

Effect of ligand modifications and varying metal-to-ligand ratio on the catalyzed hydrolysis of phosphorus triesters by bimetallic tetrabenzimidazole complexes

Kortney L. Klinkel, Lauren A. Kiemele, Douglas L. Gin*, John R. Hagadorn**

Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO 80309, USA

Received 1 September 2006; received in revised form 15 November 2006; accepted 15 November 2006

Available online 23 November 2006

Abstract

Systematic ligand modifications on certain elements of the binucleating tetrabenzimidazole ligand *N,N,N',N'*-tetrakis[(2-benzimidazolyl)methyl]-2-hydroxy-1,3-diaminopropane ($^1\mathbf{L}$) (i.e., certain functional groups, bridging linker length) and changes to the metal-to-ligand ratio were investigated as avenues for improving the catalytic performance of its dizinc(II) complex for the hydrolysis of the model phosphorus triester, *p*-nitrophenyl diphenyl phosphate (PNPDPP). Collectively, these studies showed that homobimetallic zinc(II) complexes made from the toluoyl-ester modified ligand ($^2\mathbf{L}$) with 2 equiv. of ZnCl_2 show the highest overall reactivity (nearly an order of magnitude improvement over the analogous dizinc complex of $^1\mathbf{L}$). Addition of a one- to four-fold excess of ZnCl_2 to these systems led to an observed rate enhancement of nearly two orders of magnitude, making these systems the fastest known metal-based hydrolysis catalysts for phosphorus triesters currently known. The results obtained, in turn, were able to provide certain clues to the nature of the mechanism of action of these new bimetallic tetrabenzimidazole complexes. © 2006 Elsevier B.V. All rights reserved.

Keywords: Phosphorus triester; Hydrolysis; Bimetallic; Benzimidazole; Zinc catalyst

1. Introduction

The development of metal-containing catalysts for the rapid hydrolysis of organophosphorus triesters is of current interest in the context of insecticide and nerve agent degradation because of the high toxicity and persistence of these latter compounds in the environment [1,2]. Phosphorus triester compounds include common pesticides (e.g., Paraoxon), and they are also used as mimics of G-type chemical warfare nerve agents (e.g., sarin, soman) [1,2]. Several hydrolytic enzymes in nature, including phosphatases, utilize two metals in a cooperative fashion to accelerate the hydrolytic degradation of phosphorous esters [3–5]. The search for simple, yet effective, synthetic metal-containing catalysts which mimic metalloenzymes and may exploit their synergistic effects, is a significant goal in chemistry [6–9]. Benzimidazole-containing ligands are often used in the preparation of model metal-based catalyst complexes because

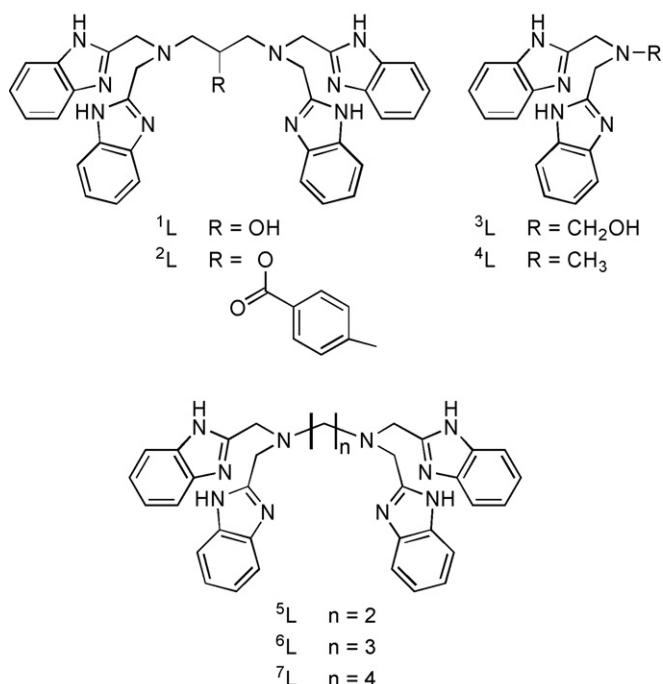
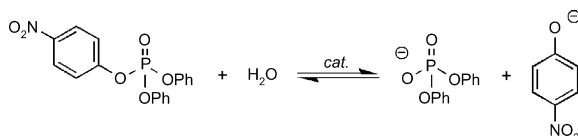
these ligands are structurally related to the biologically important imidazole group found in histidine [10].

Our research group recently reported in a communication that new homo- and heterobimetallic Cu(II), Co(II) and Zn(II) complexes of the tetrabenzimidazole ligands $^1\mathbf{L}$ and $^2\mathbf{L}$ (Scheme 1) catalyze the hydrolysis of the commonly used model phosphorus triester substrate, *p*-nitrophenyl diphenyl phosphate (PNPDPP, Scheme 2), in buffered aqueous ethanol solutions (pH 8.7). These new catalysts exhibit relative rates that, in many cases, exceed those achieved by the most effective known transition-metal-based catalyst systems [11]. In these preliminary studies we found that homo- and heterobimetallic complexes of $^2\mathbf{L}$ were generally more active than related complexes formed using $^1\mathbf{L}$. For example, the complex formed from $^2\mathbf{L}$ and 2 equiv. of ZnCl_2 , $^2\mathbf{L}\cdot\text{Zn}_2$, was found to catalyze the hydrolysis of PNPDPP at a rate that was 13 times greater than that of the related complex formed using $^1\mathbf{L}$. These results inspired us to probe further into how molecular level effects with respect to the structure of the ligand and the overall nature of the bimetallic metal coordination complex affect the activity of the catalyst system. Herein, we investigate how modifying certain elements of the tetrabenzimidazole ligand (i.e., the presence of certain functional

* Corresponding author. Tel.: +1 303 492 7640; fax: +1 303 492 4341.

** Corresponding author.

E-mail addresses: gin@spot.colorado.edu (D.L. Gin), hagadorn@colorado.edu (J.R. Hagadorn).

Scheme 1. Benzimidazole ligands used in catalytic studies with ZnCl₂.

Scheme 2. Catalyzed hydrolysis of PNPDPP.

groups, bridging linker length) and changing the metal-to-ligand ratio can be used to improve catalytic performance for PNPDPP hydrolysis. For the sake of simplicity in experimental design, these structure-reactivity studies were focused exclusively on the homobimetallic dizinc complexes because the dizinc complexes were previously found to be among the most active of all the homo- and heterobimetallic Cu(II), Co(II), and Zn(II) catalyst combinations [11]. These results obtained, in turn, were able to provide certain clues to the nature of the mechanism of action of these new bimetallic tetrabenzimidazole complexes.

2. Experimental

2.1. General information

All synthetic reactions were performed air-free using standard Schlenk techniques, unless stated otherwise. 4-Nitrophenyldiphenyl phosphate (PNPDPP) was prepared and purified as described previously in the literature [12]. The tetraacetic acids, ethylenediaminetetraacetic acid, 1,3-diamino-2-hydroxypropane-*N,N,N',N'*-tetraacetic acid, and 1,3-diaminopropane-*N,N,N',N'*-tetraacetic acid were purchased from Fisher, Aldrich, and Fluka, respectively. 1,4-Diaminobutane-*N,N,N',N'*-tetraacetic acid was prepared from 1,4-diaminobutane, 1-bromoacetic acid, and lithium hydroxide (all from Aldrich) in a procedure analogous to that used by Suzuki and coworkers [13]. Methyliminodiacetic acid and *N*-(2-hydroxyethyl)iminodiacetic

acid were purchased from Sigma–Aldrich, and TCI, respectively. 4-Ethylmorpholine and *p*-toluoyl chloride were purchased from Sigma–Aldrich and used as received. Tetrahydrofuran (THF) was passed through a column of activated alumina and sparged with N₂ prior to use. The water used in hydrolysis experiments was doubly distilled and deionized (18 MΩ cm⁻¹) prior to use. *N,N*-Dimethylformamide (DMF) was purchased from Sigma–Aldrich and used as received. Anhydrous ZnCl₂ was stirred with Me₃SiCl and dried under reduced pressure. All pH measurements were determined at 20 °C using a Corning 320 pH Meter. UV–vis absorption spectra were obtained at 21 ± 1 °C using an Agilent 8453 spectrophotometer.

2.2. Preparation of ligands

Ligand ¹L [14], its toluoyl ester derivative ²L [11], 2-[bis-(1*H*-benzimidazol-2-ylmethyl)-amino]-ethanol (³L) [15], bis-(1*H*-benzimidazol-2-ylmethyl)-methyl-amine (⁴L) [10], *N,N,N',N'*-tetrakis-(1*H*-benzimidazol-2-ylmethyl)-ethane-1,2-diamine (⁵L) [16], *N,N,N',N'*-tetrakis-(1*H*-benzimidazol-2-ylmethyl)-propane-1,3-diamine (⁶L) [17], and *N,N,N',N'*-tetrakis-(1*H*-benzimidazol-2-ylmethyl)-butane-1,4-diamine (⁷L) [18] were all prepared as described in published procedures. In brief, all the binucleating ligands were prepared by combining the appropriate alkyldiamine tetraacetic acid with 4 equiv. of finely powdered 1,2-diaminobenzene at 175 °C until effervescence ceased. The resulting glassy solids were then dissolved in 4 M HCl. The flocculent solids produced were then isolated, dissolved in water, and subsequently neutralized with aqueous ammonia to yield the crude products, which were then recrystallized from acetone. The mononucleating ligands were prepared analogously. Ligand ¹L was easily modified with 1 equiv. of toluoyl chloride in the presence of K₂CO₃ to afford ²L in good yield.

2.3. General procedure for PNPDPP hydrolysis studies

To a 1-cm cuvette was added 3.000 mL of a 4-ethylmorpholine buffer solution (67.3 mM, 30% EtOH) with a pH of 8.70. To this solution was added the bimetallic catalyst (0.126 μmol in 40.4 μL DMF) and then PNPDPP (0.505 μmol in 1.000 mL of 50% EtOH). Assuming a final volume of 4.040 mL, the initial concentrations of the reactants were set at: [PNPDPP] = 0.125 mM, [catalyst] = 0.0313 mM, [metal] = 0.0625 mM, [buffer] = 50 mM. The cuvette was then sealed and shaken resulting in a homogeneous solution. The rate of hydrolysis was monitored at 401 nm ($\epsilon(p\text{-nitrophenolate ion, pH 8.70}) = 1.6 \times 10^4 \text{ cm}^{-1} \text{ M}^{-1}$) for at least 3 half-lives [19]. A pseudo-first-order rate constant was determined for each reaction from a linear plot of $\ln(A_e - A_t)$ versus time (where A_e is the equilibrium absorbance at 401 nm) [20]. All reported data and standard deviations were determined by sampling over at least three independent runs. Statistically identical results were obtained for runs using isolated (crystalline) catalysts and mixtures prepared *in situ* by mixing ligand and metal salts together in EtOH. For the monometallic systems (³L·Zn and ⁴L·Zn), the same general procedure was followed except the

[metal] = [catalyst] = 0.0625 mM. All kinetic studies were run in at least triplicate, so the interpolated constants are listed as average values with error bars of ± 1 standard deviation. For all studies presented hereafter, with the exception of the catalyst ratio variation studies in section 2.4, 25 mol% catalyst in relation to substrate was used.

2.4. Effect of catalyst concentration on PNPDP hydrolysis rate

The rate of PNPDP hydrolysis catalyzed by all seven catalyst systems ($^1\text{L}\cdot\text{Zn}_2$ – $^7\text{L}\cdot\text{Zn}_2$) was studied as a function of catalyst concentration. The reactions were monitored as described above. Reactions were run using catalyst concentrations that varied between 1.25×10^{-5} M and 1.25×10^{-4} M. By plotting the negative log of the concentration of catalyst versus the negative log of the resulting rate constant, the order dependence on catalyst was determined from the slope of the resulting line. The order dependence on catalyst was found to be approximately first order for all systems studied. The actual order dependences are as follows: 1.27 ± 0.01 for $^1\text{L}\cdot\text{Zn}_2$, 1.1 ± 0.2 for $^2\text{L}\cdot\text{Zn}_2$, 1.30 ± 0.07 for $^3\text{L}\cdot\text{Zn}_2$, 1.07 ± 0.05 for $^4\text{L}\cdot\text{Zn}_2$, 1.35 ± 0.05 for $^5\text{L}\cdot\text{Zn}_2$, 1.10 ± 0.03 for $^6\text{L}\cdot\text{Zn}_2$, and 1.04 ± 0.04 for $^7\text{L}\cdot\text{Zn}_2$. Graphical data for $^1\text{L}\cdot\text{Zn}_2$ and $^2\text{L}\cdot\text{Zn}_2$ can be found in Ref. [11]. Data for the remaining systems can be found in the [supplementary information](#).

3. Results and discussion

3.1. Effect of benzimidazole ligand modifications on catalytic activity

Zinc complexes of the toluoyl-ester ligand ^2L , displayed greatly enhanced reactivity for the hydrolysis of PNPDP compared to the related complexes of ^1L [11]. Due to this fact, we wanted to investigate the nature of this effect and also determine whether additional molecular level ligand modifications could be used to further increase reactivity and/or shed light onto the mechanism of action for these systems.

3.1.1. Effect of ligand functional groups on catalyzed hydrolysis of PNPDP

To evaluate the effects of the alcohol and ester functionalities (of ^1L and ^2L) in the context of PNPDP hydrolysis, the related ligand ^6L was synthesized, and its corresponding complex with 2 equiv. of ZnCl_2 was formed. This new ligand features an unreactive propylene linker. The reactivity of $^6\text{L}\cdot\text{Zn}_2$ for the hydrolysis of PNPDP was evaluated using established methods [11]. The kinetic studies were conducted by monitoring the formation of the *p*-nitrophenolate ion at 401 nm using UV–vis spectrophotometry for greater than 3 half-lives as described above in Section 2.3. For each of the reactions, the kinetics for the formation of *p*-nitrophenolate are consistent with a pseudo-first-order reaction coming to equilibrium. Studies performed using varied catalyst concentrations indicated that the reaction order in catalyst is approximately first-order [11]. As a result, higher reactivity can be achieved by using larger amounts of catalyst and moreover all second-order rate constants are approximations. The catalytic activities of simple metal salts and non-complexed ligands were examined as controls. They showed no discernible effect on the hydrolysis rate of PNPDP compared to the reaction in the absence of added catalyst.

Table 1 shows the effect on the catalytic activity for the hydrolysis of PNPDP when 2 equiv. of Zn(II) are complexed with tetrabenzimidazole ligands containing a different functionality on the linker between halves of the ligand. The dizinc complex prepared with the toluoyl-ester-containing ligand, ^2L ($^2\text{L}\cdot\text{Zn}_2$) showed the highest activity for the hydrolysis of PNPDP. For comparison, the dizinc complex of the hydroxyl-containing ^1L was the slowest, and the complex of the alkyl-linked ^6L featured intermediate activity. These results indicate that the hydroxy functionality inhibits the desired reactivity, whereas the toluoyl ester does not. It is likely that the hydroxyl group in the ligand linker unit strongly coordinates to one of the metal centers to tie up a coordination site needed for substrate binding, thereby slowing reactivity. Replacing the hydroxy-containing linker with the ester-based linker frees up a coordination site which is essential for substrate and/or water

Table 1
Effect of functionality on the catalyzed hydrolysis of PNPDP^a

Entry	Catalyst ^b	$t_{1/2}$ (h)	k_{obs} ($\times 10^5 \text{ s}^{-1}$)	k_{obs} ($\text{M}^{-1} \text{ s}^{-1}$) ^c	Rel. rate ^d	Functional group on linker
1	None	30 ± 6	0.7 ± 0.1	n.a.	1	–
2	ZnCl_2	31 ± 5	0.6 ± 0.1	n.a.	0.9	–
3	$^1\text{L}\cdot\text{Zn}_2$	14.1 ± 0.1	1.4 ± 0.1	0.4 ± 0.1	2.1	Alcohol
4	$^2\text{L}\cdot\text{Zn}_2$	1.5 ± 0.1	12.8 ± 0.8	4.1 ± 0.3	19.5	Ester
5	$^6\text{L}\cdot\text{Zn}_2$	2.8 ± 0.3	7.0 ± 0.7	2.2 ± 0.2	10.6	Alkyl

^a Conditions: 35% EtOH, 50 mM 4-ethylmorpholine buffer, pH 8.7, 21 ± 1 °C, [PNPDP] = 1.25×10^{-4} M, [metal] = 0.0625 mM.

^b Abbreviated catalysts are mixtures of ligands and metal chlorides (e.g., $^1\text{L}\cdot\text{Zn}_2$ is formed from ^1L and 2 equiv. of ZnCl_2).

^c Second-order rate constants are calculated with [catalyst] = 0.0313 mM.

^d Based on the observed pseudo-first-order rate constants.

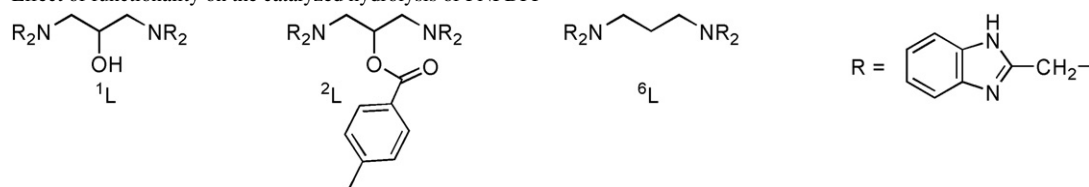


Table 2
Effect of linker length on the catalyzed hydrolysis of PNPDPDPP^a

Entry	Catalyst ^b	$t_{1/2}$ (h)	k_{obs} ($\times 10^5 \text{ s}^{-1}$)	k_{obs} ($\text{M}^{-1} \text{ s}^{-1}$) ^c	Rel. rate ^d	Alkyl linker length
1	⁵ L·Zn ₂	2.1 ± 0.1	9.4 ± 0.6	3.0 ± 0.2	14.2	Ethyl
2	⁶ L·Zn ₂	2.8 ± 0.3	7.0 ± 0.7	2.2 ± 0.2	10.6	Propyl
3	⁷ L·Zn ₂	1.8 ± 0.4	10 ± 1	3.3 ± 0.5	15.8	Butyl

^a Conditions: 35% EtOH, 50 mM 4-ethylmorpholine buffer, pH 8.7, 21 ± 1 °C, [PNPDPP] = 1.25 × 10⁻⁴ M, [metal] = 0.0625 mM.

^b Abbreviated catalysts are mixtures of ligands and metal chlorides (e.g., ⁵L·Zn₂ is formed from ⁵L and 2 equiv. of ZnCl₂).

^c Second-order rate constants are calculated with [catalyst] = 0.0313 mM.

^d Based on the observed psuedo-first-order rate constants.

activation, (see Ref. [11] for solid-state crystal structures). The modest increase in activity for ²L relative to ³L, suggests that hydrophobic effects play a secondary but significant role in this system. This is not surprising given that micelle-based catalysts have previously been shown to be much more active for the hydrolysis of PNPDPDPP and related substrates in aqueous solutions [21–24].

3.1.2. Effect of ligand linker length on the catalyzed hydrolysis of PNPDPDPP

In order to explore whether changes in conformational flexibility in the tetrabenzimidazole ligand would afford a more active bimetallic catalyst, ligands ⁵L and ⁷L containing two-carbon and four-carbon linkers, respectively, were synthesized. These ligands were then complexed with 2 equiv. of ZnCl₂ and the activity of the resulting dizinc complexes (⁵L·Zn₂ and ⁷L·Zn₂) towards the hydrolysis of PNPDPDPP was compared to that of the dizinc complex of ⁶L, the previously described tetrabenzimidazole ligand containing a three-carbon alkyl linker.

As can be seen in Table 2, the dizinc complexes of the tetrabenzimidazole ligands containing two-, three-, and four-carbon alkyl linkers show essentially no statistical difference with respect to the rate of PNPDPDPP hydrolysis under the same reaction conditions. All three dizinc complexes exhibit very similar reactivities, implying that the system is relatively insensitive to the spacer length between the binucleating pairs of benzimidazole units. These data may be interpreted to suggest that each

metal center of a bimetallic complex operates independently. This is inconclusive, however, since the three backbones are all flexible and may simply display similar reactivity, even for a cooperative bimetallic mechanism.

3.1.3. Effect of mononucleating versus binucleating ligand structure on the catalyzed hydrolysis of PNPDPDPP

In order to help elucidate the importance of the bimetallic nature of the catalysts, the mononucleating ligands ³L and ⁴L were prepared, and their monozinc(II) complexes were tested. As shown in Table 3, the 1:1 (mol/mol) zinc complexes of ³L and ⁴L were also found to be effective catalysts for PNPDPDPP hydrolysis. These monometallic catalysts show similar reactivity (at equal metal concentration) to the bimetallic ¹L·Zn₂ complex, but are less reactive than the analogous bimetallic systems (²L·Zn₂, ⁵L·Zn₂, ⁶L·Zn₂, and ⁷L·Zn₂). The fact that the bimetallic complexes show higher normalized catalytic activity compared to monometallic homologues, is not an ideal or conclusive indicator of a synergistic interaction between the two metals in the bimetallic systems [25]. However, these results and comparisons do suggest that perhaps the metal centers in the dizinc complexes of the binucleating ligands ²L, ⁵L, ⁶L, and ⁷L do mutually interact in some fashion that enhances overall catalytic activity. However, this mutual enhancement or “cooperativity” is strictly an observed performance result based on the kinetic data (i.e., a greater than expected increase in performance above and beyond that expected by simply increasing the number or

Table 3
Effect of structure on the catalyzed hydrolysis of PNPDPDPP^a

Entry	Catalyst ^b	$t_{1/2}$ (h)	k_{obs} ($\times 10^5 \text{ s}^{-1}$)	k_{obs} ($\text{M}^{-1} \text{ s}^{-1}$) ^c	Rel. rate ^d	Functional group on ligand
1	¹ L·Zn ₂	14.1 ± 0.1	1.4 ± 0.1	0.4 ± 0.1	2.1	Alcohol
2	⁶ L·Zn ₂	2.8 ± 0.3	7.0 ± 0.7	2.2 ± 0.2	10.6	Alkyl
3	³ L·Zn	6.4 ± 0.3	3.0 ± 0.1	0.48 ± 0.02	4.6	Alcohol
4	⁴ L·Zn	3.7 ± 0.1	5.2 ± 0.1	0.8 ± 0.1	7.8	Alkyl

^a Conditions: 35% EtOH, 50 mM 4-ethylmorpholine buffer, pH 8.7, 21 ± 1 °C, [PNPDPP] = 1.25 × 10⁻⁴ M, [metal] = 0.0625 mM, [L] = 0.0313 M for entries 1 and 2, and [L] = 0.0625 M for entries 3 and 4.

^b Abbreviated catalysts are mixtures of ligands and metal chlorides (e.g., ¹L·Zn₂ is formed from ¹L and 2 equiv. of ZnCl₂).

^c Second-order rate constants are calculated with [catalyst] = 0.0313 mM for entries 1 and 2, while [catalyst] = 0.0625 mM for entries 3 and 4.

^d Based on the observed psuedo-first-order rate constants.

concentration of metal centers). By using these terms, we do not wish to imply or suggest a specific mechanism of action for how this greater than expected rate enhancement occurs, given the complexity and dynamic nature of the catalyst system in solution. It is also interesting to note that the two alcohol-containing ligands (mononucleating ^3L and binucleating ^1L) (entries 1 and 3, Table 3) afford complexes that show the poorest reactivity of all ligands tested. This finding supports our earlier discovery that hydroxyl groups in these ligand systems inhibit catalytic reactivity.

3.2. Effects of excess metal ion on catalytic activity

In order to determine the amount of ZnCl_2 necessary to yield optimal reactivity, the molar amount of ZnCl_2 was varied systematically between 0.1 and 6 equiv. relative to the amount of ligand; and the catalytic activity of the resulting systems was examined. Due to the inhibitory nature of hydroxyl groups in the benzimidazole ligand, only zinc complexes with ligands containing a non-functionalized spacer, (^5L , ^6L , and ^7L) or a relatively non-coordinating ester moiety (^2L) were examined in this study. Data for the zinc complexes of the remaining ligands are presented in Figure S1 in the supporting information, for comparison purposes. The observed second-order rate constants for this experiment as a function of zinc ion-to-ligand molar ratio are depicted graphically in Fig. 1.

As can be seen in Fig. 1, the catalytic activity of the $\text{L}\cdot\text{Zn}_n$ systems does not show any appreciable value until the Zn/L ratio reaches approximately 2, which is indicative of the $\text{L}\cdot\text{Zn}_2$ complex expected for a binucleating ligand. However, as the Zn/L ratio is increased above 2, the catalytic activity continues to increase substantially and then begins to level off between 3 and 5 equiv. of added ZnCl_2 relative to ligand. Keeping in mind that control experiments showed that free, uncomplexed ZnCl_2 and uncomplexed free L are not effective catalysts for the reaction of interest, this increase in catalytic activity with increasing ZnCl_2 to binucleating L ratio suggests several possibilities: (1) the complexes are in equilibrium between uncomplexed

$\text{L} \rightleftharpoons \text{L}\cdot\text{Zn}_1 \rightleftharpoons \text{L}\cdot\text{Zn}_2$, and adding excess free $\text{Zn}(\text{II})$ ion shifts the equilibrium towards formation of more $\text{L}\cdot\text{Zn}_2$ active species; (2) formation of a third as yet unidentified stoichiometric zinc complex with more than two zinc centers per mole of L that is also catalytically active; (3) formation of a micelle-like aggregate species with the excess $\text{Zn}(\text{II})$ that enhances transport of substrate to the active zinc sites.

It has been previously demonstrated by solid-state crystal structures as well as solution-phase NMR studies that $^1\text{L}\cdot\text{Zn}_2$ and $^2\text{L}\cdot\text{Zn}_2$ form discrete, stable complexes in the solid state and in solution, respectively, when 2 equiv. of metal are present [11]. When 2 equiv. of metal are present, the ligand is fully occupied. By analogy, ^5L , ^6L , and ^7L should also exhibit similar properties towards zinc. Additionally, studies have shown that the mononucleating ligands ^3L and ^4L are strong chelators of first-row transition metals and therefore also form stable monometallic complexes in solution [26–28]. As a result, the increased reactivity with greater than 2 equiv. of zinc(II) present is not likely due to the presence of a larger percentage of filled ligand (case (1)), or the formation of a new, stoichiometric higher order zinc complex with the ligands (case (2)). Case (3) for enhanced reactivity with excess zinc ions is that excess ZnCl_2 forms a type of micelle-like aggregate species in which reactivity is increased. However, the reaction mixtures appear completely optically transparent to the eye, which suggests that no micelles or aggregate species are present. Consequently, the increased activity with excess ZnCl_2 in the system may be due to an electronic effect of the excess zinc ions interacting with the outer-sphere, or non-coordinated nitrogens of the benzimidazole ligands. This interaction could behave in a “push–pull” fashion where the outer-sphere zinc centers donate or withdraw electron density as needed through the conjugated ligand system from the primary zinc center (UV–vis spectrophotometric evidence for this phenomenon can be found in the supplemental information, Figure S2). Currently, work is being conducted in order to determine the nature of the resulting catalyst and the basis of the increased reactivity.

Since the catalytic activity of the $\text{L}\cdot\text{Zn}_n$ systems reach a maximum after 4 equiv. of ZnCl_2 are added to the binucleating ligand, we also wanted to compare the activity of these non-stoichiometric but highly active catalyst mixtures to the original systems and other metal catalysts in the literature. Table 4 depicts the rate of hydrolysis of PNPDPDP when 4.5 total equivalents of

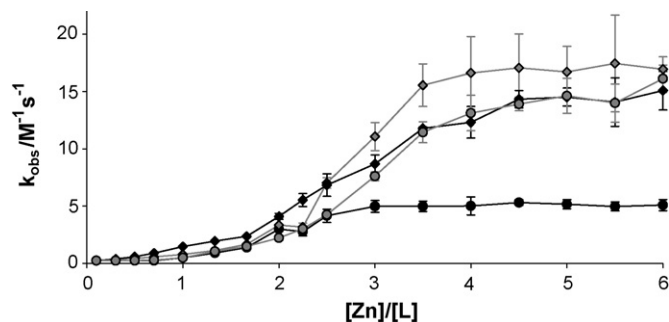


Fig. 1. Effect on reaction rate constant of varying metal-to-ligand ratio for binucleating ligands. Conditions: 35% EtOH, 50 mM 4-ethylmorpholine buffer, pH 8.7, $21 \pm 1^\circ\text{C}$, $[\text{PNPDPP}] = 1.25 \times 10^{-4}\text{ M}$, $[\text{L}] = 3.13 \times 10^{-5}\text{ M}$, $[\text{ZnCl}_2]$ ranged from $3.13 \times 10^{-6}\text{ M}$ to $1.88 \times 10^{-4}\text{ M}$. Plotted data are the second-order rate constants (calculated from the pseudo-first-order rate constants with $[\text{L}] = 3.13 \times 10^{-5}\text{ M}$) with error bars of ± 1 standard deviation. $^2\text{L}\cdot\text{Zn}_2$ = black diamonds, $^5\text{L}\cdot\text{Zn}_2$ = black circles, $^6\text{L}\cdot\text{Zn}_2$ = gray circles, and $^7\text{L}\cdot\text{Zn}_2$ = gray diamonds.

Table 4
Effect of additional ZnCl_2 on the catalyzed hydrolysis of PNPDPDP^a

Entry	Catalyst ^b	$t_{1/2}$ (h)	k_{obs} ($\times 10^5\text{ s}^{-1}$)	Rel. rate ^c
1	None	30 ± 6	0.7 ± 0.1	1
2	ZnCl_2	31 ± 5	0.6 ± 0.1	0.9
3	$^2\text{L} + 4.5\text{ ZnCl}_2$	0.43 ± 0.02	45 ± 2	68
4	$^5\text{L} + 4.5\text{ ZnCl}_2$	1.16 ± 0.05	17 ± 1	26
5	$^6\text{L} + 4.5\text{ ZnCl}_2$	0.44 ± 0.02	43 ± 2	65
6	$^7\text{L} + 4.5\text{ ZnCl}_2$	0.36 ± 0.05	53 ± 7	80

^a Conditions: 35% EtOH, 50 mM 4-ethylmorpholine buffer, pH 8.7, $21 \pm 1^\circ\text{C}$, $[\text{PNPDPP}] = 1.25 \times 10^{-4}\text{ M}$, $[\text{L}] = 0.0313\text{ mM}$, $[\text{metal}] = 0.141\text{ mM}$.

^b Abbreviated catalysts are mixtures of ligands and metal chlorides. In this case 4.5 total equivalents of ZnCl_2 are present.

^c Based on the observed pseudo-first-order rate constants.

ZnCl₂ relative to the binucleating ligands, are present. Relative rates approaching two orders of magnitude faster in relation to the non-catalyzed reaction are achieved under these conditions. Due to the aforementioned fact that these reactions are approximately first-order in catalyst, a 10-fold increase in catalyst concentration should lead to reaction rates nearly four orders of magnitude greater than those achieved with no catalyst present. To our knowledge, these hydrolysis rates surpass those achieved by the best reported metal-based solution catalysts for PNPDPDP [11,29–33].

3.2.1. Comparison of the rate of reaction with 2 equiv. of ZnCl₂ to that with 1 equiv.

In Section 3.1.3, comparisons with the monozinc complexes of the mononucleating ligands ³L and ⁴L suggested perhaps the dizinc complexes of binucleating ligands ²L as well as ⁵L, ⁶L and ⁷L operated in a synergistic fashion. From the study presented above in Section 3.2, in which the metal concentration is varied with respect to the binucleating ligand concentration, one can glean information on the presence or lack of two metal cooperativity for the catalytic hydrolysis of PNPDPDP [25,34]. Table 5 depicts the relative rate of hydrolysis when 2 equiv. of ZnCl₂ are present with binucleating tetrabenzimidazole ligand versus only one. Data for the two mononucleating ligands are also present for comparison purposes (entries 3 and 4).

Based on simple statistics, one can assume that when 1 equiv. of zinc is present, a 1:2:1 mixture of completely uncomplexed binucleating ligand (L), half-filled binucleating ligand (L·Zn₁), and completely filled binucleating ligand (L·Zn₂) will exist in solution, if the complexation strength for Zn(II) is the same in each case. In the above experiment, the metal concentrations are held constant between the mono- and binucleating systems. As a result, in the control case of the mononucleating ligands (³L and ⁴L) a 1:1 mixture of filled and unfilled ligand exists in solution when 1 equiv. of metal is present. In this case, it is expected that the catalyst solution containing 2 equiv. of metal for every 2 equiv. of ligand will perform twice as fast as the cat-

Table 5
Relative rate of hydrolysis when 2 equiv. of metal are present vs. 1 equiv.^a

Entry	Catalyst ratio ^b	k_2/k_1^c
1	¹ L·Zn ₂ / ¹ L·Zn ₁	1.8
2	² L·Zn ₂ / ² L·Zn ₁	2.8
3	³ L·Zn ₂ / ³ L·Zn ₁	1.8
4	⁴ L·Zn ₂ / ⁴ L·Zn ₁	2.1
5	⁵ L·Zn ₂ / ⁵ L·Zn ₁	6.1
6	⁶ L·Zn ₂ / ⁶ L·Zn ₁	4.6
7	⁷ L·Zn ₂ / ⁷ L·Zn ₁	4.3

^a Conditions: 35% EtOH, 50 mM 4-ethylmorpholine buffer, pH 8.7, 21 ± 1 °C, [PNPDPDP] = 1.25 × 10⁻⁴ M. For entries 1, 2 and 5–7 [ligand] = 0.0313 mM, for entries 3 and 4 [ligand] = 0.0625 mM.

^b Abbreviated catalysts are mixtures of ligands and metal chlorides (e.g., ¹L·Zn₂ is formed from ¹L and 2 equiv. of ZnCl₂). Based on statistics, it is assumed that L·Zn₁ will consist of a 1:2:1 ratio of L:L·Zn₁:L·Zn₂ for the binucleating ligands.

^c k_1 denotes the observed first order rate constant when 1 equiv. of metal is present (0.0313 mM) and k_2 denotes the observed first order rate constant when 2 equiv. of metal are present (0.0625 mM).

alyst solution containing only one metal for every two ligands. Not surprisingly, this is what is observed in Table 2 entries 3 and 4. The alcohol-containing mononucleating ligand ³L complexed with 2 equiv. of metal, is found to be 1.8 times faster than the system with only 1 equiv. of metal present. Similarly, mononucleating ⁴L is 2.1 times faster at hydrolyzing PNPDPDP when 2 equiv. of zinc are present versus 1 equiv. of zinc. As expected, neither of these mononucleating systems shows cooperativity. Likewise, the zinc complex of binucleating ligand ¹L also appears to show no synergistic effects in this study. The rate of hydrolysis of PNPDPDP with 2 equiv. of zinc is 1.8 times faster than when 1 equiv. of zinc is present for ¹L. This finding is possibly due to the interference effects of the coordinating hydroxy group which also retards activity. Conversely, for the zinc complexes of the binucleating ligands ²L, ⁵L, ⁶L, and ⁷L, the reaction rate observed after the introduction of the second equivalent of metal is significantly larger than two times that observed when 1 equiv. of zinc is present. This result also suggests that the metal ions complexed by the two halves of the binucleating ligands interact cooperatively in some fashion to enhance overall reactivity.

3.2.2. Effects of excess ligand on catalytic activity

As a final study to probe the possibility of a cooperative bimetallic nature for zinc complexes of binucleating ligands ²L, ⁵L, ⁶L, and ⁷L, excess equivalents of ligand were added to the system and the results monitored. The concentration of ligand was varied systematically from 1.56 × 10⁻⁵ M to 6.25 × 10⁻⁵ M. The results are depicted below in Figs. 2 and 3.

Assuming that the two metal-binding sites are independent and of equal binding strength for the binucleating ligands studied, one can expect a statistical mixture of uncomplexed ligand (L), half-filled ligand (L·Zn₁), and fully-filled ligand (L·Zn₂) if all three possible species are interchanging at equilibrium. As more and more free ligand is added to these systems, the proportion of L·Zn₂ would be expected to decrease with respect to that for L and L·Zn₁. In the case of the mononucleating ligands, ³L and ⁴L, one can expect simply a mixture of uncomplexed ligand and fully-filled ligand. As more free ligand is added to these sys-

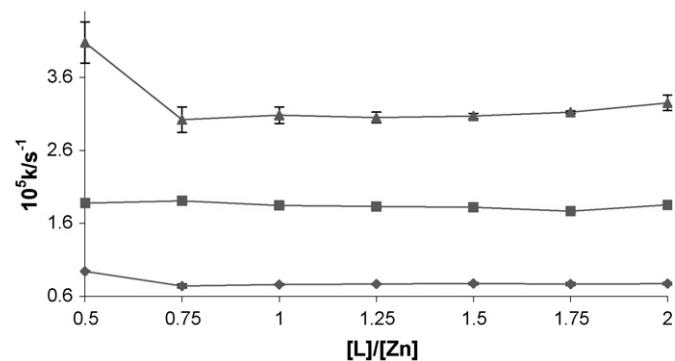


Fig. 2. The effect of excess ligand on the rate of PNPDPDP hydrolysis for the ZnCl₂ complexes of mononucleating ligand systems ³L and ⁴L; and the binucleating ligand system ¹L. Conditions: 35% EtOH, 50 mM 4-ethylmorpholine buffer, pH 8.7, 21 ± 1 °C, [PNPDPDP] = 1.25 × 10⁻⁴ M, [Zn] = 3.13 × 10⁻⁵ M, [L] ranges from 1.56 × 10⁻⁵ M to 6.25 × 10⁻⁵ M. ¹L = diamonds, ³L = squares, ⁴L = triangles.

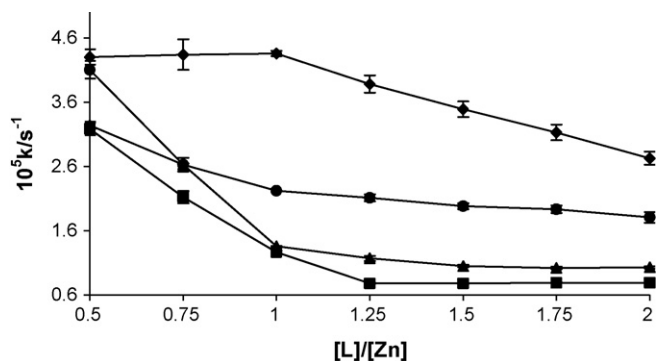


Fig. 3. The effect of excess ligand on the rate of PNPDP hydrolysis for the ZnCl_2 complexes of the binucleating ligand systems. Conditions: 35% EtOH, 50 mM 4-ethylmorpholine buffer, pH 8.7, 21 ± 1 °C, $[\text{PNPDP}] = 1.25 \times 10^{-4}$ M, $[\text{Zn}] = 3.13 \times 10^{-5}$ M, $[\text{L}]$ ranges from 1.56×10^{-5} M to 6.25×10^{-5} M. ^2L = diamonds, ^5L = squares, ^6L = triangles, ^7L = circles.

tems, the proportion of fully-filled mononucleating ligand will not change. As can be seen in Fig. 2, the two mononucleating ligands ^3L and ^4L and binucleating ligand ^1L exhibit nearly perfectly linear graphs as more ligand is added, meaning that one metal, despite its location relative to any others, acts independently to hydrolyze PNPDP. This trend is expected for the two mononucleating ligands given that they should not be able to act in a synergistic fashion. In contrast, the reactivity of ^2L , ^5L , ^6L , and ^7L all decrease as more free-ligand is added to the system (Fig. 3). This phenomenon suggests that the $\text{L}\cdot\text{Zn}_2$ species plays a significant role in the hydrolysis of PNPDP for these systems.

4. Conclusions

Collectively, systematic ligand modification studies demonstrate that homobimetallic zinc(II) complexes with the ester-containing ligand ^2L show the highest reactivity. This phenomenon may be due to the increased hydrophobicity introduced by the toluoyl group. The linker length between the two halves of the molecule appears to have no discernable effect on the rate of the reaction when 2 equiv. of ZnCl_2 are present. This trend is likely due to the flexible nature of the linkers. By removing the linker all together as in the mononucleating (half) ligands ^3L and ^4L , no rate enhancement is observed. These systems display poorer reactivity than the bimetallic systems containing no hydroxyl group. This finding suggests that perhaps some sort of cooperative mechanism between the metal ions is the mode of action for the binucleating dizinc systems. Furthermore, it appears that a hydroxyl group in the vicinity of the metal's coordination sphere hinders reactivity as seen with $^1\text{L}\cdot\text{Zn}_2$ and $^3\text{L}\cdot\text{Zn}$. Therefore, hydroxyl groups in this system should be avoided in order to optimize reactivity.

Additionally, studies comparing the rate of hydrolysis when 2 equiv. of metal are present versus one, and studies where excess ligand is present, both suggest that these dizinc-tetrabenzimidazole systems function synergistically. By adding a one- to four-fold excess of ZnCl_2 to these systems, a rate enhancement of nearly two orders of magnitude is observed,

making these systems the fastest known metal-based hydrolysis catalysts for phosphorus triesters currently known. Comparisons to the monozinc complexes of analogous mononucleating ligands also strongly suggest that the dizinc complexes formed with binucleating ligand ^2L , as well as with ligands ^5L , ^6L , and ^7L , operate with some sort of cooperativity.

All the data presented gives speculative clues to how complex and dynamic these metal-benzimidazole coordination systems are. Due to the complexity of these systems, it is difficult to obtain exact analysis on these dynamic complexation systems. However, additional work is currently being performed to elucidate the mode of action when additional metal is present in the system. Additionally, these and related catalysts are being incorporated onto solid nanostructured supports to aid in catalyst recovery and to potentially further amplify reactivity.

Acknowledgments

Primary financial support for this work was provided by the National Science Foundation in the form of a Graduate Research Fellowship to K.L.K. Partial support was also provided by the NSF in the form of a grant to D.L.G. (DMR-0111193).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molcata.2006.11.032.

References

- [1] Y.-C. Yang, J.A. Baker, J.R. Ward, Chem. Rev. 92 (1992) 1729.
- [2] Y.-C. Yang, Acc. Chem. Res. 32 (1999) 109.
- [3] M.J. Jędrzejak, P. Setlow, Chem. Rev. 101 (2001) 607.
- [4] D.E. Wilcox, Chem. Rev. 96 (1996) 2435.
- [5] N. Stråßer, W.N. Lipscomb, T. Kablunde, B. Krebs, Angew. Chem. Int. Ed. Engl. 35 (1996) 2025.
- [6] J. DuBois, T.J. Mizoguchi, S.J. Lippard, Coord. Chem. Rev. 200–202 (2000) 443.
- [7] B. Bosnich, Inorg. Chem. 38 (1999) 2554.
- [8] R.D. Adams, F.A. Cotton (Eds.), Catalysis by Di- and Polynuclear Metal Cluster Complexes, Wiley-VCH, New York, 1998.
- [9] G.J. Rowlands, Tetrahedron 57 (2001) 1865.
- [10] H. Adams, N.A. Bailey, J.D. Crane, D.E. Fenton, J.M. Latour, J.M. Williams, J. Chem. Soc., Dalton Trans. (1990) 1727.
- [11] K.L. Klinkel, L.A. Kiemle, D.L. Gin, J.R. Hagadorn, Chem. Commun. (2006) 2919.
- [12] W.M. Gulick Jr., D.H. Geske, J. Am. Chem. Soc. 88 (1966) 2928.
- [13] H. Furutachi, M. Murayama, A. Shiohara, S. Yamazaki, S. Fujinami, A. Uehara, M. Suzuki, S. Ogo, Y. Watanabe, Y. Maeda, J. Chem. Soc., Chem. Commun. (2003) 1900.
- [14] V. McKee, M. Zvagulis, J.V. Dagdigian, M.G. Patch, C.A. Reed, J. Am. Chem. Soc. 106 (1984) 4765.
- [15] Y. Nishida, K. Takahashi, J. Chem. Soc., Dalton Trans. (1988) 691.
- [16] H.M.J. Hendriks, W.O.T.B. Huinink, J. Reedijk, Recl. Trav. Chim. Pays-Bas 98 (1979) 499.
- [17] S.M. Wang, P.J. Huang, H. Chang, C.Y. Cheng, S.L. Wang, N.C. Li, Inorg. Chim. Acta 182 (1991) 109.
- [18] T. Sakurai, H. Kaji, A. Nakahara, Inorg. Chim. Acta 67 (1982) 1.
- [19] The hydrolysis reaction was monitored for at least three half-lives. Most systems were monitored in excess of five half-lives, the exception being the slow reactions such as the control experiments, which were monitored for 3 half-lives. Monitoring reactions out to at least 3 half-lives appears to

- be adequate for prior phosphorus triester hydrolysis kinetics studies in the literature. See:
- (a) R.W. Hay, N. Govan, *Polyhedron* 17 (1998) 463;
(b) S. Douderc, J. Toullec, *Langmuir* 17 (2001) 3819;
(c) L.Y. Kuo, N.M. Perera, *Inorg. Chem.* 39 (2000) 2103.
- [20] The equation $\ln(A_\infty - A_t) = -kt + \ln A_\infty$ describes the kinetics for a first-order system coming to equilibrium. See:
R.B. Jordan, *Reaction Mechanism of Inorganic and Organometallic Systems*, Oxford Press, 1991, pp. 3–4.
- [21] S. Couderc, J. Toullec, *Langmuir* 17 (2001) 3819.
- [22] R.W. Hay, N. Govan, K.E. Parchment, *Inorg. Chem. Commun.* 1 (1998) 228.
- [23] P. Scrimin, G. Ghirlanda, P. Tecilla, R.A. Moss, *Langmuir* 12 (1996) 6235.
- [24] F.M. Menger, L.H. Gan, E. Johnson, D.H. Durst, *J. Am. Chem. Soc.* 109 (1987) 2800.
- [25] M. Leivers, R. Breslow, *Bioorg. Chem.* 29 (2001) 345.
- [26] R.W. Hay, T. Clifford, P. Lightfoot, *Polyhedron* 17 (1998) 3575.
- [27] H. Adams, N.A. Bailey, J.D. Crane, D.E. Fenton, *J. Chem. Soc., Dalton Trans.* (1990) 1727.
- [28] M.J. Young, D. Wahnnon, R.C. Hynes, J. Chin, *J. Am. Chem. Soc.* 117 (1995) 9441.
- [29] Meaningful comparisons of catalyst activities can only be made between systems using identical phosphorus ester substrates.
- [30] R.A. Moss, P.K. Gong, *Langmuir* 16 (2000) 8551, and references therein.
- [31] P. Gómez-Tagle, A.K. Yatsimirsky, *J. Chem. Soc., Dalton Trans.* (2001) 2663.
- [32] J.K. Yang, S. Chang II, S.G. Ryu, Y.S. Yang, *Bull. Korean Chem. Soc.* 15 (1994) 261.
- [33] J.R. Morrow, W.C. Troglér, *Inorg. Chem.* 28 (1989) 2330.
- [34] F. Mancin, E. Rampazza, P. Tecilla, U. Tonellato, *Eur. J. Org. Chem.* (2004) 281.