A Systematic Evaluation of Molecular Recognition Phenomena. 2. Interaction between Phosphates and Nucleotides with Hexaazamacrocyclic Ligands Containing Diethylic Ether Spacers

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The host−guest interactions between ortho- (Ph), pyro- (Pp), and tripolyphosphate (Tr) anions together with ATP (At), ADP (Ad), and AMP (Am) nucleotides and the two hexaazamacrocyclic ligands 1,15-dioxa-4,8,12,-18,22,26-hexaazacyclooctacosane (Pn) and 1,13-dioxa-4,7,10,16,20,24-hexaazacyclohexacosane (Op) have been investigated by potentiometric equilibrium methods. Ternary complexes are formed in aqueous solution as a result of hydrogen bond formation and Coulombic attraction between the host and the guest. Formation constants for all the species obtained are reported. The selectivity of the Pn and Op ligands with regard to the different phosphate and nucleotide substrates is discussed and illustrated with total species distribution diagrams. A comparison is also carried out, with the results obtained in this work and those obtained previously with three other closely related hexaazamacrocyclic ligands. This comparison manifests the importance of ligand basicity, rigidity, and π-stacking capability in order to understand their binding and selectivity.

Introduction

The design of host molecules as receptors for the recognition of substrate anion guest molecules in aqueous solution is a very important target from an environmental, industrial, and health-related point of view with multiple potential applications.[1,2]

Phosphate type of anions are ubiquitous in biological structure, function, and regulation; thus, their interaction with the corresponding receptors is of special interest.[3]

Coulombic interactions, hydrogen bonding, and $\pi-\pi$ stacking forces are the main elements of the bonding between receptors and nucleobase type of guests, which have to overcome the strong solvation energies of their corresponding individual molecules in polar solvents, especially in aqueous solution.[4] A fundamental condition for a strong host−guest interaction will strongly depend on the geometrical fit of the substrate with respect to the receptor.

Polyazamacrocyclic ligands, in their protonated forms, have been shown to bind certain neutral molecules and anions including phosphates in aqueous solution and in the solid state.[5−7] Furthermore, such macrocyclic ligands have also been reported to catalyze biologically significant reactions of their bounded guests[8] such as ATP hydrolysis and formation.

In a previous report,[6] we have described a systematic evaluation of host−guest interactions between the protonated forms of two hexaazamacrocyclic ligands containing xylylic spacers but with different cavity sizes, 3,7,11,19,23,27-hexaazatricyclo[27.3.1.1^{13,17}]tetratriaconta-1(32),13,15,17(34),-29(33),30-hexaene (Bn) and 3,6,9,17,20,23-hexaazatricyclo-[23.3.1.1^{11,15}]triaconta-1(29),11(30),12,14,25,27-hexaene (Bd) and the series of anions derived from phosphoric, diphosphoric, and triphosphoric acid together with ATP, ADP, and AMP (see Chart 1).

In the present paper we present a systematic evaluation of host−guest binding interactions between three analogous mac-

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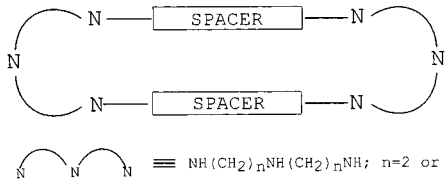
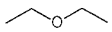
Chart 1. Ligand Abbreviations Used in the Present Work



SPACER	n	Ligand (L)	# ru[a]
	33/33	Bn	28
	22/22	Bd	24
	33/33	Pn	28
	33/22	Op	26
	22/22	Ob	24

[a] Number of ring units.

rocyclic ligands with different cavity sizes but all containing ethyl ether spacers namely, 1,15-dioxa-4,8,12,18,22,26-hexaazacyclooctacosane (Pn), 1,13-dioxa-4,7,10,16,20,24-hexaazacyclohexacosane (Op), 1,13-dioxa-4,7,10,16,19,22-hexaazacyclotetracosane (Ob), and the phosphate type of substrates mentioned above.

The complete set of data related to the recognition constants for the different protonated species of these five macrocycles with the six phosphate and nucleotide substrates makes it possible to obtain a quantitative assessment of the different factors governing the host−guest binding interactions. Furthermore, it allows one to quantitatively evaluate the selectivity of a ligand receptor toward two different substrates or a substrate toward two different ligand receptors.

Experimental Section

Materials. The ligands Pn and Op were prepared as a colorless hexabromide salts according to published procedures.[9] GR grade KCl was obtained from EM Chemical Co., and CO_2-free Dilut-it ampules of KOH were purchased from J. T. Baker Inc. Reagent grade potassium dihydrogen phosphate and tetrasodium pyrophosphate were purchased from Fischer Scientific Co. and purified by recrystallization from distilled water. Sodium tripolyphosphate (technical grade, 85%) was purchased from Aldrich Chemical Co. and was purified by repeated crystallization from aqueous solution by the addition of methanol.[10] Adenosine-5′-monophosphate, adenosine-5′-diphosphate sodium salt hydrate, and adenosine-5′-triphosphate disodium salt hydrate were purchased from Aldrich Chemical Co. ATP was recrystallized from methanol and water, and ADP and AMP were used as received. The KOH solution was standardized by titration against standard potassium acid phthalate with phenolphthalein as indicator and was checked periodically for carbonate content (<2%).[11a]

Potentiometric Titrations. Potentiometric measurements were conducted in a jacketed cell thermostated at 25.0 °C and kept under an inert atmosphere of purified argon. A Corning model 350 pH meter fitted with glass and calomel reference electrodes was used. KCl was employed as supporting electrolyte to maintain the ionic strength at 0.10 M. The apparatus was calibrated in terms of $-\log [H^+]$, designated as p[H], by titration of a small quantity of diluted HCl at 0.10 M ionic strength and 25 °C followed by adjustment of the meter so as to minimize the calculated p[H] vs observed values. $\log K_w$ for the system, defined in terms of $\log([H^+][OH^-])$, was found to be -13.78 at the ionic strength employed[12] and was maintained fixed during refinements.

Potentiometric measurements of solutions containing equimolecular amounts of Bn and the appropriate phosphate or nucleotide anion were made at concentrations approximately 1 mM and ionic strength μ = 0.10 M (KCl). Each titration utilized at least 10 points per neutralization of a hydrogen ion equivalent with titrations being repeated until satisfactory agreement was obtained. A minimum of three sets of data was used in each case to calculate the overall stability constants and their standard deviations. The standart devaitions obtianed for the different recognition constants are ±0.02 of the last significant figure reported in Table 3. The range of accurate p[H] measurements was considered to be 2−12. Equilibrium constants and species distribution diagrams were calculated using the programs BEST[11a] and SPEXY, respectively.[11b]

^{1}H NMR Experiments. The ^{1}H NMR spectra were recorded on a Bruker 200 MHz spectrometer in D_2O using DSS as internal standard (chemical shifts are reported downfield from DSS). The measurements were carried out on equilibrated nucleotide substrates (1.0×10^{-3} M) with the ligand Bn (1.0×10^{-3} M) at 300 K. The pH was adjusted to the desired value upon addition of small amounts of a 0.1 M KOD solution in D_2O.

Table 1. Logarithm of Protonation Constants of Related Hexaazamacrocyclic Ligands

equilib quotient	Pn	Bn	Op	Ob	Bd
$K^H_1 = [HL]/[L][H]$	10.38	10.33	10.14	9.58	9.51
$K^H_2 = [H_2L]/[HL][H]$	9.73	9.73	9.20	8.89	8.77
$K^H_3 = [H_3L]/[H_2L][H]$	8.82	8.56	8.44	8.26	7.97
$K^H_4 = [H_4L]/[H_3L][H]$	8.05	7.77	7.71	7.64	7.09
$K^H_5 = [H_5L]/[H_4L][H]$	7.36	7.22	6.95	3.79	3.79
$K^H_6 = [H_6L]/[H_5L][H]$	6.80	6.64	3.74	3.36	3.27
$\sum \log K^H_i$	51.14	50.24	46.18	41.52	40.40
ref	9	5c	9	8c	7a

Results and Discussion

Protonation Constants of the Ligands and Substrates. The systems described in the present work consist of hexaazamacrocyclic ligands with all six amines being secondary. The macrocycles differ from one another in the following: (a) the length of their arms and the number of methylenic units bonding the secondary amines; (b) in the nature of their spacers which can be either the 1,5-dimethylenebenzene or the diethylene ether group as shown in Chart 1.

Table 1 presents the logarithms of the stepwise protonation constants obtained for the ligands studied from the mathematical

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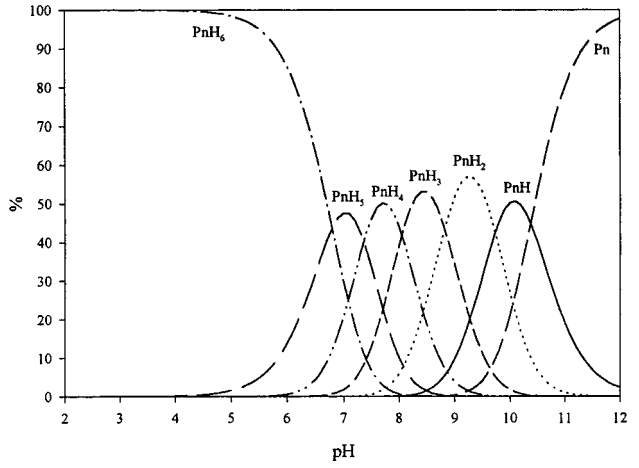
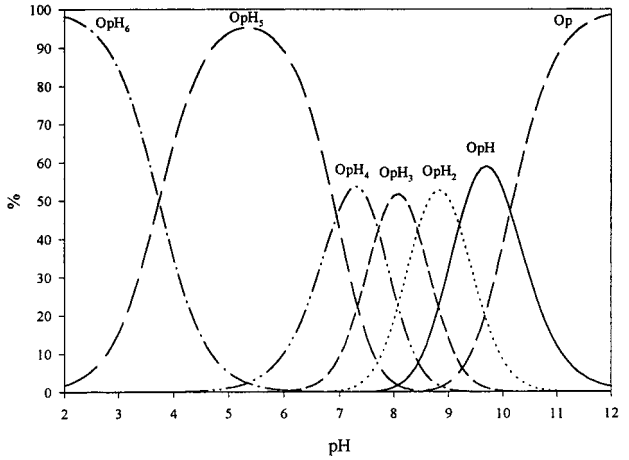


a



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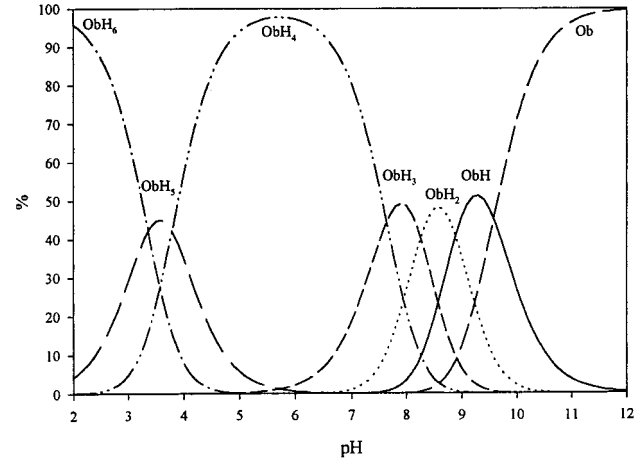
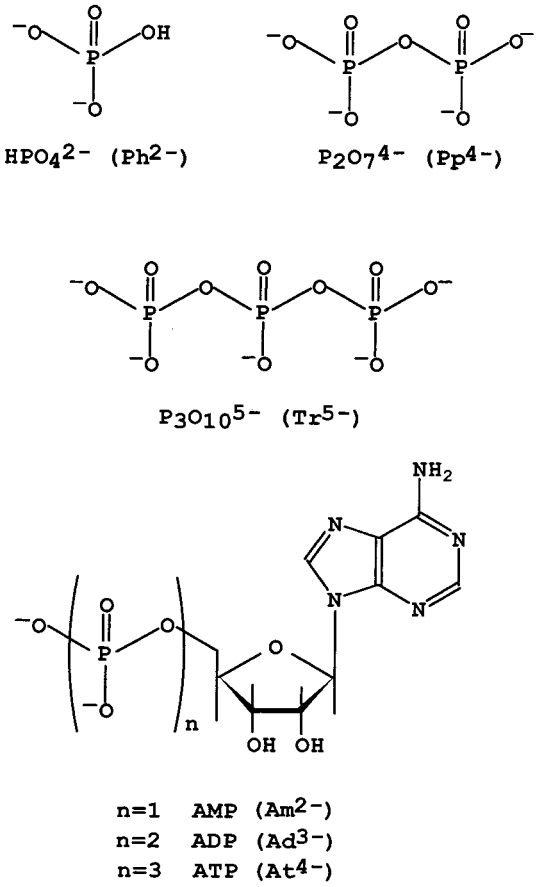


c

Figure 1. Species distribution diagrams for the Pn, Op, and Ob ligands as a function of p[H].

treatment of their respective potentiometric titrations. For Pn and Bn, 33/33 type of ligands, all six amino nitrogens are moderately to strongly basic, whereas, for Ob and Bd (22/22), they have four amino nitrogens which behave as strong to moderate bases while two of them behave as weak bases. Finally, Op (22/33) has five strong to moderate and one weakly basic amino nitrogens. As can be deduced from Table 1, the basicity of the ligand is mainly determined by the number of

Chart 2. Phosphate and Nucleotide Substrates and Their Corresponding Abbreviations Used in the Present Work



methylenic units bonded within each secondary amine with a lesser effect produced by the spacer. As the number of methylenic units increases, the basicity of the ligands also increases due to the inductive effects of the CH_2 units and also because the protonated ammonium groups lie further apart from one another (each methylenic unit contributes roughly by 2.5 log units to the overall log β_6 ($\sum$log K^{H}_i)).

Figure 1 shows the species distribution diagrams for Pn, Op, and Ob on the basis of the constants reported in Table 1. Similar diagrams for Bn and Bd have previously been reported.[6] For Pn and Bn (33/33) the H_6L^{6+} species predominate from the p[H] range 2−5 and the H_5L^{5+} species does not significantly start to form until p[H] = 5. At higher p[H] (7−10) the remaining lower protonation species are expressed. In sharp contrast for Ob and Bd (22/22) the zone of predominance of the H_6L^{6+} is dramatically reduced and the zone of predominance of the H_5L^{5+} decreases by roughly 2 p[H] units with regard to the 33/33 type of ligands.

When macrocycles are compared with the same arm length but with different spacers, it is found that the diethyl ether group always produces larger protonation constants than the *m*-xylyl group. This is due to the higher flexibility of the alkyl ether group compared to the aromatic *m*-xylyl group that makes it possible for the positive charges to be further apart. Also, there is a slightly higher electron-withdrawing effect exerted by the *m*-xylyl group compared to the alkyl ether group (log K^{H}_1-(benzylamine) 9.49 and log K^{H}_1(methoxyethylamine) 9.62, both measured at 25 °C with μ = 1 M).[12]

The substrates (S) used in the present work include three inorganic polyphosphates and their homologous nucleotides (see Chart 2).

Table 2. Logarithm of Protonation Constants of the Phosphate and Nucleotide Substrates[12]

equilib quotient	Tr	Pp	Ph	At	Ad	Am
$K^{H}_1 = [HS]/[S][H]$	7.79	8.42	11.63	6.50	6.35	6.21
$K^{H}_2 = [H_2S]/[HS][H]$	5.51	6.00	6.75	3.90	3.88	3.81
$K^{H}_3 = [H_3S]/[H_2S][H]$	1.86	1.69	1.89			

Table 2 presents the protonation constants for all six substrates. As it can be observed, in all of them only the unprotonated and monoprotonated species are moderate to strongly basic. The diprotonated species in all cases have log *K* values lower than 2 indicating that they are very weak bases. The fourth protonation constants for diphosphate and triphosphate are below the range of accurate measurement by regular potentiometry.

Given the specific nature of the monophosphate substrate and the experimental working conditions, only its second and third protonation constants will be relevant for the description of ternary complexes. Thus the Ph abbreviation in the ternary complexes described in the present work is used to symbolize the HPO_4^{2-} species.

Formation of Ternary Species H:Pn:S. With the individual protonation constants for Pn and for each substrate precisely known then the potentiometric data (Figure S1) of a solution containing an equimolecular amount of ligand and substrate can be resolved giving the nature and log K^R values of the species generated (see Table 3).

For the Pn−Tr system ($\sigma_{fit} = 0.0036$) the presence of five equilibrium species detected can be expressed as follows:

$$H_4Pn^{4+} + Tr^{5-} \rightleftarrows H_4PnTr^{-} \qquad \log K^{R}_4 = 4.67 \quad (1)$$

$$H_5Pn^{5+} + Tr^{5-} \rightleftarrows H_5PnTr \qquad \log K^{R}_5 = 7.06 \quad (2)$$

$$H_6Pn^{6+} + Tr^{5-} \rightleftarrows H_6PnTr^{+} \qquad \log K^{R}_6 = 9.27 \quad (3)$$

$$H_6Pn^{6+} + HTr^{4-} \rightleftarrows H_7PnTr^{2+} \qquad \log K^{R}_7 = 6.71 \quad (4)$$

$$H_6Pn^{6+} + H_2Tr^{3-} \rightleftarrows H_8PnTr^{3+} \qquad \log K^{R}_8 = 4.51 \quad (5)$$

Here K^R_i are the recognition constant of protonation degree *i* and are listed in order of appearance from high to low p[H].

Figure 2 shows the species distribution diagram as a function of p[H] obtained for the 1:1 Pn−substrate (for both inorganic phosphates and nucleotides) systems. For the Pn−Tr system, it is interesting to note that over the p[H] range 2−9 the predominant species are always H_iPnTr complexes rather than the individual species derived from the protonation of Pn ligand and the Tr^{5-} anion. It is also worth noting that for this system even at p[H] 2 the complex species, H_8PnTr^{3+}, has an abundance of over 80% while the free ligand, H_6Pn^{6+}, is only about 15%.

The recognition constant values obtained for this system lay within the range of previously reported host−guest interactions with hexaazamacrocyclic amines and phosphates containing aromatic[13,14] and aliphatic[15] spacers as shown also in Table 3.

The highest equilibrium constant for the present ternary recognition complexes H:Pn:Tr described in eqs 1−5 corresponds to the formation of the species H_6PnTr^{+}, log $K^R_6 = 9.27$.

Table 3. Logarithm of Recognition Constants, log K^R_i, for the Pn, Bn, Op, Ob, and Bd Ligands with Phosphate and Nucleotide Substrates

stoich L:S:H	equilib quotient	Pn	Bn	Op	Ob	Bd
	A. Triphosphate (Tr)					
1:1:1	[HLTr]/[HL][Tr]					3.51
1:1:3	$[H_3LTr]/[H_3L][Tr]$					4.71
1:1:4	$[H_4LTr]/[H_4L][Tr]$	4.67	4.56	5.46		6.47
1:1:5	$[H_5LTr]/[H_5L][Tr]$	7.06	6.61	7.64		10.85
1:1:6	$[H_6LTr]/[H_6L][Tr]$	9.27	8.60	10.93		14.19
1:1:7	$[H_7LTr]/[H_6L][HTr]$	6.71	6.76	7.66		11.06
1:1:8	$[H_8LTr]/[H_6L][H_2Tr]$	4.51	4.52	4.78		7.57
	$1000\sigma_{fit}$ or ref	3.6	6	3.7		7a
	B. Pyrophosphate (Pp)					
1:1:1	[HLPp]/[HL][Pp]				2.07	
1:1:2	$[H_2LPp]/[H_2L][Pp]$				2.41	
1:1:3	$[H_3LPp]/[H_3L][Pp]$		2.57		3.44	
1:1:4	$[H_4LPp]/[H_4L][Pp]$	4.35	4.12	4.98	5.21	5.73
1:1:5	$[H_5LPp]/[H_5L][Pp]$	6.50	6.13	7.06	9.35	9.94
1:1:6	$[H_6LPp]/[H_6L][Pp]$	8.55	7.85	10.19	12.56	13.07
1:1:7	$[H_7LPp]/[H_6L][HPp]$	5.38	5.25	6.57	8.80	6.14
1:1:8	$[H_8LPp]/[H_6L][H_2Pp]$	2.42	2.93	2.99	4.86	
	$1000\sigma_{fit}$ or ref	3.8	6	4.0	15e	7a
	C. Monophosphate (Ph)					
1:1:1	[HLPh]/[HL][Ph]	2.04		1.78		
1:1:2	$[H_2LPh]/[H_2L][Ph]$	1.93		1.98	1.49	
1:1:3	$[H_3LPh]/[H_3L][Ph]$	2.10		2.18	1.85	
1:1:4	$[H_4LPh]/[H_4L][Ph]$	2.36	2.13	2.70	2.64.	2.87
1:1:5	$[H_5LPh]/[H_5L][Ph]$	3.29	2.96	3.34	5.29	5.47
1:1:6	$[H_6LPh]/[H_6L][Ph]$	3.89	3.50	5.42	6.97	7.36
1:1:7	$[H_7LPh]/[H_6L][HPh]$	1.86		2.35		
	$1000\sigma_{fit}$ or ref	4.1	6	1.9	15a	7a
	D. ATP (At)					
1:1:3	$[H_3LAt]/[H_3L][At]$		2.59	2.52		3.35
1:1:4	$[H_4LAt]/[H_4L][At]$	3.68	4.07	4.17	4.80	5.27
1:1:5	$[H_5LAt]/[H_5L][At]$	5.33	5.76	5.76	8.15	8.69
1:1:6	$[H_6LAt]/[H_6L][At]$	7.02	7.13	8.27	11.00	11.16
1:1:7	$[H_7LAt]/[H_6L][HAt]$	4.45	4.97	5.46	7.85	7.88
1:1:8	$[H_8LAt]/[H_6L][H_2At]$	3.77	4.20	4.19	6.75	5.42
	$1000\sigma_{fit}$ or ref	4.8	6	5.9	8c	15d
	E. ADP (Ad)					
1:1:3	$[H_3LAd]/[H_3L][Ad]$					3.07
1:1:4	$[H_4LAd]/[H_4L][Ad]$	2.86	3.39	3.27	3.40	4.37
1:1:5	$[H_5LAd]/[H_5L][Ad]$	4.14	4.54	4.19	6.20	7.42
1:1:6	$[H_6LAd]/[H_6L][Ad]$	5.11	5.37	6.29	8.30	9.47
1:1:7	$[H_7LAd]/[H_6L][HAd]$	2.57	2.98	3.38	5.60	6.24
	$1000\sigma_{fit}$ or ref	7.2	6	6.6	8c	15d
	F. AMP (Am)					
1:1:1	[HLAm]/[HL][Am]					2.1
1:1:2	$[H_2LAm]/[H_2L][Am]$					2.2
1:1:3	$[H_3LAm]/[H_3L][Am]$			1.88		2.7
1:1:4	$[H_4LAm]/[H_4L][Am]$	1.89	2.42	2.20	2.85	3.33
1:1:5	$[H_5LAm]/[H_5L][Am]$	2.62	3.11	2.76	5.50	5.6
1:1:6	$[H_6LAm]/[H_6L][Am]$	3.22	3.62	4.22	6.95	7.12
1:1:7	$[H_7LAm]/[H_6L][HAm]$					3.8
	$1000\sigma_{fit}$ or ref	6.5	6	7.8	8c	15d

Taking into consideration that the first protonation constant of Tr^{5-} is 7.97 (Table 2) which is higher than the sixth protonation constants of Pn (6.80; Table 1) and that the fifth, fourth, and third Pn protonation constants are relatively close (Table 2), there is another possible set of equilibriums that can lead to the formation of H_iPnTr ($i = 4-6$) ternary species:

(13) (a) Nation, D. A.; Martell, A. E.; Caroll, R. I.; Clearfield, A. *Inorg. Chem.* **1996**, *35*, 7246. (b) Lu, Q.; Riebenspies, J. H.; Caroll, R. I.; Martell, A. E.; Clearfield, A. *Inorg. Chim. Acta* **1998**, *270*, 207. (c) Lu, Q.; Riebenspies, J. H.; Martell, A. E.; Motekaitis, R. J. *Inorg. Chem.* **1996**, *35*, 2630.

(14) Bazzicalupi, C.; Bencini, A.; Bianchi, A.; Fusi, V.; Giorgi, C.; Granchi, A.; Paoletti, P.; Valtancoli, B. *J. Chem. Soc., Perkin Trans.* 2 **1997**, 775.

(15) (a) Jurek, P. E.; Martell, A. E.; Motekaitis, R. J.; Hancock. R. D. *Inorg. Chem.* **1995**, *34*, 1823. (b) Motekaitis, R. J.; Martell, A. E. *Inorg. Chem.* **1994**, *33*, 1032. (c) English, J. B.; Martell, A. E.; Motekaitis, R. J.; Murase, I. *Inorg. Chim. Acta* **1997**, *258*, 183. (d) Dietrich, B.; Hosseini, M. W.; Lehn, J.-M. *Helv. Chim. Acta* **1985**, *68*, 289. (e) Motekaitis, R. J.; Martell, A. E. *Inorg. Chem.* **1982**, *60*, 2403.

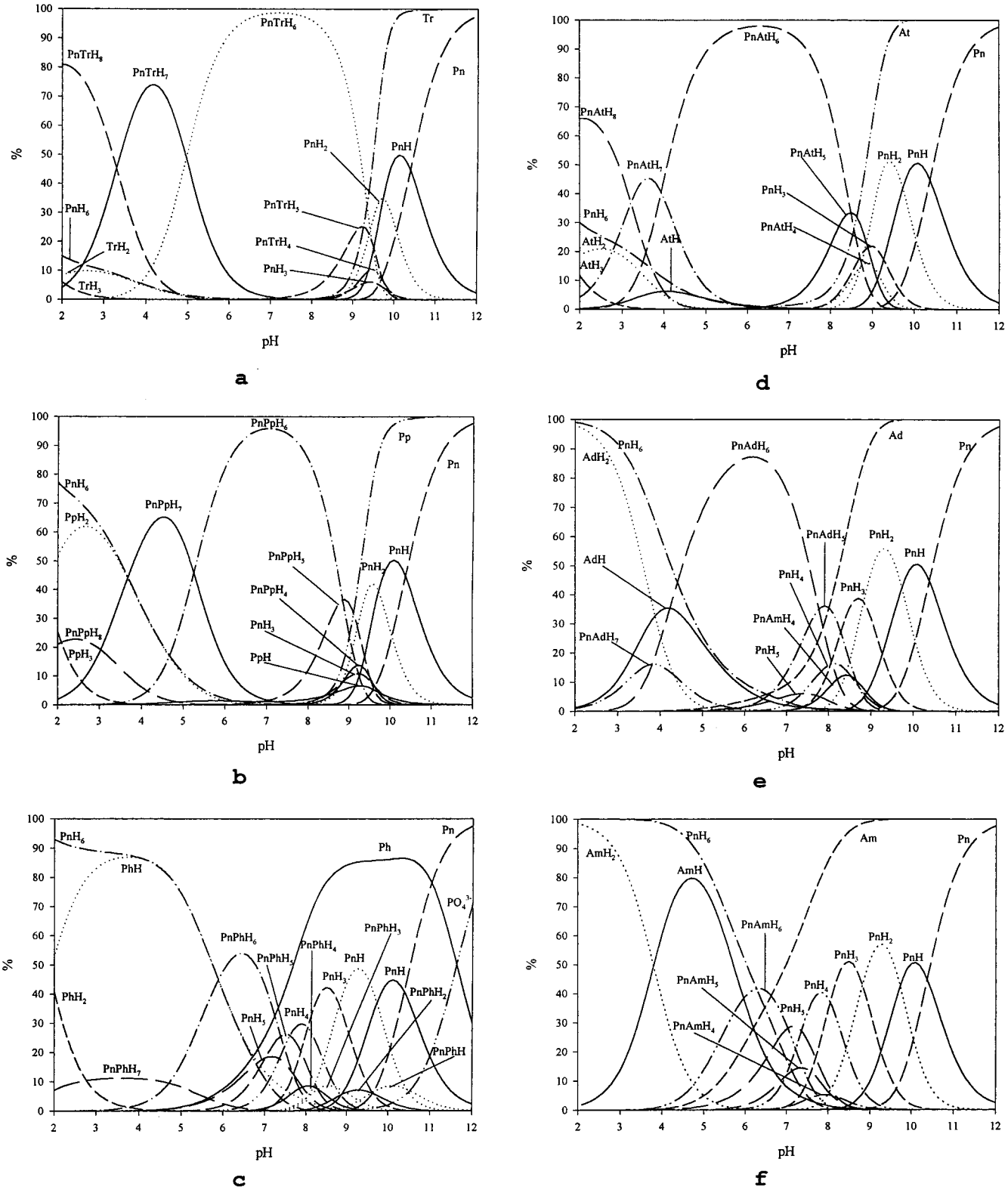


Figure 2. Species distribution diagrams as a function of p[H] for the six Pn−substrate systems.

$$H_3Pn^{3+} + HTr^{4-} \rightleftarrows H_4PnTr^{-} \qquad \log K^{R}_{4} = 3.75 \quad (6)$$

$$H_4Pn^{4+} + HTr^{4-} \rightleftarrows H_5PnTr \qquad \log K^{R}_{5} = 6.45 \quad (7)$$

$$H_5Pn^{5+} + HTr^{4-} \rightleftarrows H_6PnTr^{+} \qquad \log K^{R}_{6} = 8.55 \quad (8)$$

For each species both equilibriums simultaneously operate and their relative importance is a function of p[H]. Similar arguments can be used to propose the following alternative equilibrium, for the formation of H_7PnTr^{2+}:

$$H_5Pn^{5+} + H_2Tr^{3-} \rightleftarrows H_7PnTr^{2+} \qquad \log K^{R}_{7} = 8.01 \quad (9)$$

The interaction of the Pn ligand with the other substrates namely diphosphate, monophosphate and the nucleotides At, Ad and Am has also been studied, and the formation constants for the species obtained in each case are reported in Table 3.

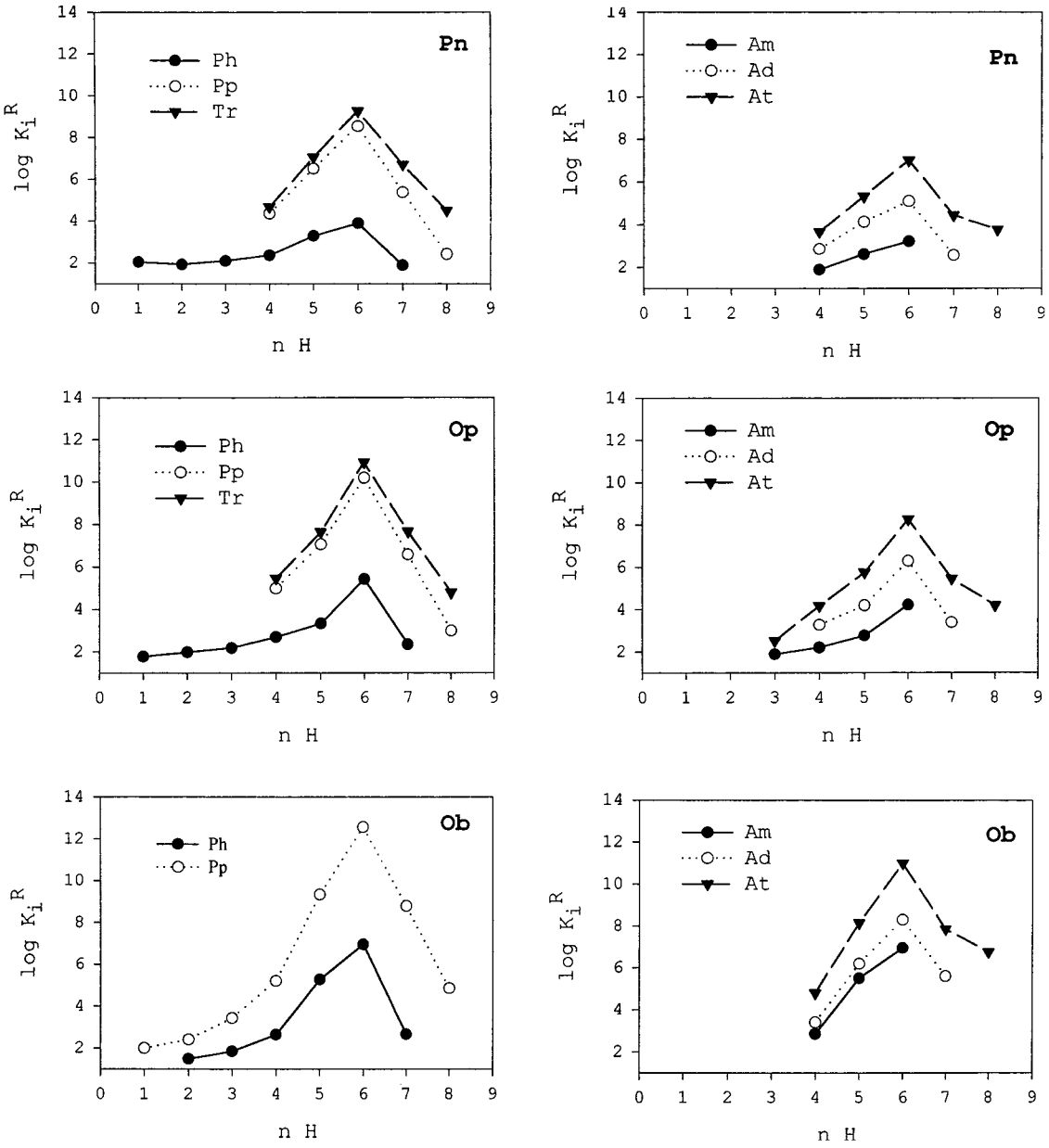


Figure 3. Plots of the log K^R_i versus *n*H (the different ternary species with various degree of protonation) obtained for the (A) Pn−S systems, (B) the Op−S systems, and (C) the Ob−S systems.

Comparison of the formation constants, on the basis of eq 3, for species with general formula H_6PnS^{n+} (S = Tr, Pp, Ph, At, Ad, Am) indicate that log *K* decrease in the manner Tr (9.27) > Pp (8.55) > Ph (3.89) for the phosphates and At (7.02) > Ad (5.11) > Am (3.22) for the nucleotides. This trend is maintained over the ternary species with substrates having the same degree of protonation H_iPnS. This can be graphically observed in Figure 3A where log K^R_i (obtained from the corresponding eqs 1−5) are plotted versus *n*H, the species with various degree of protonation. In the graph it is also clearly seen that the ternary species containing six protons H_6PnS^{n+} always have the higher recognition constant irrespective of the substrate type, either phosphate or nucleotide. The latter suggests that in the present case Coulombic interactions play a predominant role in the molecular recognition phenomenon.

The decrease of the log *K* values observed for the Pp and Ph substrates in the Pn−Pp and Pn−Ph systems is manifested with a decrease of the zone of predominance of their ternary complexes as shown in Figure 2 (Pn−Pp from p[H] 3.5 to p[H] 9.0; Pn−Ph from p[H] 6.0 to 7.0). A similar trend is found for the distribution diagrams of the Pn−nucleotide systems.

Competitive Diagrams and Selectivity. Figure 4a,a′ presents calculated species distribution diagrams for systems with equimolecular amounts of the Pn ligand and two substrates (Tr and Pp) together with their corresponding total species distribution diagrams (this useful type of diagram for selectivity evaluation was first reported in the literature in ref 8b). For the Pn−Tr−Pp competitive system, H:Pn:Tr species predominate over H:Pn:Pp species within the p[H] range 2−9, as a consequence of the higher binding constants found for the Pn−Tr system with regard to the Pn−Pp system. The total species distribution diagram gives a graphical view of the selectivity of the Pn ligand for two different substrates as a function of p[H]. For instance for the Pn−Tr−Pp competitive system (Figure 4a′) at p[H] 2, 88% of the Pn ligand is complexed with the two substrates, 82% forming H:Pn:Tr species and 6% forming H:Pn:Pp species. That implies a selectivity of 93.2% in favor of the Tr complexation against Pp (the selectivity at a

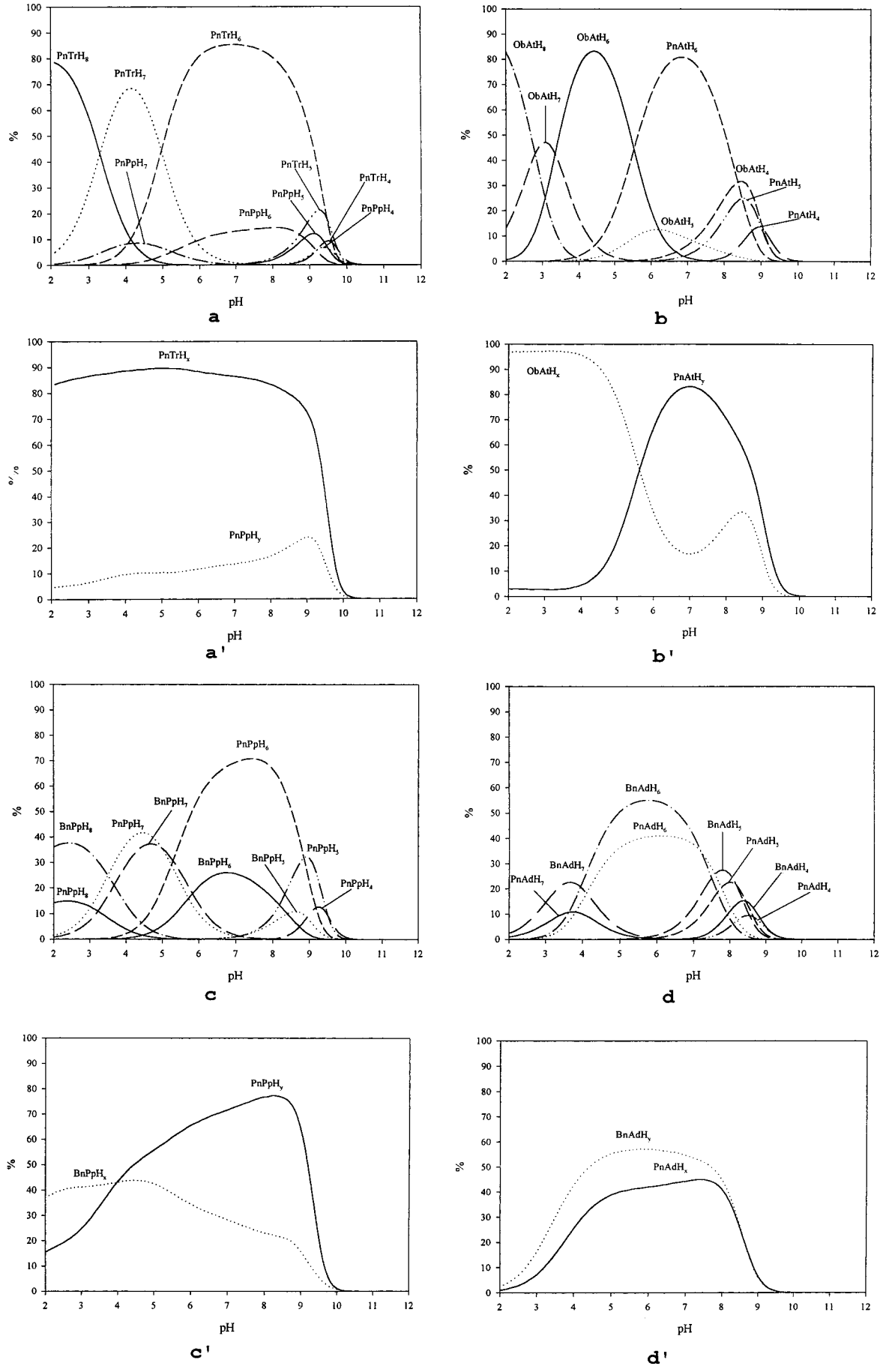


Figure 4. Competitive calculated species distribution diagrams and total species distribution diagrams for the following systems: (a) Pn−Tr vs Pn−Pp; (b) Pn−At vs Ob−At; (c) Bn−Pp vs Pn−Pp; (d) Bn−Ad vs Pn−Ad.

Table 4. $\Delta\log K^{R}_{6}$ ($\log K^{R}_{6}(H_6LS) - \log K^{R}_{6}(H_6L'S)$) for One Substrate and Two Different Macrocyclic Ligands L and L′

L−L′	Tr	Pp	Ph	At	Ad	Am
Op−Pn	1.66	1.64	1.53	1.25	1.18	1.00
Ob−Op		2.37	1.55	2.73	2.01	2.73
Ob−Pn		4.01	3.08	3.98	3.19	3.73
Bn−Pn	−0.67	−0.70	−0.39	0.11	0.26	0.40
Bd−Ob		0.51	0.18	0.64	1.17	0.10

given p[H] for the formation of H_i:Pn:Tr species over H_i:Pn:Pp is defined here as [(% H_i:Pn:Tr)/((% H_i:Pn:Tr) + (% H_i:Pn:Pp))]100)).

When the recognition constants for the complexation of anionic substrates are compared, Table 3 clearly shows that the less basic the ligand the stronger the complexes formed. This can also be graphically observed in Figure 3. For instance, the $\log K^{R}_{6}$ for the At substrate is 7.02 with Pn, 8.27 with Op, and 11.00 with Ob. Given the similarity of the ligands and substrates studied and the parallelism obtained for their respective recognition constants (Table 3, Figure 3) it is worth to analyze their $\Delta\log K^{R}_{6}$, presented in Table 4, defined for a particular substrate and two different macrocyclic ligands (L and L′) as follows:

$$\Delta\log K^{R}_{6} = \log K^{R}_{6}(H_6LS) - \log K^{R}_{6}(H_6L'S)$$

From Table 4, it can be observed that for the Pn−Op ligands, that is 28- and 26-member-ring macrocyclic ligands, respectively, their recognition constants for the substrates differ in 1.00−1.66 log units. For the Op−Ob ligands, a 26 and 24-member-ring macrocycles, the difference now jumps to 2.01−2.73 log units except for the monophosphate system. This increase is clearly an effect attributable solely to the size and nature of the macrocyclic cavity. Thus Ob having a smaller cavity is capable of better fitting the substrate and therefore ends up forming relatively stronger ternary complexes. This is further corroborated by the very similar $\Delta\log K^{R}_{6}$ values obtained for the monophosphate substrate, which is the smallest substrate with negligible fitting effect.

From Figure 4b,b′ it is clear that the selectivity of the competitive system Pn−Ob−S clearly depends on the p[H] even if the differences in the formation constants for the H:Pn:S species and H:Ob:S are always in favor of the latter by up to more than 4 log units (see Tables 3 and 4). This is due to the different basicities of the ligands Pn and Ob as shown in Figure 1. For Pn the H_5Pn^{5+} species does not start to significantly form until p[H] 5 whereas for Ob at p[H] 5 the predominant species is already H_4Ob^{4+}. As a consequence H:Ob:S species predominate from p[H] 2 to 5 while H:Pn:S species predominate from p[H] 5 to 9.

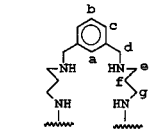
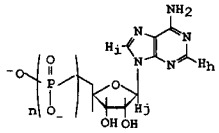
As discussed above for macrocyclic ligands with diethylic ether spacers, the more basic the ligand the weaker their recognition constants (K^{R}_{i}). The same trend is observed for ligands with benzylic spacers where K^{R}_{i} for the Bd ligand are always higher than for Bn (see Table 3 and ref 6). In sharp contrast, when K^{R}_{i} for Pn and Bn ligands are compared, it turns out that the relative strength of the complexes formed depends on the type of substrate whether it is a phosphate or a nucleotide (Tables 3 and 4). The most basic ligand Pn has higher recognition constants than the less basic ligand Bn when complexed with phosphates whereas the opposite happens with the nucleotides. The competitive species and total species distribution diagram (Figure 4c,c′,d,d′) graphically shows this effect. This is due to the fact that the Pn ligand is more flexible than Bn and is therefore capable of better wrapping around the substrate and thus forming stronger bonds even if it is more basic than Bn. With the nucleotides, Bn forms stronger bonds than with Pn due to the $\pi-\pi$ stacking interactions that the nucleotides have with Bn but not with Pn. These $\pi-\pi$ stacking interactions have been further corroborated through NMR spectroscopy. Significant upfield shifts are observed throughout the p[H] range 2.0−9.0, in which the complexes are formed, for practically all the aromatic and benzylic protons of the ligand and nucleotides and also for the nucleotide H_j anomeric protons (see Table 5). For the Bn aliphatic protons a much smaller effect is observed but always in the opposite direction. It is worth mentioning that the benzylic protons for the Bn−Ad and Bn−Am cases besides suffering a remarkable upfield shift, as in the Bn−At case, they also suffer a change in the spin system from A_2 to AB with a geminal coupling of $J_{AB} = 13.01$ Hz and $J_{AB} = 12.99$ Hz, respectively. This result had been previously observed for a related system[5a] and is attributed to the blocking of the chain movement in that region of the molecule for the Bn−Ad and Bn−Am complexes.

It is also worth noting that $\Delta\log K^{R}_{6}$ for the Bn−Pn system increase in the order At < Ad < Am. This is a consequence of the fact that in this sequence the relative degree of bonding due to $\pi-\pi$ interactions with regard to the bonding due to Coulombic interactions and hydrogen bonding reaches a maximum in the Am case.

All the other potential competitive systems bearing any of two ligands or two substrates presented in Charts 1 and 2 can be interpreted using the same arguments used above and are presented as Supporting Information.

In conclusion, for ligand receptors with similar flexibility and no aromatic groups such as Pn, Op, and Ob, the degree of binding with the phosphate and nucleotide substrates to form

Table 5. ^{1}H NMR Chemical Shifts (δ) for the Bn−Nucleotide Complexes Measured at pH = 6.10, Together with the Complexation-Induced ^{1}H NMR Chemical Shifts (CIS, ppm)[a] for Selected Protons

H-labeling scheme	^{1}H NMR chemical shifts and (CIS)/δ								
	Complex	a	b,c	d	d'	e,g	f	h	i	j
	H_6BnAt^{2+}	7.60 (0.02)	7.38 (-0.20)	4.13 (-0.19)	4.13 (-0.19)	3.20 (0.04)	2.18 (0.05)	8.32 (-0.23)	8.06 (-0.21)	5.97 (-0.19)
	H_6BnAd^{3+}	7.52 (-0.07)	7.40 (-0.18)	4.16 (-0.16)	4.06 (-0.26)	3.17 (0.01)	2.16 (0.04)	8.34 (-0.19)	8.05 (-0.21)	5.99 (-0.16)
	H_6BnAm^{4+}	7.43 (-0.15)	7.40 (-0.18)	4.18 (-0.14)	4.06 (-0.26)	3.12 (0.04)	2.12 (0.01)	8.39 (-0.14)	8.03 (-0.23)	6.02 (-0.13)

[a] Negative CIS values are upfield.

ternary complexes (H:L:S) is directly related to their acidity and to the size of their cavity. This can also be extrapolated to the Bn and Bd ligands taking into account that now those ligands have the possibility to enhance the bonding of their corresponding ternary complexes via $\pi-\pi$ interactions. The latter interaction has a key effect that can reverse selectivity on competitive systems bearing ligands with similar basicity but containing or not containing phenyl rings (Pn−Bn−S). Selectivity of a given ligand receptor for two substrates (L−S−S′) is clearly dominated by the log K^R of their corresponding substrates, and although it is p[H] dependent, it does not change strongly with p[H] and even less changes the direction of the selectivity. In sharp contrast, in competitive systems with two ligands and one substrate (L−L′−S) the selectivity of a given ligand for a particular substrate is strongly dependent on the p[H], and it actually can be reversed. This happens even if the affinity of one ligand for a given substrate is 4 orders of magnitude higher than the affinity of another ligand for the same substrate as is the case with Pn−Ob−S. Finally, the conclusions drawn here agree well with those obtained previously for related macrocyclic receptors and phosphates.[5−8]

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Supporting Information Available: Figures S1−S5, showing experimental and calculated curves obtained for potentiometric titrations and species distribution diagrams. This material is available free of charge via the Internet at http://pubs.acs.org.

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