Reactivity of $[Fe_4S_4(SR)_4]^{2-,3-}$ Clusters with Sulfonium Cations: Analogue Reaction Systems for the Initial Step in Biotin Synthase Catalysis

Christopher J. A. Daley and R. H. Holm*

Department of Chemistry and Chemical Biology, Harvard University, Cambridge, Massachusetts 02138

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The first step in catalysis by a class of iron−sulfur enzymes that includes biotin synthase is the one-electron reductive cleavage of the obligatory cofactor *S*-adenosylmethionine by an $[Fe_4S_4]^+$ cluster to afford methionine and the deoxyadenosyl radical (DOA•). To provide detailed information about the reactions of sulfonium ions with $[Fe_4S_4]^{2+,+}$ clusters, the analogue reaction systems $[Fe_4S_4(SR')_4]^{2-,3-}/[PhMeSCH_2R]^+$ (R′ = Et (**4**, **6**), Ph (**5**, **7**); R = H (**8**), COPh (**9**), *p*-C_6H_4CN (**10**)) were examined by ^1H NMR spectroscopy. Sulfonium ions **8**−**10** react completely with oxidized clusters **4** and **5** to afford PhSMe and R′SCH_2R in equimolar amounts as a result of electrophilic attack by the sulfonium ion on cluster thiolate ligands. Reactions are also complete with reduced clusters **6** and **7** but afford, depending on the substrate, the additional products RCH_3 (R = PhCO, *p*-C_6H_4CN) and the ylid PhMeS═CHR or (*p*-$NCC_6H_4CH_2)_2$. Redox potentials of **9** and **10** allow electron transfer from **6** or **7**. The reaction systems **6**/**9**,**10** and **7**/**9**,**10** exhibit two reaction pathways, reductive cleavage and electrophilic attack, in an ca. 4:1 ratio inferred from product distribution. Cleavage is a two-electron process and, for example in the system **6**/**9**, is described by the overall reaction $2[Fe_4S_4(SR')_4]^{3-} + 2[PhMeSCH_2R]^+ \rightarrow 2[Fe_4S_4(SR')_4]^{2-} + PhSMe + RCH_3 + PhMeS{=}CHR$. This and other reactions may be summarized as $[PhMeSCH_2R]^+ + 2e^- + H^+ \rightarrow PhSMe + RCH_3$; proposed reaction sequences parallel those for electrochemical reduction of sulfonium ions. This work demonstrates the intrinsic ability of $[Fe_4S_4]^+$ clusters with appropriate redox potentials to reductively cleave sulfonium substrates in overall two-electron reactions. The analogue systems differ from the enzymes in that DOA• is generated in a one-electron reduction and is sufficiently stabilized within the protein matrix to abstract a hydrogen atom from substrate or an amino acid residue in a succeeding step. In the present systems, the radical produced in the initial step of the reaction sequence, $[Fe_4S_4(SR')_4]^{3-} + [PhMeSCH_2R]^+ \rightarrow [Fe_4S_4(SR')_4]^{2-} + PhSMe + RCH_2•$, is not stabilized and is quenched by reduction and protonation.

Introduction

New functional roles of iron−sulfur clusters in biology beyond classical electron transfer are increasingly recognized, and include inter alia substrate binding and catalysis, regulation, and sensing of dioxygen, nitric oxide, and iron.[1−4] Among these developments is the ongoing elucidation of a class of enzymes whose common theme is that their clusters are implicated in the formation of radicals by the reduction of the obligatory cofactor *S*-adenosylmethionine (AdoMet, **1**) in the first step of catalysis. Biotin is an essential vitamin that is synthesized by microorganisms and plants. As demonstrated by genetics studies, the biotin biosynthetic pathway requires the product of the *bioB* gene, referred to as biotin synthase.[5−7] This enzyme transforms dethiobiotin (**2**) to biotin (**3**) in the final step of biotin biosynthesis by means of a remarkable insertion of a sulfur atom between an unactivated methyl group and a methylene group attached to a ureide ring. Scheme 1 provides a current summary of the various steps postulated or proven in the overall mechanism.[5−8] The reduced $[Fe_4S_4]^+$ cluster[9] reduces AdoMet to methionine and the deoxyadenosyl radical (DOA•), which abstracts a hydrogen atom, apparently from the 9-position of **2**.[8] At this point, but in an unestablished order, a sulfur atom is bound, a second radical is generated by DOA•, and the tetrahydrothiophene ring is closed to afford **3**. Two equivalents of AdoMet are required per turnover. On the basis of ^{34}S and ^{35}S isotope labeling, the apparent source of the inserted sulfur is the iron−sulfur cluster.[10,11]

On the basis of Scheme 1, the iron−sulfur cluster has two functions: (i) a one-electron reductant for specific cleavage of one S−C bond in AdoMet, generating DOA•, which in turn engenders a carbon-based radical of **2**; (ii) source of the sulfur atom inserted in dethiobiotin yielding biotin. Under this scheme, the cluster is a reagent rather than a catalyst, inasmuch as it must be rebuilt before the next turnover. These two functions have not been demonstrated independently. Function (i) extends to other enzymes such as anaerobic ribonucleotide reductase activating enzyme,[12] lysine-2,3-aminomutase,[13] and pyruvate formate lyase activating enzyme.[14] Function (ii) is currently best established with biotin synthase, but may also intervene in

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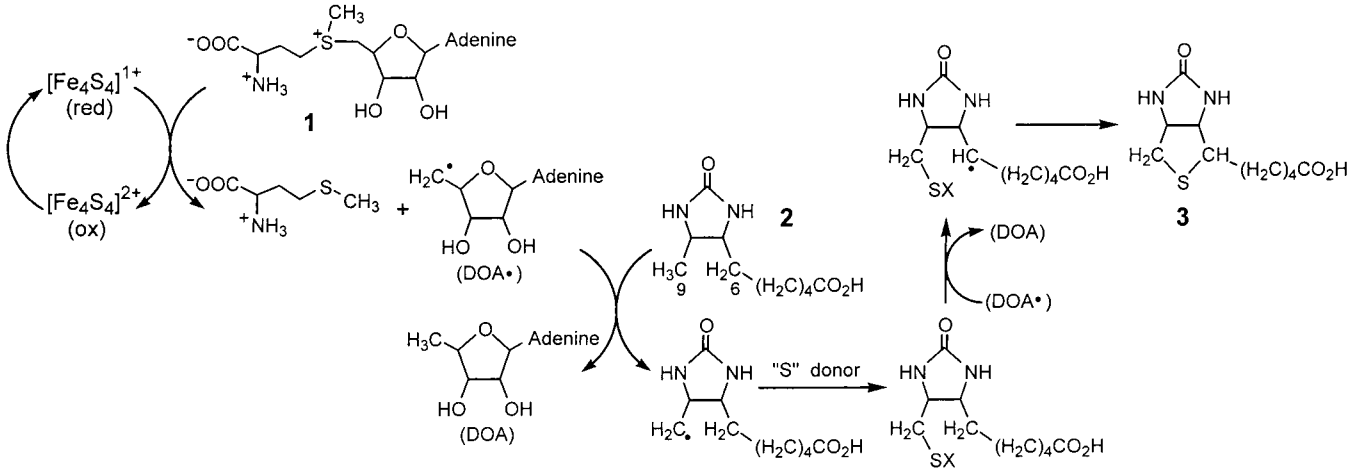
Scheme 1. Postulated Biosynthetic Pathway of Biotin Synthase



lipoate synthase.[15] Using analogue clusters, we are engaged in an investigation of both functions. We report here our initial results on the reactions of oxidized and reduced Fe_4S_4 clusters with sulfonium salts.

Experimental Section

Materials and Procedures. All operations were carried out under a pure dinitrogen atmosphere using standard Schlenk and glovebox techniques. Acetonitrile, dichloromethane, ether, and THF were dried using an Innovative Technology solvent purification system. The compounds triethylamine (CaH_2) and benzenethiol (bp 80 °C/20 mm) were purified by distillation in a dinitrogen atmosphere. The compounds $(Et_4N)_2[Fe_4S_4(SPh)_4]$,[16] $(Et_4N)_3[Fe_4S_4(SPh)_4]$,[17] $(Bu_4N)_2[Fe_4S_4(SEt)_4]$,[16] $(Et_4N)_3[Fe_4S_4(SEt)_4]$,[18] $(Et_4N)(SPh)$,[19] $[PhSMe_2](CF_3SO_3)$,[20] $[PhMeSCH_2$-p-$C_6H_4CN](BF_4)$,[20] $[PhMeSCH_2COPh](BF_4)$,[20] and PhMeS=CHCOPh[21] were prepared by published methods. Other reagents were commercial samples used as received. NMR data used to characterize reactants and products in the systems that follow are available as Supporting Information.

Synthesis of Sulfides. The compounds $RSCH_2COPh$ and $RSCH_2$-p-C_6H_4CN (R = Me, Et, Ph) were prepared by procedures analogous to that for $MeSCH_2COPh$. To a solution of 49.7 mg (0.250 mmol) of $BrCH_2COPh$ in 5 mL of acetonitrile at 0 °C was added dropwise a solution of 17.5 mg (0.250 mmol) of NaSMe in 5 mL of acetonitrile. The solution was stirred for 10 min, warmed to room temperature, and stirred for an additional 20 min. The reaction mixture was filtered through a Celite plug, and the solvent was removed under reduced pressure. The oily residue was dissolved in dichloromethane and passed through a Celite plug to remove NaBr. After solvent removal, the product was obtained as 35.6 mg (86%) of a colorless liquid. The other compounds were obtained in yields of 70−90% and were identified by ^{1}H NMR.

Reactions. All reactions were carried out in acetonitrile. Solvent removal steps were performed in vacuo. Products were identified by comparison of ^{1}H NMR spectra with those of authentic samples. The reactions described below were carried out at 6−8 mM cluster and substrate and were repeated 4−6 times. Products derived from initial sulfonium salts were quantitated by integration vs internal standards: bibenzyl and *p*-tolunitrile for systems containing $[PhMeSCH_2COPh](BF_4)$, bibenzyl and acetophenone for systems containing $[PhMeSCH_2$-p-$C_6H_4CN](BF_4)$, and bibenzyl and toluene for systems using $[PhSMe_2](CF_3SO_3)$. Product amounts are reported as percent formed from the initial sulfonium cation and are average values from multiple runs.

(1) Sulfonium and Thiolate Salts. The following procedure is typical. A solution of 0.498 g (2.50 mmol) of $[PhMeSCH_2COPh](BF_4)$ in 5 mL of acetonitrile was added dropwise to a stirred slurry of 0.211 g (2.51 mmol) of NaSEt in 5 mL of acetonitrile. The reaction mixture became homogeneous and was stirred for 5 min. Solvent was removed and the residue was analyzed by ^{1}H NMR, which demonstrated a complete reaction with equimolar formation of PhSMe and $EtSCH_2$-COPh. Equivalent results were obtained with the equimolar reaction systems NaSEt/$[PhMeSCH_2R](BF_4)$ and $(Et_4N)(SPh)/[PhMeSCH_2R]$-$(BF_4)$, which yielded the products PhSMe and $EtSCH_2R$ or $PhSCH_2R$ (R = COPh, p-C_6H_4CN).

(2) $[Fe_4S_4(SR')_4]^{2-}$ and Sulfonium Salts. (a) Without Chloride. The following procedure is typical. A solution of 0.121 g (0.314 mmol) of $[PhMeSCH_2$-p-$C_6H_4CN](BF_4)$ in 5 mL of acetonitrile was added dropwise to a stirred solution of 0.340 g (0.314 mmol) of $(Bu_4N)_2$-$[Fe_4S_4(SEt)_4]$ in 100 mL of acetonitrile. Stirring was continued for 30 min. The reaction mixture was filtered to remove a black insoluble solid (0.048 g). The filtrate was layered with 250 mL of ether and maintained at −20 °C. A black crystalline solid (0.210 g) was collected and identified as the starting cluster compound, recovered as 62% of the starting amount. Solvent was removed and the products were shown to be PhSMe and $EtSCH_2$-p-C_6H_4CN formed in quantitative yield based on the starting sulfonium salt. In this and other cases, NMR-scale reactions were also performed in order to obtain an accurate account of all products formed. Equivalent results were obtained with the following reaction systems: $(Bu_4N)_2[Fe_4S_4(SEt)_4]/[PhMeSCH_2COPh]$-$(BF_4)$ or $[PhSMe_2](CF_3SO_3)$, products PhSMe and $EtSCH_2COPh$ or EtSMe; $(Et_4N)_2[Fe_4S_4(SPh)_4]/[PhMeCH_2R](BF_4)$, products PhSMe and $PhSCH_2R$ (R = COPh, p-C_6H_4CN); $(Et_4N)_2[Fe_4S_4(SPh)_4]/[PhSMe_2](CF_3$-$SO_3)$, product PhSMe. In each reaction system, initial cluster was recovered and a small amount of unidentified insoluble black byproduct was formed.

(b) With Chloride. Reactions were performed in an analogous manner, but with the addition of 2 equiv of Et_4NCl to the cluster solution prior to the addition of the sulfonium salt. The same products were formed in the same ratios as in the systems without chloride. However, no insoluble byproduct was observed. Instead signals from the clusters $[Fe_4S_4(SR')_{4-n}Cl_n]^{2-}$ were clearly evident: R = Et (SCH_2), $n = 1$ (δ 13.45), $n = 2$ (δ 14.35); R = Ph (*m*-H), $n = 1$ (δ 8.24), 2 (δ 8.32).

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Table 1. Reactions in the Systems $[Fe_4S_4(SR')_4]^{2-,3-}/[PhMeSCH_2R]^+$ (R′ = Et, Ph; R = COPh, *p*-C_6H_4CN) in Acetonitrile

(1)	$[Fe_4S_4(SR')_4]^{2-} + [PhMeSCH_2R]^+ \rightarrow (1/x)(\text{“}[Fe_4S_4(SR')_3]^- \text{”})_x + PhSMe + R'SCH_2R$
(2)	$[Fe_4S_4(SR')_4]^{2-} + [PhMeSCH_2R]^+ + Cl^- \rightarrow [Fe_4S_4(SR')_3Cl]_2 + PhSMe + R'SCH_2R$
(3)	$[Fe_4S_4(SPh)_4]^{3-} + [PhMeSCH_2R]^+ + PhSH \rightarrow [Fe_4S_4(SPh)_4]^{2-} + PhSMe\ (100\%) + PhSCH_2R\ (57-58\%) + RCH_3\ (43\%) + PhSH(60\%)$[a]
(4)	$[Fe_4S_4(SR')_4]^{3-} + [PhMeSCH_2R]^+ \rightarrow [Fe_4S_4(SR')_4]^{2-} + PhSMe + RCH_2\bullet$
(5)	$[Fe_4S_4(SR')_4]^{3-} + RCH_2\bullet \rightarrow [Fe_4S_4(SR')_4]^{2-} + RCH_2{:}^-$
(6)	$RCH_2{:}^- + PhSH \rightarrow RCH_3 + PhS^-$
(7)[b]	$2[Fe_4S_4(SR')_4]^{3-} + [PhMeSCH_2R]^+ + PhSH \rightarrow 2[Fe_4S_4(SR')_4]^{2-} + PhSMe + RCH_3 + PhS^-$
(8)	$RCH_2\bullet + PhSH \rightarrow RCH_3 + PhS\bullet$
(9)	$[Fe_4S_4(SR')_4]^{3-} + PhS\bullet \rightarrow [Fe_4S_4(SR')_4]^{2-} + PhS^-$
(10)	$2PhS\bullet \rightarrow PhSSPh$
(11)	$[Fe_4S_4(SR')_4]^{3-} + (1/2)PhSSPh \nrightarrow [Fe_4S_4(SR')_4]^{2-} + PhS^-$
(12)	$RCH_2{:}^- + [PhMeSCH_2R]^+ \rightarrow RCH_3 + PhMeS{=}CHR$
(13)	$RCH_2\bullet + [PhMeSCH_2R]^+ \rightarrow RCH_3 + [PhMeS{=}(C\bullet)HR]^+$
(14)	$[Fe_4S_4(SR')_4]^{3-} + [PhMeS{=}(C\bullet)HR]^+ \rightarrow [Fe_4S_4(SR')_4]^{2-} + PhMeS{=}CHR$
(15)[c]	$2[Fe_4S_4(SR')_4]^{3-} + 2[PhMeSCH_2R]^+ \rightarrow 2[Fe_4S_4(SR')_4]^{2-} + PhSMe + RCH_3 + PhMeS{=}CHR$
(16)	$2RCH_2\bullet \rightarrow RCH_2CH_2R$
(17)	$RCH_2{:}^- + [PhMeSCH_2R]^+ \rightarrow PhSMe + RCH_2CH_2R$
(18)[d]	$2[Fe_4S_4(SR')_4]^{3-} + 2[PhMeSCH_2R]^+ \rightarrow 2[Fe_4S_4(SR')_4]^{2-} + 2PhSMe + RCH_2CH_2R$
(19)	$[PhMeSCH_2R]^+ + 2e^- + H^+ \rightarrow PhSMe + RCH_3$

[a] Approximate quantitation based on PhS*H* signal. [b] (4) + (5) + (6). [c] (4) + (5) + (12). [d] 2(4) + (16) or (4) + (5) + (17).

(3) $[Fe_4S_4(SR')_4]^{3-}$ and Sulfonium Salts. (a) Without Thiol. The following procedure is typical. A solution of 0.881 g (0.267 mmol) of $[PhMeSCH_2COPh](BF_4)$ in 5 mL of acetonitrile was added dropwise to a stirred solution of 0.315 g (0.267 mmol) of $(Et_4N)_3[Fe_4S_4(SPh)_4]$ in 50 mL of acetonitrile. Stirring was continued for 20 min. Solvent was removed and the dark residue was washed thoroughly with ether. The washings were combined and filtered. The remaining black solid (0.192 g) was shown to be $(Et_4N)_2[Fe_4S_4(SPh)_4]$, recovered as 68% of the expected amount assuming 100% reduction of the starting cluster. Ether was removed from the filtrate; the residue was dissolved in acetonitrile and analyzed by ^1H NMR. In addition to $(Et_4N)(BF_4)$, the products listed in Table 2 were detected for this and other reaction systems. Percentages represent the distribution of products derived from the initial sulfonium salt.

(b) With Thiol. Reactions were performed in an analogous manner, but with the addition of 1 equiv of benzenethiol to the cluster solution prior to the addition of the sulfonium salt. In addition to $(Et_4N)(BF_4)$ and $(Et_4N)_2[Fe_4S_4(SPh)_4]$ (the only cluster species observed), two reaction systems afforded the products in Table 2.

Physical Measurements. Unless stated otherwise, all ^1H NMR spectra were measured in CD_3CN solutions at 22 °C on Varian Unity 500 or Varian Mercury 400 spectrometers. Electrochemical measurements were made at room temperature with a Princeton Applied Research Model 263 potentiostat/galvanostat in acetonitrile solutions using a Pt working electrode, 50 mV/s scan rate, and 0.1 M $(Bu_4N)(PF_6)$ supporting electrolyte. Potentials are referenced to the SCE.

Results and Discussion

In the following sections, compounds are designated according to Chart 1. Reactions of the clusters **4**−**7**, accurate structural and electronic representations of protein-bound $[Fe_4S_4]^{2+,+}$ sites, with the sulfonium cations **8**−**10** have been examined in acetonitrile solutions and monitored by ^1H NMR spectroscopy. These clusters have the potentials $E_{1/2} = -1.30$ V (**4**/**6**) and −1.00 V (**5**/**7**) for the couples $[Fe_4S_4(SR')_4]^{2-/3-}$ in acetonitrile. The redox[17,22] and NMR[18,23,24] properties of the clusters are described elsewhere. The selection of sulfonium salts was made on the basis of their irreversible reduction potentials $E_{pc} = -0.91$ V (**9**) and −0.92 V (**10**), which indicate that the clusters are thermodynamically competent to reduce these cations. These species are dialkylarylsulfonium ions with a methyl group in common with AdoMet, which itself is a trialkylsulfonium ion and insoluble in acetonitrile. Ions more closely related to AdoMet such as $[Me_3S]^+$ ($E_{pc} = -1.85$ V, water)[25] and **8** ($E_{pc} = -1.64$ V, acetonitrile) have much more negative potentials. In general, reduction potentials of less than ca. −1.0 V in aprotic solvents apply to cations with one or two aryl substitutents and/or strongly electron-withdrawing substituents.[20,25−28] The reactions described below are set out in Table 1.

Chart 1. Designation of Compounds

S-adenosylmethionine	**1**
dethiobiotin	**2**
biotin	**3**
$[Fe_4S_4(SEt)_4]^{2-}$	**4**[16,22,23]
$[Fe_4S_4(SPh)_4]^{2-}$	**5**[16,22,23]
$[Fe_4S_4(SEt)_4]^{3-}$	**6**[18]
$[Fe_4S_4(SPh)_4]^{3-}$	**7**[17,24]
$[PhMeSCH_2R]^+$ [20]	R = H **8**, COPh **9**, *p*-C_6H_4CN **10**
PhMeS=CHCOPh	**11**[21]
$RSCH_2COPh$	R = Me **12**, Et **13**, Ph **14**
$RSCH_2$-*p*-C_6H_4CN	R = Me **15**, Et **16**, Ph **17**

Reaction of $[Fe_4S_4(SR')_4]^{2-}$ with Sulfonium Cations. Because of the potential nucleophilic reactivity of coordinated thiolate ligands, the reactions of **4** and **5** with sulfonium cations were examined prior to the reactions of reduced clusters with the same substrates. Treatment of **4** or **5** with cations **9** or **10** in acetonitrile results in an immediate reaction complete within 10 min or less. Reactions with **8** are substantially slower, requiring ca. 40 min for completion at the concentrations used. All reactions were accompanied by the formation of a small amount of black insoluble product. NMR spectra of the six

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reaction systems indicated in each case complete reaction of the sulfonium cation and the formation of PhSMe. The systems **4**/**8**, **4**/**9**, and **4**/**10** afforded the additional products EtSMe, **13**, and **16**, respectively. Similarly, the systems **5**/**8**, **5**/**9**, and **5**/**10** gave PhSMe, **14**, and **17**, respectively. The same organic products in the same equimolar ratios were found in the reactions of NaSEt and $(Et_4N)(SPh)$ with **8**−**10** as for the reaction systems involving the two clusters and the sulfonium ions. With **9** and **10** as substrates, the methyl sulfides **12** and **15** were not detected in any system. The relatively slow reactions of **8** are attributed to decreased electrophilic character owing to the absence of electron-withdrawing substituents.

Evidently, thiolate ligands are abstracted from the clusters in reaction 1 by electrophilic attack of the sulfonium cations to afford the two product sulfides and deligated clusters, which separate as insoluble solids. When the equimolar reaction system **4**/**10** was performed on a preparative basis, PhSMe and **16** were formed in quantitative yield and 62% of initial $(Et_4N)_2$[**4**] was recovered in substance. Cluster recovery is approximately consistent with the necessary thiolate equivalents supplied by 0.25 cluster equivalent, but does not require the removal of one thiolate per cluster. Rapid ligand exchange between singly or multiply deligated clusters can lead to the formation of soluble salts of **4** or **5** and insoluble material.

When the same reactions were performed in the presence of 2 equiv of Et_4NCl, no precipitates were observed. The NMR spectra of four completed reactions provided in Figures 1 and 2 demonstrate that the same organic products are formed in the same ratios as in the absence of chloride. Thiolate-deligated clusters are solubilized by binding chloride to form the mixed-ligand species $[Fe_4S_4(SR')_{4-n}Cl_n]^{2-}$ ($n = 1, 2$), which are readily detected by a characteristic set of SCH_2 (Figure 1) or *m*-H (Figure 2) resonances shifted downfield by hyperfine contact interactions.[24] These same signals are observed upon titration of **4**[29] and **5** with acetyl or pivaloyl chloride in acetonitrile. Chloride was used as the solubilizing cluster ligand because it is insufficiently nucleophilic to attack the sulfonium cations. The results are consistent with reaction 2 (R′ = Et, Ph; R = COPh, *p*-C_6H_4CN), which in all cases proceeds to completion. For simplicity, the reaction is written with the dominant mixed-ligand cluster formed. Reaction 2 further exemplifies the reactivity of cluster thiolate ligands with electrophiles, which include protic acids,[30,31] acid chlorides,[30,32,33] and $(MeO)_3PO$.[34]

Reaction of $[Fe_4S_4(SR')_4]^{3-}$ with Sulfonium Cations. The reactions of reduced clusters with sulfonium substrates in acetonitrile are more involved than are reactions 1 or 2. The reaction of **6** or **7** with **9** and **10** in acetonitrile is immediate and results in the total consumption of cation and the formation of soluble oxidized cluster (**4**, **5**) and a small amount of black precipitate. The expected organic products of a one-electron reduction of the sulfonium cation are PhSMe and the radical RCH_2• (R = COPh, *p*-C_6H_4CN), whose possible fates include coupling to give the combination product RCH_2CH_2R, hydrogen atom abstraction from solvent to form RCH_3, and reduction by reduced cluster to a transient carbanion that is discharged in a follow-up reaction. In the case of hydrogen atom abstraction from acetonitrile, products derived from $NCCH_2$• would also be anticipated, but have not been observed. The reaction systems that follow afford three or four organic products. These are specified in Table 2 in terms of average percentages formed from the initial sulfonium substrate in multiple runs.

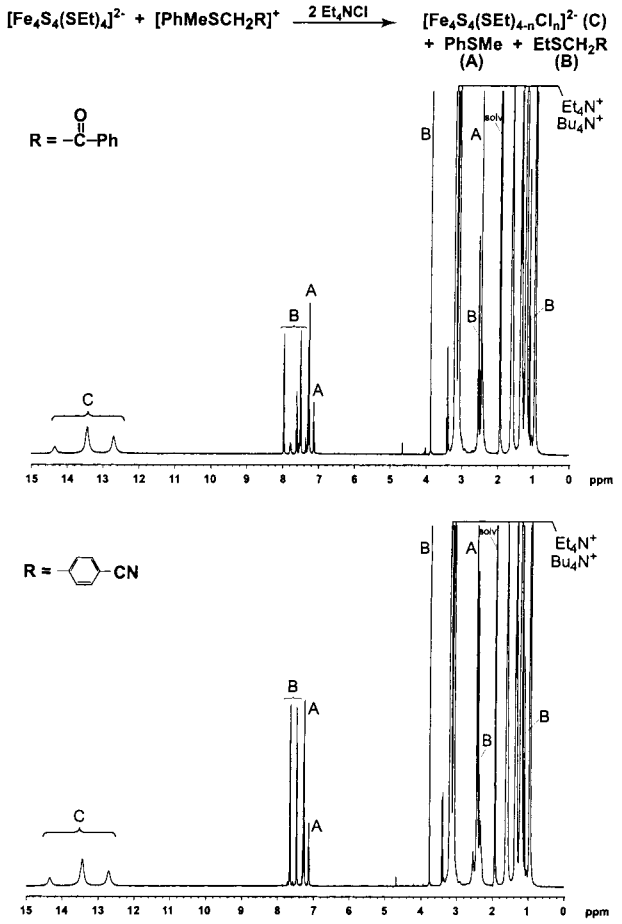


Figure 1. ^1H NMR spectra of the reaction systems $[Fe_4S_4(SEt)_4]^{2-}$/$[PhMeSCH_2R]^+$/2Et_4NCl (R = COPh (upper) and *p*-C_6H_4CN (lower)) in acetonitrile solutions. Signal assignments are indicated; in order of increasing chemical shift, the three downfield SCH_2 signals arise from $n = 0$, 1, and 2 clusters.

(a) Systems $[Fe_4S_4(SPh)_4]^{3-}$/$[PhMeSCH_2R]^+$/PhSH. Noting the probable intervention of radicals and carbanions in the electrochemical reductive cleavage of sulfonium cations,[25,27,28] equimolar reaction systems **7**/**9**/PhSH and **7**/**10**/PhSH were investigated. These contained benzenethiol as a potential quencher of such species by hydrogen atom and/or proton donation.[35] This thiol was selected because it is easier to handle and quantitate than is ethanethiol. Consequently, the systems examined contain benzenethiolate cluster **7**. Reactions are immediate and the cation is fully consumed. The outcome of these reactions can be seen from the spectra in Figure 3, where all signals are identified. Note that signal C corresponds to oxidized cluster **5**. It is immediately evident that substrate undergoes *two* reactions: electrophilic attack on coordinated thiolate[36] (attack) and reductive cleavage with rupture of the

(29) Hagen, K. S.; Watson, A. D.; Holm, R. H. *J. Am. Chem. Soc.* **1983**, *105*, 3905−3913.
(30) Johnson, R. W.; Holm, R. H. *J. Am. Chem. Soc.* **1978**, *100*, 5338−5344.
(31) Henderson, R. A.; Oglieve, K. E. *J. Chem. Soc., Dalton Trans.* **1993**, 1467−1472.
(32) Wong, G. B.; Bobrik, M. A.; Holm, R. H. *Inorg. Chem.* **1978**, *17*, 578−584.
(33) Stack, T. D. P.; Holm, R. H. *J. Am. Chem. Soc.* **1988**, *110*, 2484−2494.
(34) Wilker, J. J.; Lippard, S. J. *Inorg. Chem.* **1999**, *38*, 3569−3574.
(35) At high $[PhSH]/[Fe_4S_4(SPh)_4]^{3-}$ mole ratios, dihydrogen is evolved and the cluster is oxidized: Yamamura, T.; Christou, G.; Holm, R. H. *Inorg. Chem.* **1983**, *22*, 939−949; Grönberg, K. L. C.; Henderson, R. A.; Oglieve, K. E. *J. Chem. Soc., Dalton Trans.* **1998**, 3093−3104. These conditions do not apply in the present system.
(36) Any PhS^- formed in this reaction can attack the sulfonium ion.

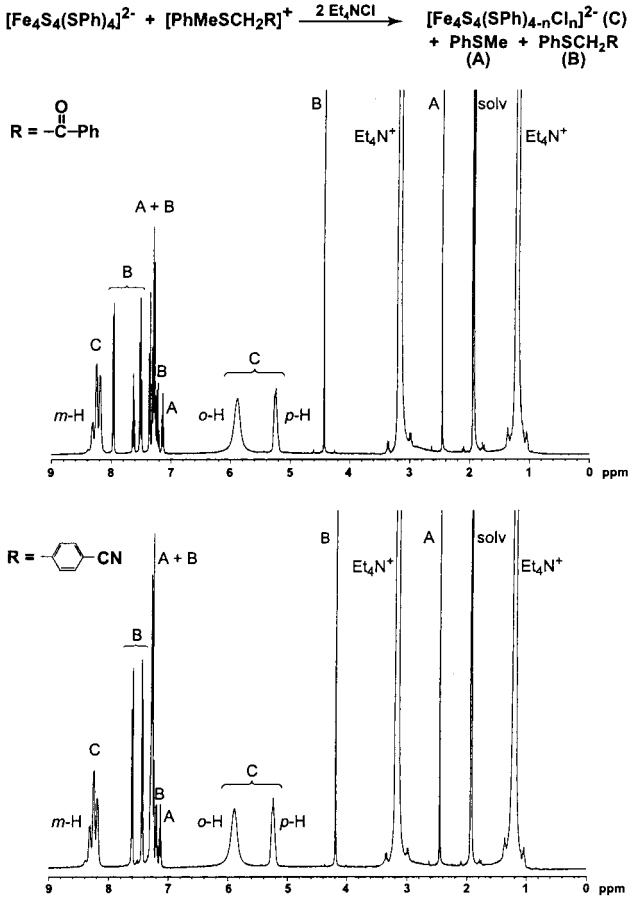


Figure 2. ^{1}H NMR spectra of the reaction systems $[Fe_4S_4(SPh)_4]^{2-}$/$[PhMeSCH_2R]^+$/2Et_4NCl (R = COPh (upper) and *p*-C_6H_4CN (lower)) in acetonitrile solutions. Signal assignments are indicated; in order of increasing chemical shift, the three downfield *m*-H signals arise from $n = 0$, 1, and 2 clusters.

Table 2. Product Distributions in the Reaction Systems $[Fe_4S_4(SR')_4]^{3-}$/$[PhMeSCH_2R]^+$ in Acetonitrile Solution (R′ = Et (**6**), Ph (**7**); R = COPh (**9**), *p*-C_6H_4CN (**10**))[a]

system	PhSMe	R′SCH_2R	RCH_3	PhMeS=CHR	RCH_2CH_2R
7/**9**/PhSH	100	57	43		
7/**10**/PhSH	100	58	43		
6/**9**	72	21	36	22	
7/**9**	60	22	39	45	
6/**10**	88	18	24	b	18
7/**10**	90	24	20	b	22

[a] Yields determined by ^{1}H NMR integration (±2−4%) against internal standards and are reported based on the initial amount of sulfonium cation used (**9** or **10**). [b] Unidentified product signals G in Figure 5 have been observed in ^{1}H NMR on treatment of **10** with NEt_3 in acetonitrile after heating at 60 °C. The implication is that it is the product of proton abstraction or nucleophilic attack on **10** and accounts for the absence of the unknown ylid (PhMeS=CH-*p*-C_6H_4CN).

same S−C bonds as in reactions 1 and 2 (cleavage). The results are summarized as reaction 3. The columns R′CH_2SR and RCH_3 in Table 2 give the percentages of substrate that undergo attack and cleavage reactions, respectively. In these two systems, attack and cleavage pathways occur in a ratio of 1.3:1.

Careful examination of the spectra did not detect either RCH_2-CH_2R or PhSSPh, or in the case of **9** the ylid **11** (vide infra), among the reaction products. Consequently, we propose reaction sequence 4−6 (R′ = Ph; R = COPh, *p*-C_6H_4CN) for the cleavage pathway. Cleavage occurs by electron transfer in the first step with generation of PhSMe, followed by reduction of the radical RCH_2• and protonation of the resultant carbanion to

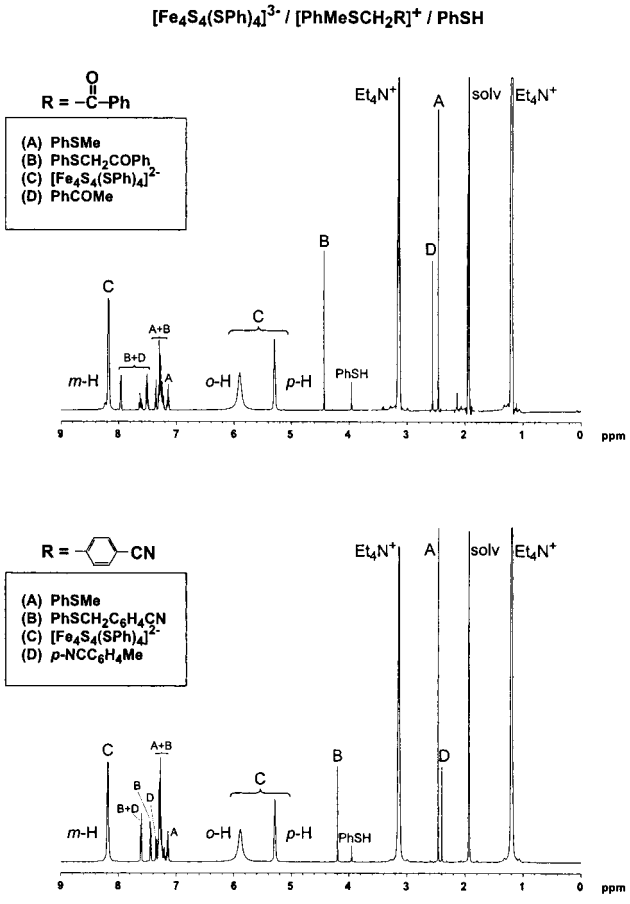


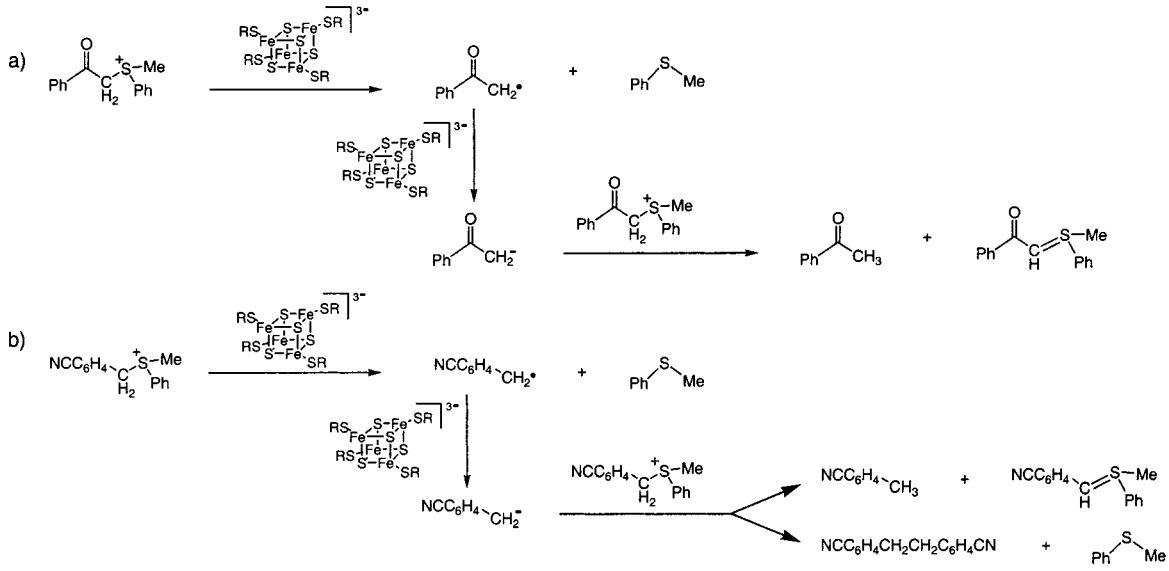
Figure 3. ^{1}H NMR spectra of the reaction systems $[Fe_4S_4(SPh)_4]^{3-}$/$[PhMeSCH_2R]^+$/PhSH (R = COPh (upper) and *p*-C_6H_4CN (lower)) in acetonitrile solutions. Reactants were present in equimolar concentration. Signal assignments are indicated.

afford a second product RCH_3 (acetophenone, *p*-tolunitrile). While the cleavage event itself is obviously a one-electron process, the complete reaction 7 requires that cleavage of the sulfonium substrate is a two-electron process. An alternative scheme considered is the quenching of the radical in reaction 5 by reaction 8. The newly formed benzenethiyl radical could then undergo further reduction to form benzenethiolate (reaction 9) or dimerize to form disulfide (reaction 10). The disulfide was not detected nor was benzenethiolate (reaction 7 and/or 9), which we assume was taken up by either deligated cluster generated in the attack pathway or by direct attack on cation as is observed in the reactions of $(Et_4N)(SPh)$ with **9** or **10**. It must be noted that $[Fe_4S_4(SPh)_4]^{3-}$ cannot reduce the disulfide in subsequent steps (reaction 11) as the reduction potential of the latter (E_{pc} = −1.54 V (DMF),[37] −1.48 V (MeCN)[38] vs SCE) is too low. Owing to the large thermodynamic stability of disulfide bond formation, that no disulfide is formed strongly suggests that the reaction pathways 4 + 8 + 9 and 4 + 8 + 10 are not significant contributions to the reductive cleavage pathway. From the information available, we cannot tell whether the oxidized or reduced clusters (or both) participate in the attack reaction. Attention is next turned to reaction systems that do not contain thiol and, as a result, generate additional products.

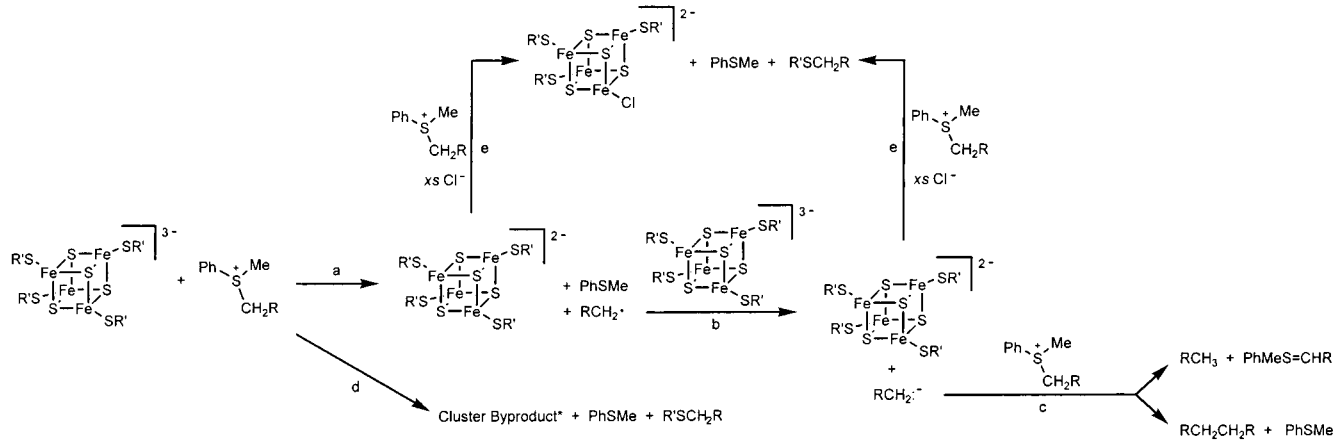
(b) Reaction Systems $[Fe_4S_4(SR')_4]^{3-}$/$[PhMeSCH_2COPh]^+$. The NMR spectra in Figure 4 convey the outcome of systems **6**/**9** and **7**/**9**. The compounds PhSMe, the sulfides **13** or **14**, and

(37) Krüger, H.-J.; Holm, R. H. *Inorg. Chem.* **1989**, *28*, 1148−1155.
(38) Samide, M. J.; Peters, D. G. *J. Electrochem. Soc.* **1998**, *145*, 3374−3378.

Scheme 2. Reduction Pathways of **9** (Path a) and **10** (Path b)



Scheme 3. Reaction Pathways in the Systems $[Fe_4S_4(SR')_4]^{3-}/[PhMeSCH_2R]^+$ [a]



[a] (a) Electron transfer reaction. (b) Second (fast) electron transfer reaction. (c) Quenching of sulfonium cation. (d) Terminal thiolate attack from reduced cluster. (e) Terminal thiolate attack from oxidized cluster (cluster decomposition observed in absence of added chloride to reaction mixture). *Unidentified.

acetophenone appear as reaction products, and together with the oxidized cluster account for signals A−D. The two sulfides **13** and **14** arise from sulfonium cation attack on coordinated thiolate. Product quantitation indicates an ca.4:1 ratio of cleavage and attack pathways; i.e., on average 21−22% of the clusters are deligated by sulfonium attack, assuming for simplicity that one thiolate per cluster reacted. With signals A−D readily identified, the signals E remain in both systems. Here we heed examples of electrochemical reduction of sulfonium cations in aprotic solvents that result in substrate quenching by proton abstraction.[25,27,28] This behavior occurs when the cation carries a relatively acidic α-hydrogen; cation **9**, with its electron-withdrawing phenacyl group, is one such species.[27] The ylid **11** was prepared and found to account for all signals E in the two systems.

From the product quantitation in Table 2, the PhMeS fragment of **9** is well accounted for in the products of both systems (94%, 105%) as is the $PhCOCH_2$ fragment in the **7**/**9** system (106%), but the latter is somewhat low in the **6**/**9** system.[39] These results express our general experience that reactions are cleaner and quantitations more satisfactory in systems based on cluster **7**. From the reproducible observation that the ratio of ylid to acetophenone is ca. 1:1, we propose reactions 4, 5, and 12 (R = COPh) to account for sulfonium cleavage and ylid formation. Acetophenone and ylid formation occur by reaction 12, in which the carbanion abstracts an α-H from the sulfonium cation. The net reaction 15 requires 2 equiv of cluster for reductive cleavage of the sulfonium cation and formation of 1 equiv each of acetophenone and ylid. Reactions 7 and 15 have the common feature of involvement of proton donors, leading to the two-electron stoichiometry. An alternative formation of the ylid to that proposed is the reaction sequence 4 + 13 + 14. This mechanism differs only in the positioning of the second electron reduction without changing the overall net reaction 15. We favor the first sequence proposed (4 + 5 + 12) based on the lack of any ylid and disufide byproduct formation in the thiol-added reaction system.

(c) Reaction Systems $[Fe_4S_4(SR')_4]^{3-}/[PhMeSCH_2$-*p*-$C_6H_4$-CN$]^+$. The products formed in systems **6**/**10** and **7**/**10** are revealed by the NMR spectra in Figure 5. As in reactions of these clusters with **9**, the spectra indicate complete consumption of **10** and cluster oxidation to **4** or **5**. The organic products

(39) One small residual signal (δ 3.86, t) has not been identified and may contribute to the lower quantitation of the ylid.

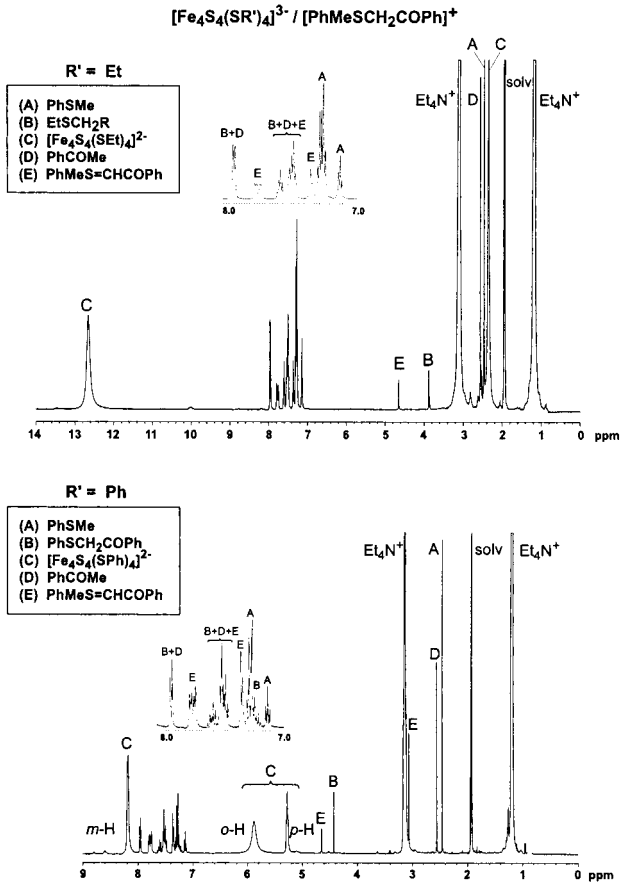


Figure 4. ^{1}H NMR spectra of the reaction systems $[Fe_4S_4(SR')_4]^{3-}$/$[PhMeSCH_2COPh]^+$ (R′ = Et (upper) and Ph (lower)) in acetonitrile solutions. Reactants were present in equimolar concentration. Signal assignments are indicated; insets show expanded aromatic proton regions.

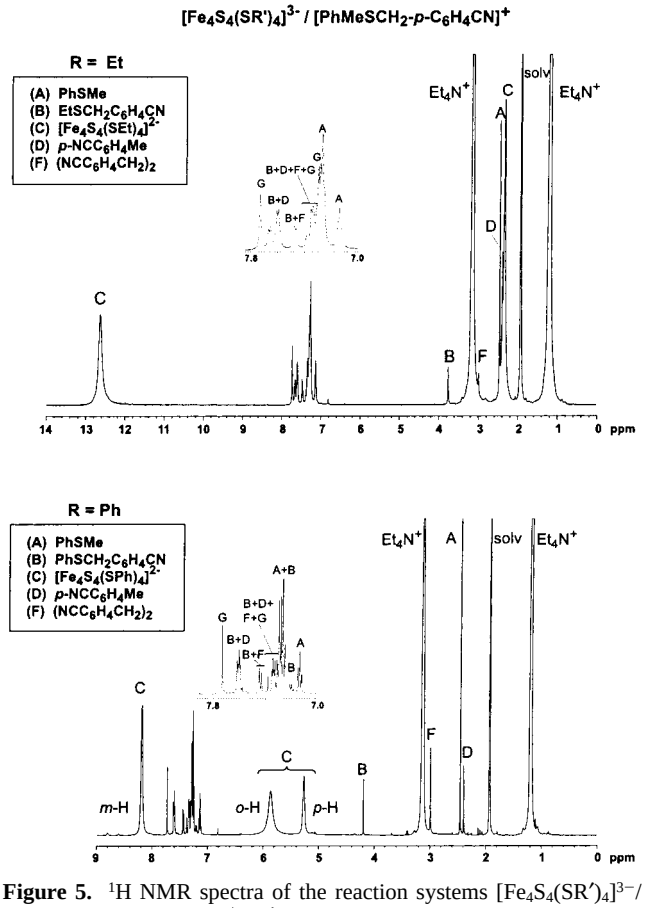


Figure 5. ^{1}H NMR spectra of the reaction systems $[Fe_4S_4(SR')_4]^{3-}$/$[PhMeSCH_2$-p-$C_6H_4CN]^+$ (R′ = Et (upper) and Ph (lower)) in acetonitrile solutions. Reactants were present in equimolar concentration. Signal assignments are indicated; insets show expanded aromatic proton regions.

include PhSMe, p-NCC_6H_4Me, and the sulfides **16** and **17**. The cleavage-to-attack ratio is ca. 4:1. Quantitation of the PhMeS (88%, 90%) and p-$NCC_6H_4CH_2$ (78%, 88%) fragments are somewhat low but better in the system containing **7**. Unlike the previous systems, the combination product RCH_2CH_2R (R = p-C_6H_4CN) is observed here. All NMR signals except those labeled G were identified. Attempts to synthesize the (unknown) ylid $[PhMeS{=}CH$-p-$C_6H_4CN]^+$ were unsuccessful. When cation **10** and 2 equiv of Et_3N were heated at 60 °C in acetonitrile, the set of signals G was observed among the products, which also included a large amount of PhSMe but no p-tolunitrile. Given that **10** is expected to be less acidic than **9** and the putative ylid formed by deprotonation does not have a stabilizing α-carbonyl group, it is perhaps not surprising that it is unstable or not formed at all. We propose the occurrence of reaction sequence 4 + 5 + 12 (R = p-C_6H_4CN), with reaction 12 the origin of signals G by means of ylid decomposition (or an unidentified process). The net reaction 15 accounts for the formation of p-tolunitrile but not $(p$-$NCC_6H_4CH_2)_2$. The reaction sequences 2(4) + 16 and/or 4 + 5 + 17 lead to overall reaction 18, which affords $(p$-$NCC_6H_4CH_2)_2$ but not p-tolunitrile. Reactions 12 and 17 result in substrate quenching. Reactions 15 and 18 are cleavage processes requiring two electrons, but the latter leads to 2 equiv of PhSMe compared to 1 equiv in reaction 15. Because of the substantially larger quantities of PhSMe formed in these systems compared to those in **6**/**9** and **7**/**9** (Table 2), in which RCH_2CH_2R is absent, reaction 18 must contribute to the product distribution. However, as no combination product nor any PhSSPh's are observed when thiol is added to these reactions, it is apparent that reaction 16 cannot be contributing to any significant extent and that reaction 17 must be the dominant, if not exclusive, pathway to the formation of the combination product $(p$-$NCC_6H_4CH_2)_2$ by means of overall reaction 18 for the reasons described earlier.

(d) Reaction Systems $[Fe_4S_4(SR')_4]^{3-}$/$[PhMe_2S]^+$. The reactions of cation **8** with clusters **6** and **7** are complete within 10 min and result in the equimolar formation of PhSMe and R′SMe (R′ = Et or Ph) and some black precipitate. No other products of **8** were observed nor was any cluster species identifiable by NMR. Attempts to stabilize the deligated clusters by the addition of chloride or Pr^i_3P[40] were unsuccessful. The redox potential of **8** (E_{pc} = −1.64 V) places it out of range for reductive cleavage by **6** or **7**. The lack of product cluster identification does not preclude the fact that the organic products are those expected from electrophilic attack by **8** on the thiolate ligands of the clusters. This finding is consistent with the formation of both attack and cleavage products in other systems containing substrates susceptible to both reaction pathways.

Summary

Provided in Figure 6 is a graphical account of experimental and calculated product distributions in the reaction systems

(40) Tertiary phosphines have been shown to stabilize reduced clusters, including $[Fe_4S_4(PPr^i_3)_4]$: Goh, C.; Segal, B. M.; Huang, J.; Long, J. R.; Holm, R. H. *J. Am. Chem. Soc.* **1996**, *118*, 11844−11853.

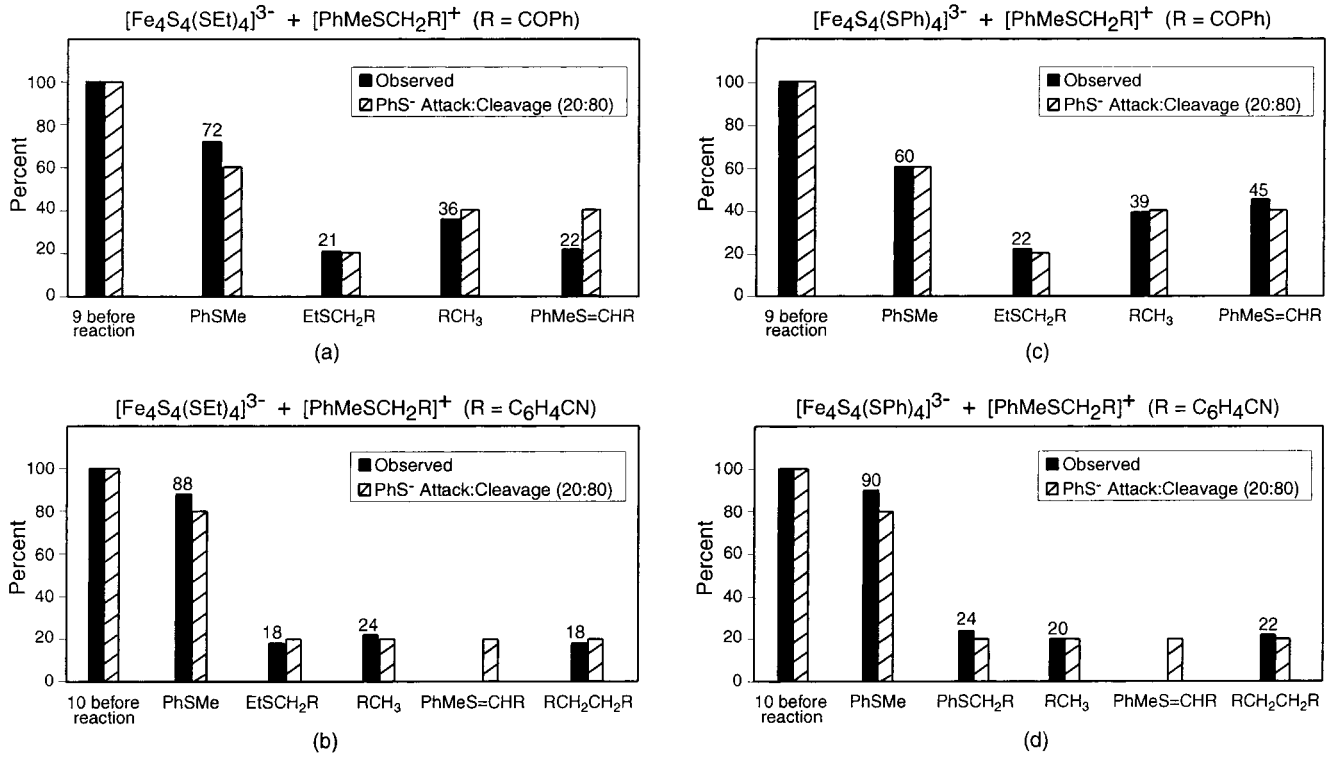


Figure 6. Graphical representations of the observed and predicted product distributions in four reaction systems $[Fe_4S_4(SR')_4]^{3-}/[PhMeSCH_2R]^+$ (R′ = Et, Ph; R = COPh, *p*-C_6H_4CN) in acetonitrile, based on a 4:1 ratio of reductive cleavage to electrophilic attack of sulfonium cation on cluster thiolate ligands.

$[Fe_4S_4(SR')_4]^{3-}/[PhMeSCH_2R]^+$. Scheme 2 exclusively depicts the reductive reaction pathways of both sulfonium salts, while Scheme 3 is a summary of reaction pathways in a generalized system. The calculated values are based on a 4:1 cleavage-to-attack ratio indicated by the data in Table 2 and on reaction stoichiometries that require two electrons in the overall reductive cleavage reactions. It is apparent that quenching of a radical produced in a reaction such as 4 by follow-up reactions of reduction and protonation necessitate a two-electron process. Reactions 7 and 15 may be represented by minimal reaction 19, in which the two electrons are used to cleave an S−C bond and reduce the radical product and the proton donor is an added acid or the substrate itself. These reactions parallel those proposed in the electrochemical reduction of sulfonium cations.[25,27,28] Reaction 18 differs in that no protonated product RCH_3 is formed.

This work demonstrates the intrinsic ability of $[Fe_4S_4]^+$ clusters of appropriate redox potential to reductively cleave sulfonium cations in overall two-electron reactions. Consistent with other findings,[20,25−28,41] the bond cleaved affords the most stable radical, resulting in the consistent formation of PhSMe. The occurrence of electrophilic attack as a minority pathway is an obvious consequence of freely diffusing reactants that are strongly electrophilic and nucleophilic. At present, the biotin synthase cluster is seemingly best described as $[Fe_4S_4(S\cdot Cys)_4]$ from ^{57}Fe isomer shifts[9] and resonance Raman shifts;[42] we are unaware of any indication of electrophilic attack by AdoMet on a cysteinate ligand. The two-electron reductions observed in the present systems are due to an inability to stabilize the radical product prior to the fast second reduction forming the carbanion. In biotin synthase, the DOA• radical is apparently sufficiently stabilized so as to allow it to abstract a hydrogen atom from dethiobiotin (Scheme 1). In anaerobic ribonucleotide reductase[12,43] and pyruvate formate lyase,[14] DOA• produces a glycyl radical that is stable enough to be identified.

The recent selenium EXAFS investigation of lysine-2,3-aminomutase,[44] a member of the enzyme family to which biotin synthase is thought to belong, under dithionite-reducing conditions and in the presence of the selenium derivative of AdoMet (*S*-adenosyl-L-selenomethionine) afforded a selenium−iron interaction at 2.67 Å.[45] It is proposed that AdoMet interacts at the open site of a $[Fe_4S_4(S\cdot Cys)_3]$ cluster. While biotin synthase is described as having complete cysteinate ligation, it may be that cysteinate becomes detached during catalysis, followed by AdoMet activation. Investigation of the reaction of AdoMet, or sulfonium salt analogues, with a 3:1 site-differentiated analogue cluster such as $[Fe_4S_4(LS_3)(SR)]^{2-}$ [33] may yield further information on the specific interaction between these species, whether directly related to both biotin synthase and lysine-2,3-aminomutase or solely the latter. As described at the outset, reduction of AdoMet is one of two functions of the Fe_4S_4 cluster in biotin synthase and extends to certain other enzymes operating through radical intermediates.

Finally, because our conclusions of probable stepwise reaction pathways are predicated on product analysis and not on

(41) Beak, P.; Sullivan, T. A. *J. Am. Chem. Soc.* **1982**, *104*, 4450−4457.
(42) Duin, E. C.; Lafferty, M. E.; Crouse, B. R.; Allen, R. M.; Sanyal, I.; Flint, D. H.; Johnson, M. K. *Biochemistry* **1997**, *36*, 11811−11820.
(43) Sun, X.; Ollagnier, S.; Schmidt, P. P.; Atta, M.; Mulliez, E.; Lepape, L.; Eliasson, R.; Fontecave, M.; Fontecave, M.; Reichardt, P.; Sjöberg, B.-M. *J. Biol. Chem.* **1996**, *271*, 6827−6831.
(44) Cosper, N. J.; Booker, S. J.; Ruzicka, F. J.; Frey, P. A.; Scott, R. A. *Biochemistry* **2000**, *39*, 15668−15673.
(45) The 2.7 Å peak was successfully modeled as a first row transition element. K-edge XAS of the Zn(II) site also present in the enzyme did not change at any stage of catalysis, leading to the conclusion of a Se−Fe interaction.

observations of individual reactions, we cannot insist upon such pathways. The distinction between electron transfer, carbanion formation, and protonation vs radical hydrogen atom abstraction is difficult to draw. We have presented observations and precedents that in our view favor the former, embodied in minimalist reaction 19. In a further investigation, the use of peptide-bound $[Fe_4S_4]^+$ clusters in media in which AdoMet and biotin are soluble will be examined.

Acknowledgment. This research was supported by NIH Grant GM 28856.

Supporting Information Available: ^{1}H NMR chemical shifts of the compounds in Chart 1 and others relevant to this investigation. This material is available free of charge via the Internet at http://pubs.acs.org.

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