*Dinuclear Complexes*

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# Efficient Phosphodiester Binding and Cleavage by a $Zn^{II}$ Complex Combining Hydrogen-Bonding Interactions and Double Lewis Acid Activation**

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Considerable efforts have been invested in the development of small synthetic metallonucleases for the cleavage of RNA, or more ambitiously DNA, as a result of their potential application as therapeutic agents and robust, versatile replacements for nucleases as laboratory tools.[1] Currently, these complexes are still very inefficient relative to enzymes and this behavior limits their practical applications. More fundamentally, the development of artificial systems both tests and expands our understanding of how catalysis works under biological conditions. These compounds are also important for the development of artificial receptors for biologically important phosphates that are effective under

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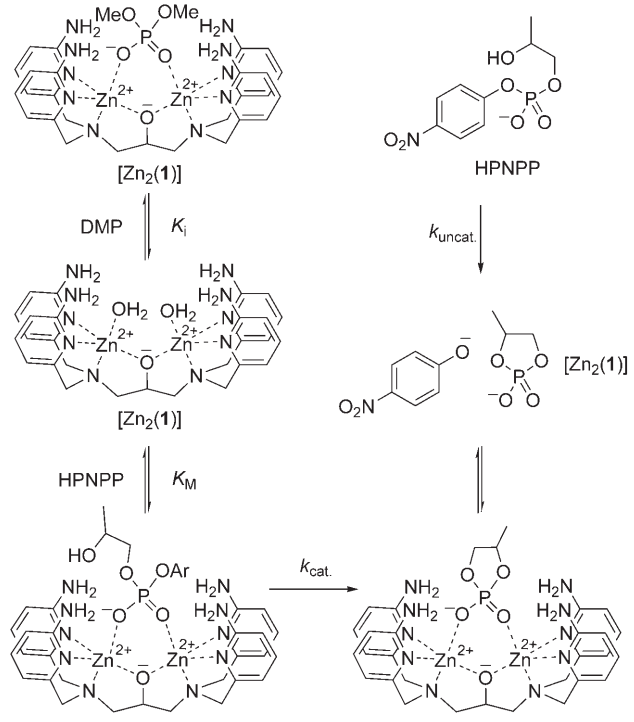
these conditions.[2] Inspired by the structures of dinuclear metalloenzymes,[3] many studies have focused on the synthesis of dinuclear or polynuclear metal-ion complexes. Typically, these complexes bind phosphates more strongly and are significantly more reactive for the cleavage of phosphate esters than their corresponding mononuclear metal-ion complexes.[2,4] However, many metalloenzymes that catalyze the cleavage of phosphate esters also use amino acid side chains to stabilize the transition state directly as well as providing a scaffold for the metal-ion cofactor.[3] Recently, several ligands have been designed to enhance the properties of their corresponding mononuclear metal complexes through the second coordination sphere.[5–7] Generally, in artificial systems designed to efficiently bind and catalyze the cleavage of phosphate esters, these ligands have been functionalized by hydrogen-bond donors, such as ammonium, amino, or guanidine groups.[6,7] We have now combined both strategies to make a much more efficient dinuclear $Zn^{II}$ complex and report our findings herein.

Specifically, the addition of 2-amino substituents to the pyridine units of metal-ion-binding ligands has been shown to be an effective modification for enhancing the binding and catalytic activity of mononuclear $Cu^{II}$,[7c] $Co^{III}$,[6a] and $Zn^{II}$[6c,7f,g] complexes. Interestingly, we showed that the introduction of aminopyridyl hydrogen-bond donors to a mononuclear $Zn^{II}$ complex is as effective as the introduction of a second $Zn^{II}$ ion for enhancing its activity towards the transesterification of phosphodiesters.[8] On the basis of these data, we designed the dinuclear $Zn^{II}$ complex $[Zn_2(\mathbf{1})]$[9] (Scheme 1), which introduces four aminopyridyl hydrogen-bond donors positioned to interact with substrates bound to the metal-ion centers, to assess the effectiveness of this combination of strategies.




***Scheme 1.*** Structures of complexes and substrates, and a proposed mechanistic scheme.

Crystallization of $[Zn_2(\mathbf{1})]$ in the presence of 4-nitrophenyl phosphate (PNPP) revealed that the phosphate binds to the complex by bridging the two $Zn^{II}$ ions as required for

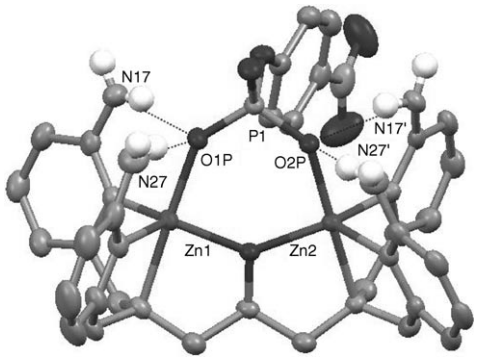



***Figure 1.*** Structure of the $[Zn_2(\mathbf{1})]\cdot PNPP^+$ cation, with thermal ellipsoids drawn at 30% probability. Only N-bound hydrogen atoms (white) are shown for clarity; these were located in different maps and refined freely. Hydrogen-bonding interactions [Å]: N(17)···O(1P) 2.818(4), N(27)···O(1P) 2.884(4), N(17′)···O(2P) 2.882(4), N(27′)···O(2P) 2.872(4).

double Lewis acid activation (Figure 1). This structure is consistent with previous studies of $Zn^{II}$ complexes containing the same core dinucleating ligand structure.[10] This behavior places the phosphoryl oxygen atoms in hydrogen-bonding distance of the amino groups so that they can assist the binding of phosphate substrates to the $Zn^{II}$ ion centers and in stabilizing the transition state.

We started our studies with HPNPP (Scheme 1), which has been widely used as a convenient model for RNA-like phosphodiesters. Initial experiments confirmed that $[Zn_2(\mathbf{1})]$ was highly reactive, with a 0.2 mM solution decreasing the half-life of HPNPP (0.05 mM) to about a minute at pH 7.4 and 25 °C—thus corresponding to an observed fold-rate acceleration of approximately $10^6$ and showing that this is the most effective artificial $Zn^{II}$ complex for catalyzing this reaction reported to date.[1d,4b] $^{31}P$ NMR spectroscopic analysis showed that the catalyst cleanly converts HPNPP into propylene phosphate with a complete turnover of 5 mM of substrate by 1 mM of complex. To characterize this reactivity, the dependence of the initial rate of the reaction on HPNPP concentration was measured. As shown in Figure 2, higher concentrations led to saturation of the catalyst, and the data could be fitted to the Michaelis–Menten reaction scheme [Scheme 1; Eq. (1)], thus giving $k_{cat.} = 0.017 \pm 0.001\ s^{-1}$ and $K_M = 0.32 \pm 0.03$ mM at pH 7.4.

$$\text{Initial rate} = k_{cat.}[Zn_2(\mathbf{1})]\frac{[HPNPP]}{(K_M + [HPNPP])} \quad (1)$$

To further explore the catalytic turnover qualitatively observed by $^{31}P$ NMR spectroscopy, we gathered kinetic data by using UV/Vis spectroscopy (Figure 3). These data confirm that 5- and 10-fold excesses of substrate were rapidly turned over by the catalyst. These curves can be fit to a first-order curve; however, this analysis is only appropriate if the

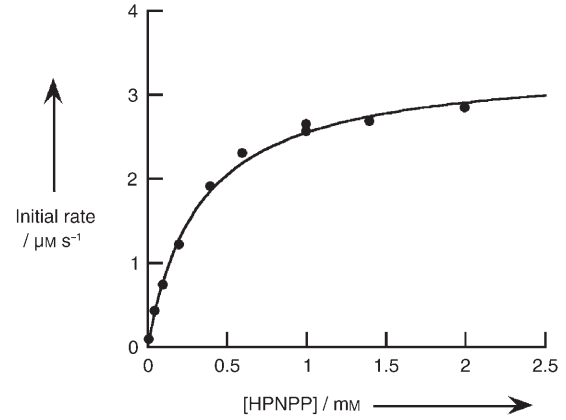



***Figure 2.*** Saturation kinetic studies for the transesterification of HPNPP catalyzed by [$Zn_2$(**1**)] (0.2 mM) at pH 7.4 (50 mM HEPES buffer; HEPES = 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid) and 25 °C. The solid line is the least-squares fit of Equation (1).

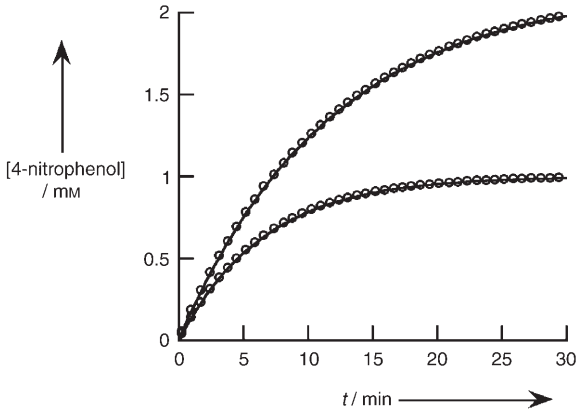



***Figure 3.*** Turnover studies of the transesterification of 1 and 2 mM HPNPP catalyzed by [$Zn_2$(**1**)] (0.2 mM) at pH 7.4 (50 mM HEPES buffer) and 25 °C. Solid lines show the predicted reaction progress as described in the text.

substrate is at subsaturating concentrations—and in both cases, the initial substrate concentration is significantly greater than the value for $K_M$ that we had determined. An alternative explanation is that the reaction is inhibited by product formation; if the $K_i$ value is similar to the $K_M$ value then apparent first-order behavior is also expected. This expectation is reasonable as the reaction generates a phosphate diester product that will have a similar affinity for the complex as the substrate. We tested this proposal by measuring how dimethyl phosphate (DMP) inhibited the transesterification of HPNPP. Figure 4 shows a plot of normalized first-order rate constant ($k/k_0$) for the transesterification of HPNPP at pH 7.4 with increasing concentration of DMP. The catalyzed reaction is clearly inhibited by DMP and is fit to Equation (2), which is derived for competitive inhibition (Scheme 1).

$$\frac{k}{k_0} = \frac{K_i}{(K_i + [\mathrm{DMP}])} \qquad (2)$$

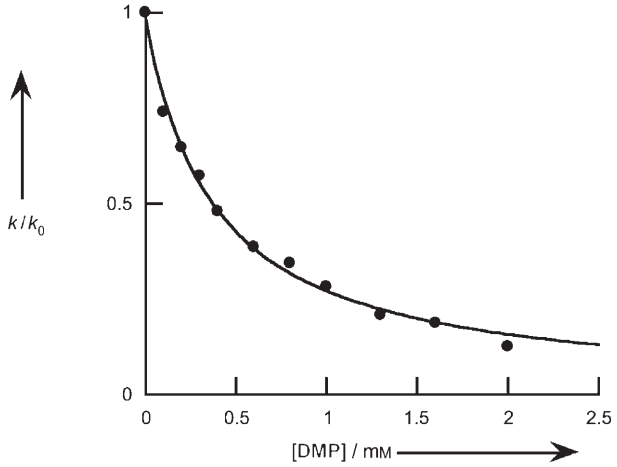



***Figure 4.*** Variation in the ratio of the rate constants for transesterification of HPNPP catalyzed by 0.2 mM [$Zn_2$(**1**)] in the presence of DMP ($k$) to the rate in the absence of DMP ($k_0$) at pH 7.4 (50 mM HEPES). The solid line is the least-squares fit of Equation (2).

The value obtained for $K_i$ ($0.37 \pm 0.07$ mM) is in good agreement with the value for substrate binding to the complex. [$Zn_2$(**1**)] binds remarkably strongly to the diester monoanion in fully aqueous solution.[11] Using these parameters ($k_{cat.}$, $K_M$, and $K_i$) to simulate the expected progress curve for the complete reaction gives excellent agreement with the experimental data (solid lines in Figure 3).

Combining these parameters gives $k_{cat.}/K_M$, the apparent second-order rate constant for catalysis by [$Zn_2$(**1**)], as $53\,M^{-1}s^{-1}$. The value for the analogous dinuclear complex without 2-amino groups on the pyridine moieties is $0.073\,M^{-1}s^{-1}$,[8,12] so this modification enhances reactivity by almost three orders of magnitude. This increase is divided roughly equally between enhanced binding of the substrate and higher reactivity of the substrate–catalyst complex.

Although HPNPP transesterification has been widely used as a convenient model for RNA cleavage, it has a less effective intramolecular nucleophile and much better leaving group than RNA. These differences mean that a catalyst that can catalyze the hydrolysis of HPNPP may not be a good catalyst for RNA cleavage.[4b,13] Therefore, we investigated whether [$Zn_2$(**1**)] is also effective for catalyzing the cleavage of uridyl(3′-5′)uridine (UpU). At pH 7.3 and 25 °C, 1 mM [$Zn_2$(**1**)] does efficiently catalyze the hydrolysis of UpU (0.06 mM). The loss of UpU was monitored by reverse-phase HPLC and fitted to a first-order curve (Figure 5), thus giving an observed pseudo-first-order rate constant of $1.2 \pm 0.1 \times 10^{-4}\,s^{-1}$. This finding corresponds to a rate acceleration of approximately $10^6$-fold,[14] which is similar to that observed for the catalysis of HPNPP cleavage—and so the high activity is not confined to the activated substrate.

A similar observation was reported by Richard and coworkers using a triazacyclononane-based dinuclear $Zn^{II}$ complex that has the same bridging group but which under the same reaction conditions gives $k_{obs} = 0.7 \times 10^{-6}\,s^{-1}$.[14] This complex has similar reactivity to the pyridyl-based ligand with no 2-amino substituents, so the amino groups provide an additional rate acceleration of two orders of magnitude for

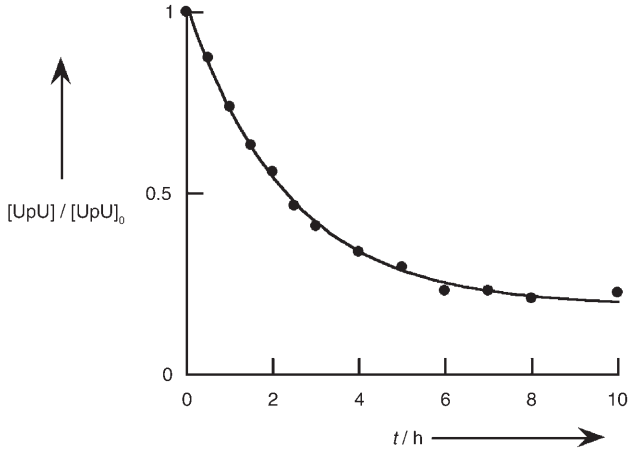



***Figure 5.*** The hydrolysis of UpU (0.06 mM) catalyzed by [$Zn_2$(**1**)] (1 mM) in HEPES buffer (50 mM, pH 7.3) at 25 °C. The solid line is the least-squares fit to a first-order decay.

cleavage of unactivated substrates relative to double Lewis acid activation by the $Zn^{II}$ ions alone.[15] As shown by the crystal structure in Figure 1, the phosphoryl moiety can bridge the two $Zn^{II}$ ion centers to benefit from double Lewis acid activation, thus simultaneously placing the phosphoryl oxygen atoms in hydrogen-bonding distance of the amino groups. This enhances binding to the substrate, which is substantially tighter than in comparable complexes, and to the transition state, which leads to the high reactivity.

In summary, we have combined two strategies to achieve tight phosphate binding and high catalytic activity for phosphodiester transesterification with a synthetic $Zn^{II}$ complex. Our kinetic studies show that this dinuclear complex shows remarkable rate enhancement both for the activated substrate HPNPP and UpU. This efficiency comes from the cooperation of double Lewis acid activation through two $Zn^{II}$ ions and the hydrogen-bonding environment provided by the ligand. We are currently exploring whether these approaches can be used even more effectively to extend the limits of activity of small molecules to rival biological catalysts and to develop phosphate receptors with new practical applications.[16]

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[11] Binding of monoanionic phosphate diesters to [$Zn_2$(**1**)] in water is even stronger than to $Co^{III}$ complexes.[6a]

[12] This rate constant is slightly less than that measured by Richards and co-workers ($0.25\,M^{-1}s^{-1}$) for the catalysis of the transesterification of HPNPP by a triazacyclononane-based dinuclear Zn complex through double Lewis acid activation.[4c] [$Zn_2$(**1**)] is about 200-fold more effective under similar reaction conditions.

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[15] Cleavage of ApA by the $Zn^{II}$ complex of the pyridyl-based ligand with no 2-amino substituents has been reported at 50 °C and pH 7.0 with a concentration of 2.5 mM for the complex (M. Yashiro, A. Ishikubo, M. Komiyama, *Chem. Commun.* **1995**, 1793), thus giving $k_{obs} \approx 2 \times 10^{-5}\,s^{-1}$. Allowing for the different conditions, a similar value is estimated to that reported in reference [14] for our reaction conditions.

[16] Experimental details can be found in the Supporting Information.

  