JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
Communication
pubs.acs.org/JACS

Primary Amine Stabilization of a Dicopper(III) Bis(μ-oxo) Species: Modeling the Ligation in pMMO

Cooper Citek,[†] Bo-Lin Lin,[†] Tim E. Phelps,[‡] Erik C. Wasinger,[‡] and T. Daniel P. Stack*[,†]

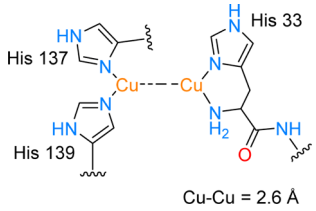
[†]Department of Chemistry, Stanford University, Stanford, California 94305, United States
[‡]Department of Chemistry and Biochemistry, California State University, Chico, California 95929, United States

Supporting Information

ABSTRACT: Here we report the formation of the first examples of dicopper(III) bis(μ-oxo) complexes ligated by the primary amines, propylenediamine, and *N,N,*-dimethyl propylenediamine. Stabilization of these new compounds is effected at −125 °C by "core capture"— introduction of exogenous ligand to a preformed dicopper(III) bis(μ-oxo) complex supported by the peralkylated tetramethyl propylenediamine. Primary amine ligation in these compounds matches the single primary amine coordination of the putative active site of particulate methane monooxygenase (pMMO) and polysaccharide monooxygenase. Reactivity studies presented here show primary amine ligated cores are competent oxidants, capable of activating C−H bonds by an H-atom abstraction mechanism. Trends in spectroscopy, structure, and reactivity provide hints to the potential role of primary amine ligation in pMMO: increased substrate accessibility to the redox active orbitals of the Cu_2O_2 core and greater stabilization of the oxidant without attenuation of oxidizing power.

Important recent studies have indicated a binuclear copper-site to be the possible active site of the oxidative enzyme particulate methane monooxygenase (pMMO) (Chart 1).[1−5]

Chart 1. Proposed Active Site of pMMO



pMMO mediates the remarkable oxidative transformation of methane to methanol in methanotrophic bacteria under ambient conditions.[6] X-ray crystallographic investigations by the group of Rosenzweig identify an asymmetric binuclear site, in which one copper is coordinated by the two imidazole groups of histidine residues and the other is chelated by an imidazole and primary amine of an N-terminal histidine.[7] Sequence homology between pMMO, particulate butane monooxygenase (pBMO), and the biologically widespread, copper-dependent ammonia monooxygenases (AMO) implicate similar active-site oxidants in their respective transformations.[8,9] Primary amine ligation of copper in an enzyme is rare, and the only other crystallographically characterized primary amine ligation to copper in an active site is that in the polysaccharide monooxygenase GH61, a copper- and dioxygen-dependent enzyme that oxidatively cleaves glycosidic bonds in cellulose; again an N-terminal histidine group chelates the copper center.[10]

Though several examples of secondary amine ligation are known in synthetic copper-dioxygen coordination complexes,[11−16] no study has trapped and fully characterized a copper-dioxygen species with primary amine ligation, as N−H bonds presumably initiate or enhance irreversible decay pathways. To our knowledge, only one primary amine ligated $Cu-O_2$ intermediate, a mononuclear superoxide, has been observed transiently in stopped-flow experiments.[17] It is thus uncertain what type of coordination modes with dioxygen and oxidation state(s) of copper can be stabilized by primary amine ligation in various ligand architectures. An outstanding issue in biological inorganic chemistry is the precise role of primary amine ligation in these oxidative enzymes.

Here we report the first fully characterized examples, among all synthetic or biological $Cu-O_2$ structures, of stable complexes supported by primary amine ligation (**2** and **3**). Not only are these complexes structurally relevant to the active site of pMMO, they oxidatively activate C−H bonds through a mechanism best described as H-atom abstraction. The complexes discussed here are in fact milestones in the field of $Cu-O_2$ chemistry, in that they bear the *simplest* ligation currently known to the literature.

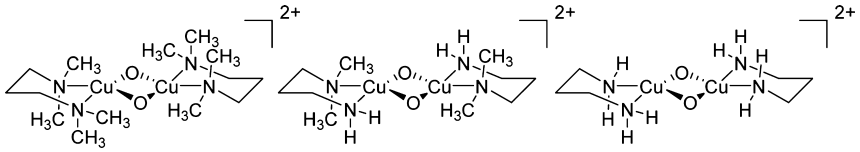
Oxygenation of $[Cu(CH_3CN)_4]^+X^-$ salts with tetramethyl propylenediamine (TMPD) in a variety of aprotic solvents is known to quickly form a fully characterized, stable dicopper(III) bis(μ-oxo) core (Cu_2O_2) at −80 °C (**1**).[18] However, direct injection of a solution of $[Cu(CH_3CN)_4]SbF_6$ with *N,N*-dimethyl propylenediamine (DMPD) or propylenediamine (PD) to dioxygen-saturated 2-MeTHF at −125 °C quickly leads to a light blue mixture without detectable accumulation of recognizable $Cu-O_2$ compounds, as monitored by *in situ* UV−vis spectroscopy. Even at this extreme solution temperature, $Cu-O_2$ intermediates on the path to characterizable compounds appear to be short-lived and highly reactive. Literature precedent of simple ligand exchange from parent $Cu-O_2$ coordination compounds by exogenously introduced ligands lead us to believe we could avoid potentially reactive intermediates by capturing the Cu_2O_2 core from a preformed, stable complex by the

Received: August 21, 2014
Published: September 30, 2014

ACS Publications © 2014 American Chemical Society

dx.doi.org/10.1021/ja508630d | J. Am. Chem. Soc. 2014, 136, 14405−14408

Table 1. Physical Properties of Compounds 1−3



1 2 3

	UV−vis λ, nm	EXAFS[b]			DFT[c]			calcd
	(ε, mM^{-1} cm^{-1})[a]	Cu−Cu (Å)	Cu−O (Å)	Cu−N (Å)	Cu−Cu (Å)	Cu−O (Å)	Cu−N (Å)	ΔG (H$^+$/e$^-$)[d]
1	403 (29.4)	2.85	1.84	2.02	2.83	1.81	2.00	0 kcal mol^{-1}
	302 (18.7)							
2	390 (27.8)	2.82	1.85	2.01	2.75	1.81	1.98	−1.3 kcal mol^{-1}
	290 (20.3)					1.80	1.94	
3	375 (22.6)	2.77	1.86	2.00	2.71	1.80	1.94	−2.9 kcal mol^{-1}
	278 (21.0)							

[a]MeTHF, −125 °C. [b]Compounds **1**−**3** show pre-edge absorption features at 8980.2, 8981.1, and 8980.7 eV, respectively, supporting a Cu(III) oxidation state. [c]Optimized at m06/tzvp level of theory. [d]Relative to compound **1**.

introduction of a ligand featuring a primary amine.[19−21] Indeed, 2 mol equiv of DMPD, injected to a solution of **1** equilibrated in 2-MeTHF at −125 °C, quickly capture the Cu_2O_2 core and stabilize the new compound **2**, a fully characterized Cu_2O_2 dimer (*vide infra*). Additionally, PD is similarly capable of supporting the high-valent Cu_2O_2 complex **3**.

The new compounds **2** and **3** appear to be indefinitely stable at −125 °C but quickly decay in a first-order manner at elevated temperatures (e.g., −100 °C). Their optical absorption spectra exhibit two characteristically intense UV transitions of Cu_2O_2 cores[11,22] but are markedly blue-shifted compared to those of the parent compound **1** (Table 1). Stepwise addition of exogenous DMPD or PD to **1** gives tight isosbestic transformations of **1** to **2** or **3**, respectively. Cu K-edge X-ray absorption spectroscopy of **1**−**3** show a clean pre-edge feature indicative of Cu(III) complexes.[23,24] Extended X-ray absorption fine structure (EXAFS) analyses indicate a systematic contraction of the Cu−Cu separation of **1**−**3** (285 to 280 to 277 pm), consistent with incremental reduction of ligand steric demands imposed by ligand alkylation (Table 1). DFT computed structures and time-dependent DFT calculations support identification of **2** and **3** as the products of ligand core capture by recapitulating the experimental metrical parameters (Table 1) and predicting a systematic blue shift of the electronic transitions across the series (Figure S11).

Core capture by DMPD and PD occurs upon mixing and appears to be nearly quantitative. However, even slight excesses beyond 2 mol equiv lead to slow but complete decay of **2** or **3**. Reductive titrations of Cu_2O_2 complexes are a rigorous method of evaluating the degree of formation of such compounds;[25] stepwise reaction of compounds **1**−**3** with 5,6-isopropylidene ascorbic acid, a reductive titrant possessing a weak O−H bond,[26] indicates concentrations of **2** are identical to the parent **1** after core capture while **3** are consistently >90% (Figures S2−S7).

Typically ligand basicity is used as a proxy for the strength of a metal−ligand σ-bond interaction. **2** and **3** provide counter-intuitive examples in which overall less basic ligands displace a more basic one in a metal−ligand interaction. The phenomenon is actually well-known and understood on simple steric arguments: PD presents fewer alkyl substituents to the copper center than does DMPD or TMPD, thereby forming closer, *more donating* ligand−metal interactions to the high-valent Cu(III) center. Ligand competition experiments further corroborate this conclusion from a thermodynamic perspective. Injection of PD into a solution of **2** leads to rapid Cu_2O_2 core transfer, yielding **3**, as assessed by its characteristic optical features. However, excess equivalents of TMPD or DMPD are not capable of displacing PD from complex **3**. The ligand of lower basicity yields a more thermodynamically stable complex. Thermodynamically calibrated DFT calculations correlate with these observations; theoretical isodesmic equilibrium reactions involving Cu_2O_2 core capture indicate ligand exchange is favored with the less alkylated ligand (Scheme 1).

Scheme 1. Isodesmic Ligand Exchange Reactions

1 + 2·DMPD ⇌ **2** + 2·TMPD ΔG = -4.2 kcal^{-1} mol^{-1}
1 + 2·PD ⇌ **3** + 2·TMPD ΔG = -5.1 kcal^{-1} mol^{-1}
2 + 2·PD ⇌ **3** + 2·DMPD ΔG = -0.9 kcal^{-1} mol^{-1}

While **3** is more stable than **1** in the presence of excess TMPD, isodesmic reactions involving the computed structures of **1** and **3** and their corresponding 1H$^+$/1e^{-1} reduced species also clearly indicate that the PD ligand creates a more powerful 1H$^+$/1e^{-1} oxidant in **3** than in **1** (Scheme 2), by substituting primary amine for tertiary amine ligation.

Scheme 2. Isodesmic H-Atom Transfer Reactions

$[\mathbf{1}]^{2+}$ + $[\mathbf{1{\bullet}H}]^{2+}$ ⇌ $[\mathbf{1{\bullet}H}]^{2+}$ + $[\mathbf{1}]^{2+}$ ΔG = 0 kcal^{-1}mol^{-1}
$[\mathbf{2}]^{2+}$ + $[\mathbf{1{\bullet}H}]^{2+}$ ⇌ $[\mathbf{2{\bullet}H}]^{2+}$ + $[\mathbf{1}]^{2+}$ ΔG = -1.3 kcal^{-1}mol^{-1}
$[\mathbf{3}]^{2+}$ + $[\mathbf{1{\bullet}H}]^{2+}$ ⇌ $[\mathbf{3{\bullet}H}]^{2+}$ + $[\mathbf{1}]^{2+}$ ΔG = -2.9 kcal^{-1}mol^{-1}

Evidence for a stronger metal−ligand interaction is discernible in the incremental blue shifting of the UV transitions across the **1**−**3** series (Figure S1). Time-dependent DFT calculations predict that the increased donation of the PD primary amine significantly raises the energy of the accepting orbitals, thus *increasing* the energy of the characteristic transitions (Figures S11−17). EXAFS metrical parameters also indicate a closer association of N-based ligand with Cu across the series and contraction of the cores (Table 1).

The Cu_2O_2 core of **3** is remarkably exposed compared to **1** (Figure 1) and oxidizes exogenous C−H bearing substrates, which the more sterically encumbered **1** does not. At −125 °C, **3** rapidly reacts with 9,10-dihydro-methylacridine (C−H bond dissociation energy (BDE) ≈ 74 kcal mol^{-1})[27] in a pseudo-first-

dx.doi.org/10.1021/ja508630d | J. Am. Chem. Soc. 2014, 136, 14405−14408

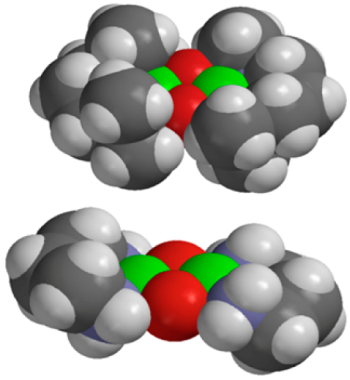


Figure 1. Space-filling models of **1** (top) and **3** (bottom).

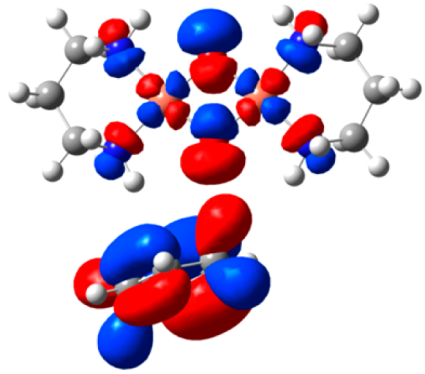


Figure 2. Reaction trajectory of 1,4-cyclohexadiene with **3**, including the frontier orbitals of **3** (top, LUMO) and 1,4-cyclohexadiene (bottom, HOMO).

order manner (4−32 equiv per **3**) to produce the dehydrogenated acridinium product, as determined by optical spectroscopy (Figure S8b).[28,29] The substantial kinetic isotope effect ($KIE_{H/D} \approx 31$) from reaction with the deuterated substrate analog[30] firmly establishes a rate limiting C−H(D) scission.[31] Under identical conditions, <1% decrease in the optical spectrum of **1** is observed over the course of an hour in the presence of 16 equiv of methylacridine, placing the difference in oxidation rate well over 1000-fold. An intermediate decay rate is observed for **2** with methylacridine ($k_{PD}/k_{DMPD} \approx 25$), positively demonstrating reactivity is enhanced by reduced steric encumbrance. Using 25−100 equiv, **3** reacts with stronger C−H bonds such as 1,4-cyclohexadiene (C−H BDE ≈ 76 kcal mol^{-1}) and dihydroanthracene (BDE ≈ 76 kcal mol^{-1}, $KIE_{H/D} \approx 25$)[32] under a pseudo-first-order decay (Figures S9−10).[33] No reaction is observed between **1** and 1,4-cyclohexadiene or dihydroanthracene under identical conditions.[18]

DFT transition-state analysis predicts that the most favorable reaction path of **3** with 1,4-cyclohexadiene proceeds along a singlet surface with H-atom abstraction by the Cu_2O_2 core at a transition state only ≈3 kcal mol^{-1} higher than the reactants, followed by relaxation to a strongly antiferromagnetically coupled biradical intermediate; the cyclohexadiene radical couples to the Cu_2OOH core through the antibonding OH σ-bond. Broken symmetry calculations of this intermediate species place its energy significantly below that of a triplet system or a restricted singlet (Figure S19). A triplet H-atom abstraction process for C−H bond cleavage is excluded by DFT as the barrier is estimated to be at least 20 kcal mol^{-1} higher than the singlet pathway.

This reaction profile correlates well with the differential reactivity of **1** and **3** with C−H substrates described above. A frontier orbital analysis of the singlet reaction path of **3** with cyclohexadiene shows the oxygen-based, electrophilic accepting orbital to be oriented along the O−O bond vector (Figure 2); the methyl groups of **1** preclude access of the substrate to these reactive orbitals. In **1**, available vacant oxygen-based orbitals above the Cu_2O_2 plane, where the steric demands of the ligand alkyl substituents are minimized, are of too high energy or are of inappropriate symmetry to facilitate electrophilic oxidative reactivity with C−H bonds; no transition state on the singlet or triplet surface could be found for **1**. Substrate accessibility to the Cu_2O_2 core is fundamentally important to the oxidative reactivity of **3**.

We have introduced two new compounds, the first fully characterized, stable Cu-dioxygen complexes in biology or synthetic chemistry bearing ligation by a primary amine. Complexes **2** and **3** are the only known biomimetic models of the primary amine ligation found in the active site of pMMO. **3** facilitates the oxidation of O−H and C−H bonds. Oxidation of substrates by a Cu_2O_2 model complex with primary amine ligation, even at the extremely low solution temperatures reported here, instigates speculation of the active-site oxidant in pMMO. The close separation (260 pm) of the copper ions in the pMMO crystal structure could be consistent with a dicopper(III) state, in that oxygenation of the dicopper active site would require minimal structural rearrangement to achieve the Cu_2O_2 coordination mode (Cu−Cu ≈ 280 pm),[34] yet spectroscopic evidence of such a core in biology is lacking currently. The electronic isomer, μ-η^2:η^2 dicopper(II) peroxide (Cu−Cu ≈ 360 pm), is well characterized in the structurally homologous active sites of tyrosinase, hemocyanin, and NspF[4]. However, numerous synthetic model studies underline the disparity in reactivity of the dicopper(II) peroxide toward H-atom abstraction in comparison to the dicopper(III) bis(μ-oxide).[35−37] It may be that a unique high-valent biological active site oxidant is in fact required to oxidize the strong C−H bonds in methane.

We have shown here that primary amines are capable of increasing the reactivity of a bis(μ-oxo) core through several factors. Primary amines are strong ligands and therefore stabilize the Cu(III) oxidation state more than do tertiary amines. Additionally, the limited steric demands of the primary amine in comparison to those of a peralkylated amine further enhance reactivity by affording substrate access to the oxidant. Compound **3** clearly exhibits oxidative reactivity toward C−H bonds, while **1** does not, because the redox-active orbitals of **3** are accessible to the substrate while those in **1** are not. Inspection of the calculated energies of H^+/e^- reduction of **1**−**3** also reveals that while a primary amine is a more donating ligand capable of stabilizing a Cu(III) species, it does not attenuate the potential of the oxygenated complex to react with C−H bonds; indeed, DFT calculations suggest an increase in the potential for a $1H/1e^-$ process. These results suggest that Nature may have incorporated this unusual ligand in pMMO in order to achieve an active site oxidant capable of transforming a recalcitrant substrate.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and results, computational details, and spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

dx.doi.org/10.1021/ja508630d | J. Am. Chem. Soc. 2014, 136, 14405−14408

AUTHOR INFORMATION

Corresponding Author

stack@stanford.edu

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

Financial support for the work was provided by NIH (GM50730). C.C. would also like to thank the William R. and Sara Hart Kimball Stanford Graduate Fellowship for financial support for his contribution to this study. Use of the Stanford Synchrotron Radiation Lightsource, SLAC National Accelerator Laboratory, is supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences under Contract No. DE-AC02-76SF00515. The SSRL Structural Molecular Biology Program is supported by the DOE Office of Biological and Environmental Research, and by the National Institutes of Health, National Institute of General Medical Sciences (including P41GM103393). The contents of this publication are solely the responsibility of the authors and do not necessarily represent the official views of NIGMS or NIH.

REFERENCES

(1) Lieberman, R. L.; Shrestha, D. B.; Doan, P. E.; Hoffman, B. M.; Stemmler, T. L.; Rosenzweig, A. C. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 3820−3825.

(2) Balasubramanian, R.; Smith, S. M.; Rawat, S.; Yatsunyk, L. A.; Stemmler, T. L.; Rosenzweig, A. C. *Nature* **2010**, *465*, 115−119.

(3) Smith, S. M.; Rawat, S.; Telser, J.; Hoffman, B. M.; Stemmler, T. L.; Rosenzweig, A. C. *Biochemistry* **2011**, *50*, 10231−10240.

(4) Solomon, E. I.; Heppner, D. E.; Johnston, E. M.; Ginsbach, J. W.; Cirera, J.; Qayyum, M.; Kieber-Emmons, M. T.; Kjaergaard, C. H.; Hadt, R. G.; Tian, L. *Chem. Rev.* **2014**, *114*, 3659−3853.

(5) Solomon, E. I.; Sundaram, U. M.; Machonkin, T. E. *Chem. Rev.* **1996**, *96*, 2563−2606.

(6) Bollinger, J. M., Jr. *Nature* **2010**, *465*, 40−41.

(7) Lieberman, R. L.; Rosenzweig, A. C. *Nature* **2005**, *434*, 177−182.

(8) Lawton, T. J.; Ham, J.; Sun, T.; Rosenzweig, A. C. *Proteins: Struct., Funct., Bioinf.* **2014**, *82*, 2263−2267.

(9) Sayavedra-Soto, L. A.; Hamamura, N.; Liu, C.-W.; Kimbrel, J. A.; Chang, J. H.; Arp, D. J. *Environ. Microbiol. Rep.* **2011**, *3*, 390−396.

(10) (a) Quinlan, R. J.; Sweeney, M. D.; Lo Leggio, L.; Otten, H.; Poulsen, J.-C. N.; Johansen, K. S.; Krogh, K. B. R. M.; Jørgensen, C. I.; Tovborg, M.; Anthonsen, A.; Tryfona, T.; Walter, C. P.; Dupree, P.; Xu, F.; Davies, G. J.; Walton, P. H. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*, 15079−15084. (b) Kjaergaard, C. H.; Qayyum, M. F.; Wong, S. D.; Xu, F.; Hemsworth, G. R.; Walton, D. J.; Young, N. A.; Davies, G. J.; Walton, P. H.; Johansen, K. S.; Hodgson, K. O.; Hedman, B.; Solomon, E. I. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111*, 8797−8802.

(11) Mirica, L. M.; Ottenwaelder, X.; Stack, T. D. P. *Chem. Rev.* **2004**, *104*, 1013−1045.

(12) Komiyama, K.; Furutachi, H.; Nagatomo, S.; Hashimoto, A.; Hayashi, H.; Fujinami, S.; Suzuki, M.; Kitagawa, T. *Bull. Chem. Soc. Jpn.* **2004**, *77*, 59−72.

(13) Mirica, L. M.; Vance, M.; Jackson Rudd, D.; Hedman, B.; Hodgson, K. O.; Solomon, E. I.; Stack, T. D. P. *J. Am. Chem. Soc.* **2002**, *124*, 9332−9333.

(14) Mirica, L. M.; Vance, M.; Rudd, D. J.; Hedman, B.; Hodgson, K. O.; Solomon, E. I.; Stack, T. D. P. *Science* **2005**, *308*, 1890−1892.

(15) Liang, H.-C.; Zhang, C. X.; Henson, M. J.; Sommer, R. D.; Hatwell, K. R.; Kaderli, S.; Zuberbühler, A. D.; Rheingold, A. L.; Solomon, E. I.; Karlin, K. D. *J. Am. Chem. Soc.* **2002**, *124*, 4170−4171.

(16) Schatz, M.; Becker, M.; Walter, O.; Liehr, G.; Schindler, S. *Inorg. Chim. Acta* **2001**, *324*, 173−179.

(17) Schindler, S. *Eur. J. Inorg. Chem.* **2000**, 2311−2326.

(18) Mahadevan, V.; DuBois, J. L.; Hedman, B.; Hodgson, K. O.; Stack, T. D. P. *J. Am. Chem. Soc.* **1999**, *121*, 5583−5584.

(19) Sanyal, I.; Karlin, K. D.; Strange, R. W.; Blackburn, N. J. *J. Am. Chem. Soc.* **1993**, *115*, 11259−11270.

(20) Citek, C.; Lyons, C. T.; Wasinger, E. C.; Stack, T. D. P. *Nat. Chem.* **2012**, *4*, 317−322.

(21) Maiti, D.; Woertink, J. S.; Narducci Sarjeant, A. A.; Solomon, E. I.; Karlin, K. D. *Inorg. Chem.* **2008**, *47*, 3787−3800.

(22) Henson, M. J.; Mukherjee, P.; Root, D. E.; Stack, T. D. P.; Solomon, E. I. *J. Am. Chem. Soc.* **1999**, *121*, 10332−10345.

(23) Kau, L. S.; Spira-Solomon, D. J.; Penner-Hahn, J. E.; Hodgson, K. O.; Solomon, E. I. *J. Am. Chem. Soc.* **1987**, *109*, 6433−6442.

(24) DuBois, J. L.; Mukherjee, P.; Collier, A. M.; Mayer, J. M.; Solomon, E. I.; Hedman, B.; Stack, T. D. P.; Hodgson, K. O. *J. Am. Chem. Soc.* **1997**, *119*, 8578−8579.

(25) Herres-Pawlis, S.; Verma, P.; Haase, R.; Kang, P.; Lyons, C. T.; Wasinger, E. C.; Florke, U.; Henkel, G.; Stack, T. D. P. *J. Am. Chem. Soc.* **2009**, *131*, 1154−1169.

(26) Warren, J. J.; Tronic, T. A.; Mayer, J. M. *Chem. Rev.* **2010**, *110*, 6961−7001.

(27) Zhu, X.-Q.; Li, H.-R.; Li, Q.; Ai, T.; Lu, J.-Y.; Yang, Y.; Cheng, J.-P. *Chem.−Eur. J.* **2003**, *9*, 871−880.

(28) Fukuzumi, S.; Kitano, T.; Mochida, K. *Chem. Lett.* **1989**, *18*, 2177−2180.

(29) Taki, M.; Itoh, S.; Fukuzumi, S. *J. Am. Chem. Soc.* **2001**, *123*, 6203−6204.

(30) Manring, L. E.; Peters, K. S. *J. Am. Chem. Soc.* **1983**, *105*, 5708−5709.

(31) Extrapolating the classical KIE limit at 298 K of a C−H(D) bond scission due to stretching along a linear C−H(D) vibration (ca. KIE = 6.5) to 148 K (ca. KIE = 44) suggests that it is not necessary to invoke tunneling in the reaction of compound **3** with the substrate.

(32) Goldsmith, C. R.; Jonas, R. T.; Stack, T. D. P. *J. Am. Chem. Soc.* **2002**, *124*, 83−96.

(33) Luo, Y. R. *Handbook of Bond Dissociation Energies in Organic Compounds*; CRC Press: Boca Raton, FL, 2003.

(34) Himes, R. A.; Barnese, K.; Karlin, K. D. *Angew. Chem., Int. Ed.* **2010**, *49*, 6714−6716.

(35) Lewis, E. A.; Tolman, W. B. *Chem. Rev.* **2004**, *104*, 1047−1076.

(36) Himes, R. A.; Karlin, K. D. *Curr. Opin. Chem. Biol.* **2009**, *13*, 119−131.

(37) Itoh, S. Chemical Reactivity of Copper Active-Oxygen Complexes. In *Copper-Oxygen Chemistry*; John Wiley & Sons, Inc., 2011; pp 225−282.