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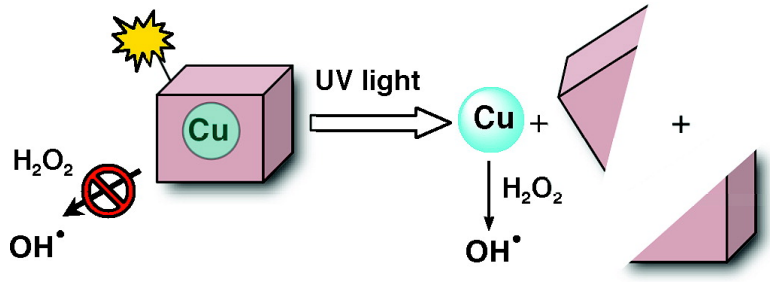

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A Photolabile Ligand for Light-Activated Release of Caged Copper

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The redox activity of copper makes it an essential cofactor in numerous enzymes critical for life, but also renders it potentially toxic by promoting the formation of reactive oxygen species (ROS) that lead to cellular oxidative stress.[1] Understanding the trafficking pathways by which cells and organisms acquire, maintain, and utilize copper while suppressing its toxicity has important ramifications for health and disease.[2] Copper's pro-oxidant property is also medicinally promising if it can be harnessed to induce oxidative stress as a cancer chemotherapy strategy.[3] New reagents that could deliver copper intracellularly in a site and time specific manner would therefore be useful both for targeted delivery of ROS-active agents and for delineating copper trafficking and utilization pathways. Toward these goals, we present here a caged copper complex in which a photoactive nitrophenyl group is incorporated into the backbone of a tetradentate chelator with high affinity for copper. Activation with UV light induces bond cleavage that releases bidentate components with low affinity for copper (Scheme 1).

The concept of light-activated caged metal ions was first introduced for Ca^{2+}.[4] Caged calcium, in which stable coordination complexes are activated by UV light to release Ca^{2+} ions intracellularly, have found widespread use in understanding the many roles of Ca^{2+} in neurotransmission, muscle contraction, and other biological processes.[5] The carboxylate-rich chelators DM-nitrophen and NP-EGTA used to cage Ca^{2+} have also been used for Sr^{2+}, Ba^{2+}, Mg^{2+}, Cd^{2+}, Mn^{2+}, and Co^{2+},[6] while photocleavable cryptands have been reported to release alkali ions[7] and a photoactive crown ether shows modest reversible photorelease of Sr^{2+}.[8] To the best of our knowledge, uncaging biologically important d-block metal ions like iron, zinc, and copper using photoactive ligands has not been reported. Because Cu^{2+}–carboxylate complexes can be pro-oxidant and are themselves photoactive to release CO_2 and carbon-centered radicals,[9] carboxylate ligands are not ideal for caging copper. We therefore chose a nitrogen-rich bispyridylamide ligand (H_2cage) for our first-generation caged copper.

The ligand H_2cage is obtained in a one-pot, two-step synthesis starting from 3-amino-3-(2-nitrophenyl)propionic acid. It is very soluble in methanol and ethanol and moderately soluble in water. Potentiometric titration of H_2cage shows that it contains no dissociable protons between pH 2 and 12, as expected since pK_a values of similarly substituted pyridines are below 2 and those of amides are above 12.[10] In the presence of Cu^{2+}, however, protons are released from H_2cage at pH 3.3 and 4.8, consistent with deprotonation of both amides. Analysis of the pH-dependent spectrophotometric titration of a 1:1 mixture of H_2cage and Cu^{2+} shows that the predominant species in solution from pH 5−12 is the neutral compound [Cu(OH$_2$)(cage)], with an overall stability constant of log $\beta = 10.8$ (Supporting Information, (SI)). This value converts to a conditional dissociation constant, K_D, at pH 7.4 for Cu-cage of 16 pM, which was further confirmed by a competition

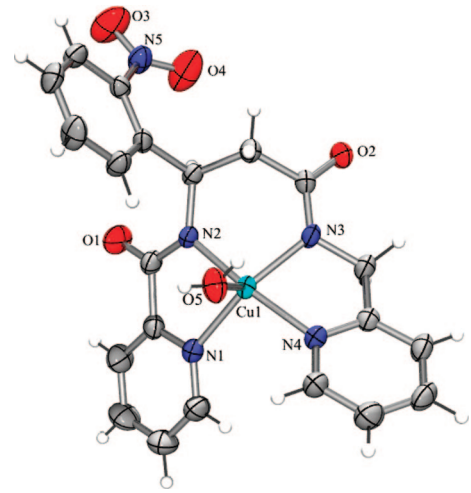
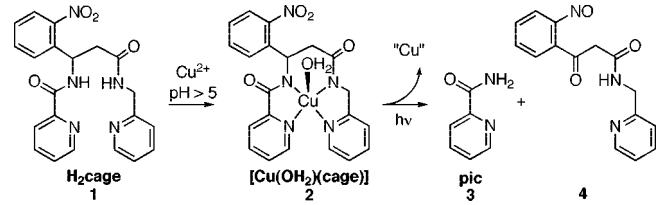


Figure 1. ORTEP plot of [Cu(OH$_2$)(cage)] showing 50% thermal ellipsoids.

Scheme 1



reaction between H_2cage and the common chelator nitrilotriacetic acid (NTA), which has a K_D of 23 pM for Cu^{2+} at this pH (SI).

Recrystallization of [Cu(OH$_2$)(cage)] from acetone in the presence of base confirmed that 2 deprotonated amide nitrogens and 2 pyridyl nitrogens coordinate Cu^{2+} in a distorted trigonal bipyramidal geometry, with a water molecule lying in the trigonal plane at a Cu−O distance of 2.299(3) Å. The average Cu−N_{amide} distance of 1.943 Å and Cu−$N_{pyridine}$ distance of 2.034 Å are similar to other bispyridylamide Cu^{2+} complexes.[11]

When solutions of [Cu(OH$_2$)(cage)] in pH 7.4 phosphate buffer are exposed to 350 nm UV light, changes in its UV−vis spectra are apparent within seconds, with a shift in the d−d band centered at 580 nm indicating a reorganization of the coordination sphere (SI). The quantum yield of photolysis decreases from 0.73 for H_2cage to 0.32 for [Cu(OH$_2$)(cage)] (SI), indicating that coordination by Cu^{2+} decreases photolysis efficiency but does not prevent it. Under our photolysis conditions, cleavage of the ligand backbone is complete within 4 min, as confirmed by LC−MS analysis shown in Figure 2 and in the SI. The peak for the intact Cu complex **2** disappears and is replaced by peaks corresponding to the expected photoproducts **3** and **4**, as confirmed by their mass spectra and by comparison to a picolinamide standard for **3**. The uncaged copper

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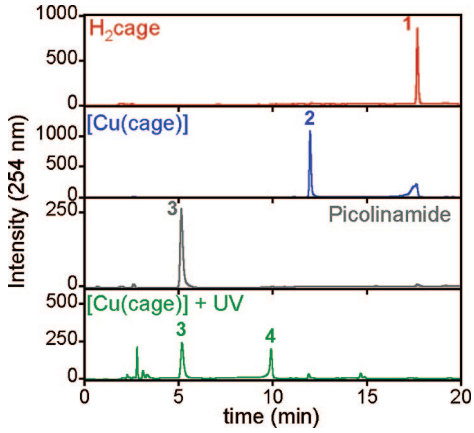

Figure 2. Chromatography traces for H_2cage (top), [Cu(cage)], authentic picolinamide, and [Cu(cage)] after 4 min of UV photolysis (bottom). Mass spectra corresponding to the LC peaks confirm the identity of ligand **1**, copper complex **2**, and photoproducts **3** and **4** (see SI).

is likely bound to these photoproducts in solution, but with significantly diminished affinity compared to the intact ligand **1**, as the log β values for picolinamide are only 2.87 and 5.40 for the 1:1 and 1:2 species.[12] Reduction to Cu^{1+} is also possible as a result of photolysis, although it would likely reoxidize to Cu^{2+} under these experimental conditions.

To show that photolysis of [Cu(OH_2)(cage)] causes a change in the reactivity of the caged versus uncaged copper, we monitored the ability of the compounds pre- and postphotolysis to generate $OH^{\bullet}$ radicals by subjecting them to the deoxyribose assay. Hydroxyl radicals, which are generated in this assay by Fenton-like conditions of copper, ascorbic acid, and H_2O_2, degrade deoxyribose to give thiobarbituric acid (TBA)-reactive products with absorbance at 532 nm (SI). Ligands added to the reaction mixture attenuate the amount of TBA-reactive species by altering the coordination environment around copper. As shown in Figure 3, our caging ligand provides 50% protection of deoxyribose degradation compared to the background reaction of Cu^{2+} alone. The photoproducts, on the other hand, increase the level of $OH^{\bullet}$ produced. The reactivity of the photoproducts matches that of control reactions run with 1 or 2 equiv of picolinamide, indicating that these bidentate ligands improve the catalytic properties of the metal with respect to Fenton-like chemistry. NTA, which has a similar affinity for Cu^{2+} as H_2cage at this pH, also promotes $OH^{\bullet}$ production by copper (Figure 3). This result highlights the fact that thermodynamic stability alone does not dictate Fenton reactivity of a metal complex.

In conclusion, we have presented a new photoactive ligand that can cage copper in a tetracoordinate binding site. Activation with UV light uncages the metal cargo by cleaving the ligand backbone to release photoproducts with diminished affinity for Cu^{2+}. The ability of copper to undergo Fenton-like reactivity and promote $OH^{\bullet}$ formation increases by 160% following light-induced uncaging. This is a promising step in developing compounds that are triggered by light to increase oxidative stress, which is the reverse strategy to our other efforts to develop chelating agents that can be triggered to inhibit oxidative stress.[13] The caged copper is a neutral compound with a molecular weight under 500, which may be favorable for cellular permeability. The 16 pM affinity of our first-generation caged copper, while significant, may not be strong enough to keep copper sequestered in the presence of endogenous copper-binding proteins; for example, human serum albumin binds Cu^{2+} with 1 pM affinity at pH 7.4.[14] Future work is focused on improving the stability of caged copper complexes, as well as applying photoactive

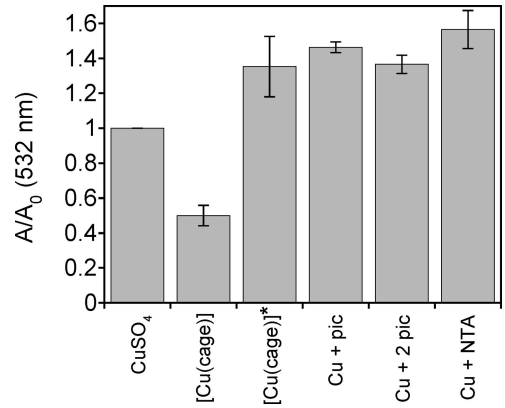

Figure 3. Uncaging copper from [Cu(cage)] in pH 7.4 phosphate buffer by UV photolysis increases $OH^{\bullet}$ production as measured by the increase in A_{532} for deoxyribose degradation. A and A_0 are the absorbance with and without ligand, so $A/A_0 = 1$ for $CuSO_4$ alone; lower values indicate an inhibitory effect and higher values indicate a promotional effect of the ligand with respect to copper's reactivity for $OH^{\bullet}$ production. [Cu(cage)]* was photolyzed for 4 min.

ligands to other biologically interesting metals. These new reagents will be valuable tools for on-demand delivery of metal ions to study mechanisms of metal ion trafficking, as well as applications such as chemotherapy where toxic metal ions could be released to induce cell death.

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Supporting Information Available: Full experimental details and X-ray crystallographic data including CIF files. This material is available free of charge via the Internet at http://pubs.acs.org.

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