Optically Active Hexaazamacrocycles: Protonation Behavior and Chiral-Anion Recognition

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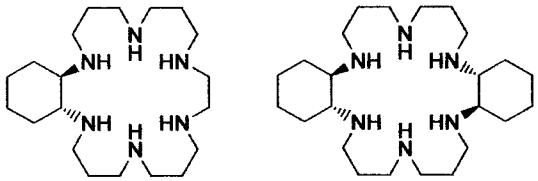
The protonation features of two optically active 22-membered hexaazamacrocycles possessing one (**L1**) or two (**L2**) (*R*,*R*)-cyclohexane-1,2-diamine moieties have been studied by means of potentiometric ^{1}H- and ^{13}C-NMR techniques. This study allows the determination of the basicity constants and the stepwise protonation sites. The presence of the cyclohexane decreases the protonation ability, and this effect can be explained in terms of conformational and electrostatic factors. Binding of different chiral dicarboxylates has been studied by potentiometry. Macrocycle **L2** presents higher anion-complexation equilibrium constants than **L1**. The stability of the diastereoisomeric complexes depends on the pH, and the structures of the macrocycles and anions. Receptor $\mathbf{L1}\cdot 6\,H^+$ shows moderate D-selectivity towards tartrate anion, whereas $\mathbf{L2}\cdot 6\,H^+$ exhibits a good preference for *N*-Ac-D-aspartate. Both protonated **L1** and **L2** form strong complexes with *N*-Ac-glutamate, and the stoichiometry of the complex depends on the degree of protonation and the absolute configuration of the anion. For this last anion, both azamacrocycles exhibit a clear D-preference.

1. Introduction. – Over the last few decades, the development of organic receptor molecules capable of binding anionic species has elicited wide interest in both chemical and biological fields [1]. Several approaches are based on the use of protonated polyammonium macrocycles [2], and their anion-coordination properties have been extensively explored [3]. The anion-complexation units of these receptor molecules consist of several positively charged binding sites, and, consequently, structural factors such as the cavity size of the macrocycle and the number of N-atoms or their relative disposition play an important role in their anion-binding features [3][4]. Thus, protonated hexa- and heptaazamacrocycles having 18- to 24-membered rings have been shown to be excellent receptors for organic phosphates [3a][3d–g] and polycarboxylates [3b–c][4], some of which are involved in biological processes, such as excitatory amino acids [5], nucleotides, or nucleic acids [6].

However, in spite of the importance of chirality in living organisms, examples of suitable ligands for the selective complexation of chiral anions are scarce (for chiral recognition of dicarboxylic acids, but not dicarboxylate anions, see [7]). Cyclodextrins have shown themselves to be excellent ligands for the recognition of helicity [8], but only discrete selectivities have been obtained with compounds bearing chiral centers [9]. Sapphyrin-based receptors [10] have been successfully used for the recognition and transport of aspartate and glutamate derivatives. Just recently, in our group, we achieved a good enantiomeric and diastereoisomeric discrimination of optically active dioxatetraazamacrocycles towards chiral 1,2-dicarboxylates [11].

In a previous paper, we reported an easy synthesis of two enantiomerically pure hexaazamacrocycles **L1** and **L2** (*Fig. 1*) by a chemoenzymatic strategy [12]. These

compounds display certain interesting characteristics: *i*) they both display 22-membered rings, a convenient size for the complexation of polycarboxylates [3–5]; *ii*) they have six N-atoms, which form four propylene-1,3-diamine moieties, and, therefore, increase their protonation abilities at neutral or weakly acidic pH with respect to those azamacrocycles with ethylene-1,2-diamine units [13]; *iii*) their chirality carrier is (*R*,*R*)-cyclohexane-1,2-diamine, a very useful compound in the asymmetric synthesis (for some examples, see [14]) and enantiomeric and diastereoisomeric recognition of peptides [15]; *iv*) compound **L1** presents C_2 symmetry, whereas **L2** possesses three orthogonal binary axes to give it overall D_2 symmetry. Here, we report the study of their protonation abilities by potentiometry and NMR methods, and their chiral-anion-recognition behavior.



L1 **L2**

Fig. 1. *Molecular structures of the macrocycles studied in this work*

2. Results and Discussion. – 2.1. *Protonation Features. Potentiometric Studies.* The values of the logarithm of the basicity constant for each protonation step of **L1** and **L2** in 0.1M Me_4NCl solution at 298 K are shown in *Table 1*. This table also presents the difference (Δ) between successive protonation constants for each compound, a parameter that describes the ease of protonation at each protonation step. The higher the Δ value, the more disfavored is the corresponding protonation process. Taking this into account, the fifth protonation step is the most difficult in both systems, as expected from the reported general protonation properties of macrocyclic polyamines [13]. Comparison of both compounds shows that **L1** is more basic than **L2**, the largest differences lying in the fifth protonation step, where **L1** has a basicity constant ten times higher than **L2**.

Table 1. *Logarithm of the Protonation Constants of* **L1** *and* **L2**, *Measured by Means of Potentiometric Titrations in a 0.1M Me_4NCl Aqueous Solution and at 298 K*

Reaction[a])	**L1**		**L2**		Δ log K (**L1-L2**)
	log K	Δ	log K	Δ	
$L + H = HL$	11.49(3)[b])		11.32(4)		0.17
$HL + H = H_2L$	10.07(3)	1.42	9.93(4)	1.39	0.14
$H_2L + H = H_3L$	9.17(3)	0.90	9.22(4)	0.71	– 0.05
$H_3L + H = H_4L$	7.80(3)	1.37	7.69(4)	1.53	0.11
$H_4L + H = H_5L$	5.55(3)	2.25	4.55(4)	3.14	1.00
$H_5L + H = H_6L$	3.95(3)	1.60	3.49(4)	1.06	0.46

[a]) Charges have been omitted for clarity. [b]) Values in parentheses correspond to the standard deviation in the last significant figure.

From the basicity constants we obtained the distribution curves of the protonated species, represented in *Fig. 2*. The species distribution shows three clearly defined sections for the compound **L2**. From pH 2–5, there is a mixture of hexa-, penta-, and tetraprotonated forms. In the pH range of 5–7.5 the tetraprotonated compound

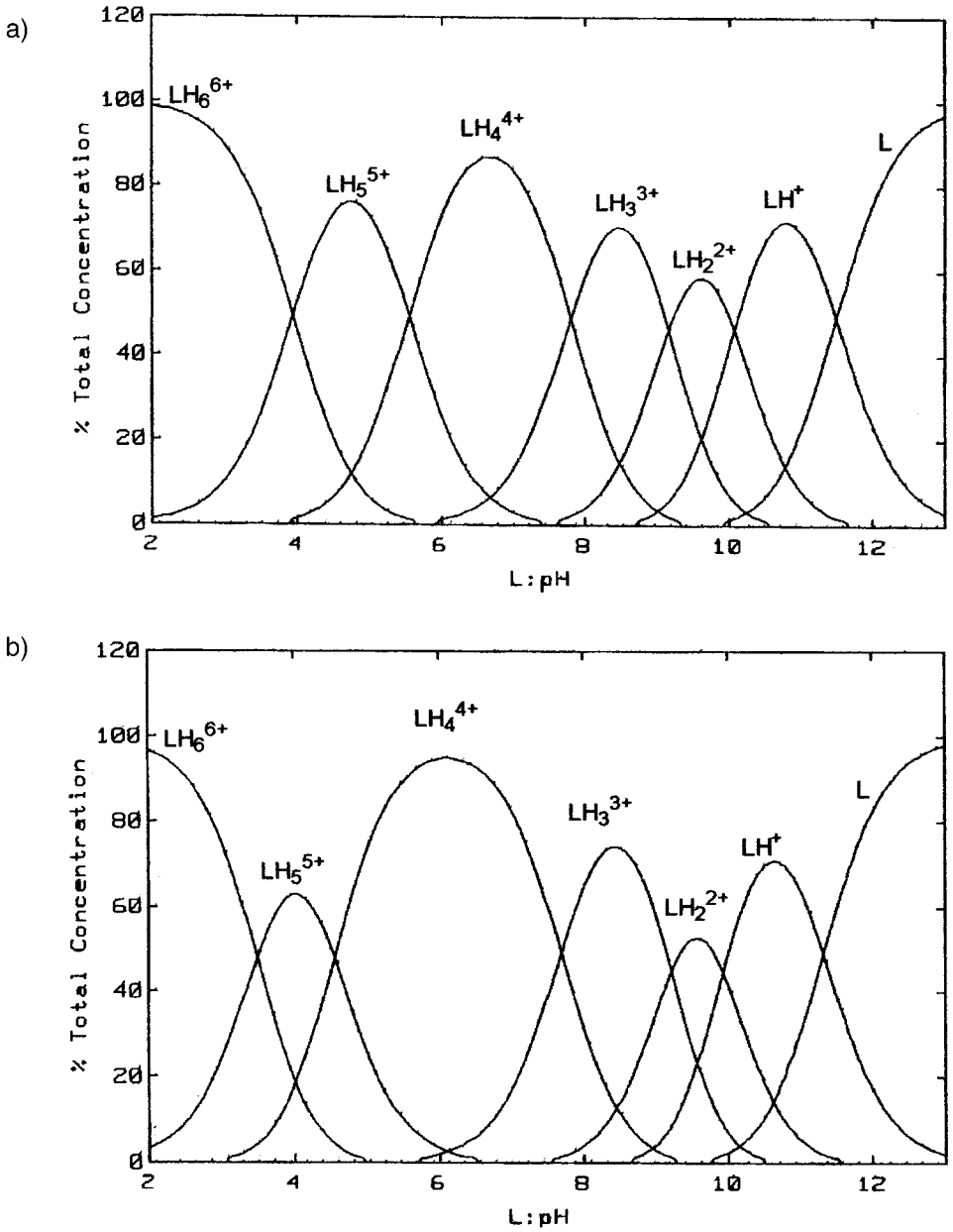


Fig. 2. *Plot of the distribution of protonated species of* a) **L1** *and* b) **L2**

prevails, while, from there to pH 13, mixtures of protonated species coexist. The plot of the species distribution of **L1** is, however, clearly different from that of **L2**, because, in this case, the tetraprotonated form does not prevail so clearly. In other words, the fifth proton is attached more easily to **L1** than to **L2**. Thus, for **L1** at a pH of *ca.* 4.7, the pentaprotonated species exists almost exclusively.

In general, the successive introduction of protons leads to the species presenting the maximum dispersion of positively charged ammonium sites. In addition, the presence of propylenic spacers between N-atoms yields higher basicity ($\Delta = 1.67$ for propylenediamine) than that of the ethylenic counterparts ($\Delta = 2.70$ for ethylenediamine) because of the reduced repulsions between polyammonium sites. As *Fig. 2* shows, the main species in solution near neutral pH are the tetraprotonated forms for both compounds. Furthermore, we can predict the probable protonation sites, which are stabilized by intramolecular H-bonds with the unprotonated N-atoms (see *Fig. 3*). These effects are equal in both azamacrocycles, and consequently, the higher basicity of **L1** with respect to **L2** in the fifth protonation step must have some other reason. Upon inspection of both structures, it becomes clear that the answer lies in the different protonation abilities of the ethylenediamine and *trans*-cyclohexane-1,2-diamine bridging moieties. This proves to be the case; the values of $\Delta = 3.17$ and 2.70 for *trans*-cyclohexane-1,2-diamine and ethylenediamine, respectively, agree with the basicity tendencies of these moieties incorporated in the azamacrocycles.

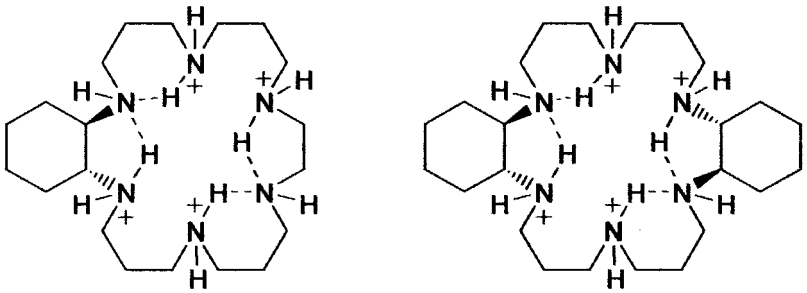


Fig. 3. *Proposed tetraprotonated species of* **L1** *and* **L2**

On the other hand, two different structures can be proposed (*Scheme 1*) for the pentaprotonated form of **L1**. The first (**I** in the *Scheme*) would be obtained from **L1** · $4\,H^+$ by protonation of the ethylenediamine fragment, whereas protonation in the cyclohexanediamine would lead to species **II**.

To determine the most favored species, we have followed the variation of the ^{1}H- and ^{13}C-NMR chemical shifts with acid concentration (*Figs. 4* and *5*). The ^{1}H- and ^{13}C-NMR spectra of both compounds show the expected averaged symmetry in all the pD values tested (C_2 for **L1** and D_2 for **L2**). This implies fast prototropic processes on the NMR time scale. In addition, broad signals are obtained in the ^{1}H-NMR spectra of both azamacrocycles, suggesting conformationally dynamic systems. However, a detailed analysis of the variations in the chemical shifts allowed us to establish the protonation sequence. As a general rule, when pD is increased, α-H signals move upfield and those of C(β) downfield [16].

Scheme 1. *Possible Protonation Sites for* **L1** · 5 H^+

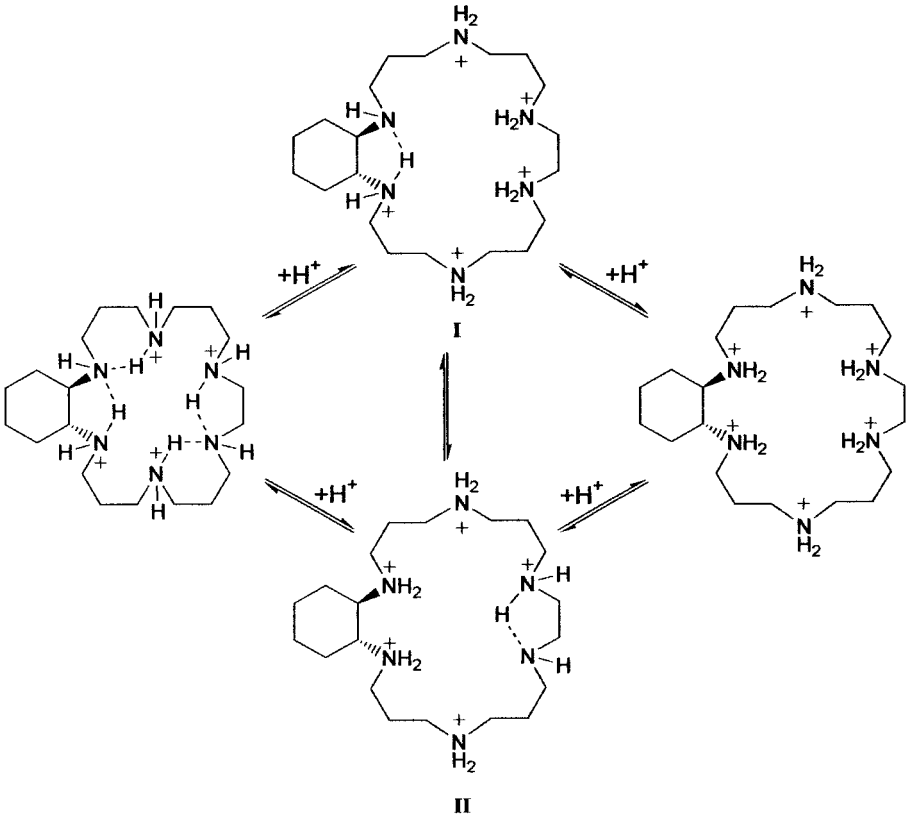


Fig. 4 shows a plot of the ^{1}H- and ^{13}C-NMR chemical shifts of **L2** *vs.* the pD value. When pD is decreased from 12 to 10, the first two protonation processes take place, and these affect all the signals of the spectra. This effect can be explained by fast prototropic processes, favored by intramolecular H-bonds and the low charge density. From pD 10–6 the most important variations are shown by H(3), H(4), and C(3). In this section, **L2** · 2 H^+-**L2** · 3 H^+-**L2** · 4 H^+ species are formed, and the spectra indicate the presence of the tetraprotonated species as proposed in *Fig. 2*. Between pD 6 and 4 **L2** · 4 H^+ and **L2** · 5 H^+ are the dominant species in solution. The ^{1}H-NMR spectra show significant deshielding of H(1), H(2), H(5ax) and H(6ax), while in ^{13}C-NMR the signals of C(1), C(3), C(5) and C(6) are shifted upfield. In the pD range 4–2, the last proton binds to the macrocycle, and only slight downfield shifts are observed for the signals of H(1), H(2), and H(5ax) signals. These results suggest that the two last protonation processes mainly involve the cyclohexanediamine substructure, which was previously mono-protonated.

We have also analyzed the ^{1}H- and ^{13}C-NMR spectra of **L1** at different pD values to establish a comparison between **L1** and **L2**. *Fig. 5* shows the plot of some selected spectral signals. A complete assignment and study was impossible due to the presence

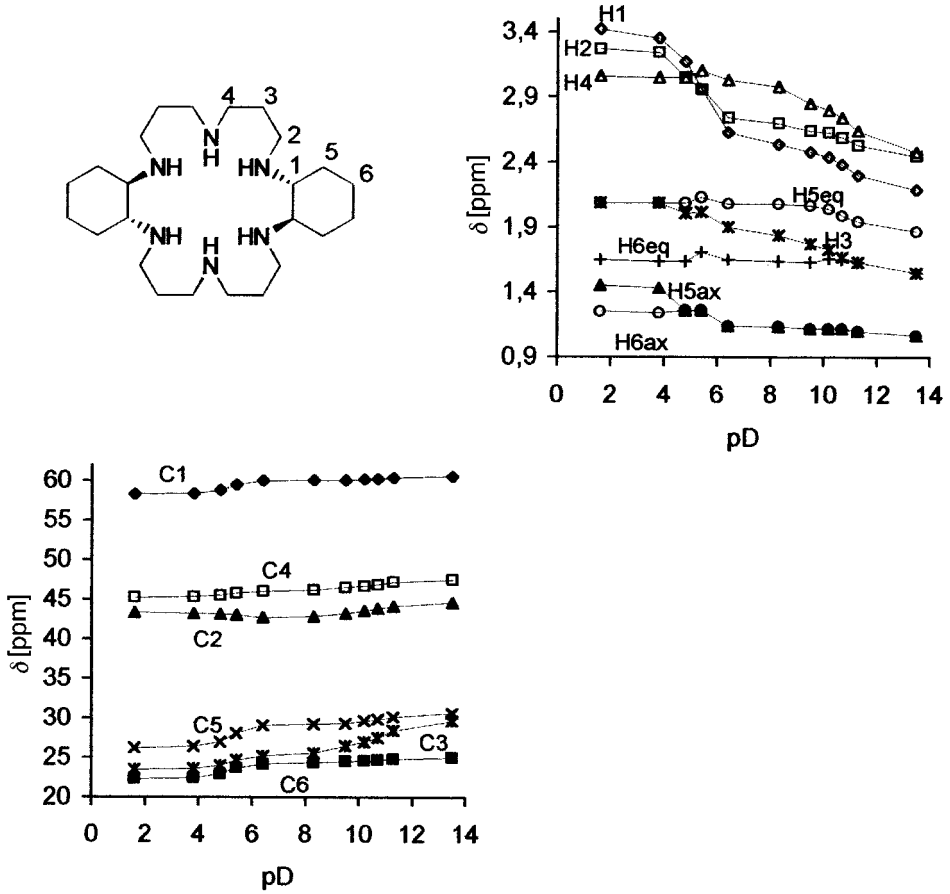


Fig. 4. *Experimental [1]H- and [13]C-NMR chemical shifts of* **L2** *as a function of pD (arbitrary numbering of atoms)*

of overlapping bands. The protonation sequence of **L1** at up to pD 8 is quite similar to **L2**, as expected from the protonation constant values. In the pD range of 8–6, where the $\mathbf{L1}\cdot 3\,H^+$-$\mathbf{L1}\cdot 4\,H^+$-$\mathbf{L1}\cdot 5\,H^+$ equilibrium prevails, H(8) is clearly deshielded in the ^{1}H-NMR specta. In the ^{13}C-NMR spectra, the signal of C(8) is shifted upfield, while those of C(1), C(4)/C(5), and C(9) do not shift significantly. All these data suggest that the fifth proton binds to the ethylenediamine moiety of the macrocycle. Finally, the last protonation takes place between pD 5 and 4, and this is clearly reflected in the NMR spectra. Downfield shifts are observed for the signals of H(1), H(9ax), and H(10ax), but the signal of H(8) remains unchanged. These data suggest that the sixth proton is attached to the cyclohexanediamine moiety. This hypothesis is confirmed by the ^{13}C-NMR spectra recorded in the same pD range, which show shielding of C(1) and C(9), while the chemical shifts of C(4), C(5), and C(8) remain unchanged.

To elucidate the conformation of the six-membered ring in the hexaprotonated species, we recorded a ROESY spectrum of **L2** at pD 1.9, where $\mathbf{L2}\cdot 6\,H^+$ is the main

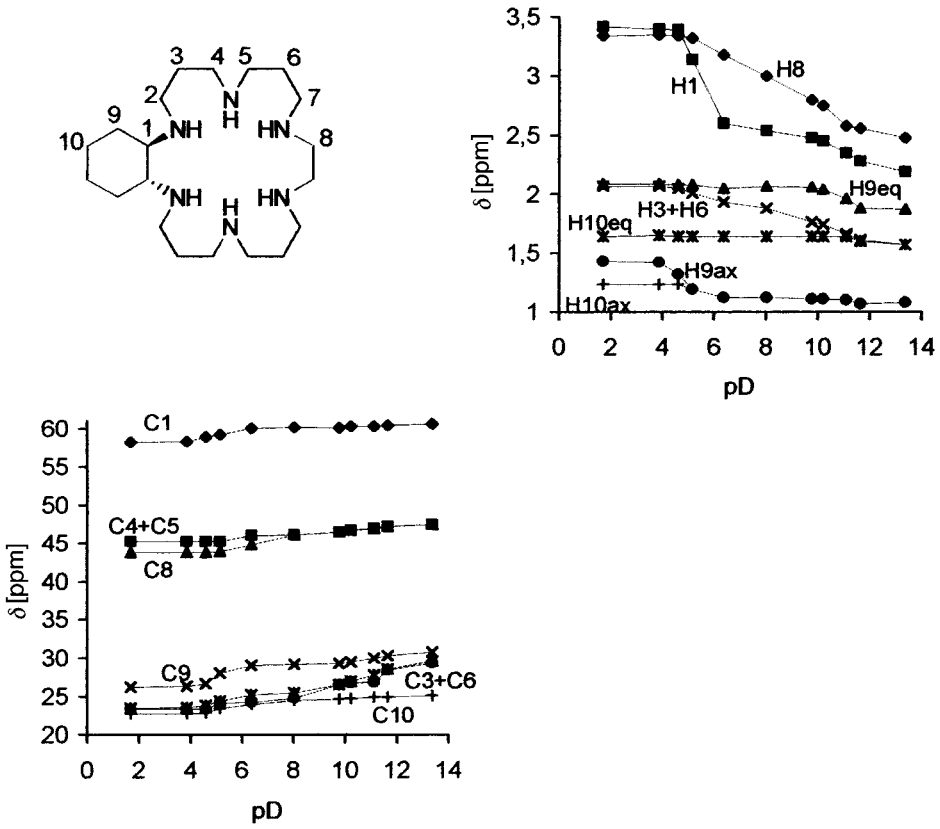


Fig. 5. *Experimental ^{1}H- and ^{13}C-NMR chemical shifts of some selected signals of* **L1** *as a function of pD (arbitrary numbering of atoms)*

species in solution. This spectrum (*Fig. 6*) shows a cross-peak between H(1) and H(6ax), and this peak is possible only when H(1) is in axial position, demonstrating the diequatorial conformation of the cyclohexanediammonium substructure.

With all these experimental data, we can conclude that the fifth protonation step in **L1** involves mainly the ethylenediamine fragment, and the difference in basicity comes from the greater ease of this moiety to be completely protonated. This behavior can be explained by electrostatic arguments. Molecular-mechanics calculations [17] of **L1**·5 H^+, with the ethylene fragment in the *gauche* or *anti*-conformations, show a difference of energy of only 0.45 kcal mol^{-1} in favor of the *anti*-conformer, suggesting the flexibility of this fragment (*Fig. 7*). In contrast, the fifth proton in **L2** must be attached to a monoprotonated *trans*-diequatorial-cyclohexane-1,2-diamine, setting two positive charges very close and increasing the electrostatic repulsion. As shown by the ROESY spectrum, the change to diaxial conformation, which would prevent this repulsion, does not happen under our experimental conditions. The whole effect is a destabilization of the protonated form and, therefore, a lower basicity of **L2**. To illustrate this point, we have performed molecular-mechanics calculations [17] of the two extreme situations of

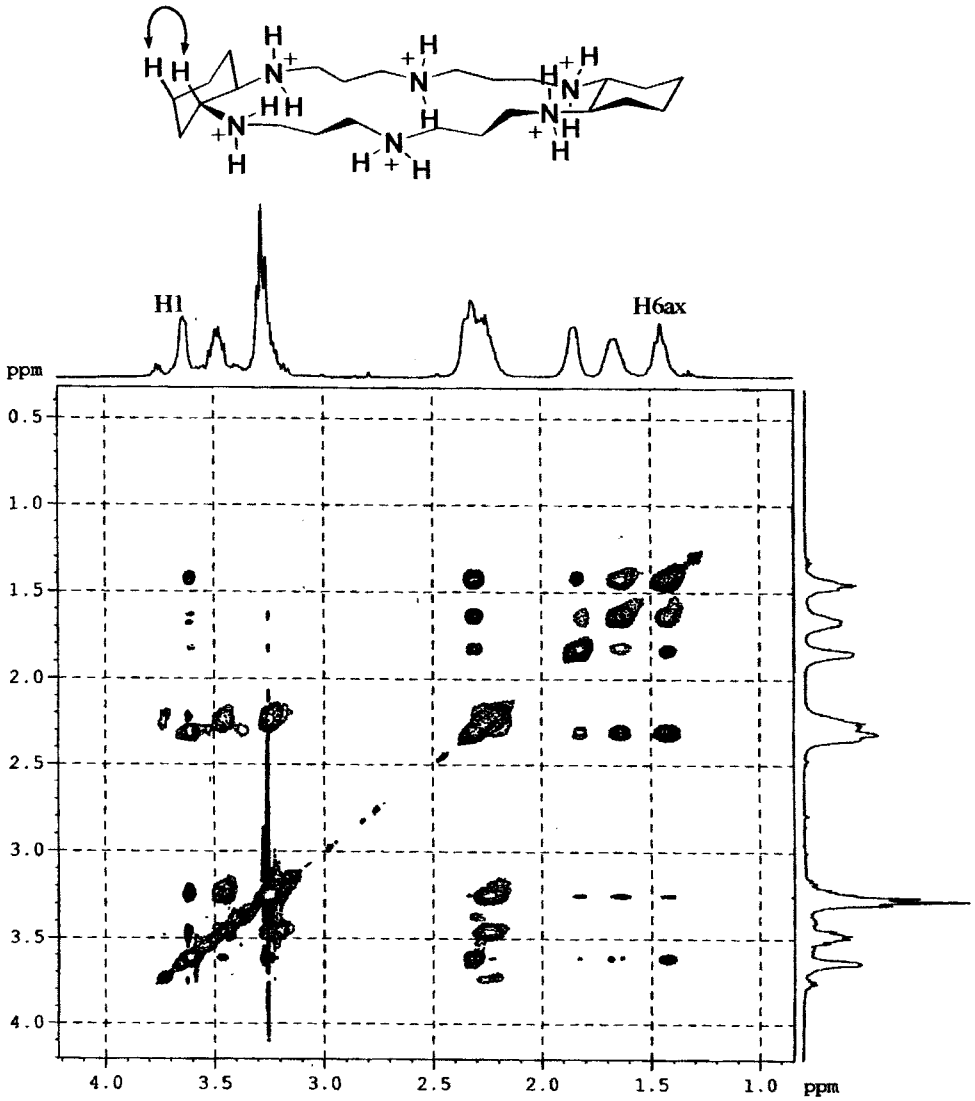


Fig. 6. *ROESY Spectrum of* **L2**·*6 H*$^+$ *in* D_2O

L2, where the two cyclohexane structures are either in the diaxial or diequatorial conformation, but maintain the observed D_2 symmetry (*Fig. 8*). The difference in energy strongly depends on the polarity of the medium, 18.1 kcal mol^{-1} favoring the axial/axial conformer in vacuum, whereas the equatorial/equatorial conformer is by 2.6 kcal mol^{-1} more stable in H_2O. The real situation would be even more favorable to this last conformation due to the presence of the supporting electrolyte, which increases the ionic strength of the solution.

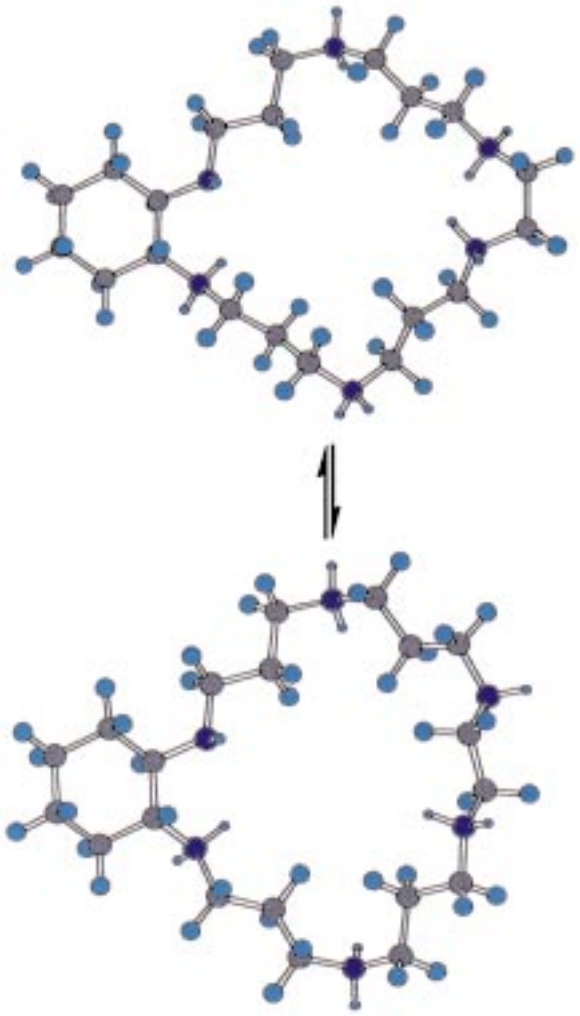
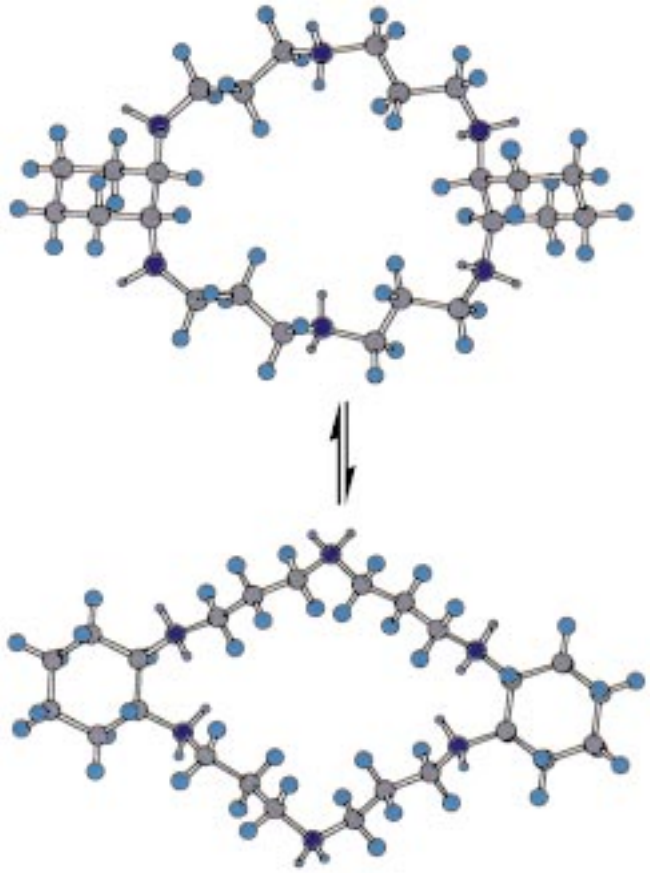


Fig. 7. *Energy-minimized structures for* **L1** · *5* H^+

In addition, a very interesting effect is observed for the ^{1}H-NMR signals of the cyclohexane ring in both azamacrocycles. When the first two protons bind to the macrocycle, the equatorial protons of the six-membered ring are deshielded, but the signals of the axial protons are shifted downfield significantly upon hexaprotonation. This effect must be due to geometrical reasons and may originate from the conformational preferences of cyclohexane. The diequatorial conformation of the $\mathbf{L} \cdot 6\,H^+$ species positions the ammonium groups towards the inner side of the macrocyclic cavity, and accordingly the minimized structure (see *Fig. 8*) shows two nonequivalent protons for each N-atom. One pseudoaxial proton (or in *exo*) forms an angle of *ca.* 180° with the CH of the stereogenic center and another interior proton (or in *endo*), as is shown schematically in *Scheme 2*. On the other hand, the free amine shows a



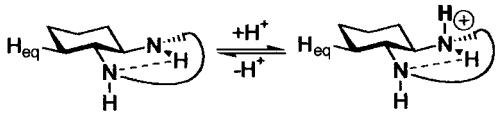
Medium	ΔH_{conf} (eq/eq-ax/ax) [kcal·mol^{-1}]
Vacuum	18.1
H_2O	–2.6

Fig. 8. *Energy-minimized structures for* **L2**$\cdot 6\,H^+$

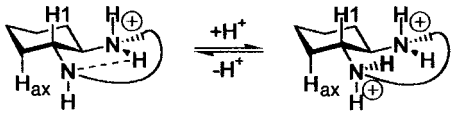
diequatorial conformation with an intramolecular H-bond between amine groups. Taking this into account, the monoprotonated form would have one *endo* and two *exo* protons, allowing the intramolecular H-bond (see *Scheme 2*) between amino and ammonium groups. As the changes in the chemical shifts produced by charges depend on both the distance and the geometry, it is possible to calculate these variations theoretically [18][1]). *Scheme 2* shows a comparison of the calculated and experimental

[1]) The variation of the chemical shift produced by punctual charges can be calculated by the equation $\Delta\sigma = 0.125\ \Sigma_i\ [(\Delta Q_i/R_i^2)\cos\theta] - 0.170\ [\Sigma_i\ (\Delta Q_i/R_i^2)]^2$, where $\Delta\sigma$ is the shielding suffered by the affected proton (H), ΔQ_i the excess of elemental charge of the i-atom, R_i is the distance [nm] between the i-atom and H, and θ is the angle between the CH bond and the vector i, H. The geometrical parameters (R_i, θ) were obtained from the minimized structures (*Figs. 6* and *7*).

Scheme 2. *Calculated and Experimental ^{1}H-NMR Chemical Shifts of the Cyclohexane Substructure*



H_{eq}: $\Delta\delta_{theor}$ = 0.27 ppm
$\Delta\delta_{exp}$ = 0.21 ppm (**L2**)
$\Delta\delta_{exp}$ = 0.20 ppm (**L1**)



H(1): $\Delta\delta_{theor}$ = 0.92 ppm
$\Delta\delta_{exp}$ = 0.88 ppm (**L2**)
$\Delta\delta_{exp}$ = 0.82 ppm (**L1**)

H_{ax}: $\Delta\delta_{theor}$ = 0.30 ppm
$\Delta\delta_{exp}$ = 0.31 ppm (**L2**)
$\Delta\delta_{exp}$ = 0.30 ppm (**L1**)

values for each protonation step. The agreement between these values gives an explanation for the changes in the chemical shifts of the six-membered-ring signals and supports the diequatorial conformation of the cyclohexane moiety.

2.2. *Chiral Dicarboxylate Anion Binding.* Protonation of **L1** and **L2** gives charged species, which are able to form stable complexes with different chiral dicarboxylate anions. The formation of these species is pH-dependent and, therefore, the relevant equilibria can be studied by potentiometric titrations. *Table 2* shows the stability constants (K_s) of the complexes formed by azamacrocycles **L1** and **L2** with different optically active dicarboxylates in 0.1 mol dm^{-3} Me_4NCl solution at 298 K. The enantiomeric discrimination of the receptors is defined as the difference of the logarithm of the stability constants ($\Delta \log K_s$) of the diastereomeric complexes obtained with a given receptor and both enantiomers of the anion (see *Table 2*).

By the examination of the values of the equilibrium constants, several overall trends emerge. All the anions studied form stable complexes with the hexa-, penta-, and tetraprotonated species of the azamacrocycles, and the three isomers of tartrate anion interact with the triprotonated receptors except for the system **L2**-*meso*-tar, for which at least four protons are required in the macrocycle for the interaction to be detected. For a given macrocycle-anion pair, the strength of the interaction generally increases with its degree of protonation. This suggests that the process of complexation is mainly electrostatically driven. Thus, an increase in the number of protons in the macrocycle increases the receptor's ability to give charge-charge and H-bonding interactions with the anion. Similar behavior is generally observed with other polyammonium receptors.

Comparison of the stability-constant values of both azamacrocycles shows a higher anion-binding ability of **L2** with respect to **L1**. This may be ascribed to a higher charge

Table 2. *Values of log* K_s *for the Azamacrocycles* **L1** *and* **L2** *and Different Chiral Anions* (measured by potentiometric titration in 0.1M Me_4NCl aqueous solution at 298 K)

Receptor	Anion	n[a])=6		$n=5$		$n=4$		$n=3$	
		log K_s	Δ log K_s[b])	log K_s	Δ log K_s	log K_s	Δ log K_s	log K_s	Δ log K_s
L1	D-tar	4.10(2)[c])		3.34(2)		2.92(2)		2.49(3)	
L1	L-tar	3.49(2)	0.61	2.71(2)	0.63	2.33(3)	0.59	2.01(3)	0.48
L1	*meso*-tar	4.49(2)		3.12(2)		2.34(3)		1.74(5)	
L1	D-mal	4.50(2)		2.64(4)		1.71(5)		–	
L1	L-mal	4.47(2)	0.03	2.79(3)	−0.15	1.92(5)	−0.31	–	
L1	*N*-Ac-D-asp	4.52(2)		2.67(4)		1.76(5)		–	
L1	*N*-Ac-L-asp	4.14(2)	0.38	2.69(2)	−0.02	1.99(3)	−0.23	–	
L1	*N*-Ac-D-glu	[10.79(1)][d])		[8.43(1)]		3.63(1)			
L1	*N*-Ac-L-glu	4.41(2)	n.a.[e])	[5.23(2)]	3.20	1.82(4)	1.81		
L2	D-tar	4.27(3)		3.60(3)		2.89(2)		2.29(4)	
L2	L-tar	4.08(5)	0.19	3.49(3)	0.11	2.71(3)	0.18	2.09(4)	0.20
L2	*meso*-tar	4.93(3)		3.27(4)		1.94(7)		–	
L2	D-mal	5.38(3)		3.23(5)		1.57(9)		–	
L2	L-mal	5.32(3)	0.06	3.53(4)	−0.30	2.09(6)	−0.52	–	
L2	*N*-Ac-D-asp	5.34(3)		3.77(3)		2.53(3)		–	
L2	*N*-Ac-L-asp	4.57(4)	0.77	3.40(3)	0.37	2.23(3)	0.20	–	
L2	*N*-Ac-D-glu	[12.22(2)][d])		[9.97(1)]		4.14(1)		2.82(2)	
L2	*N*-Ac-L-glu	5.19(2)	n.a.	[5.94(2)]	4.03	2.17(3)	1.97	–	n.a.

[a]) n = Number of protons involved in the complex. [b]) $\Delta \log K_s = \log K_s(\text{D}) - \log K_s(\text{L})$. [c]) Values in parentheses correspond to the standard deviation in the last significant figure. [d]) Values in brackets correspond to 1:2 macrocycle/anion stoichiometric complexes. [e]) n.a. = Not applicable.

density and a less-solvated receptor in case of **L2**. The presence of cyclohexane rings increases the hydrophobicity and prevents the ammonium groups from interaction with solvent. This leaves the ammonium binding sites more accesible to the anion. Besides, the *trans*-diequatorial conformation of the six-membered ring positions the ammonium groups towards the inner part of the macrocyclic ring, increasing their proximity and giving a higher charge density.

The enantiomeric discrimination strongly depends on the macrocycle and anion structures, as well as on the number of protonated sites within the complex. Poor results are obtained with the malate anion for both azamacrocycles. Compound **L1** shows a moderate D-preference with tartrate that is practically constant as a function of the degree of protonation ($\Delta \log K_s = 0.64 - 0.48$; $\Delta\Delta G = 0.88 - 0.66$ kcal mol^{-1} for $n = 6-3$). However, it should be noted that the presence of a second (*R*,*R*)-cyclohexane-1,2-diamine moiety in **L2** decreases the enantioselectivity with the same anion. The binding of *N*-Ac-asp to **L1** clearly changes with the number of protons: **L1** is D-selective for $n = 6$ and L-selective for $n = 4$, although the difference of stability constants is low in both cases. A better result is obtained with **L2**$\cdot 6\,H^+$ and *N*-Ac-asp, which shows quite a good enantiomeric preference for the D-enantiomer ($\Delta \log K_s = 0.77$; $\Delta\Delta G = 1.06$ kcal mol^{-1}). This enantiomeric selection is obviously decreased by the participation of the less selective penta- and tetraprotonated complexes. However, this significant result suggests the possible participation of the amide C=O group in the interaction as a H-bond acceptor (see *Fig. 9*). It is well-known that to obtain enantiomeric selection, a receptor must have a minimum of three points of interaction

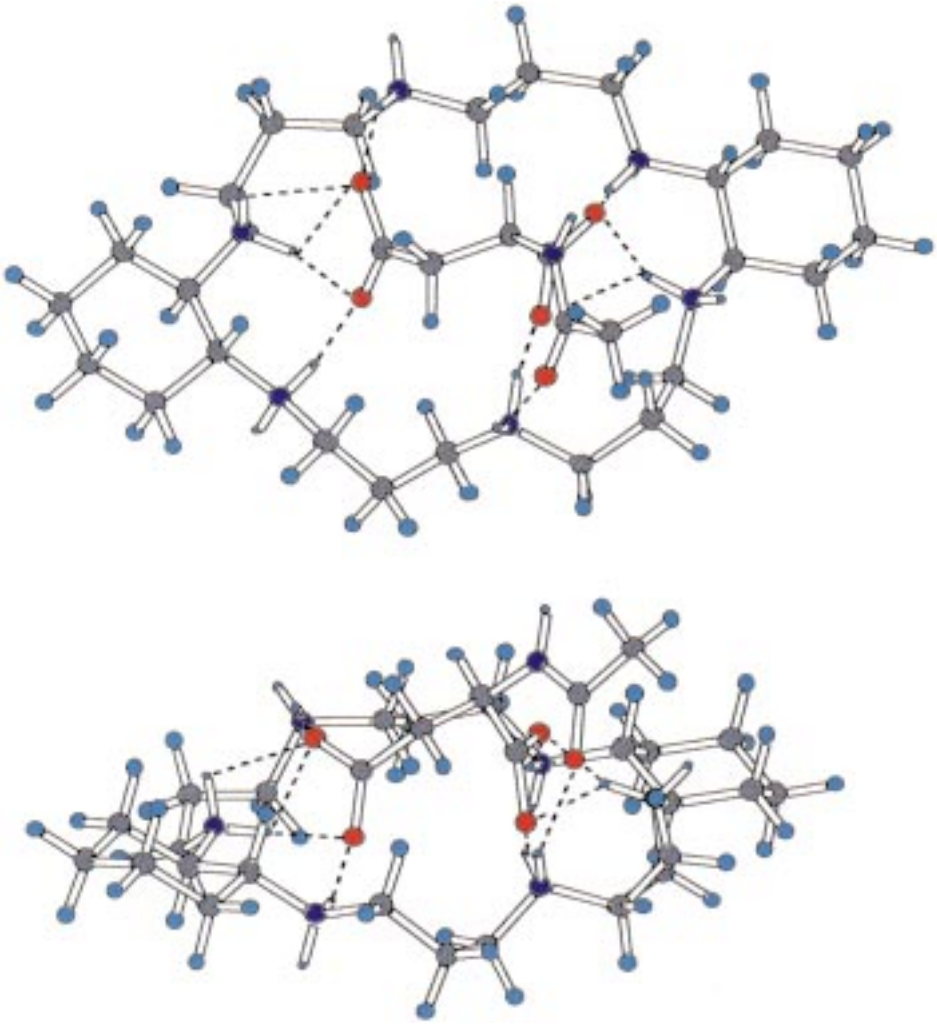


Fig. 9. *Energy-minimized structure of the complex* **L2**·6 H^+·N-*Ac*-D-*asp*

with the substrate, and at least one of them must be stereochemically dependent [19]. Two binding points must be due to carboxylate-diammonium coulombic interactions, and the third can be a H-bond between the amide C=O group of the anion and an ammonium group of the macrocycle. We tried to support this proposal with NMR experiments, but, unfortunately, NOE measurements between anion and receptor signals were not possible due to unfavorable correlation times, even in the case of spin-lock techniques like ROESY.

The most surprising example was found with *N*-Ac-glutamate, for which 1:2 (macrocycle/anion) stoichiometric complexes were detected (see *Table 2*) by NMR titration of **L2** with *N*-Ac-D-glu. Accordingly, we obtained the best fit of the experimental and calculated titration curves by introducing the possibility of 1:2

complexes. Both receptors follow similar tendencies, and thus, 1:2 complexes are detected with the pentaprotonated macrocycles, while the hexaprotonated species bind to two anion molecules of the D-enantiomer, but only to one of the L-isomer. In addition to this, both **L1** and **L2** are D-selective, although the selectivity of **L2** is higher again. In addition, the strength of the interaction suggests that the best geometric complementarity is between the macrocycles and 1,3-dicarboxylates. A proposal for the structure of the 1:2 complex, obtained by a molecular-mechanics calculation, is shown in *Fig. 10*. A more-detailed structural analysis by NMR (NOE measurements) was not possible in this case, either. However, it must be pointed out that ROESY experiments with **L2** and an excess of anion showed again a crosspeak between H(1) and $H(6_{ax})$, supporting the diequatorial conformation of the cyclohexane ring. Unfortunately, we were unable to obtain intermolecular cross-peaks, which would give some information about the host-guest structure.

3. Conclusions. – The measurement of the protonation constants of compounds **L1** and **L2** reveals that they possess four N-atoms that are very basic, and two others with a lower relative basicity. Comparison of the protonation abilities shows that the presence of the cyclohexane-1,2-diamine substructure decreases the basicity, in agreement with the protonation constants of ethylenediamine and *trans*-cyclohexane-1,2-diamine. The largest differences between **L1** and **L2** appear in the fifth protonation step, for which **L1** is ten times more basic than **L2**. The study of ^{1}H- and ^{13}C-NMR spectra at different pD values allows us to determine the sequence of the protonation sites and suggests that the fifth protonation of **L1** mainly involves the ethylenediamine fragment. The flexible ethylene fragment allows **L1** to relax the electrostatic repulsions of full protonation, while the cyclohexane-1,2-diammonium moiety holds its diequatorial conformation in our experimental conditions, increasing the charge-charge repulsion in $\mathbf{L2}\cdot 5\,H^+$.

Both azamacrocycles can be used in their protonated forms as receptors for chiral anions, leading to stable complexes in aqueous solution. The strength of the interaction increases with increasing protonation of the receptors. Macrocycle **L2** forms more stable complexes than **L1**, probably due to a higher charge density. The enantiomeric selectivity depends on the degree of protonation and the structures of both the anion and the receptor. Macrocycle **L1** shows a moderate D-preference with the tartrate anion being practically independent on the number of protons, while $\mathbf{L2}\cdot 6\,H^+$ binds to *N*-Ac-D-aspartate more strongly than to the corresponding L-isomer. The most-surprising results were obtained with the *N*-Ac derivative of glutamate anion, which forms very stable complexes with both **L1** and **L2**. The stoichiometry of these complexes can be 1:1 or 1:2 (receptor/anion), depending on the number of the protons and the enantiomer of the anion. Both macrocycles form more-stable complexes with *N*-Ac-D-glutamate than with its enantiomer.

Experimental Part

The synthesis of the macrocycles **L1** and **L2** was carried out as described in [12], and gave satisfactory spectroscopic and analytical data. ^{1}H- and ^{13}C-NMR spectra were obtained on a *Bruker AC-300* (^{1}H: 300 and ^{13}C: 75.5 MHz), *AC-200* (^{1}H: 200 and ^{13}C: 50.3 MHz), or *AMX-400* (^{1}H: 400 and ^{13}C: 100.7 MHz) spectrometers. For the NMR titrations, the samples were prepared with known amounts of the macrocycles, the pD was adjusted by addition of DCl or NaOD solns. in D_2O and the correction $pD = pH^* + 0.4$ was used,

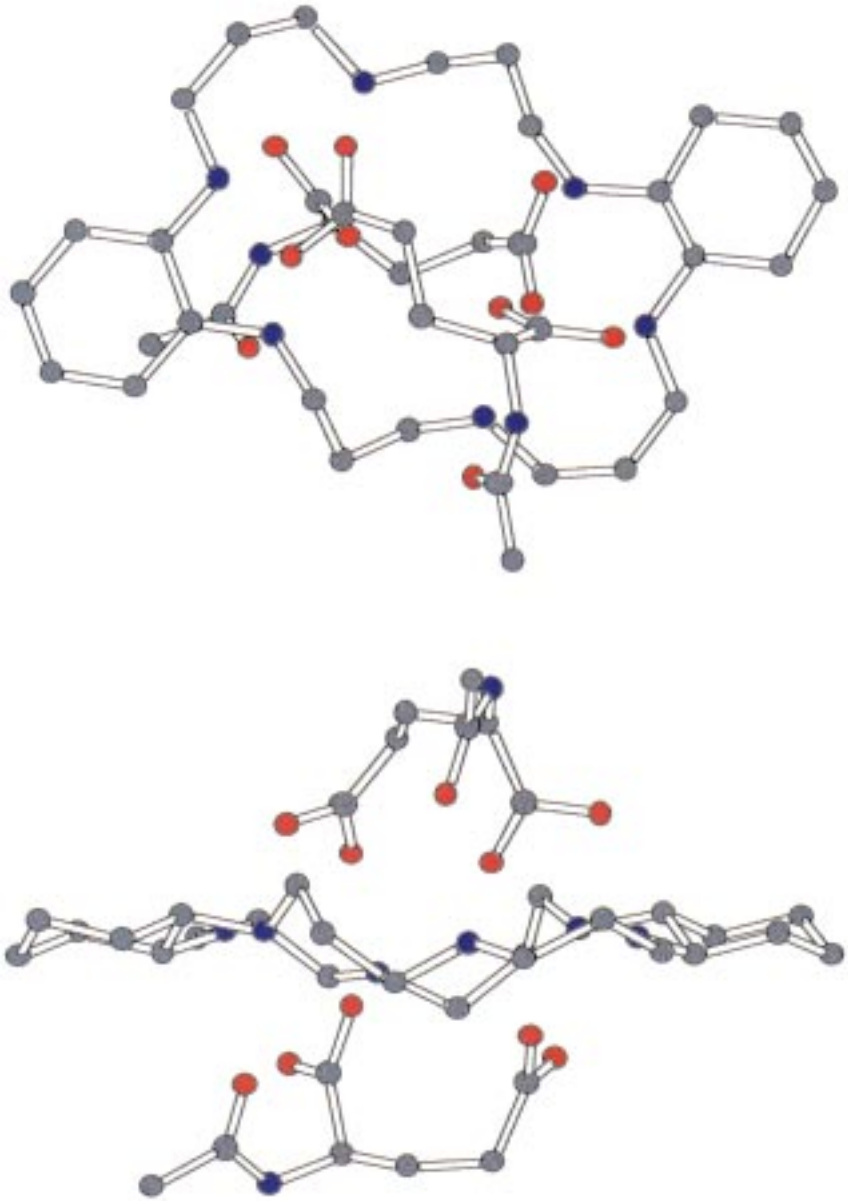


Fig. 10. *Energy-minimized structure of the complex* **L2**·6 H^+·2 N-*Ac*-D-*glu* (H-atoms have been omitted for clarity)

where pH* is the direct measurement of a pH-meter calibrated with non-deuterated buffer solns. All the ^{1}H- and ^{13}C-NMR signals were assigned by homonuclear ^{1}H,^{1}H-COSY and heteronuclear ^{1}H,^{13}C-HMQC and HMBC correlation experiments at selected pD values. For the pH-metric titrations, a *Metrohm TITROPROCESSOR-636* titrimeter was used, the reference electrode was an Ag/AgCl electrode in sat. aq. KCl, the cell was thermostated at 298 ± 0.1 K, the soln. stirred, and all the measurements were performed under N_2. The

protonation constants were determined by titration with 0.1N NaOH of a soln. containing typically 10^{-3} M of the HCl salt of the azamacrocycle in the presence of Me_4NCl (0.1M). The log K_s values of the complexes were determined by titration with 0.1N NaOH of a soln. containing 10^{-3} M of the HCl salt of the polyamine and 5×10^{-3} M of the desired dianions in the presence of 0.1M Me_4NCl. All the measurements with each system were carried out at least twice, and the data analysis was performed with the computer program *SUPERQUAD* [20].

REFERENCES

[1] (a) J.-M. Lehn, 'Supramolecular Chemistry', VCH, Weinheim, 1995; b) A. Bianchi, K. Bowman-James, E. García-España, 'The Supramolecular Chemistry of Anions', VCH, Weinheim, 1997; c) B. Dietrich, *Pure Appl. Chem.* **1993**, *65*, 1457; d) C. Seel, A. Galán, J. de Mendoza, *Top. Curr. Chem*. **1995**, *175*, 102; e) F. P. Schmidtchen, M. Berger, *Chem. Rev.* **1995**, *97*, 1609.

[2] R. M. Izatt, K. Pawlak, S. S. Bradshaw, R. L. Bruening, *Chem. Rev.* **1995**, *95*, 2529.

[3] a) B. Dietrich, M. W. Hosseini, J.-M. Lehn, R. B. Sessions, *J. Am. Chem. Soc.* **1981**, *103*, 1282; b) M. W. Hosseini, J.-M. Lehn, *Helv. Chim. Acta* **1986**, *69*, 587; c) M. W. Hosseini, J.-M. Lehn, *Helv. Chim. Acta* **1988**, *71*, 749; d) E. Kimura, A. Sakonaka, T. Yatsunami, M. Kodama, *J. Am. Chem. Soc.* **1981**, *103*, 3041; e) E. Kimura, M. Kodama, T. Yatsunami, *J. Am. Chem. Soc.* **1982**, *104*, 3182; f) E. Kimura, A. Sakonaka, *J. Am. Chem. Soc.* **1982**, *104*, 4984; g) E. Kimura, Y. Kuramoto, T. Koike, K. Fujioka, M. Kodama, *J. Org. Chem.* **1990**, *55*, 42.

[4] a) A. Bencini, A. Bianchi, M. I. Burguete, E. García-España, S. V. Luis, *J. Am. Chem. Soc.* **1992**, *114*, 1919; b) A. Bencini, A. Bianchi, M. I. Burguete, P. Dapporto, A. Doménech, E. García-España, S. V. Luis, P. Paoli, J. A. Ramírez, *J. Chem. Soc., Perkin Trans. 2* **1994**, 569.

[5] a) G. J. Siegel, B. W. Agranoff, R. W. Albers, P. B. Molinoff, 'Basic Neurochemistry: Molecular, Cellular and Medical Aspects', 5th edn., Raven Press, Ltd., New York, 1994, 367; b) J. I. Yeh, H. P. Biemann, G. G. Prive, J. Pandit, D. E. Koshland Jr., S. H. Kim, *J. Mol. Biol.* **1996**, *262*, 186.

[6] L. Stryer, *'Biochemistry'*, W. H. Freeman and Company, New York, 1988.

[7] P. Lustenberger, E. Martinborough, T. Mordasini-Denti, F. Diederich, *J. Chem. Soc., Perkin Trans. 2* **1998**, 747, and ref. cit. therein.

[8] K. Kano, H. Kamo, S. Negi, T. Kitae, R. Takaoka, M. Yamaguchi, H. Okubo, M. Hirama, *J. Chem. Soc., Perkin Trans. 2*, **1999**, 15.

[9] T. Kitae, H. Takashima, K. Kano, *J. Incl. Phenom.* **1999**, *33*, 345.

[10] J. L. Sessler, A. Andrievsky, V. Kral, V. Lynch, *J. Am. Chem. Soc.* **1997**, *119*, 9385.

[11] I. Alfonso, F. Rebolledo, V. Gotor, *Chem. Eur. J.* **2000**, 6, 3331.

[12] I. Alfonso, F. Rebolledo, V. Gotor, *Tetrahedron: Asymm.* **1999**, 10, 367.

[13] B. Dietrich, M. W. Hosseini, J. M. Lehn, R. B. Sessions, *Helv. Chim. Acta* **1983**, *66*, 1262.

[14] a) B. M. Trost, R. C. Bunt, *Angew. Chem., Int. Ed.* **1996**, *35*, 99; b) B. M. Trost, R. Radinov, E. M. Grenzer, *J. Am. Chem. Soc.* **1997**, *119*, 7879; c) M. Palucki, N. S. Finney, P. J. Pospisil, M. L. Güler, T. Ishida, E. N. Jacobsen, *J. Am. Chem. Soc.* **1998**, *120*, 948; d) S. E. Schaus, J. Branalt, E. N. Jacobsen, *J. Org. Chem.* **1998**, *63*, 403.

[15] a) N. Farrell, D. M. Kiley, W. Schmidt, M. P. Hacker, *Inorg. Chem*. **1990**, *29*, 397; b) K. Skog, O. Wennerström, *Tetrahedron Lett*. **1992**, *33*, 1751; c) S. S. Yoon, W. C. Still, *J. Am. Chem. Soc.* **1993**, *115*, 823; d) W. C. Still, *Acc. Chem. Res.* **1996**, *29*, 155; e) J. Reedijk, *Chem. Commun.* **1996**, 801; f) Z. Pan, W. C. Still, *Tetrahedron Lett*. **1996**, *37*, 8699; g) F. Gasparrini, D. Misiti, W. C. Still, C. Villani, H. Wennemers, *J. Org. Chem.* **1997**, *62*, 8221.

[16] a) A. Bencini, A. Bianchi, E. García-España, V. Fusi, M. Micheloni, P. Paoletti, J. A. Ramírez, A. Rodríguez, B. Valtancoli, *J. Chem. Soc., Perkin Trans. 2* **1992**, 1059; b) J. A. Aguilar, A. Bianchi, E. García-España, S. V. Luis, J. M. Llinares, J. A. Ramírez, C. Soriano, *J. Chem. Soc., Dalton Trans.* **1994**, 637; c) M. A. Bernardo, J. A. Guerrero, E. García-España, S. V. Luis, J. M. Llinares, F. Pina, J. A. Ramírez, C. Soriano, *J. Chem. Soc., Perkin Trans. 2* **1996**, 2335.

[17] MMX Force field as implemented in PCMODEL, Serena Software, Bloomington, IN, USA.

[18] H. Günther, 'NMR Spectroscopy: Basic Principles, Concepts and Applications in Chemistry'; John Wiley and Sons, 2nd ed., 1995.

[19] T. H. Webb, C. S. Wilcox, *Chem. Soc. Rev.* **1993**, 383.

[20] P. Gans, A. Sabatini, A. Vacca, *J. Chem. Soc., Dalton Trans.* **1985**, 1195.

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