Supporting Information for

Physical and Kinetic Analysis of the Cooperative Role of Metal Ions in Catalysis of Phosphodiester Cleavage by a Dinuclear Zn(II) Complex.

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Experimental.

General Procedures and Methods. All reagents were of analytical reagent grade and were used without further purification, unless otherwise noted. Aqueous solutions were prepared using Millipore MILLI-Q water. ^{1}H NMR spectra were recorded on an Inova 500 XL spectrometer. An UVIKON-XL spectrophotometer by Bio-Tek instruments with a thermostatted cell transfer compartment was used for all kinetic measurements.

Synthesis of [Zn(L1OH)(Br)](Br) for Crystallography Studies. The salt **L1OH**·3HBr (0.15 mmol) was dissolved in 1 mL water and $Zn(Br)_2$ (0.15 mmol) was added to this solution. The solution pH was adjusted to 9.1 with 1 M NaOH aqueous solution. Colorless parallelepipeds, suitable for X-ray crystallography, were obtained by slow evaporation of the solution.

Synthesis of $[Zn_2(L2O)(Cl)(H_2O)_2](ClO_4)_2$ for Crystallography Studies. The free base form of the ligand **L2OH** was generated by dissolution of the hydrochloride salt **L2OH**·6HCl in water (0.36 mmol/0.6 mL) and adjustment of the pH to 13 with NaOH pellets. The aqueous solution was extracted with $CHCl_3$ (5x6 mL) and $CHCl_3$ was removed to yield the free base form of **L2OH** as an oily residue. After dissolution of **L2OH** in 7 mL ethanol, a solution of $Zn(ClO_4)_2·6H_2O$ in ethanol (0.83 mmol/2 mL) was

added. A white precipitate appeared with time. The solution was stirred overnight at 50°C. The solvent was evaporated and the residue was dissolved in 9 mL water. The solution pH was adjusted to 6.0 with 1 M NaOH and 1 M HCl aqueous solutions. Colorless parallelepipeds, suitable for X-ray crystallography, were obtained by slow evaporation of the solution.

Potentiometric pH Titrations. Potentiometric pH titrations were performed using the electrode system Orion Research Digital Ionalyzer/501 and Orion Research Ross Combination pH Electrode 8115BN. The system was calibrated using standard buffers at pH 4, 7, and 10. All solutions were prepared with freshly boiled Millipore MILLI-Q water cooled in an argon stream. A 0.1 M NaOH solution was prepared from dilution of a J. T. Baker dilute-it ampule and standardized by using potassium hydrogenphthalate.[1]

The potentiometric pH titrations of **L1OH**·3HBr (1.0 mM) and **L2OH**·6HCl (1.0 mM) in the absence and the presence of 1 and 2 equivalents of Zn(II), respectively, were carried out at 25 °C with I = 0.1 M $NaNO_3$ under an argon atmosphere. At least two independent titrations were made for each system. The computer program HYPERQUAD 2000 was used to calculate both protonation and stability constants from pH values.[2] The value for the ionic product of water ($K_w = [H^+][OH^-]$) under our experimental conditions, 25 °C and I = 0.1 M ($NaNO_3$), was found to be $10^{-13.79}$.[3] The mixed stability constants for the formation of the monohydroxo complexes were defined as follows:

$$Zn(L1OH)^{2+} + H_2O \qquad Zn(L1OH)(OH)^{+} + H^{+}$$

$$K_{a1} = [Zn(L1OH)(OH)^{+}]a_{H+} / [Zn(L1OH)^{2+}]$$

$$Zn_2(L2O)^{3+} + H_2O \qquad Zn_2(L2O)(OH)^{2+} + H^+$$

$$K_{a2} = [Zn_2(L2O)(OH)^{2+}]a_{H+} / [Zn_2(L2O)^{3+}]$$

where a_{H+} is the activity of H^+.

^{1}H NMR Spectroscopic Measurements. The pH-meter readings measured in D_2O were corrected to the respective pD value with the equation pD = [pH-meter reading] + 0.4.[4] Final concentrations in the NMR tube were 5mM Zn(II) complex and 0.1 M $NaNO_3$.

Kinetics of the Uncatalyzed Transesterification of HPNP. The rate of transesterification of the phosphate diester HPNP was measured spectrophotometrically by following the increase in absorbance at 400 nm due to the release of 4-nitrophenolate. The pseudo-first-order rate constants for transesterification of HPNP in the absence of the catalyst (k_{uncat}) were determined by the method of initial rates (< 5% conversion) and are listed in Table S1. The concentration of HPNP used in these experiments was 0.05 mM. We observed less than 15% increase in pseudo-first-order rate constants by doubling the buffer concentration, therefore extrapolation to zero buffer concentration was not necessary.

References.

1) Titrimetric Analysis. *Vogel's Textbook of Quantitative Inorganic Analysis*; Bassett, J.; Denney, R. C.; Jeffery, G. H.; Mendham, J.; Eds.; John Wiley& Sons: New York, 1978.

2) Gans, P.; Sabatini, A.; Vacca, A. *Talanta* **1996**, *43*, 1739-1753.

3) Aoki, S.; Kimura, E. *J. Am. Chem. Soc*. **2000**, 122, 4542-4548.

4) Glasoe, P.K.; Long, F.A. *J. Phys. Chem.* **1960**, 64, 188-190.

Figure S1. Potentiometric titration curve of 1.0 mM **L2OH**·6HCl (a) and 1.0 mM **L2OH**·6HCl + 2.0 mM $Zn(NO_3)_2$ (b) at 25 °C with I = 0.1 M $NaNO_3$, where ***a*** is the moles of base added per mole of ligand present.

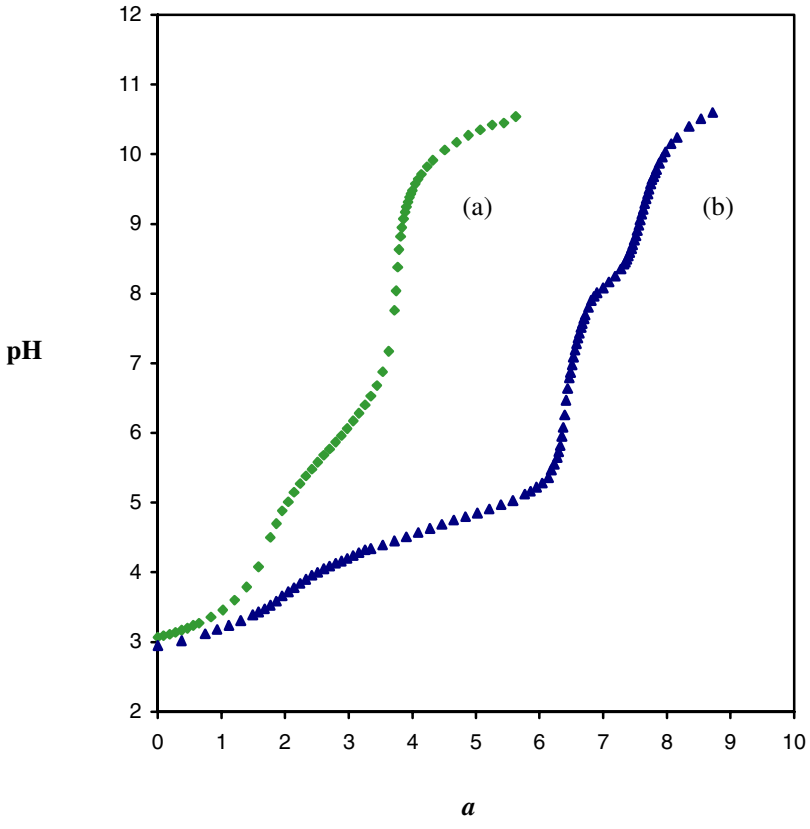


Figure S2. Potentiometric titration curve of 1.0 mM **L1OH**·3HBr (a) and 1.0 mM **L1OH**·3HBr + 1.0 mM $Zn(NO_3)_2$ (b) at 25 °C with I = 0.1 M $NaNO_3$, where ***a*** is the moles of base added per mole of ligand present.

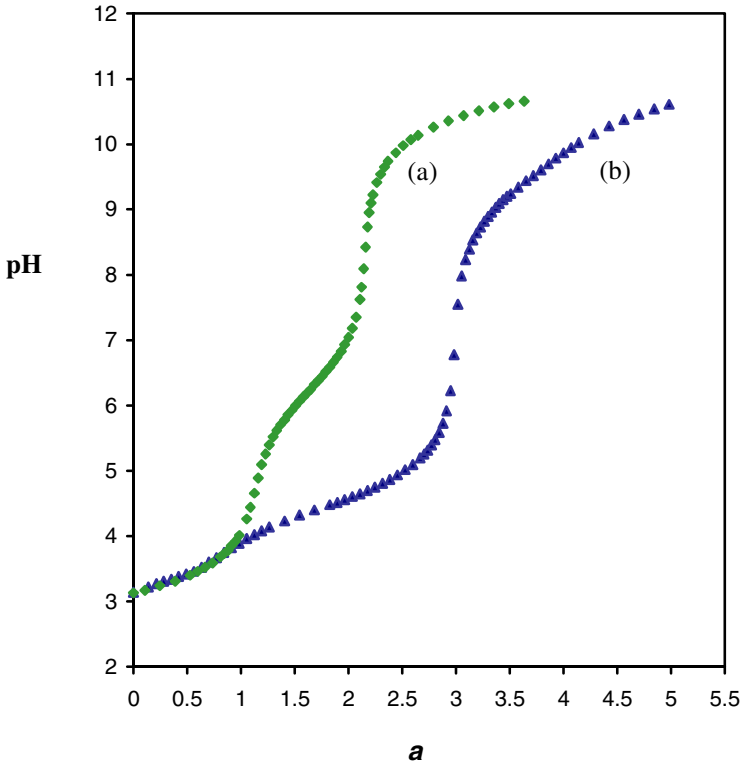
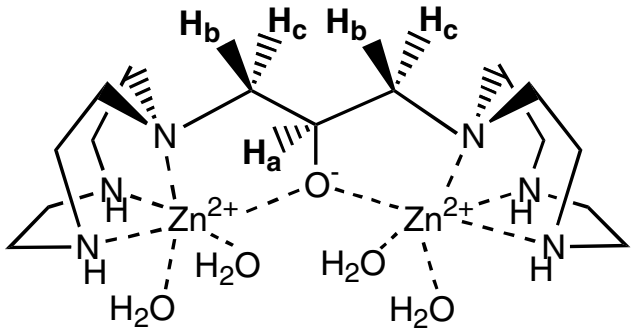


Figure S3. ^{1}H NMR spectra of the complex **Zn_2(L2O)** ([Zn_2(L2O)] = 5.0 mM) at different pD values with I = 0.1 M $NaNO_3$. The resonance of the protons H_a, H_b, and H_c did not shift substantially in the pD range 6.9 – 9.9, while the resonances of the methylene groups in the 1,4,7-triazacyclononane units did.



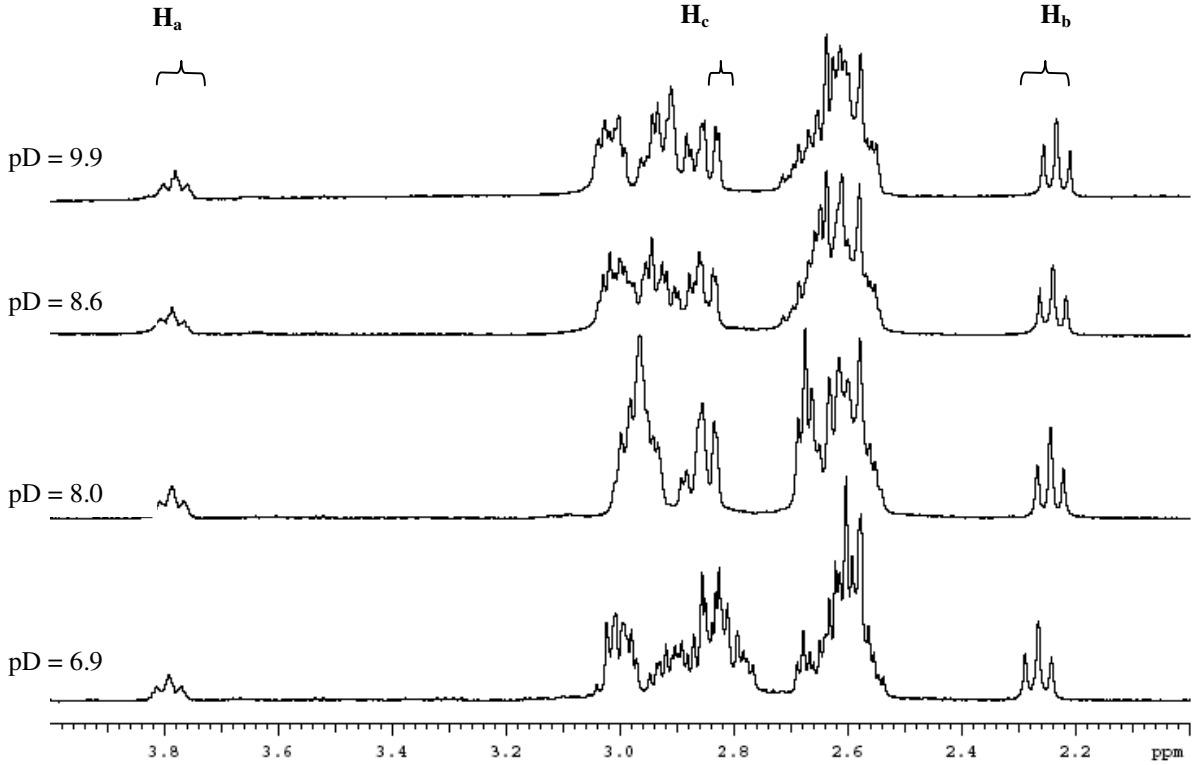
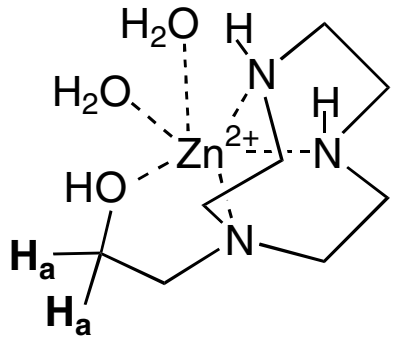


Figure S4. ^{1}H NMR spectra of the complex **Zn(L1OH)** ([Zn(L1OH)] = 5.0 mM) at different pD values with I = 0.1 M $NaNO_3$. The resonance of the protons H_a did not shift markedly in the pD range 7.8 – 10.4.



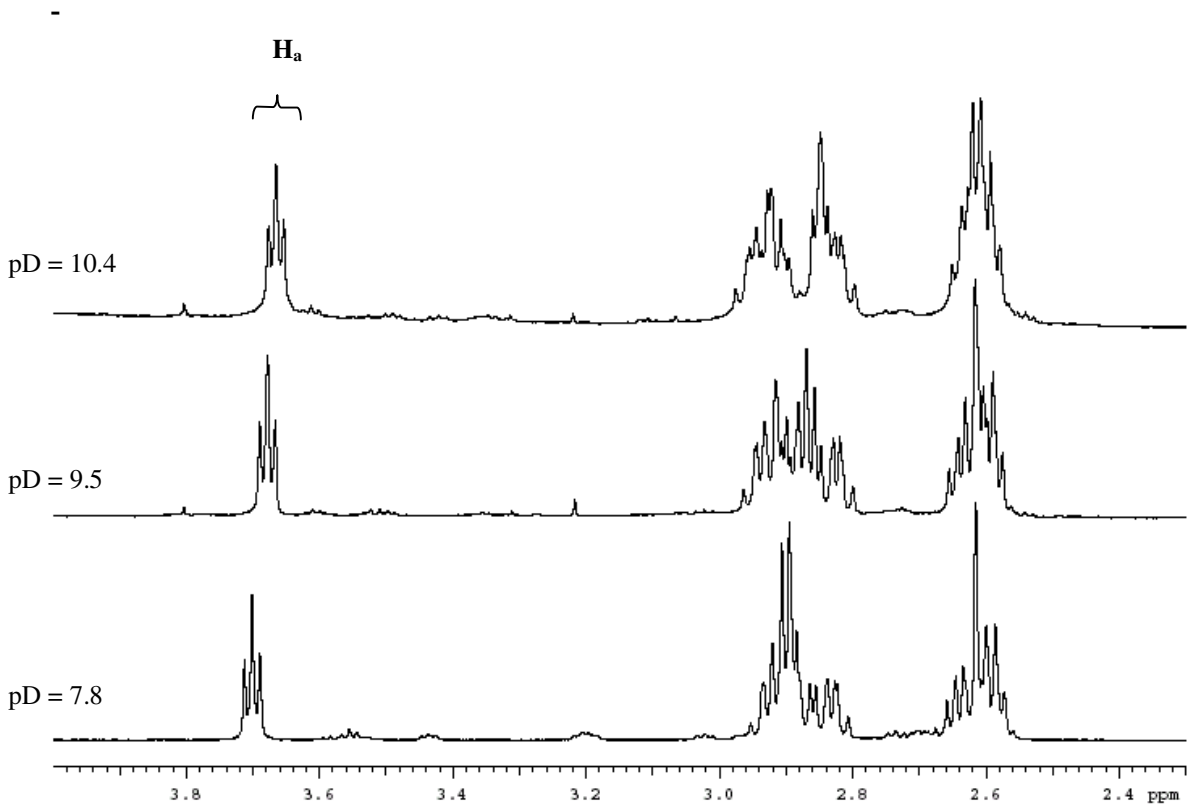


Table S1. Pseudo-first-order rate constants, k_{uncat}, for HPNP transesterification in the absence of Zn(II) complexes at 25 °C, I = 0.1 M ($NaNO_3$), and 20 mM buffer.

Buffer	**pK_a Buffer**	**pH**	**k_{uncat} / 10^{-7} (s^{-1})**
EPPS	8.00	8.03	1.2
		8.42	2.6
CHES	9.30	8.94	9.0
		9.33	16
CAPS	10.40	10.03	95