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A Water-Soluble Calix[4]arene-Based Ligand for the Selective Linear Coordination and Stabilization of Copper(I) Ion in Aerobic Conditions

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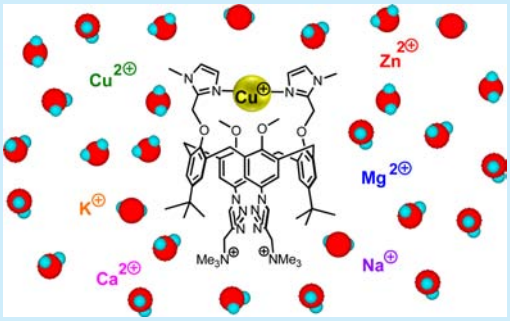
Supporting Information

ABSTRACT: A number of serious diseases are linked to copper homeostasis dysfunction. The design of copper(I)-selective chelators is of particular interest not only for the creation of therapeutic objects but also as useful tools to gain insights into the coordination of copper(I) in a biological medium. A water-soluble Cu^{I}-selective ligand that associates strong Cu^{I} binding at pH = 7.4 (10^{14} M^{-1}), insensitivity to air, and selectivity toward Cu^{II} and other biologically relevant cations is described.



Metal ions are involved in a large number of biological processes in living organisms. As an essential part of metalloproteins, they carry out catalytic, regulatory, or structural roles.[1] These essential metal ions require tight regulation systems, as they can promote cytotoxic reactions. For instance, a copper homeostasis dysfunction is responsible for several diseases, such as Menkes' syndrome (MS)[2] and Wilson's disease (WD).[3] Copper is also suspected to be involved in neurological dysfunctions, such as in Alzheimer's disease (AD).[4] In the case of AD, the formation of amyloid-β (Aβ) plaques containing redox active metal ions such as copper is suspected to be a key event in the development of the disease. Some therapeutic approaches are based on the use of specific chelators, in order to regulate the copper concentration at the defective sites.[3,5] The design and synthesis of copper chelators with high affinity and selectivity is an important issue, not only for therapy but also for analytical probes and tools for mechanism investigation. Whereas a large number of copper-(II)-selective chelators have been described in the literature,[6] relatively few examples of their copper(I)-selective counterpart can be found. Inspired by copper transporters,[7,8] selective fluorescent probes for Cu^{I} have been designed using polythioether-based ligands.[6] These were revealed to be highly selective for the soft Cu^{I} ion but display lower affinity than two- or three-thiolate based ligands.[3] Delangle et al. have designed a series of chelators based on small pseudopeptides containing cysteines that bind Cu^{I} with a remarkable affinity.[3] Quite interesting is the case of a two-cysteinate-containing small peptide, rigidified by a cyclic scaffold, that was shown to bind Cu^{I} in a linear geometry with strong selectivity vs a number of different cations.[7] The linear geometry is also adopted in Aβ peptide fragments or small peptide models, although with a different coordination sphere composed of two imidazole groups from two histidine residues (Figure 1a).[9] Two-coordinate linear Cu^{I} complexes with imidazole molecules were previously described (Figure 1b).[10] A few years later, our group published the X-ray structure of a Cu^{I} complex based on the calix[4]arene core functionalized by two imidazole arms at its small rim. The ligand (**1**) also binds Cu^{I} in a linear mode, thus mimicking the coordination sphere of the Cu^{I}-Aβ complex (Figure 1c).[11] Quite interestingly, the complex appeared unusually resistant to oxygen interaction and was stable in air for weeks in organic solvents. More recently, several other groups, following different objectives, have reported closely related calix[4]arene-based ligands able to chelate Cu^{I}.[12]

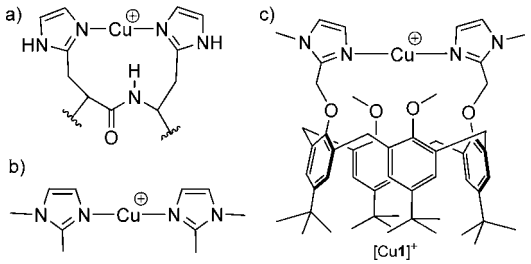


Figure 1. Schematic representations of the coordination mode of Cu^{I} as characterized with (a) Aβ peptide fragments and related small peptide,[9] (b) complex obtained with 1,2-dimethylimidazole,[10] and (c) complex obtained with the calix[4]arene-based bis-imidazole ligand **1**.[11]

Wanting to design a water-soluble ligand that was selective toward the Cu^{I} state, we thought of using the calix[4]arene bis-imidazole motif in order to take advantage of the two-coordinate linear environment that is capable of stabilizing Cu^{I},

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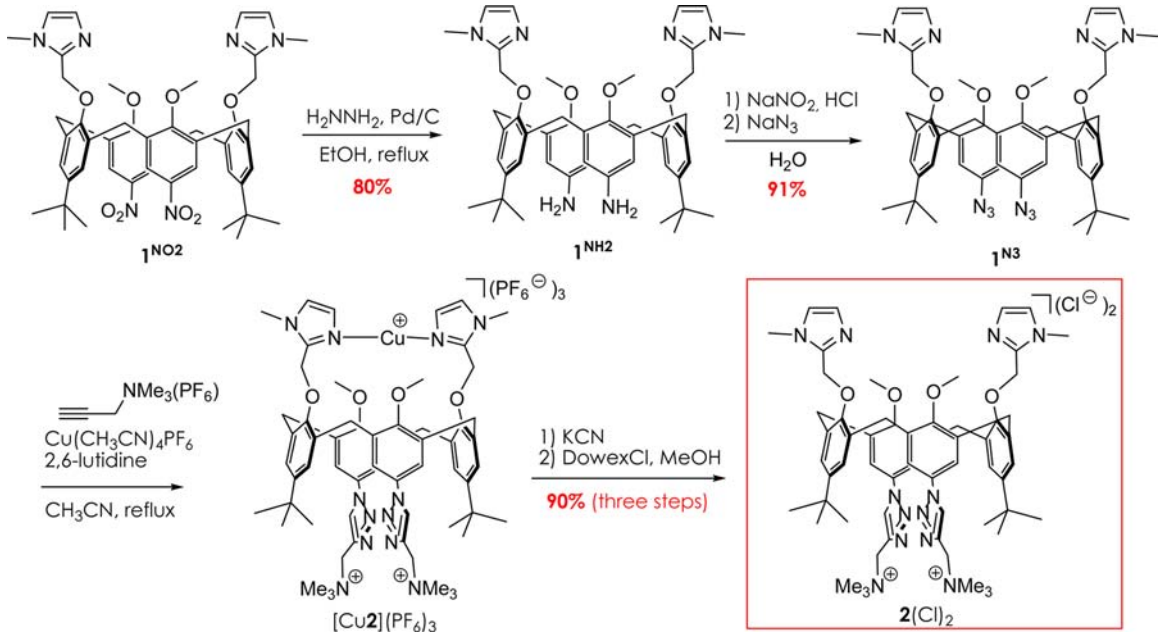
Scheme 1. Synthesis of the Water-Soluble Ligand $2(Cl)_2$[a]



[a]The broadness of the ^{1}H NMR spectra of compounds $\mathbf{1}^{X}$ and $\mathbf{2}(Cl)_2$ is likely due to the existence of several conformations in equilibrium. For clarity, all the structures are presented in a cone conformation.

whereas Cu^{II} chelation classically requires four- to five-coordinate environments.

Here we report the synthesis and binding properties of a water-soluble version of the above-mentioned ligand **1**. We show that the linear bis-imidazole binding motif allows strong coordination of Cu^{I} in water at physiological pH and prevents autoxidation as well as disproportionation reactions. Furthermore, this ligand appears to be selective vs other biologically relevant metal ions, as well as vs Cu^{II}.

Synthesis of the Water-Soluble Ligand $2(Cl)_2$. In order to obtain a water-soluble version of ligand **1**, hydrophilic substituents have to be introduced on the calixarene macrocycle. In order to perturb as little as possible the coordination properties of the bis-imidazole core at the small rim, our synthetic strategy consisted of introducing hydrophilic substituents at the opposite rim of the calix[4]arene, far away from the coordination site. For this, two key reactions were selected: first, functionalization of the large rim through *ipso*-nitration;[13] second, Cu^{I}-catalyzed Azide−Alkyne Cycloaddition methodology (CuAAC)[14] for the introduction of the hydrophilic groups. In order to ensure good water solubility for the ligand, quaternary ammonium groups were selected. Indeed, we previously experienced problems with sulfonate groups, due to charge neutralization upon coordination of metal cations, which reduced the water solubility of the corresponding complexes. Hence, the four-step synthesis of the water-soluble ligand $\mathbf{2}(Cl)_2$ from the previously reported $\mathbf{1}^{NO2}$ calixarene is described in Scheme 1.[11,13] The reduction of the two nitro groups with hydrazine in the presence of Pd/C gave the corresponding bis-anilino derivative, $\mathbf{1}^{NH2}$, in good yield. The latter was then reacted with $NaNO_2$ in acidic medium, which was followed by the addition of NaN_3 to give the bis-azido derivative, $\mathbf{1}^{N3}$. Finally, the quaternary ammonium groups were introduced through the CuAAC, using propargyl trimethylammonium in the presence of 1.5 equiv of $[Cu(CH_3CN)_4](PF_6)$ and 2,6-lutidine in acetonitrile. It is important to note that more than 1 equiv of Cu^{I} was required for the catalyzed reaction to proceed, which is due to the coordination of 1 equiv at the imidazole core, apparently leading to the deactivation of the Cu^{I} catalyst. After the addition of water, the resulting crude product was isolated by simple filtration of the reaction mixture and was identified as the PF_6 salt of the corresponding Cu^{I} complex, $[Cu\mathbf{2}](PF_6)_3$. Demetalation with potassium cyanide followed by anion metathesis ($PF_6^- \rightarrow Cl^-$) provided the water-soluble free ligand, $\mathbf{2}(Cl)_2$. The overall yield of the four-step synthesis is 65%. The solubility of the ligand in water lies in the millimolar range.

Coordination of Copper(I). The capability of ligand $\mathbf{2}(PF_6)_2$ to coordinate copper(I) in organic solvent was evidenced by the isolation of a metal complex after the CuAAC step. As its parent compound **1**, free ligand $\mathbf{2}^{2+}$ displayed a poorly defined ^{1}H NMR spectrum at room temperature (Figure 2b) that did not appreciably sharpen upon heating, either in MeCN or in water (Figure S15). This is attributable to the conformational motion that the macrocyclic

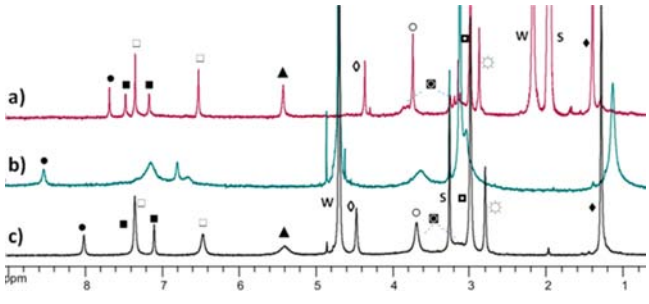


Figure 2. ^{1}H NMR (250 MHz, 300 K) spectra of (a) $[Cu\mathbf{2}](PF_6)_3$ (CD_3CN), (b) $\mathbf{2}(Cl)_2$ (D_2O), and (c) $[Cu\mathbf{2}](Cl)_3$ (D_2O). (●) H_{tria}, (□) H_{Ar}, (■) H_{Im}, (□) H_{Artria}, (▲) OCH_2, (◊) CH_2Me_3, (○) NCH_3, (◙) $ArCH_2$, (◘) $N(CH_3)_3$, (☼) OCH_3, (◆) *t*Bu. w = water, s = solvent.

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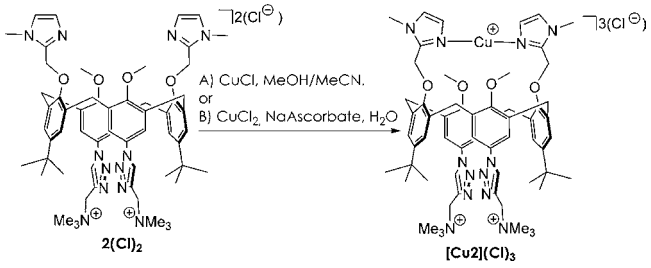
structure undergoes at the time scale of the ^{1}H NMR spectroscopy. In contrast, the ^{1}H NMR signature of its isolated Cu^I complex (Figure 2a) displays sharp resonances in CD_3CN, which were attributed by 2D NMR experiments (COSY, HMBC, HSQC). This is due to the rigidification of the calixarene core upon coordination of the metal ion to the two imidazole arms. The complex exhibits C_{2v} symmetry, consistent with a cone conformation of the calixarene, which was further confirmed by a ROESY experiment (Figure S9). The imidazole resonances are split and shifted, which attests to their coordination. Likewise, the OCH_2 peak is downfield shifted. The resonances of the aromatic protons are well separated (by ca. 1.8 ppm), which indicates a pinched conformation. Finally, the triazole protons, which appear at ca. 8.7 ppm for the free ligand, undergo an upfield shift of more than 1 ppm. They are initially in the endo position relative to the *t*Bu groups and are projected in the exo position once the imidazole groups are bound to the metal ion. This spectrum is actually very similar to that of the parent complex $[Cu\mathbf{1}](PF_6)$ (see Figure 1) and is fully consistent with the corresponding crystal structure previously reported.

The coordination properties of ligand $\mathbf{2}(Cl)_2$ were then investigated in water. First, complex $[Cu\mathbf{2}](Cl)_3$ was synthesized by reacting a stoichiometric amount of CuCl with ligand $\mathbf{2}(Cl)_2$ in a MeOH/MeCN mixture, in order to ensure good solubility of both reactants (Scheme 2). The cuprous

Scheme 2. Synthesis of the Cu(I) Complex $[Cu\mathbf{2}](Cl)_3$



complex was quantitatively isolated by precipitation with diethyl ether and characterized by ^{1}H NMR and ESI HRMS. The complex is soluble in water, and its ^{1}H NMR spectrum exhibits well-defined resonances (Figure 2c) that are very similar to those recorded in CH_3CN for the corresponding PF_6 salt (Figure 2a). This indicates that Cu^I is bound in the same environment and that the nature of the solvent does not significantly influence the structure and conformational mobility of the Cu^I complexes. The trimethylammonium groups at the large rim do not interfere with the coordination site of the ligand, meaning the triazole spacer is well adapted[15] and prevents any electrostatic repulsion between the cationic groups which could have jeopardized the coordinating ability of ligand $\mathbf{2}^{2+}$. The copper(I) complex could also be generated in situ by the addition of 1 equiv of $CuCl_2$ and 0.5 equiv of sodium ascorbate as a reductant. The formation of the Cu(I) complex is attested by the ^{1}H NMR obtained which is superimposable with the isolated complex (Figure S16). The formation of $[Cu\mathbf{2}](Cl)_3$ takes place within the time necessary for mixing and recording the spectrum (<3 min). Finally, the Cu^I complex was found to be very stable both in the solid state and in solution, even when exposed for several days to air (Figure S17).

The affinity constant of ligand $\mathbf{2}(Cl)_2$ for Cu^I was determined in buffered water (HEPES buffer, 20 mM) at physiological pH = 7.4, by spectrophotometric competition titrations with bathocuproine disulfonate (BCS, see the Supporting Information (SI)). Such a method has been outlined in the literature.[16] BCS forms a stable red Cu^I complex (λ_{max} = 483 nm, ε = 13 300 M^{-1} cm^{-1}), with a 1:2 stoichiometry and an affinity constant $\beta_2 = 10^{19.8}$ according to eq 1.[17]

$$Cu^+ + 2BCS^{2-} \leftrightarrows [Cu(BCS)_2]^{3-} \quad \beta_2 = \frac{[Cu(BCS)_2{}^{3-}]}{[Cu][BCS^{2-}]^2} \tag{1}$$

Ligand $\mathbf{2}^{2+}$ binds Cu^I with a 1:1 stoichiometry. Its competition with BCS is expressed by eq 2, from which the apparent affinity constant of ligand $\mathbf{2}^{2+}$ for Cu^I, K'_a, can be calculated according to eq 3.

$$[Cu\mathbf{2}]^{3+} + 2BCS^{2-} \leftrightarrows \mathbf{2}^{2+} + [Cu(BCS)_2]^{3-}$$

$$K' = \frac{[\mathbf{2}^{2+}][Cu(BCS)_2{}^{3-}]}{[(Cu\mathbf{2})^{3+}][BCS^{2-}]^2} \tag{2}$$

$$\mathbf{2}^{2+} + Cu^+ \leftrightarrows [Cu\mathbf{2}]^{3+} \quad K'_a = \frac{[(Cu\mathbf{2})^{3+}]}{[[\mathbf{2}^{2+}][Cu]]} = \frac{\beta_2}{K'} \tag{3}$$

As $[Cu\mathbf{2}]^{3+}$ does not absorb at 483 nm, measurement of the absorbance at this wavelength is directly proportional to the total concentration of $[Cu(BCS)_2]^{3-}$ in the solution and thus allows for the determination of the equilibrium constant K'. Two different titration experiments were conducted: one by addition of BCS to a solution of complex $[Cu\mathbf{2}](Cl)_3$, the other by addition of ligand $\mathbf{2}(Cl)_2$ to a solution of complex $[Cu(BCS)_2]^{3-}$. It is worth noting that in each case, and in between each addition, spectra were collected until two consecutive spectra were superimposable, attesting to the equilibration of the system, which generally required at least 60 min. Both procedures gave similar K' values (see the SI), the average of which is $K' = (2.1 \pm 0.9) \times 10^5$ M^{-1}, yielding an apparent stability constant $K'_a = (3.12 \pm 0.14) \times 10^{14}$ M^{-1} at pH 7.4.

The selectivity of ligand $\mathbf{2}^{2+}$ for Cu^I vs other biologically relevant metal ions was qualitatively assessed by ^{1}H NMR spectroscopy. The addition of 1 equiv of Na^+, K^+, Mg^{2+}, Ca^{2+}, or Zn^{2+} to a D_2O solution containing the ligand did not change the ill-defined spectrum. In another experiment, the addition of these metal salts into a mM solution of the complex in water was monitored by ^{1}H NMR. In no case did these additions (up to 250 equiv) affect the NMR spectrum of the complex (see SI). With the paramagnetic Cu^{II} ion, up to 5 equiv were added without perturbation of the spectrum, after which broadening of the resonances due to its paramagnetism prevented any data analysis. By UV−vis titration of, or with, $CuSO_4$, no change in the d−d absorption was observed. Consistent with this observation, cyclic voltammetry experiments with the Cu^I complex of $\mathbf{2}^{2+}$ showed an irreversible redox process, suggesting the rapid dissociation of the copper ion upon oxidation (Figures S22−S24).

In conclusion, we have described here the successful water solubilization of a calix[4]arene-based ligand, while preserving its remarkable binding properties for Cu^I. The corresponding complex, $[Cu\mathbf{2}](Cl)_3$, is soluble in water at millimolar concentration and stable in air, at physiological pH, for days. Such resistance to autoxidation and disproportionation is quite

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exceptional in view of the coordinative unsaturation displayed by the metal ion that is bound to only two donors, which furthermore are neutral. Such inertness was previously reported for its organo-soluble analog, [Cu**1**](PF$_6$), in organic solvents and can be attributed to the good preorganization of the bidentate ligand that favors coordination in a linear fashion. Indeed, it has been observed for other bis(imidazole) donors, based on peptides for example, that such a linear environment can deactivate Cu^{I} for CO binding and/or a dioxygen redox reaction.[9b,11] One condition however appears to be that the system is rigid enough to disfavor bending of the coordination bonds, which would open a reactive site and allow interaction with a third donor. Quite impressively, the apparent stability constant that is higher than most polythioethers, even the cyclic ones.[3,6,18] Only 2- and 3-cysteine based ligands appear to be stronger chelates with *K* values in the 10^{15}−10^{16} M^{-1} range.[4,9a] Hence, as a neutral donor, the imidazole group appears better than thioether at stabilizing Cu^{I}, but not as strong a donor as the thiolate anion. These results also show that when good preorganization is provided, two histidine residues may indeed also offer a strong binding site for Cu^{I}, which may be relevant to neurological diseases such as AD. Of additional importance, ligand **2**(Cl)$_2$ offers a binding motif that is different from those of other chelating agents, ensuring Cu^{I} coordination in a 1:1 stoichiometry, which stands in contrast to classical bidentate donors presenting a bent bidentate site, thus leading to tetrahedral complexes with a 1:2 stoichiometry. It is complementary to the above-mentioned sulfur-based chelators in terms of strength, thus providing a new tool for biological studies relative to Cu^{I}. They also offer the possibility to work under air, which is not possible with thiolates in view of their high oxidizability. Finally, the calixarene motif is a highly functionalizable platform that can be chemically modified to gain further interesting properties. Hence, this ligand design may find echoes for various applications and be a good candidate for the design of selective probes or in chelation therapy. We are currently exploring these possibilities.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, characterization, and spectral data for all the compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

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Notes

The authors declare no competing financial interest.

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