

TOWARD A MODEL OF THE COFACTOR OF NITROGENASE: RECENT  
DEVELOPMENTS IN THE CHEMISTRY OF  $\text{MoFe}_3\text{S}_4$  CUBANE-TYPE CLUSTERS

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SUMMARY: The synthesis and selected properties of clusters containing cubane-type  $\text{MoFe}_3\text{S}_4$  units are described, with emphasis on the recently obtained clusters containing one such unit. Certain aspects of these clusters are considered in relation to the Fe-Mo-S aggregate that is the cofactor of nitrogenase.

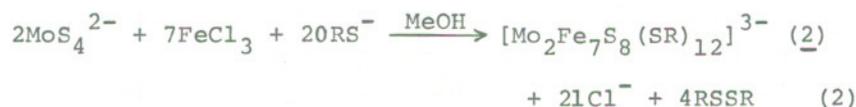
INTRODUCTION: Polynuclear metal groupings are now well-recognized constituents of certain proteins<sup>1,2)</sup>. The most widespread and thoroughly elucidated are the clusters containing the binuclear and cubane-type tetranuclear core units  $\text{Fe}_2(\mu_2\text{-S})_2$  and  $\text{Fe}_4(\mu_3\text{-S})_4$ , respectively. A recent addition to this group is the cyclic trinuclear unit  $\text{Fe}_3(\mu_2\text{-S})_3$  crystallographically identified in A. vinelandii ferredoxin I<sup>3)</sup>. The trinuclear site in at least one other protein<sup>4)</sup> may not have the same structure, however. Perhaps the most intriguing metal cluster in biology is the Fe-Mo-S entity which occurs as the cofactor (FeMo-co) of nitrogenase. This cluster was first obtained in 1977 by extraction of acid-denatured FeMo protein with N-methylformamide<sup>5)</sup>. In the native enzyme FeMo-co may act as the binding and activating site for dinitrogen and other substrates, and catalyze reduction when supplied with a flux of electrons and protons. FeMo-co has not been crystallized or otherwise isolated in substance and, consequently, is not a well characterized species. Current analytical data afford the atom ratios 7-8 Fe/4-6S/Mo<sup>5-7)</sup>. Spectroscopic

investigations have resulted in partial elucidation of certain properties of FeMo-co in solution and in the native proteins. These include the findings that six Fe atoms and one Mo atom form a spin-coupled cluster with a  $S = 3/2$  ground state and an EPR spectrum unique in biology<sup>8-10</sup>). Analysis of Mo atom EXAFS provided the first definite evidence of a cluster structure<sup>11</sup>). The latest results indicate 3 Fe atoms at 2.68 Å, 3 S atoms at 2.36 Å, and 3 O,N atoms at 2.09 Å from the Mo atom (K. O. Hodgson, private communication). The properties of FeMo-co have been reviewed<sup>12</sup>).

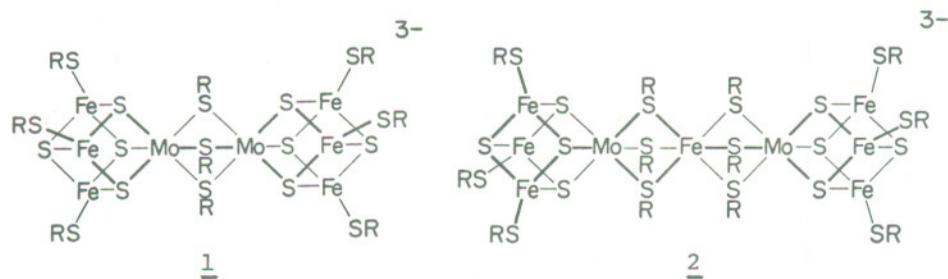
We are engaged in a search for Fe-Mo-S clusters that may serve as suitable representations of FeMo-co. The synthetic analogue approach<sup>1</sup>) to this problem has been taken and is similar to that utilized in our synthesis of the active sites of ferredoxins<sup>13,14</sup>). One difference, evident from the foregoing information, is that the structure of FeMo-co is unknown in detail. Consequently, the approach is necessarily one of speculative rather than corroborative modeling of a protein prosthetic group. The results achieved through mid-1981 and the methodology employed are set out elsewhere<sup>2</sup>). In this account experimental results obtained in the last two years are emphasized.

1. DOUBLE-CUBANE CLUSTERS: Clusters of this type, containing two  $\text{MoFe}_3\text{S}_4$  subclusters bridged through the Mo atoms by various groups, were the first obtained in our exploratory synthesis of polynuclear Fe-Mo-S species.

1.1. SYNTHESIS: Reactions (1) and (2), involving simple reagents, proceed spontaneously at ambient temperature and result in the assembly of clusters 1 and 2, which may be

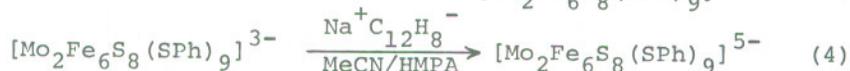
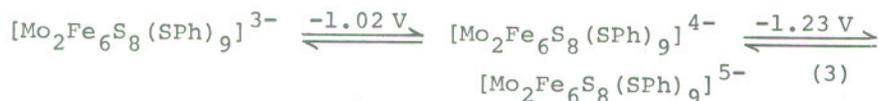


obtained in purified yields of >60% as quaternary ammonium

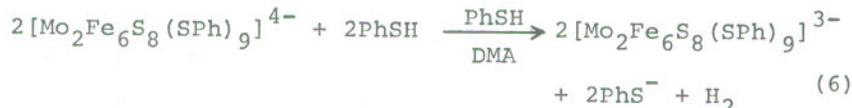
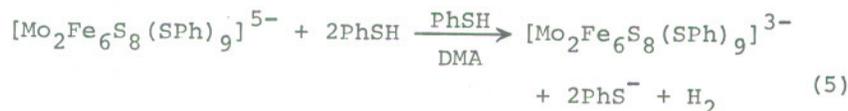


salts<sup>15-17</sup>). In addition, the clusters  $[\text{Mo}_2\text{Fe}_7\text{S}_8(\text{SR})_{12}]^{4-}$ ,  $[\text{Mo}_2\text{Fe}_6\text{S}_8(\mu_2\text{-S})(\mu_2\text{-SR})_2(\text{SR})_6]^{3-}$ , and  $[\text{Mo}_2\text{Fe}_6\text{S}_8(\mu_2\text{-OMe})_3(\text{SR})_6]^{3-}$  have been prepared by assembly reactions conducted under somewhat different conditions<sup>15-19</sup>). The structures of representative members of each cluster type have been established by x-ray diffraction<sup>15,20,21</sup>). Typical subcluster Mo-S and Mo...Fe bond distances are 2.35 and 2.71 Å, respectively. These values suggest that the MoFe<sub>3</sub>S<sub>3</sub> portion of a subcluster is structurally similar to the Mo atom coordination environment in FeMo-co as deduced from EXAFS. Indeed, one of the original structural postulations for the Mo atom site in FeMo-co involved a cubane-type MoFe<sub>3</sub>S<sub>4</sub> unit<sup>11,22</sup>). In this structure the presence of a S atom diagonally opposite the Mo atom is conjectural inasmuch as it is too distant (at ~3.9 Å in synthetic clusters) to contribute to the EXAFS. Although the synthetic clusters do not possess the composition of FeMo-co, the structural similarities of their Mo atom sites have encouraged further investigation, as has the fact that they are the only mixed-metal  $\text{M}'\text{M}_n\text{S}_4$  cubane clusters thus far isolated in the extensive field of inorganic cluster chemistry.

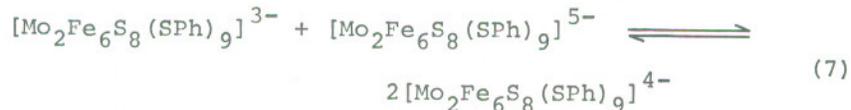
1.2. REACTIONS: All double-cubane clusters exhibit electron transfer series containing species in lower oxidation levels than those isolated in synthesis. Series (3) applies to 1 (R = Ph); potentials vs. s.c.e. in DMF solution<sup>19</sup>) are indicated for the two chemically reversible one-electron steps. The 5- cluster has been isolated by reaction (4) using sodium acenaphthylenide as the reductant and structurally characterized by x-ray analysis<sup>19</sup>). The increase of ~3% in



subcluster volumes, as well as the larger  $^{57}\text{Fe}$  isomer shifts, insures that the added electrons are located in the subclusters and have appreciable Fe orbital character. Thus the 5-cluster presents one of the few cases of a single molecule containing two essentially uncoupled, low-potential electrons possibly transferable to a two-electron substrate. The simplest such reaction is  $\text{H}^+ \xrightarrow{2e^-} \text{H}^-$ ; protonation of  $\text{H}^-$  will lead to a hydrogen-evolving system. Such systems have been realized with  $[\text{Mo}_2\text{Fe}_6\text{S}_8(\text{SPh})_9]^{5-}$  and PhSH or  $\text{Et}_3\text{NH}^+$  in N,N-dimethylacetamide (DMA) solution at ambient temperature<sup>23</sup>. Reaction (5) affords ~100%  $\text{H}_2$  yield based on cluster in the presence of a large excess of PhSH after 20 hr; under similar conditions reaction (6) gives a 86% yield. Evolution of  $\text{H}_2$  from other



protic sources and the 5- cluster was also observed; the most efficient system contained 2 equiv of  $\text{Et}_3\text{NH}^+$  and produced a 83%  $\text{H}_2$  yield after 20 hr. The 3- cluster proved inactive in  $\text{H}_2$  evolution. Inasmuch as reaction (5) will inevitably form the 4- cluster by the comproportionation process (7) for which  $K_{\text{eq}} = 10^{3.56}$ , reaction (6) was selected for kinetic analysis.



The time course of  $\text{H}_2$  yield for several systems initially containing n equiv of PhSH is shown in Figure 1. From these and

similar data for systems inhibited by PhS<sup>-</sup> and the 3- cluster an experimental rate law was obtained. This was found to be

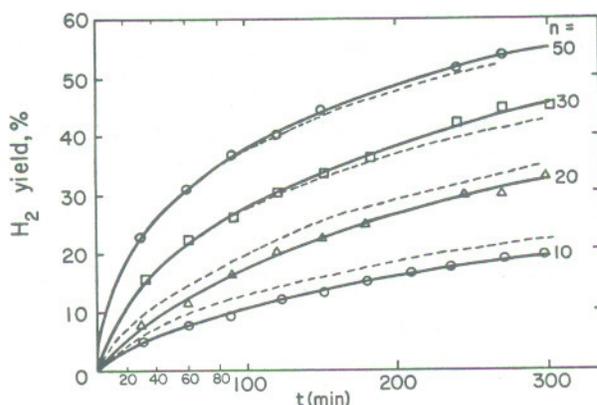
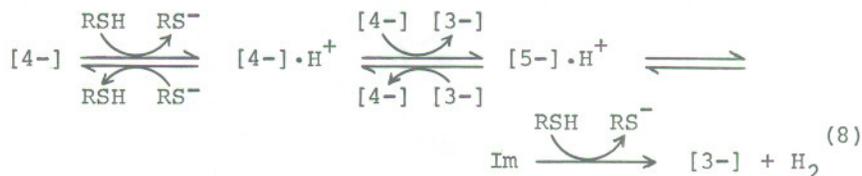


Fig. 1. Time dependence of H<sub>2</sub> formation in the system [Mo<sub>2</sub>Fe<sub>6</sub>S<sub>8</sub>(SPh)<sub>9</sub>]<sup>4-</sup>/n = 10-50 equiv PhSH/DMA (—); calculated yield curves based on reaction scheme (8) (-----).<sup>23</sup> (Reprinted with permission from ref. 23)

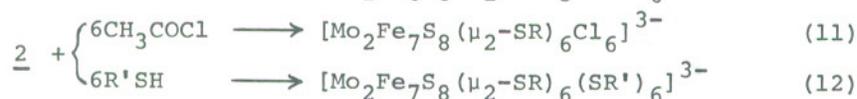
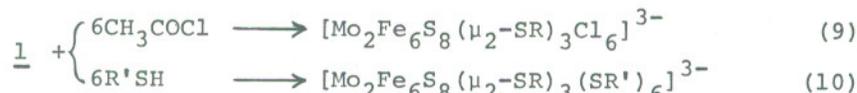
entirely consistent with the proposed reaction scheme (8) (R = Ph, Im = intermediate), which conforms to the stoichiometry of



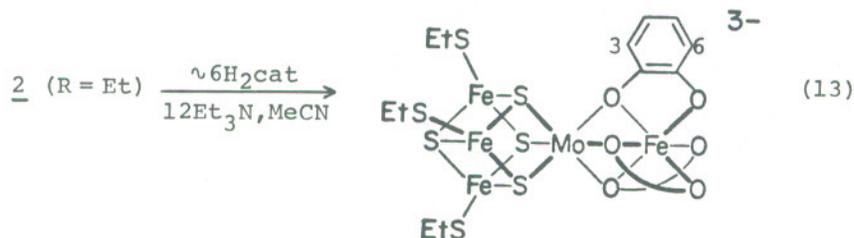
reaction (6). The process is initiated by cluster protonation (presumably at a thiolate ligand), reduction of a protonated by an unprotonated cluster, Im formation, and irreversible reaction of Im with thiol to produce H<sub>2</sub>. Im is the final kinetically resolvable intermediate prior to H<sub>2</sub> formation. A reasonable formulation of it is considered to be the hydride species [3-]·H<sup>-</sup>, produced by intramolecular two-electron transfer and transient stabilization of hydride at a metal site. In this event the advantage of a two-electron reductant is evident. Using experimentally evaluated elementary rate constants in the theoretical rate law for scheme (8) the experimental H<sub>2</sub> yield curves can be reproduced fairly well,

particularly in systems with the larger thiol concentrations. The experimental rate law and details of the kinetic analysis are given elsewhere<sup>23)</sup>.

Clusters 1 and 2 undergo clean ligand substitution reactions analogous to those of  $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-}$ <sup>13,14)</sup>. With stoichiometric or small excess of acetyl chloride or thiol reactions (9)-(12) proceed to completion<sup>18)</sup>. Under conditions



which preserve (sub)cluster structure only terminal ligands are replaced; the bridge regions remain intact. The first reaction found to disrupt the bridge portion of a double-cubane structure was (13) (cat = catecholate)<sup>24)</sup>. The



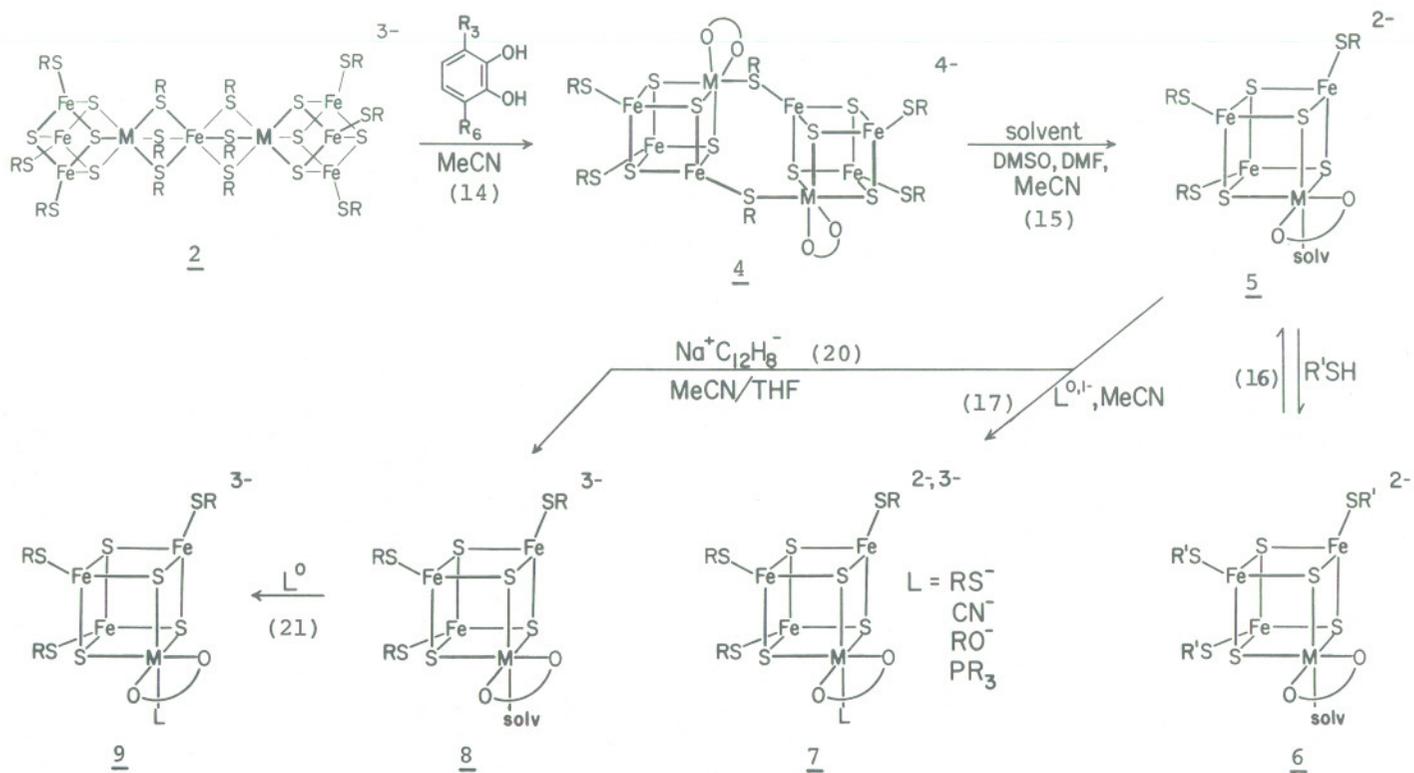
reaction product 3 contains a single  $\text{MoFe}_3\text{S}_4$  unit to which is appended through three  $\mu_2\text{-O}$  bridges the paramagnetic  $\text{Fe}(\text{cat})_3^{3-}$  fragment. This cluster provides a very good Mo EXAFS model for  $\text{FeMo-co}^{25)$ . As yet means have not been devised to remove  $\text{Fe}(\text{cat})_3^{3-}$  and liberate a single-cubane species. However, as shown in the following section, 3 has proven of value in devising a route to these species.

2. SINGLE-CUBANE CLUSTERS: Although reaction schemes involving ligand protonation followed by internal electron transfer affording a transient metal hydride can be entertained in  $\text{H}_2$ -evolving systems based on reduced clusters, any reductive transformations of other nitrogenase substrates such as

acetylene and N<sub>2</sub> are likely to require initial binding to a cluster metal site. The nature of the enzyme binding site -- mono- or bi(poly)nuclear, Mo and/or Fe atoms -- is unknown. There exists a considerable body of evidence derived from abiological systems<sup>26-28)</sup> that Mo can act as such a site. For example, the only mononuclear dinitrogen complexes which give high yields of ammonia upon protolysis are Mo-phosphine species<sup>29,30)</sup>. While these and certain other Mo complexes that interact with N<sub>2</sub>, acetylene, and other molecules resembling enzyme substrates are of a purely synthetic nature, the possibility that one or two Mo atoms, in a ligation environment similar to that in the enzyme, can effect reductive substrate transformations in the presence of electron donors, must be tested. Double-cubanes such as 1 and 2 are unsuitable for this purpose, at least in the deliberate sense, owing to coordination saturation at the Mo atoms by non-labile ligands. As shown below, a synthetic route to single-cubanes having a labile binding site has been developed. An additional desirable feature of these clusters is the absence of a neighboring cluster or paramagnetic fragment as in 1-3, thereby allowing investigation of the unperturbed electronic properties of individual MoFe<sub>3</sub>S<sub>4</sub> units.

2.1. SYNTHESIS AND STRUCTURES: Examination of the structure of 3 reveals that a 3-substituent on the catecholate ring, when about the size of a methyl group or larger, will encounter substantial steric interactions with the ethanethiolate ligand opposite it. This elementary observation has led to the reaction scheme in Fig. 2 which affords single cubanes<sup>31-34)</sup>. Reaction (14), similar to reaction (13) but with 3,6-di-*n*-propylcatechol (Pr<sub>2</sub>catH<sub>2</sub>) or 3,6-diallylcatechol (al<sub>2</sub>catH<sub>2</sub>), leads to the isolation of the doubly-bridged double-cubanes [Mo<sub>2</sub>Fe<sub>6</sub>S<sub>8</sub>(μ<sub>2</sub>-SR)<sub>2</sub>(SR)<sub>4</sub>(R<sub>2</sub>cat)<sub>2</sub>]<sup>4-</sup> a). The structure of a representative cluster 4 is shown in Fig. 3. Dimensions of the MoFe<sub>3</sub>S<sub>4</sub> unit are similar to those in 1-3. The most interesting structural features are the two Mo-S(Et)-Fe bridges,

a) The reactions in Fig. 2 give analogous Mo and W clusters; discussion is restricted to the Mo clusters.



$\text{L} = \text{PR}_3, \text{CO}$

Fig. 2. Synthesis and Reactions of Single-Cubane  $\text{MFe}_3\text{S}_4$  Clusters ( $\text{M} = \text{Mo}, \text{W}$ )<sup>34</sup>.

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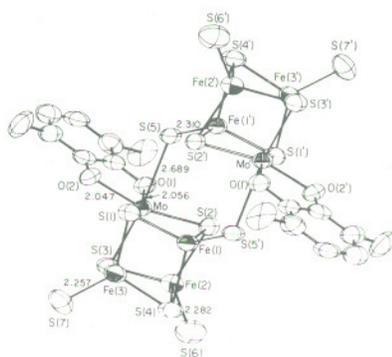


Fig. 3. Structure of  $[\text{Mo}_2\text{Fe}_6\text{S}_8(\text{SET})_6(\text{Pr}_2\text{cat})_2]^{4-}$  (4) as its  $\text{Et}_4\text{N}^+$  salt<sup>31,33</sup>). Primed and unprimed atoms are related by an inversion center; ethyl groups of the thiolate and catecholate ligands are omitted.

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unprecedented in Fe-Mo-S clusters of the cubane<sup>2)</sup> and other types<sup>35,36</sup>). Bridge Mo-S and Fe-S bond distances are  $\sim 0.04 - 0.10 \text{ \AA}$  longer than known or estimated terminal distances, suggesting bridge cleavage by suitable competing ligands. Reaction (15) occurs spontaneously upon dissolution of salts of 4 in coordinating solvents<sup>31,33</sup>). The resultant clusters 5 undergo complete substitution of all thiolate ligands in reaction (16) to afford 6. The cluster with  $\text{R}' = \text{p-C}_6\text{H}_4\text{Cl}$ , among others, can be obtained in this way. Its <sup>1</sup>H NMR spectrum in acetonitrile, presented in Fig. 4 (upper), consists of a single set of isotropically shifted o-H and m-H resonances whose chemical shifts are consistent with ligation at Fe sites. This property is exhibited by all clusters 5 and 6 with a variety of R(R') substituents. Attempts to isolate these clusters results in the reversal of reaction (15) and crystallization of salts of the double-cubanes 4.

Proof of the existence of the single-cubane structure has been accomplished by reaction (17). The clusters 5 in acetonitrile react quantitatively with 1 equivalent of the indicated ligands to afford the ligated clusters 7, a number of which have been isolated as crystalline salts<sup>32,34</sup>). The structure of one of these,  $[\text{MoFe}_3\text{S}_4(\text{S-p-C}_6\text{H}_4\text{Cl})_4(\text{al}_2\text{cat})]^{3-}$ , is set out

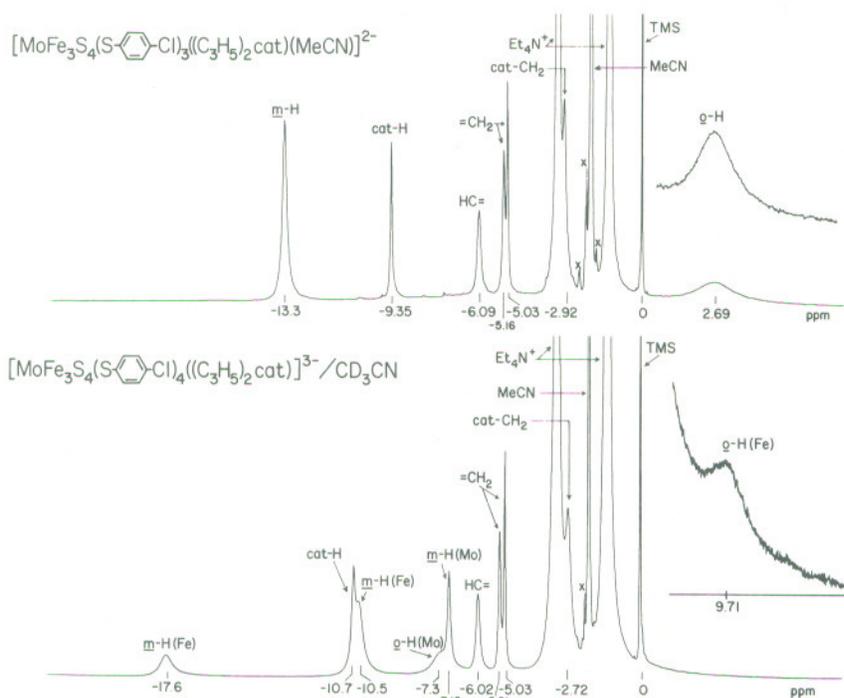


Fig. 4.  $^1\text{H}$  NMR spectra (300 MHz) of  $[\text{MoFe}_3\text{S}_4(\text{S-p-C}_6\text{H}_4\text{Cl})_3(\text{al}_2\text{cat})(\text{MeCN})]^{2-}$  (6, upper) and  $[\text{MoFe}_3\text{S}_4(\text{S-p-C}_6\text{H}_4\text{Cl})_4(\text{al}_2\text{cat})]^{3-}$  (7, lower) in  $\text{CD}_3\text{CN}$  at  $\sim 27^\circ\text{C}$ <sup>33</sup>.

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in Fig. 5. The Mo-SR bond distance is 2.600(3) Å, 0.089 Å longer than that of 4 in Fig. 3. The mean value of the terminal Fe-S bond distances, 2.271(11) Å, exceeds the bridge Fe-S distance in 4 by 0.04 Å. As noted above, bridge bond elongation is a likely contributing factor to the facile bridge rupture reaction (15). The  $^1\text{H}$  NMR spectrum of the cluster, given in Fig. 4 (lower), contains two  $\underline{m}$ -H resonances in an intensity ratio of 1:2. The highest idealized symmetry of the cluster is  $C_s$ , in which case the mirror plane bisects the catecholate chelate ring and contains the atoms Mo, Fe(1), S(1), and S(4). The Fe atoms divide into one ( $\underline{m}$ ) in the mirror plane and two ( $\underline{m}'$ ) related by it. The signals at -17.6 and -10.5 ppm correspond to the  $\underline{m}$  and  $\underline{m}'$  environments, respectively. All ligated clusters 7 exhibit analogous NMR spectra<sup>34</sup>. The existence of one set of Fe-SR signals in the spectra of the solvated

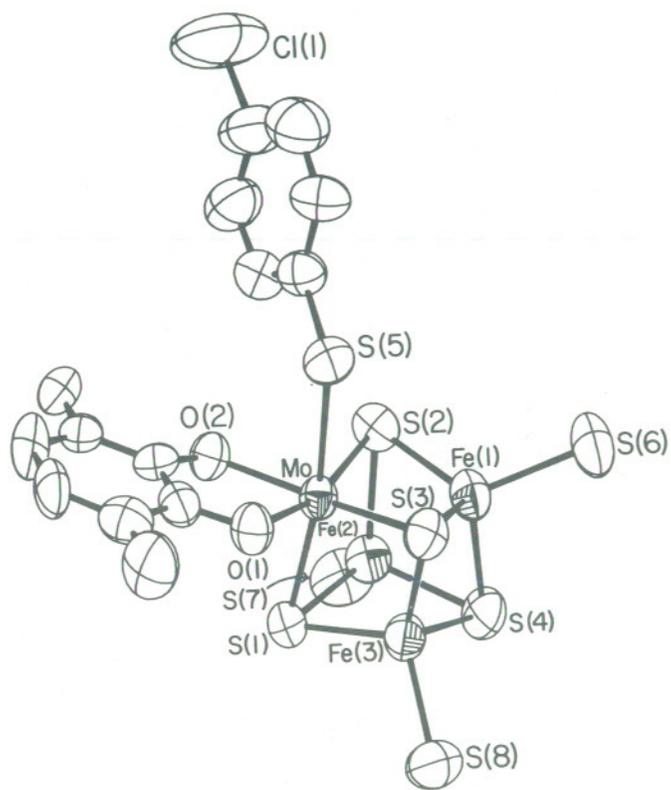


Fig. 5. Structure of  $[\text{MoFe}_3\text{S}_4(\text{S-}p\text{-C}_6\text{H}_4\text{Cl})_4\text{-(al}_2\text{cat)}]^{3-}$  as its  $\text{Et}_4\text{N}^+$  salt<sup>32,34</sup>;  $p\text{-C}_6\text{H}_4\text{Cl}$  and vinyl groups of certain ligands are omitted.

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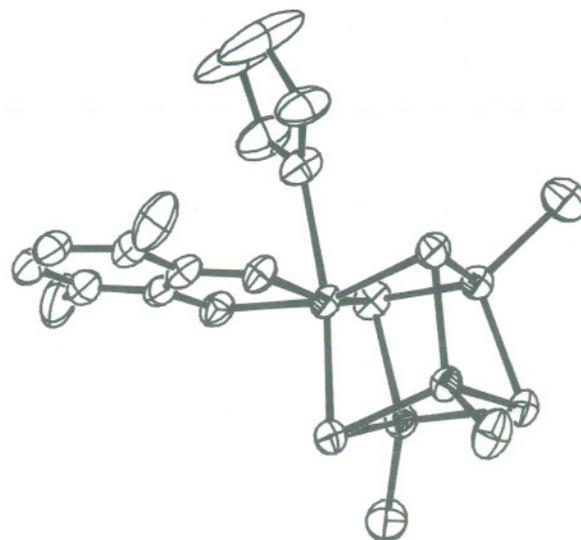


Fig. 6. Structure of  $[\text{MoFe}_3\text{S}_4\text{Cl}_3\text{-(al}_2\text{cat)(THF)}]^{2-}$  as its  $\text{Et}_4\text{N}^+$  salt<sup>37</sup>; the vinyl groups of the catecholate ligand are omitted.

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clusters 5 and 6 can be satisfactorily explained by rapid exchange of bound and bulk solvent molecules. Dissociation of a solvent molecule and degenerate reorientation of the catecholate ring to one of three possible dispositions roughly parallel to a cluster face will produce time-averaged trigonal symmetry. These processes are evidently rapid enough to equilibrate *m* and *m'* sites; the fast exchange limit is maintained at temperatures down to  $-40^{\circ}\text{C}$ <sup>33</sup>). Shortly after the structure of the thiolate-ligated cluster was solved the product of reaction (18) was obtained and its  $\text{Et}_4\text{N}^+$  salt was recrystallized from butyronitrile/THF<sup>37</sup>). The structure, provided in

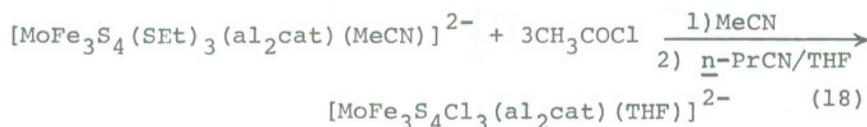
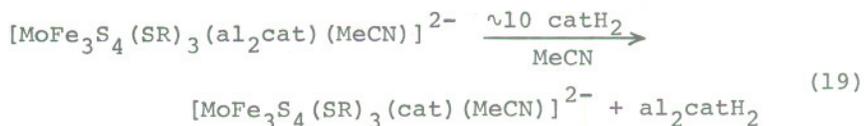


Fig. 6, contains a  $\text{MoFe}_3\text{S}_4$  unit of standard dimensions, chloride bound to Fe atoms instead of thiolate, and THF coordinated to the Mo atom with a (long) Mo-O distance of 2.352(6) Å. This structure obviously substantiates the solvate formulation of 5 and 6 in solution.

2.2. REACTIONS: Achievement of the solvated single-cubanes as well-defined solution species meets the objective of a cluster containing a Mo atom in a coordination environment related to that in FeMo-co and possessing a labile coordination site. Several reactions of cluster 5 have already been noted: thiolate ligand substitution, reactions (16) and (18); the ligation reactions (17) resulting in solvent displacement and binding of L to the Mo site. The latter reactions are best carried out in acetonitrile; dissociation of L is enhanced in more strongly coordinating solvents such as DMF and DMSO. For example, addition of as little as 10 equiv of DMSO to a 40 mM solution of 7 ( $\text{R} = \text{Ph}$ ,  $\text{L} = \text{PhS}^-$ ) produces detectable amounts of 5. Addition of 200 equiv causes complete conversion to the solvated cluster, which is easily distinguished from 7 by NMR. The catecholate ligand may also be substituted by another, as in reaction (19) which does not affect the thiolate ligands. Thus it is possible to carry out substitution reactions



specific to Fe or Mo atom sites, an aspect of reactivity that should prove useful in the construction of modified clusters, including new types of double-cubanes.

Particular interest attends the properties of reduced solvated clusters for these have the potentiality of effecting reductive substrate transformations. At potentials of  $\sim -2$  V vs. s.c.e. the clusters 5 exhibit a single, chemically reversible, one-electron reduction. Reaction (20) has been shown to generate the clusters 8 in acetonitrile solution<sup>34</sup>). Subsequently, the species with R = p-C<sub>6</sub>H<sub>4</sub>Cl has been crystallized; an x-ray structure determination is in progress. As seen in the typical NMR spectrum in Fig. 7 (upper), reduced solvated clusters exhibit a single set of Fe-SR resonances, demonstrating the persistence of the rapid solvent exchange process found with the oxidized clusters. Compared to these clusters reduced species show a greatly diminished affinity for anionic ligands. For example, a 20-fold excess of p-ClC<sub>6</sub>H<sub>4</sub>S<sup>-</sup> in a  $\sim 10$  mM solution of [MoFe<sub>3</sub>S<sub>4</sub>(S-p-C<sub>6</sub>H<sub>4</sub>Cl)<sub>3</sub>(al<sub>2</sub>cat)(MeCN)]<sup>3-</sup> caused no change in the spectrum in Fig. 7. In contrast, reaction of this cluster with 1 equiv of PET<sub>3</sub> results in essentially quantitative formation of the phosphine adduct 9 by means of reaction (21). The NMR spectrum of [MoFe<sub>3</sub>S<sub>4</sub>(S-p-C<sub>6</sub>H<sub>4</sub>Cl)<sub>3</sub>(al<sub>2</sub>cat)(PET<sub>3</sub>)]<sup>3-</sup> in Fig. 7 (lower) demonstrates non-labile binding of phosphine by virtue of the appearance of m-H signals corresponding to the m (-20.6 ppm) and m' (-14.2 ppm) environments. The affinity of reduced clusters for  $\pi$ -acid ligands was further examined in the system [MoFe<sub>3</sub>S<sub>4</sub>(S-p-C<sub>6</sub>H<sub>4</sub>Cl)<sub>3</sub>(al<sub>2</sub>cat)(MeCN)]<sup>3-</sup>/CO. In the presence of 1-2 equiv of CO in Ar a species with an intense carbonyl band at 1810 cm<sup>-1</sup> was formed. The infrared spectrum did not change appreciably over 160 min. With larger amounts of CO and longer reaction times the 1810 cm<sup>-1</sup> feature disappeared and was replaced by a multiple band spectrum at 1840-1980 cm<sup>-1</sup>. While the reaction system is obviously complicated, the 1810

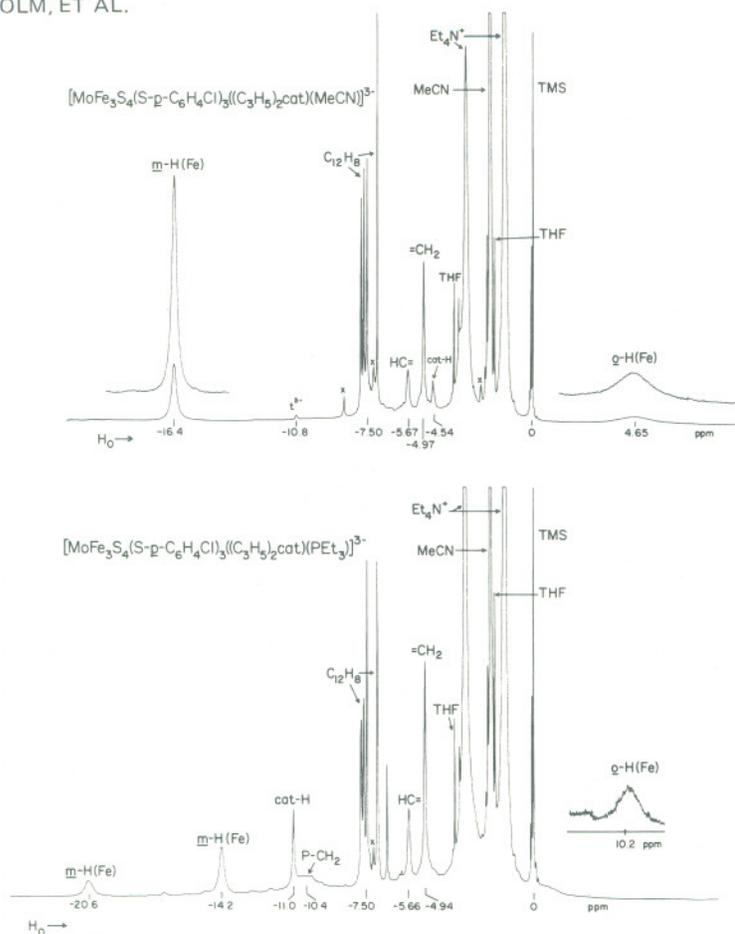


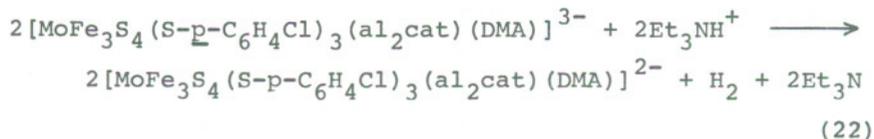
Fig. 7.  $^1\text{H}$  NMR spectra (300 MHz) of  $[\text{MoFe}_3\text{S}_4(\text{S-p-C}_6\text{H}_4\text{Cl})_3(\text{al}_2\text{cat})(\text{MeCN})]^{3-}$  (8, upper) and  $[\text{MoFe}_3\text{S}_4(\text{S-p-C}_6\text{H}_4\text{Cl})_3(\text{al}_2\text{cat})(\text{PEt}_3)]^{3-}$  (9, lower) in  $\text{CD}_3\text{CN}$  at  $\sim 27^\circ\text{C}$ <sup>34</sup>.

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$\text{cm}^{-1}$  species is thought to be the mono-CO adduct 9 based on the small quantity of CO required for its formation and its appearance as the first CO complex in the system. If this formulation is accepted the  $\nu_{\text{CO}}$  value indicates that  $[\text{MoFe}_3\text{S}_4(\text{S-p-C}_6\text{H}_4\text{Cl})_3(\text{al}_2\text{cat})]^{3-}$  is a  $\pi$ -donor comparable with, e.g.,  $\text{Mo}(\text{S}_2\text{CNET}_2)(\text{dpe})$  ( $1790\text{ cm}^{-1}$ )<sup>38</sup>,  $\text{Mo}(\text{dpe})_2$  ( $1807\text{ cm}^{-1}$ )<sup>39</sup>, and  $\text{Mo}(\text{N}_2)(\text{dpe})_2$  ( $1799\text{ cm}^{-1}$ )<sup>39</sup> ( $\text{dpe} = \text{Ph}_2\text{PCH}_2\text{CH}_2\text{PPh}_2$ ). The oxidized solvated clusters 5 or 6 do not react with CO.

Investigation of the reactivity of reduced solvated clusters

8 is at an early stage. Initial experimentation has shown that the H<sub>2</sub> evolution reaction (22) proceeds in DMA solution. After 20 hr reaction time with systems containing 1-4 equiv of Et<sub>3</sub>NH<sup>+</sup>



as the protic source, yields of H<sub>2</sub> are ~30%. Reactions of acetylenes and N<sub>2</sub> with the clusters 8 are under investigation.

3. COMPARISON OF CLUSTERS AND COFACTOR: Having obtained oxidized and reduced clusters solvated at the Mo atom, a reasonable first step in the development of synthetic representations of FeMo-co, it is appropriate to compare certain properties of the synthetic and native clusters. Spectroscopic results appear to set the minimum Fe/Mo atom ratio at 6:1<sup>8-10</sup>); chemical analysis gives a 7-8:1 ratio<sup>5-7</sup>). Obviously the synthetic clusters, with a 3:1 ratio, are deficient in Fe content compared to FeMo-co. Both the cofactor<sup>12</sup>) and the synthetic clusters are anions. The latter contains cationic core units, [MoFe<sub>3</sub>S<sub>4</sub>]<sup>3+</sup> and [MoFe<sub>3</sub>S<sub>4</sub>]<sup>2+</sup> in the oxidized and reduced forms, respectively. The net negative charge arises from incorporation of thiolate and catecholate ligands. FeMo-co is reported to contain no endogenous organic material<sup>12</sup>) and EXAFS results rule out terminal (Mo=O) oxo ligands<sup>11,22</sup>). Using the 6:1 atom ratio for FeMo-co, estimating the mean oxidation state Fe<sup>+2.5</sup> from the isomer shift<sup>8</sup>), and taking the sulfur content as entirely sulfide, a minimum of 10-11 S atoms is required for an anionic species if the Mo atom oxidation state is in the range +3 to +6. If the (mean) oxidation states are the same as those in our preferred description of [MoFe<sub>3</sub>S<sub>4</sub>]<sup>3+</sup> based on isomer shift data, viz., Fe<sup>+2.67</sup> and Mo<sup>+3</sup> 19), a minimum of 10 S atoms is required for FeMo-co to be anionic. These elementary considerations are offered with due regard to a possible lack of definitiveness in assays for anionic (organic) components and to the possible inclusion of bridging and terminal hydroxide and bridging oxide ligands. These constituents cannot be discounted on the basis of current evidence. A



ambient temperature have given  $\mu_{\text{MO}} \cong 3.8 \text{ BM}^{41}$ , consistent with a spin-quartet state. All cubane clusters having the  $[\text{MoFe}_3\text{S}_4]^{3+}$  core oxidation level possess  $S = 3/2$  states, as shown by magnetic determinations ( $\mu = 3.9 - 4.1 \text{ BM}^{32,33}$ ), high field magnetization measurements at cryogenic temperatures (unpublished observations), and EPR spectra<sup>31-33</sup>. A representative EPR spectrum, shown in Fig. 8 (upper) for a ligated cluster 7, is that of a spin-quartet species with transitions in the  $m_S = |\pm 1/2\rangle$  Kramers doublet at  $g \cong 4.6$  and  $2.0$ . The line-width of the low field feature is large ( $\sim 450 \text{ G}$ ), in part because of an unresolved resonance at  $g \sim 3.5$  which is expected for a system with rhombic symmetry. The weak feature at  $g \cong 5.9$  is presumably a forbidden transition in the  $m_S = |\pm 3/2\rangle$

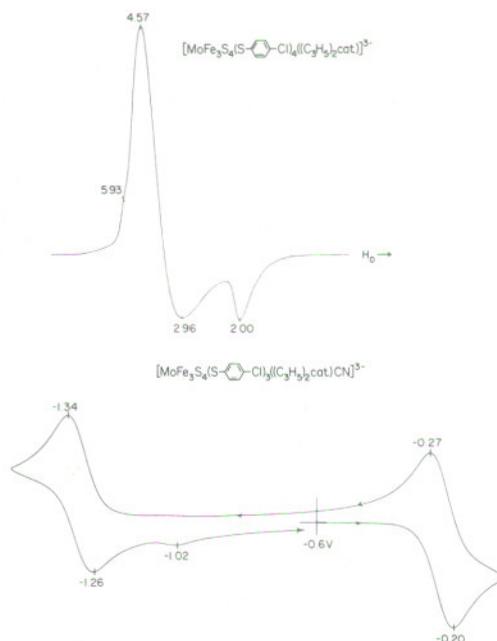
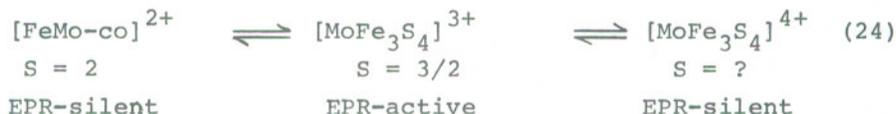
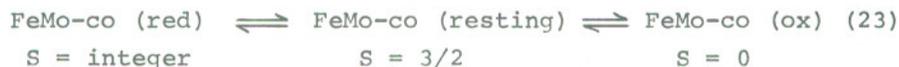


Fig. 8. X-band EPR spectrum ( $\sim 6 \text{ K}$ , acetonitrile) of  $[\text{MoFe}_3\text{S}_4(\text{S}-p\text{-C}_6\text{H}_4\text{Cl})_4(\text{al}_2\text{cat})]^{3-}$  (7, upper); cyclic voltammogram (100 mV/s, acetonitrile) of  $[\text{MoFe}_3\text{S}_4(\text{S}-p\text{-C}_6\text{H}_4\text{Cl})_3(\text{al}_2\text{cat})\text{CN}]^{3-}$  (7, lower)<sup>32</sup>. Apparent  $g$ -values and peak potentials vs. s.c.e. are indicated. The feature at  $-1.02 \text{ V}$  is due to a small amount of the solvated cluster 5 formed in the redox cycle.

(Reprinted with permission from ref. 32)

Kramers doublet. The spectrum of FeMo-co is similar but is better resolved, showing the three rhombic transitions at  $g \approx 4.6, 3.3, 2.0$  as well as a weak feature at  $g \approx 5.9$ . By the EPR criterion the clusters and FeMo-co have related electronic structures. The latter, when examined in native FeMo proteins, exhibits the three-membered redox series (23) in which the states are interrelated by  $1e^-/\text{Mo}$  atom. The existence of a



three-membered series (24) for the synthetic clusters is demonstrated by the typical cyclic voltammogram of a ligated cluster 7 in Fig. 8. Solvated clusters behave similarly. The correspondence of spin states (where known) and EPR activity is evident and emphasizes further the electronic similarity (but not, of course, an identity owing to different compositions) between synthetic clusters and the cofactor. A more detailed description of comparative properties is available elsewhere<sup>2)</sup>.

The results presented herein provide a brief summary of our activities directed toward the attainment of a functional synthetic representation of FeMo-co. For more details the recent publications from this laboratory may be consulted<sup>2,18,19,23,31-34,41)</sup>. The principal approach taken thus far has emphasized the synthesis and structural, electronic, and reactivity characterization of single-cubane  $\text{MoFe}_3\text{S}_4$  clusters having a substitutionally labile binding site at the Mo atom. We are cognizant that another approach utilizing Fe or Fe-Mo sites may be advisable. To this effect we quote from our earlier account<sup>2)</sup>: "In the present context, it should be remembered that a comparable effort has not been expended by the inorganic chemists to devise dinitrogen-fixing systems that are based on iron complexes." Indeed, the  $\text{H}_2$ -evolving systems based on  $[\text{Mo}_2\text{Fe}_6\text{S}_8(\text{SPh})_9]^{4-,5-}$  may be cases in which substrate

reduction occurs at a Fe site. A Japanese group has recently reported H-D exchange and reduction of certain nitrogenase substrates, including N<sub>2</sub> to NH<sub>3</sub>, by the electrochemical reduction products of [Mo<sub>2</sub>Fe<sub>6</sub>S<sub>8</sub>(SPh)<sub>9</sub>]<sup>3-</sup> (and [Fe<sub>4</sub>S<sub>4</sub>(SPh)<sub>4</sub>]<sup>2-</sup>) in protic media<sup>42-44</sup>. These results are encouraging but, given our observations of the instability of reduced clusters in such media, it cannot yet be asserted that intact reduced forms of the initial clusters are the actual reactants. Ongoing activities in this laboratory include a thorough exploration of substrate reductions by the reduced solvated clusters and the synthesis of new types of double-cubanes that potentially can bind a substrate species to the Mo atoms of two subclusters. This situation should facilitate concerted two-electron reductions of substrates.

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