further purification. All glassware used in the following procedures were cleaned in a bath of freshly prepared 3:1 HCl:HNO_3 (aqua regia) and rinsed thoroughly in milli-Q grade water prior to use. The Ag nanoparticles were prepared by sodium citrate reduction of $AgNO_3$:[9] $AgNO_3$ (18 mg) was dissolved in H_2O (100 mL) and brought to reflux. A solution of 1% trisodium citrate (2 ml) was added and the solution was kept at reflux for about 1 h. The final volume was adjusted to 500 mL. The Ag sols prepared were greenish yellow. The Ag plasmon absorbance maxima was at approximately 440 nm.

In a typical synthesis, as-prepared Ag sols (5 mL) was diluted with water (5 mL), then a freshly prepared plating solution of 0.02 M NH_2OH (0.10 mL) and 0.1% $HAuCl_4$ (0.25 mL) was added with stirring. The solution was then heated at reflux for about 10 min before cooling it down to room temperature. It was now ready for TEM sample preparation (sample 1). Samples 2 and 3 were prepared by adding 0.10 and 1.0 mL of a 0.1% solution of $HAuCl_4$ to the aliquot Ag sols, respectively, and then following the same procedure as for sample 1. The initial ratios of the concentration of the $HAuCl_4$ solution were 2.5:1:10 for samples 1, 2, and 3, respectively.

Samples for TEM were prepared by placing a drop of solution (for sol) on or transferring as-prepared thin film to a carbon-coated copper grid. Samples were examined by using a JEOL 2010 transmission electron microscopy operated at 200 kV. Analysis of the X-ray photoelectron spectra (XPS) was performed on a ESCLAB MKII using Mg as the exciting source.

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The First Water-Soluble Copper(I) Calix[6]arene Complex Presenting a Hydrophobic Ligand Binding Pocket: A Remarkable Model for Active Sites in Metalloenzymes**

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Because of the unique properties of water, the synthesis of biomimetic complexes that are soluble and stable under physiological conditions is an attractive goal. Paradoxically, most metalloenzyme models have been developed in organic solvents.[1] The first reason is that the ligands used to mimic the biological active site often lead to water-insoluble complexes, and their solubilization through synthetic procedures is not an easy task. For instance, tris(pyrazolyl)borate, which is the basis for countless biomimetic complexes,[2] is sensitive to hydrolysis, and hydrophilic derivatives would not survive in aqueous solvent.[3] The second reason is that biologically relevant species are often reactive to water and must be protected and/or stabilized. In the specific case of copper, few water-soluble cuprous complexes have been described,[3, 4] and to the best of our knowledge none of them can be considered biomimetic. Indeed, it is well known that Cu^I centers are thermodynamically unstable in water and disproportionate in the absence of an appropriate coordinating environment. Another major difficulty stems from the fact that metalloenzyme model complexes must present a vacant or a labile site on the metal center to allow the coordination and/or activation of a guest.

In the course of modeling type 2 active sites of copper enzymes, we have been interested in developing a supramolecular system that can stabilize a copper(I) center within a biomimetic environment.[5–7] We have now extended our previous work to demonstrate a possible route to transform the calix[6]arene derivatives soluble in organic solvents into compounds soluble in water. Here, we describe the synthesis of the first water-soluble biomimetic cuprous complex.

The selectively functionalized calix[6]arene derivative **1**[8] was first *O*-alkylated with three imidazole arms to yield compound **2** (Scheme 1).[6] The next step consisted of the *ipso* sulfonation of the three anisole rings by treating **2** with concentrated sulfuric acid. The resulting triacid **[3]** · H_3 was neutralized with sodium hydroxide, giving rise to the trisodium salt **[3]** · Na_3. This new ligand is highly soluble in water and, to a lesser extent, in methanol, ethanol, and 2-propanol.

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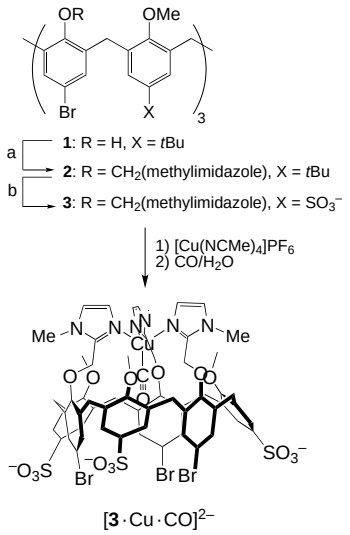

Scheme 1. Synthesis of the biomimetic water-soluble cuprous complex $[\mathbf{3}\cdot Cu\cdot CO]^{2-}$. a) 2-chloromethyl-1-methyl-1*H*-imidazole, NaH, THF, 55% yield; b) conc. H_2SO_4; aq. NaOH, 72% yield.

Its ^1H NMR spectrum attests to a very flexible molecule displaying structural interconversion (Figure 1a).

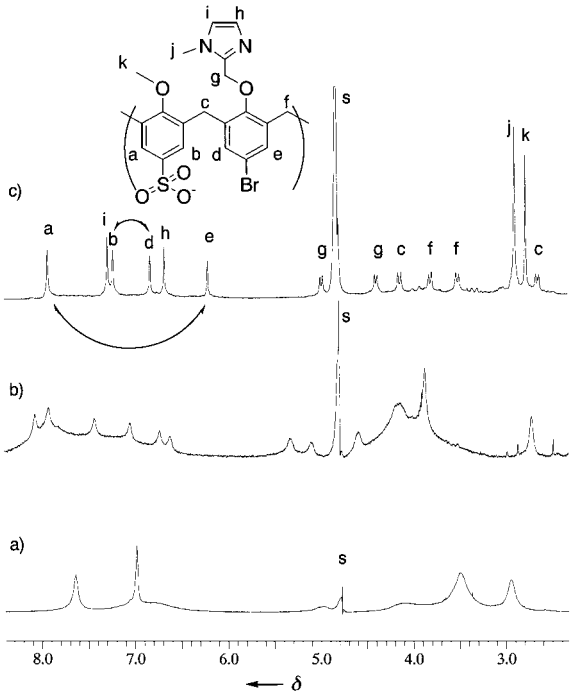

Figure 1. 500 MHz ^1H NMR spectra in D_2O: a) ligand $[\mathbf{3}]\cdot Na_3$ at 300 K, b) complex $[\mathbf{3}\cdot Cu]\cdot Na_2$ at 320 K, and c) complex $[\mathbf{3}\cdot Cu\cdot CO]\cdot Na_2$ at 300 K. Concentrations are about 10^{-3} mol L^{-1}. The signal of the undeuterated solvent was irradiated and the remaining peak is labeled "s". Important NOE effects are indicated by arrows. Labeling of the protons is indicated on the structure.

Complex $[\mathbf{3}\cdot Cu^+]\cdot Na_2$ was obtained by mixing stoichiometrically ligand $[\mathbf{3}]\cdot Na_3$ and $[Cu(NCMe)_4]PF_6$ in ethanol. This new complex, which spontaneously precipitated out of the ethanolic solution, is readily soluble in water. Like its organic-solvent soluble analogue,[5] complex $[\mathbf{3}\cdot Cu^+]\cdot Na_2$ is resistant to air autoxidation since no paramagnetic signal was detected by EPR after the solution was exposed to air for one day. The ^1H NMR spectrum in water shows very broad signals, on the top of which a set of well-defined resonances is observed (Figure 1b). The relative importance of the latter increased upon raising the temperature (from 4 to 80 °C) or upon dilution. This suggests that the broad resonances are due to an ill-defined aggregated state. In contrast, the set of relatively narrow peaks agrees with a C_3-symmetrical monomeric species, in which the cuprous ion is bound in a trigonal fashion to the three imidazole moieties of the ligand.[9] The complex reacts with carbon monoxide in either water or methanol. The subsequent solution IR spectrum, displaying an absorption at 2075 cm^{-1}, confirms the presence of CO as a fourth ligand.[10] The new adduct displays a distinct and better resolved ^1H NMR spectrum (Figure 1c). The conserved C_3 symmetry indicates that the three imidazolyl groups are still bound to the metal ion. Each proton resonance was assigned based on COSY, NOESY, HMBC, and HSQC experiments, and a proposed structure is displayed in Scheme 1.

The coordinating imidazole moieties wrap the cuprous ion in a helical fashion. As a result, the complex exists as two C_3-symmetrical enantiomers. The helicity is transmitted to the arene rings as shown by the large diastereotopic splitting of their ^1H resonances ($\Delta\delta = 0.70$ (H_a,H_b) and 0.62 (H_d,H_e), see Figure 1c). NOESY correlations were observed between each pair of aromatic protons, thereby substantiating a *cone* conformation for the calixarene structure.[11] The signals of the deshielded phenoxy protons correspond to the anisole moieties which indicates that the hydrophilic sulfonate groups are pointing outward from the cavity, toward the solvent.[12, 13] Finally, no exchange peaks between the diastereoisomeric protons were observed in the 2D experiments (NOESY and ROESY, $\tau_m = 200$ to 500 ms). Neither were the proton resonances broadened upon heating up to 350 K. This indicates that the exchange rate between the two helical enantiomeric conformations of $[\mathbf{3}\cdot Cu\cdot CO]\cdot Na_2$ is particularly slow. At a high temperature, however, slow release of CO was observed.

This novel water-soluble calixarene-based system displays some remarkable features. 1) It allows the formation of a N_3Cu^I complex with a fourth binding site that is accessible for an exogeneous ligand with a strong affinity for the metal ion such as CO. 2) In spite of this labile site and owing to its protection by the calixarene structure, the cuprous complex is resistant to disproportionation and to air autoxidation. It also shows that the coordinating environment provided by three imidazole moieties which mimics the tris(histidine) binding site often encountered among enzymes is indeed capable of stabilizing a cuprous center in water. 3) The calix[6]arene adopts a relatively rigid *cone* structure that encloses a well-defined hydrophobic cavity. This is unusual for sulfonated calix[6]arenes. Indeed, the hexasulfonated calix[6]arene derivatives have been described as highly flexible molecules with a preferred 1,2,3-*alternate* conformation.[14] In our system, the rigidity of the host results from the binding of a metal cation and the *cone* conformation is favored by the relatively smaller electrostatic repulsion on the large rim bearing only three sulfonates.

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The highly restricted mobility of the helical arrangement of the imidazolyl groups is more surprising. A tentative explanation can be proposed. First, the complex displays some large hydrophobic domains and solvation effects may increase the interconversion energy barrier. Second, electrostatic repulsion between the anionic sulfonates together with some stabilizing hydrophobic interactions between the three "*in*" *p*-bromophenyl groups may freeze the movements of the molecule. Indeed, we have already shown that, in these systems, the structural information is very efficiently conveyed from one side of the molecule to the other.[5] As a consequence, restricted mobility of the large rim may be responsible for the freezing of the imidazolyl helix of at the narrow rim.

This work demonstrates the successful transposition of an organic-solvent soluble biomimetic system into aqueous solvent. Careful functionalization of the calixarene-based ligand has allowed the retention of the most important properties of the complex: the stabilization of the cuprous center despite the presence of a free valence, the protection of the metal site in the concave area of a hydrophobic cavity, and the host behavior of the complex toward a small coordinating guest. This novel supramolecular system reproduces not only the first coordination sphere encountered in many enzymes and the frequently hydrophobic microenvironment of the active site, but also the aqueous macroenvironment of a physiological medium. We are currently exploring its behavior as a selective hydrophobic receptor[15] for a variety of guest molecules in water. Future developments may also lead to "green" catalysts.[16]

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[11] The C_3 symmetry is also compatible with an 1,3,5-*alternate* conformation. Despite the fact that this conformation has been envisaged in numerous papers, it has never been retained on the basis of structural analysis.

[12] The $\Delta\delta$ shifts for H_a vs H_d and H_b vs H_e are 1.09 and 1.01, respectively. This is much higher than the shift by ca. 0.5 expected for a simple electronic effect due to the bromo and sulfonato substituents. For *para*-substituted toluenes the *ortho* protons (relative to Br or SO_3^-) have chemical shifts of $\delta = 7.35$ ($CDCl_3$) and 7.85 (D_2O), respectively. Therefore, we can assume that the largest $\Delta\delta$ observed for the CuCO complex reflects conformational effects with H_{Ar} being in either *in* or *out* position relative to the aromatic walls of the calixarene cone (see ref. [11]).

[13] Among the *tert*-butylated organic-solvent soluble analogues, the complexes bearing three *O*-methyl groups were shown to include one of their *t*Bu moieties into the calixarene pocket which lead to a dissymetric conformation. This move is obviously disfavored by the replacement of *t*Bu groups by sulfonates. Hence, the overall properties the water-soluble CuCO complex are more reminiscent of those reported for the organic-solvent soluble parent compound bearing three *O*-ethyl substituents (see ref. [5b]). However, the relative *alternate* position of the phenoxy walls is now reversed and corresponds to that depicted for the Cu^I-nitrilo complex in ref. [5a]. This may be due to the hydrophobic effect, which pushes the methoxy groups toward the cavity away from water.

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