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ARTICLE *in* HELVETICA CHIMICA ACTA · SEPTEMBER 2003

Impact Factor: 1.14 · DOI: 10.1002/hlca.200390253

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Potentiometric, NMR, and Fluorescence-Emission Studies on the Binding of Adenosine 5′-Triphosphate (ATP) by Open-Chain Polyamine Receptors Containing Naphthylmethyl and/or Anthrylmethyl Groups

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The interaction in aqueous solution of adenosine 5′-triphosphate (ATP) with a series of open-chain polyamines linked at one or both ends to anthrylmethyl or naphthylmethyl fragments was followed by potentiometric titration, ^{1}H-, ^{13}C-, and ^{31}P-NMR spectroscopy, and by steady-state fluorescence measurements. The results revealed greater stabilities for the compounds containing one anthracene moiety than for those with one naphthalene moiety, the stabilities of the compounds with both ends *N*-substituted with naphthylmethyl groups being close to those containing just one anthrylmethyl unit. The ^{1}H-NMR spectra showed that in all systems, there is involvement of π-π stacking interactions in the stabilization of the adduct species. The competitive effect of the anions afforded by the supporting electrolyte was checked in some of the studied systems working at two different ionic strenghts (0.15M and 1.0M NaCl). The joint analysis of the spectrofluorimetric titrations and pH-metric species-distribution curves showed that for all the ATP–receptor systems, a quenching of the fluorescence occurred upon protonation of the adenine N(1)atom. Steady-state fluorescence and time-correlated single-photon-counting analysis of a system made up of ATP and a bis-chromophoric polyamine receptor containing anthracene and naphthalene fluorophores established that the energy-transfer process between the naphthalene and anthracene moieties is still operative despite the presence of ATP.

1. Introduction. – Because of the increasing awareness of the important role that anionic species play in many fields, different research groups have focused their investigations on anion detection and quantification [1–6]. Within this context, nucleotide recognition and sensing in H_2O is an important challenge in supramolecular chemistry because of its many biological implications [7][8]. To achieve this goal, a binding unit for the nucleotide and a signaling unit have to be incorporated into the receptor molecule. However, binding of anionic species in H_2O finds an important competitor in the solvent itself, which buffers the interaction between the partners through forces like H-bonding. Therefore, multifunctional receptors with electronic and steric characteristics able to develop different binding contributions like electrostatic charge–charge attractions, H-bond formation, and hydrophobic or π-π interactions usually need to be included in the design [8–12]. In this sense, polyammonium receptors containing aromatic moieties are adequate receptors, since they can interact with the anionic guest through their positively charged ammonium groups, through H-bonding by their ammonium and amino groups, as well as through

hydrophobic and stacking interactions with adenine, provided the aromatic fragments they incorporate are in the correct orientation [11][12]. In addition, the fused aromatic rings present in the receptors may serve as luminescent probes for detecting and quantifying the interaction. Indeed, fluorescence emission is one of the most useful signaling properties, since its magnitude is highly sensitive to the binding of the guest species. Within this framework, we have studied the possibilities as sensors of metal ions and anions in H_2O of a series of compounds containing one or two anthrylmethyl or naphthylmethyl groups attached to the termini of different polyamine chains [13]. In a previous paper we reported the characteristics of the anthracene derivatives **L11**–**L14** for nucleotide recognition and sensing [14]; here we extend these studies to the naphthalene derivatives **L1**–**L4** containing two naphthylmethyl groups at the termini and to the analog receptors **L5**–**L8** with only one aromatic moiety. Additionally, we check the thermodynamic and photochemical behavior of the mixed compound **L9**, in which a donor naphthalene moiety and an acceptor anthracene moiety are linked at both termini of a triethylenetetraamine chain [15]. For purposes of comparison, we additionally study the receptor compound **L10** with just one terminal anthracene moiety.

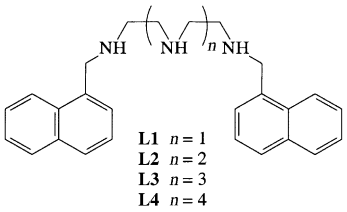
2. Results and Discussion. – 2.1. *Speciation Studies. Table 1* gives the protonation constants of the newly prepared ligands **L7**, **L8**, and **L10** as well as those of **L2** and **L6** reported previously at 298.1 K in 0.15M NaCl and those calculated in this work at the same temperature but at an ionic strength of 1M NaCl. *Tables 2* and *3* report the stability constants determined at 298.1 K in 0.15M NaCl for the interaction of ATP with the receptors **L1**–**L4** and **L5**–**L8**, respectively, as well as those determined in 1M NaCl for the bis(naphthylmethyl)-substituted receptor **L2** and its counterpart **L6** with just one naphthylmethyl group. The corresponding values for the 'hetero-bis-chromophoric' receptor **L9** are also given in *Table 2*. *Table 4* summarizes the data for the mono(anthrylmethyl)-substituted receptors **L10**, **L12**, and **L13** determined at 298.1 K in 0.15M NaCl.

Table 1. *Logarithms of the Stepwise Protonation Constants of* **L2**, **L6** *and ATP Determined in 0.15M and 1M NaCl at 298.1 K*. Values for **L7**, **L8**, and **L10** determined in 0.15M NaCl are also included. Numbers in parentheses are standard deviations in the last significant digit.

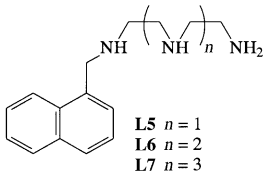
Reaction[a])	**L2**[b])	**L2**[c])	**L6**[b])	**L6**[c])	ATP[b])	ATP[c])	**L7**[b])	**L8**[b])	**L10**[b])
$L + H \rightleftarrows HL$	9.12(2)	9.55(3)	9.68(1)	9.84(2)	6.67(1)	6.19(2)	9.65(2)	10.49(2)	10.18(2)
$HL + H \rightleftarrows H_2L$	8.22(2)	8.55(3)	8.73(1)	9.17(2)	4.11(2)	4.18(2)	9.11(2)	9.37(2)	8.82(2)
$H_2L + H \rightleftarrows H_3L$	6.01(3)	6.66(3)	6.26(2)	6.90(3)	<2	>2	7.92(2)	8.80(3)	6.08(4)
$H_3L + H \rightleftarrows H_4L$	3.18(3)	4.32(6)	3.25(3)	3.97(4)			5.07(4)	7.47(3)	2.6(1)
$H_4L + H \rightleftarrows H_5L$							2.69(5)	5.25(4)	
$H_5L + H \rightleftarrows H_6L$							–	2.41(6)	
$\log \beta$	26.53	29.07	27.92	29.87			34.45	43.79	27.68

[a]) Charges omitted for clarity; L = polyamine receptor or ATP anion. [b]) In 0.15M NaCl. [c]) In 1M NaCl.

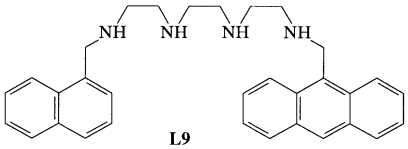
The protonation constants obtained for **L7**, **L8**, and **L10** (*Table 1*) follow the expected trend observed in previous work on this topic [16]. These trends can be largely explained in terms of minimization of electrostatic repulsions between same-



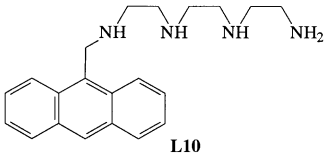
L1 $n = 1$
L2 $n = 2$
L3 $n = 3$
L4 $n = 4$



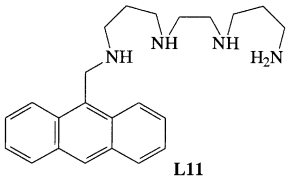
L5 $n = 1$
L6 $n = 2$
L7 $n = 3$
L8 $n = 4$



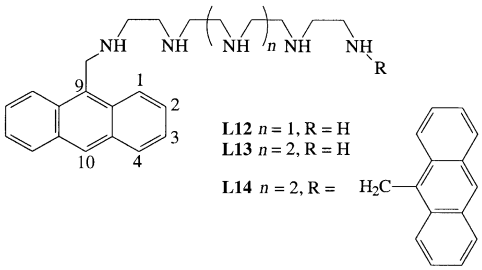
L9



L10



L11



L12 $n = 1$, R = H
L13 $n = 2$, R = H
L14 $n = 2$, R =

sign charges and the lower basicity exhibited by the bis-chromophoric compounds with regard to the mono-chromophoric ones [13]. Indeed, a comparison of **L7** and **L8** with **L3** and **L4** [13a–d], denotes that the mono-chromophoric receptors show not only

Table 2. *Stability Constants* ($\log K$) *for the Interaction of the Bis-Chromophoric Receptors* **L1**–**L4**, *and* **L9** *with ATP Determined in 0.15*M *NaCl at 298.1* ± *0.1 K.* Numbers in parentheses are standard deviations in the last significant digit.

Reaction[a])	**L1**	**L2**	**L2**[b])	**L3**	**L4**	**L9**
$A + H + L \rightleftarrows HAL$	11.61(9)	13.49(3)	13.42(3)	13.36(1)	13.74(9)	13.61(2)
$A + 2H + L \rightleftarrows H_2AL$	20.05(4)	22.25(3)	22.38(3)	22.30(1)	23.04(5)	21.93(3)
$A + 3H + L \rightleftarrows H_3AL$	26.96(5)	29.88(4)	29.76(4)	30.45(1)	31.58(6)	29.44(3)
$A + 4H + L \rightleftarrows H_4AL$	31.80(5)	35.98(4)	35.52(4)	37.39(1)	39.45(5)	35.55(3)
$A + 5H + L \rightleftarrows H_5AL$	35.54(5)	40.51(4)	40.35(5)	43.03(1)	46.22(5)	40.14(4)
$A + 6H + L \rightleftarrows H_6AL$	–	43.55(5)	43.79(5)	47.05(1)	51.87(6)	43.46(4)
$A + 7H + L \rightleftarrows H_7AL$	–	–	–	50.01(1)	55.99(6)	–
$A + 8H + L \rightleftarrows H_8AL$	–	–	–	–	59.06(8)	–
$A + HL \rightleftarrows HAL$	3.2	4.4	3.9	4.0	3.9	4.8
$A + H_2L \rightleftarrows H_2AL$	3.9	4.9	4.3	4.4	4.4	5.1
$A + H_3L \rightleftarrows H_3AL$	7.0	6.5	5.0	5.2	4.8	6.7
$A + H_4L \rightleftarrows H_4AL$	–	–	–	–	6.0	–
$HA + H_2L \rightleftarrows H_3AL$	4.1	5.9	5.5	5.9	6.3	6.0
$HA + H_3L \rightleftarrows H_4AL$	5.1	6.0	4.6	5.4	6.0	6.1
$H_2A + H_2L \rightleftarrows H_4AL$	4.8	–	–	–	–	–
$HA + H_4L \rightleftarrows H_5AL$	–	–	5.1	6.3	6.1	7.4
$H_2A + H_3L \rightleftarrows H_5AL$	4.8	6.4	5.2	7.0	–	6.6
$HA + H_5L \rightleftarrows H_6AL$	–	–	–	7.6	7.3	–
$H_2A + H_4L \rightleftarrows H_6AL$	–	6.3	4.4	6.2	7.7	6.6
$H_2A + H_5L \rightleftarrows H_7AL$	–	–	–	6.5	7.3	–
$H_2A + H_6L \rightleftarrows H_8AL$	–	–	–	–	8.2	–

[a]) Charges omitted for clarity; A = anion, L = polyamine receptor. [b]) I = 1M NaCl.

greater overall basicity but also slightly greater basicity in all successive protonation steps.

Another aspect that deserves some comment is the choice of the supporting electrolyte and the ionic strength. Since the concentration of the supporting electrolyte has to be in large excess with respect to that of the analyte to keep the ionic strength constant, the anion contained in the electrolyte will always interfere somewhat with the anionic species under study. In the measurements here presented, the ionic strength was kept constant at 0.15M NaCl and, therefore, the obtained values are internally self-consistent and allow comparison with other systems studied under the same experimental conditions. However, to analyze the influence of the ionic strength on the stability constants, we have studied the systems ATP · **L2** and ATP · **L6** also in 1M NaCl. Therefore, the protonation constants for ATP and the receptors had to be redetermined under these experimental conditions. Whereas the stepwise protonation constants of the receptors become higher upon going from 0.15M to 1M ionic strength, the protonation constants of ATP decrease (*Table 1*). In turn, the association constants ATP · L generally decrease significantly when the ionic strength grows. The values of the constants at 1M ionic strength are almost 100 times lower than those obtained at 0.15M ionic strength (*vide infra*). This clearly reflects the competitive effect exerted by the chloride anions. Nevertheless, the use of this supporting electrolyte and ionic

Table 3. *Stability Constants* (log$K$) *for the Interaction of the Mono-Chromophoric Receptors* **L5**–**L8** *and* **L10** *with ATP Determined in 0.15*M *NaCl at 298.1* ± *0.1 K*. Numbers in parentheses are standard deviations in the last significant digit.

Reaction[a])	**L5**	**L6**	**L6**[b])	**L10**	**L7**	**L8**
$A + H + L \rightleftarrows HAL$	13.39(2)	12.82(3)		13.6(1)	13.36(4)	13.5(1)
$A + 2H + L \rightleftarrows H_2AL$	22.33(2)	22.17(2)	22.33(3)	23.27(5)	22.69(3)	23.37(4)
$A + 3H + L \rightleftarrows H_3AL$	29.23(2)	29.91(2)	30.05(3)	31.23(5)	31.21(3)	32.39(5)
$A + 4H + L \rightleftarrows H_4AL$	34.05(3)	36.10(2)	35.86(4)	37.50(5)	38.25(3)	40.71(3)
$A + 5H + L \rightleftarrows H_5AL$	37.60(3)	40.48(3)	40.41(5)	41.98(5)	43.81(3)	47.58(3)
$A + 6H + L \rightleftarrows H_6AL$	–	43.75(3)	43.58(3)	45.20(5)	47.73(3)	52.88(4)
$A + 7H + L \rightleftarrows H_7AL$	–	–			50.58(5)	56.51(5)
$A + 8H + L \rightleftarrows H_8AL$	–	–			–	58.9(1)
$A + HL \rightleftarrows HAL$	3.6	3.1	–	3.4	3.7	3.0
$A + H_2L \rightleftarrows H_2AL$	4.3	3.8	3.3	4.3	3.9	3.5
$A + H_3L \rightleftarrows H_3AL$	–	5.2	4.1	6.2	4.5	3.7
$HA + H_2L \rightleftarrows H_3AL$	4.5	4.8	4.8	5.6	5.8	5.9
$A + H_4L \rightleftarrows H_4AL$	–	–	–	–	6.5	4.6
$HA + H_3L \rightleftarrows H_4AL$	5.4	5.2	3.3	6.1	4.9	5.4
$H_2A + H_2L \rightleftarrows H_4AL$	5.2	6.9	–	–	–	–
$A + H_5L \rightleftarrows H_5AL$	–	–	–	–	–	6.2
$HA + H_4L \rightleftarrows H_5AL$	–	5.9	4.3	7.6	5.4	4.8
$H_2A + H_3L \rightleftarrows H_5AL$	4.9	5.0	4.1	6.1	6.4	–
$HA + H_5L \rightleftarrows H_6AL$	–	–	–	–	6.6	4.8
$H_2A + H_4L \rightleftarrows H_6AL$	–	5.1	3.3	6.7	5.2	6.0
$HA + H_6L \rightleftarrows H_7AL$	–	–	–	–	–	–
$H_2A + H_5L \rightleftarrows H_7AL$	–	–	–	–	5.4	4.4
$H_2A + H_6L \rightleftarrows H_8AL$	–	–	–	–	–	4.4

[a]) Charges omitted for clarity; A = anion, L = polyamine receptor. [b]) I = 1M NaCl.

strength is relevant because of the higher solubility of the receptors in this media and because of the similarity of this concentration with that found in biological tissues.

To analyze the ATP · L adduct-formation constants for the different systems shown in *Tables 2–4*, care must be exerted in comparing the right equilibria and values of stability constants. Since both the substrate and the receptors participate in overlapping proton-transfer processes, translating the cumulative stability constants at the top entries of both tables into representative stepwise constants is not always straightforward. To do so, one has to consider the basicities of ATP and of the different ligands and assume that the interaction will not affect much the pH range of existence of the protonated species of ATP and L. If this is taken into account, the stepwise constants shown in the bottom entries of *Tables 2–4* can be deduced. Such constants show that the interaction of the bis-chromophoric receptors with ATP is larger than that experienced by the corresponding mono-chromophoric compounds. On the other hand, the comparison of the constants of the mono(naphthylmethyl) and mono(anthrylmethyl) derivatives shows higher values for the second kind of compound. However, the most unambiguous way to compare the relative stabilities of the different systems and to establish selectivity ratios is to use conditional constants. The conditional constants K_{cond} are calculated at each pH value as the quotient between the overall

Table 4. *Stability Constants* (log$K$) *for the Interaction of the Mono(anthrylmethyl)substituted Receptors* **L10**, **L12**, *and* **L13** *with ATP Determined in 0.15*M *NaCl at 298.1* ± *0.1 K*. Numbers in parentheses are standard deviations in the last significant digit.

Reaction[a])	**L10**	**L12**	**L13**
$A + H + L \rightleftarrows HAL$	13.6(1)	14.22(4)	13.0(1)
$A + 2H + L \rightleftarrows H_2AL$	23.27(5)	23.70(3)	22.74(3)
$A + 3H + L \rightleftarrows H_3AL$	31.23(5)	32.25(3)	31.75(3)
$A + 4H + L \rightleftarrows H_4AL$	37.50(5)	39.29(3)	39.88(2)
$A + 5H + L \rightleftarrows H_5AL$	41.98(5)	45.16(3)	46.57(3)
$A + 6H + L \rightleftarrows H_6AL$	45.20(5)	49.41(3)	51.86(3)
$A + 7H + L \rightleftarrows H_7AL$	–	52.40(5)	55.62(3)
$A + 8H + L \rightleftarrows H_8AL$	–	–	57.83(5)
$A + HL \rightleftarrows HAL$	3.4	4.0	2.9
$A + H_2L \rightleftarrows H_2AL$	4.3	4.3	3.5
$A + H_3L \rightleftarrows H_3AL$	6.2	5.0	4.1
$HA + H_2L \rightleftarrows H_3AL$	5.6	6.4	–
$A + H_4L \rightleftarrows H_4AL$	–	–	5.5
$HA + H_3L \rightleftarrows H_4AL$	6.1	5.7	5.9
$H_2A + H_2L \rightleftarrows H_4AL$	7.7	–	–
$HA + H_4L \rightleftarrows H_5AL$	7.6	6.6	5.9
$H_2A + H_3L \rightleftarrows H_5AL$	6.1	–	–
$HA + H_5L \rightleftarrows H_6AL$	–	–	7.0
$H_2A + H_4L \rightleftarrows H_6AL$	6.7	7.0	–
$H_2A + H_5L \rightleftarrows H_7AL$	–	6.9	7.0

[a]) Charges omitted for clarity; A = anion, L = polyamine receptor.

amount of complexed species and the overall amounts of free receptor and substrate independently of their protonation degree (*Eqn. 1*).

$$K_{cond} = \Sigma[H_{i+j}LA]/\Sigma[H_jL]\Sigma[H_iA] \quad (1)$$

Figs. 1,*a* and *b*, are plots of the logarithms of the conditional constant *vs.* pH for the bis- and mono-chromophoric receptors, respectively. Although, as already pointed out, the bis(naphthylmethyl) ones exhibit larger constants than the corresponding mono-(naphthylmethyl) receptors, the trends in both series are not equal. For the bis-chromophoric receptors, the sequence shows its maximum constants for the hexamine **L4**, while, for the mono-chromophoric ligands, the maximum interaction is observed for the pentamine **L7** although the differences between the different systems are not very large. An explanation of these trends may rest on the presumably different conformational arrangements adopted by both types of receptors.

Although charge–charge interactions seem to be the most important factors regulating the magnitude of the interaction, the data in *Tables 2* and *3* show that other factors like π-π stacking interactions have to be considered. To gain insight on this point, *Fig. 2* compares logarithms of the conditional constant for the tetramines **L2**, **L6**, **L9**, and **L10**. First, it can be observed that the compounds containing two naphthylmethyl groups (**L2**), one anthrylmethyl and one naphthylmethyl group (**L9**), and only one anthrylmethyl group (**L10**) display rather comparable conditional constants over the entire pH range. However, the receptor with only one naphthyl-

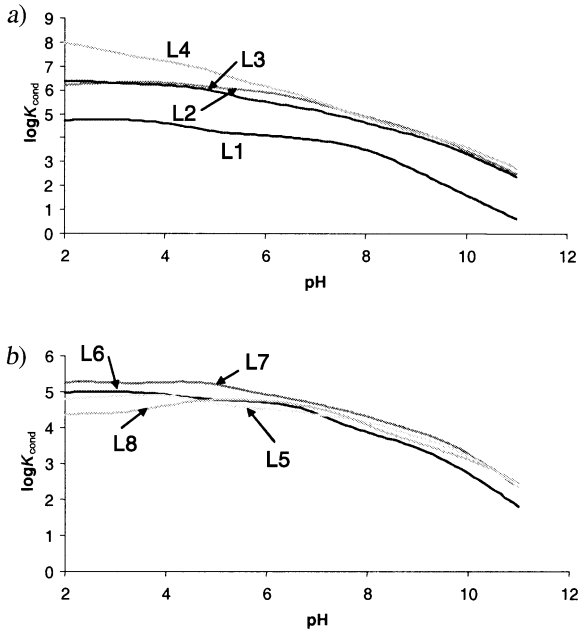


Fig. 1. *Plot of the logarithms of the conditional constants* K_{cond} *for the interaction of ATP with* a) *bis(naphthylmethyl) derivatives* **L1**–**L4**, *and* b) *mono(naphthylmethyl) derivatives* **L5**–**L8**. All systems have been calculated for $[L] = [ATP] = 10^{-3}$ M and 0.15M NaCl.

methyl group linked (**L6**) presents a much reduced interaction with ATP. All the bis-chromophoric receptors present a similar affinity independently of possessing two naphthalene moieties (**L2**) or one naphthalene and one anthracene moiety (**L9**). This last point can be described with a sandwich-type disposition of the adenine ring between the two aromatic-ring systems of the receptor with a distance from each one of the aromatic-ring systems that would depend on the conformational flexibility of the receptor species. Moreover, the plot of $\log K_{cond}$ for the system ATP · **L2** at ionic strength 0.15M and 1M confirm the above-commented competition of the chloride anions.

2.2. *NMR Studies.* To gain insight into the nature of the interaction of ATP with the different receptors, we have carried out a ^{1}H- and ^{31}P-NMR study on several of these systems. All the mono- and bis-chromophoric systems show, following the ATP-receptor interaction, significant upfield shifts of the ^{1}H-NMR signals of the protons H–C(2) and H–C(8) of the adenine moiety, of the anomeric proton H–C(1′) of the sugar moiety, and of the naphthalene or anthracene protons (for the labelings, see ATP). For instance, in *Fig. 3*, such changes can be seen in the ^{1}H-NMR spectra of ATP, of **L6**, and of the ATP · **L6** recorded at pH 6.

Table 5 gives the changes observed in the chemical shifts ($\Delta\delta = \delta_{complexed} - \delta_{free}$) for the signals of H–C(2), H–C(8), and H–C(1′) of ATP in the systems ATP · **L6**, ATP · **L8**, ATP · **L11**, and ATP · **L13** (molar ratio ATP/L 1:1) at various pH values. It is worth mentioning that the variation in the chemical shifts are observed over the entire pH

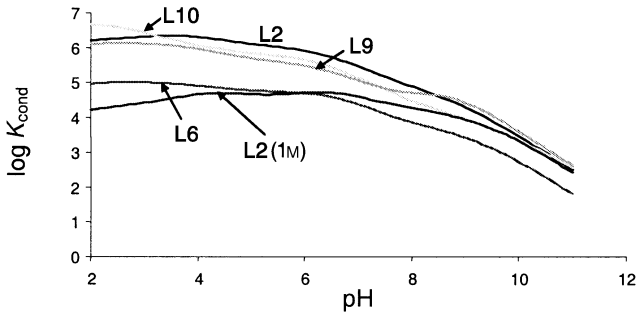


Fig. 2. *Plot of the logarithms of the conditional constants* K_{cond} *for the interaction of ATP with the tetramines* **L2**, **L6**, **L9**, *and* **L10**. [L] = [ATP] = 10^{-3} M and 0.15M NaCl. For **L2**, the values calculated at I = 1M NaCl are also included.

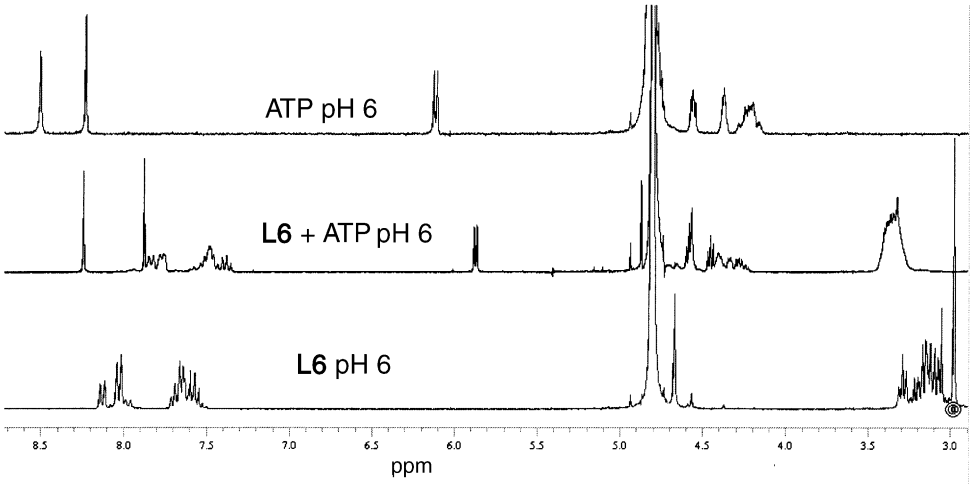
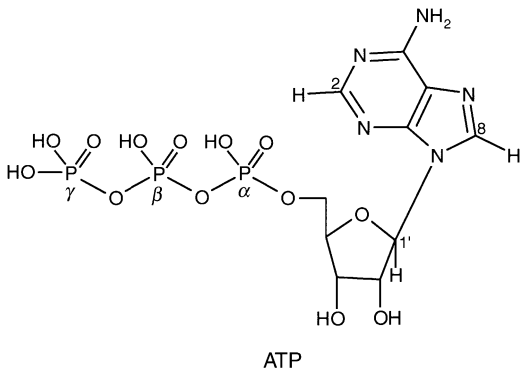


Fig. 3. *^{1}H-NMR Spectra in D_2O of the systems ATP*, **L6**, *and ATP* · **L6**, *recorded at pH 6*



range where the interaction occurs, as it can be seen in *Fig. 4* where the $\Delta\delta$ of H–C(2), H–C(8), and H–C(1′) are plotted conjointly with the species-distribution diagram obtained from the pH-metric data for the system ATP · **L6**. On the other hand, these changes are larger for the receptor containing the anthracene fragment (**L13**) than for that with the naphthalene ring (**L8**). A similar situation is observed for all other anthracene and naphthalene receptors sharing the same polyamine chains. ^{1}H-NMR NOE Experiments performed with the mono-chromophoric receptor **L13** shows intense NOE effects between H–C(10) of anthracene and H–C(2) of ATP as well as a less-intense effect with H–C(8) (ATP); additionally, H–C(2) and H–C(3) of anthracene give a moderate NOE effect with H–C(2) (ATP). These connectivities allowed us to propose an initial docking for the model shown in *Fig. 5*. Electrostatic forces and π-π stacking interactions contribute to the large stabilization of the adduct species in this kind of system [9–12][17–20].

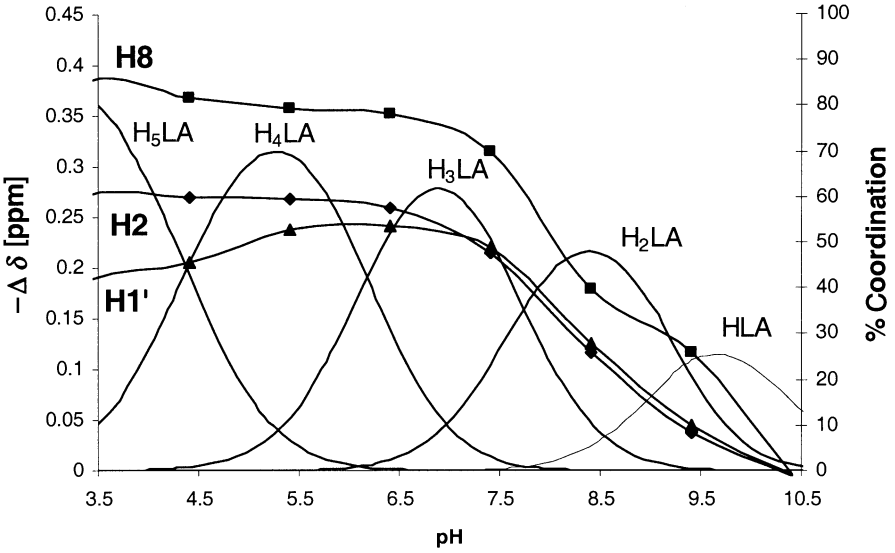


Fig. 4. *Distribution diagram of the species existing in equilibria in the system ATP* · **L6** (right-hand ordinate) *and variation with pH of the observed changes in chemical shifts* ($-\Delta\delta$) *of the signals of H–C(2)* (= H2), *H–C(8)* (= H8), *and H–C(1′)* (= H1′), *of ATP on coordination with* **L6** (left-hand ordinate). $\Delta\delta = \delta_{complexed} - \delta_{free}$, see text.

The larger constants found for the interaction of the anthracene receptors with ATP can be due to the fact that the more-hydrophobic local environment of anthracene will favor electrostatic interactions and H-bonding. Also, a greater π-π stacking contribution in the anthracene system could be argued.

^{31}P-NMR Spectra provide some complementary information. As is well known, the ^{31}P-NMR signals of ATP show important downfield shifts in correspondence with the protonation of P(γ). The net effect of the interaction of ATP with a receptor L is a reduction of its actual basicity, yielding a displacement of the downfield shift experienced by the P-atoms of ATP towards more-acidic pH values. As a matter of

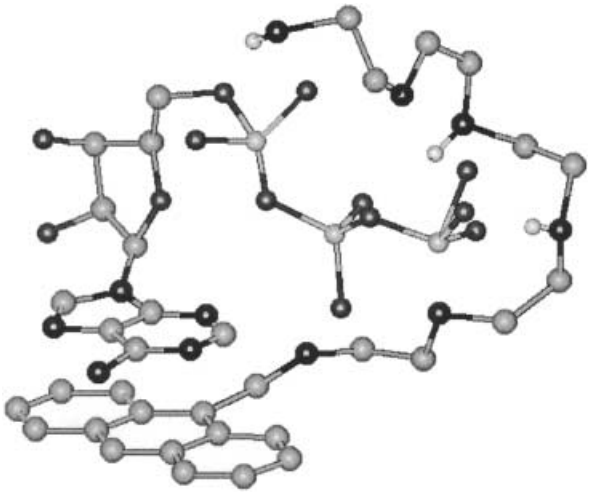


Fig. 5. *Proposed model for the interaction ATP* · **L13**

fact, in our systems, the maximum observed shift on complexation is almost the same for all the systems ATP · L studied ($\Delta\delta(P(\gamma)) \sim 3.1$ and $\Delta\delta(P(\beta)) \sim 2.3$), and what changes from one to another is the pH range in which the jump between both limiting values is produced. The larger the interaction is, the more acidic becomes ATP and the more basic the receptor. However, it has to be taken into account that conformational factors can affect the variation in these signals, and a clear-cut interpretation cannot be offered.

Finally, the system ATP · **L9** with one anthracene and one naphthalene moiety deserves some comment. In this system, the extent of the $\Delta\delta(H)$ observed for H–C(2), H–C(8), and H–C(1′) of ATP ($\Delta\delta(H-C(2)) = -0.39$, $\Delta\delta(H-C(8)) = -0.51$, and $\Delta\delta(H-C(1')) = -0.26$, pH 3.1) are intermediate between those observed for ATP · **L10** ($\Delta\delta(H-C(2)) = -0.63$, $\Delta\delta(H-C(8)) = -0.71$, and $\Delta\delta(H-C(1')) = -0.40$, pH 3.1) and ATP · **L6** ($\Delta\delta(H-C(2)) = 0.39$, $\Delta\delta(H-C(8)) = 0.41$, and $\Delta\delta(H-C(1')) = 0.24$, pH 3.1) (*Table 5*), although closer to ATP · **L6** with one naphthalene moiety. On the other hand, no clear-cut intramolecular NOE effects or cross-peaks in ROESY experiments with ATP · **L9** are detected between the protons of the adenosine and those of the anthracene moiety. These results offer evidence that a differentiated situation is produced that presumably can be related to a sandwich disposition of adenine between both aromatic-ring systems of **L9** with a larger resting time of the adenine closer to the naphthalene ring.

2.3. *Photochemical Studies.* The fluorimetric titration curves performed for the different systems studied here denote the same general pattern we have previously reported for the systems ATP · **L11**, ATP · **L12**, ATP · **L13**, and ATP · **L14** [14]. The curves show a bell-shaped profile in which light emission is strongly quenched at acidic pH values and at basic pH values. In *Fig. 6*, the fluorimetric titration curve for the system ATP · **L9** is presented as an example. The decrease in emission at high pH values corresponds, analogously to what happened with the free receptors [13], to photo-

Table 5. *Changes* $\Delta\delta$ [ppm] *in Observed Chemical Shifts for the Interaction of ATP with Polyamines* **L6**, **L8**, **L13** *and* **L11** *in Molar Ratio L/ATP 1:1.* $\Delta\delta = \delta_{complexed} - \delta_{free}$.

	pH	H–C(2) (ATP)	H–C(8) (ATP)	H–C(1′) (ATP)
ATP · **L6**	2.0	– 0.21	– 0.29	– 0.13
	3.0	– 0.27	– 0.38	– 0.19
	4.0	– 0.27	– 0.37	– 0.21
	5.0	– 0.27	– 0.36	– 0.24
	6.0	– 0.26	– 0.35	– 0.24
	7.0	– 0.22	– 0.31	– 0.22
	8.0	– 0.12	– 0.18	– 0.12
	9.0	– 0.04	– 0.12	– 0.04
	10.0	– 0.01	– 0.00	– 0.00
ATP · **L8**	2.16	– 0.26	– 0.34	– 0.20
	3.03	– 0.28	– 0.38	– 0.22
	4.0	– 0.34	– 0.42	– 0.28
	5.09	– 0.32	– 0.40	– 0.30
	6.06	– 0.31	– 0.39	– 0.30
	6.85	– 0.27	– 0.35	– 0.27
	8.01	– 0.26	– 0.33	– 0.25
	8.97	– 0.19	– 0.26	– 0.19
	10.0	– 0.11	– 0.16	– 0.10
ATP · **L13**	2.16	– 0.45	– 0.46	– 0.31
	3.03	– 0.46	– 0.52	– 0.34
	4.0	– 0.43	– 0.52	– 0.34
	5.09	– 0.42	– 0.52	– 0.39
	6.06	– 0.36	– 0.46	– 0.36
	6.84	– 0.32	– 0.42	– 0.32
	8.01	– 0.30	– 0.38	– 0.29
	8.97	– 0.22	– 0.30	– 0.22
	10.0	– 0.22	– 0.30	– 0.22
ATP · **L11**	2.0	– 0.48	– 0.47	– 0.42
	3.11	– 0.63	– 0.65	– 0.56
	6.08	– 0.53	– 0.58	– 0.52
	8.31	– 0.55	– 0.52	– 0.42
	8.87	– 0.33	– 0.41	– 0.33

induced electron-transfer processes from the lone pairs of the amino groups to the molecular orbitals of the excited fluorophores. The CHEQ effect (chelation-enhanced quenching) observed at acidic pH occurs always in correspondence with the formation of the adduct species with $N+2$ protons, N being the maximum number of protons the ligand can take up by itself. The adduct $H_{N+1}LA$ exhibits an emission identical to free compound L, leading to the conclusion that, in this form, ATP is not capable of quenching. Taking into account the pH at which the species $H_{N+2}LA^{(N-2)+}$ is formed in the different systems (see *Fig. 6* for the system ATP · **L9**) and the protonation constants of ATP (*Table 1*), such $N+2$ protonation must affect the adenine ring and, more concretely, protonation at N(1) of the heterocycle (see *Formula* of ATP). Therefore, since as confirmed by the NMR studies, π-π stacking is present throughout the entire pH range over which complexation occurs, the quenching of the emission has to be related not only to the existence of π-π stacking, as we had previously postulated [14], but also to the withdrawal of electron density from the adenine ring produced by its

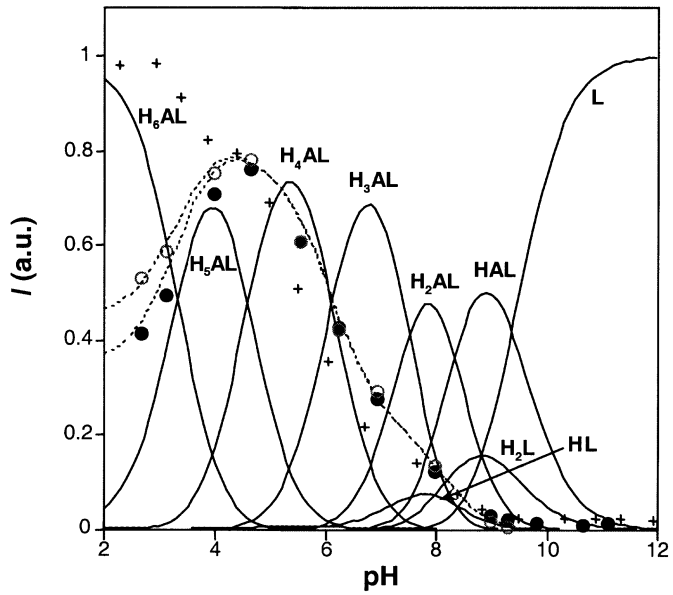


Fig. 6. *Steady-state fluorescence emission of compound* **L9** *in the presence of three-fold excess of ATP, followed at 418 nm upon excitation at 295* (○) *and 334 nm* (●). The same in the absence of ATP (+).

protonation. Our attribution was made in analogy to previous results reported in the literature for other ATP receptors [1–4]. However, we have now observed that π-π stacking is maintained over a large range of pH values, including the one in which fluorescence is restored. An alternative explanation is the possibility of an electron or energy transfer from the excited chromophore to the protonated adenine. Concerning energy transfer, the overlap integral is practically zero, and, thus, singlet–singlet energy transfer should be excluded. However, both adenine and purine can be reduced only in acidic media [21], and, thus, a possible explanation for the quenching in acidic media is the existence of an electron-transfer process from the excited naphthalene (or anthracene) to the protonated adenine moiety of ATP. This reaction is favorable by 4.2 and 2.97 eV for naphthalene and anthracene derivatives, respectively, as estimated by available data for 1-methylnaphthalene, 9-methylanthracene, and adenine. The fitting of the curves intensity *vs.* the amount of added ATP (see *Fig. 7,a* and *b*, for the systems ATP/**L10** and ATP/**L6**, resp.) allows determination of the association constant of the ATP · receptor. For ATP · **L6** at pH 2, the value obtained was $\log K = 3.8$ and for ATP · **L10** $\log K = 4.7$. Interestingly enough, although the constant with the anthracene receptor **L10** is larger, the quenching produced in the naphthalene-containing receptor **L6** is more efficient than that produced in **L10** (*Fig. 7*, *b* and *a*). This behavior can be explained by the larger driving force in the case of naphthalene. In the case of **L6**, almost full quenching is observed. On the other hand, the calculated constants confirm the greater interaction of the receptors containing anthracene units as well as the competing effect of the chloride anions. With respect to the latter point, taking into

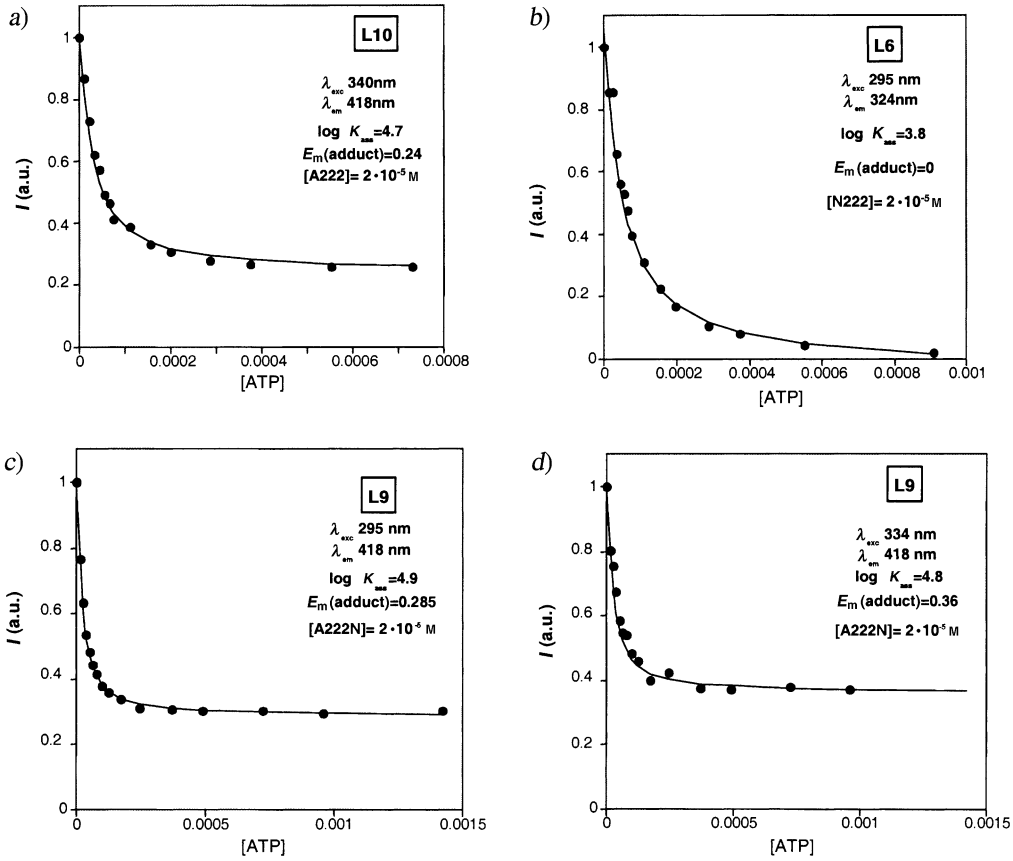


Fig. 7. *Plots of the intensity of emission with respect to the amount of added ATP for* a) **L10** (λ_{exc} 340 nm, λ_{em} 418 nm), b) **L6** (λ_{exc} = 295 nm, λ_{em} 324 nm), c) **L9** (λ_{exc} 295 nm, λ_{em} 418 nm), *and* d) **L9** (λ_{exc} 334 nm, λ_{em} 418 nm). A 222 = **L10**, N 222 = **L6**, and A 222 N = **L9**.

account that the concentration of the naphthalene derivative used was $2 \cdot 10^{-5}$ M and that a 0.01M concentration of HCl was added to fix the pH, this corresponds to a 500-fold excess of chloride anion. Therefore, the values obtained are between those calculated by potentiometry in 0.15M NaCl (chloride excess *ca.* 150-fold, log $K = 5.05$) and 1M (chloride excess *ca.* 1000-fold, log$K = 3.33$).

A more-detailed analysis of the system ATP · **L9** was performed to obtain information about the effect of ATP on the energy transfer we previously reported for this system [15]. In *Fig. 8*, the fluorescence emission of $[\mathbf{L9}] = 2 \cdot 10^{-5}$ M, upon addition of ATP up to 10^{-3} M, at pH 2.0, is represented. At λ_{exc} 295 nm (*Fig. 8,a*), the light is shared between the naphthalene, *ca.* 90%, and anthracene, 10%, moieties. As the ATP concentration is raised, quenching of both naphthalene and anthracene emissions occur until a plateau is reached (*Fig. 8,a* and *b*); a further increase in [ATP] does not affect the fluorescence-emission intensity. This is clear evidence for a static

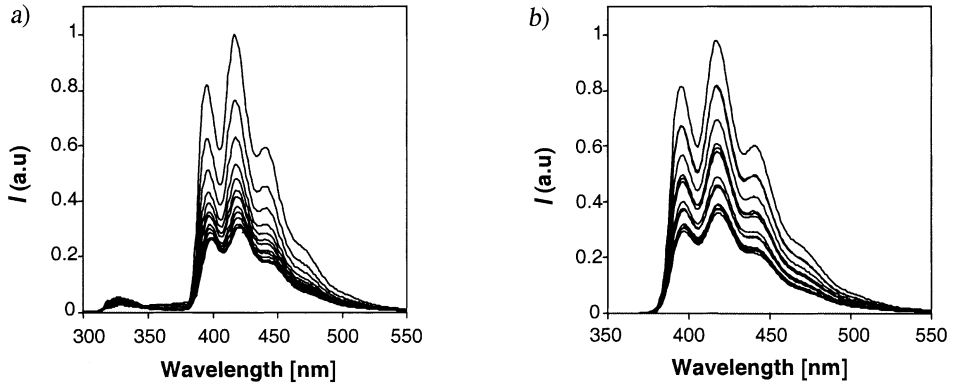


Fig. 8. *Steady-state fluorescence emission titration curve of compound* **L9** *in the presence of increasing quantities of ATP, up to* 10^{-3} M, *at pH 2.0:* a) λ_{exc} *295 nm and* b) λ_{exc} *340 nm*

quenching mechanism, due to ground-state association between L and ATP. Upon excitation at 295 nm, the fluorescence intensity at the plateau for the naphthalene emission is 0.45 of the initial value (in the absence of ATP), while the anthracene emission is 0.29 (*Fig. 7,c*). On the other hand, at λ_{exc} 334 nm (see *Fig. 7,d*), the plateau obtained for selective anthracene excitation is 0.36.

In previous work [15], we verified that, when the free polyamine is fully protonated, the system exhibits a very efficient ($\eta = 0.94\%$) energy-transfer process from the naphthalene unit to the anthracene unit. Fluorescence decays for compound **L9** were obtained at naphthalene (330 nm) and anthracene (450 nm) emission (λ_{exc} 295 nm) in the absence of ATP. The decays at 330 nm are single exponential with a decay time of 1.2 ns, and at 450 nm are fitted with a sum of two exponentials with $\tau_1 = 1.2$ ns and $\tau_2 =$ 11.4 ns. The amplitude of the short decay time (τ_1) at 330 nm is negative, showing that the anthracene excited state is populated through energy-transfer from naphthalene.

From the ratio ϕ_0/ϕ ($\phi_0 =$ quantum yield in the absence of ATP, $\phi =$ quantum yield at the plateau) for naphthalene ($\phi_0/\phi = 2.22$, λ_{exc} 295 nm) and anthracene ($\phi_0/\phi = 2.78$, λ_{exc} 334 nm), the intramolecular-quenching rate constant in the complex, k_q, can be obtained for both fluorophores from *Eqn. 2*, where τ_0 is the fluorescence decay time of each chromophore in the absence of ATP (1.2 ns for the naphthalene moiety and 11.4 ns for the anthracene moiety). This calculation yields quite different rate constants for the quenching of the two moieties by ATP inside the complex: $k_q = 2.4 \cdot 10^8\ s^{-1}$ for anthracene and $k_q = \cdot 10^9\ s^{-1}$ for naphthalene.

$$\phi_0/\phi = 1 + k_q\, \tau_0 \tag{2}$$

To check these values, fluorescence decays for compound **L9** were measured in the presence of $[ATP] = 3.62 \cdot 10^{-3}$ M, *i.e.*, at the plateau. The decay time associated with the naphthalene moiety in the complex is reduced to 0.48 ns, and the decay time of the anthracene moiety is reduced to 3.6 ns, yielding $k_q = 1.9 \cdot 10^8\ s^{-1}$ for anthracene and

$k_q = 1.3 \cdot 10^9\ s^{-1}$ for naphthalene, in excellent agreement with the values obtained from the steady-state data.

In conclusion, ATP is capable of quenching the emission from both fluorophores, but the quenching of naphthalene is one order of magnitude faster. However, energy transfer from naphthalene to anthracene is still very important. If the anthracene emission obtained at λ_{exc} 295 nm is considered, discounting the contribution through its direct excitation, which is $0.1 \cdot 0.36 = 0.036$ (10% of 0.36), there is still a fraction of 0.254 ($= 0.29 - 0.036$) that is due to the energy-transfer process ($\eta > 0.78$).

3. Conclusions. – The herein-reported studies on the interaction of ATP with several series of naphthalene- and anthracene-containing open-chain polyamines allow one to derive some general conclusions. First, for the receptors sharing a given polyamine chain, those containing one anthracene moiety interact much more strongly with ATP than those with just one naphthalene moiety. However, they present stability-constant values comparable to those containing naphthalene moieties at both ends. On the other hand, the chloride anions compete effectively with the nucleotide for the binding of the receptor.

NMR Studies show that π-π stacking is present throughout the entire pH range where interaction occurs. NOE Experiments confirm the proximity of the aromatic-ring systems of the receptors and ATP and have allowed to propose preliminary models for the interaction.

Steady-state fluorescence measurements with theses systems show the existence at acidic pH of a quenching effect of the emission of the fluorophores following the protonation of the adenine ring. The analysis of the data makes it possible to attribute this quenching to an electron transfer from the naphthalene or anthracene moieties to the protonated adenine ring, this process being more favorable for naphthalene. Time-correlated single-photon counting shows that, even in the presence of ATP, there is an important energy transfer from naphthalene to anthracene in the bis-chromophoric-receptor **L9**.

Experimental Part

Materials. The NaCl used as background electrolyte was a *Merck* 'suprapur' product. CO_2-Free NaOH solns. were prepared as described in [10]. The sodium salts of ATP, ADP, and AMP were from *Fluka* (purity > 99%). The synthesis of receptors **L1** – **L3** and **L5** – **L6** was accomplished as described in [13d], of **L9** as reported in [15], of **L4** and **L14** as described in [13b], and of **L12** and **L13** as reported in [13a]. For **L7**, **L8**, and **L10**, similar procedures to those described in [13a] and [13d] were used (see below). The compounds were handled as their hydrochloride salts.

N-*{2-[(2-Aminoethyl)amino]ethyl}*-N′-*{2-[(naphthalen-1-ylmethyl)amino]ethyl}ethane-1,2-diamine Pentakis(hydrochloride)* (**L7** · 5 HCl). Tetraethylenepentamine (= *N*-(2-aminoethyl)-*N*′-{2-[(2-aminoethyl)amino]-ethyl}ethane-1,2-diamine; 5.68 g, 30 mmol) and naphthalene-1-carboxaldehyde (0.94 g, 6 mmol) were stirred for 72 h in EtOH (75 ml). $NaBH_4$ (0.38 g, 10 mmol) was then added and the resulting soln. stirred overnight. The EtOH was evaporated, the resulting residue treated with H_2O, and the product repeatedly extracted with CH_2Cl_2 (3×30 ml). The org. phase was dried (Na_2SO_4) and evaporated to yield the free amine, which was dissolved in EtOH and precipitated as the hydrochloride salt: **L7** · 5 HCl (90%). M.p. 239 – 242°. ^{1}H-NMR (D_2O): 3.18 – 3.55 (*m*, 16 H); 4.72 (*s*, 2 H); 7.40 – 7.59 (*m*, 4 H); 7.85 – 8.01 (*m*, 3 H). ^{13}C-NMR (D_2O): 35.5; 43.0; 43.6; 43.7; 43.9; 44.9; 48.9; 50.1; 51.8; 122.8; 125.9; 126.2; 127.1; 127.9; 129.4; 129.9; 131.1; 133.9. Anal. calc. for $C_{19}H_{36}Cl_5N_5$: C 44.59, H 7.09, N 13.68; found: C 44.9, H 7.2, N 13.7.

N-*(Naphthalen-1-ylmethyl)-3,6,9,12-tetraazatetradecane-1,14-diamine Hexakis(hydrochloride)* (**L8**·6 HCl). As described for **L7**, starting from pentaethylenehexamine (= 3,6,9,12-tetraazatetradecane-1,14-diamine): **L8**·6 HCl (20%). M.p. 247–250°. ^{1}H-NMR (D_2O): 2.79–3.60 (*m*, 20 H); 4.80 (*s*, 2 H); 7.49–7.70 (*m*, 4 H); 7.91–8.19 (*m*, 3 H). ^{13}C-NMR (D_2O): 35.6; 43.1; 43.7; 44.9; 49.0; 49.3; 50.2; 51.9; 52.9; 122.8; 125.9; 126.2; 127.1; 127.9; 129.4; 129.9; 131.1; 133.9. Anal. calc. for $C_{21}H_{42}Cl_6N_6$: C 42.66, H 7.16, N 14.21; found: C 42.4, H 7.3, N 14.3.

N-*(2-Aminoethyl)*-N′-*{2-[(anthracen-9-ylmethyl)amino]ethyl}ethane-1,2-diamine Tetrakis(hydrochloride)* (**L10**·4 HCl). As described for **L7**, with triethylenetetramine (= *N*,*N*′-(2-aminoethyl)ethane-1,2-diamine) and anthracene-9-carboxaldehyde: **L10**·4 HCl (64%). M.p. 240–244°. ^{1}H-NMR (D_2O): 3.32–3.43 (*m*, 12 H); 5.16 (*s*, 2 H); 7.49 (*t*, 2 H); 7.60 (*t*, 2 H); 8.01 (*d*, 2 H); 8.12 (*d*, 2 H); 8.51 (*s*, 1 H). ^{13}C-NMR (D_2O): 35.8; 43.6; 43.8; 44.0; 44.3; 44.7; 120.0; 122.6; 125.7; 128.0; 129.7; 130.4; 130.9. Anal. calc. for $C_{21}H_{32}Cl_4N_4$: C 52.29, H 6.69, N 11.62; found: C 52.4, H 6.91, N 11.3.

Spectrophotometric and Spectrofluorimetric Titrations. Absorption spectra were recorded on a *Perkin-Elmer Lambda-6* spectrophotometer and fluorescence emission on a *SPEX F111-Fluorolog* spectrofluorimeter. $HClO_4$ and NaOH were used to adjust the pH values that were measured with a *Metrohm 713* pH meter. The linearity of the fluorescence emission *vs.* concentration was checked in the concentration range used (10^{-4}–10^{-6} M). The absorbance of the excitation wavelength was maintained at lower than 0.15. When excitation was carried out at wavelengths different than the isosbestic points, a correction for the absorbed light was performed. Fitting of the emission intensity *vs.* [substrate]/[receptor] curves was performed as described in [10].

Fluorescence lifetimes were measured by the time-correlated single-photon-counting technique (TCSPC), as described elsewhere [15]. The fluorescence decays were analyzed with the method of modulating functions implemented by *Stricker*, with automatic correction for the photomultiplier 'wavelength shift' [22]. All measurements were made in the presence of oxygen to reproduce the conditions under which steady-state fluorescence data was obtained.

emf *Measurements.* The potentiometric titrations were carried out at 298.1 ± 0.1 K in 0.15M NaCl. The experimental procedure used (burette, potentiometer, cell, stirrer, microcomputer, *etc.*) has been fully described elsewhere [23]. The acquisition of the *emf* data was performed with the computer program PASAT [24]. The reference electrode was an Ag/AgCl electrode in sat. KCl soln. The glass electrode was calibrated as a hydrogen-ion-concentration probe by titration of previously standardized amounts of HCl with CO_2-free NaOH solns. and determining the equivalent point by *Gran*'s method [25], which gives the standard potential, E^0, and the ion product of water ($pK_w = 13.73(1)$). The concentrations of the different metal ions employed were determined gravimetrically by standard methods.

The computer program HYPERQUAD [26], was used to calculate the protonation and stability constants. At least three titration curves were performed for each system (*ca.* 100 exper. points). The pH range investigated was 2.5–10.5, and the concentration of ATP and receptors ranged from $3 \cdot 10^{-4}$ M to a maximum value of $1.5 \cdot 10^{-3}$ M). The constants of ATP were measured at ionic strengths 0.15M and 1.0M NaCl to evaluate the influence of the ionic strength on the constants for the ATP-adduct formation (*Table 1*). The protonation constants of the receptors **L1**–**L6** and **L11**–**L14** were taken from [13][14] and those of **L9** from [15]. The protonation constants of **L7**, **L8**, and **L10** were determined in this work in 0.15M NaCl at 298.1 K. Finally, the protonation constants of **L2** and **L6** were also determined in 1M NaCl at 298.1 K to calculate the ATP-adduct stabilities at this ionic strength (*Table 1*).

The different titration curves for each system (at least two titrations) were treated either as a single set or as separate curves without significant variations in the values of the stability constants. The sets of data were merged and treated simultaneously to give the final stability constants. Moreover, several measurements were made both in formation and in dissociation (from acid to alkaline pH and *vice versa*) to check the reversibility of the reactions.

NMR Measurements. ^{1}H- and ^{13}C-NMR Spectra: *Varian Unity-300* spectrometer; at 299.95 (^{1}H) and 75.43 MHz (^{13}C); δ in ppm; dioxane as reference (= 67.4 ppm) for ^{13}C and solvent for ^{1}H. The concentrations of ATP and of the receptors for the NMR measurements were in the range $2.5 \cdot 10^{-4} - 10^{-3}$ M. Within the concentration range used, dilution experiments showed maximum shifts of the aromatic signals of *ca.* 0.005 ppm discarding significant self-aggregation processes in anyone of the studied systems. ^{31}P-NMR Spectra: *Bruker Avance-DPX-300-MHZ* spectrometer; at 121.495 MHz; δ rel. to an external reference of 85% H_3PO_4. NOE Connectivities: at pD 4 with a *Varian Unity-400* spectrometer; at 399.95 MHz for ^{1}H. ROESY (rotating-frame spectroscopy): *Bruker Avance-DPX-500* spectrometer; at 500.130 MHz and with three different mixing times, 100, 150 and 600 ms; pH 4, *T* 298 K. Adjustments to the desired pH were made with drops of DCl or NaOD solns. The pD was calculated from the measured pH values with the correlation pH = pD – 0.4 [27].

We thank Prof. *H.-J. Schneider*, University of Saarbrücken, for helpful comments and revision of the manuscript. Financial support from *DGICYT*, project BQU2000-1424 (Spain), and *Fundação para a Ciência e Tecnologia*, project 32442/99 (Portugal), PRAXIS/QUI/10137/98, HPRN-CT-2000-29 (EC), is gratefully acknowledged. *M. T. A.*, *R. A.*, and *P. D.* want to thank *Generalitat Valenciana*, *Ministerio de Educación Cultura y Deporte*, and *Ministerio de Ciencia y Tecnología* for their respective PhD grants.

REFERENCES

[1] J.-M. Lehn, *Angew. Chem., Int. Ed.* **1988**, *27*, 89; J.-M. Lehn, 'Supramolecular Chemistry. Concepts and Perspectives', VCH, Weinheim, 1995.
[2] F. P. Schmidtchen, *Top. Curr. Chem.* **1986**, *132*, 101.
[3] M. E. Huston, E. U. Akkaya, A. W. Czarnik, *J. Am. Chem. Soc.* **1989**, *111*, 8735.
[4] B. Dietrich, *Pure Appl. Chem.* **1993**, *65*, 1457.
[5] A. Bianchi, K. Bowman-James, E. Garcia-España, 'Supramolecular Chemistry of Anions', Wiley-VCH, New York, 1997.
[6] P. D. Beer, *Acc. Chem. Res.* **1998**, *31*, 71; P. D. Beer. J. Cadman, *Coord. Chem. Rev.* **2000**, *205*, 131; P. D. Beer, P. A. Gale, *Angew. Chem., Int. Ed.* **2001**, *40*, 486; P. A. Gale, *Coord. Chem. Rev.* **2001**, *213*, 79.
[7] J. Edwards, R. Sprung, R. Sprague, D. Spence, *Analyst* **2001**, *126*, 1257; R. Marczak, V. T. Hoang, K. Noworyta, M. E. Zandler, W. Kutner, F. D'Souza, *J. Mat. Chem.* **2002**, *12*, 2123; A. Denessiouk, V.-V. Rantanen, M. S. Johnson, *Proteins: Struct., Funct., Genet.* **2001**, *44*, 282; T. Sakakibara, S. Murakami, N. Hattori, M. Nakajima, K. Imai, *Anal. Biochem.* **1997**, *250*, 157; T. Kamidate, S. Niwa, N. Nakata, *Anal. Chim. Acta* **2000**, *424*, 169; P. Ronner, E. Friel, K. Czerniawski, S. Fraenkle, *Anal. Biochem.* **1999**, *257*, 208; F. Dai, J. A. Kelley, H. P. Zhang, N. Malinowski, M. F. Kavlick, J. Lietzau, L. Welles, R. Yarchoan, H. Ford Jr., *Anal. Biochem.* **2001**, *288*, 52.
[8] J. Rebek Jr., *Science (Washington, D.C.)* **1987**, *235*, 1478; A. D. Hamilton, D. J. Van Engen, *J. Am. Chem. Soc.* **1987**, *109*, 5035; M. W. Hosseini, J.-M. Lehn, M. P. Mertes, *Helv. Chim. Acta* **1983**, *66*, 2454; M. Shionaya, T. Ikeda, E. Kimura, S. Motoo, *J. Am. Chem. Soc.* **1994**, *116*, 3848.
[9] S. Claude, J.-M. Lehn, F. Schmidt, J.-P. Vigneron, *J. Chem. Soc., Chem. Commun.* **1991**, 1182.
[10] P. Cudic, M. Zinic, V. Tomisic, V. Simeon, J.-P. Vigneron, J.-M. Lehn, *J. Chem. Soc., Chem. Commun.* **1995**, 1073.
[11] M. W. Hosseini, A. J. Blacker, J.-M. Lehn, *J. Am. Chem. Soc.* **1990**, *112*, 3896; H. Fenniri, M. W. Hosseini, J.-M. Lehn, *Helv. Chim. Acta* **1997**, *80*, 786.
[12] J. A. Aguilar, E. García-España, J. A. Guerrero, S. V. Luis, J. M. Llinares, J. A. Ramírez, C. Soriano, *J. Chem. Soc., Chem. Commun.* **1995**, 2237; J. A. Aguilar, E. García-España, J. A. Guerrero, S. V. Luis, J. M. Llinares, J. A. Ramírez, C. Soriano, *Inorg. Chim. Acta* **1996**, *246*, 287.
[13] a) S. Alves, F. Pina, M. T. Albelda, E. García-España, C. Soriano, S. V. Luis, *Eur. J. Inorg. Chem.* **2001**, 405; b) M. A. Bernardo, S. Alves, F. Pina, J. Seixas de Melo, M. T. Albelda, E. Garciá-España, J. M. Llinares, C. Soriano, S. V. Luis, *Supramol. Chem.* **2001**, *13*, 435; c) M. T. Albelda, M. A. Bernardo, P. Díaz, E. García-España, J. Seixas de Melo, F. Pina, C. Soriano, S. V. Luis, *J. Chem. Soc., Chem. Commun.* **2001**, 1520; d) J. S. de Melo, M. T. Albelda, P. Díaz, E. García-España, C. Lodeiro, S. Alves, J. C. Lima, F. Pina, C. Soriano, *J. Chem. Soc., Perkin Trans. 2* **2002**, *5*, 991.
[14] M. T. Albelda, M. A. Bernardo, E. García-España, M.-L. Godino, S. V. Luis, M. J. Melo, F. Pina, C. Soriano, *J. Chem. Soc., Perkin Trans. 2* **1999**, *11*, 2545.
[15] M. T. Albelda, P. Díaz, E. García-España, J. C. Lima, C. Lodeiro, J. S. de Melo, A. J. Parola, F. Pina, C. Soriano, *Chem. Phys. Lett.* **2002**, *353*, 63.
[16] A. Bencini, A. Bianchi, E. García-España, M. Micheloni, J. A. Ramírez, *Coord. Chem. Rev.* **1999**, *188*, 97.
[17] A. V. Eliseev, H.-J. Schneider, *J. Am. Chem. Soc.* **1994**, *116*, 6081.
[18] C. A. Hunter, *Chem. Soc. Rev.* **1994**, *23*, 101.
[19] C. Bazzicalupi, A. Beconcini, A. Bencini, V. Fusi, C. Giorgi, A. Massotti, B. Valtancoli, *J. Chem. Soc., Perkin Trans. 2* **1999**, *8*, 1675.
[20] J. A. Aguilar, B. Celda, V. Fusi, E. García-España, S. V. Luis, M. C. Martínez, J. A. Ramírez, C. Soriano, R. Tejero, *J. Chem. Soc., Perkin Trans. 2* **2000**, *7*, 1323.
[21] D. L. Smith, P. J. Elving, *J. Am. Chem. Soc.* **1962**, *84*, 1412.
[22] G. Stricker, V. Subramaniam, C. A. M. Seidel, A. Volkmer, *J. Phys. Chem. B* **1999**, *103*, 8612; M. Goldenberg, J. Emert, H. Morawez, *J. Am. Chem. Soc.* **1978**, *100*, 7171.

[23] E. García-España, M.-J. Ballester, F. Lloret, J.-M. Moratal, J. Faus, A. Bianchi, *J. Chem. Soc., Dalton Trans.* **1988**, *1*, 101.
[24] M. Fontanelli, M. Micheloni, 'Proceedings of the I Spanish – Italian Congress on Thermodynamics of Metal Complexes', Diputación de Castellón, Castellón, Spain, 1990.
[25] G. Gran, *Analyst* **1952**, *77*, 881; F. J. Rossotti, H. Rossotti, *J. Chem. Educ.* **1965**, *42*, 375.
[26] A. Sabbatini, A. Vacca, A. Gans, P. Gans, *Coord. Chem. Rev.* **1992**, *120*, 389; P. Gans, A. Sabatini, A. Vacca, *Talanta* **1996**, *43*, 1739.
[27] A. K. Convington, M. Paabo, R. A. Robinson, R. G. Bates, *Anal. Chem.* **1968**, *40*, 700.

Received March 31, 2003