Preparation of Fe_4S_4 Iron–Sulphur Protein Analogues with Hydrophobic Macrocyclic Tetrathiol Ligand Anchored to a 38-Membered Cyclophane Type Skeleton

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(Received September 18, 1989)

Abstract

The preparation of Fe_4S_4 cubane type active site analogues for iron–sulphur proteins in which the active core is surrounded by an intramolecular hydrophobic domain formed by a 38-membered ring consisting of a cyclophane skeleton is described. An efficient synthesis of the macrocyclic tetrathiol ligands, bis[*N*,*N*′-bis(4-mercaptobenzoyl)-*N*,*N*′-octamethylene-4,4′-diaminodiphenylmethane] (**3a**), bis[*N*,*N*′-bis{4-(mercaptomethyl)benzoyl}-*N*,*N*′-octamethylene-4,4′-diaminodiphenylmethane] (**3b**) and bis[*N*,*N*′-bis(3-mercapto-3-methylbutanoyl)-*N*,*N*′-octamethylene-4,4′-diaminodiphenylmethane] (**3c**) is achieved. Reaction of the cyclic tetrathiol ligand **3** with $[Fe_4S_4(SBu^t)_4]^{2-}$ (**1c**) afforded $[Fe_4S_4\{cyclo\text{-}(XN\text{-}p\text{-}C_6H_4\text{-}p\text{-}CH_2C_6H_4\text{-}XN[CH_2]_8)_2\}]^{2-}$ [X = *p*-SC_6H_4CO (**2a**), *p*-$SCH_2C_6H_4CO$ (**2b**), $SC(CH_3)_2CH_2CO$ (**2c**)]. Thus the new clusters embedded in the cyclophane environment are obtained in good yields (70–90%) as black powders with melting points >300 °C. They dissolve in DMF, DMSO and propylene carbonate, but are hardly soluble in most common organic solvents and water.

Introduction

In the course of our extensive work on non-heme iron–sulphur protein analogues, we have been studying environmental effects on the active site core, and previously reported in a preliminary form the synthesis [1, 2] and characterization [3] of the Fe_4S_4 clusters with macrocyclic tetrathiol ligands as well as their application to carbon dioxide fixation as electron carriers [4, 5].

Non-heme iron–sulphur proteins are widely distributed in living organisms from bacteria to mammals, taking a very important role in various biological redox reactions such as photosynthesis, biosynthesis of steroidal hormones, metabolism of fatty acids and sulphur, nitrogen fixation reactions, and so on [6]. Of those, high-potential proteins [7–9] showed their redox potentials (1–/2–) near +0.35 V (versus NHE at pH 7 in water), whereas the structure of the 4-Fe active site itself is very close to that of low-potential 4-Fe ferredoxins which have their most stable redox potentials (2–/3–) near −0.4 to −0.6 V [6, 10]. It has been shown that the active site cores in high-potential proteins are surrounded by proteins consisting of largely hydrophobic amino acids [11]. This suggests the hydrophobic environment is important for stabilizing the Fe_4S_4 cores, especially for high-potential proteins. Eventually, in the model compounds, stabilization of the 1– state by the use of bulky alkyl ligands [12, 13] and hydrogen bonding [14], and the investigation of Fe_4S_4 clusters in different environments were described [15]. We have examined environmental effects on the Fe_4S_4 core using macrocyclic tetrathiol ligands which provide intramolecular hydrophobic domains instead of the conventional small alkyl- and aryl-thiols (for example, **1a**–**1c**). Consequently, we describe here the synthesis of tetrathiol ligands anchored to a 38-membered ring consisting of cyclophane type **3a**–**3c** macrocycles, and their application to the cubane type tetranuclear clusters (Fig. 1).

Experimental

General Methods

Melting points are uncorrected. Manipulations and measurements involving Fe–S clusters and thiols were carried out under an atmosphere of N_2 or Ar. Flash chromatographic separations were carried out as described in ref. 16 on 230–400 mesh silica gel 60. THF and diethyl ether were distilled from sodium benzophenone ketyl; DMF, CH_2Cl_2, CH_3CN, benzene, hexane and $CHCl_3$ were distilled from CaH_2. EtOH and MeOH were distilled from Mg, and AcOEt and acetone were purified by distillation. Other materials were purchased from appropriate sources and used as received. Absorption spectra were recorded on a Cary 219 spectrophotometer. NMR

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0020-1693/90/$3.50

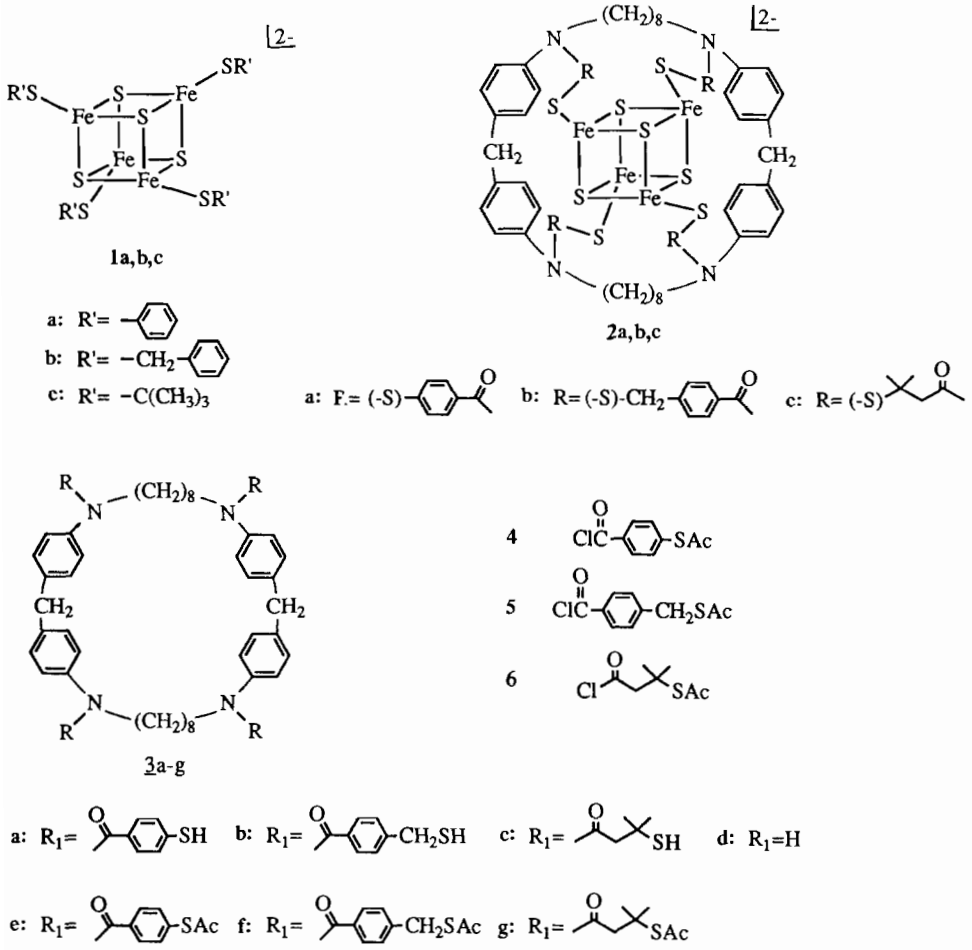

Fig. 1. Structure of clusters and ligands.

spectra were determined on a JEOL JMN GX-270 or a JEOL FX-100 spectrometer, and chemical shifts are relative to Me_4Si internal reference. The clusters **1a**, **1b** and **1c** were prepared according to literature procedures [17].

Bis[N,N'-bis{4-(acetylthio)benzoyl}-N,N'-octamethylene-4-4'-diaminodiphenylmethane (3e)

To a mixture of compound **3d** (400 mg, 0.648 mmol) and NEt_3 (543 μl, 3.89 mmol) in CH_2Cl_2 (10 ml) the acid chloride **4** (843 mg, 3.89 mmol) was gradually added at 0 °C. After stirred at room temperature (r.t.) for 3 h, CH_2Cl_2 (100 ml) was added into the mixture, and the resultant solution was washed with sat. $NaHCO_3$, 5% HCl and brine, and dried (Na_2SO_4). The crude product was then purified by silica gel chromatography eluted with ether–$CHCl_3$ (4:3) followed by recrystallization from $CHCl_3$–petroleum ether to afford colorless needles (826 mg, 96%) melting point (m.p.) 222–223 °C. IR(neat): 1700, 1640 cm^{-1}. NMR ($CDCl_3$): 2.38 (s, 12H), 3.80–3.86 (m, 12H), 6.90 (d, 8H, J = 8.4 Hz), 6.97 (d, 8H, J = 8.4 Hz), 7.20 (d, 8H, J = 8.4 Hz), 7.28 (d, 8H, J = 8.4 Hz). *Anal.* Calc. for $C_{78}H_{80}N_4O_8S_4/H_2O$: C, 69.51; H, 6.13; N, 4.16; S, 9.52. Found: C, 69.44; H, 6.11; N, 4.00; S, 9.65%.

Bis[N,N'-bis{4-(acetylthiomethyl)benzoyl}-N,N'-octamethylene-4,4'-diaminodiphenylmethane] (3f)

Compound **5** (701 mg, 3.06 mmol) was added at 0 °C to a CH_2Cl_2 solution (50 ml) containing **3d** (300 mg, 0.479 mmol) and NEt_3 (325 mg, 3.21 mmol). The mixture was reacted at r.t. for 2.5 h, and in the same manner described above, **3f** was isolated as colorless crystals (530 mg, 80%), m.p. 61–63 °C ($CHCl_3$–petroleum ether). IR(neat): 1680, 1640 cm^{-1}. NMR ($CDCl_3$): 2.32 (s, 12H), 3.78–3.83 (m, 12H), 4.01 (s, 8H), 6.90 (d, 8H, J = 8.4 Hz), 6.97 (d, 8H, J = 8.4 Hz), 7.06 (d, 8H, J = 8.4 Hz), 7.19 (d, 8H, J = 8.4 Hz). *Anal.* Calc. for $C_{82}H_{88}N_4O_8S_4$: C, 71.07; H, 6.40; N, 4.04; S, 9.25. Found: C, 71.27; H, 6.46; N, 3.83; S, 9.09%.

Bis[N,N'-bis(3-acetylmercapto-3-methylbutanoyl)-N,N'-octamethylene-4,4'-diaminodiphenylmethane] (3g)

To a mixture of **3d** (500 mg, 0.80 mmol) and K_2CO_3 (387 mg, 2.8 mmol) in CH_2Cl_2 (25 ml) **6** (933 mg, 4.8 mmol) in CH_2Cl_2 (25 ml) was added dropwise over a period of 20 min at 0 °C. The mixture was stirred at r.t. for 2 h. Colorless crystals (837 mg, 84%) were obtained after the same work-up procedures described above, m.p. 165–166 °C ($CHCl_3$–petroleum ether). IR(nujol) 1680, 1640 cm^{-1}. NMR ($CDCl_3$): 1.19–1.26 (m, 16H), 1.51 (s, 24H), 1.26–1.55 (m, 8H), 2.16 (s, 12H), 2.63 (s, 8H), 3.50–3.70 (m, 12H), 6.98, 7.06, 7.20, 7.28 (ABq, 16H, J = 8.3 Hz). *Anal.* Calc. for $C_{70}H_{96}N_4O_8S_4$: C, 67.27; H, 7.74; N, 4.48; S, 10.26. Found: C, 67.55; H, 7.81; N, 4.35; S, 10.19%.

Bis[N,N'-bis(4-mercaptobenzoyl)-N,N'-octamethylene-4,4'-diaminodiphenylmethane] (3a)

A solution of **3e** (20.4 mg, 0.015 mmol) in deaerated 1 N HCl/MeOH (5 ml) was heated at 50 °C for 2 h under Ar. After removal of the solvent *in vacuo*, the residue was dissolved in $CHCl_3$ (20 ml), washed with brine and dried (Na_2SO_4). Evaporation of the solvent gave pale yellow crystals (17.5 mg, 98%), m.p. > 320 °C ($CHCl_3$–MeOH). IR(neat): 2500 cm^{-1}. NMR ($CDCl_3$): 1.26 (m, 16H, CH_2), 1.56 (m, 8H, N_β-CH_2), 3.42 (s, 4H, SH), 3.82 (m, 8H, N_α-CH_2), 3.85 (s, 4H, $ArCH_2Ar$), 6.89 (d, 8H, J = 8.4 Hz), 6.97 (d, 8H, J = 8.4 Hz), 7.01 (d, 8H, J = 8.4 Hz), 7.12 (d, 8H, J = 8.4 Hz). *Anal.* Calc. for $C_{70}H_{72}N_4O_4S_4/H_2O$: C, 71.27; H, 6.32, N, 4.75; S, 10.87. Found: C, 71.49; H, 6.13; N, 4.71; S, 10.61%.

Bis[N,N'-bis{4-mercaptomethyl)benzoyl}-N,N'-octamethylene-4,4'-diaminodiphenylmethane] (3b)

Compound **3f** (350 mg, 0.253 mmol) was treated in the same way as **3e** for 4 h at 50 °C. Pale yellow crystals (265 mg, 86%) were obtained, m.p. 81.5–83 °C ($CHCl_3$–MeOH). IR(nujol): 2550, 1640 cm^{-1}. NMR ($CDCl_3$): 1.25 (m, 16H, CH_2), 1.57 (m, 8H, N_β-CH_2), 1.68 (t, 4H, J = 7.5 Hz, SH), 3.64 (d, 8H, J = 7.7 Hz, SCH_2), 3.81 (m, 8H, N_α-CH_2), 3.86 (s, 4H, $ArCH_2Ar$), 6.91 (d, 8H, J = 8.4 Hz), 6.97 (d, 8H, J = 8.4 Hz), 7.10 (d, 8H, J = 8.1 Hz), 7.21 (d, 8H, J = 8.1 Hz). *Anal.* Calc. for $C_{74}H_{80}N_4O_4S_4$: C, 72.89; H, 6.62; N, 4.60; S, 10.53. Found: C, 72.43; H, 6.67; N, 4.33; S, 10.12%.

Bis[N,N'-bis(3-mercapto-3-methylbutanoyl)-N,N'-octamethylene-4,4'-diaminodiphenylmethane] (3c)

A similar treatment as above by heating **3g** (400 mg, 0.320 mmol) at 50 °C for 3 h yielded pale yellow crystals (313 mg, 90%), m.p. 208–210 °C ($CHCl_3$–MeOH). IR(nujol): 2530, 1640 cm^{-1}. NMR ($CDCl_3$): 1.22 (m, 16H, CH_2), 1.43 (m, 8H, N_β-CH_2), 1.47 [s, 24H, $C(CH_3)_2$], 2.35 (s, 8H, $COCH_2$), 2.67 (s, 4H, SH), 3.62 (m, 8H, N_α-CH_2), 4.04 (s, 4H, $ArCH_2Ar$), 7.05, 7.14, 7.22, 7.25 (ABq, 16H, J = 4.8 Hz). *Anal.* Calc. for $C_{62}H_{88}N_4O_4S_4/2H_2O$: C, 66.63; H, 8.30; N, 5.01; S, 11.47. Found: C, 67.03; H, 8.46; N, 4.79; S, 10.97%.

Bis(tetramethylammonium)-bis(N,N'-octamethylene-4,4'-diaminodiphenylmethane)-N,N,N',N'-tetrakis-(p-oxobenzenethiolato-μ_3-sulphido-iron) (2a)

To a DMF (15 ml) solution of **1c** (142 mg, 0.16 mmol), **3a** (200 mg, 0.170 mmol) in DMF (15 ml) was added dropwise, and the liberated t-BuSH was distilled off *in vacuo* at 40 °C for 30 min. Then AcOEt–hexane (1:6, 700 ml), and CH_3CN and CH_2Cl_2 were added to the mixture, and the mixture was kept at –20 °C overnight. The precipitate was collected by filtration, washed subsequently with MeOH, CH_2Cl_2 and ether to afford 238 mg (90%) of black powder, m.p. > 300 °C (DMF–THF). NMR (DMSO-d_6): 1.14–1.40 [m, 24H, CH_2(skeleton)], 3.18–3.26 [br, 24H, N_α(cation-CH_3], 3.8 [br, 8H, N_α(skeleton)-CH_2], 5.3 [br, 8H, arom (*ortho* to S)], 6.90–7.00 (br, 16H, N-C_6H_4), 7.95 [br, 8H, arom (*meta* to S)]. Vis(DMF), λ_{max} (nm) ($\epsilon \times 10^{-3}$): 456 (16.8), 380 (sh, 22.6).

Bis(tetramethylammonium)-bis(N,N'-octamethylene-4,4'-diaminodiphenylmethane)-N,N,N',N'-tetrakis-(p-oxophenylmethanethiolato-μ_3-sulphido-iron) (2b)

2b (105 mg, 75%) was obtained using **1c** (72 mg, 0.0822 mmol, 10 ml DMF) and **3b** (100 mg, 0.0822 mmol, 10 ml DMF); 300 ml THF and CH_3CN and CH_2Cl_2; m.p. > 300 °C (DMF–THF). NMR (DMSO-d_6): 1.13–1.37 [m, 24H, CH_2(skeleton)], 3.16–3.17 [br, 24H, N_α(cation)-CH_3], 3.84 [br, 8H, N_α(skeleton)-CH_2], 7.03–7.44 (br, 32H, arom), 13.2 (br, 8H, ph-CH_2-S). Vis(DMF), λ_{max} (nm) ($\epsilon \times 10^{-3}$): 415 (17.3), 313 (sh, 21.8).

Bis(tetramethylammonium)-bis(N,N'-octamethylene-4,4'-diaminodiphenylmethane)-N,N,N',N'-tetrakis(3-oxo-1,1-dimethylpropanethiolato-μ_3-sulphido-iron (2c)

2c (103 mg, 71%) was obtained using **1c** (81 mg, 0.092 mmol, 7.5 ml DMF) and **3c** (100 mg, 0.092 mmol, 5 ml DMF); THF–hexane (1:1), and CH_3CN and CH_2Cl_2; m.p. > 300 °C (DMF–THF). NMR (DMSO-d_6): 1.17–1.34 [br, 24H, CH_2(skeleton)], 2.50 (s, 8H, CO-CH_2), 2.61 [br, 24H, CH_3(ligand)], 3.21–3.23 [br, 24H, N_α(cation)-CH_3], 7.1 (br, 16H, arom). Vis(DMF), λ_{max} (nm) ($\epsilon \times 10^{-3}$): 416 (16.2), 303 (21.4).

Colorimetric SH Determination of 3a–3c by DTNB [18]

A total of 3.0 ml of **3a** (0.774 mg, 0.660 μmol in 20.0 ml n-PrOH) was mixed with phosphate buffer

(2.0 ml; 0.1 M, pH 8.0) and water (5.0 ml). To a solution of the above mixture (3.0 ml), DTNB (20 μl; 10 mM in phosphate buffer, pH 7.0) was added, and the absorbance at 412 nm was determined after 40 min at r.t. The same procedures were used for the other complexes using for **3b** 1.070 mg, 0.879 μmol, 20.0 ml n-PrOH; for **3c** 0.953 mg, 0.881 μmol, 20.0 ml n-PrOH.

Results and Discussion

Synthesis of the Macrocyclic Tetrathiol Ligands (3a–3c)

We have previously developed an efficient route to the corresponding cyclic tetra-amine with cyclophane skeleton as a key intermediate [19]. To obtain the appropriate ligands, an introduction of thiol functions has been carried out into the macrocyclic tetra-amine **3d**. The acid chloride derivatives bearing thiol functions, i.e. acetylthiobenzoyl chloride (4), acetylthiomethylbenzoyl chloride (**5**) and acetylmercapto-3-methyl butanoyl chloride (**6**) were synthesized according to the procedures reported earlier [2]. The tetrathiol derivatives **3e–3g** (Fig. 1) were then synthesized by acylation of the macrocycles **3d** with the corresponding acid chlorides. The acylating reactions at four sites simultaneously with the acid chlorides **4** and **5** were performed smoothly to afford **3e** and **3f** in the presence of NEt_3 as a base. When **6** is used as an acylating agent, no desired product was obtained with NEt_3, but compound **3g** can be obtained with an excellent yield with K_2CO_3 in CH_2Cl_2. The results are summarized in Table 1.

Deprotection (deacetylation) of **3e–3g** was readily achieved under basic conditions. However the produced thiol groups are susceptible to oxidation to form the disulfide, and manipulations under inert atmosphere are absolutely necessary. The removal of the acetyl groups from **3e–3g** is readily accomplished under mild acidic conditions with HCl/MeOH–$CHCl_3$, and the products **3a–3c** can be obtained without difficulty. These results are summarized in Table 2.

TABLE 1. Synthesis of 3e–3g

Compound	Substrates	Base	Yield (%)
3e	3d + 4 (1.2 eq.)	Et_3N	96
3f	3d + 5 (1.6 eq.)	Et_3N	80
3g	3d + 6 (1.5 eq.)	K_2CO_3	84

In the IR spectra the presence of SH and amide groups is shown around 2500 and 1640 cm^{-1} respectively (Table 3). The SH stretching bands in the cyclophane derivatives (**3a–3c**) are very weak. However, both NMR spectra and colorimetric measurements with DTNB [5,5′-dithiobis(2-nitrobenzoic acid)] [18] clearly exhibited the appropriate numbers of SH groups in these molecules. The 270 MHz ^1H NMR spectra of **3a–3c** in $CDCl_3$ show singlet signals due to SH groups in **3a** and **3c**, respectively, at 3.42 and 2.67 ppm, and the triplet one appears at 1.68 (J = 7.7 Hz) in **3b** with the appropriate integration. Moreover, the number of SH groups in the molecule was also determined by colorimetry using DTNB, the results showing very close agreement. These are summarized in Table 3.

Fe_4S_4 Clusters with Macrocyclic Tetrathiol Ligands (2a–2c)

The novel Fe_4S_4 active site analogues **2a–2c** were then prepared by a ligand substitution reaction [20] using the above macrocyclic tetrathiol compounds **3a–3c**, and $[Fe_4S_4(SBu^t)_4]^{2-}$ (**1c**) [21]. The reaction was performed in DMF, and all manipulations were carried out under pure nitrogen. Typical experimental procedures are as follows.

TABLE 2. Synthesis of 3a–3c by deacetylation

Compound	Acid/Solvent	Temperature (°C)	Time (h)	Yield (%)
3a	3 N HCl/MeOH–$CHCl_3$ (1:5)	50	2	98
3b	3 N HCl/MeOH–$CHCl_3$ (1:5)	50	4	86
3c	3 N HCl/MeOH–$CHCl_3$ (1:5)	50	3	90

TABLE 3. Determination of SH groups in 3a–3c by IR, NMR and colorimetry

Compound	IR (cm^{-1})	NMR (ppm)	Colorimetry no of SH
3a	2500 (w), 1620	3.42 (s, 4H)	4.02
3b	2550 (v.w.), 1640	1.68 (t, 4H, J = 7.7 Hz)	3.88
3c	2530 (v.w.), 1640	2.67 (s, 4H)	3.94

A slight excess amount (X1.02 eq.) of **3a–3c** in DMF is added into a solution of **1c**, and the mixture is kept at 40 °C for 30 min under reduced pressure with stirring to remove the liberated t-BuSH. The reaction could proceed according to the reported process [20]. A rapid color change (brown to reddish brown) is observed after the addition of **3a**, which suggests fast ligand exchange with the cyclic phenyl ligand to form the cyclic cluster **2a**. However, for the corresponding benzyl and alkyl thiol derivatives, no remarkable color change is observed. The product was then precipitated out by the addition of THF, washed subsequently with MeOH, CH_2Cl_2 and ether, and was purified by two reprecipitations from DMF–THF.

Thus, a series of new Fe_4S_4 clusters with macrocyclic tetrathiol ligands (**2a–2c**) were obtained in good yields (70–90%) as black powders with m.p.s > 300 °C. The clusters dissolve in DMF, DMSO and propylene carbonate, but are practically insoluble in most common organic solvents such as CH_3CN, AcOEt, CH_2Cl_2, ether, THF, EtOH and MeOH. Work to obtain single crystals suitable for X-ray analysis is in progress, since the possibility of polymer formation cannot be ruled out completely.

Acknowledgements

Work by K. Uoto was carried out at Hokkaido University, Faculty of Pharmaceutical Sciences. We thank Ms Yuuko Sasaki for technical assistance and Professor O. Yonemitsu for encouragement. The authors are indebted to the Analytical Center of Hokkaido University for elemental analyses and NMR measurements.

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