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Thermodynamic stereoselectivity assisted by weak interactions in metal complexes. Chiral recognition of L/D-amino acids by the copper(II) complex of 6-deoxy-6-[4-(2-aminoethyl)imidazolyl]-cyclomaltoheptaose

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Potentiometric and spectroscopic methods were used to investigate the stability and bonding details of the copper(II) complexes of β-cyclodextrin (β-CD) functionalized by histamine through the imidazole N-1 (CDmh) and a number of enantiomeric pairs of amino acidates (AaO^-) where AaO^- is alaninate ($AlaO^-$), phenylalaninate ($PheO^-$) or tryptophanate ($TrpO^-$). Stability constant values were determined at 25 °C and $I = 0.1$ mol dm^{-3} (KNO_3). Enantioselectivity was observed in the copper(II) mixed complex formation of L/D-$TrpO^-$ in aqueous solution and $[Cu(CDmh)(L\text{-}TrpO)]^+$ was seen as being favoured over $[Cu(CDmh)(D\text{-}TrpO)]^+$. In contrast, the $AlaO^-$ systems did not show any differences in the stability of copper(II) diastereomeric ternary complexes. The c.d. spectra of the complexes containing aromatic L-amino acids showed much higher intensity ($\Delta\varepsilon$) compared with the spectra for the corresponding D-amino acids, the difference $\Delta(\Delta\varepsilon)$ increasing in proportion to the size of the side chain. Subtle differences were found to exist between the ESR parameters of the diastereomeric complexes with $TrpO^-$. However, these tended to disappear upon the addition of a less polar solvent. The results obtained are consistent with a model where the complexation of the L enantiomer to copper already bound to the histamine residue is favoured by the inclusion of the aromatic side chain in the cyclodextrin cavity, this in turn being due to the preferential *cis* arrangement of the amino nitrogens in the two ligands.

1.0 Introduction

Molecular recognition is the key step in a wide range of controlled separation and chemical transformation processes.[1–6] Nevertheless, it remains difficult to rationalize or even predict stereospecific recognition. The design of receptors which can recognize amino acids stereospecifically continues to attract considerable interest since therapeutic drugs which may be developed from chiral amino acid intermediates are increasingly required in an enantiomerically pure form. While earlier studies prompted the development of efficient receptors based on small molecules,[7,8] the binding of amino acids with high, predictable stereospecificity remains a difficult task to achieve.[9–11]

Cyclodextrins (CDs) are cyclic oligosaccharides normally consisting of six (α-CD), seven (β-CD) or eight (γ-CD) α(1→4)-linked D-(+)-glucopyranose units. In water, their inner cavity is considerably hydrophobic and the molecules can form inclusion complexes with a large range of guests.[12] The presence of chiral carbon atoms and a chiral cavity have also led to the use of CDs as chiral abiotic receptors.[13] Functionalized cyclodextrins have instead been employed as multi-site receptors where the discrimination ability of the hydrophobic cavity is greatly enhanced.[14–16]

The chiral recognition properties of mono- and bi-functionalized β-cyclodextrins have been improved over the past ten years with the formation of metal complexes which heighten the non-covalent interaction between the amino acid side-chain and the CD-cavity.[17–26] A number of examples may be mentioned. In particular, the copper(II) complex of β-CD functionalized by histamine attached *via* the amino nitrogen (CDhm) was found to show chiral selectivity towards aromatic amino acids and a molecular recognition mechanism was proposed on the basis of thermodynamic and spectroscopic investigation.[17] In another study, HPLC separation[18] of the enantiomers of unmodified aromatic amino acids (AaO^- = $PheO^-$, $TrpO^-$, and $TyrO^-$) was obtained using the complex $[Cu(CDhm)]^{2+}$ as an additive to the eluent in reverse phase chromatography. The elution order $k'_D < k'_L$ was ascribed to the difference between the stability constants of the two ternary complexes $[Cu(CDhm)(L\text{-}AaO)]^+$ and $[Cu(CDhm)(D\text{-}AaO)]^+$. The enantiomer which is involved in the more stable ternary complex in the mobile phase thus elutes first.[18]

Preliminary results for a copper(II) complex of a 6-derivative of β-cyclodextrin with histamine, bound through the imidazole N-1, *i.e.* 6-deoxy-6-[4-(2-aminoethyl)imidazolyl]cyclomaltoheptaose (CDmh),[27] showed reverse behaviour in the order of elution of the tryptophanate enantiomers in comparison to that found for the copper(II) complex with (CDhm). To explain this reverse behaviour in chiral ligand exchange chromatography, thermodynamic and spectroscopic investigations were carried out for the complexes of $[Cu(CDmh)]^{2+}$ with couples of amino acid enantiomers, *i.e.* $AlaO^-$, $PheO^-$, and $TrpO^-$. A new synthetic route was used to obtain the β-cyclodextrin derivative (Chart 1) with good yield.

2.0 Results and discussion

2.1 Conformational analysis of unprotonated and protonated CDmh species

The ^{1}H NMR spectra of the mono-, di- and un-protonated species of CDmh (see Fig. 1) were assigned by COSY, TOCSY

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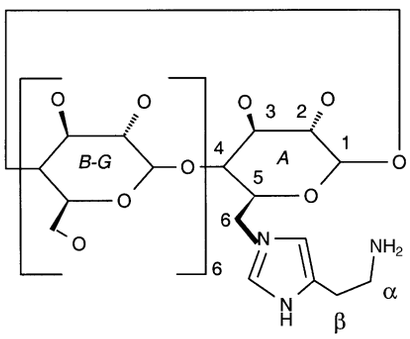

Chart 1

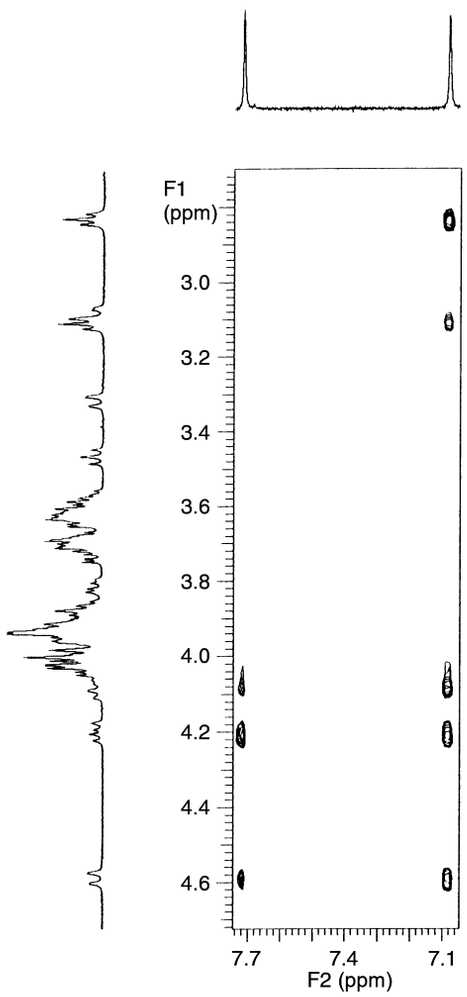

Fig. 1 Partial contour plot of the T-ROESY spectra of CDmh at pH = 9.

and T-ROESY. The ^{13}C spectra were assigned by HSQC. As previously described,[27] the functionalization of the A ring determines the downfield shift of the 6-A diastereotopic protons and a slight downfield shift in 5-A. The coupling constant values $J_{6\text{-A},5\text{-A}} = 1.8$ Hz and $J_{6'\text{-A},5\text{-A}} = 9$ Hz indicate that a *gauche–trans* conformation is preferred in the case of the A ring. The H-1 region is also modified as a consequence of functionalization which induces spreading of these signals in several groups. Together with the proton signals of the ethylenic chain of histamine, the proton signals of one of the unfunctionalized CD rings are also evident. On the basis of the ROESY experiment this ring was identified as the B ring. The evident upfield shift in the 6-H and 5-H protons of this ring can be ascribed to the ring current effect of the imidazole which is near the B ring. The 2D experiment also made it possible to assign the protons of the G ring, this being in proximity to the functionalized ring.

The 6-G protons are diastereotopic and one of them is seen to shift downfield (3.9 ppm). This shift may be due to the imidazole ring current. In the ROESY spectra, NOE correlations are evident between the 6-A and the 5-A protons and the protons of the imidazole ring. Furthermore, the NOE correlation with the 6-H and 5-H protons of the G ring and both the imidazole protons suggests that, in accordance with the evident upfield shift of the 6-B protons, the imidazole preferentially arranges itself almost perpendicular to the cavity but that it rotates with the 5-H of the imidazole near the 6-G protons so that one of these finds itself on the same plane as the imidazole ring. A similar arrangement has also been found in other studies on CDs functionalized with imidazole derivatives.[28–30]

The Corey–Pauling–Koltun models suggest that a hydrogen bond may form between the 3-N imidazole and the 6-OH proton of the B ring, thus causing the imidazole residue to move closer to the B glucose ring in keeping with the rigid arrangement of the imidazole. A similar H-bond has been described elsewhere in the solid state characterization of a CD functionalized with a small biomolecule bound through an imidazole ring.[29]

As far as the protonated species is concerned, the first protonation step involves the amino group. The effects of protonation on the ^1H NMR can clearly be seen in the downfield shift of the methylene protons. Very slight downfield shifts due to protonation are observed for the 6-H and 5-H protons of the unfunctionalized B ring. The arrangement of the imidazole ring in relation to the B ring is not seen to change as a consequence of amino group protonation. The ^{13}C spectra show how the ethylenic carbon in the β position to the 3-N proton of the imidazole ring shifts upfield after protonation. The second protonation step involves the imidazole nitrogen atom and a further downfield shift of the methylene and the 6-A, 5-A and 1-A protons alike is induced. Once again, there is a downfield shift in the 6-B protons. However, this is less evident in comparison with the shift seen in the unprotonated form. The ROESY spectra of $[(CDmh)H_2]^{2+}$ show an NOE correlation between the imidazole protons and the cyclodextrin moiety protons. In this particular species the correlation of the 5-H proton of the imidazole with the 5-A proton is stronger than that seen for the 2-H proton, thus suggesting that the imidazole ring is arranged slightly differently in relation to the CD cavity. It may be that the imidazole ring moves away from the B ring after protonation. If we hypothesize a hydrogen bond between the 6-OH proton of the B ring and the 3-N in the CDmh species, the break up of the H-bond after imidazole protonation determines the movement of the imidazole ring away from the cavity. The ^{13}C NMR spectrum of the diprotonated species clearly shows how protonation has caused a further upfield shift of the 6-A carbon atom and of the carbon atom in the β position to NH_2.

2.2 Proton and copper(II) binary complexes: formation and bonding details

The generalized formation reaction of the ligand with protons and copper(II) ions is given in eqn. (1), where L is CDmh. For

$$m\mathrm{Cu}^{2+} + h\mathrm{H} + l\mathrm{L} \rightleftharpoons \mathrm{Cu}_m\mathrm{H}_h\mathrm{L}_l \qquad (1)$$

the sake of clarity, charges on the copper(II) complexes have been omitted.

The stability constant β_{mhl} is defined in eqn. (2).

$$\beta_{mlh} = \frac{[\mathrm{Cu}_m\mathrm{H}_h\mathrm{L}_l]}{[\mathrm{Cu}^{2+}]^m[\mathrm{H}]^h[\mathrm{L}]^l} \qquad (2)$$

Table 1 Stability constant values for proton and copper(II) complexes of CDmh, CDhm and hm[a] at 25 °C and $I = 0.1$ mol dm^{-3} (KNO_3)

Equilibrium	log K[b]	Ref.
$CDmh + H^+ \rightleftharpoons [(CDmh)H]^+$	9.61(3)	This work
$[(CDmh)H]^+ + H^+ \rightleftharpoons [(CDmh)H_2]^{2+}$	5.17(3)	This work
$CDmh + Cu^{2+} \rightleftharpoons [Cu(CDmh)]^{2+}$	8.98(3)	This work
$[Cu(CDmh)]^{2+} + CDmh \rightleftharpoons [Cu(CDmh)_2]^{2+}$	7.54(3)	This work
$CDhm + H^+ \rightleftharpoons [(CDhm)H]^+$	8.01	57
$[(CDhm)H]^+ + H^+ \rightleftharpoons [(CDhm)H_2]^{2+}$	5.81	57
$CDhm + Cu^{2+} \rightleftharpoons [Cu(CDhm)]^{2+}$	7.26	57
$[(CDhm)H]^+ + Cu^{2+} \rightleftharpoons [Cu(CDhm)H]^{3+}$	2.94	57
$hm + H^+ \rightleftharpoons [(hm)H]^+$	9.79	58
$[(hm)H]^+ + H^+ \rightleftharpoons [(hm)H_2]^+$	6.07	58
$hm + Cu^{2+} \rightleftharpoons [Cu(hm)]^{2+}$	9.56	58
$[Cu(hm)]^{2+} + hm \rightleftharpoons [Cu(hm)_2]^{2+}$	6.56	58
$[(hm)H]^+ + Cu^{2+} \rightleftharpoons [Cu(hm)H]^{3+}$	3.07	58

[a] hm = histamine. [b] 3σ in parentheses.

Analysis of the titration data for CDmh in the absence of copper(II) provides the formation constants for amino and imidazole nitrogen protonation (eqns. (3) and (4)).

$$H^+ + L \rightleftharpoons HL^+ \quad (3)$$

$$2H^+ + L \rightleftharpoons H_2L^{2+} \quad (4)$$

The proton and copper(II) complex formation constants determined in this study are given in Table 1 together with those found for CDhm and histamine. The equilibria necessary to fit the experimental titration curves for the solutions of copper(II) complex formation with the CDmh under study are given in eqns. (5) and (6).

$$Cu^{2+} + L \rightleftharpoons CuL^{2+} \quad (5)$$

$$Cu^{2+} + 2L \rightleftharpoons CuL_2^{2+} \quad (6)$$

From pH = 3 to pH = 6 the major species was $[CuL]^{2+}$, while a lower percentage of $[CuL_2]^{2+}$ was formed.

The log K for the amino group protonation of CDmh (9.61) was seen to be slightly lower than that for hm (9.79) but significantly higher than that of CDhm (8.01). The decrease in the amino nitrogen basicity of this latter CD isomer derivative was attributed to the existence of a hydrogen bond which is destroyed in the formation of the monoprotonated species. The amino nitrogen protonation constant of CDmh does not require hydrogen bond formation; the NMR results thus show no evidence for this. In the imidazole group of CDmh (5.17), basicity decreases in comparison to the log K values associated with those of CDhm (5.81) and hm (6.07). The protonation constants may also be interpreted on the basis of the NMR results. The protonation of N-3 gives rise to a modification in the imidazole ring arrangement due to the break-up of the hypothesized hydrogen bond.

Formation of the $[Cu(CDmh)]^{2+}$ complex is seen to be favoured less than that of the $[Cu(hm)]^{2+}$ species but more than that of $[Cu(CDhm)]^{2+}$. In contrast, the stability constant value of the $[Cu(CDmh)_2]^{2+}$ complex is higher than that of the $[Cu(hm)_2]^{2+}$ species.

Comparison of the magnetic parameters (see Table 2) of $[Cu(CDmh)]^{2+}$ with those of both $[Cu(CDhm)]^{2+}$ and $[Cu(hm)]^{2+}$ suggests that the apical perturbation indicated by the low $A_{\parallel}$ value in the $[Cu(CDhm)]^{2+}$ species is absent in $[Cu(CDmh)]^{2+}$. Consequently, interaction with the oxygen atoms of the cyclodextrin cavity, previously thought to explain the large difference in the parallel hyperfine coupling constants of $[Cu(CDhm)]^{2+}$ and $[Cu(hm)]^{2+}$, can be ruled out. Similarly, it is possible to exclude the possibility that the equatorial plane

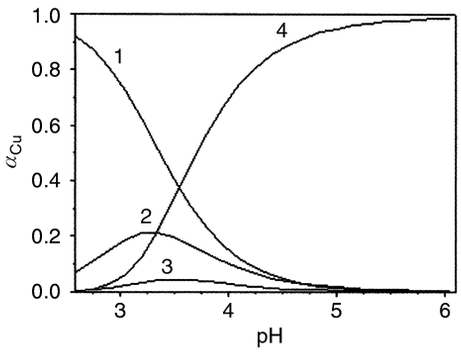

Fig. 2 Distribution diagram for the CuCDmh–L-Trp system: 1 free metal ion; 2 $[Cu(TrpO)]^+$; 3 $[Cu(CDmh)(TrpO)H]^{2+}$; 4 $[Cu(CDmh)(TrpO)]^+$.

distorts into a tetrahedral arrangement in the formation of the bis complex, $[Cu(CDhm)_2]^{2+}$ (which behaves in a similar way to $[Cu(hm)_2]^{2+}$). This suggests that the coordination ability of the histamine moiety is practically unaffected by the presence of the cyclodextrin cavity.

2.3 Thermodynamic stereoselectivity in ternary complexes of $[Cu(CDmh)]^{2+}$ with amino acidate

The reaction of CDmh (L) with copper(II) and amino acids (L′) is represented in eqn. (7) where L′ is the amino acidate. Charges have been omitted for the sake of simplicity. The stability constant $\beta_{mll'h}$ is defined in eqn. (8).

$$mCu^{2+} + hH^+ + lL + l'L' \rightleftharpoons Cu_mH_nL_lL'_{l'} \quad (7)$$

$$\beta_{mll'h} = \frac{[Cu_mH_hL_lL'_{l'}]}{[Cu^{2+}]^m[H]^h[L]^l[L']^{l'}} \quad (8)$$

In the pH range explored in the present work, $[Cu(CDmh)(AaO)]^+$ was seen to be the major ternary species, whereas one protonated species was identified in the acidic region (Fig. 2). The stability constant values of these two species are reported in Table 3 together with those for the analogous ternary complexes with Cdhm and hm. These values show two distinct types of behaviour. When the amino acidate has an aromatic moiety, the mixed complexes (*i.e.* the unprotonated species) with the L enantiomer are seen to be significantly more stable than those with the D enantiomer. In contrast, for the copper(II) alaninate ternary complexes, stereoselectivity is either absent or insignificant.

As shown in the equilibria defined in eqn. (9) where CDDA is

$$[Cu(CDDA)]^{2+} + AaO^- \rightleftharpoons [Cu(CDDA)(AaO)]^+ \quad (9)$$

either CDhm or CDmh, the formation of copper(II) ternary complexes with CDmh is favoured over those with CDhm. The highest differences are seen with $TrpO^-$. In particular, according to eqn. (9) (log $K = 10.01$), the maximum difference value (0.8 l.u.) is found when comparing the two more stable ternary species, $[Cu(CDmh)(L\text{-}TrpO)]^+$ and $[Cu(CDhm)(D\text{-}TrpO)]^+$ (log $K = 9.21$). A lower value (0.3 l.u.) is instead calculated for the two less stable complexes, $[Cu(CDmh)(D\text{-}TrpO)]^+$ (log $K = 9.16$) and $[Cu(CDhm)(L\text{-}TrpO)]^+$ (log $K = 8.86$). The enantioselective formation of metal complexes with L- and D-amino acids with [Cu(CDhm)] in aqueous solution has previously been reported elsewhere.[18] Using a combined thermodynamic and spectroscopic approach, the chiral recognition process in Cu–CDhm–AaO^- systems was explained by hypothesizing the preferential *cis* arrangement of the amino nitrogens.[18] This arrangement was thought to assist the preferential inclusion of the amino acid aromatic residue of the

Table 2 Spin Hamiltonian parameters for copper(II) complexes with CDmh, CDhm and hm in water–methanol (95 : 5) solution

Complex	$g_{\parallel}$	$A_{\parallel}$ [a]	$g_{\perp}$	$A_{\perp}$ [a]	Ref.
$[Cu(CDmh)]^{2+}$	2.300(2)	173(1)	2.060(3)	16(3)	This work
$[Cu(CDmh)_2]^{2+}$	2.227(2)	200(2)	2.055(3)	18(3)	This work
$[Cu(CDhm)]^{2+}$	2.304	153	2.079	—	57
$[Cu(CDhm)_2]^{2+}$	2.246	190	2.061	20	57
$[Cu(hm)]^{2+}$	2.305	171	2.068	15	57
$[Cu(hm)_2]^{2+}$	2.229	200	2.049	20	57

[a] Hyperfine coupling constants are given in units of 10^4 cm^{-1}.

Table 3 Stability constants log β_{1110} and log β_{1111} of copper(II) ternary complexes of CDmh, CDhm and hm with L/D-alaninate or -tryptophanate at 25 °C and $I = 0.1$ mol dm^{-3} (KNO_3) [a]

AaO^-	*mll′h*	CDmh [b]	CDhm [c]	hm [d]
L-$AlaO^-$	1110	17.11(3)	15.53	17.32
D-$AlaO^-$	1110	17.12(6)	15.51	—
L-$AlaO^-$	1111	21.33(6)		
D-$AlaO^-$	1111	21.32(6)		
L-$TrpO^-$	1110	18.99(6)	16.12	18.05
D-$TrpO^-$	1110	18.14(6)	16.47	—
L-$TrpO^-$	1111	21.46(6)		

[a] 3σ in parentheses. [b] This work. [c] Ref. 57. [d] Ref. 31.

Table 4 log K and log K_c values for $[Cu(DA)(L\text{-}TrpO^-)]^+$ [a] and $[Cu(CDDA)(L/D\text{-}TrpO^-)]^+$ [b] systems

AaO^-	hm	ampy	CDhm	CDmh	Cdampy
L-$AlaO^-$	0.0	0.0	0.0	0.0	0.0
D-$AlaO^-$	0.0	0.0	0.0	0.0	0.0
L-$TrpO^-$	0.60 [c]	0.48 [c]	0.5 [d]	1.8 [e]	1.1 [f]
D-$TrpO^-$	—	—	0.90 [d]	0.9 [e]	1.7 [f]

[a] Calculated according to standard values reported in ref. 31. [b] Calculated according to eqn. (11) with $[Cu(CDDA)(L/D\text{-}AlaO)]^+$ and $[Cu(DA)(L\text{-}AlaO)]^+$ as standards. [c] Ref. 31. [d] Ref. 18. [e] This work. [f] Ref. 19.

D enantiomer in the cyclodextrin cavity with the side chain of the L coordinated amino acid enantiomer protruding out of the cavity. The data for CDmh complexes show that the complex with L-$TrpO^-$ is more stable than the complex with D-$TrpO^-$. This is entirely in keeping with the previous hypothesis (*i.e.* a *cis* arrangement of the amino nitrogens). Only in the case of the L-enantiomer can the indole ring interact with the hydrophobic cavity of the β-cyclodextrin. Furthermore, chiral discrimination seems to be more efficient for the derivative with the histamine attached to the β-CD *via* the imidazole nitrogen than in the derivative with the histamine attached *via* the amino nitrogen. In fact, the difference in stability constant values for ternary complexes with the two enantiomeric tryptophanate anions is 0.35 l.u. for CDhm, whereas it is significantly higher, *i.e.* 0.85 l.u., for CDmh.

The structure and stabilization brought about as a result of the stacking interaction in ternary copper(II) complexes containing an aromatic amino acidate (AaO^-) and aromatic diamine (DA), have been investigated elsewhere. Various studies using potentiometric, spectroscopic and X-ray diffraction methods have looked specifically at AaO^- = L-phenylalaninate, L-tyrosinate and L-tryptophanate and DA = histamine.[31–34] In one of the papers concerned,[31] stabilization was seen to be due to the intramolecular non-covalent bonding between the L-amino acid side chain and the imidazole within these ternary copper(II) complexes and was evaluated using the significantly positive log K values for the following equilibrium (10) where

$$[Cu(DA)(L\text{-}AlaO)]^+ + [Cu(en)(L\text{-}AaO)]^+ \overset{K}{\rightleftharpoons} [Cu(DA)(L\text{-}AaO)]^+ + [Cu(en)(L\text{-}AlaO)]^+ \quad (10)$$

en = ethylenediamine. A stacking interaction has also been established in two copper(II) complexes, [Cu(hm)(L-PheO)-(ClO_4)] and [Cu(hm)(L-TyrO)(ClO_4)], in the solid state using X-ray crystal structure analysis.[32] The side–chain aromatic rings of the amino acid were located above the coordination plane in which the amino groups occupy a *cis* position, the shortest distances between the phenyl and imidazole rings varying from 3.49 to 3.45 Å for $PheO^-$ and $TyrO^-$, respectively. In addition, the mixed complexes are seen to exhibit close Cu–aromatic ring contacts with the shortest distances being 3.14 and 3.20 Å for $PheO^-$ and $TyrO^-$, respectively.

Stabilization effects due to the inclusion of the indole residue in the CD cavity within the ternary complexes of copper(II) with β-CDs functionalized with aromatic diamines (CDDA) such as histamine (CDmh, CDhm) or 2-aminomethylpyridine (CDampy), and L/D-$TrpO^-$ were evaluated according to eqn. (11), which is analogous to the equilibrium of eqn. (10) used by Yamauchi *et al.*[31,34] to give evidence of stacking interactions:

$$[Cu(DA)(TrpO)]^+ + [Cu(CDDA)(AlaO)]^+ \overset{K_c}{\rightleftharpoons} [Cu(DA)(AlaO)]^+ + [Cu(CDDA)(TrpO)]^+ \quad (11)$$

The positive values of log K_c (Table 4) show how the aromatic side-chain interaction with the hydrophobic cavity of a β-CD contributes to stabilization. Comparison with the previously reported log K values related to the non-covalent interaction between the side chain residues in the analogous copper(II) ternary complexes (Table 4), shows the more favourable contribution of the intramolecular inclusion of the indole side-chain. The log K_c values are also positive for the less stable diastereomeric complexes where the hypothesized structures would not allow an interaction of the indole residue with the cavity. As a consequence it would appear necessary to suppose that an equilibrium is set up between the *cis* and *trans* arrangement of amino nitrogens (Fig. 3). Similar conclusions have been reached by other authors in the study of bis-amino acidate copper(II) complexes.[35–38] However, other contributions which stabilize the ternary complexes of these functionalized CDs such as non-covalent interactions between the imidazole and the indole residues or between the d electrons of copper(II) ion and the π system of imidazole could be considered. Indeed, other studies have drawn similar conclusions as found for the mixed complex $[Cu(CDhm)(L\text{-}TrpO)]^+$ in the solid state.[39] In fact in this species, the indole and imidazole residues face each other in an approximately parallel fashion, the angle between these planes being 25°. The angles between the copper(II) coordination square plane (in which the amino nitrogens are again in a *cis* arrangement) and the plane of the imidazole ring and side chain rings of tryptophanate are instead 18° and 16°, respectively. There is therefore close contact between the metal ion and the two carbon atoms of the indole residue, the distances being 3.19 and 3.30 Å. These π–π and d–π interactions

stabilize the diastereoisomeric ternary complex [Cu(CDhm)-(L-TrpO)]$^+$ in which the preferential *cis*-arrangement of the amine nitrogens does not favour the inclusion of the indole residue in the CD cavity.

2.4 Spectroscopic evidence for stereoselectivity

Electronic, ESR and c.d. spectra were recorded to gain further information on the molecular recognition processes of [Cu-(CDmh)]$^{2+}$. The spin-Hamiltonian parameters reported in Table 5 are characteristic of copper(II) complexes ($g_\parallel > g_\perp >$ 2.04) in their typical elongated octahedral geometry with a $d_{x^2-y^2}$ ground state. In the frozen solution spectra a super-hyperfine (s.h.f.) structure is seen to be superimposed on the fourth copper line which unfortunately falls in the perpendicular region. This structure, which is well resolved, is due to the interaction of the copper(II) odd electron with the nitrogen nuclei present in the copper(II) coordination sphere (there being approximately seven s.h.f. lines ascribable to three nitrogen donor atoms). The $g_\parallel$ and $A_\parallel$ values reflect the nature of the ligands coordinated to the copper(II) ion. Comparison of these values with those associated with similar copper(II) systems previously reported[40–43] provides a further indication of the coordination of the histamine and amino acidate moieties to copper. As clearly shown in Table 5, no difference was observed in the magnetic parameters associated with the mixed complexes containing L- or D-AlaO$^-$ in frozen solution, irrespective of methanol content. In contrast, in the case of the analogous complexes with D- or L-TrpO$^-$, ESR spectra showed subtle differences both in the $g_\parallel$ value and in the parallel hyperfine coupling constant. The differences in the $A_\parallel$ values were seen to disappear when the methanol percentage reached 50%. Similar behaviour has been previously found for the copper(II)–CDhm–TrpO$^-$ system.[39] The subtle differences in the magnetic parameters obtained from the frozen aqueous solution with a low percentage (up to 10%) of methanol may be explained in a number of ways. They might be due to weak forces such as stacking interactions between the indole and imidazole residues; hydrophobic interactions of amino acidate side chain residues with the CD cavity, or d–π interactions between the π-cloud of the TrpO$^-$ indole group and the pertinent d orbitals of copper. Similar interactions may also provide an explanation for the differences observed in the Cu(II)–CDmh–TrpO$^-$ systems, although here the trend between the mixed copper(II) complexes with the two D- and L-diasteroisomers is reversed. As stated above, a similar difference in magnetic parameters was not observed for the ternary copper(II) complexes containing L- or D-AlaO$^-$, which have methyl groups as side chain residues. Furthermore, even if the differences in the $g_\parallel$ and in the $A_\parallel$ values for the diastereoisomeric complexes containing TrpO$^-$ are subtle, the fact that the values change according to the amount of methanol present suggests that they may be significant. It is well known that methanol has a levelling effect on the magnetic parameters of diastereomeric complexes when weak forces are operating. Yamauchi *et al.* have, however, shown that the addition of less polar organic solvents decreases the stacking interaction between the imidazole and the indole residues in [Cu(hm)-(L-TrpO)]$^+$.[31,33]

Table 5 ESR magnetic parameters from frozen solution of spectra of copper(II) complexes with CDmh and L- or D-AlaO$^-$, or L- or D-TrpO$^-$ at pH around 7

Amino acid	%CH_3OH	$g_\parallel$[a]	$A_\parallel$[a,b]	$g_\perp$[a]	$A_\perp$[a,b]
L-AlaO$^-$	10	2.240(1)	194(1)	2.049(3)	14(3)
D-AlaO$^-$	10	2.240(1)	192(2)	2.046(3)	15(3)
L-AlaO$^-$	50	2.241(1)	196(1)	2.053(3)	13(3)
D-AlaO$^-$	50	2.240(1)	196(1)	2.052(3)	13(3)
L-TrpO$^-$	10	2.237(1)	189(1)	2.042(3)	15(3)
D-TrpO$^-$	10	2.234(1)	192(1)	2.043(3)	15(3)
L-TrpO$^-$	50	2.236(1)	191(1)	2.045(3)	15(3)
D-TrpO$^-$	50	2.240(1)	191(1)	2.047(3)	13(3)

[a] Errors on the last digit in $g_\parallel$ and $A_\parallel$ values are the standard deviations from a series of measurements in which the experimental conditions were slightly varied around the Cu^{2+} : L : L′ = 1 : 1 : 1 ratio and pH = 7. [b] Hyperfine coupling constants are given in units of 10^4 cm^{-1}.

The UV-vis and c.d. spectral data of the ternary complexes [Cu(CDmh)(AaO)]$^+$ together with those of the simple copper(II) complex with CDmh are summarized in Table 6. The copper(II) mixed complexes with L/D-PheO$^-$ were also studied to evaluate the effect of the increased dimension of the amino acid side-chain residues.

According to the distribution data, obtained by means of the

Table 6 UV-vis and c.d. spectral parameters for [Cu(CDmh)]$^{2+}$, and for the ternary complexes [Cu(CDmh)(AaO)]$^+$ (AaO$^-$ = L/D-AlaO$^-$, L/D-PheO$^-$ or L/D-TrpO$^-$) at pH = 7

	UV-vis λ/nm (ε/M^{-1} cm^{-1})	c.d. λ/nm ($\Delta\varepsilon$/M^{-1} cm^{-1})
[Cu(CDmh)]$^{2+}$	218(6940), 626(72)	204(−1.21), 544(0.03)
L-AlaO$^-$	220(8436), 604(71)	225(−1.44), 540(0.014), 644 (0.011)
D-AlaO$^-$	220(8360), 604(69)	244(0.51), 552(0.012), 644(0.013)
L-PheO$^-$	208(18724), 242(4722), 606(104.6)	218(−13.52), 246(4.30), 298(0.094), 588(−0.68)
D-PheO$^-$	208(13589), 248(4722), 606(104.6)	217(7.96), 253(0.78), 298(0.018), 578(0.26)
L-TrpO$^-$	222(32028), 270(6563), 600(69)	224(−18.20), 246(10.07), 578(−1.15)
D-TrpO$^-$	222(31810), 272(6577), 600(69.33)	209(7.80), 252(−2.67), 570(0.64)

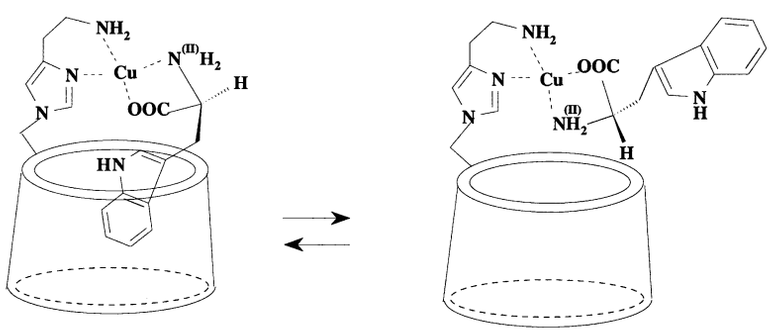

Fig. 3 *cis–trans* equilibrium in copper(II) ternary complexes of CDmh.

Table 7 Difference between the absolute values of the molar c.d. coefficients $\Delta(\Delta\varepsilon)$ of the [Cu(CDmh)(L-AaO)]$^+$ and [Cu(CDmh)-(D-AaO)]$^+$ complexes

AaO^-	λ/nm (L)	λ/nm (D)	$\Delta(\Delta\varepsilon)$
$AlaO^-$	644	644	0.02
$PheO^-$	588	578	0.42
$TrpO^-$	578	570	0.50

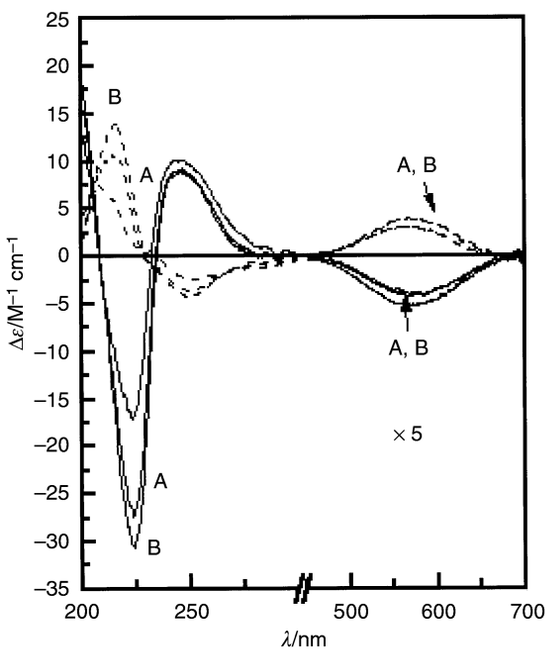

Fig. 4 c.d. spectra of [Cu(CDmh)(L-TrpO)]$^+$ (—) and [Cu(CDmh)-(D-TrpO)]$^+$ (----) at pH 6.8 alone (1×10^{-3} M) and in the presence of 1-adamantanol: 1×10^{-3} M (A) and 1×10^{-2} M (B).

DISDI program,[44] the ternary complexes alone contribute to the spectral bands, these being the only species existing at pH = 7.

If we compare both Schugar's[45,46] analysis of the electronic spectra of various copper(II) imidazole chromophores and the UV-vis and c.d. spectra displayed by binary and ternary copper(II) complexes of L-histidine and related ligands[47] with those exhibited by our systems, the similarity of the spectral pattern is evident. The UV bands may consequently be attributed to LMCT absorptions and the broad maxima to d–d transitions. This also suggests that all the donor atoms are involved in the coordination to the metal centre. (The UV-visible spectra derived from aliphatic and aromatic amino acid copper(II) complexes have been reported elsewhere along with their c.d. spectral data.[48–50])

For the mixed complexes containing $TrpO^-$, the c.d. spectra are seen to be remarkably different from each other both in the visible and UV regions. This behaviour is similar to that found in the [Cu(CDhm)(AaO)]$^+$ complexes[17] but, in this case, the c.d. absorption of the ternary complex with the L-enantiomer is much higher than that seen for the D-enantiomer (Fig. 4). On the basis of the above-mentioned hypotheses regarding the structural features of these ternary compounds,[17] this enhancement of the c.d. intensity observed for [Cu(CDmh)-(L-TrpO)]$^+$ may be attributed either to the deeper inclusion of the copper(II) chromophore in the CD cavity or to a more rigid conformation with the amino acid side chain which perturbs the copper(II) geometrical arrangement.

Furthermore, if we take the difference between the molar c.d. coefficients at maximum wavelengths in the visible region as a parameter to differentiate the diastereomeric species, we can observe an increase in the bulkiness of the aromatic amino side chain (Table 7). The progressive differentiation in the c.d. spectra of the diastereomeric complexes may be related to stereoselective interactions between the coordinated ligands. Since such interactions involve the amino acid side chains, they can only be of a non-covalent nature within the CD cavity. As in the case of stacking interactions, it is expected that the inclusion of the aromatic side chain in the cavity restricts the conformational mobility of the participating groups. This would explain the enhancement of the molar c.d. coefficient for [Cu(CDmh)(L-AaO)]$^+$ complexes. The addition of 1-adamantanol, the inclusion tendency of which is well known, shows a decrease in $\Delta\varepsilon$ values in the visible and UV regions (Fig. 4) for the complexes with the L-enantiomers. This is due to competition for the cavity between the aromatic residues of the amino acids and the adamantane ring. In contrast, an increase in $\Delta\varepsilon$ is observed for the complexes with the D-enantiomers, this probably being due to a more favourable stacking interaction between the imidazole residue and the indole ring, as previously found for analogous systems.[39]

3.0 Experimental

3.1 Chemicals

Copper(II) nitrate was prepared from copper(II) basic carbonate by adding a slight excess of HNO_3. Stock solution concentration was determined by EDTA titrations with a murexide indicator. Copper(II) sulfate pentahydrate was obtained from Aldrich. The concentrations of HNO_3 and KOH stock solutions were determined by titration with the primary standard tris(hydroxymethyl)aminomethane (THAM) and potassium hydrogen phthalate, respectively. Potassium nitrate (Suprapur Merck) was used without further purification. All solutions were prepared with doubly distilled water. All amino acids (Aldrich or Fluka) were high purity products used without further purification. Purity was checked by means of potentiometric titrations with a standard KOH solution and was always found to be higher than 99.8%. Polarimetric tests gave substantially identical results.

3.2 Synthesis of 6-deoxy-6-[4-(2-aminoethyl)imidazolyl]cyclomaltoheptaose (CDmh)

6-Deoxy-6-[4-(*N*-*tert*-butoxycarbonyl-2-aminoethyl)imidazolyl]cyclomaltoheptaose was obtained as previously reported.[28] A 500 mg solution in 2 ml CF_3COOH was stirred under nitrogen at room temperature. After 1 h the solvent was evaporated and the solid obtained was purified by elution from a 30 × 500 mm column of CM-Sephadex C-25 resin (in NH_4^+ form). Water followed by a 0–0.2 M gradient of NH_4HCO_3 aqueous solution (total volume: 6 dm^3) was used as an eluent. The product was then isolated pure (yield: 90%) $R_f = 0.16$ (PrOH–H_2O–AcOEt–NH_3 5 : 3 : 1 : 1). FAB MS, *m*/*z* 1228 (M + 1). ^{1}H NMR (500 MHz, D_2O, pH = 9): δ 7.71 (s, 1H, H-2 of Im), 7.08 (s, 1H, H-5 of Im), 5.12–5.07 (m, 5H, H-1 of CD), 5.06 (m, 2H, H-1A, H-1B), 4.59 (d, 1H, H-6A), 4.20 (dd, 1H, H-6′A), 4.08 (t, 1H, H-5A), 4.05–3.80 (m, 22H, H-5,-6,-3), 3.79–3.56 (m, 14H, H-2,-4, -5B), 3.46 (t, 1H, H-4A), 3.31 (d, 1H, H-6B), 3.12–3.07 (m, 3H, CH_2 of histamine, H-6′B), 2.82 (t, 2H, CH_2). ^{1}H NMR (500 MHz, D_2O, pH = 7): δ 7.92 (s, 1H, H-2 of Im), 7.22 (s, 1H, H-5 of Im), 5.15–5.07 (m, 7H, H-1 of CD), 4.64 (d, 1H, H-6A), 4.28 (dd, 1H, H-6′A), 4.12 (t, 1H, H-5A), 4.05–3.80 (m, 22H, H-5,-6,-3), 3.79–3.57 (m, 14H, H-2,-4,-5B), 3.46 (t, 1H, H-4A), 3.34 (d, 3H, H-6B, and CH_2NH_2), 3.15 (dd, 1H, H-6′B), 3.00 (t, 2H, CH_2). ^{1}H NMR (500 MHz, D_2O, pH = 5): δ 8.41 (s, 1H, H-2 of Im), 7.55 (s, 1H, H-5 of Im), 5.13–5.09 (m, 6H, H-1 of CD), 5.08 (d, 1H, H-1B), 4.82 (d, H-6A), 4.52 (dd, 1H, H-6′A), 4.21 (t, 1H, H-5A), 4.09–3.86 (m, 23H, H-5,-6,-3), 3.78–3.58 (m, 12H, H-2,-4), 3.55 (t, 1H, H-4B), 3.46 (t, 1H, H-4A), 3.44–3.32 (m, 4H, H-6B, CH_2 of histamine, H-6′B), 3.19 (t, 2H, CH_2). ^{13}C NMR (50.3 MHz, D_2O, pH = 9): δ

141.1 (C-2 of Im), 138.5 (C-4 of Im), 121.1 (C-5 of Im), 104.6 (C-1 of CD), 85.9 (C-4A), 83.9 (C-4), 75.7–74.8 (C-3,C-5,C-2), 73.6 (C-5A), 62.9 (C-6), 62.1 (C-6B), 50.4 (C-6A), 42.2 (C α to NH_2), 29.3 (C β to NH_2). ^{13}C NMR (50.3 MHz, D_2O, pH = 7): δ 141.2 (C-2 of Im), 138.6 (C-4 of Im), 121.4 (C-5 of Im), 104.6 (C-1 of CD), 85.9 (C-4A), 84.1, 83.8, 83.6 (C-4), 75.7–74.8 (C-3,C-5,C-2), 73.6 (C-5A), 63.2, 62.9 (C-6), 62.0 (C-6B), 50.4 (C-6A), 41.8 (C α to NH_2), 27.8 (C β to NH_2). ^{13}C NMR (50.3 MHz, D_2O, pH = 5): δ 138.7 (C-2 of Im), 131.9 (C-4 of Im), 123.6 (C-5 of Im), 104.6 (C-1 of CD), 85.9 (C-4A), 84.2, 83.9 (C-4), 75.7–74.8 (C-3,C-5,C-2), 72.3 (C-5A), 63.4, 63.0 (C-6), 62.6 (C-6B), 52.5 (C-6A), 40.4 (C α to NH_2), 25.0 (C β to NH_2).

3.3 Equilibria measurements

Stability constants for proton and copper(II) complexes were calculated from potentiometric titrations carried out at 25 °C (total volumes: 2.5 cm^3). The KOH solution was added using Hamilton burettes equipped with 0.25 or 0.50 cm^3 syringes. pH changes were measured using a combined Metrohm 125 microelectrode, standardized on the pH = $-\log [H^+]$ scale by titrating HNO_3 solutions with CO_2-free KOH. The ionic strength of all solutions was 0.1 mol dm^{-3} (KNO_3). The concentrations of the β-cyclodextrin derivative (L) and the amino acids (L′) ranged from 0.002 to 0.005 mol dm^{-3}. Duplicate or triplicate titrations were carried out for simple complexes at 1 : 1 and 1 : 2 Cu/L ratios and for mixed complexes at a 1 : 1 : 1 Cu/L/L′ ratio. Other details were as previously described.[51]

3.4 Electronic and c.d. spectroscopy

Absorption spectra were recorded on a Hewlett-Packard HP 8452 spectrophotometer. A JASCO J-600 dichrograph was used for the circular dichroism spectra. Calibration of the c.d. instrument was performed with a 0.06% solution of ammonium camphorsulfonate in water ($\Delta\varepsilon$ = 2.40 M^{-1} cm^{-1} at 290.5 nm). The spectral range between 200 and 700 nm was covered by using quartz cells of various path lengths. Dilution of the solution was therefore not required. Results are reported in terms of ε (molar adsorption coefficient) and $\Delta\varepsilon$ (molar c.d. coefficient) in M^{-1} cm^{-1}.

3.5 NMR spectroscopy

^{1}H NMR spectra were recorded at 25 °C in D_2O on a Varian Inova 500 spectrometer at 499.883 MHz. The ^{1}H NMR spectra were measured using standard pulse programs from the Varian library. In all cases the length of the 90° pulse was *ca.* 7 μs. 2D experiments were carried out using 1K data points, 256 increments and a relaxation delay of 1.2 s. T-ROESY spectra were obtained with a 300 ms spin-lock time. ^{13}C NMR spectra were recorded at 25 °C in D_2O on a Bruker AC-200 spectrometer at 50.9 MHz. The sodium salt of 3-(trimethylsilyl)-1-propane sulfonic acid was used as an external standard. Mono- and di-protonated CDmh solutions were prepared by adding the stoichiometric amount of DCl to the D_2O solution.

3.6 ESR spectroscopy

Frozen aqueous solution ESR spectra were acquired on a Bruker ER 200D X-band spectrometer driven by a Bruker ESP 3220 data system equipped with standard low-temperature apparatus. All spectra were recorded at 150 K using quartz tubes with 3 mm inner diameters. Microwave frequency was standardized against the diphenylpicrylhydrazyl radical (g = 2.0036), and the magnetic field was monitored using a Bruker ER 035 M gauss meter.

Aqueous solutions of the copper(II) mono complex were obtained by mixing solutions of $^{63}Cu(NO_3)_2$ with the appropriate ligand in a 1 : 1 metal to ligand ratio and adjusting the pH to 6. To promote the formation of the bis species, a four-fold excess of the ligand was added to a 2.5×10^{-3} mol dm^{-3} copper(II) nitrate solution and the pH was adjusted to 7. Up to 10% methanol was added to this aqueous solution in order to increase spectral resolution. In the case of the copper(II) ternary complexes (Cu^{2+} : L : L′ = 1 : 1 : 1 ratio and pH = 7) up to 50% methanol was added to estimate the influence of an organic solvent on the magnetic parameters. Parallel spin-Hamiltonian parameters for the frozen solution spectra were obtained directly from the experimental spectra. These were recorded on an enlarged scale and were calculated from the 2nd and 3rd lines in order to avoid second order effects.[52] Perpendicular magnetic parameters were calculated using the field of an overshoot in all the spectra as previously reported.[53]

3.7 Calculations

Calculations for the electrode system $E°$ values, ligand purity, and HNO_3 excess in the metal stock solution were performed using the Gran method[54] or the least-squares ACBA computer program.[55] All other potentiometric data were handled using the program SUPERQUAD,[56] thus minimizing the error-square sum of the differences between measured and calculated electrode potentials. In this paper, errors in stability constant values are expressed as three times standard deviations.

4.0 Concluding remarks

The thermodynamic stereoselectivity found in copper(II) ternary complexes of amino acids with aromatic residues provides an explanation for the chromatographic behaviour of the $[Cu(CDmh)]^{2+}$ abiotic receptor. Its chiral recognition, as opposed to that of $[Cu(CDhm)]^{2+}$, implies the preferential *cis* arrangement of amino nitrogens which differentiates the non-covalent interactions of the aromatic residues with the β-CD cavity. A combined thermodynamic and spectroscopic approach confirms what was previously found for a *cis–trans* equilibrium which more or less shifted towards the *cis* form depending on other non-covalent forces such as d→π and π–π stacking interactions. As a consequence, the different chiral recognition features, as indicated by thermodynamic and/or spectroscopic parameters, are the result of a balance between different weak forces in the diastereomeric copper(II) ternary complexes.

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