Copper(II) complexes of macrocyclic and open-chain pseudopeptidic ligands: synthesis, characterization and interaction with dicarboxylates†

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Mono- and dinuclear Cu(II) complexes were prepared with pseudopeptidic open chain and macrocyclic ligands, respectively. They were characterized by UV–vis spectroscopy, EPR, HRMS and X-ray diffraction. The Cu(II) cation is coordinated by two amines and two deprotonated amides, in a slightly distorted square planar coordination geometry. The complexes interact with several substituted dicarboxylates, as shown by UV–vis titrations and EPR experiments. The interaction of both mono- and dinuclear complexes with very similar dicarboxylates of biological interest (malate and aspartate) resulted in strikingly different outcomes: in the first case a ternary complex [ligand⋯metal⋯dicarboxylate] was obtained almost quantitatively, while in the latter, the Cu(II) displacement to form Cu(Asp)₂ was predominant.

Introduction

The Cu(II) cation plays a key role in bioinorganic chemistry as it is present in many enzymes with a variety of essential functions.1,2 Copper enzymes usually contain metal ions bound to a specific amino acid residue or directly to the amide group – carbonyl or nitrogen – in the peptide backbone, offering different coordination environments.3 For example, naturally-occurring cyclic peptides form mono- and dinuclear Cu(II) complexes, with different coordination geometries.4 The Cu(II) ion also interacts with α-synuclein (αSyn), and fragments of Park9 encoded protein from the Parkinson’s disease gene.5 Additionally, Cu(II) ionophores have been studied recently as potential therapeutic agents.6 Thus, an appropriate design of small molecules with suitable structural and functional characteristics could be important for biomimetic and recognition studies. In this regard, the design and synthesis of ligands functionalized to achieve metal complexation through a biomimetic approach is a challenge of current interest. The use of oligopeptides7 and simple ligands that contain amino acid residues8 is a successful strategy to provide the Cu(II) ions with a coordination environment similar to that found in metalloproteins.

In this context, (amino amide) compounds9 are promising ligands for several reasons: (i) they contain two kinds of nitrogen atoms, with different coordination capabilities, connected through a chiral backbone; (ii) their properties can be tuned by the variation of the substituents and the spacers and (iii) they can form stable metal complexes with transition metals.10 On the other hand, different bis(amine amides) have been used as building blocks for the construction of macrocyclic or macrobicyclic structures. Some of these systems display interesting features, acting as in vivo fluorescent pH probes,11 or as selective receptors for substrates of biological relevance.12

Recently, the coordination ability of some C₂ symmetrical bis(amine amides) derived from valine towards Cu(II) and Zn(II) ions has been reported13 and, for the same family of ligands, the effect of changes in the amino acidic residue towards the coordination of Cu(II) was evaluated.14 Here, we present a study of the Cu(II) binding ability of two open-chain and two macrocyclic (amino amide) ligands derived from phenylalanine. This particular amino acid was selected because some phenylalanine-derived receptors have been shown to be better hosts for cations than those obtained from valine.15 In view of the multiple and important roles played by dicarboxylate anions in living systems,16 we also studied
interaction of the obtained Cu(II) complexes with a series of dicarboxylates with similar structures and of high biological interest, such as malate and aspartate.

Results and discussion

Synthesis and characterization of the ligands

For this study we selected four ligands (L1–L4) with closely related structures. Thus, L1 and L2 are open-chain bis(amino amide) receptors that differ slightly in the length of the aliphatic spacer connecting the amide nitrogens. In principle, they are designed to coordinate Cu(II) forming mononuclear complexes. On the other hand, L3 and L4 are tetra(amino amide) macrocyclic ligands with different geometries of the aromatic spacer that connect the amine nitrogens. They are designed to coordinate Cu(II) forming dinuclear complexes. Overall, the complexes derived from these ligands allowed: (i) comparison of mononuclear and dinuclear Cu(II) complexes, determining which molecular architecture was more efficient in the further coordination of dicarboxylates and (ii) an evaluation of the possible effect of the length of the spacer.

The general structure and synthetic pathways of the considered ligands are displayed in Scheme 1. Primary diamines 1 and 2, derived from phenylalanine, are easily prepared starting from the corresponding N-Cbz protected phenylalanine N-hydroxysuccinimidy ester, coupled with either 1,2-diaminoethane or 1,3-diaminopropane and final N-deprotection following previously reported procedures.17 Diamines 1 and 2 were subjected to a reductive amination process with benzaldehyde in methanol, affording the new ligands L1 and L2 in good yields. On the other hand, macrocyclic ligands L3 and L4 were obtained by reaction of diamine 1 with either terephthaldehyde or isophthaldehyde in the presence of a suitable template agent (tetrabutylammonium terephthalate or isophthalate, respectively), in a macrocyclization reaction that has already been described.18

The new ligands L1 and L2 were characterised using standard techniques (1H-NMR, 13C-NMR, HRMS) and in the case of L1, crystals suitable for single-crystal X-ray diffraction were obtained. A rather concentrated solution of L1 in methanol was left evaporating slowly at room temperature. After four days, colourless elongated crystals of good quality were obtained.

Synthesis and characterization of the copper(II) complexes

The copper(II) complexes of both open-chain and macrocyclic ligands were prepared by applying slight modifications to a previously reported procedure.14 Bis(amino amide) ligands L1 and L2 were reacted with one equivalent of CuSO4 in methanol, in the presence of two equivalents of NaOH, to ensure the

![Figure 1](https://via.placeholder.com/150)

**Fig. 1** X-ray crystal structure of ligand L1. Hydrogens are omitted for clarity (top). Asymmetric unit of ligand L1 (bottom). Carbon (grey), oxygen (red), nitrogen (blue), hydrogen bond (pale blue).
complete deprotonation of the ligand amide groups. Bright purple solutions were obtained, while a white solid was discarded. Solutions were concentrated to afford the mononuclear complexes Cu\textsubscript{L1} and Cu\textsubscript{L2} as purple solids. Similarly, tetra(amino amide) ligands L\textsubscript{3} and L\textsubscript{4} were reacted with two equivalents of CuSO\textsubscript{4} and four equivalents of NaOH, affording dinuclear complexes Cu\textsubscript{2}L\textsubscript{3} and Cu\textsubscript{2}L\textsubscript{4}. The structures of the four complexes are displayed in Scheme 2.

Table 1 Absorption maxima (λ\textsubscript{max}) and molar absorptivity (ε) of the corresponding Cu(ii) complexes of L\textsubscript{1}–L\textsubscript{4} in methanol

<table>
<thead>
<tr>
<th>Complex</th>
<th>λ\textsubscript{max} (nm)</th>
<th>ε (M\textsuperscript{-1} cm\textsuperscript{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu\textsubscript{L1}</td>
<td>510</td>
<td>226</td>
</tr>
<tr>
<td>Cu\textsubscript{L2}</td>
<td>498</td>
<td>150\textsuperscript{a}</td>
</tr>
<tr>
<td>Cu\textsubscript{L3}</td>
<td>526</td>
<td>365</td>
</tr>
<tr>
<td>Cu\textsubscript{L4}</td>
<td>519</td>
<td>319</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Value obtained at the lowest studied concentration (1 × 10\textsuperscript{-4} M) since hyperchromicity was observed upon dilution.

The ε value of Cu\textsubscript{L2} is concentration-dependent, as further confirmed by a dilution study (Fig. S70\textsuperscript{†}), indicating that this complex undergoes self-aggregation in methanol. This unique feature of Cu\textsubscript{L2} complicated the study of its binding properties by UV-vis spectroscopy.

Besides, upon the Cu(ii) complex formation, the IR band corresponding to the stretching of the carbonyl amide of the ligands suffers a shift (54–63 cm\textsuperscript{-1}) to lower wavenumbers (Fig. S18–S21\textsuperscript{†}). This indicates the participation of the deprotonated amide groups in the coordination to Cu(ii), in agreement with the data obtained by EPR and the X-ray diffraction of the crystal structure of Cu\textsubscript{L2} (see below).

The X-band EPR spectrum of a frozen methanol solution at 103 K of Cu(ii) complexes is shown in Fig. 2. The Hamiltonian parameters obtained from the simulated spectra of these compounds are summarized in Table 2 (entries 1–4).

The simulated parameters show excellent agreement with the experimental spectra. All the spectra show a characteristic axial symmetry (g\textsubscript{∥} > g\textsubscript{⊥} > g\textsubscript{e} = 2.0023), and indicate that the copper ions have a square planar coordination. In all cases g\textsubscript{∥} and A\textsubscript{∥} parameters are typical of neutral CuN\textsubscript{4} units according to the Peisach and Blumberg plot\textsuperscript{19} (Fig. S32\textsuperscript{†}), pointing out that the metal centres are equatorially coordinated by four nitrogen atoms (two amines and two deprotonated amides). The values of the g\textsubscript{∥}/A\textsubscript{∥} ratio can be used as a convenient empirical index of tetrahedral distortion in Cu(ii) complexes.\textsuperscript{20}
in the studied cases, values of $g_{\perp}/A_{\perp}$ fall within the 105–135 cm$^{-1}$ range, typical of Cu(II) complexes possessing a non-distorted square-planar structure with a $d_{x^2-y^2}$ ground state. Finally, the large similarity between EPR spectra of mononuclear and dinuclear complexes, and the absence of a half-field signal at around 1600 G due to the $\Delta_{ms} = \pm 2$ transition in both frozen methanol (107 K) and DMF (103 K) solutions (Fig. S27 and S28†) rule out any Cu–Cu dipolar interaction; i.e., dinuclear Cu(II) complexes Cu$_2$L$_3$ and Cu$_2$L$_4$ behave as mononuclear complexes. This is also confirmed by the absence of the specific bands of the triplet state in the spectrum of the Cu$_2$L$_3$ complex with a 1:1 stoichiometry. The Cu$_2$L$_4$ complex crystallizes with a 1:1 stoichiometry. The complex crystallizes with one molecule of methanol that establishes hydrogen bonds with one amine group of the same molecule and with the amide carbonyl group of another molecule (Fig. S24†). The copper(II) cation is coordinated by two amine groups and two deprotonated amide groups, in a slightly distorted square planar geometry, and displays a torsion angle of 11.84° (between the plane containing N4–Cu–N3 and that containing N3–Cu–N2, see Fig. S25†).

The Cu–Namide distances are slightly shorter than the corresponding Cu–Namide being 1.919 and 2.043 Å on average, respectively. This fact was previously observed for analogous Cu–peptidic complexes and was attributed to the anionic coordination from the deprotonated Namide donors instead of the neutral donation from the Namide groups. The Cu–N angles contained in the five membered rings (N1–Cu–N2 and N3–Cu–N4) are ca. 10° smaller than the others (N2–Cu–N3 and N1–Cu–N4), as expected for a smaller ring size with a tighter environment. These values are slightly shorter than those obtained for an analogous copper complex with a three methylene chain spacer between the amide groups. The corresponding N1–Cu–N3 and N2–Cu–N4 distances are shorter than linear symmetry and also slightly shorter than in a previous analogous copper complex.†

Unfortunately, we were not able to obtain suitable crystals for X-ray diffraction with the Cu(II) complexes of the other ligands. However, the very similar UV-vis and EPR spectroscopic data support that the coordination environment of the copper ion is virtually identical for all the complexes.

Interaction of the copper complexes with dicarboxylates

Many short-chain dicarboxylates play a role in biotransformations. For example, succinate (Sue) acts as an electron donor in the citric acid cycle and plays a crucial role in adenosine triphosphate (ATP) generation in mitochondria. Malate (Mal) also takes part in the citric acid cycle and is a source of CO$_2$ in the Calvin cycle. Aspartate (Asp) is the precursor to several amino acids, including four that are essential for humans.

![Fig. 3 X-ray crystal structure of CuL2. Carbon (grey), oxygen (red), nitrogen (blue) and copper (orange).](image-url)
methionine, threonine, isoleucine, and lysine; it is also an
important neurotransmitter.

Among others, a variety of receptors and sensors for dicarboxylates containing two Cu(II) centers have been reported due to the bidentate nature of these anions and the good affinity of the carboxylate group for the Cu(II) ion. Most of the reported systems are dicopper complexes of polyamine macrocycles and cryptands. In this study we investigate the coordination properties of Cu(II) complexes coordinated by both amine and amide nitrogens present in pseudopeptidic ligands, in an environment that resembles more closely a metalloprotein.

We started our study with CuL3, as we reasoned that the presence of two Cu(II) ions at a close distance in its structure would make this complex a good receptor for short-chain dicarboxylate anions. We carried out an initial screening of various dicarboxylates (aliphatic dicarboxylic acids, amino acids and hydroxy acids) as potential guests for the complex by comparing the absorbance of the CuL3 complex alone and in the presence of 2 equivalents of dicarboxylate. Interestingly, in most cases the absorption spectrum was not affected by the addition of the dicarboxylate, while in a few cases the initial purple solution turned light blue or light green. We then focused on some particular cases and studied them in depth, by accurate UV-vis titration experiments in methanol. Unfortunately, solubility issues of the metal complexes prevented us from performing this study in water. Also, all the dicarboxylate substrates were used as the corresponding bis(tetrabutylammonium) (TBA) salts for a better solubility in methanol.

The most striking cases were those of Mal, Asp and Glu (throughout the study, the naturally occurring t isomers were always used). Titration of CuL3 with Mal showed a bathochromic shift in λmax from 526 to 575 nm (Fig. 4, top right) resulting in a change in colour from intense purple to light green. A similar trend from purple to pale blue was observed in the case of the corresponding TBA salts of Asp and Glu: the λmax shifted to 628 nm (Fig. 4, top left) and 604 nm, respectively. In contrast, addition of succinate had no effect on the absorption spectrum of the system (Fig. S34†). The case of N-acetyl-L-aspartate (N-Ac-Asp) was intermediate: the intensity of the absorption band at 526 nm decreased, but only to a low extent (≈20% of the initial value). A general trend clearly emerged from these titrations: complex CuL3 was able to coordinate dicarboxylates with a heteroatom in the α position to one COO− group. However, the dicarboxylates having the protected heteroatom were weakly coordinated and those lacking the heteroatom were not coordinated at all.

Following this, the titration curves of Mal and aspartate were analysed using Job plots. Job plots were built by reporting ΔAbs/ΔXanion as a function of Xanion (calculated with respect to Cu). In the case of Mal, the plot shows a maximum for Xanion = 0.50 (Fig. S38†) thus indicating that the Cu : Mal ratio in the formed species is 1 : 1. Accordingly, this result implies the binding of two equivalents of Mal per CuL3 complex molecule. Moreover, comparison of the absorption spectrum of Cu(Mal)2 obtained by addition of 2.0 equivalents of Mal to a solution of CuSO4 was that obtained after the addition of the deprotonated L3 ligand rendered two significantly different spectra. The first has a λmax = 691 nm (ε = 43 M−1 cm−1) while the second has a λmax = 649 nm (ε = 41 M−1 cm−1) (Fig. S39†). These combined data are consistent with the formation of a ternary complex with the formula CuL3Mal2.

The case of aspartate is different: the Job plot shows a maximum for Xanion = 0.56 (Fig. S44†). This value is intermediate between 0.5 and 0.66, suggesting the simultaneous presence of different species, with Cu : Asp ratios of 1 : 1 and 1 : 2.† The comparison of the absorption spectrum of CuAsp2 obtained by addition of 2.0 equivalents of Asp to a solution of CuSO4 with that obtained after the addition of the deprotonated L3 ligand rendered two almost identical spectra: the first has λmax = 634 nm (ε = 47 M−1 cm−1) while the second has λmax = 635 nm (ε = 49 M−1 cm−1) (Fig. S45†). These combined data suggest the presence of both CuL3Asp2 and CuAsp2 species, the latter being predominant. In other words, aspartate is able to displace a high fraction of the Cu(II) ion from the macrocyclic ligand L3.

These conclusions are supported by the EPR studies. Addition of Suc did not affect the EPR spectrum of the CuL3 complex, while addition of a small excess (ca. 3 eq.) of Mal and Asp altered it (Table 2, entries 3, 5–7 and Fig. 5). The value of A∥ decreased from 190.4 G to 162.8 G (Mal) and 168.2 G (Asp) while that of g∥ increased from 2.1884 to 2.2422 (Mal) and 2.2609 (Asp). These changes of the EPR parameters imply a modification of the coordination environment of the metal.

†Analysis of the titration data with Glu rendered the same result (Job plot maximum for Xanion = 0.60).
Cu(II) ions from the observation that Mal has a minor tendency to displace L3 (middle) provoked a similar transformation on the signals of Fig. S71, and 3.30 ppm, doublets of doublets at 2.75 and 2.95 ppm with the Cu(II) ion. Upon the addition of 2.0 equivalents of Mal, we undertook a 1H-NMR study of Cu2L3, but to a lesser extent. This difference supports the observation that Mal has a minor tendency to displace Cu(II) ions from L3, as it mainly forms the ternary species Cu2L3Mal2.

Ligand L4 is a macrocyclic structure that slightly differs from L3, due to the meta instead of the para substitution of the aromatic spacer. In principle, L4 would set the Cu(ii) ions at a closer distance. To find out if this small variation had an effect on the interaction with dicarboxylates, we investigated the coordination properties of the CuL4 complex. However, both EPR and UV-vis spectra showed no substantial differences.

Although less studied, mononuclear Cu(ii) complexes have also been employed in dicarboxylate binding.30 For this reason, and to gain further insight into the binding characteristics of the complexes, the coordination abilities of the open-chain mononuclear analogue of Cu2L3, CuL1, were studied.

According to UV-vis titrations, the coordination properties of CuL1 towards Suc, Mal and Asp are very similar to those of Cu2L3, apart from the differences in the stoichiometry of the binding.

Titration of CuL1 with Mal showed a bathochromic shift in λmax from 508 to 646 nm, resulting in a change in color from intense purple to light green (Fig. S56†). In the case of Asp, it changed to light blue and the λmax shifted to 631 nm (Fig. S61†). In contrast, addition of succinate had no effect on the absorption spectrum of the system (Fig. S54†). However, the titration curves for Mal and Asp (Fig. 6) are different; the first one is much steeper and reaches a plateau for 1.25 equivalents of Mal, while the latter one shows that saturation, if reached, requires a larger number of equivalents. The difference between Mal and Asp titration curves is clearer from the respective Job plots (inset in Fig. 6). Job plots were built by reporting ΔAbs/χanion as a function of χanion. In the case of Mal, the plot shows a maximum for χanion = 0.50 thus indicating that the Cu:Mal ratio in the formed species is 1:1 (Fig. S58†). We also compared the absorption spectrum of CuMal2 obtained by addition of 2.0 equivalents of Mal to a solution of CuSO4 in methanol, with that obtained after the addition of one equivalent of the deprotonated L1 ligand. The two spectra are different: the first has λmax = 697 nm while the second has

![Fig. 5 X-band EPR spectra of a frozen methanol solution (103 K) of Cu2L3 alone and in the presence of different dicarboxylate anions (black: experimental; red: simulated).](View Article Online)

![Fig. 6 Changes in the normalized absorbance at 508 nm of CuL1 upon addition of dicarboxylates (black: Suc; blue: Asp; red: Mal). Inset: Job plots for CuL1 + Asp (blue) and CuL1 + Mal (red).](View Article Online)
The case of Asp is quite different: the Job plot shows a maximum for $X_{\text{anion}} = 0.60$ and the comparison of the absorption spectrum of CuASP$_2$ with that obtained after the addition of one equivalent of the deprotonated L$_1$ ligand rendered two almost identical spectra (Fig. S63). Similarly to its macrocyclic analogue, the combined UV-vis data suggest the co-existence of a ternary complex (CuL1Asp) together with the CuASP$_2$ complex. However, the percentage of CuASP$_2$ in this case should be higher (the Job plot maximum now is 0.60; for CuL3 it was 0.56). Not surprisingly, it would be easier for Asp to displace the Cu(n) ion from the open-chain ligand L$_1$ than from the macrocyclic ligand L$_3$.

The $^1$H-NMR study of CuL$_1$ and its aspartate and malate adducts rendered similar results to those found for its macrocyclic counterpart CuL$_3$ (Fig. S72). The different aspect of the spectra supports the hypothesis that Mal forms mainly the ternary species CuL$_1$Mal, while Asp has a higher tendency to displace Cu(n) ions from the pseudopeptidic ligand, leading mainly to the formation of the CuASP$_2$ complex, together with a small amount of CuL$_1$Asp ternary species.

Detailed analysis of the interaction of CuL$_1$ with Asp and Mal

In view of the intricacy of the different equilibria taking place between the studied Cu(n) complexes and dicarboxylate anions, we undertook a detailed and quantitative study of the interaction between the simple mononuclear CuL$_1$ complex and the Asp/Mal pair. First of all, we determined the binding constants and the stoichiometry of binding of Asp and Mal anions to the Cu(n) cation under our experimental conditions. In fact, amino acids and hydroxy acids are known to form stable complexes with Cu(n), with a CuL$_2$ general formula. A solution of CuSO$_4$ in methanol was titrated with Asp or Mal. The UV-vis spectral variations regarding the titration with Asp are shown in Fig. S67.† The results are compatible with the formation of two complexes, CuASP and CuASP$_2$. The absorption is blue shifted upon complexation giving a maximum around 700 nm for the 1:1 complex and 637 nm for the 1:2 complex. The stability constants obtained by fitting the data to this equilibrium pattern are reported in Table 3 (entries 1–3).

Similar results were obtained regarding the Cu(n) complexation with Mal (Fig. S68†). In this case the 1:1 complex absorbs mainly in the 700–800 nm region whereas the 1:2 complex has a maximum around 694 nm (Table 3, entries 4–6). In both cases, the stability constants are high enough to allow the formation of the 1:2 complexes [CuL$_2$] under the conditions used for the UV-vis study ([Cu$^{2+}$] $\approx 1 \times 10^{-3}$ M; [dicarboxylate] up to $4 \times 10^{-3}$ M). Besides, the stability of the complexes formed by Cu(n) with Asp was seen to be slightly higher than those formed with Mal.

Following this, we determined the equilibrium constant for the formation of the CuL$_1$ complex (entries 7–9 in Table 3). The titration revealed the formation of a transient complex with a 2:1 stoichiometry, Cu$_2$L$_1$. Finally the titrations of CuL$_1$

\[ \lambda_{\text{max}} = 666 \text{ nm (Fig. S59)} \]. These combined data suggest the formation of a ternary complex with the formula CuL$_1$Mal as the predominant species.

The 1H-NMR study of CuL$_1$ and its aspartate and malate adducts rendered similar results to those found for its macrocyclic counterpart CuL$_3$ (Fig. S72). The different aspect of the spectra supports the hypothesis that Mal forms mainly the ternary species CuL$_1$Mal, while Asp has a higher tendency to displace Cu(n) ions from the pseudopeptidic ligand, leading mainly to the formation of the CuASP$_2$ complex, together with a small amount of CuL$_1$Asp ternary species.

The stability constants reported in Table 3 and [Cu] = [L$_1$] $= 1 \times 10^{-3}$ M.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reaction$^a$</th>
<th>Log $\beta$</th>
<th>Log $K$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cu + Asp $\rightarrow$ CuAsp</td>
<td>6.08 (3)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Cu + 2Asp $\rightarrow$ CuAsp$_2$</td>
<td>10.75 (3)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>CuAsp + Asp $\rightarrow$ CuASP$_2$</td>
<td></td>
<td>4.67</td>
</tr>
<tr>
<td>4</td>
<td>Cu + Mal $\rightarrow$ CuMal$_1$</td>
<td>5.77 (4)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Cu + 2Mal $\rightarrow$ CuMal$_2$</td>
<td>10.08 (4)</td>
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<tr>
<td>6</td>
<td>CuMal + Mal $\rightarrow$ CuMal$_2$</td>
<td></td>
<td>4.31</td>
</tr>
<tr>
<td>7</td>
<td>Cu + L$_1$ $\rightarrow$ CuL$_1$</td>
<td>5.40 (4)</td>
<td></td>
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<tr>
<td>8</td>
<td>2Cu + L$_1$ $\rightarrow$ CuL$_1$</td>
<td>9.25 (4)</td>
<td></td>
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<tr>
<td>9</td>
<td>Cu + CuL$_1$ $\rightarrow$ CuL$_1$</td>
<td></td>
<td>3.85</td>
</tr>
<tr>
<td>10</td>
<td>Cu + L$_1$ + Asp $\rightarrow$ CuL$_1$Asp</td>
<td>9.90 (1)</td>
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<td>11</td>
<td>CuL$_1$ + Asp $\rightarrow$ CuL$_1$Asp</td>
<td></td>
<td>4.50</td>
</tr>
<tr>
<td>12</td>
<td>Cu + L$_1$ + Mal $\rightarrow$ CuL$_1$Mal</td>
<td>11.01 (3)</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>CuL$_1$ + Mal $\rightarrow$ CuL$_1$Mal</td>
<td></td>
<td>5.61</td>
</tr>
</tbody>
</table>

$^a$ Charges have been omitted for simplicity.
hypothesis, as they showed the formation, in both cases, of Cu(II) species with CuN₂O₂ coordination, and overall zero charge (Table 2, entries 1, 8, 9 and Fig. S26†). In particular, the EPR spectrum of the CuL₁/Asp system is very similar to that of CuAsp₂ (obtained by mixing CuSO₄ and Asp in methanol) (Fig. S30†) with close spectral parameters (entry 10, Table 2). In the case of Mal, the EPR spectrum (Fig. S31†) shows two components of different intensities, both assignable to CuN₂O₂ coordination. This may be due to two different dispositions of the Mal anion in the ternary complex; in fact, the carboxylate ligand can be in a cis or trans position relative to the amino ligand in the Cu complex. The structure proposed for the ternary supramolecular complex CuL₁Mal also fits well the ATR-IR spectrum (Fig. S22†), which shows for the ternary complex a broad band with two major components: 1650 and 1593 cm⁻¹, relative to free and metal-coordinated amides, respectively. The complexity of the equilibria prevented us to undertake the same detailed study for the dinuclear CuL₃ complex. In particular, the stability constant of the macroyclic CuL₃ complex was too high to be accurately determined by UV-vis titrations in the required concentration range. However, the main difference between CuL₁ and CuL₃ seems to be the lower tendency of the macroyclic ligand to transfer the metal to Asp anions, probably due to the higher stability of the CuL₃ complex due to the macroyclic effect.

## Experimental

### Synthesis of the ligands

Primary diamines 1 and 2 were prepared as already described.¹⁷

**L₁:** To a solution of 1 (360 mg, 1.01 mmol) in methanol (15 ml) was added benzaldehyde (454 µl, 4.46 mmol) and the resulting solution was stirred at room temperature for 6 hours. Sodium borohydride (470 mg, 12.2 mmol) was added in an ice bath and the reaction mixture was stirred at room temperature for 16 hours. Aqueous 4 M HCl was added, and a white solid formed. After 1 hour the solvents were evaporated at reduced pressure, affording a solid that was suspended in aqueous 4 M NaOH. The product was extracted twice with DCM and once with AcOEt. Organic layers were combined, dried over MgSO₄ and concentrated at reduced pressure. The resulting thick oil was suspended in diethyl ether, triturated, filtered and washed with additional diethyl ether, affording L₁ as a white solid (440 mg, 0.82 mmol, 81% yield).

¹H-NMR (400 MHz, CDCl₃) δ 7.29 (bs, 2H), 7.26–7.15 (m, 12H), 7.12–7.09 (m, 4H), 7.05 (dd, J = 7.5, 1.9 Hz, 4H), 3.66 (d, J = 13.4 Hz, 2H), 3.48 (d, J = 13.4 Hz, 2H), 3.37–3.21 (m, 4H), 3.29 (dd, J = 9.4, 1.8 Hz, 2H), 3.13 (dd, J = 13.8, 4.7 Hz, 2H), 2.71 (dd, J = 13.8, 9.1 Hz, 2H), 1.65 (bs, 2H). ¹³C-NMR (101 MHz, CDCl₃) δ 174.4, 139.2, 137.5, 129.3, 128.8, 128.6, 128.0, 127.3, 127.0, 63.2, 52.7, 39.5, 39.3. HRMS (ESI-TOF) m/z [L₁ – H]⁺ Calcd for [C₃H₇₂N₄O₂] 535.3073, found 535.3080. IR (KBr, ν cm⁻¹): 3345, 3282, 1652, 1522, 745, 697.

**L₂:** Obtained following the same procedure described for L₁, using 2 (400 mg, 0.82 mmol) as the starting material. L₂ was obtained as a waxy solid (240 mg, 0.82 mmol, 44% yield).

¹H-NMR (400 MHz, CDCl₃) δ 6.75 (t, J = 5.8 Hz, 2H), 6.40–6.32 (m, 2H), 7.35–7.18 (m, 12H), 6.14 (d, J = 6.6 Hz, 4H), 7.07 (d, J = 7.4 Hz, 4H), 2.55 (dd, J = 13.4 Hz, 4H), 2.53 (d, J = 13.4 Hz, 2H), 3.53 (dd, J = 9.3, 1.8 Hz, 2H), 3.20–3.09 (m, 4H), 2.73 (dd, J = 13.8, 9.3 Hz, 2H), 1.75 (bs, 2H). ¹³C-NMR (101 MHz, CDCl₃) δ 174.0, 139.2, 137.4, 129.1, 128.7, 128.5, 128.0, 127.1, 126.8, 63.2, 52.6, 39.4, 35.6, 30.0. HRMS (ESI-TOF) m/z [L₂ – H]⁺ Calcd for [C₁₃H₁₉N₃O₆Cl] 547.3076, found 547.3073. IR (KBr, ν cm⁻¹): 3415, 3229, 1638, 1617, 1398.

L₃ and L₄ were prepared as already described.¹⁸ **L₃:** IR (KBr, ν cm⁻¹): 3415, 1653, 1521, 702. **L₄:** IR (KBr, ν cm⁻¹): 3417, 3232, 1643, 1520, 1397, 699.

### Synthesis of the Cu(II) complexes

**CuL₁:** To a solution of L₁ (105 mg, 0.197 mmol) in MeOH (20 ml) were added CuSO₄·5H₂O (50 mg, 0.200 mmol) and NaOH (16 mg, 0.40 mmol). A colour change from blue to purple was observed. The reaction mixture was stirred for 30 minutes at room temperature and then centrifuged for 10 minutes. The white solid was discarded while the purple solution was concentrated at reduced pressure. CuL₁ was obtained as a purple solid (94 mg, 0.157 mmol, 80% yield). UV-vis (1 × 10⁻³ M, MeOH) λₙₐₘₚ = 510 nm; ε = 226 M⁻¹ cm⁻¹. HRMS (ESI-TOF) m/z [CuL₁ + Cl]⁻ Calcd for [C₃H₇₂N₄O₂Cl] 630.1823, found 630.1899; [CuL₁ + HCOO]⁻ Calcd for (C₅H₅N₅)Cu₂O₄ 640.2111, found 640.2189. IR (KBr, ν cm⁻¹): 3406, 1598, 1454, 751, 700. Elemental analysis: found: C: 64.75%, H: 5.93%; N: 8.75%; calculated for CuL₁ + 2H₂O: C: 65.0%, H: 6.38%; N: 8.86%.

**CuL₂:** Prepared following the same procedure described for CuL₁. CuL₂ was obtained as a pink solid. 76% yield. UV-vis (2.4 × 10⁻³ M, MeOH) λₙₐₘₚ = 498 nm; ε = 94 M⁻¹ cm⁻¹. HRMS (ESI-TOF) m/z [CuL₂ + Cl]⁻ Calcd for (C₃H₇₂N₄O₂Cl) 644.1979, found 644.2072; [CuL₂ + HCOO]⁻ Calcd for (C₅H₅N₅)Cu₂O₄ 644.2072.
The UV-vis titrations All the titrations were carried out in methanol. UV-vis spectra were recorded on a SpectraMax M5 spectrophotometer. The association constants were calculated using the HypSpec 1.1.33 software for Windows. Species distributions of Fig. 7 were made with the HySS software (http://www.hyperquad.co.uk/hyss.htm).

Formation of CuAsp2 and CuMal2: a solution of 1.4 × 10^{-3} M CuSO4·5H2O was titrated respectively with 1.4 × 10^{-2} M (TBA)2-L-aspartate and 1.4 × 10^{-2} M (TBA)2-L-malate (from 0 to 3.0 equivalents). The absorption variations from 500 to 800 nm were fitted to 1 : 1 and 1 : 2 models.

Formation of CuL1: a solution of CuSO4·5H2O was titrated in batch with a solution of L1 7.0 × 10^{-4} M containing 2.2 equivalents of TBAOH. The next procedure was followed: several samples containing 1 mL of the Cu(n) solution and increasing amounts of the L1 solution (from 0 to 3.0 equivalents) were prepared and left to equilibrate for 24 hours prior to the measurement in the spectrophotometer.

**Crystal structure determination**

Data for all structures were collected on a STOE IPDS II twocircle diffractometer with a Genix Microfocus tube with mirror optics using MoKα radiation (λ = 0.71073 Å) and were scaled using the frame scaling procedure in the X-AREA program system.

The structures were solved by direct methods using the program SHELXS and refined against F² with full-matrix least-squares techniques using the program SHELXL-97.

All H atoms in L1 were refined using a riding model. Due to the absence of anomalous scatterers, the absolute configuration could not be determined.

The H atoms bonded to N and O in CuL2 were freely refined. The absolute configuration was determined by refining the Flack-x-parameter, x = -0.025(13) (Table 4).

CCDC 1059976 (L1) and CCDC 1059975 (CuL2) contain the supplementary crystallographic data for this paper.

### Table 4 Crystal data and structure refinement for L1 and CuL2

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<th>Compounds</th>
<th>L1</th>
<th>CuL2</th>
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<tr>
<td>Empirical formula</td>
<td>C_{64}H_{138}N_{26}O_{22}</td>
<td>C_{66}H_{142}N_{28}O_{24}</td>
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<tr>
<td>Form. weight [g cm⁻³]</td>
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<td>642.27</td>
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<td>Crystal system</td>
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<td>Space group</td>
<td>P1 (no. 1)</td>
<td>P2₁2₁2₁ (no. 19)</td>
</tr>
<tr>
<td>a [Å]</td>
<td>9.631(5)</td>
<td>10.094(5)</td>
</tr>
<tr>
<td>b [Å]</td>
<td>10.027(3)</td>
<td>15.905(10)</td>
</tr>
<tr>
<td>c [Å]</td>
<td>15.531(4)</td>
<td>19.5472(13)</td>
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<tr>
<td>α [°]</td>
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<td>90</td>
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<tr>
<td>β [°]</td>
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<td>90</td>
</tr>
<tr>
<td>γ [°]</td>
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<td>90</td>
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<tr>
<td>Volume [Å³]</td>
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<td>3138.7(4)</td>
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<tr>
<td>Absorption coeff.</td>
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<td>Reflections collected</td>
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<td>13 262</td>
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<tr>
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<td>5861</td>
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<tr>
<td>R(int)</td>
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<td>0.0591</td>
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<tr>
<td>R₁ [I &gt; 2σ(I)]</td>
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<td>wR₁ [all data]</td>
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<tr>
<td>Diff. hole [e Å⁻³]</td>
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<td>-0.451</td>
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Conclusions

The present study reports the synthesis and the characterization of mononuclear Cu(II) complexes with bis(amino amide) ligands (L1 and L2) and dinuclear Cu(II) complexes with tetra-amino amide) ligands (L3 and L4). The metal ion always displays the square planar coordination geometry, being connected to four nitrogen atoms (two amines and two deprotonated amides).

The complexes interact with hydroxy- and amino-dicarboxylate substrates, and this event provokes a strong change in the visible absorption spectra. In particular, the interaction with two biologically important dicarboxylates possessing very similar structures (malate and aspartate) has a completely different outcome. In the case of malate, a ternary complex [ligand···metal···dicarboxylate] is formed almost quantitatively; while in the case of aspartate, the anion is able to displace Cu(II) from the ligand to form, mainly, the Cu(Asp)2 complex. These complexes are model compounds that mimic the ligand exchange observed in natural systems and especially the function of metal ionophores. In fact, ionophores are specific ligands that bind a metal cation, transport it across a cellular membrane, and then release it in response to different stimuli (for example lower pH or presence of competitive ligands).

We are currently exploiting the modularity of the structure of the pseudopeptidic ligands, that allow changes in the spacer, the amino acid residue and the amine substituent, in order to improve the solubility of the Cu(II) complexes in water and tune their stability.

Acknowledgements

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Notes and references


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