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Cubane-Type Fe_4S_4 Clusters with Chiral Thiolate Ligation: Formation by Ligand Substitution, Detection of Intermediates by ^{1}H NMR, and Solid State Structures Including Spontaneous Resolution Upon Crystallization

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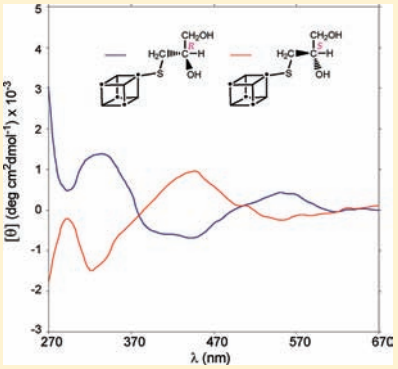
Supporting Information

ABSTRACT: Cubane-type clusters $[Fe_4S_4(SR^*)_4]^{2-}$ containing chiral thiolate ligands with R* = CH(Me)Ph (**1**), $CH_2CH(Me)Et$ (**2**), and $CH_2CH(OH)CH_2OH$ (**3**) have been prepared by ligand substitution in the reaction systems $[Fe_4S_4(SEt)_4]$/R*SH (**1**−**3**, acetonitrile) and $[Fe_4S_4Cl_4]^{2-}$/NaSR*(**3**, Me_2SO). Reactions with successive equivalents of thiol or thiolate generate the species $[Fe_4S_4L_{4-n}(SR^*)_n]^{2-}$ (L = SEt, Cl) with n = 1−4. Clusters **1** and **2** were prepared with racemic thiols leading to the possible formation of one enantiomeric pair (n = 1) and seven diastereomers and their enantiomers (n = 2−4). Reactions were monitored by isotropically shifted ^{1}H NMR spectra in acetonitrile or Me_2SO. In systems affording **1** and **2** as final products, individual mixed-ligand species could not be detected. However, crystallization of $(Et_4N)_2$[**1**] afforded **1**-[*SS*(*RS*)(*RS*)] in which two sites are disordered because of occupancy of *R* and *S* ligands. Similarly, $(Et_4N)_2$[**2**] led to **2**-[*SSSS*], a consequence of spontaneous resolution upon crystallization. The clusters **3**-[*RRRR*] and **3**-[*SSSS*] were obtained from enantiomerically pure thiols. Successive reactions lead to detection of species with n = 1−4 by appearance of four pairs of diastereotopic SCH_2 signals in both acetonitrile and Me_2SO reaction systems. Identical spectra were obtained with racemic, *R*-(−), and *S*-(+) thiols, indicating that ligand−ligand interactions are too weak to allow detection of diastereomers (e.g., [*SSSS*] vs [*SSRR*]). The stability of **3** in Me_2SO/H_2O media is described.



INTRODUCTION

The units $[Fe_4S_4(S\text{-}Cys)_{4-n}X_n]$ (n = 0, 1; X = non-Cys amino acid side chain or exogenous ligand) containing cubane-type Fe_4S_4 clusters in proteins[1] are rendered chiral by coordination of *L*-cysteinate residues and consequent inclusion of these units in folded protein structures. Manifestations of the chirality of such units in the $[Fe_4S_4]^{2+}$ oxidation level include multifeatured visible circular dichroism spectra[2−4] and prominent diastereotopic splittings of isotropically shifted cysteinate β-CH_2S protons in ^{1}H NMR spectra.[5−7] Synthetic analogues of protein-bound clusters are nearly always of the types $[Fe_4S_4(SR)_{4-n}X_n]^{2-}$ (n = 0, 1; X = nonthiolate ligand). Despite the large number of such species that have been prepared,[8] relatively few contain ligands with chirality centers. Enantiomers or racemic or diasteromeric mixtures are most simply produced by inclusion of one or more centers in the terminal ligands. The first such cluster, $[Fe_4S_4(S\text{-}L\text{-}Cys(Ac)NHMe)_4]^{2-}$, was obtained as a enantiomer;[9] thereafter, $[Fe_4S_4(SCH_2CH(OH)Me)_4]^{2-}$ was prepared from the racemic ligand.[10] More common has been the use of synthetic peptides of varying lengths containing *L*-Cys residues to prepare enantiomeric clusters,[9,11−19] for certain of which circular dichroism spectra have been reported. Clusters have also been prepared from α- and β-cyclodextrin mono- and dithiolates.[20,21] It might also be noted that cluster chirality can arise from sources other than single-atom chiral centers. $[Fe_4Se_4(Me_2LS_3)Cl]^{2-}$, containing a trigonally symmetric trithiolate ligand, undergoes spontaneous partial resolution when crystallized as the Ph_4P^+ salt.[22] Another conceivable means of forming a chiral Fe_4S_4 cluster, by binding four different ligands, is highly improbable given the pronounced tendency of clusters toward ligand scrambling in polar media.[23] Although not a single cubane, the edge-bridged tetracubanes $[Fe_{16}S_{16}(PR_3)_8]$ possess D_4 symmetry and thus are chiral,[24,25] but have not been isolated as single enantiomers. In contrast, the edge-bridged dicubanes $[Fe_8S_8L_6]^z$ are centrosymmetric and nonchiral.[24−26]

In our recent investigation of the stability of $[Fe_4S_4(SR)_4]^{2-}$ clusters in partially aqueous media, we have prepared clusters derived from an α-cyclodextrin monothiolate and a dithiolate.[21] The clusters are chiral because the ligands are composed of six *D*-glucopyranoside units arranged in a toroidal shape with $-CH_2S^-$ groups bonded to asymmetric carbon atoms in the 6^A,6^D positions. The clusters display visible circular dichroism spectra and isotropically shifted $-CH_2S$ ^{1}H NMR signals whose multiplicity

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(five or six resonances) is not readily explained. These and other observations raise questions about properties of chiral Fe_4S_4 clusters either as pure enantiomers or as mixtures. Clusters of this type have not been investigated in terms of methods of formation, structures, and other properties including observable interactions of chiral ligands. In this work, we have examined three clusters with chiral thiolate ligands, one of which has been obtained as separate enantiomers, with particular emphasis on their formation by ligand substitution reactions and the presence or absence of detectable intracluster ligand−ligand interactions as probed by NMR methods.

EXPERIMENTAL SECTION

Preparation of Compounds. All reactions and manipulations were carried out under a pure dinitrogen atmosphere in an inert atmosphere box or by standard Schlenk techniques. Solvents were passed through an Innovative Technology or MBraun solvent purification system and degassed prior to use. Deuterated acetonitrile and dimethyl sulfoxide (Cambridge Isotope Laboratories, Inc.) were dried over activated 4 Å molecular sieves. Solvent removals were done in vacuo; filtrations were performed through Celite. In the ^{1}H NMR data below, cation resonances are omitted.

$(Et_4N)_2[Fe_4S_4(SCH(Me)Ph)_4]$. To a solution of $(Et_4N)_2[Fe_4S_4(SEt)_4]$[27] (20.0 mg, 0.023 mmol) in 5 mL of acetonitrile was added 1-phenylethanethiol (13.6 mg, 0.098 mmol). The reaction mixture was subjected to partial vacuum with stirring for 3−5 min to remove ethanethiol (bp 35 °C) and was filtered. Ether was diffused into the filtrate causing separation of black needle-like crystals over several days. This material was washed with ether and dried to afford the product as 20.0 mg (74%) of black crystalline solid. Absorption spectrum (acetonitrile): λ_{max} (ε_M) 296 (23,300), 417 (17,150) nm. ^{1}H NMR (CD_3CN): δ 3.09 (3, CH_3), 6.94 (1, *p*-H), 7.40 (2, *o*-H), 7.45 (2, *m*-H), 11.32 (1, CH). *Anal.* Calcd. for $C_{48}H_{76}Fe_4N_2S_8$: C, 49.66; H, 6.60; N, 2.41. Found: C, 48.96; H, 6.71; N, 2.23.

$(Et_4N)_2[Fe_4S_4(SCH_2CH(Me)Et)_4]$. The preceding method was used on a 0.049 mmol cluster scale with 0.40 mmol of 2-methyl-1-butanethiol and a reaction time of 5−10 min. Vapor diffusion of ether into the filtrate gave the product as 31.0 mg (62%) of black crystalline solid. Absorption spectrum (acetonitrile): λ_{max} (ε_M) 308 (20,500), 421 (17,400) nm. ^{1}H NMR (CD_3CN): δ 1.05 (3, $CHCH_3$), 1.50 (3, CH_2CH_3), 1.76 (1, CH), 1.91 (2, CH_2CH_3); 12.95 (1, SCH_aH_b), 13.12 (1, SCH_aH_b). *Anal.* Calcd. for $C_{36}H_{84}Fe_4N_2S_8$: C, 42.19; H, 8.26; N, 2.73. Found: C, 41.99; H, 7.92; N, 2.68.

***(R)*-(−)- and *(S)*-(+)-2,3-dihydroxypropane-1-thiol.** *R*-(+)- and *S*-(−)-glycidol (2,3-epoxy-1-propanol) (Sigma-Aldrich) were purified by silica gel chromatography (ethyl acetate:hexane 3:2 v/v), and optical rotations were determined in chloroform: (*R*)-(+) isomer, $[\alpha]_D^{25}$ +18.7° (*c* 1.0,); (*S*)-(−) isomer, $[\alpha]_D^{24}$ −15.2° (*c* 1.35). The enantiomeric forms of the thiol were prepared by an adaptation of a published procedure.[28] $Ba(OH)_2\cdot 8H_2O$, 1.92 g (6.09 mmol) for (*R*)-(+)-glycidol and 3.82 g (12.1 mmol) for (*S*)-(−)-glycidol, was added to 80−100 mL of water, and H_2S was gently bubbled through the aqueous suspension with stirring for 90 min until most of the solid dissolved. A solution of 451 mg (6.09 mmol) of (*R*)-(+)-glycidol or 897 mg (12.1 mmol) of (*S*)-(−)-glycidol in 5 mL of methanol solution was slowly added to the aqueous solution over 5 min and H_2S was passed through the reaction mixture for 90 min. Thereafter, CO_2 was bubbled through the mixture with stirring for 60−90 min. The cloudy white suspension was filtered, and the filtrate was reduced to dryness. The residue was extracted with ethyl acetate (10−15 mL), the extract was filtered, and the filtrate was reduced to a slightly yellow oily residue. This material was purified by silica gel chromatography (dichloromethane/methanol 40:1 v/v). Removal of solvent afforded the product as a colorless oil. (*R*)-(−) isomer: 0.475 g (72%), $[\alpha]_D^{23}$ −8.47° (*c* 9.25, methanol) (lit.[29] $[\alpha]_D^{25}$ −8° (*c* 11, ethanol)). (*S*)-(+) isomer: 0.405 g (31%), $[\alpha]_D^{23}$ +8.29° (*c* 5.9, methanol); the optical rotation of this isomer has not been previously reported. The isomers obtained by this procedure gave ^{1}H and ^{13}C NMR spectra identical to each other and to the racemic thiol. ^{1}H NMR (CD_3OD): δ 3.60 (1, quintet, J = 5.5 Hz), 3.52 (2, dd, J = 5.5, 11.2 Hz), 2.60 (1, dd, J = 6.0, 13.2 Hz), 2.51 (1, dd, J = 6.5, 13.2 Hz). ^{13}C NMR (CD_3OD): δ 74.36 (SCH_2), 65.20 (CHOH), 28.16 (CH_2OH).

$(Et_4N)_2[Fe_4S_4(SCH_2CH(OH)CH_2OH)_4]$. This compound was prepared from racemic, (*R*)-(−)-, and (*S*)-(+)-2,3-dihydroxypropane-1-thiol by ligand substitution. A solution of $(Et_4N)_2[Fe_4S_4(SEt)_4]$ in acetonitrile (5 mL) was treated with the thiol (4.2 equiv). The reaction was conducted under dynamic vacuum to remove ethanethiol and drive the reaction to completion. The reaction mixture was filtered after a reaction time of 30 min. Solvent removal gave the product as a black oily material which proved difficult to crystallize whether prepared from the racemic or optically pure thiol. Absorption spectrum (acetonitrile): λ_{max} (ε_M) 305 (20,910), 405 (16,940) nm. ESI-MS: m/z 909.0, $\{(Et_4N)[Fe_4S_4(C_3H_7O_2S)_4]\}^-$. Compounds prepared from the (*R*)-(−) or racemic ligand gave identical absorption and mass spectra. ^{1}H NMR (CD_3CN): δ 4.05 (1, *C*HOH), 4.12 (2, CH_2OH), 11.95 (1, SCH_aH_b), 12.47 (1, SCH_aH_b). ^{13}C-{^{1}H} NMR (CD_3CN): δ 103.04 (SCH_2 + *C*HOH), 71.20 (CH_2OH).

Table 1. Crystallographic Data for $(Et_4N)_2[Fe_4S_4(SR)_4]$ Clusters[a]

	R = CH(Me)Ph	R = CH_2CH(Me)Et
formula	$C_{48}H_{76}Fe_4N_2S_8$	$C_{36}H_{84}Fe_4N_2S_8$
formula weight M	1160.99	1024.93
crystal system	monoclinic	monoclinic
space group	$P2_1/n$	$C2$
a (Å)	11.5438 (6)	15.857 (1)
b (Å)	22.664 (1)	19.592 (1)
c (Å)	21.534 (1)	16.271 (1)
$\alpha = \gamma$(deg)	90	90
β (deg)	92.934 (3)	90.944 (4)
V (Å^3)	5626.5 (5)	5054.3 (6)
d_{calc} (g/cm^3)	1.371	1.347
Z	4	4
GOF	1.09	1.01
R^b ($wR2$)^c	0.078 (0.190)	0.062 (0.168)

[a] Mo Kα radiation (λ = 0.71073 Å), T = 100 K. [b] $R_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$. [c] $wR^2 = \{\Sigma[w(F_o^2 - F_c^2)^2/\Sigma(F_o^2)^2]\}^{1/2}$.

X-ray Structure Determinations. X-ray structures of the two compounds in Table 1 were determined using crystals of moderate diffraction quality obtained by crystallization from acetonitrile/ether. Data were collected with a Bruker-AXS Smart Apex CCD diffractometer equipped with an Oxford Cryostream 700 series low-temperature apparatus operating at 100 K. Single crystals were coated with Paratone-N oil and mounted anaerobically on a nylon CryoLoop. Unit cells were determined with APEX II software.[30] The data collection method involved 0.5° scans in ω at 28° in 2θ. Neither crystal showed significant decay during data collection. Raw data were integrated down to 0.82 Å resolution with reflection spot size optimization and were corrected for Lorentz and polarization effects using Bruker SAINT V7.46 A.[30] Absorption corrections were made with the program SADABS. Structures were solved by direct methods and refined by least-squares methods against F^2 using SHELXS-97 and SHELXL-97.[31] All non-hydrogen atoms including disordered fragments were located in difference-Fourier maps and refined anisotropically. Restraints on bond

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lengths and constraints of atom displacement parameters on each pair of disordered fragments of cations (SADI and EADP instructions of SHELXL-97), as well as restraints of other atom displacement parameters (SIMU/DELU instructions of SHELXL97) if necessary, have been applied to disorder refinements. Hydrogen atoms were introduced at calculated positions and refined using a riding model. Crystallographic data are collected in Table 1.[32]

NMR Spectra. All 1D and 2D NMR experiments were performed on Varian UNITY/NOVA 500 and 600 spectrometers using anaerobic solutions. Both proton-coupled and proton-decoupled ^{13}C spectra were obtained to distinguish unambiguously methylene and methine carbon atoms. The longitudinal (spin−lattice) relaxation times T_1 were measured by the inversion−recovery method. 2D $^{1}H-^{13}C$ HSQC (Heteronuclear Single Quantum Coherence) experiments were carried out without using pulse-field-gradients and with decreased coherence transfer times to compensate for the fast relaxation of some protons. 2D TOCSY (Total Correlation Spectroscopy) experiments were done by varying the mixing time from 10 to 80 ms to monitor the progress of coherence transfers.

Other Physical Measurements. All measurements were made under anaerobic conditions. Absorption spectra were measured on a Varian Cary 50 Bio spectrophotometer and circular dichroism spectra (at 20 °C) on a Jasco J-715 spectropolarimeter. Electrospray ionization mass spectrometry (ESI-MS) was performed at the Boston College Mass Spectrometry Facility and recorded using a Waters LCT instrument. Samples were dissolved in LC/MS CHROMASOLV grade (≥99.9%) acetonitrile. Mass spectra were measured for 10−100 μM sample solutions in the negative ion mode; samples were infused into the spectrometer using a Harvard syringe pump operating at a flow rate of 10 μL/min. Typical instrumental conditions were 3.5 kV capillary voltage, 5 V cone voltage, and a desolvation temperature of 120 °C; data were recorded using the electrospray method.

RESULTS AND DISCUSSION

Three clusters **1**−**3** with chiral ligands have been selected for investigation; clusters **4**[27,33] and **5**[34] were used as starting materials in ligand substitution reactions:

$[Fe_4S_4(SCH(Me)Ph)_4]^{2-}$ **1**

$[Fe_4S_4(SCH_2CH(Me)Et)_4]^{2-}$ **2**

$[Fe_4S_4(SCH_2CH(OH)CH_2OH)_4]^{2-}$ **3**

$[Fe_4S_4(SEt)_4]^{2-}$ **4**

$[Fe_4S_4Cl_4]^{2-}$ **5**

Clusters **1**−**3** were prepared from **4** by ligand substitution reaction 1 conducted under dynamic vacuum to remove ethanethiol and shift the reaction to formation of the fully substituted product ($n = 4$). Reaction 2 is not reversible and, as will be seen, is also used to generate substituted species in solution.

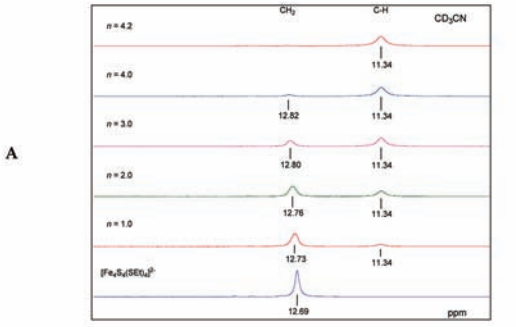
$$[Fe_4S_4(SEt)_4]^{2-} + n\ R^*SH \leftrightarrows [Fe_4S_4(SEt)_{4-n}((SR^*)_n]^{2-} + n\ EtSH \quad (1)$$

$$[Fe_4S_4Cl_4]^{2-} + n\ R^*S^- \rightarrow [Fe_4S_4Cl_{4-n}(SR^*)_n]^{2-} + n\ Cl^- \quad (2)$$

Both of these reactions are amply precedented and have been discussed elsewhere.[21,35,36] All three clusters were prepared from racemic thiols. One enantiomer each of the ligands in **1** and **2** have been described.[37,38] However, both enantiomers of 2,3-dihydroxypropane-1-thiol are accessible. The (*R*)-(−) isomer was reported earlier[29] and the (*S*)-(+) isomer has been prepared in this work, leading to the formation of the enantiomeric clusters **3**-[*RRRR*] and **3**-[*SSSS*]. The formation and properties of clusters **1** and **2**, and of **3** prepared from the racemic and enantiomeric forms of the thiol, are considered next and involve NMR observations. A large body of prior data demonstrates that isotropic shifts of $[Fe_4S_4(SR)_4]^{2-}$ clusters typified by **4** are dominantly contact in origin, arising from partial occupation of an excited spin-triplet state, and that SCH_2 chemical shifts occur in the 10−16 ppm range at room temperature.[21,33,39] In the following sections we use the index n for both the number of equiv of reactant chiral thiol R*SH and the number of thiolate ligands R^*S^- bound. The removal of ethanethiol in reaction 1 conducted under dynamic vacuum renders the reaction nearly stoichiometric in most cases.

$[Fe_4S_4(SCH(Me)Ph)_4]^{2-}$. Preparation of cluster **1** is illustrated by reaction 3 in Figure 1A. The course of the reaction was examined in increments of n = 1.0−4.2 equiv of racemic thiol in acetonitrile. The SCH_2 signal of initial cluster **4** at 12.69 ppm decreases

$$[Fe_4S_4(SEt)_4]^{2-} + n\ Ph(Me)CHSH \rightarrow [Fe_4S_4(SEt)_{4-n}(SCH(Me)Ph)_n]^{2-} + n\ EtSH\uparrow \quad (3)$$



$$[Fe_4S_4(SEt)_4]^{2-} + n\ Et(Me)CHCH_2SH \rightarrow [Fe_4S_4(SEt)_{4-n}(SCH_2CH(Me)Et)_n]^{2-} + n\ EtSH\uparrow \quad (4)$$

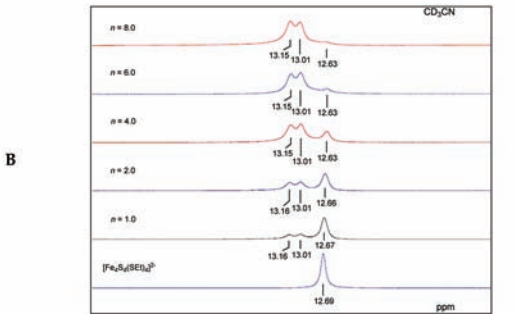


Figure 1. ^{1}H NMR spectra (600 MHz) of the reaction systems $[Fe_4S_4(SEt)_4]^{2-}$/R*SH with R* = CH(Me)Ph (A) and $CH_2CH(Me)Et$ (B) in CD_3CN at ambient temperature in the SCH and SCH_2 regions with n equiv of racemic thiol added. Reactions were conducted under dynamic vacuum to remove ethanethiol. Signal assignments are indicated. Other ligand resonances occur at higher fields.

Table 2. Enantiomers and Diastereomers of the Clusters $[Fe_4S_4(SR)_{4-n}(SR^*)_n]^{2-}$ (R Nonchiral, R* Chiral)

n	diastereomers/enantiomer		
1	*S/R*	(enantiomers only)	
2	*SS/RR*	*SR/RS*	
3	*SSS/RRR*	*SSR/RRS*	
4	*SSSS/RRRR*	*SSSR/RRRS*	*SSRR/RRSS*

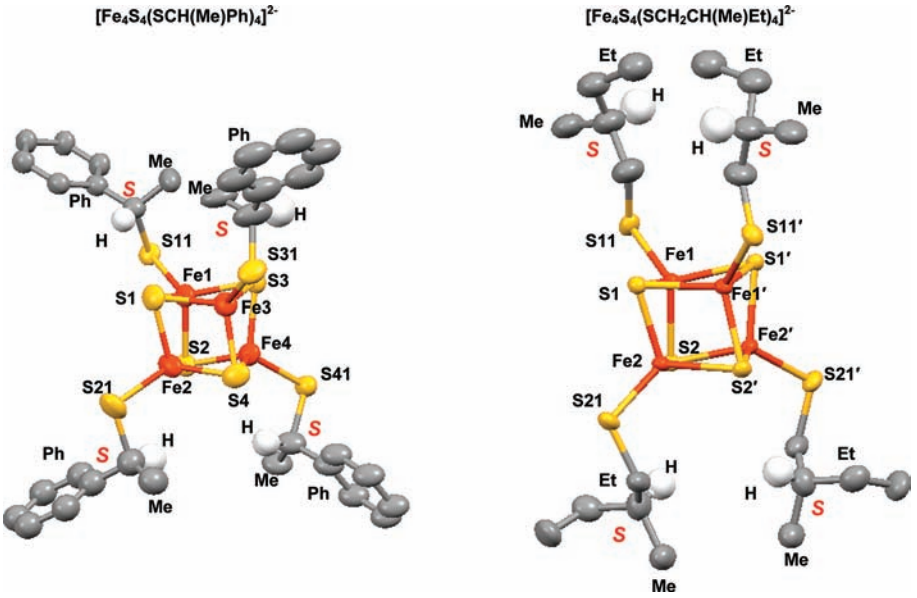


Figure 2. Structures of $[Fe_4S_4(SCH(Me)Ph)_4]^{2-}$ (**1**, left) and $[Fe_4S_4(SCH_2CH(Me)Et)_4]^{2-}$ (**2**, right) showing atom labeling schemes and 50% probability ellipsoids. For **1**, the structure of one member (*SSSS*) of the set of clusters with RS disorder at two sites is shown. The structure of **2** is chiral (*SSSS*) and has an imposed C_2 axis which passes through opposite faces Fe1Fe1′S1S1′ and Fe2Fe2′S2S2′ and relates primed and unprimed atoms. One of two independent clusters is shown. Core dimensional ranges and mean values (Å): **1** − Fe−S 2.242(2)−2.311(2), 2.28(3);Fe−Fe 2.719(1)−2.773-(1), 2.73(3); **2** − (two independent clusters) Fe−S 2.239(2)−2.317(2), 2.29(4), Fe−Fe 2.710(1)−2.786(2), 2.74(3); Fe−S 2.242(2)−2.321(2), 2.29(4), Fe−Fe 2.713(1)−2.772(1), 2.74(3).

and the SC*H* resonance of bound phenylethanethiolate at 11.34 ppm increases in intensity as *n* is increased. Substitution is complete at *n* = 4.2 resulting in the possible diastereoisomeric mixture of three species and their enantiomers. At *n* = 1 one enantiomeric pair can occur and at *n* = 2 and 3 two diastereoisomeric pairs are possible, generating a total of eight potentially detectable species over the complete range of substitution. Diastereomers and enantiomers are specified in Table 2. The SC*H* shift does not depend on the number of ethanethiolate ligands bound, while the SCH_2 signal responds with small downfield shifts as the number of ethanethiolate species decreases. Despite the often exceptional sensitivity of thiolate contact shifts of $[Fe_4S_4]^{2+}$ clusters to small structural and ligand differences,[40,41] neither signal is resolved into components over the *n* = 1.0−3.0 equiv range where mixed ligand clusters are formed, even at 600 MHz. Also, a single methyl resonance was observed at 3.09 ppm over this range with a faster T_1 relaxation time in a 1D inversion recovery experiment.[42]

Crystallization of **1** as the Et_4N^+ salt afforded diffraction-quality crystals which were manually selected by shape. Nearly all contained clusters with extensively disordered thiolates highly suggestive of binding of *R* and *S* ligands; these were not further pursued. However, crystals were found in the nonchiral space group $P2_1/n$ containing two independent cations and one independent cluster with the structure in Figure 2. All four thiolate ligands and the cations are disordered, but their positions could be located in difference Fourier maps. The crystal packing places the thiolates in two surrounding environments that can be likened to cavities or void volumes (Supporting Information, Figure S1[32]).[43] In the smaller cavities (157, 167 Å^3) the phenyl ring is not free to reorient relative to the chiral center, but the positions of methyl group and hydrogen atoms can be flipped. This leads to mixed (*RS*) coordination of two thiolates in the final disorder model. In the larger cavities (177, 184 Å^3) the extra space allows the phenyl rings to rotate without flipping the methyl and hydrogen substituents; these two ligands are unambiguously of the same absolute configuration. The cluster is described as **1**-[*SS(RS)(RS)*]. In this space group, the other cluster molecule generated by symmetry operations has the opposite configuration, **1**-[*RR(SR)(SR)*]. In these clusters, two sites contain *R* or *S* ligands with minor conformational disorder, and the other two sites are disordered because of occupancy of *R* and *S* ligands. The structure depicted in Figure 2 is one of the actual species present, **1**-[*SSSS*]. Because the X-ray evidence is consistent with the binding of *R* and *S* ligands, the failure to detect individual diastereomers by ^{1}H NMR must be a consequence of very weak or nil interactions between or among the chiral ligands of **1** in solution.

$[Fe_4S_4(SCH_2CH(Me)Et)_4]^{2-}$. The cluster was prepared by reaction 4 in Figure 1B. Addition of *n* = 1.0−4.0 equiv of 2-methylbutane-1-thiol to a solution of **4** in acetonitrile diminishes the intensity of the 12.69 ppm signal with a very slight upfield shift and develops a doublet of increasing intensity at 13.16 and 13.01 ppm. The reaction is essentially complete at *n* = 8 equiv and its product is identical to the isolated Et_4N^+ salt of **2**. The doublet arises from the diastereotopic splitting of the SCH_2 group of the chiral ligand. This splitting (0.14 ppm) is much smaller than that ascribed to $[Fe_4S_4(S\text{-}L\text{-}Cys(Ac)NHMe)_4]^{2-}$ in Me_2SO (1.2 ppm),[9] the first example of such splitting in a $[Fe_4S_4]^{2+}$ cluster. (*L*-amino acids have the *S* absolute configuration.) The remaining ligand signals occur at 1−2 ppm (Experimental Section). The two methyl signals were distinguished by 2D COSY and 2D TOCSY experiments (Supporting Information, Figure S2).[44−46]

Crystallization of $(Et_4N)_2$[**2**] from acetonitrile/ether gave a small population of crystals in chiral space group *C*2. In the asymmetric unit there are two cations, one of which shows disorder, and two half-Fe_4S_4 core units. The structure of one cluster is presented in Figure 2; Both clusters exhibit a compressed

$$[Fe_4S_4Cl_4]^{2-} + n\ (R)\text{-}HOCH_2CH(OH)CH_2SH + n\ NaOH \rightarrow [Fe_4S_4(SEt)_{4-n}(SCH_2CH(OH)CH_2OH)_n]^{2-} + n\ NaCl + n\ H_2O \quad (5)$$

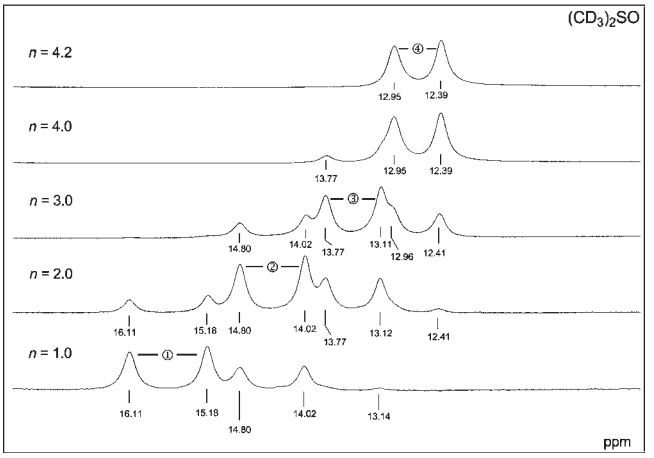


Figure 3. ^{1}H NMR spectra of the reaction system $[Fe_4S_4Cl_4]^{2-}$/*S*-$HOCH_2CH(OH)CH_2SH$/NaOH in $(CD_3)_2SO$ at ambient temperature in the SCH_2 region with n = 1.0−4.2 equiv of thiol and base added. Other ligand resonances occur at higher fields.

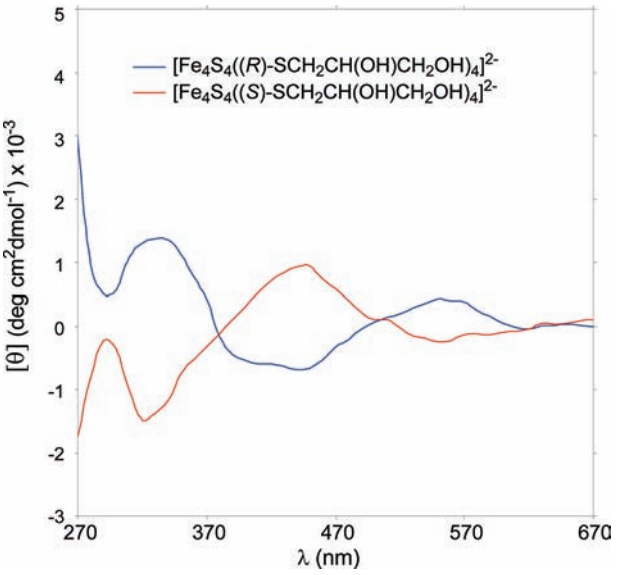


Figure 4. Circular dichroism spectra (270−670 nm) of the clusters **3**-[*RRRR*] (blue) and **3**-[*SSSS*] (red) in Me_2SO solutions at room temperature.

tetragonal core shape with four short and eight long Fe−S distances approximately parallel and perpendicular to the C_2 axis, a common distortion of $[Fe_4S_4]^{2+}$ cores from idealized T_d symmetry.[8] These distances are 2.240(1) Å and 2.311(6) Å for one cluster and 2.244(3) Å and 2.314(6) Å for the other. This distortion is approached but is not as clearcut in **1**. In both clusters and in **1**, bond distances and angles are unexceptional. One of the thiolate ligands in each half-core fragment is well-ordered and the other (in the slightly bigger cavity) shows some disorder mainly with the ethyl group that does not affect the chiral center (Supporting Information, Figure S3). The cluster is chiral with the configuration **2**-[*SSSS*], a result of spontaneous resolution upon crystallization.

$[Fe_4S_4(SCH_2CH(OH)CH_2OH)_4]^{2-}$. The pure clusters **3**-[*RRRR*] and **3**-[*SSSS*] and a presumed diastereomeric mixture of clusters were obtained from the enantiomeric and racemic thiols, respectively, by reaction 5 of Figure 3. The Et_4N^+ salts were isolated as sticky or oily products which, despite numerous attempts, could not be obtained in crystalline form. We note that clusters with monohydroxythiolate ligands and different cations have been crystallized and structures determined.[10,47−49] The majority of these clusters carry *n*-alkylthiolate ligands with a terminal hydroxyl group. An exception is $[Fe_4S_4(SCH_2CH(OH)Me)_4]^{2-}$ which crystallizes with the [*RS*(*RS*)(*RS*)] configuration.[10] Apparently, the conformational flexibility of the ligand is unfavorable to crystallization of the cluster salt whose collective spectroscopic properties (Experimental Section and Supporting Information, Figure S4) satisfactorily identify the cluster as **3**.

The enantiomeric relationship between clusters prepared from the (*R*)-(−) and (*S*)-(+) thiols is supported by the circular dichroism spectra in Figure 4. Their inverse relationship is apparent but is not exact, probably because of small differences in enantiomeric purity of the thiols and in chemical purity inasmuch as the cluster salts were not amenable to crystallization. Their isotropically shifted ^{1}H NMR spectra, however, are identical (see below).

The formation of **3** has been examined by the sets of consecutive reactions 5 (Figure 3) and 6 (Figure 5) monitored by ^{1}H NMR. Signals due to CH_3, CH, and CH_2OH occur at higher fields.

$$[Fe_4S_4(SEt)_4]^{2-} + n\ (S)\text{-}HOCH_2CH(OH)CH_2SH \rightarrow [Fe_4S_4(SEt)_{4-n}(SCH_2CH(OH)CH_2OH)_n]^{2-} + n\ EtSH\uparrow \quad (6)$$

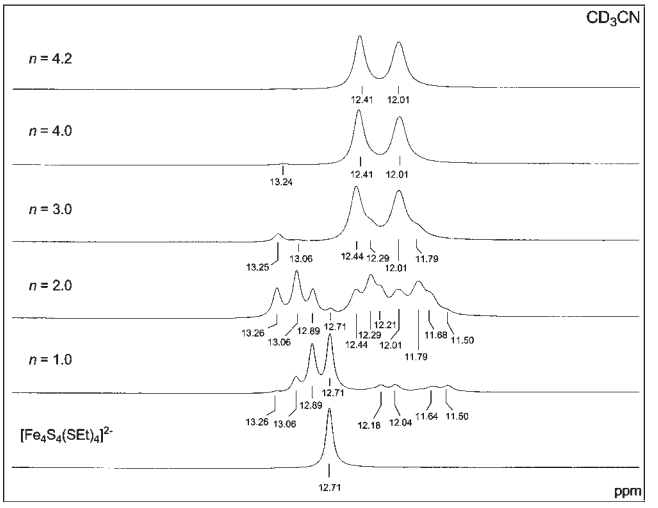


Figure 5. ^{1}H NMR spectra of the reaction system $[Fe_4S_4(SEt)_4]^{2-}$/S-$HOCH_2CH(OH)CH_2SH$ in CD_3CN at ambient temperature in the SCH_2 region with n = 1.0−4.2 equiv of thiol added. Reactions were conducted as in Figure 1; other ligand resonances occur at higher fields. Signals at 12.71 ppm and lower fields arise from SCH_2CH_3 groups while the signals at higher field are due to the 3-mercaptopropane-1,2-diol ligand in n = 1−4 clusters.

In terms of SCH_2 signal multiplicity, reaction 5 involving chloride displacement from **5**, is simpler and is considered first. Intensity changes over the interval n = 1.0−4.2 equiv of thiolate lead to the assignment of the SCH_2 diastereotopic signal pair for each species (circled). The relative intensities for a given value of n do not follow a statistical distribution because of the much higher thiolate versus chloride binding affinity. In system shown, the (*R*)-(−) thiol was employed, and the diastereotopic splitting of **3**-[*RRRR*] (0.56 ppm) is considerably larger than for **2**.

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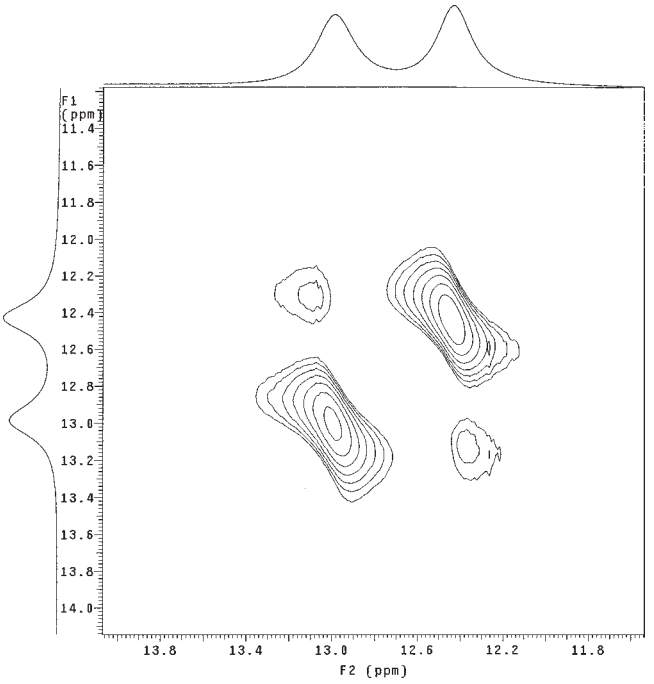

Figure 6. 2D TOCSY spectrum of $(Et_4N)_2[Fe_4S_4(R\text{-}SCH_2CH(OH)CH_2OH)_4]$ in the downfield region at ambient temperature in CD_3CN showing SCH_2 resonances.

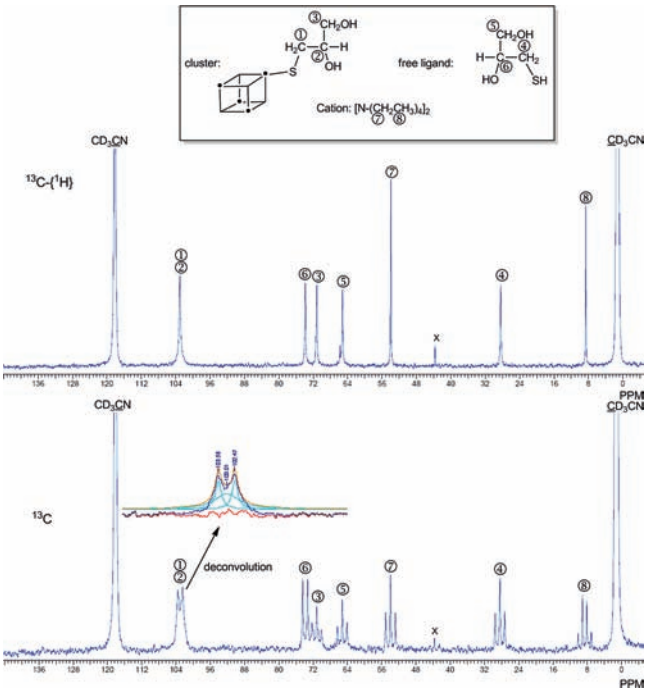

Figure 7. ^{13}C NMR spectrum of $(Et_4N)_2[Fe_4S_4(SCH_2CH(OH)CH_2OH)_4]$ in CD_3CN at ambient temperature. Upper: proton-decoupled $^{13}C\text{-}\{^1H\}$ spectrum. Lower: ^{13}C spectrum. Signal assignments are numerically designated. The cluster was prepared from the racemic thiol.

Identical spectra for all *n*-values were found with the (*S*)-(+) and the racemic thiol.

Reaction system 6 in Figure 5 is considerably more complicated because multiple SCH_2 signals are generated by starting cluster **4**, final product **3**, and three mixed-ligand species. Over the *n* = 1.0−4.2 equiv range, it appears that the signals at fields below 12.7 ppm are composed of four broad singlets of constant chemical shifts while those at higher field appear as eight features whose shifts show small variations in different mixed ligand clusters. It was shown by the addition of a small amount of D_2O that none of the signals in Figure 3 are from hydroxyl groups. As *n* is increased, the 12.71 resonance of **4** decreases in intensity while those at 12.89, 13.06, and 13.26 ppm change in a manner consistent with the assignments to clusters with *n* = 1, 2, and 3, respectively.

Examination of the relative intensities of signals at fields above 12.71 ppm over the range of added thiol leads to the following assignment of diastereotopic pairs:

$n = 1$ 11.50, 12.04 ppm $n = 2$ 11.64−11.68, 12.18−12.21 ppm

$n = 3$ 11.79, 12.29 ppm $n = 4$ 12.01, 12.41−12.44 ppm

At *n* = 1.0 equiv, the pair at 11.50 and 12.04 ppm arise from the *n* = 1 species while at *n* = 4.2 equiv the limiting spectrum of **3** is reached with the pair at 12.01 and 12.41 ppm. At *n* = 3.0 equiv the weak signal at 13.25 ppm supports assignment of the pair at 11.79 and 12.29 ppm to the *n* = 3 cluster. Shifts of the SCH_2 protons move to higher field with increasing substitution while diastereotopic splittings remain nearly constant in the range 0.40−0.54 ppm. Proof that the signal pair in the spectrum attributed to **3** arises from a diastereotopic methylene group follows from the spectra in Figure 6. The 2D TOCSY spectrum reveals cross peaks correlating the signals at 12.01 and 12.41 ppm, indicating the protons are bonded to the same carbon atom. 2D COSY is expected to produce similar cross peaks connecting two coupled J-signals. However, fast relaxation times of the two peaks suppress the coherence transfer between these peaks in the COSY experiment. Fast relaxation also prevents coherence transfer between the two protons and their directly bonded carbon atom in a 2D HSQC[50] experiment. However, the spectra do show a peak correlating a ^{13}C resonance at 103 ppm to a proton resonance at 4.0 ppm, assigned as the methine proton of the bound thiolate (Supporting Information, Figure S5).

Shown in Figure 7 are ^{13}C NMR spectra of **3** taken with and without proton broadband decoupling. Few such spectra have been reported for synthetic iron−sulfur clusters. Despite the well-resolved spectra, one of the three cluster resonances appears to be absent in the decoupled spectrum. Without decoupling, we find that the 103 ppm signal can be deconvoluted into a sharp spin−spin doublet and an underlying broad feature. The integrated area of this signal is apparently twice as large as that of the other cluster peak, a triplet at 72 ppm which arises from the CH_2OH group. The ^{13}C relaxation times are short enough that integration of signals provides a reliable indication of the number of carbon atoms associated with each signal. The doublet at 103 ppm is due to the methine carbon atom and the remaining intensity to the SCH_2 group, whose signal is broadened and $^1H-^{13}C$ spin coupling abolished by paramagnetic relaxation effects of the cluster. The solution also deliberately contained free ligand, allowing an estimate of apparent ^{13}C isotropic shifts produced by cluster paramagnetism. For the SCH_2 group, the shift is 75 ppm and much smaller for the *C*H (29 ppm) and CH_2OH (7 ppm) sites where unpaired spin delocalization proceeds through a saturated framework.

The appearance in Figures 3 and 5 of four doublets over the range of *n* = 1.0−4.2 equiv of thiol added proves that at least four different species are formed, culminating in **3**. Increasing

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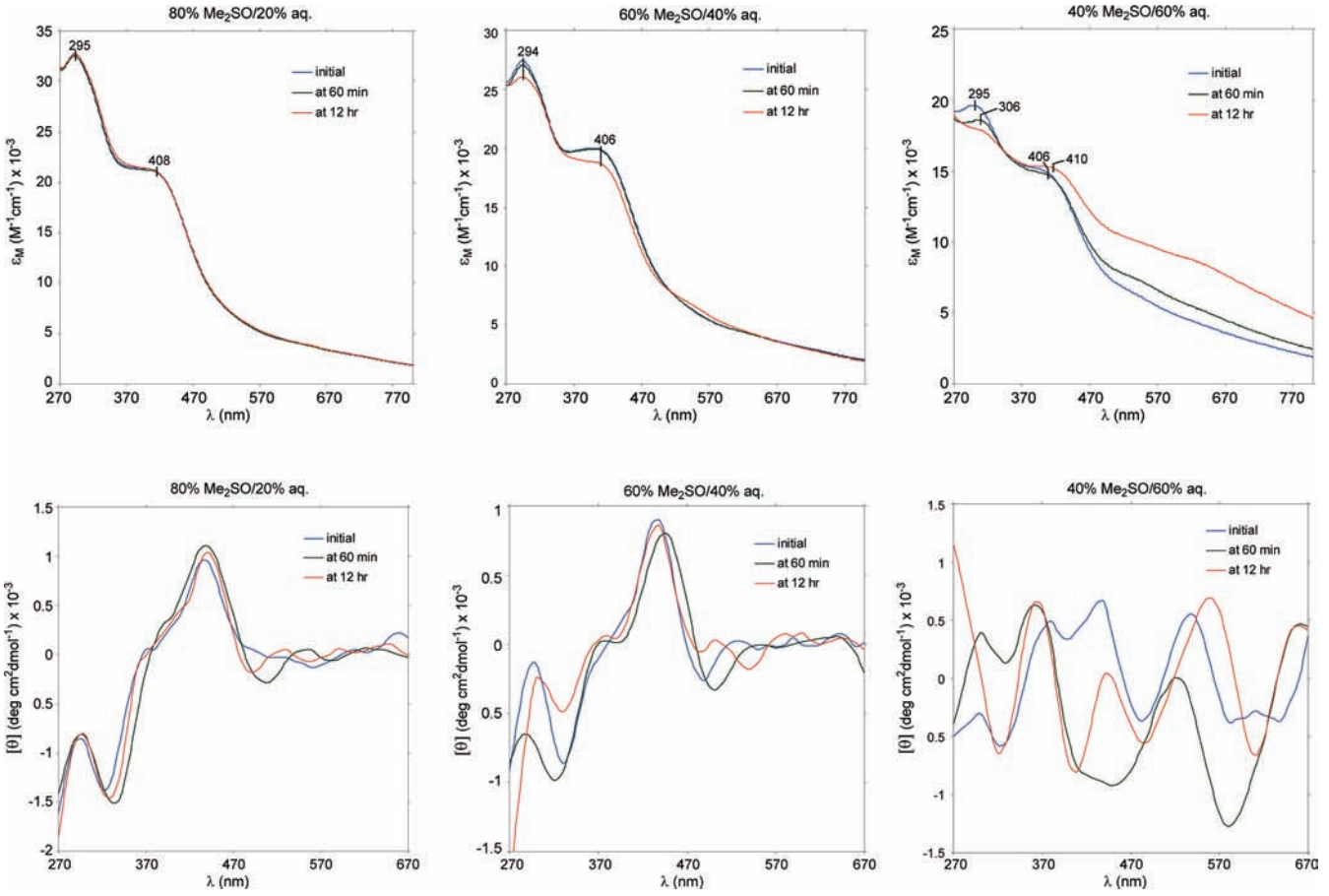

Figure 8. Absorption spectra (upper) and circular dichroism spectra (lower) of $(Et_4N)_2$-$[Fe_4S_4(SCH_2CH(OH)CH_2OH)_4]$ in 80/20, 60/40, and 40/60 Me_2SO/H_2O solutions (v/v). Cluster concentrations are 0.19−0.37 mM; the aqueous component containing 3.5 mM phosphate buffer (pH 7.5). Spectra were measured immediately after solution preparation (blue) and at 60 min (green) and at 12 h (red). The cluster **3**-[*SSSS*] was used in the circular dichroism measurements.

substitution of the original clusters with 2,3-dihydroxypropane-1-thiolate allows resolution of signals from all species, a fortunate circumstance that does not arise in the reaction system of Figure 1B leading to **2**. We cannot demonstrate that all possible diastereomers are formed, however, because the spectra in Figures 3 and 5 are identical whether (*R*)-(−), (*S*)-(+), or racemic thiol is used. Even with a ligand capable of hydrogen-bond interactions with another ligand in the same or a different cluster, diastereomeric interactions are evidently too slight to be resolved in ^{1}H or ^{13}C NMR spectra. For protein-bound $\{Fe_4S_4(S_{Cys})_4\}$ clusters in the same oxidation state, inequivalent β-CH_2 resonances of individual cysteinyl residues are generally well-resolved with much larger differences than the 0.40 ppm diastereotopic splitting of **3**. This situation arises from the angular dependence of electron−proton coupling constants associated with the more rigid protein structures and specific environmental effects of these structures.[5−7,51] Clusters **2** and **3** present chiral ligands but with conformational mobility of the alkyl chains.

Cluster Stability in Partially Aqueous Media. As explained elsewhere,[21,52] $[Fe_4S_4(SR)_4]^{2-}$ clusters that remain intact in partially aqueous media are of interest in reconstituting scaffold proteins which in turn influence restoration of mitochondrial function when iron−sulfur cluster formation is impaired. Because **3** contains more hydrophilic substituents than any previously tested cluster, its stability was examined spectrophotometrically in aqueous Me_2SO media by a procedure described recently[21] and with inclusion of circular dichroism measurements of **3**-[*SSSS*]. The results, obtained under anaerobic conditions, are set out in Figure 8. The two-band spectrum with maxima near 295 and 408 nm is entirely typical of the clusters with alkylthiolate substituents.[21,27,53] Spectra in 80/20 and 60/40 Me_2SO/water are nearly identical to each other and to spectra in pure Me_2SO, and indicate a high degree of stability up to 12 h in these media. Absorption spectra in 40/60 Me_2SO/water suggest partial retention of the original chromophore at 60 min or less, but the increased absorption into the visible region at 12 h indicates substantial alteration of the initial cluster, probably by ligand solvolytic substitution reactions.[21,54,55] Circular dichroism spectra in this medium lead to a similar interpretation, and the spectrum at 12 h, being very different from the initial and 60 min spectra, suggests the formation of a different core structure which at present is unknown. In 20/80 Me_2SO/water, spectral changes are more rapid and extensive (not shown). The solubility and stability of cluster **3** are sufficient to make it possibly useful in protein reconstitution in media containing about 50% water. Such experiments are in progress.

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

(1) Three clusters $[Fe_4S_4(SR^*)_4]^{2-}$ (**1**−**3**) containing chirality centers (R* = CH(Me)Ph (**1**), $CH_2CH(Me)Et$ (**2**),

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$CH_2CH(OH)CH_2OH$) (**3**)) are prepared from racemic thiols, and one cluster has been obtained in enantiomeric forms (**3**-[*RRRR*], **3**-{*SSSS*]) from optically enriched thiols. It is the only synthetic or biological Fe_4S_4 cluster isolated as separate enantiomers.

(2) $(Et_4N)_2$[**1**] crystallizes as **1**-[*SS*(*RS*)(*RS*)] and its opposite configuration, having *R*/*S* disorder at two sites and demonstrating mixed configuration binding. A portion of crystalline $(Et_4N)_2$[**2**] occurs in chiral space group *C*2 as **2**-[*SSSS*], an infrequent instance of spontaneous resolution upon crystallization.

(3) The clusters in (1) form in sequential substitution reactions of $[Fe_4S_4L_4]^{2-}$ with *n* equiv of R*SH (acetonitrile, L = EtS^-, **1**−**3**) or R^*S^- (Me_2SO, L = Cl^-, **3**). Intermediate species $[Fe_4S_4\text{-}L_{4-n}(SR^*)_n]^{2-}$ and final products ($n \geq 4$) were identified by isotropically shifted ^{1}H NMR spectra. All clusters exhibit well-resolved diastereotopic splittings of $-CH_2S$ signals which for **3** were identified in a 2D TOCSY spectrum.

(4) In the formation of **3** using racemic, *R*-(−), or *S*-(+) thiol, ^{1}H NMR spectra of the reactions in (3) are *identical*, indicating that at all values $n = 2-4$ diastereomers are not detectable. Similarly, no evidence for diastereomers is found in systems generating **1** and **2** from racemic thiols. Evidently, larger and perhaps more rigid R* groups are required for detectable ligand−ligand interactions.

(5) Absorption and circular dichroism spectra of **3** in anaerobic aqueous Me_2SO indicate cluster stability up to at least 60 min in the medium with 40% aqueous content. Circular dichroism spectra suggest the formation of a different core structure in 40/60 Me_2SO/water solution after 12 h.

■ ASSOCIATED CONTENT

Supporting Information. X-ray crystallographic files in CIF format for the compounds in Table 1; Figures S1−S5 showing structures and the void volumes of cavities (Å^3) for each coordinated ligand of clusters **1** and **2**; mass spectra and 2D NMR spectra of clusters **2** and **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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