Multifunctional Molecular Recognition of ATP, ADP and AMP Nucleotides by the Novel Receptor 2,6,10,13,17,21-Hexaaza[22]metacyclophane

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The novel cyclophane receptor 2,6,10,13,17,21-hexaaza[22]metacyclophane **L** presents a molecular architecture which enables recognition in aqueous solution of ATP, ADP and AMP through electrostatic, hydrogen bonding and π-stacking interactions; electrostatic interactions occur between the polyammonium sites of **L** and the phosphate chain of the nucleosides, and π-stacking interactions occur between the *m*-phenylene subunit incorporated in the receptor as a non-pendant integral part of the macrocyclic framework and the adenine ring of the nucleotides.

Molecular recognition of nucleotides by macrocyclic receptors has received increasing attention during the last two decades.[1] It has been postulated that multifunctional receptors have to be built in order to achieve selective complexation of target species.[2,3] As far as complexation of nucleotides is concerned this goal may be achieved by taking advantage of the hydrophilic and hydrophobic characteristic of these guest species. While the polyphosphate chain represents a good electrostatic binding point, the nucleoside part may operate as an adequate site for π-stacking interactions with appropriate functionalities of the ligand. Lehn and his group[3] reported in 1988 one of the first synthetic ligands able to behave as a multifunctional receptor for nucleotides. It consisted of the well-known polyazaoxamacrocycle bisdien N-monofunctionalized with an acridine subunit. Kimura *et al.* have also shown a multipoint binding between a zinc(II) complex of acridine-pendant cyclen and deoxythymidine and uridine coordinated to the metal as exogenous ligands.[4] Recently Schneider *et al.*[5] have shown the combination of hydrophobic and electrostatic effects in the interaction of several aminocyclodextrins with nucleotides. Here we report on the novel cyclophane receptor 2,6,10,13,17,21-hexaaza[22]metacyclophane **L** which presents a molecular organization which enables molecular recognition in aqueous solution of ATP, ADP and AMP through electrostatic, hydrogen bonding and π-stacking interactions. Electrostatic interactions occur between the polyammonium sites of **L** and the phosphate chain of the nucleotides, and π-stacking interactions occur between the *m*-phenylene subunit incorporated in the receptor as a non-pendant integral part of the macrocyclic framework and the adenine ring of the nucleotides. NMR studies provide unambiguous evidence for this behaviour.

The receptor **L** was synthesized by reaction in DMF of 1,3-bis(bromomethyl)benzene with the sodium salt of the tosylated open-chain polyamine *N,N′,N″,N‴,N⁗,N⁗′*-hexakis(*p*-tolysulfonyl)-1,5,9,12,16,20-hexaazacosane. Detosylation was carried out with HBr–HOAc–PhOH to give **L** as its hydrobromide salt.[6]†

The structure of **L** favours extensive protonation of the receptor even around neutral pH‡ [at pH around neutrality (5–7) the free macrocycle would be already in its pentaprotonated form, H_5L^{5+}] and prompts **L** to interact electrostatically with charged anionic species like ATP, ADP and AMP. The basicity constants of macrocycle **L**, determined by potentiometric titration are, on the other hand, very close to those of the related open-chain counterpart 4,8,11,15-tetraazaoctadecane-1,18-diamine (**L1**).[7]

L and **L1** form adduct species with ATP, ADP and AMP [$A^{a-} + H_pL^{p+} = AH_pL^{(p-a)}$; A = ATP^{4-}, ADP^{3-}, AMP^{2-}, L = **L**, **L1**] with protonation degrees (*p*) varying, for both ligands, from 3 to 8 for ATP, from 3 to 7 for ADP and from 4 to 7 for AMP. In all the systems, the largest stabilities are found for the adducts with protonation degree $p = 6$, $[A(H_6L)]^{(6-a)}$, which are the main species in solution over a wide pH range around neutrality. A lower number of protonated polyammonium groups in the receptors considerably reduces the stability, while protonation degrees over 6 necessarily imply the protonation of the nucleotides [$H_nA^{(n-a)} + H_6L^{6+} = AH_pL^{(p-a)}$; $n = 1$ or 2] which therefore decreases their negative charges and their ability to interact electrostatically with the fully protonated

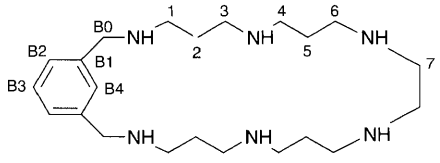
Table 1 Stability constants (log *K*) for the interaction of ATP, ADP and AMP with **L1** and **L** and ^{1}H NMR shifts (δ)[a] for the metacyclophane **L** adducts and complexation-induced ^{1}H NMR chemical shifts (CIS, ppm)[b] for selected protons

	log K[d]		^{1}H NMR chemical shifts and (CIS)/δ							
Reaction[c]	**L1**	**L**	H-7	B-0(H*a*)	B-0(H*b*)	B-4	B-2–B-3	H-8	H-2	H-1′
ATP + H_5L → $ATPH_5L$	4.6	5.2								
ATP + H_6L → $ATPH_6L$	6.7	7.6	3.32 (+0.3)	3.94 (−0.27)	3.94 (−0.27)	7.41 (−0.13)	7.14 (−0.30)	8.20 (−0.19)	7.95 (−0.15)	5.84 (−0.17)
ATP + H_7L → $ATPH_7L$	5.1	5.6								
ADP + H_5L → $ADPH_5L$	3.4	4.2								
ADP + H_6L → $ADPH_6L$	4.9	6.2	3.30 (+0.3)	3.89 (−0.32)	3.79 (−0.42)	7.22 (−0.32)	7.07 (−0.37)	8.19 (−0.18)	7.94 (−0.16)	5.84 (−0.16)
ADP + H_7L → $ADPH_7L$	3.5	4.1								
AMP + H_5L → $AMPH_5L$	3.0	2.9								
AMP + H_6L → $AMPH_6L$	4.2	4.2	3.16 (+0.1)	3.99 (−0.22)	3.86 (−0.35)	7.24 (−0.30)	7.18 (−0.26)	8.25 (−0.07)	7.98 (−0.09)	5.91 (−0.07)
AMP + H_7L = $AMPH_7L$	3.3	2.9								

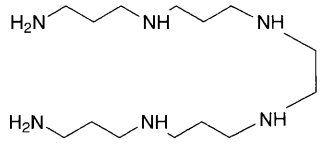
[a] Measured at 300 K in D_2O. [b] Negative CIS values are upfield. [c] Charges have been omitted for clarity. [d] Standard deviations are ±0.1 in the last significant figure.

receptors. Table 1 also shows that, for a given protonation degree and receptor, the binding strength follows the order ATP > ADP > AMP. Another interesting feature is that the stability constants for the cyclic receptor **L** are, particularly in the cases of ATP and ADP, significantly larger than those of the open-chain counterpart **L1**.

The variations in the ^{31}P NMR chemical shifts caused by the interaction with the nucleotides are very similar for both receptors **L** and **L1**. For instance, for the interaction of **L** with the three nucleotides at pH *ca.* 6 where the largest extent of complexation occurs, upfield shifts of 1.60, 1.00 and 0.1 ppm are observed for the signals of P_γ, P_β and P_α of ATP, while the signals of P_β and P_α of ADP shift 0.5 ppm upfield and that of the single phosphorous atom of AMP shifts *ca.* 0.30 ppm with respect to the signals of the free nucleotides at the same pH.

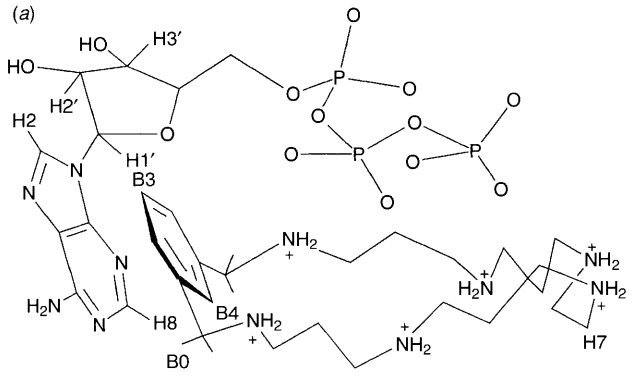


L



L1

Fig. 1 The ligands **L** and **L1**



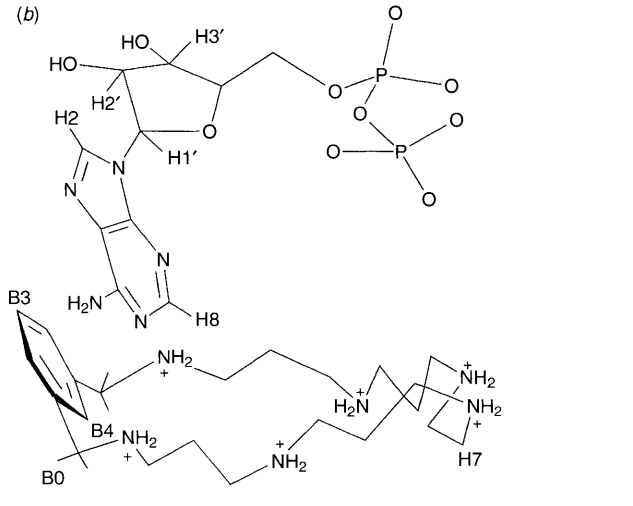


Fig. 2 Schematic representation for the interaction of H_6L^{6+} with (*a*) ATP^{4-} and (*b*) ADP^{3-}

^1H NMR spectra§ provide unambiguous evidence for the participation of π-stacking interactions in the stabilization of the adduct species of **L**. However in **L1** the lack of the aromatic ring precludes this type of interactions and no NMR evidence of π-stacking has been found. For **L**, throughout the pH range 2.5–9.5 in which interaction occurs, significant upfield displacements are observed for the resonances of the adenine protons H-2, H-8 and for the anomeric proton H-1′ of the nucleotides, as well as for the signals of the aromatic (B-2, B-3, B-4) and benzylic protons (B-0) of **L** (see Table 1, for the labelling see Fig. 1). However, some interesting differences depend on the nucleotide studied. Interaction of **L** with ATP strongly displaces the singlet signal of the benzylic protons, while interaction with ADP and AMP in addition to producing a remarkable variation in the chemical shift, also changes the spin system of the benzylic protons from A_2 to AB with geminal coupling of $J_{AB} = -12.8$ Hz (see Table 1). This result indicates that both nucleotides block the flip movement of the chain in that region of the molecule. Interaction with ATP induces significant upfield shifts of protons B-2 and B-3 pointing outwards from the macrocyclic cavity ($\Delta\delta = 0.3$ ppm) and a much reduced displacement in the chemical shift of proton B-4 ($\Delta\delta = 0.1$ ppm), pointing towards the macrocyclic cavity, whereas interaction with ADP and AMP produce similar remarkable variations ($\Delta\delta = 0.3$ ppm) in all three types of magnetically different aromatic protons. Additionally, interaction with the nucleotides produces downfield shifts of the protons of the aliphatic chain of **L**, the variation observed for the signal of the protons corresponding to the central ethylenic chains (H-7) being particularly high (Table 1). Minor changes (*ca.* 0.1 ppm) are also observed for protons H-2′ and H-3′ of the sugar moiety.

All these data suggest a partial inclusion of the nucleoside moieties of ADP and AMP in the macrocyclic cavity. Such inclusion allows for the simultaneous involvement of electrostatic and π-stacking interactions in the stabilization of the adduct and, additionally, adenine nitrogens and/or the hydroxy groups of the ribose subunits could be correctly oriented to hydrogen bond with the polyammonium benzylic groups of the receptor, reducing the mobility of this part of the molecule (see Fig. 2). This hydrogen bonding would explain the splitting of the benzylic signals of the receptor.

The larger size of ATP precludes its inclusion in the molecular cavity of **L**. π-Stacking would take place from outside and so hydrogen bonding would not be possible, in this case allowing for a higher degree of conformational freedom. Such an explanation would account both for the different effects observed on the aromatic protons of **L** and for the lack of splitting of the singlet signal of its benzylic hydrogens.

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Footnotes

† All products have been fully characterized using spectroscopic methods, and gave satisfactory elemental analysis.

‡ The logarithms of the stepwise basicity constants [$H^+ + H_pL^{p+} = H_pL^{(p+1)+}$] of **L** determined in 0.15 mol dm^{-3} $NaClO_4$ at 298.1 K by using the program SUPERQUAD (P. Gans, A. Sabatini and A. Vacca, *J. Chem. Soc., Dalton Trans.*, 1985, 1195) are: log K_{HL} = 10.78(2), log K_{H_2L} = 10.08(2), log K_{H_3L} = 9.00(2), log K_{H_4L} = 7.93(2), log K_{H_5L} = 7.30(2), log K_{H_6L} = 5.24(3). Those of **L1**, taken from ref. 7, are: log K_{HL} = 10.83(2), log K_{H_2L} = 10.15(5), log K_{H_3L} = 9.30(4), log K_{H_4L} = 8.45(5), log K_{H_5L} = 7.30(5), log K_{H_6L} = 4.98(6). A description on the equipment used in the potentiometric titrations can be found in ref. 7.

§ The ^1H, ^{13}C and ^{31}P NMR spectra were recorded in D_2O on Varian Unity 300 and 400 MHz spectrometers. The signals were assigned on the basis of ^1H–^1H and ^1H–^{13}C two-dimensional correlation experiments. NOE measurements were not possible due to unfavourable correlation times even with

the use of spin-lock techniques like ROESY. The solution pH was calculated from the measured pD values by using the relationship pH = pD − 0.4.

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