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Squaramide-Based Reagent for Selective Chromogenic Sensing of Cu(II) through a Zwitterion Radical

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ABSTRACT

A minimalist squaramide-based chemodosimeter for Cu^{2+} is described. Upon selective chelation to 2, Cu^{2+} induces the formation of a highly colored zwitterionic radical, which is kinetically stable for hours. The presence of a radical is confirmed by EPR and ESI-MS. It is then possible to use reagent 2 for visual and selective sensing of Cu^{2+} at neutral pH.

Copper-induced inhibition of important metabolic processes in plants[1] and in humans indicates that upregulated levels of copper are causative in several neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases, atherosclerosis, and diabetes.[2] Moreover, because of the widespread use of copper for industrial, agricultural, and household uses, episodes of Cu contamination of natural waters are relatively frequent. Although organically bound copper appears to be less toxic, free solvated Cu^{2+} is particularly damaging as it catalyzes the formation of reactive organic species (ROS) that cause damage to most biomolecules.[3] A large number of chemodosimeter[4] and chemosensing[5] devices for copper have been proposed so far. However, it is still highly advisible to develop new or improved methods for the selective in situ evaluation of solvated Cu^{2+} in aqueous environments. In this regard, visible detection of excess Cu^{2+} in untreated natural waters with a copper-selective chromogenic sensor offers the possibility of detecting copper contamination events in their early stages.

Properties required for an effective Cu^{2+} chemodosimeter include selectivity over other metal ions, high sensitivity with minimum manipulation of samples, and structural and synthetic simplicity.

In this work, we present a minimalist turn-on copper chemodosimeter featuring high selectivity and sensitivity for Cu^{2+}. The new radical-based[5u] reagent for Cu^{2+} combines a Wurster-type amine, namely *N*,*N*-dimethylaminophenylenedi-

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amine (DMPD), as redox reporter with a squarate ligand for Cu^{2+}. Related Wurster-based derivatives have been described as redox sensors for alkaline cations[6] and for anions.[7]

The preparation of the sodium salt of amidosquaric acid **2** is outlined in Figure 1. Although structurally very simple, this reagent has not previously been described. It was obtained in good yield via direct condensation of squaric acid with DMPD in refluxing water, followed by treatment with aqueous NaOH. Compounds **3**−**5** were prepared for comparison purposes by standard methods (see the Supporting Information).

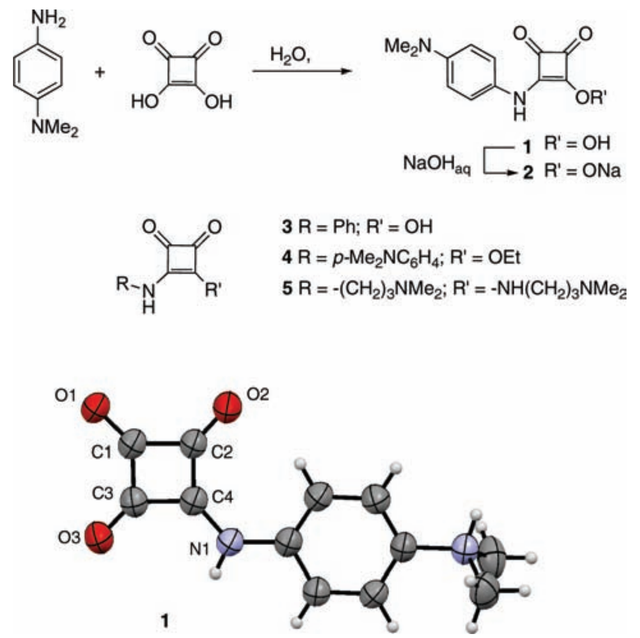
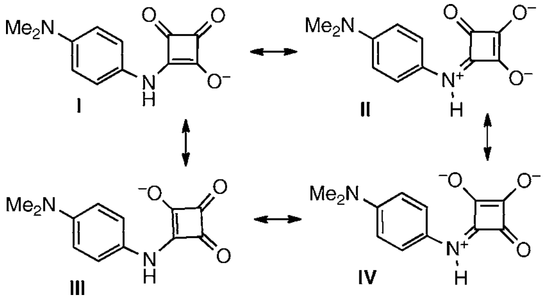


Figure 1. Preparation of amidosquaric acid and ORTEP view of **1** showing its zwitterionic nature in the solid state. Some structural information is provided: Bond distances (Å): C2−O2 = 1.238(4); C3−O3 = 1.244(4); C1−O1 = 1.251(4); C4−N1 = 1.343(4). Two molecules of disordered water have been omitted for clarity. Displacement ellipsoids are drawn at the 50% probability level.

The structure of this amidosquaric acid has been established unambiguously by X-ray difraction of single crystals obtained from aqueous solutions of **1** by slow-driven crystallization. The crystal structure confirms the zwitterionic nature of **1** in the solid state and evidence the coplanarity of the squarate-NH-aromatic system. Accordingly, the length of the C1−O1 bond (1.251 Å), which is conjugated with the aromatic NH, is larger than the lengths observed in aliphatic secondary squaramides (Figure 1).[8] The close bond distances found for C2−O2 and C3−O3 (1.244 and 1.238 Å, respectively) also support the delocalization of the negative charge in **1**. The active reagent, the sodium salt **2**, could not be crystallized. Remarkably, **2** displays negative solvatochromism, which is revealed by the hypsochromic shift ($\Delta\lambda = -12$ nm) of the most intense band from 330 nm in MeCN to 318 nm in H_2O. In principle, the negative solvatochromism is a sign of a dipolar ground-state structure, where the charge of the dipole heads decreases on excitation. Taken together, these observations suggest the contribution of polar and dipolar forms I−IV to the structure of **2** (Scheme 1).

Scheme 1. Resonance Structures of Amidosquarate Anion **2**



Compound **1**, formally an amino acid, should have two pK_a's. A potentiometric titration afforded a pK_a of 5.21. However, the protonation at pH < 2 could not be accurately studied by potentiometry. NMR titrations do not suffer from these limitations and revealed two protonation steps at 1.15 and 4.99 in water (Figures S1 and S2, Supporting Information). These results confirm the strong acidic nature of amidosquaric acid **1**.[9] Remarkably, the two pK_a's are, roughly, 1−2 units lower than those reported for dialkylaminoanilines related to DMPD at around 2.8 and 6.3, respectively.[10] As a consequence, the redox active monoanion **2** is the prevalent species in aqueous solutions at pH around 7 used in this study.

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The UV−vis spectra of **2** recorded in MeCN−H_2O (1:1 v/v) at room temperature is very different from that of DMPD in the same solvent mixture. The spectrum of **2** exhibits an absorption band at λ_{max} 325 nm which is related to the presence of the amidosquarate moiety (Figure 2).[11] When a solution of **2** (5×10^{-5} M) at pH 6.8−7.0 (Tris buffer) is treated with a solution containing excess Cu^{2+} (4 equiv), changes in the UV−vis spectrum are apparent within seconds. The initially colorless solution becomes deep purple due to the appearance of a very strong absorption band around 536 nm (Figure 2). This abrupt change does not occur when the amidosquarate anion is blocked as in the amidosquarate ester **4** or in absence of the redox portion as in model compounds **3** and **5**. Remarkably, a solution of DMPD in the same solvent mixture gives comparatively weak bands at 515 and 553 nm, respectively (Figure 2).

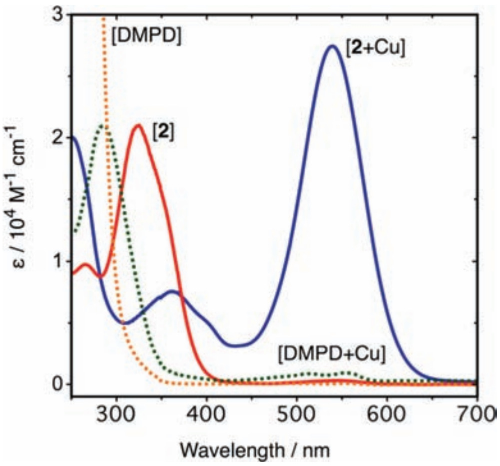


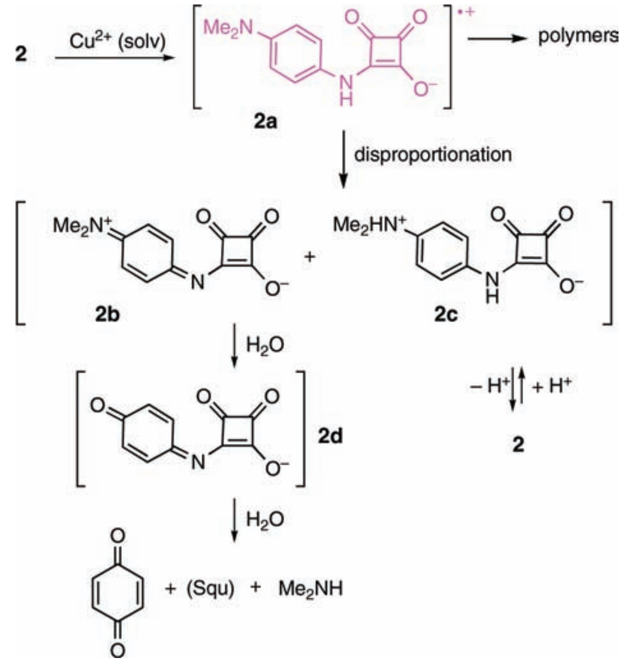
Figure 2. UV/vis spectra of a solution of **2** (5.0×10^{-5} M) in MeCN−H_2O (1:1 v/v) (red line) and spectral changes observed upon addition of 4 equiv of $CuCl_2$ (blue line). The corresponding spectra of DMPD before (orange dotted line) and after (green dotted line) the addition of Cu^{2+}, are also shown for comparison.

The absorption band around 536 nm is solvent- and time-dependent. Therefore, while addition of excess Cu^{2+}(4 equiv) to **2** (5.0×10^{-5} M) in pure MeCN triggers an intense band at 573 nm with concomitant disappearance of the amidosquarate absorption at 330 nm, in water or MeOH the solution remains almost colorless with changes in the UV absorption region of **2** which are assigned to complexation of Cu^{2+} to **2**.[12] On the other hand, the intensity of the long wavelength band in MeCN or MeCN−H_2O mixtures decays with time until it reaches a minimum value after 2 h (Figure S3, Supporting Information). In this case, the UV−vis spectra show partial recovery of the starting reagent **2** as evidenced by the reappearance of a purple coloration upon addition of additional solid $CuCl_2$ to the above solution. It is interesting to observe that the new band forms irrespective of the counterion of the copper salts used (SO_4^{2-}, AcO^-, ClO_4^-, NO_3^-, Cl^-) and that the addition of 8-hydroxyquinoleine, an effective Cu^{2+} chelating agent, inhibited the whole process.

All these observations support the involvement of an initial adduct between amidosquarate anion **2** and solvated Cu^{2+}, which then undergoes metal-chelation-controlled electron transfer with formation of a highly colored zwitterionic radical **2a**. This view is consistent with previous experimental work on the oxidation of DMPD with several oxidants[13] and also of related Wurster-type amines by $Cu(ClO_4)_2$ leading to the formation of cation radicals in MeCN but not in H_2O or MeOH, due to mistmatch of redox potentials in these solvents.[14]

The zwitterionic radical can evolve by two routes (Scheme 2). One by degradative radical coupling that leads to polymerization. The other by disproportionation[16] and subsequent hydrolysis to *p*-benzoquinone.[17] As a matter of fact, benzoquinone is the main product isolated after hydrolyis and workup of crude mixtures of **2** with Cu^{2+}.

Scheme 2. Proposed Formation and Degradation Pathways of Zwitterionic Radical Obtained from **2** [Cu^{2+}(solv)] Stands for Cu^{2+} Solvates of Variable Composition $[Cu(H_2O)_n(MeCN)_{6-n}]^{2+}$ with $n = 1 - 6$, Assuming that $CuCl_2$ Is Fully Ionized[15]



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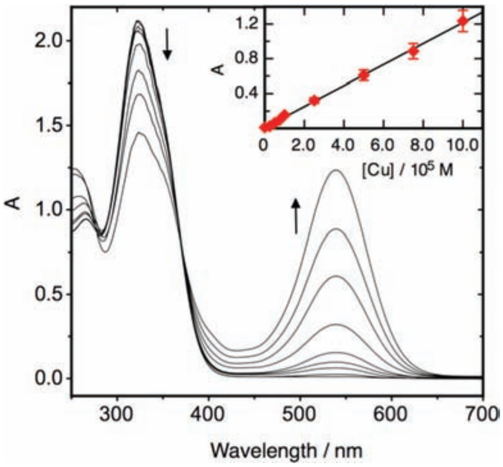

Figure 3. Absorption spectra of **2** upon addition of Cu^{2+} ($CuCl_2$) until a final concentration of 1.0, 2.5, 5.0, 7.5, 10.0, 25.0, 50.0, 75.0, and 100.0 × 10^{-6} M, from bottom to top, respectively. All spectra are taken 2 min after mixing the two components. (Inset) Lineal response of **2** (1.0 × 10^{-4} M, Tris buffer, pH 7) with increasing concentration of Cu^{2+}.

To check the formation of a radical, X-band EPR spectra of solutions containing anion **2** and Cu^{2+} were recorded in acetonitrile at −79 °C (Figure S4, Supporting Information). The initial EPR spectrum shows a signal with $g_\infty = 2.00$ and $g_{||} = 2.09$ that can be attributed to a Cu^{2+} ion with axial geometry. This signal elapses with time appearing a more isotropic new signal with $g = 2.01$ that can be unambigously ascribed to a radical species.

Further evidence obtained by electrospray ionization (ESI-MS) supports a redox reaction of Cu^{2+}.[18] In the presence of $CuCl_2$, formation of different mono- but also of dinuclear complexes of copper was evident in the mass spectra of solutions of $CuCl_2$ with **2** in H_2O, MeCN, or mixtures of both solvents (Figure S4, Supporting Information). Comparison of the measured isotope pattern with the calculated ones supports the composition of the base peak at *m/z* 355.16, which is assigned to $[Cu\mathbf{2d}(H_2O)_5]^+$ and two prominent fragments at *m/z* 253.20 and 255.23 to $[\mathbf{2b} + Na]^+$ and $[\mathbf{2c} + Na]^+$, respectively, among others.

Since the redox chromogenic event is easily visible in H_2O−MeCN mixtures, the sensitivity and selectivity of compound **2** for Cu^{2+} was evaluated by mixing aqueous samples of Cu^{2+} with **2** (1.0 × 10^{-4} M) dissolved in MeCN and diluting with water to a constant 1:1 v/v mixture of both solvents (Figure 3). Under these conditions, the lower limit for detection of Cu^{2+} is 1.0 × 10^{-6} M and the upper lineal limit is 1.0 equiv (1.0 × 10^{-4} M).

The chromogenic response of **2** upon exposure of **2** (5.0 × 10^{-5} M, Tris buffer, pH 7) to different metals (2.0 × 10^{-4} M) are depicted in Figure 4. Remarkably, at neutral pH, compound **2** displays very good selectivity for Cu^{2+} over other potentially competing metals. The pale yellowish tonality observed for Fe^{3+} is the natural color of a 20 μM solution of $FeCl_3{\cdot}6H_2O$ in MeCN−H_2O (50:50 v/v) used in the above experiment (Figure 4).

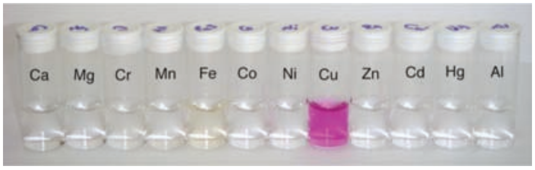

Figure 4. Visual response of **2** (5.0 × 10^{-5} M, Tris buffer, pH 6.9) upon adddition of different metal ions: Ca^{2+}, Mg^{2+}, Cr^{3+}, Mn^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Al^{3+} (2.0 × 10^{-4} M). The picture was taken 2 min after the addition of Cu^{2+}.

In summary, we set out to design a minimalist sensor for Cu^{2+} that works on the principle of chelation-induced free-radical formation. The chromogenic and selective response of compound **2** at neutral pH is very well suited for the redox chemodosimetric evaluation of nonorganically bound Cu^{2+} in natural waters.

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

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