1,10-Dimethyl-1,4,7,10,13,16-hexaazacyclooctadecane L and 1,4,7-Trimethyl-1,4,7,10,13,16,19-heptaazacyclohenicosane L1: Two New Macrocyclic Receptors for ATP Binding. Synthesis, Solution Equilibria and the Crystal Structure of $(H_4L)(ClO_4)_4$

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The synthesis of two new methylated ligands 1,10-dimethyl-1,4,7,10,13,16-hexaazacyclooctadecane **L** and 1,4,7-trimethyl-1,4,7,10,13,16,19-heptaazacyclohenicosane **L1** is described. Basicity constants and protonation enthalpies of both ligands have been determined by potentiometric and microcalorimetric measurements in 0.15 mol dm^{-3} $NaClO_4$ at 298.1 K. The protonated forms of these cyclic polyamines bind ATP in solution. The equilibrium constants of the species formed have been determined (0.15 mol dm^{-3} $NaClO_4$, 298.1 K). The results presented for **L** and **L1** are compared with those previously obtained for the related ligands 1,4,7,10,13,16-hexaazacyclooctadecane, 1,4,7,10,13,16,19-heptaazacyclohenicosane and 1,4,7,13-tetramethyl-1,4,7,10,13,16-hexaazacyclooctadecane. Among these macrocycles **L** is a selective receptor for ATP binding.

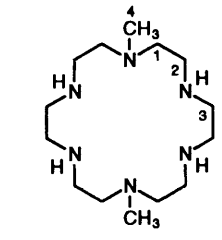
The crystal structure of the compound $(H_4L)(ClO_4)_4$ [space group $P2_1/c$, $a = 9.257(4)$, $b = 8.600(2)$, $c = 17.990(10)$ Å, $\beta = 101.74(4)°$, $V = 1402(1)$ Å^3, $Z = 2$] shows that the four charged ammonium groups point inside the macrocyclic cavity.

There is a continuing interest in the chemistry of polyazamacrocycles because of their ability to interact with both cationic and anionic species.[1] At present special attention is being focused on their effectiveness in the binding and cleavage of nucleotidic phosphate anions in solution.[2–4] Cyclic polyamines undergo extensive protonation in solution, forming highly charged polyammonium cations which give rise to strong interactions with nucleotides, such as ATP, ADP and AMP in their anionic forms, *via* electrostatic forces and hydrogen bonding. However, the presence of unprotonated amino groups promotes nucleotide dephosphorylation by nucleophilic attack of terminal phosphate groups.

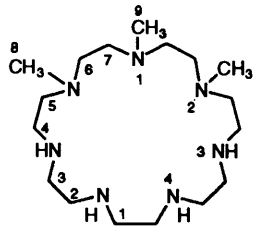
In recent papers[4,5] we have shown that nitrogen methylation alters significantly the properties of such macrocycles. In particular it has been observed that the tetramethylated polyamine 1,4,7,13-tetramethyl-1,4,7,10,13,16-hexaazacyclooctadecane **L2** is more effective in ATP binding and dephosphorylation than the unmethylated analogous 1,4,7,10,13,16-hexaazacyclooctadecane **L3**.

The properties of nucleotidic complexes of polyammonium cations have been interpreted on the basis of charge density and disposition in the ligand, structural features of polyammonium cations, and nucleophilicity of amino groups.[2–4]

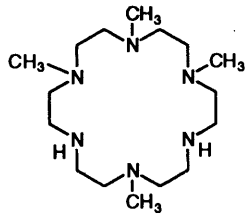
In order to gain further insight into this aspect of anion coordination we have synthesized the partially methylated ligands **L** and **L1**. We report here on the synthesis and characterization of these polyazamacrocycles as well as on their basicity properties and binding ability towards ATP.



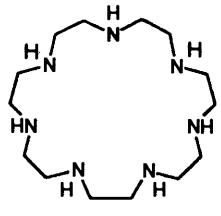
L



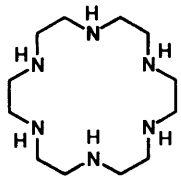
L1



L2



L4



L3

Results and Discussion

Crystal Structure of $H_4L(ClO_4)_4$.—The molecular structure consists of discrete $[H_4L]^{4+}$ cations and perchlorate anions. Fig. 1 shows an ORTEP[6] drawing of the cation with the atom labelling. The six nitrogen atoms of the macrocyclic ring, which lie around the crystallographic inversion centre, are fairly coplanar, the deviations from the mean plane (PARST[7]) being 0.279(4), −0.307(4) and 0.346(4) Å for N(1), N(2) and N(3), respectively. The four acidic protons have been localized on the secondary nitrogen atoms of the macrocycle which adopts an elliptical shape with intramolecular distances between

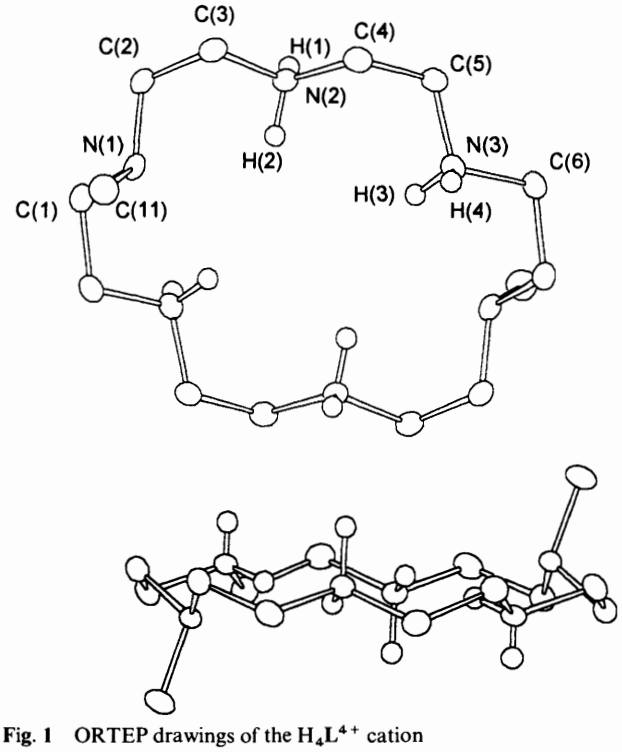


Fig. 1 ORTEP drawings of the H_4L^{4+} cation

symmetry related nitrogen atoms of 5.58, 5.64 and 6.73 Å. The N–C–C–N torsion angles are of *gauche* type, while the C–N–C–C chains are in a *trans* conformation. It is noteworthy that, in spite of the electrostatic repulsion between positive charges, all the ammonium groups, as well as the amino groups, of H_4L^{4+} converge into the macrocyclic cavity forming intramolecular contacts, *via* H-bonds, which involve the H(2) and H(3) acidic protons and the N(1) and N(1)′ methylated nitrogen atoms [N(2)–H(2)···N(1) 2.59(5) Å and N(3)–H(3)···N(1)′ 2.58(5) Å]. Moreover, the longest elongation of the H_4L^{4+} cation corresponds to the separation between the unprotonated amino groups [N(1), N(1)′]. This feature, which seems to contrast with the principle of electrostatic repulsion minimization, has been already observed for the analogous unmethylated H_4L3^{4+} cation [8] as well as for the octaprotonated form of the decaazamacrocycle 1,4,7,10,13,16,19,22,25,28-decaazacyclotriacontane.[9]

The overall conformation of the H_4L3^{4+} cation, in the crystal structure of $H_4L3(NO_3)_2Cl_2{\cdot}H_2O$,[8] is similar to that of H_4L^{4+}. The tetraprotonated cation in this case also adopts an elliptical shape with similar intramolecular distances between symmetry related nitrogen atoms (5.12, 5.48 and 6.88 Å). However, in the crystal structure of $H_6L3(NO_3)_4Cl_2$ the hexaprotonated cation shows an inversion of the ammonium groups which, in this case, point outside the cavity.[10] As a consequence the macrocycle experiences a considerable expansion (intramolecular distances between symmetry related nitrogens: 5.67, 6.89 and 8.28 Å) with respect to the tetraprotonated forms and four N–C–C–N chains pass from *gauche* to *trans* conformation. This conformational change produces a better minimization of the electrostatic repulsion.

Strong H-bonds between one of the symmetry related perchlorate anions and H_4L^{4+} [O(22)···H(2), H(3), 2.05(4), 2.28(5) Å, O(24)···H(2), 2.60(4) Å, respectively] contribute to the crystal packing of $H_4L(ClO_4)_4$.

Protonation.—The stepwise protonation constants of **L** and **L1**, obtained from the potentiometric data analysis using the computer program SUPERQUAD,[11] are presented in Table 1.

Table 1 Logarithms of the stepwise protonation constants of **L**, **L1**, **L2**, **L3** and **L4** in 0.15 mol dm^{-3} $NaClO_4$ at 298.1 K

	log K				
Reaction	**L**[a]	**L1**[a]	**L2**[b]	**L3**[c]	**L4**[c]
$L + H^+ = LH^+$	9.78(1)[d]	9.27(1)	9.75	10.15	9.76
$LH^+ + H^+ = LH_2^{2+}$	9.09(1)	8.95(1)	9.11	9.48	9.28
$LH_2^{2+} + H^+ = LH_3^{3+}$	7.76(1)	7.97(1)	7.53	8.89	8.63
$LH_3^{3+} + H^+ = LH_4^{4+}$	3.84(1)	5.42(1)	2.59	4.27	6.42
$LH_4^{4+} + H^+ = LH_5^{5+}$		2.98(1)		2.21	3.73
$LH_5^{5+} + H^+ = LH_6^{6+}$		1.78(1)		1.0	2.13
$LH_6^{6+} + H^+ = LH_7^{7+}$					2.0

[a] This work. [b] From ref. 12. [c] From ref. 13. [d] Values in parentheses are standard deviations in the last significant figure.

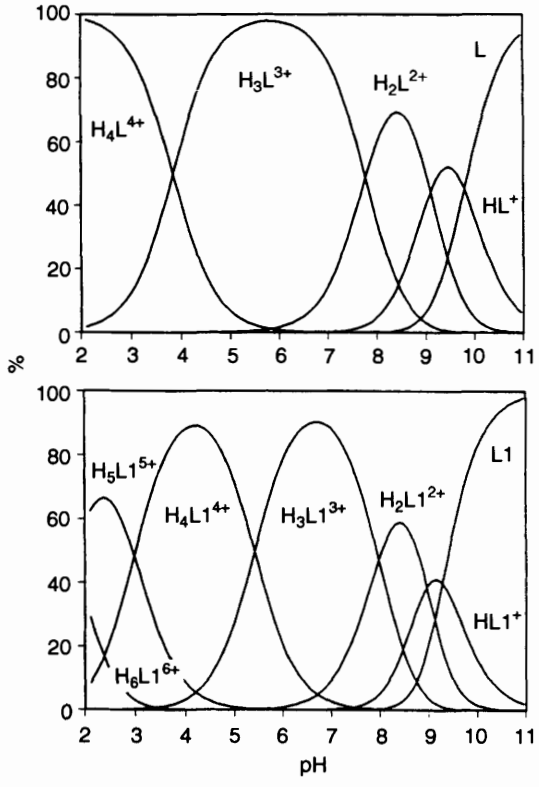


Fig. 2 Calculated distribution diagrams of the species formed by **L** and **L1** as a function of pH

As can be seen the presence of tertiary amino groups reduces the basicity of **L** and **L1**, at each protonation step, with respect to the unmethylated analogues **L3** and **L4** (1,4,7,10,13,16,19-heptaazacyclohenicosane)[12,13] (Table 1). In the pH range considered (2.5–10.5) both **L** and **L1** are unable to form the fully protonated species behaving, at most, as tetra- and hexa-protic bases, respectively (Fig. 2). Methylation of polyamino ligands largely reduces the ability of water to interact *via* hydrogen bonding with the ammonium groups formed upon protonation. As a consequence the protonated groups are weakly shielded from each other by solvent molecules and the rapidly growing electrostatic repulsion prevents the formation of highly charged cations. Similar behaviour was observed for the tetramethylated azamacrocycle **L2**[12] (Table 1).

It is noteworthy that the presence of two or four tertiary amino groups produces the same basicity in the two first protonation steps of **L** and **L2**. The first protonation constants of these hexaaza ligands ($\log K_1$ = 9.78 and 9.75) are significantly higher than that of the trimethylated heptaaza-macrocycle **L1** ($\log K_1$ = 9.27), being equal to that of the

Table 2 Thermodynamic parameters for the protonation of **L** and **L1** in 0.15 mol dm^{-3} $NaClO_4$ at 298.1 K

	$-\Delta H°$/kcal mol^{-1}		$T\Delta S°$/kcal mol^{-1}	
Reaction	L	L1	L	L1
$L + H^+ = LH^+$	8.5(1)[a]	7.79(5)	4.8(1)	4.9(1)
$LH^+ + H^+ = LH_2^{2+}$	10.4(1)	9.91(5)	2.0(1)	2.3(1)
$LH_2^{2+} + H^+ = LH_3^{3+}$	9.9(1)	9.84(6)	0.7(1)	1.0(1)
$LH_3^{3+} + H^+ = LH_4^{4+}$	12.3(2)	10.27(7)	−7.1(2)	−2.9(1)
$LH_4^{4+} + H^+ = LH_5^{5+}$		7.9(1)		−3.9(1)
$LH_5^{5+} + H^+ = LH_6^{6+}$		10.1(1)		−7.5(1)

[a] Values in parentheses are standard deviation in the last significant figure.

unmethylated **L4** (log K_1 = 9.76). As can be seen from Table 2, which reports the thermodynamic parameters determined by microcalorimetry for the protonation of **L** and **L1**, the high basicity of **L**, with respect to **L1**, in the first step of protonation is due to a greater enthalpic contribution, the entropic one being equal within experimental error for both ligands.

The enthalpy changes determined for each protonation step of **L** and **L1** are similar to the values generally observed for polyamino ligands,[13,14] varying within 7.79 and 12.3 kcal mol^{-1},* and do not exhibit any particular trend. However, the entropic contribution to the stepwise protonation decreases progressively, becoming unfavourable from the fourth step (Table 2). The initial favourable entropic contributions are determined by the large number of water molecules released by the bound H^+ ions; however, as the positive charge on the ligand increases, a larger and larger number of solvent molecules are gathered within the solvation sphere of the polycation, being subjected to stronger and stronger attractive forces (electroconstriction), thus determining an important loss in translational entropy. It is noteworthy that the fourth protonation steps of both **L** and **L1** present the highest enthalpy changes; in the case of **L1** the enthalpic contribution is particularly favourable and corresponds to a very unfavourable entropic term. These thermodynamic data strongly suggest the structural characteristics of H_4L^{4+} observed in the crystal structure of $H_4L(ClO_4)_4$ are maintained in solution. In fact, as observed in the crystal structure, such a conformation of the ligand allows the formation of considerable intramolecular hydrogen bonds, stabilizing the protonated species. In addition the high charge densities of H_4L^{4+} in the *endo* conformation produce a strong solvent electroconstriction, leading to a more exothermic, but entropically unfavourable, protonation reaction.

In order to obtain structural information on the protonated species formed by **L** and **L1** we have recorded the ^1H and ^{13}C NMR spectra over a wide pH range. ^1H–^1H and ^1H–^{13}C 2D correlation experiments have been performed to assign all the signals.

The ^1H spectrum of **L** free amine, at high pH values, exhibits [Fig. 3(*a*)] two singlets at δ 2.05 and 2.60 corresponding to H4 and H3 hydrogen atoms of C4 and C3 (H_i refers to hydrogen atoms linked to C_i carbon atom),† respectively, and an A_2B_2 spin system (δ 2.38 and 2.58) for the hydrogens of the ethylenic chain C1–C2, indicating a C_s symmetry mediated on the NMR time-scale. In accord with the above symmetry the ^{13}C spectrum presents [Fig. 3(*b*)] only four signals at δ 56.35 (C1), 46.50 (C2), 48.50 (C3) and 42.52 (C4). On lowering the pH, as the protonated HL^+ and H_2L^{2+} species are formed (pH = 8–10,

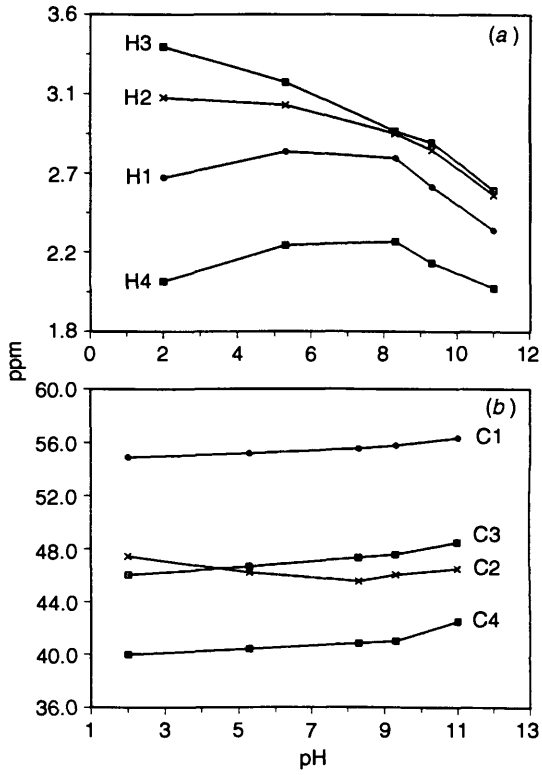


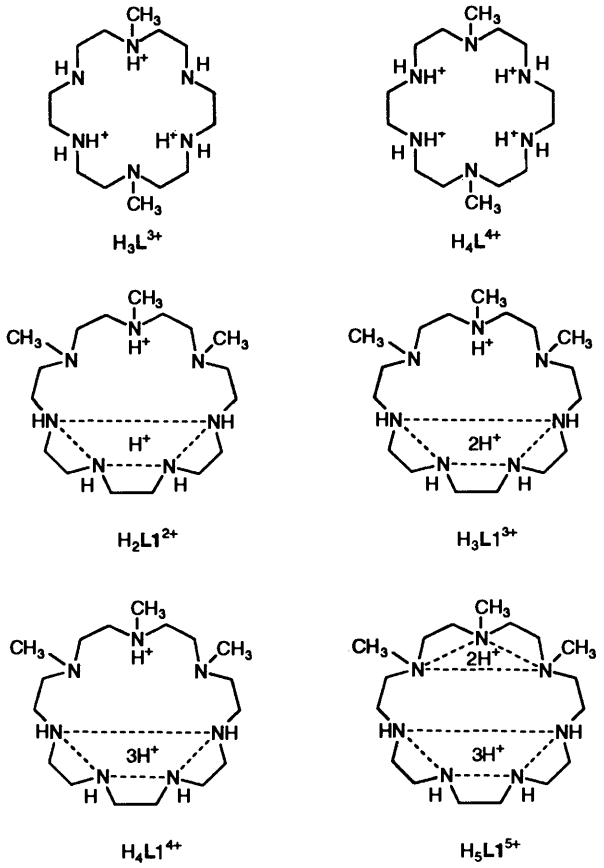
Fig. 3 Experimental ^1H (*a*) and ^{13}C (*b*) NMR chemical shifts of **L** as a function of pH in D_2O

Fig. 2), all the ^1H resonances undergo a downfield shift while those for ^{13}C move upfield revealing that the first two protonation steps involve all the nitrogen atoms of the molecule. As expected on the basis of a simple electrostatic model, and reflected by the protonation constants, the three protons of H_3L^{3+} are located on one tertiary and two secondary nitrogens of **L** in alternate positions (Scheme 1). Indeed, in the pH range 5–8 the ^1H signals for the hydrogen atoms of both C2 and C3, located in the α position with respect to the secondary nitrogens, experience a considerable downfield shift, while all the other proton signals remain unchanged. In this third protonation step only the resonances for the carbon atoms in the β position with respect to the secondary amino group (C1, C3) shifts downfield (β effect [15]). At lower pH values, where the tetraprotonated species is formed, the ^1H signals for the hydrogens H2 and H3 continue shifting downfield while those of H1 and H4 experience a considerable displacement in the opposite direction indicating deprotonation of the tertiary amino groups. This is confirmed by the ^{13}C spectrum of H_4L^{4+} in which the resonance of the carbon atom (C2) in the β position with respect to the tertiary nitrogen is seen at lower field than for H_3L^{3+}. From these NMR results we deduce that the four H^+ ions of H_4L^{4+} are localized on the four secondary nitrogen atoms (Scheme 1). This localization is maintained in the solid state as demonstrated by the crystal structure of $H_4L(ClO_4)_4$. In more acidic solutions the tertiary amino groups undergo protonation according to the observed changes in the chemical shifts.

As far as the protonation pattern of the heptaaza ligand **L1** is considered we can firstly note that at pH 11, where the free amine exists, the ^1H spectrum consists of a single resonance at δ 2.16 for the hydrogen atoms of all the methyl groups (C8, C9), of two other singlets at δ 2.50 and 2.65 corresponding to the hydrogens of C6, C7 and of C1, C2, C3, respectively, and of a A_2B_2 spin system, with ν_A = 2.48 ppm and ν_B = 2.62 ppm, for the protons of C5 and C4. At this pH the ^{13}C spectrum (Fig. 4) reveals nine signals indicating a C_s symmetry mediated on

* 1 cal = 4.184 J.

† The numbering of the carbon atoms in the structures given does not correspond to systematic numbering.



Scheme 1

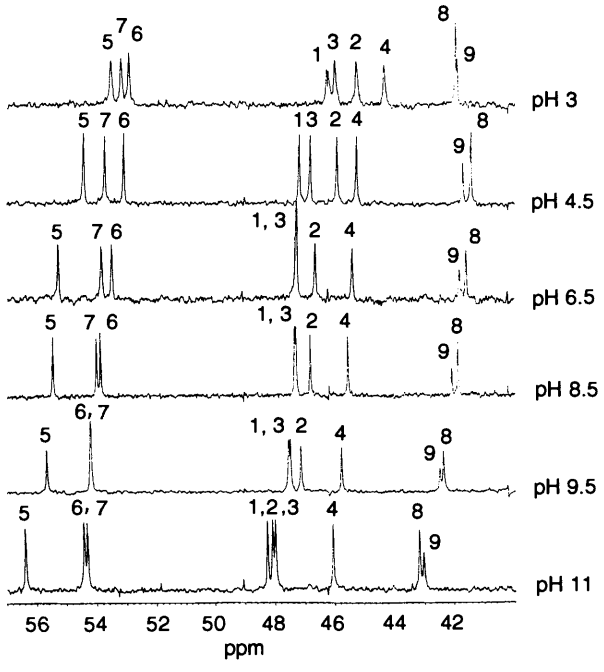


Fig. 4 ^{13}C NMR spectra of **L1** at different pH in D_2O

the NMR time scale. At lower pH values (pH = 8.5), where H_2L1^{2+} is the main species in solution, we observe the formation of an AA'BB' spin system (ν_A 2.74 ppm, ν_B 2.82 ppm) from the singlets previously found for the hydrogen atoms of C6 and C7 in free **L1**, as well as the down-field displacement (2.40 ppm) of the signals corresponding to the hydrogens of C9 suggesting that one proton is located on N1. However, the other protonation involves the secondary amino groups (Scheme 1) according to the shift of the ^1H resonances for the protons of C2 and C3 (2.92 ppm), and the shift of the ^{13}C signals (Fig. 4) for all the carbon atoms located in the β position with respect to N3 and N4 (C1, C2, C3, C5). As shown by the chemical shifts in the ^1H and ^{13}C spectra, the third protonation (pH = 6.5) also involves all the secondary nitrogens of **L1** (Scheme 1) giving rise to an AA'BB' spin system for the protons of C2 and C3. A down-field displacement of the signal corresponding to the protons of C9 (2.58 ppm) is also observed at this pH value. The only exception is the ethylenic chain containing C1 and C1', whose protons produce a singlet in the ^1H spectrum over all of the pH range investigated; all the other ethylenic chains of the H_3L1^{3+} species give rise to AA'BB' spin systems. In the pH region where H_4L1^{4+} is formed (Fig. 2) all the protons, except those of C8, undergo a slight down-field displacement, while the ^{13}C resonances of C2, C3, C5 and C6 shift upfield (Fig. 4) indicating that one out of the four H^+ ions in H_4L1^{4+} is localized on N1 and that the remaining three are on the secondary nitrogens (Scheme 1). In more acidic solution a fifth H^+ ion binds to N2 as indicated by the downfield shift of the resonances of the hydrogen atoms bound to C8 (2.62 ppm at pH = 3), and the significant displacement of the ^{13}C signals of the carbon atoms (C4, C7) in the β position with respect to N2 (Scheme 1).

From both NMR and thermodynamic results we deduce that the protonation patterns of **L** and **L1** are mainly determined by the higher basicity of the secondary amino groups in solution than the tertiary ones, as well as by the repulsion between positive charges which produces the localization of the H^+ ions as far as possible from each other.

ATP Complexation.—Species selection (speciation) and equilibrium constant determination in L/ATP (L = **L**, **L1**) systems have been determined from potentiometric data by using the computer program SUPERQUAD.[11] Table 3 reports the constants determined for **L** and **L1**, together with those previously obtained for the analogous hexaaza- **L2**, **L3** and heptaaza-**L4** macrocycles.[3,4] From these results we can observe that all the anion complexes formed present 1 : 1 stoichiometry. This seems to be the preferred binding mode for highly charged anions,[16] in particular this is true for bulky nucleotides, presenting many sites for hydrogen bonding anchorage to the macrocycles. Nevertheless, electrostatic attraction is the main force in determining the nucleotide–receptor interaction. Indeed for each ligand the stability of the ATP complexes increases with the degree of protonation and for a given charge on the macrocycles the hexaaza ligands (**L**, **L2**, **L3**) form more stable complexes than larger ligands with seven amino groups (**L1**, **L4**).

As far as the effect of nitrogen methylation on the receptors on ATP binding is concerned, we observe that the equilibrium constants for the overall reactions $L + nH^+ + ATP^{4-} = \{H_n[(ATP)L]\}^{(n-4)+}$ decrease, for a given n degree of protonation, with increasing number of tertiary nitrogens (Table 3). This behaviour is principally due to the greater basicity of unmethylated ligands. In fact, eliminating the basicity contribution by considering the complexation reactions in the form $H_nL^{n+} + ATP^{4-} = \{H_n[(ATP)L]\}^{(n-4)+}$ we note that (Table 3) the ability of the protonated species of the heptaaza ligands to coordinate to ATP is almost unaffected by methylation, while a considerable increase in the binding constants is brought about by methylation of **L3**. Nevertheless, a method of comparing the ability of such receptors in ATP binding could be misleading since one or more protons might be bound to the nucleotide. Very recently it has been suggested[4,17] that an appropriate way to overcome all these problems is to consider a ternary system containing ATP and a couple of ligands in equimolar concentrations and calculate the overall percentages

Table 3 Logarithms of the equilibrium constants of the ATP (A) complexes with L, L1, L2, L3 and L4 in 0.15 mol dm^{-3} $NaClO_4$ at 298.1 K

Reaction	log *K*				
	L[a]	L1[a]	L2[b]	L3[c]	L4[c]
$L + 2H^+ + A^{4-} = H_2LA^{2-}$	21.87(5)[d]				
$L + 3H^+ + A^{4-} = H_3LA^-$	30.45(3)		29.66	30.99	30.26
$L + 4H^+ + A^{4-} = H_4LA$	37.86(3)	36.30(1)	36.41	38.70	38.63
$L + 5H^+ + A^{4-} = H_5LA^+$	42.71(3)	42.10(1)	40.93	43.92	45.10
$L + 6H^+ + A^{4-} = H_6LA^{2+}$	46.23(3)	46.38(2)	44.18		50.48
$L + 7H^+ + A^{4-} = H_7LA^{3+}$		50.47(1)			54.85
$L + 8H^+ + A^{4-} = H_8LA^{4+}$					58.53
$H_2L^{2+} + A^{4-} = H_2LA^{2-}$	3.00(6)				
$H_3L^{3+} + A^{4-} = H_3LA^-$	3.82(4)		3.30	2.47	2.59
$H_4L^{4+} + A^{4-} = H_4LA$	7.39(4)	4.69(2)	7.48	5.91	4.54
$H_5L^{5+} + A^{4-} = H_5LA^+$		7.54(2)		8.92	7.28
$H_6L^{6+} + A^{4-} = H_6LA^{2+}$		10.04(3)			10.52
$H_7L^{7+} + A^{4-} = H_7LA^{3+}$					12.93

[a] This work. [b] From ref. 4. [c] From ref. 3. [d] Values in parentheses are standard deviation in the last significant figure.

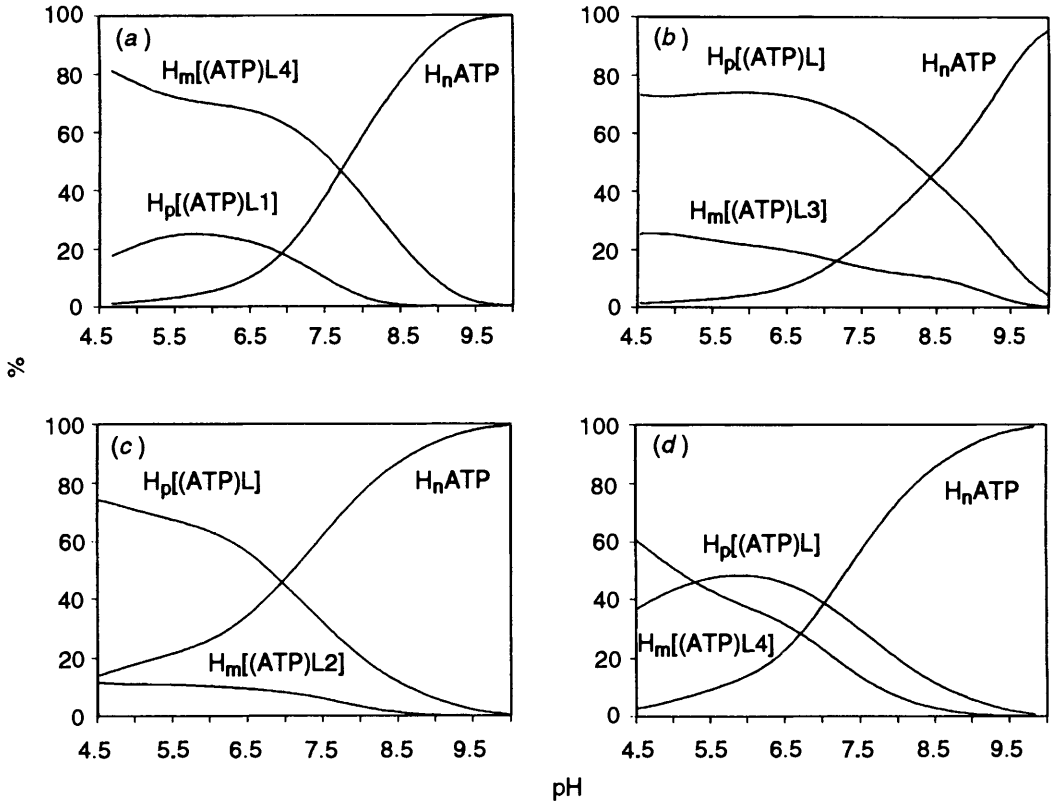


Fig. 5 Overall percentages of ATP complexed species formed as a function of pH in competing systems containing L1–L4 (*a*), L–L3 (*b*), L–L2 (*c*) and L–L4 (*d*). Percentages are calculated with respect to ATP.

of ATP bound to each one of the two receptors over a wide pH range. Plots of the percentages *versus* pH produce species distribution diagrams from which the binding ability of both receptors can be interpreted in terms of selectivity.[4,17] In Fig. 5(*a*), for example, we show a similar diagram calculated for the ATP–L1–L4 system. As can be seen the formation of ATP adducts with L4 prevails over all of the useful pH range. Note that the mere comparison of the equilibrium constants for the reaction $H_nL^{n+} + ATP^{4-} = \{H_n[(ATP)L]\}^{(n-4)+}$ does not reveal this substantial difference. Similar plots [Fig. 5(*b*), (*c*)] evidence a greater ability of L, in ATP binding, than L3 and L2. It is also of interest that in the ATP–L–L4 system [Fig. 5(*d*)] the ATP adducts of L prevail for pH values greater than 5.3 while the reverse trend is observed in more acidic media, producing an isoselectivity point in the diagram [Fig. 5(*d*)].

The present analysis of equilibrium data reveals that, among the ligands considered here, L is the most appropriate receptor for ATP recognition in solution over a wide pH range. Nevertheless, as we have already observed,[4] a marked tendency presented by a receptor for ATP binding is not indicative of a particular effectiveness of the receptor itself in promoting ATP dephosphorylation. Further study is being undertaken to elucidate this aspect of L and L1 catalytic properties.

Experimental

Synthesis of 1,10-*Dimethyl*-1,4,7,10,13,16-*hexaazacyclooctadecane* L *and* 1,4,7-*Trimethyl*-1,4,7,10,13,16,19-*heptaazacyclohenicosane* L1.—1,4-Bis(*p*-tolysulfonyl)-1,4-diazabutane **1**, bis(2-chloroethyl)methylamine **2**, 1,14-bis(methylsulfonyloxy)-3,6,9,12-tetrakis(*p*-tolysulfonyl)-3,6,9,12-tetraazatetradecane **3** and 1,4,7-trimethyl-1,4,7-triazaheptane **4** have been synthesized

following the procedures reported in refs. 18–21, respectively.

1,10-*Dimethyl*-4,7,13,16-*tetrakis*(p-*tolysulfonyl*)-1,4,7,10,13-16-*hexaazacyclooctadecane* **5**.—All reactions were carried out in a nitrogen atmosphere. A solution of sodium (0.12 mol) in dry ethanol (150 cm^3) was added to a hot solution of compound **1** (0.054 mol) in dry ethanol (150 cm^3). The resulting suspension was refluxed for about 30 min and then the solvent evaporated under reduced pressure to give the disodium salt of **1**. This was dissolved in dry DMF (200 cm^3) and to the resulting solution, heated at 110 °C, was added compound **2** (0.054 mol) in 200 cm^3 of dry DMF with stirring over a period of about 2 h. The solution was maintained at 110 °C for a further hour, concentrated to 150 cm^3 and poured into a 1 dm^3 water–ice mixture with mechanical stirring. The crude compound **5** was filtered off, washed several times with hot ethanol and dried *in vacuo* to give pure compound **5** (9.2 g, 38%), m.p. 280 °C (decomp.) (Found: C, 55.7; H, 6.4; N, 9.3. Calc. for $C_{42}H_{58}N_6O_8S_4$: C, 55.85; H, 6.47; N, 9.30%).

1,10-*Dimethyl*-1,4,7,10,13,16-*hexaazacyclooctadecane* **L**.—Compound **5** (9.2 g, 0.010 mol) was dissolved in 20 cm^3 of 96% H_2SO_4 and the resulting solution kept at 100 °C for 72 h. The solution was cooled and added dropwise to about 500 cm^3 of diethyl ether with stirring to give a solid compound which was filtered, washed with diethyl ether and dried *in vacuo*. The product was converted into the free amine **L** by means of an ionic exchange resin (Dowex 1 × 8) and purified as its hexahydrochloride salt (4.2 g, 83%) (Found: C, 33.1; H, 7.9; N, 16.6. Calc. for $C_{14}H_{40}Cl_6N_6$: C, 33.28; H, 7.98; N, 16.63%).

1,4,7-*Trimethyl*-10,13,16,19-*tetrakis*(p-*tolylsulfonyl*)-1,4,7,-10,13,16,19-*heptaazacyclohenicosane* **6**.—All reactions were carried out in a nitrogen atmosphere. Compound **3** (0.042 mol) was dissolved in 900 cm^3 of dry acetonitrile containing K_2CO_3 (58 g). The suspension was heated at reflux and compound **4** (0.042 mol) in 50 cm^3 of dry acetonitrile was added. Reflux was maintained over about 24 h; the resulting suspension was cooled, filtered, and extracted with chloroform. The crude product **6**, obtained after evaporation of the solvent, was chromatographed over a neutral alumina column (200 g, 4 cm diameter) with a 100:2 chloroform–ethyl acetate mixture. The eluted solution was evaporated to dryness and the residue recrystallized from hot toluene (9.5 g, 24%), m.p. 243–245 °C (Found: C, 55.6; H, 6.9; N, 10.0. Calc. for $C_{45}H_{65}N_7O_8S_4$: C, 56.28; H, 6.82; N, 10.2%).

1,4,7-*Trimethyl*-1,4,7,10,13,16,19-*heptaazacyclohenicosane* **L1**.—Compound **6** (9.0 g, 0.0094 mol) was dissolved in 18 cm^3 of 96% H_2SO_4 and the resulting solution kept at 100 °C for 60 h. The solution was cooled and added dropwise to about 150 cm^3 of ethanol under stirring. Diethyl ether (250 cm^3) was added to the mixture and a white solid separated. This compound was filtered, washed with diethyl ether and dried *in vacuo*. The product was converted into the free amine **L1** by means of an ionic exchange resin (Dowex 1 × 8) and purified as its tetrahydroperchlorate salt (5.6 g, 80%) (Found: C, 27.6; H, 6.1; N, 13.2. Calc. for $C_{17}H_{45}Cl_4N_7O_{16}$: C, 27.40; H, 6.08; N, 13.15%).

Potentiometric Measurements.—The pH-metric measurements (pH = $-\log[H^+]$) were carried out in 0.15 mol dm^{-3} $NaClO_4$ at 298.1 ± 0.1 K, using the equipment and methodology already described.[22] At least three measurements (about 100 data points each) were performed for each system in the pH range 2.5–10.5 for ligand protonation and 3.5–10.5 in the presence of ATP due to the high hydrolytic rate of the nucleotide at lower pH values. In all experiments the ligand concentration was about 2×10^{-3}; while for ATP complexation the concentration of nucleotide was varied in the range $[L] \leqslant [ATP] \leqslant 2[L]$. The computer program SUPERQUAD[11] was used to calculate equilibrium constants from EMF data. The protonation constants of ATP employed in the calculations were previously determined.[23]

Microcalorimetric Measurements.—The enthalpies of protonation of **L** and **L1** were determined in 0.15 mol dm^{-3} $NaClO_4$ by means of a Thermometric AB thermal activity monitor (model 2277) microcalorimeter equipped as previously reported.[24] Typically 1.5 cm^3 of a 5×10^{-3} mol dm^{-3} acidic ligand solution in 0.15 mol dm^{-3} $NaClO_4$ were charged into the calorimetric ampoule. After thermal equilibration, 0.015 cm^3 additions of 0.15 mol dm^{-3} NaOH standard solution were delivered. Under the reaction conditions and employing the determined protonation constants, the concentrations of the species present in solution before and after addition were calculated and the corresponding enthalpies of reaction were determined from the calorimetric data by means of the KK88 program.[25] At least three titrations (about 30 points each) were performed for each system. The titration curves for each system were treated either as a single set or as separated entities without significant variation in the values of the enthalpy changes.

Crystal Data.—$C_{14}H_{38}Cl_4N_6O_{16}$, M = 688.30. Monoclinic, a = 9.257(4), b = 8.600(2), c = 17.990(10), β = 101.74(4)°, V = 1402(1) Å^3 (by least-squares refinement of diffractometer setting angles of 25 carefully centred reflections, λ = 0.710 69 Å), space group $P2_1/c$, Z = 2, D_c = 1.63 g cm^{-3}. Prismatic colourless crystals. Crystal dimensions 0.4 × 0.4 × 0.6 mm, μ-(Mo-Kα) = 0.500 mm^{-1}.

Data Collection and Processing.[26]—Enraf–Nonius CAD4 X-ray diffractometer, θ–2θ scan mode with θ scan width = 0.6 + 0.35 tan θ, θ speed 4.1 deg min^{-1}, graphite monochromatized Mo-Kα radiation; 2471 reflections measured ($2.64 < \theta < 25°$, $\pm h, k, l$), 2377 unique with $I < 2.0\,\sigma(I)$. Two standard reflections monitored: no loss of intensity observed. Lorentz and polarization effects correction applied.

Structure Analysis and Refinement.*—Direct method. Absorption correction applied after structure resolution. Full-matrix least-squares refinement with all the non-hydrogen atoms anisotropic, methyl and methylene hydrogens in calculated positions with overall thermal parameters U = 0.061 Å^2. All the ammonium hydrogen atoms localized by ΔF map and isotropically refined. Function minimized: $\Sigma w(|F_o|^2 - |F_c|^2)^2$, with the weighting scheme calculated in agreement with the resolution program (weighting factors 0.1399 and 2.6834). 208 Refined parameters. Final R and wR^2 values are 0.077 and 0.21, respectively. Programs used and source of atomic scattering factors and anomalous dispersion corrections from ref. 26. Details of positional and thermal parameters, atomic coordinates, bond lengths and angles have been deposited at the Cambridge Crystallographic Data Centre.†

NMR Spectroscopy.—^{1}H and ^{13}C NMR spectra were recorded at 299.95 MHz and 75.43 MHz, respectively, on a Varian Unity 300-MHz instrument in D_2O solutions with dioxane as reference standard (δ 67.4). The solution pH was calculated from the measured pD values by using the relationship pH = pD − 0.40.[27]

* Computer used: DEX-486 DX.

† For details of the deposition scheme, see 'Instruction for Authors (1994)', *J. Chem. Soc., Perkin Trans. 2*, 1994, issue 1.

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