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# Metal-Containing Trifurcate Receptor that Recognizes and Senses Citrate in Water

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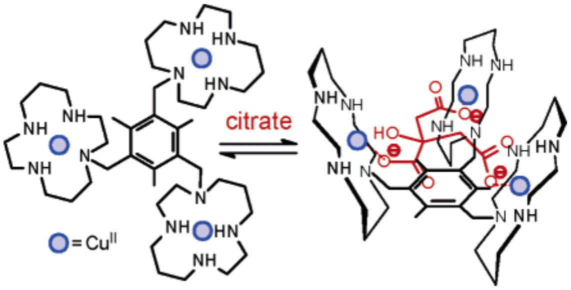
## ABSTRACT



**The binding tendencies toward carboxylates of a trifurcated receptor containing three copper(II)–cyclam subunits have been investigated in pure water, through the displacement of a fluorescent indicator. The receptor is tailor-made for the recognition of tricarboxylates, e.g., citrate, whose three negatively charged oxygen atoms interact with the three coordinatively unsaturated Cu$^{II}$ centers.**

Artificial receptors for anions can be neutral or positively charged. Neutral systems typically contain N−H fragments (from amides,[1] sulfonamides,[2] ureas,[3] pyrroles),[4] which establish directional hydrogen bonding interactions with oxoanions and fluoride: in most cases, these receptors cannot compete with water as H-bond donors, and their use is confined to aprotic solvents such as $CHCl_3$, MeCN, DMSO, a feature that limits their analytical applications. Positively charged receptors may contain ammonium,[5] alkylammonium,[6] pyridinium,[7] guanidinium,[8] and imidazolium[9] groups, which have been strategically placed within a receptor's cavity, to satisfy the geometrical requirements of the anion (whether spherical, linear, triangular, tetrahedral, etc.).[10] The electrostatic interactions established between receptor and anion may be strong enough to compensate dehydration terms, thus allowing the occurrence of the recognition process in pure water or aqueous mixtures. On the other hand, we have reported a number of receptors containing coordinatively unsaturated metal ions (mainly Cu$^{II}$), which are capable of selective interactions with anions and ionizable analytes, including amino acids, in water.[11] The high energy

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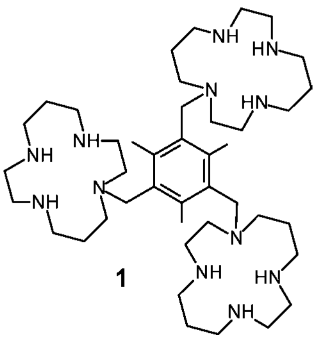
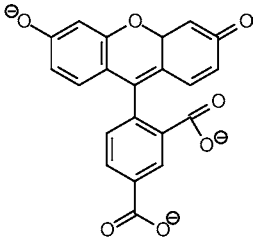
and the directional nature of the metal−ligand interaction have provided to the recognition process new and useful elements of selectivity. For instance, in a recent example, we have described the selective inclusion of dicarboxylates into a dicopper(II) bis-tren cryptate:[12] the dimetallic receptor was able to recognize the length of both aromatic and aliphatic dicarboxylates, a feature that allowed specific recognition and sensing of glutamate in the presence of a variety of anionic neurotransmitters.[13]



**1**

In the context of extending the metal−ligand approach to the recognition of tricarboxylates, we have considered the design of a receptor containing three unsaturated $Cu^{II}$ centers. In particular, we synthesized system **1**, in which three cyclam subunits have been implanted on a 1,3,5-mesityl platform. Then, the corresponding tricopper(II) complex was synthesized through reaction of **1** with $Cu^{II}(CF_3SO_3)_2$. Copper(II) complexes with cyclam derivatives tend to coordinate one $X^-$ anion, giving a stable five-coordinate species of square pyramidal geometry, in which $X^-$ occupies the apical position.[14] Very conveniently, the $Cu^{II}$ ion cannot be removed from the cyclam ring by any competing ligand, due to the kinetic macrocyclic effect.[15] Such a feature ensures the stability of the $[Cu^{II}_3(\mathbf{1})]^{6+}$ receptor even in the presence of a huge excess of anionic analyte. On the other hand, the 1,3,5-trialkylbenzene scaffold, when appropriately functionalized, favors the formation of a bowl (or "flytrap"),[16] suitable for the inclusion of anions. In particular, such a platform had been previously functionalized with three arms containing guanidinium groups, to generate a tricationic receptor, which formed a stable complex with the citrate anion in water ($\log K_{ass} = 3.8$).[17] Affinity toward citrate has been recently increased through the use of side chains containing the guanidiumcarbonyl pyrrole functionality ($\log K_{ass} = 4.9$ at pH = 7).[18] We have now found that the sexicationic receptor $[Cu^{II}_3(\mathbf{1})]^{6+}$, in an aqueous solution buffered at neutral pH, forms with citrate a 1:1 complex with $\log K_{ass} = 5.59 \pm 0.01$, thus becoming the most efficient artificial receptor for the binding of citrate in water reported thus far.

The interaction of $[Cu^{II}_3(\mathbf{1})]^{6+}$ with carboxylates was investigated by taking advantage of the fluorescent indicator displacement paradigm.[19] According to this approach, the receptor does not bind too strongly and fully quenches the emission of an anionic fluorescent indicator. Then, in a titration experiment, the envisaged anion displaces from the receptor's cavity the indicator, which can release its natural fluorescence, thus signaling through an off/on mechanism the occurrence of the recognition process.[20]



**2**

As a fluorescent indicator, we chose 5-carboxy-fluorescein (**2**), which possesses two carboxylate groups and a phenolate oxygen atom, capable of interacting with the $Cu^{II}$ centers of the receptor. **2** is strongly fluorescent in its anionic form, which is the dominating species in an aqueous solution adjusted to pH = 7. Complete quenching of the 5-carboxy-fluorescein emission was observed ($\lambda_{exc} = 450$ nm, $\lambda_{em} = 517$ nm) on titrating a degassed solution of the indicator ($5 \times 10^{-7}$ M), buffered to pH 7 (HEPES 0.01 M), with a standard solution of $[Cu^{II}_3(\mathbf{1})]^{6+}$ (see in Figure 1 the family of spectra recorded over the course of the titration).

Curve fitting of the titration profile (fluorescent intensity vs concentration of $[Cu^{II}_3(\mathbf{1})]^{6+}$, see inset of Figure 1) by a nonlinear least-squares procedure was consistent with the formation of a 1:1 receptor−indicator complex, with an association constant $\log K_{in} = 5.78 \pm 0.01$. Quenching is ascribed to the occurrence of an intramolecular electron or energy transfer process that involves the photoexcited fluorescein subunit and the paramagnetic $Cu^{II}$ center within the complex. The chemosensing ensemble consisted of an aqueous solution $2 \times 10^{-5}$ M in $[Cu^{II}_3(\mathbf{1})]^{6+}$ and $5 \times 10^{-7}$ M in **2**, and buffered to pH 7 with HEPES (0.01 M). Then, such a solution was titrated with an aqueous solution of 1,3,5-benzene-tricarboxylate, **3**, whose progressive addi-

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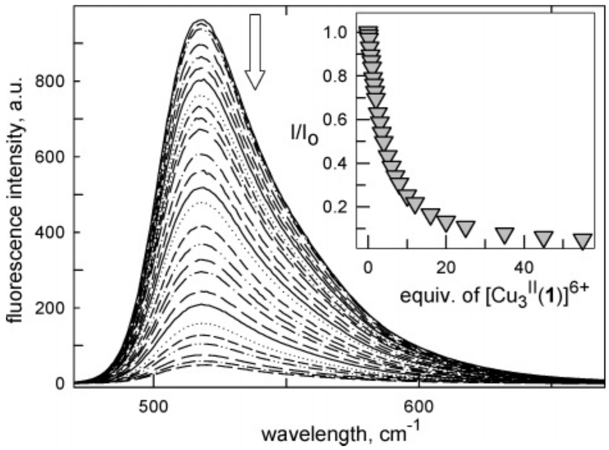
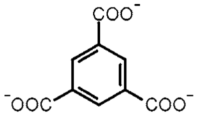
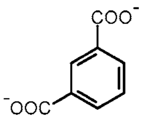


**Figure 1.** Emission spectra taken on titration of an aqueous solution of 5-carboxy-fluorescein ($5.0 \times 10^{-7}$ M), buffered at pH 7 with HEPES 0.01 M, with an aqueous solution of $[\mathrm{Cu^{II}}_3(\mathbf{1})]^{6+}$ ($\lambda_{\mathrm{exc}} = 450$ nm,).

tion induced a complete recovery of the 5-carboxy-fluorescein emission.



**3**



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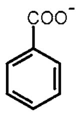

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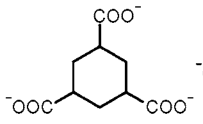

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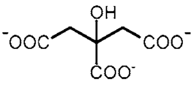
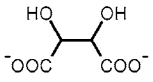
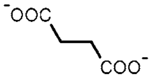


**7**

**8**



**9**



**10**

The corresponding titration profile (fluorescent intensity vs anion equiv) is shown in Figure 2. This result indicates the successful competitive binding of the $[\mathrm{C_6H_3(COO)_3}]^{3-}$ anion and displacement of the indicator from the host cavity.

On treatment of titration data with the HYPERQUAD program,[21] using the previously determined value of $K_{\mathrm{in}}$, a log $K_{\mathrm{ass}} = 5.81 \pm 0.01$ was calculated for the 1:1 complex between $[\mathrm{Cu^{II}}_3(\mathbf{1})]^{6+}$ and $[\mathrm{C_6H_3(COO)_3}]^{3-}$. Such a high value of the association constant seems to reflect the geometrical complementarity of the $[\mathrm{Cu^{II}}_3(\mathbf{1})]^{6+}$ receptor and the $[\mathrm{C_6H_3(COO)_3}]^{3-}$ anion, both possessing a $C_{3v}$ symmetry. In particular, it is suggested that the negatively charged oxygen atom of each carboxylate group establishes a coordinative interaction with the copper(II) center of each metallocyclam subunit. It has to be noted that the bidentate anion 1,3-benzenedicarboxylate (isophthalate, **4**) and the monodentate benzoate (**5**) gave much smoother titration profiles (see Figure 2), to which much smaller values of log $K_{\mathrm{ass}}$ correspond (<3), a feature that demonstrates the natural affinity of the trifurcate $[\mathrm{Cu^{II}}_3(\mathbf{1})]^{6+}$ receptor for triangular, triply negatively charged anions. The other triangular anion *cis,cis*-1,3,5-cyclohexanetricarboxylate (**6**) formed with $[\mathrm{Cu^{II}}_3(\mathbf{1})]^{6+}$ a complex ca. 6-fold less stable (log $K_{\mathrm{ass}} = 5.00 \pm 0.01$, see the smoother titration profile in Figure 2). Notice that also the trifurcate receptor containing guanidinium-carbonyl-pyrrole arms showed a distinctly higher affinity for **3** than for **6**, a feature that was ascribed to the occurrence of an additional $\pi-\pi$ interaction between the aromatic rings of the receptor and of anion **3**.[18]

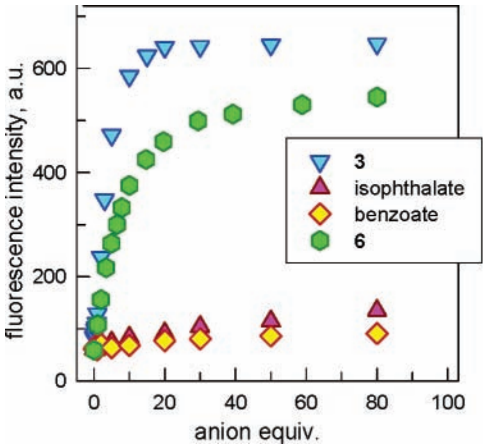


**Figure 2.** Profiles obtained over the course of the titration with a given anion of an aqueous solution $2 \times 10^{-5}$ M in $[\mathrm{Cu^{II}}_3(\mathbf{1})]^{6+}$ and $5 \times 10^{-7}$ M in **2**, and buffered to pH 7 with HEPES (0.01 M).

Most interestingly, receptor $[\mathrm{Cu^{II}}_3(\mathbf{1})]^{6+}$ forms a very stable 1:1 complex with the tricationic anion citrate (**7**, log $K_{\mathrm{ass}} = 5.59 \pm 0.01$, see titration profile in Figure 3), whose three carboxylate groups can coordinate the three $\mathrm{Cu^{II}}$ centers. Analogous titration experiments were carried out on a variety of dicarboxylates, none of which could compete with citrate, as shown by the smoother titration profiles reported in Figure 3. This is most likely due to the fact that dicarboxylates can interact with only two of the three available copper(II) centers. However, affinity toward $[\mathrm{Cu^{II}}_3(\mathbf{1})]^{6+}$ varies significantly with the nature of the dicarboxylate anion. In particular, maleate (**8**) forms a fairly stable complex with $[\mathrm{Cu^{II}}_3(\mathbf{1})]^{6+}$ (log $K_{\mathrm{ass}} = 4.5 \pm 0.1$), more stable than tartrate (**9**, log $K_{\mathrm{ass}} = 4.1 \pm 0.1$) and succinate (**10**, log $K_{\mathrm{ass}} = 3.8 \pm 0.1$). Notice that in the three anions **8**−**10**, the two COO− groups are separated by the same number of carbon atoms, thus providing a similar bite length for encompassing the metallocyclam subunits of two arms: the observed affinity sequence (**8** > **9** > **10**) seems to reflect the decrease of the rigidity of the anion framework and the consequent increasing loss of conformational entropy, which occurs on complex formation. Very poor affinity toward $[\mathrm{Cu^{II}}_3(\mathbf{1})]^{6+}$ is displayed by the spherical chloride and by the trigonal nitrate, for which $K_{\mathrm{ass}}$ values $<10^3$ could be estimated.

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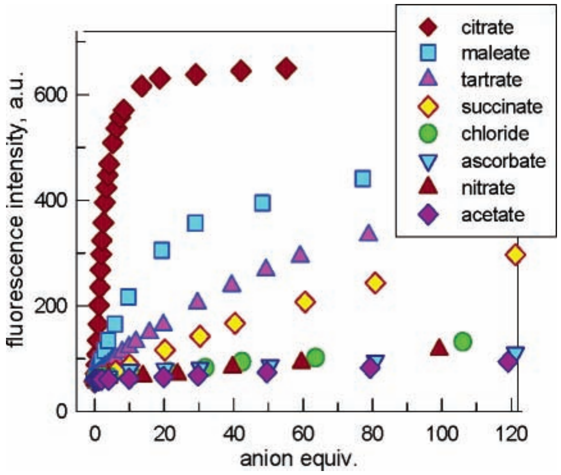

**Figure 3.** Profiles obtained over the course of the titration with a given anion of an aqueous solution $2 \times 10^{-5}$ M in $[\mathrm{Cu^{II}}_3(\mathbf{1})]^{6+}$ and $5 \times 10^{-7}$ M in **2**, and buffered to pH 7 with HEPES (0.01 M).

Indeed, $NO_3^-$ possesses a symmetry complementary to that of the investigated trimetallic receptor. However, $NO_3^-$ itself is a poorly coordinating ligand for 3d metal ions, also when acting as a monodentate. Other unfavorable factors include the low electrical charge formally available on each oxygen atom (−1/3) and the small size, which would not fit the large opening offered by $[\mathrm{Cu^{II}}_3(\mathbf{1})]^{6+}$. In particular, such a cavity is tailor-made for the much larger 1,3,5-benzene-tricarboxylate ion. Values of association constants $K_{ass}$ for all the investigated anions are reported in Table 1.

In conclusion, it has been further demonstrated that, due to the relatively high intensity of coordinative interactions, receptors containing transition metal ions ensure selective anion recognition in pure water. In particular, the metallocyclam subunit appears especially convenient, as (i) it is coordinatively unsaturated and leaves room for the binding of one anion donor atom, without any particular steric constraint, and (ii) it is kinetically inert and cannot be demetalated even in the presence of a huge excess of substrate.

**Table 1.**

| anion, $X^{n-}$ | log $K_{ass}$[a] |
|---|---|
| citrate, **7** | 5.59(1)[b] |
| **3** | 5.81(1)[b] |
| **6** | 5.00(1)[b] |
| maleate, **8** | 4.5(1)[c] |
| tartrate, **9** | 4.1(1)[c] |
| succinate, **10** | 3.8(1)[c] |
| isophthalate (**4**), ascorbate, $C_6H_5COO^-$, $CH_3COO^-$, $Cl^-$, $NO_3^-$ | <3 |

[a] $K_{ass}$ is the apparent constant for the equilibrium: $[\mathrm{Cu^{II}}_3(\mathbf{1})]^{6+} + X^{n-} \leftrightharpoons [\mathrm{Cu^{II}}_3(\mathbf{1})\cdots X]^{(6-n)+}$, in aqueous solution, buffered at pH 7, at 25 °C; the uncertainty on the last value is shown in parentheses. [b] Calculated through the HYPERQUAD program.[21] [c] Determined by a standard algorithm for competitive binding in the presence of an anion excess using the previously determined value of $K_{in}$.[22]

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**Supporting Information Available:** Syntheses of **1** and of $[\mathrm{Cu^{II}}_3(\mathbf{1})](CF_3SO_3)_6$ and selected emission spectra taken over the course of titration experiments with anions. This material is available free of charge via the Internet at http://pubs.acs.org.

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