Introduction

Hemicryptophanes have revealed to be attractive molecular receptors and they have been used for the design of supramolecular catalysts and molecular propellers or gyroscopic systems. In particular, the introduction of a vanadium atom in the cavity of hemicryptophanes has for instance been found to yield oxidovanadium-hosts acting as haloperoxidase enzyme mimics catalyzing sulfoxidation reactions.

As a general statement, the versatility and modularity of these host compounds have proven particularly suitable to mimic enzymatic systems by combining a lipophilic cavity and a catalytic site. By this way, original nanoreactors can be synthesized and give rise to artificial metallo-enzymes. Such systems have been already studied in organic solvents. With its spectroscopic characterization and redox properties.

Recently, we have reported several supramolecular systems, based on hemicryptophane ligands, combining a biomimetic copper(u) coordination core and a lipophilic cavity. Cu(u) complexes have been found to be efficient catalysts in organic solution for the conversion of cyclohexane to cyclohexanol/cyclohexanone, using H₂O₂ as the oxygen source. Both the stability and the selectivity of the catalyst have been improved by encapsulating the active site. We have also described the first synthesis of a water-soluble hemicryptophane host and its recognition properties toward choline in a basic aqueous medium.

By combining both water solubility and Cu(u) complexation in a unique cage molecule we expected a water-soluble metallo-enzyme model, presenting a copper site encaged in a closed-shell cavity. Herein, we report the synthesis of the first water-soluble copper@hemicryptophane complex together with its spectroscopic characterization and redox properties.

Results and discussion

Synthesis of the water-soluble complex

Hemicryptophane 1 was synthesized in 6 steps, following a previously published procedure (Scheme S-1†). The strongly basic conditions (pH = 12) which are required to solubilize 1 in water are not compatible with its use in biological environments. The substitution of the phenol groups in 1 by carboxylate moieties was performed in order to obtain the hemicryptophane 3, which was found to be soluble in water at physiological pH (pH ≈ 7). The strategy used to synthesize compound 3 is depicted in Scheme 1. The first step involves the reaction of compound 1 with tert-butyl-bromoacetate in DMF in the presence of Cs₂CO₃ to yield the protected tri-ester derivative 2. Deprotection of both the amine and the ester...
functions in 2, using trifluoroacetic acid, afforded the watersoluble hemicryptophane 3. Following this synthetic pathway, host 3 has been obtained from commercially available products in 8 steps with an overall yield of 6%.

The $^1$H NMR spectrum of 3 in D$_2$O (pD $\approx$ 7) indicates that this molecule is on average of C$_3$ symmetry. It displays the expected signals for the cyclotribenzylene unit (Fig. 1): two singlets for the aromatic protons, and the characteristic AB system for the ArCH$_2$ bridges. Two doublets for the aromatic protons of the linkers and the multiplets for the OCH$_2$ groups are also observed in the spectra. The NCH$_2$ protons of the tren unit appear as a complex pattern between 2.0 and 2.25 ppm.

The hemicryptophane copper(II) complex was then synthesized by the reaction of 3 with a stoichiometric amount of copper(II) perchlorate in water to produce Cu(II)@3 isolated in a 50% yield as a pale green solid (Scheme 2).

**Electronic spectroscopy**

The near-IR/vis absorption spectrum of the hemicryptophane complex Cu(II)@3 in CH$_2$Cl$_2$ displays a broad asymmetrical band centred at 850 nm which is a diagnostic signature supporting the trigonal-bipyramidal geometry of the complexed copper(II) ions (Fig. 2). These observations are fully consistent with those previously observed with the parent Cu(II)@4 complex soluble in organic solvents (Scheme 2(b)).

**EPR measurements**

The EPR spectrum of the Cu(II)@3 complex was recorded in frozen water at 70 K (Fig. 3). A pseudo-axial EPR signal ($g_1$ = 2.015, $g_2$ = 2.135 and $g_3$ = 2.220) with a resolved hyperfine structure ($A_1$ = 193 MHz, $A_2$ = 158 MHz and $A_3$ = 363 MHz) was obtained, giving a $R$ value ($(g_2-g_1)/(g_3-g_2)$) equal to 1.41. These values ($g_1$ < 2.04 and $R$ > 1) are typical for a N$_4$X coordination sphere with a distorted geometry close to trigonal-bipyramidal geometry. The $g$-values are very similar to that previously obtained with the Cu(II)@4 complex ($g_1$ = 2.010, $g_2$ = 2.136, $g_3$ = 2.220; $R$ = 1.50), indicating that water solubilisation did not induce strong distortion of the initial geometry. Complexes Cu(II)@3 and Cu(II)@4 present a five-coordination sphere with the copper ion bound to the four nitrogen atoms of the tren unit and to one solvent molecule.
Electrochemical studies

The CV curve recorded for Cu(i)@3 in an electrolytic aqueous medium exhibits a fully irreversible reduction wave \( E_{pc} = -0.59 \text{ V} \) attributed to the formation of Cu(i)@3 at the electrode interface (Fig. 4). This irreversible feature, observed at all the investigated scan rates, i.e., from 0.02 to 10 V s\(^{-1}\), is due to the existence of an isomerisation process triggered by the electrochemical reduction of the Cu(i) center. The associated EC process\(^{19}\) can indeed be modelled as a classic square scheme involving changes in the coordination sphere around the Cu(i) center in the pentacoordinated Cu(i)@3 species to produce a more stable tetrahedral Cu(i) complex noted Cu(i)@3'.\(^{20}\) These hypotheses are supported by the observation of an irreversible oxidation wave at \( E_{pa} = 0.14 \text{ V} \) on the reverse scan, the large amplitude of the potential shift seen between \( E_{pc} \) and \( E_{pa} \) being in agreement with a net decrease of the number of coordinated atoms on the copper center, from 5 in Cu(i)@3 to 4 in Cu(i)@3'. It should be mentioned that a similar EC process was postulated for Cu(i)@4 in dichloromethane; the reduction wave and the associated re-oxidation being observed under these conditions at \( E_{pc} = -0.91 \text{ V} \) ECS and \( E_{pa} = 0.45 \text{ V} \) ECS, respectively.\(^{14}\)

These differences observed between organic and aqueous media highlight the key role of water which is most probably involved both in the solvation and coordination shells of the cupric and cuprous complexes.

Conclusion

In conclusion, the synthesis of the first water soluble copper@-hemicryptophane complex has been described. This represents a rare example of water-soluble cage compound trapping a copper ion in the confined space of its cavity. The physicochemical studies indicated that the solubilisation in water affects only weakly the geometry of the complex, but strongly modifies its redox behavior. This novel water-soluble metalloenzyme model featuring a copper(ii) site encaged in a closed-shell cavity opens up the way to new bio-inspired catalytic systems.

Experimental section

General methods

All reactions were carried out under argon by means of an inert gas/vacuum double manifold and standard Schlenk techniques. Dichloromethane was first dried over molecular sieves and then passed through an activated alumina column followed by an argon flush on a solvent station.\(^1\)H and \(^1\)C NMR spectra were recorded at 500.10 MHz and 125.76 MHz, respectively. Chemical shifts are reported relative to the residual protonated solvent signal (CDCl\(_3\)), or relative to residual ethanol for the spectra in D\(_2\)O. Mass spectra were recorded by the Centre de Spectrométrie de Masse, Institute of Chemistry, Lyon. Compound 1 was prepared according to the published procedure.\(^{15}\) EPR spectra were obtained from a Bruker EMX spectrometer and fitted with Easyspin\(^{21}\) software.

Cyclic voltammetry (CV) data were recorded using an ESP-300 Biologic potentiostat equipped with a 1 A/48 V booster and an analog linear scan generator. All the experiments were conducted under an argon atmosphere in a standard one-compartment, three-electrode electrochemical cell placed in a faraday cage. An automatic ohmic drop compensation procedure was systematically implemented prior to recording CV data. All the electrodes were purchased from ALS Co. Ltd.
Vitreous carbon (Φ = 3 mm) working electrodes were polished with 1 mm diamond paste before each recording. A saturated ECS electrode was used as a reference. HPLC grade water + sodium nitrate (0.1 M) was used as the electrolyte.

**Syntheses**

**Hemicryptophane 2.** Hemicryptophane 1 (257 mg, 0.212 mmol) was dissolved in anhydrous DMF (20 mL) and C₈H₃CO₃ (276 mg, 0.848 mmol) was added to the solution which was stirred at 60 °C for 15 minutes. Then tert-butylbromoacetate (0.141 mL, 0.945 mmol) was added and the mixture was stirred at 60 °C for 2 hours, then at room temperature overnight. The solvent was removed and CHCl₃ (20 mL) and H₂O (20 mL) were added to the residue. The two layers were separated and the aqueous phase was extracted with CHCl₃ (20 mL). The combined organic solutions were washed with H₂O (2 × 20 mL) and dried over anhydrous Na₂SO₄. The solvent was removed. Milli-Q water (1.5 mL) and NaOH (19.6 mg, 490 µmol) were added to the residue and the mixture was stirred for 30 minutes. Water was then evaporated to give 3 as a yellow solid (quantitative). ESIMS m/z observed 1551.7991 ([M + H]⁺, calculated 1551.7991 for C₆₀H₆₅N₄O₁₅). 1H NMR (D₂O, 298 K, 400 MHz): δ 7.13–6.91 (br, 6H, ArH), 6.91–6.82 (br, 6H, ArH), 6.82–6.66 (br, 6H, ArH), 4.65 (d, 3H, J = 13.7 Hz, ArCH₂Ar), 4.56 (d, 3H, J = 16.0 Hz, CH₂COO), 4.48–4.40 (m, 21H, CH₂COO; O(CH₂)₂O; O(CH₃)₃), 3.80 (d, 3H, J = 15.4 Hz, OCH₂), 3.72 (d, 3H, J = 13.6 Hz, ArCH₂N), 3.17 (d, 3H, J = 13.6 Hz, ArCH₂N), 3.07 (m, 3H, O(CH₂)₂O), 3.01 (m, 2H, O(CH₂)₂O), 2.86 (t, 2H, O(CH₂)₂O), 2.25–2.00 (m, 12H, N(CH₂)₂N), 1.52 (s, 3H, ArH), 1.32 (br, 54H, C(CH₃)₃). 13C NMR (CDCl₃, 298 K, 100.61 MHz): δ 157.97, 155.92 and 155.36 (ν(CaR), 145.85 (CaR), 145.47 (CaR), 133.41 (CaR), 132.79 (CaR), 130.00 (CaH), 129.27 (CaH), 116.38 (CaH), 115.82 (CaH), 114.19 (CaH), 69.12 (O(CH₂)₂O), 66.71 (O(CH₂)₂O), 66.32 (O(CH₃)₂O), 52.92 (N(CH₂)₂N), 50.51 (ArCH₂N), 44.79 (N(CH₂)₂N), 35.26 (ArCH₂N). Mp: 219.3 °C. IR ν = 3400, 2923, 2856, 1681, 1609, 1425, 1335, 1259, 1204, 1176, 1127 cm⁻¹.

**Complex CuII@hemicryptophane 3.** To a solution of hemicryptophane 3 (15.6 mg, 14 µmol) in milli-Q water (3 mL) were added Cu(ClO₄)₂⋅6H₂O (5.1 mg, 14 µmol) and Et₃N (9 µL, 67 µmol). The solution was stirred for 5 minutes and water and Et₃N were removed under vacuum. Milli-Q water (1.5 mL) was added and the blue solid was triturated in this solvent. The solution was centrifuged and the supernatant was removed. This last step was repeated with 1 mL of milli-Q water and the residue was dried under vacuum. Pale green solid was obtained (7.9 mg, 50%). ESI-MS m/z observed 1142.3466 ([M + H]⁺, calculated 1142.3591 for C₆₀H₆₅CuN₄O₁₅). EPR (H₂O, 298 K; pH = 8; 70 K) gₓ = 2.015 (Aₓ = 193 MHz); g₁ = 2.135 (A₁ = 158 MHz); g₂ = 2.220 (A₂ = 363 MHz). Vis-near IR spectroscopy (H₂O, 298 K) λmax = 850 nm, ε = 237 M⁻¹ cm⁻¹, shoulder band around 700 nm, ε = 165 M⁻¹ cm⁻¹. Cyclic voltammetry: Epa = -0.34 V and Epa = 0.30 V vs. E₁/₂ (FcMeOHF⁺) (H₂O, NaOH (1.5 mM), 12-CuI (5 × 10⁻⁴ M), NaNO₃ (0.1 M), working electrode: carbon, 50 mV s⁻¹).

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