Synthetic Analogues Relevant to the Structure and Function of Zinc Enzymes

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1. Introduction

Zinc is essential to all forms of life,[1−4] with an average adult human containing ca. 3 g of zinc.[5] For example, zinc is indispensable for effective growth and development, has beneficial therapeutic and preventative effects on infectious diseases such as malaria and pneumonia,[6] and has been proposed to shorten the length of the common cold in adults.[7] The influence of zinc derives from its roles in enzymes, with functions that are both structural and catalytic. Indeed, there are approximately 300 zinc enzymes, with representatives known for each of the fundamental enzyme classes (oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases).[1d]

Much of the importance of zinc enzymes derives from their peptidase and amidase activity involving the cleavage of RC(O)−NH(R′) amide bonds. For example, with respect to peptidase activity, zinc enzymes include both endopeptidases (cleaving peptides or proteins at positions within the chain) and exopeptidases (cleaving a terminal amino acid from the chain), as illustrated in Figure 1. Of the exopeptidases, zinc enzymes function as both carboxypeptidases (which removes a C-terminal amino acid) and aminopeptidases (which remove a N-terminal amino acid). Other examples of zinc enzymes that function by cleaving amide bonds are (i) β-lactamases that destroy β-lactams (such as penicillin) by hydrolyzing and cleaving the four-membered lactam ring and (ii) matrix metalloproteinases that degrade extracellular matrix components such as collagen.

In addition to the cleavage of amide bonds, zinc enzymes play an important role in the cleavage of the P−OR bond in phosphates, $[(RO)PO_3]^{2-}$ and $[(RO)_2PO_2]^-$, as exemplified by their nuclease activity pertaining to the hydrolysis of DNA and RNA (Figure 2). The importance of zinc enzymes is not, however, resticted to their role in cleaving amide and phosphate bonds, with two other important zinc enzymes being carbonic anhydrase (a lyase) which hydrolyzes CO_2, and alcohol dehydrogenase which oxidizes alcohols to aldehydes or ketones.

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Gerard Parkin received his B.A., M.A., and D.Phil. degrees from the Queen's College, Oxford University. Both his graduate and undergraduate research was carried out under the guidance of Professor Malcolm L. H. Green. In 1985, he moved to the California Institute of Technology as a NATO postdoctoral fellow to work with Professor John E. Bercaw. He joined the faculty of Columbia University as Assistant Professor in 1988 and was promoted to Associate Professor in 1991 and to Professor in 1994. He served as Chairman of the Department from 1999 to 2002. Among other awards, he is a recipient of the Presidential Faculty Fellowship Award, the American Chemical Society Award in Pure Chemistry, and the Royal Society of Chemistry Corday Morgan Medal. His principal research interests are in the areas of synthetic, structural, and mechanistic inorganic chemistry.

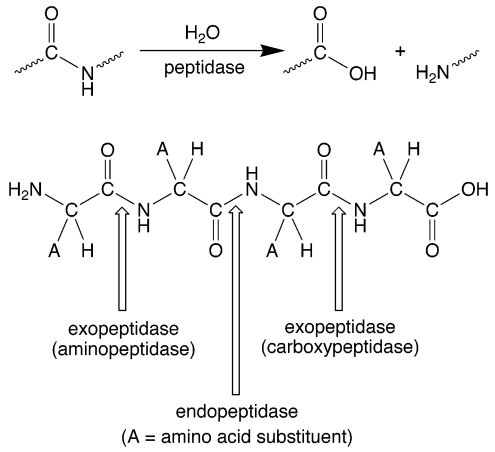


Figure 1. Peptidase function of zinc enzymes.

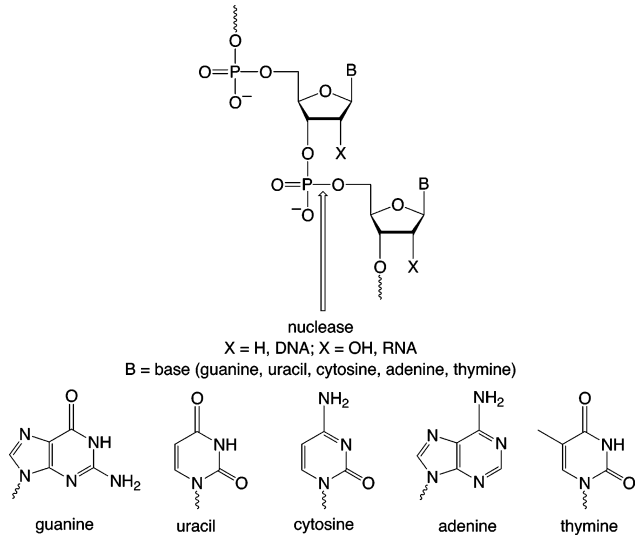


Figure 2. Nuclease function of zinc enzymes.

It is, therefore, evident that zinc plays multifaceted roles in biological systems, and a detailed understanding of these roles requires a correspondingly detailed appreciation of how the chemistry of zinc is modulated by its coordination environment. In this regard, Vallee and Auld have stated that "there is a great need to inspect the chemical properties of zinc complex ions containing combinations of nitrogen, oxygen, and sulfur ligands, as well as those that only contain sulfur ligands."[1c] This review describes advances towards this objective by highlighting recent studies that afford an understanding of the bioinorganic chemistry of zinc by investigating synthetic analogues that mimic both the structure and function of the active sites of zinc enzymes, an area that has been previously reviewed from a variety of persepectives.[8,9]

2. Zinc Enzymes: An Overview of the Structures, Functions, and Mechanisms of Action

2.1. Coordination Motifs and Functions

The most common structural motif in zinc enzymes is one in which a tetrahedral zinc center is attached to the protein backbone by three amino acid residues, with the fourth site being occupied by the catalytically important water (or hydroxide) ligand, $[\{XYZ\}Zn^{II}-OH_n]$, as illustrated in Figure 3.[1-10] The residues which bind zinc to the protein are typically a combination of His (N), Glu (O), Asp (O), or Cys (S), which provide nitrogen, oxygen, and sulfur donors (Table 1), with His being the most commonly encountered.[11-14] Histidine coordinates via its imidazole substituent as a neutral donor, while glutamate and aspartate coordinate via their deprotonated anionic carboxylate substituents. In contrast to histidine, glutamate and aspartate, the protonation state of cysteine groups is not generally known with certainty. Thus, while it is commonly considered that the $[Cys_2His_2Zn]$ core encountered in zinc fingers is neutral, such that the cysteines are coordinated in their deprotonated form, there is controversy as to whether all cysteines in $[Cys_3HisZn]$ and $[Cys_4Zn]$ cores are deprotonated.[15] However, recent calculations suggest that the cysteine residues in all zinc proteins are likely to be deprotonated, especially if

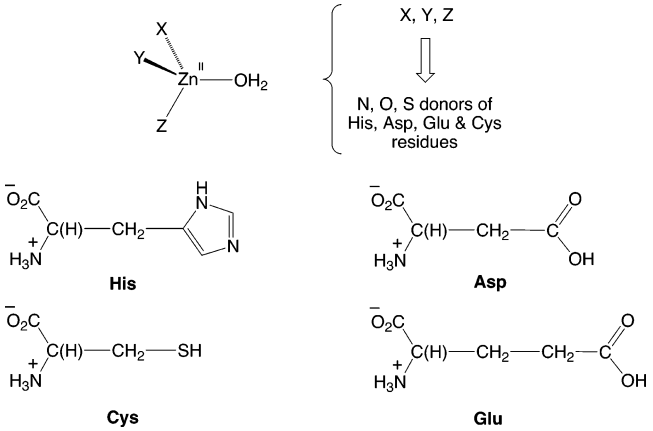


Figure 3. Common structural feature of zinc enzymes.

Table 1. Coordination Motifs in Representative Mononuclear [{XYZ}$Zn^{II}(OH_2)$] Zinc Enzymes and Their Functions

X, Y, Z	enzyme	function
His, His, His	carbonic anhydrase	hydration of CO_2
His, His, Glu	carboxypeptidase	exopeptidase
	thermolysin	endopeptidase
	neutral protease	endopeptidase
His, His, Asp	protease from *Streptomyces caespitosus*	endopeptidase
His, His, Cys	bacteriophage T7 lysozyme	cleavage of the amide bond between L-alanine and *N*-acetylmuramate moieties in polysaccharides
His, Asp, Cys	farnesyl protein transferase	transfer of a farnesyl isoprenoid to a cysteine residue
His, Cys, Cys	alcohol dehydrogenase	oxidation of alcohols to aldehydes and ketones
	cytidine deaminase	hydrolytic deamination of cytidine to uridine
Cys, Cys, Cys	5-aminolevulinate dehydratase	synthesis of porphobilinogen from 5-aminolevulinic acid

peptide residues such as lysine and arginine can form N−H···S hydrogen bonds to stabilize the Zn−Cys interaction.[15]

Despite the overall similarity of many zinc enzyme active sites in terms of their common tetrahedral [{XYZ}Zn^{II}−OH_2] structures, each zinc enzyme performs a different function (Table 1). In this respect, it is important to note that not only are the unique catalytic properties of each enzyme determined by the nature of the donor groups, but the different amino acid spacer lengths between the coordinating residues also play an important role in modulating the reactivity of the active site. Typically, two of the coordinating groups are separated by 1−3 amino acid residues, while the third is separated by a "long" spacer of 5−196 residues.[1e,11a]

While the [{XYZ}Zn^{II}−OH_n] motif with the zinc aqua(hydroxide) group is the most prominent active functionality in zinc enzymes, there are classes of zinc proteins and enzymes in which the activity centers on the reactivity of a zinc thiolate linkage. For example, alkylation of zinc cysteine thiolates is involved in the mechanisms of action of several enzymes, of which the first to be discovered is the Ada DNA repair protein;[16] other zinc proteins that involve zinc thiolate alkylation in their mechanisms of action include: methionine synthase, methanol: coenzyme M methyltransferase, farnesyl transferase, and geranylgeranyl transferase.[17] Likewise, the Zn−Cys group is the essential feature of matrix metalloproteinases (matrixins), an important group of zinc enzymes responsible for degradation of the extracellular matrix components such as collagen.[18] However, the Zn−Cys group in these enzymes represent a latent form, which is converted to the active Zn−OH_2 species by either proteolytic cleavage, oxidation, and/or a conformational change of the propeptide.[1d]

Tetrahedral zinc sites also play an important structural role by influencing the stability and conformation of enzymes. Cysteine residues, in particular, are a prominent component of structural sites, as illustrated by the [$Zn(Cys)_4$] structural sites in alcohol dehydrogenase and aspartate transcarbamoylase, and the [$Cys_\alpha His_\beta Zn^{II}$] ($\alpha + \beta = 4$) sites in zinc fingers.[1,19−21]

In addition to simple tetrahedral coordination, it is important to note that other coordination geometries are also known due to the ability of ligands such as glutamate and aspartate to bind in a bidentate manner. Indeed, it has been noted that carboxylate coordination to zinc (both in proteins and small molecules) is highly flexible with a continuous range between monodentate and bidentate coordination.[22] Computational studies suggest that the carboxylate coordination mode is influenced by interactions with other protein residues.[23]

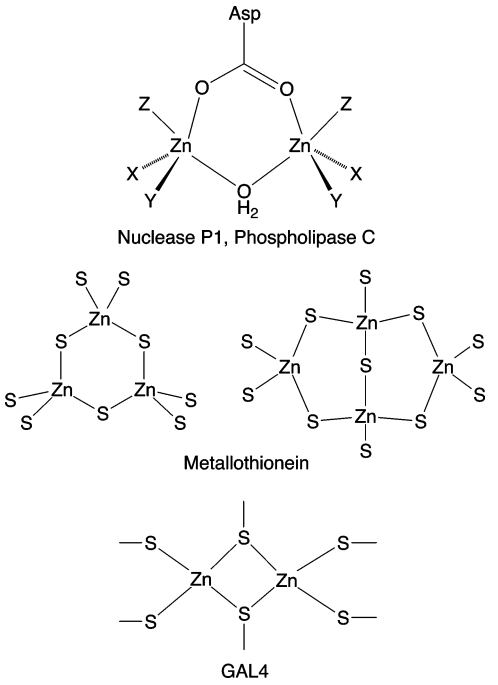


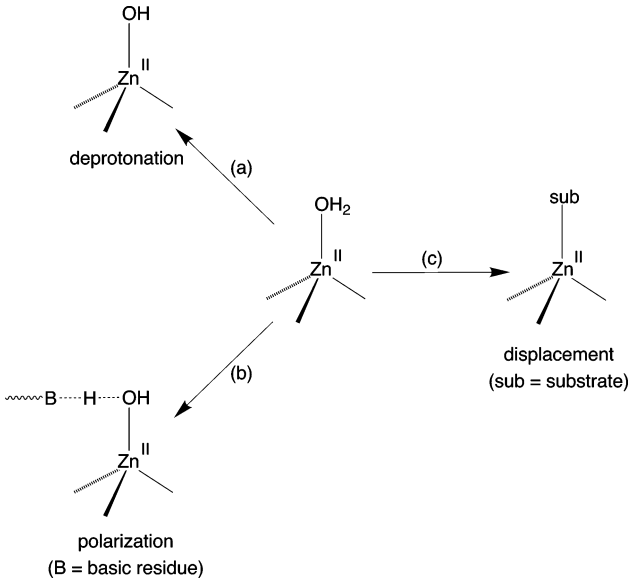
Figure 4. Polynuclear zinc sites in enzymes and proteins.

It is important to emphasize that not all zinc enzymes have mononuclear active sites and there are a variety with polynuclear sites, as illustrated by alkaline phosphatase, nuclease P1, phospholipase C, and aminopeptidases. The zinc centers in such enzymes are often bridged by a water molecule and an aspartate residue (Figure 4).[1−4,24] Cysteine residues also provide a means for linking two or more zinc centers together, as illustrated by the metallothioneins, so-called because of their high sulfur (cysteine) content, which contain two distinct clusters of zinc/cadmium atoms. The metal centers in each of these clusters are tetrahedrally coordinated to bridging and terminal cysteine residues (Figure 4). The role of metallothioneins has been postulated to be concerned with zinc storage and scavenging free radicals.[3,10a] A zinc thiolate cluster analogous to those of the metallothioneins is also observed in the GAL4 transcription factor (Figure 4).[1]

2.2. Mechanisms of Action

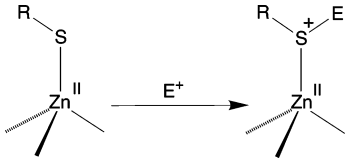
The mechanism of action of the majority of zinc enzymes centers around the $Zn-OH_2$ function,[1-4] which participates in the catalytic cycle by a variety of means, as illustrated in Scheme 1.[1e,25,26] Most commonly, the Lewis acidic Zn^{II} center activates the coordinated water towards deprotonation, thereby generating hydroxide species close to neutral pH (Scheme 1a),[27] as exemplified by the mechanism of action of carbonic anhydrase. A second means of activation is observed for enzymes such as carboxypeptidase that incorporate an anionic glutamate residue at the active site. As a result of the negative charge associated with the glutamate ligand, deprotonation of the zinc-bound water molecule is less facile than that for carbonic anhydrase and it may not be fully deprotonated at neutral pH. For such situations, further activation is necessary, and this is achieved by interaction with an adjacent protein glutamate residue, thereby generating an incipient zinc hydroxide species (Scheme 1b). A third type of participation involves complete displacement of the coordinated water to allow access of the substrate to the zinc center (Scheme 1c). This type of mechanism is exemplified by liver alcohol dehydrogenase, in which the coordinated water is displaced by an alcohol. Liver alcohol dehydrogenase favors a displacement mechanism because the presence of two negative cysteinyl ligands in LADH inhibit deprotonation.[1e]

Scheme 1. Basic mechanisms of action involving zinc aqua species: (a) deprotonation to generate a nucleophilic zinc hydroxide ligand, (b) additional activation by a basic residue to generate an incipient zinc hydroxide, and (c) displacement of the coordinated water molecule by the substrate, thereby enhancing it towards nucleophilic attack



In addition to the activation of coordinated water, zinc has also been shown to play a role in activating thiols towards nucleophilic attack (Scheme 2). For example, the alkylation of zinc cysteine thiolates is a step in the mechanisms of action of the Ada DNA repair protein, methionine synthase, methanol:coenzyme M methyltransferase, farnesyl transferase, and geranylgeranyl transferase.[16,17]

Scheme 2. Alkylation of cysteine thiolate groups occurs in reactions involving Ada DNA repair protein, methionine synthase, methanol:coenzyme M methyltransferase, farnesyl transferase, and geranylgeranyl transferase



Considering the fact that the prevalence of zinc in biological systems is disproportionate to its abundance in the earth's crust, one has to consider why Nature has selected zinc to perform such a variety of functions in biological systems. The properties of zinc that are most pertinent to its role in zinc enzymes are summarized in Table 2. Of particular note, (i) zinc is an element of borderline hardness,[28] so that nitrogen, oxygen, and sulfur ligands interact favorably, with the result that zinc binds strongly to many proteins; (ii) the divalent zinc ion is exceptionally stable with respect to oxidation and reduction, and so it does not participate in redox reactions; (iii) zinc shows a strong preference in enzymes for tetrahedral over octahedral coordination, thereby enhancing both the Lewis acidity of the zinc center and the Brønsted acidity of a coordinated water molecule; and (iv) as a result of the d^{10} configuration of Zn^{2+}, zinc complexes are not subject to ligand field stabilization effects. Thus, coordination number and geometry are

Table 2. Properties of Zn^{II} That Are Pertinent to Its Role in Enzymes

redox properties	The divalent zinc ion is exceptionally stable with respect to oxidation and reduction, and so it does not participate in redox reactions, in contrast to Mn, Fe, and Cu.
coordination geometries	The d^{10} configuration of Zn^{2+} indicates that zinc complexes are not subject to ligand field stabilization effects, and so coordination number and geometry are only dictated by ligand size and charge. In enzymes, zinc shows a strong preference for tetrahedral coordination which enhances both the Lewis acidity of a zinc center and the Brønsted acidity of a coordinated water molecule. Only Cu^{II} is a better Lewis acid.
ligand binding properties	Zinc is an element of borderline hardness, so that nitrogen, oxygen and sulfur ligands can all be accommodated, in contrast to magnesium and calcium which favor binding to oxygen. Therefore, zinc binds strongly to many proteins.
ligand exchange	The flexibility in coordination geometry makes ligand exchange more facile than for Ni or Mg and enhances the ability of zinc to effect a catalytic cycle.
ligand nucleophilicity	Anions such as OH^-, OR^- and SR^- retain nucleophilic character when coordinated to zinc. Only Mn^{II}, Fe^{III}, and Cu^{II} are better in this regard.

only dictated by ligand size and charge, and this flexibility facilitates ligand exchange and enhances the ability of zinc to effect a catalytic cycle.

3. Synthetic Analogues of Zinc Enzymes

Insight into the structures and mechanisms of action of enzymes is often obtained by studying synthetic analogues, i.e. small molecules that resemble the structural and functional sites of the enzymes.[29] Such studies are important because synthetic analogues are more amenable to structural, spectroscopic, and mechanistic studies than are the enzymes themselves. Furthermore, synthetic analogues are also more amenable to fine-tuning by systematic substituent effects than are the active sites of their enzyme counterparts, so that it is possible to examine a variety of factors that influence reactivity. Thus, the detailed information that is obtained by studying synthetic analogues defines the chemistry of zinc in a well-defined coordination environment and thereby provides a basis for understanding in more detail the structures and mechanisms of actions of the enzymes themselves.

In an ideal world, the coordination chemist could merely select the amino acid residues of interest and use the amino acids themselves to mimic the active site of interest. However, such an approach is problematic for several reasons. First, rather than merely coordinating via the amino acid substituent such as imidazole or thiol, the amino and carboxyl groups may also (or preferentially) interact with the metal;[30,31] second, even if protected forms of the amino acids were used it is unlikely that stable active site mimics could be obtained due to the steric demands of the amino acid groups being insufficient to prevent the formation of hydroxide bridges between two zinc centers; and third, the possibility of ligand dissociation and redistribution in solution would complicate mechanistic analyses.

The construction of accurate synthetic analogues is therefore nontrivial, and considerable attention must be given to ligand design in order to achieve a coordination environment which is similar to that enforced by the unique topology of a protein. A simple illustration of the problems that are encountered is provided by the fact that whereas the majority of active sites in zinc enzymes have a pseudotetrahedral coordination geometry, the structural characterization of simple zinc complexes in the solid state exhibits higher coordination numbers: 3 (0.2%), 4 (42%), 5 (19%), 6 (35%), 7 (4%).[32,10e] Furthermore, octahedral complexes such as $[Zn(OH_2)_6]^{2+}$ are even more prevalent in solution,[33] and an octahedral aqua complex $[\{Me_3[9]aneN_3\}Zn(OH_2)_3]^{2+}$ has even been isolated using the tridenate trimethyltriazacyclononane nitrogen donor ligand.[34] The protein environment thus provides a means of stabilizing four-coordination with respect to higher coordination in zinc enzymes, an attribute that the synthetic analogue must also emulate.

In addition to stabilizing tetrahedral structures, the protein also inhibits binuclear degradatory pathways by burying the zinc center in its interior, a factor that must additionally be considered when

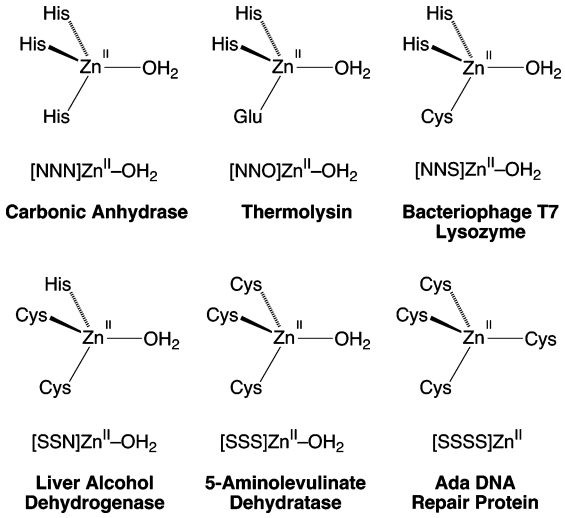


Figure 5. Active-site coordination motifs in representative zinc enzymes.

constructing a synthetic analogue. For example, while the mononuclear tetrahedral zinc hydroxide entity is a ubiquitous feature of zinc enzymes, this type of structure is not common in small-molecule chemistry because the hydroxide ligand is typically observed to bridge two zinc centers.[33] As an illustration, the four-coordinate terminal hydroxide complex $[\{Me_3[9]aneN_3\}Zn(OH)]^+$ has not been isolated, but rather exists as the hydroxide-bridged dinuclear species $\{[\{Me_3[9]aneN_3\}Zn(\mu-OH)]\}_2^{2+}$.[35] In principle, the most elegant synthetic analogues would feature the use of small peptides as the donor ligands; however, not surprisingly, simple di- and tripeptides often give polynuclear zinc complexes.[36]

It is, therefore, evident that the construction of synthetic analogues requires a judicious choice of ligands to prevent the formation of octahedral and dinuclear complexes. The difficulty associated with this problem has been noted by Kimura, who stated that, "It has been an extremely challenging task to tailor appropriate ligands that (a) form stable and discrete complexes with zinc(II) at physiological pH (~7), (b) leave catalytic sites open on zinc(II), and (c) offer structurally and/or functionally similar environments around zinc(II) as found in enzymes."[9] Fortunately, the combined efforts of a number of research groups have enabled significant advances to be made with respect to defining the bioinorganic chemistry of zinc,[8,9] the results of which are reviewed in this article. This chemistry will be discussed according to the composition of the protein residues at the active site, i.e. the zinc coordination environment, which ranges from nitrogen rich in carbonic anhydrase, $[(His)_3Zn^{II}-OH_2]$, to sulfur rich in the Ada DNA repair protein, $[(Cys)_4Zn^{II}]$, as illustrated in Figure 5.

3.1. Mononuclear Zinc Enzymes

A common approach for obtaining synthetic analogues of the type $[\{XYZ\}Zn^{II}-L]$ (e.g., L = OH, H_2O, Cys) involves the application of tridentate ligands which incorporate the requisite X, Y, and Z donor groups to mimic the three protein residues that bind

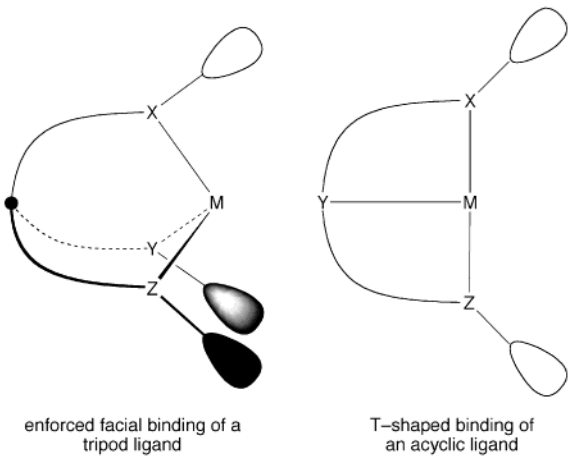


Figure 6. Comparison of the facial coordination of a tripodal ligand with the T-shaped binding of an acyclic ligand.

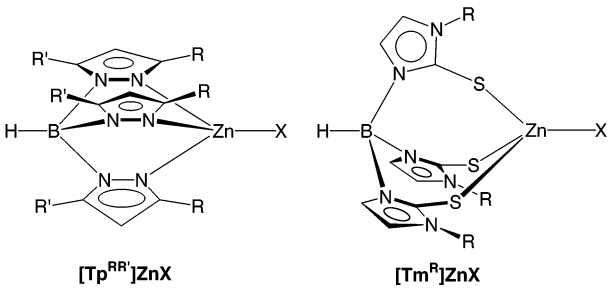


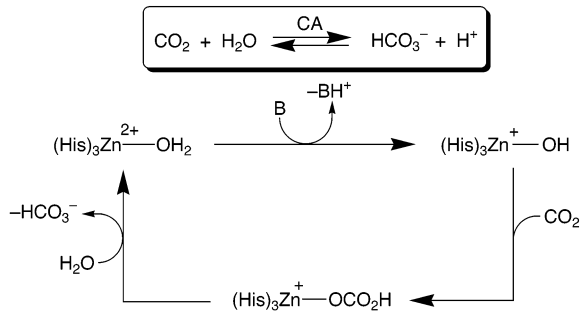
Figure 7. Tris(pyrazolyl)hydroborato and tris(mercaptoimidazolyl)borate systems, $[Tp^{RR'}]ZnX$ and $[Tm^{R}]ZnX$.

zinc at the active site. In particular, tripodal ligands in which the X, Y, and Z groups are attached to a common tetrahedral (or trigonal pyramidal) center have proven to be of particular benefit for several reasons. First, tripodal ligands enforce the "facial" binding that is required to create a tetrahedral metal center (Figure 6); in contrast, acyclic tridentate ligands frequently bind in a "T-shaped" manner that has no biological relevance with respect to zinc enzymes. Second, tripodal ligands typically possess only a single relevant binding conformation, whereas macrocyclic ligands are more conformationally flexible. Third, as a consequence of the directional nature of tripodal ligands, it is possible to incorporate substituents that directly influence the steric environment about the metal center; in contrast, the shape of macrocyclic ligands is such that substituents are not generally located in positions that enable them to have a marked steric effect on the coordination pocket. Exemplary illustrations of tripodal ligands that have received considerable attention with respect to modeling zinc enzymes are the tris(pyrazolyl)borate, $[Tp^{RR'}]$,[37] and tris(mercaptoimidazolyl)borate, $[Tm^{R}]$,[38] ligands (Figure 7), which respectively provide three nitrogen and three sulfur donors to emulate zinc enzymes with nitrogen-rich and sulfur-rich active sites. Also of importance, the substituents on these ligands can be readily modified to provide a means to influence both the size of the coordination pocket and the electronic properties of the metal center.

3.1.1. The $[(His)_3Zn^{II}–OH_2]$ Motif: Carbonic Anhydrase, Matrix Metalloproteinases, and Dihydroorotase

3.1.1.1. Carbonic Anhydrase. *(i) Structure and Mechanism of Action of Carbonic Anhydrase.* Of all zinc enzymes, carbonic anhydrase has played the most pivotal role in the development of enzymology.[39] Specifically, carbonic anhydrase was the first enzyme recognized to contain zinc,[40] is ubiquitous (occurring in animals, plants and bacteria), and is one of the most efficient enzymes known. It also has widespread occurrence in prokaryotes and has therefore been classified as an "ancient" enzyme.[41] The essential physiological function of the enzyme is to catalyze the reversible hydration of carbon dioxide (Scheme 3) and thus plays an important role in respiration,

Scheme 3. Simplified mechanism of action for carbonic anhydrase (B = base)



transporting CO_2 between metabolizing tissues and the lungs, and intracellular CO_2/HCO_3^- equilibration. Dorzolamide hydrochloride (Trusopt), a topical carbonic anhydrase inhibitor,[39e,42] has recently been introduced for the treatment of glaucoma and ocular hypertension,[43] and simple zinc complexes of carbonic anhydrase inhibitors have been isolated.[44,45,46] In addition to its physiological function, carbonic anhydrase also catalyzes non-physiological reactions such as hydration of aldehydes and esters.

X-ray diffraction studies on a variety of forms of the enzyme have demonstrated that the zinc ion is located at the bottom of a conical cavity (ca. 15 Å deep) and is coordinated to the protein by the imidazole groups of three histidine residues, with the remaining tetrahedral site being occupied by a water molecule (or hydroxide ion, depending upon pH); the coordinated water molecule is also involved in a hydrogen-bonding interaction with a Thr residue that in turn is hydrogen bonded to a Glu residue (Figure 8).

The overall features of the mechanism of action of carbonic anhydrase are illustrated in Scheme 3,[39] comprising the following steps: (i) deprotonation of the coordinated water with a $pK_a \approx 7$ (via a His-64 shuttle[47]) to give the active zinc hydroxide derivative $[(His)_3Zn{-}OH]^+$, (ii) nucleophilic attack of the zinc-bound hydroxide at the carbon dioxide substrate to give a bicarbonate (more correctly termed hydrogen carbonate) intermediate $[(His)_3Zn{-}OCO_2H]^+$, and (iii) displacement of the bicarbonate anion by H_2O to complete the catalytic cycle. However, details concerned with the nature of the bicarbonate intermediate and the means by which it is displaced from the zinc center are more speculative, due to an inability

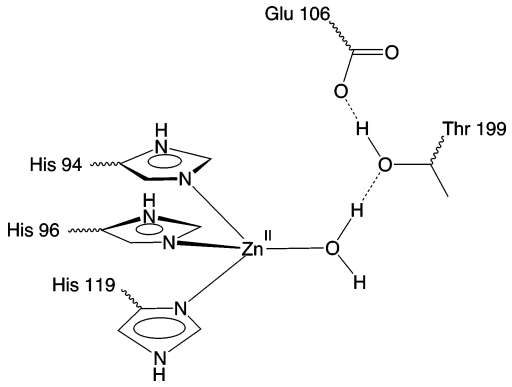


Figure 8. Active site of carbonic anhydrase.

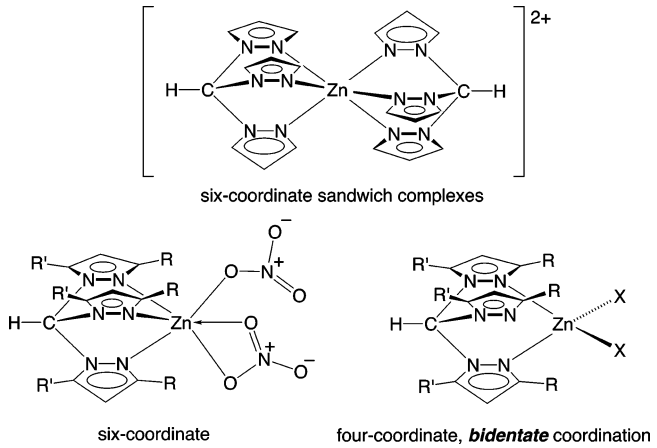
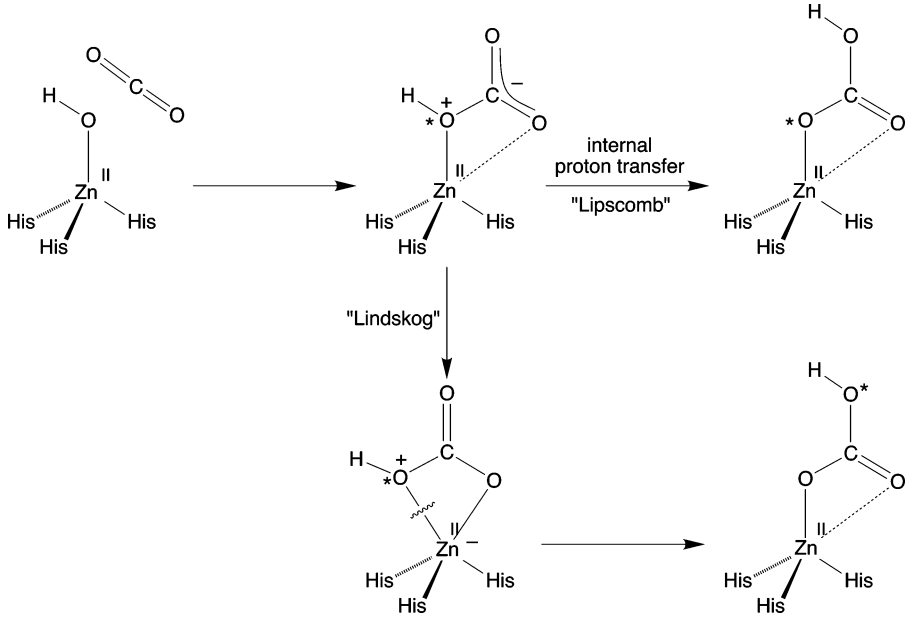


Figure 9. Examples of ligands that do not support mononuclear tetrahedral zinc hydroxide complexes.

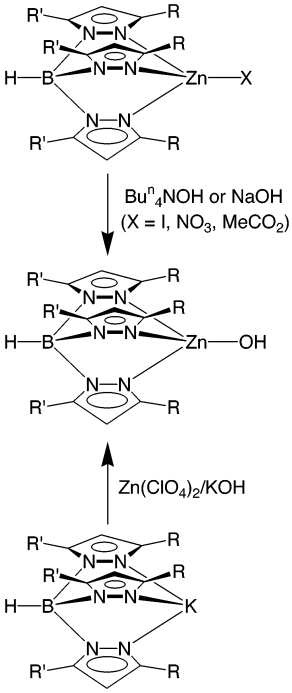
to experimentally observe such species. Two mechanisms have been proposed, namely the "Lipscomb" and "Lindskog" mechanisms (Scheme 4), which may be distinguished according to whether the original zinc-bound oxygen atom (labeled *) retains the hydroxide proton. Recent theoretical studies suggest that a Lindskog-type mechanism operates.[48,49]

(ii) Synthesis and Structural Characterization of Synthetic Analogues of Carbonic Anhydrase. Perhaps not surprisingly, tetrahedral zinc hydroxide and aqua complexes have not been isolated by using simple pyrazole or imidazoles, which instead afford $[ZnL_4]^{2+}$ and $[ZnL_6]^{2+}$ derivatives.[50] A large variety of tridentate ligands has, therefore, been employed in efforts to obtain synthetic analogues of carbonic anhydrase. In particular, considerable attention has been given to the application of tridentate nitrogen donor ligands such as tris(imidazolyl)phosphine, tris(imidazolyl)-carbinol, and tris(imidazolyl)alkane derivatives, among others, to mimic the role of the three histidine imidazole groups.[8,9,51−69] However, despite this effort, few studies have produced structurally characterized tetrahedral zinc hydroxide and aqua complexes that mimic the active site of carbonic anhydrase. For example, tris(pyrazolyl)methane and tris(pyridyl)-methanol ligands do not give tetrahedral complexes of the types $\{[\eta^3\text{-}HC(pz)_3]ZnX\}^+$ and $\{[\eta^3\text{-}HOC(py)_3]\text{-}ZnX\}^+$, but rather form the sandwich-type complexes $\{[\eta^3\text{-}HC(pz)_3]_2Zn\}^{2+}$ and $\{[\eta^3\text{-}HOC(py)_3]_2Zn\}^{2+}$ (Figure 9).[70] Furthermore, even highly substituted tris(pyrazolyl)methane ligands $[HC(pz^{RR'})_3]$ have failed to support mononuclear cationic $\{[\eta^3\text{-}HC(pz^{RR'})_3]ZnX\}^+$ complexes, yielding preferentially (i) six-coordinate derivatives, such as $\{[\eta^3\text{-}HC(pz^{RR'})_3]_2Zn\}^{2+}$ and $[\eta^3\text{-}HC(pz^{RR'})_3]Zn(NO_3)_2$, and (ii) four-coordinate halide derivatives $[\eta^2\text{-}HC(pz^{RR'})_3]ZnX_2$, in which the tris(pyrazolyl)methane ligand coordinates in a bidentate manner (Figure 9).[71] It is also evident that steric protection is important for preventing oligomerization via either hydroxide bridges or via hydrogen-bonding interactions. For example, the triazacyclononane derivative $[\{Me_3[9]aneN_3\}Zn(\mu\text{-}OH)]^+$ is unstable and exists as the hydroxy-bridged dimer $\{[\{Me_3[9]aneN_3\}Zn(\mu\text{-}OH)]\}_2^{2+}$,[35] while the triazacyclododecane derivative $[\{[12]aneN_3\}Zn(OH)\}]_3(ClO_4)_3\cdot(HClO_4)$ exists as a hydrogen-bonded trimer

Scheme 4. Two mechanisms for the formation of the bicarbonate intermediate; theoretical studies suggest that the Lindskog-type mechanism operates



Scheme 5. Syntheses of [Tp$^{RR'}$]ZnOH, the first mononuclear tetrahedral zinc hydroxide synthetic analogues of carbonic anhydrase



in the solid state in which a molecule of perchloric acid participates in the oligomerization.[72]

(A) Tris(pyrazolyl)borato Ligands, [Tp$^{RR'}$]. In view of the above discussion, it is significant that sterically demanding tris(pyrazolyl)borato ligands afforded the first isolation of monomeric tetrahedral zinc hydroxide complexes of the type [Tp$^{RR'}$]ZnOH.[73] For example, [Tp$^{Bu^t,Me}$]ZnOH has been synthesized by (i) the direct reaction between Zn(ClO$_4$)$_2$·6H$_2$O, K[Tp$^{Bu^t,Me}$] and KOH in methanol, and (ii) metathesis of [Tp$^{Bu^t,Me}$]ZnI with Bu$^n_4$NOH (Scheme 5). The monomeric and tetrahedral nature of the hydroxide complexes was determined by X-ray diffraction studies on [Tp$^{Bu^t,Me}$]ZnOH and [Tp^{Cum,Me}]ZnOH, as illustrated for the former complex in Figure 10. A comparison of the Zn−O and Zn−N bond lengths and angles of these and other synthetic analogues with those of carbonic anhydrase is provided in Table 3.

The hydroxide ligands in [Tp$^{RR'}$]ZnOH are well characterized spectroscopically, as illustrated by the IR and NMR spectroscopic data summarized in Table 4. For example, the hydroxide ligand of [Tp$^{Bu^t,Me}$]-ZnOH is observed as a sharp signal at δ −0.07 in the ^{1}H NMR spectrum and as a broad resonance at δ −8 in the ^{17}O NMR spectrum. Furthermore,

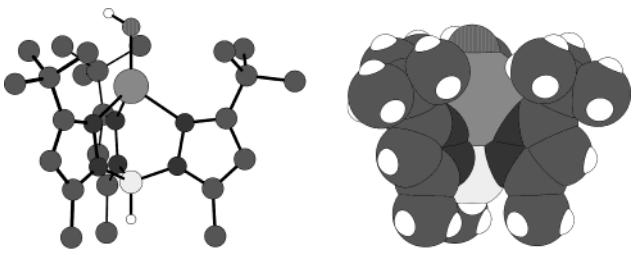


Figure 10. Molecular structure of [Tp$^{Bu^t,Me}$]ZnOH, a structural analogue of the active site of carbonic anhydrase.

Table 3. Comparison of Zn−O and Zn−N Bond Lengths in Carbonic Anhydrase with Those of Tetrahedral Synthetic Analogues

	d(Zn−O)/Å	d(Zn−N)/Å	ref
{[Pim$^{Bu^t,Pr^i}$]ZnOH}$^+$	1.86	2.08	*a*
[Tp$^{Bu^t,Me}$]ZnOH	1.85	2.05	*b*
[Tp^{Cum,Me}]ZnOH	1.85	2.05	*c*
{[Tp$^{Bu^t,Me}$]Zn(OH$_2$)}$^+$	1.94	2.02	*d*
[(X$_6$Et$_3$ImEt$_3$)Zn(OH$_2$)·(OH$_2$)]$^{2+}$	1.97	2.00	*e*
CAI	1.9	1.9	*f*
CAII	2.05	2.11	*g*

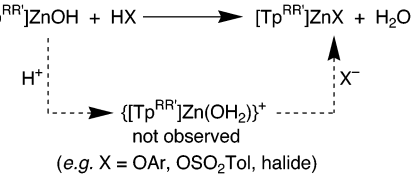
[a] Kimblin, C.; Allen, W. E.; Parkin, G. *J. Chem. Soc., Chem. Commun.* **1995**, 1813. [b] Alsfasser, R.; Trofimenko, S.; Looney, A.; Parkin, G.; Vahrenkamp, H. *Inorg. Chem.* **1991**, *30*, 4098. [c] Ruf, M.; Vahrenkamp, H. *Inorg. Chem.* **1996**, *35*, 6571. [d] Bergquist, C.; Fillebeen, T.; Morlok, M. M.; Parkin, G. *J. Am. Chem. Soc.* **2003**, *125*, 6189. [e] Sénèque, O.; Rager, M.-N.; Giorgi, M.; Reinaud, O. *J. Am. Chem. Soc.* **2001**, *123*, 8442. [f] Kannan, K. K. *Photon Fact. Act. Rep. Jpn.* **1990**, *8*, 94. [g] Håkansson, K.; Carlsson, M.; Svensson, L. A.; Liljas, A. *J. Mol. Biol.* **1992**, *227*, 1192.

Table 4. Spectroscopic Data for [Tp$^{RR'}$]ZnOH Complexes

[Tp$^{RR'}$]ZnOH	ν(O−H)/cm^{-1}	δ ^{1}H	δ^{17}O
[Tp$^{Bu^t,Me}$]ZnOH	3676	−0.07	−8
[Tp$^{Pr^i_2}$]ZnOH	3668	−0.29	−36
[Tp^{Ar_2}]ZnOH[a]	3558		
[Tp^{Cum,Me}]ZnOH	3647		

Data taken from Parkin, G. *Adv. Inorg. Chem.* **1995**, *42*, 291. [a] Ar = *p*-C$_6$H$_4$Bu^t.

Scheme 6. Protonation of a zinc hydroxide is normally accompanied by displacement of water, thereby preventing isolation of the aqua complex {[Tp$^{RR'}$]Zn(OH$_2$)}$^+$



[Tp$^{Bu^t,Me}$]ZnOH has also been investigated by solid-state ^{67}Zn NMR spectroscopy.[74]

The importance of bulky substituents in the isolation of [Tp$^{RR'}$]ZnOH derivatives is demonstrated by the fact that phenyl substituents do not provide sufficient protection, with the hydroxide complex [Tp^{Ph}]ZnOH having been reported to be unstable.[75] It is also worth noting that tris(pyrazolyl)borate ligands have been used to synthesize related thiol derivatives, e.g. [Tp$^{Bu^t}$]ZnSH[76] and [Tp^{Ar,Me}]ZnSH.[77]

While the hydroxide species is considered to be the active form of carbonic anhydrase with respect to reaction with CO_2, it is also of importance to study synthetic analogues of the aqua form from which they are generated by deprotonation. In principle, aqua complexes should be readily obtainable by protonation of the hydroxide ligand of [Tp$^{RR'}$]ZnOH. However, even though protonation of [Tp$^{RR'}$]ZnOH derivatives is facile, the incipient aqua ligand is typically irreversibly displaced by the counterion (Scheme 6),[78] as illustrated by the fact that [Tp$^{Bu^t,Me}$]ZnOH reacts with *p*-TsOH to give [Tp$^{Bu^t,Me}$]ZnOTs.[78c] It is, therefore, noteworthy that (C$_6$F$_5$)$_3$B(OH$_2$), a strong Brønsted acid with a strength comparable to that of HCl

Scheme 7. Synthesis of the aqua complex $\{[Tp^{Bu^t,Me}]Zn(OH_2)\}^+$ by employing $(C_6F_5)_3B(OH_2)$; the conjugate base $[HOB(C_6F_5)_3]^-$ does not displace the aqua ligand, but rather, hydrogen bonds to it

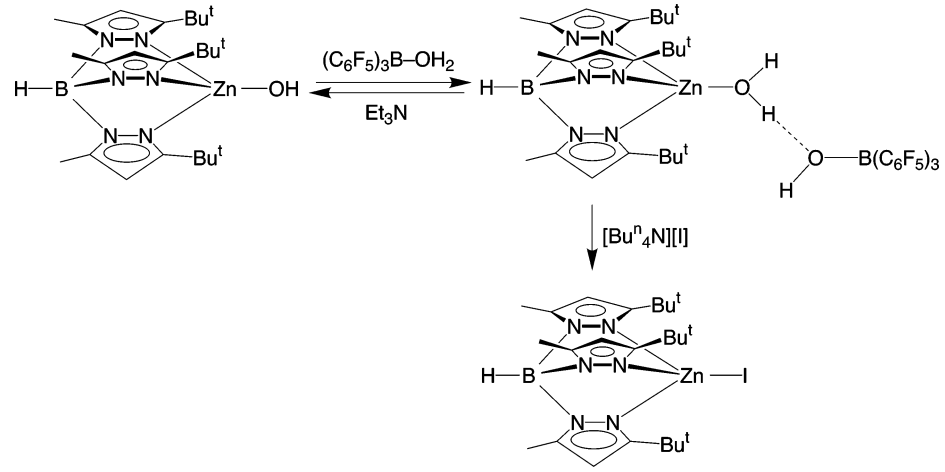


Table 5. Comparison of Zn−O and O···O Bond Lengths in Hydroxide and Aqua Complexes

	d(Zn−O)/Å	d(O···O)/Å	ref
	Zn−OH		
$[Tp^{Bu^t,Me}]ZnOH$	1.85		*a*
$[Tp^{Cum,Me}]ZnOH$	1.85		*b*
$\{[Pim^{Bu^t,Pr^i}]ZnOH\}^+$	1.86		*c*
$[\{[12]aneN_3\}Zn(OH)]^+$	1.94		*d*
$\{[\eta^4\text{-}N\{CH_2[(C_6H_3N)NHCH_2Bu^t]\}_3]ZnOH\}^+$	1.95		*e*
$\{[\eta^4\text{-}N\{CH_2CH_2NC(O)NHBu^t\}_3]ZnOH\}^{2-}$	2.02		*f*
	Zn−OH_2		
$\{\{[Tp^{Cum,Me}]Zn\}_2(H_3O_2)\}^+$	1.87	2.40	*g*
$\{\{[Tp^{3-Py,Me}]Zn\}_2(H_3O_2)\cdot H_2O\}^+$	1.87, 1.92	2.45	*g*
$\{\{[Tp^{6-MePy,Me}]Zn\}_2(H_3O_2)\}^+$	1.87, 1.89	2.42	*g*
$\{\{[Tp^{Ph,Me}]Zn\}_2(H_3O_2)\}^+$	1.90	2.41	*h*
$\{[Tp^{Bu^t,Me}]Zn(OH_2)\}[HOB(C_6F_5)_3]$	1.94	2.48	*i*
$[(X_6Et_3ImEt_3)Zn(OH_2)\cdot(OH_2)]^{2+}$	1.97	2.54	*j*
$[(TriMIm)Zn(OH_2)]X_2$	2.13 (X = BF_4), 2.03 (X = NO_3)		*k*
$[(2\text{-}NH_2,5\text{-}EtSC_2N_2)_3Zn(OH)_2]^{2+}$	1.98		*l*
$\{[N(CH_2bimH)_3]Zn(OH)_2\}^{2+}$	2.01		*m*
$\{[MeC(O)S\}_3Zn(OH_2)\}^-$	2.08		*n*

[a] Alsfasser, R.; Trofimenko, S.; Looney, A.; Parkin, G.; Vahrenkamp, H. *Inorg. Chem.* **1991**, *30*, 4098. [b] Ruf, M.; Vahrenkamp, H. *Inorg. Chem.* **1996**, *35*, 6571. [c] Kimblin, C.; Allen, W. E.; Parkin, G. *J. Chem. Soc. Chem. Commun.* **1995**, 1813. [d] Kimura, E.; Shiota, T.; Koike, T.; Shiro, M.; Kodama, M. *J. Am. Chem. Soc.* **1990**, *112*, 5805. [e] Yamaguchi, S.; Tokairin, I.; Wakita, Y.; Funahashi, Y.; Jitsukawa, K.; Masuda, H. *Chem. Lett.* **2003**, *32*, 406. [f] MacBeth, C. E.; Hammes, B. S.; Young, V. G.; Borovik, A. S. *Inorg. Chem.* **2001**, *40*, 4733. [g] Ruf, M.; Weis, K.; Vahrenkamp, H. *J. Am. Chem. Soc.* **1996**, *118*, 9288. [h] Puerta, D. T.; Cohen, S. M. *Inorg. Chim. Acta* **2002**, *337*, 459. [i] Bergquist, C.; Fillebeen, T.; Morlok, M. M.; Parkin, G. *J. Am. Chem. Soc.* **2003**, *125*, 6189. [j] Sénèque, O.; Rager, M.-N.; Giorgi, M.; Reinaud, O. *J. Am. Chem. Soc.* **2001**, *123*, 8442. [k] Voo, J. K.; Incarvito, C. D.; Yap, G. P. A.; Rheingold, A. L.; Riordan, C. G. *Polyhedron*. In press. [l] Ishankhodzhaeva, M. M.; Umarov, B. B.; Kadyrova, Sh. A.; Parpiev, N. A.; Makhkamov, K. K.; Talipov, S. A. *Russ. J. Gen. Chem.* **2000**, *70*, 1113. [m] Ichikawa, K.; Nakata, K.; Ibrahim, M. M.; Kawabata, S. *Stud. Surf. Sci. Catal.* **1998**, *114*, 309−314; Brandsch, T.; Schell, F. A.; Weis, K.; Ruf, M.; Muller, B.; Vahrenkamp, H. *Chem. Ber./Recl.* **1997**, *130*, 283. [n] Sampanthar, J. T.; Deivaraj, T. C.; Vittal, J. J.; Dean, P. A. W. *J. Chem. Soc., Dalton Trans.* **1999**, 4419.

in MeCN,[79] is capable of protonating the hydroxide ligand of $[Tp^{Bu^t,Me}]ZnOH$ to give an aqua complex $\{[Tp^{Bu^t,Me}]Zn(OH_2)\}[HOB(C_6F_5)_3]$ in which the water molecule is *not* displaced by the counterion (Scheme 7).[80] The importance of employing the $[(C_6F_5)_3BOH]^-$ counterion to stabilize the zinc aqua moiety is underscored by the fact that the coordinated water is readily displaced by addition of $[Bu^n_4N][I]$ to give $[Tp^{Bu^t,Me}]ZnI$ (Scheme 7).

The molecular structure of $\{[Tp^{Bu^t,Me}]Zn(OH_2)\}[HOB(C_6F_5)_3]$ has been determined by X-ray diffraction. Of particular note, the Zn−O bond [1.937(2) Å] is significantly longer than that in the parent hydroxide $[Tp^{Bu^t,Me}]ZnOH$ [1.850(8) Å], in accord with the fact that the hydroxide ligand has been protonated. Furthermore, the Zn−O bond length in $\{[Tp^{Bu^t,Me}]Zn(OH_2)\}^+$ is also longer than the values in dinuclear $\{[Tp^{RR'}]Zn\}$ complexes with bridging $[H_3O_2]$ moieties [1.872(6)−1.916(6) Å (Table 5)].[81,82] It is also interesting that the Zn−OH_2 bond length in $\{[Tp^{Bu^t,Me}]Zn(OH_2)\}[HOB(C_6F_5)_3]$ is actually shorter than that of the Zn−OH bond length [2.024(2) Å] in $\{[\eta^4\text{-}N\{CH_2CH_2NC(O)NHBu^t\}_3]ZnOH\}^{2-}$.[83] This discrepancy is a consequence of the Zn−O bond of $\{[\eta^4\text{-}N\{CH_2CH_2NC(O)NHBu^t\}_3]ZnOH\}^{2-}$ being exceptionally long for a hydroxide derivative due to (i) the zinc center being five-coordinate and anionic and (ii) the hydroxide oxygen being a hydrogen-bond receptor for two of the urea substituents.

The aqua complex $\{[Tp^{Bu^t,Me}]Zn(OH_2)\}^+$ exhibits a hydrogen-bonding interaction with the $[(C_6F_5)_3BOH]^-$ anion, characterized by an O···O separation of 2.480(3)

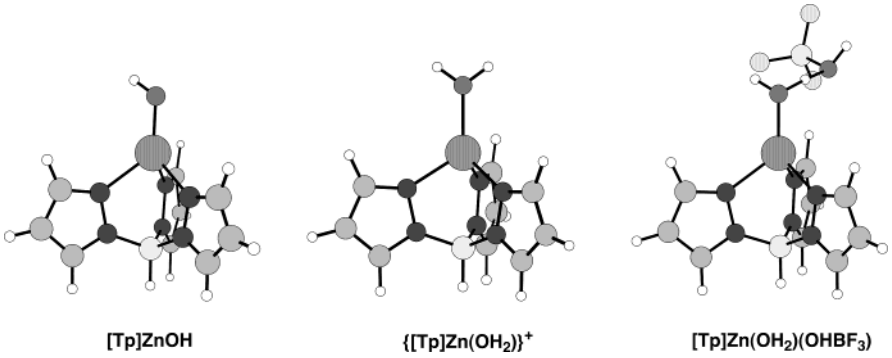


Figure 11. DFT (B3LYP) geometry optimized structures of [Tp]ZnOH, $\{[Tp]Zn(OH_2)\}^+$, and $\{[Tp]Zn(OH_2)]\}[HOBF_3]$.

Å, which is notable because it bears analogies to the active site in carbonic anhydrase, for which the zinc water ligand participates in a hydrogen bond with Thr-199.[39e] This interaction has been shown to be important to the functioning of the enzyme, with Thr-199 having been described as a "doorkeeper" that helps to block the displacement of the aqua ligand by certain inhibitors that cannot form a hydrogen bond.[39e] In addition to the interaction with Thr-199, the zinc-bound water of carbonic anhydrase is also part of a hydrogen-bonding network involving additional water molecules which mediate as a proton shuttle to His-64, prior to proton transfer to the surrounding medium.[84]

DFT (B3LYP) calculations on the model species [Tp]ZnOH and $\{[Tp]Zn(OH_2)\}^+$ (Figure 11) have been performed to complement the experimental study and provide information pertaining to the structural changes involved in protonation of a zinc hydroxide ligand.[80b] The zinc coordination geometry calculated for [Tp]ZnOH corresponds very closely to the experimental structure of $[Tp^{Bu^t,Me}]$ZnOH. Thus, the Zn−OH bond length calculated for [Tp]ZnOH [1.863 Å] is virtually identical to that for the experimental structure of $[Tp^{Bu^t,Me}]$ZnOH [1.850(8) Å]. Comparison of the calculated structures of [Tp]ZnOH and $\{[Tp]Zn(OH_2)\}^+$ indicates that protonation of the hydroxide ligand lengthens the Zn−O bond in $[TpZn(OH_2)]^+$ substantially to 2.072 Å. While this general change is reflected in the experimental structures of $[Tp^{Bu^t,Me}]$ZnOH and $\{[Tp^{Bu^t,Me}]Zn(OH_2)\}^+$, it is evident that the lengthening of the Zn−O bond in $\{[Tp^{Bu^t,Me}]Zn(OH_2)\}^+$ is not as large as that predicted for $[TpZn(OH_2)]^+$. Since a possible explanation for this difference resided with the fact that the $\{[Tp^{Bu^t,Me}]Zn(OH_2)\}^+$ is involved in the aforementioned hydrogen-bonding interaction with the $[(C_6F_5)_3BOH]^-$ counterion, DFT calculations were performed on the hypothetical species $\{[Tp]Zn(OH_2)\}[HOBF_3]$. Significantly, the Zn−O (1.944 Å) bond length in $\{[Tp]Zn(OH_2)\}[HOBF_3]$ is reduced from that of the free cation $\{[Tp]Zn(OH_2)\}^+$ and is comparable to the experimental value in $\{[Tp^{Bu^t,Me}]Zn(OH_2)\}[HOB(C_6F_5)_3]$ [1.937(2) Å].

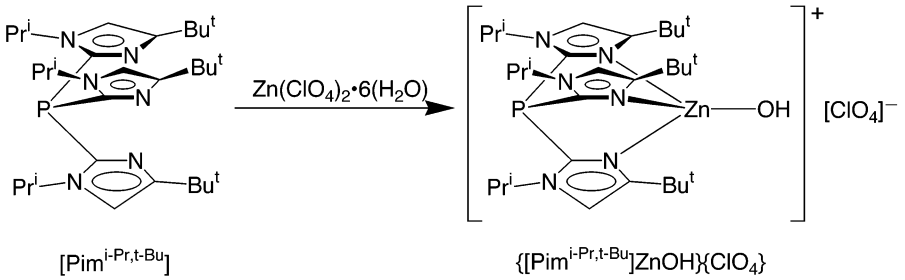
Tris(pyrazolyl)borate ligands that incorporate hydrogen-bond-accepting ester substituents have also been applied to modeling aspects of bioinorganic zinc chemistry. For example, the $[Tp^{CO_2Et,Me}]$ ligand has been used to synthesize a series of aqua and hydroxide complexes, namely, $[Tp^{CO_2Et,Me}]Zn(OAc)(OH_2)$, $\{[Tp^{CO_2Et,Me}]Zn(OH_2)_3\}^+$, and $[\{[Tp^{CO_2Et,Me}]Zn\}_2(\mu\text{-}OH)]^+$.[85] Although the $[Tp^{CO_2Et,Me}]$ ligand does not allow for the generation of tetrahedral hydroxide and aqua complexes akin to $[Tp^{Bu^t,Me}]$ZnOH and $\{[Tp^{Bu^t,Me}]Zn(OH_2)\}^+$, a notable feature of $[Tp^{CO_2Et,Me}]Zn(OAc)(OH_2)$, $\{[Tp^{CO_2Et,Me}]Zn(OH_2)_3\}^+$ and $[\{[Tp^{CO_2Et,Me}]Zn\}_2(\mu\text{-}OH)]^+$ is that the protons of the hydroxide and aqua ligand participate in hydrogen-bonding interactions with the carbonyl groups of the ester substituents. Another interesting aspect of the hydroxide $[\{[Tp^{CO_2Et,Me}]Zn\}_2(\mu\text{-}OH)]^+$ is that solutions in methanol result in transesterification with the formation of $[\{[Tp^{CO_2Me,Me}]Zn\}_2(\mu\text{-}OH)]^+$. Thus, it appears that the bridging hydroxide complex is capable of "catalyzing" self-transesterification.

Finally, the terminal hydroxide complexes $[Tp^{Ar,Me}]$ZnOH (Ar = Ph, Cum) have also been used to prepare a variety of zinc−nucleobase complexes,[78e,86,87] and to investigate the coordination chemistry of zinc with simple amino acids and their protected forms.[88]

(B) Tris(imidazolyl)phosphine Ligands, $[Pim^{RR'}]$. Although the hydroxide and aqua complexes $[Tp^{RR'}]$ZnOH and $\{[Tp^{Bu^t,Me}]Zn(OH_2)\}^+$ provided an important breakthrough in the bioinorganic chemistry of zinc, a structural deficiency is that the nitrogen donors are not of the imidazolyl type, but are rather pyrazolyl-based. In this regard, the next advance in this area came with the application of the sterically demanding neutral tris[2-(1-isopropyl-4-*tert*-butylimidazolyl)]phosphine ligand, $[Pim^{Pr^i,Bu^t}]$, that enabled isolation of the hydroxide complex $\{[Pim^{Pr^i,Bu^t}]ZnOH\}^+$ by reaction of $[Pim^{Pr^i,Bu^t}]$ with $Zn(ClO_4)_2{\cdot}6H_2O$ (Scheme 8).[89] The molecular structure of $\{[Pim^{Pr^i,Bu^t}]ZnOH\}^+$ has been determined by X-ray diffraction, thereby demonstrating the close similarity to that of $[Tp^{Bu^t,Me}]$ZnOH. More recently, the $[Pim^{Me,p-Tol}]$ ligand that incorporates *p*-tolyl substituents has been used to prepare a series of zinc complexes $\{[Pim^{Me,p-Tol}]ZnX\}^+$ (X = Cl, Br, I, NO_3), although the hydroxide complex has not been isolated.[90]

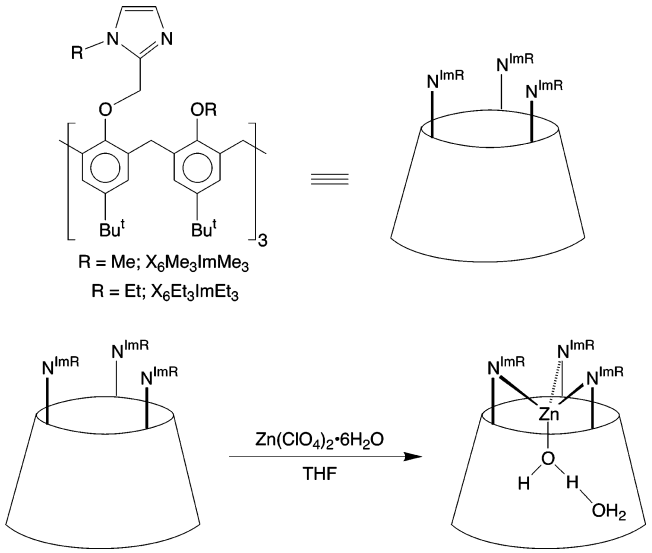
(C) Calixarene, Cavitand, and Other Ligands. A most significant recent development with respect to modeling the active site of carbonic anhydrase resides with the application of $X_6R_3ImR_3$, calixarene-based ligand that possesses three imidazole donors. Specifically, addition of $X_6Et_3ImMe_3$ and $X_6Et_3ImEt_3$ to $[Zn(OH_2)_6][ClO_4]_2$ yields $[(X_6Et_3ImMe_3)Zn(OH_2)]^{2+}$ and $[(X_6Et_3ImEt_3)Zn(OH_2)]^{2+}$, respectively, of which the latter has been structurally characterized by

Scheme 8. Synthesis of $\{[Pim^{Pr^i,Bu^t}]ZnOH\}^+$, a synthetic analogue of carbonic anhydrase that features imidazole coordination



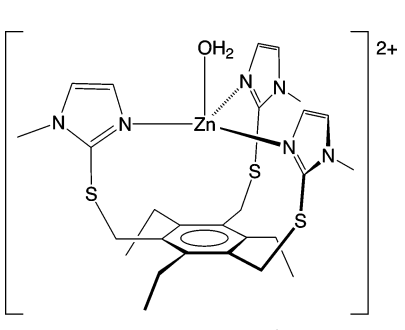
$[Pim^{i\text{-}Pr,t\text{-}Bu}]$ $\{[Pim^{i\text{-}Pr,t\text{-}Bu}]ZnOH\}\{ClO_4\}$

Scheme 9. Application of calixarene ligands for the preparation of tetrahedral zinc aqua complexes



X-ray diffraction (Scheme 9).[91] The X-ray diffraction study demonstrates that there are two molecules within the calixarene cavity. Thus, in addition to the molecule directly coordinated to zinc with a Zn−O distance of 1.974(2) Å, there is a second molecule of water that is hydrogen bonded to it with an O···O separation of 2.539(7) Å.

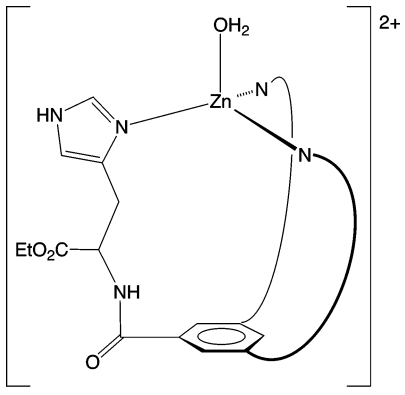
More recently, a tris(imidazolyl) cavitand ligand, TriMim, has been used to prepare the zinc aqua complex $[(TriMIm)Zn(OH_2)]X_2$ (X = BF_4, NO_3), as illustrated in Figure 12.[92] In contrast to the zinc aqua complexes $\{[Tp^{Bu^t,Me}]Zn(OH_2)\}^+$ and $[(X_6Et_3ImEt_3)\text{-}Zn(OH_2)\cdot(OH_2)]^{2+}$ where the geometries are distorted tetrahedral, the coordination geometry of $[(TriMIm)\text{-}Zn(OH_2)]^{2+}$ is best described as trigonal pyramidal. Furthermore, the Zn−O bond length of $[(TriMIm)\text{-}Zn(OH_2)]^{2+}$ (2.13 Å for BF_4^- derivative and 2.03 Å for NO_3^- derivative) is significantly longer than those of $\{[Tp^{Bu^t,Me}]Zn(OH_2)\}^+$ (1.94 Å) and $[(X_6Et_3ImEt_3)\text{-}Zn(OH_2)\cdot(OH_2)]^{2+}$ (1.97 Å). It is also notable that, depending upon the choice of counterion, the Zn−OH_2 bond lengths in $[(TriMIm)Zn(OH_2)]^{2+}$ may differ by 0.1 Å. This difference has been attributed to the different degrees of hydrogen bonding between the coordinated water and the counterion; the nitrate ligand is a better hydrogen-bond acceptor than BF_4^- and thereby shortens the Zn−O bond as the hydrogen-bonding interaction causes a shift towards a hydroxide derivative. While the TriMIm ligand enables isolation of a four-coordinate terminal aqua complex, the hydroxide ligand is observed to bridge in $\{[(TriMIm)Zn](\mu\text{-}OH)\}^{3+}$ which has also been structurally characterized by X-ray diffraction.



[(TriMIm)Zn(OH2)]2+

Figure 12. $[(TriMIm)Zn(OH_2)]^{2+}$, a trigonal pyramidal zinc aqua complex.

A benzene platform has also been used to prepare a tripodal pseudopeptide ligand with three imidazole donors (THB); the zinc complex $[(THB)Zn(OH_2)]^{2+}$ (Figure 13) has been generated in solution and the pK_a of the water molecule determined to be 6.2.[93]



[(THB)Zn(OH2)]2+

Figure 13. $[(THB)Zn(OH_2)]^{2+}$, a structural analogue of carbonic anhydrase.

Finally, tripodal peptides,[94] a cyclic heptapeptide,[95] and other small peptides[96] have also been used to generate active site models for carbonic anhydrase, but such species have not been structurally characterized by X-ray diffraction.

(iii) Modeling the Mechanism of Action and Function of Carbonic Anhydrase. **(A) Reversible Proton Transfer and the Interconversion of $\{[Tp^{Bu^t,Me}]\text{-}Zn(OH_2)\}^+$ and $[Tp^{Bu^t,Me}]ZnOH$.** The first critical step in the proposed mechanism of action of carbonic

Table 6. pK_a's of Selected Zinc Complexes

	coordination environment	pK_a	ref
H_2O	–	15.74	
$[\{Me_3[9]aneN_3\}Zn(OH_2)_3]^{2+}$	$[N_3O_2]Zn(OH_2)$	10.9	*a*
$[(phenolate\text{-}pendant\text{-}[12]aneN_3]Zn(OH_2)]^{+}$	$[N_3O]Zn(OH_2)$	10.7	*b*
$\{[N(CH_2CH_2NH_2)_3]Zn(OH_2)\}^{2+}$	$[N_4]Zn(OH_2)$	10.7	*c*
$[\{[14]aneN_4\}Zn(OH)_2]^{2+}$	$[N_4]Zn(OH_2)$	9.8	*d*
$[(DMAM\text{-}PMHD)Zn(OH_2)]^{+}$	$[N_3]Zn(OH_2)$	9.2	*e*
$[Zn(OH_2)_6]^{2+}$	$[O_5]Zn(OH_2)$	9.0	*e*
$\{[N(CH_2CH_2NMe_2)_3]Zn(OH_2)\}^{2+}$	$[N_4]Zn(OH_2)$	8.9	*c*
$[\{HN(C_2H_4NH_2)_2\}Zn(OH_2)]^{2+}$	$[N_3]Zn(OH_2)$	8.9	*f*
$[\{HN(C_2H_4NH_2)(C_3H_6NH_2)\}Zn(OH_2)]^{2+}$	$[N_3]Zn(OH_2)$	8.9	*f*
$[\{\eta^4\text{-}NH_2C_2H_4NHC_2H_4NHC_2H_4NH_2\}Zn(OH_2)]^{2+}$	$[N_4]Zn(OH_2)$	8.8	*g*
$[(CR)Zn(OH_2)]^{2+}$	$[N_4]Zn(OH_2)$	8.7	*e*
$\{[N(CH_2BIM)_3]Zn(OH_2)\}^{2+}$	$[N_4]Zn(OH_2)$	8.6, 8.0	*h,i*
$[\{HN(C_3H_6NH_2)_2\}Zn(OH_2)]^{2+}$	$[N_3]Zn(OH_2)$	8.6	*f*
$[Me_4\{[14]aneN_4\}Zn(OH)_2]^{2+}$	$[N_4]Zn(OH_2)$	8.4	*d*
$[\{EtN(CH_2Im^{Me})_2\}Zn(OH_2)]^{2+}$	$[N_3]Zn(OH_2)$	8.3	*j*
$[\{[11]aneN_3\}Zn(OH_2)]^{2+}$	$[N_3]Zn(OH_2)$	8.2	*d*
$[(ImH)_3Zn(OH_2)]^{2+}$	$[N_3]Zn(OH_2)$	8.0	*j*
$[\{(C_6H_9)(NH_2)_3\}Zn(OH_2)]^{2+}$	$[N_3]Zn(OH_2)$	8.0	*f*
$[\{[12]aneN_4\}Zn(OH_2)]^{2+}$	$[N_4]Zn(OH_2)$	8.0	*d*
$[\{[12]aneN_3\}Zn(OH_2)]^{2+}$	$[N_3]Zn(OH_2)$	7.3	*e*
$[iso\text{-}\{[12]aneN_3\}Zn(OH_2)]^{2+}$	$[N_3]Zn(OH_2)$	7.3	*d*
$[(C-PMHD)Zn(OH_2)]^{+}$	$[N_3O]Zn(OH_2)$	7.1	*e*
$\{[Tp^{Bu^t,Me}]ZnOH_2)\}^{+}$	$[N_3]Zn(OH_2)$	6.5 (est.)	*h*
$[(THB)Zn(OH_2)]^{2+}$	$[N_3]Zn(OH_2)$	6.2	*k*
$[(PATH)Zn(OH_2)]^{+}$	$[N_sS]Zn(OH_2)$	7.7	*l*

[a] Silver, G. C.; Gantzel, P.; Trogler, W. C. *Inorg. Chem.* **1995**, *34*, 2487. [b] Kimura, E.; Koike, T.; Toriumi, K. *Inorg. Chem.* **1988**, *27*, 3687. [c] Canary, J. W.; Xu, J.; Castagnetto, J. M.; Rentzeperis, D.; Marky, L. A. *J. Am. Chem. Soc.* **1995**, *117*, 11545. [d] Kimura, E.; Shiota, T.; Koike, T.; Shiro, M.; Kodama, M. *J. Am. Chem. Soc.* **1990**, *112*, 5805. [e] Bertini, I.; Luchinat, C. In *Bioinorganic Chemistry*; Bertini, I., Gray, H. B., Lippard, S. J., Valentine, J., Eds.; University Science Books: Mill Valley, California, 1994. [f] Fujii, Y.; Itoh, T.; Onodera, K.; Tada, T. *Chem. Lett.* **1995**, 305. [g] Itoh, T.; Fujii, Y.; Tada, T.; Yoshikawa, Y.; Hisada, H. *Bull. Chem. Soc. Jpn.* **1996**, *69*, 1265. [h] Vahrenkamp, H. *Acc. Chem. Res.* **1999**, *32*, 589. [i] Ichikawa, K.; Nakata, K.; Ibrahim, M. M.; Kawabata, S. *Advances in Chemical Conversions For Mitigating Carbon Dioxide*; Elsevier: New York, 1998; Vol. 114, p 309. [j] Sigel, H.; Martin, R. B. *Chem. Soc. Rev.* **1994**, *23*, 83. [k] Gelinsky, M.; Vogler, R.; Vahrenkamp, H. *Inorg. Chem.* **2002**, *41*, 2560. [l] diTargiani, R. C.; Chang, S. C.; Salter, M. H.; Hancock, R. D.; Goldberg, D. P. *Inorg. Chem.* **2003**, *42*, 5825.

anhydrase involves reversible proton transfer interconverting the aqua and hydroxide forms of the active site, $[(His)_3Zn-OH_2]^{2+}$ and $[(His)_3Zn-OH]^{+}$, (Scheme 3).[97] Indeed, the feasibility of this step proved to be an early concern for the proposed mechanism of action of carbonic anhydrase because it was not known whether it would be possible for a zinc-bound water molecule to have a pK_a as low as 7 to enable it to be sufficiently deprotonated at neutral pH such that it could play a catalytically important role;[98] for example, the pK_a of $[Zn(OH_2)_6]^{2+}$ is 9.0, such that it is not sufficiently deprotonated at neutral pH. However, it is now recognized that the pK_a of zinc-bound water molecules is strongly influenced by the coordination environment and that pK_a values as low as 7 are achievable. Specifically, studies on model species[33,52,72] and calculations[99−103] indicate that the pK_a is dictated strongly by the coordination number and charge of the complex, with the pK_a decreasing with decreasing coordination number and increasing positive charge (Table 6). While these pK_a changes are in accord with conventional notions, it is interesting to note that certain ligand modifications influence the acidity of a coordinated water in a manner that is counterintuitive, as illustrated by the fact that the hexamethyl complex $\{[N(CH_2CH_2NMe_2)_3]Zn(OH_2)\}^{2+}$ is *more* acidic than unsubstituted $\{[N(CH_2CH_2NH_2)_3]Zn(OH_2)\}^{2+}$ (Figure 14).[104] Normally one would expect that electron-donating methyl groups would lower the acidity. An explanation that has been put forth to rationalize the lower acidity of the unsubstituted

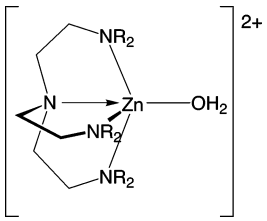


Figure 14. $\{[N(CH_2CH_2NR_2)_3]Zn(OH_2)\}^{2+}$ (R = H, Me).

$\{[N(CH_2CH_2NH_2)_3]Zn(OH_2)\}^{2+}$ derivative is that $N-H\cdots OH_2$, hydrogen bonding within $\{[N(CH_2CH_2NH_2)_3]Zn(OH_2)\}^{2+}$, stabilizes the complex and hence lowers its acidity. However, since none of the species involved has been structurally characterized, an alternative possibility that should not be discounted is that $\{[N(CH_2CH_2NR_2)_3]Zn(OH_2)\}^{2+}$ and $\{[N(CH_2CH_2NR_2)_3]Zn(OH)\}^{+}$ may have different coordination numbers due to interaction with either the counteranion or solvent.

As noted above, the hydroxide ligand of $[Tp^{Bu^t,Me}]ZnOH$ may be protonated by $(C_6F_5)_3B(OH_2)$ to give the aqua complex $\{[Tp^{Bu^t,Me}]Zn(OH_2)\}[HOB(C_6F_5)_3]$ in which the water molecule is *not* displaced by the counterion (Scheme 7). The formation of $\{[Tp^{Bu^t,Me}]Zn(OH_2)\}^{+}$ is, as expected, reversible, and subsequent treatment with Et_3N regenerates $[Tp^{Bu^t,Me}]ZnOH$ (Scheme 7). Furthermore, ^1H NMR spectroscopic studies of a solution of $[Tp^{Bu^t,Me}]ZnOH$, to which less than one equivalent of $(C_6F_5)_3B(OH_2)$ has been added, indicate that proton transfer between $[Tp^{Bu^t,Me}]ZnOH$

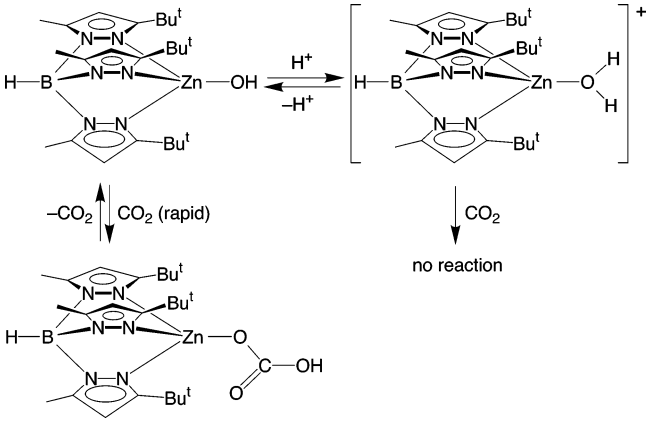
and $\{[Tp^{Bu^t,Me}]Zn(OH_2)\}[HOB(C_6F_5)_3]$ is rapid on the NMR time scale. Thus, the interconversion of $\{[Tp^{Bu^t,Me}]Zn(OH_2)\}^+$ and $[Tp^{Bu^t,Me}]ZnOH$ provides an exemplary illustration of the first step of the proposed mechanism of action of carbonic anhydrase.

In principle, an investigation of the interconversion of $\{[Tp^{Bu^t,Me}]Zn(OH_2)\}[HOB(C_6F_5)_3]$ and $[Tp^{Bu^t,Me}]ZnOH$ should enable the pK_a of the coordinated water to be determined. For example, $\{[Tp^{Bu^t,Me}]Zn(OH_2)\}[HOB(C_6F_5)_3]$ is readily deprotonated by Et_3N (Scheme 9), and measurement of this equilibrium constant would readily yield the pK_a of $\{[Tp^{Bu^t,Me}]Zn(OH_2)\}^+$ since that of $[Et_3NH]^+$ is known. However, the equilibrium constant for deprotonation of $\{[Tp^{Bu^t,Me}]Zn(OH_2)\}^+$ by Et_3N is sufficiently great that it is immeasurable; as such the pK_a of $\{[Tp^{Bu^t,Me}]Zn(OH_2)\}^+$ can only be shown to be considerably less than that of $[Et_3NH]^+$, i.e. 10.72 in aqueous solution.[105] Likewise, the equilibrium constant for protonation of $[Tp^{Bu^t,Me}]ZnOH$ by $(C_6F_5)_3B(OH_2)$ is sufficiently great that it indicates that the pK_a of $\{[Tp^{Bu^t,Me}]Zn(OH_2)\}^+$ is considerably greater than that of $(C_6F_5)_3B(OH_2)$, which has been estimated to be less than ca. 0.9 in aqueous solution.[79] Neither of these experiments, therefore, is capable of determining the pK_a of $\{[Tp^{Bu^t,Me}]Zn(OH_2)\}^+$, although they indicate that it lies in the rather unsatisfactorily large range of 0.9−10.7. For a routine pK_a determination, this would simply mean that a different acid or base should be chosen to determine the equilibrium constant. However, as discussed above, the zinc aqua ligand is readily displaced by anions, so the choice of suitable acids and bases is severely limited. Nevertheless, an estimate of ca. 6.5 has been cited for the pK_a of $\{[Tp^{Bu^t,Me}]Zn(OH_2)\}^+$, although specific details of the experiment to determine this value were not provided.[8c]

An important notion of the proposed mechanism of action of carbonic anhydrase (Scheme 3) is that the coordinated water is deprotonated prior to reaction with CO_2.[1−4,106] The isolation of both $[Tp^{Bu^t,Me}]ZnOH$ and its conjugate acid $\{[Tp^{Bu^t,Me}]Zn(OH_2)\}[HOB(C_6F_5)_3]$ provides a unique opportunity to study such a proposition in a well-defined system. It is therefore significant that, while $[Tp^{Bu^t,Me}]ZnOH$ reacts rapidly on the NMR time scale with CO_2 to give bicarbonate derivative $[Tp^{Bu^t,Me}]ZnOC(O)OH$ (discussed in more detail below),[73c] its conjugate acid $\{[Tp^{Bu^t,Me}]Zn(OH_2)\}[HOB(C_6F_5)_3]$ does *not* react with CO_2 under comparable conditions (Scheme 10). Specifically, since lifetime broadening is not observed for $\{[Tp^{Bu^t,Me}]Zn(OH_2)\}^+$ in the presence of CO_2, its reactivity towards CO_2 has been estimated to be at least a factor of 10^2 less than that of $[Tp^{Bu^t,Me}]ZnOH$. Such direct comparison provides an excellent demonstration that deprotonation of the zinc-bound water is indeed an essential step in the mechanism of action of carbonic anhydrase.

(B) Reactivity of CO_2 towards Zinc Hydroxide Complexes. The second key step of the proposed mechanism that follows deprotonation involves the reaction of CO_2 with the zinc hydroxide function. An excellent precedent for this reaction is provided by the reaction of $[Tp^{Bu^t,Me}]ZnOH$ with CO_2 to yield the

Scheme 10. Differential reactivity of $[Tp^{Bu^t,Me}]ZnOH$ and $\{[Tp^{Bu^t,Me}]Zn(OH_2)\}^+$ towards CO_2; the hydroxide complex reacts rapidly on the NMR time scale, whereas the aqua complex does not



bicarbonate derivative $[Tp^{Bu^t,Me}]Zn(OCO_2H)$ (Scheme 10).[73c] The bicarbonate ligand of $[Tp^{Bu^t,Me}]Zn(OCO_2H)$ is proposed to be unidentate on the basis that the IR spectrum exhibits absorptions at 1302 and 1675 cm^{-1}. Further support for the unidentate nature of the interaction is provided by the fact that the methyl carbonate ligand in $[Tp^{Bu^t,Me}]Zn(OCO_2Me)$ also exhibits unidentate coordination.[73b]

The formation of $[Tp^{Bu^t,Me}]Zn(OCO_2H)$ is reversible, such that removal of the CO_2 regenerates $[Tp^{Bu^t,Me}]ZnOH$. Importantly, this interconversion is rapid, even on the NMR time scale. Thus, upon addition of CO_2 to $[Tp^{Bu^t,Me}]ZnOH$, the sharp ^1H NMR spectroscopic signal attributed to the hydroxide ligand broadens substantially and merges into the baseline; upon progressive removal of the CO_2 atmosphere the signal for the hydroxide ligand returns to its original width, as illustrated in Figure 15. The

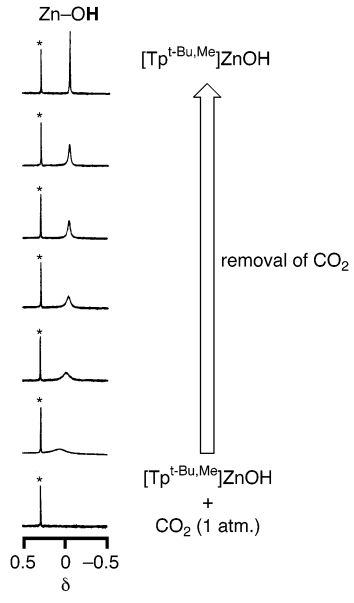
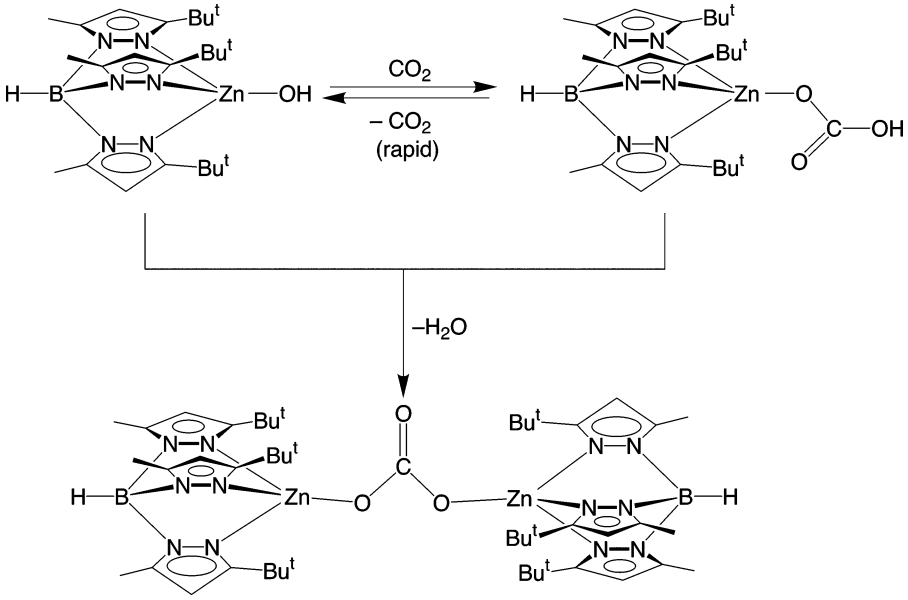


Figure 15. ^1H NMR spectroscopic evidence for rapid reaction between $[Tp^{Bu^t,Me}]ZnOH$ and CO_2 on the NMR time scale. The sharp resonance of the zinc hydroxide function (top spectrum) is broadened into the baseline in the presence of 1 atm of CO_2; upon progressive removal of the CO_2, the resonance associated with the zinc hydroxide returns to its original line width (∗ internal calibrant).

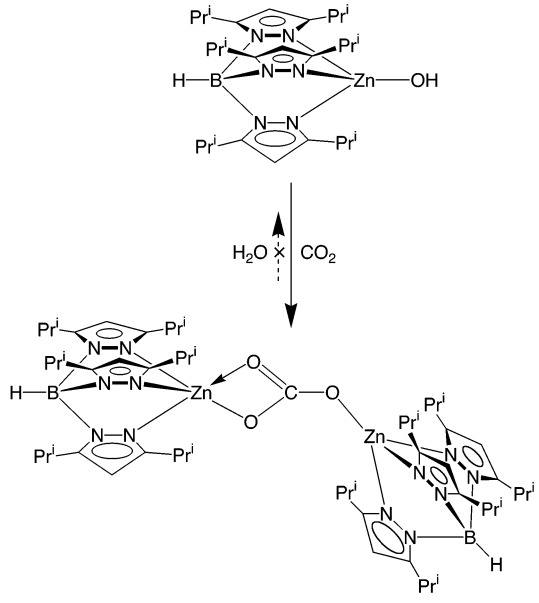
Scheme 11. Condensation of $[Tp^{Bu^t,Me}]ZnOH$ and $[Tp^{Bu^t,Me}]Zn(OCO_2H)$ to form a bridging carbonate derivative



rapid interconversion between $[Tp^{Bu^t,Me}]Zn(OCO_2H)$ and $[Tp^{Bu^t,Me}]ZnOH$ has precluded isolation of the former compound. Specifically, condensation between $[Tp^{Bu^t,Me}]Zn(OCO_2H)$ and $[Tp^{Bu^t,Me}]ZnOH$ yields the bridging carbonate complex $\{[Tp^{Bu^t,Me}]Zn\}_2(\mu\text{-}\eta^1,\eta^1\text{-}CO_3)$, which may be isolated over a period of days by virtue of its lower solubility (Scheme 11). The bridging carbonate complex $\{[Tp^{Bu^t,Me}]Zn\}_2(\mu\text{-}\eta^1,\eta^1\text{-}CO_3)$ is, however, extremely sensitive towards water, thereby regenerating the hydroxide derivative $[Tp^{Bu^t,Me}]ZnOH$.

The course of the reaction between CO_2 and $[Tp^{RR'}]ZnOH$ derivatives is strongly influenced by the nature of the pyrazolyl substituents. For example, the reaction between $[Tp^{Pr^i_2}]ZnOH$ and CO_2 proceeds *immediately* to the dinuclear bridging carbonate complex $\{[Tp^{Pr^i_2}]Zn\}_2(\mu\text{-}\eta^1,\eta^2\text{-}CO_3)$ (Scheme 12). Thus, by comparison to $[Tp^{Bu^t,Me}]Zn(OCO_2H)$, the postulated initially formed bicarbonate complex $[Tp^{Pr^i_2}]Zn(OCO_2H)$ is insufficiently stable to have a finite existence with respect to undergoing a condensation reaction with $[Tp^{Pr^i_2}]ZnOH$ to form $\{[Tp^{Pr^i_2}]Zn\}_2(\mu\text{-}\eta^1,\eta^2\text{-}CO_3)$. The higher reactivity of $[Tp^{Pr^i_2}]Zn(OCO_2H)$ is presumably a consequence of the reduced steric demands of the Pr^i versus Bu^t substituents. A second manifestation of the reduced steric demands is that the coordination mode of the carbonate ligands in $\{[Tp^{Bu^t,Me}]Zn\}_2(\mu\text{-}\eta^1,\eta^1\text{-}CO_3)$ and $\{[Tp^{Pr^i_2}]Zn\}_2(\mu\text{-}\eta^1,\eta^2\text{-}CO_3)$ are different. Specifically, the carbonate ligand in $\{[Tp^{Bu^t,Me}]Zn\}_2(\mu\text{-}\eta^1,\eta^1\text{-}CO_3)$ binds in a symmetric manner, with unidentate coordination to each zinc center, whereas that in $\{[Tp^{Pr^i_2}]Zn\}_2(\mu\text{-}\eta^1,\eta^2\text{-}CO_3)$ binds asymmetrically, with unidentate coordination to one zinc center and bidentate coordination to the other zinc center (Scheme 12). It is also significant that the different coordination modes of the carbonate ligands influences its reactivity towards water. Specifically, $\{[Tp^{Pr^i_2}]Zn\}_2(\mu\text{-}\eta^1,\eta^2\text{-}CO_3)$ is stable towards water, whereas $\{[Tp^{Bu^t,Me}]Zn\}_2(\mu\text{-}\eta^1,\eta^1\text{-}CO_3)$ reacts instantaneously to give $[Tp^{Bu^t,Me}]ZnOH$. This difference in reactivity is important because it demonstrates how the coordination mode of a carbonate ligand and, by inference, that of the bicarbonate ligand influences the stability of the complex towards water. In addition to the bridging carbonate complexes described above, a variety of other coordination modes for bridging carbonate are known for dinuclear,[107] trinuclear,[108] and tetranuclear complexes,[109] as illustrated in Figure 16.

Scheme 12. Reaction of $[Tp^{Pr^i_2}]ZnOH$ with CO_2 to give immediately the dinuclear bridging carbonate complex $\{[Tp^{Pr^i_2}]Zn\}_2(\mu\text{-}\eta^1,\eta^2\text{-}CO_3)$, with a coordination mode which differs from that of $\{[Tp^{Bu^t,Me}]Zn\}_2(\mu\text{-}\eta^1,\eta^1\text{-}CO_3)$



The reactivity of the trigonal bipyramidal hydroxide complex $\{[\eta^4\text{-}N\{CH_2[(C_6H_3N)NHCH_2Bu^t]\}_3]ZnOH\}^+$ towards CO_2 has been examined, and NMR spectroscopic evidence has been presented for the reversible formation of a bicarbonate complex, $\{[\eta^4\text{-}N\{CH_2[(C_6H_3N)NHCH_2Bu^t]\}_3]ZnOCO_2H\}^+$.[110] Another example of the formation of a zinc bicarbonate complex is obtained from the five-coordinate aqua complex $\{[N(CH_2bim)_3]Zn(OH_2)\}^{2+}$; on the basis of IR spectroscopy (ν_{CO} = 1440 and 1675 cm^{-1}),

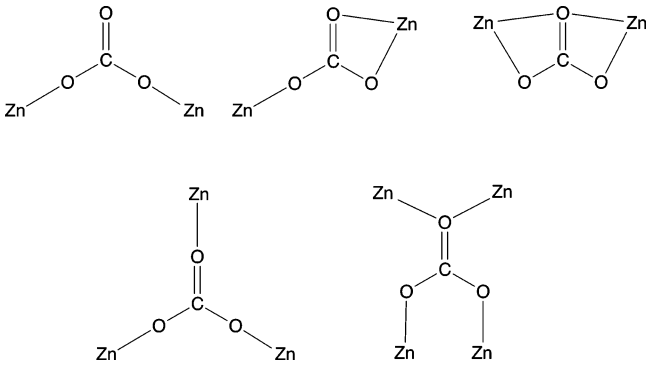


Figure 16. Coordination modes of the bridging carbonate ligand in multinuclear zinc complexes.

the bicarbonate ligand is also proposed to be unidentate.[111]

The facile displacement of the bicarbonate ligand is clearly an essential requirement for carbonic anhydrase activity, and the above observation suggests that factors which promote bidentate coordination of a bicarbonate ligand could inhibit the catalytic cycle. Support for this suggestion is provided by the fact that the greater catalytic activity of [{[12]aneN$_4$}-ZnOH]$^+$ towards hydration of CO_2, as compared to that of [{[12]aneN$_3$}ZnOH]$^+$, has been attributed to the greater tendency of the former to form a unidentate bicarbonate intermediate.[106,118] It is also worth noting that crystallographic studies on various forms of carbonic anhydrase indicate that there is not a strong preference for a specific coordination mode of bicarbonate, with both "unidentate"[112] and "bidentate"[113] structures having been observed.[48] As such, it is evident that the stability of a bidentate coordination mode in carbonic anhydrase would be insufficient to inhibit turnover.

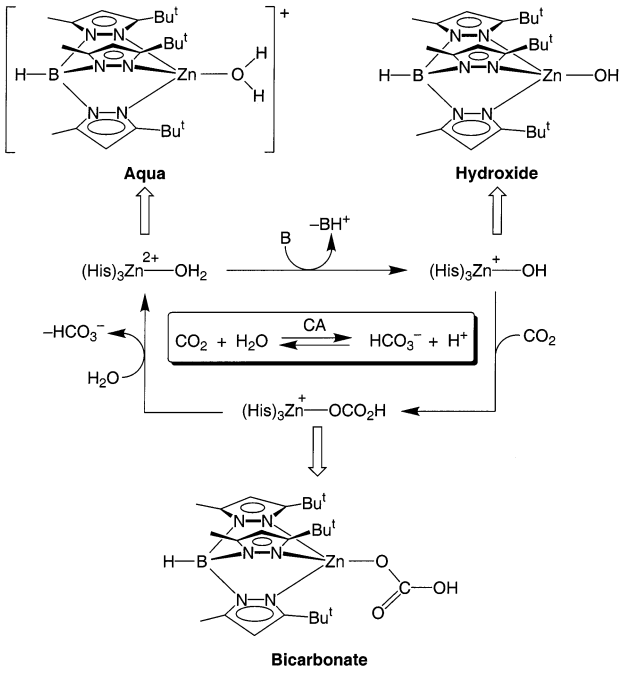
(C) Functional Models for Carbonic Anhydrase. The hydroxide complex [Tp$^{Bu^t,Me}$]ZnOH has also been shown to be a functional analogue of carbonic anhydrase. Specifically, the functional equivalence has been established by using ^{17}O NMR spectroscopy which demonstrates that [Tp$^{Bu^t,Me}$]ZnOH is capable of catalyzing the exchange of oxygen atoms between CO_2 and $H_2{}^{17}O$ (eq 1),[73c] a reaction that is also catalyzed by carbonic anhydrase.

$$CO_2 + H_2{}^{17}O \rightleftarrows CO^{17}O + H_2O \qquad (1)$$

Of direct relevance to the isotopic exchange reaction involving [Tp$^{Bu^t,Me}$]ZnOH and CO_2, the hydroxide complex [Tp^{Ph,Me}]ZnOH reacts with CS_2 to give [Tp^{Ph,Me}]ZnSH and COS, in which S/O exchange is observed.[114]

It is, therefore, evident from the above discussion that studies on the tris(pyrazolyl)borate system have provided the single most comprehensive investigation pertinent to the mechanism of action of carbonic anhydrase (Scheme 13). Thus, not only have the three key intermediates, namely aqua, hydroxide, and bicarbonate complexes, been observed, but their interconversion has also been shown to be facile as demonstrated by the fact that [Tp$^{Bu^t,Me}$]ZnOH is a catalyst for the exchange of oxygen atoms between CO_2 and $H_2{}^{17}O$.

Scheme 13. {[Tp$^{Bu^t,Me}$]Zn} system models the three key steps of the mechanism of action of carbonic anhydrase



The potential for [Tp$^{RR'}$]MX (M = Co, Ni, Cu, Zn, Cd; X = Cl, NO_3, CH_3CO_2) derivatives to participate in the reversible hydration of CO_2 has also been studied by stopped-flow techniques.[115] Other systems that have been shown to exhibit a functional equivalence to carbonic anhydrase are exemplified by five-coordinate tris(benzimidazolyl)zinc aqua derivative {[N(CH$_2$bimH)$_3$]Zn(OH$_2$)}$^{2+}$ (Figure 17),[78c,111,116] the water-soluble sulfonated version {[N(CH$_2$bimH−SO$_3$)$_3$]Zn(OH$_2$)}$^-$ (Figure 17),[117] and the macrocyclic complexes [{[12]aneN$_3$}Zn(OH)]$^+$ and [{[12]aneN$_4$}-Zn(OH)]$^+$.[72,118] Model studies employing {[12]aneN$_4$} derivatives have provided evidence that the inhibition of carbonic anhydrase by carboxamides is due to preferential coordination of the carboxamide, rather than water, to the zinc site, which thereby prevents the formation of the catlytically active zinc hydroxide.[119] The preferential coordination of carboxamides is observed in both acidic media (where

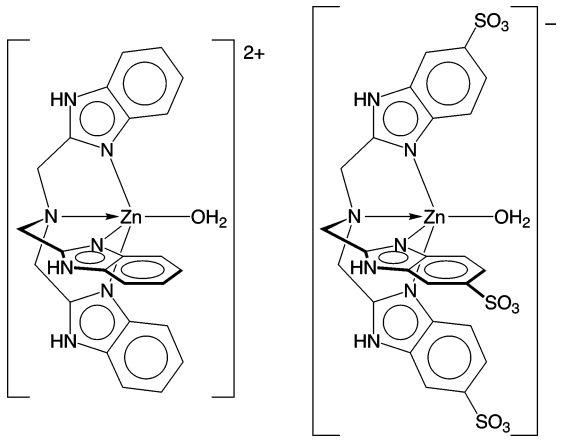
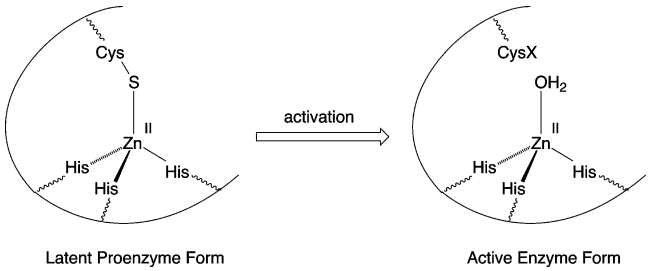


Figure 17. {[N(CH$_2$bimH)$_3$]Zn(OH$_2$)}$^{2+}$ and {[N(CH$_2$bimH−SO$_3$)$_3$]Zn(OH$_2$)}$^-$, five-coordinate zinc aqua complexes that show a functional equivalence to carbonic anhydrase.

Scheme 14. Activity of matrix metalloproteinases is controlled by secreting the enzymes in inactive proenzyme forms, and activation is achieved by converting the noncatalytic [$(His)_3Zn(Cys)$] zinc center to one with a [$(His)_3Zn(OH_2)$] motif that possesses protease activity

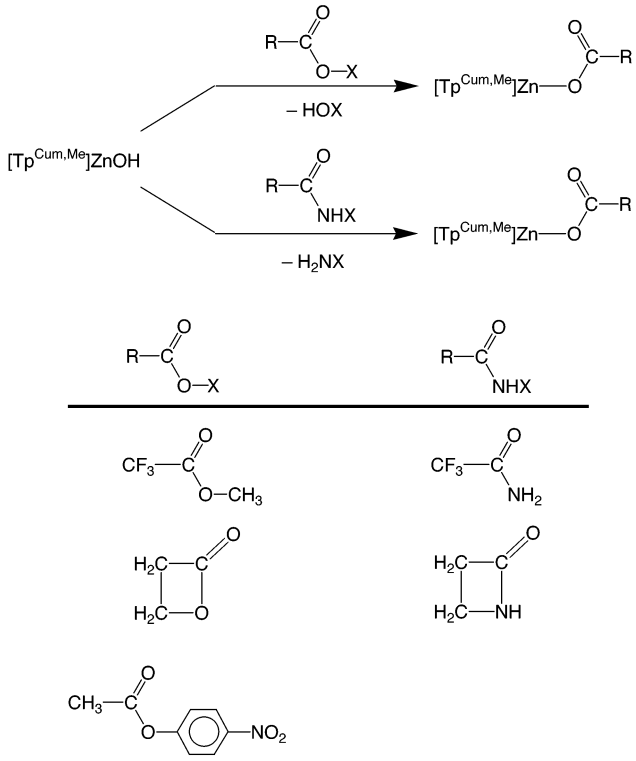


coordination occurs via the amide oxygen) and alkaline media (where coordination occurs via a deprotonated nitrogen atom).

In addition to the hydration of CO_2, carbonic anhydrase catalyzes the hydration of acetaldehyde. Comparison of the efficiencies of $\{[N(CH_2CH_2NH_2)_3]Zn(OH_2)\}^{2+}$ and $\{[N(CH_2CH_2NMe_2)_3]Zn(OH_2)\}^{2+}$ as catalysts for the hydration of acetaldehyde indicates that $\{[N(CH_2CH_2NH_2)_3]Zn(OH_2)\}^{2+}$ is an active catalyst, whereas the more sterically demanding derivative $\{[N(CH_2CH_2NMe_2)_3]Zn(OH_2)\}^{2+}$ is inert.[120] The inertness of $\{[N(CH_2CH_2NMe_2)_3]Zn(OH_2)\}^{2+}$ has been interpreted in terms of the catalytic cycle requiring zinc to expand its coordination number, with the increased steric demands provided by the methyl substituents of $\{[N(CH_2CH_2NMe_2)_3]Zn(OH_2)\}^{2+}$ inhibiting the increase in coordination number. Steric interactions in this case are therefore proposed to play a more important role in inhibiting the hydrolysis step, rather than promoting displacement of the hydrolyzed fragment.

3.1.1.2. Matrix Metalloproteinases. The matrix metalloproteinases (matrixins, MMPs) are a relatively recently discovered, but growing, class of zinc enzymes.[18] These enzymes are extremely important, with their specific role being the degradation of extracellular matrix components, such as collagen and proteoglycans, which are essential for embryonic development, wound healing, bone and growth development, and other physiological remodeling processes. The matrix components are degraded by hydrolytic cleavage of their amide bonds. Under normal conditions, the activity of matrix metalloproteinases is controlled by secreting the enzymes in inactive proenzyme forms and activation is achieved by a "cysteine switch" or "velcro" mechanism which converts the noncatalytic zinc center with a [$(His)_3Zn(Cys)$] motif to one with a [$(His)_3Zn(OH_2)$] motif that possesses protease activity (Scheme 14). In this way, the potential danger that may ensue from an organism secreting potent proteinases which can digest its supporting framework is minimized. However, to counter the adverse pathological effects of enhanced matrix metalloproteinase activity, inhibitors of these enzymes are required and have been the target of the pharmaceutical industry.[18d,121] In particular, synthetic peptides with hydroxamic acid groups are effective matrix metalloproteinase inhibitors by virtue of the fact that the hydroxamic acid groups

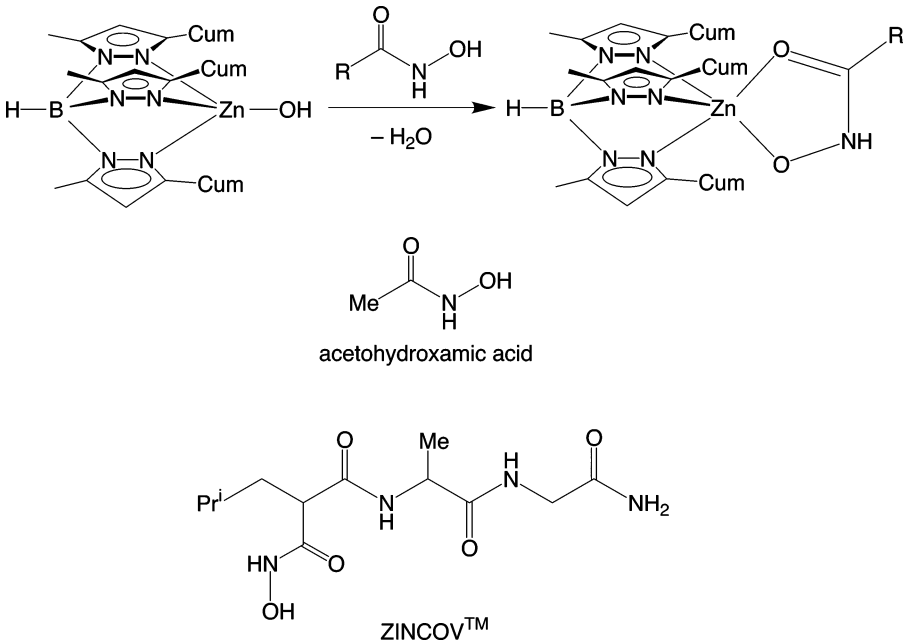
Scheme 15. Stoichiometric cleavage of activated amides and esters by $[Tp^{Cum,Me}]ZnOH$



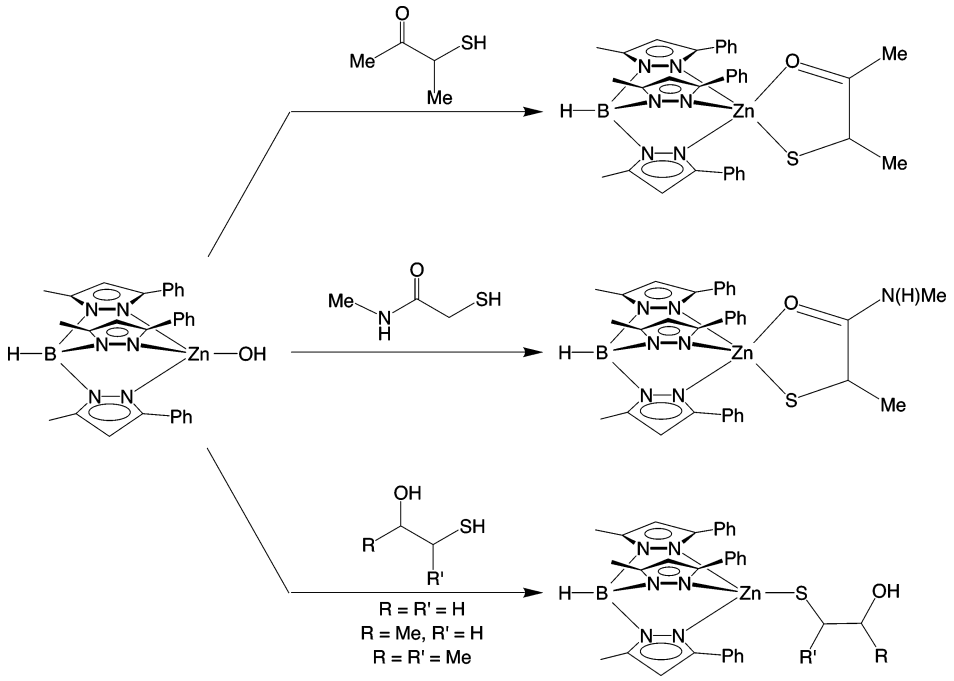
coordinate strongly to zinc and thereby prevent subsequent hydrolytic activity at the zinc center.[121] In addition to hydroxamic acid groups, carboxylic acids, phosphinic acids, and thiols have also been employed as the zinc binding groups (ZBG) that cause binding to the matrix metalloproteinase and thereby result in inhibition. To mimic the activity of matrix metalloproteinase inhibition, a variety of $[Tp^{RR'}]Zn-$ZBG complexes have been synthesized where ZBG refers to the zinc binding functional group of the inhibitor.

For example, the hydroxide complex $[Tp^{Cum,Me}]$-ZnOH has been shown to cleave activated amides and esters in a stoichiometric fashion, as illustrated in Scheme 15,[78b,122] and has been selected as a synthetic analogue to prepare mimics of enzyme inhibitors. Indeed, $[Tp^{Cum,Me}]ZnOH$ reacts with hydroxamic acids RC(O)N(H)OH to give hydroxamate derivatives $[Tp^{Cum,Me}]Zn\{ON(H)C(O)R\}$ in which both oxygen atoms of the ligand coordinate to the zinc center (Scheme 16).[78d] Structurally related complexes are obtained for both the simple acetohydroxamic acid and the more complex ZINCOV which is a commercially available inhibitor for matrix metalloproteinases.[123] The closely related zinc hydroxide $[Tp^{Ph,Me}]ZnOH$ likewise reacts with acetohydroxamic acid MeC(O)N(H)OH to give $[Tp^{Ph,Me}]Zn\{ON(H)C(O)Me\}$.[124] In addition to hydroxamic acids and related compounds with hydroxy/ketone functions, e.g., 1-hydroxy-2(1*H*)-pyridone,[125] the reactivity of $[Tp^{Ph,Me}]$-ZnOH towards simple thiol derivatives that have zinc binding groups of relevance to matrix metalloproteinase inhibitors, and specifically, β-mercaptoketones, β-mercaptoamides, and β-mercapto alcohols, was investigated (Scheme 17).[124] Interestingly, while both β-mercaptoketones and β-mercaptoamides yielded

Scheme 16. Mimicking inhibition of matrix metalloproteinases using hydroxamic acid derivatives



Scheme 17. Mimicking inhibition of matrix metalloproteinases using β-mercaptoketones, β-mercaptoamides, and β-mercapto alcohols

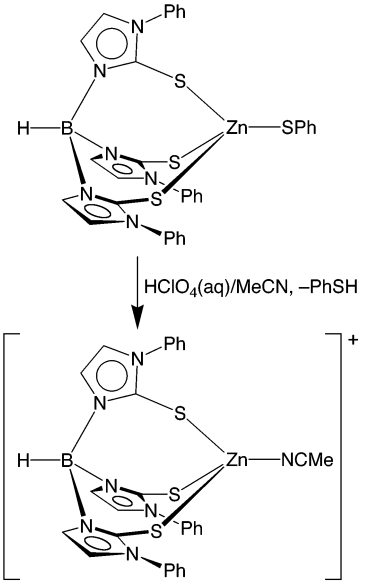


products in which the ligands coordinated in a bidentate manner, β-mercapto alcohols gave products in which the ligands coordinated in a unidentate manner via the sulfur atom. On the basis of this observation, it has been suggested that previously postulated bidentate coordination modes for related matrix metalloproteinase inhibitors may be incorrect.[124]

One means of activating matrix metalloproteinases is via protonolysis of the zinc thiolate bond (Scheme 14), and a chemical model for this activation mechanism is provided by the reactivity of [Tm^{Ph}]ZnSPh towards H^+. Specifically, treatment of [Tm^{Ph}]ZnSPh with $HClO_4$ in acetonitrile results in the rapid elimination of PhSH at room temperature and formation of {[Tm^{Ph}]Zn(NCMe)}(ClO_4), as illustrated in Scheme 18.[126] While a more biologically relevant transformation would yield a zinc aqua species, the above transformation is of relevance since it clearly demonstrates that proteolytic cleavage of a Zn−SPh moiety is facile.

Details concerning the geometrical changes that occur at the catalytic zinc center in the hydrolytic reactions catalyzed by zinc enzymes have been obtained by a Bürgi−Dunitz-type structure correlation analysis of a series of four- and five-coordinate [Tp$^{RR'}$]-Zn(X,Y) complexes.[127] The analysis suggests that the trajectory involves the initial approach of the oxygen atom of a substrate along a developing three-fold axis of a trigonal bipyramid, followed by Berry

Scheme 18. Mimicking activation of matrix metalloproteinases by hydrolytic cleavage of a zinc−thiolate bond

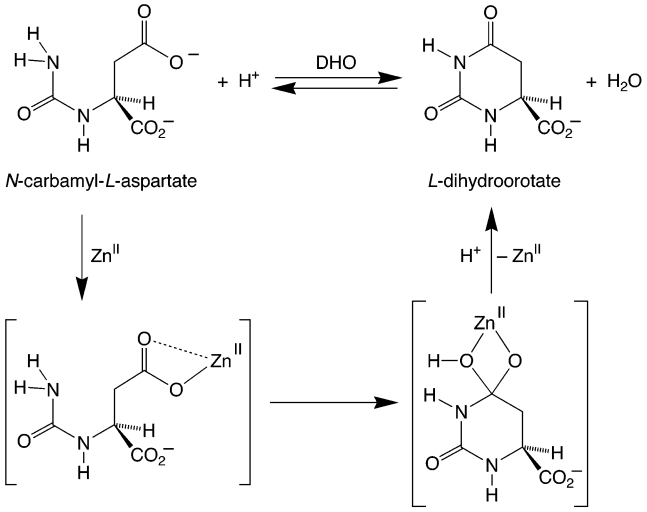


pseudorotation placing the substrate oxygen in an equatorial position and the original "hydroxide" oxygen (which is now coordinated to substrate) in an axial position prior to being displaced from zinc (Figure 18).

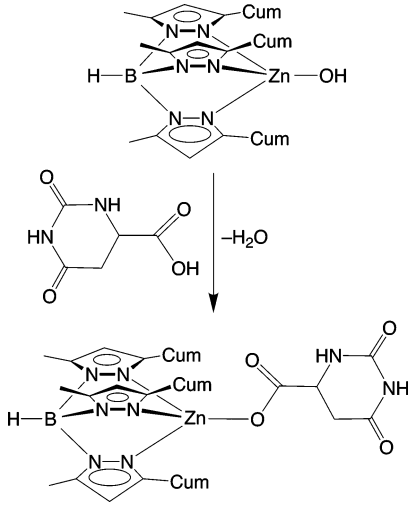
3.1.1.3. Dihydroorotase. Dihydroorotase (DHO) is a zinc enzyme that catalyzes the formation of L-dihydroorotate by cyclization of *N*-carbamyl-L-aspartate (Scheme 19).[128,129] As such, the role of the enzyme is to form, rather than cleave, an amide bond. The active site of dihydroorotase is analogous to that of carbonic anhydrase, with the zinc center being coordinated to three histidine residues. The mechanism of action is not as well established as that of carbonic anhydrase, but the pH profile indicates the involvement of a catalytic group with a pK_a of ca. 7; a postulated intermediate is illustrated in Scheme 19, in which zinc may be viewed as stabilizing a tetrahedral oxyanion transition state.

In view of the presence of three histidine residues at the active site, tris(pyrazolyl)borate ligands have also been used to model aspects of dihydroorotase chemistry. For example, the acetate complex [Tp$^{Bu^t,Me}$]-ZnO_2CMe has been suggested to model the binding of *N*-carbamyl-L-aspartate to the zinc center in dihydroorotase.[130] The hydroxide [Tp^{Cum,Me}]ZnOH reacts with L-dihydroorotic acid to give [Tp^{Cum,Me}]Zn(L-

Scheme 19. Proposed mechanism of action of dihydroorotase



Scheme 20. Reaction of [Tp^{Cum,Me}]ZnOH with L-dihydroorotic acid



dihydroorotate) in which the L-dihydroorotate ligand is bound in a unidentate manner via the carboxylate group adjacent to the [NHC(O)NH_2] fragment (Scheme 20).[78e] Furthermore, the dihydroorotate ligands in Zn(L-dihydroorotate)$_2$(OH_2)$_2$ coordinate in a similar unidentate manner.[131] However, since the mechanism of action of DHO involves an interaction between zinc and the other carboxylate group (Scheme 19), these L-dihydroorotate derivatives do not correspond to intermediates of the catalytic cycle. In addition to studying the reactivity of dihydroorotate

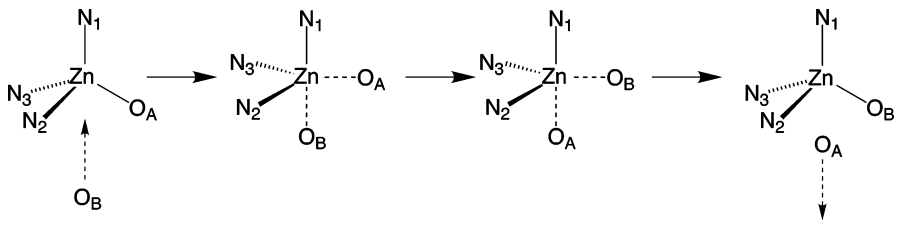


Figure 18. Bürgi−Dunitz-type structure correlation suggests the geometrical changes that occur at the catalytic zinc center in the hydrolytic reactions catalyzed by zinc enzymes: the initial approach of the oxygen atom of a substrate is along a developing three-fold axis of a trigonal bipyramid, followed by Berry pseudorotation, placing the substrate oxygen in an equatorial position and the original "hydroxide" oxygen (which is now coordinated to substrate) in an axial position prior to being displaced from zinc.

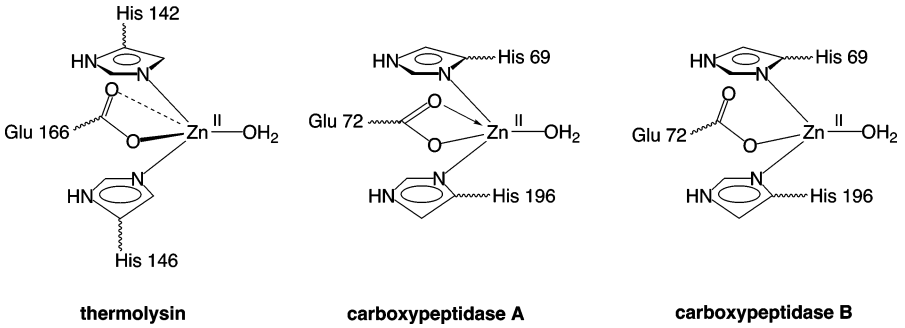
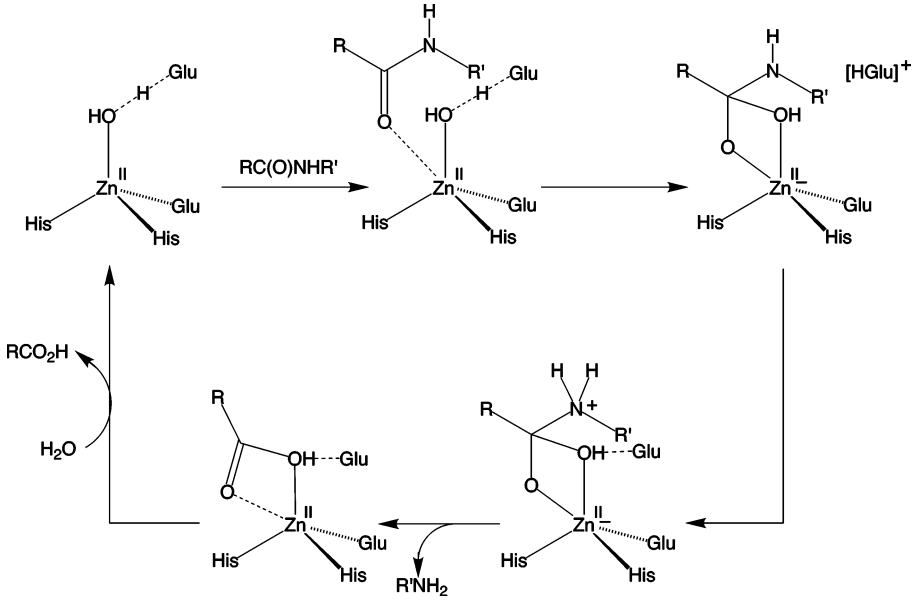


Figure 19. Active sites of thermolysin and carboxypeptidase A and B.

Scheme 21. "Hydroxide mechanism" for thermolysin reactivity (the negative charge on zinc is merely the formal charge resulting from deprotonation)



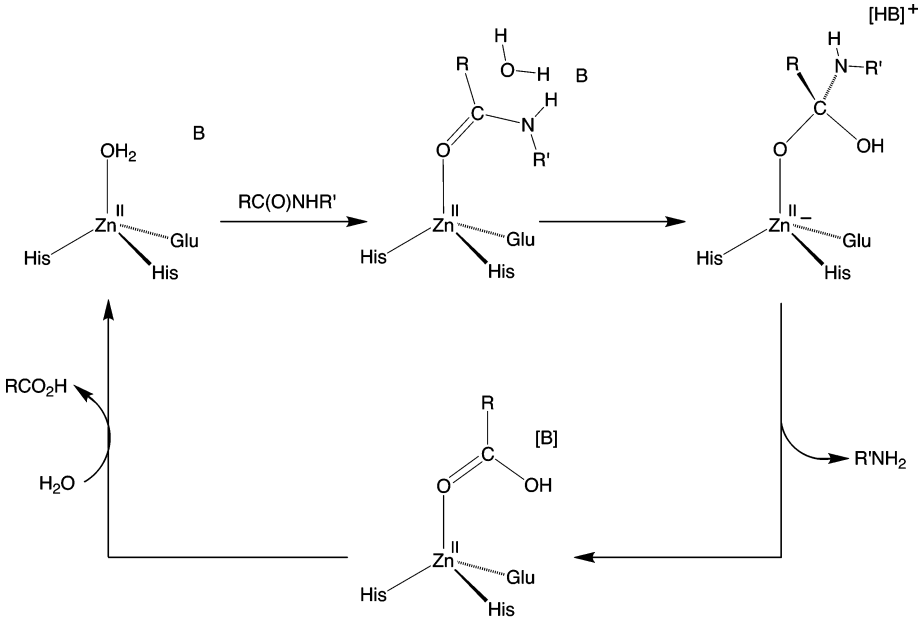
derivatives, zinc orotate complexes have also been investigated.[132]

3.1.2. The [(His)$_2$(Glu)Zn^{II}–OH$_2$] and [(His)$_2$(Asp)Zn^{II}–OH$_2$] Motifs: Thermolysin, Carboxypeptidase, and Neutral Protease

Thermolysin (TLN), carboxypeptidase (CP), and neutral protease (Bacillolysin) are three closely related zinc proteases that are responsible for catalyzing the hydrolysis of peptide bonds (Figure 1).[133] Thermolysin and neutral protease are endopeptidases, while carboxypeptidase is an exopeptidase that displays selectivity towards *C*-terminal amino acid residues. In addition to their similar peptide cleavage function, the active sites of carboxypeptidase[134,135] and thermolysin[136] bear a close resemblance, with the zinc centers of each being bound to the protein by a combination of one glutamate and two histidine residues,[137] a ligand combination that is common to many other metallopeptidases.[138] The similarity is further emphasized by the fact that the glutamate residue of each enzyme is capable of binding in both a unidentate and bidentate manner.[139] Thus, carboxypeptidase A exhibits bidentate glutamate binding,[134d] whereas carboxypeptidase B exhibits unidentate coordination,[134c] as illustrated in Figure 19. Thermolysin has likewise been reported to crystallize with either unidentate or bidentate coordination, depending upon pH.[136a] Neutral protease (Bacillolysin) also possesses a structure similar to that of thermolysin, but with a unidentate aspartate residue coordinating to zinc instead of glutamate.[140] It is, however, important to emphasize that not all zinc proteases possess [(His)$_2$(Glu/Asp)Zn^{II}–(OH$_2$)] motifs. For example, adamalysin II is an endopeptidase derived from snake venom[141−143] that has an active site in which the zinc is tetrahedrally coordinated to three histidine groups, i.e., [(His)$_3$Zn(OH$_2$)], similar to that of carbonic anhydrase. Furthermore, astacin is an endopeptidase for which the active site has an unusual trigonal bipyramidal geometry, with the zinc coordinated to three histidine residues, a tyrosine residue, and a water molecule.[144,145]

The mechanisms of action of carboxypeptidase and thermolysin are controversial, with there presently being two proposals which are principally differentiated according to whether hydrolysis of the peptide linkage occurs via attack of a zinc bound "hydroxide" or by attack of a noncoordinated water molecule (Schemes 21 and 22). In one proposal, the "hydroxide mechanism" (Scheme 21), the zinc serves two roles, namely (i) activating the water towards deprotonation by a glutamate residue and (ii) activating the carbonyl group of the peptide unit towards nucleophilic attack.[2b,134e,137,146,147] Subsequent transfer of the incipient hydroxide to the carbonyl group, followed

Scheme 22. "Reverse protonation mechanism" for thermolysin reactivity (the negative charge on zinc is merely the formal charge resulting from deprotonation)



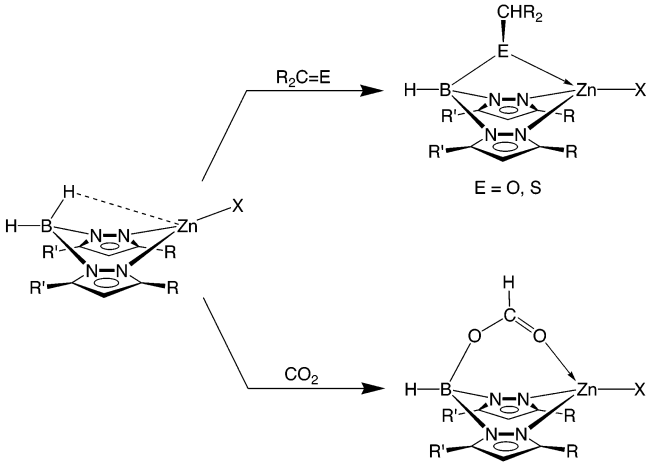
B = General base (His-231 for CPA; substrate carboxylate for TLN)

by a return transfer of the proton from the glutamate residue to the nitrogen, achieves the peptide cleavage. An alternative proposal involves "reverse protonation" catalysis, in which complete displacement of the active-site water by the oxygen atom of the peptide carbonyl group is followed by a general base-assisted attack of H_2O on the carbonyl group, as illustrated in Scheme 21.[149] In the case of thermolysin, the role of the general base is provided by His-231, whereas in the case of carboxypeptidase it is provided by the carboxylate group of the substrate.

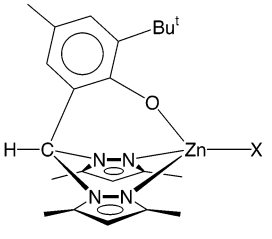
A variety of tridentate ligands with $[N_2O]$ donor arrays has been introduced to model zinc enzymes such as carboxypeptidase and thermolysin,[8b,e,150−154] as have $[N_3O]$ donors.[108g,155−157] Many of these ligands, however, do not enforce tetrahedral coordination geometries akin to those in the enzymes. For example, bis[(3,5-diisopropylpyrazolyl)ethyl]ether, $O(CH_2CH_2pz^{Pr^i_2})_2$, binds to zinc with a meridional (i.e., a "T-shaped") configuration, rather than the desired facial configuration which would mimic that in the enzymes.[151] Facial binding may, nevertheless, be enforced by using a ligand in which the [NNO] donor array is appended to a common tetrahedral center. For example, suitable [NNO] donor ligands may be constructed directly on the zinc center by insertion of R_2CO (R = H, Ph) or CO_2 into the B−H bond of a bis(pyrazolyl)hydroborato derivative (Scheme 23).[150,152] The formate derivative $[\eta^3\text{-}(HCO_2)Bp^{Bu^t,Pr^i}]ZnCl$ is particularly significant since it is the first structurally characterized tetrahedral zinc complex of a tridentate [NNO] ligand in which the O-donor is a carboxylate group. Since only one oxygen coordinates to zinc, the complex is better regarded as a synthetic analogue of thermolysin or carboxypeptidase B rather than of carboxypeptidase A.

More recently, tripod ligands using carbon as the linker atom instead of boron have allowed isolation

Scheme 23. Formation of [NNO]ZnX and [NNS]ZnX complexes by functionalization of a B−H bond



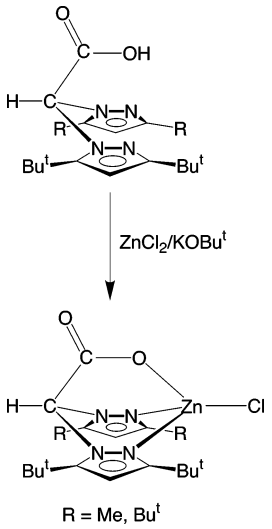
of some closely related zinc complexes, $[HC(pz^{Me_2})_2(C_6H_2MeBu^tO)]ZnX$ (X = Cl, Me) (Figure 20).[154] Furthermore, another interesting class of [NNO] donor ligands is one which incorporates a carboxylate donor group, namely, bis(pyrazolyl)methane acetate derivatives, $[Bpa^{RR'}]H$ (Scheme 24). In addition to symmetric ligands that feature the same substituents on the pyrazolyl groups, asymmetric ligands with different pyrazolyl groups may also be obtained.[158]



X = Cl, Me

Figure 20. $[HC(pz^{Me_2})_2(C_6H_2MeBu^tO)]ZnX$ derivatives which feature an [NNO] tripodal ligand.

Scheme 24. [NNO] donor ligand that incorporates a carboxylate donor group



A tripod ligand employing benzimidazole and acetate donors has allowed the complex $\{[N(CH_2BIM)_2(CH_2CO_2)]Zn(OH_2)\}^+$ to be prepared (Figure 21),[159] as have related derivatives.[155,156] However, in contrast to the $[(HCO_2)Bp^{Bu^t,Pr^i}]$ and $[HC(pz^{Me_2})_2(C_6H_2MeBu^tO)]$ ligands, the central nitrogen of the $[N(CH_2BIM)_2(CH_2CO_2)]$ tripod also interacts with the zinc center (ca. 2.36 Å), and so $\{[N(CH_2BIM)_2(CH_2CO_2)]Zn(OH_2)\}^+$ is best described as five-coordinate. Nevertheless, the complex is an active catalyst for the hydrolysis of β-lactam to β-alanine.

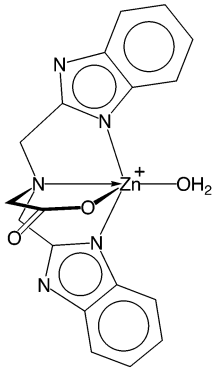


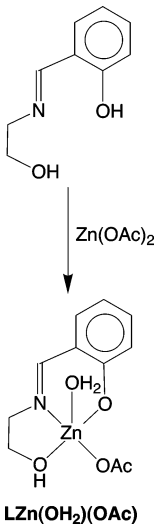
Figure 21. $\{[N(CH_2BIM)_2(CH_2CO_2)]Zn(OH_2)\}^+$, a five-coordinate zinc aqua complex that features an $[N_3O]$ donor ligand.

Finally, it is worth noting that a tridentate [NOO] ligand has recently been utilized to indicate how the chemistry of zinc behaves in an oxygen-rich environment. For example, the five-coordinate zinc aqua complex has been synthesized (Scheme 25) and structurally characterized by X-ray diffraction, thereby demonstrating that the $Zn-OH_2$ bond length [2.006(2) Å] is comparable to those of the Zn−OAr [2.025(2) Å] and Zn−OAc [1.960(2) Å] interactions but is much shorter than the Zn−O(H)R bond length [2.419(2) Å].[160]

3.1.3. The [(His)$_2$(Cys)Zn^{II}–OH$_2$] Motif: Bacteriophage T7 Lysozyme and Peptide Deformylase

Bacteriophage T7 lysozyme is a zinc enzyme which destroys bacteria by cleaving the amide bond between

Scheme 25. Synthesis of a five-coordinate zinc complex employing an [NOO] donor ligand



L-alanine and *N*-acetylmuramate moieties of polysaccharide components within their cell walls.[161] X-ray diffraction studies of a mutant lysozyme (AK6) reveal that the active site is located in a cleft within the protein which is ca. 22−26 Å long and 10−11 Å deep. The tetrahedral zinc center of the active site is bound to the protein backbone via one sulfur and two nitrogen donors of cysteine (Cys-130) and histidine (His-17 and His-122) residues; the fourth site is occupied by a water molecule (Figure 22).[162] Peptide deformylase is an enzyme that is related to T7 lysozyme by virtue of the common coordination of one cysteine and two histidine donors to the zinc center of the active site.[163,164] However, recent studies sug-

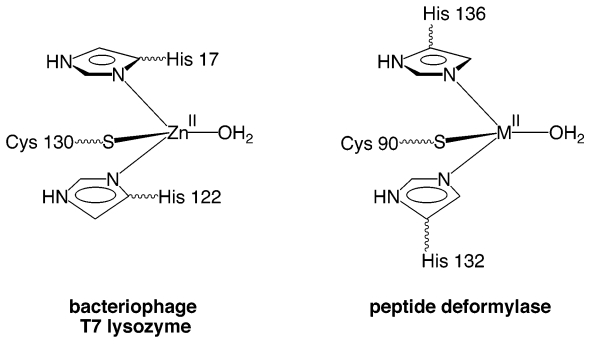
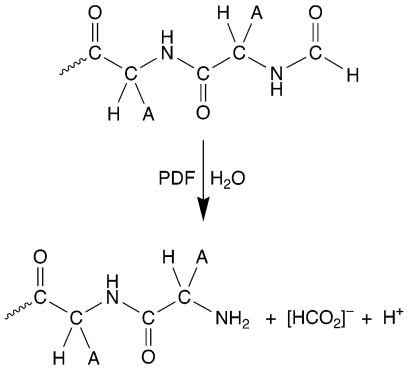
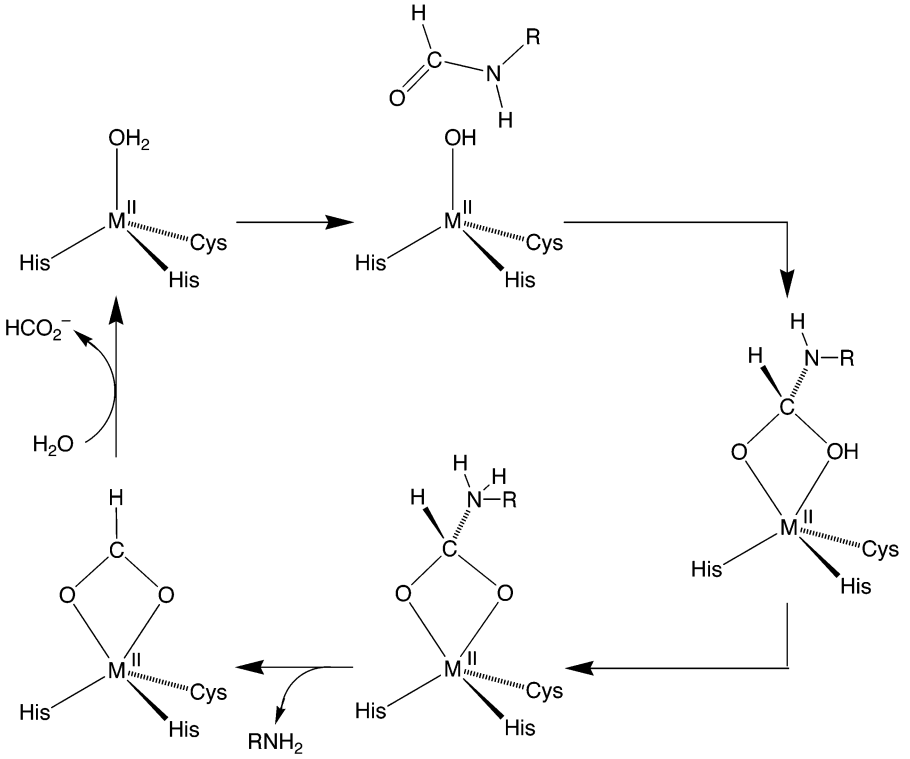


Figure 22. Active sites of bacteriophage T7 lysozyme and peptide deformylase.

Scheme 26. Reaction catalyzed by peptide deformylase



Scheme 27. Essential features of the proposed mechanism of action of peptide deformylase



gest that the zinc form of peptide deformylase has low activity and that the active form which is responsible for the hydrolytic cleavage of a formyl group (Scheme 26) is actually an *iron* enzyme.[165−167] A simplified version of the most recently proposed mechanism of action, focusing attention on changes at the metal center, is illustrated in Scheme 27.[165]

Tridentate [N_2S] ligands that support monomeric tetrahedral zinc centers analogous to the active sites of peptide deformylase and T7 lysozyme are not common. For example, *N*-(2-mercaptoethyl)picolylamine (MEPAH) and *N*-(2-mercaptophenyl)picolylamine have not yielded structually characterized monomeric tetrahedral zinc complexes.[168] Indeed, the zinc chemistry derived from MEPAH is found to be very complex, with polymeric $[(MEPA)ZnX]_n$ (X = Cl, Br), trinuclear $[(MEPA)_4Zn_3]X_2$ (X = BF_4, ClO_4, NO_3), and dinuclear $[(MEPA)Zn(OAc)]_2$ complexes having been isolated. Likewise, bis(benzimidazolyl)thioethers yield trinuclear and octanuclear zinc clusters.[169] It is, therefore, evident that one of the principal reasons for the difficulty in isolating mononuclear [N_2S]ZnX complexes is the propensity of sulfur to act as a bridge between metal centers. A second problem in obtaining synthetic analogues is that the ability of sulfur to coordinate to zinc is very sensitive to the nature of the ligand. For example, whereas the bis(pyrazolylethyl)ether ligand $O(CH_2CH_2pz^{Pri_2})_2$ binds to zinc in $[O(CH_2CH_2pz^{Pri_2})_2]Zn(NO_3)_2$ via both the pyrazolyl and ether functions, the sulfur atom of the thioether counterpart $[S(CH_2CH_2pz^{Me_2})_2]ZnCl_2$ does *not* coordinate to zinc.[170] Likewise, a variety of bis(imidazolyl)thioether[171,172] and bis(pyrazolyl)(thienyl)methane[173] ligands have been shown to form zinc complexes in which the sulfur does not coordinate to zinc. The low tendency of sulfur to bind to zinc in these thioether complexes, therefore, appears to be a rather general observation. However, the use of a [N_2S] donor ligand in which "facial" binding is preferred, assists the formation of a tetrahedral [{N_2S}ZnX] derivative. By analogy with the facially tridentate [N_2O] ligands [{$R_2C(H)O$}-Bp^{Bu^t,Pr^i}] described above, the sulfur counterpart [{$Ph_2C(H)S$}Bp^{Bu^t,Pr^i}] also stabilizes a monomeric tetrahedral zinc center. Specifically, [η^3-{$Ph_2C(H)S$}-Bp^{Bu^t,Pr^i}]ZnI may be synthesized by insertion of Ph_2CS into a B−H bond of [Bp^{Bu^t,Pr^i}]ZnI (Scheme 23).[174] Related complexes derived from [N_2S] ligands that use a carbon, rather than a boron, atom linker have also been synthesized, e.g., $[HC(pz^{Me_2})_2(C_6H_2MeBu^tS)]$-ZnSPh and $[HC(pz^{Me_2})_2(CMe_2S)]$ZnX (Figure 23).[154,175] [N_3S] donor ligands have also been investigated.[176]

An [N_2S] ligand, 2-methyl-1-[methyl-(2-pyridin-2-yl-ethyl)amino]propane-2-thiol (PATH−H) in which the sulfur donor is a thiolate has been used to prepare monomeric complexes such as (PATH)ZnBr, (PATH)ZnNCS, and (PATH)ZnMe (Scheme 28).[177,178] This is a rare example of the application of an acyclic tridentate ligand to afford monomeric zinc complexes pertinent to the biological chemistry of zinc. In this respect, acyclic *S*-benzyl-*β*-N-(2-pyridyl)-methylene-

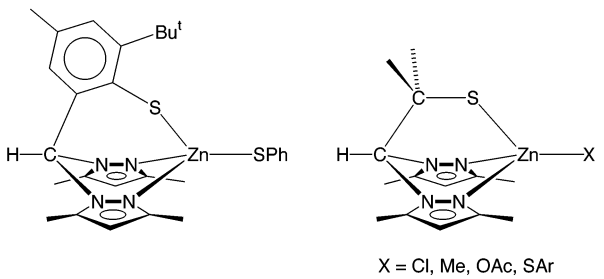
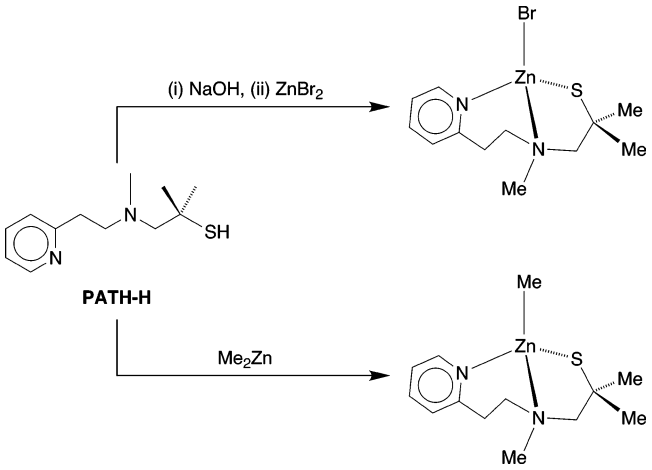
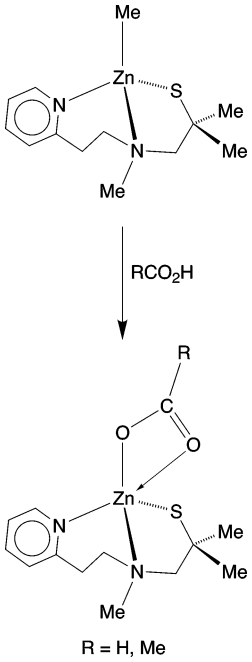


Figure 23. $[HC(pz^{Me_2})_2(C_6H_2MeBu^tS)]ZnSPh$ and $[HC(pz^{Me_2})_2(CMe_2S)]ZnX$, two complexes that feature tripodal [NNS] donor ligands.

Scheme 28. Synthesis of zinc complexes using an acyclic [NNS] donor ligand



Scheme 29. Synthesis of zinc formate and acetate complexes with an [NNS] donor co-ligand

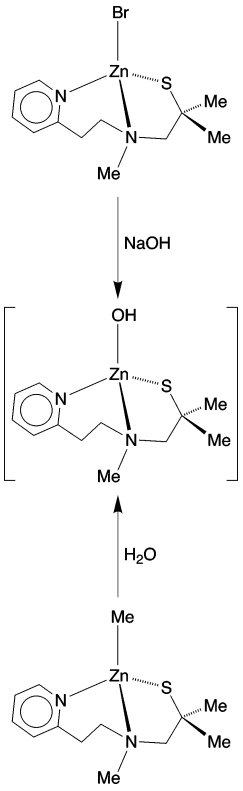


dithiocarbazate (HNNS) yields a six-coordinate $[NNS]_2Zn$ complex.[179]

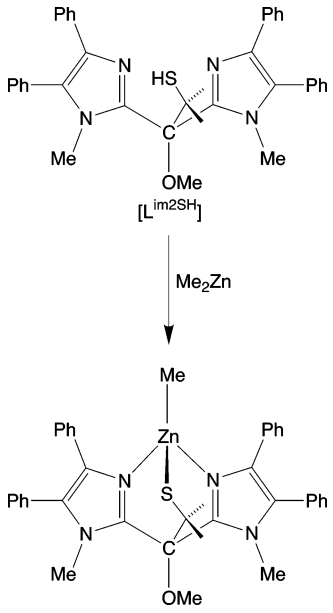
The methyl derivative (PATH)ZnMe also reacts with HCO_2H and $MeCO_2H$ to give formate and acetate derivatives, respectively (Scheme 29).[178] The isolation of the formate complex (PATH)Zn(O_2CH) is significant because formate species have been postulated to play a role in the mechanism of action of peptide deformylase.[165] The most notable feature of the structure of (PATH)Zn(O_2CH) is that the formate ligand coordinates in an anisobidentate mode with a difference in Zn−O bond lengths of 0.61 Å. In contrast, a related acetate complex [(L_3S)Zn(O_2CMe)] of a tripodal ligand exhibits unidentate coordination with a difference in Zn−O bond lengths of 0.94 Å.[175] It has been suggested that strong binding of the formate ligand could inhibit the catalytic cycle of peptide deformylase.

Although the hydroxide counterpart (PATH)ZnOH has not been isolated, evidence for its generation in

Scheme 30. Generation of an [NNS]ZnOH complex in aqueous solution



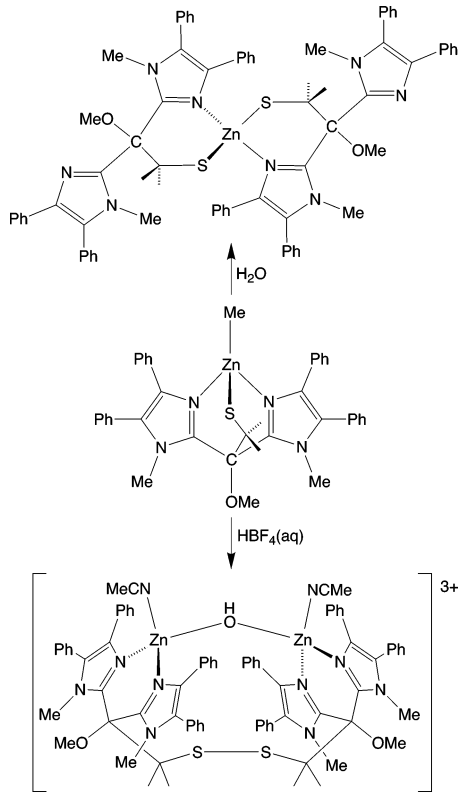
Scheme 31. Synthesis of an [NNS]ZnMe complex



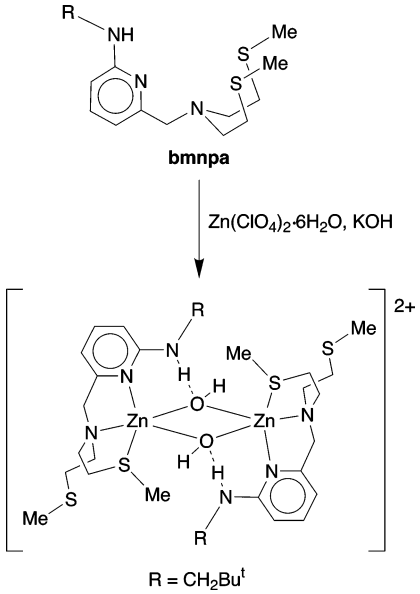
solution has been obtained by (i) addition of aqueous NaOH to a methanol solution of (PATH)ZnBr and (ii) dissolution of the methyl complex (PATH)ZnMe in D_2O (Scheme 30).[180] The pK_a of the aqua species $[(PATH)Zn(OH_2)]^+$ has been determined to be 7.7(1) by potentiometric titrations.[180] The hydroxide is a catalyst for the hydrolysis of *p*-nitrophenyl acetate, and the kinetic pK_a of 8.05(5) compares favorably with the thermodynamic measurement.

Mononuclear zinc hydroxide complexes of the type {[N_2S]ZnOH} have yet to be structurally characterized, with dinuclear species being formed as a result

Scheme 32. Reactivity of $[L^{Im2S}]ZnMe$ towards H_2O and $HBF_4(aq)$



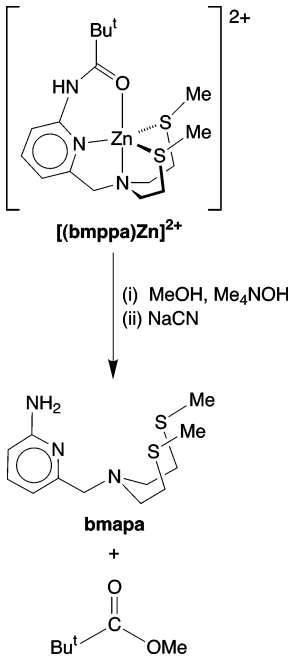
Scheme 33. Formation of a five-coordinate bridging hydroxide complex using an $[N_2S_2]$ donor ligand



of the proclivity of hydroxide ligands to bridge two zinc centers in the absence of sterically demanding substituents. For example, the methyl complex $[L^{Im_2S}]ZnMe$ (Scheme 31) reacts with H_2O to form $[\eta^2\text{-}L^{Im_2S}]_2Zn$ (and presumably $Zn(OH)_2$) via ligand redistribution (Scheme 32). Furthermore, treatment of $[L^{Im_2S}]ZnMe$ with aqueous HBF_4 yields the hydroxide-bridged complex $[\mu\text{-}\eta^2,\eta^2\text{-}(L^{Im_2S})_2]Zn_2(MeCN)_2(\mu\text{-}OH)]^{3+}$, where the $[L^{Im_2S}]$ ligand has experienced a S−S coupling reaction.[181]

Likewise, it is worth noting that the complex $\{[(bmnpa)Zn(\mu\text{-}OH)]_2\}^{2+}$ also contains bridging hydroxide ligands and that only one of the two thioether linkages of the $[N_2S_2]$ bmnpa ligand coordinates to each zinc center, as illustrated in Scheme 33 [bmnpa = *N*-bis-2-(methylthio)ethyl-*N*-(6-neopentylamino-2-pyridyl-methyl].[182] The structural characterization of the hydroxide-bridged complexes $\{[(bmnpa)Zn(\mu\text{-}OH)]_2\}^{2+}$ and $[\mu\text{-}\eta^2,\eta^2\text{-}(L^{Im_2S})_2]Zn_2(MeCN)_2(\mu\text{-}OH)]^{3+}$ underscores the difficulty associated with isolating well-defined terminal hydroxide complexes of the class $\{[N_2S]ZnOH\}$.

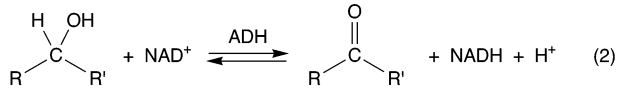
Scheme 34. Methanolysis of the bmppa ligand coordinated to zinc



The amide linkage in the zinc complex $[(bmppa)Zn]^{2+}$ undergoes methanolyis in the presence of Me_4NOH, releasing the bmpa ligand (Scheme 34).[183]

3.1.4. The [(His)(Cys)$_2$Zn^{II}–OH$_2$] Motif: Liver Alcohol Dehydrogenase

3.1.4.1. Structure and Mechanism of Action of Liver Alcohol Dehydrogenase. Alcohol dehydrogenases (ADH) are a class of zinc enzymes that catalyze the biological oxidation of primary and secondary alcohols via the formal transfer of a hydride anion to the oxidized form of nicotinamide adenine dinucleotide (NAD^+), coupled with the release of a proton (eq 2).[184]



(2)

Of these enzymes, liver alcohol dehydrogenase (LADH) is the most widely investigated, with X-ray diffraction studies having demonstrated that LADH consists of two similar subunits, each of which contains two zinc sites. However, only one site within each subunit is catalytically active, namely that in which the zinc is coordinated in a distorted tetrahedral manner to a histidine and two cysteine residues of a single polypeptide chain, with a water molecule occupying the fourth coordination site (Fig-

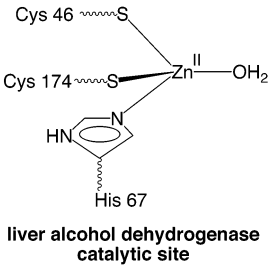
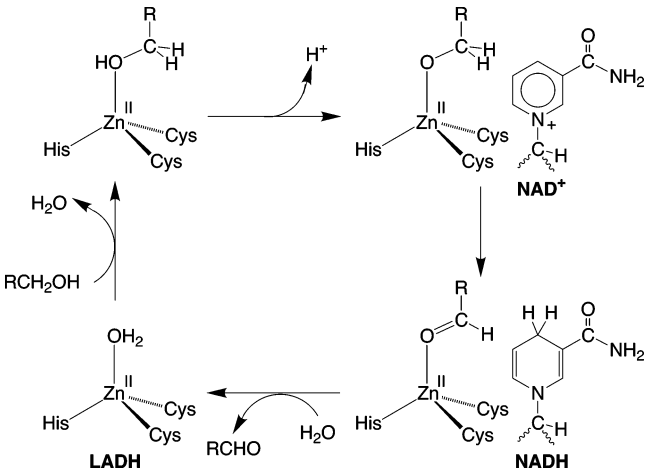


Figure 24. Active site of LADH.

ure 24). The remaining zinc, which is coordinated tetrahedrally to four cysteine residues, plays only a structural role. As with other zinc enzymes, the proposed mechanism of action of LADH has been modified over the years, with the currently accepted simplified version focusing on changes at the metal center being summarized in Scheme 35.[185] The es-

Scheme 35. Essential features of the mechanism of action of liver alcohol dehydrogenase



sential features of the catalytic cycle involve (i) binding NAD^+, (ii) displacement of the water molecule by alcohol, (iii) deprotonation of the coordinated alcohol affording a zinc alkoxide intermediate, (iv) hydride transfer from the alkoxide to NAD^+ giving a zinc-bound aldehyde, (v) displacement of the aldehyde by water, and (vi) release of NADH. The principal role of the zinc in the dehydrogenation reaction is, therefore, to promote deprotonation of the alcohol and thereby enhance hydride transfer from the incipient alkoxide species. Conversely, the role of the zinc in the reverse reaction (hydrogenation) is to enhance the electrophilicity of the carbonyl carbon atom. Fine details of the mechanism are, however, controversial. For example, it has been questioned whether the zinc center is capable of reducing the pK_a of an alcohol from ca. 16 to ca. 6, so that it is capable of being deprotonated upon coordination.[27] It has also been questioned whether the zinc remains four-coordinate during the catalytic cycle, but the consensus appears to be that the water is displaced completely by the substrate.[185]

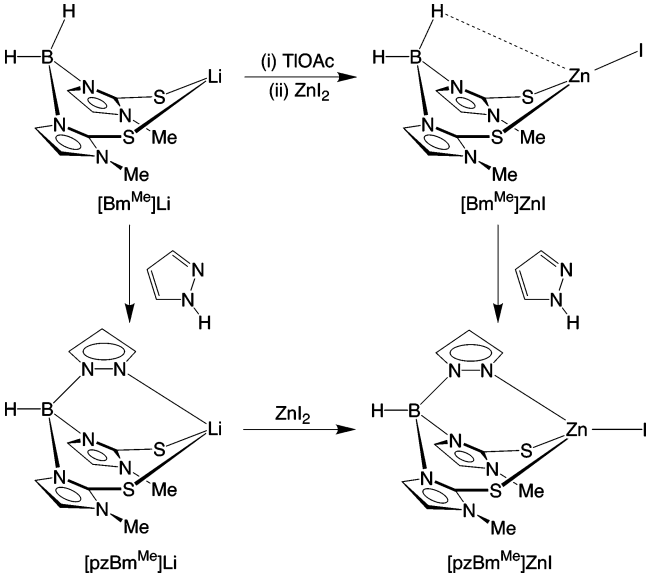
3.1.4.2. Synthesis and Structural Characterization of Synthetic Analogues of LADH. The sulfur-rich composition of the active site of LADH is quite distinct from that of most other zinc enzymes, such as carbonic anhydrase and carboxypeptidase, in which the zinc coordination environment consists solely of nitrogen and oxygen donors. Notable exceptions, however, are spinach carbonic anhydrase[186] and cytidine deaminase,[187] which have recently been shown to bind zinc at their active sites via one histidine and two cysteine residues. Furthermore, the carbonic anhydrase from the red alga, *Porphyridium purpureum*, has an unusual $[Cys_2(Asp)(His)Zn]$ active site composition, with no coordinated water molecule.[188] The proposed mechanism of action of this enzyme involves a step that involves the generation of a zinc hydroxide function by reaction of H_2O with the aspartate residue.

The distinctive coordination sphere about the catalytic zinc center in LADH has prompted the suggestion that it is critical for the effective function of the enzyme. The coordination environment of the catalytic site in LADH, however, is atypical and not well precedented in zinc chemistry, despite the fact that a variety of tridentate $[NS_2]$ donor ligands have been synthesized with a view to modeling LADH.[189−191] The coordination chemistry of small oligopeptides with cysteine residues to zinc has also been studied.[192] However, none of these investigations has yielded structurally characterized mononuclear tetrahedral complexes that mimic the active site of LADH, mainly due to the aforementioned proclivity of thiolate groups to act as bridging ligands and form multinuclear complexes, such as dinuclear $[\{\eta^3\text{-}(C_5H_3N)(CH_2CPh_2S)_2\}Zn]_2$[189b,c] and tetranuclear $\{[S(C_6H_4)NHCH_2CH_2S]Zn\}_4$.[191] Ligands such as $[(C_5H_3N)(CH_2SEt)_2]$ yield mononuclear zinc complexes but are unsuitable for modeling LADH because they bind in a meridional (i.e., non-tetrahedral) fashion and thereby promote the formation of five-coordinate complexes, e.g., $[(C_5H_3N)(CH_2SEt)_2]\text{-}ZnBr_2$.[193] In view of the difficulty in synthesizing suitable tridentate $[NS_2]$ donor ligands, tetradentate $[N_2S_2]$ ligands have also been invoked as models for the active site of LADH.[194]

In view of the above discussion, it is noteworthy that a suitable tidentate $[NS_2]$ donor ligand, namely, $[pzBm^{Me}]$, has been constructed by a reaction of pyrazole with a bis(mercaptomethylimidazolyl)borate derivative (Scheme 36).[195] As noted above, the use of a tetrahedral center as a point of attachment for the donor groups also serves to enforce a facial (rather than "T-shaped") array of nitrogen and sulfur donors, thereby favoring a tetrahedral geometry at zinc. The zinc iodide complex, $[pzBm^{Me}]ZnI$, has been structurally characterized by X-ray diffraction which demonstrates that it is indeed mononuclear with a distorted tetrahedral coordination geometry about zinc. Furthermore, the Zn−N and Zn−S bond lengths in $[pzBm^{Me}]ZnI$ are comparable to those within the enzyme.

A significant extension of this approach was to construct the $[(pz^{Ph,Me})Bm^{o-An}]$ ligand from the reaction of 1-(*o*-anisyl)-2-thioimidazole, 3-phenyl-5-methylpyrazole and KBH_4, from which the ethanol complex $\{[(pz^{Ph,Me})Bm^{o-An}]Zn(HOEt)\}\{ClO_4\}\cdot EtOH$ is obtained by subsequent reaction with $Zn(ClO_4)_2$ in EtOH (Scheme 37).[196] Since $[(pz^{Ph,Me})Bm^{o-An}]Zn\text{-}(HOEt)\}^+$ also features coordination of ethanol, it

Scheme 36. Construction of an $[NS_2]$ ligand and a synthetic analogue for liver alcohol dehydrogenase



Scheme 37. Synthesis of $[(pz^{Ph,Me})Bm^{o-An}]Zn(HOEt)\}^+$, a synthetic analogue of the alcohol adduct of LADH

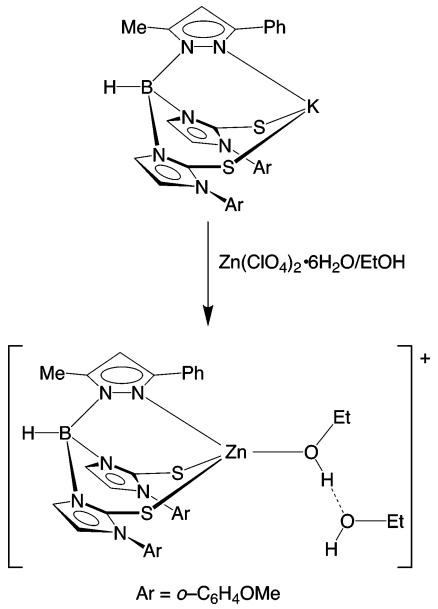


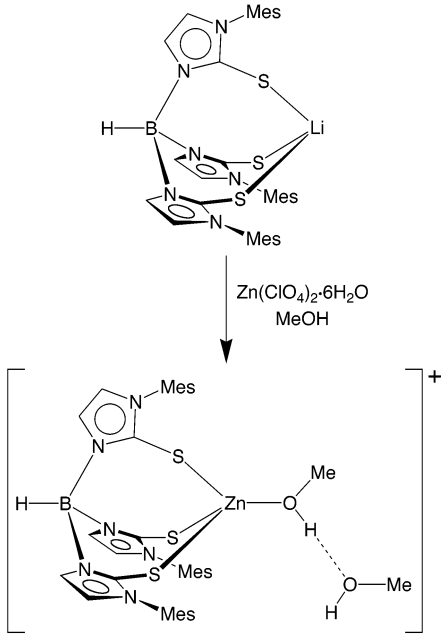
Table 7. Comparison of the Zinc Coordination Environments in $\{[(pz^{Ph,Me})Bm^{o-An}]Zn(HOEt)\}^+$ and LADH·DMSO

	$\{[(pz^{Ph,Me})Bm^{o-An}]Zn(HOEt)\}^+$	LADH·DMSO
Zn−N/Å	2.012(3)	2.02
Zn−O/Å	1.970(3	2.13
Zn−S/Å	2.282(1), 2.314(1)	2.19, 2.25
N−Zn−O/deg	116.8(1)	94.2
N−Zn−S/deg	100.2(1), 111.7(1)	107.4, 113.1
O−Zn−S/deg	105.4(1), 109.8(1)	102.5, 103.3
S−Zn−S/deg	113.2(1)	129.4

[a] Data taken from Seebacher, J.; Shu, M. H.; Vahrenkamp, H. *Chem. Commun.* **2001**, 1026.

provides by far the best structural model to date for LADH. Furthermore, the Zn−X (X = N, O, S) bond lengths are also similar to that of the enzyme, as illustrated in Table 7.

Scheme 38. Synthesis of $\{[Tm^{Mes}]Zn(HOMe)\}^+$, a methanol adduct in a sulfur-rich coordination environment



A related methanol complex $\{[Tm^{Mes}]Zn(HOMe)\}^+$ that features a tridentate sulfur donor ligand has been obtained by reaction of $Li[Tm^{Mes}]$ with $Zn(ClO_4)_2$ in methanol (Scheme 38).[197] The zinc coordination environment in $\{[Tm^{Mes}]Zn(HOMe)\}^+$ also resembles aspects of that in LADH. For example, the Zn−O and average Zn−S bond lengths in $\{[Tm^{Mes}]Zn(HOMe)\}^+$ are 1.99 and 2.32 Å, respectively. Furthermore, the hydroxyl group of the coordinated alcohol of $\{[Tm^{Mes}]Zn(HOMe)\}^+$ participates in a hydrogen-bonding interaction with an additional molecule of methanol, which may be viewed as mimicking the hydrogen-bond network at the active site of LADH. Thus, the hydrogen-bonded O···O separation of 2.58 Å between $\{[Tm^{Mes}]Zn(HOMe)\}^+$ and MeOH is effectively identical to that between the zinc-bound alcohol at the active site of LADH and Ser-48 (2.6 Å).

In contrast to the isolation of a methanol adduct $\{[Tm^{Mes}]Zn(HOMe)\}^+$, the corresponding reaction of $[Tm^{But}]K$ with $Zn(ClO_4)_2$ in methanol did not yield $\{[Tm^{But}]Zn(HOMe)\}^+$ but rather gave the perchlorate compound $[Tm^{But}]Zn(OClO_3)$, as illustrated in Scheme 39.[198] Most interestingly, however, an ethanol complex $\{[Tm^{But}]Zn(HOEt)\}^+$ may be isolated if the reaction is performed in ethanol. Furthermore, the ethanol complex $\{[Tm^{But}]Zn(HOEt)\}^+$ may be obtained by addition of ethanol to $[Tm^{But}]Zn(OClO_3)$. These observations, therefore, suggest that the order of ligand binding strengths towards $\{[Tm^{But}]Zn\}^+$ is MeOH < $[ClO_4]^-$ < EtOH.

The isolation of the tetrahedral alcohol complexes $\{[(pz^{Ph,Me})Bm^{o-An}]Zn(HOEt)\}^+$, $\{[Tm^{But}]Zn(HOEt)\}^+$, and $\{[Tm^{Mes}]Zn(HOMe)\}^+$ for these sulfur-rich ligand systems provides a contrast with the fact that the tripodal $[N_3]$ donor tris(imidazolyl)phosphine ligand $[Pim^{But,Pri}]$ yields a zinc hydroxide complex, $\{[Pim^{But,Pri}]ZnOH\}^+$, upon reaction with $Zn(ClO_4)_2$ in methanol.[89] These observations strongly indicate that

Scheme 39. Displacement of a perchlorate ligand by ethanol; in contrast, methanol does not coordinate

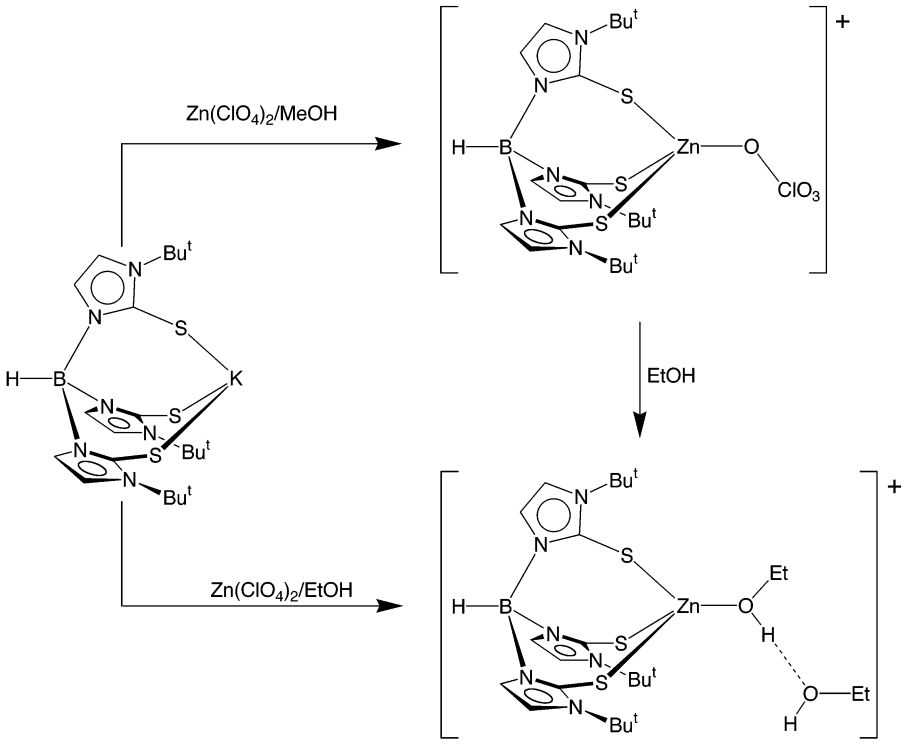


Table 8. Zn−Alcohol Bond Lengths in Selected Compounds[a]

	d(Zn−O)/Å
$[(bmapa)Zn(MeOH)]^{2+}$	2.077(1)
$[(bmpa)Zn(MeOH)]^{2+}$	2.073(1), 2.078(1)
$\{[Tm^{Mes}]Zn(MeOH)\}^{+}$	1.993(3)
$\{[(pz^{Ph,Me})Bm^{o-An}]Zn(HOEt)\}^{+}$	1.970(3)
$[(X_6Me_3ImMe_3)Zn(EtOH)]^{2+}$	1.984(5)
LADH-PFB	2.0

[a] Data taken from Makowska-Grzyska, M. M.; Jeppson, P. C.; Allred, R. A.; Arif, A. M.; Berreau, L. M. *Inorg. Chem.* **2002**, *41*, 4872.

the sulfur-rich coordination environment provided by $[Tm^{Mes}]$ and $[(pz^{Ph,Me})Bm^{o-An}]$ stabilizes alcohol binding to zinc and thereby suggests that one of the reasons why LADH utilizes a sulfur-rich coordination environment is to increase the stability of the required alcohol intermediate with respect to that of an aqua species. However, it should be noted that a most interesting example of a zinc ethanol complex derived from a ligand based on a *tert*-butylcalix[6]-arene framework with three imidazole donors, namely, $[(X_6Me_3ImMe_3)Zn(EtOH)]^{2+}$, has been prepared and structurally characterized.[199] A comparison of the Zn−O bond lengths for a variety of zinc alcohol complexes is summarized in Table 8.

$[NS_2]$ donor ligands that feature thioether donors also provide coordination environments that mimic the active site of LADH. For example, the $[NS_2]$ donor $[Ph(pz^{Bu^t})Bt^{Bu^t}]$ ligand has been used to synthesize $[Ph(pz^{Bu^t})Bt^{Bu^t}]ZnX$ (X = Me, Br, SPh) derivatives (Figure 25).[200]

In addition to modeling LADH, the above $[NS_2]$ donor ligands also offer potential for investigating synthetic analogues of other zinc enzymes that utilize $[NS_2]$ coordination, e.g., spinach carbonic anhydrase and cytidine deaminase. Although zinc aqua complexes supported by a tridentate $[NS_2]$ donor ligand have not yet been isolated, complexes in which zinc has a $Zn[N_2SO]$ motif are precedented.[201]

A variety of other ligands that are not of the $[NS_2]$ class have also been used to provide useful information pertaining to LADH. For example, comparison of the chemistry of two closely related ligands, bmpa and bmapa (Figure 26), that differ by the presence of a hydrogen-bonding functionality in the latter, provides a means for assessing the influence of the hydrogen-bond donor on the binding properties of a zinc center in a five-coordinate $[ZnS_2N_2O]$ environment.[202] Comparison of the two systems provides a basis for starting to investigate how a single hydrogen-bond donor (cf. Ser-48 in LADH) will influence the binding of a neutral oxygen donor ligand. For example, comparison of the Zn−O distances in $[(bmpa)Zn(HOMe)]^{2+}$ (average 2.076 Å) and $[(bmapa)Zn(HOMe)]^{2+}$ (2.077 Å) illustrates that the hydrogen bond in the latter exerts a negligible influence on the Zn−O bond distance.

In contrast to the negligible lengthening of the Zn−methanol bond length in the presence of a hydrogen-

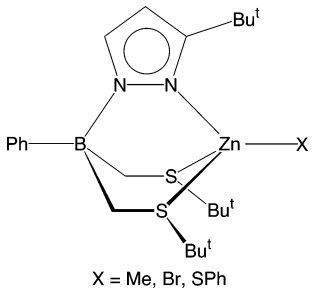


Figure 25. $[Ph(pz^{Bu^t})Bt^{Bu^t}]ZnX$ complexes that feature a tripodal [NSS] donor ligand.

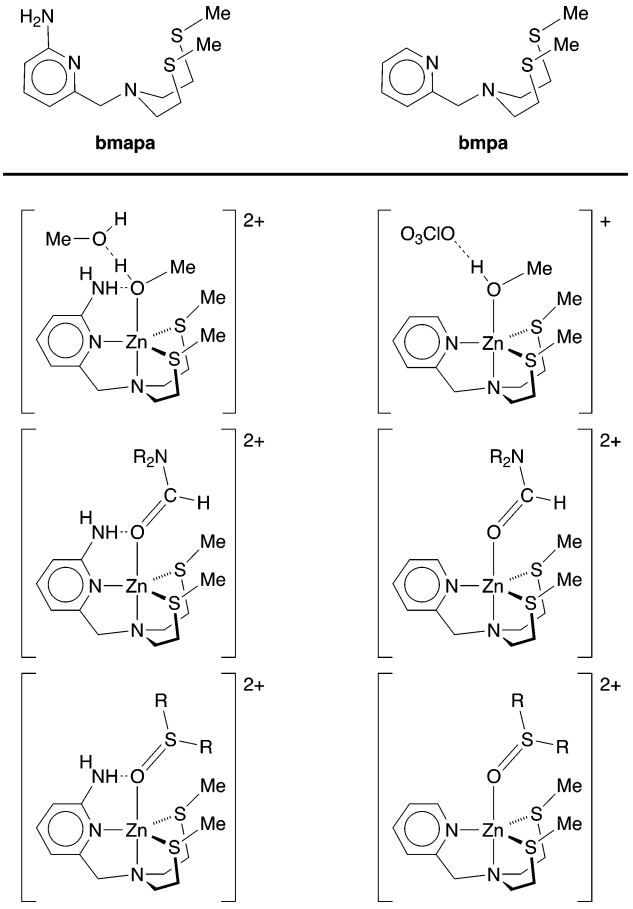


Figure 26. Zinc complexes of the $[N_2S_2]$ donor ligands, bmpa and bmapa, that differ by the presence of a hydrogen-bonding functionality in the latter.

bonding interaction, the Zn−formamide interactions in the hydrogen-bonded derivatives $[(bmapa)Zn(R_2NCHO)]^{2+}$ are lengthened by ca. 0.02 Å over those of $[(bmpa)Zn(R_2NCHO)]^{2+}$ that are devoid of hydrogen bonding. This small lengthening has been interpreted as the hydrogen-bonding interaction withdrawing electron density from the formamide carbonyl oxygen, thereby weakening the interaction with the zinc center (Figure 26).

Finally, zinc aldehyde complexes are also of importance to the mechanism of action of LADH, and a variety of such complexes have been prepared, tetrahedral $ZnX_2(ArCHO)_2$ (X = halide) and octahedral $[Zn(ArCHO)_4(OH_2)_2]^{2+}$, as illustrated in Figure 27.[201,203] Interesting examples of mixed ethanol aldehyde complexes such as $[Zn(ArCHO)_2(EtOH)_2(OH_2)_2]^{2+}$ have also been obtained. Although simple aliphatic or aromatic aldehydes do not react with the zinc hydroxide complex $[Tp^{Cum,Me}]ZnOH$, aldehydes with electronegative substituents, RCHO ($R = CCl_3$, C_6F_5) react to give α-hydroxyalkoxide derivatives $[Tp^{Cum,Me}]$-Zn{OCH(OH)R} (Figure 28).[204] Aldehyde functions

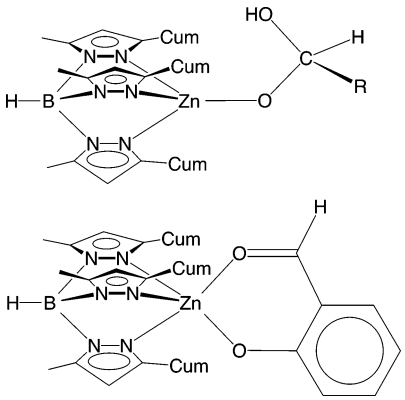


Figure 28. Complexes derived from the reaction of $[Tp^{Cum,Me}]ZnOH$ with various aldehydes.

do, nevertheless, coordinate to the zinc center if it is part of a chelate derived from *o*-hydroxy aldehydes (Figure 28).[204]

3.1.4.3. Modeling the Mechanism of Action of LADH. *(i) Formation of Alkoxide Derivatives.* An essential step in the catalytic cycle of LADH involves the generation of a four-coordinate zinc alkoxide intermediate (Scheme 35). However, until recently there was little precedent for the formation of simple aliphatic alkoxide complexes from either zinc aqua or hydroxide derivatives, despite the fact that activated alcohols with sufficient acidity (e.g., phenols and trifluoroethanol) are reactive.[205] As a result of the paucity of accurate structural models for LADH, studies designed to address this issue have been forced to focus on zinc complexes that do not exhibit the required pseudotetrahedral $\{[NS_2]Zn^{II}X\}$ motif.

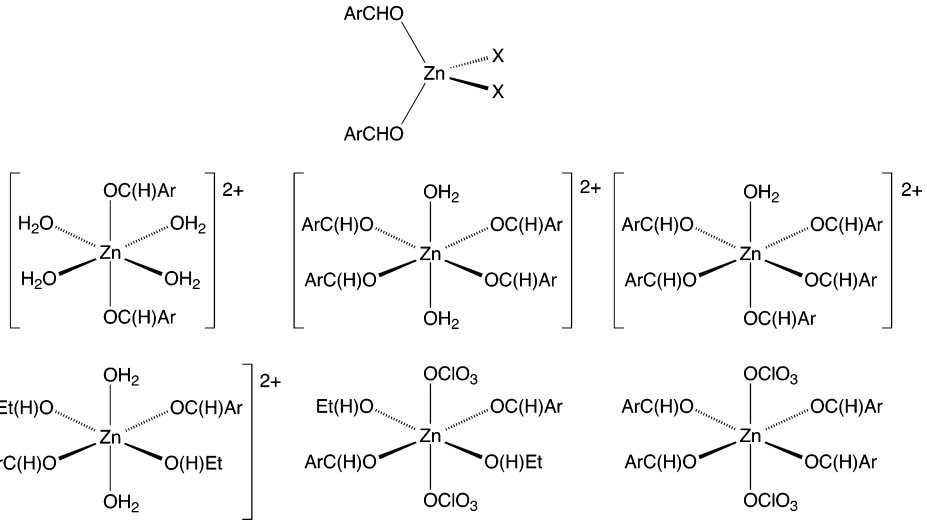
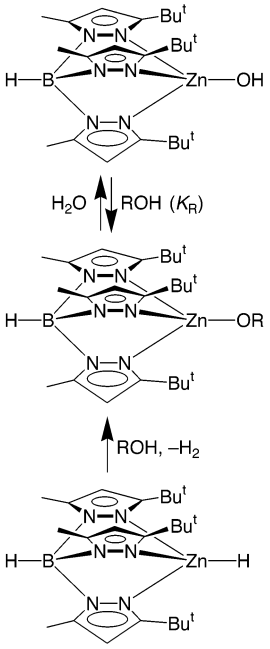


Figure 27. Representative zinc aldehyde complexes.

Scheme 40. Synthesis of a tetrahedral zinc alkoxide complex by reaction of the hydride complex [Tp$^{Bu^t,Me}$]ZnH with ROH; the alkoxide complexes can also be generated via reaction of the hydroxide complex [Tp$^{Bu^t,Me}$]ZnOH with ROH, but the equilibrium lies in favor of [Tp$^{Bu^t,Me}$]ZnOH



Despite this deficiency, these studies have nevertheless provided useful information pertaining to the mechanism of action of LADH. In particular, ^{1}H NMR spectroscopy has been used to demonstrate that the zinc hydroxide [Tp$^{Bu^t,Me}$]ZnOH complex does indeed react with ROH (R = Me, Et, Pr^i, Bu^t) to generate [Tp$^{Bu^t,Me}$]ZnOR (Scheme 40);[206] however, in contrast to the reactions of [Tp$^{RR'}$]ZnOH with phenols and trifluoroethanol, the alkoxide complexes are formed as equilibrium mixtures with the hydroxide. Although the equilibrium concentrations of [Tp$^{Bu^t,Me}$]ZnOR complexes are such that their isolation is prohibitive, they may be isolated from the reaction of the hydride complex [Tp$^{Bu^t,Me}$]ZnH[76] with the respective alcohol (Scheme 40), and the molecular structure of [Tp$^{Bu^t,Me}$]ZnOEt has been determined by X-ray diffraction. The methoxide complex [Tp^{Ph,Me}]ZnOMe·2MeOH has also been obtained as a minor byproduct in the synthesis of [Tp^{Ph,Me}]ZnOH and has been structurally characterized by X-ray diffraction.[207] The Zn−O bond length in [Tp^{Ph,Me}]ZnOMe [1.874(2) Å][207] is comparable to that in [Tp$^{Bu^t,Me}$]ZnOEt [1.826(2) Å].[206]

Table 9. Equilibrium Constants and Enthalpy Data for Alcoholysis Reactions of [Tp$^{Bu^t,Me}$]ZnOH[a]

ROH	K (300 K)
Bu^t	$\sim 10^{-8}$
Pr^i	$3(1) \times 10^{-5}$
Et	$9(2) \times 10^{-4}$
Me	$1.4(2) \times 10^{-3}$
C_6H_4OMe	4.2(9)
$C_6H_4Bu^t$	4.8(10)
C_6H_4Me	4.9(10)
C_6H_5	$1.0(2) \times 10^{1}$
C_6H_4I	$9(2) \times 10^{1}$
$C_6H_4CO_2Me$	$2.7(6) \times 10^{2}$
C_6H_4COMe	$3.1(6) \times 10^{2}$
$C_6H_4NO_2$	$3.5(8) \times 10^{3}$

[a] Data taken from Bergquist, C.; Storrie, H.; Koutcher, L.; Bridgewater, B. M.; Friesner, R. A.; Parkin, G. *J. Am. Chem. Soc.* **2000**, *122*, 12651.

The factors that influence the stability of tetrahedral zinc alkoxide complexes are of considerable relevance with respect to the mechanism of action of LADH. This issue has been addressed by determining the equilibrium constants for a large series of alcoholysis equilibria. The data presented in Figure 29 and Table 9 indicate that equilibrium constants for the reactions of [Tp$^{Bu^t,Me}$]ZnOH with ROH (R = Me, Et, Pr^i, Bu^t) lie strongly in favor of the hydroxide, with the alcoholysis equilibrium constants decreasing across the series MeOH > EtOH > Pr^{i}OH > Bu^{t}OH.[208] This variation is a result of both steric and electronic influences, and so in an effort to identify the relative importance of these components, the reactions of [Tp$^{Bu^t,Me}$]ZnOH with a series of para-substituted phenols, *p*-XC$_6$H$_4$OH, for which electronic substituent parameters (e.g., Hammett σ constants) are available, have been studied.[208] The results demonstrate that the alcoholysis reactions are very sensitive to electronic influences, being strongly

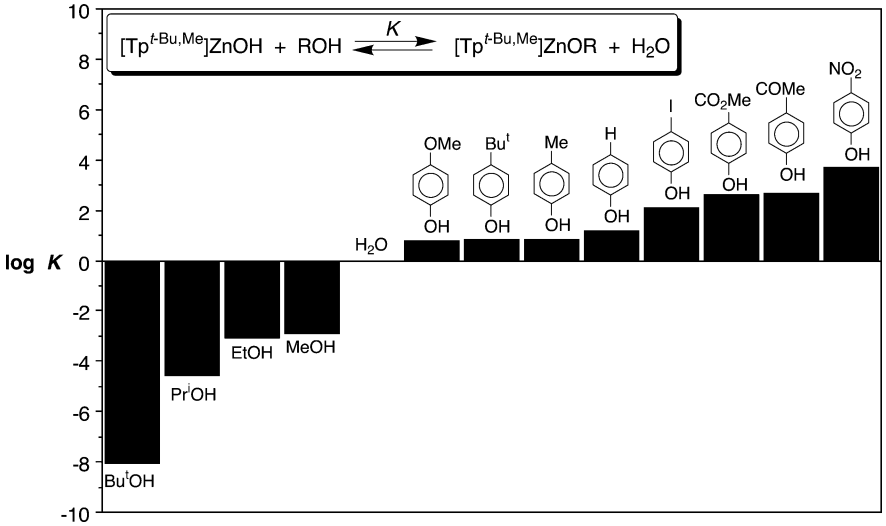


Figure 29. Variation in alcoholysis equilibrium constant as a function of R.

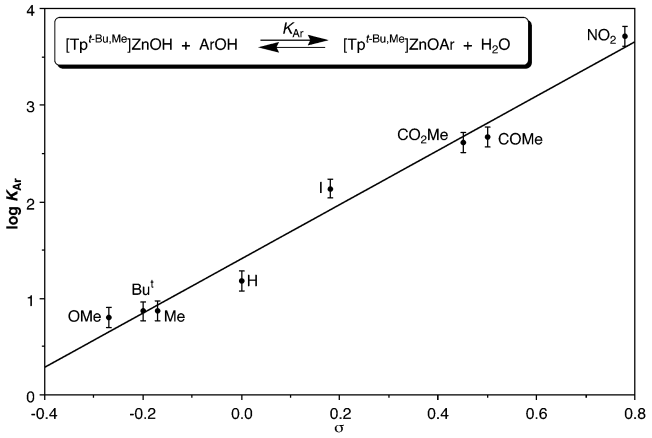


Figure 30. Hammett plot of log K versus σ for the reaction of $[Tp^{Bu^t,Me}]ZnOH$ with XC_6H_4OH. Electron-withdrawing substituents promote formation of the zinc aryloxide complex.

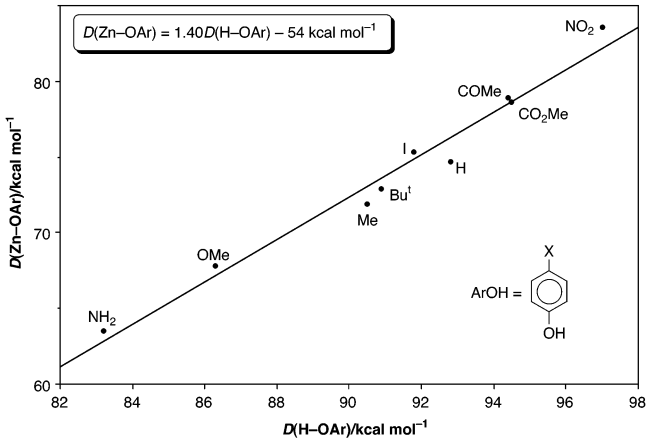


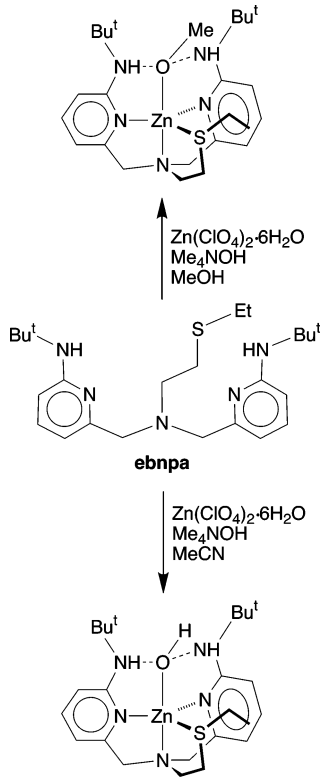
Figure 31. Correlation of calculated homolytic Zn−OAr and H−OAr BDEs. The Zn−OAr bond energies are more sensitive to the nature of the para substituent (X) than are the H−OAr bond energies.

favored for electron-withdrawing substituents (Figure 29), with a Hammett plot of log K versus σ exhibiting a good linear correlation with a ρ value of 2.8 (Figure 30).

The alcoholysis reactions have also been studied computationally using DFT calculations (B3LYP), which demonstrated that the trend illustrated in the Hammett plot is a consequence of electron-withdrawing substituents increasing Zn−OAr BDEs to a greater extent than the corresponding H−OAr BDEs.[208] Thus, whereas a 1:1 correlation between M−X and H−X bond energies has been reported for other systems,[209] the Zn−OAr BDE is substantially more sensitive to the para substituent than is the H−OAr BDE (Figure 31), i.e., D(Zn−OAr) = 1.48, D(H−OAr) − 61 kcal mol^{-1}.[208] The greater influence of an electron-withdrawing substituent on the Zn−OAr versus H−OAr BDE is proposed to be a consequence of the $Zn^{\delta+}-OAr^{\delta-}$ bond being more polar than the $H^{\delta+}-OAr^{\delta-}$ bond, i.e. an electron-withdrawing substituent would exert a greater influence in stabilizing the partial negative charge on the oxygen atom in $[Tp^{Bu^t,Me}]ZnOAr$ than in ArOH.

The fact that the simple zinc alkoxide complexes are very sensitive to hydrolysis suggests that the

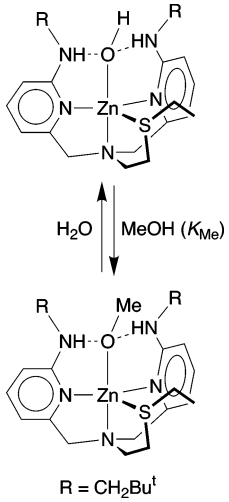
Scheme 41. Formation of zinc methoxide and hydroxide complexes using ebnpa, an $[N_2S_2]$ donor ligand



active-site environment most likely plays an important role in promoting the formation of a zinc alkoxide species. For example, hydrogen bonding may provide a mechanism for stabilizing zinc alkoxide species, as illustrated by recent studies which suggest that the benzyl alkoxide ligand forms a hydrogen bond with the hydroxyl group of Ser-48 in horse liver alcohol dehydrogenase.[210]

In this respect, studies employing the ebnpa ligand that features two hydrogen-bond donors are particularly interesting, for which both hydroxide and methoxide derivatives, $[(ebnpa)ZnOH]^+$ and $[(ebnpa)ZnOMe]^+$, have been isolated (Scheme 41). Specifically, the equilibrium constant for methanolysis of $[(ebnpa)ZnOH]^+$ to give $[(ebnpa)ZnOMe]^+$ (Scheme 42) has been determined to be orders of magnitude greater than that for the corresponding reaction of $[Tp^{Bu^t,Me}]ZnOH$ with MeOH.[211] For example, the equilibrium constant for $[(ebnpa)ZnOH]^+$ is 3.0×10^{-1} at 304 K, while that for $[Tp^{Bu^t,Me}]ZnOH$ is 1.4×10^{-3} at 300 K. Furthermore, the temperature dependence of the equilibrium constant allows determination of ΔH which is −0.9 kcal mol^{-1} for $[(ebnpa)ZnOH]^+$ and 1.2 kcal mol^{-1} for $[Tp^{Bu^t,Me}]ZnOH$. The observation that the thermodynamics of alcoholysis of the zinc hydroxide function can be modified significantly by the coordination environment is most relevant to the mechanism of action of LADH. For the present comparison, the two systems differ in several ways: (i) the zinc center of $[Tp^{Bu^t,Me}]ZnOH$ is four-coordinate, whereas that of $[(ebnpa)ZnOH]^+$ is five-coordinate, (ii) the $[Tp^{Bu^t,Me}]$ ligand is a tridentate $[N_3]$ donor, whereas the ebnpa ligand is a tetradentate $[N_3S]$ donor, and (iii) the ebnpa ligand provides two

Scheme 42. Reversible methanolysis of $[(ebnpa)ZnOH]^+$



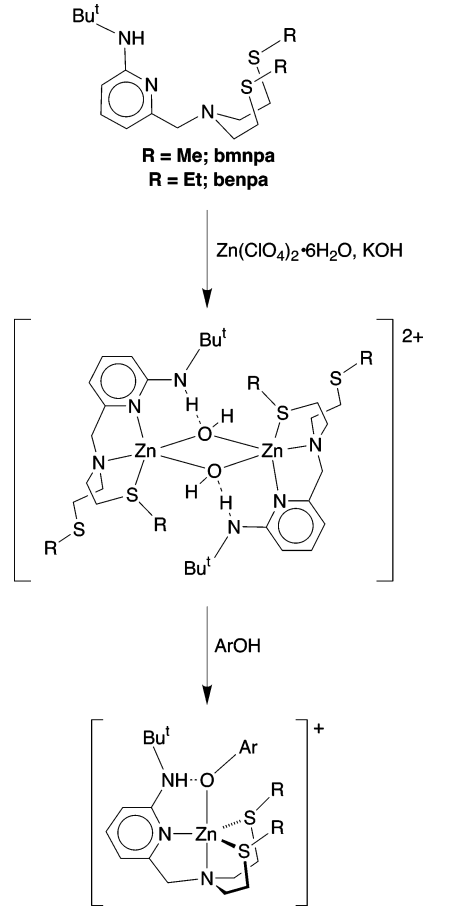
hydrogen-bond donors for the hydroxide and methoxide ligands, whereas the $[Tp^{Bu^t,Me}]$ ligand is devoid of such donors. In view of the many differences, it is difficult to decipher unambiguously which is the most important factor for modifying the reactivity in such a manner, but preliminary calculations emphasize the importance of hydrogen-bonding interactions.

In addition to studies employing two hydrogen-bond donors, the influence of ligands that feature only one hydrogen-bond donor have been investigated. For example, the dinuclear bridging hydroxide complexes, $\{[(bmnpa)Zn(\mu\text{-}OH)]_2\}^{2+}$ and $\{[(benpa)Zn(\mu\text{-}OH)]_2\}^{2+}$, react with ArOH to give a series of aryloxide complexes that exhibit an intramolecular hydrogen-bonding interaction that has been proposed to mimic that involving Ser-48 at the active site of LADH (Scheme 43).[212]

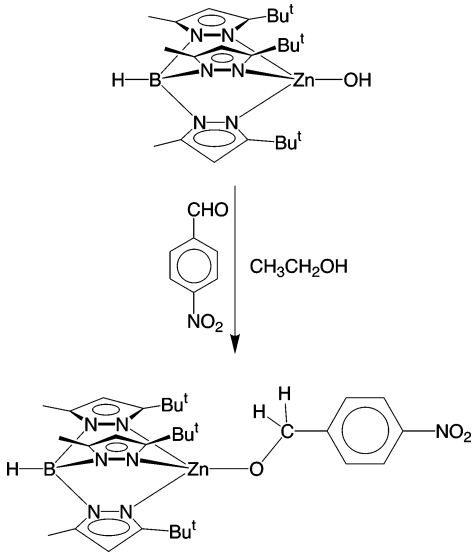
(ii) Hydride Transfer. A critical role of the proposed zinc alkoxide intermediates is to transfer "hydride" to NAD^+. Thus, an important issue is whether the zinc alkoxide intermediates $[Tp^{Bu^t,Me}]ZnOH$ are sufficiently activated to allow "hydride" transfer to a substrate. Evidence that the alkoxide complexes $[Tp^{Bu^t,Me}]ZnOR$ (R = Et, Pr^i) are capable of such a transformation is provided by studies employing *p*-nitrobenzaldehyde as a NAD^+ hydride acceptor mimic. Specifically, $[Tp^{Bu^t,Me}]ZnOEt$ reacts with ArCHO (Ar = $p\text{-}C_6H_4NO_2$) in benzene to yield $[Tp^{Bu^t,Me}]ZnOCH_2Ar$ and MeCHO. Furthermore, solutions of ArCHO in ROH (R = Me, Et, Pr^i) yield $ArCH_2OH$ at ca. 90 °C in the presence of $[Tp^{Bu^t,Me}]$-ZnOH (Scheme 44). *p*-Nitrobenzaldehyde has also been used as a NAD^+ mimic to demonstrate that the trinuclear zinc hydroxide species $[\{[12]aneN_3\}Zn(\mu\text{-}OH)]_3(OTf)_3\cdot(TfOH)$ is an effective catalyst for hydrogen transfer from Pr^iOH and EtOH.[213]

Since an aldehyde is a rather poor model for NAD^+, more realistic systems employ nicotinamide and acridinium derivatives which conserve the pyridinium ring present in NAD^+ (Figure 32). 1-Benzylnicotinamide chloride, (BNA)Cl, in particular, has previously been investigated as a hydride acceptor in zinc chemistry, but was found to be inefficient.[198,213,214] For example, $[Tp^{Cum,Me}]ZnOPr^i$ reacts

Scheme 43. Synthesis of zinc aryloxide complexes that feature a single hydrogen-bonding interaction



Scheme 44. Hydride transfer from ethanol to *p*-nitrobenzaldehyde mediated by $[Tp^{Bu^t,Me}]ZnOH$



with (BNA)Cl in Pr^iOH to generate Me_2CO in low yield (10−20%) and the chloride complex $[Tp^{Cum,Me}]$-ZnCl; (BNA)H (14%) and $(BNA)_2$ were also formed.[214a] Likewise, the *p*-nitrophenolate complex $[Tm^{Cum}]$-$ZnOC_6H_4NO_2$ has been shown to act as a catalyst for the reaction between (BNA)Cl and Pr^iOH to give (BNA)H and Me_2CO, but forcing conditions were required.[198] The reverse reaction, i.e. transfer of hydrogen from (BNA)H, has also been studied in a

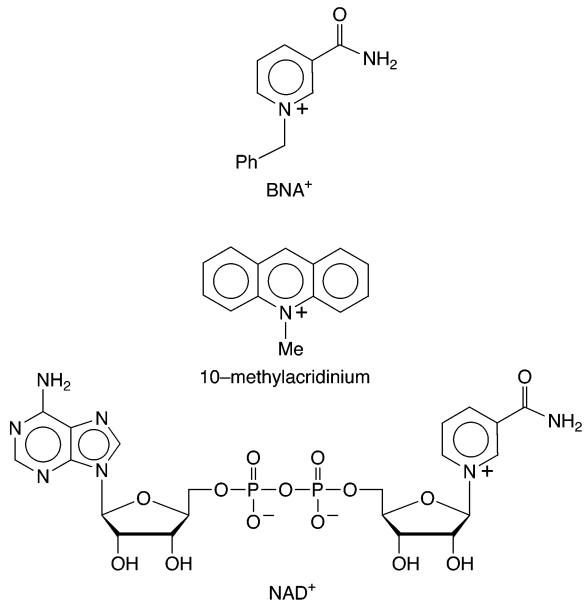
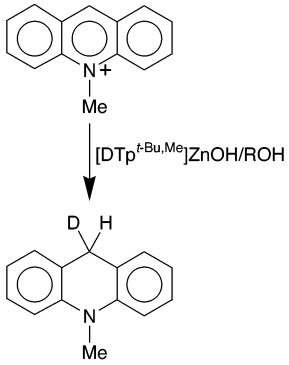


Figure 32. Chemical models for NAD^+.

Scheme 45. Reduction of 10-methylacridinium to 10-methylacridan by solutions of $[Tp^{Bu^t,Me}]ZnOH$ in ROH (R = Me, Et, Pr^i) at 80 °C; deuterium labeling indicates that the source of the hydride is the B−H group and not the alcohol



related system; specifically, $[Tm^{Bu^t}]ZnOClO_3$ has also been shown to be a catalyst for the transfer of hydrogen from (BNA)H to the electron-poor aldehydes C_6F_5CHO and pyrCHO.[198]

In view of the inefficiency of (BNA)Cl, attention was given to employing 10-methylacridinium perchlorate as a NAD^+ mimic on the basis that (i) the pyridinium nucleus of 10-methylacridinium (−0.43 V) is more susceptible to reduction than that of 1-benzylnicotinamide (−1.08 V), and (ii) perchlorate counterion would show less tendency than chloride to coordinate to zinc and thereby favor coordination of the alcohol. Indeed, 10-methylacridinium perchlorate was efficiently reduced to 10-methylacridan by solutions of $[Tp^{Bu^t,Me}]ZnOH$ in ROH (R = Me, Et, Pr^i) at 80 °C (Scheme 45).[215] However, an unanticipated result was that the reactions employing deuterated solvents (i.e., d^4-methanol, d^6-ethanol, and d^8-2-propanol) generated 10-methylacridan that is devoid of deuterium. Such absence of deuterium incorporation clearly calls into question the notion that the reduction of 10-methylacridinium in this system could involve hydride transfer via a zinc alkoxide complex with a mechanism analogous to that employed by LADH. Since $[Tp^{Bu^t,Me}]ZnOH$ and $[Tp^{Bu^t,Me}]$-ZnOR decomposed during the reaction, it was postulated that the B−H group could be the hydride source, a suggestion that was confirmed by deuterium-labeling studies (Scheme 45). It is speculated that the mechanism involves hydride transfer from the $[Tp^{Bu^t,Me}]^-$ ligand to 10-methylacridinium to generate 10-methylacridan and $B(pz^{Bu^t,Me})_3$; proteolytic cleavage of $B(pz^{Bu^t,Me})_3$ with ROH would be expected to release the pyrazole, $Hpz^{Bu^t,Me}$. Although hydride transfer from the $[Tp^{R,R'}]$ ligand is not of relevance to the catalytic cycle of LADH, it is of interest because it is an unprecedented form of reactivity for the ubiquitous $[Tp^{R,R'}]$ ligand system, despite the well-known ability of borohydride derivatives to act as reducing agents. The observation therefore provides a caveat for the often assumed inertness of such ligands towards hydride transfer, especially in the presence of reactive cationic species.

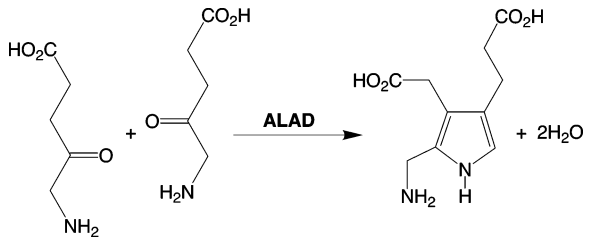
Other model studies that are of relevance to the mechanism of action of LADH include: (i) the observation of a hydrogen-bonding interaction between methanol and a thiolate ligand in five-coordinate zinc complexes with $[N_3S_2]Zn$ coordination, which has led to the suggestion that the alcohol substrate may interact with the cysteine groups in a similar manner and that this interaction may possibly promote deprotonation before interaction with the zinc center,[216] and (ii) aldehyde complexes of zinc thiolates, e.g., $[(RCHO)Zn(SC_6F_5)_2]_\infty$, have been synthesized.[201]

3.1.5. The $[(Cys)_3Zn^{II}–OH_2]$ Motif: 5-Aminolevulinate Dehydratase

3.1.5.1. Structure and Mechanism of Action of 5-Aminolevulinate Dehydratase. 5-Aminolevulinate dehydratase (ALAD) is an important enzyme that is present in all organisms which synthesize tetrapyrroles (including heme, chlorophyll, and cobalamins). ALAD is also referred to as porphobilinogen synthase (PBGS), and its specific role is to catalyze the dimerization of 5-aminolaevulinic acid (ALA) to porphobilinogen, a monopyrrole (Scheme 46).[217] ALAD is a zinc-dependent enzyme that contains both catalytic and structural zinc sites.[218] Of most interest from the perspective of the active site, the zinc center has the uncommon composition of $[(Cys)_3Zn^{II}(OH_2)]$ (Figure 33),[219] although EXAFS structures initially assigned the active site to a $[(Cys)_4Zn^{II}]$ composition.[220]

An essential feature of the ALAD-catalyzed reaction is that the dimerization proceeds in an asymmetric manner. Although many details of the cata-

Scheme 46. Dimerization of 5-aminolaevulinic acid (ALA) to porphobilinogen catalyzed by 5-aminolevulinate dehydratase (ALAD)



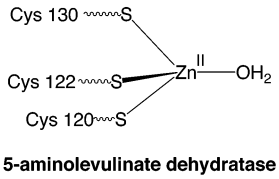
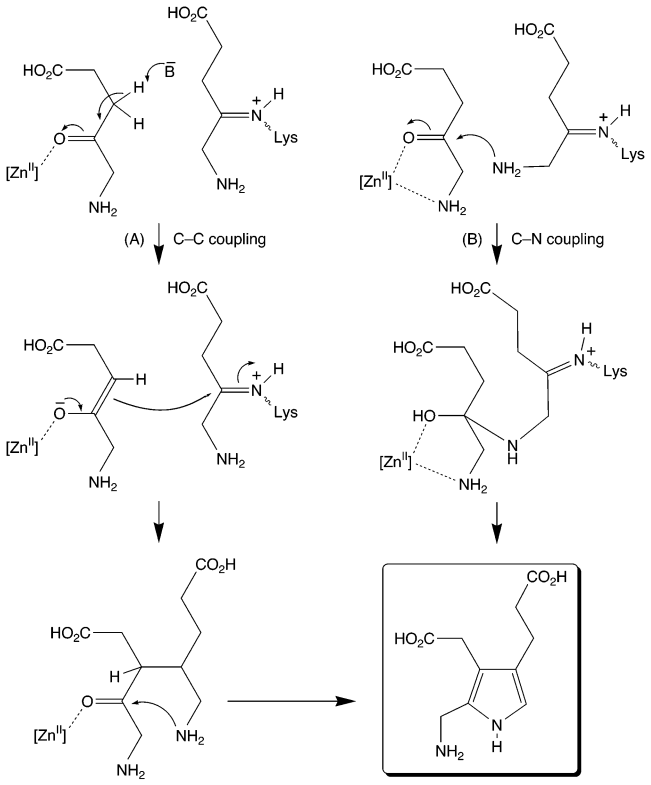


Figure 33. Active sites of 5-aminolevulinate dehydratase (ALAD).

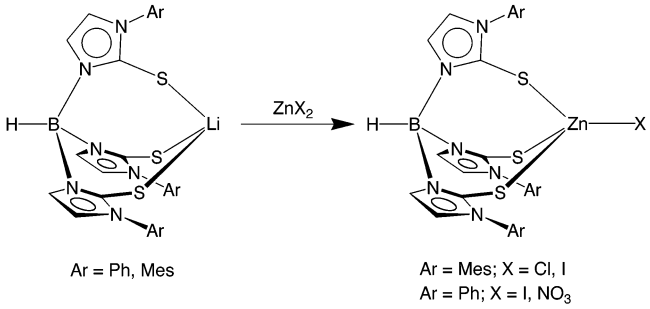
Scheme 47. Two possible mechanisms of action of ALAD, differing according to whether C−C or C−N bond formation occurs first



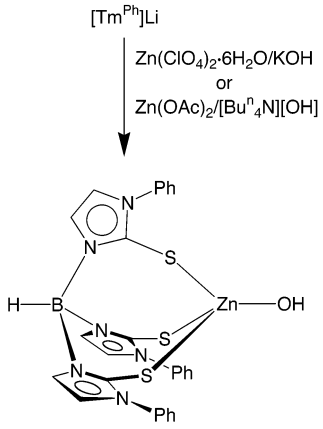
lytic cycle are unknown, the enzyme is considered to achieve this interesting selectivity by having different binding sites, termed A and P (referring to the origin of the acetate and propionate chains in the product, respectively), that orient the two molecules of ALA for coupling. The first step is proposed to involve the formation of a Schiff base linkage between one of the ALA molecules and a lysine residue of the enzyme at the P-site, while the zinc center at the A-site is believed to activate the second ALA molecule prior to condensation of the two fragments (Scheme 47). Following this initial assembly, two possible pathways for dimerization have been considered in the literature, which principally differ according to whether C−C or C−N bond formation occurs first.[217b,221] It has also been suggested that, in the absence of zinc at the active site, the mechanism could involve the formation of Schiff base linkages at both P and A sites.[222]

3.1.5.2. Synthesis and Structural Characterization of Synthetic Analogues of ALAD. The $[(Cys)_3Zn^{II}(OH_2)]$ composition of the ALAD active site must be regarded as truly unusual since the active sites (as opposed to structural sites) of most zinc enzymes include at least one histidine ligand.[1−3]

Scheme 48. Synthesis of $[Tm^{Ar}]ZnX$ complexes in which the $[Tm^{Ar}]$ ligand emulates the coordination of the three cysteine groups in ALAD



Scheme 49. Synthesis of the hydroxide complex $[Tm^{Ar}]ZnOH$, a synthetic analogue for ALAD

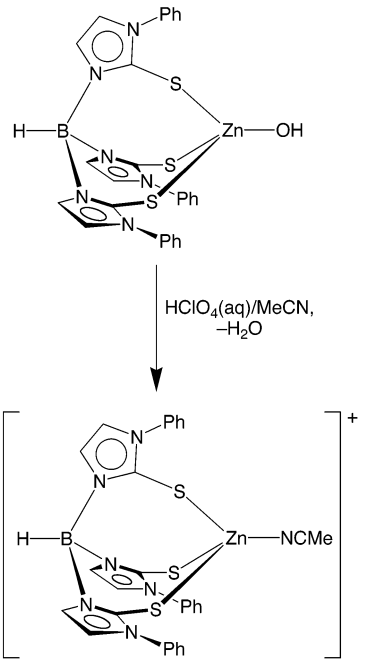


Nevertheless, precedent for a tetrahedral $[ZnS_3O]$ motif is provided by $[\{MeC(O)S\}_3Zn(OH_2)]^-$, although the complex was not discussed in terms of an ALAD mimic.[223] The tris(mercaptoimidazolyl) ligand system $[Tm^{Ar}]$ has, however, been used to emulate the coordination of the three cysteine residues in ALAD. Thus, several $[Tm^{Ar}]ZnX$ derivatives, e.g., $[Tm^{Ph}]ZnX$ (X = I, NO_3) and $[Tm^{Mes}]ZnX$ (X = Cl, I), have been synthesized (Scheme 48).[197] Of most significance, the zinc hydroxide complex, $[Tm^{Ph}]ZnOH$, may be obtained by (i) the reaction of $[Tm^{Ph}]Li$ with $Zn(ClO_4)_2$ in the presence of KOH and (ii) the reaction of $[Tm^{Ph}]Li$ with $Zn(OAc)_2$ in the presence of $[Bu^n{}_4N]$-OH, as illustrated in Scheme 49.[224] A series of other $[Tm^R]ZnX$ complexes have also been prepared, but the hydroxide counterparts have not been reported.[198,225−227]

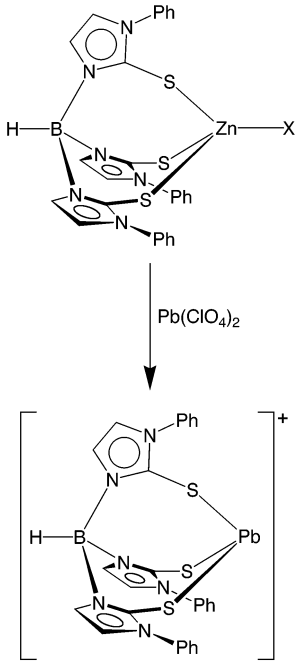
The molecular structure of $[Tm^{Ph}]ZnOH$ has been determined by X-ray diffraction. Significantly, $[Tm^{Ph}]$-ZnOH is the first tetrahedral zinc hydroxide complex supported by a $[S_3]$ donor ligand to be structurally characterized by X-ray diffraction. The Zn−OH bond length [1.896(4) Å] does, nevertheless, compare favorably with those for other monomeric tetrahedral zinc hydroxide complexes that have been structurally characterized, namely the tris(pyrazolyl)hydroborato derivatives, $[Tp^{Bu^t,Me}]ZnOH$ [1.85 Å] and $[Tp^{Cum,Me}]$-ZnOH [1.85 Å], and the tris(imidazolyl)phosphine complex $\{[Pim^{Bu^t,Pr^i}]ZnOH\}^+$ [1.86 Å] (Table 5).

It is pertinent to compare the structure of $[Tm^{Ph}]$-ZnOH with that of the active site of ALAD, for which several structures are available.[219,228,229] For example, the Schiff base complex of yeast ALAD with levulinic

Scheme 50. Displacement of the hydroxide ligand in [Tm^{Ar}]ZnOH upon protonation



Scheme 51. Facile substitution of zinc in synthetic analogues of ALAD by lead



acid exhibits Zn−S$_{av}$ and Zn−O bond lengths of 2.17 and 1.87 Å, respectively.[229] Interestingly, while the Zn−O bond length of the enzyme (1.87 Å) compares favorably with that of [Tm^{Ph}]ZnOH [1.896(4) Å], the Zn−S bond length of the enzyme (2.17 Å) is significantly shorter than that of [Tm^{Ph}]ZnOH [2.3514(7) Å].

While it is possible that the discrepancy with the structure of [Tm^{Ph}]ZnOH reflects the electronic difference between the thione-like donors of the synthetic analogue and the cysteine thiolate donors of the enzyme, it should be recognized that the discrepancy may also be a consequence of the errors inherent to protein crystallography.[230] Indeed, it should be noted that the individual Zn−S bond lengths in ALAD span a significant range (2.03−2.30 Å), the shortest of which is shorter than any zinc complex listed in the Cambridge Structural Database.

Another notable feature of [Tm^{Ph}]ZnOH is that the hydroxide ligand participates in a hydrogen-bonding interaction with an extended layer of water molecules [d(O···O) = 2.65 Å], an observation that is of relevance since hydrogen-bonding interactions are a common feature of the active sites of many zinc enzymes, including that of ALAD. For example, the Schiff base complex of yeast ALAD with levulinic acid exhibits an O···O hydrogen-bonding distance of 2.73 Å,[229] comparable to that in [Tm^{Ph}]ZnOH.

3.1.5.3. Modeling the Mechanism of Action of ALAD. The mechanism of action of ALAD is proposed to involve displacement of the aqua ligand by the substrate, ALA (Scheme 47).[217] Precedent for such displacement of an aqua ligand in an {[S$_3$]Zn^{II}−OH$_2$} species is provided by protonation of [Tm^{Ph}]ZnOH by HClO$_4$ in acetonitrile. Under these conditions, the incipient aqua ligand is displaced by MeCN to give {[Tm^{Ph}]Zn(NCMe)}$^+$ (Scheme 50).[224,231]

3.1.5.4. ALAD and Lead Poisoning. An important aspect of the chemistry of ALAD is concerned with lead poisoning. Lead is the most commonly encountered toxic metal pollutant in the environment,[232] and considerable effort is, therefore, being directed towards solving this environmental problem.[233] The toxicological properties of lead are associated with its interactions with proteins and, in particular, ALAD.[234,235] The influence of lead on the latter enzyme is particularly harmful because ALAD is responsible for the asymmetric dimerization of 5-aminolevulinic acid (ALA) to porphobilinogen, a monopyrrole which is essential for heme synthesis.[236−238] Thus, not only does inactivation of ALAD result in anemia because it inhibits the formation of heme and, hence, hemoglobin, but it also results in a build-up of ALA, a neuropathogenic agent.[237,239]

The existence of a series of [Tm^{Ar}]ZnX derivatives has enabled the replacement of zinc by lead in complexes which mimic aspects of the coordination environment in the active site of ALAD to be studied. Significantly, [Tm^{Ph}]ZnOH, [Tm^{Ph}]ZnI, and {[Tm^{Ph}]Zn(NCMe)}(ClO$_4$) react rapidly with Pb(ClO$_4$)$_2$·xH$_2$O to give the lead complex {[Tm^{Ph}]Pb}(ClO$_4$) (Scheme 51).[231] Notably, the molecular structure of the cation {[Tm^{Ph}]Pb}$^+$, as determined by X-ray diffraction, exhibits a trigonal pyramidal geometry that bears a close correspondence to the active site of Pb^{II}−ALAD.[239] For example, {[Tm^{Ph}]Pb}$^+$ and Pb^{II}−ALAD have very similar average Pb−S bond lengths of 2.7 and 2.8 Å, respectively.

Another important aspect of the structure of {[Tm^{Ph}]Pb}$^+$ is that the three-coordinate lead geometry is in marked contrast to that of tetrahedral zinc in {[Tm^{Ph}]Zn(NCMe)}$^+$. This observation clearly indicates that trigonal pyramidal lead centers have a reduced tendency to bind an additional ligand compared to that of zinc. The reduced Lewis acidity of a trigonal pyramidal Pb^{II} versus Zn^{II} center is presumably associated with the stereochemically active Pb^{II} lone pair which tempers its electrophilicity. This

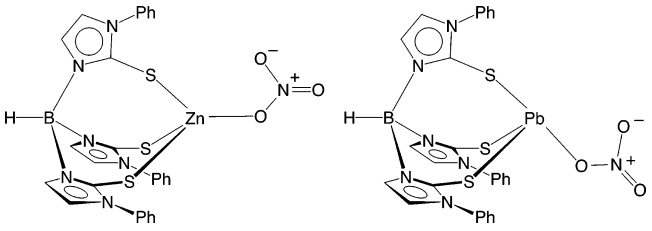


Figure 34. Markedly different structures for the zinc and lead complexes $[Tm^{Ph}]Zn(NO_3)$ and $[Tm^{Ph}]Pb(NO_3)$.

observation is of considerable significance to the inactivity of Pb^{II}−ALAD. Specifically, of the two mechanisms of action that have been proposed for ALAD (Scheme 47), both involve activation of ALA by interaction of the ketone group with the Zn^{II} center. The degree of such activation is clearly a function of the Lewis acidity of the metal center,[1e] which is considerably greater for a trigonal pyramidal Zn^{II} center than for a corresponding Pb^{II} center. As such, the formation of a tetrahedral species of the type $[(Cys)_3Pb^{II}$−ALA], a required intermediate in the proposed mechanisms of action of ALAD, would be inhibited.

A further illustration of the difference in coordination geometry preferences of Zn^{II} and Pb^{II} in this system is provided by the fact that, whereas nitrate binds to the zinc center in $[Tm^{Ph}]Zn(NO_3)$ resulting in a tetrahedral coordination,[197] the corresponding lead complex $[Tm^{Ph}]Pb(NO_3)$ exhibits a saw-horse geometry due to the presence of a stereochemically active "lone-pair" (Figure 34).[240]

The relative binding preferences of the $[Tm^{Ph}]$ ligand to Pb^{II} and Zn^{II} has been determined by measurement of the equilibrium involving ligand exchange between $\{[Tm^{Ph}]Pb\}(ClO_4)$ and $Zn(ClO_4)_2$ in MeCN (eq 3). Significantly, the preference of $[Tm^{Ph}]$ to coordinate Pb^{II} over Zn^{II} in this system is ca. 500:1, a value that is substantially greater than the ca. 25:1 relative affinity of these metals to reside at the active site of human erythrocyte ALAD.[241]

$$\{[Tm^{Ph}]Pb\}^+ + Zn^{2+} + MeCN \overset{K}{\rightleftharpoons} \{[Tm^{Ph}]Zn(NCMe)\}^+ + Pb^{2+} \quad (3)$$

However, despite the fact that $[Tm^{Ph}]$ prefers to bind to Pb^{II} rather than Zn^{II}, the lead in $\{[Tm^{Ph}]Pb\}^+$ may be replaced by zinc by addition of NaI to a solution of $\{[Tm^{Ph}]Pb\}(ClO_4)$ in acetonitrile. Thus, the reaction results in the formation of $\{[Tm^{Ph}]Zn(NCMe)\}^+$ due to the equilibrium being shifted to the right by precipitation of Pb^{II} as PbI_2. The significance of this observation is that an effective means to reverse the toxic effects of lead in the human body are not yet known, despite efforts to develop lead complexing agents.[242]

3.1.6. The [(Cys)$_4$Zn^{II}] Motif: The Ada DNA Repair Protein

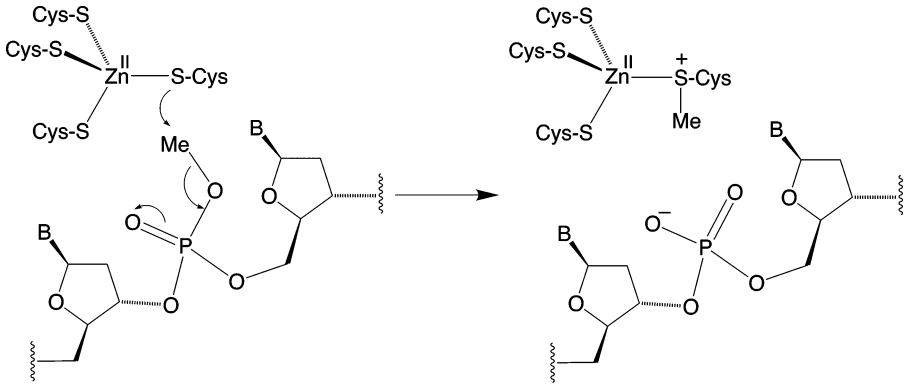
3.1.6.1. Structure and Mechanism of Action the Ada Protein. While the geometries of the zinc centers in both structural and functional sites are commonly tetrahedral, a long-held distinction between the two types of sites was the presence of a water molecule, the essential catalytic component, at the functional site, i.e., $[\{XYZ\}Zn^{II}-OH_2]$, where X, Y, and Z are three protein residues. Tetrahedral structural sites typically only involve coordination by the protein. Cysteine residues, in particular, are a prominent component of structural sites, as illustrated by the $[Cys_\alpha His_\beta Zn^{II}]$ ($\alpha + \beta = 4$) sites in zinc fingers[1,19,20] and the $[Cys_4Zn^{II}]$ structural site in liver alcohol dehydrogenase.[1] More recently, however, a class of zinc proteins and enzymes with tetrahedral "nonaqua" functional zinc sites have started to emerge in which the activity centers on the reactivity of a zinc thiolate linkage rather than that of a zinc aqua or hydroxide ligand. Of these, the first to be discovered is the Ada DNA repair protein which possesses a $[(Cys)_4Zn]$ motif,[16] but other examples that involve reactivity at zinc cysteine thiolate linkages are methionine synthase, methanol:coenzyme M methyltransferase, farnesyl transferase and geranylgeranyl transferase, as discussed in more detail below.[17]

The Ada protein repairs damage to DNA as a result of methylation.[16] The Ada protein achieves the repair by undergoing sacrificial alkylation of one of the zinc cysteine thiolate ligands (Scheme 52); in this sense Ada is not acting as an enzyme, but rather as a reagent. A similar, but structural, $[(Cys)_4Zn]$ motif is present in the XPA (xeroderma pigmentosum group A) protein which is responsible for the repair of damaged DNA in humans.[243] Inhibition of such DNA repair is now recognized as a reason for metal-induced carcinogenesis.[244] A mutant of Ada (C38G) has been synthesized in which the Cys38 residue (i.e. the cysteine residue that is alkylated) is replaced by Gly, such that the active site is converted from a $[S_4Zn^{II}]$ motif to one in which one of the cysteine groups has been replaced by an aqua ligand, i.e., $\{[S_3]Zn^{II}(OH_2)\}$.[245] Significantly, this change in active-site composition allows the C38G Ada mutant to function in a true catalytic manner if an external thiol (such as MeSH) is used as the sacrificial species to be alkylated rather than a protein cysteine residue.

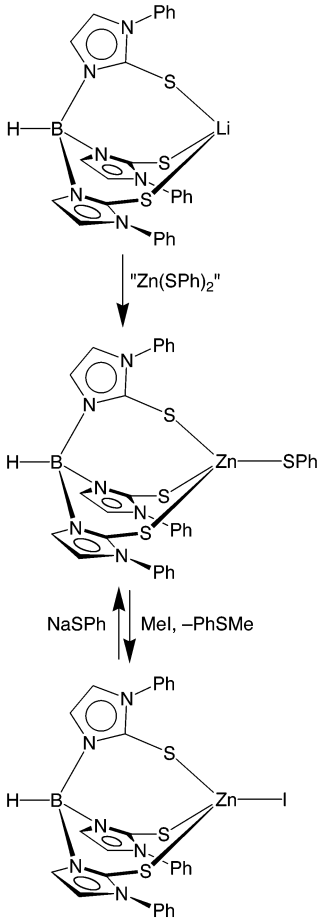
The nucleophilic reactivity of the zinc cysteine thiolate moiety of the Ada protein is particularly interesting in view of the otherwise inertness when it is a component of a structural site. As such, it has been postulated that N−H···S hydrogen-bonding interactions between the thiolate sulfur and amide groups of other residues provide a mechanism to modulate the reactivity of the zinc cysteine thiolate moiety.[20,246]

3.1.6.2. Synthesis and Structural Characterization of Synthetic Analogues of the Ada Protein. The active site of the Ada DNA repair protein possesses a $[Cys_4Zn^{II}]$ motif, as do the structural sites in many other zinc enzymes. Structurally characterized examples of mononuclear complexes with tetrahedral $[S_4Zn^{II}]$ motifs are not, however, well precedented, despite the fact that the tetra(phenylthiolate) complex $[Zn(SPh)_4]^{2-}$ was first structurally characterized in 1978.[247] While $[Zn(SPh)_4]^{2-}$ may be expected to have an idealized tetrahedral zinc coordination environment, it has been noted that $[M(SPh)_4]^{2-}$ derivatives exist with two conformations that differ according to the positions of the phenyl

Scheme 52. Repair of damaged DNA by sacrificial alkylation of one of the zinc cysteine thiolate ligands of the Ada protein



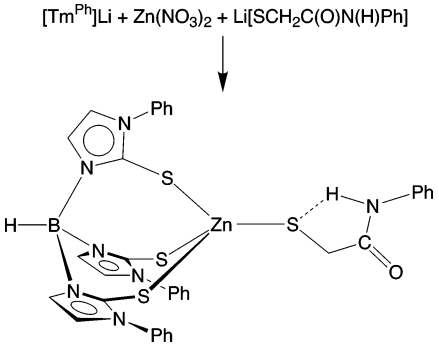
Scheme 53. Synthesis of [Tm^{Ph}]ZnSPh, a synthetic analogue of the Ada protein; by analogy to the Ada protein, the thiolate ligand in [Tm^{Ph}]ZnSPh may also be alkylated



groups; the two conformations have idealized S_4 and D_{2d} symmetry.[248] Steric interactions cause a distortion of the MS_4 core from tetrahedral geometry[249] such that the S_4 isomer is tetragonally compressed along the S_4 axis, while the D_{2d} isomer is tetragonally elongated. Due to increased steric interactions, distortions from idealized tetrahedral coordination (109.5°) are expected to be greater for the D_{2d} isomer. Thus, the S−Zn−S bond angles in $[Ph_4P]_2[Zn(SPh)_4]$ with D_{2d} geometry spread the substantial range of 97−121°,[247] while those in $[Me_4N]_2[Zn(SPh)_4]$ with S_4 geometry span the range 100−117°.[250] It is, therefore, evident that $[Zn(SPh)_4]^{2-}$ does not portray an idealized tetrahedral $[ZnS_4]$ coordination environment. Furthermore, the incorporation of different aryl groups can have a profound effect; thus, $[Et_4N]_2[Zn(SAr)_4]$ (Ar = *o*-C_6H_4Ph) exhibits rigorous S_4 symmetry, and the S−Zn−S angles are close to tetrahedral (106−116°),[248a] whereas $[Et_3NH]_2[Zn(SAr)_4]$ (Ar = *o*-$C_6H_4pyr^{Me_2}$) deviates substantially from tetrahedral coordination (95−119°).[251,252]

A structural analogue for the [(Cys)$_4$Zn] motif of the Ada protein is provided by the thiolate complex [Tm^{Ph}]ZnSPh (Scheme 53) in which the [Tm^{Ph}] ligand mimics the three cysteine residues that remain bound to zinc during the course of the alkylation reaction.[126] Related cadmium complexes, namely, [Tm^{p-Tol}]CdSR (R = Bz, Ph, *p*-Tol), have also been isolated.[253] In addition to phenylthiolate derivatives, a thiolate derived from 2-mercapto-*N*-phenylacetamide, namely, [Tm^{Ph}]ZnSCH$_2$C(O)N(H)Ph, has been obtained by the reaction of $Zn(NO_3)_2$ with [Tm^{Ph}]Li and Li[SCH$_2$C(O)N(H)Ph] (Scheme 54).[254]

Scheme 54. Synthesis of [Tm^{Ph}]ZnSCH$_2$C(O)N(H)Ph, a complex with an intramolecular N−H···S hydrogen bond



The Zn−S bond lengths of [Tm^{Ph}]ZnSPh [2.258(1) Å] and [Tm^{Ph}]ZnSCH$_2$C(O)N(H)Ph [2.267(1) Å] are comparable to the mean terminal Zn−SPh bond length [2.29 Å] for complexes listed in the Cambridge Structural Database, and also to the Zn−SEt bond length [2.203(3) Å] in the related tris(pyrazolyl)-hydroborato complex [Tp^{Ph}]ZnSEt.[255] These bond lengths are, however, noticeably shorter than the Zn−SPh bond length in $[Zn(SPh)_4]^{2-}$ [2.35 Å].[247,250] The most interesting feature of [Tm^{Ph}]ZnSCH$_2$C(O)N-(H)Ph is that the 2-mercapto-*N*-phenylacetamide ligand incorporates a N−H group that participates

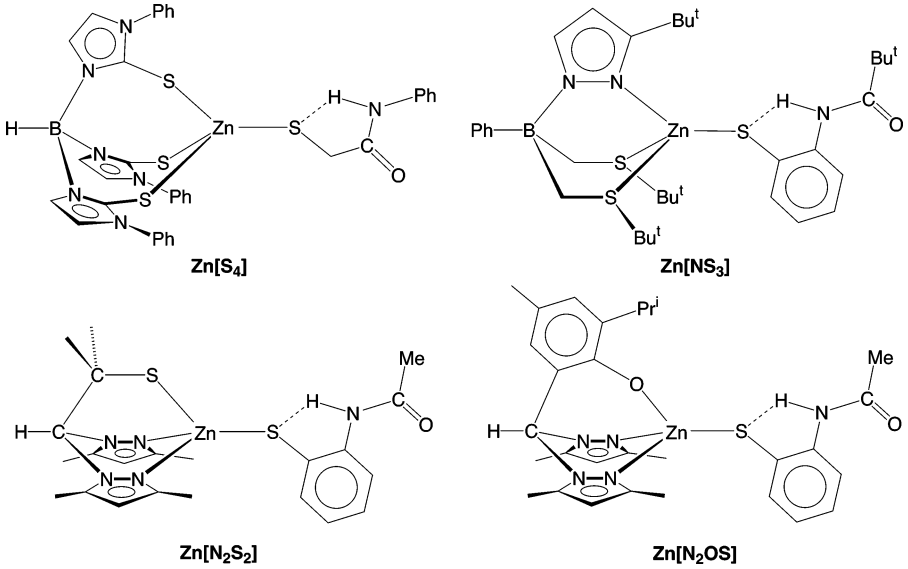


Figure 35. Examples of zinc thiolate complexes with N−H···S hydrogen bonds.

in an intramolecular N−H···S hydrogen-bonding interaction with the thiolate sulfur, as illustrated in Scheme 54.[254] Thus, the N−H···S hydrogen bond is characterized by a N···S separation of 2.99 Å and a S···H separation of 2.46 Å. Although [Tm^{Ph}]ZnSCH$_2$C(O)N(H)Ph is the first example of such an interaction for zinc complexes with a tetrahedral [ZnS$_4$] geometry, N−H···S interactions of this type in zinc complexes have been previously noted for other complexes, e.g, [HC(pz^{Me_2})$_2$(C$_6$H$_2$MePr^{i}O)]ZnS[C$_6$H$_4$-*o*-NHC(O)Me],[256] [HC(pz^{Me_2})$_2$(Me$_2$CS)]ZnS[C$_6$H$_4$-*o*-NHC(O)Me],[256] and [Ph(pz$^{Bu^t}$)Bt$^{Bu^t}$]ZnS[C$_6$H$_4$-*o*-NHC(O)Bu^t],[200b] which vary in the sulfur content of the zinc coordination sphere, as illustrated in Figure 35. In addition, a complex that possesses two N−H···S hydrogen-bonding interactions, namely Zn[*S*-2-C$_6$H$_4$N(H)C(O)Ph]$_2$(1-MeImH)$_2$,[257] is also known (Figure 36).

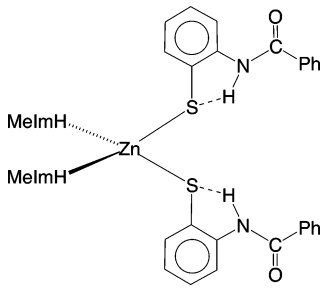
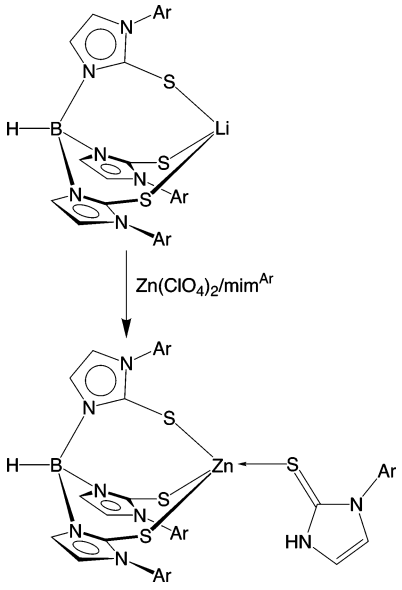


Figure 36. Zn[S-2-C$_6$H$_4$N(H)C(O)Ph]$_2$(1-MeImH)$_2$, a complex with two N−H···S hydrogen-bonding interactions.

Structurally related tetrahedral [ZnS$_4$] complexes that employ tris(2-mercapto-1-arylimidazolyl)hydroborato ligands [Tm^{Ar}] ligands are provided by mercaptoimidazole adducts {[Tm^{Ar}]Zn(mim^{Ar})}$^+$ which are synthesized by the reaction between [Tm^{Ar}]Li, mim^{Ar}, and Zn(ClO$_4$)$_2$ (Scheme 55).[258] For both {[Tm^{Ph}]Zn(mim^{Ph})}$^+$ and {[Tm^{p-Tol}]Zn(mim^{p-Tol})}$^+$, the Zn−S(mim^{Ar}) bond lengths [2.326(1), R = Ph; 2.324(1) Å, R = *p*-Tol] are longer than those of Zn−SPh and Zn−SCH$_2$C(O)N(H)Ph, consistent with the dative covalent nature of the interaction with the mercaptoimidazole ligands. The coordination of the mim^{Ar} ligands is very similar to that of the analogous groups in the [Tm^{Ar}] ligand. Thus, not only are the Zn−S bond lengths comparable, but so are the C−S bond lengths and Zn−S−C bond angles. It is, therefore, evident that the attachment of the three mim^{Ar} groups to boron exerts very little perturbation on the ability of the mim^{Ar} groups to bind to zinc.

Scheme 55. Synthesis of {[Tm^{Ar}]Zn(mim^{Ar})}$^+$



In addition to [Tm^{Ph}]ZnSPh, a related phenylthiolate complex employing the phenyltris[(*tert*-butylthio)methyl]borate ligand, [PhTt$^{Bu^t}$]ZnSPh, has been iso-

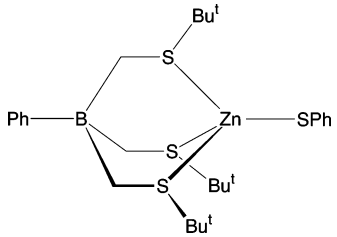
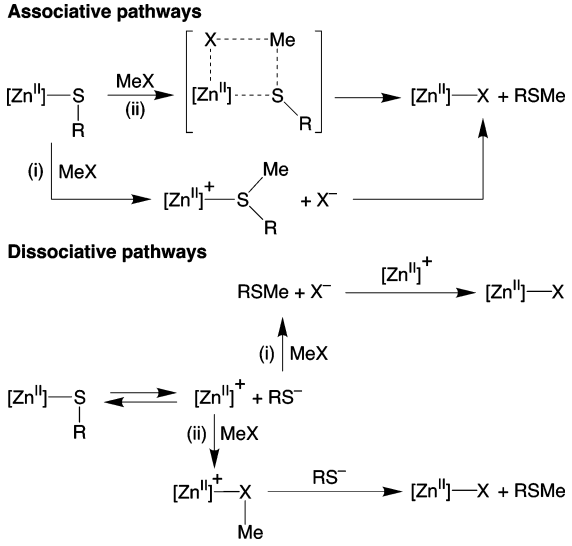


Figure 37. [PhTt$^{Bu^t}$]ZnSPh, a thiolate complex with a [ZnS$_4$] motif.

Scheme 56. Manifold of mechanisms for zinc thiolate alkylation



lated (Figure 37).[200] Other structurally characterized mononuclear complexes with a tetrahedral $[S_4Zn^{II}]$ coordination geometry include: $[Bm^{Me}]_2Zn$,[259] $[\eta^2\text{-}Tm^{Bu^t}]_2Zn$,[198] [(2-mercapto-1-methylimidazole)$_4$Zn]$^{2+}$,[260] and [(*N*-methyl-2-thioxopyrrolidine)$_4$Zn]$^{2+}$.[261] Finally, pentadentate $[NS_4]$ ligands have also been investigated for providing sulfur-rich environments to mimic zinc enzymes.[262]

3.1.6.3. Modeling the Mechanism of Action of the Ada DNA Repair Protein. The key step in the mechanism of action of the Ada DNA repair protein involves alkylation of a cysteine thiolate residue. Alkylation of a zinc thiolate function can, in principle, occur via a manifold of mechanisms of which the two extremes are associative and dissociative (Scheme 56), and studies on synthetic analogues have provided evidence for both types. The [(Cys)$_4$Zn] motif of the active site of the Ada repair protein was first functionally modeled by the anion $[Zn(SPh)_4]^{2-}$.[247,250] Specifically, $[Me_4N]_2[Zn(SPh)_4]$ was observed to be alkylated by $(MeO)_3PO$ to form PhSMe, $(MeO)_2PO_2^-$, and $[Zn(SPh)_3]^-$ (Scheme 57), and the mechanism was proposed to proceed via initial heterolytic dissociation generating an incipient thiolate anion.[263] In an effort to establish why Ada employs a sulfur-rich active site, related compounds in which the phenylthiolate ligand has been replaced by a neutral methylimidazole donor (MeImH), i.e., $[Zn(SPh)_3(MeImH)]^-$ and $Zn(SPh)_2(MeImH)_2$ have been investigated. Notably, the reactivity of the zinc thiolate complex towards alkylation was markedly dependent on the nature of the complex, decreasing as the anionic charge is reduced. Thus, the reactivity decreases in the sequence $[Zn(SPh)_4]^{2-} > [Zn(SPh)_3(MeImH)]^- > Zn(SPh)_2(MeImH)_2$, as summarized in Table 10. The lack of reactivity of the thiolate linkage in $Zn(SPh)_2(MeImH)_2$ is in accord with the prominent structural role that is played by $[Zn(Cys)_2(His)_2]$ sites, as exemplified by zinc fingers. In contrast to the inertness of the thiolate linkage in $Zn(SPh)_2(MeImH)_2$, the reactivity of $[Zn(SPh)_4]^{2-}$ is comparable to that of "free" PhS^- in the form of $[Me_4N][SPh]$. On the basis of the above observations, the role of zinc in the Ada protein may be viewed as providing a facile source of nucleophilic cysteine thiolate when needed. In its resting state, coordination to zinc serves to prevents the thiolate from being protonated that would otherwise destroy its nucleophilicity.

Scheme 57. Dissociative mechanism proposed for alkylation of $[Zn(SPh)_4]^{2-}$

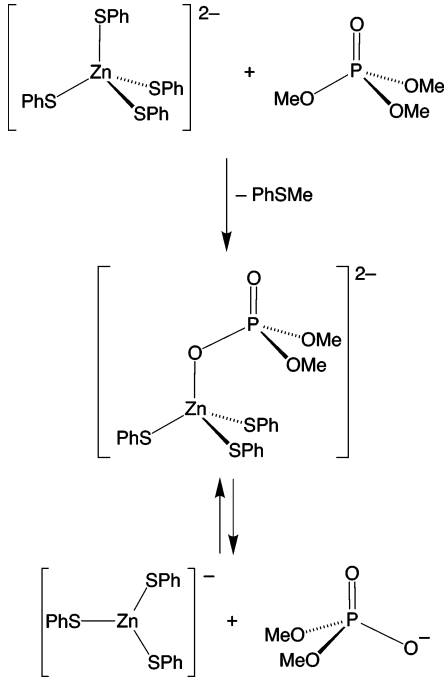


Table 10. Pseudo-First-Order Rate Constants for Reactions of Phenylthiolate Derivatives with $(MeO)_3PO$[a]

compound	k (s^{-1})
$[Me_4N]_2[Zn(SPh)_4]$	8×10^{-5}
$[Me_4N]_2[Zn(SPh)_3(MeImH)]$	6×10^{-6}
$Zn(SPh)_2(MeImH)_2$	$\leq 5 \times 10^{-8}$
$[Me_4N][SPh]$	1×10^{-4}
$[Me_4N]_2[Co(SPh)_4]$	4×10^{-5}
$[Me_4N]_2[Cd(SPh)_4]$	3×10^{-5}

[a] Data taken from Wilker, J. J.; Lippard, S. J. *J. Am. Chem. Soc.* **1995**, *117*, 8682.

In contrast to $[Zn(SPh)_4]^{2-}$, alkylation of a variety of neutral zinc thiolate complexes has been proposed to proceed via an associative mechanism. For example, alkylation of $[Tp^{RR'}]ZnSR$ derivatives with MeI was reported to occur without prior dissociation of RS^-,[88c,264] as was the corresponding reaction of the parent thiol $[Tp^{Ph,Me}]ZnSH$ with MeI to give $[Tp^{Ar,Me}]ZnI$ and MeSH.[77] Furthermore, the exchange reactions of $[Tp^{Ph,Me}]ZnSBz$ with ArSH to form $[Tp^{Ph,Me}]ZnSAr$ have likewise been proposed to occur without initial dissociation of thiolate.[207] Similar conclusions pertaining to the reactivity of zinc thiolate linkages have been reported for $[Ph(pz^{Bu^t})Bt^{Bu^t}]ZnSAr$[200] and for reactions of the chelated sulfur ligand of $[HC(pz^{Me_2})_2(CMe_2S)]ZnX$[265] and a series of other zinc thioates.[154,266–268] By analogy, the reaction of MeI with $[Tm^{Ph}]ZnSPh$, which features a $[ZnS_4]$ coordination environment, to give PhSMe and $[Tm^{Ph}]ZnI$ is also considered to be associative. The importance of these studies is that they clearly indicate that zinc-bound thiolates *may* be nucleophilic and that dissociation is not a prerequisite for alkylation. Another

Table 11. Influence of N−H···S Hydrogen-Bonding Interactions on the Reactivity of Zinc Thiolate Bonds with MeI in MeCN at 25 °C[a]

compound	k (s^{-1})
$[HC(pz^{Me_2})(C_6H_2MePr^iO)]ZnS[C_6H_4$-*o*-$NHC(O)Me]$	3.4×10^{-5}
$[HC(pz^{Me_2})(C_6H_2MePr^iO)]ZnSPh$	1.4×10^{-3}
$[HC(pz^{Bu^t,Me})_2(CO_2)]ZnS[C_6H_4$-*o*-$NHC(O)Me]$	5.9×10^{-6}
$[HC(pz^{Bu^t,Me})_2(CO_2)]ZnSPh$	1.4×10^{-5}

[a] Data taken from Smith, J. N.; Shirin, Z.; Carrano, C. J. *J. Am. Chem. Soc.* **2003**, *125*, 868.

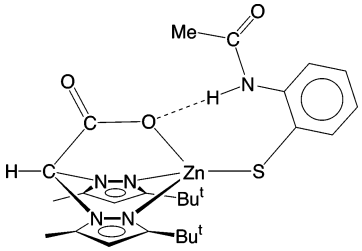
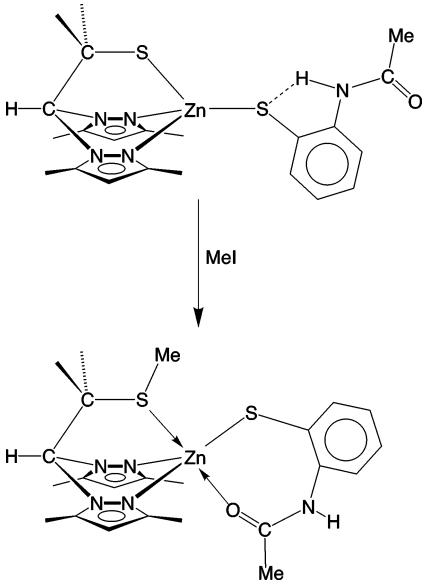


Figure 38. $[HC(pz^{Bu^t,Me})_2(CO_2)]ZnS[C_6H_4$-*o*-$NHC(O)Me]$, a complex that preferentially forms a N−H···O rather than N−H···S interaction.

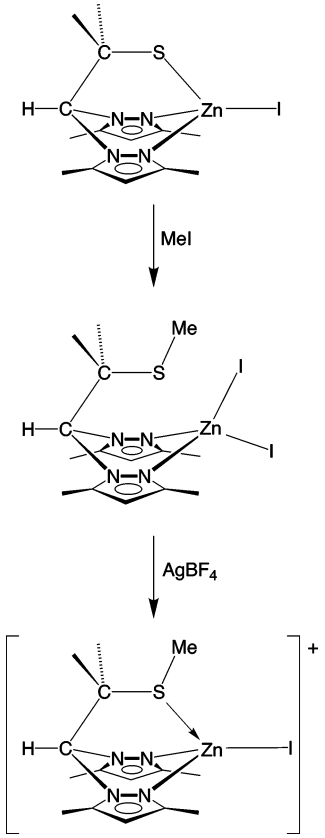
notable feature of the reaction between $[Tm^{Ph}]ZnSPh$ and MeI is that the reaction occurs specifically with the sulfur of the phenylthiolate ligand, with the sulfur atoms of the $[Tm^{Ph}]$ ligand being inert to alkylation. Indeed, DFT calculations (B3LYP) indicate that the HOMO possesses a large degree of [PhS] sulfur lone pair character, with the sulfur lone pairs of the thione groups being located at lower energies.[126] As such, the thiolate sulfur atom would be expected to possess greater nucleophilicity. The calculations indicate that the thiolate sulfur bears a greater negative charge, which would thereby also contribute to its greater nucleophilicity.

The reactivity exhibited by the zinc cysteine thiolate moiety of the Ada protein is particularly interesting in view of the otherwise inertness of this ligand when it is a component of a structural site. As such, it has been postulated that N−H···S hydrogen-bonding interactions between the thiolate sulfur and amide groups of other residues provide a mechanism to modulate the reactivity of the zinc cysteine thiolate moiety.[20,269] In this regard, complexes such as $[Tm^{Ph}]$-$ZnSCH_2C(O)N(H)Ph$,[254] $[HC(pz^{Me_2})_2(C_6H_2MePr^iO)]$-$ZnS[C_6H_4$-*o*-$NHC(O)Me]$,[256] $[HC(pz^{Me_2})_2(Me_2CS)]ZnS$-$[C_6H_4$-*o*-$NHC(O)Me]$,[256] and $[Ph(pz^{Bu^t})Bt^{Bu^t}]ZnS$-$[C_6H_4$-*o*-$NHC(O)Bu^t]$,[200] which feature intramolecular N−H···S hydrogen-bonding interactions (Figure 35) offer the potential for investigating this postulate. For example, comparison of the reactivity of $[HC(pz^{Me_2})_2$-$(C_6H_2MePr^iO)]ZnS[C_6H_4$-*o*-$NHC(O)Me]$ with the simple phenylthiolate derivative $[HC(pz^{Me_2})_2(C_6H_2MePr^iO)]$-$ZnSPh$ indicates that the hydrogen-bonding interaction reduces the reactivity by ca. 2 orders of magnitude (Table 11). In contrast, $[HC(pz^{Bu^t,Me})_2(CO_2)]ZnS$-$[C_6H_4$-*o*-$NHC(O)Me]$ and $[HC(pz^{Bu^t,Me})_2(CO_2)]ZnSPh$ do not exhibit such a large difference in reactivity, an observation that was attributed to the fact that the hydrogen-bonding interaction in the former compound is not with the sulfur, but is actually with the oxygen of the ligand (Figure 38).[256] Another illustration of the influence of the hydrogen-bonding interaction is that $[HC(pz^{Me_2})_2(Me_2CS)]ZnS[C_6H_4$-*o*-$NHC(O)$-Me] undergoes selective alkylation of the sulfur of the $[HC(pz^{Me_2})_2(Me_2CS)]$ ligand, which is not involved in a hydrogen-bonding interaction (Scheme 58).

Scheme 58. Preferential alkylation of the thiolate sulfur that is not involved in a hydrogen-bonding interaction



Scheme 59. Thiolate alkylation resulting in a coordinated thioether



Another aspect of $[HC(pz^{Me_2})_2(Me_2CS)]ZnS[C_6H_4$-*o*-$NHC(O)Me]$ which is notable is the fact that the alkylated sulfur remains coordinated to the zinc center. This observation is interesting because thioether coordination is a feature that is also observed in the Ada protein following alkylation. Obvi-

ously, the fact that the thioether remains coordinated is in part due to the fact that it is tethered to the ligand.

A related example in which a thioether is coordinated to zinc is provided by $\{[HC(pz^{Me_2})_2(Me_2CSMe)]ZnI\}^+$. However, this product is not obtained directly upon methylation of $[HC(pz^{Me_2})_2(Me_2CS)]ZnI$ with MeI, which gives initially $[\eta^2\text{-}HC(pz^{Me_2})_2(Me_2CSMe)]ZnI_2$ in which the thioether is dissociated (Scheme 59). However, $\{[HC(pz^{Me_2})_2(Me_2CSMe)]ZnI\}^+$ is obtained following abstraction of iodide from $[\eta^2\text{-}HC(pz^{Me_2})_2(Me_2CSMe)]ZnI_2$ using $AgBF_4$ (Scheme 59). Alternatively, $\{[HC(pz^{Me_2})_2(Me_2CSMe)]ZnI\}^+$ may be obtained directly from $[HC(pz^{Me_2})_2(Me_2CS)]ZnI$, employing $[Me_3O][BF_4]$ as the methylating agent.

3.1.7. Other Proteins That Mediate Alkylation of Cysteine Thiolate Residues: Methionine Synthase, Methanol:Coenzyme M Methyltransferase, Methylcobamide:Coenzyme M Methyltransferase, Farnesyl Transferase, and Geranylgeranyl Transferase

In addition to the Ada protein, there are a variety of enzymes that feature cysteine thiolate alkylation as an important component of their mechanisms of action. Such enzymes include methionine synthase, methanol:coenzyme M methyltransferase, methylcobamide:coenzyme M methyltransferase, farnesyl transferase, and geranylgeranyl transferase.[17] The protein residues that coordinate to zinc in a selection of these enzymes are summarized in Table 12, from which it is evident that cysteine is a prominent component. The alkylating groups involved in these transferase reactions from range from simple methyl to complex groups such as farnesyl and geranylgeranyl (Figure 39). For example, methionine synthase catalyzes the biosynthesis of the amino acid methionine by methylation of homocysteine (Scheme 60), while farnesyltransferase (FTase) catalyzes the transfer of the farnesyl group from farnesyl pyrophosphate (FPP) to a cysteine residue (Scheme 61).[270]

Table 12. Zinc Ligands in Selected Proteins That Participate in Thiolate Alkylation[a]

	L_1	L_2	L_3	L_4
farnesyl transferase	Cys	His	Asp	H_2O
geranylgeranyl transferase	Cys	His	Asp	H_2O
methylcobamide:coenzyme M methyltransferase	Cys	His	nd[b]	nd
cobalamin-independent enzyme (MetH)	Cys	Cys	His	N/O
cobalamin-dependent enzyme (MetE)	Cys	Cys	Cys	N/O
betaine−homocysteine methyltransferase	Cys	Cys	nd	nd
Ada	Cys	Cys	Cys	Cys

[a] Data taken from Hightower, K. E.; Fierke, C. A. *Curr. Opin. Chem. Biol.* **1999**, *3*, 176. [b] nd = not determined.

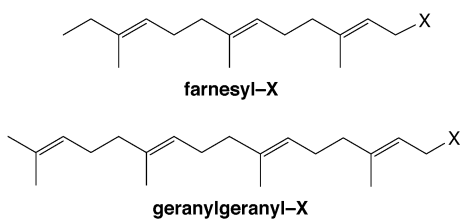
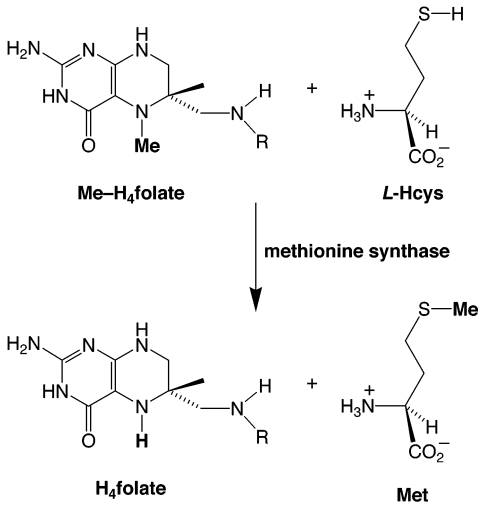
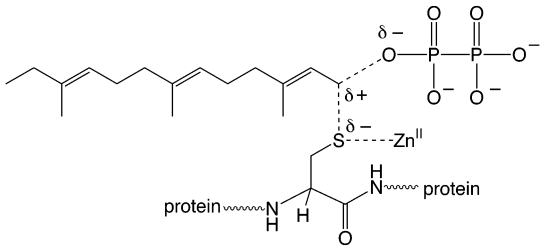


Figure 39. Farnesyl and geranylgeranyl groups that are transferred to cysteine thiolates by farnesyl transferase and geranylgeranyl transferase.

Scheme 60. Methionine synthase-catalyzed biosynthesis of the amino acid methionine (Met) by methylation of homocysteine

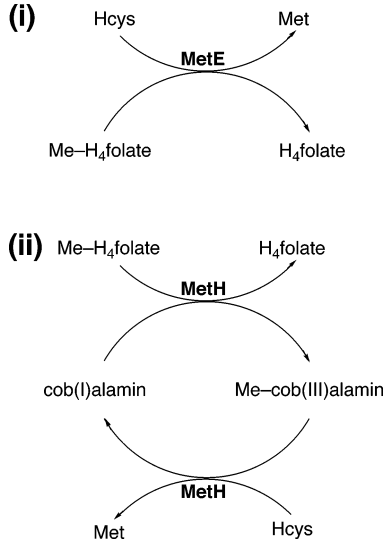


Scheme 61. Farnesyltransferase (FTase)-catalyzed transfer of the farnesyl group from farnesyl pyrophosphate (FPP) to a cysteine residue



The methyl group required for the synthesis of methionine from homocysteine is provided by methyltetrahydrofolate (Me-H_4folate), as illustrated in Scheme 60. This is a challenging reaction because not only must the thiol group with a pK_a of ca. 10 be activated by deprotonation at neutral pH, but the methyltetrahydrofolate must also be sufficiently activated by protonation to enable dissociation of the tetrahydrofolate leaving group. Biochemically, this transformation may be achieved by two pathways which involve a cobalamin-dependent enzyme (MetH) and a cobalamin-independent enzyme (MetE). The mechanism for the cobalamin-independent enzyme (MetE) involves direct transfer of the methyl group from Me-H_4folate to HCys, whereas the reaction that is mediated by cobalamin-dependent enzyme MetH involves a two step sequence, namely (i) initial transfer of the methyl group from Me-H_4folate to cob(I)almin giving Me-cob(III)almin, followed by (ii) methylation of HCys by Me-cob(III)almin (Scheme 62). Thus, the principal difference of the two pathways is whether the Hcys is methylated by Me-H_4folate or by Me-cob(III)alamin. Interestingly, zinc is required for both MetH and MetE enzymes. EXAFS studies indicate that the active site of cobalamin-independent MetE is composed of two sulfur ligands and two nitrogen/oxygen ligands, i.e., $Zn[S_2(N/O)_2]$; in the presence of Hcys, the active site has the

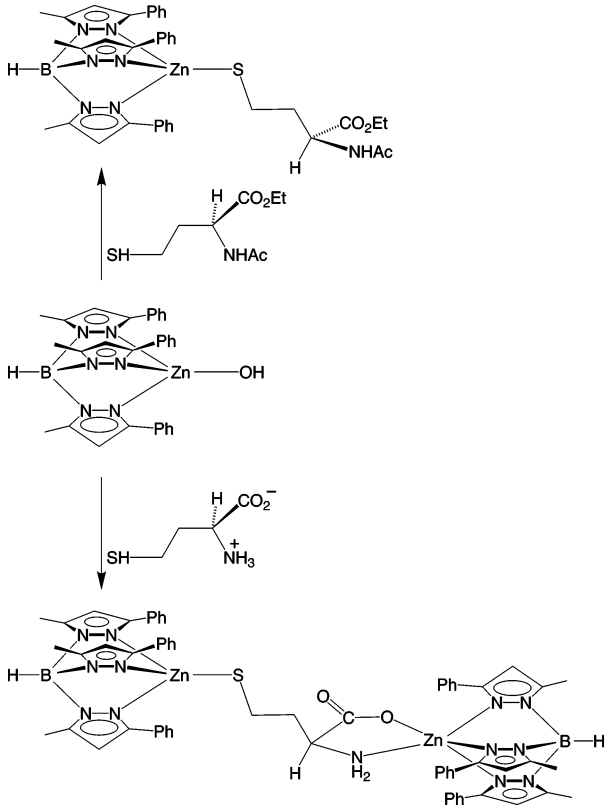
Scheme 62. Catalytic cycles for (i) cobalamin-independent MetE involving direct transfer of the methyl group from Me-H_4folate to HCys and (ii) cobalamin-dependent MetH involving initial transfer of the methyl group from Me-H_4folate to cob(I)alamin giving Me-cob(III)alamin, followed by methylation of HCys by Me-cob(III)alamin; thus, the principal mechanistic difference is whether the Hcys is methylated by Me-H_4folate or by Me-cob(III)alamin



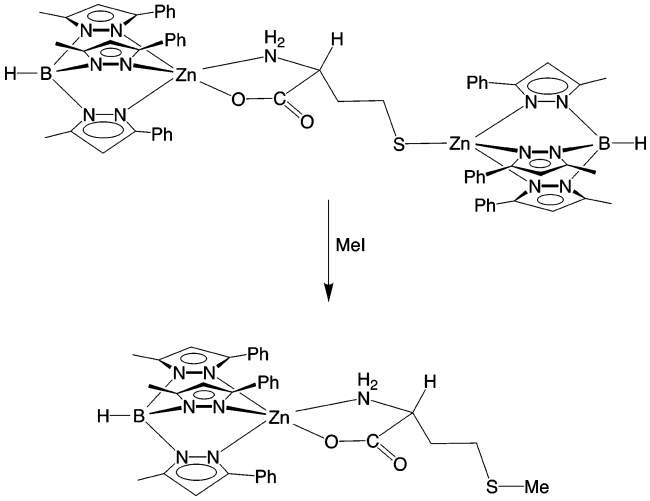
composition Zn[S_3(N/O)], i.e. the Hcys replaces one of the oxygen/nitrogen donors.[17d,271] On the basis of additional information derived from studying mutations, it has been postulated that the residues which coordinate to zinc in the native enzyme are two cysteines and one histidine. The cobalamin-dependent MetH has also been characterized by EXAFS studies which demonstrate that it possesses an active site that is coordinated by three sulfur ligands and one nitrogen/oxygen ligand, i.e., Zn[S_3(N/O)]; in the presence of Hcys, the active site becomes Zn[S_4]. Thus, the principal difference in the active sites of MetE and MetH is that the latter is more sulfur rich.

Many of the studies described above for the Ada protein are also directly pertinent to methionine synthase and the other enzymes listed in Table 12, since each involves reactivity of a zinc thiolate linkage. Several studies, however, have been discussed specifically in the context of methionine synthase. For example, derivatives of the *O*-ethyl and *N*-acetyl protected form of homocysteine, H[HCys(OEt)(NAc)], and the unprotected form, H_2[HCys], have been preprared from the zinc hydroxide complex [$Tp^{Ph,Me}$]ZnOH (Scheme 63).[88c] In the case of the protected form H[HCys(OEt)(NAc)], a simple mononuclear thiolate derivative [$Tp^{Ph,Me}$]Zn[HCys(OEt)(NAc)] analogous to the protected cysteine counterpart [$Tp^{Cum,Me}$]Zn[Cys(OEt)(NAc)][88b] was obtained, while a dinuclear complex {[$Tp^{Ph,Me}$]Zn}$_2$(HCys) was obtained from the reaction with homocysteine itself due to additional reaction with the carboxyl group (Scheme 63). Most interstingly, alkylation of {[$Tp^{Ph,Me}$]-Zn}$_2$(HCys) with MeI resulted in the formation of the methionine derivative, thereby providing an excellent example of the facile formation of methionine from homocysteine on a zinc center (Scheme 64). Thus, the above tris(pyrazolyl)hydroborato system demonstrates that (i) homocysteine reacts readily with a zinc hydroxide to form a thiolate and that (ii) the thiolate so obtained is activated towards electrophilic attack by MeI, thereby resulting in the formation of a methionine derivative.

Scheme 63. Reactivity of [$Tp^{Cum,Me}$]ZnOH towards homocysteine and its *O*-ethyl and *N*-acetyl protected form



Scheme 64. Methylation of {[$Tp^{Ph,Me}$]Zn}$_2$(HCys) forming a methionine derivative



While the above system provides an excellent precedent for the steps proposed in the mechanism of action of cobalamin-independent methionine synthase, a deficiency is that the [$Tp^{Ph,Me}$] ligand does not contain any sulfur donors. In this respect, the [Ph(pz^{Bu^t})Bt^{Bu^t}] ligand which comprises an [NS_2] donor set provides a better structural model for methionine synthase, as illustrated by the thiolate

Table 13. Relative Rate Constants for Alkylation of $[Ph(pz^{Bu^t})Bt^{Bu^t}]ZnSAr$ Derivatives with $PhCH_2Br$ in Toluene at 60 °C[a]

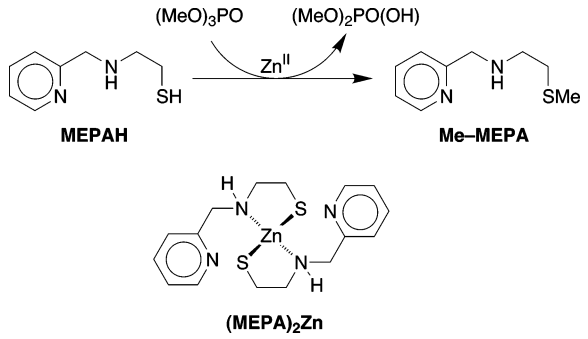
	k_{rel}
$[Ph(pz^{Bu^t})Bt^{Bu^t}]ZnSPh$	21.5
$[Ph(pz^{Bu^t})Bt^{Bu^t}]ZnS[C_6H_4$-*o*-$NHC(O)Bu^t]$	1.0
$[Ph(pz^{Bu^t})Bt^{Bu^t}]ZnS[C_6H_4$-*o*-$NDC(O)Bu^t]$	3.0
$[Ph(pz^{Bu^t})Bt^{Bu^t}]ZnS[C_6H_4$-*p*-$NHC(O)Bu^t]$	33.8

[a] Data taken from Chiou, S.-J.; Riordan, C. G.; Rheingold, A. L. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 3695.

complex $[Ph(pz^{Bu^t})Bt^{Bu^t}]ZnSPh$.[200] In addition to providing a better structural model for methionine synthase, this system has also generated interesting results pertaining to the influence of N−H···S hydrogen-bonding interactions. As noted above, N−H···S hydrogen-bonding interactions inhibit thiolate alkylation, and this trend is also observed in comparison of $[Ph(pz^{Bu^t})Bt^{Bu^t}]ZnS[C_6H_4$-*o*-$NHC(O)Bu^t]$ and $[Ph(pz^{Bu^t})Bt^{Bu^t}]ZnSPh$. Specifically, measurement of the kinetics of the reactions of $[Ph(pz^{Bu^t})Bt^{Bu^t}]ZnS[C_6H_4$-*o*-$NHC(O)Bu^t]$ and $[Ph(pz^{Bu^t})Bt^{Bu^t}]ZnSPh$ towards $PhCH_2Br$ indicates that the hydrogen-bonding interaction inhibits the reactivity by a factor of 21.5 (Table 13).[200] Evidence that this inhibition is due to the hydrogen-bonding interaction and is not merely an electronic effect due to the incorporation an amide substituent is provided by the fact that incorporation of the amide into the para position (where intramolecular hydrogen bonding is no longer feasible) actually increases the reactivity. A most interesting observation is that incorporation of deuterium into the amide group of $[Ph(pz^{Bu^t})Bt^{Bu^t}]ZnS[C_6H_4$-*o*-$NDC(O)Bu^t]$ increases the reactivity, such that the alkylation is characterized by an inverse kinetic isotpe effect of $k_H/k_D = 0.33$. While an inverse kinetic isotope effect could be taken as evidence for an equilibrium between hydrogen-bonded and non-hydrogen-bonded species, with preferential reactivity of the non-hydrogen-bonded form, the magnitude of the effect is not readily rationalized by the anticipated energy differences resulting from zero-point energy differences associated with the N−H and N−D bonds of the hydrogen-bonded and non-hydrogen-bonded species. In this regard, a neglible kinetic isotope effect is observed in the reactions of the N−H···S hydrogen-bonded complexes $[Tm^{Ph}]ZnSCH_2C(O)N(H)Ph$ and $[Tm^{Ph}]ZnSCH_2C(O)N(D)Ph$ with methyl iodide.[254]

A further illustration of how Zn^{II} promotes alkylation of thiols is provided by the methylation of *N*-(2-mercaptoethyl)picolylamine (MEPAH) by $(MeO)_3PO$.[272] Specifically, a variety of zinc salts, namely $ZnCl_2$, $Zn(NO_3)_2$, and $Zn(OAc)_2$, catalyze the methylation of MEPAH by $(MeO)_3PO$, as illustrated in Scheme 65. In refluxing methanol, each of the catalysts give the methylated product Me-MEPA in virtually quantitative yield, whereas at room temperature the efficiency depends markedly on the nature of the zinc catalyst as illustrated by the following yields: $ZnCl_2$ (0%), $Zn(NO_3)_2$ (8%), and $Zn(OAc)_2$ (25%). Since no reaction is observed in the absence of Zn^{II} under comparable conditions, it is evident that Zn^{II} is playing a catalytic role and that its ability to do so depends markedly

Scheme 65. Zn^{II}-catalyzed methylation of MEPAH by $(MeO)_3PO$



on the supporting ligands. Two factors have been proposed to be responsible for the different catalytic efficiencies: (i) the structures of [MEPAZnX] complexes depend on the identify of X,[168] and (ii) the various zinc salts have different solubilities at room temperature in methanol. The reactivity of preformed [MEPAZnX] complexes was also investigated. Thus, while methylation catalyzed by $(MEPA)_2Zn$ (Scheme 65) proceeded quantitatively at reflux and with 36% conversion at room temperature, the chloride derivative $[MEPAZnCl]_\infty$ was only effective under reflux conditions. The lower reactivity of $[MEPAZnCl]_\infty$ was attributed to its polymeric nature resulting from each sulfur atom bridging two zinc centers.[168]

Finally, a model for the reaction sequence mediated by cobalamin-dependent methionine synthase (MetH) is provided by an investigation of vitamin-B_{12}-derived Co^I complexes illustrated in Figure 40.[273] Modeling the first stage of the mechanism of action involving cobalamin-dependent MetH was achieved by demonstrating that cobalt(I) complexes of the type LCo^I may be alkylated by MeI to give $[LCo^{III}Me]^+$, as illustrated in Scheme 66. Furthermore, $[LCo^{III}Me]^+$ could also be generated from Me_2NPh by reaction of LCo^I generated *in situ* by reduction of $[LCo^{II}](ClO_4)$ with Zn. The second stage of the reaction, namely the zinc-mediated methyl transfer from cobalt to a thiol has also been demonstrated. Specifically, the yield of RSMe obtained by reaction of $[LCo^{III}Me]^+$ with RSH (R = hexyl) in the presence of pyridine as a base was found to be considerably improved by the presence of $ZnCl_2$ (Scheme 66). It was also demonstrated that *in situ* generated LCo^I in the presence of $ZnCl_2$ was capable of catalyzing the transfer

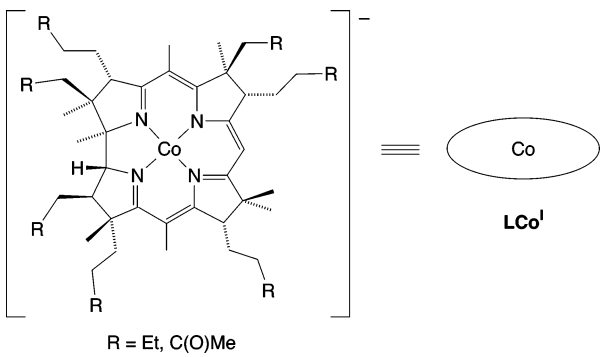
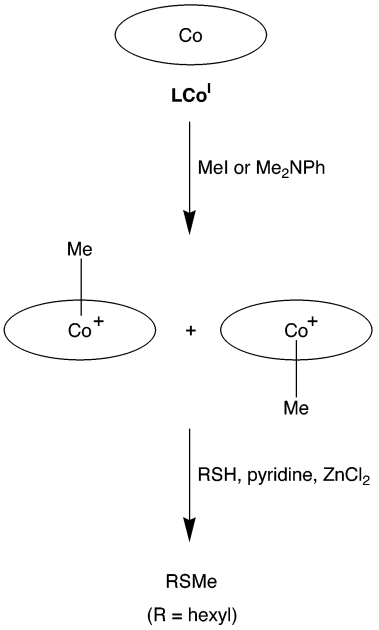


Figure 40. Vitamin-B_{12}-derived Co^I complexes used for modeling cobalamin-dependent methionine synthase (MetH).

Scheme 66. Zn^{II}-promoted transfer of methyl from cobalt to a thiol as a mimic of cobalamin-dependent methionine synthase (MetH)



of methyl transfer from Me_2NPh to RSH, albeit in low yield.

3.2. Multinuclear Zinc Enzymes

In addition to the mononuclear zinc enzymes described above, there are a range of multinuclear zinc enzymes.[1−4,24,274] Examples of enzymes that incorporate two zinc centers include (i) metallo-β-lactamases,[275] (ii) aminopeptidases such as bovine lens leucine aminopeptidase (*bl*LAP) and aminopeptidase A,[276] and (iii) alkaline phosphatase (AP),[277] as illustrated in Figures 41 and 42.[24,274] Enzymes that incorporate three zinc centers are also known, as exemplified by phospholipase C and nuclease P1 (Figure 42); however, the third zinc centers of phospholipase C and nuclease P1 are not directly associated with the dizinc units. For example, while the Zn···Zn separation of the principal dizinc unit in phospholipase C is 3.3 Å, the distances between these zinc centers and the third zinc center are 4.7 and 6.0 Å.[24b] Likewise, the corresponding separations in nuclease P1 are 3.2, 4.7, and 5.8 Å.[24b] As illustrated in Figure 42, alkaline phosphatase exhibits a structural similarity to phospholipase C and nuclease P1 in which the third zinc is replaced by magnesium. The magnesium center was originally proposed as not particpating directly in the catalytic cycle, but more recent studies suggest that its role is to provide a magnesium hydroxide ligand that acts as a general base to deprotonate a serine residue for nucleophilic attack on the phosphorus atom.[277e]

A diverse assortment of ligands has been employed to model aspects of multinuclear zinc enzymes.[278,279] Thus, approaches to incorporate two zinc centers in a molecule include: (i) tethering two or more zinc bringing ligands together by a linker that may range from a simple aliphatic chain to a complex calixarene, (ii) coordinating two zinc centers in a macrocycle or cryptand, and (iii) the application of ligands with donor groups that directly bridge two zinc centers. Examples of each of these approaches are illustrated in Figures 43, 44, 45, and 46. Bridging ligands that facilitate bringing two zinc centers together include phenolate (Figure 45),[280] carboxylate (Figures 46 and 47),[280b,281−283] phthalazine (Figure 48),[284] and pyrazolyl (Figure 49).[285] Of these, the bridging carboxylate is the most biologically pertinent in view of the occurrence of this bridge in enzymes such as phospholipase C and nuclease P1 (Figure 42), and modeling this feature in small molecules has been achieved by using both bulky aryl substituents[281] and the dicarboxylic acid H_2XDK, "*m*-xylenediamine bis(Kemp's triacid imide)",[282] as illustrated in Figures 46 and 47.

A notable aspect of the pyrazolyl bridging ligands is that, depending on the length of the linker between the pyrazolyl ring and the amine donors, either a bridging hydroxide or a bridging $[H_3O_2]$ species may be isolated, namely, $\{[pz\{CH_2N(CH_2)_3NMe_2\}_2]ZnOH\}^{2+}$ and $\{[pz\{CH_2N(CH_2)_2NEt_2\}_2]Zn(H_3O_2)\}^{2+}$ (Scheme 67).[285] The latter complex may be viewed as a hydrogen-bonded aqua hydroxide species. Most interestingly, the reactivity of these complexes towards CO_2 varies dramatically. Thus, whereas no reaction was observed between the hydroxide complex $\{[pz\{CH_2N(CH_2)_3NMe_2\}_2]ZnOH\}^{2+}$ and CO_2, the aqua hydroxide complex $\{[pz\{CH_2N(CH_2)_2NEt_2\}_2]Zn(H_3O_2)\}^{2+}$ reacted readily to give a bridging bicarbonate complex $\{[pz\{CH_2N(CH_2)_2NEt_2\}_2]Zn(\mu\text{-}O_2COH)\}^{2+}$ that was structurally characterized by X-ray diffraction. The enhanced reactivity of $\{[pz\{CH_2N(CH_2)_2NEt_2\}_2]Zn(H_3O_2)\}^{2+}$ has been interpreted as a result of displacement of water providing a substrate binding site that would facilitate nucleophilic attack by hydroxide. As such, it provides a good model for a variety of dinuclear zinc enzymes.

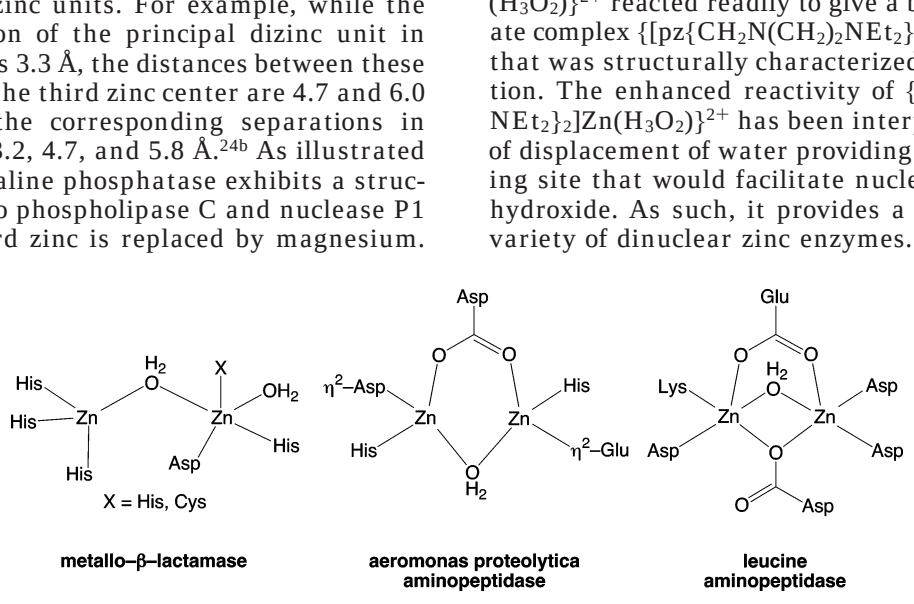


Figure 41. Active sites of representative dinuclear zinc enzymes: metallo-β-lactamases and aminopeptidases.

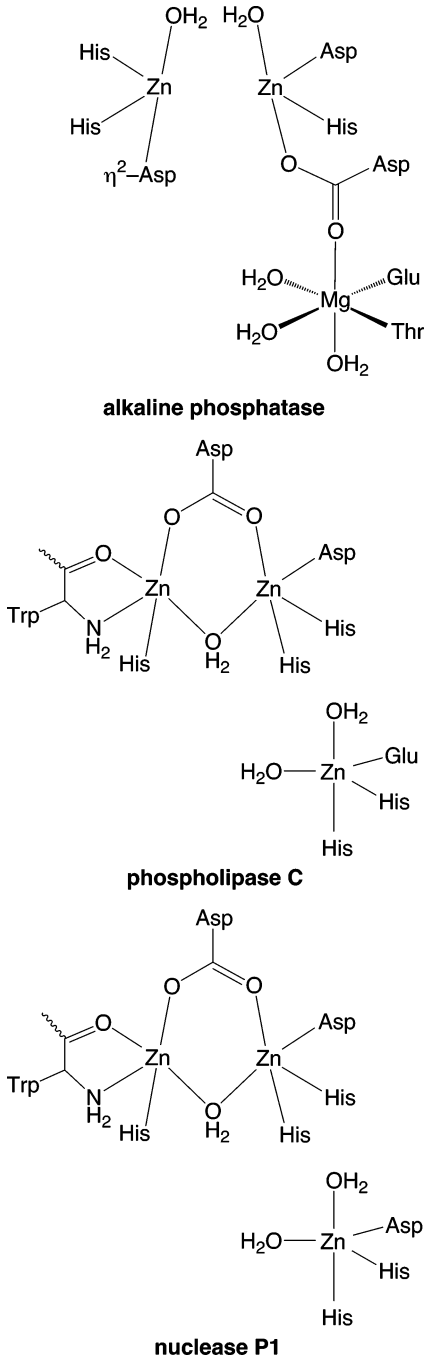


Figure 42. Active sites of representative trinuclear zinc enzymes: alkaline phosphatase (2 Zn's and 1 Mg), phospholipase C (3 Zn's), and nuclease P1 (3 Zn's).

3.2.1. Metallo-β-lactamases

β-Lactams are the single most important class of antiobiotics.[275] Despite this claim, bacterial resistance to β-lactams is becoming widespread so that their efficacy is becoming seriously compromised. One of the principal means by which this resistance is achieved is through the expression of β-lactamases which are enzymes that destroy β-lactams (including penicillins, cephalosporins, and carbapenems) by hydrolyzing and cleaving the four-membered ring (Scheme 68). There are four classes (A, B, C, and D) of β-lactamases, of which the most recent to be discovered, the so-called metallo-β-lactamases (or class B β-lactamases), are dinuclear zinc enzymes.

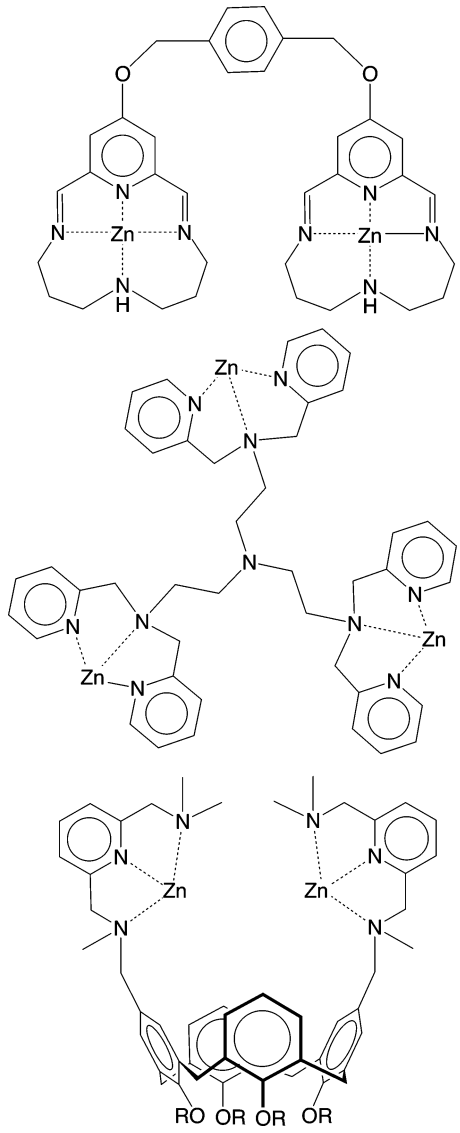


Figure 43. Representative examples of tethers used to link two or more zinc centers together.

The molecular structures of several metallo-β-lactamases have been determined, and while the intimate details may vary, there are several features in common to each enzyme, as illustrated in Figure 41. Specifically, (i) one of the zinc centers is tetrahedral with a [$(His)_3Zn(\mu$-OH)] motif, of which the hydroxide ligand serves as a bridge to the second zinc center, and (ii) the second zinc center is trigonal bipyramidal with a [(His)(Asp)(X)Zn(OH_2)(μ-OH)] motif where, X = Cys or His. Interestingly, while the various metallo-β-lactamases contain two zinc centers, activity is still observed for one of the variants in a monozinc form.[286] The mechanisms of action of the various metallo-β-lactamases are not known with certainty, but it is generally considered to be analogous to the possibilities proposed for carboxypeptidase. The role of the second zinc is likewise uncertain, although it has been postulated that coordination via the nitrogen (Scheme 69) serves to (i) position the substrate for nucleophilic attack, (ii) polarize further the N−C(O) bond, and (iii) stabilize the negative charge on the nitrogen leaving group.[287] Calculations pertaining to the mechanism of action of metallo-β-

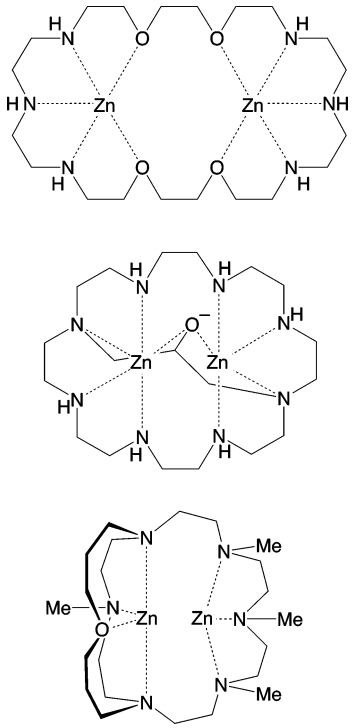


Figure 44. Representative examples of macrocyclic and cryptand ligands employed to bring two zinc centers into proximity.

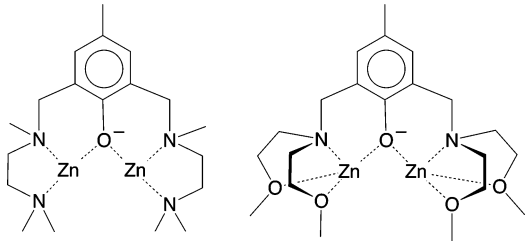


Figure 45. Representative examples of phenolate ligands employed to bring two zinc centers into proximity.

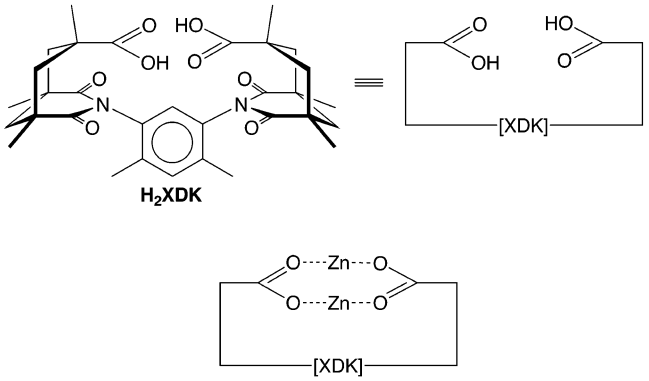


Figure 46. Application of the dicarboxylic acid H_2XDK, "*m*-xylenediamine bis(Kemp's triacid imide)" to form complexes in which two zinc centers are in close proximity.

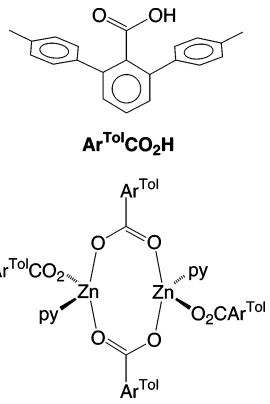


Figure 47. Application of bulky carboxylate ligands to form complexes in which two zinc centers are in close proximity.

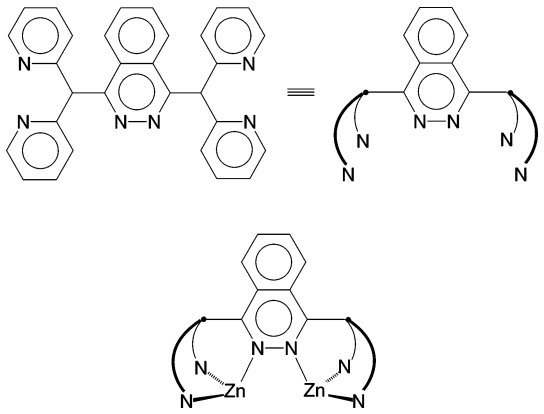


Figure 48. Application of phthalazine ligands to form complexes in which two zinc centers are in close proximity.

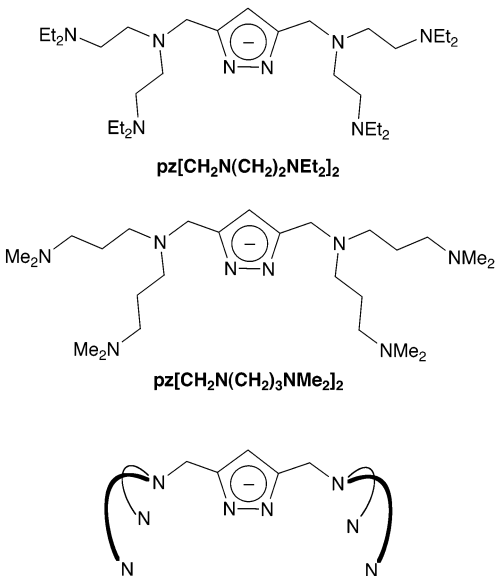
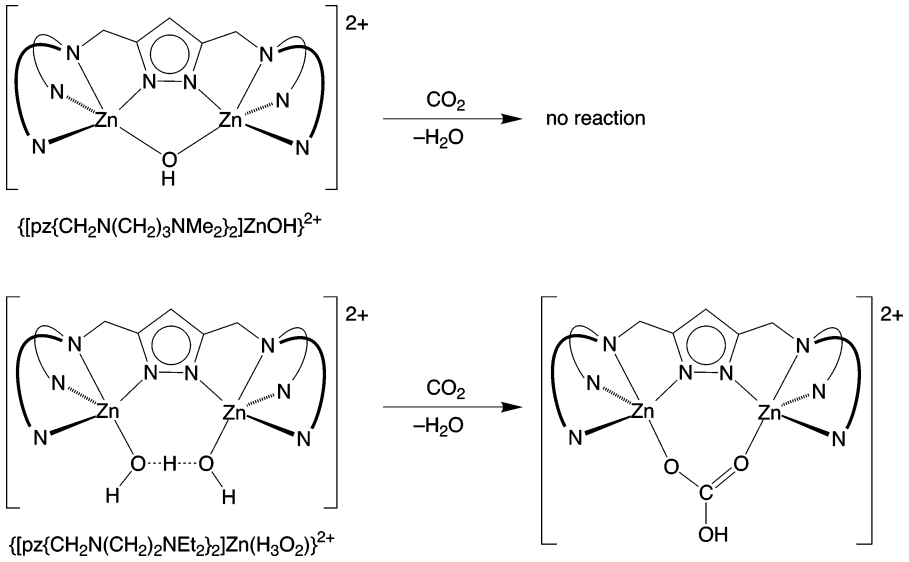


Figure 49. Application of pyrazolyl ligands to form complexes in which two zinc centers are in close proximity.

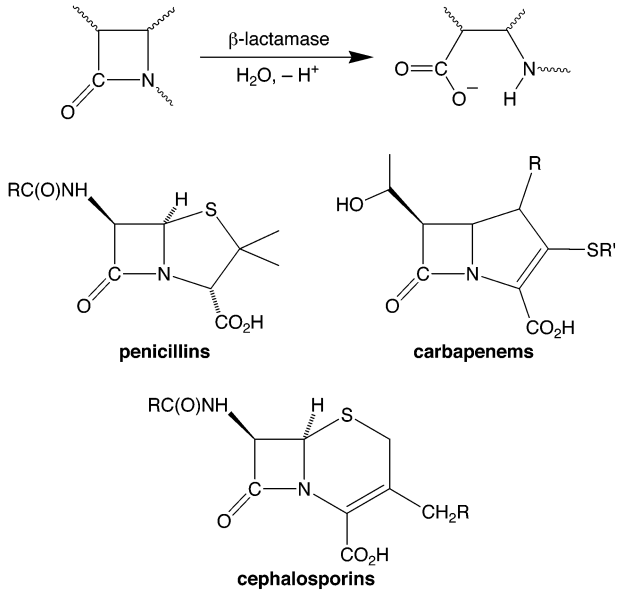
lactamases have focused on reactivity at a single zinc site.[288]

Early model studies demonstrated the ability of various metal ions and simple mononuclear complexes to promote lactam hydrolysis.[159,289,290] More recently, attention has been given to an investigation of dinuclear zinc complexes as catalysts for lactam hydrolysis. Examples of dinuclear zinc complexes that have been proposed as synthetic analogues for metallo-β-lactamases are illustrated in Figure 50.[291] Mechanistic studies employing dinuclear zinc complexes have been performed using nitrocefin as a substrate (Scheme 70) because the reaction may be conveniently monitored by UV−vis spectroscopy.[291] Studies on $[Zn_2(BPAN)(\mu\text{-}OH)(\mu\text{-}O_2PPh_2)]^{2+}$ indicate that the bridging hydroxide is not very reactive

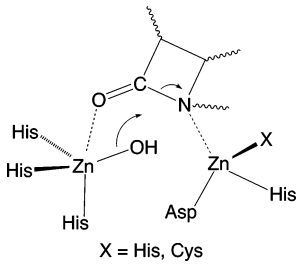
Scheme 67. Formation of a bridging hydroxide versus a bridging $[H_3O_2]$ species depending upon the length of the linker from the pyrazolyl ring to the amine donors; the two complexes exhibit markedly different reactivity towards CO_2



Scheme 68. Hydrolytic cleavage of the β-lactam ring in penicillins, cephalosporins, and carbapenems by metallo-β-lactamases



Scheme 69. Possible mechanism of action of metallo-β-lactamases



because (i) the bridging hydroxide and the coordinated substrate do not align properly to facilitate nucleophilic attack and (ii) the nucleophilicity of the hydroxide is diminished because it coordinates to two zinc centers.[291a] In solution, $[Zn_2L_1(\mu\text{-}NO_3)(NO_3)_2]$ generates the aqua hydroxide species $[Zn_2L_1(\mu\text{-}OH)(OH_2)_2]$ which is also a catalyst for hydrolysis of nitrocefin.[291b,c] The mechanism is proposed to involve coordination of the β-lactam carboxylate group to one of the zinc centers, followed by rate-limiting nucleophilic attack by the bridging hydroxide at the coordinated substrate. However, since mononuclear complexes such as (cyclen)$Zn(NO_3)_2$ and (bpta)$Zn(NO_3)_2$ have a comparable activity, it is evident that the second zinc center is not a requirement for catalytic activity. In fact, it is worth noting that the Zn^{2+}-catalyzed methanolysis of nitrocefin has been reported to occur via two mechanisms, one of which involves a single zinc center and one of which involves two zinc centers.[292] In this regard, the mononuclear zinc hydroxide complex $[Tp^{Cum,Me}]ZnOH$ has been shown to react with a four-membered ring lactam (Scheme 15).[78b,122]

In view of the deleterious effects of metallo-β-lactamases, inhibitors are actively being sought in an effort to overcome bacterial resistance to β-lactams.[275] Since most metallo-β-lactamases contain at least one cysteine residue, it has been considered that selective oxidation of these cysteine residues could provide a method for inhibiting β-lactamase activity.[207] In particular, oxidation by disulfides Ar_2S_2 was considered as a means of deactivation on the basis that zinc thiolate complexes of the type $[Tp^{Ph,Me}]ZnSR$ undergo exchange reactions with Ar_2S_2 to give

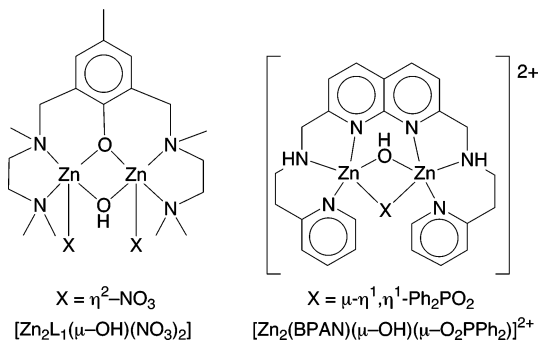
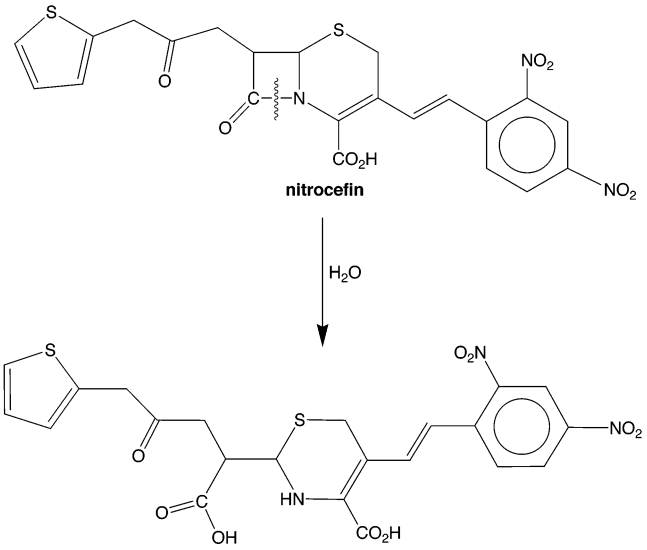
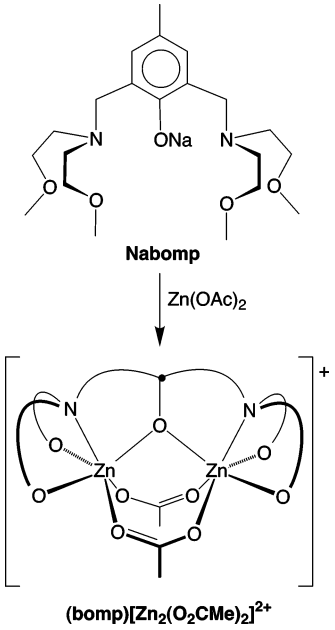


Figure 50. Synthetic analogues for metallo-β-lactamases.

Scheme 70. Nitrocefin as a model substrate for monitoring β-lactamase activity



Scheme 71. Synthesis of $[(bomp)Zn_2(CO_2Me)_2]^+$, a dinuclear zinc complex with bridging acetate ligands that models aspects of aminopeptidases



$[Tp^{Ph,Me}]ZnSAr$ and RSSAr. Thus, several disulfides (Ar_2S_2) have been shown to be irreversible inhibitors of the metallo-β-lactamase CcrA from *Bacteroides fragilis*, and evidence that the mechanism involves oxidation of a zinc thiolate is provided by mass spectrometric data which indicate that the protein experiences an increase in mass corresponding to one-half of the disulfide used, i.e., ArS.[207]

3.2.2. Aminopeptidases

Aminopeptidases are exopeptidases that remove the N-terminal amino acid from proteins (Figure 1). As such, aminopeptidases are counterparts to carboxypeptidase that remove C-terminal amino acids. In contrast to carboxypeptidases, however, aminopeptidases require active sites with *two* zinc centers to achieve such cleavage. The two zinc centers at the active site of aminopeptidases are typically linked by bridging carboxylate ligands, as illustrated in Figure 41. In this respect, the dinuclear zinc complex $[(bomp)Zn_2(CO_2Me)_2]^+$ (Scheme 71) that features bridging acetate ligands has been introduced as a model for aminopeptidases.[280a,293] Although the observed calatlytic activity was low, the aminopeptidase function of $[(bomp)Zn_2(CO_2Me)_2]^+$ was nevertheless established by using *N*-*p*-nitrophenyl-L-leucine as a substrate. A closely related bridging acetate complex $[(bdaip)Zn_2(CO_2Me)_2]^+$, but with five-coordinate zinc

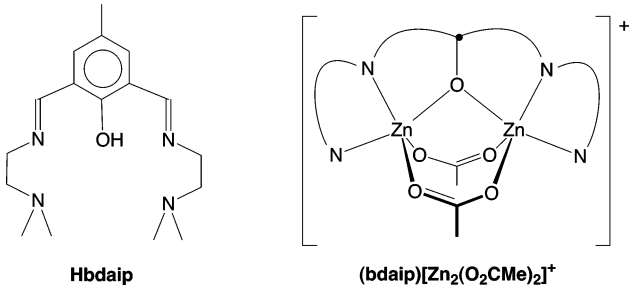


Figure 51. Synthetic analogue for aminopeptidases that features bridging acetate ligands.

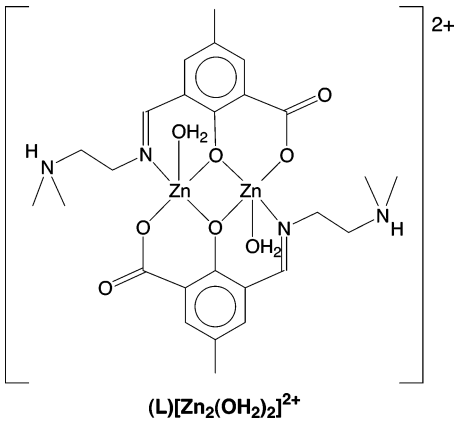


Figure 52. Synthetic analogue for aminopeptidases that features a coordinated water molecule on each zinc center.

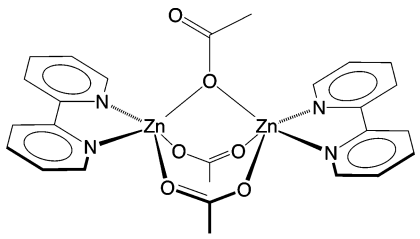


Figure 53. Complex with both unidentate and bidentate bridging acetate ligands as a synthetic analogue for leucine aminopeptidase.

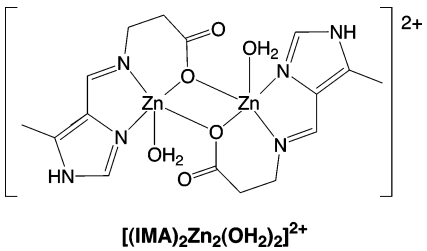
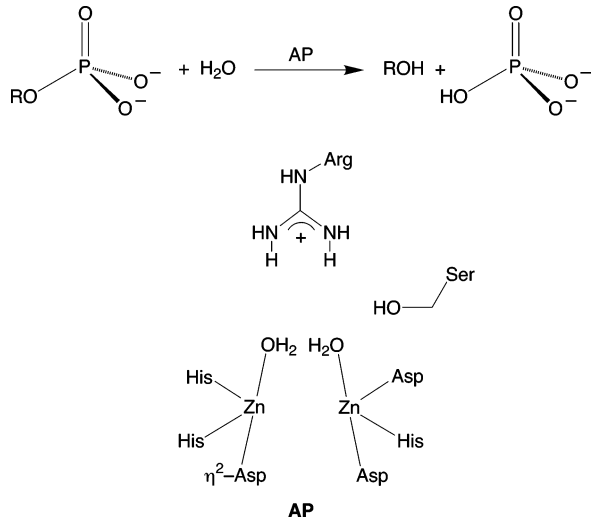


Figure 54. Complex with two unidentate bridging carboxylate ligands.

Scheme 72. Hydrolysis of phosphate monoesters by alkaline phosphatase (AP)



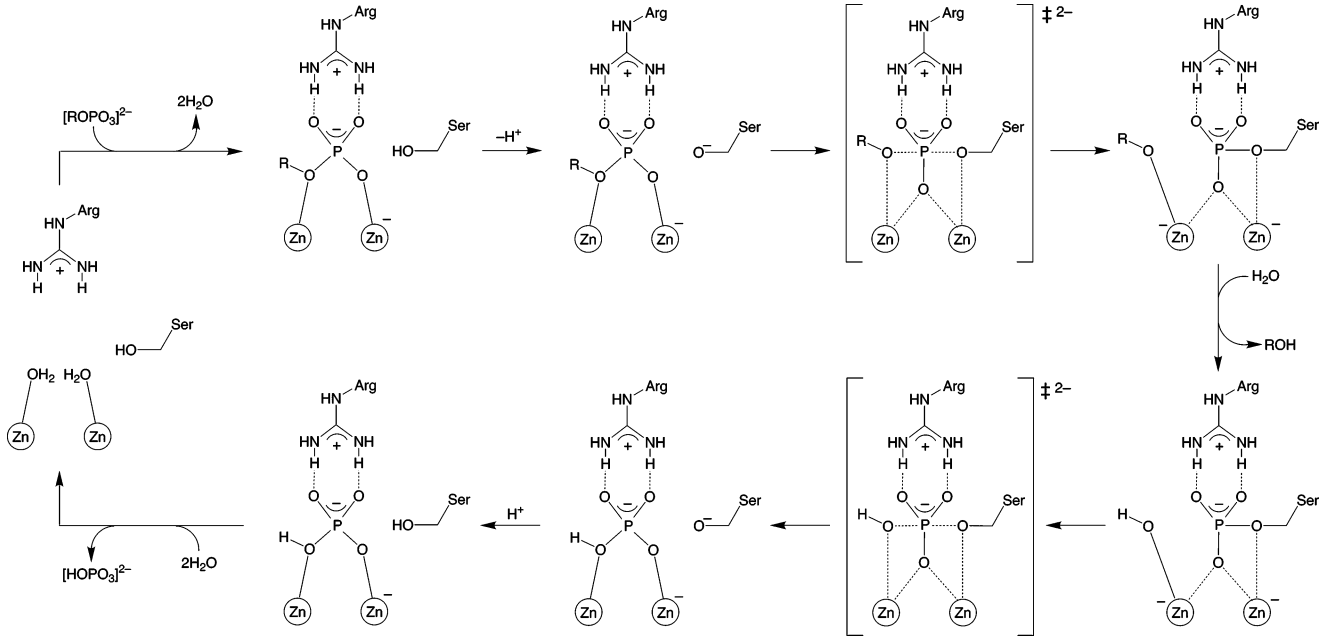
centers, has also been obtained (Figure 51), and is a catalyst for the hydrolysis of glycine ethyl ester.[294] In contrast to the dinuclear nature of the acetate complex $[(bdaip)Zn_2(CO_2Me)_2]^+$, the hydroxide counterpart $[(bdaip)Zn_2(OH)_2]^+$ is polymeric.[295] A dinuclear complex with water molecules on each zinc center, $[LZn_2(OH_2)_2]^{2+}$ (Figure 52), has, however, been obtained using a related phenoxy-carboxylic acid ligand,[296] and catalytic hydrolysis of *p*-nitrophenyl acetate by a dinuclear zinc complex has also been discussed in terms of the mechanism of action of aminopeptidases.[297]

The structure of leucine aminopeptidase exhibits both unidentate and bidentate bridging carboxylate ligands (Figure 41). Unidentate carboxylate coordination to two metal centers is not common, but an example of a zinc complex that shows both types of coordination is provided by $[(bipy)_2Zn_2(\mu\text{-}\eta^1\text{-}O_2CMe)(\mu\text{-}\eta^2\text{-}O_2CMe)_2]^+$ (Figure 53).[298] Furthermore, the tridentate [(4-methyl-5-imidazol-1-yl)methylidene]-β-alanine ligand (IMA) has been used to prepare a dinuclear zinc complex $[(IMA)_2Zn_2(OH_2)_2]^{2+}$ in which the carboxylate donor bridges the two zinc centers in a unidentate fashion (Figure 54).[299] The interconversion of carboxylate coordination modes in tetranuclear zinc complexes $[Zn_4(bdamp)_2(O_2CR)_6]$, where Hdamp = 1,3-bis(dimethylamino)-2-propanol, has been investigated by variable-temperature NMR spectroscopy.[300]

3.2.3. Alkaline Phosphatase

Alkaline phosphatase cleaves phosphate from phosphate monoesters in a nonspecific manner under alkaline conditions (Scheme 72).[24,274,277,301] The essential features of the proposed mechanism of action of alkaline phosphatase are illustrated in Scheme 73. The first stage of the catalytic reaction involves the monophosphate $[ROPO_3]^{2-}$ coordinated to the two zinc centers in an η^2-manner, accompanied by dissociation of water; the two oxygen atoms of $[ROPO_3]^{2-}$ which do not coordinate to the zinc centers interact with an arginine residue via two hydrogen bonds. The phosphorus of the coordinated phosphate ligand is attacked by a Ser-O^- residue in a S_N2 manner, thereby cleaving the P−OR bond and transferring the phosphate group to the enzyme. In this regard, the two zinc centers play several roles. First, by coordinating the OR group, one of the zinc centers activates the P−OR bond towards cleavage. Second, coordination of the Ser-OH group to the other zinc centers serves to facilitate deprotonation which generates the incipient Ser-O^- nucleophile. Once formed, the zinc alkoxide group is hydrolyzed to release ROH and generate a zinc hydroxide species. Subsequent nucleophilic attack of the hydroxide ligand on the phosphoenzyme intermediate cleaves the phosphorus−enzyme bond, thereby forming a bridging phosphate $[HOPO_3]^{2-}$ complex. Displacement of $[HOPO_3]^{2-}$ by water completes the catalytic cycle. This two-step sequence results in overall retention of configuration, a result in accord with the experimental observations.

Scheme 73. Essential features of the mechanism of action of alkaline phosphatase



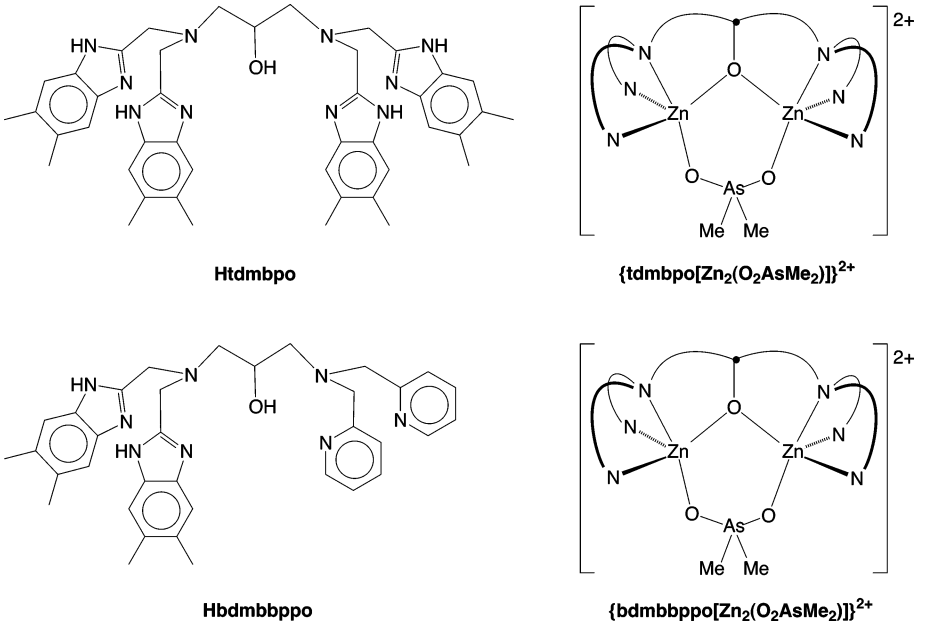
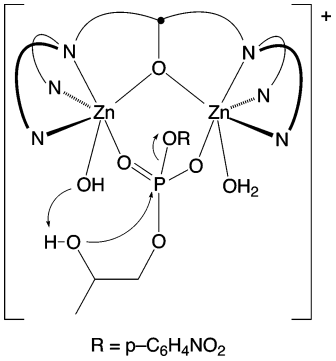
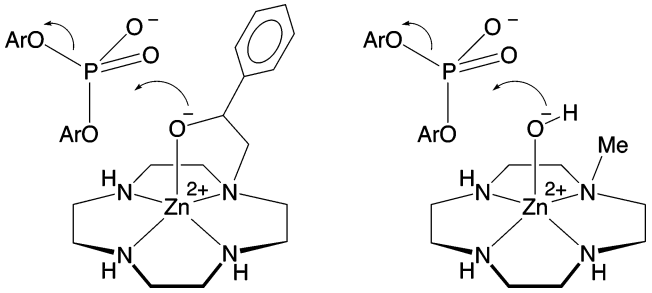


Figure 55. Dinuclear zinc complexes proposed to be synthetic analogues for alkaline phosphatase, phospholipase C, and nuclease P1.

Scheme 74. *In situ* generated $[LZn_2OH]^{2+}$ species are catalysts for the intramolecular transesterification of 2-(hydroxypropyl)-4-nitrophenyl phosphate

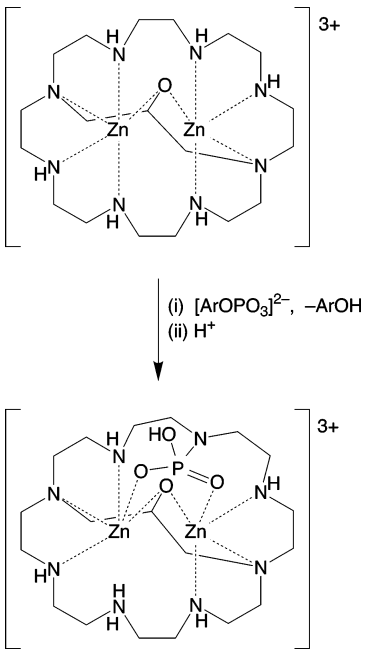


Scheme 75. Nucleophilic reactivity of the zinc alkoxide unit towards $[(ArO)_2PO_2]^-$ (Ar = *p*-$C_6H_4NO_2$) is substantially greater than that of the corresponding hydroxide species



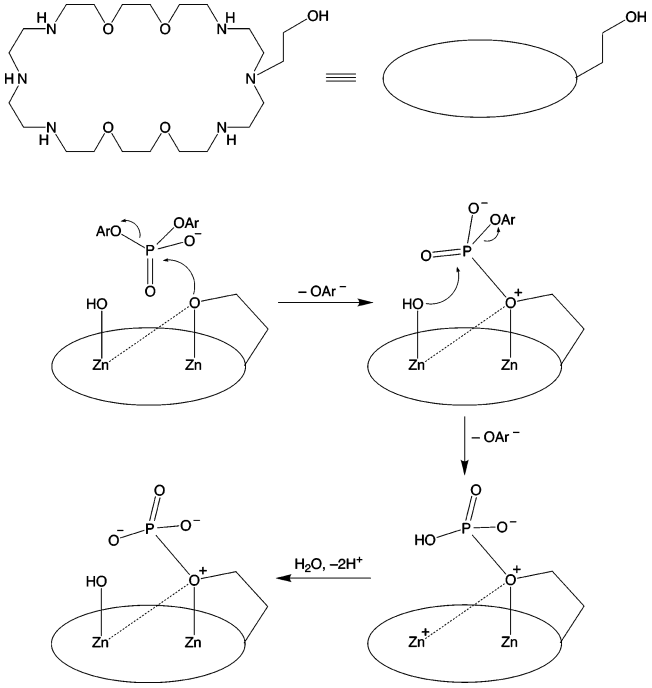
The dinuclear zinc complexes {tdmbpo[Zn_2-(O_2AsMe_2)]}$^{2+}$ and {bdmbbppo[$Zn_2(O_2AsMe_2)$]}$^{2+}$ (Figure 55) have been proposed to serve as models for alkaline phosphatase, phospholipase C, and nuclease P1.[302] Furthermore, *in situ* generated $[LZn_2OH]^{2+}$ are catalysts for the intramolecular transesterification of 2-(hydroxypropyl)-4-nitrophenyl phosphate, and the proposed mechanism is illustrated in Scheme 74.

Scheme 76. Phosphate ester cleavage by a zinc cryptate complex involving reaction with a ligand NH group



A variety of ligands have been used to model aspects of the chemistry of alkaline phosphatase, including studies on mononuclear zinc enzymes. For example, macrocyclic amine complexes with a pendant alkoxide group have been used to mimic the role of the serine residue in alkaline phosphatase.[303] Importantly, this study demonstrated that the nucleophilic reactivity of the zinc alkoxide unit towards $[(ArO)_2PO_2]^-$ (Ar = *p*-$C_6H_4NO_2$) is substantially greater than that of the corresponding hydroxide species (Scheme 75). As an extension of these studies, a cryptate analogue that encapsulates two zinc centers has also been prepared; this complex also reacts with $[ArOPO_3]^{2-}$ (Ar = *p*-$C_6H_4NO_2$), but attack

Scheme 77. Macrocyclic ligand capable of binding two metals with a pendant alkoxide ligand promotes the hydrolysis of $[(ArO)_2PO_2]^-$ (Ar = *p*-$C_6H_4NO_2$) by a mechanism proposed to involve initial nucleophilic attack by the alkoxide ligand followed by nucleophilic attack by hydroxide, analogous to that for alkaline phosphatase



occurs by one of the NH groups rather than by the bridging alkoxide oxygen (Scheme 76).[304]

A more recent development has employed a macrocyclic ligand capable of binding two metals with a pendant alkoxide ligand (Scheme 77).[305] The alcohol group deprotonates with a pK_a value of 6.9, and the alkoxide is proposed to bridge the two zinc centers. The zinc aqua ligand has a pK_a of 8.5, and under alkaline conditions the complex provides a system in which two zinc centers have alkoxide and hydroxide ligands. The complex promotes the hydrolysis of $[(ArO)_2PO_2]^-$ (Ar = *p*-$C_6H_4NO_2$) (Scheme 77), and the mechanism which has been proposed involves a two-step sequence comprising initial nucleophilic attack by the alkoxide ligand followed by nucleophilic attack by hydroxide. As such, the proposed mechanism has a close similarity to the mechanism of action of alkaline phosphatase.

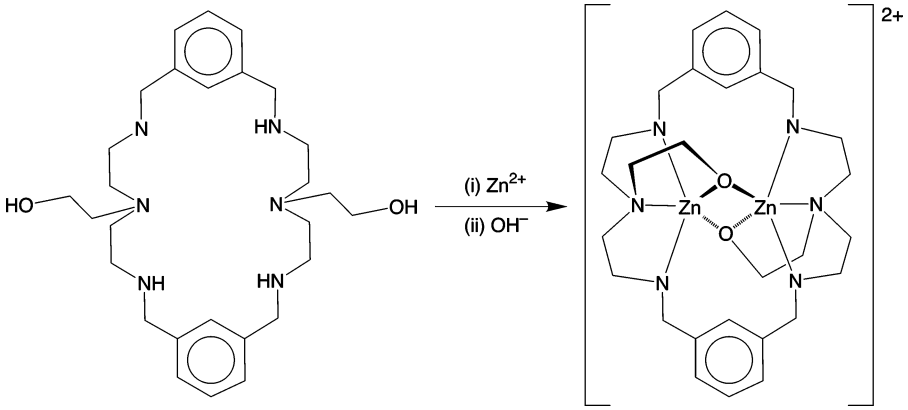
An extension of the above investigation incorporates two pendant alkoxide groups, as illustrated in Scheme 78.[306] The zinc complex is not an effective catalyst for hydrolysis of phosphate monoesters but is a catalyst for the hydrolysis of *p*-nitrophenyl acetate. The corresponding system devoid of the pendant alcohol groups has also been investigated and has allowed the isolation of a dinuclear zinc complex $[LZn_2\{\mu\text{-}O_2P(OPh)_2\}_2(MeOH)_2]^{2+}$ with bridging phosphate ester ligands (Scheme 79),[307] an observation that is of relevance to the fact that coordination of this type has been postulated to play a role in phosphate ester activation and hydrolysis. The bridging hydroxide complex $[LZn_2OH]^{3+}$ has also been isolated and structurally characterized by X-ray diffraction. Both the monohydroxide $[LZn_2OH]^{3+}$ and the dihydroxide $[LZn_2(OH)_2]^{3+}$ are catalysts for the hydrolysis of $MeCO_2Ar$ (Ar = *p*-$C_6H_4NO_2$).

The reactivity of several mononuclear zinc complexes towards phosphates has been used to model aspects of phosphatase chemistry; for example, $[Tp^{RR'}]ZnOH$ derivatives,[78b,d,f,127,308] $[\{N(CH_2C_5H_4N)_3Zn(\mu\text{-}OH)\}_2]^{2+}$,[309] simple amino acid derivatives,[310] and other complexes[311] have been studied in this respect. As an illustration, the reactivity of $[Tp^{Pri_2}]ZnOH$ towards a series of phosphate esters, $(ArO)_xP(O)(OH)_{3-x}$ (Ar = *p*-$C_6H_4NO_2$; $x = 1-3$) has been investigated, as illustrated in Scheme 80.[78f] Thus, (i) $[Tp^{Pri_2}]ZnOH$ reacts with the monoester $(ArO)P(O)(OH)_2$ to give $\{[Tp^{Pri_2}]ZnO\}_2P(O)(OAr)$, presumably via $[Tp^{Pri_2}]ZnOP(O)(OAr)(OH)$; (ii) $[Tp^{Pri_2}]ZnOH$ reacts with 1 equiv of the diester $(ArO)_2P(O)(OH)$ to give $[Tp^{Pri_2}]ZnOP(O)(OAr)_2$, but in the presence of excess $[Tp^{Pri_2}]ZnOH$ further reaction occurs to give $\{[Tp^{Pri_2}]ZnO\}_2P(O)(OAr)$; and (iii) $[Tp^{Pri_2}]ZnOH$ reacts with 1 equiv of the triester $(ArO)_3P(O)$ to give $[Tp^{Pri_2}]ZnOAr$ and $(ArO)_2P(O)(OH)$. The related hydroxide complex $[Tp^{Cum,Me}]ZnOH$ has also been reported to react with the less reactive aliphatic phosphate $H(MeO)_2P(O)$ and diphosphates $[(RO)_2P(O)]_2O$ (R = Et, Ph) to give $[Tp^{Cum,Me}]ZnOP(O)(OMe)H$ and $[Tp^{Cum,Me}]ZnOP(O)(OR)_2$, respectively (Scheme 81).[78b]

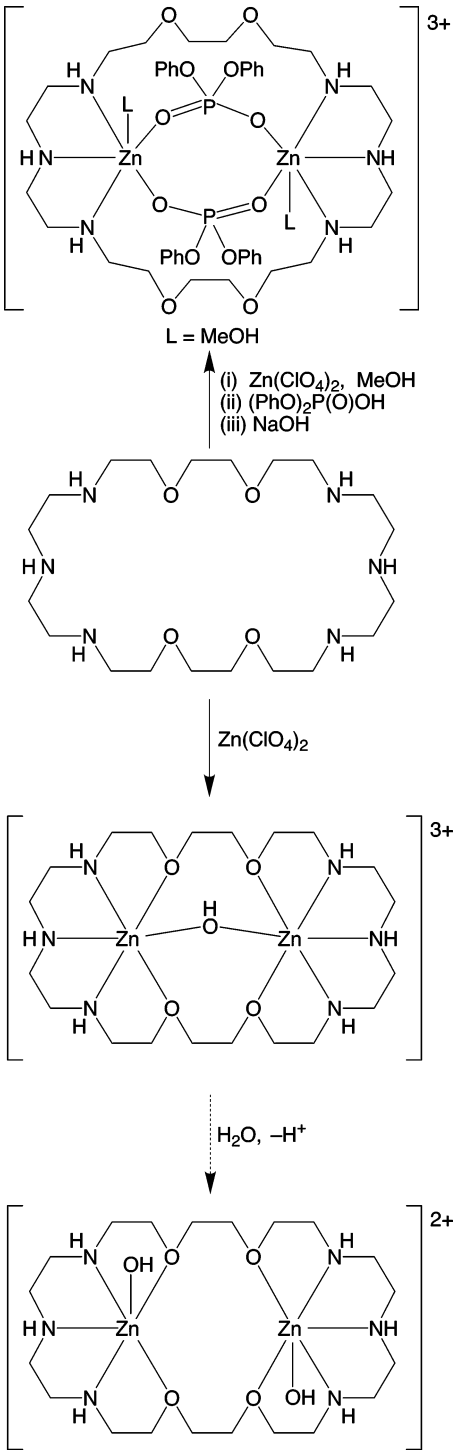
3.2.4. Purple Acid Phosphatase

Purple acid phosphatases (PAPs) are counterparts to alkaline phosphatase in the sense that they are

Scheme 78. Macrocyclic ligand with two pendant alkoxide groups

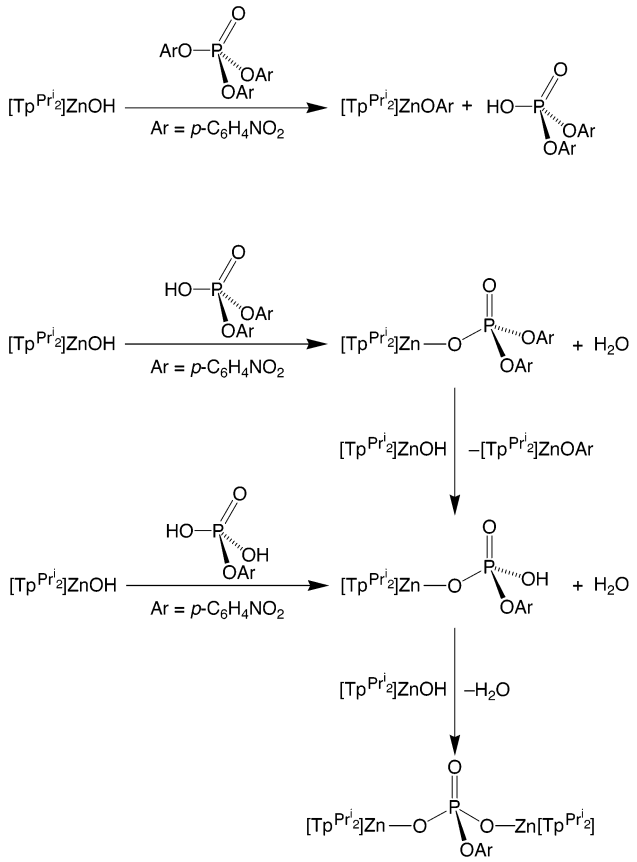


Scheme 79. Synthesis of dinuclear hydroxide and aqua complexes employing a macrocyclic ligand

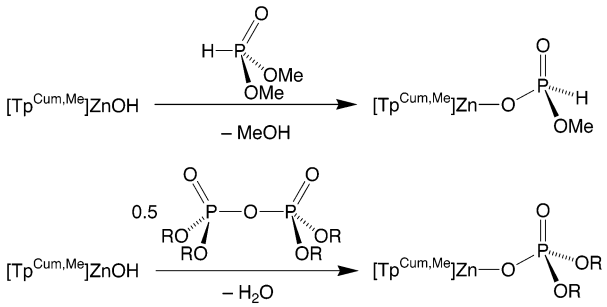


responsible for hydrolyzing phosphate monoesters at low pH.[24,274,312] The purple color of these enzymes is associated with a tyrosine→Fe^{III} charge-transfer absorption. The active form of mammalian PAP is a dinuclear Fe^{II}—Fe^{III} enzyme in its active form, while the divalent site of kidney bean PAP is occupied by zinc. The active site of kidney bean PAP is illustrated in Figure 56, which indicates that the Zn^{II} and Fe^{III} centers are bridged by a hydroxide ligand and a unidentate aspartate ligand; the iron center is also postulated to possess a terminal hydroxide ligand, while the zinc bears an aqua ligand.[313]

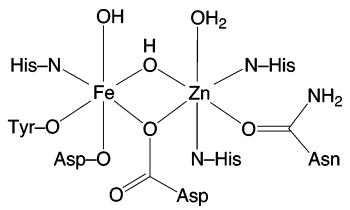
Scheme 80. Reactivity of [$Tp^{Pr^i_2}$]ZnOH towards a series of phosphate esters, $(ArO)_xP(O)(OH)_{3-x}$ (Ar = *p*-$C_6H_4NO_2$; *x* = 1 − 3)



Scheme 81. Reactivity of [$Tp^{Cum,Me}$]ZnOH towards $H(MeO)_2P(O)$ and $[(RO)_2P(O)]_2O$ (R = Et, Ph)



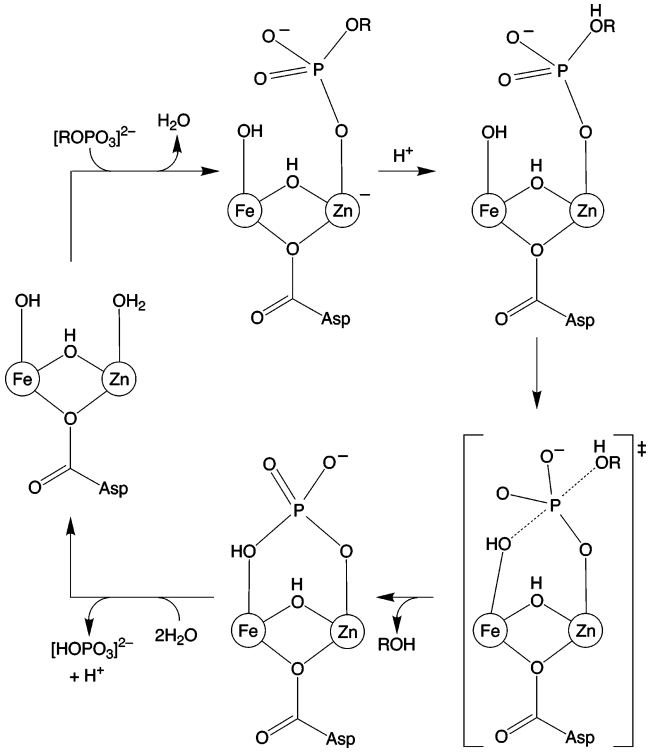
The mechanism of action proposed for kidney bean PAP involves coordination of the alkyl phosphate ligand $[ROPO_3]^{2-}$ to the zinc center via one of the oxo groups which serves to orient the substrate for in-line nucleophilic attack by the Fe^{III}—OH ligand (Scheme 82).[313a] Other variants of this mechanism have also been considered.[278k] For example, a mech-



kidney bean purple acid phosphatase

Figure 56. Active site of kidney bean purple acid phosphatase.

Scheme 82. Essential features of the mechanism of action of kidney bean purple acid phosphatase



anism that is closely related to the mechanism involving direct nucleophilic attack of the Fe^{III}−OH ligand at phosphorus (Figure 57A) is one in which the terminal iron hydroxide ligand serves as a general base to deprotonate a second water molecule and thereby generate an incipient hydroxide ion which attacks the zinc-bound phosphate ester (Figure 57B).[314] However, a very different type of mechanism that involves nucleophilic attack by the *bridging* hydroxide ligand has also been proposed. Specifically, it has been postulated that the phosphate ester bridges the two metals and that the phosphorus of the bridging phosphate ester is attacked by the bridging hydroxide ligand (Figure 57C).[315] Arguing against this mechanism, however, is the fact that a bridging hydroxide ligand has a much lower nucleophilicity than that of a terminal hydroxide ligand. It has, therefore, been suggested that this mechanism first requires the bridging hydroxide ligand to shift towards the Zn^{II} center and become a "quasi-terminal" and more nucleophilic moiety.[315]

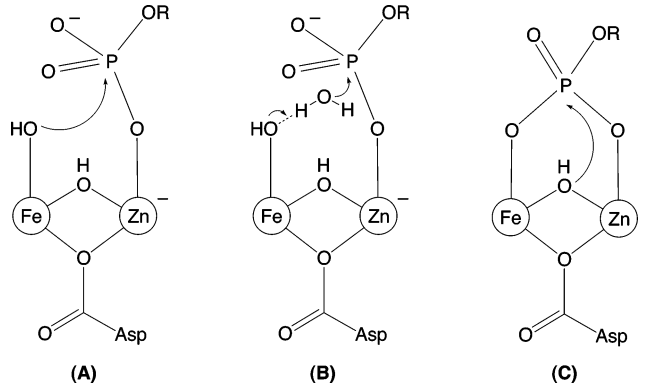


Figure 57. Other variants of the mechanism of action of kidney bean purple acid phosphatase that have been discussed: (A) direct nucleophilic attack of the Fe^{III}−OH ligand at phosphorus; (B) the terminal iron hydroxide ligand serves as a general base to deprotonate a second water molecule and thereby generate an incipient hydroxide ion which attacks the zinc-bound phosphate ester; and (C) nucleophilic attack by the *bridging* hydroxide ligand.

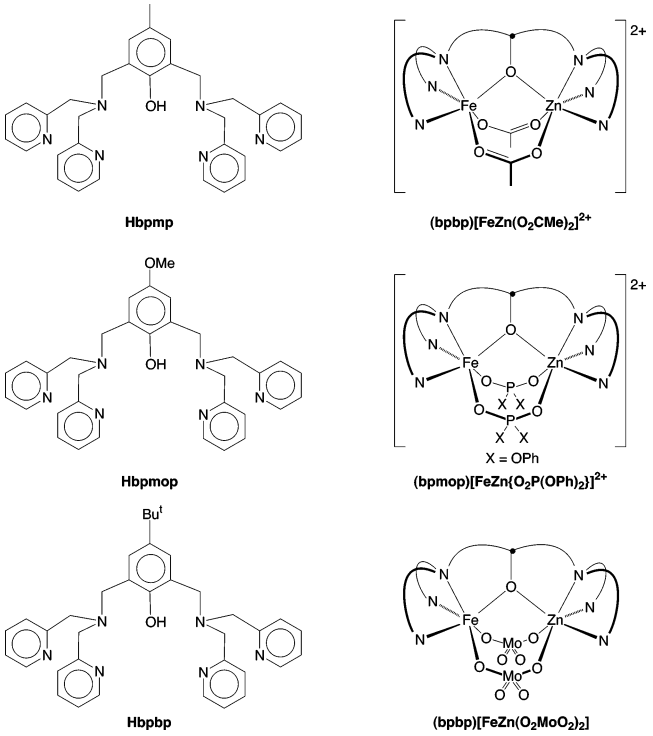


Figure 58. Synthetic analogues of kidney bean purple acid phosphatase that feature symmetric bridging phenolate ligands with pyridine donors.

Regardless of whether the attack at phosphorus proceeds via A, B, or C (Figure 57), each mechanism predicts inversion of configuration at phosphorus, a result that is in accord with experiments employing the diiron form of the enzyme.[316,317] As such, the mechanism of action of PAP is markedly different from that of alkaline phosphatase for which overall retention of configuration is observed.

Considerable attention has been directed towards the synthesis of dinuclear Fe^{III}/Zn^{II} complexes to mimic the active site of kidney bean purple acid phosphatase and examples of some complexes that have been structurally characterized are illustrated in Figures 58−61.[302,318] In addition to employing symmetric ligands, asymmetric ligands that incorporate oxygen donors have also been investigated in an effort to reflect the asymmetry of the Fe^{III} and Zn^{II} coordination environments in kidney bean purple acid phosphatase. Of particular note, both $L^1[FeZn\{O_2P(OPh)_2\}]$[318e] and $\{bpbpmp[FeZn(O_2CMe)_2]\}^+$ [318f] exhibit an oxygen-rich coordination environment about Fe^{III} (Figure 59); furthermore, the terminal $Fe-O_{phenolate}$ ligands resemble the $Fe-O_{Tyr}$ interaction in the enzyme. Evidence has been presented that aqueous solutions of $\{bpbpmp[FeZn(O_2CMe)_2]\}^+$ undergo dissociation of the acetate ligands and generate species proposed to have a composition of the type $[Fe^{III}(OH)Zn^{II}(OH_2)]$ that are catalysts for the

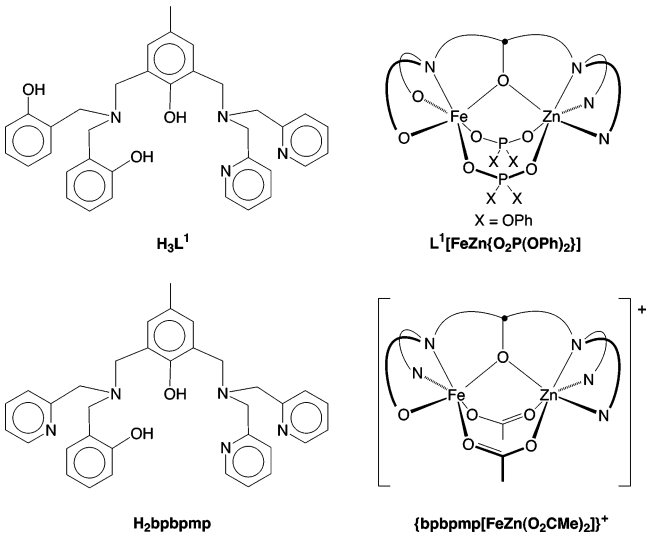


Figure 59. Synthetic analogues of kidney bean purple acid phosphatase that feature asymmetric bridging phenolate ligands with nitrogen and oxygen donors.

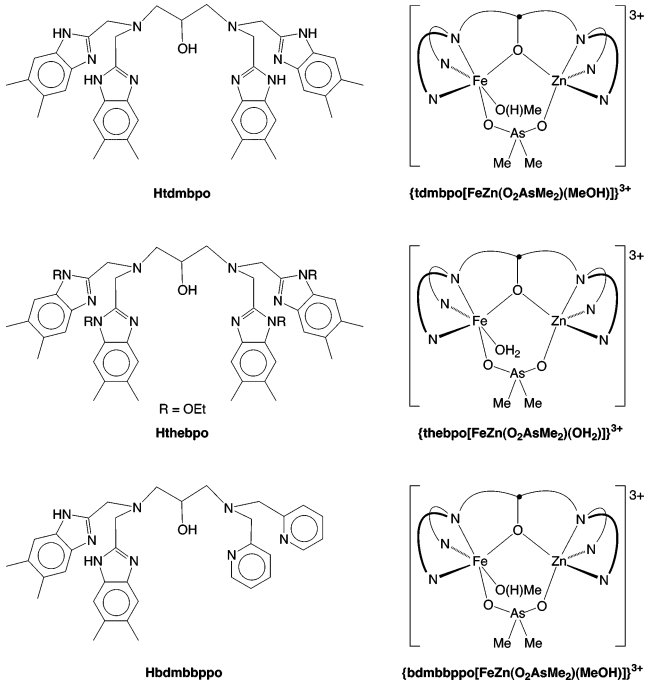


Figure 60. Synthetic analogues of kidney bean purple acid phosphatase employing bridging alkoxide ligands.

hydrolysis of the phosphate diester 2,4-bis(dinitrophenyl)phosphate.[318f]

$\{L[FeZn(O_2CMe)_3]\}^+$ (Figure 61) is a catalyst for the hydrolysis of $(ArO)_3PO$ and $(ArO)_2P(O)OH$ (Ar = p-$C_6H_4NO_2$) in aqueous DMF.[318h] The ability of $\{L[FeZn(O_2CMe)_3]\}^+$ to hydrolyze $(ArO)_2P(O)OH$ is in marked contrast to the corresponding homonuclear dizinc complex which is an ineffective catalyst for the transformation.

Studies to model aspects of purple acid phosphatase have also focused on the diiron form of the enzyme.[319,320] Likewise, the chemistry of related dizinc[278k] and dicobalt[321] complexes have been investigated, with studies on the latter providing evidence for the feasibility that phosphate hydrolysis

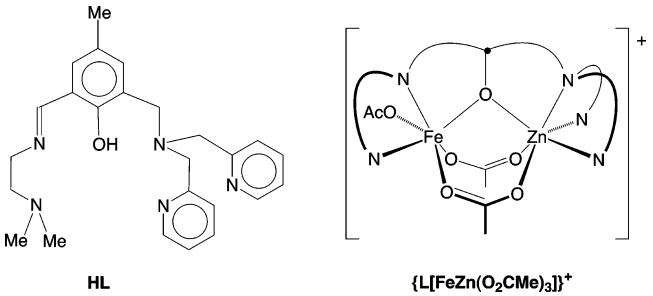


Figure 61. Synthetic analogues of kidney bean purple acid phosphatase that is a catalyst for hydrolysis of $(ArO)_3PO$ and $(ArO)_2P(O)OH$ (Ar = p-$C_6H_4NO_2$).

could involve attack on phosphorus by a bridging oxo group.

3.2.5. Nuclease P1 and Phospholipase C

Nuclease P1 and phosphlipase C are two structurally related zinc enzymes (Figure 42). Nuclease P1 is a diesterase that catalyzes the hydrolysis of single-stranded DNA and RNA; furthermore, it is also a monoesterase for hydrolysis of the 5′-terminal phosphate of the initially cleaved oligonucleotide fragment (Figure 2).[24,278,322] The active site of the enzyme features three zinc centers (Figure 42), and evidence for the importance of such a motif is provided by the observation that a trinuclear zinc complex is more active with respect to hydrolysis of diribonucleotides than related dinuclear and mononuclear complexes (Figure 62).[323,324] Likewise, calixarene derivatives designed to bind one, two, and three zinc centers (Figure 63) exhibit increasing catalytic activity towards cleavage of 2-hydroxypropyl-p-nitrophenyl phosphate as a model substrate for RNA (Scheme 83).[325]

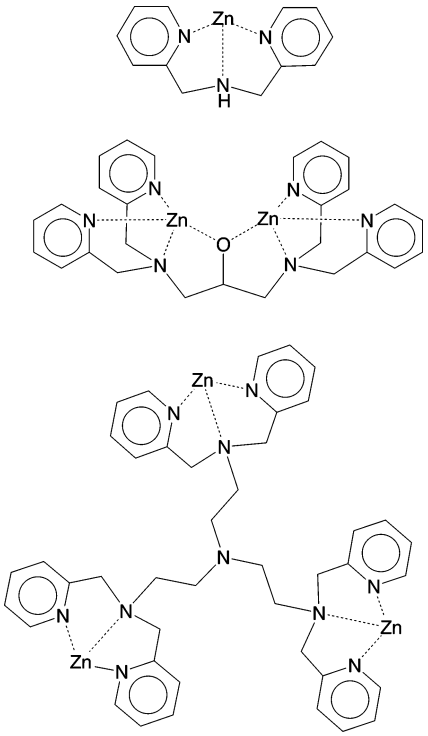


Figure 62. Trinuclear complex (bottom) that is more active than dinuclear and mononuclear counterparts with respect to hydrolysis of diribonucleotides.

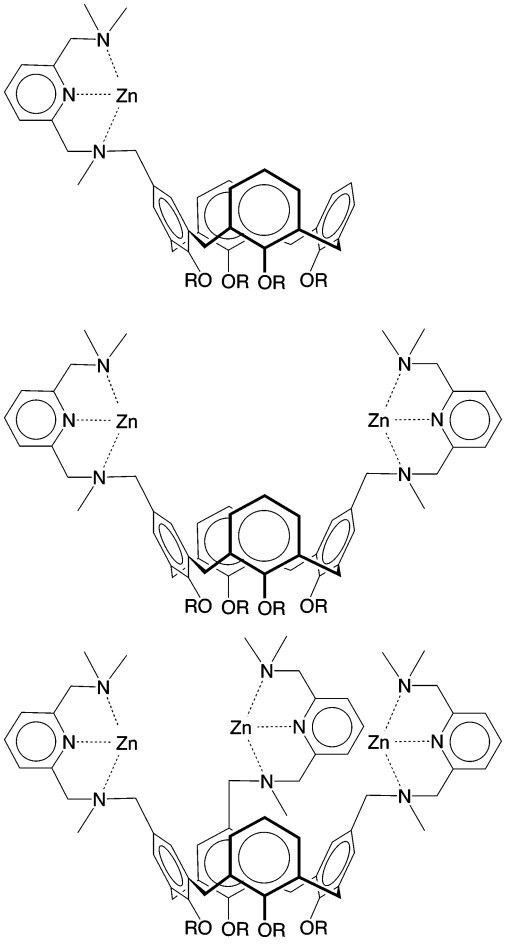
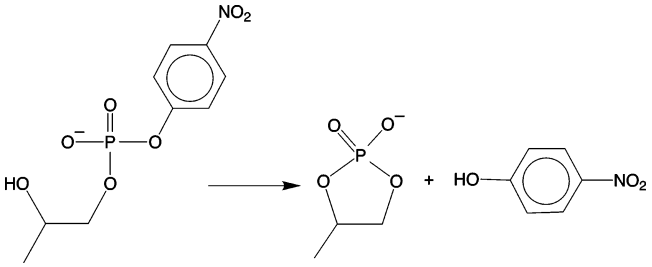


Figure 63. Mono-, di-, and trinuclear calixarene complexes that exhibit increased catalytic activity towards cleavage of 2-hydroxypropyl-*p*-nitrophenyl phosphate as a function of nuclearity.

Scheme 83. Transesterification of 2-hydroxypropyl-*p*-nitrophenyl phosphate as a model substrate for RNA

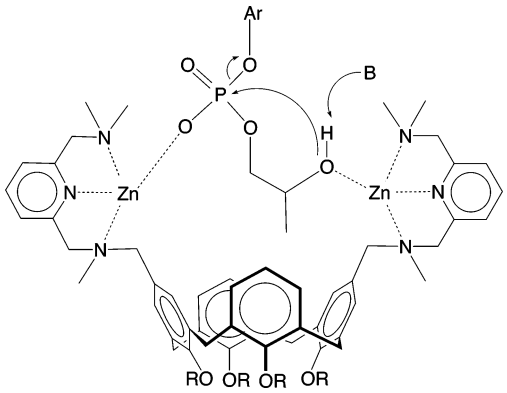


A possible bifunctional mechanism to rationalize the rate enhancement for the dinuclear system is illustrated in Scheme 84.

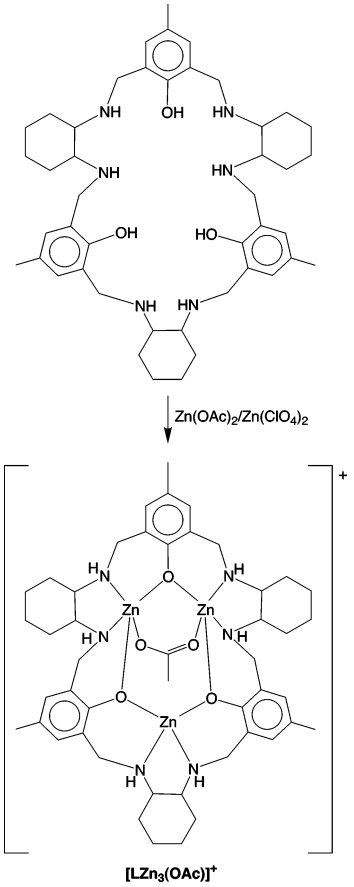
A structural model for the trizinc moiety of nuclease P1 that also features a bridging acetate ligand has been obtained by the reaction of a macrocyclic hexaamino triphenolate ligand with a mixture of $Zn(OAc)_2$ and $Zn(ClO_4)_2$, as illustrated in Scheme 85.[326]

In addition to the application of ligands that coordinate three zinc centers, other systems have also been investigated with respect to providing information pertaining to nuclease activity catalyzed by zinc centers.[327] For example, the tris(2-pyridylmethyl)-amine complex $[(TPA)Zn(OH_2)]^{2+}$ reacts with the phosphate diester $(ArO)_2P(O)OH$ ($Ar = p\text{-}C_6H_4NO_2$) in the presence of Et_3N to give $[(TPA)ZnOP(O)(OAr)_2]^+$.[327a]

Scheme 84. A possible bifunctional mechanism to rationalize the rate enhancement of the cleavage of 2-hydroxypropyl-*p*-nitrophenyl phosphate as a model substrate for RNA by a dinuclear system



Scheme 85. Synthesis of a structural model for the trizinc moiety of nuclease P1 that also features a bridging acetate ligand



In addition to being structurally similar to nuclease P1, phospholipase C is also a diesterase, but with the function of cleaving phospholipids rather than DNA and RNA. Consequently, many of the studies described above for nuclease P1 are also of direct relevance to phosphlipase C, but the dinuclear complex $\{bbap[Zn_2(\mu\text{-}O_2CMe)(OH_2)]\}^{2+}$ (Figure 64), which also possesses a coordinated water molecule, has been

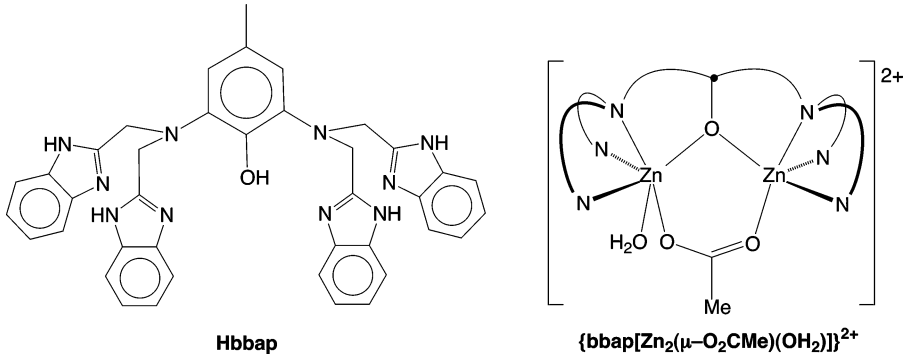


Figure 64. $\{bbap[Zn_2(\mu\text{-}O_2CMe)(OH_2)]\}^{2+}$, a dinuclear complex with a coordinated water molecule that has been proposed as a structural model for phosphlipase C.

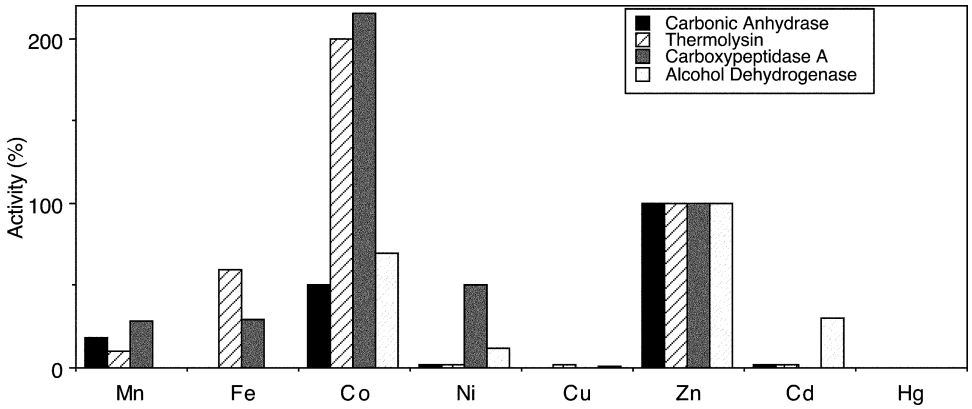


Figure 65. Relative catalytic activity of metal-substituted zinc enzymes (carbonic anhydrase, thermolysin, carboxypeptidase A, and alcohol dehydrogenase).

specifically proposed as a structural model for phosphlipase C.[328]

4. Metal Ion Substitution as a Probe of Structure and Mechanism of Action of Zinc Enzymes

Considerable effort has been directed towards investigating enzymes in which the zinc has been replaced by various other metals.[136a,329−340] There are several purposes for such investigations. First, the diamagnetic d^{10} Zn^{II} center of the native enzymes offers little in terms of a spectroscopic probe, with neither electronic, ESR, nor ^{67}Zn NMR spectroscopies being useful[341] (although recent studies indicate significant advances in the application of ^{67}Zn NMR spectroscopy). As a result of the poor spectroscopic properties of Zn^{II}, it is difficult to obtain information pertaining to the structure of the active site and the nature of intermediates. Substitution by divalent metal ions which have useful spectroscopic probes, such as Co^{II} (UV−vis)[342] and Cd^{II} (NMR), enables structural information of the metal-substituted carbonic anhydrase active site to be obtained and thereby provides an important reason to study metal-substituted enzymes. For example, studies on Co^{II}−carbonic anhydrase provided evidence for five-coordinate intermediates in the catalytic mechanism of carbonic anhydrase.[343]

A second reason for studying metal-substituted enzymes is concerned with the fact that such derivatives often exhibit markedly different activities (Figure 65), and understanding the origin of these differences provides information concerned with details of the mechanisms of action. Cobalt is the only element for which significant activity is maintained, but of most interest, not all enzymes are influenced in the same manner; thus, for carbonic anhydrase and alcohol dehydrogenase the Co^{II}−enzyme is ca. 50−70% less active than the native enzyme, whereas for thermolysin and carboxypeptidase A, the Co^{II} derivatives are substantially more active than the native enzyme by a factor of ca. 2. The use of metal ion substitution to provide insight into the structures and mechanisms of action of zinc enzymes is critically dependent on a knowledge of the chemistry of the various ions in coordination environments that are similar to those of the enzyme active sites. For this reason, it has been of importance to compare the chemistry of a variety of metals in coordination environments related to those of zinc enzymes.

Finally, a third reason for studying metal-substituted zinc enzymes pertains to the fact that other metals have been shown to play a role in enzymes that resemble zinc enzymes. Thus, a recent impetus to study the chemistry of cadmium in biologically relevant coordination spheres is provided by the discovery of the first cadmium-specific enzyme.[344] Specifically, in conditions where zinc is scarce, the marine diatom *Thalassiosira weissflogii* synthesizes a *cadmium* carbonic anhydrase. Furthermore, peptide deformylase, which was originally proposed to be a zinc enzyme, is now recognized to be an *iron* enzyme.[165−167]

4.1. Influence of the Metal on the Structure and Composition of Synthetic Analogues

Cobalt and cadmium are two of the metals that are most commonly substituted into "spectroscopically silent" zinc enzymes to provide structural information. Thus, Co^{II} is typically employed because it has distinct electronic spectroscopic properties and also has a pronounced tendency to form tetrahedral complexes, while Cd^{II} is employed because of the accessibility of ^{113}Cd NMR spectroscopy. Detailed structural studies, however, on a series of closely related metal complexes indicate that substitution of zinc in enzymes by other metals is actually likely to have a significant impact on the structure of the active site,[345,346] as has also been demonstrated for certain zinc enzymes, e.g., thermolysin[136a] and carbonic anhydrase.[329a] Consideration of the basic structural preferences for different M^{II} ions indicate that such structural changes are not to be unexpected and that the active-site structures of metal-substituted zinc enzymes need not be the same as those of the native zinc enzymes themselves. For example, of the carbonic anhydrase synthetic analogues $\{[Tp^{Pri_2}]M(\mu-OH)\}_n$, the zinc complex $[Tp^{Pri_2}]ZnOH$ exists as a tetrahedral terminal hydroxide derivative, whereas the manganese, iron, cobalt, nickel, and copper derivatives exist as five-coordinate dinuclear complexes with bridging hydroxide ligands, $\{[Tp^{Pri_2}]M(\mu\text{-}OH)\}_2$ (M = Mn, Fe, Co, Ni, Cu) (Figure 66).[73d] A cadmium hydroxide complex of composition "$[Tp^{Me_2}]CdOH\cdot H_2O$" has been reported to be obtained as an intractable material from the reactions of both $(Et_2NCH_2CH_2NEt_2)CdCl_2$ and $(Ph_3P)_2CdCl_2$ with $K[Tp^{Me_2}]$ in acetone and is not well characterized.[347] Bulkier substituents are, however, capable of stabilizing tetrahedral terminal hydroxide ligands of iron and cobalt, namely, $[Tp^{But,Pri}]FeOH$,[348] $[Tp^{But,Me}]CoOH$,[349] and $[Tp^{But,Pri}]CuOH$.[350] Thus, given a sufficiently sterically demanding environment, a series of structurally related terminal hydroxide analogues corresponding to the active site of metal-substituted zinc enzymes may be obtained.

Comparison of the zinc and cadmium complexes $\{[Pim^{Pri,But}]ZnOH\}^+$ and $\{[Pim^{Pri,But}]Cd(OH_2)(OClO_3)\}^+$ (Figure 67), obtained by the reactions of $[Pim^{Pri,But}]$ with $M(ClO_4)_2\cdot 6H_2O$,[351] provides a particularly instructive example of how metal ion substitution may perturb the structure of the active site of a zinc enzyme. Thus, whereas the zinc complex exists as a simple tetrahedral hydroxide derivative, the cadmium counterpart is a *five*-coordinate *aqua* complex. The two complexes thus differ not only in coordination number but also in the protonation state of the hydroxide/aqua ligand. The higher coordination number of the metal center in $\{[Pim^{Pri,But}]Cd(OH_2)(OClO_3)\}^+$ is undoubtedly a reflection of the larger size of cadmium versus zinc, but the observation that a ligand as weakly coordinating as perchlorate binds is of particular note since it indicates that biologically more pertinent anions should also coordinate to a tetrahedral $[\{N_3\}Cd^{II}(OH_2)]$ center. As such, it suggests that Cd^{II}–carbonic anhydrase may not be tetrahedral.

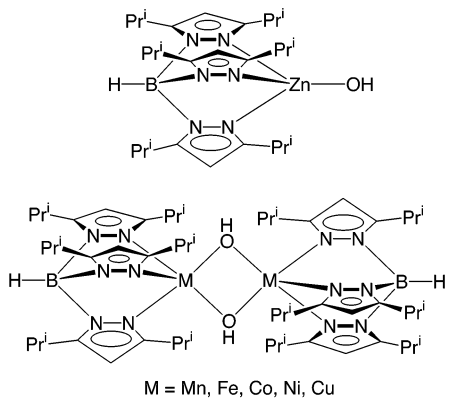


Figure 66. Mononuclear and dinuclear metal hydroxides as a function of metal.

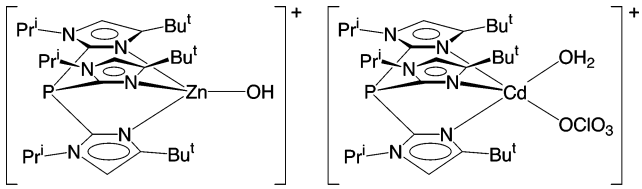
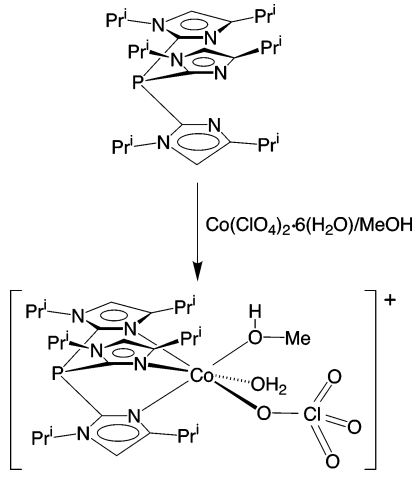


Figure 67. Comparison of the structures of $\{[Pim^{Pri,But}]ZnOH\}^+$ and $\{[Pim^{Pri,But}]Cd(OH_2)(OClO_3)\}^+$. The cadmium complex exists as a four-coordinate hydroxide, whereas the cadmium counterpart is five-coordinate with a coordinated water molecule.

The structure of $\{[Pim^{Pri,But}]Cd(OH_2)(OClO_3)\}^+$ provides a rationalization for the reduced activity of Cd^{II}–carbonic anhydrase. Specifically, since the acidity of a coordinated water molecule is reduced considerably upon the binding of an anionic ligand,[99,100] the pK_a of the monocation $\{[Pim^{Pri,But}]Cd(OH_2)(OClO_3)\}^+$ would be expected to be greater than that of dicationic $\{[Pim^{Pri,But}]Zn(OH_2)\}^{2+}$. As a result of more facile deprotonation of $\{[Pim^{Pri,But}]Zn(OH_2)\}^{2+}$, a hydroxide complex is isolated for the zinc system, whereas an aqua complex is isolated for the cadmium system. In accord with this observation, Cd^{II}–carbonic anhydrase only exhibits significant activity at higher pH which is necessary to generate the requisite hydroxide species; the pK_a of the metal-bound water molecule of Cd^{II}–CA is ~9, compared to a value of ~7 for the zinc enzyme. Furthermore, comparison of a pair of zinc and cadmium complexes of a macrocyclic oxo polyamine ligand indicates that cadmium and zinc complexes have different coordination geometries and that the cadmium complex has a higher pK_a (10.6) than that of the zinc counterpart (7.3).[352]

Although a four-coordinate aqua complex $\{[Pim^{Pri,But}]Cd(OH_2)\}^{2+}$ was not isolated for the $[Pim^{Pri,But}]$ system, a four-coordinate aqua complex $[(TriMIm)Cd(OH_2)][BF_4]_2$ was prepared using a cavitand ligand and is structurally analogous to the zinc complex $[(TriMIm)Zn(OH_2)]^{2+}$ (Figure 12).[92] It is particularly noteworthy that the $[BF_4]^-$ counterion does not coordinate to the cadmium center in $[(TriMIm)Cd(OH_2)]^{2+}$, whereas it does coordinate in $\{[Pim^{Pri,But}]Cd(OH_2)(OClO_3)\}^+$. It is also interesting that the $Cd-OH_2$ bond distance in four-coordinate $[(TriMIm)Cd(OH_2)]^{2+}$ [2.340(2) Å] is intermediate

Scheme 86. Synthesis of an aqua−methanol−perchlorate complex of cobalt, $\{[Pim^{Pr^i_2}]Co(OH_2)(HOMe)(OClO_3)\}^+$



between that in five-coordinate $\{[Pim^{Pr^i,Bu^t}]Cd(OH_2)(OClO_3)\}^+$ [2.297(9) Å] and six-coordinate tris(pyridyl)amine complex $\{(TPA)Cd(OH_2)(O_2NO)\}^+$ [2.41(1) Å].[353]

A further simple illustration of how the coordination geometry preferences of cadmium are different from those of zinc is provided by the fact that $[Bp^{Bu^t,R}]ZnI$ is a monomer, whereas cadmium analogue $\{[Bp^{Bu^t,R}]Cd(\mu\text{-}I)\}_2$ is a halide-bridged dimer.[354] Likewise, $[PhTt^{Bu^t}]ZnBr$ is a monomer, whereas $[PhTt^{Bu^t}]Cd(\mu\text{-}Cl)\}_2$ is a dimer.[200]

A related cobalt system also indicates a reluctance of cobalt to form simple tetrahedral cobalt hydroxide complexes. Thus, reaction of $[Pim^{Pr^i_2}]$ with $Co(ClO_4)_2 \cdot 6H_2O$ in methanol yields the *six*-coordinate aqua−methanol−perchlorate complex $\{[Pim^{Pr^i_2}]Co(OH_2)(HOMe)(OClO_3)\}^+$ (Scheme 86).[355] Interestingly, the methanol and water ligands in $\{[Pim^{Pr^i_2}]Co(OH_2)(HOMe)(OClO_3)\}^+$ are not bound strongly to cobalt and dissociate to yield the tetrahedral perchlorate complex $\{[Pim^{Pr^i_2}]Co(OClO_3)\}^+$.

Significant differences are also observed in the tendencies of various metals to form complexes with tetrahedral $M[S_4]$ coordination akin to the $[(Cys)_4Zn]$ structural sites in enzymes such as LADH and the active site of the Ada DNA repair protein. For example, examination of the structures of a series of bis(2-mercapto-1-methylimidazolyl)(pyrazolyl)hydroborato derivatives $[pzBm^{Me}]_2Zn$, $[pzBm^{Me}]_2Co$, and $[pzBm^{Me}]_2Cd$ indicates that the zinc exhibits a greater preference to adopt tetrahedral $M[S_4]$ coordination geometries. Thus, while $[pzBm^{Me}]_2Zn$ exhibits a tetrahedral structure, the cobalt derivative exhibits a trigonal bipyramidal $Co[S_3NH]$ structure in which one of the pyrazolyl groups and one of the B−H groups coordinate to cobalt, while the cadmium complex exhibits a six-coordinate $Cd[S_4H_2]$ structure in which both B−H groups interact with the cadmium center (Figure 68). These comparisons clearly emphasize that zinc has a greater preference for tetrahedral $M[S_4]$ coordination than does either cobalt or cadmium, an observation that is in accord with the prevalent role of zinc in the structural sites of enzymes. Furthermore, the fact that the zinc

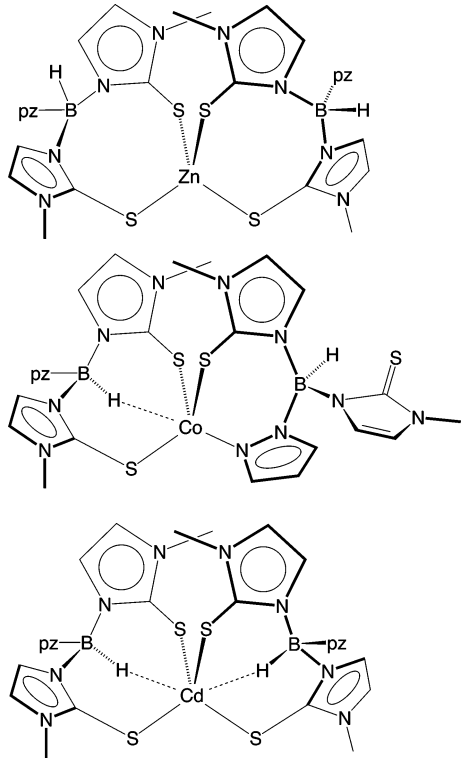


Figure 68. Structures of $[pzBm^{Me}]_2M$ (M = Zn, Co, Cd) indicating the greater preference of zinc to adopt tetrahedral $M[S_4]$ coordination.

complex $[pzBm^{Me}]_2Zn$ exhibits a different structure from those of the cobalt and cadmium complexes is of relevance to the use of these metals as spectroscopic probes for studying zinc enzymes, as noted above. Thus, even though cobalt and zinc show a strong preference for tetrahedral coordination, comparison of the structures of $[pzBm^{Me}]_2Zn$ and $[pzBm^{Me}]_2Co$ indicates that the preference for tetrahedral $M[S_4]$ coordination is actually greater for zinc. Likewise, although cadmium-substituted enzymes are studied due to the NMR spectroscopic properties of ^{111}Cd and ^{113}Cd, it must be recognized that the coordination geometries of the Cd active site may not be similar to those of the native zinc enzymes, thereby contributing to the dramatic differences in activity of the substituted enzymes.

The $[Tm^{Ph}]$ ligand likewise exhibits interesting coordination preferences. For example, only two of the sulfur donors of each ligand coordinate to the metal in the "sandwich" complexes, $[Tm^{Ph}]_2Fe$ and $[Tm^{Ph}]_2Co$, the coordination sphere being completed by interaction with the two B−H groups (Figure 69). In contrast, higher-valent metal centers favor tridentate sulfur binding. Thus, the iron(III) derivative $\{[Tm^{Ph}]_2Fe\}[ClO_4]$ adopts an octahedral structure in which both $[Tm^{Ph}]$ ligands coordinate via their full complement of sulfur donors (Figure 69).

The structures of a series of zinc, cadmium, and mercury complexes, $[Tm^R]MX$ (M = Zn, Cd; R = Bz, *p*-Tol),[225] $[Tm^{Me}]MX$ (M = Zn, Cd, Hg),[226] and $[Tm^{Bu^t}]MX$ (M = Zn, Cd, Hg),[227] have been determined; a notable feature of the $[Tm^{Me}]MX$ derivatives is that the halide in the cadmium and mercury derivatives is displaced from the C_3 axis of the $[Tm^{Me}]M$ moiety.

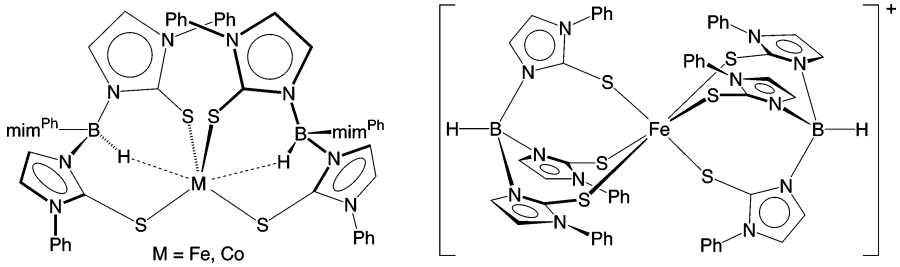
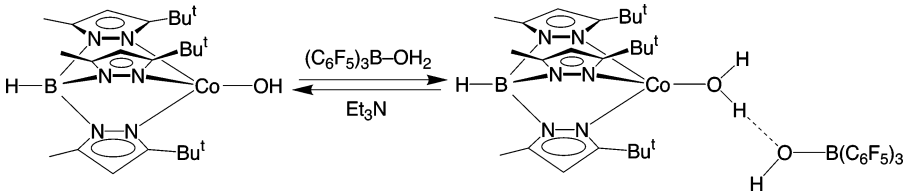


Figure 69. Influence of charge and oxidation state on the coordination preferences of the [Tm^{Ph}] ligand.

Scheme 87. Synthesis of the aqua complex, {[Tp$^{Bu^t,Me}$]Co(OH$_2$)}$^+$, an analogue of Co^{II}−carbonic anhydrase



A series of bis(mercaptoimidazolyl)complexes, [Bm^R]$_2$M (M = Zn, Cd, Hg; R = Me, Bz, Bu^t, *p*-Tol) have been structurally characterized, thereby demonstrating that while the methyl-substituted zinc complex [Bm^{Me}]$_2$Zn exhibits a geometry that is close to tetrahedral, incorporation of *tert*-butyl-substituents results in a notable distortion from tetrahedral coordination with Zn···H−B interactions.[356] The cadmium and mercury derivatives also show pronounced distortions from tetrahedral due to the formation of two M···H−B interactions.

The recent application of ^{67}Zn QCPMG solid-state NMR spectroscopy[74,357] suggests that zinc enzymes may be studied directly by this spectroscopic technique in the future, thereby removing ambiguities due to structural changes resulting from the presence of different metal atoms.

4.2. Influence of the Metal on the Reactivity of Synthetic Analogues

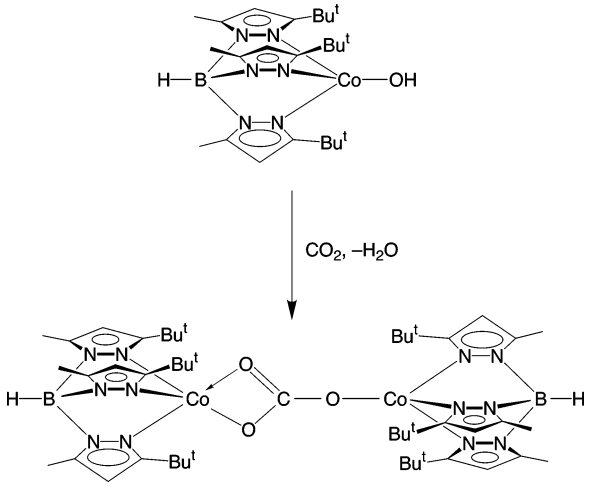
4.2.1. Comparison of the Zinc and Cobalt Hydroxide and Aqua Complexes [Tp$^{Bu^t,Me}$]MOH and {[Tp$^{Bu^t,Me}$]M(OH$_2$)}$^+$

The only pair of structurally related tetrahedral zinc and cobalt hydroxide complexes of the type {[N$_3$]MOH} are the tris(pyrazolyl)hydroborato derivatives [Tp$^{Bu^t,Me}$]ZnOH[73a] and [Tp$^{Bu^t,Me}$]CoOH.[349] An X-ray diffraction study also indicates that the zinc and cobalt complexes have very similar coordination environments, as illustrated by comparison of their respective M−O and M−N bond lengths: Zn−O [1.850(8) Å] and Co−O [1.859(3) Å]; Zn−N$_{av}$ (2.10 Å) and Co−N (2.04 Å).[80b] The Co−O bond length in [Tp$^{Bu^t,Me}$]CoOH [1.859(3) Å] is also similar to that in five-coordinate {[P(CH$_2$CH$_2$PPh$_2$)$_3$]CoOH}$^+$ [1.873(7) Å];[358] these Co−OH bond lengths are, however, considerably shorter than that in the five-coordinate anionic species {[η^4-N{CH$_2$CH$_2$NC(O)NHBu^t}$_3$]-CoOH}$^{2-}$ [2.052(3) Å], presumably due to the fact that hydroxide oxygen in the latter complex is also a hydrogen-bond receptor for two of the urea substituents.[83]

Analogous to [Tp$^{Bu^t,Me}$]ZnOH, the cobalt derivative [Tp$^{Bu^t,Me}$]CoOH reacts with (C$_6$F$_5$)$_3$B(OH$_2$) to give the aqua complex {[Tp$^{Bu^t,Me}$]Co(OH$_2$)}[HOB(C$_6$F$_5$)$_3$] (Scheme 87), which has been structurally characterized by X-ray diffraction. Comparison between the structures of [Tp$^{Bu^t,Me}$]CoOH and {[Tp$^{Bu^t,Me}$]Co(OH$_2$)}-[HOB(C$_6$F$_5$)$_3$] indicates that protonation results in geometrical changes similar to those observed for the zinc system. Specifically, (i) the Co−O bond length in the aqua cation {[Tp$^{Bu^t,Me}$]Co(OH$_2$)}$^+$ [1.963(2) Å] is longer than that in the hydroxide [Tp$^{Bu^t,Me}$]CoOH [1.859(3) Å], and (ii) the B−O bond of the anion [1.495(3) Å] is shorter than that in (C$_6$F$_5$)$_3$B(OH$_2$) [1.597(2) Å]. There is also a hydrogen bond between the cobalt aqua and the boron hydroxide ligands, with an O···O separation of 2.498(2) Å. In addition to the structural similarity between {[Tp$^{Bu^t,Me}$]Zn(OH$_2$)}-[HOB(C$_6$F$_5$)$_3$] and {[Tp$^{Bu^t,Me}$]Co(OH$_2$)}[HOB(C$_6$F$_5$)$_3$], they also exhibit similar reactivity towards deprotonation and displacement of the [(C$_6$F$_5$)$_3$BOH]$^-$ anion. Thus, the cobalt complex is deprotonated by Et$_3$N to regenerate [Tp$^{Bu^t,Me}$]CoOH and reacts with [Bu$^n_4$N][I] to give [Tp$^{Bu^t,Me}$]CoI.

The catalytic properties of carbonic anhydrase are not only influenced by the pK_a of the metal bound water molecule but also by the pK_a of a histidine residue in the vicinity of the active site (His-64 for bovine CAII and His-64 or His-200 for CAI) which serves as a shuttle to transfer a metal aqua proton to the reaction medium.[359] The protonation state of this histidine residue also influences the pK_a of the coordinated water. In view of the fact that two ionizing groups are involved in the catalysis, i.e., M^{II}−OH$_2$ and His−H$^+$, analysis of the activity in terms of a single apparent acid dissociation constant is problematic in terms of interpretation. For example, a comparative activity study of Zn^{II} and Co^{II} bovine carbonic anhydrase has identified that the pK_a associated with a single apparent acid dissociation constant is smaller for the Co^{II} enzyme (6.6) than that for the Zn^{II} enzyme (6.9) when determined by consideration of the pH profile of k_{cat}; however, the pK_a is larger for the Co^{II} enzyme (7.2) than that for the Zn^{II} enzyme (7.0) when determined by consideration of the pH profile of k_{cat}/K_M.[360,361] It is, therefore, worthwhile to determine exactly how the pK_a of an aqua ligand in a well-defined tetrahedral {[N$_3$]M^{II}(OH$_2$)} complex depends on whether the

Scheme 88. Synthesis of a bridging carbonate complex of cobalt

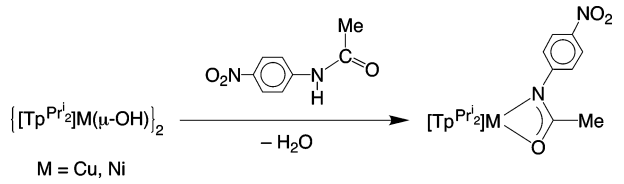


metal is zinc or cobalt, but such studies have been hampered by a lack of systematic studies as a function of coordination geometry and ligand environment.[342]

The isolation of $\{[Tp^{Bu^t,Me}]Zn(OH_2)\}[HOB(C_6F_5)_3]$ and $\{[Tp^{Bu^t,Me}]Co(OH_2)\}[HOB(C_6F_5)_3]$ provides a system that should enable determination of the manner by which substitution of zinc by cobalt influences the pK_a of the coordinated water in synthetic analogues of carbonic anhydrase. However, in view of the complications described above concerning the role of non-innocent counteranions, pK_a studies of this type are non-trivial (especially in aqueous solution).[362] Therefore, DFT calculations have been performed to determine how the metal center influences the pK_a of the zinc and cobalt aqua complexes, $\{[Tp]Zn(OH_2)\}^+$ and $\{[Tp]Co(OH_2)\}^+$.[363] The calculations indicate that the solution-free energies of deprotonation of $\{[Tp]Zn(OH_2)\}^+$ and $\{[Tp]Co(OH_2)\}^+$ are comparable, with that for the cobalt complex being only 1.05 kcal mol^{-1} more endothermic (i.e., less acidic), corresponding to a modest pK_a difference of 0.77 units. The calculations thus indicate that the pK_a values of $\{[Tp]Zn(OH_2)\}^+$ and $\{[Tp]Co(OH_2)\}^+$ are comparable, a result that is in line with the aforementioned reports of the aqua ligand of Co^{II}—carbonic anhydrase being both slightly more and slightly less acidic than that of the zinc enzyme. The similarity of the calculated pK_a values of $\{[Tp]Zn(OH_2)\}^+$ and $\{[Tp]Co(OH_2)\}^+$ is also in accord with the observation that Co^{II} is a successful substitute for Zn^{II} in carbonic anhydrase.[330,342]

As described above, in the presence of CO_2, $[Tp^{Bu^t,Me}]ZnOH$ is in rapid equilibrium with the bicarbonate derivative $[Tp^{Bu^t,Me}]ZnOC(O)OH$ but slowly forms a bridging carbonate complex $\{[Tp^{Bu^t,Me}]Zn\}_2(\mu\text{-}\eta^1,\eta^1\text{-}CO_3)$ (Scheme 11). The cobalt hydroxide complex $[Tp^{Bu^t,Me}]CoOH$ also reacts with CO_2 to form a bridging carbonate complex $\{[Tp^{Bu^t,Me}]Co\}_2(\mu\text{-}\eta^1,\eta^2\text{-}CO_3)$, presumably via the initial formation of a bicarbonate derivative (Scheme 88), but an interesting difference between the zinc and cobalt systems, however, pertains to the coordination mode of the bridging carbonate ligands. Thus, whereas the carbonate ligand of the zinc complex $\{[Tp^{Bu^t,Me}]Zn\}_2(\mu\text{-}\eta^1,\eta^1\text{-}CO_3)$ bridges in a unidentate manner to each zinc center (Scheme 11), the carbonate ligand in the cobalt counterpart $\{[Tp^{Bu^t,Me}]Co\}_2(\mu\text{-}\eta^1,\eta^2\text{-}CO_3)$ is unidentate to one cobalt center and bidentate to the other (Scheme 88). The difference in the coordination geometries of $\{[Tp^{Bu^t,Me}]Zn\}_2(\mu\text{-}\eta^1,\eta^1\text{-}CO_3)$ and $\{[Tp^{Bu^t,Me}]Co\}_2(\mu\text{-}\eta^1,\eta^2\text{-}CO_3)$ provides an illustration of how Co^{II} promotes bidentate coordination compared to that of zinc (vide infra). This observation is of significance in light of the proposition that bidentate coordination of a bicarbonate ligand could inhibit its displacement and thereby reduce the efficiency of carbonic anhydrase catalytic cycle,[73c,345] a suggestion that is consistent with the facts that Co^{II}—carbonic anhydrase is less active than the zinc enzyme and that the bicarbonate ligand in the Co^{II} derivative coordinates in a bidentate fashion.[325c,364] It is also noteworthy that the carbonate coordination mode of $\{[Tp^{Bu^t,Me}]Co\}_2(\mu\text{-}\eta^1,\eta^2\text{-}CO_3)$ contrasts with that of $\{[Tp^{Pr^i_2}]Co\}_2(\mu\text{-}\eta^2,\eta^2\text{-}CO_3)$ which features bidentate coordination to both cobalt centers as a result of the reduced steric demands.

Scheme 89. Reactivity of $\{[Tp^{Pr^i_2}]M(\mu\text{-}OH)\}_2$ towards *p*-nitroacetanilide



4.2.2. Comparison of the Reactivity of Other Metal Hydroxide Complexes

The copper and nickel hydroxide complexes $\{[Tp^{Pr^i_2}]M(\mu\text{-}OH)\}_2$ (M = Ni, Cu) and *p*-nitroacetanilide to give $[Tp^{Pr^i_2}]M\{\eta^2\text{-}MeC(O)NC_6H_4NO_2\}$ (Scheme 89).[365] However, the corresponding zinc, cobalt, and manganese hydroxo complexes were unreactive towards *p*-nitroacetanilide, an inertness that was proposed to be associated with a different coordination mode for the $[MeC(O)NC_6H_4NO_2]$ ligand. Specifically, it was suggested that since copper and nickel exhibit a greater tendency than zinc, cobalt, and manganese to coordinate ligands in a bidentate fashion, then the formation of strongly bound bidentate complexes for the nickel and copper systems $[Tp^{Pr^i_2}]M\{\eta^2\text{-}MeC(O)NC_6H_4NO_2\}$ (M = Ni, Cu) would promote the specific reaction for these derivatives.

Another example of how the structures of zinc and cadmium hydroxide complexes differ is provided by comparison of $\{[(bmnpa)Zn(\mu\text{-}OH)]_2\}^{2+}$ and $\{[(bmnpa)Cd(\mu\text{-}OH)]_2\}^{2+}$ (Figure 70).[366] Thus, whereas only one of the thioether donors of the bmnpa ligand coordinates to zinc such that the zinc centers of $\{[(bmnpa)Zn(\mu\text{-}OH)]_2\}^{2+}$ are five-coordinate, both thioether donors of the bmnpa ligand coordinate to cadmium, thereby resulting in six-coordinate cadmium centers. The cadmium complex $\{[(bmnpa)Cd(\mu\text{-}OH)]_2\}^{2+}$ reacts with CO_2 to give a bridging carbonate complex, $\{[(bmnpa)Cd(\mu\text{-}\eta^2,\eta^2\text{-}CO_3)]_2\}^{2+}$ (Scheme 90); likewise, the closely related zinc complex $\{[(benpa)Zn(\mu\text{-}OH)]_2\}^{2+}$ reacts with CO_2 to give $\{[(benpa)Zn(\mu\text{-}\eta^2,\eta^2\text{-}CO_3)]_2\}^{2+}$ (Scheme 91). The reac-

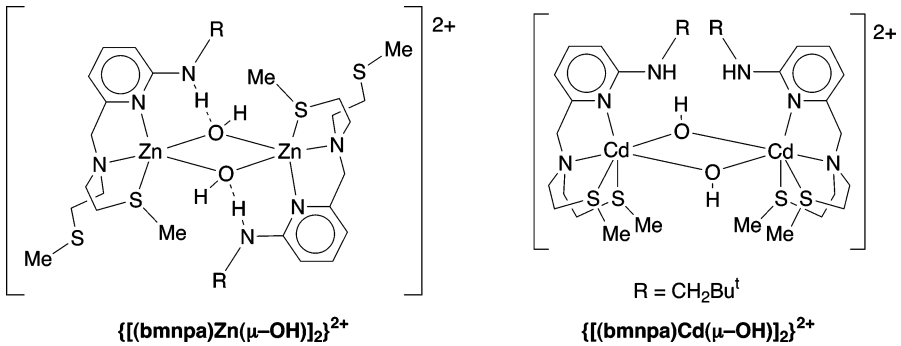
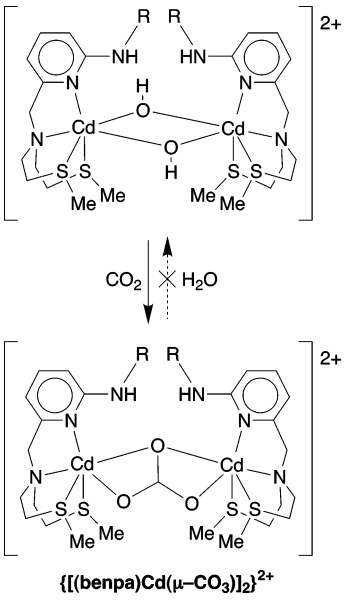
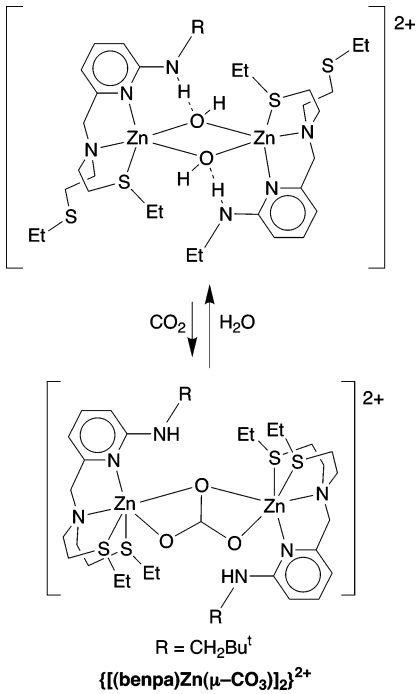


Figure 70. Comparison of the structures of the bridging hydroxide complexes, $\{[(bmnpa)Zn(\mu\text{-}OH)]_2\}^{2+}$ and $\{[(bmnpa)Cd(\mu\text{-}OH)]_2\}^{2+}$. Only one of the thioether donors of the bmnpa ligand coordinates to zinc such that the zinc centers of $\{[(bmnpa)Zn(\mu\text{-}OH)]_2\}^{2+}$ are five-coordinate, whereas both thioether donors of the bmnpa ligand coordinate to cadmium, thereby resulting in six-coordinate cadmium centers.

Scheme 90. Irreversible reaction of the cadmium hydroxide complex $\{[(bmnpa)Cd(\mu\text{-}OH)]_2\}^{2+}$ with CO_2



Scheme 91. Reversible reaction of the zinc hydroxide complex $\{[(benpa)Zn(\mu\text{-}OH)]_2\}^{2+}$ with CO_2



tivity of the zinc and cadmium complexes towards water are very different. Thus, whereas the zinc complex $\{[(benpa)Zn(\mu\text{-}\eta^2,\eta^2\text{-}CO_3)]_2\}^{2+}$ reacts rapidly with water to regenerate the hydroxide complex $\{[(benpa)Zn(\mu\text{-}OH)]_2\}^{2+}$ (Scheme 91), the cadmium carbonate complex is stable under comparable conditions (Scheme 90). The stability of the cadmium carbonate linkage suggests that this could also be one of the factors why Cd^{II}−CA is not as active as the zinc enzyme.

4.2.3. Comparison of the Reactivity of Metal Thiolate Compounds

Comparison of the reactivity of the zinc and cadmium phenylthiolate complexes $[Ph(pz^{But})Bt^{But}]ZnSPh$ and $[Ph(pz^{But})Bt^{But}]CdSPh$ towards alkylation by MeI indicates that cadmium thiolate is inherently more nucleophilic than the zinc thiolate (by a factor of 17 in rate constant).[200] In contrast, the opposite result was observed for alkylation of $[Zn(SPh)_4]^{2-}$ and $[Cd(SPh)_4]^{2-}$ with $(MeO)_3PO$, with the zinc thiolate complex reacting more rapily than the cadmium counterpart (by a factor of 2.5 in rate constant); likewise, the cobalt complex $[Cd(SPh)_4]^{2-}$ is also less reactive than the zinc analogue (Table 10).[263] The differences between the $[Ph(pz^{But})Bt^{But}]MSPh$ and $[Zn(SPh)_4]^{2-}$ systems have been rationalized on the basis of the operation of different mechanisms. Thus, it has been proposed that the reduced Lewis acidity of cadmium versus zinc causes the phenylthiolate sulfur to be more nucleophilic in $[Ph(pz^{But})Bt^{But}]$-CdSPh than in $[Ph(pz^{But})Bt^{But}]ZnSPh$, resulting in the cadmium complex being more reactive with respect to an associative mechanim; in contrast, the reactions of $[M(SPh)_4]^{2-}$ (M = Zn, Co, Cd) proceed via a dissociative pathway, and so the rate of alkylation is dictated by the rate of cleavage of the Zn−SPh and Cd−SPh bonds; cobalt and cadmium have been suggested to have a higher affinity for sulfur ligands, which correspondingly inhibits a dissociative pathway.[200]

4.2.4. Other Comparisons Pertinent to Metal-Substituted Enzymes

Phenanthroline-linked dihydronicatinamide ligands (Figure 71) in the presence of M^{2+} have been used to study aspects of hydride transfer to 2-pyridinecar-

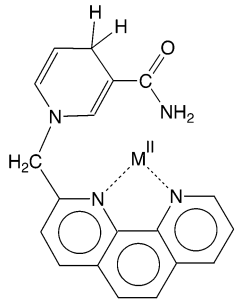


Figure 71. Phenanthroline-linked dihydronicatinamide ligand that has been used to study aspects of hydride transfer to 2-pyridinecarboxaldehyde (C_5H_4NCHO), demonstrating that the order of activity is $Zn^{2+} \gg Mg^{2+}$, $Ni^{2+} > Co^{2+} > Cd^{2+}$.

boxaldehyde (C_5H_4NCHO), which indicates that the order of activity is $Zn^{2+} \gg Mg^{2+}$, $Ni^{2+} > Co^{2+} > Cd^{2+}$.[367] The interaction between Zn^{II} or Cd^{II} and nucleoside monophosphates has been determined by calorimetric means, demonstrating that the ΔH values for coordination are similar for the two metals.[368] In a study of tris(pyridyl)amine and related complexes, it was demonstrated that the use of a piperidine scaffold greatly influences the binding preferences of Zn^{II} and Cu^{II} by a factor of 10^4.[369,370]

4.3. Nitrate and Acetate Ligands as a Probe for Trends in Bicarbonate Coordination in Metal-Substituted Carbonic Anhydrases

The final step of the carbonic anhydrase catalytic cycle involves displacement of the bicarbonate ligand by water. The coordination mode of the bicarbonate ligand undoubtedly plays an important role in determining the facility of this transformation, as illustrated by the influence of carbonate coordination mode on the hydrolytic stability of bridging carbonate complexes, as described above. Specifically, a unidentate carbonate ligand is more susceptible to hydrolysis than a related bidentate carbonate ligand. A knowledge of the different tendencies for various metals to favor unidentate versus bidentate coordination of a bicarbonate ligand is, therefore, of most relevance to the interpreation of the relative activities of metal-substituted carbonic anhydrases. However, due to the general instability of bicarbonate complexes, it is presently not possible to study the variation of bicarbonate coordination mode as a function of metal in an environment which mimics the active sites of zinc enzymes. Indeed, there are presently no structurally characterized mononuclear zinc bicarbonate complexes. To circumvent this problem, it has been proposed that the nitrate ligand may be employed as a probe to provide an indication of the structural variations that would be expected for a series of bicarbonate complexes[73c,345] Thus, because bicarbonate and nitrate ligands are isoelectronic and sterically similar, and both ligands can bind metals by either one or two oxygen atoms, the *variation* in nitrate coordination mode for a series of analogous metal complexes is expected to correlate with the *trend* for analogous bicarbonate complexes. Note that this statement is not intended to imply that a nitrate ligand coordination mode will be identical to that in a corresponding bicarbonate complex, but is rather intended to suggest that for a group of closely related nitrate and bicarbonate complexes, the variations in denticity will be similar for the two series.

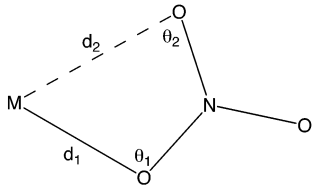


Figure 72. Parameters used in classifying nitrate coordination mode.

Table 14. Comparison of Nitrate Coordination Mode of $LM(NO_3)^{Q+}$ [a] with Activity of M^{II}−CA[b]

M	M^{II}−CA activity (%)	Δd (Å)		$\Delta\theta$ (deg)	
		$[Pim^{Pr^i,Bu^t}]$	$[Tp^{Bu^t,R}]$[c]	$[Pim^{Pr^i,Bu^t}]$	$[Tp^{Bu^t,R}]$[c]
Zn	100	0.53	0.60	24.4	29.6
Co	50	0.27	0.34	10.8	15.8
Ni	2	−	0	−	0
Cu	0	0.13	0	5.9	0
Cd	2	0.13	0.23	7.4	1.8
Hg	0	0.39	−	19.3	

[a] $Q = 1$ for $L = [Pim^{Pr^i,Bu^t}]$; $Q = 0$ for $L = [Tp^{Bu^t,R}]$. [b] Data taken from Kimblin, C.; Murphy, V. J.; Hascall, T.; Bridgewater, B. M.; Bonanno, J. B.; Parkin, G. *Inorg. Chem.* **2000**, *39*, 967. [c] R = H for M = Zn, Co, Cu, Ni; R = Me for M = Cd.

The variation in nitrate ligand coordination mode (Figure 72) has been examined for a series of complexes that employ tridentate nitrogen coordination to mimic the three histidine groups that bind zinc in carbonic anhydrase. The two most complete systems studied are the tris(pyrazolyl)hydroborato and tris(imidazolyl)phosphine complexes, $[Tp^{Bu^t,R}]M(NO_3)$ and $\{[Pim^{Pr^i,Bu^t}]M(NO_3)\}^+$,[345] although other closely related systems have also been briefly examined.[371,372] The data summarized in Table 14 indicate that the nitrate ligand binding mode, as evaluated by the difference in the two M−O bond lengths (Δd) and M−O−N bond angles ($\Delta\theta$) varies considerably among the complexes. Nitrate ligand coordination modes may be classified as either bidentate ($\Delta d < 0.3$ Å; $\Delta\theta < 14°$), anisobidentate ($0.3 < \Delta d < 0.6$ Å; $28 < \Delta\theta < 14°$), or unidentate ($\Delta d > 0.6$ Å; $\Delta\theta > 28°$), depending upon the degree of asymmetry, and the data listed in Table 14 indicate that the bidenticity increases in the sequence Zn < Hg < Co < Cu ≈ Ni ≈ Cd. While these changes reflect the different electronic properties of the metal, it is important to emphasize that steric effects are also important in influencing the coordination mode. For example, reducing the steric demands of the $[Tp^{RR'}]$ ligand results in a decrease in the asymmetry of the nitrate coordination mode across the series $[Tp^{Bu^t}]Zn(NO_3)$,[345a] $[Tp^{Ph}]Zn(NO_3)$,[255,373] and $[Tp]Zn(NO_3)$.[374] The influence of pyrazolyl substituents on the coordination mode of nitrate ligands in $[Tp^{RR'}]Co(NO_3)$ complexes has also been discussed.[375]

With the exception of mercury, the nitrate coordination mode in the above complexes correlates reasonably well with the activity of metal-substituted

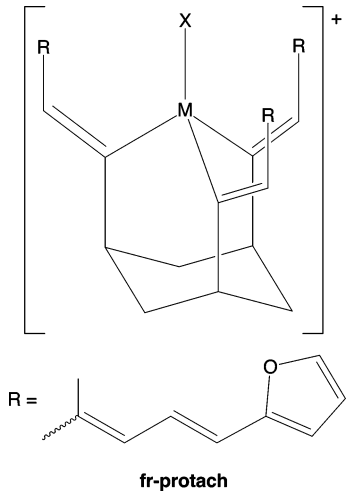


Figure 73. Tridentate *cis*-1,3,5-tris[3-(2-furyl)prop-2-enylideneamino]cyclohexane ligand that has been used to probe acetate and coordination mode as a function of metal.

carbonic anhydrases: Zn > Co ≫ Cu ≈ Ni ≈ Cd ≈ Hg (Table 14). Specifically, those metals with almost symmetric bidentate coordination are inactive, whereas those with a significant asymmetry (Zn and Co) are active. The notion that nitrate coordination modes in these complexes reflect the trend in bicarbonate coordination modes is provided by X-ray diffraction studies on the enzymes. Specifically, the X-ray structure of human CA I complexed with bicarbonate indicates that the bicarbonate ligand is coordinated to zinc in a unidentate fashion,[112] whereas the bicarbonate ligand in cobalt CA II binds more symmetrically.[329c] Further support for the notion that the variation in nitrate coordination mode is a good indication of the variation of bicarbonate coordination mode is provided by the observation that the carbonate ligand in the complexes $\{[Tp^{Pr^i_2}]M\}_2(\mu\text{-}CO_3)$ (M = Mn, Fe, Co, Ni, Cu) also exhibits varying degrees of asymmetry that closely parallel the series of nitrate complexes described above.[73d]

The anomaly provided by the mercury complex, with the nitrate ligand being distinctly asymmetric, but yet Hg^{II}−carbonic anhydrase being inactive, presumably indicates that another step of the mechanism is responsible for inhibiting the enzyme. One possibility is that the mercury center of Hg^{II}−carbonic anhydrase does not bind water efficiently, as has been observed for Hg^{II}−carboxypeptidase A.[376] In this regard, it is worth noting that the chemistry of mercury is often anomalous to that of other group 12 elements.[377]

Acetate ligands have also been used as a probe for bicarbonate ligand coordination mode in complexes that employ the tridenate *cis*-1,3,5-tris[3-(2-furyl)-prop-2-enylideneamino]cyclohexane ligand, as illustrated in Figure 73.[53] For example, the zinc complex $\{[fr\text{-}protach]Zn(\eta^1\text{-}OAc)\}^+$ contains a unidenate acetate ligand, whereas the cobalt counterpart $\{[fr\text{-}protach]Co(\eta^2\text{-}OAc)(MeOH)\}^+$ possesses a bidentate acetate ligand *and* a coordinated methanol molecule. This observation reinforces the notion that cobalt favors bidentate coordination to a greater extent than does zinc. In contrast to the isolation of the tetrahedral acetate complex $\{[fr\text{-}protach]Zn(\eta^1\text{-}OAc)\}^+$, the nitrate analogue is octahedral $\{[fr\text{-}protach]Zn(\eta^2\text{-}O_2NO)(MeOH)\}^+$ with a bidentate nitrate ligand and a methanol ligand, similar to that of $\{[fr\text{-}protach]Co(\eta^2\text{-}OAc)(MeOH)\}^+$. It is, therefore, evident that the nitrate ligand shows a greater preference to be bidentate than does the acetate ligand.

4.4. Spectroscopic Models

Metal-substituted synthetic analogues have been used to provide spectroscopic signatures to facilitate the assignment of the coordination environments of the active sites of metalloenzymes.[378,379] As an illustration, the similarity of the electronic spectrum of blue $[Tp^{Bu^t}]CoCl$ (λ = 547−659 nm, ϵ = 496 M^{-1} cm^{-1})[345b] with that of the high pH form of Co^{II}−carbonic anhydrase is consistent with the notion that the active site exhibits a pseudotetrahedral coordination geometry.[330] Likewise, comparison of the electronic spectra of the four-, five-, and six-coordinate nickel complexes, purple $[Tp^{Bu^t}]NiCl$, yellow $[Tp^{Bu^t}]Ni(\eta^2\text{-}O_2NO)$, and green $[Tp^{Me_2}]Ni(\eta^2\text{-}O_2NO)(THF)$, with that of Ni^{II}−carbonic anhydrase supports the notion that nickel is six-coordinate in the enzyme at neutral pH. In addition, comparison of the electronic spectrum of Cu^{II}−carbonic anhydrase with those of the four- and five-coordinate complexes $[Tp^{Bu^t}]CuCl$ and $[Tp^{Bu^t}]Cu(\eta^2\text{-}O_2NO)$ indicates a greater similarity to the five-coordinate structure. X-ray diffraction studies on the Co^{II}-, Ni^{II}-, and Cu^{II}-substituted enzymes are in accord with these proposals.[329a] EPR spectroscopic studies of Co^{II} doped into Zn^{II} complexes has indicated that the *g*-values are very sensitive to small structural differences, an observation that is also of use for ascertaining the structures of metal-substituted enzymes.[380]

5. Future Directions

The research described in this review highlights the advances that have been made in the study of synthetic analogues of zinc enzymes. These investigations have laid the foundation of the bioinorganic chemistry of zinc that allows one to understand the mechanisms of action of zinc enzymes. However, "perfect" synthetic analogues that mimic all aspects of zinc enzymes, i.e., structure, function, and mechanism, are yet to be obtained. For example, many structural models lack functional equivalence, while many functional models have little structural equivalence. Further advances should be directed towards the synthesis of analogues that combine both these properties and have both excellent structural and functional equivalence to the enzymes; this will also include the incorporation of groups that mimic protein residues in the vicinity of the active site that are postulated to play a role in the mechanism of action.

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