Synthesis of a $[Fe_4S_4]$−S−Ferriheme Bridged Assembly Containing an Isobacteriochlorin Component: A Further Analogue of the Active Site of Sulfite Reductase

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The catalytic site of sulfite reductase consists of exchange-coupled cubane-type Fe_4S_4 cluster and siroheme components bridged by a cysteinate sulfur atom in the *Escherichia coli* enzyme and, presumably, by sulfide in certain other bacterial enzymes. A synthetic analogue of the latter in the form of a sulfide-bridged assembly has been synthesized by the reaction of [Fe(OEiBC)Cl] and the site-differentiated cluster $[Fe_4S_4(LS_3)(SSiMe_3)]^{2-}$ (**4**) in benzene/acetonitrile. Demonstration of the $[Fe_4S_4(LS_3)-S-Fe^{III}(OEiBC)]^{2-}$ (**5**) formulation follows from spectroscopic evidence. The Mössbauer spectrum proves the $[Fe_4S_4]^{2+}$ core oxidation state. ^{1}H NMR spectra demonstrate a close juxtaposition of macrocycle and cluster owing to the appearance of doubled cluster resonances when two diastereomers of **5** are prepared from an isomeric mixture of [Fe(OEiBC)Cl]. Isotropic shifts of **5** are dominantly contact in origin and are 7−11 times larger than in **4**, a typical $[Fe_4S_4]^{2+}$ cluster, owing to spin delocalization involving Fe(III). These shifts exhibit a $1/T$ dependence whereas those of **4** are non-Curie. Spin delocalization requires the presence of a covalently bridged structure. Assembly **5** is a closer analogue of the native site than other bridged assemblies prepared in this laboratory because the OEiBC macrocycle, as that in siroheme, is at the isobacteriochlorin oxidation level. This work contributes to an experimental protocol for coupling cluster and heme components into bridged assemblies. (LS_3 = trianion of 1,3,5-tris((4,6-dimethyl-3-mercaptophenyl)thio)-2,4,6-tris(*p*-tolylthio)benzene; OEiBC = dianion of octaethylisobacteriochlorin.)

Introduction

We have recently emphasized the existence of a subset of active sites in metallobiomolecules termed bridged biological metal assemblies.[1] These assemblies consist of two discrete fragments that are juxtaposed wholly or partially by one or more covalent bridges. Among the structurally and/or spectroscopically defined examples are the assimilatory sulfite reductases (SiR), which catalyze the overall reaction $SO_3^{2-} + 7H^+ + 6e^- \rightarrow HS^- + 3H_2O$ without release of intermediate substrate oxidation states to the environment.[2] The cumulative spectroscopic results of Siegel and co-workers[3] on *Escherichia coli* SiR, an $\alpha_8\beta_4$ hemoflavoprotein, have demonstrated that the catalytic site of the enzyme consists of an Fe_4S_4 cubane-type cluster covalently bridged to a siroheme. The two fragments are exchange-coupled in the three accessible oxidation states of the site. The structural nature of the site was largely confirmed by an initial X-ray structure determination which, however, was not able to identify securely the bridge atom.[4] Very recently, these classic studies on *E. coli* SiR have culminated in the solution of the structure of the hemoprotein subunit β at 1.6 Å.[5] The site is depicted in Figure 1 in the phosphate-bound form. The Fe(III) atom is tightly bound to the phosphate (Fe−O = 1.85 Å) in a high-spin configuration and is displaced 0.29 Å toward the oxygen atom from the N_4 mean plane of the S_4-ruffled macrocycle. The siroheme and cubane cluster are bridged through a cysteinate sulfur atom with a notably long $Fe^{III}-S$ interaction of 2.85 Å. When phosphate is replaced by sulfite and the structure determined at 2.2 Å resolution,[5] the substrate binds through sulfur to low-spin siroheme with a Fe−S bond distance of 2.26 Å. The Fe−(μ-S) distance shortens to 2.46 Å, and the Fe(III) atom is 0.09 Å from the N_4 mean plane in the direction of substrate.

In addition to cysteinate-bridged active sites, there is evidence consistent with the existence of sulfide-bridged SiR sites. The most probable example of this case, although not proven by protein crystallography, is the assimilatory-type enzyme of *Desulfovibrio vulgaris*, which also contains a coupled siroheme and Fe_4S_4 cluster.[6,7] Observations supporting a sulfide bridge include an analytical atom ratio Fe:S ≈ 1:1[6] and radiolabel exchange by one sulfur atom,[7a] implying a structural difference between it and more slowly exchanging sulfur atoms. There are, however, sufficient cysteinyl residues (seven or eight)[7c] to provide the four cysteinyl ligands of the cluster. The dissimila-

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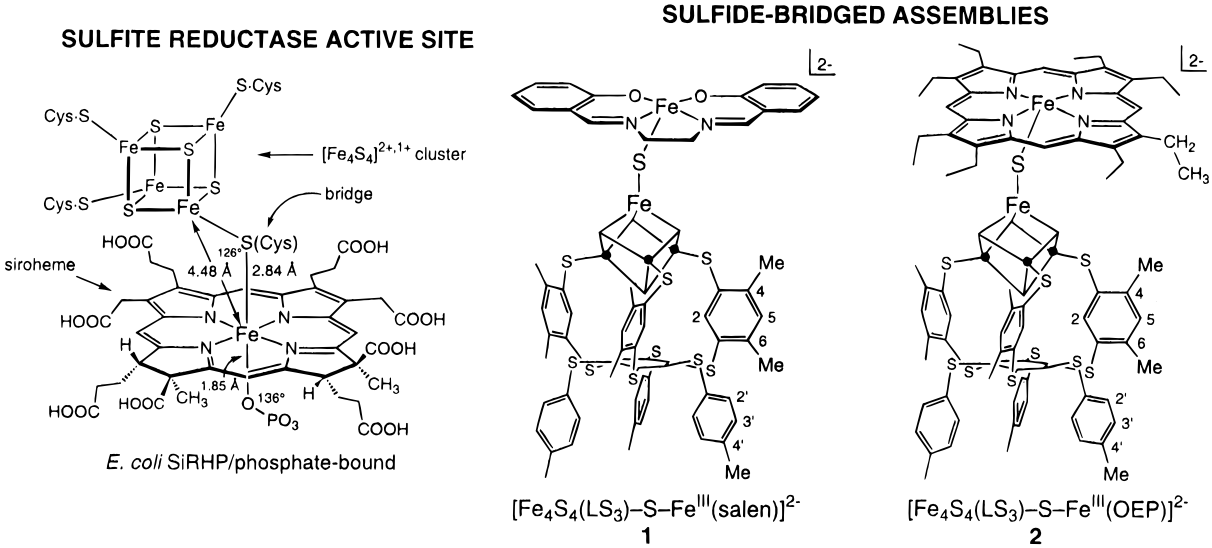

Figure 1. Left: structure of the *E. coli* sulfite reductase hemoprotein active site in the phosphate-bound form.[5] Right: depictions of two previously prepared sulfide-bridged assemblies **1** and **2** based on the Fe^{III}(salen) and Fe^{III}(OEP) fragments.[9,10]

tory SiR from *D. vulgaris* also carries a coupled siroheme-cluster entity,[8] but the nature of the bridge is unknown.

As initial synthetic analogues of the SiR site, we have recently prepared the bridged assemblies **1** and **2** in Figure 1.[9,10] Assembly **1** contains the acyclic salen ligand, while **2** includes the synthetic porphyrin OEP;[11] both Fe(III) sites are five-coordinate and high-spin. Bridge formation was demonstrated by a marked enhancement of the isotropic ^{1}H NMR shifts of the cluster ligand owing to spin delocalization across the bridge. The native porphyrinic iron component is specialized beyond common biological porphyrins. Siroheme is an iron complex of sirohydrochlorin,[12] which, with adjacent pyrroline rings, is at the isobacteriochlorin oxidation level. The biological function of the reduced macrocyclic ring system remains somewhat obscure but may be related to its relative ease of oxidation[13,14] and/or its increased N_4 hole size and attendant conformational flexibility,[15] which may be beneficial in substrate binding. Consequently, the formation of an analogue with enhanced fidelity to the native site, as either a structural or reactivity analogue, requires inclusion of an iron−isobacteriochlorin fragment. We report here the construction and certain properties of a sulfide-bridged assembly containing such a fragment. The macrocycle employed is octaethylisobacteriochlorin,[13] iron complexes of which have been prepared and examined to elicit structure−property relationships relevant to biological function in sulfite and nitrite reductases.[14−19]

Experimental Section[20]

Preparation of Compounds. All operations were carried out under a pure dinitrogen atmosphere. Benzene and hexane were distilled from sodium, and acetonitrile was distilled from CaH_2. H_2(OEiBC),[13] [Fe(OEiBC)Cl],[14] the protonated tridentate ligand $L(SH)_3$,[21] and $(Bu_4N)_2$-$[Fe_4S_4(LS_3)(OC_6H_4$-*p*-Br)][22] were prepared by published procedures. The diastereomeric mixture of H_2(OEiBC) was chromatographically resolved into the *ttt* and *tct* forms, and [Fe(*ttt*-OEiBC)Cl] was obtained as described.[17] Absorption spectrum of [Fe(*ttt*-OEiBC)Cl] (acetonitrile): λ_{max} (ϵ_M) 378 (49 100), 553 (8970), 601 (13 900), 760 (1340) nm. Because H_2(OEiBC) and its iron complexes are light-sensitive, they were prepared and manipulated under subdued illumination.

$(Bu_4N)_2[Fe_4S_4(LS_3)(SSiMe_3)]$. To a stirred solution of 150 mg (76.7 μmol) of $(Bu_4N)_2[Fe_4S_4(LS_3)(OC_6H_4$-*p*-Br)] in 40 mL of acetonitrile was added 70 μL (0.33 mmol) of $(Me_3Si)_2S$ (*Caution*: stench). The reaction mixture was stirred overnight, and 40 mL of ether was slowly added. The mixture was stored overnight at −20 °C. The solid was collected by filtration, washed with ether, and dried in vacuo to give 102 mg (69%) of product as a dark brown solid, pure by an ^{1}H NMR criterion. ^{1}H NMR (CD_3CN): δ 8.16 (5-H), 7.12 (2′-H), 6.82 (3′-H), 5.08 (2-H), 3.85 (6-Me), 3.71 (4-Me), 2.23 (4′-Me), 0.75 ($SiMe_3$). This cluster has been previously generated in solution[22] but not isolated.

$(Bu_4N)_2[Fe_4S_4(LS_3)$−S−Fe(*ttt*-OEiBC)]. A solution of 10.0 mg (5.17 μmol) of $(Bu_4N)_2[Fe_4S_4(LS_3)(SSiMe_3)]$ in 0.3 mL of acetonitrile was treated dropwise with a solution of 3.25 mg (5.17 μmol) of [Fe(*ttt*-OEiBC)Cl] in 0.2 mL of benzene. The reaction mixture was stirred overnight, and the volatiles removed in vacuo. The product was obtained as a black solid; its ^{1}H NMR spectrum indicated *ca.* 95% purity. Absorption spectrum (acetonitrile): λ_{max} (ϵ_M) 376 (51 100), 558 (sh, 14 400), 593 (18 000) nm. ^{1}H chemical shifts are listed in Table 1. The diastereomeric mixture of this compound (*ttt* + *tct*) was prepared analogously.

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Table 1. Selected Chemical (ppm) and Isotropic[a] Shifts of Cluster **4** and Bridged Assemblies **2** and **5** in Acetonitrile (298 K)

ligand	position	**4**	**2**[b]	**5**[c]
LS_3	2-H	5.08 (+1.64)	−11.3 (+18.0)	−11.1 (+17.8)
	4-Me	3.71 (−1.47)	13.6 (−11.4)	13.8 (−11.6)
	5-H	8.16 (−1.52)	17.4 (−10.8)	17.2 (−10.6)
	6-Me	3.85 (−1.61)	16.1 (−13.9)	16.0 (−13.8)
	2′-H	7.12	8.45	8.63
	3′-H	6.82	5.92	6.02
	4′-Me	2.23	2.42	2.49
OEP/OEiBC[d]	CH_2		31.3 (−27.1)	34.9, 32.8(2),
			22.9 (−18.7)	30.6(2), 30.0,
				23.8, 22.6,
				20.1, 18.7
	meso-H		−38.1 (+48.1)	−14.1 (+21.8)
				−58.4 (+65.9)
				−80.5 (+88.2)

[a] $(\Delta H/H_0)_{iso} = (\Delta H/H_0)_{dia} - (\Delta H/H_0)_{obs}$; isotropic shifts in parentheses. Diamagnetic shifts in CD_3CN, from $(Bu_4N)_3(LS_3)$: 2-H, 6.72; 4- and 6-Me, 2.24; 5-H, 6.64. [b] Data from ref 10. [c] *ttt* isomer, assignment incomplete; at least two signals do not arise from pyrrole CH_2. [d] Diamagnetic shifts from H_2(OEiBC) in $CDCl_3$.[13]

Physical Measurements. All measurements were performed under strictly anaerobic conditions. Absorption spectra were recorded in a Cary 219 spectrophotometer. ^{1}H NMR spectra were obtained with a Bruker AM 500 spectrometer. Mössbauer spectroscopic measurements of powdered samples were made as previously described;[23] isomer shifts are relative to iron metal at room temperature.

Results and Discussion

The following species are of principal interest in this work:

$[Fe_4S_4(LS_3)-S-Fe(salen)]^{2-}$	**1**
$[Fe_4S_4(LS_3)-S-Fe(OEP)]^{2-}$	**2**
$[Fe_4S_4(LS_3)(OC_6H_4\text{-}p\text{-}Br)]^{2-}$	**3**
$[Fe_4S_4(LS_3)(SSiMe_3)]^{2-}$	**4**
$[Fe_4S_4(LS_3)-S-Fe(OEiBC)]^{2-}$	**5**

Preparation of the Bridged Assembly. Bridged assembly **2** was prepared by six independent reactions as part of its structure proof in the absence of an X-ray structure determination.[10] All approaches involved the use of the site-differentiated clusters $[Fe_4S_4(LS_3)L']^{2-}$, in which an initial substitution reaction is confined to the unique iron site[22,24] because of 3-fold coordination by the semirigid cavitand ligand LS_3.[21] Four of these methods involve coupling of the $Fe_4S_4(LS_3)$ and Fe^{III}(OEP) fragments via the functionalized cluster $[Fe_4S_4(LS_3)(SH)]^{2-}$ in the presence of coordinated or free base. When applied to the present problem, these reactions resulted in varying extents of assembly formation, but the desired product proved difficult to purify. After considerable experimentation, the method of Figure 2 was found to be the most satisfactory.

Octaethylisobacteriochlorin is obtained as a mixture of two predominant diastereomers having C_2 (*ttt*) and C_s (*tct*) symmetries.[13,25] Any five-coordinate complex [M(OEiBC)X] will exist as a mixture of three diastereomers, two of which derive from the *tct* macrocycle. This situation introduces considerable complexity in ^{1}H NMR spectra, there being 16 signals alone for the diastereotopic pyrrole methylene groups. In the following reactions, we have utilized a mixture of OEiBC isomers and the *ttt* form alone. The latter has been separated from the mixture by medium-pressure liquid chromatography.[17] We have reproduced this procedure, which in our hands usually affords *ca.* 20 mg of *ttt* isomer of ≳95% purity from 50 mg of H_2(OEiBC). Given that large-scale preparation of H_2(OEiBC) is not practicable[13] and isomer separation is laborious and is not readily conducted on larger than the indicated scale, reactions of the OEiBC component which follow were performed on ≲10 mg. Product characterization is necessarily spectroscopic.

The cluster component of the bridged assembly was obtained first by conversion in good yield of previously isolated phenolate cluster **3** to the trimethylsilanethiolate cluster **4** by reaction with hexamethylsilathiane. The latter is coupled with [Fe(OEiBC)Cl] by means of reaction 1 to afford the bridged assembly **5**. The

$$[Fe_4S_4(LS_3)(SSiMe_3)]^{2-} + [Fe(OEiBC)Cl] \rightarrow [Fe_4S_4(LS_3)-S-Fe(OEiBC)]^{2-} + Me_3SiCl \quad (1)$$

formation of **4** and **5** takes advantage of the large energy differences between Si−S and Si−O (*ca.* 60 kcal/mol) and Si−Cl (*ca.* 40 kcal/mol) bonds.[26] By ^{1}H NMR analysis, assembly **5** was obtained in substantial purity (≳95%), the most persistent impurity being $[Fe^{II}(OEiBC)]$.[14] The electronic absorption spectrum of isomerically pure **5**, presented in Figure 3, retains the Soret and visible features of [Fe(*ttt*-OEiBC)Cl] with small shifts in the band maxima. Crystalline samples of $(Bu_4N)_2$[**5**] have not been achieved.

Structure and Spin Delocalization. The Mössbauer spectrum of $(Bu_4N)_2$[**5**] at 77 K is shown in Figure 4 and was fitted as two overlapping quadrupole doublets. When analyzed under a 4:1 intensity constraint, the major doublet has $\delta = 0.42$ mm/s and $\Delta E_Q = 1.20$ mm/s, parameters nearly identical to those of **2** (0.46, 1.14 mm/s) and otherwise highly similar to those of $[Fe_4S_4(SR)_4]^{2-}$ clusters.[9,10] The minor doublet is described by $\delta = 0.36$ mm/s and $\Delta E_Q = 0.61$ mm/s, values typical of high-spin ferric heme complexes.[27] Consequently, the $[Fe_4S_4]^{2+}$ core oxidation state with $S = 0$ has been maintained in assembly formation and the porphyrinic Fe(III) site is very probably five-coordinate, as indicated in Figure 2.

Proof of covalent bridge formation follows from consideration of ^{1}H NMR spectra. As we have demonstrated at length,[10,21,22,24] chemical shifts of 4-Me, 5-H, and 6-Me of the coordinating arms of the LS_3 ligand system are extremely responsive, separately or collectively, to the nature of the ligand at the unique site. This arises because of thermal population of an excited paramagnetic state and the attendant sensitivity of isotropic shifts which, on the basis of the signs of these shifts in a large variety of $[Fe_4S_4(SR)_4]^{2-}$ clusters, are overwhelmingly contact in origin. The spectral features of a diastereomeric mixture of assembly **5** are quite complex owing to line widths and signal overlap. The situation is improved somewhat when **5** is prepared from [Fe(*ttt*-OEiBC)Cl]; its spectrum is presented in Figure 5 (lower) and chemical shifts are listed in Table 1. Eight signals are observed in the 18−35 ppm range, two of which are *ca.* twice the intensity of the others. The first six downfield signals are most likely those of the pyrrole methylene groups; the remainder and certain other upfield signals derive from the pyrroline ring substitutents.[17] Their multiplicity indicates diastereotopism engendered by an axial ligand. Meso proton resonances occur in a 1:2:1 intensity pattern similar to that of [Fe(*ttt*-OEiBC)Cl].[17]

Resonances of the LS_3 cluster ligand are considerably more informative. When **5** is prepared from a diastereomeric mixture

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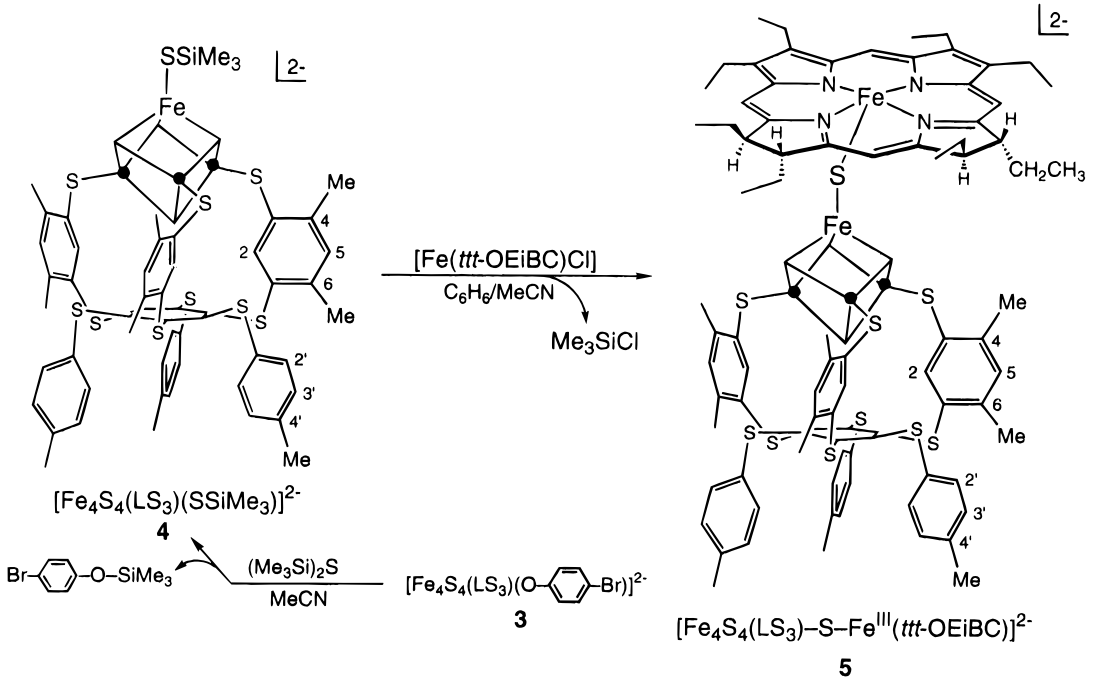

Figure 2. Synthesis of the bridged assembly $[Fe_4S_4(LS_3)-S-Fe(OEiBC)]^{2-}$ (**5**) from cluster precursors **3** and **4** and [Fe(OEiBC)Cl]. The *ttt* and *tct* diastereomers of the assembly are formed from an isomeric mixture of the Fe(III) complexes; the *ttt* isomer is depicted. The cluster ligand numbering scheme is indicated.

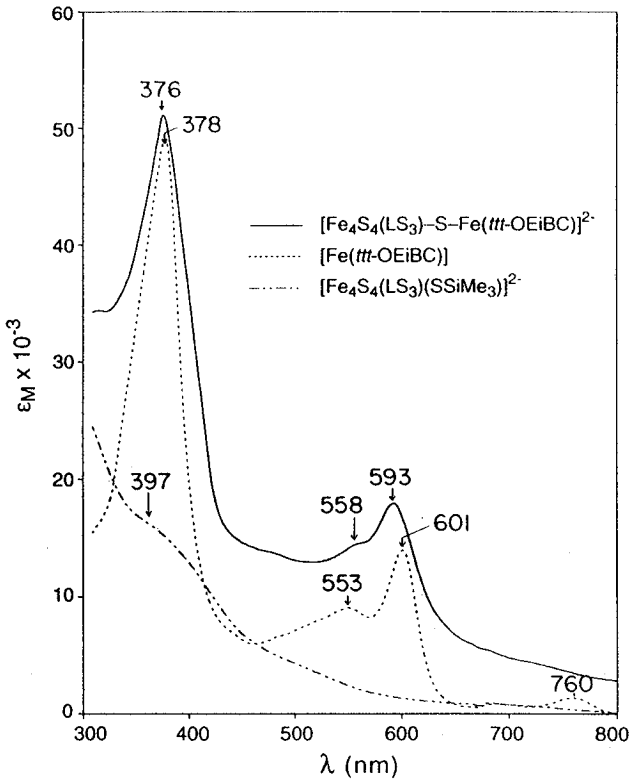

Figure 3. Absorption spectra of [Fe(*ttt*-OEiBC)Cl] (···), $[Fe_4S_4(LS_3)(SSiMe_3)]^{2-}$ (—··—), and $[Fe_4S_4(LS_3)-S-Fe(ttt\text{-}OEiBC)]^{2-}$ (—) in acetonitrile solution. Band maxima are indicated.

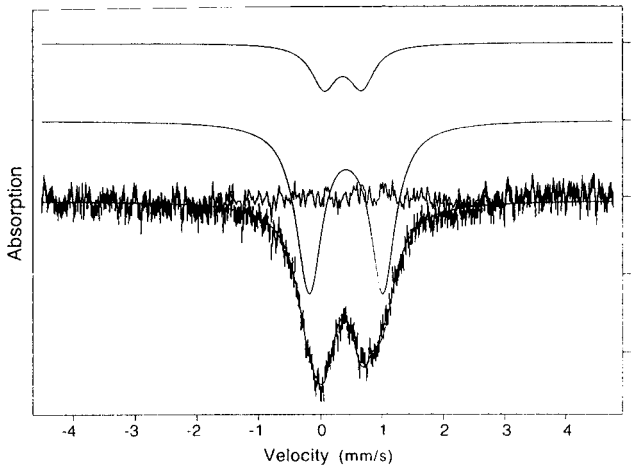

Figure 4. Zero-field Mössbauer spectrum of $(Bu_4N)_2[Fe_4S_4(LS_3)-S-Fe(OEiBC)]$. The solid line is a fit to the data using the parameters in the text; subspectra of the high-spin Fe^{III}(OEiBC) (upper) and $[Fe_4S_4]^{2+}$ (middle) fragments are shown.

of [Fe(OEiBC)Cl], the spectrum in Figure 5 (upper left) is obtained; signals of 4-Me, 5-H, and 6-Me appear doubled. The effect is clearest with 4-Me, whose signals are split by 0.18 ppm. When the synthesis is repeated with the *ttt* complex, the less intense component of each signal is barely discernible (Figure 3, upper right), proving that the doubled signals arise from the *ttt* + *tct* isomeric mixture. Because the diastereomers are sensed by resonances in the *cluster* fragment, it follows that this fragment and the Fe(OEiBC) group are rather closely juxtaposed through a covalent bridge.

Further indication of a bridged structure is provided by the values of the chemical and isotropic shifts of the ligand resonances summarized in Table 1. The shifts of **4** are typical of $[Fe_4S_4]^{2+}$ clusters with arenethiolate ligands. Isotropic shifts are $\lesssim|2|$ ppm and the pattern of signs is consistent with the propagation of positive-spin density in the π-HOMO of the odd-alternate ligand and the consequent contact shifts.[28] In this manner, the spin at C(2,4,6) is parallel to the applied field, such that the isotropic shift of 2-H is positive (upfield) while the shifts of 4-Me and 6-Me are negative (downfield). Negative spin arises at C(5) from spin correlation effects. The same signs of isotropic shifts are exhibited by assembly **5**, but the magnitudes of the shifts are 7−11 times larger. Note further that these are nearly identical to those of **1**[10] and **2** (Table 1). As shown previously with $[Fe_4S_4(LS_3)(SH)]^{2-}$,[10] the absolute values of the isotropic shifts of $[Fe_4S_4]^{2+}$ clusters increase with

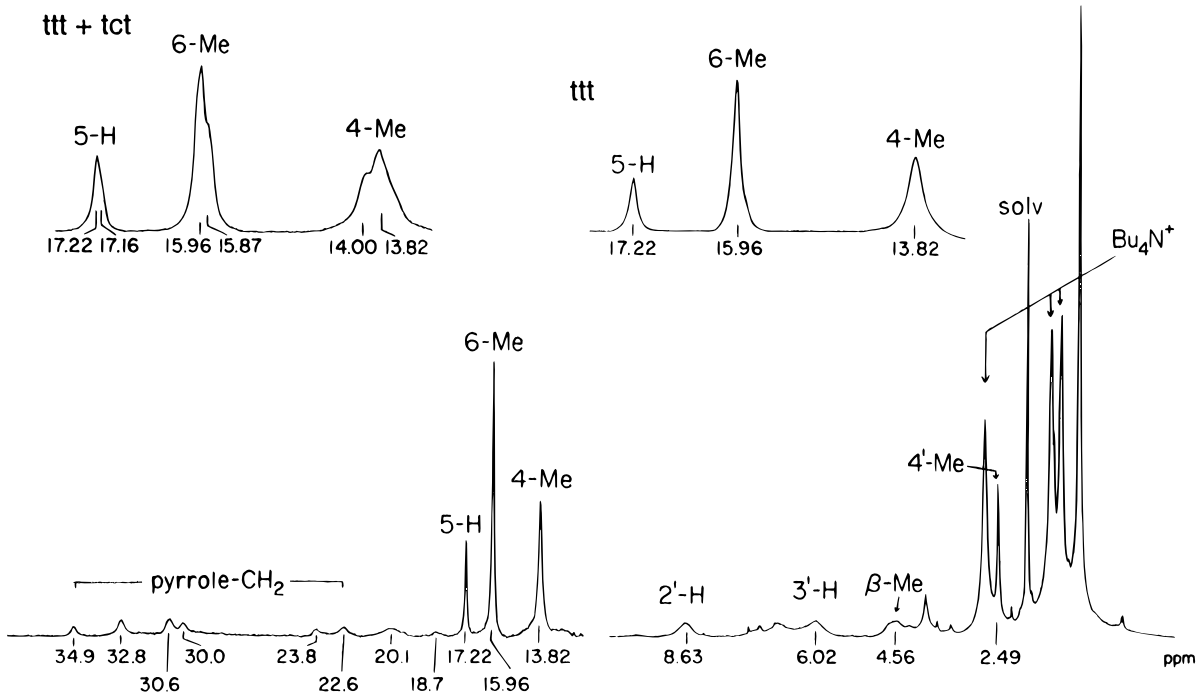

Figure 5. ^{1}H NMR spectra in CD_3CN solutions at 298 K. Lower: $[Fe_4S_4(LS_3)-S-Fe(ttt\text{-}OEiBC)]^{2-}$; the upfield region is omitted. Upper: diastereomeric mixture and the *ttt* isomer of $[Fe_4S_4(LS_3)-S-Fe(OEiBC)]^{2-}$ showing the signals of substituents of the coordinated arms of the LS_3 ligand system.

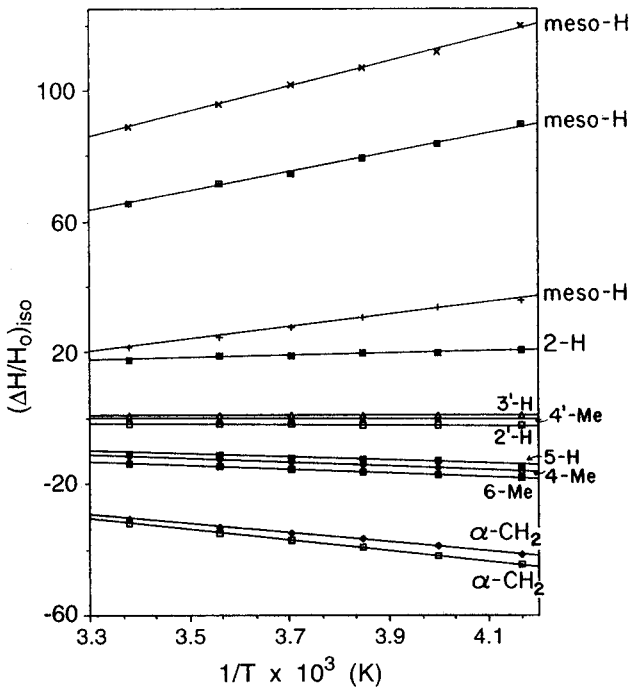

Figure 6. Temperature dependencies of the isotropic shifts of $[Fe_4S_4(LS_3)-S-Fe(OEiBC)]^{2-}$ in CD_3CN solution at 235−300 K. The signals α-CH_2 refer to the pyrrole methylene groups. Because of the complexity of the OEiBC methylene and methyl resonances, only the shifts of the most downfield (and fully resolved) methylene signals are plotted.

increasing temperature as the excited paramagnetic state is increasingly populated. In contrast, the temperature dependencies of the isotropic shifts of **5**, displayed in Figure 6, exhibit the $1/T$ behavior of eq 2 for contact shifts of a Curie paramag-

$$(\Delta H/H_0)_{con} = -[A_i/\hbar\gamma_H][g\mu_B S(2S+1)/3kT] \qquad (2)$$

net with spin S; other symbols have their usual meanings.[28] Assembly **2** behaves similarly.[10,29] The Fe(III) spin, designated as positive, is the frame of reference in the NMR experiments because it is aligned parallel to the magnetic field. In effect, the cluster is part of an extended Fe(III) ligand system and is subject to spin delocalization effects as is any ligand in a paramagnetic complex, orbital symmetry permitting. Indeed, the NMR properties of **1**, **2**, and **5** are sufficiently similar as to require the same bridge, which from the methods of synthesis must be sulfide.

The existence of ^{57}Fe magnetic hyperfine interactions at all iron sites in the cluster of oxidized *E. coli* SiR establishes exchange coupling between the cluster and siroheme.[3a] Because the closest noncovalent contact between cluster and siroheme is 3.63 Å,[5] it is likely that the exchange interaction is transmitted primarily through the covalent bridge. Recently, Bominaar *et al.*[30] have described spin coupling in the oxidized active site by a theoretical treatment which includes double exchange and vibronic coupling. They conclude that the Fe(III) site in siroheme and the cluster are mildly perturbed by exchange coupling, which mixes the first excited triplet state of $[Fe_4S_4]^{2+}$ into its diamagnetic ground state. The theory is couched in magnetic and vibronic interactions; at this point it is not readily translated into NMR contact shifts. From the NMR point of view, the relatively large isotropic shifts of **1**, **2**, and **5** arise from contact interactions resulting from electron delocalization across the sulfide bridge. While we do not know the orbital pathway(s), the net result is to place positive spin equally in p-type orbitals of three cluster ligand sulfur atoms which is delocalized in the HOMO of the phenyl rings. This effect

(28) Bertini, I.; Luchinat, C. *NMR of Paramagnetic Molecules in Biological Systems*; Benjamin/Cummings Publishing Co.: Reading, MA, 1986; Chapter 2. The signs of the contact shifts follow from the relationship $A_i = Q\rho^{\pi}_C$ where A_i is the electron−nuclear coupling constant of nucleus i, ρ^{π}_C is the spin density in a C pπ orbital, and $Q = Q_{CH}$ (negative) or Q_{CMe} (positive).

(29) Note that, in Figure 10 of ref 10, the ordinate pertaining to the temperature-dependent shifts of **2** should be labeled $-(\Delta H/H_0)_{iso}$; in Figure 6, the shifts (rather than their negative values) are plotted.

(30) Bominaar, E. L.; Hu, Z.; Münck, E.; Girerd, J.-J.; Borshch, S. A. *J. Am. Chem. Soc.* **1995**, *117*, 6976.

outweighs the intrinsic contact shifts of the cluster by roughly an order of magnitude. Further, the effect is so pronounced that the chemical shifts of 2′-H, 3′-H, and 4′-Me, distantly located on noncoordinating ligand "legs", are influenced relative to **4**.

Summary. An analogue of the oxidized catalytic site of a subset of sulfite reductases containing coupled Fe_4S_4 and siroheme components has been prepared. It differs from that developed earlier[9,10] by incorporation of an Fe(III) isobacteriochlorin fragment, which has the same macrocycle oxidation level as, and presumably approaches the flexibility and core size of, siroheme. In the absence of proof by X-ray diffraction, the existence of a sulfide bridge is collectively supported by the method of synthesis, detection of diastereomeric assemblies by cluster ^{1}H NMR signal multiplicities, and greatly enhanced proton isotropic shifts of the cluster ligand. Assembly **5** differs from the native oxidized site by having a high-spin heme Fe(III) center, whereas the heme is low-spin in the *D. vulgaris* enzyme,[6] the ligand distal to the bridge likely being a histidyl imidazole group.[31,32] Other than the simple observation that Mössbauer spectroscopic parameters of clusters are similar, the difference in heme spin states prevents meaningful comparisons between native site and analogue spectroscopic properties.[34] In both **2** and **5**, the delocalized spin density affecting isotropic shifts would contribute to hyperfine interactions at the cluster iron atoms. Unfortunately, we have not been able to prepare a stable thiolate-bridged Fe_4S_4−heme assembly in order to compare the influence of sulfide vs thiolate in the context of spin delocalization, isotropic shifts, and ^{57}Fe hyperfine interactions.

The successful preparation of **5** further substantiates an experimental protocol[10] for coupling cluster and heme fragments into bridged assemblies and the utilization of site-differentiated clusters in the process. Both **2** and **5** (with possible enrichment in ^{57}Fe) should find application in determining the extent of magnetic hyperfine interactions by exchange coupling across a sulfide bridge. It remains to be fully determined if these assemblies are sufficiently robust for reactivity studies and if assemblies of different ligand design can improve stability and afford crystalline compounds necessary for complete structure definition.

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(31) Cowan, J. A.; Sola, M. *Inorg. Chem.* **1990**, *29*, 2176.

(32) There is insufficient sequence homology between the *E. coli* SiR hemoprotein (570 residues, 6 Cys)[5,33] and *D. vulgaris* SiR (217 residues, 7 or 8 Cys)[7c] to permit any deduction as to the presence or absence of a bridging cysteinate ligand in the latter.

(33) Ostrowski, J.; Wu, J.-Y.; Rueger, D. C.; Miller, B. E.; Siegel, L. M.; Kredich, N. M. *J. Biol. Chem.* **1989**, *264*, 15726.

(34) Attempts to stabilize a low-spin Fe(III) heme fragment in **2** by coordination of axial ligands L led instead to an internal redox reaction forming $[Fe^{II}(OEP)L_2]$ and the persulfide-bridged double cubane $\{[Fe_4S_4(LS_3)]_2S_2\}^{4-}$.[10]