for their comments. We thank Scott Buckingham for experimental assistance.

**Supplementary Material Available:** ORTEP illustrations of disorder in pyrazine moieties and solvent molecules and tables of crystallographic parameters, calculated atomic coordinates, anisotropic displacement parameters, and peripheral bond distances and angles (8 pages); a listing of observed and calculated structure factors for $Cu_2(NBA)_2(\mu\text{-}2,5\text{-}Me_2pyz)\cdot 4CH_2Cl_2$ (9 pages). Ordering information is given on any current masthead page.

Contribution from the Department of Chemistry, D-006, University of California at San Diego, La Jolla, California 92093

# Hydrolysis of Phosphodiesters with Ni(II), Cu(II), Zn(II), Pd(II), and Pt(II) Complexes

Mark A. De Rosch and William C. Trogler*

*Received January 6, 1989*

The hydrolysis of bis(4-nitrophenyl) phosphate (**1**) is catalyzed by $Ni(tren)^{2+}$ in aqueous solution at 75 °C. The activity of the catalyst remains constant for 85 turnovers and thereafter decreases. Antitumor complexes of Pd(II) and Pt(II) were also examined but did not show turnover in the hydrolysis of ethyl 4-nitrophenyl phosphate (**2**). Catalytic rate enhancement in the hydrolysis of **1** by $Ni(tren)(OH)(OH_2)^+$ was 1200 at pH 8.6 and of **2** by $1 \times 10^{-4}$ M $Pd(bpy)^{2+}$ was 49 at pH 6.0 over spontaneous hydrolysis under the same conditions. The pH–rate profile of $Ni(tren)^{2+}$-catalyzed hydrolysis of **1** shows a pH-dependent region from pH 8.0 to pH 10.8 and a pH-independent region from pH 6.0 to pH 8.0. Ni(II) complexes of tren and bpy were compared to their corresponding Cu(II) and Zn(II) analogues. The pH–rate profile of the Pd(II)- and Pt(II)-accelerated hydrolysis of **2** shows a pH dependence from pH 6.0 to pH 7.5. The rate enhancement becomes negligible with respect to spontaneous hydrolysis at alkaline pH, which is attributed to the formation of hydroxy-bridged polymers. A mechanism involving intramolecular hydroxide attack on a metal-bound phosphate is proposed. Of the $M(bpy)^{2+}$ and $M(tren)^{2+}$ complexes examined ($M = Ni^{2+}$, $Cu^{2+}$, $Zn^{2+}$) only the $Cu(bpy)^{2+}$ complex was effective in nicking supercoiled plasmid DNA. The inhibition of DNA nicking by $Ce^{4+}$ for the latter complex suggests that nicking occurs by a redox process rather than by hydrolysis.

## Introduction

The development of artificial nucleases for use in molecular genetics and genetic engineering remains a challenging research problem because of the stability of the phosphate diester backbone and its resistivity to hydrolytic cleavage. A majority of the efforts have concentrated on the development of sequence-specific DNA binding agents attached to Fenton reagent analogues for nicking DNA.[1,2] Fenton-like systems cut DNA through production of hydroxide radicals by a proposed mechanism that involves oxidation of the deoxyribose moiety followed by breakage of the sugar–phosphate backbone.[3] Complexes that hydrolytically cleave DNA would be the preferred method for DNA cleavage, and ideally they should be catalytic. This problem reduces to effecting the hydrolytic cleavage of phosphodiesters. Recently the hydrolytic cleavage of supercoiled plasmid DNA has been reported using $Cu^{2+}$, $Zn^{2+}$, $Cd^{2+}$, and $Pb^{2+}$ complexed to a DNA-binding ruthenium(II) tris(phenanthroline) derivative.[4] Nonredox-active metals such as Ni(II) and Zn(II) are potentially of interest as hydrolytic cleaving agents, and their reactivity in model systems may lead to functional DNA cleaving molecules.

Two reports exist of turnover in the hydrolysis of phosphate diesters. One is the $Co(tme)_2(OH)(OH_2)^{2+}$ catalyzed hydrolysis of ethyl 4-nitrophenyl methylphosphonate, which is complicated by rapid reactions of the Co(III) complex with $CO_2$. This results in a short-lived catalyst.[5] The second is the $Cu(bpy)(OH)(OH_2)^+$-catalyzed hydrolysis of bis(4-nitrophenyl) phosphate to greater than 1000 turnovers.[6] Previous examples of metal-catalyzed hydrolysis of phosphate diesters were limited to substrates that contain a neighboring group, which participates in the hydrolysis.[7,8] Metallic[9] and nonmetallic[10] micelles also accelerate the rate of hydrolysis of simple phosphate diesters. Because the presently available catalysts exhibit rates several orders of magnitude too slow to be useful in DNA hydrolysis,[11] a better understanding of mechanistic features might be helpful in designing more effective systems.

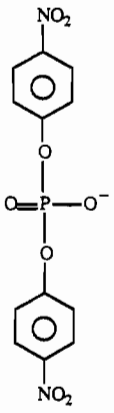
Many reports exist in the literature of metal ion promoted hydrolysis of phosphate monoesters[12–14] and triesters.[15–17] A majority of these reports are based on the hydrolysis of phosphate mono- and triesters by polyamine Co(III),[12,13] Ir(III),[15] and Rh(III)[15a] ions as the active metal centers as either monomers or dimers. Accounts also exist of a macrocyclic Zn(II) complex catalyzed hydrolysis of diphenyl 4-nitrophenyl phosphate by an intramolecular mechanism.[16] Additionally, diamine Zn(II) and diamine Cu(II) complexes have been shown to catalyze the hydrolysis of tris(4-nitrophenyl) phosphate.[17] A problem that hinders

(1) (a) Sugiyama, H.; Xu, C.; Murugesan, N.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *Biochemistry* **1988**, *27*, 58–67. (b) Baker, B. F.; Dervan, P. B. *J. Am. Chem. Soc.* **1985**, *107*, 8266–8268. (c) Taylor, J. S.; Schultz, P. G.; Dervan, P. B. *Tetrahedron* **1984**, *40*, 457–465. (d) Schultz, P. G.; Taylor, J. S.; Dervan, P. B. *J. Am. Chem. Soc.* **1982**, *104*, 6861–6863. (e) Hertzberg, R. P.; Dervan, P. B. *Biochemistry* **1984**, *23*, 3934–3945.
(2) (a) Baker, B. F.; Dervan, P. B. *J. Am. Chem. Soc.* **1989**, *111*, 2700–2712. (b) Veal, J. M.; Rill, R. L. *Biochemistry* **1989**, *28*, 3243–3250.
(3) Marfey, P.; Robinson, E. *Mutat. Res.* **1981**, *86*, 155–191.
(4) Basile, L. A.; Raphael, A. L.; Barton, J. K. *J. Am. Chem. Soc.* **1987**, *109*, 7550–7551.
(5) Kenley, R. A.; Fleming, R. H.; Laine, R. M.; Tse, D.; Winterle, J. S. *Inorg. Chem.* **1984**, *23*, 1870–1876.
(6) Morrow, J. R.; Trogler, W. C. *Inorg. Chem.* **1988**, *27*, 3387–3394.
(7) (a) Chin, J.; Zou, X. *Can. J. Chem.* **1987**, *65*, 1882. (b) Steffens, J. J.; Siewers, I. J.; Benkovic, S. J. *Biochemistry* **1975**, *14*, 2431–2440.
(8) (a) Eichhorn, G. L.; Tarien, E.; Butzow, J. J. *Biochemistry* **1971**, *10*, 2014–2018. (b) Butzow, J. J.; Eichhorn, G. L. *Biochemistry* **1971**, *10*, 2019–2027. (c) Ikenga, H.; Inoue, Y. *Biochemistry* **1974**, *13*, 577–582.
(9) Menger, F. M.; Gan, L. H.; Johnson, E.; Durst, D. M. *J. Am. Chem. Soc.* **1987**, *109*, 2800–2803.
(10) (a) Bunton, C. A.; Ionescu, L. G. *J. Am. Chem. Soc.* **1973**, *95*, 2912–2917. (b) Buist, G. J.; Bunton, C. A.; Robinson, L.; Sepulveda, L.; Stam, M. *J. Am. Chem. Soc.* **1970**, *92*, 4073–4078.
(11) Chin, J.; Banaszczyk, M.; Jubian, V.; Zou, X. *J. Am. Chem. Soc.* **1989**, *111*, 186–190.
(12) (a) Rawji, C. H.; Milburn, R. M. *Inorg. Chim. Acta* **1988**, *150*, 227–232. (b) Jones, D. R.; Lindoy, L. F.; Sargeson, A. M. *J. Am. Chem. Soc.* **1984**, *106*, 7807–7819. (c) Jones, D. R.; Lindoy, L. F.; Sargeson, A. M. *J. Am. Chem. Soc.* **1983**, *105*, 7327–7336. (d) Anderson, B.; Milburn, R. M.; Harrowfield, J. Mac B.; Robertson, G. B.; Sargeson, A. M. *J. Am. Chem. Soc.* **1977**, *99*, 2652–2661.
(13) Harrowfield, J. Mac B.; Jones, D. R.; Lindoy, L. F.; Sargeson, A. M. *J. Am. Chem. Soc.* **1980**, *102*, 7733–7741.
(14) Herschlag, D.; Jencks, W. P. *J. Am. Chem. Soc.* **1987**, *109*, 4665–4674.
(15) (a) Hendry, P.; Sargeson, A. M. *Aust. J. Chem.* **1986**, *39*, 1177–1186. (b) Hendry, P.; Sargeson, A. M. *J. Chem. Soc., Chem. Commun.* **1984**, 164–165.
(16) (a) Breslow, R.; Singh, H. *Bioorg. Chem.* **1988**, *16*, 408–417. (b) Gellman, S. H.; Petter, R.; Breslow, R. *J. Am. Chem. Soc.* **1986**, *108*, 2388–2394.
(17) Clewly, R. G.; Slebocka-Jilk, H.; Brown, R. S. *Inorg. Chim. Acta* **1989**, *157*, 233–238.

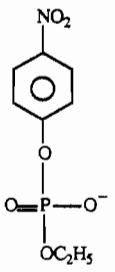
0020-1669/90/1329-2409$02.50/0 

studies using Zn(II) complexes is precipitation of zinc hydroxo species at alkaline pH.

We present here kinetic studies of the catalytic hydrolysis of bis(4-nitrophenyl) phosphate (**1**) by $Ni(tren)^{2+}$ in aqueous solution and show that other Ni(II) complexes also accelerate the hydrolysis of **1**. The effectiveness of $Ni(tren)^{2+}$ and $Ni(bpy)^{2+}$ is compared



**1**



**2**

to that of their Cu(II) and Zn(II) analogues. To our knowledge this is the first example of such catalysis by Ni(II). The effect of Pt(II) and Pd(II) antitumor complexes on the hydrolysis of phosphate diester **2** is also discussed.

## Experimental Section

Disodium methyl phosphate (Sigma), disodium 4-nitrophenyl phosphate (Sigma), bis(4-nitrophenyl) phosphate (free acid, Sigma), reagent grade inorganic salts, 2,2′-bipyridine (bpy, Aldrich), and Sigma biological buffers MES (*N*-morpholineethanesulfonic acid), HEPES (*N*-(2-hydroxyethyl)piperazine-*N*′-ethanesulfonic acid), EPPS (*N*-(2-hydroxyethyl)piperazine-*N*′-propanesulfonic acid), CHES (2-(cyclohexylamino)ethanesulfonic acid), and CAPS (3-(cyclohexylamino)-1-propanesulfonic acid) were purchased from commercial sources and used without purification. The 4-nitrophenyl diethyl phosphate was prepared by a literature method[18a] and distilled in vacuo (~1 Torr at 140–142 °C). Lithium 4-nitrophenyl ethyl phosphate[18b] was prepared from the triester by treating an acetone solution with lithium chloride and refluxing the solution overnight. Addition of a 1:2 mixture of hexanes and diethyl ether to the cooled acetone solution yielded the solid diester on standing overnight. The lithium salt was recrystallized from ethanol–acetone.[6] Bis(4-nitrophenyl) phosphate was recrystallized from ethanol–water. Amine ligands 1,2-diaminoethane (en) and tris(2-aminoethyl)amine (tren) were purchased from Aldrich Chemical Co. and vacuum-distilled before use. All other ligands used were purchased from Aldrich and used without further purification. $Ni([15]aneN_4)(NO_3)_2$ was synthesized according to literature methods by the procedure reported for $Ni([14]aneN_4)(ClO_4)_2$.[19] $Pd(bpy)Cl_2$,[20a] $Pd(en)Cl_2$,[20b] $Pt(en)Cl_2$,[20c] and *cis*-$Pt(NH_3)_2Cl_2$[20d] were prepared by literature methods, and cationic species were generated in solution by addition of $AgNO_3$. Fisher HPLC grade water was used for all solutions.

The concentration of phosphate diester in stock solutions was determined by spectrophotometric measurement of the 4-nitrophenolate released on complete acid hydrolysis, followed by basification to pH > 10. The concentration of $Ni^{2+}$ was determined by titration against ethylenediaminetetraacetic acid with murexide as an indicator.[21] Reaction solutions were prepared by combining appropriate amounts of metal, ligand, buffer, salt, and phosphate diester solutions and diluting with water to the correct volume.

An Orion Model 501 research digital ion analyzer equipped with a temperature compensation probe was used for pH measurements and titrations. Kinetic measurements were performed with the use of an IBM 9420 UV–vis spectrometer equipped with a thermostated cell compartment. All measurements were made at 75 °C.

**Kinetics.** The initial rate of production of 4-nitrophenolate ($\epsilon$ = 18 700 $M^{-1}$ $cm^{-1}$) was monitored spectrophotometrically at 400 nm. Reactions performed at pH < 9 were corrected for the degree of ionization of 4-nitrophenolate ($K_a = 2.14 \times 10^{-7}$)[6] at 75 °C. The Sigma biological buffers MES, HEPES, EPPS, CHES, and CAPS were used to maintain a constant pH in the range 6.0–10.8. The pH of the solutions was adjusted with NaOH or $HNO_3$ and checked at 75 °C. The pH values are within ±0.05 pH units. Ionic strength was maintained at 0.1 M by $NaNO_3$. Inhibition studies were performed by adding an equivalent amount of inhibitory agent and phosphate diester.

First-order rate constants were calculated from the slope of the linear plots of absorbance against time by converting to concentration units and dividing by the initial phosphate diester concentration. Metal-assisted hydrolysis rates are uncorrected for spontaneous hydrolysis. Rates of spontaneous (control) hydrolysis of the phosphate diesters were measured by using identical conditions, except $M^{2+}$ and L were omitted, and are included in the tables for comparison purposes. All points were from an average of at least three measurements. Standard deviations are included in the tables.

**DNA Cleavage Studies.** Plasmid DNA experiments were conducted at 37 °C with the use of a constant-temperature circulator bath. The supercoiled plasmid pSP64[22] (2999 base pairs) was isolated from *Escherica coli*[23] and provided by Prof. D. Donoghue. The pH of the DNA solutions was adjusted to 8.0 by addition of an aliquot of EPPS buffer previously adjusted to pH 8.0. The final concentrations were $5 \times 10^{-4}$ M metal–ligand, $1 \times 10^{-2}$ M EPPS, and 25 μg/mL pSP64, which corresponds to a concentration of $4 \times 10^{-5}$ M in base pairs. Samples were incubated at 37 °C for 3 h. Reactions were quenched by addition to a bromophenol blue–xylene cyanol–glycerol solution. Electrophoresis was conducted on 1% agarose (Fisher Scientific, high-melting DNA grade) in a 10-cm horizontal minigel apparatus at 65 V for 1 h at a gel thickness of 7 mm. Electrophoresis buffer contains 0.08 M tris(hydroxymethyl)-ammoniomethane phosphate, 0.002 M ethylenediaminetetraacetate (pH 8.0), and 0.3 μg/mL ethidium bromide (Sigma) as a DNA stain.[23]

**Table I.** Representative Observed Pseudo-First-Order Rate Constants, $k_0$, for the Hydrolysis of Sodium Bis(4-nitrophenyl) Phosphate (**1**) in Water at 75 °C[a]

| catalyst | | pH 7.00 | pH 8.60 | | pH 10.00 |
|---|---|---|---|---|---|
| $M^{2+}$ | L | $10^6 k_0$, $s^{-1}$ [b] | $10^6 k_0$, $s^{-1}$ [b] | $10^6 k_0$ $s^{-1}$ [c] | $10^6 k_0$, $s^{-1}$ [c] |
| Ni | tren | 0.17 ± 0.01 | 3.70 ± 0.10 | 0.44 ± 0.07 | 7.80 ± 0.34 |
| | bpy | 0.46 ± 0.04 | [d] | 0.83 ± 0.01 | 5.10 ± 0.20 |
| | $(bpy)_2$ | | [d] | 0.90 ± 0.03 | |
| | $(bpy)_3$ | | [d] | 0.36 ± 0.03 | |
| Cu | tren | 0.045 ± 0.001 | 0.22 ± 0.02 | 0.17 ± 0.01 | 4.00 ± 0.11 |
| | bpy | 13.6 ± 1.1 | [d] | 2.78 ± 0.35 | 5.22 ± 0.21 |
| Zn | tren | 0.051 ± 0.003 | 0.25 ± 0.01 | 0.17 ± 0.02 | 3.88 ± 0.11 |
| | bpy | 1.39 ± 0.11 | [d] | 0.25 ± 0.01 | 4.14 ± 0.11 |
| | $(bpy)_2$ | 0.39 ± 0.02 | [d] | | |
| control | | 0.026 ± 0.004 | 0.13 ± 0.02 | | 3.68 ± 0.13 |

[a] pH measurement made at 75 °C with temperature compensation probe, $\mu$ = 0.1 M ($NaNO_3$), 0.01 M buffer, tren = tris(2-aminoethyl)amine, bpy = 2,2′-bipyridine, $K_w = 2.0 \times 10^{-13}$, and hydrolysis values are uncorrected for spontaneous (control) hydrolysis. The control values were obtained by using identical conditions except $M^{2+}$ and L were omitted. [b] $1.0 \times 10^{-3}$ M $ML^{2+}$ concentration. [c] $1.0 \times 10^{-4}$ M $ML^{2+}$ concentration. [d] Precipitate formed at this concentration.

**Table II.** Representative Observed Pseudo-First-Order Rate Constants, $k_0$, for the Hydrolysis of Sodium Ethyl 4-nitrophenyl Phosphate (**2**) in Water at 75 °C at pH 6.05[a]

| catalyst (0.1 mM)[b] | $10^7 k_0$, $s^{-1}$ | catalyst (0.1 mM)[b] | $10^7 k_0$, $s^{-1}$ |
|---|---|---|---|
| $Pd(bpy)^{2+}$ | 5.9 ± 0.7 | $Pt(en)^{2+}$ | 1.2 ± 0.1 |
| $Pd(en)^{2+}$ | 4.5 ± 0.9 | control | 0.12 ± 0.02 |

[a] pH measurement made at 75 °C with temperature compensation probe, $\mu$ = 0.1 M ($NaNO_3$), 0.01 M MES, bpy = 2,2′-bipyridine, en = ethylenediamine, $K_w = 2.0 \times 10^{-13}$, and hydrolysis values are uncorrected for spontaneous (control) hydrolysis. [b] Generated by adding 2 equiv of $AgNO_3$ to a $M(L_2)Cl_2$ solution.

(18) (a) Kirby, A. J.; Younas, M. *J. Chem. Soc. B* **1970**, 1165–1172. (b) De Roos, A. M.; Toet, H. J. *Recl. Trav. Chim. Pays-Bas* **1958**, *77*, 946–955.
(19) Bosnich, B.; Tobe, M. L.; Webb, G. A. *Inorg. Chem.* **1965**, *4*, 1109–1112.
(20) (a) McCormick, B. J.; Jaynes, E. N., Jr.; Kaplan, R. I. *Inorg. Synth.* **1972**, *13*, 217–218. (b) McCormick, B. J.; Jaynes, E. N., Jr.; Kaplan, R. I. *Inorg. Synth.* **1972**, *13*, 216–217. (c) Johnson, G. L. *Inorg. Synth.* **1966**, *8*, 242–244. (d) Kauffman, G. B.; Cowan, D. O. *Inorg. Synth.* **1963**, *7*, 241–242.
(21) Vogel, A. I. *Vogel's Textbook of Quantitative Inorganic Analysis*; Longman: London, 1978.
(22) Melton, D. A.; Krieg, P. A.; Rebagliati, M. R.; Maniatis, T.; Zinn, K.; Green, M. R. *Nucl. Acids Rev.* **1984**, *12*, 7035–7056.
(23) Maniatis, T.; Fritsch, E. F.; Sambrock, J. *Molecular Cloning*; Cold Spring Harbor Laboratory: Cold Spring Harbor, NY, 1982.

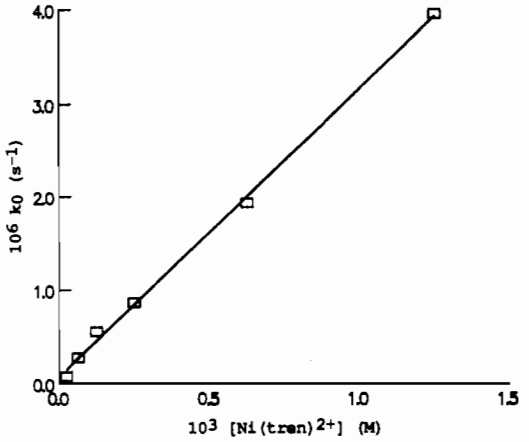


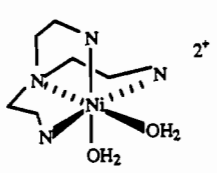

**Figure 1.** Dependence of $k_0$ for the hydrolysis of **1** on Ni(tren)$^{2+}$ concentration (75 °C, pH 8.60, 0.01 M CHES, $\mu$ = 0.1 M ($NaNO_3$), correlation coefficient 0.998, observed rate corrected for spontaneous hydrolysis).

Titrations to determine the p$K_a$ of the water bound to metal complexes were performed on 10 mL of $1 \times 10^{-3}$ M solution of metal–ligand complex and titrated with $1 \times 10^{-2}$ M NaOH solution. Ionic strength was maintained at 0.1 M with $NaNO_3$. All p$K_a$'s were reproducible to within 0.05 units.

## Results and Discussion

In Tables I and II are contained observed pseudo-first-order rate constants, $k_0$, for the hydrolysis of bis(4-nitrophenyl) phosphate (**1**) and ethyl 4-nitrophenyl phosphate (**2**) by metal complexes in aqueous buffered solution at 75 °C. The alkaline hydrolysis of phosphodiesters is not first-order in sodium hydroxide, which makes a direct comparison of intrinsic rate constants difficult because of the complex nature of the pH–rate profiles for alkaline hydrolysis.[24] These data suggested that square-planar Cu(II) and octahedral Ni(II) amine complexes significantly accelerate phosphate diester hydrolysis. Our detailed studies of the Cu(II) amine catalyzed reactions also suggested the active species was a square-planar *cis*-hydroxo(diaryl phosphato) complex.[6] Since the redox activity of Cu(II) presents problems for applications to DNA hydrolysis,[4] we report mechanistic studies here for octahedral Ni(II) and limited observations for its periodic analogues Pd(II) and Pt(II), which adopt square-planar structures.

**Ni(II)-Catalyzed Phosphodiester Hydrolysis.** Many Ni(II) complexes with mono- and polydentate amine ligands (2 and 1 equiv, respectively) exhibited rate acceleration in the hydrolysis of **1** (Table III). The use of nonmacrocyclic polydentate ligands led to a greater rate acceleration then did the monodentate ligands, which roughly parallels the stability constants of the complexes in aqueous solution.[25] In several cases the formation of precipitates prevents a quantitative comparison. The complex with the tetradentate ligand tren, **3**, exhibited the greatest rate acceleration and was chosen for more detailed mechanistic studies.




**3**

The tren ligand can only adopt a cis structure around an octahedral metal ion to form a high-spin, paramagnetic complex,

**Table III.** Representative Pseudo-First-Order Rate Constants, $k_0$, for the Hydrolysis of Bis(4-nitrophenyl) Phosphate (**1**) by Ni(II) Complexes in Water at 75 °C at pH 8.60

| complex[a] | $10^7 k_0$, $s^{-1}$ | log $K$[c] |
|---|---|---|
| Ni([15]aneN$_4$)$^{2+}$ | 1.79 ± 0.11 | 22.2[d] |
| Ni$^{2+}$ [b] | 2.35 ± 0.18 | −0.22 |
| Ni(NH$_2$OH)$_2^{2+}$ [b] | 2.59 ± 0.05 | 1.47 |
| Ni(pd)$_2^{2+}$ [b] | 2.82 ± 0.44 | [e] |
| Ni(pr)$_2^{2+}$p [b] | 2.90 ± 0.64 | 1.8 |
| Ni(NH$_2$py)$_2^{2+}$ [b] | 2.94 ± 0.41 | 1.85[f] |
| Ni(en)$^{2+}$ | 5.82 ± 0.43 | 7.35 |
| Ni(bpy)$^{2+}$ | 8.31 ± 0.08 | 7.0 |
| Ni(pao)$^{2+}$ | 9.92 ± 0.10 | 9.4 |
| Ni(trien)$^{2+}$ | 9.94 ± 0.09 | 13.8 |
| Ni(tren)$^{2+}$ | 10.0 ± 0.7 | 14.6 |
| control | 1.27 ± 0.11 | |

[a] 0.01 M CHES; $\mu$ = 0.1 M ($NaNO_3$), $K_w = 2.0 \times 10^{-13}$, $NH_2py$ = 2-aminopyridine, pr = pyrazole, pd = pyridazine, en = ethylenediamine, bpy = 2,2′-bipyridine, pao = 2-pyridinealdoxime, trien = triethylenetetramine, tren = tris(2-aminoethyl)amine, [15]aneN$_4$ = 1,4,8,12-tetraazacyclopentadecane; all Ni complexes are dications as nitrate salts, [Ni$^{2+}$] = $1 \times 10^{-4}$ M, hydrolysis values are uncorrected for spontaneous (control) hydrolysis. [b] Precipitates formed. [c] Smith and Martell;[25] values are the log of stability constants at 25 °C in water, $\mu$ = 0.1 M; K is for the reaction $Ni^{2+} + L \rightleftharpoons NiL^{2+}$. [d] Value is for [14]aneN$_4$ from ref 27 ([14]aneN$_4$ = 1,4,8,11-tetraazacyclotetradecane). [e] Stability constant was not available. [f] Stability constant for pyridine; the value for 2-aminopyridine was not available.

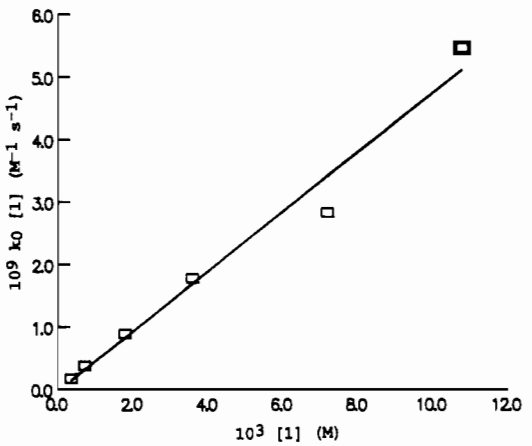



**Figure 2.** Dependence of $k_0$ for the hydrolysis of **1** by Ni(tren)$^{2+}$ on phosphate diester concentration (75 °C, pH 8.60, 0.05 M CHES, $\mu$ = 0.1 M ($NaNO_3$), [Ni(tren)$^{2+}$] = $1 \times 10^{-4}$ M, correlation coefficient 0.976, observed rate corrected for spontaneous hydrolysis).

which is consistent with its short wavelength absorbance at 365 nm.[26] This geometry maintains open cis sites in the octahedral complex to allow both phosphate diester and hydroxide coordination for intramolecular hydroxide attack on the coordinated phosphate diester. Our previous work on the Cu(bpy)$^{2+}$-catalyzed hydrolysis of phosphate diesters suggested formation of a Cu(bpy)(OH)[$O_2P(OR)_2$] complex as a key intermediate.[6] The rate enhancement for the Ni(tren)$^{2+}$ (1 mM, pH 8.60) catalyzed hydrolysis of **1** over spontaneous hydrolysis is 28. Saturation kinetics could not be observed because of the low solubility of **1**, so the maximum rate enhancement could not be determined. The rate of hydrolysis of **1** was first-order in Ni(tren)$^{2+}$ to $1 \times 10^{-3}$ M (Figure 1), in contrast to Cu(bpy)$^{2+}$, which demonstrated first-order behavior only below $5 \times 10^{-5}$ M. Above this concentration the formation of Cu(bpy)$^{2+}$ hydroxy-bridged dimers was significant. The rate of Ni(tren)$^{2+}$-catalyzed hydrolysis of **1** is also first-order in phosphate diester up to its solubility limit

(24) (a) Kirby, A. J.; Younas, M. *J. Chem. Soc. B* **1970**, 510–511. (b) Khan, S. A.; Kirby, A. J. *J. Chem. Soc. B* **1970**, 1172–1182. (c) Bunton, C. A.; Mhala, M. M.; Oldham, K. G.; Vernon, C. A. *J. Chem. Soc.* **1960**, 3293–3301.

(25) Smith, R. M.; Martell, A. E., Eds. *Critical Stability Constants*; Plenum: New York, 1974; Vol. 4.

(26) (a) Fabbrizzi, L. *Inorg. Chem.* **1977**, *16*, 2667–2668. (b) Wilkins, R. G.; Yelin, R.; Margerum, D. W.; Weatherbarn, D. C. *J. Am. Chem. Soc.* **1969**, *91*, 4326. (c) Jørgensen, C. K. *Acta Chem. Scand.* **1957**, *11*, 399–400. (d) Trien also adopts a cis structure about an octahedral metal ion.

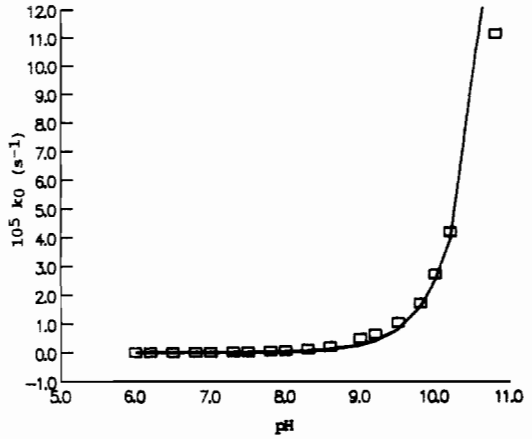
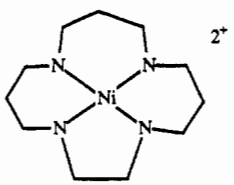



**Figure 3.** Dependence of $k_0$ on pH for the hydrolysis of **1** by Ni(tren)$^{2+}$ (75 °C, 0.01 M buffer, $\mu$ = 0.1 M ($NaNO_3$), [Ni(tren)$^{2+}$] = $3.8 \times 10^{-4}$ M, observed rate corrected for spontaneous hydrolysis). The theoretical line is calculated from eq 3 with $C = 3.5 \times 10^{-3}$.

(Figure 2). A slight inhibition (10%) in the rate was observed on increasing the concentration of CHES or CAPS buffer from 0.01 M to 0.1 M. This probably arises from weak coordination of the buffer to the catalyst.

The tetradentate macrocyclic ligand [15]aneN$_4$ enforces a square-planar structure about Ni(II) to form **4**, a low-spin, diamagnetic complex.[27] With this geometry, incoming ligands are




**4**

forced to bind in the axial positions (which would result in a high-spin complex) of the square-planar complex. Because this binding mode is weak, we would expect metal-promoted hydrolysis to be retarded. Complex **4** also should be blocked from an intramolecular hydrolysis pathway because of the absence of cis binding sites. The rate of hydrolysis of **1** by Ni([15]aneN$_4$)$^{2+}$ ($k_0 = 1.79 \times 10^{-7}$ $s^{-1}$) is less than that of Ni$^{2+}$ ($k_0 = 2.35 \times 10^{-7}$ $s^{-1}$) and only slightly larger than that of spontaneous hydrolysis ($k_0 = 1.27 \times 10^{-7}$ $s^{-1}$).

The pH profile (Figure 3) of the Ni(tren)$^{2+}$-catalyzed hydrolysis of **1** shows a dependence above pH 8.0 and approaches a pH-independent plateau below pH 8.0. In contrast, the Cu(bpy)$^{2+}$-catalyzed hydrolysis of ethyl 4-nitrophenyl phosphate (**2**) shows two pH-independent regions, one below pH 6.4 and another above pH 7.8, and an intermediate pH-dependent range. These observations can be explained by the differing $K_a$ equilibria expected for the coordinated water molecules of Cu(bpy)$^{2+}$ and Ni(tren)$^{2+}$ (p$K_a$'s of 7.0[28] and 12.1,[29] respectively) to form the metal–aquo–hydroxy complexes. We could not determine the rate of hydrolysis of **1** above pH 10.8, since the pH decreases with increasing temperature and pH 10.8 at 75 °C corresponds to pH 12.4 at 25 °C, the limit of available buffer systems. By analogy to Cu(bpy)$^{2+}$ we would expect the rate to become pH independent above the first p$K_a$ of Ni(tren)(OH$_2$)$_2^{2+}$. The Ni(tren)$^{2+}$-catalyzed hydrolysis of methylglycinate has been shown to have a similar dependence in the pH range 8.25–9.50, which was the limit for coordination of the amino acid ester to Ni(tren)$^{2+}$ ($T$ = 25 °C, $\mu$ = 0.1 M).[29] Similar observations were made in the Ni(II)-catalyzed hydrolysis of ethyl *N*,*N*-diacetoxyglycinate.[30]

**Table IV.** Stability Constants (log $K$)[a] and p$K_a$'s of Metal–Ligand Complexes at 25 °C and $\mu$ = 0.1 M

| $M^{2+}$ | L | log $K$ | p$K_a$ |
|---|---|---|---|
| Ni | tren | 14.6 | 12.1[b] |
| | bpy | 7.0 | 8.9[c] |
| | $(NO_3)_2$ | −0.5 | 9.9[d] |
| Cu | tren | 18.5 | 9.4[e] |
| | bpy | 6.33 | 7.8[f,g] |
| | $(NO_3)_2$ | −0.6 | 8.0[d] |
| Zn | tren | 14.5 | 10.3[e] |
| | bpy | 5.13 | 8.1[c] |
| | $(NO_3)_2$ | −0.18 ($\mu$ = 0.5) | 9.0[d] |

[a] Stability constants ($K$) are from Smith and Martell[24] for the reaction $M^{2+} + L \rightleftharpoons ML^{2+}$; tren = tris(2-aminoethyl)amino, bpy = 2,2′-bipyridine. [b] Reference 29. [c] This work. [d] Reference 49. [e] Reference 50. [f] Reference 25, $\mu$ = 1 M. [g] p$K_a$ = 7.0 at 75 °C.[6]

The Ni(tren)$^{2+}$ complex exhibited catalysis in solutions containing a large excess of **1**, where the rate of hydrolysis was constant up to 85 turnovers and thereafter decreased. The rate was not inhibited by addition of sodium 4-nitrophenyl phosphate (**5**) or inorganic phosphate (1 mol/mol of substrate), which is a hydrolysis product of **5**. The turnover number was corrected for secondary hydrolysis of the monoester, **5**, which is also accelerated by Ni(tren)$^{2+}$.

A comparison of the phosphate diester hydrolysis rates by Ni(II), Cu(II), and Zn(II) complexes with tren and bpy yielded interesting results (Table I). The complexes of Cu(tren)$^{2+}$ and Zn(tren)$^{2+}$ showed hydrolysis rates of **1** only slightly above control values, while Ni(tren)$^{2+}$-catalyzed hydrolysis of **1** showed a rate enhancement of almost 30 over control values.[31] This observation can be attributed to tridentate coordination of tren to square-planar Cu(II) and tetrahedral Zn(II), leaving only one coordination site open to hydroxide or phosphate binding. Therefore, the intramolecular hydrolysis pathway is inhibited and rates are only slightly above the controls. However, with tetradentate coordination of tren to octahedral Ni(II), cis coordination sites are open to both hydroxide and phosphate coordination simultaneously, thus allowing an intramolecular pathway to occur.

The metal complexes with the ligand bpy show a reactivity trend different from that of the corresponding tren complexes. Bipyridine forms a bidentate complex with each of the three metal ions in question, which in all three complexes leaves cis coordination sites open to simultaneous hydroxide and phosphate binding. The important factor now becomes the p$K_a$ of the bound water molecule in each of the metal–ligand complexes. The p$K_a$'s of the M(bpy)(OH$_2$)$_2^{2+}$ complexes are 7.8, 8.1, and 8.9 at 25 °C for Cu, Zn, and Ni, respectively (Table IV). Thus, at pH 7.00, Cu(bpy)$^{2+}$ and Zn(bpy)$^{2+}$ are better at hydrolyzing **1** than Ni(bpy)$^{2+}$, as a higher concentration of the copper and zinc complexes exist in the monohydroxo form than for the nickel complex. The Cu(bpy)$^{2+}$ complex is only slightly better than Ni(bpy)$^{2+}$ at pH 8.60 and statistically equivalent to Ni(bpy)$^{2+}$ at pH 10.00 owing to the increased formation of inactive copper dihydroxides and dimers and the increased concentration of the monohydroxo form of Ni(bpy)$^{2+}$. Above pH 8.1, Zn(bpy)$^{2+}$ forms precipitates with a corresponding decrease in catalytic efficiency.

At pH 8.60, the rates of hydrolysis of **1** by Ni(bpy)$^{2+}$ and Ni(bpy)$_2^{2+}$ are similar; both complexes have open cis coordination sites (Ni(bpy)$_2^{2+}$ is an octahedral cis complex). Also, both complexes show rates of hydrolysis of **1** greater than that of Ni(tren)$^{2+}$. In contrast to Ni(bpy)$^{2+}$ and Ni(bpy)$_2^{2+}$, the rate of hydrolysis of **1** by coordinatively saturated Ni(bpy)$_3^{2+}$ is decreased compared to either the mono- or bis(ligand) complex. Although the rate is greater than spontaneous (control) hydrolysis, this probably

(27) Hinz, F. P.; Margerum, D. W. *Inorg. Chem.* **1974**, *13*, 2941–2949.
(28) The value was calculated from the rate expression for the hydrolysis of ethyl 4-nitrophenyl phosphate and a least-squares fit of the observed data from ref 4b.
(29) Newlin, D. E.; Pellack, M. A.; Nakon, R. *J. Am. Chem. Soc.* **1977**, *99*, 1078–1082.
(30) Bedell, S. A.; Nakon, R. *Inorg. Chem.* **1977**, *16*, 3055–3059.
(31) Data are from Table I, $1 \times 10^{-3}$ M Ni(tren)$^{2+}$ and pH 8.60
(32) Milburn, R. M.; Gautam-Basak, M.; Tribolet, R.; Sigel, H. *J. Am. Chem. Soc.* **1985**, *107*, 3315–3321.

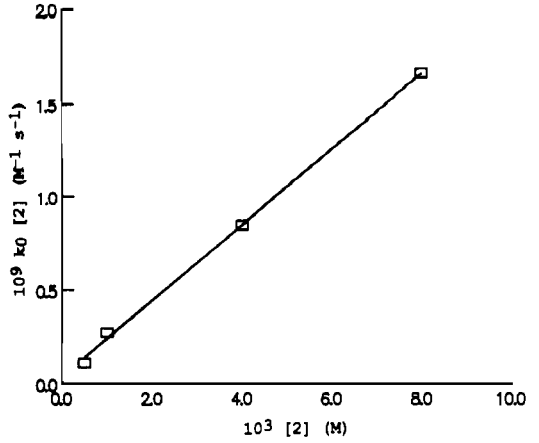



**Figure 4.** Dependence of $k_0$ for the hydrolysis of **2** by $Pd(bpy)^{2+}$ on phosphate diester concentration (75 °C, pH 6.05, 0.01 M MES, $\mu$ = 0.1 M ($NaNO_3$), $[Pd(bpy)^{2+}] = 1 \times 10^{-4}$ M, correlation coefficient 0.999, observed rate corrected for spontaneous hydrolysis).

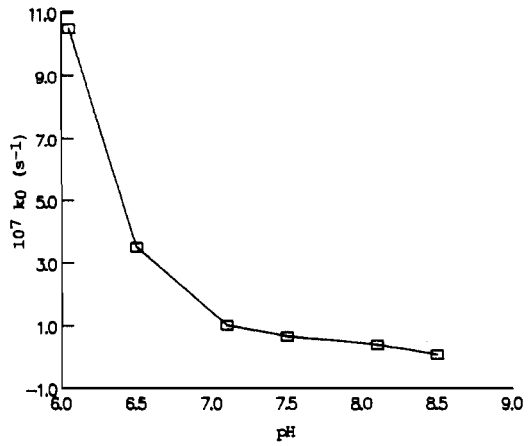



**Figure 5.** Dependence of $k_0$ on pH for the hydrolysis of **2** by $Pd(bpy)^{2+}$ (75 °C, 0.01 M buffer, $\mu$ = 0.1 M ($NaNO_3$), $[Pd(bpy)^{2+}] = 1 \times 10^{-4}$ M, observed rate corrected for spontaneous hydrolysis). The line is drawn for clarity and is not a theoretical fit.

arises from the equilibrium concentrations of $Ni(bpy)_2^{2+}$ and $Ni(bpy)^{2+}$.

At pH 7.00, addition of 2 equiv of bipyridine to $Zn^{2+}$ shows a decrease in the rate of hydrolysis of **1** from that of $Zn(bpy)^{2+}$. This result may be attributed to partial saturation of the coordination sites on Zn(II) to give a distribution of $Zn^{2+}$, $Zn(bpy)^{2+}$, and $Zn(bpy)_2^{2+}$ species. The only catalytically active species is $Zn(bpy)^{2+}$; any formation of $Zn(bpy)_2^{2+}$ decreases the concentration of active catalyst with a corresponding decrease in rate of hydrolysis of **1**.

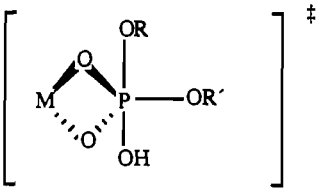
In our previous work on the Cu(II) system,[6] we demonstrated that addition of 2 equiv of bipyridine to $Cu^{2+}$ decreased the rate of hydrolysis of **2**. Additionally, a further increase in the ratio of bipyridine to $Cu^{2+}$ resulted in a corresponding decrease in hydrolysis rates. The effect was not linear due to complex equilibria between $Cu^{2+}$, $Cu(bpy)^{2+}$, and $Cu(bpy)_2^{2+}$. The optimal rate of hydrolysis was determined to have resulted from the 1:1 metal–ligand complex, $Cu(bpy)^{2+}$.

The order of catalytic activity for the metal–tren complexes is Zn(II) = Cu(II) ≪ Ni(II) at every pH tested, which is consistent with the requirement for cis binding sites about the metal center. For metal–bpy complexes at neutral pH, the order of activity is Ni(II) < Zn(II) < Cu(II), which is the same order of activity observed for the metal ion promoted hydrolysis of nucleoside 5′-triphosphates at neutral pH.[31] At pH 8.60, the order of reactivity is Zn(II) < Ni(II) < Cu(II). At pH 10.00, the order of reactivity is Zn(II) < Cu(II) ≈ Ni(II).

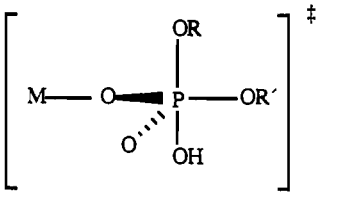
**Pd(II)- and Pt(II)-Accelerated Phosphodiester Hydrolysis.** Because of the increased interest in antitumor reagents[33] and the Pt(II)-promoted hydrolysis of inorganic polyphosphates,[34] we also investigated the effect of various Pd(II) and Pt(II) diamine complexes on the hydrolysis of **1** and **2** (Table II). Since the chloro complexes were generally inactive, cationic species were generated by the addition of $AgNO_3$. Since pH-dependent equilibria result in a variety of aquo and hydroxo species in solution, formulas such as $Pt(NH_3)_2^{2+}$ will be used as abbreviations for the mixture. The antitumor drug derived, *cis*-$Pt(NH_3)_2^{2+}$,[35] failed to demonstrate activity in the hydrolysis of **1** or **2**;[36] however, $Pt(en)^{2+}$ exhibited some activity near neutral pH. The greater activity of the bidentate ligand complexes can be attributed to the need for maintaining open cis sites on the square-planar metal center. The $Pd(bpy)^{2+}$ and $Pd(en)^{2+}$ complexes exhibit much greater activity than the Pt(II) analogues, which could arise from the enhanced kinetic lability of Pd(II) versus Pt(II).[37] Bipyridine may be a better ligand for the Pd(II)-catalyzed hydrolysis of phosphate diesters because the $\pi$ acidity of 2,2′-bipyridine may favor the anionic ligands.[38] The reaction is first-order in phosphate (Figure 4). The pH–rate profile of the hydrolysis of **2** promoted by $Pd(bpy)^{2+}$ (Figure 5) shows a pH dependence with an enhancement of 49 (Table II) at pH 6.05 over spontaneous hydrolysis. The decreased activity at high pH can be explained by the formation of hydroxy-bridged polymers (eq 1), if one assumes the polymers are less active than the monomers as catalysts.[39a-c]

**Scheme I**

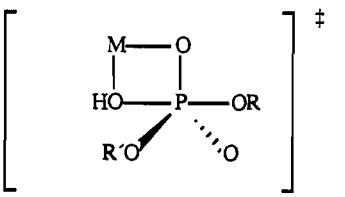
1. External attack on bidentate phosphate




2. External attack on monodentate phosphate




3. Intramolecular attack




(33) (a) Köpf-Maier, P.; Köpf, H. *Chem. Rev.* **1987**, *87*, 1137–1152. (b) Sherman, S. E.; Lippard, S. J. *Chem. Rev.* **1987**, *87*, 1153–1181. (c) Sigel, H., Ed. *Metal Ions in Biological Systems*; Dekker: New York, 1980; Vol. 10.

(34) (a) Bose, R. N.; Cornelius, R. D.; Viola, R. E. *Inorg. Chem.* **1985**, *24*, 3989–3996. (b) Bose, R. N.; Cornelius, R. D.; Viola, R. E. *Inorg. Chem.* **1984**, *23*, 1182–1183.

(35) (a) Naser, L. J.; Pinto, A. L.; Lippard, S. J.; Essigmann, J. M. *Biochemsitry* **1988**, *27*, 4357–4367. (b) Hacker, M. P.; Douple, E. B.; Krakoff, I. H., Eds. *Platinum Coordination Complexes in Cancer Chemotherapy*; Nijhoff: Boston, MA, 1984. (c) Spiro, T. G., Ed. *Nucleic Acid-Metal Ion Interactions*; Wiley: New York, 1980.

(36) *trans*-$Pt(NH_3)_2^{2+}$, a less effective antitumor agent than *cis*-$Pt(NH_3)_2^{2+}$, also failed to demonstrate hydrolytic activity.

(37) Cotton, F. A.; Wilkinson, G. *Advanced Inorganic Chemistry*, 4th ed.; Wiley: New York, 1980.

(38) Sigel, H. *Angew. Chem., Int. Ed. Engl.* **1975**, *14*, 394–402.

Under some conditions $Ag^+$ adducts may also form.[39a]

$$Pd(bpy)(OH)(OH_2)^+ \overset{K_d}{\rightleftharpoons} [Pd(bpy)(OH)]_x^{2+} + xH_2O \qquad (1)$$

Catalytic turnover is not observed for the Pd(II)- or Pt(II)-accelerated hydrolysis of phosphate diesters. Inhibition studies show a 75% reduction in rate by added inorganic phosphate and a 15% reduction in rate by added sodium methyl phosphate. No inhibitory effect was observed for added 4-nitrophenol. The lack of catalytic turnover could result from binding of the products of the hydrolysis reaction to the metal.

Recent evidence suggests an initial binding of *cis*-$Pt(NH_3)_2^{2+}$ simultaneously to the N7 position on a Guanine residue of DNA and to a 5′-phosphate group before binding of *cis*-$Pt(NH_3)_2^{2+}$ to the second Guanine residue of a d(pGpGp) sequence.[40] This recognition of the 5′-phosphate group by *cis*-$Pt(NH_3)_2^{2+}$ is thought to accelerate the binding reaction of *cis*-$Pt(NH_3)_2^{2+}$ to the DNA moiety.

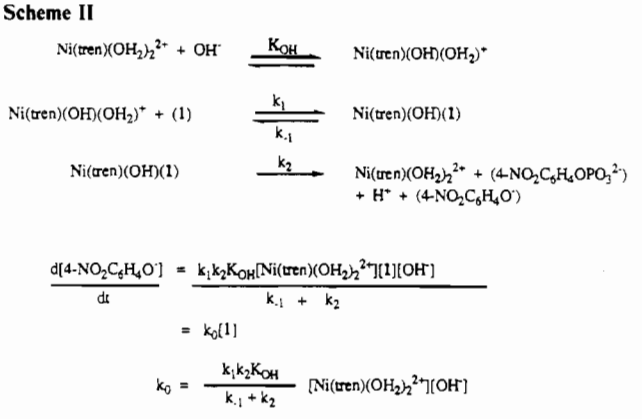
**Mechanism of Metal-Catalyzed Hydrolysis of Phosphodiesters.** Three possible reaction pathways for the hydrolysis of phosphate diesters are shown in Scheme I. One involves phosphate diester coordination to the metal ion as a bidentate ligand followed by external hydroxide attack. The second involves monodentate coordination of the phosphate diester to the metal ion followed by external hydroxide attack. The third involves monodentate phosphate diester coordination to a hydroxymetal complex followed by intramolecular hydroxide transfer from the metal to the phosphate.

Several reports exist in the literature of an intramolecular pathway for the hydrolysis of phosphate diesters. The Co(tetraamine)$^{3+}$-catalyzed hydrolysis of ethyl 4-nitrophenyl methylphosphonate[5,41] and bis(4-nitrophenyl) phosphate[11,42] and the Cu(bpy)$^{2+}$-catalyzed hydrolysis of ethyl 4-nitrophenyl phosphate[6] were both proposed to proceed through formation of a pentacoordinate phosphorane intermediate by attack of a cis hydroxide on a monodentate phosphate ester. Product is formed through pseudorotation of the pentacoordinate intermediate. Five-coordination phosphoranes are likely intermediates in the mechanism of base hydrolysis of organic phosphate esters.[43,44]

Intramolecular attack of cis hydroxide on a coordinated phosphate ester has also been reported for *cis*-$[Ir(en)_2(OH)(O_2P(OR)_2)]^+$, where the phosphate diester ethyl 4-nitrophenyl phosphate or bis(4-nitrophenyl) phosphate is precoordinated to the Ir(III) center.[45] A four-membered phosphorane intermediate decays to yield 4-nitrophenoxide and a monodentate phosphate monoester. This report notes both a first- and second-order dependence on hydroxide due to both intra- and intermolecular pathways.

The Ni(tren)$^{2+}$-catalyzed hydrolysis of methyl glycinate (MeGly) has been proposed[29] to occur by binding of the amino acid ester nitrogen to the metal ion to form Ni(tren)(MeGly)$^{2+}$, followed by external hydroxide attack on the uncoordinated carbonyl group of the amino acid ester. This mechanism (analogous to path 2) agrees with the evidence that Ni(tren)$^{2+}$ was only catalytically active in the pH range 8.40–9.00, where little Ni(tren)(OH)(OH$_2$)$^+$ forms, and that a proton promoted ester hydrolysis to a greater degree than Ni(tren)$^{2+}$. External attack of hydroxide on the bidentate ester has been proposed for the Ni(II)-catalyzed hydrolysis of ethyl *N*,*N*-diacetoxyglycinate.[30] The amino acid ester is coordinated through both the amine nitrogen and carbonyl oxygen; hydroxide then attacks the carbonyl carbon to produce the observed products. This mechanism (analogous to path 1) was suggested because the observed trends of ligand-exchange reaction rates for a series of metal ions parallel the trend of the metal ion promoted hydrolysis of ethyl *N*,*N*-diacetoxyglycinate. In contrast, our studies suggest that Ni(tren)$^{2+}$ is catalytically active in phosphodiester hydrolysis to at least pH 10.8, which makes an intramolecular mechanism (path 3) more likely.

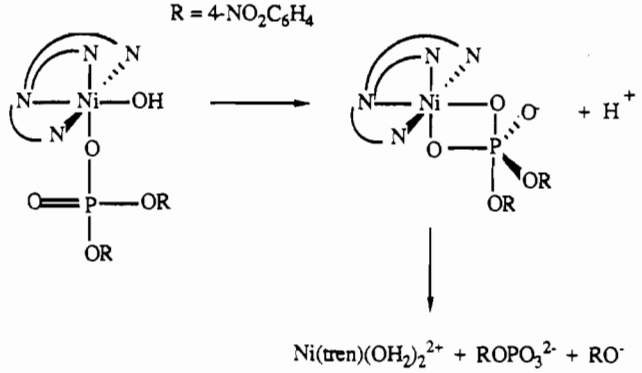
**Scheme II**




$$\frac{d[4\text{-}NO_2C_6H_4O^-]}{dt} = \frac{k_1k_2K_{OH}[Ni(tren)(OH_2)_2^{2+}][1][OH^-]}{k_{-1} + k_2} = k_0[1]$$

$$k_0 = \frac{k_1k_2K_{OH}}{k_{-1} + k_2}[Ni(tren)(OH_2)_2^{2+}][OH^-]$$

**Scheme III**




We propose that a preequilibrium is first established in the removal of a proton from a metal-coordinated water molecule to form Ni(tren)(OH)(OH$_2$)$^+$ as the active catalytic species at alkaline pH (Scheme II). The Ni(tren)(OH$_2$)$_2^{2+}$ complex appears to be catalytically inactive, since the metal-catalyzed hydrolysis is negligible below pH 8.0. The preequilibrium is followed by a reversible replacement of the coordinated water molecule with the monodentate phosphate diester ligand, as shown in Scheme II. The last step is rate-determining and assumed to be irreversible because of the poor nucleophilicity of 4-nitrophenolate. Unless a concerted mechanism is operable, products form through a metallophosphorane intermediate (Scheme III), which can pseudorotate (if necessary) to give 4-nitrophenolate. Phosphate ligand is then replaced by aquo ligands to reenter the catalytic cycle.

Because we cannot directly observe the metal–phosphate complex, the value of the $K_1$ equilibrium is unknown. No metal–phosphate complex was observable by $^{31}P$ NMR spectroscopy. Without $K_1$ we cannot accurately determine the value of the catalytic rate constant, $k_2$. The value of $K_{OH}$ for Ni(tren)(OH$_2$)$_2^{2+}$ at 75 °C has a lower limit of 72.4, as estimated from the value of $K_{OH}$ at 25 °C[29] (the value of $K_w$ increases with increasing temperature from $10^{-14}$ at 25 °C to $2.0 \times 10^{-13}$ at 75 °C;[6] the value of $K_{OH}$ should follow a similar trend). At higher hydroxide concentration, the Ni(tren)(OH)(OH$_2$)$^+$ concentration composes

(39) (a) Lim, M. C.; Martin, R. B. *J. Inorg. Nucl. Chem.* **1976**, *38*, 1911–1914. (b) Coley, R. F.; Martin, D. S., Jr. *Inorg. Chim. Acta* **1973**, *7*, 573–577. (c) Rochon, F. D.; Morneau, A.; Melanson, R. *Inorg. Chem.* **1988**, *27*, 10–13. (d) Rochon, F. D.; Melanson, R. *Acta Crystallogr., Sect. C* **1988**, *C44*, 474–477.
(40) (a) Verma, C.; Green, M.; Wing, R. M. *J. Chem. Soc., Chem. Commun.* **1988**, 884–886. (b) Orbell, J. D.; Taylor, M. R.; Birch, S. L.; Lawton, S. E.; Vilkins, L. M.; Keefe, L. J. *Inorg. Chim. Acta* **1988**, *152*, 125–134.
(41) See refs 34 and 36–39 in ref 5.
(42) (a) Chin, J.; Zou, X. *J. Am. Chem. Soc.* **1988**, *110*, 223–225. (b) Chin, J.; Banaszczyk, M.; Jubian, V. *J. Chem. Soc., Chem. Commun.* **1988**, 735–736. (c) Reference 7a.
(43) (a) Westheimer, F. H. *Acc. Chem. Res.* **1968**, *1*, 70–78. (b) Kumamoto, J.; Cox, J. R., Jr.; Westheimer, F. H. *J. Am. Chem. Soc.* **1956**, *78*, 4858–4860.
(44) (a) Gillespie, P.; Ramirez, F.; Ugi, I.; Marquarding, D. *Angew. Chem., Int. Ed. Engl.* **1973**, *12*, 91–172. (b) Mislow, K. *Acc. Chem. Res.* **1970**, *3*, 321–332.
(45) Hendry, P.; Sargeson, A. M. *J. Am. Chem. Soc.* **1989**, *111*, 2521–2527.

a larger portion of the total nickel concentration. The steady state derived rate expression is shown in eq 2. At hydroxide concentrations well below the $pK_a$ of the aquo complex, the simplified rate expression in Scheme II can be used since $[Ni]_T \approx [Ni(tren)(OH_2)_2^{2+}]$.

$$\frac{d[4\text{-}NO_2C_6H_4O^-]}{dt} = \frac{k_1k_2[\mathbf{1}]}{k_{-1} + k_2}\left(\frac{K_{OH}[Ni]_T[OH^-]}{1 + K_{OH}[OH^-]}\right) \quad (2)$$

The concentration of the active catalyst, $Ni(tren)(OH)(OH_2)^+$, can be calculated from the $pK_a$ of the diaquo complex. Concentration dependence studies were conducted at pH 8.60, where approximately 0.03% of the total nickel concentration is present as catalytically active $Ni(tren)(OH)(OH_2)^+$. The rate enhancement at pH 8.60 is estimated as 1200 on the basis of the $Ni(tren)(OH)(OH_2)^+$ concentration but only 34 on the basis of the total nickel concentration.[46]

A computer simulation of the pH dependence using eq 3 was performed where $1/K_{OH} = 0.0138$ (from pH 6.0 to pH 10.8). The

$$y = C\frac{[OH^-]}{1/K_{OH} + [OH^-]} \quad (3)$$

calculated theoretical curve from this equation (one parameter varied) was plotted over the experimental data points with $C = 3.5 \times 10^{-3}$ (Figure 3) to give excellent agreement with the observed pH–rate profile.

The role of the metal ion in catalytic phosphate ester hydrolysis is that of a Lewis acid to activate the phosphorus toward nucleophilic attack. A key factor in determining catalytic activity is the $pK_a$ of the bound water molecule, which is 7.0 for $Cu(bpy)(OH_2)_2^{2+}$ and 12.1 for $Ni(tren)(OH_2)_2^{2+}$. This corresponds to $[Ni(tren)(OH)(OH_2)_+] = 1.6 \times 10^{-7}$ M and $[(Cu(bpy)(OH)(OH_2)^+] = 9.1 \times 10^{-5}$ M at pH 8.0 and a total metal complex concentration of $1 \times 10^{-4}$ M. In the hydrolysis of **1**, $k_0 = (2.2 \pm 0.1) \times 10^{-7}$ s$^{-1}$ for $Ni(tren)^{2+}$ (Table IX, supplementary material) and $k_0 \approx (1.8 \pm 0.2) \times 10^{-6}$ for $Cu(bpy)^{2+}$.[47] Thus, Ni(II) could be judged as a more effective catalyst than Cu(II) at pH 8.0 on the basis of the estimated amount of catalyst present in the monohydroxy form. The $Cu(bpy)(OH)(OH_2)^+$ complex is an active species in a pH range where spontaneous hydrolysis is minimal, which results in a high rate enhancement. The $Cu(bpy)(OH_2)_2^{2+}$ complex remains active at pH < 7 (Table I), where the $Ni(tren)(OH_2)_2^{2+}$ complex in inactive; however, Ni(II) has an advantage over Cu(II) because Ni(II) complexes are less redox active and Ni(II) complexes are stable to a higher pH than the corresponding Cu(II) complexes.

**Plasmid DNA Experiments.** The rate enhancements for phosphate diester hydrolysis by Zn(II), Cu(II), and Ni(II) complexes are not estimated to be sufficient for DNA hydrolysis; however, in light of the results of Basile, Raphael, and Barton[4] we screened for activity of the tren and bpy metal complexes for nicking supercoiled DNA. In Figure 6 is shown the effects of these metal–ligand complexes on supercoiled plasmid DNA cleavage. Only $Cu(bpy)^{2+}$ cleaves DNA from the supercoiled form (form I) to the nicked form (form II). The cleavage reaction most likely proceeds through an oxidative mechanism as evident by the suppression of the cleavage reaction on addition of a small amount of Ce(IV) ion to the solution (Figure 6, upper panel, lanes 3 and 4).

$$Cu(bpy)^+ + Ce^{4+} \rightarrow Cu(bpy)^{2+} + Ce^{3+}$$

Copper(II) complexes may react with adventious reductants to produce Cu(I) complexes, which are known to cleave DNA by an oxidative mechanism.[48] Therefore, the addition of Ce(IV) helps to maintain the Cu(II) oxidation state and interferes with the redox reaction. Because the DNA plasmid concentration is low ($10^{-8}$ M), small amounts of redox-active impurities can be important.

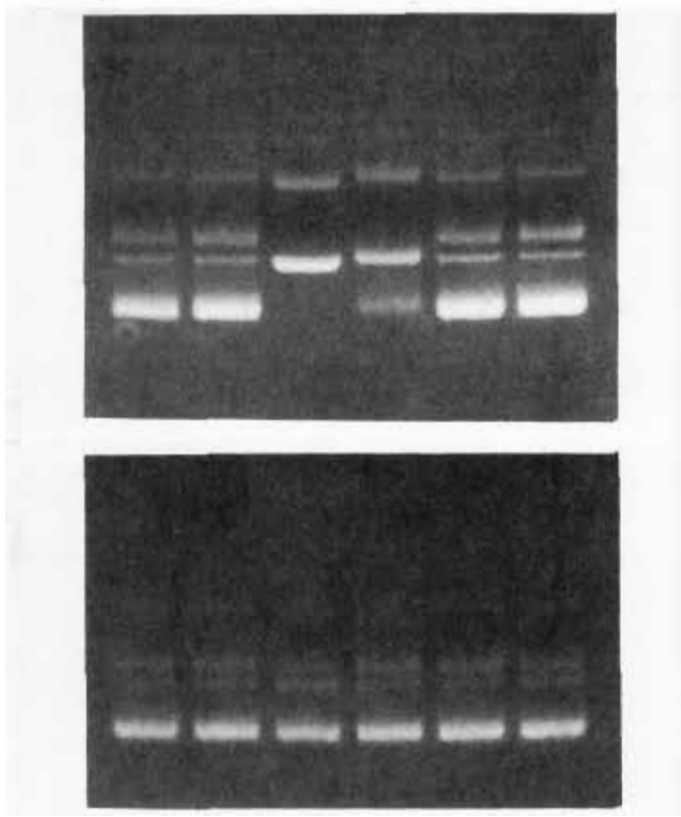


**Figure 6.** Effect of $5 \times 10^{-4}$ M $ML^{2+}$ complexes on supercoiled pSP64 plasmid DNA after incubation for 3 h at 37 °C ($4 \times 10^{-5}$ M in base pairs). Top panel (lanes from left to right): control, $Ni(bpy)^{2+}$, $Cu(bpy)^{2+}$, $Cu(bpy)^{2+}$ + 0.2 equiv of $(NH_4)_2Ce(NO_3)_6$, $Zn(bpy)^{2+}$, and control. Bottom panel (lanes from left to right): control, $Ni(tren)^{2+}$, $Cu(tren)^{2+}$, $Cu(tren)^{2+}$ + 0.2 equiv of $(NH_4)_2Ce(NO_3)_6$, $Zn(tren)^{2+}$, and control. The lowermost band is the supercoiled form.

Plasmid DNA cleavage was not evident with any of the metal–tren complexes tested (Figure 6). Somewhat surprising is the lack of plasmid DNA cleavage by $Cu(tren)^{2+}$. This may be explained by the difference between bpy and tren as donor ligands to the copper metal center. The $\pi$ acidity of bpy should stabilize the Cu(I) oxidation state, whereas $Cu(tren)^{2+}$ is more likely to remain in the 2+ oxidation state.

Barton's group[4] used the tren ligand attached to a DNA-binding $Ru^{2+}$ complex to effect hydrolytic DNA cleavage with a variety of simple metal ions. This observation is quite surprising, since the active metal ions Co(II), Zn(II), Cd(II), Cu(II), and Pb(II) were attached to the DNA binding agent by the tren ligand. Our kinetic studies of the hydrolysis of activated model phosphate diesters suggest that the tren complexes react too slowly to effect DNA hydrolysis. For example, $Cu(tren)^{2+}$ and $Zn(tren)^{2+}$ have little detectable activity as catalysts for hydrolysis of $(NO_2C_6H_4O)_2PO_2^-$ (Table I) at the same temperature as those used for the reported DNA hydrolyses. One explanation for these observations is that the binding–intercalating agent plays a nonin-

(46) The value of 0.03% is an estimate for the concentration of $Ni(tren)(OH)(OH_2)^+$ at pH 8.60 based on a value of 72.4 for $K_{OH}$. See supplementary material for calculations.

(47) The value for $Cu(bpy)^{2+}$-catalyzed hydrolysis of **1** is calculated from the apparent second-order rate constant reported in Table VI in ref 6 and from calculation of the pseudo-first-order rate constant by using eq 3 in ref 6 with the assumption that $k_2 \gg k_1$.

(48) (a) Veal, J. M.; Rill, R. L. *Biochemistry* **1989**, *28*, 3243–3250. (b) Stubbe, J.; Kozarick, J. W. *Chem. Rev.* **1987**, *87*, 1107–1153. (c) Chen, C. B.; Sigman, D. S. *Science* **1987**, *237*, 1197–1201. (d) Goldstein, S.; Czapski, G. *J. Am. Chem. Soc.* **1986**, *108*, 2244–2250. (e) Fleisher, M. B.; Waterman, K. C.; Turro, N. J.; Barton, J. K. *Inorg. Chem.* **1986**, *25*, 3549–3551. (f) Tullius, T. D. *Science* **1985**, *230*, 679–681. Note added in proof: Recent experiments (varying the concentration of bpy and added catalase) suggest that small equilibrium concentrations of $Cu(bpy)_2^{2+}$ are responsible for redox nicking of the $Cu(bpy)^{2+}$ system.

(49) Perrin, D. D. *Ionization Constants of Inorganic Acids and Bases in Aqueous Solution*, 2nd ed.; Pergamon Press: Oxford, England, 1982.

(50) Coates, J. H.; Gentle, G. J.; Lincoln, S. F. *Nature* **1974**, *249*, 773–775.

nocent role and induces strain in the supercoiled double helix to accelerate phosphate diester hydrolysis. Another factor could be the proximity effect of holding the metal ion near the phosphate diester backbone of DNA, which may accelerate the hydrolysis reaction.

## Conclusions

We have presented evidence that the $Ni(tren)^{2+}$-catalyzed hydrolysis of phosphate diesters occurs by an intramolecular mechanism in contrast to that proposed for amino acid hydrolysis. The reaction is first-order in phosphate ester and in $Ni(tren)^{2+}$. Below pH 8.0 little activity is observed. Above pH 8.0 the rate increase is consistent with the generation of $Ni(tren)(OH)(OH_2)^+$ as the active catalyst. Nickel(II) complexes are much better in promoting hydrolysis than either Pd(II) or Pt(II) complexes in the order Ni(II) > Pd(II) > Pt(II). By contrast, $Cu(tren)^{2+}$ and $Zn(tren)^{2+}$ are ineffective as catalysts. The Pt(II) antitumor complexes are ineffective as catalysts. The relative catalytic activities of $Ni(bpy)^{2+}$, $Cu(bpy)^{2+}$, and $Zn(bpy)^{2+}$ parallel the $pK_a$ of a metal-bound water molecule. The $Ni(tren)(OH)(OH_2)^+$ complex exhibits catalytic behavior to 85 turnovers and thereafter decreases in rate. The Pd(II) or Pt(II) complexes show no turnover activity. Rates of hydrolysis of phosphate diesters by the divalent cations examined are not by themselves sufficient to be useful in DNA hydrolysis.

**Acknowledgment**. This research was supported by the Army Research Office (Grant DAAG29-85-K0263). We thank Johnson-Matthey for a loan of platinum and palladium through their metal loan program. We thank Prof. Daniel Donoghue for a gift of plasmid DNA and for helpful discussions.

**Supplementary Material Available:** Tables and figures of kinetic data for hydrolysis of phosphate diesters by $Ni(tren)^{2+}$, $Pd(bpy)^{2+}$, $Pd(en)^{2+}$, and controls, kinetic schemes containing equations and text, a computer program with data from a simulation, and figures of typical titration curves (20 pages). Ordering information is given on any current masthead page.

---

Contribution from the Department of Pharmaceutical Science, School of Pharmacy, Hokuriku University, 3 Ho Kanagawa-machi, Kanazawa 920-11, Japan

# Base Binding to Zinc Picket Fence Porphyrins. Attractive Intramolecular Interactions in Organic Solvents[1]

Hiroyasu Imai and Eishin Kyuno*

*Received September 20, 1989*

Thermodynamic values for the binding of pyridine and isoquinoline to $\alpha^4$ and trans-$\alpha^2$ atropisomers of four kinds of zinc(II) picket fence porphyrins, which contain pivalamido, (isopropylcarbonyl)amino, (neopentylcarbonyl)amino, or (butylcarbonyl)amino pickets, were determined in toluene. The binding constants $K$ of the trans-$\alpha^2$ complexes were remarkably larger than those of the corresponding $\alpha^4$ complexes, depending on both shape and size of pickets; the stability enhancements by pivalamido pickets were a factor of 20 and 34 in $K$ for pyridine and isoquinoline, respectively, while those by (butylcarbonyl)amino pickets were only a factor of 2.6 and 3.7. These stabilizations of the trans-$\alpha^2$ complexes were due to increased binding energy ($-\Delta\Delta H$ = 0.8–2.3 kcal/mol), although accompanied by a slight decrease of entropy ($-\Delta\Delta S$ = 0.1–2.2 eu). It was concluded that the stability enhancements of the trans-$\alpha^2$ complexes can be ascribed as the attractive intramolecular CH–$\pi$ interactions induced by the London dispersion force in base adducts rather than solvation effects in the weakly polar solvent. Comparisons of the shape of picket fences with both thermodynamic values and $^1H$ NMR chemical shifts suggested that preorganization of the cavity is essential to the stability enhancements. The variation of binding constants in several noncoordinating solvents also suggested that desolvation processes upon the ligation of free base considerably affect the stability factor $K(\text{trans-}\alpha^2)/K(\alpha^4)$ in this case.

## Introduction

Hydrophobic interactions are well-recognized to occur in certain biomolecules and provide the flexibility and specificity required in biochemical processes.[2] Recently, attention has also been directed toward the interactions in artificial systems containing a metal ion in order to understand the fundamental nature of substrates binding to metalloenzymes.[3,4] The contribution of these noncovalent interactions to binding energy ($\Delta H$) may be small but critical to the organic inclusion complex formation[5-7] and also to the stereoselectivity or stability enhancements of metal complexes.[8,9] The nature of hydrophobic interactions are, however, not clear, since such attractive interactions have been observed only in water or polar nonaqueous solvents. This is probably due to the greater solvent liberation driving forces, although the London dispersion force may also play a significant role.[6,7] On the other hand, few works have dealt with porphyrin complexes for the purpose of studying the interactions, despite the importance of those compounds as native metalloenzymes in biological systems. Rather, the chemical behavior of most model porphyrin complexes has been investigated to mimic the biological functions of natural hemoproteins.

Picket fence porphyrin Fe(II) and Co(II) complexes, developed by Collman et al., are an excellent model of natural hemoproteins, and their static and dynamic properties have been studied extensively by using a variety of physicochemical methods.[10-12] These compounds have four atropisomers by the restricted rotation of the phenyl rings,[11] and some interesting points have appeared

(1) Preceding paper: Imai, H.; Kyuno, E. *Inorg. Chim. Acta* **1988**, *153*, 175.
(2) Frieden, E. *J. Chem. Educ.* **1975**, *52*, 754.
(3) Meade, T. J.; Busch, D. H. *Prog. Inorg. Chem.* **1985**, *33*, 59.
(4) Ōkawa, H. *Coord. Chem. Rev.* **1988**, *92*, 1.
(5) (a) Takeuchi, K. J.; Busch, D. H. *J. Am. Chem. Soc.* **1983**, *105*, 6812. (b) Meade, T. J.; Kwik, W.; Herron, N.; Alcock, N. W.; Busch, D. H. *Ibid.* **1986**, *108*, 1954. (c) Meade, T. J.; Takeuchi, K. J.; Busch, D. H. *Ibid.* **1987**, *109*, 725.
(6) (a) Tabushi, I.; Kiyosuke, Y.; Sugimoto, T.; Yamamura, K. *J. Am. Chem. Soc.* **1978**, *100*, 916. (b) Tabushi, I.; Kuroda, Y.; Mizutani, T. *Tetrahedron* **1984**, *40*, 545. (c) Tabushi, I.; Kuroda, Y.; Mizutani, T. *J. Am. Chem. Soc.* **1986**, *108*, 4514.
(7) For example: Diederich, F.; Dick, K.; Griebel, D. *J. Am. Chem. Soc.* **1986**, *108*, 2273.
(8) (a) Fischer, B. E.; Sigel, H. *J. Am. Chem. Soc.* **1980**, *102*, 2998. (b) Sigel, H.; Fischer, B. E.; Farkas, E. *Inorg. Chem.* **1983**, *22*, 925. (c) Liang, G.; Tribolet, R.; Sigel, H. *Ibid.* **1988**, *27*, 2877.
(9) Ōkawa, H.; Ueda, K.; Kida, S. *Inorg. Chem.* **1982**, *21*, 1594.
(10) Collman, J. P.; Halbert, T. R.; Suslick, K. S. In *Metal Ion Activation of Dioxygen*; Spiro, T. G., Ed.; Wiley: New York, 1980; Chapter 1.
(11) Collman, J. P.; Gagne, R. R.; Reed, C. A.; Halbert, T. R.; Lang, G.; Robinson, W. T. *J. Am. Chem. Soc.* **1975**, *97*, 1427.
(12) Collman, J. P.; Brauman, J. I.; Doxsee, K. M.; Halbert, T. R.; Hayes, S. E.; Suslick, K. S. *J. Am. Chem. Soc.* **1978**, *100*, 2761.

0020-1669/90/1329-2416$02.50/0 