SUPPLEMENTARY SECTION

Sensing and Liquid–Liquid Extraction of Dicarboxylates Using Dicopper Cryptates

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Index

1. Synthesis of L2 .................................................................................................................. 3

2. Potentiometric and pH-spectrophotometric titrations ................................................. 3
   2.1. Protonation constants of L1 ..................................................................................... 3
   2.2. Protonation constants of L2 ..................................................................................... 4
   2.3. Complexation of L1 with Cu(II) ............................................................................. 5
   2.4. Complexation of L2 with Cu(II) ............................................................................. 6
   2.5. pH-spectrophotometric titration of L1 with Cu(II) ................................................. 6
   2.6. pH-spectrophotometric titration of L2 with Cu(II) ................................................. 7
   2.7. Potentiometric titrations on H$_2$suc and H$_2$glut in dioxane/water 20% v/v ............... 8

3. Spectrophotometric and fluorimetric titrations ............................................................... 9
   3.1. UV-vis. titrations of [Cu$_2$(L1)]$^{4+}$ in aqueous solution at pH .................................. 9
   3.2. Studies with the fluorescent indicator 5-FAM ......................................................... 15
   3.3. UV-vis. titrations of [Cu$_2$(L2)]$^{4+}$ in dioxane/water 20% v/v at pH 7 ..................... 17
   3.4. UV-vis. titration of [Cu$_2$(L2)]$^{4+}$ with succinate in dichloromethane ..................... 21

4. X-Ray diffraction studies ............................................................................................... 21

5. Extraction experiments ................................................................................................. 25
   5.1 UV-vis. spectrum of extracting solution .................................................................. 25
   5.2 HPLC-UV chromatographic analysis ...................................................................... 26
   5.3 $^1$H-NMR study ...................................................................................................... 28

6. Characterization of L2 .................................................................................................. 30
   6.1. HRMS-ESI spectra of L2 ......................................................................................... 30
   6.2. HRMS-ESI spectra of [Cu$_2$(L2)(suc)]$^{2+}$ ................................................................ 311
   6.3. NMR spectra of L2 ................................................................................................. 32

References ......................................................................................................................... 34
1. Synthesis of L2

Figure S1. Template reaction of 1,5-di(hexyloxy)naphthalene-2,6-dicarboxaldehyde with tris(2-aminoethyl)amine to form L2.

2. Potentiometric and pH-spectrophotometric titrations

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 S2.

Table S1. Protonation equilibria and the corresponding constants for L1 in water/methanol 30% v/v (T=25 °C). Standard deviation in parentheses.

| Equilibria       | Logβ  |
|------------------|-------|
| L1 + H⁺ ⇄ L1H⁺   | 9.31(4) |
| L1 + 2H⁺ ⇄ L1H₂²⁺| 17.75(5) |
| L1 + 3H⁺ ⇄ L1H₃³⁺| 26.13(4) |
| L1 + 4H⁺ ⇄ L1H₄⁴⁺| 33.74(4) |
| L1 + 5H⁺ ⇄ L1H₅⁵⁺| 38.53(8) |
| L1 + 6H⁺ ⇄ L1H₆⁶⁺| 43.44(4) |
Figure S2. Distribution diagram of species present at equilibrium over the course of the potentiometric titration of L1 (5 \times 10^{-4} \text{ M}), L in the figure, with standard NaOH in water/methanol (30\% v/v) (T=25 °C).

2.2. Protonation constants of L2

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 S2) allowed calculation of the species distribution diagram (as % abundance with respect to L2 vs. pH) reported in Figure S3.

Table S2. Protonation equilibria and the corresponding constants for L2 in dioxane/water 20\% v/v (T=25 °C). Standard deviations are shown in parentheses.

| Equilibria               | Log$\beta$  |
|--------------------------|-------------|
| $\text{L2} + \text{H}^+ \rightleftharpoons \text{L2H}^+$ | 9.03(8)     |
| $\text{L2} + 2\text{H}^+ \rightleftharpoons \text{L2H}_2^{2+}$ | 17.74(4)    |
| $\text{L2} + 3\text{H}^+ \rightleftharpoons \text{L2H}_3^{3+}$ | 25.72(6)    |
| $\text{L2} + 4\text{H}^+ \rightleftharpoons \text{L2H}_4^{4+}$ | 32.66(6)    |
| $\text{L2} + 5\text{H}^+ \rightleftharpoons \text{L2H}_5^{5+}$ | 39.25(6)    |
| $\text{L2} + 6\text{H}^+ \rightleftharpoons \text{L2H}_6^{6+}$ | 44.50(7)    |
Figure S3. Distribution diagram of species present at equilibrium over the course of the potentiometric titration of L2 (4 × 10^{-4} M), L in the figure, with standard NaOH in dioxane/water (20% v/v) (T=25 °C).

2.3. 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: [Cu(L1H3)]^{5+}, [Cu_2(L1)]^{4+}, [Cu_2(L1)(OH)]^{3+}, [Cu_2(L1)(OH)_2]^{2+}. The corresponding cumulative constants (as Logβ) are shown in Table S3. The distribution diagram as % abundance vs. pH is reported in the main text (see Figure 2). The constants relative to the formation of the mono- and di-hydroxide complexes [Cu_2(L1)(OH)]^{3+} and [Cu_2(L1)(OH)_2]^{2+}, shown in Table S4, have been calculated from the corresponding cumulative constants reported in Tables S1 and S3.

Table S3. Complexation constants for L1 with Cu(II) in water/methanol 30% v/v (T=25 °C). Standard deviations are shown in parentheses.

| Equilibria | Logβ |
|------------|------|
| L1 + Cu^{2+} + 3H^{+} ⇋ [Cu(L1H3)]^{5+} | 33.90(4) |
| L1 + 2Cu^{2+} ⇋ [Cu_2(L1)]^{4+} | 22.28(4) |
| L1 + 2Cu^{2+} + H_2O ⇋ [Cu_2(L1)(OH)]^{3+} + H^{+} | 12.83(9) |
| L1 + 2Cu^{2+} + 2H_2O ⇋ [Cu_2(L1)(OH)_2]^{2+} + 2H^{+} | 3.41(8) |

Table S4. Equilibria obtained from the data reported in Tables S1 and S3. Standard deviations are shown in parentheses.

| Equilibria | LogK |
|------------|------|
| L1H_3^{3+} + Cu^{2+} ⇋ [Cu(L1H_3)]^{5+} | 7.77(8) |
| [Cu_2(L1)]^{4+} + H_2O ⇋ [Cu_2(L1)OH]^{3+} + H^{+} | -9.5(1) |
| [Cu_2(L1)OH]^{3+} + H_2O ⇋ [Cu_2(L1)(OH)_2]^{2+} + H^{+} | -9.4(1) |
2.4. Complexation of L2 with Cu(II)

Best fitting was obtained by assuming the formation of the following complex species over the course of the titration: \([\text{Cu}(\text{L2H}_3)^{5+}], \ [\text{Cu}_2(\text{L2})^{4+}], \ [\text{Cu}_2(\text{L2})(\text{OH})^{3+}], \ [\text{Cu}_2(\text{L2})(\text{OH})_2]^{2+}\). The corresponding cumulative constants (as Log\(\beta\)) are shown in Table S5. The distribution diagram (as % abundance vs. pH) is reported in Figure S6. The constants relative to the formation of the mono- and di-hydroxide complexes \([\text{Cu}_2(\text{L2})(\text{OH})]^{3+}\) and \([\text{Cu}_2(\text{L2})(\text{OH})_2]^{2+}\), shown in Table S6, have been calculated from the corresponding cumulative constants reported in Tables S2 and S5.

Table S5. Complexation constants for L2 with Cu(II) in dioxane/water 20% v/v (T=25 °C). Standard deviations are shown in parentheses.

| Equilibria                                  | Log\(\beta\)  |
|--------------------------------------------|-------------|
| \(\text{L2} + \text{Cu}^{2+} + 3\text{H}^+ \rightleftharpoons [\text{Cu}(\text{L2H}_3)]^{5+}\) | 35.37(3)   |
| \(\text{L2} + 2\text{Cu}^{2+} \rightleftharpoons [\text{Cu}_2(\text{L2})]^{4+}\) | 24.60(7)   |
| \(\text{L2} + 2\text{Cu}^{2+} + \text{H}_2\text{O} \rightleftharpoons [\text{Cu}_2(\text{L2})(\text{OH})]^{3+} + \text{H}^+\) | 12.4(1)    |
| \(\text{L2} + 2\text{Cu}^{2+} + 2\text{H}_2\text{O} \rightleftharpoons [\text{Cu}_2(\text{L2})(\text{OH})_2]^{2+} + 2\text{H}^+\) | 1.8(1)     |

Table S6. Equilibrium constants deriving from the overall constants shown in Tables S2 and S5. Standard deviations are shown in parentheses.

| Equilibria                                  | Log\(K\)     |
|--------------------------------------------|-------------|
| \(\text{L2H}_3^{3+} + \text{Cu}^{2+} \rightleftharpoons [\text{Cu}(\text{L2H}_3)]^{5+}\) | 9.65(9)     |
| \([\text{Cu}_2(\text{L2})]^{4+} + \text{H}_2\text{O} \rightleftharpoons [\text{Cu}_2(\text{L2})(\text{OH})]^{3+} + \text{H}^+\) | -12.2(1)    |
| \([\text{Cu}_2(\text{L2})(\text{OH})]^{3+} + \text{H}_2\text{O} \rightleftharpoons [\text{Cu}_2(\text{L2})(\text{OH})_2]^{2+} + \text{H}^+\) | -10.6(2)    |

2.5. pH-spectrophotometric titration of L1 with Cu(II)

Figure S4. Spectra taken over the course of the pH-spectrophotometric titration of a solution of \(4 \times 10^{-4} \text{ M}\) in L1 and \(8 \times 10^{-4} \text{ M}\) in Cu(II). The red line highlights the initial spectrum (at about pH 3), while the green and blue lines correspond to the spectra recorded ad pH 7 and 11, respectively.
2.6. pH-spectrophotometric titration of L2 with Cu(II)

Figure S5. Spectra taken over the course of the pH-spectrophotometric titration of a solution of $4 \times 10^{-4}$ M in L2 and $8 \times 10^{-4}$ M in Cu(II). The red line highlights the initial spectrum (at about pH 3), while the green and blue lines correspond to the spectra recorded at pH 7 and 12, respectively.

Figure S6. Distribution diagram of the species present at the equilibrium over the course of the titration with standard NaOH of a solution $4 \times 10^{-4}$ M in L2 and $8 \times 10^{-4}$ M in Cu(II) with the superimposed pH-spectrophotometric profiles. Symbols: red triangles, superimposed plot of Mol Abs at 885 nm; green triangles, superimposed plot of Mol Abs at 650 nm. The lines in the diagram correspond to the species: $\text{H}_2\text{L}_2^{2+}$, grey; $\text{H}_2\text{L}_2^{3+}$, orange; $[\text{Cu(L}_2\text{H}_3)]^{3+}$, cyan; $[\text{Cu}_2\text{L}_2]^{4+}$, red; $[\text{Cu}_2\text{L}_2(\text{OH})]^{3+}$, green; $[\text{Cu}_2\text{L}_2(\text{OH})_2]^{2+}$, blue.
2.7. Potentiometric titrations on H2suc and H2glut in dioxane/water 20% v/v

Potentiometric titrations were also performed on two representative dicarboxylic acids, H2suc (succinic acid) and H2glut (glutaric acid), in order to determine their pKₐ values in dioxane/water mixture 20% v/v (0.05 M [TBA]NO₃, 25°C). In a typical experiment, 15 mL of a 1 × 10⁻³ M dicarboxylate solution were treated with an excess of a 1.0 M HNO₃ standard solution. Titrations were performed by addition of 10 μL aliquots of carbonate-free standard 0.1 M NaOH, recording 80-100 points for each titration. In pure water at pH 7, both acids are largely deprotonated, with pKₐ₁ and pKₐ₂: 4.21 and 5.41 for H2suc; 4.34 and 5.22 at 25°C for H2glut.¹ In the chosen mixture, deprotonation to form the dicarboxylate anion is significantly shifted toward basic pH values for both H2suc and H2glut. The potentiometric titrations allowed estimation of pKₐ values, which were found to be pKₐ₁ = 6.89(4) and pKₐ₂ = 8.74(6) for H2suc, pKₐ₁ = 7.55(5) and pKₐ₂ = 8.36(7) for H2glut. The corresponding distribution diagram of the species, as % abundance vs. pH, are reported in Figures S7-S8.

![Figure S7. Distribution diagram of the species present at the equilibrium over the course of the potentiometric titration of H2suc (1 × 10⁻³ M) with standard NaOH in dioxane/water 20% v/v (T=25 °C).](image-url)
3. Spectrophotometric and fluorimetric titrations

3.1. UV-vis. titrations of [Cu₂(L₁)]⁴⁺ in aqueous solution at pH

For the determination of binding constants, the solution of the azacryptate was titrated with at least 100-fold more concentrated solution of the acid of the titled anion. For the titration with phthalates, the corresponding Na⁺ salts were used.

Table S7. Binding constants (as Log\(K_{11}\) values) obtained by UV-vis titrations on [Cu₂(L₁)]⁴⁺ in water with HEPES buffer 0.05 M at pH 7 (T=25 °C). (a) Anion-promoted demetallation of the dicopper complex. Standard deviations are shown in parentheses.

| anionic guest | \(\text{Log}K_{11}\)   |
|-------------|----------------------|
| oxalate    | (a)                  |
| malonate   | (a)                  |
| pimelate   | 2.64(8)              |
| phthalate  | 2.54(1)              |
| terephthalate | 3.54(1)             |
| citrate    | 5.70(1)              |
Figure S9. Left: UV-vis. spectra taken upon titration of $[\text{Cu}_2(L1)]^{4+}$ 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 4 eqv. of the added anion, respectively. Right: titration profile at 666 nm (blue triangles) vs. equivalents of the added guest.

Figure S10. Left: UV-vis. spectra taken upon titration of $[\text{Cu}_2(L1)]^{4+}$ 0.50 mM with isophthalate (as Na+ salt) 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 (blue triangles) vs. equivalents of the added guest.
Figure S11. Left: UV-vis. spectra taken upon titration of \([\text{Cu}_2(\text{L1})]^4+\) 50 \(\mu\)M 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 7 eqv. of the added anion, respectively. Right: titration profile at 687 nm (red triangles) with superimposed distribution diagram of the species \([\text{Cu}_2(\text{L1})]^4+\) (solid line) and \([\text{Cu}_2(\text{L1})(\text{glut})]^2+\) (dotted line) vs. equivalents of the added guest, calculated for Log\(K_{11}=5.39(1)\).

Figure S12. Left: UV-vis. spectra taken upon titration of \([\text{Cu}_2(\text{L1})]^4+\) 0.5 mM with pimelate 0.2 M 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 605 nm (red triangles) with superimposed distribution diagram of the species \([\text{Cu}_2(\text{L1})]^4+\) (solid line) and \([\text{Cu}_2(\text{L1})(\text{pim})]^2+\) (dotted line) vs. equivalents of the added guest, calculated for Log\(K_{11}=2.64(8)\).
Figure S13. UV-vis. titration of $[\text{Cu}_2(\text{L1})]^{4+}$ 20 µM with $\alpha$-ketoglutarate 5 mM in water at pH 7 (HEPES 0.050 M, path length 10 cm): titration profile at 665 nm (red triangles) with superimposed distribution diagram of the species $[\text{Cu}_2(\text{L1})]^{4+}$ (solid line) and $[\text{Cu}_2(\text{L1})(\alpha$-ketoglutarate)$]^2^{+}$ (dotted line) vs. equivalents of the added guest, calculated for $\log K_{11}=6.00(1)$.

Figure S14. UV-vis. titration of $[\text{Cu}_2(\text{L1})]^{4+}$ 0.55 mM with adipate 50 mM in water at pH 7 (HEPES 0.05 M, path length 1 cm): titration profile at 830 nm (red triangles) with superimposed distribution diagram of the species $[\text{Cu}_2(\text{L1})]^{4+}$ (solid line) and $[\text{Cu}_2(\text{L1})(\text{adi})]^2^{+}$ (dotted line) vs. equivalents of the added guest, calculated for $\log K_{11}=2.58(1)$. 
Figure S15. UV-vis. titration of $[\text{Cu}_2(\text{L1})]^{4+}$ 0.50 mM with phthalate (as Na$^+$ salt) 50 mM water at pH 7 (HEPES 0.25 M, path length 1 cm): titration profile at 868 nm (red triangles) with superimposed distribution diagram of the species $[\text{Cu}_2(\text{L1})]^{4+}$ (solid line) and $[\text{Cu}_2(\text{L1})(\text{ph})]^{2+}$ (dotted line) vs. equivalents of the added guest, calculated for Log$K_{11}$=2.59(1).

Figure S16. UV-vis. titration of $[\text{Cu}_2(\text{L1})]^{4+}$ 0.50 mM with terephthalate (as Na$^+$ salt) 50 mM water at pH 7 (HEPES 0.25 M, path length 1 cm): titration profile at 880 nm (red triangles) with superimposed distribution diagram of the species $[\text{Cu}_2(\text{L1})]^{4+}$ (solid line) and $[\text{Cu}_2(\text{L1})(\text{tereph})]^{2+}$ (dotted line) vs. equivalents of the added guest, calculated for Log$K_{11}$=3.54(1).
Figure S17. UV-vis. titration of \([\text{Cu}_2(L_1)]^{4+}\) 0.50 mM with maleate 50 mM in water at pH 7 (HEPES 0.05 M, path length 1 cm): titration profile at 870 nm (red triangles) with the superimposed distribution diagram of \([\text{Cu}_2(L_1)]^{4+}\) (solid line) and \([\text{Cu}_2(L_1)(\text{male})]^{2+}\) (dotted line) vs. equivalents of the added guest, calculated for LogK_{11}=2.78(1).

Figure S18. UV-vis. titration of \([\text{Cu}_2(L_1)]^{4+}\) 20 µM with acetylenedicarboxylate 5 mM in water at pH 7 (HEPES 0.050 M, path length 10 cm): titration profile at 680 nm (red triangles) with the superimposed distribution diagram of the species \([\text{Cu}_2(L_1)]^{4+}\) (solid line) and \([\text{Cu}_2(L_1)(\text{ace})]^{2+}\) (dotted line) vs. equivalents of the added guest, calculated for LogK_{11}=5.90(1).
3.2. Studies with the fluorescent indicator 5-FAM

Figure S19. UV-vis. titration of 5-FAM 1 µM with a solution of the in-situ prepared [Cu$_2$(L1)]$^{4+}$ complex. (0.050 M HEPES pH 7, path length 1 cm). Red and blue lines: initial and final spectra, respectively.

Figure S20. a) Family of emission spectra taken over the course of the titration of the indicator displacement assay (0.1 µM 5-FAM, 5 µM [Cu$_2$(L1)]$^{4+}$) with fumarate in 0.05M HEPES at pH 7 ($\lambda_{\text{exc}}$ = 473 m; $\lambda_{\text{em}}$ =520 nm); b) titration profile at 516 nm, superimposed to the distribution diagram of the species, calculated for an 1:1 association constant with fumarate of 8.21 log units ($\log K_{11} = 7.2$, receptor:5FAM). Solid line: % free 5-FAM; dashed line: % [Cu$_2$(L1)(5-FAM)]$^+$; red triangles: $I/I_{\text{max}}$ vs. equivalents of the added anion ($I_{\text{max}}$= maximum emission intensity of the indicator, taken in the same conditions and in the absence of the dicopper complex)
Figure S21. Titration of the indicator displacement assay (0.1 μM 5-FAM, 5 μM [Cu₂(L1)]^{4+}) with succinate in 0.05M HEPES at pH 7 (λ.exc = 473 m; λ.em = 520 nm), red triangles: titration profile as I/I_{max} at 516 nm vs. equivalents of the added anion (I_{max} = maximum emission intensity of the indicator, taken in the same conditions and in the absence of the dicopper complex), superimposed to the distribution diagram of the species, calculated for an 1:1 association constant with succinate of 7.38 log units (logK_{11} = 7.2, receptor:5FAM). Solid line: % free 5-FAM; dashed line: % [Cu₂(L1)(5-FAM)]^{4+}.

Figure S22. Titration of the indicator displacement assay (0.1 μM 5-FAM, 5 μM [Cu₂(L1)]^{4+}) with isophthalate in 0.05M HEPES at pH 7 (λ.exc = 473 m; λ.em = 520 nm), red triangles: titration profile as I/I_{max} at 516 nm vs. equivalents of the added anion (I_{max} = maximum emission intensity of the indicator, taken in the same conditions and in the absence of the dicopper complex), superimposed to the distribution diagram of the species, calculated for an 1:1 association constant with isophthalate of 7.20 log units (logK_{11} = 7.2, receptor:5FAM). Solid line: % free 5-FAM; dashed line: % [Cu₂(L1)(5-FAM)]^{4+}. 

S16
Figure S23. Titration of the indicator displacement assay (0.1 μM 5-FAM, 5 μM [Cu₂(L₁)]⁺⁺) with α-ketoglutarate in 0.05M HEPES at pH 7 (λ<sub>exc</sub> = 473 m; λ<sub>em</sub> = 520 nm), red triangles: titration profile as I/I<sub>max</sub> at 516 nm vs. equivalents of the added anion (I<sub>max</sub>= maximum emission intensity of the indicator, taken in the same conditions and in the absence of the dicopper complex), superimposed to the distribution diagram of the species, calculated for an 1:1 association constant with α-ketoglutarate of 5.95 log units (logK₁₁ = 7.2, receptor:5FAM). Solid line: % free 5-FAM; dashed line: % [Cu₂(L₁)(5-FAM)]⁺⁺.

3.3. UV-vis. titrations of [Cu₂(L₂)]⁺⁺ in dioxane/water 20% v/v at pH 7

Figure S24. Left: UV-vis. spectra taken upon titration of [Cu₂(L₂)]⁺⁺ 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 4 eqv. of the added anion, respectively. Right: titration profile at 400 nm (red triangles) with superimposed distribution diagram of the species [Cu₂(L₂)]⁺⁺ (solid line) and [Cu₂(L₂)(suc)]²⁺ (dotted line) vs. equivalents of the added guest (dotted line), calculated for logK₁₁=5.60(3).
Figure S25. Left: UV-vis. spectra taken upon titration of \([\text{Cu}_2(\text{L}2)]^{4+}\) 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 4 eqv. of the added anion, respectively. Right: titration profile at 643 nm (red triangles) with superimposed distribution diagram of the species \([\text{Cu}_2(\text{L}2)]^{4+}\) (solid line) and \([\text{Cu}_2(\text{L}2)(\text{fum})]^2+\) (dotted line) vs. equivalents of the added guest, calculated for \(\text{Log}K_{1/2}=5.76(2)\).

Figure S26. Left: UV-vis. spectra taken upon titration of \([\text{Cu}_2(\text{L}2)]^{4+}\) 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 7 eqv. of the added anion, respectively. Right: titration profile at 900 nm (red triangles) with the superimposed distribution diagram of the species \([\text{Cu}_2(\text{L}2)]^{4+}\) (solid line) and \([\text{Cu}_2(\text{L}2)(\text{adip})]^2+\) (dotted line) vs. equivalents of the added guest, calculated for \(\text{Log}K_{1/2}=3.47(1)\).
Figure S27. UV-vis. titration of [Cu$_2$(L$_2$)]$^{4+}$ 0.20 mM with glutarate 10 mM in dioxane/water 20% v/v at pH 7 (HEPES 0.025 M, path length 1 cm): titration profile at 600 nm (red triangles) with the superimposed distribution diagram of the species [Cu$_2$(L$_2$)]$^{4+}$ (solid line) and [Cu$_2$(L$_2$)(glut)]$^{2+}$ (dotted line) vs. equivalents of the added guest, calculated for Log$K_{11}=4.14(3)$.

Figure S28. UV-vis. titration of [Cu$_2$(L$_2$)]$^{4+}$ 50 μM with α-ketoglutarate 12.5 mM in dioxane/water 20% v/v at pH 7 (HEPES 0.025 M, path length 10 cm). Titration profile at 660 nm (red triangles) with the superimposed distribution diagram of the species [Cu$_2$(L$_2$)]$^{4+}$ (solid line) and [Cu$_2$(L$_2$)(α-keto)]$^{2+}$ (dotted line) vs. equivalents of the added guest, calculated for Log$K_{11}=5.90(4)$. 
Figure S29. UV-vis. titration of $[\text{Cu}_2(\text{L}_2)]^{4+}$ 50 µM with isophthalate (as Na$^+$ salt) 12.5 mM in dioxane/water 20% v/v at pH 7 (HEPES 0.025 M, path length 10 cm): titration profile at 880 nm (red triangles) with superimposed distribution diagram of the species $[\text{Cu}_2(\text{L}_2)]^{4+}$ (solid line) and $[\text{Cu}_2(\text{L}_2)(\text{isoph})]^{2+}$ (dotted line) vs. equivalents of the added guest, calculated for $\log K_7=4.98(4)$.

Figure S30. UV-vis. titration of $[\text{Cu}_2(\text{L}_2)]^{4+}$ 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): titration profile at 900 nm (red triangles) with superimposed distribution diagram of the species $[\text{Cu}_2(\text{L}_2)]^{4+}$ (solid line) and $[\text{Cu}_2(\text{L}_2)(\text{male})]^{2+}$ (dotted line) vs. equivalents of the added guest, calculated for $\log K_7=3.65(5)$. 
3.4. UV-vis. titration of [Cu$_2$(L2)]$^{4+}$ with succinate in dichloromethane

![UV-vis spectra and titration profile](image)

Figure S31. Left: UV-vis. spectra taken upon titration of [Cu$_2$(L2)]$^{4+}$ 30 μM 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) vs. equivalents of the added guest.

4. X-Ray diffraction studies

The crystal structure of the [Cu$_2$(L1)(ace)](CF$_3$SO$_3$)$_2$·4(H$_2$O) compound showed positional disorder for one of the two triflate counterions and for a water solvent molecules. Disorder resulted in two alternative positions for these molecular species. The alternative positions were mutually exclusive and occurring with the same statistical probability. Atom sites belonging to the two alternative positions of the triflate counterion were refined with soft restraints on the molecular geometry (SAME) and on the atom displacement parameters (ISOR and DELU).

The crystal structure of the [Cu$_2$(L1)(glut)](CF$_3$SO$_3$)$_2$·4(H$_2$O) compound was refined with soft restraints (ISOR and DELU) for the atom displacement parameters of atom sites defining the triflate counterions. Also in this crystal, one of the two independent triflate counterions resulted disordered over two alternative positions, mutually exclusive and with the same statistical probability. The final cycles of structure refinements were done using soft restraints on the molecular geometry (SAME) for atom sites defining the alternative positions of the triflate counterion.

The crystal structure of the [Cu$_2$(L1)(α-keto)](CF$_3$SO$_3$)$_2$·5(H$_2$O) compound was isostructural to the one of the [Cu$_2$(L1)(glut)](CF$_3$SO$_3$)$_2$·4(H$_2$O) compound. The alpha-ketoglutarate ion was disordered over two alternative positions, mutually exclusive and with the same statistical probability. The two alternative molecular structures differ only in the position of the carbonyl oxygen of the ketone functional group, which appears as disordered between the two alpha positions of the organic backbone. Soft restraints on the atom displacement parameters (DELU) and on the molecular geometry (SAME) were applied to atom positions belonging to the triflate counterions.
The crystal structure of the [Cu$_2$(L1)(isoph)]$_3$(CF$_3$SO$_3$)$_6$·6(H$_2$O) compound resulted quite complicated for the presence of three similar but not symmetrically equivalent [Cu$_2$(L1)(isoph)]$^{2+}$ anionic complexes. The poor X-ray diffraction quality of this synthetic crystal imposed the use of a great number of restraints during the least-square procedures. The crystal structure 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 [Cu$_2$(L1)]$^{4+}$ dimetallic cage to show similar geometrical features (by using the SAME instruction). Further restraints on the atom displacement parameters (DELU and ISOR) were applied for atom positions showing large and elongated thermal ellipsoids.

Figure S32. Couples of simplified sketches of the other two independent [Cu$_2$(L1)(isoph)]$^{2+}$ molecular cations occurring in the [Cu$_2$(L1)(isoph)]$_3$(CF$_3$SO$_3$)$_6$·6(H$_2$O) crystal.
Figure S3. Plots showing thermal ellipsoids for $[\text{Cu}_2(\text{L1})(\text{glut})]^{2+}$ (top left), $[\text{Cu}_2(\text{L1})(\alpha\text{-keto})]^{2+}$ (top center), $[\text{Cu}_2(\text{L1})(\text{ace})]^{2+}$ (top right) and for the three independent $[\text{Cu}_2(\text{L1})(\text{isoph})]^{2+}$ cations (bottom). Ellipsoids are drawn at the 30% probability level.

Table S8. Crystal data for the studied compounds.

| Formula | $[\text{Cu}_2(\text{L1})(\text{glut})]$ $(\text{CF}_3\text{SO}_3)_2 \cdot 4(\text{H}_2\text{O})$ | $[\text{Cu}_2(\text{L1})(\alpha\text{-keto})]$ $(\text{CF}_3\text{SO}_3)_2 \cdot 5(\text{H}_2\text{O})$ | $[\text{Cu}_2(\text{L1})(\text{isoph})]$ $(\text{CF}_3\text{SO}_3)_6 \cdot 6(\text{H}_2\text{O})$ | $[\text{Cu}_2(\text{L1})(\text{ace})]$ $(\text{CF}_3\text{SO}_3)_2 \cdot 4(\text{H}_2\text{O})$ |
|---------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| $M$    | 1376.42                         | 1408.42                         | 4123.20                         | 1357.35                         |
| Crystal system | monoclinic                    | monoclinic                    | triclinic                     | triclinic                     |
| Space group | $P 2_1/c$ (no. 14)               | $P 2_1/c$ (no. 14)               | $P -1$ (no. 2)                  | $P -1$ (no. 2)                  |
| $a$ (Å)    | 18.611(2)                       | 18.4812(13)                    | 18.547(3)                      | 10.2103(6)                     |
Table S9. Cu(II)···Cu(II) intermetallic distance (Å), bond distances (Å) and bond angles (°) for the metal centers in [Cu₂L₁(glut)]²⁺, [Cu₂L₁(α-keto)]²⁺, in the three not symmetrically equivalent [Cu₂L₁(isoph)]²⁺ and in [Cu₂L₁(ace)]²⁺ molecular cations.

|               | [Cu₂L₁(glut)]²⁺ | [Cu₂L₁(α-keto)]²⁺ | [Cu₂L₁(isoph)]²⁺ | [Cu₂L₁(ace)]²⁺ |
|---------------|-----------------|-------------------|------------------|----------------|
| Cu(II)···Cu(II)| 8.952(1)        | 8.983(1)          | 8.923(3)         | 8.808(4)       |
| Cu(1)-O(1)    | 1.935(4)        | 1.944(4)          | 1.957(9)         | 1.977(10)      |
| Cu(1)-N(1)    | 2.031(5)        | 2.047(5)          | 2.061(12)        | 1.98(2)        |
| Cu(1)-N(2)    | 2.380(5)        | 2.339(5)          | 2.371(14)        | 2.349(14)      |
| Cu(1)-N(3)    | 2.148(5)        | 2.143(5)          | 2.105(11)        | 2.16(2)        |
| Cu(1)-N(4)    | 2.128(5)        | 2.142(5)          | 2.097(13)        | 1.93(2)        |
| Cu(2)-O(3)    | 1.923(4)        | 1.933(8)          | 1.933(8)         | 1.952(10)      |
| Cu(2)-N(5)    | 2.035(6)        | 2.038(5)          | 2.035(12)        | 2.11(2)        |
| Cu(2)-N(6)    | 2.402(6)        | 2.371(5)          | 2.445(11)        | 2.377(13)      |
| Cu(2)-N(7)    | 2.128(6)        | 2.135(5)          | 2.101(10)        | 2.074(14)      |
| Cu(2)-N(8)    | 2.147(5)        | 2.159(5)          | 2.156(10)        | 2.09(2)        |
| O(1)-Cu(1)-N(1)| 168.6(2)      | 169.12(2)         | 168.15(5)        | 166.5(7)       |
| O(1)-Cu(1)-N(2)| 109.6(2)     | 108.8(2)          | 110.5(4)         | 115.3(5)       |
| O(1)-Cu(1)-N(3)| 91.1(2)       | 91.9(2)           | 92.2(4)          | 92.1(5)        |
| O(1)-Cu(1)-N(4)| 95.2(2)       | 93.9(2)           | 95.3(4)          | 92.0(6)        |
| N(1)-Cu(1)-N(2)| 81.7(2)       | 82.12(2)          | 81.5(5)          | 77.9(7)        |
| N(1)-Cu(1)-N(3)| 84.4(2)       | 84.4(2)           | 83.5(5)          | 86.8(9)        |
| N(1)-Cu(1)-N(4)| 83.4(2)       | 83.6(2)           | 83.0(5)          | 82.3(9)        |
| N(2)-Cu(1)-N(3)| 107.2(2)      | 109.12(2)         | 109.0(6)         | 104.3(6)       |
5. Extraction experiments

5.1 UV-vis. spectrum of extracting solution

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 S33). 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, $[\text{Cu}_2(\text{L}_2)(\text{suc})]^2^+$, is confirmed by comparing the spectra shown in Fig. S33 with those obtained upon titration of $[\text{Cu}_2(\text{L}_2)]^{4+}$ with $[\text{TBA}]_2\text{suc}$ in DCM (see Fig. S30).

![UV-vis. spectra](image)

Figure S34. UV-vis. spectra of the solution of $[\text{Cu}_2(\text{L}_2)]^{4+}$ (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).
5.2 HPLC-UV chromatographic analysis

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 S34.

Figure S35. 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 S35.
Figure S36. 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 S10.
Table S10. Results from the seven independent extraction experiments. The measured average extraction yield resulted to be $20 \pm 4\%$ ($n=7$, $\alpha=0.05$).

| 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               |

5.3 $^1$H-NMR study

Figure S37. $^1$H-NMR spectrum of a D$_2$O solution of succinate (0.05M PBS, pH 7) in presence of glycine as internal reference. The concentration of succinate was determined using the Topspin Eretic2 package (details are reported in the Experimental section, see the main text).
Figure S38. $^1$H-NMR spectrum of the D$_2$O solution of succinate (0.05M PBS, pH 7). The spectrum was recorded after stirring (10 min.) the solution in contact with an equal volume of DCM (6% DMSO). Internal reference: glycine.

Figure S39. $^1$H-NMR spectrum of the D$_2$O solution of succinate (0.05M PBS, pH 7). The spectrum was recorded after stirring (10 min.) the solution in contact with an equal volume of a solution of the dicopper complex (0.42 mM) in DCM (6% DMSO). Internal reference: glycine.
6. Characterization of L2

6.1. HRMS-ESI spectra of L2

Figure S40. HRMS-ESI spectrum of L2 in methanol. Peaks found at m/z 1350.0529 (+1), 675.5296 (+2) and 450.6889 (+3) are attributable to the species [M+H+], [M+2H+]2+, [M+3H+]3+. The corresponding calculated values for the formula CsH132N8O6 are 1350.0348, 675.5208 and 450.6829, respectively.
6.2. HRMS-ESI spectra of $[\text{Cu}_2(\text{L}_2)(\text{suc})]^{2+}$

Figure S41. HRMS-ESI spectrum of a solution of $[\text{Cu}_2(\text{L}_2)]^{4+}$ in CH$_3$CN, containing 1 eqv. of Na$_2$Suc. Up: zoom scan of the peak at 796.5492 m/z obtained from the experimental HRMS-ESI spectrum. Bottom: calculated spectrum of the adduct $[\text{Cu}_2(\text{L}_2)(\text{suc})]^{2+}$. 
6.3. NMR spectra of L2

Figure S42. $^1$H-NMR spectrum of L2 (10 mM) in d$_6$-DMSO, recorded at 80°C and after the addition of aliquots of HNO$_3$ (in D$_2$O) 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 (see the sketch).

Figure S43. $^1$H-$^1$H COSY spectrum of L2 in d$_6$-DMSO (+ HNO$_3$) recorded at 80°C.
Figure S44. $^1$H-$^1$C HSQC spectrum of L2 in d$_6$DMSO (+ HNO$_3$) recorded at 80°C
Figure S45. $^{13}$C-NMR of L2 in d$_6$-DMSO recorded at 25°C.

References

[1] (a) Dawson, R. M. C., et al., *Data for Biochemical Research*, 3rd ed., 1986, Oxford University Press, New York, NY; (b) Lide, D. R. *CRC Handbook of Chemistry and Physics*, 84th ed., 2003, CRC Press, Boca Raton, FL.

[2] Xie, G. Y.; Jiang, L.; Lu, T. B. Discrimination of Cis-Trans Isomers by Dinuclear Metal Cryptates at Physiological pH: Selectivity for Fumarate vs. Maleate. Dalton Trans. 2013, 42 (39), 14092–14099.