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Metal-Catalyzed Intramolecular Hydrolysis of Phosphate Esters

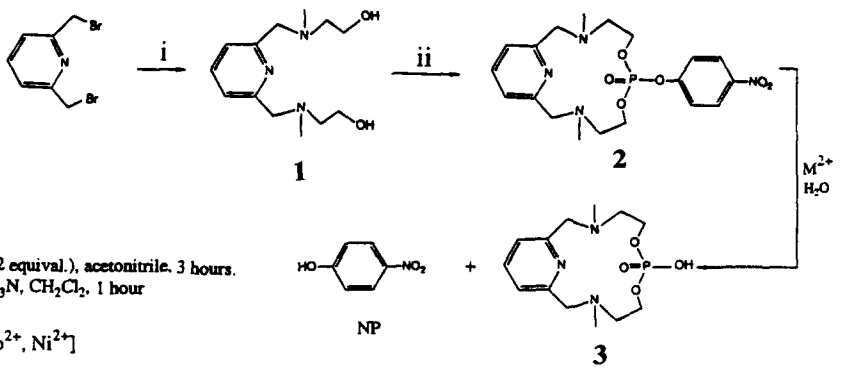
Ismail O. Kady* and Biao Tan

Department of Chemistry, East Tennessee State University, Johnson City, TN 37614-0695, USA

Abstract: A tridentate water-soluble phosphotriester ligand has been synthesized. Kinetics studies have shown that divalent metal ions dramatically enhance the rate of hydrolysis of such phosphotriester at 25 °C and pH 8.0 via intramolecular nucleophilic attack by a metal bound hydroxide.

Acceleration of enzymic reactions can be attributed to specific effects such as entropic advantage, transition-state binding, and chemical catalysis by neighboring groups.[1] The study of simple model systems has served to probe the relative importance of such effects and as a test of perceived insight into a particular mechanism.[2] Many of the enzymes that catalyze the hydrolysis of phosphate esters or phosphoryl group transfer reactions require divalent metal ions for activation.[3-5] Although there have been numerous reports on phosphate esterase model systems that addressed the role of metal ions in the hydrolysis of phosphomonoesters and phosphodiesters,[6-10] surprisingly only few model systems addressed hydrolysis of neutral phosphotriesters.[11-13] As part of our investigation of the effect of neighboring metal complexes on the rate of hydrolysis of phosphotriesters, we wish to report the synthesis of a water soluble phosphotriester **2** in which a dramatic rate acceleration of hydrolysis by metal ions has been observed. In such model systems, metal ions are strongly chelated to the tridentate amine ligand and believed to initiate nucleophilic attack at phosphorus by a metal-coordinated water molecule or hydroxy group, resulting in rate enhancements of 10^5-10^6 folds relative to uncatalyzed reactions.

Scheme 1



i) $CH_3NHCH_2CH_2OH$ (2 equival.), acetonitrile, 3 hours.
ii) $OPCl_2(OPh\text{-}NO_2)$, Et_3N, CH_2Cl_2, 1 hour

[M^{2+} = Zn^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}]

Phosphotriester **2** was synthesized from 2,6-bis-(bromomethyl)pyridine upon treatment with two equivalents of 2-(methylamino)ethanol in anhydrous acetonitrile to produce a clear oil 2,6-bis-[(2-hydroxyethyl)methylamino-methyl]pyridine **1**. Compound **1** was converted into the desired cyclic phosphotriester upon treatment with one equivalent of 4-nitrophenylphosphoro dichloridate in anhydrous methylene chloride under high dilution conditions (Scheme 1). Analytical and spectral data for compounds **1** and **2** are consistent with the assigned structures.[14]

Formation constants for the 1:1 complexes of **2** with divalent metal ions were calculated according to the method of Pjerrum[15] and are presented in Table I. Thermodynamic deprotonation constants of metal-bound water, $pK_a(H_2O)$, were determined by potentiometric pH-titration of the metal complexes.

Table I. Formation Constants for Metal Complexes of **2** at 25 °C, and Observed (and Relative) Rate Constants for the Hydrolysis of **2**-M at 25 °C and pH 8.0, $\mu = 0.2$ ($NaClO_4$).

Metal	K_f (M^{-1})[a]	$pK_a(H_2O)$[b]	k_{obs} (s^{-1})	k_{rel}
None	----	---	2.4×10^{-9}	1.0
Zn^{2+}	2.5×10^{5}	8.1	1.5×10^{-3}	6.3×10^{5}
Cu^{2+}	2.9×10^{9}	7.8	1.8×10^{-2}	7.5×10^{6}
Co^{2+}	1.3×10^{7}	7.5	1.1×10^{-3}	4.6×10^{5}
Ni^{2+}	1.9×10^{6}	8.3	1.7×10^{-3}	7.0×10^{5}

[a] Determined at pH 8.0 and [metal] = [Ligand] = 1 mM.
[b] pK_a for the reaction: **2**-M(H_2O) $\rightleftharpoons$ **2**-M(OH) + H^+.

Rate enhancement of the hydrolysis of **2** by Zn^{2+}, Cu^{2+}, Co^{2+}, and Ni^{2+} was studied at 25 °C and pH 8.0. The reaction progress was monitored by following the changes in absorbance at 405 nm (λmax of 4-nitrophenolate anion, NP^-). A typical hydrolysis procedure involves immediate monitoring of the absorbance after rapid injection of 1.5 mL of 0.02 mM aqueous solution of **2** into 1.5 mL of HEPES buffer solution containing 0.02 mM of the metal ion (the ionic strength was maintained at 0.2 with $NaClO_4$). Reference experiments were carried out under identical conditions except in the absence of metal ions. Reactions were generally followed to more than 90% completion. Based on ^{31}P NMR spectroscopy, subsequent hydrolysis of the produced phosphodiester **3** was too slow to be detected under these conditions. Observed rate constants (k_{obs}) of the hydrolysis reactions were determined by the log plot method with correlation coefficients of >0.99. Kinetics results are summarized in Table I. Possibility

of hydrolysis of the phosphotriester by uncomplexed metal ions was ruled out since under similar reaction conditions free metal ions failed to hydrolyze diethyl(4-nitrophenyl)phosphate, an analogous phosphotriester lacking the tridentate ligand. Moreover, intermolecular catalysis of diethyl(4-nitrophenyl)phosphate by metal complexes of 2,6-bis-(aminomethyl)pyridine have shown only 10^2-10^3 fold enhancements over uncatalyzed hydrolysis.[16]

pH-Dependence of the hydrolysis reactions was studied over a pH range of 6.5-9.0. All reactions displayed sigmoidal pH-rate profiles with inflection points around the $pk_a(H_2O)$ of the metal complex, of the type observed in a number of phosphate ester hydrolyses promoted by metal complexes.[17] Such profiles are indicative of the involvement of a metal-hydroxy intermediate in the rate determining step.

The results of this work have shown that (1) dramatic rate enhancements of phosphate ester hydrolysis (10^5-10^6) are observed in metal complexes in which the metal is forced to lie in the vicinity of the phosphate group; (2) the kinetically reactive species is the metal-hydroxide complex. These observations should be considered in the design of future model systems.

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References and Notes:

1. Lipscomb, W. N. *Acc. Chem. Res.* **1982**, 15, 232-238.
2. Breslow, R. In *Bioorganic Chemistry*; Gould, R. F., Ed.; American Chemical Society: Washington, D.C., 1971; Advanced Chemical Series No. 100, Chapter 2.
3. Benkovic, S. J.; Schray, K. J. In *The Enzymes*; Boyer, P. D.,Ed.; Academic Press: New York, 1973; Vol. 8, p 201.
4. (a) Spiro, T. G. In *Inorganic Biochemistry*; Eichhorn, G. L., Ed.; American Elsevier: New York, 1973; p 549. (b) Coleman, J. E.; Gettins, P. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1983**, 55, 381.
5. O'Sullivan, W. J. In *Inorganic Biochemistry*; Eichhorn, G. L., Ed.; American Elsevier: New York, 1973; p 582.
6. (a) Tafesse, F.; Massoud, S. S.; Milburn, R. M. *Inorg. Chem.* **1985**, 24, 2591. (b) Chin, J.; Banaszczyk, M.; Jubian, V.; Zou, Z. *J. Am. Chem. Soc.* **1989**, 111, 186. (c) Chung, Y.; Akkaya, E. U.; Venkata-chalam, T. K.; Czarnik, A. W. *Tetrahedron Lett.* **1990**, 31, 5413.
7. Hendry, P.; Sargeson, A. M. *Inorg. Chem.* **1990**, 29, 92.
8. Fife, T. H.; Pujari, M. P. *J. Am. Chem. Soc.* **1990**, 112, 5551.
9. Rosch, M. A. D.; Trogler, W. C. *Inorg. Chem.* **1990**, 29, 2409
10. Breslow, R.; Huang, D. *J. Am. Chem. Soc.* **1990**, 112, 3686.
11. Gellman, S. H.; Breslow, R. *J. Am. Chem. Soc.* **1986**, 108, 2388.
12. Norman, P. R.; Tate, A.; Rich, P. *Inorg. Chem. Acta* **1988**, 145, 211
13. Tohru, K.; Kimura, E. *J. Am. Chem. Soc.* **1991**, 113, 8935.

14. Data for compound **1**: colorless oil; MS: (EI) m/z 253 (M^+,17), 207 (100), 147 (15), 135 (15), 79 (74), 73 (88); IR (film): 3100-3500 (broad), 3050, 2800, 1600, 1500, 1250, 1150 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.30 (s, 6H), 2.55 (t, 4H), 3.60 (t, 4H), 3.70 (s, 4H), 4.80 (s, 2H), 7.10-7.40 (m, 3H); ^{13}C NMR ($CDCl_3$): δ 42.2, 58.6, 62.0, 62.7, 121.0, 136.6, 157.8. Anal. Calcd (found) for $C_{13}H_{23}N_3O_2$: C, 61.62 (61.48); H, 9.17 (9.22); N, 16.59 (16.51).
Data for compound **2**: colorless oil; MS: (EI) m/z 436 (M^+, 4), 298 (21), 273 (15), 207 (100), 147 (27), 138 (74), 135 (22); IR (film): 3050, 2800, 1600, 1550, 1320, 1260, 1180, 1160 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.40 (s, 6H), 2.60 (t, 4H), 3.80 (s, 4H), 4.40 (t, 4H), 7.10-7.30(m, 3H), 7.40 (d, 2H),8.25 (d, 2H); ^{13}C NMR ($CDCl_3$): δ 43.3, 60.0, 63.5, 65.0, 123.0, 124.9, 127.5, 137.5, 158.4, 162.3, 166.7; ^{31}P NMR ($CDCl_3$): δ -7.1. Anal. Calcd (found) for $C_{19}H_{25}N_4O_6P$: C, 52.28 (52.25); H, 5.79 (5.82); N, 12.84 (12.81).

15. (a) Hartley, F. R.; Burgess, C.; Alcock, R. M.in *Solution Equilibria*; Ellis Horwood Limited: Chichester, West Sussex, England, 1980. (b) Martell, A. E.; Calvin, N. in *Chemistry of Metal Chelate Compounds*; Prentice-Hall: New York, 1952, p 78.

16. Kady, I.; Tan, B.; Ho, Z.; Scarborough, T. *J. Chem. Soc., Chem. Commun.*, in press.

17. (a) Koike, T.; Kimura, E. *J. Am. Chem. Soc.* **1991**, 113, 8935.
(b) Burstyn, J.; Deal, K. A. *Inorg. Chem.* **1993**, 32, 3585.
(c) Raivji, G. H.; Milburn, R. M. *Inorg. Chim. Acta* **1988**, 150, 227.
(d) Fife, T. H.; Pujari, M, P. *J. Am. Chem. Soc.* **1988**, 110, 7790.

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