Aryl-bis-(scorpiand)-aza receptors differentiate between nucleotides by combination of aromatic, hydrogen bond and electrostatic interactions

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Supplementary material includes:

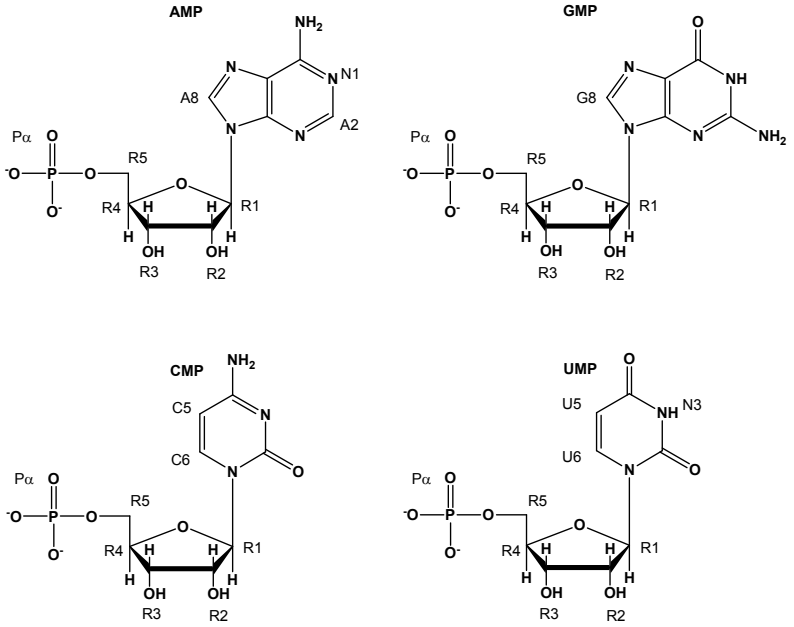

Chart 1. Structure of studied nucleotides and atom labelling

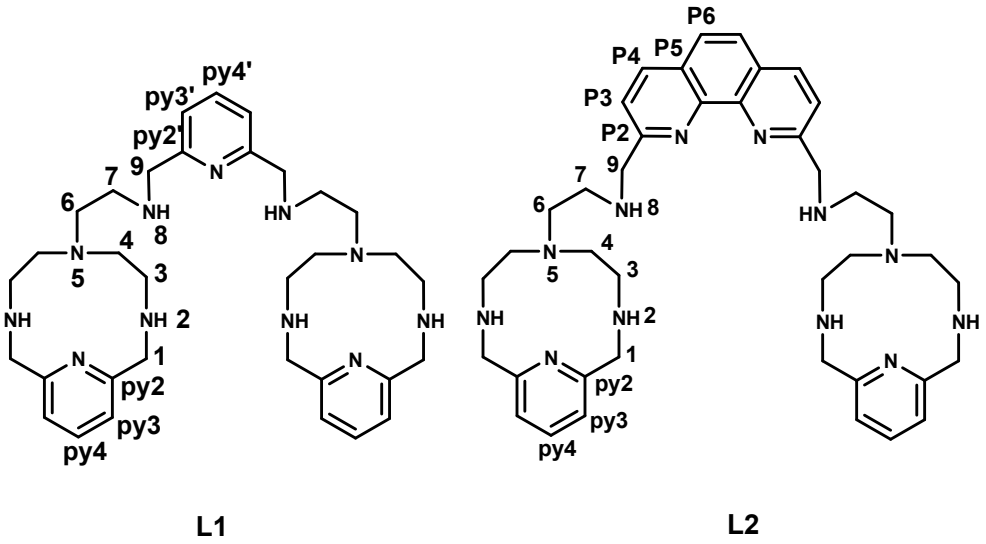

Chart 2. Structure of studied receptors and atom labelling

Table S1.- Logarithms of stepwise protonation constants for the protonation of nucleotide monophosphates (AMP, GMP, CMP and UMP) determined by pH-metric studies and by NMR spectroscopy respectively.

Reaction[a]	Technique	AMP	GMP	CMP	UMP
$H + H_{-1}A \rightleftharpoons A$	*Pot.*[b]		9.45(2)[d]		9.39(1)
$H + A \rightleftharpoons HA$	*Pot.*	6.26(1)	6.30(3)	6.27(1)	6.13(1)
	NMR[c]	6.81(1)	6.62(1)	6.84(1)	6.86(1)
$H + HA \rightleftharpoons H_2A$	*Pot.*	3.87(1)	2.rrrfv 55(1)	4.43(1)	
	NMR	4.25(6)	2.84(3)	4.78(7)	

[a] Charges omitted for clarity. [b] Obtained by pH-metric measurements using NaCl 0.15 M as supporting electrolyte at 298.1 ±0.1 K. [c] Obtained by ^{1}H / ^{31}P NMR. [d] Values in parenthesis are standard deviation in the last significance figure.

Tabla S2. Logarithms of the cumulative stability constants for the formation complexes of nucleotide monophosphates (AMP, GMP, CMP and UMP) with **PYPOD** and **PHENPOD** calculated in 0.15 M NaCl at 298.1 ± 0.1 K.

	PYPOD				**PHENPOD**			
	AMP	GMP	CMP	UMP	AMP	GMP	CMP	UMP
$L + H_{-1}A \rightleftharpoons H_{-1}LA$		3.91(4)[b]						
$L + A \rightleftharpoons LA$		14.14(4)	3.38(3)	13.24(3)		13.91(7)[b]		13.1(1)
$H + L + A \rightleftharpoons HLA$	13.38(3)	24.05(3)	14.19(2)	23.27(1)		23.10(7)	13.52(2)	23.19(4)
$2H + L + A \rightleftharpoons H_2LA$	23.28(1)	33.18(3)	23.91(2)	32.45(2)		32.79(8)	23.34(1)	32.48(9)
$3H + L + A \rightleftharpoons H_3LA$	32.14(2)	41.79(3)	32.70(2)	41.14(1)		41.39(7)	32.33(2)	41.49(5)
$4H + L + A \rightleftharpoons H_4LA$	40.06(2)	49.45(3)	40.49(2)	48.83(2)	39.39(7)	49.65(7)	40.70(2)	49.87(5)
$5H + L+A \rightleftharpoons H_5LA$	47.07(2)	56.38(2)	47.38(2)	55.62(1)	47.17(2)	57.09(4)	47.93(2)	57.17(5)
$6H + L + A \rightleftharpoons H_6LA$	53.52(2)	62.61(3)	53.76(1)	62.06(2)	53.62(2)	63.65(4)	54.20(2)	63.45(5)
$7H + L + A \rightleftharpoons H_7LA$	58.80(2)		58.90(2)		58.88(3)	68.75(5)	59.87(2)	69.05(6)
$8H + L + A \rightleftharpoons H_8LA$							63.99(3)	

[a] Charges omitted for clarity. [b] Values in parenthesis are standard deviation in the last significance figure.

Table S3.- Logarithms of the stepwise constants for the interaction of **PYPOD** and **PHENPOD** with AMP, GMP, CMP and UMP calculated in 0.15 M NaCl at 298.1 ± 0.1 K.

Reaction[a]	**PYPOD**-AMP	**PYPOD**-GMP	**PYPOD**-CMP	**PYPOD**-UMP	**PHENPOD**-AMP	**PHENPOD**-GMP	**PHENPOD**-CMP	**PHENPOD**-UMP
HL + $H_{-1}A$ ⇌ LA		4.18(4)		3.28(3)		3.90(5)[b]		3.0(1)
HL + A ⇌ HLA	3.42(3)[b]	4.57(4)	4.23(2)	3.79(1)		3.26(7)	3.48(2)	3.35(4)
H_2L + A ⇌ H_2LA	3.80(1)	5.19(4)	4.43(2)	4.46(2)		4.06(8)	3.50(1)	3.76(9)
H_3L + A ⇌ H_3LA	4.15(2)		4.71(2)	5.50(1)		4.52(7)	3.61(2)	4.62(5)
H_4L + A ⇌ H_4LA	4.42(2)	4.35(3)	4.85(2)	3.76(1)		3.22(6)	3.83(2)	3.38(5)
H_3L+HA ⇌ H_4LA	5.81(2)							
H_5L + A ⇌ H_5LA	4.65(2)	4.36(3)	4.96(2)	3.80(2)	4.77(1)	3.33(7)	4.28(2)	3.61(5)
H_4L + HA ⇌ H_5LA	5.17(2)	4.51(3)	5.48(2)	3.81(1)		3.99(7)	4.79(2)	4.14(5)
H_5L + HA ⇌ H_6LA	4.84(2)		5.08(1)		5.12(2)		4.28(2)	
H_6L + A ⇌ H_6LA	5.16(2)	4.80(3)	5.40(1)	4.31(2)	5.43(2)	4.60(8)	4.60(2)	4.46(5)
H_6L + HA ⇌ H_7LA	4.18(2)	4.44(3)	4.28(2)	4.12(2)		4.25(6)	4.00(2)	4.28(5)
H_5L + H_2A ⇌ H_7LA			5.78(2)		5.10(1)	3.40(5)		3.93(6)
H_6L + H_2A ⇌ H_8LA					5.59(2)		3.69(3)	

[a] Charges omitted for clarity. [b] Values in parenthesis are standard deviation in the last significance figure.

Table S4.- Signal shifts variation at pD 6.8 for **PYPOD**:MNPs and **PHENPOD**:NMPs systems

	PYPOD:AMP	**PHENPOD**:AMP	**PYPOD**:GMP	**PHENPOD**:GMP	**PYPOD**:CMP	**PHENPOD**:CMP	**PYPOD**:UMP	**PHENPOD**:UMP
R1	0,14[a]	0,54	0,15	0,68	0,17	0,51	0,11	0,40
R3	0,10	0,18	0,15		0,08	0,29	0,13	0,25
R4	0,07	0,13	0,06		0,12	0,26		
R5	0,03	0,02	0,02	0,05	0,07	0,17	0,08	0,11
A2	0,13	0,48						
A8	0,12	0,47						
G8			0,13	0,40				
C5					0,15	0,06		
C6					0,28			
U5							0,14	0,49
U6							0,17	0,42
py4	0,30	0,10	0,12		0,04		0,04	-0,02
py3	0,30	0,08	0,12	0,01	0,04	-0,04	0,04	-0,03
py4'			0,21		0,06		0,06	
py3'	0,30		0,19		0,06		0,06	
P3		0,24				0,04		0,00
P4		0,31		0,19		0,04		0,02
P6		0,40				0,05		0,19
1	0,05	-0,01	0,05	-0,01	0,27	0,11	0,11	0,02
9	0,024	0,14	0,14	0,43	0,05	0,03	0,08	-0,01
3	0,251	0,162	0,073	0,007	0,041	0,01	0,04	-0,004
4	0,272	0,1335	0,069	-0,093	0,047	-0,015	0,057	-0,012
6		0,125	0,055	-0,039	0,018	-0,015	0,035	-0,02
7	0,224	0,098	0,062	-0,156	0,046	-0,033	0,078	-0,021

Shifts measured in ppm and positive value indicates upfield shift while negative value indicates downfield shift.

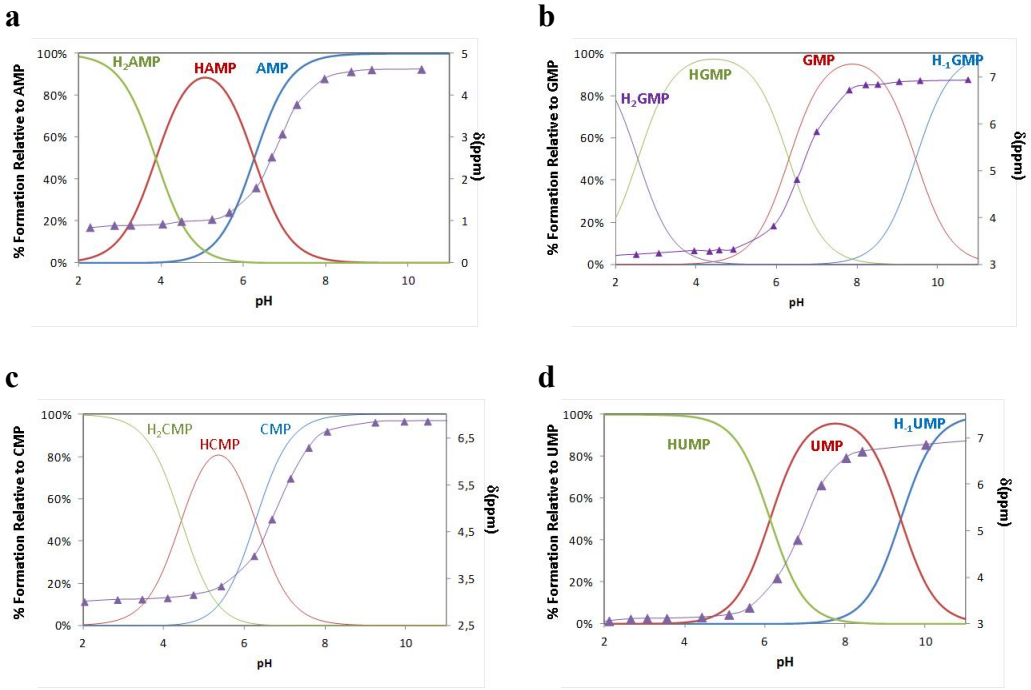

Figure S1.- Distribution diagrams of the protonation of mononucleotides with the ^{31}P NMR signals overlapped at different pD values **AMP** (a), **GMP** (b), **CMP** (c) and **UMP** (d).

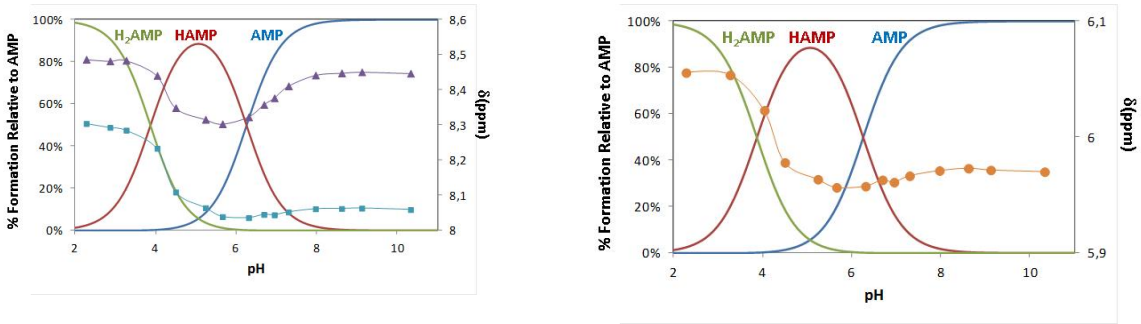

Figure S2.- Distribution diagrams for AMP protonation obtained by pH-metric titrations with ^{1}H NMR chemical shifts of AMP (▲H_{A2}, ■H_{A8} and ●H_{R1}) at different pD values.

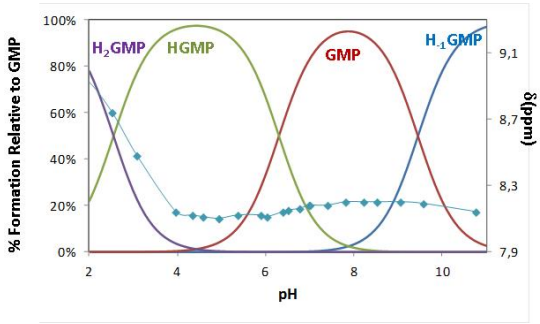

Figure S3.- Distribution diagram for GMP protonation obtained by pH-metric titrations with ^{1}H NMR chemical shifts of GMP signal ♦H_{G8} at different pD values.

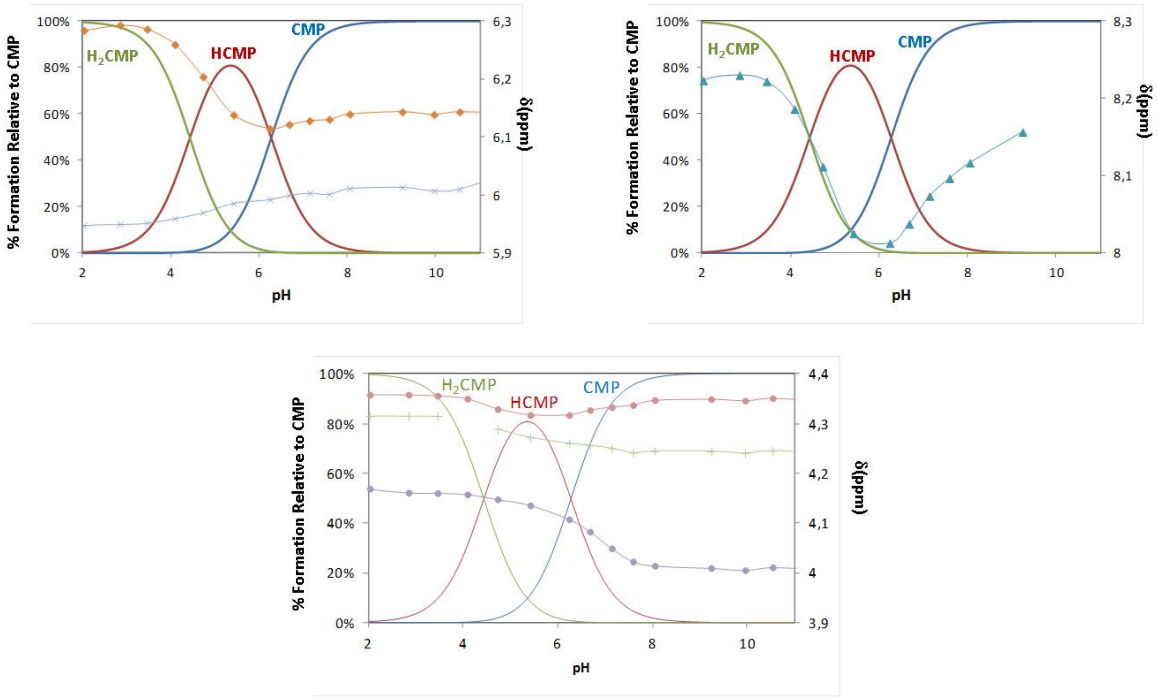

Figure S4.- Distribution diagrams for CMP protonation obtained by pH-metric titrations with ^{1}H NMR chemical shifts of CMP signals ▲H_{C6}, ♦H_{C5} ×H_{R1} ●H_{R3}, +H_{R4} and ●H_{R5} at different pD values.

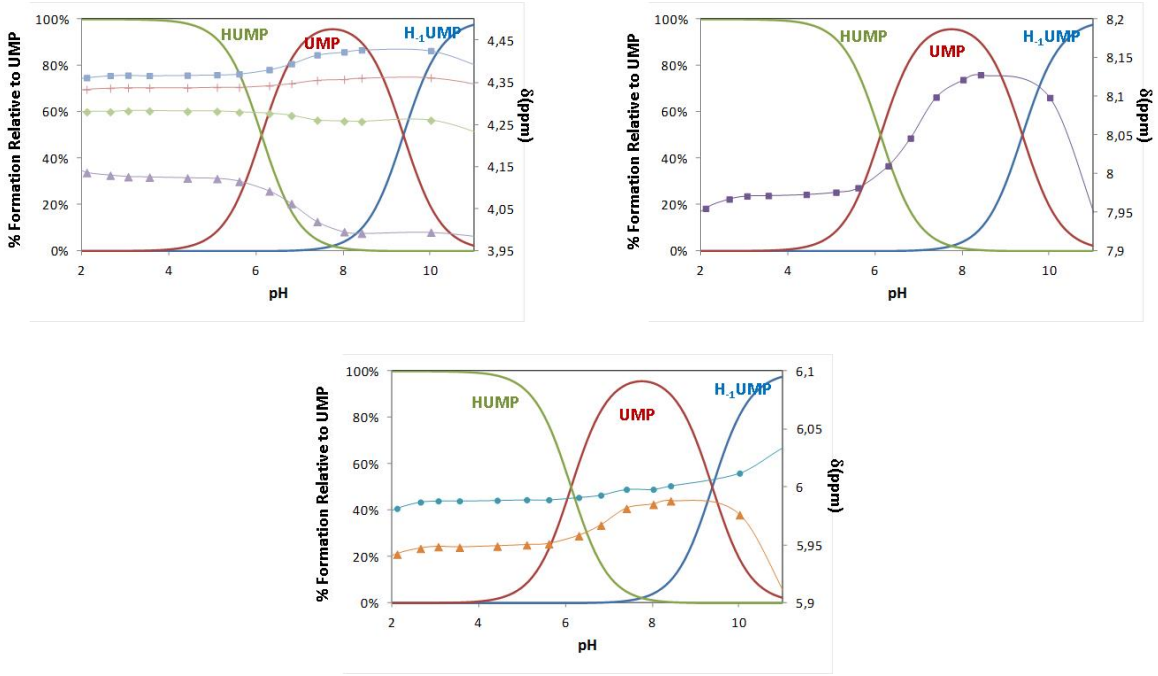

Figure S5.- Distribution diagrams for UMP protonation obtained by pH-metric titrations with ^{1}H NMR chemical shifts of UMP signals ■H_{U6}, ■H_{R2}, +H_{R3}, ♦H_{R4}, ▲H_{R5} ▲H_{U5} and ●H_{R1}.at different pD values.

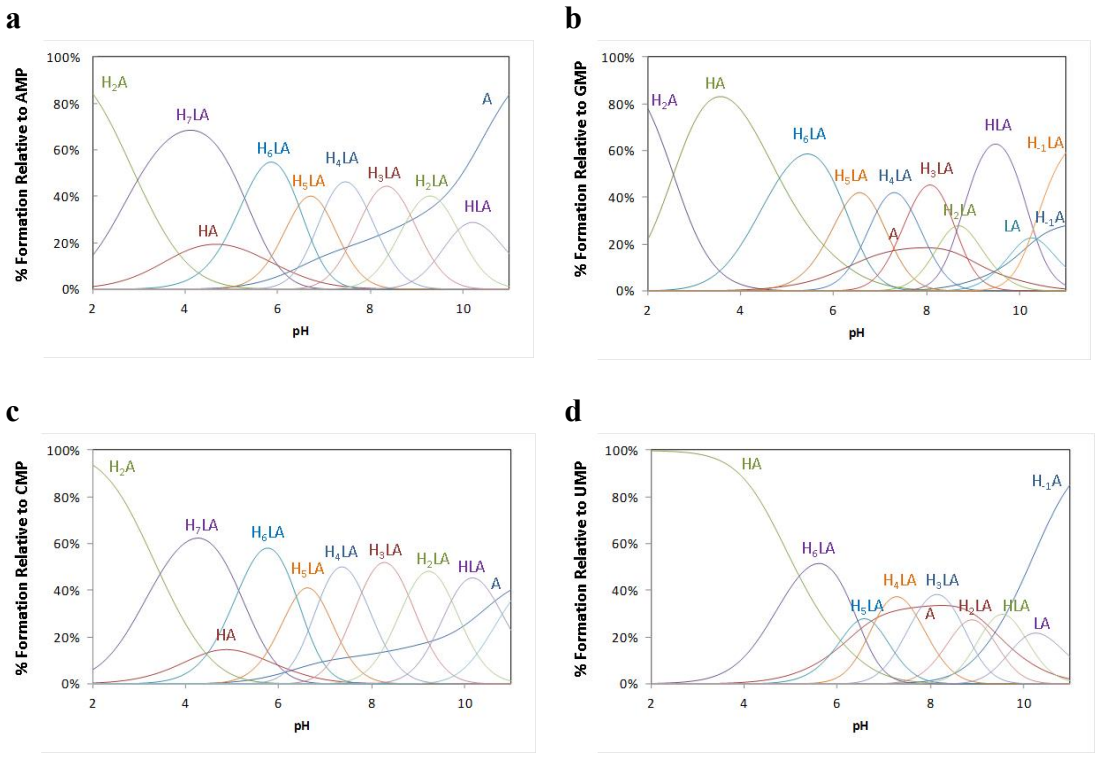

Figure S6. Distribution diagrams for the protonation of **PYPOD** with AMP (a), GMP (b), CMP (c) and UMP (d) determined in 0.15 mol dm^{-3} NaCl at 298.1 ± 0.1 K.

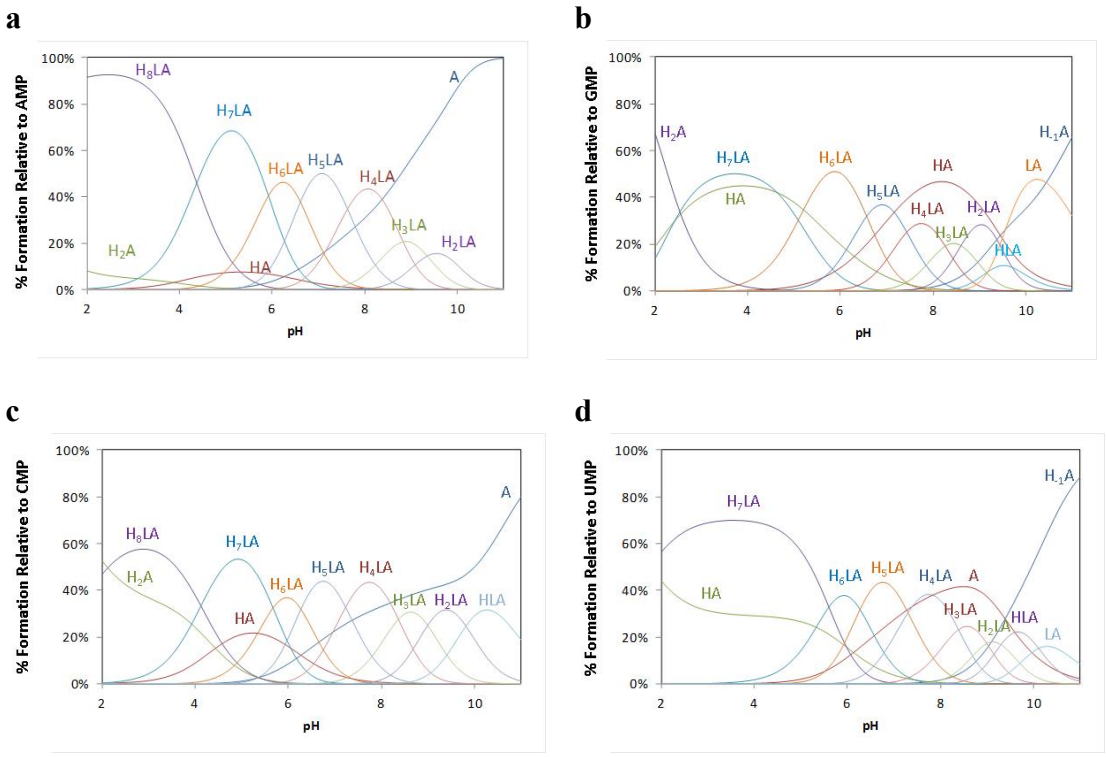

Figure S7. Distribution diagrams for the protonation of **PHENPOD** with AMP (a), GMP (b), CMP (c) and UMP (d) determined in 0.15 mol dm^{-3} NaCl at 298.1 ± 0.1 K.

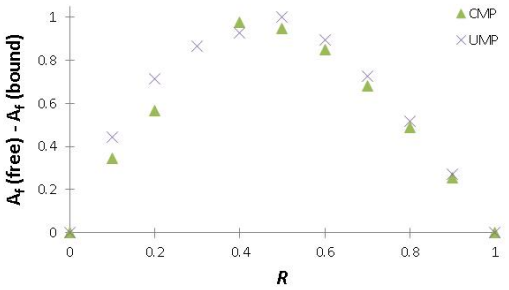

Figure S8. Job plot for complex formation between **PHENPOD** and CMP (▲) or UMP (×). Spectra were recorded at 369 nm in a pH 5.0 solution (0.05 M cacodylate buffer) at a total concentration of [**PHENPOD**] + [NMP] = 5×10^{-5} M

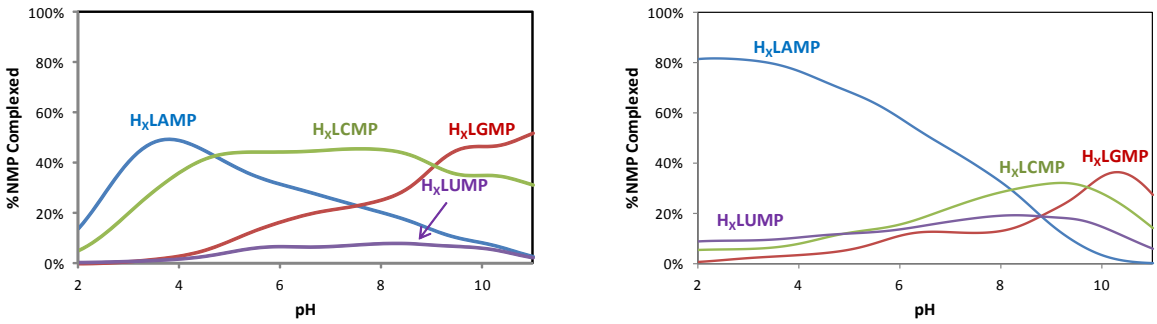

Figure S9. Plot of the percentages of complexation of each nucleotide *vs.* pH of **PYPOD** system (left) and **PHENPOD** system (right)

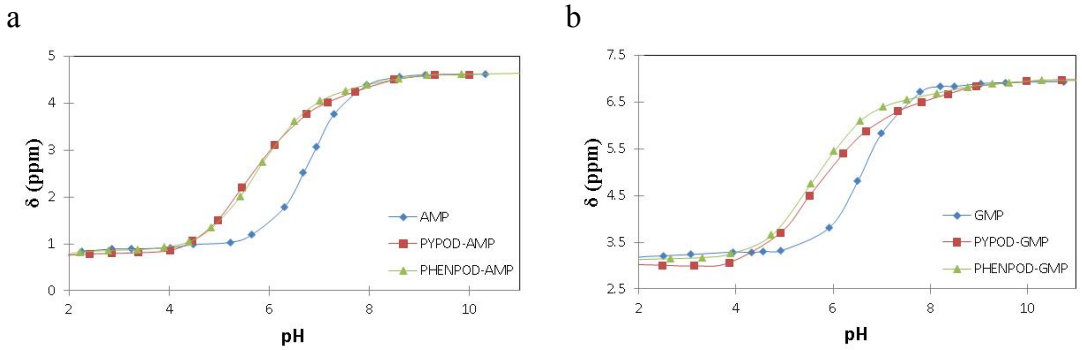

Figure S10. Plot of ^{31}P NMR δ (ppm) *vs.* pH for free mononucleotides (●) AMP (a) and GMP (b) and for the complexes formed with **PYPOD** (■) and **PHENPOD** (▲).

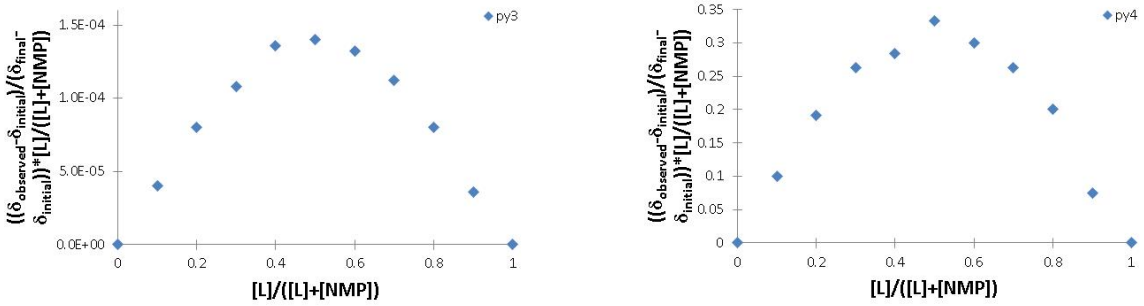

Figure S11. Job Plot of **PYPOD** with CMP in D_2O at pD 7.0. The total concentration of **PYPOD** and CMP was 2 mM.

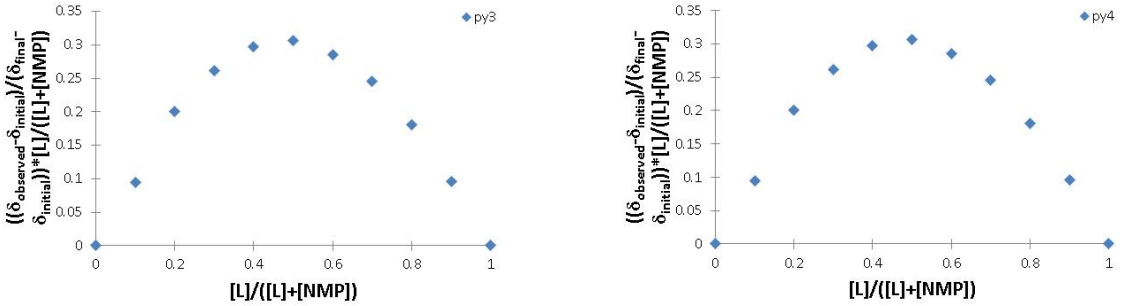

Figure S12. Job Plot of **PYPOD** with AMP in D_2O at pD 7.0. The total concentration of **PYPOD** and AMP was 2 mM.

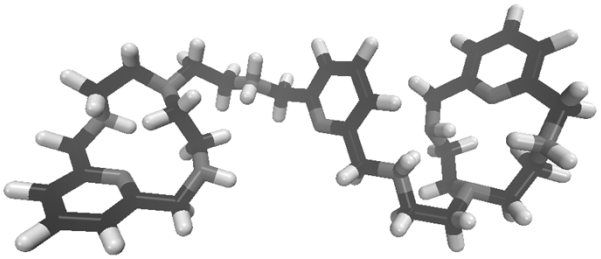

Figure S13. PYPOD structure with four positive charges at aliphatic nitrogens (according to the **PYPOD** protonation constants[1]) was constructed and minimised in aqueous medium.

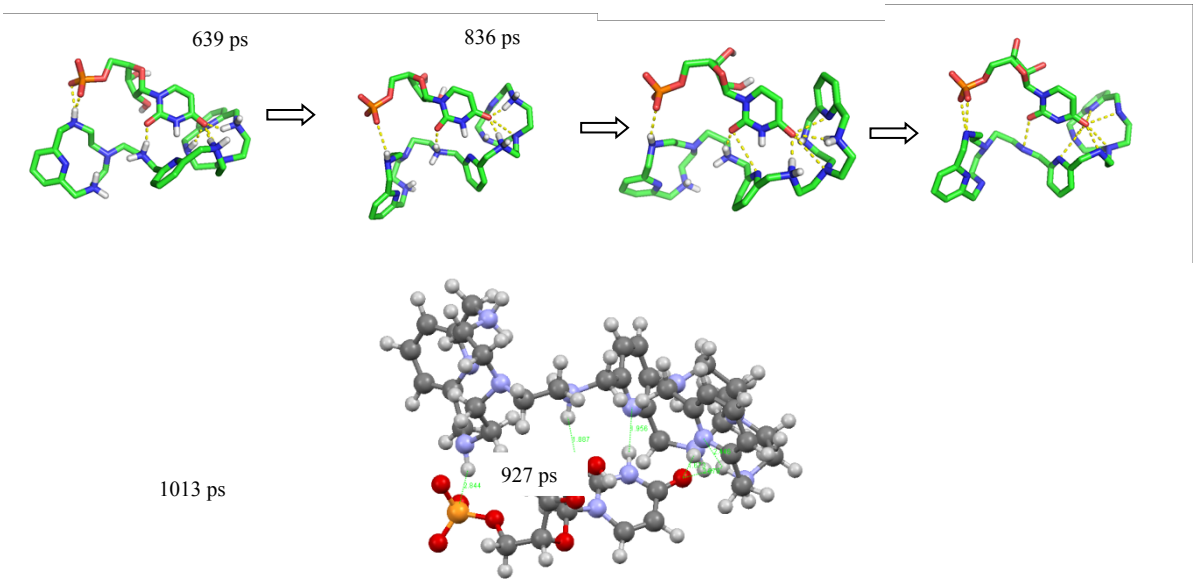

Figure S14. Major conformations of **PYPOD-**UMP (UP-progress in molecular dynamics simulations noted by arrows and picosecond (ps) time scale of each structure); DOWN side-view of end-conformation with green line-labelled binding interactions.

[1] J. Gonzalez, J. M. Llinares, R. Belda, J. Pitarch, C. Soriano, R. Tejero, B. Verdejo, E. García-España, *Org. Biomol. Chem.*, 2010, **8**, 2367.