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Formation, Spectroscopic Characterization, and Solution Stability of an $[\text{Fe}_4\text{S}_4]^{2+}$ Cluster Derived from a β -Cyclodextrin Dithiolate

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Abstract

The formation and solution properties, including stability in mixed aqueous- Me_2SO media, have been investigated for an $[\text{Fe}_4\text{S}_4]^{2+}$ cluster derived from a β -cyclodextrin (CD) dithiolate. Clusters of the type $[\text{Fe}_4\text{S}_4(\text{SAr})_4]^{2-}$ ($\text{Ar} = \text{Ph}$, $\text{C}_6\text{H}_4\text{-3-F}$) are generated in Me_2SO by redox reactions of $[\text{Fe}_4\text{S}_4(\text{SEt})_4]^{2-}$ with 2 equiv of ArSSAr . An analogous reaction with the intramolecular disulfide of 6^A,6^D-(3-NHCOC₆H₄-1-SH)₂-6^A,6^D-dideoxy- β -cyclodextrin (**14**), whose synthesis is described, affords a completely substituted cluster formulated as $[\text{Fe}_4\text{S}_4\{\beta\text{-CD-(3-NHCOC}_6\text{H}_4\text{-1-S)}_2\}_2]^{2-}$ (**15**). Ligand binding is indicated by a circular dichroism spectrum and also by UV/visible and isotropically shifted ¹H NMR spectra and redox behavior convincingly similar to $[\text{Fe}_4\text{S}_4(\text{SPh})_4]^{2-}$. One formulation of **15** is a single cluster to which two dithiolates are bound, each in bidentate coordination. There being no proven precedent for this binding mode, we show that the cluster $[\text{Fe}_4\text{S}_4(\text{S}_2\text{-}m\text{-xyl})_2]^{2-}$ is a single cubane whose *m*-xylyldithiolate ligands are bound in a bidentate arrangement. This same structure type was proposed for a cluster formulated as $[\text{Fe}_4\text{S}_4\{\beta\text{-CD-(1,3-SC}_6\text{H}_4\text{-S)}_2\}_2]^{2-}$ (**16**; Kuroda et al., *J. Am. Chem. Soc.* **1988**, *110*, 4049–4050) and reported to be water-stable. Clusters **15** and **16** are derived from a similar ligands differing only in the spacer group between the thiolate binding site and the CD platform. In our search for clusters stable in aqueous or organic-aqueous mixed solvents that are potential candidates for reconstitution of scaffold proteins implicated in cluster biogenesis, **15** is the most stable cluster we have thus far encountered under anaerobic conditions in the absence of added ligand.

INTRODUCTION

We have described elsewhere¹ the potential use of iron-sulfur clusters in treating human diseases arising from impaired cluster biogenesis that disrupts cellular iron homeostasis and leads to mitochondrial failure. Friedreich's ataxia is the most prominent of these diseases^{2–5} An untested approach involves delivery of intact iron-sulfur clusters to organelles for the purpose of reconstituting scaffold proteins involved in cluster biogenesis as part of a process that restores mitochondrial function.⁴ The clusters required for implementation of this approach must be soluble and stable in water or mixed organic-aqueous media and, ideally, largely impervious to destructive reactions at physiological dioxygen concentrations. Given that active mitochondrial aconitase and cytosolic aconitase IRP1 both contains $[\text{Fe}_4\text{S}_4]$ clusters,^{6,7} the problem devolves to a search for suitable clusters of this type.

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Within the large family of known $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-}$ clusters ($\text{R} = \text{alkyl, aryl}$),⁸ nearly all of which have been isolated as quaternary ammonium salts, very few have been prepared that are water-soluble. This property is enhanced by hydrophilic substituents such as $\text{R} = \text{CH}_2\text{CH}_2\text{OH}$,^{1,9} $\text{CH}_2\text{CH}(\text{OH})\text{Me}$,¹⁰ and $\text{CH}_2\text{CH}_2\text{CO}_2^-$.¹¹ For stability in water, such clusters require excess ligand to diminish the rate of solvolytic decomposition.¹² Certain dendritically encapsulated clusters are described as soluble in water and Me_2SO /water media; stability information was not reported.¹³

A recent approach in this laboratory has utilized clusters derived from doubly deprotonated α -cyclodextrindithiol, **1** in Figure 1.¹ The hydrophilic nature of cyclodextrins (CDs), which contain 6–8 D-glucopyranoside units linked in a toroidal topology,¹⁴ together with $[\text{Fe}_4\text{S}_4]^{2+}$ core shielding by a large ligand, are expected to promote aqueous solubility and stability. This ligand and the related α -cyclodextrin monodithiolate are generated in solution by base hydrolysis of the corresponding thioesters. Cluster binding occurs by ligand substitution of a preformed cluster, for example $[\text{Fe}_4\text{S}_4\text{Cl}_4]^{2-}$, in a polar solvent such as Me_2SO , and was demonstrated by uv-visible and circular dichroism spectra and by ^1H NMR signals paramagnetically shifted into the downfield range (10–16 ppm) diagnostic of SCH_2 protons in clusters with the core oxidation state $[\text{Fe}_4\text{S}_4]^{2+}$.¹ These clusters were formulated as $[\text{Fe}_4\text{S}_4\{\alpha\text{-CD-CH}_2\text{S}\}_4]^{2-}$ and $[\text{Fe}_4\text{S}_4\{\alpha\text{-CD-(CH}_2\text{S)}_2\}_2]^{2-}$ to emphasize complete substitution by thiolate, but formation of oligomeric species (more than one $[\text{Fe}_4\text{S}_4]^{2+}$ unit per molecule) could not be eliminated. The clusters showed modestly improved stability compared to, e.g., $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{CH}_2\text{OH})_4]^{2-}$, in aqueous Me_2SO solvent mixtures with no added ligand.

The investigation of α -CD thiolate clusters was motivated in part by the work of Kuroda et al.¹⁵, who prepared the β -CD bis(3-thiophenyl-1-thiol) **2** (Figure 1) and the related monothiol. They proposed the formation of $[\text{Fe}_4\text{S}_4\{\beta\text{-CD-(1,3-SC}_6\text{H}_4\text{S)}\}_4]^{2-}$ and $[\text{Fe}_4\text{S}_4\{\beta\text{-CD-(1,3-SC}_6\text{H}_4\text{S)}_2\}_2]^{2-}$ by reaction of $[\text{Fe}_4\text{S}_4(\text{S}^t\text{Bu})_4]^{2-}$ with, apparently, four and two equiv of monothiol and dithiol, respectively. The UV-visible spectrum and 2-/3-redox potential of the dithiol reaction product are entirely consistent with formation of a cluster $[\text{Fe}_4\text{S}_4(\text{SAr})_4]^{2-}$. However, the formulation $[\text{Fe}_4\text{S}_4\{\beta\text{-CD-(1,3-SC}_6\text{H}_4\text{S)}_2\}_2]^{2-}$ requires that the dithiolate function as a bidentate chelating ligand to the same cluster core, a rare, if not unprecedented, behavior in $[\text{Fe}_4\text{S}_4]$ cluster chemistry. The molecular weight measured by light scattering was consistent with this formulation. It was also reported that this cluster was remarkably stable in aqueous phosphate buffer (pH 7.0) with no added ligand, displaying a half-life of greater than 120 hours. In the context of water-soluble clusters, the structural and stability features of the cluster derived from **2** have engaged our attention. Our results and conclusions for a related anaerobic $[\text{Fe}_4\text{S}_4]^{2+}$ cluster system based on the β -CD bis(3-carboxamidobenzene-1-thiol) **3** are described here.

EXPERIMENTAL SECTION

Preparation of Compounds

$(\text{Et}_4\text{N})_2[\text{Fe}_4\text{S}_4(\text{SR})_4]$ ($\text{R} = \text{Et, Ph}$)¹⁶ and $(\text{Et}_4\text{N})_2[\text{Fe}_4\text{S}_4(\text{S}_2\text{-}m\text{-xyl})_2]$ ¹⁷ ($\text{S}_2\text{-}m\text{-xyl} = m\text{-xylyl-}\alpha,\alpha'\text{-dithiolate(2-)}$) were prepared as previously described. The method of synthesis of the desired ligand in oxidized form (**14**, β -cyclodextrin disulfide—an oxidation product of dithiol **3**) is set out in Scheme 1.

2^A,2^B,2^C,2^D,2^E,2^F,2^G,3^A,3^B,3^C,3^D,3^E,3^F,3^G,6^B,6^C,6^E,6^F,6^G-Nonadeca-O-acetyl-6^A,6^D-diazido-6^A,6^D-dideoxy- β -cyclodextrin (7)

To a solution of β -cyclodextrin (**4**, 10.0 g, 8.8 mmol) in anhydrous pyridine (100 mL) was added dropwise a solution of 4,4'-biphenyldisulfonyl chloride¹⁸ (3.09 g, 8.8 mmol) in

anhydrous pyridine (30 mL), and the reaction mixture was stirred at 50 °C for 2 h. Acetic anhydride (40 mL) was added and the reaction was continued overnight to cause conversion of **5** to **6**. The mixture was concentrated under reduced pressure; the residue was dissolved in ethyl acetate (250 mL) and sequentially washed with aqueous HCl (2 N, 2 × 100 mL), saturated NaHCO₃ (2 × 100 mL), and 10% brine (1 × 100 mL). After being dried with anhydrous Na₂SO₄, the organic solution was evaporated to afford a mixture containing the capped disulfonate **6**. The residue was dissolved in DMF (100 mL), and NaN₃ (5.7 g, 88 mmol) was added. After heating at 70 °C overnight, the solution was concentrated under reduced pressure; the residue was extracted with ethyl acetate (250 mL), the solution was washed with saturated brine (1 × 100 mL), dried over anhydrous Na₂SO₄, and evaporated. The crude mixture was purified by column chromatography on silica gel using a mixture of 35% ethyl acetate – hexane as the eluent to afford the diazide **7** (5.42 g, 31%) as a white solid. $[\alpha]_D^{20}$: +71° (*c* 0.82, MeOH). ¹H NMR (400 MHz, CDCl₃): δ 5.39 – 5.19 (m, 7H, 7 × H-3), 5.18 – 5.10 (m, 4H, 4 × H-1), 5.08 (d, *J* = 3.8 Hz, 1H, H-1), 5.04 (d, *J* = 3.5 Hz, 1H, H-1), 5.03 (d, *J* = 3.7 Hz, 1H, H-1), 4.88 – 4.74 (m, 7H, 7 × H-2), 4.66 – 4.51 (m, 5H, 5 × H-6a-CH₂H_bOAc), 4.34 – 4.00 (m, 12H, 7 × H-5 + 5 × H-6b-CH₂H_bOAc), 3.84 – 3.62 (m, 11H, 7 × H-4 + 2 × H-6a-CH₂H_bN₃ + 2 × H-6b-CH₂H_bN₃), 2.18 – 2.02 (m, 57H, 19 × OAc). ¹³C NMR (100 MHz, CDCl₃) δ 170.86, 170.81, 170.73, 170.68, 170.56, 170.56, 170.52, 170.48, 170.43, 170.35, 170.27, 169.35 (CO), 97.11, 96.89 (× 2), 96.80 (× 2), 96.72, 96.41 (C-1), 77.59, 77.23 (× 2), 76.74, 76.61, 76.54, 76.22 (C-4), 71.30, 71.24, 71.13 (× 2), 71.05, 70.80, 70.66, 70.62, 70.54, 70.45, 70.24 (× 2), 70.17 (× 2), 70.11, 69.91, 69.66, 69.63, 69.57 (× 2), 69.34 (C-2, C-3, C-5), 62.71, 62.65, 62.61 (× 2), 62.46 (C-6-CH₂H_bOAc), 50.82, 50.68 (C-6-CH₂H_bN₃), 20.89-20.65 (19 × OAc). High-resolution MS (ESI): calcd *m/z* for C₈₀H₁₀₆N₆O₅₂Na (M+Na)⁺: 2005.57268; found: 2005.56980.

6^A,6^D-Diazido-6^A,6^D-dideoxy-β-cyclodextrin (**8**)

A solution of the per-*O*-acetylated diazide **7**, (1.0 g, 0.50 mmol) in methanol (10 mL), water (2.0 mL) and concentrated ammonia (2.0 mL) was heated to 50 °C for 24 h, and then concentrated under reduced pressure. The residue was purified by reverse-phase chromatography on C18 using a gradient of water-methanol to afford the fully deacetylated diazide (**8**) which was freeze-dried (0.573 g, 96%). $[\alpha]_D^{20}$: +55° (*c* 0.45, MeOH). ¹H NMR (400 MHz, D₂O) δ 5.14 – 5.08 (m, 7H, 7 × H-1), 4.08 – 3.80 (m, 27H, 7 × H-3 + 7 × H-5 + 5 × H-6a-CH₂H_bOH + 5 × H-6b-CH₂H_bOH + 2 × H-6a-CH₂H_bN₃), 3.75 – 3.55 (m, 16H, 7 × H-2 + 7 × H-4 + 2 × H-6b-CH₂H_bN₃). ¹³C NMR (100 MHz, D₂O) δ 101.81 (× 3), 101.78 (× 2), 101.56 (× 2), 81.98 (× 2), 81.23 (× 2), 81.17, 81.16, 81.10 (C-4), 72.99, 72.94, 72.76, 71.96, 71.89, 71.70, 70.46 (C-3, C-5, C-2), 60.32, 60.26 (× 2), 51.00 (× 2) (C-6). High resolution MS (ESI): calcd *m/z* for C₄₂H₆₈N₆O₃₃Na (M+Na)⁺: 1207.37195; found: 1207.37022. This compound has been briefly described previously.^{19,20}

6^A,6^D-Diamino-6^A,6^D-dideoxy-β-cyclodextrin acetate (**9**)

A solution of the diazide (**8**, 200 mg, 0.169 mmol) and triphenylphosphine (177 mg, 0.675 mmol) in pyridine (8.0 mL) and water (2.0 mL) was heated to 50 °C overnight. The solvent was removed under reduced pressure, and coevaporated with water to remove the residual amount of pyridine. The residue was re-dissolved in H₂O (10 mL), neutralized with a few drops of acetic acid. The aqueous solution was washed with dichloromethane (3 × 5 mL), and freeze-dried to afford the previously known 6^A,6^D-diamino derivative,²¹ isolated as the acetate salt (**9**, 189 mg, ~96% yield). ¹H NMR (400 MHz, D₂O): δ 5.02 (m, 3H, 3 × H-1), 4.98 (m, 4H, 4 × H-1), 3.99 – 3.69 (m, 24H), 3.65 – 3.38 (m, 14H), 3.32 (dd, *J* = 13.6, 2.7 Hz, 2H, 2 × H-6a-CH₂H_bNH₃⁺), 3.07 (dd, *J* = 13.7, 7.6 Hz, 2H, 2 × H-6a-CH₂H_bNH₃⁺), 1.82 (s, 6H, 2 × Ac). ¹³C NMR (100 MHz, D₂O) δ 101.80 (× 3), 101.77 (× 3), 101.36 (C-1), 82.74, 82.74 (× 2), 81.17 (× 3), 81.09, 80.72 (C-4), 73.03, 72.91, 72.58, 72.01, 71.91, 71.79,

68.97 (C-2, 3, 5), 60.41, 60.36, 60.26, 60.20 ($\times 2$), 40.42 ($\times 2$), 23.26 (OAc). High-resolution MS (ESI): calcd m/z for $C_{42}H_{73}N_2O_{33}$ (M+H) $^+$: 1133.40956; found: 1133.40617.

3-(Pivaloylthio)benzoic acid (11)

A solution of 3,3'-dithiodibenzoic acid (**10**, 3.0 g, 9.8 mmol) and triphenylphosphine (2.83 g, 10.78 mmol) in THF (30 mL) and water (1.5 mL) was stirred for 1 h at room temperature, and evaporated to dryness under reduced pressure. The residue was re-dissolved in a mixture of THF (30 mL) and anhydrous pyridine (15 mL), and treated with pivaloyl chloride (7.23 mL, 58.7 mmol) for 30 min. To hydrolyze the formed anhydride, water (15 mL) was added and the mixture was stirred for 1 h at 50°C. The reaction mixture was concentrated under reduced pressure and the residue was partitioned using a mixture of ethyl acetate (100 mL) and 2 N HCl (100 mL); the organic solution was separated, dried over anhydrous Na_2SO_4 , and concentrated. The desired acid (3.45 g, 74%) was obtained by column chromatography on silica gel using a mixture of 0.5% methanol-dichloromethane as the eluent. 1H NMR (400 MHz, $CDCl_3$): δ 11.97 (br, 1H, COOH), 8.21 – 8.11 (m, 2H, H-2 + H-6), 7.65 (ddd, J = 1.6, 1.6, 7.8 Hz, 1H, H-4), 7.50 (dd, J = 8.0, 8.0 Hz, 1H, H-5), 1.35 (s, 9H, $(CH_3)_3CCO$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.31 (CO), 147.99, 140.34, 136.59, 130.80, 130.22, 129.17 (Ar), 47.10 ($(CH_3)_3CCO$), 27.35 ($(CH_3)_3CCO$). High-resolution MS (ESI): calcd m/z for $C_{12}H_{14}O_3SNa$ (M+Na) $^+$: 261.05618; found: 261.05558.

2,5-Dioxopyrrolidin-1-yl 3-(pivaloylthio)benzoate (12)

The protected acid **11** (565 mg, 2.37 mmol) and *N*-hydroxysuccinimide (273 mg, 2.37 mmol) were dissolved in ethyl acetate (10 mL) with heating, and *N,N*-dicyclohexylcarbodiimide (689 mg, 3.28 mmol) was added to the hot solution. After stirring for 3 h at room temperature, the precipitate was filtered off and washed with ethyl acetate. The combined solution was evaporated to dryness. The residue was recrystallized from isopropanol to afford the desired ester **12** (620 mg, 78%). 1H NMR (400 MHz, $CDCl_3$): δ 8.14 (m, 2H, H-2 + H-6), 7.71 – 7.66 (ddd, J = 1.3, 1.7, 7.8 Hz, 1H, H-4), 7.56 (dd, J = 7.8, 7.8 Hz, 1H, H-5), 2.87 (br s, 4H, NHS), 1.31 (s, 9H, $(CH_3)_3CCO$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 203.27 ($(CH_3)_3CCOS$), 169.13 (ArCO), 161.20 (CO_NHS), 141.47, 136.63, 130.98, 129.87, 129.51, 126.12 (Ar), 47.11 ($(CH_3)_3CCO$), 27.30 (NHS), 25.64 ($(CH_3)_3CCO$). High-resolution MS (ESI): calcd m/z for $C_{16}H_{17}NO_5SNa$ (M+Na) $^+$: 358.07196; found: 358.07147.

β -Cyclodextrin Disulfide (14)

Diazide **8** (200 mg, 0.169 mmol) was reacted with triphenylphosphine (177 mg, 0.675 mmol) in a mixture of pyridine (8.0 mL) and water (2.0 mL) at 50 °C overnight. The activated *N*-hydroxysuccinimide ester **12** (170 mg, 0.506 mmol) was added, and the mixture was maintained at 50°C for 24 h. The reaction mixture (presumably containing the intermediate diamide **13**) was concentrated by evaporation. Methanol (3.0 mL), water (1.0 mL), and concentrated ammonia (1.0 mL) were added to remove the pivalate thioester. After heating at 50 °C overnight, the solution was concentrated under reduced pressure, and the residue was purified by column chromatography, first on silica gel using a mixture of isopropanol–water–aqueous ammonia (7/1/1 v/v/v) as the eluent, followed by reverse phase chromatography on a C18 Sep-Pak cartridge to provide disulfide **14**, which was freeze-dried (102 mg, 43%). $[\alpha]_D^{20}$: +38.5° (c 0.19, MeOH). 1H NMR (600 MHz, CD_3OD): δ 8.08 (t, J = 1.7 Hz, 1H, H-2' _Ar), 7.85 (t, J = 1.7 Hz, 1H, H-2' _Ar), 7.76 (m, 2H, 2 $\times$ H-6' _Ar), 7.72 (ddd, J = <1, <1, 7.6 Hz, 1H, H-4' _Ar), 7.69 (ddd, J = <1, 1.7, 7.8 Hz, 1H, H-4' _Ar), 7.51 (dd, J = 7.8, 7.8 Hz, 2H, 2 $\times$ H-5' _Ar), 5.05 (d, J = 3.8 Hz, 1H, H-1), 5.04 (d, J = 3.6 Hz, 1H, H-1), 5.03 (d, J = 3.8 Hz, 1H), 5.01 – 4.99 (m, 4H, 4 $\times$ H-1), 4.42 (dd, J = <1, 12.9 Hz, 1H, H-6a_ CH_2H_bNH), 4.32 (dd, J = <1, 12.4 Hz, 1H, H-6a_ CH_2H_bNH), 4.10 (ddd, J = 2.4,

2.4, 9.9 Hz, 1H, H-5), 3.98 (dd, $J = 12.4, 2.8$ Hz, 1H, H-6a-CH_aH_bOH), 3.95 – 3.49 (m, 33H, $7 \times \text{H-2} + 7 \times \text{H-3} + 6 \times \text{H-5} + 4 \times \text{H-6a-CH}_a\text{H}_b\text{OH} + 4 \times \text{H-6b-CH}_a\text{H}_b\text{NH} + 5 \times \text{H-4}$), 3.47 (dd, $J = 1.5, 12.2$ Hz, 1H, H-6b-CH_aH_bOH), 3.28 (dd, $J = 9.3, 9.3$ Hz, 1H, H-4), 3.24 (dd, $J = 9.3, 9.3$ Hz, 1H, H-4), 3.08 (dd, $J = 14.0, 10.4$ Hz, 2H, $2 \times \text{H-6b-CH}_a\text{H}_b\text{NH}$). ¹³C NMR (150 MHz, CD₃OD) δ 168.24 (CO), 167.94 (CO), 137.74, 137.57, 135.54, 135.02, 132.15, 129.25, 129.15, 129.05, 126.65, 126.32, 125.80, 124.99 (Ar), 102.94 ($\times 2$), 102.40, 102.31, 102.22, 102.17, 102.14 (C-1), 85.17, 84.99, 81.25, 81.11, 81.05, 80.91, 80.64 (C-4), 73.33 ($\times 3$), 73.27, 73.24, 73.18, 73.07, 72.93, 72.81, 72.65 ($\times 2$), 72.55, 72.51, 72.38, 71.98, 71.92, 71.89, 71.74 ($\times 2$), 71.17, 70.56 (C-2, C-3, C-5), 60.17, 60.11, 59.81, 59.79, 59.67 (C-6-CH_aH_bOH), 41.85, 41.58 (C-6-CH_aH_bNH). High-resolution MS (ESI): calcd m/z for C₅₆H₇₈N₂O₃₅S₂Na (M+Na)⁺: 1425.37187; found: 1425.36793.

X-ray Structure Determination

The compound (Et₄N)₂[Fe₄S₄(S₂-*m*-xyl)₂] was crystallized from DMF/ether. Owing to the very small size of crystals (*ca.* 0.02 $\times$ 0.02 $\times$ 0.001 mm), data were acquired at Argonne National Laboratory utilizing the Advanced Photon Source. Data were collected at 95 K with a high-brilliance synchrotron X-ray source and a Bruker-Apex II CCD detector. The unit cell was determined with Bruker SMART software. The crystal did not show any significant decay during data collection (*ca.* 1 h). Data were corrected for Lorentz and polarization effects (Bruker SAINT V7.46A) and absorption corrections were applied (SADABS). The space group was assigned based on symmetry analysis and systematic absences (XPREF). The structure was solved by direct methods and refined by least-squares on F^2 (SHELXL-97²²). All non-hydrogen atoms were located in difference-Fourier maps and refined anisotropically. Hydrogen atoms were introduced at calculated positions using a riding model. Crystallographic data are collected in Table 1.²³

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. NMR spectra were recorded on a Varian 500 or a Varian 600 MHz spectrometer. High-resolution mass spectra (ESI-TOF) were obtained on an Agilent 6520-Q-TOF spectrometer. Electrochemical measurements were made with a Bioanalytical Systems potentiostat/galvanostat in Me₂SO solutions using a glassy carbon working electrode, 0.1 M (Bu₄N)(PF₆) supporting electrolyte, and an SCE reference electrode. Mössbauer spectra were determined with a constant acceleration spectrometer; isomer shifts are referenced to iron metal at room temperature.

RESULTS AND DISCUSSION

Ligand Synthesis

Initially we wished to reexamine the results of Kuroda et al.¹⁵ involving Fe₄S₄ cluster binding to doubly deprotonated **2**. With reference to Scheme 1, the 6^A,6^D-capped disulfonate **5** was first prepared by a published procedure.¹⁸ Unfortunately, several attempts to synthesize the previously reported 6^A,6^D-diarylthiol **2** from **5** by nucleophilic attack with excess benzene-1,3-dithiol monoanion were unsuccessful. Consequently, the alternate synthetic target **3** was designed which employs the carboxamide linkage to connect a 6^A,6^D-diamino-substituted β -CD to the arylthio groups.

We first sought the 6^A,6^D-diamino scaffold **9** using **5** as a key intermediate. This compound was obtained from β -CD **4** and 4,4'-biphenyldisulfonyl chloride in pyridine, and was isolated in pure form (15–20%) by reverse-phase chromatography on C18 silica gel. We

found that if direct acylation was carried out after sulfonylation, the presumed intermediate **6** could be directly reacted with NaN_3 in DMF (70°C) to afford the diazide **7**. This compound was isolated by normal phase chromatography on silica gel (31%); the acetyl protecting groups were smoothly removed in methanol-water by concentrated ammonia (50°C) to provide **8** (96%). The azido groups were simultaneously reduced with Ph_3P in pyridine-water (50°C) to obtain the diamine **9**²¹ which was isolated in the acetate salt form. Unfortunately, the EDC-mediated coupling of **9** with the diacid **10** to give the desired **14** was unsuccessful despite numerous attempts. Given the possibility that the difficulty might originate with dual reactive sites in both **9** and **10**, it was decided to reduce the disulfide bond of **10** to provide a carboxylic acid with one reactive group.

The disulfide linkage of **10** was reduced with Ph_3P ,²⁴ and the intermediate thiol was directly protected with pivaloyl chloride to afford **11** (74%). This compound was converted to the activated *N*-hydroxysuccinimide ester **12** (78%). Attempts to functionalize **9** with either **11** (using EDC as a reagent) or **12** in the presence of base (Et_3N , 4-*N,N*-dimethylaminopyridine) were unproductive. The ultimately successful method utilized reduction of the diazide **8** to the diamine followed by treatment in situ with ester **12** to generate S-pivaloyl protected **13** (not isolated) and deprotection with ammonia. Normal phase column chromatography followed by reverse phase chromatography on C18 silica gel yielded the 6^A,6^D-disubstituted β -CD disulfide **14** (43%), the oxidized form of dithiol **3** formed under the aerobic purification conditions.

The structure of **14** was confirmed by several methods, including 1D ¹H and ¹³C as well as 2D COSY and HSQC NMR experiments.²³ The two phenyl groups were found to be magnetically inequivalent by the appearance of eight aromatic proton and twelve aromatic carbon signals in CD_3OD and $(\text{CD}_3)_2\text{SO}$ solutions. The ¹H NMR spectrum in the downfield region in $(\text{CD}_3)_2\text{SO}$, displayed in Figure 2, reveals two well-resolved NH resonances, two *p*-H signals from different rings, three *o*-H signals (four are resolved CD_3OD) and a *m*-H doublet of doublets. This spectral region is relevant to cluster formation. The cyclic voltammograms of two phenyldisulfides, one containing electron-withdrawing substituents, and **14** are presented in Figure 3. Irreversible reduction ($2\text{ArS}^- \rightarrow \text{ArSSAr} + 2\text{e}^-$) and oxidation (reverse reaction) steps are observed, a behavior typical of compounds of this type in aprotic solvents.^{25,26} The 3-fluoro substituents cause a positive shift of E_{pc} , in agreement prior observations of 4-substituted compounds.²⁷ The same redox pattern is observed for **14** at nearly the same potentials as for PhSSPh , confirming the presence of an S-S bond. The structure of **14** was further confirmed by high resolution mass spectrometry.

Thiolate Cluster Ligation

Variation of terminal thiolate ligation in $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-}$ clusters is conventionally accomplished by displacement of halide (e.g., in $[\text{Fe}_4\text{S}_4\text{Cl}_4]^{2-}$) with thiolate or by substitution of bound thiolate by reaction with another, usually more acidic, thiol.^{8,28} To avoid conversion of disulfide **14** to dithiol **3** required for either method, we have utilized redox reaction 1 which finds a near-precedent in the formation of $[\text{Fe}_4\text{S}_4(\text{SePh})_4]^{2-}$ from $[\text{Fe}_4\text{S}_4(\text{SBu}^t)_4]^{2-}$ and PhSeSePh .²⁹ Reactions with both disulfides have been monitored by ¹H NMR as seen in Figure 4. At $n = 1$ and 2 equiv, multiple downfield signals (12.5–13.5 ppm) due to methylene protons in the species

$[\text{Fe}_4\text{S}_4(\text{SEt})_{4-n}(\text{SAr})_n]^{2-}$ are evident and the characteristically contact-shifted aromatic ring proton signals appear at 5.0–8.3 ppm. At $n = 2.1$ –2.2 equiv and up to 36 h reaction time reaction 1 is complete for both disulfides.

In the following sections, CD-derived clusters are designated as indicated. See also Figure 1. These formulations are not intended to imply molecularity.

With the feasibility of reaction 1 demonstrated, reaction 2 was conducted in order to generate cluster **15** in Me₂SO solution. The reactions were followed by ¹H NMR with variable equiv of **14**. The spectra in Figure 5 are similar to those of reaction 1; one mixed ligand cluster (13.05 ppm) was detected. As *n* equiv of the disulfide increase, the three ring

proton resonances emerge, and at *n* = 2.0 and 16 h the limiting spectrum, ascribed to cluster **15**, is reached. Resonances of *m*-H and *p*-H were correlated by a COSY experiment. Shifts of *m*-H are close to those of [Fe₄S₄(SAr)₄]²⁻, thus assuring cluster binding. Shifts of *o*-H and *p*-H indicate smaller contact contributions to their chemical shifts than in the arylthiolate clusters. This arises from differences in spin delocalization possibly due to phenyl group orientations controlled by ligand structure and by the carboxamide substituent. The spectrum of **16** would be an interesting comparison but it was not reported.¹⁵

Because a crystalline reaction product has not been isolated from reaction 2, additional evidence for cluster binding to reduced **14** has been sought from other observations. Relevant absorption spectra in Me₂SO are collected in Figure 6. The spectrum of the product of reaction 2 has a prominent band at 440 nm which is the counterpart of the 457 nm feature of [Fe₄S₄(SPh)₄]²⁻. These spectra distinguish arylthiolate from alkylthiolate clusters such as [Fe₄S₄(SEt)₄]²⁻ (λ_{max} 297, 419 nm). Treatment of the reaction product with *ca.* 25 equiv of benzenethiol in reaction 3 generates a spectrum identical with that of authentic [Fe₄S₄(SPh)₄]²⁻ and corresponds to 98% of the initial cluster concentration in reaction 2.

We conclude that (barring an unlikely core disassembly/assembly event) reaction 2 proceeds with retention of the [Fe₄S₄]²⁺ core of the starting cluster. Note also the circular dichroism spectra in Figure 7 where the effect is induced by one and two equiv of the β-CD disulfide. Cluster **18** with four α-CD monothiolate ligands also shows a strong circular dichroism signal.¹ Disulfide **14**, as CD itself, does not exhibit a circular dichroism spectrum in the same region. The circular dichroism spectrum constitutes further evidence for the occurrence of reaction 2, whose chromophoric product is necessarily chiral.

Lastly, the voltammetry of the 2-/3- redox couples of [Fe₄S₄(SPh)₄]²⁻ and the product of reaction 2 are compared in Figure 8. Note that both are chemically reversible (*i*_{pc}/*i*_{pa} ≈ 1) and differ by only 30 mV, a result that requires arylthiolate coordination in the reaction product. Alkylthiolate clusters such as [Fe₄S₄(SEt)₄]²⁻ (−1.33 V) exhibit 2-/3- couple as substantially more negative potentials.³⁰

Structural Considerations

Results from four physical techniques not only establish a reaction between the β-CD disulfide and [Fe₄S₄(SEt)₄]²⁻ in Me₂SO solution but require arylthiolate binding to the iron sites, rather than any other form of interaction. These results do not demonstrate the molecularity of the reaction product nor is the property known in the solid state for we have

not yet achieved diffraction-quality crystals. The dianion reduction product of **14** can bind to the $[\text{Fe}_4\text{S}_4]^{2+}$ core as a bridging or terminal ligand in the stoichiometry of **15**. The formation μ_2 -SR bridges would lead to oligomeric species, of which there is one documented example.³¹ Binding of one thiolate at an iron site would afford a single cluster with two bidentate ligands in chelate-like ligation, as proposed for **16** apparently based on a molecular weight from light scattering.¹⁵ This type of binding is found with the Fe protein of nitrogenase, which consists a cluster ligated by two cysteinate residues from each of two α -subunits.^{32,33} We have sought an example of this binding mode in a synthetic cluster closer to the problem at hand.

Attention has turned to the previously reported cluster $[\text{Fe}_4\text{S}_4(\text{S}_2\text{-}m\text{-xyl})_2]^{2-}$, formulated on the basis of absorption and ^1H NMR spectral features and redox behavior but lacking structure proof.^{17,30} In the original work, the *m*-xylyldithiolate ligand was selected because its bite distance appeared appropriate to span two iron sites. We verified that the cluster possesses the $[\text{Fe}_4\text{S}_4]^{2+}$ oxidation state from its Mössbauer parameters ($\delta = 0.41$ mm/s, $\Delta E_Q = 1.05$ mm/s at 90 K) whose values are typical of that state.⁸ As is evident in Figure 9, cluster has idealized S_4 symmetry. The two aromatic rings are held above opposite Fe_2S_2 core faces at a dihedral angle of 20.74° ; the least-mean-squares planes of these faces are disposed at 15.00° . The shortest ring carbon distance to a face is 3.14 Å. The core unit has distorted tetrahedral iron sites and a compressed tetragonal arrangement with 4 short (2.236(5) Å) + 8 long (2.313(8) Å) Fe-S bonds, a frequently observed distortion from T_d symmetry.⁸ Mean bond lengths are very close to those of $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{Ph})_4]^{2-}$.¹⁶ The $\text{S}\equiv\text{S}$ distance (6.25 Å) between terminal ligands is smaller than the values in the latter cluster (6.30, 6.49 Å) because of constraints of the bidentate ligand structure.

The structure of $[\text{Fe}_4\text{S}_4(\text{S}_2\text{-}m\text{-xyl})_2]^{2-}$ provides the first demonstration of the unidentate terminal binding mode by a bidentate dithiolate. At the least, it offers a precedent for a similar binding arrangement in **15** and well as **16** and **17**. We note that the $\text{S}\equiv\text{S}$ separations in **15** are expected to be similar to those of $[\text{Fe}_4\text{S}_4(\text{S}_2\text{-}m\text{-xyl})_2]^{2-}$ and comparable to the cavity diameter of β -CD (6.0–6.5 Å).³⁴ While this size range does not strongly constrain thiolate sulfur positions, benzenethiolate substituents at 6^A, 6^D positions on the rim of the structure can accommodate the $\text{S}\equiv\text{S}$ distances of a synthetic cluster.

Solution Stability

In the examination of stability, reaction 2 was conducted in Me_2SO solution after which there were added varying volumes of aqueous phosphate buffer to give solutions with the indicated solvent compositions (v/v). The spectrum in aqueous buffer alone was obtained by removing Me_2SO and dissolving the residue in buffer. Spectra are assembled in Figure 10 for anaerobic solutions containing 80-0% Me_2SO and no excess disulfide **14** and measured at times up to 150 hours after the initial spectra. For each system, spectra were recorded at 4–6 intermediate times as well, but for clarity only the initial and final spectra and those at 60 min and 12 hours are shown. Spectra in the different solvent media were measured in the concentration ranges given in the figure. Stability is qualitatively assessed by departure from the spectrum in pure Me_2SO (Figure 6).

The spectrum of cluster **15** in 80% Me_2SO is virtually identical to that in the pure solvent. The cluster is completely stable in this medium at 72 hr and at 150 hr and evidences only *ca.* 6% decrease in intensity of the 440 nm band at 150 hr. At 60% Me_2SO , the cluster is fully stable at 60 min and slowly decays at 440 nm to 80% of the original intensity after 150 hr. The spectra at 40% and 20% Me_2SO are further progressions but with significant increases in absorbance in the 470–770 nm region. Increased visible absorbances are observed for α -CD thiolate clusters **17** and **18** and also for $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{CH}_2\text{OH})_4]^{2-}$ and $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{CH}_2\text{CO}_2)_4]^{6-}$ at .50% Me_2SO . However, their appearance is a certain sign of

(partial) cluster degradation. CD spectra at 270–770 nm (not shown) confirm stability at 60–100% Me₂SO for up to 12 hr, and reveal substantial changes in ellipticity and several new features at higher aqueous buffer content after 1 hr. Comparison of spectra at 80–20% Me₂SO indicate a somewhat improved stability of **15** in these media at 60 min to 12 hr compared to clusters **17** and **18**, with the former somewhat more stable than the latter.¹

Clusters **15** and **16**, derived from β-CD ligands **3** and **2**, respectively, are potentially capable of chelate-type ligation. Both possess essentially identical visible absorption features having maxima at 440 nm (Me₂SO) and 441 nm (DMF)¹⁵ with $\mu_M \approx 17,500$, suggestive of related structures. Conceivably, the cluster structures in their entirety differ primarily by the connector (-S-, -NHCO-) from the 6-CH₂ group of rings A and D to the 3-C₆H₄S binding sites. We have not attempted to determine the stability half-life of **15** in aqueous buffer as has been reported for **16** (120 hours) on the basis of an absorbance decrease at 420–470 nm. We do note that the absorbance of **15** at 440 nm is unchanged at 1 hour and decays by 15% over 120 hours with an increase in absorbance at longer wave lengths. The latter aspect, not just the absorbance at or near the band maximum, should be monitored to detect cluster decay. The results when compared to those for [Fe₄S₄(SCH₂CH₂OH)₄]²⁻ and [Fe₄S₄(SCH₂CH₂CO₂)₄]⁶⁻ disclose a moderate stability enhancement owing to the presence of the large, apparently protective α- and β-CD ligand platforms. Thus we concur with Kuroda et al.¹⁵ that an unusual property of cyclodextrin-based clusters is their aqueous stability. In our hands, **15** is the most stable cluster produced thus far.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Schematic structures of cyclodextrin dithiols: 6^A,6^D-(CH₂SH)₂-6^A,6^D-dideoxy-α-cyclodextrin (**1**), 6^A,6^D-(3-SC₆H₄-1-SH)₂-6^A,6^D-dideoxy-β-cyclodextrin (**2**), 6^A,6^D-(3-NHC(O)C₆H₄-1-SH)₂-6^A,6^D-dideoxy-β-cyclodextrin (**3**). Abbreviations of doubly deprotonated forms: α-CD-(CH₂S)₂, β-CD-(1,3-SC₆H₄S)₂, β-CD-(3-NHC(O)C₆H₄-1-S)₂. CD = cyclodextrin.

Figure 2. Schematic structure of the β -cyclodextrin disulfide and designation of aromatic protons, and the ^1H NMR spectrum (600 MHz) of the downfield region in $(\text{CD}_3)_2\text{SO}$. Signal assignments are indicated; solv = DMF.

Figure 3. Cyclic voltammograms (100 mV/s) in Me_2SO of diphenyl disulfide (blue, upper) and bis(3-fluorophenyl)disulfide (red, upper) and β -cyclodextrin disulfide (lower). Peak potentials are indicated.

Figure 4.

^1H NMR spectra (600 MHz) in the downfield region for the reaction between 14 mM $[\text{Fe}_4\text{S}_4(\text{SEt})_4]^{2-}$ and n equiv of (a) diphenyl disulfide and (b) bis(3-fluorophenyl)disulfide in $(\text{CD}_3)_2\text{SO}$ at $\sim 25^\circ\text{C}$. Signal assignments of bound arylthiolate ligands are indicated; solv = DMF.

Figure 5.

(a) ^1H NMR spectra (600 MHz) in the downfield region for the reaction between 2.3 mM $[\text{Fe}_4\text{S}_4(\text{SET})_4]^{2-}$ and n equiv of β -cyclodextrin disulfide at various times in $(\text{CD}_3)_2\text{SO}$ at $\sim 25^\circ\text{C}$. (b) COSY spectrum of the $n = 2.0$ equiv product at 16 h reaction time showing the correlation between H_m and H_p .

Figure 6.

Absorption spectra in Me_2SO of $[\text{Fe}_4\text{S}_4(\text{SEt})_4]^{2-}$ (red), the reaction product of $[\text{Fe}_4\text{S}_4(\text{SEt})_4]^{2-}$ and $\beta\text{-CD}$ disulfide (reaction 2, blue), the reaction product plus 25 equiv of benzenethiol (orange), and $\beta\text{-CD}$ disulfide (green).

Figure 7. Circular dichroism spectra of the reaction product between 0.51 mM $[\text{Fe}_4\text{S}_4(\text{SEt})_4]^{2-}$ and 1.0 equiv (blue) and 2.0 equiv (red) of β -cyclodextrin disulfide in Me_2SO . The disulfide (green, 1.4 mM) did not produce an effect at 270–770 nm.

Figure 8.

Cyclic voltammograms (100 mV/s) of $[\text{Fe}_4\text{S}_4(\text{SEt})_4]^{2-}$ (upper) and the reaction product between 1.2 mM $[\text{Fe}_4\text{S}_4(\text{SEt})_4]^{2-}$ and 2.1 equiv of β -cyclodextrin disulfide after 16 hr (reaction 2, lower). The inset diagrams show the redox potentials obtained by differential pulse voltammetry (pulse width 50 ms, pulse period 200 ms). Voltammograms were recorded in Me_2SO solutions at $\sim 25^\circ\text{C}$; peak potentials are indicated.

Figure 9.

The structure of $[\text{Fe}_4\text{S}_4(\text{S}_2\text{-}m\text{-xyl})_2]^{2-}$ with 50% probability ellipsoids and the atom numbering scheme. The 4 short (2.253(1)–2.268(1) Å) and 8 long (2.297(1)–2.318(1) Å) Fe–S bond distances define the indicated ranges. The mean of Fe–Fe distances (2.698(1)–2.756(1) Å) is 2.73(3) Å.

Solution Stability: **$[\text{Fe}_4\text{S}_4\{\beta\text{-CD-(1,3-NHCOC}_6\text{H}_4)_2\}_2]^{2-}$**

Figure 10.

Absorption spectra of the product of reaction 2 in specified Me_2SO /aqueous buffer solutions (v/v) in which the aqueous component is 3.55 mM phosphate buffer (pH 7.5–7.6). Spectra were recorded under anaerobic conditions at 0 to 120 or 150 hr after reaction at the following concentrations: 80% Me_2SO /20% aq., 0.27–0.41 mM; 60% Me_2SO /40% aq., 0.20–0.31 mM; 40% Me_2SO /60% aq., 0.14–0.21 mM; 20% Me_2SO /80% aq.; 100% aq., 0.39 mM. Band maxima or shoulders are indicated.

Scheme 1.

Scheme for the synthesis of β -cyclodextrin disulfide **14** via intermediates **4–8**, **13**, and **14**.

Abbreviations: DCC = dicyclohexylcarbodiimide, EDC = *N*-ethyl-*N*-(3-dimethylaminopropyl)carbodiimide, NH₃ · H₂O = conc. ammonia, Piv = pivaloyl.

Table 1Crystallographic Data for (Et₄N)₂[Fe₄S₄(S₂-*m*-xyl)₂]

formula	C ₃₂ H ₅₆ Fe ₄ N ₂ S ₈
formula weight <i>M</i>	948.70
crystal system	monoclinic
space group	<i>Cc</i>
<i>a</i> (Å)	18.2095 (6)
<i>b</i> (Å)	11.2828 (4)
<i>c</i> (Å)	21.8275 (7)
α = γ (deg)	90
β (deg)	111.866 (1)
<i>V</i> (Å ³)	4161.9 (2)
<i>d</i> _{calc} (g/cm ³)	1.514
<i>Z</i>	4
GOF	1.256
<i>R</i> ^{<i>b</i>} (<i>wR</i> 2) ^{<i>c</i>}	0.0403 (0.142)

^{*a*} Synchrotron radiation source (λ = 0.39364 Å), T = 95 K.^{*b*} $R_1 = \sum ||F_o| - |F_c|| / \sum |F_o|$.^{*c*} $wR^2 = \{ \sum [w(F_o^2 - F_c^2)^2 / \sum w(F_o^2)^2] \}^{1/2}$