Spring 2005

Approaches Towards the Syntheses of New Molybdenum Complexes as Models for the Active Site in Nitrogenase Enzymes

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Approaches Towards the Syntheses of New Molybdenum Complexes as Models for the Active Site in Nitrogenase Enzymes

Jessica Hilborn
April 2005
This thesis has been approved for honors recognition in the Department of Natural Sciences by:

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April 2005
Acknowledgements

I would like to thank Dr. Michelle Millar at the State University of New York at Stony Brook, for all of her help and guidance through the duration of this project. I am grateful to her for allowing me to work in her lab over the summer of 2004 as an REU participant. The experience was one that aided in my decision to attend graduate school in chemistry. I would also like to thank Dr. Stephen Koch and the other group members for their assistance and explanations regarding my research. Also, a big thanks to my lab partner for the summer, Edythe Maa. Her companionship was invaluable and her work ethic was one to be coveted. All other REU participants in New York also deserve recognition—Katie, Luciano, Chris, Scott, Diana, Kunil, and Rob—we all made it through the good times and the bad times at chemistry camp. Thanks also to the NSF for providing the funding for this REU program. The overall experience was invaluable to my education and my appreciation of chemistry.

I would also like to thank my chemistry professors at Carroll—Dr. Salzsieder, Dr. Wilde, Dr. Strode, and Dr. Bregel—who have guided me in my undergraduate experience and have watched my personal transformation from freshman year to the present day. Their time and effort in writing the many letters of recommendation, as well as their advice on classes, REUs, graduate school, and life in general is greatly appreciated. I know that I would not have made it to where I am today without their support. Thank you also to Dr. Shields for his continued help in the thesis writing process. All of my other professors deserve gratitude as well for their positive influence in my college experience.

And most importantly, I would like to thank my family for everything. Their love and support have taken me to heights I never thought possible in my life and have given me everything I could ever need. Thank you and I love you.
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Abstract

The present study aims to mimic the catalytic properties of the Mo center in the active site of the nitrogenase enzyme. The phosphorous-sulfur ligands PS3, PS3' and PS3* were used to synthesize Mo-thiolate complexes of the formula [Mo(PS3)L2] where L is NO, N2 or a related molecule. The starting material [Mo(NO)2Cl2] allows for the study of structure and reactivity at the Mo center via IR spectroscopy of the NO region. Reactions of the starting material in various solvent systems to form complexes of the formulation [Mo(NO)2Cl2L2] confirmed the stationary nature of the cis NO ligands. Reactions with PS3 and PS3' worked best in methanol, giving clean spectra with two NO peaks, as expected for a cis conformation; the reaction with PS3* worked best in THF. A crystal structure of air-stable [Mo(NO)2Cl2(C2H5CN)2] was successfully obtained and analyzed using X-ray crystallography. The bond angles in the structure compare favorably to accepted octahedral complexes.
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Introduction

N₂ makes up about seventy-five percent of the composition of air. However, it is so stable that it is practically unusable to many living organisms. To compensate, nitrogenase, an enzyme found in some biological systems, converts N₂ to NH₃ in a process called nitrogen fixation to provide a nitrogen source for the building of amino acids and nucleic acids.¹ It also reduces other compounds such as C₂H₂ to C₂H₄, and HCN to CH₄ and NH₃.² Plants utilize the enzyme in their roots to reduce nitrogen and extract nutrients.³ Industry also utilizes the fixation of nitrogen in the commercial production of NH₃ for fertilizers and other nitrogen based products.¹ Further interest in the nitrogenase enzyme comes from the fact that the reaction is exergonic (-33 kJ), and can occur at near room temperature (290 K) and pressure (0.08 atm).¹ Comparatively, the Haber-Bosch process, a well known industrial process, requires iron catalysts and very harsh conditions, about 600 K+, 500 atm.¹ The rate-determining step in biological nitrogen fixation is thought to be the dissociation of the protein dimer of the enzyme after electron/proton shuttle action for the reduction of iron.¹ The rate-determining step in the industrial nitrogen fixation is thought to be the activation of the N₂ before it splits.¹ The difference in these reaction conditions gives rise to the need for further study: why is the nitrogenase enzyme so efficient and how can we model it?

X-ray crystallographic and spectroscopic studies indicate that the active site of the nitrogenase enzyme contains a unique inorganic cluster composed of one molybdenum, seven iron, and nine sulfur atoms, as shown in Figure 1.³ It is not known where the N₂ activation site is located on the cofactor: the Fe or the Mo.³
Focused studies on molybdenum coordination chemistry have produced several successful model systems that are capable of converting N\textsubscript{2} to NH\textsubscript{3}. For example, the Chatt system uses an Mo(0) catalytic center coordinated to four phosphine ligands.\textsuperscript{2} The Schrock system uses an Mo(III) center coordinated to an amine and three amide ligands, creating complexes of the form [N(N)\textsubscript{3}MoL].\textsuperscript{2}

The active site was modeled to mimic the catalytic properties of the nitrogenase enzyme via the Mo portion of the cofactor. To more closely represent the Fe-Mo center in nitrogenase, which contains sulfur centers, analogous ligands to the Schrock system were used to synthesize new Mo-thiolate complexes. Past studies have indicated that the ligands used must also effectively block the formation of Mo-N-N-Mo bridges between active sites, which make the sites robust to reduction.\textsuperscript{2} To prevent this problem, the polydentate ligands PS3, PS3', PS3* (Figs. 2-4), available in the lab group of Dr. Michelle Millar at the State University of New York at Stony Brook, were used to synthesize model compounds of the formulation [Mo(PS3)L\textsubscript{2}], where L is N\textsubscript{2}, NO, or a related molecule.

A better understanding of the activation at the nitrogenase enzyme active site will be reached through the study of the model complexes.
One can model the Mo site and use it as a base unit for further modification through the ligand attachments. NO binds similarly to N₂, and is easy to work with in the study of the nitrogen fixation models. Studying enough reactions involving NO groups will offer a better understanding of what may occur at the molybdenum center in the enzyme with regard to N₂ activation. The results may indicate if the molybdenum is the active site in the cofactor. NO is also an important ligand, as it is involved with many biological complexes itself, possibly leading to further understanding of the biological systems containing the nitrogenase enzyme.⁹,¹⁰

Experimental

The starting material, [Mo(NO)₂Cl₂]ₙ, reacted with ligands to provide a monomeric molybdenum center⁴,⁵ possessing NO ligands. The synthesis of the starting material provided high yields via a reaction of Mo(CO)₆ and NOCl.⁶ Since NOCl is not available commercially, it was synthesized by reacting NaNO₂ with HCl⁷ under nitrogen using the equipment set-up shown in Fig. 5. An addition funnel containing sodium nitrite and water was attached to a side neck of a 2000-mL 3-neck round bottom flask (RBF)
containing HCl. An angled distillation tube for product purification was attached to one of the side necks. A purification tube was attached containing sodium nitrite powder (HCl purification), KCl powder (NO₂ purification), and calcium sulfate (drying agent) separated by glass wool. The purification column led to a smaller RBF immersed in a dry ice/acetone slurry at -78°C, and a nujol bubbler to prevent build-up of pressure. The middle neck was stoppered. The sodium nitrite solution was added drop-wise over a period of several hours while being continuously stirred and closely monitored. The brown NOCl gas that evolved passed through the purification column, liquefied (bp=-6°C), and solidified (fp=-61°C) in the cooled RBF. Once collected, the NOCl was warmed and sublimed into another cooled RBF to ensure purity. The product was stored in dry ice in the freezer until used.

Figure 5. Equipment set-up for the synthesis of NOCl.
Figure 6. Equipment set-up for the synthesis of [Mo(NO)₂Cl₂].

Mo(CO)₆ was then reacted with the NOCl⁶ in a closed system (Fig. 6).

NOCl was dissolved in methylene chloride (referred to as CH₂Cl₂ or MeCl₂ herein) and placed in an addition funnel attached to a 2000-mL 3-neck RBF and added drop-wise to a solution of the Mo(CO)₆ in methylene chloride while stirring. On one side neck was a large column attached to the N₂ inlet. The other neck was attached through a column to a nujol bubbler to prevent CO gas build-up. The system was opened to air for a few seconds to initiate the reaction. After the desired green flocculent precipitate, [Mo(NO)₂Cl₂]ₙ, settled out, it was filtered using a coarse frit in a separate closed system.

To check for solvent interactions, [Mo(NO)₂Cl₂] was then reacted under nitrogen with several solvent systems: THF, MeOH, EtCN, PrCN, iPrOH, and EtOH. In each
case, about 0.100 g of the Mo complex was completely dissolved in 3-4 mL of solvent. The solutions had no change in appearance after 1 hour, 24 hours, or 3 days. IRs were collected and analyzed (see Fig. 7 and Table 1). The reaction of [Mo(NO)2Cl2] and EtCN was repeated. The reaction tube was heated with a heat gun for a few minutes, and green crystals were allowed to settle out overnight. X-ray crystallography indicated that perfect, single crystals had been obtained.

[Mo(NO)2Cl2] was also reacted 1:1, with the aforementioned ligands PS3, PS3', and PS3* (Figs. 2-4) in both MeOH and THF solvent systems. The reactions were allowed to react overnight and IR spectra were collected the following day (see Figs. 8-11 and Tables 2-5). A pure product was not isolated.

**Results**

A nujol mull IR spectrum was taken of the [Mo(NO)2Cl2] to compare to previously recorded NO peak values in similar experiments. IR spectra were collected for the reactions of [Mo(NO)2Cl2] with various solvento ligands (Fig. 7, peak assignments in Table 1) and with PS3 in MeOH and THF (Figs. 8 and 9, peak assignments in Tables 2 and 3). The IR spectra were also collected for the reaction of [Mo(NO)2Cl2] and PS3' in MeOH (Fig. 10, peak assignments in Table 4) and with PS3* in THF (Fig. 11, peak assignments in Table 5). Crystallographic data were obtained for the structure of [Mo(NO)2Cl2(C2H5CN)2] (Fig. 12, Tables 6 and 7).
Figure 7. NO region of IR spectra of \( [\text{Mo(NO)}_2\text{Cl}_2\text{L}_2] \) (L = THF, MeOH, \( \text{C}_2\text{H}_5\text{CN}, \text{CH}_3\text{CN}, \) iPrOH, EtOH).

Table 1. Peak shift values for IR spectra of \( [\text{Mo(NO)}_2\text{Cl}_2\text{L}_2] \) (L = THF, MeOH, \( \text{C}_2\text{H}_5\text{CN}, \text{CH}_3\text{CN}, \) iPrOH, EtOH).

<table>
<thead>
<tr>
<th>L</th>
<th>NO Peak Shifts (cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nujol mull(^{6,8})</td>
<td>1805</td>
</tr>
<tr>
<td>THF</td>
<td>1790</td>
</tr>
<tr>
<td>MeOH</td>
<td>1796</td>
</tr>
<tr>
<td>( \text{C}_2\text{H}_5\text{CN} )</td>
<td>1803</td>
</tr>
<tr>
<td>( \text{CH}_3\text{CN} )</td>
<td>1806</td>
</tr>
<tr>
<td>iPrOH</td>
<td>1797</td>
</tr>
<tr>
<td>EtOH</td>
<td>1796</td>
</tr>
</tbody>
</table>
Figure 8. NO region of IR spectra of $[\text{Mo(NO)}_2\text{Cl}_2]$ in MeOH (top) and $[\text{Mo(NO)}_2\text{Cl}_2]$ with PS3 in MeOH (bottom).

Table 2. Peak shift values for IR spectra of $[\text{Mo(NO)}_2\text{Cl}_2]$ in MeOH and $[\text{Mo(NO)}_2\text{Cl}_2]$ with PS3 in MeOH.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>NO Peaks (cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[\text{Mo(NO)}_2\text{Cl}_2]$ in MeOH</td>
<td>1796 1683</td>
</tr>
<tr>
<td>$[\text{Mo(NO)}_2\text{Cl}_2]$ with PS3/MeOH</td>
<td>1736 1650</td>
</tr>
</tbody>
</table>
Figure 9. NO region of IR spectra of $[\text{Mo(NO)}_2\text{Cl}_2]$ in THF (top) and $[\text{Mo(NO)}_2\text{Cl}_2]$ with PS3 in THF (bottom).

Table 3. Peak shift values for IR spectra of $[\text{Mo(NO)}_2\text{Cl}_2]$ in THF and $[\text{Mo(NO)}_2\text{Cl}_2]$ with PS3 in THF.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>NO Peaks (cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[\text{Mo(NO)}_2\text{Cl}_2]$ in THF</td>
<td>1789, 1675</td>
</tr>
<tr>
<td>$[\text{Mo(NO)}_2\text{Cl}_2]$ with PS3/THF</td>
<td>1751, 1724, 1663, 1637</td>
</tr>
</tbody>
</table>
Table 4. Peak shift values for IR spectra of [Mo(NO)$_2$Cl$_2$] in MeOH and [Mo(NO)$_2$Cl$_2$] with PS3' in MeOH.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>NO Peaks (cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Mo(NO)$_2$Cl$_2$] in MeOH</td>
<td>1796 1684</td>
</tr>
<tr>
<td>[Mo(NO)$_2$Cl$_2$] with PS3'/MeOH</td>
<td>1737 1638</td>
</tr>
</tbody>
</table>

Figure 10. NO region of IR spectra of [Mo(NO)$_2$Cl$_2$] in MeOH (top) and [Mo(NO)$_2$Cl$_2$] with PS3' in MeOH (bottom).
Figure 11. NO region of IR spectra of \([\text{Mo(NO)}_2\text{Cl}_2]\) in THF (top) and \([\text{Mo(NO)}_2\text{Cl}_2]\) with PS3* in THF (bottom).

Table 5. Peak shift values for IR spectra of \([\text{Mo(NO)}_2\text{Cl}_2]\) in THF and \([\text{Mo(NO)}_2\text{Cl}_2]\) with PS3* in THF.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>NO Peaks (cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>([\text{Mo(NO)}_2\text{Cl}_2]) in THF</td>
<td>1789 1676</td>
</tr>
<tr>
<td>([\text{Mo(NO)}_2\text{Cl}_2]) with PS3*/THF</td>
<td>1719 1630</td>
</tr>
</tbody>
</table>
Figure 12. Crystallographic structure of $[\text{Mo(NO)}_2\text{Cl}_2(\text{C}_2\text{H}_5\text{CN})_2]$. 
Table 6. Experimental bond lengths of [Mo(NO)$_2$Cl$_2$(C$_2$H$_5$CN)$_2$].

<table>
<thead>
<tr>
<th>Bond</th>
<th>Distance (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mo1-N1</td>
<td>1.816(7)</td>
</tr>
<tr>
<td>N1-O1</td>
<td>1.160(8)</td>
</tr>
<tr>
<td>Mo1-N2</td>
<td>2.113(10)</td>
</tr>
<tr>
<td>N2-C1</td>
<td>1.123(16)</td>
</tr>
<tr>
<td>Mo1-N3</td>
<td>2.142(10)</td>
</tr>
<tr>
<td>N3-C4</td>
<td>1.140(14)</td>
</tr>
<tr>
<td>Mo1-Cl1</td>
<td>2.432(2)</td>
</tr>
</tbody>
</table>

Table 7. Experimental bond angles of [Mo(NO)$_2$Cl$_2$(C$_2$H$_5$CN)$_2$].

<table>
<thead>
<tr>
<th>Bond</th>
<th>Angle (deg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl1-Mo1-Cl1</td>
<td>89.60(12)</td>
</tr>
<tr>
<td>N1-Mo1-N1</td>
<td>88.5(4)</td>
</tr>
<tr>
<td>N2-Mo1-N3</td>
<td>173.4(4)</td>
</tr>
<tr>
<td>Mo1-N1-O1</td>
<td>178.4(7)</td>
</tr>
</tbody>
</table>

Discussion

A model complex of the Mo site of the nitrogenase enzyme was synthesized and reactions were performed to begin to understand the activity at the Mo center. All reactions confirmed activity within the Mo coordination sphere as evidenced by NO peak shifts measured with IR spectroscopy.

The starting material is valuable for the following reasons: 1) it has defined solubility properties, being soluble in non-aqueous solvents such as alcohols, acetone, acetonitrile, and THF,$^3$ and not soluble in benzene, CCl$_4$, CHCl$_3$, and CH$_2$Cl$_2$, and alkanes,$^6$ which make reaction parameters easy to set up; 2) it is relatively stable upon reaction with solvent ligands,$^6$ 3) it can be produced in high yields,$^6$ and 4) it possesses NO ligands.

The NO ligand binds similarly to N$_2$, a target ligand, and also acts as an IR probe.
for the study of the structure and reactivity of the Mo coordination sphere. Since the reactions were monitored with IR spectroscopy and analyzed specifically for the NO functionality peaks and recognizable shifts, any movement in the location of the peaks would indicate that a reaction had occurred. NO is also an important ligand as it is involved with many biological processes itself.\textsuperscript{9,10}

In the present study, most of the ligand solvents were electron donors and gave rise to a NO peak field shift in the IR region (Fig. 7). The two distinct peaks indicated that the NO functionalities are arranged \textit{cis} to one another because they are equivalent in nature.\textsuperscript{3} Cotton\textsuperscript{6} obtained similar results in his studies with dinitrosyldihalide-Mo complexes, with peaks at 1805 and 1690 cm\textsuperscript{-1}. When his metal complex was reacted with different ligands, he produced [Mo(NO)\textsubscript{2}X\textsubscript{2}L\textsubscript{2}] compounds which could be monitored with IR spectroscopy.\textsuperscript{6} The experimental results found herein (Table 1) were similar when [Mo(NO)\textsubscript{2}Cl\textsubscript{2}] was reacted with various solvents. NO peaks in this study shifted up-field in all reactions, indicating good back-donation of electrons from the \textit{s}- and \textit{p}-shells of the ligand to the metal \textit{\pi}-orbitals. The NO peaks shift up- or down-field depending on the presence of electron back-donation; a larger electron donor will produce a more noticeable shift.

Since the NO elements stayed in a \textit{cis} orientation, further exploration with more complex ligands requiring \textit{cis} configurations of base molecules for stereospecific reactions may be performed. The double humped peaks in the reaction with PS3 in THF could be a result of isomers forming in solution or poor resolution of the IR detector between the peaks of those isomers. These peaks could also be a result of molecular bridging at the Mo site, creating a molecule with \textit{four} NO signals. From the limited data presented here, it was decided that MeOH was the best solvent for the reactions because
it produced clean spectra, and that the more bulky ligand, PS3*, worked best in THF, producing the cleanest spectra in comparison to MeOH.

The final reaction of Mo(NO)₂Cl₂ in C₂H₅CN produced rich, green, single [Mo(NO)₂Cl₂(C₂H₅CN)₂] crystals that settled out overnight. They appeared to be stable in air with no observed color changes. This result was ideal because the starting material was very air sensitive. The crystal structure (Fig. 12), bond lengths, and angles (Tables 4 and 5) compare favorably to accepted theoretical measurements for octahedral complexes. As predicted, the EtCN ligands are *trans* and the NO and Cl remain *cis*.

The successful synthesis of [Mo(NO)₂Cl₂L₂] complexes will provide air-stable reactants for further reactions to model the Mo center, particularly in the activation of N₂. The reactions completed also provide inspiration for further work in the field. When the chemistry of the Mo center is more fully understood, it may lead to better industrial nitrogen fixation processes that are more efficient and that reduce energy and resource consumption.
References


