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Formation, Spectroscopic Characterization, and Solution Stability of an $[Fe_4S_4]^{2+}$ Cluster Derived from β-Cyclodextrin Dithiolate

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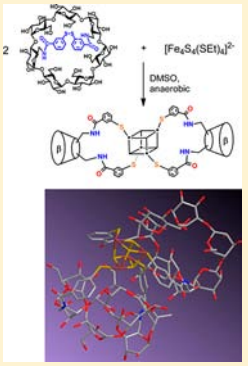
Supporting Information

ABSTRACT: The formation and solution properties, including stability in mixed aqueous–Me_2SO media, have been investigated for an $[Fe_4S_4]^{2+}$ cluster derived from β-cyclodextrin (CD) dithiolate. Clusters of the type $[Fe_4S_4(SAr)_4]^{2-}$ (Ar = Ph, C_6H_4-3-F) are generated in Me_2SO by redox reactions of $[Fe_4S_4(SEt)_4]^{2-}$ with 2 equiv of ArSSAr. An analogous reaction with the intramolecular disulfide of $6^A,6^D$-(3-$NHCOC_6H_4$-1-SH)$_2$-$6^A,6^D$-dideoxy-β-cyclodextrin (**14**), whose synthesis is described, affords a completely substituted cluster formulated as $[Fe_4S_4\{\beta$-CD-(1,3-$NHCOC_6H_4S)_2\}_2]^{2-}$ (**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 $[Fe_4S_4(SPh)_4]^{2-}$. One formulation of **15** is a single cluster to which two dithiolates are bound, each in bidentate coordination. With there being no proven precedent for this binding mode, we show that the cluster $[Fe_4S_4(S_2$-*m*-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 $[Fe_4S_4\{\beta$-CD-(1,3-$SC_6H_4S)_2\}_2]^{2-}$ (**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 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 the reconstitution of scaffold proteins implicated in cluster biogenesis, **15** is the most stable cluster that we have thus far encountered under anaerobic conditions in the absence of added ligand.



1. INTRODUCTION

We have described elsewhere[1] 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 the 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 contain $[Fe_4S_4]$ clusters,[6,7] the problem devolves to a search for suitable clusters of this type.

Within the large family of known $[Fe_4S_4(SR)_4]^{2-}$ clusters (R = 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 R = CH_2CH_2OH,[1,9] $CH_2CH(OH)Me$,[10] and $CH_2CH_2CO_2^-$.[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 $[Fe_4S_4]^{2+}$ core shielding by a large ligand, is 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, $[Fe_4S_4Cl_4]^{2-}$ 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 $[Fe_4S_4]^{2+}$.[1] These clusters were formulated as $[Fe_4S_4\{\alpha$-CD-$CH_2S\}_4]^{2-}$ and $[Fe_4S_4\{\alpha$-CD-$(CH_2S)_2\}_2]^{2-}$ to emphasize complete substitution by thiolate, but the formation of oligomeric species (more than one $[Fe_4S_4]^{2+}$ unit per molecule) could not be eliminated. The clusters showed modestly improved stability compared to, e.g., $[Fe_4S_4(SCH_2CH_2OH)_4]^{2-}$, in aqueous Me_2SO solvent mixtures with no added ligand.

The investigation of α-cyclodextrin thiolate clusters was motivated, in part, by the work of Kuroda et al.,[15] who prepared the β-cyclodextrin bis(3-thiophenyl-1-thiol) **2** (Figure 1) and the

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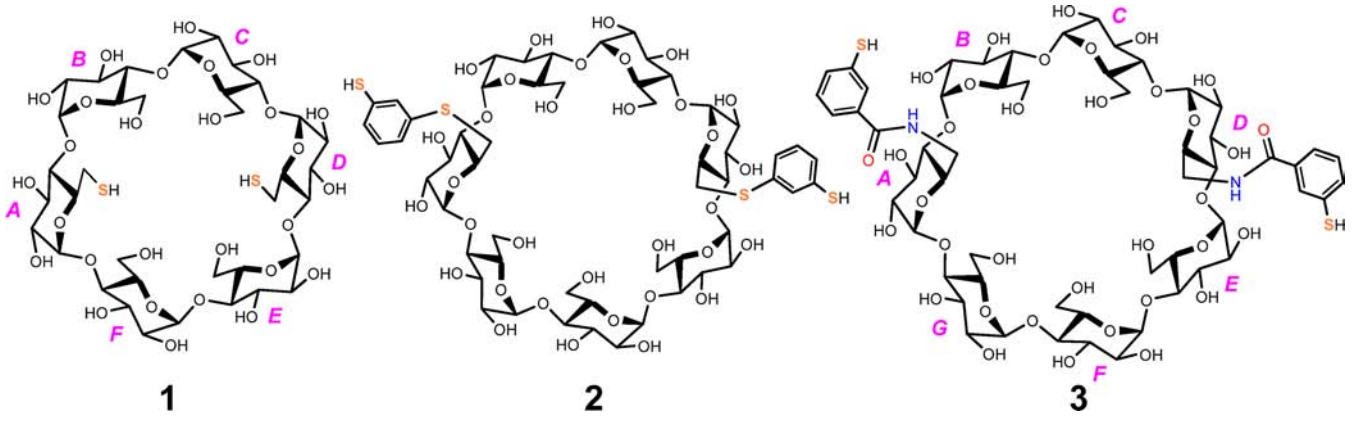

Figure 1. Schematic structures of cyclodextrin dithiols: $6^A,6^D$-$(CH_2SH)_2$-$6^A,6^D$dideoxy-α-cyclodextrin (**1**), $6^A,6^D$-$(3\text{-}SC_6H_4\text{-}1\text{-}SH)_2$-$6^A,6^D$-dideoxy-$\beta$-cyclodextrin (**2**), $6^A,6^D$-$(3\text{-}NHC(O)C_6H_4\text{-}1\text{-}SH)_2$-$6^A,6^D$-dideoxy-$\beta$-cyclodextrin (**3**). Abbreviations of doubly deprotonated forms: α-CD-$(CH_2S)_2$, β-CD-$(1,3\text{-}SC_6H_4S)_2$, β-CD-$(3\text{-}NHC(O)C_6H_4\text{-}1\text{-}S)_2$. CD = cyclodextrin.

related monothiol. They proposed the formation of $[Fe_4S_4\{\beta\text{-CD-}(1,3\text{-}SC_6H_4S)\}_4]^{2-}$ and $[Fe_4S_4\{\beta\text{-CD-}(1,3\text{-}SC_6H_4S)_2\}_2]^{2-}$ (**16**) by the reaction of $[Fe_4S_4(SBu^t)_4]^{2-}$ with, apparently, 4 and 2 equiv of monothiol and dithiol, respectively. The UV–visible spectrum and 2–/3– redox potential of the dithiol reaction product are entirely consistent with the formation of a cluster $[Fe_4S_4(SAr)_4]^{2-}$. However, the formulation **16** requires that the dithiolate function as a bidentate chelating ligand to the same cluster core, a rare, if not unprecedented, behavior in $[Fe_4S_4]$ 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 an aqueous phosphate buffer (pH 7.0) with no added ligand, displaying a half-life of greater than 120 h. 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 $[Fe_4S_4]^{2+}$ cluster system based on the β-cyclodextrin bis(3-carboxamidobenzene-1-thiol) **3** are described here.

2. EXPERIMENTAL SECTION

Preparation of Compounds. $(Et_4N)_2[Fe_4S_4(SR)_4]$ (R = Et, Ph)[16] and $(Et_4N)_2[Fe_4S_4(S_2\text{-}m\text{-xyl})_2]$[17] [$S_2$-*m*-xyl = *m*-xylyl-$\alpha,\alpha'$-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 given 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-$\beta$-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 chloride (3.09 g, 8.8 mmol)[18] 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 $NaHCO_3$ (2 × 100 mL), and 10% brine (1 × 100 mL). After being dried with anhydrous Na_2SO_4, the organic solution was evaporated to afford a mixture containing the capped disulfonate **6**. The residue was dissolved in *N*,*N*-dimethylformamide (DMF; 100 mL), and NaN_3 (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), and the solution was washed with saturated brine (1 × 100 mL), dried over anhydrous Na_2SO_4, 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). ^{1}H NMR (400 MHz, $CDCl_3$): 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_CH_aH$_b$OAc), 4.34–4.00 (m, 12H, 7 × H-5 + 5 × H-6b_CH$_a$$H_b$OAc), 3.84–3.62 (m, 11H, 7 × H-4 + 2 × H-6a_CH_aH$_b$N$_3$ + 2 × H-6b_CH$_a$$H_bN_3$), 2.18–2.02 (m, 57H, 19 × OAc). ^{13}C NMR (100 MHz, $CDCl_3$): 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, and C-5), 62.71, 62.65, 62.61 (×2), 62.46 (C-6_CH$_a$H$_b$OAc), 50.82, 50.68 (C-6_CH$_a$H$_b$N$_3$, 20.89–20.65 (19 × OAc). High-resolution MS (ESI). Calcd for $C_{80}H_{106}N_6O_{52}Na$ [(M + Na)$^+$]: *m*/*z* 2005.57268. Found: *m*/*z* 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). ^{1}H NMR (400 MHz, D_2O): 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_CH_aH$_b$OH + 5 × H-6b_CH$_a$$H_b$OH + 2 × H-6a_C$H_aH_bN_3$), 3.75–3.55 (m, 16H, 7 × H-2 + 7 × H-4 + 2 × H-6b_CH$_a$$H_bN_3$). ^{13}C NMR (100 MHz, D_2O): 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, and C-2), 60.32, 60.26 (×2), 51.00 (×2, C-6). High-resoluton MS (ESI). Calcd for $C_{42}H_{68}N_6O_{33}Na$ [(M + Na)$^+$]: *m*/*z* 1207.37195. Found: *m*/*z* 1207.37022. This compound was 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 redissolved in water (10 mL) and 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). ^{1}H NMR (400 MHz, D_2O): 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 and 2.7 Hz, 2H, 2 × H-6a_CH_aH$_b$NH$_3^+$), 3.07 (dd, *J* = 13.7 and 7.6 Hz, 2H, 2 × H-6a_CH$_a$$H_bNH_3^+$), 1.82 (s, 6H, 2 × Ac). ^{13}C NMR (100 MHz, D_2O): 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, C-3, and C-5), 60.41, 60.36, 60.26, 60.20 (×2), 40.42 (×2), 23.26 (OAc). High-resolution MS (ESI). Calcd for $C_{42}H_{73}N_2O_{33}$ [(M + H)$^+$]: *m*/*z* 1133.40956. Found: *m*/*z* 1133.40617.

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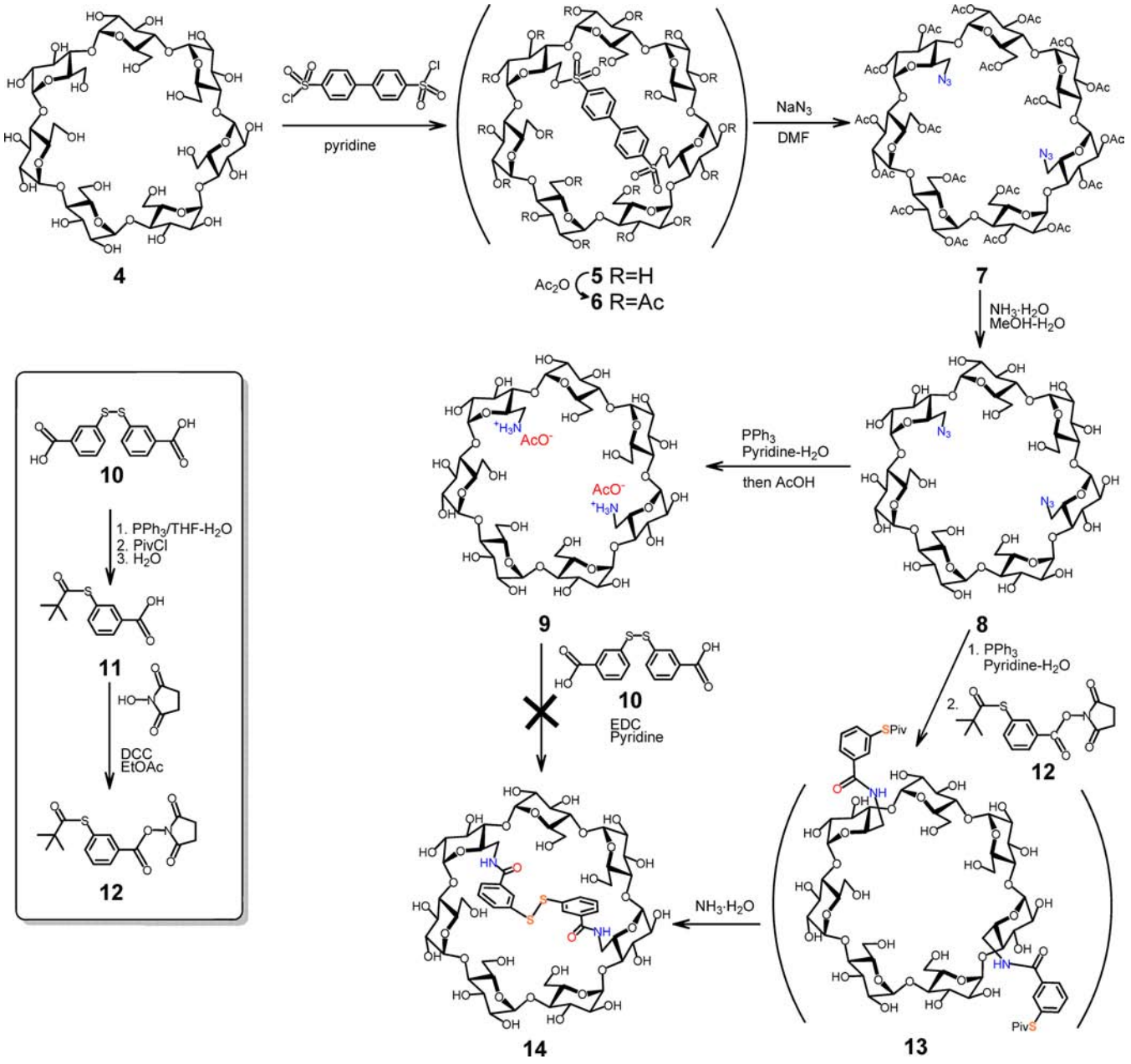
Scheme 1. Scheme for the Synthesis of β-Cyclodextrin Disulfide 14 via Intermediates 4−8, 13, and 14[a]



[a]Abbreviations: DCC = dicyclohexylcarbodiimide, EDC = *N*-ethyl-*N*′-[3-(dimethylamino)propyl]carbodiimide, $NH_3·H_2O$ = concentrated ammonia, and Piv = pivaloyl.

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 tetrahydrofuran (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 redissolved 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. ^{1}H 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, and 7.8 Hz, 1H, H-4), 7.50 (dd, *J* = 8.0 and 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 for $C_{12}H_{14}O_3SNa$ [$(M + Na)^+$]: *m*/*z* 261.05618. Found: *m*/*z* 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 isopropyl alcohol to afford the desired ester **12** (620 mg, 78%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14 (m, 2H, H-2 + H-6), 7.71−7.66 (ddd, *J* = 1.3, 1.7, and 7.8 Hz, 1H, H-4), 7.56 (dd, *J* = 7.8 and 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),

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Table 1. Crystallographic Data for $(Et_4N)_2[Fe_4S_4(S_2\text{-}m\text{-}xyl)_2]$[a]

formula	$C_{32}H_{56}Fe_4N_2S_8$
formula weight M	948.70
cryst syst	monoclinic
space group	Cc
a (Å)	18.2095(6)
b (Å)	11.2828(4)
c (Å)	21.8275(7)
$\alpha = \gamma$ (deg)	90
β (deg)	111.866(1)
V (Å^3)	4161.9(2)
d_{calc} (g/cm^3)	1.514
Z	4
GOF	1.256
R1[b] (wR2)[c]	0.0403 (0.142)

[a]Synchrotron radiation source (λ = 0.39364 Å); T = 95 K. [b]R1 = $\sum||F_o| - |F_c||/\sum|F_o|$. [c]wR2 = $\{\sum[w(F_o^2 - F_c^2)^2/\sum(F_o^2)^2]\}^{1/2}$.

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 for $C_{16}H_{17}NO_5SNa$ $[(M + Na)^+]$: m/z 358.07196. Found: m/z 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 isopropyl alcohol–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). ^{1}H 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 × H-6′_Ar), 7.72 (ddd, J = <1, <1, and 7.6 Hz, 1H, H-4′_Ar), 7.69 (ddd, J = <1, 1.7, and 7.8 Hz, 1H, H-4′_Ar), 7.51 (dd, J = 7.8 and 7.8 Hz, 2H, 2 × 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 × H-1), 4.42 (dd, J = <1 and 12.9 Hz, 1H, H-6a_CH_aH$_b$NH), 4.32 (dd, J = <1 and 12.4 Hz, 1H, H-6a_CH_aH$_b$NH), 4.10 (ddd, J = 2.4, 2.4, and 9.9 Hz, 1H, H-5), 3.98 (dd, J = 12.4 and 2.8 Hz, 1H, H-6a_CH_aH$_b$OH), 3.95–3.49 (m, 33H, 7 × H-2 + 7 × H-3 + 6 × H-5 + 4 × H-6a_CH_aH$_b$OH + 4 × H-6b_CH_aH$_b$NH + 5 × H-4), 3.47 (dd, J = 1.5 and 12.2 Hz, 1H, H-6b_CH_aH$_b$OH), 3.28 (dd, J = 9.3 and 9.3 Hz, 1H, H-4), 3.24 (dd, J = 9.3 and 9.3 Hz, 1H, H-4), 3.08 (dd, J = 14.0 and 10.4 Hz, 2H, 2 × H-6b_CH_aH$_b$NH). ^{13}C NMR (150 MHz, CD_3OD): 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 (×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 (×3), 73.27, 73.24, 73.18, 73.07, 72.93, 72.81, 72.65 (×2), 72.55, 72.51, 72.38, 71.98, 71.92, 71.89, 71.74 (×2), 71.17, 70.56 (C-2, C-3, and C-5), 60.17, 60.11, 59.81, 59.79, 59.67 (C-6_CH_aH$_b$OH), 41.85, 41.58 (C-6_CH_aH$_b$NH). High-resolution MS (ESI). Calcd for $C_{56}H_{78}N_2O_{35}S_2Na$ $[(M + Na)^+]$: m/z 1425.37187. Found: m/z 1425.36793.

X-ray Structure Determination. The compound $(Et_4N)_2[Fe_4S_4(S_2\text{-}m\text{-}xyl)_2]$ was crystallized from DMF–ether. Owing to the very small size of the crystals (ca. 0.02 × 0.02 × 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 synchroton X-ray source and a Bruker ApexII 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*, version 7.46A), and absorption corrections were applied (*SADABS*). The space group was assigned based on symmetry analysis and systematic absences (*XPREP*). The structure was solved by direct methods and refined by least squares on F^2 (*SHELXL-97*[22]). 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]

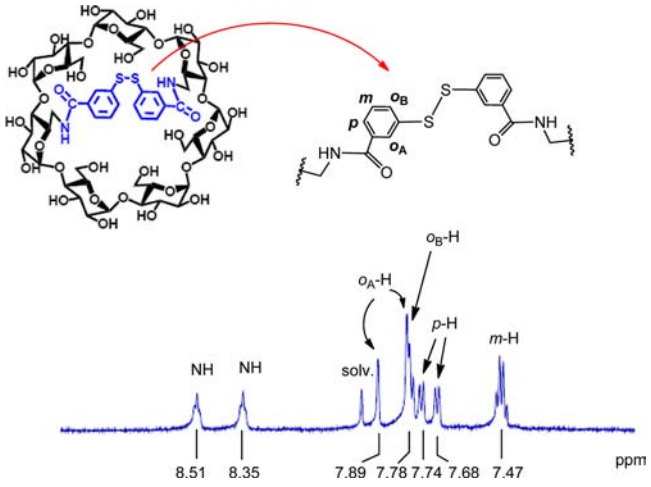

Figure 2. Schematic structure of β-cyclodextrin disulfide and designation of the aromatic protons and the ^{1}H NMR spectrum (600 MHz) of the downfield region in $(CD_3)_2SO$. Signal assignments are indicated; solv = DMF.

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 (MS) spectra (electrospray ionization time-of-flight, ESI-TOF) were obtained on an Agilent 6520-Q-TOF spectrometer. Electrochemical measurements were made with a Bioanalytical Systems potentiostat/galvanostat in Me_2SO solutions using a glassy carbon working electrode, a 0.1 M $(Bu_4N)(PF_6)$ supporting electrolyte, and a saturated calomel reference electrode. Mössbauer spectra were determined with a constant-acceleration spectrometer; isomer shifts are referenced to iron metal at room temperature.

3. RESULTS AND DISCUSSION

Ligand Synthesis. Initially, we wished to reexamine the results of Kuroda et al.[15] involving Fe_4S_4 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, an 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

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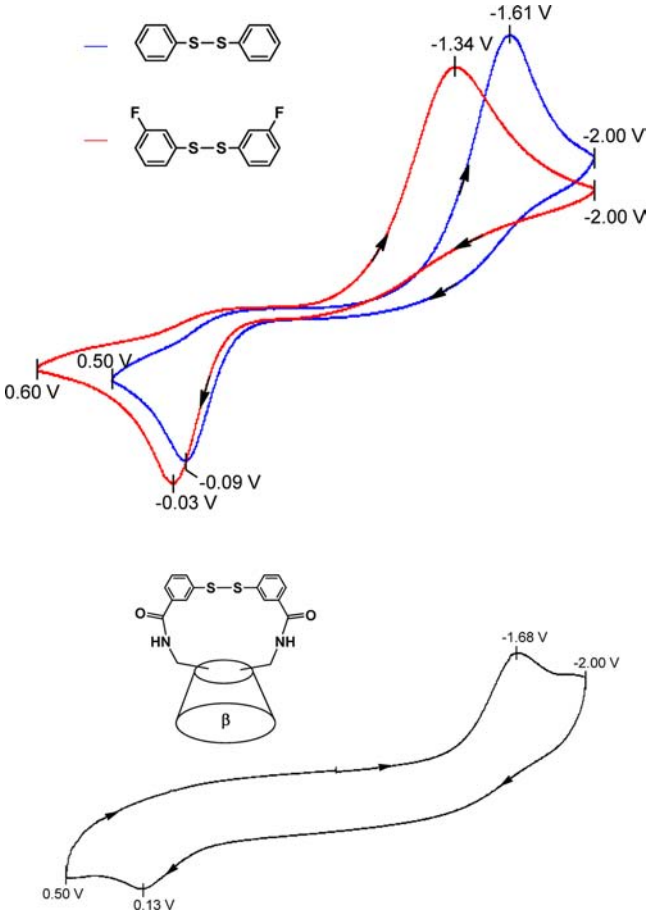

Figure 3. Cyclic voltammograms (100 mV/s) in Me_2SO of diphenyl disulfide (blue, upper), bis(3-fluorophenyl) disulfide (red, upper), and β-cyclodextrin disulfide (lower). Peak potentials are indicated.

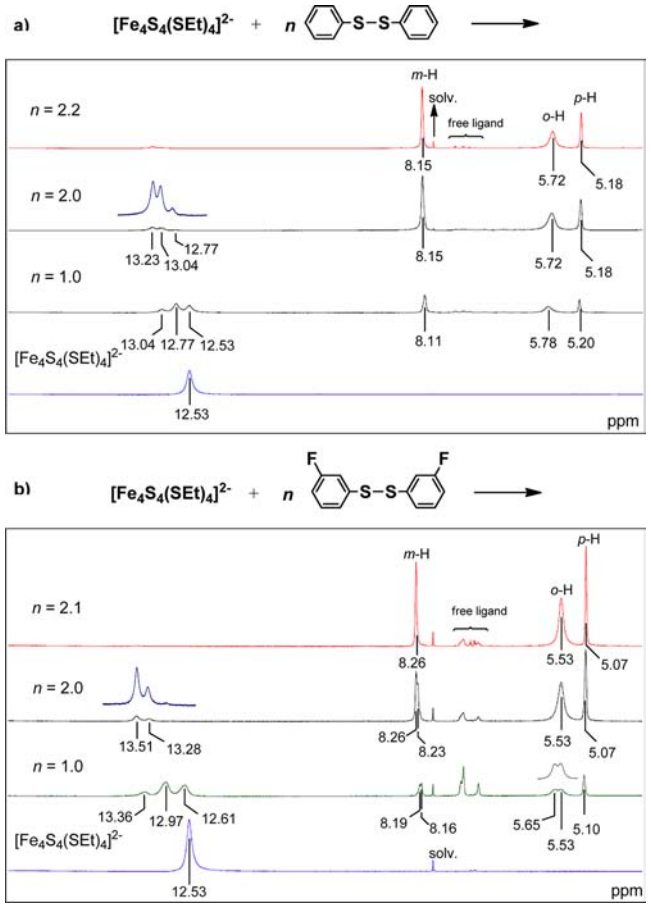

Figure 4. ^1H NMR spectra (600 MHz) in the downfield region for the reaction between 14 mM $[Fe_4S_4(SEt)_4]^{2-}$ and n equiv of (a) diphenyl disulfide and (b) bis(3-fluorophenyl) disulfide in $(CD_3)_2SO$ at ~25 °C. The signal assignments of bound arylthiolate ligands are indicated; solv = DMF.

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*-dimethylamino)pyridine] 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-cyclodextrin 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 ^{13}C as well as 2D COSY and HSQC NMR experiments.[23] The two phenyl groups were found to be magnetically inequivalent by the appearance of 8 aromatic proton and 12 aromatic carbon signals in CD_3OD and $(CD_3)_2SO$ solutions. The ^1H NMR spectrum in the downfield region in $(CD_3)_2SO$, 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 ($2ArS^- \rightarrow ArSSAr + 2e^-$) and oxidation (reverse reaction) steps are observed, a behavior typical of compounds of this type in aprotic solvents.[25,26] The 3-fluoro substitutents cause a positive shift of $E_{p,c}$ in agreement with prior observations of 4-substituted compounds.[27] The same redox pattern is observed for **14** at nearly the same potentials as those for PhSSPh, confirming the presence of an S−S bond. The structure of **14** was further confirmed by high-resolution MS.

Thiolate Cluster Ligation. Variation of the terminal thiolate ligation in $[Fe_4S_4(SR)_4]^{2-}$ clusters is conventionally accomplished by the displacement of halide (e.g., in $[Fe_4S_4Cl_4]^{2-}$) with thiolate or by the 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 $[Fe_4S_4(SePh)_4]^{2-}$ from $[Fe_4S_4(SBu^t)_4]^{2-}$ 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 $[Fe_4S_4(SEt)_{4-n}(SAr)_n]^{2-}$ 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 of reaction time, reaction (1) is complete for both disulfides.

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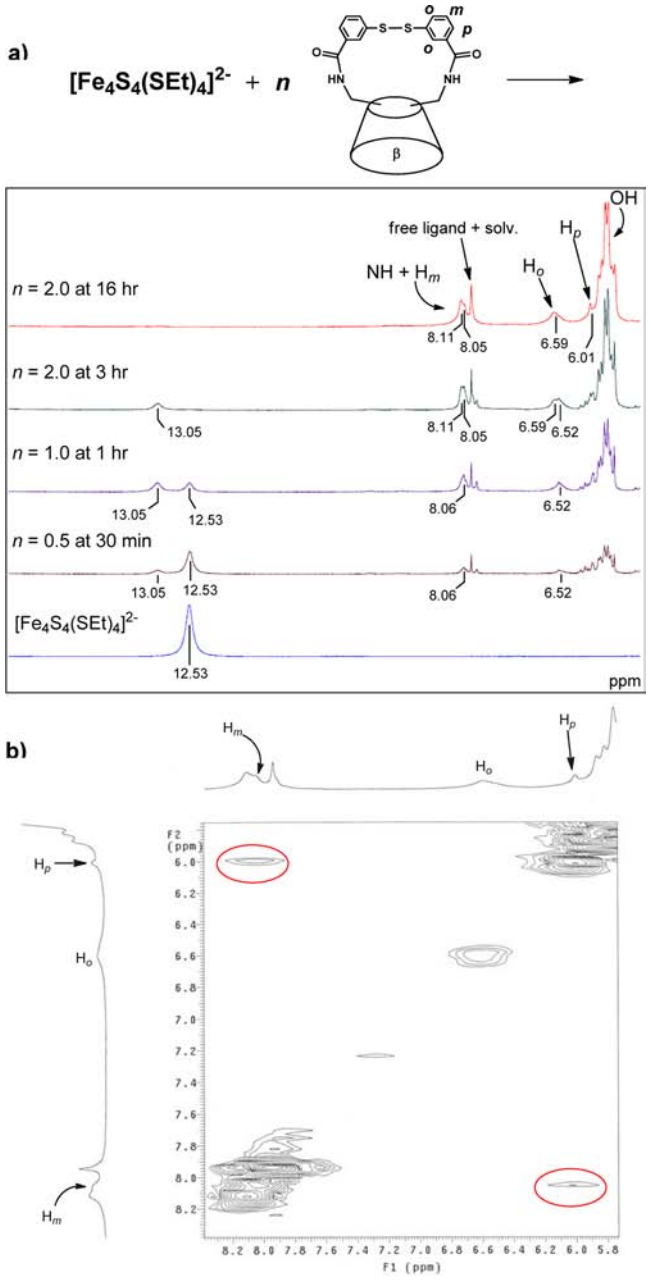

Figure 5. (a) ^{1}H NMR spectra (600 MHz) in the downfield region for the reaction between 2.3 mM $[Fe_4S_4(SEt)_4]_{2-}$ and n equiv of β-cyclodextrin disulfide at various times in $(CD_3)_2SO$ at ~25 °C. (b) COSY spectrum of the n = 2.0 equiv product at 16 h of reaction time showing the correlation between H_m and H_p.

$$[Fe_4S_4(SEt)_4]^{2-} + 2ArSSAr \rightarrow [Fe_4S_4(SAr)_4]^{2-} + 2EtSSEt \quad (1)$$

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

$[Fe_4S_4\{\beta\text{-CD-}(1,3\text{-}NHCOC_6H_4S)_2\}_2]^{2-}$ **15**
$[Fe_4S_4\{\beta\text{-CD-}(1,3\text{-}SC_6H_4S)_2\}_2]^{2-}$ **16**[15]
$[Fe_4S_4\{\alpha\text{-CD-}(CH_2S)_2\}_2]^{2-}$ **17**[1]
$[Fe_4S_4\{\alpha\text{-CD-}(CH_2S)\}_4]^{2-}$ **18**[1]

With the feasibility of reaction (1) demonstrated, reaction (2) was conducted in order to generate cluster **15** in a Me_2SO solution. The reactions were followed by ^{1}H NMR with variable equivalents 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 is increased, the three phenyl 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 $[Fe_4S_4(SAr)_4]^{2-}$, 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 the ligand structure and by the carboxamide substituent. The spectrum of **16** would be an interesting comparison, but it was not reported.[15]

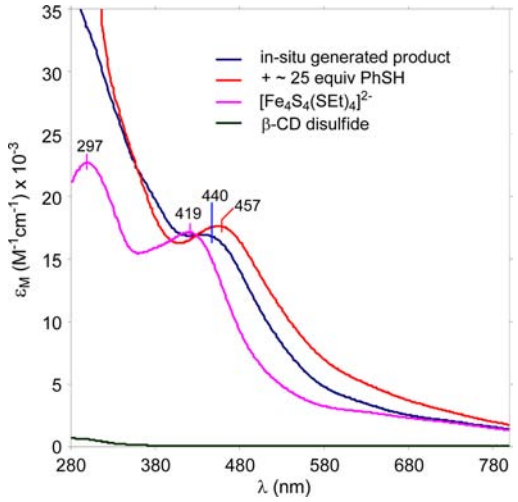

Figure 6. Absorption spectra in Me_2SO of $[Fe_4S_4(SEt)_4]^{2-}$ (red), the reaction product of $[Fe_4S_4(SEt)_4]^{2-}$ and β-cyclodextrin disulfide [reaction (2), blue], the reaction product plus 25 equiv of benzenethiol (orange), and β-cyclodextrin disulfide (green).

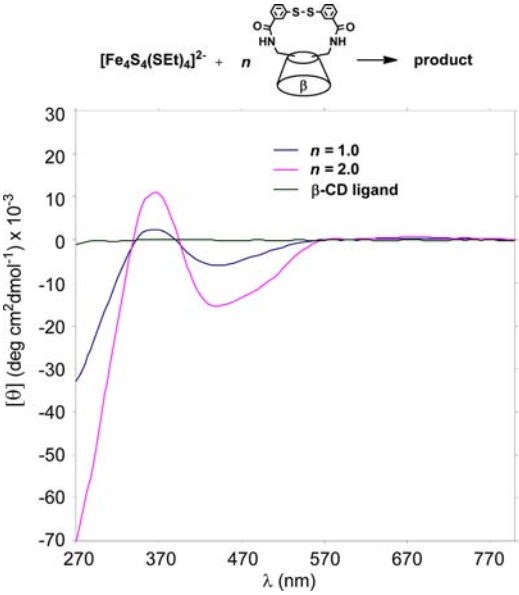

Figure 7. Circular dichroism spectra of the reaction product between 0.51 mM $[Fe_4S_4(SEt)_4]^{2-}$ and 1.0 equiv (blue) and 2.0 equiv (red) of β-cyclodextrin disulfide in Me_2SO. The disulfide (green, 1.4 mM) did not produce an effect at 270−770 nm.

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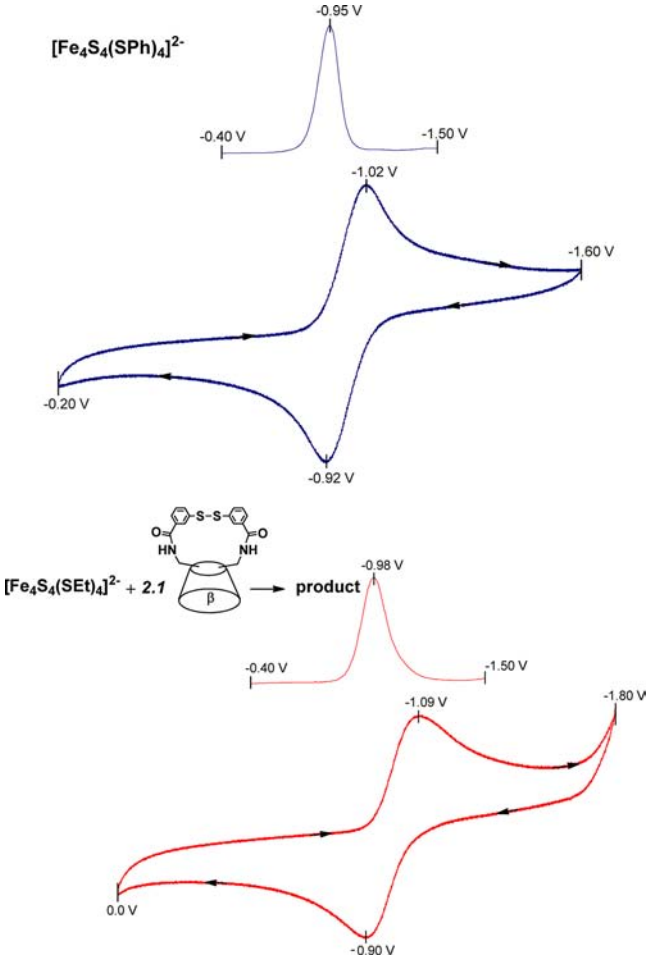

Figure 8. Cyclic voltammograms (100 mV/s) of $[Fe_4S_4(SEt)_4]^{2-}$ (upper) and the reaction product between 1.2 mM $[Fe_4S_4(SEt)_4]^{2-}$ and 2.1 equiv of β-cyclodextrin disulfide after 16 h (reaction 2, lower). The inset diagrams show the redox potentials obtained by differential pulse voltammetry (pulse width 50 ms; pulse period 200 ms). Voltammograms were recorded in Me_2SO solutions at ~25 °C; peak potentials are indicated.

$$[Fe_4S_4(SEt)_4]^{2-} + 2\beta\text{-CD-(1,3-NHCOC}_6H_4S)_2 \rightarrow [Fe_4S_4\{\beta\text{-CD-(1,3-NHCOC}_6H_4S)_2\}_2]^{2-} + 2EtSSEt \quad (2)$$

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 Me_2SO 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 $[Fe_4S_4(SPh)_4]^{2-}$. These spectra distinguish arylthiolate from alkylthiolate clusters such as $[Fe_4S_4(SEt)_4]^{2-}$ (λ_{max} = 297 and 419 nm). Treatment of the reaction product with ca. 25 equiv of benzenethiol in reaction (3) generates a spectrum identical with that of authentic $[Fe_4S_4(SPh)_4]^{2-}$ and corresponds to 98% of the initial cluster concentration in reaction (2).

$$[Fe_4S_4\{\beta\text{-CD-(1,3-NHCOC}_6H_4S)_2\}_2]^{2-} + 4PhSH \rightarrow [Fe_4S_4(SPh)_4]^{2-} + 2\beta\text{-CD-(1,3-NHCOC}_6H_4SH)_2 \quad (3)$$

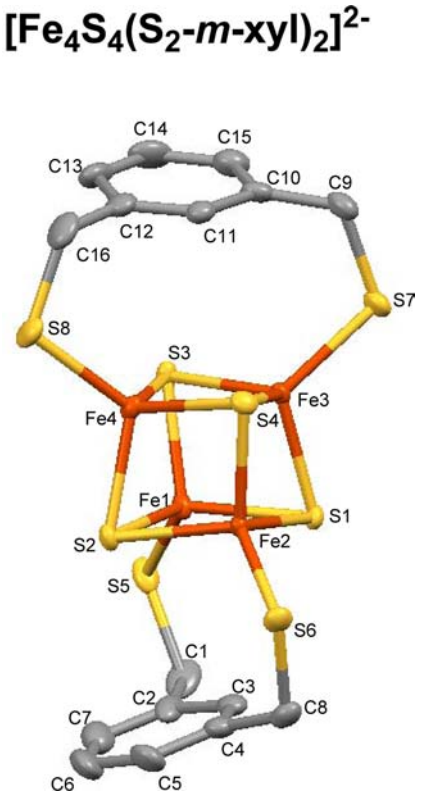

Figure 9. Structure of $[Fe_4S_4(S_2\text{-}m\text{-xyl})_2]^{2-}$ with 50% probability ellipsoids and the atom numbering scheme. The four short [2.253(1)–2.268(1) Å] and eight long [2.297(1)–2.318(1) Å] Fe–S bond distances define the indicated ranges. The mean of the Fe–Fe distances [2.698(1)–2.756(1) Å] is 2.73(3) Å.

We conclude that (barring an unlikely core dissembly/assembly event) reaction (2) proceeds with retention of the $[Fe_4S_4]^{2+}$ core of the starting cluster. Note also the circular dichroism spectra in Figure 7, where the effect is induced by 1 and 2 equiv of β-cyclodextrin disulfide. Cluster **18** with four α-cyclodextrin 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 voltammetries of the 2–/3– redox couples of $[Fe_4S_4(SPh)_4]^{2-}$ and the product of reaction (2) are compared in Figure 8. Note that both are chemically reversible ($i_{pc}/i_{pa} \approx 1$) and differ by only 30 mV, a result that requires arylthiolate coordination in the reaction product. Alkylthiolate clusters such as $[Fe_4S_4(SEt)_4]^{2-}$ (−1.33 V) exhibit 2–/3– couples at substantially more negative potentials.[30]

Structural Considerations. The results from four physical techniques not only establish a reaction between β-cyclodextrin disulfide and $[Fe_4S_4(SEt)_4]^{2-}$ in a Me_2SO 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 that 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 $[Fe_4S_4]^{2+}$ core as a bridging or terminal ligand in the stoichiometry of **15**. The formation of μ_2-SR bridges would lead to oligomeric species, of which there is one documented example.[31] The binding of one thiolate at an iron site would afford a single cluster with two

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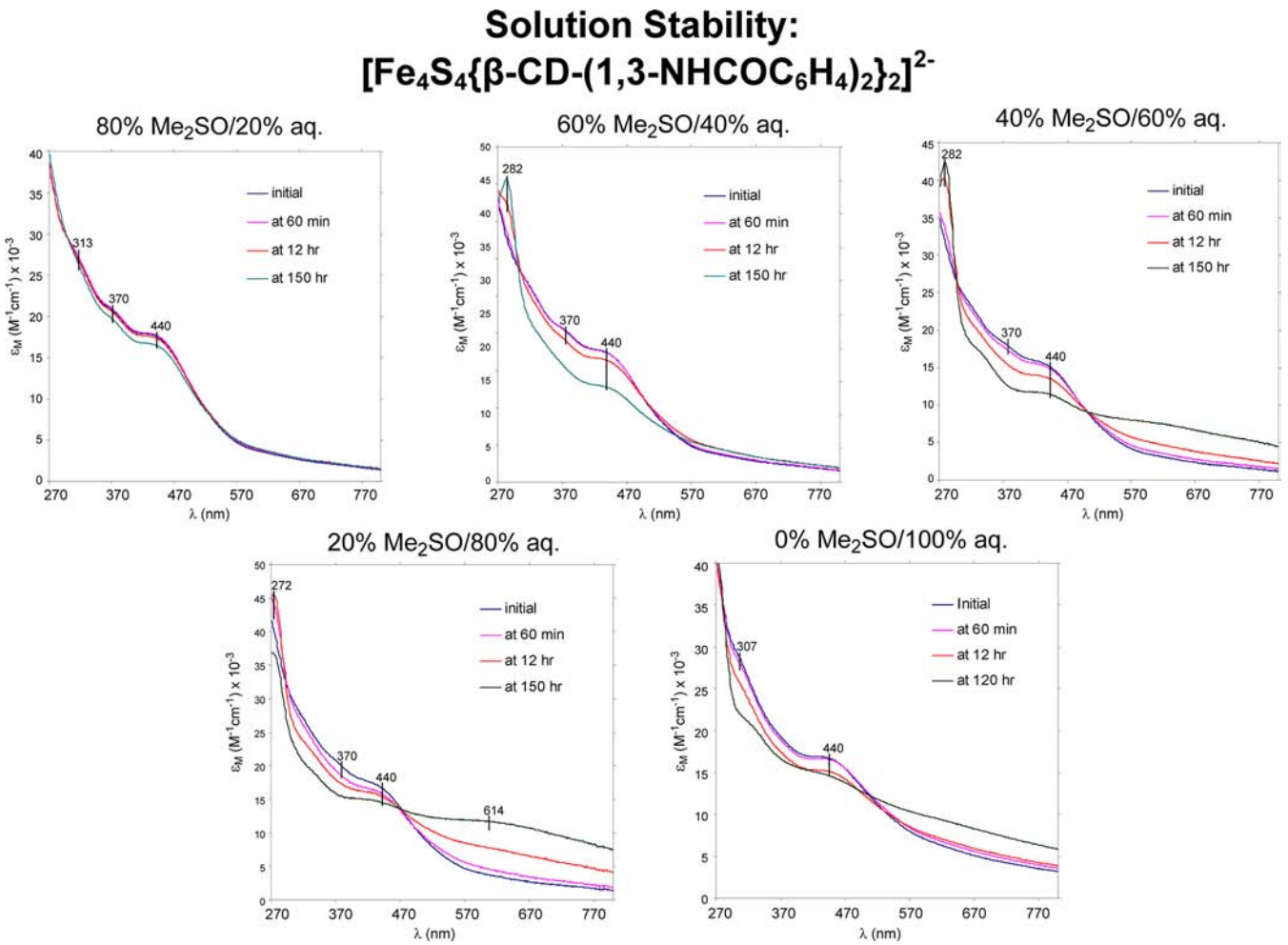

Figure 10. Absorption spectra of the product of reaction (2) in specified Me_2SO–aqueous buffer solutions (v/v) in which the aqueous component is 3.55 mM phosphate buffer (pH 7.5–7.6). Spectra were recorded under anaerobic conditions at 0–120 or 150 h after reaction at the following concentrations: 80% Me_2SO–20% aqueous, 0.27–0.41 mM; 60% Me_2SO–40% aqueous, 0.20–0.31 mM; 40% Me_2SO–60% aqueous, 0.14–0.21 mM; 20% Me_2SO–80% aqueous; 100% aqueous, 0.39 mM. Band maxima or shoulders are indicated.

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 iron protein of nitrogenase, which consists of a cluster ligated by two cysteinate residues from each of the 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 $[Fe_4S_4(S_2\text{-}m\text{-xyl})_2]^{2-}$, formulated on the basis of absorption and ^{1}H NMR spectral features and redox behavior but lacking structural 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 $[Fe_4S_4]^{2+}$ oxidation state from its Mössbauer parameters (δ = 0.41 mm/s, ΔE_Q = 1.05 mm/s at 90 K) whose values are typical of that state.[8] As is evident in Figure 9, the 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 four short [2.236(5) Å] + eight 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 $[Fe_4S_4(SCH_2Ph)_4]^{2-}$.[16] The S–S distance (6.25 Å) between terminal ligands is smaller than the values in the latter cluster (6.30 and 6.49 Å) because of constraints of the bidentate ligand structure.

The structure of $[Fe_4S_4(S_2\text{-}m\text{-xyl})_2]^{2-}$ provides the first demonstration of the unidentate terminal binding mode by a bidentate dithiolate. At the very least, it offers a precedent for a similar binding arrangement in **15** as well as **16** and **17**. We note that the S–S separations in **15** are expected to be similar to those of $[Fe_4S_4(S_2\text{-}m\text{-xyl})_2]^{2-}$ 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 the 6^A,6^D positions on the rim of the structure can accommodate the S–S distances of a synthetic cluster.

Solution Stability. In the examination of the stability, reaction (2) was conducted in a 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 an aqueous buffer alone was obtained by removing Me_2SO and dissolving the residue in the 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 h after the initial spectra. For each system, spectra were recorded at four to six intermediate times as well, but for clarity,

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only the initial and final spectra and those at 60 min and 12 h are shown. Spectra in the different solvent media were measured in the concentration ranges given in the figure. The 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 with that in the pure solvent. The cluster is completely stable in this medium at 72 and 150 h and evidences only ca. 6% decrease in the intensity of the 440 nm band at 150 h. 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 h. The spectra at 40% and 20% Me_2SO are further progressions but with significant increases in the absorbance in the 470−770 nm region. Increased visible absorbances are observed for α-cyclodextrin thiolate clusters **17** and **18** and also for $[Fe_4S_4(SCH_2CH_2OH)_4]^{2-}$ and $[Fe_4S_4(SCH_2CH_2CO_2)_4]^{6-}$ at 0−50% Me_2SO. However, their appearance is a certain sign of (partial) cluster degradation. Circular dichroism spectra at 270−770 nm (not shown) confirm stability at 60−100% Me_2SO for up to 12 h and reveal substantial changes in the ellipticity and several new features at higher aqueous buffer content after 1 h. A comparison of spectra at 80−20% Me_2SO indicates a somewhat improved stability of **15** in these media at 60 min to 12 h 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 (Me_2SO) and 441 nm (DMF)[15] with $\varepsilon_M \approx 17500$, suggestive of related structures. Conceivably, the cluster structures in their entirety differ primarily by the connector (−S− and −NHCO−) from the 6-CH_2 group of rings A and D to the 3-C_6H_4S binding sites. We have not attempted to determine the stability half-life of **15** in an aqueous buffer, as has been reported for **16** (120 h) 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 h and decays by 15% over 120 h with an increase in the absorbance at longer wavelengths. 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 $[Fe_4S_4(SCH_2CH_2OH)_4]^{2-}$ and $[Fe_4S_4(SCH_2CH_2CO_2)_4]^{6-}$ 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.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

NMR and Mössbauer spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Lo, W.; Scott, T. A.; Zhang, P.; Ling, C.-C.; Holm, R. H. *J. Inorg. Biochem.* **2011**, *105*, 497−508.
(2) Lill, R.; Muhlenhoff, U. *Annu. Rev. Biochem.* **2008**, *77*, 669−700.
(3) Rouault, T. A.; Tong, W. H. *Trends Genet.* **2008**, *24*, 398−407.
(4) Ye, H.; Rouault, T. A. *Biochemistry* **2010**, *49*, 4945−4956.
(5) Rouault, T. A. *Dis. Models Mech.* **2012**, *5*, 155−164.
(6) Robbins, A. H.; Stout, C. D. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 3639−3643.
(7) Dupuy, J.; Volbeda, A.; Carpentier, P.; Darnault, C.; Moulis, J.-M.; Fontecilla-Camps, J. C. *Structure* **2006**, *14*, 129−139.
(8) Rao, P. V.; Holm, R. H. *Chem. Rev.* **2004**, *104*, 527−559.
(9) Hill, C. L.; Renaud, J.; Holm, R. H.; Mortenson, L. E. *J. Am. Chem. Soc.* **1977**, *99*, 2549−2557.
(10) Davies, S. C.; Evans, D. J.; Henderson, R. A.; Hughes, D. L.; Longhurst, S. *J. Chem. Soc., Dalton Trans.* **2002**, 3470−3477.
(11) Job, R. C.; Bruice, T. C. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 2478−2482.
(12) Bruice, T. C.; Maskiewicz, R.; Job, R. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 231−234.
(13) Sharma, A. K.; Kim, N.; Cameron, C. S.; Lyndon, M.; Gorman, C. B. *Inorg. Chem.* **2010**, *49*, 5072−5079.
(14) Harata, K. *Chem. Rev.* **1998**, *98*, 1803−1827.
(15) Kuroda, Y.; Sasaki, Y.; Shiroiwa, Y.; Tabushi, I. *J. Am. Chem. Soc.* **1988**, *110*, 4049−4050.
(16) Averill, B. A.; Herskovitz, T.; Holm, R. H.; Ibers, J. A. *J. Am. Chem. Soc.* **1973**, *95*, 3523−3534.
(17) Holm, R. H.; Phillips, W. D.; Averill, B. A.; Mayerle, J. J.; Herskovitz, T. *J. Am. Chem. Soc.* **1974**, *96*, 2109−2117.
(18) Tabushi, I.; Yamamura, K.; Nabeshima, T. *J. Am. Chem. Soc.* **1984**, *106*, 5267−5270.
(19) Fujita, K.; Yamamura, H.; Imoto, T. *J. Org. Chem.* **1985**, *50*, 4393−4395.
(20) Cotner, E. S.; Smith, P. J. *J. Org. Chem.* **1998**, *63*, 1737−1739.
(21) Bonomo, R. P.; Pedotti, S.; Vecchio, G.; Rizzarelli, E. *Inorg. Chem.* **1996**, *35*, 6873−6877.
(22) Sheldrick, G. M. *Acta Crystallogr.* **2009**, *A64*, 112−122.
(23) See the paragraph at the end of this article for the Supporting Information available.
(24) Humphrey, R. E.; Hawkins, J. M. *Anal. Chem.* **1964**, *36*, 1812−1814.
(25) Persson, B. *J. Electroanal. Chem.* **1978**, *86*, 313−323.
(26) Christensen, T. B.; Daasbjerg, K. *Acta Chem. Scand.* **1997**, *51*, 307−317.
(27) Daasbjerg, K.; Jensen, H.; Benassi, R.; Taddei, F.; Antonello, S.; Gennaro, A.; Maran, F. *J. Am. Chem. Soc.* **1999**, *121*, 1750−1751.
(28) Holm, R. H. In *Biocoordination Chemistry*; Que, L., Jr., Tolman, W. A., Eds.; Elsevier: Oxford, U.K., 2004; pp 61−90.
(29) Que, L., Jr.; Bobrik, M. A.; Ibers, J. A.; Holm, R. H. *J. Am. Chem. Soc.* **1974**, *96*, 4168−4178.
(30) DePamphilis, B. V.; Averill, B. A.; Herskovitz, T.; Que, L., Jr.; Holm, R. H. *J. Am. Chem. Soc.* **1974**, *96*, 4159−4167.

(31) Rao, P. V.; Bhaduri, S.; Jiang, J.; Hong, D.; Holm, R. H. *J. Am. Chem. Soc.* **2005**, *127*, 1933−1945.
(32) Georgiadis, M. M.; Komiya, H.; Chakrabarti, P.; Woo, D.; Kornuc, J. J.; Rees, D. C. *Science* **1992**, *257*, 1653−1659.
(33) Schlessman, J. L.; Woo, D.; Joshua-Tor, L.; Howard, J. B.; Rees, D. C. *J. Mol. Biol.* **1998**, *280*, 669−685.
(34) Saenger, W.; Jacob, J.; Gessler, K.; Steiner, T.; Hoffman, D.; Sanbe, H.; Koisumi, K.; Smith, S. M.; Takaha, T. *Chem. Rev.* **1998**, *98*, 1787−1802.