Supplementary Data

Application of the Cluster Expansion Method

The NMR data were analyzed by the Cluster Expansion Method[1]. Starting from the macrostates L^{10-}, HL^{9-}, H_2L^{8-}, H_3L^{7-}, H_4L^{6-} and H_5L^{5-}, we can construct 32 microstates depending on the particular pattern of protonation of the phosphate groups. These different microstates are specified by introducing a two-valued state variable S*i* for each individual site i, such that S*i* = 1 if the site is protonated and S*i* = 0 if the site is deprotonated. Hence, the 32 possible microstates are: {0,0,0,0,0}, {1,0,0,0,0}, {0,1,0,0,0}, {0,0,1,0,0}, {0,0,0,1,0}, {0,0,0,0,1}, {1,1,0,0,0}, {1,0,1,0,0}, {1,0,0,1,0}, {1,0,0,0,1}, {0,1,1,0,0}, {0,1,0,1,0}, {0,1,0,0,1}, {0,0,1,1,0}, {0,0,1,0,1}, {0,0,0,1,1}, {1,1,1,0,0}, {1,1,0,1,0}, {1,1,0,0,1}, {1,0,1,1,0}, {1,0,1,0,1}, {0,1,1,1,0}, {0,1,1,0,1}, {0,0,1,1,1}, {0,1,0,1,1}, {1,0,0,1,1}, {1,1,1,1,0}, {1,1,0,1,1}, {1,1,1,0,1}, {1,0,1,1,1}, {0,1,1,1,1}, {1,1,1,1,1}. Taking into account the symmetry of the system under consideration, the cluster expansion model applied was:

$$\delta_{1/3}=\delta_{1/3}^{(0)}+\left(\frac{1}{1+\beta_1 10^{-pH}+\beta_2 10^{-2pH}+\beta_3 10^{-3pH}+\beta_4 10^{-4pH}+\beta_5 10^{-5pH}}\right)\left\{\begin{array}{c}A\left(\beta_1 10^{-pH}\right)+B\left(\beta_2 10^{-2pH}\right)+C\left(\beta_3 10^{-3pH}\right)+\\ +D\left(\beta_4 10^{-4pH}\right)+E\left(\beta_5 10^{-5pH}\right)\end{array}\right\} \quad (1)$$

$$\delta_{4/6}=\delta_{4/6}^{(0)}+\left(\frac{1}{1+\beta_1 10^{-pH}+\beta_2 10^{-2pH}+\beta_3 10^{-3pH}+\beta_4 10^{-4pH}+\beta_5 10^{-5pH}}\right)\left\{\begin{array}{c}F\left(\beta_1 10^{-pH}\right)+G\left(\beta_2 10^{-2pH}\right)+H\left(\beta_3 10^{-3pH}\right)+\\ I\left(\beta_4 10^{-4pH}\right)+J\left(\beta_5 10^{-5pH}\right)\end{array}\right\} \quad (2)$$

$$\delta_5=\delta_5^{(0)}+\left(\frac{1}{1+\beta_1 10^{-pH}+\beta_2 10^{-2pH}+\beta_3 10^{-3pH}+\beta_4 10^{-4pH}+\beta_5 10^{-5pH}}\right)\left\{\begin{array}{c}K\left(\beta_1 10^{-pH}\right)+L\left(\beta_2 10^{-2pH}\right)+M\left(\beta_3 10^{-3pH}\right)+\\ N\left(\beta_4 10^{-4pH}\right)+O\left(\beta_5 10^{-5pH}\right)\end{array}\right\} \quad (3)$$

where $\delta_{1/3}^{(0)}$, $\delta_{4/6}^{(0)}$ and $\delta_5^{(0)}$ are the chemical shifts for P1 (or P3), P4 (or P6) and P5 respectively, when all groups are deprotonated, β_1, β_2, β_3, β_4 and β_5 are the logarithms of the overall protonation constants, and A, B, C, D, E, F, G, H, I, J, K, L, M, N and O are defined as:

$$A = (\Delta_{11}+\Delta_{13})\pi(\{1,0,0,0,0\})+(\Delta_{14}+\Delta_{16})\pi(\{0,0,1,0,0\})+\Delta_{15}\left(1-2\pi(\{1,0,0,0,0\})-2\pi(\{0,0,1,0,0\})\right) \quad (4)$$

$$\begin{aligned}B = {} & (\Delta_{11}+\Delta_{13})\left(\pi(\{1,1,0,0,0\})+\pi(\{1,0,0,0,1\})+\pi(\{1,0,0,1,0\})+\pi(\{1,0,1,0,0\})\right)+\\ & +(\Delta_{16}+\Delta_{14})\left(\pi(\{1,0,1,0,0\})+\pi(\{1,0,0,0,1\})+\pi(\{0,0,0,1,1\})+\pi(\{0,0,1,0,1\})\right)+\\ & +(\Delta_{15})\left(2\pi(\{1,0,0,1,0\})+2\pi(\{0,0,0,1,1\})\right)\end{aligned} \quad (5)$$

$$\begin{aligned}C = {} & (\Delta_{11}+\Delta_{13})\left(2\pi(\{1,1,1,0,0\})+\pi(\{1,1,0,1,0\})+\pi(\{0,1,1,1,0\})+\pi(\{0,1,1,0,1\})+\pi(\{1,0,1,1,0\})\right)+\\ & +(\Delta_{16}+\Delta_{14})\left(\pi(\{1,1,1,0,0\})+\pi(\{1,0,1,1,0\})+2\pi(\{0,1,1,0,1\})+\pi(\{0,1,1,1,0\})+\pi(\{0,0,1,1,1\})\right)+\\ & +(\Delta_{15})\left(\pi(\{1,1,0,1,0\})+2\pi(\{1,0,1,1,0\})+2\pi(\{0,1,1,1,0\})+\pi(\{0,0,1,1,1\})\right)\end{aligned} \quad (6)$$

$$\begin{aligned}D = {} & (\Delta_{11}+\Delta_{13})\left(1-\pi(\{1,0,1,1,1\})\right)+(\Delta_{16}+\Delta_{14})\left(1-\pi(\{1,1,1,1,0\})\right)+\\ & +(\Delta_{15})\left(2\pi(\{1,1,1,1,0\})+2\pi(\{1,0,1,1,1\})\right)\end{aligned} \quad (7)$$

$$E = \Delta_{11}+\Delta_{13}+\Delta_{14}+\Delta_{15}+\Delta_{16} \quad (8)$$

$$F = (\Delta_{41}+\Delta_{43})\pi(\{1,0,0,0,0\})+(\Delta_{44}+\Delta_{46})\pi(\{0,0,1,0,0\})+\Delta_{45}\left(1-2\pi(\{1,0,0,0,0\})-2\pi(\{0,0,1,0,0\})\right) \quad (9)$$

$$\begin{aligned} G = {} & (\Delta_{41}+\Delta_{43})\left(\pi(\{1,1,0,0,0\})+\pi(\{1,0,0,0,1\})+\pi(\{1,0,0,1,0\})+\pi(\{1,0,1,0,0\})\right)+ \\ & +(\Delta_{46}+\Delta_{44})\left(\pi(\{1,0,1,0,0\})+\pi(\{1,0,0,0,1\})+\pi(\{0,0,0,1,1\})+\pi(\{0,0,1,0,1\})\right)+ \\ & +(\Delta_{45})\left(2\pi(\{1,0,0,1,0\})+2\pi(\{0,0,0,1,1\})\right) \end{aligned} \quad (10)$$

$$\begin{aligned} H = {} & (\Delta_{41}+\Delta_{43})\left(2\pi(\{1,1,1,0,0\})+\pi(\{1,1,0,1,0\})+\pi(\{0,1,1,1,0\})+\pi(\{0,1,1,0,1\})+\pi(\{1,0,1,1,0\})\right)+ \\ & +(\Delta_{46}+\Delta_{44})\left(\pi(\{1,1,1,0,0\})+\pi(\{1,0,1,1,0\})+2\pi(\{0,1,1,0,1\})+\pi(\{0,1,1,1,0\})+\pi(\{0,0,1,1,1\})\right)+ \\ & +(\Delta_{45})\left(\pi(\{1,1,0,1,0\})+2\pi(\{1,0,1,1,0\})+2\pi(\{0,1,1,1,0\})+\pi(\{0,0,1,1,1\})\right) \end{aligned} \quad (11)$$

$$\begin{aligned} I = {} & (\Delta_{41}+\Delta_{43})\left(1-\pi(\{1,0,1,1,1\})\right)+(\Delta_{46}+\Delta_{44})\left(1-\pi(\{1,1,1,1,0\})\right)+ \\ & +(\Delta_{45})\left(2\pi(\{1,1,1,1,0\})+2\pi(\{1,0,1,1,1\})\right) \end{aligned} \quad (12)$$

$$J = \Delta_{41}+\Delta_{43}+\Delta_{44}+\Delta_{45}+\Delta_{46} \quad (13)$$

$$K = (2\Delta_{51})\pi(\{1,0,0,0,0\})+(2\Delta_{54})\pi(\{0,0,1,0,0\})+\Delta_{55}\left(1-2\pi(\{1,0,0,0,0\})-2\pi(\{0,0,1,0,0\})\right) \quad (14)$$

$$\begin{aligned} L = {} & (2\Delta_{51})\left(\pi(\{1,1,0,0,0\})+\pi(\{1,0,0,0,1\})+\pi(\{1,0,0,1,0\})+\pi(\{1,0,1,0,0\})\right)+ \\ & +(2\Delta_{54})\left(\pi(\{1,0,1,0,0\})+\pi(\{1,0,0,0,1\})+\pi(\{0,0,0,1,1\})+\pi(\{0,0,1,0,1\})\right)+ \\ & +(\Delta_{55})\left(2\pi(\{1,0,0,1,0\})+2\pi(\{0,0,0,1,1\})\right) \end{aligned} \quad (15)$$

$$\begin{aligned} M = {} & (2\Delta_{51})\left(2\pi(\{1,1,1,0,0\})+\pi(\{1,1,0,1,0\})+\pi(\{0,1,1,1,0\})+\pi(\{0,1,1,0,1\})+\pi(\{1,0,1,1,0\})\right)+ \\ & +(2\Delta_{54})\left(\pi(\{1,1,1,0,0\})+\pi(\{1,0,1,1,0\})+2\pi(\{0,1,1,0,1\})+\pi(\{0,1,1,1,0\})+\pi(\{0,0,1,1,1\})\right)+ \\ & +(\Delta_{55})\left(\pi(\{1,1,0,1,0\})+2\pi(\{1,0,1,1,0\})+2\pi(\{0,1,1,1,0\})+\pi(\{0,0,1,1,1\})\right) \end{aligned} \quad (16)$$

$$N = (2\Delta_{51})\left(1-\pi(\{1,0,1,1,1\})\right)+(2\Delta_{54})\left(1-\pi(\{1,1,1,1,0\})\right)+(\Delta_{55})\left(2\pi(\{1,1,1,1,0\})+2\pi(\{1,0,1,1,1\})\right) \quad (17)$$

$$O = 2\Delta_{51}+2\Delta_{54}+\Delta_{55} \quad (18)$$

On the other hand, the cluster parameter Δ_{lm} is the chemical shift increment representing the change in the chemical shift of a phosphorus nucleus l, while a given site m changes its state from deprotonated to protonated, and $\pi(\{S_i\})$ is the conditional probability of finding a particular microstate within its macrostate. A nonlinear regression was performed, fitting the experimental points to the Eqs. (1), (2) and (3). Then, starting from the values obtained for A–O, the equation system (4)–(18) was solved, setting the inputs from the data previously

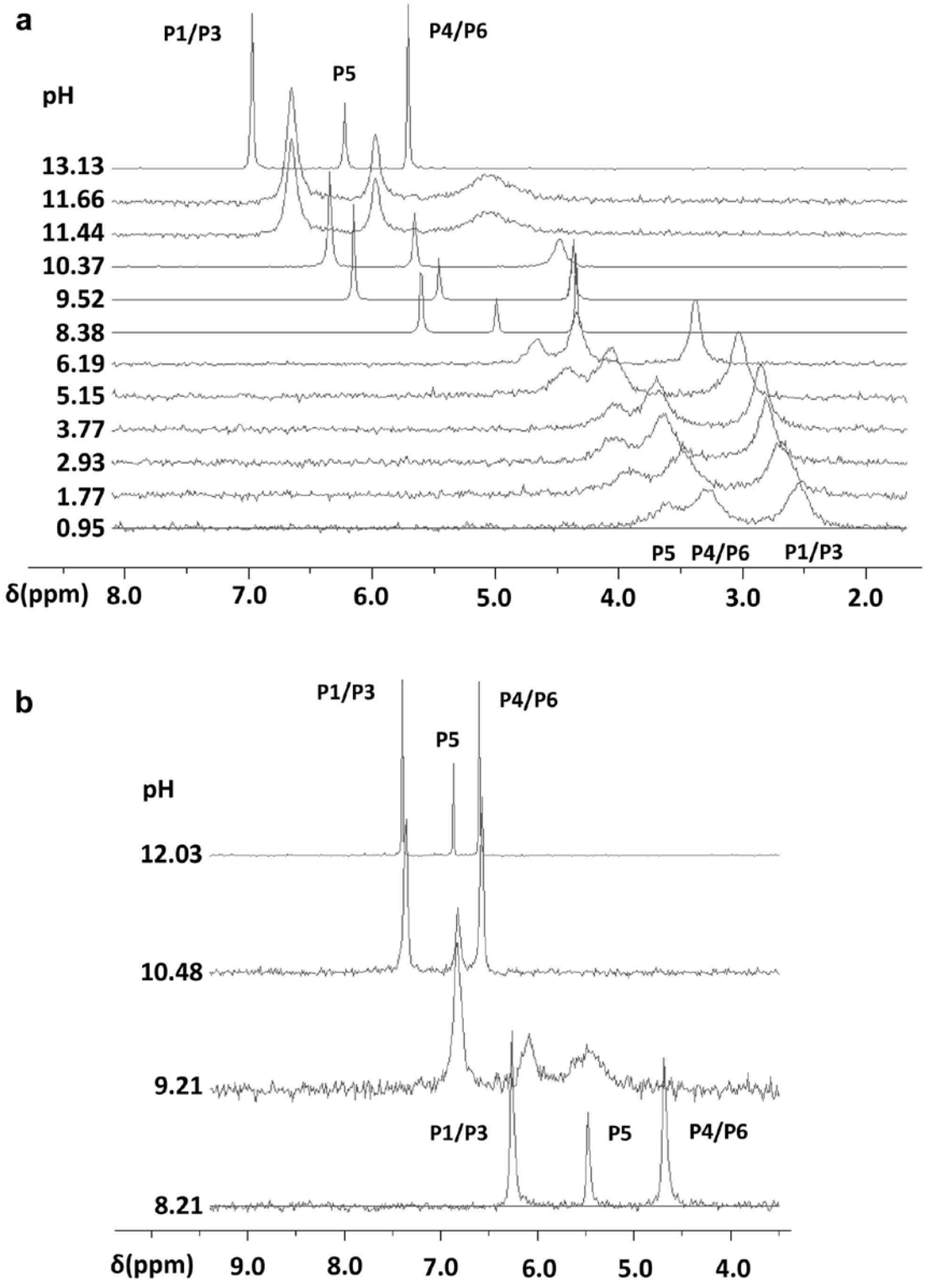
reported for Ins(1,2,3)P_3^2, and with the restriction that the $\pi(\{S_i\})$ must take values between 0 and 1. Obtained results are depicted in Table S1.

Cluster parameters			**Microprotonation constants**									
			i	**log k_i**	***pi***	**log k_{pi}**	***ppi***	**log k_{ppi}**	***pppi***	**log k_{pppi}**	***ppppi***	**log k_{ppppi}**
Δ_{11}	-4.810		1/3	11.94	13/31	10.82	134/136	9.13	1346/1364	7.58	14563/34561	5.83
Δ_{13}	0.195		4/6	12.60	14/36	11.13	135	9.24	1345/1365	7.41	13456/13564	5.75
Δ_{14}	0.425		5	12.28	15/35	10.76	143/361	8.82	1354/1356	7.30	1346	5.58
Δ_{15}	-0.638				16/34	10.60	145/365	8.93	3451/1563	7.83		
Δ_{16}	0.675				41/63	10.47	146/364	9.05	3456/1564	7.75		
Δ_{41}	0.155				43/61	9.94	153/351	9.30	3461/1463	7.35		
Δ_{43}	0.375				45/65	10.45	154/356	9.30	3465/1465	7.10		
Δ_{44}	-2.630				46/64	11.05	156/354	8.77	1453/3561	7.30		
Δ_{45}	-1.180				51/53	10.42	163/341	9.35	1456/3564	7.22		
Δ_{46}	1.200				54/56	10.77	164/346	9.58	4561/4563	6.95		
Δ_{51}	0.012						165/345	8.93				
Δ_{54}	0.551						541/563	8.42				
Δ_{55}	-3.380						543/561	8.95				
							546/564	9.22				
$\pi(\{1,0,0,0,0\})$	0.113						461/463	8.47				
$\pi(\{0,0,1,0,0\})$	0.324						465	8.62				
$\pi(\{1,1,0,0,0\})$	0.010											
$\pi(\{1,0,0,0,1\})$	0.077											
$\pi(\{1,0,0,1,0\})$	0.104											
$\pi(\{1,0,1,0,0\})$	0.027											
$\pi(\{0,0,0,1,1\})$	0.094											
$\pi(\{1,1,1,0,0\})$	0.031											
$\pi(\{1,1,0,1,0\})$	0.012											
$\pi(\{0,1,1,1,0\})$	0.012											
$\pi(\{0,1,1,0,1\})$	0.144											
$\pi(\{1,0,1,1,0\})$	0.192											
$\pi(\{1,0,1,1,1\})$	0.087											
$\pi(\{1,1,1,1,0\})$	0.264											
$\pi(\{1,1,1,1,1\})$	1.000											

Table S1. Adjusted values of the cluster expansion parameters, Δ_{lm} and $\pi(\{S_i\})$, and logarithms of the microprotonation constants (k) for Ins(1,3,4,5,6)P_5 in NMe_4Cl 0.15 M at 37.0 ˚C. The subscript *i* represents the phosphate site being protonated while *p* denotes the sites already protonated.

^{31}P NMR spectra

The ^{31}P NMR spectra registered for Ins(1,3,4,5,6)P_5 in the absence and presence of Na^+ and Mg^{2+} are shown in Figure S1.



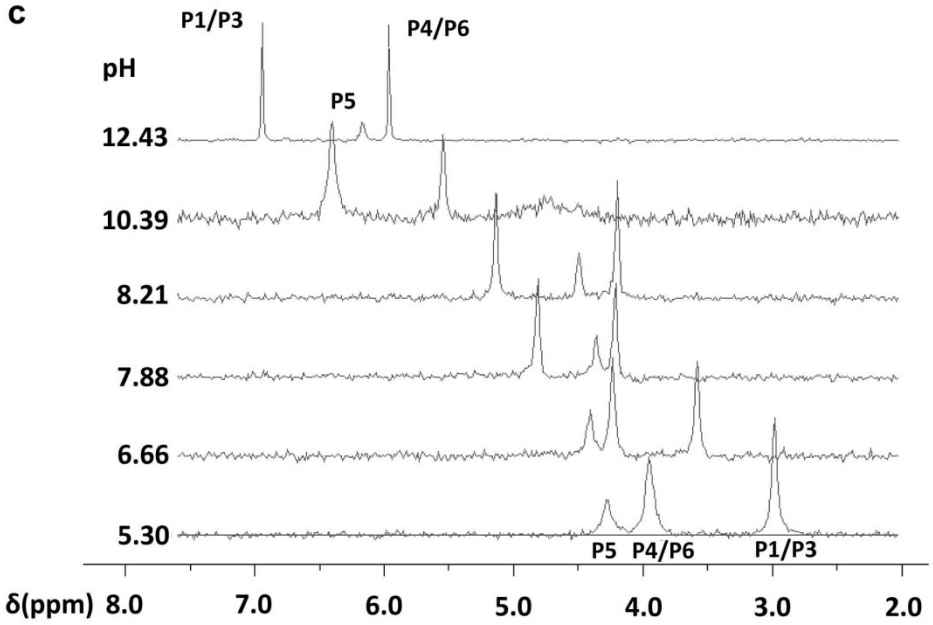


Figure S1. ^{31}P NMR spectra for Ins(1,3,4,5,6)P_5 as a function of pH in 0.15 M NMe_4Cl at 37.0 ˚C. (a) [Ins(1,3,4,5,6)P_5] = 10.6 mM; (b) [Ins(1,3,4,5,6)P_5] = 4.8 mM, [Na^+] = 0.5 M; [Ins(1,3,4,5,6)P_5] = 2.5 mM, [Mg^{2+}] = 1.0 mM.

Structural aspects of Ins(1,3,4,5,6)P_5-Mg^{2+} interaction

Based on the ^{31}P NMR information, the figure S2 depicts the most probable sites for the metal ion and the protons on the ligand in the 1:1 Ins(1,3,4,5,6)P_5-Mg^{2+} complexes.

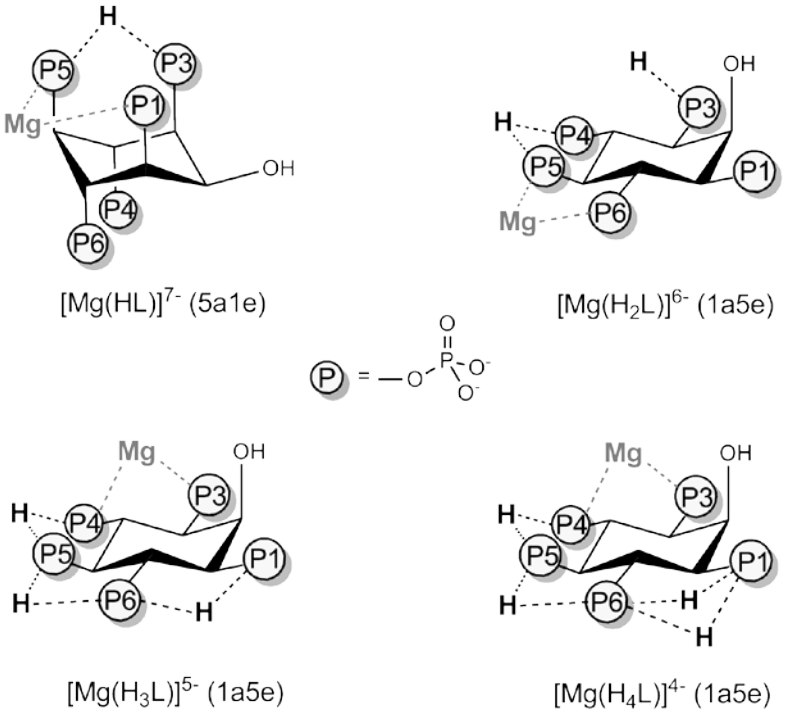


Figure S2. Scheme of the most probable protonation and complexation patterns for the 1:1 Ins(1,3,4,5,6)P_5-Mg^{2+} complexes as provided by the ^{31}P NMR data.

Comparative fit of alternative chemical models for the Ins(1,3,4,5,6)P_5-Na^+ system

In the case of sodium containing system, the best chemical model for the NMR experimental data fit is that included in the section "Coordination properties of Ins(1,3,4,5,6)P_5 towards Na^+". Other alternatives were assayed during the model adjustment steps. However, those options failed in fitting the registered chemical shifts. As an example, figure S3 depicts the comparison between the experimental and predicted ^{31}P NMR chemical shifts as the pH varies. Using a model without sodium complexes leads to a very poor adjustment of the experimental data (Figure S3a). Even more, all the three Na-species detected are essential. As it is shown in Figure S3b, if just one of the sodium complexes, $[Na_2(H_2L)]^{6-}$, is not taken into account, the model shows an inadequate behaviour for pH values below 10.5 where this species is formed.

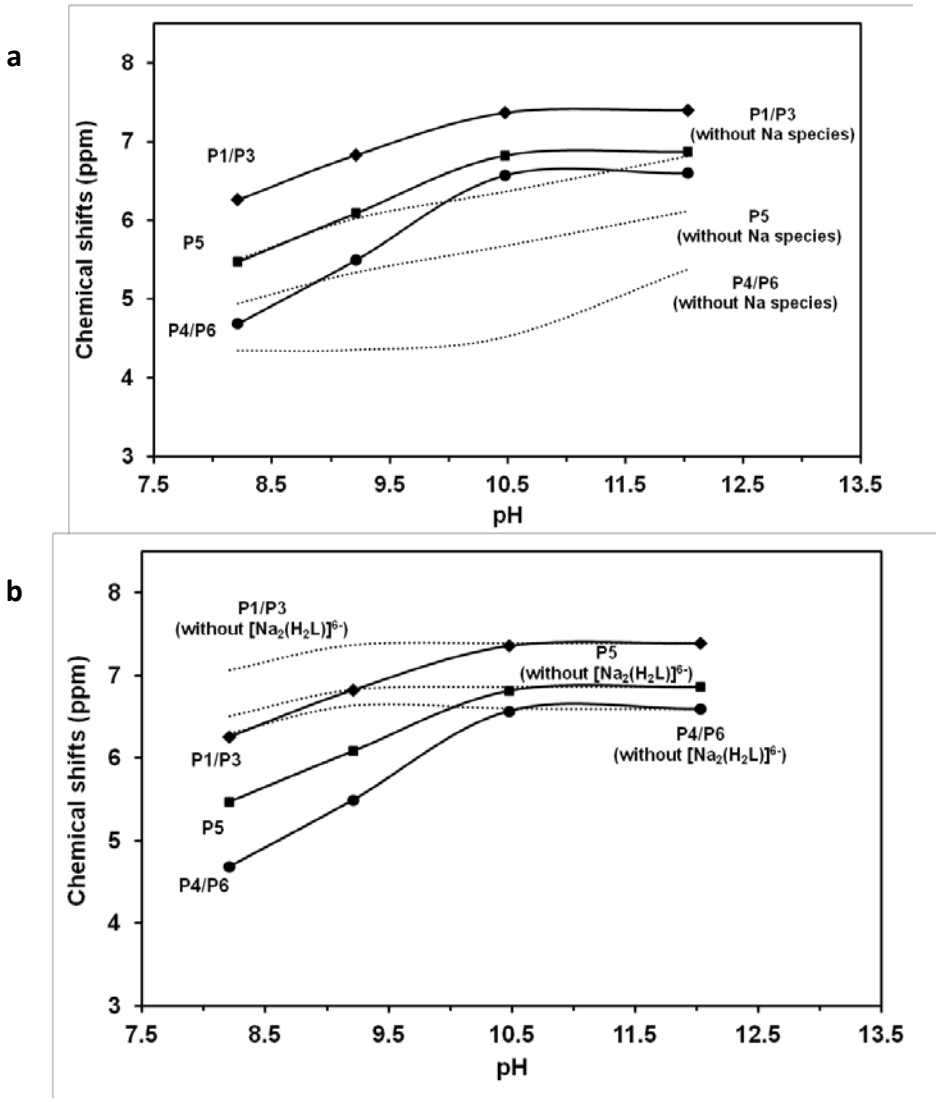


Figure S3. ^{31}P NMR chemical shifts for Na^+-Ins(1,3,4,5,6)P_5 system. The experimental data are shown as filled points. The solid lines show the HypNMR expected tendency according to the model presented in this work. The calculated trend for the chemical shifts without taking the Na^+ complexes into account (a) or when $[Na_2(H_2L)]^{6-}$ is ignored (b) are depicted as dotted lines.

References

1 M. Borkovec and G. J. M. Koper, *Anal. Chem.*, 2000, **72**, 3272-3279.
2 N. Veiga, J. Torres, G. González, K. Gómez, D. Mansell, S. Freeman, S. Domínguez and C. Kremer, *J. Mol. Struct.*, 2011, **986**, 75-85.