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A thermodynamic insight into the recognition of hydrophilic and hydrophobic amino acids in pure water by aza-scorpion type receptors†

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Interactions of different hydrophilic (His, Asp, Glu,) and hydrophobic (Ala, Phe, Tyr, Trp) amino acids in water with a scorpion aza-macrocyclic (**L1**) containing a pyridine group in the ring and its derivative (**L2**) bearing a naphthalene group in the tail have been analysed by potentiometric and calorimetric measurements. Theoretical calculations corroborate that major attractive forces that hold the adduct together are hydrogen bonds and salt-bridges, even though other interactions such as π -stacking or $\text{NH}^+\cdots\pi$ may contribute in the case of hydrophobic amino acids and **L2**. Calorimetric measurements indicate that the interactions between **L1** and the different amino acids are principally driven by entropy, often associated with solvation/desolvation processes.

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Introduction

Molecular recognition implies complementarity of electronic and structural features between the receptor and the target substrate.¹ In the case of biological substrates, the forces involved in the recognition processes are mostly electrostatic attractions (charge–charge, dipole–charge, dipole–dipole, and H-bonds). Such electrostatic forces are markedly weakened in polar solvents, in particular, in water, which, apart from having high polarity, is also an efficient donor and acceptor of H-bonds. Hence, molecular recognition in water requires high complementarity between substrates and receptors and the presence of many binding sites on both, as actually occurs in biological systems. On the other hand, water favours hydrophobic association, which is another important aspect of the biological world. Accordingly, the design of synthetic receptors for the recognition of biological substrates in water is very challenging, but it is also a very attractive objective since water is the solvent of life. To obtain a clear view of the binding event in water, enthalpic and entropic contributions give fundamental information. Nonetheless, these types of data related to pure water are very scarce in the literature.²

Here we report on the interactions of a selection of hydrophilic (His, Asp, Glu) and hydrophobic (Ala, Phe, Tyr, Trp)

amino acids with two scorpion aza-macrocycles (**L1** and **L2**, see Chart 1). Amino acid recognition is of great interest in different fields like drug delivery,³ protein–nucleic acid recognition⁴ or amino acid sensing.⁵ This process is particularly challenging, since they have a zwitterionic nature over a wide pH range and often require host species having functionalities able to simultaneously interact with their positive ammonium and negative carboxylate sites. In this respect, in spite of being relatively small molecules, **L1** and **L2** contain different binding sites (amine and/or ammonium groups, hydrophobic moieties) and have the ability to self-adapt to the guest amino acid due to the flexibility afforded by their hanging arms. Indeed, such an induced-fit ability is a key point for successful recognition in many biological systems.

L1 and **L2** have been chosen to achieve selectivity with amino acids of different hydrophobicity due to their different hydrophilic/hydrophobic balance.⁶ In the case of the more water soluble **L1**, a complete set of thermodynamic binding data is provided.

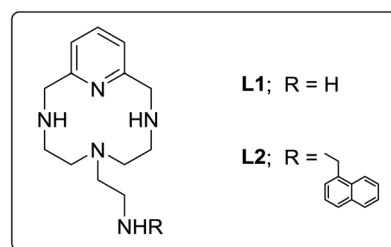


Chart 1

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Results and discussion

Protonation of L1 and L2

The equilibrium constants for the protonation of **L1** and **L2** were reported in a previous paper where it was anticipated that **L2** undergoes important conformational changes upon protonation.⁶ In the mono- and diprotonated forms, the molecule assumes a folded conformation stabilized by intramolecular hydrogen bonds, involving the amino group of the pendant arm and the ammonium group(s) of the macrocyclic moiety, and π -stacking interaction between the two aromatic units. Further protonation of **L2**, occurring on the amino group of the pendant arm, causes the opening of such a conformation with loss of all intramolecular interactions to form a more expanded structure where the three positive charges of $[\text{H}_3\text{L}_2]^{3+}$ are brought as far apart as possible to minimize the electrostatic repulsion between them.⁷

This was clearly evidenced by NMR spectra recorded for ligand solutions at different pH values and by the crystal structures obtained for **L2**, $[\text{H}(\text{L}_2)]\text{ClO}_4 \cdot \text{H}_2\text{O}$ and $[\text{H}_3(\text{L}_2)](\text{H}_2\text{PO}_4)_3 \cdot \text{H}_2\text{O}$.⁶ Furthermore, stacking of pyridine and naphthalene moieties for **L2** was also evidenced by changes in the UV-Vis spectra as a function of pH (see Fig. 1).

The calorimetric study performed in this work showed that such conformational changes are clearly manifested by the enthalpic and entropic contributions to ligand protonation, in contrast to the free energy change (stability constants) which

Table 1 Thermodynamic data for the protonation of **L1** and **L2** in 0.15 M NaClO_4 , at 298.1 K

Reaction	Log <i>K</i>	ΔG° (kcal mol^{-1})	ΔH° (kcal mol^{-1})	$T\Delta S^\circ$ (kcal mol^{-1})
L1 + $\text{H}^+ \rightleftharpoons [\text{HL1}]^+$	10.19(6) ^a	−13.90(8)	−10.19(7)	3.7(1)
$[\text{HL1}]^+ + \text{H}^+ \rightleftharpoons [\text{H}_2\text{L1}]^{2+}$	9.19(7)	−12.54(9)	−12.05(9)	0.5(1)
$[\text{H}_2\text{L1}]^{2+} + \text{H}^+ \rightleftharpoons [\text{H}_3\text{L1}]^{3+}$	7.94(5)	−10.83(7)	−11.46(5)	−0.6(1)
L2 + $\text{H}^+ \rightleftharpoons [\text{HL2}]^+$	10.01(1)	−13.65(1)	−8.6(4)	5.1(4)
$[\text{HL2}]^+ + \text{H}^+ \rightleftharpoons [\text{H}_2\text{L2}]^{2+}$	8.71(1)	−11.88(2)	−13.2(6)	−1.3(6)
$[\text{H}_2\text{L2}]^{2+} + \text{H}^+ \rightleftharpoons [\text{H}_3\text{L2}]^{3+}$	7.27(1)	−9.92(2)	0.1(7)	10.0(5)

^a Numbers in parentheses are standard deviations in the last significant figure.

appears to be almost insensitive to these structural modifications (Table 1). As shown in Table 1, successive protonation of **L1** is accompanied by invariably favourable enthalpy changes, while the entropic contributions become less and less favourable with increasing ligand protonation and consequent increasing solvation, according to a general trend observed for polyamines.⁸ A similar behaviour is found for the first two protonation stages of **L2**, while the third one is an athermic process moved by a largely favourable entropy contribution (Table 1): the breaking of intramolecular bonds, occurring at this stage, compensates the favourable enthalpy contribution coming from protonation of the amine group but gives the molecules a greater conformation freedom.

Therefore, it appears that to achieve a closed conformation, both intramolecular hydrogen bonding and π -stacking interaction need to occur simultaneously in this kind of receptors. Taking into account that these types of intramolecular interactions are precluded to **L1**, it can be assumed that this receptor is not able to adopt a “closed” conformation.

Binding of amino acids by L1 and L2

The equilibrium constants for the interaction of protonated forms of **L1** and **L2** with Ala, Phe, Tyr, Trp, His, Asp and Glu were determined by means of pH-metric titrations in 0.15 M NaClO_4 solutions at 298.1 K. Analyses of the titrations curves by means of the HYPERQUAD⁹ program afforded the equilibrium constants for the general reaction (1):



where A^{n-} is the amino acid in its completely deprotonated form ($n = 1$ for Ala, Phe, His and Trp; $n = 2$ for Tyr, Asp and Glu). It has been shown for many similar assemblies that the supramolecular interaction does not alter significantly the protonation patterns and the distribution of the charged groups in the interacting species; that is, the interacting partners maintain their identity, and no significant redistribution of protons occurs.¹⁰ Accordingly, the m protons of the $\text{H}_m\text{LA}^{(m-n)+}$ complexes were assumed to be localized on the ligand or on the amino acid in accordance with their basicity, and the relevant binding equilibria were derived as reported in Table 2 for

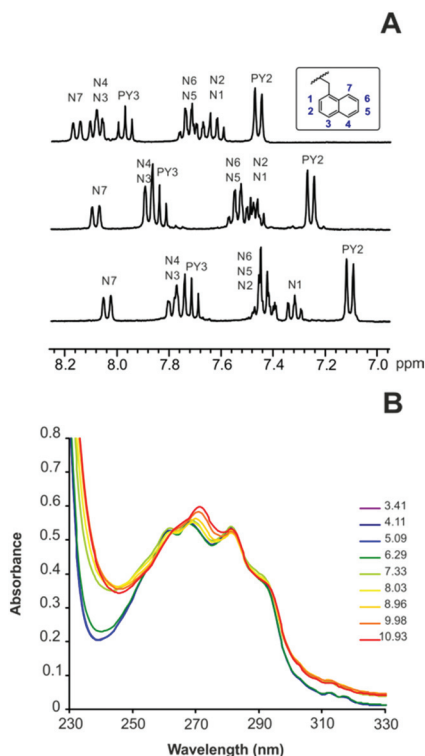


Fig. 1 (A) ^1H NMR spectra in D_2O of **L2** recorded from the top to the bottom at $\text{pD} = 4.1$, $\text{pD} = 8.2$ and $\text{pD} = 9.4$. (B) pH dependence of the absorption spectra of **L2**.



Table 2 Thermodynamic data for the formation of selected amino acid complexes with **L1** in 0.15 M NaClO₄ at 298.1 K

Reaction	Log <i>K</i>	ΔH° (kcal mol ⁻¹)	$T\Delta S^\circ$ (kcal mol ⁻¹)
HL ⁺ + Tyr ²⁻ $\rightleftharpoons$ [HL(Tyr)] ⁻	3.27(1) ^a	9.0(4)	13.5(4)
HL ⁺ + HTyr ⁻ $\rightleftharpoons$ [HL(HTyr)]	3.61(1)	5.0(3)	9.9(3)
H ₂ L ²⁺ + HTyr ⁻ $\rightleftharpoons$ [H ₂ L(HTyr)] ⁺	3.79(1)	7.2(4)	12.3(4)
H ₂ L ²⁺ + H ₂ Tyr $\rightleftharpoons$ [H ₂ L(H ₂ Tyr)] ²⁺	3.31(1)	6.2(3)	10.7(3)
H ₃ L ³⁺ + H ₂ Tyr $\rightleftharpoons$ [H ₃ L(H ₂ Tyr)] ³⁺	3.39(1)	7.0(4)	11.6(4)
HL ⁺ + Asp ²⁻ $\rightleftharpoons$ [HL(Asp)] ⁻	3.2(1)	5.1(2)	9.5(2)
HL ⁺ + HAsp ⁻ $\rightleftharpoons$ [HL(HAsp)]	3.48(6)	3.4(2)	8.2(2)
H ₂ L ²⁺ + HAsp ⁻ $\rightleftharpoons$ [H ₂ L(HAsp)] ⁺	3.47(6)	4.1(2)	8.9(2)
H ₃ L ³⁺ + HAsp ⁻ $\rightleftharpoons$ [H ₃ L(HAsp)] ²⁺	3.72(6)	4.6(2)	8.3(2)
H ₃ L ³⁺ + H ₂ Asp $\rightleftharpoons$ [H ₃ L(H ₂ Asp)] ³⁺	4.26(6)	4.3(2)	10.2(2)

^a Numbers in parentheses are standard deviations in the last significant figure.

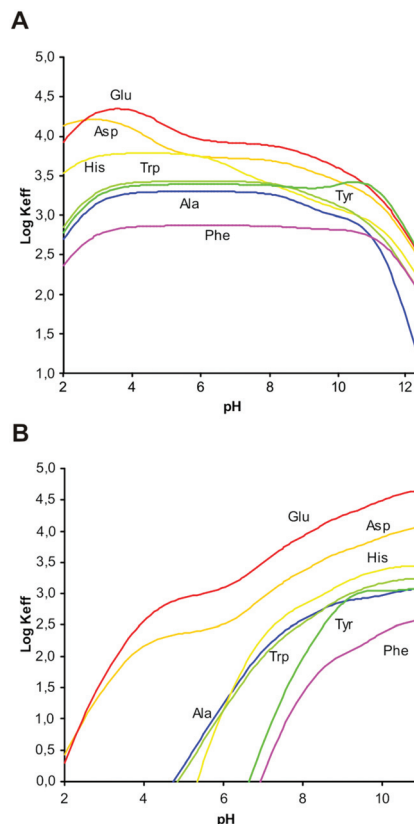
Tyr and Asp with **L1** and in Tables S1 and S2† for all the amino acids with both ligands.

A comparison of the equilibrium information collected in these tables evidence significant differences in the binding properties of the two ligands towards amino acids. Very impressive is the ability of the neutral (not protonated and not charged) **L2** ligand to form stable complexes with anionic forms of amino acids. These are the most stable complexes formed by **L2**, the stability of the other complex species decreasing with increasing ligand protonation. Conversely, **L1** requires at least one positive charge, [HL1]⁺, to interact with the amino acids and the stability of its complexes generally increases with ligand protonation. Accordingly, **L2** is a better receptor for amino acids in neutral-to-alkaline media while **L1** extends its binding properties to the acidic region. To better visualize and quantify such differences, the stability constants reported in Tables S1 and S2† can be used to calculate effective stability constants (K_{eff} ,¹¹ eqn (2)) for complexation equilibria involving total amounts of reactants and products in the form:

$$K_{\text{eff}} = [\text{H}_{x+y}\text{LA}]/[(\text{H}_x\text{L})][(\text{H}_y\text{A})] \quad (2)$$

Plots of log K_{eff} as a function of pH for all the complex systems here studied are shown in Fig. 2. As can be seen, for **L1** all complexes show the same general trend with a slight increase of log K_{eff} at around pH 3, where deprotonation of the carboxylic group present in the amino acid takes place.

This interaction remains constant in a wide pH range until total deprotonation of **L1** occurs at around pH 9, a value from which a marked decline in log K_{eff} is observed. If the overall charge of the amino acids is considered, we observe that complex stability is not determined by merely electrostatic attractions. For instance, the equilibrium constant for the interaction of [H₃L1]³⁺ with H₂Asp (log K = 4.26, Table 2) is higher than the constant for the interaction of the same ligand form with the more charged HAsp species (log K = 3.72, Table 2), in contrast to electrostatic expectations, while the opposite trend, obeying Coulomb's law, is observed when

**Fig. 2** Log K_{eff} vs. pH for complexes with **L1** (A) and **L2** (B).

HTyr⁻ and H₂Tyr (log K = 3.79 and 3.31, respectively, Table 2) are considered. Other examples of this type can be found in Tables S1 and S2 (see ESI†). Evidently, other forces than charge-charge interactions contribute to determine the stability of these amino acid complexes.

However, for **L2** a clearly different behaviour is observed depending on the hydrophilic/hydrophobic character of the amino acids studied. As can be seen in Fig. 2B, in comparison with Ala, amino acids like Glu or Asp show an increasing interaction from acidic to basic pH values that can be attributed to the additional carboxylate group present in the side chain. Nevertheless, interaction with amino acids containing aromatic moieties (Phe, Tyr, Trp, His) is observed only for pH values above 5. This behaviour can be principally ascribed to the π -stacking interactions between the aromatic rings present in the amino acid side chain and the naphthalene moiety of **L2**.

¹H NMR spectra recorded with solutions containing **L2** and Glu showed that opening of the ligand structure upon protonation ([H₂L2]²⁺ $\rightarrow$ [H₃L2]³⁺) occurs also in the presence of amino acids (Fig. S1†). As already discussed for the free ligand, such a conformational change has a considerable enthalpic cost which is overcompensated by the entropic gain originating from the increased freedom achieved by the ligand when passing from closed to open conformation. Nevertheless, if the open ligand conformation is constrained by interaction with an amino acid, this favourable entropic gain vanishes and



the corresponding complex loses stability. Most likely, this is the reason why the amino acid complexes with **L2** in open conformation ($[\text{H}_3\text{L2}]^{3+}$), when formed, are the less stable complexes produced by this ligand. In contrast, **L1**, maintaining an open conformation in all its forms, does not experience a similar depression of complex stability in acidic solution and, instead, the greater positive charge of its highly protonated forms enhances the complex stability.

In agreement with the ^1H NMR information, UV-vis spectroscopic data obtained for **L2** and its Glu and Ala complexes confirm that the presence of the amino acid, in $[\text{H}_3\text{L}(\text{GluH})]^{2+}$ species, forces the ligand to remain longer in its open conformation, while in the presence of Ala the effect is more reduced.^{12,13}

Theoretical calculations

To rationalize these behaviours, we performed a modeling study on the complexes formed by **L1** and **L2**. The modeling was performed using simulated annealing procedures based on a molecular mechanics method. The empirical force field was used as implemented in the HyperChem program.¹⁴ In all cases, the solvent effects were implicitly simulated. The minimum energy structures of the different complexes formed by **L1** with Tyr and by **L2** with Glu, which are used here as an example, are shown in Fig. 3 and 4, respectively, while those for the other complex systems are shown in Fig. S2–S7.†

A general observation coming from the analysis of the minimum energy structures of the complexes formed with all amino acids is that when the amino acid contains an

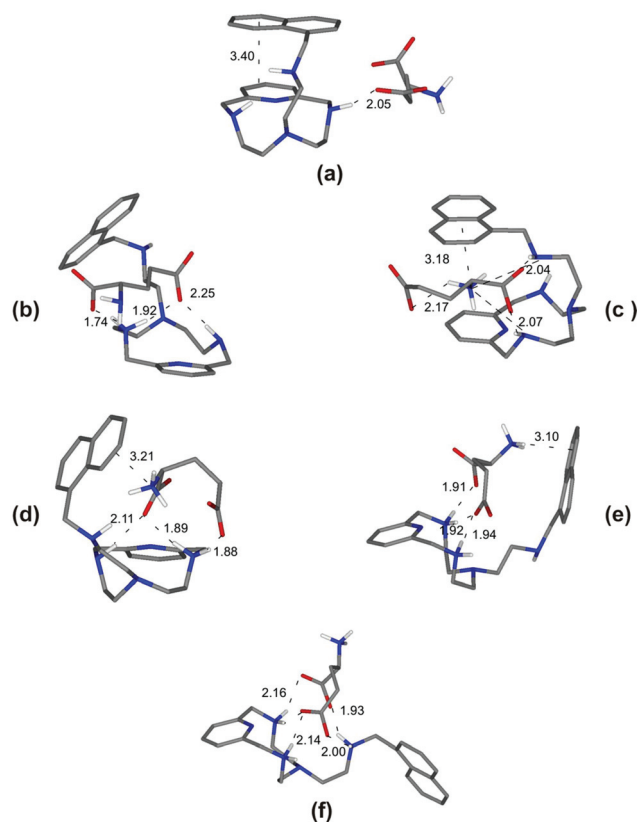


Fig. 4 Minimum energy structures calculated for the complexes $[\text{L2}-(\text{Glu})]^{2-}$ (a), $[\text{HL2}(\text{Glu})]^{-}$ (b), $[\text{L2}(\text{HGlu})]^{-}$ (c), $[\text{HL2}(\text{HGlu})]^{-}$ (d), $[\text{H}_2\text{L2}(\text{HGlu})]^{+}$ (e), and $[\text{H}_3\text{L2}(\text{HGlu})]^{2+}$ (f).

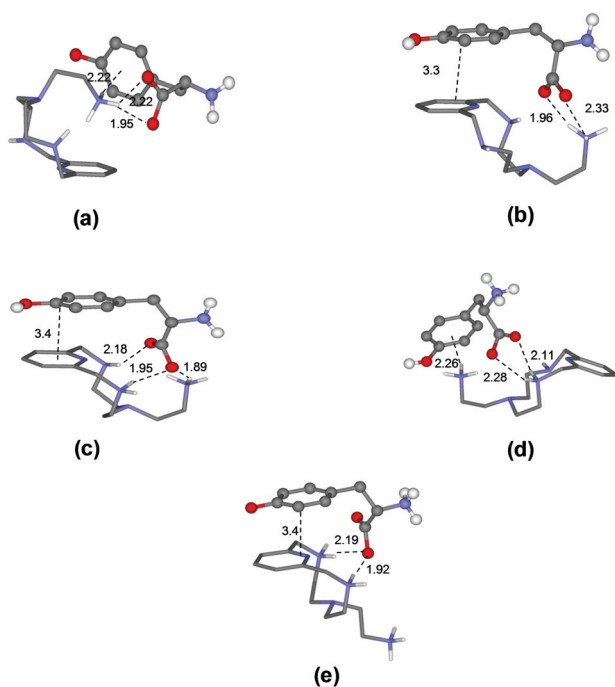


Fig. 3 Minimum energy structures calculated for the complexes $[\text{HL1}-(\text{Tyr})]^{-}$ (a), $[\text{HL1}(\text{HTyr})]$ (b), $[\text{H}_2\text{L1}(\text{HTyr})]^{+}$ (c), $[\text{H}_2\text{L1}(\text{H}_2\text{Tyr})]^{2+}$ (d), and $[\text{H}_3\text{L1}(\text{H}_2\text{Tyr})]^{3+}$ (e).

ammonium group, the interacting partners approach each other in such a way as to locate the positively charged ammonium groups as far apart as possible. Hence, although at a macroscopic level (stability constants) electrostatic forces appear not to play a fundamental role, at the molecular level they can be fundamental in regulating the mutual orientation of the interacting partners within the complex.

As a specific example, the minimum energy structures for the complexes with tyrosine are shown in Fig. 3. According to the calculated structure, the $[\text{HL1}(\text{Tyr})]^{-}$ complex, formed by $[\text{HL1}]^{+}$ with the completely deprotonated, 2- charged form of tyrosine, is held together by a bifurcated hydrogen bond involving the two tyrosine carboxylate oxygen atoms and a single proton of the ligand ammonium group, and by a $\text{NH}^{+}-\pi$ interaction between this ammonium group and the aromatic ring of the amino acid (Fig. 3a). Upon the protonation of the phenolate tyrosine oxygen to give $[\text{HL1}(\text{HTyr})]$, strengthening of the complex is observed thanks to the formation of two salt-bridges between the oxygen atoms of the carboxylate groups of tyrosine and two hydrogen atoms of the ligand ammonium groups accompanied by π -stacking interaction between the aromatic residues of the interacting partners (Fig. 3b). Such structural features are consistent with the fair increase of complex stability observed in solution (from $\log K = 3.27$ to $\log K = 3.61$, Table 2). Protonation of the ligand to form the $[\text{H}_2\text{L1}(\text{HTyr})]^{+}$



Depending on the amino group where protonation occurs, two different closed conformations are possible. If protonation occurs over one of the amino groups of the macrocyclic core to give $[\text{HL2}(\text{Glu})]^-$, a rearrangement can be observed accompanied by the formation of two new salt-bridges between the oxygen atoms of the carboxylate groups of Glu and the hydrogen atoms of the receptor ammonium group (Fig. 4b). However, if protonation occurs over the Glu's amino group, a most favourable $[\text{L2}(\text{HGlU})]^-$ complex is formed due to the presence of an additional $\text{NH}^+ \cdots \pi$ interaction between the ammonium group of amino acids and the naphthalene moiety, thereby strengthening the complex (Fig. 4c). Such structural features are consistent with the fair increase of

^aNumbers in parentheses are standard deviations in the last significant figure.

The formation of amino acid complexes with **L1** was also studied by means of isothermal titration calorimetry in 0.15 M NaClO₄, at 298.1 K, to obtain the enthalpy changes and

derived entropic terms reported in Tables 2 and S1.† Unfortunately, an analogous study was not possible with **L2**, since its complexes are not enough soluble to analyse with the apparatus described in the Experimental section. As can be seen in Tables 2 and S1,† most of the complexation reactions are endothermic and promoted by favourable entropy changes, although there are a number of complexation equilibria, mostly related to the formation of histidine and alanine complexes, that are weakly exothermic or nearly athermic. The enthalpy changes associated with similar interactions result from a subtle combination of favourable and unfavourable contributions deriving from the formation of weak bonds (favourable) and desolvation effects (unfavourable) the latter being strictly related to the local neutralization of charge occurring upon substrate–receptor interaction. Desolvation effects are normally dominating when oppositely charged species are involved, leading to positive (unfavourable) enthalpy changes and positive (favourable) entropic gains.² The favourable entropic contribution caused by the release of solvent molecules occurring upon charge neutralization is normally large enough, as in the present case, to overcome the loss of entropy due to substrate–receptors association. As shown by the thermodynamic parameters in Tables 2 and S1,† these effects give rise to some compensation between the enthalpy and entropy changes for the relevant complexation reactions. A good linear $\Delta H^\circ - T\Delta S^\circ$ correlation (correlation coefficient (R) of 0.98) is obtained for the complete set of data reported in Table S1† (Fig. 5).

Similar $\Delta H^\circ - T\Delta S^\circ$ compensatory relationships hold in general for complexation reactions involving weak interactions, *i.e.*, van der Waals, hydrogen bonding, dipole–dipole, and ion–dipole interactions, and have been used to obtain quantitative estimations of ligand conformational changes resulting from complex formation, according to the general observation that the slope α of the $\Delta H^\circ - T\Delta S^\circ$ plots ($T\Delta S^\circ = \alpha\Delta H^\circ + I$) increases with the adaptability of the ligand binding sites to the specific substrates.¹⁶ From this point of view, the high α value (1.02(4)) obtained for the amino acid complexes with

L1 compares well with the values (0.89–0.92) previously obtained for the binding of phosphate and pyrophosphate anions by acyclic and macrocyclic polyammonium receptors,¹⁷ suggesting, by analogy with the latter, a good adaptability of **L1** in all its protonated forms to the amino acidic substrates, despite the ligand stiffening occurring upon accumulation of positive charge on the ligand.

Experimental

EMF measurements

The potentiometric titrations were carried out at 298.1 ± 0.1 K using 0.15 M NaClO₄ as the supporting electrolyte. The experimental procedure (burette, potentiometer, cell, stirrer, micro-computer, *etc.*) has been fully described elsewhere.¹⁸ The acquisition of the emf data was performed with the computer program PASAT.¹⁹ The reference electrode was a Ag/AgCl electrode in saturated KCl solution. The glass electrode was calibrated as a hydrogen-ion concentration probe by titration of previously standardized amounts of HCl with CO₂-free NaOH solutions and the equivalent point was determined by Gran's method,²⁰ which gives the standard potential, E° , and the ionic product of water ($pK_w = 13.73(1)$).

The computer program HYPERQUAD was used to calculate the protonation and stability constants.⁸ The pH range investigated was 2.5–11.0 and the concentration of the amino acids and of the ligands ranged from 1×10^{-3} to 5×10^{-3} mol dm^{−3} with the A : L molar ratio 1 : 1. The different titration curves for each system (at least two) were treated either as a single set or as separated curves without significant variations in the values of the stability constants. Finally, the sets of data were merged together and treated simultaneously to give the final stability constants.

Calorimetric studies

The enthalpies of ligand protonation and anion binding were determined in the same ionic media as those of potentiometric measurements by means of an automated system composed of a Thermometric AB thermal activity monitor (model 2277) equipped with a perfusion–titration device and a Hamilton Pump (model Microlab M) coupled with a 0.250 cm³ gas-tight Hamilton syringe (model 1750 LT). The microcalorimeter was checked by determining the enthalpy of reaction of strong base (KOH) with strong acid (HCl) solutions. The value obtained ($-56.7(2)$ kJ mol^{−1}) was in agreement with the literature values.²¹ In a typical experiment, a NaOH solution (0.10 M, addition volumes 15 μ L) was added to acidic solutions of the ligand (5×10^{-3} M, 1.2 cm³), containing equimolar quantities of the amino acid in the binding experiments. Corrections for heats of dilution were applied. The corresponding enthalpies of reaction were determined from the calorimetric data by means of the AAAL program.²² ΔH° and $T\Delta S^\circ$ values for the protonation of anions were redetermined under our experimental conditions (Table S3, ESI†).

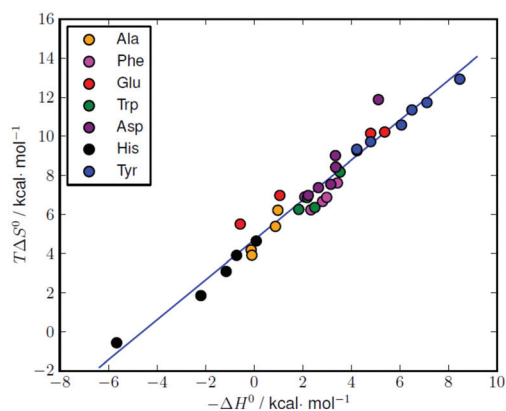


Fig. 5 Enthalpy–entropy compensation plot for the complexation of different amino acids with **L1** (see Table S1† for the original data). Correlation: $T\Delta S^\circ = (1.02 \pm 0.04)(-\Delta H^\circ) + (4.69 \pm 0.15)$, $r = 0.98$.



Theoretical calculations

Molecular modelling investigations on the adducts formed by different protonated forms of **L1** and **L2** with some amino acids were performed according to the location of acidic protons on the interacting partners reported in Tables 2, 3 and S1† (tables of stability constants). The potential energy surface of all systems was explored by means of simulated annealing (empirical force field method AMBER3,²³ $T = 600$ K, equilibration time = 5 ps, run time = 10 ps and cooling time = 15 ps, time step = 1.0 fs, atomic partial charges evaluated at the PM3²⁴ semi-empirical level of theory). For each system, 80 conformations were sampled.

In the case of **L1**, adducts formed with Ala, Asp, His, Glu, Phen, Tyr and Trp were studied using an implicit simulation of the aqueous environment ($\epsilon = 4\epsilon$). In the case of ligand **L2**, only adducts formed with Ala and Glu were studied. Due to the formation of adducts between the amino acids and the ligand in its completely deprotonated form, calculations were preliminarily performed in a vacuum, restraining to a given value the interaction distances between donor/acceptor groups (N–H...O distance 2.0 Å, an additional force constant of 7 kcal mol^{−1} Å^{−2}).

Conclusions

Interaction of several hydrophilic (His, Asp, Glu) and hydrophobic (Ala, Phe, Tyr, Trp) amino acids in water with two scorpion aza-macrocycles (**L1** and **L2**) has been analysed and two clearly differentiated behaviours have been observed. The stability of **L1** complexes with all amino acids remains almost constant in a wide pH range until total deprotonation of the receptor forces it to decrease. Conversely, **L2** complexes show an increasing stability at high pH values that can be attributed to the action of the naphthalene moiety attached to the pendant arm of the receptor.

In all cases, theoretical calculations show that the main interactions take place through the carboxylate groups of the amino acids. In this sense, the major attractive forces that hold the adduct together are hydrogen bonds and salt-bridges, even though other interactions such as π -stacking or $\text{NH}^+ - \pi$ may give a significant contribution, particularly for hydrophobic amino acids and **L2**.

Calorimetric measurements indicate that the interaction between **L1** and the different amino acids is principally driven by entropy changes, often associated with solvation/desolvation processes.

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