Selective EPR Detection of Primary Amines in Water with a Calix[6]azacryptand-Based Copper(II) Funnel Complex

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S *Supporting Information*

ABSTRACT: A water-soluble calix[6]arene-based azacryptand was synthesized. The corresponding tren [tris(2-aminoethyl)-amine] cap grafted at the small rim coordinates strongly a copper(II) ion over a wide range of pH. The host–guest properties of the complex were explored by EPR spectroscopy. Due to second coordination sphere effects and the hydrophobic effect ascribed to the calixarene cavity, this funnel complex selectively binds neutral molecules (alcohols, nitriles, amines) versus anions in water near physiological pH. Among the coordinating guests, hydrophobic primary amines are preferentially recognized thanks to the combined effect of the better metal–ligand interaction and hydrogen bonding to the oxygen atoms present at the small rim. Hence, this Cu(II) calix[6]arene-based funnel complex behaves as a sensitive and selective EPR probe for primary amines, including biologically important molecules such as tyramine and tryptamine, in water, over a large pH window.
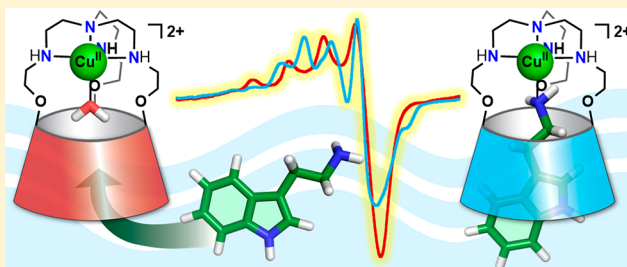
INTRODUCTION

The design of a molecular receptor for obtaining probes or sensors that are specific of one molecular target is an important field of research. This is particularly true in biology, where new tools are needed in order to get qualitative and quantitative information for understanding a mechanism or for diagnostics. For this purpose, water-soluble molecular receptors are required for the specific detection of biologically active compounds. In that field, biological amines are important targets, as they are correlated to various diseases.[1,2] Primary alkylamines are also widely used in industry[3] in spite of their toxicity for the environment and aquatic organisms.[4] To play the role of a probe, these receptors must be equipped with a reporting tool. Classically, fluorescence or luminescence are chosen due to their sensitivity.[5,6] However, they suffer several limitations such as bleaching or sensitivity to the environment and difficult quantification without internal reference. Their use is also restricted by the penetration of photons. Electrochemical probes is another strategy, but it is limited as well by the intrinsic response to the contact with the electrode. Besides host–guest studies involving nitroxide spin probes,[7] very little has been reported on EPR as a detection tool.[8] This technique

offers several advantages: when based on the intrinsic response of a stable metal ion such as Cu^{II}, it may allow ex and in vivo monitoring and be little affected by the biological environment, provided it is not reduced, i.e. has a low redox potential. This is particularly important in cells, where Fe^{III} presents a very distinct signal. Nevertheless, it has to be very specific, particularly vs anions that classically bind preferentially to the metal ion over the targeted molecules such as amines. It also must overpass severe obstacles: the water solvent is a good competitive ligand and readily provides hydroxide and protonates amines.

Over the last few years, following a biomimetic strategy, we have developed molecular receptors that associate the coordination properties of a metal ion in a well-defined environment to the hosting properties of a cavity (Figure 1).[9]

The biomimetism stems from the nitrogenous core grafted at one rim of the macrocycle that is open to the solvent for guest-binding in a confined environment. It also allows controlling
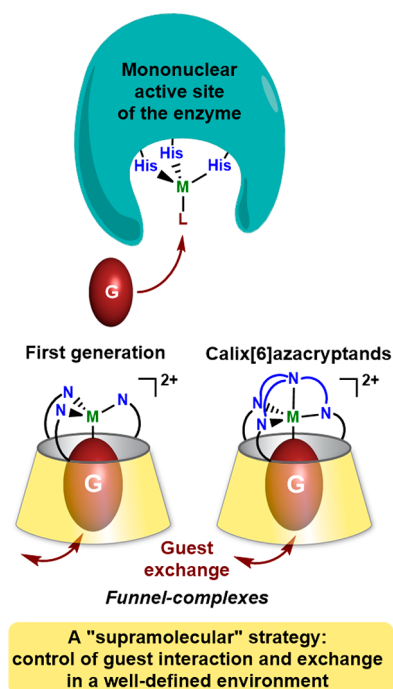
Figure 1. Funnel-shaped biomimetic molecular receptors (M = Zn, Cu).

the first coordination sphere of the metal ion and the guest exchange process. The selectivity of the receptor is based on the relative affinity of the guest for (i) the metal ion, (ii) the second coordination sphere, and (iii) the shape and size of the macrocycle. The latter point appears crucial for recognition. With calix[6]arene presenting a coordination core at the small rim, there is only space for one guest binding inside the cavity and the oxygen-rich small rim surrounding the coordination site makes it prone at hosting hydrogen-bond donors. As a result, the calix system displays very high affinity for primary amines and disfavors anion coordination. Indeed, primary amines fit very well in the cone. They are excellent donors, bind strongly to the metal ion, and have the benefit of two strong hydrogen bonds at the small rim (Figure 2).[10]

Our first attempt to design a water-soluble version of such a molecular receptor was conducted with a calix[6]arene-tris(imidazole) derivative. The strategy for the water-solubilization consists in replacing three lipophilic *t*Bu substituents at the large rim of the calix by three hydrophilic cationic "legs" (denoted as X in Figure 2). Quite remarkably, it was shown that the corresponding tricationic ligand binds Zn[II] and a hydro-

phobic primary amine synergistically at physiological pH in spite of the protonated state of free amines at this pH.[11] However, this system suffers a relatively low sensitivity, which is due to the moderate binding constant of the metal ion in the competitive water medium. Therefore, we turned to develop funnel complexes that display much stronger binding constants to metal ions, namely the calix[6]azacryptands (Figure 1). These hosts present a calix covalently capped by a *N*-tripod, which presents two merits: it preorganizes the metal ion binding site through a strong chelate effect and constrains the calix into a cone, well-shaped for guest inclusion. As a result, the metal coordination is much stronger, which is important in water, and the guest affinity much higher, which is important for sensitivity. The first water-soluble calix[6]azacryptand was the one equipped with a tmpa [tmpa: tris(2-pyridylmethyl)-amine] cap and three quaternary ammonium (-NMe₃⁺) directly grafted at the aromatic cores.[12] However, host−guest studies revealed very different properties as compared to calix[6]arene-tris(imidazole): strong affinity for anions and low affinity for primary amines.[13] The reason for such a difference stems from the different calix conformation. With the flexible tris-(imidazole) system, the phenoxyl units connected to the *N*-donors are projected toward the center of the cavity. As a result, the corresponding oxygen lone pairs are well oriented for H-bonding to guest primary amines and are repulsive for anions (Figure 3).[14] With the rigid tmpa cap, the calix adopts the opposite conformation: the phenoxyl units connected to the *N*-donor site are pushed into the exo position, directing their lone pair toward the solvent. H-Bonding to the guest is much less favored, and electrostatic repulsion with anionic donors is much lower (Figure 3).

We thus turned to the calix[6]tren system,[15−17] where a tren unit [tren: tris(2-aminoethyl)amine] covalently caps the small rim of the calixarene. Indeed, the tren cap is much more flexible than the tmpa cap, and as a result, the corresponding metal complexes adopt the same relative conformation as the one adopted by the tris(imidazole)-based complexes and are thus reluctant to bind anions (Figure 3). On the one hand, we reported the successful grafting of the corresponding Cu[II] complex onto an electrode and showed that it could be used as a selective electrochemical probe for hydrophobic primary amines in water. One limitation, however, is that it is effective only at basic pH (8.5).[18] On the other hand, we showed that, when incorporated into micelles, the calix[6]tren-based Zn[II] complex maintains its hosting properties toward primary amines at physiological pH.[19] The next step was then to obtain a soluble version of this ligand, which is presented in this
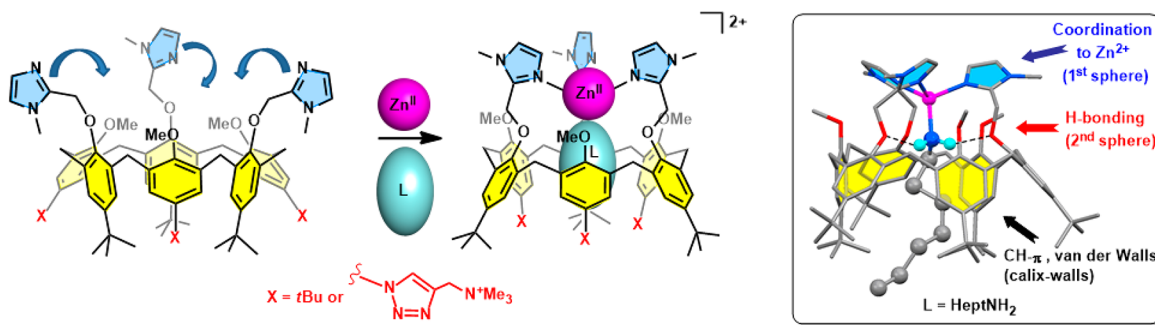

Figure 2. Recognition of a heptylamine by a calix[6]arene-tris(imidazole) zinc complex (first generation of funnel complexes). Inset: X-ray structure of the Zn-complex hosting heptylamine.
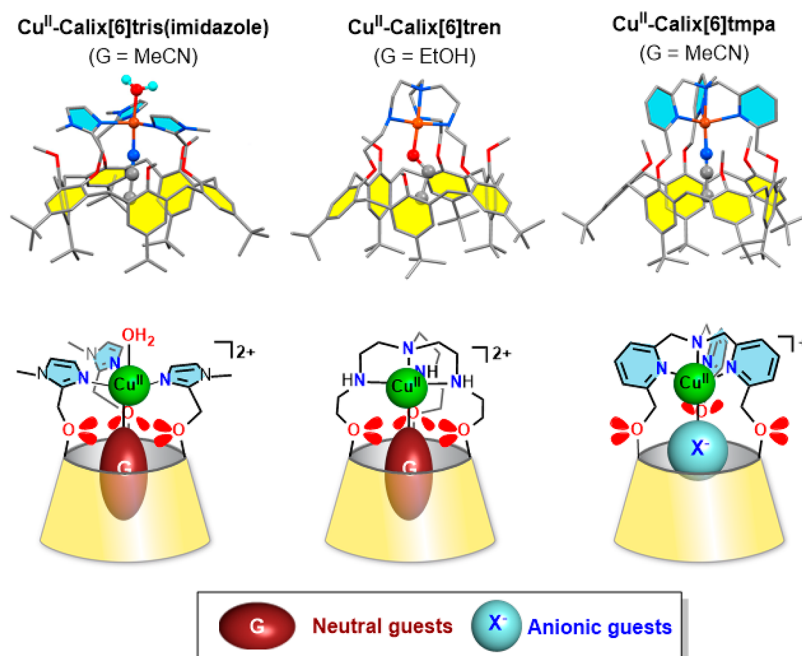
Figure 3. Top: X-ray structures of representative Cu^{II} complexes. Bottom: second sphere effect on the recognition of the guest.

article. Here, we show that the corresponding Cu^{II} complex behaves as an efficient EPR probe for hydrophobic primary amines at physiological pH.

■ RESULTS AND DISCUSSION

Synthesis and characterization of the water-soluble ligand $(4)(Cl)_3$. The water-soluble ligand $(4)(Cl)_3$ was synthesized in seven steps from the parent calix[6]tren 1[15] (Scheme 1). As previously described, the key azido intermediate 2 was first obtained through the selective nitration

Scheme 1. Synthesis of Water-Soluble Calixarene $(4)(Cl)_3$ and Its Copper(II) Complex $[Cu·4](Cl)_5$.
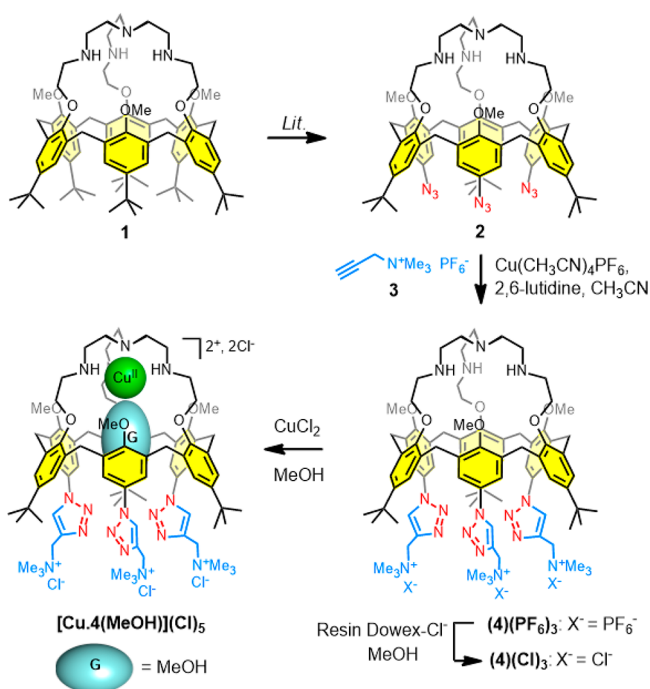
of the acetyl N-protected calix[6]tren followed by a reduction–deprotection–diazotation sequence.[20] Calixarene 2 was thus treated with alkyne N,N,N-trimethylprop-2-yn-1-ammonium hexafluorophosphate 3 in the presence of a Cu^I catalyst and 2,6-lutidine in acetonitrile. Note that more than 1 equiv of the copper catalyst is necessary for the reaction to occur since 1 equiv of the Cu^I salt is deactivated by complexation at the level of the tren cap. Demetalation of the copper complex was then achieved through addition of potassium cyanide, and subsequent addition of aqueous $NaPF_6$ led to the precipitation of $(4)(PF_6)_3$.[21] This later was characterized by electrospray mass spectrometry (ES-MS) and infrared spectroscopy (IR).[22] In particular, the absence of the N_3 band at 2100 cm^{-1} in the IR spectrum and the intense peak at m/z = 889.3 corresponding to the dicationic species $[(H·4)(PF_6)_2]^{2+}$ in the ES-MS analysis attested to the complete trifunctionalization of 2. Calix[6]tren $(4)(PF_6)_3$ is not soluble in water. Anion metathesis ($PF_6^- \rightarrow Cl^-$) using an ion-exchange resin was thus performed and monitored by IR spectroscopy until the complete disappearance of the PF_6^- band at 830 cm^{-1}. Final water-soluble calix[6]arene $(4)(Cl)_3$ was isolated in an overall 90% yield from tris(azido)-calix[6]arene 2.

Whereas the new tris-cationic ligand $(4)(Cl)_3$ was unambiguously identified by mass spectrometry, its characterization by NMR spectroscopy in water appeared very tricky. An initial survey of its spectrum in D_2O as a function of pH at room temperature showed sharp resonances at either low pH (ca. 1) or high pH (ca. 13) that vanished at intermediate pH.[22] Varying the temperature at neutral pH allowed detecting some peaks emerging at high T, but the whole spectrum did not allow us to fully characterize the compound.[22] Such a behavior is typical of amphiphilic compounds that undergo aggregation in water. We thus conducted the full characterization of ligand $(4)(Cl)_3$ in acidic medium (pH = 1.1). But here also we observed an unusual behavior. A variable T study revealed the presence of two different species, the proportion of which varied with T (Figure 4). At 358 K, the ^{1}H spectrum shows sharp resonances that are characteristic of a C_{3v}-symmetrical
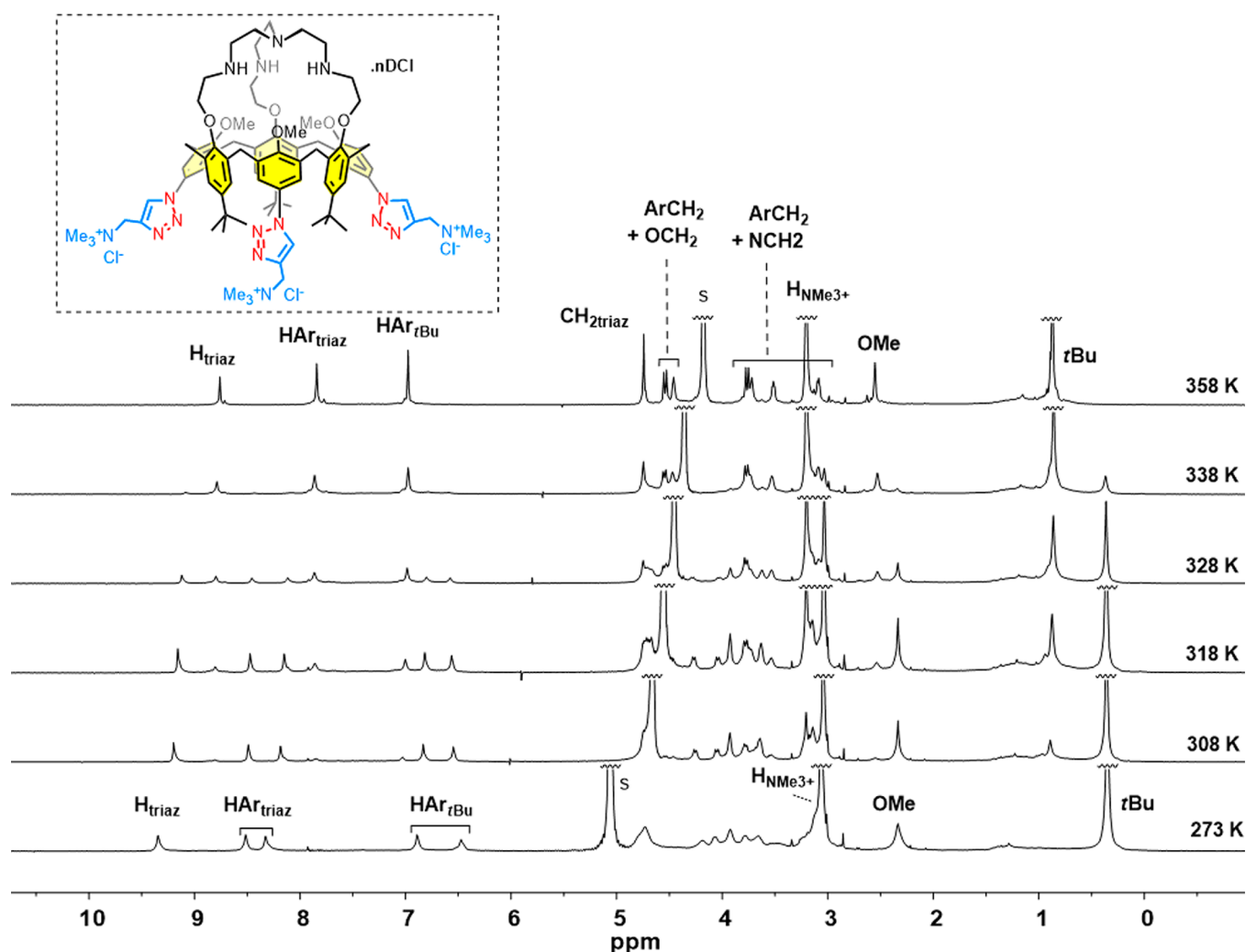
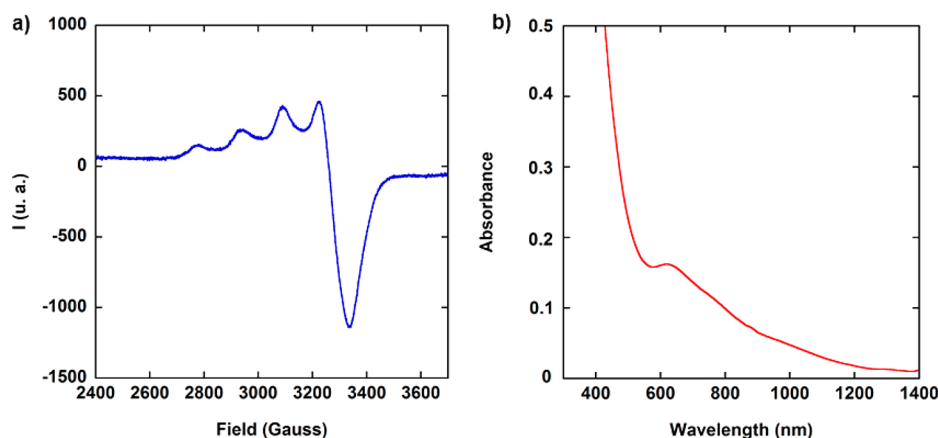**Figure 4.** ^{1}H NMR spectra $(D_2O + DCl, pH = 1.1, 600 MHz)$ of $(\mathbf{4})(\mathbf{Cl})_3$ at different temperatures.

Figure 5. (a) EPR spectra (X band, 20 mM, pH = 7.0) at 100 K and (b) UV−vis spectra (1.4 mM, pH = 7.4) at 298 K of $[\mathbf{Cu\cdot4}](\mathbf{Cl})_5$ in water.

calixarene in a flattened cone conformation $(\Delta\delta_{HAr} = 0.86$ ppm) (see structure displayed in Figure 4). At 328 K, a second species started to emerge, presenting a reduced symmetry, i.e. C_3 with an even more flattened conformation $(\Delta\delta_{HAr} = 1.5$ ppm). The corresponding tBu and OMe groups are high field shifted $(\delta = 0.3$ and 2.3 ppm, respectively), which indicates their tilting toward the cavity. Such a flattened cone conformation is classically observed when the calix[6]arene is

hosting a small guest, and it may well be due to the embedment of water molecules in the calix cavity at low T. Full characterization was conducted at high T (358 K), with 2D ^{1}H and ^{13}C spectra, which confirmed the structure and the purity of the compound.[22]

Synthesis and characterization of the copper(II) complex [Cu·4](Cl)$_5$. The Cu^{II} complex was prepared by mixing an equimolar amount of CuCl$_2$ and $(\mathbf{4})(\mathbf{Cl})_3$ in
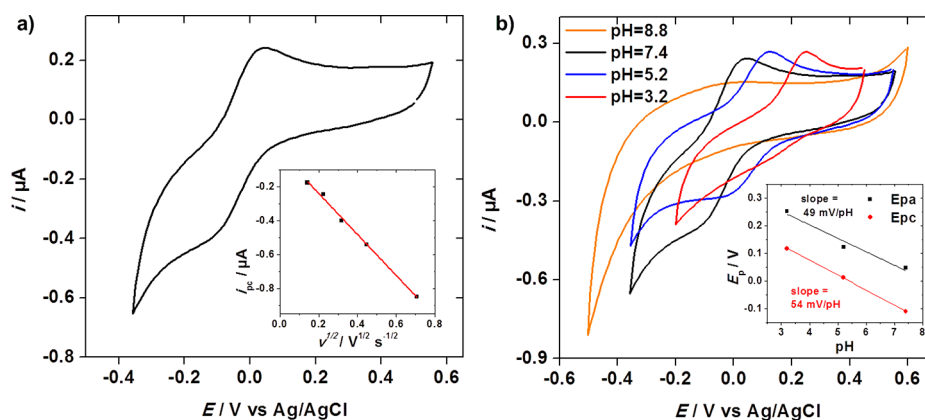
Figure 6. (a) Cyclic voltammogram (v = 0.1 V/s, 298 K) at a glassy carbon electrode of complex $[\mathbf{Cu \cdot 4}]^{5+}$ (0.3 mM) in 12.5 mM phosphate buffer, pH = 7.4. Inset: Plots of cathodic peak current i_{pc} against $v^{1/2}$ for 0.02 V/s < v < 0.5 V/s; (b) CVs of complex $[\mathbf{Cu \cdot 4}]^{5+}$ upon variation of pH. Inset: variation of cathodic (E_{pc}) and anodic (E_{pa}) peaks with pH.

Scheme 2. Host−Guest Properties of Complex $[\mathbf{Cu \cdot 4}](Cl)_5$ in Phosphate Buffer, pH = 7.4
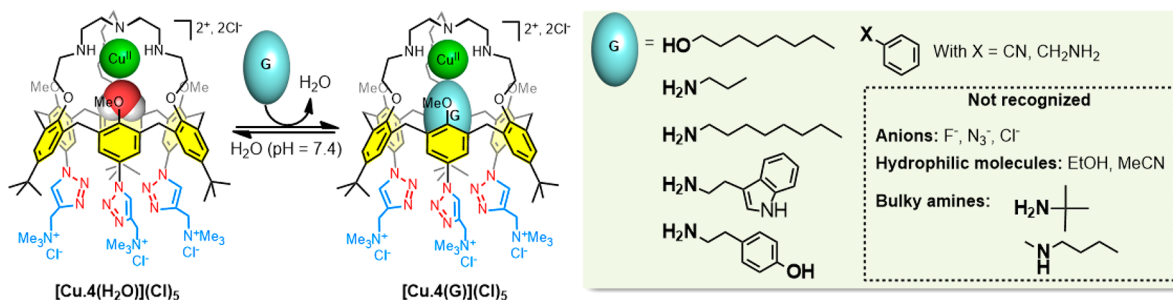
methanol at room temperature (Scheme 1). The resulting green complex was found to be nicely soluble in water up to at least 40 mM. The corresponding EPR spectrum, recorded at 100 K in water (Figure 5a) displays an axial signal characterized by $g_{//}$ = 2.24, $g_{\perp}$ = 2.06 ($A_{//}$ = 160.0 × 10^{-4} cm^{-1}). The UV−vis absorption spectrum of the complex in H_2O displayed a maximum absorption at 620 nm (ε = 116 M^{-1}cm^{-1}) (Figure 5b). These values are typical of a 5-coordinate environment due to the N_4 aza core and a solvent molecule (here water) embedded in the cavity at the level of the small rim, as for all other Cu^{II} complexes of the same family.[17] In contrast, however, with its organo-soluble analog, which presented a distorted trigonal bipyramidal (TBP) geometry, the spectroscopic parameters here denote a square-based pyramidal (SBP) environment. This may be attributed to the different microenvironment provided by water (compared to CH_2Cl_2), possibly through second coordination sphere effects.[23] Finally, a very broad absorption at λ < 500 nm is suggestive of the presence of aggregates as noticed for the free ligand by NMR spectroscopy (*vide supra*). Since the resulting spectrum is little informative,[24] further studies have been conducted, mostly by EPR spectroscopy.

The acid−base stability of complex $[\mathbf{Cu \cdot 4}]^{5+}$ (2 mM) was thus monitored by EPR spectroscopy.[22,25] The signature and the intensity of the EPR spectrum did not change significantly in the 3−12 pH window, even after several days, whereas the solution remained green during the pH sweep. This shows qualitatively that the copper complex is stable in this pH window under these experimental conditions. The EPR signature of the complex was also not affected by the addition of a phosphate buffer.

The Cu^{II} complex was also characterized by electrochemistry. At pH 7.4, in phosphate buffer, it displayed a quasi-reversible system at $E_{1/2}$ = −0.02 V vs Ag/AgCl (ΔE_p = 130 mV at v = 0.1 V/s) (Figure 6a). The Cu$^{II/I}$ redox system remained reversible in the 0.02 V/s to 0.5 V/s scan rate range (Figure 6b and S2.4). Plots of cathodic peak current i_{pc} against $v^{1/2}$ displayed a linear trend (Figure 6a, inset), indicating that the process is diffusion-limited (no adsorption on the electrode surface). Such a reversible behavior stands in strong contrast with the organo-soluble parent complex, $[\mathbf{Cu \cdot 1}]^{2+}$. Indeed, in a noncoordinating solvent (CH_2Cl_2), the CV was totally irreversible due to the fast release of the coordinated water molecule upon reduction of Cu^{II} into Cu^{I}.[17] The reversibility observed for the water-soluble system suggests that the water guest remains either coordinated to the Cu^{I} center or in its vicinity for fast rebinding (relative to the time scale of the voltammetric experiments). Such a behavior was previously evidenced for the closely related water-soluble calix[6]tmpa system[12] and Cu-calix[6]tren complex immobilized onto a gold surface by CuAAC click-chemistry.[18] The reduction potential of $[\mathbf{Cu \cdot 1}]^{2+}$ at neutral pH is significantly higher (>300 mV) than that of its analogous complex $[Cu(tren)(H_2O)]^{2+}$ deprived of a cavity (Figure S2.4) This effect is ascribed to the presence of the calix[6]arene moiety which inhibits solvation of the encapsulated Cu ion and disfavors stabilization at the Cu(II) redox state (vs Cu(I)), as previously observed in organic and aqueous media for the Cu-calix[6]tmpa and Cu-tmpa complexes.[12,26] Variation of the pH (3.2 < pH < 11.5) induced a drift of the redox system according to a 54 mV per unit pH, close to the 58 mV slope expected for a 1H$^+$,1e$^-$ Nernstian process (Figure 6b). Above pH 8.8, the redox system, which is pH-dependent, is no longer detected because it is probably shrouded in the water reduction wave.
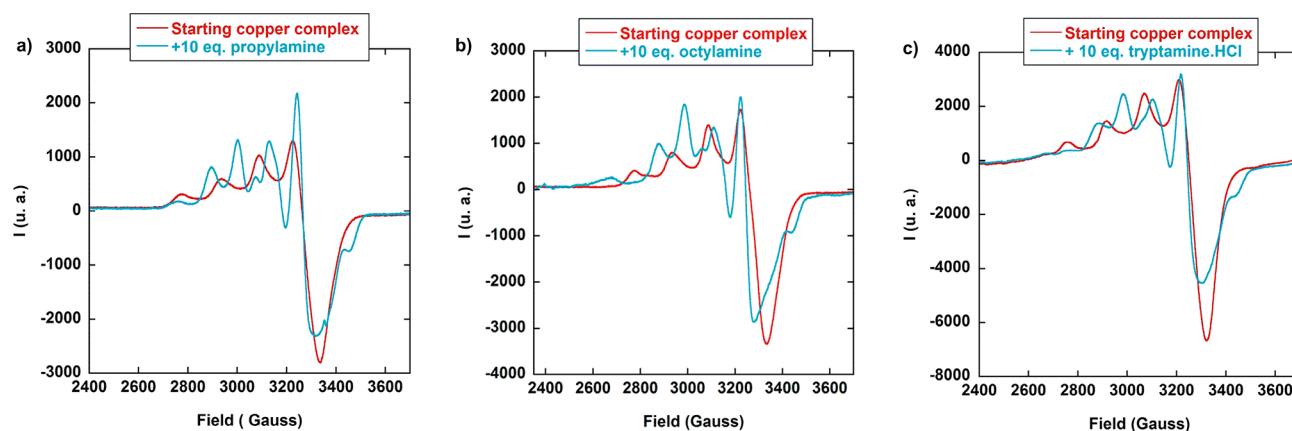
Figure 7. EPR spectra (phosphate buffer, pH = 7.4, 6, 6, and 9 mM of starting complex, respectively, X band, 100 K) of $[\textbf{Cu·4}]^{5+}$ (a) after addition of 10 equiv of propylamine; (b) after addition of 10 equiv of octylamine; (c) after addition of 10 equiv of tryptamine·HCl.

Recognition properties. The host−guest properties of the complex $[\textbf{Cu·4}]^{5+}$ toward various guests were investigated in a phosphate buffer at pH = 7.4 by EPR spectroscopy (Scheme 2).

Anions. As for its organo-soluble parent,[17] complex $[\textbf{Cu·4}]^{5+}$ did not respond to anions. Indeed, the addition of NaF, NaN_3 and NaCl (up to 30 equiv) did not change the EPR spectrum of the complex (6 mM).[22] This singular behavior was previously evidenced with organo-soluble funnel complexes and rationalized by the electrostatic repulsion exerted by the lone pairs of electrons of the oxygen atoms at the small rim (Figure 3).[14]

Alcohols and nitriles. Whereas $[\textbf{Cu·1}]^{2+}$ was found to be very sensitive to small coordinating molecules such as EtOH and MeCN in organic solvents, $[\textbf{Cu·4}]^{5+}$ was not. Indeed, more than 100 equiv of EtOH or MeCN had to be added to a 40 mM solution of the complex in phosphate buffer to generate a change of the EPR spectra.[22] In contrast, the addition of 10 equiv of octanol to a 20 mM solution of the copper complex led to a new EPR signature indicating the quantitative formation of the inclusion complex $[\textbf{Cu·4(octanol)}]^{5+}$ ($K_{octanol} > 50\ M^{-1}$),[27] which highlights the crucial role played by the hydrophobic effect. Similar results were obtained with the addition of 10 equiv of PhCN to a 20 mM solution of the copper complex ($K_{PhCN} > 50\ M^{-1}$).[22] It is worthy to note that supramolecular calixarene-based systems able to recognize alcohols in water had not yet been described in the literature. Actually, only a few examples of recognition of hydrophobic alcohols in water have been reported so far.[28]

Amines. It is well-known that primary amines are among the best guests for funnel complexes, due to their strong donor ability for metal ions. Indeed, in spite of its high hydrophilicity, propylamine readily led to the formation of the corresponding adduct, $[\textbf{Cu·4(propylamine)}]^{5+}$. This is attested to by the new rhombic EPR signature[23] (Figure 7a), obtained upon addition of 10 equiv of $PrNH_2$ to a 6 mM solution of $[\textbf{Cu·4}]^{5+}$ in phosphate buffer at pH 7.4.[29] More lipophilic guests such as octylamine and tryptamine also readily reacted with the complex to give rise to EPR signatures similar to that obtained with propylamine, consistent with the formation of amino-complexes (Figure 7b,c, Table 1). Although highly sensitive to primary amines, the receptor displayed the selectivity expected of its conic cavity shape.[16] Indeed, no response was obtained upon addition of either secondary or bulky amines such as *N*-butylmethylamine and *tert*-butylamine under the same experimental conditions (up to 20 equiv of amine in a 6 mM solution of the complex at pH 7.4).[22] The selectivity is ascribed

Table 1. EPR Data of $[\textbf{Cu·4}]^{5+}$ (phosphate buffer, pH = 7.4) in Water and in the Presence of 10 equiv of Various Amino-Guests

Guest	EPR parameters (X band, 100 K) ($A/10^{-4}\ cm^{-1}$)
H_2O	$g_{//} = 2.24$ (160.0), $g_\perp = 2.06$
Propylamine	$g_1 = 2.008$ (64.3), $g_2 = 2.151$ (81.5), $g_3 = 2.198$ (124.4)
Octylamine	$g_1 = 2.008$ (64.0), $g_2 = 2.125$ (49.0), $g_3 = 2.200$ (120.0)
Tryptamine	$g_1 = 2.002$ (59.2), $g_2 = 2.110$ (31.7), $g_3 = 2.202$ (123.4)

to a steric clash between the *N*-substituent and the calix small rim or bad fit between the cavity cone and the guest shape.

A second set of experiments was aimed at evaluating the importance of the lipophilicity in the recognition process. Competition experiments between primary amines could not be monitored by EPR spectroscopy because of the strong similarity of the EPR spectra. We thus proceeded by comparing the detection limit for a given guest. With propylamine, 5 equiv were required to see the appearance of a second EPR signature indicating the coexistence of the aqua and amino complex, and 11 equiv led to a single signature attesting to the quantitative formation of $[\textbf{Cu·4(propylamine)}]^{5+}$ (9 mM) (Figure 8a). In strong contrast, the EPR signature of the amino adduct was observed after the addition of only 0.25 equiv of octylamine and 1 equiv gave rise to the quantitative formation of $[\textbf{Cu·4(octylamine)}]^{5+}$ (9 mM) (Figure 8b). From these experiments, the value of the relative affinity constant $K_{octylamine}/K_{propylamine}$ was evaluated to be ca. 100 with $K_{octylamine} > 10000\ M^{-1}$. Likewise, the binding constants of tyramine and tryptamine were evaluated to be higher than 15000 M^{-1} and 10000 M^{-1}, respectively.[22]

Acid−base stability of the amino-complexes. Having shown that the new water-soluble receptor $[\textbf{Cu·4}]^{5+}$ recognizes amines at physiological pH, we were then interested in evaluating the stability of the corresponding inclusion complexes at different pH values. This pH study was undertaken with complex $[\textbf{Cu·4(octylamine)}]^{5+}$ (2 mM) formed in the presence of 10 equiv of the amine, and variation of pH was performed by addition of NaOH or HCl. The resulting EPR spectra showed that the signature of the complex $[\textbf{Cu·4(octylamine)}]^{5+}$ was preserved between pH = 12.2 and pH = 3.1.[22] Besides, the EPR signature of the complex at these extreme pH values did not change after a few days, highlighting its thermodynamic stability. At pH < 2, the signature of the inclusion complex was lost, but subsequent addition of NaOH
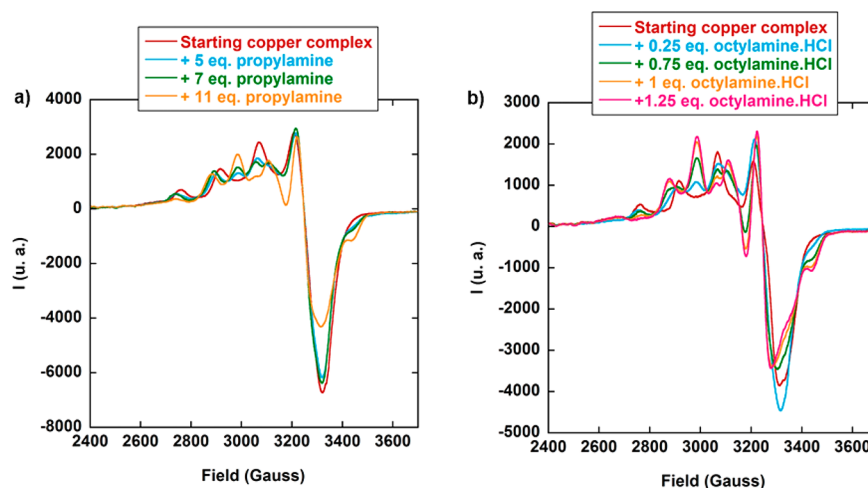
Figure 8. EPR spectra (phosphate buffer 0.36 M, pH = 7.4, 9 mM of starting complex, X band, 100 K) for the titration of $[\mathbf{Cu \cdot 4}]^{5+}$ by (a) propylamine; (b) octylamine·HCl.

until pH = 5.1 restored it, showing the reversibility of the coordination/decoordination process.

Relative strength of complexation. Finally, the relative strength of complexation of $[\mathbf{Cu \cdot 4}]^{5+}$ toward different families of neutral guests was evaluated. To a phosphate buffer solution of the inclusion complex $[\mathbf{Cu \cdot 4(octanol)}]^{5+}$ (20 mM) formed with 20 equiv of octanol, heptylamine was added. The addition of 10 equiv of heptylamine to the solution led to a new EPR spectrum, suggesting the recognition of the latter.[22] In that case, competitive experiments could be conducted by EPR spectroscopy since the signatures associated with different donors were quite different. It was found that heptylamine readily displaces octanol, and the corresponding relative affinity constant $K_{octylamine}/K_{heptanol}$ was evaluated to be >20, whereas another competition experiment showed the following relative affinity order: $PhCH_2NH_2$ > PhCN > $PhCH_2OH$.[22]

DISCUSSION

The receptor properties of funnel complexes based on calix[6]arene depend on a number of points: (i) the Lewis acidity of the bound metal ion, tuned by the first coordination sphere implemented at the small rim, (ii) the solvent that can coordinate the metal center, (iii) the rigidity of the calixarene macrocycle for which covalent capping plays a key role, (iv) the substitution pattern at the aromatic units (at both large and small rims). Ligands **1** and **4** present very similar properties. Indeed, a tren unit insures strong binding of the metal ion and maintains the calixarene into a cone conformation with an increased rigidity compared to the tris-imidazole system, and the same substitution pattern is present at the small rim, which gives rise to similar second coordination sphere effects. The major structural difference stems from the replacement of three *t*Bu groups by triazole units connected to quaternary ammonium moieties. Such a substitution pattern has been shown to enlarge the hydrophobic cavity of the calixarene.[11,30] Actually, the most important difference between both systems stems from the solvent in which they are studied. With calix **1**, a noncoordinating solvent can be chosen, and only residual water competes with the guest for the fifth coordination site embedded in the calix cavity. With calix **4**, water is the solvent, and it strongly competes for the coordination to the metal ion. It is also a protic medium that can interact with the guest, transforming the amino-guests, for example, into ammoniums

that are deprived of coordination properties. Quite remarkably, in spite of these obstacles, the Cu^{II} complex based on ligand **4** revealed itself to be an excellent receptor for various molecules. Like $[\mathbf{Cu \cdot 1}]^{2+}$, it selectively binds neutral ligands such as nitriles or alcohols with no detectable interaction with competitive anions. This is attributed to the similar small rim pattern. It also selectively binds primary amines, in spite of their protonated state in water at pH = 7. The *pseudo*-pK_a value corresponding to the couple $[\mathbf{Cu \cdot 4(RNH_2)}]^{5+}$ / RNH_3^+ was estimated for octylamine to be as low as 3.4. This, compared to the pK_a value of free octylamine, 10.6,[31] indicates a pK_a shift of more than 7 units. This impressive result shows the extraordinary stabilization of the amine due to its complexation to the metal cation inside the calixarene cavity.

Hence, receptor $[\mathbf{Cu \cdot 4}]^{5+}$, although in water, displays selectivity similar to that reported for its organo-soluble analog, $[\mathbf{Cu \cdot 1}]^{2+}$, but it benefits from the triazole-extended cavity and from the hydrophobic effect: the longer the apolar chain, the better the recognition ($K_{octylamine}/K_{propylamine}$ = ca. 100), which is the converse in organic solvent.

When compared with water-soluble Zn^{II} funnel complexes obtained with the first generation of calix[6]-ligands (bearing three imidazole arms instead of a tren unit, see Figures 1 and 2), a number of improved properties are highlighted: (i) the metal complex $[\mathbf{Cu \cdot 4}]^{5+}$ is stable even in the absence of a guest, (ii) this receptor is responsive not only to amines but also to weaker donors such as alcohols and nitriles, (iii) hydrophilic amines such as propylamine are also recognized, and finally (iv) the pH window for the complexation of amines is much wider [(3−12) vs (6−10)]. Quite interestingly, the pK_a shift estimated for primary ammoniums in the presence of receptor $[\mathbf{Cu \cdot 4}]^{5+}$ is similar to the one found for the tris-imidazole-based receptor (also 7 units).[11] This suggests that major roles in the hosting process are played by the calix-core: H-bonding at the small rim, CH−π interactions with the aromatic walls, and the hydrophobic effect. It is noteworthy that such polyvalence in water for a receptor has been rarely described in the literature and recognition of amines in acidic conditions (pH = 3) is also very rare. It is also interesting to compare this water-soluble copper complex receptor studied in pure water with the organo-soluble complex $[\mathbf{Zn \cdot 1}]^{2+}$ studied in micelles.[19] In micelles, it was found that the selectivity, albeit different from the one observed in organic solvents, was still in favor of

smaller hydrophilic amines ($K_{\text{heptylamine}}/K_{\text{propylamine}}$ is 0.5 and 0.25×10^{-3} in micelles and in chloroform, respectively).

CONCLUSION

A water-soluble copper(II) complex presenting a labile coordination site embedded in a hydrophobic cavity was successfully synthesized and characterized. The host–guest properties of this complex could be evaluated by EPR spectroscopy, which revealed the complex to behave as an effective receptor for different types of ligands in a large pH window (3 to 12). It displayed a good selectivity for hydrophobic primary amines with a positive response to biologically important molecules such as tyramine and tryptamine. These remarkable features may be exploited for the development of a selective sensor for biologically relevant amines such as polyamines, tryptamine derivatives, and lysine rich-peptides, with EPR spectroscopy as an efficient detection tool, which is complementary to electrochemistry and fluorescence at presenting very different advantages and drawbacks.

EXPERIMENTAL SECTION

General experimental methods. All the reactions were performed under an Ar atmosphere. Solvents were dried by conventional methods and distilled prior to use. ^{1}H spectra were recorded at 600 MHz. ^{13}C NMR spectra were recorded at 100 MHz. 2D NMR spectra (COSY, HSQC, HMBC) were recorded to complete signal assignments. ^{1}H NMR spectra were referenced to residual protiated solvents (4.79 ppm for D$_2$O). Chemical shifts of the HDO signals as a function of temperature were referenced according to values found in the literature.[32] High resolution mass spectra (HRMS) were obtained with a QTOF spectrometer. EPR spectra were recorded using a Bruker Elexsys spectrometer (X-band) at T = 100 K. UV–vis spectroscopy was performed with a V670 JASCO spectrophotometer. The electrochemical studies have been performed with a home-designed three-electrode cell connected to an Autolab PGSTAT 100 (Ecochemie) potentiostat monitored by a computer. The reference electrode used was a silver/silver chloride (NaCl 3M) system (Ag/AgCl). The working electrode (glassy carbon) was polished on alumina (3 μm) before use, and the auxiliary electrode was a platinum wire. Phosphate buffer (12.5 mM) was used as supporting salt (with ca. 10^{-4}–10^{-3} M in electroactive species), and the experiments were performed by bubbling N$_2$ into the solution. As for the EPR pH studies, the copper complex (~1 mg) was dissolved in 300 μL of water and the pH of the solution was measured. 100 μL of this solution was taken for the EPR measurement. The solution in the EPR tube was then recombined with the mother solution, the pH of which was modified by addition of HCl/NaOH.

(4)(Cl)$_3$. To a stirred solution of ^{N3}X$_6$Tren **2** (0.035 g, 0.029 mmol) in CH$_3$CN (4 mL) were added propargyl trimethylammonium hexafluorophosphate **3** (0.025 g, 0.10 mmol) and 2,6-lutidine (0.005 g, 0.044 mmol). Argon was bubbled through the solution and then Cu(CH$_3$CN)$_4$.PF$_6$ (0.016 g, 0.044 mmol) was added. The reaction mixture, which was initially brown, turned green over time and was refluxed overnight. The reaction was monitored by IR spectroscopy and was stopped as soon as the 2100 cm^{-1} band (N$_3$) fully disappeared. The mixture was cooled to r.t. and then an aqueous solution of KCN (15 equiv) was added which led to the vanishing of the green color. Aqueous NaPF$_6$ was added to the resulting mixture, leading to the precipitation of the calixarene product that was abundantly washed with H$_2$O. The precipitate was dissolved in MeOH (4 mL) and Cl$^-$ exchange resin (60 equiv) was added. The mixture was gently stirred at r.t. for 1 h and then filtered. The anion metathesis was monitored by IR spectroscopy (disappearance of the PF$_6^-$ band at 830 cm^{-1}). The filtrate was concentrated under reduced pressure, yielding **(4)(Cl)$_3$** as a brown solid (0.042 g, 0.026 mmol, 90% yield based on **2**). mp = 210–212 °C (*dec.*). IR (cm^{-1}): 3399, 2955, 1602,

1484, 1235, 1206, 1114, 1051, 1011, 898. ^{1}H NMR (600 MHz, D$_2$O+DCl, pH = 1.1, 358 K): δ 0.87 (s, 27H, (C(CH$_3$)$_3$)), 2.55 (s, 9H, OCH$_3$), 3.09 (bdd, J = 7–8 Hz, 6H, NCH$_2$), 3.20 (s, 27H, (N$^+$(CH$_3$)$_3$), 3.52 (bdd, J = 7–8 Hz, 6H, CH$_2$NH), 3.72 (bm, 6H, CH$_2$NH), 3.76 (d, J = 15.4 Hz, 6H, ArCH$_2$Ar), 4.46 (bt, J = 5.1 Hz, 6H, OCH$_2$), 4.54 (d, J = 15.3 Hz, 6H, ArCH$_2$Ar), 4.74 (s, 6H, CH$_{2\text{triaz}}$), 6.98 (s, 6H, HAr$_{\text{tBu}}$), 7.84 (s, 6H, HAr$_{\text{triaz}}$), 8.76 (s, 3H, H_{triaz}) ppm. ^{13}C NMR (100 MHz, D$_2$O+DCl, pH = 1.1, 358 K): 157.7 (C_{Ar}OCH$_3$), 150.8 (C_{Ar}OCH$_2$), 149.0 (C_{Ar} C(CH$_3$)$_3$), 137.0 ($C_{Ar\text{-}triaz}$CH$_2$), 136.2 (C_{Ar}CH$_2$), 133.5 (C_{Ar}CH$_2$), 132.9 (C_{Ar}N), 127.6 ($C_{Ar\text{-}triaz}$H), 125.1 (C_{Ar}H), 124.4 (C_{Ar}H), 69.5 (OCH$_2$), 61.3 (OCH$_3$), 60.6 (CH$_{2\text{triaz}}$), 53.5 (N$^+$(CH$_3$)$_3$), 52.3 (NCH$_2$), 48.2 (CH$_2$NH), 48.0 (CH$_2$NH), 34.2 (C(CH$_3$)$_3$), 30.7 (C(CH$_3$)$_3$), 30.1 (ArCH$_2$Ar) ppm. HRMS (ESI+): calc. for C$_{87}$H$_{123}$N$_{16}$O$_6$ [(**4**)]$^{3+}$ 495.9931 found 495.9904.

[Cu·4](Cl)$_5$. (4)(Cl)$_3$. (0.022 g, 0.014 mmol) and anhydrous CuCl$_2$ (0.002 g, 0.014 mmol) were mixed in MeOH (2 mL) and stirred for 2 h. The solvent was removed under reduced pressure to give [**Cu·4(MeOH)**](Cl)$_5$ (0.024 g, 0.014 mmol, quant.) as a green solid. mp = 255–260 °C (*dec.*). IR (cm^{-1}): 3373, 2928, 2859, 1648, 1601, 1481, 1244, 1210, 1052, 1008, 897. EPR (H$_2$O, 100 K): g$_\perp$ = 2.06, g$_{//}$ = 2.24, A$_{//}$ = 160.0 G. UV–vis (H$_2$O), λ_{max} = 620 nm, ε = 116 M^{-1} cm^{-1}. HRMS (ESI+): calc. for C$_{87}$H$_{123}$CuN$_{16}$O$_6$ [**Cu·4**]$^{5+}$ 310.1836, found 310.1787.

ASSOCIATED CONTENT

S **Supporting Information**

The Supporting Information is avaible free of charge via the Internet on the Web site The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.inorgchem.7b02541.

^{1}H, ^{13}C, and 2D NMR, and IR and ES-MS spectra of the ligand and the complex, EPR spectra of the host–guest studies (PDF)

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(22) See the Supporting Information.

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coordinating guest molecule (168.8 instead of 180° in the case of the EtOH complex). This was attributed to hydrogen-bonding of the guest EtOH to one oxygen of the calixarene skeleton, at the small rim (see ref 18). For a comparison of the EPR parameters of the Cu^{II} complexes in water herein described and those measured for the analogous complexes in CH$_2$Cl$_2$, see the Supporting Information.

(24) Diffusion phenomena, very likely due to the formation of small aggregates in water, overlay part of the dd absorption, and no exploitable data could be extracted from our tentative titrations using UV-absorption, even at a lower concentration. Indeed, the formation of aggregates certainly depends on the presence/absence of guest ligand, as has been reported in our previous work with water-soluble calixarenes. See: Houmadi, S.; Coquière, D.; Legrand, L.; Fauré, M.-C.; Goldmann, M.; Reinaud, O.; Rémita, S. *Langmuir* **2007**, *23*, 4849−4855. Bize, C.; Garrigues, J.-C.; Blanzat, M.; Rico-Lattes, I.; Bistri, O.; Colasson, B.; Reinaud, O. *Chem. Commun.* **2010**, *46*, 586−588.

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