Partial synthetic models of FeMoco with sulfide and carbyne ligands: Effect of interstitial atom in nitrogenase active site

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Nitrogen-fixing organisms perform dinitrogen reduction to ammonia at an Fe-M (M = Mo, Fe, or V) cofactor (FeMco) of nitrogenase. FeMco displays eight metal centers bridged by sulfides and a carbyne having the MFe7S8C cluster composition. The role of the carbyne ligand, a unique motif in protein active sites, remains poorly understood. Toward addressing how the carbon bridge affects the physical and chemical properties of the cluster, we isolated synthetic models of sulfide MoFe7S8C displaying sulfides and a chelating carbyne ligand. We developed synthetic protocols for structurally related clusters, [Tp*MFe2S2X]2−, where M’ = Mo or W, the bridging ligand X = CR, NR, or S, and TP* = Tris(3,5-dimethyl-1-pyrazolyl)-hydroborate, to study the effects of the identity of the heterometal and the bridging X group on structure and electrochemistry. While the nature of M’ results in minor changes, the chelating, µ2-bridging carbyne has a large impact on reduction potentials, being up to 1 V more reducing compared to nonchelating N and S analogs.

FeMco model | nitrogenase | carbide clusters | reduction potential | iron-sulfur clusters

Biological dinitrogen conversion to ammonia is performed by nitrogenases, a class of enzymes displaying several complex iron-sulfur clusters (1). The site of N2 reduction in the most efficient nitrogenase is a heterometallic cluster displaying Fe and Mo, the iron-molybdenum cofactor (FeMco) (1). Two other nitrogenases are known where Fe or V are found at the Mo position. FeMco consists of Fe5S2C and MoFe7S8C cubanes with µ3-sulfides joined together by a shared interstitial µ4-carbide and three additional sulfides that bind in µ2-fashion (Fig. 1) (2). The impact of the carbide ligand on the electronic structure and reactivity of the cofactor, and therefore its role in the catalytic cycle of N2-to-NH3 conversion, is unclear (3). The carbide ligand is not lost during catalysis, and it has been suggested that it becomes protonated before N2 activation (3). To address the effect of carbon-based ligands for N2 activation, such as providing electronic stabilization and structural flexibility to accommodate multielectron redox processes, synthetic models have included arene (4), N-heterocyclic carbene (5), aryl (6), and alkyl (7, 8) donors in mononuclear iron complexes.

Bi- and multimetallic synthetic analogs focused on interrogating the role of the interstitial atom and multimetallic effects have been targeted (7, 9–23), but complexes that display bridging carbide (11, 24–27) or even carbyne (9, 10, 28) are rare. Carbide-containing Fe clusters display four to six metal centers but invariably are rich in CO ligands (24, 25, 27). The presence of this strong field donor limits the comparison to FeMco given the significantly different electronic structure conferred by the weak field sulfides. Moreover, the formal oxidation state of the Fe centers is significantly more reduced, between Fe0 and Fe1+, than in the protein, between FeII and FeIII (2). Recent promising advances have been made toward the incorporation of sulfide ligands into carbide-containing iron carbonyl clusters (10, 11). In order to gain a more accurate understanding of the impact of the carbide on the properties of clusters related to FeMco, metal complexes structurally related to the biological active site that are multimetallic, have multiple sulfide ligands and few CO ligands, and display bridging carbon-based ligands and oxidation states of FeII-FeIII are desirable.

Toward developing synthetic methodologies to structures analogous to FeMco that include a bridging carbon donor, we focus our initial efforts on the cubane subsite, MoFe7S8C (Fig. 1, Top Row). Because the nature of the µ2-bridging ligands in FeMco is variable, with sulfide, selenide (29), CO (30), or NH (31) (for FeVco) moieties at these positions as characterized by crystallography, the primary target was to match the composition of the cubane core. In this work, we present the preparation of a series of heterometallic iron-sulfur cubane-type clusters containing Mo or W with biologically relevant µ2 bridging ligands X (X = N, NR, CR, and S) incorporated at the Fe3 face—including examples bearing a bridging CR ligand. These variations in the bridging ligand result in a large shift in the biologically relevant MFe31+/ MFe3++ redox couple of up to 2 V, with the most reducing system occurring for the cluster bearing a bridging carbyne. These results suggest an important role of the interstitial carbide ligand in FeMco in modulating the electronic properties of the cluster toward rendering it more reducing and potentially more reactive in N2 activation and conversion into NH3.


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Results and Discussion

To rationally incorporate different ligands at the \( \mu_3 \)-bridging position corresponding to the carbide, a \( \text{WF}_6\text{S}_1 \) cluster supported by a \( \text{W}^- \)-coordinated \( \text{Tp}^- \) ligand, \( 1\text{-W} \), was selected as precursor bearing a \( \mu_3\text{-Cl} \) at the carbide position (Fig. 2) (32). Although heterometallic iron-sulfur clusters of the \( \text{MFe}_3 \) types have been reported with \( \text{M} = \text{V}, \text{Mo}, \) and \( \text{W} \), they typically display a \( \mu_3\text{-S} \) vertex opposite the heterometal that is difficult to substitute with other donor types relative to chloride (33, 34). Indeed, starting from a \( \mu_3\text{-Cl} \) precursor offers a versatile route to incorporating biologically relevant light atoms at the bridging position by ligand metathesis reactions (32). As an example, the \( \mu_3\text{-Cl} \) ligand can be substituted with \( \mu_3\text{-S} \) or \( \mu_3\text{-NSiMe}_3 \) by oxidative metathesis with \( \text{S}_8 \) or \( \text{Me}_3\text{SiN}_3 \), respectively (32).

For the installation of a carbon-based ligand at the \( \mu_3 \) position, we were inspired by the utilization of the strained carbene bis(diisopropylamino)cyclopropenylidene (BAC) (35) for promoting \( \text{C}^- \)atom transfer to the \( \text{Fe}^\text{IV} \) nitride \( \{\text{PhB}([\text{Pr}_2\text{Im}]_3)\} \text{Fe}(\text{N}) \) (\( \text{Pr}_2\text{Im} = 1,2\text{-diisopropylimidazolylidene} \)) (36). This ultimately generated a cyanide ligand, with the release of alkyne \( \text{Pr}_2\text{NC}≡\text{CN}\text{Pr}_2 \) as the side product. Mixing \( 1\text{-W} \) with 3 equivalents of BAC in tetrahydrofuran (THF) in the presence of \( \text{NaBPh}_4 \) as a chloride abstracting agent results in the gradual disappearance of the insoluble \( 1\text{-W} \) to form a dark red solution, along with the precipitation of a colorless solid, assigned as \( \text{NaCl} \) (Fig. 2). Upon filtration, the vapor diffusion of pentane into the filtrate over one day leads to the formation of dark purple needles. A single crystal X-ray diffraction (XRD) study of these crystals confirmed the structure of the product, where the three terminal chlorides have been substituted with BAC to give a monocationic cluster, \( 2\text{-W} \), with a \( \text{BPh}_3 \) counterion (Fig. 2). Although \( \text{MFe}_3\text{S}_3 \) clusters supported by carbene ligands have not been structurally characterized, the Fe-C distances are in the range of Fe-S distances supported by NHC ligands (15, 37).

In order to promote the delivery of a C atom or CR group, at least one C-C bond has to be cleaved, which can be achieved by methods such as heating (38), photolysis (38), or reduction (36, 39). While \( 2\text{-W} \) remains unchanged when irradiated with a 75-W Xe lamp and decomposes when heated at reflux in THF under an inert atmosphere, reduction with one equivalent of a strong reducing agent like potassium naphthalenide leads to the new cluster \( 3 \). Instead of generating a neutral, one-electron reduced form of \( 2\text{-W} \) and \( \text{KBPh}_4 \) as byproduct, product 3 loses

![Fig. 1.](image1.png)

![Fig. 2.](image2.png)
the μ3-Cl ligand as KCl likely driven by precipitation, leaving an open triangular Fe₃ face, as demonstrated by XRD characterization (Fig. 2).

Cluster 3 possesses a rare incomplete cubane geometry for iron-sulfur clusters. The related [Fe₃S₄] geometry has only been reported in the anion [Fe₂S₃(NO)₂]⁻ of Roussin’s black salt (40) in inorganic compounds, and an oxygen-tolerant [NiFe] hydrogenase in biology (41). Incomplete heterometallic cubanes of the form MFe₂S₃ have only been observed for M = Mo in a synthetic system, where the Fe atoms are ligated by multiple CO ligands (42). The open-face Fe₃ triangle resembles the sulfide-free triiron systems supported by multinucleating trisamide ligands, which can bind μ3-nitride or μ3-imide moieties (14, 43). Thus, the open nature of the Fe₃ cluster face in 3 makes it a promising platform for the rational installation of various bridging ligands in a μ3 mode.

Cluster 3 can further be reduced with an excess of KC₈ to form the neutral, Et₂O soluble cluster 4-W. Gratifyingly, under these highly reducing conditions, the C-C bond in the BAC ligand is cleaved and the cyclopropene ring opens, delivering a carbyne ligand to the bridging position. The cluster loses its C₃ symmetry, resulting in two Fe atoms ligated by BAC and a unique Fe center, to which the rest of the ring-opened BAC ligand anchors as a vinyl fragment. This is an example of a synthetic iron-sulfur cluster without CO ligands that displays a carbyne donor. Aside from the bridging carbyne ligand, the terminal hydrocarbyl ligand is also notable, given the role of such ligands in SAM enzymes (44) and their scarcity in synthetic iron-sulfur cluster chemistry (45). Convenienly, 4-W can also be synthesized directly from 2-UW using an excess of KC₈ or potassium naphthalene without isolating 3. This reaction stops at 3 if conducted at –78 °C for 1 h, while appreciable conversion to 4-W can only be achieved at room temperature over longer reaction times, suggesting that the ring opening and rearrangement of the BAC ligand is rate-limiting. The vinyl ligand in 4-W can be alkylated with MeOIT, leading to a five-membered amine-carbyne chelate with NPr₃ bound to the unique Fe (Fig. 2). This cluster is reminiscent of a putative NH₃-bound form of FeMoco, as it displays a bridging C-based ligand and a nitrogen donor at one of the Fe centers.

Toward preparing structural analogs of the μ3-carbyne ligand, 3 was investigated as a precursor to a cluster bearing N or S at the bridging position. Treatment of 3 with NBu₃N₃, Me₂SiN₃, and PPH₃ (or S₅) leads to the formation of the corresponding nitride-(6), imide-(7), and sulfide-(8) bridged clusters (Fig. 3). Complexes 6, 7, and 8 are isoostructural, with a WFe₂S₃(N = N or S) cubane supported by Tp* at W and one BAC ligand bound to each iron center (Fig. 4). The presence of three BAC ligands is a distinct feature relative to 4-W. Targeting a carbyne analog with the same number of BAC donors, compound 5 was treated with BAC; however, no reaction was observed, likely due to a combination of steric constraints and stability of the chelate.

For closer similarity to FeMoco, a Mo variant of the above clusters was targeted. The Mo-containing precursor 1-Mo was conveniently synthesized from [NEt₄][Tp**MoS₃] via [NEt₄][Tp**MoS₃] generated by sulfur abstraction with PPh₃ (St Appendix). Adapting the synthetic protocol developed for 2-W, chloride substitution with BAC from 1-Mo allowed for the isolation of 2-Mo. Ring opening upon reduction with KC₈ resulted in the formation of 4-Mo (Fig. 2). Notably, the Mo₃S₃=Fe₃C cluster core of 4-Mo reproduces one half of the structure of FeMoco, including the bridging carbon donor. Furthermore, the geometry of the unique Fe in the C-bridged clusters 4-W, 4-Mo, and 5 reproduces the four-coordinate, distorted trigonal pyramidal geometry found in the belt sites of FeMoco (Fig. 2). The S-Fe-S-C and S-Fe-S-N torsion angles in 4-W (173.2°) and 5 (154.2°) approach 180°, bringing these four atoms close to coplanar, which corresponds to a distorted trigonal pyramidal geometry at Fe, leaving the axial site open for potential substrate coordination, as has been previously invoked for N₂ binding in FeMoco (17). In addition, the Fe-N distance in 5 is 2.16 Å, close to the Fe-N bond length in the previously characterized NH-bound FeVco (2.01 ± 0.04 Å) (31). Further studies are being conducted to investigate reactivity at this site.

A comparison of the structural aspects of the reported clusters and the corresponding subsite of FeMoco is informative (SI Appendix, Table S3). The W/Mo-S distances vary modestly (2.36 to 2.39 Å) in the series of cubane complexes, suggesting that the metal oxidation state remains unchanged. Although the total redox state of the metal core varies from (M⁴⁺) to (M⁵⁺), it is likely that the formal oxidation state for M lies within the 3+/4+ range, based on literature assignments for MoFe₃S₄ (47) and WFe₃S₄ (48) in two redox states, (MFe₃)⁴⁺ and (MFe₃)⁵⁺, as well as the trend in M⁻S bond length as a function of oxidation states of M from related species (SI Appendix, Table S4). Comparison of bond lengths within the organic fragment supporting the carbyne ligand reveals notable differences in 4-W/4-Mo versus 5. In 4-W/4-Mo, the C₁₀-N₁₁ (average 1.36 Å) and C₉-N₁₂ (average 1.46 Å) distances are significantly different, suggesting multiple bonding character in the carbyne. The C₉-C₁₀ bond, increasing the N-C bond order (49), the possible changes in the character of the carbyne makes oxidation states ambiguous, but for consistency, herein the carbyne is assigned in the same way in all of the compounds. It is worth noting that the nature of the carbyd ligand in FeMoco may also vary as a function of changes in the interactions with the other, remote metal centers. Additional experiments will be necessary to determine the overall redox states and distribution between metals; nevertheless, these compounds are in the range assigned for FeMoco (2). The structural parameters for the W and Mo analogs 4-W and 4-Mo are very similar, which suggests analogous redox distribution within the cluster despite different heterometals M. The Fe-C distances in 4-W, 4-Mo, and 5 are in the range of 1.94 to 1.95 Å, which are close to the average Fe-C bond length in FeMoco of 2.00 Å.
In order to probe the impact of structure on the redox potentials of the cubane models of FeMoco, we carried out a comparative cyclic voltammetry (CV) study of compounds 4 to 8 (Fig. 5). Each cluster displays at least one oxidation and one reduction event, both reversible. To assign the redox waves to the corresponding redox couple, starting from the structurally characterized complexes, the open-circuit potential of the system was determined prior to scanning reductively. For 6, 7, and 8, the two CV features are assigned to the \((M'^0\text{Fe}_3)^{11+}/(M'^0\text{Fe}_3)^{10+}\) and \((M'^0\text{Fe}_3)^{10+}/(M'^0\text{Fe}_3)^{9+}\) couples. For 4-W, 4-Mo, and 5, they correspond to \((M'^0\text{Fe}_3)^{12+}/(M'^0\text{Fe}_3)^{11+}\) and \((M'^0\text{Fe}_3)^{11+}/(M'^0\text{Fe}_3)^{10+}\). Compounds 4-W and 4-Mo show an additional reversible event at more positive potentials, assigned to \((M'^0\text{Fe}_3)^{13+}/(M'^0\text{Fe}_3)^{12+}\), which might be an indication of the carbyne ligand’s ability to accommodate expanded redox capabilities. Compounds 4 to 8 can be compared using the \((M'^0\text{Fe}_3)^{11+}/(M'^0\text{Fe}_3)^{10+}\) couple (highlighted by boxes in Fig. 5), which they all display.

Although the compared redox event corresponds to the same formal oxidation state and metal coordination number across all clusters, there are several structural changes that can impact the reduction potentials and convolute interpretation: the identity of the bridging atom (C versus N versus S), the presence and nature of a chelate attached to the bridge, and the character of the bridging ligand stemming from its substituents (i.e., rotation of the amine and delocalization of its lone pair). Within the carbyne-containing clusters there is little impact of the identity of the Group 6 metal (M' = Mo versus W) on the reduction potentials, with a slight increase in redox potential of 70 mV on changing W to Mo, although the other biologically relevant Fe or V variants remain to be pursued. In biomimetic group transfer chemistry with Mo and W, a similarly modest increase in potentials of about 120 mV is also observed for a nicotinic acid hydroxylase synthetic analog when Mo is replaced with W (51). The redox couple shifts positively by about 0.75 V between 4-W and 5, and a combination of structural changes support this trend: the positive charge of 5, the weaker electron donating capability of the NiPr2 group compared to the vinyl ligand, and the donation of the amine lone pair into the olefin π system. The size of the carbyne chelate may also impact redox chemistry by changing the electronic...
impact of the chelating C- versus N-based, though nonchelating, ligands. Changing the donor from nitride (6) to imide (7) or sulfide (8) shifts the redox potential less than 200 mV, highlighting the similar effect of S and N donors on the redox chemistry, in contrast to the chelating carbyne.

These electrochemical results suggest that the interstitial carbon ligand in FeMoco may play an important role in increasing the reducing power of the clusters. While the specific oxidation states of the metal centers cannot be verified without additional spectroscopic studies, the reduced form, (MFe3)10+, corresponds to an average metal oxidation state of 2.5 (or 2 if a Fischer carbyne resonance is considered), close to the resting state of FeMoco as Fe3Fe10Mo0III (average metal oxidation state 2.6) (2). Additional reduction steps lower the average formal oxidation state of FeMoco, but not below 2, and do not bring it in the range typically observed for mononuclear Fe complexes studied for N2 activation (53). Therefore, the ability of the biological cofactor to perform N2 activation at high oxidation states is unusual. A possible explanation is charge redistribution within the cluster to increase reducing equivalents at the site of substrate binding or electronic communication between different metal sites (21–23, 54). We find here that the chelating carbyne ligand has a remarkable impact on the cluster reduction potentials, with very reducing potentials for relatively high biologically relevant metal oxidation states. Moreover, the chelating carbyne clusters are significantly more reducing for the same redox state compared to N and S analogs. It is important to note that the chelation present in all carbynes reported here and the delocalization of amine lone pair in some of them may have a substantial effect on the redox chemistry by tuning the electronic properties of the carbon ligand; conceptually related, changes in the coordination environment of distal iron centers in FeMoco may have similar effects on the carbyne.

Toward addressing the effect of the interstitial ligands of FeMoco, we have reported studies of tetranuclear Fe clusters with μ2-fluoride and oxide ligands (21). In those cases, the oxide makes the cluster about 1 V more reducing compared to fluoride for the same redox state while also promoting NO activation. Additionally, remote metal centers affect reactivity through interactions with the bridging moiety (O or F). In those systems, the charge of the interstitial atom could play a role in changing the potential. Here, this series of clusters accounts for changes in ligand charge. Chelating carbyne (5) and nitride (6) ligands have the same formal charge but result in ~1 V difference in reducing power, in contrast with the nitride (6), imide (7), and sulfide (8) species that have reduction potentials within 300 mV. The ability of the chelating carbyne to increase the reduction power is likely a consequence of its stronger interaction with the metal centers, an aspect that will be pursued in a separate study, including nonchelated targets.

In the context of N2 reduction, the redox tuning observed here suggests that the interstitial carbon may allow FeMoco to access higher reducing power, enabling more facile transfer of electrons to the N2 substrate for conversion to NH3. This parallels the results from experiments using synthetic iron catalysts for N2 reduction, many of which require strong external reducing agents like KC8 (8, 55). Considering the potential impact of replacing the bridging carbyne with a more biologically inexpensive sulfide, a much less reducing cluster (8) is generated for the same redox state, (MFe3)10+, and even an additional reducing equivalent in (MFe3)9+ does not match with the (MFe3)10+ carbyne system. Therefore, an interstitial sulfide may not provide sufficient reducing power to efficiently convert N2 into NH3, leading instead to the preference for the unusual bridging carbide motif.

In summary, we have described the synthesis of a series of heterometallic iron-sulfur clusters of the form M′Fe3S3X (M′ =
Mo or W and X = CR, N, NR, and S) with the cubane geometry matching the structure of the MFe5S4C6 subisite of FeMoco. These include examples of iron-sulfur clusters containing a chelating carbon-based ligand bridging the Fe3 face. Importantly, electrochemical studies indicate that the presence of a bridging C-donor in combination with electronic tuning (by chelation and amine lone pair delocalization) allows the clusters to reach highly reducing states, with potential implications for N2 reduction chemistry. These studies shed light on possible structural and electrochemical roles of the interstitial carbide ligand in nitrogenase.

Materials and Methods

All reactions were performed at room temperature in a N2-filled MBraun glovebox or using standard Schlenk techniques unless otherwise specified. Solvents were dried by sparging with N2 for at least 15 min and then passing through a column of activated alumina under positive N2 pressure and stored over molecular sieves prior to use. All experimental conditions and procedures, spectrophotometric data, and details of characterization of compounds [NEt4][Tp*MoS3] and 1 to 8 are given in SI Appendix. CV experiments were performed with a Pine Instrument Company AFCBP1 bipotentiostat in a three-electrode cell, which consisted of glassy carbon (working; φ = 3.0 mm), Ag wire (reference), and bare Pt wire (counter), in a N2-saturated electrolyte solution.

Data Availability. Atomic coordinates and structure factors data have been deposited in the Cambridge Structural Database (CSD) of the Cambridge Crystallographic Data Centre (https://www.ccdc.cam.ac.uk/structures). CSD reference numbers are as follows: 2084246 (1-Mo), 2081620 (2-W), 2084269 (2-Mo), 2081619 (3), 2081616 (4-W), 2084247 (4-Mo), 2081621 (5), 2081617 (6), 2081622 (7), and 2084054 (8). All other study data are included in the article and/or SI Appendix.

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