

## Transesterification of a Phosphate Diester by Divalent and Trivalent Metal Ions

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The transesterification of the 2-(4-nitrophenyl phosphate) ester of propylene glycol (**1**) was promoted by  $\text{La}^{3+}$ ,  $\text{Nd}^{3+}$ ,  $\text{Eu}^{3+}$ ,  $\text{Gd}^{3+}$ ,  $\text{Tb}^{3+}$ ,  $\text{Yb}^{3+}$ ,  $\text{Lu}^{3+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Mg}^{2+}$ , and  $\text{Ca}^{2+}$  at 37 °C in 0.01 M Hepes buffer (*N*-(2-hydroxyethyl)piperazine-*N'*-ethanesulfonic acid), pH 6.85. The ability of metal ions to promote transesterification of **1**, as measured by apparent-second-order rate constants, followed the order  $\text{Tb}^{3+} > \text{Gd}^{3+} > \text{Yb}^{3+} > \text{Eu}^{3+} > \text{Nd}^{3+} > \text{Pb}^{2+} > \text{Lu}^{3+} > \text{La}^{3+} > \text{Cu}^{2+} > \text{Zn}^{2+} > \text{Co}^{2+} > \text{Mn}^{2+} > \text{Ni}^{2+} > \text{Mg}^{2+} > \text{Ca}^{2+}$ . The transesterification of **1** by  $\text{La}^{3+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Co}^{2+}$ , or  $\text{Mn}^{2+}$  exhibited saturation kinetics, consistent with the formation of a reactive metal ion complex with **1**. Metal ion association constants ( $K$ ) for formation of the metal-1 complex and a first-order catalytic rate constant ( $k_{\text{cat}}$ ) for decomposition of the metal-1 complex were determined for metal ions that showed saturation kinetics.  $K$  varied by only a factor of 5, whereas  $k_{\text{cat}}$  varied by a factor of 29. Transesterification of **1** bound to  $\text{Pb}^{2+}$ ,  $\text{La}^{3+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Mn}^{2+}$ , or  $\text{Ni}^{2+}$  occurred  $2.1 \times 10^4$ ,  $1.4 \times 10^4$ ,  $4.6 \times 10^3$ ,  $3.6 \times 10^3$ ,  $9.2 \times 10^2$ ,  $7.7 \times 10^2$ , or  $7.7 \times 10^2$  times more rapidly, respectively, than the transesterification of free **1**. Properties of metal ions that may affect their ability to promote transesterification of phosphate esters are discussed; these include the Lewis acidity of the metal ion, the formation of metal hydroxide complexes, metal ion radius, and stabilization of a hypervalent phosphorus(V) intermediate. These metal ion model studies are put in context with metal ion requirements in self-cleaving RNAs.

### Introduction

Many of the enzymes that catalyze nucleophilic displacement reactions at phosphorus(V) require metal ions for activity.<sup>1</sup> Investigation of metal ion model systems has helped to elucidate the role of metal ions in these reactions which include ATP hydrolysis,<sup>2</sup> phosphate diester<sup>3</sup> and phosphate monoester hydrolysis,<sup>4</sup> and phosphoryl transfer.<sup>5</sup> Metal ion catalyzed displacement reactions at phosphorus(V) are also involved in reactions catalyzed by RNA enzymes and self-splicing and self-cleaving RNAs;<sup>6</sup> hydrolysis, intermolecular transesterification, or intramolecular transesterification of phosphate diesters are the types of reactions that are catalyzed by these biomacromolecules. Several studies have focused on metal ion requirements in self-splicing or self-cleaving RNAs,<sup>7-9</sup> and it appears that at least in some systems several different divalent metal ions may promote cleavage.<sup>9</sup> The site-specific cleavage of phenylalanine t-RNA, a reaction that resembles that of self-cleaving RNAs, is promoted by several metal ions, including  $\text{Pb}^{2+}$ ,<sup>10,11</sup>  $\text{Eu}^{3+}$ ,<sup>12</sup>  $\text{La}^{3+}$ ,<sup>13</sup>  $\text{Zn}^{2+}$ ,<sup>12</sup>  $\text{Fe}^{2+}$ ,<sup>14</sup>  $\text{Cu}^{2+}$ ,<sup>13</sup> and  $\text{Mg}^{2+}$ .<sup>15</sup> For certain catalytic RNAs, metal ions involved

in catalytic roles and those involved in structural roles have been identified.<sup>7</sup> As with metal ion models of reactions of protein catalysts, metal ion model studies of RNA transesterification or hydrolysis reactions would aid in the assessment of the catalytic role of various metal ions and clarify the mechanism of metal ion catalysis.

Many metal ions promote the transesterification of RNA, including  $\text{La}^{3+}$ ,<sup>16</sup>  $\text{Bi}^{3+}$ ,<sup>17</sup>  $\text{Al}^{3+}$ ,<sup>17</sup>  $\text{Pb}^{2+}$ ,<sup>10,11,18</sup>  $\text{Eu}^{3+}$ ,<sup>12</sup>  $\text{Zn}^{2+}$ ,<sup>12,17,19-21</sup>  $\text{Cu}^{2+}$ ,<sup>19,21</sup>  $\text{Ni}^{2+}$ ,<sup>19,21</sup>  $\text{Co}^{2+}$ ,<sup>21</sup>  $\text{Mn}^{2+}$ ,<sup>21,22</sup> and  $\text{Mg}^{2+}$ .<sup>15</sup> However, many of these systems, especially those containing  $\text{Pb}^{2+}$  or  $\text{La}^{3+}$ , are not amenable to mechanistic study because of the ready precipitation of metal hydroxides or metal nucleotide complexes. As a complement to studies of metal ion promoted RNA cleavage, we have undertaken a kinetic study of transesterification of the 4-nitrophenyl phosphate ester of propylene glycol by several divalent and trivalent metal ions in order to gain insight into metal ion catalysis of transesterification reactions at phosphate esters. This phosphate ester has been used previously as an RNA model compound to examine the effect of base,<sup>23</sup> zinc(II) complexes,<sup>24,25</sup> or imidazole<sup>25</sup> on phosphate ester transesterification. Previous model studies<sup>26</sup> of metal ion promoted reactions that involve intramolecular nucleophilic attack on a phosphate diester suggested the importance of metal ion radius and invoked the formation of a metal-bound hypervalent phosphorus(V) intermediate. These and other metal ion properties that are pertinent to metal ion catalysis of phosphate ester transesterification are analyzed.

### Experimental Section

The 4-nitrophenyl phosphate ester of propylene glycol (**1**) was synthesized according to literature procedures.<sup>23</sup> A single <sup>31</sup>P resonance was observed at -4.07 ppm in D<sub>2</sub>O. Solutions of **1** were analyzed by spec-

- (1) (a) Walsh, C. *Enzymatic Reaction Mechanisms*; W. H. Freeman: San Francisco, CA, 1979; Chapters 6-8. (b) *Advances in Inorganic Biochemistry*; Eichhorn, G. L., Marzilli, L. G., Eds.; Elsevier: Amsterdam, 1979; Vol. 1.
- (2) (a) Sigel, H.; Hofstetter, F.; Martin, R. B.; Milburn, R. M.; Scheller-Krattiger, V.; Scheller, K. H. *J. Am. Chem. Soc.* **1984**, *106*, 7935-7946. (b) Sigel, H.; Amsler, P. E. *J. Am. Chem. Soc.* **1976**, *98*, 7390-7400. (c) Hediger, M.; Milburn, R. M. *J. Inorg. Biochem.* **1982**, *16*, 165-182.
- (3) (a) Morrow, J. R.; Trogler, W. C. *Inorg. Chem.* **1988**, *27*, 3387-3394. (b) Hendry, P.; Sargeson, A. M. *J. Am. Chem. Soc.* **1989**, *111*, 2521-2527. (c) Chin, J.; Banaszczuk, M.; Jubian, V.; Zou, X. *J. Am. Chem. Soc.* **1989**, *111*, 186-190. (d) Chin, J.; Zou, X. *Can. J. Chem.* **1987**, *65*, 1882-1884.
- (4) (a) Jones, D. R.; Lindoy, L. F.; Sargeson, A. M. *J. Am. Chem. Soc.* **1983**, *105*, 7327-7336. (b) Farrell, F. J.; Kjellstrom, W. A.; Spiro, T. G. *Science* **1969**, *164*, 320-321. (c) Chin, J.; Banaszczuk, M. *J. Am. Chem. Soc.* **1989**, *111*, 4103-4105.
- (5) Cooperman, B. S. *Met. Ions Biol. Syst.* **1976**, *5*, 79.
- (6) For leading references: (a) Cech, T. R. *Science* **1987**, *236*, 1532-1539. (b) McCorkle, G. M.; Altman, S. J. *Chem. Educ.* **1987**, *64*, 221-226.
- (7) (a) Grosshans, C. A.; Cech, T. R. *Biochemistry* **1989**, *28*, 6888-6894. (b) Dahm, S. C.; Uhlenbeck, O. C. *Biochemistry* **1991**, *30*, 9464-9469.
- (8) Uhlenbeck, O. C. *Nature* **1987**, *328*, 595-600.
- (9) Prody, G. A.; Bakos, J. T.; Buzayan, J. M.; Schneider, I. R.; Bruening, G. *Science* **1986**, *231*, 1577-1580.
- (10) (a) Werner, C.; Krebs, B.; Keith, G.; Dirheimer, G. *Biochim. Biophys. Acta* **1976**, *432*, 161-175. (b) Brown, R. S.; Hingerty, B. E.; Dewan, J. C. *Nature* **1983**, *303*, 543-546. (c) Brown, R. S.; Dewan, J. C.; Klug, A. *Biochemistry* **1985**, *24*, 4785-4801.
- (11) Behlen, L. S.; Sampson, J. R.; Drenzo, A. B.; Uhlenbeck, O. C. *Biochemistry* **1990**, *29*, 2515-2523.
- (12) Rorodorf, B. F.; Kearns, D. R. *Biopolymers* **1976**, 1491-1504.
- (13) Morrow, J. R. Unpublished results.
- (14) Kean, J. M.; White, S. A.; Draper, D. E. *Biochemistry* **1985**, *24*, 5062-5070.

- (15) Wintermeyer, W.; Zachau, H. G. *Biochim. Biophys. Acta* **1973**, *299*, 82-90.
- (16) (a) Eichhorn, G. L.; Butzow, J. J. *Biopolymers* **1965**, *3*, 79-94. (b) Baumann, E.; Trapman, H.; Fischler, F. *Biochem. Z.* **1954**, *328*, 89-96.
- (17) Dimroth, K.; Witzel, H.; Hulsens, W.; Mirbach, H. *Justus Liebigs Ann. Chem.* **1959**, *620*, 94-108.
- (18) Farkas, W. R. *Biochim. Biophys. Acta* **1967**, *155*, 401-409.
- (19) Stern, M. K.; Bashkin, J. K.; Sall, E. D. *J. Am. Chem. Soc.* **1990**, *112*, 5357-5359.
- (20) (a) Eichhorn, G. L.; Butzow, J. J. *Biochemistry* **1971**, *10*, 2014-2019. (b) Butzow, J. J.; Eichhorn, G. L. *Biochemistry* **1971**, *10*, 2019-2024.
- (21) (a) Butzow, J. J.; Eichhorn, G. L. *Biopolymers* **1965**, *3*, 95-107. (b) Ikenaga, H.; Inoue, Y. *Biochemistry* **1974**, *13*, 577-582.
- (22) Dange, V.; Van Atta, R. B.; Hecht, S. M. *Science* **1990**, *248*, 585-588.
- (23) Brown, D. M.; Usher, D. A. *J. Chem. Soc.* **1965**, 6558-6564.
- (24) Breslow, R.; Berger, D.; Huang, D. *J. Am. Chem. Soc.* **1990**, *112*, 3686-3687.
- (25) Breslow, R.; Huang, D.; Anslyn, E. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 1746-1750.
- (26) (a) Steffens, J. J.; Sampson, E. J.; Siewers, I. J.; Benkovic, S. J. *J. Am. Chem. Soc.* **1973**, *95*, 936-938. (b) Steffens, J. J.; Siewers, I. J.; Benkovic, S. J. *Biochemistry* **1975**, *14*, 2431-2440.

**Table I.** Apparent-Second-Order Rate Constants for the Transesterification of **1** in Water at 37 °C (pH 6.85) by Metal Ions

metal <sup>a</sup>	$k_2,^b \text{ M}^{-1} \text{ s}^{-1}$	metal <sup>a</sup>	$k_2,^b \text{ M}^{-1} \text{ s}^{-1}$
La <sup>3+</sup>	$1.3 \times 10^{-1}$	Pb <sup>2+</sup>	$2.0 \times 10^{-1}$
Nd <sup>3+</sup>	$2.6 \times 10^{-1}$	Cu <sup>2+</sup>	$4.0 \times 10^{-2}$
Eu <sup>3+</sup>	$3.2 \times 10^{-1}$	Zn <sup>2+</sup>	$1.8 \times 10^{-2}$
Gd <sup>3+</sup>	$5.4 \times 10^{-1}$	Co <sup>2+</sup>	$2.6 \times 10^{-3}$
Tb <sup>3+</sup>	$5.7 \times 10^{-1}$	Mn <sup>2+</sup>	$2.4 \times 10^{-3}$
Yb <sup>3+</sup>	$4.0 \times 10^{-1}$	Ni <sup>2+</sup>	$1.6 \times 10^{-3}$
Lu <sup>3+</sup>	$1.7 \times 10^{-1}$	Mg <sup>2+</sup>	$7.4 \times 10^{-5}$
		Ca <sup>2+</sup>	$4.8 \times 10^{-5}$

<sup>a</sup>Nitrate salts of metal ions were used except for the case of Ca<sup>2+</sup> or the lanthanide ions, where chloride salts were used. <sup>b</sup>In 0.01 M Hepes buffer,  $\mu = 0.100 \text{ M}$ , from five or more metal concentrations ranging from  $1.00 \times 10^{-4}$  to  $5.00 \times 10^{-4} \text{ M}$  for Pb<sup>2+</sup>, La<sup>3+</sup>, Nd<sup>3+</sup>, Eu<sup>3+</sup>, Gd<sup>3+</sup>, Tb<sup>3+</sup>, Yb<sup>3+</sup>, and Lu<sup>3+</sup>,  $6.00 \times 10^{-4}$  to  $1.40 \times 10^{-3} \text{ M}$  for Zn<sup>2+</sup>,  $1.00 \times 10^{-3}$  to  $4.00 \times 10^{-3} \text{ M}$  for Co<sup>2+</sup>, Ni<sup>2+</sup>, and Mn<sup>2+</sup>,  $5 \times 10^{-5}$  to  $3 \times 10^{-4} \text{ M}$  for Cu<sup>2+</sup>, and 0.1 to 0.6 M for Ca<sup>2+</sup> and Mg<sup>2+</sup>.

trophotometric measurement of the amount of 4-nitrophenolate produced upon complete base hydrolysis. A single phosphorus-containing product was observed at the end of kinetic runs by <sup>31</sup>P NMR spectroscopy (18.60 ppm, D<sub>2</sub>O). It was identified as the cyclic phosphate ester (**2**) by comparison of its <sup>31</sup>P NMR spectrum to that of an authentic sample.<sup>27</sup> <sup>31</sup>P NMR spectra were obtained at 169 MHz by use of a Varian 400 XL spectrometer. The external reference was 85% phosphoric acid, and chemical shifts downfield of the standard are positive. Solutions were treated either with cation-exchange resin or with sodium oxalate (for lanthanides) to remove paramagnetic metal ions prior to NMR studies.

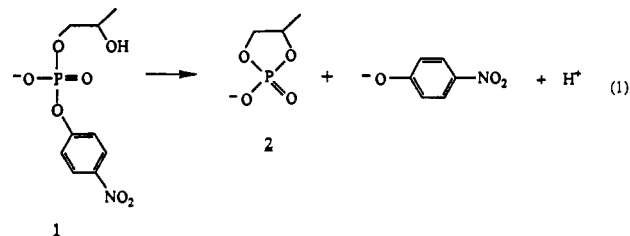
TbCl<sub>3</sub>, EuCl<sub>3</sub>, YbCl<sub>3</sub>, LuCl<sub>3</sub>, GdCl<sub>3</sub>, NdCl<sub>3</sub>, LaCl<sub>3</sub>, Zn(NO<sub>3</sub>)<sub>2</sub>, Cu(NO<sub>3</sub>)<sub>2</sub>, Pb(NO<sub>3</sub>)<sub>2</sub>, Mn(NO<sub>3</sub>)<sub>2</sub>, Ni(NO<sub>3</sub>)<sub>2</sub>, Co(NO<sub>3</sub>)<sub>2</sub>, Mg(NO<sub>3</sub>)<sub>2</sub>, and CaCl<sub>2</sub> were reagent grade. The concentration of metal ions was determined by titration with EDTA with either Eriochrome Black T, Fast Sulphon Black F, or Xylenol Orange as indicator.<sup>28</sup> Hepes buffer (*N*-(2-hydroxyethyl)piperazine-*N'*-ethanesulfonic acid) and Tris buffer (tris(hydroxymethyl)aminomethane) were reagent grade and were purchased from Sigma Chemicals. All solutions were made with Milli-Q water. Solution pHs were measured at 37 °C by use of an Orion digital pH meter equipped with temperature compensation probe. Either Hepes or Tris buffer was used to maintain solution pH at 6.85, and reaction solutions were tested at the end of kinetic runs to ensure that the pH had remained constant ( $\pm 0.01$  pH unit). Although Tris buffer has a relatively low buffering ability at pH 6.85, a large excess of Tris over **1** or use of initial rates allowed us to perform studies at constant pH over the kinetic run.

The rates of transesterification of **1** by metal ions were measured spectrophotometrically by following the increase in absorbance at 400 nm due to the production of 4-nitrophenolate. A Hewlett-Packard 5420 diode array UV-vis spectrophotometer equipped with a thermostated cell was used for measurements. Typically, the reaction was initiated by injection of 30  $\mu\text{L}$  of a concentrated solution of **1** into 5 mL of a buffered reaction solution of pH 6.85 and  $\mu = 0.100 \text{ M}$  (NaNO<sub>3</sub>) maintained at 37 °C. Reactions with trivalent lanthanide catalysts or Pb<sup>2+</sup> showed good pseudo-first-order kinetics for greater than 4 half-lives. Pseudo-first-order rate constants were calculated by using an iterative curve fit (Hewlett-Packard Kinetics software package) to the following equation:  $A = A_\infty + K(e^{-k_{\text{obs}}t})$ . Because reactions were slow and high metal concentrations caused the formation of precipitates, first-order rate constants for transesterification of **1** by all other metal ions were determined by the method of initial rates. Plots of absorbance versus time were converted to concentration units ( $\epsilon = 18500$ ) and divided by the concentration of **1** to obtain first-order rate constants. The pK<sub>a</sub> of 4-nitrophenol at 37 °C was determined by titration to be 6.90; this value was used to correct for the degree of 4-nitrophenol ionized at pH 6.85. Transesterification of **1** by metal ions showed a first-order dependence on **1** for concentrations of **1** from  $1.00 \times 10^{-4}$  to  $1.00 \times 10^{-3} \text{ M}$ . Transesterification of **1** showed a first-order dependence on metal concentration for the concentration ranges listed in Table I except for the lanthanides where reaction order was somewhat less than 1 (La<sup>3+</sup>, 0.869; Gd<sup>3+</sup>, 0.824; Tb<sup>3+</sup>, 0.825; Yb<sup>3+</sup>, 0.821; Lu<sup>3+</sup>, 0.750; Eu<sup>3+</sup>, 0.702; Nd<sup>3+</sup>, 0.771). Apparent-second-order rate constants were obtained from plots of  $k_{\text{obs}}$  versus metal concentration (correlation coefficients >0.990). Saturation experiments were performed with excess **1** instead of working under conditions with excess

metal to avoid precipitation of metal ions; the method of initial rates was used in these experiments. All experiments were run in duplicate or triplicate, and tabulated data represent the average of these experiments. Rate constants were reproducible to  $\pm 10\%$ . Control experiments containing **1** and buffer, pH 6.85 and  $\mu = 0.100 \text{ M}$ , were run alongside experiments with metal ion catalysts; rates of transesterification in the absence of metal ions were subtracted from rates of catalytic transesterification where transesterification of the control was not negligible. The rate of transesterification of the control for solutions  $1.10 \times 10^{-3}$  to  $5.00 \times 10^{-3} \text{ M}$  in **1** at pH 6.85;  $\mu = 0.100 \text{ M}$  (NaNO<sub>3</sub>), 0.01 M Hepes, measured by initial rates as described above, was  $1.3 \times 10^{-7} \text{ s}^{-1}$ .

## Results

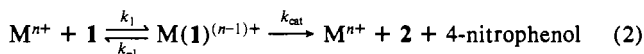
The kinetics of transesterification of the 4-nitrophenyl phosphate ester of propylene glycol (**1**) at 37 °C, pH 6.85, by metal ions (eq 1) was monitored by following the production of the 4-



nitrophenolate anion by use of UV-vis spectroscopy. For all metal ion promoted reactions the cyclic phosphate (**2**) was the major phosphorus-containing product. Metal ion concentrations and other experimental conditions are summarized in Table I. Reactions were recorded at pH 6.85 as a compromise between performing experiments at near-neutral pH to mimic physiological conditions and preventing precipitation of metal hydroxide species that occurred at more basic pHs. Apparent-second-order-rate constants in Table I were calculated from plots of pseudo-first-order rate constants ( $k_{\text{obs}}$ ) versus metal ion concentration. A  $k_{\text{obs}}$  of  $1.3 \times 10^{-7} \text{ s}^{-1}$  at pH 6.85, 0.01 M Hepes,  $\mu = 0.100 \text{ M}$ , was determined for the transesterification of **1** in the absence of metal ion catalysts by the method of initial rates as described in the Experimental Section.

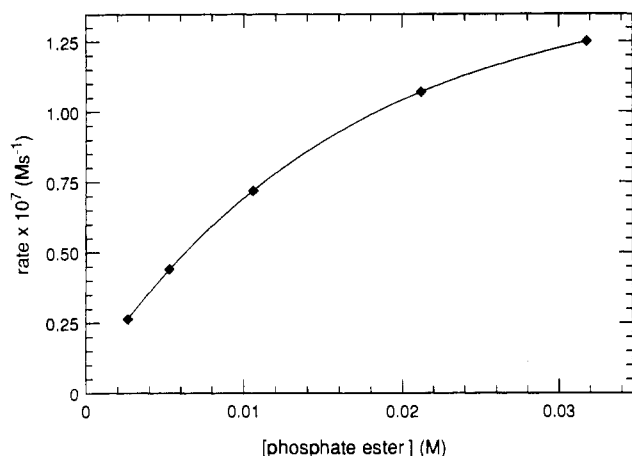
Most studies were performed in solutions 0.01 M in Hepes buffer at pH 6.85, but a few studies were done to assess the effect of buffer concentration and buffer type. In general, the transesterification of **1** by transition metals and Zn<sup>2+</sup> proceeded more rapidly as the concentration of buffer was lowered. The pseudo-first-order rate constants for transesterification of **1** by Cu<sup>2+</sup>, Zn<sup>2+</sup>, Co<sup>2+</sup>, Mn<sup>2+</sup>, or Ni<sup>2+</sup> in 0.0015 M Hepes buffer, pH 6.85, were at least 2-fold greater than those in 0.01 M Hepes. Pseudo-first-order rate constants for transesterification of **1** by Pb<sup>2+</sup> or La<sup>3+</sup> did not vary substantially in 0.0015 M Hepes buffer from values recorded in 0.01 M Hepes buffer. Tris buffer is also commonly used in studies of RNA cleavage involving metal ions, and a few kinetic studies were performed in 0.01 M Tris at pH 6.85 to determine the effect of this buffer. In Tris buffer, none of the transition metals when used in the highest concentrations listed in Table I promoted transesterification of **1**, and the rate of transesterification of **1** by 1 mM Zn<sup>2+</sup> was reduced 5-fold compared to that in 0.01 M Hepes buffer. In contrast, Tris buffer did not have as dramatic an effect on La<sup>3+</sup>- or Pb<sup>2+</sup>-promoted transesterification of **1**; the rate of transesterification by these metal ions was approximately 2-fold less in 0.01 M Tris buffer than in 0.01 M Hepes buffer. Similarly, Tris buffer in 10-fold excess (2.0 M) over Ca<sup>2+</sup> or Mg<sup>2+</sup> reduced the rate of transesterification of **1** by these metal ions 3-fold and 2-fold, respectively, over that recorded in 2.0 M Hepes buffer. Kinetic experiments without buffer proved impractical with our methods because of the sensitivity of the reaction to pH and because of the ready formation of precipitates with some metal ions without buffer.

The observed kinetics for transesterification of **1** by La<sup>3+</sup>, Pb<sup>2+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup>, Ni<sup>2+</sup>, Mn<sup>2+</sup>, or Co<sup>2+</sup> can be described by the pre-equilibrium formation of a reactive complex (eq 2). Trans-



(27) Ukita, T.; Nagasawa, K.; Irie, M. *Pharm. Bull. (Tokoyo)* **1957**, *5*, 121-126.

(28) Vogel, A. I. *Vogel's Textbook of Quantitative Inorganic Analysis*; Longman: London, 1978.



**Figure 1.** Dependence of the rate of transesterification of **1** by  $3.00 \times 10^{-4}$  M  $\text{Cu}^{2+}$  on the concentration of **1** at 37 °C, pH 6.85.

**Table II.** Rate Constants for the Transesterification of **1** in Water at 37 °C (pH 6.85) by Metal Ions<sup>a</sup>

catalyst	$K, \text{M}^{-1}$	$k_{\text{cat}}, \text{s}^{-1}$	catalyst	$K, \text{M}^{-1}$	$k_{\text{cat}}, \text{s}^{-1}$
$\text{Pb}^{2+}$	76	$2.7 \times 10^{-3}$	$\text{Mn}^{2+}$	25	$1.0 \times 10^{-4}$
$\text{La}^{3+}$	73	$1.8 \times 10^{-3}$	$\text{Co}^{2+}$	22	$1.2 \times 10^{-4}$
$\text{Cu}^{2+}$	64	$6.0 \times 10^{-4}$	$\text{Ni}^{2+}$	16	$1.0 \times 10^{-4}$
$\text{Zn}^{2+}$	38	$4.7 \times 10^{-4}$			

<sup>a</sup> 0.01 M HEPES buffer,  $\mu = 0.100$  M. Concentration of **1** ranged from  $5.00 \times 10^{-3}$  to  $5.00 \times 10^{-2}$  M,  $[\text{Pb}^{2+}] = 2.50 \times 10^{-4}$  M,  $[\text{La}^{3+}] = 5.12 \times 10^{-4}$  M,  $[\text{Cu}^{2+}] = 3.03 \times 10^{-4}$  M,  $[\text{Zn}^{2+}] = 1.00 \times 10^{-3}$  M,  $[\text{Mn}^{2+}] = 2.00 \times 10^{-3}$  M,  $[\text{Co}^{2+}] = 2.00 \times 10^{-3}$  M, and  $[\text{Ni}^{2+}] = 3.00 \times 10^{-3}$  M.

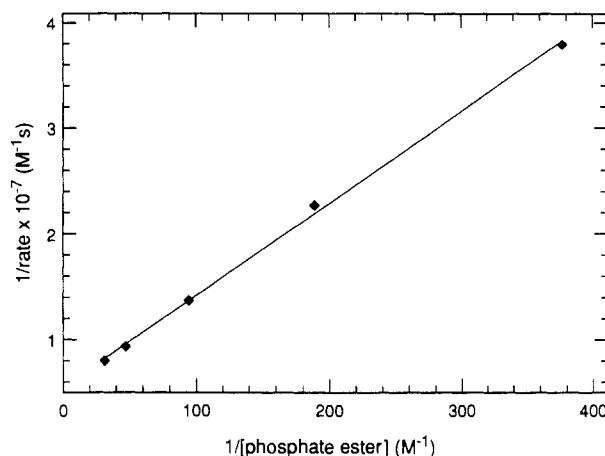
esterification of **1** by these seven metal ions, initially first-order in **1**, begins to saturate at high concentrations of **1**. A typical example of this behavior is shown in Figure 1 for the  $\text{Cu}^{2+}$  catalyst. Kinetic data can be expressed by eq 3 where  $[\text{M}^{n+}]$  is the total

$$\text{rate} = (k_{\text{cat}}K[\text{1}][\text{M}^{n+}]) / (1 + K[\text{1}]) \quad K = k_1/k_{-1} \quad (3)$$

concentration of metal ion,  $K$  is the metal ion association constant, and  $k_{\text{cat}}$  is the first-order catalytic rate constant. Data were fit to a linear form of eq 3 ( $1/\text{rate}$  versus  $1/[\text{1}]$ ), correlation coefficients  $>0.995$  to determine values for  $K$  and  $k_{\text{cat}}$  (Figure 2). Values for  $K$  and  $k_{\text{cat}}$  are listed in Table II. From comparison of  $k_{\text{cat}}$  to  $k_{\text{obs}}$  for transesterification of **1** in the absence of metal ions ( $1.3 \times 10^{-7} \text{ s}^{-1}$ ), rate accelerations of 21 000, 14 000, 4600, 3600, 770, 920, and 770 are calculated for  $\text{Pb}^{2+}$ ,  $\text{La}^{3+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Co}^{2+}$  and  $\text{Ni}^{2+}$ , respectively.

## Discussion

All trivalent and divalent metal ions studied here promoted transesterification of the phosphate ester **1**. Apparent-second-order-rate constants vary by a factor of  $10^4$ , with  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$  the least effective and the trivalent lanthanides and  $\text{Pb}^{2+}$  the most effective metal ions. Metal ion promoted transesterification of **1** is highly sensitive to the type of buffer, as are many metal ion promoted phosphorus(V) substitution reactions.<sup>29</sup> Kinetic studies without buffer were not possible here; however, two commonly used biological buffers, Hepes and Tris, gave very different rates of transesterification by metal ions. Complexation of metal ions by buffer might be expected to slow catalytic transesterification by competing for available coordination sites on the metal ion. That Tris buffer in excess over metal ion completely inhibited catalysis by transition metals is not surprising because Tris as a primary amine is probably a relatively good ligand for transition-metal ions. Despite this ability of Tris buffer to inhibit catalysis by transition metals, Tris buffer is commonly used in studies of metal ion requirements of self-cleaving RNAs.<sup>7-9</sup> What



**Figure 2.** Plot of data from Figure 1 as  $1/\text{rate} = 1/(k_{\text{cat}}K[\text{Cu}^{2+}][\text{1}]) + 1/(k_{\text{cat}}[\text{Cu}^{2+}])$ .

is the basis for the observed ordering of metal ions in the transesterification of **1**? Differences in rates of ligand exchange have been invoked to explain differences in rates of metal ion promoted RNA transesterification.<sup>21a</sup> However, metal ions used here undergo relatively fast dissociation of simple anionic ligands<sup>30,31</sup> compared to transesterification of **1** ( $k_{-1} \gg k_{\text{cat}}$ ); thus it is unlikely that ligand exchange is the rate-limiting step. Furthermore, there is no correlation between the relative rates of metal ion catalyzed RNA cleavage and rates of ligand exchange for the metal ions here.<sup>32</sup> Thus, properties other than ligand-exchange kinetics of metal ions determine their ability to promote transesterification of phosphate esters; some of these are discussed below.

Transesterification of phosphate esters is catalyzed by  $\text{H}^+$  as well as by a large number of divalent and trivalent metal ions. Similar to protons, metal ions may act as electrophilic catalysts to activate phosphorus(V) to nucleophilic attack by coordination to the phosphate ester or by binding to the leaving group to aid leaving-group departure. Neutralization of the charge on the phosphate ester by metal ion binding may overcome an electrostatic barrier if the attacking hydroxyl group is deprotonated. Factors such as the overall charge of the metal ion and its ability to polarize an oxygen-phosphorus bond (Lewis acidity) may be important. In reactions where metal ions act as Lewis acids<sup>33</sup> with esters of carbon<sup>34</sup> as substrates or with phosphorus(V) compounds as substrates,<sup>2,3a,26,35</sup>  $\text{Cu}^{2+}$  is generally the best divalent transition-metal ion, probably at least in part because of the relatively high formation constants of  $\text{Cu}^{2+}$  complexes and the superior ability of  $\text{Cu}^{2+}$  to act as a Lewis acid. For similar reasons, the transition-metal cations and  $\text{Zn}^{2+}$  are generally better at promoting reactions involving Lewis acid catalysis than are  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$ . There are few examples where the trivalent lanthanide ions have been compared to transition-metal ions as catalysts; however, the ability of  $\text{La}^{3+}$  to hydrolyze phosphate esters<sup>36</sup> and the rapid cleavage of transfer RNA<sup>12</sup> by  $\text{Eu}^{3+}$  are examples of the enormous potential of these metal cations to catalyze phosphorus(V) nucleophilic displacement reactions. An overall charge of 3+ on

(29) (a) Gellman, S. H.; Petter, R.; Breslow, R. *J. Am. Chem. Soc.* **1986**, *108*, 2388–2394. (b) Sigel, H.; Amsler, P. *J. Am. Chem. Soc.* **1976**, *98*, 7390–7400.

(30) Burgess, J. *Metal Ions in Solution*; Wiley and Sons: New York, 1978; see also references within.  
 (31) Burgess, J. *Metal Ions in Solution: Basic Principles of Chemical Interactions*; Wiley and Sons: New York, 1988; see also references within.  
 (32) Eigen, M. *Pure Appl. Chem.* **1963**, *6*, 97–115.  
 (33) Bender, M. L. *Mechanisms of Homogeneous Catalysis from Protons to Proteins*; Wiley and Sons: New York, 1971; Chapter 8 and references within.  
 (34) (a) Steinberger, R.; Westheimer, F. H. *J. Am. Chem. Soc.* **1951**, *73*, 429–435. (b) Prue, J. E. *J. Chem. Soc.* **1952**, 2331–2338.  
 (35) Courtney, R. C.; Gustafson, R. L.; Westerback, S. J.; Hyytiäinen, H.; Chaberek, S. C., Jr.; Martell, A. E. *J. Am. Chem. Soc.* **1957**, *79*, 3030–3037.  
 (36) (a) Butcher, W. W.; Westheimer, F. H. *J. Am. Chem. Soc.* **1955**, *77*, 2420–2424. (b) Milburn, R. M.; Gautam-Basak, M.; Tribolet, R.; Sigel, H. *J. Am. Chem. Soc.* **1985**, *107*, 3315–3321.

the lanthanide ion may be beneficial for catalysis; however, at neutral pH a water ligand may be deprotonated to reduce the overall charge of the lanthanide complex. Unfortunately, there is a large range of  $pK_a$  values reported in the literature<sup>30</sup> for the trivalent lanthanides; values for  $La^{3+}$  range from 9.3 to 7.4 and for  $Lu^{3+}$  range from 8.2 to 3.5 with intermediate  $pK_a$  values for the remaining lanthanides. (A smooth decrease in  $pK_a$  from  $La^{3+}$  to  $Lu^{3+}$  is generally observed.) Alternatively, a metal ion may catalyze transesterification of **1** by a pathway not possible for  $H^+$ : a metal hydroxide may act as a general base to deprotonate the incoming nucleophile.  $Pb^{2+}$ ,  $Cu^{2+}$ , and possibly the lanthanides may be especially effective catalysts because they are present in hydroxide form at neutral pH. This possibility is discussed further below.

For several metal complexes that promote RNA cleavage, a metal hydroxide is the most active catalytic species;<sup>19,37,38</sup> similarly, a metal hydroxide may be the active catalyst for some metal ions in the transesterification of **1**. A metal hydroxide in transesterification is relegated to the role of general base in deprotonating the 2'-hydroxyl group of RNA rather than acting as a nucleophile as often occurs in phosphate ester hydrolysis. However, because metal hydroxide complexes promote transesterification much more rapidly than do organic bases with similar  $pK_a$ 's, it is unlikely that metal hydroxide complexes act simply as general bases to deprotonate the incoming nucleophile, but instead promote transesterification by a combination of Lewis acid/general base catalysis.<sup>37,38</sup> In support of this postulate, a metal hydroxide complex with no available coordination sites,  $Co(NH_3)_5(OH)^{2+}$  (5 mM, pH 6.85, 37 °C), does not promote transesterification of **1**. At neutral pH, the only metal hydroxides that are present in substantial quantity<sup>30</sup> are those of  $Cu^{2+}$  ( $pK_a = 6.5$ ) and  $Pb^{2+}$  ( $pK_a = 7.2$ ) and perhaps some of the trivalent lanthanide ions (see discussion above). It may be significant that these metal ions are among the best catalysts. However  $Zn^{2+}$ , the remaining transition-metal ions,  $Mg^{2+}$ , and  $Ca^{2+}$  do not contain hydroxide ligands yet promote transesterification. Hence, the presence of a metal hydroxide ligand may be an important but not essential property of an efficient phosphate ester transesterification catalyst. Many of the metal ions used here in the concentrations given in Table I precipitated from solution at pHs where their metal hydroxide would be the predominant species in solution.<sup>30</sup> Hence kinetic experiments to obtain a pH-rate profile that ordinarily might provide evidence to support a metal hydroxide as the active catalyst were not performed.

The efficiency of a metal ion in promoting nucleophilic displacement reactions at phosphate diesters has been shown to correlate to ionic radius.<sup>26b</sup> Correlation of metal ion size to reaction rate constants has been observed where precise geometrical constraints are dictated by the formation of a bidentate complex as reaction intermediate. In phosphate diester transesterification, bidentate chelation might occur in the complexation of a hypervalent phosphorus(V) intermediate (**3**). Alternately, the metal

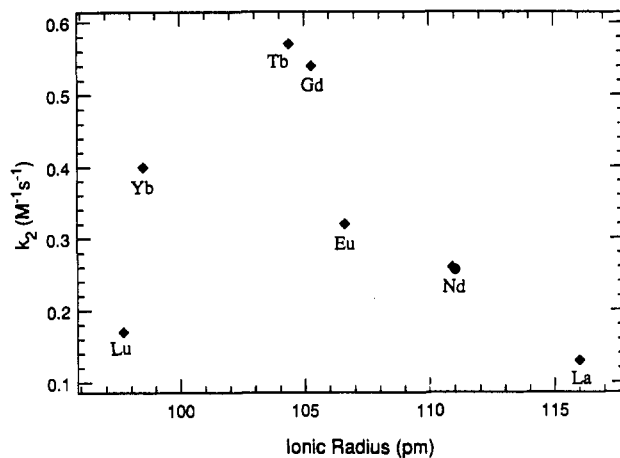
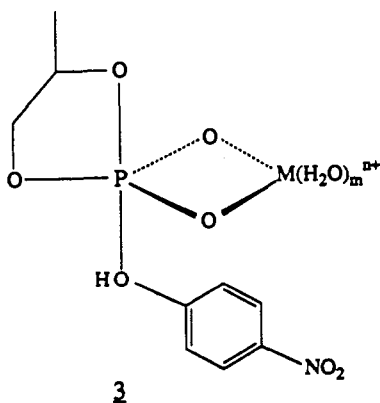


Figure 3. Dependence of apparent-second-order rate constant ( $k_2$ ) on metal ion radius for trivalent lanthanide ions.

ion may coordinate through an equatorial oxygen and the leaving-group oxygen.<sup>26</sup> The lanthanides are an ideal series for the study of the effect of ionic radius on catalysis because of the regular decrease in radius<sup>39</sup> from  $La^{3+}$  (116 pm) to  $Lu^{3+}$  (97.7 pm). A small variation in apparent-second-order rate constants ( $k_2$ ) is observed for transesterification of **1** by lanthanide ions. The lanthanides at the extreme ends of the series,  $La^{3+}$  and  $Lu^{3+}$ , have the smallest  $k_2$  values with a maximum  $k_2$  at  $Tb^{3+}$  (Figure 3); the optimal radius appears to be 104.0 pm. Thus, there may be a minor dependence of the transesterification of **1** on ionic radius for the lanthanide series. What is most striking, however, is the small magnitude of the variation in rate constants in traversing the lanthanide series. Physical properties such as the  $pK_a$  of a metal-bound water molecule, coordination number, and ionic radius change dramatically through the series,<sup>39</sup> and we had expected that these would have had more of an effect on the efficiency of various lanthanide ions in promoting the transesterification of **1**.

Kinetic data from saturation studies resolved apparent-second-order rate constants into a metal ion association constant ( $K$ ) and a catalytic rate constant ( $k_{cat}$ ) for the formation and decomposition of the metal-phosphate ester complex, respectively (Table II). These data allow further analysis of the ability of different metal ions to catalyze transesterification. First, we consider the formation constants obtained here. A relatively large metal ion association constant contributes to a greater apparent-second-order rate constant of metal ion promoted transesterification (eq 3). From Table II, the four best catalysts that exhibited saturation kinetics,  $La^{3+}$ ,  $Pb^{2+}$ ,  $Cu^{2+}$ , and  $Zn^{2+}$ , have the four highest formation constants; however, the variation in  $K$  is less than a factor of 5. Note that the Irving-Williams order is not followed for  $Ni^{2+}$ ,  $Co^{2+}$ , and  $Mn^{2+}$ , but formation constants for these metal ions are not very different. Generally, association constants between these metal ions and monodentate anionic oxygen ligands vary little. For example, formation constants<sup>40</sup> for binding of  $Mn^{2+}$ ,  $Co^{2+}$ , and  $Ni^{2+}$  to acetate at 25 °C are 25, 29, and 27  $M^{-1}$ , respectively. Few formation constants for phosphate diesters and metal ions have been reported probably because phosphate diesters are such weak ligands; however, the formation constants here are comparable to those of other phosphate diester complexes with  $Cu^{2+}$  (20  $M^{-1}$ )<sup>3a</sup> or with  $Mn^{2+}$  (11 or 20  $M^{-1}$ ).<sup>26</sup>

The catalytic rate constant,  $k_{cat}$ , largely determines the ordering of the metal ions as catalysts;  $k_{cat}$  varies by a factor of 29. A correlation between formation constant of metal substrate complex and the rate of reaction would suggest that the metal substrate complex bears some resemblance to the transition state of the

(37) Shelton, V. M.; Morrow, J. R. *Inorg. Chem.*, in press.

(38) Matsumoto, Y.; Komiyama, M. *J. Chem. Soc., Chem. Commun.* **1990**, 1050-1051.

(39) *Lanthanide Probes in Life, Chemical and Earth Sciences*; Bunzli, J.-C. G., Choppin, G. R., Eds.; Elsevier: New York, 1989; Chapter 1.

(40) *Critical Stability Constants*; Martell, A. E., Smith, R. M., Eds.; Plenum: New York, 1974; Vol. 2.

reaction.<sup>33</sup> As  $K$  increases, so does  $k_{\text{cat}}$  for metal ions in Table II, but no linear correlation is observed; in particular, the catalytic rate constants of  $\text{La}^{3+}$  and  $\text{Pb}^{2+}$  are larger than one would expect from the trend set by the transition metals and  $\text{Zn}^{2+}$ . Thus, as one might expect, the transition state does not resemble the initial complex formed between metal ion and **1**. It has been proposed that stabilization of hypervalent phosphorus(V) transition states or intermediates by metal ions may be important in metal ion catalysis of phosphorus(V) substitution reactions,<sup>26</sup> and perhaps it is the ability of the metal ion to bind strongly to a hypervalent phosphorus(V) species that governs its ability to catalyze transesterification of **1**. Studies on metal complexes of hypervalent phosphorus(V) species may shed light on this question.<sup>41</sup> Other explanations for the superior ability of the trivalent lanthanides and  $\text{Pb}^{2+}$  to catalyze transesterification might involve the large number of coordination sites of these metal ions<sup>30</sup> (8 or 9) and their relatively loose geometrical constraints compared to those of the transition-metal ions.

**RNA Cleavage.** Many metal salts studied here have been shown to promote RNA cleavage by transesterification. Comparison of the relative abilities of metal ions to cleave RNA is complicated by the heterogeneous nature of many of the cleavage reactions. In addition, metal ion promoted RNA cleavage is dependent on the nitrogenous base sequence of RNA,<sup>20a,21b</sup> although the base-sequence selectivity of cleavage is not high. Kinetic data for metal ion promoted RNA cleavage, although not as complete as here, show similar trends in the relative abilities of metal ions as catalysts. For example, first-order rate constants for cleavage of UpUp(3') at 62.1 °C in solutions  $5 \times 10^{-4}$  M in  $\text{Zn}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Co}^{2+}$ , and  $\text{Ni}^{2+}$  are 207, 107, 7.2, and  $4.6 \text{ h}^{-1}$ , respectively.<sup>21b</sup> In addition, the extent cleavage of polyadenylic acid by  $\text{Mn}^{2+}$  at 64 °C is similar<sup>21a</sup> to that of  $\text{Ni}^{2+}$  or  $\text{Co}^{2+}$ , and the extent cleavage at 37 °C of oligoribonucleotides by metal ions follows the order  $\text{Zn}^{2+} > \text{Cu}^{2+} \gg \text{Ni}^{2+}$ .<sup>19</sup> The difference in the observed order for  $\text{Cu}^{2+}$ -promoted RNA cleavage compared to  $\text{Cu}^{2+}$ -promoted cleavage of **1** may occur because of the insolubility of many  $\text{Cu}^{2+}$  polynucleotide complexes or copper(II) hydroxide species; low concentrations of  $\text{Cu}^{2+}$  were used with **1** to avoid the formation of precipitates. All of the trivalent lanthanides, with the exception of  $\text{Pm}^{3+}$ , which was not examined, and  $\text{Pb}^{2+}$  cleave dinucleotides of RNA at 37 °C much more rapidly<sup>42</sup> than does  $\text{Zn}^{2+}$ . Similarly, phenylalanine transfer RNA (t-RNA<sup>phe</sup>) is cleaved more rapidly<sup>12</sup> at 50 °C by  $\text{Pb}^{2+}$ ,  $\text{Eu}^{3+}$ , or  $\text{La}^{3+}$  than by  $\text{Zn}^{2+}$  or  $\text{Cu}^{2+}$ ,<sup>13</sup> and cleavage of t-RNA<sup>phe</sup> by  $\text{Mg}^{2+}$  occurs only at alkaline pH.<sup>15</sup>

Most RNA enzymes and self-cleaving or self-splicing RNAs have a requirement for metal cations;<sup>6-9</sup> intermolecular or intramolecular transesterification of a phosphate ester as well as hydrolysis of a phosphate ester may be involved. Several types

of small RNAs derived from plant viral satellite RNAs undergo site-specific self-cleavage; cleavage occurs by intramolecular attack of the 2'-hydroxyl group of ribose to form a 2',3'-cyclic phosphate diester in a reaction similar to the one described in this paper. In these self-cleaving RNAs, metal ions may perform structural roles or participate in active-site chemistry. Metal ions in catalytic roles presumably bind to a pocket formed by several nucleotide residues in a site proximal to the phosphate ester undergoing nucleophilic attack.  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{Pb}^{2+}$  meet metal ion requirements in the self-cleavage of small satellite RNAs.<sup>9</sup> In other self-cleaving RNAs,<sup>8</sup> metal ions other than  $\text{Mg}^{2+}$  or  $\text{Mn}^{2+}$ , such as  $\text{Zn}^{2+}$ , do not form functional self-cleaving RNAs. Correct binding and orientation of the metal ion in the active site are crucial to these RNA self-cleavage reactions, and the coordination properties of  $\text{Zn}^{2+}$  may not meet requirements in the latter case. In this context, it is interesting that the site-specific cleavage of t-RNA<sup>phe</sup> by metal ions, a reaction that bears a resemblance to the self-cleavage of RNAs, is promoted by a large number of metal ions including  $\text{Mg}^{2+}$ ,<sup>15</sup>  $\text{Pb}^{2+}$ ,<sup>10,11</sup>  $\text{La}^{3+}$ ,<sup>13</sup>  $\text{Eu}^{3+}$ ,<sup>12</sup>  $\text{Zn}^{2+}$ ,<sup>12</sup>  $\text{Fe}^{2+}$ ,<sup>14</sup> and  $\text{Cu}^{2+}$ .<sup>13</sup> In t-RNA cleavage, a metal-binding pocket adjacent to the nucleotide being cleaved may be important or, alternatively, the conformational flexibility of the region of RNA being cleaved may be an important factor in the observed specificity of cleavage. The relative rates of t-RNA cleavage with metal ions ( $\text{Eu}^{3+} > \text{Pb}^{2+} \gg \text{Zn}^{2+} \gg \text{Mg}^{2+}$ ) mirror those of our model studies here, and similar mechanistic considerations may be appropriate. Once the metal is bound in the active site, it may promote cleavage by electrophilic mechanisms, and those metals that are good Lewis acids should promote most rapid cleavage. As in the transesterification of **1**, a hypervalent phosphorus intermediate may be stabilized by complexation to the metal ion, perhaps in a bidentate fashion. Stabilization of this intermediate and the facilitation of its breakdown to products may accelerate RNA cleavage. Alternatively, if a metal hydroxide is available, it may act as a general base to deprotonate the 2'-hydroxyl; this has been proposed to occur<sup>10</sup> in t-RNA cleavage by  $\text{Pb}^{2+}$ . As we have noted,  $\text{Pb}^{2+}$  and  $\text{Cu}^{2+}$  are probably the only divalent metals that will be present in hydroxide form at neutral pH, although nucleotide ligands bound to the metal ion may certainly influence the  $pK_a$  of remaining water ligands.

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**Registry No.** **1**, 87174-80-9;  $\text{TbCl}_3$ , 10042-88-3;  $\text{EuCl}_3$ , 10025-76-0;  $\text{YbCl}_3$ , 10361-91-8;  $\text{LuCl}_3$ , 10099-66-8;  $\text{GdCl}_3$ , 10138-52-0;  $\text{NdCl}_3$ , 10024-93-8;  $\text{LaCl}_3$ , 10099-58-8;  $\text{Zn}(\text{NO}_3)_2$ , 7779-88-6;  $\text{Cu}(\text{NO}_3)_2$ , 3251-23-8;  $\text{Pb}(\text{NO}_3)_2$ , 10099-74-8;  $\text{Mn}(\text{NO}_3)_2$ , 10377-66-9;  $\text{Ni}(\text{NO}_3)_2$ , 13138-45-9;  $\text{Co}(\text{NO}_3)_2$ , 10141-05-6;  $\text{Mg}(\text{NO}_3)_2$ , 10377-60-3;  $\text{CaCl}_2$ , 10043-52-4.

(41) Morrow, J. R.; Perri, A. J. *Inorg. Biochem.* **1989**, *361*, Abs. K013.  
 (42) Shelton, V. M.; Morrow, J. R. Manuscript in preparation.