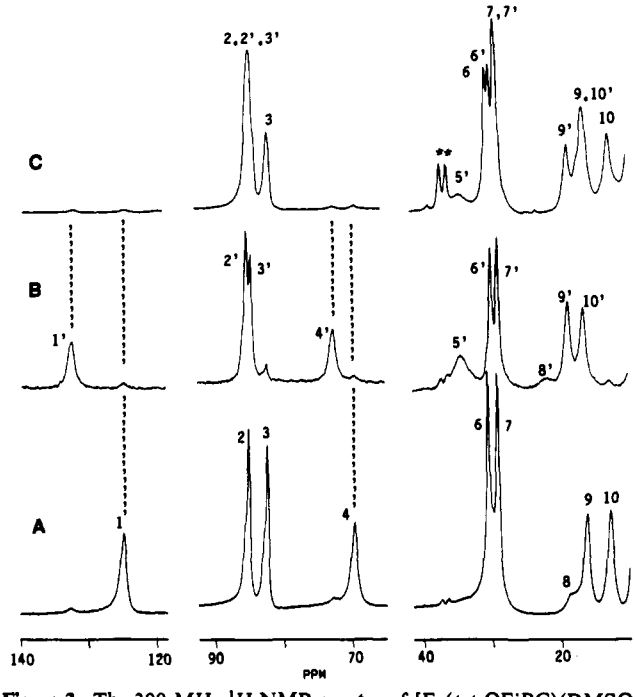

Figure 3. The 300-MHz ^{1}H NMR spectra of [Fe(*tct*-OEiBC)(DMSO-d_6)$_2$]$^+$[CF_3SO_3]$^-$ (A), [Fe(*ttt*-OEiBC)(DMSO-d_6)$_2$]$^+$[CF_3SO_3]$^-$ (B), and a mixture of *ttt* and *tct* isomers of [Fe(OEiBC-*2,3,7,8,10,15,20-d*$_7$)-(DMSO-d_6)$_2$]$^+$[CF_3SO_3]$^-$ (C). All three spectra were recorded at 25 °C. The samples were dissolved in 50:50 (v/v) dichloromethane-d_2/dimethyl sulfoxide-d_6. The peaks marked with asterisks are due to [Fe(*t*-OEC)(DMSO-d_6)$_2$]$^+$[CF_3SO_3]$^-$.

Spectral Assignments and Isotropic Shifts. Variable-temperature ^{1}H NMR spectra of Fe(OEP)Cl in chloroform-d[31] and in dichloromethane-d_2[80] have been previously reported. In these solvents, the diastereotopic methylene protons give rise to separate signals, differing by ~5 ppm at 298 K. In this study, we report the spectrum of Fe(OEP)Cl in toluene-d_8 for the first time. The spectrum exhibited only a single methylene resonance at 293 K. However, two separate signals were observed at subambient temperatures, each of which obeyed the Curie law (see Table I).

Variable-temperature ^{1}H NMR spectra of Fe(*t*-OEC)Cl in dichloromethane-d_2 have been reported as part of a previous study

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Table I. Proton NMR Spectral Parameters[a]

resonance label[b]	assignment (integrated intens.)	δ_{293}, ppm	T_1,[c] ms	Curie plot parameters: (10^{-3}) slope, ppm·K	Curie plot parameters: δ at $1/T = 0$, ppm	resonance label[b]	assignment (integrated intens.)	δ_{293}, ppm	T_1,[c] ms	Curie plot parameters: (10^{-3}) slope, ppm·K	Curie plot parameters: δ at $1/T = 0$, ppm
					Fe(OEP)Cl						
	CH_2 (8)	39.8	2.7	18.1	−21.4		CH_3 (24)	7.7	*d*	*d*	*d*
	CH_2 (8)	39.8	2.7	21.5	−33.7		meso (4)	−55.6	0.30	*d*	*d*
					Fe(*t*-OEC)Cl						
1	pyrrole CH_2 (1)	50.4	2.1			b	pyrroline H (1)	21.6	0.85		
2	pyrrole CH_2 (1)	46.3	2.6			c	pyrroline H (1)	20.3	0.86		
3, 4, 5	pyrrole CH_2 (3)	45.3	(2.2)			d	pyrroline CH_2 (1)	18.6	1.0		
6	pyrrole CH_2 (1)	43.8	2.5			e	pyrroline CH_2 (1)	15.4	0.74		
7	pyrrole CH_2 (1)	42.9	2.6			f	pyrroline CH_2 (1)	10.9	0.99		
8	pyrrole CH_2 (1)	40.9	2.5			w	15 or 20 meso (1)	−44.3	0.31		
9	pyrrole CH_2 (1)	38.6	2.5			x	15 or 20 meso (1)	−53.2	0.30		
10, 11	pyrrole CH_2 (2)	35.5	(2.4)			y	5 or 10 meso (1)	−82.0	0.31		
12	pyrrole CH_2 (1)	34.7	2.1			z	5 or 10 meso (1)	−91.4	0.32		
a	pyrroline CH_2 (1)	26.7	0.93								
					Fe(*ttt*-OEiBC)Cl						
1	pyrrole CH_2 (1)	70.1	2.6	25.4	−15	n	pyrroline CH_2 (1)	27.2	(0.89)	9.8	−6
2	pyrrole CH_2 (1)	61.8	2.5	21.6	−10	b	pyrroline H (1)	25.9	*d*	*d*	*d*
3	pyrrole CH_2 (1)	56.8	2.7	17.7	−2	c	pyrroline H (1)	20.0	0.79	9.0	−10
4	pyrrole CH_2 (1)	51.7	2.7	16.8	−4	d	pyrroline H (1)	4.3	*d*	*d*	*d*
5	pyrrole CH_2 (1)	40.9	(2.2)	13.1	−5	o	pyrroline CH_2 (2)	18.2	(1.1)	4.6	3
a	pyrroline H (1)	40.7	*d*	*d*	*d*	p	pyrroline CH_2 (2)	14.1	(0.65)	3.4	3
6	pyrrole CH_2 (1)	39.9	2.1	12.9	−3	q	pyrroline CH_2 (2)	8.0	(1.3)	1.9	4
7	pyrrole CH_2 (1)	37.6	2.6	13.4	−7	x	15 meso (1)	−38.4	0.34	−12.5	4
8	pyrrole CH_2 (1)	34.6	2.4	9.3	4	y	10, 20 meso (2)	−77.7	0.28	−23.1	0
m	pyrroline CH_2 (1)	29.7	1.1	11.8	−10	z	5 meso (1)	−116.2	0.34	−35.4	3
					Fe(*tct*-OEiBC)Cl						
1′	pyrrole CH_2 (1)	68.2				n′	pyrroline CH_2 (1)	24.0			
2′	pyrrole CH_2 (1)	62.4				d′	pyrroline H (1)	16.3			
3′	pyrrole CH_2 (1)	57.2				o′	pyrroline CH_2 (2)	15.9			
4′	pyrrole CH_2 (1)	50.4				p′	pyrroline CH_2 (2)	14.3			
5′	pyrrole CH_2 (1)	44.6				q′	pyrroline CH_2 (2)	10.5			
6′	pyrrole CH_2 (1)	41.3				x′	15 meso (1)	−31.1			
a′	pyrroline H (1)	38.7				x″	15 meso (1)	−48.8			
7′	pyrrole CH_2 (1)	35.0				y′	10, 20 meso (2)	−69.8			
8′	pyrrole CH_2 (1)	34.5				y″	10, 20 meso (2)	−90.2			
b′	pyrroline H (1)	33.0				z′	5 meso (1)	−111.3			
m′	pyrroline CH_2 (1)	28.0				z″	5 meso (1)	−126.8			
c′	pyrroline H (1)	28.0									
					[Fe(OEP)(DMSO-d_6)$_2$]$^+$[CF_3SO_3]$^-$						
	pyrrole CH_2 (16)	47.9	4.8	17.1	−10		pyrrole CH_3 (24)	7.7	*d*	3.6	−5
	meso (4)	40.1	0.75	18.5	−22						
					[Fe(*t*-OEC)(DMSO-d_6)$_2$]$^+$[CF_3SO_3]$^-$						
1	pyrroline H (2)	81.3	1.4	28.9	−15	6	pyrrole CH_2 (2)	35.5	2.8	12.8	−7
2	pyrrole CH_2 (4)	60.6	3.4	20.5	−8	7	15, 20 meso (2)	31.7	0.73	14.3	−16
3	pyrrole CH_2 (2)	54.3	3.3	18.8	−8	8	5, 10 meso (2)	29.6	0.72	14.0	−17
4	pyrrole CH_2 (2)	52.8	3.1	18.1	−7	9	pyrroline CH_2 (2)	16.5	1.3	5.8	−2
5	pyrrole CH_2 (2)	36.4	2.9	14.3	−11	10	pyrroline CH_2 (2)	8.6	1.6	2.1	−2
					[Fe(*ttt*-OEiBC)(DMSO-d_6)$_2$]$^+$[CF_3SO_3]$^-$						
1′	pyrroline H (2)	131.0	1.5			7′	pyrrole CH_2 (2)	29.4	2.6		
2′	pyrrole CH_2 (2)	84.3	3.4			8′	15 meso (1)	21.4	*d*		
3′	pyrrole CH_2 (2)	83.7	3.3			9′	pyrroline CH_2 (2)	19.1	1.3		
4′	pyrroline H (2)	72.6	1.3			10′	pyrroline CH_2 (2)	17.1	1.3		
5′	10, 20 meso (2)	34.2	0.76				5 meso (1)	0.7[e]	*d*		
6′	pyrrole CH_2 (2)	30.6	2.7								
					[Fe(*tct*-OEiBC)(DMSO-d_6)$_2$]$^+$[CF_3SO_3]$^-$						
1	pyrroline H (2)	125.1		39.3	−8	7	pyrrole CH_2 (2)	29.2		11.0	−8
2	pyrrole CH_2 (2)	85.4		26.4	−4	8	15 meso (1)	17.4		7.7	−7
3	pyrrole CH_2 (2)	82.6		24.9	−1	9	pyrroline CH_2 (2)	16.2		4.6	1
4	pyrroline H (2)	69.8		24.2	−12	10	pyrroline CH_2 (2)	12.8		2.2	5
5	10, 20 meso (2)	29.8[e]		*d*	*d*		5 meso (1)	0.7[e]		*d*	*d*
6	pyrrole CH_2 (2)	30.6		12.1	−10						

[a] Solvents used were toluene-d_8 for Fe(P)Cl complexes and 50:50 (v/v) dichloromethane-d_2/dimethyl sulfoxide-d_6 for [Fe(P)(DMSO-d_6)$_2$]$^+$[CF_3SO_3]$^-$ complexes. [b] The resonance labels are the ones used in Figures 2–5. [c] Measured at 293 K. [d] Unable to measure. [e] Value taken from ^{2}H NMR spectrum.

carried out in this laboratory.[8o] In addition, room-temperature spectra of Fe(*t*-OEC)Cl and Fe(OEiBC)Cl (mixture of isomers) in chloroform-*d* have been briefly described.[10] An important recent paper from La Mar's laboratory describes the NMR spectral behavior of related high-spin Fe(III) complexes Fe(CHL)Cl and [Fe(CHL)(DMSO-d_6)$_2$]$^+$[NO_3]$^-$, where CHL is the naturally occurring chlorin derivative pyropheophorbide *a* methyl ester.[8m] Among other things, La Mar's study confirmed an earlier sug-

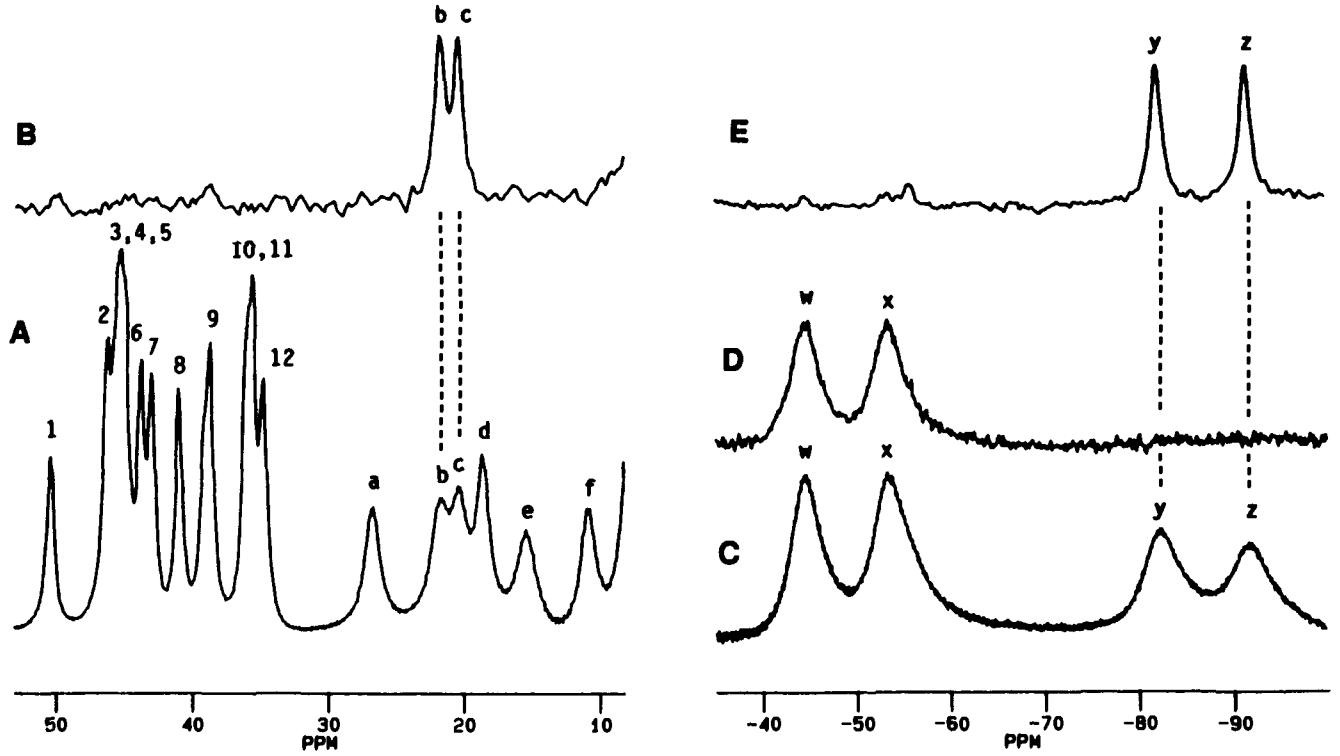

Figure 4. The 300-MHz ^{1}H NMR spectra of Fe(*t*-OEC)Cl (A and C) and Fe(*t*-OEC-*5,10-d*$_2$)Cl (D) and 46-MHz ^{2}H NMR spectra of Fe(*t*-OEC-*7,8,15,20-d*$_4$)Cl (B) and Fe(*t*-OEC-*5,10-d*$_2$)Cl (E). All of the spectra were recorded at 25 °C. Samples for ^{1}H NMR were dissolved in toluene-d_8, while samples for ^{2}H NMR were dissolved in natural abundance toluene.

gestion[80] that π spin delocalization into the "a_{1u}" MO is significant for chlorins.

The assignments for resonances in Figures 2–5 are made based on (i) integrated intensity of the resonances, (ii) deuterium substitution, and (iii) analogy to spectra reported in our previous study.[80] For Fe(*t*-OEC)Cl, the assignments are pyrrole methylene protons, resonances 1–12; pyrroline protons, resonances b and c; pyrroline methylene protons, resonances a and d–f; and meso protons, resonances w–z. Resonances y and z are due to the meso protons adjacent to the pyrroline ring (the 5 and 10 positions). These are the meso positions that are subject to more rapid electrophilic substitution, which suggests a higher π electron density than positions 15 and 20 as well as a higher π electron density than the meso positions of Fe(OEP)Cl.[18] This probably accounts for the much larger isotropic shifts exhibited by the 5,10 protons relative to the 15,20 protons or relative to the meso protons of five-coordinate high-spin Fe(III) porphyrins.[32,33]

For [Fe(*t*-OEC)(DMSO-d_6)$_2$]$^+$[CF_3SO_3]$^-$, the assignments are pyrrole methylene protons, resonances 2–6; pyrroline protons, resonance 1; pyrroline methylene protons, resonances 9 and 10; and meso protons, resonances 7 (positions 15 and 20) and 8 (positions 5 and 10). Note the very large change in isotropic shifts exhibited by the meso protons and the pyrroline protons on going from five- to six-coordination, in contrast with the relatively invariant isotropic shifts exhibited by methylene protons in both types of complexes. A change in sign for meso proton isotropic shifts has also been observed for homologous porphyrin complexes.[32,33]

For Fe(*ttt*-OEiBC)Cl and Fe(*tct*-OEiBC)Cl, the assignments are (primed letters and numbers for the latter complex) pyrrole methylene protons, resonances 1–8; pyrroline protons, resonances a–d; pyrroline methylene protons, resonances m–q; and meso protons, resonances x–z. Note the very large isotropic shift range exhibited by each type of proton. For example, for Fe(*ttt*-OEiBC)Cl, the ranges at 293 K are 35–70 ppm for pyrrole methylene protons, 4–40 ppm for pyrroline protons, 8–30 ppm for pyrroline methylene protons, and −38 to −116 ppm for meso protons. Resonances z, z′, and z″ are due to the meso proton between the two pyrroline rings (5 position) and exhibit the largest isotropic shifts for these complexes. Resonances y, y′, and y″ are due to the meso protons at positions 10 and 20, while resonances x, x′, and x″ are due to the meso proton at position 15. As was seen for Fe(*t*-OEC)Cl, the meso proton isotropic shifts correlate with the rates of electrophilic substitution at the meso positions, since both phenomena are related to the amount of π electron density at the respective meso carbon atoms.

For [Fe(*ttt*-OEiBC)(DMSO-d_6)$_2$]$^+$[CF_3SO_3]$^-$ and [Fe(*tct*-OEiBC)(DMSO-d_6)$_2$]$^+$[CF_3SO_3]$^-$, the assignments are (primed numbers for the former complex) pyrrole methylene protons, resonances 2, 3, 6, and 7; pyrroline protons, resonances 1 and 4; pyrroline methylene protons, resonances 9 and 10; and meso protons, resonances 5 (positions 10 and 20) and 8 (position 15). Consistent with the changes observed for the chlorin complexes, there is a very large change in isotropic shifts exhibited by the meso protons (changes up to 127 ppm) and the pyrroline protons (potential changes up to 120 ppm) on going from five- to six-coordination.

As reviewed in our earlier paper,[80] Curie plots for five-coordinate high-spin Fe(III) porphyrin complexes should not be strictly linear. The presence of zero-field splitting gives rise to a dipolar contribution to the isotropic shift with a $1/T^2$ dependence.[34,35] However, the departure from linearity is generally very small when the axial ligand is Cl^-.[36] Furthermore, zero-field splitting is expected to be smaller in six-coordinate high-spin Fe(III) complexes than in analogous five-coordinate complexes.[37] In any

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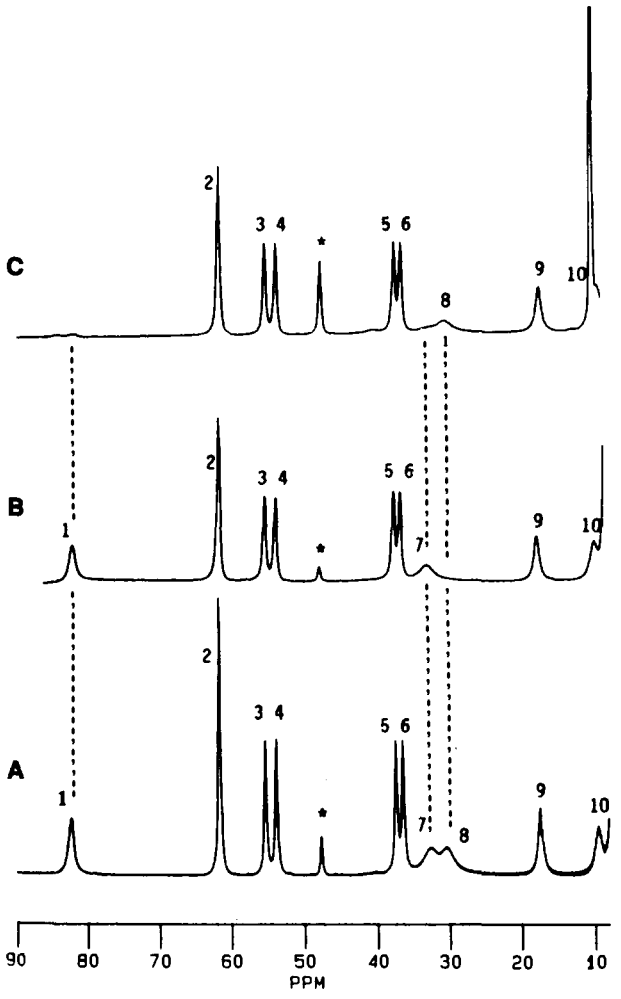

Figure 5. The 300-MHz ^1H NMR spectra of [Fe(*t*-OEC)(DMSO-d_6)$_2$]$^+$[CF_3SO_3]$^-$ (A), [Fe(*t*-OEC-*5,10-d*$_2$)(DMSO-d_6)$_2$]$^+$[CF_3SO_3]$^-$ (B), and [Fe(*t*-OEC-*7,8,15,20-d*$_4$)(DMSO-d_6)$_2$]$^+$[CF_3SO_3]$^-$ (C). All three spectra were recorded at 25 °C. The samples were dissolved in 50:50 (v/v) dichloromethane-d_2/dimethyl sulfoxide-d_6. The peaks marked with asterisks are due to [Fe(OEP)(DMSO-d_6)$_2$]$^+$[CF_3SO_3]$^-$.

event, we have observed that the Curie plots for the complexes in this study are approximately linear over the temperature range 220–298 K.[26]

Pyrroline Protons. The pyrroline rings of hydroporphyrins distinguish them from porphyrins. If one could identify resonances due to pyrroline ring protons in green heme and siroheme enzymes, they would provide a new and potentially valuable spectroscopic handle. The NMR spectral behavior of the protons on the pyrroline rings in Fe(TPC)Cl,[11] Fe(TPC)($OTeF_5$), and Fe(*t*-OEC)Cl has been described in detail.[8o] In that study it was found that the inequivalent pairs of pyrroline protons in Fe(TPC)($OTeF_5$) and Fe(TPC)Cl exhibited similar isotropic shifts—in the former complex $\Delta\delta$ is 7.9 ppm at 293 K, while in the latter complex the two resonances are unresolved. In Fe(*t*-OEC)Cl, $\Delta\delta$ is only 1.3 ppm at 293 K (see Figure 4 and Table I). Thus, we were prepared to find that the pyrroline protons in Fe(*ttt*-OEiBC)Cl and Fe(*tct*-OEiBC)Cl would also exhibit a narrow range of shifts. However, the shift ranges at 293 K were very large, from 4.3 to 40.7 ppm in Fe(*ttt*-OEiBC)Cl and from 16.3 to 38.7 ppm in Fe(*tct*-OEiBC)Cl. Despite this difference between the chlorin and the iBC, T_1 values for the pyrroline protons in Fe(*t*-OEC)Cl (0.85 and 0.86 ms) and in Fe(*ttt*-OEiBC)Cl (0.79 ms) are not very different.

The change in ligation state from five- to six-coordinate shifts the pyrroline protons for both chlorins and iBCs far downfield. Since resonance 1 in Figure 5 (δ 81.3) has been unambiguously assigned by deuterium substitution as due to two symmetry equivalent pyrroline protons, we can confirm the suggestion made by La Mar and co-workers that two resonances at δ 75 and 95 in the spectrum of [Fe(CHL)(DMSO-d_6)$_2$]$^+$[NO_3]$^-$ are due to pyrroline protons.[8m] The T_1 values for pyrroline protons are 50–75% larger in the six-coordinate complexes than in the five-coordinate complexes.

Conclusions. We have discovered a number of NMR spectral features for high-spin Fe(III) isobacteriochlorins that should allow for easier and less ambiguous interpretation of siroheme enzyme spectra. Various pyrrole methylene protons, of which there are eight in siroheme, exhibit a wide range of isotropic shifts in our model compounds. These shifts are in the same spectral window for both five- and six-coordinate complexes. Pyrroline protons and pyrroline methylene protons also exhibit a wide range of shifts but are in another region of the spectrum. In the case of pyrroline methylene protons, the shifts are similar for five- and six-coordinate complexes. It now appears possible that pyrroline and pyrrole methylene protons can be distinguished by their isotropic shifts as well as by their T_1 values, which differ by a factor of $\sim$2. This statement applies to both isobacteriochlorin and chlorin complexes.

The result of greatest significance in this study is that pyrroline protons undergo a diagnostic shift (they are shifted downfield) as a high-spin Fe(III) hydroporphyrin complex switches from five- to six-coordinate. We suggest that this result can be immediately applied to help elucidate the coordination state of the heme iron atom in fully oxidized siroheme enzymes. Of course, this would require definite assignments of pyrroline proton resonances in enzyme NMR spectra. The differences in T_1 values measured in this study will allow pyrroline protons and pyrroline methylene protons to be distinguished from pyrrole methylene protons. However, there is a more important reason that such assignments will likely be made: considering that iBC pyrroline protons undergo relatively facile deuterium substitution,[16] exchange of them with D_2O at mildly basic pD can be anticipated (note that the polar nature of the substituents in siroheme (see Figure 1) as well as the ionic nature of its natural substrates, sulfite and nitrite, suggest access to the prosthetic group by solvent and small ions such as OD^-).

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Supplementary Material Available: Figure S1 of electronic spectra of [Fe(OEP)(DMSO)$_2$]$^+$[CF_3SO_3]$^-$, [Fe(*t*-OEC)(DMSO)$_2$]$^+$[CF_3SO_3]$^-$, and [Fe(*ttt*-OEiBC)(DMSO)$_2$]$^+$[CF_3SO_3]$^-$ (1 page). Ordering information is given on any current masthead page.