

Communication

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Copper(I) Complex Mediated Nitric Oxide Reductive Coupling: Ligand Hydrogen Bonding Derived Proton Transfer Promotes N₂O_(g) Release

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Supporting Information Placeholder

ABSTRACT: A cuprous chelate bearing a secondary sphere hydrogen bonding functionality, $[(PV-tmpa)Cu^{1}]^{+}$, transforms $NO_{(g)}$ to $N_{2}O_{(g)}$ in high-yields in methanol. Ligand derived proton transfer facilitates N–O bond cleavage of a putative hyponitrite intermediate releasing $N_{2}O_{(g)}$, underscoring the crucial balance between H-bonding capabilities and acidities in (bio)chemical $NO_{(g)}$ coupling systems.

Transition metal mediated reductive coupling of nitric oxide $(\cdot NO_{(g)})$ to nitrous oxide $(N_2O_{(g)})$ has been implicated in a number of critical chemical processes including the denitrification enzymatic cascade within the geochemical nitrogen cycle, as well as the detoxification of automobile and industrial exhausts by catalytic convertors.¹ Further, pathogens utilize nitric oxide reductase (NOR) activity in debilitating host defense activities exerted by 'NO(g).² Bacterial NORs may possess a heme/non-heme diiron (or heme-copper; HCO's) active site.^{1b,c,3} Also, widely occurring (bacteria, archaea and protozoa) flavin dependent diiron (non-heme binuclear)⁴ and fungal P450nor (i.e., with heme-thiolate active sites)⁵ enzymes are important. The corresponding chemical transformation $(2 \cdot NO_{(g)} + 2H^+ + 2e^- \rightarrow N_2O_{(g)} + H_2O)$ involves two key mechanistic steps: (1) N–N bond formation following the reduction of $NO_{(g)}$ to give hyponitrite $(N_2O_2^{2-}; HN)$,^{1b,6} and (2) N–O bond cleavage, with or without the assistance of protons. In that, the corresponding metal centers and adjacent water molecules/amino acid side chains have been proposed to act as electron and proton sources, respectively.^{1b,7} However, precise details concerning the N-N coupling process leading to the HN intermediate, the varying possible metal binding mode(s) (Chart 1) to this species (or is it $N_2O_2^{1-}$ or neutral N_2O_2 ^{8,9} and the exact timing of metal redox shuttling and protonation events are to-date not well understood.^{4a,7e,10} In-depth comprehension of such details has significant impacts in rational design of NOR-related therapeutics and managing key green house gases such as $N_2O_{(g)}$.^{2,11}

In this regard, small-molecule models are fundamentally important in shedding light on a number of these mechanistic unknowns.1d,10b,12 Our own efforts have focused on combinations of heme, non-heme Fe- and Cu-containing 'NO_(g) coupling systems.^{1b,13} The latter are not only of interest with regard to heme-copper oxidases (vide supra), $^{1b,3c-e}$ but also (1) Cu-dependent nitrite reductases that transform 'NO(g) to $N_2O_{(g)}$ under large 'NO_(g) concentrations,^{1a,b} (2) Cu^I/·NO_(g) interactions that lead to $\cdot NO_{(g)}$ -disproportion-ation (Cu^I + $3 \cdot NO_{(g)} \rightarrow N_2O_{(g)} + Cu^{II}(NO_2^{-}))$,^{1b,9a,14} and/or physiological peroxynitrite (-OON=O) formation,¹⁵ and (3) with regard to environmentally-benign next generation 'NO(g)detoxification catalysts.^{1d-f,16}

Chart 1. Different Hyponitrite Binding Modes Involving Two Metal Ion Centers. $^{\rm 1d,4e}$



Our previous account has revealed that in protic solvents (i.e., MeOH) [(tmpa)Cu^I(MeCN)]⁺ (tmpa = tris(2-pyridylmethyl)amine) can efficiently couple 'NO_(g), giving a dicopper(II) *trans*-hyponitrite-bridged species, ([{(tmpa)Cu^{II}}₂(μ -N₂O₂²⁻)]²⁺), which undergoes redox reversal in aprotic solvents leading to Cu^I-mediated 'NO_(g)-disproportionation.¹⁷ The present work, in accordance with other reports^{6b,12f,18} highlights the importance of non-covalent interactions in choreographing selective/efficient pathways for metal-mediated 'NO_(g) reduction. In support, hydrogen bonding: (1) has been proposed for **HN**-level intermediates of HCO's,^{3c,6b} (2) is thought to mediate proton transfer during NOR turnover,¹⁹ and (3) has been shown to stabilize the Fe^{III}– μ -oxo–Fe^{III} resting state of NOR.²⁰ The latter observation also suggests the possibility of H-bonding interactions with the ligated atoms of a putative **HN** intermediate in NOR. Moreover, the balance between acidity and H-bonding strength is imperative for NOR activity, where protonation of the **HN** intermediate is thought to lead to N–O bond cleavage, releasing N₂O_(g).^{4h,7e,12f}

Herein, we report the first example of a complete Cu-only functional model of NOR that stoichiometrically couples $\cdot NO_{(g)}$ to $N_2O_{(g)}$ assisted by an intramolecular hydrogen bonding derived proton transfer event. The complex [(PV-tmpa)Cu^I](B(C₆F₅)₄)²¹ promotes efficient coupling of $\cdot NO_{(g)}$ generating a putative H-bonded **HN** intermediate ([{(PV-tmpa)Cu^{II}}₂(μ -N₂O₂²⁻)]²⁺; **A**; Figure 1) in MeOH at RT, which upon protonation by the in-built pivalamido groups quantitatively releases $N_2O_{(g)}$, producing the structurally-characterized copper(II) product complex **B** (Figure 1) supported by the deprotonated PV-tmpa ligand.



Figure 1. Proposed reaction landscape for $[(PV-tmpa)Cu^{I}]^{+}$ with excess 'NO_(g) in MeOH under ambient conditions (top). The CF₃-analogue couples 'NO_(g) instantaneously, with no observable intermediate (bottom).

When a pale yellow MeOH solution of $[(PV-tmpa)Cu^{l}]^{+}$ (λ_{max} ($\epsilon/M^{-1}cm^{-1}$) = 370 nm (3300)) is treated with 'NO(g) at RT, a rapid color change to forest green was observed (**A**; $\lambda_{max}(\epsilon) = 260(17800)$, 320(5900), 645(160) and 760(150) nm), which gradually turns into a sea green solution (**B**; $\lambda_{max}(\epsilon) = 260(19100)$, 320(6600), 645(145) and 760(140) nm) over two hours (Figure 2). The EPR spectral analysis of both the intermediate and the final species indicates the presence of a single Cu^{II} species within a trigonal bipyramidal (TBP) geometry (i.e., $g_{\perp} > g_{||}$ with a d_z^2 ground state; Figure 2) around the copper center;^{17} the g and A values closely resemble those of previously published species [(PV-tmpa)Cu^{II}(OOH)]⁺.²² Moreover, the optimized Cu^I: 'NO(g) stoichiometry was found to be 1:1.5 (essentially a 1:1 reaction²³) as observed for [(tmpa)Cu^{II}(MeCN)]⁺.¹⁷



Figure 2. Top: Electronic absorption spectral changes during a reaction of 0.1 mM [(PV-tmpa)Cu^I]⁺ (red) with 1.5 equiv. 'NO_(g) at RT, rapidly producing **A** (green), which then slowly decays to **B** (blue). Insets: Absorbance changes for the same reaction when 2 mM [(PV-tmpa)Cu^I]⁺ is used, and the respective time trace for 645 nm. Bottom: EPR spectra of **A** (green) and **B** (blue) at 20K (2 mM in MeOH/EtOH = 1:1 glass; $g_{\perp} = 2.214$, $A_{\perp} = 135$ G; $g_{\parallel} = 2.044$, $A_{\parallel} = 71$ G).

The final Cu^{II} product, **B** was isolated in high yield (>98%),²⁴ and was unambiguously characterized via single crystal X-ray crystallography. In agreement with its solution EPR spectrum (Figure 2), the crystal structure (Figure 3) reveals a pentacoordinate Cu^{II} center within a distorted TBP geometry ($\tau = 0.77$).²⁵ In that, the pivalamido group is deprotonated (no residual electron density peak could be found near N5 in the difference Fourier map) and the Cu^{II} ion is iminoxide-ligated.²⁶ In further support, (1) only a single $B(C_6F_5)_4^$ counteranion is found in the asymmetric unit, indicating that the ligand-Cu^{II} fragment is overall +1 charged; (2) comparison of N5...C19, C19...O1, and O1...Cu distances (1.317(2), 1.280(2), and 1.8801(13) Å) in **B** with the previously published X-ray structures of $[(PV-tmpa)Cu^{II}(N_3^{-})]^+$ and [(PV-tmpa)Cu^{II}]²⁺ (1.36,²⁷ 1.21,²⁷ and 1.94 Å²⁸)²⁹ unambiguously establish the iminoxide formation (i.e., the contraction and elongation of the N5...C19 and C19...O1 bond 1

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lengths, respectively), thus the anionic nature of the axially Cu^{II}-coordinated *O*-atom in **B** (Figure 3).



Figure 3. Displacement ellipsoid plot (50% probability level) of the monocationic complex **B** at 110(2) K. The H atoms and the noncoordinating $B(C_6F_5)_4$ - counteranion are omitted for clarity.

The formulation and yield of **B** was further confirmed by comparing its spectroscopic properties to those of an authentic compound, prepared by reacting $[(PV-tmpa)Cu^{II}]^{2+}$ complex²⁹ with 1 equiv (*t*-Bu₄N⁺)(OH⁻) in acetonitrile at RT.²⁴ These findings clearly elucidate that $[(PV-tmpa)Cu^{I}]^{+}$ reacts with 1 equiv $NO_{(g)}$ to stoichiometrically generate **B** in MeOH under ambient conditions, with the intermediacy of a distinct, unstable intermediate (Figure 2).

Interestingly, without the in-built pivalamido moiety, [(tmpa)Cu^I(MeCN)]⁺ generates the stable hyponitrito complex $[{(tmpa)Cu^{II}}_2(\mu N_2O_2^{2-})]^{2+}$ under identical conditions which can subsequently react with 2 equiv HCl stoichiometrically producing $[(tmpa)Cu^{II}(Cl)]^+$, N₂O_(g), and H₂O.¹⁷ This body of evidence leads us to the proposal summarized in Figure 1, where $[(PV-tmpa)Cu^{I}]^{+}$ reacts with $\cdot NO_{(g)}$ generating the hyponitrito complex, $[{(PV-tmpa)Cu^{II}}_2(\mu-N_2O_2^{2-})]^{2+}$, which upon two ligand derived proton transfer events from each PV-tmpa ligand fragment produces 2 equiv **B**, $N_2O_{(g)}$, and H₂O. In strong agreement, [(PV-tmpa)Cu^I]⁺ reacts with ~1.5 equiv of $\cdot NO_{(g)}$ to liberate $N_2O_{(g)}$ in ~86% yield as detected and quantified by headspace gas chromatographic analysis;²⁴ no nitrites were detected in solution under these conditions, ruling out a Cu^I-mediated NO_(g) disproportionation pathway.¹⁴ Therefore, we do attribute the metastable³⁰ specobserved intermediate A to [{(PVtroscopically tmpa)Cu^{II} $_{2}(\mu$ -N₂O₂²⁻)]²⁺, with an *O*-bound hyponitrito ligand as in $[{(tmpa)Cu^{II}}_2(\mu-N_2O_2^{2-})]^{2+.31}$

Further, when the pivalamido group of the supporting ligand was replaced by CF₃C(O)NH- having a far more acidic amide-hydrogen (by \geq 8 pKa units),³² the Cu^I complex (X-ray structure determined),²⁴ reacts far more rapidly with \cdot NO_(g) giving N₂O_(g) in ~96% yield (t_{1/2} \approx 0.5 min), with no observable intermediate even when the transformation is carried out at –40 °C (Figure 1).²⁴ Protons are known to be crucial to the NOR cycle. 7a,7b,12a,12g

Our formulation of $[{(PV-tmpa)Cu^{II}}_2(\mu-N_2O_2^{2-})]^{2+}$ (A) is further supported by its spectroscopic features: (1) The electronic absorption characteristics of A and B are strikingly similar (Figure 2), revealing a close similarity in their Cu^{II} primary coordination spheres and geometries (i.e., N_4O^- as found in **B**; see Figure 3); thus, in support of an O-bound hyponitrito ligand in A (also see below). (2) The EPR spectrum (Figure 2) of A (as well as in B) indicates the presence of siteisolated (i.e., magnetically and/or electronically) Cu^{II} centers within a TBP geometry (also see the SI)²⁴ as in the case of $[\{(tmpa)Cu^{II}\}_2(\mu N_2O_2^{2-})]^{2+.17}$ (3) Although electronic absorption features of A could be altered by the potential Hbonding interactions between the hyponitrito moiety and PVtmpa ligand framework, we note that its charge-transfer feature at 320 nm (Figure 2) is still comparable to that of $[\{(tmpa)Cu^{II}\}_2(\mu-N_2O_2^{2-})]^{2+}$ at 310 nm.¹⁷

To further probe the geometric-electronic properties of the putative hyponitrito complex (**A**), we independently synthesized and characterized the related cupric nitrito complex, $[(PV-tmpa)Cu^{II}(NO_2)]^+$, via a metathesis reaction between $[(PV-tmpa)Cu^{II}(CI)]^+$ and AgNO₂.²⁴ The electronic absorption energies of $[(PV-tmpa)Cu^{II}(NO_2)]^+$ are very similar to those of **A** and **B**, as expected, especially the charge-transfer peak at 320 nm.²⁴ As well, the EPR spectrum of [(PV $tmpa)Cu^{II}(NO_2)]^+$ is also comparable to those of **A** and **B**. All of these findings fully reflect close similarities in coordination geometries about their Cu^{II} centers in solution.²⁴

The solid-state structure of $[(PV-tmpa)Cu^{II}(NO_2)]^+$ is unique, possessing a pseudo-bidentate nitrito ligand.²⁴ Markedly, the proximal *O*-atom of the nitrite ligand is patently Hbonded with the amide group of the PV-tmpa ligand framework with a N_{amide}····O_{nitrite} distance of 2.877(2)Å (NH_{amide}···O_{nitrite} = 2.025(19)Å), and a N–H_{amide}–O_{nitrite} angle of 162.0(19)°.³³ This observation, along with unambiguous literature evidence that H-bonding occurs between the N-H moiety of the ligand pivalamido group and the proximal Xatom in [LCu^{II}-X]⁺ complexes (X⁻ = superoxide, peroxide, hydroxide, hydroperoxide, azide;)²⁴ supports our claim of analogous H-bonding interaction in [{(PV-tmpa)Cu^{II}}₂(μ -N₂O₂²⁻)]²⁺ (**A**), as depicted in Figure 1.

To further interrogate the geometric properties of the putative cupric hyponitrito species, $[{(PV-tmpa)Cu^{II}}_2(\mu N_2O_2^{2-})]^{2+}$ (**A**), we carried out Density Functional Theory (DFT) computations along with COSMO continuum solvent model. In light of our knowledge of the X-ray structure of $[{(tmpa)Cu^{II}}_2(\mu N_2O_2^{2-})]^{2+,17}$ the structure of **A** was optimized with (and without) a MeOH molecule placed near each O_{HN} atom (*vide infra*).³⁴ The distorted TBP pentacoordinated Cu^{II} centers are bridged by an *O*,*O*' bound *trans*-hyponitrito ligand,³⁵ Cu...Cu = 5.861 Å (Figure 4), comparable to that in $[\{(tmpa)Cu^{II}\}_2(\mu-N_2O_2^{2-})]^{2+}$ (5.5648 Å).¹⁷ The Cu--distal $N_{\rm HN}$ distance is larger in A (3.057 Å) compared to that in $[\{(tmpa)Cu^{II}\}_2(\mu-N_2O_2^{2-})]^{2+}$ (2.851 Å; $\tau = 0.55$), leading to a TBP-like geometry in **A** (τ = 0.71). These unique structural features of **A** compared to $[{(tmpa)Cu^{II}}_2(\mu-N_2O_2^{2-})]^{2+}$ are presumably due to the enhanced steric encumbrance exerted by the large pivalamido groups, the H-bonding MeOH molecules, and intramolecular H-bonding modifications. For A, Cu–N and Cu–O_{HN} distances of 2.0 – 2.2 and 1.967 Å were observed, respectively, both in close resemblance to $[\{(tmpa)Cu^{II}\}_{2}(\mu-N_{2}O_{2}^{2-})]^{2+}.^{24}$ Most importantly, the Cubound O_{HN} atoms are strongly H-bonded in A, with an N_{a-} _{mide}···· O_{HN} distance of 2.874 Å (NH_{amide}···· O_{HN} = 1.862 Å). The hyponitrito N=N and N-O distances are 1.260 and 1.356 Å, respectively, in excellent agreement with previously observed and/or calculated values.^{1d,6b,12e,12f,17,36}



Figure 4. DFT optimized structure of **A**, where a MeOH molecule and N_{amide} are H-bonded to each O_{HN} atom. Non-H-bonding hydrogen atoms are omitted for clarity. Also, see the text.

Comparison of single-point energies of geometry optimized structures revealed further insights into this system. Firstly, the overall stability of A is increased by as much as 15.7 kcal/mol with the addition of the two MeOH molecules as described above, indicating strong H-bonding interactions (Figure 4)³⁷ between the MeOH molecules and $O_{\rm HN}$ atoms in addition to the $NH_{amide} \cdots O_{HN}$ interaction; the $O_{MeOH} \cdots O_{HN}$ distance is 2.859 Å (OH_{MeOH} $\bullet \bullet O$ _{HN} = 1.882 Å) and the N_a- $_{\text{mide}} \bullet \bullet O_{\text{HN}}$ distance is 2.882 Å (NH_{amide} $\bullet \bullet O_{\text{HN}} = 1.920$ Å). This observation is in-line with the computationally observed hydrogen bonding interactions of the hyponitrito intermediate of HCO's that included an H-bonded active site water molecule.^{6b,18a} Secondly, the comparison of single point energies of A, B, $N_2O_{(g)}$, and H_2O infer a 90.9 kcal/mol overall free energy change for the conversion: $\mathbf{A} \rightarrow 2\mathbf{B} + N_2O_{(g)} + H_2O$ (second step in Figure 1), clearly illustrating the favorable

energetics driving its spontaneity, which results in the impaired stability of ${\bf A}$ even in protic solvents.

In conclusion, we describe the first example of a copperonly complete NOR model system with in-built, acidic Hbonding functionalities, which protonates the putative hyponitrite intermediate to efficiently couple ' $NO_{(g)}$, leading to $N_2O_{(g)}$ production. This work underscores the critical importance of fine-tuned acidities of active site components and/or proximal water molecules (i.e., the H-bonding vs. protonation capabilities) in governing the efficient ' $NO_{(g)}$ reductive coupling in NOR. Further interrogations are warranted for the vivid understanding of the timing of protonation and/or redox shuttling events implicated with N–N bond formation, and N–O bond cleavage.

ASSOCIATED CONTENT

The Supporting Information is available free of charge on the ACS Publications website at http://pubs.acs.org.

Synthetic and analytical details (methodologies and UV-Vis, EPR, and FT-IR spectra; gas chromatograms); X-ray diffraction data collection details and CIF files.

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The authors declare no competing financial interest.

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23. Typically for Cu: $NO_{(g)}$ 1:1 reactions, a slight excess of $NO_{(g)}$ is required due to its equilibration between the reaction mixture and the headspace of the cuvette. Also see Ref 17.

24. See Supporting Information.

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27. These bond distances are the same for both [(PV-tmpa)Cu^{II}(N₃⁻)]⁺ and [(PV-tmpa)Cu^{II}]²⁺ structures.

28. In $[(PV-tmpa)Cu^{II}]^{2+}$, the pivalamido *O*-atom is coordinated to the Cu(II) center.

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30. $t_{\rm l_2}$ = 30 min in MeOH at RT when prepared with 1.5 equiv of *NO(g).

31. A reviewer pointed out that for non-heme iron reactivity with $NO_{(g)}$ i.e. the release of $N_2O_{(g)}$ and formation of a bridging

Fe–O–Fe complex alone provides enough driving force for the reaction (see refs 4(d)–4(f)). In fact, this has also been observed for copper, where 2 (L)Cu¹ + 2 ·NO_(g) gives a μ -oxo dicopper(II) complex + N₂O_(g), i.e., without the need for protons. See the following references: (a) Paul, P. P.; Karlin, K. D. Functional Modeling of Copper Nitrite Reductases: Reactions of NO₂⁻ or NO with Copper(I) Complexes *J. Am. Chem. Soc.* **1991**, *113*, 6331. (b) Obias, H.V.; Lin, Y.; Murthy, N.N.; Pidcock, E.; Solomon, E.I.; Ralle, M.; Blackburn, N.J.; Neuhold, Y.M.; Zuberbuhler, A.D.; Karlin, K.D. Peroxo-, Oxo-, and Hydroxo-Bridged Dicopper Complexes: Observation of Exogenous Hydrocarbon Substrate Oxidation *J. Am. Chem. Soc.* **1998**, *120*, 12960.

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34. Despite the presence of H-bonding pivalamido group, MeOH was still necessary for the stabilization of the putative HN intermediate. This is presumably due to the theoretically observed large stabilization **A** acquires by H-bonding with MeOH solvent. In acetonitrile, tetrahydrofuran etc, the reaction followed the common pathway involving $NO_{(g)}$ disproportionation, yielding nitrite. See reference 14.

35. Our multiple attempts failed to produce an energy minimized geometry optimized structure for an N,N'-hyponitrito dicopper species and such a sterically encumbered species is not likely to form. An analogous N,N'-hyponitrito species was previously found to be 30 kcal/mol higher in energy than the O-bound form. See Reference 4h.

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TOC Graphic

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