

New branched macrocyclic ligand and its side-arm, two urea-based receptors for anions: synthesis, binding studies and crystal structure†

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The synthesis and characterization of the two new hosting molecules for anions 4(*N*),10(*N*)-bis-[2-(4-nitrophenylureido)acetamido]-1,7-dimethyl-1,4,7,10-tetraazacyclododecane (**L1**) and 1-((diethylcarbamoyl)methyl)-3-(4-nitrophenyl)urea (**L2**) are reported. **L1** is a branched tetraazamacrocyclic bearing two *p*-nitrophenylureido groups as side-arms, whereas **L2** has the same linear chain and binding moiety of **L1** side-arm. The best synthetic routes for use in obtaining **L1** were explored, affording the synthesis of the new intermediate **4**, a versatile building block for further functionalized branched macrocyclic hosts. The binding properties of both ligands towards the halides series and acetate anions (**G**) were investigated by NMR and UV-Vis spectroscopy in a dimethyl sulfoxide–0.5% water solution. Both ligands interact with F[−], Cl[−] and AcO[−] while Br[−] and I[−] did not. The NMR experiments proved that the binding occurs *via* H-bond to the ureido fragments. Fluoride anion is basic enough to deprotonate the ureido group of both ligands, thus preventing the determination of the addition constants to both ligands; this was instead possible for Cl[−] and AcO[−]. **L1** forms **G**–**L** species of 1 : 1 ([**GL1**]) and 2 : 1 ([**G₂L1**]) stoichiometry while **L2** forms only the 1 : 1 [**GL2**] species. The higher value of the formation constant of the [**AcOL1**][−] vs. the [**AcOL2**][−] species (log *K* = 5.5 vs. 2.8 for the reaction AcO[−] + **L** = AcOL[−]) suggested that both side-arms of **L1** cooperate in binding acetate; this does not occur with Cl[−]. The results confirmed that this tetraaza-macrocyclic base acts as a preorganizing scaffold for side-arms when they are linked to it *via* an amide function. The crystal structure of **L2**·H₂O is also reported.

Introduction

Since the first report on the preparation of a synthetic anion receptor by Park and Simmons in 1968, the development of anion receptors has become a field of great interest and activity, with many groups involved worldwide.^{1,2} The continuing interest in the development of new selective anion receptor systems stems from their potential use as membrane transport carriers,³ chemosensors,⁴ and reaction catalysts.⁵ As a result, considerable attention has been focused upon the designing of synthetic receptors for the detection of biologically relevant anions⁶ such as chloride,⁷ which plays a specific role in the interaction with haemoglobin in the “chloride shift” effect,^{8a} whilst substantial alteration in the plasma chloride level has been associated with many pathological conditions. For instance, an increase in chloride concentration can be found in renal insufficiency, renal tubule acidosis, hyperparathyroidism, dehydration and over-treatment with saline solution.^{8b} Decreased levels have been linked to gastrointestinal

diseases, renal insufficiency, overtreatment with diuretics, chronic respiratory acidosis, diabetic acidosis and adrenal insufficiency. The fluoride ions are also important anions because of their central role in dental care and the clinical treatment of osteoporosis.^{9,10} Thus, development of sensors for the direct detection of fluoride ions in aqueous media is an important target for their potential application in clinical and environmental analyses.

Sulfate is involved in activation and detoxification of a variety of endogenous and exogenous substances such as xenobiotics, steroids, neurotransmitters, and bile acids.¹¹ Sulfate conjugation is essential for the biosynthesis of a large number of structural proteins such as sulfated glycosaminoglycans (a major component of cartilage), cerebroside sulfate (a constituent of the myelin membranes in the brain) and heparin sulfate (which is required for anticoagulation).¹²

Molecular recognition of phosphate anions is crucial to a myriad of biological processes involving gene regulation, metabolism, antibiotic resistance, signal transduction, *etc.*¹³

In addition, important anions, such as lactate, pyruvate and glutamate play an important role in neurological diseases,¹⁴ as well as the free carboxylic function of the dipeptide D-Ala-D-Ala, which has an important role in the formation of the supramolecular complex with Vancomycin, inhibiting the cross-linking of the bacterial cell wall.¹⁵

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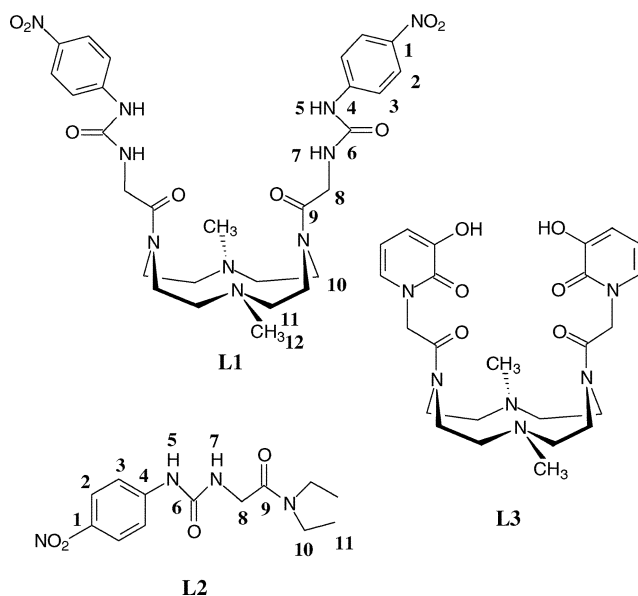


Chart 1 Ligands with labels for the NMR experiments.

Several approaches can be used to obtain anion receptors. For example, the most exploited strategies are: (i) metal complexes, in which the metal centre shows an unsaturated coordination environment, and can bind an anion *via* classic coordination chemistry;¹⁶ (ii) positively charged hosts, as the polyammonium class, which can interact with the anion mainly *via* charge–charge interactions;¹⁷ (iii) neutral non-metallic systems, with the aim to mimic several proteins in their anion binding.^{2g,18} In these latter systems, the forming supramolecular adducts are mainly stabilized *via* weak interaction forces such as H-bonding, π -stacking and others. Looking at the neutral non-metallic systems, a way in designing these hosts has been to introduce several and suitable hydrogen bond donor groups in the scaffold in order to improve the interaction with the anion. However, in this type of system, the “host–guest” binding affinity is modulated by many factors including the solvent, counterions, ionic strength, *etc.*, and the best results are obtained when the binding sites are structurally organized for anion complexation.

As emerged from the current literature,¹⁹ a high degree of structural organization is obtained when the host is able to adopt a conformation in which all binding sites are positioned in such way as to be structurally complementary to the guest. Furthermore, the host should exhibit a limited number of stable conformations and the binding conformation should be low in energy relative to other possible forms.⁴ Thus, preorganization and complementarity are pivotal concepts for the correct design for anion receptors. Although, the design and preparation of simple molecular systems that are able to exhibit these properties is not a simple task, in general, host structures that possess a rigid scaffold and urea,^{20a} thiourea^{20b} or guanidinium^{20c} functional groups as side-arms have been reported to be very effective in the binding of anions.

Recently, we reported on new cation receptors (**L3** in Chart 1) able to selectively bind NH_4^+ and primary ammonium

cations such as MeNH_3^+ , discriminating them from the secondary or tertiary ammonium cations, as well as to bind the alkali, alkaline earth, Ga^{3+} , Cu^{2+} , and Zn^{2+} ions.²¹ The most interesting structure had a 1,7-dimethyl-1,4,7,10-tetraazacyclododecane unit ($\text{Me}_2[12]\text{aneN}_4$)²² as macrocyclic scaffold, upon which we attached two 3-(hydroxyl)-1-(carbonylmethylene)-2(1*H*)-pyridone (HPO) moieties as side-arms. Molecular modelling and crystal studies highlighted that the way in which the two HPO groups are connected to the macrocyclic $\text{Me}_2[12]\text{aneN}_4$ is fundamental in stiffening and preorganizing the host; the two $\text{N}=\text{C}=\text{O}$ amide groups, which link each HPO moiety to the 12-member ring, draw the two side-arms to occupy the same region with respect to the macrocyclic base.

Taking advantage of these observations, we have now designed and studied a new anion receptor **L1** (Chart 1) based on the use of the same macrocyclic $\text{Me}_2[12]\text{aneN}_4$ base, upon which we have attached two ureido groups *via* a methylene carbonyl chain. Studies of binding with anions, such as F^- and Cl^- , as well as the acetate anion were performed and compared with the results obtained with its analogous linear ligand **L2** (Chart 1), in order to verify the concept of preorganization and to enhance the binding affinity of the **L1** receptor.

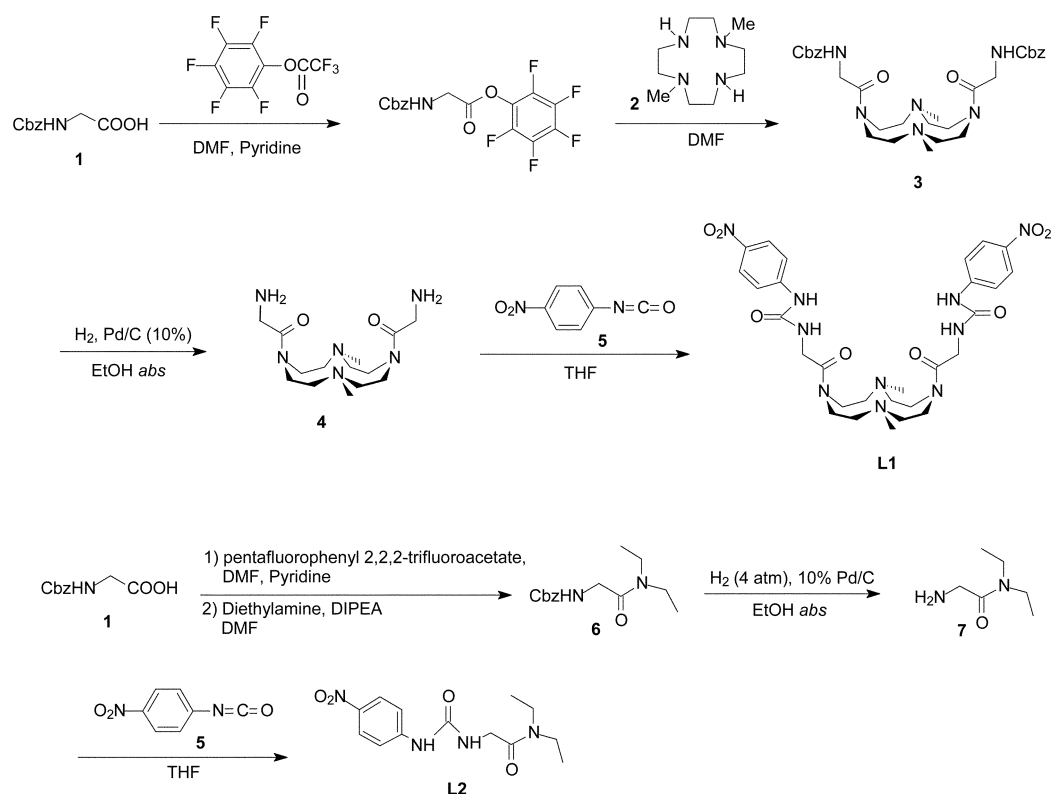
Results and discussion

Synthesis

Scheme 1 outlines the synthesis of the new multidentate ligand **L1**, which was based on the coupling of the protected glycine derivative **1** with the 1,7-dimethyl-1,4,7,10-tetraazacyclododecane **2** followed by the removal of the amine function protection obtaining the intermediate **4**; finally, the latter was reacted with the isocyanate **5**, affording the final compound **L1**.

The coupling step was studied in some detail, employing different coupling reagents, and the most significant results are reported in Table 1. Initial attempts to achieve coupling between the *N*-Cbz glycine **1**, as the corresponding succinimide derivative,²³ and the tetraamine **2** gave the bis-acylated product **3** in moderate yields (40%, entry 1, Table 1).

The use of other coupling agents, such as the DCCl,²⁴ the EDCI/HOBT²⁵ or the 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate/1-hydroxybenzotriazole (HBTU/HOBT)²⁶ systems were ineffective, whilst *N,N'*-carbonyl-diimidazole (CDI)²⁷ gave mainly the monoacylated product together with compound **3** and the starting material in a ratio of 20/60/20 as determined by liquid chromatographic–mass spectroscopy (LC–MS) on the crude reaction mixture. A mixture of monoacylated and the bis-acylated product **3** (25/65 ratio) was obtained using of 2,4,6-trichlorotriazine (TCT),²⁸ whereas a complete conversion of **1** into **3** was achieved in the presence of 2-(7-aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU),²⁹ although the isolation of the product required a long and tedious careful purification by column chromatography. Finally, a high yield (89%) and clean reaction occurred when the *N*-Cbz glycine **1** was activated as the pentafluorophenyl ester.³⁰ Among the coupling agents used, the

Scheme 1 Synthesis of **L1** and **L2**.

pentafluorophenyl trifluoroacetate turned out to be the best for use as one-pot “by-product-free” activation/coupling agent. Indeed, it allowed convenient high yield and easy purification of **L1** sample.

Thus, treatment of **1** with pentafluorophenyl trifluoroacetate/Py in DMF gave, in almost quantitative yield, the corresponding activated ester, which was immediately reacted with **2** in dry DMF, in the presence of *i*-Pr₂EtN at room temperature overnight. A single crystallisation of the crude reaction mixture gave the bis-acylated product **3** in 77% yield, which was used without any further purification. Additional product (12%) was isolated from the residue by column chromatography. Subsequent removal of the benzyloxycarbonyl protecting group by catalytic hydrogenolysis was achieved in EtOH using a pressure of 4 atm, in the presence of 10% Pd/C cat. The free diamine was treated with two equivalents of *p*-nitrophenylisocyanate **5** in dry THF to give the desired bis-urea **L1** in 61% yield.

Table 1 Coupling reagents for the activation of the carboxylic group function of **1** to give **3**

Entry	Coupling reagent	Yield (%) 3
1	<i>N</i> -Hydroxysuccinimide/DCCl	40
2	DCCl	<5
3	EDCI/HOBT	15
4	HBTU/HOBT	<5
5	CDI	60
6	TCT	65
7	HATU	92
8	EEDQ	62
9	CF ₃ COOC ₆ F ₅ /Py	89

The linear ligand **L2** was prepared according to the Scheme 1 by using the same synthetic route of **L1** but substituting the macrocyclic Me₂[12]aneN₄ with the *N,N*-diethylamine.

Interestingly, while compound **L2** was soluble in common organic solvents, compound **L1**, which appears to be particularly prone to self-association through intra- and/or intermolecular hydrogen bonding interactions with the urea groups, exhibits poor solubility in organic solvents other than DMSO.

Description of the L2·H₂O structure

The asymmetric unit of **L2**·H₂O contains one molecule of **L2** and one water molecule. As expected on the basis of its functional groups, **L2** shows an extended conjugation in that the whole molecule appears quite planar, with the obvious exception of the terminal methyl groups which are in the same hemisphere with respect to the mean plane containing **L2** (see Fig. 1) [the maximum deviation from the mean plane defined by the backbone atoms is 0.126(2) Å for N(3)]. All the N_{amide}–C_{sp²} bond distances are affected by the usual nitrogen lone pair delocalization (see Table 2), which causes the urea moiety to be almost coplanar with the aromatic ring and then with the NO₂ group: the angle formed by the mean plane containing the urea–aromatic ring (P1) and the atoms of the NO₂ is 11.4°.

In addition, the mean plane (P2) defined by the atoms C(2), C(4), N(1), C(5), O(1), C(6) also lies on the mean plane defined by P1, notwithstanding the single-bond character of C(5)–C(6); the small value of the angle between P1 and P2 (6.73(5)°) may be due to the short hydrogen bond contact

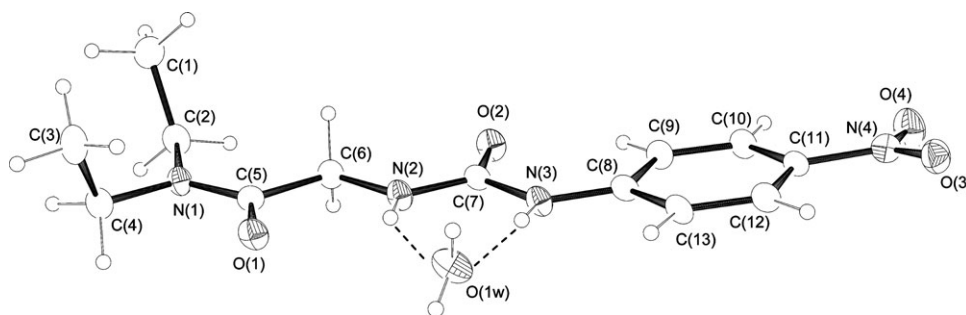


Fig. 1 ORTEP 3 view of **L2**·H₂O species. Ellipsoids are drawn at 30% probability.

involving the O(1) atom and the amidic hydrogen atom H(2n) (see Table 3). The O(1), C(5), C(6), N(2) and H(2n) atoms, as a consequence, are well within a plane, the maximum deviation from the mean plane being 0.03(2) Å [H(2n)].

Due to the planar disposition of **L2**, a network of weak intramolecular hydrogen bonds is present.³¹

The co-crystallized water molecule links together three symmetry-related **L2** molecules: the oxygen atom O(1w) acts as a donor atom towards the hydrogen atoms H(2n) and H(3n) bonded to N(2) and N(3) of the same **L2** molecule (see Fig. 1), while at the same time the hydrogen atoms of the water molecule interact with the oxygen atoms O(1) and O(2) of two different **L2** molecules (see Table 3).

Finally, the urea moiety and the aromatic ring of two symmetry-related **L2** molecules (the symmetry operation is $1 - x, -2 - y, 2 - z$) overlap, giving rise to a dimer (the distance between the mean planes containing the urea-aromatic ring moiety of each molecule is 3.4048(5) Å) (see Fig. 2),³² these dimers are connected by means of a network of weak hydrogen bonds of C–H···O type whose lengths fall in the 2.50(2)–2.93(2) Å range with angles ranging from 101(2) to 148(2)°.

Table 2 Bond distances (Å) and angles (°) for **L2**·H₂O

O(1)–C(5)	1.237(3)	C(2)–N(1)–C(4)	117.7(2)
O(2)–C(7)	1.231(3)	C(2)–N(1)–C(5)	124.0(2)
O(3)–N(4)	1.230(2)	C(4)–N(1)–C(5)	118.3(2)
O(4)–N(4)	1.230(2)	C(6)–N(2)–C(7)	121.4(2)
N(1)–C(2)	1.462(3)	C(7)–N(3)–C(8)	128.2(2)
N(1)–C(4)	1.471(3)	O(3)–N(4)–O(4)	122.7(2)
N(1)–C(5)	1.337(3)	O(3)–N(4)–C(11)	118.9(2)
N(2)–C(6)	1.438(3)	O(4)–N(4)–C(11)	118.5(2)
N(2)–C(7)	1.344(3)	N(1)–C(2)–C(1)	112.7(2)
N(3)–C(7)	1.375(3)	N(1)–C(4)–C(3)	112.9(2)
N(3)–C(8)	1.384(3)	O(1)–C(5)–N(1)	122.7(2)
N(4)–C(11)	1.462(3)	O(1)–C(5)–C(6)	119.0(2)
C(1)–C(2)	1.519(4)	N(1)–C(5)–C(6)	118.3(2)
C(3)–C(4)	1.523(4)	N(2)–C(6)–C(5)	108.0(2)
C(5)–C(6)	1.522(3)	O(2)–C(7)–N(2)	122.3(2)
C(8)–C(9)	1.401(3)	O(2)–C(7)–N(3)	123.4(2)
C(8)–C(13)	1.398(3)	N(2)–C(7)–N(3)	114.3(2)
C(9)–C(10)	1.376(4)	N(3)–C(8)–C(9)	123.9(2)
C(10)–C(11)	1.369(3)	N(3)–C(8)–C(13)	117.9(2)
C(11)–C(12)	1.384(3)	C(9)–C(8)–C(13)	118.2(2)
C(12)–C(13)	1.366(3)	C(8)–C(9)–C(10)	119.8(2)
		C(9)–C(10)–C(11)	120.3(2)
		N(4)–C(11)–C(10)	119.3(2)
		N(4)–C(11)–C(12)	119.4(3)
		C(10)–C(11)–C(12)	121.3(3)
		C(11)–C(12)–C(13)	118.5(3)
		C(8)–C(13)–C(12)	121.8(3)

Solution studies

The anion coordination properties of both ligands **L1** and **L2** were tested in relation to the series of halides as well as the acetate anions (G); the main aim was not only to test the intrinsic binding properties of both ligands but also to determine whether the side-arms in **L1** were able to cooperate in binding anions. The binding experiments were carried out *via* ¹H NMR and UV-Vis titrations.

The interaction between ligands L (L = **L1** or **L2**) and the anions G (G = F[−], Cl[−], Br[−], I[−] and AcO[−]) was investigated in DMSO–0.5% water solution at 298 K. In the ¹H NMR experiments, the addition of G to L produced a significant ¹H NMR shift of some resonances of both **L1** and **L2** systems for G = F[−], Cl[−] and AcO[−] while the spectra remain substantially unchanged following the addition of G = Br[−] and I[−] (see Fig. 3). This aspect indicates that both ligands, under these experimental conditions, are able to interact with the anions F[−], Cl[−] and AcO[−] while Br[−] and I[−] do not interact with these receptors. Moreover, Fig. 3 also reports the spectrum obtained by adding NMe₄OH as a base which clearly highlights the deprotonation of the urea groups and the complete disappearance of the ureido proton H5 and the large broadening of H7; this, together with the shift of the phenyl protons can be explained by a deprotonation process mainly involving the nitrogen atoms closer to the *p*-nitrophenyl group.

Analysis of the ¹H NMR spectra of both **L1** and **L2** systems revealed that the resonances perturbed by the presence of the interacting anions are the same in both ligands and namely those of the phenyl, the urea and the CH₂, in α -position to the latter (see Fig. 3), while the macrocyclic portion of **L1** remains unchanged when guests are added. These data, as predicted, indicate that only the ureido backbone is involved in the interaction with the anions in both ligands, producing an NMR shift also in the nearest groups.

Table 3 Intra- and Intermolecular hydrogen bonds

X–H···Y	H···Y distance (Å)	X–H···Y angle (°)
N(2)–H(2n)···O(1)	2.18(3)	108(2)
N(2)–H(2n)···O(1w)	2.29(3)	146(2)
N(3)–H(3n)···O(1w)	1.98(2)	167(2)
O(1w)–H(1wa)···O(1) ^a	2.07(3)	160(3)
O(1w)–H(1wb)···O(2) ^b	1.91(4)	158(3)

^a = 2 – x, –1 – y, 2 – z. ^b = 1 + x, y, z.

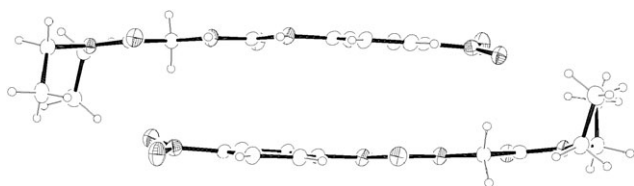


Fig. 2 ORTEP 3 view of **L2** dimer. Ellipsoids are drawn at 30% probability.

As shown in Fig. 3 and 4, the resonances shifting downfield in the presence of all interacting **G** are those of the ureido unit H5 and H7 as well as those of the H3 phenyl ring nearest to the urea group; on the contrary, those of CH₂ (H8) and those of the H2 phenyl ring undergo an upfield shift.

The addition of fluoride to **L1** leads (Fig. 3) to a shifting and broadening of both signals of the urea group which completely disappear at low F[−] to **L** molar ratio; similar spectra were also obtained with **L2**. In particular, the resonances of the amide NH protons in the F[−]/**L** systems broadened to the point of being unrecognizable after 0.5 equiv. of F[−]. These effects are not uncommon during titration with fluoride salts, as it was discovered³³ that fluoride is sufficiently basic to deprotonate these protons. This observation was supported by the fact that after the addition *ca.* 2.5 equiv. of F[−], the peak attributed to [FHF][−] began to appear at ~16.0 ppm³⁴ and sharpened to a perfect triplet at 16.2 ppm after the addition of 4.0 equiv. of F[−]. Consequently, we were unable to accurately determine binding constants *via* monitoring of these changes in the ¹H NMR spectra.

For the Cl[−] and AcO[−] anions, no deprotonation process was observed even when large amounts of **G** were added; in these cases all data are in agreement with NH...**G** hydrogen bonding interactions and indicate a fast equilibrium process involving the free and complexed forms of the ligands.

¹H-NMR titrations, adding different amounts of **G** (**G** = AcO[−], Cl[−] or F[−]) to a solution of **L** (**L** = **L1** or **L2**), were carried out to quantify the interaction as addition constant of **G** to **L1** or **L2**; the data obtained were analyzed using the

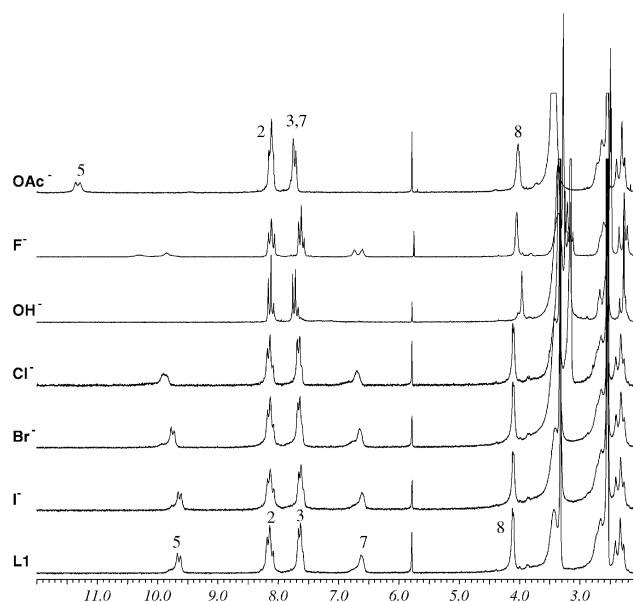


Fig. 3 ¹H NMR spectra of **L1** in DMSO–0.5% water solution at 298 K by adding 3 equivalents of guest with respect to **L1**, with the exception of F[−] where only 0.3 eq. were added.

HypNMR computer program³⁵ and the equilibrium constants (log *K*) obtained are reported in Table 4. Unfortunately, the disappearance of the NH signals H5 and H7 observed during the titration of both ligands **L1** and **L2** with F[−], also at low **G** to **L** molar ratio, prevented quantification of the process occurring in this case (see below).

For example, the titration spectra obtained with the system AcO[−]/**L2** are reported in Fig. 4. The Job plot (inset to Fig. 4) of the shift of H5 *vs.* the equivalents of acetate added shows, in agreement with the fitting of the data, the formation of only one AcO[−]–**L2** complexed species with 1 : 1 stoichiometry; similar spectral features were obtained for the addition of Cl[−] to **L2**. Furthermore, in addition to the 1 : 1 also a 2 : 1 molar ratio for **G**/**L1** systems was found (see Table 4).

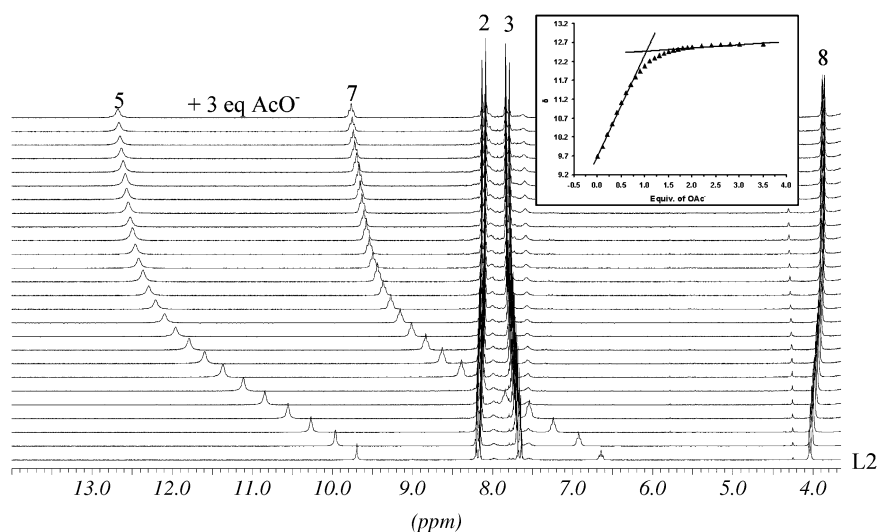


Fig. 4 ¹H NMR titration of the **L2**–AcO[−] system in DMSO–0.5% water solution at 298 K obtained by adding several amounts of Bu₄NAcO to **L2** solution; Inset: variation of the resonance H5 as a function of AcO[−] added.

Table 4 Logarithms of the addition constants of the G guests ($G = \text{Cl}^-$ and AcO^-) to L ($L = \text{L1}$ and L2), determined by NMR and UV-Vis titration in DMSO–0.5% water solution at 298 K

Reaction	log K			
	Cl^-		AcO^-	
	L1	L2	L1	L2
$L + G = \text{LG}$	$1.5(1)^{ac}$ – $1.6(1)^{bc}$	$1.4(1)^{ac}$ – $1.5(1)^{bc}$	$5.6(1)^{ac}$ – $5.3(2)^{bc}$	$2.9(1)^{ac}$ – $2.8(1)^{bc}$
$\text{LG} + G = \text{LG}_2$	$<1^{ab}$	—	$2.9(1)^a$ – $3.2(2)^b$	—

^a Values obtained by NMR. ^b UV-Vis experiments. ^c Values in parentheses are the standard deviation to the last significant figure.

Similar titration experiments were carried out using UV-Vis absorption experiments; two examples are shown in Fig. 5 and 6, which reports the UV-Vis spectra in the range where the *p*-nitrophenyl chromophore absorbs, obtained by adding increasing amounts of solution containing $G = \text{F}^-$ or AcO^- to **L2**, respectively; the spectrum obtained by adding an excess of NMe_4OH to **L2** solution is also reported in Fig. 5. As shown in Fig. 5, the spectral profile of free **L2** is completely different from that obtained by adding NMe_4OH ; this, as reported above for the NMR spectra, is due to the deprotonation of the ureido group which, occurring to the closer nitrogen atom, affects the absorption properties of the chromophore. The free **L2** shows a spectrum with a main band with λ_{max} at 352 nm ($\epsilon = 13\,400 \text{ cm}^{-1} \text{ mol}^{-1} \text{ dm}^3$), while the spectrum obtained by adding NMe_4OH shows a main band with λ_{max} at 482 nm ($\epsilon = 36\,000$); the bands can be attributed to the neutral and deprotonated forms of the *p*-nitrophenylurea group, respectively.

When increasing amounts of F^- to **L2** were added, some spectral changes were observed and the titration should be divided in two steps: the first one due to the addition of F^- up to 2 equivalents and the second one due to the addition of a larger amount of F^- with respect to **L2** (more than 5 equivalents). In fact, while the UV/Vis spectra of Fig. 5 show that the band recorded in the presence of 5 equivalents of Bu_4NF is similar to that of the deprotonated one, it is also possible to

observe that, during the titration (up to 2 eq.), there is the formation of new band centred around $\lambda_{\text{max}} = 482 \text{ nm}$ but with a lower absorbance with respect to the deprotonated one and a shift of the main band towards lower energy ($\lambda_{\text{max}} = 363 \text{ nm}$) with a decrease in absorptivity (see Fig. 5). This profile is similar to that obtained at the end of the titration with acetate (see Fig. 6). Similar spectral profiles to that observed after adding up to 2 equivalents of F^- to **L2** are also observable with **L1**; in addition, this profile also occurs with the other G/L systems in which, as already reported for the NMR experiments, no deprotonation process (*i.e.* formation of the deprotonated band) was observed in the presence of a large amount of guest (see also Fig. 6). Furthermore, it must be taken into account that the increase in the band centered at $\lambda_{\text{max}} = 482 \text{ nm}$, as depicted in inset of Fig. 5, is not linear. This behaviour cannot be attributed merely to a deprotonation process driven by the basicity of the fluoride anion but rather could be better explained by two processes occurring simultaneously in the F^-/L systems: (i) a true H-bond interaction between the ligand and the fluoride anion, (ii) the deprotonation of the ureido group. Evidence for the same interaction is lost using the ^1H NMR technique probably because of the different NMR time scale with respect to UV/Vis spectroscopy.

In other words, as already reported for similar interaction studies, both **L1** and **L2** show two processes in the presence of

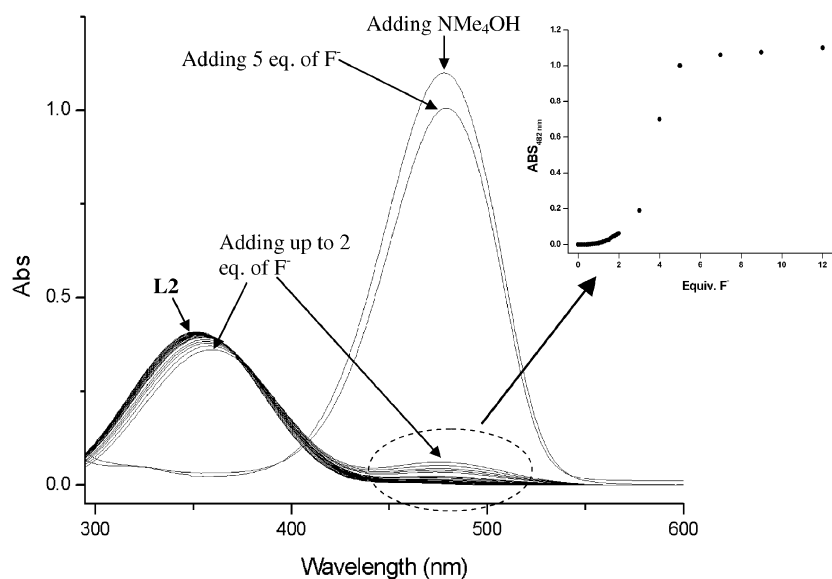


Fig. 5 UV-Vis spectra of **L2** in DMSO–0.5% water solution at 298 K [**L2**] = $3.0 \times 10^{-5} \text{ M}$ and those obtained by adding several amounts of F^- up to 2 equivalent with respect to [**L2**] (inset) and 5 equivalent or an excess of NMe_4OH . Inset: variation of the absorbance at 482 nm as a function of F^- added.

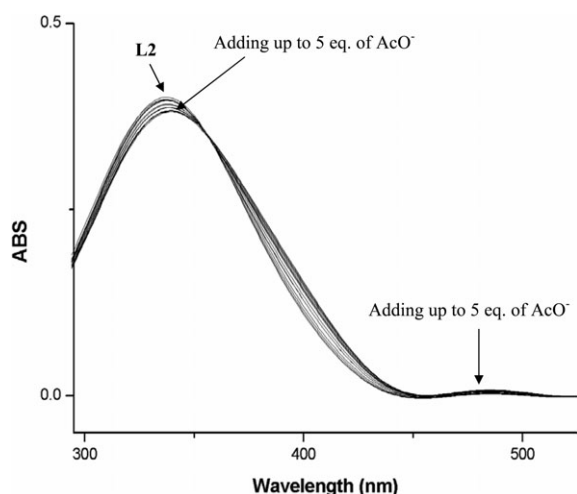


Fig. 6 UV-Vis spectra of **L2** in DMSO–0.5% water solution at 298 K [**L2**] = 3.0×10^{-5} M and those obtained by adding several amounts of AcO^- up to 5 equivalent with respect to [**L2**].

the F^- guest; one due to the association between F^- and **L** occurring *via* H-bonding along with a deprotonation process both involving the urea group of **L** systems. On the contrary, only the association behavior is detected in the presence of Cl^- and AcO^- , as depicted in Fig. 6; moreover, the addition of Br^- and I^- even in a large excess did not perturb the spectral profile of the free ligands, once again highlighting the absence of interaction with these two guests.

The interaction as addition constant of **G** to **L1** or **L2** evaluated by elaborating the UV-Vis titration data of both ligands is also reported in Table 4. Unfortunately, even when using this method, it was impossible to measure both the deprotonation constants as well as the addition constants induced by the addition of F^- to both **L** systems with a reliable precision.

On the other hand, the values of the addition constants reported in Table 4 for the interaction of **L1** and **L2** with the guests AcO^- and Cl^- are calculated by two different methods and they are in agreement, thus supporting the values and the speciation reported. A key aspect concerns the values as well as the speciation found with the macrocyclic ligand **L1** with respect to **L2**. In fact, while **L2** forms only $[\text{GL}]$ complexes having a 1 : 1 stoichiometry with both guests Cl^- and AcO^- , **L1** is able to form the $[\text{G}_2\text{L1}]$ as well as the $[\text{GL1}]$ species. In other words, **L1** is also able to bind two equivalents of guests under these experimental conditions, even though the constant value for the formation of the dichloride $[\text{Cl}_2\text{L1}]^{2-}$ species is too low to be calculated with certainty.

Comparison of the values of the constants leads us to specific considerations: (i) acetate is better bound by both systems than is chloride; (ii) the formation of the $[\text{GL}]$ species is similar for chloride in both **L** systems ($\log K$ is about 1.5 in both cases) while it is very different for acetate ($\log K = 5.6$ and 2.9 for **L1** and **L2**, respectively); (iii) the constant value for the addition of the second acetate to $[\text{AcOL1}]^-$ species is lower with respect to the first one and it is similar to the that of AcO^- to **L2**.

The first situation is usually observed in hosts having urea as interacting function; this is due to the different H-bond inter-

action related to the different form of the two anions, V-shaped *vs.* spherical for acetate and chloride, respectively, that permits both acetate oxygen atoms to form H-bonds with both ureido protons.³⁶

The second point, which is the key for our aims, can be attributed to the different way of stabilization of the two guests by the two ligands; while the chloride anion, showing similar constant values with both hosts, is stabilized by the urea fragment of a side-arm of **L1** in a similar coordination environment as in **L2**, this cannot be affirmed for acetate. The higher constant values found for the formation of $[\text{AcOL1}]^-$ with respect to the $[\text{AcOL2}]^-$ species suggests a different interaction environment. In particular, we can suppose that both side-arms of **L1** with both ureido groups are involved in stabilizing AcO^- *via* H-bonding. This occurs when the two side-arms are face-to-face, occupying the same region with respect to the macrocycle plane and leading to the cooperation of both urea groups to stabilize, *via* H-bond, the V-shaped acetate but not the small spherical chloride guest. This can be attributed to the way of linking the side-arms to the $\text{Me}_2[12]\text{aneN}_4$ scaffold; in detail, as previously demonstrated,²¹ the two $\text{N}-\text{C}=\text{O}$ groups which link each side-arm to the twelve membered ring play a fundamental role in stiffening and preorganizing the host. These two amide moieties force the two side arms to occupy the same region with respect to the macrocycle and yield **L1**, a preorganized host for acetate. This hypothesis is supported by the value of the addition constant of AcO^- to **L1**, which is higher (about 2–3 logarithmic units) than those reported for hosts containing only a ureido group as binding unit.^{36a}

At this time, despite repeated attempts, we have not been able to obtain crystals of the **L1** species suitable for X-ray analysis, but the crystal structure of Fig. 1, in which an oxygen atom of a water molecule is bound *via* H-bonding by both ureido protons of **L2**, suggests a possible pathway for the disposition of acetate in the $[\text{AcOL1}]^-$ complex. In fact, considering the V-shape of acetate, we can suppose that each oxygen atom of acetate interacts with a urea fragment of one side-arm in similar way of Fig. 1, thus acetate can interact simultaneously with both side-arms and this V-shaped guest is located in a bridge disposition between them. A proposal for the interaction between AcO^- and **L1** and **L2** in the $[\text{AcOL}]^-$ species is shown in Fig. 7.

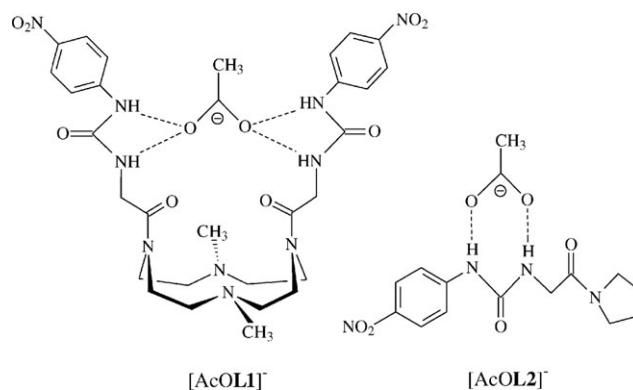


Fig. 7 Proposed interaction models between AcO^- and **L** in $[\text{AcOL1}]^-$ and $[\text{AcOL2}]^-$ complexes.

At last, the lower value for the addition of the second acetate underlines that its binding is not preferred in the same way as the first one. This can be attributed to the fact that both urea groups are involved in the stabilization of the first AcO^- , hindering the binding of the second acetate, once again indicating the cooperation of both side-arms in binding the first AcO^- .

It is difficult to suggest a coordination environment for the two guests in the $[(\text{AcO})_2\text{L1}]^{2-}$ species, although the value similar to that of **L2** found for the addition of the second acetate suggests a similar interaction pathway between acetate and side-arm. In this case, each side-arm of **L1** should be involved in the stabilization of only one AcO^- guest, however other interaction pathways could be suggested.

Conclusions

The synthesis of the two new hosts for anions **L1** and **L2** is reported. **L1** is a branched macrocyclic ligand containing two equal side-arms attached on a tetraaza macrocyclic scaffold; each side-arm includes the *p*-nitrophenylureido moieties as interacting group. **L2** shows the same chain and binding moiety of the side-arm of **L1** and it was synthesized for comparison with **L1**. Different synthetic routes were explored to afford the best yield; **L1** was synthesized from the intermediate compound **4** that can be considered as a versatile building block to obtain further functionalized branched macrocyclic hosts.

The hosting properties of both ligands, tested with the halide series and with the acetate anions by NMR and UV-Vis spectroscopy, allow them to interact with the same anion guests investigated in dimethyl sulfoxide–0.5% water solution. In particular, under our experimental conditions both hosts bind the F^- , Cl^- and AcO^- guests while Br^- and I^- did not. The NMR experiments proved that the binding occurs *via* H-bond at the ureido fragments. The fluoride anion also showed enough basicity to deprotonate the ureido group of both ligands, preventing the determination of the addition constants for this guest which were possible for Cl^- and AcO^- . Both spectroscopic methods gave rise to similar results for the complexed species formed as well as for the value of the addition constants reported as $\log K$.

L1 forms G-L species of 1 : 1 ($[\text{GL1}]$) and 2 : 1 ($[\text{G}_2\text{L1}]$) stoichiometry while **L2** forms only the 1 : 1 $[\text{GL2}]$ species. The high values of the constant for the formation of the $[\text{AcOL1}]^-$ species suggested that both side-arms cooperate in binding this guest, while this probably does not occur with the spherical Cl^- . This aspect can be attributed to both the preorganization of the side-arms and the V-shape of acetate. The first characteristic is due to the way of attaching side-arms to the tetraaza-macrocyclic base, in that the amide groups stiffened the molecular skeleton and forced the two side-arms to stay in the same part of the macrocycle ring. In this way, the whole molecule appears to be preorganized for interaction with other species having the capacity to form simultaneously H-bonding with both ureido groups such as the V-shape of the acetate anion, which should be located in a bridge disposition between the two ureido fragments. The crystal structure reported supports this hypothesis. On the contrary, small guests such

as the chloride cannot be simultaneously bonded and thus the side-arms of **L1** act separately in binding them.

In conclusion, as already demonstrated in an analogous way for the attaching of side-arms, the tetraaza-macrocyclic base acts as a scaffold to preorganize them when the side-arms are linked *via* amide function. This allows the presence of an area richer in H-bond sites in **L1** than that given by only one urea moiety as in **L2**. Guests having the ability to form several H-bonds in this area, such as the acetate, can be strongly stabilized.

The promising results obtained with **L1** as host for acetate make it an attractive host for molecular recognition studies of guests showing carboxylic functions as well as separated H-bonding sites acceptor.

Experimental

General methods

IR spectra were recorded on a Shimadzu FTIR-8300 spectrometer. Melting points were determined on a Buchi melting point B 540 apparatus and are uncorrected. EI-MS spectra (70 eV) were recorded with a Fisons Trio 1000 spectrometer. The HPLC system (Waters Alliance 2795) was coupled with a photodiode array detector (Waters 2996 PDA), followed by an electrospray mass spectrometer detector (ESI-MS) (Waters-Micromass ZQ) worked by Mass Lynx 4.1 SP4 software. The ESI-MS analyses were performed in a positive mode under the following conditions: source and desolvation temperature 100 and 250 °C; capillary and cone voltage 2.5 kV and 25 V; cone and desolvation flow (nitrogen gas) 40 and 400 L/h. HPLC analyses were performed on a 25 cm × 4.6 mm Discovery C-18, 5 cm column (Supelco, Bellefonte, PA) equipped with a Supelguard Discovery C-18 guard column (2 cm × 4 mm, 5 cm). Solvents A (MeOH) and C (5 mM ammonium acetate) were run at a flow rate of 1 mL/min. The running gradient was adjusted to 10% A (2 min), increasing to 90% A (18 min), and then 100% C (2 min), followed by a re-equilibration at 10% A (15 min). All solvents were HPLC-grade (Aldrich-Sigma) and water was purified *via* a Millex Q-plus system (Millipore).

All chemicals were purchased from Aldrich, Fluka and Lancaster in the highest quality commercially available. All the solvents were dried prior to use. Chromatographic separations were performed on silica gel columns by flash chromatography (Kieselgel 60, 0.040–0.063 mm, Merck). TLC analyses were performed on precoated silica gel on aluminium sheets (Kieselgel 60 F254, Merck). The 1,7-dimethyl-1,4,7,10-tetraazacyclododecane **2** was prepared as previously described.²²

Synthesis

4(N),10(N)-Bis-[N-(benzyloxycarbonyl)glycyl]-1,7dimethyl-1,4,7,10-tetraazacyclododecane (3). Pyridine (8.5 mmol, 0.7 mL) and pentafluorophenyl 2,2,2-trifluoroacetate (1.66 mL, 10 mmol) were added to a solution of *N*-(benzyloxycarbonyl)-glycine **1** (1.61 g, 7.70 mmol) in DMF (5 mL). The reaction mixture was stirred for 1 hour at room temperature, diluted with AcOEt (300 mL) and washed with 0.1 N aqueous HCl (3 × 50 mL), 5% aqueous NaHCO_3 (3 × 50 mL) and brine

(50 mL). The organic solution was dried over Na_2SO_4 and concentrated under reduced pressure to give 2.8 g (98%) of the desiderate pentafluorophenyl ester.

A solution of the above pentafluorophenyl ester **4** in dry DMF (5 mL) was slowly added dropwise over 30 min at 0 °C to a stirred solution of 1,7-dimethyl-1,4,7,10-tetraazacyclododecane **2** (0.77 g, 3.86 mmol) and *i*-Pr₂EtN (2.69 mL, 0.0155 mol) in dry DMF (2 mL). The reaction mixture was stirred for 12 hours at room temperature. The organic yellow solution was concentrated under reduced pressure and the yellow oily residue was diluted in CHCl_3 and precipitated by the addition of Et₂O to give **5** (2.03 g, 77%) as a colorless solid. The organic solution was concentrated under reduced pressure and the residue was purified by silica gel chromatography (CH_2Cl_2 saturated with NH_3 /methanol 99 : 1) to give additional **3** (0.3 g, 12%; 89% overall yield); mp 105–106 °C (decomp.).

The latter dissolved in DMF (5 mL) was slowly added dropwise to a solution of tetramine (3.2 mmol, 640 mg) and DIPEA (2.7 mL) in DMF (2 mL). The mixture was stirred at room temperature for 36 hours, after which the solvent was removed under vacuum and the residue purified by flash chromatography on alumina using a mixture of methylene chloride/methanol 95 : 5 as eluent to give 1.35 g (65% yield) of the desired product as a white solid.

Rotamer mixture: mp = 171–173 °C FTIR (KBr) 3268, 3066, 2986, 2940, 1726, 1642, 1550, 1454, 1255, 1167, 1049, 1006, 743, 653 cm^{-1} ; ¹H NMR (CDCl_3) δ 2.24–2.38 (6H, m), 2.63–2.71 (8H, m), 3.28–3.46 (8H, m), 4.05–4.07 (4H, m), 5.10–5.11 (4H, m), 5.82–5.89 (2H, m), 7.30–7.40 (10H, m); ¹³C NMR (CDCl_3) δ 42.8, 43.1, 46.6, 55.0, 65.9, 127.2, 129.0, 141.2, 156.3, 164.3 ppm; MS (+ESI) 583.7 ($\text{M} + 1$); Anal. for $\text{C}_{30}\text{H}_{42}\text{N}_6\text{O}_6$ (582.70): Calcd C 61.84, H 7.27, N 14.42; Found C 62.0, H 7.2, N 14.3.

4(N),10(N)-Bis-glycyl-1,7-dimethyl-1,4,7,10-tetraazacyclododecane (4). 10% Pd/C (400 mg) was added to a solution of bis-carbamate **3** (1.35 g, 2.3 mmol) in EtOH *abs* (20 mL). The mixture was then hydrogenated at 4 atm in an autoclave for 16 hours at room temperature, then filtered through Celite and the solvent was removed under vacuum to give **4** (710 mg, purity = 98% by HPLC) which was used without any further purification.

Rotamer mixture: ¹H NMR (D_2O , pH = 3) 2.41–2.79 (10H, m), 2.95–3.15 (4H, m), 3.65–3.45 (8H, m), 3.75–3.70 (4H, m); ¹³C NMR (D_2O , pH = 3), 40.8, 41.4, 41.8, 45.1, 46.1, 46.2, 46.4, 57.7, 58.2, 58.5, 59.4 ppm; MS (+ESI) 315, 2 (MH^+); Anal. for $\text{C}_{14}\text{H}_{30}\text{N}_6\text{O}_2$ (314.43): Calcd C 53.48, H 9.62, N 26.73; Found C 53.6, H 9.5, N 26.9.

4(N),10(N)-Bis-[2-(4-nitrophenylureido)acetamido]-1,7-dimethyl-1,4,7,10-tetraazacyclododecane (L1). *p*-Nitrophenylisocyanate **5** (3.17 mmol, 530 mg) was added to a solution of diamine **4** (500 mg, 1.58 mmol) in dry THF (6 mL). The mixture was stirred at room temperature for 16 hours. The solvent was removed under vacuum and the residue was treated with diethyl ether to remove any apolar substances. The solid thus obtained was purified on alumina using CH_2Cl_2 /MeOH (92 : 8) as eluent to give **L1** as pale yellow solid in 48% yield (480 mg).

Rotamer mixture: mp = 246–248 °C; FTIR (film) 3390, 2924, 1680, 1648, 1559, 1325, 1269, 1174, 1110 cm^{-1} ; ¹H NMR (DMSO) δ 2.25–2.36 (6H, m), 2.55–2.68 (8H, m), 3.28–3.46 (8H, m), 4.02–4.07 (4H, m), 6.52–6.62 (2H, m), 7.50–7.60 (4H, m), 8.00–8.15 (4H, m), 9.60–9.70 (2H, m); ¹³C NMR (DMSO) δ 41.3, 41.6, 46.6, 55.6, 116.6, 125.1, 140.3, 147.0, 154.2, 168.8 ppm; MS (+ESI) 643.3 (MH^+); Anal. for $\text{C}_{28}\text{H}_{38}\text{N}_{10}\text{O}_8$ (642.67): Calcd C 52.33, H 5.96, N 21.79; Found C 52.2, H 6.1, N 21.7.

N-(Benzyloxycarbonyl)glycine-*N,N*-diethylamide (6)

Pyridine (0.7 mL, 8.5 mmol) and perfluorophenyl 2,2,2-trifluoroacetate (10 mmol, 1.66 mL) were added to a solution of *N*-(benzyloxycarbonyl)glycine **1** (1.61 g, 7.70 mmol) in DMF (5 mL).

The mixture was stirred at room temperature for 1 hour, diluted with EtOAc (300 mL) and washed with an aqueous solution of HCl 0.1 N (2×50 mL), NaHCO_3 5% (2×50 mL) and brine (1×50 mL). The organic mixture was dried over Na_2SO_4 and evaporated under vacuum to give 2.8 g (98%) of the pentafluorophenyl ester.

A solution of the above pentafluorophenyl ester in dry DMF (5 mL) was added dropwise over 30 min at 0 °C to a stirred solution of *N,N*-diethylamine (0.8 mL, 7.7 mmol) and DIPEA (2.7 mL, 15.5 mmol) in DMF (2 mL). The mixture was stirred at room temperature for 36 hours, and the solvent was removed under vacuum and the residue purified by flash chromatography on silica using cyclohexane/ethyl acetate 40–60 as eluent to furnish 1.41 g (69% yield) of **6** as a sticky oil.

Rotamer mixture: FTIR (film) 3268, 3066, 2986, 2940, 1726, 1642, 1550, 1454, 1255, 1167, 1049, 1006, 743, 653 cm^{-1} ; ¹H NMR (CDCl_3) δ 1.13 (t, $J = 7.2$ Hz), 1.19 (t, $J = 7.2$ Hz), 3.26 (q, $J = 7.2$ Hz), 3.40 (q, $J = 7.2$ Hz), 4.02 (d, $J = 4.2$ Hz), 5.13 (s), 5.85 (br s, NH), 7.30–7.37 (m, PhH); ¹³C NMR (CDCl_3) δ 12.7, 13.7, 40.2, 40.7, 42.3, 66.5, 127.7, 127.8, 128.2, 136.4, 156.0, 166.7; MS (+ESI) 265.1 (MH^+ , 100); Anal. for $\text{C}_{14}\text{H}_{20}\text{N}_2\text{O}_3$ (264.32): Calcd C 63.62, H 7.63, N 10.60; Found C 63.6, H 7.6, N 10.5.

2-Amino-*N,N*-diethylacetamide (7)

10% Pd/C (180 mg) was carefully added to a solution of *N*-(benzyloxycarbonyl)glycine-*N,N*-diethylamide **6** (1.41 g, 5.3 mmol) in EtOH (20 mL). The mixture was then hydrogenated at 4 atm in autoclave for 16 hours at room temperature. The reaction mixture was then filtered through Celite and the solvent was removed by rotary evaporation to give 530 mg of clean product with 85% yield. The product was immediately used in the next step.

FTIR (film): 3395, 2932, 1632, 1567, 1325, 1269, 1172, 1110 cm^{-1} ; ¹H NMR (DMSO) δ 1.02 (3H, m), 3.20 (2H, m), 3.42 (2H, m); ¹³C NMR (DMSO) δ 13.0, 14.0, 39.7, 40.2, 41.0, 163.8; MS (+ESI) 131.0 (MH^+) 153 (MNa^+).

1-((Diethylcarbamoyl)methyl)-3-(4-nitrophenyl)urea (L2)

p-Nitrophenylisocyanate **5** (1 mmol, 177 mg) was added to a solution of 2-amino-*N,N*-diethylacetamide (130 mg, 1 mmol) in THF (3 mL). The mixture was stirred at room temperature for

16 hours. The solvent was removed under vacuum and the residue was treated with ethyl ether to remove any apolar substances. The solid so obtained was crystallized by ethanol to obtain **L2** as pale yellow solid in 51% yield.

Rotamer mixture: mp = 168–169 °C FTIR (film): 3389, 2924, 1701, 1628, 1559, 1325, 1269, 1172, 1174, 1110, 751 cm⁻¹; ¹H NMR (DMSO) δ 1.02 (3H, t, J = 7.2 Hz), 1.12 (3H, t, J = 7.2 Hz), 3.24 (2H, q, J = 7.2 Hz), 3.32 (2H, q, J = 7.2 Hz), 3.98 (2H, d, J = 4.8 Hz), 6.61 (1H, br s, NH), 7.60 (2H, d, J = 9.4 Hz), 8.13 (2H, d, J = 9.4 Hz), 9.69 (1H, br s); ¹³C NMR (DMSO) δ 12.9, 13.8, 39.7, 40.4, 41.0, 116.7, 125.2, 140.5, 147.0, 154.3, 167.3; MS (+ESI) 295.1 (MH⁺, 100); Anal. for C₁₃H₁₈N₄O₄ (294.31): Calcd C 53.05, H 6.16, N 19.04; Found C 53.0, H 6.3, N 19.1.

Crystals suitable for X-ray analysis were obtained by slow evaporation of a DMSO/H₂O solution of **L2**.

X-Ray crystallography

Intensity data for **L2**·H₂O were collected on an Oxford Diffraction Xcalibur diffractometer equipped with a CCD area detector, using Mo-K α radiation (0.71069 Å), monochromated with a graphite prism. Data were collected using the CrysAlis CCD program³⁷ and the reduction was carried out with the CrysAlis RED program.³⁸ Absorption correction was performed with the program ABSPACK in CrysAlis RED. Structure was solved with the direct methods of the SIR97³⁹ package and refined by full-matrix least squares against F^2 with the SHELX97 program.⁴⁰

Geometrical calculations were performed by PARST97⁴¹ and molecular plots were produced by the ORTEP3 program.⁴²

All the non-hydrogen atoms were refined anisotropically and all the hydrogen atoms were found in the Fourier difference map and refined isotropically.

Crystallographic data and refinement parameters for **L2**·H₂O are reported in Table 5.

UV-Vis and NMR experiments

¹H and ¹³C NMR spectra were recorded on a Bruker Avance 200 instrument, operating at 200.13 and 50.33 MHz, respectively, and equipped with a variable temperature controller.

Table 5 Crystal and structure refinement data for **L2**·H₂O

Chemical formula	C ₁₃ H ₂₀ N ₄ O ₅
M_r	312.33
Crystal system, space group	Triclinic, $P\bar{1}$
Cell parameters (Å, °)	a = 6.793(2), α = 117.05(4) b = 11.479(5), β = 93.32(3) c = 12.229(2), γ = 105.24(3)
V (Å ³)	761.5(5)
Z	2
D_c (g cm ⁻³)	1.362
λ (Å)	0.71069
μ (mm ⁻¹)	0.106
Temperature (K)	150
Reflections collected/unique	7383/2767 [$R(\text{int})$ = 0.0335]
Refinement method	Full-matrix least-squares on F^2
Data/parameters	2767/279
Final R indices [$I > 2\sigma(I)$]	$R1$ = 0.0454, $wR2$ = 0.0988
R indices (all data)	$R1$ = 0.0949, $wR2$ = 0.1087

The temperature of the NMR probe was calibrated using 1,2-ethanediol as calibration sample. For the spectra recorded in D₂O, the peak positions are reported with respect to HOD (4.75 ppm) for ¹H NMR spectra, while dioxane was used as reference standard in ¹³C NMR spectra (δ = 67.4 ppm). For the spectra recorded in CDCl₃ and DMSO-*d*₆ the peak positions are reported with respect to TMS. ¹H–¹H and ¹H–¹³C correlation experiments were performed to assign the signals. Chemical shifts (δ scale) are reported in parts per million (ppm values) relative to the characteristic peak of the solvent; coupling constants (J values) are given in hertz (Hz).

NMR titrations were carried out in a DMSO-*d*₆-0.5% water mixture; 0.5% of water was added to DMSO to avoid the not uniform absorption of water from the atmosphere by anhydrous DMSO during the titration. In a typical experiment, a 5×10^{-2} mol dm⁻³ DMSO–0.5% water solution of the anion was added in 0.1 eq at a time to a 1×10^{-2} mol dm⁻³ DMSO–0.5% water solution of the ligand directly in the NMR tube; the tube was then kept for 5 min at a temperature of 298 K before starting the acquisition of the spectrum. The anions tested were added as their tetrabutylammonium salts. The monitoring of the shift of the signals in the ligand spectra (see Discussion) permitted evaluation of the association constants ligand-anion using the HYPNMR computer program.³⁵

UV-Vis absorption spectra were recorded at 298 K with a Varian Cary-100 spectrophotometer equipped with a temperature control unit. The interaction of anions with ligands **L1** and **L2** was studied in similar conditions of the NMR titration experiments using DMSO–0.5% water mixture as solvent; solution containing the anion G (F⁻, Cl⁻, Br⁻, I⁻ or AcO⁻) up to 5 equivalents with respect to the ligand was added to the solution containing L ($[L1] = 1.5 \times 10^{-5}$ M; $[L2] = 3.0 \times 10^{-5}$ M). At least three sets of spectrophotometric titration curves for each G/L system were performed. All sets of curves were treated either as single sets or as separate entities, for each system; no significant variations were found in the values of the determined constants. The HYPERQUAD computer program was used to process the spectrophotometric data.^{35b}

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