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Copper-Hydroperoxo Mediated *N*-Debenzylation Chemistry Mimicking Aspects of Copper Monooxygenases

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Abstract

Substantial oxidative *N*-debenzylation reaction along with PhCH=O formation occurs from a hydroperoxo copper(II) complex which has a dibenzylamino substrate (-$N(CH_2Ph)_2$ appended as a substituent on one pyridyl group of its tripodal tetradentate TMPA {≡ TPA ≡ tris(2-pyridylmethyl) amine)} ligand framework. During the course of the ($\mathbf{L^{N(CH_2Ph)_2}}$)$Cu^{II}(^{-}OOH)$ reactivity, formation of a substrate and ^{-}OOH (an oxygen atom) derived alkoxo $Cu^{II}(^{-}OR)$ complex occurs. The observation that the same $Cu^{II}(^{-}OR)$ species occurs from Cu^{I}/PhIO chemistry suggests the possibility that a copper-oxo (cupryl) reactive intermediate forms during alkoxo species formation, and new ESI-MS data obtained provides some further support for this high-valent intermediate. Net H-atom abstraction chemistry is proposed, based on kinetic isotope effect studies provided here and that previously published for a closely related $Cu^{II}(^{-}OOH)$ species incorporating dimethylamine (-$N(CH_3)_2$) as the internal substrate (*J. Am. Chem. Soc.* **2007**, *129*, 6720-6721); the Cu^{I}/PhIO reactivity, with similar isotope effect results, provides further support. The reactivity of these chemical systems closely resembles proposed oxidative *N*-dealkylation mechanisms effected by the copper-monooxygenases dopamine β-monooxygenase (*DβM*) or peptidylglycine-*α*-hydroxylating monooxygenase (*PHM*).

Introduction

The study of copper(I)-dioxygen adducts and their (reduced) derivatives is of considerable current interest.[1-8] In large part these efforts comprise the desire to elucidate fundamental aspects such as structural type along with associated spectroscopy, electronic structure, and of course reactivity patterns. Inspirations of such investigations also come from aspects of bioinorganic chemistry with interest to provide fundamental information which may be relevant to metalloenzymes with copper containing active sites where the processing of molecular oxygen occurs. Included in the latter are the copper monooxygenases peptidylglycine-*α*-hydroxylating monooxygenase (*PHM*) and dopamine β-monooxygenase (*DβM*), mammalian proteins serving in the biosynthesis of various neuropeptides or hormones.[9-12] Figure 1 outlines the specific reactions carried out, each involving insertion of one oxygen atom (from O_2) into the substrate, for *DβM* a net benzylic hydroxylation while for *PHM* hydroxylation is followed by *N*-dealkylation. The active sites of each enzyme are known to be very similar and each contains two copper ions. Previous biophysical studies have shown these to be relatively far apart and more recent X-ray crystallographic structures on *PHM* indeed show them to be this way, ~ 11 Å apart.[12] Thus, the mechanism of action focus on copper chemistry taking place at one of the two copper ions ($Cu_M \equiv Cu_B$) while the other ($Cu_H \equiv Cu_A$) serves to transfer electrons.

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With a history of biochemical/biophysical studies on these enzymes, and in the context of considering various copper-dioxygen derived species, either known or unknown in inorganic chemistry, a hydroperoxo-copper(II) $Cu^{II}(^{-}OOH)$, a superoxo-copper(II) $Cu^{II}(O_2^{\bullet-})$; derived from initial Cu^{I}-O_2 chemistry or high-valent cupryl [Cu^{II}-O•] have been discussed as species which might be responsible for an initial hydrogen-atom abstraction reaction.[2,4,8,9,11-18] For some time a hydroperoxo complex was suggested, but more recently both experimental and computational (bio)chemistries rather suggest that the $Cu^{II}(O_2^{\bullet-})$ moiety is relevant.[4,9-11,13] Further, one crystal structure of *PHM* reveals what is best described as a $Cu_M^{II}(O_2^{\bullet-})$ moiety perfectly juxtaposed to the relevant substrate C–H bond. Still other theoretical treatments prefer a prior (rather then subsequent) O-O cleavage from $Cu^{II}(^{-}OOH)$ leading to a high-valent $[Cu\text{-}O]^{2+}$ or $[Cu\text{-}O]^{+}$ (equiv to Cu^{II}-O•) moieties ("cupryl") which effects H-atom transfer.[14,15] In part to obtain answers or provide insights, synthetic bioinorganic chemists have been actively pursuing the chemistry of mononuclear copper-dioxygen derived complexes, especially in reactivity toward substrates.[2,19-27] Which species may effect H-atom abstractions followed by the net transfer of an oxygen atom to the substrate, i.e., monooxygenase activity, Scheme 1?

In biomimetic studies approached via coodination chemistry efforts, two types of hydroperoxide-copper species have to date been produced, μ-1,1-hydroperoxo-dicopper(II) and mononuclear complexes. We have recently demonstrated substrate C–H activation chemistries starting from well-characterized dinuclear μ-$^{-}$OOH-dicopper(II) complexes, oxidative *N*-dealkylation or $RCH_2C{\equiv}N$ oxidative cleavage (to the RCH=O aldehyde + cyanide).[28,29] Suzuki has also observed a hydrocarbon attack from a $Cu^{II}_2(^{-}OH)_2 + H_2O_2$ reaction, giving a Cu^{II}-$^{-}OOR_{ligand\text{-}substrate}$ product.[30] A number of rather well characterized mononuclear $Cu^{II}(^{-}OOH)$ complexes have been described,[31-37] however substrate oxidations has been limited to organic sulfides[37] or olefins (but in low yields).[32] In our own labs, we have in the last year been able to demonstrate C–H activation and thus oxygenation from $Cu^{II}(^{-}OOH)$ complexes, as further discussed. There have been notable advances in the generation of mononuclear $Cu^{II}(O_2^{\bullet-})$ or $Cu^{III}(O_2^{2-})$ species,[2,24,25,38] but chemistry where such entities attack substrate C–H bonds have yet to be described. There are as yet no discrete examples or direct evidence for mononuclear high-valent copper-oxo species, however see further discussion below.

Within the context of the introduction given above, we here describe the chemistry of a new ligand $\mathbf{L^{N(CH_2Ph)_2}}$ and its copper complex promoted oxidative chemistry. While the main focus is on a mononuclear $Cu^{II}(^{-}OOH)$ species, $[(\mathbf{L^{N(CH_2Ph)_2}})Cu^{II}(^{-}OOH)]^{+}$ (**2**), we complement it with (ligand)Cu^{I}-O_2 reactivity and a separate attempt to interrogate the possible involvement of cupryl chemistry. The work follows our recent report where we employed a close analogue $[(\mathbf{L^{N(CH_3)_2}})Cu^{II}(^{-}OOH)]^{+}$ (**3**), where both ligands are the derivatives of TMPA {≡ TPA ≡ tris (2-pyridylmethyl)amine)}, showing that the hydroperoxo group coordinated to copper(II) can effect oxidative *N*-dealkylation chemistry on the juxtaposed dimethylamino substrate.[27] In this report the chemistry of the $\mathbf{L^{N(CH_2Ph)_2}}$ analogue is fully described.

Experimental Section

Materials and Methods

Unless otherwise stated all solvents and chemicals used were of commercially available analytical grade. Acetone, dietyhlether, pentane, propionitrle, methanol and tetrahydrofuran (THF) were used after passing them through a 60 cm long column of activated alumina (Innovative Technologies, Inc.) under argon. Deoxygenation of solvents was effected by either repeated freeze/pump/thaw cycles or bubbling with argon for 30 - 45 minutes. Dioxygen was dried by passing through a short column of supported P_4O_{10} (Aquasorb, Mallinkrodt). Preparation and handling of air sensitive compounds were performed under an argon atmosphere using standard Schlenk techniques or in an MBraun Labmaster 130 inert

atmosphere (< 1 ppm O_2, < 1 ppm H_2O) drybox filled with nitrogen. Elemental analyses were performed by Desert Analytics (Tucson, AZ). ^{1}H-NMR and ^{13}C-NMR spectra were measured on a Bruker 400 MHz spectrometer. Chemical shifts were reported as δ values relative to an internal standard (Me_4Si) and the residual solvent proton peak. UV-vis spectra were recorded on a Hewlett-Packard Model 8453A diode array spectrophotometer equipped with two-window quartz H.S. Martin Dewar filled with cold MeOH (25 °C to −85 °C) maintained and controlled by a Neslab VLT-95 low temp circulator. Spectrophotometer cells used were made by Quark Glass with column and pressure/vacuum side stopcock and 2 mm path length. The copper complexes are ClO_4^- and $B(C_6F_5)_4^-$ salt complexes unless otherwise stated. *While we have experienced no problems in working with perchlorate compounds, they are potentially explosive and care must be taken not to work with large quantities*. ESI mass spectra were acquired using a Finnigan LCQDeca ion-trap mass spectrometer equipped with an electrospray ionization source (Thermo Finnigan, San Jose, CA). For meta-stable species (as described below), samples were introduced from low temperature solutions with a liquid-N_2 precooled plastic syringe, and quickly injected into the instrument sample port which feeds the instrument syringe pump operating at 10μL/min via a silica capillary line. The heated capillary temperature was 250 °C and the spray voltage was 5 kV. X-ray diffraction was performed at the X-ray diffraction facility at the Johns Hopkins University. The X-ray intensity data were measured on an Oxford Diffraction Xcalibur3 system equipped with a graphite monochromator and an Enhance (Mo) X-ray Source (λ = 0.71073Å) operated at 2 kW power (50 kV, 40 mA) and a CCD detector. The frames were integrated with the Oxford Diffraction *CrysAlisRED* software package. X-band electron paramagnetic resonance (EPR) spectra were recorded on a Bruker EMX CW-EPR spectrometer controlled with a Bruker ER 041 XG microwave bridge operating at X-band (~9 GHz). The low temperature experiments were carried out via $N_2(l)$ finger dewar.

Synthesis of $[(L^{N(CH_2Ph)_2})Cu^{II}(H_2O)(ClO_4)]^+$ (1)

The synthesis and characterization of the ligand $\mathbf{L^{N(CH_2Ph)_2}}$ was recently reported.[39] Ligand $\mathbf{L^{N(CH_2Ph)_2}}$ (0.250 g, 0.515 mmol) was treated with $Cu^{II}(ClO_4)_2.6H_2O$ (0.191 g, 0.516 mmol) (Aldrich) in acetone (10 mL) and stirred for 25 min at RT. The complex was precipitated as greenish blue solid upon addition of Et_2O (140 mL). The supernatant was decanted and the resulting crystalline solid was washed two times with Et_2O and dried under vacuum to afford $[(\mathbf{L^{N(CH_2Ph)_2}})Cu^{II}(H_2O)(ClO_4)]ClO_4$.acetone **(1)** (0.305 g, 72% yield). Single crystals were obtained by vapor diffusion of Et_2O into a solution of the complex in acetone. Anal. Calcd. $[(\mathbf{L^{N(CH_2Ph)_2}})Cu^{II}(H_2O)(ClO_4)]ClO_4$.(acetone) **(1)**; $C_{35}H_{39}Cl_2CuN_5O_{10}$: C, 51.01; H, 4.77; N, 8.50. Found: C, 50.88; H, 4.88; N, 8.37. EPR spectrum: X-band spectrometer in acetone at 77 K: $g_\parallel = 2.252$, $g_\perp = 2.047$, $A_\parallel = 170$ G, $A_\perp = 29.5$ G.

Generation of $[(L^{N(CH_2Ph)_2})Cu^{II}(^-OOH)]^+$ (2) and reactivity study

In 20 mL acetone solution, complex **1** (0.337 g, 0.409 mmol) was dissolved and was cooled to −80 °C using an acetone/dry-ice bath. Addition of Et_3N (0.412 g, 4.08 mmol) and 50 wt % H_2O_2 (252 μL, 4.09 mmol) (Aldrich) led to the formation of green complex $[(\mathbf{L^{N(CH_2Ph)_2}})Cu^{II}(^-OOH)]ClO_4$ (**2**), λ = 380 nm (ε = 1400 $M^{-1}cm^{-1}$). When an aliquot of this −80 °C green solution was injected into the mass spectrometer, a parent peak cluster at *m/z* 581.03 corresponding to the positive ion $[(\mathbf{L^{N(CH_2Ph)_2}})Cu^{II}(OOH)]^+$ was observed. When $H_2{}^{18}O_2$ was used for the formation of **2**, the positive ion peak clusters shifted to *m/z* 585.09. The alkoxide product $[(\mathbf{L^{N(CH_2Ph)_2}})Cu^{II}(OOH)]^+$ (**2**) was detected with a parent peak cluster at *m/z* 563.05 corresponding to the positive ion $[(\mathbf{L^{N(CH_2Ph)(PhCHO\text{-})}})Cu^{II}]^+$ (**4**). An EPR spectrum of the reaction mixture containing **4** reveals typical Cu(II) axial species supporting its mononuclear formulation. When the formation of **2** was carried out with $H_2{}^{18}O_2$, the positive ion peak clusters of **4** shifted to *m/z* 565.20. X-band EPR of **2**, in acetone at 77 K: $g_\parallel = 2.240$, $g_\perp = 2.041$, $A_\parallel = 180$ G, $A_\perp = 27$ G. The hydroperoxo species $[(\mathbf{L^{N(CH_2Ph)_2}})Cu^{II}(^-OOH)]ClO_4$ (**2**) was kept at −80 °C and stirred for 15 min. Upon warming, the solution was carefully concentrated

to 4 mL under vacuum and the ESI-MS of the reaction solution was recorded. The resulting copper solution was washed three times (200 mL) with Et_2O whereupon the Et_2O solutions were combined and dried over Na_2SO_4. The Et_2O solution was analyzed by GC and GC-MS (see below, for conditions) confirming the formation of PhCHO (authentic commercial PhCHO was also used for comparison) (yield, 43 %). When the formation of **2** was carried out with $H_2{}^{18}O_2$ and similar follow-up procedures were performed, ~ 65 % ^{18}O-atom incorporation in PhCHO occurs.

Isolation of Organic Products

The residual material after Et_2O washing was treated with 250 mL saturated Na_2EDTA/H_2O solution and the organic part was extracted by using 80 mL CH_2Cl_2. The $CH_2Cl_2/Na_2EDTA/H_2O$ extraction was repeated three times to ensure complete demetallation. The organic CH_2Cl_2 solution was dried over anhydrous Na_2SO_4, filtered, and concentrated by rotory evaporation. ESI-MS of the organic extract was recorded. Isolation and purification by column chromatography (with Al_2O_3, 2% MeOH + CH_2Cl_2) revealed unreacted ligand $\mathbf{L^{N(CH_2Ph)_2}}$ (0.032 g, ~16 %) and the *N*-debenzylated product $\mathbf{L^{NH(CH_2Ph)}}$ (0.077 g, ~48%). The products were characterized by ESI-MS, ^{1}H-NMR and ^{13}C-NMR spectroscopies and thin-layer chromatography (TLC). Formation of $\mathbf{L^{N(CH_2Ph)(COPh)}}$ and $\mathbf{L^{N(CH_2Ph)(COPh)}}$ (0.014 g, ~7%; ESI-MS, 522.53, M+Na and 536.31, M+Na respectively) and $\mathbf{L^{NH(COPh)}}$ (0.004 g, ~3%) ESI-MS (410.65, M+H and 432.51, M+Na) were also observed. Several attempts for isolation of these organic products in absolute pure form were unsuccessful due to their comparable R_f values, however their formulation can be confirmed from both pre and post demetallation. We did not find any evidence for $\mathbf{L^{NH2}}$ formation.

Experiment adding only one equiv peroxide

Triethylamine, Et_3N (0.041 g, 0.408 mmol) and 50 wt % H_2O_2 (25 μL, 0.408 mmol) was added to the 20 mL acetone solution (−80 °C) of complex **1** (0.337 g, 0.409 mmol). Following procedures similar to that described above showed the production of unreacted ligand $\mathbf{L^{N(CH_2Ph)_2}}$ (0.130 g, ~65 %), $\mathbf{L^{NH(CH_2Ph)}}$ (0.026 g, ~16%), $\mathbf{L^{N(CH_2Ph)(COPh)}}$ plus $\mathbf{L^{N(CH_2Ph)(COPh)}}$ (0.002 g, ~1%). A similar reaction in methanol as solvent lead to formation of ~14 % $\mathbf{L^{NH(CH_2Ph)}}$, <1% $\mathbf{L^{N(CH_2Ph)(COPh)}}$ and $\mathbf{L^{N(CH_2Ph)_2}}$ in ~68 % yield. The starting ligand $\mathbf{L^{N(CH_2Ph)_2}}$ was obtained in ~64 % yield along with ~14% $\mathbf{L^{NH(CH_2Ph)}}$ and <1% $\mathbf{L^{N(CH_2Ph)(COPh)}}$ in propionitrile as solvent for an analogous $[(\mathbf{L^{N(CH_2Ph)_2}})Cu^{II}(H_2O)(ClO_4)]^+$ (**1**)/H_2O_2/Et_3N reaction.

$\mathbf{L^{NH(CH_2Ph)}}$: ^{1}H-NMR ($CDCl_3$): δ 3.70 (s, 2H, CH_2Py), 3.89 (s, 4H, 2CH_2Py), 4.48 (d, 2H, CH_2Ph), 5.13 (s, 1H, NH), 6.22 (d, 1H), 6.83 (d, 1H), 7.09 (t, 2H), 7.11-7.44 (m, 5H), 7.45-7.68 (m, 5H), 8.49 (d, 2H). ^{13}C-NMR ($CDCl_3$): δ 46.51, 60.21, 77.39, 104.74, 112.04, 121.86, 122.83, 126.79, 127.41, 128.55, 136.38, 137.89, 139.43, 143.28, 148.99, 157.76, 158.29, 159.84. ESI-MS (396.68, M+H; 418.33, M+Na). R_f = 0.23, Alumina, 2% MeOH + CH_2Cl_2.

Reaction of $[(\mathbf{L^{N(CH_2Ph)_2}})Cu^I]^+$ (6) with O_2

The synthesis and characterization of copper(I)-complex, $[(\mathbf{L^{N(CH_2Ph)_2}})Cu^I]B(C_6F_5)_4$ (**6**) was previously reported.[39] Formation of bis(μ-oxo)dicopper(III) complex, $[\{(\mathbf{L^{N(CH_2Ph)_2}})Cu^{III}\}_2(O^{2-})_2](B(C_6F_5)_4)_2$ (**9**) from O_2-reactivity of **6** was confirmed by low-temperature UV-vis and rR spectroscopy. For the determination of ligand oxidation chemistry, a solution of $[(\mathbf{L^{N(CH_2Ph)_2}})Cu^I]^+$ (**6**) (0.125 g, 0.101 mmol) in a 25 mL Schlenk flask with 6 mL Et_2O was prepared in the glovebox. Removal to the benchtop and cooling to −80 °C, followed by bubbling O_2 via a long syringe needle (20 s) was carried out, and this was followed by removal of excess dioxygen via evacuation and purging with argon. After 30 mins, the solution was warmed to RT and demetallation procedures with $Na_2EDTA/H_2O/CH_2Cl_2$ was performed; this

results in the production of dipicolylamine, (0.001 g, ~ 4%), $\mathbf{L^{NH(CH_2Ph)}}$ (0.002 g, ~ 5%) and unreacted $\mathbf{L^{N(CH_2Ph)_2}}$ (0.037 g, ~ 75%).

Synthesis of $[(L^{NH(CH_2Ph)})Cu^{II}(Cl)]^+$ (10)

Here, we followed synthetic procedures previously employed to generate crystalline copper (II)-chloride complexes via ligand-Cu(I)-$CHCl_3$ chemistry.[27,40] Isolated $\mathbf{L^{NH(CH_2Ph)}}$ (0.050 g, 0.126 mmol) and $[Cu^I(CH_3CN)_4]ClO_4$ (0.042 g, 0.128 mmol) were placed in a 25 mL Schlenk flask under argon. Dioxygen-free CH_3CN (2 mL) was added under argon to form a yellow solution and this was stirred for 10 min. Deoxygenated $CHCl_3$ (0.3 mL) was then added under argon whereupon the solution ceased to look transparent, and its color turned to green. After 2 h, 20 mL of Et_2O was added in order to precipitate the copper complex. X-ray quality crystals of this compound $[(\mathbf{L^{NH(CH_2Ph)}})Cu^{II}(Cl)]^+$ (**10**) were obtained after precipitation prompted by Et_2O, from the reaction solution. The green precipitate was recrystallized two times from CH_3CN/Et_2O. Anal. Calcd. $C_{25}H_{29}Cl_2CuN_5O_6$: C, 47.66; H, 4.64; N, 11.12. Found: C, 47.71; H, 4.34; N, 11.08. After vacuum-drying the green crystals weighed 0.054 g (68%).

Reaction of $[(L^{N(CH_2Ph)_2})Cu^I]B(C_6F_5)_4$ (6) with PhIO

$[(\mathbf{L^{N(CH_2Ph)_2}})Cu^I]B(C_6F_5)_4$ (**6**)[39] (0.018 g, 0.015 mmol) was dissolved in 18 mL CH_3CN inside the drybox and was taken in a 25 mL Schlenk flask. In a separate Schlenk flask, PhIO (0.033g, 0.150 mmol) was taken with 4.0 mL CH_3CN under argon and was stirred for 30 min and cooled to −40 °C. With a syringe, 1 mL of this CH_3CN solution was introduced anaerobically into the −40 °C solution of **6**. ESI-MS of this low temperature copper solution was recorded, showing the initial strong peak at *m/z* 564.25 due to high-valent $[(\mathbf{L^{N(CH_2Ph)_2}})Cu^{III}{=}O]^+$ species formation. The decomposition of this 564 species was followed with respect to time, which leads to the alkoxo species, $[(\mathbf{L^{N(CH_2Ph)(PhCHO^-)}})Cu^{II}]^+$ (**4**) (*m/z* = 563.18). When the formation of **4** was carried out with PhIO plus $H_2{}^{18}O$, the positive ion peak clusters of **4** shifted to *m/z* = 565.34.

Reaction of $[(L^{N(CH_3)_2})Cu^I]B(C_6F_5)_4$ (7)[39] with PhIO

$[(\mathbf{L^{N(CH_3)_2}})Cu^I]B(C_6F_5)_4$ (**7**) (0.015 g, 0.014 mmol) was dissolved in 15 mL CH_3CN inside the drybox and was taken in a 25 mL Schlenk flask. In a separate Schlenk flask, PhIO (0.031 g, 0.140 mmol) was taken with 5.0 mL CH_3CN under argon and was stirred for 30 mins. With a syringe, 1 mL of this CH_3CN solution (−40 °C) was introduced anaerobically into the −40 °C solution of **7**. ESI-MS of the low temperature copper solution was recorded, showing the strong peak at *m/z* 411.18. When a similar reaction was carried out with $[(\mathbf{L^{N(CH_3)(CD_3)}})Cu^I]^+$ and PhIO, the positive ion peak clusters contains dominant peak at *m/z* = 413.24 and 414.14 due to the generation of $[(\mathbf{L^{N(CH_3)(CD_2O^-)}})Cu^I]^+$ and $[(\mathbf{L^{N(CD_3)(CH_2O^-)}})Cu^I]^+$, respectively.

Chemistry of $[(L^{N(CH_3)_2})Cu^{II}(^-OOH)]^+$ (3) and its Reactivity Study

The synthesis, characterization and reactivity studies of copper(II)-hydroperoxo complex, $[(\mathbf{L^{N(CH_3)_2}})Cu^{II}(^-OOH)]ClO_4$ (**3**) were previously reported.[27] Via further examination in the present study, we were able to isolate a small amount of one decomposition product $[(\mathbf{L^{N(CH_3)_2}})Cu^{II}(HCOO^-)(ClO_4)Cu^{II}\text{-}(\mathbf{L^{NH(CH_3)}})](ClO_4)_2$ (**11**) with dicopper(II) centers bridged by ($HCOO^-$) group from the reactivity of **3** in acetone. Compound **11** was characterized crystallographically and its EPR and ESI-MS characterization studies were performed (see Supporting information). We also previously described the chemistry of $[(\mathbf{L^{N(CH_3)(CD_3)}})Cu^{II}(^-OOH)]^+$ (**3-CD$_3$**) [27] for which now we find that the extracted unreacted ligand has its isotope label scrambled (see discussion below).

Results and Discussion

Ligand Design and Hydroperoxo-Copper(II) Complexes

We have previously carried out and published extensive studies concerning the dioxygen reactivity of the parent ligand copper(I) complex $[(TMPA)Cu^{I}(CH_3CN)]^{+}$ along with a variety of analogs.[41-43] Masuda and coworkers[44,45] elaborated on the TMPA framework inputting potential hydrogen-bonding moieties, placing them in 6-pyridyl positions. In a series of studies, they showed that ligand-copper(I) compounds react with O_2 to form binuclear copper-dioxygen adducts, formally μ-1,2-peroxodicopper(II) complexes, which do show altered UV-vis and resonance Raman (rR) spectroscopic properties due to H-bonding.[44,45] Further, Masuda generated a variety of hydroperoxo-copper(II) complexes via {ligand-Cu^{II} + H_2O_2 + base} reactions, including those shown in Chart 2.[33-36] As indicated one of these could be characterized by X-ray crystallography.[33]

However, oxidative reactivity of these mononuclear $Cu^{II}(^{-}OOH)$ species (with tetradentate chelates) towards exogenous substrates was not observed. One reason for such inactivity could be that potential substrates could not achieve an appropriate proximity (either spatially or temporarily) to the $Cu^{II}(^{-}OOH)$ moiety. Another explanation could be that stabilization of the $Cu^{II}(^{-}OOH)$ unit with H-bonding comes at the cost of reactivity. In the last year, concerning hydroperoxo-Cu(II) compounds, we reported two examples where we 'mounted' a potentially oxidizable substrate within the ligand framework: $N(CH_3)_2$, i.e., $\mathbf{L^{N(CH_3)_2}}$ (Chart 1)[27] or a hydrocarbon aryl group,[26] thus fixed by covalent bonding to be potentially juxtaposed to a $Cu^{II}(^{-}OOH)$ moiety or a species derived from it via O-O cleavage. As mentioned in the Introduction, here we detail here the overall oxidative copper ion chemistry with $\mathbf{L^{N(CH_2Ph)_2}}$.

Copper(II) Complex $[(L^{N(CH_2Ph)_2})Cu^{II}(H_2O)(ClO_4)]ClO_4$ (1)

For the purpose of generating a hydroperoxo complex by starting with Cu(II) and adding H_2O_2, we straightforwardly synthesized complex **1** (see Experimental Section). X-ray quality crystals were obtained, and the structure is described here, Figure 2.

The copper(II) ion is five-coordinate. The oxygen atom (O1) from a water molecule along with the alkylamino nitrogen (N2) and pyridyl groups not substituted in the 6-position (N1 and N3) comprise the basal plane of the structure found in a pyramidal geometry around copper center. One of the two perchlorate anions coordinates as the fifth axial ligands, $Cu1\text{-}O2_{perchlorate}$ = 2.3515(11) Å. A simple geometric analysis indicates the overall coordination geometry as a slightly distorted square pyramid (τ = 0.156, where τ = 0.00 for a perfect square pyramid and τ = 1.00 for a trigonal bipyramid).[46] The copper ion (Cu1) in fact lies in the best least squares basal plane. The pyridyl substituents arm (with N4) is moved and twisted away from the copper ion (Cu…N4 = 2.9345(13) Å, most likely because of steric issues with the 6-dibenzylamino substituent.[40,47] The structure is likely maintained in solution, as a typical axial EPR is observed (Figure 3a).

Hydrogen Peroxide Reactivity of $[(L^{N(CH_2Ph)_2})Cu^{II}(H_2O)(ClO_4)]ClO_4$ (1)

Generation of $[(L^{N(CH_2Ph)_2})Cu^{II}(^{-}OOH)]^{+}$ (2)—The green product solution which forms following addition of 2-3 equiv H_2O_2/Et_3N using 50 % $H_2O_{2(aq)}$ to a greenish blue acetone solution of **1** at −80 °C is formulated as the hydroperoxide species $[(\mathbf{L^{N(CH_2Ph)_2}})Cu^{II}(^{-}OOH)]^{+}$ (**2**). The band at λ_{max} = 380 nm (ε 1400 $M^{-1}cm^{-1}$) is assignable to a ^{-}OOH –> Cu^{II} LMCT absorption based on the correspondence with a number of literature examples.[27,33-37] An axial Cu^{II} EPR spectrum is also observed for **2**, consistent with a single mononuclear species formulation, but which is distinguishable from **1** (Figure 3b).

Direct evidence for [($\mathbf{L^{N(CH_2Ph)_2}}$)$Cu^{II}(^{-}OOH)]^+$ (**2**) comes from Electrospray Ionization Mass Spectrometry. Injection of a −80 °C acetone solutions of **2** gives a highest molecular weight parent peak cluster with *m/z* = 581.03 and expected ^{63,65} Cu pattern, corresponding to the monocation as formulated. As indicated (Figure 4a), the most prominent ESI-MS peak at *m/z* = 548.59 matches a complex having lost (H)OOH, [($\mathbf{L^{N(CH_2Ph)_2}}$)$Cu^{II}]^+$. When formation of **2** was instead carried out using $H_2{}^{18}O_2$, the positive ion peak shifts to 585.09 due to formation of [($\mathbf{L^{N(CH_2Ph)_2}}$)$Cu^{II}(^{-18}O^{18}OH)]^+$ (**2-^{18}O**); fitting of the parent peak pattern around *m/z* = 585 indicates > 99 % 18-O incorporation.

Hydrogen peroxide or $^{-}$OOH are known to attack the carbonyl group of acetone and Itoh and coworkers[21] recently observed a thus-formed alkylperoxo adduct bound to copper(II) in related but tridentate ligand-copper complex chemistry. However, by ESI-MS, we see no evidence for such a hydroperoxo-acetone-copper(II) complex. Also, see further discussion below.

Ligand Oxidative Reactivity for [($\mathbf{L^{N(CH_2Ph)_2}}$)$\mathbf{Cu^{II}(^{-}OOH)]^+}$ (2)—Hydroperoxo-copper (II) species **2** is stable in solution at −80 °C, but warming results in a change to a darker green color. Thin-layer chromatography (TLC) and ESI-MS data obtained from the product solution which has been stripped of copper ion (by addition of $Na_2EDTA_{(aq)}$ and extraction into CH_2Cl_2; see Experimental Section) shows that the formation of several new organic products has occurred. Thus, the singly *N*-dealkylated (i.e., *N*-debenzylation) compound $\mathbf{L^{NH(CH_2Ph)}}$ {(*m/z* = 396.48 (M+H), 418.49 (M+Na)}, the singly oxygenated organic compound $\mathbf{L^{N(CH_2Ph)(COPh)}}$ {(*m/z* = 500.32 (M+H), 522.35 (M+Na)}, the over-oxidized/oxygenated amide derived from benzoic acid $\mathbf{L^{NH(COPh)}}$ {(*m/z* = 410.47 (M+H), 432.41 (M+Na)} and the doubly-oxygenated (bis-amide) parent ligand $\mathbf{L^{N(COPh)_2}}$ {(*m/z* = 514.22 (M+H), 536.27 (M +Na)}. Unreacted ligand $\mathbf{L^{N(CH_2Ph)_2}}$ {(*m/z* = 486.36 (M+H), 418.49 (M+Na)} is also present. The absolute yields (average of three runs) are summarized in Scheme 2.

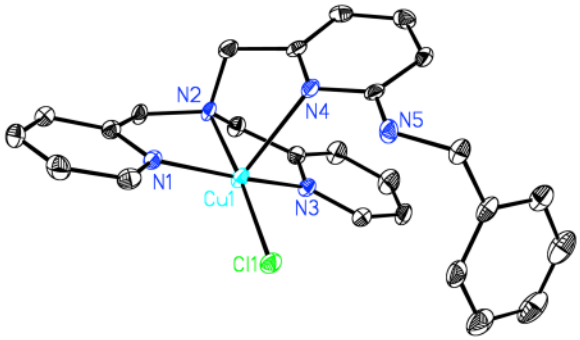
We also observed over-oxidized products, i.e., more than a single two-electron substrate oxidation (peroxide as oxidizing/oxygenating agent) in our previously published results with $\mathbf{L^{N(CH_3)_2}}$ and [($\mathbf{L^{N(CH_3)_2}}$)$Cu^{II}(^{-}OOH)]^+$ (**3**).[27] However, here, no corresponding $\mathbf{L^{NH_2}}$ product derived from **2** was observed. In the present chemistry, chromatographic separation/isolation reveals that only ~ 16 % yield of the original $\mathbf{L^{N(CH_2Ph)_2}}$ ligand remains after reactions. The major (~48 %) new organic product formed is the oxidatively singly *N*-debenzylated compound $\mathbf{L^{NH(CH_2Ph)}}$ (Scheme 2). As would be expected in an *N*-dealkylation reaction, a corresponding aldehyde should form, and indeed we observe a ~ 43 % yield of benzaldehyde as determined by GC and GC-MS spectroscopy. When labeled hydrogen peroxide is used in the reaction giving [($\mathbf{L^{N(CH_2Ph)_2}}$)$Cu^{II}(^{-18}O^{18}OH)]^+$ (**2-^{18}O**), 18-O labelled PhC(^{18}O)H is formed with ~65% of the theoretically expected O-18 incorporated. Benzaldehyde, with its pretty exchangeable (with water) carbonyl group, is well-documented to not always retain a high degree of 18-O incorporation.[48,49] As mentioned and from the yields obtained (Scheme 2), small but significant amounts of formally four or eight electron over-oxidized products form, ketones $\mathbf{L^{N(CH_2Ph)(COPh)}}$ plus $\mathbf{L^{N(COPh)_2}}$ (7%) and $\mathbf{L^{NH(COPh)}}$ (3%) (Scheme 2). These are likely to derive from the reaction of initial two-electron oxidation products with excess hydrogen peroxide (see also below).

When only one equiv H_2O_2/Et_3N is used to generate [($\mathbf{L^{N(CH_2Ph)_2}}$)$Cu^{II}(^{-}OOH)]^+$ (**2**), warming and workup leads to considerably more (~ 65%) unreacted $\mathbf{L^{N(CH_2Ph)_2}}$, the yield of primary mono *N*-debenzylated ligand-substrate $\mathbf{L^{NH(CH_2Ph)}}$ drops to 16 %; only 1 % (total) of $\mathbf{L^{N(CH_2Ph)(COPh)}}$ plus $\mathbf{L^{N(COPh)_2}}$ are obtained. No $\mathbf{L^{NH(COPh)}}$ or $\mathbf{L^{NH_2}}$ are observed. Thus, the major reaction product is again the two-electron (from one H_2O_2) oxidatively *N*-dealkylated ligand, $\mathbf{L^{NH(CH_2Ph)}}$, is formed in a "dose" dependent manner. Following our comments above, we further wanted to rule out the possible involvement of solvent to activate the hydroperoxo species. Thus, we carried out the formation of [($\mathbf{L^{N(CH_2Ph)_2}}$)$Cu^{II}(^{-}OOH)]^+$ (**2**) in both

propionitrile and methanol solvents, separately. In both cases, product distributions were found to be the similar as were found for acetone. We conclude that the $Cu^{II}(^{-}OOH)]^{+}$ moiety truly effects the oxidations observed.

Some further insights into the overall chemistry occurring in this system come from direct ESI-MS reaction solution, following warming to RT, but prior to extraction of the copper ion with EDTA (vide supra). The ESI-MS data (in Supporting Information) reveal the presence of several copper complexes, $[(\mathbf{L^{NH(CH_2Ph)}})Cu]^{+}$ (*m/z* = 457.48) and $[(\mathbf{L^{NH(CH_2Ph)}})Cu(acetone)]^{+}$ (*m/z* = 517.27), unreacted $[(\mathbf{L^{N(CH_2Ph)_2}})Cu]^{+}$ (*m/z* = 548.53). In addition, a prominent (intense) ESI-MS cluster is found at *m/z* = 563.05l, corresponding to alkoxide species $[(\mathbf{L^{N(CH_2Ph)(PhCHO-)}})Cu]^{+}$ (**4**), see Scheme 2. In a corresponding experiment with $[(\mathbf{L^{N(CH_2Ph)_2}})Cu^{II}(^{-18}O^{18}OH)]^{+}$ (**2-^{18}O**), 18-O does appear in this alkoxide product, $[(\mathbf{L^{N(CH_2Ph)(PhCH(18)O-)}})Cu]^{+}$ (*m/z* = 565.19; 94 % 18-O incorporation). As will be described below, this finding is relevant to the biomimetic nature of the reaction chemistry and also provides some insight to mechanistic considerations.

X-ray Crystal Structure of $[(\mathbf{L^{NH(CH_2Ph)}})Cu^{II}(Cl)]ClO_4$ (10)—We wished to further verify our major organic product assignment, and thus for $\mathbf{L^{NH(CH_2Ph)}}$ as isolated and purified via column chromatography, we formed a copper complex product. Under Ar, reaction of $\mathbf{L^{NH(CH_2Ph)}}$ and $Cu^{I}(MeCN)_4(ClO_4)$ in acetonitrile followed by addition of $CHCl_3$ leads to the chloro-copper(II) complex, $[(\mathbf{L^{NH(CH_2Ph)}})Cu^{II}(Cl)]^{+}$ (**10**) (see Experimental Section). An X-ray structure of the pentacoordinate complex was obtained (see diagram), and it confirms the presence and identity of $\mathbf{L^{NH(CH_2Ph)}}$. The full X-ray structure is presented in Supporting Information.



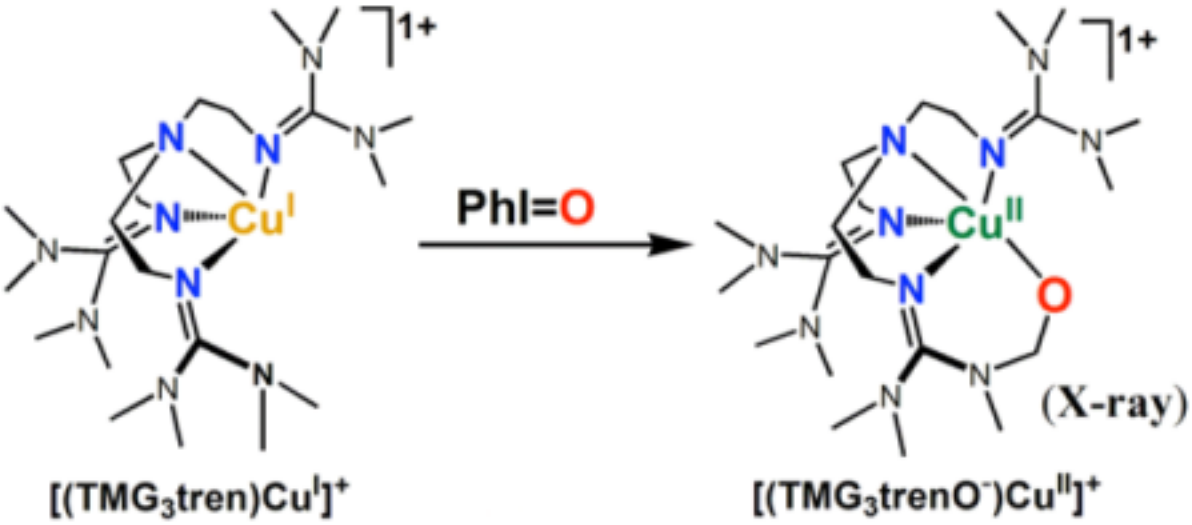
Corresponding Cu^{I}/O_2 Chemistry with $L^{N(CH_2Ph)_2}$—While the results so far presented implicate the $Cu^{II}(^{-}OOH)$ moiety as being responsible for the observed reactivity (Scheme 2), as before[27] we have examined the product(s) of O_2 reaction with $[(\mathbf{L^{N(CH_2Ph)_2}})Cu^{I}]B(C_6F_5)_4$ (**6**), to determine if copper(I)-dioxygen might be responsible. This seems to not be the case, *vide infra*.

In the context of other works, the **6**/O_2 reactions result in the formation of the metastable bis(μ-oxo)dicopper(III) complex, $[\{(\mathbf{L^{N(CH_2Ph)_2}})Cu^{III}\}_2(O^{2-})_2](B(C_6F_5)_4)_2$ (**9**), which could in fact be characterized by low-temperature UV-vis and rR spectroscopy.[39] Warming such solutions to RT and following $Na_2EDTA/H_2O/CH_2Cl_2$ demetallation procedures, the organic products and yields of these (which are low) are found to be quite different from the $[(\mathbf{L^{N(CH_2Ph)_2}})Cu^{II}(H_2O)(ClO_4)]^{+}$ (**1**)/H_2O_2/Et_3N chemistry, Scheme 2. Some oxidative *N*-dealkylation product $\mathbf{L^{NH(CH_2Ph)}}$ is observed but only in ~ 5% total yield. A small amount of new product, dipicolylamine (~ 4% yield) is also observed; this must be derived from

$Cu^{III}_2(O^{2-})_2$ attack on the benzylic position of the substituted (i.e., with $N(CH_2Ph)_2$) pyridyl moiety, analogous to that previously seen by Suzuki and coworkers.[50,51] Most of the organic recovered (~ 75%) is unreacted starting ligand, $\mathbf{L^{N(CH_2Ph)_2}}$ (Scheme 2). Cu^I/O_2 reaction would normally progress through a copper-superoxo $[Cu^{II}(O_2^{-})]$ initial species,[6,7] thus the observed oxidative chemistry of $[(\mathbf{L^{N(CH_2Ph)_2}})Cu^{II}(H_2O)(ClO_4)]^+$ (**1**)/H_2O_2/Et_3N does not proceed via a superoxo species. The lack of effective *N*-debenzylation reaction of $\mathbf{L^{N(CH_2Ph)_2}}$ by $[Cu^{III}_2(O^{2-})_2]^{2+}$ is most likely due to the axial positioning of the *N*,*N*-dibenzyl arm which prevents the oxo-atom attack. Suzuki and coworkers demonstrated such an axial ligand elongation for 6-substituted 2-pyridyl ligand arms in $[\{(6\text{-}Me_2TPA)Cu^{III}\}_2(O^{2-})_2]^{2+}$ (X-ray, Me_2TPA = (2-pyridylmethyl)bis(6-methyl-2-pyridylmethyl)amine).[50]

Reaction of $[(L)Cu^I]^+$ with PhIO—One might anticipate that $[(\mathbf{L^{N(CH_2Ph)_2}})Cu^{II}(^{-}OOH)]^+$ (**2**) potentially could undergo O-O cleavage chemistry giving a high-valent copper-oxo species, something which is sought after but not known (see further discussion below). There is a very broad and more established Fe^{III}-OOR (R = H or alkyl or acyl; either heme or non-heme iron) homolytic or heterolytic O-O cleavage (bio)chemistry.[52-57] In this context, iodosylbenzene or analogue donors have been widely utilized with reduced metal complexes to generate higher valent metal-oxo complexes, {i.e., PhIO + ligand-M^{n+}; (M = heme, non-heme Fe, Mn, …) —> ligand-M^{n+2}=(O^{2-}) + PhI}.[58-61] As referred to in the Introduction, a copper-oxo moiety is seen to either be responsible for H-atom abstraction in *PHM* or *DβM*, or form later in the reaction sequence/mechanism.

In fact we were previously successful in effecting C-H activation and ligand methyl group hydroxylation leading to an alkoxo product $[(TMG_3trenO^{-})Cu^{II}]^+$, see diagram {$TMG_3$tren = tris(2-(N-tetramethylguanidyl)ethyl)amine}.[24]

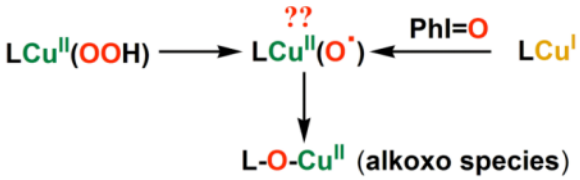


Most interestingly, for the present system, reaction of PhIO with the cuprous complex $[(\mathbf{L^{N(CH_2Ph)_2}})Cu^I]^+$ (**6**) did produce $[(\mathbf{L^{N(CH_2Ph)(PhCHO^{-})}})Cu^{II}]^+$ (**4**) (Scheme 2), as detected by ESI-MS (*m/z* = 563.18) as corroborated when employing $PhI^{18}O$ (*m/z* = 565.34 due to $[(\mathbf{L^{N(CH_2Ph)(PhCH(18)O^{-})}})Cu^{II}]^+$ (**4-^{18}O**) in complementary experiments (see Supporting Information). One thus could suggest that the cupryl species $[(\mathbf{L^{N(CH_2Ph)_2}})Cu^{II}\text{-}O\bullet]^+$ (**8**) formed during the reaction and effected the hydroxylation. Caution is required however, since

it might be that an iodosylbenzene bound copper complex (e.g., [($\mathbf{L^{N(CH_2Ph)_2}}$)Cu^{I}-OIPh]$^{+}$) is the true oxygenating agent and that prior O-O cleavage does not occur.

Potentially exciting observations occurred when we followed the [($\mathbf{L^{N(CH_2Ph)_2}}$)Cu^{I}]$^{+}$ (**6**) / PhIO chemistry using ESI-MS as a function of time; the reaction was carried out at −40 °C and aliquot samples were injected into the mass spectrometer while initially at this temperature. Most interestingly, early on in the reaction, ~ 1 minute after addition of PhIO to [($\mathbf{L^{N(CH_2Ph)_2}}$)Cu^{I}]$^{+}$ (**6**) (CH_3CN solvent, −40 °C) the predominant higher molecular weight ESI-MS species detected occurs with strongest peak at *m/z* = 564.25 (Figure 5), which in fact correspond to a cupryl species, [($\mathbf{L^{N(CH_2Ph)_2}}$)Cu^{III}=O]$^{+}$ (**8**, Cu^{III}=O ≡ Cu^{II}-O•). This peak is only observed early on, as the product alkoxo complex [($\mathbf{L^{N(CH_2Ph)(PhCHO^{-})}}$)$Cu^{II}$]$^{+}$ (**4**) (*m/z* = 563.18) comes in and dominates. Notice that theoretical fitting of this early time mass spectrum best corresponds to a mixture of 70 % cupryl (**8**) and 30 % alkoxo product **4** (Figure 5). In the Supporting Information we show a succession of mass spectra (as a function of time), showing how the putative cupryl is there initially but disappears over ~ 5-6 minutes; it is almost not there at all after ~ 3 minutes. The yields of the organics from the reaction of [($\mathbf{L^{N(CH_2Ph)_2}}$)Cu(I)]$B(C_6F_5)_4$ (**6**) with PhIO could not be determined due to insufficient separation and 'tailing', observed in column and thin-layer chromatography.

Similarly we here carried out a PhIO reaction with the previously studied ligand complex with dimethylamino substituents/substrate, [($\mathbf{L^{N(CH_3)_2}}$)Cu^{I}]$^{+}$ (**7**).[27,39] ESI-MS data indicate that the corresponding alokoxide product forms, i.e. [($\mathbf{L^{N(CH_3)(CH_2O^{-})}}$)$Cu^{II}$]$^{+}$ (**5**) (*m/z* = 411.18) (see Supporting Information). This forms from [($\mathbf{L^{N(CH_3)_2}}$)Cu^{II}($^{-}$OOH)]$^{+}$ (**3**) chemistry also, as reported.[27] Interestingly when the partially deuteriated ligand complex [($\mathbf{L^{N(CH_3)(CD_3)}}$)$Cu^{I}$]$^{+}$ (**7-CD$_3$**) was reacted with PhIO, the ESI-MS analysis leads to the conclusion that for this reaction, the kinetic isotope effect (k_H/k_D) is 1.9 based on the relative formation of [($\mathbf{L^{N(CD_3)(CH_2O^{-})}}$)$Cu^{II}$]$^{+}$ (*m/z* = 414.14) and [($\mathbf{L^{N(CH_3)(CD_2O^{-})}}$)$Cu^{II}$]$^{+}$ (*m/z* = 413.24) (Figure 6). This may be compared to the finding that k_H/k_D ~ 2.2 for the [($\mathbf{L^{N(CH_3)(CD3)}}$)$Cu^{II}$($^{-}$OOH)]$^{+}$ (**3**) reaction.[27] Considering that 1.9 and 2.2 are nearly within experimental error, we suggest that both the [(L)Cu^{II}($^{-}$OOH)]$^{+}$ and [Cu^{I}]$^{+}$/PhIO chemistry proceed through a common reactive intermediate, the cupryl species [(L)Cu^{II}-O•]$^{+}$ (see diagram). The data are suggestive but probably not good enough to yet firmly establish cupryl chemistry.



To summarize this section of discussion, we employed PhIO reactions with reduced complexes [(L)Cu^{I}]$^{+}$ to probe the possibility that high-valent curpyl species are involved in the reactions of [(L)Cu^{II}($^{-}$OOH)]$^{+}$, i.e., following hemolytic O-O bond cleavage. For both the ligand complexes with $\mathbf{L^{N(CH_2Ph)_2}}$ and $\mathbf{L^{N(CH_3)_2}}$, PhIO reactions do give the expected alkoxo products (Scheme 2) and the mass spectrometric data (Figure 5) appear to give direct evidence for the presence or formation of the cupryl complex for the one case, [($\mathbf{L^{N(CH_2Ph)_2}}$)Cu^{II}-O•]$^{+}$ (**8**). We do not wish to overly strongly state that we have detected the first copper cupryl. The overall reaction yields are not exceptional for the PhIO reaction chemistry, although this is probably not the ideal oxo-transfer agent. Also, while detecting the cupryl species in the ESI-MS experiments using PhIO, for some reason it was not observed when employing $PhI^{18}O$; however that final product alkoxide did contain 18-O. In addition to the chemistry described here (and the initial report),[27] Cu^{II}-O-OR homolytic cleavage has been suggested to lead to a

Cu^{II}-O• transient reactive species which may effect substrate oxidation/oxygenation chemistry.[20,22,24,62-64] Scheme 4 depicts some of the recent synthetic examples.[27,20,22]

Mechanistic Discussion—Oxidative *N*-dealkylation is a relatively common reaction in the biotransformation of alkylamines; it is also catalyzed by cytochrome P-450 monooxygenases. Considerable experimental effort has gone into differentiating between conventional hydrogen-atom transfer (*HAT*) versus a sequential reaction where a single-electron transfer (*SET*) oxidation is followed by deprotonation.[57,65-67] Oxidative *N*-dealkylation of the ligand bound to copper in $Cu^{III}_2(O^{2-})_2$ compounds have been observed where mechanistic studies favored initial *HAT*.[7] We also have reported on cases where a $Cu^{III}_2(O^{2-})_2$ (or side-on bound peroxo-dicopper(II) species in equilibrium with this) oxidatively *N*-dealkylate subsititued dimethylanilines but where a shift in mechanism from ET to *HAT* was observed, depending on the ease of substrate one-electron oxidation.[68]

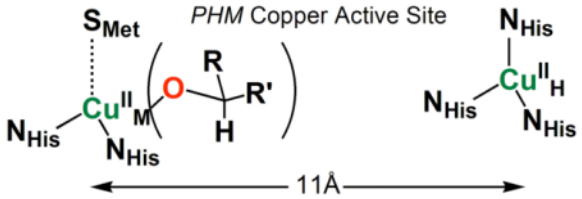
As discussed above for the complexes *N*,*N*-dimethyl group at the 6-position of one arm of TMPA derived ligand, observed KIE values are ~2.2 from $[(\mathbf{L^{N(CH_3)(CD_3)}})Cu^{II}(^{-}OOH)]^+$ (**3-CD$_3$**)[27] chemistry and ~1.9 from $[(\mathbf{L^{N(CH_3)(CD_3)}})Cu^{I}]^+$ (**7-CD$_3$**)/PhIO chemistry. These results suggest a net H-atom abstraction occurs. Organic products isolated (i.e., recovered dialkyl ligand) from $[(\mathbf{L^{N(CH_3)(CD_3)}})Cu^{II}(^{-}OOH)]^+$ chemistry included those due to scrambling of isotopes via radical intermediates, and $\mathbf{L^{N(CH_3)(CD_3)}}$, $\mathbf{L^{N(CH_3)(CD_2H)}}$, $\mathbf{L^{N(CH_3)(CDH_2)}}$ and $\mathbf{L^{N(CH_3)_2}}$ were all observed. However, more studies would be required to differentiate between *HAT* and a *SET* step.

Based on the results described here, we outline our proposed mechanism of reaction for $[(\mathbf{L^{N(CH_2R')_2}})Cu^{II}(^{-}OOH)]^+$ chemistry (Scheme 5). Initial net *HAT* (either directly from the hydroperoxide or occurring following O-O cleavage) substrate H-atom abstraction would lead to a high-valent Cu^{II}-O• (≡ Cu^{III}=O) and substrate imminium radical cation species; 'rebound' gives the alkoxide (*Path A*). Most often in the synthetic system and always in the enzyme, protonation (from active-site solvent) releases the organic products observed, the amine and corresponding aldehyde.

In Scheme 5, we also show (*Path B*) how an over-oxidized product amide would most likely form in the model synthetic system, via facile nucleophilic attack of a 2nd equivalent of hydroperoxide anion. In connection with other over-oxidation product chemistry, we recently isolated a small amount of a decomposition product, $[(\mathbf{L^{N(CH_3)_2}})Cu^{II}(HCOO^{-})(ClO_4)Cu^{II}(\mathbf{L^{NH(CH_3)}})](ClO_4)_2$ (**11**), for which we were able to obtain an X-ray structure (Figure 7) (See Supporting Information); this originates from previously described chemistry of $[(\mathbf{L^{N(CH_3)_2}})Cu^{II}(^{-}OOH)]^+$ (**3**). This odd dicopper(II) complex possesses a bridging formate ($HCOO^{-}$) group, wherein one of the copper ligands is $\mathbf{L^{N(CH_3)_2}}$, i.e., unreacted starting ligand, and the other copper ion is supported by a singly *N*-demethylated product $\mathbf{L^{NH(CH_3)}}$. An EPR spectrum of the isolated crystals then dissolved in acetone indicates the presence of two independent Cu (II) ions, with overlapping axial spectra (see Supporting Information). Further, an ESI-MS of the material contains two strong peaks; the one at *m/z* = 427.04 corresponds to $[(\mathbf{L^{NH(CH_3)}})Cu^{II}(HCOO^{-})]^+$ and the other at m/z = 441.04 corresponds to $[(\mathbf{L^{N(CH_3)_2}})Cu^{II}(HCOO^{-})]^+$. Generation of formate ($HCOO^{-}$) can be envisaged since formaldehyde was earlier on detected as a product derived from **3**. Similarly, we are able to detect a small ESI-MS peak at *m/z* = 579.17 from the decomposition product mixture of $[(\mathbf{L^{N(CH_2Ph)_2}})Cu^{II}(^{-}OOH)]^+$ (**2**), which corresponds to a copper complex species containing benzoate, $[(\mathbf{L^{NH(CH_2Ph)}})Cu^{II}(PhCOO^{-})]^+$, i.e. a further oxidation product of benzaldehyde which was a primary product derived from **2**. All of these over-oxidized products are obtained in very small yields. Never-the-less they are indicative of the extensive chemistry occurring in the manner proposed.

***PAM* Chemistry Mimic—**We have shown here that the hydroperoxo group reaction in $[(\mathbf{L^{N(CH_2Ph)_2}})Cu^{II}(^{-}OOH)]^{+}$ (**2**) leads to the formation of oxidatively *N*-dealkylated $\mathbf{L^{NH(CH_2Ph)}}$ plus PhCH=O. The $-N(CH_2Ph)_2$ substrate (ligand substituent) resides in close proximity to the $Cu^{II}(^{-}OOH)$ moiety (Scheme 3, R′ = Ph) and essentially identical chemistry occurs for R′ = H.[27] As described above, a copper(II)-alkoxide complex forms prior to organic product release (Scheme 3).

Thus, the present model systems (R′ = H, Ph) very closely resemble much of the proposed oxidative *N*-dealkylation reaction mechanism effected by *PAM* (Figure 1). There, an active site reactive species forms at Cu_M with His_2Met coordination, ideally juxtaposed to the prohormone peptide substrate C–H group of the terminal glycine. Further, our cupric-alkoxide complex, formed following from $[(\mathbf{L^{N(CH_2Ph)_2}})Cu^{II}(^{-}OOH)]^{+}$ (**2**) or $[(\mathbf{L^{N(CH_2Ph)_2}})Cu^{I}]^{+}$ (**6**)/PhIO reactivity mimics the ‘product substrate Cu^{II}-alkoxide complex’ discussed for both *PHM* and *DβM* (see diagram).[9] However, as stated in the Introduction, current thinking is that a copper-superoxo complex may be the active species. Or it could be a cupryl species derived from $Cu^{II}(^{-}OOH)$ O-O bond cleavage. Our results, with reactivity pattern so close to that of *PHM/PAM*, rather favors the view that the reactive species is a hydroperoxo or cupryl group.



Summary

This report summarizes our advances in oxidative *N*-dealkylation chemistry with hydroperoxo copper(II) complexes which have an dialkylamino substrate ($-N(CH_2Ph)_2$ or $-N(CH_3)_2$) appended as a substituent on one pyridyl group of the tripodal tetradentate TMPA ligand framework. In the previous Communication and from the present full report, we can argue that a $Cu^{II}(^{-}OOH)$ or species generated from it (i.e., a high-valent copper-oxo species) can initiate useful substrate hydroxylation reactions, those in fact occurring in the enzyme *PAM* (*PHM* + *PAL*) (Figure 1). Net substrate H-atom abstraction occurs starting with the $Cu^{II}(^{-}OOH)$ moiety and the likely structure of an intermediate substrate organic radical species is proposed, accounting for the products observed. The formation of a substrate and ^{-}OOH (an oxygen atom) derived alkoxo $Cu^{II}(^{-}OR)$ complex has been proven; the latter has also been proposed in *PHM* mechanisms. The fact that the alkoxo complex forms either from $Cu^{II}(^{-}OOH)$ or Cu^{I}/PhIO chemistry suggests the possibility that a high-valent copper-oxo reactive intermediate forms, and ESI-MS data provides some further support. Clearly, however, further studies on this or other systems need to be carried out to truly substantiate the existence of a cupryl species, and to elucidate the full chemistry of already existing $Cu^{II}(^{-}OOH)$ and $Cu^{II}(O_2^{-}\bullet)$ complexes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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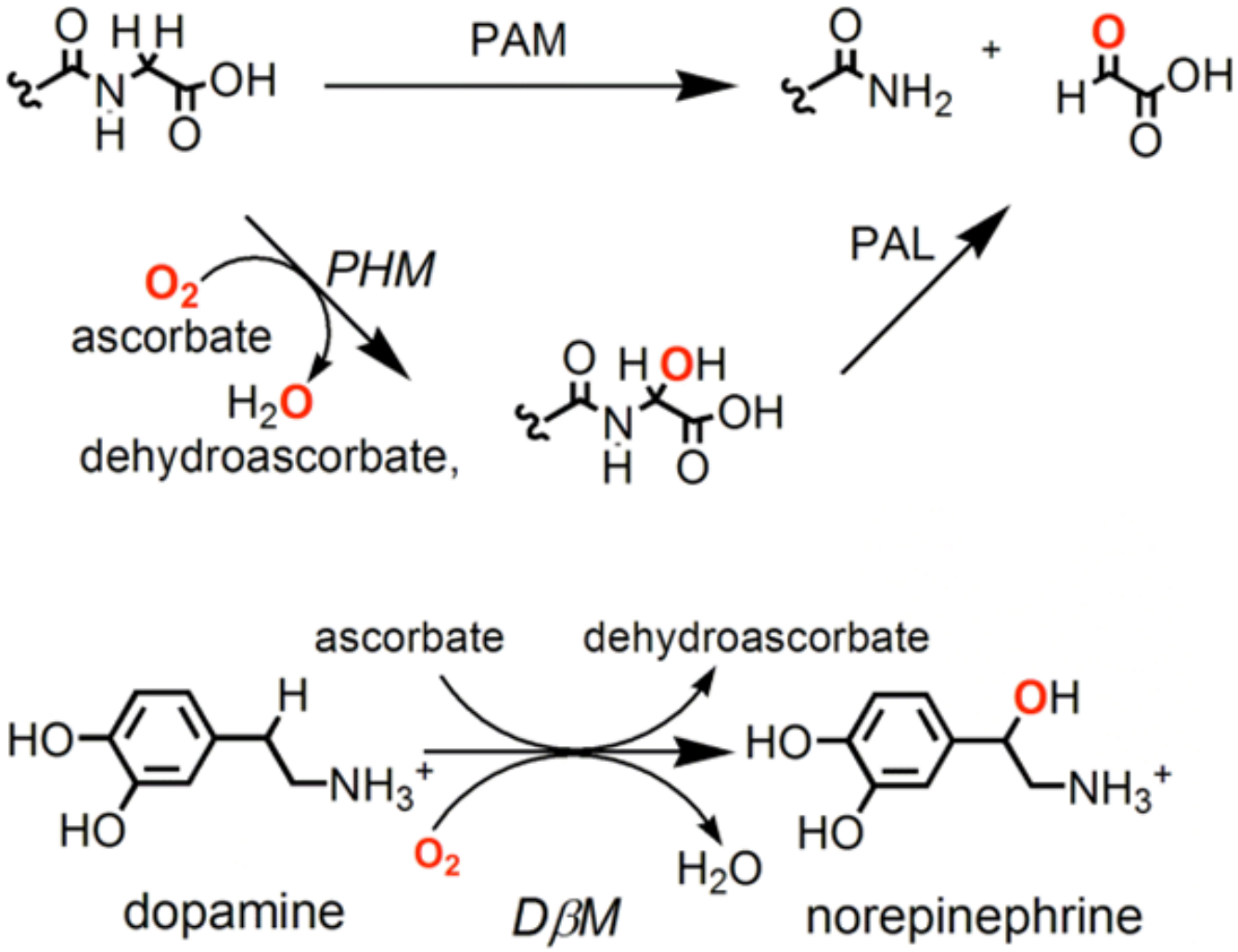


Figure 1.
Homologus copper proteins DβM (bottom) transform dopamine to norepinephrine and *PHM* (top) hydroxylate the C-terminus of the peptide backbone followed by *N*-dealkylation by PAL.

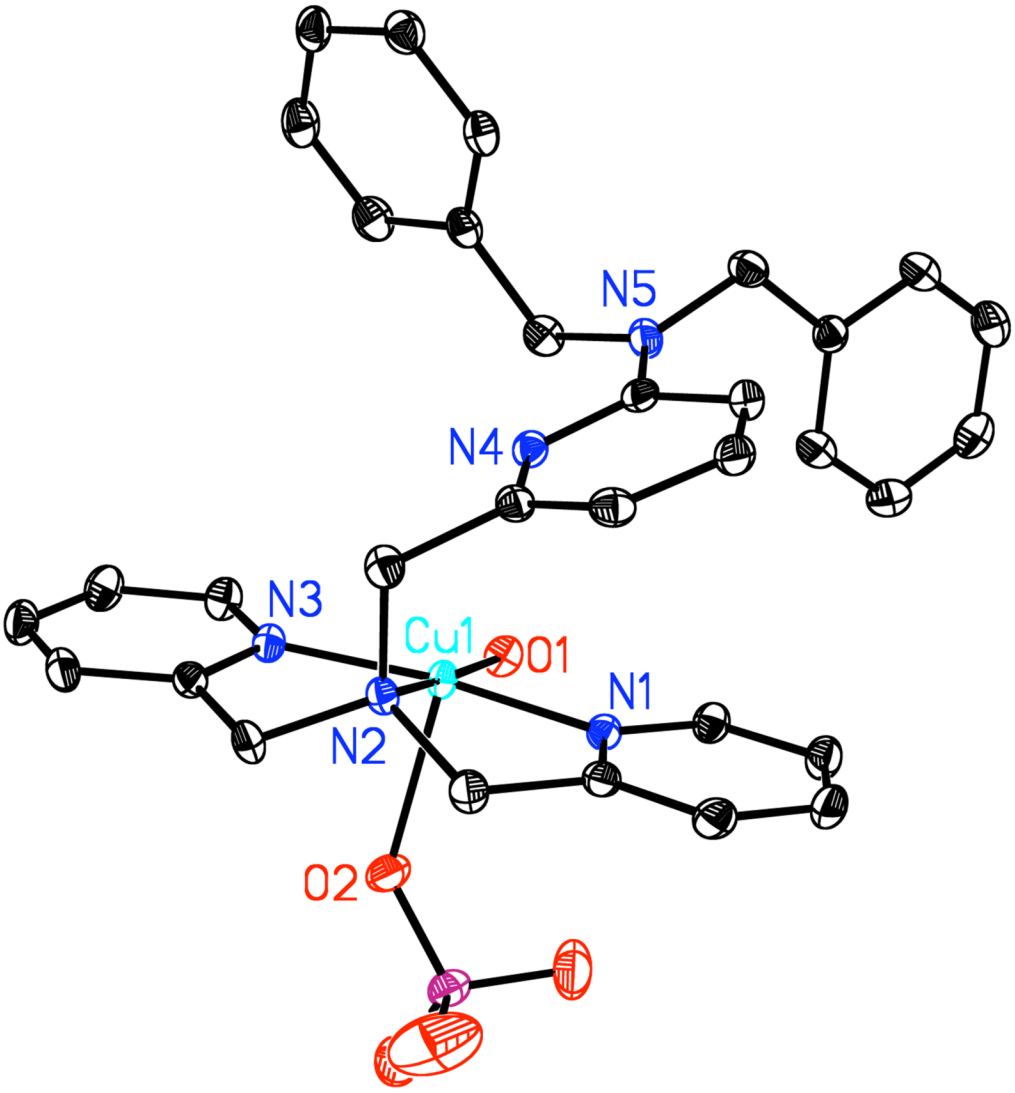


Figure 2.

ORTEP diagram of [($\mathbf{L}^{\mathbf{N(CH_2Ph)_2}}$)$Cu^{II}(H_2O)(ClO_4)$]($ClO_4$) (**1**). Thermal ellipsoids are drawn by ORTEP and represent 50% probability surfaces. Cu(1)-O(1) = 1.972 Å; Cu(1)-N(1) = 1.989 Å; Cu(1)-N(3) = 1.997 Å; Cu(1)-N(2) = 2.010 Å; ∠ O(1)-Cu(1)-N(1) = 94.34°; ∠ O(1)-Cu(1)-N(3) = 99.06°; ∠ N(1)-Cu(1)-N(3) = 166.31°; Cu1-N4 = 2.935 Å.

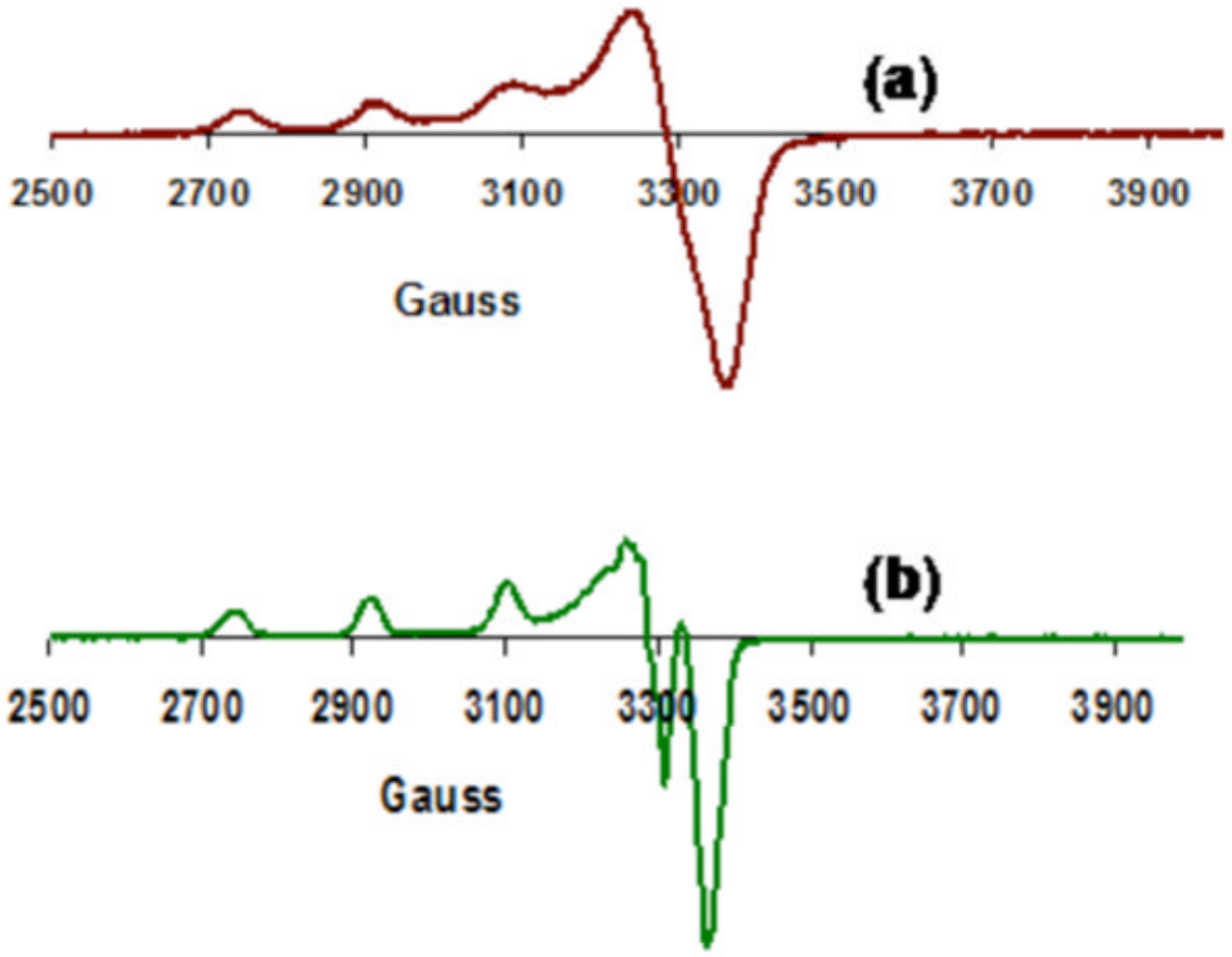


Figure 3.

EPR (X-band, 77K, acetone) spectra of (**a**) [($\mathbf{L^{N(CH_2Ph)_2}}$)$Cu^{II}(H_2O)(ClO_4)](ClO_4)$ (**1**), $g_{\|}$ = 2.252, $g_{\perp}$ = 2.047, $A_{\|}$ = 170 G, $A_{\perp}$ = 29.5 G. (**b**) [($\mathbf{L^{N(CH_2Ph)_2}}$)$Cu^{II}(^{-}OOH)](ClO_4)$ (**2**) generated from reaction of **1** with H_2O_2, $g_{\|}$ = 2.240, $g_{\perp}$ = 2.041, $A_{\|}$ = 180 G, $A_{\perp}$ = 27 G.

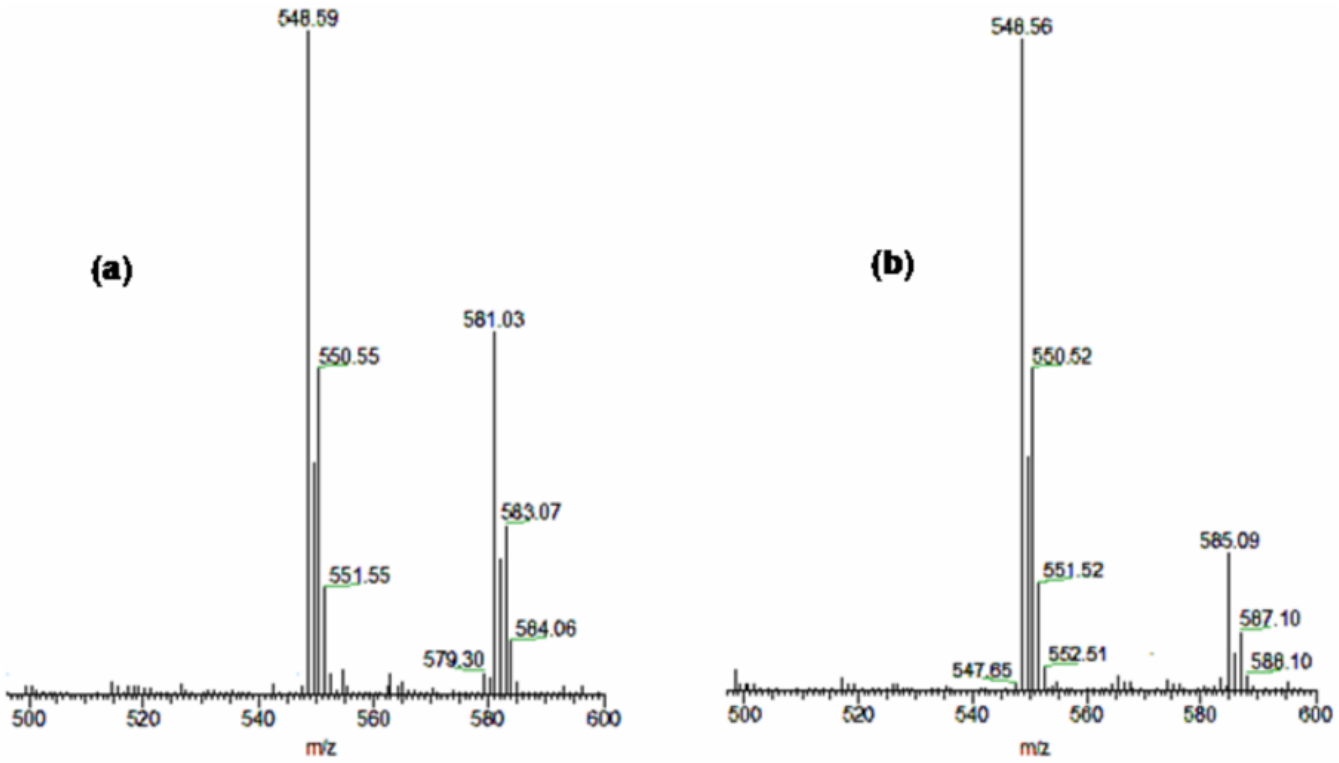


Figure 4.
ESI-MS spectra of (**a**) [(**L**$^{\mathbf{N(CH_2Ph)_2}}$)Cu^{II}($^{-}$OOH)](ClO_4) (**2**) from the reaction of **1** with H_2O_2 and (**b**) [(**L**$^{\mathbf{N(CH_2Ph)_2}}$)Cu^{II}($^{-18}O^{18}OH$)](ClO_4) (**2-^{18}O**) generated by **1**/$H_2{}^{18}O_2$.

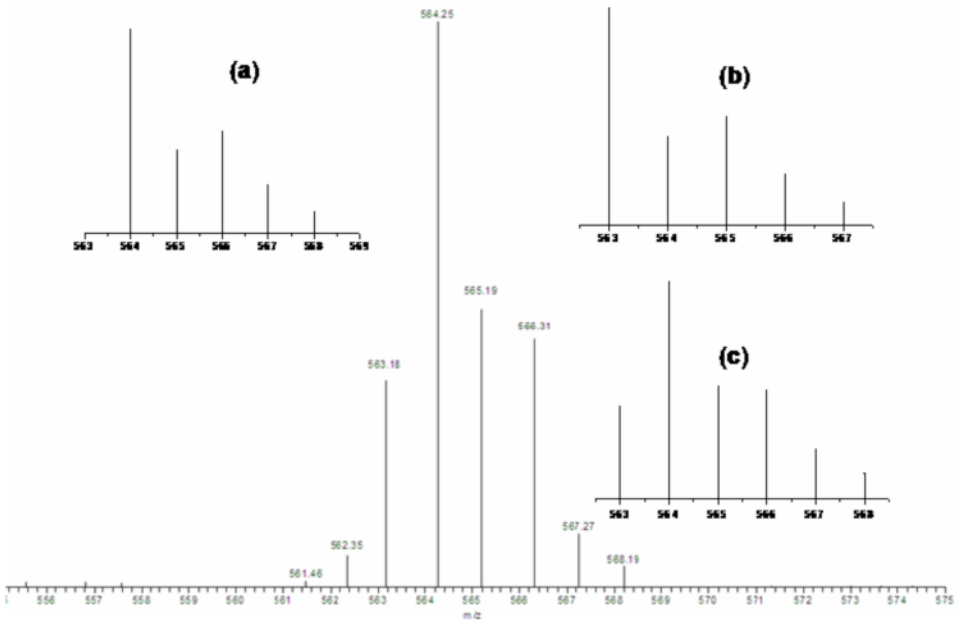


Figure 5.

ESI-MS spectra from the reaction of $[(\mathbf{L^{N(CH_2Ph)_2}})Cu^{I}]^{+}$ (**6**) with PhIO suggesting the transient formation of the cupryl complex $[(\mathbf{L^{N(CH_2Ph)_2}})Cu^{II}\text{-}O\bullet]^{+}$ (**8**) at *m/z* = 564.25 (~70% formation) and $[(\mathbf{L^{N(CH_2Ph)(PhCHO^{-})}})Cu^{II}]^{+}$ (**4**) at *m/z* = 563.18 (~30% formation). Insets showing the expected pattern for (**a**) 100% formation of **8** and (**b**) 100% formation of **4**, and the best fitting to the data with (**c**) 70% formation of **8** plus 30% formation of **4**. See text for further discussion.

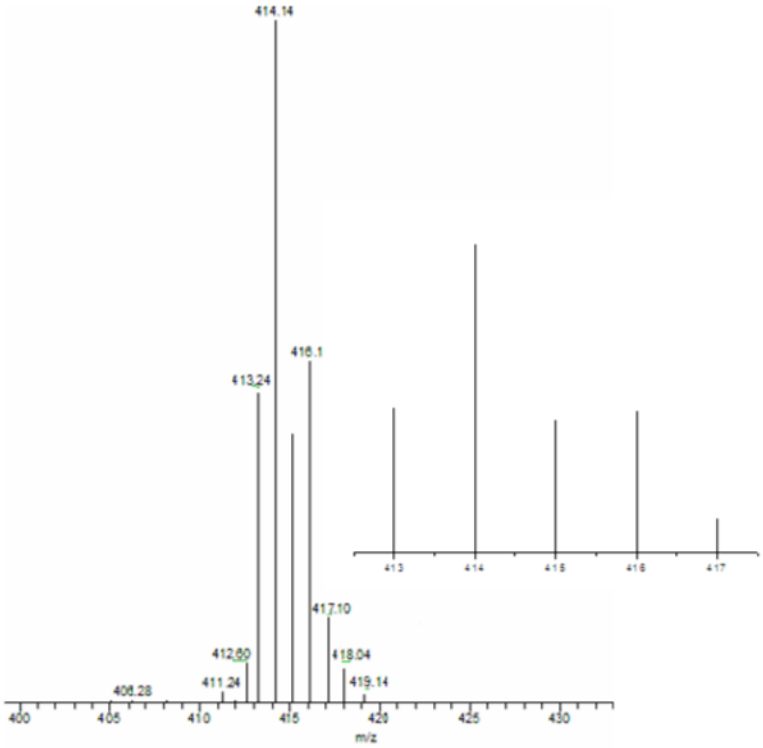


Figure 6.

ESI-MS spectra obtained from the reaction of [($\mathbf{L}^{\mathbf{N(CH_3)(CD_3)}}$)$Cu^{I}$]$^{+}$ (**7**) with PhIO. The ratio of formation of [($\mathbf{L}^{\mathbf{N(CD_3)(CH_2O^-)}}$)$Cu^{II}$]$^{+}$ (*m/z* = 414.14) versus [($\mathbf{L}^{\mathbf{N(CH_3)(CD_2O^-)}}$)$Cu^{II}$]$^{+}$ (*m/z* = 413.24) suggest that $k_H/k_D \sim 1.9$. The inset shows the expected (calculated) ESI-MS spectral pattern for a reaction which would lead to $k_H/k_D \sim 1.9$.

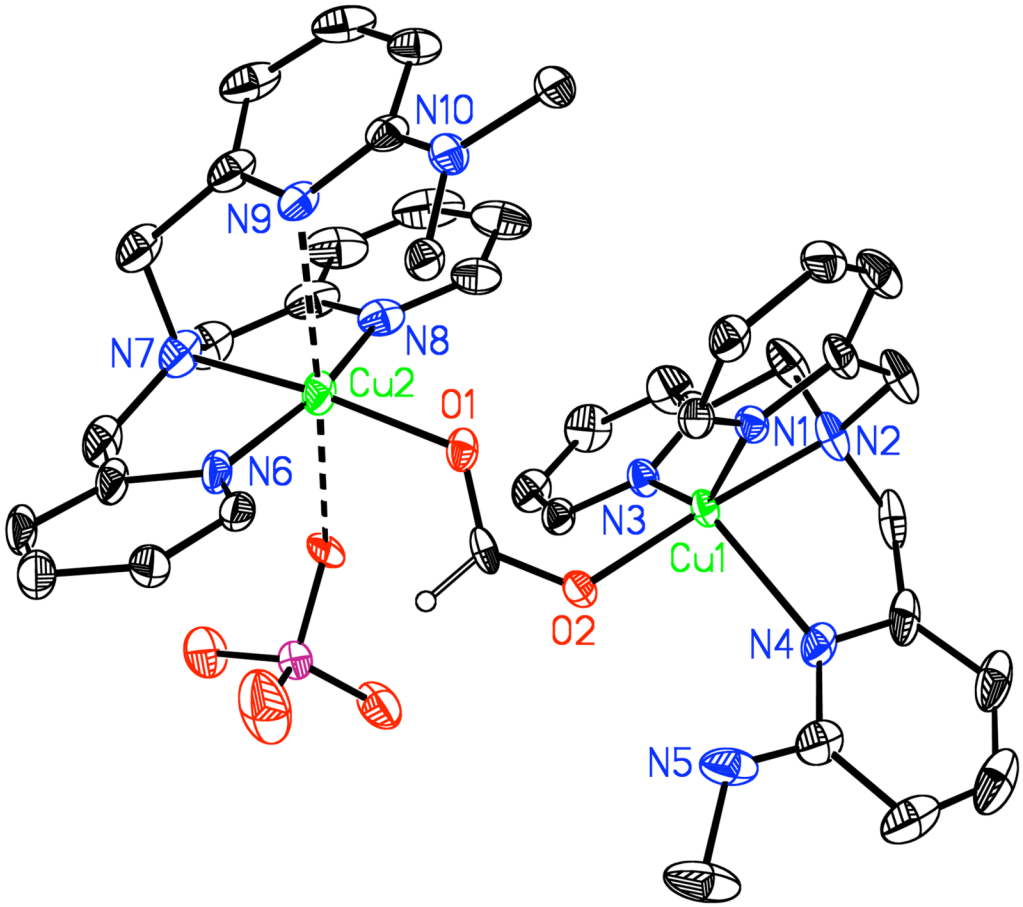
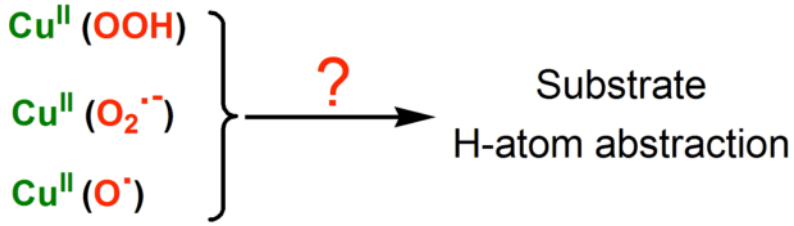
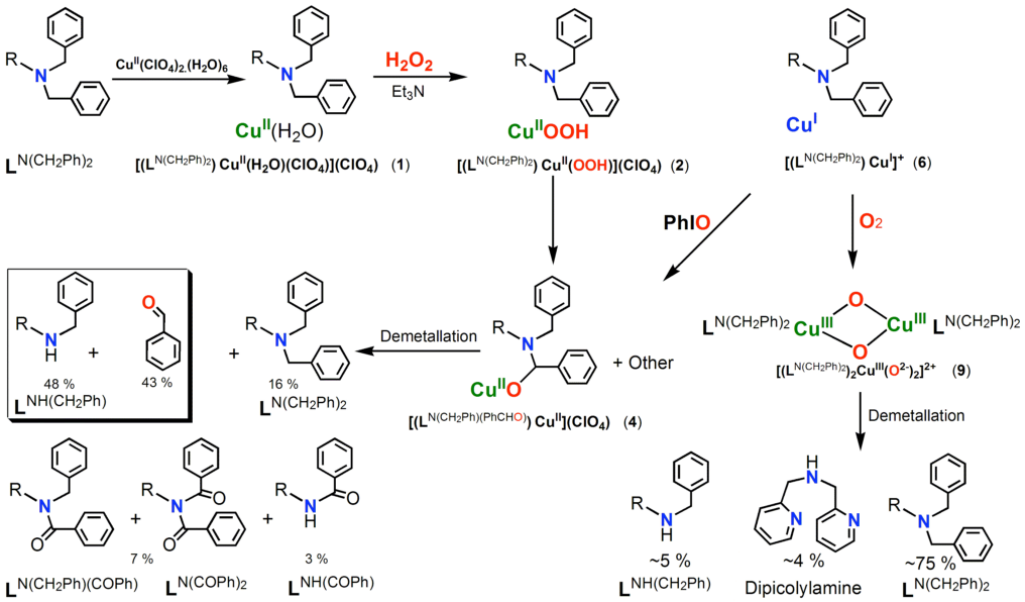


Figure 7.

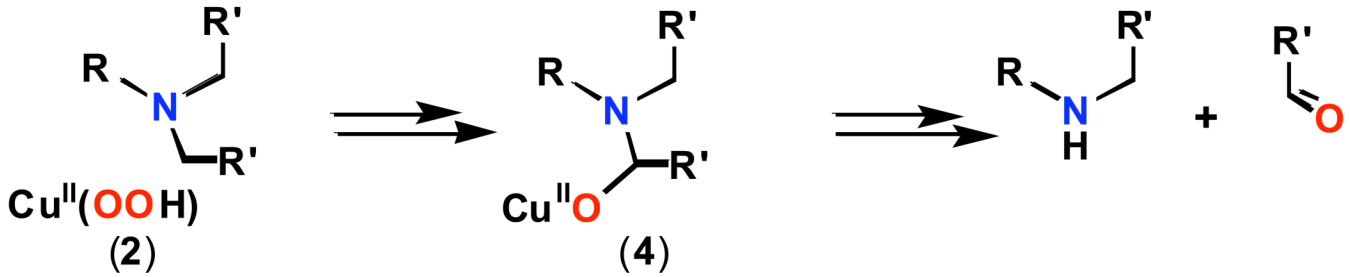
ORTEP diagram of the cationic component of [($\mathbf{L}^{\mathbf{N(CH_3)_2}}$)$Cu^{II}$(HCOO$^-$)($ClO_4$) Cu^{II}($\mathbf{L}^{\mathbf{NH(CH_3)}}$)]($ClO_4$)$_2$ (**11**). Thermal ellipsoids are drawn by ORTEP and represent 50% probability surfaces. Cu(1)-O(2) = 1.942 Å; Cu(1)-N(3) = 2.020 Å; Cu(1)-N(2) = 2.022 Å; Cu (1)-N(1) = 2.023; ∠ O(2)-Cu(1)-N(3) = 96.00°; ∠ O(2)-Cu(1)-N(2) = 175.51°; ∠ N(3)-Cu (1)-N(2) = 82.15°; ∠ O(2)-Cu(1)-N(1) = 99.39°.



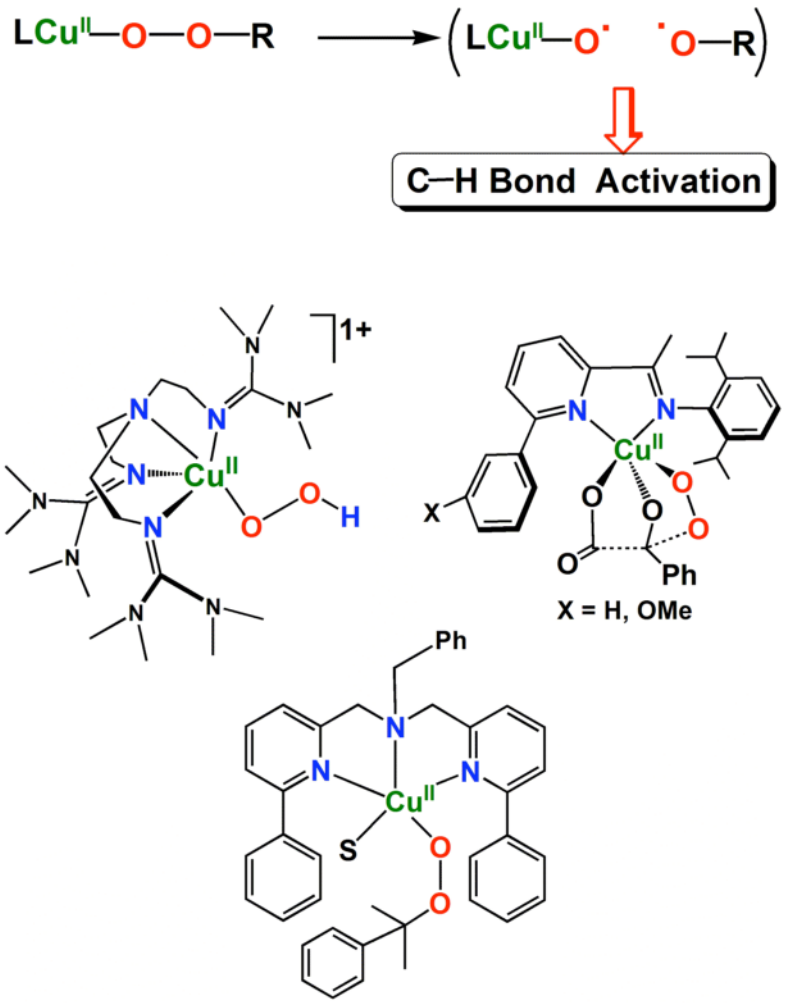
Scheme 1.



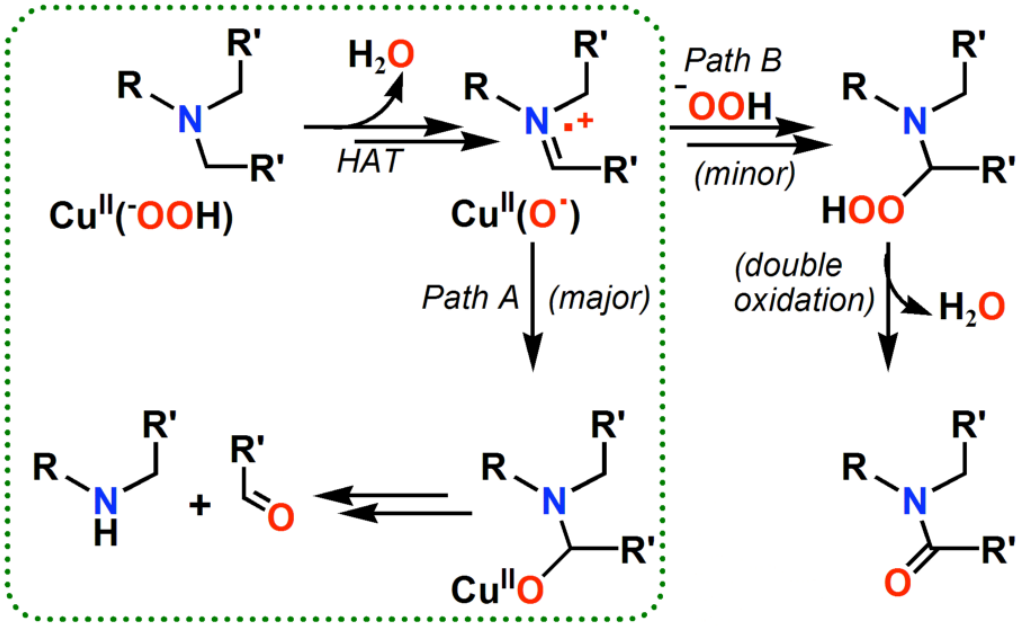
Scheme 2.



Scheme 3.



Scheme 4.



Scheme 5.

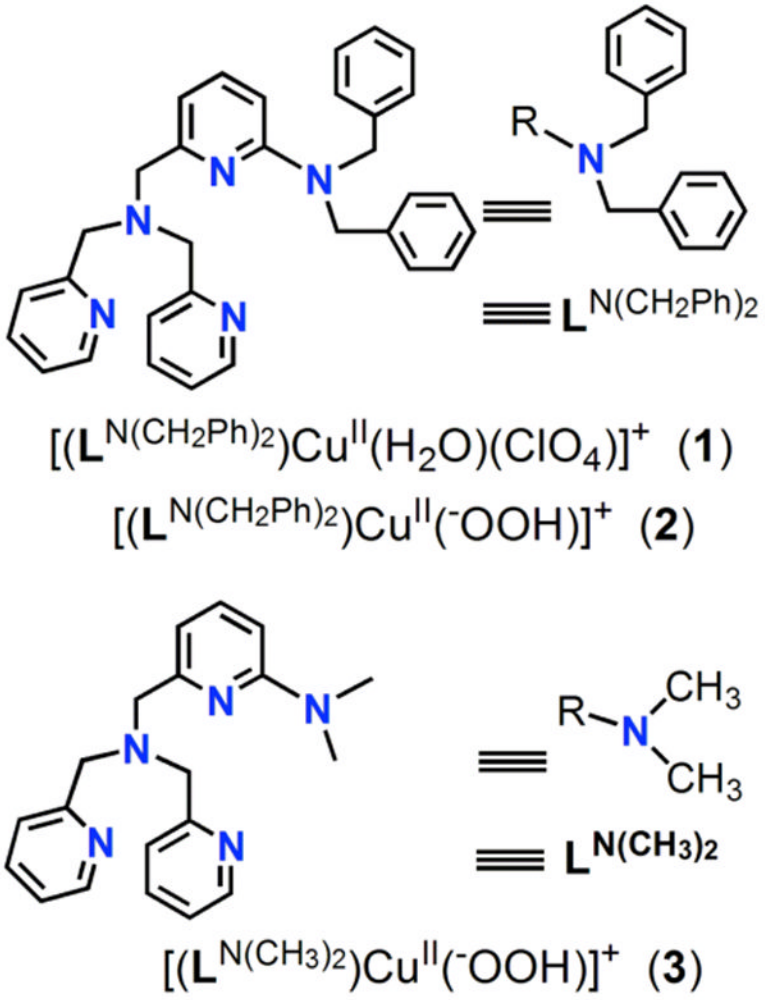


Chart 1.

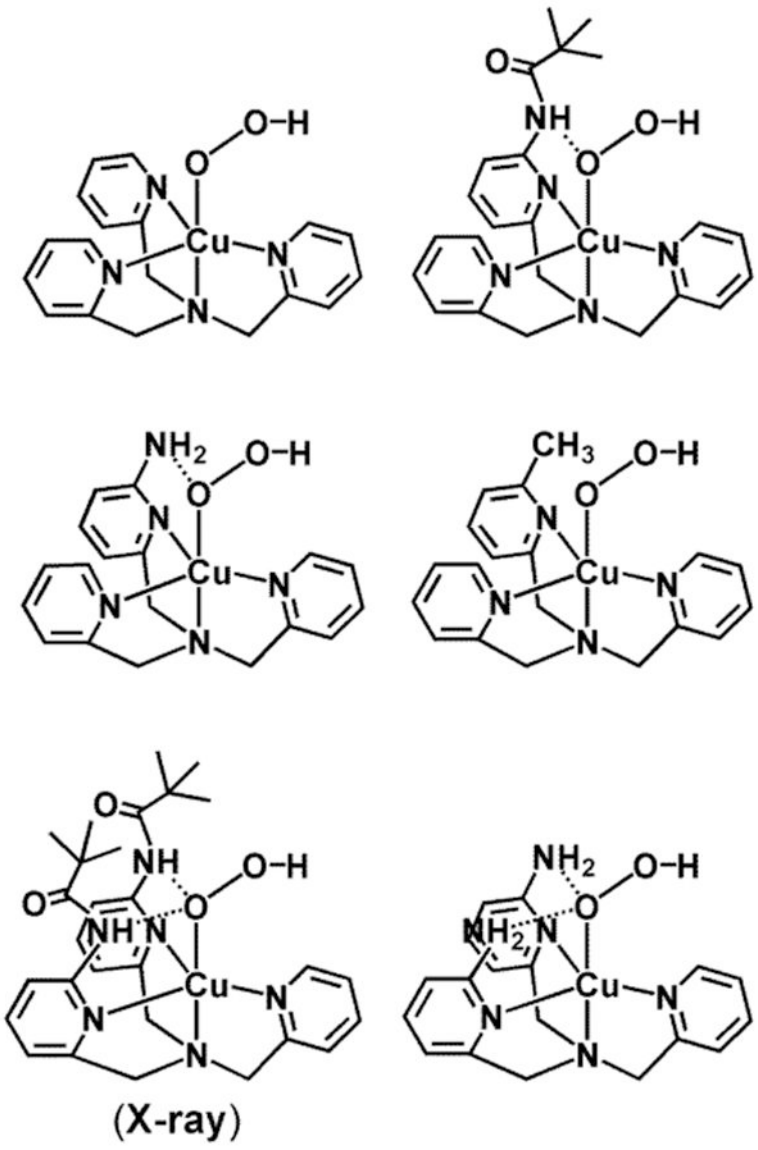


Chart 2.