SYNTHETIC MODELING OF NITRIC OXIDE (NO) AND NITROXYL (HNO) REACTIVITY AT COPPER SITES

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ABSTRACT

The N-O functionality is ubiquitous endogenously. The wide range of oxidation states of the nitrogen atom, (+5 to -3), is attributed to the various biologically relevant congeners containing the NO functionality. The simplest form of the N-O moiety is nitric oxide (NO’), a stable free radical, which plays an important role in several biological processes such as neurotransmission, immune function, and oxidative stress. The one electron reduced form of nitric oxide is nitroxyl (HNO). Nitroxyl functionality is similar to but distinct from nitric oxide and some report the endogenous formation of NO via nitric oxide synthase (NOS) actually produces a nitric oxide precursor, presumably HNO. The actual production of nitric oxide requires co-factor copper-zinc superoxide dismutase (CuZnSOD), which has been shown to react with HNO to produce NO at a rate of \( k = 9 \times 10^4 \text{ M}^{-1}\text{s}^{-1} \) in the same manner as superoxide dismutases convert \( \text{O}_2^- \) to \( \text{O}_2 \).

This work seeks to study the interaction between copper and nitroxyl and the formation of a copper-nitroxyl ([Cu](HNO)) complex via computational studies and utilization of analogous nitrosobenzene (PhNO). Three binding modes (κ^1-N, κ^1-O, and η^2-ON) were identified for both copper β-diketiminate nitroxyl ([Cu](HNO)) and nitrosobenzene ([Cu](PhNO)) complexes. The two viable binding modes (κ^1-N and η^2-ON) were relatively close in energy and are in exchange at room temperature. The binding of nitrosobenzene to copper results in changes to the copper oxidation state as suggested by x-ray crystallography, cyclic voltammetry, and x-ray absorption
studies. All of these studies suggest copper-nitrosobenzene complexes may be best classified as copper(II) compounds bound to nitrosobenzene where the ON bond order is 1.5.

The β-diketiminate copper nitrosobenzene complexes can be synthesized via (a) the addition of nitrosobenzene to corresponding copper(I) acetonitriles (b) the double deprotonation of N-phenylhydroxylamine by copper(II) hydroxides and (c) via the disproportionation of N-phenylhydroxylamine via corresponding copper(I) acetonitriles. This work proposes a mechanism for the disproportionation of N-phenylhydroxylamine and investigates the requisite copper(II) nitrooxide intermediates. The mechanistic insights and isolated intermediates put forth in this work will add potential inferences for larger related systems and can give insights into how to effect these biological NO processesing.
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1.1. Introduction

Nitroso containing compounds have been linked to a multitude of biological processes such as immune function, signaling, neurotransmission, antioxidant defense, cardiological activity and many more. Thus, a detailed understanding of the biological reactivity of the NO functionality is vital to understand its key roles health and disease. The issue is complex, for many different NO containing compounds are involved in nitric oxide reactivity and metabolism: nitric oxide (NO\(^{\cdot}\)), nitroxy (HNO), hydroxylamine, (R\(_2\)NOH), C-nitroso (RNO), nitroxides (R\(_2\)NO\(^{\cdot}\)) compounds, nitrite (NO\(_{2}^{\cdot}\)) and S-nitrosothiols (RSNO) (Figure 1.1). Moreover, the consumption and production of any one particular NO containing compound can be either deleterious or beneficial depending on its concentration and form.

Figure 1.1. Biological functions and congeners of nitric oxide.
Often, under physiological conditions, interconversions between reactive nitrogen oxide species (rNOS)\(^5-6\) (Table 1.1) are facilitated by metal cofactors essential for various biological processes.\(^7-8\)

<table>
<thead>
<tr>
<th>nitric oxide</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>nitroxyl</td>
<td>HNO</td>
</tr>
<tr>
<td>nitrogen dioxide</td>
<td>NO(_2)</td>
</tr>
<tr>
<td>peroxynitrite</td>
<td>ONOO(^-)</td>
</tr>
<tr>
<td>dinitrogen trioxide</td>
<td>N(_2)O(_3)</td>
</tr>
</tbody>
</table>

**Table 1.1.** Most common and reported on reactive nitrogen oxide species.

Copper is crucial for a number of biological functions through processes enabled by copper containing enzymes and ions.\(^9\) In particular, copper proteins play an integral role in NO metabolism because of their ability to react with NO. The reactivity of copper proteins with other NO derivatives is also quite important,\(^10-12\) especially because of the extremely low physiological concentration of NO (1 µM – 10 nM).\(^13-14\) In fact, before the identification of diverse roles for NO in biology, NO has been used extensively as a spin probe to study copper protein centers, especially copper(II) centers that possess one unpaired electron.\(^15\) Acting as a spin probe, nitric oxide often distinguish between different types of copper active sites (\textit{vide infra}). It can also act as a redox probe and has led to the elucidation of redox reaction mechanisms and the nature of copper proteins such as cytochrome C oxidase and laccase.\(^12,15-16\) For instance, NO allowed for the observation of an unstable cytochrome C (CcO) conformation previously unseen but physiologically relevant to understanding the role of CcO in electron transport.\(^15\)

While the first identification of copper in humans (more specifically, human brains), was reported in 1901, copper as an essential component of living systems was not widely accepted\(^14\) until 1921.\(^17\) Since then copper has been implicated in a number of biological functions, including immune function, cardiovascular health, antioxidant defense, electron transport, and connective
the biological misregulation of copper has been linked to a myriad of diseases including osteoporosis, Wilson’s, Parkinson’s, Huntington’s, Alzheimer’s and Menkes diseases.\textsuperscript{9}

The environments of the copper active sites within enzymes are variations of one of seven basic types: mononuclear type 1 (T1), mononuclear type 2 (T2), binuclear type (T3), trinuclear type 2+3, post-synthetically modified Cu\textsubscript{B}, binuclear Cu\textsubscript{A}, and tetranuclear Cu\textsubscript{Z} copper sites (Figure 1.2).

Ceruloplasmin and azurin both contain T1 copper active sites (Figure 1.3), though azurin has a variant of the typical T1 active site that possesses a weakly bound glutamine oxygen that renders the metal geometry more trigonal bipyramidal.\textsuperscript{19} Type 2 (T2) sites are comprised of three histidine imidazole nitrogen donors and a fourth ligand that may be water, a substrate, or product (Figure 1.2).\textsuperscript{20} Copper based nitrite reductase (Cu-NiR), a key enzyme in the denitrification cycle contains both T1 and T2 sites. Cu-NiR is a homogenous trimer in which each trimeric unit contains a T1 copper site to funnel electrons to the T2 copper site to reduce NO\textsubscript{2} to NO (Figure 1.3). Type 3 (T3) copper active sites are dinuclear copper ions in which each copper is supported by 3 imidazole N donors and the Cu centers are close enough to act in concert on substrates such as O\textsubscript{2}. For instance, hemocyanin (Figure 1.3) possesses a T3 copper site in which the Cu centers are separated by 3.6 – 4.6 Å and collaboratively to achieve oxygen transport via [Cu]_{2}(\mu-\eta^{2}:\eta^{2}-O\textsubscript{2}) intermediates.\textsuperscript{21}
Figure 1.2. Biological copper sites classified by coordination environment.
Figure 1.3. Active sites in selected copper proteins.
1.2. Nitric Oxide (NO)

1.2.1. Physiological aspects of NO

Nitric oxide, the simplest NO containing compound, was considered only as a toxic gas until its discovery as an endogenous product released by endothelial cells connected to heart contractility and smooth muscle relaxation in 1980.\textsuperscript{22} Since then nitric oxide has been implicated in a wide array of functions important in health and disease such as vasodilation, melanogenesis, immune function, septic shock, and neurotransmission.\textsuperscript{23-24}

Nitric oxide is a gas with low water solubility and can permeate biological membranes relatively easily which contributes to its efficacy as a signaling molecule.\textsuperscript{25} Its signaling role along with a few other small molecules such H\textsubscript{2}O\textsubscript{2}, O\textsubscript{2}•−, HO•, ONOO−\textsuperscript{26} brought about the concept of redox signaling, involving cellular signal transduction networks which rely on reactive, redox active species as messengers.\textsuperscript{27}

Nitric oxide can act in various physiological capacities: as a protective agent, a regulator, a signaling molecule and, in high concentrations, a deleterious cytotoxin. As a protective agent, nitric oxide can act as an antioxidant that rapidly reacts with high energy oxygen, nitrogen, and carbon based radicals. For highly reactive radicals such as alkoxy and alkyl hydroperoxyl Fig 1.3. (continued) Active sites in selected copper proteins.
radicals, NO reacts with such species at the rate of diffusion.\textsuperscript{28} The protective nature of nitric oxide also extends to cells by preventing cellular apoptosis.\textsuperscript{29} As a regulator, NO plays an important role in heme homeostasis and even its own production by inhibiting iron based enzymes such as the globin enzymes, guanylate cyclase and nitric oxide synthase (NOS).

In high concentrations, nitric oxide can be cytotoxic. NO can induce apoptosis depending on the cell type and conditions. Also, its inhibition of iron enzymes has been known to lead to various forms of anemia and disruption of iron enzymes processes. Furthermore, though NO does not react with DNA itself, NO metabolites such as N\textsubscript{2}O\textsubscript{3} and HNO\textsuperscript{4} damage DNA and can cause mutations in bacterial and mammalian cells. The counteraction of NO cytotoxicity in bacteria and fungi occurs via nitric oxide reductase, a dinuclear flavodiiron enzyme which catalyzes the conversion of NO to N\textsubscript{2}O in the last step of the denitrification process.\textsuperscript{30-31}

\textbf{1.2.2. Formation of NO}

The biological production of nitric oxide occurs via the oxidation of L-arginine by isoforms of nitric oxide synthase (NOS) (Scheme 1.1).\textsuperscript{32} Under normal physiological conditions, the available concentration of free NO is on the nanomolar level and has a very short lifetime in the blood of about 3-5 seconds.\textsuperscript{15, 33} Because of its short lifetime, NO is often produced on-site. Under certain pathological conditions, large amounts of NO may be required and subsequently supplied via donor molecules such as organic nitroso molecules.\textsuperscript{34-35}
Various NO congeners allow for the transport of NO activity over long distances within an organism, especially when air-stable in contrast to NO itself. Through reaction with carbon, oxygen, and nitrogen based radicals, NO can transform into C-nitroso compounds (C-NO) (Chapter 3), nitrite (O-NO) (Chapter 5) and N-nitrosamines (N-NO), respectively. The oxidation and reduction of NO, producing nitrosonium (NO$^+$) and nitrosyl (NO$^-$) ions, respectively, augment the number forms in which NO can stably exist and travel within the body. While each also serves as a source of NO$_{gas}$, S-nitrosothiols (S-NO) (Chapter 5) represent an endogenous source of NO$^+$ and nitroxyl (HNO) serves as a source for NO$^-$. Other nitric oxide donors and donor drugs outside the purview of this chapter have been extensively reviewed by (but not limited to) Yamamoto, Wang, and Miller. While each of these redox forms has their own biological function and relevance, there is also great overlap in terms of net physiological effect.

Scheme 1.1. Synthesis of NO by nitric oxide synthase.
1.2.4. Metal – nitrosyl (NO) complexes

1.2.4.a. NO binding to transition metals

Nitric oxide can bind to metals exhibiting either a bent (120 – 140°) or linear (160 – 180°) geometry (Figure 1.4). For bent metal-nitrosyls, they often exhibit NO⁺ or NO⁻ reactivity while linear nitrosyls often serve as N-based electrophiles with NO⁺ reactivity. Unfortunately, nomenclature for NO ligands based on their easily observed various geometric structures complexes has not been firmly established; all [M](NO) compounds are simply metal nitrosyls. On the other hand, metal-nitrosyls are commonly distinguished by their Enemark-Feltham notation that sums the valence electrons at both the metal (d-orbital) and NO ligand (π*) levels. For example the notation \{Cu(NO)\}⁺⁺ is used to signify both copper(I) (d¹⁰) bound to nitric oxide radical (1 π⁺ e⁻) (e.g. Cu⁺(NO⁺)) as well as a copper(II) center (d⁹) bound to the NO anion (2 π⁺ e⁻) (e.g. Cu²⁺(NO⁻)). The Enemark-Feltham notation circumvents the assignment of oxidation state in the metal-nitrosyl complex which is complicated by the redox non-innocence of the NO ligand. Instead, the d-electron count of the metal fragment [M] is typically employed (dⁿ), formally adding one additional electron that gives rise to metal nitrosyls [M]-NO described as \{M(NO)\}ⁿ⁺¹ species in Enemark-Feltham notation.

Metal nitrosyl complexes are generally synthesized via the addition of NO gas, NO⁺ and NO⁻ salts to precursor metal complexes. Nitrosonium tetrafluoroborate (NOBF₄), nitrosonium hexafluorophosphate (NOPF₆) and nitrosyl chloride (NOCl) are good sources for NO⁺. Angeli’s
salt and Pilothy’s acid are the more classic NO/HNO donors which are detailed in chapter 2, section 2.1.2.b.

1.2.4.b. Copper – nitrosyl model complexes

There are several copper containing enzymes known to react directly with nitric oxide including copper nitrite reductase, hemocyanin, tyrosinase, ceruloplasmin, ascorbate oxidase (cytochrome c oxidase), laccase, and some mononuclear blue copper proteins such as azurin and halocyanin. Copper nitrite reductases (Cu-NiR), found in bacteria, are a vital component to the denitrification process in soil and are responsible for the conversion of nitrite (NO$_2^-$) to NO. Tocheva et al. reported the isolation of side-on NO bound Cu-NiR via the reduction of NO$_2^-$ in 2004 and in 2007 found side-on NO for both Cu$^1$ and Cu$^{11}$-NiR. Hemocyanin, necessary for respiratory function in mollusks and arthropods such as horseshoe crabs and tarantulas, are the copper equivalent of human hemoglobin and provide oxygen transport for many invertebrates. Also possessing a T3 site, tyrosinase, found in humans, fungi, and plants, catalyzes the production of melanin through oxidation of a tyrosine side-chain to a catechol. Laccases contain four copper atoms in T1, T2 and binuclear T3 copper sites and found in fungi and some higher form plants are responsible for pigmentation, delignification in certain plants, and used in the food industry for beer and fruit juice stabilization. Azurin and halocyanin, both of which have a T1 active site, and cytochrome c oxidase, which has a Cu$_B$ active site, are all involved in electron transport for various biological processes. Possessing both T1 and T3 Cu sites, ceruloplasmin is involved in iron and copper homeostasis, energy and connective tissue production. Ceruloplasmin is the greatest source of copper in the plasma constituting more than 95% of available copper in this medium.
Biomimetic complexes also allow the study of the NO interaction with copper centers found in a variety of different coordination environments in biology. Such coordination complexes simulate biological systems, but employ much more synthetically accessible models to allow: (a) the study of biochemical mechanisms without required (co)factors, (b) facile active site modification to assess varied aspects of a biochemical enzyme, as well as (c) potential substitution of the natural product in therapeutic applications.\(^{57-58}\) In particular, Kitajima, Karlin, and Tolman each have made seminal contributions to understanding the reactivity of mono-, bi- and trinuclear copper enzymatic sites through the use of tris(pyrazolyl)borate, chelating poly(pyridyl)s, and β-diketiminate ligands (Figure 1.5).\(^{59}\)

![Figure 1.5. Popular ligands used to model enzymatic active sites.](image)
1.3. Nitroxyl (HNO)

1.3.1. Physiological aspects of HNO

Nitroxyl, the one electron reduced form of NO⁺, elicits a similar physiological response as does NO with regard to vasorelaxation and neurotransmission, but has the opposite effect as NO when it comes to myocardiological inotropic effects. It is also very resistant to superoxide scavenging and is highly reactive toward thiols (Scheme 1.2).60 These separate modes of biological activity may be due to fundamental chemical differences between HNO and NO. HNO and NO differ by an electron and a proton: in water, the redox potential between NO⁺, H⁺/HNO is -0.55 V (vs. NHE).61 HNO possesses a very weak H-NO bond (BDE = 46-47 kcal/mol).61 HNO may be oxidized to NO either by abstraction of a hydrogen atom from NO (H-atom abstraction: HAA) or through a coupled transfer of proton and electron (proton-coupled electron transfer: PCET). It is of note that the NO⁻ possesses a triplet ground state that must undergo a spin state change to diamagnetic HNO.

Various reviews and papers have experimentally and theoretically explored potential mechanistic steps between the most stable endpoints: ¹HNO and ³NO⁻.62-65

\[
\begin{align*}
\text{O}_2^- + & \text{HNO} \\ 
\text{O}_2^- + & \text{HNO} \\
\text{RSH} + & \text{HNO} \to \text{RSNHOOH} \to \text{H}_2\text{NOH} + \text{RSSR'}
\end{align*}
\]

**Scheme 1.2.** Comparing nitroxyl and nitroxide reactivity with superoxide radical and thiols.
Nitroxyl (HNO), unlike NO\(^*\), is an elusive molecule because of its rapid dimerization (\(k = 8 \times 10^6 \text{ M}^{-1}\text{s}^{-1}\)) and reorganization to produce nitrous oxide (\(\text{N}_2\text{O}\)) and water (Scheme 1.3).\(^{66}\) HNO can react with oxygen to produce NO\(^*\) and hydroperoxyl radical (HO\(\cdot\)) at a moderate rate of \(3 \times 10^3 \text{ M}^{-1}\text{s}^{-1}\), and subsequently react with NO\(^*\) resulting in \(\text{N}_2\text{O}\) and nitrous acid (HNO\(_2\)) at a rate of \(5 \times 10^6 \text{ M}^{-1}\text{s}^{-1}\).\(^{67-68}\) Because of its elusiveness, HNO is usually produced via donor molecules. The most prevalent HNO donors are Piloty’s acid (Scheme 1.4(a)) and Angeli’s salt (Scheme 1.4(b)) which are very stable, water soluble solids whose decomposition to HNO is well studied and understood.\(^{66}\)

![Scheme 1.3. Nitroxyl dimerization and reactivity with nitric oxide and dioxygen.](image)

![Scheme 1.4. Under basic conditions Piloty’s acid releases HNO (a), while Angeli’s salt produces HNO under acidic conditions (b).](image)
1.3.2. Formation of HNO

The biological formation of HNO has not yet been unequivocally documented, but there are several candidate reactions that have been considered which may participate in the generation of biological HNO reactivity (Scheme 1.5).\textsuperscript{5,69-70} For instance, HNO can be formed via the oxidation of hydroxylamine and via reaction between S-nitrosothiols (RSNO) and thiols (RSH) resulting in disulfide formation.\textsuperscript{69} Additionally, the (pen)ultimate product of NO formation via NOS is thought to be nitroxyl, rather than nitric oxide.\textsuperscript{10} Copper zinc superoxide dismutase (Cu-ZnSOD) has been found as a required cofactor to complete the formation of NO; it is thought Cu-ZnSOD performs a one electron oxidation on HNO to effect NO as it similarly acts upon superoxide radicals (O$_2^•$-) to effect conversion to O$_2$.\textsuperscript{69,71}

\[
\begin{align*}
\text{H}_2\text{NOH} \quad &\xrightarrow{\text{peroxidase}} \quad \text{HNO} \\
\text{RSNO} + \text{R'SH} \quad &\xrightarrow{\text{CuZnSOD} \quad \text{& cofactors}} \quad \text{HNO} + \text{RSSR'}
\end{align*}
\]

\textbf{Scheme 1.5.} Possible reactions that may lead to the endogenous formation of HNO.
1.3.3. Metal – nitrosyl (HNO) complexes

1.3.3.a. HNO binding to transition metals

Nitroxyl undergoes reaction with many metal centers, but the most biologically relevant are Fe(III), Cu(II), and Mn(III) (Scheme 1.6). Nitroxyl reacts very quickly with oxygenated hemoglobin and myoglobin (k ≈ 10^8 M^{-1}s^{-1}) and in the case myoglobin produces its oxidized Fe^{3+} form, metmyoglobin.\textsuperscript{72} CuZnSOD reacts with HNO to produce NO (k = 9 × 10^4 M^{-1}s^{-1}),\textsuperscript{64} as does ceruloplasmin, which is only slightly more efficient than CuZnSOD.\textsuperscript{72} Reaction with manganese-based SOD (MnSOD) results in a [Mn](NO) product which slowly loses NO over a long period of time.\textsuperscript{69}

\[
\begin{align*}
\text{Cu}^{2+}\text{ZnSOD} + \text{HNO} & \rightarrow \text{Cu}^{+}\text{ZnSOD} + \text{H}^+ + \text{NO}^- \\
\text{Mn}^{3+}\text{SOD} + \text{HNO} & \rightarrow \text{Mn}^{2+}\text{SOD} + \text{H}^+ \\
\text{Ceruloplasmin}^{2+} + \text{HNO} & \rightarrow \text{Ceruloplasmin}^+ + \text{H}^+ + \text{NO}^-
\end{align*}
\]

Scheme 1.6. Oxidation of nitroxyl by metalloproteins to produce nitric oxide.

Because such rapid metal-HNO reactivity that leads to metal-nitrosyls or other species, it is very challenging to isolate metal-HNO complexes.\textsuperscript{73} Indeed, there are only five isolated and crystallographically characterized metal-HNO complexes: Mb(Fe),\textsuperscript{74} Ru,\textsuperscript{73, 75} and Os\textsuperscript{76} (Figure 1.6). To date, all metal-HNO structures exhibit the κ^1-N binding mode, in contrast to a wider range of bonding modes for mononuclear C-nitroso compounds which include κ^1-O, κ^1-N, and η^2-ON
linkages (Figure 1.7). HNO is known to bind to many different metals, but those that have been isolated are stable, kinetically inert low spin d\textsuperscript{6} octahedral complexes.\textsuperscript{46, 63} Metal bound HNO complexes exhibit smaller ν\textsubscript{NO} (1335 cm\textsuperscript{-1} – 1493 cm\textsuperscript{-1}) compared to HNO alone (1565 cm\textsuperscript{-1}) due to π-backbonding.\textsuperscript{63} Yet, the most characteristic feature of each of these structures is an extremely downshifted \textsuperscript{1}H NMR signal between 14-15 ppm indicative of the nitroxyl-\textit{H}.

\textbf{Figure 1.6.} Fully characterized metal HNO complexes.
1.3.3.b. Copper – HNO model complexes

To date, there are no reports of a bonafide [Cu](HNO) structure. There are a few reports of copper ions that may be considered to be bound to NO$^-$ (Figure 1.8(a); (b)).$^{32,77-78}$ The first metallic copper nitrosyl to be structurally characterized was $[\text{Cu}_2(\text{XYL-O})(\mu-\text{NO})]^ {2+}$ (XYL-O = 2,6-bis(bis(2-pyridylethyl)aminomethyl)phenolate) formally a Cu-$d^9$ nitrosyl complex synthesized by Karlin et al (Figure 1.8(a)).$^{46,78}$ The $[^{1} \text{BuH} \text{Tp}]\text{Cu(NO)}$ synthesized by Tolman is a 19-electron species, also classified as a $\{\text{Cu(NO)}\}^{11}$ species (Figure 1.8(b)).$^{46,77}$ The nitrosyl anion can bind to either [Cu$^{II}$] or [Cu$^{III}$] resulting in the respective Enemark-Feltham notations, $\{\text{Cu(NO)}\}^{11}$ and $\{\text{Cu(NO)}\}^{10}$ (Figure 1.8). Thus far, reported structures of the $\{\text{Cu(NO)}\}^{11}$ configuration exhibit nearly linear Cu-NO structures and may be best classified as Cu$^{I}$-NO$^-$ rather than Cu$^{II}$-NO$^-$ (Figure 1.8).$^{46,79-80}$ Theoretical studies of copper nitrite reductase (CuNIR) comparing both end-on and side-on versions of $\{\text{Cu(NO)}\}^{11}$ evince the electronic structure to be Cu$^{I}$-NO$^-$ rather than a spin-coupled system of Cu$^{II}$-NO$^-$. $^{81}$

\[ \text{M} \equiv \text{N} \equiv \text{H} \quad \text{M} \equiv \text{N} \equiv \text{H} \quad \text{M} \equiv \text{N} \equiv \text{H} \]

$\kappa^1$-O \quad $\kappa^1$-N \quad $\eta^2$-ON

**Figure 1.7.** Potential binding modes for mononuclear nitroxyl complexes.
In 2010, Hayton, *et al.* reported the first structure of a copper nitrosyl complex with a {Cu(NO)}\(^{10}\) configuration which was synthesized via the addition of nitrosonium hexafluorophosphate to copper powder (Figure 1.8(c)). The Cu-N-O bond angle of 121.0(3)° suggests sp\(^2\) hybridization at the nitrosyl N donor atom indicating a [Cu\(^{III}\)]-NO\(^-\) formalism; however, the ν\(_{\text{NO}}\) (1993 cm\(^{-1}\)) and NO bond length (1.109(5) Å) led the authors to classify this as a spin-coupled [Cu\(^{II}\)]-NO\(^-\) species.\(^82\) In fact, this species readily reacts with mesitylene to give the charge-transfer complex [mesitylene-NO]\(^+\) suggesting its possible formulation as [Cu\(^1\)]-NO\(^+\).

**Figure 1.8.** Copper nitrosyl complexes.
1.4. Hydroxylamines (H$_2$NOH)

1.4.1. Physiological aspects of H$_2$NOH

Hydroxylamines participate in various biological processes and act as NO and HNO donors. For instance, acetohydroxamic acid is used to treat chronic urinary tract infection.$^{83}$ Hydroxyurea is used to treat patients with sickle cell and works on the premise of slow release of HNO through bioactivation (Figure 1.9).$^{84-85}$

![Figure 1.9. Biologically relevant hydroxylamines.](image)

Hydroxylamines are known to be toxic and mutagenic. As a mutagen, hydroxylamine shows a high degree of specificity toward cytosine of all the DNA base, adding across the imine bond in a Markovnikov fashion.$^{86-87}$ The modified cytosine directs the incorporation of adenine rather than guanine in newly synthesized DNA strands.$^{88}$ Upon further oxidation produce nitroso- and nitro-compounds which are equally toxic,$^{87}$ and its in vitro conversion to NO can activate guanylate cyclase, a major NO target, and induce vascular relaxation and inhibit programmed cell death.$^{89-90}$

1.4.2. Formation of H$_2$NOH

The parent hydroxylamine (H$_2$NOH) can be formed as a byproduct of the formation of NO via NOS and adenosine metabolism.$^{69, 89, 91-92}$ Mainly, N-substituted hydroxylamines, such as N-phenylhydroxylamine and N-sulfamethoxazole, can occur in the oxidation of amines or during the reduction of nitrites, nitrates, nitroso compounds.$^{93}$
1.4.3. Conversion of $H_2NOH$ to other NO congeners

Enzymes known to interact with hydroxylamines generally have iron-based active sites and result in a number of different species. Reactions with hemoglobin can lead to the formation of nitrooxides;\textsuperscript{94} peroxidases and cytochrome P-450 enzymes result in the formation of the corresponding nitroso compounds.\textsuperscript{95} As part of the nitrification cycle, ammonia-oxidizing bacteria (AOB) and other chemoautotrophs possess hydroxylamine oxidoreductase (HAO) as a key respiratory component.\textsuperscript{96} This 24-heme homotrimeric enzyme catalyzes the oxidation of hydroxylamine to nitrite.\textsuperscript{97} Conversely, anaerobic ammonium oxidation (anammox) uses nitrite as an electron acceptor and is reduced to $N_2$ via hydroxylamine and hydrazine. Hydroxylamine itself is considered to be a donor for nitroxyl and nitric oxide.\textsuperscript{89} Catalase is able to convert hydroxylamine to HNO via dehydrogenation,\textsuperscript{66} and even go all the way to nitric oxide.\textsuperscript{89} Nitric oxide is also byproduct of the oxidation of hydroxylamine by HAO.\textsuperscript{98} Conversely, HNO is known to react with thiols (RSH) to produce the corresponding disulfide and hydroxylamine.\textsuperscript{99}

1.4.4. Metal – hydroxylamine ($H_2NOH$) complexes

1.4.4.a. $R_2NOH$ binding to transition metals

Hydroxylamine can undergo a potentially explosive disproportionation to effect ammonia, dinitrogen, and nitrous oxide (Scheme 1.7).\textsuperscript{100} In the presence of metal ions, $H_2NOH$ can decompose to give $NH_3$, $H_2O$, $N_2$, $N_2O$, and a small amount of NO, and $H_2$.\textsuperscript{101} Despite this

\[
3 \text{NH}_2\text{OH} \rightarrow \text{NH}_3 + \text{N}_2 + 3 \text{H}_2\text{O} \quad \Delta H = -134.2 \text{ kcal mol}^{-1}
\]

Scheme 1.7. Explosive disproportionation of pure hydroxylamine above 100 °C.

reactivity, there are four crystals structures of parent hydroxylamine bound to metals. In Ir,\textsuperscript{102} Re,\textsuperscript{103} Ru,\textsuperscript{104} and Pt\textsuperscript{105} hydroxylamine complexes, all are N-bound with N-O distances over the tight range of 1.431(6) Å – 1.45(1) Å (Figure 1.10). Hydroxylamine reactivity with metal
complexes is typically of the acid/base variety given its low pKa’s (H₂NOH = ca. 6,[106] [H₃NOH]⁺ = 13.7[100]) rather than cleavage of the weak N-O bond (BDE ≈ 60 kcal/mol)[101] (Scheme 1.8).

Calculations of hydroxylamine show the OH bond (BDE = 74 kcal/mol) is slightly weaker than that of NH (BDE 81 kcal/mol).[101] Wang et al. calculate the formation of zwitterionic ammonium oxide O⁺NH₃ form of H₂NOH, as the first step toward either uni- or bimolecular thermal decomposition.[101] In the presence of metals, however, we suspect the first step of decomposition to include a nitroxide intermediate (H₂NO⁺), which is further explored in Chapter 4.

\[
\begin{align*}
\text{NH}_2\text{OH} & \quad \rightarrow \quad \text{H}^+ \quad + \quad \text{NH}_2\text{O}^- \quad \text{pKa} = 6.0 \\
[\text{NH}_3\text{OH}]^+ & \quad \rightarrow \quad \text{H}^+ \quad + \quad \text{NH}_2\text{OH} \quad \text{pKa} = 13.7
\end{align*}
\]

**Scheme 1.8.** Relative pKa values with hydroxylamine.
Figure 1.10. Metal hydroxylamine complexes.

\[
[\text{mer, trans-} \text{Re(CO)}_3(\text{NH}_2\text{OH})-(\text{PPh}_3)_2^+][\text{SOCF}_3^-] \\
\text{Hillhouse 1997}
\]

\[
[\text{Ir(}\mu\text{-SC}_6\text{H}_3\text{MeCH}_2\text{Cl}(\text{PPh}_3)(\text{NH}_2\text{OH})]_2 \\
\text{Hidai 2002}
\]

\[
[\text{Ru(CO)(Et}_2\text{dtc})(\text{PPh}_3)_2(\text{NH}_2\text{OH})](\text{OTf})] \\
\text{Wong 2000}
\]
1.4.4.b. Copper – $R_2$NOH interactions

To date, no reports cite the structural characterization of a copper hydroxylamine complex. Nonetheless, copper ions have been implicated in hydroxylamine transformations to nitroxy$^{107}$ and nitroso compounds. Fujimoto and Imamura performed kinetic studies in the 1970’s demonstrating that one hydroxylamine is able to reduce two copper(II) to copper(I) atoms and suggest the transformation goes through a nitroxide intermediate ultimately producing nitrous oxide.$^{108}$ The Baudisch reaction, used to synthesize aromatic nitroso compounds, employs copper(II) salts and hydroxylamine (Scheme 1.9). Studies of this reaction report spectroscopic evidence of a putative CuSO$_4$-$H_2$NOH-HCl complex (1:2) with UV-vis peaks at $\lambda_{\text{max}} = 230$ nm and 273 nm.$^{109}$

1.5. C-nitroso compounds (RNO)

![Scheme 1.9](image_url)

X = F, Cl, OH, Me

**Scheme 1.9.** Baudisch reaction discovered in 1939 by Oskar Baudisch.

1.5.1. Physiological aspects of RNO

Though related to HNO, C-nitroso compounds (RNO) are far more chemically stable compounds and thus exhibit longer-lived biological effects such as the inhibition of platelet aggregation$^{37,110}$ and aldehyde dehydrogenase.$^{111}$ For example, nitrosobenzene binds appreciably stronger to hemoglobin (Hb) than oxygen leading to methemoglobinemia and hemolytic anemia.$^{112}$ Furthermore, RNO’s can be introduced to the body as prodrugs and NO donor drugs as the decomposition of RNO can lead to the release of all forms of NO: NO$^+$, NO$^-$, and NO$^-$ or HNO.$^{34,111}$ The favored derivative of NO lost from C-nitroso compounds along with its rate can be
manipulated by the R group. Due to the stability of the NO radical, aliphatic C-nitroso compounds possess relatively weak C-N bonds. For instance, the C-NO BDE for Me-NO and 1Bu-NO is 40.0(8) and 37(2) kcal/mol, respectively. Generally, C-NO bond enthalpies in aliphatic R-NO compounds (R = Me, 1Bu, neo-pentyl, iPr) range from 34 – 40 kcal/mol. Due to the greater stability of allylic and benzylic radicals, their C-NO BDE are somewhat depressed: the C-NO BDE’s for α-nitrosotoluene and 1-nitrosopropene are 29(1) and 26(1) kcal/mol, respectively.

Monomeric C-nitroso compounds exhibit a blue or green color in solution due a n → π* transition of electrons localized on the nitrogen. This Lapport forbidden absorption is of low intensity (ε = 1 – 60 M⁻¹cm⁻¹) and within the visible spectrum ranging from 630 – 790 nm. Another low intensity band (ε = 80 M⁻¹cm⁻¹) around 270 – 300 nm is due to n → π* transition of electrons localized on the oxygen. The relatively accessible empty π* orbital enables C-nitroso compounds to act as relatively good π-acids which may attribute to its efficacy in hemoglobin degradation.

Dimeric C-nitroso compounds are formed via the reversible dimerization (Eₐ = 6-10 kcal/mol for tertiary C-nitroso compounds) of monomeric RNO’s (Scheme 1.10). The dimer is colorless and displays double bond character between the two N atoms, forcing the resulting azodioxy species to be in either a trans or cis conformation. For unencumbered aliphatic nitroso compounds, low temperatures (-80 °C) kinetically favor the cis-dimer, which is not the case for tertiary nitroso

\[
\begin{align*}
\text{O}^- & \quad \text{NR}_3 \quad \text{CR}_3 + \quad \text{N} \quad \text{N} + \quad \text{O}^- \quad \xrightarrow{E_a = 20 - 36 \text{ kcal mol}^{-1}} \quad \text{O}^- & \quad \text{NR}_3 \quad \text{CR}_3 \quad \text{cis} \quad \text{E_a = 6 - 10 kcal mol}^{-1} \\
\text{trans} & \quad 2 \quad \text{NR}_3 \quad \text{CR}_3 & \quad \text{cis} \quad \text{NR}_3 \quad \text{CR}_3
\end{align*}
\]

**Scheme 1.10.** Reversible dimerization of tertiary C-nitroso compounds and corresponding activation energies.
compounds. The thermodynamically preferred trans-dimer for aliphatic nitroso compounds is always preferred for the tertiary compounds.\textsuperscript{110} The dissociation of dimeric tertiary nitroso compounds is a first order process with activation energies ranging from 20 - 36 kcal mol\textsuperscript{-1}. Aromatic C-nitroso compounds can act as a radical trap for nitric oxide as the two N atoms can couple to form a nitrosobenzene-nitric oxide adduct of similar structure to a diazeniumdiolate.\textsuperscript{113} The dimeric form is less reactive toward nitric oxide than the monomer.\textsuperscript{113}

1.5.2. Formation of RNO

The endogenous formation of C-nitroso compounds occurs via the oxidation of amines or the reduction of hydroxylamines and nitro compounds at enzymes such as nitroreductases and Cu-ZnSOD (Scheme 1.11).\textsuperscript{35,95} Additionally, C-nitrosation by endogenous nitrite is another method for the biological production of C-nitroso comounds.\textsuperscript{114-115} Noguchi et al. identified a copper containing enzyme capable of C-nitrosation,\textsuperscript{114} commenting on the dearth of biosynthetic enzymes for aromatic and aliphatic C-nitroso compounds.

1.5.3. Conversion of RNO to other NO congeners

\[
\begin{align*}
\text{H}_2\text{NR} & \rightleftharpoons \text{HRNOH} & \text{O} & \rightleftharpoons \text{R-NO}_2 \\
\text{reduction} & & \text{oxidation} & \\
\end{align*}
\]

\textbf{Scheme 1.11.} Oxidation of hydroxylamines and amines and the reduction of nitro- compounds to produce C-nitroso compounds.

C-nitroso derivatives of phenolic compounds have been shown to act as nitrosating agents of amines to produce N-nitroso compounds or nitrosamines. In fact, the phenolic compounds themselves are known to catalyze the N-nitrosation of amines via nitrite \textit{in vivo}.\textsuperscript{115} Davies et al. suggest the nitrosating agent is formed via the quinone monoxime tautomer and nitrous acid.\textsuperscript{116} Reports from the late 1970’s to early 1980’s discuss a C-nitrosoreductase enzyme extracted from porcine cytosol (liver and heart) that is very similar to alcohol dehydrogenase and capable of
reducing C-nitroso compounds such as p-nitrosophenol to the corresponding amino derivatives.\textsuperscript{117-118} This enzyme is unreactive towards N-nitroso compounds and performs a 4-electron reduction of C-nitroso compounds that is unattributable to alcohol dehydrogenase.\textsuperscript{119-120}

\subsection*{1.5.4. Metal – C-nitroso (RNO) complexes}

\subsubsection*{1.5.4.a. RNO binding to transition metals}

Because C-nitroso compounds are known to affect iron homeostasis, heme and non-heme Fe(C-NO) adducts have been avidly targeted as models for understanding the toxicity of C-nitroso compounds. Richter-Addo \textit{et al.} published in 2013 the first human hemoglobin (Hb) structures bound to MeNO and EtNO, each bonded to Fe in \( \kappa^1\)-N conformations.\textsuperscript{112} The \( \kappa^1\)-N-bound conformation is the most typically seen of the 3 major binding modes for mononuclear C-nitroso compounds (Figure 1.11).\textsuperscript{121} The other two are \( \kappa^1\)-O and \( \eta^2\)-ON similar to that of HNO. The \( \kappa^1\)-O binding mode has been seen in several complexes containing Zn(II), Sn(IV), Fe(III), Mn(III) and Cu(I), while the \( \eta^2\)-ON has been seen in Mo(IV), W(VI), Ru(II), Pt(II), and Cu(I).\textsuperscript{35,121-122}
Activation of the N-O bond via $\pi$-backbonding can vary dramatically and lead to ambiguous determinations of metal oxidation states.\textsuperscript{2,42-43} The N-O bond distance for monomeric aliphatic C-nitroso compounds can range from 1.10 Å – 1.22 Å, but upon $\kappa^1$-N-binding to monometallic centers, can lengthen to 1.209 Å – 1.296 Å.\textsuperscript{35} The $\eta^2$-ON binding mode leads to even greater activation with N-O distances ranging from 1.386 Å – 1.432 Å.\textsuperscript{35} Monomeric aromatic ArNO compounds result in stretching frequencies between 1488 cm\textsuperscript{-1} – 1513 cm\textsuperscript{-1} which, upon binding to metals, usually drops.\textsuperscript{35} Sometimes the drop is modest, as in the case of platinum complex series ($v_{N-O} = 1487$ cm\textsuperscript{-1} – 1495 cm\textsuperscript{-1}) with PhNO (free $v_{N-O} = 1506$ cm\textsuperscript{-1}) derivatives,\textsuperscript{35,95} while in other cases the drop is large, as in the case of Figueroa and co-workers’ palladium complex ($v_{N-O} = 1317$ cm\textsuperscript{-1}).\textsuperscript{123}

\[ \text{[Cu(6-amino-1,3-dimethyl-5-nitrosoaril)\textsubscript{2}(bipy)\textsubscript{2}(H\textsubscript{2}O)\textsuperscript{+}}] } \]

\[ \text{[Cu(\alpha,\omega-Bis((1,3-dimethyl-5-nitrosoaril-6-yl)amine)propyl)\textsubscript{2}(H\textsubscript{2}O)\textsuperscript{+}]3H\textsubscript{2}O} \]

\textbf{Figure 1.12.} Examples of metal R-NO compounds that exhibit disorder within the O-N nitroso bonds.

The greater changes seen in the $\eta^2$-ON binding mode support the decrease in N-O bond order and greater $\pi$-backbonding by metal centers and greater orbital overlap. The inconclusive
determination of the $\nu_{N-O}$ in $\kappa^1-O$ binding mode to metals is partly due disorder within the structures and misassignment of $\nu_{N-O}$ bands (Figure 1.12).\textsuperscript{35}

1.5.4.b. Copper – RNO metal complexes

All three binding modes for mononuclear Cu C-nitroso compounds ($\kappa^1-N$, $\kappa^1-O$, $\eta^2-ON$) appear in structurally characterized in synthetic copper model complexes which are further explored in Chapter 3. Unfortunately, little has been reported on the biological interactions of copper ions or enzymes with C-nitroso compounds. In 1999, Ohkuma reported oxidative DNA damage in the presence of NADH and Cu$^{II}$ ions.\textsuperscript{124-125} Noguchi identified a copper enzyme, $NspF$, that catalyzes the biosynth of the C-nitroso compound 4,3-HNBAm from the corresponding aromatic amine in *Streptomyces murayamaensis* (Scheme 1.12).\textsuperscript{114, 126} Of special interest is the role that the dicopper site plays to first activate dioxygen that is ultimately the source of the N-O moiety in the product.

![Scheme 1.12.](image)

**Scheme 1.12.** Formation of C-nitroso 4,3-HNBAm by $NspF$ from *Streptomyces murayamaensis*. 
1.6. Nitroxides (R₂NO⁺)

1.6.1. Physiological aspects of R₂NO⁺

Nitroxides play a valuable role in protecting living organisms from reactive oxygen species (ROS) by exhibiting SOD behavior and can inhibit the propagation of aerobic lipid peroxidation (Scheme 1.13). Low molecular weight nitroxides have proved valuable in this regard in relation to closed-head injuries and other bodily injuries due to their ability to cross cell membranes as well as the blood brain barrier that prohibits ROS from further deleterious reactivity. In fact, all three redox forms: nitroxide, hydroxylamine, and oxoammonium, are present in brain tissue (Figure 1.13). 

![Figure 1.13. Hydroxylamine, nitroxide, and oxoammonium.](image)

Soule et al. suggest the anti-oxidant nature of nitroxides provides a unique opportunity to study disparate diseases related free radicals e.g. hypertension, weight control, and Parkinson’s.

![Scheme 1.13. Mechanism of low molecular weight nitroxides acting as a metal free SOD mimic.](image)
1.6.2. Formation of $R_2NO^\cdot$

Biological nitroxides are formed from hydroxylamines by either H-atom abstraction or by a one-electron oxidation / deprotonation. For example, the two-electron oxidation of $N$-phenylhydroxylamine to nitrosobenzene at methemoglobin goes through a nitroxide intermediate. The metabolism of arylhydroxylamines by peroxidases and microsomal incubations can also produce nitroxide intermediates (Scheme 1.14). Conversely, Ohkuma suggests that oxidative DNA damage elicited by nitrosobenzene in the presence of NADH and Cu$^{II}$ ions goes through the intermediacy of a phenylnitroxide radical (Scheme 1.14).

\[
\begin{align*}
R \text{N} & \xrightleftharpoons{\text{Mb oxidation}} \xrightleftharpoons{\text{NADH}} \text{NO}^\cdot \\
R \text{N} & \xrightleftharpoons{\text{Cu$^{II}$ reduction}} \text{O}
\end{align*}
\]


1.6.3. Conversion of $R_2NO^\cdot$ to other NO congeners

1.6.3.a. Hydroxylamines

Quite often the reduction of nitroxide to corresponding hydroxylamines is reported on as a result of a spin probe study of other biologically relevant molecules, and not as a biologically relevant reduction process. Nonetheless, there are some reports of metal catalysis of reduction of hydroxylamine to nitroxides. In vivo EPR studies showed the nitroxide and hydroxylamine forms to be in equilibrium. In erythrocytes, hepatocytes, and kidney cells, ascorbate plays a role in the reversible reduction of nitroxide. Dakilov et al. mention their study on the spontaneous free radical formation of nitroxides from amyloid β peptides could aid in better understanding the metal-catalyzed (iron and copper) oxidation of free radicals including the hydroxylamine to nitroxide transformation.
1.6.3.b. Oxoammonium

The oxidation of nitroxides to the oxammonium derivative (Figure 1.12) comprises the first step of the accepted mechanism by which nitroxides, themselves, convert superoxide into dioxygen. Nitroxides have been shown to inhibit anti-oxidant enzymes such as SOD, catalase, and laccase, but the inhibition stems more from the nitroxide reactivity with the radical substrate rather than the enzyme itself.

1.6.4. Metal – nitroxide (R\textsubscript{2}NO\textsuperscript{•}) complexes

The interconversion of nitroxide, oxoammonium, and hydroxylamine forms are not only facilitated by ascorbate/ascorbic acid, but also by chelated metals. There is evidence that DNA chelated Fe\textsuperscript{II} has been shown to react with nitroxides to produce the corresponding hydroxylamine and Fe\textsuperscript{III} complex. This transformation is presumed to occur at endogenous Cu\textsuperscript{II} ions, as well. This presumption is based on the simplistic model that endogenous free iron and copper can both become ligated in the case of site specific injury, and the Bar-On study demonstrates several viable ligands that accomplish the transformation at Fe\textsuperscript{II}. TEMPO, (2,2,6,6-tetramethylpiperidin-1-yl)oxy, one of the most utilized free radical nitroxides, has been complexed to several iron and copper compounds which are further explored in Chapter 4.

1.7. Nitrite (NO\textsubscript{2}−)

1.7.1. Physiological aspects of NO\textsubscript{2}−

Though previously considered physiologically inert, several in vivo and in vitro studies have shown nitrite to be a potent vasodilator and enzyme regulator. Various sources suggest differing ranges of normal nitrite circulation concentrations in the plasma from 50 – 300 nM to 150 – 1000 nM. van Faassen et al. suggest the variability in physiological nitrite concentrations are due to differences in dietary habits, lifestyle (e.g. tobacco consumption), and physical exercise prior to
One study suggests an infusion of nitrite (~ 36 μM) into the human forearm leads to a local increase blood flow by 175% and (after an infusion of $[\text{NO}_2^-] \sim 200 \ \mu\text{M}$) the substantial increase of in venous levels of iron-nitrosylated hemoglobin ($\sim 750 \ \text{nM}$) and $S$-nitrosohemoglobin ($\sim 200 \ \text{nM}$) in less than 10 seconds. The conversion of nitrite to NO under hypoxic conditions has become an important component to cardiovascular health, ischemia-reperfusion injury, and hypertension.

Nitrite has been implicated in a number of beneficial tissue responses and cellular mitochondria has been identified as one of the major targets, implying nitrite regulation of mitochondrial function. By reversibly binding to the binuclear copper$_B$/heme$_{a3}$ site, it prevents the binding of dioxygen and hampers respiration. This process could be vital to oxygen conservation during ischemic episodes.

Nitrite itself, without having to be reduced to NO, has a significant impact on some physiological processes. A large dietary intake of nitrite is known to lead to significant and even lethal methemoglobinemia. Furthermore, nitrite is known to inhibit a number of enzymes including catalase, cytochrome C oxidase, and certain peroxidases and can react with oxy-hemoglobin to produce nitrate and methemoglobin.

1.7.2. Formation of $\text{NO}_2^-$

Nitrites and nitrates are components of many vegetables, such as lettuce and spinach, and preservatives for food stuffs, especially cured meats and bacon. The consumption of such food introduces the anions to the body. With a half-life of 5 – 6 hours in the plasma, the normal circulation concentrations of nitrate are 20 - 40 μM. 75% of nitrates consumed are expelled from the body via excretion. The rest of the nitrates is saved for the benefit of benign salivary bacteria and used as an alternative to oxygen as an electron acceptor source during respiration resulting in
the formation of nitrite.\textsuperscript{142} Though this only accounts for about 30\% of endogenous nitrite formation, the symbiotic relationship is beneficial as humans are deficient of enzymes to convert nitrate to nitrite. To a much smaller extent, xanthine oxidoreductase, a mammalian molybdenum enzyme in the liver can, too, convert nitrate to nitrite, but only under hypoxic conditions.\textsuperscript{146}

The majority of nitrite is formed via the oxidation of NOS generated NO and can occur via several pathways. The third-order autooxidation of NO is relatively slow ($2 \times 10^6$ M$^{-2}$s$^{-1}$)\textsuperscript{147} compared to that of the two electron oxidation of nitrate by heme proteins ($8 \times 10^7$ M$^{-1}$s$^{-1}$).\textsuperscript{145} The oxidation can also be catalyzed by ceruloplasmin and cytochrome c oxidase in the plasma and tissues, respectively.\textsuperscript{145}

1.7.3. Conversion of NO$_2^-$ to other NO congeners

1.7.3.a. Conversion of nitrate to nitrite

The conversion of nitrate to nitrite is a very important part of the nitrogen cycle (denitrification) and best accomplished under hypoxic conditions, which are usually found in substrata soil and stagnant water. But more recently enteric bacteria have garnered researchers’ attention due to their ability to convert nitrate to nitrite. Studies show during anaerobic respiration, \textit{Escherichia coli}, one of the most well understood enteric bacteria, first convert nitrate to nitrite via a molybdenum based enzyme, which can subsequently be reduced to ammonia via a heme nitrite reductase.\textsuperscript{148}
1.7.3.b. Conversion of nitrite to nitric oxide

The interconversion of nitrite (+3 N oxidation state) and nitric oxide (+2 N oxidation state) occurs in blood and tissues via enzymatic and non-enzymatic pathways. In the stomach and gastrointestinal tract, nitrite can be protonated to nitrous acid (pKa ~ 3.3), which can dimerize and eliminate water to produce dinitrogen trioxide and then decompose to produce nitric oxide (Scheme 1.15).\(^{149}\) Nitrous acid reactivity with polyphenols and ascorbate can also produce nitric oxide and phenoxy radicals or dehydrogenated ascorbate, respectively.\(^{142}\)

\[
\begin{align*}
\text{NO}_2^- + \text{H}^+ & \rightleftharpoons \text{HNO}_2 \\
2 \text{HNO}_2 & \rightleftharpoons \text{H}_2\text{O} + \text{N}_2\text{O}_3 \\
\text{N}_2\text{O}_3 & \rightleftharpoons \text{NO}^\cdot + \text{NO}_2^\cdot 
\end{align*}
\]

**Scheme 1.15.** Metal free formation of nitric oxide in the stomach from nitrite.

Enzymatic interconversion of nitrite to nitric oxide is possible at (met)hemoglobin, (met)myoglobin, xanthine oxidoreductase, ceruloplasmin, and copper nitrite reductase.\(^{145}\) The nitrite reductase reactivity of globin proteins has recently captured researchers attention, though Doyle *et al.* reported this type of reactivity with human hemoglobin in 1981.\(^{150}\) Nitrite was previously thought to be physiologically inactive and nitrite reactivity at methemoglobin is known to induce methemoglobinemia by binding so tightly to metHb as to render it nearly ineffective.\(^{145,\ 151}\) Methemoglobin reversibly reduces nitrite to nitric oxide \(k = 2.9 \text{ M}^{-1} \text{s}^{-1}, 25 \degree \text{C}, \text{pH } 7.0\) under hypoxic conditions.\(^{145,\ 152}\)
1.7.4. Metal – nitrite (NO$_2^-$) complexes

1.7.4.a. NO$_2^-$ binding to transition metals

Nitrite bound metal complexes exhibit one of nine bonding modes which include five mononuclear and four dinuclear binding modes (Figure 1.14 and 1.15). Figure 1.14.a and 1.14.d. are both M(κ$^1$-NO$_2$), despite their differing classifications as a M-nitro (κ-N) and M-nitrito (κ-O), respectively.\textsuperscript{153}

**Figure 1.14.** Geometry of mononuclear metal nitrite complexes.

Crystal structures of nitrite bound globin proteins exhibit the relatively uncommon [Fe](κ$^1$-O-NO$_2$) binding mode (Figure 1.14.d.), which is in contrast with the [Fe](κ$^1$-N-NO$_2$) bonding mode (Figure 1.14.a) found in nitrite bound cytochrome reductases.\textsuperscript{154} Copper nitrite reductase exhibits the O-binding mode in its resting, oxidized state (Figure 1.14.c.).\textsuperscript{32} Richter-Addo suggested in 2008 that the difference is due to differing distal pocket interactions that direct the nitrite binding mode.\textsuperscript{151} In 2009, Lehnert confirmed the side-on NO binding mode reported by Tocheva in 2004.\textsuperscript{32} Based on DFT studies, Lehnert *et al.* suggested that a nearby isoleucine is responsible for the side-on binding of a copper(I) nitrosyl in crystallized samples of CuNIR which is not maintained in solution.
1.7.4.b. Copper – NO\textsuperscript{−} metal complexes

In an effort to better understand copper nitrite reductase, many researchers have used various ligands to model the enzyme and synthesize both Cu\textsuperscript{I} and Cu\textsuperscript{II} nitrite complexes. Generally, the synthesis of copper nitrite complexes are achieved via the addition of stoichiometric or excess NO\textsuperscript{−} salts, such as NaNO\textsubscript{2} or Ba(NO\textsubscript{2})\textsubscript{2}.\textsuperscript{155-156} The addition of NaNO\textsubscript{2}, however, frequently results in decomposition when added to [Cu\textsuperscript{I}] rather than formation of the desired [Cu\textsuperscript{I}](NO\textsubscript{2}), but success is found using bis(triphenylphosphine)iminium nitrite ([(PPh\textsubscript{3})\textsubscript{2}N]NO\textsubscript{2}), instead.\textsuperscript{157-159}

All nine nitrito binding modes have been seen at copper (Figure 1.16), despite the method of synthesis being generally the same.\textsuperscript{160-161} Halfen et al. suggests the softer Cu\textsuperscript{I} center should generally prefer N-binding, while the harder Cu\textsuperscript{II} would prefer O-binding, but more recent studies via EPR and X-ray crystallography show that both O- and N-binding modes can be present simultaneously, which suggests a low-energy barrier equilibrium between the two.\textsuperscript{156, 158}

The release of NO from model copper nitrite complexes, often confirmed via GC analysis of reaction headspace, has been achieved via the addition of acid, such as trifluoroacetic (pKa = 0.52) or acetic (pKa = 4.76) acids. This reaction, performed at room temperature, supports the proposed mechanism nitrite protonation to initiate NO release.\textsuperscript{157, 162}

![Figure 1.15. Geometry of dinuclear metal nitrite complexes.](image-url)
Figure 1.16. Copper nitrite complexes in various binding modes.
1.8. S-nitrosothiols (RSNO)

1.8.1. Physiological aspects of RSNO

In 1981, the first report of the biological relevance of S-nitrosothiols (also known as thionitrites) detailed their contribution to bioactivities as pharmacological NO donors. In 1997, Stamler and co-workers demonstrated that RSNOs have a distinct role outside of that from nitric oxide itself. For instance, they reported that exogenous RSNOs as capable of relaxing vascular smooth muscles during copper catalysis. Later, RSNOs were found to play an intricate role in other physiological processes including neuronal signaling, inflammation, and anti-platelet activity. There are multiple reports specifying varying concentrations of S-nitrosothiols in human plasma ranging from 0.250 - 11.1 µM. Concentrations of S-nitrosocysteine and S-nitrosoglutathione, mainstay components within the blood stream, range from 0.2 – 0.3 and 0.02 – 0.2 µM, respectively. It is now accepted that S-nitrosothiols (RSNOs) serve as storage and transport molecules for endogenous NO.

Biologically relevant RSNOs are stable molecules that have a slightly polar, stable bond between the sulfur and nitrogen that is not very susceptible to homolysis. This means that RSNOs are relatively resistant to superoxide, especially when compared to nitric oxide. This resistance is valuable in protecting tissues against oxidative damage during ischemia/reperfusion. The resulting superoxide generated by ischemia/reperfusion facilitates the synthesis of SNO-albumin which is known for its protective capabilities.

S-nitrosothiols are also known to inhibit certain enzymes and can alter enzymatic activity via S-nitrosation or S-nitrosylation of protein thiols. Some of the proteins susceptible to modification are creatine kinase, certain proteases (caspases), papain, and protein disulfide
isomerase (PDI). In the case of PDI, its S-nitrosylation leads to dysregulated protein folding which eventually promotes neuronal cell death.

1.8.2. Formation of RSNO

The formation of RSNO’s, synthetically and biologically, often takes the route of either S-nitrosation or S-nitrosylation with varying supporting catalysts, such as copper and iron. S-nitrosation is generally considered the transfer of NO\(^+\) to a thiol (RS\(^-\)), whereas S-nitrosylation involves the addition of NO radical to metal-thiolate to produce RSNO (Scheme 1.16). The homolytic dissociation enthalpies of the S-N bond, determined theoretically and experimentally, range from 20-30 kcal mol\(^{-1}\) and S-nitrosothiols, which are thermally and photochemically sensitive, can be considered a source of NO gas and NO\(^+\).

\[
\begin{align*}
\text{S-nitrosation} & : \quad \text{RSH} + \text{XNO} \rightarrow \text{RSNO} + \text{X}^- + \text{H}^+ \\
\text{Transnitrosation} & : \quad \text{RSNO} + \text{R'S}^- \rightarrow \text{R'SNO} + \text{RS}^- \\
\text{S-nitrosylation} & : \quad \text{RS} + \text{M}^{n\text{NO}} \rightarrow \text{RSNO} + \text{M}^{n+1} + \text{H}^+
\end{align*}
\]

Scheme 1.16. Formation of S-nitrosothiols.

1.8.3. Conversion of RSNO to other NO congeners

1.8.3.a. Nitric Oxide

Very often the production or the addition of NO gas involves a third component, such as metal thiolates or disulfides, to account for the radical nature of nitric oxide (Scheme 1.17). NO cannot nitrosylate thiols at a reasonable biological rate by itself, and initially \(\text{O}_2\) was first proposed to generate S-nitrosothiols from a mixture of \(\text{O}_2\), NO, and \(\text{RSH}\). Biological and synthetic copper thiolates have been shown to reversibly bind nitric oxide gas. T1 copper proteins such as ceruloplasmin, ascorbic oxidase are known to decay in the presence of NO. Yet upon purging
of the NO gas, the copper thiolate site is restored. This reversible binding was synthetically replicated and studied by Shiyu Zhang from our laboratory in which he demonstrated two possible binding modes for the addition of NO to copper thiolates \([\text{Cu}^1(\kappa^1\text{-N(O)SR})]\) and \([\text{Cu}^1(\kappa^1\text{-S(NO)R})]\).\(^{144, 174}\)

Alternatively, the spontaneous release of NO from low molecular weight RSNOs, such as \(S\)-nitroso-\(N\)-acetylcysteine (SNAC), \(S\)-nitrosoglutathione (GSNO), and \(S\)-nitroso-\(N\)-acetylpenicillamine (SNAP), have been explored (Figure 1.17).\(^{175}\) Because controlling the release duration and levels are so vital, others have explored adapting these low molecular weight RSNOs to various frameworks such as polymers, silica, and hydrogels and their relative kinetics.\(^{175-177}\)

1.8.3.b. Nitroxyld

![Scheme 1.17. Pathways to interconvert RSNO to NO.](image)

![Figure 1.17. Molecular structures of SNAC, GSNO, and SNAP.](image)
In 1998, Nagasawa et al. reported the reaction of S-nitrosoglutathione and glutathione to be a second order process and suggested the initial products to be nitroxyl and the corresponding disulfide (Scheme 1.18). Hogg identifies this particular decomposition as S-thiolation, which can be catalyzed by dithiocarbamates as well as metals.

\[
\text{GSH} + \text{GSNO} \rightarrow \text{GSSG} + \text{HNO}
\]

**Scheme 1.18.** Generation of HNO from reaction between thiol and S-nitrosothiol.

### 1.8.4. Metal – S-nitrosothiol (RSNO) complexes

#### 1.8.4.a. RSNO binding to transition metals

Though S-nitrosothiols, themselves, are very stable, their decomposition in the presence of metals, even at low concentrations, can be swift. Copper and iron contaminants in buffer solutions or deionized water are able to catalytically decompose RSNOs to NO and corresponding disulfides. Williams tested an array of metals for catalytic activity including Zn\(^{2+}\), Ca\(^{2+}\), Mg\(^{2+}\), Ni\(^{2+}\), Co\(^{2+}\), Mn\(^{2+}\), Cr\(^{3+}\), Fe\(^{3+}\), none of which show measureable activity. Hg\(^{2+}\), Ag\(^{+}\), Cu\(^{2+}\), Cu\(^{+}\), and Fe\(^{2+}\), however, do show catalytic activity.
Because of their thermal and photochemical sensitivity there are few structures of metal-S-nitrosothiol complexes. Currently, the only isolated S-nitrosothiol structures are those of iridium and copper by the Doctorovich\textsuperscript{180} and Warren\textsuperscript{174} labs, respectively, both of which exhibit a syn RSNO conformation (Figure 1.18).\textsuperscript{144, 174, 180}

1.8.4.b. Copper – S-nitrosothiol interactions

Copper is involved in both the formation and decomposition of S-nitrosothiols. There is evidence for RSNO generation by copper as ceruloplasmin has been shown to increase the formation of GSNO from a mixture of NO and glutathione.\textsuperscript{167} Yet, CuZnSOD catalytically releases NO from GSNO.\textsuperscript{174} Additionally, S-nitrosothiols are susceptible to copper-containing cell-surface proteins which catalyze the degradation of RSNOs and release nitric oxide.\textsuperscript{169} Recently, Zhang et al. reported the reversible binding of NO to $[^{\text{Mes}}\text{Tp}]\text{Cu}$ thiolate, mimicking reversible NO binding observed at multicopper oxidases including ceruloplasmin.\textsuperscript{144} Theoretical calculations and experimental data support the formation of RSNOs at copper via the insertion of NO into the copper thiolate bond (Scheme 1.19(a)).\textsuperscript{144}
Studies of the mechanism for the generation of RSNOs were often muddled by the catalytic decomposition by the same catalysts, and vice versa. In 2013, however, Zhang et al. reported the reversible decomposition and formation of RSNOs at copper and suggested the mechanism to go through a $[\text{Cu}(\text{NO})]$ intermediate by way of a $[\text{Cu}(\kappa^2-(\text{SR})\text{N(O)SR}')]$ transition state (Scheme 1.19(b)).

Scheme 1.19. The formation of RSNO via NO insertion into copper(II) thiolate bond (a) and the reversible formation of disulfide from addition of RSNO to copper(II) thiolate (b).

Studies of the mechanism for the generation of RSNOs were often muddled by the catalytic decomposition by the same catalysts, and vice versa. In 2013, however, Zhang et al. reported the reversible decomposition and formation of RSNOs at copper and suggested the mechanism to go through a $[\text{Cu}(\text{NO})]$ intermediate by way of a $[\text{Cu}(\kappa^2-(\text{SR})\text{N(O)SR}')]$ transition state (Scheme 1.19(b)).

1.9. Conclusion

The selected nitric oxide derivatives reviewed in this chapter are certainly not the only NO containing compounds that are vital for a true comprehensive understanding of biological NO metabolism. Some not included in this main chapter are nitrate ($\text{NO}_3^-$), N-nitrosoamines ($\text{R}_2\text{NNO}$), and peroxynitrite (ONOO$^-$), each of which attracts attention for their biological importance. The complexity of nitric oxide biochemistry leaves many open doors for detailed investigation of interconversion pathways among various NO congeners that more closely connects individual compounds to the ultimate physiological effects observed.
CHAPTER 2

COMPUTATIONAL STUDIES ON COPPER NITROXYL COMPLEXES

2.1. Introduction

HNO is an elusive, highly reactive molecule that poses significant challenges to those who attempt to experimentally investigate its physiological, biological, and chemical effects. Due to its extremely rapid dimerization to N₂O and H₂O (Scheme 2.2), biological and chemical studies of HNO typically require the use of substances that can slowly release HNO or the use of specially designed molecular or electrochemical sensors that can quantify HNO.\(^{182-184}\) The addition of HNO is accomplished via HNO donor molecules, such as Piloty’s acid or Angeli’s salt (Section 2.1.2).\(^{66}\)\(^{182}\) The identification and quantification of HNO can be accomplished via subsequent HNO reactivity either with itself (Section 2.1.2) or metal complexes (Section 2.1.3).\(^{31, 184-185}\) These experimental challenges motivate computational approaches to understand the reactivity of HNO with biological targets and metal complexes.\(^{63, 67, 186}\)

We note that nomenclature regarding the triatomic molecule HNO can be confusing.\(^{182}\) Though we follow the generally accepted term “nitroxy” for HNO, nitroxy has also been used to describe nitroxides (R₂NO•).\(^{187}\) Others have used the term nitrosyl hydride \(^{187-188}\) and azanone\(^{67, 189}\) to describe HNO.

2.1.1. Physical and chemical properties of HNO

Nitroxy is an small molecule that displays a bent structure about the central N atom, the H-N=O angle being about 109 °, and has a predicted NO bond distance of 1.211 Å.\(^{67}\) Calculations by Dixon \textit{et al.} that predict the heat of formation to be 26.7 ± 0.8 kcal/mol,\(^{190}\) are quite close to experimental values of 24.5 kcal/mol.\(^{190}\) HNO possesses a very weak H-NO bond, due to the stability of the free radical •NO. Dixon \textit{et al.} report the H-NO dissociation energy to be 47.03 ±
0.03 kcal/mol,\(^{191}\) derived from spectroscopic and thermochemical data along with quantum calculations.

The electronic ground state of HNO is a singlet (\(^{1}\)HNO).\(^{192}\) Deprotonation of HNO leads to the nitrosyl anion NO\(^{-}\) which possesses a triplet ground state (\(^{3}\)NO\(^{-}\)).\(^{188}\) This lead to an initially reported pK\(_{a}\) of 4.7, but was re-determined to be 11.4 after the spin-state change was taken into account.\(^{187}\) The greatest consequence of this higher pK\(_{a}\) of 11.4 is that HNO should exist as a neutral molecule under most biological conditions near pH = 7.4. Deprotonation is also kinetically hampered by this spin conversion due to the nuclear reorganization between the N and O atoms which must occur concomitant with the spin conversion (Scheme 2.1).\(^{187}\)

\[
^{1}\text{HNO} \rightleftharpoons ^{3}\text{NO}^{-} + \text{H}^{+}
\]

**Scheme 2.1.** Deprotonation of nitroxyl.

The re-evaluated pKa necessitated the re-evaluation of corresponding electrochemical predictions that also took into account the spin state conversion. Previously, a facile reduction of NO to NO\(^{-}\) (E\(_{\text{red}}\)^{\circ} = 0.3 V vs. NHE) was predicted, but the re-evaluated redox calculations for NO/\(^{3}\)NO\(^{-}\) (-0.8 V vs NHE) which better correlated with experimental data.\(^{187}\) Also, the negative redox value for NO, H\(^{+}\)/\(^{1}\)HNO (E\(_{\text{red}}\)^{\circ} = -0.55 V) better correlates with preferred HNO species under biological/physiological conditions.\(^{61}\)

**2.1.2. Organic nitroxyl reactivity**

While there are a myriad of pharmacological and biological reactions and interactions involving HNO that result in varying physical consequences (highlighted in Section 1.3), there are quite a number of organic transformations pertinent to synthetic manipulations of HNO.
2.1.2.a. Nitroxyl consumption reactions

Among nitroxyl’s fastest reactions is its dimerization \((k = 8 \times 10^6 \text{ M}^{-1} \text{s}^{-1})\).\(^{187}\) This is the simple reason why HNO cannot be stored, purified, or concentrated and why so many have used the presence of \(\text{N}_2\text{O}\) as an indication for the generation of HNO (Scheme 2.2).\(^{66, 187, 193}\)

\[
2 \text{HNO} \rightarrow \text{HONNOH} \rightarrow \text{N}_2\text{O} + \text{H}_2\text{O}
\]

Scheme 2.2. Irreversible dimerization of nitroxyl.

Fast reactions of HNO with certain thiols have been reported in which nitroxyl acts as an electrophile (Scheme 2.3).\(^{194}\) This is an important mode of reactivity that clearly distinguishes HNO from NO in the biological milieu. The ubiquitous biological thiol glutathione (typical concentrations \(\sim 0.3 - 3 \text{ mM})\),\(^{195-196}\) reacts with nitroxyl relatively easily \((k = 2 \times 10^6 \text{ M}^{-1} \text{s}^{-1})\) to create the corresponding N-hydroxysulfenamide.\(^{66}\)

\[
\begin{align*}
\text{N} &= \text{H} \\
\text{H} &= \text{O} \\
\text{O} &= \text{N} \\
\text{H} &= \text{S} \\
\text{S} &= \text{R} \\
\text{R} &= \text{H}
\end{align*}
\]

Scheme 2.3. Nitroxyl reactivity with thiols to produce hydroxylamines.

Nitroxyl can also react with biological radicals such as •NO to yield nitrous oxide and nitrous acid at a rate of \(5 \times 10^6 \text{ M}^{-1} \text{s}^{-1}\) (Scheme 2.4).\(^{67-68}\) On the other hand, nitroxyl is resistant towards

\[
\begin{align*}
\text{N} &= \text{H} \\
\text{H} &= \text{O} \\
\text{O} &= \text{N} \\
\text{N} &= \text{O} \\
\text{O} &= \text{H}_2\text{O} \\
\text{N} &= \text{N} \\
\text{N} &= \text{O}
\end{align*}
\]

Scheme 2.4. Nitroxyl reactivity with dioxygen and nitric oxide.
superoxide even though the reaction of nitric oxide with superoxide to produce peroxynitrite is diffusion limited. HNO reacts at a moderate rate \( (k = 3 - 8 \times 10^3 \text{ M}^{-1}\text{s}^{-1}) \) \(^{67}\) with dioxygen, \(^{197}\) though the products are ambiguous. However, the interaction between the nitrosyl anion \((\text{NO}^-)\) and dioxygen \( (k = 2.7 \times 10^9 \text{ M}^{-1}\text{s}^{-1}) \) \(^{67}\) to produce peroxynitrite is not disputed. \(^{198}\) Rates of reaction of HNO with \( \text{O}_2 \) are slower than between \( \text{NO}^- \) and \( \text{O}_2 \) due to the spin forbidden process of the former, predicted to be physiologically very slow. \(^{187-188}\) The reaction between HNO and \( \text{O}_2 \) produces an active, 2-electron oxidant, though many believed that the product was not peroxynitrite. \(^{197}\) Shafirovich and Lamar reported evidence for the production of nitric oxide and hydroperoxyl radical at a rate of \( 3 \times 10^3 \text{ M}^{-1}\text{s}^{-1} \). \(^{199}\) In 2014, however, Smulik et al. reported unambiguous evidence for the formation of peroxynitrite as a result of the interaction between dioxygen and HNO at a rate of \( 1.8 \pm 0.3 \times 10^4 \text{ M}^{-1}\text{s}^{-1} \) at pH 7.4 (Scheme 2.4). \(^{198}\)

\[
\begin{align*}
\text{N} &= \text{O} \\
\text{H} \\
\text{N} \\
\text{H} + \text{O}_2 &\rightarrow \text{NO}^- + \text{HO}_2^-
\end{align*}
\]

\[
\begin{align*}
\text{N} &= \text{O} \\
\text{H} \\
\text{N} \\
\text{H} + \text{O}_2 &\rightarrow \text{ONOO}^-
\end{align*}
\]

\[
\begin{align*}
\text{N} &= \text{N} \text{O} \text{O} \\
\text{H} &\text{N} + \text{NO}^- &\rightarrow \text{N} &= \text{N} \text{O} \text{O}^- \\
\text{H} &\rightarrow \text{N} &= \text{N} \text{O} \text{O}^- &\rightarrow \text{N}_2\text{O} + \text{HONO}
\end{align*}
\]

Scheme 2.4. Nitrooxyl reactivity with dioxygen and nitric oxide.
2.1.2.b. HNO donors

There are several extensive reviews by Miranda, King, and Nakagawa that report exhaustively on HNO donors. Herein, we discuss just a few donors that are relevant to work described in this thesis (Scheme 2.5). Angeli’s salt (Na$_2$N$_2$O$_3$) is the most widely employed HNO donor, spontaneously decomposing under physiological conditions to release HNO and NO (Scheme 2.5). Requiring protonation to expel HNO, low pH conditions accelerate the generation of HNO. Other HNO donor molecules that find widespread use are Piloty’s acid (C$_6$H$_5$S(O)$_2$NHOH) and N-hydroxycyanamide (NCNHOH). Less often used HNO donors include corresponding amides and dienes.
Scheme 2.5. Generation of HNO from HNO donors.

(a) Angeli’s salt

(b) Piloty’s acid

(c) N-hydroxycyanamide

d. Hydroxamic Acid

e. N-acyl-3,6-dihydro 1,2-oxazine

\[
\text{Na}_2 \begin{array}{c}
\text{O} \\
\text{N} \\
\text{N} \\
\text{O} \\
\text{O}
\end{array} \rightarrow \text{HNO} + \text{NO}_2^- + \text{H}^+
\]

b. base

\[
\text{HNO} \rightarrow \text{HNO} + \text{SO}_3^- + \text{phenyl}
\]
2.1.3. Metal(HNO) model complexes

HNO reactivity through the use of HNO donors has been observed with a number of important enzymes such as peroxidases, hemoglobin (Hb), myoglobin (Mb), catalases, manganese (MnSOD) and copper-zinc superoxide dismutase (CuZnSOD). Of these proteins, myoglobin is the only enzyme to be isolated with HNO bound (Figure 2.1). In this structure, the HNO ligand is bound κ¹-N to the Fe atom and the hydrogen of HNO can be seen via ¹H NMR (δ 14.8 ppm) and the NO bond distance determined to be 1.24(1) Å. The lability of HNO ligand is decreased by a strong H-bond between the nitroxylic oxygen and hydrogen of a distal histidine (His64).
2.1.3.a. Synthetic [(M)(HNO)] model complexes

The only published structures of metal nitroxyl compounds are by Ibers, Hillhouse, Hess, and Wieghardt containing osmium,\textsuperscript{76} iridium,\textsuperscript{203} ruthenium,\textsuperscript{75} and molybdenum\textsuperscript{73, 204} metals, respectively (Figure 2.2). Crystal structures of the osmium, iridium, and ruthenium complexes exhibit an octahedral or pseudo-octahedral confirmation about the metal and are \textit{N}-bound to HNO, while the molybdenum structure exhibits an \(\eta^2\)-ON-HNO binding mode. The N-O distances for the \(\kappa^1\)-N-HNO bound complexes do not vary widely, but they do differ significantly from the \([\text{Mo}(\eta^2\text{-ON-HNO})] \cdot 2 \text{H}_2\text{O}\) structure [(a): not listed, (b): 1.235(11) Å, (c): 1.422(9) Å, (d): 1.242(9) Å, (e): 1.193(7) Å].

Each of these compounds overcomes the principle synthetic challenge to isolating a M(HNO) species which is the dissociation of HNO from the metal \(k_{\text{off}}\) allowing for irreversible nitroxyl

\[\text{Figure 2.2. Crystallographically characterized metal HNO complexes.}\]
dimerization (Scheme 2.6). Each of the synthetic models is able to trap HNO generating electronically saturated, 18-electron species with kinetically inert, low-spin \(d^6\) configurations.

\[
[M](HNO) \xrightleftharpoons[k_{\text{dissociation}}]{k_{\text{association}}} [M] + HNO
\]

\[
2 \text{HNO} \quad k = 8 \times 10^6 \text{M}^{-1}\text{s}^{-1} \quad \text{N}_2\text{O} + \text{H}_2\text{O}
\]

**Scheme 2.6.** Formation and dissociation of \([M](HNO)\) complexes.

Other metal nitrosyl compounds have only spectroscopic confirmation of the formation of M(HNO) via H-N IR bands and \(^1\)H-NMR. These structures incorporate metals such as cobalt,\(^{41}\) ruthenium,\(^{73}\), \(^{205}\) rhodium,\(^{206}\) rhenium,\(^{103}\), \(^{207-208}\) molybdenum,\(^{209}\) osmium,\(^{210-211}\) and iron.\(^{74}\) The downfield \(H\)-NO signal of Mb(HNO) (Figure 2.1) at 14.8 ppm is more greatly shifted than Richter-Addo’s ruthenium complex at 13.6 ppm (Figure 2.2(a)).\(^{73}\) The reported \(\nu_{\text{NO}}\) and \(\nu_{\text{NH}}\) stretching frequencies for these compounds widely range from 1358\(^{75}\) cm\(^{-1}\) to 1650\(^{209}\) cm\(^{-1}\) with \(\nu_{\text{NH}}\) from 2810\(^{203}\) cm\(^{-1}\) – 3280\(^{206}\) cm\(^{-1}\) compared to free HNO (\(\nu_{\text{NO}} = 1565\) cm\(^{-1}\), \(\nu_{\text{HN}} = 2717\) cm\(^{-1}\))\(^{212}\) (Table 2.1). Differences in the degree of \(\pi\)-backbonding from the metal center to the HNO ligand principally accounts for the range of N-O bond activation revealed by IR spectroscopy.

<table>
<thead>
<tr>
<th>(\nu_{\text{NO}}) (cm(^{-1}))</th>
<th>(\delta) HNO (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HNO</td>
<td>1565</td>
</tr>
<tr>
<td><a href="HNO">Os</a></td>
<td>1410</td>
</tr>
<tr>
<td><a href="HNO">Ir</a></td>
<td>1493</td>
</tr>
<tr>
<td><a href="HNO">Ru</a></td>
<td>1358</td>
</tr>
<tr>
<td><a href="HNO">Ru(heme)</a></td>
<td>1380</td>
</tr>
<tr>
<td><a href="HNO">Mb(heme)</a></td>
<td>1385</td>
</tr>
</tbody>
</table>

**Table 2.1.** Stretching frequencies (\(\nu_{\text{NO}}\)(cm\(^{-1}\))) and \(^1\)H-NMR (\(\delta\) (ppm)) shifts for selected [M](HNO) complexes.
Specifically, the work of Richter-Addo et al.\textsuperscript{73, 203} and Hillhouse et al.\textsuperscript{203, 208} substantiates the ability of the HNO ligand (and its C-nitroso analogues R-NO) to serve as a strong $\pi$-acid capable of significant $\pi$-backbonding with a metal center.

\subsection*{2.1.3.b. Synthesis of [M](HNO) compounds}

A variety of synthetic routes lead to metal-nitroxyl compounds (Scheme 2.7). Hydride (H\textsuperscript{-}) attack on an electrophilic metal-nitrosyl \{[M]-NO\}\textsuperscript{+} using borohydride salts can form the corresponding [M](HNO) species.\textsuperscript{73, 75} Also, [M](HNO) compounds can be formed via formal protonation (addition of H\textsuperscript{+}) of [M](NO\textsuperscript{-}) complexes\textsuperscript{41, 76, 203, 206, 208-210} or the addition of a hydrogen atom (addition of H\textsuperscript{+}) to [M](NO\textsuperscript{-}).\textsuperscript{211} Alternatively, NO\textsuperscript{+} can insert into the metal-hydride bond in [M]-H complexes to form [M](HNO) species.\textsuperscript{207} Proton abstraction from a coordinated H\textsubscript{2}NO ligand can also produce a [M](HNO) complex.\textsuperscript{73} Oxidation of coordinated hydroxylamine (H\textsubscript{2}NOH) can also result in the formation of [M](HNO) species.\textsuperscript{103} Hydroxylamine is an endogenous molecule that is a byproduct of NO formation via NOS and can readily be used as an HNO donor.\textsuperscript{66, 71, 188} The oxidation of NH\textsubscript{2}OH/HNO, 2 H\textsuperscript{+} (0.3 V vs NHE) is less facile than the electrochemical formation of nitroxyl from nitric oxide and proton (HNO/NO, H\textsuperscript{+}) (0.55 V vs NHE).\textsuperscript{187}

\begin{align*}
[M]&(NO^*) \\
[M](NO^+) + H^- &\rightarrow [M](HNO) \\
[M](NO^+) + H^+ &\rightarrow [M](NO^-) \\
[M]-H + NO^+ &\rightarrow [M]-ONH_2 \\
[M]-H &\rightarrow [M](HNO) \\
[M](NO^-) &\rightarrow -H^+ \\
[M](HNO) &\rightarrow [M]-ONH_2 \\
\end{align*}

\textbf{Scheme 2.7.} Synthetic methods for formation of [M](HNO) complexes.
It should be noted that some cases of formal protonation (usually with HCl) resulted in spectroscopic observations of what was thought to be both [M](HNO) and [M](NOH) species. This is significant because HNO (singlet) and NOH (triplet) have different preferred ground states. Calculations show that free $^3\text{NOH}$ and $^1\text{NOH}$ are less stable than $^1\text{HNO}$ by 26 - 31 kcal/mol and 40 - 42 kcal/mol, respectively.

2.1.3. c. Detecting and trapping HNO with metal complexes

Metal nitroxy traps have taken advantage of HNO’s relatively fast complexation with oxidized metals ($>10^5 \text{ M}^{-1} \text{ s}^{-1}$) though many are not specific to nitroxyl, and react in the presence of NO$^\cdot$ and N$_2$O. Boron et al. reported a Mn$^{\text{III}}$ protoporphyrinate IX complex which is able to trap HNO discriminately and quantify HNO concentrations as low as 10 nM via UV-Vis (Scheme 2.8). The resulting Mn$^{\text{II}}$(NO) complex is surprisingly air stable and forms faster than nitroxyl’s dimerization and reaction with dioxygen. Probably the most widely used fluorescent nitroxyl trap is a

\[
\begin{align*}
\text{Mn}^{\text{III}} \text{protoporphyrinate IX} + \text{HNO} & \rightarrow \text{Mn}^{\text{II}}(\text{NO}) + \text{H}^+ \\
\text{Cu}^{\text{II}}[\text{BOT1}] + \text{HNO} & \rightarrow \text{Cu}^{\text{II}}(\text{NO}) \\
\text{Cu}^{\text{II}}[\text{BOT1}] + \text{NO}^\cdot, \text{NO}_2^-, \text{ONO}_2^-, \text{H}_2\text{O}_2, \text{OCl}^- & \rightarrow \text{Cu}^{\text{II}}[\text{BOT1}] \\
\text{Co}^{\text{III}}(\text{P}) + \text{HNO} & \rightarrow \text{Co}^{\text{III}}(\text{P})(\text{NO})^-$
\end{align*}
\]

Scheme 2.8. Metal complexes (a) Mn$^{\text{III}}$ protoporphyrinate (b) Cu$^{\text{II}}$[BOT1] and (c) Co porphyrin that trap HNO with great specificity.
trap, however, was reported by Lippard et al. in 2010. The Cu\textsuperscript{II}BOTI complex took inspiration from CuZnSOD and allows for fluorescent (and thus \textit{in vivo} and \textit{in vitro}) quantification of HNO (Scheme 2.8). The non-heme fluorescent probe, BOT1, binds Cu\textsuperscript{II} at its tripodal binding site and transforms to Cu\textsuperscript{I} upon reacting with \textsuperscript{1}HNO/\textsuperscript{3}NO\textsuperscript{-}; Cu\textsuperscript{II}BOT1 is able to detect cellular production of nitroxyl after only 1 h of incubation.\textsuperscript{67, 185} Doctorovich developed the leading electrochemical method of HNO detection which utilized a cobalt porphyrin covalently bound through Au-S bonds.\textsuperscript{214} This probe allows for time resolved electrochemical quantification of HNO in concentrations as low as 1 – 1000 nM.\textsuperscript{215}

\textit{2.1.3.d. Coordination of HNO at copper}

Harrop has suggested that the complexation of NO\textsuperscript{-} to metals can provide insight into [M](O\textsubscript{2}) complexes, including the preferred binding mode, reactivity, and redox behavior of such metal dioxygen complexes.

Mononuclear binding modes of copper dioxygen complexes

$$\text{[Cu]}\text{O} \quad \text{[Cu]}\text{O}$$

Dinuclear binding modes of copper dioxygen complexes

$$\text{[Cu]}\text{O} \quad \text{[Cu]}\text{O} \quad \text{[Cu]} \quad \text{[Cu]} \quad \text{[Cu]}\text{O} \quad$$

\textbf{Figure 2.3.} Mononuclear and dinuclear dioxygen binding modes to copper.

dioxygen complexes.\textsuperscript{216} Similarly, O\textsubscript{2} binding modes should give insight into possible binding modes for HNO (Figure 2.3). Despite the differing ground states (\textsuperscript{3}O\textsubscript{2} and \textsuperscript{1}HNO),\textsuperscript{63} the otherwise isoelectronic nature of HNO and O\textsubscript{2} could guide possible modes of interactions of HNO with metal centers known to bind O\textsubscript{2}. 

55
Copper complexes are known to exhibit a diverse range of dioxygen binding modes. The nature of the supporting ligand often dictates the binding modes observed. Using tris(pyrazolyl)borate (Tp) ligands, the first synthetic dioxygen complexes were reported by Kitajima et al. who described a mononuclear Cu(II) superoxo complex and a dinuclear copper(II) side-on peroxy species in 1994 and 1992, respectively (Figure 2.4b and 2.4d). Another important class of supporting ligands for copper dioxygen chemistry are β-diketimates. Generally strongly donating, they offer a versatile and tunable monoanionic bidentate framework that allows for a range of steric and electronic profiles that lead to dicopper(III) bis(μ-oxo) species [Cu<sup>III</sup>]<sub>2</sub>(μ-O)<sub>2</sub>. In 2001, Dai and Warren isolated a [Cu<sup>II</sup>]<sub>2</sub>(μ-OH)<sub>2</sub> species proposed to proceed via [Cu<sup>III</sup>]<sub>2</sub>(μ-O)<sub>2</sub> dimer (Figure 2.4c). Later, Tolman published spectroscopic and structural data outlining these dinuclear bis(μ-oxo) species along with mononuclear copper dioxygen complexes [Cu](η<sup>2</sup>-O<sub>2</sub>) (Figure 2.4(a) and (c)). The use of very sterically demanding β-diketiminate ligands enabled the isolation of mononuclear species [Cu](η<sup>2</sup>-O<sub>2</sub>) (Figure 2.4(a) and (b)). The mononuclear structure exhibits an η<sup>2</sup>-O<sub>2</sub> structure, which by XAS spectroscopy features Cu<sup>III</sup> characteristics inferring a Cu<sup>III</sup>-peroxo structure. Since the interaction of the κ<sup>3</sup>-Tp ligand with a copper(I) center is inherently less directional than the κ<sup>2</sup>-β-diketiminate ligands, TpCu complexes feature less destabilization of the d-orbital used to engage the O<sub>2</sub> ligand and thus less activation of the O-O bond in {TpCu}<sub>2</sub>(μ-η<sup>2</sup>:η<sup>2</sup>-O<sub>2</sub>) and TpCu(η<sup>2</sup>-O<sub>2</sub>) complexes.
Synthetic models of copper nitroxyl compounds \([\text{Cu}](\text{HNO})\) would be an important step toward understanding how copper might play a role in the processing of nitric oxide and its derivatives such as HNO. Yet, the facile dimerization of HNO that leads to synthetic difficulties to isolate all but the most kinetically inert \([\text{M}](\text{HNO})\) species suggests computational investigations as a reasonable first step to gain insight into \([\text{Cu}](\text{HNO})\) species. Herein, we report DFT studies of \([\text{Cu}](\text{HNO})\) and \([\text{Cu}_2](\text{HNO})\) species as well as preliminary evidence for a \([\text{Cu}](\text{HNO})\) species via the oxidation of hydroxylamine.

**Figure 2.4.** Previously isolated copper dioxygen complexes.
2.2. Results and Discussion

The DFT calculations for the [Cu](HNO) and [Cu]_2(HNO) complexes employed the same level of theory employed to examine the electronic structure of the related dinuclear C-nitroso adduct \([\text{Me}_2\text{NN}][\text{Ni}]_2(\mu-\eta^2:\eta^2-\text{ONAr})\) (Ar = 3,5-Me_2C_6H_3)\(^{125}\) as well as mononuclear \([\text{Me}_2\text{NN}]\text{Cu-O}^\text{tBu}\)\(^{228}\) and \([\text{Me}_2\text{NN}]\text{Cu-SCPh}_3\)\(^{229}\) complexes. The Becke-Perdew (BP86) exchange correlation functional was used within the Amsterdam Density Functional suite of programs (ADF 2007.01).\(^{230-234}\) Slater-type orbital basis sets employed for C, N, and F were of triple - \(\zeta\) quality augmented with two polarization functions (ZORA/TZ2P) while an improved triple - \(\zeta\) basis set with two polarization functions (ZORA/TZ2P+) was employed for the copper atom. Scalar relativistic effects were included by virtue of the zero order regular approximation (ZORA).\(^{235}\) The 1s electrons of C, N, O, and F as well as the 1s and 2p electrons of Cu were treated as a frozen core. The electronic energies used to compare the relative stabilities of individual structures were not corrected for zero-point and entropic considerations.

2.2.1. DFT of mononuclear [Cu](HNO) complexes

We sought to identify the lowest energy binding mode of HNO at copper(I) \(\beta\)-diketiminate complexes that have successfully bound isoelectronic O_2. Based on our laboratory’s experience

![Figure 2.5. \(\beta\)-diketiminate copper complexes and three respective binding modes.](image)
with [Me₂NN]Cu^{125,223,236} and [Cl₂NN]Cu^{228,237} complexes (Figure 2.5), we examined the binding of HNO to these copper(I) complexes to give mononuclear [Cu(HNO)] complexes with the $\kappa^1$-$N$, $\kappa^1$-$O$, and $\eta^2$-$ON$ binding modes inspired by previous [M](HNO) complexes (Figure 2.5). In each case, the $\kappa^1$-$N$ binding mode was found to be lowest in energy, followed by the $\eta^2$-$ON$ binding mode that is only 2.8 and 2.4 kcal/mol higher in energy for [Me₂NN]Cu and [Cl₂NN]Cu, respectively (Table 2.2). The $\kappa^1$-$O$ binding mode is over 14 kcal/mol higher in energy, rendering this binding modes not synthetically likely. Importantly, the $\eta^2$-$ON$ binding mode leads to significant N-O bond activation (N-O: 1.341 and 1.337 Å) compared to the $\kappa^1$-$N$ binding mode (N-O: 1.251 and 1.251 Å) at [Me₂NN]Cu and [Cl₂NN]Cu, respectively, in all cases longer than the calculated N-O bond distance for free HNO (N-O: 1.211 Å).^{67}

<table>
<thead>
<tr>
<th>Complex</th>
<th>O-N bond distance</th>
<th>$\kappa^1$-$N$-HNO</th>
<th>$\eta^2$-$ON$-HNO</th>
<th>$\kappa^1$-$O$-HNO</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Me₂NN]Cu</td>
<td>O-N bond distance</td>
<td>1.251 Å</td>
<td>1.341 Å</td>
<td>1.278 Å</td>
</tr>
<tr>
<td></td>
<td>Relative energies (kcal/mol)</td>
<td>0.0</td>
<td>2.8</td>
<td>14.5</td>
</tr>
<tr>
<td>[Cl₂NN]Cu</td>
<td>O-N bond distance</td>
<td>1.251 Å</td>
<td>1.337 Å</td>
<td>1.273 Å</td>
</tr>
<tr>
<td></td>
<td>Relative energies (kcal/mol)</td>
<td>0.0</td>
<td>2.4</td>
<td>14.6</td>
</tr>
</tbody>
</table>

Table 2.2. Relative optimized energies of [Cu](HNO) and respective O-N bond distance.

Despite the more electron-rich nature of the [Me₂NN]Cu complex compared to [Cl₂NN]Cu (Section 4.2.4.c.), calculations reveal little difference in binding mode energetics or degree of N-O bond activation. This finding is interesting as the greater orbital overlap between the most destabilized copper d orbital and the entire HNO $\pi^*$ orbital in the $\eta^2$-$ON$ binding mode could confer greater stabilization for this binding mode (Figure 2.6). This is the binding mode observed in analogous $\beta$-diketiminato C-nitroso complexes [Me₂NN]Cu($\eta^2$-ONPh),^{125}
[Cl₂NN]Cu(η²-ONPh), [Me₂NNF₆]Cu(η²-ONPh) (chapter 3). In fact, the LUMO for [Cl₂NN]Cu(η²-ON-HNO) (-2.83 eV) is much higher in energy than the LUMO for [Cl₂NN]Cu(κ¹-N-HNO) (-3.30 eV), suggesting stronger π-backbonding in the more electron-rich [Me₂NN]Cu(η²-ON-HNO). One manifestation of the increased overlap is the greater efficacy of π-backbonding into the N-O anti-bonding orbital resulting in the lengthening of the O-N bond, which is seen in both η²-ON structures, attesting to Richter-Addo and Hillhouse’s evidence of HNO acting as a π-acid.

And yet, despite the increased overlap between the copper d and π* orbitals, most of the isolated [M](HNO) complexes exhibit a κ¹-N-HNO binding mode (section 2.1.3.a). This binding mode may be preferred because the sp² hybridized N lone pair can provide greater σ donation to copper than can the NO bonding π electrons. In this manner, N lone pair (higher in

Figure 2.6. LUMO of [Cu](κ¹-N-HNO) and [Cu](η²-ON-HNO).
energy than the NO bonding π electrons) and the still accessible NO π* orbital can result in the lowest energy [Cu](HNO) complex.

2.2.2. DFT of dinuclear copper complexes [Cu]₂(HNO)

The greater π-backbonding opportunities from two copper(I) centers as compared to a single copper(I) center offer the possibility to bind HNO more tightly. Such dinuclear trapping of HNO could offer important synthetic opportunities to isolate a copper HNO complex. It is essential to identify a structure type with a very high affinity for HNO to circumvent irreversible HNO loss via its rapid dimerization. Additionally, dinuclear trapping of HNO at copper would provide additional steric hindrance towards electrophilic attack on bound HNO as well as the possibility of associative exchange mechanisms that could result in loss of HNO.

The five binding modes investigated were inspired by those observed in dinuclear dioxygen complexes (section 2.1.3.d). The three most viable binding modes are the same for both [Me₂NN]Cu and [Cl₂NN]Cu and could feasibly interconvert at room temperature (Table 2.3). The calculations show the binding of a second [Cu] structure via the O atom in the μ-η²:η¹-ONH binding mode is only slightly more favorable (3.2 and 2.6 for [Me₂NN]Cu and [Cl₂NN]Cu, respectively) than the breaking of the ON double bond in the (μ-O)(μ-NH) structure, but both the μ-η²:η¹-ONH and (μ-O)(μ-NH) are impractical. The lowest energy binding mode (μ-η¹:η²) has

<table>
<thead>
<tr>
<th>Structure</th>
<th>μ-η¹:η²-ONH</th>
<th>μ-1,2-ONH</th>
<th>μ-η²:η²-ONH</th>
<th>μ-η²:η¹-ONH</th>
<th>(μ-O)(μ-NH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Me₂NN]Cu</td>
<td>0.0</td>
<td>1.3</td>
<td>3.7</td>
<td>11.5</td>
<td>14.7</td>
</tr>
<tr>
<td>[Cl₂NN]Cu</td>
<td>0.0</td>
<td>1.8</td>
<td>1.8</td>
<td>9.6</td>
<td>12.2</td>
</tr>
</tbody>
</table>

Table 2.3. Relative optimized energies of [[Cu]₂](HNO).
been observed in analogous dinuclear copper C-nitroso compounds $\{[\text{Me}_2\text{NN}]\text{Cu}\}_2(\eta^2-\text{ONAr})$ (Ar = Ph or 3,5-Me$_2$C$_6$H$_3$) and the $\mu$-$\eta^2:\eta^2$ binding mode has been observed in corresponding $\{[\text{Me}_2\text{NN}]\text{Ni}\}_2(\eta^2-\text{ONAr})$ (Ar = Ph or 3,5-Me$_2$C$_6$H$_3$) compounds.$^{125}$
2.2.3. Stretching frequency calculations of copper HNO complexes

As described in the introduction to this chapter (Section 2.1.3.a), IR spectroscopy is a powerful way to identify the extent of N-O bond activation in [M](HNO) complexes. Since the [Cu](κ¹-N-HNO) and [Cu](η²-ON-HNO) binding modes have such disparate O-N distances, IR spectroscopy should be able to distinguish between these two binding modes. Due to their greater computational cost, we carried out computationally intensive frequency calculations on small models (no β-diketiminato substituents) of the most energetically favorable [Cu](HNO) and [Cu]₂(HNO) binding modes discussed above. Calculations for [H₅C₃N₂]Cu(κ¹-N-HNO) and [H₅C₃N₂]Cu(η²-ON-HNO) reveal $\nu_{\text{NO}} = 1441$ and $1089 \text{ cm}^{-1}$, respectively. The η²-ON-HNO complex is predicted to have a $\nu_{\text{NO}}$ stretching frequency very similar to that experimentally observed in the C-nitroso adduct [Me₂NN]Cu(η²-ONPh) with $\nu_{\text{NO}} = 1113 \text{ cm}^{-1}$. Unfortunately, pure N-O stretching frequencies could not be determined unambiguously in the dinuclear structures due to heavy mixing with other vibrational modes (Figure 2.5).

![Figure 2.5. Stretching frequencies ($\nu_{\text{NO}}$, cm⁻¹) and N-O bond distances (Å) for the most energetically favorable [Cu](HNO) and ([Cu]₂(HNO) complexes.](image)

$\nu_{\text{NO}} = 1441 \text{ cm}^{-1}$  
N-O: 1.24 Å

$\nu_{\text{NO}} = 1089 \text{ cm}^{-1}$  
N-O: 1.34 Å

N-O: 1.36 Å  
N-O: 1.34 Å  
N-O: 1.30 Å
2.2.4. Solvent stability of mono- and dinuclear copper HNO complexes

Due to the Lewis acidity of the copper centers, synthetic β-diketiminato copper(I) complexes [Me₂NN]Cu and [Cl₂NN]Cu are not isolated as two coordinate copper complexes. Rather, they are isolated as Lewis base solvento adducts [Cu](solvent) with bases such as acetonitrile (solvent = NCMe) or benzene (solvent = η²-benzene). To gain a sense of binding affinity of HNO at [Me₂NN]Cu and [Cl₂NN]Cu complexes in comparison to solvents found in synthetically accessible starting materials [Cu](solvent), we computationally optimized related [Cu](solvent) (solvent = NCMe, THF, η²-benzene) complexes. Computational access to these solvento structures [Cu](solvent) allowed us to estimate the thermodynamics of HNO binding in comparison to the solvents. Comparing electronic energies of individual species in the gas phase to generate overall energies of reaction, we find that HNO binds considerably stronger to give [Cu](κ¹⁻N-HNO) adducts than these common solvents. Even HNO displacement by acetonitrile is enthalpically uphill by 13.8 and 14.9 kcal/mol for [Me₂NN]Cu(κ¹⁻N-HNO) and [Cl₂NN]Cu(κ¹⁻N-HNO), respectively (Table 2.3). Thus, HNO binding should be highly favored at mononuclear β-diketiminato complexes.

<table>
<thead>
<tr>
<th></th>
<th>NCMe</th>
<th>THF</th>
<th>η²-benzene</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Me₂NN]Cu</td>
<td>13.8</td>
<td>29.9</td>
<td>23.2</td>
</tr>
<tr>
<td>[Cl₂NN]Cu</td>
<td>14.9</td>
<td>28.6</td>
<td>24.3</td>
</tr>
</tbody>
</table>

Table 2.3. Energy difference of ligand (HNO vs solvent) exchange in mononuclear [Cu] structures.
Similarly, we examined the relative thermodynamic stability of the lowest energy dinuclear species \([\text{Cu}]_2(\mu-\eta^1:\eta^2-\text{ONH})\) towards conversion to the corresponding mononuclear species \([\text{Cu}](\kappa^1-N\text{-HNO})\) along with a solvent bound species \([\text{Cu}](\text{solvent})\) (Table 2.4). These studies reveal that dissociation of dinuclear \([\text{Cu}]_2(\text{HNO})\) species to mononuclear \([\text{Cu}](\text{HNO})\) complexes may be difficult to prevent under experimental conditions. Use of solvents that bind weakly to the \([\text{Cu}]\) fragment such as THF, however, represents one possible strategy.

\[
\begin{align*}
\text{[Cu]}(\eta^1:\eta^2:\text{ONH}) + \text{solvent} & \rightleftharpoons \text{[Cu]}(\eta^2:\text{N}) + \text{[Cu]}(\text{solvent}) \\
\Delta E_{\text{rxn}} \text{ (kcal/mol)} & \\
\text{potential solvent} & \\
\text{NCMe} & +10.1 & +3.4 \\
\text{THF} & -6.0 & +9.2 \\
\text{Benzene} & -4.5 & +4.9
\end{align*}
\]

**Table 2.4.** Energy difference of ligand (HNO vs solvent) exchange in dinuclear \([\text{Cu}]\) structures.

2.2.5. Preliminary experimental evidence for the formation of a \([\text{Cu}](\text{HNO})\) complex

We generated preliminary experimental evidence for the formation of a \([\text{Cu}](\text{HNO})\) complex upon the addition of hydroxylamine to \([\{\text{Me}_2\text{NN}\text{Cu}\}_2(\mu-\text{OH})\}_2\) at -80 °C in THF. Following this reaction via UV-Vis spectroscopy (Figure 2.6) resulted in loss of the band at \(\lambda = 410\) nm due to \([\{\text{Me}_2\text{NN}\text{Cu}\}_2(\mu-\text{OH})\}_2\) and the formation of a fleeting new species at \(\lambda = 550\) nm. Even at -80 °C this new band at \(\lambda_{\text{max}} = 550\) nm decays over 30 minutes. Analogous copper C-nitroso compounds, \([\text{Me}_2\text{NN}\text{Cu}(\eta^2-\text{ONAr})\] (Ar = Me$_2$C$_6$H$_3$) and \([\text{Me}_2\text{NN}\text{Cu}(\eta^2-\text{ONPh})\), further explored in chapter three, show a similar UV-Vis absorption at 583 nm and 588 nm in Et$_2$O, respectively.\textsuperscript{125}
Unfortunately, neither the decrease in the $\lambda_{\text{max}} = 410$ nm nor the increase of $\lambda_{\text{max}} = 550$ nm fit perfectly into any rate law manipulations and more in depth kinetic studies are needed.

Though Cu$^{II}$ ions have previously been shown to produce NO upon the addition of hydroxylamine,$^{107}$ the proposed mechanism by which this transformation occurs begins with a hydronitroxide (\'NHOH) radical, which is then further oxidized to HNO and NO.$^{4, 66, 107}$ We propose an alternative to the hydronitroxide (\'NHOH) free radical. We suggest a copper (II) nitroxide ([Cu]-ONH$_2$) intermediate based on the H$_2$NOH pKa values and calculations of the decomposition of free H$_2$NOH, which suggest \'ONH$_2$ as the first step, we investigate this intermediate in more detail in chapter four.

![Figure 2.6](image)

**Figure 2.6.** Reaction between $\{[\text{Me}_2\text{NN}]\text{Cu}\}_2(\mu-\text{OH})_2$ and 1 equiv. hydroxylamine in THF at -80 °C. UV-vis spectra taken every 7 s.
2.3 Conclusions

After computationally examining several binding modes for both [Cu](HNO) and 
{[Cu]}2(HNO), we find 5 binding modes that are viable: two mononuclear (κ¹-N-HNO and η²-
NO-HNO) and three are dinuclear (μ-η¹:η²-ONH, μ-1,2-ONH, and μ-η²:η²-ONH). The 
mononuclear binding modes are both very close in energy, though the κ¹-N-HNO is slightly 
more stable by about 4 kcal/mol for both [Cl₂NN]Cu and [Me₂NN]Cu β-diketiminate scaffolds. 
These two binding modes can be spectroscopically distinguished by νNO. Using a pared version 
of the β-diketiminate ligand, we find the νNO for κ¹-N-HNO to be higher (1441 cm⁻¹) than that for 
η²-NO-HNO (1089 cm⁻¹).

The dinuclear {{[Cu]}2}(HNO) complexes can provide an opportunity to slow the kinetic 
lability of HNO and hamper ligand loss via HNO dimerization. Of the three most stable 
dinuclear binding modes, the μ-η¹:η²-ONH is the most stable, but by only 1-2 kcal/mol in both 
[Cl₂NN]Cu and [Me₂NN]Cu complexes.

The solvent used to isolate a copper nitroxyl complex can also impact kinetic lability. 
Surprisingly, we calculate HNO binds more tightly to both [Cl₂NN]Cu and [Me₂NN]Cu 
complexes than either NCMe, THF, and benzene in the case of the mononuclear compounds. 
Using the most stable κ¹-N-HNO mononuclear binding mode, we find the exchange of HNO for 
NCMe is slightly disfavored by 14-15 kcal/mol. In the case of the dinuclear {{[Cl₂NN]Cu}2(μ-
η¹:η²-ONH) and {{[Me₂NN]Cu}2(μ-η¹:η²-ONH), we find that the dissociation of the second [Cu] 
structure is slightly favored by 4.5 and 6 kcal/mol.

Future efforts in the isolation of a copper nitroxyl complex may employ a larger β-
diketiminate framework that insulates the HNO ligand from further reactivity. Additionally, a β-
diketiminate framework with greater π-backbonding capacity may aid in the isolation of a
[Cu](HNO species. The influence of π-backbonding on [Cu](ONR) structures is further explored in chapter 3.
2.4. Experimental Procedures

2.4.1. DFT calculation details

The DFT calculations employed the Becke-Perdew exchange correlation functional using the Amsterdam Density Functional suite of programs (ADF 2007.01).\textsuperscript{230-234} ADFView was used to prepare the three dimensional representations of the structures shown.\textsuperscript{240} Slater-type orbital basis sets employed for C, N, and F were of triple - $\zeta$ quality augmented with two polarization functions (ZORA/TZ2P) while an improved triple - $\zeta$ basis set with two polarization functions (ZORA/TZ2P+) was employed for the copper atom. Scalar relativistic effects were included by virtue of the zero order regular approximation (ZORA).\textsuperscript{235} The 1s electrons of C, N, O, and F as well as the 1s and 2p electrons of Cu were treated as a frozen core. The VWN (Vosko, Wilk, and Nusair) functional was used for LDA (local density approximation).\textsuperscript{241} Default convergence ($\Delta E = 1 \, \text{Å} \sim 10^{-3} \text{hartree}$, max. gradient = $1 \, \text{Å} \sim 10^{-2} \text{hartree} / \text{Å}$, max. Cartesian step = $1 \, \text{Å} \sim 10^{-2} \text{Å}$) and integration (4 significant digits) parameters were employed for geometry optimizations. The calculations are of electronic energies only in which zero point and entropic considerations were excluded.

2.4.2. General experimental procedures

All experiments were carried out in a dry nitrogen atmosphere using an MBraun glovebox and/or standard Schlenk techniques. 4 A molecular sieves were activated in vacuo at 180 °C for 24 h. Dry THF and benzene was purchased from Aldrich and stored over activated 4 A molecular sieves. Diethyl ether was first sparged with nitrogen and then dried by passage through activated alumina columns. Pentane was first washed with conc. HNO$_3$ / H$_2$SO$_4$ to remove olefins, dried by passage through activated alumina columns, and stored over CaCl$_2$ and activated 4 A molecular sieves. All deuterated solvents were sparged with nitrogen, dried over activated 4A molecular
sieves and stored under nitrogen. Celite was dried overnight at 200 °C under vacuum.

UV-Vis spectra were recorded on either a Varian Cary 50 or 100 spectrophotometer, using cuvettes with screw-cap tops.

All reagents were obtained commercially unless otherwise noted and typically stored over activated 4A molecular sieves. \([\text{Me}_2\text{NN}]\text{H}^{238}\) and \([\text{Me}_2\text{NN}]\text{Cu}\) were prepared using literature methods.

2.4.3. Preparation and characterization of compounds

\([\text{Me}_2\text{NN}]\text{Cu}\)\(^2\)\((\mu\text{-OH})_2\). \([\text{Me}_2\text{NN}]\text{Cu}\)\(^2\)(\(\mu\text{-OH})_2\) was synthesized according to a modified procedure reported.\(^{224}\) A vial containing a yellow solution of \([\text{Me}_2\text{NN}]\text{Cu}\)\(^2\) in THF was prepared in the glovebox. After the vial was pumped out of the glovebox, the cap was loosened so as to slowly expose the solution to air. The resulting brown solution was purged with \(\text{N}_2\) for 5 min and reintroduced to the glovebox and dried in vacuo and stored at -40 °C for future use.

**UV-Vis reaction with \([\text{Me}_2\text{NN}]\text{Cu}\)\(^2\)(\(\mu\text{-OH})_2\) and hydroxylamine**

\([\text{Me}_2\text{NN}]\text{Cu}\)\(^2\)(\(\mu\text{-OH})_2\) (0.0645 g, mol) was dissolved in 7 mL of THF and then the volume was increased to 10.00 mL via a volumetric flask using THF as solvent to make a 10.00 mL stock solution of 8.38 mM of \([\text{Me}_2\text{NN}]\text{Cu}\)\(^2\)(\(\mu\text{-OH})_2\). 355 μL of the 8.38 mM \([\text{Me}_2\text{NN}]\text{Cu}\)\(^2\)(\(\mu\text{-OH})_2\) stock solution and 2.565 mL THF were added to a UV-Vis cuvette. The collection of uv-vis spectra began immediately after placing the cuvette in the sample holder. After 5 scans, 80 μL of hydroxylamine (50% by wt in \(\text{H}_2\text{O}\)) were added to the cuvette and the reaction monitored was over 3 h.
CHAPTER 3

STRUCTURES AND REACTIVITY OF NEW COPPER-NITROSO ARENE COMPLEXES

3.1. Introduction

Nitrosoarenes and N-arylhydroxylamines are endogenous metabolites of amines. Formed via the N-oxygenation of aromatic amines or the reduction of nitro aromatics, the oxidized amines can have important reactivity with biological targets, often times with cytotoxic results.\(^\text{35, 242-246}\) For instance, the binding affinity of nitrosobenzene to hemoglobin is much stronger than that of dioxygen.\(^\text{247}\) N-phenylhydroxylamine (PhHNOH), the two-electron reduced congener of nitrosobenzene, can be formed through prolonged exposure of nitrosobenzene within red blood cells.\(^\text{248}\) The phenylhydronitroxide radical (PhHNO\(^\cdot\)), a metabolic product of PhNO\(^\text{244-246}\) (as well as PhNH\(_2\), PhHNOH and PhNO\(_2\)),\(^\text{243}\) can be formed from ONPh in the presence of both NADH and Cu\(^{\text{II}}\) ions,\(^\text{124}\) which causes oxidative DNA damage\(^\text{124}\) and oxidation of thiol residues within red blood cells.\(^\text{243}\)

Nitrosobenzene and N-phenylhydroxylamine can serve as synthetic analogues for the more reactive nitroxyl (HNO)\(^\text{60, 182, 249}\) and hydroxylamine (H\(_2\)NOH)\(^\text{111, 250}\) and can aid in understanding the structure and reactivity of metal intermediates and metal assisted conversions between the two.\(^\text{111, 251}\) Nitroxyl and hydroxylamine, also produced endogenously, are byproducts of nitric oxide production via nitric oxide synthase (NOS)\(^\text{10, 60}\) and upon reaction of S-nitrosothiols with excess thiols.\(^\text{99, 252-253}\)
3.1.1. Physical and chemical properties of ArNO

Nitrosoarenes, often used as analogues for nitroxyl, contain the N=O functionality and inherency to dimerize like that of HNO. Unlike nitroxyl, however, the dimerization of nitrosoarenes is reversible with activation energies in the range of 20-25 kcal/mol that results in the formation of either cis or trans azodioxy species (PhN(O)=N(O)Ph) (Scheme 3.1).\textsuperscript{60, 182, 249, 254-255} There are several factors that affect this dimerization in solution including concentration and position of substituents. Concentrations at or below $10^{-4}$ M result in the majority of monomers in solution, while concentrations of $10^{-1}$ M or greater exhibit dimeric forms.\textsuperscript{242} The dimeric form is also favored when substituents are present at the 2- and 2,6-positions for the resulting diminished resonance between the aromatic system and NO group encourages the subsequent limited conjugation resulting from the formation of the N-N bond.\textsuperscript{242} Steric hindrances are circumvented by the twisting out of coplanarity from the central C$_2$N$_2$O$_2$ structure.\textsuperscript{254} Both cis and trans azodioxy species show the N-N bond has significant double bond character as the azodioxy N-N bond length generally lies between 1.30 Å and 1.32 Å, in between that of a single (1.45 Å) and double (1.25 Å) N-N bond.\textsuperscript{256} Crystals of many isolated ArNO species exist as colorless dimers\textsuperscript{255} and dimerization studies show the dimer to have absorption bands at 280 nm and 306 nm while the monomer exhibits a weak band from 630-790 nm.\textsuperscript{35, 242} Solutions possessing monomeric ArNO species are often colored blue or green from a resulting weak, Laporte forbidden $n \rightarrow \pi^*$ transition.\textsuperscript{255} The N-O bond distance for aromatic C-nitroso compounds is generally longer in
dimers than in monomers due to the O-N single bond character of the azodioxy form. The monomeric N-O bond distance in PhN=O is calculated to be 1.223 Å\textsuperscript{126}, while the cis-dimer is crystallographically determined to be 1.268(4) Å\textsuperscript{35}. Infrared spectroscopy shows the trans dimers to have NO stretching frequencies ($\nu_{NO} = 1200 – 1300$ cm\textsuperscript{-1}) much lower than that of corresponding monomeric species ($\nu_{NO} = 1500 – 1600$ cm\textsuperscript{-1})\textsuperscript{254}. UV-Vis spectra of free aromatic C-nitroso compounds display 3 main electronic features around 280 nm, 305 nm, and 730 nm\textsuperscript{126}. Almost all C-nitroso compounds are colored, ranging from blue to emerald green to green in solution\textsuperscript{254}. The n (N) $\rightarrow$ $\pi^*$ (NO) transition (ca. 630 – 790 nm) is responsible for the low energy transition which results in the color of free, monomeric C-nitroso compounds. Another low intensity band ($\varepsilon$ = 80 M\textsuperscript{-1}cm\textsuperscript{-1}) around 270 – 300 nm is due to n $\rightarrow$ $\pi^*$ transition of electrons localized on the oxygen\textsuperscript{110, 257}.

\subsection*{3.1.2. Metal RNO model complexes}

\subsubsection*{3.1.2.a. Endogenous formation nitrosoarenes (RNO)}

Nitrosoarenes are endogenously formed via the N-oxygenation of aromatic amines or the reduction of nitroaromatics at enzymes such as nitroreductase, cytochrome P-450, and CuZnSOD (Scheme 3.2).\textsuperscript{35, 95, 242, 248} Additionally, in 2010, Noguchi \textit{et al.} discovered a dicopper enzyme, \textit{NspF}, from \textit{Streptomyces murayamaensis}, which after forming a peroxy-dicopper(II) complex upon reacting with dioxygen, metabolizes amino substrates to form the corresponding nitroso derivatives\textsuperscript{126}. Furthermore, industry has long understood that certain flavoenzymes biodegrade

\begin{center}
\includegraphics[width=\textwidth]{scheme32.png}
\end{center}

\textbf{Scheme 3.2}. The formation of C-nitroso compounds from amine and nitro functionalities at various enzymes.
nitro explosives forming nitroso compounds and corresponding azodioxy species. The mechanism by which this transformation occurs has not yet been fully determined, but two potential routes include either an aziridine N-oxide or polarized diradical intermediate (Scheme 3.3).

![Scheme 3.3. Potential mechanism of nitroso ene reaction via polarized diradical or aziriding intermediate.](image)

Though these oxidized amines can be less reactive, they are still biologically relevant and are often deleterious. For example, nitrosobenzene (PhNO) binds to hemoglobin and myoglobin more strongly than dioxygen and can act as an inhibitor to platelet action and to certain enzymes, which has engendered its use as a prodrug. Additionally, the prolonged exposure of red blood cells to nitrosobenzene leads to the formation of N-phenylhydroxylamine. The phenylnitroxide radical (PhNHO•), a metabolic product of nitrosobenzene formed in the presence of NADH and CuII ions, causes oxidative DNA damage and oxidation of thiol
residues within red blood cells. H$_2$NOH can react with oxyhemoglobin to produce H$_2$NO$^\cdot$ radical, as well, which is also capable of oxidative damage (Scheme 3.4).

3.1.2.b. Copper nitrosoarene complexes

Additionally, copper adducts of nitrosoarenes play vital roles in catalysis. Copper(I) nitrosoarene complexes are involved in the catalytic formation of C-N bonds. The crystal structure of the Cu$^\text{I}$-nitrosoarene (Figure 3.1.a) resulting from the addition of N-aryl hydroxylamines to [Cu(NCMe)$_4$]PF$_6$ is used as a catalyst for the amination of allylic substrates and exhibits a $\kappa^1$-$N$ binding mode typical for these compounds. Additionally, copper nitrosoarenes have been considered in asymmetric Diels-Alder reactions of electron-poor nitrosoarenes with dienes. Changing the environment of the copper center can change the binding mode of the nitroso compound. For example, a tripodal ligand such as [Me$_6$tren] changes the structure to reveal a $\kappa^1$-$O$ binding mode (Figure 3.1.c. and 3.1.d.), which is suggested as a mimic for end on superoxide binding to mononuclear copper enzymes, and an $\eta^2$-ON binding mode, respectively.
The N-O bond distance in [Cu(Et$_2$NPhNO)$_3$]PF$_6$ (I) that exhibits κ$^1$-$N$ coordination is 1.2646(16) Å, shorter than N-O distances in the nitrosoarene ligands depicted in Figure 1 that range from 1.320(4) Å– 1.337(3) Å. The N-O bond distance can give some indication of the activation of the N-O bond as well as provide insight into the oxidation state of copper. Nonetheless, among the κ$^1$-$O$ and η$^2$-ON bound nitrosobenzene complexes in Figure 1, the N-O distances are relatively unchanged while the oxidation state of each copper may not be uniform. The κ$^1$-$N$ [Cu(Et$_2$NPhNO)$_3$]PF$_6$ (I) compound is classified as a Cu$^I$ for which there is not much ambiguity. The oxidation state of the κ$^1$-$O$ complexes, however, may be considered either Cu$^I$ and Cu$^{II}$ and the η$^2$-ON β-diketiminate structure shows spectroscopic features for both Cu$^{II}$ and Cu$^{III}$.2

3.1.2.c. Binding modes and spectroscopy of M(RNO) compounds

The κ$^1$-$N$, κ$^1$-$O$, and η$^2$-ON binding modes for mononuclear metal nitrosoarene compounds have all been observed at copper centers (Figure 3.2). These include the κ$^1$-$N$ bonding mode in [Cu(Et$_2$NPhNO)$_3$]PF$_6$ reported by Srivastava, the κ$^1$-$O$ motif in [Me$_6$trenCu(PhNO)]OTf and [Me$_6$trenCu(PhNO)]SbF$_6$ reported by Askari et al., as well as η$^2$-ON coordination in [Me$_2$NN]Cu(η$^2$-ONAr) reported by Wiese et al. Not only can the supporting ligand affect the nitroso binding mode, but so can the C-nitroso N-substituent. In many cases, spectroscopic

![Figure 3.2. Binding modes of copper nitroso compounds.](image-url)

![Figure 3.3. Ambiguity of copper oxidation state.](image-url)
determination of the binding mode may be best observed through IR spectroscopy. N-O stretching frequencies for unbound, monomeric, aromatic nitroso compounds may range from 1488 cm$^{-1}$ – 1513 cm$^{-1}$, with free nitrosobenzene displaying the N-O stretch at 1506 cm$^{-1}$.

The stretching frequencies for each of the mononuclear copper compounds are considerably less than that of free nitrosobenzene [3: 1119 cm$^{-1}$; 4: 1160 cm$^{-1}$; 10: 1113 cm$^{-1}$], suggesting reduction in the N-O bond order due to backbonding to the N-O $\pi^*$ of the C-nitroso ligand. Especially for the $\eta^2$-ON binding mode, the extent of N-O activation can lead to some ambiguity in the formal Cu oxidation state that has been examined with XAS and high level multireference DFT calculations (Figure 3.3).

Described herein is the synthesis of new family $\beta$-diketiminato copper C-nitrosoarene complexes [Cu]($\eta^2$-ONPh) featuring varied electronic and steric properties of the $\beta$-diketiminate ligand. We describe a number of different synthetic pathways to these $\eta^2$-ON C-nitrosoarene complexes [Cu]($\eta^2$-ONPh) that include the addition of nitrosobenzene to [CuI] complexes, the disproportionation of PhNHOH by [CuI] as well as the reaction of PhNHOH with [[Cu$^{II}$]$\mu$(OH)$_2$. We also examine the reactivity of [Cu]($\eta^2$-ONPh) complexes with nitric oxide to give the corresponding diazeniumdiolate [Cu$^{II}$]($\kappa^2$-O$_2$N$_2$Ph).
3.2. Results and Discussion

3.2.1. Formation of $[\text{Cu}]({\eta}^2\text{-ONPh})$ compounds

The addition of 1 equiv. PhNO to $[\text{Cl}_2\text{NN}]\text{Cu(NCMe)}$ (5) and $[\text{Me}_2\text{NN}_{\text{F6}}]\text{Cu(NCMe)}$ (7) in diethyl ether results in the displacement of NCMe and the formation of the corresponding copper C-nitroso compound $[\text{Cl}_2\text{NN}]\text{Cu}({\eta}^2\text{-ONPh})$ (9) and $[\text{Me}_2\text{NN}_{\text{F6}}]\text{Cu}({\eta}^2\text{-ONPh})$ (11), which can be isolated as green-purple crystals from pentane in 46% and 43% yield, respectively (Scheme 3.5).

The formation of $[\text{Pr}_2\text{NN}_{\text{F6}}]\text{Cu}({\eta}^2\text{-ONPh})$ (12) does not result from the addition of PhNO to $[\text{Pr}_2\text{NN}]\text{Cu(NCMe)}$ (8), however. We suspect that the more electron-withdrawing copper(I) center in 8 that results from coordination of the electron-poor $\beta$-diketiminate $[\text{Pr}_2\text{NN}]$ enhances the binding of the generally labile MeCN ligand, leading to the failure of the PhNO ligand to replace it. In situ formation of $[\text{Pr}_2\text{NN}]\text{Cu}$ by combination of $[\text{Pr}_2\text{NN}]\text{H}$ and Cu$^{\text{I}}\text{O'Bu}$, followed by addition of PhNO, however, results in the formation of $[\text{Pr}_2\text{NN}]\text{Cu}({\eta}^2\text{-ONPh})$ (12) as purple-green crystals in 54% yield.

**Scheme 3.5.** Synthesis of 9 – 12 via the addition of PhNO.
3.2.2. Structural characterization and comparison of [Cu](η²-ONPh) compounds

3.2.2.a. X-ray diffraction studies and comparisons of [Cu](η²-ONPh)

X-ray diffraction studies reveal that side-on binding of nitrosobenzene occurs in all copper β-diketiminato compounds 9 – 12 despite electronic and steric differences within the β-diketiminate ligand (Figure 3.5 – Figure 3.7). The N-O bond lengths in 9, 11, and 12 [9: 1.354(3) Å; 11: 1.330(4) Å; 12: 1.332(2) Å] are very similar to that found in the two crystallographically independent molecules of 10 [1.334(5) Å and 1.338(5) Å]. Careful inspection reveals an interesting trend:
we find direct comparisons of the N-O bond of any 2 structures generally lengthens as the electron-donating ability of the β-diketiminate ligand increases (Figure 3.4). The nitrosarene Cu-O and Cu-N distances in 9, 11, and 12 [9: 1.850(3) Å, 1.923(4) Å; 11: 1.861(3) Å, 1.942(3) Å; 12: 1.843 Å,

**Figure 3.4.** Depiction of the general lengthening of the NO bond as the electron donating capacity of β-diketiminate ligand increases.
1.938 Å, respectively] do not differ appreciably from those found in 10 [Cu-O: 1.853(3) Å, 1.855(3) Å; Cu-N: 1.923(4) Å, 1.935(3) Å].
Figure 3.5. Fully labeled thermal ellipsoid diagram of [Cl₂NN]Cu(η²-ONPh) (9) (all H atoms omitted). Selected bond distances (Å) and angles (deg): N3-O 1.354(3), Cu-N3 1.923(4), Cu-O 1.850(3), Cu-N1 1.890(3), Cu-N2 1.885(3), N3-C18 1.426(5), N3-Cu-O 41.71(11), N1-Cu-N2 98.92(13), N1-Cu-O 105.81(11), N2-Cu-N3 113.51(12).
Figure 3.6. Fully labeled thermal ellipsoid diagram of $\text{[Me}_2\text{NN}_6\text{]}\text{Cu}(\eta^2-\text{ONPh})$ (11) (all H atoms omitted). Selected bond distances (Å) and angles (deg): N3-O 1.330(4), Cu-N3 1.942(3), Cu-O 1.861(3), Cu-N1 1.907(3), Cu-N2 1.898(3), N3-C22 1.443(5), N3-Cu-O 40.88(12), N1-Cu-N2 99.77(12), N1-Cu-O 106.640(12), N2-Cu-N3 112.94(13).
Figure 3.7. Fully labeled thermal ellipsoid diagram of [Pr$_2$NN$_6$]Cu(η^{2}-ONPh) (12) (all H atoms omitted). Selected bond distances (Å) and angles (deg): N3-O 1.332(2), Cu-N3 1.9511(16), Cu-O 1.8689(13), Cu-N1 1.9104(16), Cu-N2 1.9048(15), N3-C30 1.426(2), N3-Cu-O 40.73(6), N1-Cu-N2 99.52(6), N1-Cu-O 103.56(6), N2-Cu-N3 116.09(7).
Despite the differences in steric and electronic properties that result from the range of \( \beta \)-diketiminate ligands in 9 – 12, all possess \( \eta^2 \)-ON binding modes as revealed by X-ray crystallography. Even in the case of compound 12 possessing the sterically demanding \( N \)-aryl \( \text{tPr} \) groups which appear to be bent back in response to the nitrosoarene aryl ring (Figure 3.7), the \( \eta^2 \)-ON binding mode is observed although a shift to \( \kappa^1 \)-N binding would relieve this steric strain. In contrast to \( \beta \)-diketiminate compounds 9 – 12, the tris(pyrazolyl)borato copper(I) complex \([\text{Pr}^2 \text{TpCu}] (\kappa^1 \text{-N(O)Ph}) \) (14) features the \( \kappa^1 \)-N binding mode. The N-O bond lengths found in the two crystallographically independent molecules of 14 (1.250(6) \( \text{Å} \) and 1.236(7) \( \text{Å} \)) are considerably shorter than those found in the \( \eta^2 \)-ONPh adducts of 9 – 12, but similar to Srivastava’s \([\text{Cu}(\kappa^1 \text{-N(O)Ar})_3]^+ \) [1.252(2) \( \text{Å} \) - 1.265(2) \( \text{Å} \); \( \text{Ar} = 4\text{-Et}_2\text{NC}_6\text{H}_4 \)].\(^{260, 265}\) Apparently, the \( \beta \)-diketiminate copper(I) fragment \([\beta \text{-diketiminate}] \text{Cu}^1 \) promotes stronger \( \pi \)-backbonding than the TpCu\(^1 \) fragment, perhaps due to a more directional interaction with the d-orbital directly influenced by the \( \beta \)-diketiminate \( N, N \)-donors.

3.2.2.b. Infrared spectroscopy of \([\text{Cu}](\eta^2 \text{-ONPh})\) complexes 9 - 12

IR spectroscopy may be used to determine the extent of backbonding to the N=O \( \pi^* \) orbital of the nitrosoarene that results in a weakening of the N-O interaction. A comparison of the IR spectra of \([\text{Me}_2\text{NN}] \text{Cu}(\eta^2 \text{-ONPh}) \) (10) and \([\text{Me}_2\text{NN}_{\text{F}_6}] \text{Cu}(\eta^2 \text{-ONPh}) \) (11) suggests that weaker donation by the more electron deficient \( \beta \)-diketiminate ligand in 11 affects the nitrosoarene N-O interaction. The weaker \( \pi \)-backbonding ability of the electron-poor \([\text{Me}_2\text{NN}_{\text{F}_6}] \text{Cu} \) fragment in 11 results in a higher \( \nu_{\text{NO}} \) stretching frequency of 1122 cm\(^{-1} \) (\( \nu_{15\text{NO}} = 1103 \text{ cm}^{-1} \)) relative to 1113 cm\(^{-1} \) (\( \nu_{15\text{NO}} = 1093 \text{ cm}^{-1} \)) found in the more electron rich 10.
3.2.2.c. NMR studies of [Cu](η⁶-ONPh)

Incongruous to the crystal structure which possesses four distinct N-aryl o-Me groups, the NMR spectra of each of these compounds at room temperature reveal completely symmetrized N-aryl substituents from the β-diketiminate ligands. The NMR spectrum of [Me₂NNF₆]Cu(η²-ONPh) (11) displays one N-aryl methyl peak (δ = 2.13 ppm) (Figure 3.8). Lowering the temperature, however, results in a spitting of this single resonance into four separate peaks (Figure 3.8). The NMR spectrum of 11 in toluene-ᴅ₈ at -80 °C possesses four N-aryl o-Me groups at δ = 2.45, 2.44, 2.34 and 1.21 ppm (Figure 3.8). At -60 °C the four peaks coalesce into two, suggesting a facile C₂ᵥ-like symmetrization via rotation of the ONPh moiety about the Cu-ON centroid with a modest activation barrier of ∆G‡ = 10.4(2) kcal/mol (Scheme 3.6(a)). Possibly, weaker π-backbonding and longer Cu-O and Cu-N bond lengths in 11 allow for less hindered rotation of the ONPh moiety.
about the Cu-ON centroid than in 10 ($\Delta G^\ddagger = 11.4(2)$ kcal/mol at -44(2) °C). The barrier to rotation of the ONPh moiety about the Cu-ON centroid in 11 is similar to that found for rotation of the alkene moiety in [Me$_2$NN]Cu($\eta^2$-styrene) ($\Delta G^\ddagger = 10.7(3)$ kcal/mol at -58 °C).$^{224}$ Careful inspection reveals, however, that only two sets of $\beta$-diketiminate N-aryl-Me groups would coalesce under this scenario. The easiest method by which all four methyl groups can become equivalent is via isomerization of the binding mode from $\eta^2$-ON to $\kappa^1$-N, enabling free and rapid rotation about the Cu-N bond which gives effective C$_{2v}$ symmetry (Scheme 3.6(b)). DFT studies (Section 3.2.2.f.) suggest that there is only a small energetic difference between the $\eta^2$-ONAr to $\kappa^1$-N binding modes, consistent with the effective C$_{2v}$ symmetry seen in NMR spectra of 11 at room temperature.

Figure 3.8. Variable Temperature $^1$H NMR (400 MHz, toluene-$d_8$) spectra of [Me$_2$NN]$\text{Fe}_6$Cu($\eta^2$-ONPh) (11). * denotes Ar-Me peaks which undergo symmetrization.
3.2.2.d. Cyclic voltammetry of [Me$_2$NN]Cu(η$^2$-ONPh) (10)

The interaction between the aryl nitroso ligand and the β-diketiminato copper(I) fragment is strong enough to drastically affect the oxidation potential of the copper center. Cyclic voltammetry of [Me$_2$NN]Cu(η$^2$-ONPh) (10) in NCMe revealed an irreversible oxidation wave at ca. +1.1 V vs NHE, referenced against an internal ferrocene standard (Figure 3.9). This value is staggering when compared to the Cu$^1$ complex [Me$_2$NN]Cu(NCMe) whose oxidation potential is +0.45 V vs NHE, and yet, if the oxidation state of [Cu](η$^2$-ONPh) could be considered as +3, maybe the observed redox value is less extraordinary.$^{219}$

3.2.2.e. X-ray absorption spectroscopy and comparisons of [Cu](η$^2$-ONPh) compounds

As mentioned in Section 3.1.2.c., the determination of the Cu oxidation state in [Cu](L$_2$) compounds, where the 2p-orbitals of the L$_2$ π-backbonding ligand interact with copper d orbitals, can be quite challenging. Traditional indicators of the extent of activation of the 2p orbitals and subsequent copper oxidation states have been bond lengths and vibrational frequencies, but
changes in the coordination mode ($\eta^2$ or $\kappa^1$), ligand field effects, and diamagnetism complicate matters.$^2$ For example, the O-N bond lengths in 9-12 all fall between the classic O-N single (1.47 Å) and double bonds (1.20 Å), suggesting an approximate bond order of 1.5 (Table 3.1). This may suggest the ONPh ligand is formally a radical anion (PhON$^-$), producing a [Cu$^{II}$]-d$^9$ species, but all of the [Cu]( $\eta^2$-ONPh) species are diamagnetic (Figure 3.x). The O-N vibrational frequency of

$$\text{Table 3.1. Bond lengths and } v_{\text{NO}} \text{ of } [\text{Cu}](\eta^2-\text{ONPh}) \text{ compounds.}$$

<table>
<thead>
<tr>
<th></th>
<th>O-N bond length (Å)</th>
<th>$v_{\text{NO}}$ (cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PhNO</td>
<td>1.223</td>
<td>1506</td>
</tr>
<tr>
<td>9</td>
<td>1.354(3)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1.334(5), (1.338(5))</td>
<td>1113</td>
</tr>
<tr>
<td>11</td>
<td>1.330(4)</td>
<td>1122</td>
</tr>
<tr>
<td>12</td>
<td>1.373</td>
<td></td>
</tr>
</tbody>
</table>

Figure 3.10. Possible ONPh $\eta^2$-bonding modes and corresponding copper d-electron count.

10 and 11 are more akin to the single bond stretching frequency in N-phenylhydroxylamine (PhHNOH) which suggests a O-N bond order of 1. In such a case, the copper center would be assigned as d$^8$ [Cu$^{III}$] (Figure 3.10). While this classification may align with observed diamagnetism, Cu$^{III}$ species are quite rare and often require careful substantiation. As a case in point, [iPr$_2$NN]Cu($\eta^2$-O$_2$) (15) published by Tolman and co-workers was originally classified as a copper(III) side-on peroxo species [Cu$^{III}$]($\eta^2$-O$_2^-$). This report spurred several computational
studies by different groups which ultimately lead to its re-classification as an antiferromagnetically coupled copper(II) superoxo complex \([\text{Cu}^{II}](\eta^2\text{O}_2^-)\).\textsuperscript{2,219,225}

In collaboration with our research group, an array of \([\text{Cu}](\eta^2)\) complexes were investigated via XAS and computational studies by Tomson et al.\textsuperscript{2} Of particular importance here is the family of isoelectronic of ligands (\(\text{O}_2\), ONPh, \(\text{Ar}^\text{F}N=N\text{Ar}^\text{F}\)) bound to the copper \(\beta\)-diketiminate framework \([\text{Me}_2\text{NN}]\text{Cu}\) that includes \textbf{10}, \textbf{15}, and \([\text{Me}_2\text{NN}]\text{Cu}(\eta^2\text{Ar}^\text{F}N=N\text{Ar}^\text{F})\) (\textbf{16}) (\(\text{Ar}^\text{F} = 3,5\)-bis(trifluoromethyl)phenyl) (Section 3.2.2.f. and Figure 3.12). Each of these compounds exhibit square planar geometry about the copper atom and have modest variations in the Cu-N\(\beta\)-dik lengths. The corresponding O-N, O-O, and N-N bond lengths in \textbf{10}, \textbf{15}, and \textbf{16} all lie in between that of their corresponding single and double bonds which suggests the ligands to have radical anion character and be bound to Cu\textsuperscript{II} (Figure 3.12).

X-ray absorption studies (XAS) is a spectroscopic technique with exquisite atom specificity that provides a vast amount of structural information including physical oxidation states of metal centers. K-edge XAS involves the excitation of a 1s electron and follows its transition through empty or singly occupied valence levels in its pre-edge and rising edge absorptions. The pre-edge feature in XAS details the 1s → 3d transition, observed for Cu\textsuperscript{II} and Cu\textsuperscript{III} compounds as a low-energy, low intensity feature at \(\sim 8979\text{ eV} (+0.4/-0.6\text{ eV})\), but not seen for d\textsuperscript{10} Cu\textsuperscript{I} compounds due to the completely filled d-shell. The pre-edge features for Cu\textsuperscript{III} complexes are shifted \(\sim 2\text{ eV}\) higher to \(\sim 8981 \pm 0.5\text{ eV}\).

XANES spectra of \textbf{10}, \textbf{15}, and \textbf{16} were collected by Dr. Steven Sproules in collaboration with Prof. Serena DeBeer and Prof. Karl Wieghardt.\textsuperscript{[Tomson, 2015 #1161]} XANES spectra along with an expanded view of the pre-edge region of \textbf{10} (red), \textbf{15} (black), and \textbf{16} (blue) (Figure 3.11) show that \textbf{10} and \textbf{16} are quite similar in their near and rising edge, while the edge transition of \textbf{15} is at a
much higher energy, even more than that of a classic square planar Cu\textsuperscript{II} complex (Figure 3.11). The pre-edge transitions for 15, 10, and 16 appear at 8980.6 eV, 8980.7 eV and 8980.9 eV, respectively. These values, when compared to a classic square planar Cu\textsuperscript{II} species (~ 8979.5 eV), have been proposed as an indication of \([\text{Cu}^{\text{III}}]\) species. The presence of any pre-edge features that originates from 1s → 3d transitions eliminates the possibility of classifying these species as \(d^{10}\) \([\text{Cu}^{\text{I}}]\)(\(\eta^2\)) compounds.

The rising edge feature, indicative of a 1s → 4s transition, lies at 8985.5 eV and 8985.3 eV for 10 and 16, respectively. While both the rising edge feature for 10 and 16 are lower in energy than that of 15, which appears at 8986.4 eV, the rising edge feature for all three compounds is nearer
that expressed for a classic square planar Cu\textsuperscript{II} compound (8985.4 eV) rather than Cu\textsuperscript{III} (8980.2 eV(calculated)).

![Diagram showing the LUMO for Cu(η\textsuperscript{2}-ONPh) compounds]

**Figure 3.12.** Graphical representations of DFT-derived LUMOs for 10, 15, and 16 and experimental O-O, O-N, and N-N bond lengths of compounds [Cu](η\textsuperscript{2}-L\textsubscript{2}) (N-aryl substituents have been graphically truncated for clarity.) Ar\textsuperscript{F} = 3,5-bis(trisfluoromethyl)phenyldiazene.

3.2.2.f. *DFT and computational studies of [Cu](η\textsuperscript{2}-ONPh) compounds*

DFT studies of the extent of orbital mixing from η\textsuperscript{2} bound ligands to copper β-diketiminate frameworks were also investigated by Tomson *et al.* A comparison of the corresponding LUMOs of 15, 10, and 16 reveal contributions from copper d\textsubscript{xz}, respective ligand π*, and σ-donation from the β-diketiminate ligand (Figure 3.12). As the identities of ligating atoms change, generally decreasing in electronegativity (O=O → O=NPh → Ar\textsuperscript{F}=N=NAr\textsuperscript{F}), we find the metal character of the LUMO decreases. These studies were also performed to better understand the origin of
different features of the XAS spectra through predictions of specific 1s → valence states that result from the DFT calculations on 10, 15, and 16.2

Additional DFT studies performed at Georgetown (neglecting zero point energies and entropic contributions) reveal that the η2-ON and κ1-N for [Me2NNF6]Cu(η2-ONPh) (11) are very close in energy and the κ1-N binding mode is favored only by 4.8 kcal/mol (Table 3.2). Our findings mirror those of Cundari and coworkers who reported that the η2-ON and κ1-N copper nitrosobenzene structures were nearly degenerate using an unsubstituted β-diketiminate copper fragment [H5C3N2]Cu that has a slight preference for the η2-ON form (Table 3.2).266 Despite the prevalence of the κ1-N binding mode, the atypical η2-ON binding mode is likely due to the relatively strong π-backbonding interaction and the copper β-diketiminate fragment due to the destabilization of the d orbital that interacts with the β-diketiminate N-donors.

DFT calculations suggest differences in the [Cu](ONPh) electronic structure based on the particular binding mode. For instance, calculations predict NO distances of 1.253 Å and 1.330 Å in 11-κ1 and 11-η2, respectively. The LUMOs of 11-κ1 and 11-η2, expected to have appreciable mixing with the ligand orbitals,2 reflect the stronger π-backbonding interaction and are primarily

Table 3.2. Calculated relative energies (kcal/mol) of κ1-N and η2-ON bound copper complexes.
composed of the ON \( \pi^* \) orbital interacting with the Cu d-orbital most destabilized by the \( \sigma \)-donation from the \( \beta \)-diketiminato N-atoms (Figure 3.13). The LUMO energy is higher by 0.34 eV in \( \text{11-} \eta^2 \) (-3.63 eV) relative to \( 2-\kappa^1 \) (-3.97 eV), which reflects stronger \( \pi \)-backbonding to the \( \eta^2 \)-NO bound nitrosobenzene ligand, further evidenced by the calculated O-N bond distances.

### 3.2.3. Reactivity studies: additional routes to \([\text{Cu}(\eta^2-\text{ONPh})]\) and NO reactivity

#### 3.2.3.a. Reactivity of \([\text{Cu}(\eta^2-\text{ONPh})]\) with ArNO

Investigations of binding studies with ONPh and ONAr (Ar = 3,5-dimethylphenyl) were conducted to determine which nitroso compound binds best to \([\text{Me}_2\text{NN}]\text{Cu}\) (Scheme 3.7). The addition of 1 equiv. ONPh to \([\text{Me}_2\text{NN}]\text{Cu(}\eta^2-\text{ONAr})\) (2) yielded both \([\text{Me}_2\text{NN}]\text{Cu(}\eta^2-\text{ONAr})\) (2) and \([\text{Me}_2\text{NN}]\text{Cu(}\eta^2-\text{ONPh})\) (10) in a 1:1.4 ratio, respectively (Scheme 3.7; Figure 3.14). This result suggests that there is a very slight preference for organonitroso compounds ArNO that possess less
electron-donating C-substituents. Moreover, exchange of nitroso compounds at β-diketiminate copper(I) fragments \([\text{Cu}^I]\) appears quite facile.

Scheme 3.7. Equilibrium between 2 (s, 1H, backbone: 4.772) and 10 (s, 1H, backbone 4.762).

Figure 3.14. \(^1\)H-NMR (benzene-\(d_6\)) of equilibrium formed between \([\text{Me}_2\text{NN}]\text{Cu(\(\eta^2\)-ONAr)} (s, 1H, backbone:4.772) and \([\text{Me}_2\text{NN}]\text{Cu(\(\eta^2\)-ONPh)} (s, 1H, backbone: \(\delta\) 4.762 ppm) depicted above (inset).
3.2.3.b. Disproportionation of PhHNOH by [Cu$^{I}$]

Our initial investigation into the formation of β-diketiminato [Cu](η$_2$-ONPh) complexes from N-phenylhydroxylamine began with the addition of PhHNOH to [Me$_2$NN]Cu(NCMe) (6). This reaction led to several products including [Me$_2$NN]Cu(η$_2$-ONPh) (10), aniline (PhNH$_2$), water, and a significant amount of the free β-diketimine ligand [Me$_2$NN]H (Scheme 3.9; Figure 3.15).

![Scheme 3.8](image)

**Scheme 3.8.** The protonation and release of copper ions of the β-diketiminate ligand.

While we were excited to see the ability of the [Cu$^{I}$] complex to disproportionate PhHNOH to bound PhNO along with free PhNH$_2$ and H$_2$O, a significant amount of protonation of the β-diketiminate supporting ligand occurred. This result is most likely due to the basicity of the β-diketiminate backbone in the [Me$_2$NN] ligand. There can be considerable electron density on the backbone of the β-diketiminate ligand, demonstrated by resonance, which causes the backbone to be especially susceptible to attack by electrophiles, especially acidic substrates (Scheme 3.8).$^{229}$
Scheme 3.9. The addition of PhHNOH to [Me₂NN]Cu(NCMe) (6) to produce free ligand, the desired product 10, aniline, and water.

Figure 3.15. $^1$H NMR (400 MHz, 298K, benzene-$d_6$) of reaction of PhHNOH and [Me₂NN]Cu(NCMe) (6) which exhibits backbone-$H$ of free ligand ([Me₂NN]H) (δ 4.825), 6 (δ 4.785) and 10 (δ 4.776).
Seeking to limit reactivity at the β-diketiminato backbone by influence of strongly electron-withdrawing backbone CF$_3$ groups, we examined the reaction of PhNOH with the electron-poor [Me$_2$NN$_6$F$_6$]Cu(NCMe) (7). Addition of 2 equiv. PhHNOH to [Me$_2$NN$_6$F$_6$]Cu(NCMe) (7) in benzene-$d_6$ leads to the clean formation of [Me$_2$NN$_6$F$_6$]Cu(η$^2$-ONPh) (11) in 93% yield by $^1$H NMR, as well as H$_2$O and H$_2$NPh (Scheme 3.10; Figure 3.16). These observations are consistent with the disproportionation of PhNHOH at [Me$_2$NN$_6$F$_6$]Cu.

**Scheme 3.10.** Disproportionation of N-phenylhydroxylamine by [Cu$^+$].
Figure 3.16. $^1$H NMR (400 MHz, 298K, benzene-$d_6$) of reaction of [Me$_2$NN$_6$F$_6$]Cu(NCMe) (7) and 2 equiv. PhHNOH resulting in [Me$_2$NN$_6$F$_6$]Cu($\eta^2$-ONPh) (11) (peaks designated by (*)), H$_2$NPh (peaks designated by (≠)), H$_2$O(☉), and unbound NCMe (♭) with 1,2,4,5-tetrachlorobenzene (#) as an internal standard.
3.2.3.c. Reaction of PhNHOH with \([[\text{Cu}]_2(\mu-\text{OH})_2\]

The addition of 1 equiv. PhHNOH to \([[\text{Me}_2\text{NN}]\text{Cu}]_2(\mu-\text{OH})_2\) (13) also results in the formation of \([\text{Me}_2\text{NN}]\text{Cu}(\eta^2-\text{ONPh})\) (10) (Scheme 3.11). Indeed, the $^1$H NMR spectrum of a preliminary experiment involving these two species in benzene-$d_6$ exhibits the backbone-$H$ and $N$-aryl methyl peaks corresponding to 10. The ability of PhNHOH to react in an acid-base manner with a \([\text{Cu}^{II}]\)-OH moiety informs our proposed mechanism by which PhHNOH is dismutated by \([\text{Cu}^I]\) further explored in Section 3.2.3.e.

Scheme 3.11. Formation of \([\text{Me}_2\text{NN}]\text{Cu}(\eta^2-\text{ONPh})\) via addition of PhHNOH to \([[\text{Me}_2\text{NN}]\text{Cu}]_2(\mu-\text{OH})_2\).
3.2.3.d. Reactions of \([\text{Cu}\,(\eta^2-\text{ONAr})]\) with hydroxylamine

In addition to observing the exchange of C-nitroso compounds at copper resulting in an equilibrium of the copper C-nitroso adducts, we find that equilibrium can be readily established from the interaction between the copper C-nitroso compounds and \(N\)-phenylhydroxylamine (Scheme 3.12; Figure 3.17). We propose the potential mechanism by which this exchange can take place goes through a bis(nitroxide) intermediate \([\text{Cu}^{III}](\text{ONAr})(\text{ONPh})\).
**Scheme 3.12.** Addition of N-phenylhydroxylamine to 2 (s, 1H, backbone: 4.772) resulting in the formation of 10 (s, 1H, backbone: 4.762).

**Figure 3.17.** $^1$H-NMR spectra in benzene-$_d_6$ of equilibrium established between [Me$_2$NN]Cu($\eta^2$-ONAr) (s, 1H, backbone: 4.772) and N-phenylhydroxylamine resulting in the formation of [Me$_2$NN]Cu($\eta^2$-ONPh) (s, 1H, backbone: 4.762) depicted above, inset.
3.2.3.e. *Mechanism of disproportionation of N-phenylhydroxylamine at [CuI]*

UV-vis studies were employed to investigate the formation of 10 via the reaction of 7 with N-phenylhydroxylamine (Figure 3.18). Analysis of preliminary kinetic data taken at room temperature suggest that the reaction is first order with respect to 7. Unfortunately, more in depth kinetic studies involving excess PhHNOH at low temperatures were inconclusive, and we suspect the impediment to our studies lies in the fact that the product, 10, is also able to catalyze the reaction. At some stage, however, the N-O bond of PhHNOH bond must be cleaved.

![UV-Vis spectrum of disproportionation of hydroxylamine from [Me₂NNF₆]Cu(NCMe) to [Me₂NNF₆]Cu(η²-ONPh) (λₘₐₓ = 588) after 5 h in THF at rt.](image)

**Figure 3.18.** UV-Vis spectrum of disproportionation of hydroxylamine from [Me₂NNF₆]Cu(NCMe) to [Me₂NNF₆]Cu(η²-ONPh) (λₘₐₓ = 588) after 5 h in THF at rt.

Perhaps the PhHN-OH bond is unimolecularly cleaved as preliminary data suggests, to give [CuII]-OH and ’NHPH species in a similar manner to the reaction of ’BuOO’Bu at the related copper (I) β-diketiminate [Cl₂NN]Cu, which results in direct formation of the [Cl₂NN]Cu-O’Bu and ’OtBu species.228 It is possible that [CuII]-OH and [CuII]-NPH species (the latter being a likely product of the radical capture of ’NPH by [CuI]) could react with another equiv. HONHPh to create [Cu](η²-ONPh) and [Cu], H₂O, and aniline (Figure 3.19). A proposed stepwise mechanism
for this transformation is shown in (Scheme 13). Reaction of PhHNOH with \([\{\text{Me}_2\text{NNF}_6\text{Cu}\}_2(\mu-\text{OH})_2]\) to give \([\text{Me}_2\text{NNF}_6\text{Cu}(\eta^2-\text{ONPh})](11)\) to 13 as is suggested in step 4.

Scheme 3.13. Proposed mechanism of disproportionation of N-phenylhydroxylamine.
Figure 3.19. $^1$H-NMR spectra in benzene-$d_6$ of disproportionation of 2 equiv. $N$-phenylhydroxylamine by $[\text{Me}_2\text{NNF}_6]\text{CuNCMe}$ (7) and resulting in the formation of $[\text{Me}_2\text{NNF}_6]\text{Cu}(\eta^2\text{-ONPh})$ (11) (s, 1H, backbone: 4.762) and aniline.
3.2.3.3 \textit{Reactions of copper C-nitroso compounds with nitric oxide}

[Me$_2$NN$_{F6}$]Cu(η$^2$-ONPh) (11), like [Me$_2$NN]Cu(η$^2$-ONPh) (10), reacts with a slight excess of NO in pentane to form the corresponding diazeniumdiolate (NONOate), [Me$_2$NN$_{F6}$]Cu(κ$^2$-O$_2$N$_2$Ph) (17) which can be isolated as red brown crystals in 72% yield (Scheme 3.14). X-ray analysis shows the κ$^2$ coordination of the NONOate with square planar conformation about the copper center that is very much like that of its more electron-rich counterpart [Me$_2$NN]Cu(κ$^2$-O$_2$N$_2$Ph) (18). [Me$_2$NN$_{F6}$]Cu(κ$^2$-O$_2$N$_2$Ph) (17) possesses Cu–O1 and Cu–O2 distances of 1.9544(10) and 1.9666(11) Å, respectively, with a O1–Cu–O2 angle of 79.55(4)$^\circ$.\cite{125} A weak absorption band is observed by UV-Vis at $\lambda_{\text{max}} = 609$ nm ($\varepsilon = 130$ M$^{-1}$ cm$^{-1}$) and a relatively intense shoulder at $\lambda_{\text{max}} = 455$ nm ($\varepsilon = 910$ M$^{-1}$ cm$^{-1}$). In benzene solution at room temperature the square planar copper(II) compound yields the typical EPR spectrum whose isotropic simulation centered about $g_{\text{iso}} = 2.094$ shows hyperfine coupling to $^{63/65}$Cu ($A_{\text{iso}}^{63/65}$Cu = 228 MHz) and two β-diketiminate N donors ($A_{\text{iso}}^{14}$N = 38 MHz) (Figure 3.20). These EPR parameters are similar to those found for [Me$_2$NN]Cu(κ$^2$-O$_2$N$_2$Ar) (17) [$g_{\text{iso}} = 2.10$; $A_{\text{iso}}^{63/65}$Cu = 230 MHz $A_{\text{iso}}^{14}$N = 38 MHz].
Figure 3.20. Isotropic X-band EPR spectrum and simulation for [Me₂NN₆₆]Cu(κ²-O₂N₂Ph) (16) (benzene-d₆, RT): 8.906468 GHz, ModWidth = 0.12 mT, Power = 1.0 mW, time constant = 0.1 s, Simulation (Gaussian lineshape with 10.0 G linebroadening) gives $g_{\text{iso}} = 2.094$, $A^{(63}\text{Cu)} = 228$ MHz, $A^{(14}\text{N)} = 35$ MHz for 2 N atoms. Quadratic ($c_2 = 6$ G/MHz) and linear ($\epsilon = 0.01$ G²/MHz) strain were employed to reproduce the copper nuclear quantum number dependence of the linewidth.
3.3. Conclusion

We have synthesized a family of copper C-nitroso compounds by a number of different methods that provide insight into how C-nitroso compounds RNO as well as the corresponding reduced hydroxylamines RNHOH react with copper(I) centers. Addition of PhNO to β-diketiminate copper(I) complexes [CuI], disproportionation of PhNOH at [CuI], as well as reaction of PhNOH with {[Cu]2}(µ-OH)2 all result in b-diketiminato copper complexes [Cu](η2-ONPh). All of the compounds in this family possess distinct η2-ON coordination of the nitrosobenzene ligand irrespective of steric and electronic environment provided by the β-diketiminate supporting ligand. Nonetheless, DFT studies of the suggest the κ1-N and experimentally observed η2-ON binding modes are close in energy for [Me2NNF6]Cu(η2-ONPh), which results in a facile pathway for the symmetrization observed in the variable temperature NMR spectra.

Computational data and analysis of the XAS Cu K-edge transitions result in ambiguous classification of the oxidation state of Cu in [Me2NN]Cu(η2-ONPh). The high pre-edge transition energy of in [Me2NN]Cu(η2-ONPh) suggests a CuIII complex, while the edge transition energy is very near that of a classic square planar CuII complex. DFT studies show LUMO to be predominated by the ligand π* orbitals and subsequent increasing electronegativity of the copper bound ligand atoms play a role in increasing the oxidation state of the copper atom.

Swift capture of NO by [Me2NNF6]Cu(η2-ONPh) to give the corresponding copper(II) diazeniumdiolate [Me2NNF6]Cu(κ2-O2N2Ph) (17) occurs, similar to the reaction previously observed with the more electron-rich [Me2NN]Cu(η2-ONPh) (10) that leads to [Me2NN]Cu(κ2-O2N2Ph).125 Related capture of NO by metal nitrosoarene adducts has been observed with both more sterically encumbered [Pr2Tp]Cu(κ1-ONPh) and dinickel {[Me2NN]Ni}(µ-η2:η2-ONAr) to produce [Pr2Tp]Cu(κ2-O2N2Ph) and {[Me2NN]Ni}(κ2-O2N2Ph).125, 236
Considering the similarities between PhNO and HNO (as well as PhHNOH and H₂NOH), the reactivity pathways discussed in this Chapter that describe the formation of [Cu](η²-ONPh) complexes along with their reactivity with NO to give copper(II) diazeniumdiolates may foreshadow discrete reactivity patterns that the more fleeting, simplest reduced nitroso compound, HNO, may exhibit at copper centers.
3.4. Experimental Procedures

3.4.1. General experimental procedures

All experiments were carried out in a dry nitrogen atmosphere using an MBraun glovebox and/or standard Schlenk techniques. 4A molecular sieves were activated in vacuo at 180 °C for 24 h. Dry benzene, THF, and CH₂Cl₂ were purchased from Aldrich and stored over activated 4A molecular sieves. Diethyl ether was first sparged with nitrogen and then dried by passage through activated alumina columns. Pentane was first washed with conc. HNO₃ / H₂SO₄ to remove olefins, stored over CaCl₂ and then distilled before use from sodium/benzophenone. All deuterated solvents were sparged with nitrogen, dried over activated 4A molecular sieves and stored under nitrogen. Celite was dried overnight at 200 °C under vacuum.

¹H and ¹³C NMR spectra were recorded on a 400 MHz Inova Spectrometer (400 and 100.47 MHz respectively). All NMR spectra were recorded at a room temperature unless otherwise noted and were indirectly referenced to residual solvent signals or TMS as internal standards. UV-Via spectra were measured on a Varian Cary 50 or 100 spectrometer, using cuvettes with screw-cap tops. GC-MS spectra were recorded on a Varian Saturn 3900 and ESI-MS analyses was done using Varian 500 Ion Trap Trap LC/MS using positive mode.

All reagents were obtained commercially unless otherwise noted and typically stored over activated 4A molecular sieves. H[Me₂NNF₆]²⁶⁷ and [Me₂NNF₆]Cu(NCMe)²¹⁹ were prepared using literature methods. ¹⁵N-labeled PhNO was prepared by oxidation Ph¹⁵NH₂.¹²⁵
3.4.2. Preparation of [Cu] compounds and corresponding spectra

[Me₂NN₆]Cu(η²-ONPh) (11). A light green solution of nitrosobenzene (0.050 g, 0.467 mmol) in diethyl ether (3 mL) was added to a red solution of [Me₂NN₆]Cu(NCMe) (0.250 g, 0.467 mmol) also in diethyl ether (7 mL). Immediately upon addition, a color change to dark purple was observed and the solution was allowed to stir for 30 min. The resulting solution was passed through Celite, concentrated, and cooled to -35°C to afford 0.130 g (46% yield). UV-Vis (-80 °C, pentane): \( \lambda_{\text{max}} = 594 \text{ nm} \) (1880 M⁻¹cm⁻¹). \(^1\)H NMR (400 MHz benzene-d\(_6\)) \( \delta \) 6.91 (m, 9H), 6.65 (t, 2H), 6.09 (s, 1H-backbone), 2.13 (s, 12H); \(^1\)H NMR (400 MHz benzene-d\(_6\)) \( \delta \) 161.1, 145.7, 131.4, 131.3, 129.6, 128.2, 127.9, 126.0, 122.2, 121.2, 119.4, 18.6. ESI-MS (m/z) Calculated base peak for C\(_{27}\)F\(_6\)H\(_{24}\)CuN\(_3\)O\(_1\): 583.1[M]⁺ Found: 583.4 [M]⁺

[Me₂NN₆]Cu(η²-O\(^{15}\)NPh). Was made in accordance with the aforementioned procedure using Ph\(^{15}\)NO producing a \(^1\)H NMR identical to that of [Me₂NN₆]Cu(η²-ONPh).
Figure 3.21. $^1$H NMR (400 MHz, 298K, C$_6$D$_6$) of [Me$_2$NN$_6$F$_6$]Cu($\eta^2$-ONPh).

Figure 3.22. Beer’s Law plot for [Me$_2$NN$_6$F$_6$]Cu(NCMe) (7) at $\lambda_{max} = 594$ nm ($\varepsilon = 1880$ M$^{-1}$ cm$^{-1}$) in THF at 193 K.
Figure 3.23. Solid State IR (nujol mull) of [Me$_2$NN$_6$]Cu($\eta^2$-ONPh). Calculated $\nu^{15}$NO = 1102 cm$^{-1}$ for $\nu^{14}$NO = 1122 cm$^{-1}$.
[Cl₂NN]Cu(η²-ONPh) (9). Was made in accordance with the aforementioned procedure similar to 11. ¹H NMR (400 MHz benzene-d₆) δ 7.04 (d, 4H), 6.83 (t, 2H), 4.84 (s, 1H-backbone), 1.56 (s, 6H).

[iPr₂NNF₆]Cu(η²-ONPh) (12). Was made in accordance with the aforementioned procedure similar to 11. ¹H NMR (400 MHz benzene-d₆) δ 7.08 (d, 4H), 6.96 (t, 2H), 6.12 (s, 1H-backbone), 3.252 (quintet, 4, iPr-H), 1.27 (d, 24H, iPr).

[Me₂NN]Cu(ArF=N=ArF) (16). A solution of ArF=N=ArF (0.110 g, 0.33 mmol) in 5 mL of toluene was added dropwise to a solution of [Me₂NN]Cu(ethylene) (0.130 g, 0.34 mmol) in 5 mL of toluene, the solution color changed from yellow to red-brown immediately. After stirred overnight, the volatiles were removed in vacuo and the residue was extracted with pentane (15 mL). The mixture was filtered through Celite, and the filtrate was concentrated and cooled to –35 °C to afford 0.180 g (78 %) red crystals, and crystals are suitable for X-ray diffraction. ¹H NMR (THF-d₈, RT, 400MHz): δ 7.96 (s, 2H, p-H-diazene), 7.48 (s, 4H, o-H-diazene), 7.12 (m (broad), 6H, Ar-H), 5.03 (s, 1H, H-backbone), 2.46 (s, 6H, Me-backbone), 1.53 (s, 12H, Me-Ar) ¹³C NMR (THF-d₈, RT): 165.21, 153.95, 148.44, 133.31, 132.56, 129.72, 126.07, 122.52, 97.72, 23.25 ¹⁹F NMR (THF-d₈, RT): δ -62.03. Anal. Calc’d for C₃⁷H₄₁N₄F₁₂Cu: C, 53.99; H, 3.80; N, 6.81. Found: C, 53.73; H, 3.77, N, 6.66.
Figure 3.24. Beer’s law plot of $[\text{Me}_2\text{NN}]\text{Cu}(\text{N}_2\text{Ar}^2)$, 16, in Et$_2$O at 25°C, monitored at 421 nm.
[Me₂NNF₆]Cu(κ²-O₂N₂Ph) (17). A dark green solution of [Me₂NNF₆]Cu(κ²-O₂N₂Ph) (100.0 mg, 0.171 mmol) in pentane (10 mL) was prepared in nitrogen atmosphere. Nitric oxide gas (4 eq, 4.35 mL at 298 K, 1 atm.) was bubbled through the solution resulting in color change to a red brown. The solution was filtered, concentrated and stored at -35°C to afford red-brown crystals, collected with a yield of 72% (78 mg). ESI-MS (m/z) Calculated for C₂₇F₆H₂₄CuN₄O₂: 613.1[M]⁺, 614.1 [M+1]⁺ Found: 613.8 [M]⁺ and 614.6 [M+1]⁺
Figure 3.25. Beer’s Law plots for $[\text{Me}_2\text{NNF}_6]\text{Cu}($κ²-O₂N₂Ph$)$ (7) in ether at 298 K: $\lambda_{\text{max}} = 369$ nm ($\varepsilon = 2.07 \times 10^4$ M⁻¹cm⁻¹), $\lambda_{\text{max}} = 455$ nm ($\varepsilon = 910$ M⁻¹cm⁻¹), $\lambda_{\text{max}} = 603$ nm ($\varepsilon = 130$ M⁻¹cm⁻¹), $\lambda_{\text{max}} = 766$ nm ($\varepsilon = 41$ M⁻¹cm⁻¹).
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<td><em>P2</em>_{1}/<em>n</em></td>
<td><em>P2</em>_{1}/<em>c</em></td>
<td><em>P2</em>_{1}/<em>c</em></td>
</tr>
<tr>
<td>a (Å)</td>
<td>11.2809(10)</td>
<td>12.935(5)</td>
<td>17.5607(18)</td>
</tr>
<tr>
<td>b (Å)</td>
<td>9.9062(9)</td>
<td>17.826(7)</td>
<td>11.7422(12)</td>
</tr>
<tr>
<td>c (Å)</td>
<td>21.2330(19)</td>
<td>11.412(5)</td>
<td>16.3392(17)</td>
</tr>
<tr>
<td>α (deg)</td>
<td>90</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>β (deg)</td>
<td>95.521(2)</td>
<td>104.162(5)</td>
<td>97.0058(14)</td>
</tr>
<tr>
<td>γ (deg)</td>
<td>90</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>Z</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>θ range (deg)</td>
<td>2.27 - 28.73</td>
<td>1.62 - 27.00</td>
<td>1.168 - 25.429</td>
</tr>
<tr>
<td>measd reflns</td>
<td>3843</td>
<td>21450</td>
<td>25504</td>
</tr>
<tr>
<td>unique reflns</td>
<td>3522</td>
<td>5563</td>
<td>6166</td>
</tr>
<tr>
<td>R(int)</td>
<td>0.0395</td>
<td>0.0690</td>
<td>0.0340</td>
</tr>
<tr>
<td>GOF of F^2</td>
<td>0.907</td>
<td>1.042</td>
<td>1.020</td>
</tr>
<tr>
<td>R1 (I &gt; 2σ(I))</td>
<td>0.0344</td>
<td>0.0555</td>
<td>0.0301</td>
</tr>
<tr>
<td>wR2 (all data)</td>
<td>0.0840</td>
<td>0.1519</td>
<td>0.0738</td>
</tr>
<tr>
<td>Largest diff. peak and hole e^-</td>
<td>0.558 and -0.527</td>
<td>1.110 and -0.412</td>
<td>0.381 and -0.328</td>
</tr>
</tbody>
</table>

**Table 3.3.** Crystallographic data tables for 9, 11, 12, and 16.
3.4.3. Stoichiometric addition of 2 equiv PhHNOH to [Me$_2$NN$_6$]Cu(NCMe). To a red solution of [Me$_2$NN$_6$]Cu(NCMe) (35.5 mg, 0.0676 mmol) and 1,2,4,5-tetrachlorobenzene, used as an internal standard, (15.1 mg, 0.0699 mmol), a solution of PhHNOH (14.7 mg, 0.135 mmol) in benzene-$d_6$ (500 μL). The resulting green solution was vigorously shaken and produced the $^1$H NMR spectrum shown in Figure 3.17.
3.4.4. DFT calculation details

The DFT calculations employed the Becke-Perdew exchange-correlation functional\textsuperscript{230-232} using the Amsterdam Density Functional suite of programs (ADF 2007.01).\textsuperscript{233-234, 268} Slater-type orbital (STO) basis sets employed for H, C, and N atoms were of triple-\(\xi\) quality augmented with two polarization functions (ZORA/TZ2P) while an improved triple-\(\xi\) basis set with two polarization functions (ZORA/TZ2P+) was employed for the Cu atom. Scalar relativistic effects were included by virtue of the zero order regular approximation (ZORA).\textsuperscript{269-271} The 1s electrons of B, C, N, O, and F as well as the 1s – 2p electrons of Cu were treated as frozen core. The VWN (Vosko, Wilk, and Nusair) functional was used for LDA (local density approximation).\textsuperscript{272} Default convergence (\(\Delta E = 1 \times 10^{-3}\) hartree, max. gradient = \(1 \times 10^{-2}\) hartree / Å, max. Cartesian step = \(1 \times 10^{-2}\) Å) and integration (4 significant digits) parameters were employed for geometry optimizations.

Experimental X-ray coordinates for [Me\(_2\)NN\(_6\)]Cu(\(\eta^2\)-ONPh) were used as the starting point for the geometry optimization of these species. The geometry of [Me\(_2\)NN\(_6\)]Cu(\(\kappa^1\)-N(O)Ph) was optimized after placing the nitrosobenzene ligand in a geometry that enables efficient Cu-N \(\pi\)-backbonding when coordinated to Cu solely by the N atom. ADFview\textsuperscript{268} was used to prepare the three-dimensional representations of the structures as well as to render the Kohn-Sham MOs.
**Figure 3.26.** DFT structures of [Me$_2$NN$_6$]Cu(κ$^1$-N(O)Ph) and [Me$_2$NN$_6$]Cu(η$^2$-ONPh) (ADF ZORA BP/TZ2P(+)). DFT optimized distances are also collected.

<table>
<thead>
<tr>
<th></th>
<th>[Me$_2$NN$_6$]Cu(κ$^1$-N(O)Ph)</th>
<th>0 kcal/mol</th>
<th>[Me$_2$NN$_6$]Cu(η$^2$-ONPh) + 4.8 kcal/mol</th>
</tr>
</thead>
<tbody>
<tr>
<td>O-N3</td>
<td>1.253 Å</td>
<td></td>
<td>O-N3</td>
</tr>
<tr>
<td>Cu-N3</td>
<td>1.860 Å</td>
<td></td>
<td>Cu-N3</td>
</tr>
<tr>
<td>Cu-N1</td>
<td>1.942 Å</td>
<td></td>
<td>Cu-N1</td>
</tr>
<tr>
<td>Cu-N2</td>
<td>1.942 Å</td>
<td></td>
<td>Cu-N2</td>
</tr>
</tbody>
</table>

**Table 3.4.** Selected calculated bond distances and relative electronic energies (neglecting entropic and zero-point effects).
CHAPTER 4

REVERSIBLE BINDING OF REDOX NON-INNOCENT NITROXIDES TO COPPER(I)

4.1. Introduction

TEMPO ((2,2,6,6-tetramethylpiperidin-1-yl)oxy), a stable nitroxide free radical, has been involved in a wide range of reactivity. Capable of abstracting hydrogen atoms from weak C-H bonds driven by the O-H bond strength (69.7 kcal/mol)\(^2\) of the diamagnetic hydroxylamine (TEMPO-H) (Figure 4.1), TEMPO can act as a metal free catalyst to effect the oxidation of alcohols.\(^2\) TEMPO is also used as a co-catalyst with copper to the same effect\(^3\) and with some key advantages.\(^2\) The Cu / TEMPO co-catalytic system can utilize a dioxygen, representing a greener oxidant, and has the potential to mitigate unwanted side reactions. An understanding of the mechanism by which TEMPO and copper salts accomplish alcohol oxidation can provide potential insight into enzymatic mechanisms such as that of tyrosinase, galactose oxidase,\(^2\) and laccase\(^3\) which is further explored in Section 4.1.2.a.

![Figure 4.1. Addition/loss of hydrogen atom to effect TEMPO-H and TEMPO.](image)

As a prototypical nitroxide, TEMPO can serve as a model\(^4\) for the parent ‘ONH\(_2\) involved in the oxidation of hydroxylamine to HNO that has been suspected at copper sites.\(^5\) Our evidence of the dismutation of \(N\)-substituted hydroxylamines, e.g. PhHNOH (Chapter 3) and Et\(_2\)N-OH (Chapter 5) at copper sites, also, leads us to suspect such transformations occur through a copper nitroxide intermediate [Cu\(^{II}\)](\(\eta^2\)-ONR\(_2\)). TEMPO can also act in analogy to phenoxy radicals •OAr. The aryloxy radical that attracts much attention is the tyrosyl radical found in galactose
oxidase (GaO) which is excreted extracellularly for some unknown biological function by several fungi species. GaO can oxidize alcohols to corresponding aldehydes and ketones, which is accomplished by cooperation of its Tyr radical and a proximate T2 copper active site.

The importance of such [CuII](η2-ONR2) species in biological and synthetic processes led us to further investigate the synthesis and characterization of copper nitroxide species.

4.1.1. Physical and chemical properties of TEMPO

TEMPO, discovered in 1962, is a stable nitroxide radical that is a red-orange solid at room temperature.282 Further stability is conferred to this particular nitroxide by the absence of α-H’s that can result in a disproportionation reaction via intermolecular hydrogen atom abstraction (HAA) to the corresponding hydroxylamine and nitrone compounds.127,282 TEMPO, like nitric oxide, can exist as either an cation (TEMPO+) (TEMPOnium), radical (TEMPO), or anion (TEMPO−) (Scheme 4.1). The O-N bond length and stretching frequency track with the bond order. TEMPO+ has an O-N bond order of 2, O-N bond length of 1.184(10) Å and stretching frequency of 1626 cm⁻¹.283 TEMPO and TEMPO− have O-N bond lengths of 1.284(8) Å and 1.459(2) Å, respectively and the O-N stretching frequency for TEMPO is measured at 1465 cm⁻¹ (Scheme 4.1).283-284 While the stretching frequencies of the anion have been measured284 and reported at 1339 cm⁻¹,285 DFT studies by Kubala et al. report the excitation of the O-N bond in TEMPO− as a mixture of several different vibrations: N-O, C-H (including overlapping σ* (C-C), σ* (C-H)), and N-pyramidalization vibrations.286
The redox couples of TEMPO$^{\pm}$ and TEMPO$^{-/-}$ have been studied in depth as TEMPO has been used as radical spin traps and as an oxidizing agent especially in regard to alcohol oxidation. The oxidation of TEMPO to TEMPO$^{+}$ is a relatively slow ($k_0 = 8.4 \times 10^{-1} \text{ cm s}^{-1}$)\textsuperscript{283}, but relatively easy process ($E_{1/2} = 0.76 \text{ V vs NHE}$)\textsuperscript{287-288} compared to the reduction of TEMPO to TEMPO$^{-}$ ($k_0 = \text{ cm s}^{-1}$; $E_{1/2} = -1.48 \text{ V vs NHE}$)\textsuperscript{289} (Scheme 4.1). The 6-membered ring conformation lessens the angle strain upon a change in hybridization at N from sp$^3$ to sp$^2$ associated with the conversion of TEMPO to TEMPO$^{+}$ which facilitates this oxidation.

**Scheme 4.1.** Disproportionation of TEMPO under acidic conditions with corresponding bond lengths, stretching frequencies, and redox potentials.

The use of TEMPO in several applications, including catalysis, synthesis, magnetic studies, and photochemistry, has produced a number of metal TEMPO complexes. The complexation of TEMPO to metals can impact reduction potentials, alter magnetic properties and affect the ground spin state which can enable detailed investigation of metal adducts via NMR (diamagnetic) or EPR (paramagnetic).\textsuperscript{44, 290-291} Similar to C-nitroso compounds, the observed binding conformations of TEMPO to metals have been either κ$^1$-$O$ or η$^2$-$ON$ each with varying degrees of activation of the
O-N bond. First row transition metal-TEMPO complexes exhibit both binding modes and varying degrees of activation of the O-N bond (Figure 4.2; Table 4.1).
Figure 4.2. First row transition metal-TEMPO complexes and selected bond distances.

Table 4.1. First row transition metal-TEMPO complexes and selected bond distances.

<table>
<thead>
<tr>
<th></th>
<th>Ligand</th>
<th>O-N</th>
<th>M-O</th>
<th>M-N</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(TEMPO)Mn(CO)₃</td>
<td>η²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>PhB(MesIm)₃Fe(TEMPO)</td>
<td>κ¹</td>
<td>1.449(2)</td>
<td>1.8710(1)</td>
</tr>
<tr>
<td>3</td>
<td>(TEMPO)Co(CO)₂</td>
<td>η²</td>
<td>1.379(5)</td>
<td>1.845(3)</td>
</tr>
<tr>
<td>4</td>
<td>Ni(η²-TEMPO)₂</td>
<td>η²</td>
<td>1.4136(12)</td>
<td>1.8404(11)</td>
</tr>
<tr>
<td>5</td>
<td>Ni(η²-TEMPO)(κ¹-TEMPO)(CN⁴Bu)</td>
<td>η²</td>
<td>1.3850(10)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Ni(η²-TEMPO)(κ¹-TEMPO)(CCPh)</td>
<td>η²</td>
<td>1.3869(10)</td>
<td>1.8868(7)</td>
</tr>
<tr>
<td>7</td>
<td>(dbpe)Ni(Cl)(O,N;η²-TEMPO)</td>
<td>η²</td>
<td>1.385(2)</td>
<td>1.839(2)</td>
</tr>
<tr>
<td>8</td>
<td>(PNP)Ni(TEMPO)</td>
<td>η²</td>
<td>1.4020(13)</td>
<td>1.8113(9)</td>
</tr>
<tr>
<td>9</td>
<td>Ni(SDmp)(PPh₃)(O,N;η²-TEMPO)</td>
<td>η²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>CuBr₂•TEMPO</td>
<td>η²</td>
<td>1.304(8)</td>
<td>1.860(5)</td>
</tr>
<tr>
<td>11</td>
<td>CuCl₂(TEMPO)</td>
<td>η²</td>
<td>1.276(2)</td>
<td>1.940(1)</td>
</tr>
<tr>
<td>12</td>
<td>4-Me₂Si-Ar*Zn(TEMPO)</td>
<td>η²</td>
<td>1.528*</td>
<td>1.838*</td>
</tr>
</tbody>
</table>
4.1.2.a. Copper-TEMPO models

Previously reported copper-TEMPO complexes were synthesized via the addition of TEMPO to copper (I) or copper(II) salt precursors and exhibit both \( \eta^2\)-ON and \( \kappa^1\)-O binding conformations (Figure 4.3).\textsuperscript{296, 299, 302} The TEMPO O-N bond distances for each of these structures (1.304(8) Å (10) and 1.276(2) Å (11)) is squarely within the range of the free TEMPO radical (Table 4.1). Investigation into the electronic structure of 10 revealed electronic exchange between copper and TEMPO suggests both Cu(I)(TEMPO\(^+\)) and Cu(II)(TEMPO) formalisms.\textsuperscript{299} Studies of 11 suggests the stability of the copper-TEMPO adducts are solvent dependent and the binding mode can have great impact on subsequent reactivity.\textsuperscript{296}

![Figure 4.3. Copper-TEMPO complexes.](image)

4.1.2.b. Reactivity of copper-TEMPO complexes

Solution studies of 11 ultimately led to the conclusion that the active species in alcohol oxidations was in fact the copper-TEMPO adduct rather than free TEMPO itself.\textsuperscript{296} The mechanism by which Cu(II) and TEMPO work to achieve catalytic oxidation reactions is not yet fully understood, but it is widely accepted that both copper(II) and TEMPO are reduced by 1 electron to achieve a 2-electron oxidation of the substrate (Scheme 4.2).\textsuperscript{1, 303-304}
GaO, an extracellular T2 copper enzyme found in fungus *Dactylium dendroides*, catalyzes the oxidation of primary alcohols to corresponding aldehydes using molecular oxygen and has inspired many to utilize copper-TEMPO complexes to study the mechanism for alcohol oxidation.\(^{278, 305}\)

Though not yet fully elucidated, an inner sphere process containing a Cu(II)(TEMPO) intermediate appears crucial (Scheme 4.2).\(^{1, 303, 306}\)
**4.2. Results and Discussion**

**4.2.1. New copper-TEMPO complexes**

We describe herein the formation of three new [Cu](TEMPO) complexes via the addition of TEMPO to copper(I) β-diketiminate complexes (Figure 4.4). Through solid state structures, solution behavior including UV-vis and EPR studies, we reveal the redox non-innocent behavior of TEMPO. The π-backbonding strength and oxidation potentials of the corresponding copper(I) β-diketiminate complexes have an important effect on the ultimate activation of the O-N TEMPO bond. Furthermore, the acid-base reactivity of TEMPO-H at copper(II) complexes such as [Cu$$^{II}$$]-O'Bu as prototypical hydroxylamine is also explored in this chapter.

**Scheme 4.2.** Proposed mechanism of galactose oxidase (a) and Cu/TEMPO alcohol oxidation (b).
4.2.2. Formation of $\text{[Cu}^{II}\text{]}(\eta^2\text{-TEMPO})$ compounds

In both pentane and diethyl ether, the addition of TEMPO to copper(I) $\beta$-diketimines $\text{[Cu}^1\text{]}$ results in the formation of copper(II)-TEMPO complexes $\text{[Cu}^{II}\text{]}(\eta^2\text{-TEMPO})$ (Scheme 4.3).

$$\begin{align*}
\text{[Me}_2\text{NN}]\text{Cu(}\eta^2\text{-TEMPO)} & \quad \text{13} \\
\text{[Cl}_2\text{NN}]\text{Cu(}\eta^2\text{-TEMPO)} & \quad \text{14} \\
\text{[Me}_2\text{NN}_F_6\text{]}\text{Cu(}\eta^2\text{-TEMPO)} & \quad \text{15}
\end{align*}$$

**Figure 4.4.** New copper-TEMPO complexes.

$$\begin{align*}
\text{L} = (\eta^2\text{-TEMPO}) \\
\text{O-N bond distance (Å)} \\
\text{[Me}_2\text{NN}]\text{CuL} & = 1.4104(15) \\
\text{[Cl}_2\text{NN}]\text{CuL} & = 1.4053(14) \\
\text{[Me}_2\text{NN}_F_6\text{]}\text{CuL} & = 1.3963(3)
\end{align*}$$

**Table 4.2.** O-N bond distances, reduction potentials, and wavenumbers showing extent of backbonding within $\beta$-diketiminate copper complexes.

$$\begin{align*}
\text{L} = (\text{NCMe}) \\
\text{E}_{1/2} \text{ vs NHE (mV)} \\
\text{[Me}_2\text{NN}]\text{CuL} & = +308 \\
\text{[Cl}_2\text{NN}]\text{CuL} & = +478 \\
\text{[Me}_2\text{NN}_F_6\text{]}\text{CuL} & = +659
\end{align*}$$

$$\begin{align*}
\text{L} = (\text{CNAr}) \\
\nu_{\text{CN}} \text{ (cm}^{-1}\text{)} \\
\text{[Me}_2\text{NN}]\text{CuL} & = 2123 \\
\text{[Cl}_2\text{NN}]\text{CuL} & = 2141 \\
\text{[Me}_2\text{NN}_F_6\text{]}\text{CuL} & = 2149
\end{align*}$$

4.2.2. *Formation of $\text{[Cu}^{II}\text{]}(\eta^2\text{-TEMPO})$ compounds*

In both pentane and diethyl ether, the addition of TEMPO to copper(I) $\beta$-diketimines $\text{[Cu}^1\text{]}$ results in the formation of copper(II)-TEMPO complexes $\text{[Cu}^{II}\text{]}(\eta^2\text{-TEMPO})$ (Scheme 4.3).

$$\begin{align*}
\text{[[Me}_2\text{NN}]\text{Cu}}_2 + \text{TEMPO} & \rightarrow \text{[Me}_2\text{NN}]\text{Cu(}\eta^2\text{-TEMPO)} \\
\text{[[Cl}_2\text{NN}]\text{Cu}}_2(\mu\text{-benzene}) + \text{TEMPO} & \rightarrow \text{[Cl}_2\text{NN}]\text{Cu(}\eta^2\text{-TEMPO)} \\
\text{[Me}_2\text{NN}_F_6\text{]}\text{H} + \text{Cu}^{+\text{-O}^\prime\text{Bu}} + \text{TEMPO} & \rightarrow \text{[Me}_2\text{NN}_F_6\text{]}\text{Cu(}\eta^2\text{-TEMPO)}
\end{align*}$$

**Scheme 4.3.** Synthesis of $\text{[Me}_2\text{NN}]\text{Cu(}\eta^2\text{-TEMPO)}$ (13), $\text{[Cl}_2\text{NN}]\text{Cu(}\eta^2\text{-TEMPO)}$ (14), and $\text{[Me}_2\text{NN}_F_6\text{]}\text{Cu(}\eta^2\text{-TEMPO)}$ (15).

Typically base-free, dimeric copper(I) complexes such as $\text{[[Me}_2\text{NN}]\text{Cu}_2$ or monomeric, Lewis
base adducts $[\text{Cu}^1](L)$ ($L = \text{NCMe}, \text{benzene}$) of the $[\text{Me}_2\text{NN}]\text{Cu}$, $[\text{Cl}_2\text{NN}]\text{Cu}$, and $[\text{Me}_2\text{NN}_\text{F}_6]\text{Cu}$ fragments were employed. Addition of TEMPO to these copper(I) precursors in pentane or ether results in dark red colored solutions which yield deep red colored crystals of $[\text{Me}_2\text{NN}]\text{Cu}(\eta^2-\text{TEMPO})$ (13), $[\text{Cl}_2\text{NN}]\text{Cu}(\eta^2-\text{TEMPO})$ (14), $[\text{Me}_2\text{NN}_\text{F}_6]\text{Cu}(\eta^2-\text{TEMPO})$ (15) in 59%, 65%, and 46%, respectively. The formation of 15 from $[\text{Me}_2\text{NN}_\text{F}_6]\text{Cu}(\text{NCMe})$ proved to be challenging and finally obtained via the addition of TEMPO to a slurry of in situ formed $[\text{Me}_2\text{NN}_\text{F}_6]\text{Cu}$ via the reaction of $[\text{Me}_2\text{NN}_\text{F}_6]\text{H}$ and CuO'Bu. We suspect the NCMe ligand binds too tightly to the electron-poor $[\text{Me}_2\text{NN}_\text{F}_6]\text{Cu}$ to be displaced by TEMPO.

4.2.3. Structural characterization and comparison of $[\text{Cu}](\text{TEMPO})$ compounds

4.2.3.a. X-ray diffraction studies and comparisons of $[\text{Cu}](\text{TEMPO})$ compounds

Despite electronic differences, each of these new $[\text{Cu}](\text{TEMPO})$ compounds exhibit $\eta^2$-ON binding giving a net square planar-like coordination at copper (Figures 4.5 - Figure 4.7). The Cu-O distances of [13: 1.9026(11) Å, 14: 1.8993(9) Å, 15: 1.9050(17) Å] are a bit shorter than the Cu-N3 distances [13: 2.0685(12) Å, 14: 2.0718(11) Å, 15: 2.075(2) Å] to the coordinated TEMPO ligand. The O-N bond lengths in 13, 14, and 15, as well as the coordination geometry about the copper suggest a full, one-electron reduction of the TEMPO ligand with concomitant formation of a Cu$^{II}$ center. In this small family of compounds, the O-N bond lengths are all relatively similar [13: 1.4104(15) Å; 14: 1.4053(14) Å; 15: 1.3963(3) Å] and are more closely matched with the O-N bond length of TEMPO anion rather than TEMPO radical. Moreover, this increase in reduction of the O-N bond of TEMPO is somewhat mirrored by shorter Cu-N$_{\beta-dik}$ distances [13: 1.9338(13) and 1.9379(13) Å, 14: 1.9404(11) and 1.9471(11) Å, 15: 1.9517(17) and 1.9617(19) Å] that also suggest enhanced Cu(II) character. The only other copper structure published with an $\eta^2$-ON confirmation is Subra’s Br$_2$Cu($\eta^2$-ON-TEMPO) which displays an O-N bond distance of 1.304(8)
Å suggesting the TEMPO ligand maintains its radical character.\textsuperscript{299} Precendence for the full reduction of TEMPO by monovalent late transition metals such as Fe, Ni, and Pd are known, with corresponding TEMPO O-N bond distances of 1.449(2) (κ\textsuperscript{1}),\textsuperscript{300} 1.385(2) (η\textsuperscript{2}),\textsuperscript{298} 1.372(6) (η\textsuperscript{2}),\textsuperscript{307} respectively, but to the best of our knowledge, \textbf{13}, \textbf{14}, and \textbf{15}, are the first to exhibit the full reduction at copper.
Figure 4.5. Fully labeled thermal ellipsoid diagram of [Me$_2$NN]Cu(η$^2$TEMPO) (13) (all H atoms omitted). Selected bond distances (Å) and angles (deg): N3-O: 1.4104(15), Cu-N3 2.0685(12), Cu-O 1.9026(11), Cu-N1 1.9338(13), Cu-N2 1.9379(13), N3-Cu-O 41.34(4), N1-Cu-N2 96.47(5), N1-Cu-O 101.92(5), N2-Cu-N3 120.30(5).
Figure 4.6. Fully labeled thermal ellipsoid diagram of [Cl_NN]Cu(η²-TEMPO) (14) (all H atoms omitted). Selected bond distances (Å) and angles (deg): N3-O 1.4053(14), Cu-N3 2.0718(11), Cu-O 1.8993(9), Cu-N1 1.9471(11), Cu-N2 1.9404(11), N3-Cu-O 41.16(4), N1-Cu-N2 96.84(5), N1-Cu-O 101.33(4), N2-Cu-N3 121.12(4).
Figure 4.7. Fully labeled thermal ellipsoid diagram of [Me$_2$NN$_6$]Cu(η$^2$-TEMPO) (15) (all H atoms omitted). Selected bond distances (Å) and angles (deg): N3-O 1.396(3), Cu-N3 2.0754(18), Cu-O 1.9050(17), Cu-N1 1.9617(19), Cu-N2 1.9517(17), N3-Cu-O 40.78(7), N1-Cu-N2 96.73(7), N1-Cu-O 99.89(7), N2-Cu-N3 123.61(8).
4.2.3.b. EPR and NMR spectroscopy of [Cu](η²-TEMPO) compounds.

We find in arene solvents, TEMPO readily dissociates from [CuI](η²-TEMPO) complexes 13 - 15 to form free TEMPO and the corresponding [CuI](arene) complex (Scheme 4.4). At room temperature in benzene-$d_6$, samples of 13 - 15 each give readily interpretable $^1$H NMR and EPR spectra showing [CuI](benzene) and free TEMPO, respectively (Figure 4.5 - Figure 4.10) from the same sample tube. For example, the $^1$H NMR of 13 exhibits peaks corresponding to the [Me$_2$NN]Cu fragment (Figure 4.5).

![Scheme 4.4](image_url)

**Scheme 4.4.** The dissociation of TEMPO from [Cu] in arene solvents.
Figure 4.5. $^1$H NMR (400 MHz) (a) and isotropic X-band EPR (b) of 0.151 M [Me$_2$NN]Cu($\eta^2$-TEMPO) (13) prepared in benzene-$d_6$. 
Figure 4.6. $^1$H NMR (400 MHz) (a) and isotropic X-band EPR (b) of 0.145 M [Cl$_2$NN]Cu($\eta^2$-TEMPO) (14) prepared in benzene-$d_6$. 
Figure 4.7. $^1$H NMR (400 MHz) (a) and isotropic X-band EPR (b) of 0.115M $[\text{Me}_2\text{NN}_6\text{Cu}(\eta^2\text{-TEMPO})\text{(15)}]$ prepared in benzene-$d_6$. 
Initial room temperature EPR measurements of 13, 14, and 15 at 5 – 10 mM concentrations in toluene show a broadened three-line spectrum with g values close to that of a free electron generally associated with organic radicals (Figure 4.8). The spectra appear very similar to that of free TEMPO [$g_{iso} = 13: 2.0072, 14: 2.0046, 15: 2.0055$, free TEMPO: 2.0079]. Notably absent is any evidence of any hyperfine coupling to $^{63/65}$Cu typically seen at square planar copper(II) complexes. Thus, the radical species that we see by EPR is due to free TEMPO.

By careful control of solvent, we are able to produce EPR spectra that correspond to prototypical square planar copper(II) complexes (Figure 4.9 – Figure 4.11). The use of non-polar, non-coordinating solvents such as n-heptane and diethyl ether that do not bind strongly to copper(I)
β-diketiminate complexes is crucial. RT spectra of [Cu\textsuperscript{II}](η\textsuperscript{2}-TEMPO) compounds in a n-heptane/diethyl ether mixture clearly show coordination of TEMPO to copper due to the expected four line pattern which can be simulated to give $A(^{63/65}\text{Cu}) = 155$ MHz and $163$ MHz for 13 and 14 respectively (Figure 4.9 – Figure 4.10). At higher concentrations (0.151 M and 0.115 M for 13 and 14, respectively), however, only a broad 1-line EPR spectrum suggesting loss of coupling information. 14 at concentration of 0.145 M, retains the typical copper(II) 4-line pattern though the peaks are much less resolved. The spectra for 15, however, did not give enough information for simulation (Figure 4.11) and we suspect the equilibrium for 15 in arene solvents lies far to the right (Scheme 4.4).
Figure 4.9. Isotropic X-band EPR spectrum of [[Me$_2$NN]CuTEMPO (13) experimental (blue (c)) and simulation (red (b)) (70/30 n-heptane/Et$_2$O, 223 K): 8.902288 GHz, ModWidth = 0.10 mT, Power = 1.0 mW, time constant = 0.1s, simulation performed using 1Cu and 2N: g$_{iso}$ = 2.65, A($^{63}$Cu) = 155 MHz, A($^{14}$N) = 38 MHz.
Figure 4.10. Isotropic X-band EPR spectrum experimental (blue (c)) and simulation (red (b)) for \([\text{Cl}_2\text{NN}]\text{Cu(}\eta^2\text{-TEMPO)}\) (14) (70/30 mix of n-heptane/Et\(_2\)O, RT): 8.915097 GHz, ModWidth = 0.10 mT, Power = 1.0 mW, time constant = 0.03 s, simulation performed using 1Cu and 3N: \(g_{\text{iso}} = 2.094\), \(A(\text{63Cu}) = 163\) MHz, \(A(\text{14N}) = 37\) MHz for 2 \(\beta\)-diketiminate-N and 1 TEMPO-N atoms.
Figure 4.11. Isotropic X-band EPR spectrum for [Me₂NN₆₆]Cu(η²-TEMPO) (15) (70/30 mix of n-heptane/Et₂O, RT): a) inset: 8.961354 GHz, ModWidth = 1.0 mT, Power = 1.0 mW, time constant = 0.1 s, b) 8.961367 GHz, ModWidth = 0.25 mT, Power = 1.0 mW, time constant 0.1 s. Unfortunately, not enough information can be obtained from the spectra for simulation.
4.2.3.c. Correlating degree of O-N activation with the reducing capability of [Cu$^+$]

The degree of activation of the O-N bond in 13, 14, and 15 shows a clear dependence on the electron-donating properties of the β-diketiminate ligand. We can quantify this trend by comparing oxidation potentials of the corresponding [Cu$^+$](NCMe) complexes as well as the stretching frequencies ($\nu_{CN}$) to assess the degree of $\pi$-backbonding in the corresponding copper isocyanide adducts [Cu$^+$](CNAr) (Ar = 2,6-Me$_2$C$_6$H$_3$).

$[\text{Me}_2\text{NN}]\text{Cu(NCMe)}$ (19), $[\text{Cl}_2\text{NN}]\text{Cu(NCMe)}$ (20) (Figure 12), and $[\text{Me}_2\text{NN}_{6}]\text{Cu(NCMe)}$ (21) were synthesized via the addition of NCMe to either $[\text{Me}_2\text{NN}]\text{Cu}_2$ or a slurry of the corresponding β-diketimine ligand $\text{H}[\text{Cl}_2\text{NN}]$ or $\text{H}[\text{Me}_2\text{NN}_{6}]$ along with CuO$^+$Bu (Scheme 4.5). The redox potentials measured by cyclic voltammetry were + 308 mV, + 478 mV, + 659 mV (vs NHE) for 19, 20, and 21, respectively (Figure 4.8). Thus, the copper(I) complexes become harder to oxidize with decreasing electron-donating capability of the β-diketiminate ligand.

**Scheme 4.5.** The formation of $[\text{Me}_2\text{NN}]\text{Cu(NCMe)}$ (19), $[\text{Cl}_2\text{NN}]\text{Cu(NCMe)}$ (20), and $[\text{Me}_2\text{NN}_{6}]\text{Cu(NCMe)}$ (21) via the addition of NCMe to precursor copper compounds.
Figure 4.12. Fully labeled thermal ellipsoid diagram of [Cl\textsubscript{2}NN]CuNCMe (20) (all H atoms omitted). Selected bond distances (Å) and angles (deg): Cu-N3 1.8791(14), Cu-N1 1.9067(12), Cu-N2 2.0048(12), N3-C18 1.144(2), N1-Cu-N3 155.75(6), N2-Cu-N3 106.08(5), N1-Cu-N2 97.29(5).
**Figure 4.8.** Cyclic voltamgrams of [Me$_2$NNF$_6$]Cu(NCMe) (19) and [Cl$_2$NN]Cu(NCMe) (20) in 0.1 M [n-Bu$_4$]NBF$_4$/MeCN using a three electrode setup: the pseudo-reference and auxiliary electrodes were 0.5 mm silver wires and the working electrode 3.0 mm glassy carbon. Current was applied and monitored throughout cycling between 0 and +1000 mV relative to 0.1 mM Fc/Fc$^+$ reference and scanned at a rate of 100 mVs$^{-1}$.

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**Table 4.2.** Cyclic voltammetry of copper(I) acetonitrile complexes (* literature value).
Joining known [Me$_2$NN]Cu(CNAr) (16), new arylisocyanide complexes [Cl$_2$NN]Cu(CNAr) (17) and [Me$_2$NN$_{F_6}$]Cu(CNAr) (18) were prepared by addition of CNAr to a slurry of the free β-diketimine ligand and CuO’Bu (Scheme 4.6). Similar to previously published [Me$_2$NN]Cu(CNAr) (16), X-ray crystallography of 17 (Figures 4.13) and 18 (Figure 4.14) show a three coordinate, trigonal planar geometry about the copper center. The νC≡N stretches for 16, 17 and 18 are 2123, 2141 and 2149 cm$^{-1}$ (Figure 4.9; Table 4.3). This increase in νC≡N stretching frequency mirrors the decreasing electron-donating ability of the β-diketiminate copper(I) complexes [Me$_2$NN$_{F_6}$]Cu, [Cl$_2$NN]Cu, and [Me$_2$NN$_{F_6}$]Cu, completely consistent with trends seen via cyclic voltammetry.

$$[\text{Cl}_2\text{NN}]\text{H} + \text{Cu-O'Bu} + \text{CNAr} \rightarrow [\text{Cl}_2\text{NN}]\text{Cu(CNAr)}$$
$$[\text{Me}_2\text{NN}_{F_6}]\text{H} + \text{Cu-O'Bu} + \text{CNAr} \rightarrow [\text{Me}_2\text{NN}_{F_6}]\text{Cu(CNAr)}$$

**Scheme 4.6.** The formation of [Cl$_2$NN]Cu(CNAr) (17) and [Me$_2$NN$_{F_6}$]Cu(CNAr) (18) via the addition of CNAr to corresponding β-diketiminate ligand and CuO’Bu.
Figure 4.13. Fully labeled thermal ellipsoid diagram of [Cl₂NN]CuCNAr (17) (all H atoms were omitted). Selected bond distances (Å) and angles (deg): Cu-C18 1.8189(10), Cu-N1 1.9331(8), Cu-N2 1.9367(8), C18-N3 1.623(18), N1-Cu-C18 134.25(4), N2-Cu-C18 128.55(4), N1-Cu-N3 96.94(3).
Figure 4.14. Fully labeled thermal ellipsoid diagram of [Me₂NÑF₆]CuCNAr (18(a)) (all H atoms were omitted). Selected bond distances (Å) and angles (deg): Cu1-C22 1.820(18), Cu-N1 1.936(14), Cu-N2 1.962(15), C22-N3 1.16(2), N1-Cu-C22 137.3(7), N1-Cu-N2 99.8(6), N2-Cu-C22 121.4(7).
Figure 4.14. Fully labeled thermal ellipsoid diagram of [Me₂NNF₆]CuCNAr (18(b)) (all H atoms were omitted). Selected bond distances (Å) and angles (deg): Cu2-C52 1.830(19), Cu2-N4 1.953(16), Cu-N5 1.921(14), C52-N6 1.15(2), N1-Cu-C22 121.0(7), N1-Cu-N2 98.4(6), N5-Cu-C22 140.5(7).
Figure 4.9. Solid state IR spectrum of [Cl₂NN]Cu(CNAr) (a) and [Me₂NNF₆]Cu(CNAr) (b) (film from diethyl ether on NaCl salt plates).

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Table 4.3. IR ν_{CNAr} copper(I) 2,6 dimethyl phenyl isocyanide complexes (* literature value).
4.2.3.d. Redox non-innocence of TEMPO in $[\text{Cu}^{II}]\left(\eta^2\text{-TEMPO}\right)$ complexes

The oxidation potentials for the $[\text{Cu}^I]$(NCMe) complexes 19, 20, and 21 are much lower than the reduction potential for TEMPO/TEMPO$^-$ (-1.48 V vs NHE). Thus, the formal reduction of TEMPO to TEMPO$^-$ by copper intrinsically requires an inner-sphere interaction initiated by coordination of TEMPO to the copper(I) complex $[\text{Cu}^I]$. Coupled with the varying extent of O-N bond reduction connected with the electron-richness of the corresponding $[\text{Cu}^I]$ fragment, these observations reveal the redox non-innocent nature of TEMPO in which the electronic structure of the metal fragment determines the degree of single electron transfer from copper(I) to TEMPO in $[\text{Cu}]\left(\eta^2\text{-TEMPO}\right)$ complexes.

4.2.3.e. Formation of $[\text{Cu}]\text{(TEMPO)}$ via acid/base reactivity with $[\text{Cu}^{II}]\text{-O'Bu}$ and TEMPO-H

The hydroxylamine TEMPO-H may also be used as a precursor to $[\text{Cu}^{II}]\left(\eta^2\text{-TEMPO}\right)$ complexes via acid/base reactivity with $[\text{Cu}^{II}]$-O'Bu complexes. For instance, addition of TEMPO-H to $[\text{Me}_2\text{NNF}_6]\text{Cu-O'Bu}$ (22) in benzene-$d_6$ results in the formation of HO'Bu and $[\text{Me}_2\text{NNF}_6]\text{Cu}(\eta^2\text{-TEMPO})$ (13) (Scheme 4.7). This reaction occurs relatively quickly and quantification of HO'Bu via NMR shows a quantitative yield. As anticipated, we do not observe the formation of TEMPO-H when HO'Bu is added to $[\text{Me}_2\text{NNF}_6]\text{Cu}(\eta^2\text{-TEMPO})$. We suspect the relative BDE and pKa difference between HO'Bu (105.1 kcal/mol$^{310}$, pKa = 16.5)$^{311}$ and TEMPO-H.

Scheme 4.7. The formation of 13 via addition of TEMPO-H to $[\text{Cu}^{II}]$-O'Bu.
H (69.7 kcal/mol; pKₐ(aqueous) = 6.6²⁸⁷ (pKₐ(NCMe) = 41)²⁸⁸ is why this reaction occurs in only one direction.²⁷³, ³¹²
**Figure 4.7.** Fully labeled thermal ellipsoid diagram of \([\text{Me}_2\text{NNr}_6]\text{CuO}^\prime\text{Bu} (22)\) (all H atoms were omitted). Selected bond distances (Å) and angles (deg): Cu-O 1.7732(18), Cu-N1 1.8938(16), N1-Cu-O 131.89(5). Note: The 'Bu group is disordered over two positions whose disorder is omitted for clarity. All (*) are grown in.
4.2.4. Disproportionation of $[\text{Cu}]\langle \eta^2\text{-TEMPO} \rangle$

Like $N$-phenylhydroxylamine (Section 3.2.1.b.), the hydroxylamine TEMPO-H undergoes disproportionation at $\beta$-diketiminato $[\text{Cu}^1]$ complexes in a pathway that must involve O-N bond cleavage. Addition of 3 equiv. TEMPO-H to $[\text{Me}_2\text{NN}]\text{Cu}(\text{NCMe})$ (19) in benzene-$d_6$ at RT resulted in the formation of free amine piperidine and water as analyzed by $^1\text{H}$ NMR spectroscopy (Scheme 4.10). This decomposition pathway for TEMPO at electron-rich copper(I) centers may be important given the extensive use of TEMPO and related nitroxides in the catalytic oxidation of alcohols at copper(I) centers such as $[(\text{bipy})\text{Cu}]^+$.

![Scheme 4.8](image)

Scheme 4.8. The formation of piperidine and water via the disproportionation of TEMPO-H.

4.3 Conclusion

We have described the synthesis of three new copper-TEMPO complexes by addition of TEMPO to copper(I) $\beta$-diketimates $[\text{Cu}^1]$. Despite electronic changes in the $\beta$-diketiminate ligands, the $\eta^2$-ON TEMPO conformation persists with an elongated O-N bond consistent with a TEMPO$^-$ anion. $[\text{Me}_2\text{NN}]\text{Cu}(\eta^2\text{-TEMPO})$ (13), $[\text{Cl}_2\text{NN}]\text{Cu}(\eta^2\text{-TEMPO})$ (14), and $[\text{Me}_2\text{NNF}_6]\text{Cu}(\eta^2\text{-TEMPO})$ (15), each displays a distorted square planar geometry consistent with the presence of a copper(II) center. Nonetheless, subtle changes in the O-N bond distance that track the electron-donating ability of the $[\text{Cu}^1]$ fragment support the notion of redox non-innocence in the coordination of the TEMPO ligand. The most electron-rich copper(I) complex $[\text{Me}_2\text{NN}]\text{Cu}$ encourages disproportionation of TEMPO-H to the free amine and water, suggesting a potentially
important decomposition pathway for TEMPO and related nitroxides used in conjunction with copper(I) complexes in catalytic alcohol oxidation reactions.
4.4. Experimental Procedures

4.4.1. General experimental procedures

All experiments were carried out in a dry nitrogen atmosphere using an MBraun glovebox and/or standard Schlenk techniques. 4 A molecular sieves were activated \textit{in vacuo} at 180 °C for 24 h. Dry THF and benzene was purchased from Aldrich and stored over activated 4 A molecular sieves. Diethyl ether was first sparged with nitrogen and then dried by passage through activated alumina columns. Pentane was first washed with conc. \( \text{HNO}_3 / \text{H}_2\text{SO}_4 \) to remove olefins, dried by passage through activated alumina columns, and stored over CaCl\(_2\) and activated 4 A molecular sieves. All deuterated solvents were sparged with nitrogen, dried over activated 4A molecular sieves and stored under nitrogen. Celite was dried overnight at 200 °C under vacuum.

\(^1\text{H}\) and \(^{13}\text{C}\) NMR spectra were recorded on either a Varian 300 MHz or 400 MHz spectrometer. All NMR spectra were recorded at room temperature unless otherwise noted and were indirectly referenced to TMS using residual solvent signals as internal standards. Elemental analyses were performed on a Perkin-Elmer PE2400 microanalyzer in our laboratories, and UV-Vis spectra were recorded on either a Varian Cary 50 or 100 spectrophotometer, using cuvettes with screw-cap tops. Cyclic voltammetry was performed in 0.1 M [n-Bu\(_4\)]NBF\(_4\)/MeCN using a three electrode setup. The pseudo-reference and auxiliary electrodes were 0.5 mm silver wires and the working electrode 3.0 mm glassy carbon. Potential was applied using BASi EC Epsilon Electrochemical Workstation; current was applied and monitored throughout cycling between 0 and +1000 mV relative to 0.1 mM Fc/Fc\(^+\) reference and scanned at a rate of 100 mVs\(^{-1}\).

All reagents were obtained commercially unless otherwise noted and typically stored over activated 4A molecular sieves. [Cl\(_2\)NN]H,\(^{316}\) [Me\(_2\)NN]H,\(^{238}\) [Me\(_2\)NNF\(_6\)]H,\(^{267}\) \{[Cl\(_2\)NN]Cu\}_2(μ-benzene),\(^{239}\) \{[Me\(_2\)NN]Cu\}_2,\(^{317}\) [Me\(_2\)NNF\(_6\)]Cu(NCMe),\(^{219}\) and were prepared using literature methods.
4.4.2. Preparation of [CuII] compounds

[Me₂NN]Cu(η²-TEMPO) (13). A solution of [Me₂NN]H (0.500 g, 1.63 mmol) in 5 mL diethyl ether was added to a suspension of copper(I) tert-butoxide (0.225 g, 1.64 mmol) in 5 mL diethyl ether and allowed to for 30 min resulting in a bright orange/red color. The addition of TEMPO (0.255 g, 1.63 mmol) in 2 mL diethyl ether and subsequent stirring for ca. 2 h produced a dark red solution. The resulting solution was then filtered through Celite, concentrated and placed at -35 °C to crystallize overnight to give 0.57 g (67% yield) of the product as red crystals. Anal. Calc’d for C₂₆H₃₁Cl₄CuN₃O: C, 68.60; H, 8.25; N, 8.00. Found: C, 68.22; H, 8.15; N, 7.81.

[Cl₂NN]Cu(η²-TEMPO) (14). A solution of [Cl₂NN]H (0.500 g, 1.28 mmol) in 5 mL diethyl ether was added to a suspension of copper(I) tert-butoxide (0.175 g, 1.28 mmol) in 5 mL diethyl ether and allowed to for 30 min resulting in a bright orange/red color. The addition of TEMPO (0.200 g, 1.28 mmol) in 2 mL diethyl ether and subsequent stirring for ca. 2 h produced a dark red solution. The resulting solution was filtered through Celite, concentrated and placed at -35 °C to crystallize overnight to give 0.49 g (63% yield) of the product as red crystals. Anal. Calc’d for C₂₆H₃₁Cl₄CuN₃O: C, 51.45; H, 5.15; N, 6.92. Found: C, 51.78; H, 5.35; N, 6.82.

[Me₂NNF₆]Cu(η²-TEMPO) (15). A solution of [Me₂NNF₆]H (0.500 g, 1.21 mmol) in 5 mL diethyl ether was added to a suspension of copper(I) tert-butoxide (0.164g, 1.20 mmol) in 5 mL diethyl ether. The solution was allowed to stir for 30 min resulting in a bright orange color. The addition of TEMPO (0.158 g, 1.20 mmol) in 2 mL diethyl ether and subsequent stirring for ca. 2 h produced a dark reddish/purple solution. The resulting solution was filtered through Celite, concentrated and placed at -35 °C to crystallize overnight to give 0.35 g (46% yield) of the product as red crystals. Anal. Calc’d for C₂₆H₃₁Cl₄CuN₃O: C, 56.91; H, 5.89; N, 6.64. Found: C, 56.15; H, 5.49; N, 6.85.
[Cl2NN]Cu(CNAr) (17). A solution of [Cl2NN]H (0.500 g, 1.28 mmol) in 5 mL diethyl ether was added to a suspension of copper(I) tert-butoxide (0.229 g, 1.67 mmol) in 5 mL diethyl ether. The solution was allowed to for 6 h resulting in a dark brown color. After addition of CNAr (0.221 g, 1.68 mmol) the solution was allowed to stir for 3 h resulting in a dark purple solution. The solution was filtered through Celite, concentrated and placed at -35 °C to crystallize overnight to give 0.208 g (28% yield) of the product as yellow crystals. 1H NMR (benzene-d6, 400 MHz, 25 °C): δ 7.11 (d, 4, m-ArH), 6.62 (t, 1, p-CNArH), 6.42 (m, 3, (1) p-ArH, (2) m-CNArH), 5.08 (s, 1, CHbackbone), 1.88 (s, 6, o-CNArMe2), 1.73 (s, 6, Mebackbone) 13C{1H} NMR (benzene-d6): δ 164.07, 148.93, 134.59, 130.21, 128.11, 127.80, 127.12, 123.07, 95.45, 22.46, 17.72. Anal. Calc’d for C26H22Cl4CuN3: C, 53.67; H, 3.81; N, 7.22. Found: C, 53.41; H, 3.78; N, 7.06.

[Me2NNF6]Cu(CNAr) (18). A solution of [Me2NNF6]H (0.500 g, 1.21 mmol) in 5 mL diethyl ether was added to a suspension of copper(I) tert-butoxide (0.164 g, 1.20 mmol) in 5 mL diethyl ether. The solution was allowed to for 6 h resulting in a bright orange/red color. After addition of CNAr (0.158 g, 1.20 mmol) the solution was allowed to stir overnight. The solution was filtered through Celite, concentrated and placed at -35 °C to crystallize overnight to provide 0.308 g (42% yield) of the product as orange/red crystals. 1H NMR (benzene-d6, 400 MHz, 25 °C): δ 7.00 (d, 4, m-ArH), 6.90 (t, 2, p-ArH), 6.59 (t, 1, p-CNArH), 6.36 (d, 2, m-CNArH), 6.25 (s, 1, CHbackbone), 2.36 (s, 12, o-ArMe2), 1.56 (s, 6, o-CNArMe2) 13C{1H} NMR (benzene-d6): δ 152.43, 152.17, 149.86, 135.27, 129.74, 129.27, 127.65, 124.02, 122.54, 119.68, 84.41, 19.17, 17.88. Anal. Calc’d for C30H28CuF6N3: C, 53.67; H, 4.64; N, 6.91. Found: C, 59.14; H, 4.73; N, 6.85.

[Me2NN]Cu(NCMe) (19). [Me2NN]Cu(NCMe) was prepared according to a modified literature procedure.309 A solution of [Me2NN]H (0.500 g, 1.28 mmol) in 7 mL 70/30 acetonitrile/diethyl ether was added to a suspension of copper (I) tert-butoxide (0.179 g, 1.31 mmol) in 5 mL acetonitrile
and allowed to stir overnight. Subsequent addition of 2 mL diethyl ether lead to a precipitate which was isolated and washed with cold pentane to afford 1.22 g (92% yield) of the product as a very light reddish powder. $^1$H NMR (benzene-\textit{d}_6, 400 MHz, 25 °C): $\delta$ 7.04 (d, 4, \textit{m}-Ar\textit{H}), 6.92 (t, 2, \textit{p}-Ar\textit{H}), 4.75 (s, 1, CH\textsubscript{backbone}), 1.76 (s, 12, \textit{o}-ArMe\textsubscript{2}), 1.59 (s, 6, Me\textsubscript{backbone}), 0.46 (s, 3, NCMe). (d, 4, m-ArH). $^{13}$C\{\textit{H}\}NMR (benzene-\textit{d}_6): $\delta$ 162.52, 150.90, 130.68, 123.11, 93.22, 23.14, 18.97, 0.163.

[Cl\textsubscript{2}NN]Cu(NCMe) (20). [Cl\textsubscript{2}NN]Cu(NCMe) was prepared according to proceeding procedure of [Me\textsubscript{2}NN]Cu(NCMe) to yield 0.975 g (77% yield) of a light yellowish powder. $^1$H NMR (benzene-\textit{d}_6, 400 MHz, 25 °C): $\delta$ 7.12 (d, 4, \textit{m}-Ar\textit{H}), 6.45 (t, 2, \textit{p}-Ar\textit{H}), 4.90 (s, 1, CH\textsubscript{backbone}), 1.76 (s, 12, \textit{o}-ArMe\textsubscript{2}), 0.42 (s, 3, NCMe). $^{13}$C\{\textit{H}\}NMR (benzene-\textit{d}_6): $\delta$ 164.29, 147.79, 130.80, 128.21, 123.65, 94.80, 23.48, 0.20.

[Me\textsubscript{2}NNF\textsubscript{6}]Cu\textupsilonBu (22). The synthesis of [Me\textsubscript{2}NNF\textsubscript{6}]Cu\textupsilonBu was adapted from a literature procedure.\textsuperscript{318} A solution of [Me\textsubscript{2}NNF\textsubscript{6}]H (0.300 g, 0.724 mmol) in 5 mL diethyl ether was added to a suspension of copper(I) \textit{tert}-butoxide (0.164g, 1.20 mmol) in 5 mL diethyl ether. The solution was allowed to stir for 30 min resulting in a bright orange/red color. 5 eq di-\textit{tert}butyl peroxide (0.667 mL, 3.62 mmol) was added and allowed to stir for 2 h. The resulting purple solution was filtered through Celite, concentrated and placed at -35 °C to crystallize overnight to provide 0.434 g (66 % yield) of the product as purple crystals. UV-Vis (Et\textsubscript{2}O, -40 °C, nm (M\textsuperscript{-1} cm\textsuperscript{-1})): 338 (12800), 396 (1970). Anal. Calc’d for C\textsubscript{26}H\textsubscript{31}Cl\textsubscript{4}CuN\textsubscript{3}O: C, 54.59; H, 5.13; N, 5.09. Found: C, 54.82; H, 4.87; N, 5.31.
4.4.3. Crystallographic data tables

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Table 4.2. Crystallographic data tables 13, 14, and 15.
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**Table 4.3.** Crystallographic data tables 17, 18, 20, and 22.
4.4.4. X-band EPR spectroscopy of [Cu$^{II}$] compounds

4.4.4.a. Anisotropic EPR spectroscopy for 13, 14, and 15

![Figure 4.9](image-url)

**Figure 4.9.** Anisotropic X-band EPR spectrum experimental (blue (a)) and simulation (red (b)) for ([Me$_2$NN]CuTEMPO (13) (70/30 n-heptane/Et$_2$O, 98 K): 8.937102 GHz, ModWidth = 1.0 mT, Power = 1.0 mW, time constant = 0.03 s, simulation performed using 1Cu and 2N model: $g_1 = 2.1214$, $g_{2,3} = 2.0430$. $A_{1}^{(63\text{Cu})} = 495$ MHz, $A_{2,3}^{(63\text{Cu})} = 90$ MHz, $A_{1}^{(14\text{N})} = 70$ MHz, $A_{2,3}^{(14\text{N})} = 0$ MHz; linewidth $W_1 = 21$ mT, $W_{2,3} = 10$ mT.
Figure 4.11. Anisotropic X-band EPR spectrum experimental (blue (a)) and simulation (red (b)) for [Cl₂NN]CuTEMPO (14) (70/30 mix of n-heptane/Et₂O, 89 K): 8.914317 GHz, ModWidth = 1.0 mT, Power = 1.0 mW, time constant = 0.03 s, simulation performed using 1Cu and 2N model: $g_1 = 2.1150$, $g_{2,3} = 2.0315$. $A_{1}(^{63}\text{Cu}) = 470$ MHz, $A_{2,3}(^{63}\text{Cu}) = 110$ MHz, $A_{1}(^{14}\text{N}) = 80$ MHz, $A_{2,3}(^{14}\text{N}) = 0$ MHz; linewidth $W_1 = 18$ mT, $W_{2,3} = 12$ mT.
Figure 4.13. Anisotropic X-band EPR spectrum $[[\text{Me}_2\text{NN}_{\text{F}_6}]\text{CuTEMPO} \textbf{(15)}$ (70/30 mix of n-heptane/Et$_2$O, 50 K): 9.009618 GHz, ModWidth = 1.0 mT, Power = 1.0 mW, time constant = 0.03 s.
4.4.4.a. Isotropic EPR of \([\text{Me}_2\text{NNF}_6]\text{CuOtBu} \text{ (22)}\)

Figure 4.14. Isotropic X-band EPR spectrum (blue (a)) and simulation (red (b)) for \([\text{Me}_2\text{NNF}_6]\text{CuOtBu} \text{ (22)}\) (heptane, RT): 8.963578 GHz, ModWidth = 1.0 mT, Power = 1.0 mW, time constant = 0.03, simulation performed using 1Cu model: \(g_{\text{iso}} = 2.1100\). \(A_{1}(^{63}\text{Cu}) = 112\) MHz, linewidth \(W_{\text{iso}} = 27\) mT.
A.1. Reactivity of copper(I) with nitric oxide

A.1.1. Introduction

The copper nitrosyl (\(\{\text{Cu(NO)}\}_1\)) is a vital aspect of nitric oxide processing at copper.\(^{319}\) As explained in chapter 1, section 1.2.4.b., there are several copper enzymes that interact directly with nitric oxide. Furthermore, an isolated copper nitrosyl is an invaluable intermediate for synthetic investigations. Dr. Allan J. Cardenas describes in his thesis (chapter 3) the reactivity and spectroscopic data for the reactivity of \([\text{Cl}_2\text{NN}]\text{Cu}(\text{benzene})\) with nitric oxide.\(^{320}\) In brief summary, we find the addition of nitric oxide (in excess and with 2 equiv.) to \([\text{Cl}_2\text{NN}]\text{Cu}(\text{benzene})\) leads to the formation of \(\{[\text{Cl}_2\text{NN}]\text{Cu}\}_2(\mu-\text{OH})_2\). We suspect the mechanistic pathway for this reaction is similar to that of the Ni complex described by Bohle in 2002 which includes a nickel cis-hyponitrite and nickel oxo species.\(^{321}\) We propose the complexation of nitric oxide to copper in a 2 to 1 ratio results in the formation of a copper cis-hyponitrite which upon loss of \(\text{N}_2\text{O}\) results in the formation of a copper oxo species (Scheme 5.1).

\[
\text{Cu} + 2\text{NO} \rightleftharpoons \text{Cu-N=N} \quad \text{N}_2\text{O} \rightarrow \text{Cu=O}
\]

**Scheme 5.1.** Proposed formation of copper cis-hyponitrite and its subsequent loss of nitrous acid to form a copper oxo species.
Herein we provide supplemental data for the reaction of [Cl\(_2\)NN]Cu(benzene) with 2 equiv. nitric oxide via *in situ* IR spectroscopy. Utilization of *in situ* IR spectroscopy enables the ability to monitor a reaction in real time and elucidate any intermediates from composite spectra via subtraction of known IR spectra of starting materials and products in an air free environment.

**A.1.2. Results and Discussion**

2 equiv. NO were added to a 0.040 M solution of [Cl\(_2\)NN]Cu(NCMe) in diethyl ether held at low temperatures ranging from -47 °C - -30 °C. By subtracting the IR spectra of the initial [Cl\(_2\)NN]Cu(NCMe) solution, we observe IR peaks all below 1580 cm\(^{-1}\) (Figure 5.1). The spectrum is relatively quiet in the region we might expect to observe an NO stretch for [Cl\(_2\)NN]Cu(NO), which is predicted by DFT to be ca. 1736 cm\(^{-1}\).

![Figure 5.1. in situ IR spectrum of addition of 2 equiv. NO to [Cl\(_2\)NN]Cu(NCMe) in diethyl ether.](image)

I thought diethyl ether may be too coordinating of a solvent copper to enable the spectroscopic observation of [Cl\(_2\)NN]Cu(NO), and so performed a repeat experiment in fluorobenzene at a temperature range of -39 °C to -35 °C. The addition of 2 equiv. NO to 0.040 M of [Cl\(_2\)NN]Cu(NCMe), again, displays IR peaks below 1580 cm\(^{-1}\) (Figure 5.2). Repeating this
experiment within a closer temperature range of -30 °C to -27 °C produces nearly the same spectrum displaying new peaks at 1567 cm\(^{-1}\), 1537 cm\(^{-1}\), and 1440 cm\(^{-1}\) (Figure 5.3).

**Figure 5.2.** *in situ* IR spectrum of the addition of 2 equiv. NO to [Cl\(_2\)NN]Cu(NCMe) in fluorobenzene.

**Figure 5.3.** *in situ* IR spectrum (traditional IR view) of the addition of 2 equiv. NO to [Cl\(_2\)NN]Cu(NCMe)

Given the limitations of the *in situ* IR instrumentation, these spectra seem to support the formation of some species version of a copper hyponitrite species. DFT calculations of the truncated ß-diketiminate species [C\(_3\)N\(_2\)]Cu(κ\(^2\)-O\(_2\)N\(_2\)) display stretching frequencies from 1600 cm\(^{-1}\)
1 to 1400 cm\(^{-1}\). Previous IR studies of metal \(\text{N}_2\text{O}_2^-\) and \(\text{N}_2\text{O}_2^{2-}\) species are been generally characterized by bands between 1630-1580 cm\(^{-1}\), 1370-1200 cm\(^{-1}\) and 1000-860 cm\(^{-1}\).\(^{322-324}\) In his study of metallic dianionic hyponitrite species, Andrews reports that peaks around 1400 cm\(^{-1}\) are indicative of a bimetallic hyponitrite species.\(^{323}\)

Attempts at isolating and characterizing a copper(II) \textit{cis}-hyponitrite species continue in our lab using a more electron deficient \(\beta\)-diketiminate ([\text{Me}_2\text{NNF}_6]\text{Cu}) in hopes of stabilizing this rather elusive species.

**A.2. Reactivity of copper(II) with tert-butyl nitrite**

**A.2.1. Introduction**

Organic nitrites (RO-NO), similar to S-nitrosothiols (RS-NO), are esters of alcohols and nitrous acid and are generally prepared via \(O\)-nitrosation of a precursor alcohol.\(^{24, 35}\) Susceptible to NO loss via a 1-electron oxidation\(^{325-327}\) (homolytic BDE RO-NO: 36 – 41 kcal/mol)\(^{328}\), nitrites can be a good source of NO and used clinically as vasodilators, as is the case for butyl nitrite, \textit{tert}-butyl nitrite and amyl nitrite.\(^{24, 329}\)

Investigation of the interaction between copper and organic nitrites aids in understanding various enzymatic mechanisms involved in reductive nitrosylation (\(O\)- and S-nitrosylation) and reductive cleavage. Dr. Marie Melzer discusses a literature summary of reductive nitrosylation at copper and presents spectroscopic UV-Vis evidence in her thesis (chapter 3) of the reductive cleavage of \('\text{BuO}-\text{NO}\) by \([\text{Me}_2\text{NN}]\text{Cu}\).\(^{24}\) In summary, the addition of \('\text{BuO}-\text{NO}\) to \([\text{Me}_2\text{NN}]\text{Cu}^\text{I}\) at room temperature results in the formation of \([\text{Me}_2\text{NN}]\text{Cu}^\text{I}_{2}(\mu-\text{OH})_2\). However, upon purging the solution with \(\text{N}_2\) gas immediately after the addition of \('\text{BuO}-\text{NO}, \ [\text{Me}_2\text{NN}]\text{Cu}^\text{II}-\text{O}^\text{Bu}\) is formed in 91% yield. Only after the subsequent addition of 1 equiv. NO to \([\text{Me}_2\text{NN}]\text{Cu}^\text{II}-\text{O}^\text{Bu}\), did \([\text{Me}_2\text{NN}]\text{Cu}^\text{I}_{2}(\mu-\text{OH})_2\) form.
Herein we provide NMR data in support for the production of 'BuOH as a byproduct of the reductive cleavage of ONO'tBu and discuss preliminary investigation of the H-atom donor to eventually produce {[Me₂NN]Cu}_2(µ-OH)₂ and 'BuOH.

A.2.2. Results and Discussion

The addition of 2 equiv. NO (4.4 mL, 0.180 mmol) to [Me₂NN]CuOtBu (0.040 g, 0.090 mmol) (Scheme 5.2) in solution of toluene-d₈ (1 mL) at -41 °C led to the formation of 'BuOH in 100% yield via NMR (Figure 5.4).

\[ [\text{Cu}] + '\text{Bu}O \xrightarrow{\text{N=O}} '\text{Bu}OH \]

\[ [\text{Cu}]O't\text{Bu} + 2 \text{NO} \xrightarrow{} '\text{Bu}OH \]

Scheme 5.2. Formation of 'BuOH via the addition of 'BuONO to [Cu] and the addition of NO to [Cu]O'tBu.

Figure 5.4. ¹H NMR of resulting solution from addition of NO to [Me₂NN]CuOtBu.

yield via NMR (Figure 5.4). Also, the addition of 1.2 equiv. 'BuONO (21 µL, 0.018 mmol) to a
solution of [Me₂NN]Cu (0.054 g, 0.015 mmol) (Scheme 5.2) yielded the formation of 'BuOH in 93% yield via NMR (Figure 5.5).

![Resulting 1H NMR from addition of 1.2 equiv. 'BuONO to [Me₂NN]Cu.](image)

**Figure 5.5.** Resulting 1H NMR from addition of 1.2 equiv. 'BuONO to [Me₂NN]Cu.

The source of the protons acquired by both 'BuOH (this work) and [{[Me₂NN]Cu}₂(μ-OH)]₂ (Dr. Marie Melzer’s thesis) has not yet been identified. Currently, we suspect the solvent to be the source of protons, but preliminary isotopic investigations of the formation of 'BuOD and [{[Me₂NN]Cu}₂(μ-OD)]₂ from deuterated solvents are inconclusive. I did not have success distinguishing between 'BuOD vs 'BuOH via NMR, and thus, did not observe the formation of 'BuOD when performing this reaction in deuterated toluene. Following the same reaction via IR spectroscopy, Dr. Allan Cardenas did not observe the definitive formation of [{[Me₂NN]Cu}₂(μ-OH)]₂. This alludes to the formation of [{[Me₂NN]Cu}₂(μ-OD)]₂ without there being direct evidence for its formation. The mechanism of this reaction seems complex and may require more delicate and detailed isotopic studies.
A.3. Supplemental data pertinent to the mechanism of hydroxylamine dismutation

A.3.1. Introduction

The observation of the disproportionation of secondary and tertiary hydroxylamines is discussed extensively in chapter 3 and briefly in chapter 4. Attempts to elucidate kinetic parameters and isolate proposed key intermediates were made and are discussed herein.

A.3.2. Results and Discussion

A.3.2.a. UV-Vis of addition of HONHPh to [Me₂NNF₆]Cu

Preliminary investigations into the mechanism and kinetics of the dismutation of N-phenylhydroxylamine by [Me₂NNF₆]CuNCMe were followed via UV-vis spectroscopy. [Me₂NNF₆]CuNCMe, previously published by Tolman, displays a shoulder peak at λ_max = 471 nm.

Figure 5.6. Beers law plot of [Me₂NNF₆]CuNCMe.
in THF at -40 °C (ε = 415 M⁻¹cm⁻¹) (Figure 5.6). The addition of 2 equiv. PhHNOH lead to the disappearance of the 471 nm and the appearance of a peak at λ_{max} = 595 nm evincing the formation of [Me₂NN₆]Cu(η²-ONPh) (Figure 5.8); this finding is further explored in chapter 3. Via independent synthesis and UV-vis characterization, [Me₂NN₆]Cu(η²-ONPh) has a λ_{max} at 595 nm in THF at -40 °C (ε = 1880 M⁻¹cm⁻¹) (Figure 5.7).

Figure 5.7. Beers law plot of [Me₂NN₆]Cu(η²-ONPh).

Unfortunately, the product of this reaction, [Me₂NN₆]Cu(η²-ONPh), is also able to dismutate PhHNOH, complicating the investigation of this mechanism (Figure 5.9).
A.3.2.b. Disproportionation of HONEt$_2$

An EPR of the resulting solution of the addition of 3 equiv. HONEt$_2$ to $[\text{Me}_2\text{NNF}_6]\text{CuNCMe}$. produces a spectrum (Figure 5.9) similar to that published for the •ONEt$_2$ radical. This finding further supports the breaking of the O-N bond by copper and highlights the importance of ratio equivalence in these reactions.

Figure 5.8. Resulting uv-vis spectrum for addition of 2 equiv. PhHNOH to $[\text{Me}_2\text{NNF}_6]\text{CuNCMe}$. 
A.4. Fortuitous crystal structures and relevant spectroscopic data

A.4.1. Copper(II) oxime

A.4.1.a. Synthesis of cyclohexanone oxime

An aliquot of H$_2$NOH solution (50% by wt in H$_2$O) (5 mL, 0.075 mol) was added dropwise to an aqueous solution of cyclohexanone (5 mL, 0.048 mol) and allowed to stir overnight. After filtration of H$_2$O, the crude product was dissolved in toluene and gently warmed to aid solubility of the crude product. After which, the solution was allowed to cool overnight to afford 4.88g (90% yield) of the desired product.

Figure 5.9. X-band EPR spectrum of resulting solution from the addition of 3 equiv. HONEt$_2$ to [$\text{Pr}_2\text{NNF}_6$]CuNCMe (toluene, RT). Freq = 9.058805 GHz, ModWidth = 1.0 mT, Power = 1.0 mW, time constant = 0.03 s.
A.4.1.b. Synthesis of copper(II) cyclohexanone oxime

A colorless solution of cyclohexanone oxime (0.092 g, 0.813 mmol) in pentane (5 mL) was added to a red solution of [\(^{1}\)Pr\(_{2}\)NN]CuO'Bu (0.300 g, 0.679 mmol) also in pentane (7 mL). The resulting solution was dried \textit{in vacuo} to remove resulting HO'Bu product, dissolved in pentane (5

\textbf{Figure 5.10.} Fully labeled thermal ellipsoid diagram of [\(^{1}\)Pr\(_{2}\)NN]Cu(\(\eta^2\)-ON=C\(_6\)H\(_{10}\)) (all H atoms omitted for clarity). Selected bond distances (Å) and angles (deg): N3-O 1.366(3), Cu-N3 1.956(2), Cu-O 1.255(19), Cu-N1 1.909(2), Cu-N2 1.899(2), N3-C30 1.273(3), N3-Cu-O 41.20(8), N1-Cu-N2 97.53(9), N1-Cu-O 106.33(9), N2-Cu-N3 114.96(9).
mL) and cooled to -35 °C. Crystals of [Pr$_2$NN]Cu(ON=C$_6$H$_{10}$/0 were collected affording 0.216 g (66% yield).

**A.4.2. Copper(II) 2-pyridil thiolate**

**A.4.2.a Synthesis of copper(II) 2-pyridil thiolate**

The addition of 0.75 equiv. 2,2’-pyridil dithiol (0.109 g, 0.494 mmol) solution in diethyl ether (3 mL) to a red solution of [Me$_2$NN$_6$]CuNCMe (0.341 g, 0.659 mmol), also in diethyl ether (4 mL) resulted in the formation of a red precipitate. The precipitate was allowed to settle and the supernatant filtered through celite, concentrated *in vacuo*, and cooled to -35 °C. Crystals were collected in 29% yield affording 0.109 g of the copper thiolate.

Alternatively, copper(II) 2-pyridil thiolate can be synthesized via the addition of 2 equiv. 2-pyridil thiol (0.080 g, 0.727 mmol) in a solution of diethyl ether (3 mL) to [Me$_2$NN$_6$]CuOtBu (0.200 mg, 0.363 mmol) in diethyl ether (4 mL).

**A.4.2.b Xray crystallography of copper(II) 2-pyridil thiolate**

Preliminary xray data of the copper(II) thiolate crystals shows the pyridil nitrogen in a conformation that doesn’t suggest a stabilizing interaction with the copper (Figure 5.11(a) and 5.11(b)). This preliminary xray data provides a glimpse of the atom connectivity in the copper(II) thiolate (Figure 5.12), despite producing 2 non-positive definite atoms and 2 relatively large inexplicable U-peaks (Figure 5.13). Previous studies of β-diketiminate copper thiolates by Dr. Marie Melzer were performed using relatively short-lived copper(II) trityl thiolate. We hoped the

![Figure 5.11](image_url)

**Figure 5.11.** Expected (a), observed (b), and possible future (c) binding modes for copper (II) thiolates.
additional stabilization from the pyridil nitrogen might extend the lifetime of a copper(II) thiolate and allow for relatively easier mechanistic studies. A possible future step may include adding a –CH₂ linker between the sulfur and pyridyl ring to create a 5 membered κ²-SCH₂C₅H₄N copper species (Figure 5.11.c).
Figure 5.12. Fully labeled thermal ellipsoid diagram of [Me$_2$NN]Cu(S-C$_5$H$_4$N) (all H atoms omitted for clarity). Selected bond distances (Å) and angles (deg): Cu-S1 2.175(4), Cu-N1 2.006(13), Cu-N2 1.937(13), S1-C22 1.776(15), N1-Cu-N2 98.1(5), N1-Cu-S1 114.3(4), N2-Cu-S1 147.6(4), CuS1-C22 112.2(5).
Figure 5.13. Thermal ellipsoid diagram of two independent molecules of \([\text{Me}_2\text{NN}]\text{Cu}(\text{S-C}_5\text{H}_4\text{N})\) (all H atoms omitted for clarity). Selected bond distances (Å) and angles (deg): Cu2-S2 2.173(4), Cu2-N4 1.993(13), Cu2-N5 1.942(11), S2-C48 1.789(15), N4-Cu-N5 97.5(6), N4-Cu-S2 116.1(4), N5-Cu-S2 146.4(4), S2-Cu-C48 111.2(5).
A.4.1.c. NMR and EPR of copper(II) 2-pyridil thiolate

Crystals of the copper(II) 2-pyridil thiolate gave both NMR (Figure 5.14) and EPR (Figure 5.15) spectra suggesting an equilibrium between a copper(II) thiolate and a copper(I) thione.

Figure 5.14. $^1$H NMR of crystals of [Me$_2$NN$_6$F$_6$]CuSC$_3$H$_4$N: δ 7.55 (s, 1, 3-m-pyridil-$H$), δ 6.88 (s, 1, o-pyridil-$H$), δ 6.70 (d, 4, m-N-aryl-$H$), δ 6.59 (d, 2, p-N-aryl-$H$), δ 6.35 (s, 1, 5-m-pyridil-$H$), δ 6.28 (s, 1, p-pyridil-$H$), δ 6.20 (s, 1, β-diketiminate backbone-$H$), δ 2.29 (s, 12, N-aryl-$CH_3$).
The sulfur–carbon bond length determined by x-ray crystallography of 1.776(15) Å and 1.789(15) Å (Figure 5.13) is between classic single and double sulfur–carbon bond distances (1.82 Å and 1.60 Å, respectively). This equilibrium has been seen before with chromophoric nickel complexes.

**A.5. EPR of Frustrated Lewis Pairs**

Dr. Allan Cardenas began our lab’s investigation into frustrated lewis pairs (FLP) and discusses their relevance to capturing nitric oxide and other small molecules in his thesis (chapter 4). We’ve continued to partner with the Erker group in Germany to further the study of small molecule capture with FLPs.

**Figure 5.15.** X-band EPR spectrum and simulation for [Me₂NNF₆]CuSC₅H₄N (toluene-₉, RT): 8.945869 GHz, ModWidth = 0.3 mT, Power = 1.0 mW, time constant = 0.3 s. Simulation gives $g_{iso} = 2.1320$, $A_{(63/65Cu)} = 300$ MHz, $A_{(14N)} = 44$ MHz.
Synthesized by the Erker group, one P/B FLP is a markedly oxygen centered nitroxide radical which undergoes H-atom abstraction to give the diamagnetic P/B FLP NOH (Scheme 5.3).\textsuperscript{332} Characterized by EPR spectroscopy, we find the coupling to N, P, and B to be 22.4 MHz, 50.8 MHz, and 9.2 MHz, respectively.

\textbf{Scheme 5.3.} Synthesis of P/B FLP NOH via H-atom abstraction by P/B FLP NO.

\textbf{Figure 5.10.} X-band EPR spectrum and simulation for P/B FLP NO (dichloromethane, RT): 8.934278 GHz, ModWidth = 0.1 mT, Power = 1.0 mW, time constant = 0.1 s. Simulation (Gaussian lineshape with 1.4 G linebroadening) gives $g_{\text{iso}} = 2.0064$, $A(^{14}\text{N}) = 22.4$ MHz, $A(^{31}\text{P}) = 50.8$ MHz, $A(^{11}\text{B}) = 9.2$ MHz, $A(^{10}\text{B})$ not simulated.
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