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REACTIVITY OF MONONUCLEAR ZINC HYDROXO COMPLEX BEARING HINDERED TRIS (PYRAZOLYL) BORATE WITH ADENINE AND THYMINE

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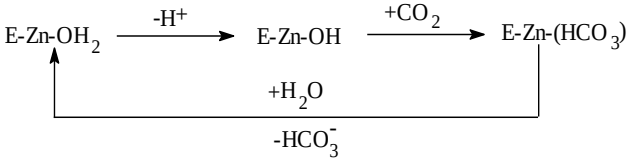
ABSTRACT
Zinc can also perform a different role in enzymes like the role it performs in carbonic anhydrase. Here the metal binds H_2O and makes it acidic enough to lose a proton and form a Zn-OH group. The zinc metal serves as a nucleophile to the substrate. Since zinc has the ability to act as an electrophile or as the source of a nucleophilic group it is incorporated and used by many enzymes (10). The carbonic anhydrases (or carbonate dehydratases) form a family of enzymes that catalyze the rapid conversion of carbon dioxide and water to bicarbonate and protons, a reaction that occurs rather slowly in the absence of a catalyst. The active site of most carbonic anhydrases contains a zinc ion; they are therefore classified as metalloenzymes. The reaction rate of carbonic anhydrase is one of the fastest of all enzymes, and its rate is typically limited by the diffusion rate of its substrates. An anhydrase is defined as an enzyme that catalyzes the removal of a water molecule from a compound, and so it is this "reverse" reaction that gives carbonic anhydrase its name, because it removes a water molecule from carbonic acid. Close-up rendering of active site of human carbonic anhydrase II, showing three histidine residues and a hydroxide group coordinating the zinc ion . The present paper describes the synthesis and characterization of mononuclear zinc hydroxo complex and its reaction products with carbon dioxide, adenine and thymine.

KEYWORDS: zinc, carbonic anhydrase, metalloenzyme, zinc-binding sites, zinc enzyme, enzyme model, catalytic hydrolysis, zinc hydroxide, carboxypeptidase, carbonic anhydrase, alcohol dehydrogenase.

INTRODUCTION
Zinc is an essential element - one that is necessary for the occurrence of reactions that are required in the metabolic processes of living organisms. It is the second most abundant transition or post-transition metal, transported by proteins (macroglobulin, transferrin and albumin), stored in a protein (thionein) and bound to proteins. It is generally bound to histidines, carboxylate-containing residues and cysteines. There are several zinc-containing hydrolytic enzymes which catalyze addition or removal of water in a substrate molecule.

Carbonic anhydrase **(CA)** is a monomeric protein of molecular mass 30 kDa. It catalyzes the conversion of carbon dioxide to bicarbonate. The catalytic mechanism has been studied extensively and is generally accepted to consist of two steps (Scheme 1).



Scheme 1

Structural studies on the enzyme have identified that the zinc center is coordinated to three histidine imidazole groups and a water molecule, $[(His)_3Zn\text{-}OH_2]^{2+}$ (His = histidine). The catalytic mechanism of carbonic anhydrase activity has been the subject of both intense experimental.[1] and theoretical investigations and these studies have led to the proposal that a zinc bicarbonate complex $[(His)_3Zn\text{-}OCO_2H]^+$ may be a key intermediate. However, structural details of the proposed bicarbonate intermediate are not known and theoretical treatments have explored the possibilities of both unidentate and bidentate coordination modes.[2] The kinetics of the carbonic anhydrase reaction are pH-dependent, being faster at high pH and under the control of a group having an apparent pK_a value near 7.0. The simplest and most widely accepted interpretation of these observations is that this reaction involves formation of a zinc-bound hydroxide ion. This proposal is also supported by the observation that many anions inhibit the reaction, presumably by competing with water ligands for the open coordination site on zinc.

Human carbonic anhydrase has a molecular weight of ~ 30, 000 and occurs in two similar but not identical forms.

The molecular structures of both are known from X-ray crystallography. The enzyme-catalyzed reaction rates are pH dependent in a way that indicates the existence of a group in the enzyme with a pK_a of ~ 7 that must be deprotonated to give that form of the enzyme E, which is required for hydration of CO_2. Conversely, an acid form EH^+ is required for the reverse reaction. It is necessary to assume that the substrate for dehydration is HCO_3^-, since the pH dependence of dehydration is the inverse of that for hydration. In effect, the enzyme makes the fast reaction $CO_2 + OH^- = HCO_3^-$, the major pathway of hydration at a pH of 7, whereas normally the slow reaction of CO_2 with H_2O would predominate at a pH of 7 and the reaction with OH^- would not become dominant until pH 10 or greater. The importance of a Zn–OH group in providing a potent attacking nucleophile is in harmony with the proposed importance of a similar feature of the carboxypeptidase mechanism. A difference between the two, however, is that here it must be assumed that hydrogen bonding to the two CO_2 oxygen atoms polarizes the C=O bonds so as to enhance the positive character of the carbon atom, whereas in carboxypeptidase, the zinc atom was also involved in the polarization of the C=O group.

Reaction mechanisms of hydrolytic metalloenzymes (e.g., carbonic anhydrase (CA), carboxypeptidase, phosphatase) and the role of the metal ions in their active centers have constantly been interesting bioinorganic subjects.[3,4] Literature also revealed that in most of the zinc-containing hydrolytic enzyme the mononuclear hydroxo is the active species. As one of the approaches, various types of metal complexes have been designed to account for or mimic the functions played by the central Zn(II) ions, a typical central metal ion. There are many research groups who have reported various zinc compounds with various types of ligands as catalyst in ester hydrolysis.[5-8] Some very recent works are described below-

Several model complexes have also been reported by various workers and their catalytic activity for the hydration of cabon dioxide have been proposed.[9-12] Gultneh et al.[13] synthesized a hydroxo-bridged dinuclear Zn(II) complex as possible model for the hydrolytic zinc enzymes. They also reported that the synthesised complex reacts reversibly in acetonitrile solution with CO_2. Rombach et al.[14] reported that $Tp^{Ph,Me}$Zn-H and $Tp^{Ph,Me}$Zn-OH undergo insertion reactions with CO_2. Bergquist and Parkin,[15] described the protonation of the hydroxide ligand in a synthetic analogue of carbonic anhydrase, [$Tp^{tBu,Me}$] ZnOH and demonstrated that the protonation inhibits the reactivity towards CO_2. Looney et al.[16] reported the tris(pyrazolyl)hydroboratozinc hydroxide complexes as functional models for carbonic anhydrase and performed the study on the nature of the bicarbonate intermediate. Nakata et al.[17] reported the synthesis of water soluble zinc complex and used it in the study of CO_2 hydration. Doring et al.[18] described bi- and tetranuclear Zn complexes of the tridentate N,N,O ligand [{[2-(2-pyridyl)ethyl]imino}methyl]phenol (HL) and their enzyme-like reactions are described. The coordinated H_2O molecules in a dizinc complex of this ligand can be deprotonated reversibly similar to those in the resting states of Zn enzymes. The resulting tetranuclear complex bearing bridging hydroxide ions allows the reversible uptake of CO_2 whereas the coordination of H carbonate was not observed. Also some synthesised hydroxo complexes of zinc (II) were used for the reaction with various nucleic acid bases and their nucleosides.[19-21]

The above reported literatures revealed that in most of the hydrolytic enzymes, the mononuclear hydroxo species are involved at some stages of their catalytic cycle.

Experimental

MATERIALS AND METHODS

All solvents used were purified by literature methods.[22] and treated under nitrogen prior to use. The reagents of the highest grade commercially available were used without further purification. The preparations of the complexes were performed under nitrogen by standard Schlenk techniques. KHB(3-Bu^t-5-Pr^{i}pz)$_3$ was prepared by the method described previously.[23]

Physical Measurements

^{1}H-NMR spectra were recorded on a JEOL-GX-270 (270 MHz) spectrometer at 25 ^{o}C. The chemical shifts are reported as values (δ, ppm) downshifted from the internal standard Me_4Si. FD-MS spectra were recorded on a Hitachi M-80 spectrometer. Carbon, hydrogen and nitrogen were analyzed with a Vario EL III elemental analyzer. Infrared spectra (400-4000 cm^{-1}) of solid samples were recorded on a Perkin-Elmer model 1600 FT-IR spectrometer as KBr disks. Electro Spray Ionization mass spectra (ESI-MS) in the positive ion mode were recorded on a Hewlett-Packard HP 5989 mass spectrometer. The used solvent is given in parentheses. The intensity and possible composition of the peaks is between brackets. Temperature dependent magnetic susceptibilities of powdered samples were measured by using a SQUID magnetometer (Quantum Design) at 1.0 T (2-290 K). Corrections for underlying diamagnetism were made by using tabulated Pascal constants.

Synthesis of 3-tert-butyl-5-isopropylpyrazole (2a): A solution of pinacolin (136.50 g., 1.36 mol) in 100 ml of diethyl ether was added drop-wise to a stirred suspension of lithium amide (50.00 g., 2.17 mol) in 250 ml diethyl ether over 1hr.. Ethylisobutyrate (196.54 g., 1.69 mol) in 100 ml diethyl ether was added dropwise to the resulting mixture over 1 hr.. After the mixture was refluxed for 7 hrs., the thick sludge was hydrolyzed by a dilute HCl aqueous solution and the compound was extracted with diethyl ether (3 x 100 ml). The ether layer was treated with a saturated NaCl aqueous solution several times and drying over $MgSO_4$. After drying diethyl ether was

removed by evaporation. The resulting solution was distilled under reduced pressure, at 70 ^{o}C affording 139.30 g. 2,2-dimethyl-6-methyl-3,5-heptanedione.

Hydrazine monohydrate (69.65 g., 1.39 mol) was added dropwise to a solution of 2,2-dimethyl-6-methyl-3,5-heptanedione (139.30 g., 0.81 mol) in 100 ml ethanol. After 6 hrs. of refluxing, the solution was treated with 200 ml saturated NaCl aqueous solution and was extracted with diethyl ether (3 x 100 ml). After drying over $MgSO_4$ overnight, the solvent was evaporated to dryness. The resulting white solid was dissolved in acetonitrile and allows to stand overnight at –20 ^{o}C. The white needles in 74% yield (100.00 g., 0.60 mol), were filtered off and dried under vacuum. Anal. Calcd for $C_{10}H_{18}N_2$: C, 72.24; H, 10.91; N, 16.85. Found: C, 71.89; H, 11.04; N, 16.63. IR (KBr, cm^{-1}): ν(NH) 3173, ν(NH) 3098, ν(CH) 2963. ^{1}H NMR ($CDCl_3$, δ ppm): 9.07 (br, 1H, NH), 5.88 (s 1H, Pz), 2.97 (sept, J = 7 Hz, 1H, C*H*Me$_2$), 1.31 (s, 9H, C*Me*$_3$), 1.26 (d, J = 7Hz, 6H, CH*Me*$_2$).

Synthesis of hydrotris(3-tert-butyl-5-isopropyl-1-pyrazolyl)borate K[HB (3-Bu^t-5-Pr^{i}pz)$_3$] (2b): A mixture of 3-tert-butyl-5-isopropylpyrazole (35.60 g., 0.21 mol) and KBH_4 (3.85 g., 0.07 mol) was heated in an oil bath with stirring. The temperature was elevated gradually. After the temperature of oil bath reached 260 ^{o}C, heating was continued at the same temperature until no hydrogen evolution has been observed. The mixture was allowed to cool to room temperature, and the resulting solid was dissolved in dicholoromethane, filtered over celite. The solvent was evaporated under vacuum, the white solid was dissolved in 25 ml of acetonitrile and allowed to stand overnight at –20 ^{o}C. Then it was filtered off and dried under vacuum. Yield is 62% (73.48 g., 0.13 mol). Anal. Calcd for $C_{30}H_{52}N_6BK$: C, 65.91; H, 9.59; N, 15.37. Found: C, 64.99; H, 9.52; N, 16.64. IR (KBr, cm^{-1}): ν(CH) 2962, ν(BH) 2476. ^{1}H NMR (C_6D_6, δ ppm): 5.77 (s, 3H, Pz), 3.09 (sept, J = 7 Hz, 3H, CH*Me*$_2$), 2.02 (s, 3H, *Me*CN), 1.16 (s, 27H, C*Me*$_3$), 1.00 (d, J = 7Hz, 18H, CH*Me*$_2$).

Zn(HB(3-Bu^t-5-Pr^{i}pz)$_3$)(NO$_3$) (2c): A mixture of $Zn(NO_3)_2.6H_2O$ (0.2974g, 1.0 mmol) and K[HB(3-Bu^t-5-Pr^{i}pz)$_3$] (0.5078g, 1.0 mmol) was stirred in a mixture of 25.0 ml dichloromethane and 5.0 ml acetone for 3 hrs.. After removal of salt by filteration, solvent was evaporated under vacuum. The resulting colorless crystalline compound was obtained in 51% yield (0.320g). Anal calcd. for $C_{30}H_{52}N_7O_3BZn$: C, 56.75; H, 8.25; N, 15.44: Found C, 56.35; H, 8.17; N, 15.41. IR (KBr, cm^{-1}): 2566 (ν BH). ^{1}H NMR (C_6D_6, 270 MHz): 1.18 (d, J=7Hz, 18 H, CHMe$_2$), 1.51 (s, 27H, CMe$_3$), 3.16 (sept, J=7Hz, 18H, CHMe$_2$), 6.00 (s, 3H, pz). FD-MS(m/z): 633.

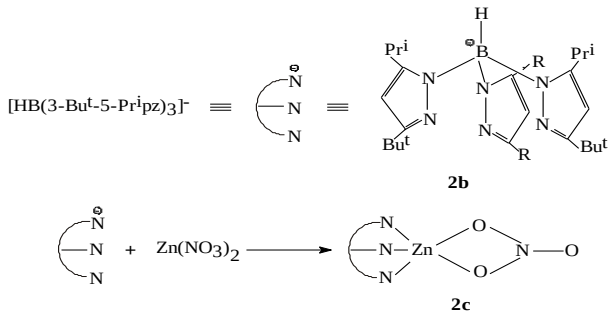
Zn(HB(3-Bu^t-5-Pr^{i}pz)$_3$)(OH) (2d): A Toluene solution of **2c** (0.30g, 0.47 mmol) in 20.0 ml was stirred with 10.0 ml of 1N aquous NaOH for 30 min.. Toluene phase was separated and dried over sodium sulphate for 2 hrs.. Sodium sulphate was removed by filteration and the solvent was dried under vacuum to give pure sample of **2d** as a white crystalline solid in 76% yield (0.22g). Anal calcd. for $C_{30}H_{53}N_6OBZn$: C, 61.07; H, 9.05; N, 14.24 : Found C, 61.65; H, 9.27; N, 14.55. IR(KBr, cm^{-1}): 3686(ν OH), 2566 (ν BH). ^{1}H NMR (C_6D_6, 270 MHz, δ ppm): 1.12 (d, J=7Hz, 18 H, CHMe$_2$), 1.57 (s, 27H, CMe$_3$), 3.53 (sept, J=7Hz, 3H, CHMe$_2$), 5.98 (s, 3H, pz). FD-MS(m/z): 588.

Zn(HB(3-Bu^t-5-Pr^{i}pz)$_3$)(adeninate) (2e): A suspension of adenine (0.291g, 1.32 mmol) in 10 ml of methanol was added to a solution of **2d** (0.778g, 1.32 mmol) in 15 ml of dichloromethane.After overnight stirring solution is filtered and filterate is evaporated to dryness in an oil pump vacuum.Yield: 0.774g (83%). Anal calcd. For $C_{35}H_{56}N_{11}BZn$: C, 59.42; H, 7.98; N, 21.78: Found C, 58.96; H, 7.46; N, 21.63. IR (KBr, cm^{-1}): 3476, 3122 (ν NH) ; 2555 (ν BH).

Zn(HB(3-Bu^t-5-Pr^{i}pz)$_3$)(thyminate) (2f): A suspension of thymine (0.175g, 1.39 mmol) in 10 ml of methanol was added to a solution of **2d** (0.852, 1.39 mmol) in 15 ml of dichloromethane.After overnight stirring a clear solution is obtained. Solution was evaporated to dryness under vacuum. Yield: 0.864g (89%). Anal calcd. For $C_{35}H_{57}N_8O_2BZn$: C, 60.2; H, 8.23; N, 16.05: Found C, 58.93; H, 8.68; N, 16.68. IR (KBr, cm^{-1}): 3133 (ν NH); 2544 (ν BH); 1676 (ν C_2=O); 1657 (ν C_4=O).

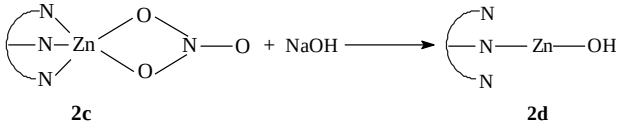
RESULTS AND DISCUSSION

The reaction of $Zn(NO_3)_2.6H_2O$ with KHB(3-Bu^t-5-Pr^{i}pz)$_3$) resulted the formation of complex **2c** [Scheme 2].



Scheme 2

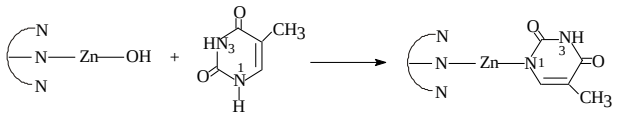
The complex **2c** is five coordinate species with N_3O_2 donor set. The presence of $\nu_{as}(NO_3)$ at 1530 cm^{-1} and $\nu_s(NO_3)$ at 1261 cm^{-1} in the i.r. spectrum suggested the bidentate character of nitrate group coordinated to zinc. The reaction of **2c** (toluene solution) with 1N NaOH resulted in the formation of **2d** as shown in scheme 3.



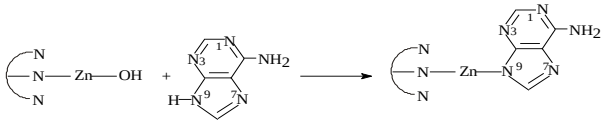
Scheme 3

The presence of ν OH at 3686 cm^{-1} suggested the presence of hydroxo group. Due to the high solubility of **2d** in most of the solvents, its crystal structure could not be determined but on the basis of other analogous monomeric zinc hydroxo complexes with a hindered tris(pyrazolyl)borate ligand available in the literatures [24-26] and also on the basis of mass determination, we infer that **2d** is also monomeric species. Based on the spectral data (including i.r., n.m.r. and mass spectra) in present study, the proposed coordination environment of **2d** is very similar to the active site of carbonic anhydrase where zinc center is bound to three histidine imidazole groups and a water molecule $[His_3\text{-}Zn\text{-}OH_2]^{2+}$ (His = histidine) and the initial states of the catalytic cycle are considered to involve initial deprotonation of the coordinated water to give the active zinc hydroxide derivative $[His_3\text{-}Zn\text{-}OH]^{+}$, followed by reaction with CO_2 to give a zinc bicarbonate derivative $[His_3Zn\text{-}OCO_2H]^{+}$.
In *de novo* synthesis, the pyrimidine nucleotides originate from amino acids via heterocyclic carboxylic acids. The decarboxylation of the pyrimidine nucleotides after attachment of the sugar and phosphate units resulted to uridine monophosphate, which is the precursor of the other pyrimidine nucleotides.[27] Zinc enzymes are involved in several steps of this sequence including the formation of oligonucleotides and polymerase activity. Also several metal complexes of zinc, have been found to act as drugs.

I. R. data also suggested that the thymine is bound to zinc via N1 [Scheme 4]as in the case of uracil while N9 seems to be preferred donor atom in case of zinc complex of adenine [Scheme 5].



Scheme 4



Scheme 5

X-ray Powder Diffraction Studies

The X-ray powder diffraction (not shown) of the complexes have been indexed according to the Ito's method [28] [Table 1 and Table 2]. The indexing pattern yield the lattice constants a = b = 13.38, c = 15.07 Å for **2j**; a = b = 14.31, c = 19.24 Å for **2k** indicating tetragonal symmetry for all these complexes.

Similar structure for Zn-uracil complexes has been proposed by Vahrenkamp et al with different pyrazolylborate ligand.[29]

Table 1: X-Ray diffraction data for the complex (HB(3-Bu^t-5-Pr^{i}pz)$_3$)Zn-adeninate (2j).

Powdered Pattern line	2θ	d value	Qobs	Qcalc	hkl
(1)	(2)	(3)	(4)	(5)	(6)
1	6.5	13.3840	0.0055	0.0055	100
2	8.7	10.0471	0.0099	0.0099	101
3	9.3	9.4082	0.0112	0.0111	110
4	10.9	8.0446	0.0154	0.0154	111
5	12.5	7.0273	0.0202	0.0223	200
6	14.4	6.1109	0.0267	0.0266	201
7	18.9	4.6733	0.0457	0.0452	212
8	21.5	4.1166	0.0590	0.0601	312
9	22.5	3.9368	0.0645	0.0668	213
10	23.7	3.7411	0.0714	0.0725	320
11	26.2	3.3909	0.0869	0.0891	303
12	26.7	3.3288	0.0902	0.0898	322
13	28.4	3.1341	0.1018	0.1004	330
14	30.5	2.9237	0.1169	0.1177	332
15	33.4	2.6770	0.1395	0.1393	333
16	34.2	2.6163	0.1460	0.1451	510
17	35.1	2.5515	0.1535	0.1527	225
18	39.6	2.2721	0.1937	0.1941	531
19	44.7	2.0246	0.2439	0.2449	406
20	50.7	1.7954	0.3102	0.3090	605
21	53.2	1.7171	0.3391	0.3405	650
22	55.4	1.6542	0.3654	0.3660	407
23	57.0	1.6117	0.3849	0.3845	546
24	59.0	1.5619	0.4098	0.4097	654
25	60.2	1.5337	0.4250	0.4264	804
26	61.3	1.5089	0.4391	0.4395	409
27	62.9	1.4765	0.4586	0.4577	910
28	69.8	1.3450	0.5527	0.5523	657
29	71.5	1.3172	0.5763	0.5755	862
30	78.4	1.2179	0.6741	0.6740	739
31	80.6	1.1902	0.7058	0.7075	809
32	85.3	1.1363	0.7743	0.7735	858

Table 2: X-Ray diffraction data for the complex (HB(3-Bu^t-5-Pr^{i}pz)$_3$)Zn-thyminate (2k).

Powdered Pattern line	2θ	d value	Qobs	Qcalc	hkl
(1)	(2)	(3)	(4)	(5)	(6)
1	6.1	14.3153	0.0048	0.0048	100
2	7.6	11.5185	0.0075	0.0075	101
3	8.8	9.9992	0.0100	0.0097	110
4	10.6	8.2840	0.0145	0.0124	111
5	11.1	7.9142	0.0159	0.0155	102
6	12.3	7.1684	0.0194	0.0195	200
7	14.3	6.1721	0.0262	0.0270	211
8	17.3	5.1101	0.0382	0.0390	220
9	18.1	4.8775	0.0420	0.0416	221
10	18.8	4.7063	0.0451	0.0465	301
11	20.3	4.3623	0.0525	0.0522	114
12	21.6	4.0968	0.0595	0.0594	312
13	22.8	3.8901	0.0660	0.0660	321
14	23.3	3.8078	0.0689	0.0678	303
15	25.1	3.5343	0.0800	0.0807	401
16	26.3	3.3803	0.0875	0.0873	323
17	27.4	3.2493	0.0947	0.0935	412
18	28.3	3.1461	0.1010	0.1019	403
19	30.5	2.9274	0.1166	0.1152	315
20	30.8	2.8965	0.1191	0.1200	216
21	36.8	2.4373	0.1683	0.1685	531
22	38.3	2.3453	0.1818	0.1805	610
23	38.9	2.3067	0.1879	0.1884	505
24	39.8	2.2603	0.1957	0.1951	620
25	44.1	2.0481	0.2383	0.2377	624
26	48.6	1.8687	0.2863	0.2860	635
27	49.3	1.8450	0.2937	0.2936	732
28	53.3	1.7156	0.3397	0.3396	716
29	69.4	1.3508	0.5479	0.5470	829
30	70.0	1.3424	0.5548	0.5544	865
31	73.9	1.2794	0.6108	0.6105	909
32	78.6	1.2148	0.6775	0.6768	974
33	80.1	1.1958	0.6992	0.7007	975
34	85.2	1.1370	0.7734	0.7739	985

CONCLUSION

The synthesis of mononuclear zinc hydroxo, Zn(OH)(HB(3-Bu^t-5-Pr^{i}pz)$_3$) by using sterically hindered pyrazolylborate ligand i.e. hydrotris(3-tert-butyl-5-isopropyl-1-pyrazolyl)borate ligand is described in this chapter. The structure of Zn(OH)(HB(3-Bu^t-5-Pr^{i}pz)$_3$) is very similar to the active site of the enzyme carbonic anhydrase. The complex Zn(OH)(HB(3-Bu^t-5-Pr^{i}pz)$_3$) was found to stabilize zinc complexes of adenine and thymine where thymine are bound as monodentate ligand via its deprotonated N1 and adenine via N9.

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