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POSSIBLE SYNTHETIC PATHWAYS TO MODELS
OF THE METAL COFACTORS OF NITROGENASE

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Major professor

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POSSIBLE SYNTHETIC PATHWAYS TO MODELS OF THE METAL COFACTORS OF NITROGENASE

By

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ABSTRACT

POSSIBLE SYNTHETIC PATHWAYS TO MODELS
OF THE METAL COFACTORS OF NITROGENASE

By

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Methods for synthesis of potential models for the metal cofactors of nitrogenase have been investigated. These include Fe_4S_4 clusters coordinated by: (1) metallocenes, $Fe_4S_4(SR)_{4-n}[(SEt)_2TiCp_2]_n^{(2-n)-}$ and $Fe_4S_4(SR)_{4-n}(S_2TiCp_2)_n^{(2+n)-}$; (2) thiomolybdate bridges, $(RS)_3S_4Fe_4(S_2MoS_2)Fe_4S_4(SR)_3^{4-}$; and (3) a tripod, $Fe_4S_4(SR)[(S\text{-}\underline{o}\text{-}C_6H_4\text{-}NH)_3P{=}O]^{2-}$. Isolation of analytically pure crystals has not yet been achieved, but the thiomolybdate-bridged entity has been observed and partially characterized spectroscopically in solution. Nmr spectroscopy shows the coordinated thiolate ligands of the Fe_4S_4 clusters may be removed upon the addition of a suitable proton donor. This technique has been used to force the Fe_4S_4 clusters to bind the selected ligands. Results from attempted syntheses of the titanocene-bound Fe_4S_4 clusters indicate that formation of polymers, varied substitution products, and rearrangements hinder preparation of the desired compounds. These conditions also hinder preparation of the thiomolybdate-bridged Fe_4S_4 clusters. A tripod ligand was synthesized to eliminate these

problems; it may provide another metal cofactor model. A discussion of future plans is included.

To
George and Betty
for their support, inspiration, and dedication
to the education of their children

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Again, I want to express my sincere admiration for my parents' devotion to my goals.

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A. INTRODUCTION

As future energy needs focus attention on the dwindling petroleum resources of our planet, research interests increasingly concentrate on industrial processes where potential energy savings may be realized. One such area is nitrogen fixation, or more specifically, the catalyzed reduction of dinitrogen to ammonia. On an indus-

$$3H_2 + N_2 \rightarrow 2NH_3 \qquad (1)$$

$T = 450°C$

$P = 350$ atm

catalyst = $Fe/Al_2O_3/K_2O$

trial scale this process is relatively inefficient (yields $\leq 20\%$) and requires large inputs of energy, often in the form of fossil fuels. The energy requirement is due both to the elevated temperatures and pressures necessary for the reaction to take place and to the requirement for hydrogen, obtained by the high temperature cracking of fossil fuels. Consequently, a great deal of recent research effort has been directed toward developing potential alternative processes. In 1974 alone over 40 million tons (two billion moles) of ammonia were produced synthetically,[1] all by the Haber-Bosch process (1). An entirely different process occurs in the biosphere. In bacteria

and algae an enzyme, nitrogenase (N_2ase), catalyzes a process that is chemically and energetically different to the Haber-Bosch process:

$$12ATP + 6e^- + 6H^+ + N_2 \rightarrow 2NH_3 + 12ADP + 6P_i \qquad (2)$$

T = ambient temperature of the cell

P = atmospheric pressure

catalyst = nitrogenase.

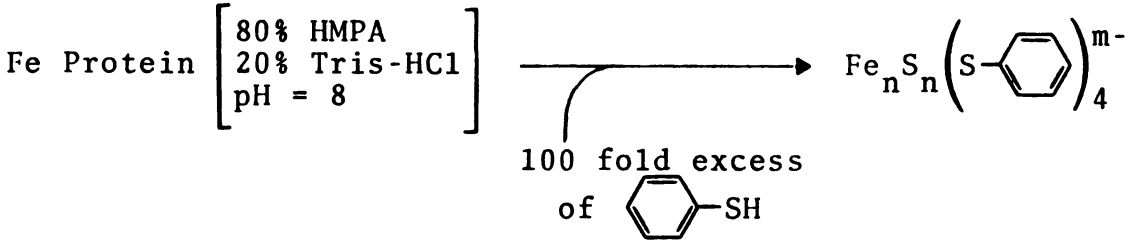
This specific and more efficient reaction (typical of most biological reactions) occurs with the transfer of six electrons and the dephosphorylation of the magnesium complex of the nucleotide, adenosine triphosphate. The fact that conservative estimates project that bacteria and algae produce 120 million tons of ammonia annually coupled with the high specificity and relative efficiency of the biological process indicate that this path is worth investigating. Understanding the catalyst, nitrogenase, is a necessary prerequisite to success in solving these economical and petrochemical problems. An in depth understanding of the enzyme's structural and mechanistic aspects is needed; until recently, research in this area has been hindered by the extreme oxygen sensitivity of nitrogenase.

Nitrogenase consists of two components: component I (the MoFe protein) and component II (the Fe protein).[2] The Fe protein has a molecular weight of about 60,000 and

is thought to contain two indistinguishable subunits.[3] Assays have shown the presence of 4Fe and $4S^{=}$ (acid labile sulfur) per molecule. Mossbauer[4] and displacement studies[6] (Figure 1) designate that the iron and sulfur are in the form of a single tetranuclear cluster.[5] The function of the Fe protein in dinitrogen fixation has been determined to be that of a one electron carrier and a binding site for MgATP.[7] However, the actual site of reduction of dinitrogen is not the Fe protein,[3] but the MoFe protein.

The MoFe protein, larger and more complex than the Fe protein, has a molecular weight of approximately 240,000; current data point to an $\alpha_2\beta_2$ structure. Assays indicate $\sim$32Fe, $\sim 32S^{=}$, and 1-2 molybdenum per molecule.[3] Unlike the relative degree of certainty that surrounds the Fe protein's structure, the distribution of Mo and Fe among the MoFe protein's subunits are unknown. Spectroscopic and displacement experiments[3] have shown the iron and sulfur to be contained in three distinct types of unit:[8] (1) P-clusters, which are tetranuclear species similar to the unit found in the Fe protein, but with one iron atom spectroscopically distinct from the others; (2) M_{EPR} clusters containing 6 to 8 Fe and 1 Mo, with the characteristic S = 3/2 EPR of the MoFe protein; and finally, (3) an unknown Fe-S species thought possibly to be dimeric (Fe_2S_2) units. Known iron-sulfur species exist in mono, di and tetranuclear forms and are prepared from simple

Figure 1. The displacement experiment[6] effects the removal of iron-sulfur (Fe-S) dimeric and tetrameric clusters from the Fe protein. Results of ligand substitution experiments[31] indicate that the Fe_4S_4 core has the greatest affinity for aryl thiol functionalities. The addition of HMPA and the Tris-HCl buffer denatures or unfolds the protein. With the protein in the unfolded state the thiol is able to enter the active site and begin the substitution process by protonating the biological ligand. Large excesses of thiophenol were employed to shift the substitution process equilibrium and favor formation of $Fe_nS_n(S\text{-}Ph)_4^{m-}$.



$Fe_nS_n(S-C_6H_5)_4^{m-}$ is a previously synthesized species, characterized spectroscopically ($n = 2, 4$; $m = 2$)

Figure 1

starting materials (Fe salt, $S^{=}$, thiolate). In fact they form readily and are stable for extended periods of time, provided their environment is strictly anaerobic. These iron-sulfur species are examples of true "synthetic analogues"[9] of biological systems. The facile synthesis, prolonged stability, and well-documented presence of these species in nitrogenase form the basis for the investigation of syntheses of potential "models" of the nitrogenase metal cofactors.

The presence of molybdenum in the MoFe protein is of significant catalytic importance. W. H. Orme-Johnson and coworkers have stated that the molybdenum is "presumed" to be part of the catalytic step, yet no direct evidence is available. They add, "no EPR ascribable to the Mo has been seen."[10] However, without molybdenum the enzyme is inactive. In a recent series of articles, Hodgson, Newton, and coworkers have employed the technique of EXAFS[11,12] (Extended X-ray Absorption Fine Structure) spectroscopy to investigate the environment surrounding the molybdenum of N_2ase. The end result was the reification of many previous ideas about the environment of the molybdenum centers in the MoFe protein of nitrogenase. The EXAFS studies suggest that the molybdenum in nitrogenase is surrounded by four sulfurs at "close" distances and approximately three irons at "near neighbor" distances; the molybdenum also has approximately three more

sulfurs at "less near neighbor" positions. Although the quantitative aspects of conclusions based on EXAFS are open to question, the qualitative information will aid in the postulation and subsequent preparation of a synthetic model or synthetic analogue of the metal cofactors of nitrogenase. The report of the isolation of the iron-molybdenum cofactor (FeMoco) of nitrogenase[13] followed closely by limited characterization data[14] (composition: ∿8Fe, ∿6$S^{=}$/Mo atom; molecular weight unknown), provided researchers with a stoichiometry and certain physical properties on which to base potential synthetic models.

Our efforts have focused on two specific areas. First was the combination of organometallic molybdenum complexes (specifically molybdocene derivatives) with iron-sulfur tetrameric clusters. The second area entailed designing a synthetic entity whose stoichiometry would closely parallel that reported for FeMoco. Researchers working in widely separated laboratories have recently synthesized and characterized molybdenum-iron-sulfur species[15,16] that are very similar; $[Mo_2Fe_6S_8(S\text{-}Ph)_9]^{3-}$ vs. $[Mo_2Fe_6S_9(SEt)_8]^{3-}$. Unfortunately these synthetic models do not mimic the FeMoco's stoichiometry. Lastly, an offshoot originating from attempts to solve the synthetic problems arising in the first two areas has led us to a potential synthetic model for the P-clusters of the MoFe protein of nitrogenase.

Knowledge of the biochemical background of nitrogenase is necessary to effect the design of potential metal cofactor models. Elucidation of the enzyme's complex mechanism depends, in part, on the study of synthetic species whose reactivity and structure model particular aspects of the nitrogenase enzyme. Indeed, solution of the problems surrounding the dinitrogen fixation process will not be possible until reasonable models for the metal cofactor are synthesized. This thesis describes the present state of progress in our laboratories toward synthesis and characterization of these models.

B. EXPERIMENTAL

1. Materials and Methods

Reagent grade tetrahydrofuran and toluene were distilled from sodium benzophenone ketyl. Acetonitrile and methanol were distilled from CaH_2 and $Mg(OCH_3)_2$, respectively. Heptane, hexanes, and toluene were distilled from sodium, while N-methylformamide was distilled *in vacuo* from BaO (bp 85°C, 10 mm Hg). All spectro-grade solvents (N,N-dimethylacetamide, acetonitrile) were used without further purification. All solvents, including deuterated solvents, were degassed by repeated evacuation and flushing with prepurified nitrogen gas (freed of O_2 and H_2O by passage through a hot tower containing BASF catalyst R-3-11 followed by passage through a tower containing Mallinckrodt moisture absorbent, Aquasorb) prior to use.

Titanocene dichloride was obtained from two sources: Dr. C. H. Brubaker, Jr. and Alfa Products. The titanocene dichloride was recrystallized from hot xylenes; after cooling to room temperature, hexanes were introduced until crystallization was imminent. All mercaptans were obtained from Aldrich Chemical Company, Inc. and used without further purification. Sodium hydrosulfide was synthesized by a literature method.[17] The 2,4,6-trimethylpyridine hydrochloride was prepared by bubbling

gaseous HCl through 2,4,6-trimethylpyridine and washing the precipitate with diethylether. The 2,4,6-trimethylpyridiniumhexafluorophosphate was prepared in a metathesis reaction with 2,4,6-trimethylpyridinehydrochloride and ammonium hexafluorophosphate. The product was checked for chloride with silver nitrate and recrystallized from isopropanol/hexanes. The tetraphenylarsonium salts of thiomolybdate and thiotungstate were recrystallized by H. C. Silvis, who also prepared the tetraethylammonium and the tetramethylammonium salts of these compounds and distilled the N-methylformamide used in these preparations. The tripod ligand, $OP(HN\text{-}C_6H_4\text{-}\underline{o}\text{-}SH)_3$, was synthesized and purified by Dr. B. A. Averill.

Electronic spectra were obtained on a Cary 17 spectrophotometer; proton magnetic resonance spectra were measured on a Varian T-60 spectrometer; and all melting points were determined on a Thomas-Hoover apparatus (melting points are uncorrected).

2. General Preparations

a. Synthesis and Purification of Precursors

Unless otherwise noted all operations were performed in prepurified nitrogen atmosphere.

i) Preparation of $Cp_2Ti(SCH_2CH_3)_2$.

The preparation was performed according to the methods of Koepf.[18] The reaction stoichiometry is:

$$Cp_2TiCl_2 + 2CH_3CH_2SH + 2Et_3N \xrightarrow{\text{toluene}} Cp_2Ti(SCH_2CH_3)_2 + 2Et_3NHCl \quad (3)$$

The product was recrystallized from toluene/heptane. Nmr data[58] were used to characterize the products: Koepf[18] (CS_2), 6.09($C_5\underline{H}_5$), 1.24($C\underline{H}_3$), 3.12($C\underline{H}_2$); found (d_6DMSO/CD_3CN), 6.02($C_5\underline{H}_5$), 1.05($C\underline{H}_3$), 2.98($C\underline{H}_2$). Intensity ratios for the peaks were 5:3:2.

ii) Preparation of $Cp_2Ti(SH)_2$.

The preparation was analogous to that of Koepf and Schmidt[19] according to the following reaction:

$$Cp_2TiCl_2 + 2H_2S + 2Et_3N \xrightarrow[\text{or toluene @ r.t.}]{Et_2O@20°C} Cp_2Ti(SH)_2 + 2Et_3NHCl \quad (4)$$

Characterization: mp, Koepf[19] 150-160°C (decomp), found 150-160°C (decomp); nmr, Koepf[19] ($CDCl_3$), 6.35($C_5\underline{H}_5$), 3.44 ($S\underline{H}$), found ($CDCl_3$), 6.35($C_5\underline{H}_5$), 3.45($S\underline{H}$), both with intensity ratio of 5:1. This reportedly air stable compound must be protected from both light and moisture during preparation and storage.

iii) Preparation of $Fe_4S_4(SR)_4^{2-}$ salts.

The method of Averill, et al.[20] was followed, except that the minimum volume of solvent necessary to dissolve the starting materials (approximately 50% of

the literature values) was employed. The salt, $(Et_4N)_2[Fe_4S_4(S\text{-}\underline{t}\text{-}Bu)_4]$, though not previously synthesized, was prepared with slight modifications of the above methods.

iv) Preparation of $(\phi_4As)_2MS_4$.

The preparation of $(WS_4^{=})$[21] and $(MoS_4^{=})$[22] followed their respective literature procedures. Progress of either reaction was monitored by changes in the electronic spectra. Hydrogen sulfide was passed through the solution until no traces of the peaks due to $MOS_3^{=}$ remained. Recrystallization was effected from an acetonitrile/methanol mixture.

v) Preparation of $(Et_4N)_2MS_4$ and $(Me_4N)_2MS_4$.

Either MoO_3 or H_2WO_4 was dissolved in an aqueous solution of Et_4NOH or Me_4NOH. H_2S was slowly bubbled through these solutions until absence of peaks due to the oxo species in the optical spectrum indicated completion of the reaction. Recrystallization was effected from a water/methanol mixture.

vi) Preparation of the tripod ligand, $OP(HN\text{-}C_6H_4\text{-}\underline{o}\text{-}SH)_3$.

This ligand was prepared in three steps from $\underline{o}$-aminothiophenol: (1) protection of the mercaptide with benzylchloride in basic solution to yield the benzylthioether; (2) condensation of the amine with hexamethylphosphoramide in refluxing $\underline{p}$-cymeme; (3) removal of the

S-benzyl protecting groups with sodium in liquid ammonia.

b. Method of Removal of Coordinated Thiolate Ligand from an Fe-S Tetrameric Cluster

$$Fe_4S_4(SR)_4^{2-} + Me_3pyrHCl \rightarrow Fe_4S_4(SR)_3^{1-} + RSH + Me_3pyr + Cl^- \quad (5)$$

A five dram vial equipped with a tight fitting rubber serum cap and a small magnetic stirring bar was repeatedly purged through a syringe needle fitted to a dry nitrogen/vacuum line. Carefully 250 mg (0.17 mmole) of $(\phi_4As)_2[Fe_4S_4(S\text{-}\underline{t}\text{-}Bu)_4]$ was introduced, and the vial was repeatedly purged with nitrogen. The same procedure was followed for the addition of 90 mg (0.34 mmole) of 2,4,6-trimethylpyridiniumhexafluorophosphate (2,4,6-$Me_3pyrHFP_6$). Degassed deuterated acetonitrile was introduced via anaerobic syringe into the vials (final concentrations: tetramer 0.05 M, pyridinium salt 0.30 M), and the solutions were stirred until dissolution was complete. As a check of the concentration and purity of the tetrameric species 20 µl was removed from the stock solution, diluted to 500 µl with degassed spectro-grade acetonitrile, and the electronic spectrum recorded. Upon observation of satisfactory concentration and absorption features,[23] 300 µl and 500 µl of the respective tetrameric and pyridinium[24]

species were introduced via syringe into another similarly prepared two dram vial. Mixing was continued for several minutes, and the reaction mixture was withdrawn from the vial and introduced into a 5 mm nmr tube. The nmr tube was previously fitted with an appropriate rubber serum cap and purged with nitrogen. This is the general procedure for preparation of samples for optical or nuclear magnetic resonance spectroscopy.

c. Method for the Preparation of the Metallocene-Bound Tetrameric Species

$$Fe_4S_4(SR)_4^{2-} + nCp_2Ti(SEt)_2 + nMe_3pyrHCl \rightarrow \{Fe_4S_4(SR)_{4-n}[(SEt)_2TiCp_2]\}_n^{(2-n)-} + nRSH + nMe_3pyr + nCl^- \quad (6a)$$

$$Fe_4S_4(SR)_4^{2-} + nCp_2Ti(SH)_2 + nEt_3N \rightarrow \{Fe_4S_4(SR)_{4-n}(S_2TiCp_2)_n\}^{(2+n)-} + nRSH + nEt_3NH^+ \quad (6b)$$

Reaction (6a). In a 100 ml round bottom flask equipped with a side arm, 2.6 grams (1.8 mmole) of $(\phi_4As)_2[Fe_4S_4(S\text{-}\underline{t}\text{-}Bu)_4]$ was dissolved in 36 mls of degassed acetonitrile. The ligand, $Cp_2Ti(SEt)_2$ (0.5 gram, 1.8 mmole), was dissolved in 6 mls of degassed spectro-grade dimethyl sulfoxide and then repeatedly degassed.

The proton donor, 2,4,6-trimethylpyridiniumhexafluorophosphate (0.5 gram, 1.8 mmole), was similarly dissolved in 6 mls of dry degassed acetonitrile. The ligand and proton donor were sequentially introduced via cannula with subsequent degassing. The proton donor was added dropwise over a period of 20-30 minutes. Stirring was continued overnight and the resulting crystals were filtered and dried *in vacuo*.

Reaction (6b). A two dram vial equipped with a tight fitting rubber serum cap and a small stirring bar magnet was repeatedly purged with nitrogen. Then 75 mg (50 mmole) of $(\phi_4As)_2[Fe_4S_4(S\text{-}\underline{t}\text{-}Bu)_4]$ was carefully added, and the vial was again degassed. A similar procedure was used for preparation of a $Cp_2Ti(SH)_2$ sample (61 mg, 0.25 mmole). One milliliter of 100 atom percent d_6-DMSO was added to the tetramer (final concentration, 0.05 M) and 0.5 ml to the titanocene derivative (final concentration, 0.5 M).

Concentration of the tetrameric cluster was checked by inspection of an electronic spectrum,[23] and aliquots were added to an anaerobic 5 mm nmr tube equipped with a rubber serum cap. To the previously prepared nmr tube were added in the following sequence: 500 µl (2.5×10^{-5} moles) of tetramer, 500 µl (2.5×10^{-5} moles) of $Cp_2Ti(SH)_2$, 5 µl (3.5×10^{-5} moles) of Et_3N, and 1 drop (10-15 µl) of TMS. The tube was agitated vigorously on a vortex mixer.

d. Method for the Preparation of the Thiomolybdate-Bridged Species

$$2Fe_4S_4(SR)_4^{2-} + MoS_4^{=} + 2Me_3pyrHCl \rightarrow (SR)_3Fe_4S_4(S_2MoS_2)Fe_4S_4(SR)_3^{4-} + 2Me_3pyr + 2Cl^- \quad (7)$$

To a 50 ml round bottom flask equipped with a side arm was added 700 mg of $(\phi_4As)_2[Fe_4S_4(S\text{-}\underline{t}\text{-}Bu)_4]$. Eight mls of previously degassed spectro-grade acetonitrile were introduced via cannula into the flask. The solution was stirred until dissolution was complete, and then degassed several times. Stock solutions of $(\phi_4As)_2MoS_4$ (7 mg in 2 ml CH_3CN) and 2,4,6-Me_3pyrHCl (25 mg in 4 mls CH_3CN) were also prepared in this manner. With a gas tight syringe 40 µl (2.38×10^{-6} mole) of the tetrameric stock solution was diluted to 1000 µl and introduced into a 2 dram vial equipped with a magnetic stirring bar and connected to a dry nitrogen/vacuum line. The thiomolybdate solution (300 µl, 1.05×10^{-6} mole) was then introduced via syringe with stirring. An aliquot (60 µl, 2.37×10^{-6} mole) of the 2,4,6-trimethylpyridinium chloride solution was then introduced dropwise via syringe with stirring.[24]

e. Method for the Preparation of the Tripod-Bound Species

$$[Fe_4S_4(S\text{-}\underline{t}\text{-}Bu)_4]^{2-} + OP(HN\text{-}C_6H_4\text{-}\underline{o}\text{-}SH)_3 \rightarrow$$
$$\{Fe_4S_4(S\text{-}\underline{t}\text{-}Bu)[(S\text{-}C_6H_4\text{-}NH)_3PO]\}^{2-} + 3\underline{t}\text{-}Bu\text{-}SH \qquad (8)$$

In a 50 ml round bottom flask equipped with a side arm, 0.5 gram (5.8×10^{-4} mole) of $(Me_4N)_2[Fe_4S_4(S\text{-}\underline{t}\text{-}Bu)_4]$ was dissolved in 35 mls of acetonitrile. In another similarly equipped 100 ml flask 0.25 gram (5.8×10^{-4} mole) of $OP(NH\text{-}C_6H_4\text{-}\underline{o}\text{-}SH)_3$ (tripod) was slurried in 25 mls of acetonitrile. The tetrameric cluster was added to the tripod slurry via cannula. The stirring was continued overnight and the mixture was occasionally evacuated to remove t-Bu-SH (bp. 62-65°C) to force the equilibrium to the right and favor formation of the tripod-bound species. The acetonitrile was removed at 40-50°C and the subsequent solid (a fine silky material with metallic lustre) was dried *in vacuo*. Recrystallization from acetonitrile was twice attempted.

3. Analyses

a. Micro-Assay for Molybdenum[25] (aerobically performed in a 20 ml test tube)

Required solutions:

i) 30% H_2O_2

ii) 4 N HCl

iii) KI (prepared as needed); 2.5 grams/5.0 mls deionized H_2O.

iv) Ascorbic acid, 20% (w/v), 20 grams acid/ 100 mls deionized H_2O.

v) Tartaric acid, 40% (w/v), 40 grams acid/ 100 mls deionized H_2O.

vi) Dithiol (3,4-mercaptotoluene): 0.5 grams of dithiol is melted and dissolved in 190 mls of deionized H_2O previously degassed with nitrogen. Add 60 mls of 1 N NaOH also degassed with nitrogen. Thioglycolic acid[26] is added dropwise (2-4 mls) until the first faint opalescence is observed.

Procedure:

i) To the Mo sample is added 100 µl of deionized H_2O and 100 µl of concentrated H_2SO_4. The solution is heated slowly at first, then vigorously with care. This step is performed in an effort to digest the extraneous material present and to "free" the Mo. It must be repeated until the entire sample is soluble in the H_2SO_4/ H_2O solution, therefore when necessary 100 µl aliquots of a 5:2:1 solution of HCl/HNO_3/HBr were added, and the mixture heated until clear[27] and one was certain dissolution was complete.

ii) After cooling the sample, 25 µl of 30% H_2O_2 is introduced. The solution is heated with care.

iii) After cooling the solution, 7 mls of 4 N HCl, 350 µl of the KI solution and a micro-stirring bar magnet are added and the solution stirred for 10 minutes.

iv) 500 µl ascorbic acid solution, 250 µl of Tartaric acid solution, and 500 µl of the dithiol solution are added, and the solution is stirred for 10 minutes.

v) 650 µl of iso-amylacetate is introduced, the mixture stirred for 10 minutes, and the organic layer pipetted into a cuvette. The Mo complex absorbs at 680 nm with a molar absorbtivity (ε) of 24,400 $M^{-1}cm^{-1}$.

b. Iodimetric Titration of the Tripod Ligand

Preparation of solutions and the general procedure were adapted from standard sources.[28,29]

Preparation of the tripod ligand as a sample was as follows:

i) To a 250 ml Erlenmeyer flask was added 0.05 gram of the tripod ligand weighed to ±0.1 mg.

ii) 15 ml of N,N-dimethylacetamide and 15 ml of deionized H_2O was added and the resulting mixture stirred until the solution was clear.

iii) 10 ml of starch solution was added and the mixture titrated against the standardized iodine solution until a pale blue color was just visible.

C. RESULTS AND DISCUSSION

1. Removal of the Coordinated Thiolate Ligand from an Fe-S Tetrameric Cluster (Reaction 3)

Combination of $[Fe_4S_4(S\text{-}\underline{t}\text{-}Bu)_4]^{2-}$ (tetramer) with thiomolybdate does not result in an observable chemical reaction[30] (Figures 2a and 2b). The desired result would be easily detected by nuclear magnetic resonance spectroscopy as the free thiolate ligand in the equilibrium,

$$[Fe_4S_4(S\text{-}\underline{t}\text{-}Bu)_4]^{2-} + L^* \rightleftarrows [Fe_4S_4(S\text{-}\underline{t}\text{-}Bu)_3L^*]^{1-} + \underline{t}\text{-}Bu\text{-}S^- \qquad (9)$$

The equilibrium apparently favors the reactants; implying that the proposed ligands have a lower affinity than $\underline{t}$-Bu-S^- for the iron-sulfur tetranuclear (Fe_4S_4) core. This is significant because the $\underline{t}$-butyl-mercaptide ion exhibits the least affinity, of all mercaptides examined,[31] for the Fe_4S_4 core. To bind thiomolybdate or the metallocene ligands to the Fe_4S_4 core, it is therefore necessary to force the equilibrium to the right. The difference between the exchange reaction

$$Fe_4S_4(SR)_4^{2-} + R'SH \rightleftarrows Fe_4S_4(SR)_3(SR')^{2-} + RSH \qquad (10)$$

and reaction (9) is that the incoming ligand in the

Figures 2a and 2b. Each figure is a nuclear magnetic resonance spectrum in d_6-DMSO (99%-d). Figure 2a is the control spectrum of the tetramer, $(\phi_4As)_2[Fe_4S_4(S\text{-}\underline{t}\text{-}Bu)_4]$. The resonance at $\delta = 7.73$ corresponds to the aryl protons; the resonance at $\delta = 2.50$ corresponds to the solvent impurity peak. Figure 2b is a spectrum of tetramer and $(\phi_4As)_2MoS_4$ [2:1]. The two resonances present in Figure 2a are visible (aryl, $\delta = 7.72$; impurity, $\delta = 2.48$), however the desired reaction has not taken place as substantiated by the lack of "free" $\underline{t}$-butyl-thiol proton resonances ($\phi \underset{\sim}{} 1.2$).

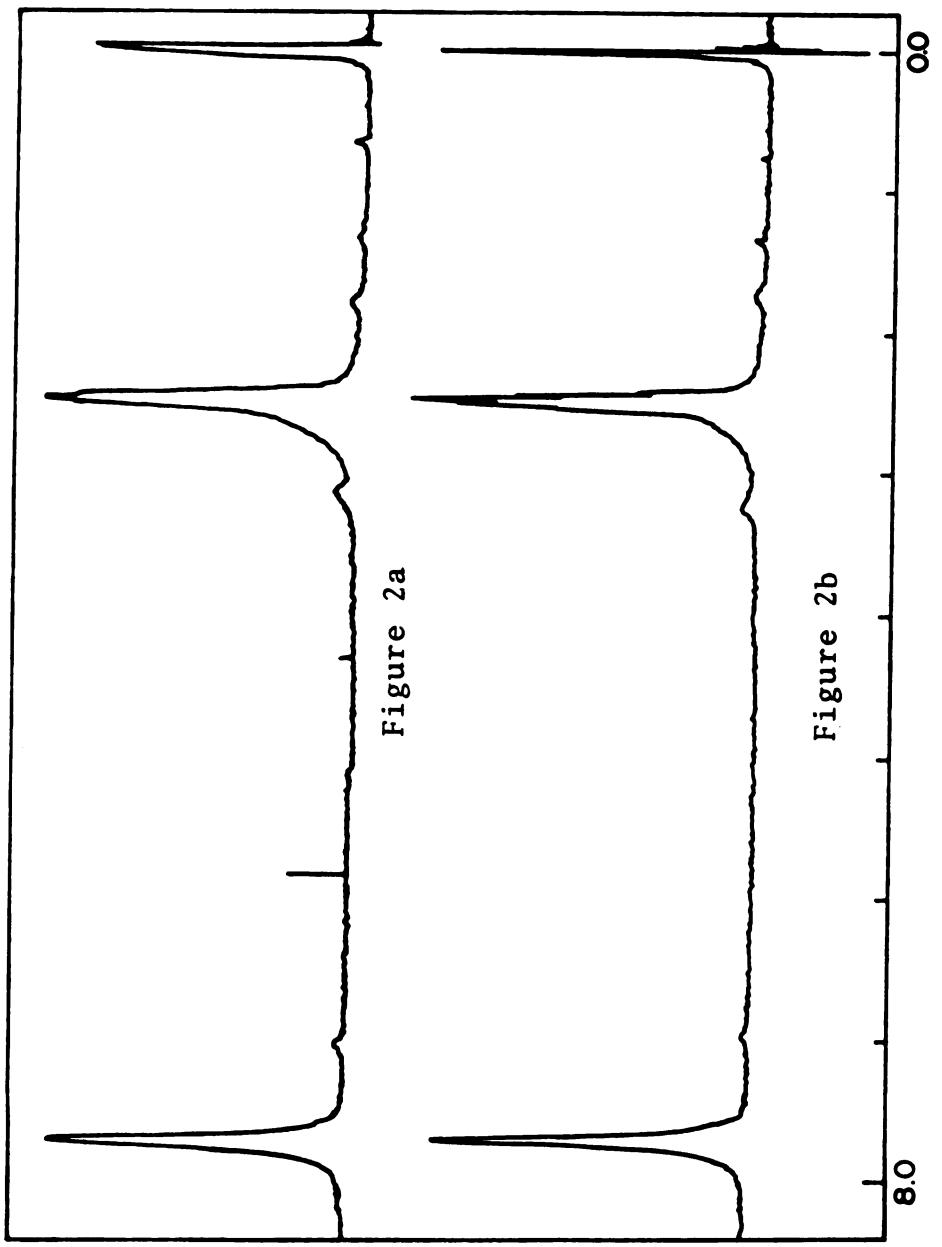


Figure 2a

Figure 2b

exchange reaction protonates the species that is leaving.[32] This suggests that the unfavorable equilibrium of reaction (9) might be driven to the right by adding a species that can donate a proton when the entering ligand cannot perform this function. To prevent coordination to the Fe_4S_4 core, the conjugate base of the proton donor selected must be a poor nucleophile. Further, the proton donor must not be a sufficiently strong acid to destroy the acid sensitive tetranuclear cluster.[33]

The donor, 2,4,6-trimethylpyridiniumhexafluorophosphate ($Me_3pyrHPF_6$), was selected for a variety of reasons. Although pyridine has a relatively low affinity for both iron (II) and iron (III),[34] 2,4,6-trimethylpyridine was used to introduce a steric factor that would further hinder binding during evaluation of this novel technique. In addition, the report of the structure of the chloro-tetramer[35] $(Fe_4S_4Cl_4)^{2-}$ and the lack of characterization data prompted the use of the hexafluorophosphate anion in lieu of chloride. Comparison of physical data was not possible until subsequent publication of the electronic spectra of the halo-clusters.[36] The primary visible absorption peak of the tetramer with alkylthiolate ligands ($\sim$420 nm) shifts to longer wavelength and is reduced in intensity on replacement of thiolate by chloride ion. This effect was not observed during the course of experiments with donors such as 2,4,6-trimethylpyridine

hydrochloride ($Me_3pyrHCl$). Comparison of spectra (Figure 3) reveals that the primary absorption peak of the tetramer does not decrease in intensity and instead simply shifts to ∿400 nm when an equivalent amount of pyridinium chloride is added. The chloro-tetramer is clearly not formed under these conditions, however, so the use of $Me_3pyrHCl$ and $Me_3pyrHPF_6$ are intermixed throughout this text. The Me_3pyrH^+ has a pK_a of 7.4[37] and therefore provides two orders of magnitude less driving force than the pyridinium ion (pK_a = 5.4) in forcing the equilibrium reaction (9) in the desired direction. This does not limit the ability of the Me_3pyrH^+ to favorably offset the equilibrium, as observation of the release of free thiolate in Figure 4 attests. In addition, the repeated evacuation and purging of the reaction vessel will also shift the equilibrium through the removal of the more volatile alkylthiols(t-butylthiol, bp 62-65° at 760 mm). Further, Me_3pyrH^+ is a sufficiently weak acid that, under these conditions, it can aid the coordination of these bidentate metallocene or tetrathiometallate species without initiating decomposition of the tetramer or the potential ligands. Also, while Me_3pyr is a better base than pyridine, the steric hindrance introduced by the o-methyl functions should prevent competition between the base and our least nucleophilic ligand, $Cp_2Ti(SEt)_2$, for the available sites on the Fe_4S_4 core.

Figure 3. The electronic spectra display the changes in the primary absorption peak of $(\phi_4As)_2[Fe_4S_4(S\text{-}\underline{t}\text{-}Bu)_4]$ upon the addition of a proton donor (pyridinium chloride). In the control spectrum (tetramer in acetonitrile, —•—•—) the $(\phi_4As)_2[Fe_4S_4(S\text{-}\underline{t}\text{-}Bu)_4]$ absorbs at 420 nm initially. On addition of a proton donor the 420 nm absorption shifts to higher energy (tetramer and pyridinium chloride in acetonitrile, ———) and absorbs at 400 nm.

Figure 3

Figures 4a and 4b. Each figure is a nuclear magnetic resonance spectrum in d_3-acetonitrile (99%-d). Figure 4a is the control spectrum of the tetramer, $(\phi_4As)_2[Fe_4S_4(S\text{-}t\text{-}Bu)_4]$. Figure 4b exhibits the addition of 1.1 equivalents of 2,4,6-trimethylpyridiniumhexafluorophosphate to 1.0 equivalent of the tetramer.

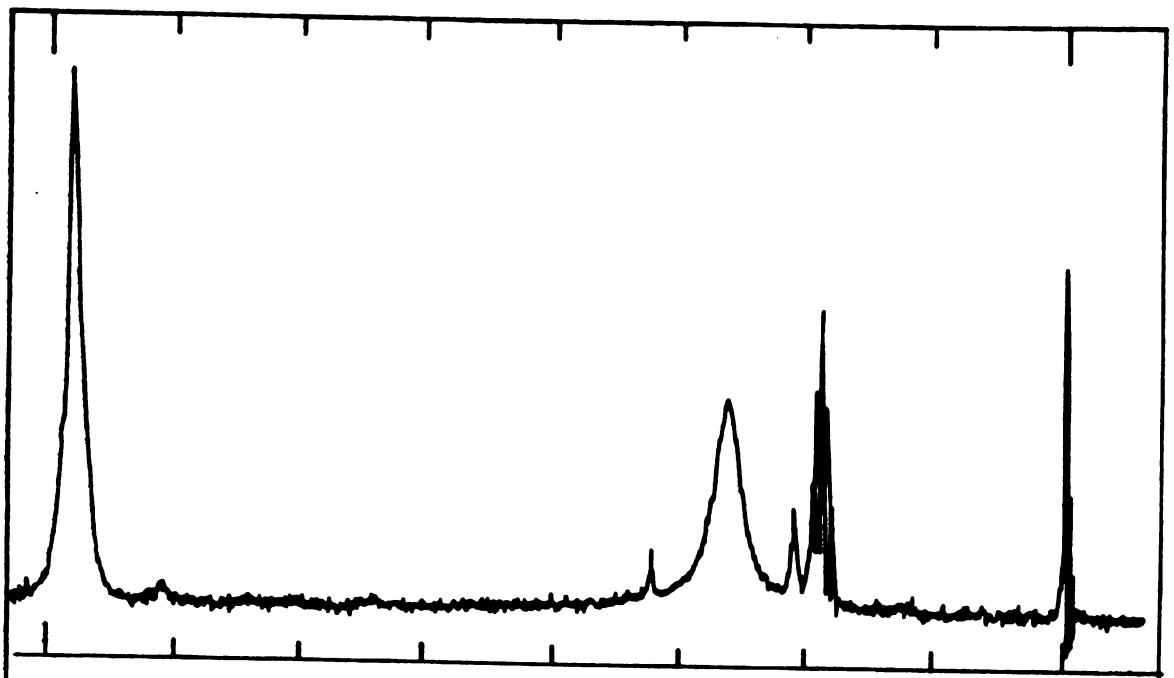


Figure 4a

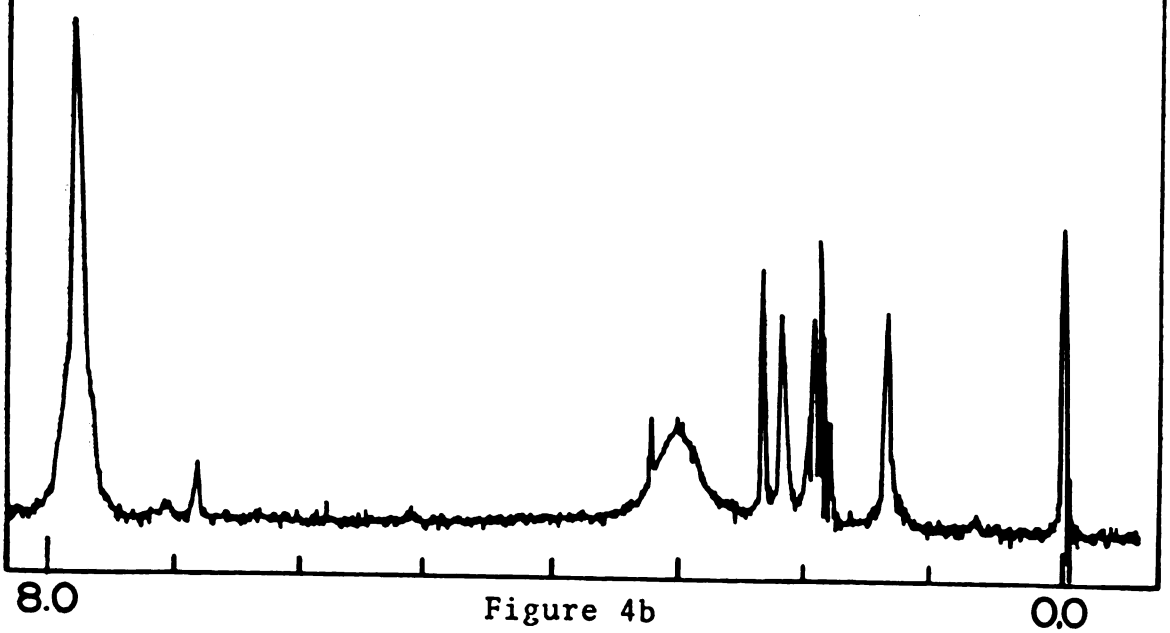


Figure 4b

Figures 4a and 4b display the release of the free thiol when $Me_3pyrHPF_6$ is combined with tetramer. Figure 4a shows the nmr spectrum of the t-butyl tetramer [d_3-acetonitrile; 7.70 ($C_6\underline{H}_5$), 2.66 (bound t-butyl-S)$^-$; 1.92, 2.11, 3.22 (solvent impurities)]; when compared to the nmr spectrum of a combination of tetramer and 1.1 equivalents of $Me_3pyrHPF_6$ in Figure 4b [d_3-acetonitrile; 6.72 (pyr$\underline{H}$), 2.35 ($\underline{o}$-Me), 2.20 ($\underline{p}$-Me), 1.36 (free thiol)], the ease with which the thiolate ligand is removed and observed is apparent. Integration of the spectrum in Figure 4b reveals that 1.1 equivalents of thiol have been released by addition of 1.1 equivalents[38] of proton donor, $Me_3pyrHPF_6$.

The utility of this technique will depend on the following considerations:

a. The ability of the solvent to stabilize the Fe_4S_4 core once the ligand is released.

b. The difference in pK_a between the leaving ligand and the proton donor.

c. There is at present no way to limit the obvious statistical distribution of products that will result from having four structurally equivalent sites available for coordination. The $Fe_4S_4(SR)_3^{1-}$ was observed to decompose on standing; consequently, release of thiolate from more than one site of an Fe_4S_4 cluster appears to accelerate the decomposition due to the

increased instability of the core. As a necessary part of the technique, a dilute solution of the proton donor must be added slowly to a more concentrated mixture of the potential ligand and tetrameric cluster. This insures the proximity of the ligand to the coordination sites as they are made available.

d. The success of any of these potential ligands as a coordinating entity will be limited by its nucleophilicity compared to that of the solvent and of the conjugate base of the proton donor. The selection of solvent, ligand, and donor must therefore be made with careful consideration of each species' relative nucleophilic capabilities.

The results of the application of this new technique follow.

2. The Metallocene-Bound Species

The first application of the technique described above involved dithiolate derivatives of titanocene. Since certain metallocenes were catalysts for early dinitrogen reduction experiments[39] and Fe_4S_4 clusters were documented as being part of the overall mechanism for dinitrogen reduction to ammonia,[3] the combination of the two entities became the subject of a synthetic investigation. Also, evidence concerning the role of molybdenum in the nitrogen fixation mechanism,[10] and recent structural

evidence[11,13] supporting the existence of Fe-S-Mo segments in nitrogenase, suggested coupling a molybdenum derivative to an Fe_4S_4 cluster. The preparation of any $Cp_2Mo(SR)_2$ species, however, is tedious, requiring a low yield synthesis of oxygen-sensitive Cp_2MoH_2 followed by conversion of Cp_2MoCl_2 (soluble only in liquid SO_2). Final conversion of the dichloride to the dithiolate involves yet another low-yield synthetic step. Titanium was utilized as a substitute for molybdenum to circumvent the synthetic problems surrounding the Mo analog. The initial plan was to work out the synthetic details with titanium analogs, whose preparation is more straightforward and inexpensive.[18,19] Since both metallocene dithiolates have shown the ability to coordinate in a bidentate manner to other metal centers,[40] extension of the synthetic scheme developed for titanium derivatives to molybdenum was expected to require only minor modifications.

a. bis(η_5-cyclopentadienyl)bis(ethanethiolate)-titanium (IV)

$$Fe_4S_4(SR)_4^{2-} + nCp_2Ti(SEt)_2 + nMe_3pyrHPF_6 \rightarrow Fe_4S_4(SR)_{4-n}[(S\text{-}Et)_2TiCp_2]_n^{(2-n)-} + nRSH + nMe_3pyr + nPF_6^- \quad (11)$$

Figure 5. Proposed structure for the metallocene-bound species (a).

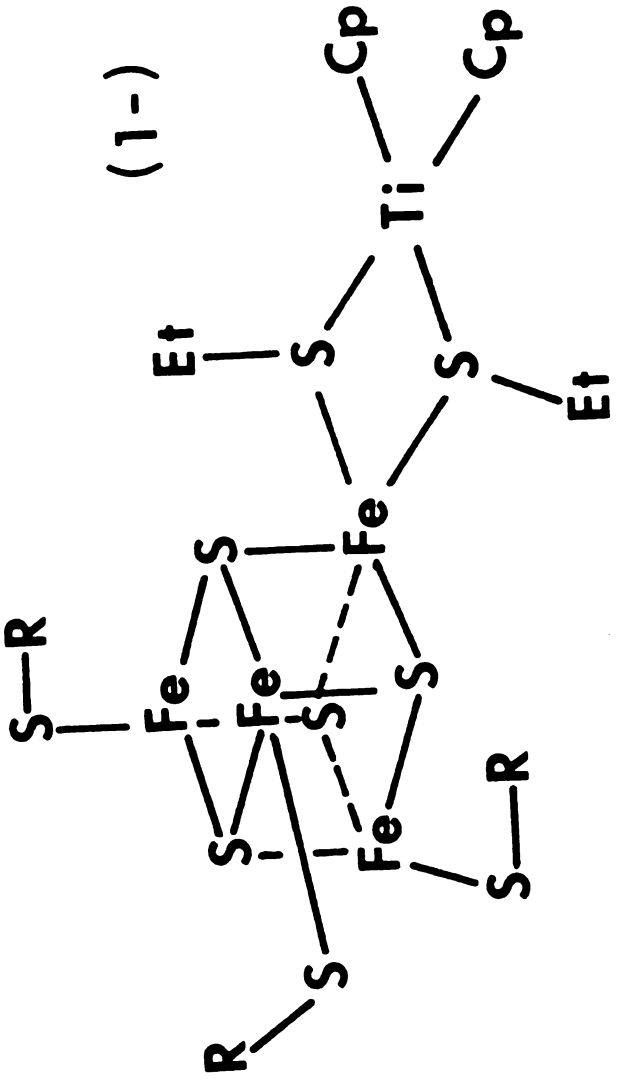


Figure 5

The $Cp_2Ti(SEt)_2$, selected because of its inexpensive starting materials and ease of preparation, acts as a neutral ligand in reaction (10). As described above, $Me_3pyrHPF_6$ is used to force an equilibrium shift and increase the likelihood of coordination. Experiments monitored by nmr and optical spectroscopy revealed neither a shift of the cyclopentadienyl or bound thiol resonances nor any change in the primary electronic absorption (417 nm, ε = 17,400 $M^{-1}cm^{-1}$) of the tetrameric cluster. A carefully designed nmr experiment showed inconsistencies in the integrations of the important proton-bearing functionalities (Figure 6). Figure 6a (d_6-DMSO) is an nmr spectrum of a mixture of 1.0 equivalent of $Cp_2Ti(SEt)_2$ [6.04 ($C_5\underline{H}_5$), 2.93 ($C\underline{H}_2$), 1.00 ($C\underline{H}_3$)] and 1.2 equivalents of $Me_3pyrHPF_6$ [7.40 (m-H), 2.56 (o-Me), 2.35 (p-Me)]. Figure 6b (d_6-DMSO) is an nmr spectrum of a solution originally containing 1.0 equivalent of $(\phi_4As)_2[Fe_4S_4(S\text{-}\underline{t}\text{-}Bu)_4]$, $Me_3pyrHPF_6$, and $Cp_2Ti(SEt)_2$. The intensities of the Me_3pyr and tetramer peaks agree with the expected 1:1 ratio, yet only 0.55 equivalents of $Cp_2Ti(SEt)_2$ are observed. The resonances of the metallocene ligand were not altered in position [6.10 ($C_5\underline{H}_5$), 1.02 ($C\underline{H}_3$), the ($C\underline{H}_2$) quartet was obscured by the bound t-butyl thiolate], and those of the conjugate base of the proton donor were not shifted either [6.78 (m-H), 2.34 (o-Me), 2.19 (p-Me); literature[41] $CDCl_3$, 6.59 (m-H), 2.37 (o-Me), 2.18 (p-Me)].

Figures 6a and 6b. Each figure is a nuclear magnetic resonance spectrum. Figure 6a is the control spectrum of 1.0 equivalent of ligand, $Cp_2Ti(SEt)_2$, and 1.2 equivalents of proton donor, 2,4,6-trimethylpyridiniumhexafluorophosphate in d_6-dimethylsulfoxide (100%-d). Figure 6b is a 1.0:1.0:0.55 equivalents ratio of tetramer, $(\phi_4As)_2[Fe_4S_4(S\text{-}t\text{-}Bu)_4]$, ligand and proton donor in d_6-dimethylsulfoxide. The resonances of either the ligand or the conjugate base of the proton donor were not shifted.

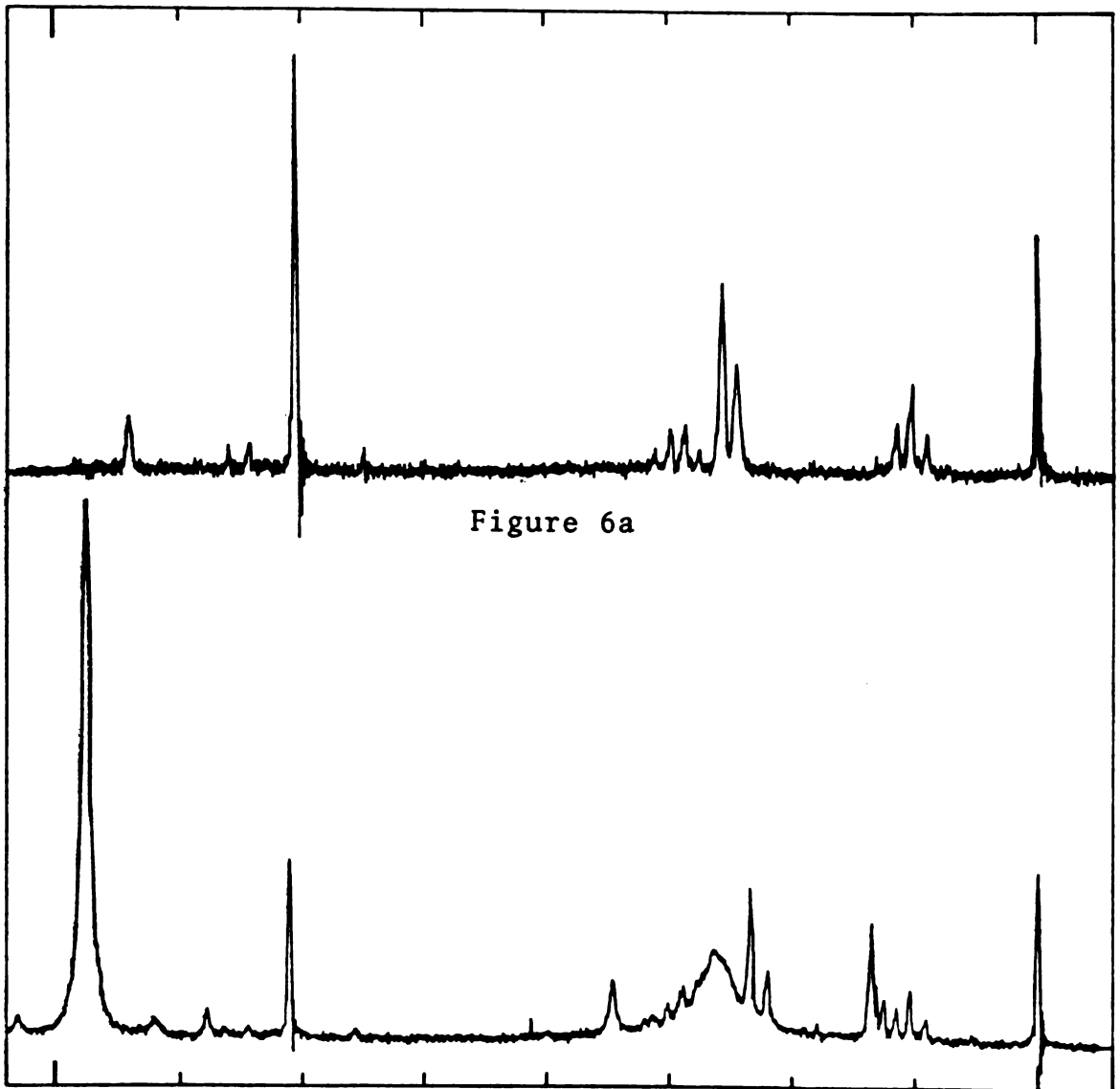


Figure 6a

Figure 6b

Therefore coordination of either the metallocene ligand or Me_3pyr is unlikely. But 0.5 equivalent of free thiol was released [1.35 ($C\underline{H}_3$)] in Figure 6b! Proximity of protons to a paramagnetic species can result in the loss of splittings[42] and shifting and broadening of peaks into the baseline,[43] suggesting that the cluster-bound metallocene might not be detectable by nmr. This last rationale was initially accepted as an explanation for the inconsistencies in the proton integrations. It was then discovered that the nmr solutions formed a dark microcrystalline precipitate on standing undisturbed for ten hours. Workup of the products of a preparative scale experiment resulted in isolation of an apparently microcrystalline substance that was insoluble[44] and pyrophoric. Further characterization proved to be impractical. This suggests that the "missing" titanocene derivative (that not observed in nmr) is being removed from solution by a reaction with (or catalyzed by) a small portion of the iron-sulfur cluster, with formation of the uncharacterized solid residue.

b. bis(η_5-cyclopentadienyl)bis(hydrogensulfide)-titanium (IV)

$$Fe_4S_4(SR)_4^{2-} + nCp_2Ti(SH)_2 + nEt_3N \rightarrow$$
$$Fe_4S_4(SR)_{4-n}(S_2TiCp_2)_n^{(2+n)-} + nRSH + nEt_3NH^+ \qquad (6b)$$

Figure 7. Proposed structure for the metallocene-bound species (b).

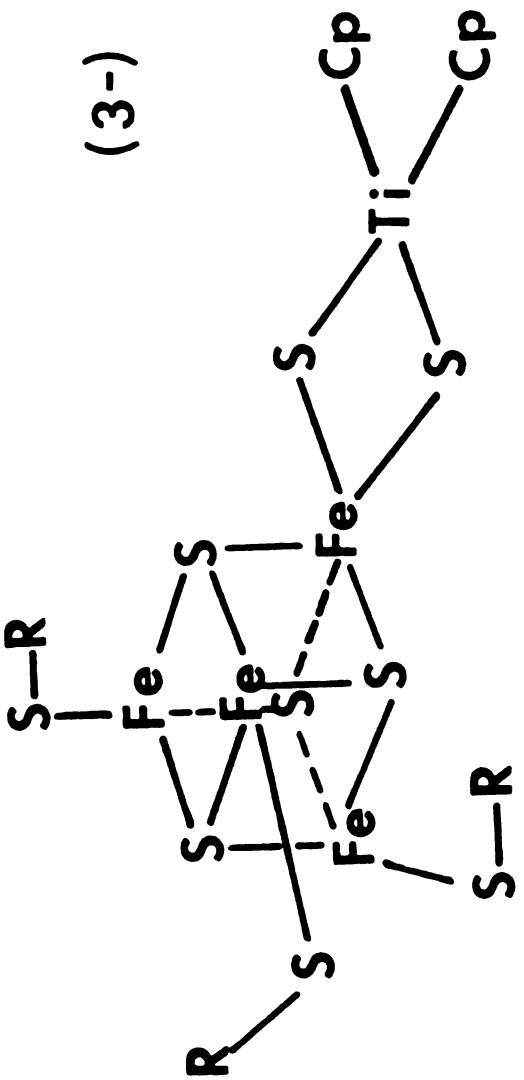


Figure 7

$Cp_2Ti(SH)_2$, whose binding to the Fe_4S_4 cluster was examined for reasons mentioned above, is unique among the ligands studied in that it is a diprotic acid, formally capable of donating a proton to the departing thiolate ligand; the removal of the second proton requires an additional equivalent of base. This will result in the titanocene derivative acting as a dinegative bidentate ligand. Upon loss of a proton, the nucleophilicity of $Cp_2Ti(SH)_2$ should be enhanced compared to $Cp_2Ti(SEt)_2$; therefore, the former should have a greater tendency to coordinate to the Fe_4S_4 core. This trend was not observed, however. Much like $Cp_2Ti(SEt)_2$, coordination studies with the bis(hydrogensulfide) derivative did not result in the observation of any shifts in the nmr peaks of either the C_5H_5 or bound t-butyl; further, no shifts or changes in intensity of optical spectral features were observed. Most importantly, a free t-butyl peak, indicative of release of coordinated thiolate, was not observed on mixing the components. This last point initiated experiments to abstract one of the thiol protons of $Cp_2Ti(SH)_2$ to generate a better nucleophile. Two common nitrogen bases were used: triethylamine (pK_b = 3.00) and proton sponge (N,N,N',N'-tetramethyl-1,8-naphthalenediamine, pK_b = 1.63);[45] neither apparently was able to abstract a proton from $Cp_2Ti(SH)_2$. Figure 8a [$CDCl_3$, 6.20 ($C_5\underline{H}_5$), 3.33 (S$\underline{H}$), intensity ratio 5:1] is that of

$Cp_2Ti(SH)_2$; the proton resonances integrate well but are shifted slightly upfield from the literature values. In Figure 8b [$CDCl_3$, 6.20 ($C_5\underline{H}_5$), 3.33 (S$\underline{H}$), 2.53 ($C\underline{H}_2$), 1.03 ($C\underline{H}_3$), intensity ratios 5:1 and 2:3] where 0.6 equivalents of triethylamine were added to the titanocene derivative, there is no shift of either the titanocene or the triethylamine resonances,[46] or is the intensity of the thiol proton resonance altered. The spectrum in Figure 8c [$CDCl_3$, ca 7.2 (3,4,5,6-$\underline{H}$), 6.90 (2,7-$\underline{H}$), 6.10 ($C_5\underline{H}_5$), 3.26 (S$\underline{H}$), 2.75 (Me) with intensity ratios 5.1:1 for the titanocene derivative and 12:2.6:4 for the proton sponge] is that of a solution containing 3.8 equivalents of proton sponge per titanocene dithiolate; again the chemical shifts and intensities correspond to those of the reactants.[47]

The above data suggest, that of the following equilibria

$$Cp_2Ti(SH)_2 \rightleftarrows Cp_2Ti(SH)S^- + H^+ \qquad pK_a > 13.0 \qquad (12)$$

$$Cp_2Ti(SH)S^- \rightleftarrows Cp_2Ti(S)_2^{=} + H^+ \qquad (13)$$

the first dissociation must be difficult, requiring an organic base with a $pK_b < 1.0$ to remove the first proton. Since the acidity of $Cp_2Ti(SH)_2$ has not been investigated, the conditions necessary for the removal of the second

Figures 8a, 8b, and 8c. Each figure is a nuclear magnetic resonance spectrum in d_1-chloroform (100%-d). Spectrum 8a is the control spectrum of $Cp_2Ti(SH)_2$ with the two resonances in an intensity ratio of 5:1 and identified in units of δ (delta). Spectrum 8b indicates that addition of triethylamine does not effect the removal of the thiol proton of $Cp_2Ti(SH)_2$, intensity ratios: 5:1 for $Cp_2Ti(SH)_2$ and 2:3 for triethylamine. Spectrum 8c indicates that addition of proton sponge does not effect the removal of the thiol proton of $Cp_2Ti(SH)_2$ either, intensity ratios: 5.1:1, $Cp_2Ti(SH)_2$ and 12:26:4 for proton sponge.

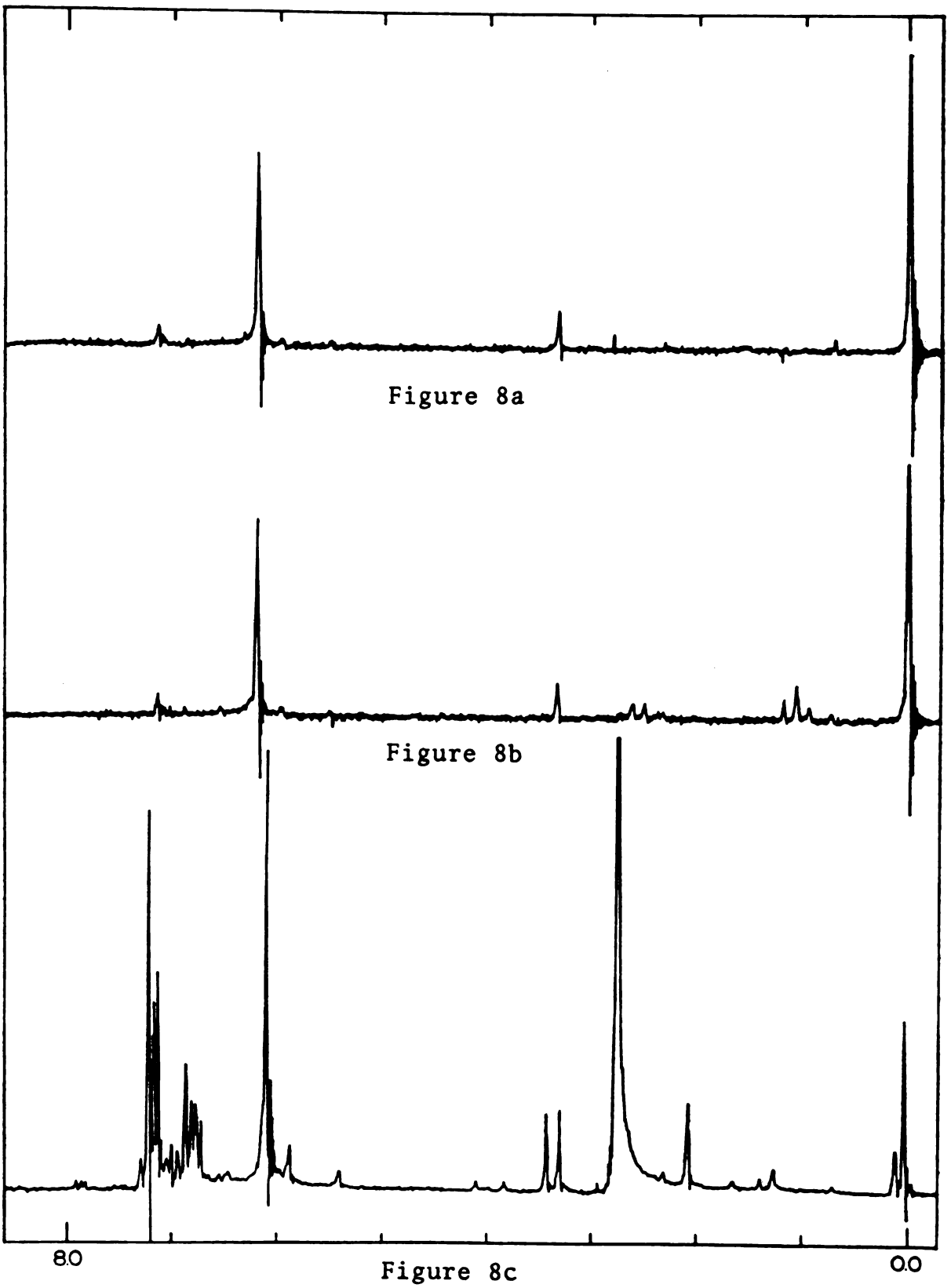


Figure 8a

Figure 8b

Figure 8c

thiol are unknown and could result in the destruction of the metallocene. However, if the $Cp_2Ti(SH)_2$ is to bind to the Fe_4S_4 core, its thiol protons must exhibit facile exchange with the organic base and the thiolate ligands of the cluster.

Unfortunately, neither of the titanocene derivatives gave any physical evidence for binding to the Fe_4S_4 cluster. The experience gained by the study of titanocene derivatives should expedite the transition to work entailing molybdocene derivatives. Indeed, the problems encountered might be due to titanium's chemical behavior (for example the $Cp_2Ti(SH)_2$ and the $Cp_2Ti(SEt)_2$ are both photo-sensitive), and parallel situations may not occur with molybdenum. Molybdenum's presence in nitrogenase and its proximity to an Fe_4S_4 core indicate that all future synthetic work deal with coupling dithiolate derivatives of molybdocene to Fe-S tetrameric clusters, in spite of the difficulties involved in the molybdocene derivatives preparation.

3. The MoS_4^{2-}-Bridged Species

$$2Fe_4S_4(SR)_4^{2-} + MoS_4^{2-} + 2Me_3pyrHCl \rightarrow$$
$$(RS)_3Fe_4S_4(MoS_4)Fe_4S_4(SR)_3^{4-} + 2RSH + 2Me_3pyr + 2Cl^- \quad (14)$$

The isolation and characterization of the iron-molybdenum cofactor (FeMoco) of nitrogenase[13,14] initiated efforts directed at synthesizing the Mo-bridged species (Figure 9) in which two tetrameric Fe-S clusters are joined by a thiomolybdate bridge. The plausibility of this species as a potential model for the metal cofactor was enhanced by the subsequent appearance of extended x-ray absorption fine structure (EXAFS) data,[12] which were interpreted as showing that the metal cofactor contained a molybdenum atom surrounded by S-Fe-S units. The conceptual drawing in Figure 9 portrays this structural unit, including the Mo-S-Fe segment described in Section 2.

The thiomolybdate anion, prepared from simple reagents in high yield,[21,22] can act as a dinegative bidentate ligand that would bridge two Fe_4S_4 clusters. Its affinity for the Fe_4S_4 core was found to be less than that of t-butyl mercaptide (see below). Consequently, to make coordination feasible a proton donor was required to release the coordinated thiolate ligand. Figure 10a (d_6-DMSO) is a spectrum of a solution containing a 2:1 mixture of $(\phi_4As)_2MoS_4$ (thiomolybdate) and $(\phi_4As)_2[Fe_4S_4(S\text{-}t\text{-}Bu)_4]$ (tetramer) [7.72 (ϕ), 2.58 (bound t-butyl), 3.25 (impurity); intensity ratio calculated for ϕ: t-butyl, 80:36; found 80:33[43]]; as predicted, the free alkyl thiol (ca. 1.30) is not observed. The addition of one equivalent of pyridine hydrochloride effects the release of an

Figure 9. Proposed structure of the thiomolybdate-bridged species.

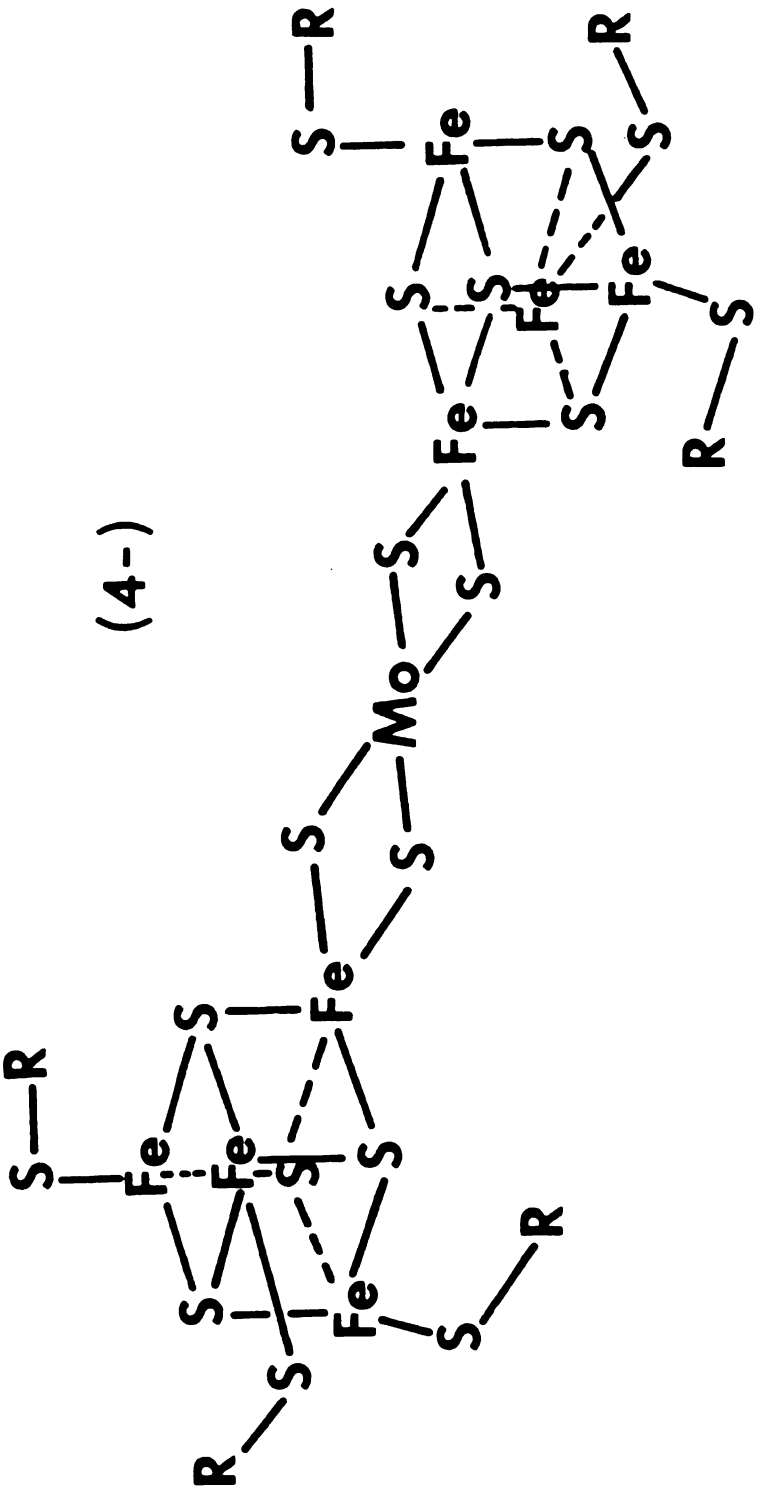


Figure 9

Figures 10a and 10b. Each figure is a nuclear magnetic resonance spectrum in d_6-dimethylsulfoxide (100%-d) with resonances in units of δ (delta). Figure 10a is the control spectrum of tetramer and thiomolybdate ligand in an equivalents ratio of 2:1. Addition of a proton donor to a mixture of tetramer and the thiomolybdate ligand effects the release of t-butyl thiol. Figure 10b is the nmr spectrum of this phenomena, intensity ratios: 8:26:11 for aryl:"bound" t-butyl:"free" alkyl thiol (the pyridine hydrogens are obscured by the large aryl resonance).

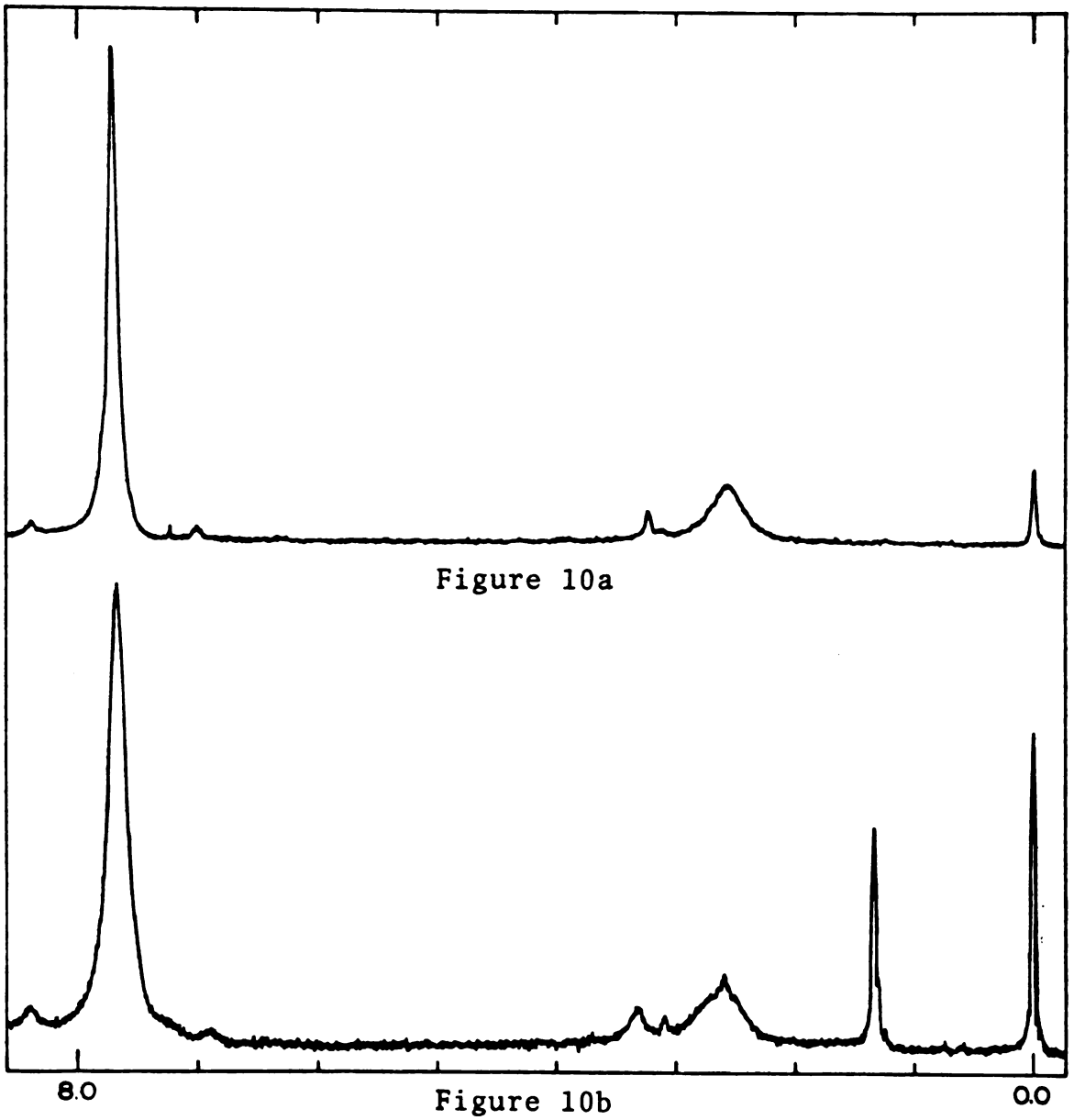


Figure 10a

Figure 10b

equivalent of free alkyl thiol [Figure 10b, d_6-DMSO, 7.76 (ϕ), 2.58 (bound t-butyl), 1.32 (free thiol), pyridine hydrogens are obscured by the large ϕ resonance; intensity ratio calculated for ϕ:bound t-butyl:free alkyl thiol, 80:27:9, found 80:26:11].

Since both reactants have unique optical features with known molar absorptivities,[49,50] binding could be monitored spectrometrically, using two distinct properties of the system. First, it is known that binding of a metal ion to two sulfides of MoS_4^{2-} induces a shift of the optical features to longer wavelength; coordination of all four sulfides will afford species in which MoS_4^{2-} acts as a bridging ligand and the optical features shift to shorter wavelength.[49] The bridged species lacks terminal Mo-S entities; therefore the optical spectrum should also lack bands due to these features. Second, changing the coordinated ligands bound to the Fe_4S_4 core causes a corresponding shift of the primary absorption peak (417 nm for most alkyl thiol ligands) of the core.[31] An optical experiment resulted in the spectra shown in Figure 11. Spectrum A is that of a solution containing 1.0 equivalent of $(\phi_4As)_2MoS_4$ [literature[49] in H_2O; nm ($M^{-1}cm^{-1} \times 10^{-4}$), 467 (1.2), 317 (1.7), 242 (2.4); found in spectro-acetonitrile; nm (ε not determined), 468, 318, 243]. Spectrum B is that of a solution containing 2.0 equivalents of $(\phi_4As)_2[Fe_4S_4(S\text{-}t\text{-}Bu)_4]$ [literature[50] in

Figure 11. All of the following optical spectra are recorded in dry, degassed acetonitrile. In spectrum A the thiomolybdate absorbs at 318 nm (ε 17,000) and 468 nm (ε 12,000), in spectrum B the tetramer absorbs at 310 nm (ε 21,800) and 419 nm (ε 16,700). Spectrum C displays the results of combining 2 equivalents of pyridinium chloride with 2 equivalents and 1 equivalent of tetramer and thiomolybdate, respectively. The tetramer primary absorption has shifted to 398 nm, while the thiomolybdate primary absorption has also shifted to shorter wavelength.

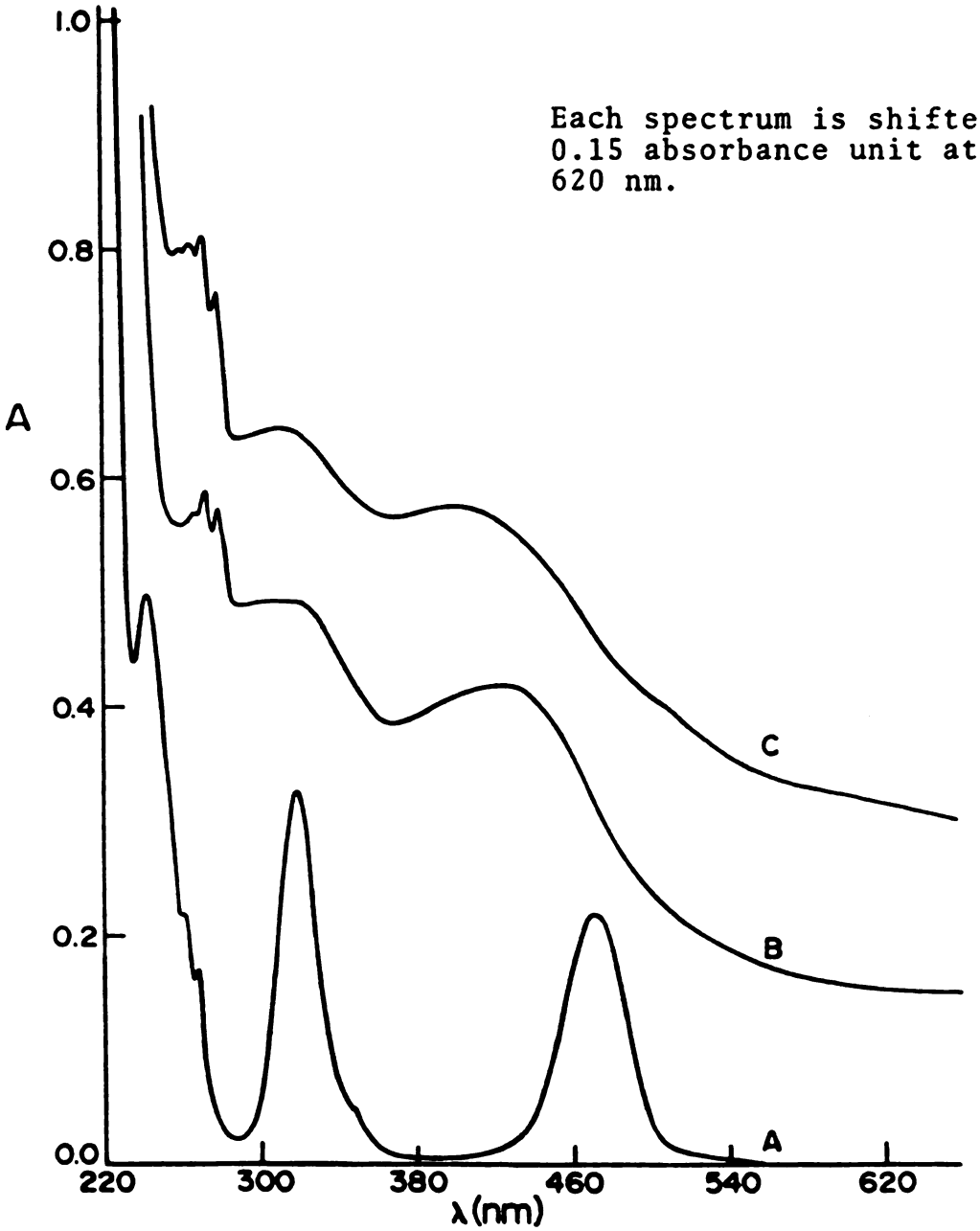

Each spectrum is shifted
0.15 absorbance unit at
620 nm.
1.0
0.8
0.6
0.4
0.2
0.0
A
C
B
A
220
300
380
460
540
620
λ(nm)

Figure 11

spectro-DMF; nm ($M^{-1}cm^{-1} \times 10^{-4}$), 417 (1.67), 303 (2.18); found in spectro-acetonitrile; nm (ε not determined), 419, 310]. Mixing of tetramer and thiomolybdate in a 2:1 mole ratio, followed by the addition of 2.0 equivalents of pyridine hydrochloride, shifted the 419 nm absorption of the tetramer to 398 nm but left the 310 nm peak and the relative intensities almost unchanged (spectrum C of Figure 11). The lack of any spectral features attributable to the thiomolybdate (the loss or shift of both the 468 nm and 318 nm absorption peaks) coupled with the shift of the tetramer's 419 nm absorption suggested that the desired species containing bridging S_2MoS_2 units may have been formed. There is no evidence for bidentate MoS_4^{2-}.[51] A preparative scale experiment was therefore carried out. A search for a solvent or a solvent system suitable for dissolving all species resulted in the use of a minimum of acetonitrile/N,N-dimethylacetamide/N-methylformamide to dissolve the tetramer and thiomolybdate. The proton donor, $Me_3pyrHCl$ (in acetonitrile), was introduced into the tetramer/thiomolybdate solution via cannula. Progress of the reaction was followed spectrophotometrically; the final electronic spectrum is shown in Figure 12. After a few hours stirring, the products were precipitated with a ten-fold volume excess of tetrahydrofuran. Initial attempts at recrystallization resulted in isolation of the starting material (tetramer) characterized by optical spectrum and melting point.

Figure 12. The electronic spectrum of 2:1:2 equivalents ratio of $(Et_4N)_2[Fe_4S_4(S\text{-}\underline{t}\text{-}Bu)_4]$, $(Et_4N)_2MoS_4$ and $Me_3pyrHCl$ in spectrograde dimethylacetamide (DMA). After initially mixing the reactants, the length of the absorption "shelf" increases with time, however subsequent attempts at recrystallizing the solid obtained from this solution resulted in loss of the absorption feature.

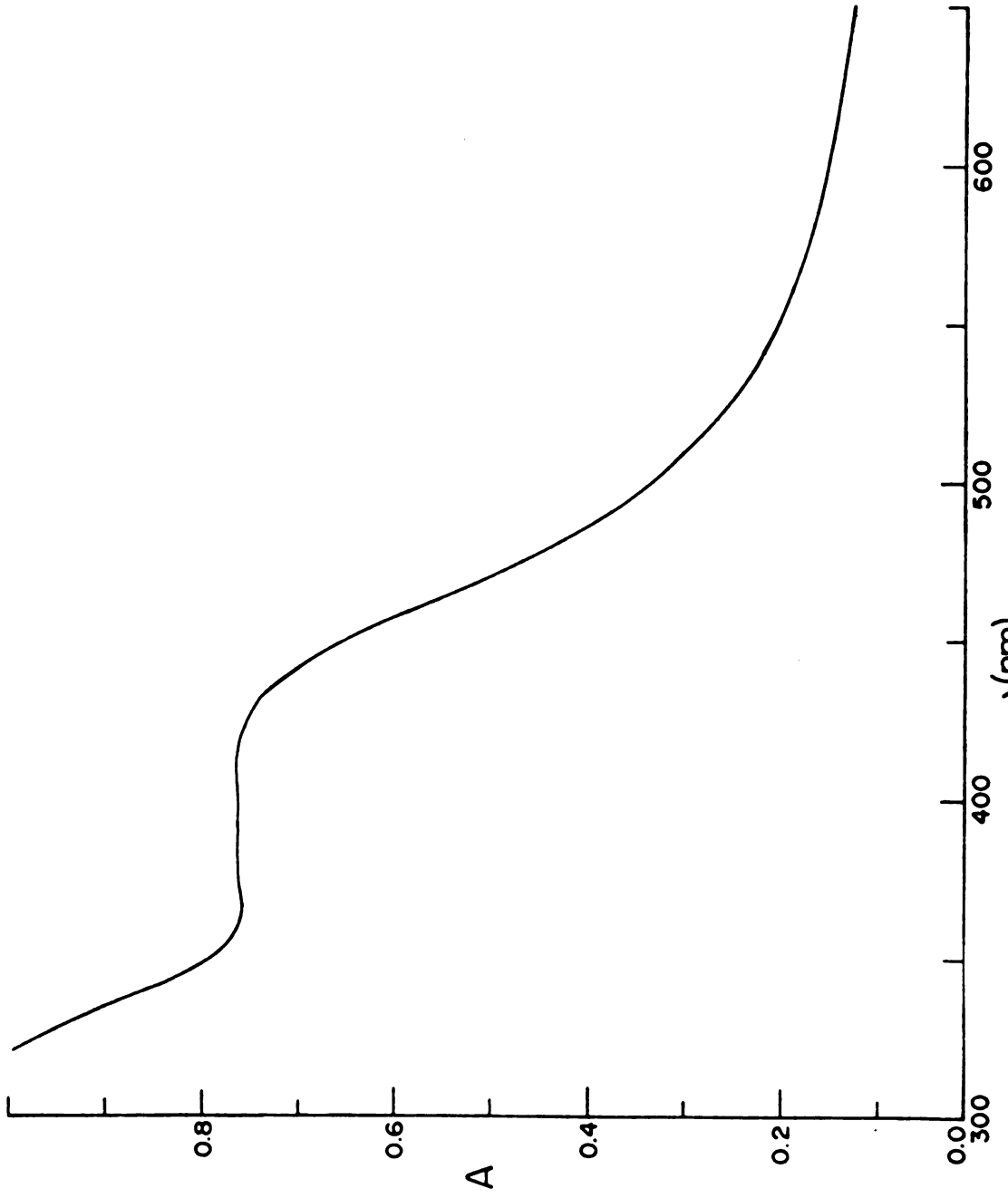


Figure 12

Was the molybdenum ever present in the form of a bridged complex, or was the disappearance of MoS_4^{2-} bands due to reduction of molybdenum to another soluble molybdenum complex? The micro-assay for molybdenum[52] was employed (with suitable controls), and the results indicated that a significant amount of molybdenum was present (theoretical in sample: 4.3×10^{-4} gram Mo, found in sample: 1.1×10^{-4} gram Mo) in the crude unrecrystallized products mentioned above. In addition, nmr spectra of the crude product also exhibited a shift of the bound thiolate ligand resonance (δ = ca. 1.2 vs. δ = ca. 2.8) to lower field, indicative of a substantial change in the environment of the Fe_4S_4 core (Figure 13). However, efforts to recrystallize this material have proved futile. The electronic spectra show shift of the primary absorption peaks back to values characteristic of starting material, indicating that rearrangements, not decomposition, are occurring. One possible solution to this problem would be to bind a tridentate (tripod) ligand to the Fe_4S_4 cluster, so as to tie up three of the four iron atoms with a high-affinity ligand. This would leave only one thiolate ligand free to participate in substitution reactions (in addition to providing a potential model for the P-clusters of nitrogenase). An attached tripod ligand could prevent polymeric species from forming and restrict rearrangements during attempts to synthesize both the molybdenum-bridged

Figures 13a and 13b. Each figure is a nuclear magnetic resonance spectrum in d_6-dimethylsulfoxide (99%-d) with resonances in units of δ (delta). Figure 13a is the control spectrum of the solvent, the resonance at 2.45 is the solvent impurity. Figure 13b is the same 2:1:2 equivalents ratio of tetramer, thiomolybdate, and trimethylpyridiniumchloride present in Figure 12. Note the shift of the "bound" t-butyl resonance from ca 2.58 to 1.32. The other proton resonances (tetraethylamine and trimethylpyridine) have been obscured by both solvent impurity and the "bound" t-butyl protons.

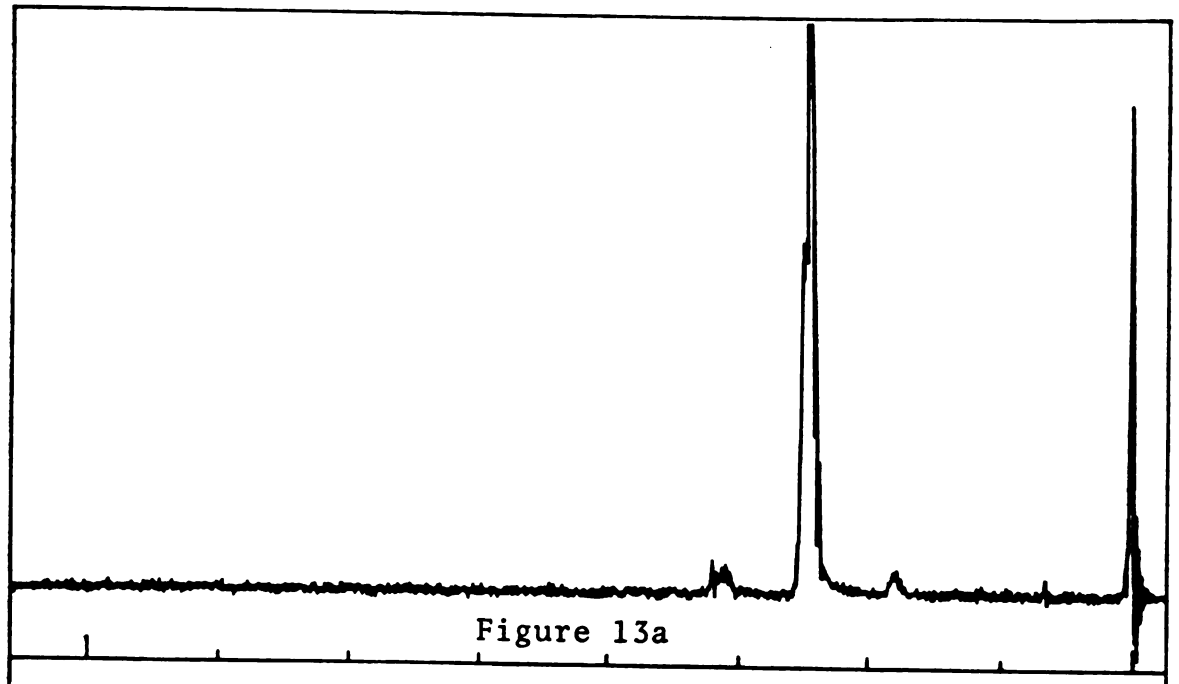


Figure 13a

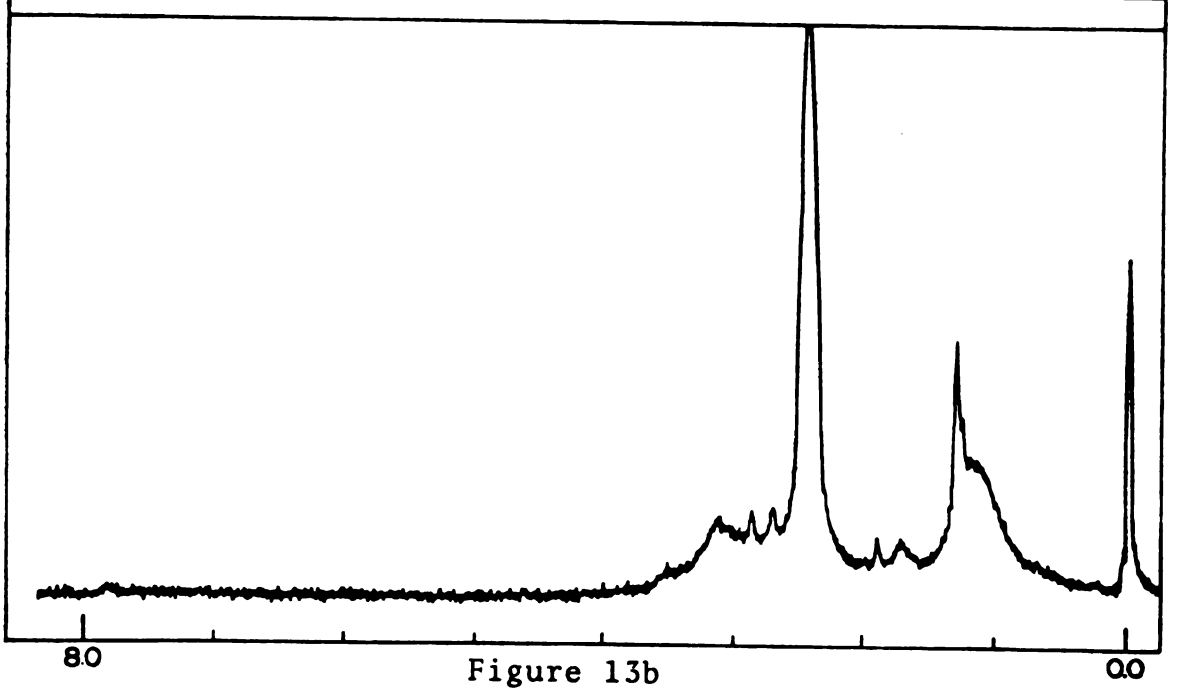


Figure 13b

and metallocene-bound species. A discussion of the tripod and biological significance of synthesis of the tripod-bound species follows.

4. The Tripod-Bound Species

$$Fe_4S_4(SR)_4^{2-} + O{=}P(NH\text{-}C_6H_4\text{-}\underline{o}\text{-}SH)_3 \rightarrow$$
$$Fe_4S_4(SR)[(S\text{-}\underline{o}\text{-}C_6H_4\text{-}NH)_3P{=}O]^{2-} + 3RSH \qquad (15)$$

The synthetic feasibility of utilizing a tridentate ligand to irreversibly replace three thiolate ligands of an Fe_4S_4 cluster has been briefly discussed. In addition, component I (MeFe protein) of nitrogenase has been shown by displacement experiments[3] to contain tetrameric Fe-S clusters (P-clusters) that appear to be tetranuclear species where one iron is found to be distinct from the others by Mössbauer spectroscopy.[54] Due to the equivalence of ligand sites on iron-sulfur tetramers, substitution of less than four thiolates results in a complete statistical distribution of products: mono, bis, tris, and tetrakis. This phenomenum prevents synthesis of a model for P-clusters through a simple ligand substitution reaction with three equivalents of thiophenol. However, successful binding of a tripod-type ligand to an Fe_4S_4 core would provide the first model of this unusual cluster type.[54] Results of ligand substitution

Figure 14. Proposed structure of the tripod-bound species.

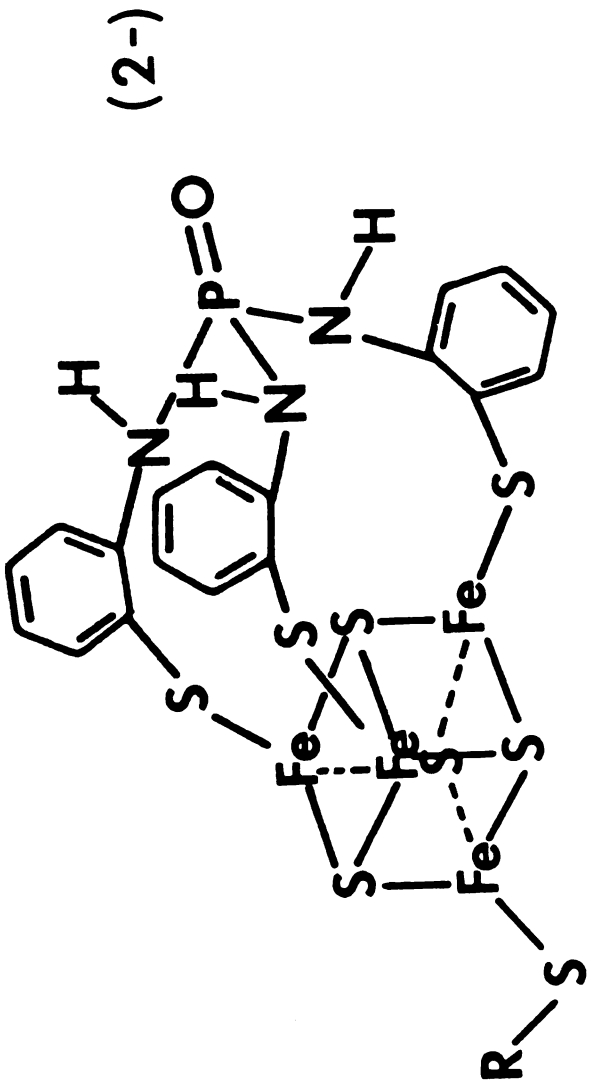


Figure 14

experiments[31] indicate that the Fe_4S_4 core has the greatest affinity for aryl thiol functionalities. This prompted the attempts at the synthesis of the species described in the experimental section. Unlike the ligands previously discussed, the tripod should spontaneously and rapidly displace the coordinated alkyl thiolate from $[Fe_4S_4(S\text{-}\underline{t}\text{-}Bu)_4]^{2-}$, with concomitant formation of three equivalents of $\underline{t}$-butylthiol.

The tripod ligand proved to be insoluble in most common organic solvents.[53] Nmr studies of the combination of the tripod ligand with the tetrameric cluster, requiring high concentrations, were therefore precluded, and the emphasis shifted to optical studies. It was here that the first evidence for reaction (15) was observed. The primary electronic absorption of the tetrameric cluster shifted from its normal position (417 nm) out to approximately 435 nm (Figure 15). This behavior is similar to that observed for the substitution of thiophenol for other ligands on Fe-S tetrameric clusters.[31] However, the spectrum displays a feature which may indicate that polymeric forms of the cluster are present:[55] a very gradual rise to the primary absorption instead of a sharper rise present in the parent compound (Figure 15). A preparative scale experiment (experimental section) showed a more significant shift of the primary absorption to 445 nm, but the long wavelength absorption was still

Figure 15. The electronic spectra display the changes in the primary absorption peak of $(\phi_4As)_2[Fe_4S_4(S\text{-}\underline{t}\text{-}Bu)_4]$ upon binding the tripod ligand, $O{=}P(\text{-}NH\text{-}C_6H_4\text{-}\underline{o}\text{-}SH)_3$. In the control spectrum (tetramer in DMA ———) the $(\phi_4As)_2[Fe_4S_4(S\text{-}\underline{t}\text{-}Bu)_4]$ initially absorbs at 417 nm (ε 16,700). Addition of the tripod ligand shifts the 417 nm absorption to a lower energy absorption, 435 nm (tetramer and tripod in DMA —·—·).

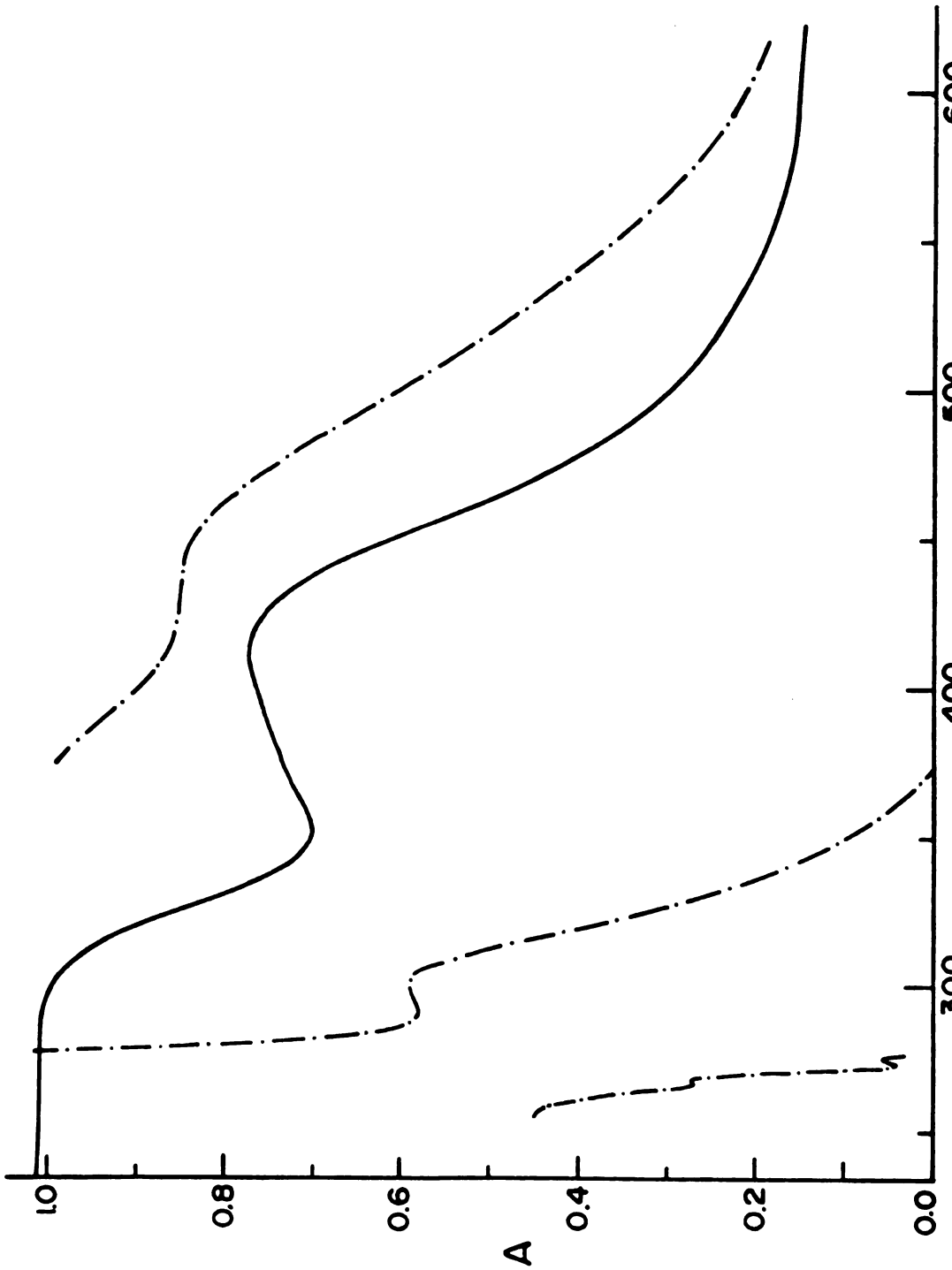


Figure 15

present. However, overnight stirring of a combination of an acetonitrile solution of tetramer with a slurry of tripod in the same solvent resulted in complete dissolution of both species, indicating that a reaction had occurred. Continued stirring produced a fine material, silky or micro-crystalline, with a metallic lustre. This material was collected but attempts at recrystallization proved futile, and the solid eventually decomposed. The possibility of the tripod ligand forming disulfides is clear. Presence of a disulfide, whether intra- or intermolecular, could inhibit the formation of the desired product and result in the occurrence of detrimental side reactions, perhaps the formation of polymers through rearrangements. Alkyl mercaptans have the ability to reduce aryl disulfides; introduction of a twenty-fold molar excess of t-butylmercaptan to a pale yellow tripod solution (acetonitrile) resulted in the instantaneous change to a clear solution. This result coupled with the long wavelength absorption of the tetrameric product prompted an iodometric titration to determine the extent to which the disulfide had formed. No further experiments were attempted because the tripod was found to contain just short of one-third of the necessary thiol functionalities. An x-ray structure study of the ligand is currently underway in order to ascertain its exact spatial configuration. Future work will entail altering the basic skeleton of the

tripod ligand in an effort to increase its solubility and decrease its ability to form disulfides. Under consideration is alkyl substitution in positions meta and para to the sulfur functionality; this would increase solubility by making the ligand's backbone hydrophobic. Also, a sufficiently large alkyl group (t-Bu or i-propyl) would create steric hindrance that could ultimately prevent formation of polymeric entities.

Solution of the problems surrounding successful binding of the tripod ligand to the Fe_4S_4 core will enable relevant synthetic investigation to continue. The monosubstitution of the cluster with metallocene derivatives and bridging of two clusters with thiometallate ligands are of special interest.

D. CONCLUSIONS AND PLANS FOR FUTURE WORK

A series of experiments has been performed in an effort to devise pathways to the preparation of compounds related to the metal cofactors of nitrogenase. As a prelude, the method for release of alkyl-thiolate ligands coordinated to the iron-sulfur tetranuclear core, necessary for the binding of ligands with potential catalytic and biological importance, has been examined in detail.

Subsequent research efforts has been directed toward these goals:

1. The synthesis of compounds that combine metal complexes of potential catalytic activity (e.g., metallocene dithiolates, tetrathiometallates) with iron-sulfur clusters that play a role in biological nitrogen reduction. Although the structure and reactivity of the desired compounds may not exactly mimic that of any biological unit, their chemical and physical properties may shed some light on the properties of similar units in biological systems; further, such compounds may be of interest in their own right as potential catalysts.

2. The synthesis of molecules that closely coincide with the available data concerning the stoichiometry of the FeMoco. Characterization and subsequent study of these compounds will help answer some of the questions involving the structure and role of the metal cofactors in biological dinitrogen fixation.

3. The preparation of a cluster bound by a large tridentate (tripod) ligand; this may provide a model for the P-clusters of nitrogenase, as well as furnish a means of avoiding the problems encountered in the preparation of the above compounds (rearrangements, polymer formation, multi-substitution).

Work on the metallocene-bound iron-sulfur clusters has not yet resulted in the isolation of the desired compounds; hence a major revision of the synthetic approach is necessary. As previously discussed, the reaction of the Fe_4S_4 core with molybdocene derivatives, in lieu of their titanocene analogs, will be examined. The potential model of the FeMoco, two iron-sulfur clusters bridged by a thiomolybdate, approximates the stoichiometry ($\sim$8Fe, $\sim$6$S^{=}$/Mo atom) and at least a portion of the proposed structure (Mo-S-Fe-S segments) of the metal cofactor more closely than the two Mo-Fe-S species reported to date.[15,16] Since the thiomolybdate bridged species has only been characterized spectroscopically in solution, efforts to produce a pure crystalline solid will continue. Included in the problems that hinder synthesis of these compounds are those of rearrangements to less suitable species, formation of insoluble polymers, and formation of multiple substitution products. The tripod ligand was designed to minimize these concerns. Insolubility of the tripod and its formation of intra- and/or intermolecular disulfides

have been major problems in its utilization. While the structure of the tripod is being determined by x-ray crystallography, the design and synthesis of a more soluble tripod ligand is being considered. The tripod-bound species should simplify the synthesis of iron-sulfur clusters with metallo-ligands by favoring substitution at only one iron.

Future work with the metallocenes may include binding the metallocene derivatives of other metals (e.g., Zr, Nb, Ta, W) to an iron-sulfur tetranuclear core and studying the effect of changing size and number of d electrons on the new systems. Since many of these metallocene ligands are known to bind small molecules [N_2, CO][39] upon reduction, the generation of lower oxidation states by electrochemical and chemical means will be investigated, as will the reactivity of any of the species produced with CO, N_2, etc. If necessary, the pentamethylcyclopentadienyl derivatives will be prepared to avoid the π-σ rearrangements common for reduced M-Cp complexes.[56] Of interest with regard to the FeMoco would be the use of thiotungstate instead of thiomolybdate in the preparation of the bridged species; the effect of the substitution on the physical and electronic properties of the metallate-bound iron-sulfur tetranuclear cluster could be examined. Some future effort will be directed toward preparation of per-substituted ($Fe_4S_4L_4^{m-}$) metallocene and metallate

complexes. Recently in our laboratory, while this thesis was being written, H. C. Silvis has been successful in preparing a tetra-substituted thiomolybdate cluster, $[Fe_4S_4(S_2MoS_2)_4]^{6-}$, using the methods outlined here. While of indirect biological significance, examination of these compounds will contribute to our understanding of the Fe-S_2-M unit. Four metallo-ligands coordinated to an Fe_4S_4 core may also prove to be of catalytic interest. Other tripod types being considered, exclusive of those previously mentioned, are those that will bind through an oxygen terminus (phenol, carboxylate, etc.) instead of through sulfur. Iron-sulfur clusters are capable of binding oxygen functionalities,[57] and while their biological significance is limited, the ability to preferentially effect other metallo-ligand substitution reactions at one iron will be of synthetic and catalytic importance.

Finally, isolation of analytically pure crystalline solids will subsequently entail many physical and chemical studies. Among studies performed will be an x-ray structure determination, electrochemical, spectroscopic, and binding studies.

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24. The pyridinium salt should be added dropwise. Approximately 1 drop of solution every 5-10 seconds.

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Triethylamine·HBr, Stadtler nmr #268, 3.20 ($C\underline{H}_2$), 1.45 ($C\underline{H}_3$).
Triethylamine·HCl, Stadtler nmr #9397, 3.21 ($C\underline{H}_2$), 1.45 ($C\underline{H}_3$).

47. N,N,N',N'-tetramethyl-1,8-naphthalenediamine, Stadtler nmr #13067, ca. 7.31 (3,4,5,6-$\underline{H}$), 6.91 (2,7-$\underline{H}$), 2.77 (Me).

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