

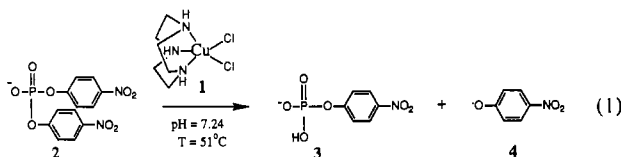
Selective Catalytic Hydrolysis of a Simple Phosphodiester by a Macrocyclic Copper(II) Complex

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Nucleases catalyze hydrolytic cleavage of the phosphodiester backbone of DNA and RNA, remarkable chemistry given the stability of the phosphodiester bond.¹ Even the activated phosphodiester bis(*p*-nitrophenyl) phosphate (**2**) has a half-life measured in years at pH 7.² There has been interest in the development of chemical nucleases,³ reagents that can recognize and cleave specific nucleic acid structures or sequences. A number of reagents have been reported to cleave DNA oxidatively,³ but these are not practical for construction of recombinant DNA molecules. Recently, considerable progress has been made in the study of metal complexes that promote phosphodiester hydrolysis. Significant rate enhancements in hydrolysis of model phosphodiester have been reported using a variety of metal complexes.⁴ Metal-promoted transesterification has received attention in light of the discovery of catalytic RNA and the need for antiviral therapy.⁵ Catalytic transesterification of simple RNA molecules has been reported,⁶ the first example of turnover observed in the cleavage of biological phosphodiester by nonenzymic catalysts. We report the selective catalytic hydrolysis of the phosphodiester **2** to the phosphomonoester **3** by a catalyst derived from Cu(9-aneN₃)Cl₂ (**1**), at neutral pH (reaction 1).



The hydrolysis of **2** in the presence of **1** is catalytic as confirmed by our observation of both rate enhancement and turnover.⁷ A rate enhancement was calculated by comparing the observed pseudo-first-order rate constant for the disappearance of **2** in the presence of the metal complex with that in the absence of metal complex, measured under identical conditions.⁸ The rate enhancement factors observed for the metal complex catalyzed reaction are significant, 10³ over the uncatalyzed reaction for 1 mM **1**. Turnover was demonstrated in the release of a greater than stoichiometric amount of product.⁹ For these reactions, a

turnover constitutes the release of 1 mol of **4**/mol of **2** added, as shown in reaction 1, and demonstrated experimentally as described below.

Analysis by reverse phase HPLC demonstrated that the products of the catalytic hydrolysis are **3** and **4**, suggesting that the hydrolysis reaction was selective for the cleavage of the phosphodiester. When a catalytic reaction was monitored over a long period by HPLC, the only peaks observed were the products **3** and **4**, eluting at 1.4 and 3.5 min, respectively, and the unreacted substrate, **2**, eluting at 4.3 min.¹⁰ The identity of the products was confirmed by co-injection of authentic standards. Both products were detectable at short reaction times, and the intensities of the two peaks grew at approximately the same rate over the entire reaction period. The rate of increase in the peak due to **3** did not slow or decrease relative to that of **4**, indicating that the monoester was not hydrolyzed under the reaction conditions. When **3** was used as a substrate in the reaction with **1**, the rate of hydrolysis was 17 times slower than with **2**.¹¹ This selectivity in the hydrolysis of phosphate esters is remarkable in light of previous observations. The hydrolysis of phosphate monoesters is generally much faster than that of diesters, and metal ions are known to promote both phosphate monoester and diester hydrolysis.¹² One therefore expects hydrolysis of both the phosphate monoester and diester to be accelerated in the presence of a metal complex, with the monoester being hydrolyzed more rapidly than the diester, as is observed in the case of Co(III)-promoted phosphoester hydrolysis.^{5a} On the other hand, the rate of metal-catalyzed phosphodiester hydrolysis exceeded the rate of metal-catalyzed monoester hydrolysis when Cu(bipy)²⁺ was used as a

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- (7) (a) The synthesis of **1** was as described. Schwindinger, W. F.; Fawcett, T. G.; Lalancette, R. A.; Potenza, J. A.; Schugar, H. J. *Inorg. Chem.* **1980**, *19*, 1379–1381. (b) Anal. Calcd for C₆H₁₅N₃Cl₂Cu: C, 27.33; H, 5.73; N, 15.94; Cl, 26.89. Found: C, 27.17; H, 5.96; N, 15.79; Cl, 27.25. (c) Single crystal X-ray diffraction is in agreement with the structure previously published in ref 7a. (d) Hydrolysis reactions were carried out at 51 °C in sealed glass cells and the production of **4** was monitored by an increase in absorbance at 400 nm in the heated cell. No other spectral changes were observed. Concentrations of **4** were determined by direct difference relative to a reference sample identical in all respects except lacking **1**. The concentration of **4** released was calculated from the extinction coefficient at 400 nm, 18 700 L mol⁻¹ cm⁻¹, with the absorbance corrected for pH. The pK_a of **4** was corrected for temperature using data from: Martell, A. E.; Smith, R. M. *Critical Stability Constants*; Plenum Press: New York, 1977; Vol. 3, p 183.
- (8) (a) Hydrolysis reactions were carried out with 5.00 mM **2** (sodium salt) as substrate and varying concentrations of **1**. The pH was maintained with HEPES (*N*-[2-hydroxyethyl]piperazine-*N'*-ethanesulfonic acid) buffer (0.050 M), pH = 7.24 ± 0.02, μ = 33 ± 0.7 mM, and the temperature was 51.0 ± 0.2 °C, stabilized by a sand bath. The total reaction time was 10 days. (b) The contribution of HEPES to the ionic strength at this temperature and pH is 55% of the molar concentration. For an explanation of the contribution of zwitterion buffers to ionic strength, see: Perrin, D. D.; Dempsey, B. *Buffers for pH and Metal Ion Control*; John Wiley & Sons: New York, 1974.
- (9) Turnovers of 3.2, 5.0, 7.5, and 10.2 were measured for [1] = 1.03, 0.515, 0.206, and 0.102 mM, respectively.
- (10) Aliquots were analyzed by reversed phase HPLC on a Alltech Econosphere C18 column (150 mm, 5 μm) using a mobile phase containing 50% methanol/50% 50 mM phosphate buffer, pH 7. Detection was by UV absorbance at 254 nm.
- (11) Analysis was by the method of initial rates, usually at less than 5% conversion of **2** to products. At the highest concentration of **1**, the conversion was 8%. All reactions were carried out at pH 7.24 ± 0.02, μ = 60 ± 5 mM, and 51 ± 0.2 °C with HEPES buffer (0.1 M) used to maintain pH and ionic strength. For **3**: initial rate, v = 4.5 × 10⁻¹¹ M s⁻¹, [1] = 2.42 mM, [3] = 0.1 mM; for **2**: initial rate, v = 7.5 × 10⁻¹⁰ M s⁻¹, [1] = 2.55 mM, [2] = 0.1 mM.
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catalyst for hydrolysis of **2** and **3**.^{5b} In the presence of $\text{Cu}(\text{bipy})^{2+}$, there was only a 3-fold difference in the observed rate constants, in contrast to the 17-fold difference observed in the presence of **1**.

The dependence of the rate constant on the concentration of **1** is consistent with catalysis by a monomeric copper complex that is in equilibrium with a dimer. First-order plots of the initial rate versus the catalyst concentration are nonlinear, with the rate decreasing with increasing concentration of catalyst. When the initial rates are plotted versus the $[\mathbf{1}]^{1/2}$, a linear plot is obtained (Figure 1). The observation of a half-order dependence on the metal complex concentration demonstrates that it is the monomer that is the hydrolysis catalyst.¹³ The presence of a monomer-dimer equilibrium in aqueous solution is consistent with the known chemistry of this and other $\text{Cu}(\text{II})$ complexes. The bis(μ -hydroxo) dimer of **1**, $(\text{Cu}[\mathbf{9}]\text{aneN}_3)_2(\text{OH})_2$, has been previously characterized.¹⁴

In summary, a catalyst derived from the well-characterized metal complex has been observed to catalytically hydrolyze phosphodiester. The reaction is selective for hydrolysis of the diester over the monoester and exhibits both turnover and rate enhancement. The monomer is the source of the active catalyst, but it is in equilibrium with an inactive dimer as indicated by the half-order dependence on the concentration of **1**. This system is ideal for mechanistic studies of phosphodiester hydrolysis, and further studies are presently underway to characterize the reaction

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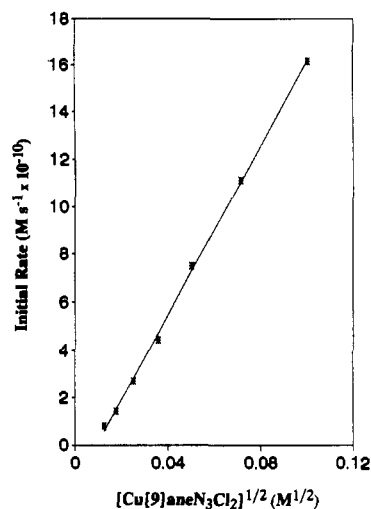


Figure 1. Plot of initial rate of hydrolysis versus $[\text{Cu}([\mathbf{9}]\text{aneN}_3)\text{Cl}_2]^{1/2}$. All points are the average of three determinations with errors <5%. Reactions were carried out with 0.1 mM **2**. All other conditions were identical to those described in ref 11. The correlation coefficient of the regressed line is 0.9976.

in detail. Evaluation of the potential application of this complex in the hydrolysis of biological phosphodiester is also in progress.

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Supplementary Material Available: A table of rate constants (1 page). Ordering information is given on any current masthead page.