The bioinorganic chemistry of zinc: synthetic analogues of zinc enzymes that feature tripodal ligands

Gerard Parkin

Department of Chemistry, Columbia University, New York, New York 10027, USA.
E-mail: parkin@chem.columbia.edu

Received (in Cambridge, UK) 15th June 2000, Accepted 18th August 2000
First published as an Advance Article on the web 29th September 2000

Zinc, as a constituent of more than 300 enzymes, plays essential roles in biological systems. The active sites of these enzymes feature a zinc center attached to the protein backbone by three or four amino acid residues, the nature of which influences the specific function of the enzyme. In order to understand why different zinc enzymes utilize different amino acid residues at the active site, it is necessary to understand how, and why, the chemistry of zinc is modulated by its coordination environment. Answers to these questions are being provided by a study of synthetic analogues of zinc enzymes, *i.e.* small molecules that resemble the enzyme active sites. This article provides an account of those studies that have specifically used tripodal ligands to mimic the active site protein residues in an effort to ascertain the bioinorganic chemistry of zinc.

1. Introduction

Once dubbed a 'boring element',[1] zinc is now experiencing a dramatic renaissance in its chemistry due to the important roles that it plays in biological systems.[2] For example, an average adult human contains *ca*. 3 grams of zinc,[3] and its bioavailability has been shown to have an effect on the occurrence of malaria, pneumonia, and also the common cold.[2,3] To a large degree, the primary influence of zinc in biological systems resides with its presence in *ca*. 300 enzymes. The active sites of many of these enzymes feature a tetrahedrally coordinated zinc center that is attached to the protein backbone by three amino acid residues, with the fourth site being occupied by a water molecule (Fig. 1).[2,4] The specific function performed by each of these enzymes is dictated by both *(i)* the nature of the residues which bind zinc to the protein, typically His (N), Glu (O), Asp (O), and Cys (S), and *(ii)* the amino acid spacer lengths between the active site residues. For example, a selection of zinc enzymes and their functions, which differ according to the composition of the active site, are illustrated in Table 1 and Fig. 2. While it is recognized that the prevalent use of zinc in biological systems is a manifestation of the unique characteristics associated with the Zn^{2+} ion (Table 2),[5] the factors that are responsible for modifying the detailed properties of the active sites of zinc enzymes are not known with certainty. For this

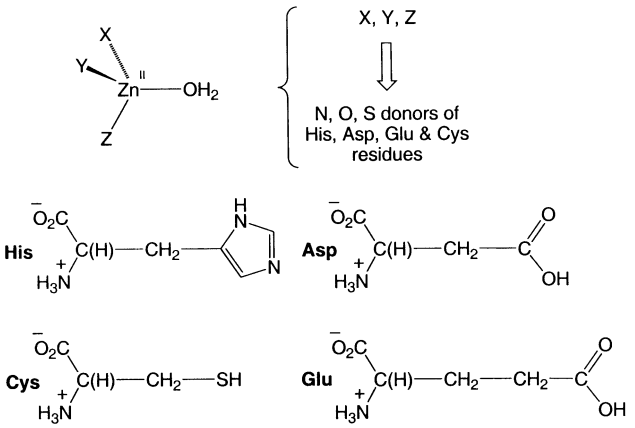


Fig. 1 A common structural feature of zinc enzymes.

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 Thermolysin Neutral protease	Exo peptidase Endo peptidase Endo peptidase
His, His, Asp	Protease from streptomyces caespitosus	Endo peptidase
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 Cytidine deaminase	Oxidation of alcohols to aldehydes and ketones Hydrolytic deamination of cytidine to uridine
Cys, Cys, Cys	5-Aminolevulinate dehydratase	Synthesis of porphobilinogen from 5-aminolevulinic acid

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 post-doctoral 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 is currently Chairman of the Department. 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.

DOI: 10.1039/b004816j

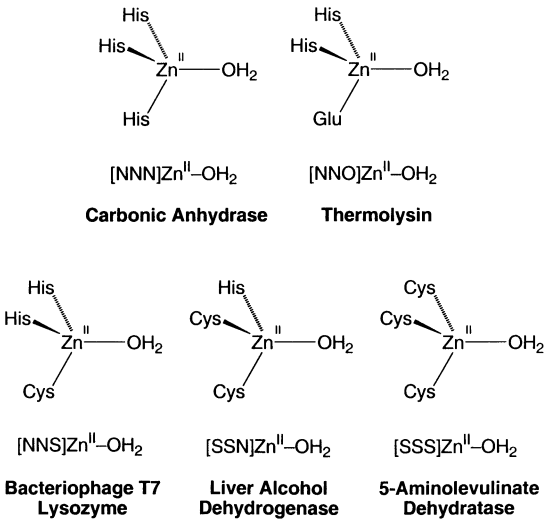


Fig. 2 Active site coordination motifs in representative zinc enzymes.

Table 2 Properties of Zn^{II} that are pertinent to its role in enzymes (information taken from refs. 2 and 3)

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 is 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.

reason, a number of research groups are actively studying the chemistry of synthetic analogues of zinc enzymes (*i.e.* small molecules that resemble the enzyme active sites) as part of a concerted effort to establish how, and why, the chemistry of zinc is modulated by its coordination environment.[6] The purpose of this article is to provide an account of those studies that have specifically used tripodal ligands to mimic the active site protein residues in an effort to ascertain the bioinorganic chemistry of zinc.

2. Tripodal ligands as mimics for protein binding

The importance of studying synthetic analogues of zinc enzymes resides with the fact that such species are more amenable to structural, spectroscopic, and mechanistic studies than the enzymes themselves. As such, these studies enable the bioinorganic chemistry of zinc to be established for well defined systems. Accurate synthetic analogues of zinc enzymes are not, however, trivial to obtain. A simple illustration of this statement is provided by the fact that whereas *pseudo*-tetrahedral coordination geometry is prevalent in zinc enzymes, higher coordination numbers are common for simple zinc complexes in both the solid state and solution.[4e,7,8] Furthermore, binuclear degradatory pathways are inhibited for the enzyme by virtue of the fact that the active sites are located in its interior. To circumvent such problems involving departures from tetrahedral coordination and oligomerization, considerable attention must be given to ligand design in order to procure synthetic analogues that mimic well the enzyme active sites. Since the active sites of most zinc enzymes are of the composition [{XYZ}Zn^{II}–OH_2], where X, Y, and Z are three protein residues (Table 1 and Fig. 2), a rational approach towards obtaining synthetic analogues is to use tridentate ligands which incorporate the requisite X, Y and Z donor groups to mimic the protein ligation. A further refinement of this approach is to use a *tripodal* ligand in which the X, Y, and Z groups are attached to a common tetrahedral (or trigonal pyramidal) center.

There are at least three advantages of using tripodal ligands, rather than acyclic or cyclic ligands, to support tetrahedral zinc centers. Specifically:

(i) As a result of its trigonal nature, a tripodal ligand enforces the 'facial' (as opposed to 'T-shaped') binding that is required to create a tetrahedral metal center (Fig. 3). In contrast, acyclic

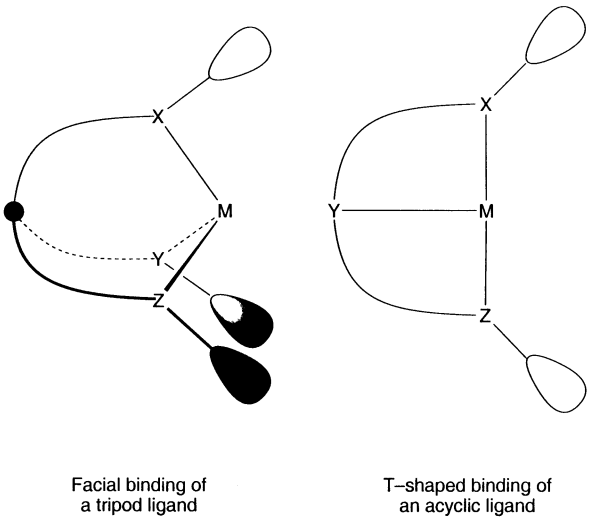


Fig. 3 Comparison of the facial coordination of a tripodal ligand with the T-shaped binding of an acyclic ligand.

tridentate ligands often bind in a 'T-shaped' manner that has no significant biological relevance.

(ii) Tripodal ligands typically possess only a single relevant binding conformation, whereas macrocyclic ligands are more conformationally flexible.

(iii) The directional nature of tripodal ligands is such that it is possible to incorporate substituents that directly influence the steric environment about the metal center. In contrast, substituents in macrocyclic ligands are not, in general, suitably placed to have a profound impact on the sterics of the coordination pocket.

Trofimenko's tris(pyrazolyl)borate ligand system, [$Tp^{RR'}$] (Fig. 4),[9] which features prominently in this article, provides an

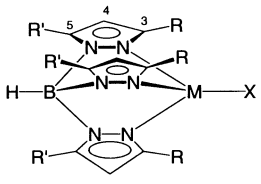


Fig. 4 The tris(pyrazolyl)hydroborato ligand system, [$Tp^{RR'}$]MX.

exemplary illustration of the above virtues of a tripodal ligand. Indeed, with bulky *tert*-butyl substituents on the 3-position of

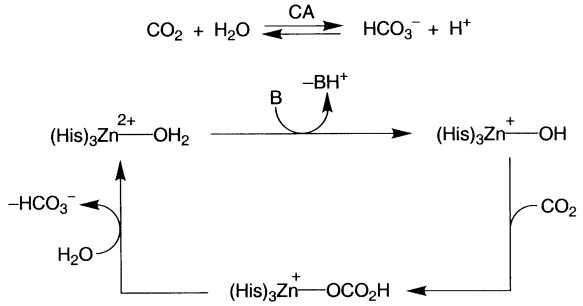
the pyrazolyl groups, the ligand has been referred to as a 'tetrahedral enforcer' due to its tendency to favor tetrahedral coordination.[10] Not only can the substituents at the 3-position be used to modify the size of the coordination pocket, they may also be used to influence the electronic properties of the metal center. For example, in addition to simple alkyl and aryl substituents, electron withdrawing perfluoroalkyl groups, *e.g.* CF_3, may be incorporated.[9] Finally, it should be noted that substituents such as Bu^t provide a valuable ^{1}H NMR spectroscopic probe that facilitates reactivity and mechanistic studies.

3. Synthetic analogues of zinc enzymes incorporating tripodal ligands

In view of the very large number of zinc enzymes known, a systematic and comprehensive investigation of synthetic analogues for all of these enzymes is yet to be conducted. For this reason, the selection of enzymes discussed in this article is eclectic, with the specific intention being to illustrate aspects of the chemistry of those enzymes which feature mononuclear tetrahedral active sites of the type [{XYZ}Zn^{II}–OH$_2$]. The chemistry will be discussed according to the nature of the {XYZ} ligand complement at the active site, ranging from nitrogen rich carbonic anhydrase, [(His)$_3$Zn^{II}–OH$_2$], to sulfur rich 5-aminolevulinate dehydratase [(Cys)$_3$Zn^{II}–OH$_2$].

(a) The [(His)$_3$Zn^{II}–OH$_2$] motif: carbonic anhydrase

Carbonic anhydrase, the first enzyme recognized to contain zinc, has played a pivotal role in the development of zinc enzymology.[11] It is recognized to be an 'ancient' enzyme since it has widespread occurrence in prokaryotes, it is ubiquitous in nature, and is one of the most efficient enzymes known. As implied by its name, its physiological function is to catalyse the reversible hydration of carbon dioxide (Scheme 1), and thus



Scheme 1

carbonic anhydrase plays an important role in respiration and intracellular CO_2/HCO_3^- equilibration. X-Ray diffraction studies demonstrate that the zinc center of the active site is coordinated to the protein by the imidazole groups of three histidine residues and a water molecule (or hydroxide ion, depending upon pH) (Fig. 5), and the overall features of the mechanism of action are illustrated in Scheme 1.[11]

Numerous studies have been performed using tridentate nitrogen donor ligands to model the structure and function of the active site carbonic anhydrase.[6] However, very few of these studies have successfully enabled the isolation of structurally-characterized mononuclear four-coordinate zinc–hydroxide or zinc–aqua complexes that mimic the active site of carbonic anhydrase. It is, therefore, significant that the first such complexes were obtained using sterically demanding tris(pyrazolyl)borato ligands, namely [Tp$^{RR'}$]ZnOH.[12] For example, [Tp$^{Bu^t,Me}$]ZnOH, the first monomeric terminal zinc hydroxide complex to be isolated, has been synthesized by (*i*) the direct reaction between equimolar amounts of $Zn(ClO_4)_2 \cdot 6H_2O$, K[Tp$^{Bu^t,Me}$] and KOH in methanol, and (*ii*) by metathesis of [Tp$^{Bu^t,Me}$]ZnI with Bu$^n_4$NOH (Scheme 2).

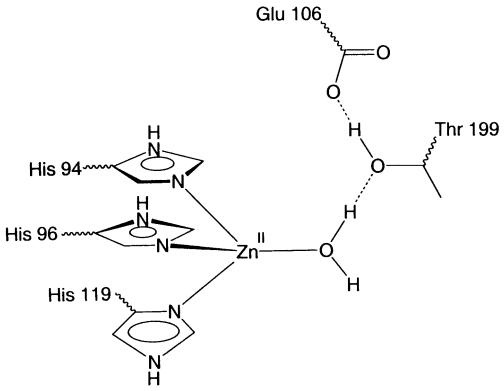
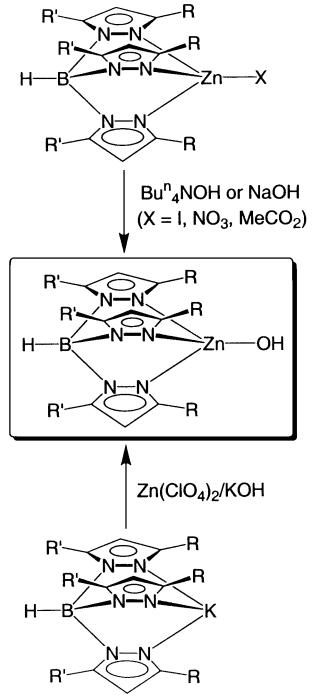


Fig. 5 Active site of carbonic anhydrase.



Scheme 2

The molecular structures of [Tp$^{Bu^t,Me}$]ZnOH and [Tp^{Cum,Me}]-ZnOH have been determined by X-ray diffraction, thereby confirming the monomeric and tetrahedral nature of the complexes, as illustrated for [Tp$^{Bu^t,Me}$]ZnOH in Fig. 6. It is important to emphasize that the presence of bulky substituents (*e.g.* Bu^t and $C_6H_4Pr^i$) is essential for the successful isolation of the four-coordinate [Tp$^{RR'}$]ZnOH terminal hydroxide complexes. For example, a simple phenyl substituent in the 3-position of [Tp^{Ph}]ZnOH does not afford a stable derivative.[13] Furthermore, the absence of bulky susbtituents on simple macrocyclic ligands results in oligomerization *via* either hydroxy-bridges, *e.g.* $\{[\{Me_3[9]aneN_3\}Zn(\mu\text{-}OH)]\}_2^{2+}$,[14] or hydrogen bonding interactions, *e.g.* $[\{[12]aneN_3\}Zn(OH)]_3(ClO_4)_3 \cdot (HClO_4)$.[15] More recently, a monomeric five-coordinate anionic zinc hydroxide with a trigonal-bipyramidal geometry, $\{[\eta^4\text{-}N\{CH_2CH_2NC(O)NHBu^t\}_3]ZnOH\}^{2-}$,[16] has been synthesized; notably, this complex also features bulky amide substituents that provide a hydrogen bonding cavity in which the hydroxide ligand resides.[17]

In addition to being structurally determined by X-ray diffraction, the hydroxide ligand in [Tp$^{RR'}$]ZnOH is well characterized spectroscopically, as illustrated by the IR and NMR spectroscopic data listed in Table 3. For example, the ^{1}H NMR spectrum of [Tp$^{Bu^t,Me}$]ZnOH in C_6D_6 illustrates that the hydroxide proton is observed as a sharp signal at δ −0.07 ppm.

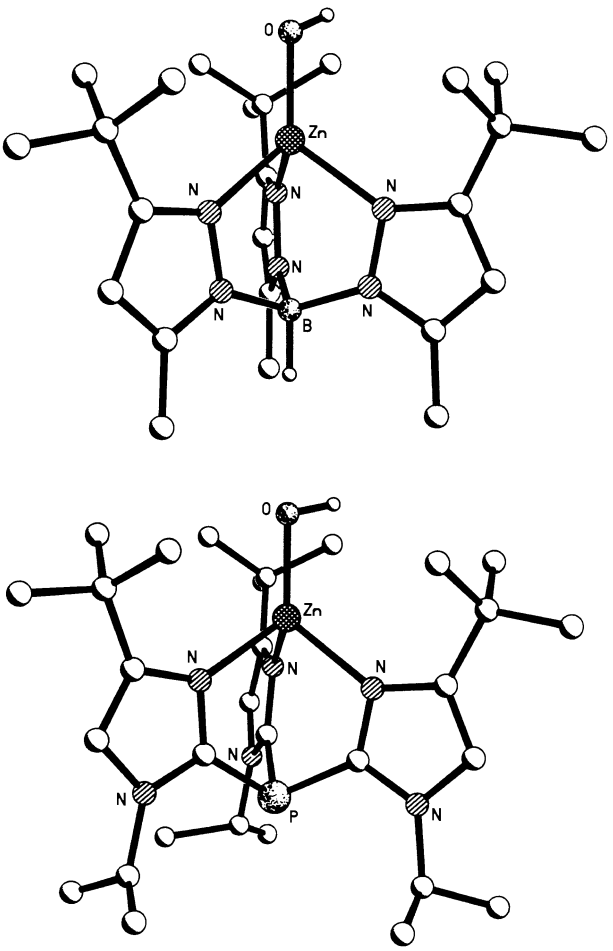


Fig. 6 Molecular structures of $[Tp^{Bu^t,Me}]ZnOH$ and $\{[Pim^{Pr^i,Bu^t}]ZnOH\}^+$.

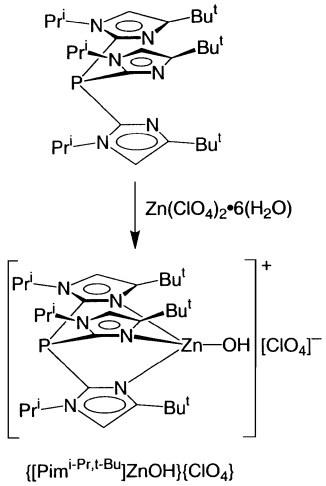
Table 3 Spectroscopic data for $[Tp^{RR'}]ZnOH$ complexes (data taken from ref. 9*b*)

$[Tp^{RR'}]ZnOH$	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		

[a] Ar = *p*-$C_6H_4Bu^t$

While the $[Tp^{RR'}]ZnOH$ complexes represent a major advance in bioinorganic zinc chemistry by providing the first well characterized tetrahedral zinc hydroxide complexes, their structural similarity to the enzyme is limited by the fact that the nitrogen donors are not of the imidazolyl type, but are rather pyrazolyl-based. Nevertheless, a tetrahedral terminal zinc hydroxide complex has been obtained by the use of the sterically demanding neutral tris[2-(1-isopropyl-4-*tert*-butylimidazolyl)]phosphine ligand, $[Pim^{Pr^i,Bu^t}]$. Specifically, the hydroxide complex $\{[Pim^{Pr^i,Bu^t}]ZnOH\}^+$ was isolated as the perchlorate derivative upon reaction of $[Pim^{Pr^i,Bu^t}]$ with $Zn(ClO_4)_2{\cdot}6H_2O$ (Scheme 3).[18] The molecular structure of $\{[Pim^{Pr^i,Bu^t}]ZnOH\}^+$ has been determined by X-ray diffraction, as illustrated in Fig. 6, which emphasizes the strong relationship with that of $[Tp^{Bu^t,Me}]ZnOH$. The Zn–O and Zn–N bond length data listed in Table 4 reinforce this similarity, and also the relationship with carbonic anhydrase. Since $\{[Pim^{Pr^i,Bu^t}]ZnOH\}^+$ is the only structurally characterized monomeric tetrahedral zinc hydroxide derivative with three imidazole moieties attached to zinc, the complex represents the best structural model for carbonic anhydrase to date.

As with the tris(pyrazolyl)borate derivatives, $[Tp^{RR'}]ZnOH$, the importance of bulky substituents in enabling the isolation of



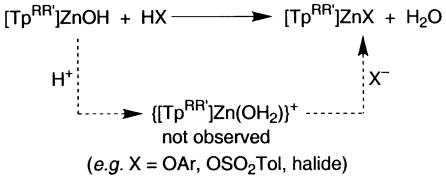
Scheme 3

Table 4 Comparison of Zn–O and Zn–N bond lengths in carbonic anhydrase with those of synthetic analogues

	d(Zn–O)/Å	*d*(Zn–N)/Å
$\{[Pim^{Bu^t,Pr^i}]ZnOH\}^+$	1.86	2.08
$[Tp^{Bu^t,Me}]ZnOH$	1.85	2.05
$[Tp^{Cum,Me}]ZnOH$	1.85	2.05
$\{[Tp^{Bu^t,Me}]Zn(OH_2)\}^+$	1.94	2.02
CAI	1.9	1.9
CAII	2.05	2.11

$\{[Pim^{Pr^i,Bu^t}]ZnOH\}^+$ is underscored by the fact that analogous zinc hydroxide complex species have not been isolated using other tris(imidazolyl)phosphine ligands with less sterically demanding substituents.[6*f*] Furthermore, tris(imidazolyl)carbinol and tris(imidazolyl)alkane ligands have likewise failed to yield structurally characterized terminal zinc hydroxide complexes.[6*f*]

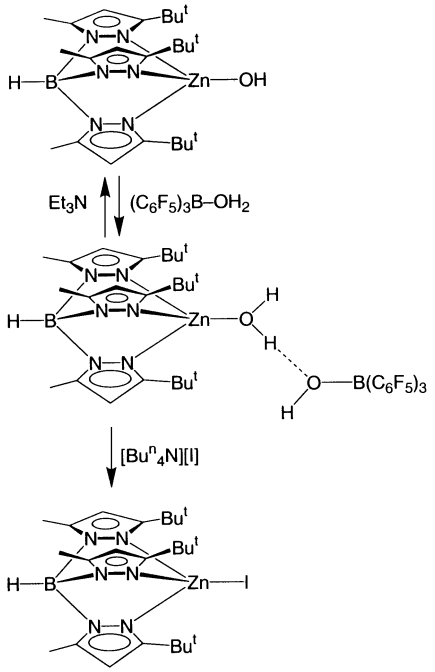
The first essential step in the proposed mechanism of action of carbonic anhydrase (Scheme 1) involves reversible proton transfer which interconverts the aqua and hydroxide forms of the active site, $[(His)_3Zn–OH_2]^{2+}$ and $[(His)_3Zn–OH]^+$. However, while several studies have addressed the factors that influence the pK_a of zinc-bound water molecules in a general sense,[8,15,19] there is to date only a single report demonstrating reversible deprotonation/protonation of a four-coordinate $[Zn–OH_2]^{2+}/[Zn–OH]^+$ pair of complexes. The paucity of such examples is due to the fact that protonation of a zinc hydroxide moiety is typically accompanied by displacement of the aqua ligand by the counter anion (Scheme 4),[20] as illustrated by the



Scheme 4

reaction of $[Tp^{Bu^t,Me}]ZnOH$ with *p*-$TolS(O)_2OH$ to give $[Tp^{Bu^t,Me}]ZnOS(O)_2Tol$.[20*d*] It is, therefore, significant that $[Tp^{Bu^t,Me}]ZnOH$ may be protonated by $(C_6F_5)_3B(OH_2)$ to give a zinc aqua derivative $\{[Tp^{Bu^t,Me}]Zn(OH_2)\}[HOB(C_6F_5)_3]$ in which the water molecule is *not* displaced by the counter ion (Scheme 5).[21] Furthermore, the reaction is reversible and subsequent treatment of $\{[Tp^{Bu^t,Me}]Zn(OH_2)\}^+$ with Et_3N regenerates $[Tp^{Bu^t,Me}]ZnOH$.

The molecular structure of $\{[Tp^{Bu^t,Me}]Zn(OH_2)\}[HOB(C_6F_5)_3]$ has been determined by X-ray diffraction, as



Scheme 5

illustrated in Fig. 7. A notable feature of the structure of {[Tp$^{Bu^t,Me}$]Zn(OH$_2$)}[HOB(C$_6$F$_5$)$_3$] is that the Zn–O bond [1.937(2) Å] is significantly longer than that in [Tp$^{Bu^t,Me}$]ZnOH

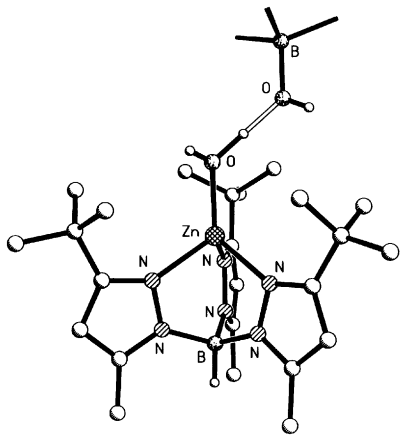
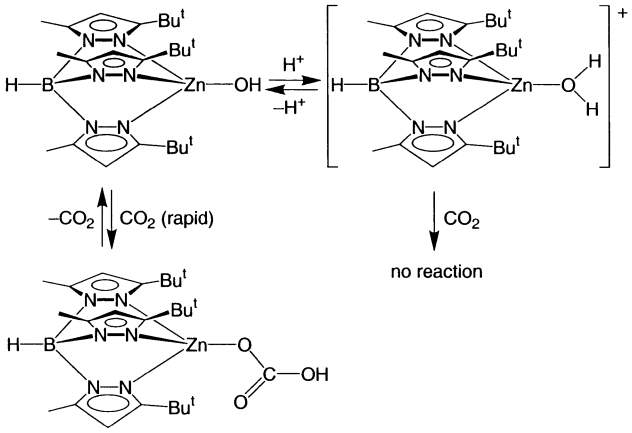


Fig. 7 Molecular structure of {[Tp$^{Bu^t,Me}$]Zn(OH$_2$)}[HOB(C$_6$F$_5$)$_3$].

[1.850(8) Å]. Such lengthening is in accord with the fact that the hydroxide ligand has been protonated. Another interesting aspect of the structure of {[Tp$^{Bu^t,Me}$]Zn(OH$_2$)}[HOB(C$_6$F$_5$)$_3$] is the existence of a hydrogen bond interaction between the coordinated water molecule and the [(C$_6$F$_5$)$_3$BOH]$^-$ counter anion, which bears an analogy to that between the zinc-bound water molecule in carbonic anhydrase and the Thr-199 residue (Fig. 5). The existence of these hydrogen bonding interactions is consistent with the water molecule attached to a tetrahedral Zn^{II} center being acidic. Although the pK_a of {[Tp$^{Bu^t,Me}$]Zn(OH$_2$)}$^+$ has not been accurately determined due to the complications mentioned above, an estimate of *ca*. 6.5 has been cited,[6b] which is comparable to the value of *ca*. 7 for carbonic anhydrase.

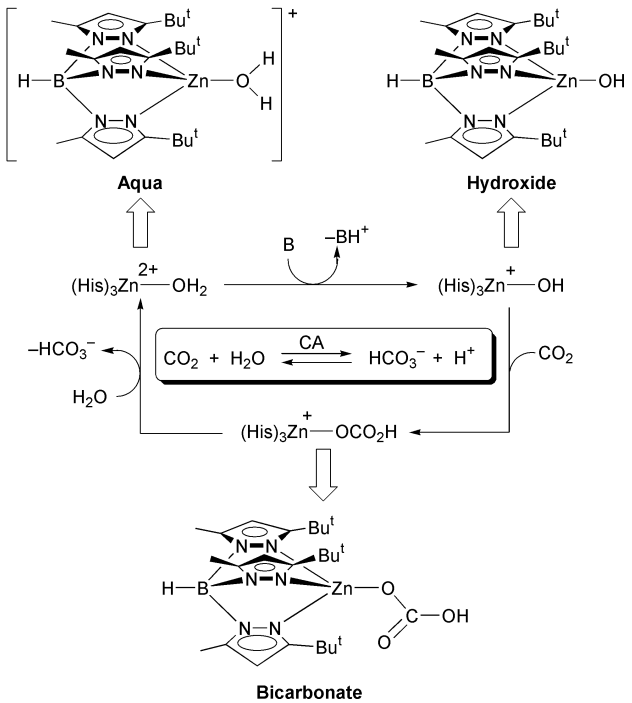
Following deprotonation of the zinc-bound water molecule, the second key step of the mechanism of action of carbonic anhydrase involves the reaction of the nucleophilic zinc–hydroxide group with carbon dioxide to give a bicarbonate intermediate. An excellent precedent for this transformation is provided by the reaction of [Tp$^{Bu^t,Me}$]ZnOH with CO_2 to generate the bicarbonate complex [Tp$^{Bu^t,Me}$]Zn(OCO$_2$H), as illustrated in Scheme 6.[12c] IR spectroscopic studies demonstrate that the bicarbonate ligand is characterized by absorptions at



Scheme 6

1302 and 1675 cm^{-1}, which are indicative of unidentate coordination.

Also of relevance to a catalytic cycle, the formation of the bicarbonate complex [Tp$^{Bu^t,Me}$]Zn(OCO$_2$H) is reversible, and removal of the CO_2 atmosphere results in regeneration of the hydroxide derivative [Tp$^{Bu^t,Me}$]ZnOH (Scheme 6).[12c] Furthermore, ^{1}H NMR spectroscopic studies demonstrate that the equilibration is rapid on the NMR time-scale. Thus, upon addition of CO_2 to [Tp$^{Bu^t,Me}$]ZnOH, the sharp signal attributed to the hydroxide ligand broadens substantially and merges into the base line. The spectroscopic transformation is reversible, and the signal of the hydroxide complex gradually reappears upon progressive removal of the CO_2 atmosphere. The tris(pyrazolyl)hydroborato system has, therefore, allowed direct observation of all three zinc species that correspond to the proposed zinc intermediates of the mechanism of action of carbonic anhydrase, namely aqua, hydroxide, and bicarbonate complexes (Scheme 7). Moreover, their interconversion has



Scheme 7

been shown to be facile. As such, {[Tp$^{Bu^t,Me}$]ZnOH$_2$}$^+$, [Tp$^{Bu^t,Me}$]ZnOH and [Tp$^{Bu^t,Me}$]Zn(OCO$_2$H) represent to date the most thoroughly characterized synthetic analogue system corresponding to carbonic anhydrase.

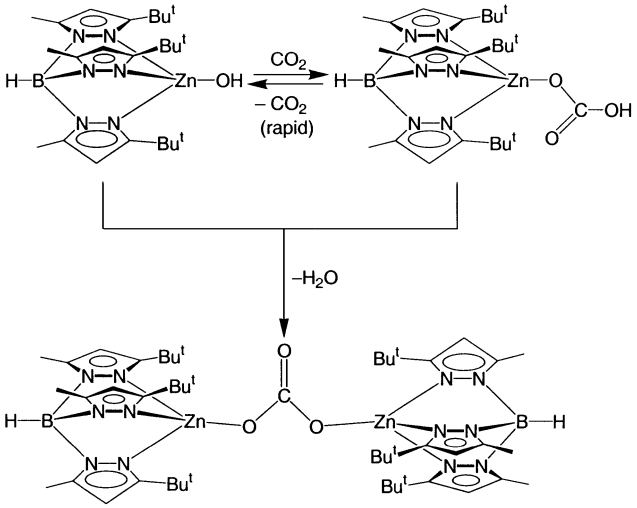
The functional equivalence of [Tp$^{Bu^t,Me}$]ZnOH as a synthetic analogue of carbonic anhydrase has been established by using

^{17}O NMR spectroscopy which demonstrates that [Tp$^{Bu^t,Me}$]Zn-OH is capable of catalyzing the exchange of oxygen atoms between CO_2 and $H_2{}^{17}O$ [eqn. (1)],[12c] a reaction that is also catalyzed by carbonic anhydrase.

$$CO_2 + H_2{}^{17}O \rightleftharpoons CO^{17}O + H_2O \quad (1)$$

Specifically, ^{17}O NMR spectroscopy indicates that, in the presence of a [Tp$^{Bu^t,Me}$]ZnOH catalyst and CO_2, the ^{17}O NMR spectroscopic signal for $H_2{}^{17}O$ in benzene solution is rapidly replaced by the signal for $CO^{17}O$. Under comparable conditions, but in the absence of a [Tp$^{Bu^t,Me}$]ZnOH catalyst, a mixture of $H_2{}^{17}O$ and CO_2 takes several days to reach isotopic equilibrium in benzene solution, thereby clearly demonstrating the efficiency of [Tp$^{Bu^t,Me}$]ZnOH as a functional carbonic anhydrase mimic. Related to the exchange of oxygen atoms between [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.[22]

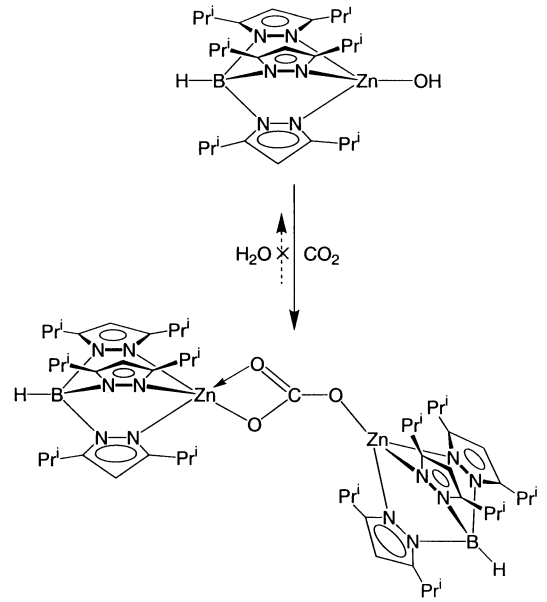
Another aspect of the reaction between [Tp$^{Bu^t,Me}$]ZnOH and CO_2 which deserves mention is that of the condensation reaction between [Tp$^{Bu^t,Me}$]Zn(OCO_2H) and [Tp$^{Bu^t,Me}$]Zn-OH.[12c] Thus, as a result of the facile interconversion between [Tp$^{Bu^t,Me}$]Zn(OCO_2H) and [Tp$^{Bu^t,Me}$]ZnOH, condensation of the latter two molecules may occur to generate a bridging carbonate complex {[Tp$^{Bu^t,Me}$]Zn}$_2$(μ-η^1,η^1-CO_3) which may be isolated over a period of days by virtue of its lower solubility (Scheme 8). The bridging carbonate complex {[Tp$^{Bu^t,Me}$]-



Scheme 8

Zn}$_2$(μ-η^1,η^1-CO_3) is, however, extremely sensitive towards water, thereby regenerating the hydroxide derivative [Tp$^{Bu^t,Me}$]ZnOH.[12c]

The course of the reaction between [Tp$^{RR'}$]ZnOH and CO_2 is strongly influenced by the nature of the pyrazolyl substituents, as illustrated by the reactivity of [Tp$^{Pr^i_2}$]ZnOH towards CO_2 (Scheme 9). Thus, in contrast to the {[Tp$^{Bu^t,Me}$]Zn} system, the bicarbonate complex [Tp$^{Pr^i_2}$]Zn(OCO_2H) is insufficiently stable to be spectroscopically detected and [Tp$^{Pr^i_2}$]ZnOH reacts rapidly with CO_2 to yield the bridging carbonate complex {[Tp$^{Pr^i_2}$]Zn}$_2$(μ-η^1,η^2-CO_3) (Scheme 9), which also differs from that of the {[Tp$^{Bu^t,Me}$]Zn}$_2$(μ-η^1,η^1-CO_3) counterpart by virtue of the nature of the carbonate bridge. In particular, whereas the carbonate ligand in {[Tp$^{Bu^t,Me}$]Zn}$_2$(μ-η^1,η^1-CO_3) binds in a symmetric manner, with unidentate coordination to each zinc center, that in {[Tp$^{Pr^i_2}$]Zn}$_2$(μ-η^1,η^2-CO_3) binds asymmetrically, with unidentate coordination to one zinc center and bidentate coordination to the other zinc center (Scheme 9); the bidentate coordination mode is presumably a consequence of the lower steric demands of the [Tp$^{Pr^i_2}$] ligand. It is also noteworthy that the reactivity of the carbonate ligand towards water is strongly influenced by the nature of the bridge. Thus,



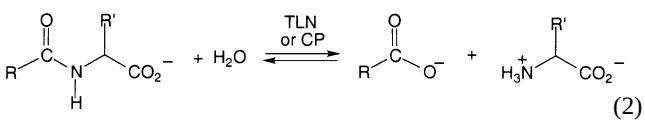
Scheme 9

{[Tp$^{Pr^i_2}$]Zn}$_2$(μ-η^1,η^2-CO_3) is stable towards water (Scheme 9), whereas {[Tp$^{Bu^t,Me}$]Zn}$_2$(μ-η^1,η^1-CO_3) reacts instantaneously to give [Tp$^{Bu^t,Me}$]ZnOH. The significant difference in reactivity emphasizes the extent to which the coordination mode of a carbonate ligand and, by inference, that of a bicarbonate ligand, dictates its stability towards water. Since the final step of the proposed mechanism of action of carbonic anhydrase involves displacement of bicarbonate by water, the above difference in reactivity of {[Tp$^{Pr^i_2}$]Zn}$_2$(μ-η^1,η^2-CO_3) and {[Tp$^{Bu^t,Me}$]-Zn}$_2$(μ-η^1,η^1-CO_3) towards H_2O suggests that factors which promote bidentate coordination of a bicarbonate ligand could inhibit the catalytic cycle. In support of this notion, the increased catalytic activity of [{[12]aneN_4}ZnOH]$^+$ over that of [{[12]aneN_3}ZnOH]$^+$ towards hydration of CO_2 has also been attributed to the greater tendency of the former to form a unidentate, rather than bidentate, bicarbonate intermediate.[23,24]

A final issue concerned with the formation of the bicarbonate intermediate of the catalytic cycle (Scheme 1) is that it requires that the coordinated water be deprotonated prior to reaction with CO_2. Comparative studies on both the hydroxide [Tp$^{Bu^t,Me}$]Zn-OH and aqua {[Tp$^{Bu^t,Me}$]Zn(OH_2)}[HOB(C_6F_5)$_3$] complexes allows this proposal to be confirmed. Thus, whereas [Tp$^{Bu^t,Me}$]-ZnOH reacts rapidly with CO_2 to yield the bicarbonate derivative [Tp$^{Bu^t,Me}$]Zn(OCO_2H), the aqua complex {[Tp$^{Bu^t,Me}$]Zn(OH_2)}$^+$ is inert under comparable conditions (Scheme 6).

(b) The [(His)$_2$(Glu)Zn^{II}–OH_2] motif: thermolysin, carboxypeptidase and neutral protease

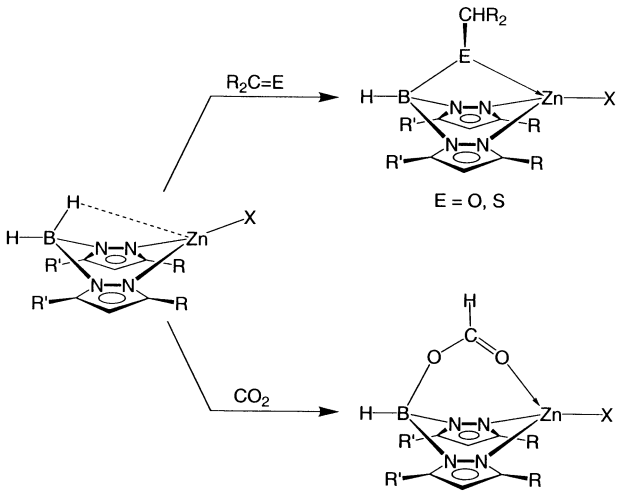
Thermolysin (TLN), carboxypeptidase (CP) and neutral protease are a class of related zinc proteases that are responsible for catalyzing the hydrolysis of peptide bonds [eqn. (2)].



(2)

The active sites of carboxypeptidase, thermolysin, and neutral protease 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. The similarity between thermolysin

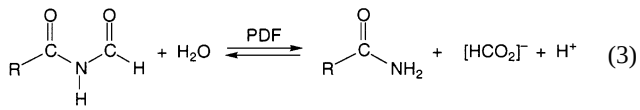
and carboxypeptidase is further emphasized by the fact that the glutamate residue of each enzyme is capable of binding in both a unidentate and bidentate manner. Efforts to obtain synthetic analogues of these enzymes have focused on the use of a variety of tridentate ligands with $[N_2O]$ donor arrays,[25–29] but many of these ligands do not enforce tetrahedral coordination geometries akin to those in the enzymes. For example, ligands such as bis[(3,5-diisopropylpyrazolyl)ethyl] ether, $O(CH_2CH_2pz^{Pr^i_2})_2$, bind with a 'T-shaped' configuration, rather than with the facial configuration required to mimic the tetrahedral coordination in enzymes.[26] The use of a $[N_2O]$ tripod ligand, however, ensures facial binding. Interestingly, such 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 10).[30] The formate derivative $[\eta^3$-$(HCO_2)Bp^{Bu^t,Pr^i}]ZnCl$ that is obtained by reaction of CO_2 with $[Bp^{Bu^t,Pr^i}]ZnCl$ is particularly significant because it is the first structurally characterized tetrahedral zinc complex of a tridentate $[N_2O]$ 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. In addition to using boron as a tripod linker, carbon has also been used as a linker atom to prepare the $[N_2O]$ donor ligand $[HC(pz^{Me_2})(C_6H_2MeBu^tO)]^-$, from which the zinc complexes $[HC(pz^{Me_2})(C_6H_2MeBu^tO)]ZnX$ (X = Cl, Me) have been synthesized (Fig. 8).[28]



Scheme 10

(c) The $[(His)_2(Cys)Zn^{II}–OH_2]$ motif: bacteriophage T7 lysozyme and peptide deformylase

The active site of bacteriophage T7 lysozyme, a zinc enzyme which destroys bacteria by cleaving the amide bond between L-alanine and *N*-acetylmuramate moieties of polysaccharide components within their cell walls, consists of a tetrahedral zinc center which is bound to the protein backbone *via* one sulfur and two nitrogen donors of cysteine and histidine residues, with the fourth site being occupied by a water molecule (Fig. 2).[31] The active site of peptide deformylase is similar to that of T7 lysozyme, but recent studies suggest that the zinc form has low activity and that the active form of the enzyme which is responsible for the hydrolytic cleavage of a formyl group [eqn. (3)] is actually an *iron* enzyme.[32]



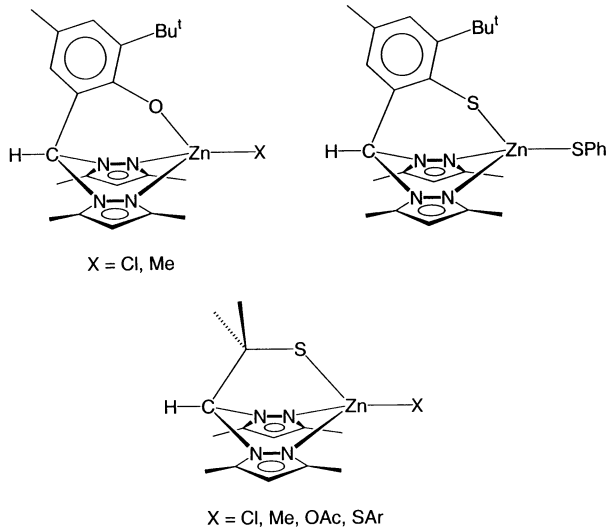
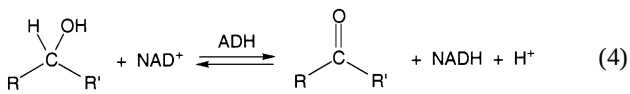


Fig. 8 [NNO] and [NNS] ligands based on the bis(pyrazolyl)methane fragment.

In part due to the propensity of sulfur to act as a bridge between metal centers, tridentate $[N_2S]$ ligands that support monomeric tetrahedral zinc centers analogous to the active sites of peptide deformylase and T7 lysozyme are not common. A second problem is that a variety of studies indicate that the ability of sulfur to coordinate to zinc is very sensitive to the nature of the ligand. For example, the sulfur atom of the thioether derivative $[S(CH_2CH_2pz^{Me_2})_2]ZnCl_2$ does *not* coordinate to zinc.[33] Nevertheless, the use of a tripod construction enforces 'facial' binding of a $[N_2S]$ donor ligand and, by analogy with the $[N_2O]$ ligands $[\{R_2C(H)O\}Bp^{Bu^t,Pr^i}]$ described above, the tetrahedral zinc complex, $[\eta^3$-$\{Ph_2C(H)S\}Bp^{Bu^t,Pr^i}]ZnI$ may be obtained by insertion of Ph_2CS into a B–H bond of $[Bp^{Bu^t,Pr^i}]ZnI$ (Scheme 10).[34] Related complexes, $[HC(pz^{Me_2})_2(C_6H_2MeBu^tS)]ZnSPh$ and $[HC(pz^{Me_2})_2(CMe_2S)]ZnX$ (Fig. 8), derived from $[N_2S]$ ligands that use a carbon, rather than boron, atom linker have also been synthesized.[28,35] Although mononuclear zinc hydroxide complexes of the type $\{[N_2S]ZnOH\}$ have yet to be structurally characterized, it is worth noting that a related dinuclear species has recently been isolated. Thus, only one of the two thioether linkages of the $[N_2S_2]$ bmnpa ligands in the bridging hydroxide complex $\{[(bmnpa)Zn(\mu\text{-}OH)]_2\}^{2+}$ coordinates to each zinc center, such that each zinc adopts a $\{[N_2S]Zn(\mu\text{-}OH)_2\}$ coordination environment [bmnpa = *N*-bis-2-(methylthio)ethyl-*N*-(6-neopentylamino-2-pyridylmethyl)amine].[36a] A dinuclear hydroxide complex with a similar motif, but in which thiosulfate $[S_2O_3]^{2-}$ provides the sulfur ligand, has also been reported.[36b]

(d) The $[(His)(Cys)_2Zn^{II}–OH_2]$ motif: liver alcohol dehydrogenase

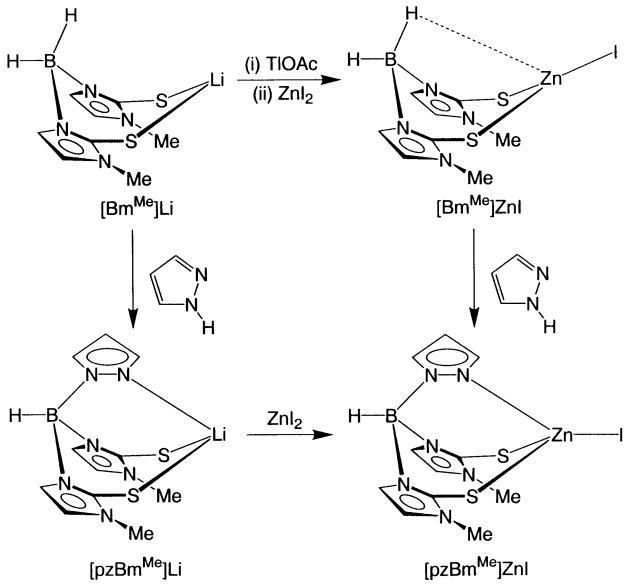
Alcohol dehydrogenases (ADH) belong to an important class of enzymes that catalyze the biological oxidation of primary and secondary alcohols. The oxidations proceed *via* the formal transfer of a hydride anion to the oxidized form of nicotinamide adenine dinucleotide (NAD^+), coupled with the release of a proton [eqn. (4)].



Liver alcohol dehydrogenase (LADH) is the most widely studied of this class of enzymes. X-Ray diffraction studies demonstrate that the active site consists of a zinc center which 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. 2). In addition to the catalytic site, there is also a zinc center that is coordinated tetrahedrally to four cysteine residues, which plays a structural role. Although the sulfur-rich composition of the active site of LADH is relatively uncommon for zinc enzymes, two other examples that have similar active sites are spinach carbonic anhydrase[37] and cytidine deaminase.[38] The coordination environment of the catalytic site in LADH is also not well precedented in zinc chemistry. For example, even though a variety of tridentate [NS_2] donor ligands have been synthesized with a view to modeling LADH,[39–41] none of these previous studies has yielded structurally characterized mononuclear tetrahedral complexes that mimic the active site of LADH. The principal problems of these ligands are associated with (*i*) the proclivity of thiolate groups to act as bridging ligands and thus form oligonuclear structures, and (*ii*) the preference of certain tridentate [NS_2] ligands to bind with a meridional (*i.e.* 'T'-shaped) geometry, rather than the facial binding required to mimic the active site of LADH.

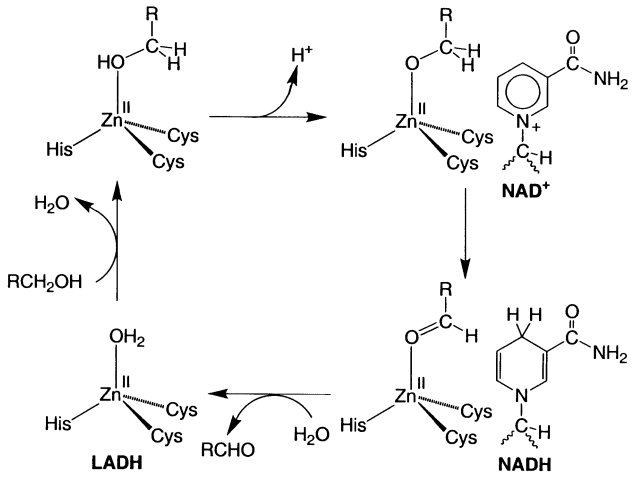
A suitable tridentate [NS_2] donor ligand has, nevertheless, been obtained by reaction of pyrazole with a bis(mercaptomethylimidazolyl)borate derivative.[42] Specifically, the reaction of $LiBH_4$ with methimazole (2 equivalents) yields [Bm^{Me}]Li, from which the [NS_2] donor [$pzBm^{Me}$]Li may be obtained by reaction with pyrazole (Scheme 11). Subsequent transfer of the [NS_2] ligand to zinc is readily achieved by treatment of [$pzBm^{Me}$]Li with ZnI_2, thereby resulting in the formation of [$pzBm^{Me}$]ZnI. Alternatively, [$pzBm^{Me}$]ZnI may be obtained *via* reaction of the [Bm^{Me}]ZnI with pyrazole (Scheme 11).



Scheme 11

The molecular structure of the zinc iodide complex, [$pzBm^{Me}$]ZnI, has been determined by X-ray diffraction which demonstrates that it is indeed mononuclear with a distorted tetrahedral coordination geometry about zinc, and Zn–N and Zn–S bond lengths that are comparable to those within the enzyme. As noted above, the ability of [$pzBm^{Me}$] to provide a facial set of donors is associated with the use of a tetrahedral center as a point of attachment for the donor groups. In this regard, Riordan and coworkers have also recently used tripodal phenylborato ligands to provide [NS_2] donation that is capable of stabilizing monomeric structures, as illustrated by [$Ph(pz)(CH_2SBu^t)_2$]ZnBr and [$Ph(pz^{Bu^t})(CH_2SBu^t)_2$]ZnSPh.[43]

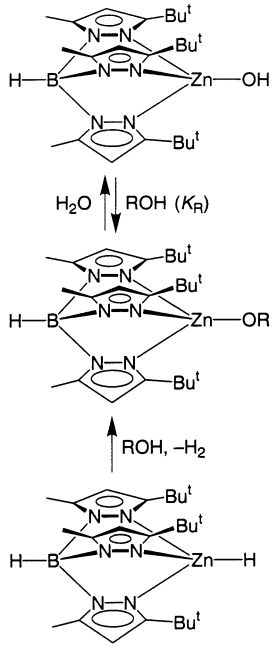
A simplified version of the postulated mechanism of action of LADH is illustrated in Scheme 12, with the essential features involving (*i*) binding NAD^+ (nicotinamide adenine dinucleotide), (*ii*) displacement of the water molecule by alcohol, (*iii*) deprotonation of the coordinated alcohol affording a zinc alkoxide intermediate,[44] *(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 role of the zinc center is, therefore, to facilitate the formation of an alkoxide and thereby enhance hydride transfer to NAD^+. Conversely, in the reverse direction, coordination of the ketone or aldehyde to the zinc center serves to enhance the electrophilicity of the carbonyl carbon atom and thereby promote reduction.



Scheme 12

Interestingly, although the generation of a four-coordinate zinc alkoxide intermediate is an essential step in the catalytic cycle of LADH (Scheme 12), until recently, there was little precedent for the formation of simple aliphatic alkoxide complexes from either zinc aqua or hydroxide derivatives. In view of the non-existence of well defined mononuclear zinc hydroxide complexes with a pseudo-tetrahedral {[NS_2]$Zn^{II}X$} structure, such studies have necessarily focused on complexes with a different structural motif, and specifically those supported by tris(pyrazolyl)hydroborato ligation for which a series of [$Tp^{RR'}$]ZnOH complexes are known. Significantly, ^{1}H NMR spectroscopy demonstrates that the zinc hydroxide [$Tp^{Bu^t,Me}$]-ZnOH complex reacts with ROH (R = Me, Et, Pr^i) to generate the alkoxides [$Tp^{Bu^t,Me}$]ZnOR (Scheme 13);[45] however, in contrast to the reactions of [$Tp^{RR'}$]ZnOH with acidic alcohols (*e.g.* phenols and trifluoroethanol),[46] the simple alkoxides [$Tp^{Bu^t,Me}$]ZnOR (R = Me, Et, Pr^i, Bu^t) are only formed as minor components in an equilibrium mixture (Fig. 9).[45] As such, the alkoxides [$Tp^{Bu^t,Me}$]ZnOR are not readily isolated from the reaction of [$Tp^{Bu^t,Me}$]ZnOH with ROH. It is, therefore, significant that the alkoxides [$Tp^{Bu^t,Me}$]ZnOR may be isolated from the reaction of the hydride complex [$Tp^{Bu^t,Me}$]ZnH with the respective alcohol (Scheme 13). The molecular structure of [$Tp^{Bu^t,Me}$]ZnOEt has been determined by X-ray diffraction, thereby demonstrating its mononuclear tetrahedral nature and confirming its relationship to the proposed intermediate in the mechanism of action of LADH.

An understanding of the factors which influence the stability of tetrahedral zinc alkoxide complexes with respect to hydrolysis is critical to understanding the mechanism of action of LADH. The data presented in Fig. 9 indicate that the equilibrium constant for formation of [$Tp^{Bu^t,Me}$]ZnOR from [$Tp^{Bu^t,Me}$]ZnOH and ROH (R = Me, Et, Pr^i, Bu^t) is highly dependent upon the nature of R, decreasing markedly in the sequence: Me > Et > Pr^i > Bu^t, a trend that is a result of steric and electronic influences (Table 5). Therefore, in an effort to identify the relative importance of these components, the equilibria involving the reactions of [$Tp^{Bu^t,Me}$]ZnOH with a series of *para*-substituted phenols, *p*-XC_6H_4OH, for which electronic substituent parameters (*e.g.* Hammett σ constants) are available, have been studied.[47] Significantly, the data



Scheme 13

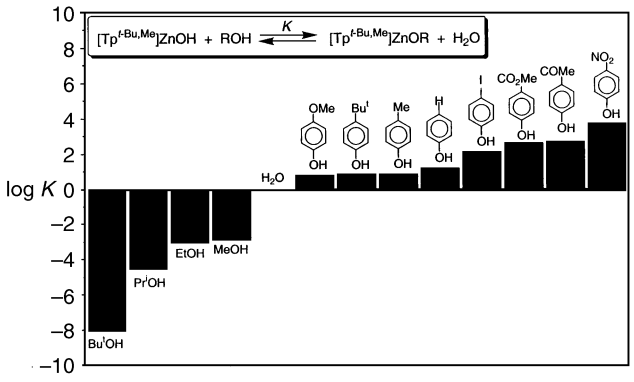


Fig. 9 Variation in alcoholysis equilibrium constant as a function of R.

Table 5 Equilibrium constant and thermodynamic data for alcoholysis reactions of [Tp$^{Bu^t,Me}$]ZnOH with ROH (data taken from ref. 45)

R	K_R(300 K)
Me	$1.4(2) \times 10^{-3}$
Et	$9(2) \times 10^{-4}$
Pr^i	$3(1) \times 10^{-5}$
Bu^t	$\approx 10^{-8}$

presented in Fig. 9 demonstrate that the alcoholysis reactions are very sensitive to electronic influences, being strongly favored for electron withdrawing substituents. Thus, a Hammett plot of log *K vs.* σ gives a good linear correlation with a ρ value of 2.8. The alcoholysis reactions have also been studied computationally using DFT calculations (B3LYP) and the results are in excellent agreement with the experimental results.[47]

The computational study demonstrates that the trend illustrated by the Hammett plot is a consequence of electron withdrawing substituents increasing Zn–OAr BDEs to a greater extent than the corresponding H–OAr BDEs.[47] Thus, rather than exhibit the 1:1 correlation between M–X and H–X bond energies that has been reported for certain other systems,[48] the Zn–OAr BDE is substantially more sensitive to the *para* substituent than is the H–OAr BDE (Fig. 10), *i.e.* D(Zn–OAr) = 1.48 D(H–OAr) − 61 kcal mol^{-1}.[47]

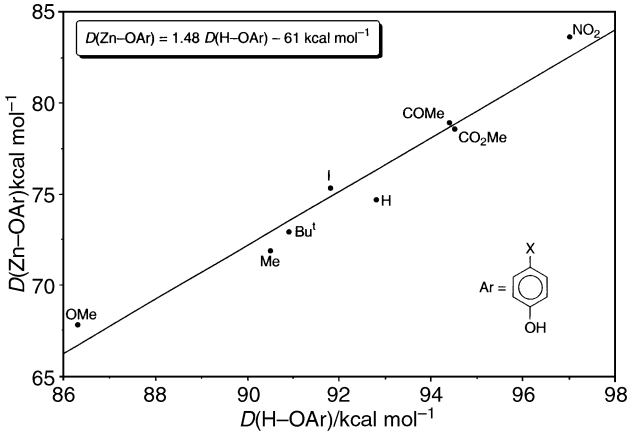


Fig. 10 Correlation of calculated [Zn]–OAr and H–OAr BDEs.

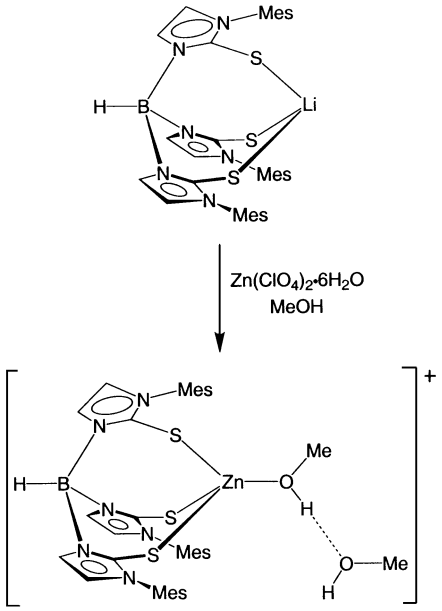
Theoretical studies on phenols have rationalized that the ability of an electron withdrawing *para* substituent to increase the H–OAr BDE is a result of preferential stabilization of the ground state by increasing the delocalization of the electron density from the oxygen atom.[49] On the other hand, electron-donating *para* substituents decrease the H–OAr BDE, but the effect is principally due to stabilization of the ArO• radical, with destabilization of ArOH contributing only to a small degree. The greater influence of an electron withdrawing substituent on the Zn–OAr *vs.* 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.

Calculations on the alkoxides [Tp$^{Bu^t,Me}$]ZnOR (R = Me, Et, Pr^i, Bu^t) indicate that the observed trend is a result of the homolytic Zn–OR BDE decreasing rapidly upon increasing the bulk of R as compared to that for the corresponding H–OR BDE. The greater sensitivity of the Zn–OR BDEs to the bulk of R has been attributed to steric interactions between R and the *tert*-butyl substituents of the [Tp$^{Bu^t,Me}$] ligand in [Tp$^{Bu^t,Me}$]Zn-OR playing a more important role that the interactions between R and H in ROH.[47]

In view of the unfavorable thermodynamics for the formation of the alkoxide derivatives [Tp$^{Bu^t,Me}$]ZnOR from [Tp$^{Bu^t,Me}$]-ZnOH, it is noteworthy that the use of the related tris(mercapto-mesitylimidazolyl)borate ligand [Tm^{Mes}], which features [S_3] rather than [N_3] coordination, favors the formation of an *alcohol* adduct. Thus, the alcohol complex {[Tm^{Mes}]Zn(HOMe)}$^+$ is obtained by reaction of Li[Tm^{Mes}] with $Zn(ClO_4)_2$ in methanol (Scheme 14).[50] This observation indicates that the sulfur rich coordination environment provided by [Tm^{Mes}] 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.

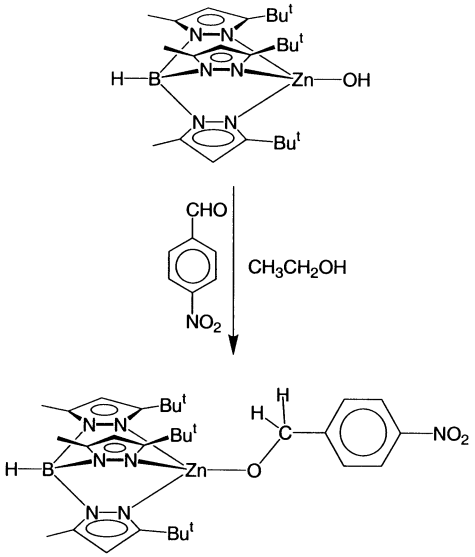
The alcohol coordination mode in {[Tm^{Mes}]Zn(HOMe)}$^+$ resembles aspects of that in LADH. For example, the Zn–O bond length in {[Tm^{Mes}]Zn(HOMe)}$^+$ (1.99 Å) is effectively identical to that in the $C_6F_5CH_2OH$ adduct of LADH (2.0 Å) and the hydroxy group of the coordinated methanol participates in a hydrogen bonding interaction with an additional molecule of methanol, which resembles the hydrogen bond network at the active site of LADH. For example, the hydrogen bonded O···O separation of 2.58 Å in {[Tm^{Mes}]Zn(HOMe)(HOMe)}$^+$ is effectively identical to that between the zinc-bound alcohol at the active site of LADH and Ser-48 (2.6 Å).

A final issue concerned with the proposed role of zinc alkoxide intermediates is their potential for 'hydride' transfer to NAD$^+$. Studies employing *p*-nitrobenzaldehyde as a NAD$^+$ hydride acceptor mimic[51] provide evidence that the alkoxide complexes [Tp$^{Bu^t,Me}$]ZnOR (R = Et, Pr^i) are indeed capable of



Scheme 14

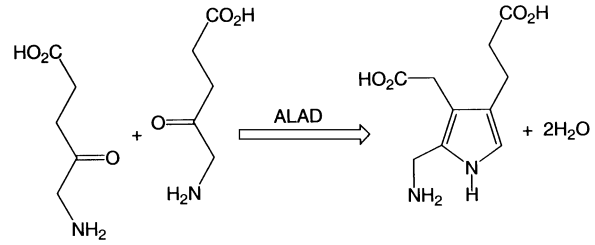
such a transformation. For example, [Tp$^{Bu^t,Me}$]ZnOEt reacts with ArCHO (Ar = *p*-$C_6H_4NO_2$) in benzene to yield [Tp$^{Bu^t,Me}$]ZnOCH_2Ar and MeCHO (Scheme 15). Furthermore, solutions of ArCHO in ROH (R = Me, Et, Pr^i) yield ArCH_2OH at *ca*. 90 °C in the presence of [Tp$^{Bu^t,Me}$]ZnOH.



Scheme 15

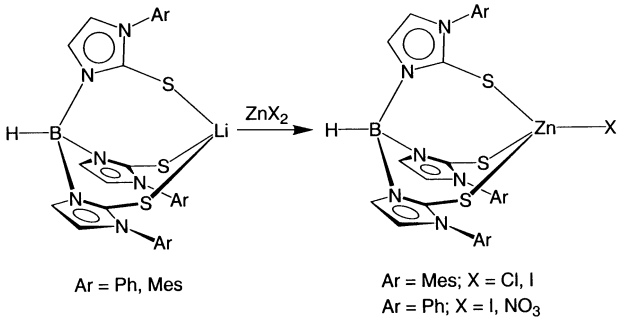
(e) The [(Cys)$_3$Zn^{II}–OH$_2$] motif: 5-aminolevulinate dehydratase

5-Aminolevulinate dehydratase (ALAD), also known as porphobilinogen synthase (PBGS), catalyzes the dimerization of 5-aminolevulinic acid (ALA) to porphobilinogen, a monopyrrole (Scheme 16).[52] This transformation is of considerable importance since it is the first step in the biological synthesis of tetrapyrroles (including heme, chlorophyll and cobalamins). ALAD is a zinc dependent enzyme, containing both catalytic and structural zinc sites, of which the catalytic site possesses the unusual composition of [(Cys)$_3$Zn^{II}(OH$_2$)] (Fig. 2).[53] As discussed above for liver alcohol dehydrogenase, due to the proclivity of sulfur containing ligands to bridge more than one zinc center, mononuclear tetrahedral zinc complexes with sulfur rich coordination environments that mimic the active site of ALAD are not common. Nevertheless, the sterically demanding



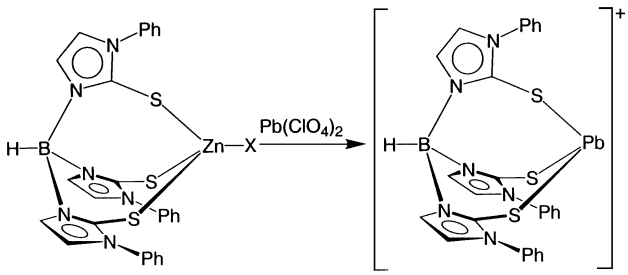
Scheme 16

tris(mercaptoarylimidazolyl)borate ligands, [Tm^{Ar}] (Ar = Ph, Mes), described above may be considered to provide the requisite motif which mimics the three cysteine residues at the active site of ALAD. Thus, a series of [Tm^{Ar}]ZnX complexes have been prepared, *e.g.* [Tm^{Ph}]ZnX (X = I, NO_3) and [Tm^{Mes}]ZnX (X = Cl, I),[50] as illustrated in Scheme 17.



Scheme 17

An important aspect of the chemistry of ALAD is concerned with lead poisoning. Specifically, lead is the most commonly encountered toxic metal pollutant in the environment,[54] and its toxicological properties are associated with its interactions with proteins and, in particular, ALAD.[55] 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, both [Tm^{Ph}]ZnI and {[Tm^{Ph}]Zn(NCMe)}(ClO_4) react rapidly with Pb(ClO_4)$_2$•*x*H_2O to give the lead complex {[Tm^{Ph}]Pb}(ClO_4) (Scheme 18).[56]



Scheme 18

The molecular structure of {[Tm^{Ph}]Pb}$^+$ has been determined by X-ray diffraction (Fig. 11), thereby demonstrating that the trigonal-pyramidal geometry bears a close correspondence to the active site of Pb^{II}–ALAD.[57] 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. It is also important to emphasize that the lead coordination environment in {[Tm^{Ph}]Pb}$^+$ is in marked contrast to that of zinc in {[Tm^{Ph}]Zn(NCMe)}$^+$, an observation which 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 three coordinate Pb^{II} center is of significance to the inactivity of Pb^{II}–ALAD since the mechanism of action has been proposed to involve activation of ALA by interaction of the ketone group with the

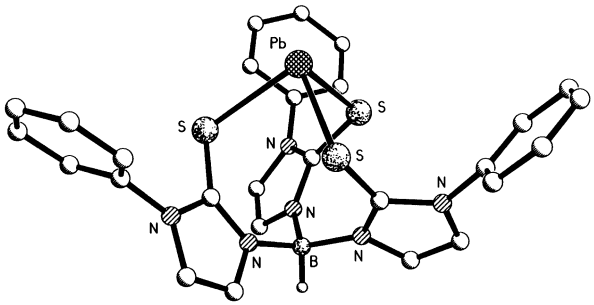


Fig. 11 Molecular structure of {[Tm^{Ph}]Pb}$^{+}$.

Zn^{II} center.[52] The reduced Lewis acidity of a three coordinate Pb^{II} center indicates that the formation of the required tetrahedral intermediate [(Cys)$_3$Pb^{II}–ALA] would be inhibited.

The binding preferences of lead and zinc to ligands which mimic the coordination motif in ALAD is of relevance to obtaining a thorough understanding of the factors responsible for the debilitating effects of lead poisoning. It is, therefore, significant that a study of the equilibrium involving ligand exchange between {[Tm^{Ph}]Pb}(ClO$_4$) and Zn(ClO$_4$)$_2$ in MeCN [eqn. (5)] indicates that the preference of [Tm^{Ph}] to coordinate Pb^{II} over Zn^{II} in this system is *ca.* 500 : 1. This value is substantially greater than the *ca.* 25 : 1 relative affinity of these metals to reside at the active site of human erythrocyte ALAD.[58]

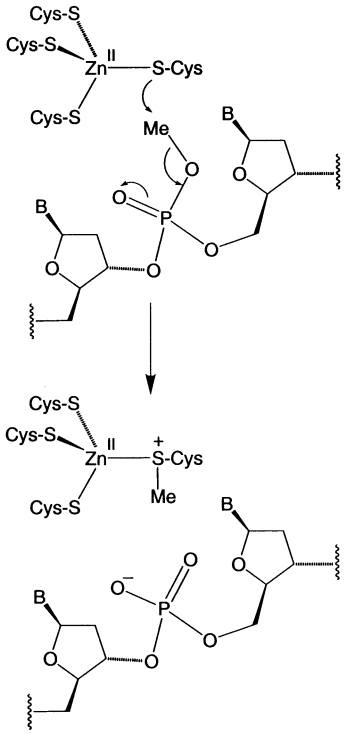
$$\{[Tm^{Ph}]Pb\}^{+} + Zn^{2+} + MeCN \overset{K}{\rightleftharpoons} \{[Tm^{Ph}]Zn(NCMe)\}^{+} + Pb^{2+} \qquad (5)$$

More interestingly, 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 in the presence of NaI. Thus, addition of NaI to a mixture of {[Tm^{Ph}]Pb}(ClO$_4$) and Zn(ClO$_4$)$_2$ in acetonitrile 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$_2$. This observation is of relevance since a completely effective means to reverse the toxic effects of lead in the human body are not yet known, despite efforts to develop lead complexing agents.[59]

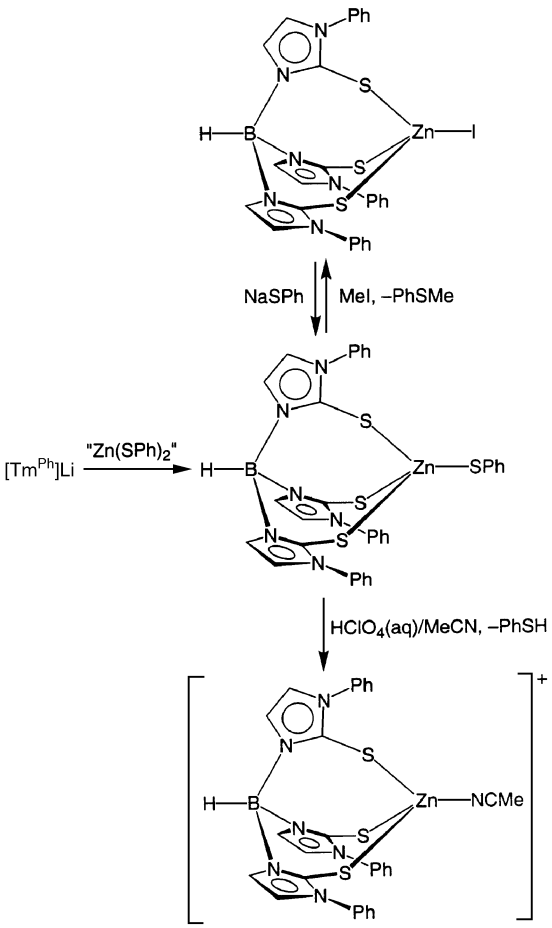
(f) The [(Cys)$_4$Zn^{II}] motif: The Ada DNA repair protein

In addition to the ubiquitous [{XYZ}Zn^{II}–OH$_2$] motif in which the coordinated water molecule plays a critical role, recent studies indicate that zinc may also play an important role by activating thiols towards nucleophilic attack. For example, alkylation of zinc thiolates has been proposed to be a step in the mechanism of action of the Ada DNA repair protein which is responsible for demethylating DNA in a stoichiometric manner (Scheme 19);[60] related zinc thiolate reactivity has also been proposed for other enzymes.[61] The active site of the Ada repair protein possesses a [(Cys)$_4$Zn] motif and has been modeled by the anion [Zn(SPh)$_4$]$^{2-}$.[62] Thus, Wilker and Lippard have reported that [Me$_4$N]$_2$[Zn(SPh)$_4$] reacts with (MeO)$_3$PO to form PhSMe, (MeO)$_2$PO$_2^-$, and [Zn(SPh)$_3$]$^-$ *via* initial heterolytic dissociation generating an incipient thiolate anion. More recently, the thiolate complex [Tm^{Ph}]ZnSPh (Scheme 20) has been introduced as a model for the Ada repair protein, in which the [Tm^{Ph}] ligand mimics the three cysteine residues that remain bound to zinc during the course of the alkylation reaction.[63] The phenylthiolate ligand in [Tm^{Ph}]ZnSPh also possesses nucleophilic character and is alkylated by MeI to give PhSMe and [Tm^{Ph}]ZnI, a transformation that is analogous to the reactivity exhibited by tris(pyrazolyl)borate complexes [Tp$^{RR'}$]ZnSR[64] and other scorpionate thiolates.[65]

Methionine synthases are a class of enzymes related to the Ada protein in that thiolate alkylation is a common feature.[61*a*] The active sites of the various methionine synthases also feature cysteine coordination, and Riordan and coworkers have recently



Scheme 19



Scheme 20

introduced the use of the phenylborato ligands [PhB(CH$_2$SBu^t)$_3$] and [Ph(pz$^{Bu^t}$)(CH$_2$SBu^t)$_2$] as [S$_3$] and [NS$_2$] donors to mimic the active sites in these enzymes (Fig. 12).[43]

In addition to alkylation of zinc–cysteine thiolates, proteolytic cleavage of such groups is of relevance to the mechanism

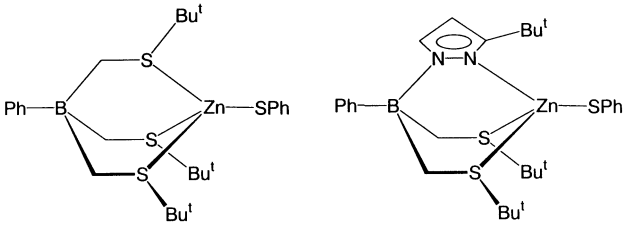


Fig. 12 Methionine synthase mimics.

of action of matrix metalloproteinases (matrixins) which are an important group of zinc enzymes responsible for degradation of the extracellular matrix components.[66] One of the mechanisms for activating the proenzyme involves proteolytic cleavage of the cysteine thiolate residue and a chemical model for this process is provided by the reactivity of $[Tm^{Ph}]ZnSPh$ towards H^+. Specifically, treatment of $[Tm^{Ph}]ZnSPh$ with $HClO_4$ in acetonitrile results in elimination of PhSH and formation of $\{[Tm^{Ph}]Zn(NCMe)\}(ClO_4)$, as illustrated in Scheme 20.

4. Metal ion substitution for probing zinc enzymes

As a result of the poor spectroscopic properties of Zn^{II}, it is difficult to obtain information concerning the structure of the active site of the enzyme and the nature of the intermediates involved in the catalytic cycle. For this reason, considerable effort has been directed towards investigating enzymes in which the zinc has been replaced by various other metals in an effort to incorporate a spectroscopic probe, *e.g.* Co^{II} (UV–vis) and Cd^{II} (NMR).[67] However, the various metal substituted enzymes often exhibit markedly different activities, and so the use of metal ion substitution to provide insight into the mechanism of action depends critically on a knowledge of the chemistry of various metal ions in coordination environments that are closely related to the enzyme active site. Therefore, in an effort to provide such information, the chemistry of other metals in coordination environments related to those of zinc enzymes has also been investigated.

(a) Influence of the metal on active site coordination geometry

Cobalt is the metal that is most commonly substituted into zinc enzymes because it has distinct electronic spectroscopic properties and also has a strong tendency to form tetrahedral complexes. However, structural studies 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,[68] as illustrated by the carbonic anhydrase synthetic analogues $\{[Tp^{Pr^i_2}]M(\mu\text{-}OH)\}_n$. Thus, of these derivatives, only the zinc complex $[Tp^{Pr^i_2}]ZnOH$ 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, $\{[Tp^{Pr^i_2}]M(\mu\text{-}OH)\}_2$ (M = Mn, Fe, Co, Ni, Cu)[12d] (Fig. 13).

Interesting differences as a function of metal are also observed for complexes obtained using tris(imidazolyl)phosphine ligands. For example, the reactions of $[Pim^{Pr^i,Bu^t}]$ with $M(ClO_4)_2{\cdot}6H_2O$ (M = Zn, Cd) yield $\{[Pim^{Pr^i,Bu^t}]ZnOH\}^+$ and $\{[Pim^{Pr^i,Bu^t}]Cd(OH_2)(OClO_3)\}^+$ which, as illustrated in Fig. 14, possess significantly different structures.[69] Thus, the zinc complex $\{[Pim^{Pr^i,Bu^t}]ZnOH\}^+$ exists as a simple tetrahedral hydroxide derivative, whereas the cadmium counterpart is a *five*-coordinate *aqua* complex, $\{[Pim^{Pr^i,Bu^t}]Cd(OH_2)(OClO_3)\}^+$. In addition to the different structures, the fact that

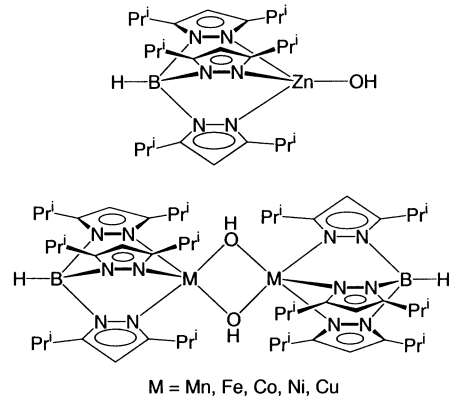


Fig. 13 Mononuclear and dinuclear metal hydroxides.

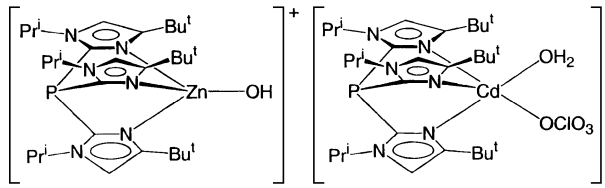


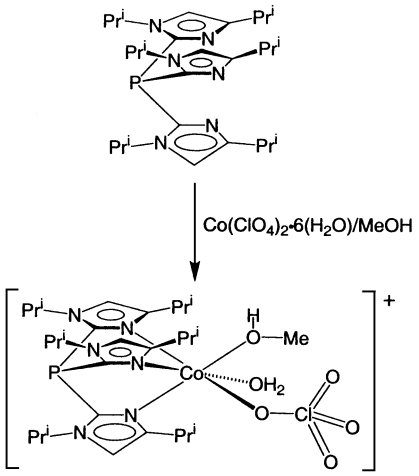
Fig. 14 Comparison of the structures of $\{[Pim^{Pr^i,Bu^t}]ZnOH\}^+$ and $\{[Pim^{Pr^i,Bu^t}]Cd(OH_2)(OClO_3)\}^+$.

the cadmium center binds a ligand as weakly coordinating as perchlorate is particularly interesting because it indicates that biologically more pertinent anions should also coordinate to a tetrahedral $[\{N_3\}Cd^{II}(OH_2)]$ center and thereby suggests that Cd^{II}–carbonic anhydrase may not be tetrahedral.

The observation that the cadmium complex $\{[Pim^{Pr^i,Bu^t}]Cd(OH_2)(OClO_3)\}^+$ also exists as an aqua species provides a rationalization for the reduced activity of Cd^{II}–carbonic anhydrase since access to a hydroxide species is required for efficient carbonic anhydrase activity. The reluctance of the aqua complex $\{[Pim^{Pr^i,Bu^t}]Cd(OH_2)(OClO_3)\}^+$ to convert to a hydroxide species is a consequence of the coordinated perchlorate ligand since the acidity of a coordinated water molecule is reduced considerably upon the binding of an anionic ligand, *i.e.* the pK_a of the monocation $\{[Pim^{Pr^i,Bu^t}]Cd(OH_2)(OClO_3)\}^+$ would be expected to be greater than that of dicationic $\{[Pim^{Pr^i,Bu^t}]Zn(OH_2)\}^{2+}$. The fact that Cd^{II}–carbonic anhydrase only exhibits significant activity at higher pH (corresponding to the ionization of a cadmium-bound water molecule with a pK_a of *ca.* 9) is in accord with the isolation of the aqua species $\{[Pim^{Pr^i,Bu^t}]Cd(OH_2)(OClO_3)\}^+$, with a higher pH being necessary to generate the requisite hydroxide species.

An investigation of the reaction of $Co(ClO_4)_2{\cdot}6H_2O$ with $[Pim^{Pr^i_2}]$ in methanol also indicates the reluctance of cobalt to form simple tetrahedral cobalt hydroxide complexes by forming the six-coordinate aqua–methanol–perchlorate complex $\{[Pim^{Pr^i_2}]Co(OH_2)(HOMe)(OClO_3)\}^+$ (Scheme 21).[70]

Structural studies on a series of complexes of the bis(2-mercapto-1-methylimidazolyl)(pyrazolyl)hydroborato $[NS_2]$ donor ligand also indicate interesting differences in coordination mode as a function of the metal. Thus, of the $[pzBm^{Me}]_2Zn$, $[pzBm^{Me}]_2Co$ and $[pzBm^{Me}]_2Cd$ derivatives (Fig. 15), only the zinc complex has a tetrahedral $M[S_4]$ structure which resembles the $[(Cys)_4Zn]$ structural sites in enzymes such as LADH. In contrast, the cobalt and cadmium derivatives, $[pzBm^{Me}]_2Co$ and $[pzBm^{Me}]_2Cd$, exhibit structures in which the B–H groups also interact with the metal center. The non-tetrahedral nature of the latter complexes indicates that zinc has a greater preference for tetrahedral $M[S_4]$ coordination, which is in accord with its prevalent role in the structural sites of enzymes.



Scheme 21

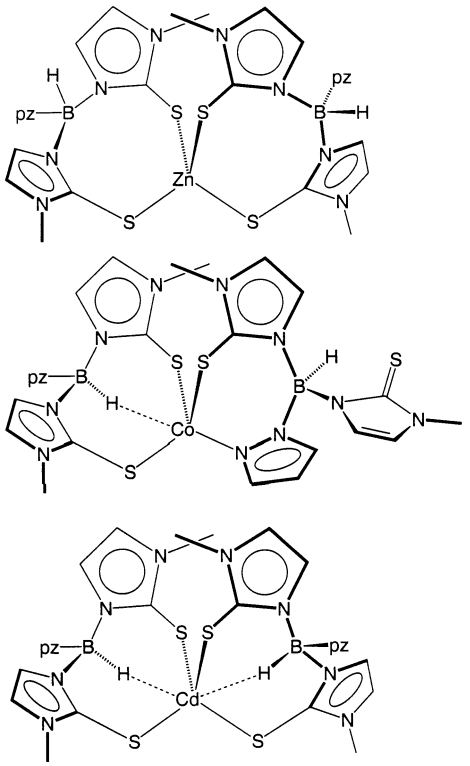


Fig. 15 Structures of $[pzBm^{Me}]_2M$ (M = Zn, Co, Cd) indicating the greater preference of zinc to adopt tetrahedral $M[S_4]$ coordination.

(b) Nitrate ligands as a probe for trends in bicarbonate coordination modes in metal-substituted carbonic anhydrases

The final step of the carbonic anhydrase catalytic cycle, *i.e.* displacement of a bicarbonate ligand by water, is undoubtedly dependent on the coordination mode of the bicarbonate ligand. For example, Co^{II}–carbonic anhydrase is less active than the zinc enzyme and X-ray diffraction studies indicate that the bicarbonate ligand binds in a bidentate manner. An appreciation of the factors that influence the coordination mode of a bicarbonate ligand is, therefore, critical to understanding the relative activities of metal-substituted carbonic anhydrases. However, such a study is presently not possible due to the general instability of bicarbonate complexes, with no zinc bicarbonate complex having been structurally characterized. For this reason, the nitrate ligand has been employed as a probe to provide an indication of the structural variations that would be expected for a series of bicarbonate complexes.[12c,68] Specifically, since bicarbonate and nitrate ligands are isoelectronic and sterically similar, the *variation* in nitrate coordination mode (Fig. 16) for a series of analogous metal complexes is anticipated to correlate with the *trend* observed for analogous bicarbonate complexes.

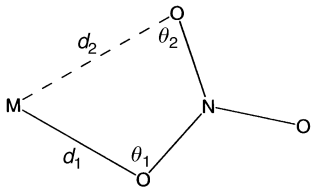


Fig. 16 Parameters used in classifying nitrate coordination mode.

The variation in nitrate ligand coordination mode has been examined for the tris(pyrazolyl)hydroborato and tris(imidazolyl)phosphine complexes, $[Tp^{Bu^t,R}]M(NO_3)$ and $\{[Pim^{Pr^i,Bu^t}]M(NO_3)\}^+$,[68] as summarized in Table 6. 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. The data listed in Table 6 indicate that the bidenticity increases in the sequence Zn < Hg < Co < Cu ≈ Ni ≈ Cd, reflecting the different electronic properties of the metals. Steric effects are also important in influencing the coordination mode, as illustrated by the observation that the asymmetry of the nitrate coordination mode decreases across the series $[Tp^{Bu^t}]Zn(NO_3)$,[68a] $[Tp^{Ph}]Zn(NO_3)$,[71] and $[Tp]Zn(NO_3)$[72]

Table 6 Comparison of nitrate coordination mode of $LM(NO_3)^{Q+a}$ with activity of M^{II}–CA (data taken from ref. 68*d*)

M	M^{II}–CA activity (%)	Δd/Å		$\Delta\theta$/°	
		$[Pim^{Pr^i,Bu^t}]$	$[Tp^{Bu^t,R}]^b$	$[Pim^{Pr^i,Bu^t}]$	$[Tp^{Bu^t,R}]^b$
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] R = H for M = Zn, Co, Cu, Ni; R = Me for M = Cd.

Interestingly, with the exception of mercury, the nitrate coordination mode in these complexes correlates with the activity of metal substituted carbonic anhydrases: Zn > Co >> Cu ≈ Ni ≈ Cd ≈ Hg (Table 6). Specifically, those metals with almost symmetric bidentate coordination are inactive, whereas those with significant asymmetry (Zn and Co) are active. In this regard, it is also noteworthy that the carbonate ligand in the complexes $\{[Tp^{Pr^i_2}]M\}_2(\mu\text{-}CO_3)$ (M = Mn, Fe, Co, Ni, Cu)[12d] exhibits varying degrees of asymmetry that closely parallel the series of nitrate complexes described above, and thereby provides further support for the notion that nitrate is a good model for bicarbonate.

(c) Spectroscopic models

Finally, another use for synthetic analogues is to provide spectroscopic signatures that enable the assignment of the coordination environments of the active sites of metalloenzymes. For example, (*i*) the similarity of the electronic spectrum of blue $[Tp^{Bu^t}]CoCl$[68b] with that of the high pH form of Co^{II}–carbonic anhydrase is consistent with the notion that the active site exhibits a pseudo-tetrahedral coordination geometry,[67] (*ii*) comparison of the electronic spectra of purple $[Tp^{Bu^t}]NiCl$, yellow $[Tp^{Bu^t}]Ni(\eta^2\text{-}O_2NO)$ and green $[Tp^{Me_2}]Ni(\eta^2\text{-}O_2NO)(THF)$,[68b] with the electronic spectrum of Ni^{II}–carbonic anhydrase supports the notion that nickel is six-coordinate in the enzyme at neutral pH, and (*iii*) comparison of the electronic spectrum of Cu^{II}–carbonic anhydrase with those

of [Tp$^{Bu^t}$]CuCl and [Tp$^{Bu^t}$]Cu(η^2-O_2NO) indicates a greater similarity to the five-coordinate structure.[68b]

Acknowledgements

I sincerely wish to thank the students and postdoctoral researchers who carried out portions of the research described in this article, which was supported by the National Institutes of Health (Grant GM46502).

Notes and references

1 H. Vahrenkamp, *Chem. Unserer Zeit*, 1988, **22**, 73.
2 (*a*) B. L. Vallee and D. S. Auld, *Acc. Chem. Res.*, 1993, **26**, 543; (*b*) W. N. Lipscomb and N. Sträter, *Chem. Rev.*, 1996, **96**, 2375; (*c*) D. S. Auld, *Struct. Bonding (Berlin)*, 1997, **89**, 29; (*d*) J. E. Coleman, *Curr. Opin. Chem. Biol.*, 1998, **2**, 222; (*e*) S. Rahuel-Clermont and M. F. Dunn, *Copper Zinc Inflammatory Degener. Dis.*, 1998, 47.
3 C. F. Mills, *Zinc in Human Biology*, Springer-Verlag, New York, 1989.
4 See, for example: I. L. Alberts, K. Nadassy and S. J. Wodak, *Protein Sci.*, 1998, **7**, 1700.
5 R. J. P. Williams, in *Zinc in Human Biology*, ed. I. Macdonald, Springer-Verlag, New York, 1989, ch 2.
6 For lead references, see: (*a*) E. Kimura and E. Kikuta, *J. Biol. Inorg. Chem.*, 2000, **5**, 139; (*b*) H. Vahrenkamp, *Acc. Chem. Res.*, 1999, **32**, 589; (*c*) H. Vahrenkamp, *Bioinorganic Chemistry—Transition Metals in Biology and their Coordination Chemistry*, Wiley-VCH, Weinheim, 1997, pp. 540–551; (*d*) R. S. Brown, *NATO ASI Ser., Ser. C*, 1990, **314**, 145; (*e*) L. Banci, I. Bertini, C. Luchinat and J. M. Moratal, *NATO ASI Ser., Ser. C*, 1990, **314**, 181; (*f*) G. Parkin, in *Probing of Proteins by Metal Ions and Their Low-Molecular-Weight Complexes, Met. Ions Biol. Syst.*, ed. A. Sigel and H. Sigel, M. Dekker, New York, vol. 38, in press.
7 C. W. Bock, A. K. Katz and J. P. Glusker, *J. Am. Chem. Soc.*, 1995, **117**, 3754.
8 H. Sigel and R. B. Martin, *Chem. Soc. Rev.*, 1994, **23**, 83.
9 For recent reviews, see: (*a*) S. Trofimenko, *Scorpionates – The Coordination Chemistry of Polypyrazolylborate Ligands*, Imperial College Press, London, 1999; (*b*) G. Parkin, *Adv. Inorg. Chem.*, 1995, **42**, 291; (*c*) N. Kitajima and W. B. Tolman, *Prog. Inorg. Chem.*, 1995, **43**, 419.
10 S. Trofimenko, J. C. Calabrese and J. S. Thompson, *Inorg. Chem.*, 1987, **26**, 1507.
11 See, for example: (*a*) S. Lindskog, *Pharmacol. Ther.*, 1997, **74**, 1; (*b*) D. N. Silverman, *Can. J. Bot.*, 1991, **69**, 1070.
12 (*a*) R. Alsfasser, S. Trofimenko, A. Looney, G. Parkin and H. Vahrenkamp, *Inorg. Chem.*, 1991, **30**, 4098; (*b*) R. Alsfasser, M. Ruf, S. Trofimenko and H. Vahrenkamp, *Chem. Ber.–Recl.*, 1993, **126**, 703; (*c*) A. Looney, R. Han, K. McNeill and G. Parkin, *J. Am. Chem. Soc.*, 1993, **115**, 4690; (*d*) N. Kitajima, S. Hikichi, M. Tanaka and Y. Morooka, *J. Am. Chem. Soc.*, 1993, **115**, 5496; (*e*) M. Ruf and H. Vahrenkamp, *Inorg. Chem.*, 1996, **35**, 6571; (*f*) A. Looney, G. Parkin, R. Alsfasser, M. Ruf and H. Vahrenkamp, *Angew. Chem., Int. Ed. Engl.*, 1992, **31**, 92.
13 F. Hartmann, W. Kläui, A. Kremer-Aach, D. Mootz, A. Strerath and H. Wunderlich, *Z. Anorg. Allg. Chem.*, 1993, **619**, 2071.
14 P. Chaudhuri, C. Stockheim, K. Wieghardt, W. Deck, R. Gregorzik, H. Vahrenkamp, B. Nuber and J. Weiss, *Inorg. Chem.*, 1992, **31**, 1451.
15 E. Kimura, T. Shiota, T. Koike, M. Shiro and M. Kodama, *J. Am. Chem. Soc.*, 1990, **112**, 5805.
16 A. S. Borovik, personal communication.
17 For the cobalt analogue, see: B. S. Hammes, V. G. Young, Jr. and A. S. Borovik, *Angew. Chem., Int. Ed.*, 1999, **38**, 666.
18 C. Kimblin, W. E. Allen and G. Parkin, *J. Chem. Soc., Chem. Commun.*, 1995, 1813.
19 (*a*) I. Bertini, C. Luchinat, M. Rosi, A. Sgamellotti and F. Tarantelli, *Inorg. Chem.*, 1990, **29**, 1460; (*b*) M. Sola, A. Lledos, M. Duran and J. Bertran, *Inorg. Chem.*, 1991, **30**, 2523.
20 (*a*) M. Ruf, K. Weis and H. Vahrenkamp, *J. Chem. Soc., Chem. Commun.*, 1994, 135; (*b*) M. Ruf, K. Weis, I. Brasack and H. Vahrenkamp, *Inorg. Chim. Acta*, 1996, **250**, 271; (*c*) M. Ruf, K. Weis and H. Vahrenkamp, *Inorg. Chem.*, 1997, **36**, 2130; (*d*) T. Brandsch, F. A. Schell, K. Weis, M. Ruf, B. Muller and H. Vahrenkamp, *Chem. Ber.–Recl.*, 1997, **130**, 283; (*e*) S. Hikichi, M. Tanaka, Y. Morooka and N. Kitajima, *J. Chem. Soc., Chem. Commun.*, 1992, 814; (*f*) U. Hartmann and H. Vahrenkamp, *Inorg. Chem.*, 1991, **30**, 4676; (*g*) U. Hartmann and H. Vahrenkamp, *Chem. Ber.*, 1994, **127**, 2381.
21 C. Bergquist and G. Parkin, *J. Am. Chem. Soc.*, 1999, **121**, 6322.
22 M. Bräuer, E. Anders, S. Sinnecker, W. Koch, M. Rombach, H. Brombacher and H. Vahrenkamp, *Chem. Commun.*, 2000, 647.
23 (*a*) X. Zhang and R. Van Eldik, *Inorg. Chem.*, 1995, **34**, 5606; (*b*) X. Zhang, R. van Eldik, T. Koike and E. Kimura, *Inorg. Chem.*, 1993, **32**, 5749.
24 (*a*) X. Zhang, C. D. Hubbard and R. van Eldik, *J. Phys. Chem.*, 1996, **100**, 9161; (*b*) S. Schindler, C. D. Hubbard and R. van Eldik, *Chem. Soc. Rev.*, 1998, **27**, 387.
25 C. Dowling and G. Parkin, *Polyhedron*, 1996, **15**, 2463 and references therein.
26 C. Dowling, V. J. Murphy and G. Parkin, *Inorg. Chem.*, 1996, **35**, 2415.
27 P. Ghosh and G. Parkin, *J. Chem. Soc., Dalton Trans.*, 1998, 2281.
28 S. S. Tandon, S. Chander, L. K. Thompson, J. N. Bridson and V. McKee, *Inorg. Chim. Acta*, 1994, **219**, 55.
29 B. S. Hammes and C. J. Carrano, *Inorg. Chem.*, 1999, **38**, 4593.
30 (*a*) C. Dowling and G. Parkin, *Polyhedron*, 1996, **15**, 2463; (*b*) P. Ghosh and G. Parkin, *J. Chem. Soc., Dalton Trans.*, 1998, 2281.
31 X. Cheng, X. Zhang, J. W. Pflugrath and F. W. Studier, *Proc. Natl. Acad. Sci. USA*, 1994, **91**, 4034.
32 (*a*) P. T. R. Rajagopalan, X. C. Yu and D. Pei, *J. Am. Chem. Soc.*, 1997, **119**, 12418; (*b*) A. Becker, I. Schlichting, W. Kabsch, D. Groche, S. Schultz and A. F. V. Wagner, *Nat. Struct. Biol.*, 1998, **5**, 1053; (*c*) S. Ragusa, S. Blanquet and T. Meinnel, *J. Mol. Biol.*, 1998, **280**, 515.
33 P. Ghosh, M. Wood, J. B. Bonanno, T. Hascall and G. Parkin, *Polyhedron*, 1999, **18**, 1107.
34 P. Ghosh and G. Parkin, *Chem. Commun.*, 1998, 413.
35 (*a*) B. S. Hammes and C. J. Carrano, *Inorg. Chim. Acta*, 2000, **300**, 427; (*b*) B. S. Hammes and C. J. Carrano, *J. Chem. Soc., Dalton Trans.*, 2000, 3304.
36 (*a*) L. M. Berreau, R. A Allred, M. M. Makowska-Grzyska and A. M. Arif, *Chem. Commun.*, 2000, 1423; (*b*) S. Baggio, M. I. Pardo, R. Baggio and M. T. Garland, *Acta Crystallogr., Sect. C*, 1997, **53**, 551.
37 M. H. Bracey, J. Christiansen, P. Tovar, S. P. Cramer and S. G. Bartlett, *Biochemistry*, 1994, **33**, 13126.
38 S. B. Xiang, S. A. Short, R. Wolfenden and C. W. Carter, *Biochemistry*, 1996, **35**, 1335.
39 (*a*) R. M. Kellogg and R. P. Hof, *J. Chem. Soc., Perkin Trans. 1*, 1996, 1651; (*b*) B. Kaptein, G. Barf, R. M. Kellogg and F. Vanbolhuis, *J. Org. Chem.*, 1990, **55**, 1890; (*c*) B. Kaptein, L. Wang-Griffin, G. Barf and R. M. Kellogg, *Chem. Commun.*, 1987, 1457; (*d*) B. Koning, R. Hulst, A. Bouter, J. Buter, A. Meetsma and R. M. Kellogg, *Chem. Commun.*, 1997, 1065.
40 N. J. Curtis and R. S. Brown, *Can. J. Chem.*, 1981, **59**, 65.
41 U. Brand and H. Vahrenkamp, *Z. Anorg. Allg. Chem.*, 1996, **622**, 213.
42 C. Kimblin, T. Hascall and G. Parkin, *Inorg. Chem.*, 1997, **36**, 5680.
43 S.-J. Chiou, J. Innocent, C. G. Riordan, K.-C. Lam, L. Liable-Sands and A. L. Rheingold, *Inorg. Chem.*, 2000, **39**, 4337.
44 The observation of a hydrogen-bond interaction between methanol and a thiolate ligand in five-coordinate zinc complexes with [N_3S_2]Zn coordination, 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. See: S. C. Shoner, K. J. Humphreys, D. Barnhart and J. A. Kovacs, *Inorg. Chem.*, 1995, **34**, 5933.
45 C. Bergquist and G. Parkin, *Inorg. Chem.*, 1999, **38**, 422.
46 (*a*) R. Walz, K. Weis, M. Ruf and H. Vahrenkamp, *Chem. Ber.–Recl.*, 1997, **130**, 975; (*b*) M. Ruf, F. A. Schell, R. Walz and H. Vahrenkamp, *Chem. Ber.–Recl.*, 1997, **130**, 101.
47 C. Bergquist, H. Storrie, L. Koutcher, B. M. Bridgewater, R. A. Friesner and G. Parkin, submitted.
48 See, for example: H. E. Bryndza, P. J. Domaille, W. Tam, L. K. Fong, R. A. Paciello and J. E. Bercaw, *Polyhedron*, 1988, **7**, 1441.
49 R. M. Borges dos Santos and J. A. Martinho Simões, *J. Phys. Chem. Ref. Data*, 1998, **27**, 707.
50 C. Kimblin, B. M. Bridgewater, D. G. Churchill and G. Parkin, *Chem. Commun.*, 1999, 2301.
51 Kimura *et al.* have also used *p*-nitrobenzaldehyde as a NAD^+ mimic in model studies. See: E. Kimura, M. Shionoya, A. Hoshino, T. Ikeda and Y. Yamada, *J. Am. Chem. Soc.*, 1992, **114**, 10134.
52 E. K. Jaffe, *Acta Crystallogr. Sect. D: Biol. Crystallogr.*, 2000, **56**, 115.
53 (*a*) P. T. Erskine, N. Senior, S. Awan, R. Lambert, G. Lewis, I. J. Tickle, M. Sarwar, P. Spencer, P. Thomas, M. J. Warren, P. M. Shoolingin-Jordan, S. P. Wood and J. B. Cooper, *Nat. Struct. Biol.*, 1997, **4**, 1025;

(*b*) P. T. Erskine, E. Norton, J. B. Cooper, R. Lambert, A. Coker, G. Lewis, P. Spencer, M. Sarwar, S. P. Wood, M. J. Warren and P. M. Schoolingin-Jordan, *Biochemistry*, 1999, **38**, 4266.

54 (*a*) M. J. Hynes and B. Jonson, *Chem. Soc. Rev.*, 1997, **26**, 133; (*b*) N. Castellino, P. Castellino and N. Sannolo, *Inorganic Lead Exposure: Metabolism and Intoxication*, Lewis Publishers, Boca Raton, FL, 1995.

55 See, for example: (*a*) I. A. Bergdahl, *Analusis*, 1998, **26**, M81; (*b*) A. C. Todd, J. G. Wetmur, J. M. Moline, J. H. Godbold, S. M. Levin and P. J. Landrigan, *Environ. Health Perspect.*, 1996, **104**, 141.

56 B. M. Bridgewater and G. Parkin, *J. Am. Chem. Soc.*, 2000, **122**, 7140.

57 M. J. Warren, J. B. Cooper, S. P. Wood and P. M. Shoolingin-Jordan, *Trends Biochem. Sci.*, 1998, **23**, 217.

58 T. J. B. Simons, *Eur. J. Biochem.*, 1995, **234**, 178.

59 See, for example: K. Abudari, T. B. Karpishin and K. N. Raymond, *Inorg. Chem.*, 1993, **32**, 3052.

60 (*a*) L. C. Myers, M. P. Terranova, A. E. Ferentz, G. Wagner and G. L. Verdine, *Science*, 1993, **261**, 1164; (*b*) L. C. Myers, F. Jackow and G. L. Verdine, *J. Biol. Chem.*, 1995, **270**, 6664

61 (*a*) R. G. Matthews and C. W. Goulding, *Curr. Opin. Chem. Biol.*, 1997, **1**, 332; (*b*) K. E. Hightower and C. A. Fierke, *Curr. Opin. Chem. Biol.*, 1999, **3**, 176; (*c*) C. L. Strickland and P. C. Weber, *Curr. Opin. Drug Discovery Dev.*, 1999, **2**, 475.

62 (*a*) J. J. Wilker and S. J. Lippard, *Inorg. Chem.*, 1997, **36**, 969; (*b*) J. J. Wilker and S. J. Lippard, *J. Am. Chem. Soc.*, 1995, **117**, 8682.

63 B. M. Bridgewater and G. Parkin, *J. Chem. Soc., Dalton Trans.*, submitted.

64 (*a*) U. Brand, M. Rombach and H. Vahrenkamp, *Chem. Commun.*, 1998, 2717; (*b*) R. Burth and H. Vahrenkamp, *Z. Anorg. Allg. Chem.*, 1998, **624**, 381.

65 C. R. Warthen, B. S. Hammes and C. J. Carrano, *J. Biol. Inorg. Chem.*, in press.

66 (*a*) W. Bode, C. Fernandez-Catalan, H. Tschesche, F. Grams, H. Nagase and K. Maskos, *Cell. Mol. Life Sci.*, 1999, **55**, 639; (*b*) H. Nagase and J. F. Woessner, Jr., *J. Biol. Chem.*, 1999, **274**, 21491.

67 See, for example: L. Banci, I. Bertini, C. Luchinat, A. Donaire, M. J. Martinez and J. M. Moratal Mascarell, *Comments Inorg. Chem.*, 1990, **9**, 245 and references therein.

68 (*a*) R. Han and G. Parkin, *J. Am. Chem. Soc.*, 1991, **113**, 9707; (*b*) R. Han, A. Looney, K. McNeill, G. Parkin, A. L. Rheingold and B. S. Haggerty, *J. Inorg. Biochem.*, 1993, **49**, 105; (*c*) A. Looney, A. Saleh, Y. Zhang and G. Parkin, *Inorg. Chem.*, 1994, **33**, 1158; (*d*) C. Kimblin, V. J. Murphy, T. Hascall, B. M. Bridgewater, J. B. Bonanno and G. Parkin, *Inorg. Chem.*, 2000, **39**, 967.

69 C. Kimblin and G. Parkin, *Inorg. Chem.*, 1996, **35**, 6912.

70 C. Kimblin, B. M. Bridgewater, D. G. Churchill and G. Parkin, *J. Chem. Soc., Dalton Trans.*, 2000, 2191.

71 (*a*) R. Alsfasser, A. K. Powell, S. Trofimenko and H. Vahrenkamp, *Chem. Ber.–Recl.*, 1993, **126**, 685; (*b*) R. Alsfasser, A. K. Powell and H. Vahrenkamp, *Angew. Chem., Int. Ed. Engl.*, 1990, **29**, 898.

72 A. Looney and G. Parkin, *Inorg. Chem.*, 1994, **33**, 1234.