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Stabilities of Cubane Type $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-}$ Clusters in Partially Aqueous Media

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Abstract

The stability of cubane-type $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-}$ clusters in mixed organic/aqueous solvents was examined as an initial step in the development of stable water-soluble cluster compounds possibly suitable for reconstitution of scaffold proteins in protein biosynthesis. The research involves primarily spectrophotometric assessment of stability in 20–80% Me_2SO /aqueous media (v/v), from which it was found that conventional clusters tend to be stable for up to 12 hours in 60% Me_2SO but are much less stable at higher aqueous content. α -Cyclodextrin mono- and dithioesters and thiols were prepared as ligand precursors for cluster binding, which was demonstrated by spectroscopic methods. A potentially bidentate cyclodextrin dithiolate was found to be relatively effective for cluster stabilization in 40% Me_2SO , suggesting (together with earlier results) that other exceptionally large thiolate ligands may promote cluster stability in aqueous media.

Keywords

iron-sulfur clusters; cyclodextrin thiolates; solution stability of clusters

1. Introduction

Iron-sulfur proteins are critical components in the key life processes of respiration, photosynthesis, and nitrogen fixation [1–3]. An ultimate fundamental understanding of their diverse cellular functions requires elucidation of the biogenesis of these proteins, which includes the means of assembly of an iron-sulfur cluster on a scaffold protein followed by its insertion into an apoprotein. There is currently much activity directed toward biogenesis of iron-sulfur proteins in both bacterial and mammalian systems [2–8]. Identification of the proteins involved and the probable order of events contributes to formulation of a biogenesis pathway. There is yet another imperative for this research, *viz.*, the emerging connection between impaired iron-sulfur cluster formation and human disease, most notably Friedreich's ataxia [7–10]. In this disease, mutations in the frataxin genes lead to a deficiency in this protein, a probable chaperone that delivers iron to scaffold proteins in cluster biosynthesis, and to reduced activities of proteins such as aconitase and succinate

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dehydrogenase in patient cells. Excess iron accumulates in the mitochondria and iron deficiency occurs in the cytosol, resulting in disruption of cellular iron homeostasis and mitochondrial failure. One conceivable but untested approach to the problem is the delivery of intact iron-sulfur clusters to organelles in order to reconstitute scaffold proteins, which in turn reconstitute receptor proteins and restore mitochondrial function [9]. This approach requires clusters that are soluble and stable in a partly or wholly aqueous environment and are adequately resistant to possible damage at physiological dioxygen concentrations. Inasmuch as active mitochondrial aconitase and cytosolic aconitase IRP1 contain $[\text{Fe}_4\text{S}_4]$ clusters [11,12] and succinate dehydrogenase has one such cluster [13], the stability properties of synthetic clusters of the type $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-}$, in which thiolate simulates cysteinate binding, are a central issue.

Although a multitude of $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-}$ clusters ($\text{R} = \text{alkyl, aryl}$) have been prepared [14], there has been no comprehensive examination of their stability in partially or wholly aqueous media. Literature reports usually caution that clusters should be handled anaerobically, with the occasional qualitative observation that they tend not to be stable over more than several hours in protic media. These clusters are normally isolated as quaternary ammonium salts, soluble in polar organic media such as acetonitrile, DMF, and Me_2SO . Aqueous solubility of R_4N^+ salts can be improved by the presence of hydrophilic groups as in $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{CH}_2\text{OH})_4]^{2-}$ [15], $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{CH}_2\text{CO}_2)_4]^{6-}$ [16,17], and $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{CH}(\text{OH})\text{Me})_4]^{2-}$ [18]. The latter cluster is reported to be stable in methanol and water for at least 2 h. Cluster salts have been examined in aqueous-organic solvent media and in pure water, usually in the presence of added ligand as a stabilizing device to repress solvolysis and cluster decomposition [15,16,19–21]. These experiments also include the addition of micellar or liposomal materials in aqueous solution to solubilize and stabilize clusters [20–22]. Clusters that are soluble in water and 15% $\text{Me}_2\text{SO}/\text{H}_2\text{O}$ have been prepared from hydrophilic dendrimeric thiolates [23]; their solution stability was not described. Another type of cluster with large hydrophilic ligands (cyclodextrin thiolates [24]) is considered in a later section.

We seek clusters that are soluble and stable in water near neutral pH and/or in mixed solvent media containing about 50% water (v/v) for several hours. The clusters must sustain ligand substitution reactions, as in the incorporation of $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{CH}_2\text{OH})_4]^{2-}$ by a large cysteinyl peptide in Tris buffer [25]. Lastly, the clusters must be stable to low concentrations of dioxygen. This investigation provides the first quantitative examination of the stability of $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-}$ clusters, beginning with relatively simple examples and proceeding to species with large hydrophilic ligands.

2. Materials and Methods

2.1. Preparation of Compounds

2.1.1. Clusters— $(\text{Et}_4\text{N})_2[\text{Fe}_4\text{S}_4\text{Cl}_4]$ was obtained by a reported procedure [26] and was used as a reactant in the preparation of other clusters. The following compounds were prepared according to known methods for examination of solution stability. $(\text{Me}_4\text{N})_2[\text{Fe}_4\text{S}_4(\text{CH}_2\text{CH}_2\text{OH})_4]$ [15,27]: absorption spectrum λ_{max} (ϵ_{M}) 293 (22,000), 403 (16,100) nm (N,N' -dimethylformamide (DMF)); 290 (23,200), 405 (16,700) nm (Me_2SO). $(\text{Et}_4\text{N})_2[\text{Fe}_4\text{S}_4(\text{SCMe}_2\text{CH}_2\text{OH})_4]$ [28]: absorption spectrum λ_{max} (ϵ_{M}) 296 (20,600), 393 (16,300) nm (DMF); 295 (21,600), 402 (17,300) nm (Me_2SO). $(\text{Et}_4\text{N})\text{Na}_5-[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{CH}_2\text{CO}_2)_4]\cdot 5\text{C}_5\text{H}_9\text{NO}$ [29]: absorption spectrum λ_{max} (ϵ_{M}) 310 (22,000), 415 (16,000) nm (Me_2SO , 20 mM $\text{HS}(\text{CH}_2)_2\text{CO}_2\text{Na}$).

$(\text{Et}_4\text{N})_2[\text{Fe}_4\text{S}_4(\text{S-p-C}_6\text{H}_4\text{CH}_2\text{OH})_4]$: A solution of $(\text{Et}_4\text{N})_2[\text{Fe}_4\text{S}_4(\text{S}^t\text{Bu})_4]$ [30] (0.50 g, 0.52 mmol) in acetonitrile was treated with $\text{HS-p-C}_6\text{H}_4\text{CH}_2\text{OH}$ (0.45 g, 3.21 mmol). The

black reaction mixture was stirred overnight, subjected to partial vacuum to remove Bu^tSH, and filtered through Celite. Ether was diffused into the filtrate, causing separation of the product over several days as a black crystalline solid (0.53 g, 87%). Absorption spectrum (Me₂SO): λ_{max} (ϵ_{M}) 365 (sh, 19,000), 457 (16,800) nm. ¹H NMR (Me₂SO-*d*₆, anion): δ 4.92 (1, OH), 5.68 (2, *o*-H), 5.87 (2, CH₂), 8.07 (2, *m*-H).

(Et₄N)₂[Fe₄S₄(SCHMeCH₂OH)₄]: To a solution of 2-mercapto-1-propanol (0.58 g, 6.29 mmol) was added a solution of (Et₄N)₂[Fe₄S₄(SBu^t)₄] (1.20 g, 1.24 mmol) in 10 mL of acetonitrile. The black reaction mixture was stirred for 1 h and placed under partial vacuum with stirring overnight. Volatile materials were removed in vacuo and the residue was washed with THF (3 × 2 mL) and dried, affording the product as a black microcrystalline solid (1.06 g, 88%). Absorption spectrum (DMF): λ_{max} (ϵ_{M}) 293 (21,800), 402 (16, 500) nm. ¹H NMR (CD₃CN, anion): δ 2.57 (3, Me), 3.63 (1, OH), 4.95 (2, CH₂), 9.15 (1, CH).

(Et₄N)₂[Fe₄S₄(SCEt₂CH₂OH)₄]: Following related procedures [31,32], 2-ethylbutyraldehyde was converted to 2-ethyl-2-mercaptobutanol. To a solution of 0.31 g (2.27 mmol) of thiol in 5 mL of acetonitrile was added a solution of 0.48 g (0.49 mmol) of (Et₄N)₂[Fe₄S₄(SBu^t)₄] in 10 mL of acetonitrile. The black reaction mixture was stirred overnight, placed under partial vacuum for 1 h to remove Bu^tSH, and filtered through Celite. Ether was diffused into the filtrate, affording over several h the product as a black crystalline solid (0.48 g, 84%). Absorption spectrum (DMF): λ_{max} (ϵ_{M}) 290 (21,600), 395 (sh, 15,300). ¹H NMR (Me₂SO-*d*₆, anion): δ 0.93 (6, Me), 1.62 (4, CH₂), 4.39 (2, CH₂OH).

2.1.2. α -Cyclodextrin thioesters and thiols—The syntheses of α -cyclodextrin monothiol **7** and the dithiol **8** are summarized in Fig. 1. Compounds **3** and **6** have been previously prepared by a different method [33]. The splitting patterns for ¹H NMR data are abbreviated as follows: singlet (s), doublet (d), doublet of doublets (dd), multiplet (m).

Mono-6-O-methanesulfonyl- α -cyclodextrin (2): 6^A-O-Methanesulfonyl-2^A,2^B,2^C,2^D,2^E,2^F,3^A,3^B,3^C,3^D,3^E,3^F,6^B,6^C,6^D,6^E,6^F-heptadeca-*O*-benzyl- α -cyclodextrin (**1**) [34] (1.900 g, 0.736 mmol) was dissolved in THF (30 mL), and the solution was diluted with H₂O (8 mL) and AcOH (1 mL). 5% Pd-C (60 mg) was added, and the reaction mixture was hydrogenated (1 atm) for 48 h. The catalyst was removed by filtration through a 0.2 μ m membrane filter to obtain a clear solution, which was concentrated. The residue was dissolved in deionized water (3.0 mL), and freeze-dried to afford pure 6-*O*-monomesylate **2** as a colorless solid (750 mg, 98%). *R*_f = 0.54 (isopropanol:H₂O, 8:2). [α]_D²⁵: +104.5° (*c* 0.31, MeOH) (*c* 1.7, CHCl₃). ¹H NMR (400 MHz, D₂O) δ 5.01 (d, *J* = 3.6 Hz, 1H, H-1), 4.99 (d, *J* = 3.5 Hz, 1H, H-1), 4.97 (d, m, 4H, 4 × H-1), 4.59 (dd, *J* = 11.6, 1.6 Hz, 1H, H-6^A), 4.45 (dd, *J* = 11.6, 6.0 Hz, 1H, H-6^{A'}), 4.03 (ddd, *J* = 1.6, 5.9, 9.8 Hz, 1H, H-5^A), 3.94 – 3.71 (m, 21H, 5 × H-5 + 5 × H-6 + 5 × H-6' + 6 × H-3), 3.62 – 3.46 (m, 12H), 3.16 (s, 3H, OMs). ¹³C NMR (100 MHz, D₂O) δ 101.57 (C-1), 101.52 (C-1), 101.45 (2 × C-1), 101.34 (C-1), 100.87 (C-1), 81.22 (C-4), 81.17 (C-4), 81.11 (3 × C-4), 80.84 (C-4), 73.37 (C-5), 73.30 (C-5), 73.17 (C-5), 73.11 (C-5), 73.06 (C-5), 72.13, 71.87, 71.86, 71.67, 71.63, 71.40 (6 × C-2 + 6 × C-3, 69.93 (C-5^A), 69.66 (C-6^A), 60.32 (C-6), 60.27 (C-6), 60.22 (C-6), 36.83 (Ms). High resolution MALDI-TOF MS: *m/z* calcd for [C₃₇H₆₂O₃₂S + Na]⁺ 1073.28371; found 1073.2834.

Mono-6-S-acetyl-6-deoxy- α -cyclodextrin (3): Mono-6-*O*-methanesulfonyl- α -cyclodextrin **2** (53 mg, 0.050 mmol) was dissolved in commercial anhydrous DMF (1 mL), and the solution was degassed by bubbling a stream of argon for 10 min. KSAc (22 mg, 0.19 mmol) was added to the solution, and the reaction mixture was heated to 50 °C overnight under argon. The solvent was removed under reduced pressure, and the residue was purified by reverse-phase column chromatography on a C18 silica gel using a gradient of MeOH–H₂O

(0 → 30%), to afford the desired compound **3** as a white solid after lyophilization (33 mg, 64% yield). $R_f = 0.58$ (isopropanol: H₂O, 8:2). $[\alpha]_{25}^{D_{25}}: +116.8^\circ$ (*c* 0.37, MeOH). ¹H NMR (400 MHz, D₂O) δ 5.05 (d, *J* = 3.5 Hz, 1H, H-1), 5.00 – 4.96 (m, 4H, 4 × H-1), 4.93 (d, *J* = 3.5 Hz, 1H, H-1), 4.00 – 3.45 (m, 37H,), 3.39 (dd, *J* = 9.1, 9.1 Hz, 1H, H-4), 2.95 (dd, *J* = 14.5, 8.8 Hz, 1H, H-6^A), 2.31 (s, 3H, OMs). ¹³C NMR (100 MHz, D₂O) δ 199.42 (SAc), 101.66 (C-1), 101.52 (C-1), 101.48 (C-1), 101.40 (C-1), 101.32 (C-1), 101.11 (C-1), 84.78 (C-4), 81.24 (C-4), 81.01 (C-1), 73.37 (C-5), 73.33 (C-5), 73.25 (C-5), 73.21 (C-5), 73.17 (C-5), 72.02, 71.99, 71.98, 71.95, 71.88, 71.70, 71.67, 71.65, 71.62 (C-2, 3), 69.95 (, 60.42 (C-6), 60.34 (C-6), 60.29 (C-6), 60.28 (C-6), 60.18 (C-6), 30.72 (C-6), 30.19 (SAc). High resolution MALDI-TOF MS: *m/z* calcd for [C₃₈H₆₂O₃₀S + Na]⁺ 1053.29388; found 1053.2925.

6^A,6^D-Di-O-methanesulfonyl- α -cyclodextrin (5): 6^A,6^D-Di-O-methanesulfonyl-2^A,2^B,2^C,2^D,2^E,2^F,3^A,3^B,3^C,3^D,3^E,3^F,6^B,6^C,6^E,6^F-hexadeca-O-benzyl- α -cyclodextrin (**4**) [35] (1.490 g, 0.580 mmol) was dissolved in THF (30.0 mL), and the solution was diluted with H₂O (10.0 mL) and AcOH (1.0 mL). 5% Pd-C (60 mg) was added, and the reaction mixture was hydrogenated for 48 h. The catalyst was removed by filtration through a 0.2 cm membrane filter to obtain a clear solution, which was concentrated. The residue was dissolved in deionized water (3.0 mL), and freeze-dried to afford pure dimesylate **5** as a colorless solid (635 mg, 97% yield). $R_f = 0.57$ (isopropanol:H₂O, 8:2). $[\alpha]_{25}^{D_{25}}: +94^\circ$ (*c* 0.37, MeOH). ¹H NMR (400 MHz, D₂O) δ 5.01 (d, *J* = 3.5 Hz, 2H, H-1), 5.00 (d, *J* = 3.5 Hz, 2H, H-1), 4.97 (d, *J* = 3.3 Hz, 2H, H-1), 4.57 (dd, *J* = 1.4, 11.5 Hz, 2H, H-6^A + H-6^D), 4.41 (dd, *J* = 6.4, 11.5 Hz, 2H, H-6^{A'} + H-6^{D'}), 4.03 (m, 2H, H-5^A + H-5^D), 3.98 – 3.70 (m, 18H, 4 × H-5 + 4 × H-6 + 4 × H-6' + 6 × H-3), 3.64 – 3.46 (m, 12H, 6 × H-2 + 6 × H-4), 3.16 (s, 6H). ¹³C NMR (100 MHz, D₂O, from HSQC) δ 101.1 (2 × C-1), 100.7 (2 × C-1), 99.9 (2 × C-1), 80.8 (6 × C-4), 72.8 (4 × C-5), 71.4 (6 × C-3), 71.3 (6 × C-2), 69.6 (2 × C-5), 69.5 (2 × C-6), 59.8 (4 × C-6), 36.4 (2 × OMs). High resolution MALDI-TOF MS: *m/z* calcd for [C₃₈H₆₄O₃₄S₂ + Na]⁺ 1151.26126; found 1151.2608.

6^A,6^D-Di-S-acetyl-6^A,6^D-dideoxy- α -cyclodextrin (6): The 6^A,6^D-di-O-methane-sulfonyl- α -cyclodextrin **5** (200 mg, 0.177 mmol) was dissolved in anhydrous DMF (3.0 mL), and the solution was degassed by bubbling a stream of argon for 10 min. KSAc (82 mg, 0.708 mmol) was added to the solution, and the reaction mixture was heated to 50 °C for 48 h under argon. The solvent was removed under reduced pressure, and the residue was purified by reverse-phase column chromatography on a C18 silica gel using a gradient of MeOH–H₂O (0 → 30%), to afford the desired compound **6** as a white solid after lyophilization (102 mg, 53% yield). $R_f = 0.64$ (isopropanol:H₂O, 8:2). $[\alpha]_{25}^{D_{25}}: +114.0^\circ$ (*c* 0.3, MeOH). ¹H NMR (400 MHz, D₂O) δ 5.01 (d, *J* = 3.5 Hz, 2H, H-1), 5.00 (d, *J* = 3.5 Hz, 2H, H-1), 4.97 (d, *J* = 3.3 Hz, 2H, H-1), 4.57 (dd, *J* = 1.4, 11.5 Hz, 2H, H-6^A + H-6^D), 4.41 (dd, *J* = 6.4, 11.5 Hz, 2H, H-6^{A'} + H-6^{D'}), 4.03 (m, 2H, H-5^A + H-5^D), 3.98 – 3.70 (m, 18H, 4 × H-5 + 4 × H-6 + 4 × H-6' + 6 × H-3), 3.64 – 3.46 (m, 12H, 6 × H-2 + 6 × H-4), 3.16 (s, 6H). ¹³C NMR (100 MHz, D₂O, from HSQC) δ 198.88 (SAc), 101.78 (C-1), 101.45 (C-1), 101.25 (C-1), 84.89 (C-4), 81.37 (C-4), 81.17 (C-4), 73.36 (C-5), 73.30 (C-5), 73.23 (C-5), 72.02, 71.92, 71.65, 71.60, 70.15 (C-2, C-3), 60.34 (C-6), 60.10 (C-6), 30.62 (C-6), 30.24 (SAc). High resolution MALDI-TOF MS: *m/z* calcd for [C₄₀H₆₄O₃₀S₂ + Na]⁺ 1111.28160, found 1111.2795.

In-situ de-S-acetylation of compounds 3 and 6: The procedures illustrated below were employed under anaerobic conditions and generated monothiol **7** and dithiol **8**. A ~0.45 M solution of NaOH in D₂O was prepared by dissolving 18 mg of NaOH·xH₂O in degassed D₂O (1.0 mL). Compound **3** (2.2 mg, 2.1 μ mol) was dissolved in 0.6 mL of D₂O. An aliquot of the NaOH solution (6.0 μ L) was added, and the reaction was monitored by ¹H NMR every 3 min. Another aliquot of the NaOH solution (3 μ L) was added after 30 min and

the ^1H NMR spectra were recorded periodically. The deacylation of **3** was assessed to be complete after 45 min by comparing the disappearance of peaks at δ 3.38 (CH_{6a}SAc) and 2.84 (CH_{6b}SAc) and the appearance of peaks of δ 2.88 (CH_{6a}SH) and 2.77 (CH_{6b}SH) of **7**.

Compound **6** (2.2 mg, 2.0 μmol) was dissolved in ~ 0.6 mL of degassed D_2O in a NMR tube. A ~ 0.45 M solution of NaOH in D_2O was prepared by dissolving 18 mg of $\text{NaOH}\cdot x\text{H}_2\text{O}$ in degassed D_2O , an aliquot of the prepared NaOH solution (11.5 μL) was added to the NMR tube, and the reaction was monitored by ^1H NMR every 3 min. Another aliquot of the NaOH solution (5 μL) was added at 30 min and 60 min each, and the ^1H NMR spectra were recorded 5 min after each addition. The reaction was continued overnight and was found to complete after 15 h by the disappearance of peaks at δ 3.41 (CH_{6a}SAc) and 2.91 (CH_{6b}SAc) of **6** and the appearance of peaks at δ 2.87 (CH_{6a}SH) and 2.81 (CH_{6b}SH) of **8**.

2.2. Designation of Clusters*

In the sections that follow, clusters are designated numerically as indicated.

$[\text{Fe}_4\text{S}_4\text{Cl}_4]^{2-}$	9	$[\text{Fe}_4\text{S}_4(\text{SCEt}_2\text{CH}_2\text{OH})_4]^{2-}$	16
$[\text{Fe}_4\text{S}_4(\text{SEt})_4]^{2-}$	10	$[\text{Fe}_4\text{S}_4(\text{SCMe}_2\text{CH}_2\text{OH})_4]^{2-}$	17
$[\text{Fe}_4\text{S}_4(\text{SPh})_4]^{2-}$	11	$[\text{Fe}_4\text{S}_4\{\beta\text{-CD}-(1,3\text{-SC}_6\text{H}_4\text{S})\}_4]^{2-}$	18
$[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{CH}_2\text{OH})_4]^{2-}$	12	$[\text{Fe}_4\text{S}_4\{\beta\text{-CD}-(1,3\text{-SC}_6\text{H}_4\text{S})_2\}_2]^{2-}$	19
$[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{CH}_2\text{CO}_2)_4]^{6-}$	13	$[\text{Fe}_4\text{S}_4\{\alpha\text{-CD-CH}_2\text{S}\}_4]^{2-}$	20
$[\text{Fe}_4\text{S}_4(\text{S-}p\text{-C}_6\text{H}_4\text{CH}_2\text{OH})_4]^{2-}$	14	$[\text{Fe}_4\text{S}_4\{\alpha\text{-CD}-(\text{CH}_2\text{S})_2\}_2]^{2-}$	21
$[\text{Fe}_4\text{S}_4(\text{SCHMeCH}_2\text{OH})_4]^{2-}$	15		

* CD = cyclodextrin.

2.3. Cluster Stability in Solution

2.3.1. Anaerobic Stability—The stabilities of Me_4N^+ or Et_4N^+ salts of clusters **12–17**, ligated with hydrophilic thiolates, were examined spectrophotometrically at room temperature in DMF and/or Me_2SO solutions containing 0–80% (v/v) aqueous component. The primary (**12–14**), secondary (**15**), and tertiary (**16, 17**) thiolate ligands were intended to increase steric shielding of the $[\text{Fe}_4\text{S}_4]^{2+}$ core in that order. The aqueous component was pure water, 3.55 mM phosphate buffer at pH 7.6–7.7, or 50 mM Tris buffer at pH 7.4. All spectra were recorded under anaerobic conditions using degassed solvents at various time intervals up to 12 h. Stability was assessed by departure from cluster spectra in pure DMF or Me_2SO where they are completely stable for days in the absence of dioxygen. Observations under a variety of conditions (solvent composition, buffer, concentration) indicated that **15–17** do not manifest significantly enhanced stability compared to **12–14**. It was further found with individual clusters that DMF vs. Me_2SO as the nonaqueous solvent component showed little effect, and that phosphate buffer was marginally more stabilizing than Tris buffer. Consequently, **12–14** were selected for more detailed study in Me_2SO /aqueous media. Solvents contained 80% to 20% (v/v) Me_2SO ; the aqueous component contained phosphate buffer. Solute concentration ranges from the highest to lowest Me_2SO content were the following: **12**, 0.79–0.19 mM cluster, 0.67–2.68 mM buffer; **13**, 0.71–0.14 mM cluster, same buffer; **14**, 0.86–0.17 mM cluster, same buffer.

Clusters ligated by α -cyclodextrin thiolates were generated in situ under anaerobic conditions in Me_2SO /aqueous media by the reaction of NaOH with the thioesters **3** and **6**, the preferred storage form of these compounds compared to thiols **7** and **8**, followed by cluster binding. Typically, a solution of 5.47 mg (5.31 μmol) of monothioester **3** was

dissolved in 0.6 mL of Me₂SO-*d*₆ and the integrity of **3** checked by ¹H NMR. This solution was treated with 2.5 equiv of NaOH (29.5 μL of a solution of 18 mg NaOH/1 mL D₂O). A solution of 1.00 mg (1.33 μmol) of (Et₄N)₂[**9**] in 0.2 mL of Me₂SO-*d*₆ was added. The final cluster solution (1.6 mM) was allowed to stand for at least 1 h prior to use for ¹H NMR and (with dilution) for circular dichroism measurements. This solution was diluted as necessary with 3.55 mM phosphate buffer to obtain 80–20% Me₂SO/aqueous (v/v) solutions for spectrophotometric stability studies. Over this range, the initial concentration of **9** was 0.26–0.064 mM and the buffer concentration was 0.67–2.68 mM. The same procedure was used with dithioester **6**. The final solution was prepared from 1.0 equiv of (Et₄N)₂[**9**] (1.33 μmol), 2.0 equiv of **6** (2.65 μmol, 2.89 mg), and 10 equiv of NaOH. Over the 80–20% solvent composition range used in stability studies, the initial concentration of **9** was 0.29–0.073 mM with the preceding range of buffer concentrations. These protocols were repeated multiple times and found to be fully reproducible.

2.3.2. Aerobic Stability—This property of (Et₄N)₂[**12**] was examined in 100–20% Me₂SO/aqueous buffer at the same concentrations as anaerobic measurements. Solutions were prepared anaerobically and placed in small vials of diameter 1.08 cm. These vials were filled to 2.68 cm with a solution surface area of 0.92 cm² and exposed to air while the solution was magnetically stirred at 1100 rpm. Absorption spectra were recorded every 10 min over a 50 min period.

2.4. Physical Measurements

Structures of the two compounds in Table 1 were determined using crystals of moderate diffraction quality obtained by vapor diffusion of ether into an acetonitrile solution ((Et₄N)₂[**14**]) or by slow addition of toluene into a concentrated acetonitrile solution at room temperature (Et₄N)₂[**10**]·PhMe). 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 Dow Corning high vacuum grease mixed with Paratone-N oil and mounted anaerobically on a glass loop. Unit cells were determined with SMART software and data reduction was performed with SAINT, which corrects for Lorentz polarization. No crystal decay was found over the course of data collections. Structures were solved and refined by direct methods using the SHELXL-97; data out to 2θ of 45° were used for both compounds because of the somewhat lower quality of the high-angle data. Cation disorders in both compounds were refined with the aid of similarity constraints on several bond distances and displacement parameters. Hydrogen atoms were introduced at calculated positions and refined using a riding model. Crystallographic data are collected in Table 1.

The following 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. ¹H NMR spectra were obtained with Varian 400/500/600 MHz spectrometers. Matrix-assisted laser desorption/ionization and time-of-flight (MALDI-TOF) mass spectra were recorded on a Bruker Daltonics AUTOFLEX III spectrometer. 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 solvents (≥99.9%) such as acetonitrile or Me₂SO. Mass spectra were measured for 10–100 μM sample solutions in the negative ion mode, and 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 desolvation temperature of 120°C; data were recorded using the electrospray method.

3. Stability of Conventional $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-}$ Clusters in Solution

3.1. Cluster Structures

The structures of two clusters, one used as a reactant in ligand substitution (**10**) and the other an object of a solution stability study (**14**), were determined and are shown in Fig. 2. Both clusters as Et_4N^+ salts show crystallographic C_2 axes passing through opposite Fe_2S_2 faces of a cubane-like $[\text{Fe}_4\text{S}_4]^{2+}$ core, resulting in six Fe-S, four Fe-Fe, and two Fe-SR independent bond lengths. Metric features of the two clusters are unexceptional; bond lengths are summarized in Fig. 2. Mean values ($\text{\AA}$) of core distances are very similar for **10/14**: Fe-S 2.26(2)/2.27(1), Fe-Fe 2.71(3)/2.71(4), but the distortions from idealized T_d symmetry differ. Neither cluster manifests the elongated D_{2d} core structure which is the most common distortion for this oxidation level [14].

3.2. Solution Stabilities

3.2.1. Anaerobic Conditions—The set of clusters **12**, **13** and **15–17**, containing a primary, secondary, or tertiary alkyl group with a hydrophilic substituent, was investigated. We have chosen to examine intrinsic stabilities in various media, i.e., without significant quantities of terminal ligands added. Also examined was cluster **14**, which carries an arylthiolate ligand with a solubilizing hydroxymethyl group. Of these, **12** and **13** have been shown to be soluble and stable for short periods in aqueous media under alkaline conditions with excess thiol present [15,16]. Solution stabilities were monitored by UV-visible spectrophotometry over various time intervals. Initial anaerobic screening of clusters under various experimental conditions revealed no significant stability advantage of an aromatic (**14**) or secondary or tertiary (**15–17**) thiolate ligands over primary thiolate ligands, and led finally to detailed examination of **12–14** in aqueous $\text{Me}_2\text{SO}/\text{H}_2\text{O}$ solvent media containing phosphate buffer. Cluster and buffer concentrations varied with the exact solvent composition; at a given composition, concentrations of different clusters were within ± 0.15 mM and buffer concentrations were the same.

In polar organic solvents, clusters with aliphatic thiolate ligands present absorption bands near 300 nm ($\epsilon_M \sim 22,000$) and 420 nm ($\epsilon_M \sim 17,000$) and those with aromatic thiolate ligands a band near 450 nm ($\epsilon_M \sim 17,000$) [36,37]. Addition of water ($\leq 10\%$ v/v) tends to cause small blue shifts of the visible feature and variable but small intensity changes. Typical results for clusters **12–14** are presented in Figs. 3–5, in which spectra were recorded immediately after solution preparation and 50 min and 12 h later in solvents ranging from 80% to 40% or 20% Me_2SO . In the three cases, spectra in 80% Me_2SO are essentially identical with those in pure Me_2SO (not shown) and with those in 60% Me_2SO , excepting a 10% decrease in visible band intensity of **14** after 12 h. With **12**, the maxima at 295 and 411 nm observed in 80% Me_2SO shift slightly in 60% Me_2SO , and to 405 nm in 40% Me_2SO with increased underlying absorption beyond *ca.* 470 nm evident after 12 h. At 20% Me_2SO , the band at 410 nm is now a shoulder, and absorbance in the visible has markedly increased at 50 min and 12 h. Cluster **13** in 40% Me_2SO at 50 min retains the 307 and 411 nm bands with a striking change in relative intensities. At 12 h there is much increased visible absorption and development of a broad feature centered near 630 nm. At 20% Me_2SO (not shown), the UV absorbance has markedly decreased and the visible region is nearly featureless. At 40% Me_2SO , cluster **14** does not show the feature at 457 nm and the spectrum overall is nearly featureless except for retention of a weak shoulder at 365 nm.

The results in Figs. 3–5 and related observations lead to the conclusion that under the anaerobic conditions employed, clusters **12–14** are adequately stable in 50–60% Me_2SO in the absence of added ligand for periods of at least two hours. This conclusion is further supported by thiol ligand exchange experiments (see below) in Fig. 3. Addition of excess

benzenethiol results in formation of **11** (λ_{max} (ϵ_{M}) 457 (17,400) nm). Based on initial cluster concentrations, 100%, 98%, and 96% of the cluster core is retained in 100%, 80%, and 60% Me₂SO, respectively, at 0–12 h, assuming the cluster core does not reassemble in the presence of excess thiol. All clusters are subject to progressive decomposition in solvents with an aqueous buffer content exceeding *ca.* 60% shortly after solution preparation. Based on the solvolytic ligand substitution reactions of [Fe₄S₄(SR)₄]^{2–/6–} clusters in aqueous media reported by Bruce and coworkers [16,19], it is probable that decomposition is similarly initiated. No stable aquo- or hydroxo-substituted cluster has ever been isolated but a monohydroxo species [Fe₄S₄(SR)₃(OH)]^{2–} of a 3:1 site-differentiated cluster has been detected electrochemically [38].

3.2.2. Aerobic Conditions—All [Fe₄S₄(SR)₄]^{2–} clusters are air-sensitive in solution, as observed by a color change from various shades of red-brown to nearly colorless over time, sometimes accompanied by separation of a black solid. The aerobic stability of **12** was examined in 100% to 20% Me₂SO. Two sets of results, in 100% and 40% Me₂SO, are included in Fig. 6, from which it is evident that even within 10 min the visible band at 410 nm shows reduced intensity. At 50 min, the spectra are nearly featureless. The behavior in 80% and 60% Me₂SO is similar. These results emphasize the air-sensitivity of these clusters by surface exposure of solutions to the atmosphere and the necessity of handling [Fe₄S₄(SR)₄]^{2–} clusters under anerobic conditions.

4. α -Cyclodextrinthiolate Clusters

4.1. Background

A conspicuous feature of [Fe₄S₄(SR)₄]^{2–} clusters is the ready variation of the R-substituent, which can influence properties such as redox potential and solubility. These clusters can sustain binding to the smallest substituent, hydrosulfide (R = H) [39,40], and to thiolates with capacious groups such as R = 1-adamantyl [20,41], 2,4,6-C₆H₂Pr^{*i*}₃ [42], and CH₂Ar [43–45]. At the limit are clusters bound to dendrimeric thiolates [23,46–48]. In these cases, R = *p*-C₆H₄X with the dendrimer anchored at X and grown outward such that any steric protection of the core comes from folding back of flexible structure near the core. In the context of large cluster ligands, we note the work of Tabushi and coworkers [24], who reported the preparation of clusters derived from β -cyclodextrin capped once with the 1,3-CH₂SC₆H₃SH group or twice (at the 6A,6D positions) with this group. We designate these clusters as [Fe₄S₄{ β -CD-(SC₆H₄S)}₄]^{2–} (**18**) and [Fe₄S₄{ β -CD-(SC₆H₄S)₂}]^{2–} (**19**), respectively, following published structural depictions. While these are unproven, absorption spectra and redox potentials are fully consistent with coordination of four arylthiolates to [Fe₄S₄]²⁺ as in [Fe₄S₄(SPh)₄]^{2–} [37]. Cluster **19** is proposed to have a structure in which two bidentate dithiolates bind to the same cluster, an arrangement unproven in any Fe₄S₄ cluster. In addition, a cluster similar to **18** with four β -CD monothiolate ligands has been reported but was not isolated [49]. Cyclodextrin-based ligands are expected to enhance cluster aqueous solubility owing to their large complement of hydroxyl groups.

Our interest in these compounds is two-fold. They are among the relatively few examples of metal complexes derived from covalently functionalized cyclodextrin ligands [50], thus indicating a nearly undefined area of metal-sulfur chemistry. Further, these clusters are reported to have half-lives in neutral phosphate buffer of 70 h (**18**) and 120 h (**19**) in the absence of added thiol [24] under an argon atmosphere. On this basis, they display the aqueous solubility and stability desired but not approached by conventional clusters. We have undertaken an examination of cyclodextrinthiolate clusters. In our initial work described here, we use a ligand platform of α -cyclodextrin, which contains six D-

glucopyranoside units instead of seven in β -cyclodextrin. In its unsubstituted form, α -cyclodextrin carries 18 hydroxyl groups.

4.2. Preparation of Clusters

Thioesters **3** and **6** were prepared by the methods in Fig. 1 and are stable storage forms of thiols **7** and **8** obtainable from them by base hydrolysis. Available amounts of the esters or thiols precluded direct synthesis and isolation of clusters. Three methods for cluster generation in solution are described as they are pertinent here and of general interest in cluster chemistry. Reactions 1 and 2 involving thiolate cluster **10** [36] were performed first in test systems; chloride cluster **9** [26] was employed in reaction 3.

4.2.1. Ligand Substitution with Thioesters

In reaction 1, cluster-bound thiolate acts as a nucleophile with the thioester, resulting in RS[−] ligation to the cluster. Reactions performed with $n = 1$ –6 equiv of n -butylthioacetate under dynamic vacuum to remove ethylthioacetate (bp ~116°C) were monitored by ¹H NMR in Fig. 7. The isotropically shifted methylene resonance of **10** at 12.56 ppm diminishes in intensity while a peak at 12.76 ppm increases with increasing n . The latter is same as the SCH₂ shift of authentic [Fe₄S₄(SBuⁿ)₄]^{2−}. No other resonances were resolved. The signal at 12.56 ppm is assigned to SCH₂CH₃ in species with $n = 0$ –3 and that at 12.76 ppm to SCH₂CH₂CH₂CH₃ in species with $n = 1$ –4. Even with excess thioester ($n = 6$) after 16 h, the reaction does not go to completion, with only 72% of the product clusters containing n -butylthiolate ligands. Similar results were obtained with other thioesters (R = Prⁿ, C₆H₁₁CH₂). Because of incomplete reaction, this method was not applied to the CD thioesters **3** and **6**.

4.2.2. Ligand Substitution with Thiols

This reaction, introduced at the outset of weak-field iron-sulfur cluster chemistry [30], examined mechanistically [51], and applied in numerous cases, proceeds to completion with R = alkyl, R' = aryl and $n = 4$ or a slight excess. However, when R and R' are alkyl groups, the system usually adopts an intermediate equilibrium position which can be displaced to the right by removal of volatile thiol. For example, clusters **18** and **19** were prepared from [Fe₄S₄(SBuⁿ)₄]^{2−} and the CD thiols [24]. Two reaction systems involving **10** and cyclohexylthiol or benzylthiol and conducted under dynamic vacuum (EtSH bp 35°C) are illustrated in Fig 8. Both reactions proceed cleanly to completion with product SCH_{1,2} chemical shifts (13.87, 9.99 ppm) well-separated from that of **10**. At certain stages, intermediates can be detected, as in the $n = 1$ reaction (left panel) where the feature centered at 12.67 ppm contains signals of $n = 0, 1$, and 2 species. Thereafter, systems containing **10** and CD thiols **7** (4 equiv) or **8** (2 equiv), generated in situ from thioesters by base hydrolysis in 95% Me₂SO followed by treatment with triflic acid, were examined by ¹H NMR. The downfield region reveals multiple signals at 11–15 ppm which include overlapping signals of **12** and the reaction product(s). Multiple runs afforded the same spectra. Signals in the region are entirely consistent with cluster Fe-SCH₂ resonances (Section 4.2.3). However, the reactions are incomplete and the amounts of CD thiolate clusters formed could not be

meaningfully quantitated from the spectra. Consequently, another approach to CD thiolate cluster binding was sought.

4.2.3. Ligand Substitution with Thiolates

Reaction 3, expressing halide lability at the tetrahedral iron sites of Fe_4S_4 clusters, is recognized but less extensively exploited for ligand substitution than reaction 2. The reaction is stoichiometric with alkyl- and arylthiolates. For example, reaction of **9** with 1 equiv of $\text{NaSCH}_2\text{CH}_2\text{OH}$ in Me_2SO produces fully resolved SCH_2 signals at 14.92 ($n = 1$), 13.90 ($n = 2$), and 13.08 ($n = 3$) ppm and a very weak signal at 12.41 ppm ($n = 4$). Further addition of thiolate results in diminution of the $n = 1,2$ signals relative to the $n = 3,4$ signals. At 4 equiv of thiolate only the $n = 4$ signal was detectable, indicating complete conversion of **9** to **12**. This procedure was then applied to **9** and the CD thioesters in reactions 4 and 5 in Me_2SO ; thiolates are generated by treatment with excess base followed by addition of the cluster (cf. Materials and Methods). It is emphasized that formulations **20** and **21** are based on reaction stoichiometries only and are not meant to imply structures or the state of oligomerization of cluster products. With this qualification, they will be employed in the sections that follow.

4.3. Cluster-Cyclodextrinthiolate Binding

CD thiolate cluster binding is established by several lines of evidence, collected in Fig. 9 for the product **20** of reaction 4 and in Fig. 10 for the product **21** of reaction 5. The absorption spectra compare very closely in band positions (293, 404 nm for **20**; 291, 411 nm for **21**) to those of **12** (295, 410 nm) albeit with increased intensity. These spectra differ completely from that of precursor cluster **9**. The products manifest strong visible circular dichroism spectra as do $[\text{Fe}_4\text{S}_4]^{2+}$ clusters in ferredoxins [52,53]. Visible circular dichroism spectra of several clusters bound to synthetic Cys-containing peptides have also been observed [54,55]. Thioesters **3** and **6** do not show circular dichroism spectra, as is the case for cyclodextrins themselves [56].

After establishing that $(\text{Et}_4\text{N})_2[\text{Fe}_4\text{S}_4(\text{SBu}^t)_4]$ could be identified by its ESI mass spectrum by showing the principal ions $[\text{Fe}_4\text{S}_4(\text{SBu}^t)_4]^{2-}$ and $\{(\text{Et}_4\text{N})[\text{Fe}_4\text{S}_4(\text{SBu}^t)_4]\}^{1-}$, cyclodextrinthiolate clusters were examined. The high-resolution spectra were in general complex. However, the feature centered near $m/z = 2346\text{--}2348$ in the spectrum of the reaction 4 contained an isotope pattern consistent with $[\text{Fe}_4\text{S}_4(\alpha\text{-CD-CH}_2\text{S})_2(\text{H}_2\text{O})]^{1-}$, implying cluster-bound thiolate.

The ^1H NMR spectrum of **20** exhibits six features at 11–16 ppm; the spectrum of **21** is similar, showing some five principal features at 10–16 ppm. This is the region where

isotropically shifted $-\text{SCH}_2$ resonances of $[\text{Fe}_4\text{S}_4]^{2+}$ clusters are always found in aprotic solvents. The following chemical shifts for $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-}$ in Me_2SO illustrate this behavior: 12.56 (**10**), 12.41 (**12**), 13.42 (**13**), 12.71 ($\text{R} = \text{Pr}^n$), 12.95 ($\text{R} = \text{Bu}^n$), 13.96 ($\text{R} = \text{CH}_2\text{Ph}$) ppm. In their structurally simplest form, **20** and **21** as formulated should each exhibit two $-\text{SCH}_2$ signals because these protons are diastereotopic. Note that $[\text{Fe}_4\text{S}_4(\text{S-L-Cys}(\text{Ac})\text{NHMe})_4]^{2-}$, one of the very few clusters ever prepared with chiral ligands, has methylene resonances at 12.4 and 13.6 ppm [57]. The spectra are somewhat similar to those of clusters bound with $(\text{Cys-Gly-Gly})_{2,3}$ peptides, which show broad, poorly resolved signals at 10–14 ppm, also in Me_2SO [57]. In this case as well as with ferredoxins, multiple methylene signals are observed owing to positional inequivalence of Cys residues, amplified by the angular dependence of SCH_2 hyperfine contact shifts in the more rigid protein structures [58–60]. While linkage of clusters through a μ_2 -SR bridge has been structurally proven [61] and could lead to methylene proton inequivalencies in cluster oligomers, such bridges are not expected to be robust. Examination of the NMR spectra of **20** and **21** at 296–373 K in Me_2SO showed retention of the multiple signals with little broadening and small downfield shifts with increasing temperature. The latter is standard behavior for $[\text{Fe}_4\text{S}_4]^{2+}$ clusters as the population of a paramagnetic excited state and hyperfine contact shifts increase.

While the protons responsible for the signals do not appear to be in question, the origin of the multiple signals is unclear. Further evidence that the cluster core remains intact in reactions 4 and 5 follows from addition of excess benzenethiol to solutions of the generated clusters. For periods up to 12 h after cluster formation, the characteristic absorption spectrum of **11** is developed as a consequence of ligand exchange reaction 2 ($n = 4$) (Figs. 9 and 10). Based on the initial concentration of chloride cluster **9**, the extent of formation of **11** for the products of reactions 4 and 5 is 88% and 94%, respectively. Excluding as before the unlikely event that cluster cores reassemble in the presence of excess benzenethiol, these values are the minimum in situ yields of the reactions. Absorption and ^1H NMR spectra substantiate thiolate binding to the $[\text{Fe}_4\text{S}_4]^{2+}$ core and circular dichroism spectra require coordination of chiral ligands. The results support the stoichiometries of **20** and **21** but do not disclose molecular structures.

4.4. Anerobic Solution Stability

The stabilities of the clusters **20** and **21** were assessed as were those of conventional clusters (Section 3.2); relevant spectra are collected in Fig. 11. The bands of **20** at 293 and 407 nm in 80% Me_2SO are preserved in the initial spectrum in 60% Me_2SO but with decreased intensity. After 12 h, absorbance in the visible region markedly increases. These changes continue in 40% Me_2SO ; at 12 h band maxima have shifted to the red and visible absorbance has further increased relative to these features. The initial spectrum in 20% Me_2SO (not shown) is nearly featureless with diminished intensity, indicative of destruction of the original chromophore. In contrast, the principal features of **21** at 293 and 408 nm in 80% Me_2SO are preserved at 60% and 40% Me_2SO at 12 h with little intensity change and a small increase in absorbance centered around 630 nm. Even in 20% Me_2SO (not shown) at 12 h the two main bands remain but are shifted to 308 and 408 nm with decreased intensity and more pronounced absorbance in the 630 nm region.

5. Summary

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

1. Compounds of the type $(\text{Et}_4\text{N})_2[\text{Fe}_4\text{S}_4(\text{SR})_4]$ with hydrophilic R substituents ($-\text{OH}$, $-\text{CO}_2^-$), several of which have been previously described, are appreciably soluble

in organic/aqueous solvent mixtures with $\leq 80\%$ Me₂SO, DMF, or acetonitrile. Clusters are stable for days in dry anaerobic aprotic solvents.

- Cluster stability is enhanced by lower aqueous solvent content and the presence of excess ligand, but is not significantly influenced by the substituent variations R = CH₂R', CHMeR', CMe₂R', and CEt₂R' adjacent to iron-sulfur interactions.
- The stability behavior in (2) is documented by spectrophotometric observation of clusters with R = CH₂CH₂OH, CH₂CH₂CO₂⁻, and *p*-C₆H₄CH₂OH in 20–80% Me₂SO/water (pH 7.6–7.7, phosphate buffer) v/v. Clusters are stable in 60% Me₂SO for periods up to 12 h; the core of [Fe₄S₄(SCH₂CH₂OH)₄]²⁻ is recovered in 96% yield as [Fe₄S₄(SPh)₄]²⁻ after 12 h. At 20–40% Me₂SO, spectral changes indicative of cluster alteration or decomposition are evident after several hours and are pronounced after 12 h.
- The air-sensitivity of clusters is exemplified by [Fe₄S₄(SCH₂CH₂OH)₄]²⁻, whose spectra in 20–100% Me₂SO are appreciably changed after several min exposure to air and rendered essentially featureless after *ca.* 50 min.
- α -Cyclodextrin mono- and dithioesters and thiols were prepared as precursors of unusually large and hydrophilic cluster ligands. The clusters [Fe₄S₄(α -CD-CH₂S)₄]²⁻ and [Fe₄S₄{ α -CD-(CH₂S)₂]₂]²⁻ were generated in solution from reaction of [Fe₄S₄Cl₄]²⁻ and the corresponding thiolates formed by base hydrolysis of thioesters. Thiolate binding was demonstrated by absorption, circular dichroism, and ¹H NMR spectra. Cluster formulations are based on reaction stoichiometries; overall structures were not determined.
- The clusters in (5) retained original spectra in 60–80% Me₂SO. [Fe₄S₄{ α -CD-(CH₂S)₂]₂]²⁻ is appreciably stable for at least 60 min in 40% Me₂SO; by the spectrophotometric criterion, this is the most stable cluster examined in this work.

The results demonstrate that cyclodextrin thiolate ligands, in particular the potentially bidentate dithiolate, stabilize clusters in partially aqueous media. This property, however, does not meet the aqueous stability behavior reported for clusters bound to mono- and dithiolates of a different design based on a β -cyclodextrin ligand platform [24]. The present results do encourage further examination of cyclodextrin thiolate clusters as well as those with different ligand types designed to improve aqueous solubility and aerobic stability. Further investigations directed toward these goals are in progress.

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References

- Beinert H, Holm RH, Münck E. Science. 1997; 277:653–659. [PubMed: 9235882]
- Johnson DC, Dean DR, Smith AD, Johnson MK. Annu Rev Biochem. 2005; 74:247–281. [PubMed: 15952888]
- Lill R. Nature. 2009; 460:831–838. [PubMed: 19675643]
- Dos Santos PC, Smith AD, Frazzon J, Cash VL, Johnson MK, Dean DR. J Biol Chem. 2004; 279:19705–19711. [PubMed: 14993221]
- Rouault TA, Tong W-H. Nature Rev Mol Cell Biol. 2005; 6:345–351. [PubMed: 15803140]
- Raulfs EC, O'Carroll IP, Dos Santos PC, Unciuleac M, Dean DR. Proc Natl Acad Sci USA. 2008; 105:8591–8596. [PubMed: 18562278]

7. Lill R, Muhlenhoff U. *Annu Rev Biochem.* 2008; 77:669–700. [PubMed: 18366324]
8. Rouault TA, Tong WH. *Trends in Genetics.* 2008; 24:398–407. [PubMed: 18606475]
9. Ye H, Rouault TA. *Biochemistry.* 2010; 49:4945–4956. [PubMed: 20481466]
10. Richardson DR, Lane DJR, Becker EM, Huang MLH, Whitnall M, Rahmanto YS, Sheftel AD, Ponka P. *Proc Natl Acad Sci USA.* 2010; 107:10775–10782. [PubMed: 20495089]
11. Robbins AH, Stout CD. *Proc Natl Acad Sci USA.* 1989; 86:3639–3643. [PubMed: 2726740]
12. Dupuy J, Volbeda A, Carpentier P, Darnault C, Moulis J-M, Fontecilla-Camps JC. *Structure.* 2006; 14:129–139. [PubMed: 16407072]
13. Yankovskaya V, Horsefield R, Törnroth S, Luna-Chavez C, Miyoshi H, Léger C, Byrne B, Cecchini G, Iwata S. *Science.* 2003; 299:700–704. [PubMed: 12560550]
14. Rao PV, Holm RH. *Chem Rev.* 2004; 104:527–559. [PubMed: 14871134]
15. Hill CL, Renaud J, Holm RH, Mortenson LE. *J Am Chem Soc.* 1977; 99:2549–2557. [PubMed: 850027]
16. Job RC, Bruice TC. *Proc Natl Acad Sci USA.* 1975; 72:2478–2482. [PubMed: 16592256]
17. Henderson RA, Sykes AG. *Inorg Chem.* 1980; 19:3103–3105.
18. Davies SC, Evans DJ, Henderson RA, Hughes DL, Longhurst S. *J Chem Soc, Dalton Trans.* 2002:3470–3477.
19. Bruice TC, Maskiewicz R, Job R. *Proc Natl Acad Sci USA.* 1975; 72:231–234. [PubMed: 16592211]
20. Kambayashi H, Nagao H, Tanaka K, Nakamoto M, Peng S-M. *Inorg Chim Acta.* 1993; 209:143–149.
21. Nakamoto M, Fukaishi K, Tagata T, Kambayashi H. *Bull Chem Soc Jpn.* 1999; 72:407–414.
22. Roy RC, Das DK, Das B. *Indian J Chem.* 2007; 46A:1252–1256.
23. Sharma AK, Kim N, Cameron CS, Lyndon M, Gorman CB. *Inorg Chem.* 2010; 49:5072–5079. [PubMed: 20450203]
24. Kuroda Y, Sasaki Y, Shiroiwa Y, Tabushi I. *J Am Chem Soc.* 1988; 110:4049–4050.
25. Laplaza CE, Holm RH. *J Am Chem Soc.* 2001; 123:10255–10264. [PubMed: 11603975]
26. Wong GB, Bobrik MA, Holm RH. *Inorg Chem.* 1978; 17:578–584.
27. Christou G, Garner CD, Drew MGB, Cammack R. *J Chem Soc, Dalton Trans.* 1981:1550–1555.
28. Mascharak PK, Hagen KS, Spence JT, Holm RH. *Inorg Chim Acta.* 1983; 80:157–170.
29. Carrell HL, Glusker JP, Job R, Bruice TC. *J Am Chem Soc.* 1977; 99:3683–3690. [PubMed: 858874]
30. Que L Jr, Bobrik MA, Ibers JA, Holm RH. *J Am Chem Soc.* 1974; 96:4168–4178. [PubMed: 4854592]
31. Chrysosou K, Pelecanou M, Pirmettis IC, Papadopoulos MS, Raptopoulou C, Terzis A, Chiotellis E, Stassinopoulou CI. *Inorg Chem.* 2002; 41:4653–4663. [PubMed: 12206688]
32. Roy B, d'Hardemare AD, Fontecave M. *J Org Chem.* 1994; 59:7019–7026.
33. Kumprecht L, Buděšinsky M, Vondrášek J, Vymětal J, Cerný J, Císarova I, Brynda J, Herzig V, Koutník P, Závada J, Kraus T. *J Org Chem.* 2009; 74:1082–1092. [PubMed: 19113826]
34. Guieu S, Sollogoub M. *Angew Chem Int Ed.* 2008; 47:7060–7063.
35. Rawal GK, Zhang P, Ling C-C. *Org Lett.* 2010; 12:3096–3099. [PubMed: 20575590]
36. DePamphilis BV, Averill BA, Herskovitz T, Que L Jr, Holm RH. *J Am Chem Soc.* 1974; 96:4159–4167. [PubMed: 4854591]
37. Cambray J, Lane RW, Wedd AG, Johnson RW, Holm RH. *Inorg Chem.* 1977; 16:2565–2571.
38. Weigel JA, Holm RH. *J Am Chem Soc.* 1991; 113:4184–4191.
39. Müller A, Schladerbeck N, Bögge H. *J Chem Soc, Chem Commun.* 1987:35–36.
40. Hoveyda HR, Holm RH. *Inorg Chem.* 1997; 36:4571–4578. [PubMed: 11670123]
41. Nakamoto M, Tanaka K, Tanaka T. *J Chem Soc, Chem Commun.* 1988:1422–1423.
42. O'Sullivan T, Millar MM. *J Am Chem Soc.* 1985; 107:4096–4097.
43. Hauptmann R, Schneider J, Köckerling M, Henkel G. *Acta Crystallogr.* 1999; C55:190–192.
44. Lenormand A, Iveson P, Jordanov J. *Inorg Chim Acta.* 1996; 251:119–123.

45. Lawson Daku LM, Pécault J, Lenormand-Foucaut A, Vieux-Melchoir B, Iveson P, Jordanov J. *Inorg Chem.* 2003; 42:6824–6850. [PubMed: 14552635]
46. Gorman CB, Hager MW, Parkhurst BL, Smith JC. *Macromolecules.* 1998; 31:815–822.
47. Gorman CB, Smith JC, Hager MW, Parkhurst BL, Sierzputowska-Gracz H, Haney CA. *J Am Chem Soc.* 1999; 121:9958–9966.
48. Chasse TL, Sachdeva R, Li Q, Li Z, Petrie RJ, Gorman CB. *J Am Chem Soc.* 2003; 125:8250–8254. [PubMed: 12837096]
49. Siegel B. *J Inorg Nucl Chem.* 1979; 41:609–610.
50. Engeldinger E, Armspach D, Matt D. *Chem Rev.* 2003; 103:4147–4173. [PubMed: 14611261]
51. Henderson RA. *Chem Rev.* 2005; 105:2365–2437. [PubMed: 15941217]
52. Stephens PJ, Thomson AJ, Dunn JBR, Keiderling TA, Rawlings J, Rao KK, Hall DO. *Biochemistry.* 1978; 17:4770–4778. [PubMed: 728385]
53. Stephens PJ, Jensen GM, Devlin FJ, Morgan TV, Stout CD, Martin AE, Burgess BK. *Biochemistry.* 1991; 30:3200–3209. [PubMed: 2009261]
54. Gibney BR, Mulholland SE, Rabanal F, Dutton PL. *Proc Natl Acad Sci USA.* 1996; 93:15041–15046. [PubMed: 8986760]
55. Grzyb J, Xu F, Weiner L, Reijerse EJ, Lubitz W, Nanda V, Noy D. *Biochim Biophys Acta.* 2010; 1797:406–413. [PubMed: 20035711]
56. Krois, D.; Brinker, UH. *Cyclodextrins and Their Complexes.* Dodziuk, H., editor. Wiley-VCH; Weinheim: 2006. p. 289-298.
57. Que L Jr, Anglin JR, Bobrik MA, Davison A, Holm RH. *J Am Chem Soc.* 1974; 96:6042–6048. [PubMed: 4416035]
58. Scrofani SDB, Brereton PS, Hamer AM, Lavery MJ, McDowall SG, Vincent GA, Brownlee RTC, Hoogenraad NJ, Sadek M, Wedd AG. *Biochemistry.* 1994; 33:14486–14495. [PubMed: 7981209]
59. Lebrun E, Simenel C, Guerlesquin F, Delepierre M. *Mag, Res Chem.* 1996; 34:873–880.
60. Bertini I, Capozzi F, Luchinat C, Piccioli M, Vila AJ. *J Am Chem Soc.* 1994; 116:651–660.
61. Rao PV, Bhaduri S, Jiang J, Hong D, Holm RH. *J Am Chem Soc.* 2005; 127:1933–1945. [PubMed: 15701028]

Synthesis of α -Cyclodextrin Mono- and Di- Thioesters and Thiols

Fig. 1. Scheme for the synthesis of α -cyclodextrin monothiol **7** and dithiol **8** via intermediate compounds **1–3** and **4–6**, respectively.

Fig. 2.

Cluster structures showing the atom labeling schemes; selected bond distances (Å) are given. $[\text{Fe}_4\text{S}_4(\text{SEt})_4]^{2-}/[\text{Fe}_4\text{S}_4(\text{SC}_6\text{H}_4\text{CH}_2\text{OH})_4]^{2-}$: core -- Fe1-S1 2.247(5)/2.256(4), Fe1-S1A 2.287(4)/2.252(4), Fe1-S2 2.222(7)/2.285(4), Fe2-S1 2.243(7)/2.278(4), Fe2-S2 2.263(5)/2.264(5), Fe2-S2A 2.266(4)/2.280(4); terminal -- Fe1-S3 2.241(6)/2.246(4), Fe2-S4 2.183(5)/2.222(5); Fe1-Fe2 2.692(4)/2.680(3), Fe1-Fe2A 2.688(4)/2.692(3), Fe1-Fe1A 2.745(4)/2.707(4), Fe2-Fe2A 2.696(4)/2.707(4). Both clusters have a crystallographically imposed two-fold axis passing through the faces Fe(1,1A)S(1,1A) and Fe(2,2A)S(2,2A)

Fig. 3. Absorption spectra reflecting the stability of $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{CH}_2\text{OH})_4]^{2-}$ in the indicated Me_2SO /aqueous solvent mixtures (v/v) at room temperature. The aqueous component contains phosphate buffer (pH 7.6–7.7). Spectra were recorded immediately after solution preparation and up to 12 h later. Addition of benzenethiol to the 80% and 60% Me_2SO solutions forms $[\text{Fe}_4\text{S}_4(\text{SPh})_4]^{2-}$ (λ_{max} 457 nm).

Fig. 4. Absorption spectra reflecting the stability of $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{CH}_2\text{CO}_2)_4]^{6-}$ in the indicated $\text{Me}_2\text{SO}/\text{aqueous}$ solvent mixtures (v/v) at room temperature. Conditions are the same as in Fig. 3.

Fig. 5. Absorption spectra reflecting the stability of $[\text{Fe}_4\text{S}_4(\text{S-}p\text{-C}_6\text{H}_4\text{CH}_2\text{OH})_4]^{2-}$ in the indicated Me_2SO /aqueous solvent mixtures(v/v) at room temperature. Conditions are the same as in Fig. 3.

Fig. 6. Spectral changes upon aerobic exposure of solutions of $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{CH}_2\text{OH})_4]^{2-}$ in 100% Me_2SO (0.98 mM, left) and 40% Me_2SO /60% aq. buffer (0.39 mM, right). Spectra were recorded every 10 min for 50 min.

Fig. 7. ^1H NMR spectra (600 MHz) in the downfield region for the reaction between $[\text{Fe}_4\text{S}_4(\text{SEt})_4]^{2-}$ and variable equivs of *n*-butylthioacetate in $\text{Me}_2\text{SO}-d_6$ at different time intervals; relative intensities of the two signals are indicated.

Fig. 8. ^1H NMR spectra (600 MHz) in the downfield region for the reaction between $[\text{Fe}_4\text{S}_4(\text{SET})_4]^{2-}$ and variable equivs of cyclohexylmethylthiol (left) and cyclohexylthiol (right).

Fig. 9.

Absorption spectrum (upper), circular dichroism spectrum (middle), and ^1H NMR spectrum (600 MHz, lower) of the reaction product of $[\text{Fe}_4\text{S}_4\text{Cl}_4]^{2-}$ and 4 equiv of $\alpha\text{-CD-(SCOMe)}$ with excess base in 96% Me_2SO .

Fig. 10. Absorption spectrum (upper) and circular dichroism spectrum (lower) of the reaction product of $[\text{Fe}_4\text{S}_4\text{Cl}_4]^{2-}$ and 2 equiv of $\alpha\text{-CD-(SCOMe)}_2$ with excess base in 95% Me_2SO .

Fig. 11. Absorption spectra reflecting the stability of $[\text{Fe}_4\text{S}_4(\alpha\text{-CD-CH}_2\text{S})_4]^{2-}$ (left) and $[\text{Fe}_4\text{S}_4\{\alpha\text{-CD-(CH}_2\text{S)}_2\}_2]^{2-}$ (right) in the indicated Me₂SO/aqueous solvent mixtures at room temperature. Conditions are the same as in Fig. 3.

Table 1Crystallographic Data for (Et₄N)₂[Fe₄S₄(SR)₄]Clusters^a

	R = Et-PhMe^b	R = <i>p</i>-C₆H₄CH₂OH
Formula	C₃₁H₆₈Fe₄N₂S₈	C₄₄H₆₈Fe₄N₂O₄S₈
Formula weight <i>M</i>	948.78	1168.88
Crystal system	monoclinic	tetragonal
Space group	<i>P</i> 2/ <i>c</i>	<i>P</i> 4 ₃ 2 ₁ 2
<i>a</i> (Å)	11.365(5)	11.985(3)
<i>b</i> (Å)	9.320(4)	11.985(3)
<i>c</i> (Å)	23.11(1)	36.009(8)
<i>α</i> = <i>γ</i> (deg)	90°	90°
<i>β</i> (deg)	100.088(6)	90°
<i>V</i> (Å ³)	2410(2)	5172(2)
<i>d</i> _{calc} (g/cm ³)	1.407	1.501
<i>Z</i>	4	4
GOF	1.017	1.200
<i>R</i> ^c (<i>wR</i> 2) ^d	0.101 (0.2114)	0.0870 (0.2113)

^aMo Kα radiation (*λ* = 0.71073 Å), T = 100 K.^bToluene monosolvate^c*R*₁ = Σ||*F*_O| - |*F*_C||/Σ|*F*_O|.^d*wR*² = {Σ[*w*(*F*_O² - *F*_C²)²/Σ(*F*_O²)²]}^{1/2}