Effect of Nitrogen Methylation on Cation and Anion Coordination by Hexa- and Heptaazamacrocycles. Catalytic Properties of These Ligands in ATP Dephosphorylation

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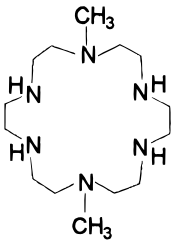
The stability constants of the complexes formed by 1,10-dimethyl-1,4,7,10,13,16-hexaazacyclooctadecane (**L**) and 1,4,7-trimethyl-1,4,7,10,13,16,19-heptaazacyclohenicosane (**L1**) with Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, and Pb^{2+}, as well as that for the formation of $Pb\mathbf{L2}^{2+}$ (**L2** = 1,4,7,13-tetramethyl-1,4,7,10,13,16-hexaazacyclooctadecane), were determined by means of potentiometric (pH-metric) titrations in 0.15 mol dm^{-3} $NaClO_4$ at 298.1 ± 0.1 K. The enthalpy changes for the formation of Cu^{2+} complexes with **L** and **L1** were measured by means of microcalorimetry. These thermodynamic data were compared with those previously reported for **L2**, 1,4,7,10,-13,16-hexaazacyclooctadecane (**L3**), and 1,4,7,10,13,16,19-heptaazacyclohenicosane (**L4**) evidencing that nitrogen methylation can produce lower or higher complex stability depending on the metal ion and the number of methylated nitrogens. The equilibria of complexation of ATP^{4-}, ADP^{3-}, AMP^{2-}, $P_2O_7^{4-}$, and $[Co(CN)_6]^{3-}$ by **L** and **L1** were studied by means of pH-metric titrations in 0.15 mol dm^{-3} $NaClO_4$ at 298.1 ± 0.1 K. The catalytic reactions of ATP dephosphorylation induced by these ligands in solution were followed by ^{31}P NMR spectroscopy at different temperature and pH values. **L** is the most appropriate receptor, among **L**−**L4**, in the recognition of the nucleotide. The catalytic efficiency of hexa- and heptaazaligands increases in the order **L** < **L3** < **L2** and **L1** < **L4**, respectively, **L4** being the most efficient. Namely, di- and tetramethylation of **L3** produces opposite effects on its catalytic properties.

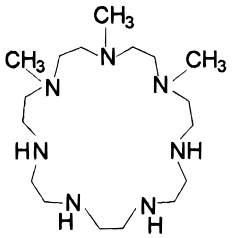
Introduction

In a preceding paper[1] we described the synthesis of the two new polyazamacrocycles 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**) and their basicity properties in aqueous solution, focusing our attention on the ability presented by the polyprotonated forms of both ligands in the recognition and binding of the anionic forms of ATP.

There is intense current interest in the chemistry of polyamino macrocyclic ligands because of their special ligational properties which give rise to both cation and anion complexation.[2] Furthermore, these ligands induce acceleration of the dephosphorylation reaction of some nucleotides (ATP, ADP) in solution and, therefore, have been used as models in ATPase and kinase mimicry.[3−5] Moreover, the presence in such molecules of large numbers of amino groups allows the modulation of their coordinative characteristics through nitrogen functionalization.



L



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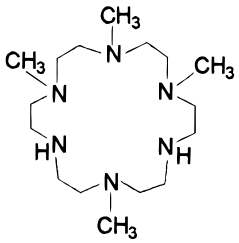

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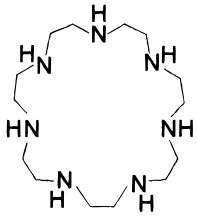

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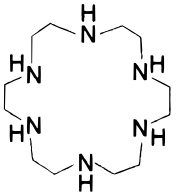

L3

[⊗] Abstract published in *Advance ACS Abstracts,* February 1, 1996.

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We have recently shown that nitrogen methylation produces significant changes in cation and anion binding features of polyazamacrocycles.[1,5,6] In particular we found that 3d transition metal complexes of the tetramethylated hexaazamacrocycle 1,4,7,13-tetramethyl-1,4,7,10,13,16-hexaazacyclooctadecane (**L2**) are less stable than those of the analogous unmethylated 1,4,7,-10,13,16-hexaazacyclooctadecane (**L3**) ligand.[6] On the other hand, considering their catalytic properties, **L2** is more effective than **L3** in enhancing the rate of ATP dephosphorylation in acidic media.[5] Moreover we observed that the dimethylated receptor **L** is more efficient than **L1**−**L4** (**L4** = 1,4,7,10,13,-16,19-heptaazacyclohenicosane) in ATP recognition in solution over a wide pH range.[1]

Aiming at further knowledge on the 2-fold nature of the coordination chemistry of polyazamacrocycles, we present here the results of a thermodynamic study on the binding of metal cations and anions, as well as the results on the kinetics of ATP-catalyzed cleavage, by **L** and **L1**.

In order to produce an homogeneous set of equilibrium data for metal ion binding, we have also determined the formation constant of Pb**L2**$^{2+}$.

Experimental Section

Materials. **L**, **L1**, **L2**, and **L4** were synthesized according to reported procedures.[1,7] **L3** was purchased from Aldrich and used as its hexahydrochloride salt. The sodium salts of ATP, ADP, and AMP were purchased from Boehringer-Mannheim. All other chemicals were Merck reagent grade and were used without further purification.

Potentiometric Measurements. All the pH-metric measurements (pH = −log [H$^+$]) were carried out in 0.15 mol dm^{-3} $NaClO_4$ at 298.1 ± 0.1 K, by using the equipment and the methodology that has been already described.[8] The reference electrode was an Ag/AgCl electrode in saturated KCl solution. The glass electrode was calibrated as a hydrogen concentration probe by titrating known amounts of HCl with CO_2-free NaOH solutions and determining the equivalent point by Gran's method[9] which allows to determine the standard potential $E°$ and the ionic product of water (pK_w = 13.73(1) at 298.15 K in 0.15 mol dm^{-3} $NaClO_4$). At least three measurements (about 100 data points each) were performed for each system in the pH ranges 2.5−10.5. In all experiments the ligand concentration [L] was about 1×10^{-3} mol dm^{-3}; metal ion concentration [M] was in the range 5×10^{-4} mol dm^{-3} ≤ [M] ≤ [L] for **L** and 5×10^{-4} mol dm^{-3} ≤ [M] ≤ 2[L] for **L1**; for anion complexation, the concentration of the anionic species [A] was varied in the range [L] ≤ [A] ≤ 2[L]. The computer program SUPERQUAD[10] was used to calculate equilibrium constants from emf data.

Due to the long time required for the equilibrium reactions between **L** and Ni^{2+}, to be reached, a batchwise potentiometric procedure was employed to determine the stability constants of the complexes formed. Twenty solutions containing different amounts of Ni^{2+}, **L**, and OH^-, or H^+, in 0.15 mol dm^{-3} $NaClO_4$ aqueous media were prepared in separate bottles and maintained at 298.1 ± 0.1 K. The value of −log-[H$^+$] for each solution was measured periodically until a constant value was reached. The computer program SUPERQUAD[10] was used to calculate also the equilibrium constants from these batchwise measurements.

The criterion for fitting e.m.f. data and the critical evaluation of the least-squares results supplied by SUPERQUAD[10] program was as reported in ref 11, footnote 18. The protonation constants of **L**, **L1**, ATP, ADP, AMP, and $P_2O_7^{4-}$ employed in the calculations were previously determined.[1,4]

Microcalorimetric Measurements. The enthalpies of protonation of **L** and **L1** have been 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.[12] Typically 1.5 cm^3 of 5 × 10^{-3} mol dm^{-3} ligand acidic solution in 0.15 mol dm^{-3} $NaClO_4$ were charged into the calorimetric ampule. After thermal equilibration, 0.015 cm^3 additions of 0.15 mol dm^{-3} NaOH standard solution were delivered. Under the reaction conditions and with the determined equilibrium constants and protonation enthalpy changes, 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 KK95 program.[13] Ligand protonation enthalpies used in calculation were previously determined. At least three titration (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. Due to the surprisingly high value found for the enthalpic contribution to the formation of Cu**L**$^{2+}$, the system Cu^{2+}/**L** was subjected to extensive control using products coming from different synthetic batches. All measurements were consistent.

NMR Measurements. The ^{31}P NMR spectra were recorded at 121.42 MHz on a Varian Unity 300-MHz instrument. 85% H_3PO_4 was used as an external reference. pH was adjusted by additions of HCl or NaOH solutions. Kinetic studies were performed by following the time-dependent change in the integrals of the resolved ^{31}P NMR signals of P_α, P_β, and P_γ of ATP and peaks for inorganic phosphate and ADP. The kinetic parameters for the conversion of ATP into ADP were calculated by following the disappearance with time of the P_β NMR signal in the samples with molar ratio ATP/macrocycle ≤1 or the signal corresponding to the formation of inorganic phosphate in samples with an excess of ATP. The initial concentrations of ATP and macrocycles varied in the range 10^{-2} to 2×10^{-2} mol dm^{-3}. An automatic array of spectra was used. Plots of log[ATP] vs time were linear for several half-lives. Calibration curves were employed when the integral ratios were not equal because of variations in the ^{31}P relaxation times. Kinetic thermodynamic parameters were calculated by means of the equation $E_a = \Delta H^* + RT = -RT \ln(kh/Tk_b)$ and $\Delta G^* = \Delta H^* - T\Delta S^*$, k_b being the Boltzman constant, h the Plank constant, and k the rate constant in s^{-1}.

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Table 1. Logarithms of the Equilibrium Constants for the Formation of **L**, **L1**, and **L2** Metal Complexes Determined in 0.15 mol dm^{-3} $NaClO_4$ at 298.1 ± 0.1 K

reaction[a]	Ni^{2+}	Cu^{2+}	Zn^{2+}	Cd^{2+}	Pb^{2+}
L + M = M**L**	21.42(4)[b]	22.49(1)	15.67(2)	16.91(2)	14.47(1)
L + M + H = MH**L**	24.75(7)	26.13(1)	20.28(4)	21.44(2)	
H**L** + M = MH**L**	14.97(4)	16.35(1)	10.43(4)	11.66(2)	
M**L** + H = MH**L**	3.33(7)	3.64(1)	5.01(5)	4.53(3)	
L1 + M = M**L1**	11.83(2)	18.32(4)	10.69(1)	15.12(1)	10.47(3)
L1 + M + H = MH**L1**	19.33(1)	26.09(3)	17.71(1)		17.21(2)
L1 + M + 2H = MH_2**L1**	24.63(2)	30.80(2)			
L1 + M + H_2O = M**L1**OH + H	0.37(4)			3.65(4)	
L1 + 2M = M_2**L1**		25.76(3)			
L1 + 2M + H_2O = M_2**L1**OH + H		20.36(4)			
M**L1** + H = MH**L1**	7.50(3)	7.77(5)	7.02(2)		6.74(3)
MH**L1** + H = MH_2**L1**	5.30(3)	4.71(4)			
M**L1** + OH = M**L1**OH	2.27(5)			2.26(5)	
M**L1** + M = M_2**L1**		7.44(6)			
M_2**L1** + OH = M_2**L1**OH		8.33(5)			
L2 + M = M**L2**					13.37(3)

[a] Charges have been omitted. [b] Values in parentheses are standard deviations on the last significant figure.

Spectrophotometric Measurements. The electronic spectra were recorded on a Perkin-Elmer Lambda 9 spectrophotometer equipped with 1-cm cell thermostated at 298.1 ± 0.1 K.

Results and Discussion

Metal Cations Complexation. Speciation of the complexes formed by **L** and **L1** with Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, and Pb^{2+} and of **L2** with Pb^{2+}, in 0.15 mol dm^{-3} $NaClO_4$ at 298.1 ± 0.1 K, was performed by computer analysis of the pH-metric data using the SUPERQUAD[10] program. The calculated equilibrium constants are listed in Table 1.

The hexaaza ligand **L** forms stable ML^{2+} complexes with all the metal ions here considered. These complexes are the predominant species in a wide pH range, while in acidic solutions, with the exception of PbL^{2+} and Pb$L2^{2+}$, they undergo protonation producing the monoprotonated MHL^{3+} species.

In a previous paper[6] we reported that tetramethylation of **L3** to give **L2** causes a general lowering in complex stability, more marked for Ni^{2+}, Cu^{2+}, and Zn^{2+} than for Cd^{2+} and Pb^{2+}. Otherwise, we observe that the insertion of only two methyl groups produces**,** depending on the metal cations, different modifications of the ligational ability of **L3**. In fact, while **L** complexes of Cu^{2+}, Zn^{2+}, and Cd^{2+} are less stable than those formed by **L3**, NiL^{2+}, and PbL^{2+} are a little more stable than Ni$L3^{2+}$ (log $K_{NiL3^{2+}}$ = 21.1)[6] and Pb$L3^{2+}$ (log $K_{PbL3^{2+}}$ = 14.13),[14] respectively. Nevertheless, all complexes of **L** are more stable than those with **L2** (Figure 1).

It has been reported that nitrogen methylation in polyazamacrocycles produces some enlargement of the macrocyclic cavity.[15] On the other hand, Hancock and co-workers[16] have shown that from the several conformations a determined polyazamacrocycle can adopt, a metal ion binds to a particular one depending, among other factors, on metal ion size. As a consequence, these authors have shown that often larger metal ions coordinate more strongly to small macrocycles than to large ones. These effects could be involved in the particular stability trends presented by Cd^{2+} and Pb^{2+} (Figure 1). For instance, **L3** complexes with Zn^{2+} and Cd^{2+} have equal stability (log $K_{ZnL3^{2+}}$ = 18.70, log $K_{CdL3^{2+}}$ = 18.80),[17,18] but the larger Cd^{2+} ion is less affected

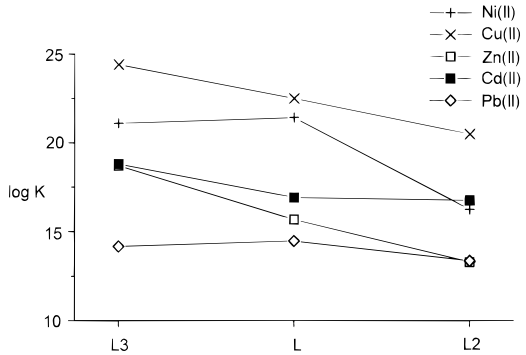

Figure 1. Logarithms of the equilibrium constants for Cu^{2+} (×), Ni^{2+} (+), Cd^{2+} (■), Zn^{2+} (□) and Pb^{2+} (◇) complexation with **L**, **L2**, and **L3**, showing the effect of increasing nitrogen methylation.

than Zn^{2+} by nitrogen methylation (log $K_{ZnL2^{2+}}$ = 13.29, log $K_{CdL2^{2+}}$ = 16.75;[6] log $K_{ZnL^{2+}}$ = 15.67, log $K_{CdL^{2+}}$ = 16.91 (Table 1)). A similar insensivity to nitrogen methylation is observed for Pb^{2+} (Figure 1).

In the case of Ni^{2+} the influence of ligand stiffening seems to be more evident. The full octahedral coordination of the ligand to Ni^{2+} in Ni$L3^{2+}$ has been extensively documented;[19−21] thus, the similarity of the stability constants of NiL^{2+} and Ni$L3^{2+}$ suggests an analogous coordination behavior for the dimethylated ligand **L**. Accordingly, NiL^{2+} and Ni$L3^{2+}$ present, in aqueous solution, three similar bands in the electronic spectra (NiL^{2+}, λ_{max} 880 (19.6), 550 (8.7), 355 (12.8) nm (ϵ dm^3 mol^{-1} cm^{-1}); N1$L3^{2+}$,[20] λ_{max} 835 (19), 530 (11), 345 (11) nm (ϵ dm^3 mol^{-1} cm^{-1})) typical for high-spin octahedral Ni^{2+} chromophores. It is worth noting that the molar absorbancy for the near-infrared band of the Ni^{2+} complexes is considerably higher than the other ones, which is considered as diagnostic for cis coordination in octahedral complexes of Ni^{2+}.[22] Such a coordination mode could be facilitated in NiL^{2+} by the binding

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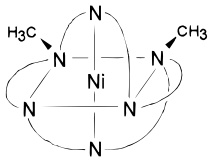

Figure 2. Proposed structure of Ni$\mathbf{L}^{2+}$.

Table 2. Thermodynamic Parameters for the Formation of Cu^{2+} Complexes of **L**−**L4** determined in 0.15 mol dm^{-3} $NaClO_4$ at 298.1 ± 0.1 K

reaction[a]	$-\Delta H°$ (kJ mol^{-1})	$T\Delta S°$ (kJ mol^{-1})
L + Cu = Cu**L**	120.5(8)[b]	8.0(8)
L + Cu + H = CuH**L**	149.0(8)	0.0(8)
Cu**L** + H = CuH**L**	28.5(8)	0.0(4)
L1 + Cu = Cu**L1**	71.1(8)	33(1)
L1 + Cu + H = CuH**L1**	102.1(8)	46.9(8)
L1 + Cu + 2H = CuH_2**L1**	138.5(8)	37(1)
L1 + 2Cu = Cu_2**L1**	88.7(8)	58.2(8)
L1 + 2Cu + H_2O = Cu_2**L1**OH + H	55.2(8)	61.1(8)
Cu**L1** + H = CuH**L1**	31.0(8)	13.4(8)
CuH**L1** + H = CuH_2**L1**	36.4(8)	−9.6(8)
Cu**L1** + Cu = Cu_2**L1**	17.6(8)	25(1)
Cu_2**L1** + OH = Cu_2**L1**OH	23.4(8)	24(1)
Cu + **L2** = Cu**L2**[c]	72.4	44.4
Cu + **L3** = Cu**L3**[d]	100	39
Cu + **L4** = Cu**L4**[d]	82.4	29

[a] Charges have been omitted. [b] Values in parentheses are standard deviations on the last significant figure. [c] From ref 6. [d] From ref 24.

of the two tertiary nitrogen atoms in *cis* positions, hence, the ligand should be arranged in *mer* disposition (Figure 2). It seems reasonable that similar coordination features of **L** and **L3** produce comparable binding properties toward Ni^{2+}. As a matter of fact, a lower ligand field interaction (λ_{max} 1074 (8.3), 587 (5.4), 368 (10.3) nm (ϵ dm^3 mol^{-1} cm^{-1})) was observed for the less stable complex, Ni$\mathbf{L2}^{2+}$,[6] in which important alteration of the ligand characteristics are expected as a consequence of tetramethylation.

It is well known that many Ni^{2+} complexes are present in solution as a mixture of octahedral high-spin and diamagnetic square-planar complexes. This "blue to yellow" equilibrium is particularly evident in Ni^{2+} complexes of tetraazamacrocyles, which produce, depending on their molecular characteristics, different amounts of the yellow diamagnetic species and of *cis*- and/or *trans*-diaquo octahedral blue complexes.[23] Formation of the yellow species is promoted by increasing temperature and ionic strength. Electronic spectra of Ni$\mathbf{L}^{2+}$ obtained from solutions containing up to 7 mol dm^{-3} $NaClO_4$ in the 298−323 K range of temperature did not show substantial changes with respect to the spectra obtained at room temperature in absence of added salt, confirming the tendency of **L** to involve a high number of donor atoms in the coordination to Ni^{2+}.

In the case of Cu^{2+} we measured the enthalpy changes for its complexation reactions with **L**; the relevant thermodynamic parameters are listed in Table 2 together with those previously determined for **L2** and **L3**.[6,24] As can be seen, the decrease in stability observed for Cu$\mathbf{L}^{2+}$ with respect to Cu$\mathbf{L3}^{2+}$ is entirely due to a large reduction of the entropic term, while the enthalpic contribution is more favorable for the formation of Cu$\mathbf{L}^{2+}$ than for the more stable Cu$\mathbf{L3}^{2+}$ (log $K_{Cu\mathbf{L3}^{2+}}$ = 24.40, ref 24). On the contrary, the lower stability of the Cu$\mathbf{L2}^{2+}$ complex is determined by a reduced enthalpy change. It is noteworthy that the value of the enthalpy change for the protonation of the complex Cu$\mathbf{L}^{2+}$ is significantly exothermic ($-\Delta H°$ = 28.5(8) kJ mol^{-1}), in comparison with the protonation enthalpy of the free amine ($-\Delta H°$ = 35.6 kJ mol^{-1} for **L** + H^+ = **L**$H^{+\,1}$), indicating that H^+ binds Cu$\mathbf{L}^{2+}$ through an uncoordinated, or weakly interacting, nitrogen donor. Hence, at most five donors of **L** should be involved in the coordination to Cu^{2+}. Similar coordination modes were deduced for the Cu^{2+} complexes of **L2** and **L3** in solution.[6,24]

The enthalpy change for the formation of Cu$\mathbf{L}^{2+}$ ($-\Delta H°$ = 120.5(8) kJ mol^{-1}) is surprisingly large, in comparison with the corresponding values determined for **L2** and **L3** (Table 2); for this reason its determination was subjected to extensive verification (see Experimental Section). The value well compares with the enthalpic contribution ($-\Delta H°$ = 135(1) kJ mol^{-1})[25] determined for the formation of the Cu^{2+} complex with the tetraazaligand cyclam (cyclam = 1,4,8,11-tetraazacyclotetradecane), which encircles the metal ion in a planar ligand disposition. At first glance, a similar coordination seems to be suitable also for **L**, which could achieve a similar arrangement if the two weaker donors, the methylated nitrogens, were not involved in the binding to the metal ion. However, the electronic spectrum of Cu(cyclam)$^{2+}$ (λ_{max} = 503 nm)[25] is quite different from that of Cu$\mathbf{L}^{2+}$, showing a main band at 651 nm (ϵ 51 dm^3 mol^{-1} cm^{-1}) and an additional very broad absorption at about 1200 nm (ϵ 13 dm^3 mol^{-1} cm^{-1}). This Cu$\mathbf{L}^{2+}$spectrum, which is typical of distorted octahedral coordination environments of Cu^{2+},[26] is similar to the spectra observed for the Cu$\mathbf{L2}^{2+}$ (λ_{max} 642 (90), 946 broad (19) nm (ϵ dm^3 mol^{-1} cm^{-1}))[6] and Cu$\mathbf{L3}^{2+}$ (λ_{max} 658 (85) nm (ϵ dm^3 mol^{-1} cm^{-1}))[24] complexes where the ligands are pentacoordinated. Therefore, as already discussed for Ni$\mathbf{L}^{2+}$, tetracoordination of **L** seems to be highly improbable also in the Cu^{2+} complex.

From these results it is evident that increasing nitrogen methylation of the ligand **L3** does not produce a monotonous alteration of its coordinative properties. The number and the location of methyl groups determine not only the donating properties of the amine groups but also the preorganization of the ligands toward different metal ions. In the case of the dimethylated hexaazaligand **L** the high enthalpic and the low entropic contribution to the formation of the Cu$\mathbf{L}^{2+}$ complex seems to be attributable to a special ligand conformation imposed by the two methyl groups, much more than to methylation itself, since a similar behavior is not observed for the tetramethylated **L2** macrocycle.

Metal ions complexation by the heptaazaligands (**L1**, **L4**) is also affected by nitrogen methylation (Table 1). With the only exception of Pb^{2+} (log $K_{Pb\mathbf{L1}^{2+}}$ = 10.47(3) vs log $K_{Pb\mathbf{L4}^{2+}}$ = 10.02[14]), all the metal ions here considered form weaker mononuclear complexes with the trimethylated macrocycle **L1**. As we have already commented for **L**, some enlargement of the macrocyclic cavity, brought about by nitrogen methylation, could be responsible for the small increase in stability of the complex Pb$\mathbf{L1}^{2+}$ with respect to Pb$\mathbf{L4}^{2+}$. The stability loss is much more marked for Ni^{2+} (log $K_{Ni\mathbf{L1}^{2+}}$ = 11.83(2) vs log $K_{Ni\mathbf{L4}^{2+}}$ = 16.56[21]) than for Zn^{2+} (log $K_{Zn\mathbf{L1}^{2+}}$ = 10.69(1) vs log $K_{Zn\mathbf{L4}^{2+}}$ = 13.33[17]), and Cd^{2+} (log $K_{Cd\mathbf{L1}^{2+}}$ = 15.12(1) vs log $K_{Cd\mathbf{L4}^{2+}}$ = 18.10[18]), while it is rather modest for Cu^{2+} (log $K_{Cu\mathbf{L1}^{2+}}$ = 18.32(4) vs log $K_{Cu\mathbf{L4}^{2+}}$ = 19.48[24]).

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Table 3. Logarithms of the Equilibrium Constants for the Formation of **L** and **L1** Anion Complexes Determined in 0.15 mol dm^{-3} $NaClO_4$ at 298.1 ± 0.1 K

reaction[a]	ATP^{4-} [b]	ADP^{3-}	AMP^{2-}	$P_2O_7^{4-}$	$[Co(CN)_6]^{3-}$
$A + 2H + \mathbf{L} = H_2\mathbf{L}A$	21.87(5)[c]	21.72(4)	21.88(4)		
$A + 3H + \mathbf{L} = H_3\mathbf{L}A$	30.45(3)	30.23(3)	29.96(4)	29.9(1)	
$A + 4H + \mathbf{L} = H_4\mathbf{L}A$	37.86(3)	37.11(3)	36.62(3)	38.31(3)	34.28(4)
$A + 5H + \mathbf{L} = H_5\mathbf{L}A$	42.71(3)	41.53(3)	40.99(3)	44.81(4)	37.63(3)
$A + 6H + \mathbf{L} = H_6\mathbf{L}A$	46.22(3)	44.68(4)	43.70(6)	49.19(4)	40.72(4)
$H_2\mathbf{L} + A = H_2\mathbf{L}A$	3.00(6)	2.85(5)	3.01(5)		
$H_3\mathbf{L} + A = H_3\mathbf{L}A$	3.82(4)	3.60(3)	3.33(5)	3.2(1)	
$H_4\mathbf{L} + A = H_4\mathbf{L}A$	7.39(4)	6.64(4)	6.15(4)	7.84(4)	3.81(5)
$A + 3H + \mathbf{L1} = H_3\mathbf{L1}A$		29.76(5)	29.67(4)		29.1(1)
$A + 4H + \mathbf{L1} = H_4\mathbf{L1}A$	36.30(1)	36.94(4)	36.52(5)	37.2(1)	35.76(6)
$A + 5H + \mathbf{L1} = H_5\mathbf{L1}A$	42.10(1)	42.82(4)	42.19(4)	44.17(6)	39.19(7)
$A + 6H + \mathbf{L1} = H_6\mathbf{L1}A$	46.38(2)	47.46(5)	46.55(4)	49.97(6)	
$A + 7H + \mathbf{L1} = H_7\mathbf{L1}A$	50.47(1)	50.89(5)	49.54(9)	54.33(6)	
$A + 8H + \mathbf{L1} = H_8\mathbf{L1}A$		54.13(4)	52.76(7)	57.33(8)	
$H_3\mathbf{L1} + A = H_4\mathbf{L1}A$		3.57(6)	3.48(5)		2.9(1)
$H_4\mathbf{L1} + A = H_4\mathbf{L1}A$	4.69(2)	5.33(5)	4.91	5.6(1)	4.15(7)
$H_5\mathbf{L1} + A = H_5\mathbf{L1}A$	7.51(2)	8.23(5)	7.59(5)	9.58(7)	4.60(8)
$H_6\mathbf{L1} + A = H_6\mathbf{L1}A$	10.01(3)	11.09(6)	10.18(5)	13.60(7)	

[a] Charges have been omitted. [b] From ref 1. [c] Values in parentheses are standard deviations on the last significant figure.

The $Ni\mathbf{L1}^{2+}$ complex shows a considerable tendency to form protonated species. It is to be noted that the equilibrium constants for the addition of H^+ to $NiH\mathbf{L1}^{3+}$ (log $K = 5.30(3)$, Table 1) is almost equal to the equilibrium constant for the protonation of the equally charged, uncomplexed ligand $H_3\mathbf{L1}^{3+}$ (log $K = 5.42$)[1] suggesting the involvement of only five, out of seven, nitrogen donors of **L1** in the formation of the $Ni\mathbf{L1}^{2+}$ complex. Otherwise, hexacoordination of **L4** to Ni^{2+} in $Ni\mathbf{L4}^{2+}$ was observed in the crystalline complex $Ni\mathbf{L4}(ClO_4)_2$ and deduced in solution.[21] The different number of nitrogen donors involved in the coordination to Ni^{2+} by **L1** (five nitrogens) and **L4** (six nitrogens) is, most likely, the principal source of loss in stability for $Ni\mathbf{L1}^{2+}$. Accordingly, the electronic spectrum of $Ni\mathbf{L1}^{2+}$ in solution (λ_{max} 952 (8.0), 568 (5.8), 355 (12) nm (ϵ dm^3 mol^{-1} cm^{-1})) reveals a weaker ligand field interaction than $Ni\mathbf{L4}^{2+}$ (λ_{max} 940 (15.7), 552 (11.2), 363 (14.3) nm (ϵ dm^3 mol^{-1} cm^{-1}))[21] and it is almost identical to the electronic spectra of $NiH\mathbf{L1}^{3+}$ and $NiH_2\mathbf{L1}^{4+}$.

As indicated above, the formation of the mononuclear Cu^{2+} complex with the heptaazaligands is not much affected by nitrogen methylation, the reduced stability observed for $Cu\mathbf{L1}^{2+}$ with respect to $Cu\mathbf{L4}^{2+}$ being produced by a lowering of both enthalpic ($-\Delta H°$) and entropic ($T\Delta S°$) contributions (Table 2). The reaction of successive binding of H^+ ions to $Cu\mathbf{L1}^{2+}$, to produce $CuH\mathbf{L1}^{3+}$ and $CuH_2\mathbf{L1}^{4+}$, are considerably exothermic, the $\Delta H°$ changes associated with these processes ($-\Delta H° = 31.0$-(8), 36.4(8) kJ mol^{-1}, Table 2) being very similar to the enthalpy changes determined for protonation of the uncomplexed ligand ($-\Delta H°$ ranges from 32.6 to 43.0 kJ mol^{-1}),[1] revealing that the two amine groups being protonated in $Cu\mathbf{L1}^{2+}$ are not bound to the metal ion. Pentacoordination of the ligand to Cu^{2+} was also proposed for the unmethylated ligand **L4**. Furthermore, also the almost identical electronic spectra of $Cu\mathbf{L1}^{2+}$ (λ_{max} 640 (223) nm (ϵ dm^3 mol^{-1} cm^{-1})) and $Cu\mathbf{L4}^{2+}$ (λ_{max} 636 (193) nm (ϵ dm^3 mol^{-1} cm^{-1}))[24] in solution suggest quite similar coordination environments of Cu^{2+} in both complexes. Hence, only one of the methylated nitrogens of **L1** should be bound to Cu^{2+} in $Cu\mathbf{L1}^{2+}$.

Potentiometric measurements revealed that **L1** is also able to form dicopper(II) complexes. Although a similar behavior was also observed for **L4**, the tendency of **L1** to bind the second Cu^{2+} ion is much lower (log $K = 7.44(6)$ for $L = \mathbf{L1}$, log $K = 11.01$ for $L = \mathbf{L4}$[24] in $CuL^{2+} + Cu^{2+} = Cu_2L^{4+}$). The absence of protonated binuclear species suggests that also for **L1** all the donor atoms are involved in the coordination of the two Cu^{2+} ions. Therefore, the reduced stability of $Cu_2\mathbf{L1}^{4+}$ with respect to $Cu_2\mathbf{L4}^{4+}$ may be ascribed to the different electronic properties of the amine groups in both ligands. The binding of the second Cu^{2+} by **L1** would imply the coordination of the remaining two tertiary amine nitrogens to produce a structure where the copper(II) atoms will be most likely coordinated by three and four nitrogen atoms, respectively. Although for the $Cu_2\mathbf{L4}^{4+}$ similar coordination environments are to be expected, in this case, all the donor atoms are secondary nitrogens.

Anions Complexation. Polyazamacrocycles produce, in aqueous solution, highly charged protonated cations which can interact with anionic species. Electrostatic attraction and hydrogen bonding are the main contributions to the stability of anion complexes. The formation of such species is strictly pH-dependent and, therefore the relevant equilibria can be studied by pH-metric titrations. Table 3 collects the equilibrium constants determined for the species formed by **L** and **L1** with ADP^{3-}, AMP^{2-}, $P_2O_7^{4-}$, and $[Co(CN)_6]^{3-}$ together with those previously reported for ATP^{4-}.[1] As can be seen from this table, the phosphate anions form complexes of higher stability than $[Co(CN)_6]^{3-}$; this applies also for ADP^{3-}, and AMP^{2-}, which present equal or smaller charge than the hexacyanocobaltate-(III) anion, accounting for the greater tendency of phosphate groups to form hydrogen bonds with ammonium groups. In addition the elongated structures of ATP^{4-}, ADP^{3-}, and $P_2O_7^{4-}$ allow the formation of multiple electrostatic and hydrogen bond interactions with the polyammonium macrocycles. In this sense we expect that the disposition as well as the electronic properties of the amino groups in the macrocycle determine the binding characteristics of the polyammonium receptors. It has been shown by ^{13}C NMR studies at different pH values that there is no observable charge localization in protonated $[3k]aneN_k$ ligands, due to the equivalence of secondary amino groups.[27] This means that, on the NMR time scale, the overall positive charge has a mediate homogeneous distribution over all the nitrogen atoms. Otherwise, nitrogen methylation reduces the basicity and the hydrogen bonding ability of the amino groups, in solution, orienting protonation on secondary nitrogens,[1,6] as illustrated in Figure 3 for the tetraprotonated forms of **L**, **L1**, and **L2**.

The different basicity between tertiary and secondary amino groups opposes the redistribution of the positive charges on the

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Table 4. Rate Constants (±10%) and Activation Parameters for the Hydrolysis of ATP (0.01 mol dm^{-3}) in the Presence of **L** and **L1** (0.01 mol dm^{-3})

	T °C	pH	10^3k	E_a	ΔH^*	ΔG^*	ΔS^*
L	25	4.0	0.0026	122 ± 2	119 ± 2	115.2 ± 0.3	13 ± 7
	45	3.0	1.1				
	65	3.0	1.3				
	80	3.0	5.6				
	60	4.0	0.44				
	70	4.0	1.7				
	80	4.0	5.0				
L1	25	2.0	62				
	25	3.0	14				
	25	4.0	7.4	94.1 ± 0.5	91.7 ± 0.5	95.5 ± 3	−13 ± 2
	30	2.5	51				
	30	4.0	13				
	45	4.0	80				
	60	4.0	220				
	60	4.5	121				
	60	5.0	35				
	60	6.5	2.2				

[a] Rate constants are in min^{-1}; E_a, ΔH^* and ΔG^* are in kJ mol^{-1}, ΔS^* is in J mol^{-1} K^{-1}.

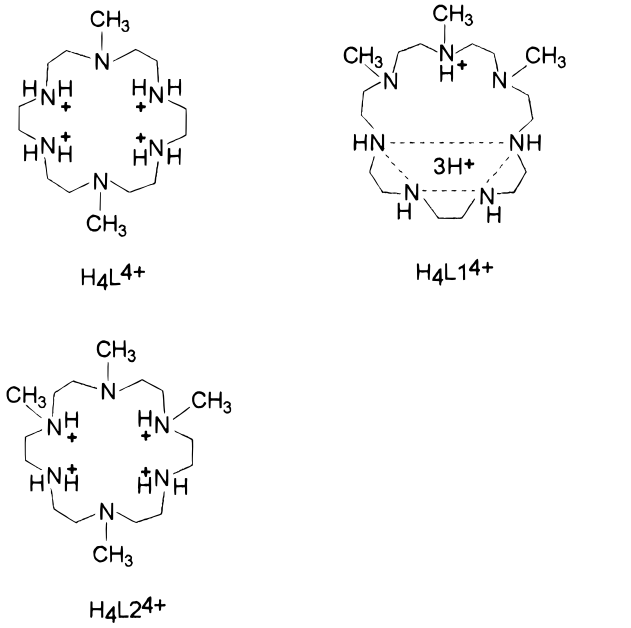

Figure 3. Localization of H^+ ions in the tetraprotonated forms of **L**, **L1**, and **L2** deduced by ^{1}H and ^{13}C NMR.[1]

macrocycle expected when the anion is approaching the polyammonium receptor. In this sense while the [3*k*]aneN$_k$ ligands offer only a size criterion in anion binding their polymethylated derivatives introduce a further topological factor, charge localization, increasing selectivity in anion recognition. Indeed, the equilibrium constant for the interaction of the species $(H_n[3k]aneN_k)^{n+}$ with ATP^{4-} decreases with increasing k number of nitrogen atoms in the macrocycle, according to a lowering in charge density, while a large variability of equilibrium constants is observed for similar reactions involving the analogous methylated ligands. Analysis of the equilibrium data for **L**−**L4**, according to a suggested method,[5,28] revealed that, among these ligands, **L** is the most appropriate receptor for ATP recognition in solution over a wide pH range.[1]

Dephosphorylation Reactions. In recent papers we have analyzed the ability of **L2**, **L3**, and **L4** in enhancing the rate of ATP dephosphorylation.[4,5] It was found that **L4** is the polyammonium receptor, among those studied up to now, inducing the largest rate enhancement with respect to free ATP in neutral and acidic solutions. Although, under similar conditions, another macrocyclic receptor, the ditopic hexaazadioxa bisdien, presents comparable catalytic ability.[3a] It was also observed there is not a parallelism between the increasing stability of ATP complexes and the rate enhancement of ATP dephosphorylation reactions, and it was suggested that the dimensions of the macrocycle are critical in determining the efficiency of the catalyst. From this point of view it seems that **L4** presents well suited structural characteristics.[4]

Conversion of ATP into ADP and inorganic phosphate promoted by **L** and **L1** was followed monitoring the ATP loss by means of ^{31}P NMR at different temperature and pH values. First order reactions with respect to ATP complexes were found (Table 4). Plots of log *k* versus 1/*T*, for the measurements performed at pH 4 in presence of **L** or **L1**, furnished the activation energy E_a from which the thermodynamic activation quantities ΔG^*, ΔH^* and ΔS^* were obtained (Table 4).

As already observed for similar macrocyclic polyammonium receptors,[3−5] the rate of ATP dephosphorylation in presence of **L** and **L1** increases in more acidic solutions (Table 4). For example, for **L1** at 60 °C, a pH change from 6.5 to 4 produces a 10^2-fold rate enhancement.

It has been proposed[3−5] that cleavage of ATP by cyclic polyammonium cations proceeds, at least in not very acidic solutions, via a nucleophilic attack on the γ-phosphorus of the nucleotide by an unprotonated nitrogen of the catalyst, forming a covalent phosphoramidate intermediate which is readily hydrolyzed. For example a small amount of such a phosphoramidate intermediate was observed in the ^{31}P NMR spectrum of **L4**/ATP mixture at pH 4.[4] General acid catalysis could play a major role in more acidic solutions, where the macrocyclic catalyst, existing in highly protonated form, facilitates the dephosphorylation process by proton transfer from an ammonium group to the P−O−P oxygen. Similar mechanisms are consistent with the present kinetic results, but no phosphoramidate intermediates were observed for **L**−**L3**.

A principal aim of this kinetic study is to investigate how the catalytic properties of **L3** and **L4** are modified by altering the electronic properties of the amino groups through methylation. In this respect it is worth noting that, although the trimethylated receptor **L1** remains one of the most efficient polyammonium catalysts for ATP cleavage in solution, it presents reduced catalytic ability ($k = (7.4 \pm 0.7) \times 10^{-3}$ min^{-1} at pH 4, 25 °C) with respect to its unmethylated analogous **L4**[4] ($k = (54 \pm 5) \times 10^{-3}$ min^{-1} at pH 4, 25 °C). Of particular

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interest are the hexaaza ligands; while tetramethylation of **L3** produces a large rate enhancement, dimethylation gives rise to a significant slowing. For instance, the dephosphorylation rate in presence of **L3** at 80 °C and pH 3 is $k = (13 \pm 1) \times 10^{-3}$ min^{-1}, [4] while under the same conditions $k = (43 \pm 4) \times 10^{-3}$ min^{-1} and $k = (5.6 \pm 0.5) \times 10^{-3}$ min^{-1} for **L2**[5] and **L**, respectively. It is to be noted that from a thermodynamic point of view **L** was recognized to be more effective than **L2** and **L3** in ATP recognition and binding in solution over a wide pH range.[1] The stability of the ATP−macrocycle complexes, whose formation is required as a first step in the catalytic pathway, is mainly controlled by the charges on the interacting partners. In particular the preorganization of the polyammonium groups in the receptor can furnish appropriate complementary structures to the nucleotide. In this respect, the presence of tertiary amino groups in the macrocycle orientates protonation on secondary nitrogens, hence producing well-defined, more localized charge distributions. For instance, in the case of the species $H_4\mathbf{L}^{4+}$, ^1H and ^{13}C NMR experiments in solution, as well as X-ray analysis in the solid state, demonstrated[1] that the tertiary nitrogens of **L** do not participate in the binding of the four H^+ ions (Figure 3). The particular stability of the ATP complexes of **L** could derive from a similar preorganization. On the other hand these rather fixed structures of the receptor could prevent a good match with ATP in the complex where the γ-phosphorus of the nucleotide could be kept away, or hindered, from the nucleophilic amine groups. In such conditions the stability of the complex could represent a negative contribution to rate enhancement in dephosphorylation reactions. Similar considerations can also be invoked to explain the higher efficiency of **L2**.

It was observed that dephosphorylation reactions of ATP in presence of this ligand are accompanied by a largely negative activation entropy, suggesting a bimolecular reaction involving an addition process as the rate-limiting step in the addition−elimination mechanism proposed for the catalyzed ATP cleavage.[5] In contrast with this behavior **L** and **L1**, similarly to **L3**, produce small activation entropies, positive ($\Delta S^* = 13 \pm 7$ J mol^{-1} K^{-1}) for the first ligand and negative ($\Delta S^* = -13 \pm 2$ J mol^{-1} K^{-1}) for the second one, which seems more indicative of a mononuclear elimination reaction as the rate-limiting step.

From these results it is evident that nitrogen methylation as a way to increase nucleophilic character of polyazamacrocycles can not be considered a general method to improve the catalytic properties of these molecules toward ATP dephosphorylation. The higher preorganization of polyammonium receptors imposed by the presence of tertiary amine groups can enhance or reduce their catalytic ability. Topological factors are, therefore, of great importance in determining the efficiency of the catalyst. Indeed it was observed that for unmethylated polyazamacrocycles there is a close relation between the dimension of the macrocyclic rings, observed in the crystal structures, and the rate of catalytic dephosphorylation. Hence, a further aspect of nitrogen methylation is the modification of ring size which can furnish opposite effects in determining the rate of catalyzed ATP dephosphorylation.

Further study is being developed to achieve more information on this aspect.

Conclusions

From the presented results we can conclude that increasing nitrogen methylation in polyazamacrocycles does not produce monotonous changes in the properties of such ligands toward cation and anion coordination. Nitrogen methylation alters the electronic properties of the amino groups and the ligand conformations determining specific matching with the coordinative requirements of metal ions. Furthermore, methyl groups orientate protonation on secondary nitrogens giving rise to the formation of preferential conformations of the protonated species, increasing selectivity in anion binding. However, a larger stability of anion complexes does not necessarily provide, in the case of ATP, higher rates of the dephosphorylation processes.

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