Synthesis and Electron Delocalization of $[Fe_4S_4]$—S—Fe(III) Bridged Assemblies Related to the Exchange-Coupled Catalytic Site of Sulfite Reductases

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Abstract: Because of the pervasive occurrence of magnetically coupled siroheme and Fe_4S_4 units in assimilatory and dissimilatory sulfite and nitrite reductases, we have undertaken the synthesis of the sulfide-bridged assembly Fe_4S_4—S—heme as a possible analogue to the active sites of certain assimilatory enzymes. The approach has utilized iron subsite-differentiated clusters of the type $[Fe_4S_4(LS_3)L']^{2-}$, which undergo regiospecific substitution at the unique subsite. Reaction of $[Fe_4S_4(LS_3)(SEt)]^{2-}$ with limited H_2S in acetonitrile affords the functionalized cluster $[Fe_4S_4(LS_3)(SH)]^{2-}$ (**4**), which exists in equilibrium with the μ-S double cubane $\{[Fe_4S_4(LS_3)]_2S\}^{4-}$ (**6**) and H_2S. Reaction of **4** and $[Fe(salen)]_2O$ gave the bridged assembly $[Fe_4S_4(LS_3)$—S—$Fe^{III}(salen)]^{2-}$ (**8**), detectable by its characteristic isotropically shifted ^1H NMR spectrum. Six routes were devised to a related heme-based assembly: directed acid–base coupling of **4** with $[Fe(OEP)]_2O$, $[Fe(OEP)(OMe)]$, $[Fe(OEP)(OC(Me)=CH_2)]$, and $[Fe(OEP)(OClO_3)]/Et_3N$; Si—S bond cleavage in the reaction of $[Fe_4S_4(LS_3)(SSiEt_3)]^{2-}$ with [Fe(OEP)F]; oxidative addition of $[Fe^{II}(OEP)]$ to the disulfide bond of the μ-S_2 double cubane $\{[Fe_4S_4(LS_3)]_2S_2\}^{4-}$ (**7**). In each case, the product was $[Fe_4S_4(LS_3)$–S–$Fe^{III}(OEP)]^{2-}$ (**9**), recognizable by UV–visible absorption and ^1H NMR spectra. Both **8** and **9** contain $[Fe_4S_4]^{2+}$ and high-spin Fe(III) fragments. Isotropic shifts mainly contact in origin that are enhanced by factors of 7-12 compared to those of precursor cluster **4**, and the Curie-type temperature dependence of the shifts of **9**, originate from extensive spin localization from the Fe(III) fragment to the cluster. This effect requires the existence of a covalent bridge between the fragments and, together with the methods of synthesis and other spectroscopic observations, provides structure proof of the bridged assemblies. These species sustain two one-electron reduction reactions; other reactions of **9**, which alter or cleave the bridge, are summarized. The electronic features of bridged assemblies such as **8** and **9** approach the intrinsic magnetic and spectroscopic properties of a structurally similar unit in the oxidized enzymes and potentially provide a means of identification of such units. (LS_3 = 1,3,5-tris[(4,6-dimethyl-3-mercaptophenyl)-thio]-2,4,6-tris(*p*-tolylthio)benzene(3–); OEP = octaethylporphyrinate(2–); salen = 1,2-bis(salicylideneamino)ethane-(2–).)

Introduction

A small but increasing number of active sites of metalloenzymes may be described as *bridged biological assemblies* inasmuch as a single metal center or a metal cluster is linked to the same or different type of unit via covalent interactions through one or more bridging atoms. A prominent example is the binuclear Cu–Fe center in eukaryotic cytochrome *c* oxidases and prokaryotic cytochrome and quinol oxidases.[1] Additional instances of bridged assemblies are found in sulfite reductases (SiR), which catalyze the reaction $SO_3^{2-} + 7H^+ + 6e^- \rightarrow HS^- + 3H_2O$. The properties of the assimilatory SiR of *Escherichia coli* have been elucidated in a now-classic series of investigations by Siegal and co-workers.[2-9]

Sulfite reductase of *E. coli* is an $\alpha_8\beta_4$ hemoflavoprotein whose minimal functional unit is the β subunit ($M_r \sim 57\,000$).[5] Protein crystallography[2] and abundant spectroscopic evidence[3-9] have shown that the active site is composed of a cubane-type Fe_4S_4 cluster bridged to siroheme, a specialized iron porphyrin at the isobacteriochlorin stage of oxidation. In all oxidation states, $[Fe_4S_4]^{2+}$—Fe^{3+}, $[Fe_4S_4]^{2+}$—Fe^{2+}, and $[Fe_4S_4]^{+}$—Fe^{2+}, the cluster and siroheme are exchange-coupled.[3,4,6,9] In fully oxidized SiR, the siroheme Fe(III) atom is high-spin ($S = 5/2$) and five-coordinate and is separated by 4.4 Å from the nearest iron atom in the $[Fe_4S_4]^{2+}$ cluster ($S = 0$).[2] The crystal structure lacked resolution sufficient to identify the bridging ligand; the electron density at and near the bridge atom site was consistent with a sulfur or oxygen atom, but is not compatible with bridging through a large side chain from residues such as Tyr or Glu. Nitrogen bridging has been eliminated by ENDOR results.[6] Janick and Siegel[4a] originally proposed a model of the active site in which the cluster and siroheme are bridged by a cysteinyl sulfur atom. More recently, an explicit model with the same basic features that is consistent with the X-ray data and all spectroscopic results has been put forward by Ostrowski *et al.*[8] This structure is shown in schematic form in Figure 1.

A second SiR species of current interest are the assimilatory-type enzymes of relatively low molecular weight ($M_r \sim 27\,000$) isolated from anaerobic bacteria.[10,11] The SiR of *Desulfovibrio*

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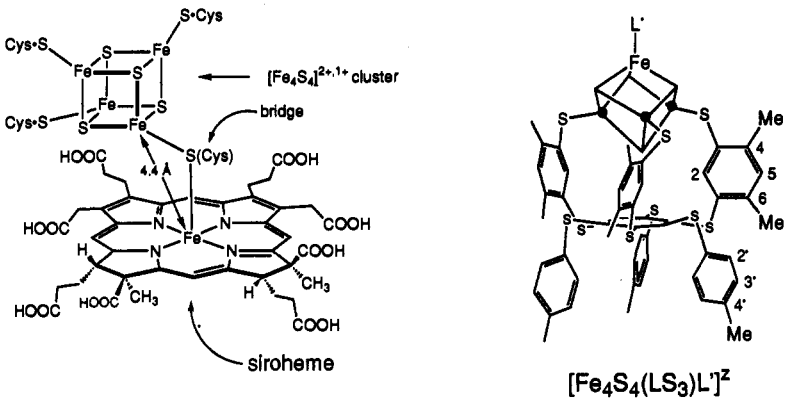

Figure 1. Schematic representations of the structures of the sulfite reductase active site with bridging cysteinate or sulfide (left) and a subsite-differentiated cluster with generalized ligand L′ at the unique subsite (right). The phenyl ring numbering scheme is used in assignments of ^{1}H NMR spectra.

vulgaris (Hildenborough) has been the most thoroughly investigated.[10,12,13] As isolated, the enzyme was reported to contain low-spin Fe(III) siroheme which from Mössbauer spectroscopic results was concluded to be exchange-coupled to an $[Fe_4S_4]^{2+}$ cluster.[10] On the basis of the analytically determined atom ratio Fe:S ≈ 1, Huynh *et al.*[10] suggested a sulfide bridge between the coupled components. More recently, Cowan and co-workers have provided NMR evidence that the axial siroheme ligand distal to the cluster is a histidyl imidazole group[13a] and have found that one sulfur atom underwent radiolabel exchange more rapidly than others,[13c] a behavior not inconsistent with the presence of a unique sulfide functioning as a bridge. The corresponding bridge structure is also represented in Figure 1.

Dissimilatory SiR from sulfate-reducing bacteria,[14] typified by $M_r \sim 200\,000$ and an $\alpha_2\beta_2$ subunit configuration, have been less thoroughly investigated. However, two such enzymes, from *Desulfovibrio baculatus* and *D. vulgaris*, have been shown to contain exchange-coupled siroheme and Fe_4S_4 clusters.[14] The identity of the bridge in these species is unknown. Additionally, there is spectroscopic evidence for magnetic interactions between these same components in SiR[15] and nitrite reductase[16] from spinach.

Given the pervasive occurrence of a coupled siroheme–Fe_4S_4 array in sulfite and nitrite reductases, we have undertaken an investigation of bridged assemblies containing an Fe_4S_4 cluster and a heme group as a means of developing, in the first stage, structural synthetic analogues of these sites. Prior to the beginning of this work, sulfido-bridged double cubanes of the type $[Fe_4S_4]^{2+}$—S—$[Fe_4S_4]^{2+}$ had been prepared;[17,18] their formation, while instructive (vide infra), presents the simpler problem of bridging identical units. In a subsequent investigation,[19] we demonstrated the formation of unsymmetrical bridged assemblies utilizing subsite-differentiated Fe_4S_4 clusters[20] that were linked to Fe(II) complexes through pyridinethiolate groups. Recently, we have shown that assemblies of the type $[Fe_4S_4]^{2+}$—S—Fe^{III} can be prepared.[21] In this report we describe in detail the synthesis of such assemblies, related reaction chemistry of Fe_4S_4 clusters, and NMR evidence that the components of the assemblies are electronically coupled.

Experimental Section

Preparation of Compounds. All operations were carried out under a pure dinitrogen atmosphere. The tridentate ligand 1,3,5-tris[(4,6-dimethyl-3-mercaptophenyl)thio]-2,4,6-tris(*p*-tolylthio)benzene ($L(SH)_3$) was prepared by a published method.[22] Solid products were collected by filtration; solvent removal and drying of solids were performed in vacuo at room temperature.

(a) Clusters. $(Ph_4P)_2[Fe_4S_4(SH)_4]$. To a solution of 0.530 g (0.416 mmol) of $(Ph_4P)_2[Fe_4S_4(SEt)_4]$[23] in 50 mL of acetonitrile was added 50 mL (2.03 mmol) of H_2S (1 atm, 298 K) by syringe. The reaction mixture was stirred for 10 min, 80 mL of ether was added while stirring was continued, and the mixture was cooled at −25 °C for 3 h. The dark precipitate was collected by filtration, washed with ether, and dried in vacuo to afford 0.400 g (83%) of dark green product. Anal. Calcd for $C_{48}H_{44}Fe_4P_2S_8$: C, 49.58; H, 3.82; Fe, 19.21; P, 5.33; S, 22.06. Found: C, 48.42; H, 3.74; Fe, 19.71; P, 5.35; S, 22.68. ^{1}H NMR (CD_3CN, anion): δ 47.6 (SH).

$(Bu_4N)_2[Fe_4S_4(SH)_4]$. Hydrogen sulfide was bubbled through a suspension of 1.07 g (0.990 mmol) of $(Bu_4N)_2[Fe_4S_4(SEt)_4]$[23] in 20 mL of acetonitrile for 20 min. The color of the reaction mixture changed from dark green to dark brown, and all solid dissolved. Addition of 250 mL of ether caused precipitation of a dark solid. This material was collected and dried to afford the product as 0.828 g (86%) of a microcrystalline dark green solid; the ^{1}H NMR spectrum of the anion is identical with that in the preceding preparation.

$(Bu_4N)_2[Fe_4S_4(LS_3)(SEt)]$. The following preparation is an improvement over that given earlier.[19] $(Bu_4N)_2[Fe_4S_4(SEt)_4]$ (0.515 g, 0.476 mmol) and $L(SH)_3$ (0.452 g, 0.476 mmol) were suspended in 20 mL of pyridine. The mixture was stirred overnight, during which time the color of the reaction mixture changed from dark green to dark brown and all solid dissolved. Ether (200 mL) was added to the stirred solution, which was allowed to stand overnight. The solid that separated was collected and dissolved in 20 mL of acetonitrile, to which was added a 10% mmol

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excess of $L(SH)_3$ over that already used (to ensure complete reaction of the original cluster). The mixture was stirred for 1 h and filtered to remove excess ligand. The filtrate was reduced to dryness to afford the pure product as a dark brown solid in essentially quantitative yield. ^1H NMR (CD_3CN, anion): δ 13.2 (SCH_2), 8.15 (5-H), 7.13 (2′-H), 6.83 (3′-H), 5.10 (br, 2-H), 3.82 (6-Me), 3.69 (4-Me), 2.40 (SCH_2CH_3).

$(Bu_4N)_2[Fe_4S_4(LS_3)(SH)]$. (a) Small Scale. To a solution of 21.6 mg (0.0117 mmol) of $(Bu_4N)_2[Fe_4S_4(LS_3)(SEt)]$ in 1.0 mL of pyridine was added 0.300 mL (0.0123 mmol) of H_2S (1 atm, 298 K) in a 2.5-mL sealed vial. The solution was stirred for 5 min, and 6.0 mL of ether was added with continued stirring. The mixture was allowed to stand overnight at ambient temperature. The solid that separated was collected and dissolved in 1.0 mL of pyridine. The same quantity of H_2S was injected into the solution, which was stirred for 5 min. Ether (6.0 mL) was added by vacuum transfer to the stirred solution maintained at −40 °C. The mixture was allowed to stand at −78 °C overnight. The product was collected as 19 mg (86%) of a dark brown solid; the ^1H NMR spectrum showed >95% purity (<5% $\{[Fe_4S_4(LS_3)]_2S\}^{4-}$ impurity). ^1H NMR (CD_3CN, anion): δ 46.5 (SH), 8.18 (5-H), 7.12 (2′-H), 6.83 (3′-H), 5.15 (br, 2-H), 3.83 (6-Me), 3.77 (4-Me), 2.24 (4′-Me). Absorption spectrum (acetonitrile): λ_{max} (ϵ_M) 482 (sh, 9500) nm.

(b) Larger Scale. To a solution of 656 mg (0.355 mmol) of $(Bu_4N)_2[Fe_4S_4(LS_3)(SEt)]$ in 20 mL of acetone was added 50 mL (2.03 mmol) of H_2S (1 atm, 298 K) in a sealed flask. The reaction mixture was stirred for 5 min, and ether (60 mL) was added as the stirring was continued. The mixture was allowed to stand overnight and was filtered to afford 571 mg (88%) of product as a dark brown solid. The ^1H NMR spectrum of the product cluster is identical with that of preparation a; impurities of ≤10% of $\{[Fe_4S_4(LS_3)]_2S\}^{4-}$ were typically encountered.

$(Bu_4N)_2[Fe_4S_4(LS_3)(SSiEt_3)]$. A suspension of 33.6 mg (0.127 mmol) of $CF_3SO_3SiEt_3$ and 111 mg (1.98 mmol) of NaSH in 5.0 mL of acetonitrile was thoroughly agitated and was filtered into a solution of 215 mg (0.117 mmol) of $(Bu_4N)_2[Fe_4S_4(LS_3)(SEt)]$ in 10 mL of acetonitrile. The remaining solid was collected and washed with acetonitrile until the washings were colorless. The washings and the filtrate were combined, and the solvents were removed. The residue was recrystallized from acetonitrile/ether (1:5 v/v) at −20 °C to give the product as 180 mg (81%) of dark brown solid. ^1H NMR (CD_3CN, anion): δ 8.17 (5-H), 7.12 (2′-H), 6.82 (3′-H), 5.07 (br, 2-H), 3.84 (6-Me), 3.71 (4-Me), 2.24 (4′-Me), 1.27–1.42 ($SiCH_2CH_3$).

$(Bu_4N)_4\{[Fe_4S_4(LS_3)]_2S\}$. To a solution of 3.50 mg (1.93 μmol) of $(Bu_4N)_2[Fe_4S_4(LS_3)Cl]$[24] in 0.50 mL of acetonitrile was added 105 μL (5.36 μmol) of a 0.051 M solution of Na_2S in methanol. The mixture was stirred for several minutes, and the solvent was removed. The residue was recrystallized from dichloromethane/hexane (1:2 v/v) at −25 °C to afford the pure product as a dark brown microcrystalline product. ^1H NMR (CD_3CN, anion): δ 8.66 (5-H), 7.21 (2′-H), 6.78 (3′-H), 4.53 (6-Me), 4.25 (br, 2-H), 4.09 (4-Me), 2.27 (4′-Me).

$(Bu_4N)_4\{[Fe_4S_4(LS_3)]_2S_2\}$. To a solution of 7.0 mg (3.9 μmol) of $(Bu_4N)_2[Fe_4S_4(LS_3)Cl]$ in 0.5 mL of acetonitrile was added a solution of 0.87 mg (7.9 μmol) of Na_2S_2 in 0.5 mL of methanol. The solution was stirred for *ca.* 40 h and filtered to remove unreacted Na_2S_2 (yellow), and the filtrate was taken to dryness. The ^1H NMR spectrum indicated 70% conversion to product, the principal impurity being the preceding compound. ^1H NMR (CD_3CN, anion): δ 8.24 (5-H), 7.12 (2′-H), 6.83 (3′-H), 5.1 (br, 2-H), 3.87 (4-Me, 6-Me), 2.23 (4′-Me).

(b) $[Fe(OEP)(OC(CH_3)=CH_2)]$. This compound was obtained previously as a synthetic byproduct.[25] To a solution of 90.5 mg (0.132 mmol) of $[Fe(OEP)(OClO_3)]$[26] in acetone was added 33.5 μL (0.132 mmol) of $(Me_2N)_3PN$-*t*-Bu (Fluka). The reaction mixture was shaken for 5 min, the solvent was removed, the residue was extracted with ether, and the ether solution was filtered. The residue was extracted twice more, the ether extracts were combined, and the solvent was removed to afford the pure product as a purple solid in almost quantitative yield. ^1H NMR (acetone): δ 70.3 (br, $=CH_2$); 32.2, 28.8 (CH_2); 12.1 (OCMe); 4.86 (Me); −38.9 (*meso*-H).

(c) Bridged Assemblies. $(Bu_4N)_2[Fe_4S_4(LS_3)—S—Fe(salen)]$. To a solution of 94.9 mg (0.0521 mmol) of $(Bu_4N)_2[Fe_4S_4(LS_3)(SH)]$ in 1.0 mL of dichloromethane was added 1 equiv of a 0.28 M solution of NaSH in methanol. The solution was shaken for 5 min. To a solution of 32.1 mg (0.0524 mmol) of $[Fe(salen)]_2O$[27] in 2.5 mL of dichloromethane was added 1 equiv of a 0.28 M solution of $(Et_3NH)(ClO_4)$ in acetonitrile. This solution was shaken for 5 min and then transferred into the first solution. The reaction mixture was stirred for 5 min and was filtered. The solid was discarded, and the filtrate was cooled to −78 °C; a volume equivalent of ether was layered on the stirred solution, and the mixture was allowed to stand overnight at −78 °C. The solid was isolated by removal of solvent via cannula, washed with cold ether, and dried in vacuo at −78 °C and then at room temperature. This material was recrystallized from dichloromethane/toluene to yield the pure product as 28 mg (25%) of dark brown solid. Absorption spectrum (acetonitrile): λ_{max} (ϵ_M) 497 (sh, 12 000) nm. ^1H NMR (CD_3CN, anion): δ 61.8 (4″-H), 35.4 (6″-H), 17.2 (5-H), 16.2 (6-Me), 14.0 (4-Me), 8.44 (2′-H), 6.19 (3′-H), 2.51 (4′-Me), −8.80 (2-H), −41.5 (5″-H), −50.3 (3″-H). Averaged integrated signal intensities gave the cation:anion ratio 2:0.90 ± 0.16.

$(Bu_4N)_2[Fe_4S_4(LS_3)—S—Fe(OEP)]$. The following procedures afforded dark brown products whose purities were assessed by ^1H NMR spectra of CD_3CN solutions. Unless otherwise noted, the impurity was the $(Bu_4N)_4\{[Fe_4S_4(LS_3)]_2S\}$.

(i) Reaction 12. To a solution of 12.7 mg (6.99 μmol) of $(Bu_4N)_2[Fe_4S_4(LS_3)(SH)]$ in 1.0 mL of dichloromethane was added 1 equiv of a 0.28 M solution of NaSH in methanol. A second solution was prepared containing 8.67 mg (7.27 μmol) of $[Fe(OEP)]_2O$[28] in 1.0 mL of dichloromethane to which was added 1.0 equiv of a 0.28 M solution of $(Et_3NH)(ClO_4)$ in dichloromethane. The solutions were shaken, and the second was added to the first by syringe. The reaction mixture was stirred for 5 min and filtered, and the filtrate was reduced to dryness to afford the pure product. Absorption spectrum (acetonitrile): λ_{max} (ϵ_M) 374 (69 000), 500 (15 000), 536 (sh, 14 000), 633 (9000). ^1H NMR (CD_3CN, anion): δ 31.3, 22.9 (CH_2); 17.4 (5-H); 16.1 (6-Me); 13.6 (4-Me); 8.45 (2′-H); 5.92 (3′-H); 4.48 (CH_2CH_3); 2.42 (4′-Me); −11.3 (2-H); −32.0 (*meso*-H). Averaged integrated signal intensities gave the cation:anion ratio 2:0.86 ± 0.14.

(ii) Reaction 13. To a solution of 3.25 mg (1.79 μmol) of $(Bu_4N)_2[Fe_4S_4(LS_3)(SH)]$ in 1.0 mL of acetonitrile/benzene (1:1 v/v) was added 1.89 mg (3.05 μmol) of $[Fe(OEP)(OMe)]$[29] in 1.0 mL of the same solvent. The mixture was stirred for 1 h at 0 °C, and the solvent was removed. The residue was extracted with acetonitrile, the extract was filtered, and the filtrate was reduced to dryness. The ^1H NMR spectrum of the solid showed >90% product purity.

(iii) Reaction 14. $(Bu_4N)_2[Fe_4S_4(LS_3)(SH)]$ (10.04 mg, 5.53 μmol) was dissolved in 0.5 mL of acetone, to which was added 1.0 equiv of a 0.28 M solution of NaSH in 10 μL of methanol. The reaction mixture was shaken vigorously for 5 min, 1.0 equiv of $(Et_3NH)(ClO_4)$ in 20 μL of acetonitrile was added, and the mixture was shaken for an additional 5 min. The resultant clear solution was cooled to −40 °C and subjected to a dynamic vacuum for 15 min (to remove H_2S). A solution of 3.96 mg (6.13 μmol) of $[Fe(OEP)(OC(Me)=CH_2)]$ in 0.5 mL of acetone was added dropwise over 15 min. The solution was allowed to warm to 0 °C and stirred for 1 h, and solvent was removed. The ^1H NMR spectrum of the solid showed >90% product purity.

(iv) Reaction 15. $(Bu_4N)_2[Fe_4S_4(LS_3)(SH)]$ (12.8 mg, 7.07 μmol) was dissolved in 0.5 mL of acetonitrile, to which 5 μL of Et_3N and 1 equiv of NaSH in 10 μL of methanol were added. The procedure of reaction 14 was followed except that a solution of 6.33 mg (9.19 μmol) of $[Fe(OEP)(OClO_3)]$ in 0.5 mL of acetonitrile was added; the procedure was then resumed. After stirring was completed, 1.3 equiv of 0.098 M KOMe in methanol was added, the mixture was shaken vigorously for 5 min, and the solvent was removed. The ^1H NMR spectrum of the solid showed >90% product purity.

(v) Reaction 16. To a solution of 6.66 mg (3.45 μmol) of $(Bu_4N)_2[Fe_4S_4(LS_3)(SSiEt_3)]$ in 0.5 mL of acetonitrile was added a solution of 2.84 mg (4.66 μmol) of $[Fe(OEP)F]$[30] in 0.5 mL of acetonitrile. The solution was stirred for 1–2 days and filtered, and the solvent was removed. The ^1H NMR spectrum of the solid showed >90% product purity.

(vi) Reaction 17. $(Bu_4N)_4\{[Fe_4S_4(LS_3)]_2S_2\}$ (9.78 mg, 2.70 μmol) was dissolved in 0.5 mL of acetonitrile, to which a solution of 1.74 mg (2.96 μmol) of $[Fe(OEP)]$[31,32] in benzene was added. The reaction mixture

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FORMATION AND REACTIONS OF HYDROSULFIDE CLUSTERS

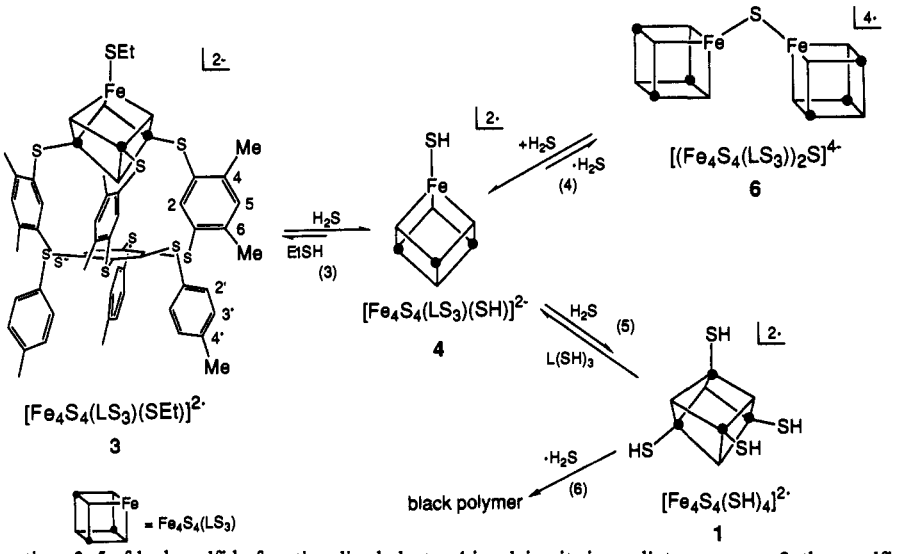

Figure 2. Equilibrium reactions 3–5 of hydrosulfide-functionalized cluster **4** involving its immediate precursor **3**, the μ-sulfido double cubane **6**, and fully hydrosulfide-substituted cluster **1**.

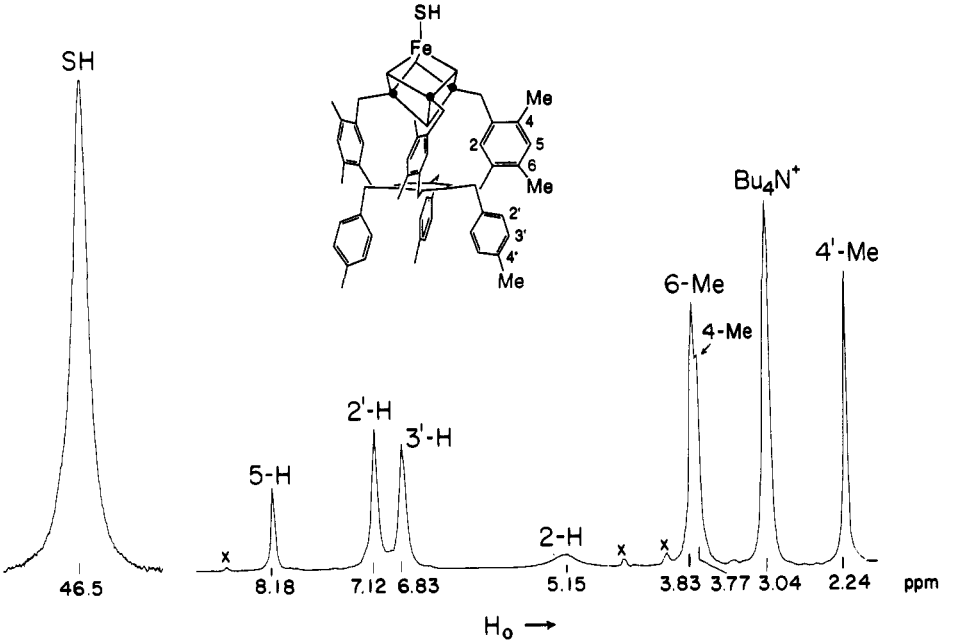

Figure 3. ^{1}H NMR spectrum of cluster **4** in CD_3CN solution at 298 K; signal assignments are indicated.

was shaken vigorously for 5 min, and the solvents were removed. The solid was extracted with acetonitrile, the extract was filtered, and the solvent was removed. The ^{1}H NMR spectrum indicated 90% of the bridged assembly and 10% $\{[Fe_4S_4(LS_3)]_2S_n\}^{4-}$ (n = 1, 2).

Physical Measurements. All measurements were made under strictly anaerobic conditions. UV–visible absorption spectra were recorded on a Cary 219 spectrophotometer. ^{1}H NMR spectra were obtained with a Bruker AM 500 spectrometer. Electrochemical and Mössbauer spectroscopic measurements were made as described;[33] potentials are referenced to the SCE and isomer shifts to Fe metal at room temperature.

Results and Discussion

In this investigation, the following cluster species are of principal interest:

$[Fe_4S_4(SH)_4]^{2-}$ **1**

$[Fe_4S_4(LS_3)Cl]^{2-}$ **2**

$[Fe_4S_4(LS_3)(SEt)]^{2-}$ **3**

$[Fe_4S_4(LS_3)(SH)]^{2-}$ **4**

$[Fe_4S_4(LS_3)(SSiEt_3)]^{2-}$ **5**

$\{[Fe_4S_4(LS_3)]_2S\}^{4-}$ **6**

$\{[Fe_4S_4(LS_3)]_2S_2\}^{4-}$ **7**

$[Fe_4S_4(LS_3)—S—Fe^{III}(salen)]^{2-}$ **8**

$[Fe_4S_4(LS_3)—S—Fe^{III}(OEP)]^{2-}$ **9**

Species **2-5** are iron subsite-differentiated clusters of general formulation $[Fe_4S_4(LS_3)L']^z$, with the structure depicted in Figure 1. Elsewhere we have reported the synthesis and properties of such clusters,[18,20,22,24,34] whose singular advantage is regiospecific

(33) Cen, W.; Lee, S. C.; Li, J.; MacDonnell, F. M.; Holm, R. H. *J. Am. Chem. Soc.* **1993**, *115*, 9515.

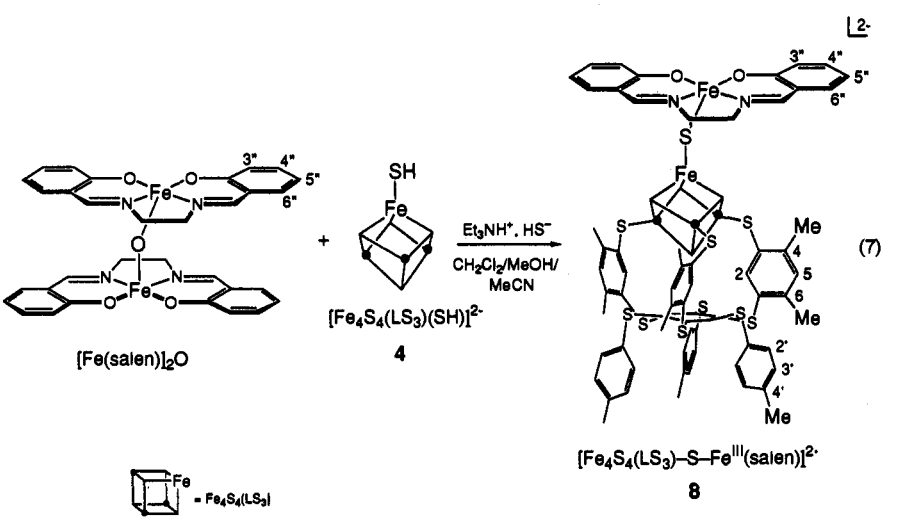

Figure 4. Formation of bridged assembly **8**; the phenyl ring numbering schemes are used in assignments of the ^{1}H NMR spectrum.

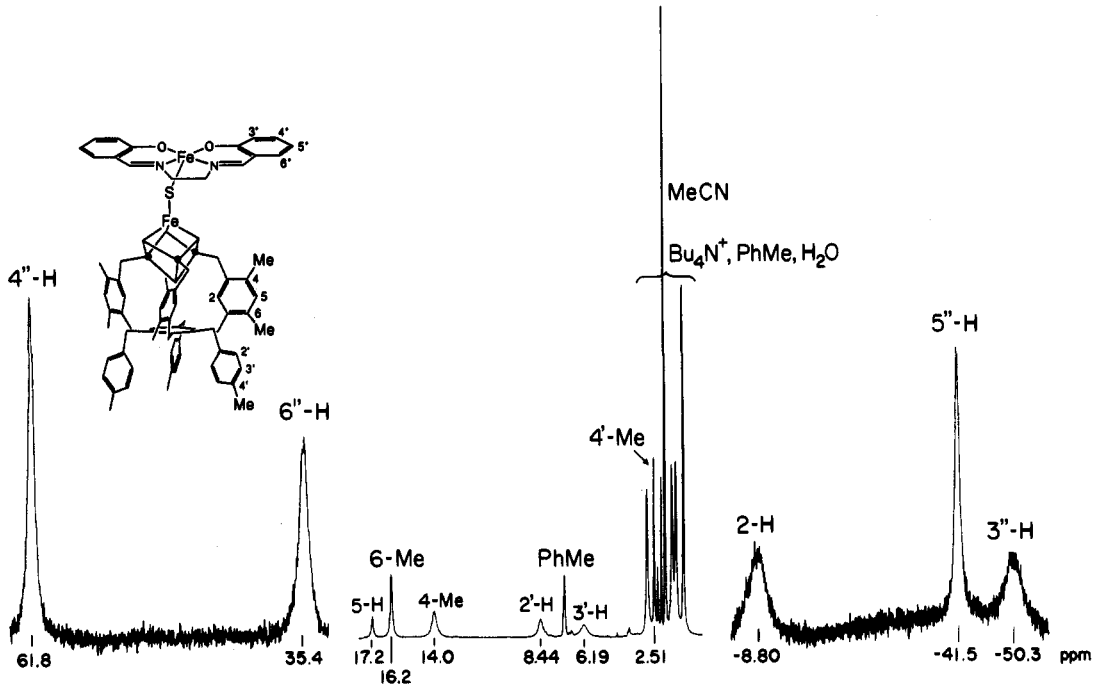

Figure 5. ^{1}H NMR spectrum of isolated bridged assembly **8** in CD_3CN solution at 298 K; signal assignments are indicated. The extreme downfield and upfield spectral portions were recorded at higher gain and different sweep widths than the central portion.

substitution at the unique subsite. The large majority of such reactions have been based on substitution of chloride in cluster **2**.[34c] Proton chemical shifts of the coordinating arms of subsite-differentiated clusters are acutely sensitive to the identity of ligand L′ at the unique subsite; because of this effect, substitution products are readily detected. Clusters **2**[24] and **3**[19] have been previously reported; an improved preparation of the latter is given here. All species **2–9** were prepared on relatively small scales and were conclusively identified by their ^{1}H NMR spectra. Some additional development of subsite-specific reactions was required to obtain the desired bridged assembly precursors **4** and **5**.

Subsite-Functionalized Clusters. Hydrosulfide cluster **4** was selected as the key functionalized precursor to sulfide-bridged assemblies. The feasibility of a terminal HS^- ligand was evident from the (serendipitous) synthesis of cluster **1**, which was identified by an X-ray structure determination.[35] As the Ph_4P^+ salt, the cluster shows the compressed tetragonal geometry common to the $[Fe_4S_4]^{2+}$ oxidation state. We have prepared two salts of **1** in 83–86% yield by reaction 1 in acetonitrile; the product is readily recognized by its SH resonance at 47.6 ppm. Cluster **4** has been previously generated in solution by reaction 2, but small to

$$[Fe_4S_4(SEt)_4]^{2-} + H_2S \rightarrow [Fe_4S_4(SH)_4]^{2-} + EtSH \quad (1)$$

$$[Fe_4S_4(LS_3)Cl]^{2-} + (Et_4N)(SH) \rightarrow [Fe_4S_4(LS_3)(SH)]^{2-} + Et_4NCl \quad (2)$$

substantial quantities of the μ-S double cubane cluster **6** always appear as an impurity.[18] The cluster was not isolated in substance.

Preparation of cluster **4** in adequate purity requires recognition of equilibria 3–5 in Figure 2, all of which we have independently

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(35) Müller, A.; Schladerbeck, N. H.; Bögge, H. *J. Chem. Soc., Chem. Commun.* **1987**, 35.

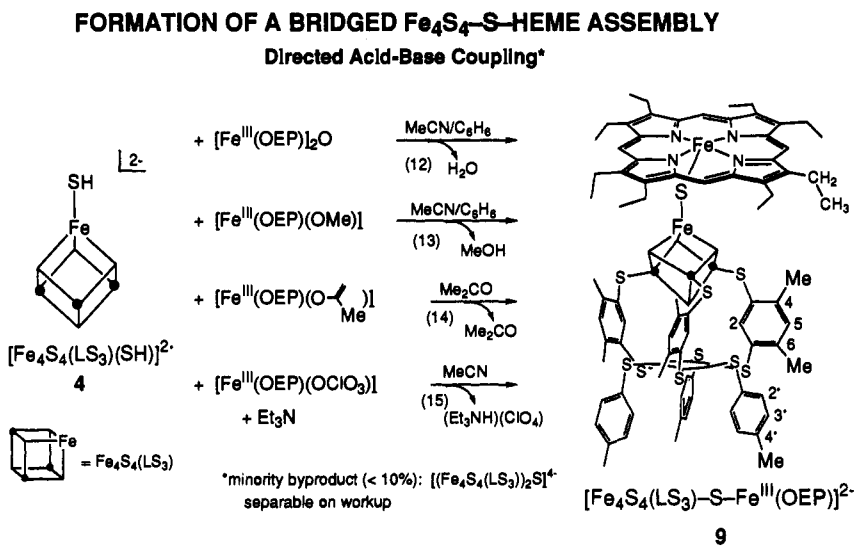

Figure 6. Formation of bridged assembly **9** from common functionalized precursor **4** by directed acid–base coupling reactions 12–15.

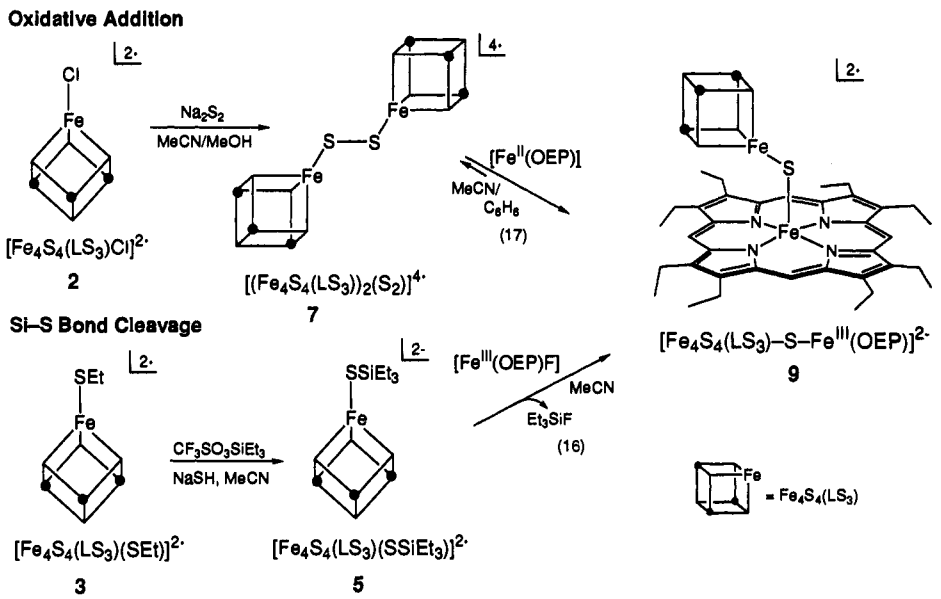

Figure 7. Formation of bridged assembly **9** by Si—S bond cleavage reaction 16 involving **5** (prepared from **3**) and [Fe(OEP)F], and by oxidative addition reaction 17 utilizing disulfide cluster **7** (prepared from **2**) and [Fe(OEP)].

demonstrated in systems monitored by ^{1}H NMR. We have found that this cluster is optimally prepared in pyridine solution, in which H_2S is quite soluble. For reaction 3, $K_{eq} = 120 \pm 20$ in pyridine-d_5 at 298 K, as determined by ^{1}H NMR. Consequently, completion of this reaction is favored by a high [H_2S]:[EtSH] mol ratio, a condition readily achieved in pyridine. Reaction 4, which is inappropriate when cluster **4** is the desired product, is shifted to the left by H_2S. However, care must be taken to minimize forward reaction 5 (Figure 2), which removes the LS_3 ligand and forms **1**. By careful precipitation with ether at low temperature in the presence of H_2S, $(Bu_4N)_2$[**4**] was consistently obtained in >95% purity. We also describe a larger scale preparation of this compound in good yield in acetone solution. In this system, purity has been more difficult to control, but products with ≥90% purity have been obtained; the NMR-detectable impurity is **6**. Cluster **4** can also be generated by reverse reaction 5, in which the H_2S concentration should be minimized. However, reaction 6 (Figure 2) is competitive at low H_2S concentrations; the black product is evidently an oligomer of $[Fe_4S_4]^{2+}$ clusters. Cluster **4** is most readily distinguished by its SH resonance at 46.5 ppm; its complete ^{1}H NMR spectrum is presented in Figure 3.

$$[Fe_4S_4(LS_3)(SEt)]^{2-} + H_2S \rightleftharpoons [Fe_4S_4(LS_3)(SH)]^{2-} + EtSH \quad (3)$$

$$2[Fe_4S_4(LS_3)(SH)]^{2-} \rightleftharpoons \{[Fe_4S_4(LS_3)]_2S\}^{4-} + H_2S \quad (4)$$

Triethylsilanethiolate cluster **5** was prepared in 81% yield in acetonitrile solution by the reaction of **3** and Et_3SiSH, generated in situ from $CF_3SO_3SSiEt_3$ and NaSH. Its 5-H signal at 8.18 ppm is typical of clusters with thiolate ligands at the unique subsite (e.g., 8.15 ppm for **3**). Prolonged reaction of **5** with 1 equiv of NaOMe in acetonitrile at 60 °C afforded **6** as the only detectable cluster product.

Synthesis of Bridged Assemblies. All attempts to prepare *thiolate*-bridged assemblies have thus far been unsuccessful. Earlier we reported that the system $[Fe_4S_4(LS_3)(SMe)]^{2-}/Fe^{II}$(acen) in acetonitrile, so constituted to avoid redox reactions and minimize steric hindrance in bridging interactions, formed a labile

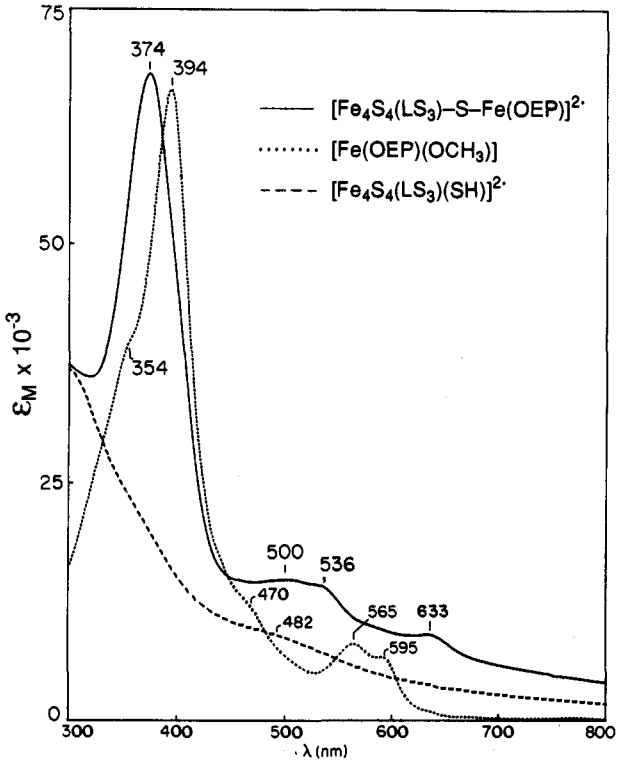

Figure 8. UV–visible absorption spectra of bridged assembly **9** and its immediate precursors [Fe(OEP)(OMe)] and **4** in reaction 13; band maxima and shoulders are indicated.

equilibrium mixture greatly favoring the separated components.[19] The system **3**/[Fe(OEP)($OClO_3$)] in acetonitrile, dichloromethane, or benzene was monitored by ^{1}H NMR; it yielded [Fe(OEP)(SEt)], [Fe(OEP)], and considerable amounts of a black precipitate after reaction times of 5 min or longer. These observations are similar to those encountered earlier in the systems [$Fe_4S_4(SPh)_4$]$^{2-}$/[Fe(OEP)X] (X = ClO_4^-, $CF_3SO_3^-$) in various solvents,[36] which also did not afford the desired thiolate-bridged species.

With these results in hand, attention was directed to *sulfide*-bridged versions of the sulfite reductase site (Figure 1). At the outset, we note that few unsupported[30] Fe—S—Fe bridges have ever been prepared, and only five are structurally authenticated.[17,37–39] Bridge angles vary over the range 102–167°. The comparison of [Fe(salen)]$_2$S (122°)[37] and [Fe(3-*t*-Busaltmen)]$_2$S (167°) was the basis of our demonstration that under steric pressure unsupported Fe—S—Fe bridge angles are deformable.[39] Thus, in neither of the target assemblies was destabilization of the bridge by means of steric interactions across it likely to be decisive. However, all five known species contain symmetric bridges, necessitating the development of new coupling reactions leading to unsymmetrical bridges.

Synthesis of Bridged Assemblies. (a) [$Fe_4S_4(LS_3)$—S—Fe^{III}(salen)]$^{2-}$. Initial reactions directed toward bridged assemblies utilized inexpensive [Fe(salen)]$_2$O as a starting material. The reaction system **7** in Figure 4 consists initially of the component solutions **4** + NaSH in CH_2Cl_2/MeOH (1:5 v/v) and [Fe(salen)]$_2$O + (Et_3NH)(ClO_4) in MeCN/CH_2Cl_2 (1:13 v/v), each containing equimolar reactants. In reactions 8–11 below, L″ = salen or OEP inasmuch as two bridged assemblies are formed in parallel systems (vide infra). In the first component system, hydrosulfide converts any chloride impurity **2**[40] to **4**. When the two solutions are mixed, reactions 8 and 9 occur simultaneously. The former generates H_2S, which assists in the displacement of reaction 4 to the left as cluster **4** is consumed in reaction 10 and/or 11. Further, we have established that triethylamine catalyzes reaction 9, which affords a species more reactive toward **4** than the μ-oxo complex, and reaction 10. Triethylamine in equivalent amounts does not deprotonate cluster **4** or promote reaction 4. Because we have not measured reaction rates, the sequence of reactions and their consequent contributions to product formation cannot be assessed. Assembly formation reactions 10 and 11 are proton-transfer processes.

$$HS^- + (Et_3NH)(ClO_4) \rightarrow H_2S + Et_3N \quad (8)$$

$$[(FeL'')_2O] + 2MeOH \rightleftharpoons 2[FeL''(OMe)] + H_2O \quad (9)$$

$$[(FeL'')_2O] + 2[Fe_4S_4(LS_3)(SH)]^{2-} \rightarrow [Fe_4S_4(LS_3)\text{—}S\text{—}FeL'']^{2-} + H_2O \quad (10)$$

$$[FeL''(OMe)] + [Fe_4S_4(LS_3)(SH)]^{2-} \rightarrow [Fe_4S_4(LS_3)\text{—}S\text{—}FeL'']^{2-} + MeOH \quad (11)$$

When reaction 7 was conducted in situ and monitored by ^{1}H NMR, assembly **8** was produced in essentially quantitative yield with negligible paramagnetic impurities. When carefully recrystallized from dichloromethane/toluene, the pure compound was recovered in 25% yield as a dark brown solid soluble in acetonitrile. The ^{1}H NMR spectrum, shown in Figure 5 and considered subsequently, is consistent with the indicated structure. The formation of **8** by an acid–base reaction was the point of departure for development of related reactions yielding a heme-based assembly of improved pertinence to the enzyme site.

(b) [$Fe_4S_4(LS_3)$—S—Fe^{III}(OEP)]$^{2-}$. The subsite-differentiated clusters [$Fe_4S_4(LS_3)L'$]^z consistently exhibit full regioselectivity in ligand substitution, but rarely form diffraction-quality crystals for X-ray structure determinations. Indeed, structures of only two such clusters are available.[22,24] Structure proof of heme-based assembly **9** has been realized in a different way, viz., by the execution of a series of independent reactions—directed acid–base coupling, Si—S bond cleavage, and oxidative addition—designed to have an unambiguous common product.

(i) Directed Acid–Base Coupling. Four reactions, 12–15, of this type are illustrated in Figure 6. Reaction 12 was conducted similarly to reaction 7 and implicates the reaction set 8–11. Observations in the Fe^{III}(salen)-based system apply here. Reaction 13 utilizes isolated [Fe(OEP)(OMe)];[29] it is more rapid than reaction 12 and is catalyzed by triethylamine. Reaction 14 is similar to 13 and involves the acetone enolate complex [Fe(OEP)-(OC(Me)=CH_2)].[25] Reaction 15 is a concerted acid–base process which utilizes the pronounced lability of axial perchlorate; it proceeds in the absence of triethylamine, but the yield is poor. After the reaction is complete, 1 equiv of KOMe in methanol was added to neutralize Et_3NH^+, whose perchlorate salt is difficult to separate from (Bu_4N)$_2$[**9**] and which decomposes the product as the solvent is removed from the reaction mixture.

(ii) Si—S Bond Cleavage. In reaction 16 of Figure 7, triethylsilanethiolate cluster **5** was treated with 35% excess of [Fe(OEP)F] in acetonitrile. This coupling reaction proceeded slowly; the Et_3SiF byproduct is volatile and easily removed in

(36) Stolzenberg, A. M.; Stershic, M. T. *Inorg. Chem.* **1985**, *24*, 3095.
(37) Dorfman, J. R.; Girerd, J.-J.; Simhon, E. D.; Stack, T. D. P.; Holm, R. H. *Inorg. Chem.* **1984**, *23*, 4407. (salen = 1,2-bis(salicylideneamino)-ethane(2-).)
(38) (a) Corazza, F.; Floriani, C.; Zehnder, M. *J. Chem. Soc., Dalton Trans.* **1987**, 709. (b) Berno, P.; Floriani, C.; Chiesi-Villa, A.; Guastini, C. *J. Chem. Soc., Dalton Trans.* **1989**, 551.
(39) Mukherjee, R. N.; Stack, T. D. P.; Holm, R. H. *J. Am. Chem. Soc.* **1988**, *110*, 1850. (3-*t*-Busaltmen = 2,3-dimethyl-2,3-bis[(3-*tert*-butylsalicylidene)amino]butane(2-).)

(40) Cluster **2** is a ubiquitous impurity in [$Fe_4S_4(LS_3)L'$]^z preparations, but usually can be converted to the desired cluster by ligand substitution; it is not an immediate precursor to bridged assemblies by the reactions in this work.

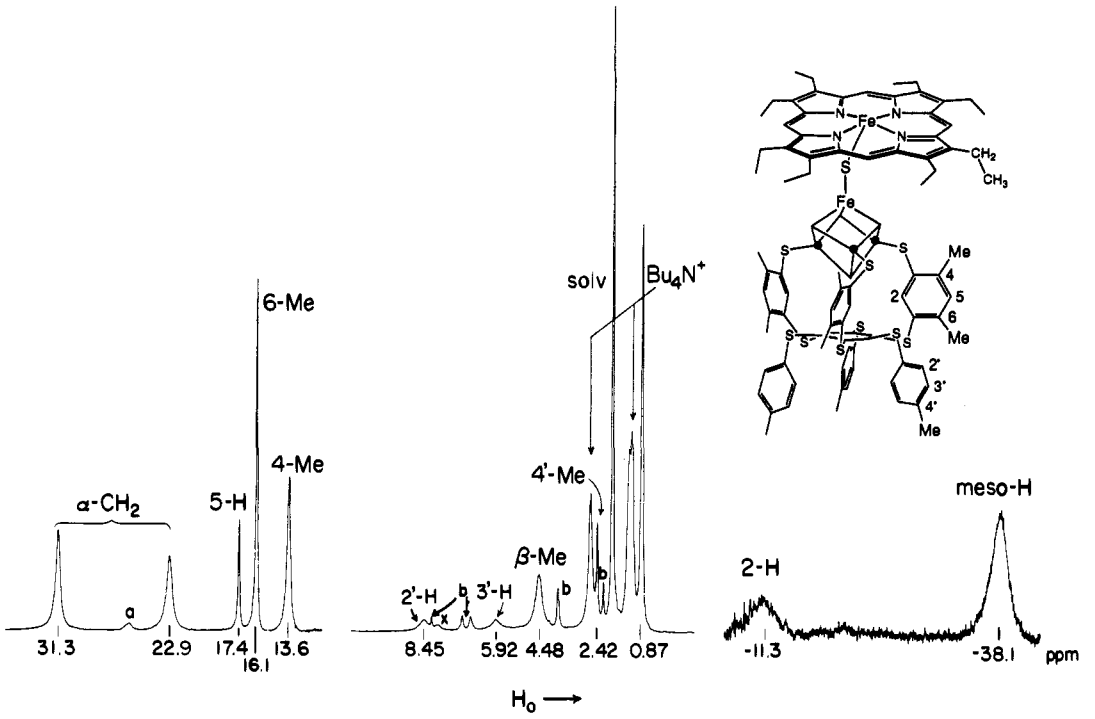

Figure 9. ^{1}H NMR spectrum of bridged assembly **9** in CD_3CN solution at 298 K; signal assignments are indicated. The upfield region was recorded at higher gain and different sweep width than the remainder of the spectrum. (In our previous report,[21] the β-CH_3 and *meso*-H chemical shifts were incorrectly labeled in Figure 2.)

vacuo. This reaction was designed to capitalize on the *ca.* 90 kcal/mol difference in Si—F vs Si—S bond energies.[41]

(iii) Oxidative Addition. Homogeneous reaction of chloride cluster **2** with Na_2S_2 in methanol/acetonitrile (1:1 v/v) (Figure 7) results in a product whose ^{1}H NMR spectrum is not clearly distinguishable from that of the starting cluster. The spectrum is definitely not that of μ-S double cubane **6**; for example, in acetonitrile the 5-H signal of the product occurs at 8.24 ppm compared with 8.66 ppm for **6**. We formulate the product as the μ-S_2 double cubane **7** on the basis of its method of formation and the occurrence of reaction 17. When 2.7 μmol of **7** and 3.0 μmol of [Fe^{II}(OEP)] were allowed to react in an acetonitrile/benzene solution (1:1 v/v), the ^{1}H NMR spectrum of the isolated product indicated 90% conversion of **7** to **9**, with the remaining 10% of cluster species being a mixture of **6** and unreacted **7**. Reaction 17 is oxidative addition to [Fe(OEP)] by reduction of the S—S bond of persulfide-bridged cluster **7**.

$$\{[Fe_4S_4(LS_3)]_2S_2\}^{4-} + 2[Fe(OEP)] \rightleftharpoons 2[Fe_4S_4(LS_3)\text{—}S\text{—}Fe(OEP)]^{2-} \quad (17)$$

When reactions 12–17 were monitored in situ or their isolated products examined, in both instances by ^{1}H NMR, bridged assembly **9** was formed in every case. Conversions of initial cluster to product of $\gtrsim$90% (in situ) and NMR purities of >90% (isolated) were achieved. The persistent impurity is μ-S cluster **6**, whose formation, presumably mainly by reaction **4**, is difficult to suppress entirely under conditions that afford high product yields and purities. As seen in Figure 8, the absorption spectrum of **9** is very different from its heme precursor in reaction 13. Band structure in the visible region has been altered, and there is a marked shift in the Soret band from 394 nm in [Fe(OEP)(OMe)] to 374 in **9**. The isotropically shifted ^{1}H NMR spectrum of **9**, set out in Figure 9, provides a second means of identification. Consistent with reversible reaction 17, very small amounts of [Fe(OEP)] (signal a) and **7** (signals b) are detected together with those of **9**.

(41) Average bond energies: Si—S = 54 kcal/mol, Si—F = 142 kcal/mol. Pawlenko, S. In *Houben-Weyl. Methoden der Organischen Chemie*; Bayer, O., Müller. E., Eds.; Thieme Verlag: Stuttgart, 1980; Vol. XIII/5, p 14.

Structure and Spin Delocalization. Diffraction-quality crystals of the two bridged assemblies were not obtained. In addition to methods of synthesis, the structures of **8** and **9** have been deduced from ^{1}H NMR and other spectroscopic observations. In developing structure proofs, it is useful to consider properties of component parts of the assemblies and of closely related compounds. Relevant data from this work and prior investigations are collected in Table 1;[18,28,37,42–44] redox potentials are those of one-electron chemically reversible processes ($i_p/i_a \approx 1$).

(a) Fe(III) Fragments. In assembly **8**, the chemical shifts of the ring protons of the [Fe^{III}(salen)] fragment are displaced to high and low field (Figure 5); the isotropic shifts given in Table 1 alternate around the phenyl ring in a manner consistent with dominant contact interactions in an odd–alternate ligand system.[45] They are somewhat smaller than but roughly comparable to those of [Fe(salen)(OAc)]. Because this and other five-coordinate [Fe^{III}(salen)L] complexes are uniformly high-spin,[46] the $S = 5/2$ state is assigned to this fragment. Prior work on [Fe^{II}(salen)L]$^-$ complexes[44] and the electrochemical behavior of [Fe(salen)]$_2$S, the only structurally authenticated Fe^{III}(salen) complex with an axial sulfide ligand, provide independent evidence that an [Fe^{III}(salen)] fragment with an axial anionic sulfur ligand can sustain reversible reduction. The first reduction potential of the assembly is within *ca.* 40 mV of that of [Fe(salen)]$_2$S, but this may be fortuitous in view of the difference in charges of the two species. All evidence is consistent with the presence of the high-spin five-coordinate [Fe^{III}(salen)S] component in **8**; in this event, the Fe(III) atom will be displaced toward the axial sulfide ligand as in structurally defined [Fe^{III}(salen)(OR)] complexes.[47]

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(43) Tang, S. C.; Koch, S.; Papaefthymiou, G. C.; Foner, S.; Frankel, R. B.; Ibers, J. A.; Holm, R. H. *J. Am. Chem. Soc.* **1976**, *98*, 2414.

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(45) Bertini, I.; Luchinat, C. *NMR of Paramagnetic Molecules in Biological Systems*; Benjamin/Cummings Publishing Co.: New York, 1986; Chapter 2.

(46) (a) Gerloch, M.; Lewis, J.; Mabbs, F. E.; Richards, A. *J. Chem. Soc. A* **1968**, 112. (b) Lewis, J.; Mabbs, F. E.; Richards, A.; Thornley, A. S. *J. Chem. Soc. A* **1969**, 1993.

(47) (a) Heistand, R. H.; Roe, A. L.; Que, L., Jr. *Inorg. Chem.* **1982**, *21*, 676. (b) Dzugan, S. J.; Goedken, V. L. *Inorg. Chim. Acta* **1986**, *119*, 43.

Table 1. Properties of Bridged Assemblies and Related Fragment Compounds

species	$(\Delta H/H_o)_{iso}$, ppm[a]	mm/s, 4.2 K δ	mm/s, 4.2 K ΔE_Q	$E_{1/2}$, V[b]
[Fe(salen)(OAc)]	+89.9 (3-H), −69.5 (4-H), +74.3 (5-H), −39.9 (6-H)[c,d]	0.49[e]	1.27[e]	−0.59
[Fe(salen)(SPh)]	+67.5 (3-H), −62.9 (4-H), +57.8 (5-H), −42.4 (6-H), +86.3 (*o*-H), −50.6 (*m*-H), +87.8 (*p*-H)[f]	0.49	1.00	−0.52[f,g]
[Fe(salen)$_2$]S	*h*	0.45	0.70	−0.67[i,j]
[Fe(OEP)(OMe)]	−26.5, −29.4 (CH_2); −4.91 (Me); +44.4 (*meso*-H)[d]	0.41	0.65[k]	−0.98
[Fe(OEP)(SH)]	−32.7, −34.5 (CH_2); −3.08 (Me); +60.1 (*meso*-H)[l]	*m*	*m*	*m*
[Fe(OEP)(SPh)]	−40.0 (CH_2), −4.03 (Me), +69.2 (*meso*-H), +127.3 (*o*-H), −65.8 (*m*-H), +122.2 (*p*-H)[n]	0.43	0.49[o]	−0.72, −1.38[n]
4	+1.57 (2-H), −1.59 (4-Me), −1.44 (5-H), −1.59 (6-Me), −50.2 (SH)[f]	0.46	1.15	−1.06[f]
6	+2.42 (2-H), −1.87 (4-Me), −1.93 (5-H), −2.25 (6-Me)[f]	0.47	1.15	−1.28, −1.50[f,p]
8	+57.4 (3″-H), −54.5 (4″-H), +48.5 (5″-H), −28.5 (6″-H), +15.7 (2-H), −11.7 (4-Me), −10.4 (5-H), −13.8 (6-Me)[f]	0.45	1.14[q]	−0.71, −0.96[f]
9	−18.6, −27.1 (CH_2); −2.52 (Me); +48.1 (*meso*-H); +18.0 (2-H); −11.4 (4-Me); −10.7 (5-H); −13.8 (6-Me)[f]	0.46	1.14[q]	−1.05, −1.32[f]

[a] $(\Delta H/H_o)_{iso} = (\Delta H/H_o)_{dia} - (\Delta H/H_o)_{obs}$. Diamagnetic references: H_2(salen), PhSH, Ni(OEP), L(SNa)$_3$ (in CD_3CN), $(Et_4N)(SH)$. [b] vs SCE, reductions. [c] Reference 42. [d] Dichloromethane. [e] 77 K. [f] MeCN. [g] Reference 44. [h] Antiferromagnetically coupled, $S = 0$ ground state.[37] [i] Reference 37; in this reference the potential was incorrectly reported as −1.27 V. [j] DMF. [k] Reference 28. [l] Generated in situ in C_6D_6. [m] Not reported or not determined in this work. [n] MeCN/C_6H_6 (1:1 v/v). [o] Reference 43. [p] Reference 18. [q] Data for cluster portion.

Table 2. Chemical Shifts[a] and Spin States of $[Fe^{III}(OEP)L_n]^z$ Complexes at ~298 K

L_n	solvent	ppm CH_2	ppm CH_3	ppm *meso*-H
$S = 5/2$				
Cl^-	CD_3CN	45.4, 40.9	6.88	−57.5
MeO^-	$CDCl_3$	33.4, 31.4	5.03	−35.0
$CH_2{=}C(Me)O^-$	CD_3CN	32.2, 28.8	4.86	−38.9
F^-	CD_3CN	39.7, 36.0	5.73	−33.1
PhS^-	C_6D_6	44.1	5.95	−59.1
Ph_3SiS^-	C_6D_6	39.0, 36.2	5.64	−57.5
HS^-	C_6D_6	38.6, 36.8	5.00	−50.0
$(MeCN)_2$	CD_3CN	38.2	6.30	−27.0
$[Fe_4S_4(LS_3)S]^{3-}$	CD_3CN	31.3, 22.9	4.48	−38.1
$S = 3/2$				
$OClO_3^-$	C_6D_6	50.2, 33.1	7.20	−18.9
$(EtOH)_2$	C_6D_6	38.1	6.76	−8.4
$S = 1/2$				
$(Im)_2$[b]	$CDCl_3$	6.07	0.32	3.1
$S = 0$				
$[Fe(OEP)]_2O$[c]	C_6D_6	6.22, 5.21	1.76	6.4

[a] Determined in this work unless otherwise noted. [b] Reference 49. For related cases, *cf.* refs 48 and 54. [c] Antiferromagnetically coupled with partial occupancy of an $S = 1$ state.[43]

In the ^1H NMR spectrum of assembly **9** (Figure 9), diastereotopic methylene signals of the ethyl substituents are observed (31.3, 22.9 ppm), consistent with the indicated structure. High-spin [Fe^{III}(OEP)L] complexes (Table 2) exhibit chemical shifts of OEP *meso*-H and ethyl substituents similar to those of **9**. In earlier work, a correlation between porphyrin shifts and Fe(III) spin state and coordination number had been briefly noted but with limited results for a given porphyrin.[48,49] With the data of Table 2, this correlation is pursued for OEP complexes in three spin states. The $S = 5/2$ spin state has been demonstrated by a combination of magnetic and EPR and Mössbauer spectroscopic results for [Fe(OEP)Cl],[28,50] [Fe(OEP)(OMe)],[28,43] and [Fe(OEP)(SPh)].[43] These compounds define a *meso*-H chemical shift range −35 to −59 ppm in the indicated solvents, allowing certain other complexes to be classified as high-spin on this basis. Just below the range is [Fe(OEP)F] (−33.1 ppm), whose structural parameters[30] meet the criteria for high-spin stereochemistry.[51] The *meso*-H shift of $[Fe(OEP)(MeCN)_2]^+$ is downfield (27 ppm); this complex is representative of the set $[Fe(P)L_2]^+$, which are six-coordinate and high-spin[49,52] and whose *meso*-H shifts are opposite to the five-coordinate high-spin case. For complexes shown to have intermediate spin $S = 3/2$,[26,53] *meso*-H shifts are upfield but smaller. Low-spin complexes tend to have small downfield chemical shifts.[48,54]

In considering the chemical shift/spin state relationships of Table 2, we note the prior report of a porphyrin complex formulated as [Fe^{III}(TAP)(SH)], which is both five-coordinate and low-spin.[55] We have not attempted to repeat the preparation of this compound but have carried out reaction 18. In benzene

$$[Fe(OEP)]_2O + 2H_2S \rightarrow 2[Fe(OEP)(SH)] + H_2O \quad (18)$$

solution at ambient temperature, a transient paramagnetic species is formed which decays to [Fe(OEP)] within 5 min; its *meso*-shift of −50.0 ppm is that of a high-spin Fe(III) species which we formulate as the hydrosulfide complex.[56] The compound is not $[Fe(OEP)]_2S$, which has not been otherwise reported and by analogy with $[Fe(salen)]_2X$ (X = O, S)[37] and $[Fe(OEP)]_2O$[42] would have much smaller isotropic shifts owing to antiferromagnetic coupling leading to an $S = 0$ ground state. With OEP, at least two species of the type [Fe^{III}(OEP)(SR)] are clearly high-spin (Table 1). Voltammetry of [Fe(OEP)(SR)] demonstrates a reversible redox process for the Fe^{III}(OEP) group with an anionic axial sulfur ligand. The *meso*-H shift of −38.1 ppm is taken to be decisive for a high-spin five-coordinate [Fe^{III}(OEP)S] component in assembly **9**. Consequently, the Fe(III) atom should be displaced from the mean porphyrin plane toward the axial sulfur atom as is the case for [Fe(OEP)X] (X = F^-,[30] MeO^- [29b]) and [Fe^{III}(P)(SR)] complexes,[43,57] including [Fe(OEP)(SPh)], in which the displacement is 0.51 Å.[57c]

(b) Fe_4S_4 Fragments. In the Mössbauer spectra of assemblies **8** and **9** in zero applied magnetic field, the features arising from

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(55) English, D. R.; Hendrickson, D. N.; Suslick, K. S.; Eigenbrot, C. W., Jr.; Scheidt, W. R. *J. Am. Chem. Soc.* **1984**, *106*, 7258. TAP = 5,10,15,-20-tetrakis(*p*-methoxyphenyl)porphyrinate(2−). This compound has a 0.33-Å displacement of the Fe(III) atom from the porphyrin plane toward the axial ligand.
(56) Attempts to prepare this compound by the reaction of [Fe(OEP)-$(OClO_3)$] and NaSH in acetonitrile at room temperature resulted in immediate formation of [Fe(OEP)].
(57) (a) Miller, K. M.; Strouse, C. E. *Inorg. Chem.* **1984**, *23*, 2395. (b) Byrn, M. P.; Strouse, C. E. *J. Am. Chem. Soc.* **1991**, *113*, 2501. (c) Miller, K. M.; Strouse, C. E. *Acta Crystallogr.* **1984**, *C40*, 1324.

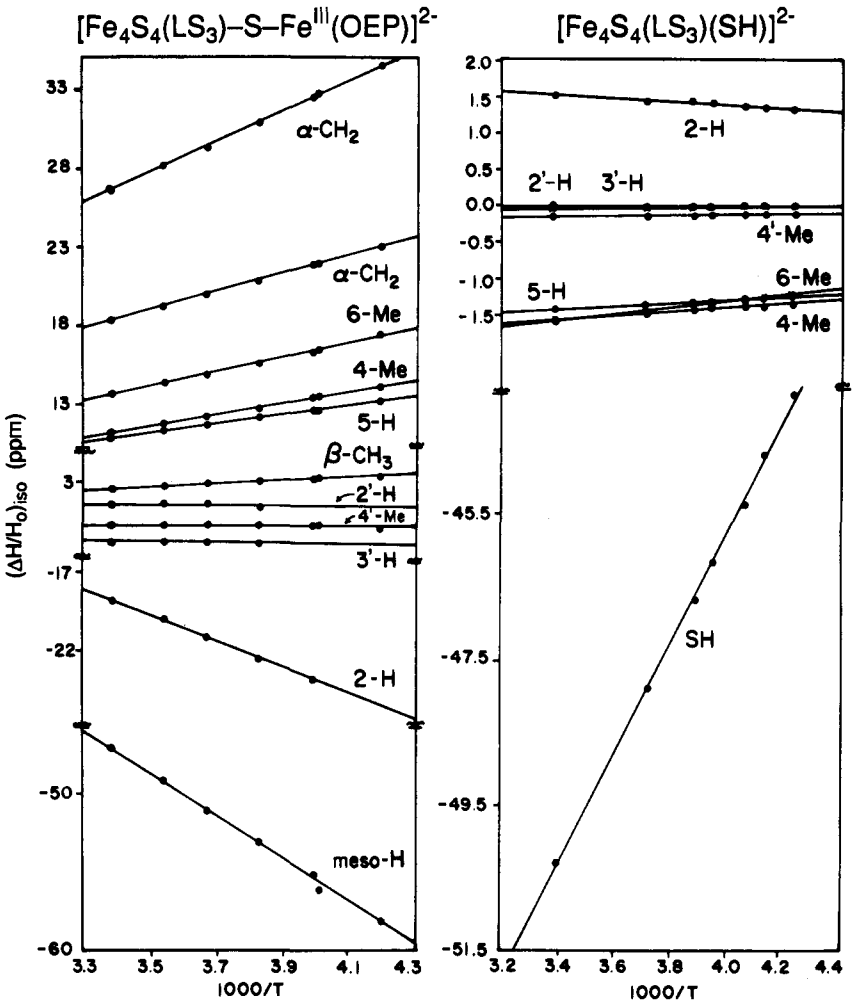

Figure 10. Temperature dependencies of the isotropic shifts of 4 (right) and 9 (left) in acetonitrile solutions over the interval *ca.* 235–300 K.

these fragments are nearly symmetric quadrupole doublets. The oxidation state of the clusters is readily determined by their isomer shifts of 0.45–0.46 mm/s. Comparison with data for other clusters in Table 1 and those of a more conventional type[58] reveals that these shifts, as well as the quadrupole splitting of 1.14 mm/s, are entirely typical of the $[Fe_4S_4]^{2+}$ core oxidation state, which has an $S = 0$ ground state. Mössbauer spectra of the Fe(III) fragments, which either are not well resolved from the cluster spectrum (**9**) or are in an intermediate relaxation regime (**8**), will be described in a future report on magnetic properties.

The most conspicuous feature of the ^1H NMR spectra of assemblies **8** and **9** (Figures 5 and 9) is the greatly enhanced paramagnetically induced shifts of the $Fe_4S_4(LS_3)$ fragments compared to those of **4**. Information detailing this behavior is given in Table 1 and Figures 10 and 11. The spectrum of **4** (Figure 3) is consistent with the normal behavior of $[Fe_4S_4]^{2+}$ clusters, all of which have an $S = 0$ ground state and small isotropic shifts arising from the population of an excited paramagnetic state.[59] The negative (downfield) shifts of 4-Me, 5-H, and 6-Me and the positive shift of 2-H are consistent with dominant contact interactions arising from the delocalization of net positive (α) spin in the π orbitals of the coordinated phenylthiolate rings of the LS_3 ligand. The SH shifts, which have not been previously observed, are very large (*ca.* 40-50 ppm) because only two bonds separate the hydrogen and iron atoms, a situation that favors a strong contact interaction. The signal assigned to the SH group disappears upon addition of a slight amount of D_2O to an acetonitrile solution. Except for SH, the temperature dependencies of the isotropic shifts of **4** are small (Figure 10). However, for all resonances $|(\Delta H/H_o)_{iso}|$ *increases* with increasing temperature, consistent with thermal population of an excited paramagnetic state. The isotropic shifts of assembly **9** have the same sign but are relatively large compared to **4** and manifest a clear linear T^{-1} behavior (Figure 10) consistent with contact shift equation 19, in which A_i is the electron–nuclear coupling constant

$$(\Delta H/H_o)_{con} = -[A_i/\hbar\gamma_H][g\mu_B S(S+1)/3kT] \quad (19)$$

of the ith proton and the remaining symbols have their usual meanings.[45] Thus, for all resonances $|(\Delta H/H_o)_{iso}|$ *decreases* with increasing temperature.

Under ligand-to-metal positive spin delocalization in an odd–alternate system described by the McConnell equation $A_i = Q\rho_{Ci}$, with Q_{CH} negative and Q_{CMe} positive, elementary contact shift theory[45] predicts negative 4-/6-Me and 5-H shifts and positive 2-H shifts, as observed (Table 1). The slopes of the shift plots for **9** are consistent with the predicted signs of the hyperfine coupling constants and thus with dominant contact interactions. Isotropic shifts for cluster **4**, the high-spin heme precursor [Fe(OEP)(OMe)], and assembly **9**, and their shift ratios at 298 K, are compared in Figure 11. While the shifts in the Fe fragment show small and unpredictable changes, the shifts of the cluster fragment are increased by factors of 7.2–11.5 compared to **4**. The shift ratios of **8** and **4** are convincingly similar to those of **9**: 4-Me = 7.36, 5-H = 7.22, 6-Me = 8.68, 2-H = 10.0. The closest of these nuclei to the Fe(III) center is 2-H, which is eight bonds

(58) Values of $\delta, \Delta E_Q$ (mm/s, 4.2 K) for typical $[Fe_4S_4]^{2+}$ clusters: $(Et_4N)_2[Fe_4S_4(SEt)_4]$, 0.44, 1.21; $(Ph_4P)_2[Fe_4S_4(SPh)_4]$, 0.47, 1.10.

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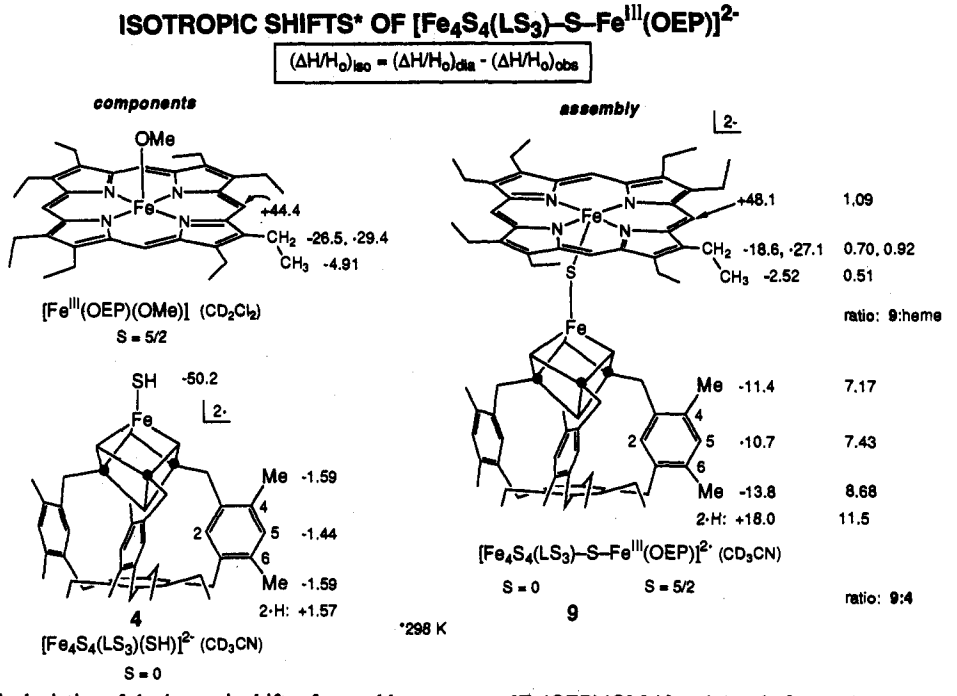

Figure 11. Schematic depiction of the isotropic shifts of assembly precursors [Fe(OEP)(OMe)] and **4** and of assembly **9**; ratios of isotropic shifts are given at the right.

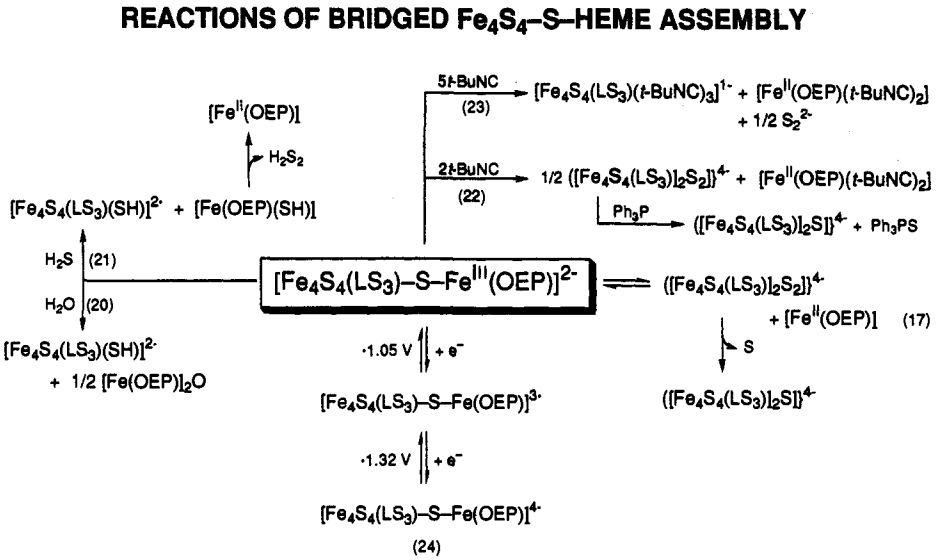

Figure 12. Summary of the reactions of bridged assembly **9**. Other reactions were conducted in acetonitrile except for reaction 17, which was carried out in acetonitrile/benzene (1:1 v/v); oxidized sulfur byproducts were not demonstrated.

removed. The effect is so large in **8** and **9** that the *p*-tolylthio ring protons and methyl groups, whose isotropic shifts are nil in **4**, experience isotropic shifts of *ca.* 0.4–1.4 ppm, although they are 15 bonds or more removed from the Fe(III) center.

The conclusion is inescapable that spin has been delocalized from the high-spin Fe(III) fragments in **8** and **9** through the bridge to the cluster and overwhelms the intrinsic shifts of the latter such that T^{-1} behavior is observed. The [$Fe_4S_4(LS_3)S$] portion is an axial ligand in the sense of benzenethiolate in [Fe(salen)(SPh)] and [Fe(OEP)(SPh)], which exhibit very large shifts dominantly contact in nature (Table 1). We cannot trace an orbital path of spin delocalization primarily because of the complexity of the cluster orbitals, but the net result is the creation of positive spin in the p-type orbitals of coordinated sulfur atoms and delocalization into the π system of the phenylthiolate rings. Isotropic shift ratios are close estimates of relative spin densities of **8** or **9** and **4** (averaged over diamagnetic and paramagnetic states) if the *g* values of the paramagnetic states of the fragments are nearly equal (eq 19). Spin delocalization requires the presence of a covalently bridged structure.

Reactions of Bridged Assemblies. Reactions 17 and 20–24 of **9** were carried out primarily in acetonitrile solutions and were monitored by ^{1}H NMR; results are summarized in Figure 12. Equilibrium reaction 17 in acetonitrile greatly favors the intact assembly at low concentrations ($\lesssim$10 mM); at higher concentrations the equilibrium shifts to the right because of the limited solubility of [Fe(OEP)]. At the same concentration in THF, the assembly is even more favored, all components of the reaction being quite soluble. As noted earlier, one basis for identification of μ-S_2 cluster **7** is its oxidative addition reaction with [Fe(OEP)]. Chloride cluster **2**, from which **7** was prepared (Figure 7), does not react with [Fe(OEP)]. On standing, **7** gradually loses sulfur to form **6**. Hydrolysis reaction 20 with a small excess of water and similar reaction 21 with H_2S result in bridge cleavage and the formation of **4**. In the latter process, we assume the formation of [Fe(OEP)(SH)], which quickly decays to the observed product

[Fe(OEP)]. These reactions identify the bridging sulfide atom as the most basic site in the assembly.

In an attempt to bind an axial ligand that would stabilize the Fe^{II}(OEP) fragment generated by reduction of the intact assembly, **9** was titrated with *t*-BuNC in reaction 22. The products were **7**, which was detected by reaction with Ph_3P, and diamagnetic [Fe(OEP)(*t*-BuNC)$_2$], which was independently generated from the isonitrile and [Fe(OEP)]. Treatment of **9** with excess *t*-BuNC led to formation of [Fe(OEP)(*t*-BuNC)$_2$] and the previously prepared cluster $[Fe_4S_4(LS_3)(t\text{-BuNC})_3]^-$, which is readily identified by its characteristic isotropically shifted ^{1}H NMR spectrum.[60] Reactions 17, 22, and 23 demonstrate that **9** is susceptible to internal redox reactions involving reduction of the $[Fe^{III}(OEP)]$ fragment by the sulfide bridge. The latter two reactions are presumably driven in large measure by the general stability of low-spin six-coordinate Fe(II) porphyrins.

As *E. coli* SiR,[3-5] both assemblies show two one-electron reduction steps at the potentials in Table 1 and in series 24 of Figure 12. In the enzyme, the Fe(III) center of siroheme is reduced before the cluster in the unligated and nearly all ligated states.[3c] Experiments designed to determine the order of reduction in bridged assemblies **8** and **9** are in progress.

Summary. The following are the principal results and conclusions of this investigation.

(1) The hydrosulfide-functionalized cluster **4** can be isolated from the reaction of H_2S with cluster **3**; subsequent successful use of **4** in synthesis requires manipulation of its equilibria with μ-S double cubane **6** and "perhydrosulfide" cluster **1**.

(2) The bridged assemblies **8** and **9**, each containing an unsupported μ-S bridge linking a high-spin Fe(III) fragment and an $[Fe_4S_4]^{2+}$ subsite-differentiated cluster, have been synthesized by a variety of routes based on functionalized cluster **4** in yields and purities exceeding 90%; the main impurity is **6**, coupled to **4** by the equilibrium in (1).

(3) Assembly **9** has been prepared by six different reactions, all designed to afford an unambiguous common product and thereby collectively to provide a structure proof of that product.

(4) The observations of isotropic shifts mainly contact in nature enhanced by factors of 7–12 in assemblies **8** and **9** vs cluster **4** and the Curie-type temperature dependence of the shifts of **9** are consistent only with extensive spin delocalization from the high-spin Fe(III) fragment to the $Fe_4S_4(LS_3)$ cluster portion. This occurs to an extent that dominates the intrinsic isotropic shifts of cluster **4**.

(5) The results in 3 and 4, together with similarities or coincidences of the properties of fragment compounds and of assemblies **8** and **9**, provide the basis of proof of the proposed bridged structures.

(6) The electronic features of **8**, **9**, and related μ-S assemblies with $[Fe^{III}$(isobacteriochlorin)] fragments approach the intrinsic magnetic and spectroscopic properties of any structurally similar unit in an oxidized enzyme and thus potentially provide a means of identification of that unit. The isotropic shifts in (4) require the existence of magnetic hyperfine interactions in the Mössbauer spectra of the $[Fe_4S_4]^{2+}$ cluster, one defining aspect of a magnetically coupled Fe(III)-cluster unit.[3,10]

(7) Assemblies **8** and **9** exhibit two one-electron reduction steps as does *E. coli* SiR in the unligated and a variety of ligated states. The order of fragment reduction in the synthetic assemblies remains to be determined.

Work in progress, based on conclusion 6, involves the synthesis of a bridged assembly containing an Fe–isobacteriochlorin fragment and elucidation of the electronic and redox properties of that and other assemblies related to **8** and **9**.

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