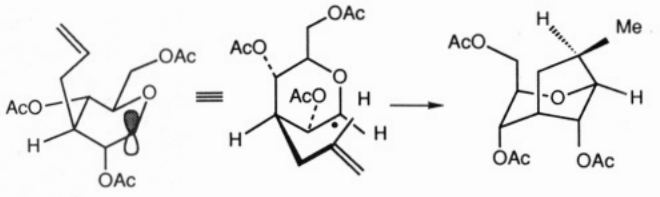
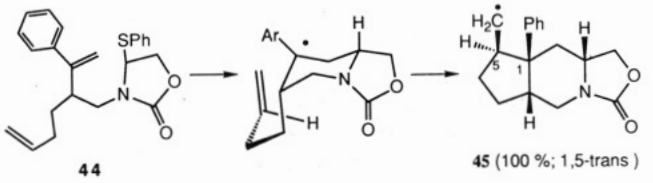


There are a number of other instances[43–45] where the heuristic models presented above can be used to rationalize unusual stereochemical outcomes in hexenyl radical cyclizations including an interesting case of a tandem cyclization (Scheme XIV).[46]

Scheme XIV
Stereochemistry of a Tandem Cyclization



Conclusions

In this study of cyclizations of conformationally rigid 2-but-3-enylcyclohexyl radicals, we have conclusively shown that the stereochemical outcome of the reaction is critically influenced by the orientation of the butenyl side chain; an equatorial butenyl group leads predominantly to 1,5-cis cyclization products, whereas an axial butenyl group preferentially gives rise to 1,5-trans products.[47] These stereochemical consequences can be satisfactorily accounted for by the cyclohexane "chair-like" transition states originally proposed by Beckwith and co-workers for *acyclic* hex-5-enyl radical cyclizations. In related but conformationally less rigid systems, the transition states having an equatorial and an axial butenyl side chain may compete. Minor ring-closure products also may arise from less favorable "boat-like" transition states. As shown in several of the sugar-derived radicals, the formation of these boat-like transition states may sometimes be helped by the configuration at the C_4 center. In the presence of a C_4 substituent, the local allylic conformation dictates the choice between the "chair-like" and "boat-like" transition states, and the one with the lowest 1,3-strain controls the course of the reaction. This results in an unprecedented control of the 1,5-stereoselectivity of the hex-5-enyl radical cyclization. In systems with C_3 and C_4 substituents, the C_4 substituent is the control element and the C_3 substituent exerts only a marginal influence on the 1,5-stereoselection. Special effects, such as the stabilization a β-CO-σ^* provides for an α-oxy radical, should be taken into account before considering these models.

Finally the predictive value of these conformational models is illustrated with several examples from the literature. We believe that these heuristic models and the carbohydrate to carbocycle conversion protocols developed during the course of these investigations will become valuable tools in planning the synthesis of highly functionalized cyclopentanoid natural products.

I acknowledge valuable discussions with Dr. T. Fukunaga. I am deeply indebted to Drs. W. A. Nugent and A. Casalnuovo for critical review of the manuscript. Dr. G. W. Parshall has been a constant source of encouragement and support, and his leadership role is gratefully appreciated. Technical assistance was provided by Mr. T. H. Kline, Jr., and Mrs. M. S. Beattie.

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(47) Since alkylation of cyclohexanones can be carried out to produce axial (kinetic) or equatorial (thermodynamic) 2-but-3-enyl ketones, this control element can be parlayed into annulation stereochemistry by the appropriate choice of radical cyclization methodology.

Developing Artificial Hydrolytic Metalloenzymes by a Unified Mechanistic Approach

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Nature has developed many hydrolytic metalloenzymes. They have evolved to hydrolyze some of the most important molecules of life including proteins, phospholipids, and DNA. Over the years numerous hydrolytic metalloenzyme models have been designed and studied. Much has been learned through elegant designs and careful analyses of simple enzyme models. However, a major difference between enzymes and their models is their reactivity. In most enzyme model studies, either the substrates are highly activated or they are permanently anchored to various catalytic groups preventing any catalytic turnover. The principal focus of this account is on true catalysts that hydrolyze unactivated substrates with catalytic turnover. A mechanisitically unified approach to developing metal complexes that hydrolyze esters,[1–3] amides,[4] nitriles,[5]

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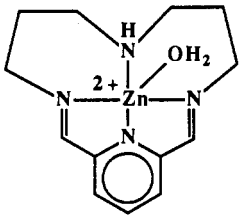
0001-4842/91/0124-0145$02.50/0

and phosphate monoesters[6] and diesters[7-11] is examined in this account.

Esters

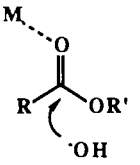
Compared to amides, nitriles, and phosphate esters, carboxylate esters are the most labile toward hydrolysis. Even so, in most metalloesterase model studies either the esters are highly activated or they are covalently linked to various metal-binding groups.[12-17] Model studies involving esters with good leaving groups must be interpreted with caution because the structural requirements of a catalyst for hydrolyzing activated esters are not necessarily the same as those for hydrolyzing unactivated esters or amides.[18,19]

It is well-known that imidazole is an efficient nucleophilic catalyst for hydrolyzing *p*-nitrophenyl acetate but not a nucleophilic catalyst at all for hydrolyzing esters with poor leaving groups.[19] Imidazole is almost as reactive as hydroxide for hydrolyzing esters with good leaving groups (e.g., *p*-nitrophenyl acetate, acetic anhydride) yet millions of times less reactive than hydroxide for hydrolyzing esters with poor leaving groups (e.g., methyl acetate, methyl trifluoroacetate). Interestingly, substitutionally labile metal-monoaqua complexes such as **1** efficiently catalyze the hydrolysis of *p*-nitrophenyl acetate and methyl trifluoroacetate but not methyl acetate.[1]

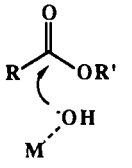


1

In principle, **1** could catalyze the hydrolysis of methyl trifluoroacetate by the Lewis acid mechanism (a) or by the metal hydroxide mechanism (b). The two mech-



a



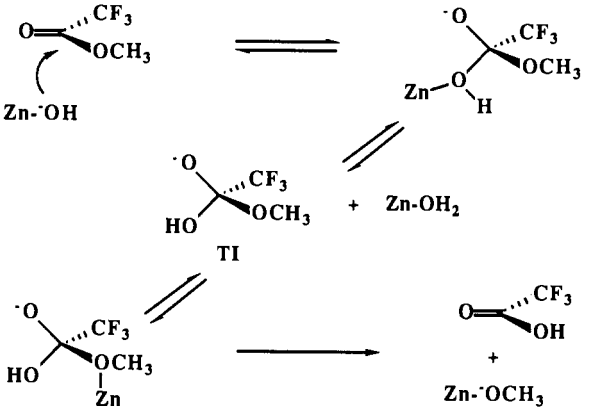
b

anisms are kinetically indistinguishable, but the Lewis acid mechanism can be ruled out on the basis of the reactivity–selectivity principle.[20] According to the reactivity–selectivity principle, selectivity decreases with increase in reactivity. Since metal-coordinated esters are more reactive toward nucleophilic attack than the corresponding free esters, hydroxide should be less selective toward the metal-corrdinated esters than toward the free esters. It follows that the less reactive the ester, the greater the expected rate acceleration upon coordination of the ester to the metal. Experimentally, a greater rate acceleration over the hydroxide rate is observed for methyl trifluoracetate hydrolysis than for methyl acetate hydrolysis, inconsistent with the Lewis acid mechanism.

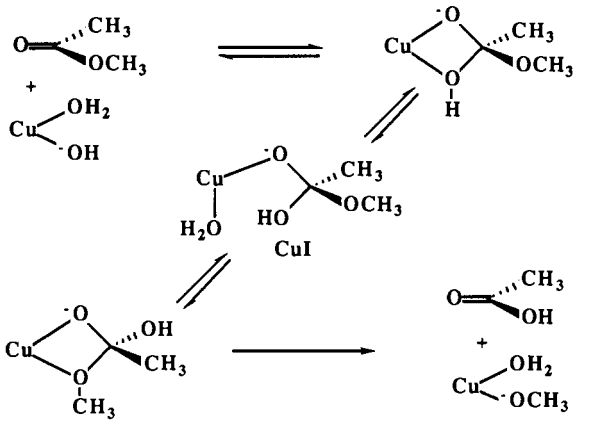
Scheme I shows a metal hydroxide mechanism for 1-catalyzed hydrolysis of methyl trifluoracetate.

Two questions may be raised at this point. (1) Why should **1** hydrolyze methyl trifluoroacetate but not methyl acetate by the mechanism shown in Scheme I? (2) What is the structural requirement of a catalyst for hydrolyzing methyl acetate? The lifetime of the tetrahedral intermediate for methyl trifluoroacetate hydrolysis (**TI**, Scheme I) is much longer than that for methyl acetate hydrolysis.[21] There is enough time for **TI** to coordinate to the zinc complex and break down to the starting material or to the product. If the breakdown of **TI** is catalytic, its formation must also be catalytic according to the principle of microscopic reversibility. Compared to the lifetime of **TI**, the life-

Scheme I



Scheme II



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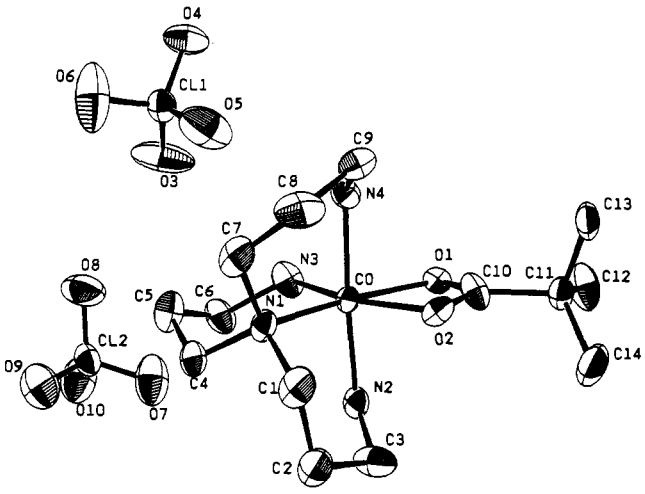
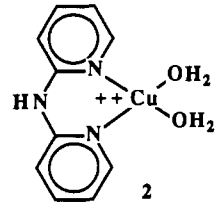


Figure 1. ORTEP diagram of the four-membered-ring pivalato complex of $[(trpn)Co(OH_2)_2]^{3+}$.

time of the corresponding tetrahedral intermediate for methyl acetate hydrolysis is too short-lived to coordinate to the zinc complex. As a result, the metal hydroxide mechanism is incompatible with methyl acetate hydrolysis. Clearly, the lifetimes of intermediates play an important role in determining the catalytic mechanism.[22-24]

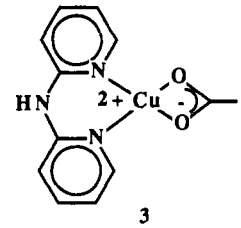
The second question above may now be addressed. For catalytic hydrolysis of methyl acetate, the lifetime of the tetrahedral intermediate corresponding to **TI** (Scheme I) should be increased and the metal migration should be made to be more efficient. Both of these goals may be realized with a *cis*-diaqua metal complex (Scheme II). The only difference between Schemes I and II is that in Scheme II the anionic oxygen of the tetrahedral intermediate is coordinated to the metal (**CuI**, Scheme II). This should stabilize the intermediate and at the same time facilitate the intramolecular metal migration. Indeed, **2** hydrolyzes methyl acetate with catalytic turnover under mild conditions.[2]



2

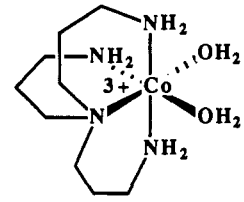
A common misconception is that catalysts that are the most reactive toward activated substrates should also be the most reactive toward unactivated substrates. **2** is slightly *less* reactive than **1** or imidazole for hydrolyzing *p*-nitrophenyl acetate, yet **2** is the most reactive by at least 2 orders of magnitude for hydrolyzing methyl acetate.

Scheme II shows the formation of four-membered-ring copper complexes for **2**-catalyzed hydrolysis of methyl acetate. Although in general four-membered rings are unstable, **2** is known to form four-membered rings easily. For example, acetate binds to **2** as a bidentate ligand (**3**). The crystal structure of **3** has been reported.[25]

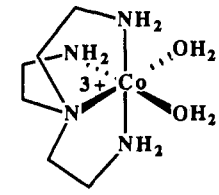


3

Like *cis*-diaqua Cu(II) complexes, *cis*-diaqua Co(III) complexes can also be made to catalyze the hydrolysis of methyl acetate.[3] *cis*-$[(trpn)Co(OH_2)_2]^{3+}$ (**4**) efficiently catalyzes the hydrolysis of methyl acetate with catalytic turnover whereas *cis*-$[(tren)Co(OH_2)_2]^{3+}$ (**5**) is not active [trpn, tris(aminopropyl)amine; tren, tris(aminoethyl)-amine]. As in **3**, sodium acetate readily forms the

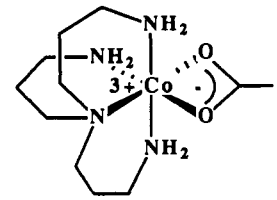


4

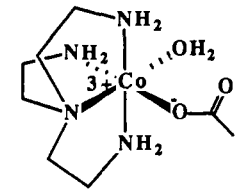


5

four-membered-ring acetato complex (**4Ac**) with **4** but it forms only the monodentate complex (**5Ac**) with **5**. This shows the importance of the tetramine ligand structure in stabilizing the four-membered ring. Figure 1 shows the crystal structure of a four-membered-ring carboxylato complex of **5**.[26]



4Ac



5Ac

Amides

Amides are thousands of times less reactive than esters toward hydroxide. For this reason peptidases are generally efficient esterases. There are many zinc peptidases in nature. The most widely studied zinc peptidase is carboxypeptidase A, an enzyme that catalyzes the hydrolysis of the amide bonds in proteins from the carboxy terminus. Much has been learned about carboxypeptidase A through kinetic and mechanistic studies,[27] site-directed mutagenesis,[28] and X-ray crystallography.[29] Other zinc proteases related to carboxypeptidase A such as angiotensin converting enzyme, enkephalinase, collagenase, and subtilisin have been the target for drug design.

Over the years there has been a great deal of interest in developing artificial enzymes that hydrolyze amides.[30-32] Due mainly to the stability of amides[33-36] and

(22) Jencks, W. P. *Acc. Chem. Res.* **1980**, *13*, 161.
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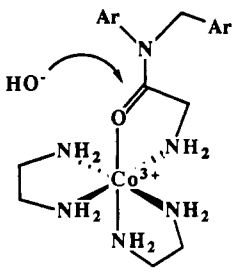
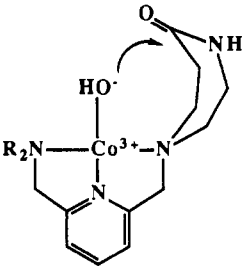
the difficulty associated with monitoring the hydrolysis reaction, most of the artificial enzymes have been tested for hydrolyzing *p*-nitrophenyl esters rather than for hydrolyzing amides. There is a real need to test the activity of artificial enzymes directly on amides since the structural requirements of a catalyst for hydrolyzing a *p*-nitrophenyl ester are not the same as those for hydrolyzing an amide. Recently, Still and Kahne[37] developed a sensitive assay for detecting trace hydrolysis of unactivated amides. Lerner and Iverson[38] developed a metallocatalytic antibody that hydrolyzes amides.

Kroll's discovery[39] in 1952 of Cu^{2+}-catalyzed hydrolysis of amino acid esters has triggered an intensive research effort into finding the mechanistic role of the metal ion in this and other hydrolysis reactions including those catalyzed by hydrolytic metalloenzymes. As in the ester hydrolysis, the Lewis acid mechanism (a) and the metal hydroxide mechanism (b) emerged as the two most likely. These two mechanisms have been the subject of much debate for several decades.[32] Buckingham et al. used substitutionally inert Co^{3+} complexes to show that both the Lewis acid and the metal hydroxide mechanisms are highly efficient for hydrolyzing glycinamide tethered to the metal ion via the amine group. More recently, research teams led by Groves[40] and by Breslow[41] studied elegant models of carboxypeptidase A. In the model designed by Breslow (**6**), the amide bond is hydrolyzed by the Lewis acid mechanism, whereas in Groves's model (**7**), the amide bond is hydrolyzed by the metal hydroxide mechanism. The amide bonds in both models have been shown to hydrolyze with spectacular rates.

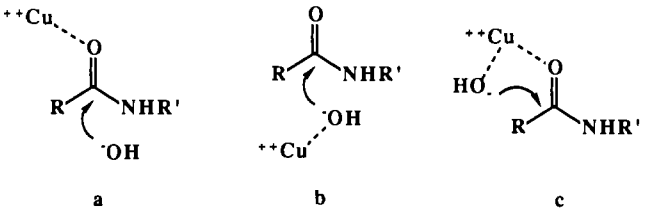


6



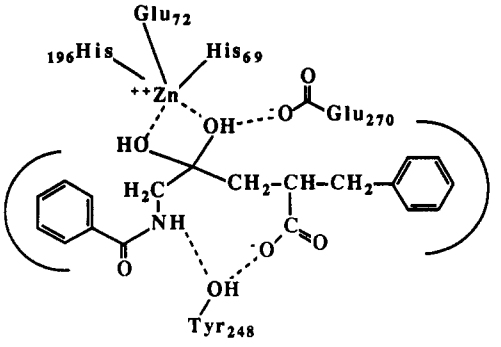
7

Our own interest[4] in determining the structural requirements of a simple metal complex for hydrolyzing unactivated amides led us to consider a thrid mechanism (c), in which the Lewis acid (a) and the metal hydroxide (b) mechanisms are combined. The reactivities of **1** and **2** for hydrolyzing formamides were compared. If either the Lewis acid mechanism or the metal hydroxide mechanism is more efficient than mechanism c, then the reactivity of monoaqua metal complexes should be comparable to that of diaqua



metal complexes. On the other hand, if mechanism c is the most efficient, then diaqua metal complexes should be more reactive than monoaqua metal complexes. **2** is over 2 orders of magnitude more reactive than **1** for hydrolyzing formamide, *N*-methyl formamide, and *N,N*-dimethylformamide (neutral pH, 100 °C), supporting mechanism c. It is unlikely that this difference in the reactivity is due to any steric effect since **1** is slightly *more* reactive than **2** in hydrolyzing *p*-nitrophenyl acetate. Furthermore, it is unlikely that the metal hydroxide in mechanism c is a general base catalyst because no solvent kinetic isotope effect was found.

Interestingly, the mechanism for **2**-catalyzed hydrolysis of formamides appears to parallel the mechanism for carboxypeptidase A catalyzed hydrolysis of peptides. Christianson and Lipscomb[42] determined the X-ray structure (**8**) of a ketone bound to carboxypeptidase A. Surprisingly, the ketone is in its hydrated



8

form with both oxygens of the *gem*-diol bound to the active-site zinc of carboxypeptidase A. **2**-catalyzed hydrolysis of formamides (mechanism c) and carboxypeptidase A catalyzed hydrolysis of peptides (**8**) both involve the formation of a four-membered-ring bidentate metal complex. Hence the crystal structure (**8**) supports mechanism c. It appears that, in some distant past, nature in its infinite wisdom chose the double activation path.

The mechanisms for **2**-catalyzed hydrolysis of unactivated esters (Scheme II) and amides (mechanism c) are analogous in that both involve coordination of the substrate to the metal followed by intramolecular metal hydroxide attack with formation of the four-membered-ring intermediate. In the ester hydrolysis, metal migration (Scheme II) follows the initial four-membered-ring formation, whereas proton transfer from oxygen to nitrogen takes place in the amide hydrolysis.[4]

Nitriles

Nitriles are quite resistant to hydrolysis. For example, the second-order rate constants for hydroxide-catalyzed hydrolysis of methyl acetate,[21] acetonitrile,[43]

(31) Buckingham, D. A.; Harrowfield, J. M.; Sargeson, A. M. *J. Am. Chem. Soc.* **1974**, *96*, 1726.
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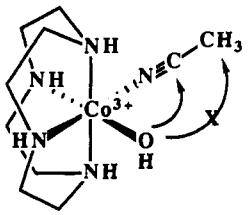
(42) Christianson, D. W.; Lipscomb, W. N. *Acc. Chem. Res.* **1989**, *22*, 62.

and acetamide[44] are 1.5×10^{-1} M^{-1} s^{-1}, 1.6×10^{-6} M^{-1} s^{-1}, and 7.4×10^{-5} M^{-1} s^{-1}, respectively. Cu^{2+}, Ni^{2+}, and Zn^{2+} have all been shown to catalyze the hydration of nitriles when the nitrile function is covalently linked to metal-coordinating functionalities.[45,46] Hence, 2-cyanopyridine and 2-cyano-1,10-phenanthroline are rapidly hydrated to the corresponding amides when Cu^{2+} is added to the aqueous reaction mixture under mild conditions. Pt^{2+} complexes have been shown to catalyze the hydration of simple nitriles that do not contain any additional metal-coordinating functionalities under reflux conditions.[47,48] Simple nitriles directly coordinated to Co(III),[43] Rh(III),[49] Ru(III),[50,51] and Ir(III)[49] complexes can be easily hydrated to the corresponding amido complexes at ambient temperature. However, there is no catalytic turnover with the substitutionally inert Co(III), Rh(III), Ru(III), and Ir(III) complexes. We[5] recently showed that $[(cyclen)Co(OH_2)_2]^{3+}$ efficiently catalyzes the hydration of acetonitrile to acetamide in neutral water at 40 °C (cyclen, 1,4,7,10-tetraazacyclododecane). Interestingly, more than 10 turnovers of the catalyst were detected. At neutral pH the turnover time is about 3×10^3 s. Although Co(III) complexes are generally substitutionally inert, the coordinated water molecules in $[(cyclen)Co(OH_2)_2]^{3+}$ can be rapidly displaced under mild conditions. The catalytic mechanism involves coordination of the nitrile functionality followed by intramolecular metal hydroxide attack on the cobalt-bound nitrile. There is twofold experimental evidence for the hydration mechanism. First, acetonitrile bound to $[(cyclen)Co(OH_2)_2]^{3+}$ (**9**) is hydrated much more rapidly than acetonitrile bound to $[(NH_3)_5Co(OH_2)_2]^{3+}$ (**10**).[43] Second, hydration of **10** but not **9** is accompanied by proton exchange of the methyl group. Interestingly, this mechanism allows for clean conversion of acrylonitrile to acrylamide without hydrating the carbon–carbon double bond (**11**, **12**).

As was the case for unactivated ester and amide hydrolysis, the most efficient mechanism for nitrile hydration involves coordination of the substrate to the metal followed by intramolecular metal hydroxide attack with formation of a four-membered-ring intermediate.

Phosphate Monoesters

Phosphate monoesters such as adenosine monophosphate (AMP) and methyl phosphate are highly resistant toward hydrolysis. The unimolecular rate for monoanionic methyl phosphate hydrolysis ($k = 2.7 \times 10^{-10}$ s^{-1} at 25 °C)[52] is comparable in value to the water rate for methyl acetate hydrolysis ($k = 3 \times 10^{-10}$ s^{-1} at 25 °C).[53] Cobalt complexes of the type *cis*-$[(N_4)Co(OH_2)_2]^{3+}$, where N_4 is any tetramine ligand, have been used extensively by various research teams as ATPase models and phosphatase models. Most of the model studies have been focused on phosphate anhydrides [e.g., adenosine triphosphate (ATP)] or phosphate monoesters with good leaving groups (e.g., *p*-nitrophenyl phosphate).[54-62] Unactivated phosphate monoesters [e.g., adenosine monophosphate (AMP), methyl phosphate] are much more resistant to hydrolysis.[63]



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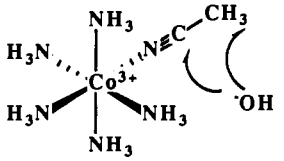

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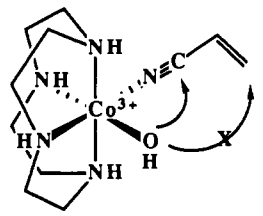

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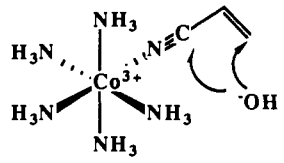

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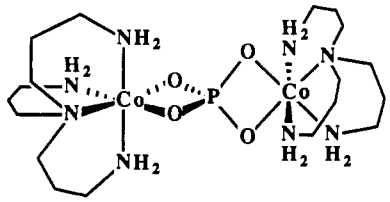
Our interest in determining the structural requirements of a simple metal complex for hydrolyzing phosphate monoesters led us to investigate $[(trpn)Co(OH_2)_2]^{3+}$ (**4**) and $[(tren)Co(OH_2)_2]^{3+}$ (**5**) promoted hydrolysis of methyl phosphate and AMP.[6] Interestingly, adding 2 equiv of **4** to methyl phosphate or AMP at pH 5, 25 °C, resulted in hydrolysis of the phosphate monoesters with concomitant formation of a novel binuclear complex (*13*) in about 20 min. The binuclear complex consists of a doubly bidentate μ-phosphato bridge. **13** can also be formed by simply adding **4** to inorganic phosphate. Adding 1 equiv of **4** to methyl



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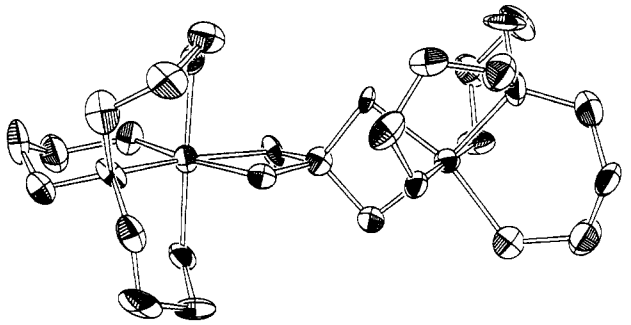
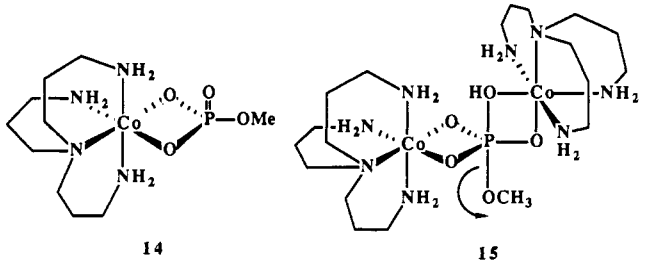


Figure 2. ORTEP diagram of compound 13.

phosphate resulted in the formation of a stable mononuclear complex (14). A second equivalent of 4 is necessary for the hydrolysis reaction to take place (15). This represents an amazing tetrafunctional activation for hydrolyzing phosphate monoesters with poor leaving groups. Bifunctional catalysis has been demonstrated for *cis*-diaqua metal complex promoted hydrolysis of phosphate monoesters with good leaving groups[54-62] as well as for hydrolysis of phosphate esters[64-65] and phosphate triesters.[66,67]

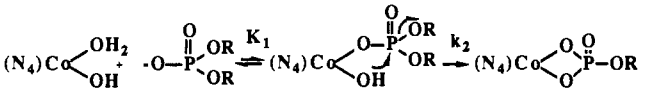


14 15

Interestingly, $[(tren)Co(OH_2)(OH)]^{2+}$ did not hydrolyze phosphate monoesters with poor leaving groups under the same conditions used to hydrolyze the esters with $[(trpn)Co(OH_2)(OH)]^{2+}$. Furthermore, in contrast to the ease of formation of $[((trpn)Co)_2PO_4]^{4+}$ (13), the corresponding cobalt complex with the tren ligand, $[((tren)Co)_2PO_4]^{4+}$, could not be synthesized.

Figure 2 shows the crystal structure of compound 13. The interesting structural features of this compound are the two four-membered rings connected at phosphorus. The two four-membered rings are planar and oriented perpendicularly to one another. Recently, Armstrong et al.[68] determined the crystal structure of a binuclear Li(I) complex that contains two four-membered rings connected at V(II). In that case, the two four-membered rings are almost coplanar. The values of the O–Co–O bond angles and the O–P–O bond angles included in the four-membered rings in compound 13 are 74° and 100°, respectively. In contrast, the O–Co–O bond angles in the four-membered-ring Co(III) carbonato complexes are 68°.[69] The O–Co–O bond angle and the O–P–O bond angle in the four-membered-ring Co(III) phosphato complex are 76.0° and 98.7°, respectively.[70]

Scheme III



The crystal structure of compound 13 reveals some valuable and interesting insights into the structural requirements of a binuclear metal complex for hydrolyzing phosphate monoesters.[71] First, two *cis*-diaqua metal complexes that easily form four-membered rings are required. Second, the two *cis*-diaqua metal complexes should be arranged *perpendicularly* to one another as in 13 or as in the proposed intermediate (15) for the hydrolysis reaction. This is indicated by the 90° dihedral angle between the two four-membered rings in 13. Such an arrangement is not found in most simple binuclear metal complexes.[72,73] Molecular mechanics calculation shows that the distance between the cobalt atoms in 13 is 5.08 Å, in excellent agreement with the corresponding crystallographic data (5.05 Å). The cobalt to cobalt distance in 15 is somewhat less (4.62 Å) according to calculations. It is interesting that 4, like purple acid phosphatases,[73-75] hydrolyzes phosphate monoesters efficiently by a bimetallic process. Furthermore, both purple acid phosphatase and the cobalt complex reach maximum efficiency for hydrolyzing phosphate monoesters at pH 5.

Although the mechanism for 4-promoted hydrolysis of methyl phosphate is different from the for *cis*-diaqua metal complex catalyzed hydrolysis of esters, amides, and nitriles, all of the mechanisms are related in that they go through four-membered-ring intermediates. To this end *cis*-diaqua metal complexes that easily form four-membered rings should be good catalysts for hydrolyzing carboxylate esters, amides, nitriles, and phosphate monoesters.

Phosphate Diesters

In the field of artificial hydrolytic metalloenzymes, the ultimate challenge is to hydrolyze phosphate diesters. In neutral water, phosphate diesters are by far the most stable compared to esters, nitriles, amides, phosphate monoesters, and phosphate triesters. The hydroxide rate for even the highly activated phosphate diester bis(*p*-nitrophenyl) phosphate (BNPP)[76,77] is 10 times slower than the hydroxide rate for acetamide[44] hydrolysis. In this sense, it is not surprising that nature chose the phosphate diester linkage for preserving the genetic material.[78] The half-life for hydrolytic cleavage of the phosphate diester bonds in DNA has been estimated[10] to be on the order of 200 million years (pH 7, 25 °C). Many DNases hydrolyze DNA within seconds. Even more remarkable is a class of DNases called re-

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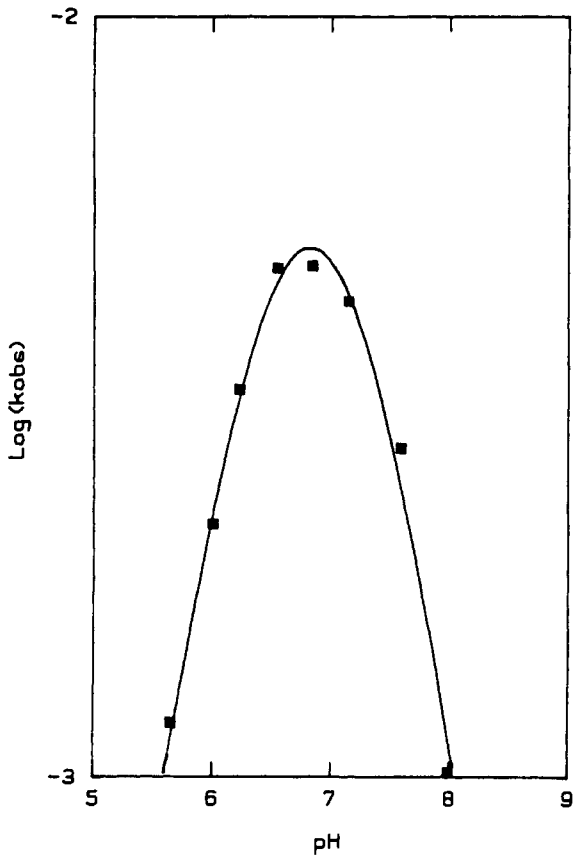
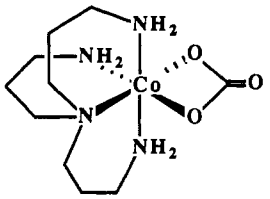


Figure 3. pH–rate profile for $[(cyclen)Co(OH_2)_2]^{3+}$ (0.01 M) promoted hydrolysis of BNPP (10^{-5} M) at 50 °C.

striction enzymes that hydrolyze DNA sequence specifically. Most DNases as well as ribozymes[79,80] are activated by metal ions.

Currently there is considerable interest in developing artificial DNases and artificial restriction enzymes.[81-85] To date there is no known artificial enzyme that can hydrolyze in neutral water simple phosphate diesters like dimethyl phosphate. The mechanism for *cis*-diaqua cobalt(III) complex promoted hydrolysis of BNPP involves rapid coordination of the ester followed by rate-determining intramolecular metal hydroxide attack (Scheme III).[8] Figure 3 shows a typical pH–rate profile for $[(cyclen)Co(OH_2)_2]^{3+}$-promoted hydrolysis of BNPP. The pK_a values of the two cobalt-bound water molecules in $[(cyclen)Co(OH_2)_2]^{3+}$ are 5.6 and 8.0, respectively. Consistent with the mechanism shown in Scheme III, maximum rate is obtained at neutral pH when the cobalt complex is in the aqua hydroxy form.

Interestingly, the rate-determining step (k_2, Scheme III) is highly sensitive to the tetramine ligand (N_4) structure. For example, **4** ($[(trpn)Co(OH_2)_2]^{3+}$) is 300 times more reactive than **5** ($[(tren)Co(OH_2)_2]^{3+}$) in hydrolyzing BNPP, even though the equilibrium constants (K_1, Scheme III) for binding of phosphate diesters to **4** and **5** are comparable. This result is striking considering that the two cobalt complexes are so closely related structurally. The k_2 step involves the formation of a four-membered ring. We recently determined the crystal structure of the four-membered-ring carbonato complex (**16**) of **4**. The crystal structure of the corresponding carbonato complex (**17**) of **5** has also been determined.[69]



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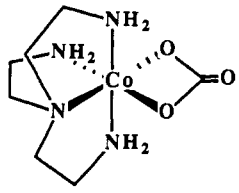

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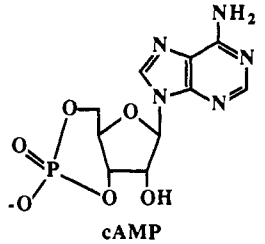
X-ray structures of $[(trpn)Co(CO_3)]^+$ and $[(tren)Co(CO_3)]^+$ reveal that the trpn ligand is better able to stabilize four-membered rings. Both O–Co–O bond angles in $[(trpn)Co(CO_3)]^+$ (68°) and $[(tren)Co(CO_3)]^+$ (68°) are highly distorted from those found in regular octahedral complexes (90°). All the N–Co–N bond angles are rigidly held (87°) with the tren ligand, whereas the N–Co–N bond angle opposite the O–Co–O bond angle in $[(trpn)Co(CO_3)]^+$ is free to expand to 100°. It appears that a major factor in stabilizing the four-membered Co(III) complexes is increasing the bond angle opposite the four-membered ring.[11]

The half-life for BNPP hydrolysis is about 100 years at neutral pH, 25 °C.[86] Under the same conditions, BNPP bound to **4** is hydrolyzed within a couple of seconds. This corresponds to about a 10 billion fold rate enhancement. Indeed, the reactivity of BNPP bound to **4** is comparable to that of BNPP bound to a real enzyme from *Enterobacter aerogenes*.[87]

cis-Diaqua Co(III) complexes are also efficient at hydrolyzing phosphate diesters with poor leaving groups. For example, the second-order rate constant for $[(trien)Co(OH_2)_2]^{3+}$-promoted hydrolysis of 3',5'-cyclic adenosine monophosphate (cAMP)is 6×10^{-6} M^{-1} s^{-1} (trien: triethylenetetramine).[7] Due to the ring strain, cAMP is more reactive than acylic phosphate diesters. Hydroxide-catalyzed hydrolysis of cAMP is about 100 times faster than hydroxide-catalyzed hydrolysis of dimethyl phosphate.[88] The challenge of developing catalysts that hydrolyze totally unactivated phosphate diesters (e.g., DNA, dimethyl phosphate) still remains.



cAMP

In summarizing, a number of key points can be made concerning the unified approach to developing artificial hydrolytic metalloenzymes. *cis*-Diaqua metal complexes are efficient catalysts for hydrolyzing unactivated esters such as methyl acetate. The catalytic mechanism involves coordination of the substrate to the metal followed by intramolecular metal hydroxide attack on the metal-bound ester forming a four-membered-ring

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intermediate. Such bifunctional activation plays an important role not only in the hydrolysis of unactivated esters but also in the hydrolysis of nitriles, amides, and phosphate mono-, di-, and triesters. Amazingly, unactivated phosphate monoesters such as methyl phosphate can be hydrolyzed by tetrafunctional activation. A unifying theme for all of the above hydrolysis reactions is that one or more *cis*-diaqua metal complexes that can easily form four-membered rings are required. The ease of octahedral *cis*-diaqua Co(III) complexes in forming four-membered rings is highly sensitive to the tetramine ligand structure. With an appropriate tetramine ligand, a cobalt complex can bring about a 10 billion fold rate enhancement in the hydrolysis of phosphate diesters. Understanding the structure–reactivity relationship of these metal complexes is important for developing artificial restriction proteases[89-91] and nucleases[92] that can hydrolyze proteins and nucleic acids sequence specifically.

I am grateful to my talented students whose names appear in the original references. Financial support was provided by the Natural Sciences and Engineering Research Council of Canada and the U.S. Army Research Office.

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RNA Pseudoknots

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The perception of ribonucleic acid (RNA) has undergone a radical change during the past 10 years. RNA is now known to be integrally involved in each step in the transmission of genetic information. The genetic code is stored in deoxyribonucleic acid (DNA) molecules and ultimately converted to the amino acid sequences of proteins. The DNA strand is first transcribed into a messenger RNA (mRNA) intermediate. The mRNA is then processed: large regions are excised by the spliceosome, a large protein and RNA complex. Finally, the mRNA is translated into a protein by the ribosome, another giant complex of RNA and protein. RNA not only serves as a carrier of the genetic information but also helps regulate and catalyze the reactions involved in protein synthesis.[1] RNA molecules alone can catalyze chemical reactions, such as cleavage and synthesis of phosphodiester bonds.[2]

The enviable specificity of biochemical reactions catalyzed by enzymes results from the specific three-dimensional structure formed by the protein's polypeptide chain. Like protein-mediated reactions, the specific biochemical functions involving RNA are guided by the three-dimensional folding of the polynucleotide. Unfortunately, relatively little is known about RNA conformations. RNA molecules form intramolecular structures; the polynucleotide chain folds back upon itself to form double-helical regions held together by Watson–Crick base pairs. The double helices are connected by single-stranded regions containing unpaired bases. The two-dimensional map of base pairs is called the secondary structure. The secondary structure of an RNA consists of double-helix regions, called stems, and single-stranded regions termed bulge loops (bulges), internal loops (bubbles), hairpin loops, and junctions (Figure 1).[3] Tertiary interactions are those between the elements of the secondary structure; they include tertiary base pairing, single strand–helix interactions, and helix–helix interactions.

A pseudoknot is a type of tertiary interaction that involves base pairing between nucleotides in a loop with nucleotides in a single-stranded region outside the loop.[4] Thus, a pseudoknot has two double-helical stem regions and two loop regions (Figure 2). The term pseudoknot was coined in order to exclude this type of interaction from the possible conformations considered by algorithms used to predict secondary structures. Inclusion of pseudoknots or knots prevents an exhaustive search of possible secondary structures (see Figure 3). If each of the ends of the RNA chain passes through a loop, then an overhand knot is formed; pulling on the two ends of the strand result in a knot. This possibility could arise only if both stems contained full turns of the helix. The biologically relevant interaction is called a pseudoknot; only one or neither of the ends extends through a loop, and a figurative pull on the ends does not lead to a knot. It is important to distinguish this common usage of the term knot from its definition in topology.[5]

Pseudoknots seem to be a widely used motif in RNA structure; they are proposed to have significant roles in a variety of RNA molecules.[6,7] They were originally

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