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Investigation of the Effect of Oxy Bridging Groups in Dinuclear Zn(II) Complexes that Catalyze the Cleavage of a Simple Phosphate Diester RNA Analogue

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Two sets of dinuclear Zn(II) complexes were prepared to determine the effect of the presence of oxyanionic bridging groups between the metal centers on the catalytic activity toward the methanolysis of the RNA analogue 2-hydroxypropyl-4-nitrophenyl phosphate (HPNPP, **2**). The $Zn(II)_2$ complexes of bis(di-(2-pyridylmethyl)amino)-*m*-xylene (**6**) and 2,6-bis(di-(2-pyridylmethyl)amino)-4-methylphenol (**7**) were compared to assess the effect of a bridging phenoxide ligand, while the $Zn(II)_2$ complex of 1,3-bis-N_1-(1,5,9-triazacyclododecyl)-propan-2-ol (**8**) was prepared to determine the effect of the 2-propoxy group compared to the previously studied complex of 1,3-bis-N_1-(1,5,9-triazacyclododecyl)-propane (**4**). Detailed kinetic studies of the cleavage of **2** including k_{obs} vs [catalyst] plots and $^{s}_{s}$pH-rate profiles were performed for each system along with potentiometric titration experiments to determine the acid dissociation constants for the catalytically relevant groups. The results show that inclusion of the phenoxy bridging group in **7**:$Zn(II)_2$ reduces the second-order catalytic rate constant (k_2^{cat}) for cleavage of **2** by a factor of 160 relative to that of **6**:$Zn(II)_2$, while the incorporation of a propoxy group in **8**:$Zn(II)_2$ reduces its efficacy by 3.7×10^4 times relative to **4**:$Zn(II)_2$. Energetics calculations reveal that **6**:$Zn(II)_2$ offers a 3.7 kcal/mol greater stabilization of the reaction transition state for the cleavage of **2** than does **7**:$Zn(II)_2$ and that **4**:$Zn(II)_2$ affords 6.5 kcal/mol greater transition state stabilization than does **8**:$Zn(II)_2$. The analyses show that the reduction in the transition state stabilization experienced with the complexes having permanently bridging oxyanion groups stems almost entirely from a weaker binding of the phosphate and catalyst, and a reduced catalytic rate constant. These results indicate that the presence of a bridging oxyanion ligand between the metal centers, a common structural element required for the successful formation of many small molecule dinuclear catalysts that show cooperative activity in water, significantly impairs the catalytic efficiency for cleavage of **2**.

Introduction

The phosphodiester linkage is exceptionally stable toward solvolytic cleavage and widely present throughout Nature as the chemically robust linker that gives great stability to DNA and RNA polymers. Many of the enzymes that facilitate the cleavage of these stable phosphate esters contain two or more metal ions (notably Zn(II) but in some cases Ca(II), Mg(II), Fe(III), and Mn(II)) in their active sites which act cooperatively, giving rate enhancements for P−O cleavage of up to 10^{17}-fold.[1] The impressive rate accelerations offered by metal ion containing phosphodiesterases have elicited considerable interest in the design of small molecule mono- and dinuclear metal complexes that catalyze the cleavage of phosphate diesters.[2] The study of such biomimetic catalysts has provided important insights into the origin of the catalysis afforded by enzymes but, at the present time, few actually approach the accelerations achieved by the naturally occurring systems. Virtually all of the catalytic systems have been investigated in aqueous media where it appears that dinuclear complexes are generally more active than their mononuclear counterparts[3−8] although this is not always so.[9,10] This might

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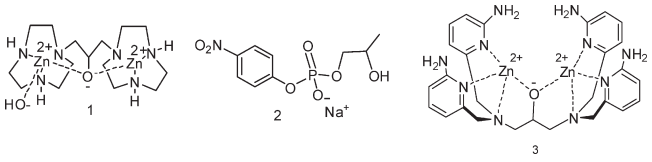
suggest that cooperative effects between two metal ions are not easily realized in water, but there are some standout cases where (1) a $Zn(II)_2$ complex of 1,3-bis-N_1-(1,3,7-triazacyclononanyl)propan-2-ol (**1**) is 120-fold more active toward the hydrolysis of an RNA model 2-hydroxypropyl-4-nitrophenyl phosphate (HPNPP, **2**) than is the mono-Zn(II) complex of 1,3,7-triazacyclononane;[6b] and (2) the dinuclear catalyst **3** is ~600-fold more active toward the hydrolysis of HPNPP than the corresponding mononuclear species.[4]

A common structural motif among dinuclear catalysts such as **1** and **3** that show metal ion cooperativity in water is the presence of a bridging alkoxy group in the linker unit connecting the two metal centers.[2,11] An oxyanion bridging group seems to be essential for cooperative catalytic activity[6b] since it serves to shield the cationic metal centers from one another and allows them to achieve a close proximity and act cooperatively.[12] Attempts to study systems lacking the bridging alkoxy group have proven difficult in water as these do not generally exhibit a catalysis that is greater than the sum of the parts, perhaps because they do not cleanly bind two metal ions because of strong electrostatic repulsions, but rather tend to form so-called "sandwich complexes" under the kinetic conditions in which both ligand groups are complexed to one metal ion.[13]



Work in this laboratory has found that the situation in the light alcohols (methanol and ethanol) is different from what one observes in water.[14] We have recently shown that the cleavage of HPNPP is accelerated by 10^{12} relative to the background reactions in methanol and ethanol in the presence of the dinuclear Zn(II) complex **4**:$Zn(II)_2$:($^-$OR).[15−17] Notably, this catalyst in methanol is 10^8 more reactive than CH_3O^- alone and 1.5×10^4 times more active for cleavage of **2** than the corresponding mononuclear complex, **5**:Zn(II):($^-$OR), indicating strong metal ion cooperativity in the dinuclear system.[15,16] The large increase in activity seems to be attributable to a medium effect imbued by the alcohol since the $Zn(II)_2$ complex of **4** is reported to be no more reactive toward the cleavage of a phosphate diester in water than its mononuclear counterpart.[18] Since highly active dinuclear complexes of **4** form in alcohol solution in the absence of a bridging oxyanion in the linker, we wished to make comparisons of the catalytic activity of $Zn(II)_2$ systems with and without that bridging group, bearing in mind that the presence of a permanent oxyanionic group is expected to alter not only the coordination number of the metal ions, but their Lewis acidity and ability to acidify coordinated $HOCH_3$ to form the catalytically active monomethoxy forms. Herein we report a kinetic study of the cleavage of **2** in methanol (proceeding via the pathway given in Scheme 1) catalyzed by the dinuclear Zn(II) complexes **6**:$Zn(II)_2$ and **7**:$Zn(II)_2$, which illustrates that the inclusion of a bridging phenoxide in the linker unit decreases the activity of the catalyst by a factor of 160. We also report a kinetic study of complex **8**:$Zn(II)_2$, which is 3.7×10^4 times less active than **4**:$Zn(II)_2$:($^-$$OCH_3$). These results indicate not only that the inclusion of the permanently fixed bridging alkoxy group is not essential for metal ion cooperativity in alcohol, but that this bridging group is in fact a significant detriment to the catalytic efficiency.

Experimental Section

Materials. Methanol (99.8%, anhydrous), $Zn(OTf)_2$ (98%), sodium methoxide (0.50 M solution in methanol, titrated against N/50 certified standard aqueous HCl solution and found to be 0.49 M), triethylamine (99%), 2-picoline (98%), 2,6-lutidine (99%), 2,2,6,6-tetramethylpiperidine (99%), di(2-picolyl)amine (97%), α,α-dibromo-*m*-xylene (97%), di-*tert*-butyl dicarbonate (1.0 M in THF), trifluoromethanesulfonic acid, and 1,3-dibromo-2-propanol (95% technical grade) were purchased from Aldrich and used without further purification. 2,4,6-Collidine was purchased from BDH Chemicals. $HClO_4$ (70% aqueous solution, titrated to be 11.40 M) was purchased from Acros Organics and used as supplied. THF and Acetonitrile were purchased from Fisher Scientific and dried prior to use on an Innovative Technology, Inc. PureSolv solvent purification system. Silica gel for chromatography (ultra pure, 230−400 mesh) was purchased from Silicycle.

Synthesis. The syntheses of bis(di-(2-pyridylmethyl)amino)-*m*-xylene (**6**)[19] and 2,6-bis(di-(2-pyridylmethyl)amino)-4-methylphenol (**7**)[20] were done as previously reported.

Synthesis of 1,3-Bis-N_1-(1,5,9-triazacyclododecyl)-propan-2-ol (8). The title compound was prepared by a modification of the published procedure for the synthesis of ligand **1**[6b] according to the procedure reported for ligand synthesis[21] to obtain the X-ray structure of the diZn(II) complex of **8**.[22]

***N*, *N*′-Bis(*tert*-butoxycarbonyl)-1,5,9-triazacyclododecane (Boc$_2$-12N3).** 1,5,9-Triazacyclododecane (12N3)[23] (1.04 g, 6.1 mmol) was dissolved in 40 mL of dry tetrahydrofuran (THF) with stirring and 1.7 mL (12 mmol, 2 equiv) of triethylamine was added. The stirring mixture was cooled in an ice bath under N_2 after which 12.1 mL of a 1.0 M solution of di-*tert*-butyl dicarbonate in THF (12 mmol, 2 equiv) was slowly added via a dropping funnel over ~10 min followed by an additional 10 mL of dry THF to wash the funnel. The reaction mixture was allowed to stir under N_2 and slowly warm to room temperature. After stirring 72 h, the solvent was removed

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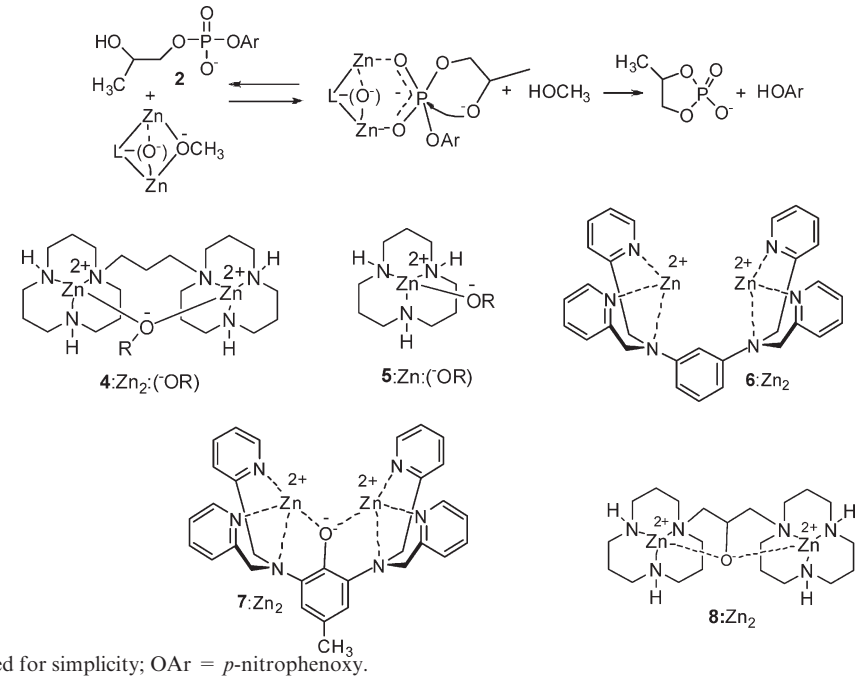
Scheme 1. Catalytic Pathway for the Catalytic Cleavage of **2**[a]



[a] Charges on Zn omitted for simplicity; OAr = *p*-nitrophenoxy.

under vacuum leaving a pale yellow viscous oil. The crude product was purified by flash chromatography on a silica column eluting with 6:1 $CHCl_3$:$HOCH_3$ (R_f = 0.43). Yield: 2.02 g (90%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.29 (q, 8H, J_{H-H} = 6 Hz), δ 2.65 (t, 4H, J_{H-H} = 6 Hz), δ 1.88 (t, 2H, J_{H-H} = 6 Hz), δ 1.76 (m, 4H), δ 1.44 (s, 18H).

1,3-Bis(5,9-di(*tert*-butoxycarbonyl)-1,5,9-triazacyclododecyl)-propan-2-ol (Boc$_4$-8). Boc_2-12N3 (1.99 g, 5.4 mmol), 1,3-dibromo-2-propanol (2.59 g, 2.7 mmol), and triethylamine (0.71 g, 7 mmol) were dissolved in 50 mL of dry acetonitrile with stirring under N_2. The reaction flask was equipped with a condenser and heated to reflux under N_2 for 72 h. The solvent was evaporated under vacuum to afford a thick oily residue which was suspended in 60 mL of 3 M NaOH and transferred to a separatory funnel. The aqueous mixture was extracted with 3 × 40 mL $CHCl_3$, and the combined organic layers were washed with 30 mL of water followed by drying over Na_2SO_4 and the solvent evaporated to yield a pale yellow oil which was purified by flash chromatography on a silica column (~20 g silica) eluting with 8:1 $CHCl_3$:$HOCH_3$ (R_f = 0.75). Yield: 1.46 g (68%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.77 (m, 1H), δ 3.39 (m, 17H), δ 2.64−2.33 (m, 11H), δ 1.92−1.76 (m, 12H), δ 1.47 (s, 36H).

1,3-Bis-*N*$_1$-(1,5,9-triazacyclododecyl)-propan-2-ol (8). Boc$_4$-**8** (1.46 g, 1.83 mmol) was dissolved in 25 mL of concentrated aqueous HCl (12 M) and allowed to stir at room temperature for 36 h. The solvent was evaporated under vacuum to give an off-white solid which was then stirred in 50 mL of ethanol for 1 h prior to filtering to give an off-white crystalline powder (the HCl salt of **8**). The hydrochloride salt (0.82 g, 1.3 mmol) was dissolved in 15 mL of 10 M aqueous NaOH and stirred for 2 h during which an orange oily residue developed on the surface of the aqueous solution. The aqueous mixture was extracted with 4 × 20 mL $CHCl_3$ and dried over Na_2SO_4. The solvent was removed under reduced pressure to afford a viscous orange oil that was dried under high vacuum with gentle heating overnight. Yield 0.50 g (96%).

^{1}H NMR (600 MHz, CD_3OD): δ4.06 (m, 1H), δ2.81 (m, 21H), δ2.56 (m, 2H), δ2.42 (m, 3H), δ2.08 (dd, 2H), δ1.85 (m, 3H), δ1.75 (m, 3H), δ1.66 (m, 6H). ^{13}C NMR (400 MHz, $CDCl_3$): δ65.60, 59.10, 55.65, 48.90, 48.57, 47.88, 24.96. HRMS (ESI-TOF): calcd for $C_{21}H_{47}N_6O$ (M-H$^+$): 399.3805; found 399.3790.

Methods. Potentiometric Titrations. Potentiometric titrations were performed[24a] in duplicate using an autotitrator equipped with an Accumet Accu-pHast combination electrode. The typical saturated KCl solution was removed from the outer jacket of the electrode, and then filled with a 1 M $LiClO_4$ solution in methanol. Solutions were titrated in a jacketed glass cell thermostatted at 25 °C while being sparged with N_2. The sodium methoxide titrant was standardized by titrating against an aqueous solution of Fisher-certified HCl (N/50). Aliquots of stock solutions were transferred into the titration cell via a glass pipet, and the total volume was brought to 20.0 mL by adding anhydrous methanol. Subsequent ^s_wpH meter readings in methanol were converted to the ^s_spH values by subtracting the δ correction factor of −2.24.[24b]

The $CH_2OH_2^+$ concentrations for the various kinetic runs were determined potentiometrically using a combination glass electrode (Radiometer model no. XC100−111−120−161) calibrated with certified standard aqueous buffers (pH = 4.00 and 10.00).

UV−visible Kinetics in Methanol. The rates of catalyzed cleavage of **2** (0.05 mM) were monitored spectrophotometrically using a UV−vis spectrophotometer thermostatted at 25.0 ± 0.1 °C. Reaction rates were determined from the rate of appearance of *p*-nitrophenol at 320 nm or *p*-nitrophenolate at 400 nm. All kinetic experiments were performed with catalyst formed in situ through sequential addition of stock solutions (typically 25 mM) of sodium methoxide, ligand and $Zn(OTf)_2$ to anhydrous methanol such that [$^-OCH_3$]:[**6**]:[$Zn(OTf)_2$] = 1:1:2 to make a total volume of 2.5 mL in quartz cuvettes, and kinetic experiments were conducted over the range 0.05 mM < [**6**:Zn_2] < 1.0 mM. Formulation of the catalyst in this way gave solutions

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with ${}^{s}_{s}$pH in the range of 9.0 ± 0.3. Kinetic studies involving **7**:Zn_2 were performed under buffered conditions ([buffer]$_{total}$ = 25 mM) using mixtures of amine (2-picoline, ${}^{s}_{s}pK_a$ = 6.50; 2,6-lutidine, ${}^{s}_{s}pK_a$ = 7.30; *i*-Pr-morpholine, ${}^{s}_{s}pK_a$ = 8.80; triethylamine, ${}^{s}_{s}pK_a$ = 11.04; 2,2,6,6-tetramethylpiperidine, ${}^{s}_{s}pK_a$ = 12.02) and $HClO_4$ in methanol to adjust the ${}^{s}_{s}$pH of the solution. Experiments were conducted over the range 0.075 mM < [**7**:Zn_2] < 0.75 mM. Kinetic experiments with catalyst **8**:Zn(II)$_2$ were conducted under similar buffered conditions, but using HOTf to prepare the buffer. Examination of the catalytic activity of **8**:Zn(II)$_2$ as a function of the time after mixing of the catalyst components showed a leveling off of the activity after 20 min indicating that full formation of the catalyst required some time. The reported values of the pseudo-first order rate constants (k_{obs}) for the production of *p*-nitrophenol-(phenolate) are the averages of duplicate runs.

Results

1. 6:Zn(II)$_2$ Promoted Cleavage of HPNPP. The formation of an active 2:1 dinuclear complex between ligand **6** and Zn(II) was confirmed through kinetic titration of the ligand with $Zn(OTf)_2$ where the k_{obs} for cleavage of **2** was monitored under buffered conditions at varying [$Zn(OTf)_2$]/[**6**] ratios at a constant [**6**] of 0.25 mM. The plot shown in Figure 1 indicates catalytic activity maximizes at [$Zn(OTf)_2$]/[**6**] = 2.0, and plateaus at higher ratios.

This kinetic titration supports the findings of an ^{1}H NMR titration that showed that addition of 1 equiv of $Zn(OTf)_2$ to a 5 mM solution of **6** containing 1 equiv of $NaOCD_3$ in CD_3OD, transformed the simple spectrum of the ligand to a complex spectrum, but addition of a second equivalent of $Zn(OTf)_2$ gave a simplified spectrum with significant broadening. Addition of a third equivalent of $Zn(OTf)_2$ left the ^{1}H spectrum unchanged (see Supporting Information)

The plot of k_{obs} against increasing [**6**:Zn(II)$_2$:($^-$OCH$_3$)] (Figure 2) is indicative of the formation of a substrate-catalyst Michaelis complex. As is the case for all our previous studies with **4**:Zn(II)$_2$:($^-$OCH$_3$) in methanol,[15,16] triflate anion is also an inhibitor of the catalysis exhibited by **6**:Zn(II)$_2$:($^-$OCH$_3$) as evidenced by a downward curving plot of the k_{obs} for the cleavage of **2** at constant [**6**:Zn(II)$_2$:($^-$OCH$_3$)] versus increasing [tetrabutylammonium triflate]. These data were analyzed (see Supporting Information eq (1S)) to give a triflate inhibition constant of 29.3 mM. The inhibition constant was used to correct the kinetic data (See Supporting Information eq 2S)) and the corrected k_{obs} versus [**6**:Zn(II)$_2$:($^-$OCH$_3$)]$_{free}$ data were fit to universal binding eq 1,[25] giving a k_{cat}^{max} of 0.097 ± 0.003 s^{-1} and K_m of (1.1 ± 0.1) × 10^{-5} M where k_{cat}^{max} is the maximum observed rate constant, and K_m is the [**6**:Zn_2:**2**] dissociation constant (taken as the reciprocal of the binding constant, K_B, from eq 1). The second-order rate constant for the cleavage of **2** catalyzed by **6**:Zn(II)$_2$ at ${}^{s}_{s}$pH 9.1 (defined as k_{cat}^{max}/K_m) is $k_2 = 8.8 \times 10^3$ M^{-1} s^{-1}.

$$k_{obs} = k_{cat}(1 + K_B \times [S] + [Cat] \times K_B - X)/(2K_B)/[S] \quad (1)$$

where

$$X = (1 + 2K_B \times [S] + 2 \times [Cat] \times K_B + K_B^2 \times [S]^2 - 2 \times K_B^2 \times [Cat][S] + [Cat]^2 \times K_B^2)^{0.5}$$

The ${}^{s}_{s}$pH-rate profile in Figure 3 for 0.5 mM **6**:Zn(II)$_2$ promoted cleavage of 0.05 mM **2** (conditions under which the catalyst-substrate complex is fully formed) was generated by adding additional $NaOCH_3$ or $HClO_4$ in 0.25 equiv portions to a catalyst formulated in situ by mixing 2 equiv of $Zn(OTf)_2$, with 1 equiv of **6** and 1 equiv of $NaOCH_3$. The k_{obs} versus ${}^{s}_{s}$pH kinetic data were fit to eq 2, derived for the process given in Scheme 2 where the active catalyst has a single methoxide, to give the constants ${}^{s}_{s}pK_a^1$ = 7.4 ± 0.1, ${}^{s}_{s}pK_a^2$ = 11.3 ± 0.1, and k_{cat}^{max} of 0.10 ± 0.01 s^{-1}. From these data and those contained in Figure 2, one can compute the second order rate constant for catalysis of the cyclization of **2** as $k_2^{cat} = k_{cat}^{max}/K_m$ = 9100 M^{-1} s^{-1}.

$$k_{obs} = \left(\frac{k_{cat}^{max}\ {}_{s}^{s}K_a^1}{{}_{s}^{s}K_a^1 + [H^+]}\right)\left(\frac{[H^+]}{{}_{s}^{s}K_a^2 + [H^+]}\right) \quad (2)$$

Potentiometric titration of **6** (1.0 mM) with $NaOCH_3$ in methanol in the presence of 2.0 equiv of $Zn(OTf)_2$ and 1.0 equiv of diphenyl phosphate (Na^+ form) shows two ionization events which, when fit using the Hyperquad 2000 NT computer program,[26] give apparent ${}^{s}_{s}pK_a$ values of 7.02 ± 0.04 and 10.82 ± 0.03 (see Supporting Information) which can be compared with first and second kinetic ${}^{s}_{s}pK_a$ values of 7.4 and 11.3 respectively found for the data in Figure 3.

2. 7:Zn(II)$_2$ Promoted Cleavage of HPNPP. As with **6**, the formation of a 2:1 complex between Zn(II) and ligand **7** was confirmed through a kinetic titration showing that the rate constant for cleavage of **2** (0.05 mM) maximizes at [$Zn(OTf)_2$]/[**7**] = 2.0 at a constant [**7**] of 0.4 mM (see Figure 1S, Supporting Information). This is consistent with ^{1}H NMR data which shows that upon addition of 1 equiv of $Zn(OTf)_2$ to a 5 mM solution of **7**, a complex spectrum results which greatly simplifies on addition of a second equivalent of $Zn(OTf)_2$, indicative of a symmetric 2:1 complex.

Contrary to what was observed in studies with **6**:Zn(II)$_2$, the ${}^{s}_{s}$pH of solutions of **7**:Zn(II)$_2$ could not be held constant simply through addition of 1 equiv of $NaOCH_3$, so the kinetic experiments with this complex were conducted under buffered conditions. The appearance of the plots of k_{obs} versus [**7**:Zn(II)$_2$] for the cleavage of **2** (0.05 mM) were pH-dependent with those determined between ${}^{s}_{s}$pH 6.5 and 11.5 exhibiting downward curvature suggestive of saturation binding, while those determined at ${}^{s}_{s}$pH > 12.0 were linear (see Supporting Information). The extent of buffer inhibition/catalysis was determined for all buffers by measuring the rate of cleavage of **2** at constant [**7**:Zn(II)$_2$] at varying [buffer], and where buffer inhibition was observed, plots of k_{obs} versus [buffer]$_{total}$ were linear with a downward slope. The plots of k_{obs} versus [**7**:Zn(II)$_2$] thus employed the rate constants that

(25) Equation 1 was obtained from the equations for equilibrium binding and for conservation of mass by using the commercially available MAPLE software, *Maple* 9.00, June 13, 2003, Build ID 13164, Maplesoft, a division of Waterloo Maple Inc. 1981−2003, Waterloo, Ontario, Canada.

(26) Gans, P.; Sabatini, A.; Vacca, A. *Talanta* **1996**, *43*, 1739.

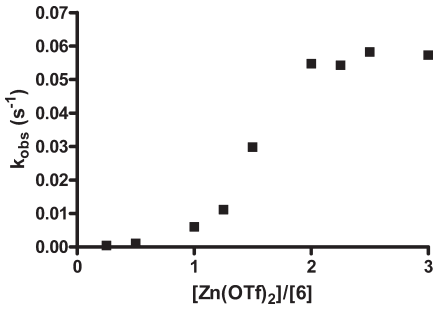


Figure 1. Dependence of the rate of methanolysis of HPNPP (0.05 mM) on the $[Zn(OTf)_2]/[\mathbf{6}]$ ratio at constant [**6**] (0.25 mM) in 25 mM *i*-Pr-morpholine buffer ($^{s}_{s}$pH = 9.1) at $T = 25.0 \pm 0.1$ °C.

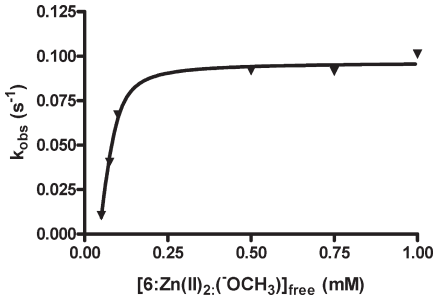


Figure 2. Plot of k_{obs} vs $[\mathbf{6}{:}Zn(II)_2]_{free}$ for cleavage of HPNPP (**2**) (5×10^{-5} M) determined from the rate of appearance of 4-nitrophenol at 320 nm, $^{s}_{s}$pH = 9.1 and $T = 25.0 \pm 0.1$ °C. Data corrected for triflate inhibition and fitting to eq 1 gives $k_{cat}^{max} = 0.097 \pm 0.003\ s^{-1}$ and $K_m = (1.1 \pm 0.1) \times 10^{-5}$ M.

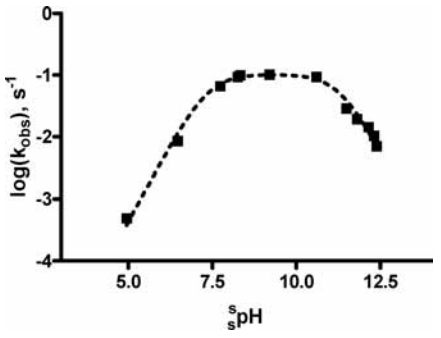
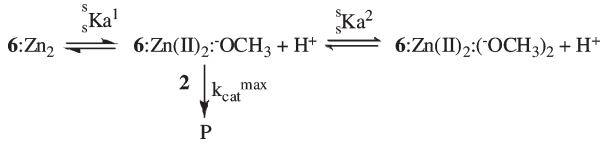


Figure 3. Plot of log k_{obs} vs $^{s}_{s}$pH for the methanolysis of HPNPP (5×10^{-5} M) catalyzed by $\mathbf{6}{:}Zn(II)_2$ (5×10^{-4} M). The best fit dashed line through the data points is calculated on the basis of eq 2 where $^{s}_{s}pK_a^1 = 7.4 \pm 0.1$, $^{s}_{s}pK_a^2 = 11.3 \pm 0.1$, and $k_{cat}^{max} = 0.10 \pm 0.01\ s^{-1}$.

were extrapolated to zero buffer concentration. Second-order rate constants (k_2^{cat}) between $^{s}_{s}$pH = 6.5 and 11.5 were assigned as k_{cat}/K_m, the individual constants being obtained from fitting the k_{obs} versus $[\mathbf{7}{:}Zn(II)_2]$ data to eq 1, while for experiments at $^{s}_{s}$pH > 12.0, the k_2^{cat} constants were taken as the gradients of the k_{obs} versus $[\mathbf{7}{:}Zn(II)_2]$ plots. Kinetic constants (k_{cat} and K_m) and second-order rate constants are summarized in Table 1. A plot of log k_2^{cat} versus $^{s}_{s}$pH for the cleavage of **2** catalyzed by $\mathbf{7}{:}Zn_2$ is given in Figure 4, from which a kinetic $^{s}_{s}pK_a$ of 7.9 and k_2^{max} of 55.8 $M^{-1}\ s^{-1}$ are obtained by fitting the data in Table 1 to eq 3.

$$k_2^{cat} = \left(\frac{k_2^{max}\ {}^{s}_{s}K_a}{{}^{s}_{s}K_a + [H^+]} \right) \qquad (3)$$

Scheme 2. Postulated $^{s}_{s}$pH Dependent Process for the Cleavage of **2** Mediated by $\mathbf{6}{:}Zn(II)_2$



3. 8:Zn(II)$_2$ Promoted Cleavage of HPNPP. Both the kinetic and ^{1}H NMR titration of ligand **8** with $Zn(OTf)_2$ confirm the formation of an active dinuclear $\mathbf{8}{:}Zn(II)_2$ complex. Figure 2S, (Supporting Information) shows a plot of observed rate constant for the cleavage of **2** versus the $[Zn(OTf)_2]/[\mathbf{8}]$ ratio at constant [**8**] which maximizes at 2.0. Kinetic experiments with $\mathbf{8}{:}Zn(II)_2$ were conducted under buffered conditions and at all $^{s}_{s}$pH values, plots of k_{obs} versus $[\mathbf{8}{:}Zn(II)_2]$ for the cleavage of **2** were linear over the [catalyst] range employed (for original data see Supporting Information). Buffer inhibition was examined by monitoring the rate of cleavage of **2** with constant $[\mathbf{8}{:}Zn(II)_2]$ at varying [buffer] (see Supporting Information). No significant inhibition was detected for 2,4,6-collidine or tetramethylpiperidine buffers; however the *i*-Pr-morpholine and triethylamine buffers exhibited weak inhibition. In the case of triethylamine the plot of k_{obs} versus $[buffer]_{total}$ was linear with downward slope from which a theoretical rate constant at zero buffer concentration was determined and used to correct the plot of k_{obs} versus $[\mathbf{8}{:}Zn(II)_2]$. With *i*-Pr-morpholine the k_{obs} versus $[buffer]_{total}$ plot exhibited downward curvature which, when fit to eq (1S) (Supporting Information), gave an inhibition constant of $K_i = 76.4$ mM. The k_{obs} versus $[\mathbf{8}{:}Zn(II)_2]$ plot was corrected for the buffer inhibition based on eq (2S) (Supporting Information).

Second-order rate constants (Table 2) for the cleavage of **2** catalyzed by $\mathbf{8}{:}Zn(II)_2$ were determined as the slope of the linear portions of the plots of k_{obs} versus $[\mathbf{8}{:}Zn(II)_2]$. Between $^{s}_{s}$pH 7.8 and 8.3 these plots are upward curving (see Supporting Information) which we attribute to dissociation of at least one metal ion from the dinuclear catalyst at low concentration giving a far less reactive or inactive mononuclear complex. Similar upward curvature of the k_{obs} versus [catalyst] plots have been observed for the $Cd(II)_2$ complex of **1**[6c] and also for the $Zn(II)_2$ complex of **4**[15,16] and rationalized in terms of incomplete formation of the active dinuclear species at low concentrations.

The plot of the log k_2^{cat} versus $^{s}_{s}$pH data from Table 2 is shown in Figure 5 which, when fit to eq 3, gave a kinetic $^{s}_{s}pK_a$ of 9.65 and k_2^{max} of 7.6 $M^{-1}\ s^{-1}$.

Discussion

To assess the consequences of a bridging oxyanion group in the linker we compared two sets of $\mathbf{L}{:}Zn_2$ systems by investigating their catalysis of the cleavage of **2**. The presence of this linker reduces the net positive charge of the dinuclear core by one unit such that one might anticipate a detrimental effect on catalysis of the cyclization of **2** via a pathway where the dianionic phosphate formed from deprotonation of the cyclizing 2-hydroxypropyl group and its dianionic transition state for cleavage as in Scheme 1. Nevertheless, the presence of such a linker seems to be a requirement to form active complexes in water where its presence acts as an electrostatic buffer to reduce the Zn(II)---Zn(II) repulsion. It has been

Table 1. Kinetic Constants (k_{cat} and K_m) and Second-Order Rate Constants for the Cleavage of **2** (0.05 mM) Catalyzed by **7**:Zn(II)$_2$ (0.075−0.75 mM) at T = 25.0 ± 0.1°C[a]

buffer	^s_spH	k_{cat} (s^{-1})	K_m (mM)	k_2^{cat} (M^{-1} s^{-1})
2-picoline	6.50	$(4.3 \pm 0.2) \times 10^{-5}$	0.03 ± 0.01	1.4 ± 0.5
2,6-lutidine	7.30	$(1.0 \pm 0.01) \times 10^{-4}$	0.0055 ± 0.0008	18 ± 3
iPr-morpholine	8.80	$(3.6 \pm 0.2) \times 10^{-3}$	0.11 ± 0.04	32 ± 11
Triethylamine	11.04	0.078 ± 0.01	1.4 ± 0.3	56 ± 13
2,2,6,6-tetramethylpiperidine	12.02	*b*	*b*	51 ± 1
2,2,6,6-tetramethylpiperidine	12.33	*b*	*b*	81 ± 2

[a] Data corrected for buffer inhibition but not for triflate inhibition which is negligibly small. [b] At these two ^s_spH values, k_2^{cat} is determined as the gradient of the linear k_{obs} vs [**7**:Zn(II)$_2$] plots.

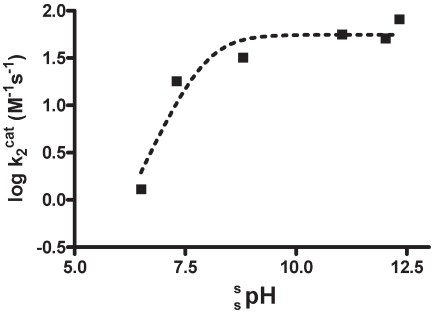


Figure 4. Plot of log k_2^{cat} vs ^s_spH for the cleavage of **2** (5×10^{-5} M) catalyzed by **7**:Zn(II)$_2$. The dashed line through the data points is calculated from the NLLSQ fit to eq 3 with ^s_spK_a = 7.9 ± 0.2 and k_2^{cat} = 55.8 ± 12.9 M^{-1} s^{-1}.

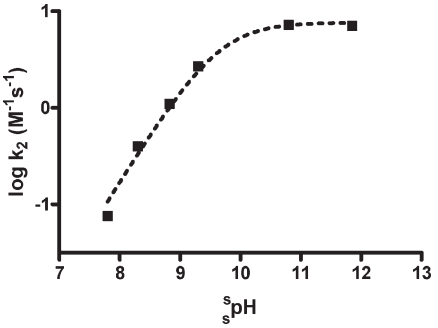


Figure 5. Plot of log k_2 vs ^s_spH for the methanolysis of 0.05 mM **2** catalyzed by **8**:Zn(II)$_2$. The dashed line through the data points is calculated from the fit of the data to eq 3 which gives a ^s_spK_a of 9.65 ± 0.09 and k_2^{max} = 7.6 ± 1.1 M^{-1} s^{-1}.

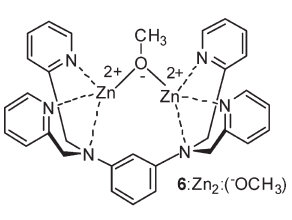
Table 2. Second-Order Rate Constants for the Cleavage of 0.05 mM **2** Catalyzed by **8**:Zn(II)$_2$ (0.075−0.75 mM) at T = 25.0 ± 0.1°C[a]

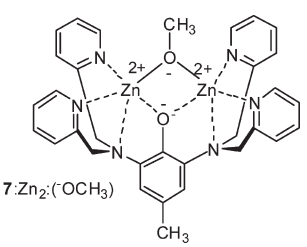
buffer	^s_spH	k_2^{cat} (M^{-1} s^{-1})
2,4,6-collidine	7.8	0.08 ± 0.003
i-Pr-morpholine	8.3	0.4 ± 0.03
i-Pr-morpholine	8.8	1.1 ± 0.1
i-Pr-morpholine	9.3	2.7 ± 0.1
triethylamine	10.8	7.3 ± 0.1
2,2,6,6-tetramethylpiperidine	11.9	7.1 ± 0.2

[a] Data are corrected for buffer effects by extrapolating to zero [buffer], but are not corrected for triflate ion inhibition which is negligibly small.

stated,[12a] on the basis of analysis of the activities of several systems,[2,6b,12b] that "for Zn(II) complexes, a bridging linker is a necessity for cooperative catalysis. Zn(II) complexes that lack this linker are barely more active than their mononuclear analogues." Among the most effective linkers in this regard are the calixeranes[2c] and 2-propoxy ones.[2a,4,6] While such linker systems with bridging anions that bind to both metal ions may well be beneficial in water, the summary of the kinetic data in Table 3 that compares the activities of **4**:Zn(II)$_2$ with **8**:Zn(II)$_2$ and **6**:Zn(II)$_2$ with **7**:Zn(II)$_2$ in methanol show that, in less polar solvents that more closely resemble the effective dielectric constants of the interiors of enzymes,[27] the presence of bridging oxyanion groups is detrimental to activity. It is also notable in the present case that, while many of the studies of dinuclear Zn(II) catalysts report on the *hydrolysis* of RNA analogues in water, the reactions monitored and their immediate products are not hydrolytic ones, but ones that result from intramolecular transesterifications as in Scheme 1 where the solvent nature, being water or alcohol, is not pertinent to the observed cleavage.

Comparison of the Rates of Cleavage of HPNPP Promoted by 6:Zn(II)$_2$:($^-$OCH$_3$) and 7:Zn(II)$_2$:($^-$OCH$_3$). The ^s_spH-rate profiles presented in Figures 3 and 4 indicate that the active catalysts in both systems are generated by ionizations having respective kinetic ^s_spK_a values of 7.4 and 7.9 that result from the deprotonation of bound methanol to form a methoxide coordinated to one or both metal ions. For the two systems that contain the oxyanion linker (**7**:Zn(II)$_2$ and **8**:Zn(II)$_2$) the ionization of the oxyanion linker is not observed in any of our kinetic studies, but the associated ^s_spK_a values should be lower than 7 by analogy with other systems investigated in water where the pK_a for ionization of the linker is lower than any ionization stemming from a metal-bound HOH.[6] The kinetic ^s_spK_a^1 determined for **6**:Zn(II)$_2$ is close to the titrimetric ^s_spK_a of 7.02 found for the complex when bound to a non-reactive diphenyl phosphate anion which supports the assertion that the kinetically active form is a ternary complex between the Zn(II)$_2$ complex and the bound substrate **2** or its kinetic equivalent **2**$^-$.





Importantly, the potentiometric titration of **7**:Zn(II)$_2$ in the presence of equimolar diphenyl phosphate (Na^+ form)

(27) (a) Richard, J. P.; Ames, T. L. *Bioorg. Chem.* **2004**, *32*, 354. (b) Cleland, W. W.; Frey, P. A.; Gerlt, J. A. *J. Biol. Chem.* **1998**, *273*, 25529. (c) Czerwinski, R. M.; Harris, T. K.; Massiah, M. A.; Mildvan, A. S.; Whitman, C. P. *Biochemistry* **2001**, *40*, 1984. (d) Plow, F.; Kowlessur, D.; Malthouse, J. P. G.; Mellor, G. W.; Hartshorn, M. J.; Pinitglang, H. P.; Topham, C. M.; Thomas, E. W.; Verma, C.; Brocklehurst, K. *J. Mol. Biol.* **1996**, *257*, 1088. (e) Cachau, R. E.; Garcia-Moreno, E. B. *J. Mol. Biol.* **1996**, *255*, 340. (f) Kanski, R.; Murray, C. J. *Tetrahedron Lett.* **1993**, 2263.

Table 3. Constants for the Various Catalysts Used to Calculate the $\Delta\Delta G^{\ddagger}_{stab}$ for **L**:Zn(II)$_2$ Binding to the Transition State of the Presumed Methoxide Reaction for Cyclization of **2**

catalyst	${}^{s}_{s}pK_a^1$	${}^{s}_{s}K_a^1/K_{auto}$ [a]	k_2^{-OMe} [b] (M^{-1} s^{-1})	$k_2^{L:Zn2:(-OMe)}$ (M^{-1} s^{-1})	$\Delta\Delta G^{\ddagger}_{stab}$ [e] (kcal/mol)
4:Zn(II)$_2$:($^-$OCH$_3$)	9.3	2.95×10^7	2.6×10^{-3}	275,000[c]	−21.1
8:Zn(II)$_2$:($^-$OCH$_3$)	9.65	1.17×10^7	2.6×10^{-3}	7.6[d]	−14.3
6:Zn(II)$_2$:($^-$OCH$_3$)	7.4	2.34×10^9	2.6×10^{-3}	9.1×10^3[d]	−21.7
7:Zn(II)$_2$:($^-$OCH$_3$)	7.9	7.41×10^8	2.6×10^{-3}	55.8[d]	−18.0

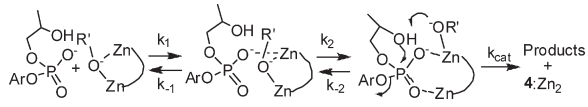
[a] Determined from the kinetic or titrimetric ${}^{s}_{s}pK_a^1$ value for the complex and the autoprotolysis constant of methanol ($10^{-16.77}$ M^2). [b] From ref 28. [c] From ref 15. [d] This work. [e] Computed from eq 4 at standard state of 1 M.

shows a well-defined ionization with a ${}^{s}_{s}pK_a$ of less than 4 which we attribute to the bridging phenoxy group. Unfortunately, the same potentiometric titrations of **7**:Zn(II)$_2$ along with equimolar diphenyl phosphate (see Supporting Information) do not give information concerning the ${}^{s}_{s}pK_a$ values for Zn(II)-bound methanols because of the apparent displacement of phosphate from the complex that occurs at increasing [methoxide], likely as a result of the weaker binding between the phosphate and catalyst complex (which already has a bridging aryloxy anion present so that the net positive charge is reduced by one unit relative to the situation with **6**:Zn(II)$_2$). This interpretation is consistent with results of kinetic experiments showing that binding between substrate and **7**:Zn(II)$_2$ became weaker as ${}^{s}_{s}$pH increased, as evidenced by the progression from saturation kinetics to linear kinetics at higher ${}^{s}_{s}$pH. For convenience, we represent the active forms of **6**:Zn(II)$_2$:($^-$OCH$_3$) and **7**:Zn(II)$_2$:($^-$OCH$_3$) as having bridging methoxides, although it is possible that this is only bound to one of the Zn(II) ions in **7**:Zn(II)$_2$:($^-$OCH$_3$).

Compared to the methoxide promoted background reaction with $k_2^{OMe} = 2.6 \times 10^{-3}$ M^{-1} s^{-1},[28] both **6**:Zn(II)$_2$:($^-$OCH$_3$) and **7**:Zn(II)$_2$:($^-$OCH$_3$) give significant rate accelerations for cleavage of **2**. At ${}^{s}_{s}$pH = 9.1, the center of the bell-shaped pH-rate profile of Figure 3, the observed rate constant of $k_{cat}^{max} = 0.10$ s^{-1} for the **6**:Zn(II)$_2$:($^-$OCH$_3$)-catalyzed reaction is 1.7×10^9 greater than the methoxide reaction at that ${}^{s}_{s}$pH. In terms of its apparent second order rate constant, $k_{cat}^{max}/K_m = k_2^{cat} = 0.1$ $s^{-1}/1.1 \times 10^{-5}$ M = 9100 M^{-1} s^{-1}, **6**:Zn(II)$_2$:($^-$OCH$_3$) is 5.2×10^6 more effective than methoxide. Similarly, above ${}^{s}_{s}$pH = 8.8 where **7**:Zn(II)$_2$:($^-$OCH$_3$) is fully formed, its k_2^{cat} of 55.8 M^{-1} s^{-1} is ~21,000 times larger than that of methoxide but ~160 times less reactive than **6**:Zn(II)$_2$:($^-$OCH$_3$) because of the presence of the bridging oxyanion.

Comparison of the Cleavage of HPNPP Promoted by 4:Zn(II)$_2$:($^-$OCH$_3$) and 8:Zn(II)$_2$:($^-$OCH$_3$). NLLSQ fitting of the ${}^{s}_{s}$pH/rate constant data shown in Figure 5 for the **8**:Zn$_2$ promoted cleavage of **2** to eq 3 gives a kinetic ${}^{s}_{s}pK_a$ of 9.65 and a $k_2^{max} = 7.6$ M^{-1} s^{-1}. Furthermore, a plot of k_{obs} versus added methoxide indicates that maximal catalytic activity is attained at a methoxide/Zn(II)$_2$ ratio of 1 with a sharp drop in rate constant when the ratio is <1. Taken together, these data indicate that two methoxides per catalyst are required for activity: one to generate the bridging propoxy anion which is fully bound to the two metal ions below ${}^{s}_{s}$pH 7 as a structural component, and a second added to the complex as

Scheme 3. Proposed Pathway for the Cleavage of Phosphate Diesters Promoted by **4**:Zn(II)$_2$:($^-$OR)[a]



[a] R = CH$_3$ or 2-hydroxypropyl; $^-$OR' = alkoxide.

OCH$_3^-$ or as a kinetic equivalent such as a deprotonated 2-oxypropyl substrate (**2**$^-$). Since none of the plots of k_{obs} versus [**8**:Zn(II)$_2$] reveals evidence of saturation substrate binding at any ${}^{s}_{s}$pH, the k_2 values reported in Table 2 are simply given as the gradients of the lines.

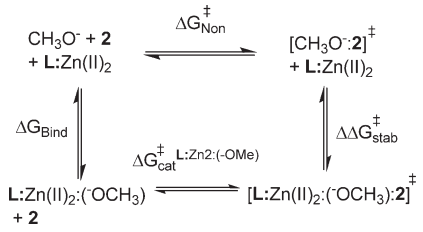
Our previous[15,16] and more recent[29] work with **4**:Zn(II)$_2$ and 2-hydroxypropyl aryl phosphates (including **2**) gave kinetic values of ${}^{s}_{s}pK_a^1 = 9.3$ and ${}^{s}_{s}pK_a^2 = 11.2$. While the cleavage of 2-hydroxypropyl aryl phosphates having poor leaving groups gave saturation kinetic profiles, that with **2**, having a good *p*-nitrophenoxy leaving group, was linear with a gradient of $k_2 = 275{,}000$ M^{-1} s^{-1} which is about 10^8 larger than the k_2^{OMe} value for the methoxide reaction.[16] Mechanistic investigations led to the proposal that the catalyzed reaction follows a multistep pathway consistent with a minimal process given in Scheme 3 with a bimolecular binding step of catalyst plus phosphate, followed by intramolecular rearrangement to form a catalytically competent complex where the phosphate is activated by binding to two metal ions. This is followed by one or more chemical steps that produce the observed phenol leaving group and the cyclic 5-membered phosphate. For catalyzed cleavage of substrates with good leaving groups like *p*-nitrophenoxy, the k_{cat} term in Scheme 3 is larger than k_{-2} so the rate limiting step is formation of the activated phosphate complex which leads to the linear form of the k_{obs} versus [catalyst] plot. With substrates having poor leaving groups, the k_{cat} term in Scheme 3 is smaller than k_{-2}, so saturation behavior is observed.

The linear appearance of the k_{obs} versus [**8**:Zn(II)$_2$] plots with **8**:Zn(II)$_2$:($^-$OCH$_3$) may involve a similar sort of two step binding process, although a more likely explanation is that the substrate binding is simply weaker because the net positive charge on **8**:Zn(II)$_2$ is less than that on **4**:Zn(II)$_2$ or because the coordination number of the metal ions is higher in **8**:Zn(II)$_2$. In terms of k_2^{cat} values presented in Table 3, the activity of **8**:Zn(II)$_2$:($^-$OCH$_3$) is 37,000 times less than that of **4**:Zn(II)$_2$:($^-$OCH$_3$).

(28) Tsang, J. S.; Neverov, A. A.; Brown, R. S. *J. Am. Chem. Soc.* **2003**, *125*, 1559.

(29) Brown, R. S.; Liu, C. T., unpublished results. By way of comparison, the first ${}^{s}_{s}pK_a$ for formation of **4**:Zn$_2$:($^-$OCH$_3$) determined by half-neutralization, is 9.41 (ref 16.).

Scheme 4. Thermodynamic Cycle Comparing **L**:Zn(II)$_2$:($^-$OCH$_3$) and $^-$OCH$_3$ Promoted Cyclization Reactions of **2**



Energetics calculations. Analysis of the energetic factors which govern the catalysis by the Zn(II)$_2$ complexes of **4**, and **6**−**8** compares the free energy of binding of the catalyst to the transition state of the presumed lyoxide-promoted reaction.[6d,30−32] In Scheme 4 is a thermodynamic cycle that allows a quantitative assessment of the ΔG for binding the catalyst to a hypothetical transition state (TS) involving methoxide plus substrate (or its kinetic equivalent of a TS involving the 2-oxypropyl aryl phosphate dianion). The $\Delta\Delta G^{\ddagger}_{stab}$ for this is given in eq 4 where ΔG_{Bind} is the free energy of binding $^-$OCH$_3$ to **L**:Zn(II)$_2$ (mathematically equivalent to the first ionization constant of the **L**:Zn(II)$_2$ complex divided by the K_{auto} of methanol ($10^{-16.77}$)), $\Delta G^{\ddagger}_{cat}{}^{L:Zn2:(-OMe)}$ and $\Delta G^{\ddagger}_{Non}$ are the activation free energies of the catalyzed and methoxide reactions calculable from their second order rate constants give in Table 3. The units of the term in square brackets in eq 4 are M^{-1} signifying that this is formally an association constant of the catalyst and the TS.[30,31]

$$\Delta\Delta G^{\neq}_{stab} = (\Delta G_{Bind} + \Delta G_{cat}{}^{L:Zn_2:(-OMe)}) - \Delta G^{\neq}_{Non} = -RT \ln\left[\frac{(k_2^{cat})({}^{s}_{s}K_a/K_{auto})}{k_2^{-OMe}}\right] \quad (4)$$

Given in Table 3 are values for the various rate and equilibrium constants used for the calculations of the $\Delta\Delta G^{\ddagger}_{stab}$ at standard state. The data clearly indicate that free energy for binding of the catalyst to the transition state consisting of $CH_3O^- + \mathbf{2}$, or its kinetic equivalent of $\mathbf{2}^-$, is more negative for the complexes without the bridging oxyanions. The origins of the effect can be gleaned from the graphical representations given in Figures 6 and 7 of the ΔG values of the three main components (ΔG_{Bind}, $\Delta G^{\ddagger}_{cat}{}^{L:Zn2:(-OMe)}$, and $\Delta G^{\ddagger}_{Non}$) contributing to the $\Delta\Delta G^{\ddagger}_{stab}$ in eq 4.

Figure 6 shows that binding of **6**:Zn(II)$_2$ or **7**:Zn(II)$_2$ with $^-$OCH$_3$ is exergonic by 12.8 and 12.2 kcal/mol, respectively, and that the $\Delta G^{\ddagger}$ for the reaction of **L**:Zn(II)$_2$:($^-$OCH$_3$) with **2**[33] is endergonic by 12.1 and 15.1 kcal/mol, respectively. Thus, while the affinity of each complex for methoxide is nearly the same because the ${}^{s}_{s}pK^{l}_{a}$ values are close, the activation energies are quite

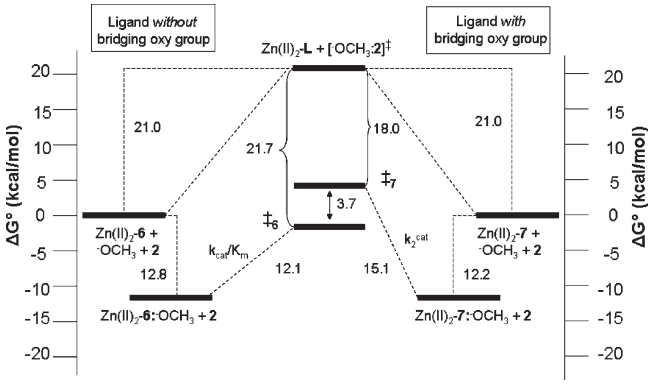


Figure 6. Free energy diagram comparing the reactions of CH_3O^-, **6**:Zn(II)$_2$:($^-$OCH$_3$), and **7**:Zn(II)$_2$:($^-$OCH$_3$) with **2** at standard state of 1.0 M and T = 25 °C showing the computed ΔG values for CH_3O^- binding to **L**:Zn(II)$_2$ and the $\Delta G^{\ddagger}$ for k_{cat}/K_m, k_2^{cat} and k_2^{-OMe} derived from the experimental rate and equilibrium constants.

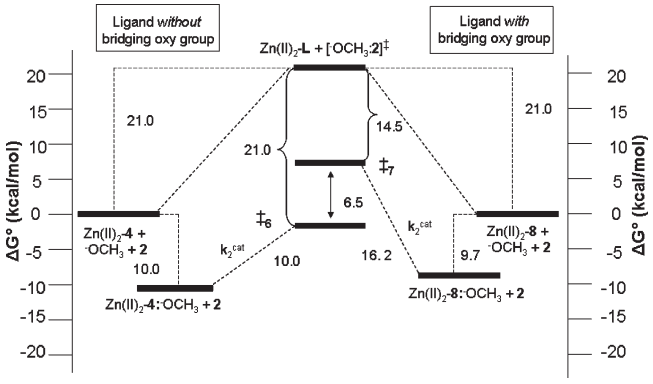


Figure 7. Free energy diagram comparing the reactions of CH_3O^-, **4**:Zn(II)$_2$:($^-$OCH$_3$), and **8**:Zn(II)$_2$:($^-$OCH$_3$) with **2** at standard state of 1.0 M and T = 25 °C showing the computed ΔG values for CH_3O^- binding to **L**:Zn(II)$_2$ and the $\Delta G^{\ddagger}$ for k_2^{cat} and k_2^{-OMe} derived from the experimental rate and equilibrium constants.

different with **7**:Zn(II)$_2$:($^-$OCH$_3$) being about 3 kcal/mol larger probably because the apparent binding constant of **2** with the catalyst is smaller for the form having the oxyanion bridge. The $\Delta G^{\ddagger}$ for the methoxide reaction in all cases is computed to be 21.0 kcal/mol. Values of $\Delta\Delta G^{\ddagger}$ = 21.7 and 18.0 kcal/mol are calculated for the transition state stabilization afforded by **6**:Zn(II)$_2$ and **7**:Zn(II)$_2$.

Figure 7 shows the analogous free energy plot for the process involving **4**:Zn(II)$_2$ and **8**:Zn(II)$_2$. Once again, the binding of methoxide to each complex is about the same at 10.0 and 9.7 kcal/mol, respectively, meaning that the presence of the oxyanion linker does not greatly perturb the ${}^{s}_{s}pK^{l}_{a}$. However the $\Delta G^{\ddagger}$ values for the k_2^{cat} for the catalyzed reactions are quite different at 10 and 16.2 kcal/mol, with the complex with the bridging alkoxy group (**8**:Zn(II)$_2$:($^-$OCH$_3$)) being larger.

The representations in Figures 6 and 7 indicate that poorer catalysis for the oxyanion containing complexes originates in their smaller second order rate constants (k_2^{cat} or k_{cat}/K_m values). These constants are made up of a pre-equilibrium binding of the substrate and catalyst, as well as the kinetic term for the intramolecular cleavage. The oxyanion could disfavor either or both of these by decreasing the Lewis acidity of the Zn(II)$_2$ complex due to reducing the net positive charge by one unit, thereby

(30) Wolfenden, R. *Nature* **1969**, *223*, 704.
(31) For applications of this energetic treatment to phosphate cleavage and other reactions see: Yatsimirsky, A. K. *Coord. Chem. Rev.* **2005**, *249*, 1997, and references therein.
(32) Yang, M.-Y.; Morrow, J. R.; Richard, J. P. *Bioorg. Chem.* **2007**, *35*, 366.
(33) $\Delta G^{\ddagger}$ for the complex or methoxide promoted reactions calculated from the Eyring equation as $\Delta G^{\ddagger} = -RT \ln(k_2^{L:Zn2:(-OMe)}/(kT/h))$ or $-RT \ln(k_2^{-OMe}/(kT/h))$ where $kT/h = 6 \times 10^{12}$ s^{-1} at 298 K.

reducing the substrate binding. In addition, since strong electrostatic interactions are an important stabilizing feature for the transition states of these sorts of reactions between positively charged catalysts and negatively charged substrates, any decrease in the net positive charge of the catalyst should reduce the electrostatic charge neutralization in the transition state and raise the activation energy of the chemical transformations. Finally, the bridging oxyanion occupies a coordination site on each Zn(II) ion, leaving fewer coordination sites available for binding of the substrate in the Michaelis complex and transition state.

Conclusions

The observation that a bridging group such as an oxyanion is an essential component of the ligand to engender cooperativity in dinuclear catalysts in water dictates the design of the majority of the newer synthetic metallonucleases that have been investigated under aqueous conditions. It is generally observed that dinucleating ligands without such bridging groups cannot form dinuclear catalysts with activities much greater than the sum of their parts,[2,12] and in many, if not most, cases they are unable to form dinuclear complexes at all unless there is an electrostatic buffer that insulates the charge repulsion of the two metal ions and fixes them in place through binding. Thus, in aqueous solution it has been a difficult task to investigate how such a linker influences the kinetics of cleavage of phosphates in comparison with systems that lack a bridging oxyanion group.

The medium effect afforded by the light alcohols such as methanol and ethanol allows us to directly examine the effect of an anionic linker on the catalytic activity by comparing two sets of dinuclear Zn(II) complexes. The data clearly indicate that the inclusion of the propoxide and phenoxide in **8**:$Zn(II)_2$ and **7**:$Zn(II)_2$ makes them inferior in terms of rate acceleration when compared to the analogous complexes lacking the oxyanion bridge (**4**:$Zn(II)_2$ and **6**:$Zn(II)_2$). The presence of the phenoxide of **7**:$Zn(II)_2$ leads to a 3.7 kcal/mol loss in transition state stabilization compared to **6**:$Zn(II)_2$ which is manifested in a 160-fold decrease in k_2^{cat} for the cleavage of **2**. The substitution of the propoxy group for the propyl linker of **4**:$Zn(II)_2$ to produce **8**:$Zn(II)_2$ leads to a 3.7×10^4-fold drop in k_2^{cat} that results from a 6.5 kcal/mol decrease in transition state stabilization. The kinetic analysis described here demonstrates that the inclusion of a bridging oxyanion between the metal centers in a dinuclear complex is not only unnecessary for catalyzed cleavage of **2** in methanol, but in fact quite counterproductive in terms of rate. It seems likely that the requirement for an anionic bridging group for these sorts of dinuclear catalysts in water is a necessary, but not optimal, compromise to ameliorate unfavorable electrostatic interactions and heavier solvation of the metal ions leading to poorer binding with the ligand and poorer catalysis.

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Supporting Information Available: Plots of kinetics data as a function of [**L**:Zn^{2+}], NMR spectra of ligand systems as a function of added [Zn(II)], potentiometric titration profiles, tables of kinetic data and methods for correction of kinetic data for buffer effects and for triflate ion inhibition (27 pages). This material is available free of charge via the Internet at http://pubs.acs.org.