

# Light-Induced Copper(II) Coordination by a Bicyclic Tetraaza Chelator through a Ligand-to-Metal Charge-Transfer Reaction

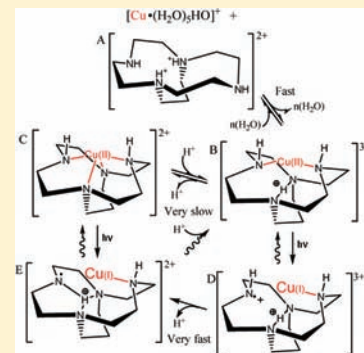
Jacob R. Holm-Jørgensen,<sup>†,‡</sup> Mikael Jensen,<sup>†</sup> and Morten J. Bjerrum<sup>\*,‡</sup>

<sup>†</sup>The Hevesy Laboratory, Risoe-DTU, Technical University of Denmark, DK-4000 Roskilde, Denmark

<sup>‡</sup>Department of Basic Sciences and Environment, Faculty of Life Science, University of Copenhagen, DK-1165 Copenhagen, Denmark

## Supporting Information

**ABSTRACT:** To enable utilization of the broad potential of copper isotopes in nuclear medicine, rapid and robust chelation of the copper is required. Bowl adamanzanes (bicyclic tetraaza ligands) can form kinetically stable copper complexes, but they are usually formed at low rates unless high pH values and high temperatures are applied. We have investigated the effects of the variation in the pH, different anions, and UV irradiation on the chelation rate. UV spectra of mixtures of Cu<sup>2+</sup> and [2<sup>4</sup>.3<sup>1</sup>]adz in water show the existence of a long-lived two-coordinated copper(II) intermediate (only counting coordinated amine groups) at pH above 6. These findings are supported by pH titrations of mixtures of Cu<sup>2+</sup> and [2<sup>4</sup>.3<sup>1</sup>]adz in water. Irradiation of this complex in the ligand-to-metal charge-transfer (LMCT) band by a diode-array spectrophotometer leads to photodeprotonation and subsequently to formation of the four-coordinated copper(II) complex at a rate up to 7800-fold higher at 25 °C than in the dark. Anions in the solution were found to have three major effects: competitive inhibition due to Cu<sup>II</sup> binding anions, inhibition of the photoinduced transchelation from UV-absorbing anions, and photoredox inhibition from acido ligands capable of acting as electron donors in LMCT reactions. Dissolved O<sub>2</sub> was also found to result in photoredox inhibition.



## INTRODUCTION

Kinetically stable (robust) copper complexes are interesting for use in radiopharmaceuticals, notably for positron emission tomography (PET) because of the versatility of the copper PET isotopes.<sup>1</sup> Robustness or kinetic inertness is central in order to avoid loss of radionuclides from the tracer molecules. Radiometal loss by transchelation (ligand exchange where one chelator replaces another) to competing chelators will typically result in liver accumulation.<sup>2</sup> The usefulness of a robust chelate is, however, limited by the need for a short preparation time under mild conditions; typically less than the half-life of the relevant isotope (minutes to hours). Therefore, the challenge is to construct a chelator able to form a sufficiently robust copper chelate within the time limit set by the half-life of the chosen isotope.

Monocyclic tetraamine chelators, such as 2,2',2'',2'''-(1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrayl)tetraacetate (dota) and 2,2',2'',2'''-(1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetrayl)-tetraacetate (teta) are able to chelate copper(II) fast and with high labeling efficiency;<sup>1</sup> however, they also have a high level of in vivo transchelation.<sup>2</sup> Bicyclic chelators, such as bowl adamanzanes,<sup>3</sup> form more robust copper(II) chelates because of the cryptate effect and their rigidity.<sup>4,5</sup> However, with high robustness also comes a low chelation rate, partly because of the increased rigidity of the ligands and partly because the ligands act as proton sponges.<sup>6,7</sup> One way to overcome this kinetic barrier has been to reflux in an ammonia/ammonium

buffer; another has been chelation in a dry basic ethanol solution at 90 °C.<sup>6,7</sup>

Here, we will show that UV irradiation from a diode-array spectrophotometer is sufficient to increase the rate of copper(II) chelation up to 7800-fold at 25 °C. This is achieved by ligand-to-metal charge transfer (LMCT), which facilitates deprotonation of the amines in the adamanzane cavity. As a result, chelation can be completed in minutes at nondenaturing conditions. However, UV radiation at low wavelengths can also damage the target molecules intended to be copper-labeled, especially with O<sub>2</sub> present in solution.

## EXPERIMENTAL SECTION

**Adamanzane Nomenclature.** The adamanzane nomenclature was described earlier and is illustrated in Figure 1 for the adamanzane [2<sup>4</sup>.3<sup>1</sup>]adz discussed in this article.<sup>6</sup> The corresponding IUPAC name for the adamanzane is 1,4,7,10-tetraazabicyclo[5.5.3]pentadecane.

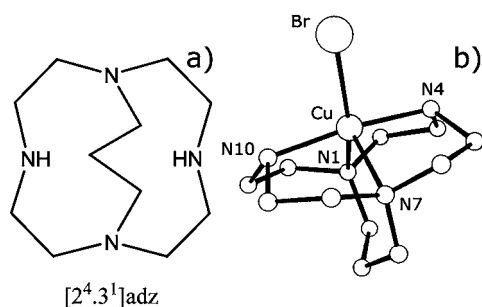
**Caution!** Mechanical handling or heating of perchlorate salts, especially of organic molecules, can be dangerous. However, we have never experienced any explosions with the presented compounds.

**Ligand Synthesis.** [2<sup>4</sup>.3<sup>1</sup>]adz·3HBr and [Cu([2<sup>4</sup>.3<sup>1</sup>]adz)Br]ClO<sub>4</sub> were prepared as described previously.<sup>6,8</sup> Solutions of [2<sup>4</sup>.3<sup>1</sup>]adz·3HClO<sub>4</sub> were prepared from 20.0 mM solutions of [2<sup>4</sup>.3<sup>1</sup>]adz·3HBr by the addition of stoichiometric amounts of a AgClO<sub>4</sub> solution followed by removal of AgBr by centrifugation. This method was also used to prepare solutions of [Cu([2<sup>4</sup>.3<sup>1</sup>]adz)ClO<sub>4</sub>]ClO<sub>4</sub> in water from

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**Figure 1.** Bowl adamanzane (a bicyclic tetraaza ligand) with the short name  $[2^4.3^1]\text{adz}$  investigated in this study. The corresponding IUPAC name for the adamanzane is 1,4,7,10-tetraazabicyclo[5.5.3]pentadecane. (a) 2D drawing of  $[2^4.3^1]\text{adz}$ . (b) Crystal structure of  $[\text{Cu}([2^4.3^1]\text{adz})\text{Br}]^+$ .

$[\text{Cu}([2^4.3^1]\text{adz})\text{Br}]\text{ClO}_4$ . Solid  $[2^4.3^1]\text{adz}\cdot 3\text{HClO}_4$  was prepared by dissolving 0.5 g of  $[2^4.3^1]\text{adz}\cdot 3\text{HBr}$  in 2 mL of water and recrystallizing by adding 1 mL of saturated  $\text{NaClO}_4$ . The white crystals were washed three times in 96% ethanol at room temperature and dried before repeating the recrystallization. Final yield: 68%.

**Spectrophotometry.** Coordination of  $\text{Cu}^{2+}$  to  $[2^4.3^1]\text{adz}$  was measured by changes in the UV–visible spectra measured on a HP8435 diode-array spectrophotometer equipped with a tungsten lamp and a DURANIUM L2D2 UV-glass see-through deuterium lamp or on a Shimadzu UV-3600 UV–vis–near-IR (NIR) spectrophotometer. All cuvettes were UV-transparent quartz cuvettes from Hellma. All data from the HP8435 diode-array spectrophotometer were analyzed using *UV Visible ChemStation* software, and all data from the Shimadzu UV-3600 UV–vis–NIR spectrophotometer were analyzed using Shimadzu *UVProbe* software.

**UV Light Intensity of the HP8435 Spectrophotometer.** The intensity of the polychromatic light from the deuterium lamp in the HP8435 spectrophotometer was estimated by a Hachard-Parker actinometer using an average quantum yield of 1.0.<sup>9</sup> The range spanned by the deuterium lamp is 190–400 nm.

A total of 125  $\mu\text{L}$  of an actinometer solution (6.4 mM  $\text{K}_3[\text{Fe}(\text{C}_2\text{O}_4)_3]$  and 50 mM  $\text{H}_2\text{SO}_4$ ) prepared in the dark was transferred to the sample cuvette, where it was exposed to radiation from the spectrophotometer.<sup>10</sup> Irradiation time: 1–10 min. A total of 100  $\mu\text{L}$  of an exposed solution was then mixed with 25  $\mu\text{L}$  of a 0.2% phenanthroline solution, 62.5  $\mu\text{L}$  of a buffer solution (600 mM  $\text{NaCH}_3\text{COO}$  and 180 mM  $\text{H}_2\text{SO}_4$ ), and 62.5  $\mu\text{L}$  of  $\text{H}_2\text{O}$  to a total volume of 250  $\mu\text{L}$  and placed in darkness for 1 h. The absorbance at 510 nm was measured in a HP8435 diode-array spectrophotometer using a 10 mm cuvette. An unexposed actinometer solution was used for the control. All handling of the actinometer solutions was done under red light. An actinometer solution exposed in a 100  $\mu\text{L}$ , 10 mm (Hellma 105.201-QS) quartz cuvette was measured to be  $6.0 \times 10^{-4}$  einstein/min from the spectrophotometer. A NaBr filter (see the Photochemistry section) reduces the intensity by 32% (Figure S1 in the Supporting Information). The 32% reduction in the intensity is in agreement with the spectral radiant intensities of similar lamps measured by Hollandt et al.<sup>11</sup>

**Mass Spectrometry (MS).** Aqueous solutions of samples were mixed with methanol (to 25%) and acetic acid (to 0.1%). All spectra were recorded at room temperature on an electrospray ionization ion-trap mass spectrometer (Esquire 4000, Bruker) and analyzed by *HYSTAR*, version 3.2, software.

**Titrations.** A total of 273  $\mu\text{mol}$  of  $[2^4.3^1]\text{adz}\cdot 3\text{HClO}_4$  was dissolved in 25 mL of a mixture (10 mM  $\text{HClO}_4$  and 990 mM  $\text{NaClO}_4$ ) and 216  $\mu\text{mol}$  of  $\text{Cu}(\text{ClO}_4)_2$  was dissolved in 25 mL of a mixture (10 mM  $\text{HClO}_4$  and 990 mM  $\text{NaClO}_4$ ). A total of 5.0 mL of these solutions was titrated with freshly prepared 10.00 mM  $\text{NaOH}$  under argon at 25 °C. A total of 2.5 mL of the  $[2^4.3^1]\text{adz}$  solution + 3.15 mL of the  $\text{Cu}(\text{ClO}_4)_2$  solution (equal to 27.25  $\mu\text{mol}$  of each) was titrated similarly. The titrations were performed in triplicate.

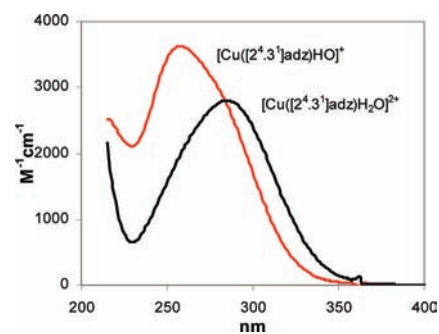
Simulations of experimental titration curves were performed using the program *CURTIPOT*, version 3.3.2 (2008), for MS-Excel by Prof. Ivano G. R. Gutz. As input, the following  $\text{p}K_a$  values were used: for  $[2^4.3^1]\text{adz}$ ,  $\text{p}K_1 = -1$ ;  $\text{p}K_2 = 3.202$ ;  $\text{p}K_3 = 7.242$ , and  $\text{p}K_4 = 15$ ; for  $\text{Cu}^{2+}(\text{aq})$ ,  $\text{p}K_1 = 6.21$  and  $\text{p}K_2 = 6.5$ .<sup>3</sup> Small amounts of hydrogencarbonate ( $\text{p}K_a = 10.33$ ) were also allowed in the simulations. No attempts were made to simulate the effect of the proposed bidentate copper(II) complex.

**Photochemistry.** Photoinduced coordination of  $[2^4.3^1]\text{adz}$  to  $\text{Cu}^{2+}$  was both induced and measured using the UV light from the deuterium lamp in a HP8435 diode-array spectrophotometer. In contrast to most spectrophotometers, a diode-array spectrophotometer irradiates the sample with polychromatic light. To ensure that as much of the sample as possible was exposed to the light source, a Hellma 105.201-QS cuvette and a sample volume of 125  $\mu\text{L}$  were used. The small volume in an ultramicrocuvette enabled the whole sample to be placed within the light beam. The samples were irradiated by the unfiltered UV light from the deuterium lamp of the spectrophotometer, unless a filter was placed between the light source and the sample to block high-energy UV light.

The time from the start of one measurement to the start of the next is the cycle time of the spectrophotometer. The integration time is the time that the spectrophotometer actually measures absorption of the sample. A HP8435 spectrophotometer has a shutter that blocks the light beam between each measurement. The shutter is open approximately 1 s longer than the integration time, so with an integration time of 1 s and a cycle time of 4 s, the samples are irradiated half of the time. When the cycle time is equal to the integration time, the shutter stays open and the sample is irradiated continuously during the whole run time.

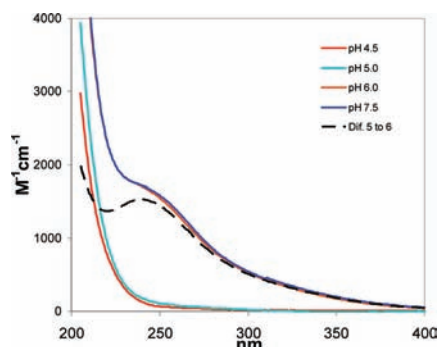
UV-light filters: a 50 mM aqueous solution of  $\text{Na}_2\text{X}$  ( $\text{X} = \text{ClO}_4^-$ ,  $\text{PO}_4^{3-}$ ,  $\text{Cl}^-$ ,  $\text{Br}^-$ , or  $\text{NO}_3^-$ ) in a 2 mm quartz cuvette, the side of a 4 mL disposable cuvette from Ratiolab, and an object glass. The filter cutoff is defined as the wavelength at which absorption is equal to 1.0, resulting in the following values:  $\text{NaClO}_4$ , 186 nm;  $\text{Na}_3\text{PO}_4$ , 188 nm (both from extrapolation);  $\text{NaCl}$ , 197 nm;  $\text{NaBr}$ , 221 nm;  $\text{NaNO}_3$ , 243 nm; cuvette side, 285 nm; object glass, 307 nm (Figure S2 in the Supporting Information).

**Coordination Rate of  $\text{Cu}^{2+}$ .** The amount of coordinated  $\text{Cu}^{2+}$  can be measured spectrophotometrically by exploiting the difference in absorption of uncoordinated and Cu-coordinated  $[2^4.3^1]\text{adz}$ . Absorption of Cu-coordinated  $[2^4.3^1]\text{adz}$  is independent of the pH at 285 nm because of an isosbestic point (Figure 2). In contrast, absorption of



**Figure 2.** UV spectra of the tetradentate chelate complex between  $\text{Cu}^{\text{II}}$  and the ligand  $[2^4.3^1]\text{adz}$  with water (black line) or hydroxide (red line) as the axial ligand. The pH in the  $[\text{Cu}([2^4.3^1]\text{adz})\text{H}_2\text{O}]^{2+}$  solution was less than 6 and higher than 10 in the  $[\text{Cu}([2^4.3^1]\text{adz})\text{HO}]^+$  solution.

“uncoordinated”  $\text{Cu}^{2+}$  is pH-dependent because of a labile copper(II) complex formed with  $[2^4.3^1]\text{adz}$  at pH above 6 (Figure 3). Therefore, in order to calculate formation of the complex  $[\text{Cu}([2^4.3^1]\text{adz})\text{H}_2\text{O}]^{2+}$ , irradiated samples were acidified with  $\text{HClO}_4$  after incubation and before measurement of the final absorption at 285 nm.



**Figure 3.** UV spectra measured immediately after mixing an 1:2 mixture of  $\text{Cu}^{2+}$  ions and  $[\text{2}^4\text{.3}^1]\text{adz}$  at different pH values. Also shown is the difference in absorption from pH 5.0 to 6.0.

The concentration of  $[\text{Cu}([\text{2}^4\text{.3}^1]\text{adz})\text{H}_2\text{O}]^{2+}$  was then calculated using  $\epsilon_{285} = 2805 \text{ M}^{-1} \text{ cm}^{-1}$ .

Solutions of  $[\text{2}^4\text{.3}^1]\text{adz}\cdot 3\text{HClO}_4$  were diluted with water, and the pH was adjusted to a predetermined pH with 10 mM NaOH [to achieve a final pH of 7.4 after the addition of anions and  $\text{Cu}(\text{ClO}_4)_2$ ].  $\text{Na}_n\text{X}$  ( $\text{X} = \text{ClO}_4^-$ ,  $\text{PO}_4^{3-}$ , or  $\text{NO}_3^-$ ) solutions or distilled water was added before mixing with a  $\text{Cu}(\text{ClO}_4)_2$  solution. Concentrations after mixing: 0.10 mM  $\text{Cu}^{2+}$ , 0.20 mM  $[\text{2}^4\text{.3}^1]\text{adz}$ , and 20 mM X at pH 7.4 at 25 °C. Reaction temperatures: 25, 40, and 55 °C. Fixed temperatures were ensured by using a thermostatted cuvette holder and preheated solutions of  $\text{Cu}^{2+}$  and  $[\text{2}^4\text{.3}^1]\text{adz}$ . The chelation rate during irradiation experiments was calculated by exploiting the difference in  $\epsilon_{285}$  (nm) between  $[\text{Cu}([\text{2}^4\text{.3}^1]\text{adz})\text{H}_2\text{O}]^{2+}$  ( $2805 \text{ M}^{-1} \text{ cm}^{-1}$ ) and the bidentate copper(II) complex formed with  $[\text{2}^4\text{.3}^1]\text{adz}$  at pH above 6 ( $700 \text{ M}^{-1} \text{ cm}^{-1}$ ).

Coordination of  $[\text{2}^4\text{.3}^1]\text{adz}$  to  $\text{Cu}^{2+}$  under argon was performed by mixing a solution of 250  $\mu\text{L}$  of 1.0 mM NaOH and 150  $\mu\text{L}$  of 1.0 mM  $[\text{2}^4\text{.3}^1]\text{adz}$  with 100  $\mu\text{L}$  of 1.0 mM  $\text{Cu}(\text{ClO}_4)_2$ , resulting in a final pH of 7. Argon was bubbled through all solutions for 1 min before mixing. After mixing, the solution was transferred to a cuvette, thermostatted to 25 °C. After 1 min, measurement under continuous irradiation from the spectrophotometer through a NaBr filter (cutoff at 221 nm) was started. Samples prepared identically, except for argon bubbling, were also prepared.

The rates in darkness with and without copper(I) present were measured by mixing two argon-bubbled solutions as described above; one of them with acid-cleaned metallic copper added. Aliquots of 125  $\mu\text{L}$  were then taken at various times for UV-vis measurement. The calculated equilibrium concentration of copper(I) is approximately 6  $\mu\text{M}$ .

**Irradiation of  $[\text{Cu}([\text{2}^4\text{.3}^1]\text{adz})\text{ClO}_4]\text{ClO}_4$ .** Solutions of 200  $\mu\text{M}$   $[\text{Cu}([\text{2}^4\text{.3}^1]\text{adz})\text{ClO}_4]\text{ClO}_4$  in water were irradiated in a HP8435 spectrophotometer at room temperature, with and without a NaBr or a NaCl filter between the sample and the light source. Experiments without oxygen were performed by purging the solution with argon for 1 min before irradiating the sample.

In acid-decomplexation studies, solutions of  $[\text{Cu}([\text{2}^4\text{.3}^1]\text{adz})\text{ClO}_4]\text{ClO}_4$  were mixed with either HCl or  $\text{HClO}_4$  to final concentrations of 200  $\mu\text{M}$  complex and 5.0 M acid, and the mixed solutions were irradiated in a HP8435 spectrophotometer at room temperature, without a filter. Identically mixed solutions were left in darkness.

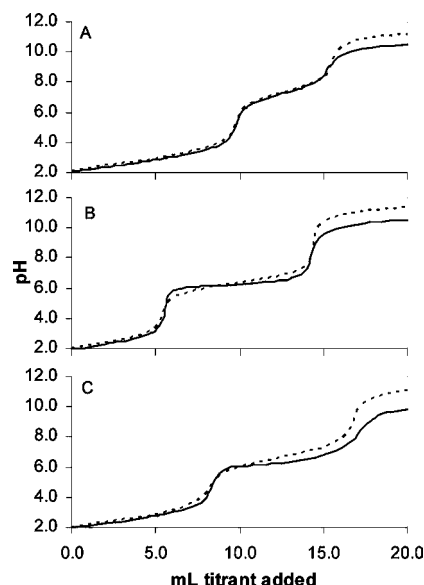
## RESULTS

**Changes in the UV Spectra of Copper(II) Complexes of  $[\text{2}^4\text{.3}^1]\text{adz}$  as a Function of the pH.** The four-coordinated copper(II) chelate of  $[\text{2}^4\text{.3}^1]\text{adz}$  has formerly been characterized with various axial ligands, forming a pentacoordinated complex (see Figure 1).<sup>3</sup> The axial ligand exchange from  $\text{H}_2\text{O}$  to  $\text{HO}^-$  is known to move  $\lambda_{\text{max}}$  of the chelate in the visible field from 600 to 632 nm.<sup>6</sup> The UV absorption spectra of  $[\text{Cu}([\text{2}^4\text{.3}^1]\text{adz})\text{H}_2\text{O}]^{2+}$  and  $[\text{Cu}([\text{2}^4\text{.3}^1]\text{adz})\text{HO}]^+$  are

shown in Figure 2. It can be seen that the ligand exchange also moves  $\lambda_{\text{max}}$  and  $\epsilon_{\text{max}}$  in the UV region from 285 nm and  $2805 \text{ M}^{-1} \text{ cm}^{-1}$  for  $[\text{Cu}([\text{2}^4\text{.3}^1]\text{adz})\text{H}_2\text{O}]^{2+}$  to 258 nm and  $3630 \text{ M}^{-1} \text{ cm}^{-1}$  for  $[\text{Cu}([\text{2}^4\text{.3}^1]\text{adz})\text{HO}]^+$ . Coincidentally, 285 nm is an isosbestic point, so even if some of the chelate has  $\text{OH}^-$  as the axial ligand, it has no effect on absorption at 285 nm. Both spectra were of the perchlorate salts of the chelates, as most other anions can replace  $\text{H}_2\text{O}$  and  $\text{HO}^-$  as axial ligands.<sup>6</sup>

Over time (hours), a nonacidic solution of  $\text{Cu}^{2+}$  and  $[\text{2}^4\text{.3}^1]\text{adz}$  will result in the formation of some  $[\text{Cu}([\text{2}^4\text{.3}^1]\text{adz})\text{H}_2\text{O}]^{2+}$ . However, shortly after mixing, it does not contain detectable amounts of  $[\text{Cu}([\text{2}^4\text{.3}^1]\text{adz})\text{H}_2\text{O}]^{2+}$  because of the slow formation of this compound (vide infra).<sup>6</sup> The UV absorption spectra of such a mixture solution at various pH values are shown in Figure 3. It is clear that these spectra are quite different from those in Figure 2. The UV spectra of  $\text{Cu}^{2+} + [\text{2}^4\text{.3}^1]\text{adz}$  are nearly the same at pH 6 and 7.5, but UV absorption at pH 5 is much lower (Figure 3). The increase in absorption from pH 5 to 6 is due to an LMCT band with  $\lambda_{\text{max}}$  and  $\epsilon_{\text{max}}$  of 240 nm and  $1525 \text{ M}^{-1} \text{ cm}^{-1}$ .

**Titration of  $[\text{2}^4\text{.3}^1]\text{adz}$ ,  $\text{Cu}^{2+}$ , and an Equimolar Mixture of the Two Solutions.** Acidic solutions of  $[\text{2}^4\text{.3}^1]\text{adz}$  were titrated with NaOH and revealed that the alkaline error starts to affect the data curve at pH above 9 (Figure 4). Acidic

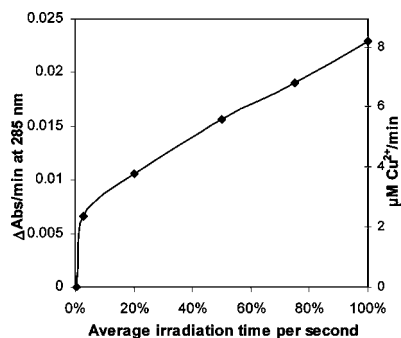


**Figure 4.** Titrations of acid solutions of (A)  $[\text{2}^4\text{.3}^1]\text{adz}$ , (B)  $\text{Cu}^{2+}$ , (C) equimolar  $[\text{2}^4\text{.3}^1]\text{adz}$ , and  $\text{Cu}^{2+}$  with NaOH under argon. Representative titrations are compared to computer-generated titration curves (dashed lines). These simulations were performed using individual  $\text{pK}_a$  values for  $\text{Cu}^{2+}(\text{aq})$  and the free ligand  $[\text{2}^4\text{.3}^1]\text{adz}$  without considering the formation of complexes. The deviation in part A is due to the alkaline error (at pH over 9). The deviation in part B is due to precipitation of  $\text{Cu}(\text{OH})_2$  and the alkaline error. The deviation in part C is due to coordination to the ligand and the alkaline error.

solutions of  $\text{Cu}(\text{ClO}_4)_2$  were titrated and revealed that the data curve has more profound inflection points than the simulation because of precipitation of  $\text{Cu}(\text{OH})_2$ . It also has a slightly larger difference between calculated and measured pH values after 20 mL of titrant. Finally, an equimolar mixture of the two solutions was titrated. This titration had a more profound primary inflection point but a less profound secondary

inflection point. Titrations of the mixture of  $\text{Cu}^{2+}$  and  $[\text{2}^4\text{.3}^1]\text{adz}$  with  $\text{NaOH}$  reveal that the mixture is more acidic than can be explained from  $\text{Cu}^{2+}$  and  $[\text{2}^4\text{.3}^1]\text{adz}$  acting separately in the solution. Furthermore, no precipitation of  $\text{Cu}(\text{OH})_2$  was observed when  $[\text{2}^4\text{.3}^1]\text{adz}$  was present in amounts equal to those of  $\text{Cu}^{2+}$  in the titration solution.

**Effect of Light on the Coordination of  $[\text{2}^4\text{.3}^1]\text{adz}$  to  $\text{Cu}^{2+}$ .** In this study, we used a photodiode-array spectrophotometer both as a light source for polychromatic irradiation and for simultaneous measurement of the absorbance. The rate of coordination of  $[\text{2}^4\text{.3}^1]\text{adz}$  to  $\text{Cu}^{2+}$  was measured in an assay, where the contents of all of the samples were identical and the only parameter changed was how often the shutter was open (exposure ratio) shown as a percentage of time (see the Experimental Section). It is evident from Figure 5 that increased



**Figure 5.** Formation rate of  $[\text{Cu}([\text{2}^4\text{.3}^1]\text{adz})\text{H}_2\text{O}]^{2+}$  due to irradiation without filter at  $25\text{ }^\circ\text{C}$  and  $\text{pH } 7.5$  as a function of the exposure ratio. Full spectra were measured at five frequencies in a manner which the shutter of the spectrophotometer was open approximately the stated fractions of time. The rate that micromolar copper chelated per minute is shown on the second  $y$  axis. The rates measured after 1.0 min are shown.

irradiation from the diode-array spectrophotometer increases the reaction speed. Between exposures of 20% (that is, 2 s of exposure to the polychromatic light from the UV lamp in the spectrophotometer every 10 s) and 100% (that is, 2 s exposure to the polychromatic light every 2 s), the reaction speed increases in a linear manner, when measured after 60 s. An increase of 10 percentage point irradiation per time corresponds to a  $0.55\text{ }\mu\text{M Cu}^{2+}/\text{min}$  faster rate. However, increasing the exposure frequency from zero to 3% of the time (2 s every 60 s) has a much more profound effect because the rate jumps from 0.0 to  $2.4\text{ }\mu\text{M Cu}^{2+}/\text{min}$  (Figure 5).

The reaction rate decreases seemingly faster over time than expected from the decrease in the reactant concentration alone (Figure S3 in the Supporting Information and data not shown).

To distinguish the effect of the polychromatic irradiation from the spectrophotometer as a function of the wavelength, cutoff filters were placed between the light source and the samples. In these experiments, a sample of  $\text{Cu}^{2+}$  and  $[\text{2}^4\text{.3}^1]\text{adz}$  (in a ratio of 1:2) was irradiated through a filter for 1 h at  $25\text{ }^\circ\text{C}$  and then the increase in absorbance at 285 nm was measured without filter. With an object glass filter (cutoff at 307 nm), the increase was only 6% higher than that of a control kept in darkness. When the filter was a disposable cuvette side (cutoff at 285 nm), the difference from the control was 14% (data not shown). Only UV light at low wavelength seems to induce accelerated chelation of  $\text{Cu}^{2+}$  by  $[\text{2}^4\text{.3}^1]\text{adz}$ .

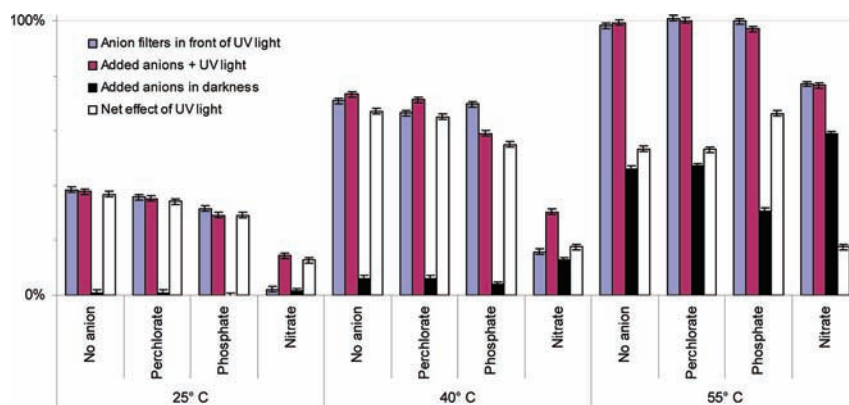
In darkness, coordination of  $[\text{2}^4\text{.3}^1]\text{adz}$  to  $\text{Cu}^{2+}$  is slow and the reaction rate increases with rising temperatures, proving the existence of a pure thermal reaction. In order to evaluate the effect of the temperature on UV-light-induced chelation, the percentage of  $\text{Cu}^{2+}$  bound in the form of complex  $[\text{Cu}([\text{2}^4\text{.3}^1]\text{adz})\text{H}_2\text{O}]^{2+}$  is calculated relative to the situation where all  $\text{Cu}^{2+}$  is bound as  $[\text{Cu}([\text{2}^4\text{.3}^1]\text{adz})\text{H}_2\text{O}]^{2+}$ . The term “maximal chelation” is hereafter used for the situation where all  $\text{Cu}^{2+}$  is bound as  $[\text{Cu}([\text{2}^4\text{.3}^1]\text{adz})\text{H}_2\text{O}]^{2+}$ , corresponding to 100%. The uncertainty due to the experimental conditions is estimated to approximately 1 percentage point, so the uncertainty of very small chelation values is high.

Irradiation for 30 min at  $25\text{ }^\circ\text{C}$  through a  $\text{NaNO}_3$  filter (cutoff at 243 nm) increases the formation of complex  $[\text{Cu}([\text{2}^4\text{.3}^1]\text{adz})\text{H}_2\text{O}]^{2+}$  from 1% of maximal chelation in darkness to 2% when exposed. The same experiment at  $40\text{ }^\circ\text{C}$  with a  $\text{NaNO}_3$  filter increases formation of the complex from 6% of maximal chelation in darkness to 16% when irradiated through the  $\text{NaNO}_3$  filter. At  $55\text{ }^\circ\text{C}$ , the formation increased from 46% of maximal chelation in darkness to 77% when irradiated through the  $\text{NaNO}_3$  filter. With no filter, the degree of chelation after irradiation for 30 min was 39% at  $25\text{ }^\circ\text{C}$ , 71% at  $40\text{ }^\circ\text{C}$ , and 98% at  $55\text{ }^\circ\text{C}$ , compared to 1% at  $25\text{ }^\circ\text{C}$ , 6% at  $40\text{ }^\circ\text{C}$ , and 46% at  $55\text{ }^\circ\text{C}$  in darkness. At  $25\text{ }^\circ\text{C}$ , there seemed to be a small effect of using phosphate or perchlorate as the filter but not at higher temperatures.

**Chemical Effects of Anions on the Chelation Rate.** Phosphate is known to bind  $\text{Cu}^{2+}$ , and as expected, it reduced the chelation in darkness, most visible at  $55\text{ }^\circ\text{C}$ . The chelation after 30 min was thus changed to 31% compared to 46% when no anion was added (Figure 6), and the same trend was seen at 25 and  $40\text{ }^\circ\text{C}$ . Added nitrate enhanced the chelation in darkness at  $55\text{ }^\circ\text{C}$  to 59%, while perchlorate had no effect (47%). The effects at 25 and  $40\text{ }^\circ\text{C}$  were similar, although nitrate seemed more effective with the chelation doubled from 6% to 12% at  $40\text{ }^\circ\text{C}$ .

The effect of added anions on the chelation rate under UV irradiation is always to reduce the chelation rate compared to samples irradiated without added anions. This effect is a combination of anions binding to copper ions and of absorption of UV light (Figure 6). The two effects can be separated by the addition of a filter with the anions in front of the sample instead of having them in solution or by incubation in darkness. Phosphate reduces the rate, whereas nitrate, and to some extent perchlorate, results in a higher final chelation when added to the solution, compared to irradiation through the anion as a filter. However, this effect was not seen at  $55\text{ }^\circ\text{C}$ .

**Temperature Effect on the Light-Induced Reaction.** To compare the effect of UV irradiation at different temperatures, we have also calculated the percentages of chelate formation in “irradiated samples with added anions” minus the percentages formed in identical samples incubated in darkness. The result is the “net effect” of irradiation (Figure 6). In all cases, the net effect of irradiation increases with increasing temperatures; although at  $55\text{ }^\circ\text{C}$ , some samples reach maximal chelation before incubation is over, giving the result that the “Net effect of UV light” decreases from 40 to  $55\text{ }^\circ\text{C}$ . Further evidence of a thermal effect on the light-induced reaction comes from the initial rate of light-induced chelation, which is doubled from 25 to  $35\text{ }^\circ\text{C}$  and from 35 to  $45\text{ }^\circ\text{C}$  and increased by 66% from 45 to  $55\text{ }^\circ\text{C}$  (Figure S4 in the Supporting Information).

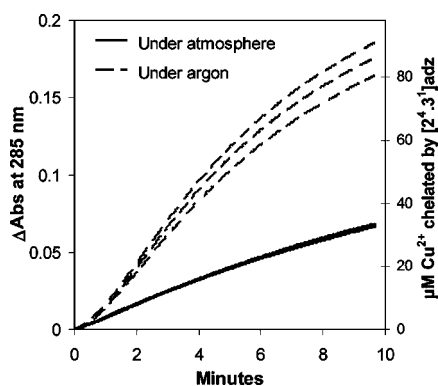


**Figure 6.** Effect of  $\text{Na}_n\text{X}$  ( $\text{X} = \text{ClO}_4^-$ ,  $\text{PO}_4^{3-}$ , or  $\text{NO}_3^-$ ) on the formation of  $[\text{Cu}([2^4,3^1]\text{adz})\text{H}_2\text{O}]^{2+}$  measured by spectrophotometry after 30 min of irradiation. Blue bars: irradiated solutions with anions used as UV-light filters. Red bars: irradiated solutions with anions added to them. Black bars: solutions incubated in darkness with anions added to them. White bars: net effect of UV light, which is the value of solutions irradiated with anions minus the corresponding solutions incubated in darkness.

**Effect of Light on Acid Decomplexation.** Solutions of  $[\text{Cu}([2^4,3^1]\text{adz})\text{ClO}_4]\text{ClO}_4$  in 5.0 M acid behaved very differently depending on the type of acid. In 5.0 M  $\text{HClO}_4$ , there is only one LMCT peak, at 285 nm, corresponding to  $[\text{Cu}([2^4,3^1]\text{adz})\text{H}_2\text{O}]^{2+}$ , and under irradiation, it declined initially ( $0-1/2$  h) by a zero-order reaction and from  $2 1/2$  h it declined by a first-order reaction (Figure S5 in the Supporting Information). Absorption at the ligand-field peak at 600 nm declined in a similar manner. Without irradiation, the LMCT peak declined by a much slower first-order reaction. In darkness, a 5% decrease in absorption at 285 nm was reached after 700 h. Under irradiation, a 5% decrease was reached after only 3.5 min and a 95% decrease was reached after 2 h.

In 5.0 M  $\text{HCl}$ , there are two LMCT peaks and the largest declines in LMCT absorption occur at 265 and 310 nm. Again, without irradiation, the reaction is similar but much slower (although the effect of irradiation is smaller than that in  $\text{HClO}_4$ ). In darkness, a 5% decline at 310 nm was reached after 16 h and after 13.5 min under irradiation.

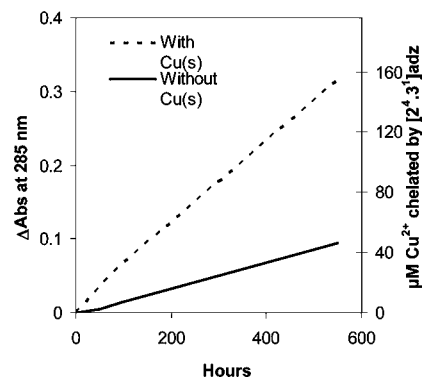
**$\text{O}_2$  Effect on the Chelation Rate.** Identical samples of  $\text{Cu}^{2+}$  mixed with  $[2^4,3^1]\text{adz}$  were irradiated through a NaBr filter (cutoff at 221 nm), either under a normal atmosphere or under argon. When the chelation rates are compared after 5 min, the average rate with  $\text{O}_2$  present was  $3.9 \mu\text{M Cu}^{2+}/\text{min}$  and that with  $\text{O}_2$  removed by argon  $10.8 \mu\text{M Cu}^{2+}/\text{min}$  (Figure 7).



**Figure 7.** Effect of  $\text{O}_2$  on the formation rate of  $[\text{Cu}([2^4,3^1]\text{adz})\text{H}_2\text{O}]^{2+}$  at pH 7 under filtered UV irradiation (cutoff at 221 nm). Full lines:  $\text{Cu}^{2+}$  and  $[2^4,3^1]\text{adz}$  irradiated under a normal atmosphere. Dashed lines:  $\text{Cu}^{2+}$  and  $[2^4,3^1]\text{adz}$  irradiated under argon.

This corresponds to a 64% reduction in the chelation rate when  $\text{O}_2$  is present.

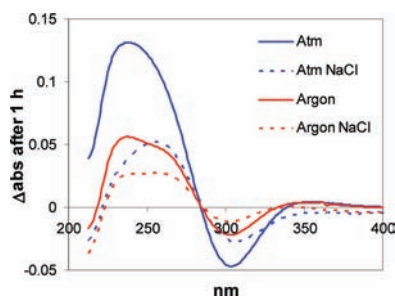
**Catalysis of Chelation by  $\text{Cu}^{\text{I}}$ .** The effect of  $\text{Cu}^{\text{I}}$  on the chelation rate in darkness was measured over 3 weeks by comparing reaction solutions under argon with and without metallic copper added to produce  $\text{Cu}^{\text{I}}$  (Figure 8). Without



**Figure 8.** Effect of  $\text{Cu}^{\text{I}}$  on the formation rate of  $[\text{Cu}([2^4,3^1]\text{adz})\text{H}_2\text{O}]^{2+}$  at pH 7 in darkness. Dashed line: chelation with  $\text{Cu}(\text{s})$  present to produce  $\text{Cu}^{\text{I}}$ . Full line: only  $\text{Cu}^{\text{II}}$ .

$\text{Cu}(\text{s})$ , the chelation rate was quite constant at  $1.4 \text{ nM Cu}^{2+}/\text{min}$ , resulting in chelation of  $46 \mu\text{M Cu}^{2+}$  after 550 h. With  $\text{Cu}(\text{s})$ , the initial chelation rate was  $5.6 \text{ nM Cu}^{2+}/\text{min}$ , and after 100 h, it declined a bit, so  $155 \mu\text{M Cu}^{2+}$  was chelated after 550 h.

**Side Effect of High-Energy UV-Light Irradiation.** To investigate whether or not UV irradiation has an effect on  $[\text{Cu}([2^4,3^1]\text{adz})\text{H}_2\text{O}]^{2+}$  besides ligand exchange, a solution of purified  $\text{Cu}([2^4,3^1]\text{adz})(\text{ClO}_4)_2$  was irradiated in the spectrophotometer with and without a NaBr filter (cutoff at 221 nm) between the sample and the light source. With the filter, there is no change of the spectrum, but without the filter, an increase in  $\epsilon_{\text{max}}$  and a decrease in  $\lambda_{\text{max}}$  are observed (Figure S6 in the Supporting Information). This was further investigated by measuring the effect of a NaCl filter (cutoff at 197 nm) and by  $\text{O}_2$  removal by argon, singly and in combination. The change in absorption resulting from irradiation is shown in Figure 9: Under a normal atmosphere and with no filter, the maximal change in absorption ( $\Delta A_{\text{max}}$ ) is 0.13 at 239 nm. With  $\text{O}_2$



**Figure 9.** Difference between the final and initial UV absorption of a  $[\text{Cu}([2^4.3^1]\text{adz})\text{H}_2\text{O}]^{2+}$  sample irradiated for 1 h in the HP8435 spectrophotometer with an initial pH of 6.53. Samples irradiated with a UV filter (NaCl, cutoff at 197 nm) in front of the sample are shown as dashed lines. Samples kept under a normal atmosphere are shown in blue, while samples kept under argon are shown in red.

removed by argon,  $\Delta A_{\text{max}}$  is still at 239 nm but is reduced by 57% to 0.056. The NaCl filter also reduces the change in absorption, but  $\Delta A_{\text{max}}$  is shifted to 258 nm, indicating a different process. With a NaCl filter,  $\Delta A_{\text{max}}$  is reduced by 60% to 0.053. The effects of  $\text{O}_2$  removal and of using a NaCl filter seem to be independent of each other. Interestingly, all spectra are isosbestic at 286 nm.

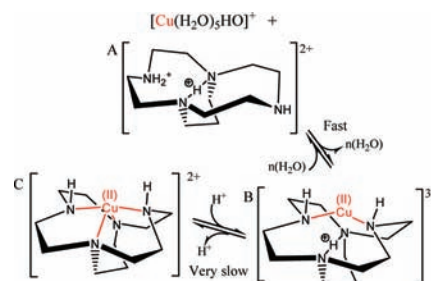
## DISCUSSION

**Formation of a Long-Lived, Intermediate Coordination Compound.** The pH dependency on the UV spectrum (Figure 3) of the reactant mixture ( $\text{Cu}^{2+}$  and  $[2^4.3^1]\text{adz}$ ) is not due to a difference in protonation of the adamanzane because its  $\text{p}K_{\text{a}3}$  of 7.24 makes it effectively double protonated at both pH 5 and pH 6.<sup>8</sup> The  $\text{Cu}^{2+}(\text{aq})$  ion, on the other hand, is 3% deprotonated at pH 5 and 24% deprotonated at pH 6. However, this deprotonation does not explain the differences between the spectra at pH 5 and 6 in Figure 3 by itself. At pH 7.5, where  $\text{Cu}^{2+}(\text{aq})$  ions precipitate without the presence of  $[2^4.3^1]\text{adz}$ , the adamanzane keeps  $\text{Cu}^{2+}$  in solution through a reaction much faster than full chelation via all four amine groups. This is supported by the deviation in the titration curve C (Figure 4), which cannot be explained by the alkaline error alone, and there was no precipitation. Therefore, the deviation is attributed to coordination of the ligand to  $\text{Cu}^{2+}$ .

The difference between the LMCT band at pH 5 and 6 of a mixture of  $\text{Cu}^{2+}$  and  $[2^4.3^1]\text{adz}$  supports the formation of an intermediate with  $\lambda_{\text{max}}$  at 240 nm at pH 6 (Figure 3). The molar absorption coefficient of an LMCT band peak  $\epsilon_{\text{max}}$  is correlated to the nature and number of coordinating ligands that are able to transfer an electron to the metal ion when absorbing a photon in the LMCT band.<sup>12</sup> On the basis of the LMCT band of  $[\text{Cu}([2^4.3^1]\text{adz})\text{H}_2\text{O}]^{2+}$ , each N– $\text{Cu}^{\text{II}}$  bond has an absorption of  $2805 \text{ M}^{-1} \text{ cm}^{-1}/4 = 701 \text{ M}^{-1} \text{ cm}^{-1}$  on average. The wry peak of the  $[\text{Cu}([2^4.3^1]\text{adz})\text{HO}]^+$  LMCT band (Figure 2) is expected because the energy required for a charge transfer from a  $\text{HO}^-$  ligand to  $\text{Cu}^{\text{II}}$  is higher (smaller wavelength) than that from an amine ligand, corresponding to the difference in the redox potential.<sup>13</sup> The nature of the N– $\text{Cu}^{\text{II}}$  bond is also affected by the increased electron density around the copper ion due to  $\text{HO}^-$ , which lowers  $\lambda_{\text{max}}$  of the N– $\text{Cu}^{\text{II}}$  LMCT. Likewise, if  $\text{Cu}^{\text{II}}$  is only coordinated by the two unprotonated amine groups in the adamanzane (and water), then the ligands will have lower electron densities (due to the remaining  $\text{H}^+$ ), resulting in lower  $\lambda_{\text{max}}$  and higher  $\epsilon_{\text{max}}$ . This fits well with the observed  $\lambda_{\text{max}}$  at 240 nm and an absorption of

$1525 \text{ M}^{-1} \text{ cm}^{-1}/2 = 763 \text{ M}^{-1} \text{ cm}^{-1}$  on average per bond of the  $\text{Cu}^{2+}$  and  $[2^4.3^1]\text{adz}$  mixture. At high pH ( $>10$ ), the UV spectrum of the mixture is shifted, resulting in lower  $\lambda_{\text{max}}$  and higher  $\epsilon_{\text{max}}$  of the peak (data not shown), similar to the shift seen for  $[\text{Cu}([2^4.3^1]\text{adz})\text{H}_2\text{O}]^{2+}$  when it deprotonates.

We suggest, on the basis of the UV spectra, the titrations, and the UV-light-induced reaction, that  $\text{Cu}^{2+}$  and  $[2^4.3^1]\text{adz}$  form a semistable intermediate complex at pH above 6 via the two secondary amine groups (N4 and N10; Figure 10B), a complex



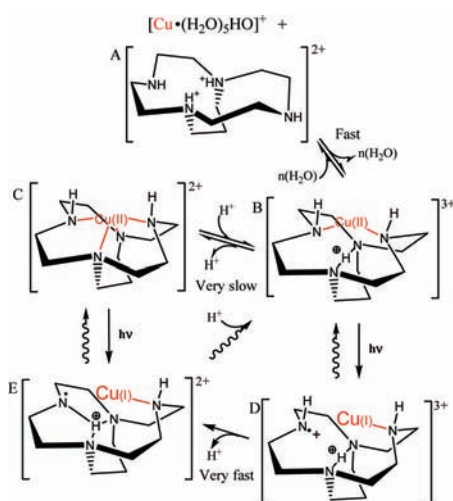
**Figure 10.** Formation of semistable intermediate (B) and of stable chelate (C). Water coordinated to  $\text{Cu}^{\text{II}}$  in B and C is omitted for clarity.

stable enough to prevent precipitation even at pH 11, as seen in the titrations (Figure 4).

**Light-Induced Chelation.** Because most small adamanzanes are proton sponges, the rate-limiting step in chelation of  $\text{Cu}^{2+}$  by adamanzanes usually is the final deprotonation of the ligand.<sup>6</sup> Especially for  $[2^4.3^1]\text{adz}$  with a  $\text{p}K_{\text{a}}$  of  $>15$ , this is an extremely slow process at pH 7 and room temperature (Figure 10,  $\text{A} \rightarrow \text{B} \rightarrow \text{C}$ ). Besides the high  $\text{p}K_{\text{a}}$ , deprotonation might be inhibited by steric hindrance when  $\text{Cu}^{\text{II}}$  coordinates to the nonbridging amine groups of the adamanzane as in B. The overall charge of B is 3+ because water can be assumed to constitute any remaining coordinating ligands, according to the UV spectrum (Figure 3).

Irradiation through a plastic cuvette side for 1 h (cutoff at 285 nm) resulted only in a 2.3% formation of  $[\text{Cu}([2^4.3^1]\text{adz})\text{H}_2\text{O}]^{2+}$ , compared to 2.0% for a control kept in darkness. Blocking the UV light with a filter with a cutoff at around 285 nm almost removes the light effect, which explains why formation of  $[\text{Cu}([2^4.3^1]\text{adz})\text{H}_2\text{O}]^{2+}$  under irradiation drops faster than expected based on the consumption of the reactants (Figure S3 in the Supporting Information); the product  $[\text{Cu}([2^4.3^1]\text{adz})\text{H}_2\text{O}]^{2+}$  has an absorption peak at 285 nm and is thus acting as an UV “inner filter”.<sup>14</sup> Even the nitrate filter with a cutoff at 243 nm removes a large part of the light effect (Figure 6). Perchlorate and phosphate have cutoffs at very low wavelengths (186 and 188 nm, respectively) and, as expected, have little effect as light filters (Figure 6).

The light-induced reaction is dependent on irradiation below 250 nm, which fits the LMCT band of the intermediate formed in a mixture of  $\text{Cu}^{2+}$  and  $[2^4.3^1]\text{adz}$  with  $\lambda_{\text{max}}$  at 240 nm at pH 6 (Figure 3). Therefore, it is most likely that the charge transfer induced by absorption at 240 nm is crucial for the reaction. A possible model for a reaction initiated by a photoinduced charge transfer is through radical-ion-pair formation, which is possible in water.<sup>15,16</sup> In that case, an electron transfer from the adamanzane ligand to  $\text{Cu}^{\text{II}}$  will produce a nitrogen radical and  $\text{Cu}^{\text{I}}$  (Figure 11D). Although the lifetime of such an ion pair is short, it is long enough for the nitrogen radical to deprotonate and it is much more acidic than the ground state because of the



**Figure 11.** Proposed reaction scheme for coordination of  $\text{Cu}^{2+}$  to  $[2^4.3^1]\text{adz}$  including the UV-light-induced reaction. Only coordination to the adamanzane is shown.  $\text{C} \rightarrow \text{E}$  is at least a two-step reaction, but other intermediates are omitted in this figure for simplification. Both  $\text{C} \rightarrow \text{E}$  and  $\text{E} \rightarrow \text{C}$  are intramolecular proton transfers, where the overall charge of the complex is kept constant.

extra charge on the nitrogen.<sup>17,18</sup> Deprotonation of D leads to E. This is still not the ground state, and when the nitrogen radical in E is reduced by  $\text{Cu}^{\text{I}}$ , two reactions are possible. The nitrogen can either receive a  $\text{H}^+$  from the solvent, reverting to B, or receive the  $\text{H}^+$  between the bridging nitrogen atoms, facilitating the formation of C. The pH and structure of the adamanzane are important for whether E becomes B or C.

If the intermediate B is rapidly converted to the final chelate C by UV irradiation, then formation of the intermediate in the irradiated area of the cuvette could become the rate-limiting step. However, the  $\text{A} \rightleftharpoons \text{B}$  equilibrium is expected to be very rapid. Therefore, the UV-mediated reaction can be assumed to be limiting for the reaction rate in this case. The reaction rate for B to C (via D and E) is thus expected to increase proportionally to the intensity of the radiation  $I$  and with temperature  $T$ .<sup>19</sup> However, this is only partly the case, as can be seen from Figure 5. The rate does increase proportionally to the radiation, but there also seems to be a constant effect of irradiation. A possible explanation could be that  $\text{Cu}^{\text{I}}$  formed by UV irradiation catalyzes chelation, as seen with porphyrin.<sup>20</sup> To test this explanation, the chelation rate in darkness and under argon was measured with and without metallic copper added to produce  $\text{Cu}^{\text{I}}$  through the equilibrium  $\text{Cu}^{2+} + \text{Cu}(\text{s}) \rightleftharpoons 2\text{Cu}^{\text{I}}$ .  $\text{Cu}^{\text{I}}$  does increase the rate (Figure 8). The problem with the  $\text{Cu}^{\text{I}}$  explanation is that in darkness at equilibrium concentrations of  $\text{Cu}^{\text{I}}$  ( $6 \mu\text{M}$ ) the rate is only increased 4-fold, and the rate under argon and irradiation is increased 7800-fold compared to that in darkness without  $\text{Cu}^{\text{I}}$  (see Figures 7 and 8, all rates at  $25^\circ\text{C}$ ). Furthermore, the mechanism of  $\text{Cu}^{\text{I}}$  catalysis proposed by Tabata and Babasaki (deformation of the ligand allowing  $\text{Cu}^{\text{II}}$  to enter from the back site) cannot be used for the adamanzanes because the back side is blocked by the cross bridge. Instead,  $\text{Cu}^{\text{I}}$  might bind on the top of the ligand, allowing (faster but still relatively slow) deprotonation, and then  $\text{Cu}^{\text{II}}$  could oxidize the bound  $\text{Cu}^{\text{I}}$ . Coming back to Figure 5, the “3%” sample was irradiated the first 2 s of the minute, so any  $\text{Cu}^{\text{I}}$  formed there had 58 s to act as a catalyst unless it was reoxidized or disproportionated before the minute had passed.

**Thermal Effect.** As expected, the chelate formation rate in darkness is temperature-dependent. However, it is somewhat unexpected that the same is the case for the light-induced reaction, when it is modified by subtracting the reaction in darkness (net effect). That is because primary quantum yields have an insignificant temperature dependence. Apparently, the intramolecular proton transfer required from E to C is favored by increasing temperature over intramolecular electron transfer from  $\text{Cu}^{\text{I}}$  to  $\text{N}^\bullet$ . If the electron transfer comes first, the result is  $\text{D} \rightarrow \text{B}$ ; if the proton transfer comes first, the result is  $\text{D} \rightarrow \text{C}$ . A light-induced temperature-dependent reaction could also be explained by  $\text{Cu}^{\text{I}}$  catalysis.

**Effects of Ligands Other Than Adamanzanes.** The concentrations of the “added anions” in solution were made to have the same average UV blocking effect as the filter of the same anion, taking into account the different absorption conditions in the cuvette: When anions are added to the reaction cuvette, only the anions in the side of the cuvette where the light enters have a full filtering effect, while anions in the opposite side of the cuvette have no filtering effect.

The presence of UV-absorbing solutes will decrease the rate unless they have an even greater enhancing effect (e.g., by coordinating to the chelate and not the reactants). Thus, most  $\text{Cu}^{\text{II}}$  coordinating ligands will reduce the rate (even without UV absorption) by competitive binding to  $\text{Cu}^{\text{II}}$ . Nonetheless, nitrate seems to facilitate coordination of adamanzanes to  $\text{Cu}^{\text{II}}$  in darkness. When this effect is combined with UV irradiation, the result is much faster chelation than expected from experiments where nitrate is used as the filter. Especially at low temperatures, the two effects combine with synergy (Figure 6). Contrary to this is phosphate, which coordinates to  $\text{Cu}^{\text{II}}$  and therefore competes with the adamanzane for coordination to  $\text{Cu}^{\text{II}}$ . The result in darkness is a  $1/3$  reduction in the reaction rate at 40 and  $55^\circ\text{C}$  (Figure 6). This is important to consider when coordinating  $\text{Cu}^{\text{II}}$  in a buffered solution because almost all buffers used for  $\text{Cu}^{\text{II}}$  coordination also coordinates  $\text{Cu}^{\text{II}}$  themselves. Choosing the right buffer, with little  $\text{Cu}^{\text{II}}$  binding, can increase the coordination rate greatly.

Upon UV irradiation, another effect is added to competitive coordination.  $\text{Cu}^{\text{II}}$  can also oxidize other coordinating ligands ( $\text{X}^-$ ) upon UV irradiation, producing  $\text{X}^\bullet$  and a  $\text{Cu}^{\text{I}}$  complex. This has been shown for UV irradiation of several  $\text{Cu}^{\text{II}}$  macrocycles.<sup>21,22</sup> Two possible reactions can follow. The  $\text{X}^\bullet$  produced can reoxidize  $\text{Cu}^{\text{I}}$  before it dissociates from the adamanzane, or  $\text{Cu}^{\text{I}}$  can dissociate from the adamanzane because  $\text{Cu}^{\text{I}}$  chelates, in general, are much more labile than  $\text{Cu}^{\text{II}}$  chelates. In both cases, the result would be slower chelation rates under UV radiation relative to systems with nonoxidizable ligands. The acid-decomplexation rates under UV irradiation are much faster with nonoxidizable  $\text{HClO}_4$  than with  $\text{HCl}$ , whereas in darkness  $\text{HCl}$  results in a higher rate than  $\text{HClO}_4$  (vide infra). These results are in favor of the rapid reoxidation explanation for the acido ligand reactions.

**Light-Induced Dechelation.** Photoexcitation of the chelate can be expected to result in breakage of the  $\text{Cu}-\text{N}$  bonds because  $\text{Cu}^{\text{I}}$  prefers a different coordination geometry than  $\text{Cu}^{\text{II}}$  and because  $\text{Cu}^{\text{I}}$  does not coordinate well to amines. Therefore, at pH less than 2, this leads to a reverse reaction, producing  $\text{Cu}^{2+}$  and free ligand from C (in Figure 10), as Granifo et al. also have shown with another tetraaza macrocycle.<sup>23</sup>

In 5.0 M  $\text{HClO}_4$ , the reaction is as expected, very slow in darkness and more than 12 000-fold faster under UV irradiation measured by a 5% decline at 285 nm. The reaction rate pattern

starting at zero order and ending at first order indicates that UV light is the limiting factor initially, but this limitation is reduced as the inner filter from  $[\text{Cu}([\text{2}^4.3^1]\text{adz})\text{H}_2\text{O}]^{2+}$  is removed by dechelation. Instead, the amount of substrate, also  $[\text{Cu}([\text{2}^4.3^1]\text{-adz})\text{H}_2\text{O}]^{2+}$ , becomes the limitation. Finally, the decline at 285 nm followed that at 600 nm. That was not the case in 5.0 M HCl. The difference in reaching 5% decline (measured at 310 nm where the decline was fastest) is only 70-fold. Moreover, the rate depends on which wavelength it is measured at. Apparently,  $\text{Cl}^-$  protects the chelate from light-induced dechelation as explained above.

It is normal to compare the kinetic stability of copper(II) complexes by acid decomplexation.<sup>24,25</sup> This is a stepwise process where the N–Cu coordination bonds one by one are broken and replaced with protonation of the amine groups. However, because photoexcitation enables a faster ligand exchange, there is a risk that the  $k_{\text{obs}}$  values reported in various papers are much too high.<sup>21,26,27</sup> This would be the case, if the dissociation of tetraazacopper complexes in an acid solution was studied kinetically by spectrophotometry, and the reaction rate was enhanced by UV irradiation. Furthermore, acido ligands like  $\text{Cl}^-$  can stabilize both the chelate and free  $\text{Cu}^{2+}$ , making the results difficult to compare.

**Effects of Solute  $\text{O}_2$  on the Light-Induced Chelation Rate.** Experiments performed under a normal atmosphere and under argon showed that chelation without  $\text{O}_2$  was about 130% higher after 600 s than chelation under a normal atmosphere (Figure 7).  $\text{O}_2$  is known to form copper(II) superoxo complexes by the oxidation of  $\text{Cu}^{\text{I}}$ .<sup>28</sup> These can be quite stable, but in this case, where  $\text{Cu}^{\text{I}}$  in D is oxidized by  $\text{O}_2$ , the  $\text{O}_2^{\bullet-}$  radical is adjacent to an  $>\text{NH}^{\bullet+}$  radical. Therefore, rapid redelivery of the electron from  $\text{O}_2^{\bullet-}$  to  $>\text{NH}^{\bullet+}$  can be expected, with B as the result. Thus, the overall result of  $\text{O}_2$  presence is a higher rate of  $\text{D} \rightarrow \text{B}$ .

**Side Reaction.** To investigate possible side reactions due to UV irradiation, pure  $\text{Cu}([\text{2}^4.3^1]\text{adz})(\text{ClO}_4)_2$  in an aqueous solution was irradiated with and without a NaBr UV filter with a cutoff at 221 nm in front of the sample (Figure S6 in the Supporting Information). Apparently, only UV light with wavelengths shorter than 220 nm is energetic enough to induce the side reaction. Maurialidharan and Ferraudi have shown that high-energy photons can open a macrocycle, forming a C–C double bond.<sup>23,29</sup> In the adamantanes, it is also possible that the cross bridge is broken, yielding a cyclen with two side chains, one of them containing a double bond. The reaction has been studied by MS with no sign of decomposition products as in accordance with this model (data not shown): monocyclic chelates with a double bond resulting from a C–C bond break will have the same molecular weight as the adamantane chelate, so this type of reaction is undetectable by MS. In contrast, imine formation, as described in a macrocycle by Chrisian et al., would be detectable by MS.<sup>30</sup> The lack of structure that can stabilize a double bond prevents the reaction at low energies.

The results of irradiation with and without  $\text{O}_2$  and through a NaCl filter and not (Figure 9) speak against cyclen formation as the sole explanation. That is because the overlapping peaks between 220 and 280 nm found without  $\text{O}_2$  change shape and intensity when  $\text{O}_2$  is present, indicating that more than one reaction is active and that one of the reactions has to involve  $\text{O}_2$ . It is possible that part of the effect of a bromide filter seen in Figure S6 in the Supporting Information is to prevent a secondary reaction involving the formation of oxygen radicals. The isosbestic point at 286 nm, within the error of measurement of the isosbestic point at 285 nm for  $[\text{Cu}([\text{2}^4.3^1]\text{adz})\text{H}_2\text{O}]^{2+}$  and

$[\text{Cu}([\text{2}^4.3^1]\text{adz})\text{HO}]^+$ , indicates that one of the products could involve deprotonation of the coordinated water.

**Consequences for Copper Radiochemistry.** Competitive binding of  $\text{Cu}^{2+}$  ions from salts and buffers is generally not considered a problem in radiochemistry, although commonly used buffers are slightly  $\text{Cu}^{\text{II}}$  binding.<sup>31,32</sup> In regular chemistry, with concentrations of  $\text{Cu}^{2+}$  and the chelator in at least the millimolar range, this is an advantage because it helps to keep the  $\text{Cu}^{2+}$  ions in solution. However, in radiochemistry, the concentration of  $\text{Cu}^{2+}$  can be in the subnanomolar range (under carrier-free conditions).<sup>31</sup> Under these conditions, competitive binding from buffers might be very important.

Presently,  $\text{Cu}^{2+}$  labeling of peptides and protein-targeting molecules relies on heat to increase the reaction speed sufficiently, with the half-life of the isotope in mind. Unfortunately, this either restricts the kinetic stability of the chelator or excludes heat-labile targeting molecules. UV-induced chelation of  $\text{Cu}^{2+}$  offers a possible alternative. However, like heat, UV irradiation can denature proteins and cause DNA cleavage so the use of a suitable UV filter is important.<sup>33,34</sup> That said, the only way UV irradiation is presently used for  $\text{Cu}^{\text{II}}$  coordination is by causing an electron transfer within the ligand, thus enabling it to coordinate to  $\text{Cu}^{\text{II}}$ , and microwaving at high temperatures, as required for some ligands, is also a harsh treatment.<sup>35,36</sup>

## CONCLUSION

The UV spectra of a mixture of  $\text{Cu}^{2+}$  and  $[\text{2}^4.3^1]\text{adz}$  in water indicates the existence of a long-lived two-coordinated copper(II) complex (not counting water ligands) with the adamantane at pH 6–7.5, which does not form at lower pH. Irradiation of the labile complex in the LMCT band leads to photodeprotonation and subsequently to the formation of  $[\text{Cu}([\text{2}^4.3^1]\text{adz})\text{H}_2\text{O}]^{2+}$  at a rate 7800-fold higher at 25 °C. At very low pH, this process is reversed because photoexcitation of the chelate results in the breakage of Cu–N bonds and the fact that  $\text{Cu}^{\text{I}}$  prefers a different coordination geometry than  $\text{Cu}^{\text{II}}$  and because  $\text{Cu}^{\text{I}}$  does not coordinate well to amines. This leads to increased acid-decomplexation rates compared to the rates in darkness.

The presence of anions in the solution during chelation was found to have three major effects: competitive inhibition from  $\text{Cu}^{\text{II}}$  binding anions, inhibition of the photoinduced transchelation from UV-absorbing anions, and photoredox inhibition from ligands able to act as electron donors in an LMCT reaction.

If UV irradiation in the LMCT band is to be used as a chelation technique, the effects of the acido anions, dissolved  $\text{O}_2$ , and high-energy UV photons have to be taken into account.

## ASSOCIATED CONTENT

### Supporting Information

Supporting Figures S1–S6. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## AUTHOR INFORMATION

### Corresponding Author

\*E-mail: [mobj@life.ku.dk](mailto:mobj@life.ku.dk)

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