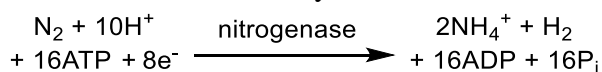


Modeling the FeMoco Cluster of Nitrogenase: Synthesis of a μ_3 -Carbide Metal Cluster

Ammonia is the second-largest synthetic chemical product worldwide, with 1.68×10^8 tons produced annually.¹ Typically, ammonia is synthesized via the Haber-Bosch process, where a heterogeneous iron oxide catalyzes the reaction between hydrogen and nitrogen.^{1,2} About 85% of ammonia produced is used towards crop fertilization; large-scale ammonia production has been credited for the quadrupling of the world's population from 1.8 to 7.4 billion in the last 100 years.² The process is typically performed in excess of 400°C at 200 atm, and is highly energy-intensive, consuming about 3–5% of the world's yearly natural gas production and 1–2% of the global energy produced. These are consequences of nitrogen's high stability: the N–N triple bond of N_2 is one of the strongest covalent bonds known, with a dissociation energy of 226 kcal/mol.³

Despite the stability of nitrogen gas, many classes of bacteria and archaea have evolved the ability to catalytically fix nitrogen via the enzyme nitrogenase, which couples the reduction of nitrogen to the hydrolysis of ATP over 8 single-electron transfer events (Scheme 1). Remarkably, nitrogenase is capable of fixing nitrogen at ambient temperatures and pressures, a feat that humans have yet to mimic.



Scheme 1. Balanced half-reaction for nitrogenase-mediated nitrogen fixation. P_i = inorganic phosphate.

Nitrogenase contains three types of metal cluster cofactors: the most complex of these is the FeMo cluster (FeMoco), where nitrogen is bound and reduced (Figure 1). FeMoco is comprised of 8 metal centers encapsulating a small interstitial atom. The interstitial atom's identity has been long debated as either C, N, or O; recent research has provided strong evidence for the identification of this atom as carbon.⁴

Much is still unknown about the mechanism through which FeMoco reduces nitrogen, including the precise N_2 binding site, the electronics of the cluster during reduction, and the role of the interstitial carbon. Computational

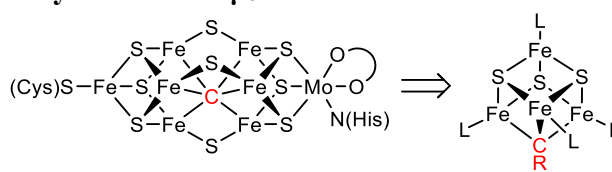


Figure 1. (left) Structure of the FeMo cluster of nitrogenase. The terminal iron is bound to cysteine; the terminal molybdenum is bound to histidine and chelated by homocitrate. (right) Proposed synthetic target, corresponding to the left cubane of FeMoco.

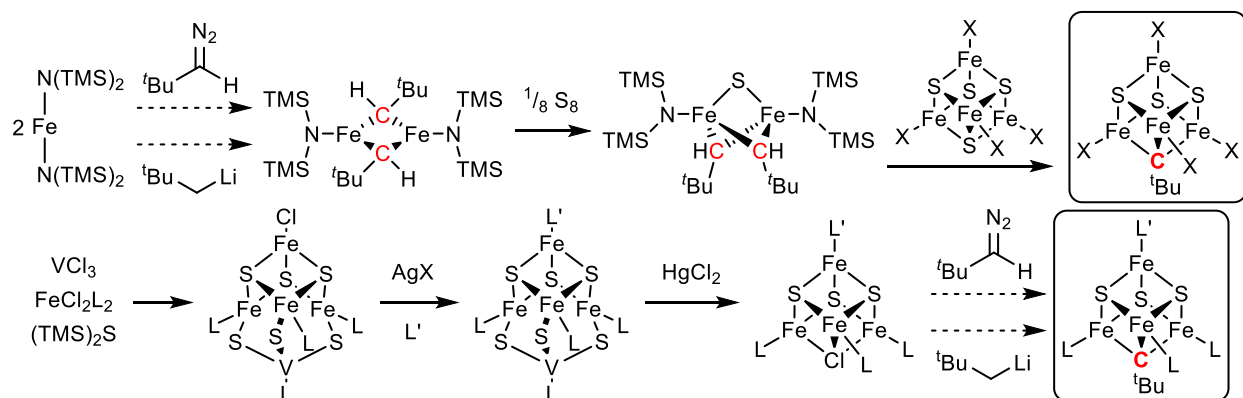
studies have attempted to calculate stable nitrogen binding sites of FeMoco and possible intermediates of the reduction.^{5,6} However, in order to determine the true mechanism, experiments on a structural model are necessary.

Due to the high complexity of FeMoco, a synthetic model adequate for detailed structure-function studies has proven elusive thus far; as of yet, no models incorporate the interstitial carbon atom. I propose to develop a synthesis of a novel cubane cluster structurally relevant to FeMoco (Figure 1). This tetrametallic cluster contains an unusual bridging carbide ligand coordinated to three iron centers.

Trimetallic bridging carbide clusters are known for various metals, including Ti,⁷ Co,⁸ Ru,⁹ and Os.¹⁰ However, these isolated complexes all contain strong-field ligands such as CO and cyclopentadienide; μ_3 -carbides are extremely rare for complexes containing low-field ligands such as sulfides, making the synthesis of a $[4Fe-3S-C]$ cluster such as this a formidable challenge.

Lee and coworkers have previously synthesized a $[4Fe-3S-N]$ cluster containing a μ_3 -imido ligand, analogous to the proposed μ_3 -carbide, from a $[4Fe-4S]$ cluster and a nitride-carrying bimetallic species.¹¹ I will use this route as a model synthetic strategy (Scheme 2). The μ_3 -imido nitrogen in Lee's system is derived from an amine; an isoelectronic reagent such as an organolithium reagent could react similarly. A carbene equivalent such as a diazo species could also serve as a carbon precursor.

An alternative synthesis draws from the work of Holm and coworkers, who synthesized pentametallic cuboidal clusters containing a



Scheme 2. Two proposed syntheses of the target cubane clusters, inspired by Lee (top) and Holm (bottom).

[4Fe-3S] moiety capped by a fifth metal through sulfide linkages.¹² By using a small phosphine such as PMe_3 as L and a bulky phosphine such as $\text{P}(\text{tBu})_3$ as L', the sulfides capping the cuboidal face could potentially be more accessible. This would allow for selective removal of the capping sulfides with a thiophilic reagent such as mercury, opening up a vertex for carbide insertion.

Although ^{13}C NMR spectroscopy could potentially be used to check for incorporation of the carbide, the paramagnetism of iron clusters diminishes the usefulness of NMR spectroscopy as a characterization tool. Instead, product characterization will focus heavily on mass spectrometry and X-ray crystallography, to determine whether the synthesis was successful. I will also use Mössbauer spectroscopy to determine the oxidation states of the iron centers.

Upon successful synthesis of the target, further experiments will be performed to fully characterize the cluster. Cyclic voltammetry will be utilized to test the redox properties of the clusters. The ability of these clusters to bind N_2 will also be examined, both under redox-neutral and reducing conditions. In the event that N_2 successfully binds, characterization to determine the binding site(s) will be carried out via X-ray crystallography. The reactivity of a nitrogen-binding species towards various nucleophiles and reductants will also be explored.

Future directions include using this carbide-containing cluster as a stepping stone towards the full synthesis of a FeMoco-like cluster. By constructing the other half of the cluster and li-

gating the clusters together, a true analog of FeMoco could be synthesized. This full model could be used in structure-function studies and would prove invaluable in determining the precise mechanism of nitrogenase.

INTELLECTUAL MERIT, BROADER IMPACTS

The proposed research would provide a synthetic route to low-field μ_3 -carbide metal clusters, which are very uncommon and not well understood. This research would also fill a wide gap in our knowledge about the mechanism of nitrogen reduction via FeMoco. Knowing the mechanism through which biological systems fix nitrogen could lead to the development of new manmade nitrogen fixation methods, boosting the production of ammonia while simultaneously reducing energy inputs.

The primary use of ammonia is as a fertilizer; a more efficient means of ammonia production would correspond to a potential increase in agricultural productivity, helping to sustain the ever-growing global population. Although this is not likely to outcompete the Haber-Bosch process, a small-scale source of readily obtainable ammonia would be incredibly useful for remote regions where fertilizer is scarce.

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