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Supplementary Information

Multiple functional group cooperation

in phosphate diester cleavage promoted by Zn(II) complexes

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1. Experimental Procedures.

General: Solvents were purified by standard methods. All commercially available reagents and substrates were used as received. TLC analyses were performed using Merck 60 F_{254} precoated silica gel glass plates. Column chromatography was carried out on Macherey-Nagel silica gel 60 (70-230 mesh). NMR spectra were recorded using a Bruker AC250F spectrometer operating at 250 MHz for ^{1}H and 62.9 MHz for ^{13}C and a Bruker AV300 operating at 300 MHz for ^{1}H and 121.5 MHz for ^{31}P. Chemical shifts are reported relative to internal Me_4Si. Multiplicity is given as follow: s = singlet, d = doublet, t = triplet, q = quartet, qn = quintet, m = multiplet, br = broad peak. Potentiometric titrations were performed using a Metrohom 716 DMS Titrino dynamic titrator. UV-Visible spectra and kinetic traces were recorded on Perkin Elmer Lambda 16 and Lambda 45 spectrophotometers equipped with thermostated multiple cell holders. $Zn(NO_3)_2$ was an analytical grade product. Metal ion stock solutions were titrated against EDTA following standard procedures. The buffer components were used as supplied by the manufacturers: acetic acid (Aldrich), 2-morpholinoethanesulfonic acid (MES, Fluka), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, Sigma), 4-(2-hydroxyethyl)-1-piperazinepropanesulfonic acid (EPPES, Sigma), 2-[N-cyclohexylamino]ethanesulfonic acid (CHES, Sigma),), 3-[cyclohexylamino]1-propanesulfonic acid (CAPS, Sigma). The bis-*p*-nitrophenyl phosphate sodium salt (BNP) was an Aldrich product, used as received. N-bis(2-pyridylmethyl)-1-hydroxy-3-propylamine[1] (L1) N-acetyl-N-(6-methyl-pyridin-2-yl)-acetamide was prepared as reported.[2]

Potentiometric titrations: Protonation constants and Zn(II) complex formation constants for ligands **L1-L3** were determined by pH potentiometric titrations (25° C, 0.10 M NaCl). Solutions approximately $1 \cdot 10^{-3}$ M of the hydrochloride salts of the ligands and, when necessary, $Zn(NO_3)_2$ were titrated using a 0.1 M sodium hydroxide solution. The electrode system was calibrated by titrating a 0.01 M solution of HCl so that the pK_w value was 13.78. The pH and the volume of

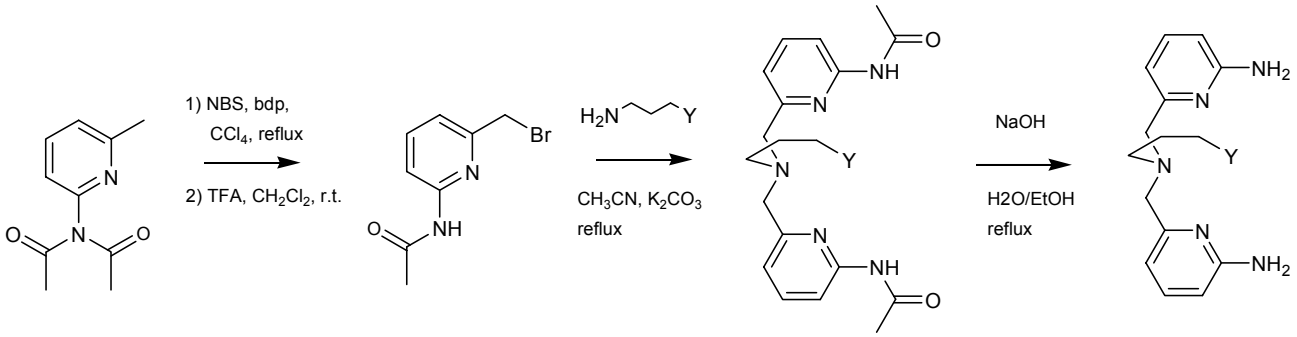
added NaOH data were fitted with the computer program BEST[3] to obtain the desired protonation and complex formation constants.

Kinetic Measurements. The kinetic traces were recorded on Perkin Elmer Lambda 16 and Lambda 45 spectrophotometers equipped with a thermostated multiple cell holders. Reaction temperature was maintained at 25±0.1 °C. The reactions were started by adding 20 μL of a $2.0 \cdot 10^{-2}$ M solution of substrate to a 2-mL solution of metal complex in the appropriate buffer and monitored by following the absorption of *p*-nitrophenoxide at 400 nm. Reactions were followed up to about 5% of substrate hydrolysis. The pseudo-first order rate constants (k_ψ) were obtained from the slope of the absorbance versus time data (the fit error was always less than 1%) divided by the molar absorbivity of the *p*-nitrophenoxide and the concentration of substrate. Apparent pH dependent second order rate constants (k'_2) were obtained by linear regression fitting of the k_ψ versus metal complex concentration data. Kinetic K^n_a values and second order rate constants (k_2) for the mono-deprotonated complexes were obtained by non-linear regression analysis of the apparent second order rate constants *vs.* pH data according to the equation: $k'_2 = k_2 \cdot (K^1_a/[H^+]+1+[H^+]/ K^2_a)$.

[1] M. J. Young, D. Wahnon, R. C. Hynes and J. Chin, *J. Am. Chem. Soc.*, 1995, **117**, 9441-9447.
[2] B. Abarca, A. Asensio, G. Jones and D. J. Mouat, *Tetrahedron* 1989, **45**, 7041-7048.
[3] A. E. Martell and R. J.Motekaitis, *Determination and Use of Stability Constants*, 2nd ed.; VCH: New York, 1992.

2. Synthesis of ligands L2 and L3

Ligands **L2** and **L3** have been prepared according to Scheme S1.



2.1 Synthesis of N-(6-Bromomethyl-pyridin-2-yl)-acetamide (2)

14.42 g (75.09 mmol) of **1** and 17.72 g (27.7mmol) g of recristallized NBS were suspended in 200 mL of CCl_4 and the mixture was heated to reflux under N_2. for 8 hours. The reaction mixture was allowed to cool and diluted with CH_2Cl_2 (200 mL), the resulting mixture was extracted with a 5% solution of Na_2CO_3 and $Na_2S_2O_3$ (2 × 100 mL). The organic phase was dried with Na_2SO_4 and the solvent evaporated. The crude product was purified by flash chromatography (divided in two batches, silica gel, eluent: CH_2Cl_2/ AcOEt 97:3). After the chromatographic separation, the product is obtained as a mixture of mono- and di- acetylated derivatives.

The mixture was dissolved in 120 ml of TFA/CH_2Cl_2 3:1 and stirred for 4 days. Saturated Na_2CO_3 was added to bring the pH of the mixture around 8, the organic phase was separated and the aqueous layer extracted with CH_2CH_2 (3 × 100 mL). The combined organic solutions were evaporated to yield 4.96 g (29%) of 2 as a white solid.

^{1}H-NMR ($CDCl_3$, 250 MHz), δ: 8.12 (d, 1H, 8 Hz), 7.88 (bs, 1H), 7.69 (t, 1H, 8 Hz), 7.14 (d, 1H, 8 Hz), 4.42 (s, 1H), 2.21 (s, 3H).

2.2 Synthesis of N-bis(6-acetamido-2-pyridylmethyl)-1-hydroxy-3-propylamine (3)

0.223 g (2.97 mmol) of 3-amino-1-propanol, 1.45 g (6.33 mmol) of **2** and 0.886 (6.41 mmol) of K_2CO_3 were suspended in 20 ml of dry CH_3CN and the resulting mixture was refluxed for 2.5 hours under N_2. The reaction mixture was allowed to cool, the unsoluble salts were filtered and washed with CH_3CN. The solvent was evaporated and the crude product purified by flash chromatography (silica gel, eluent: CH_2Cl_2/MeOH/NH_3 90:10:1). 0.87 g (79%) of **3** were obtained as a white solid.

^{1}H-NMR ($CDCl_3$, 250 MHz), δ: 8.33 (bs, 2H), 8.00 (d, 2H, 8 Hz), 7.58 (t, 2H, 8 Hz), 7.02 (d, 2H, 8 Hz), 5.64 (bs, 1H), 3.69 (t, 2H, 7Hz), 3.63 (s, 4H), 2.64 (t, 2H, 7 Hz), 2.16 (s, 6H), 1.73 (qn, 2H, 7 Hz).

2.3 Synthesis of N-bis(6-amino-2-pyridylmethyl)-1-hydroxy-3-propylamine (L2)

0.396 g (1.07 mmol) of **3** were dissolved in 100 mL of 5 M NaOH in water/ethanol 1:1 and the resulting solution was refluxed overnight. The reaction mixture was allowed to cool, ethanol was removed at the rotary evaporator and the remaining aqueous solution was extracted with $CHCl_3$ (4 × 75 mL). The combined organic phases were extracted with saturated NaHCO3 (1 × 100 mL) and water (2 × 50 ml), dried over Na_2SO_4 and evaporated. 0.284 g (93%) of **L2** were obtained as a brown oil.

^{1}H-NMR ($CDCl_3$, 250 MHz), δ: 7.40 (t, 2H, 8 Hz), 6.74 (d, 2H, 8 Hz), 6.35 (d, 2H, 8 Hz), 4.57 (bs, 4H), 3.75 (t, 2H, 7Hz), 3.62 (s, 4H), 2.69 (t, 2H, 7 Hz), 2.16 (s, 6H), 1.76 (qn, 2H, 7 Hz).

Elemental analysis, calcd. for $C_{15}H_{21}N_5O$ (287.36): C 62.70, H 7.37; N 24.37%; found: C 62.15, H 7.22, N 24.12%.

ESI-MS (m/z): 288 [M^++H^+]; 310 [M^++ Na^+]; 326 [M^++K^+].

2.4 Synthesis of N-bis(6-acetamido-2-pyridylmethyl)-propylamine (4)

0.115 g (1.94 mmol) of propylamine, 0.997 g (4.35 mmol) of **2** and 0.615 (5.80 mmol) of Na_2CO_3 were suspended in 20 ml of dry CH_3CN and the resulting mixture was refluxed for 8 hours under N_2. The reaction mixture was allowed to cool, the unsoluble salts were filtered and washed with

CH_3CN. The solvent was evaporated and the crude product purified by flash chromatography (silica gel, eluent: CH_2Cl_2/MeOH/NH_3 20:1:0.1). 0.287g (42%) of **4** were obtained as a white solid.

^{1}H-NMR ($CDCl_3$, 250 MHz), δ: 8.12 (bs, 2H), 8.02 (d, 2H, 8 Hz), 7.64 (t, 2H, 8 Hz), 7.20 (d, 2H, 8 Hz), 3.65 (s, 4H), 2.45 (t, 2H, 7 Hz), 2.17 (s, 6H), 1.51 (m, 2H, 7 Hz), 0.83 (t, 2H, 7Hz).

2.5 Synthesis of N-bis(6-amino-2-pyridylmethyl)-1-hydroxy-3-propylamine (L3)

0.287 g (1.07 mmol) of **4** were dissolved in 100 mL of 5 M NaOH in water/ethanol 1:1 and the resulting solution was refluxed overnight. The reaction mixture was allowed to cool, ethanol was removed at the rotary evaporator and the remaining aqueous solution was extracted with $CHCl_3$ (4 × 75 mL). The combined organic phases were extracted with saturated NaHCO3 (1 × 100 mL) and water (2 × 50 ml), dried over Na_2SO_4 and evaporated. 0.214 g (98%) of **L3** were obtained as a brown oil.

^{1}H-NMR ($CDCl_3$, 250 MHz), δ: 7.36 (t, 2H, 8 Hz), 6.89 (d, 2H, 8 Hz), 6.32 (d, 2H, 8 Hz), 4.51 (bs, 4H), 3.60 (s, 4H), 2.45 (t, 2H, 7 Hz),), 1.51 (m, 2H, 7 Hz), 0.83 (t, 2H, 7Hz).

Elemental analysis, calcd. for $C_{15}H_{21}N_5$ (271.36): C 66.39, H 7.80 N, 25.81%; found: C 66.65, H 7.48, N 25.35%.

ESI-MS (m/z): 272 [M^++H^+]; 294 [M^++ Na^+]; 310 [M^++K^+].

3. Potentiometric titrations

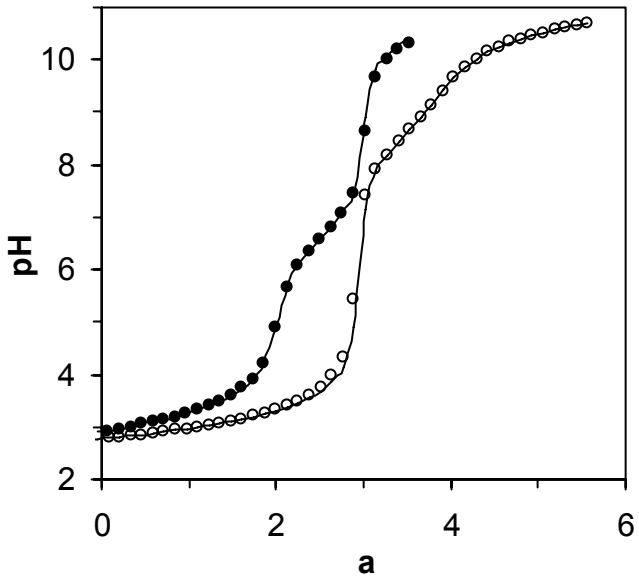


Figure S1. Potentiometric titrations of **L1**·3HCl (●) and **L1**·3HCl in the presence of one equivalent of $Zn(NO_3)_2$ 1:1 at 25° C. [**L1**] = 0.83 mM, [$Zn(NO_3)_2$] = 0.83 mM, [NaCl] = 0.1 M. a = number of added equivalents of NaOH, the lines report the calculated curves.

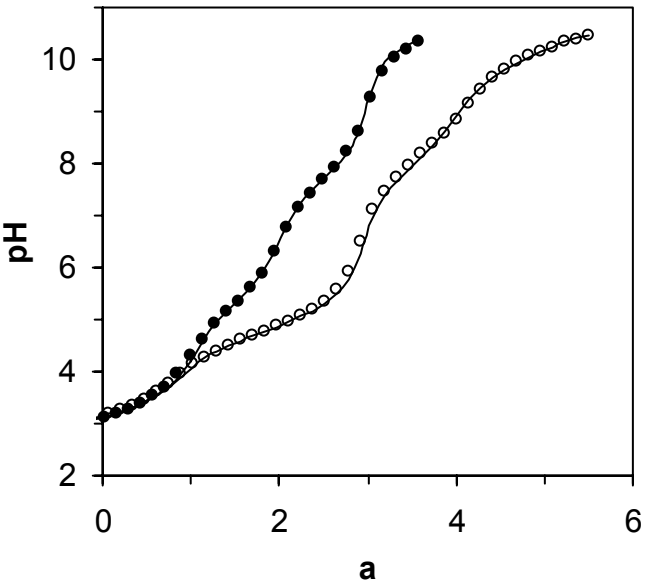


Figure S2. Potentiometric titrations of **L2**·3HCl (●) and **L2**·3HCl in the presence of one equivalent of $Zn(NO_3)_2$ 1:1 at 25° C. [**L2**] = 0.75 mM, [$Zn(NO_3)_2$] = 0.75 mM, [NaCl] = 0.1 M. a = number of added equivalents of NaOH, the lines report the calculated curves.

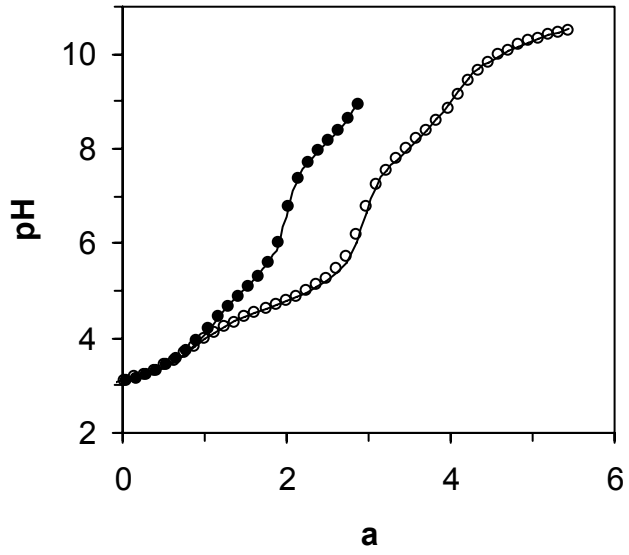


Figure S3. Potentiometric titrations of **L3**·3HCl (●) and **L3**·3HCl in the presence of one equivalent of $Zn(NO_3)_2$ 1:1 at 25° C. [**L3**] = 0.74 mM, [$Zn(NO_3)_2$] = 0.74 mM, [NaCl] = 0.1 M. a = number of added equivalents of NaOH, the lines report the calculated curves.

4. NMR experiments

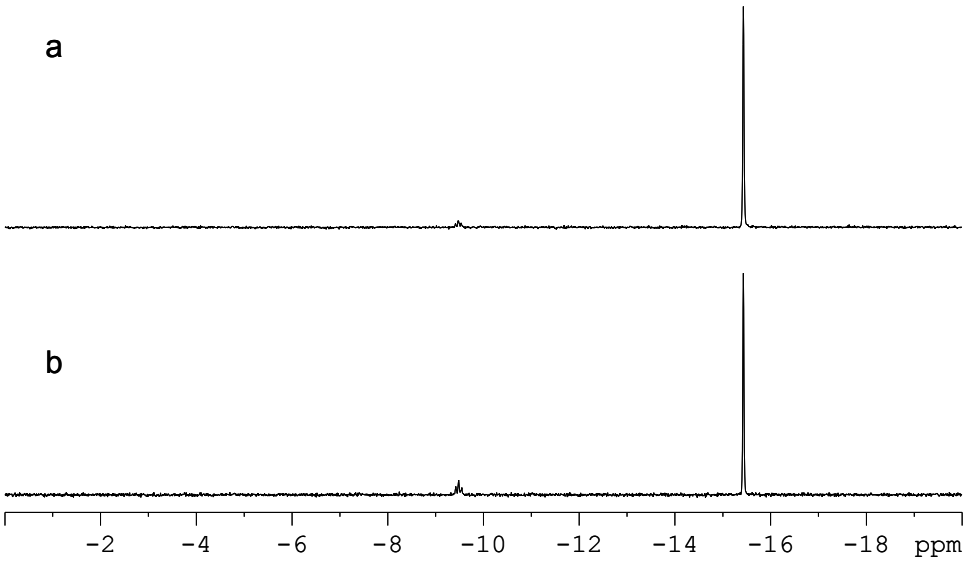


Figure S4. ^{31}P NMR spectra of a BNP solution (10% D_2O in H_2O) in the presence of **L1**·Zn(II) at pH 9.0 and 25 °C, 9 days (a) and 24 days (b) after mixing. [**L1**·Zn(II)] = 0.8 mM, [BNP] = 4 mM, [CHES buffer] = 0.05 M. The signals at -9.47 (triplet) and -15.43 (singlet) ppm correspond respectively the phosphorilated ligand and bis-*p*-nitrophenyl phosphate (BNP).

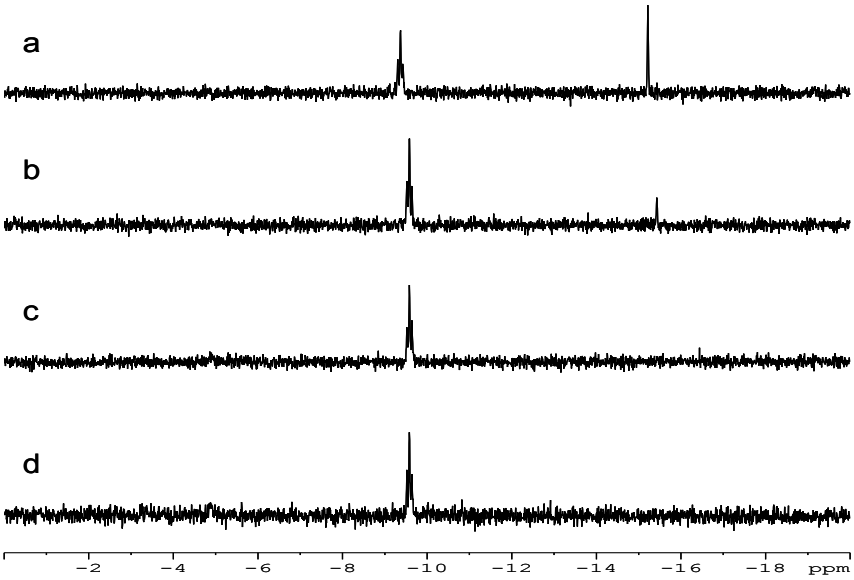


Figure S5. ^{31}P NMR spectra of a BNP solution (10% D_2O in H_2O) in the presence of **L2**·Zn(II) at pH 9.0 and 25 °C, 2 days (a), 3 days (b), 9 days (c) and 21 days after mixing. [**L2**·Zn(II)] = 0.8 mM, [BNP] = 0.8 mM, [CHES buffer] = 0.05 M. The signals at -9.58 (triplet) and -15.43 (singlet) ppm correspond respectively to the phosphorilated ligand and bis-*p*-nitrophenyl phosphate (BNP).

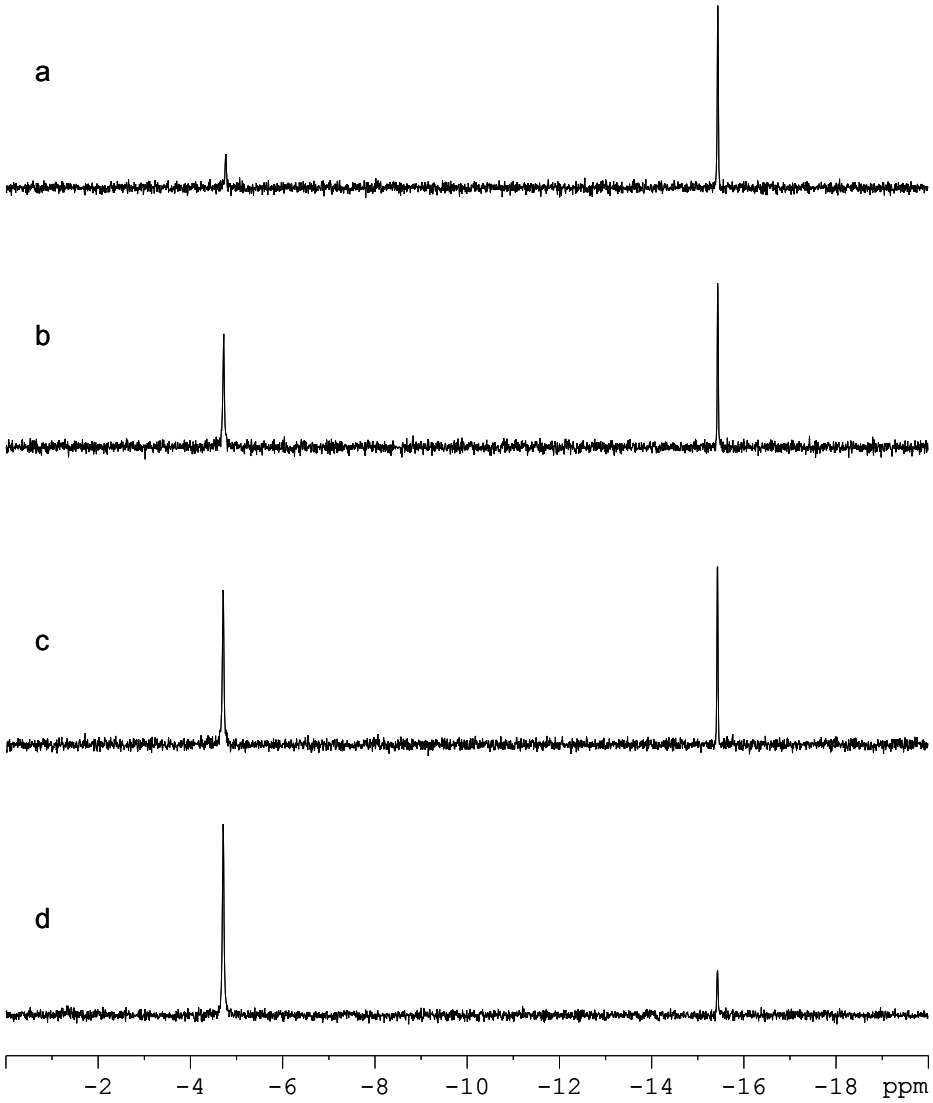


Figure S6. ^{31}P NMR spectra of a BNP solution (10% D_2O in H_2O) in the presence of **L3**·Zn(II) at pH 9.0 and 25 °C, 5 days 15 hours (a), 8 days (b), 14 days (c) and 174 days after mixing. [**L3**·Zn(II)] = 0.8 mM, [BNP] = 2 mM, [CHES buffer] = 0.05 M. The two signals at -6.06 (singlet) and -15.43 (singlet) ppm correspond respectively to *p*-nitrophenyl phosphate and bis-*p*-nitrophenyl phosphate (BNP).

5. pH dependant kinetic profiles

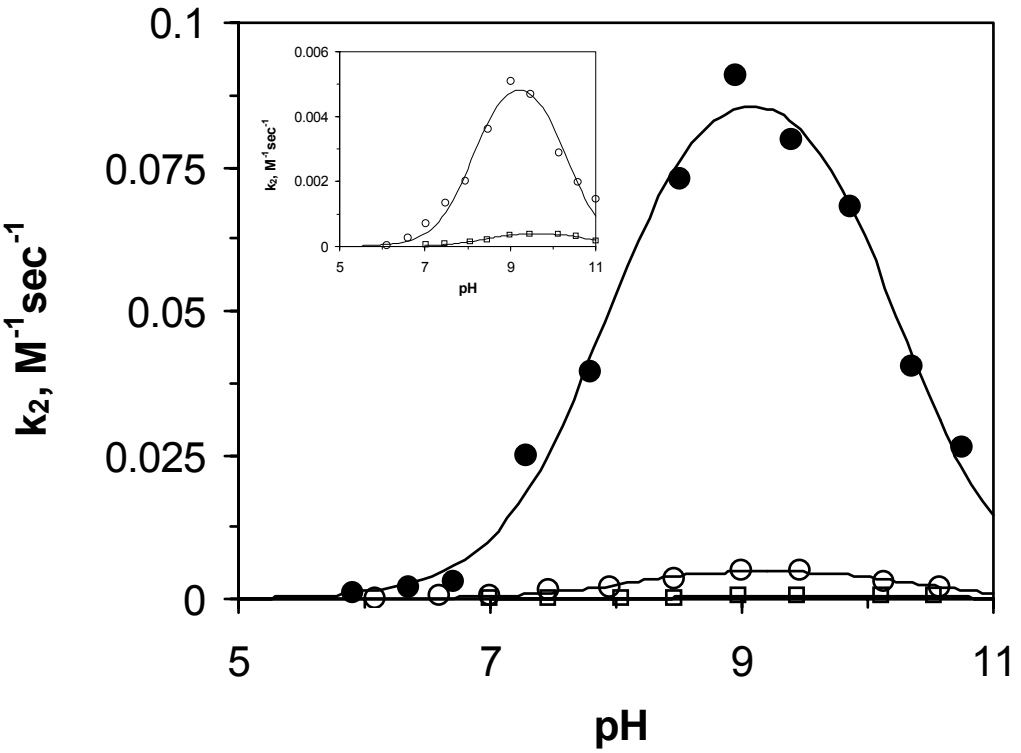


Figure S7. pH dependence of the second order rate constant for the reaction between BNP and Zn(II) complexes of ligands L1 (□), L2 (●), L3 (○) at 25° C ([buffer] = $5.0 \cdot 10^{-2}$ M). Inset: Zn(II) complexes of L1 (□) and L3 (○).