Triazacyclane-based Trithiols and Their Use in the Preparation of Site-differentiated Iron–Sulfur Clusters

David J. Evans,*[a] Gabriel Garcia,[b] G. Jeffery Leigh,*[a] Maurice S. Newton[a] and M. Dolores Santana[b]

[a] *AFRC Institute of Plant Science Research, Nitrogen Fixation Laboratory, University of Sussex, Brighton BN1 9RQ, UK*

[b] *Departamento de Química Inorgánica, Universidad de Murcia, 30071-Murcia, Spain*

The new tripodal thiol ligands 1,4,7-tris(4-mercaptobenzoyl)-1,4,7-triazacyclononane (H_3L^1) and 1,5,9-tris(4-mercaptobenzoyl)-1,5,9-triazacyclododecane (H_3L^2) have been synthesised and characterised. On reaction with iron–sulfur clusters $[Fe_4S_4(SR)_4]^{2-}$ (R = Et or Bu^t), subsite-differentiated clusters, for example $[Fe_4S_4(L^1)(SEt)]^{2-}$, are formed. Site-specific reaction at the differentiated iron is demonstrated. The novel clusters have been prepared both in solution and as isolated solids, and characterised mainly by ^{1}H NMR and Mössbauer spectroscopy.

It has long been recognised that the four metal sites in protein-bound $\{Fe_4S_4\}$ clusters may be inequivalent (site-differentiated) due to differences in the environments of the ligating groups (generally cysteine thiolato-groups) in the protein itself. A common structural motif in the protein backbone is -Cys-X-X-Cys-X-X-Cys- (Cys = cysteinyl residue, X = other amino acid residues).[1] This sequence normally binds three iron atoms of an $\{Fe_4S_4\}$ cluster, the fourth ligand generally being yet another cysteinyl residue somewhat removed from these three.

Our interest in site differentiation arose from two considerations: first, if a cluster is to mediate reactions of a substrate, presumably that substrate must be able to bind at one metal site, even if this means a substitution of ligands; secondly, in nitrogenase in particular, there are not enough cysteinyl residues in the proteins to bind all the metal vertices of all the metal clusters believed to be present. Consequently, we showed that very many amino acid residues bearing functional groups (*e.g.* tyrosine and aspartic acid) can bind to $\{Fe_4S_4\}$ clusters.[2,3] This supports the hypothesis that amino acid residues other than of cysteine are potential ligands from protein to cluster. There is some evidence of this in real systems.[4]

Unfortunately, model systems until recently have consisted of completely symmetrical molecules, not allowing easy study of site differentiation. The salient exception to this has been the work of the Holm group.[5] Based upon lengthy theoretical discussion, they designed and synthesised the ligand system H_3L' (Fig. 1).[6] This is supposed to prefer the conformation in the diagram which predisposes it to bind preferentially to three metal atoms of an $\{Fe_4S_4\}$ cluster. The trithiolate does indeed bind unequivocally to three metal sites in an $\{Fe_4S_4\}$ cluster and has allowed an impressive series of studies of site differentiation, confirmed in some cases by X-ray structural analysis.[6,7]

Unfortunately, the synthesis of the trithiol is multi-staged, of low overall yield, both in our hands and as reported,[6] and exceedingly time-consuming. We therefore decided to synthesise other more accessible trithiols, to fulful the same function as Holm's trithiol. This paper discusses our results with two such systems.

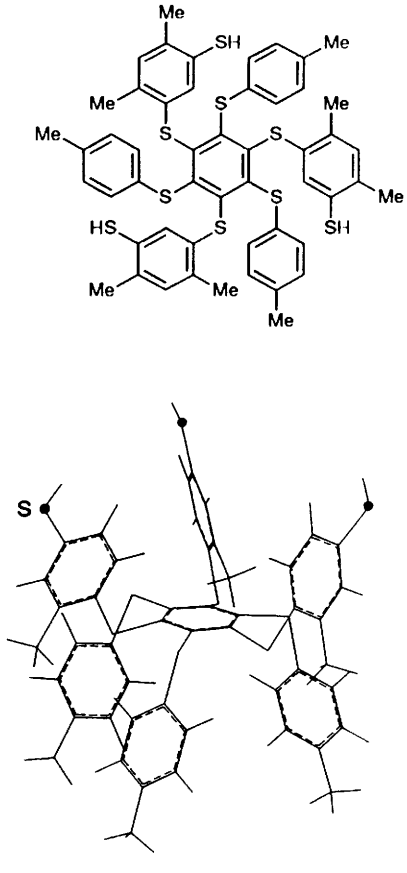
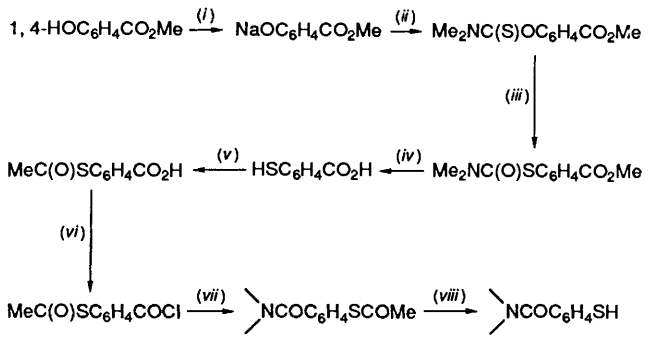


Fig. 1 Diagrammatic representations of the Holm ligand, H_3L'

Results and Discussion

Ligand Synthesis.—We based our ligand synthetic work on that of Okuno *et al.*,[8] who synthesised a series of tetrathiols designed to encapsulate $\{Fe_4S_4\}$ clusters. However, instead of using the 1,10,19,28-tetraazacyclohexatriacontane as the basal template as they did, we used 1,4,7-triazacyclononane and 1,5,9-triazacyclododecane. The sequence of reactions is represented in Scheme 1. Several of the reactions are carried out in the same vessel without isolating the intermediates so that the number of individual operations is not as large as it seems. The two products required, 1,4,7-tris(4-mercaptobenzoyl)-1,4,7-triazacyclononane (H_3L^1) and 1,5,9-tris(4-mercaptobenzoyl)-1,5,9-triazacyclododecane (H_3L^2), were obtained in overall yields of *ca.* 40%, and were characterised by elemental analysis, ^{1}H



Scheme 1 (*i*) NaH; (*ii*) $Me_2NC(S)Cl$; (*iii*) heat; (*iv*) NaOH; (*v*) $(MeCO)_2O$; (*vi*) $(COCl)_2$; (*vii*) triazane; (*viii*) HCl

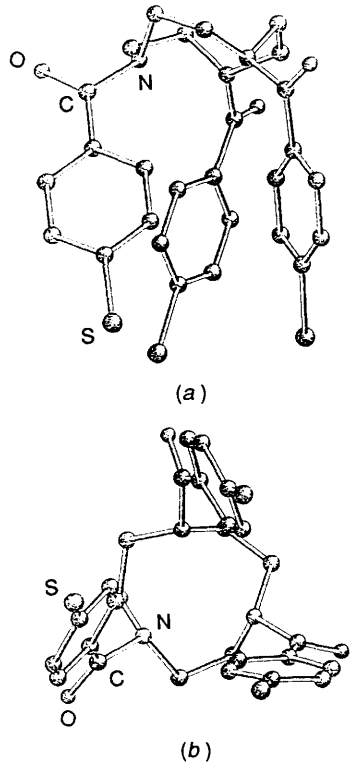


Fig. 2 Molecular model of the tridentate thiol H_3L^1, hydrogen atoms excluded: (*a*) side view; (*b*) from above

and ^{13}C NMR and IR spectroscopies (see Experimental section).

Computations.—We carried out molecular modelling studies using SYBYL software[9] to determine whether the derived trithiolates are feasible tridentate ligands towards an $\{Fe_4S_4\}$ cluster, this being rather more informative than using Carey–Pauling–Koltun models. The calculated energy minimum for the trithiol H_3L^1 has each of the arms of the trithiol aligned on the same side of the cyclononane ring (Fig. 2). There is rough three-fold symmetry about an axis through the centre of the cyclononane ring. This is no more than similar calculations on the Holm ligand show.[6,7] In fact, the ^1H NMR spectrum of H_3L^1 is consistent with this conformation in solution.

Our computations, which do not involve the metal atoms specifically, suggest that $(L^1)^{3-}$ in the presence of an $\{Fe_4S_4\}^{2+}$ cluster, produces a structure $[Fe_4S_4(L^1)Cl]^{2-}$ shown in Fig. 3, which represent an energy minimum. This has a pseudo-three-fold axis of symmetry along the Fe–Cl axis, and so $(L^1)^{3-}$ seems perfectly capable of accommodating the cluster in the required form. The system should be chiral, consistent with what has been shown for $[Fe_4Se_4(L')Cl]^{2-}$ by X-ray crystallography.[7] However, it is notable that such a cluster would not be

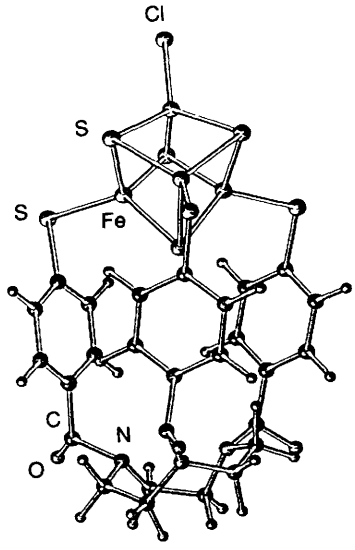


Fig. 3 The core structure of the site-differentiated cluster $[Fe_4S_4(L^1)Cl]^{2-}$, as predicted by computer molecular modelling

surrounded by the ligand $(L^1)^{3-}$, as observed in the Holm system. Rather the cluster is held at arms' length, situated on a platform with its further corner fully exposed. The distance from the cyclononane ring to the nearest sulfur atom of the cluster is *ca.* 6 Å, compared with 3–4 Å determined crystallographically for the corresponding separations from the basal benzene ring in the Holm clusters.[6,7] The ligand $(L^2)^{3-}$ seems to be very similar.

The next step is to prove that $(L^1)^{3-}$ and $(L^2)^{3-}$ can bind to the clusters, and in the required fashion, rather than randomly in three dimensions.

Cluster Complexes.—Complexes were prepared by reaction of H_3L^1 or H_3L^2 with $[Fe_4S_4(SR)_4]^{2-}$ (R = Et or Bu^t) in dimethylformamide (dmf), dimethyl sulfoxide (dmso), or acetonitrile under dynamic vacuum to remove the volatile RSH. Yields based on the product formulation $[Fe_4S_4(L^1)(SR)]^{2-}$ or $[Fe_4S_4(L^2)(SR)]^{2-}$ were 70–90%. These products were characterised by elemental analysis and by Mössbauer and ^1H NMR spectroscopies.

We were never able to obtain material suitable for X-ray crystallographic analysis. However, the materials are soluble in dmf, dmso, and, to a lesser extent, in MeCN. Their properties are fully consistent with the formation of isolated clusters in which $(L^1)^{3-}$ and $(L^2)^{3-}$ behave as tridentate ligands in the hoped-for manner. This cannot yet be proved unequivocally, but the evidence we adduce here is certainly no less convincing than that presented for similar systems in which tetradentate ligands are held to encapsulate clusters.[8,10,11] None of our data suggests that these systems are polymeric.

The new compounds $[Fe_4S_4L(SR)]^{2-}$ (L = L^1, R = Et **1** or Bu^t **2**; L = L^2, R = Et **3** or Bu^t **4**) react with an excess of PhSH to generate $[Fe_4S_4(SPh)_4]^{2-}$, as determined by ^1H NMR spectroscopy, showing that the $\{Fe_4S_4\}$ core has remained intact. Reaction of any of **1**, **2** or **4** with 2-aminobutane hydrochloride[12] in dmf yielded the chloro-derivatives $[Fe_4S_4(L)Cl]^{2-}$ (L = L^1 **5** or L^2 **6**). This hydrochloride proved to be a better reagent than the acid chlorides, such as pivaloyl chloride, used previously to effect this kind of conversion.[6]

The new complexes **1**–**6** should each contain a 'site-differentiated' iron atom. The alkanethiolate in **1** and **2** was allowed to react with 1 equivalent of the arenethiols thiophenol and 4-methylthiophenol, and of the corresponding phenolates, and the reactions monitored by ^1H NMR spectroscopy. This produced clusters $[Fe_4S_4(L^1)(XR)]^{2-}$ (X = S, R = Ph **7** or C_6H_4Me-4 **8**; X = O, R = Ph **9** or C_6H_4Me-4 **10**). The solids isolated from

Table 1 Proton NMR parameters (δ) for $[Fe_4S_4(L)X]^{2-}$ clusters in $(CD_3)_2SO$ at 293 K

	L	X	H(3)	H(2)	NCH_2	*m*-H	*o*-H	*p*-H	*p*-CH_3	Others[a]
1	L^1	SEt	8.60	6.30	3.90					13.6 SCH_2CH_3 2.38 SCH_2CH_3
2	L^1	SBu^t	8.20	5.85	3.90					2.7 SBu^t
5	L^1	Cl	8.23	6.00	3.80					
7	L^1	SPh	8.18	5.77	3.70	8.05	5.16	4.58		
8	L^1	SC_6H_4Me-4	8.07	5.64	3.58	8.22	5.42		3.94	
9	L^1	OPh	8.06	6.25	3.60	9.32	5.45	4.56		
10	L^1	OC_6H_4Me-4	8.19	6.02	3.60	9.13	5.10		4.80	
3	L^2	SEt	8.00	5.25	3.30, 1.80[b]					13.6 SCH_2CH_3[c]
4	L^2	SBu^t	8.60	5.46	3.35, 1.80[b]					2.7 SBu^t
6	L^2	Cl	8.30	5.50	3.25, 1.80[b]					

[a] Cation signals excluded. [b] CH_2 α and β to N, respectively. [c] SCH_2CH_3 concealed.

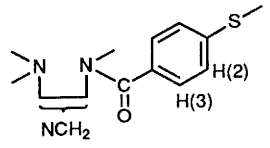


Fig. 4 Proton numbering for the tripodal thiolate L^1

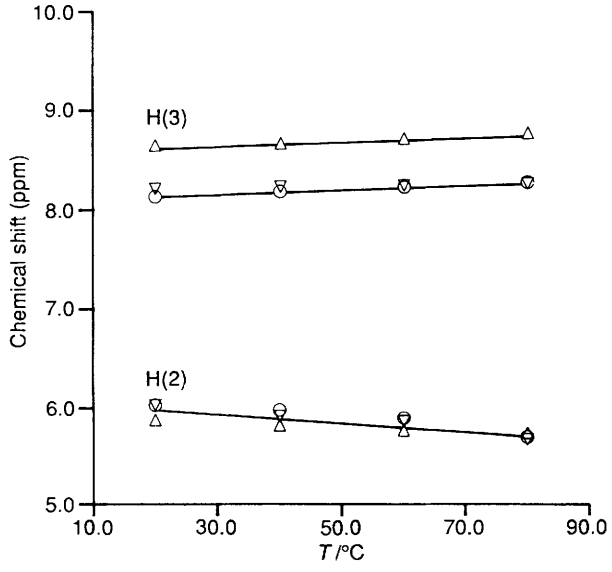


Fig. 5 Temperature dependence of ^1H NMR chemical shifts for the 'arm' protons of the site-differentiated clusters in $(CD_3)_2SO$ solutions: $[Fe_4S_4(L^1)(SEt)]^{2-}$ **1** (△), $[Fe_4S_4(L^1)(SBu^t)]^{2-}$ **2** (○) and $[Fe_4S_4(L^1)Cl]^{2-}$ **5** (▽)

these reactions have been characterised by IR and Mössbauer spectroscopies. Their solutions yielded ^1H NMR spectra identical with those observed upon mixing the reactants during the synthesis.

^1H *NMR Spectra.*—When the formation of clusters **1**–**4** in solution from a 1:1 mixture of H_3L^1 or H_3L^2 and $[Fe_4S_4(SR)_4]^{2-}$ was monitored by ^1H NMR spectroscopy the SH resonance of H_3L^1 or H_3L^2 was observed to disappear, as resonances of EtSH or Bu^tSH grew in intensity. The resonances assigned to the co-ordinated thiolate RS^- decreased in intensity, but never disappeared completely. However, on synthesising complexes **5**–**10** this thiolate resonance did disappear, to be replaced by other thiolate or phenolate resonances in the cases of **7**–**10**. The assigned resonances are collected in Table 1, which used the numbering scheme in Fig. 4, and where *o*, *m* and *p* refer to the unique phenolate or thiophenolate.

Isotropically shifted signals were assigned by comparison with reported data for $[Fe_4S_4(SR)_4]^{2-}$ and $[Fe_4S_4(OR)_4]^{2-}$ (R = aryl),[13,14] and from the temperature dependence of the

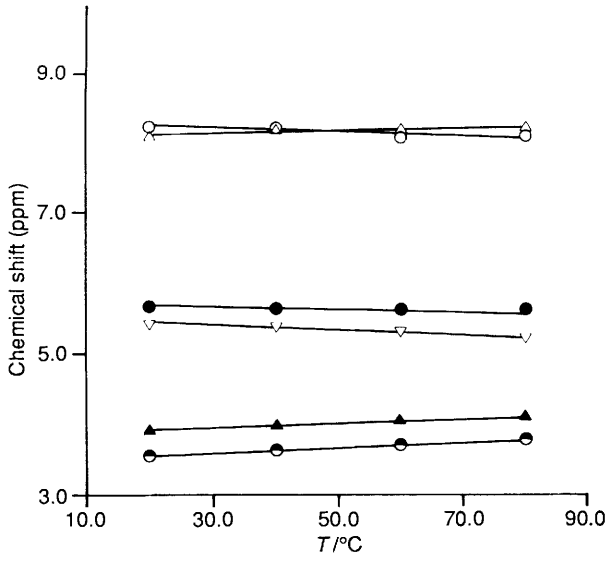


Fig. 6 Temperature dependence of ^1H NMR chemical shifts of $[Fe_4S_4(L^1)(SC_6H_4Me$-4$)]^{2-}$ **8** in $(CD_3)_2SO$ solution: (○) *m*-H, (△) H(3), (●) *o*-H, (▽) H(2), (▲) *p*-CH_3 and (◓) NCH_2

resonances. The resonances of L^1 and L^2 are consistent with trigonally symmetric conformations, and are generally less sensitive to changes in ligation at the unique site than those of the Holm ligand. The resonance assigned to H(2), closest to the cluster, exhibits the greatest isotropic shifts. The upfield and downfield shifts relative to the diamagnetic shifts of H(2) (≈ 1.5 ppm) and H(3) (≈ −1.0 ppm) are consistent with a dominant contact-shift mechanism, as found previously for ligands bound to $\{Fe_4S_4\}^{2+}$.[13] The magnitudes of these shifts are also similar to those observed for $[Fe_4S_4(SPh)_4]^{2-}$.[13] Thiolates bound to the unique site exhibit typical isotropically shifted signals: −11.39 (CH_2), −1.65 (CH_3) in **1**; −1.57 ppm (CCH_3) in **2**.

The temperature dependence of the shifts of $[Fe_4S_4(L^1)X]^{2-}$ (X = SEt **1**, SBu^t **2** or Cl **5** in Fig. 5) follows the established pattern, the shifts away from diamagnetic increasing with temperature.

The isotropic shifts of H(2) and H(3) in clusters **7**–**10** are relatively invariant irrespective of the substituent at the fourth iron site. However, the ring protons of the thiophenolate or phenolate exhibit shifts similar to those reported for the analogous site-differentiated clusters of Weigel and Holm,[15] with alternating signs around the ring. The phenolate shifts are greater than the thiophenolate shifts, this being most pronounced for *m*-H and *p*-CH_3 of complex **10** (Table 2). The larger shifts for phenolates as compared to thiophenolates have been ascribed to hyperfine interactions through a covalent Fe–O bond.[14]

The temperature dependence of these arene shifts is

Table 2 Comparison of selected ^1H isotropic shifts* (ppm) of arenethiolate- and phenolate-substituted clusters in $(CD_3)_2SO$

Cluster	*m*-H	*p*-CH_3	*T*/K	Ref.
8 $[Fe_4S_4(L^1)(SC_6H_4Me\text{-}4)]^{2-}$	−1.77	−1.91	293	This work
10 $[Fe_4S_4(L^1)(OC_6H_4Me\text{-}4)]^{2-}$	−2.66	−2.91	293	This work
$[Fe_4S_4(L')(SC_6H_4Me\text{-}4)]^{2-}$	−1.69	−1.87	297	15
$[Fe_4S_4(L')(OC_6H_4Me\text{-}4)]^{2-}$	−2.30	−2.68	297	15
$[Fe_4S_4(SC_6H_4Me\text{-}4)_4]^{2-}$	−0.89	−1.70	295	13
$[Fe_4S_4(OC_6H_4Me\text{-}4)_4]^{2-}$	−2.23	−2.77	295	14
$[Fe_4S_4(SPh)_4]^{2-}$	−0.98	—	295	13
$[Fe_4S_4(OPh)_4]^{2-}$	−2.23	—	295	14

* Isotropic shifts are relative to the diamagnetic ligand-proton resonances and are defined by $(\Delta H/H_o)_{iso} = (\Delta H/H_o)_{dia} - (\Delta H/H_o)_{obs}$.

Table 3 Mössbauer data for $Q_2[Fe_4S_4(L)X]$ and some $Q_2[Fe_4S_4X_4]$ at 77 K[a]

Q	L	X	i.s.[b]/ mm s^{-1}	q.s.[c]/ mm s^{-1}	Approximate relative intensity[c]
1 PPh_4	L^1	SEt	0.43	1.21	2
			0.43	0.76	1
2 NMe_4	L^1	SBu^t	0.42	1.04	—
5 NMe_4	L^1	Cl	0.44	0.85	3
			0.45	1.25	1
7 PPh_4	L^1	SPh	0.46	0.65	3
			0.45	1.18	1
8 PPh_4	L^1	$SC_6H_4Me\text{-}4$	0.44	0.81	2
			0.44	1.21	1
9 PPh_4	L^1	OPh	0.44	1.18	3
			0.43	0.76	1
10 PPh_4	L^1	$OC_6H_4Me\text{-}4$	0.44	0.85	3
			0.44	1.27	1
3 PPh_4	L^2	SEt	0.43	1.19	3
			0.42	0.80	1
4 PPh_4	L^2	SBu^t	0.43	0.90	3
			0.43	1.27	1
6 PPh_4	L^2	Cl	0.44	0.82	3
			0.44	1.26	1
PPh_4	L'	SPh[d]	0.46	1.04	3
			0.46	1.40	1
PPh_4		SPh[e]	0.43	0.93	—
PPh_4		SBu^t[f]	0.44	0.97	—
PPh_4		SEt	0.43	0.96	—
PPh_4		Cl[e]	0.49	0.67	—
NMe_4		SBu^t[g]	0.43	0.72	—
NMe_4		Cl[f]	0.50	0.98	—
NEt_4		OPh[h]	0.50	1.21	—

[a] Half-width at half-maxima are in the range 0.14–0.28 mm s^{-1}. [b] Referenced against iron foil at 298 K, errors less than ±1 standard deviation. [c] Errors less than ±2 standard deviations. [d] Ref. 18. [e] Ref. 19. [f] Ref. 17. [g] Ref 3. [h] Ref. 14.

anomalous (Fig. 6, which shows data from complex **8**). Protons H(2), H(3) and NCH_2 are 'normal', as are *o*-H and *p*-CH_3, but *m*-H in **8** (and in all of **7**, **9** and **10**) moves in the opposite sense. Consequently, although the room-temperature NMR spectrum is consistent with a dominant contact mechanism determining the shifts, this particular temperature dependence, in a range probably below the Néel temperature,[16] is not. We have shown previously that the *o*- and *m*-protons and α-CH and β-CH_2 protons in $[NMe_4]_2[Fe_4S_4\{OC_6H_4CH_2CH(CO_2Me)(NH_3)\}_4]Cl_4$ have resonances with a temperature dependence inconsistent with this theory.[3] Clearly, a more refined theory is required to explain the shifts and dependencies in detail.

Mössbauer Spectra.—Mössbauer spectra of $\{Fe_4S_4\}^{2+}$ clusters with a variety of ligands L, all of the same kind, show a simple quadrupole doublet with an isomer shift (i.s.) of 0.47 ± 0.03 mm s^{-1}, and a quadrupole splitting (q.s.) covering a much wider range, 0.70–1.50 mm s^{-1}. This variation in quadrupole splitting has been explained by changes in the electric field to which the equivalent iron atoms in the cluster are subjected by the solid structure.[17] For co-ordination as in our complexes with L^1 or L^2 one might hope to observe two quadrupole doublets, with relative intensities 3:1, reflecting the different ligating groups of the iron atoms. The data in Table 3 confirm this expectation. A representative spectrum is shown in Fig. 7.

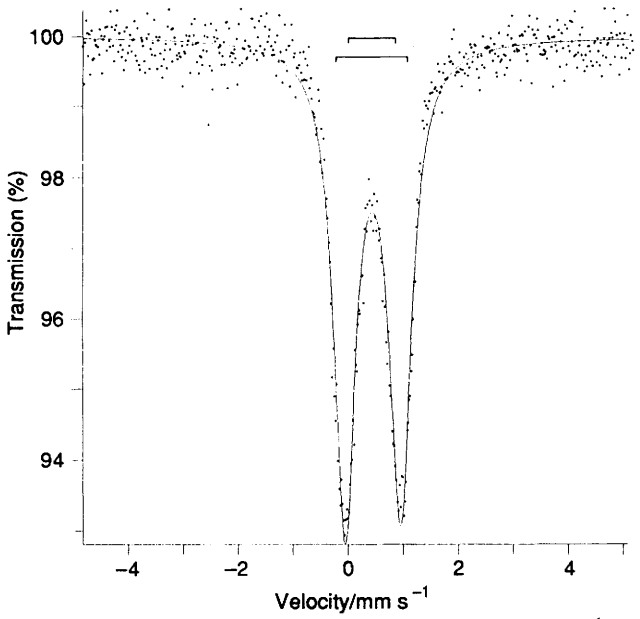


Fig. 7 The solid-state Mössbauer spectrum of $[NMe_4]_2[Fe_4S_4(L^1)Cl]$ at 77 K

In every case except $[NMe_4]_2[Fe_4S_4(L^1)(SBu^t)]$, the spectra can be resolved into two quadrupole doublets of roughly the correct relative intensities. The q.s. values in that single case seem to be accidentally the same, because in general the q.s. values cover as wide a range as those observed for symmetrically ligated clusters, and with as little obvious rationale. The quality of the data sometimes does not allow entirely satisfactory estimations of intensities, and it is just these cases which depart most from the 3:1 ratio. However, the i.s. and q.s. values are more reliable, as shown by the quoted standard deviations. Included in the Table are the available data from Holm and co-workers,[18] and some data for symmetrically co-ordinated species for comparison.

We feel that detailed analyses of these data is premature. What they show unequivocally is that, in the solid state, site differentiation occurs. Of course, this does not prove that the species are mononuclear rather than bridged, but no more would be expected. We are currently investigating the Mössbauer spectra of frozen solutions to refine further our conclusions.

Conclusion

We have shown that relatively accessible tripodal ligands are capable of binding to three apical iron atoms of an $\{Fe_4S_4\}^{2+}$ cube. The ligand need not be designed to take up a specific conformation, even if this might help. Tripodal binding seems to be preferred over random polymerisation. The products we have obtained possess normal NMR and Mössbauer properties, though some temperature dependencies of chemical shifts are inconsistent with current theory. Although our data are consistent with the presence of the hoped-for site-differentiated species, ultimate proof of this will require the preparation of suitable crystalline material. To date this has eluded us.

Experimental

General Methods and Physical Techniques.—All solvents were distilled from appropriate drying agents and degassed before use. Standard syringe and Schlenk techniques were employed when handling air-sensitive materials.

1,4,7-Triazacyclononane was prepared as published,[20] 1,5,9-triazacyclododecane trihydrobromide was purchased from Aldrich Chemical and converted into 1,5,9-triazacyclododecane. *S*-4-Methoxycarbonylphenyl dimethylthiocarbamate was prepared by an adaptation (see below) of an earlier method[21] and converted, as recently described,[8] into 4-(acetylthio)benzoyl chloride. Salts of the metal–sulfur clusters $[Fe_4S_4(SR)_4]^{2-}$ (R = Et or Bu^t) and $[Fe_4S_4Cl_4]^{2-}$ were prepared by standard procedures.[22,23] All other chemicals were purchased from Aldrich Chemical and used as supplied.

Infrared and Mössbauer spectra were recorded on Perkin Elmer 883 and ES-Technology MS105 spectrometers, respectively. Mössbauer parameters were determined at 77 K, using a 25 mCi ^{57}Co source in a rhodium matrix, and were referenced against iron foil at 298 K. The NMR spectra were recorded on a JEOL GSX270, Bruker AC 200E or Varian Unity 300 spectrometer; ^1H spectra were referenced against $SiMe_4$. Molecular modelling studies used SYBYL 5.4 software on an Evans and Sutherland workstation. Microanalyses were performed by Mr. C. J. Macdonald, Nitrogen Fixation Laboratory.

Syntheses.—S-4-*Methoxycarbonylphenyl dimethylthiocarbamate.* Small portions of a cooled solution of methyl 4-hydroxybenzoate (10 g, 66 mmol) in dmf (70 cm^3) were added to sodium hydride (1.57 g, 66 mmol). When dihydrogen evolution ceased the solution was cooled in an ice-bath and dimethylthiocarbamoyl chloride (10.56 g, 86 mmol) added in one portion. The cooling bath was removed and the mixture heated at 80 °C for 1 h. The mixture was cooled and poured into 1% aqueous potassium hydroxide solution (200 cm^3), the resultant yellow solution was saturated with sodium chloride, extracted twice (2 × 500 cm^3), with light petroleum (b.p. 60–70 °C)–benzene (4:1 v/v), the organic extracts filtered through anhydrous $MgSO_4$ and the solvent removed under reduced pressure to give crude *O*-4-methoxycarbonylphenyl dimethylthiocarbamate.

Recrystallisation from hot methanol gave white crystals (12 g, 80%), m.p. 100–102 °C (Found: C, 54.2; H, 5.3; N, 5.8. Calc. for $C_{11}H_{13}NO_3S$: C, 55.2; H, 5.5; N, 5.85%); ν_{max} 1900 (CN) and 1710 cm^{-1} (CO) (Nujol); $\delta_H(CDCl_3)$ 8.06 and 7.11 (4 H, m, aromatics), 3.99 (3 H, s, OCH_3), 3.43 (3 H, s, NCH_3) and 3.33 (3 H, s, NCH_3').

The *O*-aryl carbamate was heated at 220 °C for 80 min, and TLC and ^1H NMR spectroscopy showed complete conversion into the *S*-aryl carbamate, m.p. 90–92 °C; ν_{max} 1910 (CN) and 1712 cm^{-1} (CO) (Nujol); $\delta_H(CDCl_3)$ 8.0 and 7.55 (4 H, m, aromatics), 3.89 (3 H, s, OCH_3) and 3.02 [6 H, s, $N(CH_3)_2$].

1,4,7-*Tris*(4-*acetylthiobenzoyl*)-1,4,7-*triazacyclononane.* To a mixture of 1,4,7-triazacyclononane (0.5 g, 3.07 mmol) and triethylamine (1.65 g, 16 mmol) in dichloromethane (5 cm^3) was added, at 0 °C, 4-(acetylthio)benzoyl chloride (3.21 g, 14.97 mmol) in dichloromethane (10 cm^3). After warming the mixture was stirred at room temperature for 3 h. The yellow solution was washed three times, with each of saturated sodium hydrogencarbonate, 5% hydrochloric acid, and 10% sodium chloride solutions. After drying over anhydrous $MgSO_4$, chromatography (silica column; methanol–hexane–chloroform, 7:18:14) of the organic extract gave a white solid (1.5 g, 57%) (Found: C, 58.9; H, 5.1; N, 5.6. $C_{33}H_{37}N_3O_6S_3$ requires C, 59.7; H, 5.0; N, 6.3%); ν_{max} 1700 and 1615 cm^{-1} (CO) (Nujol); $\delta_H(CD_2Cl_2)$ 7.5–7.4 (18 H, m, aromatics), 3.75 (12 H, m, CH_2) and 2.43 (9 H, s, $COCH_3$).

1,5,9-*Tris*(4-*acetylthiobenzoyl*)-1,5,9-*triazacyclododecane.* This was prepared by a method similar to that above (83%) (Found: C, 61.9; H, 5.7; N, 5.1. $C_{36}H_{39}N_3O_6S_3$ requires C, 61.25; H, 5.6; N, 5.9%); ν_{max} 1700 and 1610 cm^{-1} (CO) (Nujol); $\delta_H(CD_2Cl_2)$ 7.47–7.35 (12 H, m, aromatics), 3.52 (12 H, br, $NCH_2CH_2CH_2N$), 2.44 (9 H, s, OCH_3) and 2.06 (6 H, br, $NCH_2CH_2CH_2N$).

1,4,7-*Tris*(4-*mercaptobenzoyl*)-1,4,7-*triazacyclononane* (H_3L^1). 1,4,7-Tris(4-acetylthiobenzoyl)-1,4,7-triazacyclononane (1.5 g, 2.26 mmol) was added to a solution of anhydrous HCl (220 mmol) in methanol (150 cm^3), and the mixture was heated to 80 °C and stirred under a dinitrogen atmosphere for 8 h. Solvent was removed *in vacuo*, the residue dissolved in chloroform (250 cm^3), washed with brine and dried over anhydrous $MgSO_4$. Evaporation of solvent gave a white solid which was recrystallised from chloroform–hexane (1.04 g, 85%), m.p. >230 °C (decomp.) (Found: C, 60.6; H, 5.1; N, 7.9. $C_{27}H_{27}N_3O_3S_3$ requires C, 60.3; H, 5.1; N, 7.8%); λ_{max}/nm (CH_2Cl_2) 260 (ε/dm^3 mol^{-1} cm^{-1} 26 450); ν_{max} 2538w (SH) and 1610s cm^{-1} (CO) (Nujol); $\delta_H(CDCl_3)$ 7.4–7.2 (12 H, m, aromatics), 3.70 (12 H, br, CH_2) and 3.54 (3 H, s, SH); $\delta_C(CDCl_3)$ 173.50 (CO), 129.61, 129.55, 128.18, 127.74 (aromatics) and 51.30 (CH_2). Colorimetric determination of SH: 3 per mol.

Crystals suitable for structure determination by X-ray crystallography were not obtained. However, on leaving the filtrate from the recrystallisation to stand at room temperature for 1 week, crystals of 1,4-bis(mercaptobenzoyl)-7-(toluene-*p*-sulfonyl)-1,4,7-triazacyclononane were obtained. Presumably this molecule was formed by the reaction of 1-(toluene-*p*-sulfonyl)-1,4,7-triazacyclononane, a very minor impurity in the 1,4,7-triazacyclononane, with 4-(acetylthio)benzoyl chloride. An X-ray crystallographic study of these crystals is reported elsewhere.[24]

1,5,9-*Tris*(4-*mercaptobenzoyl*)-1,5,9-*triazacyclododecane* (H_3L^2). This was prepared by a method similar to that described above (81%), m.p. >260 °C (decomp.) (Found: C, 61.8; H, 6.0; N, 6.9. $C_{30}H_{33}N_3O_3S_3$ requires C, 62.1; H, 5.7; N, 7.2%); λ_{max}/nm(CH_2Cl_2) 264 (ε/dm^3 mol^{-1} cm^{-1} 30 000); ν_{max} 2495w (SH) and 1610s cm^{-1} (CO) (Nujol); $\delta_H(CDCl_3)$ 7.4–7.2 (12 H, m, aromatics), 3.58 (3 H, s, SH), 3.53 (12 H, br, $NCH_2CH_2CH_2N$) and 2.21 (6 H, br, $NCH_2CH_2CH_2N$); $\delta_C(CDCl_3)$ 172.99 (CO), 129.65 and 128.06 (aromatics), 48.18, 44.55 ($NCH_2CH_2CH_2N$) and 28.48 ($NCH_2CH_2CH_2N$). Colorimetric determination of SH: 3 per mol.

Site-differentiated 4Fe-4S *clusters.* Clusters $[Fe_4S_4(L)(SR)]^{2-}$ (L = L^1 or L^2, R = Et or Bu^t) were prepared by one of any of the three methods described below in yields of 70–90%. The ^1H NMR and Mössbauer parameters are presented in Tables 1–3. Crystals suitable for X-ray structural characterisation could not be grown.

$[PPh_4]_2[Fe_4S_4(L^1)(SEt)]$. The salt $[PPh_4]_2[Fe_4S_4(SEt)_4]$ (0.56 g, 0.44 mmol) was dissolved in dmf (20 cm^3). A solution of H_3L^1 (0.24 g, 0.44 mmol) in dmf (20 cm^3) was added, and the mixture stirred at 60 °C for 2 h under dynamic vacuum. After cooling to 25 °C, tetrahydrofuran (thf) or diethyl ether was added until precipitation began, the mixture was stored overnight at −20 °C and the brown product filtered off, washed with diethyl ether and dried *in vacuo* (0.57 g, 80%) (Found: C, 53.2; H, 4.7; Fe, 15.0; N, 2.9. $C_{77}H_{69}Fe_4N_3O_3P_2S_8$ requires C, 56.9; H, 4.3; Fe, 13.7; N, 2.6%;* ν_{max} 1610 cm^{-1} (CO) (Nujol).

$[NMe_4]_2[Fe_4S_4(L^1)(SBu^t)]$. To a solution of $[NMe_4]_2[Fe_4S_4(SBu^t)_4]$ (0.2 g, 0.23 mmol) in dmso (4 cm^3) was added solid H_3L^1 (0.125 g, 0.23 mmol). The mixture was stirred at 60 °C for 2 h under dynamic vacuum. To the residue was added acetonitrile–diethyl ether (1:2 v/v, 40 cm^3). The brown precipitate was filtered off, washed with diethyl ether and dried *in vacuo* (0.18 g, 70%) (Found: C, 42.2; H, 5.2; N, 6.05. $C_{39}H_{57}Fe_4N_5O_3S_8$ requires C, 41.7; H, 5.1; N, 6.2%); ν_{max} 1610 cm^{-1} (CO) (Nujol).

$[PPh_4]_2[Fe_4S_4(L^2)(SEt)]$. To a slurry of H_3L^2 (0.34 g, 0.59 mmol) in MeCN (5 cm^3) was added a solution of $[PPh_4]_2$-

* The elemental analysis of this compound was poor; however, by all other criteria the formulation is as shown.

$[Fe_4S_4(SEt)_4]$ (0.75 g, 0.59 mmol) in MeCN (60 cm^3). After stirring for 1 h under vacuum a brown precipitate was collected, washed with diethyl ether and dried *in vacuo* (0.33 g, 75%) (Found: C, 56.2; H, 4.9; Fe, 14.1; N, 3.0. $C_{80}H_{75}Fe_4N_3O_3P_2S_8$ requires C, 57.6; H, 4.5; Fe, 13.4; N, 2.5%); ν_{max} 1610 cm^{-1} (CO) (Nujol).

$[NMe_4]_2[Fe_4S_4(L^1)Cl]$. Salts of $[Fe_4S_4(L)Cl]^{2-}$ (L = L^1 or L^2) were prepared by the general method described below. The salt $[NMe_4]_2[Fe_4S_4(L^1)(SBu^t)]$ (0.06 g, 0.053 mmol) and 2-aminobutane hydrochloride (0.006 g, 0.053 mmol) (prepared from the reaction of equimolar quantities of 2-aminobutane and trimethylsilyl chloride in dry methanol) were dissolved in dmf (7 cm^3) and stirred at room temperature for 1 h. Diethyl ether (20 cm^3) was added and the product filtered off, washed with diethyl ether and dried *in vacuo*.

Reaction at the site-differentiated iron. Clusters $[Fe_4S_4(L^1)(SR')]^{2-}$ and $[Fe_4S_4(L^1)(OR')]^{2-}$ (R' = Ph or C_6H_4-Me-4) were prepared both in $(CD_3)_2SO$ solution and as isolated solids. In both cases the product purity and identity was established by ^1H NMR spectroscopy. In addition, Mössbauer spectra of the isolated solids were recorded. Alkane thiolate substitution in solution was typically performed by adding 1.0 equivalent of HSR' or HOR' *via* syringe to a $(CD_3)_2SO$ solution of $[Fe_4S_4(L^1)(SR)]^{2-}$ (R = Et or Bu^t) (*ca.* 3 mmol). Solids were isolated (80–90% yield) by the following general procedure.

$[PPh_4]_2[Fe_4S_4(L^1)(OPh)]$. To $[PPh_4]_2[Fe_4S_4(L^1)(SEt)]$ (95.6 mg, 0.06 mmol) in dmso (2 cm^3) was added phenol (5.54 mg, 0.06 mmol) against a stream of dinitrogen. The mixture was stirred at 60 °C under dynamic vacuum for 2 h. After cooling to room temperature diethyl ether (20 cm^3) was added. The product precipitated as a black solid which was washed with diethyl ether and dichloromethane and then dried *in vacuo*. Yield 81%.

Colorimetric SH *Determination.*[25]—Known weights of H_3L^1 and H_3L^2 (*ca.* 1–2 mg) were each dissolved in dmso–water (1:5 v/v, 50 cm^3). A portion (3 cm^3) of each solution was mixed with Na_2HPO_4 buffer (2 cm^3, pH 8) and 5,5'-dithiobis(2-nitrobenzoic acid) (0.02 cm^3, 10 mmol in phosphate buffer) added. After 40 min at room temperature the absorbance at 412 nm was determined.

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References

1 J. M. Berg and R. H. Holm, in *Iron–Sulphur Proteins*, ed. T. G. Spiro, Wiley-Interscience, New York, 1982.
2 D. J. Evans and G. J. Leigh, *J. Chem. Soc., Chem. Commun.*, 1988, 395.
3 D. J. Evans and G. J. Leigh, *J. Inorg. Biochem.*, 1991, **42**, 25.
4 S. J. George, F. A. Armstrong, E. C. Hatchikian and A. J. Thomson, *Biochem J.*, 1898, **264**, 275; S. J. George, F. A. Armstrong, E. C. Hatchikian and M. G. Yates, *Pure Appl. Chem.*, 1990, **62**, 1071; R. C. Conover, A. T. Kowal, W. Fu, J.-B. Park, S. Aono, M. W. W. Adams and M. K. Johnson, *J. Biol. Chem.*, 1990, **265**, 8533; N. Okawara, M. Ogata, T. Yagi, S. Wakabayashi and H. Matsubara, *J. Biochem.*, 1988, **104**, 196; S. Wakabayashi, N. Fujimoto, K. Wada, H. Matsubara, L. Kerscher and D. Oesterhelt, *FEBS Lett.*, 1983, **162**, 21; Y. Minami, S. Wakabayashi, K. Wada, H. Matsubara, L. Kerscher and D. Oesterhelt, *J. Biochem.*, 1983, **97**, 745.
5 R. H. Holm, S. Ciurli and J. A. Weigel, *Prog. Inorg. Chem.*, 1990, **38**, 1.
6 T. D. P. Stack and R. H. Holm, *J. Am. Chem. Soc.*, 1988, **110**, 2484.
7 T. D. P. Stack, J. A. Weigel and R. H. Holm, *Inorg. Chem.*, 1990, **29**, 3745.
8 H.(Y). Okuno, K. Uoto, T. Tomohiro and M.-T. Youinou, *J. Chem. Soc., Dalton Trans.*, 1990, 3375.
9 SYBYL 5.4, Tripos Associates, St. Louis, MO, 1991.
10 K. Uoto, T. Tomohiro and H.(Y). Okuno, *Inorg. Chim. Acta*, 1990, **170**, 123.
11 C. F. Martens, H. L. Blonk, T. Bongers, J. G. M. van der Linden, G. Beurskens, P. T. Beurskens, J. M. M. Smits and R. J. M. Nolte, *J. Chem. Soc., Chem. Commun.*, 1991, 1623.
12 D. J. Evans, G. J. Leigh and J. B. Parra-Soto, *Polyhedron*, 1989, **8**, 1865.
13 R. H. Holm, W. D. Phillips, B. A. Averill, J. J. Mayerle and T. Herskovitz, *J. Am. Chem. Soc.*, 1974, **96**, 2109.
14 W. E. Cleland, D. A. Holtman, M. Sabat, J. A. Ibers, G. C. Defotis and B. A. Averill, *J. Am. Chem. Soc.*, 1983, **105**, 6021.
15 J. A. Weigel and R. H. Holm, *J. Am. Chem. Soc.*, 1991, **113**, 4184.
16 G. C. Papaefthymiou, E. J. Laskowski, S. Frota-Pessoa, R. B. Frankel and R. H. Holm, *J. Am. Chem. Soc.*, 1982, **21**, 1723.
17 D. J. Evans, A. Hills, D. L. Hughes, G. J. Leigh, A. Houlton and J. Silver, *J. Chem. Soc., Dalton Trans.*, 1990, 2735.
18 S. Ciurli, M. Carrié, J. A. Weigel, M. J. Carney, T. D. P. Stack, G. C. Papaefthymiou and R. H. Holm, *J. Am. Chem. Soc.*, 1990, **112**, 2654.
19 M. G. Kanatzidis, N. C. Baenziger, D. Coucouvanis, A. Simopoulos and A. Kostikas, *J. Am. Chem. Soc.*, 1984, **106**, 4500.
20 J. E. Richman and T. J. Atkins, *J. Am. Chem. Soc.*, 1974, **96**, 2268.
21 M. S. Newman and H. A. Karnes, *J. Org. Chem.*, 1966, **31**, 3980.
22 G. Christou and C. D. Garner, *J. Chem. Soc., Dalton Trans.*, 1979, 1093.
23 G. B. Wong, M. A. Bobrik and R. H. Holm, *Inorg. Chem.*, 1978, **17**, 578.
24 D. J. Evans, G. Garcia, D. L. Hughes, G. J. Leigh and M. D. Santana, *Acta Crystallogr., Sect. C*, in the press.
25 G. L. Ellman, *Arch. Biochem. Biophys.*, 1959, **82**, 70.

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