

# Phosphate Diester Hydrolysis by Mono- and Dinuclear Lanthanum Complexes with an Unusual Third-Order Dependence

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A series of mono- and dinuclear lanthanum complexes of 15,31-dimethyl-3,11,19,27,33,35-hexaazapentacyclo-[27.3.1.1<sup>5,9</sup>1.1<sup>13,17</sup>.1<sup>21,25</sup>]hexatriaconta-5,7,9(33),13,15,17(34),21,23,25(35),29,31,1(36)-dodecaene-34,36-diol (24RB-PyBC, L) have been defined in solution. Their ability to hydrolyze bis(4-nitrophenyl) phosphate, a phosphate diester, was studied. The various metal-coordinated hydroxide nucleophiles that form in solution attack the substrate in the hydrolysis reaction. The dihydroxo dilanthanum complex, L-2La-2(OH), is the most effective catalyst. Its rate constant is 75 times larger than the rate constant for the monohydroxo dilanthanum complex, L-2La-OH. The mononuclear complexes are not as successful as the dinuclear complexes because they have fewer metal ions per complex to act as Lewis acids. They also cannot generate hydroxide nucleophiles at low pH values like the dinuclear complexes can. The reaction has an unusual third-order dependence on the catalyst concentration which is valid for the dinuclear complexes as well as the mononuclear complexes. This implies a mechanism where a metal-coordinated hydroxide nucleophile attacks the phosphorus of the substrate on the side opposite the negatively charged oxygens.

## Introduction

Lanthanum has been a common metal ion for use in phosphodiesterase model systems.<sup>1–8</sup> While zinc is present in the active sites of some crystallographically defined phosphodiesterases,<sup>9–11</sup> other metal ions such as Co<sup>3+</sup> and La<sup>3+</sup> have made more successful model complexes for hydrolyzing phosphate diesters.<sup>12–14</sup> However, Co<sup>3+</sup> complexes are not good catalysts due to their kinetic inertness and consequent slow turnover.<sup>13</sup> The trivalent charge of La<sup>3+</sup> makes it a good Lewis acid for withdrawing electron density away from a phosphate diester substrate. It is also known for forming hydroxo species in aqueous solution by deprotonating coordinated water molecules. La<sup>3+</sup> also has a high coordination number so that it has numerous sites available to coordinate both the substrate and one or more hydroxide nucleophiles.<sup>15</sup>

One difficulty in studying free La<sup>3+</sup> and La<sup>3+</sup> complexes is their ability to dimerize, trimerize, and form multinuclear aggregates in solution. This effect has plagued many lanthanum model studies by making it very difficult to define the active catalytic species in solution, if at all.<sup>1,2,4,5,7,8,16</sup> The use of weakly coordinating buffers further complicates matters when trying to define what species exist in solution. Nonetheless, La<sup>3+</sup> complexes have much potential as artificial restriction enzymes due to their ability to selectively hydrolyze phosphate diesters.<sup>17</sup>

The macrocyclic ligand 15,31-dimethyl-3,11,19,27,33,35-hexaazapentacyclo [27.3.1.1<sup>5,9</sup>1.1<sup>13,17</sup>.1<sup>21,25</sup>]hexatriaconta-5,7,9(33),13,15,17(34),21,23,25(35),29,31,1(36)-dodecaene-34,36-diol, 24RBPYBC, L (see Figure 1), was originally synthesized in this laboratory for its potential to form both mono- and dinuclear iron complexes.<sup>18</sup> The macrocycle has eight donor sites and can, indeed, form both mono- and dinuclear complexes with various metal ions.<sup>19,20</sup> The number of donor sites is advantageous for complexing La<sup>3+</sup>, which has a higher coordination number than the first-row transition metals. The following work describes well-defined mononuclear and dinuclear lanthanum systems that can readily hydrolyze BNP, bis-(4-nitrophenyl) phosphate (Figure 2). The hydrolysis reaction is catalytic and has a highly unusual third-order dependence with respect to the catalyst concentration. The dinuclear dihydroxo species, L-2La-2(OH), is more effective than the dinuclear monohydroxo species, L-2La-OH, or any mononuclear species. Much insight is gained about the importance of

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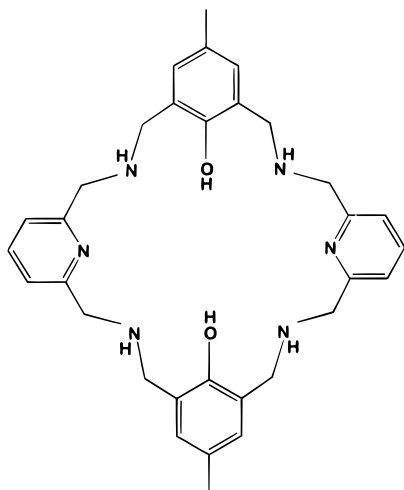


Figure 1. Macrocyclic ligand, L, that was used in this work.

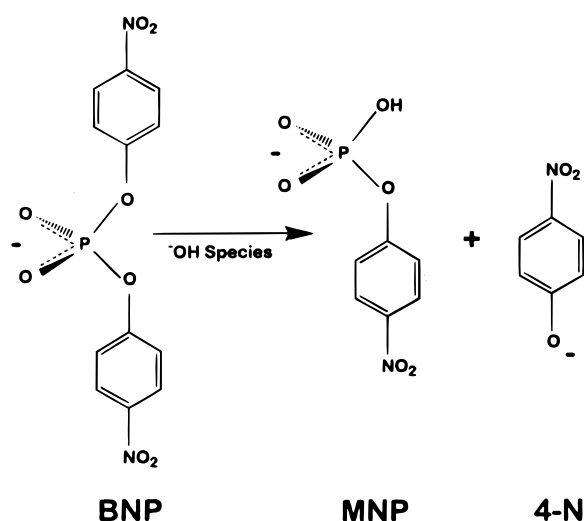


Figure 2. Hydrolysis of BNP to 4-nitrophenyl phosphate (MNP) and 4-nitrophenol (4-N).

preorganization of the metal ions and the hydroxide nucleophile in hydrolyzing phosphate diesters.

## Experimental Section

**Materials and Methods.** The following reagents were used: GR grade KCl, AC S reagent  $\text{LaCl}_3 \cdot 7\text{H}_2\text{O}$ , and bis(4-nitrophenyl) phosphate from Aldrich; 200-proof ethyl alcohol from McCormick Distilling Co., Inc.; and  $\text{CO}_2$ -free Dilut-it ampules of KOH from J. T. Baker.  $\text{K}^+\text{BNP}^-$  was made by adding an equivalent amount of solid KOH to bis(4-nitrophenyl) phosphate in EtOH and collecting the precipitate. The ligand, L, was synthesized as previously described.<sup>18</sup> Elemental analysis and potentiometry deduced a formula of  $\text{C}_{32}\text{H}_{38}\text{N}_6\text{O}_2 \cdot 4\text{HCl} \cdot 1/3\text{MeOH} \cdot 4/3\text{H}_2\text{O}$  ( $M_w = 719$  g/mol) in the sample that was used. The  $\text{La}^{3+}$  solution was standardized by adding excess  $\text{Na}_2\text{EDTA}$  and then back-titrating with standardized  $\text{Zn}^{2+}$  to an eriochromeblack T endpoint.<sup>21</sup> All UV-vis measurements were performed on a Perkin-Elmer 553 fast scan UV/vis spectrophotometer. The  $+/-$  electrospray ionization experiments were performed on a Vestec 201A Thermospray LC/MS instrument.

**Potentiometry.** Each system was investigated in a 100-mL thermostated cell and kept at 35 °C. The solvent for each system consisted of 37.5 mL of EtOH and 12.5 mL of aqueous solution. A 75% EtOH solution was chosen because the dinuclear lanthanum complex, L-2La-4Cl, precipitates out of 100% aqueous solution. The initial volume for such a 75% EtOH solution was determined volumetrically to be 49.02

mL. The  $\text{p}K_w$  under the described conditions was measured and calculated to be 14.65. The ionic strength was kept constant with 0.10 M KCl. Under such conditions, the activity of an ion parallels its concentration. Argon was bubbled through each solution to create an inert atmosphere. Direct millivolt measurements were taken on a Corning model 150 pH meter as KOH was added to the system in small increments. The electrodes were calibrated from the average calculated  $E^\circ$  of 20 separate titration points of a titration of dilute standardized HCl. Thus, the electrodes were calibrated in terms of  $\text{p}[\text{H}]$  which is defined as  $-\log [\text{H}]$ . The program BEST was used to calculate all equilibrium constants.<sup>22</sup> Species distribution diagrams were computed and plotted with the programs SPE and SPEPLOT, respectively.<sup>22</sup> The  $\text{p}K_a$ 's of L were also determined under the above conditions. The lowest four were determined potentiometrically: 4.38, 5.76, 7.39, and 8.24. The two highest  $\text{p}K_a$ 's were determined spectrophotometrically by measuring the absorbance at 308 nm of solutions at 18 different  $\text{p}[\text{H}]$  values between 12.3 and 13.6. The data were fit to a curve in which the  $\text{p}K_a$ 's and extinction coefficients could be calculated:  $\text{p}K_a(\text{L}-2\text{H}) = 12.83$ ,  $\text{p}K_a(\text{L}-\text{H}) = 12.95$ ,  $\epsilon(\text{L}-\text{H}) = 4500 \text{ M}^{-1} \text{ cm}^{-1}$ , and  $\epsilon(\text{L}) = 8900 \text{ M}^{-1} \text{ cm}^{-1}$ .

**Kinetics.** All kinetic studies were carried out in a 75% EtOH solution, with 0.10 M KCl present, and at 35 °C. The pH was kept constant by the addition of dilute KOH to the thermostated cell either manually or with a pH-stat. The pH could easily be kept constant to 0.05 pH units. The hydrolysis of BNP was followed by taking 20- $\mu\text{L}$  aliquots from the cell at appropriate times, injecting the aliquots into an HPLC instrument equipped with a UV-vis detector, and following the increase in absorption at 397 nm due to  $d[4\text{-N}]/dt$  (4-N is defined in Figure 2). A C18 Adsorbosphere column was employed, and the mobile phase consisted of a 50:50 MeOH-phosphate buffer (25 mM total,  $\text{pH} = 7.0$ ). In each kinetic trial 4-6 points were recorded that defined a straight line, each of which had a Pearson's correlation coefficient of  $>0.98$ . Hydrolysis of BNP was followed until 1-10% completion to avoid detecting hydrolysis of the product, MNP. The kinetic data were analyzed by the method of initial rates and the following rate equations:

$$1:1 \text{ system:} \quad \text{rate} = k_{\text{obs}}[\text{L-La}]_{\text{TOT}}^3[\text{BNP}]_{\text{INIT}}$$

$$1:2 \text{ system:} \quad \text{rate} = k_{\text{obs}}[\text{L-2La}]_{\text{TOT}}^3[\text{BNP}]_{\text{INIT}}$$

The equations are, indeed, first-order with respect to  $[\text{BNP}]_{\text{INIT}}$  and third-order with respect to  $[\text{L-La}]_{\text{TOT}}$  or  $[\text{L-2La}]_{\text{TOT}}$ . A typical solution in the 1:2 system contained  $[\text{BNP}]_{\text{INIT}} = [\text{L}] = 0.5[\text{La}^{3+}] = 0.2-0.8$  mM. Spontaneous hydrolysis of BNP under these conditions is  $3.4 \times 10^{-11} \text{ s}^{-1}$  at  $\text{p}[\text{H}] = 7$  and  $3 \times 10^{-9} \text{ s}^{-1}$  at  $\text{p}[\text{H}] = 10.5$ ; thus, it is considered negligible.

Multiple regression analysis<sup>23</sup> was used to fit the kinetic data in the 1:2 system from  $\text{p}[\text{H}] = 6$  to 10 to the following model:

$$\text{rate} = \{k_1[\text{L-2La-OH}]^3 + k_2[\text{L-2La-2(OH)}]^3\}[\text{BNP}]_{\text{INIT}}$$

The species distribution diagram shows that two other species are present in the  $\text{p}[\text{H}]$  range that was studied and can possibly contribute to the model:  $[\text{L-2La}]$  and  $[\text{L-2La-3(OH)}]$ . The ligand degrades rapidly at  $\text{p}[\text{H}]$  values higher than 10; thus  $[\text{L-2La-3(OH)}]$  was not included in the model due to lack of data. The F-test in a stepwise regression analysis showed that  $[\text{L-2La}]$  was not a significant variable for the model. Both independent variables,  $[\text{L-2La-OH}]$  and  $[\text{L-2La-2(OH)}]$ , are statistically significant to an  $\alpha = 0.05$  significance level. The coefficient of multiple regression,  $R^2$ , is 0.84. Thus, 84% of the total variation in the rates is explained by  $[\text{L-2La-OH}]$  and  $[\text{L-2La-2(OH)}]$ . The fourth-order rate constants with their standard errors were obtained and are shown below:

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$$k_1 = (5.2 \pm 0.8) \times 10^6 \text{ M}^{-3} \text{ s}^{-1}$$

$$k_2 = (4 \pm 1) \times 10^8 \text{ M}^{-3} \text{ s}^{-1}$$

## Results and Discussion

L forms both mononuclear and dinuclear complexes with  $\text{La}^{3+}$ . The stability constants are given in Table 1. The log  $K$  value for the formation of the dinuclear complex is 11.85 log units less than the formation constant for the mononuclear complex. This is a result of placing two trivalent ions in close proximity (3–4 Å). A number of hydroxo species form in solution. The  $\text{pK}_a$  of a water molecule bound to the mononuclear complex is 11.15. The  $\text{pK}_a$  of a water molecule bound to the dinuclear complex is 7.75. The fact that the  $\text{pK}_a$  is lowered 3.40 p[H] units in the dinuclear complex compared to the mononuclear complex implies that the water bridges between the two metal ions (Figure 3). The  $\text{pK}_a$  of a second water molecule bound to the dinuclear complex is 10.03. This value is 1.12 p[H] units below the  $\text{pK}_a$  of L-La-OH<sub>2</sub>; thus, this water also bridges between the two  $\text{La}^{3+}$  ions. A third hydroxide ligand can also form on the dinuclear complex. However, the ligand starts to rapidly degrade at p[H] values higher than 10. The lifetime of L-2La-3(OH) is quite short, and no kinetic measurements above pH = 10 were reliable. The various hydroxide species form only after the phenolic protons have been deprotonated. The overall charge on L-La-OH is 0 while the overall charge on L-2La-OH is +3.

Although numerous species form in solution, the important species to focus on are the hydroxo species. The metal-coordinated hydroxide acts as a nucleophile and can attack BNP in a hydrolysis reaction. The 1:2 system will be discussed first. A species distribution diagram for the 1:2 system is shown in Figure 4. L-2La-OH starts to form at p[H] ~ 6.5 and has a maximum concentration at p[H] = 8.9. L-2La-2(OH) starts to form at p[H] ~ 8.5 and has a maximum concentration at p[H] = 10.6.

All kinetic data for the present systems were generated using approximately equimolar concentrations of  $[\text{BNP}]_{\text{INIT}}$  and  $[\text{L-2La}]_{\text{TOT}}$  or  $[\text{L-La}]_{\text{TOT}}$ . Turnover numbers for the 1:2 system were reported earlier.<sup>24</sup> Thus, all rate constants described herein are fourth-order rate constants. The ability of the 1:2 system to hydrolyze BNP is described by the rate constants which are shown for the p[H] range 8.25–10 (Figure 4). In the p[H] range 6–8 the rate constants are measurable, but relatively small. Above p[H] = 8 the rate constants follow the formation of L-2La-2(OH). The rate law is the following:

$$\text{rate} = k_{\text{obs}} [\text{L-2La}]_{\text{TOT}}^3 [\text{BNP}]_{\text{INIT}}$$

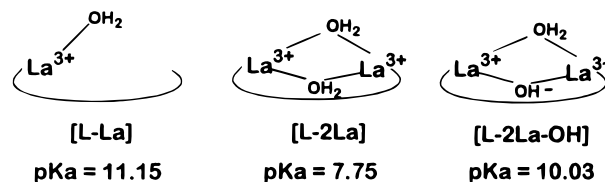
The value for  $k_{\text{obs}}$  is a collection of the total rate constants for the whole 1:2 system. The system is first-order with respect to  $[\text{BNP}]_{\text{INIT}}$ . However, the system is a highly unusual third-order with respect to  $[\text{L-2La}]_{\text{TOT}}$ . Table 2 shows how the rate is affected when only the catalyst concentration is varied. The rate increases by 9 when the catalyst concentration is doubled, and the rate increases by 30 when the catalyst concentration is tripled. This effect also occurs at p[H] = 7 and 10.

The [L-2La-2(OH)] species is clearly the most effective catalyst. The individual rate constants for L-2La-OH and L-2La-2(OH),  $k_1$  and  $k_2$ , respectively, were calculated:  $k_1 = 5.2 \times 10^6 \text{ M}^{-3} \text{ s}^{-1}$  and  $k_2 = 4 \times 10^8 \text{ M}^{-3} \text{ s}^{-1}$ . The rate constant  $k_2$  is over 75 times greater than  $k_1$ . If both hydroxide nucleophiles

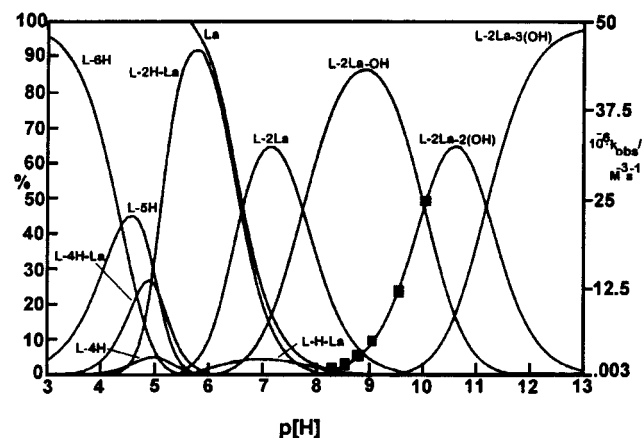
**Table 1.** Stability Constants for the Formation of Mono- and Dinuclear  $\text{La}^{3+}$  Complexes with the Ligand L<sup>a</sup>

equilibrium	log $K$
[L-La]/[L][La]	18.52
[L-2La]/[L-La][La]	6.67
[L-H-La]/[L-H][La]	14.48
[L-2H-La]/[L-2H][La]	9.31
[L-3H-La]/[L-3H][La]	6.23
[L-4H-La]/[L-4H][La]	3.56
[L-La]/[L-La-OH][H]	11.15
[L-2La]/[L-2La-OH][H]	7.75
[L-2La-OH]/[L-2La-2(OH)][H]	10.03
[L-2La-2(OH)]/[L-2La-3(OH)][H]	11.17

<sup>a</sup>  $T = 35^\circ \text{C}$ ,  $\mu = 0.10 \text{ M KCl}$ , 75% EtOH. Estimated error =  $\pm 0.08$ .



**Figure 3.** Hydroxo species of the mono- and dinuclear lanthanum complexes of L. The macrocycle has been drawn as a curved line for simplicity.



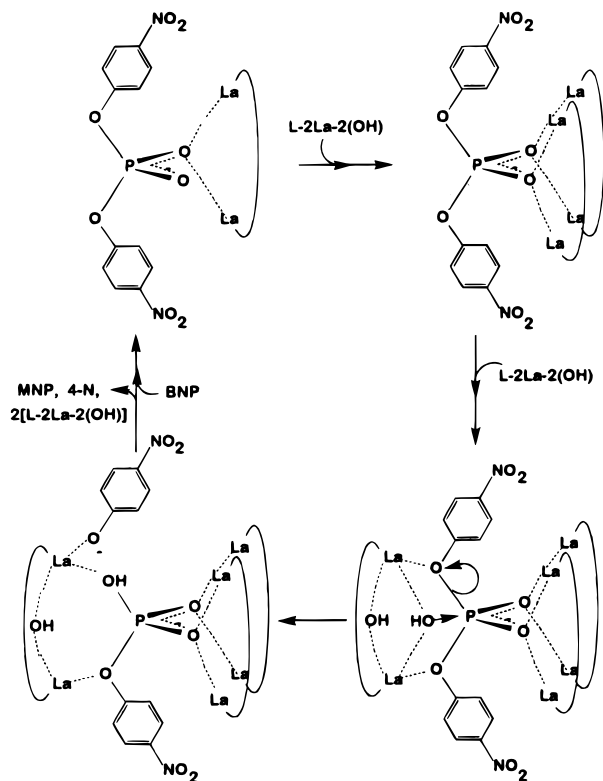
**Figure 4.** Species distribution diagram for the 1:2 system. Rate constants for the hydrolysis of BNP by the 1:2 system are given by the solid squares (■). Estimated error on kinetic data =  $\pm 10\%$ .

**Table 2.** Dependence of the Rate of BNP Hydrolysis on the Concentration of  $[\text{L-2La}]_{\text{TOT}}$  and  $[\text{L-La}]_{\text{TOT}}$ <sup>a</sup>

pH	$[\text{L-2La}]_{\text{TOT}}/\text{M}$	$[\text{BNP}]_{\text{INIT}}/\text{M}$	$10^8(\text{rate})/\text{M/s}$
8.25	0.000728	0.000531	24
8.25	0.000486	0.000539	7.3
8.25	0.000244	0.000518	0.81
pH	$[\text{L-La}]_{\text{TOT}}/\text{M}$	$[\text{BNP}]_{\text{INIT}}/\text{M}$	$10^{10}\text{Rate}/\text{M/s}$
8.00	0.000970	0.000495	49
8.00	0.000487	0.000500	8.0

<sup>a</sup> Estimated error on kinetic data =  $\pm 10\%$ .

on L-2La-2(OH) were equivalent,  $k_2$  would be expected to be about 2 times greater than  $k_1$ . Since  $k_2$  is over 75 times greater than  $k_1$ , preorganization of the hydroxide nucleophile must be a major factor. That is, the second hydroxide is better positioned to attack the substrate compared to the first hydroxide nucleophile. The La–La distance will be the same in L-2La-OH and L-2La-2(OH) since the hydroxide ions bridge the two metals in both species. The ideal metal–metal distance for binding a phosphate diester is ~5.4 Å while the approximate M–M

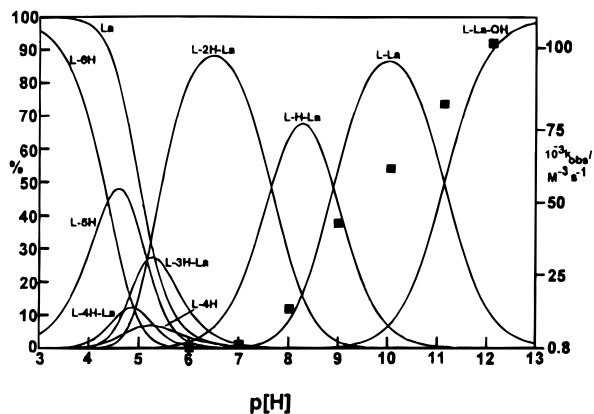


**Figure 5.** Proposed mechanism for the hydrolysis of BNP by  $[L-2La-2(OH)]^3$ . For simplicity, the ligand has been drawn as a curved line and the coordinated hydroxides of the two rightmost  $L-2La-2(OH)$  species were not included.

distance with a bridged hydroxide is 3.5 Å.<sup>25,26</sup> Since the La–La distance in  $L-2La-OH$  and  $L-2La-2(OH)$  will be approximately the same at 3.5 Å, neither has an advantage for recognizing the substrate with respect to the M–M distance.  $L-2La-OH$  does have an advantage over  $L-2La-2(OH)$  because it has a higher overall charge (+3 to +2) and has more open coordination sites available to bind the substrate. Although the process of molecular recognition favors  $L-2La-OH$ ,  $L-2La-2(OH)$  is a more effective catalyst. Thus, molecular recognition can be ruled out as a major factor why  $k_2$  is 75 times greater than  $k_1$ .

The unique third-order dependence on  $[L-2La]_{TOT}$ ,  $[L-2La-OH]$ , or  $[L-2La-2(OH)]$  sheds some light on the mechanism of the hydrolysis reaction. Figure 5 shows the proposed mechanism for the hydrolysis of BNP by  $[L-2La-2(OH)]^3$ . The aggregation of three dinuclear lanthanum complexes occurs only when BNP is present. This was demonstrated by measuring the  $pK_a$ 's of the lanthanum complexes at different concentrations (0.2 and 2.0 mM). Any interaction between individual  $La^{3+}$  complexes, such as dimerization or trimerization, would be manifested in the decrease of the  $pK_a$ 's of  $L-2La-OH_2$ ,  $L-2La-OH-OH_2$ , and  $L-La-OH_2$  as their concentration in solution increases. However, no difference within experimental error was observed for any of the stability constants in Table 1.

When a  $L-2La-2(OH)$  complex encounters a free substrate, the complex binds to one of the negatively charged oxygens of BNP. A second complex binds to the other negatively charged oxygen. The third-order dependence on the dinuclear lanthanum complex implies that the first two complexes that bind the



**Figure 6.** Species distribution diagram for the 1:1 system. Rate constants for the hydrolysis of BNP by the 1:1 system are given by the solid squares (■). Estimated error on kinetic data = ±10%.

substrate do not take part in the nucleophilic attack. Although there are four potential hydroxide nucleophiles on two  $L-2La-2(OH)$  complexes bound to the negatively charged oxygens of BNP, no nucleophilic attack occurs. The reason is that these bridging hydroxide ions are directed right at the negatively charged oxygens which resist any kind of nucleophilic attack. The initial two  $L-2La-2(OH)$  complexes can play a number of roles with respect to Lewis acid catalysis. They can withdraw electron density from the phosphorus center, stabilize the transition state, and/or stabilize the leaving group.

When a third  $L-2La-2(OH)$  complex is encountered, the complex will bind to the oxygens of the ester groups. The hydroxide nucleophiles on this third  $L-2La-2(OH)$  complex are highly preorganized to attack the phosphorus center. These hydroxide ions are directed away from the electron density cloud of the negatively charged oxygens so they encounter much less resistance when attacking the phosphorus center. Another role that the third  $L-2La-2(OH)$  complex plays is that it coordinates the oxygens of the ester groups. Chin and co-workers have established a correlation between the rate of phosphate diester hydrolysis and the basicity of the leaving group (4-N in the case of BNP hydrolysis).<sup>12,14</sup> The lower the log protonation constant of the leaving group, the faster the hydrolysis of the corresponding phosphate diester. When  $L-2La-2(OH)$  binds the ester oxygen, it lowers its basicity and, consequently, increases the rate of hydrolysis. The ester group becomes a better leaving group when a  $La^{3+}$  ion is bound. However, this is a minor factor in this system. The log stability constant for the formation of a  $La^{3+}_{free}-(4-N)$  complex is only 1.0.<sup>27</sup> The interaction is very weak; thus, the basicity of the ester group is not lowered very much by binding to the  $L-2La-2(OH)$  complex.

The fourth-order rate constants are difficult to appreciate since they are very large in magnitude. Based on the rate constants,  $k_1$  and  $k_2$ , the time required to hydrolyze 1 equiv of BNP at 35 °C can be theoretically calculated. For  $[L-2La-2(OH)] = 1$  mM, 1 equiv of BNP is hydrolyzed every 2.5 s. For  $L-2La-OH = 1$  mM, 1 equiv of BNP is hydrolyzed every 192 s. This system is very effective for hydrolyzing BNP.

The species distribution diagram for the 1:1 system is shown in Figure 6. The rate constants are given by the squares. In this system the rate constants do not follow the formation or depletion of any one species. That is, no single species is markedly more effective than any other. At each  $p[H]$  a

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combination of species with comparable rate constants make up the total observed rate constant,  $k_{\text{obs}}$ . The rate law for the 1:1 system is the following:

$$\text{rate} = k_{\text{obs}}[\text{L-La}]_{\text{TOT}}^3[\text{BNP}]_{\text{INIT}}$$

The 1:1 system is similar to the 1:2 system in that they are both first-order in  $[\text{BNP}]_{\text{INIT}}$  and third-order in catalyst concentration. The overall rate constants for the 1:1 system are lower than the rate constants for the 1:2 system. There are two major reasons for this. First, the 1:2 system has two lanthanum ions acting as Lewis acids per complex compared to only one lanthanum ion per complex in the 1:1 system. Second, dinuclear complexes generate hydroxide nucleophiles at lower  $\text{p}[\text{H}]$  values than do mononuclear complexes. Metal-coordinated hydroxide nucleophiles can hydrolyze phosphate diesters faster than coordinated aqua ions or free  $\text{OH}^-$  at low pH values. Unfortunately, multiple regression on the kinetic data of the 1:1 system could not be performed due to the amount of primary data required to solve for 4–5 independent variables.

The 1:1 and 1:2 systems are catalytic systems. The presence of deprotonated phenol groups on the ligand begs the question of whether a transesterification is occurring rather than a hydrolysis reaction. In a separate experiment involving a highly concentrated solution of  $[\text{L-2La}]_{\text{TOT}}$  and  $[\text{BNP}]$  a yellow precipitate occurred within seconds. The NMR spectrum showed a pattern characteristic of the ligand plus two doublets in the aromatic region that could be BNP, MNP, or a phosphorylated ligand. A +ESI spectrum identified the yellow solid as  $[\text{L-2La-MNP-2Cl}]^{+}$ , and a major peak was found at  $m/z = 1067$   $[\text{L-2La-MNP-Cl}]^{+}$ , and a major peak was found at  $m/z = 516$   $[\text{L-2La-MNP}]^{2+}$ . Since the hydrolysis product was isolated and not the transesterification product, a hydrolysis reaction is operative.

Last, it is known in enzymes<sup>28</sup> and has been established in model systems<sup>29,30</sup> that the distance between metal ions is a large factor influencing the rate of phosphate diester hydrolysis. This is especially important considering the fact that crystal-

lographically defined zinc-dependent phosphodiesterases contain three zinc ions that are 3–4 Å, 4–5 Å, and 5–6 Å apart ( $\text{Zn}_1-\text{Zn}_2$ ,  $\text{Zn}_2-\text{Zn}_3$ ,  $\text{Zn}_1-\text{Zn}_3$ ).<sup>9–11</sup> Although no structural data are available, the La–La distance in  $\text{L-2La-2(OH)}$  is  $\sim 3.5$  Å due to the presence of the bridging hydroxide ions. This is similar to the enzymes'  $\text{Zn}_1-\text{Zn}_2$  distance. A Zn–Zn distance of  $\sim 5.4$  Å, similar to the enzymes'  $\text{Zn}_1-\text{Zn}_3$  distance, is ideal for the coordination of a phosphate diester.<sup>25</sup> While the 3.5 Å M–M distance is advantageous for the generation of a hydroxide nucleophile at physiological pH, the longer M–M distance of 5.4 Å is advantageous for binding the substrate. Phosphodiesterases may employ three metal ions in order to benefit from both strategies.

## Conclusions

The dihydroxo dinuclear lanthanum complex,  $\text{L-2La-2(OH)}$ , is a very effective catalyst for hydrolyzing BNP. The rate constant versus  $\text{p}[\text{H}]$  profile follows the formation of  $\text{L-2La-2(OH)}$  in solution, and the individual rate constant for the hydrolysis of BNP was calculated to be  $k_2 = (4 \pm 1) \times 10^8 \text{ M}^{-3} \text{ s}^{-1}$ . The unusual third-order dependence on  $[\text{L-2La}]_{\text{TOT}}$  or  $[\text{L-La}]_{\text{TOT}}$  is key to the proposed mechanism. The third  $\text{L-2La-}n(\text{OH})$  that binds BNP is much more preorganized than the first two complexes that bind. An attack on the phosphorus center on the side opposite the negatively charged oxygens is the path of least resistance for the metal-coordinated hydroxide nucleophile. Overall rate constants for the hydrolysis of BNP by the 1:1 system were also obtained. Since they are much less than the corresponding rate constants in the 1:2 system, cooperativity between the two  $\text{La}^{3+}$  ions in the dinuclear complex is evident. The two  $\text{La}^{3+}$  ions in the complex lower the  $\text{p}K_a$  of the bridging water molecules so that hydroxide nucleophiles form at lower  $\text{p}[\text{H}]$  values. Both  $\text{La}^{3+}$  ions in the dinuclear complex take part in withdrawing electron density from the substrate. The high rate constants and third-order dependence hold much promise for future work on the hydrolysis of DNA.

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