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A Copper(II) Thiolate from Reductive Cleavage of an *S*-Nitrosothiol

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Supporting Information

ABSTRACT: *S*-Nitrosothiols RSNO represent circulating reservoirs of nitric oxide activity in the plasma and play intricate roles in protein function control in health and disease. While nitric oxide has been shown to reductively nitrosylate copper(II) centers to form copper(I) complexes and ENO species (E = R_2N, RO), well-characterized examples of the reverse reaction are rare. Employing the copper(I) β-diketiminate [Me_2NN]Cu, we illustrate a clear example in which an RS−NO bond is cleaved to release NO_{gas} with formation of a discrete copper(II) thiolate. The addition of Ph_3CSNO to [Me_2NN]Cu generates the three-coordinate copper(II) thiolate [Me_2NN]CuSCPh_3, which is unstable toward free NO.

S-Nitrosothiols RSNO play an intricate role in the control of protein function in health and disease through the post-translational modification of cysteine SH residues.[1] Low-molecular-weight *S*-nitrosothiols such as *S*-nitrosoglutathione (GSNO) represent circulating reservoirs of nitric oxide activity typically present at submicromolar concentrations in the plasma,[2] which have protective effects against myocardial[3] and lung/airway[3] injuries among other functions.[1] RSNO compounds are prone to homolytic loss of NO due to the relative weakness of the RS−NO bond (20−32 kcal/mol)[4] and the strength of the RS−SR bond (65−66 kcal/mol).[5]

Trace amounts of copper ions serve as efficient catalysts for RSNO decomposition to form NO_{gas} and RSSR (Scheme 1).[6]

Scheme 1. Copper-Catalyzed Release of NO from RSNO Compounds

$$2\ \mathrm{RSNO} \xrightarrow{\text{Cu ions}} 2\ \mathrm{NO_{gas}} + \mathrm{RSSR}$$

CuZnSOD is the most abundant source of copper in red blood cells and is effective at releasing NO from GSNO.[7] It may be inhibited with neocuproine, a copper(+) chelator, suggesting copper(I) as an active oxidation state for NO loss.[7] Moreover, medical polymers with imbedded copper(2+) ions serve as long-lived NO-generating devices via the copper-catalyzed decomposition of endogenous *S*-nitrosothiols.[8]

Copper enzymes also generate RS−NO bonds from NO via reductive nitrosylation (Scheme 2).[9] CuZnSOD has been shown to specifically *S*-nitrosylate β-Cys93 of hemoglobin.[10]

Scheme 2. Redox Interconversion of RSNO and NO

$$[\mathrm{Cu^{II}}]\text{-SR} + \mathrm{NO_{gas}} \rightleftharpoons [\mathrm{Cu^{I}}](\mathrm{RS\text{-}NO}) \rightleftharpoons [\mathrm{Cu^{I}}] + \mathrm{RS\text{-}NO}$$

reductive nitrosylation (metal reduced) →

← *reductive E-NO cleavage (metal oxidized)*

Ceruloplasmin, the enzyme carrying ca. 95% of all copper in the plasma,[11] generates GSNO from NO.[12] In other environments, NO reduces copper(2+) in cytochrome *c* oxidase[13] and laccase[14] with concomitant N−O bond formation to give nitrite upon the formal attack of water on NO^+. In a related fashion, Cu(dmp)$_2$(H_2O)$^{2+}$ reacts with NO in MeOH to give Cu(dmp)$_2^+$ and MeONO.[15] Intramolecular nitrosylation of a coordinated amine ligand bound to copper(2+) upon exposure of NO_{gas}[16] represents a chemical trigger in turn-on fluorescence-based approaches to sense NO.[17]

We recently reported the microscopic reverse of this process in the reductive cleavage of the N−NO bond in the nitrosamine Ph_2NNO by an electron-rich β-diketiminato copper(I) complex to give the copper(II) amide [Me_2NN]-CuNPh_2.[18] We describe herein cleavage of the RS−NO bond of a synthetic *S*-nitrosothiol by copper(I) to form a discrete copper(II) thiolate connected to the copper-promoted generation of NO from this important class of NO donors.

The addition of 2 equiv of Ph_3CSNO to {[Me_2NN]Cu}$_2$ (**1**) in toluene (ca. 0.1 M) at 0 °C results in the rapid (ca. 10 s) formation of a new blue species, [Me_2NN]CuSCPh (**2**), with λ_{max} = 731 nm that is unstable under the reaction conditions. Over the course of ca. 30 min, the solution turns green because of the final major product [Me_2NN]Cu(ON[Me_2NN]) (**3**) with λ_{max} = 647 nm.

Crystallization of the reaction mixture involving **1** and Ph_3CSNO allows for identification of the final green species **3**. The highly soluble **3** [λ_{max} = 647 nm (1700 $M^{-1}cm^{-1}$)] is isolated in 70% yield as green crystals from ether/hexamethyldisiloxane (Scheme 3). X-ray characterization of **3** (Figure 1) reveals a distorted square-planar copper(II) species with one "normal" and one nitrosated anionic β-diketiminate ligand in which the backbone methine hydrogen atom has undergone formal substitution by NO.[18,19] The π-delocalized nitrosated ligand coordinates through two different types of

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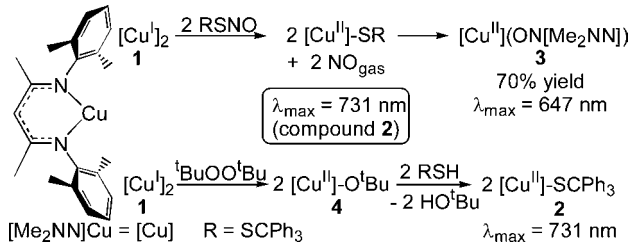
Scheme 3. Reactivity of Ph_3CSNO with 1



nitrogen atoms, including the nitrogen derived from NO [Cu–N3 = 2.037(3) Å; Cu–N5 = 1.962(3) Å], while the "normal" β-diketiminate coordination is unremarkable [Cu–N1 = 1.940(3) Å; Cu–N2 = 1.928(3) Å]. The square-planar structure is maintained in a toluene solution as judged by the large isotropic copper hyperfine A_{iso} = 223(5) MHz, which largely results from the high value of the axial parameter in the pseudoaxial frozen glass spectrum of **3** [g_1 = 2.192(3); A_1(Cu) = 567(5) MHz].

Because we anticipated the formation of **2** in the reaction of **1** with Ph_3CSNO, we sought a convenient route for its independent synthesis. The addition of 1 equiv of ^{t}BuOO^{t}Bu to **1** in toluene allows for the isolation of [Me$_2$NN]CuO^{t}Bu (**4**) on a preparative scale in 86% yield (Scheme 3) as red crystals from pentane [λ_{max} = 409 nm (1500 M^{-1}cm^{-1}) in toluene]. The X-ray crystal structure of **4** (Figure 1) reveals a planar, three-coordinate copper center due to the steric bulk of the *tert*-butoxy ligand. Related to Tolman's β-diketiminato copper(II) phenoxides[20] and our [Cl$_2$NN]CuO^{t}Bu,[21] **4** possesses particularly short Cu–O [1.788(2) Å] and Cu–N$_{\beta\text{-dik}}$ [1.879(2) and 1.890(2) Å] distances with a Cu–O–C22 angle of 123.82(12)°. The reaction of **4** with HSCPh$_3$ in toluene provides a smooth, quantitative conversion to **2** [λ_{max} = 731 nm (5800 M^{-1}cm^{-1})]. Crystals of thermally sensitive **2** may be obtained from ether at −35 °C. The X-ray structure of **2** (Figure 1) features a trigonally coordinated copper center with Cu–S [2.137(1) Å] and Cu–N$_{\beta\text{-dik}}$ distances [1.896(2) and 1.907(2) Å] along with a Cu–S–C angle of 116.80(7)° closely related to metrical parameters in Tolman's [Cu^{II}]SCPh$_3$ complex employing an *o*-isopropyl-*N*-aryl variant of the β-diketiminate ligand.[22a]

Conceptually related, the tertiary thiolate **2** and alkoxide **4** possess subtle but important differences in their electronic structure. Each exhibits a nearly axial frozen-glass electron paramagnetic resonance (EPR) spectrum [**2**, g_1 = 2.165(3), g_2 = 2.039(8), g_3 = 2.031(8); **4**, g_1 = 2.233(5), g_2 = 2.06(1), g_3 = 2.04(1); Figures S15 and S17 in the Supporting Information, SI]. The pseudoaxial hyperfine contribution from copper in alkoxide **4** [A_1(Cu) = 372(5) MHz], however, is greater than that in thiolate **2** [A_1(Cu) = 332(5) MHz], suggesting greater delocalization of the unpaired electron away from copper in the case of thiolate **2**. Density functional theory calculations corroborate this picture, indicating that thiolate **2** possesses greater spin density on the sulfur atom (Cu, 0.31 e$^-$; S, 0.39 e$^-$) than on the oxygen atom in alkoxide **4** (Cu, 0.40 e$^-$; O, 0.28 e$^-$). These trends may be rationalized by the higher orbital energies of sulfur versus oxygen that result in greater covalency in the Cu–SR interaction (Figures 2 and S12 and S13 in the SI).[23,24] Quasi-reversible cyclic voltammetry of **2** and **4** in tetrahydrofuran reveals that thiolate **2** is considerably easier to reduce ($E_{1/2}$ = −0.18 V vs NHE) than alkoxide **4** ($E_{1/2}$ = −0.52 V; Figures S10 and S11 in the SI).

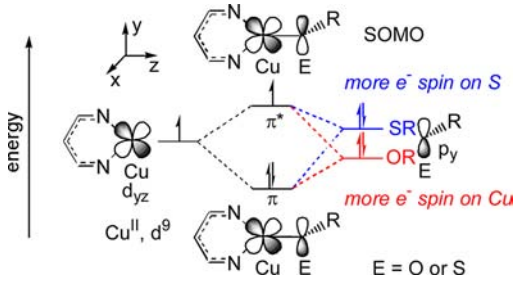


Figure 2. Orbital interactions in trigonal copper(II) thiolates and alkoxides.

The rapid conversion of **2** to other species under its synthesis conditions from Ph_3CSNO that leads to NO$_{gas}$ formation suggests that **2** is unstable toward NO (Scheme 3). We find that when the addition of 2 equiv of Ph_3CSNO to **1** at 0 °C in toluene is followed by the immediate flushing of the solution with N$_2$ to remove all NO$_{gas}$ formed in the reaction, **2** may be observed in 73% spectroscopic yield (Scheme 4). Importantly, the addition of 2 equiv of NO to pure **2** leads to **3** (79% yield) and $Ph_3CSSCPh_3$ (83% yield) (Scheme 4). We have not detected any intermediates by UV–vis spectroscopy at −80 °C in toluene during the addition of Ph_3CSNO to **1**.

In conclusion, an electron-rich copper(I) complex reacts with RSNO species to give a well-defined [Cu^{II}]SR complex with the

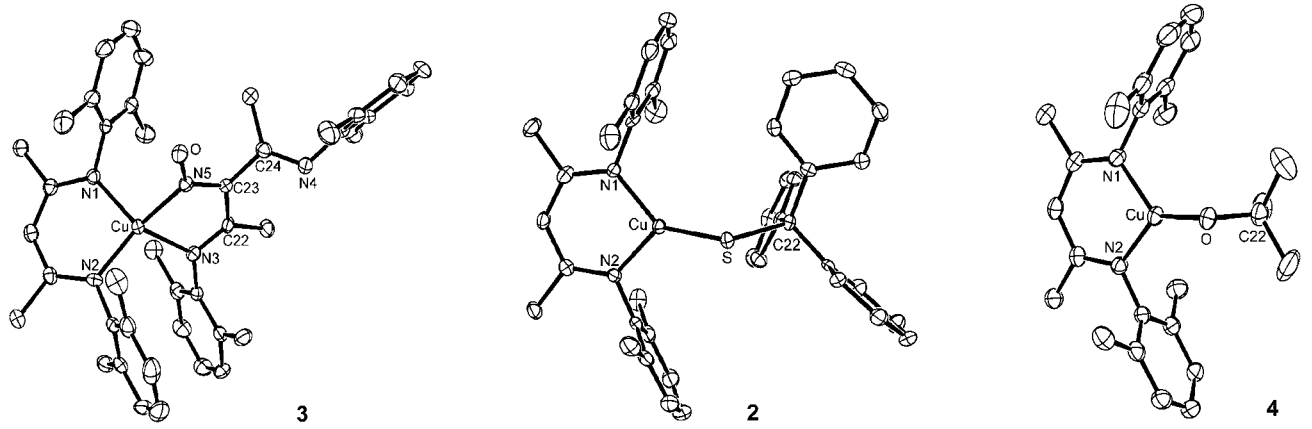


Figure 1. X-ray structures of **3**, **2**, and **4**.

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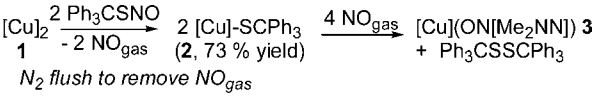
Scheme 4. Reaction of 1 with Ph_3CSNO (N_2 Flush) To Give 2 and Its Conversion to 3 with NO_{gas}



loss of NO_{gas}. This reaction represents the microscopic reverse of reductive nitrosylation commonly observed at copper(II) species upon the addition of NO_{gas} and sheds light on mechanistic possibilities in the copper-catalyzed interconversion of NO and RSNO species (Scheme 2). We note that NO reacts reversibly with oxidized type 1 copper sites in ceruloplasmin[25] and ascorbate oxidase,[26] returning to their oxidized (blue) states upon flushing with dinitrogen or argon, a coordination motif to which copper(II) thiolate **2** bears significant semblance.[22,24] Unfortunately, our copper(II) thiolate is subject to nitrosation at the central position of the β-diketiminato supporting ligand, which prevents observation of clean CuSR reductive nitrosylation with NO_{gas}. We are actively pursuing coordination environments resistant to functionalization by NO[27] to allow observation of both RSNO reductive cleavage and reductive nitrosylation at a common copper center.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental, characterization, and calculational details and X-ray crystallographic data in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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