Liquid-liquid extraction of succinate using a dicopper cryptate

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Succinate extraction from water using a cage-like dicopper complex

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ABSTRACT: The extraction of the succinate dianion from a neutral aqueous solution into dichloromethane is obtained using a lipophilic cage-like dicopper(II) complex as the extractant. The quantitative extraction exploits the high affinity of the succinate anion for the cavity of the azacryptate. The anion is effectively transferred from the aqueous phase, buffered at pH 7 with HEPES, into dichloromethane. A 1:1 extractant:anion adduct is obtained. Extraction can be easily monitored by following changes in the UV-visible spectrum of the dicopper complex in dichloromethane, and by measuring the residual concentration of succinate in the aqueous phase by HPLC–UV. Considering i) the relevance of polycarboxylates in biochemistry, as e.g. normal intermediates of the TCA cycle, ii) the relevance of dicarboxylates in the environmental field, as e.g. waste products of industrial processes, and iii) the recently discovered role of succinate and other dicarboxylates in pathophysiological processes including cancer, our results open new perspectives for research in all contexts where selective recognition, trapping and extraction of polycarboxylates is required.

Polycarboxylates are relevant in the biological, industrial and environmental fields.¹ In particular, these anionic species are natural cellular metabolites with roles as intermediates of the tricarboxylic acid (TCA) cycle.^{2,3} For these reasons, molecular systems capable of selectively recognizing target polycarboxylates have attracted attention in the supramolecular chemistry field over many years.^{4–15} Interest will undoubtedly grow because of the recent medical recognition of pathological roles of some dicarboxylates beyond normal metabolism.^{2,3,16,17} For instance, succinate plays a crucial role in the TCA cycle, where it is oxidized for electron delivery to the respiratory chain, and in heme and itaconate metabolism, and the “GABA (γ -aminobutyric acid) shunt.” Succinate has recently been recognized as an oncometabolite whose accumulation drives pathophysiological processes including cancer.^{2,3,16,18}

Molecular receptors are appreciated as powerful tools for the selective recognition and binding of anionic guests in competing media.^{7,10,19–25} Research in the field has led to the development of i) a plethora of anion sensors, anion-responsive materials, organocatalytic processes involving anion complexation, ii) systems for the selective liquid-liquid or solid-liquid extraction of the target anion, and iii) compounds to be applied as anion transporters across cell membranes.^{14,15,26,27} The latter applications have generally involved the extraction^{28–33} and/or transport of halides^{27,34–39} – chloride, in particular – or oxoanions^{40–42}. Despite strong interest in the manipulation of polycarboxylates, application of molecular receptors in the selective extraction of these relevant species is rare.^{27,43,44} Extraction of polycarboxylates from a neutral aqueous solution into an apolar solvent is both interesting and challenging. In particular, the process must be both electroneutral and selective, i.e. charge neutrality must be maintained and the extractant should discriminate among similar guests, e.g. species differing only for one methylene group on the alkyl chain.¹⁴

In this work, we demonstrate that both challenges can be overcome by implementing a cage-like dicopper(II) complex with a lipophilic framework, whose cavity is shaped to selectively accommodate the target dicarboxylate species. For this study, we chose succinate as the target anion, because of its established role as an oncometabolite through inhibition of dioxygenases involved in epigenetics and the hypoxic response.

Figure 1. Azacryptands studied in this work.

The lipophilic azacryptand **L1** (Figure 1) was synthesized by [2+3] Schiff condensation of *tren* with the proper dialdehyde, followed by reduction of the imine bonds with NaBH₄ (see S.I. for details). The final polyamine **L1** was characterized by potentiometric titrations in dioxane/water 20% v/v, [TBA]NO₃ 0.05 M, 25 °C. These studies, performed on both the ligand alone and in the presence of 2 *eqv.* Cu(NO₃)₂, allowed determination of the protonation and complexation constants reported in Table S1. The [Cu₂L1]⁴⁺ species was found to be the major complex (> 99%) in solution between pH 6 and 10 (Figure 2). The UV-vis. spectrum of [Cu₂L1]⁴⁺ is characterized by a typical d-d band around 900 nm and a shoulder at 730 nm, due to the coordination of the copper(II) ions to the *tren* subunits, with water molecules completing the penta-coordination sphere of the metal centers. The binding of Cu(II) ions to the *tren* compartments is also accompanied by the development of N_{sec} → Cu(II) (LMCT) bands near 330 nm. The profile of the molar absorbance at 885 nm vs. pH (see red triangles in Figure 2) is superimposable on the curve for percent abundance of [Cu₂L1]⁴⁺ vs. pH in the distribution diagram.

Figure 2. Distribution diagram showing species present at equilibrium during potentiometric titration of **L1** (0.4 mM) in presence of 2 *eqv.* Cu(NO₃)₂ with the superimposed pH-spectrophotometric profiles of Mol Abs at 885 and 650 nm (red and green triangles, respectively) vs. pH, in dioxane/water 20% v/v, 0.05 M [TBA]NO₃ (T 25 °C). The lines in the diagram correspond to the species: H₆L1⁶⁺, black; H₅L1⁵⁺, dark red; [Cu₂L1]⁴⁺, red; [Cu₂L1(OH)]³⁺, green; [Cu₂L1(OH)₂]²⁺, grey.

Figure 3. Family of spectra taken over the course of the UV-vis. titration of [Cu₂L1]⁴⁺ (50 μM) with a solution of H₂suc in dioxane/water 20% v/v, buffered at pH 7 by HEPES 0.025 M (T 25 °C, path length: 10 cm). Initial spectrum and final spectrum: red and blue lines, respectively. Inset figure: plot of ε (i.e. Mol Abs) at 687 nm vs. eqv. of the added guest (triangles), superimposed to the distribution diagram of the species calculated for a logK₁₁=5.60; % [Cu₂L1]⁴⁺ (red line) and [Cu₂L1(suc)]²⁺ (blue line) vs. eqv. of succinate. See S.I. for details.

The anion binding trend of [Cu₂L1]⁴⁺ was then investigated with dicarboxylates of various structures and chain lengths. UV-vis. titrations were performed in dioxane/water solution 20% v/v, buffered at pH 7 by HEPES 0.025 M (T = 25 °C). Appreciating that organic solvents in the aqueous mixture may alter dissociation equilibria, we determined pK_a values for two relevant acids, H₂suc and H₂glut, through potentiometric titrations in dioxane/water 20% v/v, 0.05 M [TBA]NO₃ (T = 25 °C; see S.I.). As expected, complete deprotonation of H₂suc and H₂glut was obtained only at pH > 8 (see S.I., Figures S2 and S3), while the abundance of the corresponding dicarboxylates in pure water was already greater than 99% at pH 7.

Even if the deprotonation of dicarboxylic acids is disfavored in the chosen aqueous mixture, the coordination of the corresponding dianion to the metal centers within the cavity of the cage may provide sufficient driving force to promote complete deprotonation. This was confirmed by UV-vis. titrations for [Cu₂L1]⁴⁺ testing the guests reported in Table 1 in dioxane/water solution 20% v/v buffered at pH 7 (HEPES 0.025 M; T = 25 °C). These studies revealed that [Cu₂L1]⁴⁺ forms 1:1 adducts with all of the investigated species (Table 1), with

greatest selectivity for dicarboxylates with a C4 chain (see Figure 3 for succinate). Reduced affinity was found for anions with longer chains, e.g. glutarate (C5) and adipate (C6).

Table 1. Conditional binding constants (as $\text{Log}K_{11}$ values) obtained by UV-vis. titrations on $[\text{Cu}_2\text{L1}]^{4+}$ and $[\text{Cu}_2\text{L2}]^{4+}$ in dioxane/water solution 20% v/v and water, respectively, buffered at pH 7 by HEPES 0.025 M (T 25 °C). Standard deviations are shown in parentheses. Titration data were processed with the Hyperquad package.⁴⁴ See S.I. for details.

anionic guest	$\text{Log}K_{11} [\text{Cu}_2\text{L1}]^{4+}$	$\text{Log}K_{11} [\text{Cu}_2\text{L2}]^{4+}$
succinate	5.60(3)	> 6
glutarate	4.14(3)	5.39(1)
adipate	3.47(1)	2.58(1)
isophthalate	4.98(4)	> 6
fumarate	5.76(2)	> 6
maleate	3.65(5)	2.78(1)

Figure 4. Simplified sketches of the $[\text{Cu}_2\text{L2}(\text{glut})]^{2+}$ molecular cation (left top and left bottom) and of one of the three similar but not symmetrically equivalent $[\text{Cu}_2\text{L2}(\text{isoph})]^{2+}$ molecular cations (right top and right bottom; hydrogen atoms omitted for clarity). Atom names are reported only for Cu, N, and O atom sites.

Further insight into the binding of dicarboxylates to $[\text{Cu}_2\text{L1}]^{4+}$ was gained from comparison with UV-vis. titration results obtained on the related model complex $[\text{Cu}_2\text{L2}]^{4+}$ in water at pH 7 (HEPES 0.025 M; T = 25 °C). **L2** is known from the literature to form stable dimetallic complexes with Zn(II) and Cu(II).⁴⁵ Our UV-vis. studies in neutral aqueous solutions showed that, among dicarboxylates with a C4 chain, fumarate (fum^{2-}) and succinate (suc^{2-}) are the privileged guests of $[\text{Cu}_2\text{L2}]^{4+}$, because of their good fit within the cavity. This result is consistent with the crystal structures of $[\text{Cu}_2\text{L2}(\text{suc})]^{2+}$ and $[\text{Cu}_2\text{L2}(\text{fum})]^{2+}$ reported by Lu et al..⁴⁵

However, also glutarate and isophthalate (i.e. glut^{2-} and isoph^{2-} , respectively) form stable 1:1 complexes with $[\text{Cu}_2\text{L2}]^{4+}$, as is shown by our results in both solution and solid state. In particular, X-ray crystal structures of $[\text{Cu}_2\text{L2}(\text{glut})]^{2+}$ and $[\text{Cu}_2\text{L2}(\text{isoph})]^{2+}$ show that both anionic guests bridge the two metal centers and reside in the cavity of the cryptate, generating cradle-like arrangements (Figure 4). These complexes are similar to those observed for fumarate and succinate. Given the longer backbones of glutarate and isophthalate ions, the Cu(II)⋯Cu(II) separation increases from 8.77(1) Å in $[\text{Cu}_2\text{L2}(\text{suc})]^{2+}$ and $[\text{Cu}_2\text{L2}(\text{fum})]^{2+}$ molecular cations to 8.95(1) Å in $[\text{Cu}_2\text{L2}(\text{glut})]^{2+}$ molecular cation and to 8.92(1), 8.81(1), 8.89(1) Å in the three symmetrically independent $[\text{Cu}_2\text{L2}(\text{isoph})]^{2+}$ molecular cations originating in the solid state (see S.I.).

The binding of suc^{2-} to the lipophilic azacryptate $[\text{Cu}_2\text{L1}]^{4+}$, with the formation of the $[\text{Cu}_2\text{L1}(\text{suc})]^{2+}$ adduct, was confirmed by HRMS-ESI spectroscopy in an acetonitrile solution of $[\text{Cu}_2\text{L1}]^{4+}$ treated with 1 *eqv.* aqueous Na_2suc . The HRMS-ESI spectrum (see S.I.) displays a peak at 796.4592 m/z, attributable to the doubly charged ion $[\text{Cu}_2\text{L1}(\text{suc})]^{2+}$.

Given its high affinity for polycarboxylate guests, we tested the cage-like complex $[\text{Cu}_2\text{L1}]^{4+}$ as a potential extractant of dicarboxylates from neutral water into an immiscible organic solvent, i.e. dichloromethane. The stability of the $[\text{Cu}_2\text{L1}(\text{suc})]^{2+}$ adduct in the chosen solvent was first verified by UV-vis. titration of $[\text{Cu}_2\text{L1}]^{4+}$ (30 μM) with $[\text{TBA}]_2\text{suc}$ in dichloromethane (path length 10 cm; T = 25 °C). This study confirmed the high affinity of suc^{2-} for the azacryptate and the formation of a receptor:anion adduct, with a 1:1 stoichiometry (see S.I.).

We then performed liquid-liquid extraction experiments (Figure 5). A 0.20 mM solution of $[\text{Cu}_2\text{L1}]^{4+}$ in dichloromethane was stirred for 10 minutes in contact with an aqueous solution of 1 mM H_2suc in HEPES 0.050 M at pH 7. The successful extraction of H_2suc from water was demonstrated by recording UV-vis. spectra of the organic phase before and after mixing. Notably, the final spectrum of the dichloromethane phase displayed the same changes in the d-d bands of the $[\text{Cu}_2\text{L1}]^{4+}$ complex as those found upon titration of the azacryptate with $[\text{TBA}]_2\text{suc}$ in this medium. This result indicates that succinate is successfully transferred from neutral water to dichloromethane, where it is stabilized by complexation with $[\text{Cu}_2\text{L1}]^{4+}$.

These initial tests were then followed by quantitative extraction experiments, in which the Suc^{2-} concentration in the aqueous layer was monitored by HPLC–UV. These experiments (see S.I. for experimental details) again employed 0.20 mM solutions of $[\text{Cu}_2\text{L1}]^{4+}$ in dichloromethane and 1.0 mM solutions (i.e. 5-fold excess vs. $[\text{Cu}_2\text{L1}]^{4+}$) of H_2suc in HEPES 0.050 M at pH 7.

Seven extraction experiments were performed independently over non-consecutive days. HPLC–UV analyses indicated that Suc^{2-} was effectively extracted into the organic phase only in the presence of the azacryptate. The measured average extraction yield was $20 \pm 4\%$ ($n=7$, α 0.05), calculated with respect to the initial concentration of Suc^{2-} in the aqueous phase (see S.I. for details). These results indicate that Suc^{2-} is quantitatively extracted into dichloromethane by the cage.

These findings were corroborated by additional trials showing that succinate dianion does not measurably enter the organic phase without complexation by $[\text{Cu}_2\text{L1}]^{4+}$: the organic phase was evaporated to dryness after extraction in the absence of cryptand. Chromatographic analysis of the residue, reconstituted with 1 mL 0.050 M HEPES buffer, showed an undetectable concentration of Suc^{2-} .

It is noteworthy that the experimental value of extraction yield (20%) as determined by HPLC–UV is in full agreement with the data obtained by UV-vis spectroscopy (i.e. by measuring the spectrum of the $[\text{Cu}_2\text{L1}]^{4+}$ in the organic phase before and after extraction). Importantly, this yield corresponds to the theoretical maximum extraction yield assuming that Suc^{2-} is extracted by complexation of the cage in a 1:1 molar ratio.

In conclusion, we demonstrate that lipophilic cage-like complexes can be successfully applied in the extraction of highly hydrated polyanionic species from water. The quantitative extraction of a dicarboxylate anion (i.e. succinate) was achieved here by employing a suitable lipophilic azacryptate, capable of stabilizing the polyanion in dichloromethane through encapsulation in the cavity. Given the relevance of polycarboxylates as normal metabolites and pathological oncometabolites, as well as industrial waste products, our results open new possibilities for research and application in selective recognition, trapping and extraction of target polycarboxylates..

Figure 5. Quantitative extraction of Suc^{2-} from an aqueous solution, buffered at pH 7 by HEPES 0.050M, to dichloromethane (DCM) using the $[\text{Cu}_2\text{L1}]^{4+}$ complex (0.20 mM) as the extractant.

ASSOCIATED CONTENT

Supporting Information

Materials, methods and experimental details. Synthesis and characterization of **L1**, NMR and HRMS-ESI spectra. Additional material regarding UV-vis. titrations, anion binding studies, potentiometric investigations, extraction experiments.

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‡These authors contributed equally. R. Mobili and S. La Cognata synthesized **L1** and **L2**, performed the UV-vis. investigations, the determination of binding constants, performed the extraction experiments and contributed to the preparation of the manuscript; M. Boiocchi and T. Recca performed X-ray diffraction and NMR studies, respectively. F. Merlo and A. Speltini were involved in the quantification of the succinate anion by HPLC–UV; L. Maher III contributed to writing.

Notes

The authors declare no competing financial interests.

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SUPPLEMENTARY SECTION

Succinate extraction from water using a cage-like dicopper complex

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1. Materials and methods

All reagents for syntheses were purchased from Sigma-Aldrich and used without further purification. All reactions were performed under dinitrogen. High-resolution mass spectra were recorded on a Thermo Scientific Q Exactive Plus instrument. ^1H - and ^{13}C -NMR spectra were recorded on Bruker AVANCE 400 (operating at 9.37 T, 400 MHz). 1,5-di(hexyloxy)naphthalene-2,6-dicarboxaldehyde, $[\text{TBA}]_2\text{suc}$ and **L2** were synthesized following known procedures.¹⁻³

1.1 Synthesis of L1

A solution of 1,5-di(hexyloxy)naphthalene-2,6-dicarboxaldehyde (0.12 g; 0.31 mmol; 3.0 eqv) in dichloromethane (DCM; 10 mL) and MeOH (70 mL) was added to a solution of AgNO_3 (0.05 g; 0.31 mmol; 3.0 eqv) in MeOH (70 mL). The reaction mixture was then refluxed under Ar atmosphere. A solution of tris(2-aminoethyl)amine (0.03 mL; 0.21 mmol; 2.0 eqv) in MeOH (50 mL) was then slowly added dropwise and the reaction was refluxed for 24 hours. The solution containing the polyimine cage compound was then transferred in a round bottom flask and DCM was evaporated under vacuum. The methanol solution was refluxed again and NaBH_4 (3.0 g) was added in small portions in order to reduce both imine bonds and Ag^+ . After 12 hours the mixture was cooled to room temperature and filtered to eliminate Ag^0 particles. The solvent was evaporated under vacuum to give a white solid that was dissolved with brine (200 mL) and extracted with DCM (4 x 50 mL). The collected organic phase was then dried with Na_2SO_4 . The solvent was evaporated under vacuum to obtain 0.13 g of a yellowish dense oil. Yield: 91%

^1H -NMR (400 MHz, d_6 -DMSO + HNO_3 , $T=80^\circ\text{C}$) δ : 7.84-7.61 (6H, naph), 4.44 (m, 12H, N-CH_2 -naph), 3.91 (m, 12H, O-CH_2 -C), 3.20–2.80 (br. m, 24H, $\text{N-CH}_2\text{CH}_2$ -N), 1.97 (m, 12H, OCH_2 - CH_2 -C), 1.51–1.27 (m, 36H, $\text{O}(\text{CH}_2)_2$ -(CH_2)₃-C), 0.91 (t, 18H, $-\text{CH}_3$); ^{13}C -NMR (100 MHz, d_6 -DMSO, $T=298\text{K}$) δ : 154.02, 129.16, 126.89, 122.22, 118.91, 76.32, 51.07, 45.68, 45.26, 31.78, 30.29, 25.53, 22.61, 14.40.

HRMS-ESI (MeOH) m/z : $[\text{M}+\text{H}^+]^+$ calculated for $\text{C}_{84}\text{H}_{132}\text{N}_8\text{O}_6$, 1350.0348, found 1350.0529; $[\text{M}+2\text{H}^+]^{2+}$ calculated 675.5208, found 675.5296; $[\text{M}+3\text{H}^+]^{3+}$ calculated 450.6829, found 450.6889.

2. Potentiometric titrations

Protonation constants of ligand **L1** were determined in dioxane/water (20% v/v) mixture, 0.05 M in [TBA]NO₃. In a typical experiment, 15 mL of a 4×10^{-4} M ligand solution was treated with an excess of HNO₃ 1.0 M. Titrations were performed by addition of 10 μ L aliquots of carbonate-free standard 0.1 M NaOH, recording 80-100 points for each titration. Complexation constants were determined by performing a potentiometric titration experiment, working in the presence of 2 *eqv.* of Cu(NO₃)₂. Prior to each potentiometric titration, the standard electrochemical potential (E°) of the glass electrode was determined in the dioxane/water mixture, by a titration experiment according to the Gran method⁴. Protonation and complexation titration data (emf vs. mL of NaOH) were processed with the Hyperquad package⁵ to estimate the equilibrium constants.

The same procedure was followed for the determination of the protonation constants of succinic and glutaric acids in dioxane/water (20% v/v) mixture, 0.05 M in [TBA]NO₃. In a typical experiment, 15 mL of a 1×10^{-3} M ligand solution were treated with an excess of a 1.0 M HNO₃ standard solution.

pH-spectrophotometric titrations were also performed on solutions of the dicopper(II) azacryptate 4×10^{-4} M in dioxane/water (20% v/v) mixture (0.05 M [TBA]NO₃, 25°C). In a typical titration, aliquots of carbonate-free standard 0.1 M NaOH were added to the solution of the azacryptate, both the electrochemical potential and the UV-vis. spectrum of the solution were recorded after each addition. Prior to the titration, the standard electrochemical potential (E°) was determined by the Gran method.

2.1. Protonation constants of L1

Best fits of the potentiometric titration profiles were obtained by assuming the presence of six protonated species at the equilibrium over the course of the potentiometric experiment. The calculated protonation constants (Table S1) allowed calculation of the species distribution diagram (as % abundance with respect to **L1** vs. pH) reported in Figure S1.

Table S1. Protonation equilibria and the corresponding constants for **L1** (L in the Table) in dioxane/water 20% v/v (T=25 °C). Standard deviation in parentheses.

Equilibria	Log β
L1 + H ⁺ $\rightleftharpoons$ L1H ⁺	9.03(8)
L1 + 2H ⁺ $\rightleftharpoons$ L1H ₂ ²⁺	17.74(4)
L1 + 3H ⁺ $\rightleftharpoons$ L1H ₃ ³⁺	25.72(6)
L1 + 4H ⁺ $\rightleftharpoons$ L1H ₄ ⁴⁺	32.66(6)
L1 + 5H ⁺ $\rightleftharpoons$ L1H ₅ ⁵⁺	39.25(6)
L1 + 6H ⁺ $\rightleftharpoons$ L1H ₆ ⁶⁺	44.50(7)

Figure S1. Distribution diagram of species present at equilibrium over the course of the potentiometric titration of **L1** (4×10^{-4} M), noted as **L** in the figure, with standard NaOH in dioxane/water (20% v/v) ($T=25$ °C).

2.2. Complexation of **L1** with Cu(II)

Best fitting was obtained by assuming the formation of the following complex species over the course of the titration: $[\text{Cu}(\text{L1H}_3)]^{5+}$, $[\text{Cu}_2(\text{L1})]^{4+}$, $[\text{Cu}_2(\text{L1})\text{OH}]^{3+}$, $[\text{Cu}_2(\text{L1})(\text{OH})_2]^{2+}$. The corresponding cumulative constants (as $\text{Log}\beta$) are shown in Table S2. The distribution diagram (as % abundance vs. pH is reported in the main text, see Figure 2).

Table S2. Complexation constants for **L1** with Cu(II) in dioxane/water 20% v/v ($T=25$ °C). Standard deviation in parentheses.

Equilibria	$\text{Log}\beta$
$\text{L1} + \text{Cu}^{2+} + 3\text{H}^+ \rightleftharpoons [\text{Cu}(\text{L1H}_3)]^{5+}$	35.37(3)
$\text{L1} + 2\text{Cu}^{2+} \rightleftharpoons [\text{Cu}_2(\text{L1})]^{4+}$	24.60(7)
$\text{L1} + 2\text{Cu}^{2+} + \text{H}_2\text{O} \rightleftharpoons [\text{Cu}_2(\text{L1})\text{OH}]^{3+} + \text{H}^+$	12.4(1)
$\text{L1} + 2\text{Cu}^{2+} + 2\text{H}_2\text{O} \rightleftharpoons [\text{Cu}_2(\text{L1})(\text{OH})_2]^{2+} + 2\text{H}^+$	1.8(1)

2.3. Potentiometric titrations on H_2suc and H_2glut in dioxane/water 20% v/v

Potentiometric titrations were also performed on two representative dicarboxylic acids, H_2suc and H_2glut , in order to determine their pK_a values in dioxane/water mixture 20% v/v (0.05 M $[\text{TBA}]\text{NO}_3$, 25°C). In pure water at pH 7, both acids are largely deprotonated, with pK_{a1} and pK_{a2} :

4.21 and 5.41 for H_2suc ; 4.34 and 5.22 at 25 °C for H_2glut .⁶ In the chosen mixture, deprotonation to form the dicarboxylate anion is significantly shifted toward basic pH values for both H_2suc and H_2glut . The potentiometric titrations allowed estimation of pK_a values, which were found to be $\text{pK}_{a1} = 6.89(4)$ and $\text{pK}_{a2} = 8.74(6)$ for H_2suc , $\text{pK}_{a1} = 7.55(5)$ and $\text{pK}_{a2} = 8.36(7)$ for H_2glut . The corresponding distribution diagram of the species, as % abundance vs. pH, are reported in Figures S2-S3.

Figure S2. Distribution diagram of the species present at the equilibrium over the course of the potentiometric titration of H_2suc (1×10^{-3} M) with standard NaOH in dioxane/water 20% v/v ($T=25$ °C).

Figure S3. Distribution diagram of the species present at the equilibrium over the course of the potentiometric titration of H_2glut (1×10^{-3} M) with standard NaOH in dioxane/water 20% v/v ($T=25$ °C).

3. Spectrophotometric titrations

UV-vis. spectra were collected using a Varian Cary 50 SCAN spectrophotometer, with quartz cuvettes of the appropriate path length (1 or 10 cm) at $25.0 \pm 0.1^\circ\text{C}$. Solvents were dried by common methods. In a typical experiment, a concentrated solution of the dicopper(II) azacryptate in DMSO was diluted with buffer at pH 7, i.e. 0.025M HEPES in either dioxane/water (20% v/v) for $[\text{Cu}_2(\text{L1})]^{4+}$ or pure water for $[\text{Cu}_2(\text{L2})]^{4+}$: the final concentration of the azacryptate ranged between 50-500 μM . The buffered solution of the azacryptate was then titrated with a standard solution of either the chosen dicarboxylic acid or the corresponding disodium salt, in the case of phthalates. After each addition of the titrant solution, the UV-vis. spectrum was recorded. It has to be noticed that, with terephthalate and phthalate anions, the formation of a precipitate over the course of the titration prevented us to safely determine the association constants. The concentration of the azacryptate solution was chosen on the basis of the p-parameter ($p = [\text{concentration of the azacryptate:anion adduct}] / [\text{maximum possible concentration of the azacryptate:anion adduct}]$), which should range between 0.2 and 0.8.⁷⁻⁸ Titration data were processed with the Hyperquad package⁵ to estimate equilibrium constants. In some cases, even working with very dilute solutions of the azacryptate, the curvature of the titration profile was too sharp to allow a safe calculation of the binding constant. In these cases, the distribution diagrams were traced assuming an estimated value of the constant (obtained using Hyperquad package).

3.1. UV-vis. titrations of $[\text{Cu}_2(\text{L1})]^{4+}$ in dioxane/water 20% v/v at pH 7

Figure S4. Left: UV-vis. spectra taken upon titration of $[\text{Cu}_2(\text{L1})]^{4+}$ 50 μM with succinate 12.5 mM in dioxane/water 20% v/v at pH 7 (HEPES 0.025 M, path length 10 cm). The red and blue lines represent the spectra at 0 and 5 eqv. of the added anion, respectively. Right: titration profile at 400 nm (red triangles) with superimposed distribution diagram of the species $[\text{Cu}_2(\text{L1})]^{4+}$ (solid line) and $[\text{Cu}_2(\text{L1})(\text{suc})]^{2+}$ vs. equivalents of the added guest (dotted line), calculated for $\text{Log}K_{11}=5.60(3)$.

Figure S5. Left: UV-vis. spectra taken upon titration of $[Cu_2(L1)]^{4+}$ 0.20 mM with glutarate 10 mM in dioxane/water 20% v/v at pH 7 (HEPES 0.025 M, path length 1 cm). The red and blue lines represent the spectra at 0 and 50 eqv. of the added anion, respectively. Right: titration profile at 600 nm (red triangles) with the superimposed distribution diagram of the species $[Cu_2(L1)]^{4+}$ (solid line) and $[Cu_2(L1)(glut)]^{2+}$ (dotted line) vs. equivalents of the added guest, calculated for $LogK_{IJ}=4.14(3)$.

Figure S6. Left: UV-vis. spectra taken upon titration of $[Cu_2(L1)]^{4+}$ 0.55 mM with adipate 0.10 M in dioxane/water 20% v/v at pH 7 (HEPES 0.025 M, path length 1 cm). The red and blue lines represent the spectra at 0 and 8.5 eqv. of the added anion, respectively. Right: titration profile at 900 nm (red triangles) with the superimposed distribution diagram of the species $[Cu_2(L1)]^{4+}$ (solid line) and $[Cu_2(L1)(adip)]^{2+}$ (dotted line) vs. equivalents of the added guest, calculated for $LogK_{IJ}=3.47(1)$.

Figure S7. Left: UV-vis. spectra taken upon titration of $[\text{Cu}_2(\text{L1})]^{4+}$ 50 μM with isophthalate 12.5 mM in dioxane/water 20% v/v at pH 7 (HEPES 0.025 M, path length 10 cm). The red and blue lines represent the spectra at 0 and 5 eqv. of the added anion, respectively. Right: titration profile at 400 nm (red triangles) with superimposed distribution diagram of the species $[\text{Cu}_2(\text{L1})]^{4+}$ (solid line) and $[\text{Cu}_2(\text{L1})(\text{isoph})]^{2+}$ (dotted line) vs. equivalents of the added guest, calculated for $\text{Log}K_{11}=4.98(4)$.

Figure S8. Left: UV-vis. spectra taken upon titration of $[\text{Cu}_2(\text{L1})]^{4+}$ 50 μM with fumarate 12.5 mM in dioxane/water 20% v/v at pH 7 (HEPES 0.025 M, path length 10 cm). The red and blue lines represent the spectra at 0 and 5 eqv. of the added anion, respectively. Right: titration profile at 643 nm (red triangles) with superimposed distribution diagram of the species $[\text{Cu}_2(\text{L1})]^{4+}$ (solid line) and $[\text{Cu}_2(\text{L1})(\text{fum})]^{2+}$ (dotted line) vs. equivalents of the added guest, calculated for $\text{Log}K_{11}=5.76(2)$.

Figure S9. Left: UV-vis. spectra taken upon titration of $[Cu_2(L1)]^{4+}$ 0.55 mM with maleate 0.10 M in dioxane/water 20% v/v at pH 7 (HEPES 0.025 M, path length 1 cm). The red and blue lines represent the spectra at 0 and 4 eqv. of the added anion, respectively. Right: titration profile at 900 nm (red triangles) with superimposed distribution diagram of the species $[Cu_2(L1)]^{4+}$ (solid line) and $[Cu_2(L1)(mal)]^{2+}$ (dotted line) vs. equivalents of the added guest, calculated for $\text{Log}K_{II}=3.65(5)$.

3.2. UV-vis. titrations of $[Cu_2(L2)]^{4+}$ in aqueous solution at pH 7

For the determination of binding constants, the solution of receptor was titrated with at least 100-fold more concentrated solution of the acid of the titled anion. For the titration with isophthalate, the corresponding Na^+ salt was used.

Figure S10. Left: UV-vis. spectra taken upon titration of $[Cu_2(L2)]^{4+}$ 50 μM with succinate 5 mM in water at pH 7 (HEPES 0.050 M, path length 10 cm). The red and blue lines represent the spectra at 0 and 5 eqv. of the added anion, respectively. Right: titration profile at 680 nm (red triangles) with the superimposed distribution diagram of the species $[Cu_2(L2)]^{4+}$ (solid line) and $[Cu_2(L2)(suc)]^{2+}$ (dotted line) vs. equivalents of the added guest, calculated for an hypothetical $\text{Log}K_{II}$ value of 6.5 units.

Figure S11. Left: UV-vis. spectra taken upon titration of $[Cu_2(L2)]^{4+}$ 50 μM with glutarate 5 mM in water at pH 7 (HEPES 0.050 M, path length 10 cm). The red and blue lines represent the spectra at 0 and 8 eqv. of the added anion, respectively. Right: titration profile at 687 nm (red triangles) with superimposed distribution diagram of the species $[Cu_2(L2)]^{4+}$ (solid line) and $[Cu_2(L2)(glut)]^{2+}$ (dotted line) vs. equivalents of the added guest, calculated for $\text{Log}K_{II}=5.39(1)$.

Figure S12. Left: UV-vis. spectra taken upon titration of $[Cu_2(L2)]^{4+}$ 0.55 mM with adipate 50 mM in water at pH 7 (HEPES 0.05 M, path length 1 cm). The red and blue lines represent the spectra at 0 and 8 eqv. of the added anion, respectively. Right: titration profile at 830 nm (red triangles) with superimposed distribution diagram of the species $[Cu_2(L2)]^{4+}$ (solid line) and $[Cu_2(L2)(adip)]^{2+}$ (dotted line) vs. equivalents of the added guest, calculated for $\text{Log}K_{II}=2.58(1)$.

Figure S13. Left: UV-vis. spectra taken upon titration of $[Cu_2(L2)]^{4+}$ 0.50 mM with isophthalate 50 mM water at pH 7 (HEPES 0.25 M, path length 1 cm). The red and blue lines represent the spectra at 0 and 3 eqv. of the added anion, respectively. Right: titration profile at 670 nm (red triangles) with superimposed distribution diagram of the species $[Cu_2(L2)]^{4+}$ (solid line) and $[Cu_2(L2)(isoph)]^{2+}$ (dotted line) vs. equivalents of the added guest, calculated for an hypothetical $LogK_{II}$ value of 6.5 units.

Figure S14. Left: UV-vis. spectra taken upon titration of $[Cu_2(L2)]^{4+}$ 50 μ M with fumarate 5 mM in water at pH 7 (HEPES 0.05 M, path length 10 cm). The red and blue lines represent the spectra at 0 and 5 eqv. of the added anion, respectively. Right: titration profile at 683 nm (red triangles) with superimposed distribution diagram of the species $[Cu_2(L2)]^{4+}$ (solid line) and $[Cu_2(L2)(fum)]^{2+}$ (dotted line) vs. equivalents of the added guest, calculated for an hypothetical $LogK_{II}$ value of 6.5 units.

Figure S15. Left: UV-vis. spectra taken upon titration of $[Cu_2(L2)]^{4+}$ 0.50 mM with maleate 50 mM in water at pH 7 (HEPES 0.05 M, path length 1 cm). The red and blue lines represent the spectra at 0 and 9 eqv. of the added anion, respectively. Right: titration profile at 870 nm (red triangles) with superimposed distribution diagram of $[Cu_2(L2)]^{4+}$ (solid line) and $[Cu_2(L2)(mal)]^{2+}$ (dotted line) vs. equivalents of the added guest, calculated for $\text{Log}K_{11}=2.78(1)$.

3.3. UV-vis. titration of $[Cu_2(L1)]^{4+}$ with succinate in dichloromethane

Figure S16. Left: UV-vis. spectra taken upon titration of $[Cu_2(L1)]^{4+}$ 30 μ M with $[TBA]_2$ succinate 5 mM in dichloromethane (path length 10 cm). The red and blue lines represent the spectra at 0 and 2.2 eqv. of the added anion, respectively. Right: titration profile at 650 nm (red triangles) with superimposed distribution diagram of $[Cu_2(L1)]^{4+}$ (solid line) and $[Cu_2(L1)(suc)]^{2+}$ (dotted line), calculated for an hypothetical $\text{Log}K_{11}$ value of 6.5 units.

4. X-Ray diffraction studies

Single crystals suitable for X-Ray diffraction studies were obtained by slow evaporation of an aqueous solution containing the relevant dicarboxylate sodium salt and the in-situ prepared complex: $[\text{Cu}_2(\text{L2})](\text{CF}_3\text{SO}_3)_4$; in a 1:1 molar ratio.

Diffraction data for $[\text{Cu}_2(\text{L2})(\text{glut})](\text{CF}_3\text{SO}_3)_2 \cdot 4(\text{H}_2\text{O})$ crystal (greenish azure, $0.30 \times 0.26 \times 0.04 \text{ mm}^3$), and $[\text{Cu}_2(\text{L2})(\text{isoph})]_3(\text{CF}_3\text{SO}_3)_6 \cdot 6(\text{H}_2\text{O})$ crystal (greenish azure, $0.42 \times 0.20 \times 0.05 \text{ mm}^3$) were collected by means of a Bruker-AXS diffractometer equipped with the SMART-APEX CCD detector. Data collection was performed at room temperature with $\text{MoK}\alpha$ X-radiation ($\lambda=0.7107 \text{ \AA}$) and crystal data were reported on Table S3.

CCD frames were processed with the SAINT software⁹ and intensities were corrected for Lorentz and polarization effects; absorption effects were empirically evaluated by the SADABS software¹⁰ and absorption correction was applied to the data. All crystal structures were solved by direct methods (SIR 97)¹¹ and refined by full-matrix least-squares procedures on F^2 using all reflections (SHELXL-2018)¹². Anisotropic displacement parameters were used for all non-hydrogen atoms. Hydrogens were placed at calculated positions and their positions refined according to a riding model.

In general, the X-ray diffraction quality of both synthetic crystals was poor and the $[\text{Cu}_2(\text{L2})(\text{isoph})]_3(\text{CF}_3\text{SO}_3)_6 \cdot 6(\text{H}_2\text{O})$ crystal diffraction was particularly poor (diffracted intensities at θ angle greater than 22° not observable with the available diffractometer). The poor diffraction quality resulted in a low accuracy for the atom positions of the triflate counterions and of the water solvent molecules. In particular, the large atom displacement parameters for atoms belonging to the counterions and to the water solvent molecules suggested the presence of unresolved positional disorder and imposed the use of soft geometrical restraints in the refinement of these atom positions. The crystal structure of the $[\text{Cu}_2(\text{L2})(\text{glut})](\text{CF}_3\text{SO}_3)_2 \cdot 4(\text{H}_2\text{O})$ compound was refined restraining the molecular geometry of the triflate counterions to be similar by using the SAME instruction. In this crystal, some alternative positions for one triflate counterion were identified. The crystal structure of the $[\text{Cu}_2(\text{L2})(\text{isoph})]_3(\text{CF}_3\text{SO}_3)_6 \cdot 6(\text{H}_2\text{O})$ was complicated by the presence of three similar but not symmetrically equivalent $[\text{Cu}_2(\text{L2})(\text{isoph})]^{2+}$ anionic complexes. This compound was refined restraining the molecular geometry of the triflate counterions to the ideal one (by using the DFIX and DANG instructions) and restraining the three arms of each $[\text{Cu}_2(\text{L2})]^{4+}$ dimetallic cage to show similar geometrical features (by using the SAME instruction). For both crystal structures, restraints on the atom displacement parameters (DELU and ISOR) were applied for atoms showing large and elongated thermal ellipsoids and positions for H atoms belonging to water solvent molecules were not determined.

Table S3. Crystal data for the studied compounds.

	$[\text{Cu}_2(\text{L2})(\text{glut})](\text{CF}_3\text{SO}_3)_2 \cdot 4(\text{H}_2\text{O})$	$[\text{Cu}_2(\text{L2})(\text{isoph})]_3(\text{CF}_3\text{SO}_3)_6 \cdot 6(\text{H}_2\text{O})$
Formula	$\text{C}_{55}\text{H}_{74}\text{Cu}_2\text{F}_6\text{N}_8\text{O}_{14}\text{S}_2$	$\text{C}_{174}\text{H}_{204}\text{Cu}_6\text{F}_{18}\text{N}_{24}\text{O}_{36}\text{S}_6$
<i>M</i>	1376.42	4123.20
Crystal system	monoclinic	triclinic
Space group	$P 2_1/c$ (no. 14)	$P -1$ (no. 2)
<i>a</i> (Å)	18.611(2)	18.547(3)
<i>b</i> (Å)	20.114(2)	21.950(4)
<i>c</i> (Å)	16.353(2)	23.907(4)

α (°)	90	100.574(2)
β (°)	96.2020(10)	102.098(2)
γ (°)	90	96.155(2)
V (Å ³)	6085.6(10)	9247(3)
Z	4	2
ρ_{calcd} (g·cm ⁻³)	1.502	1.481
θ max (°)	25	22
Measured reflections	42829	47534
Unique reflections	10700	22705
R_{int}	0.047	0.045
Strong data [$I_0 > 2\sigma(I_0)$]	7164	10718
$R1, wR2$ strong data	0.0857, 0.2437	0.1454, 0.3889
$R1, wR2$ all data	0.1221, 0.2784	0.2256, 0.4473
GoF	1.045	1.369
max/min residuals (eÅ ⁻³)	1.05/-0.84	1.28/-0.92

In spite of the poor quality of the available single crystals, the results of the crystallographic study are considered suitable to the aims of this work. In particular, the $[\text{Cu}_2(\text{L2})]^{4+}$ dimetallic cage exhibits very similar molecular geometries in all moieties occurring in the two studied crystals. Furthermore, the C5-backbone common to the two dicarboxylate ions, *i.e.* the two terminal carbon atoms of the carboxylic groups and the three intermediate carbon atoms connecting them, were similarly positioned within the cryptand cavity. Figure S17 shows simplified sketches emphasizing the similarity among the molecular cage structures containing glutarate and the three different structures of the adduct with isophthalate. Figure S18 shows thermal ellipsoids of the dimetallic cages interacting with the two dicarboxylates.

For all studied complexes, the anionic guest resides in the cryptate cavity, exhibiting a cradle-like arrangement previously observed for the $[\text{Cu}_2(\text{L2})]^{4+}$ complex interacting with fumarate and succinate ions.³ The two metal centers are bridged by the dicarboxylate ion and each Cu(II) cation is penta-coordinated by four N atoms of the *tren* subunit and by a carbonyl oxygen atom of the dicarboxylate. The incorporation of both glutarate and isophthalate ions into the receptor's cavity occurs through an increase of the Cu(II)···Cu(II) separation of the dimetallic cage respect to the value observed in presence of fumarate and succinate ions. The Cu(II)···Cu(II) separations are 8.95(1) Å in $[\text{Cu}_2(\text{L2})(\text{glut})]^{2+}$ and 8.92(1), 8.81(1), and 8.89(1) Å for the three independent $[\text{Cu}_2(\text{L2})(\text{isoph})]^{2+}$ cations, respectively, whereas the Cu(II)···Cu(II) separations are 8.77(1) Å in both $[\text{Cu}_2(\text{L2})(\text{fum})]^{2+}$ and $[\text{Cu}_2(\text{L2})(\text{suc})]^{2+}$ compounds.³ Geometrical features of the metal-center coordinations are reported in Table S4.

Figure S17. From the left, simplified sketches of the $[\text{Cu}_2(\text{L2})(\text{glut})]^{2+}$ molecular cation and of the three independent $[\text{Cu}_2(\text{L2})(\text{isoph})]^{2+}$ molecular cations forming at the solid state. Sketches are drawn along two similar viewing

directions in order to emphasize their geometrical similitudes (hydrogen atoms were omitted for clarity). Atom names are reported only for Cu, N, and O atom sites.

Figure S18. From the left, plot showing thermal ellipsoids for the $[\text{Cu}_2(\text{L2})(\text{glut})]^{2+}$ molecular cation and of the three independent $[\text{Cu}_2(\text{L2})(\text{isoph})]^{2+}$ molecular cations (ellipsoids are drawn at the 30% probability level).

As for the compounds in the literature, the best plane of the dicarboxylate moieties remains almost parallel to the best plane of the naphthalene ring forming the two lateral arms of the cage (the dihedral angles for the studied molecular compounds are in the range $0.8\text{--}14.1^\circ$). However, the separations between the centroid of the two naphthalene rings and the centroid of the dicarboxylate moieties are longer than observed, for instance, in the compound containing the unsaturated fumarate ion, thus suggesting minor face-to-face π -stacking interactions in presence of glutarate and isophthalate ions.

In particular, the two centroid-centroid separations in $[\text{Cu}_2(\text{L2})(\text{glut})]^{2+}$ compound are 3.50 and 3.51 Å, whereas those in the three independent $[\text{Cu}_2(\text{L2})(\text{isoph})]^{2+}$ molecular cations are 3.70 and 3.81 Å, 3.97 and 4.13 Å, 3.77 and 3.80 Å, respectively. These values are to be compared to the centroid-centroid separations of 3.33 and 3.37 Å occurring in the $[\text{Cu}_2(\text{L2})(\text{fum})]^{2+}$ compound.

Table S4. Bond distances (Å) and bond angles ($^\circ$) for the metal centers in the $[\text{Cu}_2(\text{L2})(\text{glut})]^{2+}$ and in the three not symmetrically equivalent $[\text{Cu}_2(\text{L2})(\text{isoph})]^{2+}$ molecular cations.

	$[\text{Cu}_2(\text{L2})(\text{glut})]^{2+}$	$[\text{Cu}_2(\text{L2})(\text{isoph})]^{2+}$		
Cu(1)-O(1)	1.934(4)	1.957(9)	1.977(10)	1.947(12)
Cu(1)-N(1)	2.036(6)	2.061(12)	1.98(2)	2.06(2)
Cu(1)-N(2)	2.377(5)	2.371(14)	2.349(14)	2.37(2)
Cu(1)-N(3)	2.144(5)	2.105(11)	2.16(2)	2.154(14)
Cu(1)-N(4)	2.133(5)	2.097(13)	1.93(2)	2.12(2)
Cu(2)-O(3)	1.918(5)	1.933(8)	1.952(10)	1.96(2)
Cu(2)-N(5)	2.043(6)	2.035(12)	2.11(2)	2.12(2)

Cu(2)-N(6)	2.402(6)	2.445(11)	2.377(13)	2.361(14)
Cu(2)-N(7)	2.128(6)	2.101(10)	2.074(14)	2.117(13)
Cu(2)-N(8)	2.155(6)	2.156(10)	2.09(2)	2.172(13)
O(1)-Cu(1)- N(1)	168.7(2)	168.1(5)	166.5(7)	168.3(8)
O(1)-Cu(1)- N(2)	109.56(18)	110.5(4)	115.3(5)	112.2(7)
O(1)-Cu(1)- N(3)	90.94(19)	92.2(4)	92.1(5)	92.8(5)
O(1)-Cu(1)- N(4)	95.40(18)	95.3(4)	92.0(6)	95.7(6)
N(1)-Cu(1)- N(2)	81.7(2)	81.5(5)	77.9(7)	79.5(7)
N(1)-Cu(1)- N(3)	84.7(2)	83.5(5)	86.8(9)	83.1(6)
N(1)-Cu(1)- N(4)	83.0(2)	83.0(5)	82.3(9)	82.0(7)
N(2)-Cu(1)- N(3)	107.3(2)	109.0(6)	104.3(6)	106.5(6)
N(2)-Cu(1)- N(4)	99.81(19)	96.7(6)	100.9(8)	100.3(8)
N(3)-Cu(1)- N(4)	148.3(2)	148.7(5)	149.8(9)	146.3(9)
O(3)-Cu(2)- N(5)	167.8(2)	170.8(4)	165.7(5)	168.1(7)
O(3)-Cu(2)- N(6)	111.7(2)	109.2(4)	113.1(4)	111.8(6)
O(3)-Cu(2)- N(7)	94.1(2)	96.8(4)	97.3(5)	96.9(5)
O(3)-Cu(2)- N(8)	91.2(2)	90.9(4)	91.9(5)	90.2(6)
N(5)-Cu(2)- N(6)	80.4(2)	79.7(4)	80.5(5)	79.7(6)
N(5)-Cu(2)- N(7)	83.8(3)	83.4(4)	83.6(7)	83.7(5)
N(5)-Cu(2)- N(8)	84.6(2)	84.4(4)	80.5(7)	83.4(6)
N(6)-Cu(2)- N(7)	100.5(2)	100.4(4)	100.9(6)	100.6(6)
N(6)-Cu(2)- N(8)	105.7(2)	104.9(4)	102.6(6)	104.8(6)
N(7)-Cu(2)- N(8)	149.1(2)	149.5(4)	148.9(6)	148.8(6)

5. HRMS-ESI spectra of $[\text{Cu}_2(\text{L1})(\text{suc})]^{2+}$

Figure S19. HRMS-ESI spectrum of a solution of $[\text{Cu}_2(\text{L1})]^{4+}$ in CH_3CN , containing 1 *eqv.* of Na_2suc . Up: zoom scan of the peak at 796.5492 m/z obtained from the experimental HRMS-ESI spectrum. Bottom: calculated spectrum of the adduct $[\text{Cu}_2(\text{L1})(\text{suc})]^{2+}$.

6. Extraction experiments.

6.1 Experimental details

Extraction experiments were performed with solutions of succinic acid (1.0 mM), prepared in aqueous buffer (0.05 M HEPES) at pH 7, and an organic phase obtained by dilution with dichloromethane (DCM) of a stock solution of $[\text{Cu}_2(\text{L1})](\text{NO}_3)_4$ (9 mM) in DMSO. The final concentration of $[\text{Cu}_2(\text{L1})]^{4+}$ in DCM was 0.20 mM [the % DMSO into the final solution being about 2% v/v].

The extraction of succinate into DCM was first verified by recording the UV-vis. spectrum of the solution, before and after mixing with the aqueous phase (Figure S20). The changes in the d-d bands at about 680 and 820 nm, before and after extraction, can be attributed to the binding of succinate to the dicopper azacryptate in DCM. The formation of the 1:1 adduct, $[\text{Cu}_2(\text{L1})(\text{suc})]^{2+}$, is confirmed by comparing the spectra shown in Fig. S20 with those obtained upon titration of $[\text{Cu}_2(\text{L1})]^{4+}$ with $[\text{TBA}]_2\text{suc}$ in DCM (see Fig. S16).

Figure S20. UV-vis. spectra of the solution of $[\text{Cu}_2(\text{L1})]^{4+}$ (0.20 mM, path length = 1 cm) in DCM before (green line) and after contact (blue line) with an aqueous solution of succinate (1.0 mM).

The extraction of succinate from water into DCM was quantitatively evaluated by comparing the dicarboxylate concentration in the buffered aqueous solution, before and after mixing with the solution of $[\text{Cu}_2(\text{L1})]^{4+}$ in DCM. The solutions were vigorously stirred for 20 minutes and then allowed to separate for 10 minutes. A blank experiment was run by mixing the aqueous solution of succinate with pure DCM under the same conditions as in the cryptate-mediated extraction. The procedure (for both extraction and blank experiments) was repeated seven times. For each repeat, the stock solution of succinic acid was freshly prepared in order to reduce experimental errors.

6.1.1. HPLC-UV chromatographic analysis

HPLC gradient grade acetonitrile and ultrapure water were purchased from VWR (Milan, Italy). The HPLC-UV apparatus consisted of a Shimadzu (Milan, Italy) LC-20AT solvent delivery module equipped with a DGU-20A3 degasser and interfaced with a SPD-20A UV detector. A Sepachrom

Adamas C18-AQ (250 × 4.6 mm, 5 μm) column coupled with a Supelco Supelguard Ascentis C18 (20 × 2.1 mm, 5 μm) guard-column was used. After an equilibration period of 5 min, 20 μL of each sample was injected in the HPLC system. The mobile phases were (a) 25 mM aqueous H₃PO₄ (pH 2.5) and (b) acetonitrile, working with an isocratic elution 10% B for 7 min, followed by a washing step (linear gradient to 70% B until 9 min, hold for 3 min). The flow rate was 0.8 mL min⁻¹ and the detection wavelength was 214 nm.

Linearity was evaluated by analysis of succinate standard solutions prepared by dissolving succinic acid in HEPES buffer (0.050 M, pH 7) in the concentration range 25-200 mg L⁻¹ starting from a 1000 mg L⁻¹ stock solution; examples of chromatograms are shown in Figure S21.

Figure S21. HPLC-UV chromatograms overlay of succinate standard solutions: 25 mg L⁻¹ (violet line), 50 mg L⁻¹ (blue line), 100 mg L⁻¹ (black line), 150 mg L⁻¹ (red line). Succinate retention time: 6.1 min (peak zoom in the inset).

Good linearity was observed on three independent five-point calibration lines ($r^2 > 0.9982$); an example of calibration curve graph is reported below:

The percent loss of succinate in the aqueous phase in the extraction experiment was quantified by comparison with the analyte chromatographic peak obtained in the blank experiments,

performed in parallel under the same conditions (but omitting the dicopper complex in the organic phase). An example of the chromatographic traces obtained in the extraction experiments is shown in Figure S22.

Figure S22. HPLC-UV chromatograms overlay of a succinate sample (in 0.050 M HEPES buffer) after contact with dichloromethane containing the dicopper cryptate (black line) and after contact with dichloromethane (2.2% v/v DMSO, no cage) as control sample (red line). Succinate retention time: 6.1 min (peak zoom in the inset).

Due to the tailing of the peak eluting after 4 min observed in all samples from the extraction experiments, the succinate response has been evaluated by analysis of succinate standard solutions prepared in 0.050 M HEPES buffer and contacted with the organic phase (containing 2.2% v/v DMSO but not the receptor), using the same experimental setup. Good linearity was observed in the working concentration range ($r^2 > 0.9944$). An example of calibration curve graph is shown below:

The succinate concentrations determined by HPLC–UV after the extraction experiments are reported in Table S5.

Table S5. Results from the seven independent extraction experiments. The measured average extraction yield resulted to be $20 \pm 4\%$ ($n=7$, α 0.05).

Succinate concentration in the aqueous phase (mg L^{-1})		
Without azacryptate	With azacryptate	Extraction yield (%)
142	101	28.4
161	134	17.1
143	113	20.9
152	127	16.8
119	91	24.2
163	132	18.7
154	128	16.7

7. Characterization

Figure S23. HRMS-ESI spectrum of **L1** in methanol. Peaks at m/z 450.6889 (+3), 675.5296 (+2) and 1350.0529 (+1) are attributable to the species $[M+3H^+]$, $[M+2H^+]$ and $[M+H^+]$, respectively.

Figure S24. ^1H -NMR spectrum of **L1** (10 mM) in d_6 -DMSO, recorded at 80°C and after the addition of aliquots of HNO_3 (in D_2O) to the solution. At room temperature and without acidification, the peaks were actually too large to allow a safe interpretation of the spectrum. Integrals are calculated on one portion of the molecule (as shown in the inset).

Figure S25. ^1H - ^1H COSY spectrum of **L1** in d_6 -DMSO (+ HNO_3) recorded at 80°C .

Figure S27. ^{13}C -NMR of **L1** in d_6 -DMSO recorded at 25°C.

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