advanced previously[46] as a necessary step in a proposed pathway for the activation and reduction of N_2 with a nitrogenase active site model that employs S^{2-} bridged $[MoFe_3S_4]$ and $[Fe_4S_4]$ centers.

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Supplementary Material Available: Tables of positional parameters, thermal parameters, and selected distances and angles for $(Et_4N)_3[Fe_3S_4(SEt)_3Mo(CO)_3]\cdot CH_3CN$ (**I**) (7 pages); table of observed and calculated structure factors for **I** (6 pages). Ordering information is given on any current masthead page. The same crystallographic data for **I** have been deposited with a previous communication[21] and can be obtained on request from the Fachinformationzentrum Energie, Physik, Mathematik GmbH, D-7514 Eggenstein-Leopoldshafen 2 (FRG), on quoting the depository number CSD-53196, the names of the authors, and the journal citation.

Mechanism of Aromatic Hydroxylation in a Copper Monooxygenase Model System. 1,2-Methyl Migrations and the NIH Shift in Copper Chemistry

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Abstract: The NIH shift mechanism appears to be operative in a copper monooxygenase model system involving dicopper ion complex mediated O_2 hydroxylation of an arene substrate. Previous studies have shown that when a dicopper(I) complex containing two tridentate PY2 units (PY2 = bis[2-(2-pyridyl)ethyl]amine) which are linked by a *m*-xylyl group, i.e., $[Cu_2(XYL\text{-}H)]^{2+}$ (**1**), is reacted with dioxygen, a Cu_2O_2 intermediate forms and hydroxylation in the intervening 2-xylyl position occurs. Here, corresponding reactions of 2-methyl substituted analogues $[Cu_2(Me_2XYL\text{-}CH_3)]^{2+}$ (**4**) and $[Cu_2(XYL\text{-}CH_3)]^{2+}$ (**5**) are described in detail. Oxygenation of these causes xylyl hydroxylation reactions producing new phenol products, with concomitant 1,2-migration of the methyl group, loss of one PY2 ligand arm, and formaldehyde formation. Manometric O_2 uptake experiments and an $^{18}O_2$ labeling study confirm that the stoichiometry of these reactions are consistent with that observed for monooxygenases. A reaction carried out using a dinucleating ligand which has been deuterated in benzylic positions confirms that the CH_2O product is derived from this carbon atom, a result also consistent with migration of the 2-methyl group. A small yield of methylbis[2-(2-pyridyl)ethyl]amine (MePY2) is consistently obtained, and experiments suggest this may be derived from the reduction of an intermediate iminium salt $\{CH_2{=}N[CH_2CH_2PY]_2\}^+$ (PY = 2-pyridyl). The hydroxylation induced 1,2-methyl migrations observed here are reminiscent of the NIH shift reactions previously observed only in iron hydroxylases and suggest that the copper ion mediated reactions proceed by the electrophilic attack of a Cu_2O_2 intermediate upon the proximate aromatic substrate. A detailed mechanism is proposed and discussed in terms of the known O_2 reactivity and structure of these dinuclear copper complexes. The biological relevance and significance of this monooxygenase model system is also discussed.

Introduction

Widely occurring copper enzymes are involved in the processing or utilization of dioxygen.[1-3] Functions performed include O_2 transport by hemocyanin (Hc),[4] substrate mono- and dioxygenation, and oxidation (dehydrogenation) of substrates with concomitant reduction of O_2 to either H_2O_2 or water (e.g., oxidases). Monooxygenases of copper have attracted a great deal of recent attention, along with iron enzymes such as (i) cytochrome P-450 monooxygenase,[5] (ii) non-heme iron methane monooxygenase (MMO),[6] and (iii) pterin-dependent phenylalanine hydroxylase.[7] This is due to the importance of the reactions they catalyze, an interest in the fundamental chemistry involved, and the hope to mimic the mild and selective biological oxygenation reactions in model systems or in synthetically useful applications. Copper monooxygenases[8-12] include tyrosinase (Tyr; *o*-phenol hydroxylase),[8] pterin-dependent *Chromobacterium violaceum* phenylalanine hydroxylase (PAH),[7,9] dopamine β-hydroxylase (benzylic hydroxylation of dopamine yielding the neurotransmitter norepinephrine),[10] and peptidylglycine α-amidating monooxygenase[11] (PAM; oxidative N-dealkylation of glycine-extended neuropeptide prohormones).

Tyr and Cu-dependent PAH effect aromatic hydroxylation reactions, which undoubtedly occur via different mechanisms since

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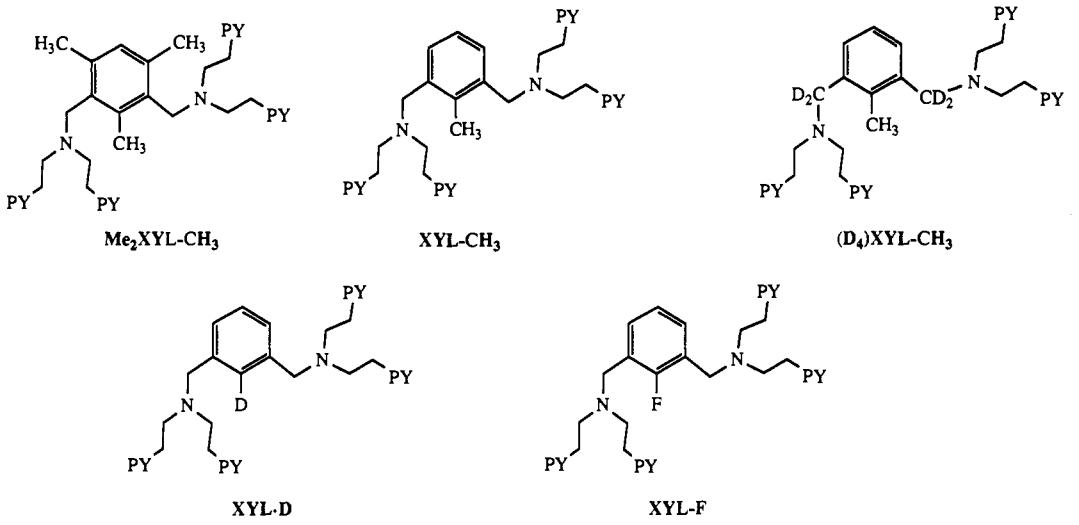
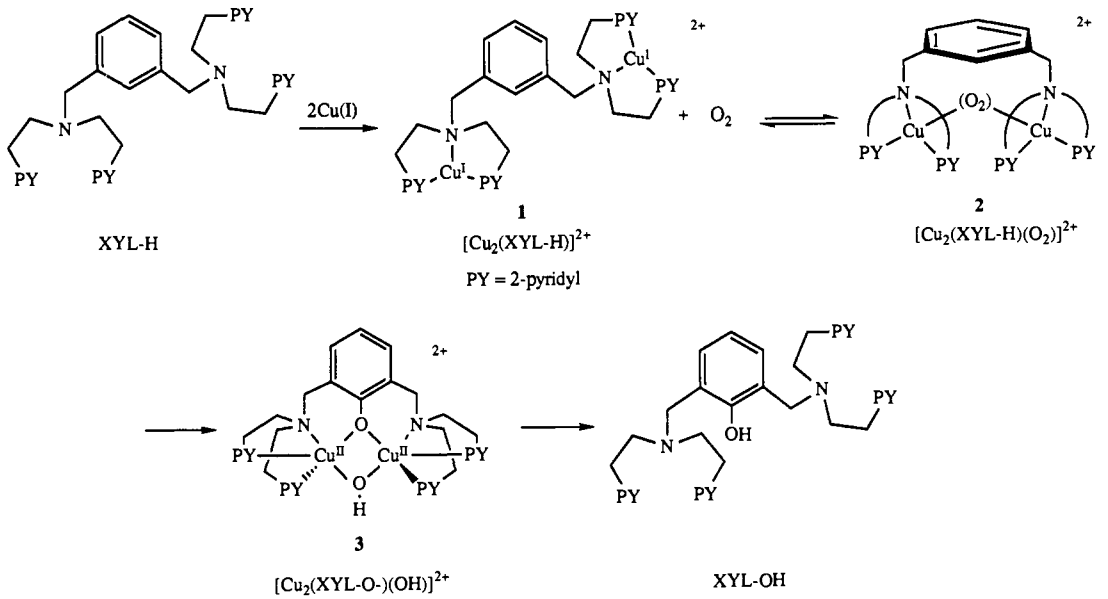


Figure 1. 2-Substituted xylyl dinucleating analogues used in this study.

Scheme I



the former contains an active site very similar to that of Hc with a dinuclear copper center, while the latter utilizes a single copper ion and pterin cofactor. We have described a copper complex mediated hydroxylation of an arene, probably best serving as a copper monooxygenase model system for Tyr, since a dicopper ion center is involved.[3,13] Dicopper(I) complex $[Cu_2(XYL\text{-}H)]^{2+}$ (**1**) possessing two tricoordinate Cu(I) moieties reacts reversibly (vide infra) with O_2 to form an intermediate O_2 complex $[Cu_2(XYL\text{-}H)(O_2)]^{2+}$ (**2**), best described as a peroxo dicopper(II) species. The peroxo group then attacks the xylyl ligand resulting in hydroxylation to give the phenoxo- and hydroxo-bridged compound $[Cu_2(XYL\text{-}O^-)(OH)]^{2+}$ (**3**). The conversion of **1** to **3** is essentially quantitative (>95% isolated yield), and isotopic labeling experiments using $^{18}O_2$ confirm that the phenoxo oxygen atom in **3** and the free phenol obtained from this complex (e.g., XYL-OH) are derived from molecular oxygen. From manometry, $Cu/O_2 = 2{:}1$, thus it is clear that the conversion of **1** to **3** represents the same stoichiometry observed in enzyme monooxygenases (e.g., eq 1), with the two electrons supplied by the two Cu(I) ions and the second oxygen atom trapped as a coordinated hydroxide ion in the product **3**.

$$RH + O_2 + 2e^- + 2H^+ \rightarrow ROH + H_2O \qquad (1)$$

Thus, a mechanistic study of the reaction **1** → **3** could provide insights into the chemical and biological activation of O_2. Along these lines, our efforts have included both kinetic and chemical studies of the reactions of compound **1** as well as complexes derived from synthetic analogues of the xylyl ligand XYL-H. In this report, we describe investigations using the 2-substituted xylyl analogues shown in Figure 1. These have provided useful insights, especially the 2-methyl substituted ligand complexes. We find that reactions of O_2 with dicopper(I) complexes of $Me_2XYL\text{-}CH_3$[14] and $XYL\text{-}CH_3$ cause 2-hydroxylation accompanied by 1,2-methyl migration. Here we present the details of these observations, including labeling and other ancillary experiments which give very useful insights concerning the nature of these reactions.

These findings allow us to propose a mechanism for these copper mediated xylyl hydroxylations. The methyl migration reactions are highly reminiscent of the "NIH shift" rearrangements [her-

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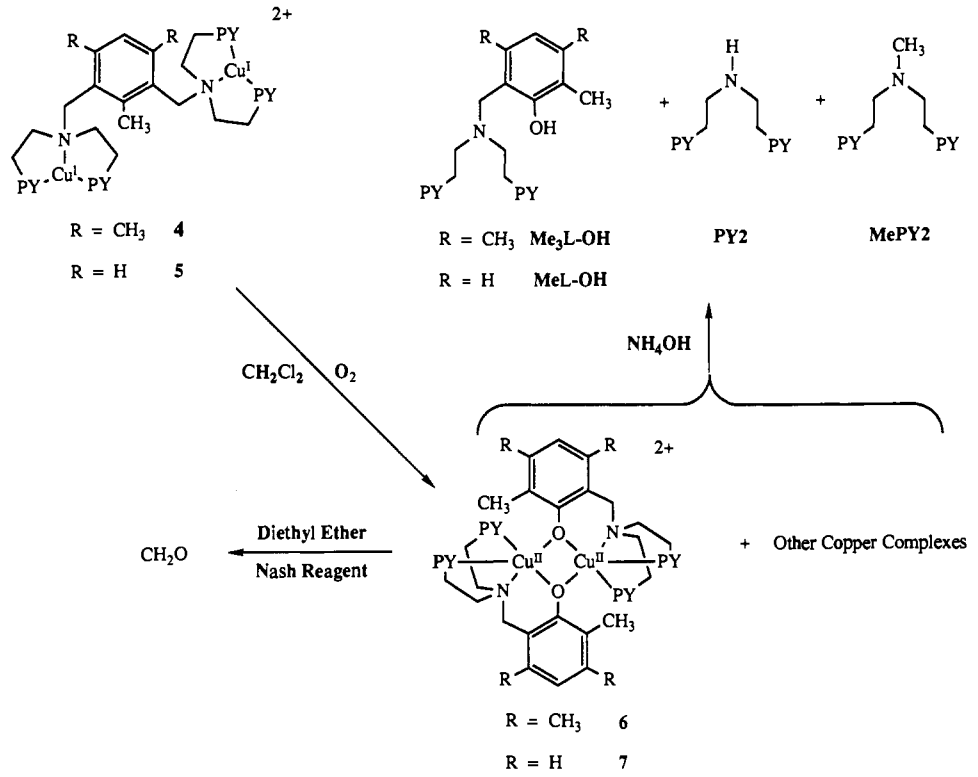

Figure 2. Summary of reactions of O_2 with 2-methyl substituted xylyl dicopper(I) complexes $[Cu_2(Me_2XYL\text{-}CH_3)]^{2+}$ (**4**) and $[Cu_2(XYL\text{-}CH_3)]^{2+}$ (**5**).

eafter referred to simply as the NIH shift] observed during iron enzyme catalyzed aromatic hydroxylations by cytochrome P-450 or PAH.[15-19] The NIH shift reaction characteristically involves cationic intermediates derived from electrophilic attack of an as yet not fully described iron–oxy species upon the arene substrate. It is thus suggested the reaction **1** → **3** proceeds by xylyl group attack of an electrophilic Cu_2O_2 oxygenating agent **2**, followed by what we believe is the first example[14] of the NIH shift in copper chemistry. These results may also be relevant to the mechanism of action of copper hydroxylases such as Tyr and PAH.

Results

Ligand and Complex Synthesis. The dinucleating ligands shown in Figure 1 were synthesized by standard procedures, as outlined in the Experimental Section. The focus of this paper is on the 2-methyl derivatives $Me_2XYL\text{-}CH_3$, $XYL\text{-}CH_3$, and $(D_4)XYL\text{-}CH_3$, which were used to probe the oxidatively induced methyl migration reactions with accompanying labeling experiments. Since results from kinetic and chemical/spectroscopic studies employing XYL-D[20] and XYL-F[21] contributed to an understanding of the hydroxylation mechanism described here, the full synthesis and characterization of these ligands and their dicopper(I) complexes are reported here. A 2-chloro ligand analogue (XYL-Cl) was also prepared; it undergoes a related oxidative dechlorination reaction which has been described.[22]

The dinuclear copper(I) complexes $[Cu_2(XYL)](PF_6)_2$ were prepared in good yield by the addition of 2 mol-equiv of $[Cu(CH_3CN)_4]PF_6$ to a dichloromethane solution of the appropriate ligand under an argon atmosphere. Precipitation followed by recrystallization afforded off-white to yellow solids (sometimes as solvates) which are stable under Ar and soluble in a variety of polar organic solvents such as CH_2Cl_2, CH_3CN, CH_3OH, or acetone. These complexes have been characterized by elemental analyses and by infrared, ^1H and ^{13}C NMR spectroscopies. Sharp spectra typical of diamagnetic compounds are observed in all cases, and ^1H and ^{13}C chemical shift data are given in the supplementary material (Tables I and II, respectively). The assignments were made in accordance with data from other related complexes and literature NMR spectroscopic tables.[23-27] There is a general trend for downfield ^1H chemical shifts of ligand resonances upon coordination to Cu(I). As expected,[26] reduced ^{13}C intensities were observed in cases where hydrogens on carbon atoms were replaced with deuterium.

Summary of O_2 Reactions of the 2-Me Substituted Complexes. Reactions of O_2 with both the trimethyl and monomethyl substituted xylyl dicopper(I) complexes $[Cu_2(Me_2XYL\text{-}CH_3)]^{2+}$ (**4**) and $[Cu_2(XYL\text{-}CH_3)]^{2+}$ (**5**) have been examined, Figure 2. Thus, when **4** is exposed to O_2 at 0 °C in dichloromethane overnight, a purple brown solution is formed. Following demetalation using $NH_4OH_{(aq)}$, analysis of the organic products (vide infra) indicated that good yields [always based on the amount of starting dinuclear complex] of the phenol $Me_3L\text{-}OH$, bis[2-(2-pyridyl)ethyl]amine (PY2), and formaldehyde (CH_2O) were obtained. In addition,

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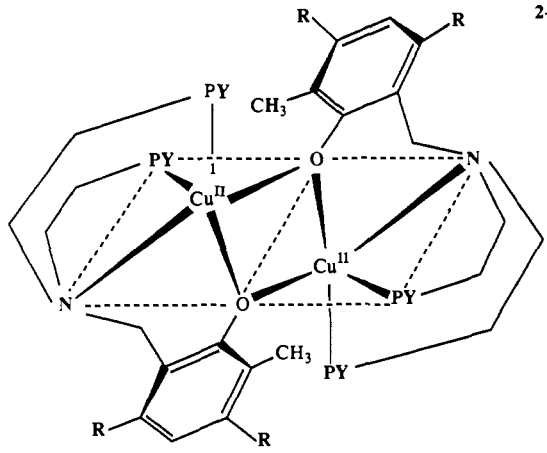
small amounts of methylbis[2-(2-pyridyl)ethyl]amine (MePY2) were also consistently observed (Figure 2). Me_3L-OH was identified by NMR and mass spectrometries, and through an X-ray crystal structure analysis of the dimeric copper(II) complex $[\{Cu(Me_3L\text{-}O^-)\}_2]^{2+}$ (**6**) formed in the reaction of **4** with O_2 (vide infra). PY2[28] and MePY2[29] were identified by comparison to authentic materials, while CH_2O was identified and quantified using the Nash reagent[30] which forms an easily identifiable adduct, a yellow crystalline solid. Good mass balance was observed in these reactions; a small amount of unreacted starting ligand $Me_2XYL\text{-}CH_3$ (e.g., <5%) was sometimes seen.[31] Analogous products are observed in reactions of $[Cu_2(XYL\text{-}CH_3)]^{2+}$ (**5**) with O_2 in either CH_2Cl_2 or dimethylformamide (DMF). Overall mass balance is again very good, but lower absolute yields (vide infra) of MeL-OH, CH_2O, and other amine products are obtained. Oxidation of **5** does afford Cu(II) products, but ligand oxygenation occurs only partially, as accounted for by the recovery of 20–35% of unreacted $XYL\text{-}CH_3$.

As discussed below, the Me_nL-OH phenol products are believed to result from oxidatively induced methyl migrations, with the phenol oxygen atom originating from O_2. The process results in loss of one of the two tridentate ligand "arms" of the xylyl dinucleating ligand. We suggest the other products originate from a common iminium cation intermediate $\{CH_2{=}N[CH_2CH_2PY]_2\}^+$ (PY = 2-pyridyl, vide infra), resulting from $C_{methylene}\text{–}C_{xylyl}$ cleavage; this either hydrolyzes upon reaction mixture workup to give PY2 and CH_2O or is reduced to give MePY2. In order to more fully understand the stoichiometry of reaction of **4** and **5** with O_2 and to better see the relationship between products and how they were formed, it was necessary to run reactions under a variety of conditions. These results are described below.

Copper Complexes and Products. The dicopper(I) complexes **4** and **5** are presumed to possess two tricoordinate Cu(I) moieties, as is found for several examples of structurally characterized compounds containing the PY2 tridentate unit.[13,29] When diethyl ether is added to the purple-brown solution resulting from oxygenation of $[Cu_2(Me_2XYL\text{-}CH_3)]$ (**4**), copper complex containing precipitates are formed, from which a dimeric phenoxide-bridged dicopper(II) complex $[\{Cu(Me_3L\text{-}O^-)\}_2]^{2+}$ (**6**) was purified and obtained in 68% isolated yield (Figure 2). As a PF_6^- salt and dichloromethane solvate, suitable crystals for an X-ray diffraction study were obtained.[14] The structure consists of typical phenoxo dibridged Cu(II) ions, each found in a distorted square pyramidal coordination environment with Cu···Cu = 3.091 Å. Consistent with this structure, **6** exhibits a strong absorption assignable to a phenoxo-to-Cu(II) LMCT transition at 486 nm (ϵ, 3700). It is EPR silent and possesses a reduced room temperature magnetic moment of μ_{RT} = 1.3 μ_B/Cu, indicating moderately strong antiferromagnetic coupling between the Cu(II) spins. Most importantly, the structural determination of **6** unambiguously proved the structure of Me_3L-OH, since it was difficult for us to determine the exact regiochemistry of the phenol oxygen and methyl groups with 100% confidence just based on NMR spectroscopic data.

A similar phenoxo-bridged dicopper(II) product $[\{Cu(MeL\text{-}O^-)\}_2]^{2+}$ (**7**) was isolated from the reaction of $[Cu_2(XYL\text{-}CH_3)]^{2+}$ (**5**) with O_2. In a typical reaction, filtration of a green precipitate from the purple-brown product solution, precipitation with Et_2O, and recrystallization afforded 32% of phenoxide complex $[\{Cu^{II}(MeL\text{-}O^-)\}_2]^{2+}$ (**7**) (see Experimental Section). We suggest that the green material is a hydroxo-bridged copper(II) complex containing $XYL\text{-}CH_3$, since the latter is recovered after $NH_4OH_{(aq)}$ treatment of the precipitate.[31] This product results from simple irreversible O_2 oxidation of Cu(I) to Cu(II) (i.e., four-electron reduction of O_2 by four Cu(I)), a process which often competes with oxygenation processes originating from Cu_2O_2 intermediates.[1a,2,3,31]



R = CH_3, $\{[Cu^{II}(Me_3L\text{-}O\text{-})]_2\}^{2+}$ **6**

R = H, $\{[Cu^{II}(MeL\text{-}O\text{-})]_2\}^{2+}$ **7**

Since one Cu(II) ion per original dinucleating ligand is found in isolated complexes **6** and **7**, additional copper ion chelates should also be present in the product solutions. This is the case; qualitative tests confirmed that PY2 and MePY2 were present (see Experimental Section), while neocuproine [2,9-dimethyl-1,10-phenanthroline] was used as a colorimetric reagent for copper ion.[32] Thus, filtrate solutions of precipitated compounds **6** and **7** turned to a greenish-brown color when exposed to neocuproine, a positive test for copper ion, probably as Cu(II), since copper(I) complexes of this reagent are intensely red.

Organic Products. In quantitating and identifying the organic products formed in these reactions, it was useful to employ two approaches. One was to completely release copper(II) ion from the ligand products; this was accomplished by the addition of $NH_4OH_{(aq)}$ followed by extraction of the organics into CH_2Cl_2. The other was to add excess diethyl ether to the product solutions, to precipitate copper-ligand products, followed by analysis of these and/or the filtrate solutions. Separation and isolation of organics was effected using preparative column chromatography.

For the reaction of $[Cu_2(Me_2XYL\text{-}CH_3)]^{2+}$ (**4**) with dioxygen in CH_2Cl_2, as much as an 88% isolated yield (i.e., after chromatography) of Me_3L-OH could be obtained after the $NH_4OH_{(aq)}$ extraction procedure, although yields in the 60–70% range were more typical. Thus, the reaction as outlined in Figure 2 does occur in an essentially stoichiometric fashion. Accounting for the other portion of the dinucleating ligand $Me_2XYL\text{-}CH_3$, the amounts of PY2 produced should be comparable and they are, with *isolated* yields in the 50–80% range. As indicated, MePY2 is also produced, and its presence should account for the average lower yield of PY2 compared to Me_3L-OH. This is the case with isolated yields of MePY2 being 10–16%. An illustrative case is given in the Experimental Section; here, the isolated yields of Me_3L-OH, PY2, and MePY2 are 88, 80, and 16%, respectively.

Since formaldehyde and PY2 products are suggested to be both derived from a common intermediate (vide supra and Discussion), the yields of CH_2O should parallel that for PY2 and be ≤84–90% to account for MePY2 product. Under a variety of conditions, *isolated* yields of 58–63% of Nash adduct[30] were obtained, when the reagent mixture was added to the original reaction product solution from which copper-ligand complexes had been precipitated with excess Et_2O; considerably lower yields were obtained from $NH_4OH_{(aq)}$ extracts. In repeated control experiments, we were only able to obtain a 76_{av}% yield of isolated Nash adduct, using reagent grade formaldehyde in similar idealized solution conditions, i.e., in CH_2Cl_2/Et_2O (1:3, v/v) mixtures. Thus, real (i.e., adjusted)

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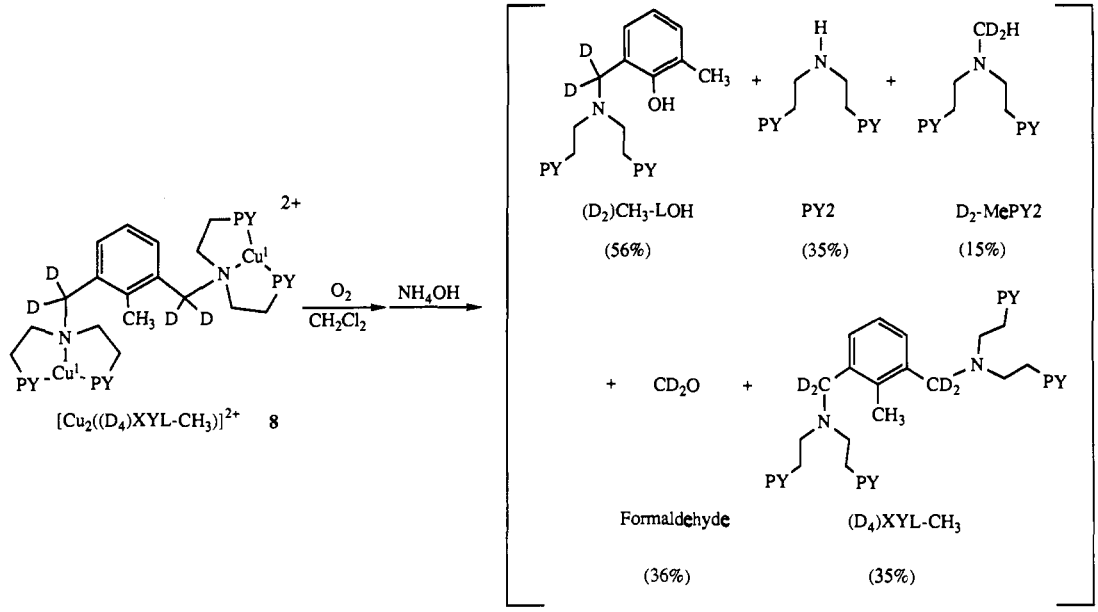


Figure 3. Reaction of dicopper(I) complex $[Cu_2((D_4)XYL\text{-}CH_3)]^{2+}$ (**8**) with O_2. The results are consistent with the 1,2-methyl migration reactions proposed and also show that formaldehyde produced derives from the ligand benzylic carbon atom.

yields of formaldehyde in reactions of O_2 with $[Cu_2(Me_2XYL\text{-}CH_3)]^{2+}$ (**4**) are closer to 77–83%, as expected and in line with the best yields of PY2.

As discussed, absolute yields of organics resulting from the hydroxylation reaction of $[Cu_2(XYL\text{-}CH_3)]^{2+}$ (**5**) are lower, but relative yields of MeL-OH, PY2, CH_2O, and MePY2 are similar to that formed for oxygenation of **4**. Thus, in a typical run in CH_2Cl_2 as solvent, isolated yields are 59, 39, 44, and 13%, respectively. Material balance was also good, with recovery of 33% yield of starting ligand XYL-CH_3. Similar results were obtained when DMF was used as the reaction solvent.

O_2 Stoichiometry. This is important to deduce when accounting for the important reaction products and to confirm the monooxygenase nature of the reaction. Firstly, the fate of the oxygen atom(s) derived from O_2 was of utmost concern, and this was determined in a reaction of $[Cu_2(Me_2XYL\text{-}CH_3)]^{2+}$ (**4**) exposed to $^{18}O_2$ (98%) in CH_2Cl_2 overnight. Workup of the reaction mixture in the usual manner resulted in a recovery of 68% yield of phenol product Me_3L-OH and mass spectrometric analysis indicated >85% incorporation of one ^{18}O atom into this material.

As was found for the conversion of **1** → **3**, a monooxygenase stoichiometry would correspond to direct addition of one O_2 to a dicopper(I) precursor compound, such that $Cu/O_2 = 2{:}1$. We normally carry out manometric measurements for all systems exhibiting dioxygen reactivity, and, for $[Cu_2(Me_2XYL\text{-}CH_3)]^{2+}$ (**4**), we found that the amount of O_2 taken up was usually larger than this, with numbers corresponding closer to $Cu/O_2 = 1{:}1$ than 2:1. We examined this effect in greater detail by carrying out the reaction of **4** with O_2 on a manometric apparatus, while quenching the reaction (with $NH_4OH_{(aq)}$) at various stages of O_2 uptake and examining the product yields.

Thus, when **4** was reacted with O_2 in CH_2Cl_2 at 0 °C until manometric measurements indicated that $Cu/O_2 = 4{:}1$, analysis by TLC indicated that ~80% of the starting ligand $Me_2XYL\text{-}CH_3$ remained unreacted, while the yield of hydroxylated product Me_3L-OH was ~20%. Clearly, the reaction was incomplete. This $Cu/O_2 = 4{:}1$ stoichiometry was checked because it corresponds to the most common reaction of Cu(I) with O_2, that corresponding to the irreversible O_2 reduction to water, i.e., $4Cu(I) + O_2 \rightarrow 2\{Cu(II)\text{–}(O^{2-})\text{–}Cu(II)\} \rightarrow 4Cu(II) + 2H_2O$.

By contrast, when the reaction was allowed to proceed until $Cu/O_2 = 2{:}1$ (~6 h at 0 °C), the conversion appeared complete. No starting $Me_2XYL\text{-}CH_3$ could be detected, and the isolated yield of phenol fragment Me_3L-OH was 68%. In two separate experiments again carried out such that $Cu/O_2 = 2{:}1$, workup via Et_2O precipitation gave isolated yields of 67 and 68% of $[\{Cu^{II}(Me_3L\text{-}O^-)\}_2]^{2+}$ (**6**), while CH_2O was isolated as the Nash reagent adduct in 63 and 60% yields.

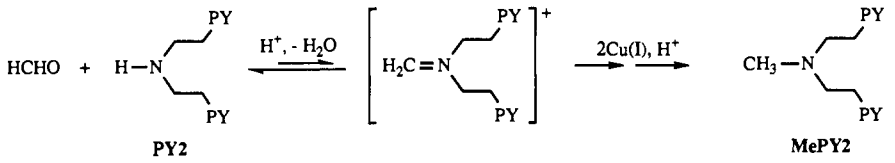
To further compare, the reaction was allowed to run until $Cu/O_2 \sim 1{:}1$ (18 h, 0 °C). Here, the yields of **6** and CH_2O were 70 and 60%, respectively, essentially the same as those observed for the reactions run in a $Cu/O_2 = 2{:}1$ stoichiometry. Thus, the true stoichiometry of reaction of **4** (or **5**) to give hydroxylated product plus amines plus formaldehyde appears to be that of a copper monooxygenase, i.e., $Cu/O_2 = 2{:}1$. We were unable to observe any other organic oxidation products which might account for the extra O_2 taken up.

Cu_2O_2 Intermediate. We also attempted to spectrophotometrically monitor the absorption of O_2 by **4** and **5** at low temperatures, since Cu_2O_2 intermediates are postulated to occur in these reactions (vide infra). However, while oxygenation of these dicopper(I) complexes does produce color changes (e.g., to green) at low temperatures, stable characterizable intermediates do not appear to be generated; warming is required to allow the reaction to proceed fully. Thus, no direct observation of low-temperature stabilized intermediates $[Cu_2(Me_2XYL\text{-}CH_3)(O_2)]$ or $[Cu_2(XYL\text{-}CH_3)(O_2)]^{2+}$ was possible, although considerable evidence for their likely formation exists, as described below.

Deuterium Labeling Experiment and Formation of MePY2. As postulated in the proposed mechanism of reaction, the 2-methyl groups in $[Cu_2(Me_2XYL\text{-}CH_3)]^{2+}$ (**4**) and $[Cu_2(XYL\text{-}CH_3)]^{2+}$ (**5**) undergo 1,2-migration reactions, and the formaldehyde produced should therefore be derived from the benzylic CH_2- group originating between tertiary amine nitrogen and arene groups. To test this hypothesis, we carried out a labeling experiment, where we deuterated the benzylic groups in XYL-CH_3 to give (D_4)-XYL-CH_3, Figure 1. A dicopper(I) complex, $[Cu_2((D_4)XYL\text{-}CH_3)]^{2+}$ (**8**), was synthesized and reacted with O_2 in CH_2Cl_2 in the usual manner. Four organic products were isolated and identified as shown in Figure 3, in yields comparable to the reaction of parent complex **5**. The results are clearly consistent with 2-methyl group migration, and the formaldehyde produced is indeed derived from the benzylic position of XYL-CH_3. Interestingly, the ^1H NMR spectrum of MePY2 isolated from the labeled precursor clearly corresponds to HD_2PY2 (Experimental Section). This is consistent with the idea that MePY2 may be derived from reduction of an intermediate iminium salt $\{D_2C{=}N[CH_2CH_2PY]_2\}^+$, with a solvent derived hydrogen atom (i.e., unlabeled) being supplied to the final product.

To provide further evidence and explanation for the formation of MePY2 from reduction of a $\{H_2C{=}N[CH_2CH_2PY]_2\}^+$ intermediate, we wished to show that such a reaction could occur under

Scheme II



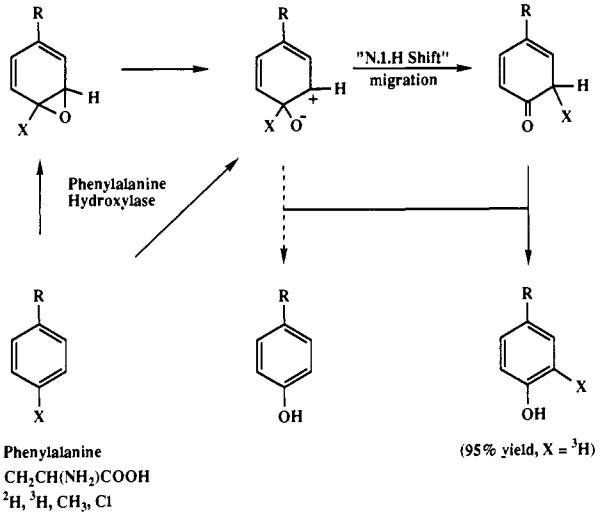


Figure 4. Basic elements of the NIH shift mechanism illustrated for non-heme iron phenylalanine hydroxylases (PAH). Attack of an iron-oxy (?) species results in electrophilic attack upon the aromatic substrate. An X-substituent migration reaction occurs from the resulting cationic intermediate, leading to products.

the reaction conditions employed or ones very similar. We therefore used conditions known to be effective in the generation of Mannich adducts,[33] also in the presence of a Cu(I) complex as possible reductant (see Experimental Section and supplementary material). We did not attempt to isolate the iminium salt, but formed it in situ by reaction of the secondary amine PY2 with formaldehyde and formic acid (as necessary catalyst) in CH_2Cl_2. Using thin-layer chromatography (TLC), no MePY2 was detected. However, when Cu^ICl was added to such a mixture in CH_2Cl_2/MeOH solvent and allowed to stir for 48 h, $NH_4OH_{(aq)}$ workup and chromatography resulted in a 7% isolated yield of MePY2. In a qualitative experiment using the dicopper(I) complex $[Cu_2(Me_2XYL\text{-}CH_3)]^{2+}$ (4) as a potential reductant, MePY2 was also detected in significant amounts. Thus, these experiments suggest that MePY2 formation could very well be explained by reduction of an iminium salt intermediate, possibly by the copper(I) complex already present.

Discussion

NIH Shift. The observed 1,2-methyl migrations occuring during oxygenation of $[Cu_2(Me_2XYL\text{-}CH_3)]^{2+}$ (4) and $[Cu_2(XYL\text{-}CH_3)]^{2+}$ (5) are reminiscent of the NIH shift (Figure 4), well-known previously for iron hydroxylases such as cytochrome P-450 and mammalian non-heme iron PAH.[15-19] For example, action of PAH on [4-^{3}H]phenylalanine produces >90% [3-^{3}H]tyrosine, while *p*-chlorophenylalanine produces 10–15% *m*-chlorotyrosine.[34] Perhaps of more direct relevance to the copper system described here, it is known that while *p*-methylphenylalanine is a relatively poor substrate for PAH (i.e., 27% is hydroxylated), 41% of that which reacts is converted to *m*-methyltyrosine,[35] while a hemin thiol ester cytochrome P-450 model system also exhibits methyl migration using *p*-methylanisole as substrate.[36] Alkyl migrations are also observed in hepatic oxygenation of methyl substituted aromatic substrates.[37]

Originally, the shift mechanism was understood in terms of arene oxide intermediates effected by some kind of electrophilic iron–oxy species,[15-19] but more recent experimental[19,38] and theoretical considerations[39] show that this need not be the case and that rearrangement via cationic (e.g., cyclohexadienyl) intermediates is likely. While electrophilic organic oxidants were shown to effect NIH shifts on aromatic substrates, for the Fenton system (e.g., $Fe(II)/H_2O_2$) or others containing an iron salt with peroxide or O_2/reductant (e.g., Udenfriend, Hamilton, or Viscontini systems), no NIH shift was observed.[17,18,40] Sharpless and Flood[41] showed that chromyl reagents CrO_2X_2 exhibit an NIH shift in aromatic hydroxylation, and some systems employing porphyrin–iron oxidants do as well.[36,42] These are likely to contain the now well-established high-valent iron–oxo species.[5] Lindsay-Smith[42] suggested that oxidation of the arene substrate all the way to the level of the two-electron oxidized cationic intermediate is important for observation of NIH shift derived products, i.e., one needs to get beyond a one-electron oxidized intermediate which could aromatize by radical processes not exhibiting a NIH shift. Protection of this cation to hydrolysis may also be critical. Thus, oxidants such as $Fe(II)/H_2O_2$ in CH_3CN solvent[42] exhibit NIH shifts probably because of the nonaqueous environment and the higher redox potential of the iron complex. A very informative recent study[19] using a variety of one-electron oxidants on substituted aromatics strongly supports the notion that to improve the relative yield of NIH shifted products, oxidation of an initially formed cyclohexadienyl radical cation (e.g., the initial one-electron oxidation product) to get to the cationic intermediate is critical. In this study, it is also concluded that arene–oxide intermediates are not important and that NIH shift efficiencies are also governed by any factor influencing the migration itself or the deprotonation–rearomatization step.

Proposed Mechanism for Xylyl Complexes/Summary. Many types of reactions occurring on aromatic compounds involve electrophilic attack to generate cationic intermediates, while Wagner–Meerwein 1,2-shifts of alkyl and other groups are well-established in organic chemistry.[33] With the suggestion or evidence for cationic intermediates in aromatic oxidation by iron hydroxylases, we suggest that the present copper monooxygenase model system involves a similar process, i.e., a NIH shift in copper chemistry, discovered prior to any such effect in copper proteins (vide infra). An outline for the proposed *general* mechanism of dicopper(I) xylyl hydroxylations by O_2 is given in Figure 5, accounting for the hydroxylation of a number of 2-Y substituted xylyl complexes, Y = H, D, Me, and F. The proposal takes into

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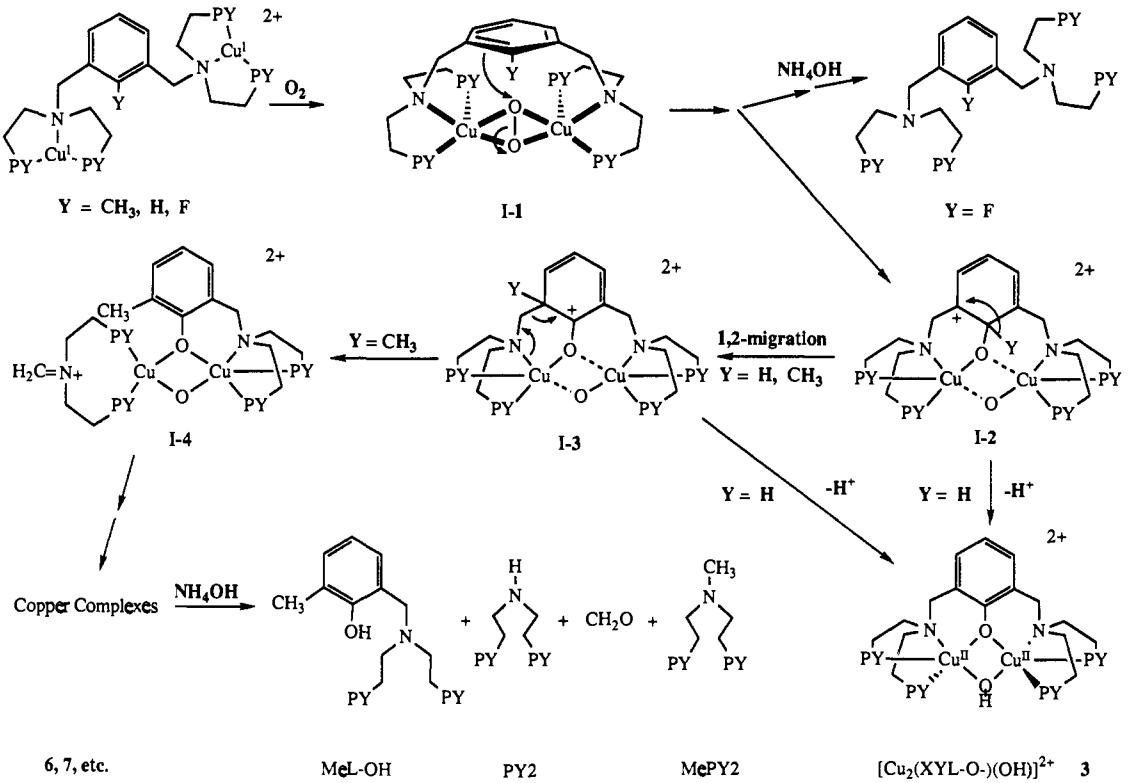

Figure 5. Proposed general mechanism for the hydroxylation of xylyl dicopper complexes, including the observed hydroxylation-induced methyl migration reactions. See text for further discussion and explanations.

account the key methyl migration reactions described in the present report as well as information from investigations described elsewhere. An initial summary is presented in the paragraph below, and this is followed by further discussion and explanation.

Summary/Overview. Oxygenation of dicopper(I) complexes $[Cu_2(XYL)]^{2+}$ leads to the formation of a $[Cu_2(XYL)(O_2)]^{2+}$ adduct, believed to have a μ-η^2:η^2 structure and a tendancy to react as an electrophile. As generally occurs in enzyme systems, the Cu_2O_2 oxygenating agent is formed in close proximity to the arene substrate, apparently perfectly poised to attack the π-system at the 2-carbon which becomes hydroxylated. Attack then generates a cationic intermediate, possibly driven by O–O cleavage and formation of an oxo–dicopper(II) intermediate. For the case of Y = H, migration and/or loss of H^+ occurs; this leads to the rearomatized final product **3** (Y = H). For 1,2-migration of the methyl group for compounds where Y = Me, rearomatization by loss of Me^+ is highly unlikely, but the lone pair electrons of the tertiary amine nitrogen from the PY2 moiety can "assist", leading to $C_{methylene}$–C_{xylyl} bond cleavage giving the Me_nL-O^- and iminium salt $\{CH_2{=}N[CH_2CH_2PY]_2\}^+$ products, presumably associated with copper ion. Under the reaction workup conditions, dinuclear complexes $[\{Cu^{II}(Me_3L\text{-}O^-)\}_2]^{2+}$ (**6**) or $[\{Cu^{II}(MeL\text{-}O^-)\}_2]^{2+}$ (**7**) and CH_2O are formed, or hydrolysis affords Me_nL-OH plus PY2 and MePY2, as described.

Cu_2O_2 Intermediate. As yet, no *direct* evidence exists for the intermediacy of Cu_2O_2 (peroxo dicopper(II)) intermediates for oxygenation reactions of $[Cu_2(Me_2XYL\text{-}CH_3)]^{2+}$ (**4**) and $[Cu_2(XYL\text{-}CH_3)]^{2+}$ (**5**). However, overwhelming evidence does exist for the very closely related xylyl analogues $[Cu_2(R\text{-}XYL\text{-}Y)]^{2+}$ (R = para 4-substituent = H, NO_2, CN, F, *t*-Bu, and Y site of hydroxylation = H; R = H and Y = D, F.)[3,20,21,43] Low-temperature stopped-flow kinetic/spectroscopic monitoring for all of these derivatives (except where Y = F, which was not examined) clearly indicates that O_2 binds in a reversible manner with a reaction stoichiometry of Cu/O_2 = 2:1, to give $[Cu_2(RXYL\text{-}Y)(O_2)]^{2+}$.[20,43] Strong and multiple UV–vis charge-transfer bands are observed (e.g., distinctive intense band in the 350–365-nm region with ϵ = 10 000–21 000), and these closely match those seen for the stable (i.e., at −80 °C in CH_2Cl_2) complexes $[Cu_2(Nn)(O_2)]^{2+}$ where Nn = $[PYCH_2CH_2]N(CH_2)_nN[CH_2CH_2PY]_2$ (n = 3, 4, or 5).[3,44,45] For all cases where the 2-Y substituent is either H or D, hydroxylation occurs, but, at −80 °C in CH_2Cl_2, $[Cu_2(R\text{-}XYL\text{-}Y)(O_2)]^{2+}$ (R = NO_2, F and Y = H) are stable for minutes to hours, allowing for facile spectral monitoring.[31] Stabilization of a closely related peroxo dicopper(II) intermediate is particularly striking in a case where we synthesized an unsymmetrical dicopper(I) analogue $[Cu_2(UN)]^{2+}$ where UN has one of its two PY2 nitrogen atoms directly connected to the arene spacer.[46] Low-temperature oxygenation provides $[Cu_2(UN)(O_2)]^{2+}$ which is so stable (−80 °C) that the O_2 can be removed, and cycling between deoxy and oxy forms is possible. Warming produces hydroxylated product $[Cu_2(UN\text{-}O\text{-})(OH)]^{2+}$.

When Y = F in $[Cu_2(XYL\text{-}F)]^{2+}$, neither hydroxylation nor F-migration occurs and mostly intact XYL-F is recovered; <3% of material is hydroxylated and dehalogenated. The lack of reactivity may be ascribed to the presence of an electronically deactivated ring and very strong C–F bond. Low-temperature oxygenation and manometric measurements indicate that a stable adduct $[Cu_2(XYL\text{-}F)(O_2)]^{2+}$ is formed, having spectral features closely matching those for $[Cu_2(N5)(O_2)]^{2+}$.[21]

While no crystal structure is yet available for any derivative $[Cu_2(XYL)(O_2)]^{2+}$ or $[Cu_2(Nn)(O_2)]^{2+}$, X-ray absorption studies[47] on the latter led us to suggest that these classes of Cu_2O_2 species possess a bent μ-η^2:η^2 structure (Cu···Cu = 3.3–3.4 Å,

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Scheme III

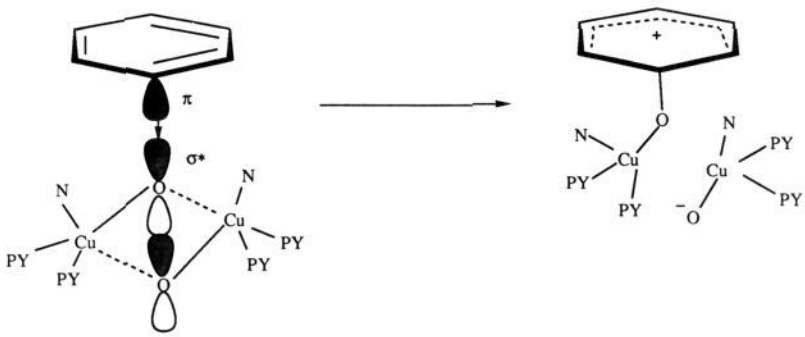
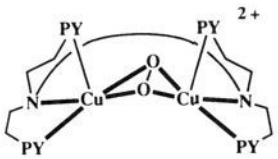


Chart I. Proposed η^2:η^2-Peroxo Structure in $[Cu_2(XYL)(O_2)]^{2+}$ and $[Cu(Nn)(O_2)]^{2+}$



depending upon n), also seen in alkyne-bridged dicopper(I) complexes.[48] The presence of a bridging peroxo group also accounts for strong magnetic coupling of Cu(II) unpaired electrons, suggested by solution properties such as EPR (silent), ^{1}H NMR ("normal"),[43] and magnetic susceptibility.[49] Kitajima and coworkers[50] have recently crystallographically described a planar μ-η^2:η^2-peroxo dicopper(II) complex. Thus, this type of peroxo coordination has now been clearly demonstrated for copper chemistry and the analogous bent formulation seems justified for XYL and Nn ligand complexes. The planar μ-η^2:η^2-O_2^{2-} structure is now considered a leading candidate for that in oxy-Hc and oxy-Tyr.[45,49-51]

To conclude, reaction of $Cu_2(Me_2XYL\text{-}CH_3)]^{2+}$ (**4**) or $[Cu_2(XYL\text{-}CH_3)]^{2+}$ (**5**) with O_2 clearly must lead to the generation of Cu_2O_2 adduct intermediate **I-1**, as indicated in the proposed mechanism, Figure 5. This is thought to have a μ-η^2:η^2-peroxo dicopper(II) structure.

Electrophilic Attack. Discussions concerning the probable intramolecular nature of the O_2 binding in $[Cu_2(XYL)(O_2)]^{2+}$, $[Cu_2(Nn)(O_2)]^{2+}$, and therefore Cu_2O_2 intermediate **I-1** (Figure 5) have been presented.[3,44] Evidence includes fast rates of complex formation (i.e., compared to mononuclear compounds), excellent solubility (i.e., nonpolymeric structures), and observations from kinetic studies of O_2 with $[Cu_2(R\text{-}XYL)]^{2+}$ complexes, with a pronounced R-dependence upon equilibrium binding parameters.[43] Thus, it can be presumed that the Cu_2O_2 intermediate is formed in close proximity to the xylyl substrate.

We have recently completed a study comparing reactivity patterns of three classes of Cu_2O_2 peroxo dicopper(II) species, including $[Cu_2(Nn)(O_2)]^{2+}$.[45] We found that the η^2:η^2-O_2^{2-} group in $[Cu_2(Nn)(O_2)]^{2+}$ behaves as a nonbasic electrophilic peroxide, in contrast to the basic or nucleophilic behavior of the two other types which possess end-on peroxo coordination, including a structurally characterized compound $[\{(TMPA)Cu\}_2(O_2)]^{2+}$ (TMPA = tris[(2-pyridyl)methyl]amine) with a *trans*-μ-1,2-O_2^{2-} group.[52] For example, in reactions with H^+, CO_2, and PPh_3, $[Cu_2(Nn)(O_2)]^{2+}$ does not protonate readily, is unreactive toward CO_2, and oxygenates PPh_3, while the others readily give H_2O_2 or carbonates or liberate O_2 (respectively) in reactions with the same reagents.

Further suggestions for the electrophilic nature of the hydroxylation reaction come from kinetic studies with $[Cu_2(XYL\text{-}D)]^{2+}$ and $[Cu_2(R\text{-}XYL)]^{2+}$ (R = NO_2, H, *t*-Bu, F).[20,43] The k_2 value observed for $[Cu_2(XYL\text{-}D)]^{2+}$ is within experimental error of that seen for the –H parent compound **1**; this lack of deuterium isotope effect is consistent with electrophilic attack of the arene substrate π-system, precluding C–H cleavage in the rate-determining step. Also in line with this conclusion is the increase in $\Delta H^{\ddagger}$ with electron-withdrawing ability of R. While linear free energy relationship analyses are problematic because of the compensating effect of activation entropies $\Delta S^{\ddagger}$, k_2 does decrease with electron-withdrawing power of R; at –80 °C, a modest ρ (versus σ^+) value of ~2.1 is obtained. Two possibilities for a relatively weak substituent effect are (i) k_2 is a composite rate constant, reflecting what must be multiple steps occurring between **2** and **3**, and (ii) the peroxo group in $[Cu_2(R\text{-}XYL\text{-}Y)(O_2)]^{2+}$ may be so reactive that it is not particularly sensitive to arene electronic effects.

$$\underset{\mathbf{2}}{[Cu_2(R\text{-}XYL\text{-}H)(O_2)]^{2+}} \xrightarrow{k_2} \underset{\mathbf{3}}{[Cu_2(R\text{-}XYL\text{-}O^-)(OH)]^{2+}}$$

While the reactivity of the peroxo group is clearly seen to be that of an electrophile, we do not see it as being an intrinsically powerful one. A more likely explanation for its reactivity and insensitivity to substituent would be the peroxo group's ideal positioning for p-π attack, possibly along the O–O vector. Stick molecular models suggest that a μ-η^2:η^2-peroxo moiety in complex **2** and **I-1** (Figure 5) may have such an orientation with respect to the xylyl substrate 2-position. Sorrell[2] has discussed the importance of the alignment of an organic or metal–peroxide O–O bond with respect to a substrate electron pair, and a recent study indicates that olefin epoxidation by peracids proceeds by HOMO π-attack at a 180° approach to the σ^* LUMO of the O–O bond.[53] Similar explanations provide a rather straightforward way in which to rationalize a variety of apparently electrophilic oxygenation reactions observed in chemical or enzyme systems. Thus, approach along the O–O bond allows for donation to the peroxo ion σ^*-orbital, weakening of the O–O bond for cleavage, and transfer of the oxygen atom to substrate. In the present case, the nucleophile is the arene π-system.

The enhanced electrophilic nature of a η^2:η^2-peroxo group (i.e., by comparison to end-on peroxo structures) has also been noted in recent theoretical studies of Ross and Solomon,[51] and this further supports the notion that the peroxide moiety in $[Cu_2(R\text{-}XYL\text{-}Y)(O_2)]^{2+}$ can mediate electrophilic reactions. This side-on bonding also has a unique interaction involving the peroxide σ^* orbital as a π acceptor from the Cu d_{xy} HOMO.[51] This weakens the O–O bond, as seen by a very low $\nu_{O\text{-}O}$ (~750 cm^{-1}) in Kitajima's complex.[50] Substrate electron donation to an electrophilic LUMO would induce cleavage of both Cu–O and O–O bonds, resulting in oxygen atom transfer. From these theoretical studies,

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however, the LUMO does not coincide with the peroxide σ^*-orbital.

Product Formation. Returning to further details of the proposed mechanism of hydroxylation of copper–xylyl complexes (Figure 5), the peroxo group in $[Cu_2(R\text{-}XYL\text{-}Y)(O_2)]^{2+}$ (**I-1**) attacks the substrate as an electrophile, thereby generating a cationic intermediate **I-2**, as shown. Structural details of the intermediates are speculative, but peroxide O–O cleavage probably occurs synchronous with arene attack, helping to drive the reaction by favorable formation of an oxo- or hydroxo-bridged Cu–O(H)–Cu product (i.e., **I-2**). In fact, we have recently[54] independently generated and characterized the stable oxo-bridged species $[Cu_2(XYL\text{-}O^-)(O)]^+$, a deprotonated version of $[Cu_2(XYL\text{-}O^-)(OH)]^{2+}$ (**2**), which closely resembles **I-2**.

At this point, rearomatization via loss of H^+ could occur directly to give the product $[Cu_2(XYL\text{-}O^-)(OH)]^{2+}$ (**2**). Alternatively, Y could undergo a Wagner–Meerwein rearrangement[33] which is the formal migration of Y^- in a *NIH shift*. This produces another resonance stabilized carbocation intermediate **I-3**. Here again, loss of Y = H as H^+ could result in the formation of **2**, but in the case of Y = Me, "assistance" by the amine nitrogen lone pair can aid the rearomatization process, producing the copper bound phenol product which dimerizes to give **6** or **7** and an iminium cation shown in **I-4**. Hydrolysis during the workup procedure could release the 2-methylphenol product and a retro-Mannich reaction leads to the observed secondary amine PY2 and formaldehyde. MePY2 is produced in small quantities at the expense of PY2 and CH_2O, probably via reduction of the iminium cation, as described in the Results section.

Modified Xylyl or Other Tyrosinase Model Systems. Sorrell[55] has studied oxygenation reactions of analogous synthetic xylyl dicopper(I) complexes containing 1-pyrazolyl or 2-imidazolyl donor groups which fully or partially replace the 2-pyridyl ligands in XYL-H. For all such complexes, no hydroxylation occurs and only four-electron reduction of O_2 to give bis(μ-hydroxy)copper(II) dinuclear compounds occurs. Also, if $-CH_2PY$ (PY = 2-pyridyl) instead of $-CH_2CH_2PY$ groups are used in the xylyl dinucleating ligands, only irreversible oxidation and no ligand hydroxylation takes place.[56] These observations are not fully understood, but electronic effects due to differences of ligand basicity and resulting Cu(II)/Cu(I) redox potentials are likely to be quite important in determining whether (i) a Cu_2O_2 complex forms, (ii) it has a sufficient lifetime or stability, and (iii) whether is is reactive enough attack the substrate. These ligand donor changes might also cause sufficient changes in structure so as to detrimentally affect the proximity/orientation of the peroxo group with respect to the substrate.

Other xylyl–copper systems studied by Casella,[57] Feringa,[58] and Martell[59] indicate that hydroxylation is sometimes possible. These systems are all derived from the condensation of various amines with isopthalaldehyde and contain bidentate Schiff base groups rather than tridentate chelating arms which extend out from the xylyl substrate groups. Hydroxylation in Casella's system (derived from N^1-methylhistamine) is sensitive to acid or protic solvent, and it is suggested that hydroperoxo intermediates may be involved. Feringa found that placement of methoxy substituents in the xylyl 2- and 5-positions of his system (derived from [2-(2-pyridyl)ethyl]amine) resulted in oxidative demethylation of the 2-OMe group, either via aryl–oxygen or alkyl–oxygen bond cleavage pathways.

Of possible relevance to tyrosinase action, there also exist a number of synthetic systems involving external phenol hydroxylation, and these have been recently reviewed.[1a] Brackman and Havinga[60] presented pioneering work in this area, finding conditions to effect the straightforward ortho hydroxylation of phenols. For example, when copper salts are reacted with phenol, O_2, and morpholine (Mp) in MeOH, morpholino-substituted *o*-benzoquinone is produced; a cupric-peroxo-phenol-Mp species is seen to be an important intermediate. Most recently, Rêglier and co-workers[61] report an interesting catalytic system in which 2,4-di-*tert*-butylphenol is converted to the corresponding *o*-quinone with up to 16 turnovers/hour when reacted with dioxygen in the presence of a dicopper(I) complex [with dinucleating ligand derived from biphenyl-2,2′-dicarbaldehyde and [2-(2-pyridyl)-ethyl]amine] and triethylamine. As observed for several other previously studied cases,[1a] prior coordination of phenol (as phenoxide) is seen to be important in effecting the ortho hydroxylation as opposed oxidative (radical) coupling reactions.

While peroxo dicopper(II) or even Cu(III)–oxo species have been invoked as intermediates in many of the cases cited above, these proposals remain highly speculative. Few, if any details have been provided concerning the spectral or structural characterization of the proposed intermediates, and little else is known concerning detailed mechanisms of action.

Biological Relevance. Studies employing $[Cu_2(XYL\text{-}Y)]^{2+}$ (Y = H, **1**; Y = Me, **4** and **5**) have shown that a dicopper(I) center is capable of forming a peroxo dicopper(II) complex competent in effecting an arene hydroxylation via an electrophilic process, including a NIH shift. Insofar as these oxygenation reactions represent copper monooxygenase model systems, we are led to the suggestion that similar processes may be occurring in tyrosinase and copper phenylalanine hydroxylase.

Ideas concerning electrophilic attack in tyrosinase have been discussed for quite some time.[8c,62,63] For the followup catacholase reaction carried out by Tyr (i.e., *o*-catechol produced is converted to *o*-quinone in a two-electron oxidation), Duckworth and Coleman[64] showed conclusively that this operates through an electrophilic mechanism, since less electron rich *o*-diphenols, although strongly bound, are less rapidly oxidized. A similarly detailed study has not yet been carried out for the prior cresolase reaction involving phenol ortho hydroxylation, but monophenols with electron withdrawing para substituents are very poor (i.e., slower) substrates, suggesting that these substrates are also subject to electrophilic attack by the peroxo dicopper(II) intermediate formed. In considering electronic properties of oxy-Tyr and the active site chemistry, more recent investigations by Solomon and co-workers[8a,51] have further lead to conclusions that an electrophilic mechanism possibly operates in Tyr. The possibility that a NIH shift mechanism might operate in tyrosinase exists but may be difficult to prove. Phenol substrates would not be expected to retain an ortho substituent such as tritium,[8c,63] and this has indeed been observed to be the case.[62] Further attempts to verify the detailed mechanism need to be undertaken.

For PAH enzyme, the NIH shift is well established for the mammalian iron enzyme, as discussed above. With *p*-methylphenylalanine as substrate, a very small amount of *p*-methyl-*m*-hydroxyphenylalanine product is also observed,[35] suggesting that hydroxyl group migration may occur. Most interestingly, recent investigations have revealed that the bacterial copper PAH does in fact exhibit the NIH shift.[65] Thus, the NIH shift is now established in copper oxygenases, after its initial discovery[14] in the chemical systems described here. Future studies aimed at

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elucidating the exact nature of the oxygenating agent will be of great interest, since a single copper ion plus pterin cofactor, rather than dicopper center, is involved.

Conclusion

The present account provides a summary of how dioxygen can be activated by copper ion toward the oxygenation of an aromatic substrate. We have outlined here the first example of the NIH shift in copper chemistry, within the framework of a well-characterized copper monooxygenase model system. Structural and mechanistic insights from this model system provide insights or suggestions for how biological oxygenation reactions may proceed, especially for the dicopper containing enzyme tyrosinase. (i) There is formed an intermediate Cu_2O_2 species which is electronically suitable [by comparison to those with other ligand donor groups (vide supra)] and reactive. (ii) This probably consists of a μ-η^2:η^2-O_2^{2-}-dicopper(II) moiety, the currently proposed structure for oxy-Hc and oxy-Tyr. (iii) The hydroxylation mechanism involves electrophilic attack and the NIH shift. (iv) Perhaps most importantly and an aspect common to all enzymes, the binding of dioxygen to the dicopper center in these xylyl complexes results in the placement of the reactive peroxo dicopper(II) group and xylyl substrate in a close and appropriate juxtaposition.

Further efforts will be directed at confirmation of hypotheses concerning the μ-η^2:η^2-O_2^{2-} structure, its orientation with respect to the substrate, and how subtle alterations affect the hydroxylation process. These latter perspectives also provide a strategy for the design of copper reagents which may be useful for practical applications of O_2-mediated hydrocarbon oxygenation.

Experimental Section

Materials and Methods. Reagents and solvents used were of commercially available reagent quality unless otherwise stated. Labeled dioxygen ($^{18}O_2$, 99%) was obtained from Icon Services, Inc. Dioxygen gas was dried initially by passing through a short column of supported P_4O_{10} (Aquasorb, Mallinkrodt) and was further dried by passing it through a copper coil tube immersed in a −80 °C cold trap. Methanol was distilled from $Mg(OMe)_2$, and anhydrous diethyl ether was used by passing it through a 50-cm long column of activated alumina or it was directly distilled from sodium/benzophenone under Ar. Dichloromethane was stirred with concentrated sulfuric acid for several days. After washing with water, Na_2CO_3 (saturated) solution, and then water again, it was dried over anhydrous $MgSO_4$ for several days. Finally, it was refluxed and distilled from CaH_2. Preparations and handling of air sensitive materials were carried out under an argon atmosphere using standard Schlenk techniques. Deoxygenation of solvents and solutions was effected by either repeated vacuum/purge cycles using argon or by bubbling (20 min) of Ar directly through the solutions. Solid samples were stored and transferred, and samples for IR and NMR spectra were prepared in a Vacuum/Atmospheres dry box filled with argon. Elemental analyses were performed by Desert Analytics, Tucson, AZ.

Infrared spectra were recorded as Nujols mulls either on a Perkin-Elmer 710B or Perkin-Elmer 283 instrument. ^1H NMR spectra were measured in CD_3NO_2, CD_3CN, CD_2Cl_2, or $CDCl_3$ on either a Varian EM360 (60 MHz) or a Varian XL-300 (300 MHz) NMR spectrometer. ^{13}C NMR spectra were recorded in CD_3NO_2, CD_2Cl_2, or $CDCl_3$ on a Varian XL-300 (300 MHz) NMR spectrometer with broad band proton decoupling. All the spectra were recorded in 5-mm o.d. NMR sample tubes (Norell). Chemical shifts are reported as δ values downfield from an internal standard of Me_4Si. Electrical conductivity measurements were carried out in DMF using a Barnstead Sybron Corporation Model PM-70CB conductivity bridge, using a Yellow Springs Instrument Co. Inc. 3403 cell. The cell constant, k, was determined using a standard aqueous KCl solution. Room temperature magnetic moments were determined using a Johnson Matthey magnetic susceptibility balance, calibrated using $Hg[Co(SCN)_4]$. Electronic absorption spectra at room temperature were recorded on a Shimadzu UV-160 spectrometer in acetonitrile. As previously described,[43a] low-temperature electronic spectroscopic studies were carried out in dichloromethane on a Perkin-Elmer Lambda Array 3840 spectrophotometer driven by an IBM PC. Field desorption mass spectra were obtained at the General Electric Company, Schenectady, NY.

Ligand Syntheses. Me_2XYL-CH_3. 2,4-Bis(chloromethyl)mesitylene (1.56 g, 7 mmol) was added to a solution of bis[2-(2-pyridyl)ethyl]amine (PY2)[28] (3.26 g, 0.0143 mol) and triethylamine (2.33 g, 0.023 mol) in 100 mL of ethyl acetate. The mixture was stirred at room temperature for 5 days whereupon it was filtered. Removal of the solvent gave a crude oil which was chromatographed using ethyl acetate on alumina (R_f 0.36) whereupon a total of 2.79 g of pure product, Me_2XYL-CH_3, was recovered (65%): MS (m/z), 599 (MH^+, 100), 492 (24), 371 (84), 279 (80), 228 ((64), PY2)), 106 (56).

XYL-CH_3. 2,6-Dicyanotoluene (10 g, 0.07 mol) was reacted with $LiAlH_4$ (6.65 g, 0.175 mol) in a THF/ether mixture and worked up by slowly adding 7 mL of water followed by 15 mL of (10%) NaOH solution (aqueous). The mixture was filtered, and the filtrate was purified by vacuum distillation at 115–120 °C (0.1 mmHg) to give a colorless oil, 2,6-bis(aminomethyl)toluene (4.54 g, 43%): ^1H NMR (300 MHz) ($CDCl_3$, δ) 1.4 (s, 4 H, NH_2), 2.2 (s, 3 H, CH_3), 3.8 (s, 4 H), 7.1 (m, 3 H).

The product (4.54 g, 0.03 mol) was refluxed with 2-vinylpyridine (25 g, 0.24 mol) in a methanolic solution of acetic acid (9 g, 0.15 mol) for 6 days. The methanol was removed, and the thick liquid obtained was washed with 100 mL (10%) sodium hydroxide solution and extracted into CH_2Cl_2. After removal of this solvent, the material was reacted with 3 g of phthalic anhydride in 150 mL of benzene solution (5 h) to remove all the primary and secondary amines. The resultant mixture was washed with 100 mL (5%) of NaOH solution and chromatographed using ethyl acetate on a 12 × 3.5 cm glass column packed with Al_2O_3 to give pure product XYL-CH_3 (5.6 g, 32%) (R_f on Al_2O_3/EtOAc ~ 0.35): MS (m/z), 571 (MH^+).

(D_4)XYL-CH_3. 2,6-Bis(aminomethyl-D_4)toluene was prepared by the same procedure as above (45% yield), except that $LiAlD_4$ was used instead of $LiAlH_4$: ^1H NMR ($CDCl_3$) 6.9 (3 H), 2.2 (s, 3 H, CH_3) 1.3 (s, 4 H, NH_2). Reaction of this with 2-vinylpyridine (as above) gave (D_4)XYL-CH_3 in 30% yield: MS (m/z), 575 (MH^+).

XYL-D. In a 250-mL, three-necked, round-bottom flask equipped with a dropping funnel, condenser, and magnetic stirrer was added 100 mL of dry THF and magnesium chips (6.56 g, 0.27 mol). 2-Bromo-*m*-xylene (25 g, 0.135 mol) was added dropwise over a period of 1 h, and the solution was stirred for 2 additional h. During this time, the solution developed a dark green color. Quenching the resulting solution with D_2O (7 mL, 0.405 mol), followed by 100 mL of H_2O, and then extracting with 300 mL of ether gave crude 2-*d*-*m*-xylene. Distillation (1 atm) afforded pure product (11.76 g, 81%): bp 130–135 °C; ^1H NMR ($CDCl_3$) 2.2 (s, 6 H), 6.6–7.1 (m, 3 H); IR (neat, cm^{-1}) 3000 (s, C-H), 2260 (s, C–D), 1900 (w), 1780 (w), 1620 (s, C=C), ca. 1480 (s), 1400 (w), 1170 (s), 1100 (s), 1040 (s), 850 (vs), ca. 750 (vs), 690 (w), 620 (vs).

Bromination of 2-*d*-*m*-xylene (11.76 g, 0.1097 mol) was carried out by refluxing for 5 h in CCl_4 (200 mL) with *N*-bromosuccinimide (39 g, 0.219 mol) in the presence of a trace of dibenzoyl peroxide. After cooling and filtering, the solvent was removed in vacuo to give a crude residue. Recrystallization from 20 mL of CCl_4 and 80 mL of hexane while standing at 0 °C for 3 days resulted in the isolation of an off-white crystalline solid, 1,3-bis(bromomethyl)-2-*d*-benzene (8.71 g, 30%): ^1H NMR ($CDCl_3$) 4.4 (s, 4 H), 7.1 (s, 3 H); IR (Nujol cm^{-1}) ca. 2900 (s, C–H), 2280 (w, C–D), 1900 (w), 1700 (w), 1580 (w, C=C), 1440 (s), 1380 (m), 1250 (s), 1300 (s), 1000 (w), 800 (s), 760 (s), 740 (w), 700 (w), 630 (s).

This product (3 g, 0.011 mol) was added to a solution of PY2 (5.14 g, 0.022 mol) and triethylamine (3.67 g, 0.036 mol) in 150 mL of ethyl acetate solution. The mixture was allowed to stir at room temperature for 5 days whereupon the solution was filtered, and removal of the solvent from the filtrate gave a crude oil (6.81 g). This oil was chromatographed on silica gel with 100% methanol (R_f 0.52), and a total of 3.64 g of pure product, XYL-D, was recovered (58%): IR (neat, cm^{-1}) ca. 3400 (br s, H_2O), ca. 2900 (s, C–H), 1680 (w), 1600 (s, C=C), 1450 (s), 1360 (s), 1250 (s), 1120 (s), 1090 (s), 1000 (s), 790 (s, br); MS (m/z), 558 (MH^+ (92)), 465 (65), 372 (23), 358 (15), 330 (100), 267 (19), 239 (58), 227 ((46), PY2)), 106 (73).

XYL-F. A 500-mL plastic bottle was placed in dry ice containing 90 g of pyridine (1.139 mol). Hydrogen fluoride (HF) (210 mL, 11.39 mol) was also added by condensing it over the frozen pyridine solution. To this frozen solution were added 2,6-dimethylaniline (30 g, 0.24 mol) and sodium nitrite ($NaNO_2$) (16.56 g, 0.24 mol). The resultant mixture was stoppered and heated to 45 °C for 2 h (to prevent 2,6-dimethylaniline loss). The mixture was then cooled to room temperature and stirred for 2 days. Water (300 mL) was added to the mixture, and this was extracted with 300 mL of diethyl ether. Removal of solvent by distillation (1 atm) afforded the desired product, 2-fluoro-*m*-xylene (5.79 g, 20%). In another method, concentrated HCl (40 mL) and distilled water (40 mL) were mixed in a 500-mL Erlenmeyer flask and cooled in an ice-salt bath to 0 °C. With stirring, 20 g (0.165 mol) of 2-amino-*m*-xylene was added, while maintaining the temperature between 0 and 5 °C. An off-white solid was formed, which was dissolved by slowly adding a 25-mL aqueous solution of $NaNO_2$ (12 g, 0.175 mol) with constant stirring, while still maintaining the temperature at 0–5 °C. Meanwhile a 50-mL water solution of $NaBF_4$ (25 g, 0.23 mol) was cooled in ice and

added to the above solution with stirring. The thick slurry formed was filtered through a coarse Buchner funnel, and the precipitates were washed with 15 mL of ice water, 10 mL of methanol, and then with 20 mL of ether. The solid was dried and heated gently in a 250-mL, round-bottomed flask inside an efficient hood. At the end, the flask was heated strongly, and the residue was vacuum distilled (~110 °C) to give a colorless product 2-*F*-*m*-xylene (8.5 g, 41%): ^{1}H NMR (60 MHz) ($CDCl_3$) 2.3 (s, 6 H), 6.8–7.4 (m, 3 H).

Bromination of 2-fluoro-*m*-xylene (5.79 g, 0.0464 mol) was carried out by refluxing in CCl_4 (90 mL) for 18 h with *N*-bromosuccinimide (16.45 g, 0.0924 mol) in the presence of a trace of dibenzoyl peroxide. After 18 h, the mixture was cooled and filtered, and the solvent was removed in vacuo to give a crude residue. Recrystallization while standing at 0 °C for 3 days from CCl_4 (20 mL) and hexane (80 mL) resulted in the isolation of an off-white solid, 1,3-bis(bromomethyl)-2-*F*-benzene (3.34 g, 26%): mp 86–87 °C; ^{1}H NMR (300 MHz) ($CDCl_3$) 4.50 (s, 4 H), 7.1–7.34 (m, 3 H); ^{13}C{^{1}H} NMR ($CDCl_3$) 125.78 (1), 160.13 (2), 156.76 (2), 125.57 (3), 131.63 (4), 124.64 (5), 25.19 (1′-CH_2).

This product (1.86 g, 6.5 mmol) was added to a solution of PY2 (3.00 g, 1.32 mol) and triethylamine (2.00 g, 1.97 mmol) in 80 mL of ethyl acetate. The mixture was allowed to stir at room temperature for 5 days whereupon the solution was filtered and removal of the solvent from the filtrate gave a crude oil. Chromatography on alumina using ethyl acetate (R_f 0.40) gave a total of 1.97 g of pure product, XYL-F (52%).

Copper Complex Synthesis. $[Cu^I_2(XYL\text{-}H)](PF_6)$ (**1**-$(PF_6)_2$). This complex was prepared by the literature method.[13]

$[Cu^I_2(Me_2XYL\text{-}CH_3)](PF_6)_2 \cdot 1.25CH_2Cl_2$ (**4**-$(PF_6)_2$). To a solution of $[Cu^I(CH_3CN)_4](PF_6)$[66] (0.52 g, 1.4 mmol) in 15 mL of CH_2Cl_2, while stirring, was added dropwise $Me_2XYL\text{-}CH_3$ (0.42 g, 0.7 mmol) dissolved in 20 mL of CH_2Cl_2. The resulting mixture was allowed to stir for 20 min before addition of 150 mL of diethyl ether. The precipitate obtained was filtered and dissolved in 40 mL of CH_2Cl_2 and reprecipitated using 200 mL of Et_2O. The resulting solid was washed with Et_2O (30 mL) and dried in vacuo to give 0.65 g (82% yield) of a yellowish-brown crystalline solid: IR (Nujol cm^{-1}) ca. 2900 (s, C–H), 1610 (s, C=C), ca. 840 (s, br, PF_6). Anal. Calcd for $Cu_2(C_{40.25}H_{48.5}Cl_{2.5}N_6)(PF_6)_2$: C, 43.06; H, 4.36; N, 7.49. Found: C, 43.07; H, 4.41; N, 7.75.

$[Cu^I_2(XYL\text{-}CH_3)](PF_6)_2$ (**5**-$(PF_6)_2$). $XYL\text{-}CH_3$ (0.4 g, 0.7 mmol) dissolved in 35 mL of CH_2Cl_2 was stirred with $[Cu^I(CH_3CN)_4]PF_6$ (0.5 g, 1.3 mmol) for 40 min under argon before adding 80 mL of deaerated Et_2O. The precipitates were dried, dissolved in 30 mL of CH_2Cl_2, and filtered through a medium porosity frit. The resultant solution was precipitated with 100 mL of ether and vacuum dried overnight to give 0.4 g of yellow powder (58%): IR (Nujol cm^{-1}) 1600 (C=C, aromatic), 840 (PF_6). Anal. Calcd for $Cu_2C_{37}H_{42}N_6P_2F_{12}$: C, 44.98; H, 4.25; N, 8.51. Found: C, 44.92; H, 4.15; N, 8.38.

$[Cu^I_2((D_4)XYL\text{-}CH_3)](PF_6)_2$ (**8**-$(PF_6)_2$). A 75-mL CH_2Cl_2 solution of $(D_4)XYL\text{-}CH_3$ (3 g, 5.226 mmol) was stirred with $[Cu^I(CH_3CN)_4]$-PF_6 (3.8 g, 10.3 mmol) for 1 h. Diethyl ether (20 mL) was added, and the whole solution was filtered through a medium porosity frit under Ar. The resultant solution was precipitated with Et_2O, washed with 20 mL of deaerated methanol under Ar, and vacuum dried overnight to give 3.5 g (67%) of yellow powder: IR (Nujol cm^{-1}) 1600 (C=C, aromatic), 840 (PF_6). Anal. Calcd for $Cu_2C_{37}H_{38}D_4N_6P_2F_{12}$: C, 44.98; H, 4.25; N, 8.51. Found: C, 44.79; H, 4.25; N, 8.04.

$[Cu^I_2(XYL\text{-}D)](PF_6)_2$. $[Cu^I(CH_3CN)_4](PF_6)$ (0.4 g, 1.077 mmol) suspended in 25 mL of THF, while stirring, was added dropwise to XYL-D (0.3 g, 0.538 mmol) dissolved in 30 mL of THF. The colorless suspension slowly dissolved before a yellow-brown powder started to form. The mixture was stirred further overnight and filtered, and the precipitate was dissolved into 40 mL of acetone and precipitated with 200 mL of Et_2O. The resulting solid was washed with Et_2O (30 mL) and dried in vacuo to give 0.34 g (65% yield) of a yellow crystalline solid: IR (Nujol cm^{-1}) ca. 2900 (s, C–H), 1605 (s, C=C), ca. 840 (s, br, PF_6), 760 (m), 710 (w). Anal. Calcd for $Cu_2(C_{36}H_{39}D_1N_6)(PF_6)_2$: C, 44.33; H, 4.04; N, 8.62. Found: C, 44.82; H, 4.27; N, 8.56.

$[Cu^I_2(XYL\text{-}F)](PF_6)_2$. To a suspension of $[Cu^I(CH_3CN)_4](PF_6)$ (1.3 g, 3.48 mmol) in 20 mL of CH_2Cl_2, while stirring, was added dropwise 1.0 g (1.74 mmol) of XYL-F dissolved in 30 mL of CH_2Cl_2. The resulting mixture was allowed to stir for 2 h. Addition of 200 mL of Et_2O gave a precipitate which was filtered and redissolved in 30 mL of CH_2Cl_2 and 80 mL of MeOH. The resulting yellow mixture was allowed to sit under vacuum for 3 h. A yellow powder formed, and this was washed twice with 50-mL portions of methanol and dried in vacuo to give 1.30 g (75%) of a yellow microcrystalline solid: IR (Nujol cm^{-1}) ca. 2900 (s, C–H), 1605 (s, C=C), ca. 840 (s, br, PF_6). Anal. Calcd for Cu_2-$(C_{36}H_{39}N_6F)(PF_6)_2$: C, 43.59; H, 3.97; N, 8.47.

Oxygenation of 4 and Isolation of $[\{Cu^{II}(Me_3L\text{-}O^-)\}_2](PF_6)_2 \cdot CH_2Cl_2$ (**6**-$(PF_6)_2$)). Complex **4** (0.75 g, 0.669 mmol) was dissolved in 35 mL of CH_2Cl_2 with stirring under argon, giving a yellow colored solution. After 20 min, this was exposed to an atmosphere of dry dioxygen (O_2) at room temperature for a period of 3 h during which time the solution slowly began to turn to a dark brownish-purple color. This was filtered and precipitated with 200 mL of Et_2O, and the resulting precipitate was dissolved in 50 mL of CH_2Cl_2, and recrystallized using 200 mL of Et_2O, while standing for 3 days at 0 °C. The resulting brownish-purple crystalline solid was washed with 30 mL of diethyl ether and air dried to give 0.56 g (67%) product $[\{Cu^{II}(Me_3L\text{-}O^-)\}_2](PF_6)_2 \cdot CH_2Cl_2$ (**6**-$(PF_6)_2$). IR (Nujol cm^{-1}) ca. 2900 (s, C–H), 1605 (s, C=C), ca. 840 (s, br, PF_6); UV–vis (CH_3CN) λ_{max} (ϵ, M^{-1} cm^{-1}) 259 (20 200), 295 (sh, 8700), 338 (sh, 4000), 486 (3700), 655 (sh, 1050) nm; molar conductivity Λ_M = 134.0 Ω^{-1} cm^2 mol^{-1}; Onsager plot 320.0 Ω^{-1} M$^{1/2}$; EPR (DMF/$CHCl_3$ (1:1) 77 K) "silent", with 5% paramagnetic impurity per Cu; magnetism (solid state, room temperature): μ_B = 1.3 ± 0.1 μ_B/Cu; MS (*m*/*z*), 584 ((100), $[Cu(Me_3LO)(PF_6)]$), 438 [(40), $[Cu(Me_3LO)]$], 375 [(95), $(Me_3LOH]$. Anal. Calcd for $Cu_2(C_{49}H_{58}Cl_2N_6O_2)(PF_6)_2$: C, 47.02; H, 4.68; N, 6.72. Found: C, 47.36; H, 4.69; N, 6.65.

^{1}H NMR spectroscopy was used to show that CH_2Cl_2 was present in **6**-$(PF_6)_2$. The complex (0.1 g, 0.08 mmol) was mixed with KCN (0.052 g, 0.8 mmol) as a reductant and stirred in 3 mL of CD_3NO_2 for 3 h. The color of the solution turned from brownish-purple to a light orange color. The mixture was then allowed to sit for 30 min to let any unreacted KCN settle to the bottom of the flask: ^{1}H NMR (60 MHz) (CD_3NO_2) 2.15 (s, br, 9 H), 2.95 (s, br, 16 H), 3.80 (s, br, 4 H), 5.40 (s, 2 H (1 CH_2Cl_2)), 6.50–7.80 (m, br, 13 H), 8.40 (d, br, 4 H (py-6)).

Oxygenation of 4 and Isolation of Me_3LOH, MePY2, and PY2. Complex **4** (1.955 g, 1.743 mmol) was dissolved in 200 mL of CH_2Cl_2 while stirring under argon. The solution was then exposed to an atmosphere of dry dioxygen (O_2) where a measured volume of O_2 corresponding to 2 Cu/2O_2 was taken up by the complex (see manometry) overnight, while at 0 °C. The resulting dark brownish-purple solution was allowed to stir vigorously for 1 h with 50 mL of aqueous ammonia (NH_4OH). After stirring for 1 h, a deep blue aqueous layer and a brownish dichloromethane layer developed. The aqueous layer was separated and further extracted with 100 mL of CH_2Cl_2. The organic extracts were combined, and the above ammonia treatment and extractions were repeated two additional times to ensure complete removal of copper. The final CH_2Cl_2 solution was reduced in volume by rotary evaporation, resulting in the isolation of a crude oil (1.18 g). This was chromatographed first on alumina with EtOAc as eluant (R_f 0.65) whereupon a total of 0.58 g of pure product, Me_3LOH, was obtained (88% yield): MS (*m*/*z*) 376 (MH^+, (100)), 228 ((16), PY2), 135 (50). A 3% yield of $Me_2XYL\text{-}CH_3$ was also recovered (R_f 0.35) in this elution.

Further elution of the column with 100% methanol resulted in the isolation of an oil which was a mixture of MePY2 and PY2 (0.61 g). This was chromatographed on alumina with 98% EtOAc/2% MeOH as eluant (R_f 0.63) and total of 0.07 g (16%) of pure MePY2 was recovered: ^{1}H NMR (300 MHz) ($CDCl_3$) 2.43 (s, br, 3 H), 2.90 (s, br 8 H), 7.00–7.55 (m, 6 H), 8.51 (d, br, 2 H (py-6)); ^{13}C{^{1}H} NMR ($CDCl_3$) 159.69 (2″-py), 122.38 (3″-py), 135.36 (4″-py), 120.25 (5″-py), 148.32 (6″-py), 41.27 (1′-CH_3), 56.79 (2′-CH_2), 35.45 (3′-CH_2); MS (*m*/*z*), 242 (MH^+, (56)), 228 ((22), PY2), 135 (89), 106 (100). Finally, flushing the column with 100% methanol as eluant (R_f 0.8) resulted in the isolation of 0.32 g (80%) of pure product PY2: ^{1}H NMR ($CDCl_3$) 2.50 (s, br, 1 H, (NH)), 2.90 (t, br, 8 H), 6.94–7.40 (m, 6 H), 8.40 (d, br, 2 H (py-6)); ^{13}C{^{1}H} NMR ($CDCl_3$), 159.91 (2″-py), 123.03 (3″-py), 136.07 (4″-py), 120.96 (5″-py), 149.02 (6″-py), 48.89 (2′-CH_2), 37.89 (3′-CH_2); MS (*m*/*z*), 228 (MH^+, (100), (PY2)), 135 (96), 106 (94).

^{18}O-Labeling. This experiment was carried out by an analogous reaction in CH_2Cl_2, in a closed system using $^{18}O_2$ (Icon Services, Inc., 98 atom% (250 mL)) with approximately triple the volume of dioxygen necessary for completion of the reaction. Complex **4** (0.217 g, 0.193 mmol) was dissolved in CH_2Cl_2 (40 mL) under Ar, and the solution was exposed to $^{18}O_2$ for 24 h, while stirring at 0 °C. The resulting brownish-purple solution was stirred vigorously with 20 mL of aqueous ammonia (NH_4OH) for 1 h, and the aqueous layer was separated and further extracted with 50 mL of CH_2Cl_2. The organic extracts were combined, and the above ammonia treatment and extractions were repeated two additional times. The final CH_2Cl_2 solution was reduced in volume by rotary evaporation, resulting in the isolation of a crude oil which was chromatographed on alumina with EtOAc as eluant (R_f 0.65) whereupon a total of 0.05 g (68%) of Me_3L-OH (with ^{18}O label) was recovered: MS (*m*/*z*), 377 (M^+, (17), (Me_3L-OH)), indicating >85% incorporation of one ^{18}O atom into this phenol.

Oxygenation of 4 in CH_2Cl_2. Isolation of MePY2 and PY2 After Removal of 6. Complex **4** (0.15 g, 1.34 mmol) was dissolved in 20 mL of CH_2Cl_2, while stirring under argon. The solution was then exposed to an atmosphere of dry dioxygen (O_2) for 3 h at room temperature. The resulting brownish-purple solution was filtered and precipitated with 100

mL of diethyl ether. The clear filtrate was concentrated by rotary evaporation to 30 mL of CH_2Cl_2 and allowed to stir vigorously for 1 h with 20 mL of aqueous ammonia (NH_4OH) whereupon a deep blue aqueous layer and a brownish dichloromethane layer formed. The aqueous layer was separated and further extracted with 100 mL of CH_2Cl_2. The organic extracts were combined, and the ammonia treatment and extractions were repeated two additional times to ensure complete removal of copper. The final CH_2Cl_2 solution was reduced in volume by rotary evaporation and then in vacuo, giving 0.007 g of a crude oil. This was found to be a mixture of MePY2 (20%) and PY2 (80%) as determined by ^1H NMR and estimated by TLC ligand analysis: ^1H NMR ($CDCl_3$), 2.35 (s, br, 0.5 H (CH_3-N) (20%, CH_3-N from Me-py2)), 2.30 (s, br, 0.8 H (NH) (80%, H-N from py2)), 2.95 (d, br, 8 H), 6.9–7.60 (m, br, 6 H), 8.40 (d, br, 2 H (py-6)).

Manometry. O_2 absorption for reactants was monitored at constant pressure as previously described.[44a] For three runs using ca. 0.5 mmol of the dicopper(I) complex **4**, at 0 °C, at a point where O_2 absorption leveled off, the Cu/O_2 ratio was found to be 1 Cu:1.1 ± 0.2 O_2.

Dioxygen Uptake: $4Cu/1O_2$. Complex **4** (0.1477 g, 0.1316 mmol) was dissolved in 67 mL of CH_2Cl_2, while stirring under argon. The solution was then exposed to an atmosphere of dry dioxygen (O_2) where a measured volume of O_2 corresponding to $4Cu/1O_2$ was taken up by the complex for 1 h at 0 °C (i.e., a total of 1.6 mL of dioxygen was added to the complex, as followed by manometry). The resulting dark brownish-purple solution was then exposed quickly to air and allowed to stir vigorously for 1 h with 50 mL of aqueous ammonia; a deep blue aqueous layer and a brownish CH_2Cl_2 layer developed. The aqueous layer was separated and further extracted with 50 mL of CH_2Cl_2. The organic extracts were combined, and the above ammonia treatment and extractions were repeated two additional times. The final CH_2Cl_2 solution was reduced in volume by rotary evaporation, resulting in the isolation of a crude oil (0.11 g). Analysis by TLC indicated that this mostly consisted of unaltered Me_2XYL-CH_3: TLC EtOAc/alumina; Me_2XYL-CH_3, R_f 0.36 (~80%); Me_3L-OH, R_f 0.62 (~20%).

Oxygen Uptake: $2Cu/1O_2$ and Isolation of Me_3L-OH. Complex **4** (0.262 g, 0.234 mmol) was dissolved in 67 mL of CH_2Cl_2, while stirring under argon. The solution was then exposed to an atmosphere of dry dioxygen (O_2) where a measured volume of O_2 corresponding to $2Cu/1O_2$ was taken up by the complex for 6 h at 0 °C (a total of 5.7 mL of dioxygen was added to the complex). The resulting dark brownish-purple solution was then exposed quickly to the air and allowed to stir vigorously for 1 h with 50 mL of NH_4OH. The aqueous layer was separated and further extracted with 50 mL of CH_2Cl_2. The organic extracts were combined, and the above ammonia treatment and extractions were repeated two times. The final CH_2Cl_2 solution was reduced in volume by rotary evaporation, resulting in the isolation of a crude oil (0.15 g). This was chromatographed on alumina with 100% EtOAc as eluant (R_f 0.64) and a total of 0.06 g of pure product, Me_3L-OH, was recovered for a total yield of 68%: ^1H NMR (60 MHz) ($CDCl_3$) 1.90 (s, 3 H), 2.05 (s, 6 H), 2.85 (s, br, 8 H), 3.70 (s, br, 2 H), 6.3–7.4 (m, 9 H), 8.35 (d, br, (2 H)(py-6)).

Formaldehyde Analysis: Nash Test Control Experiment. The quantity (run no. 1, 0.04 g) (run no. 2, 0.07 g) (~3 drops) of formaldehyde was added to 25 mL of CH_2Cl_2 and 75 mL of Et_2O and allowed to stir for 20 min. Addition of an equal volume of NASH reagent[30] (consisting of 100 mL of water with 15 g of ammonium acetate, 0.3 mL of acetic acid, and 0.2 mL of acetyl acetone) caused an immediate color change to dark yellow; the mixture was stirred for 3 h at room temperature. The intense yellow mixture was then reduced in volume by rotary evaporation whereupon 50 mL of water was added. This mixture was extracted with dichloromethane (4 × 100 mL), the organic layer was dried over $MgSO_4$ and the solvent was removed to give a yellow crystalline solid which was washed with 30 mL of Et_2O and air dried (run no. 1, 0.07 g (74%)) (run no. 2, 0.13 g (78%)) (av yield = 76%): mp (run no. 1, 210 °C) (run no. 2, 210 °C) (lit. 200 °C).[30]

Formaldehyde Quantitation: Oxygenation of 4 with Excess O_2. Complex **4** (0.23 g, 0.205 mmol) was dissolved in 25 mL of CH_2Cl_2, while stirring under argon. The solution was then exposed to an atmosphere of dry dioxygen (O_2) for 60 min at room temperature. An equal volume of Nash reagent was added to the resulting purple-brown solution. Workup as described above gave 0.08 g (64%) yield of **6**; no formaldehyde Nash adduct was detected.

Complex **4** (0.2 g, 0.178 mmol) was similarly reacted with O_2 in 25 mL of CH_2Cl_2. Following precipitation by addition of 125 mL of Et_2O, filtration, and then addition of the Nash reagent, workup gave 0.02 g (58%) yield of this formaldehyde adduct. A similar reaction of **4** (0.2 g, 0.178 mmol) was followed by precipitation with Et_2O (as above), and when the filtrate was treated with 50 mL of $NH_4OH_{(aq)}$, workup and application of the Nash text resulted in the isolation of 0.005 g of adduct, a 15% yield of CH_2O. In another experiment, complex **4** (0.2 g, 0.178 mmol) was similarly reacted with O_2 and hydrolyzed with $NH_4OH_{(aq)}$, and the organic extracts (with CH_2Cl_2) were treated with Nash reagent. The yield of adduct was 29%. TLC analysis of a crude oil isolated from the organic extracts indicated both PY2 and Me_3L-OH were present.

Oxygen Uptake ($2Cu/1O_2$): Isolation of 6 and NASH Test on Filtrate. Complex **4** (0.506 g, 0.451 mmol) was dissolved in 67 mL of CH_2Cl_2, while stirring under argon. The solution was then exposed to an atmosphere of dry dioxygen (O_2) where a measured volume of O_2 corresponding to $2Cu/1O_2$ was taken up by the complex for 5 h at 0 °C (a total of 10.9 mL of dioxygen was added to the complex). The resulting dark brownish-purple solution was then quickly precipitated with 250 mL of diethyl ether. After filtration (0.19 g, 67%) of **6**, the Nash reagent (as before) was added to the clear filtrate. The usual workup procedure gave 0.055 g (63%): mp 206–207 °C (lit. 200 °C).[30] In another run using 0.172 g (0.153 mmol) of **4**, the yields of formaldehyde (as adduct) and complex **6** were 60% and 68%, respectively.

Oxygen Uptake ($1Cu/1O_2$): Isolation of 6 and NASH Test on Filtrate. Complex **4** (0.318 g, 0.284 mmol) was dissolved in 67 mL of CH_2Cl_2, while stirring under argon. The solution was then exposed to an atmosphere of dry dioxygen (O_2) where a measured volume of O_2 corresponding to $2Cu/2O_2$ was taken up by the complex for 18 h at 0 °C (a total of 13 mL of dioxygen was added to the complex). A similar workup procedure gave 0.125 g of **6** (70%) and 0.055 g (60%) of Nash adduct.

Oxygenation of $[Cu^I_2(XYL\text{-}CH_3)](PF_6)_2$ (5-$(PF_6)_2$) in CH_2Cl_2. Isolation of $[\{Cu^{II}(MeL\text{-}O^-)\}_2](PF_6)_2 \cdot {}^1/_2CH_2Cl_2$ (7-$(PF_6)_2$). Compound **5** (3.5 g, 3.3 mmol) was dissolved in 100 mL of CH_2Cl_2 under argon, and the yellow solution obtained was stirred for 1 h. After lowering the temperature to 0 °C, it was oxygenated by exposure to an atmosphere of dry O_2 for 2 days. A purple solution with some green solid was produced. The mixture was filtered, and the filtrate was layered with 200 mL of ether. $[\{Cu^{II}(MeL\text{-}O^-)\}_2](PF_6)_2 \cdot {}^1/_2CH_2Cl_2$ (7-$(PF_6)_2$) (1.2 g, 32%) was obtained as a brown microcrystalline product: μ_B = 1.3 ± 0.1 BM/Cu; IR (Nujol; cm^{-1}) 1600 (C=C, aromatic), 840 (PF_6); UV–vis is (CH_3CN; λ_{max}, nm (ϵ, M^{-1} cm^{-1})) 254 (24 700), 285 (sh, 11 900), 325 (sh, 6070), 470 (4580), 670 (sh, 910), 799 (340). Anal. Calcd for $Cu_2C_{44.5}H_{49}N_6ClO_2P_2F_{12}$: C, 46.37; H, 4.25; N, 7.29. Found: C, 46.33; H, 4.30; N, 7.08.

^1H NMR spectroscopy was used to show that CH_2Cl_2 was present in 7-$(PF_6)_2$. The complex (0.1 g, 0.08 mmol) was dissolved in 2 mL of CD_3NO_2 in a small test tube, and 0.055 g (0.86 mmol) KCN was added along with a small stirrer. The tube was covered with a stopper and stirred at room temperature for 4 h. A yellowish-brown color was produced. The clear solution was then taken for NMR analysis: ^1H NMR (60 MHz) 2.2 (s, CH_3), 2.9 (8 H), 3.9 (s, 2 H), 5.3 (s, CH_2Cl_2), 6.7–7.6 (m, 9 H), 8.5 (d, br, 2 H, py-6″).

Reaction of 5 with O_2 in CH_2Cl_2. Isolation and Identification of MeL-OH, PY2, and MePY2 Products. Complex **5** (4.64 g, 4.71 mmol) was dissolved in 250 mL of CH_2Cl_2 under argon, and while decreasing the temperature to 0 °C, the solution was exposed to an atmosphere of dry oxygen for 24 h. Initially a greenish color was formed, which changed to dark brown with time. Some insoluble green powder also settled down. After 24 h, the solution was filtered and washed with 30 mL of CH_2Cl_2. The insoluble green precipitate was mixed with CH_2Cl_2 (40 mL) and stirred with an equal volume of aqueous ammonia for 3 h, after which the aqueous blue layer was discarded and the organic layer was dried over $MgSO_4$. The solvent was removed by rotary evaporation to give 0.9 g (33%) of an oil identified as starting ligand **5**, i.e., XYL-CH_3 [TLC in $EtOAc/Al_2O_3$; R_f ~ 0.35]: ^1H NMR (60 MHz) ($CDCl_3$) 2.1 (s, 3 H, $-CH_3$), 3.0 (s, 16 H), 3.7 (s, 4 H), 7.0–7.5 (m, 15 H), 8.5 (d, 4 H, py-6″).

To the original brown filtrate was added 75 mL of aqueous ammonia, and the solution was stirred for 3 h. Thereafter, an aqueous blue layer was removed, and the organic layer was again stirred with 50 mL of aqueous ammonia for 1 h. The organic layer was washed with water and dried over $MgSO_4$, and solvent was removed by rotary evaporation to give 1.8 g of crude oil. This crude oil is a mixture of three products (TLC in 98% ethyl acetate/2% methanol, R_f ~ 0.9, 0.5, 0.00). This mixture was chromatographed on an Al_2O_3 column. Initially, EtOAc was used as an eluant, and pure fractions of one product, MeL-OH, were collected (R_f ~ 0.85). The solvent was removed by rotary evaporation to give 0.96 g (59%) of pure product: MS (M/Z), 347 (MH^+). Two products remained, and the next was eluted by using 98% EtOAc/2% MeOH; a total of 0.14 g (13%) of MePY2 was collected (R_f in 98% EtOAc/2% MeOH, ~0.55): ^1H NMR ($CDCl_3$) 2.35 (s, br, 3 H, CH_3), 2.85 (s, br, 8 H), 6.8–7.35 (m, 6 H), 8.35 (d, br, 2 H). Next, the column was flushed with 100% methanol to give 0.42 g (39%) PY2 (R_f in 100% MeOH, ~0.8): ^1H NMR ($CDCl_3$) 2.4 (s, br, NH), 3.0 (br, 8 H), 6.8–7.6 (m, 6 H), 8.4 (d, 2 H, py-6″).

Reaction of 5 with O_2 in DMF. Complex **5** was prepared in situ in by reacting 3.57 g (6.26 mmol) of XYL-CH_3 with $[Cu^I(CH_3CN)_4]PF_6$ (4.6

g, 12.5 mmol) in 170 mL of DMF under argon. The temperature was lowered to 0 °C, and the solution was exposed to an atmosphere of dry oxygen for 24 h. The solvent was removed by rotary evaporation (using high vacuum), and the residue obtained was dissolved in 100 mL of CH_2Cl_2 and filtered. A green precipitate as well as a brown filtrate were extracted with aqueous ammonia separately. The different products were again separated as described before. XYL-CH_3 (0.7 g, 20%) was recovered, along with MeL-OH (1.27 g, 59%), MePY2 (0.2 g, 16%), and PY2 (0.42 g, 36%).

Oxygenation of 5 with O_2: Formaldehyde Quantitation. Complex **5** (0.18 g, 0.1823 mmol) was dissolved in 30 mL of CH_2Cl_2 under argon, and exposed to an atmosphere of dry oxygen for 24 h. After that the solution was precipitated with 130 mL of diethyl ether and filtered, and to the filtrate was added an equal volume of Nash reagent, as described above. Workup afforded yellow crystals of CH_2O adduct (0.0155 g, 44%): mp 210 °C; ^1H NMR (CD_3NO_2) 2.1 (m, 12 H), 3.3 (s, 3 H).

Reaction of $[Cu_2((D_4)XYL\text{-}CH_3)](PF_6)_2$ (8-$(PF_6)_2$) with O_2 in CH_2Cl_2. Isolation and Identification of Products. Complex **8** (0.54 g, 0.547 mmol) was dissolved in 50 mL of CH_2Cl_2 under argon and was oxygenated and processed in the manner described for complex **5**. Four products were similarly isolated along with ~35% starting ligand which was characterized by comparing the TLC and ^1H NMR data with a standard sample. The four products isolated were as follows: (i) d_2-MeL-OH, 0.11 g (56%) with R_f on EtOAc/Al_2O_3, ~0.7; ^1H NMR (60 MHz) ($CDCl_3$), 2.00 (s, 3 H, CH_3), 2.8 (s, 8 H), 6.2–7.3 (m, 9 H), 8.1 (d, 2 H, py-6″); ^1H NMR (300 MHz), ($CDCl_3$; δ) 2.1 (s, 3 H, –CH_3), 3.0 (s, 8 H), 6.5–7.5 (m, 9 H), 8.4 (d, 2 H, py-6″); MS (m/z), 349 (MH^+); (ii) HD_2C-PY2, 0.02 g (15%); ^1H NMR (60 MHz), ($CDCl_3$), 2.3 (1 H, CD_2H), 2.85 (br, 8 H), 6.8–7.55 (m, 6 H), 8.35 (d, br, 2 H); (iii) PY2, 0.043 g (35%); ^1H NMR (60 MHz) ($CDCl_3$), 2.4 (s, br, NH), 3.0 (br, 8 H), 6.8–7.6 (m, 6 H), 8.4 (d, 2 H); (iv) a Nash adduct (0.38 g, 36%) having two deuterium atoms incorporated; ^1H NMR (CD_3NO_2), 2.1–2.15 (m, 12 H).

Reaction of PY2 with Formaldehyde in the Presence of Formic Acid and Cu^ICl. PY2 (3 g, 13.21 mmol), HCHO (37%, 0.72 g, 8 mmol), HCOOH (0.33 g, 7.3 mmol), and Cu^ICl (0.42 g, 4.24 mmol) were mixed in a 30-mL solution of CH_2Cl_2/MeOH (1:1) and stirred for 48 h under argon. The color was initially light yellow, which changed to very light green with time. The solvent was removed by rotary evaporation, and the residue was dissolved in 50 mL of CH_2Cl_2. An equal volume of aqueous ammonia was added, and solution was stirred at room temperature. After 3 h, the aqueous layer was separated, and the organic layer was washed with water. The CH_2Cl_2 was removed by rotary evaporation to give a residue which showed two spots using TLC. The mixture was then columned on Al_2O_3 using 98% EtOAc/2% MeOH. The product with R_f ~ 0.65% was collected to give 0.15 g (7.7%) of MePY2: ^1H NMR ($CDCl_3$) 2.35 (s, CH_3), 2.85 (s, br, 8 H), 6.8–7.55 (m, 6 H), 8.35 (d, br, 2 H).

Other conditions for reactions of PY2 and HCHO are given in the supplementary material.

Reaction of $[Cu^I_2(XYL\text{-}D)](PF_6)_2$ with O_2. This complex (0.2 g, 0.205 mmol) was dissolved in 50 mL of dry CH_2Cl_2 under Ar, and the resulting yellow solution was cooled to 0 °C and exposed with dry dioxygen overnight. The green product solution was filtered under Ar, and the filtrate was layered with 80 mL of dry diethyl ether. Dark green crystals were produced after standing for 3 days under an argon atmosphere. The crystals were filtered and dried under vacuum to give 0.18 g (87%) of pure compound $[Cu_2(XYL\text{-}O^-)(OH)]^{2+}$ (**3**). Samples for IR and UV–vis spectroscopy were prepared in a drybox under an inert atmosphere and were compared with an authentic sample.[13] IR spectroscopy showed a ν(O–H) absorption at 3605 cm^{-1}. Deuterium was not found in the product.

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Registry No. 4, 110243-80-6; **5**, 139016-46-9; PY2, 15496-36-3; $Me_2XYL\text{-}CH_3$, 139016-42-5; XYL-CH_3, 139016-43-6; $(D_4)XYL\text{-}CH_3$, 139016-45-8; XYL-F, 106776-15-2; O_2, 7782-44-7; monooxygenase, 9038-14-6; 2,4-bis(chloromethyl)mesitylene, 1585-17-7; 2,6-dicyanotoluene, 2317-22-8; 2,6-bis(aminomethyl)toluene, 139016-44-7; phthalic anhydride, 85-44-9; 2-bromo-*m*-xylene, 576-22-7; 2-*d*-*m*-xylene, 38422-51-4; 1,3-bis(bromomethyl)-2-*d*-benzene, 38422-52-5; 2,6-dimethylaniline, 87-62-7; 2-fluoro-*m*-xylene, 443-88-9; 1,3-bis(bromomethyl)-2-*F*-benzene, 25006-86-4.

Supplementary Material Available: Tables I and II with ^1H and $^{13}C\{^1H\}$ NMR chemical shift data, respectively, for ligands and Cu(I) complexes and additional experimental details concerning formaldehyde chemistry (3 pages). Ordering information is given on any current masthead page.