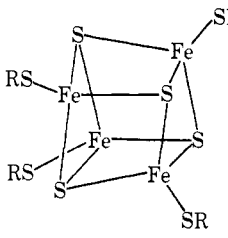
Synthetic Analogs of the Active Sites of Iron-Sulfur Proteins. IX.[1] Formation and Some Electronic and Reactivity Properties of Fe_4S_4 Glycyl-L-cysteinylglycyl Oligopeptide Complexes Obtained by Ligand Substitution Reactions

L. Que, Jr., J. R. Anglin, M. A. Bobrik, A. Davison, and R. H. Holm*

Contribution from the Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139. *Received May 16, 1974*

Abstract: The complex $[Fe_4S_4(S\text{-}t\text{-}Bu)_4]^{2-}$ in DMSO solution reacts readily with Ac-L-Cys-NHMe and the L-cysteinyl peptides *t*-BOC-Gly-Cys-Gly-Gly-Cys-Gly-Gly-Cys-Gly-NH_2 and *t*-BOC-Gly-Cys-Gly-Gly-Cys-Gly-Gly-Cys-Gly-Gly-Cys-Gly-NH_2 to afford the species $[Fe_4S_4(S\text{-}Cys(Ac)NHMe)_4]^{2-}$ (**2**), $[Fe_4S_4(9\text{-peptide})(S\text{-}t\text{-}Bu)]^{2-}$ (**3**), and $[Fe_4S_4(12\text{-peptide})]^{2-}$ (**5**) isolated as their tetraphenylarsonium salts. Reaction of **3** with Ac-L-Cys-NHMe affords the salt of $[Fe_4S_4(9\text{-peptide})(S\text{-}Cys(Ac)NHMe)]^{2-}$ (**4**). The pmr and electronic spectra and voltammetry of **2**–**5** were determined in DMSO or 80% DMSO–20% H_2O solutions. Contact shifted cysteinyl methylene proton resonances were observed at *ca.* 10–14 ppm downfield of TMS and provide further confirmation of signal assignments in oxidized ferredoxin (Fd_{ox}) and reduced high-potential (HP_{red}) proteins. Electronic spectra and 2−/3− redox potentials of **2**–**5** more closely resemble those of the proteins in aqueous solution than is the case for simple alkylthiolate tetramers such as $[Fe_4S_4(SEt)_4]^{2-}$. However, potentials for the synthetic species are estimated to be $\gtrsim$300 mV more negative than that usually found for Fd_{ox}/Fd_{red}. Significantly, spectra of **2**–**5** and that of the unfolded form of *Chromatium* HP_{red} in 80% DMSO are very similar and their redox potentials approach the upper limit estimated for the protein. These results indicate that denaturation of the protein affords the active site in a condition such that features inherent to isolated $[Fe_4S_4(S\text{-}Cys)_4]$ clusters, as exemplified by **2**–**5**, are developed.

Results reported in preceding parts of this series have demonstrated that the synthetic tetranuclear dianions, **1**, of D_{2d} stereochemistry serve as close repre-



1a, R = *t*-Bu
b, R = Ph

sentations of the $[Fe_4S_4(S\text{-}Cys)_4]$ active centers found in 4-Fe and 8-Fe bacterial proteins,[2] which include the "high-potential" (HP) and ferredoxin (Fd) types. Evidence has been presented which shows that $[Fe_4S_4(SR)_4]^{2-}$, HP_{red}, and Fd_{ox} possess a common total oxidation level[3-6] and that the structures of the R = CH_2-Ph[3,7] and Ph[8] tetramers are very similar to those of *Peptococcus aerogenes* Fd_{ox}[9,10] and *Chromatium* HP_{red}.[10]

In addition to an examination of electronic properties, current work is directed toward an elucidation of the reaction chemistry of the species **1**, which exhibits two types of reactivity. One type is oxidation–reduction, and voltammetric studies[5] have shown the existence of a four-membered electron transfer series $[Fe_4S_4(SR)_4]^z$ with z = 4−, 3−, 2−, and 1−. Electronic properties of the trianions[1] are closely related to those of Fd_{red} and the recently discovered "super-reduced" form of the *Chromatium* high-potential protein,[11] $HP_{s\text{-}red}$. The second type of reactivity, which is further elaborated in the present work, involves ligand substitution reactions of a tetramer dianion with thiols.[8,12] For monofunctional thiols the substitution process may be represented by eq 1. The following characteristics of this

$$[Fe_4S_4(S\text{-}t\text{-}Bu)_4]^{2-} + n\text{R}'\text{SH} \rightleftharpoons \underset{\textbf{1a}}{} [Fe_4S_4(S\text{-}t\text{-}Bu)_{4-n}(SR')_n]^{2-} + n\text{RSH} \quad (1)$$

reaction in acetonitrile solution at ambient temperature have been established:[8,12] (i) equilibrium is attained rapidly; (ii) rate constants for formation of n = 1 species with R′ = aryl are *ca.* $1\text{–}10^3$ M^{-1} sec^{-1},[13] (iii) substitution tendencies of thiols R′SH tend to parallel their aqueous acidities and lead to the equilibrium ligand replacement series R′ = alkyl < Ac-L-Cys-NHMe ∼ aryl; (iv) addition of 4.5–5 equiv of ArSH results in complete formation of $[Fe_4S_4(SAr)_4]^{2-}$ (*e.g.*, **1a** + PhSH → **1b**). The *tert*-butylthiolate dianion, **1a**, has been found to be equally or more prone to substitution reactions than other alkylthiolate tetramers. Its adequately soluble tetraphenylarsonium salt[8] is readily prepared by the direct tetramer synthesis[7] and serves as a convenient starting material for substitution reactions.

Previously it has been shown that $[Fe_4S_4(S\text{-}Cys(Ac)$-

(1) Part VIII. R. B. Frankel, T. Herskovitz, B. A. Averill, R. H. Holm, P. J. Krusic, and W. D. Phillips, *Biochem. Biophys. Res. Commun.*, **58**, 974 (1974).
(2) W. H. Orme-Johnson, *Annu. Rev. Biochem.*, **42**, 159 (1973).
(3) T. Herskovitz, B. A. Averill, R. H. Holm, J. A. Ibers, W. D. Phillips, and J. F. Weiher, *Proc. Nat. Acad. Sci. U. S.*, **69**, 2437 (1972).
(4) R. H. Holm, W. D. Phillips, B. A. Averill, J. J. Mayerle, and T. Herskovitz, *J. Amer. Chem. Soc.*, **96**, 2109 (1974).
(5) B. V. DePamphilis, B. A. Averill, T. Herskovitz, L. Que, Jr., and R. H. Holm, *J. Amer. Chem. Soc.*, **96**, 4159 (1974).
(6) R. B. Frankel, B. A. Averill, and R. H. Holm, results to be submitted for publication.
(7) B. A. Averill, T. Herskovitz, R. H. Holm, and J. A. Ibers, *J. Amer. Chem. Soc.*, **95**, 3523 (1973).
(8) L. Que, Jr., M. A. Bobrik, J. A. Ibers, and R. H. Holm, *J. Amer. Chem. Soc.*, **96**, 4168 (1974).
(9) E. T. Adman, L. C. Sieker, and L. H. Jensen, *J. Biol. Chem.*, **248**, 3987 (1973).
(10) C. W. Carter, Jr., J. Kraut, S. T. Freer, R. A. Alden, L. C. Sieker, E. Adman, and L. H. Jensen, *Proc. Nat. Acad. Sci. U. S.*, **69**, 3526 (1972).
(11) R. Cammack, *Biochem. Biophys. Res. Commun.*, **54**, 548 (1973).
(12) M. A. Bobrik, L. Que, Jr., and R. H. Holm, *J. Amer. Chem. Soc.*, **96**, 285 (1974).
(13) G. R. Dukes and R. H. Holm, results submitted for publication.

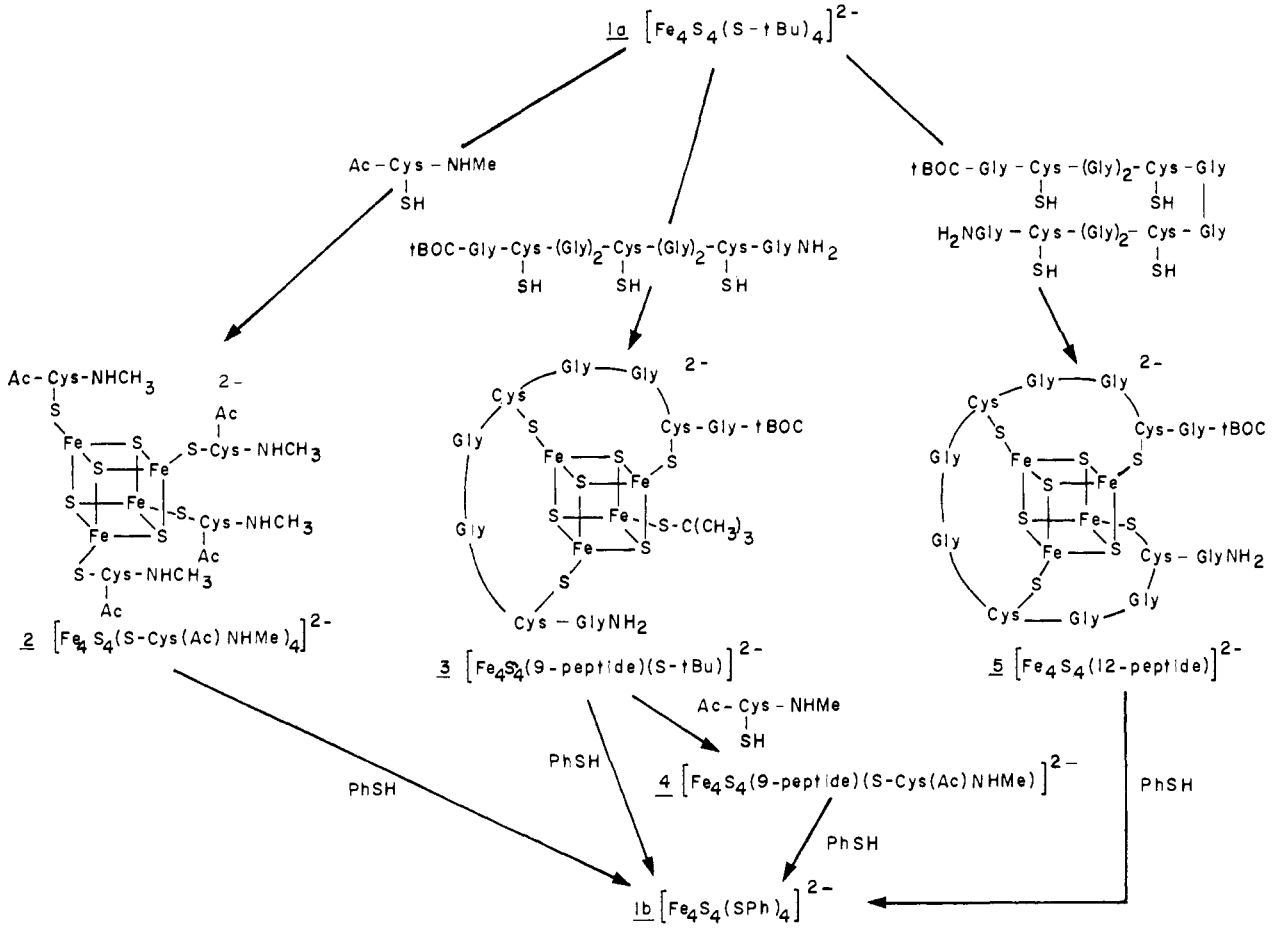


Figure 1. Ligand substitution reactions of $[Fe_4S_4(S\text{-}t\text{-}Bu)_4]^{2-}$ with *N*-acetyl-L-cysteine-*N*-methylamide and glycyl-L-cysteinylglycyl peptides, also illustrating conversion of peptide complexes to $[Fe_4S_4(SPh)_4]^{2-}$ upon reaction with benzenethiol. No absolute stereochemistry is implied for reaction products **3** and **5**.

$NHMe)_4]^{2-}$ (**2**, Figure 1) could be readily formed in solution from **1a** and the simple "monopeptide" *N*-acetyl-L-cysteine-*N*-methylamide.[5,12] The spectral and redox properties of the dianion suggest that it is the closest representation of Fd_{ox} and HP_{red} active sites among the various synthetic tetramers examined to date. However, an approximate comparison of its polarographically determined 2−/3− redox potential with E_0' values for Fd_{ox}/Fd_{red} indicates that the synthetic species reduces at substantially more negative values than the proteins.[5] In order to investigate the scope of ligand substitution reactions with polyfunctional thiols and to commence an assessment of the effects of peptide structure around the Fe_4S_4 core of **1** on redox and other properties, the reactions of **1a** with two N-protected *C*-amide peptides have been examined. The peptides employed are *t*-BOC-Gly-Cys-Gly-Gly-Cys-Gly-Gly-Cys-Gly-NH_2 and *t*-BOC-Gly-Cys-Gly-Gly-Cys-Gly-Gly-Cys-Gly-Gly-Cys-Gly-NH_2, hereafter referred to as 9-peptide and 12-peptide, respectively. These peptides represent a simple approach to portions of the native proteins inasmuch as they contain the -Cys-X-X-Cys- spacing found in one segment of two HP proteins,[2,14] and the -Cys-X-X-Cys-X-X-Cys- unit bound to one Fe_4S_4 cluster in *Peptococcus aerogenes* Fd and present in the eight 4-Fe and 8-Fe ferredoxins thus far sequenced.[2,15] They do not of course reproduce the amino acid sequence of the proteins. In related work short cysteinyl-containing segments of native rubredoxin[16] and clostridial ferredoxin[17,18] proteins have been synthesized. In one case[19] reaction of a dicysteinyl peptide with an Fe(III) salt and sulfide in aqueous solution yielded a species whose electronic spectrum was similar to that of 2-Fe Fd_{ox} proteins, but the species was not isolated or otherwise characterized.

Experimental Section

Preparation of Compounds. *N*-Acetyl-L-cysteine-*N*-methylamide[5] and $(Ph_4As)_2[Fe_4S_4(SR)_4]$ (R = *t*-Bu[7,8] and Ph[7,8]) were prepared by published methods. The peptides *tert*-butoxycarbonylglycyl-L-cysteinylglycylglycyl-L-cysteinylglycylglycyl-L-cysteinylglycylamide (*t*-BOC-Gly-L-Cys-Gly-Gly-L-Cys-Gly-Gly-L-Cys-Gly-NH_2, 9-peptide) and *tert*-butoxycarbonylglycyl-L-cysteinylglycylglycyl-L-cysteinylglycylglycyl-L-cysteinylglycylglycyl-L-cysteinylglycylamide (*t*-BOC-Gly-L-Cys-Gly-Gly-L-Cys-Gly-Gly-L-Cys-Gly-Gly-L-Cys-Gly-NH_2, 12-peptide) were prepared by block synthesis from the tripeptide *tert*-butyloxycarbonylglycyl-*S*-benzyl-L-cysteinylglycine (*t*-BOC-Gly-L-Cys(Bzl)-Gly-OH). Coupling of peptide blocks was effected through use of 3-acyloxy-2-hydroxy-*N*-

(14) S. M. Tedro, T. E. Meyer, and M. D. Kamen, *J. Biol. Chem.*, **249**, 1182 (1974).

(15) M. Tanaka, M. Haniu, K. T. Yasunobu, R. H. Himes, and J. M. Akagi, *J. Biol. Chem.*, **248**, 5215 (1973).

(16) A. Ali and B. Weinstein, *J. Org. Chem.*, **36**, 3022 (1971); A. Ali, F. Fahrenholz, J. C. Garing, and B. Weinstein, *J. Amer. Chem. Soc.*, **94**, 2556 (1972).

(17) H. Yajima, N. Shirai, and Y. Kiso, *Chem. Pharm. Bull.*, **19**, 1900 (1971).

(18) A. Schöberl, M. Rimpler, and U. Dethlefsen, *Justus Liebigs Ann. Chem.*, **1372**, 1612 (1973).

(19) Y. Sigiura and H. Tanaka, *Biochem. Biophys. Res. Commun.*, **46**, 335 (1972).

Table I. Electrochemical, Spectral, and Reactivity Properties of Fe_4S_4–Peptide and Related Tetramers in 80% DMSO–20% Water Solution

Complex	$E_{1/2}$, V[a] 2−/3−	λ_{max},[b] nm (ϵ_M)	Equiv of PhSH[f]
$[Fe_4S_4(S\text{-}Cys(Ac)NHMe)_4]^{2-}$ (**2**)	−0.91[c]	294 (22,700); 409 (16,600)	5.0
$[Fe_4S_4(9\text{-peptide})(S\text{-}t\text{-}Bu)]^{2-}$ (**3**)	−0.86	294 (23,400); 407 (17,300)	5.3
$[Fe_4S_4(9\text{-peptide})(S\text{-}Cys(Ac)NHMe)]^{2-}$ (**4**)	−0.82	291 (24,200); 404 (17,400)	5.5
$[Fe_4S_4(12\text{-peptide}]^{2-}$ [c] (**5**)	−0.80	290 (25,500); 406 (17,100)	*e*
$[Fe_4S_4(SEt)_4]^{2-}$	−1.16	298 (21,400); 414 (15,700)	4.6
$[Fe_4S_4(SPh)_4]^{2-}$ (**1b**)	−0.88[d]	458 (17,600)	

[a] *Vs.* sce. [b] Principal bands. [c] DMSO solution. [d] Aqueous component pH 9 (ref 5). [e] Not determined. [f] ±0.1, required for complete conversion to $[Fe_4S_4(SPh)_4]^{2-}$

ethylbenzamides[20] (–NEV esters) as the active esters. Accordingly, the nonapeptide was obtained by reaction of *t*-BOC-Gly-L-Cys-(Bzl)-Gly-NEV with H-Gly-L-Cys(Bzl)-Gly-Gly-L-Cys(Bzl)-Gly-NH_2 followed by S deprotection with sodium in liquid ammonia. Similarly, the dodecapeptide was prepared by the reaction of *t*-BOC-Gly-L-Cys(Bzl)-Gly-Gly-L-Cys(Bzl)-Gly-NEV with H-Gly-L-Cys(Bzl)-Gly-Gly-L-Cys(Bzl)-Gly-NH_2 followed by S deprotection with sodium in liquid ammonia. Synthetic details together with the reaction of the dodecapeptide with metal ions will be reported elsewhere.[21]

Fe_4S_4–Peptide Complexes. Tetraphenylarsonium salts of $[Fe_4S_4(S\text{-}Cys(Ac)NHMe)_4]^{2-}$, $[Fe_4S_4(9\text{-peptide})(S\text{-}t\text{-}Bu)]^{2-}$, and $[Fe_4S_4(12\text{-peptide})]^{2-}$ were prepared by adding a stoichiometric amount of peptide ligand to a solution of $(Ph_4As)_2[Fe_4S_4(S\text{-}t\text{-}Bu)_4]$ in DMSO under a nitrogen atmosphere. The *tert*-butylthiol formed was removed *in vacuo* thereby forcing the ligand substitution equilibria to complete formation of the peptide complexes. The DMSO solvent was also pumped off while maintaining the reaction solution near ambient temperature. The tetraphenylarsonium salt of $[Fe_4S_4(9\text{-peptide})(S\text{-}Cys(Ac)NHMe)]^{2-}$ was similarly prepared from Ac-L-Cys-NHMe and a DMSO solution of preformed $[Fe_4S_4(9\text{-peptide})(S\text{-}t\text{-}Bu)]^{2-}$. The red-brown solids which were obtained were not analyzed but their anionic components were identified by the spectral methods described in the text.

Physical Measurements. Because of the sensitivity of the tetranuclear dianions to oxygen, especially in solution, all manipulations and measurements were carried out under a pure nitrogen atmosphere. Proton magnetic resonance spectra at 100 MHz were measured at *ca.* 31° on a Varian XL-100 spectrometer and 270-MHz spectra were obtained using a Bruker WH-270 spectrometer. Chemical shifts were measured relative to TMS internal standard; shifts downfield of TMS are taken as negative. Electrochemical measurements were carried out with a Princeton Applied Research Model 170 electrochemistry system as described elsewhere.[5] Solutions were maintained at 25.0 ± 0.1°, and contained 0.05 *M* tetra-*n*-propylammonium perchlorate supporting electrolyte and *ca.* 10^{-3} *M* tetramer salt. Potentials were measured *vs.* an aqueous saturated calomel electrode (sce), and a dropping mercury electrode was employed as the working electrode. Reagent grade DMSO (Matheson Coleman and Bell) was stored over Linde Type 3A molecular sieves for several days before use. The aqueous component of 4:1 v/v DMSO–water solutions was unbuffered inasmuch as previous results[5] indicated that spectra and redox potentials of the tetramer dianions are not strongly pH dependent in the region pH 7–9.

As shown in Figure 1, the peptide tetramer dianions **2**–**5** can be converted to $[Fe_4S_4(SPh)_4]^{2-}$ (**1b**) upon reaction with benzenethiol. The equivalents of thiol required for full conversion of **2**, **3**, and **4** were determined by the spectrophotometric method described elsewhere.[8] Solutions of the peptide complexes in 80% DMSO–20% water (3.1–3.2 m*M*), prepared from the isolated tetraphenylarsonium salts, were titrated in duplicate runs with an ~0.3 *M* stock solution of benzenethiol in the same solvent mixture until the limiting spectrum of $[Fe_4S_4(SPh)_4]^{2-}$, determined separately (Table I), was obtained. Further addition of thiol produced no spectral changes in the 400–700-nm region where only the Fe–S chromophore has appreciable absorbance. Spectra were monitored using a Cary Model 14 instrument equipped with a 0.1-mm path length quartz titration cell. Concentrations of peptide complexes were calculated from the ϵ_{458} value of the benzenethiolate tetramer. These agreed to within 2% of the concentrations calculated from the weighed amounts of $(Ph_4As)_2[Fe_4S_4(S\text{-}t\text{-}Bu)_4]$ used in the preparation of peptide tetramer salts and were employed in determination of the intensity data given in Table I.

Results and Discussion

Previous examples of ligand substitution reactions of alkylthiolate tetramer dianions have involved reactions with monofunctional alkyl or aryl thiols,[5,8,12] usually in acetonitrile solution. Evidence has been presented that substitution of $[Fe_4S_4(S\text{-}t\text{-}Bu)_4]^{2-}$ (**1a**) with aryl thiols proceeds stepwise,[8] according to eq 1. The facile formation of $[Fe_4S_4(S\text{-}Cys(Ac)NHMe)_4]^{2-}$ (**2**) from **1a** and Ac-Cys-NHMe,[8,12] together with the lack of detectable degradation of the Fe_4S_4 core in this or any other ligand exchange process examined thus far, suggested that reactions of this type might be quite useful for the introduction of peptide structures around the core. This has proven to be the case, and the ligand substitution reactions of **1a** accomplished in this study are set out in Figure 1. The three complexes, **2**, $[Fe_4S_4(9\text{-peptide})(S\text{-}t\text{-}Bu)]^{2-}$ (**3**), and $[Fe_4S_4(12\text{-peptide})]^{2-}$ (**5**), were prepared by reaction of exactly stoichiometric amounts of **1a** and the corresponding ligands in DMSO solution followed by removal of liberated *tert*-butylthiol and solvent *in vacuo*. A fourth complex, $[Fe_4S_4(9\text{-peptide})(S\text{-}Cys(Ac)NHMe)]^{2-}$ (**4**) was obtained by the same procedure from **3** and Ac-Cys-NHMe. Complexes **2**–**5** were isolated as their tetraphenylarsonium salts, which were not subjected to elemental analysis but were characterized by spectroscopic methods. Certain spectral properties of **2**, measured in the presence of excess thiol after generation of the complex in solution by ligand substitution, have been reported.[4,5,12]

In the absence of well-characterized water soluble salts of the tetramer dianions, the solvent media selected for this work were DMSO and 80% DMSO–20% H_2O. Several proteins have been examined in these solvents, which have been found to have definite effects on electronic properties of the active sites. The pmr spectra of *C. pasteurianum* Fd_{ox}[22] indicate that the protein is denatured to some extent. A conformational change has been suggested by Cammack[11] to be responsible for the shift of the 388 nm band of HP_{red} in aqueous solution to 406 nm in 80% DMSO and for the reduction to $HP_{s\text{-}red}$ by dithionite in the latter solvent but not in water. The trianions $[Fe_4S_4(SR)_4]^{3-}$, which have the same total oxidation level as $HP_{s\text{-}red}$ and Fd_{red},[1] can be produced by reduction of the dianions in a variety of media,[1,5] including DMSO and 80% DMSO. Thus these solvents have been chosen in this initial attempt to examine the consequences of

(20) D. S. Kemp and S. W. Chien, *J. Amer. Chem. Soc.*, **89**, 2743 (1967).

(21) J. R. Anglin and A. Davison, results to be submitted for publication.

(22) C. C. McDonald, W. D. Phillips, W. Lovenberg, and R. H. Holm, *Ann. N. Y. Acad. Sci.*, **222**, 789 (1973).

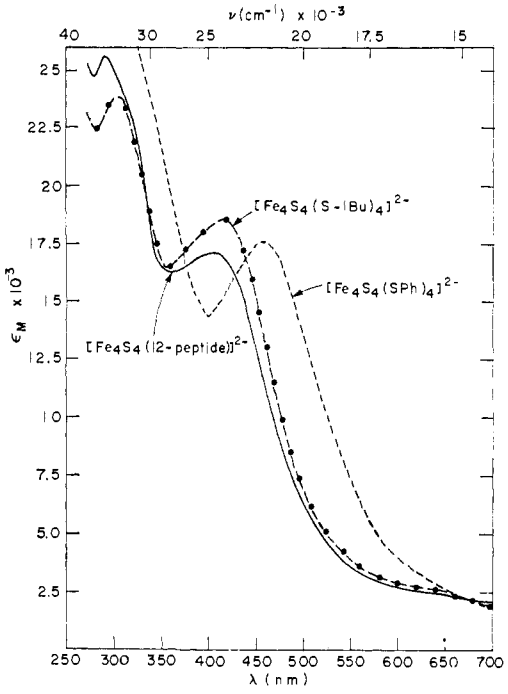


Figure 2. Electronic spectra in DMSO solution illustrating the ligand substitution reactions **1a** → **5** → **1b** (Figure 1): (-·-·-·) $(Ph_4As)_2[Fe_4S_4(S\text{-}t\text{-}Bu)_4]$ (**1a**), original complex; (——) $[Fe_4S_4(12\text{-peptide})]^{2-}$ (**5**), isolated from the reaction of **1a** with 1.0 equiv of dodecapeptide; (----), $[Fe_4S_4(SPh)_4]^{2-}$, obtained by treatment of 1.0 equiv of **5** (0.53 m*M*) with 14.6 equiv of PhSH (limiting spectrum).

peptide structure incorporation in synthetic tetranuclear dianions.

Electronic Spectra. The chromophoric properties of the previously well-characterized tetramers **1** with R = alkyl, are dominated by two principal absorptions in the 250–700 nm region. In DMF solution these occur at 297–308 nm (ϵ ~22,000–24,000) and 417–421 nm (ϵ ~16,000–18,000).[5] This spectral pattern is retained upon passing to DMSO or 80% DMSO solvent media with only small spectral changes, primarily involving blue shifts of the bands, evident. For **2** and the typical alkylthiolate tetramer, $[Fe_4S_4(SEt)_4]^{2-}$, the visible band maxima shift from 413 and 420 nm in DMF[5] to 409 and 414 nm, respectively, in 80% DMSO Spectral data are summarized in Table I, and the spectra of $[Fe_4S_4(12\text{-peptide})]^{2-}$ (λ_{max} 406 nm) and its precursor, $[Fe_4S_4(S\text{-}t\text{-}Bu)_4]^{2-}$ (λ_{max} 419 nm), in DMSO are depicted in Figure 2. The four peptide complexes show two main absorptions as well as unresolved bands at ~300–310 and 600–700 nm. All of these features are characteristic of the chromophore present in alkylthiolate tetramer dianions[5] and offer substantial support for the $[Fe_4S_4(S\text{-}Cys)_4]$ structures in Figure 1. As discussed previously,[5] a similar pair of intense bands is found in the aqueous solution spectra of HP_{red} and Fd_{ox} proteins. The lower energy charge-transfer absorption derives exclusively from the Fe–S chromophore with λ_{max} ~388 (HP_{red}) and 390–400 nm (Fd_{ox}). Extinction coefficients of the peptide complexes compare favorably with those of the proteins, *e.g.*, *B. polymyxa* Fd_{ox}[23] (16,000–17,000), *Chromatium* HP_{red}[24] (16,000), and *C. acidi-urici* Fd_{ox}[25] (15,300/active site). In addition, the absorption maximum of HP_{red} in 80% DMSO (406 nm[11]) falls within the 404–409 nm interval found for the peptide complexes in the same solvent. These results indicate that the spectral properties of the peptide-containing tetramers **2–5** in 80–100% DMSO are more closely related to those of Fd_{ox} and HP_{red} chromophores in aqueous and in aqueous or 80% DMSO solutions, respectively, than is the case for tetramer dianions substituted with simple alkyl groups.

Pmr Spectra. Proton resonance spectra of complexes **2–6** in DMSO-d_6 solutions are shown in Figures 3–6. Designation of protons of cysteinyl groups is indicated in Figure 3. Isotropic shifts calculated from the relation $(\Delta H/H_0)^{iso} = (\Delta H/H_0)^{obsd} - (\Delta H/H_0)^{dia}$ using free ligand resonance positions as the diamagnetic references are collected in Table II. These shifts were determined only for α-CH_2 and β-CH cysteinyl protons; those of other protons are substantially smaller.

Table II. Isotropic Proton Shifts of Fe_4S_4–Peptide Complexes in DMSO-d_6 Solution

Complex	$(\Delta H/H_0)^{iso}$, ppm: α-CH_2	β-CH
$[Fe_4S_4(S\text{-}Cys(Ac)NHMe)_4]^{2-}$ (**2**)[a,g]	−9.7, −10.8[h]	−1.07
$[Fe_4S_4(9\text{-peptide})(S\text{-}t\text{-}Bu)]^{2-}$ (**3**)[b]	−7.1, −8.3, −8.7, −9.8, −10.8	−2.05, −2.26, −1.41 (*t*-Bu)
$[Fe_4S_4(9\text{-peptide})(S\text{-}Cys(Ac)NHMe)]^{2-}$ (**4**)[a]	~−10.2[d]	−1.0, −2.2[c]
$[Fe_4S_4(12\text{-peptide})]^{2-}$ (**5**)[a]	~−10.2[d]	[e]
$[Fe_4S_4(SEt)_4]^{2-}$ [f]	−10.0	−1.10 (CH_3)

[a] At 100 MHz, 31°. [b] At 270 MHz, 25°. [c] Assignment to peptide β-CH uncertain. [d] Approximate center of broad resonance with half-width 225–250 Hz. [e] Not located. [f] At 22°, data from ref 4. [g] Note that the observed chemical shifts of this complex were incorrectly reported as positive instead of negative in Table II of ref 4. [h] These signals were not well resolved in previous 60-MHz spectra.[12]

An extensive pmr investigation[4] of the synthetic tetramers $[Fe_4S_4(SR)_4]^{2-}$ has led to the following two conclusions. (i) Chemical shifts of α-CH_2 protons of alkylthiolate tetramers occur in the same range as the extreme downfield resonances of Fd_{ox}[22,26–28] and HP_{red}[29] proteins (*ca.* 9–17 and 10–16 ppm, respectively, at ambient temperature in aqueous solution), thereby confirming assignment of the latter to α-CH_2 protons of the cysteinyl residues. (ii) Isotropic shifts are dominantly contact in origin and their temperature dependencies can be related to the inherent antiferromagnetism of the Fe_4S_4 core. The most important feature of the pmr spectra of **2–5** is the appearance of one or more broad signals at *ca.* 10–15 ppm downfield of TMS. These signals occur in the same region as those in (i) and are assigned to α-CH_2 protons. The

(23) N. A. Stombaugh, R. H. Burris, and W. H. Orme-Johnson, *J. Biol. Chem.*, 248, 7951 (1973).
(24) K. Dus, K. Sletten, H. DeKlerk, and R. G. Bartsch, *Biochim. Biophys. Acta*, 140, 291 (1967).
(25) J.-S. Hong and J. C. Rabinowitz, *J. Biol. Chem.*, 245, 4982 (1970).
(26) M. Poe, W. D. Phillips, C. C. McDonald, and W. Lovenberg, *Proc. Nat. Acad. Sci. U. S.*, 65, 797 (1970).
(27) M. Poe, W. D. Phillips, C. C. McDonald, and W. H. Orme-Johnson, *Biochem. Biophys. Res. Commun.*, 42, 705 (1971).
(28) W. D. Phillips, C. C. McDonald, N. A. Stombaugh, and W. H. Orme-Johnson, *Proc. Nat. Acad. Sci. U. S.*, 71, 140 (1974).
(29) W. D. Phillips, M. Poe, C. C. McDonald, and R. G. Bartsch, *Proc. Nat. Acad. Sci. U. S.*, 67, 682 (1970).

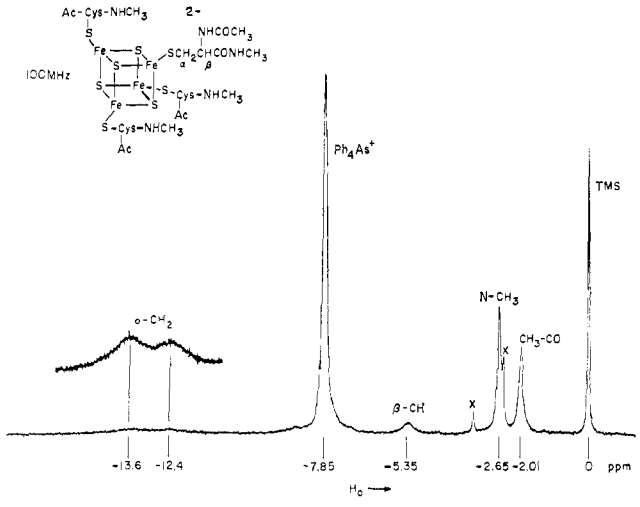


Figure 3. Pmr spectrum (100 MHz) of $(Ph_4As)_2[Fe_4S_4(S\text{-}Cys(Ac)NHMe)_4]$ (**2**) in DMSO-d_6 solution at 31°. Insert: methylene region recorded at higher gain. Proton-containing solvent impurities are indicated by ×.

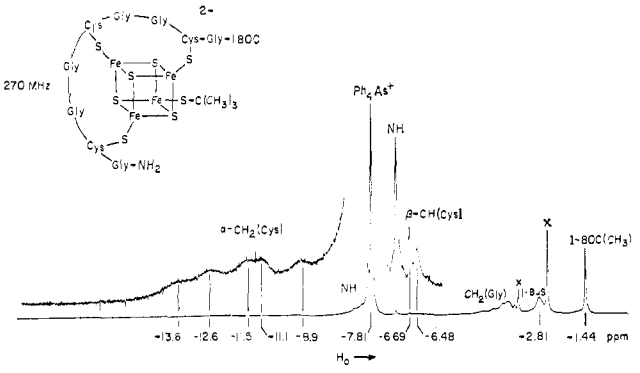


Figure 4. FT pmr spectrum (270 MHz) of $(Ph_4As)_2[Fe_4S_4(9\text{-peptide})(S\text{-}t\text{-}Bu)]$ (**3**) in DMSO-d_6 solution at 25°. Insert: methylene region recorded at higher gain. Proton-containing solvent impurities are indicated by ×.

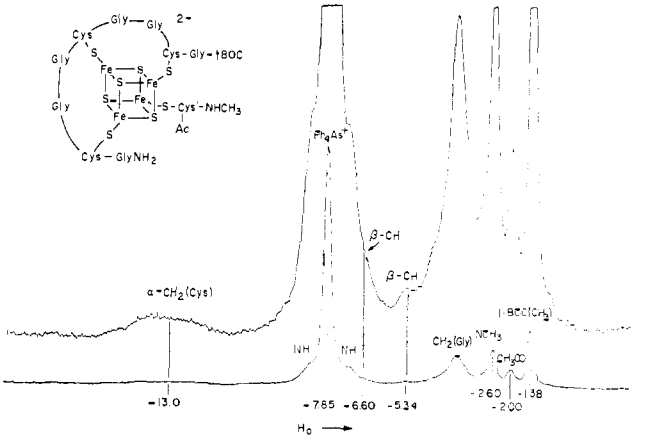


Figure 5. FT pmr spectrum (100 MHz) of $(Ph_4As)_2[Fe_4S_4(9\text{-peptide})(S\text{-}Cys(Ac)NHMe)]$ (**4**) in DMSO-d_6 solution at 31°. The upper spectrum was recorded at higher gain. Assignment of the shoulder at −6.6 ppm to β-CH of the 9-peptide ligand is tentative.

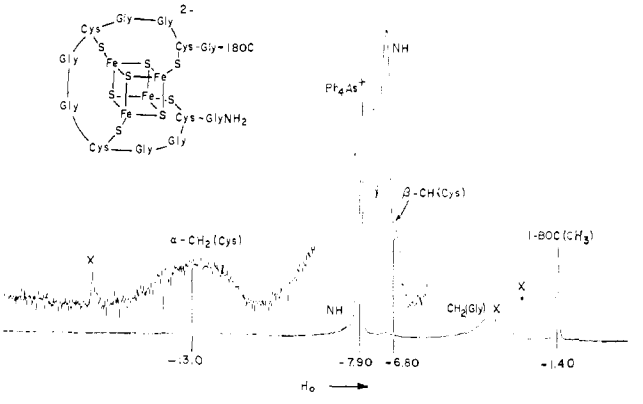
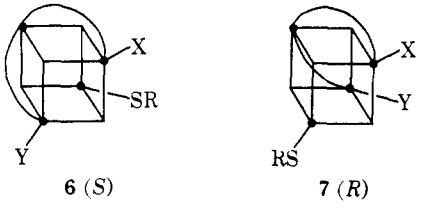


Figure 6. FT pmr spectrum (100 MHz) of $(Ph_4As)_2[Fe_4S_4(12\text{-peptide})]$ (**5**) in DMSO-d_6 solution at 31°. Insert: methylene region recorded at higher gain. Proton-containing solvent impurities are indicated by ×.

simplest case is that of $[Fe_4S_4(S\text{-}Cys(Ac)NHMe)_4]^{2-}$, for which two resonances are clearly resolved (Figure 3). These arise from the inherent inequivalence (diastereotopism) of the methylene protons effected by the adjacent chiral center. Methylene proton inequivalencies are increased in **3**, **4**, and **5** due to the chelating properties of the peptide ligands. For example, $[Fe_4S_4(9\text{-peptide})(S\text{-}t\text{-}Bu)]^{2-}$ in principle can adopt the absolute configurations **6** and **7** (in which X and Y



6 (*S*) **7** (*R*)

denote the different ends of the peptide chain), leading to the diastereomers *S*(LLL) and *R*(LLL) and 12 inequivalent methylene protons. Of this total five appear as partially resolved resonances in the 270-MHz spectrum of this complex (Figure 4). Similarly, 16 methylene signals are possible for $[Fe_4S_4(9\text{-peptide})(S\text{-}Cys(Ac)NHMe)]^{2-}$ and $[Fe_4S_4(12\text{-peptide})]^{2-}$ but their 100-MHz spectra (Figures 5 and 6) show only broad poorly resolved features centered at *ca.* −13 ppm. Relatively poor resolution has been observed in the spectra of *C. pasteurianum* Fd_{ox} in solutions containing more than 60% DMSO.[22] Here the protein is in a partially or completely unfolded state whose presumed flexibility should diminish chemical shift differences afforded by angular dependence of $\alpha\text{-}CH_2$ nuclear–electron coupling constants.[26] The higher viscosity of these media compared to pure water should also contribute to increased line widths. In the aqueous solution spectra of *C. pasteurianum*[22,26] and *C. acidi-urici*[27] Fd_{ox} at least 12 of the 16 $\alpha\text{-}CH_2$ signals of the two inequivalent Fe_4S_4 clusters are resolved at 220 MHz.

In the spectra of **2**–**5**, signals of the β-CH cysteinyl protons could be assigned with certainty in most cases. The relative isotropic shifts $\alpha\text{-}CH_2$:β-CH cover the range 1.00:(0.10–0.38), similar to the relative shifts of *n*-alkyl tetramers and consistent with the attenuation of shifts due to contact interactions.[4] From these results it appears certain that none of the β-CH resonances in Fd_{ox} and HP_{red} proteins would be shifted into the downfield region containing the signals assigned by Phillips, *et al.*,[22,26–29] to $\alpha\text{-}CH_2$.

The foregoing results establish that the Fe_4S_4 core is preserved in the ligand substitution reactions and other-

wise provide support for structures **2–5**[30] in Figure 1. Structure **4** is of some interest because of the presence of a peptide spacing and a monofunctional cysteinyl group somewhat similar to, *e.g.*, the -Cys(8)-X-X-Cys(11)-X-X-Cys(14) run and the "remote" Cys(45), respectively, associated with one cluster in *P. aerogenes* Fd.[9,10]

Reactivity with Benzenethiol. It has recently been shown that the reaction of alkylthiolate tetramers with a small excess of aryl thiol affords the corresponding arylthiolate tetramers.[5,8,12] These reactions extend to the peptide complexes **2–5**, as indicated in Figure 1. The series of spectral changes accompanying the reaction sequence $[Fe_4S_4(S\text{-}t\text{-}Bu)_4]^{2-} \rightarrow [Fe_4S_4(12\text{-peptide})]^{2-} \rightarrow [Fe_4S_4(SPh)_4]^{2-}$ is shown in Figure 2. The latter complex, whose structure has been fully characterized,[8] and other arylthiolate tetramers are readily distinguished from their alkyl counterparts by the pronounced red shift of the intense band in the visible region and the absence of a definite maximum near 300 nm. The number of equivalents of benzenethiol required for complete conversion of **2**, **3**, and **4** to $[Fe_4S_4(SPh)_4]^{2-}$ in 3 m*M* 80% DMSO solutions is given in Table II. These values are somewhat larger than those for the similar conversion of $[Fe_4S_4(SEt)_4]^{2-}$ in the same solvent and $[Fe_4S_4(S\text{-}t\text{-}Bu)_4]^{2-}$ in acetonitrile (4.5 equiv).[8] The larger number of equivalents indicate an enhanced stability of the cysteinyl-substituted tetramers. The successful conversion of **2–5** to the readily identifiable product $[Fe_4S_4(SPh)_4]^{2-}$ represents a clear precedent for removal and identification of 4-Fe clusters in proteins by treatment with benzenethiol. Several such cluster extrusion reactions have recently been achieved with proteins.[31] The binuclear complex $[FeS(SCH_2)_2C_6H_4]_2^{2-}$, a well-defined analog of the active sites of oxidized 2-Fe proteins,[32] undergoes similar reactions affording the dimers $[Fe_2S_2(SAr)_4]^{2-}$.[33] Hence these extrusion reactions offer the possibility of intact removal and subsequent identification of active site clusters in complex Fe–S proteins and enzymes.

Redox Properties. Voltammetric examination of a large series of $[Fe_4S_4(SR)_4]^{2-}$ species has shown that all possess a reversible or nearly reversible 2−/3− redox process in DMF,[5] equivalent in terms of total oxidation state changes to Fd_{ox}/Fd_{red} and $HP_{red}/HP_{s\text{-}red}$ electron transfer reactions of the proteins. Half-wave potentials of alkylthiolate tetramers are linearly related to Taft σ^* substituent parameters, with the least cathodic value found for $[Fe_4S_4(SCH_2Ph)_4]^{2-/3-}$ (−1.25 V *vs.* sce). When approximate allowance is made for experimental liquid junction potential and $E_{1/2}$ is referenced to the she, the resultant value is at least 500 mV more negative than aqueous E_0' values for Fd_{ox}/Fd_{red}, nearly all of which are near −0.40 V.[2] Introduction of cysteinyl ligands, as with $[Fe_4S_4(S\text{-}Cys(Ac)NHMe)_4]^{2-}$ generated in solution, produces less cathodic $E_{1/2}$ values in DMF (−1.07 V) and 80% DMSO (−0.91 V).[5]

The effect of cysteinyl ligands on half-wave potentials was further examined in this work by polarography of $[Fe_4S_4(SEt)_4]^{2-}$ and the isolated complexes **2–5** in DMSO or 80% DMSO. All complexes undergo a one-electron reduction with slopes of current voltage curves in the −65 to −85 mV range. The $E_{1/2}$ values of **2–5** are clearly increased relative to the ethanethiolate and other alkylthiolate tetramers, and the 40 mV difference between **3** and **4** is consistent with this behavior. Using the highest and lowest values for the peptide complexes and allowing for a +200 mV liquid junction potential, a maximum estimate,[5] apparent E_0 values of −0.86 and −0.75 V are obtained after correction to the she. These differ by −0.46 and −0.35 V from the usual −0.40 V for Fd_{ox}/Fd_{red}[34] determined by potentiometry. An accurate potential is not available for $HP_{red}/HP_{s\text{-}red}$ but Cammack[11] has estimated that the mid-point potential $E_m \leq -0.64$ V in 80% DMSO. Due care must be exercised in comparisons of potentials measured in different solvents by different methods, a problem compounded by uncertainties in liquid junction potentials.[5] However, the data are interpreted to indicate that potentials for the 2−/3− process of the peptide complexes are more negative than the usual value for Fd_{ox}/Fd_{red} by 300 mV or more. This point is supported by polarographically determined potentials of *C. pasteurianum* Fd[37] and *Veillonella alcalescens* Fd[38] in aqueous solutions, which in terms of E_0' are −0.32 V and −0.41 V, respectively.

Summary

The following principal conclusions have been reached from the results of this investigation. (i) Ligand exchange reactions of $[Fe_4S_4(S\text{-}t\text{-}Bu)_4]^{2-}$ with cysteinyl peptides provide a simple and essentially quantitative route to incorporation of peptide structure around the Fe_4S_4 core. (ii) Contact-shifted α-CH_2 cysteinyl resonances of **2–5** fall within the 9–17 ppm downfield region in which similar assignments have been made for Fd_{ox} and HP_{red}, and offer further confirmation of the protein spectral assignments in aqueous and DMSO–H_2O solutions. (iii) Relative to the simpler alkylthiolate tetramers, the peptide complexes **2–5** in DMSO or 80% DMSO more closely resemble Fd_{ox} and HP_{red} proteins in absorption spectral maxima and redox potentials; (iv) the Fe_4S_4 core may be recovered from **2–5** in the form of the easily identified and well-characterized complex $[Fe_4S_4(SPh)_4]^{2-}$ by reaction with five or more equivalents of benzenethiol. Concerning (iii) it is emphasized that the spectral and redox properties of **2–5** are more nearly similar to those of HP_{red} in 80% DMSO than to the spectrum of this protein and the spectra and potentials of Fd_{ox} in aqueous solution. From this observation it appears that the environmental effects of, and whatever structural constraints are imposed by, the normal protein conformation on the active sites in aqueous solution are substantially re-

(30) However, in the absence of X-ray structural information the available data do not definitively rule out more complicated (and, in our opinion, less likely) octameric or polymeric structures. These are readily visualized by allowing the 9-peptide or 12-peptide to link tetrameric units by functioning other than as strictly intracluster ligands.

(31) J. R. Bale and W. H. Orme-Johnson, results to be submitted for publication.

(32) J. J. Mayerle, R. B. Frankel, R. H. Holm, J. A. Ibers, W. D. Phillips, and J. F. Weiher, *Proc. Nat. Acad. Sci. U. S.*, **70**, 2429 (1973).

(33) J. J. Mayerle, S. E. Denmark, B. V. DePamphilis, J. A. Ibers, and R. H. Holm, submitted for publication to *J. Amer. Chem. Soc.*

(34) Extreme E_0' values for this process are −0.33 V (*Desulfovibrio gigas* Fd[35]) and −0.49 V (*Chromatium* Fd[36]).

(35) J. A. Zubieta, R. Mason, and J. R. Postgate, *Biochem. J.*, **133**, 851 (1973).

(36) B. Bachofen and D. I. Arnon, *Biochim. Biophys. Acta*, **120**, 259 (1966).

(37) P. D. J. Weitzman, I. R. Kennedy, and R. A. Caldwell, *FEBS (Fed. Eur. Biochem. Soc.) Lett.*, **17**, 241 (1971).

(38) H. Dalton and J. A. Zubieta, *Biochim. Biophys. Acta*, **322**, 133 (1973).

moved in the unfolded form of the protein. In this form, at least for *Chromatium* HP_{red}, features inherent to clusters of the essential $[Fe_4S_4(S\text{-}Cys)_4]$ type exemplified by **2–5** begin to emerge. Spectral and redox properties of denatured Fd_{ox} proteins have not yet been reported. Future experiments will attempt to determine the effect of protein structure on active site properties. These are planned to include voltammetric and spectral studies of synthetic analogs derived from other cysteinyl peptides and of proteins under normal aqueous and denaturing conditions.

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Stereochemistry of Polynuclear Compounds of the Main Group Elements. The Bonding and the Effect of Metal–Hydrogen–Carbon Interactions in the Molecular Structure of Cyclohexyllithium, a Hexameric Organolithium Compound[1]

Richard Zerger, Wendell Rhine, and Galen Stucky*

Contribution from the School of Chemical Sciences and the Materials Research Laboratory, University of Illinois, Urbana, Illinois 61801.
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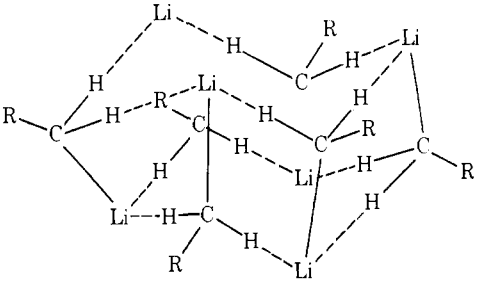
Abstract: A cyclohexyllithium benzene adduct, $[C_6H_{11}Li]_6(C_6H_6)_2$, has been isolated and characterized by infrared and single-crystal X-ray studies. As in other alkyllithium compounds, the C–H stretching frequency associated with the α-carbon atom is shifted to a lower frequency (2800 *vs.* 2900 cm^{-1} for cyclohexane). $[C_6H_{11}Li]_6(C_6H_6)_2$ crystals possess monoclinic symmetry, space group $P2_1/n$ with $a = 10.450$ (3) Å, $b = 10.443$ (4) Å, $c = 20.587$ (6) Å and $\beta = 92.40$ (1)°. The calculated density for 12 monomeric cyclohexyllithium groups and four benzene molecules per unit cell is 1.031 g/cm³. A total of 2271 unique reflections were utilized to refine atomic parameters, including hydrogen atoms, to $R_F = 0.064$ and $R_{wF} = 0.048$. Cyclohexyllithium is hexameric with a crystallographic center of inversion. The molecular symmetry closely approximates S_6. The geometry of the lithium atoms is very similar to that observed for the copper atoms in $H_6Cu_6(PPh_3)_6$ and for the ruthenium atoms in $H_2Ru_6(CO)_{18}$, with the metal atoms in a near octahedral configuration. There are six triangular lithium atom faces which have two short (2.397 (6) Å) and one long (2.968 (9) Å) distance. The former lithium–lithium atom distances are the shortest known lithium–lithium atom contact. The α-carbon atom of each cyclohexyl group is most closely associated (2.184 (3) Å) with the two lithium atoms which possess the longest lithium–lithium atom distance. A vector from the α-carbon atom to the plane of the isosceles triangle intersects the plane at the midpoint of this shortest lithium–lithium atom bond. The orientation of the cyclohexyl group is apparently determined by the interaction of α and β protons with the lithium atoms. The lithium–carbon atom distances are 2.184 (3) and 2.300 (4) Å. The lithium–hydrogen atom distances are 2.00 (5), 2.09 (5), and 2.33 (5) Å. The two benzene molecules are situated above the two equilateral triangle lithium atom faces which are transoidal. The Li–Li atom distance in these faces is 2.968 (9) Å. The bonding of organolithium hexamers is discussed in terms of a localized four-centered bond which involves a triangle of lithium atoms and the bridging carbon atoms of an alkyl ligand.

In all alkyllithium compounds which have been studied to date, the α-carbon C–H stretching modes have been shifted to a lower frequency relative to that of the parent hydrocarbon.[2] One possible explanation for this effect is the formation of three-center bonds of the form C···H···Li. The same model has been used as an explanation for the absence of ^{13}C–^{7}Li scalar coupling in hexameric *n*-butyllithium by Craubner,[3] as shown in the figure below. The results of neutron and X-ray diffraction studies of $LiB(CH_3)_4$[4] also suggest that lithium–hydrogen interactions may be important in the chemistry and stabilization of organolithium compounds. Li···H···C bonding may also be important in the structure of tetralithium octamethyldichromium(II) tetrahydrofuranate.[5] In contrast, the published structural data for organoaluminum and organolithium compounds[6,7] and the fact that a dimin-



(1) This research supported in part by the National Science Foundation under Grants GH-33634 and GP-31016.
(2) R. West and W. Glaze, *J. Amer. Chem. Soc.*, **83**, 3580 (1961).
(3) I. Craubner, *Z. Phys. Chem.*, **51**, 225 (1966).
(4) W. Rhine and G. D. Stucky, unpublished observations.
(5) J. Krausse, G. Marx, and G. Schödl, *J. Organometal. Chem.*, **21**, 159 (1970).
(6) (a) V. R. Magnuson and G. D. Stucky, *J. Amer. Chem. Soc.*, **91**, 2544 (1969); (b) J. C. Huffman and W. E. Streib, *Chem. Commun.*, 911 (1971).
(7) R. P. Zerger and G. D. Stucky, *J. Chem. Soc., Chem. Commun.*, 44 (1973).