PYRAZOLYLBORATE COMPLEXES OF Mn AND Co: SYNTHESIS, STRUCTURE AND REACTIVITY STUDIES

A THESIS

Submitted in partial fulfilment of the requirements for the award of the degree of

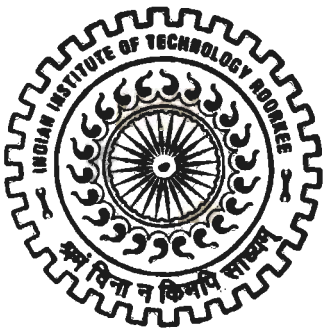
DOCTOR OF PHILOSOPHY

in

CHEMISTRY

By

ASISH KUMAR SHARMA



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CANDIDATE'S DECLARATION

I hereby certify that the work which is being presented in the thesis entitled **"PYRAZOLYLBORATE COMPLEXES OF Mn AND Co: SYNTHESIS, STRUCTURE AND REACTIVITY STUDIES"**, in Partial fulfilment of the requirement for the award of the Degree of Doctor of Philosophy and submitted in the Department of Chemistry of the Indian Institute of Technology Roorkee, Roorkee is an authentic record of my own work carried out during a period from March 2002 to May 2005 under the supervision of Dr. Udai P. Singh.

The matter presented in this thesis has not been submitted by me for the award of any other degree of this or any other Institute.

Asish

Dated: (ASISH KUMAR SHARMA)

This is certify that the above statement made by the candidate is correct to the best of my knowledge.

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ABSTRACT

The polypyrazolylborate ligand has more than 40 years old history as it was first time reported by S. Trofimenko in 1960's. In general, however, the polypyrazolylborate are tripodal ligands that include a diverse set of structure. The ligands can be tetradentate, bidentate or monodentate depending on the number of donor substituents on boron and the steric congestion around the metal. In addition, tripodal ligand can have central atoms other than boron such as carbon, phosphorus or gallium. The ligands also can bind metals through sulphur, phosphorus or other donor atoms.

The pyrazolylborates / scorpionate chemistry signify a very large and growing aera. These ligands are extremely versatile because the number of donor atoms in each ligand can be varied from two to three while going from the bidentate ligands to the potentially tridentate ligands. The steric properties of the ligands can be altered by changing the substituents on the pyrazolyl rings. These ligands have been used extensively to prepare complexes of the transition metals. The polypyrazolylborate ligands have properties lying between those of parent $[H_4\text{-}nB(pz)_n]^-$ ligands (pz = pyrazolyl) and those of the recently reported derivatives $[H_4\text{-}nB(3\text{-}Rpz)_n]^-$, where R = tert-butyl and phenyl groups. The parents ligands, in particular the derivatives with n = 3, have been extensively used in inorganic and bioinorganic chemistry. However, the chemistry is severly limited because of the tendency of the first row transition metal ions to form the bisligand complex (i.e. $M(HB(pz)_3)_2$). On the other hand, the bulky group of the 3-R derivatives prevents formation of this type of complex. The 3-tert-butyl ligand allows no further access to the metal ion and is a "tetrahedral enforcer", whereas the 3-phenyl derivative accommodates five coordination with trigonal-bipyramidal geometry.

Although bisligand complex formation is prevented, the metal ion is not readily accessible to other molecules because of the steric bulk of the 3-substituents. In contrast to cyclopentadienide ligands, with which they have often been compared, the tris(pyrazolyl)borate ligands offer much greater opportunities for modifying their coordinative features. Whereas only one substituent can be placed on $C_5H_5(C_5R_5)$ to yield a derivative ligand with retention of the original ligand symmetry, there are 15 ways of placing from one to ten identical substituents on the parent $HB(pz)_3$ ligand and still maintain their original C_{3v} symmetry. It is well established in the literature that from a structural point of view the complex chemistry of the trispryrazolylborate anion frequently resembles that of cyclopentadienyl (or pentamethylcyclopentadienyl) complexes. However, as might be expected for a tridentate nitrogen donor ligand; the trispyrazolylborate complexes are kinetically less labile than their cyclopentadienyl analogues. In the hierarchy of increasing steric hindrance around the metal in 3- or 5-substituted tris(pyrazolyl)borates, the currently known series is H< CH_3< C_6H_5 < ^{i}Pr < ^{t}Bu. In terms of forming octahedral L_2M complexes, ligands with R = H and CH_3 do so rapidly whereas those with R = C_6H_5 do so reluctantly, and those with R = ^{i}Pr do not form L_2M complexes. The ligands with R = ^{i}Pr form octahedral complexes only with rearrangement of each L to $HB(3\text{-}^{i}Prpz)_2(5\text{-}^{i}Prpz)$; they also form mixed octahedral LML complexes, provided L is a relatively unhindered tris(pyrazolyl)borate ligand [19]. Finally, ligands with R = Bu^{t} do not form octahedral complexes at all with first-row transition metals. Conversely, in terms of formation of LMX (X= halide or pseudohalide) species, ligands with R = Bu^{t} form these stable complexes with ease. With R = ^{i}Pr, LMX complexes are also readily formed. But they posses reactivity for solvation or for

displacement of X with anionic nucleophiles. The ligands with R = phenyl form LMX species, which are even more reactive towards solvation or substitution of X. When R = methyl, and even more so when R = H, the LMX species becomes progressively less stable and undergo transformation to the octahedral L_2M complexes.

Among the different types of pyrazolylborates, hydrotris(pyrazolyl)borate (Tp^R) ligand has been widely used as supporting ligand for various inorganic and organometallic compounds. It has unique structural (i.e., facially capping tridentate ligand) and electronic characteristics ($6e^-$ donating mono anionic ligand) are similar to those of the family of cyclopentadienyl (Cp^R) ligands, which are the most commonly adopted ligands in organometallic chemistry. One of advantages of the tris(pyrazolyl)borate ligands is the ease of controlling the properties of the resulting metal complexes (coordination environment and reactivity of metal centers, solubility in organic solvents, facility of crystallization, etc.) by introduction of various substituents into the pyrazole rings. It is notable that highly sterically demanding hydrotris(pyrazolyl)borate ligands containing bulky substituents at the 3-position of the pyrazole rings can stabilize a coordinatively unsaturated metal center and provide a vacant site for substrate binding. The Tp^R ligand is also able to tune the electronic and steric environment of the central metal atoms by introducing various substituents to the pyrazolyl ring. In addition to this feature, a coordination environment created by the Tp^R ligand can mimic an environment created by a set of three imidazole rings of histidine residue, which is frequently found in the metal coordination site of metalloproteins.

For the sake of the convenience, the work embodied in the thesis is presented in the following chapter:

The **First** chapter of the thesis is the general introduction and present an up to date survey of literature related to the various pyrazole and their borate salts. The different kind of metal complexes related to the present research have been posed in the context of the cited work.

The **second** chapter of the thesis deals with the synthesis of the mononuclear and binuclear of manganese complexes with 3,5-diisopropylpyazole (3,5-iPr_2pzH) and its borate salt [$KHB(3,5\text{-}Pr^i_2pz)_3$] as ligand. The reaction of 3,5-diisopropylpyazole with $MnCl_2.4H_2O$ and sodium fluorobenzoate (NaOBz-F)/sodium nitrobenzoate (NaOBz-NO_2) resulted the formation of $[Mn(F\text{-}OBz)_2(3,5\text{-}iPr_2pzH)_4]$ and $[Mn(NO_2\text{-}OBz)_2(3,5\text{-}iPr_2pzH)_4]$. $Mn(OBzF)_2(iPr_2H)_4$ and $Mn(NO_2\text{-}OBz)_2(3,5\text{-}iPr_2pzH)_4$ have six coordinated structure with intramolecular hydrozen bonding between the uncoordinated oxygen of mono-coordinated fluorobenzoate/nitrobenzoate and the free hydrogen of coordinated 3,5-iPr_2pzH. The single crystal X-ray structure also shows the CH_3-Pπ interaction between the two molecules of above complexes. A dimanganese(II) complex $[Mn(HB(3,5\text{-}Pr^i_2pz)_3)(\mu\text{-}FOBz)_3Mn(3,5\text{-}iPr_2pzH)_2]$ was prepared from a mixture of $[Mn(Cl)(HB(3,5\text{-}Pr^i_2pz)_3)]$, 3,5-$iPr_2pzH$, $MnCl_2.4H_2O$ and (NaOBz-F). The X-ray studies established the unsymmetric coordination environment of each manganese ion with a structurally unique bridging unit consisting of three carboxylate groups. Some other mononuclear complexes was also prepared for finding out the coordination mode of fluorobenzoate, azide, thiocyanate and cyanide group.

The synthesis and structural studies of binuclear cobalt complexes having different bridging ligands are presented in chapter **three**. The reaction of $Co(NO_3)_2.6H_2O$ with 3,5-diisopropylpyazole (3,5-iPr_2pzH) and sodium nitrobenzoate resulted the

formation $[Co_2(3,5\text{-}iPr_2pzH)_2(\mu\text{-}3,5\text{-}iPr_2pz)_2(OBz\text{-}NO_2)_2]$ complex. In this complex both cobalt ions are four coordinated, bridged by two 3,5-diisopropylpyrazole. The other two molecules 3,5-diisopyropylprazole are unidentately coordinated to the metal ions and also form the hydrogen bonds with un-coordinated oxygen of nitrobenzoate group. The complex $[CoNO_3(HB(3,5\text{-}Pr^i{}_2pz)_3)]$ was synthesized by the reaction of $Co(NO_3)_2.6H_2O$ with $[KHB(3,5\text{-}Pr^i{}_2pz)_3]$ which upon reaction with NaOH resulted the formation of binuclear hydroxo complex $[HB(3,5\text{-}Pr^i{}_2pz)_3Co)_2(OH)_2]$. This complex reacted with two equivalent of bis(4-nitrophenyl)phosphate and resulted the complex $[(HB(3,5\text{-}Pr^i{}_2pz)_3)_2Co_2bis(\mu\text{-phosphodiester})]$. The reaction of $[HB(3,5\text{-}Pr^i{}_2pz)_3Co)_2(OH)_2]$ with two equivalent KSCN / NaN_3 / KCN were performed in order to know the binding mode of these molecules in resultant complexes.

The chapter **four** of the thesis deals with the synthesis and characterization of some mononuclear hydroxo of the type $[(HB(3\text{-}Bu^t\text{-}5\text{-}Pr^ipz)_3)MOH]$ (M = Mn, Ni, Zn, Cd). These complexes were characterized by different available techniques and were used as a catalyst in the hydrolysis of different types of phosphate, carboxylate and sulphonate esters. The hydrolysis of different ester was monitored spectrophotometrically.

The material and reagents, synthetic procedures, experimental details and different type of spectroscopic measurement are described in chapter **five** of the thesis. Methods for the preparation of different types of ligands and their complexes with Mn(II) and Co(II) have been included.

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This work has been carried out under the supervision of **Dr. Udai P. Singh** whose suggestions, advices, ideas and criticisms at all stages of the work, right from the formulation of the problem through laboratory works to the completion of the final draft of the thesis have made it possible for me to complete this work. At the stage of my career when I paused to look back to my research period I find that at every stage his perpetual encouragement, his enthusiasm and above all morale boosting inspiration served a vital source to bring the present work to conclusion. Further his bold initiative and uncompromising gesture made a highly challenging path feasible. My heartfelt thanks to him for guiding me in successful path.

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(ASISH KUMAR SHARMA)

LIST OF PUBLICATION / CONFERENCES / SYMPOSIUM:

Publications:

1. Udai P. Singh, Preeti Babbar, **Asish K. Sharma** "Cobalt Complexes Bridge with a (μ-X) (μ- carboxylato) unit (X= OH, N_3): Synthesis and Structural Studies", Inorganica Chemica Acta, 328 (2005) 271-278 ,

2. Udai P. Singh, **Asish K. Sharma**, S. Hikichi, H. Komatsuzaki, Y. Moroko-oka and M. Akita, "Hydrogen bonding interaction between imidazolyl N-H group and peroxide: Stabilization of Mn(III)-Peroxo Complex $Tp^{iPr2}Mn(\eta^2\text{-}O_2)(im^{Me}H)$)($im^{Me}H$ = 2- methylimidazole)", Dalton Comm., 2005 (under reversion).

3. Udai P. Singh, **Asish K. Shrama** "Synthesis and molecular structure of hydrogen bonded benzoate complexes of Mn(II) and Co(II). Inorganica Chemica Acta (submitted).

Conferences/Symposium:

1. "Synthesis and Molecular Structure of Intermolecular Hydrogen Bonded Carboxylate Complexes" Seventh National Symposium in Chemistry CRSI, IACS, Kolkata, India, P -146 (2005). **Asish K. Sharma**, Udai P. Singh

2. "Synthesis and Molecular Structure Studies of some cobalt complexes" X^{th} Symposium on Modern Trend in Inorganic Chemistry, I.I.T Bombay, India, P-135, (2003). **Asish K. Sharma**, Preeti Babbar, Udai P. Singh

3. "Solution, Solid and Antitumor Studies of Some Metal Complexes with Cytosine and Its Derivatives" Twenty second Conference in Chemistry, Indian Council of Chemist, I.I.T Roorkee, India, PP-09, (2003). Rajeev Kumar, **Asish K. Sharma**, Sadhana Tyagi and Udai P. Singh.

4. "Synthesis and Molecular Structure of Mononuclear Cobalt Carboxylate Complexes" Fifth National Symposium in Chemistry, CLRI Chennai, India P-25 (2002). **Asish K. Sharma**, Udai P. Singh

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Chapter 1

GENERAL INTRODUCTION

The polypyrazolylborate ligand has more than 40 years old history as it was first time reported by S. Trofimenko in 1960's [1]. This ligands used for providing steric shielding to the metal center in complexes and also serve as reliable spectator ligands. In general, however, the polypyrazolylborate are tripodal ligands that include a diverse set of structure. The ligands can be tetradentate, bidentate or monodentate depending on the number of donor substituents on boron and the steric congestion around the metal. Nitrogen heterocycle other than pyrazole can be used such as pyrrole, imidazole, indole and indizole. In addition, tripodal ligand can have central atoms other than boron such as carbon, phosphorus or gallium. The ligands also can bind metals through sulphur, phosphorus or other donor atoms.

The pyrazole nucleus both thermally and hydrolytically is very stable and occupies a position similar to that of pyridine or ammonia in spectrochemical series. As a ligand, it coordinates to metals and metalloids through the 2-N but after deprotonation, the formed pyrazolate anions can coordinate through both nitrogen atoms as an exobidentate ligand of C_{2v} symmetry. The nucleophilicity of the nitrogens and their steric accessibility may be varied through appropriate ring substitution. Due to these attractive features the coordination chemistry of pyrazole and its derivatives has attracted much attention and several substituted pyrazoles have been reported in literature. Some substituted pyrazoles are given below.

3-tert-butylpyrazole [2], 3-tert-butyl-5-isopropylpyrazole [3], 3-tert-butyl-5-methylpyrazole, 3-isopropyl-4-bromopyrazole [4,5] 3-isopropylpyrazole [6], 3-isopropyl-5-methylpyrazole [7], 3,5-diisopropylpyrazole [8], 3-neopentylpyrazole [9], 3-thienylpyrazole [10], 3-phenylpyrazole [2], 3-phenyl-5-methylpyrazole [11], 3- diphenylmethylpyrazole [12], 3,5-diphenylpyrazole [8], 3-p-tolylpyrazole [13], 3-p-anisylpyrazole [4], 3,5-di-tert-butylphenylpyrazole [14], 3-anthrylpyrazole [15], 3-mesitylpyrazole, 3-cumyl-5-

methylpyrazole [16], 3, 5-trifluoromethylpyrazole [17]. Most of the synthesized substituted pyrazoles have been used for the synthesis of their dihydrobis-, hydrotris- and tetrakispyrazolylborate salts which have wide application in coordination chemistry, bioinorganic and organometallic chemistry.

The pyrazolylborates / scorpionate chemistry signify a very large and growing aera. These ligands are extremely versatile because the number of donor atoms in each ligand can be varied from two to three while going from the bidentate ligands to the potentially tridentate ligands. The steric properties of the ligands can be altered by changing the substituents on the pyrazolyl rings. These ligands have been used extensively to prepare complexes of the transition metals [18]. The polypyrazolylborate ligands have properties lying between those of parent $[H_4\text{-}nB(pz)_n]^-$ ligands (pz = pyrazolyl) and those of the strerically hindered derivatives $[H_4\text{-}nB(3\text{-}Rpz)_n]^-$, where R = tert-butyl and phenyl groups [2,19]. The parents ligands, in particular the derivatives with n = 3, have been extensively used in inorganic and bioinorganic chemistry. However, the chemistry is severly limited because of the tendency of the first row transition metal ions to form the bisligand complex (i.e, $M(HB(pz)_3)_2$). On the other hand, the bulky group of the 3-R derivatives prevents formation of this type of complex. The 3-tert-butyl ligand allows no further access to the metal ion and is a "tetrahedral enforcer", whereas the 3-phenyl derivative accommodates five coordination with trigonal-bipyramidal geometry. Although bisligand complex formation is prevented, the metal ion is not readily accessible to other molecules because of the steric bulk of the 3-substituents.

In contrast to cyclopentadienide ligands, with which they have often been compared, the tris(pyrazolyl)borate ligands offer much greater opportunities for modifying their

coordinative features. Whereas only one substituent can be placed on $C_5H_5(C_5R_5)$ to yield a derivative ligand with retention of the original ligand symmetry, there are 15 ways of placing from one to ten identical substituents on the parent $HB(pz)_3$ ligand and still maintain their original C_{3v} symmetry. It is well established in the literature that from a structural point of view the complex chemistry of the trispryrazolylborate anion frequently resembles that of cyclopentadienyl (or pentamethylcyclopentadienyl) complexes. However, as might be expected for a tridentate nitrogen donor ligand; the trispyrazolylborate complexes are kinetically less labile than their cyclopentadienyl analogues.

In the hierarchy of increasing steric hindrance around the metal in 3- or 5-substituted tris(pyrazolyl)borates, the currently known series is $H < CH_3 < C_6H_5 < {}^iPr < {}^tBu$. In terms of forming octahedral L_2M complexes, ligands with R = H and CH_3 do so rapidly whereas those with $R = C_6H_5$ do so reluctantly and those with $R = {}^iPr$ do not form L_2M complexes [6]. The ligands with $R = {}^iPr$ form octahedral complexes only with rearrangement of each L to $HB(3\text{-}{}^iPrpz)_2(5\text{-}{}^iPrpz)$; they also form mixed octahedral LML complexes, provided L is a relatively unhindered tris(pyrazolyl)borate ligand [20]. Finally, ligands with $R = Bu^t$ do not form octahedral complexes at all with first-row transition metals. Conversely, in terms of formation of LMX (X= halide or pseudohalide) species, ligands with $R = Bu^t$ form these stable complexes with ease, with $R = {}^iPr$, LMX complexes are also readily formed. But they posses reactivity for solvation or for displacement of X with anionic nucleophiles.The ligands with R = phenyl form LMX species, which are even more reactive towards solvation or substitution of X. When R = methyl, and even more so when R = H, the LMX species becomes progressively less stable and undergo transformation to the octahedral L_2M complexes.

Among the different types of pyrazolylborates, hydrotris(pyrazolyl)borate (Tp^R) ligand has been widely used as supporting ligand for various inorganic and organometallic compounds. It has unique structural (i.e., facially capping tridentate ligand) and electronic characteristics ($6e^-$ donating mono anionic ligand) are similar to those of the family of cyclopentadienyl (Cp^R) ligands, which are the most commonly adopted ligands in organometallic chemistry. One of advantages of the tris(pyrazolyl)borate ligands is the ease of controlling the properties of the resulting metal complexes (coordination environment and reactivity of metal centers, solubility in organic solvents, facility of crystallization, etc.) by introduction of various substituents into the pyrazole rings. It is notable that highly sterically demanding hydrotris(pyrazolyl)borate ligands containing bulky substituents at the 3-position of the pyrazole rings can stabilize a coordinatively unsaturated metal center and provide a vacant site for substrate binding. In addition, a hydrophobic shading pocket formed by the bulky substituents surrounding the metal center may kinetically stabilize unstable functional groups such as O-O and metal-carbon bond moieties.

The Tp^R ligand is also able to tune the electronic and steric environment of the central metal atoms by introducing various substituents to the pyrazolyl ring. In addition to this feature, a coordination environment created by the Tp^R ligand can mimic an environment created by a set of three imidazole rings of histidine residue, which is frequently found in the metal coordination site of metalloproteins. Moro-oka's et al. have reported the use of Tp^R for modeling the structure and function of the metal centers in various metalloproteins [in particular, hydridotris(3,5-diisopropylpyrazolyl)borate (Tp^{iPr2}) [21, 22] and hydrotris(3-tert-butyl-5-ispropyl-pyrazolyl)borate ($Tp^{tBu, iPr}$)]. Also, hydrotris(pyrazolyl)borate ligands having various bulky substituents on pyrazolyl ring have been prepared and demonstrated to be

advantageous supporting chelates in organometallic and bioinorganic studies. Metal complexes of some of the sterically hindered hydrotris(pyrazolyl)borate ligands relevance to the present work are given below:

Hydrotris(3-tert-butylpyrazol-1-yl)borate [=HB(3-^{t}Bu-pz)$_3$=Tp^{tBu}]

Its coordinative behaviour reflected the severe screening of the metal in the Tp^{tBu}M fragment, so that with first row transition metals (Mn to Zn) only four coordinate tetrahedral complexes of type Tp^{tBu}MX were obtained (X=Cl, NCS, NCO, N_3), which resisted solvation. No (Tp^{tBu})$_2$M species could be obtained, in contrast to Tp and Tp* [2]. The structure of Tp^{tBu}CoNCS was established by X-ray crystallography.

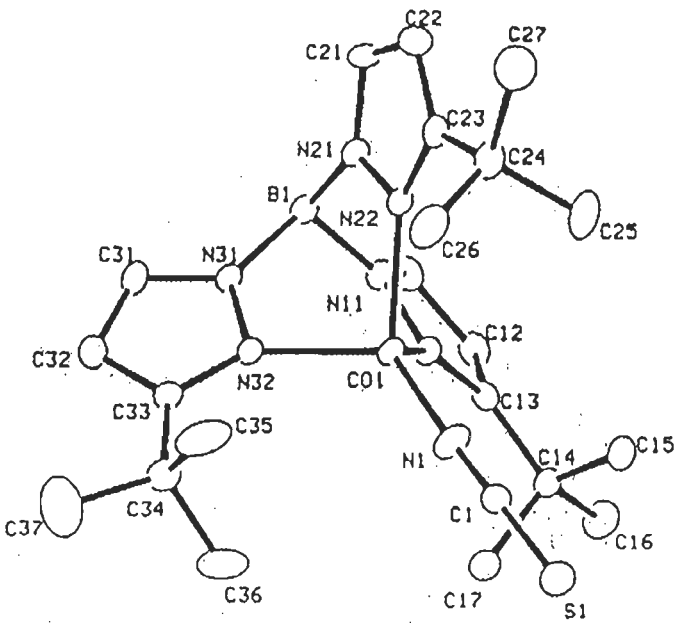


Fig. 1-1 Crystal Structure of [Tp^{tBu}CoNSC]

Hydrotris(3-tert-butyl-5-methylpyrazol-1-yl)borate[=HB(3-^{t}Bu-5-Mepz)$_3$=Tp^{tBu,Me}]

It is very similar to Tp^{tBu}, but the presence of the 5-methyl groups offers steric protection to the B-H bond; moreover, the nonbonding repulsions of the three 5-Me groups are likely to somewhat tighten the bite of the ligand at the metal end. The fact is that Tp^{tBu,Me}MX derivatives show greater stability than their Tp^{tBu} analogs. The structure of

$Tp^{tBu,Me}NiNCS$ as determined by X-ray crystallography showed that the Ni is four coordinated [4]. The complex $[Tp^{tBu,Me}Cu]_2$ has a dimeric structure, similar to that of $[Tp^{tBu}Cu]_2$, while the structure of $Tp^{t\text{-}Bu,Me}CuCl$ is different from that of $Tp^{tBu}CuCl$. Also characterized by X-ray crystallography were tetrahedral complexes $Tp^{tBu,Me}HgI$, $Tp^{tBu,Me}CdMe$, and the five coordinate $Tp^{tBu,Me}Cd(NO_3)$.

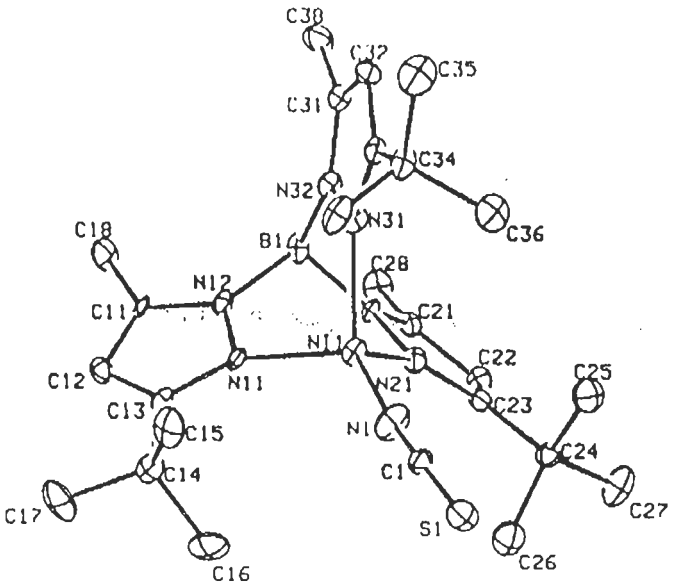


Fig. 1-2 Crystal structure of $[Tp^{tBu,Me}NiNCS]$

Alsfasser et al. [23] reported the formation of $Tp^{tBu,Me}Zn(OH)$ as an analog of enzyme carbonic anhydrase. This compound absorbed CO_2 and yielded to the isolable bicarbonate complex, which upon further reaction resulted the formation of the carbonate complex. They determined the structure of this complex, and of $Tp^{tBu,\ Me}ZnO(CO)OMe$ by X-ray crystallography [24].

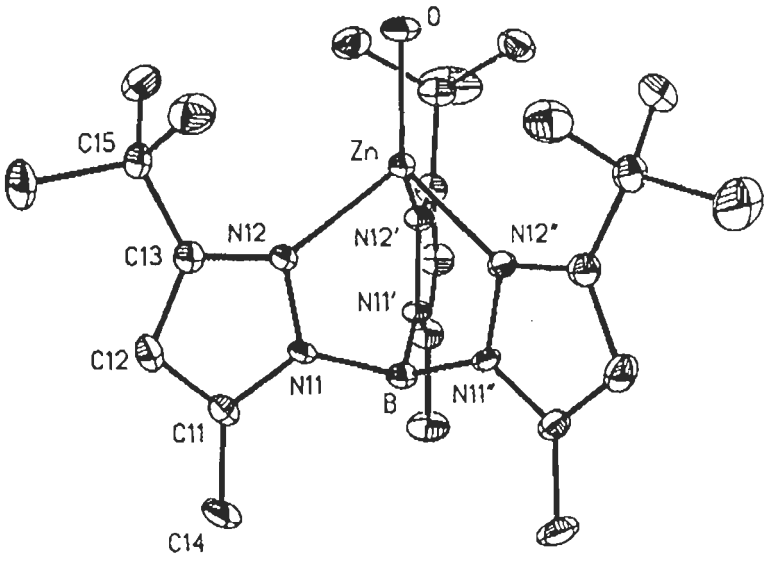


Fig 1-3; Crystal structure of $Tp^{tBu,Me}Zn(OH)$

$Tp^{tBu,Me}CoCl$ upon reduction with Mg gave the complex $Tp^{tBu,Me}CoN_2$ which reacted with oxygen and resulted the formation of the superoxo complex [5].

Berquist et al. [25] studied the protonation of $[(Tp^{tBu,Me})]ZnOH$ by $(C_6F_5)_3B(OH_2)$ and structurally characterized by X-ray diffraction, The protonation studies resulted in a lengthening of the Zn-O bond by ca. 0.1 Å. The cobalt hydroxide complex $[Tp^{tBu,Me})]CoOH$ after similar protonation reaction resulted the product, which was isostructural with the zinc complex.The aqua complexes $[Tp^{tBu,Me}]M(OH)_2][HOB(C_6F_5)_3]$ (M = Zn, Co) exhibited a hydrogen bonding interaction between the metal aqua and boron hydroxide moieties, which may be viewed as analogous to that between the aqua ligand and Thr-199 at the active site of carbonic anhydrase. The cobalt hydroxide $[Tp^{tBu,Me}]CoOH$ reacted with CO_2 and gave the bridging carbonate complex $[(Tp^{tBu,Me})]CoOH]_2(\mu\text{-}\eta^1, \eta^2\text{-}CO_3)$. The coordination mode of the carbonate ligand in this complex was bidentate to one cobalt center and unidentate to the other wheras in the zinc counterpart $[[Tp^{tBu,Me})]ZnOH]_2(\mu\text{-}\eta^1, \eta^2\text{-}CO_3)$, carbonate ligand bridged in a unidentate manner to both zinc centers. The above finding about different coordination mode may be a possible reason that the lower activity of Co(II)-carbonic

anhydrase is associated with enhanced bidentate coordination of bicarbonate inhibiting its displacement.

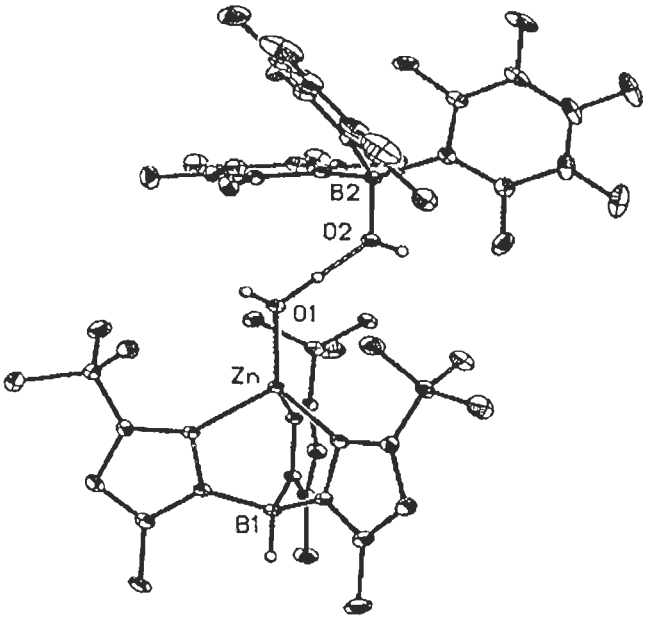


Fig. 1-4 The Crystal sture of $[Tp^{tBu,Me})]Zn(OH)_2][HOB(C_6F_5)_3]$

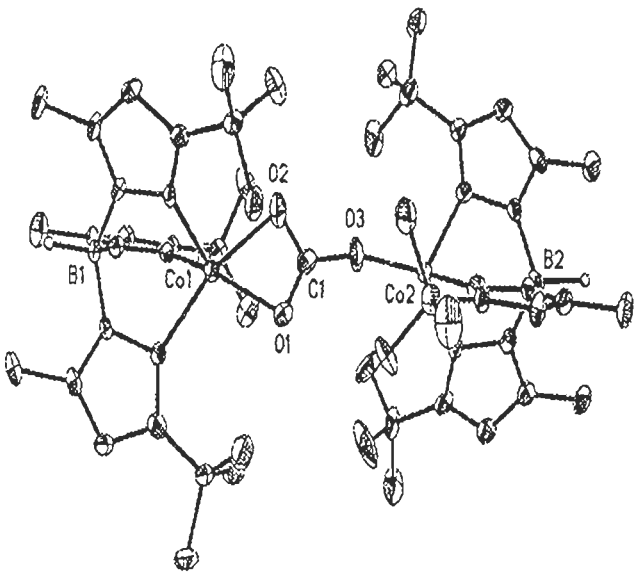


Fig. 1-5 Crystal Structure of $[Tp^{tBu,Me})]CoOH]_2(\mu\text{-}\eta^1, \eta^2\text{-}CO_3)$

Hydrotris(3-phenylpyrazol-1-yl)borate [= $HB(3Phpz)_3 = Tp^{Ph}$]

In contrast to Tp^{tBu}, it readily formed five coordinate solvated species (which retains solvent tenaciously), such as $Tp^{Ph}CoNCS(THF)$, the structure of which was determined by X-ray ctystallography. The structures of $Tp^{Ph}ZnX$ (X = NO_3, Me, SEt) were determined by X-ray crystallography, the NO_3 ligand is asymmetrically bidentate, with one long O-Zn bond, so that Zn is five coordinate [26]. In contrast to Tp^{tBu}, Tp^{Ph} is capable of forming octahedral complexes, such as $Tp^{Ph2}M$ (M = Fe, Mn) in the absence of strongly coordinating anions. The Tp^{Ph} ligand was used in the preparation of the structurally characterized, mixed Cu-protein complex [27]. Kremer-Aach el al. [28] reported the synthesis of blood-red bis(ligand) complex [$Co(\eta^3\text{-}Tp^{Ph})(\eta^2\text{-}Tp^{Ph})$] by the reaction of cobalt(II) perchlorate with 1 equivalent each of potassium hydroxide and potassium hydrotris(3-phenylpyrazolyl)borate (KTp^{Ph}). As shown in molecular structure, contains a square Co-N (5) pyramid with an agostic BH-Co interaction of 2.17(2) Å. One Tp^{Ph} ligand acts tridentate; the other, bidentate. The reaction of KTp^{Ph} with zinc and cobalt halides gave a series of heteroleptic halogeno complexes [$(Tp^{Ph})MX$]. The zinc species were all tetrahedral, while in the case of cobalt (II), the UV-vis spectra indicated equilibrium of tetra- and pentacoordinated species depending on the anion size and the donor properties of the solvent. Metathesis reactions with carboxylates yielded mononuclear complexes [$(Tp^{Ph})M(O_2CR)$]; M = Zn(II), Co(II); $RCOO^-$ = acetate, benzoate, 4-fluorobenzoate, and 4-nitrobenzoate. The infrared bands $\nu_{as}(CO_2)$ and $\nu_s(CO_2)$ indicated monodentate carboxylate ligands in the zinc complexes in the solid state and in solution. They also prepared the 2-aminobenzoate (anthranilate) complexes [$(Tp^{Ph})M$(anthranilate)], M = Zn(II), Co(II). X-ray study suggested that anthranilate acts as a chelating oxygen ligand with Zn-O distances of 1.932(2) and 2.460(2) Å and the amino group was not involved in

metal coordination. The reaction of the chloro complexes [$Tp^{Ph}MCl$] with sodium or potassium acetylacetonate gave isotypic zinc and cobalt complexes [(η^3)-Tp^{Ph})M(η^2)-acac)]. Both crystallize in the monoclinic space group P2(1)/c, with Z = 4 and the structures were best described as slightly distorted trigonal bipyramids with the two axial positions occupied by one of the acetylacetonate oxygen and one of the tripodal N donor atoms.

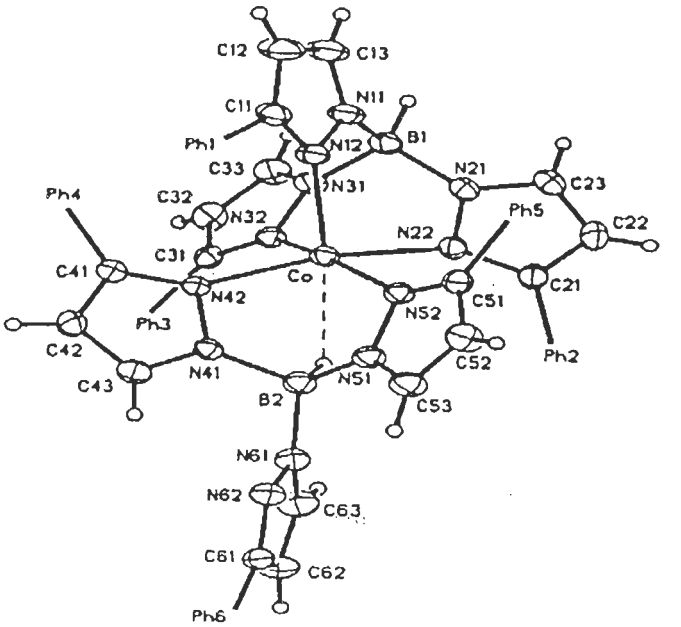


Fig. 1-6 Crystal structure of [Co(η^3-Tp^{Ph})(η^2-Tp^{Ph})]

Hydrotris(3,5-diphenylpyrazol-1-yl)borate [= $HB(3Ph_2pz)_3$ = Tp^{Ph2}]

It was converted to the Tp^{Ph2}CuCo complex, which reacted with PMe_3 but not with PPh_3 and yielded the $Tp^{Ph2}CuPR_3$ derivative [29].

Hydrotris(3-(p-tolyl)pyrazol-1-yl)borate [= $HB[3(p\text{-tolyl})pz]_3$ = Tp^{pTol}]

It was characterized as the Tl salt and was converted to a variety of tetrahedral $Tp^{pTol}MX$ complexes (M = Co Ni, Zn; X = NCS, NCO, N_3) the ligand field spectra of which were studied [4].

Hydrotris(3-isopropylpyrazol-1-yl)borate [= $HB(3\text{-}^{i}Prpz)_3$ = Tp^{iPr}] and

Hydrotris(3-isopropyl-4-bromopyrazol-1-yl)borate [= $HB(3\text{-}^{i}Pr\text{-}4Brpz)3$ = $Tp^{iPr,\,4Br}$]

The steric requirements of the 3-isopropyl group are intermediate between those of 3-tert-butyl and 3-phenyl. The effect of the 4-bromo substituent makes the $Tp^{iPr,4Br}$ derivatives more crystalline. Both ligands, Tp^{iPr} and $Tp^{iPr,4Br}$, yielded tetrahedral derivatives $Tp^{iPr}MX$ and $Tp^{iPr,Br}MX$. These complexes formed five coordinate solvates with the unhindered solvents (e.g. with MeOH but not with ^{i}PrOH), but loses solvent slowly and reverted to the tetrahedral complex [6]. Both regiospecifically pure ligands formed octahedral L^*_2M complexes but this occured with rearrangement of each ligand to $HB[(3\text{-}^{i}Prpz)_2(5\text{-}^{i}Prpz)]$ and $HB(3\text{-}^{i}Pr\text{-}4Brpz)_2(5\text{-}^{i}Pr\text{-}4\text{-}Brpz)]$, respectively as was proven by NMR studies of the paramagnetic Co(II) complexes, and by X-ray crystallography. The structure of $Tp^{iPr,4Br}CoCl$ determined by X-ray crystallography [30]. The stable $Tp^{iPr,Br}MCl$ complexes were very suitable for the preparation of hetroleptic compounds, $Tp^{iPr,\,4Br}ML$ via reaction with L^- ligands [20].

Brunker et al. [31] synthesised the 4-coordinate Tp'MCl complexes (where M = Fe, Mn and Tp' = hydrotris(3-isopropyl-4-bromopyrazolyl)borate). The single-crystal X-ray structures showed that the metal centers have distorted tetrahedral coordination. Analogous reaction of $CrCl_2(MeCN)_2$ with TlTp' gave $Cr(\kappa^3\text{-}Tp')(\kappa^2\text{-}Tp')$ as the initial product. The 5-coordinate structure was assigned by single-crystal X-ray crystallography, and it was found that the κ^3 ligand had isomerized to hydro(3-isopropyl-4-bromopyrazolyl)$_2$(5-isopropyl-4-bromopyrazolyl)borate). This was labile in solution and slowly converted to the 6-coordinate isomer $Cr(\kappa^3\text{-}Tp')$ in pentane determined by X-ray crystallography.

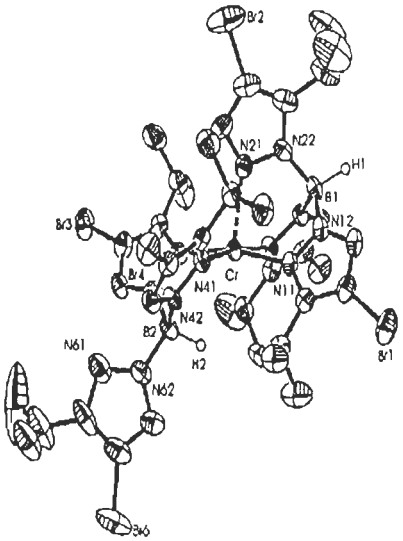


Fig 1-7 Crystal Structure of $Cr(\kappa^3\text{-}Tp')_2$

Hydrotris(3,5-diisopropylpyrazol-1-yl)borate [= $HB(3,5\text{-}^{i}Pr_2pz)_3$ = Tp^{iPr2}]

In contrast to Tp^{iPr} or $Tp^{4Br,iPr}$, where the isopropyl group straddles the pz plane with the methyl pointed either towards the metal (as in $Tp^{iPr}CoNCS$) or away from the metal (as in $TpCoTp^{4Br,iPr}$), in all hitherto reported Tp^{iPr2} complexes the isopropyl methyl groups pointed toward the 4-H. The symmetrical substitution with isopropyl groups precludes rearrangements observed with Tp^{iPr}, $Tp^{iPr,4Br}$, and $Tp^{iPr,Me}$ which would, anyway, be degenerate.Much of the Tp^{iPr2} coordination chemistry deals with copper, manganese, cobalt, iron and nickel. The $Tp^{iPr2}Cu$ complex, obtained from KTp^{iPr2} with CuCl, bonds to CO, yielded $Tp^{iPr2}CuCO$, which, unlike $Tp^{Ph2}CuCO$, reacted with triphenylphosphine, yielded $Tp^{iPr2}CuPPh_3$ [32] .

The ligand Tp^{iPr2} readily formed tetrahedral $Tp^{iPr2}CuCl$ upon reaction with $CuCl_2$. The structure of $Tp^{iPr2}CuCl$, and of its readily formed five coordinate DMF adduct, was established by X-ray crystallography. Treatment of $Tp^{iPr2}CuCl$ with NaSR caused reduction of the former, but $Tp^{iPr2}CuS^{t}Bu$ could be prepared by the reaction of $HS^{t}Bu$ with the

dinuclear $[Tp^{iPr2}Cu(OH)]_2$ [32]. This complex was claimed to have the closest resemblance in its type 1 CT and ESR spectral features, as compared with the actual enzyme [33].

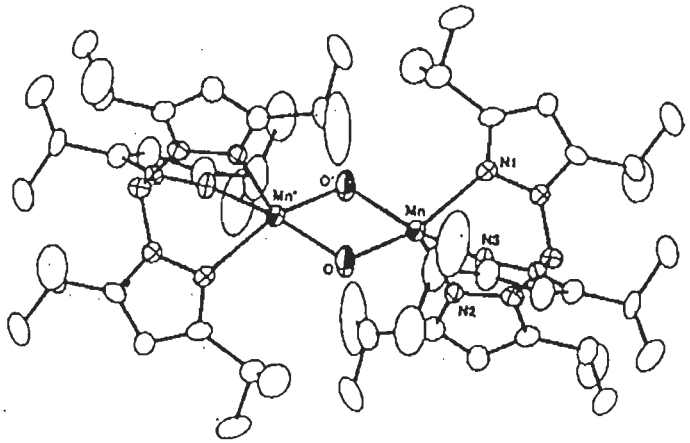


Fig. 1-8 Crystal structure of $[Tp^{iPr2}CuOH]_2$]

$Tp^{iPr2}CuOH]_2$ reacted with H_2O_2 and resulted μ-η^2:η^2-peroxobinuclear copper(II) complex. The following structure of this, containing the unusual side-on bridging by O_2, was confirmed by X-ray crystallography [34].

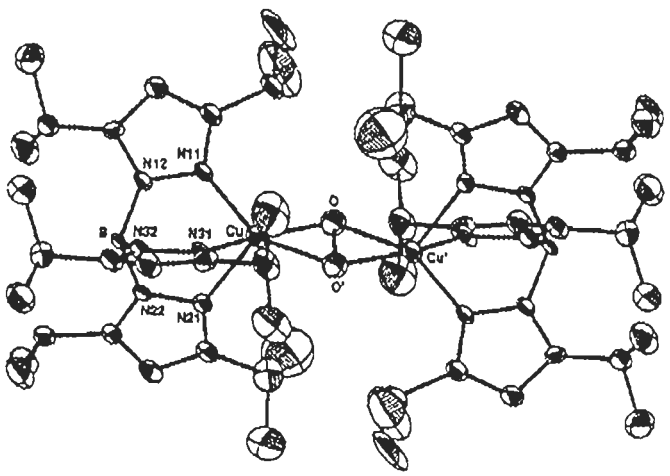


Fig.1-9 Crystal structure of $[Tp^{iPr2}Cu(O)]_2$

The dinuclear complex $[Tp^{iPr2}Mn(OH)]_2$, with a structure analogous to that of the dinuclear copper complex, was prepared by treating the five coordinate complex $Tp^{iPr2}MnCl(3,5\text{-}^{i}Pr_2PzH)$ with NaOH. Oxidation of $[Tp^{iPr2}Mn(OH)]_2$ with either $KMnO_4$ or O_2 afforded the dark red $Tp^{iPr2}Mn(\mu\text{-}O)_2MnTp^{iPr2}$, containing Mn(III), the structure of which was established by X-ray crystallography [35]. While the anaerobic reaction with $KMnO_4$ produced only $Tp^{iPr2}Mn(\mu\text{-}O)_2MnTp^{iPr2}$, oxidation with oxygen yielded two types of Mn(III) complexes, the already described $Tp^{iPr2}Mn(\mu\text{-}O)_2MnTp^{iPr2}$ and a complex, containing the $Tp^{iPr2}Mn\text{-}O\text{-}MnTp^{iPr2}$ core, but also with additional Mn-O bridges from each Mn to one isopropyl carbon per Tp^{iPr2} ligand [36]. This was regarded as a dioxygenase-type oxidation.

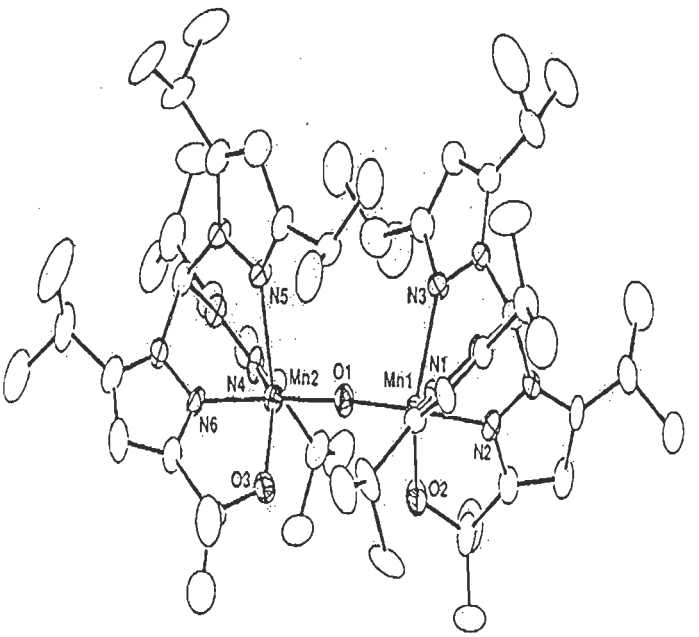


Fig. 1-10 Crystal structure of $[Tp^{iPr2}Mn\text{-}O\text{-}MnTp^{iPr2}]$

Replacement of chloride in $Tp^{iPr2}FeCl$ with benzoate ion produced the five coordinate benzoate complex, which was still coordinatively unsaturated, binding oxygen reversibly, and which on addition to added pyridine or acetonitrile form octahedral complexes [37].

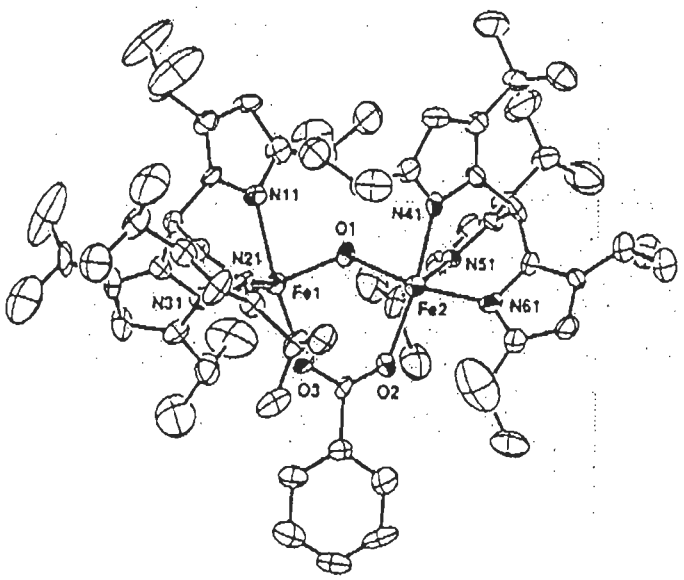


Fig. 1-11 Crystal structure of [$Tp^{iPr2}Fe(OH)(OBz)$]

The complex $Tp^{iPr2}Zn(OH)$ was converted via cleavage of tris- or bis-phosphate esters to the structurally characterized dinuclear, phosphate bridged $(Tp^{iPr2}Zn)_2(\mu\text{-}ArOP(O)O_2)$ and to related monomeric complexes [38].

Mariae et al. [39] reported the reaction of $[UI_3(THF)_4]$ with 1 equivalent of KTp^{iPr2} in toluene in the presence of several neutral coligands allowed the synthesis of a novel family of mono-Tp^{iPr2} complexes, $[UI_2Tp^{iPr2}L_x]$ [L = $OPPh_3$, x = 1; L = C_5H_5N, x = 2; L = $Hpz^{t(Bu,Me)}$, x = 2; and L = bipy, x = 1]. The adduct with THF, $[UI_2Tp^{iPr2}(THF)_2]^{3-}$, could also be isolated by reacting $[UI_3(THF)_4]$ with 1 equivalent of KTp^{iPr2} in tetrahydrofuran.

Hydrotris(3-(2-thienyl)pyrazol-1-yl)borate [= HB(3-Tnpz)$_3$ = Tp^{Tn}]

The ligand Tp^{Tn} showed much more resemblance in its coordination behavior to Tp than to Tp^{Ph}. The complexes $Tp^{Tn2}M$ are formed very readily, while attempts to prepare tetrahedral $Tp^{Tn}Mx$ species produced $Tp^{Tn2}M$ only.

Hydrotris(3-neopentylpyrazol-1-yl)borate [= HB(3-neopentylpz)$_3$ = TP^{Np}]

Compounds of type Tp^{Np}MX were easily prepared, were stable and readily produced hetroleptic Tp^{Np}ML complexes, e.g. Tp^{Np}CoTp. For steric reasons, the neopentyl group in tetrahedral Tp^{Np}CoNCS was oriented with the tertbutyl groups almost perpendicular to the pz plane, and all pointed either clockwise or counterclockwise, when viewed along the B-M axis [40]. In contrast to Tp^{iPr} and Tp^{iPr,4Br}, unrearranged Tp^{Np2}M complexes was isolated. The structure of Tp^{Np2}Ni, established by X-ray crystallography, was octahedral. The equatorial compression of the six neopentyl groups twisted them into a conformation where the tert-butyl groups were turned away from the metal, and the CH_2-CMe_3 bond was almost parallel with the pz plane.The unrearranged Tp^{Np2}Co complex was probably five coordinate in the solid, and tetrahedral in solution. On heating it became octahedral, via ligand rearrangement, as did the complex Tp^{Np2}Fe. All the rearranged octahedral complexes were isomorphous [40]. In its effective steric blocking, the neopentyl 3-substituents was very similar to 3-isopropyl, so that the steric hindrance hierarchy for Tp^R ligands was R = H < 2-theinyl < Me < Ph < ^{i}Pr ≈ neopentyl < ^{t}Bu.

Hydrotris(3-mesitylpyrazol-1-yl)borate [HB(3-mesitylpz)$_3$ = Tp^{Ms}]

To prevent or restrict rotation of a planar aromatic substiuent in the 3-position of a Tp ligand, homoscorpionate ligands were prepared containing 2, 6-substituents on the 3-phenyl which would prevent coplanarity with the pz ring (possible with R = phenyl), and which would tend force the phenyl ring to be orthogonal to the pz plane. This would eliminate complications arising from oxidative addition of the 3-phenyl group to a coordinatively unsaturated metal. One such ligand was Tp^{Ms}, prepared from KBH_4 and 3-mesitylpyrazole

and it was converted to tetrahedral derivatives, $Tp^{Ms}MX$, which did not produced crystal suitable for X-ray crystallography [16].

Hydrotris(3-(9-anthryl)pyrazol-1-yl)borate [= HB[3-(9-anthryl)pz]$_3$ = Tp^{Ant}]

Another ligand with an aromatic 3-substiuent, orthogonal to the pz ring is Tp^{Ant}, containing a 9-anthryl group. $Tp^{Ant}Tl$, $Tp^{Ant}CoCl$, and $Tp^{Ant}CoNCS$ were determined by X-ray crystallography. In all of them the anthryl group was almost orthogonal to the pz plane, but total orthogonality was prevented by nonbonding interaction of the 2,3- and 6,7-hydrogens. The anthryl groups provided extensive side shielding of the metal, but at the same time, permited considerable frontal access [15].

Hydrotis(3-diphenylmethylpyrazole)borate[=HB[(3-diphenylmethyl)pz]$_3$ = Tp^{CHPh2}]

Rheingold et al. [12] synthesised the new ligand, hydrotris[3-(diphenylmethyl)pyrazol-1-yl]borate, Tp^{CHPh2}, and its coordination chemistry was compared with that of the analogous Tp^{iPr}. The new ligand converted to a variety of complexes, such as $M[Tp^{CHPh2}]X$ (M = Co, Ni, Zn; X = Cl, NCO, NCS), $Pd[Tp^{CHPh2}][\eta^3$-methallyl], $Co[Tp^{CHPh2}]$(acac), and $Co[Tp^{CHPh2}]$(scorpionate ligand). Compounds $Tl[Tp^{CHPh2}]$, $Co[Tp^{CHPh2}]Cl$, $Co[Tp^{CHPh2}](NCS)(DMF)$, $Ni[Tp^{CHPh2}](NCS)(DMF)_2$, $Co[Tp^{CHPh2}]$(acac), $Co[Tp^{CHPh2}][Ph_2Bp]$, $Co[Tp^{CHPh2}][Bp^{Ph}]$, $Co[Tp^{CHPh2}][Tp]$ and $(Ni[Tp^{CHPh2}])_2[C_2O_4](H_2O)_2$ were structurally characterized.

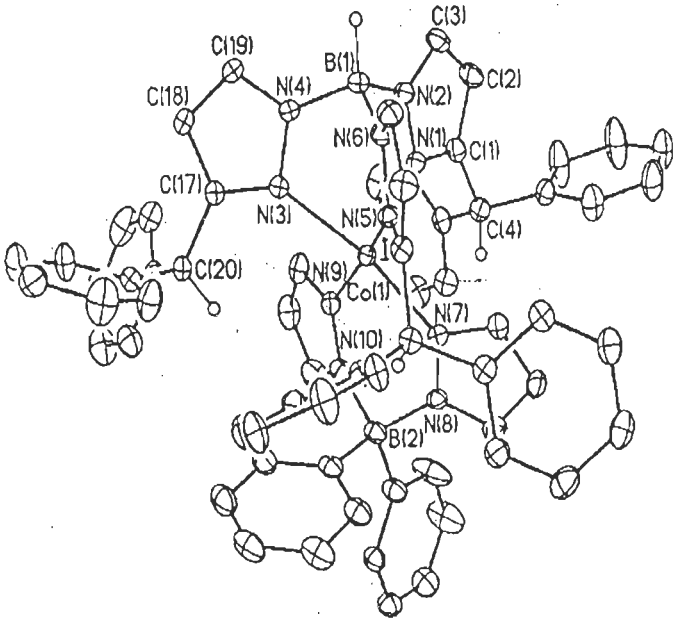


Fig. 1-12 Crystal Structure of $Co[Tp^{CHPh2}][Bp^{Ph}]$

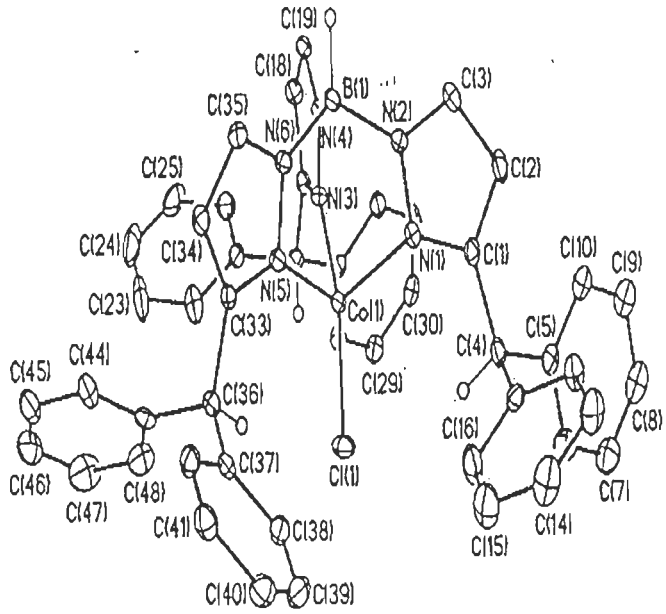


Fig. 1-13 Crystal Structure of $Co[Tp^{CHPh2}]Cl$

Hydrotris(3,5-ditrifluormethylpyrazol)borate[=HB[(3,5-ditrifluoromethyl)pz]$_{3=}$ $Tp^{2(CF3)}$]

Dias et al. [41] synthesized the fluorinated tris(pyrazolyl)borate ligands $[HB(3,5\text{-}(CF_3)_2Pz)_3]^-$ and $[HB(3\text{-}(CF_3)Pz)_3]^-$ (where Pz = pyrazolyl) as their sodium salts from the corresponding pyrazoles and $NaBH_4$ in high yield. These sodium complexes and the related $[HB(3,5\text{-}(CF_3)_2Pz)_3]K(DMAC)$ were used as ligand transfer agents in the preparation of the copper and silver complexes $[HB(3,5\text{-}(CF_3)_2Pz)_3]Cu(DMAC)$, $[HB(3,5\text{-}(CF_3)_2Pz)_3]CuPPh_3$, $[HB(3,5\text{-}(CF_3)_2Pz)_3]AgPPh_3$, and $[HB(3\text{-}(CF_3)_2Pz)_3]AgPPh_3$. The metal complexes of the fluorinated $[H(3,5\text{-}(CF_3)_2pz)_3]^-$ ligand, have highly electrophilic metal sites relative to their hydrocarbon analogs. This was evident from the formation of stable adducts with neutral oxygen donors such as H_2O, dimethylacetamide, or THF. Furthermore, the metal compounds derived from fluorinated ligands showed fairly long-range coupling between fluorines of the trifluoromethyl groups and the hydrogen, silver, or phosphorus. The solid state structures showed that the fluorines were in close proximity to these nuclei, thus suggested a possible through-space coupling mechanism. Crystal structures of the sodium adduct exhibited significant metal-fluorine interactions. The treatment of $[HB(3,5\text{-}(CF_3)_2pz)_3]Na(H_2O)$ with Et_4NBr led to $[Et_4N][HB(3,5\text{-}(CF_3)_2pz)_3]$, which contains a well-separated $[Et_4N]^+$ cation and the $[HB(3,5\text{-}(CF_3)_2pz)_3]^-$ anion in the solid state.

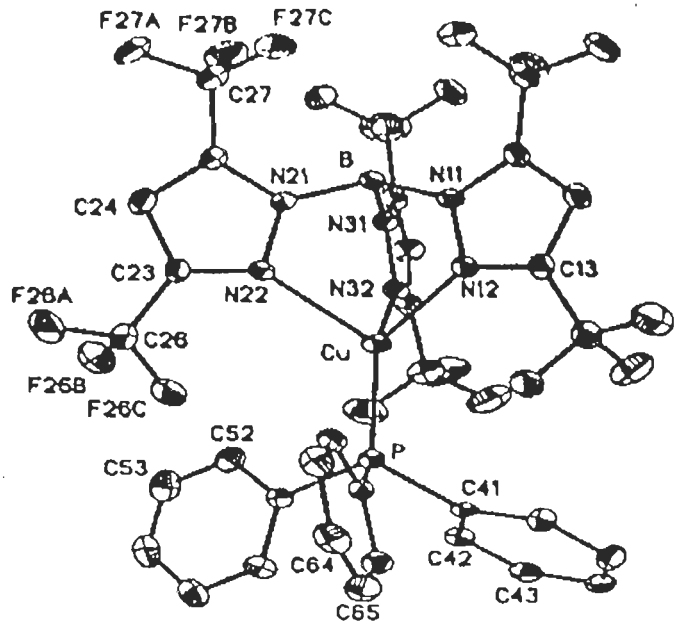


Fig. 1-14 Crystal structure of $[HB(3,5\text{-}(CF_3)_2Pz)_3]CuPPh_3$

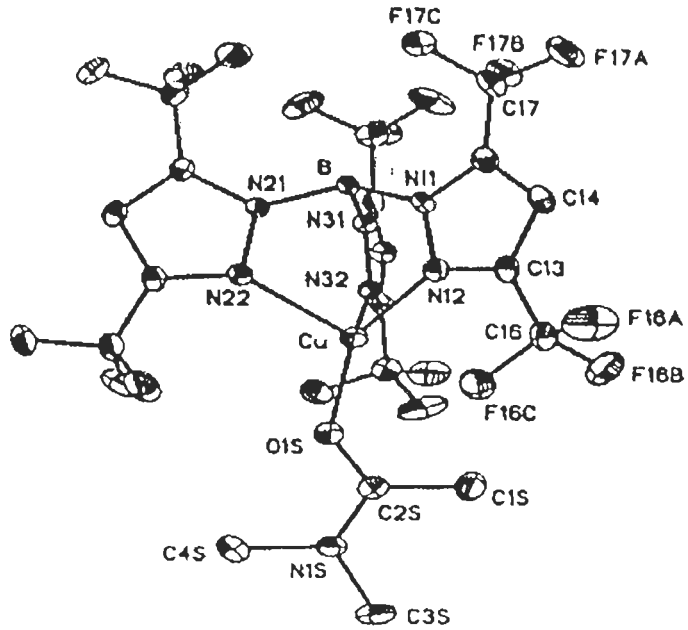


Fig. 1-15 Crystal structure of $[HB(3,5\text{-}(CF_3)_2Pz)_3]Cu(DMAC)$

Steric effects on metal complex properties were particularly profound in these systems due to orientation of the R^3 substituents, which enclose the bound metal ion in highly protected pocket.

From a bioinorganic point of view, the coordination environment provided by tris(pyrazolyl)borate ligand is fascinating, since the geometry and the ligand donor set reasonably mimic the coordination of three histidyl nitrogen atoms in a pyramidal array often found at the metal sites in proteins. Such coordination structures have been established by X-ray crystallography for a number of metalloproteins which include hemocyanin [42], hemerythrin [43], ascorbate oxidase [44], Cu, Zn-SOD, Fe/Mn-SOD [45], carbonic anhydrase and the non heme iron at the photosynthetic reaction center [46]. Moreover numerous other proteins are known to contain a metal ion ligated by one or two histidyl nitrogen atoms [47].

We and other workers have recently demonstrated a much higher synthetic utility of more hindered trispyrazolylborate ligand $[Tp^{iPr2}]^-$ and $[Tp^{tBu,iPr}]^-$ over $BH(pz)_3^-$ and $[TP^{Me2}]^-$ [9, 36, 38, 47, 48].

The advantageous characteristics of this ligand are as follows:

1. Preventing formation of bis-chelated complexes completely.
2. High solubility in non-coordinating solvents such as toluene and pentane.
3. Highly shielding effect of isopropyl groups to stabilize unusual coordinating structures.
4. Moderate steric hindrance to allow formation of dimeric complexes.
5. Strong electron donating property.

Owing to these properties, we have succeeded in preparing some manganese and cobalt of Mn and Co complexes with KTp^{iPr2} and $KTp^{tBu,\ iPr}$, some of which may serve as models for the active sites of these metal-containing proteins.

References:

1. (a)Trofimenko, S., "Boron-pyrazole chemistry" J. Am. Chem. Soc., **88**, 2445 (1966). (b) Trofimenko, S., "Boron-pyrazole chemistry II. Poly(1-pyrazolyl)-borate" J. Am. Chem. Soc., **89**, 3170 (1967). (c)Trofimenko, S., "The coordination chemistry of pyrazole-derived ligands", Chem. Rev., **72**, 497 (1972).
2. Trofimenko, S., Calabrese, J. C. and Thompson, J. S., "Novel polypyrazolylborate ligands: Coordination control through 3-substituents of the pyrazole rings", Inorg. Chem., **26**, 1507 (1987).
3. Imai, S., Fujisawa, K., Kobayashi, T., Shirasawa, N., Fuju, H., Yoshimura, T., Kitajima, N. and Moro-oka, Y., "^{63}Cu NMR study of copper(I) carbonyl complexes with various hydrotris(pyrazolyl)borates: Correlation between ^{63}Cu chemical shifts and CO stretching vibrations", Inorg. Chem., **37**, 3066 (1998).
4. Trofimenko, S., Calabrese, J. C., Kochi, J. K., Wolowiece, S., Hulsbergen, R. B. and Reedijk, J., "Spectroscopic analysis, coordination geometry and X-ray structure of nickel(II) compounds with sterically demanding `tris(pyrazolyl)borate ligands and azide or (thio)cyanate anions. Crystal and molecular structure of bis [(μ-thiocyanato-N,S) (hydrotris(3-isopropyl-4-bromopyrazol-1-l)borato)nickel(II)]–3-heptane and (thiocyanato-N)(hydrotris(3-tert-butyl-5-methylpyrazol-1-yl)borato)nickel(II)]", Inorg. Chem., **31**, 3943 (1992).

5. Egan, J. W., Haggerty, B. S. Jr., Rheingold, A. L., Sendlinger, S. C. and Theopold, K. H., "Crystal structure of a side-on superoxo complex of abstraction by a reactive terminal oxo ligand", J. Am. Chem. Soc., **112**, 2445 (1990).

6. Trofimenko, S., Calabrese, J. C., Domaille, P. J. and Thompson, J. S., "Steric effect in polypyrazolylborate ligands poly(3-isopropylpyrazolyl)borates: Ligands of intermediate steric requirements", Inorg. Chem., **28**, 1091 (1989).

7. Cano, M., Heras, J. V., Jones, C. J., McCleverty, J. A. and Trofimenko, S., "Sterically induced rearrangements of the tripodal ligand $HB(^{i}PrMeC_3N_2H)_3$ in some molybdenum mononitrosyl derivatives", Polyhedron, **9**, 619 (1990).

8. Kitajima, N., Fujisawa, K., Fujimoto, C., Moro-oka, Y., Hashimoto, S., Kitagawa, T., Toriumi, K., Tatsumi, K. and Nakamura, A., "A new model for dioxygen binding in hemocyanin. Synthesis, characterization and molecular structure of the μ-η^2:η^2 peroxo dinuclear copper(II) complexes, $[Cu(HB(3,5\text{-}R_2pz)_3)]_2(O_2)$ (R = iPr and Ph)", J. Am. Chem. Soc., **114**, 1277 (1992).

9. Kitajima, N., "Synthetic approach to the structure and function of copper proteins", Adv. Inorg. Chem., **39**, 1 (1992).

10. Calabrese, J. C., Domaille, P. J., Trofimenko, S. and Long, G. J., "Hydrotris[3-(2'-thienyl)pyrazol-1-yl]borate: A ligand of remarkably low steric requirements", Inorg. Chem., **30**, 2795 (1991).

11. Rheingold, A. L., Ostrander, R., Haggerty, B. S. and Trofimenko, S., "Homoscorpionate tris (pyrazolyl) borate ligands containing tethered 3-phenyl groups", Inorg. Chem., **33**, 3666 (1994).

12. Rheingold, A. L., Liable-Sands, L. M., Golen, J. A., Yap, G. P. and Trofimenko, S., "The coordination chemistry of the hydrotris(3-diphenylmethylpyrazol-1-yl)borate (TpCHPh2) ligand", J. Chem. Soc., Dalton Trans., 598 (2004).

13. Ferguason, G., Jennings, M. C., Laor, F. J. and Shanahan, C., "Structure of hydrotris [3-(para-tolyl) pyrazolyl]boratothalium (I)", Acta.Crystallogr. Ser., **C47**, 2079 (1991).

14. Libertini, E., Yoon, K. and Parkin, G., "The synthesis of $HB(3,5\text{-}(Bu^tPh)_2pz)_3]^-$ [^{t}BuPh = p-$C_6H_4{}^tBu$], a new tris(pyrazolyl)hydroboratoligand: The crystal and molecular structure of $Tl\{\eta^3\text{-}HB[3,5\text{-}(Bu^tPh)_2pz)_3]\}$", Polyhedron, **12**, 2539 (1993).

15. Han, R., Parkin, G. and Trofimenko, S., "The tris [3-(9-anthryl) pyrazol-1-yl]hydroborato ligand, [Tp(Ant)]: Compositional disorder between a vacancy and a chain of three atoms", Polyhedron, **14**, 387 (1995).

16. Rheingold, A. L., White, C. B. and Trofimenko, S., "Hydrotris (3-mesitylpyrazol-1-yl)borate and hydrobis(3-mesitylpyrazol-1-yl)(5-mesitylpyrazo-1-yl)borate: Symmetric and asymmetric ligands with rotationally restricted aryl substituents", Inorg. Chem., **32**, 3471 (1993).

17. Dias, H. V. R., "Chemistry of Trifluoromethylated tris(pyrazolyl)borate: Synthesis and characterization of carbonyl and isonitrile adducts of silver(I)", J. Am. Chem. Soc., **117**, 11381 (1995).

18. Trofimenko, S., "Coordination chemistry of pyrazole-derived ligands", Prog. Inorg. Chem., **34**,115 (1986). (b) Trofimenko, S., "Recent advances in poly(pyrazolyl) borate (scorpionate) chemistry", Chem. Rev., **93**, 943 (1993).

19. Calabrese, J. C., Trofimenko, S. and Thompson, J. S., "A new class of polypyrazolylborate ligands", J. Chem. Soc., Chem. Commun., 1122 (1986).

20. Calabrese, J. C., Domaille, P. J., Thompson, J. S. and Trofimenko, S., "Steric effects in polypyrazolylborates: Mixed complexes MHB(3-isopropyl-4-bromo- pyrazolyl)$_3$)L", Inorg. Chem., **29**, 4429 (1990).

21. Kitajima, N. and Moro-oka, Y., "Copper dioxygen complexes. Inorganic and bioinorganic perspectives", Chem. Rev., **94**, 739 (1994).

22. (a) Kitajima, N. and Tolman, W. B., "Coordination chemistry with sterically hindered hydrotris(pyrazolyl)borate ligands: Organometallic and bioinorganic perspectives", Prog. Inorg. Chem., **43**, 419 (1995). (b) Reger, D. L., "Poly(pyrazolyl)borate complexes of gallium and indium", Coord. Chem. Rev., **147**, 57 (1996).

23. Alsfasser, R., Trofimenko, S., Looney, A., Parkin, G. and Vahrenkamp, H., "A mononuclear zinc hydroxide complex stabilized by a highly substituted tris(pyrazolyl)hydroborato ligand: Analoges with enzyme carbonic anhydrase", Inorg. Chem., **30**, 4098 (1991).

24. Looney, A., Parkin, G., Alsfasser, R., Ruf, M. and Vahrenkamp, H., "Zinc pyrazolylborate complexes relevant to the biological function of carbonic anhydrase", Angew. Chem., Int. Ed. Engl., **31**, 92 (1992).

25. Bergquist, C., Fillebeen, T., Morlok, M.M. and Parkin, G., "Protonation and reactivity towards carbon dioxide of the mononuclear tetrahedral zinc and cobalt hydroxide complexes, [$Tp^{But,Me}$]ZnOH and [$Tp^{But,Me}$]CoOH: Comparison of the reactivity of the metal hydroxide function in synthetic analogues of carbonic anhydrase", J. Am. Chem. Soc., **125,** 6189 (2003).

26. Alsfasser, R., Powell, A. K. and Vahrenkamp, H., "Functional tetrahedral zinc complexes", Angew. Chem., Int. Ed. Engl., **29**, 898 (1990).

27. Perkinson, J., Brodie, S., Yoon, K., Mosny, K., Carroll, P. J., Morgan, T. V. and Burgmeyer, S. J. N., "Preparation and properties of transition-metal protein complexes models for the metal site in phenylanine hydroxylase", Inorg. Chem., **30**, 719 (1991).

28. Kremer-Aach, A., Klaui, W., Bell, R., Strerath, A., Wunderlich, H., Mootz, D., "Cobalt as a probe for zinc in metalloenzyme model compounds? A comparison of spectroscopic features and coordination geometry of four- and five-coordinate complexes. Crystal and molecular structures of $[Co(\eta^3\text{-}Tp(Ph))(\eta^2\text{-}Tp(Ph))]$, $[\eta^3\text{-}Tp(Ph))Zn(anthranilate)]$, and $[(\eta^3\text{-}Tp(Ph))M(\eta^2\text{-}acac)]$ (Tp^{Ph} = Hydrotris(3-phenylpyrazol-1-yl)borate, acac = Pentane-2,4-dionate, and M = Zn, Co)", Inorg. Chem., **36**, 3787 (1997).

29. Kitajima, N., Fujisawa, K., Fujimoto, C. and Moro-oka, Y., "Preparation of copper (I) complexes ligated with novel hindered pyrazolylborates", Chem. Lett., 421 (1989).

30. Olson, M. D., Rettig, S. J., Storr, A., Trotter, J. and Trofimenko, S., "Structure of a tetrahedral pyrazolylborato cobalt (II) complex, $Co[HB(3\text{-}iPr,4\text{-}Brpz)_3]Cl$", Acta Crystallogr., **C47**, 1543 (1991).

31. Brunker, T. J., Hascall, T., Cowley, A. R., Rees L. H. and O'Hare, D., " Variable coordination modes of hydrotris(3-isopropyl-4-bromopyrazolyl)borate (Tp') in Fe (II), Mn (II), Cr (II), and Cr (III) complexes: Formation of MTp'Cl (M = Fe and Mn), structural isomerism in CrTp'(2), and the observation of Tp'^- as an uncoordinated anion.", Inorg. Chem., **40**, 3170 (2001).

32. Kitajima, N., Fujisawa, K. and Moro-oka, Y., "Tetrahedral copper (II) complexes supported by hindered pyrazolylborate: Formation of the thiolato complex, which

closely mimics the spectroscopic characteristics of blue copper proteins", J. Am. Chem. Soc., **112**, 3210 (1990).

33. Brader, M. L., Bochardt, D. and Dunn, M. F., "Ligand effects on the blue copper site, spectroscopic studies of an insulin-stabilized copper (II) chromophore incorporating an exogenous thiolate ligand", J. Am. Chem. Soc., **114**, 4480 (1992).
34. Kitajima, N., Fujisawa, K. and Moro-oka, Y., "μ-η^2: η^2 – peroxobinuclear copper complex, [Cu(HB(3,5-$iPr_2pz)_3$](O_2)", J. Am. Chem. Soc., **111**, 8975 (1989).
35. Kitajima, N., Singh, U. P., Amagai, H., Osawa, M. and Moro-oka, Y., "Oxidative conversion of a Mn(μ-OH)$_2$Mn moiety. Synthesis and molecular structures of a (μ-hydroxo)dimanganese (II,II) and (μ-oxo)-dimanganese (III,III) complex with a hindered N_3 ligand", J. Am. Chem. Soc., **113**, 7757 (1991).
36. Kitajima, N., Osawa, M., Tanaka, M. and Moro-oka, Y., "A novel dioxygenase type oxygen insertion. CH bond oxidation of isopropyl groups in a dimanganese complex with molecular oxygen", J. Am. Chem. Soc., **113**, 8952 (1991).
37. Kitajima, N., Fukui, H., Moro-oka, Y., Mizutani, Y. and Kitagawa, T., "Synthetic model for dioxygen binding sites of non-heme iron proteins. X-ray structure of Fe(OBz)(MeCN)HB(3,5-$iPr_2pz)_3$) and resonance Raman evidences for reversible formation of a peroxo adduct", J. Am. Chem. Soc., **112**, 6402 (1990).
38. Hikchi, S., Tanaka, M., Moro-oka, Y. and Kitajima, N., "Phosphate ester cleavage with zinc hydroxide complex. Formation and crystal structure of a dinuclear zinc complex bridged with a phosphate mono ester", J. Chem. Soc., Chem. Commun., 814 (1992).

39. Maria, L., Domingos, A., Galvao, A., Ascenso, J. and Santos, I., "The role of neutral coligands on the stabilization of mono-$Tp^{iPr2}U$ (III) complexes", Inorg. Chem., **43,** 6426 (2004).

40. Calabrese, J. C. and Trofimenko, S., "Hydrotris(3-neopentylpyrazol-1-yl)borates: A new type of sterically hindered poly(pyrazolyl)borate", Inorg. Chem., **31**, 4810 (1992).

41. Dias, H. V., Jin, W., Kim, H. J. and Lu, H. L.,"Polyfluorinated Tris(pyrazolyl) borates. Synthesis and spectroscopic and structural characterization of Group I and Group II metal complexes of $[HB(3,5\text{-}(CF_3)_2Pz)_3]^-$ and $[HB(3\text{-}(CF_3)Pz)_3]^-$", Inorg. Chem., **35**, 2317 (1996).

42. Gaykema, W. P. J., Hol, W. G. J., Vereijken, J. M., Soeter, N. M., Bak, H. J. and Beinteme, J. J., "3.2 Å stucture of the copper-containing, oxygen carrying protein. Planulirus interruptus hemocyanin", Nature, **309**, 23 (1984).

43. Stemkamp, R. E., Sieker, L. C. and Jenson, L. H., "Binuclear iron complexes in methemerythrin and azidomethemerythrin at 2.0 Å resolution", J. Am. Chem. Soc., **106**, 618 (1984).

44. Meseerschmidt, A., Rossi, A., Ladenstein, R., Huber, R., Bologenesi, M., Gatti, G., Marchesini, A., Petruzelli, R. and Finnazzi-Argo, A., "X-ray crystal structure of the blue oxidase ascorbate oxidase from Zucchini: A preliminary analysis of the polypeptide fold and a model of the copper sites and ligands", J. Mol. Biol., **206**, 513 (1989).

45. Stalling, W. C., Pattridge, K. A., Strong, R. K. and Ludwig, M. L., "The structure of manganese superoxide dismutase from *Thermu thermophilus* HB8 at 2.4 Å resolution", J. Biol. Chem., **260**, 16424 (1985).

46. Deisenhofer, J., Epp, O., Miki, K., Huber, R. and Michel, H., "Structure of the protein subunit in the photosynthetic reaction center of *Rhodo-pseudomonas viridis* at 3.0 Å resolution", Nature, **318**, 618 (1985).

47. Osawa, M., Singh, U. P., Tanaka, M., Moro-oka, Y. and Kitajima, N., "Synthesis and molecular structure of an unsymmetrical di-manganese(II) carboxylato complexes", J. Chem. Soc., Chem. Commun., 310 (1993)

48. (a) Osawa, M., Tanaka, M., Fujisawa, K., Kitajima, N. and Moro-oka, Y., "A monomeric zinc complex ligated by an unsymmetric hydrotris-(pyrazolyl)borate containing an OH group", Chem. Lett., 397 (1996). (b) Hikchi, S., Komatsuzaki, H., Kitajima, N., Akita, M., Mukai, M., Kitagawa, T. and Moro-oka, Y., "Characterization of a μ-η^2:η^2- peroxo dinuclear cobalt(II) complex", Inorg. Chem., **36**, 226 (1997). (c) Hikchi, S., Ogihara, T., Fujisawa, K., Kitajima, N., Akita, M. and Moro-oka, Y., "Synthesis and characterization of the benzoylformato ferrous complexes with the hindered trispyrazolylborate ligand as a structural model for mononuclear non-heme iron enzymes", Inorg. Chem., **36**, 4539 (1997). (d) Hikchi, S., Komatsuzaki, H., Akita, M.and Moro-oka, Y., "Aliphatic C-H bond oxygenation by the Co(II)-OOX species with the hindered hydrotris(pyrazolyl)borate ligands (X = Co(II), Alkyl, H)", J. Am. Chem. Soc., **120,** 4699 (1998). (e) Hikchi, S., Yoshizawa, M., Sasakura, Y., Akita, M. and Moro-oka, Y., "First synthesis and structural characterization of dinuclear M(III) bis(μ-oxo) complexes of nickel and cobalt with hydrotris(pyrazolyl)borate ligand", J. Am. Chem. Soc., **120**, 10567 (1998). (f) Fujisawa, K., Imai, S., Kitajima, N. and Moro-oka, Y., "Preparation, spectroscopic characerization and molecular structure of copper(I) aliphatic thiolate complexes", Inorg. Chem., **37**,168 (1998). (g) Komatsuzaki,

H., Ichikawa, S., Hikchi, S., Akita, M. and Moro-oka, Y., "Characterization of a dinuclear Mn(II) (μ-hydroxo)(μ-pyrazolato) complex and hydrolytic equilibrium of the bridging pyrazolate ligand", Inorg. Chem., **37**, 3652 (1998). (h) Komatsuzaki, H., Nagasu, Y., Suzuki, K., Shibasaki, T., Satoh, M., Ebina, F., Hikchi, S., Akita, M. and Moro-oka, Y., "Dioxygen activation via a novel manganese(II) thiolate complex with hydrotris(3,5-diisopropylpyrazolyl)borate ligand", J. Chem. Soc., Dalton Trans., 511 (1998). (i) Kosugi, M., Hikchi, S., Akita, M. and Moro-oka, Y., "The first evidence for activation of exogenous O_2 on a vanadium(V) center: Synthesis and characterization of a peroxo vanadium(V) complex with hydrotris(3,5-diisopropyl-1-pyrazolyl)borate", J. Chem. Soc., Dalton Trans., 1369 (1999). (j) Hikchi, S., Yoshizawa, M., Sasakura, Y., Komatsuzaki, H., Akita, M. and Moro-oka, Y., "Oxygenation of saturated hydrocarbylgroups in the dinuclear nickel(III) bis(μ-oxo) complexes with the hydrotris(pyrazolyl)borate ligands Tp^R (R = Me_3 and $^iPr_2$)", Chem. Lett., 979 (1999). (k) Fujita, K., Hikchi, S., Akita, M. and Moro-oka, Y., "Novel monoanionic tripodal ligands methylbis(1-methyl-2-imidazolyl)(1-pyrazolyl)borate (= $MeB(Im^{N\text{-}Me})_2(Pz^R)^-$): Synthesis and structural characterization of their nickel complexes and carboxylate shift from nickel to boron", J. Chem. Soc., Dalton Trans., 117 (2000).

Chapter 2

SYNTHESIS AND STRUCTURAL CHARACTERISATION OF MONONUCLEAR AND BINUCLEAR COMPLEXES OF MANGANESE

Metal ions play essential roles in about one third of enzymes [1]. These enzymes provide an arrangement of side chain functional groups having an appropriate sized hole with the preferred groups on enzyme side chains needed to bind the required metal ion. The metal ions may be bound by main-chain amino and carbonyl groups, but specific binding is achieved by the amino acid side chains, particularly the carboxylate groups of aspartic and glutamic acid and the ring nitrogen N atom of histidine. Other side chains that bind metals ions include tryptophan (ring nitrogen), cysteine (thiol), methionine (thioether), serine, threonine, tyrosine (hydroxyl groups) and asparagines and glutamine (carbonyl groups, less often amino groups) [2].The crystal structures of many proteins available in literature gives useful information on the binding of metal ions in the active sites of enzymes and also help in understanding mechanism of the action of the enzyme.

Manganese is unique amongst the transition metal which is required for the growth and survival of most living organism. The Mn (II) is pumped in vesicles of plants where it competitive with proteins. It is essential for certain metabolic pathways also for example, oxygenic photosynthesis in cyanobacteria requires a tetra-Mn cluster present in the reaction centre complex of photosystem II [3] and a number of enzymes, including Mn-SOD, manganese-catalase and arginase, speciecally require Mn (II) for activity [4]. In addition, glycolysis cannot proceed fully without 3-phosphoglycerate mutase (PGM) which, in several Gram-positive endospore-forming bacteria, is active only when associated with Mn (II) [5]. The pH-dependent dissociation of Mn (II) from PGM represents a novel signaling mechanism for rapid enzyme inactivation. It has been suggested that similar mechanisms may be responsible for the regulation of other strictly Mn (II) - dependent enzymes, such as arginase [6]. In addition, it appears that Mn has an important role in bacterial signal

transduction. It is specifically required for the regulation of reactive species by enzyme such as superoxide dismutase in prokaryotic mitochondria and in several types of bacterial catalase.

The Mn (II) ion plays an important role in biological systems as a constituent of a selected group of proteins. The details of some manganese-containing proteins / enzyme are given below:

Arginase catalyses the hydrolytic cleavage of arginine to urea and ornithine. It contains 4 Mn (II) ions per enzyme molecule and is found in the mitochondria [7].

Concanavalin A is a manganese-calcium metalloprotein which has been structurally characterized [8]. It exists as a dimmer at pH = 6 and a tetramer at pH > 7. Protomer has two binding sites, a) a site S1 for transition metal ions, b) a site specific for calcium ions. Mn (II) is bound to protein through Glu, H_2O, H_2O, His, Asp, and Asp. The binding of the metal ions converts the apoprotein into a form that exhibits specific binding sites for small carbohydrates. Calcium can be replaced with another Mn (II) ion and subsequent EPR spectroscopy demonstrates weak antiferromagnetic coupling between the two manganese facilated by a bridging carboxylate. The biological function associated with this activity remains uncertain.

Glutamine synthetase and Glutamate synthetase act together. The first one catalyses the synthesis of glutamine in virtually all organisms while glutamate synthetase which is not present in humans but it is found in bacteria catalyses the formation of glutamate. This coupled enzyme system is used by the blue green algae and is found in the mitochondria. It requires two Mn (II) ions associated with the active site of the enzyme. The

distance between the Mn (II) sites is between 8-10 Å and Mn (II) is bound to N or (and) O donor sites [9].

Phosphoenolpyruvate carboxykinase enzyme converts irreversible cytoplasmic oxaloacetate to phsophoenolpyruvate when only the ATP concentration is high. In this enzyme Mn (II) may directly involved in interaction with substrate in the catalytic cycle and is bound to the protein through O [10].

Isopropylmalate synthase catalyses the formation of malate from glyoxylate in the presence of acetylcoenzyme A. It has been suggested that the Mn (II) center is bound to S-H group near site is involved in the reaction [11].

Isocitrate dehydrogenase catalyses the conversion of isocitrate to 2-oxoglutarate by an oxidative decarboxylation reaction. In this enzyme Mn (II) or Mg (II) is bound [12].

Pyruvate carboxylase the first metalloenzyme shown to contain manganese [13] is a mitochondrial enzyme catalyzing the conversion of pyruvate to oxaloacetate [14]. The overall catalytic process is the sum of two reactions; the biotin carboxylase binds 4 Mn (II) very tightly without the Mn (II) to be involved directly in the catalytic cycle. The interatomic distances are too great [15] to allow direct coordination of pyruvate to metal ion. It is proposed that a molecule of water-buried in the protein is tightly bound to Mn (II) and this coordinated water acts as an electrophile to active pyruvate. Mn (II) is believed to have a role in maintaining the integrity of the active site and can be replaced with divalent magnesium with only a small decrease in activity.

There are numerous enzymes that not only have a specific requirement for manganese but also utilize the redox capabilities of this element. Manganese redox enzymes are as follows:

Manganese peroxidase (MnP) is one of the two known enzymes capable of the oxidative degradation of lignin [16]. The MnP is unique in that it absolutely requires Mn (II) to complete its catalytic cycle [17]. The enzyme can be oxidized to an Fe (IV) porphyrin radical by H_2O_2. This radical then reduced twice by Mn (II) giving two free Mn (III) species. Certain α-hydroxy acids promote this reaction through the chelation of Mn (III). In the absence of these acids manganese dioxide is formed. The resulting Mn (III) species then proposed to be responsible for diffusion into the lignin matrix and initiation of phenolic radical decomposition of the polymer.

Manganese thiosulphate oxidase catalyses the oxidation of thiosulfate to sulfate[18]. EPR evidences indicated a binuclear Mn (II) site and the Mn (II) bound to the S-H group near site is involved in the reaction.

Manganese superoxide dismutase (Mn-SOD) catalyzes the dismutation of superoxide ion (O^{2-}) [19] and protects living cells against the dioxygen dependent toxicities of viologens, quinones, hypervalent compounds and benzofurans [20]. The enzyme is found in mitochondria, chloroplasts and prokaryotes. The crystal structure of Mn-SOD from *Thermus Thermophilus* [21] shows that it is tetramer. The Mn in Mn-SOD adopts a trigonal-bipyramidal coordination geometry with a N_3O_2 ligand donor set, one histidyl nitrogen occupies the apical position while water is suggested to sit at the opposite site. The carboxylate oxygen from aspartate is bound to the manganese unidentatly, constructing the basal plane with two other histidyl nitrogen atoms as shown in figure 2-1. The non liganding oxygen from the aspartate forms a hydrogen bond with an amino acid residue or the peptide backbone. The manganese is believed to cycle between +3 and +2 oxidation in a ping-pong type mechanism.

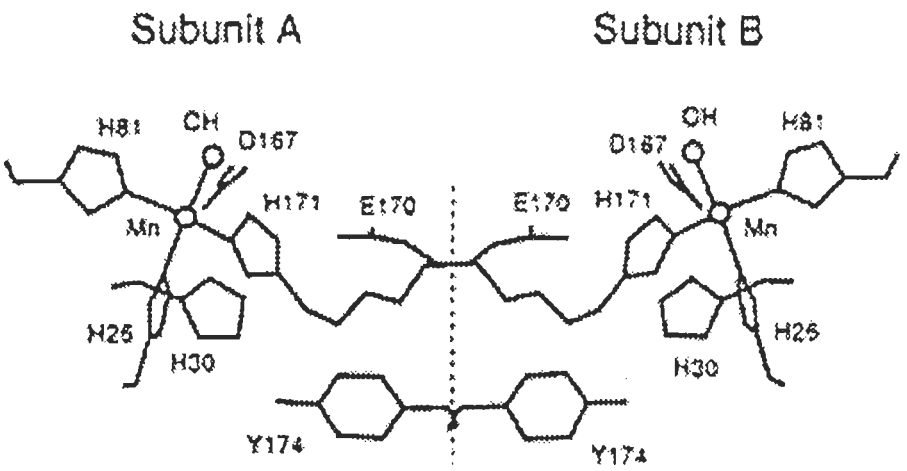


Fig. 2-1 Systematic representation of interconnected two subunit of Mn-SOD

Manganese catalase enzymes are present in most aerobic forms of life and are responsible for the decomposition of hydrogen peroxide to molecular oxygen and water. The most extensively studied manganese catalases are from extremely thermophlic *Thermus thermophilus* and lactate requiring *Lactobacillus plantarumn* [22]. The enzyme exists as hexamer of six equivalent subunit, each with a molecular mass of 35 kDa. A 3Å X-ray structure of *Thermus thermophilus* Mn catalase has revealed a four helices bundle motif as the major secondary and tertiary proteins folding element in each unit, each subunit contain a pair of Mn ions separated by 3.6 Å in a reduced state of the enzyme [23]. The manganese catalase enzyme contains a dinuclear center and is believed to cycle between the Mn II / Mn II and Mn III / Mn III oxidation level during catalysis [24].

Ribonucleotide reductase is the key enzyme in all proliferating cells, catalyzes the irreversible reduction of the four common ribonucleoside-5′-phosphates to the corresponding 2-deoxyribonucleotide-5′-phosphates and thus provides the deoxyribonucleotide precursor necessary for DNA synthesis [23]. Recently a manganese-containing ribonucleotide reductase [24] was isolated from *Brevibacterium ammoniagemes* consisting of a dimmer of

M. W. 100,000 and a monomer of M. W. 80,000. The structural details about these two enzymes remain unclear; both enzymes are suggested to function with a Mn (II, II) / (III, III) redox couple [25]. On the basis of the X-ray structure of non heme iron ribonucleotide reductase [26] and the close resemblance in optical spectra of the Mn (III, III) state of these proteins to those of Mn (III, III) complexes having a (μ-oxo)bis(μ-carboxylato) bridge [27, 28], the dinuclear site in Mn catalase or ribonucleotide reductase has been suggested to possess a bridging unit comprised of oxo (or hydroxo) and carboxylate groups.

Acid phosphatases catalyse the non-specific hydrolyses of various phosphomonoester in acidic condition and are present in sweet potato and soyabean. The Mn-containing acid phosphatase consists of two subunit of M. W. 55000. The determination of Mn by the atomic absorption method clearly shows the presence of one atom of Mn per enzyme molecule. Electronic, circular dichromism and ESR findings reveal that the Mn-valence state of the native enzyme is trivalent. Resonance Raman and chemical evidence suggests at least tyrosine phenolate and cystein sulphahydryl group as the Mn (III) active site ligands [29].

Oxygen evolving complex is the name of the water oxidation center in photosystem II and present in the thyalkoid membranes of chloroplasts in plants. This manganese center [30] appears to contain four manganese atoms while the presence of calcium and chloride ions is required for proper functioning. Most of the oxygen in the atmosphere, which supports aerobic life on earth, is generated by plants, algae and cynobacteria by the photoinduced oxidation of water to dioxygen.

$$2H_2O \longrightarrow O_2 + 4e^- + 4H^+$$

The Mn-OEC exhibits a periodicity in oxygen evolution corresponding to one molecule of molecular oxygen released every four quanta of light absorbed by the complex. Five states of the complex i.e. S_0-S_4 can be identified. The S_2 state is ESR active showing a multi line signal at g = 2 indicative of at least two coupled manganese atoms [31] and a broad signal at g = 4.1. Extended X-ray absorption fine structure (EXAFS) studies agree that at least two Mn-Mn vectors at 2.7 Å and one 3.3 Å vector are present. The 2.7 Å distances are generally agreed to correspond to high-valent $Mn_2(\mu\text{-}O)_2$ units, which have been shown to have similar Mn-Mn distances. Each Mn atom is bound to either oxygen or nitrogen donors with distance 1.8 and 1.9 Å. X-ray absorption near edge structure (XANES) measurements suggest 3.0 and 3.25 average oxidation state per manganese in the lower S states. Based on these data a number of structural proposals have been discussed (dimmer of dimmers, trimmer / monomer, distorted cubane, butterfly, dimmer / monomer). The structure and chemistry of the intermediates has been the subject of intense interest and still some critical questions related to the process of photosynthetic water oxidation are unsolved i.e. what are the oxidation states and structural changes in the manganese complex as the OEC proceeds through the S state cycle ? and what is the mechanism [32] by which four electrons are removed from two water molecules by the Mn complex to produce an oxygen molecule ? Photosystem is unique among this group of enzymes in that other transition metals have not been found to function in place of Mn, whereas alternate naturally occurring form of superoxide dismutase and catalase exit which contain Fe instead of Mn.

A common structural feature found in most of above proteins and enzymes is the presence of one or more bridging (μ) carboxylates derived from aspartate or glutamate side chains of the protein. The bridging carboxylates probably serve a functional role beyond

merely that of a passive structural bridge to bring the metal ions together. Their size and negative charge enables them to spatially separate and electrically screen the metal ions so that the degree of intermetallic electronic coupling is small. $Mn_2^{II,II}$(μ-carboxylate) [31, 33] complexes are relevant examples with weak electronic coupling. The coordination chemistry of metal carboxylato species is attractive subject from the bioinorganic standpoint because the carboxylate group of glutamate and aspartate works as a supporting ligand for the metal centers in various metalloproteins. The carboxylate groups also suggested to play an important role for structural holding and proton transfer via hydrogen-bonding interaction in proteins. Especially, a bimetallic μ-carboxylato complex is the most attractive synthetic target because the carboxylato-bridged bimetallic core is the common structural motif of the O_2-metabolic non-heme Fe and Mn-proteins such as MMO, RR, Hr(Fe) and catalase (Mn) [34]. Recent advance of X-ray crystallographic works for metalloproteins reveals the variety of the coordination mode of the carboxylate ligands and formation of hydrogen-bonding interaction with the other protic group [35].

Hydrogen bonding is very important interaction which is involved in providing the protein not only secondary and tertiary but also quaternary structure along with other interaction like hydrophobic and electrostatic forces etc.. The role of hydrogen bonding in stabilization of structure as well as in function of various metalloprotein is well knows in biochemistry [36]. The carboxylate oxygen from aspratate Mn-SOD is bound to the manganese unidentately and nonliganding oxygen from the aspartate forms hydrogen bond with an amino acid residue or the peptide backbone (Fig. 2-2) which is important for the activity of enzyme.

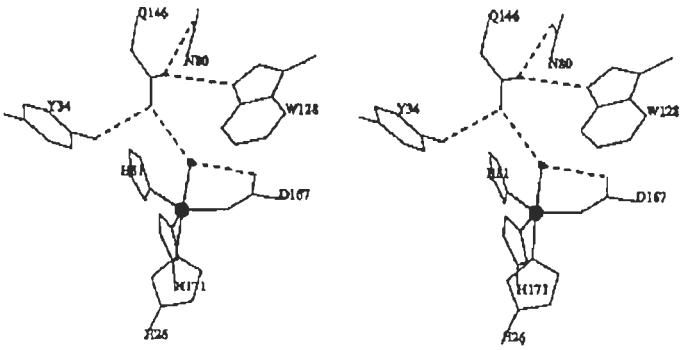


Fig. 2-2 Systematic representation of hydrogen bonded Mn-SOD

Hakansson et al. [37] demonstrated that in all catalytic sites of zinc – containing enzymes, the zinc ion functions as a Lewis acid. The chemical nature of the direct ligands and the structure of the surrounding hydrogen bond network (Fig. 2-3) are crucial for both the activity of carbonic anhydrase and the metal ion affinity of the zinc-binding site. Hydrogen bonding also play important role in Zn – containing carbonic anhydrase.

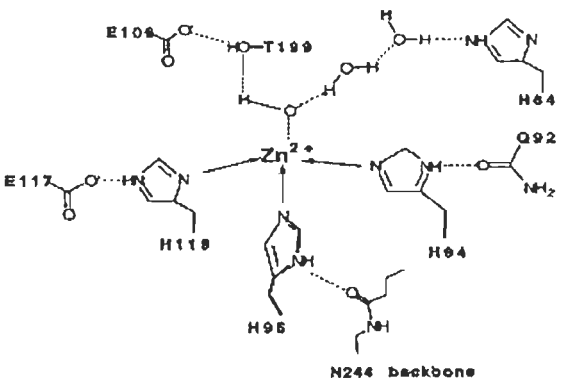
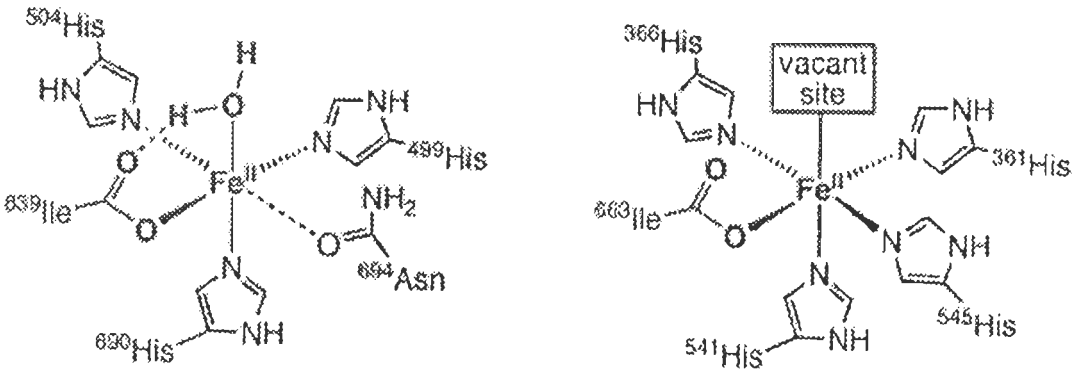


Fig. 2-3 Systematic representation of the active site for Carbonic anhydrase II

Mononuclear non-heme iron enzymes, lipooxygenases, which are present in plants and in animals, catalyses the peroxidation of polyunsaturated fatty acids containing the cis, cis-1,4-diene moiety to the corresponding 1-hydroperoxy-trans, cis-2,4-diene. The crystal structure the plants lipoxygenases (Fig. 2-4a) at 1.4 Å resolution has revealed that the iron (II) core has six-coordinate octahedral geometry with one carboxylato from the C-terminal

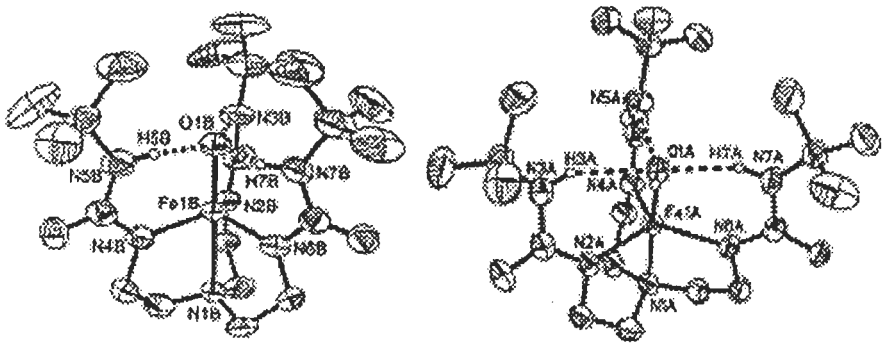
isoleucine (Ile839), one carboxyamidato carbonyl oxygen atom from asparagines (Asn694) which is weakly associated with the iron atom (Fe---O = 3.05 A), three imidazoles from the histidines (His499, His504 and His690), and one water molecule (which forms an intramolecular hydrogen bond with Ile893). On the other hand, the crystal structure of the mammalian lipooxygenase at 2.4 Å (Fig. 2-4 b) [38] has revealed that the iron (II) core structure also has octahedral coordination geometry with one carboxylato from the C-terminal isoleucine (Ile663), four imidazoles from the histidines (His361, His366, His541, and His545), and one vacant coordination site capable of accommodating and exogenous ligand such as water molecule.



(a) (b)

Fig. 2-4 Systematic representations of core structure of catalytically inactive iron (II) species of (a) a plant lipoxygenase (soybean lipoxygenase-1) and (b) a mammalian lipoxygenase (15-rabbit lipoxygenase).

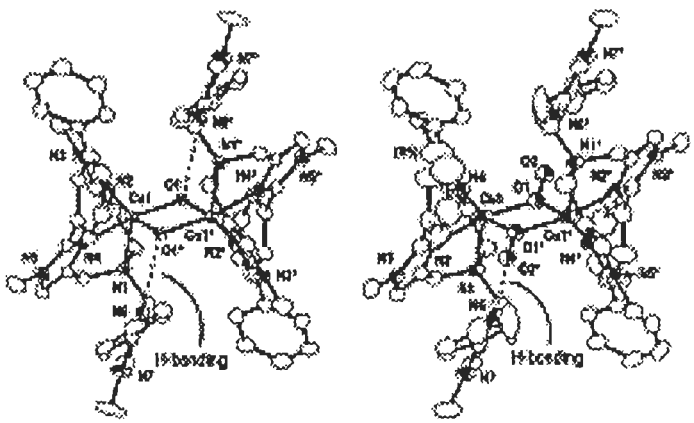
Model complexes showing hydrogen bonding are also available in literature. MacBeth et al. [39] reported the synthesis and properties for a series of mononuclear iron (III) and manganese (III) complexes with terminal oxo and hydroxo ligands by using the tripodal ligand [tris[(N'-tert-butylurealato)-N-ethylaminato]($[H_31]^{3-}$), which created a protective hydrogen bond cavity around the M (III)-O(H) units (M (III) = Fe and Mn) as shown in figure 2-5.



(a) (b)

Fig. 2-5 Crystal structure of (a) $[Fe^{III}H_31\text{-}b(O)]^{2-}$ (b) $[Fe^{III}H_31\text{-}a(OH)]^{-}$

Itho et al. [40] synthesized the binuclear hydroxo and hydroperoxo of copper - $[Cu_2(L3)_2(OH)_2]^{2+}$ **1** and $[Cu_2(L3)_2(OOH)\text{-}(OH)]^{2+}$ **2** by using the tetradentate tripodal ligand, tris(1-methyl-2-phenyl-4-imidazolylmethyl)amine **L3** and showed the hydrogen bonding between the hydroperoxide and the imidazole nitrogen of an uncoordinated pendant arm in one side and the hydroxide and the imidazole nitrogen of an uncoordinated pendant arm in the other side as shown in the figure 2-6.



(a) (b)

Fig. 2-6 Crystal structure of (a) $[Cu_2(L3)_2(OH)_2]^{2+}CF_3SO_3.CH_3CN$),

(b) $[Cu_2(L3)_2(OOH)\text{-}(OH)]^{2+}\text{-}BPh_4$

Garner et al. [41] reported the synthesis and characterization of several mononuclear zinc aryloxide complexes with N,N-bis-2-(methylthio)ethyl-N-(6-neopentyl-amino-2-pyridylmethyl)amine (bmnpa) and (benpa) ligand. They found that in the solid state structure

of [(bmnpa)Zn($OC_6H_4NO_2$)]ClO_4 , [(benpa)Zn(*p*-OC_6H_4Br)]ClO_4 and [(benpa)Zn(OC_6H_4CN)]ClO_4 , a hydrozen bonding interaction is present between N(3)-H of the supporting bmnpa / benpa ligand and the Zn-bound oxygen atom of the aryloxide ligand {N(3)----O(1)} ~ 2.78 Å.

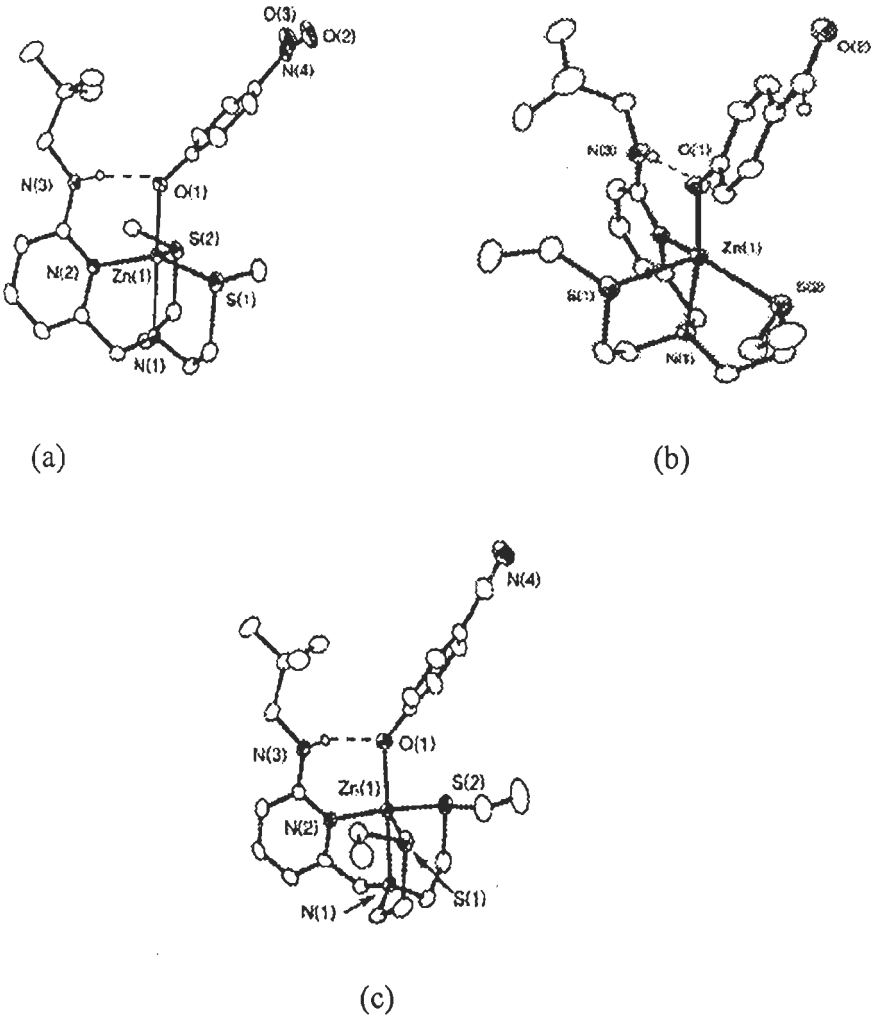


Fig. 2-7 Crystal structure of (a) [(bmnpa)Zn($OC_6H_4NO_2$)]ClO_4 (b) [(benpa)Zn(*p*-OC_6H_4Br)]ClO_4 (c) [(benpa)Zn(OC_6H_4CN)]ClO_4

Ogo et al. [42] reported the synthesis and structural studies of [Fe^{III}(tnpa)(OH)(RCO_2)]ClO_4 1 [tnpa = tris(6-neopentylamine-2-pyridemethyl)amine), R = C_6H_5] and [Fe^{III}(tnpa)(OH)(RCO_2)]ClO_4 2 [R=CH_3] as an active site model for

lipoxygenases. The crystal structure of 1 and 2 (Fig. 2-8) suggested that these complexes have a cis-configuration between the OH^- ligand and RCO_2^- or three NH ligands of tnpa to form four intramolecular hydrogen bond, for example, for 2 O(1)–H(2) = 1.99 Å, O(1)-H(3) = 2.08 Å, O(1)-H(4) = 1.87 Å and O(2)-H(1) = 1.61 Å (Fig. 2-8). These hydrogen bonds may contribute to stabilization of the (hydroxo)iron (III) core structure of 1 and 2.

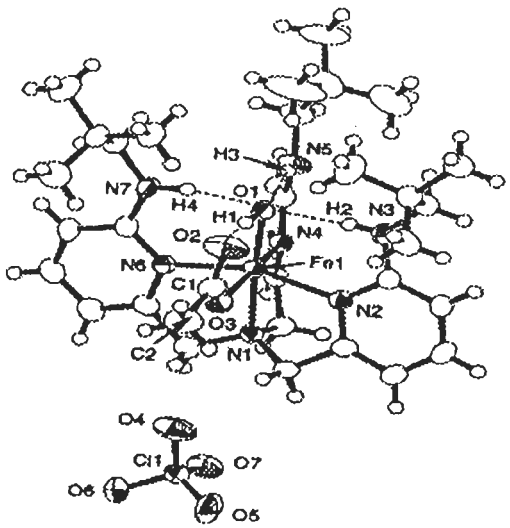
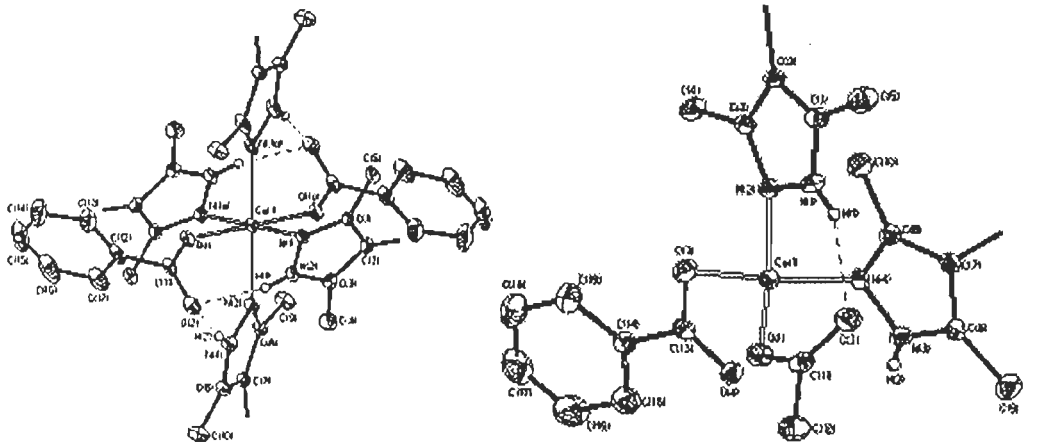


Fig. 2-8 Crystal structure of $[Fe^{III}(tnpa)(OH)(RCO_2)]ClO_4$

Boldog et al. [43] designed 3,3′, 5,5′-tetramethyl-4,4′-bipyrazolyl (4,4′-bpz) as an angular bridging unit and reported the synthesis of $[Co(4,4'\text{-bpz})_2\{C_6H_5CO_2\}_2].2C_6H_5Br$ **1** and $[Co(4,4'\text{-bpz})_2\{C_6H_5CO_2\}\{CH_3CO_2\}].2C_6H_5Br$ **2**. The crystal structure of complex **1** exhibited 3-D structure containing rectangular channels whereas complex **2** exits as a hybrid coordination / hydrogen - bonded porous network which incorporates two guest bromo benzene molecule per metal atom.



(a) (b)

Fig. 2-9 Crystal structure of (a) $[Co(4,4'\text{-}bpz)_2\{C_6H_5CO_2\}_2].2C_6H_5Br$ (b) $[Co(4,4'\text{-}bpz)_2\{C_6H_5CO_2\}\{CH_3CO_2\}].2C_6H_5Br$

Singh et al. [44] reported the synthesis of a dinuclear Mn (II) tri(μ-carboxylato) complexes, $Tp^{iPr2}Mn\text{-}(\mu\text{-}OBz)_3\text{-}Mn(Tp^{iPr2}H)$ where Tp^{iPr2} is hydrotris(3,5-diisopropyl-1-pyrazolyl)borate. The X-ray crystallography studies of this complex revealed the unsymmetrical coordination environments for the manganese centers. One of the two Tp^{iPr2} ligands, which bound to the five-coordinated Mn center, is protonated by the action of the third carboxylic acid the resulting non-Mn-binding N-H moiety forms an intramoleclar hydrogen bonding with the oxygen donor of a carboxylate ligand as shown in figure 2-10.

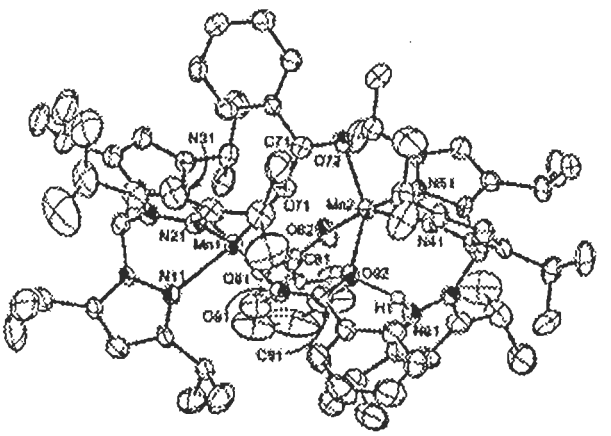


Fig. 2-10 Crystal structure of $Tp^{iPr2}Mn\text{-}(\mu\text{-}OBz)_3\text{-}Mn\ (Tp^{iPr2}H)$

Mehn et al. [45] reported the synthesis of several iron (II) complexes using Tp^{Ph2} (3,5-diphenylpyrazolyl-1-borate). They also found that in crystal structure of $[Fe(Tp^{Ph2})(OAc)(3,5\text{-}Ph_2pzH)]$, hydrogen bonding interaction exist between hydrogen of the free pyrazole and unligand oxygen of acetate anion.

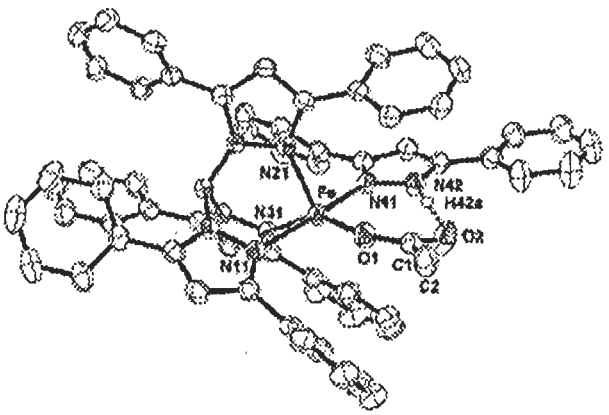
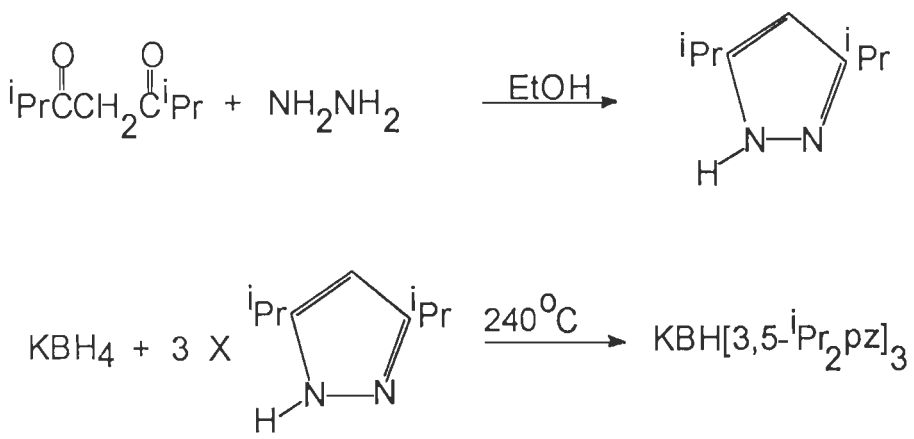


Fig. 2-11 Crystal structure of $[Fe(Tp^{Ph2})(OAc)(3,5\text{-}Ph_2pzH)]$

Beside above hydrogen bonded carboxylate complexes, some mononuclear and binuclear carboxylate complexes of manganese are also available in literature [28, 46]. The present chapter deals with the synthesis and characterization of some mononuclear and binuclear carboxylate complexes of manganese.

Results and Discussion

The 3,5-diisopropylpyrazole, [3,5-iPr_2pzH] **2a** and potassium hydrotris(3,5-diisopropyl-1-pyrazolyl)borate [$(KHB(3,5\text{-}iPr_2pz)_3)$] **2b** have been prepared by the modified method of Kitajima et al. as describe in chapter 5. (Scheme 2-1).



Scheme 2-1

The synthesis of manganese complexes in +2 oxidation state have been tried with both **2a** and **2b** ligands. The **2b** has (Fig. 2-1) has been chosen for present study due to the following properties.

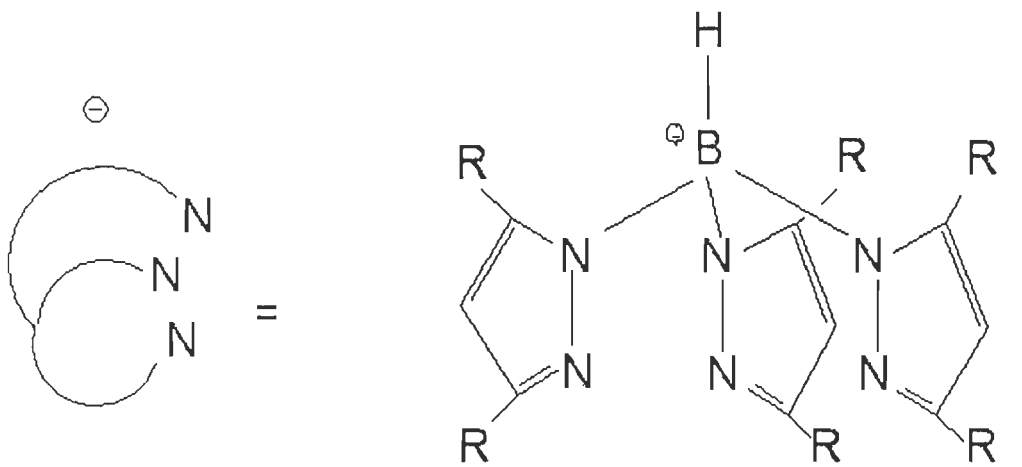


Fig. 2-1

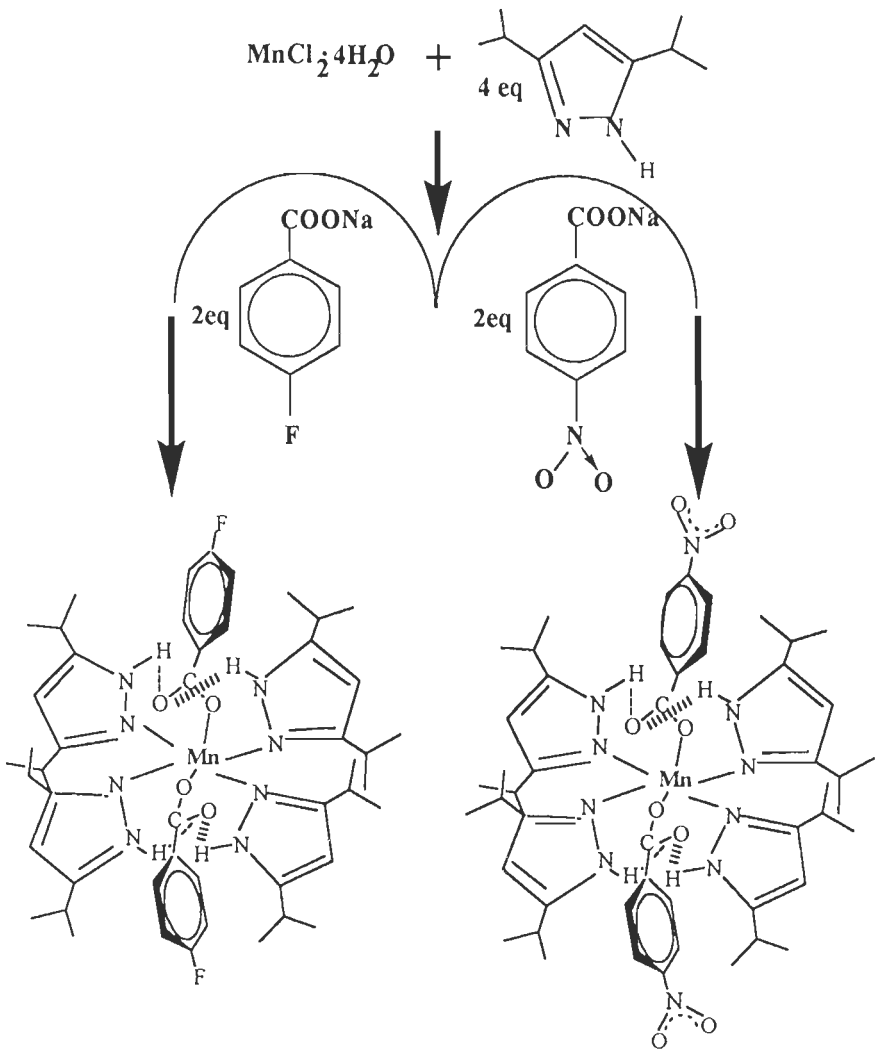
It is:

1. N_3 ligand donor set;
2. Strong electron donor;
3. Highly soluble in CH_2Cl_2, toluene, pentane etc.;
4. Successfully preventing the formation of L_2M;
5. Flexible for variety of coordination geometries.

The reaction of $MnCl_2$ with 4 equivalent of **2a** and 2 equivalent of sodium nitrobenzoate \ sodium fluorobenzoate resulted the formation six coordinated mononuclear high spin complex **2c** and **2d** (Scheme 2-2).

The crystal structure of **2c** and **2d** are shown in figure 2-2 and 2-5 and their unit cell packing is shown in figure 2-3 and 2-6 respectively. As shown X- ray structure both the complexes are six coordinated with octahedral geometry. The manganese-nitrogen and manganese-oxygen bond distances are Mn1-N11, 2.329(2), Mn1-N21, 2.327(2), Mn1-O1, 2.175(2) Å (are same) for complex **2c** and **2d**. The crystal structure of both complexes show that there is hydrogen bonding (Fig. 2-4 and 2-7) between uncoordinated oxygen atom of benzoate group and hydrogen atom of the pyrazole. Figures 2-4 and 2-7 also show that in both of these complexes, two molecules of **2c** and **2d** have CH_3\Pi interaction during crystal packing in unit cell. The hydrogen bonded mononuclear manganese benzoate complexes are very rare (as given in introduction part of this chapter) but play important role in biological system. Due to six coordinated structure the complexes **2c** and **2d** are stable under air in the solid and even in solution state, they do not react with O_2 and CO_2. The SOD activity studies for these complexes are under progress as the coordination sphere around the manganese in

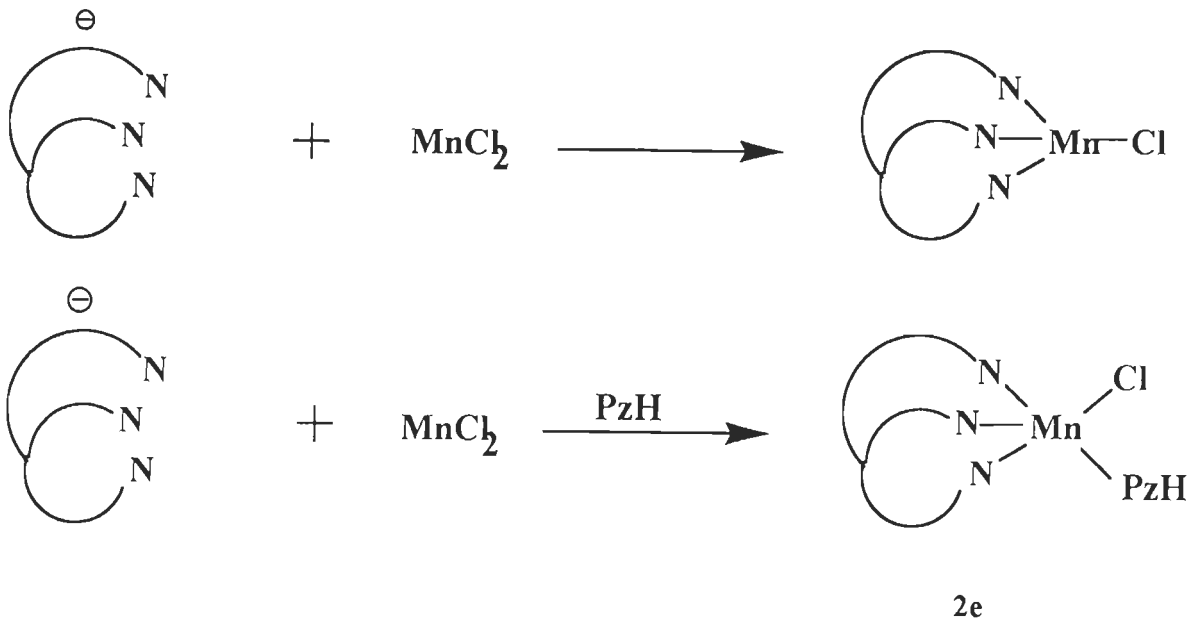
both complexes are very close to the coordination sphere around the manganese in manganese-containing SOD enzyme [47].



2c 2d

Scheme 2-2

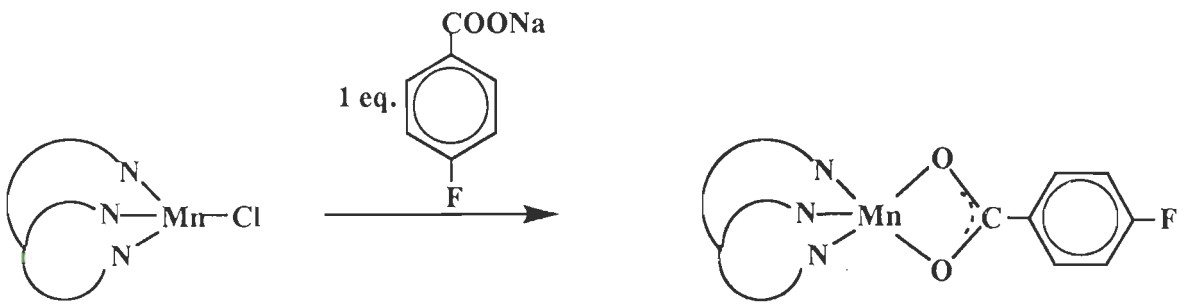
The reaction of $MnCl_2$ and ($KHB(3,5\text{-}iPr_2pz)_3$) (Scheme 2-3) gives a four coordinated mononuclear complex **2e**. The same complex has been reported previously by Kitajima et al. [48] and has been used as starting material for the synthesis of binuclear hydroxo and oxo complexes of manganese.



2e

Scheme 2-3

The five coordinate non hydrogen bonded mononuclear benzoate complex **2f** has been prepared by the reaction of **2e** with one equivalent of sodium fluorobenzoate (scheme 2-4) for the reaction with hydrogen peroxide in order to generate peroxo bonded binuclear benzoate complex.

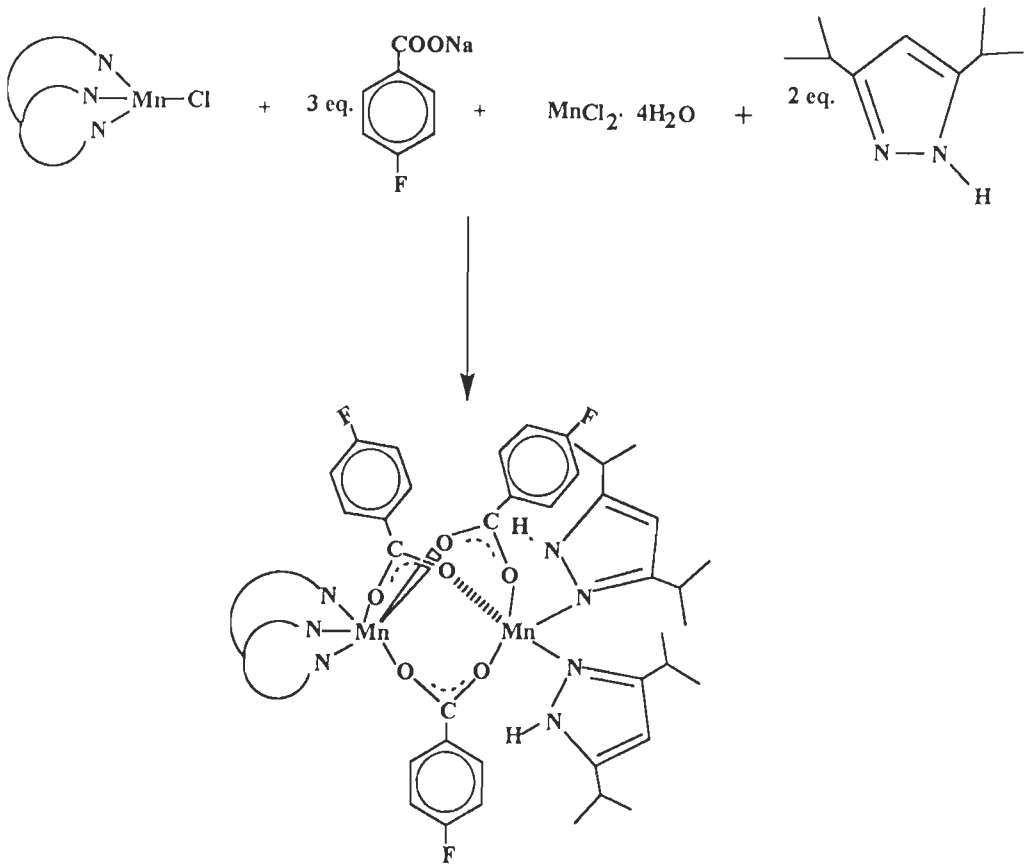


2f

Scheme 2-4

The spectroscopic characterization revealed that benzoate group is coordinating in bidentate fashion in this complex.

We have also prepared the complex **2g** by using scheme 2-5.



2g

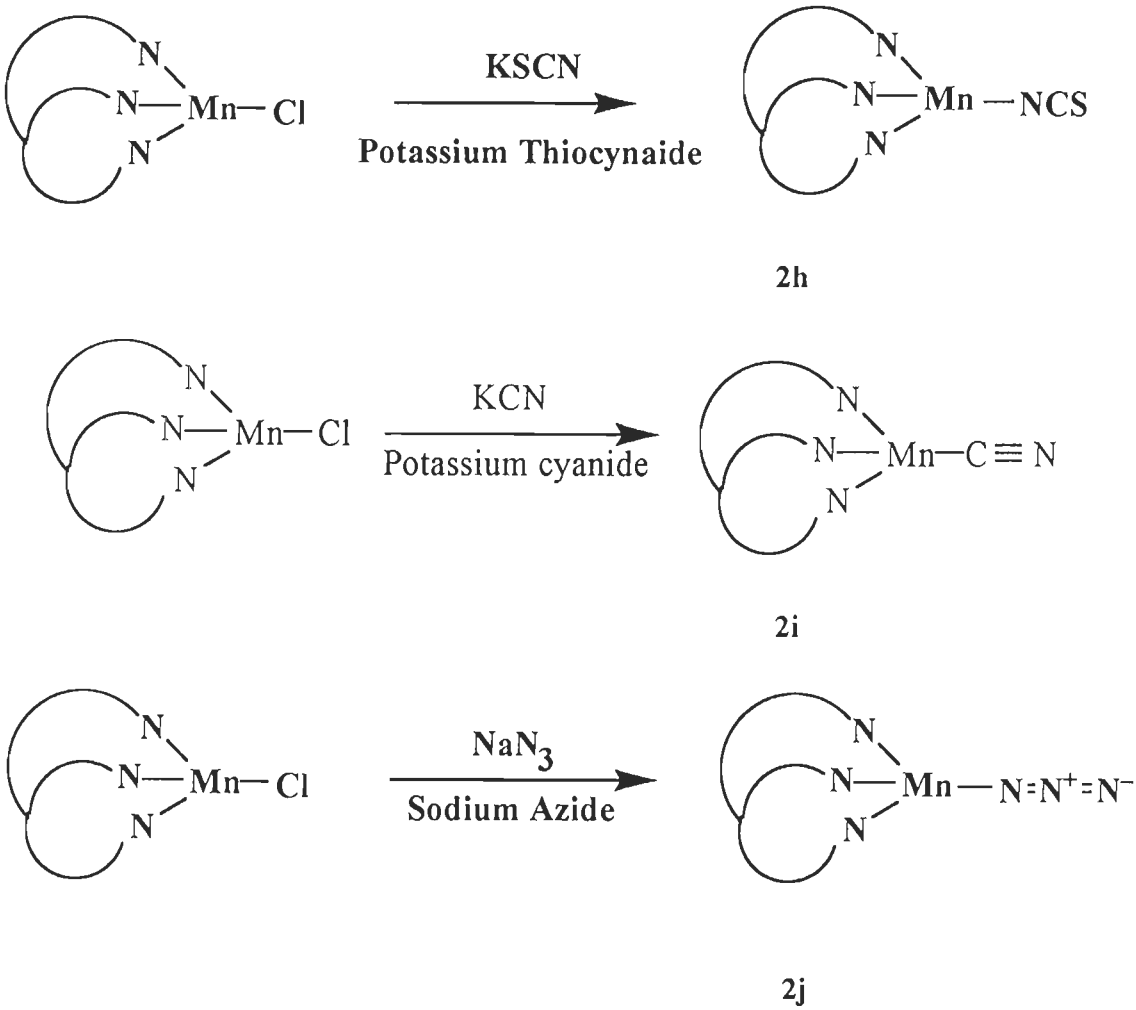
Scheme 2-5

The anaerobic reaction of **2e** with **2a**, $MnCl_2.4H_2O$ and sodium fluorobenzoate gave an unsymmetric dimanganese (II) complex **2g**. The existence of the protonated form of the pyrazole in 2g is evident from the strong IR band observed at 3297 cm^{-1} which is assigned as νN-H stretching band. Complex **2g** is relatively air-stable even in solution. The molecular structure of **2g** was determined by X-ray crystallography and its ORTEP view is given in figure 2-8 and unit cell packing in figure 2-9. As shown in figure 2-8, both manganese (II) ions are in different environment. Mn1 is six coordinated and Mn2 is five coordinated. Mn1 is coordinated by three nitrogens from the tris(pyrazolyl)borate ligand, where as Mn2 is coordinated by two nitrogen from the pyrazole. The two manganese (II) ions are bridged

with three benzoate groups, forming the dinuclear complex. All the three benzoate groups are symmetrically bridging with both metal ions and behaving as bidentate. Similar binuclear manganese complexes having three benzoate group has been reported by Osawa et al. [49] but in this complex both manganese ions are in octahedral environment and the two of the μ-benzoate groups are symmetrically bridging, whereas the other one adopts a unique coordination mode; only one oxygen from the carboxylate serves as a bridging ligand, while the other one binds to Mn2 terminally.

A structurally similar (μ-carboxylato)$_3$ unit has been found in a linear trinuclear manganese(II) carboxylato complex [50]. The Mn-Mn separation found in complex **2g** is 3.852(2) Å, is comparable to (μ-aqua)(bis(μ-carboxylato)dimanganese(II) complexes 3.739(2) Å [51], 3.612(2) Å [52] rather than a symmetric tris(μ-carboxylato)$_3$ complex 4.034(2) Å [53]. The magnetic susceptibility of the powdered sample of **2g** is 8.34 B.M. , indicative of a weak antiferromagnetic property.

As reported in the literature that some enzymes which are unable to synthesize heme can never the less produces a catalase. The catalase which in contrast to heme-containing catalase is not inhibited by CN^-, $N3^-$, has been called pseudocatalase [54]. Pseudocatalase was first observed in Pediococci. It is called azide insensitive. In this chapter, the efforts were also made to synthesize the CN^-, SCN^- and N_3^- coordinated complexes of Mn(II) by using **2b** as tridentate ligand. When **2e** was allowed to react with one equivalent of KSCN \ KCN \ NaN_3, the complexes **2h**, **2i**, **2j** were obtained (Scheme 2-6)



Scheme 2-6

All these complexes give satisfactory elemental analysis and other spectroscopic data including FD-MS. The single crystal X-ray structure of **2h** (Fig. 2-10) shows that manganese is six coordinated with three nitrogen from the ligand, one nitrogen from SCN^- and two nitrogen from acetonitrile solvent which are present as coordinating solvent. The Mn-N bond distances i.e., Mn-N1, 2.219(8); Mn1-N4, 2.218(9); Mn1-N5, 2.246(8) Å of the pyrazolylborate are longer than the Mn-N bond distance of coordinated SCN^- (Mn1-N8, 2.159(9) Å) but are shorter than the Mn-N bond distances from the coordinated acetonitrile (Mn1-N7, 2.311(8) Å and Mn1-N9, 2.313(9) Å). The elemental analysis data and other

spectroscopic studies suggested that complexes **2i** amd **2j** are four coordinated. The efforts to get the single crystal structure for these complexes are in progress.

Summary

The reaction of 3,5-diisopropylpyrazole 3,5-iPr_2pzH with $MnCl_2.4H_2O$ and sodium nitrobenzoate \ sodium fluorbenzoate resulted the formation of complex **2c** and **2d** which have octahedral geometry with hydrogen bonding. In both **2c** and **2d** complexes the uncoordinated oxygen atom of benzoate group form the hydrogen bond with hydrogen atom of 3,5-disoprppylpyrazole. Complexes **2c** and **2d** may act as a model compound for the Mn-containing super oxide dismutase as such type of hydrogen bonding present in native enzyme. The effort for performing SOD activity test for complexes **2c** and **2d** are in progress.

The reaction of potassium hydrotris(3,5-diisopropyl-1-pyrazolyl)borate, (KHB(3,5-$iPr_2pz)_3$) with $MnCl_2.4H_2O$ resulted the formation of four coordinated complex **2e** which has been used for the synthesis of five coordinated unsaturated **2f** complex. Complex **2f** is air sensitive and not stable in solution. Reaction of **2f** with H_2O_2 at low temperature resulted the formation of peroxo complex. The detail characterization of peroxo complexes is in progress.

The complex **2g** has been synthesized from the mixture of **2e**, $MnCl_2.4H_2O$, 3,5-iPr_2pzH and sodium fluorobenzoate. The X-ray analysis of **2g** established the unsymmetrical coordination environment about each manganese ions with structurally symmetrical bridging unit consisting of three carboxylate groups although its biological relevance is not certain. The reaction of **2e** with 1 equivalent KSCN gave six coordinated complex **2h** where three nitrogen comes from the pyrazolyl borate ligand, one nitrogen from SCN^- and two nitrogen from acetonitrile which are present as crystallization solvent.

Table 2-1 Selected Bond Distance (Å) and Bond Angles(deg) for [(3,5-$iPr_2pzH)_4$ $Mn(NO_2\text{-}OBz)_2$] 2c

Interatomic Bond Distances			
Mn1-N11	2.329(2)	Mn1-N21	2.327(2)
Mn1-O1	2.175(2)	O1-C1	1.251(2)
O2-C1	1.263(3)		
Interatomic Bond Angles			
O1-Mn1-O1	180.00(0)	O1-Mn1-N11	90.92(6)
O1-Mn1-N11	89.08(6)	O1-Mn1-N21	90.68(6)
O1-Mn1-N21	89.32(6)	N11-Mn-N11	180.00(0)
N21-Mn1-N21	180.00(0)		

Table 2-2 Crystal Data and Collection Details of $[(3,5\text{-}iPr_2pzH)_4Mn(NO_2\text{-}OBz)_2]$ 2c

Emperical formula	$C_{50}H_{72}N_{10}O_8Mn$
Formula weight	996.12
Crystal System	triclinic
Space Group	P-1(No. 2)
Lattice Parmeters	a = 10.715(6)Å
	b = 11.334(4)Å
	c = 12.747(8)Å
	$\alpha = 100.33(3)^0$
	$\beta = 107.16(2)^0$
	$\gamma = 105.97(3)^0$
Cell volume	$1364(1)^0(Å)^3$
Z_{Value}	1
D_{cal}	1.212 g/cm^3
Diffractometer	Rigaku RAXIS- IV Imaging Plate Radiation
	Graphite monochromated
	$MoK_\alpha(0.71073)$
μ(Mo K_α) / cm^{-1}	0.300
$2\theta_{max}$	27.4
No. of Measured reflection	5582
No. of Observed reflection	4752
No. of parameter refined	329
R(based on F)	0.0625
R_w	0.1756

Table 2-3 Selected Bond Distance (Å) and Bond Angles (deg.) for $[(3,5\text{-}iPr_2pzH)_4 Mn(F\text{-}OBz)_2]$

Interatomic Bond Distances			
Mn1-N11	2.329(2)	Mn1-N21	2.327(2)
Mn1-O1	2.175(2)	O1-C1	1.251(2)
O2-C1	1.263(3)		
Interatomic Bond Angles			
O1-Mn1-O1	180.00(0)	O1-Mn1-N11	90.92(6)
O1-Mn1-N11	89.08(6)	O1-Mn1-N21	90.68(6)
O1-Mn1-N21	89.32(6)	N11-Mn-N11	180.00(0)
N21-Mn1-N21	180.00(0)		

Table 2-4 Crystal Data and Collection Details of $[(3,5\text{-}iPr_2pzH)_4Mn(F\text{-}OBz)_2]$ 2d

Emperical formula	$C_{50}H_{72}N_8O_4F_2Mn$
Formula weight	942.10
Crystal System	monoclinic
Space Group	P1(No. 2)
Lattice Parmeters	a = 10.948(2)Å
	b = 12.663(3)Å
	c = 18.695(3)Å
	$\alpha = 90.00(0)^0$
	$\beta = 96.035(9)^0$
	$\gamma = 90.00(0)^0$
Cell volume	$2577.5(9)^0(Å)^3$
Z_{Value}	2
D_{cal}	1.214 g/cm^3
Diffractometer	Rigaku RAXIS- IV Imaging Plate
Radiation	Graphite monochromated
	$MoK_\alpha(0.71073)$
μ(Mo K_α) / cm^{-1}	0.313
$2\theta_{max}$	27.5
No. of Measured reflection	5613
No. of Observed reflection	4352
No. of parameter refined	311
R(based on F)	0.0588
R_w	0.1664

Table 2-5 Selected Bond Distance (Å) and Bond Angles (deg.) for [(HB(3,5-Pr^{i}_{2}pz)$_3$ $Mn_2(\mu$-FOBz)$_3$(3,5-iPr_2pzH)$_2$]. CH_3CN 2g

	Interatomic Bond Distances		
Mn1-Mn2	3.732(4)	Mn1-O2	2.133(3)
Mn1-O4	2.193(3)	Mn1-O6	2.255(3)
Mn1-N3	2.238(3)	Mn1-N5	2.240(3)
Mn1-N1	2.293(3)	Mn2-O3	2.096(3)
Mn2-O1	2.107(3)	Mn2-O5	2.271(2)
Mn2-N7	2.216(3)	Mn2-N9	2.272(3)
	Interatomic Bond Angles		
O2-Mn1-O4	88.80(7)	O2-Mn1-O6	91.69(7)
O4-Mn1-O6	93.10(7)	N3-Mn1-O6	169.71(8)
N5-Mn1-O6	87.31(8)	O2-Mn1-N1	94.40(8)
O4-Mn1-N1	174.02(8)	O3-Mn2-N7	91.82(9)
O1-Mn2-N7	88.63(9)	N7-Mn2-O5	113.94(8)
O3-Mn2-N9	96.62(8)	O5-Mn2-N9	81.00(7)
O1-Mn2-N9	171.60(8)	N7-Mn2-N9	88.06(9)

Table 2-6 Crystal Data and Collection Details of $[(HB(3,5\text{-}Pr^{i}_{2}pz)_3Mn_2(\mu\text{-}FOBz)_3 (3,5\text{-}iPr_2pzH)_2].CH_3CN$ 2g

Emperical formula	$C_{68}H_{90}BF_3Mn_2N_{11}O_6$
Formula weight	1335.20
Crystal system	triclinic
Space group	P-1(No. 2)
Lattice parmeters	a = 12.3069(16)Å
	b = 13.4695(18)Å
	c = 24.205(3)Å
	$\alpha = 81.3(2)^0$
	$\beta = 80.22(2)^0$
	$\gamma = 64.15(2)^0$
Cell volume	3544.87(8) $(Å)^3$
Z_{Value}	2
D_{cal}	1.251 g/cm^3
Diffractometer	Bruker SMART
Radiation	Graphite monochromated
	$MoK_\alpha(0.71073)$
μ(Mo K_α) / mm^{-1}	0.420
$2\theta_{max}$	28.02 °C
No. of Measured reflection	13064
No. of Observed reflection	10845
No. of parameter refined	841
R(based on F)	0.0579
R_w	0.1591

Table 2-7 Selected Bond Distance (Å) and Bond Angles (deg.) for [(HB(3,5-$Pr^{i}_{2}pz)_3$)MnNCS $(CH_3CN)_2$].]. CH_3CN 2h

Interatomic Bond Distances					
Mn1-N1	2.219(8)	Mn1-N4	2.218(9)	Mn1-N5	2.246(8)
Mn1-N7	2.311(8)	Mn1-N8	2.159(9)	Mn1-N9	2.313(9)
Interatomic Bond Angles					
N8-Mn1-N4	95.42(9)		N8-Mn1-N1	96.30(8)	
N8-Mn1-N6	179.55(9)		N8-Mn1-N7	88.46(9)	
N8-Mn1-N9	88.72(9)		N4-Mn1-N9	175.85(9)	
N1-Mn1-N7	175.02(8)		N6-Mn1-N8	179.55(9)	
N4-Mn1-N1	84.31(8)		N4-Mn-N6	85.00(8)	
N1-Mn-N6	83.89(8)		N8-C27-S1	178.89(28)	

Table 2-8 Crystal Data and Collection Details of $[(HB(3,5\text{-}Pr^{i}_{2}pz)_3)MnNCS].3CH_3CN$

Emperical formula	$C_{38}H_{39}BMnN_{12}S$
Formula weight	761.62
Crystal System	triclinic
Space Group	P-1(No. 2)
Lattice Parmeters	a = 11.643(6)Å
	b = 11.856(6)Å
	c = 18.178(9)Å
	$\alpha = 88.33(1)^0$
	$\beta = 81.73(1)^0$
	$\gamma = 66.35(1)^0$
Cell volume	2273.43(190) $(Å)^3$
Z_{Value}	2
D_{cal}	1.113 g/cm^3
Diffractometer	Bruker SMART
Radiation	Graphite monochromated
	$MoK_\alpha(0.71073)$
μ(Mo K_α) / cm^{-1}	0.374
$2\theta_{max}$	28.16
No. of Measured reflection	8249
No. of Observed reflection	6654
No. of parameter refined	543
R(based on F)	0.0543
R_w	0.1654

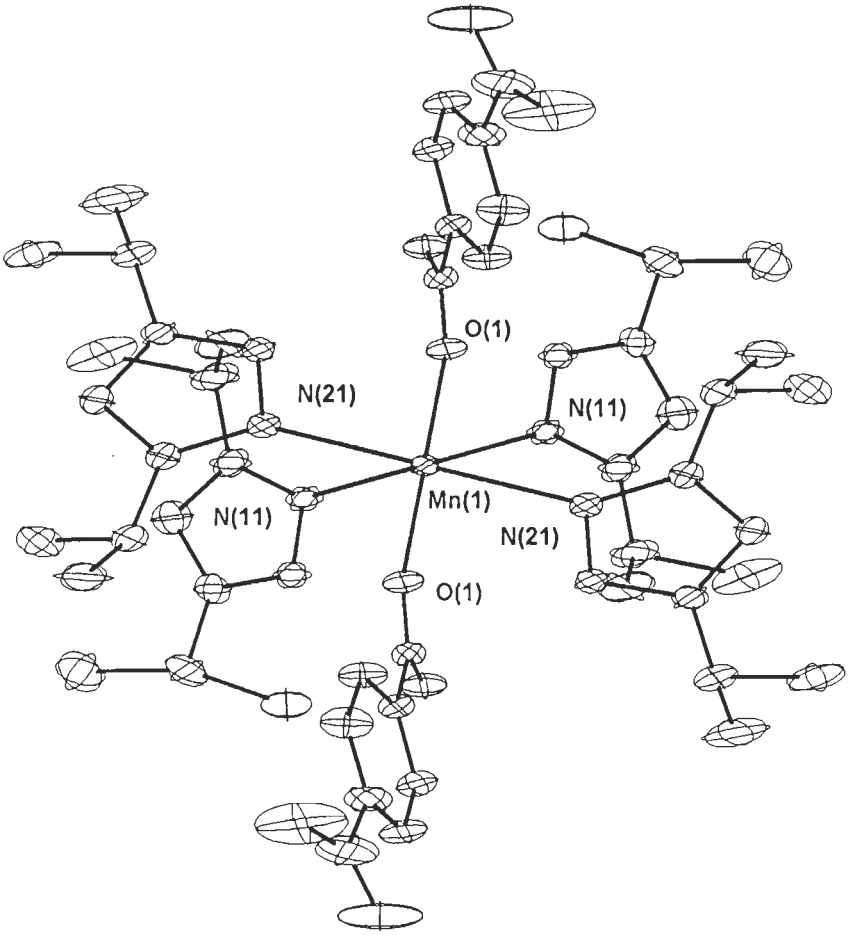


Fig. 2-2 ORTEP view of $[(3,5\text{-}iPr_2pzH)_4Mn(NO_2\text{-}OBz)_2]$ **2c**

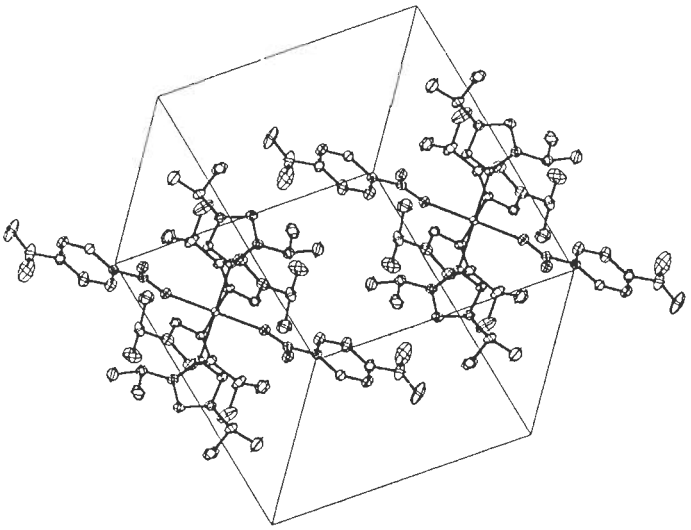


Fig. 2-3 Unit cell packing of $[(3,5\text{-}iPr_2pzH)_4Mn(NO_2\text{-}OBz)_2]$ **2c**

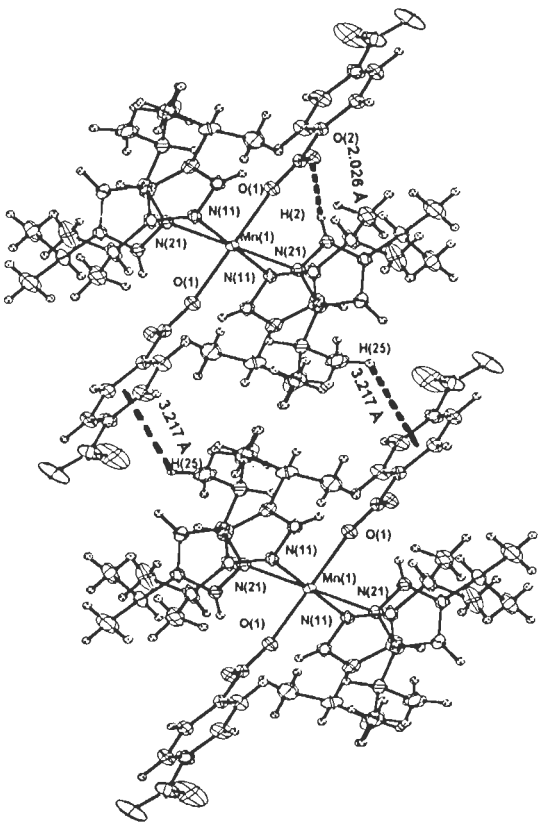


Fig. 2-4 ORTEP view of Intramoleclar hydrogen bonding and CH_3 / Pi Interaction $[(3,5\text{-}iPr_2pzH)_4Mn(NO_2\text{-}OBz)_2]$ **2c**

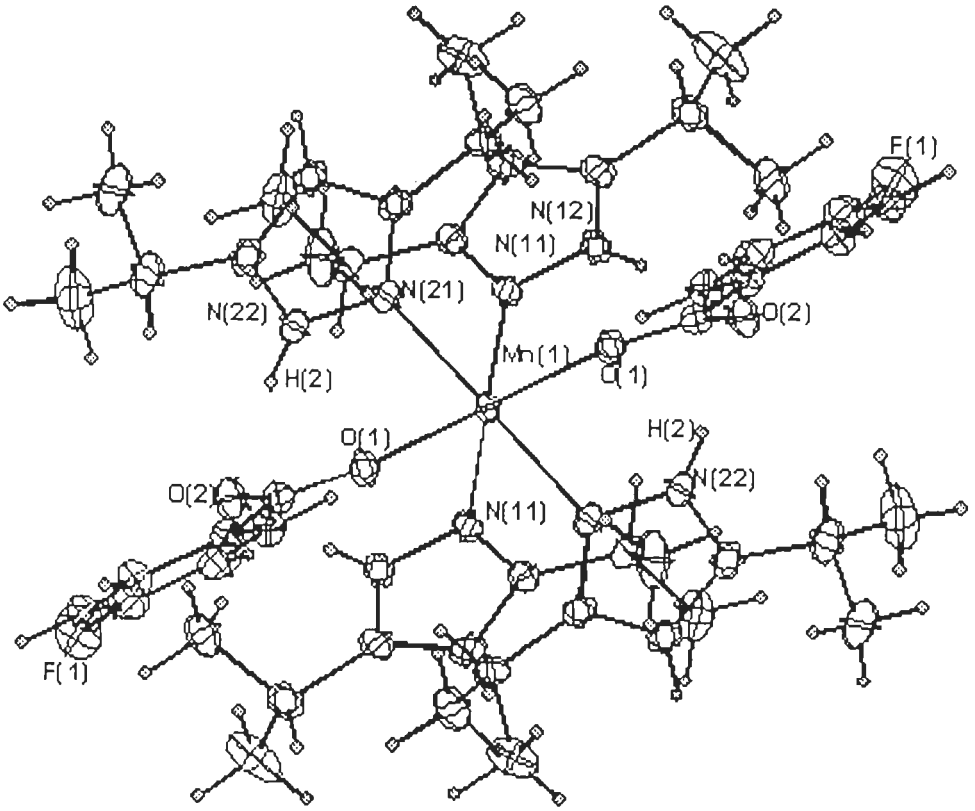


Fig. 2-5 ORTEP view of $[(3,5\text{-}iPr_2pzH)_4Mn(F\text{-}OBz)_2]$ **2d**

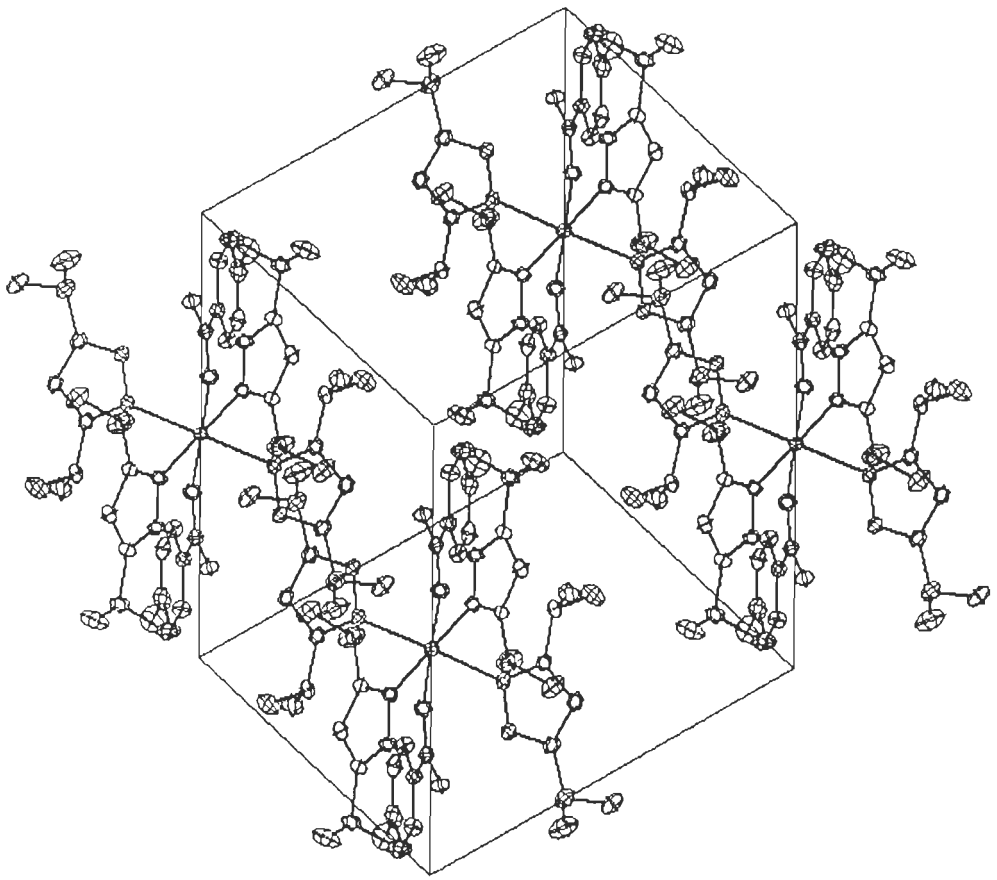


Fig. 2-6 Unit cell packing of $[(3,5\text{-}iPr_2pzH)_4Mn(F\text{-}OBz)_2]$ **2d**

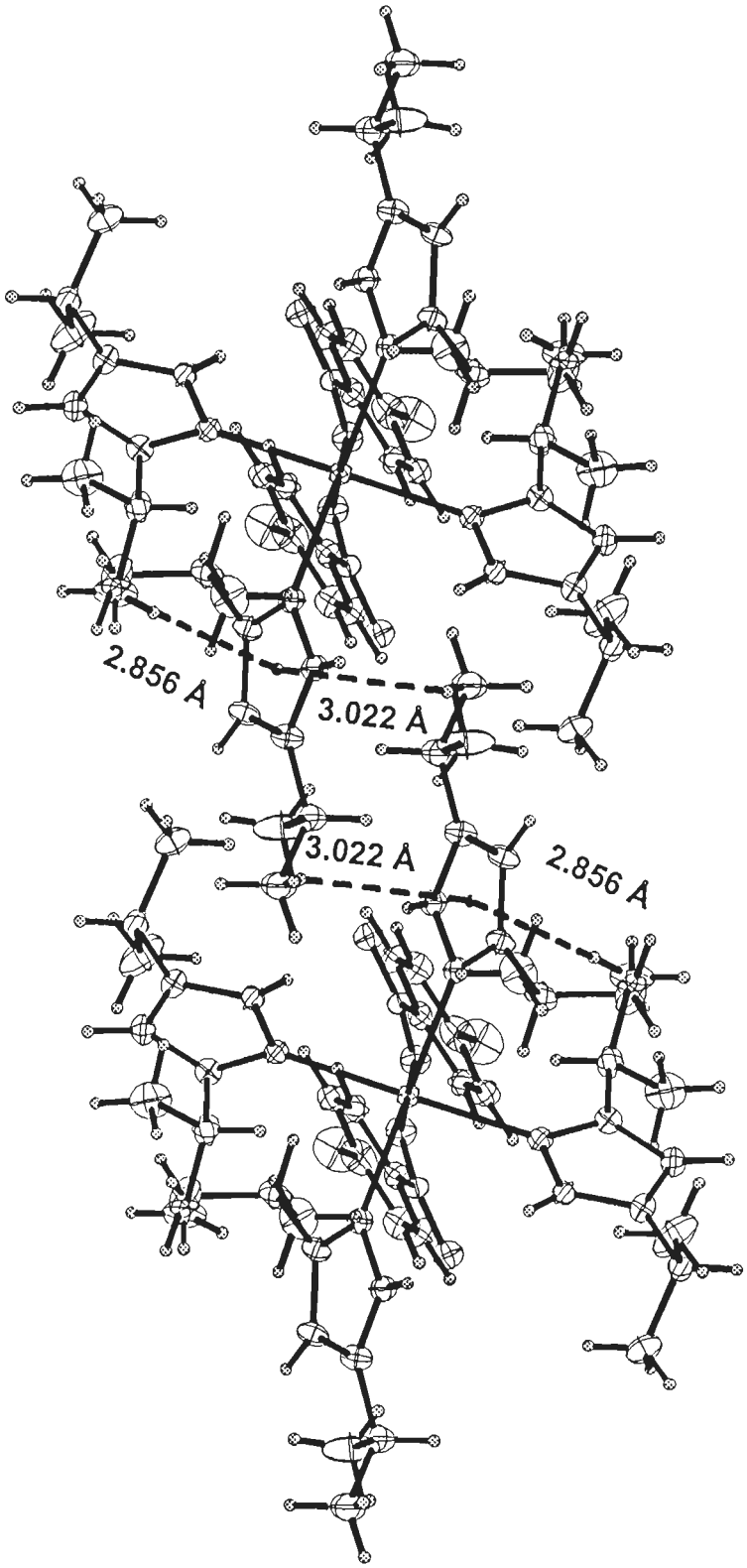


Fig. 2-7 ORTEP view of Intramoleclar hydrogen bonding and CH_3 / Pi Interaction $[(3,5\text{-}iPr_2pzH)_4Mn(F\text{-}OBz)_2]$ **2d**

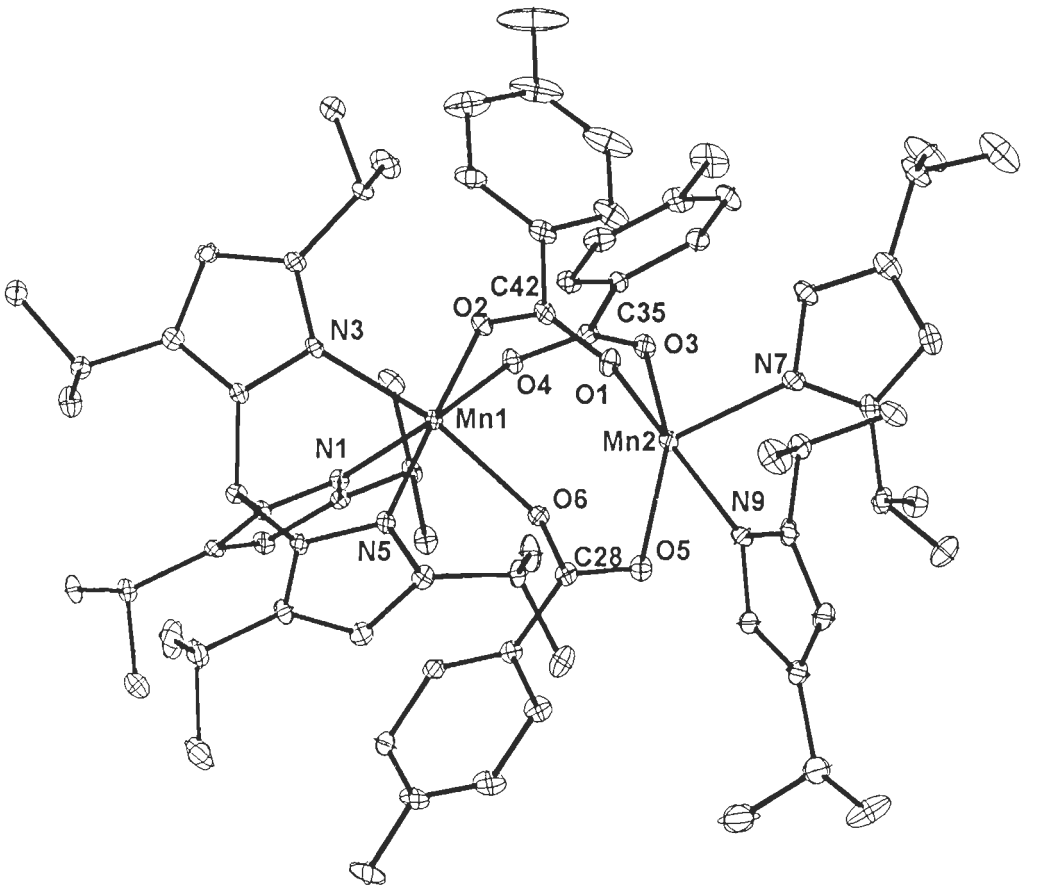


Fig. 2-8 ORTEP view of $[(HB(3,5\text{-}Pr^{i}_{2}pz)_3Mn_2(\mu\text{-}FOBz)_3(3,5\text{-}iPr_2pzH)_2]$ **2g**

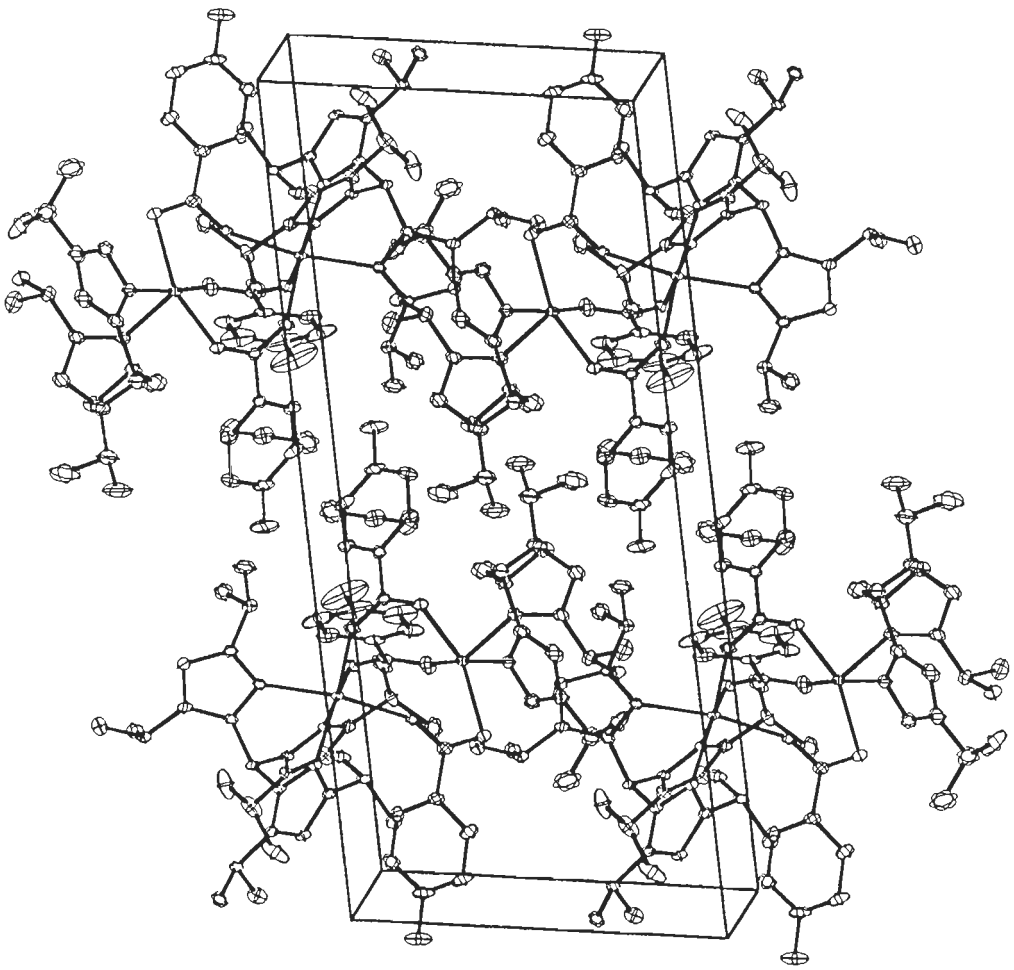


Fig. 2.9 Unit cell packing of [(HB(3,5-Pr^{i}_{2}pz)$_3$Mn$_2$(μ-FOBz)$_3$(3,5-iPr_2pzH)$_2$] **2g**

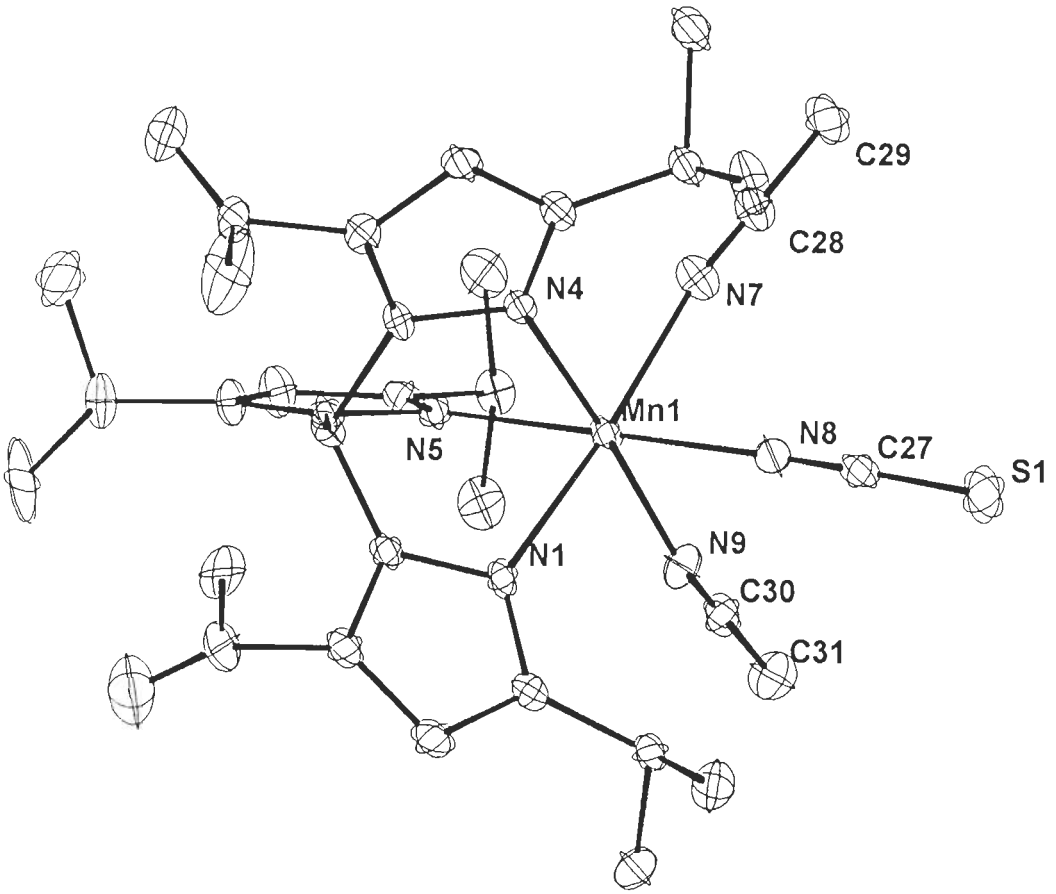


Fig. 2-10 ORTEP view of $[(HB(3,5\text{-}Pr^{i}{}_{2}pz)_{3})MnNCS].2CH_{3}CN$

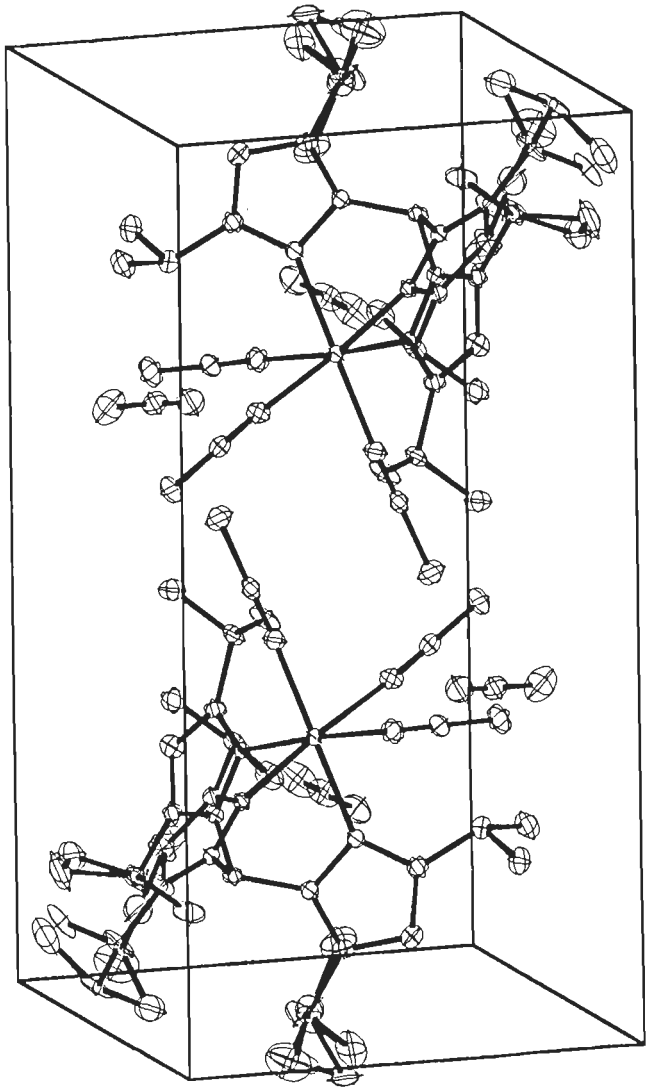


Fig. 2-11 Unit cell packing of [(HB(3,5-Pr^{i}_{2}pz)$_3$)MnNCS].2CH_3CN

References:

1. Silva, J. J. R. F. D. and Williams, R. J. P., "The biological chemistry of the elements", Clarendon Press: Oxford, (1991).
2. Glusker, J. R., "Structural aspects of metal liganding to functional groups in proteins", Adv. Protein Chem., **42**, 1 (1991).
3. Yocum, C. F. and Pecoraro, V. L., "Recent advance in the understanding of the biological chemistry of manganese", Curr. Opin. Chem. Biol., **3**, 182 (1999).
4. Christianson, D. W., "Structural chemistry and biology of manganese metalloenzymes", Prog. Biophys. Mol. Biol., **67**, 217 (1997).
5. Chander, M., Setlow, B. and Seltow, P., "The enzymatic activity of phosphoglycerate mutase from gam-positive endospore-forming bacteria requires Mn(II) and pH sensitive", Can. J. Microbiol., **44**, 759 (1998).
6. Kun, N. J., Seltow, B., Seltow, P., Cammack, R. and Williams, R., "Cooperative manganese (II) activation of 3-phosphglycerate mutase of *Bacillus megaterium*: a biological pH-sensing mechanism in bacterial spore formation and germination", Arch. Biochem. Biophys., **320**, 35 (1995).
7. Carvajal, N., Venegas, A., Oestreicher, G. and Plaza, M., "Effect of manganese on the quaternary structure of human liver arginase", Biochim Biophys Acta, **250**, 437 (1971).
8. Hardmann, K. D., Aggarwal, R. C. and Frieser, M. J., "Manganese and calcium binding sites of concanavalin A", J. Mol. Biol., **157**, 69 (1982).
9. Murphy, J. E., Ma, S. L. and Kantrowitz, E. R., "Trapping and visualization of a covalent enzyme-phosphate intermediate", Nature Struct. Biol., **4**, 618 (1997).

10. Matte, A., Goldie, H., Sweet, R. M. and Delbaere, L.T. J., "Crystal structure of *Escherichia coli* phosphoenolpyruvate carboxykinase: a new structural family with the P-loop nucleoside triphosphate hydrolase fold", J. Mol. Biol., **256**, 126 (1996).

11. Kroymann, J., Textor, S., Tokuhisa, J. G., Falk, K. L., Bartram, S., Gershenzon, J. and Mitchell-Olds, T., "A gene controlling variation in arabidopsis glucosinolate composition is part of the methionine chain elongation pathway", Plant Physiol, **127**, 1077 (2001).

12. (a) Stoddard, B. L., Dean, A. and Jr. Koshland, D. E., "The structure of isocitrate dehydrogenase with isocitrate, NADP, and calcium at 2.5 A^0 resolution: a pseudo-Michaelis ternary complex. ", Biochemistry, **32**, 9310 (1993). (b) Chen, R. D., Grobler, J. A., Hurley, J. H. and Dean, A. M., "Second-site suppression of regulatory phosphorylation in *Escherichia coli* isocitrate dehydrogenase", Protein Sci., **5**, 287 (1996). (c) Hurley, J. H., Dean, A. M., Sohl, J. L., Jr. Koshland, D. E. and Stroud, R. M., "Regulation of an enzyme by phosphorylation at the active site", Science, **249**, 1012 (1990).

13. Schramm, V. L. and Wedler, F. C., "In Manganese in metabolism and enzyme function", Academic Press, Orlando, FL, USA (1986).

14. Hansson, O., Aasa, R. and Vanngard, T., "The origin of the multiline and g = 4.1 electron paramagnetic resonance signals from the oxygen evolving system of photosystem II", Biophys. J., **51**, 825 (1987).

15. Balakrishnan, M. S. and Villafranka, J. J., "Distance determination between the metal ion sites of *Escherichia coli* glutamine synthetase by electron paramagnetic resonance using Cr(III)-nucleotides as paramagnetic substrate analogues", Biochemistry, **17**, 3531 (1978).

16. Gold, M. H., Wariishi, H. and Valli, K., "Biocatalysis in agricultural biotechnology", Chap. 9, Am. Chem. Soc., Washington, D. C., 127 (1989).

17. (a) Wariishi, H., Khadar, V. and Gold, M. H., "Oxidative cleavage of phenolic diarylpropane lignin model dimmer by manganese peroxidase from phanerochaete chrysosporium", Biochemistry, **28**, 6017 (1989). (b)Wariishi, H. and Gold, M. H., "Lignin peroxidase compound III", J. Biol. Chem., **265**, 2070 (1990).

18. Cammack, R., Chapman, A., Lu, W.-P., Karagouni, A. and Kelly, D. P., "Evidence that protein B of the thiosulphate-oxidising system of *Thiobacillus versutus* contains a binuclear manganese cluster", FEBS Lett., **253**, 239 (1989).

19. Valentine, J. S. and Mota de Freitas, D., "Copper-zinc superoxide dismutase. A unique biological "ligand" for bioinorganic studies", J. Chem. Educ., **62**, 990 (1985).

20. (a) Hassan, H. M. and Fridovich, I., "Intracellular production of superoxide radical and of hydrogen peroxide by redox active compounds", Arch. Biochem. Biophys., **196**, 385 (1979). (b) Takahashi, M., Nagano, T. and Hirobe, M., "Dioxathiadiaza- heteropentalenes mediate superoxide and hydrogen peroxide production in *Escherichia-coli*", Arch. Biochem. Biophys. **268**, 137 (1989). (c) Takabatake, T., Hasegawa, M., Nagano, T. and Hirobe, M., "Formation of superoxide by benzofurazans in *Escherichia-coli* under aerobic incubation", Chem. Pharm. Bull. 39, 1352 (1991). (d) Takabatake, T., Hasegawa, M., Nagano, T. and Hirobe, M., "Difference in superoxide toxicity between 4,7-dicyanobenzofurazan and daraquat", J. Biol. Chem., **267**, 4613 (1992).

21. Stallings, W. C., Pattridge, K. A., Strong, R. K. and Ludwig, M. L., "The structure of manganese superoxide dismutase from *Thermus thermophilus* HB8 at 2.4 Å resolution", J. Biol. Chem., **260**, 16424 (1985).

22. Kono, Y. and Fridovich, I., "Isolation and characterization of the pseudocatalase of *Lactobacillus plantarum*. A new manganese containing enzyme", J. Biol. Chem., **258**, 6015 (1983).

23. (a) Barynin, V. V., Vagin, A. A., Melik-Adamyn, V. R., Grebenko, I., Khangulov, S. V., Popov, A. N., Andrianova, M. E. and Vainshtein, B. K., "3-dimensional structure of the T-catalase at 3.0 Å resolution", Dolk. kad. Nauk. SSSR (Crystallogr.), **288**, 877 (1986). (b) Nordlund, P., Sjoberg, B. M. and Eklund, H., "Three dimensional structure of the free radical protein of ribonucleotide reductase", Nature, **345**, 593 (1990).

24. (a) Penner-Hahn, J. E., "In Manganese Redox enzymes", V. L. Pecoraro, (Ed.), VCH Publishers, Inc., New York, pp 29 (1992). (b) Stube, J., "Ribonucleotide reductases. Amazing and confusing", J. Biol. Chem., **265**, 5329 (1990). (c) Lynch, J. B., Juarez-Garcia, C., Munck, E. and Que, L., Jr., "Mossbauer and EPR studies of the binuclear iron center in ribonucleotide reductase from *Escherichia coli*", J. Biol. Chem., **264**, 8091 (1989).

25. (a) Dismukes, G. C., "Manganese enzymes with binuclear active sites", Chem. Rev., **96**, 2909 (1996). (b) Meier, A. E., Whittaker, M. M. and Whittaker, J. W., "EPR polarization studies on Mn catalase from *Lactobacillus plantarum*", Biochemistry, **35**, 348 (1996).

26. Atta, M., Nordlund, P., Aberg, A., Eklund, H. and Fontecave, M., "Substitution of manganese for iron in ribonucleotide reductase from Escherichia coli", J. Biol. Chem., **267**, 20684 (1992).

27. (a) Wieghardt, K., Bossek, U., Ventur, D. and Weiss, J., "Assembly and structural characterization of binuclear μ-oxo-di-μ-acetato bridged complexes of manganese (III) analogues of the di-iron (III) center in emerythrin" J. Chem. Soc. Chem. Commun., 347 (1985). (b) Sheats, J.E., Czernuszewicz, R. S., Dismukes, G. C., Rheingold, A. L.,

Petrouleas, V., Stubbe, J., Armstrong , W. H., Beer, R. H. and Lippard, S. J., "Binuclear manganese(III) complexes of potential biological significance", J. Am. Chem. Soc., **109**, 1435 (1987). (c) Menage, S., Girerd, J- J., and Gleizes, A., "A $[Mn_2^{III}O(MeCO_2)_2(H_2O)(bipy)]^{2+}$ (bipy = 2,2'-bipyridine) unit with accessible coordination sites. Contribution to the modeling of the photosynthetic oxygen evolving center", J. Chem. Soc. Chem. Commun., 431 (1988). (d) Bossek, U., Wieghardt, K., Nuber, B. and Weiss, J., "Manganese complexes: inorganic model complexes for active site in Mn-containing catalase. Crystal structures of $[L'_2Mn^{II}_2(\mu\text{-}OH)(\mu\text{-}O_2CCH_3)_2](PF_6).CH_3OH$ and $[L'_2Mn^{II}_2(\mu\text{-}OH)(\mu\text{-}O_2CCH_3)_2](I_3)I.H_2O$", Inorg. Chim. Acta, **165**, 123 (1989). (e) Wieghardt, K., Bossek, U., Bonvoisin, J., Beauvillian, G. J. J., Nuber, B., Weiss, J. and Heinze, J., "Dinuclear manganese (III,IV) model complexes for the active center of the metalloprotein photosystem II. Synthesis, magnetism and crystal structure of $[LMn^{III}(\mu\text{-}O)(\mu\text{-}O_2CCH_3)_2Mn^{IV}L](ClO_4)_3$ (L = N, N', N"-trimethyl-1,4,7-Tri- azacyclononane)", Angew Chem. Int. Ed. Engl. **25**, 1030 (1986). (f) Wieghardt, K., Bossek, U., Zsolnai, L., Huttner, G., Blondin, G., Girerd, J. J. and Babonneau, F., "A novel mixed valent Mn^{III}-Mn^{IV}-dimer, $[L_2Mn_2(\mu\text{-}O)(\mu\text{-}MeCO_2)][BPh_4]_2.MeCN$: Crystal structure, magnetic properties and ESR spectrum (L = 1,4,7- triazacyclononane)", J. Chem. Soc., Chem. Commun., 651 (1987).

28. (a) Wieghardt, K., "The active sites in manganese-containing metalloproteins and inorganic model complexes", Angew. Chem. Int. Ed. Engl., **28**, 1153 (1989). (b) Pecoraro V. L., Baldwin, M. J. and Gelasco, A., "Interaction of manganese with dioxygen and its reduced derivatives", Chem. Rev., **94**, 807 (1994). (c) Ruttinger, W. and Dismukes, G. C., "Synthetic water oxidation catalysis for artificial photosynthetic water oxidation", Chem. Rev., **97**, 1 (1997).

29. (a) Sugiura, Y., Kawabe, H, Tanaka, H., Fujimoto, S. and Ohara, A.,"Purification, enzymatic properties and active site environment of a novel manganese(III)-containing acid phosphatase", J. Biol. Chem., **256**, 10664 (1981). (b) Sugiura, Y., Kawabe, H. and Tanaka, H., "New manganese(III)-containing acid phosphatase evidence for an intense charge-transfer band and tyrosine phenolate coordination", J. Am. Chem. Soc., **102**, 6581 (1980). (c) Sugiura, Y., Kawabe, H, Tanaka, H., Fujimoto, S. and Ohara, A., "First evidence for manganese binding to sulfur donor group in metalloprotein, Mn (III)-containing acid phosphatase", J. Am. Chem. Soc., **103**, 963 (1981).

30. (a) Dismukes, G. C., "The metal centers of the photosynthetic oxygen-evolving complex", Photochem. Photobiol., **43**, 99 (1986). (b) Pecoraro, V. L., "Structural proposals for the manganese centers of the oxygen evolving complex. An inorganic chemists perspective", Photochem. Photobiol., **48**, 249 (1988).

31. (a) Guiles, R. D., Yachandra, V. K., McDermott, A. E., Cole, J. L., Dexheimer, S. L., Britt, R. D., Sauer, K. and Klein, M. P., "The So state of photosystem II induced by hydroxylamine. Difference between the structure of the manganese complex in the S_o and S_1 states determined by X-ray absorption spectroscopy", Biochemistry, **29**, 486 (1990). (b) George, G. N., Prince, R. C. and Cramer, S. P., "The manganese site of the photosynthetic water-splitting enzyme", Science, **243**, 789 (1989). (c) Penner-Hahn, J. E., Fronko, R. M., Pecoraro, V. L., Yocum, C. F., Betts, S. D. and Bowlby, N. R., "Structural characterization of the manganese sites in the photosynthetic oxygen-evolving complex using X-ray absorption spectroscopy", J. Am. Chem. Soc., **112**, 2549 (1990). (d) Tamura, N., Ikeuchi, M. and Inoue, Y., "Assignment of histidine residues in D1 protein as possible ligands for functional manganese in photosynthetic water-oxidising complex", Biochim. Biophys. Acta, **973**, 281

(1989). (e) Andreasson, L.-E., "Is nitrogen liganded to manganese in the photosynthetic oxygen-evolving system?. EPR studies after isotopic replacement with ^{15}N", Biochim. Biophys. Acta, **973**, 465 (1989).

32. For recent reviews of the OEC, see: (a) Debus, R. J., "The manganese and calcium ions of photosynthetic oxygen evolution", Biochim. Biophys. Acta **1102**, 269 (1992). (b) Rutherford, A. W., Zimmermann, J.-L. and Boussac, A., "In the photosystems: Structure, function and molecular biology", Barber, J., (Ed.), Elsveir, Amsterdam, pp 179 (1992). (c) Babcock, G. T., In new comprehensive biochemistry in photo-synthesis, Amesz, J. (Ed.), Elsvier Amsterdam, **15**, 125 (1987). (d) Britt, R. D., In oxygenic photosynthesis: The light reactions, Ort, D. R. and Yocum, C. F., (Eds.), Kluwer Academic Publishers, Dordrecht, pp 137 (1996).
33. Christou, G., "Manganese carboxylate chemistry and its biological relevance", Acc. Chem. Res., **22**, 328 (1989).
34. Feig, A. L. and Lippard, S. J., " Reactions of non-heme iron(II) centers with dioxygen in biology and chemistry", Chem. Rev., **94**, 759 (1994). (b) Wallar, B. K. and Lipscomb, J. D., "Dioxygen activation by enzymes containing binuclear nonheme iron clusters", Chem. Rev., **96**, 2625 (1996). (c) Dismukes, G. C., "Manganese enzymes with binuclear active sites", Chem. Rev., **96**, 2909 (1996).
35. (a) Lippard, S. J. and Berg, J. M., "Principles of bioinorganic chemistry", University Science Books, Mill Valley, CA 94941 (1994). (b) Holm, R. H., Kennepohl, P. and Solomon, E. I., "Preface: bioinorganic enzyme-biology", Chem. Rev., **96**, 2237 (1996).
36. Edwards, R. A., Baker, H. M., Whittaker, M. M., Whittaker, J. W., Jameson, G. B. and Baker, E. N., "Crystal structure of *Escherichia coli* manganese superoxide dismutase at 2.1-A^0 resolution", J. Biol. Inog. Chem., **3**, 161 (1998).(b) Barynin V.V., Whittaker M. M.,

Antonyuk S. V., Lamzin, V. S., Harrison, P. M., Artymiuk, P. J. and Whittaker, W., "Crystal structure of manganese catalase from *lactobacillus plantarum*", Structure, **9**, 725 (2001).

37. Hakansson, K., Carlsson, M., Svensson, L. A. and Liljas, A., "Structure of native and apo carbonic anhydrase II and some of its anion-ligand complexes", J. Mol. Biol., **227**, 1192 (1992).

38. (a) Funabiki, T., "In Oxygenases and Model Systems", Funabiki, T., Ed., Kluwer Academic Publishers: Dordrecht, The Netherlands, pp 69-77, 140-145 (1997). (b) Nelson, M. J., Seitz, S. P., "In Active Oxygen in Biochemistry", Valentine, J. S., Foote, C. S., Greenberg, A., Liebman, J. F., Eds.; Chapman & Hall: London, 1995; pp 276-312. (b),Skrzypczak-Jankun, E., Bross, R. A., Carroll, R. T., Dunhan, W. R., Funk, M. O., Jr," Three-dimensional structure of a purple lipoxygenase," J. Am. Chem. Soc., **123**, 10814 (2001).

39. MacBeth, C. E., Gupta, R., Mitchell-Koch, K. R., Jr. Young, V. G., Lushington G. H., Thompson, W. H., Hendrich, M. P. and Borovick, A. S., " Utilization of hydrogen bonds to stabilize M-O(H) units: synthesis and properties of monomeric iron and manganese complexes with terminal oxo and hydroxo ligands", J. Am. Chem. Soc., **126**, 2556 (2004).

40. Itho, K., Hayashi, H., Furutachi, H., Matsumoto, T., Nagatomo, S., Tosha, T., Terada, S., Fujinami, S., Suzuki, M. and Kitagawa T., "Synthesis and reactivity of a (μ-1,1-hydroperoxo)(μ-hydroxo)dicopper (II) complex: Ligand hydroxylation by a bridging hydroperoxo ligand", J. Am. Chem. Soc., **127**, 5212 (2005).

41. Garner, D. K., Allred, R. A., Tubbs, K. J., Arif, A. M., and Berreau, L. M.," Synthesis and characterization of mononuclear zinc aryloxide complexes supported by nitrogen / sulfur ligands possessing an internal hydrogen bond donor", Inorg. Chem., **41**, 13, 3533 (2002).

42. Ogo, S., Yamahara, R., Roach, M., Suenobu, T., Aki, M., Ogura, T., Kitagawa, T., Masuda, H., Fukuzumi, S. and Watanade, Y.,"Structural and spectroscopic feature of a cis(hydroxo)-Fe^{III}-(carboxylato) configuration as an active model for lipoxygenases", Inorg. Chem., **41**, 21, 5513 (2002).

43. Boldog, I., Rusanov, E. B., Chernega A. N., Sieler, J. and Domasevitch K. V., "Coordination polymers of Co^{II} and 3,3',5,5'-tetramethyl-4,4'-bipyrazolyl: A noval metal-organic three-dimensional network with four-coordinated planner vertices", J. Chem. Soc., Dalton Trans., 893 (2001).

44. Singh, U. P., Singh, R., Hikichi, S., Akita, M., Moro-oka, Y., "Characterization of a dinuclear Mn (II) tri(μ-carboxylato) complex with the hindered hydrotris(3,5-diisopropyl-1-pyrazolyl)borate (= Tp^{iPr2}) ligand: Intramolecular hydrogen bonding interaction between the protonated Tp^{iPr2} and Mn-coordinating carboxylate ligands", Inorg. Chim. Acta., **162**, 297 (2000).

45. Mehn, M. P., Fujisawa, K., Hegg, E. L., and Jr, L.Q., "Oxygen activation by nonheme iron (II) complexes: α-keto carboxylate versus carboxylate", J. Am. Chem. Soc., **125**, 7828 (2003).

46. (a) Kessissoglou, D. P., Butler, W. M. and Pecoraro, V. L., "Characterization of mono and binuclear manganese(II) schiff base complexes with metal-disulfide ligation", Inorg. Chem., **26**, 495 (1987). (b) Wieghardt, K., Bossek, U., Nuber, J., Weiss, J., Bonovoisn, M., Corbella, M., Vitols, S. E. and Girerd, J. J., "Synthesis, crystal structures, reactivity and magnetochemistry of a series of binuclear complexes of manganese (II), -(III) and -(IV) of biological relevance. The crystal structure of $[LMn^{IV}(\mu\text{-}O)_3Mn^{IV}L']$[$PF_6$].$H_2O$ containing an unprecedented short Mn...Mn distance of 2.296 Å", J. Am. Chem.Soc., **110**, 7398 (1988). (c)

Caneschi, A., Ferraro, F., Gatteschi, D., Melandri, M. C., Rey, P. and Sessoli, R., "Synthesis, structure and magnetic properties of a dinuclear manganese(II) complex with one μ-aqua and two μ-carboxylato bridges", Angew. Chem. Int. Ed. Eng., **28**, 1365 (1989). (d) Kitajima, N., Fujisawa K. and Moro-oka, Y., "Formation and characterization of a mononuclear (acylperoxo)copper(II) complex", Inorg. Chem., **29**, 357 (1990). (e) Rardin, R. L., Tolman, W. B. and Lippard, S. J., "Monodentate carboxylate complexes and the carboxylate shift-implications for polymetalloprotein structure and function", New J. Chem., **15**, 417 (1991). (f) Aurangzeb, N., Hulme, C. E., McAuliffe, C. A., Pritchard, R. G., Watkinson, M., Garcia-Deibe, A., Bermejo, M. R. and Sousa, A., "The crystal structure of $[Mn(salpn)(acetate)]_2(H_2O)_3$; The first example of a manganese(III) schiff base polymeric complex containing a dimeric repeat unit (Salpn = N, N'-bis(salcylidene)-1,3-diamino-propane", J. Chem. Soc., Chem. Commun., 1524 (1992). (g) Yu, S.-B., Lippard, S. J., Shweky, I. and Bino, A., "Dinuclear manganese(II) complexes with water and carboxylate bridges", Inorg. Chem., **31**, 3501 (1992). (h) Oberhausen, K. J., Brein, R. J. O., Richardson, J. F., Buchanan, R. M., Costa, R., Latour, J. M., Tsai, H. L. and Hendrickson, D. N., "Synthesis and characterization of a (μ-oxo)(μ-carboxylato)dimanganese(III) polyimidazole complex", Inorg. Chem., **32**, 4561 (1993). (i) Tanase, T., Lippard, S. J., "Dinuclear manganese(II) complexes with the $\{Mn_2(\mu\text{-carboxylato})_2\}^{2+}$ core and their transformation to (μ-Oxo)bis(μ-carboxylato)dimanganese(III) complexes.", Inorg. Chem., **34**, 4682 (1995). (j) Yamami, M., Tanaka, M., Sakiyama, H., Koga, T., Kobayashi, K., Miyasaka, H., Ohba, M., and Okawa, H., "Dinuclear complexes of Mn^{II}, Co^{II} and Zn^{II} triply bridged by carboxylate groups: properties and catalase-like function" J. Chem. Soc., Dalton Trans., 4595 (1997). (k) Lewis, E. A., Smith, J. R. L., Walton, P. H., Archibald, S. J., Foxon, S. P., and Giblin, M. P.,

"Tuning the metal-based redox potentials of manganese cis,cis-1,3,5-triaminocyclohexane complexes.", J. Chem. Soc., Dalton Trans., 1159 (2001).

47. Stallings, W. C., Pattridge, K. A., Strong, R. K. and Ludwig, M. L., "The structure of manganese superoxide dismutase from *Thermus thermophilus* HB8 at 2.4 Å resolution", J. Biol. Chem., **260**, 16424 (1985).

48. N. Kitajima, Udai P. Singh, H. Amagai, M. Osawa, Y. Moro-oka, "Oxidative Conversion of $Mn(\mu\text{-}OH)_2Mn$ to $Mn(\mu\text{-}O)_2Mn$ Moiety. Synthesis and Molecular Structures of a μ-Hydroxo Dimanganese (II) and μ-Oxo Dimanganese (III) Complexes with a Hindered N_3 Ligand", J. Am. Chem. Soc., **113**, 7757 (1991)

49. Osawa, M., Singh, U. P., tanaka, M., Moro-oka, Y. and Kitajima, N. " Synthesis and molecular structure of an unsymmetric dimanganese(II) carboxylato complex", J. Chem. Soc, Chem. Commun., 310 (1993).

50. Rardin, R. L., Poganiuch, P., Bino, A., Goldberg, D. P., Tolman, W. B., Liu, S. and Lippard, S. J., "Synthesis and characterization of novel trinuclear iron(II) and manganese(II) carboxylate complexes: structural trends in low valent iron and manganese carboxylates", J. Am. Chem. Soc., **114**, 5240 (1992).

51. Caneschi, A., Ferraro, F., Gatteschi, D., Melandri, M. C., Rey, P. and Sessoli, R, Synthesis, structure, and magnetic properties of binuclear manganese(II) complexes with one μ-aqua and two μ-carboxylate-bridges", Angew. Chem., Int. Ed. Eng., **28**, 1365 (1989).

52. Yu, S.-B., Lippard, S. J., Shweky, I. and Bino, A., "Dinuclear manganese(II)

complexes with water and carboxylate bridges", Inorg. Chem., **31**, 3502 (1992).

53. Wieghardt, K., Bossek, U., Nuber, B., Weiss, J., Bonovoisn, J., Corbella, M., Vitols, S. E. and Girerd, J. J., "Synthesis, crystal structures, reactivity, and magneto chemistry of a series of binuclear complexes of manganese(II), -(III), and -(IV) of biological relevance. The crystal structure of [L'MnIV(μ-O)3MnIVL'](PF6)2.H2O containing an unprecedented short Mn···Mn distance of 2.296 .ANG.", J. Am. Chem. Soc., **110**, 7398 (1988).

54. Kono, Y. and Fridovich, I., "Isolation and characterization of the Pseudocatalase of *Lactobacillus plantarum*. A new manganese containing enzyme", J. Biol. Chem., **258**, 6015 (1983).

Chapter 3

SYNTHESIS AND STRUCTURAL CHARACTERISATION OF BINUCLEAR COBALT COMPLEXES WITH DIFFERENT BRIDGING LIGANDS

Cobalt is a transition metal that occupies a position in the periodic table between iron and nickel and is an essential element for life although it does not participate in O_2 metabolism. Although cobalt is less frequently encountered in metalloenzymes than the other first-row transition metals, it is nevertheless an important cofactor in vitamin-B_{12}-dependent enzymes. Vitamin B_{12} [1] contains cobalt in a substituted corrin macrocycle (a porphyrin relative). In contrast, only a few proteins containing noncorrin cobalt have been characterized. Because cobalt has characteristic spectra correlated with structural properties (such as observed co-ordination numbers for Co (II) and Co (III), it has also served as a spectroscopic probe in metalloenzymes. Substituting cobalt for zinc has often been a useful tool to investigate the structural basis of catalytic properties in zinc enzymes and the co-ordination environment of active zinc sites in other proteins [2]. Non-corrin cobalt is receiving increased interest not only in bioinorganic chemistry but also in biotechnology and its availability and remarkable chemical versatility makes cobalt an invaluable catalyst in the chemical industry. Details about vitamin B_{12} and some noncorrin-cobalt-containing enzymes are given below.

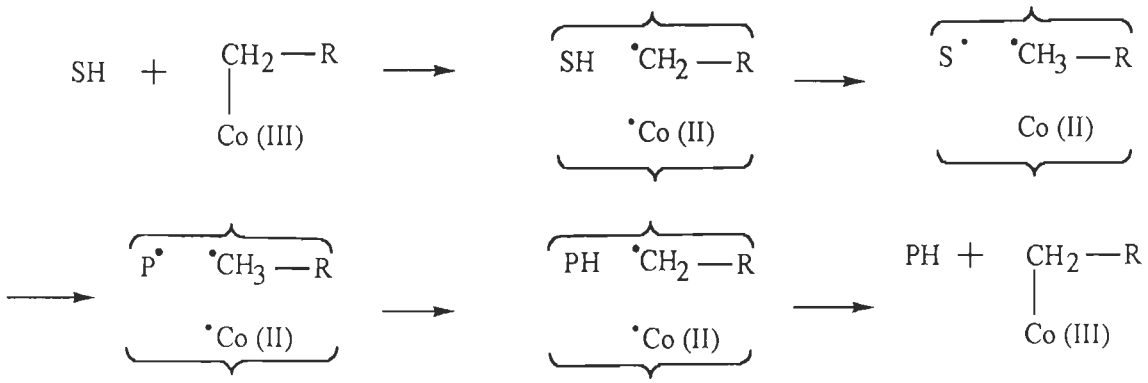
The vitamin B_{12} is a cyano complex, but a methyl or methylene group replaces CN in native enzymes. Its deficiency causes the severe disease of pernicious anemia in humans, which indicates the critical role of cobalt. Increased dietary concentrations of cobalt and of vitamin B_{12}, or injections of vitamin B_{12}, have been shown to result in improved weight gains in growing catles in comparision to untreated control animals. Cobalt and copper, when added to diets of growing cattle, increased the ether extract, fibre and cellulose digestibilities of the diets [3-5]. Methionine complexes of cobalt and other trace elements in the diet were

found to be more effective than the inorganic salts of the elements in promoting increased milk yield on cows.

Vitamin B_{12}, an antipernicious anemia factor in liver, was isolated by Ricks et al. [6], Smith and Parkar [7] in 1948 in the form of dark red crystals. The structure of the vitamin was revealed in 1956 by the X-ray crystallographic works of Hodgkin and coworkers [8], together with chemical studies [9]. In 1958, a biochemically active form B_{12} (vitamin B_{12} coenzyme) was discovered by Barker et al. [10]. The cobalt in vitamin B_{12} is coordinated to five N atoms, four contributed by a tetrapyrrole (corrin: the sixth ligand is C, provided either by C_5 of deoxyadenosine in enzymes such as methylmalonbyl-CoA mutase (fatty acid metabolism) or by a methyl group in the enzyme that synthesizes the amino acid methionine in bacteria. The vitamin is qauite commonly isolatd in a form called cyanocobalamin, in which CN occupes this position. The similarity to the porphyrin ring systems of the tetradentate ring, called a corrin ring, should be obvious, but there is a key difference, namely, that one of the bridges between pyrrole rings is a direct ring-to-ring bond instead of an HC or CH_3C unit. The reason why the corrin system rather than a porphyrin one is employed may be that it's greater flexibility [11] leads to steric forces that eject the 5′ -deoxyadenosyl radical, thus initiating the catalytic cycle.

There are many B_{12}-dependent enzymes, which differ in the substrate upon which they operate [12], but the role of coenzyme B_{12} appears uniformly to be the same: by means of Co-C homolytic bond cleavage, a formal Co (II) form of the coenzyme and the adenosyl radical are formed [13]. The relative importance of various factors in enhancing the homolytic Co-C bond cleavage is still to be determined [14]. However, once this step has occurred the adenosyl radical (denoted later as RCH_2) accept a hydrogen atom from the

substrate (SH) to generate a substrate radical (S˙). The enzyme causes this to rearrange to the product radical (P˙) and this then abstracts a hydrogen atom from RCH_3 to give product PH and the coenzyme is recovered, ready for another cycle. The details of the enzyme-catalyzed conversion, S˙-P˙., are still in need of clarification. This general sequence of events is summarized in the scheme 3-1 where the curly brackets indicate intermediates whose association is mediated by the enzyme.



Scheme 3-1

Like usual Co (III) complexes, the majority of the B_{12} compounds are six-coordinate octahedral Co (III) complexes, although certain Co (II) corrinoids exist as five-coordinate square pyramidal complexes [15]. The corrinoids which contain the nucleotide loop consisting of phosphate, ribose and an organic base, like B_{12} itself, are designated complete corrinoids, whereas the corrinoids lacking the nucleotide loop are referred to as imcomplete corrinoids. The cobalamins in which the cobalt ion is reduced to divalent and monovalent states are called cob (II)alamin (B_{12r}) and cob(I)alamin(B_{12s}), respectively. These Co (II) and Co (I) corrinoids are important intermediates in B_{12}-dependent enzymetic reactions. The coordination linkage between cobalt ion and the corrin ring is so strong that it is impossible to remove cobalt chemically from vitatmin B_{12} without destroying the corrin ring. Certain photosynthetic bacteria [16] and *streptomuyces olivaceus* [17] produce metal-free corrinoids.

In addition to cobalt other metals viz., zinc, copper, rhodium, manganese and iron [18] can be inserted into these metal-free corrinoids. The rhodium analogues of adenosyl-B_{12} and methyl-B_{12} have been synthesized [19] and it has been reported that the Rh-C bond is less sensitive to light than the Co-C bond.

Adenosyl-B_{12} and methyl-B_{12} are two coenzyme forms of vitamin B_{12}. The former participate in enzymatic reactions involving the common transfer of a hydrogen atom, whereas the latter is involved as an intermediate in enzymatic methyl transfer reactions. The Co-C bond of adenosyl-B_{12} dissociates reversibly in the enzymatic rearrangements and reduction involving hydrogen transfer, i.e., the Co-C bond becomes activated and is cleaved by the binding to apoenzymes, regenerating the catalytic center.

Methionine aminopeptidase is the only protein containing noncorrin cobalt that has been crystallized and studied by X-ray crystallography [20]. It cleaves the N-terminal methionine from many newly translated polypeptide chains in both prokaryotes and eukaryotes. Methionine aminopeptidase is an important catalyst for the N-terminal modification to be involved in functional regulation, intracellular targeting and protein turnover [21]. All forms of methionine aminopeptidase examined to date appear to be metalloproteins and are activated by, or are sensitive to, metals such as Mn (II), Co (II) and Zn (II). The *E. coli* methionine aminopeptidase is a monomeric protein of 29 kDa consisting of 263 residues that binds two Co (II) ions in its active site. In the enzyme, the cobalt ions are co-ordinated by the residues Asp97, Asp108, His171, Glu204 and Glu235 (Fig. 3-1).

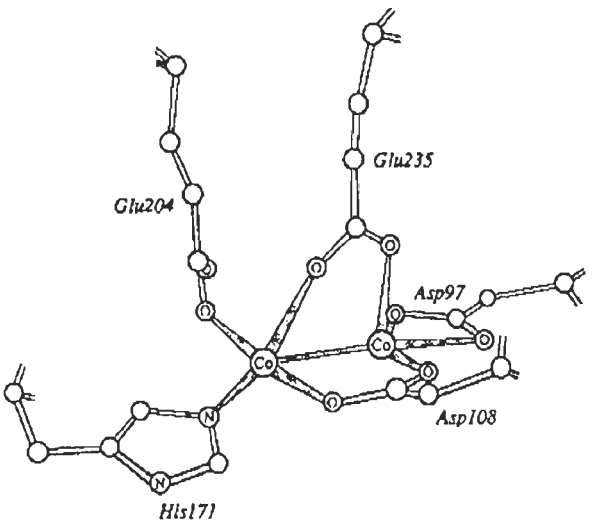


Fig. 3-1 Structure of the catalytic bimetallic core of the *Escherichia coli* methionine amino peptidase.

These five amino acid residues are conserved in the corresponding sequence of four other methionine aminopeptidases, with the exception of Glu235, which is substituted by Gln in the *Bacillus subtilis* enzyme. The distance between the cobalt ions is somewhat larger than twice the covalent radius for cobalt (2.32 Å) and is similar to that between the two zinc atoms of leucine aminopeptidase (Zn (II) – Zn (II); 2.9 Å). The presence of two Co (II) ions at the active site of the *E. coli* enzyme demonstrates the special ability of such two metal centers to catalyze hydrolytic reactions. The protein-cobalt and cobalt-cobalt interactions for each cobalt atom in methionine aminopeptidase are organized in an approximately octahedral geometry, with the sixth position unoccupied in the crystal structure. The two cobalt ions and six of the ligand atoms (i.e., ligands other than the two oxygen atoms of Glu235 that directly bind to two cobalts) are very nearly coplanar [20]. Solvent molecules may be bound to both cobalt ions in the direction of the `absent' octahedral ligands.

The catalytic mechanism of methionine aminopeptidase is not known in detail but it appears to share common features with other metalloproteases, particularly, the bovine

leucine aminopeptidase containing two zinc ions [20]. Recently, Wilce et al. [22] reported the crystal structure and proposed reaction mechanism of proline aminopeptidase from *E. coli*, which contains two manganese ions per subunit and shows similarity to the *E. coli* methionine aminopeptidase in sequence and structure. Based on these findings, a possible reaction mechanism of the methionine aminopeptidase, which does not require direct co-ordination of the substrate to the cobalt ions, can be proposed.

Nitrile hydratase, catalyzes hydration of nitriles to amides, is a key enzyme involved in the metabolism of the toxic compounds [23].

$$R{-}C{\equiv}N + H_2O \rightarrow R{-}(C{=}O){-}NH_2$$

This enzyme is also industrially used in the kiloton-scale production of acrylamide and nicotinamide from the corresponding nitriles [23-25]. An actinomycete, *Rhodococcus rhodochrous* J1 produces two kinds of nitrile hydratases, a high-molecular mass enzyme (520 kDa) and a low-molecular mass form (130 kDa) depending on the inducer added to the culture. Both enzymes are composed of α and β subunits: the larger enzyme has nine or 10 of each subunit, and the smaller enzyme has two of each. The cobalt K-edge spectrum of the high-molecular mass nitrile hydratase also suggests six co-ordinate cobalt and a distorted octahedral symmetry of the cobalt center in the enzyme. The native protein is EPR-silent, as is expected for low-spin Co (III). Chemical reduction of the native enzyme by dithionite and the artificial dye methylviologen results in a species with the EPR signature of a low-spin Co (II) complex [26]. Consequently, the cobalt-containing nitrile hydratase is the first example of a native protein that contains a noncorrin Co (III) ion with a mixed S and N ligand field of constituent amino acid of the enzyme. Very recently, another cobalt-containing nitrile hydratase, which exhibits enantioselectivity for nitriles such as 2(S)-(4′ -chlorophenyl)-3-

methylbutyronitrile as a substrate, was isolated from *P. putida* [27]. In this enzyme, cobalt exists as a noncorrin low-spin Co (III) ion with a distortion from the octahedral symmetry. This enzyme is similar to the *R. rhodochrous* J1 nitrile hydratases and contains the CXXCSC sequence conserved in all sequenced nitrile hydratases, suggesting that these three cysteines and serine were co-ordinated to the Co (III) ion. This sequence motif was also found in thiocyanate hydrolase from *Thiobacillus thioparus Thi*115 [28], which catalyzes the conversion of thiocyanate ($^{-}$SCN) to carbonyl sulfide (S = C = O) and ammonia, although the involvement of metals in the latter enzyme remains unknown. Whether the cobalt center of the cobalt-containing nitrile hydratases is the site for nitrile-binding and hydrolytic cleavage of a CN triple bond in the substrate is unknown, but the EPR spectra described above strongly suggest a role for the cobalt ion as a Lewis acid in the catalytic reaction.

Prolidase, also known as, proline dipeptidase is widespread in nature and has been isolated from bacteria [29]. Very recently, prolidase from *P. furiosus* has been found to be another cobalt-containing member (together with methionine aminopeptidase) of the binuclear N-terminal exopeptidase family [29]. This enzyme is a homodimer (39.4 kDa per subunit) and contains one cobalt atom per subunit. Its catalytic activity requires the addition of Co (II) ions, indicating that the enzyme has a second metal ion binding site. The second Co (II) can be replaced by Mn (II) (resulting in a 25% decrease in activity), but not by other heavy metals such as Mg (II), Fe (II), Zn (II), Cu (II) and Ni (II). In summary, prolidase has at least two binding sites per subunit for Co (II) ions: one is an integral part of the protein (and not removed through purification or dialysis); and the other has an association constant of approximately 0.3 mm and is essential for catalysis. In this regard, the *P. furiosus* prolidase is similar to members of binuclear metallohydrolases represented by the N-terminal

exopeptidases, the active site of which contains two metal ions. Interestingly, although the amino acid sequence of the *P. furiosus* prolidase shows no significant similarity to those of methionine aminopeptidases, all five of the cobalt-coordinating residues are conserved in the former enzyme (Asp209, Asp220, His280, Glu313 and Glu327) [29].

Glucose isomerase is one of the most highly used enzymes in industry [30] and catalyses the reversible isomerization of d-glucose to d-fructose. Although this enzyme requires divalent cations for the activity, its specific requirement depends on the source of enzyme. Co (II) is essential for glucose isomerase production by *Streptomyces* strain YT-5 [31] and *Streptomyces albus* [32]. The enzyme from S. *albus* has been characterized in detail, and was the first noncorrin-cobalt containing enzyme reported. It is tetrameric (16.5 kDa) and contains one noncorrin cobalt, which shows a low-spin Co (II) complex in EPR measurements. The cobalt ion can be removed by EDTA treatment, resulting in a loss of activity. Extended X-ray absorption fine-structure analyses showed a distortion of the octahedral symmetry of the cobalt center, a binding to N-ligands or O-ligands of protein donor atoms (constituent amino acids of the enzyme), and an absence of S-ligands, while electron nuclear double resonance spectra suggested only one type of N-ligand, which appeared to be imidazoles nitrogens from histidine residues. The visible spectrum of the enzyme exhibits only one shoulder peak at 410 nm and a weak maximum at 735 nm, in agreement with a distorted octahedral co-ordination of Co (II). When treated with cyanide in the presence of dithionite, the shoulder shifts from 410 nm to 385 nm. These spectra were also observed for the cyanide derivative of Co (II)-substituted carbonic anhydrase which showed a low-spin complex, indicating that the absent octahedral ligand of the glucose isomerase is replaced by cyanide.

Methylmalonyl-CoA carboxytransferase. The biotin-containing enzyme methylmalonyl-CoA carboxytransferase (transcarboxylase) as found in *Propionibacterium shermanii* [33] is a complex multisubunit enzyme (26S) composed of three types of subunits (1.3S, 5S, and 12S) that catalyzes the transfer of a carboxyl group from methylmalonyl-CoA to pyruvate to form propionyl-CoA and oxaloacetate. The 5S (120 kDa) subunit is a homodimer containing Co (II) and Zn II) (1 mol of each per monomer), and assembles with two 1.3S (12 kDa) biotin containing subunits to form a stable complex termed the 6S subunit. The 12S subunit is a hexamer composed of six identical monomers and combines with 6S subunits to form active methylmalonyl-CoA carboxytransferase. There are three types of cobalt in methylmalonyl-CoA carboxytransferase [33]. The first type is catalytic cobalt. Detection of the tightly-bound cobalt in the enzyme at approximately 12K by EPR measurements is consistent with high-spin Co (II) in a distorted octahedral environment. Two rapidly exchanging water ligands are proposed to be coordinated to the enzyme-bound Co (II) [34]. The second type consists of six cobalt ions (per 26S enzyme) that are detected when the methylmalonyl-CoA carboxytransferase is incubated with exogeneous Co (II) at pH 8, during which some Co (II) becomes tightly bound. The third type of cobalt in the enzyme is more weakly bound and is in equilibrium with the free Co (II), which must be maintained at about 2 mm in order to stabilize the enzyme at alkaline pH. This weakly bound Co (II) is involved in the equilibrium of the association and dissociation of the enzyme [33]. Although the three-dimensional structure of the 1.3S subunit of the *P. shermanii* methylmalonyl-CoA carboxytransferase [35] is known, that of Co (II)-containing 5S subunit is not. The structure of the corresponding biotin carboxylase of *E. coli* [36] has been determined.

Aldehyde decarbonylase. The cobalt-containing enzyme aldehyde decarbonylase converts a fatty aldehyde to a hydrocarbon and CO [37]. This enzyme is responsible for a key step in the biosynthesis of hydrocarbon copounds, which is ubiquitous in living organism. The aldehyde decarbonylase in the green colonial alga *Botryococcus braunii* consists of α and β subunits (66 and 55 kDa, respectively) and consists of one cobalt-porphyrin per α β pair of subunits [37].

Lysine 2, 3-aminomutase catalyzes the reversible isomerization of l-lysine into l-b-lysine [38], a reaction in which the hydrogen in the 3- pro-*R* position of lysine is transferred to the 2-pro-*R* position of b-lysine and the 2-amino group of lysine migrates to carbon-3 of b-lysine. The enzyme contains three cofactors - pyridoxal phosphate, Fe-S centers and cobalt or zinc. S-Adenosylmethionine is required to activate the enzyme and this activation is thought to take place by a reaction with a reduced metal center, leading to the formation of a reactive adenosyl-metal cofactor. The enzyme purified from cells cultured in media supplemented with $CoCl_2$ contains higher levels of cobalt than that from cells grown in media without added cobalt. The enzyme seems to contain either one or two Co (II) per dimer of subunits and for *Clostridium* SB4, the level of cobalt in the enzyme depends on the concentration of $CoCl_2$ in the growth medium [39]. Electron paramagnetic resonance measurements suggest a high-spin Co(II) in the enzyme and therefore an octahedral co-ordination.

The enzyme does not contain, and is not activated by, vitamin B_{12}. The absence of ^{59}Co-hyperfine splitting in the EPR spectrum of the Fe-S cluster in lysine 2,3-aminomutase rules out the possibility that cobalt and iron coexist within a mixed metal-sulfur cluster [39].

Bromoperoxidase from *Pseudomonas putida* [40], which catalyzes the formation of a carbon-bromine bond in the presence of peroxides, is activated by incubation with cobalt

ions but not with other transition metals such as iron, nickel, zinc and vanadate.The enzyme is dimeric, 68 kDa in size and contains cobalt (0.035 nm ± 0.1 mol.mol enzyme^{-1}), while bromoperoxidase from the marine red alga *Corallina pilulifera* contains non-heme iron, and bromoperoxidase from the fungus *Curvularia inaequalis* contains zinc and iron. The low concentration of cobalt in the *P. putida* enzyme can be explained by the partial dissociation of cobalt from the enzyme during the purification [40]. Although the enzyme is likely to contain cobalt as an essential metal for bromination [40], further studies are required to elucidate its function.

The above studies on native enzymes revealed that most of above the cobalt-containing proteins have bridging group (i.e., carboxylate, thiocyante or phophate) between two cobalt metal centers. Several workers have described cobalt complexes as model for the above enzymes. Some pertinent to present works are given below.

Horrocks et al. [41] reported the complexes of Co (II) and Zn (II) involving monodentate coordination of two alkyl carboxylate and two imidazole ligands in a slightly distorted tetrahedral fashion. Theses complexes have visible and magnetic circular dichroism spectra remarkably similar to the Co (II)-substituted proteolytic enzymes thermolysin and carboxypeptidase. Bauer and Wang [42] synthesized the imidazole-bridged Co (III) pentaamine complexes and studied their interactions with DNA oligonucleotides investigated by circular dichroism (CD) and NMR spectroscopy. CD studies of the titrations of d(A2C15G15T2) with $[(NH_3)_5Co(Im)Co(NH_3)_5]Br_5$ and $[(NH_3)_5Co(Im)_2Co(NH_3)_4]Br_7$ showed that the former metal compound indeed is more effective than $[Co(NH_3)_6]^{3+}$ in inducing the transition from B- to A-DNA. Lee et al. [43] reported the synthesis of a series of dicobalt (II), dicobalt (III), dinickel (II) and dizinc (II) complexes by using m-terphenyl-

derived carboxylate ligands. Structural analysis of these complexes revealed that additional bridging ligands can be readily accommodated within the $[M_2(\mu\text{-}O_2CAr^{Tol})_2]^{2+}$ core, allowing a wide distribution of M...M distances from 2.5745(6) to 4.0169(9) Å.

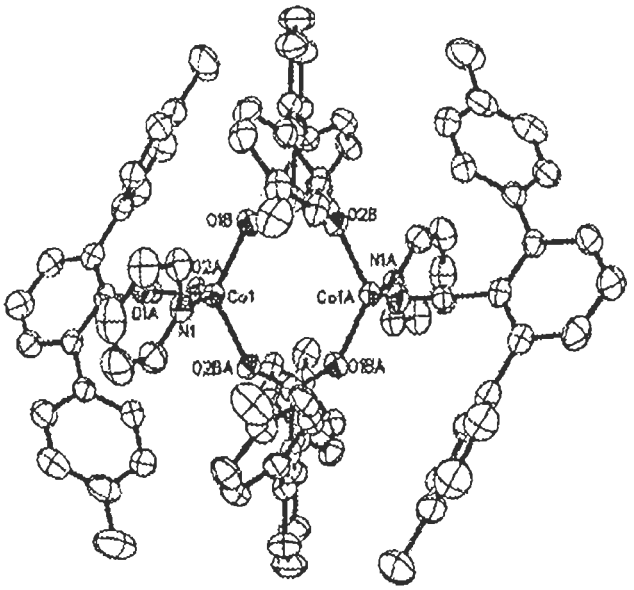


Fig. 3-2 Crystal structure of $[Co_2(\mu\text{-}O_2CAr^{Tol})_2(O_2CAr^{Tol})_2(C_5H_5N)_2]$

Gavrilova et al. [44] reported the crystal structure of dicobalt complex, $[Co^{2+}(\mu\text{-}OH)(oxapyme)Co^{2+}(H_2O)]^{+}$. It contains an unsymmetrical binucleating ligand (oxapyme) which provides five- and six-coordinate metal sites when a hydroxide bridge is introduced. They alos studied the oxygenated product at low temperatures.

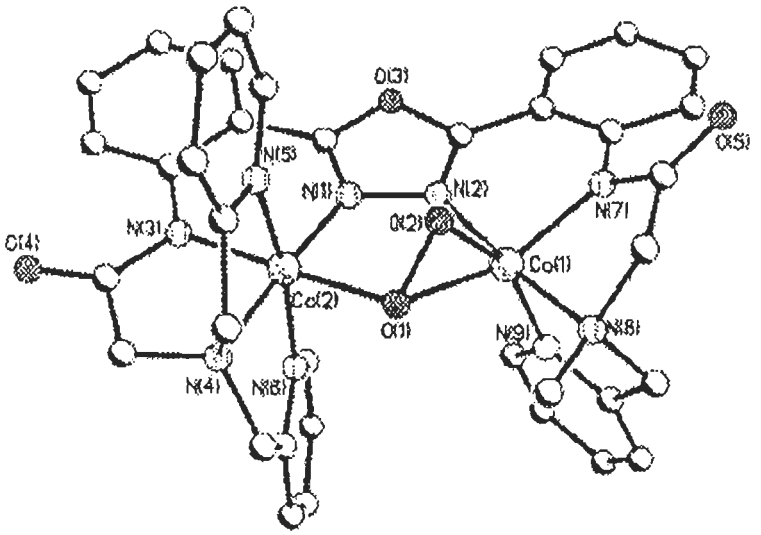


Fig. 3-3 Crystal Structure of [Co (μ,η^1: η^2- O_2)(oxapyme)Co^{2+}]$(PF_6)_2CH_3CN$

Kersting and Steinfeld [45] prepared a series of new dicobalt complexes of the permethylated macrocyclic hexaamine dithiophenolate ligand $H_2L(Me)$ and investigated in the context of ligand binding and oxidation state changes. They also suggested that octadentate ligand is an effective dinucleating ligand that supports the formation of bioctahedral complexes with a central $N_3Co(\mu\text{-}SR)_2(\mu\text{-}X)CoN_3$ core structure, leaving a free bridging position X for the coordination of the substrates.Theacetato- and cinnamato-bridged complexes $[(L^{Me})(Co^{II})_2(\mu\text{-}O_2CMe)]^+$ **2** and $(L^{Me})(Co^{II})_2(\mu\text{-}O_2CCH{=}CHPh)]^+$ **5** were prepared by reaction of the μ-Cl complex $[(L^{Me})Co^{II}2(\mu\text{-}Cl)]^+$ **1** with the corresponding sodium carboxylates in methanol.

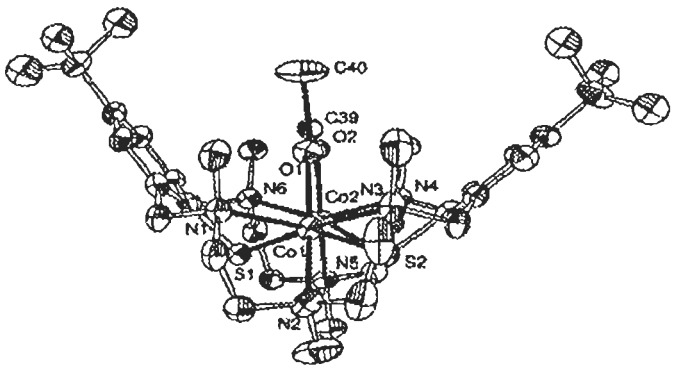
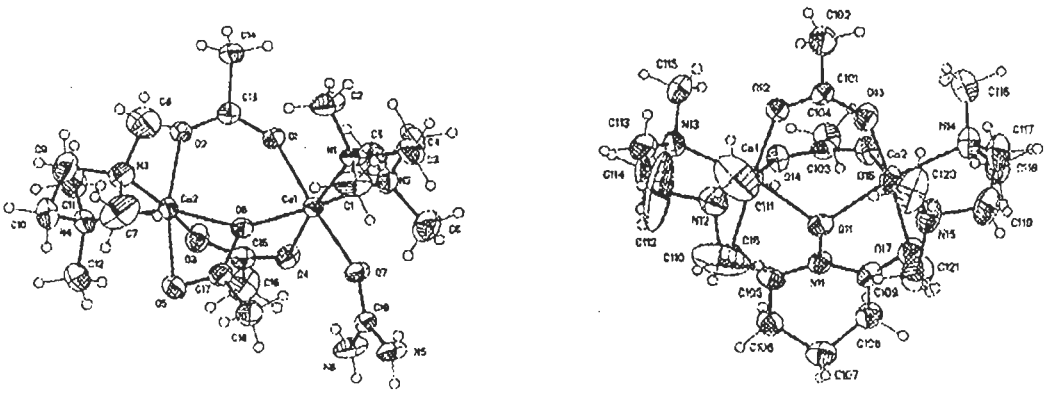


Fig. 3-4 Crystal Structure of $[(L^{Me})Co^{II}2(\mu\text{-}O_2CMe)$ .BPh4.

Bill et al. [46] reported the molecular and electronic structure of four- and five-coordinate cobalt complexes containing two o-phenylenediamine- or two o-aminophenol-type ligands at various oxidation levels. They also performed an experimental, density functional, and correlated ab initio study.Uehara et al.[47] reported xenophilic complexes bearing a Tp^R ligand, [Tp^RM--M'L(n)] [Tp^R=Tp^{iPr2}, Tp ($Tp^{Me2,4-Br}$)); M = Ni, Co, Fe, Mn; M'L(n) = $Co(CO)_4$, $Co(CO)_3(PPh_3)$, $RuCp(CO)_2$]. In these complexes the two metal centers are held together not by covalent interaction but by electrostatic attraction. Hausmann et al. [48] reported the synthesis, structures, and magnetic properties of the complexes [(LMe)Ni_2(mu-L')]$^{n+}$ with L'=NO_3^-, NO_2^-, N_3^-, N_2H_4, pyridazine, phthalazine, pyrazolate, and benzoate. Ruf et al. [49] prepared 3, 5-di-tert-butyl-1,2-semiquinonate (3,5-DBSQ) complexes of Co (II), Cu (II), and Zn (II) that contain the coligand. hydrotris(cumenylmethyl-pyrazolyl)borate ($Tp^{Cum,Me}$ coligand. $Tp^{Cum,Me}$Zn(3,5-DBSQ) and $Tp^{Cum,Me}$Cu(3,5-DBSQ) were prepared by treating the parent hydroxide, $Tp^{Cum,Me}$M(OH), M = Cu and Zn, with 3,5-di-tert-butylcatechol whereas $Tp^{Cum,\ Me}$ Co (3,5-DBSQ) was prepared by a reaction between $(Tp^{(Cum,Me)})_2$Co and 3,5-DBCat. Garcia-Teran et. al [50] reported the reaction of nucleobases (adenine or purine) with a metallic salt in the presence of potassium oxalate in an aqueous solution yields one-dimensional complexes of formulas $[M(\mu\text{-ox})(H_2O)(pur)]_n$ (pur = purine, ox = oxalato ligand ($^{2-}$); M = Cu (II) [1], Co (II) [2], and Zn(II) [3]), $[Co(\mu\text{-ox})(H_2O)(pur)(0.76)(ade)(0.24)](n)_4$ and $([M(\mu\text{-ox})(H_2O)(ade)].2(ade).(H_2O)_n$ (ade = adenine; M = Co(II) [5] and Zn(II) [6]). They also reported their X-ray single-crystal structures, variable-temperature magnetic measurements, thermal behavior, and FT-IR spectroscopy.

Brown et. al [51] reported the magnetic, spectroscopic, and structural studies of dicobalt hydroxamates and model hydrolases.The Co (II) urease model complex [$Co_{(2)}$(μ-

$OAc)_3(urea)(tmen)_2][OTF]$ **2** prepared from the cobalt model hydrolase $[Co_2(\mu\text{-}H_2O)(\mu\text{-}OAc)(OAc)_2(tmen)_2]$ **1** undergoes facile reaction with acetohydroxamic acid (AHA) to give the monobridged hydroxamate complex $[Co_2(\mu\text{-}OAc)_2(\mu\text{-}AA)(urea)(tmen)_2][OTF]$ while **1** gives the dibridged hydroxamate complex $[Co_2(\mu\text{-}OAc)(\mu\text{-}AA)_2(tmen)_2][OTF]$ **4**. The structures and Co-Co distances of the hydroxamate derivatives of **1** and **2** are very close to those of their nickel analogues and suggest that hydroxamic acids can also inhibit cobalt-based hydrolases as well as inhibiting urease.



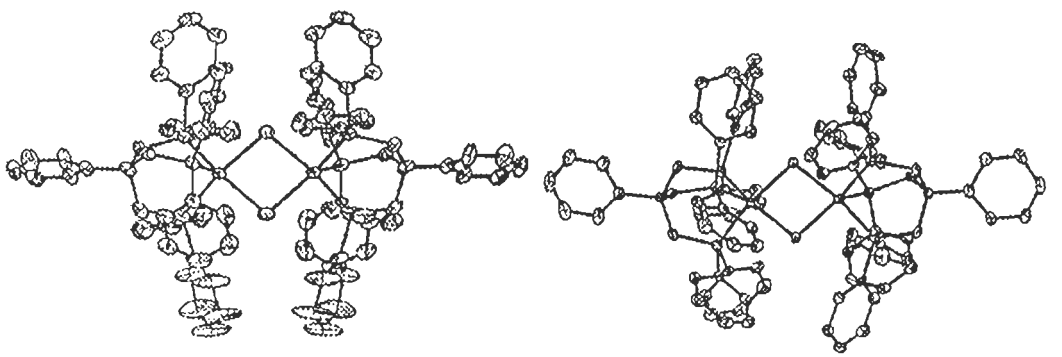
(a) (b)

Fig 3-5 Crystal Structure (a) $[Co(_2)(\mu\text{-}OAc)_3(urea)(tmen)_2[OTF]$

(b) $[Co(_2)(\mu\text{-}OAc)(\ \mu\text{-}\ AA)_2]\ )(tmen)_2[OTF]$.

Ross et. al [52] reported that the reactions of the LCr(III) unit with an insitu prepared $M(PyA)_3^{n-}$ ion, where L represents 1,4,7-trimethyl-1,4,7-triazacyclononane and PyA^- is the monoanion of pyridine-2-aldoxime, yielded heterodinuclear complexes of general formula $[LCr(III)(PyA)_3M]^{(2+/3+)}$ as perchlorate salts, where M = Cr (II), Mn (II), low-spin Fe (II), Ni (II), Cu (II), Zn (II), and low-spin Co (III) (7). These compounds contain three oximato anions as bridging ligands. Sastri et al.[53] reported four new mixed-ligand complexes, namely $[Co(phen)_2(qdppz)]^{3+}$, $[Ni(phen)_2(qdppz)]^{2+}$, $[Co(phen)_2(dicnq)]^{3+}$ and $[Ni(phen)_2(dicnq)]^{2+}$ (phen = 1,10-phenanthroline, qdppz = naptho[2,3-a]dipyrido[3,2-

H:2',3'-f]phenazine-5, 18-dione and dicnq = dicyanodipyrido quinoxaline), were synthesized and characterized by FAB-MS, UV/Vis, IR, 1H NMR, cyclic voltammetry and magnetic susceptibility methods. Absorption and viscometric titration as well as thermal denaturation studies revealed that each of these octahedral complexes is an avid binder of calf-thymus DNA. Higgs et. al [54] reported the synthesis and characterization of a series of edge-sharing octahedral-tetrahedral-octahedral linear trinuclear complexes $[M_3(L1O)_4]^{2+}$, Where M = Mn (II), Co (II), Ni (II), Cu (II)' and Zn(II) and L1OH is the "heteroscorpionate" ligand 2-hydroxyphenyl)bis(pyrazolyl) methane. Jenkins et al. [55] prepared a series of divalent Co (II) complexes supported by the $[PhBP_3]$ ligand ($[PhBP_3] = [PhB(CH_2PPh_2)_3]^-$ to probe certain structural and electronic phenomena that arise from this strong field, anionic tris(phosphine) donor ligand. The solid-state structure of the complex $[PhBP_3]CoI$ **1**, accompanied by SQUID, EPR, and optical data, indicates that it is a pseudotetrahedral Co (II) species with a doublet ground state-the first of its type. Complex 1 provided a useful precursor to the corresponding bromide and chloride complexes, $\{[PhBP_3]Co(\mu\text{-}Br)\}_2$ **2**, and $\{[PhBP_3]Co(\mu\text{-}Cl)\}_2$, **3**. These complexes were similarly characterized and shown to be dimeric in the solid-state.



(a) (b)

Fig. 3-6 Crystal Structure (a) $\{[PhBP_3]Co(\mu\text{-}Br)_2$ (b) $\{[PhBP_3]Co(\mu\text{-}Cl)_2$

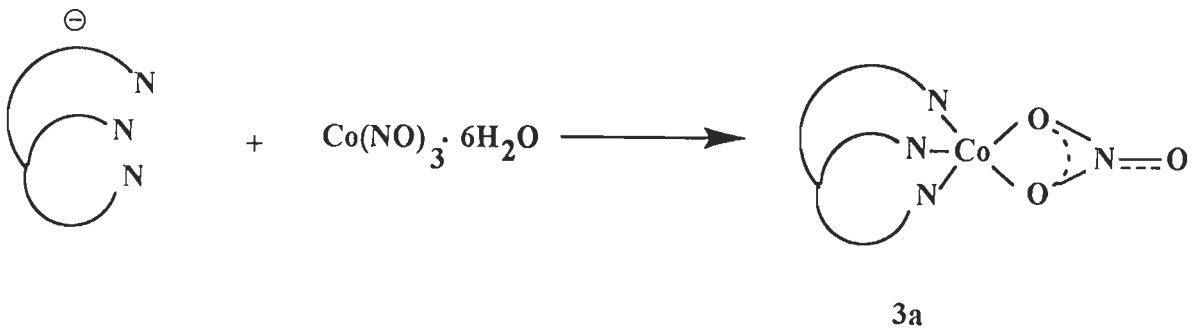
Ware et al. [56] prepared Co (III) complexes $[Co(R_2dtc)_2(L)]^+$ containing two dithiocarbamate ligands (R = Me, Et, pyrrolidine) and a bidentate nitrogen mustard ligand (L) as potential hypoxia-selective cytotoxins. They synthesized the complexes by treatment of the binuclear precursor $[Co_2(R_2dtc)_5]^+$ with the diamine mustards N,N'-bis(2-chloroethyl)ethylenediamine (BCE) and N,N-bis(2-chloroethyl)ethylenediamine (DCE), or their non-alkylating analogues [N,N-diethylethylenediamine (DEE) and N,N'-diethylethylenediamine (BEE)]. Cyclic voltammetry of the complexes in MeCN revealed quasi-reversible behaviour for the Co (III) / Co (II) couple, with E1/2 increasing in the order DCE < DEE approximately BCE < BEE. In MeCN/H_2O electrochemical reduction is irreversible, indicating rapid substitution of H_2O into the coordination sphere of the Co (II) intermediate. Incarvito et al. [57] described the preparation of an unsymmetrical binucleating ligand bearing a bridging oxadiazole ring flanked on one side by three ligands and on the other by four ligands. When bound to two metals, the ligand forms complexes where the metals are in 5- and 6-coordinate sites after the incorporation of an exogenous bridging ligand. Deak et al. [58] synthesized the Co (II) and Ni (II) cupferronato (N-nitroso-N-phenylhydroxylaminato) mixed-ligand complexes of 2-aminopyridine (2-(NH_2)py) $[Co(PhN_2O_2)_2(2\text{-}NH_2py)_2]$ **1** , $[Ni(PhN_2O_2)_2(2\text{-}NH_2py)_2]$ **2** and 2,6-diamino-4-phenyl-1,3,5-triazine (dpt) $[Co(PhN_2O_2)_2(dpt)_2].[Co(PhN_2O_2).2(EtOH)_2]$ **3** and characterized by X-ray diffraction analysis and suggested that the cobalt **1** and nickel **2** complexes are isostructural. Tyler et al. [59] reported the preparation of the two cobalt complexes, namely, $(Et_4N)[Co(PyPepS)_2]$ and $Na[Co(PyPepRS)_2]$ as the first examples of Co (III) complexes with carboxamido nitrogens and thiolato sulfurs as donors as models for Co-containing nitrile hydratase. The average Co (III)-N_{amido} and Co (III)-S distances in these complexes lie in the

range 1.90-1.92 and 2.22-2.24 Å, respectively. Reaction of H_2O_2 with both complexes readily affords $Na[Co(PyPepSO_2)_2]$ and $Na[Co(PyPepRSO_2)_2]$ species in which the thiolato sulfurs are converted to sulfinato (SO_2) groups. Hong et al. [60] prepared two new one-dimensional single azide-bridged metal (II) compounds $[[M(5\text{-methylpyrazole})_4(N_3)]_n](ClO_4)_n(H_2O)_n$ [M = Co (1a), Ni (2a)] by treating an M (II) ion with stoichiometric amount of sodium azide in the presence of four equivalents of the 3, 5-methylpyrazole ligand. The isostructural compounds 1a and 2a crystallize in the monoclinic space group P2(1)/n. The azide bridging ligands have a unique end-to-end coordination mode that brings two neighboring metal centers into a cis-position with respect to the azide unit to form single end-to-end azide-bridged Co (II) and Ni (II) chains. The two neighboring metal atoms at inversion centers adopt octahedral environments with four equatorial 3, 5-methylpyrazole ligands and two axial azide bridges. The present chapter deals with the synthesis and characterisation of binuclear cobalt complexes having different bridging ligands.

Result and Discussion:

The same ligands i.e., 3,5-diisopropylpyrazole **2a** and hydrotris(3,5-diiospropyl-1 pyrazolyl) borate **2b** have been used in present chapter also for the preparation of binuclear cobalt complexes with different bridging ligands. In general, hydrotris(pyrazolyl) borate ligands containing substituents at the 3- and 5- positions of the pyrazole ring form coordinatively unsaturated metal center and provide a vacant site for substrate binding.

The reaction of $Co(NO_3)_26H_2O$ and **2b** gives a mononuclear $[HB(3,5\text{-}iPr_2pz)_3Co(NO_3)]$ complex **3a** (Scheme 3.1).

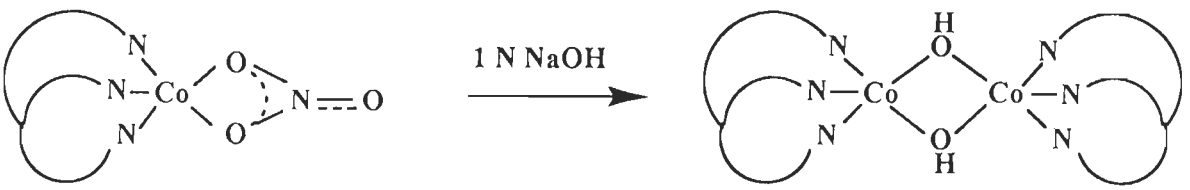


3a

Scheme 3-1

Complex **3a** has also been reported previously by Singh et al. [61] where X-ray studies suggested six coordinated structure. In complex **3a**, cobalt is coordinated by three nitrogen atoms from pyrazolylborate ligand, two oxygen atoms from nitrate group and one nitrogen was from acetonitrile. Coordination of acetonitrile to cobalt is suggested on the basis of IR band observed at 2280 cm^{-1}, attributable to υ(CN) of the coordinated acetonitrile, free acetonitrile gives the band at 2255cm^{-1}. The complex 3a is ideally suited as a starting material for the synthesis of other mononuclear and dinuclear complexes by substitution of the nitrate ion with other anions.

The binuclear hydroxo complex of cobalt 3b has been prepared by the reaction of 3a with 1N aqueous NaOH in toluene (Scheme 3-2)



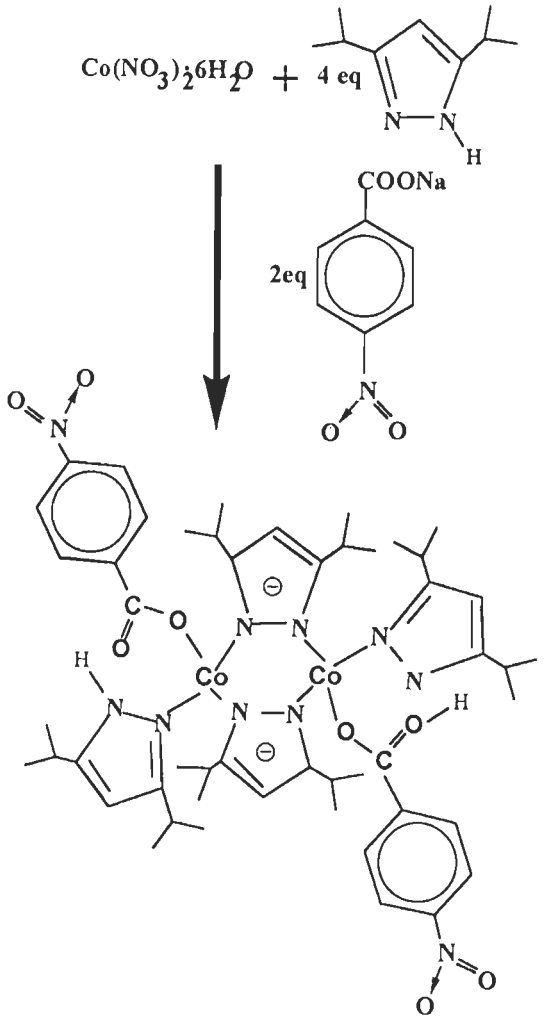
3a **3b**

Scheme 3-2

The crystal structure **3b** has been reported by Kitajima et al. [62] where they have clearly demonstrated the presence of two hydroxo groups bridging both cobalt ions. Complex

3b has a characteristic IR band at 3707 cm^{-1} due to υ(OH).

The $[(3,5\text{-}iPr_2pzH)_2Co_2(\mu\text{-}3,5\text{-}iPr_2pz)_2(OBz\text{-}NO_2)_2]$ complex, **3c** has been prepared by performing the reaction of $Co(NO_3)_2.6H_2O$, 3,5-iPr_2pzH (4.0 equivalent) and sodium nitro benzoate (2.0 equivqlent) in a mixture of dichloromethane and acetonitrile (Scheme 3-3). Single crystal structure of **3c** was obtained by crystallization from acetonitrile at -20^{0}C. Given in figure 3-1 is an Ortep view of **3c**, representing a novel di(μ-pyrazolato) dicobalt core. The selected bond distances and bond angles are summarized in table 3-1. Cobalt-nitrogen bond distances of both bridging pyrazolato are same i.e., Col-N4, 1.986(2) Å; Col-N5, 1.987(2) Å. whereas the cobalt-nitrogen bond distance for terminally coordinated pyrazole are slightly longer (Co1-N2, 2.004(2) Å than the bridging one. As shown in figure 3-1 that both nitrobenzoate groups are coordinated unidentately with cobalt metal ions at Co1-O1 bond distance of 1.943(2) Å. The uncoordinated oxygen atom of both nitrobenzoate group forms hydrogen bond with the hydrogen atom of 3,5-iPr_2pzH (Fig. 3-3). The hydrogen-oxygen bond distance is 2.002(3) Å



3c

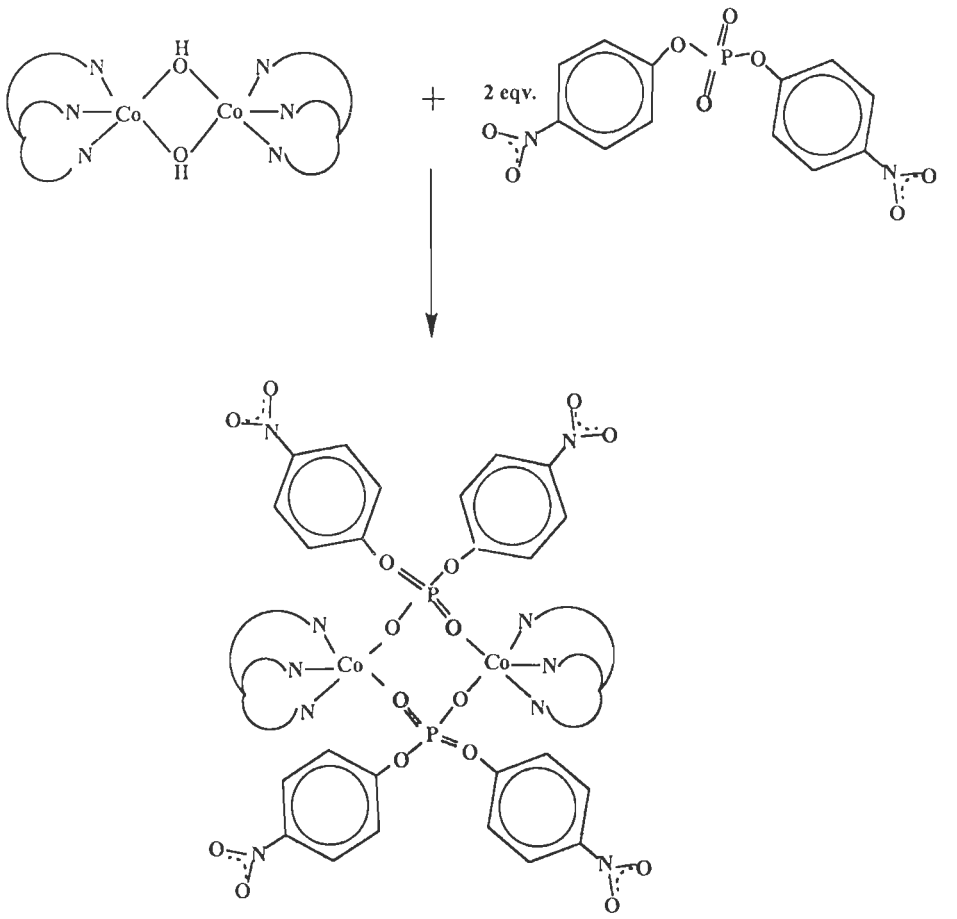
Scheme 3-3

which clearly indicates the presence of hydrogen bonding in complex **3c**. The Col-Col separation in **3c** is 3.597(4) Å which is longer than the metal-metal separation in other binuclear cobalt complexex [63].

We have examined the reaction of binuclear hydroxo complex **3b** with different bridging ligands under various reaction conditions in order to replace one or both hydroxo group from **3b**. The reaction of **3b** with two equivalent of bis(4-nitrophenyl)phosphate hydrate in toluene and methanol under nitrogen yielded a novel di(μ-phosphate) complex 3d (scheme 3-4).

The crystal structure of 3d is presented in figure 3-4 and selected bond lengths and

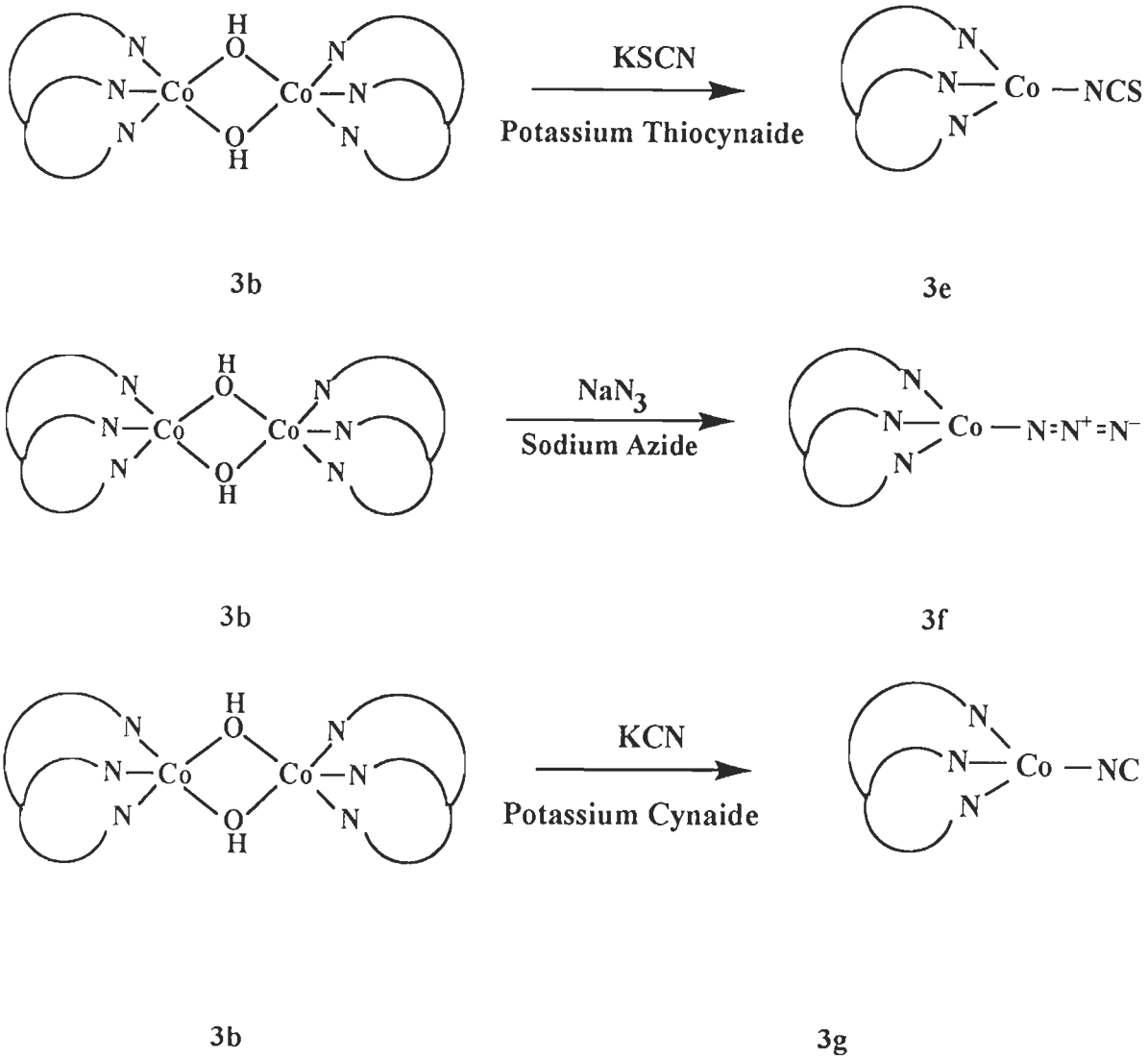
bond angles are given in tables 3-3. As shown in figure 3-4, both cobalt(II) ions are five coordinated with a N_3O_2 ligand-donor set. The coordination geometry of each metal ion is best described as square pyramidal. Although several other metal complexes [63] containing bridging phosphate group are known, to our knowledge this is the first example of cobalt with pyrazolylborate ligand where both cobalt ions are solely bridged with phosphate. The cobalt-cobalt separation in 3d is 5.387(1) Å which is shorter that the similar zinc complex [63]. The cobalt nitrogen bond distances are Col-N11, 2.082(4)Å; Col-N21, 2.086 (3) Å; Co1-N31, 2.098 (3) Å which are very close to other dinuclaer pyrazolylborate complexes [63].



3d

Scheme 3-4

An attempt to replace both hydroxide ligands, the reaction of **3b** with excess of KSCN \ NaN_3 \ KCN (Scheme 3-5) gave **3e**, **3f** and **3g** (Scheme 3-5).



3b **3e**

3b **3f**

3b **3g**

Scheme 3-5

The results including elemental analysis and spectroscopic data suggested that the complexes **3e**, **3f** and **3g** are all mononuclear four coordinated species. During reaction the binuclear hydroxo dissociated and gave the mononuclear complex. The IR spectrum of these

complexes suggested the mondentate coordination of the SCN^-\$N_3^-$\$CN^-$ group to cobalt. The efforts for getting the single crystals of **3e**, **3f** and **3g** are in progress in order to confirm the coordination mode of these groups and geometry of the resultant complexes.

Summary

The reaction of hydrotris(3,5-diiospropyl-1 pyrazolyl) borate **2b** with $Co(NO_3)_2 \cdot 6H_2O$ resulted the formation of complex **3a** which has vacant coordination sites on cobalt for important reactivity studies. The complex **3a** has been used for the preparation of complex **3b** which upon reaction with two equivalent of bis(4-nitrophenyl)phosphate hydrate in a mixture of toluene and methanol gave complex **3d**. The complex **3d** has bridging phosphate group.

In other reaction we have prepared binuclear cobalt complex with pyrazloato bridging **3c** by using 3,5-diisopropylpyrazole as ligand in place of **2b**. In complex **3c**, there is hydrogen bonding between uncoordinated oxygen of nitrobenzoate and hydrogen atom of treminally bonded pyrazole ligand. Both cobalt ions are bridged by pyrazolato ligands.

In other experiments, the reaction of **3b** with two equivalent of KSCN/ NaN_3/ KCN were performed in order to know the binding sites of these biologically important molecules with Co(II) ion and to isolate the binuclear bridging complexes but the resultant complexes **3e**, **3f** and **3g** are mononuclear as suggested by elemental analysis and other data. Further efforts on the X-ray structure determination of these complexes are in progress.

Table 3-1 Selected Bond Distance (Å) and Bond Angles (deg.) for [(3,5-$iPr_2pzH)_2$ $Co_2(\mu$-3,5-$iPr_2pz)_2(OBz\text{-}NO_2)_2$] 3c

Interatomic Bond Distances			
Co1-Co_1	3.597(4)	Co1-O1	1.943(2)
Co1-N2	2.004(2)	Co1-N5	1.987(2)
Co1-N4	1.986(2)	O2-H3N	2.002(3)
Interatomic Bond Angles			
N4-Co1-N_5	111.67(7)	N4-Co1-N2	107.60(7)
O1-Co1-N4	109.24(8)	O1-Co1-N_5	106.86(7)
O1-Co1-N2	114.68(8)	N_5-Co1-N2	106.84(7)

Table 3-2 Crystal Data and Collection Details of $[(3,5\text{-}iPr_2pzH)_2Co_2(\mu\text{-}3,5\text{-}iPr_2pz)_2(OBz\text{-}NO_2)_2]$ 3c

Emperical formula	$C_{50}H_{70}Co_2N_{10}O_8$
Formula weight	1057.02
Crystal System	triclinic
Space Group	P-1(No. 2)
Lattice Parmeters	a = 10.3603(8)Å
	b = 11.3682(8)Å
	c = 13.9441(10)Å
	$\alpha = 67.83(10)^0$
	$\beta = 70.85(10)^0$
	$\gamma = 88.54(10)^0$
Cell volume	$1427.61(18)(Å)^3$
Z_{Value}	1
D_{cal}	1.229 g/cm^3
Diffractometer	Bruker SMART
Radiation	Graphite monochromatic
	$MoK_\alpha(0.71073)$
μ(Mo K_α) / cm^{-1}	0.637
$2\theta_{max}$	27.40
No. of Measured reflection	5272
No. of Observed reflection	4732
No. of parameter refined	324
R(based on F)	0.0410
R_w	0.1163

Table 3-3 Selected Bond Distance (Å) and Bond Angles (deg.) for [[(HB(3,5-$Pr^{i}_{2}pz)_3)Co_2(\mu\text{-}BPNPP)_2]$. CH_2Cl_2 3d

Interatomic Bond Distances			
Co1-Co	5.387(1)	Co1-O1	2.029(3)
Co1-O2	2.017(3)	Co1-N11	2.082(4)
Co1-N21	2.086(3)	Co1-N31	2.098(3)
P1-O1	1.472(3)	P1-O4	1.596(3)
O3-C41	1.386(5)	O4-C51	1.399(5)
Interatomic Bond Angles			
O1-Co1-O2	89.0(1)	O1-Co-N11	103.2(1)
O1-Co1-N21	163.0(1)	O1-Co1-N31	90.6(1)
O2-Co1-N11	104.7(1)	O2-Co1-N21	91.4(1)
O2-Co1-N31	163.7(1)	N11-Co1-N21	93.1(1)
N11-Co1-N31	91.2(1)	N21-C0-N31	84.3(1)
Co1-O1-P1	159.6(2)	Co1-O2-P1	106.9(2)
P1-O3-C41	125.2(3)	P1-O4-C51	122.8(3)
O1-P1-O2	120.6(2)	O1-P1-O3	112.0(2)
O1-P1-O4	104.5(2)	O2-P1-O3	104.2(2)
O2-P1-O4	111.5(2)	O3-P1-O4	102.7(2)

Table 3-4 Crystal Data and Collection Details of $[(HB(3,5\text{-}Pr^{i}_{2}pz)_3)Co_2(\mu\text{-}BPNPP)_2].CH_2Cl_2$ 3d

Emperical formula	$C_{79}H_{110}Co_2N_{16}O_{16}B_2P_2Cl_2$
Formula weight	1812.18
Crystal System	monoclinic
Space Group	P1(No. 2)
Lattice Parmeters	a = 23.978(3) Å
	b = 13.327(4) Å
	c = 14.686(10) Å
	$\alpha = 90(0)^0$
	$\beta = 94.76(1)^0$
	$\gamma = 90(0)^0$
Cell volume	$4677(2)^0(Å)^3$
Z_{Value}	2
D_{cal}	1.29 g/cm^3
Diffractometer	Rigaku AFC-5
Radiation	Graphite- monochromatic
	MoKα (0.71068 Å)
μ(MoKr)/CM^{-1}	5.14
$2\theta max$	50 ^{0}C
No of measured reflections	8973
No of observed reflection	8614
No. of parameter refined	6018
R(based on F)	0.0549
R_w	0.0575

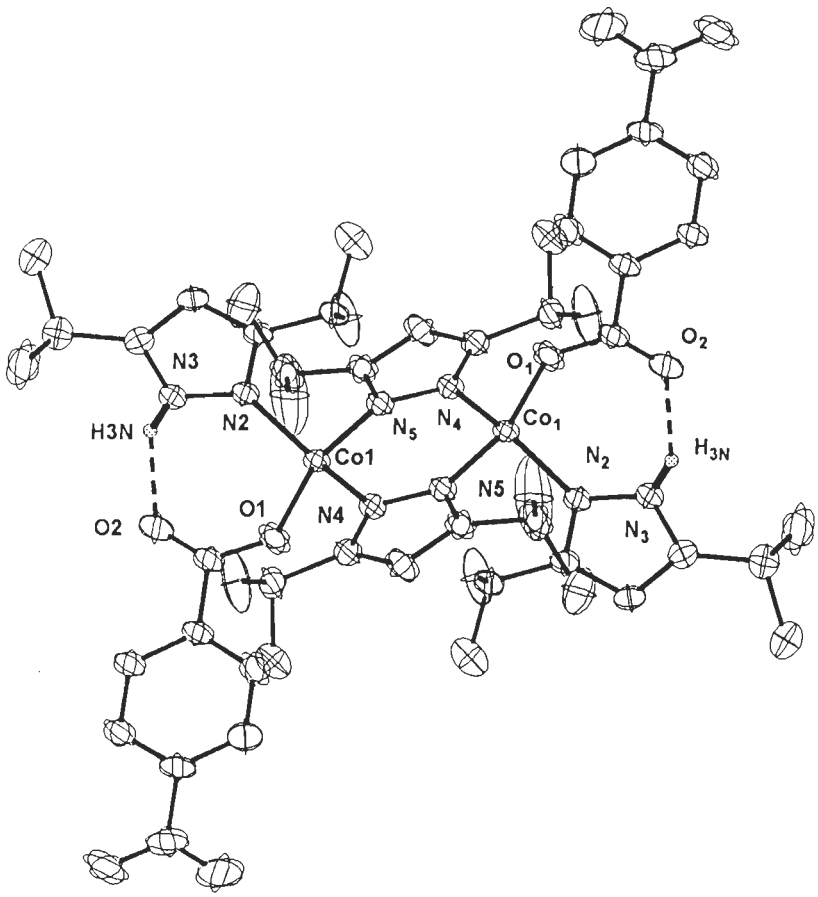


Fig. 3-1 ORTEP view of $[(3,5\text{-}iPr_2pzH)_2Co_2(\mu\text{-}3,5\text{-}iPr_2pz)_2(OBz\text{-}NO_2)_2]$ **3c**

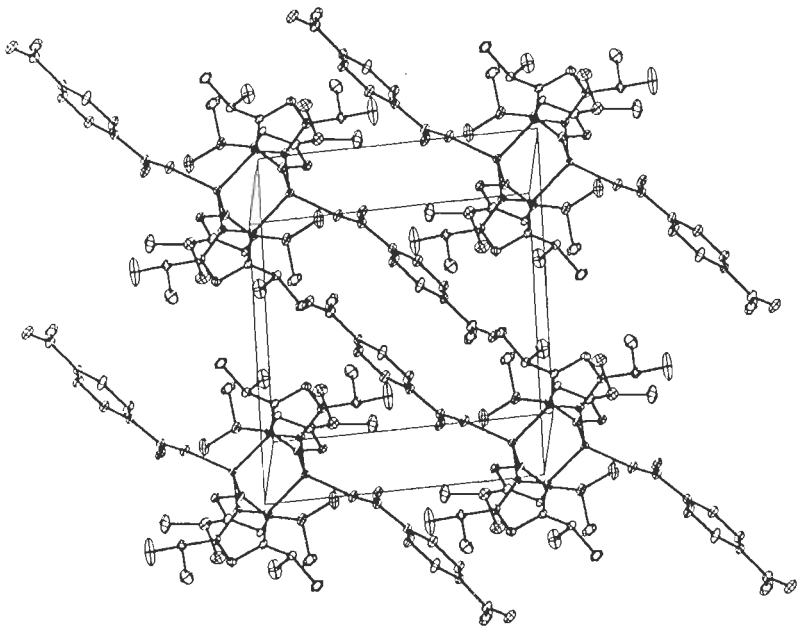


Fig. 3.2 Unit cell of $[(3,5\text{-}iPr_2pzH)_2Co_2(\mu\text{-}3,5\text{-}iPr_2pz)_2(OBz\text{-}NO_2)_2]$ **3c**

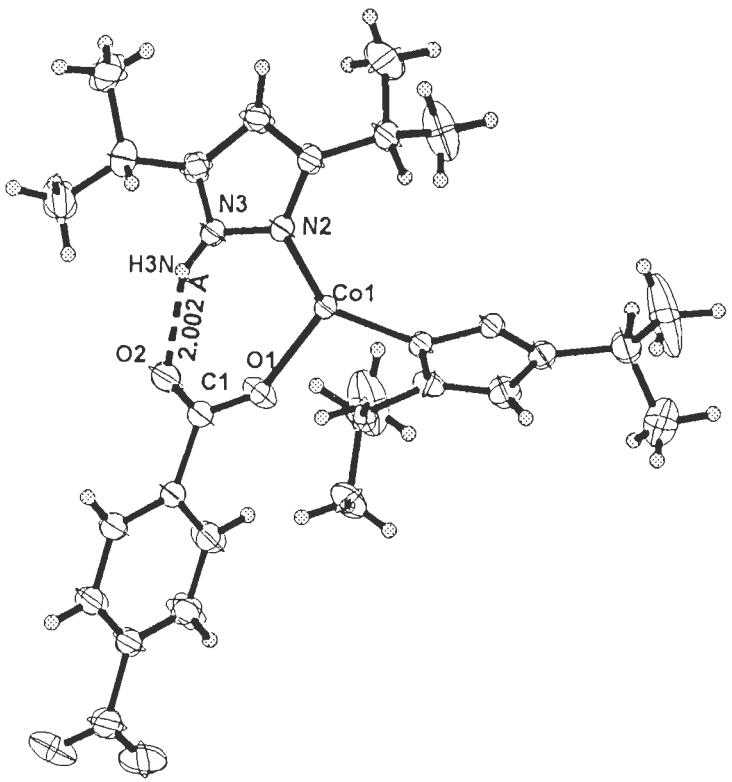


Fig. 3-2 Intramoleclar hydrogen bonding view of $[(3,5\text{-}iPr_2pzH)_2Co_2(\mu\text{-}3,5\text{-}iPr_2pz)_2(OBz\text{-}NO_2)_2]$ **3c**

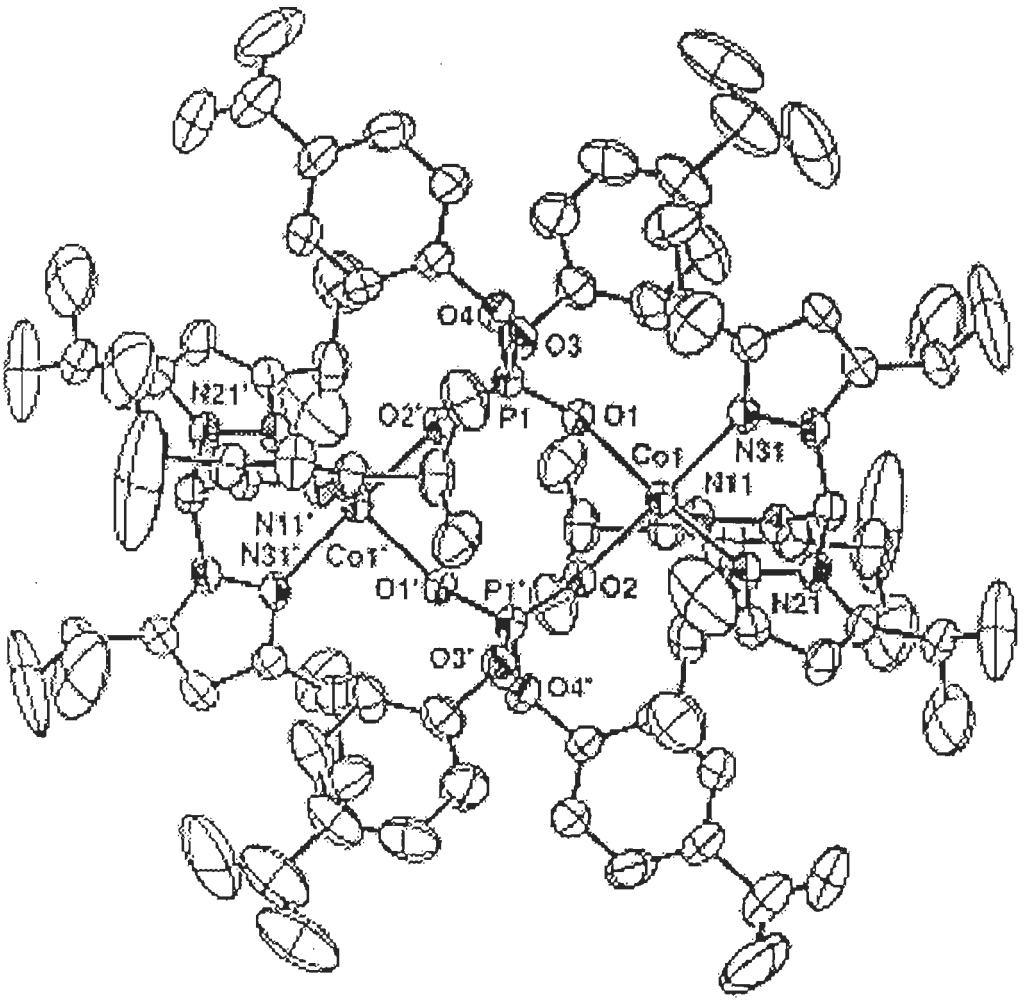


Fig. 3-4 ORTEP view of $[(HB(3,5\text{-}Pr^{i}{}_{2}pz)_3)Co_2(\mu\text{-}BPNPP)_2]$ **3d**

References:

1. Battersby, A. R., "Biosynthesis of vitamin B_{12}", Acc. Chem. Res., **26**, 15 (1993).
2. Maret, W. and Vallee, B. L., "Cobalt as probe and label of proteins", Meth. Enzymol., **226**, 52 (1993).
3. Saxena, K. K., " A note on the effect of cobalt and copper supplementation on the digestibility of organic nutrients and mineral balances in Hariana calves", Indian J. Anim. Sci., **48**, 539 (1978).
4. Saxena, K. K. and Ranjhan, S. K., "Effect of cobalt and copper supplementation, separately and in combination, on the digestibility of organic nutrients and mineral balances in Hariana calves", Indian J. Anim. Sci., **48**, 566 (1978).
5. Saxena, K. K. and Ranjhan, S. K., " A note on the effect of cobalt and copper supplementation on in vivo cellulose digestion by nylon-bag technique in Hariana calves", Indian J. Anim. Sci., **48**, 833 (1978).
6. Ricks, E. L., Brink, N. G., Koniuszy, F. R., Wood, T. R. and Folkers, K., "Crystalline vitamin B_{12}", Science, **107**, 396 (1948).
7. Smith, E. L. and Parker, L. F. G., "Purification of anti-pernicious anaemia factor", Biochem. J., **43**, 8 (1948).
8. Hodgkin, D. C., Kamper, J., Mackay, M., Pickworth, J., Trueblood, K. N. and White, J. G., "Structure of vitamin B_{12}", Nature, **178**, 64 (1956).
9. Bonnett, R., Cannon, J. R., Clark, V. M., Johanson, A. W., Parker, L. F. J., Smith, E. L. and Todd, A. R., "Chemistry of the vitamin B_{12} group. V. The Structure of the chromatographic grouping", J. Chem. Soc., 1158 (1958).

10. Barker, H. A., Weissbach. H. and Smyth, R. D., "A coenzyme containing pseudovitamin B_{12}", Proc. Natl. Acad. Sci. USA, **44**, 1093 (1958).

11. Geno, M. K. and Halpern, J., "Why does nature not use the porphyrin ligand in vitamin B_{12}?", J. Am. Chem. Soc., **109**, 1238 (1987).

12. Pratt, J. M., "The B_{12}-dependent isomerase enzymes; how the protein controls the active site", Chem. Soc. Rev., **14**, 161 (1985).

13. Halpern, J., "Mechanisms of coenzyme B_{12}-dependent rearrangements", Science, **227**, 869 (1987).

14. Bax, A., Marzilli, L. G. and Summers, M. F., "New insights into the solution behaviour of cobalamins. Studies of the base-off form of coenzyme B_{12} using modern two-dimentional NMR methods", J. Am. Chem. Soc., **109**, 566 (1987).

15. Firth, R. A., Hill, H. A. O., Mann, B. E., Prett, J. M., Thorp, R. G. and Williams, R. J. P., "The chemistry of vitamin B_{12}. IX. Evidence for five co-ordinate cobalt (II) complexes", J. Chem. Soc. **A**, 2419 (1968).

16. Toohey, J. I., "A vitamin B_{12} compound containing no cobalt", Proc. Natl. Acad. Sci. USA, **54**, 934 (1965).

17. Sato, K., Shimizu, S. and Fukui, S., "A cobalt-free corrinoid compound in *streptomyces olivaceus*", Biochem. Biophy. Res. Commun., **39**, 170 (1970).

18. Bieganowski, R. and Freidrich, W., "Preparation and some properties of ferribalamin, the Fe (II)-analogue of vitamin B_{12}", FEBS Lett., **97**, 325 (1979).

19. Koppenhagen, V. B., Elsenhans, B., Wagner, F. and Pfiffner, J. J., "Methylrhodibalamin and 5'-deoxyadenosylrhodibalamin, the rhodium analogues of methylcobalamin and cobalamin coenzyme", J. Biol. Chem., **249**, 6532 (1974).

20. Roderick, S. L. and Matthews, B.W., "Structure of the cobalt dependent methionine aminopeptidase from *Escherichia coli*: a new type of proteolytic enzyme", Biochemistry, **32**, 3907 (1993).

21. Bazan, J. F., Weaver, L. H., Roderick, S. L., Huber, R. and Matthews, B. W. , "Sequence and structure comparison suggest that methionine aminopeptidase, prolidase, aminopeptidase P, and creatinase share a common fold", Proc. Natl Acad. Sci. USA, **91**, 2473 (1994).

22. Wilce, M. C., Bond, C. S., Dixon, N. E., Freeman, H. C., Guss, J. M., Lilley, P. E. and Wilce, J. A. "Structure and mechanism of a proline-specific aminopeptidase from *Escherichia coli*." Proc. Natl. Acad. Sci. USA, **95**, 3472 (1998).

23. Kobayashi, M., Nagasawa, T. and Yamada, H. "Enzymatic synthesis of acrylamide: a success story not yet over", Trends Biotechnol., **10**, 402 (1992).

24. Yamada, H. and Kobayashi, M., "Nitrile hydratase and its application to industrial production of acrylamide", Biosci. Biotechnol. Biochem., **60**, 1391 (1996).

25. Kobayashi, M. and Shimizu, S., "Metalloenzyme nitrile hydratase: structure, regulation, and application to biotechnology", Nature Biotechnol., **16**, 733 (1998).

26. Brennan, B. A., Alms, G., Nelson, M., Durney, L. T. and Scarrow, R. C., "Nitrile hydratase from *Rhodococcus rhodochrous* J1 contains a non-corrin cobalt ion with two sulfur ligands", J. Am. Chem. Soc., **118**, 9194 (1996).

27. Payne, M. S., Wu, S., Fallon, R. D., Tudor, G., Stieglitz, B., Turner, I. M. and Nelson, M. J., "A stereoselective cobalt-containing nitrile hydratase", Biochemistry, **36**, 5447 (1997).

28. Katayama, Y., Matsushita, Y., Kaneko, M., Kondo, M., Mizuno, T. and Nyunoya, H., "Cloning of genes coding for the three subunits of thiocyanate hydrolase of *Thiobacillus thioparus* Thi115 and their evolutionary relationships to nitrile hydratase", J. Bacteriol., **180**, 2583 (1998).

29. Ghosh, M., Grunden, A. M., Dunn, D. M., Weiss, R. and Adams, M. W., "Characterization of native and recombinant forms of an unusual cobalt-dependent proline dipeptidase (prolidase) from the hyperthermophilic archaeon Pyrococcus furiosus", J. Bacteriol. **180**, 4781 (1998).

30. Bhosale, S. H., Rao, M. B. and Deshpande, V. V., "Molecular and industrial aspects of glucose isomerase", Microbiol. Rev., **60**, 280 (1996).

31. Takasaki, Y. and Tanabe, O., "Studies on sugar isomerisation enzyme: Production and utilization of glucose isomerase from *Streptomyces* spp" Agric. Biol. Chem., **30**, 1247 (1966).

32. Hemker, J., Kleinschmitdt, L. and Witzel, H., "Glucose isomerase, obviously the first native cobalt enzyme", Recueil des Travaux Chimiques des Pays-Bas, **106**, 350 (1987).

33. Harmon, F. R., Goss, N. H. and Wood, H. G., "Stabilization of the quaternary structure of transcarboxylase by cobalt (II) ions", Biochemistry, **21**, 2847 (1982).

34. Fung, C. H., Mildvan, A. S. and Leigh, J. S. Jr,"Electron and nuclear magnetic resonance studies of the interaction of pyruvate with transcarboxylase", Biochemistry, **13**, 1160 (1974).

35. Reddy, D. V., Rothemund, S., Shenoy, B. C., Carey, P. R. and Sonnichsen, F. D., "Structural characterization of the entire 1.3 S subunit of transcarboxylase from Propionibacterium shermanii", Protein Sci., 7, 2156 (1998).

36. Waldrop, G. L., Rayment, I. and Holden, H. M., "Three-dimensional structure of the biotin carboxylase subunit of acetyl-CoA carboxylase", Biochemistry, **33**, 10249 (1994).

37. Dennis, M. and Kolattukudy, P. E., "A cobalt-porphyrin enzyme converts a fatty aldehyde to a hydrocarbon and CO", Proc. Natl Acad. Sci. USA, **89**, 5306 (1992).

38. Reed, G. H. and Ballinger, M. D., "Characterisation of a radical intermediate in the lysine 2, 3- aminomutase reaction", Meth. Enzymol.", **258**, 362 (1995).

39. Petrovich, R. M., Ruzicka, F. J., Reed, G. H. and Frey, P. A., "Metal cofactor of lysine-2,3-aminomutase", J. Biol. Chem., **266**, 7656 (1991).

40. Itho, N., Morrinaga, N. and Kouzai, T., "Purification and characterization of a novel metal-containing nonheme bromoperoxidase from *Pseudomonas puida"*, biochem. Biophys. Acta, **1207**, 208 (1994).

41. Horrocks, W. D. Jr., Ishley, J. N., Holmquist, B., Thompson, J. S., "Structural and electronic mimics of the active site of cobalt (II)-substituted zinc metalloenzymes", J Inorg. Biochem., **12**, 131(1980).

42. Bauer, C., Wang, A. H., "Bridged cobalt amine complexes induce DNA conformational changes effectively", J. Inorg. Biochem., **68**, 129 (1997).

43. Lee, D., Hung, P. L, Spingler, B. and Lippard, S. J. "Sterically hindered carboxylate ligands support water-bridged dimetallic centers that model features of metallohydrolase active sites", Inorg Chem., **41**, 521 (2002).

44. Gavrilova, A, L., Qin, C. J., Sommer, R. D., Rheingold, A. L. and Bosnich, B., "Bimetallic reactivity. One-site addition two-metal oxidation reaction of dioxygen with a bimetallic dicobalt (II) complex bearing five- and six-coordinate sites", J. Am. Chem. Soc., **124**, 1714 (2002).

45. Kersting, B and Steinfeld, G., "Carboxylate and alkyl carbonate coordination at the hydrophobic binding site of redox-active dicobalt amine thiophenolate complexes", Inorg. Chem., **41**, 1140 (2002).

46. Bill, E., Bothe, E., Chaudhuri, P., Chlopek, K., Herebian, D., Kokatam, S., Ray, K, Weyhermuller, T., Neese, F., and Wieghardt, K., "Molecular and electronic structure of four- and five-coordinate cobalt complexes containing two o-phenylenediamine- or two o-aminophenol-type ligands at various oxidation levels: an experimental, density functional and correlated ab initio study", Chem. Eur. J., **11**, 204 (2004).

47. Uehara, K., Hikichi, S., Inagaki, A. and Akita, M., "Xenophilic Complexes Bearing a Tp^{R} Ligand, [Tp^{R} M--M'L(n)] [Tp^{R}=Tp^{iPr2} , Tp'^{R} ($Tp^{(Me2,4Br)}$)); M=Ni, Co, Fe, Mn; M'Ln=$Co(CO)_4$, $Co(CO)_3(PPh_3)$, $RuCp(CO)_2$]: The two metal centers are held together not by covalent interaction but by electrostatic attraction", Chem. Eur. J., **11**, 2788 (2005).

48. Hausmann, J., Klingele, M.H., Lozan, V., Steinfeld, G., Siebert, D., Journaux, Y., Girerd, J.J. and Kersting, B., "Relisation of unusual ligand binding motifs in the metalated container molecules; synthesis, strucrues and magnetic properties of the complexes $[(LMe)Ni_2(mu\text{-}L')^{n+}$ with L'= NO^{3-}, NO^{2-}, N^{3-},N_2H_4, Pyridazine , pthalazine, pyrazolate and benzoate", Chem. Eur. J., **10,** 1716 (2004).

49. Ruf, M., Noll, B. C., Groner, M. D., Yee, G. T. and Pierpont, C. G., "Pocket semiquinonate complexes of Cobalt (II), Copper (II), and Zinc (II) prepared with the hydrotris(cumenylmethylpyrazolyl)borate ligand", Inorg. Chem., **36**, 4860 (1997).

50. Garcia-Teran, J. P., Castillo, O., Luque, A., Garcia-Couceiro, U., Roman, P. and Lloret, F., "One-dimensional oxalato-bridged Cu (II), Co (II), and Zn (II) complexes with purine and adenine as terminal ligands", Inorg. Chem., **43**, 5761 (2004).

51. Brown, D. A., Errington, W., Glass, W. K, Haase, W., Kemp, T. J., Nimir, H., Ostrovsky, S. M., Werner, R., "Magnetic, spectroscopic and structural studies of dicobalt hydroxamates and model hydrolases", Inorg. Chem., **40**, 5962 (2001).

52. Ross, S., Weyhermuller, T., Bill, E., Wieghardt, K. and Chaudhuri, P., "Tris(pyridinealdoximato)metal complexes as ligands for the synthesis of asymmetric heterodinuclear Cr (III)M species [M = Zn (II), Cu (II), Ni (II), Fe (II), Mn (II), Cr (II), Co (III)]: a magneto-structural study", Inorg. Chem., **40**, 6656 (2001).

53. Sastri, C. V., Eswaramoorthy, D., Giribabu, L. and Maiya, B. G., "DNA interactions of new mixed-ligand complexes of cobalt (III) and nickel (II) that incorporate modified phenanthroline ligands", J. Inorg. Biochem. **94**, 138 (2003).

54. Higgs, T. C, Spartalian, K., O' Connor, C, J., Matzanke, B. F., Carrano, C. J., "Synthesis and characterization of a series of edge-sharing octahedral-tetrahedral-octahedral linear trinuclear complexes $[M_3(L_1O)_4]^{2+}$, Where M = Mn (II), Co (II), Ni (II), Cu (II), and Zn (II) and L_1OH Is the "Heteroscorpionate" Ligand(2-Hydroxyphenyl)bis(pyrazolyl)methane",Inorg Chem., **37**, 2263 (1998).

55. Jenkins, D. M., Di Bilio, A. J., Allen, M. J., Betley, T. A. and Peters, J. C. "Elucidation of a low spin cobalt (II) system in a distorted tetrahedral geometry", J. Am. Chem. Soc., **124**, 15336 (2002).

56. Ware, D. C., Palmer, H. R., Pruijn, F. B., Anderson, R. F., Brothers, P. J., Denny , W. A. and Wilson, W. R., "Bis(dialkyl)dithiocarbamato cobalt (III) complexes of bidentate nitrogen mustards: synthesis, reduction chemistry and biological evaluation as hypoxia-selective cytotoxins", Anticancer Drug Des., **13**, 81 (1998).

57. Incarvito, C., Rheingold, A. L., Gavrilova, A. L., Qin, C. J. and Bosnich, B., "Bimetallic reactivity. One-site addition two-metal oxidation reactions using a di-Co(II) complex of a binucleating ligand with 5- and 6-coordinate sites", Inorg. Chem., **40**, 4101 (2001).

58. Deak, A., Kalman, A., Parkanyi, L. and Haiduc, I., "Hydrogen-bonded hexagonal and pseudo-hexagonal grid motifs in supramolecular cobalt (II) and nickel (II) cupferronato complexes incorporating neutral N-donors with intermolecular NH_2 connectors and solvent molecules", Acta. Crystallogr. B., **57**, 303 (2001).

59. Tyler, L. A., Noveron, J. C., Olmstead, M. M. and Mascharak, P. K. "Co (III) complexes with coordinated carboxamido nitrogens and thiolato sulfurs as models for Co-containing nitrile hydratase and their conversion to the corresponding sulfinato species", Inorg. Chem., **39**, 357 (2000).

60. Hong, C. S., Koo, J. E., Son, S. K., Lee, Y. S, Kim, Y. S. and Do, Y. "Unusual ferromagnetic couplings in single end-to-end azide-bridged cobalt (II) and nickel (II) chain systems",Chem. Eur. J., **7**, 4243 (2001).

61. Singh, U. P., Babbar, P. and Sharma A. K., "Cobalt Complexes Bridged with a (μ-X)(μ – Carboxylato) unit (X = OH, N_3): Synthesis and structural studies", Inorg. Chim. Acta, **271**, 358 (2005).

62. Kitazima, N., Hikichi, S., Tanaka, M. and Moro-oka, Y., "Fixation of atmospheric CO_2 by a series of hydroxo complexes of divalent metal ions and the implication foe catalytic role of metal ions in carbonic anhydrase. Synthesis, Characterisation, and molecular structure of $[LM(OH)_n$ (n = 1 or 2) and $LM(\mu\text{-}CO_3)ML$ (M (II) = Mn, Fe, Co, Ni, Cu, Zn; L = $(HB(3,5\text{-}iPr_2pz)_3)$" J. Am. Chem. Soc. **115**, 5497 (1993).

63. Hikichi, S., Tanaka, M., Moro-oka, Y. and Kitajima, N., "Phosphate ester cleavage with a zinc hydroxide complex. Formation and crystal structure of a dinuclear zinc complex bridged with a phosphate monoester", J. Chem. Soc., Chem. Commun. 814 (1992).

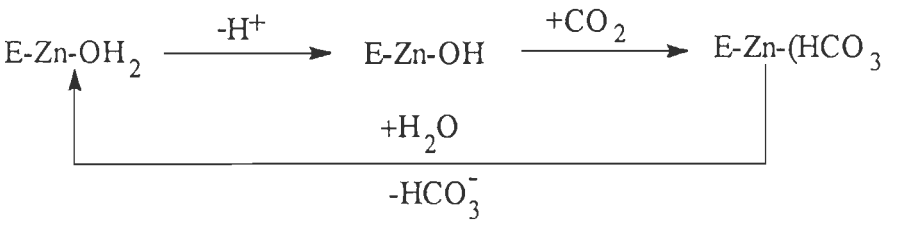
Chapter 4

SYNTHESIS OF SOME HYDROXO COMPLEXES AND THEIR USES IN ESTER HYDROLYSIS

The hydrolysis of ester or amides is an important reaction in many biological systems. Many enzymes that catalyze the hydrolytic cleavage of different ester or amides contain one or two metals ions in their active site. In nature there are many enzyme that hydrolyze phosphate monoesters. Enzymes that hydrolyses phosphate esters include alkaline phosphatase, purple acid phosphatase, inositol monophophatase, and D-fructose 1,6-biphosphate 1-phosphatase etc.. The details about some hydrolytic enzymes are given below.

Purple acid phosphatases (PAPs) are dinuclear metalloproteins containing a Fe (III)-Me (II) core (Me (II) = Fe, Zn or Mn) in their active site [1]. PAPs, which are found in mammals, plants and microorganisms, hydrolyze a wide range of activated *phosphomonoesters*, anhydrides and phosphorylated peptides. Their characteristic purple colour is due to an Tyr-Fe (III) charge transfer transition. Mammalian PAPs contain a diiron center which is active in the mixed-valent Fe (III)-Fe (II) state only. The oxidation state of the divalent iron can be reversibly changed with mild oxidants and reductants, respectively. Strong reductants like dithionite led to reduction of the trivalent iron, resulting in quick loss of an Fe-ion and subsequent denaturation of the protein [2]. It is possible to exchange the divalent iron with zinc [3]. The resulting enzyme is redox-stable, with respect to the divalent site and resembles the well studied PAP from red kidney bean [4].

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 4-1).



Scheme 4-1

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 [5] 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 [6].

The kinetic of the carbonic anhydrase reactions 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.

Carboxypeptidase represents a classic case in bioinorganic chemistry of the application of metal-substitution probes. Because Zn (II) is magnetically and spectroscopically silent, extensive studies of various metal-substituted derivatives have been carried out. The cobalt (II) derivative, in particular, can be prepared from the apoprotein and is more active than the native enzyme in hydrolyzing peptides and equally good at ester hydrolysis.

Carboxypeptidase A, a pancreatic enzyme, cleaves the carboxyl terminal amino acid from a peptide chain by hydrolyzing the amide linkage (Scheme 4-2).

$$\text{. . . Pro-Leu-Glu-Phe} \xrightarrow[\text{carboxypeptidase A}]{H_2O} \text{. . . Pro-Leu-Glu + phenylalanine}$$

Scheme 4-2

The enzyme consists of a protein chain of 307 amino acid residues plus one Zn (II) ion to yield a molecular weight of about 34,600. The molecule is roughly egg-shaped, with a maximum dimension of approximately 5000 pm and a minimum dimension of about 3800 pm. There is a cleft on one side that contains the zinc ion, the active site. The metal is coordinated approximately tetrahedrally to two nitrogen atoms and an oxygen atom from three amino acids (His 69, Glu 72, His 169) in the protein chain. The fourth coordination site is free to accept a pair of electrons from a donor atom in the substrate to be cleaved. The coordination geometry is completed by a water molecule, resulting in pentacoordinate Zn (II). In the presence of substrates or inhibitors, the Glu-72 carboxylate group becomes coordinated in a more nearly unidentate fashion. The ability of Glu or Asp residues to rearrange in such a manner, a process that has been termed the carboxylate shift, allows maintenance of a constant or nearly constant coordination number even when various forms of substrate are bound to the metal. There are hydrogen bonds between the coordinated water molecule and Glu-270. Several arginine and tyrosine residues are positioned in the active site in a way that allows them to participate in substrate binding and activation. The enzyme is thought to act through coordination of the zinc atom to the carbonyl group of the amide linkage. In addition, a nearby hydrophobic pocket envelops the organic group of the amino acid to be cleaved and those amino acids with aromatic side groups react most readily. Accompanying these events is a change in conformation of the enzyme. The arginine side chain moves about 200 pm closer to the carboxylate group of the substrate and the phenolic group of the tyrosine comes within hydrogen bonding distance of the imido group of the C-terminal amino acid, a shift of 1200 pm [7]. The hydrogen bonding to the free carboxyl group (by

arginine) and the amide linkage (by tyrosine) not only holds the substrate to the enzyme but helps in breaking the N–C bond. Nucleophilic displacement of the amide group by an attacking carboxylate group from a glutamate group could form an anhydride link to the remainder of the peptide chain. Hydrolysis of this anhydride could then complete the cycle and regenerate the original enzyme. More likely, the glutamate acts indirectly by polarizing a water molecule that attacks the amide linkage.

Alkaline Phosphatase cleaves phosphate monoester non-specifically. This enzyme contains two zinc ions at the active site. The catalytic role of zinc ions is ascribed to the binding and activation of substrates, whereas deprotonation of co-ordinated water to produce a nucleophilic zinc hydroxide is also proposed as an essential catalytic hydrolysis function. Other function of this enzyme is to transfer a phosphate ester to the external alcohol [8]. In alkaline phosphatase, a pair of zinc (II) ions binds the terminal phosphate group of a substrate, typically a monoester such as p-nitrophenyl phosphate. A serine hydroxyl group at the active site then attacks the phosphoryl group, cleaving the ester functionality; in the process, the phosphate is transferred to the enzyme, forming a phosphorylserine residue. Hydrolysis of this phosphate ester by coordinated hydroxide ion completes the catalytic cycle. The pair of zinc ions serve as a general Lewis acid, polarizing the substrate and rendering it a better electrophile. Positioning of the phosphate ester at the active site by the arginine residue is analogous to Arg-145 facilitated terminal carboxyl orientation of substrate in carboxypeptidase. Interestingly, in alkaline phosphatase, there is a third metal, a magnesium ion, within ~ 5.0 Å of one zinc and 7.0 Å of the other. Although this metal ion does not appear to participate directly in

the catalytic mechanism, it may contribute by shaping the structure and the electrostatic potential of the active site.

The Bacterial Phosphotriesterase (PTE) catalyzes the hydrolysis of organophosphates with turnover numbers that approach the diffusion-controlled limit for the best substrates. The structure of this protein has been determined to high resolution by X-ray crystallography. A coupled binuclear metal complex of two zinc ions has been shown to be required for the delivery of the hydroxide to the phosphorus center during substrate turnover. Site directed mutagenesis has been used to systematically alter the substrate and stereochemical specificity of this enzyme. The wild-type enzyme hydrolyzes the P-F bond of diisopropyl fluorophosphate (DFP) with a turnover number of 41 per second. Mutagenesis of two phenylalanine residues within the leaving group pocket to histidine residues and replacement of the Zn (II) with Co (II) enhances the turnover of DFP by a factor of 80. Ethyl p-nitrophenyl phosphate is hydrolyzed by the native enzyme 100,000 times more slowly than the triester, diethyl p-nitrophenyl phosphate. The diminished activity of the diester can be partially rescued by the addition of small alkyl amines to the reaction medium. These results suggest that the amine binds to the active site and neutralizes the negative charge of the diester.

The biological importance of phosphate transfer, specifically by zinc enzymes, has attracted many coordination chemistry research groups. Numerous mechanistic studies [9] and model complexes [10] have been published. Similar studies related to the metabolism of phosphate-bearing substrates have been less popular among chemists so far. 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 [11, 12]. Literature also revealed that in most of the hydrolytic enzyme the mononuclear hydroxo is the active species. However the structurally well characterized mononuclear hydroxo complexes are limited. As one of the approaches to mimic the functions played by the metal ion in hydrolytic enzymes, various types of metal complexes have been designed [13-47]. Some of the recent works have been described below.

Koerner and Brown [48] reported the the synthesis of a tris(4,5-di-n-propyl-2-imidazolyl)phosphine-Zn^{2+} complex and its uses in the hydrolysis of an activated ester. Albedyhl et al. [49] synthesized dinuclear zinc (II) - Iron (III) and iron (II) - iron (III) complexes as models for purple acid phosphatases. They used these complexes in cleavage of 2-hydroxypropyl p-nitrophenyl phosphate (HPNP) and found that the diiron complex from BPMOP (HBPMOP = 2,6-bis[(bis(2-pyridylmethyl)amino)methyl]-4-methoxyphenol) is 4-fold more reactive than the homologous complex from BPMP (methyl analog of BPMOP). Xia et al.[50] performed the complexation study on the Zn (II) complexes of asymmetric tripodal ligand, 2-[bis(2-aminoethyl)amino]ethanol (L) and suggested that the alcoholic OH group of complex ZnL exhibits remarkable acidity with a very low pKa value of 7.7 at 25 ^{0}C. Ibrahim et al. [51] synthesised tren based zinc (II) complexes as structural phosphatase models for phosphoester hydrolysis. Su et al. [52] reported the synthesis of Zn (II) complexes of six new functionalized phenanthrolines and examined as catalysts for the hydrolysis of 4-nitrophenyl acetate (NA). Verge et al. [53] designed new μ-oxo-diferric complexes for hydrolysis of phosphodiesters as a model for the diiron active site of purple acid phosphatase. Kou et al. [54] studied the comparative kinetics of carboxylic esters hydrolysis catalyzed by the zinc (II) complex of a

macrocyclic Schiff base ligand and suggested that the difference in hydrolytic rates may be attributed to the difference of hydrolytic mechanisms by which the PNPP (para – nitrophenyl piconilate) and PNPA (para - nitrophenyl acetate) operate. Um et al. [55] studied the kinetics and mechanism for the reaction of 4-nitrophenyl 2-thiophene carboxylate with secondary alicyclic amines by measuring the second-order-rate constants (kN) spectrophotometrically. Suh et al. [56] performed the catalysis studies of binuclear zinc ions in ester hydrolysis and suggested that the Zn (II) catalyzed hydrolysis of 6-carboxy-O-(acetyloxy)-2-pyridinecarboxaldoxim consists of two reaction paths. They also found that rates of the paths were proportional to [Zn(II)][OH^-] or to [Zn(II)][OH^-]* but they did not observed catalysis by the Cu (II) ion in the hydrolysis of same ester. Ye et al. [57] reported the formation of a novel tetranuclear iron (III) complex $[Fe_2(tren)_2(\mu\text{-}O)]_2(\mu_4\text{-}PO_4][ClO_4]_5.2.5H_2O$ [tren = tris(2-aminoethyl)-amine] by reacting 4-nitrophenyl phosphate and a methanolic solution of Fe^{III} and tren. The X-ray structure as given below shows that all the four iron atoms are linked by $\mu_4\text{-}PO_4^{3-}$ which is hydrolytic product from 4-nitrophenyl phosphate.

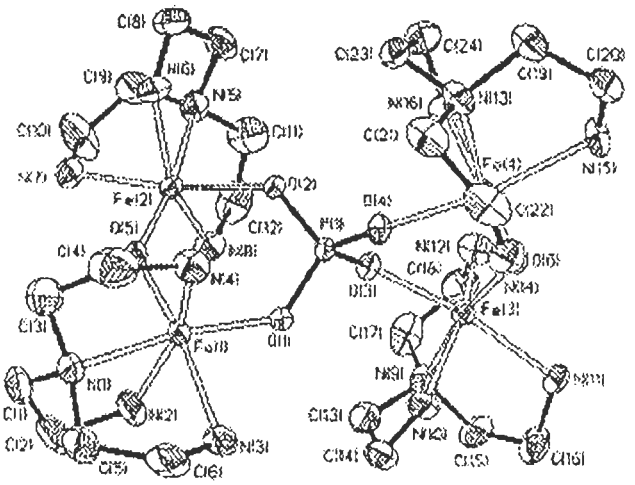


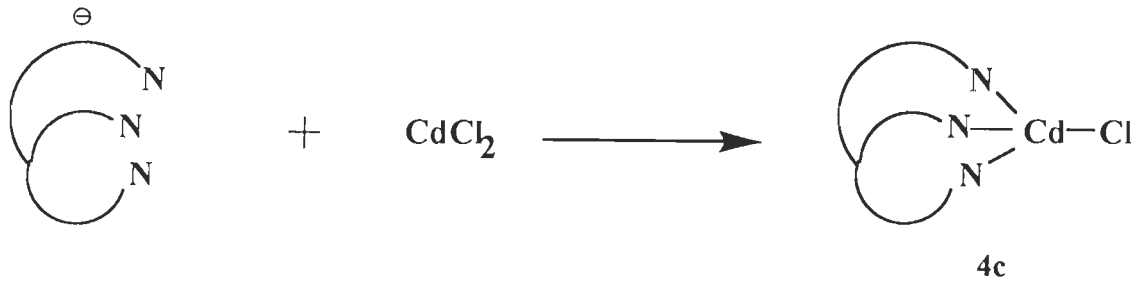
Figure 4.1 $[Fe_2(tren)_2(\mu\text{-}O)]_2(\mu_4\text{-}PO_4][ClO_4]_5.2.5H_2O$

Yin et al. (58) performed the studies on artificial hydrolytic metalloenzymes by catalyzing carboxylic ester hydrolysis using cobalt (II), copper (II) and zinc (II) complexes of the tripod ligand, tris(2-benzimidazylmethyl)amine. The result indicated that the hydrolytic metalloenzyme activity of different metal complexes increases with the electrophilicity of the metal ions. Wahnon et al. [59] reported the hydrolysis of a phosphate diester doubly coordinated to a dinuclear cobalt (III) complex and reported a novel mechanism for this reaction. Hegg et al. [60] investigated the ability of a series of three related copper complexes to hydrolyze the activated phosphodiesters, bis(4-nitrophenyl) phosphate (BNPP) and ethyl 4-nitrophenyl phosphate (ENPP). They also suggested that as the ligand size increases, the dimer formation constant (K(f)) decreases due to steric constraint, thereby increasing the concentration of active species and hence the rate of hydrolysis. Jancso et al. [61] reported the phosphodiester cleavage of ribonucleoside monophosphates and polyribonucleotides by homo- and heterodinuclear metal complexes of a cyclohexane-based polyamino-polyol. Echizen et al. [62] reported the nucleophilic reaction by carbonic anhydrase model zinc compound and characterization of intermediates for CO_2 hydration and phosphoester hydrolysis. Yashiro et al. [63] reported the synthesis of some dinuclear Zn^{2+}complexes in the hydrolysis of the phosphodiester linkage in a diribonucleoside monophosphate diester. Knight et al. [64] reported the synthesis of carboxylic acid functionalized cobalt (III) cyclen complexes for catalytic hydrolysis of phosphodiester bonds. Hix et al. [65] reported the synthesis and structural characterization of $Zn(O_3PCH_2OH)$, a new microporous zinc phosphonate formed by reaction of zinc acetate with diethyl hydroxymethylphosphonate and suggested that the acidity of the zinc solution affects the hydrolysis of the

phosphonate to produce phosphonic acid *insitu*. Frey et al. [66] performed the hydrolysis of unactivated esters and acetonitrile hydration by a hydroxo-dicopper (II) complex. Kinoshita et al. [67] investigated the recognition of phosphate monoester dianion by an alkoxide-bridged dinuclear zinc (II) complex (Zn_2L^{3+}) (L = alkoxide species of 1,3-bis[bis(pyridin-2-ylmethyl)amino]propan-2-ol). The X-ray crystal analysis of the dizinc (II) complex with p-nitrophenyl phosphate showed that the phosphate dianion binds as a bridging ligand to the two zinc (II) ions. Santana et al. [68] prepared the bis(phosphate diester)-bridged complexes $[\{Ni([12]aneN_3)(\mu\text{-}O_2P(OR)_2)\}_2](PF_6)_2$ {[12]aneN$_3$=Me$_3$[12] aneN$_3$, 2,4,4-trimethyl-1,5,9-triazacyclododec-1-ene; R=Me 1, Bu 2, Ph 3, Ph-4-NO_2 4; [12]aneN$_3$=Me$_4$[12]aneN$_3$, 2,4,4,9-tetramethyl-1,5,9-triazacyclododec-1-ene; R=Me **5**, Bu **6**, Ph 7, Ph-4-NO_2 (**8**)} by hydrolysis of the phosphate triester with the hydroxo complex $[\{Ni([12]aneN_3)(\mu\text{-}OH)\}_2](PF_6)_2$ or by acid-base reaction of the dialkyl or diaryl phosphoric acid and the above hydroxo complex. Xie et al. [69] performed the studies on the reaction kinetics and the mechanism of hydrolysis of bis(4-nitrophenyl) phosphate (BNPP) catalyzed by oxamido-bridged dinuclear copper (II) complexes in micellar solution. Ge et al. [70] performed potentiometric determination for knowing the role of trinuclear Zn (II) complexes in promoting the hydrolysis of 4-nitrophenyl acetate and suggested that trinuclear Zn (II) complexes of the four tripods 1,3,5-tri(2′,5′-diazahexyl)benzene (L1), 1,3,5-tri(2′,5′-diazaheptyl)benzene (L2), 1,3,5-tri(2′,5′-diazaoctyl)benzene (L3), and 1,3,5-tri(2′,5′-diazanonyl)benzene (L4) could be potential hydrolytic catalysts. The present chapter deals with the synthesis and characterisation of some hydroxo complexes. After characterisation these complexes have been used in hydrolysis of different esters.

Results and Discussion

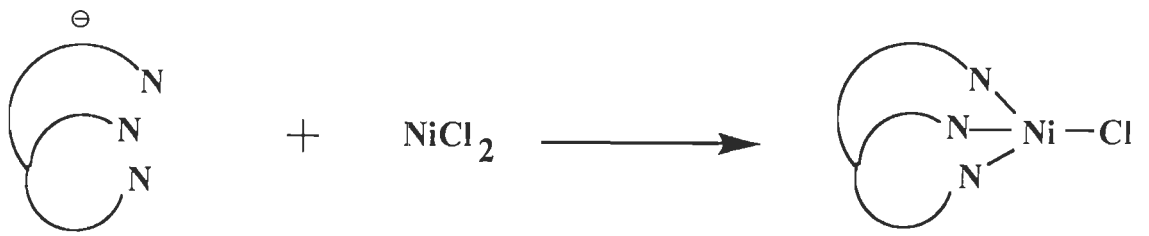
The reaction between cadmium (II) and **4b** in a mixture of dichloromethane and methanol resulted the formation of **4c** complex in 75% yield (Scheme 4.1)



4c

Scheme 4.1

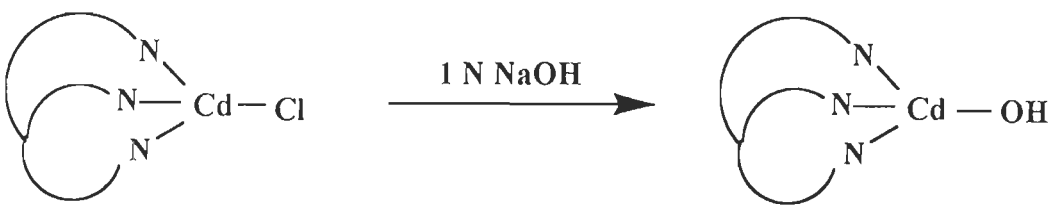
It was not possible to get the crystal structure of **4c** but the elemenatl analysis data and other spectroscopic data including FD-MS suggested four coordinated structure for **4c**. The complexes **4d** and **4e** have been prepared in similar method as applied for complex **4c** (Scheme 4.2) and their characterization studies also suggested four coordinated structure for the complexes **4d** and **4e**.



4d, 4e

Scheme 4.2

The complex **4c, 4d and 4e** were used as a starting material for the preparation of hydroxo complex. The treatment of **4c** and **4e** with 1N aqueous solution of NaOH in toluene yielded the hydroxo complex **4g** and **4h** (Scheme 4.3)

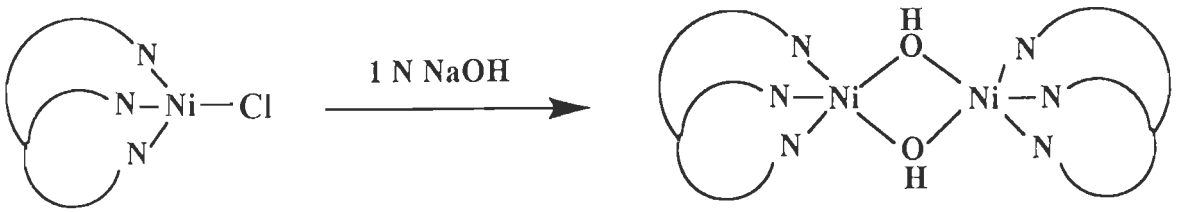


4c **4h**

Scheme 4.3

Efforts were made to iobtain the single crystals of **4g** and **4h** suitable for X-ray analysis but were not successful. The elemental analysis and other spectroscopic data including FD-MS suggested that these complexes are four coordinated with possibly tetrhedral geometry. As shown in scheme 4.3, complex **4h** has one Cadmium center coordinated by three nitrogen atoms of the ligand and the fourth position is occupied by hydroxo group. The further more evidence for the presence of the hydroxo group was provided by the appearance of a band at 3674 cm^{-1} in **4g** and 3674 cm^{-1} in the IR spectrum of complex **4h** attributable to OH stretching. The mononuclear terminal hydroxo cobalt (II) [71] has been prepared with same **4b** ligand suggested that the ligand **4b** due to its more sterically hindered nature gives mononuclaer hydrox complexes. On the other hand, the formation of only dinuclear μ-hydrox complexes were observed with less sterically hindered ligand, $HB(3,5\text{-}iPr_2pz)_3$ [72].

The complex **4d** on treatment of with 1N aqueous solution of NaOH in toluene yielded the binuclear nickel (II) hydroxo complex **4f** (Scheme 4.4).



4d **4f**

Scheme 4.4

The structure of **4f** has already been reported by Kitajima et al.[72]. They have suggested square pyramidal geometry around each nickel ion. As shown in scheme 4.4, each nickel is five coordinate with N3O2 donor set and the complex 4f has νOH at 3702 cm^{-1}.

As reported in literature that mononuclear hydroxo complexes are catalytically active species in various metal containing hydrolytic enzymes but well characterized mononuclear hydroxo complexes are very limited to our knowledge. X-ray structures of Fe (II), Cu (II) and Zn(II) mononuclear hydroxo complexes are only available in the literature [73a, b, c]. In order to asses the catalytic abilities of these

synthesized hydroxo complexes, **4f, 4g** and **4h** were tested for their catalytic in ester hydrolysis. The catalytic properties of these complexes in ester hydrolysis were monitored spectrophotometrically

The Complexes **4f**, **4g** and **4h** were used as catalysts in various ester hydrolysis namely, tris(4-nitrophenyl)phosphate,4-nitrophenylpropionate, 4-nitrophenylcaprylate, 4-nitrophenylleurate and 4-nitrophenyltrifluoroacetate. The course of ester's cleavage was monitored spectrophotometrically by the appearance of 4-nitrophenolate anion. The rate constants were calculated by using the plots between log (A_{int}-A_t) versus time and provided estimates of the pseudo-first order rate constants.

The rate of the generation of 4-nitrophenolate (at 360nm) was increasing with time. A prolonged (time vary from ester to ester) reaction at room temperature did not change the spectra, indicating that no more hydrolysis occurs (Fig. 4-1(a) – 4-12(l)). Similar type of ester hydrolysis by using metal-bound hydroxo species has been performed by several workers using different metal ions [74-82]. But to the best of our knowledge, no literature is available on the use of mononuclear hydroxo complexes of nickel (II) and cadmium(II) as a catalyst in ester hydrolysis. From the tables 4.1 to 4.9, it is clears that the rate of ester hydrolysis vary from ester to ester and also from catalyst to catalyst. The rate of the hydrolysis of esters used in present chapter are greater with binuclear hydroxo nickel complexes in comparison to mononuclear nickel and cadmium hydroxo complexes. The efforts were also made to observe the rate with different concenntration of ester. And we also observe the rate constant of different mono nuclear and bi-nuclear hydroxo with the esters (See in Table 4-10).

Table 4-1 Pseudo First order rate constants for tris(4-nitrophenyl)phosphate by **4h** at different concentration

Ester Concentration (Mol/Liter)	Rate Constant (K_{obs}/S^{-1})
0.000138	0.0053
0.000276	0.0088
0.000415	0.009
0.000553	0.0138
0.000691	0.0238

Table 4-2. Pseudo First order rate constants for 4-nitrophenylpropionate by **4h** at different concentration

Ester Concentration (Mol/Liter)	Rate Constant (K_{obs}/S^{-1})
0.000138	0.0051
0.000276	0.004
0.000415	0.0044
0.000553	0.005
0.000691	0.0055

Table 4-3. Pseudo first order rate constants for 4-nitrophenylcaprylate by **4h** at different concentration

Ester Concentration (Mol/Liter)	Rate Constant (K_{obs}/S^{-1})
0.000138	0.005
0.000276	0.0053
0.000415	0.0048
0.000553	0.0045
0.000691	0.0051

Table 4-4. Pseudo first order rate constants for 4-nitrophenyllaurate by **4h** at different concentration

Ester Concentration (Mol/Liter)	Rate Constant (K_{obs}/S^{-1})
0.000138	0.0039
0.000276	0.0087
0.000415	0.0052
0.000553	0.0043
0.000691	0.0047

Table 4-5. Pseudo first order rate constants for (4-nitrophenyl)trifluoroacetate by **4h** at different concentration

Ester Concentration (Mol/Liter)	Rate Constant (K_{obs}/S^{-1})
0.000144	0.0048
0.000287	0.0053
0.000430	0.0050
0.000573	0.0041
0.000716	0.0046

Table 4-6. Pseudo First order rate constants for 4-nitrophenylpropionate by **4f** at different concentration

Ester Concentration (Mol/Liter)	Rate Constant (K_{obs}/S^{-1})
0.0003	0.00727
0.0004	0.01887
0.00045	0.016332
0.00048	0.01311

Table 4-7. Pseudo First order rate constants for 4-nitrophenylcaprylate by **4f** at different concentration

Ester Concentration (Mols/Liter)	Rate Constant (K_{obs}/S^{-1})
0.0000087	0.00513
0.0000174	0.00157
0.00002615	0.0000207
0.00004358	0.002483

Table 4-8. Pseudo first order rate constants for 4-nitrophenyllaurate by **4f** at different concentration

Ester Concentration (Mol/Liter)	Rate Constant (K_{obs}/S^{-1})
0.0000087	0.00474
0.0000174	0.005
0.00002615	0.0061
0.0000349	0.005
0.0000435	0.003

Table 4-9. Pseudo first order rate constants for (4-nitrophenyl)trifluoroacetate by **4f** at different concentration

Ester Concentration (Mol/Liter)	Rate Constant (K_{obs}/S^{-1})
0.000053	0.006909
0.000107	0.007642
0.000161	0.007642
0.000215	0.00732
0.000268	0.00681

Figure 4-1 Dependencies of K_{obs} for the hydrolysis of tris(4-Ntrophenyl)phosphate by **4h** at different concentration

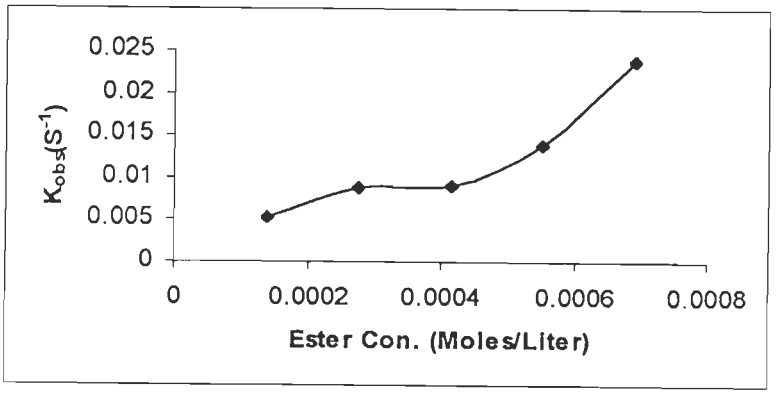


Figure 4-2 Dependencies of K_{obs} for the hydrolysis of 4-Ntrophenylpropionate by **4h** at different concentration

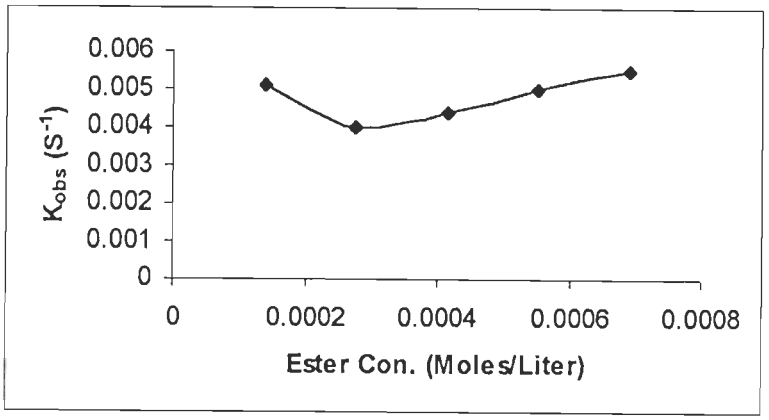


Figure 4-3 Dependencies of K_{obs} for the hydrolysis of 4-Nitrocaprylate by **4h** at different concentration

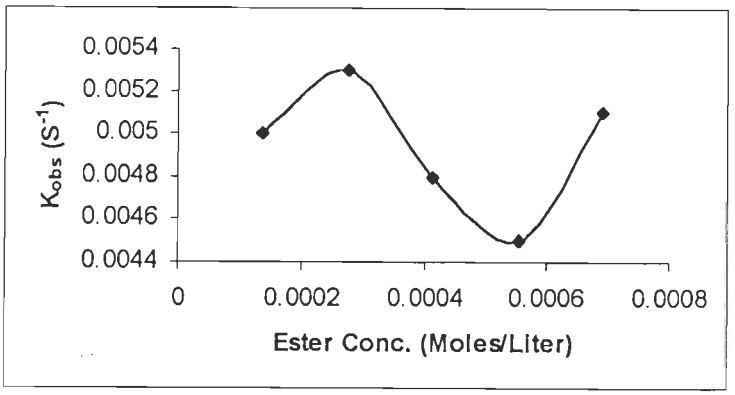


Figure 4-4 Dependencies of K_{obs} for the hydrolysis of 4-Nitroleurate by **2h** at different concentration

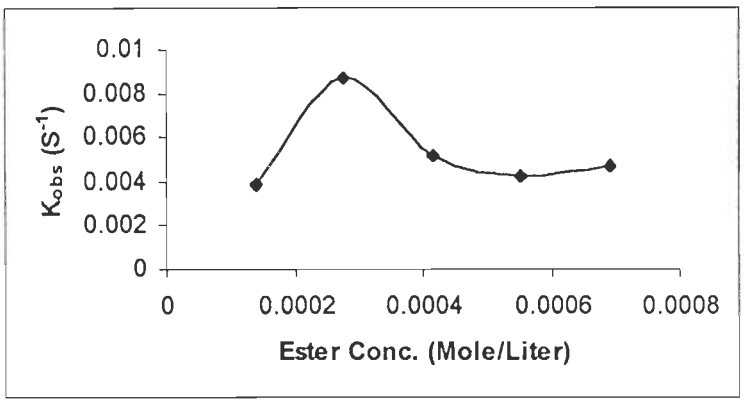


Figure 4-5 Dependencies of K_{obs} for the hydrolysis of 4-Ntrophenyltrifluoroacetate by **2h** at different concentration

Figure 4-6 Dependencies of K_{obs} for the hydrolysis of 4-nitrophenylpropionate by **4f** at different concentration

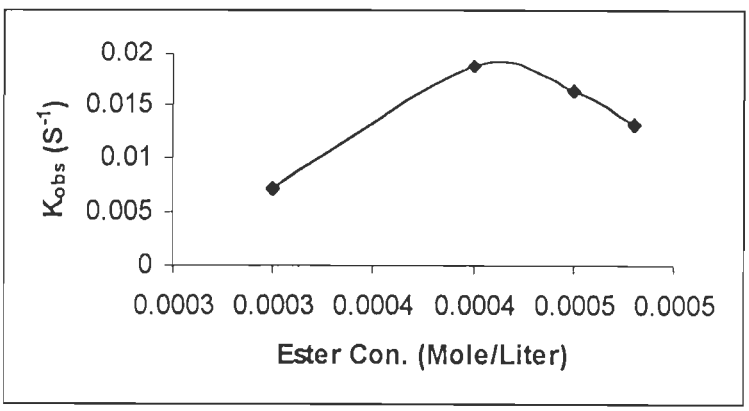


Figure 4-7 Dependencies of K_{obs} for the hydrolysis of 4-nitrophenylcaprylate by **4f** at different concentration

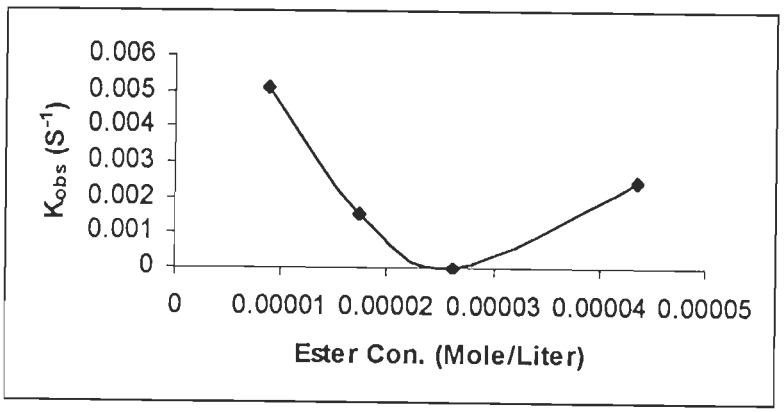


Figure 4.8. Dependencies of K_{obs} for the hydrolysis of 4-nitrophenyllaurate by **4f** at different concentration

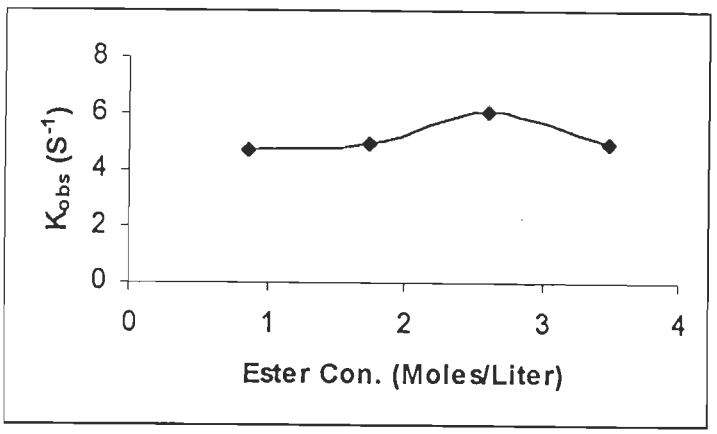
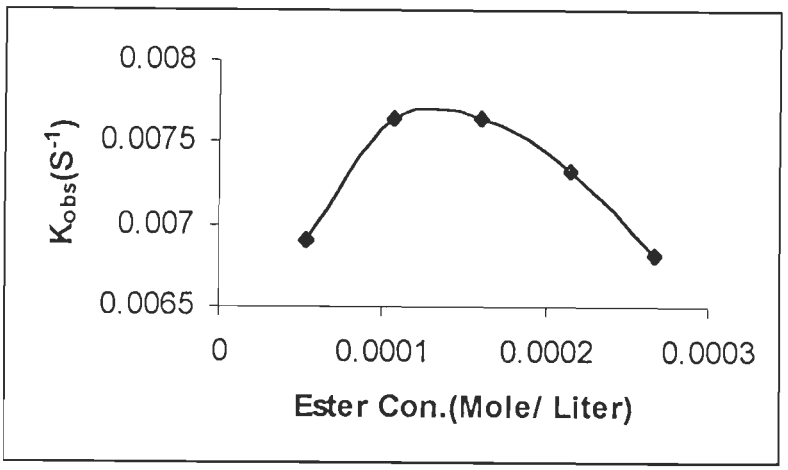


Figure 4.9. Dependencies of K_{obs} for the hydrolysis of 4-Ntrophenyltrifluoroacetate by **4f** at different concentration



Summary

By using the sterically more hindered hydrotris (pyrazolyl) borate ligand ($Tp^{tBu,iPr}$) mononuclear chloro cadmium (II) **4c** and chloro nickel (II) **4d, 4e** complexes have been prepared. The reaction of **4c** and **4e** with 1N NaOH resulted the formation of mononuclear hydroxo nickel (II) **4g** and mononuclear hydroxo cadmium (II) **4h** complexes. These complexes were characterized by different spectroscopic techniques. The efforts to get single crystals for X-ray data collection were not successfull. The complexes **4f**, **4g** and **4h** were tested for their catalytic properties in hydrolysis of tris(4-nitrophenyl)phosphate, 4-nitrophenylpropionate, 4-nitrophenylcaprylate, 4-nitrophenylleurate and 4-nitrophenyltrifluoroacetate and it was found that these complexes catalysed the above ester hydrolysis efficiently with different pseudo-first order rate constants.

Table 4-10.

Ester → / Hydroxo ↓	Mono(4-Nitrophenyl) Phosphate	Bis(4-Nitrophenyl) Phosphate	Tris(4-Nitrophenyl) Phosphate	P-(Nitrophenyl) Acetate	(4-Nitrophenyl) Propionate	(4-Nitrophenyl) Caprylate	(4-Nitrophenyl) Laurate	(4-Nitrophenyl) TFA
LMnOH	8.66×10^{-3}	5.33×10^{-3}	4.90×10^{-3}	7.35×10^{-3}	2.10×10^{-3}	1.99×10^{-3}	1.20×10^{-3}	5.23×10^{-3}
LNiOH	1.15×10^{-2}	3.18×10^{-3}	2.15×10^{-3}	6.35×10^{-3}	4.1×10^{-3}	3.0×10^{-3}	1.99×10^{-3}	3.5×10^{-3}
LZnOH	6.0×10^{-3}	7.25×10^{-3}	5.10×10^{-3}	6.20×10^{-3}	4.90×10^{-3}	3.50×10^{-3}	3.20×10^{-3}	4.99×10^{-3}
LCdOH	6.24×10^{-3}	7.82×10^{-3}	5.3×10^{-3}	6.89×10^{-3}	5.1×10^{-3}	5.0×10^{-3}	3.9×10^{-3}	4.8×10^{-3}
$L'_2Co_2(OH)_2$	1.75×10^{-3}	4.63×10^{-3}	1.45×10^{-2}	1.26×10^{-3}	4.14×10^{-3}	5.10×10^{-3}	5.75×10^{-3}	4.61×10^{-3}
$L'Ni_2(OH)_2$	1.04×10^{-2}	4.03×10^{-3}	5.00×10^{-2}	8.25×10^{-3}	7.25×10^{-3}	4.94×10^{-3}	4.74×10^{-3}	5.30×10^{-3}

L' - $(HB(3,5\text{-}Pr^i{}_2pz)_3$;
L - $(HB(3\text{-}Bu^t\text{-}5\text{-}Pr^i{}_2pz)_3$

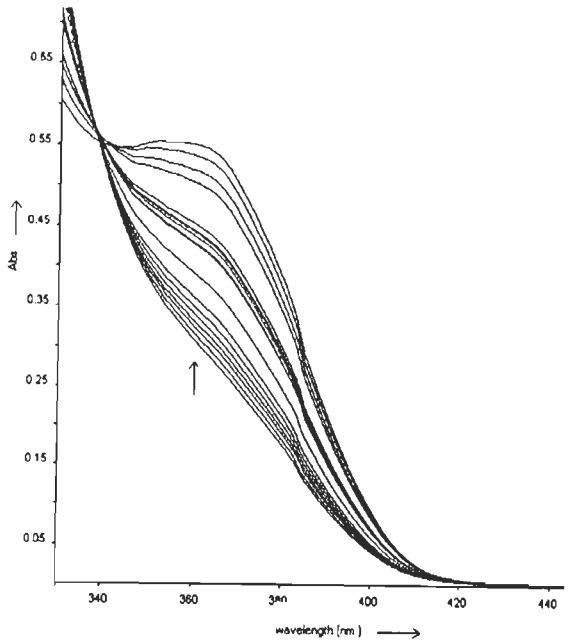


Fig. 4-1(a) - Change in Uv-spectrum due to [(HB(3-Bu^t-5-Pr$^i_2$pz)$_3$CdOH] promoted hydrolysis of tris(p-nitrophenyl)phosphate at room tempearture, recorded at 2 min. interval.

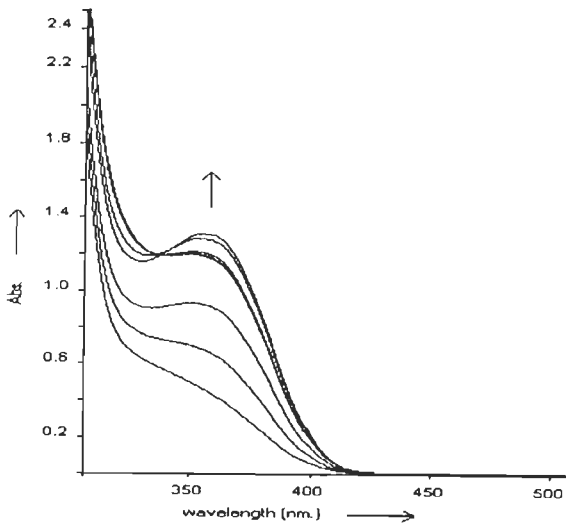


Fig. 4-2(b) - Change in Uv-spectrum due to [(HB(3-Bu^t-5-Pr$^i_2$pz)$_3$CdOH] promoted hydrolysis oft (p-nitrophenyl)propionate at room tempearture, recorded at 2 min. interval.

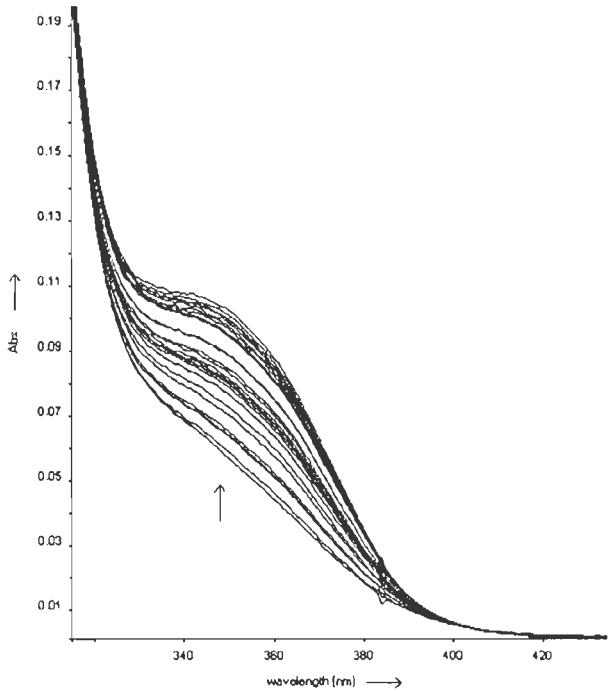


Fig. 4-3(c) - Change in Uv-spectrum due to [(HB(3-Bu^t-5-Pr$^i_2$pz)$_3$CdOH] promoted hydrolysis of p-nitrophenyllaurate at room tempearture, recorded at 2 min. interval.

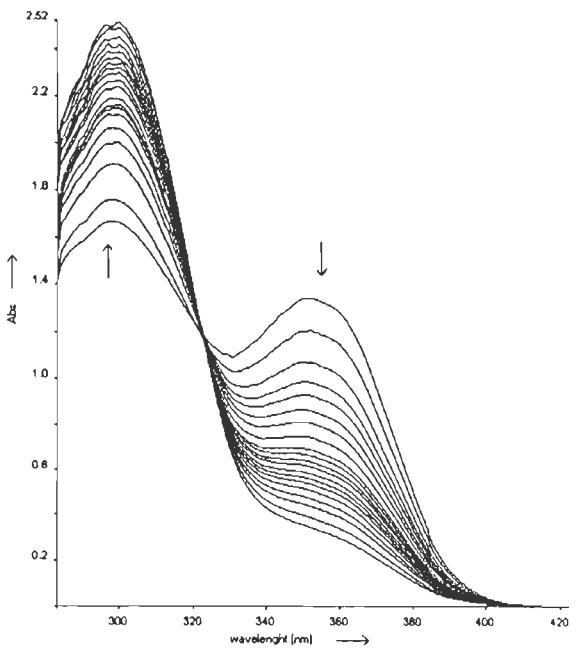


Fig. 4-4(d) - Change in Uv-spectrum due to [(HB(3-Bu^t-5-Pr^i $_2$pz)$_3$CdOH] promoted hydrolysis of p-nitrophenyltrifluoroacetae at room tempearture, recorded at 2 min. interval.

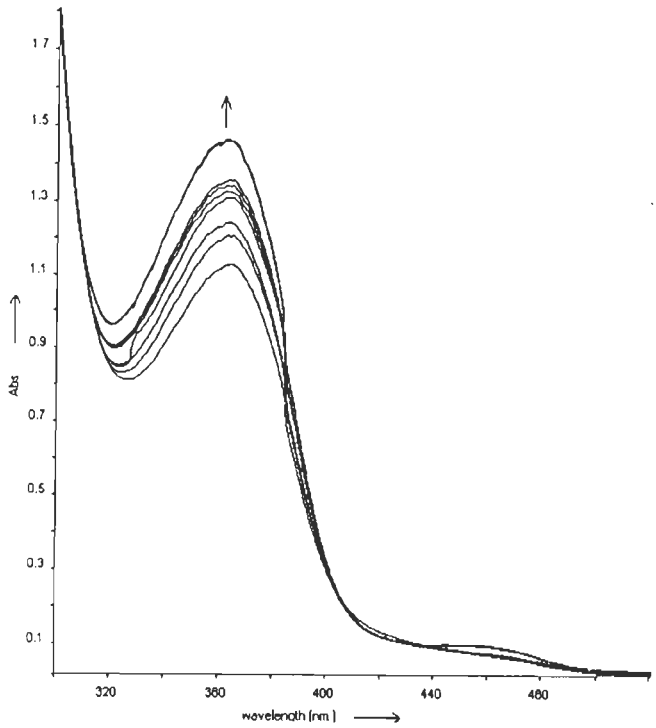


Fig. 4-5(e) - Change in Uv-spectrum due to $(HB(3,5\text{-}Pr^{i}{}_{2}pz)_{3}Ni)_{2}(OH)_{2}$ promoted Hydrolysis of p-nitrophenylpropionateat room tempearture, recorded at 2 min.interval. (Increasing)

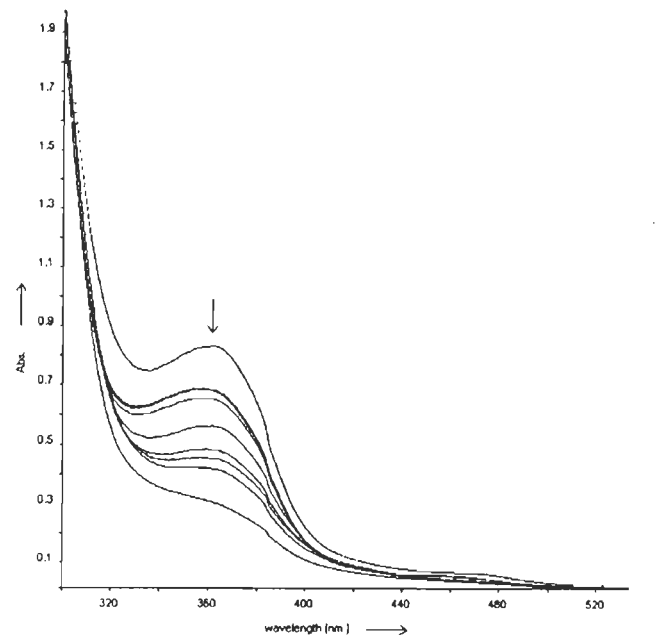


Fig. 4-6 (f) - Change in Uv-spectrum due to $(HB(3,5\text{-}Pr^{i}{}_{2}pz)_{3}Ni)_{2}(OH)_{2}$ promoted hydrolysis of p-nitrophenylpropionateat room tempearture, recorded at 2 min.interval.(deceasing)

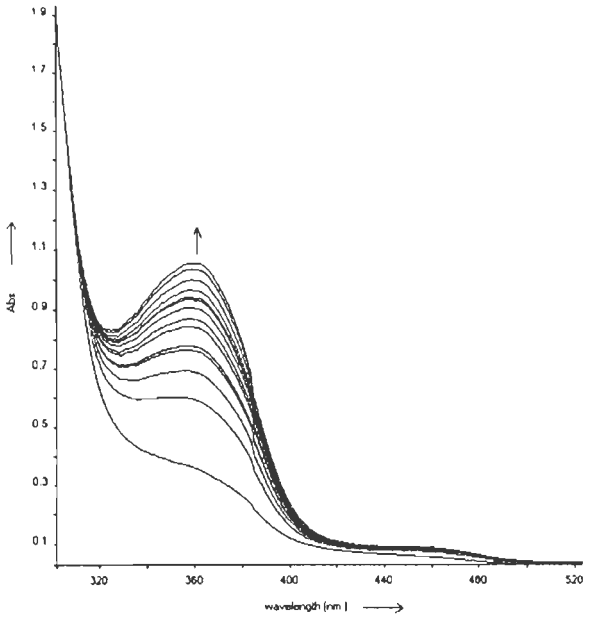


Fig. 4-7(g) - Change in Uv-spectrum due to $(HB(3,5\text{-}Pr^{i}{}_{2}pz)_{3}Ni)_{2}(OH)_{2}$ promoted hydrolysis of p-nitrophenylcaprylate at room tempearture, recorded at 2 min.interval.

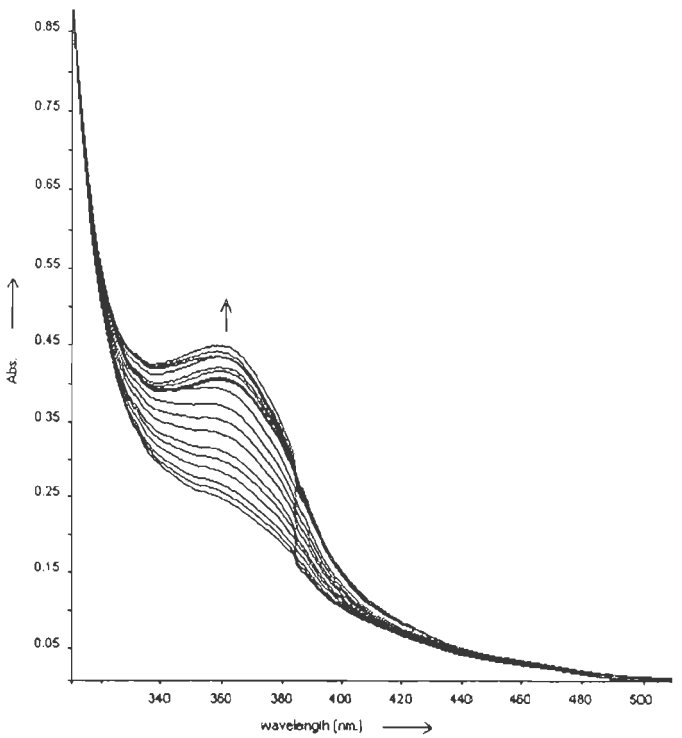


Fig. 4-8(h) - Change in Uv-spectrum due to $(HB(3,5\text{-}Pr^{i}{}_{2}pz)_{3}Ni)_{2}(OH)_{2}$ promoted hydrolysis of p-nitrophenyllaurate at room tempearture, recorded at 2 min.interval.

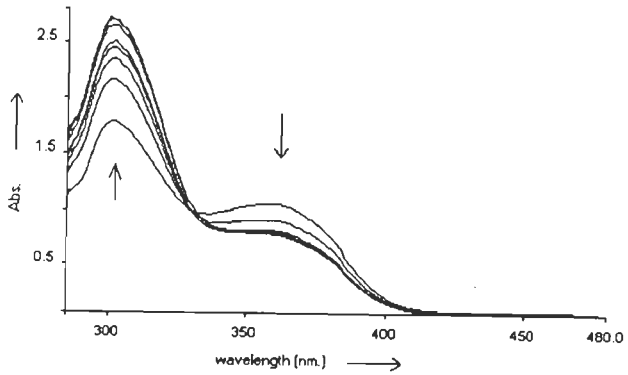


Fig. 4-9(i) - Change in Uv-spectrum due to $(HB(3,5\text{-}Pr^{i}{}_{2}pz)_{3}Ni)_{2}(OH)_{2}$ promoted hydrolysis of p-nitrophenyltrifluoroacetate at room temperature, recorded at 2min. interval.

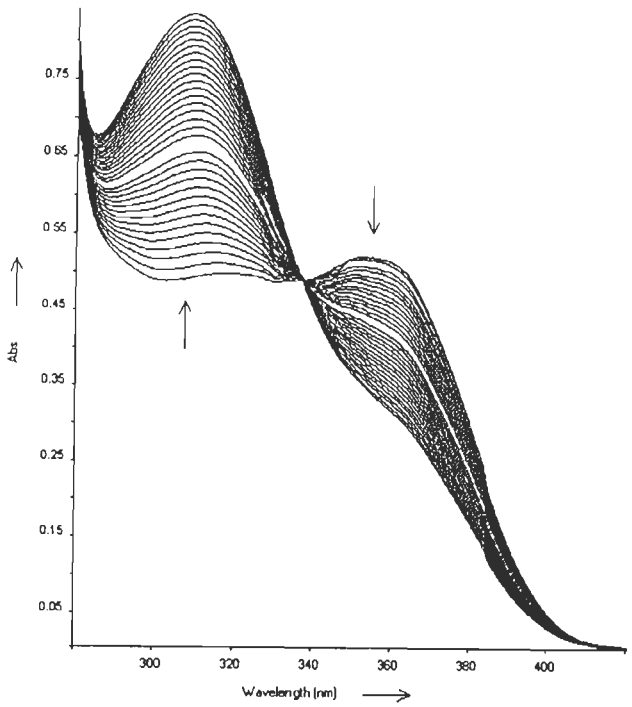


Fig. 4-10(j) - Change in Uv-spectrum due to $[(HB(3\text{-}Bu^{t}\text{-}5\text{-}Pr^{i}{}_{2}pz)_{3}ZnOH]$ promoted hydrolysisof p-nitrophenyltrifluoroacetate at room tempearture, recorded at 2 min. interval.

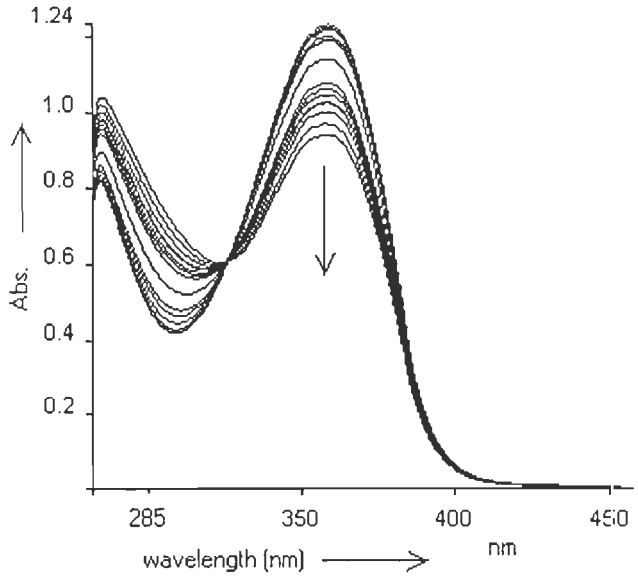


Fig. 4-11(k) - Change in Uv-spectrum due to [(HB(3-Bu^t-5-Pr$^i_2$pz)$_3$NiOH] Promoted hydrolysis of p-nitrophenyltrifluoroacetate at room temperature, recorded at 2 min. interval.

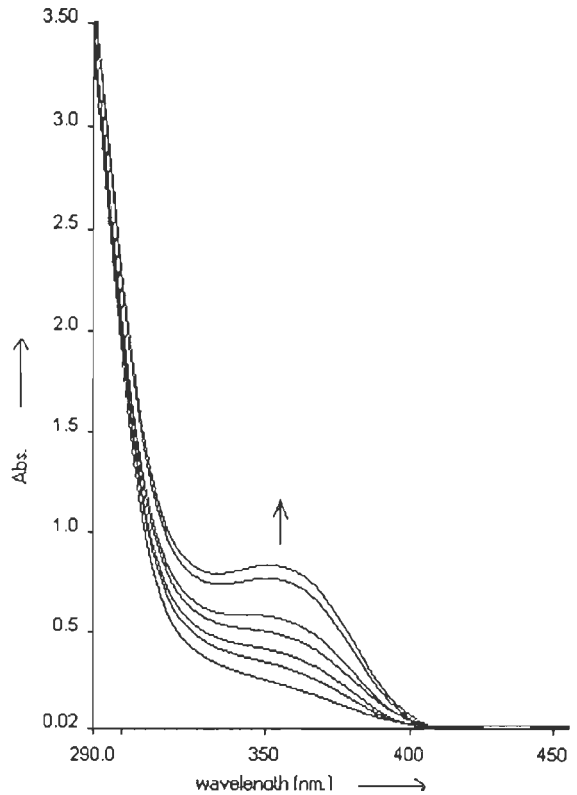


Fig.4-12(l) - Change in Uv-spectrum due to [(HB(3-Bu^t-5-Pr$^i_2$pz)$_3$ZnOH] promoted hydrolysis oft tris(p-nitrophenyl)phosphate at room temperature, recorded at 2 min. interval

References:

1. Vogel, F., Spener, and Krebs, B., in Handbook of Metalloproteins, A. Messerschmidt, R. Huber, T. Poulos and K. Wieghardt (Eds.), Vol. **2**, John Wiley & Sons, Chichester, pp. 752-767 (2001).
2. Suerbaum, H., Körner, M., Witzel, H., Althaus, E., Mosel, B. D. and Müller-Warmuth W., "Zn-exchange and mossbauer studies on the [Fe-Fe] derivatives of the purple acid Fe (III)-Zn (II)-phosphatase from kidney beans", Eur. J. Biochem., **214**, 313 (1993).
3. Davis, J. C. and Averill, B. A., "Evidence for a spin-coupled binuclear iron unit at the active site of the purple acid phosphatase from beef spleen", Proc. Natl. Acad. Sci. USA, **79**, 4623 (1982).
4. Sträter, N., Klabunde, T., Tucker P., Witzel, H. and Krebs, B., "Crystal structure of a purple acid phosphatase conatining a dinuclear Fe (III)-Zn (II) active site", Science, **268**, 1489 (1995).
5. (a) Silverman, D. N. and Lindskog, S., "The catalytic mechanism of carbonic anhydrase: Implications of a rate-limiting protolysis of water", Acc. Chem. Res., **21**, 30 (1988). (b) Woolley, P., "Models for metal ion function in carbonic anhydrase", Nature, **258**, 677 (1975). (c) Lindskog, S., "Carbonic anhydrase", Spiro, T. G. (ed.), "Zinc Enzymes", Wiley, New York, 77 (1983). (d) Lindskog, S. and Coleman, J. E., "The catalytic mechanism of carbonic anhydrase (metalloenzymes / enzyme mechanism / hydration of CO_2)", Proc. Natl. Acad. Sci. U.S.A., 70, 2505 (1973). (e) Yachandra, V., Powers, L. and Spiro, T. G., "X-ray absorption spectra and the coordination number of Zn and Co carbonic anhydrase as

a function of pH and inhibitor binding", J. Am. Chem. Soc., **105**, 6596 (1983). (f) Khalifah, R. G., "The carbon dioxide hydration activity of carbonic anhydrase. I. Stop-flow kinetic studies on the native human isoenzymes B and C", J. Biol. Chem., **246**, 2561 (1971). (g) Khalifah, R. G., "Carbon dioxide hydration activity of carbonic anhydrase: Paradoxical consequences of the unusually rapid catalysis", Proc. Natl. Acad. Sci. U.S.A., **70**, 1986 (1973).

6. (a) Merz, K. M., Jr., Hoffman, R. and Dewar, M. J. S., "Mode of action of carbonic anhydrase", J. Am. Chem. Soc., **111**, 5636 (1989). (b) Jacob, O., Cardenas, R. and Tapia, O., "An ab initio study of transition structures and associated products in $[ZnOHCO_2]^+$, $[ZnHCO_3H_2O]^+$ and $[Zn(NH_3)_3HCO_3]^+$ hypersurfaces. On the role of zinc in the catalytic mechanism of carbonic anhydrase", J. Am. Chem. Soc., **112**, 8692 (1990). (c) Liang, J.-Y. and Lipscomb, W. N., "Hydration of carbon dioxide by carbonic anhydrase: Internal proton transfer of Zn^{2+}-bound HCO_3^-", Biochemistry, **26**, 5293 (1987).
7. Lipscomb, W. N., "Relationship of the three dimensional structure of carboxypeptidase A to catalyze possible intermediate and pH effects", Tetrahedron, **30**, 1725 (1974).
8. Frey, P. A., Whitt, S. A. and Tobin, J. B., "A low-barrier hydrogen bond in the catalytic triad of serine proteases", Science, **264**, 1927 (1994).
9. (a) Linkletter, B. and Chin, J., "Rapid hydrolysis of RNA with a Cu (II) complex", Angew. Chem., Int. Ed. Engl., **34**, 472 (1995). (b) Bazzicalupi, C., Bencini, A., Bianchi, A., Fusi, V., Giorgi, C., Paoletti, P., Valtancoli, B. and Zanchi, D., "Carboxy and phosphate esters cleavage with mono- and dinuclear zinc (II)

macrocyclic complexes in aqueous solution, crystal structure of $[Zn_2L1(\mu\text{-}PP)_2(MeOH)_2](ClO_4)_2$ (L1 = $[30]aneN_6O_4$; PP – equals diphenyl phosphate)", Inorg. Chem., **36**, 2784 (1997). (c) Koike, T., Kajitani, S., Nakamura, I., Kimura, E. and Shiro, M., "The catalytic carboxyester hydrolysis by a new zinc (II) complex with an alcohol-pendant cyclane (1-(2-hydroxyethyl)-1,4,7,10-tetraazacyclodecane): A novel model for indirect activation of the serine nucleophile by zinc (II) in zinc enzymes", J. Am. Chem. Soc., **117**, 1210 (1995). (d) Adams, H., Bailey, N. A., Fenton, D. E. and He, Q. -Y., "Zinc (II) complexes of tripodal ligands providing phenolate and pyridine donors: Formation, structure and hydrolytic activity", J. Chem. Soc., Dalton Trans., 2857 (1996). (e) Yashiro, M., Ishikubo, A. and Komiyama, M., "Efficient and unique cooperation of three zinc (II) ions in the hydrolysis of diribonucleotides trinuclear zinc (II) complex", J. Chem. Soc., Chem. Commun., 83 (1997).

10. (a) Adams, H., Bailey, N. A., Fenton, D. E. and He, Q. -Y., "Mononuclear zinc (II), homodinuclear zinc(II) and heterodinuclear zinc (II)-copper (II) complexes derived from tris[(2-pyridyl)methyl]amine", J. Chem. Soc., Dalton Trans., 1533 (1997). (b) Schepers, K., Bremer, B., Krebs, B., Henkel, G., Althaus, E., Mosel, B. and Müller-Warmuth, W., "Zn (II)-Fe (III) and Fe(II)-Fe (III) complexes containing a novel (μ-phenoxo)bis(μ-diphenylphosphato)-dimetal (II,III) core as model complexes for active sites of purple phosphatases", Angew. Chem., **29**, 531 (1990). (c) Wilker, J. J. and Lippard, S. J., "Alkyl transfer to metal thiolates: Kinetics, active species identification and relevance to the DNA methyl phosphotriester repair center of *Escherichia coli* Ada", Inorg. Chem., **36**, 969 (1997). (d) Koike, T., Inoue, M.,

Kimura, E. and Shiro, M., "Novel properties of cooperative dinuclear zinc (II) ions: The selective recognition of phosphomonoesters and their P-O ester bond cleavage by a new dinuclear zinc (II) cryptate", J. Am. Chem. Soc., **118**, 3091 (1996). (e) Priggemeyer, S., Eggers-Borkenstein, P., Ahlers, F., Henkel, G., Körner, M., Witzel, H., Nolting, H. F., Hermes, C. and Krebs, B., "XAS investigations on the iron-zinc center of purple acid phosphatase from red kidney beans", Inorg. Chem., **34**, 1445 (1995).

11. Coleman, J. E., "Zinc Enzymes", Birkhäuser: Boston, M. A., Chapter 4, 49 (1986).
12. (a) Ochiai, E., "Uniqueness of zinc as a bioelement- principles and applications in bioinorganic chemistry-III", J. Chem. Edu., **65**, 943 (1988). (b) Vallee, B. L. and Galdes, A., "The metallobiochemistry of zinc enzymes", Adv. Enzymol., **56**, 283 (1984).
13. Meier, M. M., Karloh, P., Terenzi, H. and Szpoganicz, B., "Equilibrium and hydrolysis studies of phosphate esters model molecules and DNA catalyzed by obisdien-Zn(II) complexes", Southern Brazilian J. Chem., **7**, 11 (1999).
14. Kaminskaia, N. V., Spingler, B. and Lippard, S. J., "Hydrolysis of β-lactam antibiotics catalyzed by dinuclear zinc (II) complexes: functional mimics of metallo-β-lactamases", J. Am. Chem. Soc., **122**, 6411 (2000).
15. Erxleben, A. and Hermann, J., "Di- and poly-nuclear zinc (II) Schiff base complexes: synthesis, structural studies and reaction with a α-amino acid ester", J. Chem. Soc., Dalton Trans., 569 (2000).

16. Mancin, F., Tecilla, P. and Tonellato, U., "Metallomicelles made of Ni (II) and Zn (II) complexes of 2-pyridinealdoxime-based ligands as catalyst of the cleavage of carboxylic acid esters", Langmuir, **16**, 227 (2000).

17. Bencini, A., Berni, E., Bianchi, A., Fedi, V., Giorgi, C., Paoletti, P. and Valtancoli, B., "Carboxy and diphosphate ester hydrolysis promoted by dinuclear zinc (II) macrocyclic complexes. Role of Zn (II) bound hydroxide as the nucleophilic function", Inorg. Chem., **38**, 6323 (1999).

18. Kim, J. and Lim, H., "Preparation and study of dinuclear Zn (II) complex for the hydrolysis of phosphate esters", Bull. Korean Chem. Soc., **20**, 491 (1999).

19. Manseki, K., Sakiyama, H., Sakamoto, M., Nishida, Y., Sadaoka, Y., Ohba, M. and Okawa, H., "Hydrolysis of phosphate ester by zinc (II)-lanthanide (III) complexes of end-off type unsymmetric dinucleating ligand", Kidorui, **34**, 162 (1999).

20. Suh, J., Son, S. J. and Suh, M. P., "Kinetics of hydrolysis of phenyl acetates catalyzed by the zinc (II) complex of 1,5,9-triazacyclododecane. Evidence for attack of water or hydroxide ion at the coordinated ester", Inorg. Chem., **37**, 4872 (1998).

21. Breslow, R. and Nesnas, N., "Burst kinetics and turnover in an esterase mimic", Tetrahedron Lett., 40, 3335 (1999).

22. Yatsimirsky, A. K., Gomez-Tagle, P., Escalante-Tovar, S. and Ruiz-Ramirez, L., "Kinetics and mechanism of ester hydrolysis by metal complexes of 2,6-diacetylpyridine dioxime", Inorg. Chim. Acta, **273**, 167 (1998).

23. Weis, K., Rombach, M., Ruf, M. and Vahrenkamp, H., "(Pyrazolylborate) zinc organophosphate complexes resulting from hydrolytic cleavage of phosphate esters", Eur. J. Inorg. Chem., **2**, 263 (1998).

24. Lin, H.-K., Kou, F.-P., Zhu, S.-R. and Chen, R.-T., "Carboxylate ester hydrolysis promoted by new zinc (II) macrocyclic tetraamine complexes with a phenol pendant", Gao. Xue. Hua. Xue., **18**, 513 (1997).

25. Bazzicalupi, C., Bencini, A., Bianchi, A., Fusi, V., Giorgi, C., Paoletti, P., Valtancoli, B. and Zanchi, D., "Carboxy and phosphate esters cleavage with mono- and dinuclear zinc(II) macrocyclic complexes in aqueous solution. Crystal structure of $[Zn_2L^1(\mu\text{-}PP)_2(MeOH)_2](ClO_4)_2$ (L^1 = [30]aneN_6O_4, PP^- = Diphenyl Phosphate)", Inorg. Chem., **36**, 2784 (1997).

26. Rammo, J. and Schneider, H.-J., "Supramolecular complexes of transition-metal (II) ions for the hydrolysis of phosphate esters and of DNA", Inorg. Chim. Acta, **251**, 125 (1996).

27. Kimura, E., Hashimoto, H. and Koike, T., "Hydrolysis of lipophilic esters catalyzed by a zinc (II) complex of a long alkyl-pendant macrocyclic tetraamine in micellar solution", J. Am. Chem. Soc., **118**, 10963 (1996).

28. Bruice, T. C., Tsubouchi, A., Dempcy, R. O. and Olson, L. P., "One- and two-metal ion catalysis of the hydrolysis of adenosine 3'-alkyl phosphate esters. Models for one- and two-metal ion catalysis of RNA hydrolysis", J. Am. Chem. Soc., **118**, 9867 (1996).

29. Ruf, M. and Vahrenkamp, H., "Hydrolysis of esters and amides by the metallo nucleophile $Tp^{Cum,Me}$Zn-OH", Chem. Ber., **129**, 1025 (1996).

30. Wendelstorf, C., Warzeska, S., Koevari, E. and Kraemer, R., "Carboxy ester hydrolysis catalyzed by a dinuclear, hexaazamacrocyclic zinc (II) complex. A model for zinc (II) aminopeptidases", J. Chem. Soc., Dalton Trans., 3087 (1996).

31. Hu, C.-C., Chen, W.-H., Liu, C.-Y. and Chen, J.-L., "Hydrolysis of phosphate esters with polymer-supported catalysts containing cyclodextrin pendant group", J. Inclusion Phen. and Mol. Recognition in Chem., **23**, 289 (1996).

32. Canary, J. W., Gibb, B. C., Lajmi, A. R., Liu, Z. H., Qian, H. T. and Santos, O. D., "Models of the superstructure in zinc enzyme active sites", Book of Abstracts, 212th ACS National Meeting, Orlando, FL, August 25-29 (1996).

33. Tanase, T., Yun, J. W. and Lippard, S. J., "Mono- and Dinuclear Zinc Complexes of XDK, H_2XDK = m-Xylenediamine Bis(Kemp's triacid imide), and their Reaction with phosphate esters", Inorg. Chem., **34**, 4220 (1995).

34. Tsubouchi, A. and Bruice, T. C., "Phosphonate ester hydrolysis catalyzed by two lanthanum ions. Intramolecular nucleophilic attack of coordinated hydroxide and lewis acid activation", J. Am. Chem. Soc., **117**, 7399 (1995).

35. Chapman, W. H. Jr. and Breslow, R., "Selective hydrolysis of phosphate esters and nitrophenyl phosphates by dimeric zinc complexes depends on the spacer length", J. Am. Chem. Soc., **117**, 5462 (1995).

36. Rodriguez de Barbarin, C. O., Bailey, N. A., Fenton, D. E. and He, Q., "The synthesis, structure and hydrolytic activity of a functional pentacoordinate zinc complex", Inorg. Chim. Acta, **219**, 205 (1994).

37. Scrimin, P., Tecilla, P. and Tonellato, U., "Leaving group effect in the cleavage of picolinate esters catalyzed by hydroxy-functionalized metallomicelles", J. Org. Chem., **59**, 18 (1994).

38. Viladkar, S., Kamaluddin and Nath, M., "Hydrolysis of 4-nitrophenyl phosphate by (amino acidato)zinc complexes", Bull. Chem. Soc. Japan, **66**, 2922 (1993).

39. Breslow, R. and Chipman, D., "Mixed metal complexes as enzyme models. I. Intracomplex nucleophilic catalysis by an oxime anion", J. Am. Chem. Soc., **87**, 4195 (1965).

40. Yatsimirskii, K. B. and Tikhonova, L. P., "Effect of zinc ions on the rate of alkaline hydrolysis of cysteine ethyl ester", Zhurnal Neorganicheskoi Khimii, **12**, 417 (1967).

41. Hay, R. W. and Porter, L. J., "Interaction of cysteine methyl ester with metal ions. II. Metal ion catalyzed base hydrolysis of cysteine methyl ester", J. Chem. Soc., **1**, 127 (1969).

42. Chapman, P. H., Houghton, R. P. and Williams, C. S., "Reactions of coordinated ligands. V. Mechanisms of the hydrolysis, ester-exchange reactions, deuteriations, and racemization of the copper (II), zinc (II), magnesium (II), and palladium (II) complexes of N-(alkoxycarbonylalkyl)salicylideneimines", J. Chem. Soc., **9**, 1183 (1970).

43. Norman, P. R., "Enhanced hydrolysis of neutral phosphate esters by the zinc (II) complex of 1,4,7,10-tetraazacyclododecane", Inorg. Chim. Acta, **130**, 1 (1987).

44. Breslow, R. and Singh, S., "Phosphate ester cleavage catalyzed by bifunctional zinc complexes: comments on the "p-nitrophenyl ester syndrome"", Bioorg. Chem., **16**, 408 (1988).

45. Weijnen, J. G. J., Koudijs, A. and Engbersen, J. F. J., "Carboxylic and phosphate ester hydrolysis catalyzed by bivalent zinc and copper metallosurfactants", J. Chem. Soc., Perkin Trans., 1121 (1991).

46. Koike, T. and Kimura, E., "Roles of zinc (II) ion in phosphatases. A model study with zinc (II)-macrocyclic polyamine complexes", J. Am. Chem. Soc., **113,** 8935 (1991).

47. Ogino, K., Inoue, K. and Tagaki, W., "Ester substrate p-nitrophenyl isoquinoline-3-carboxylate (PNIQ) as a novel fluorescent probe for the study of acylation and deacylation steps in ester hydrolysis catalyzed by lipophilic imidazole-zinc(II) complexes in micelles", Tetrahedron Lette., **33,** 4191 (1992).

48. Koerner, T. B. and Brown, R. S., "The hydrolysis of an activated ester by a tris(4,5-di-n-propyl-2-imidazolyl)phosphine-Zn^{2+} complex in neutral micellar medium as a model for carbonic anhydrase", Canadian J. Chem., **80,** 183 (2002).

49. Albedyhl, S., Averbuch-Pouchot, M. T., Belle, C., Krebs, B., Pierre, J. L., Saint-Aman, E. and Torelli, S., "Dinuclear zinc (II) -iron (III) and iron (II) -iron (III) complexes as models for purple acid phosphatases", Eur. J. Inorg. Chem., **6,** 1457 (2001).

50. Xia, J., Xu, Y., Li, S.-A., Sun, W.-Y., Yu, K.-B. and Tang, W.-X., "Carboxy ester hydrolysis promoted by a zinc (II) 2-[Bis(2-aminoethyl)amino]ethanol complex: A

new model for indirect activation on the serine nucleophile by zinc (II) in zinc enzymes", Inorg. Chem., **40,** 2394 (2001).

51. Ibrahim, M. M., Shimomura, N., Ichikawa, K. and Shiro, M., "Phosphoester hydrolysis using structural phosphatase models of tren based zinc (II) complexes and X-ray crystal structures of $[Zn(tren)(H_2O)](ClO_4)_2$ and $[Zn(tren)(BNPP)]ClO_4$", Inorg. Chim. Acta, **313,** 125 (2001).

52. Su, X.-C., Sun, H.-W., Zhou, Z.-F., Lin, H.-K., Chen, L., Zhu, S.-R. and Chen, Y.-T., "Kinetics and mechanism of carboxy ester hydrolysis using Zn (II) complexes with functionalized phenanthroline complexes", Polyhedron, **20,** 91 (2001).

53. Verge, F., Lebrun, C., Fontecave, M., Me´nage Ste´p., "Hydrolysis of phosphodiesters by diiron complexes: Design of nonequivalent iron sites in purple acid phosphatase models", Inorg. Chem., **42,** 2, 499 (2003).

54. Kou, X., Meng, X., Xie, J., and Zeng, X., "Comparative kinetics of carboxylic esters hydrolysis catalyzed by the zinc (II) complex of a macrocyclic schiff base ligand", Trans. Metal Chem., **28,** 777 (2003).

55. Um, Ik-H., Lee, Eun-Ju., and Lee, Jong-Pal., "Kinetics and mechanism for the reaction of 4-nitrophenyl 2-thiophenecarboxylate with secondary alicyclic amines", Bull. Korean Chem. Soc, **23,** 3381 (2002).

56. Suh J., Han O. and Chang, B., "Catalysis by binuclear zinc ions in ester hydrolysis", J. Am. Chem. Soc. , **108,** 1839 (1986).

57. Ye, Bao-Hui, Xiao-Yuan Li,* Xueb, Feng and Makb, Thoms, C. W., "Formation of a novel tetranuclear iron (III) complex with a m_4-PO_4 bridge from 4-nitrophenyl phosphate", Chem. Commun., 2407 (1997).

58. Yin, X., Lin, C., Zhou, Z., Chen, W., Zhu, S., Lin, H., Su, X., and Chen, Y., "Studies of artificial hydrolytic metalloenzymes: The catalyzed carboxyester hydrolysis by copper (II), zinc (II) and cobalt (II) complexes of the tripod ligand tris(2-benzimidazylmethyl)amine", Trans. Metal Chem., **24**, 537 (1999).

59. Wahnon, D., Lebuis, Anne-Marie, Chin, J., "Hydrolysis of a phosphate diester doubly coordinated to a dinuclear cobalt (III) complex: A novel mechanism", Ang. Chem. Int. Edit. Eng., **34**, 2412 (1995).

60. Hegg, E. L, Mortimore, S. H., Cheung, C. L., Huyett, J. E., Powell, D. R., Burstyn, J. N., "Structure-reactivity studies in copper (II) -catalyzed phosphodiester hydrolysis", Inorg. Chem., **38**, 2961(1995).

61. Jancso, A., Mikkola, S., Lonnberg, H., Hegetschweile, K., Gajda, T., "Phosphodiester cleavage of ribonucleoside monophosphates and polyribonucleotides by homo- and heterodinuclear metal complexes of a cyclohexane-based polyamino-polyol ligand", Chem. Eur. J., **9,** 5404 (2003).

62. Echizen, T., Ibrahim, M. M., Nakata, K., Izumi, M., Ichikawa, K., Shiro M.,"Nucleophilic reaction by carbonic anhydrase model zinc compound: characterization of intermediates for CO_2 hydration and phosphoester hydrolysis", J. Inorg. Biochem. **98,** 1347 (2004).

63. Yashiro, M., Kaneiwa, H., Onaka, K., Komiyama, M., "Dinuclear Zn (II) complexes in the hydrolysis of the phosphodiester linkage in a diribonucleoside monophosphate diester," J. Chem. Soc., Dalton Trans., 605 (2004).

64. Knight, D. A., Delehanty, J.B., Goldman, E. R., Bongard, J., Streich, F., Edwards, L.W., Chang, E. L., "Carboxylic acid functionalized cobalt (III) cyclane complexes

for catalytic hydrolysis of phosphodiester bonds", J. Chem. Soc., Dalton Trans., 2006 (2004).

65. Hix, G. B., Kariuki, B. M., Kitchin, S. and Tremayne, M., "Synthesis and structural characterization of $Zn(O_3PCH_2OH)$, a new microporous zinc phosphonate", Inorg. Chem., **40,** 1477 (2001).

66. Frey, S. T., Murthy, N. N., Weintraub, S. T., Thompson, L. K., Karlin, K. D.,"Hydrolysis of unactivated esters and acetonitrile hydration by a hydroxo-dicopper (II) complex", Inorg. Chem., **36,** 956 (1997).

67. Kinoshita, E., Takahashi, M., Takeda, H., Shiro, M., Koike, T.,"Recognition of phosphate monoester dianion by an alkoxide-bridged dinuclear zinc (II) complex", J. Chem. Soc., Dalton Trans.,118 (2004).

68. Santana, M. D., García, G., Lozano, A. A., López, G., Tudela, J., Pérez, J., García, L., Lezama, L., Rojo, T., "Pentacoordinate nickel (II) complexes double bridged by phosphate ester or phosphinate ligands: spectroscopic, structural, kinetic, and magnetic studies", Chem. Eur. J., **10**, 1738 (2004).

69. Xie, J.-Q., Jiang, B-Y., Kou, X.-M., Hu, C.-W., Zeng, X.-C. and Li, Y.-T., "Studies on the reaction kinetics and the mechanism of hydrolysis of bis(4-nitrophenyl) phosphate (BNPP) catalyzed by oxamido-bridged dinuclear copper (II) complexes in micellar solution", Transtion. Met. Chem.., **28**, 782 (2003).

70. Ge, Q.-C., Guo, Y.-H., Lin, H., Gao, D.-Z., Lin, H.-K. and Zhu, S.-R., "Role of trinuclear Zn (II) complexes in promoting the hydrolysis of 4-nitrophenyl acetate", Canadian J. Chem., **82**, 409 (2004).

71. Singh, U. P. and Babbar, P. "Synthesis and molecular structure of mononuclear

cobalt hydroxo complex and its uses in ester hydrolysis", under preparation.

72. Kitajima, N., Hikichi, S., Tanaka, M. and Moro-oka, Y, J. Am. Chem. Soc., **115**, 5496 (1993).

73. (a) Alsfasser, R., Trofimenko, S., Looney, A., Perkin, G., and Vahrenkamp, H., "A mononuclear zinc hydroxo complex stabilized by a highly substituted tris(pyrazolyl)hydroborato ligand: Analogies with the enzyme carbonic anhydrase", Inorg. Chem., **30**, 4098 (1991). (b) Hikichi, S., Ogihara, T., Fujisawa, K., Kitajima, N., Akita, M. and Moro-oka, Y., "Synthesis and characterization of the benzoylformato ferrous complexes with the hindered tris(pyrazolyl)borate ligand as a structural model for mononuclear non-heme iron enzymes", Inorg. Chem., **36**, 4539 (1997). (c) Fujisawa, K., Kobayashi, T., Fujita, K., Kitajima, N., Moro-oka, Y., Miyashita, Y., Yamada, Y. and Okamoto, K., "Mononuclear copper(II) hydroxo complex: Structural effect of a 3-position of tris(pyrazolyl)borates", Bull. Chem. Soc. Jpn., **73**, 1997 (2000).

74. Kenley, R. A., Fleming, R. H., Laine, R. M., Tse, D. S., and Winterle, J. S., "Cobalt catalyzed hydrolysis of phosphorus esters", Inorg. Chem. **23**, 1870 (1984).

75. Kimura, E., Kodama, Y., Koike, T., and Shiro, M., "Phosphodiester hydrolysis by a new zinc(II) macrocyclic tetramine complex with an alcohol pendant: Elucidation of the role of Ser-102 and Zinc(II) in alkaline phosphatase", J. Am. Chem. Soc., **117**, 32 (1995).

76. Wahnon, D., Lebuis, A-M. and Chim, J., "Hydrolysis of a phosphate diester doubly coordinated to a dinuclear cobalt(III) complexes: A noval mechanism", Angew. Chem. Int. Ed. Engl. **34**, 21 (1995).

77. Ito, M., Sakai, K., Tsubomura, T. and Takita, Y. -S, "Transesterification by (hydroxo)nickel complex in the presence of external alcohol", Bull. Chem. Soc. Jpn., **72**, 239 (1999).

78. Ichikawa, K., Uddin, M. K. and Nakata, K., "Zinc complexes of artificial histidine-containing dipeptides as catalysts of hydrolyses of p-nitrophenyl phosphates", Chem. Lett., 115 (1999).

79. Rombach, M., Maurer, C., Weis, K., Keller, E. and Vahrenkamp, H., "Evidences for a trajectory of hydrolytic reactions brought about by [L3Zn-OH] species". Chem. Eur. J., **5**, 1013 (1999).

80. Jones, D. R., Lindoy, L. F. and Sargeson, A. M., "Hydrolysis of phosphate esters bound to cobalt(III). Kinetics and mechanisms of intramolecular attack of hydroxide on coordinated 4-nitrophenyl phosphate", J., Am. Chem. Soc., **105**, 7327 (1983).

81. Gellman, S. H., Petter, R. and Breslow, R., "Catalytic hydrolysis of a phosphate trimester by tetracoordinated zinc complexes", J. Am. Chem. Soc., **108**, 2388 (1986).

82. Clewley, R. G., Slebocka-tilak, H. and Brown, R. S., "Mono and dinuclear M2+ chelates catalysts for the hydrolysis of organophosphate triesters", Inorg. Chim. Acta, **157**, 233 (1989).

Chapter 5

EXPERIMENTAL

Materials:

All manipulations were carried out under an inert atmosphere by using standard Schlenk tube techniques unless otherwise stated. Dichloromethane, toluene, methanol, acetonitrile, diethyl ether and pentane were carefully purified and distilled under nitrogen prior to use by the literature method [1]. $MnCl_2.4H_2O$ and $Co(NO_3)_2.4H_2O$ (reagent grade) was purchased from E. Merck. Other reagents were of the highest grade commercially available and used without further purification.

^{1}H-NMR spectra were recorded on JEOL-GX-270 (270 MHz). The chemical shifts are reported as values (δ, ppm) down shifted from the internal standard Me_4Si (TMS). IR spectra were obtained on a Thermo Nikolet Nexus FTIR spectrometer in KBr and FD-MS spectra were obtained on a Hitachi M-80 mass spectrometer. Room temperature magnetic susceptibility measurements were done on a Cahn Faraday magnetic susceptibility balance using cobalt mercury tetrathiocyanate as a calibrant and the experimental magnetic susceptibilities were corrected for dimagnetism using the procedure described by Figgis and Lewis [2]. The electronic spectra were recorded on Perkin Elmer Lamda 35, spectrophotometer. The X-ray data collection and processing for **2c**, **2d** were performed on a Rigaku RAXIS-IV imaging plate area detector with MoKα radiation (λ = 0.71070 Å). In the reduction of data Lorentz and polarization corrections and emperical absorptions were made [3]. The structure analysis was performed on an IRIS O2 (Silicon Graphics) using the teSan structure solving program system obtained from the Rigaku Corp., Tokyo, Japan [4]. Neutral scattering factors were obtained from the standard source [5]. The structures were solved by a combination of the direct methods (SHELXS – 86) [6] and Fourier synthesis (DIRDIF 94) [6, 7]. Least-squares refinements were carried out using SHELEX –97 [6] linked to teXsan.

All non – hydrogen atoms were refined aniostropically. Single crystal diffraction studies for **2g, 2h** were carried out on a Brucker SMART CCD diffractometer with MoKα (λ = 0.71073 Å) sealed tube at 25 °C. Crystal structures were solved by direct methods and in anisotropic approximation refined using the SHELX TL package [8, 9]. Hydrigen atoms were constrained by rigid model.

Chapter-2

Synthesis of 3,5-diisopropylpyrazole [3,5-iPr_2pzH] (2a)

Diisobutrylmethane used for the preparation of 3,5-diisopropylpyrazole was synthesized by the following method:

Lithium amide (25.00 g., 1.08 mol) and diethyl ether (80 ml) were taken three necked flask round bottom flask. To the mixture was added dropwise a solution of isopropylmethylketone (58.82 g., 0.68 mol) over a 60 minutes period. Methylisobutyrate (56.62 g., 0.55 mol) was then added dropwise to the above mixture over a period of 90 minutes. After being refulxed for 10 hrs., a dilute HCl solution was added to the mixture to hydrolyze the unreacted lithium amide and the water layer was extracted with diethyl ether (3 x 100 ml). The ether layer was washed three times with saturated NaCl aqueous solution. After drying over $MgSO_4$, diethyl ether was removed by evaporation. The resulting solution was distilled under reduced pressure at 140 °C affording 44.96 g. diisobutrylmethane.

In a two-necked round bottom flask, aqueous hydrazine monohydrate (23.00 g., 0.46 mol) was added dropwise to a solution of diisobutrylmethane (42.49 g., 0.27 mol) dissolved in 60 ml ethanol. After 10 hrs. refluxing, the mixture was allowed to cool at room temperature, added 100 ml saturated NaCl aqueous solution and compound was extracted

with diethyl ether (3 x 100 ml). The organic layer was washed with saturated NaCl solution (three times). After being dried over $MgSO_4$ for 6 hrs. the solvent was evaporated to dryness. The resultant white solid was recrystallized from acetonitrile, afforded 3,5-diisopropylpyrazole as white needles (yield 28.24%). Anal. Calcd for $C_9H_{16}N_2$; C, 71.01; H, 10.59; N 18.40; Found: C, 70.33; H, 10.49; N, 17.95. ^1H NMR ($(CD_3)_2CO$, 270 MHz) δ, 1.22 (d, 12 H, CH*Me*2), 2.91 (m, 2 H, *CH*Me2), 5.80 (s, 1 H, Pz), 11.31 (s, br, 1 H, NH).

Synthesis of potassium hydrotris (3,5-diisopropyl-1-pyrazolyl)borate [(KHB(3,5-$iPr_2pz)_3$)] (2b)

The mixture of 3, 5-diisopropylpyrazole (13.00 g., 0.08 mol) and KBH_4 (1.53 g., 0.028 mol) was was warmed gradually while monitoring hydrogen evolution. The mixture was heated to 240 °C and heating continued until no hydrogen evolution was observed. The solution was allowed to cool at room temperature, the solid mass was dissolved in dichloromethane and filtered over celite. The solvent was evaporated under vaccum and the resultant solid was carefully recrystallized from acetonitrile, affording [(KHB(3,5-$iPr_2pz)_3$)] as a white crystalline solid (yield 15.45%). Anal. Calcd for $C_{27}H_{46}N_6BK$; C, 64.27; H, 9.19; N, 16.65; Found: C, 64.20 ; H, 9.26; N,16.65. IR (KBr, cm^{-1}) ν(BH) 2453. ^1H NMR ($(CD_3)_2CO$, 270 MHz)δ, 1.00 (d, 18H, CH*Me*$_2$), 1.05 (d, 18H, CH*Me*$_2$), 2.66 (m, 3H, *CH*Me_2), 3.14 (m, 3H, *CH*Me_2), 5.67 (s, 3H, Pz).

[(3,5-$iPr_2pzH)_4Mn(NO_2$-OBz)$_2$] (2c)

(0.197 g., 1.0 mmol) $MnCl_2.4H_2O$ and (0.604 g., 4.0 mmol) 3,5-iPr_2pzH were stirred in 25 ml CH_2Cl_2 + 5 ml CH_3OH for 1 hour. To this solution added 5 ml acetonitrile solution of sodium nitrobenzoate (0.378 g., 2.0 mmol) and stirred the whole reaction mixture for 12 hrs. at room temperature. The mixture was filtered over celite and the solvent evaporated to

dryness. The compound was dissolved in 10 ml CH_3CN and colourless crystals were obtained at -20 ^{0}C (yield 84%). Anal. Calcd for $C_{50}H_{72}N_{10}O_8Mn$: C, 60.26; H, 7.28; N, 14.06; Found: C, 59.69; H, 7.31; N, 13.42. IR (KBr, cm^{-1}), ν(NH of free pyrazole) 3211, ν_{as}(COO) 1576, ν_s(COO) 1473, μ_{eff} = 6.28 B.M. at 295 K.

[(3,5-iPr_2pzH)$_4$Mn(F-OBz)$_2$] (2d)

(0.197 g., 1.0 mmol) $MnCl_2.4H_2O$ and (0.604 g., 4.0 mmol) 3,5-iPr_2pzH were stirred in 30 ml CH_2Cl_2 + 5 ml CH_3OH for 1 hour. To this solution added 5 ml acetonitrle solution of sodium fluorobenzoate (0.324, 2.0 mmol) and stirred for 12 hrs.. The mixture was filtered over celite and solvent was evaporated upto dryness. The compound was dissolved in 5 ml acetonitrile and colourless crystals were obtained by slow cooling at -20 ^{0}C (yield 84%). Anal. Calcd for $C_{50}H_{72}N_8O_4F_2Mn$: C, 63.72; H, 7.70; N, 11.89; Found: C, 63.49; H, 7.67; N, 11.44. IR (KBr, cm^{-1}) ν(BH) 2556, ν(NH of free pyrazole) 3297, ν_{as}(COO) 1570, ν_s(COO) 1442, μ_{eff} = 5.78 B.M. at 295 K.

[(HB(3,5-iPr_2pz)$_3$)MnCl] (2e)

(0.197 g., 1.0 mmol) $MnCl_2.4H_2O$ and (0.504 g., 1.0 mmol) KHB(3,5-iPr_2pz)$_3$ were stirred in 25 ml CH_2Cl_2 + 5 ml CH_3OH for 1 hr. The mixture was filtered over celite and solvent was evaporated to dryness under vacuum. The compound was recrystallized from toluene at -20 ^{0}C in 80% yield. Anal. Calcd for $C_{27}H_{46}N_6BClMn$: C, 58.32; H, 8.34; N, 15.12; Cl, 6.59; Found: C, 58.12 ; H, 8.45; N,15.62; Cl, 6.47. IR (KBr, cm^{-1}) ν(BH) 2535.

[(HB(3,5-iPr_2pz)$_3$)Mn(F-OBz)] (2f)

To a 30 ml toluene solution of **2e** (0.489 g., 0.882 mmol) added 10 ml acetonitrile solution of sodium fluorobenzoate (0.143 g., 0.882 mmol) and stirred the reaction mixture for 16 hrs.. The mixture was filtered over celite and solvent was evaporated under vacuum. The

colourless microcrystalline solid was obtained by slow cooling of acetonitrile solution at -20 ^{0}C in 45% yield. Anal. Calcd for $C_{34}H_{51}N_6BFO_2Mn$: C, 61.82; H, 7.78; N, 12.27; Found: C, 61.65; H, 7.90; N, 12.07. IR (KBr, cm^{-1}), ν(BH) 2541, ν_{as}(COO)1600, ν_s(COO) 1469. μ_{eff} = 6.03 B.M. at 295 K. FD-MS (m\z) = 660.

[(HB(3,5-iPr$_2$pz)$_3$Mn$_2$(μ-FOBz)$_3$(3,5-iPr$_2$pzH)$_2$] (2g)

$MnCl_2.4H_2O$ (0.168 g., 0.855 mmol), complex **2e** (0.474 g., 0.855 mmol), sodium fluorobenzoate (0.4191 g., 2.57 mmol) and 3,5-iPr$_2$pzH (0.260g., 1.710 mmol) in a mixture of toluene and acetonitrile (1:1) were stirred for 12 hrs. at room temperature. The mixture was filtered over celite and solvent was evaporated to dryness. The colourless crystals were obtained by slow cooling of acetonitrile solution at -20 ^{0}C in 65 % yield. Anal. Calcd $C_{66}H_{93}N_{10}BF_3O_6Mn_2$; C, 60.96; H, 7.20; N, 10.77; Found: C, 60.85; H, 6.95; N, 10.85. IR(KBr, cm^{-1}), ν(BH) 2538, ν_{as}(COO) 1594, ν_s(COO) 1469. μ_{eff} = 6.36 / Mn^{2+} B.M.at 295 K.

[(HB(3,5-iPr$_2$pz)$_3$)MnNCS] (2h)

The 10 ml methanolic solution of KSCN (0.082 g., 0.846 mmol) was added to a dichloromethane solution (20 ml) of complex **2e** (0.470 g., 0.846 mmol) and stirred for 6 hrs. at room temperature. The reaction mixture was filtered over celite and evaporated upto dryness under vacuum. The colourless crystals were obtained from the acetonitrile solution at -20 ^{0}C in 82% yield. Anal. Calcd for $C_{32}H_{52}N_9BSMn$: C, 58.17; H, 7.93; N, 19.08; Found: C, 58.05; H,7.70; N, 18.90. IR (KBr, cm^{-1}), ν(BH) 2550, ν(NCS) 2041, ν(CN) 2279. μ_{eff} = 6.14 B.M. at 295 K. FD-MS (m\z) = 660

[(HB(3,5-iPr$_2$pz)$_3$)MnCN] (2i)

To 20 ml dichloromethane solution of **2e** (0.450 g., 0.810 mmol) added 10 ml methanolic solution of potassium cyanide (0.053 g., 0.810 mmol) and stirred the whole reaction mixture for 6 hrs. at room temperature. The reaction mixture was filtered over celite and evaporated upto dryness under vaccum. The colourless microcrystalline soild was obtained from the acetonitrile solution at -20 ^{0}C in 60 % yield. Anal. Calcd for $C_{28}H_{46}N_7BMn$: C, 61.54; H, 8.48; N, 17.94; Found: C, 61.20; H, 8.30; N, 17.30. IR (KBr, cm^{-1}), ν(BH) 2533; ν(CN) 2044. μ_{eff} = 6.25 B.M. at 295 K. FD-MS (m\z) = 546.

[(HB(3,5-iPr$_2$pz)$_3$)MnN$_3$](2j)

An aqueous solution (5 ml) of NaN_3 (0.0523 g., 0.805 mmol) was added to a dichloromethane solution (20 ml) of complex **2e** (0.447 g., 0.805 mmol) and the mixture was stirred for 1 hour at room temperature. The water layer was removed and to the dichloromethane layers, sodium sulphate anhydrous (~ 1.00 g.) was added and stirred for 1 hour. Sodium sulphate was removed by fitration over celite and the filtrate was evaporated upto dryness under vacuum. The colourless microcrystalline solid was obtained by slow cooling of acetonitrile solution at -20 ^{0}C in 85% yield. Anal. Calcd for $C_{27}H_{46}N_9BMn$: C, 57.65; H, 8.24; N, 22.44; Found: C, 57.50; H, 8.66; N, 22.65. IR (KBr, cm^{-1}), ν(BH) 2522.; ν(N_3) 2077. μ_{eff} = 5.93 B.M. at 295 K. FD-MS (m\z) = 563.

Chapter 3

[(HB(3,5- iPr$_2$pz)$_3$)CoNO$_3$] (3a)

(0.2912 g., 1.0 mmol) $Co(NO_3)_2.6H_2O$ and (0.504 g., 1.0 mmol) KHB(3,5-iPr$_2$pz)$_3$ were stirred in 30 ml CH_2Cl_2 for 6 hrs.. The mixture was filtered over celite and solvent was evaporated upto dryness under vacuum. The compound was recrystallized from acetronitrile

at at -20 ^{0}C and the purple crystals were obtained in 75% yield. Anal. Calcd for $C_{29}H_{49}N_8O_3BCo$: C, 55.51; H, 7.87; N, 17.86; Found: C, 55.12; H, 7.65; N, 16.97. IR (KBr, cm^{-1}) ν(BH) 2535, ν(CN) 2280, $\nu_{as}(NO_3)$ 1530, $\nu_s(NO_3)$ 1261. UV-vis (toluene, nm, # /M^{-1}cm^{-1}) 572 (209), 732 (21). μ_{eff} = 3.60 B.M. at 295 K.

[(HB(3,5-iPr$_2$pz)$_3$Co)$_2$(OH)$_2$] (3b)

The 10 ml aqueous solution of NaOH (1N) was added to dichloromethane solution (25 ml) of complex **3a** (0.51 g., 0.81 mmol). The mixture was stirred at room temperature for 1 hour. Sodium hydroxide layer was removed and to the toluene layer, sodium sulphate anhydrous (~ 1.0 g.) was added and stirred for 1hour. Sodium sulphate was removed by filtration over celite and the filtrate was evaporated under vacuum. The purple coloured crystalline compound was obtained from dichloromethane (5 ml) and at -20 ^{0}C. Anal. Calcd for $C_{54}H_{94}N_{12}B_2O_2Co_2$: C, 59.89; H, 8.75; N, 15.52; Found: C, 59.32; H, 8.35; N, 15.65. IR (KBr, cm^{-1}) ν(BH) 2547, ν(OH) 3707. UV-vis (toluene, nm, #/M^{-1}cm^{-1}) 451 (70), 552 (42), 732 (19). μ_{eff} = 6.10 B.M. at 295 K.

[(3,5-iPr$_2$pzH)$_2$Co$_2$(μ-3,5-iPr$_2$pz)$_2$(OBz-NO$_2$)$_2$] (3c)

$Co(NO_3)_2.6H_2O$ (0.291 g., 1.0 mmol), 3,5-iPr$_2$pzH (0.604 g., 4.0mmol) and sodium nitrobenzoate (0.378 g., 2.0 mmol) were stirred in a mixture of dichloromethane and acetonitrile in 4:1 ratio for 12 hrs.. The solution was filtered over celite and the solvent was evaporated to dry ness under vacuum. The prismatic violet coloured crystals were obtained by slow cooling of acetonitrile solution at -20 ^{0}C in 75% yield. Anal. Calcd for $C_{50}H_{70}N_{10}O_8Co_2$: C, 56.81; H, 6.67; N, 13.25; Found: C, 56.51; H, 6.75; N, 13.10. IR (KBr, cm^{-1}), ν(NH of free pyrazole) 3195, ν_{as}(COO) 1591, ν_s(COO) 1471. μ_{eff}=5.78 B.M. at 295 K.

$[(HB(3,5\text{-}iPr_2pz)_3)_2Co_2bis(\mu\text{-}BPNPP)]$ (3d)

The complex **2b** (0.849 g., 0.785 mmol) and bis(4-nitrophenyl)phosphate hydrate (0.534 g., 1.57 mmol) were stirred in a mixture of toluene and methanol in 4:1 ratio for 4 hrs.. The mixture was filtered over celite and solvent was evaporated upto dryness under vacuum. The blue coloured crystals were obtained from the mixture of acetonitrile and dichloromethane at -20 ^{0}C in 60% yield. Anal. Calcd for $C_{78}H_{108}N_{16}O_{16}B_2P_2Co_2$: C, 54.23; H, 6.30; N, 12.97; Found: C, 54.12; H, 6.32; N, 12.80; IR (KBr, cm^{-1}) ν(BH) 2535, ν (NO) 1595, ν (NO) 1340.

$[(HB(3,5\text{-}iPr_2pz)_3)CoNCS]$(3e)

A methanolic solution (10 ml) of KSCN (0.149 g., 1.54 mmol) was added to the dichloromethane solution (20 ml) of complex **2b** (0.833 g., 0.770 mmol) and the whole solution was stirred for 6 hrs. The mixture was filtered over celite and solvent was evaporated upto dryness under vacuum. The bluish-green coloured microcrystalline solid was obtained from acetonitrile solution at -20 ^{0}C in 65% yield. Anal. Calcd for $C_{28}H_{46}N_7BSCo$: C, 57.73; H, 7.95; N, 16.83; Found: C, 57.52; H, 8.04; N, 16.52. IR (KBr, cm^{-1}) ν(BH) 2535, ν(NCS) 2058. FD-MS((m\z) = 582.

$[(HB(3,5\text{-}iPr_2pz)_3)_2CoN_3]$(3f)

The mixture of complex **2b** (0.831 g., 0.768 mmol) and sodium azide (0.099 g., 1.53 mmol) were stirred in dichloromethane (20 ml) and methanol (10 ml) for 6 hrs.. The solution was filtered over celite and solvent was evaporated upto dryness under vacuum. The bluish-green coloured microcrystalline compound was obtained by slow cooling of acetonitrile solution at -20 ^{0}C in 60% yield. Anal. Calcd for $C_{27}H_{46}N_9BCo$: C, 57.24; H, 8.18; N, 22.25; Found: C, 58.12; H, 8.04; N, 21.82. IR (KBr, cm^{-1}) ν(BH) 2535, ν(N-N-N) 2076. FD-MS((m\z) = 566.

[(HB(3,5-iPr$_2$pz)$_3$)$_2$CoCN](3g)

The methanolic solution (10 ml) of KCN (0.098 g., 1.52 mmol) was added to dichloromethane solution (20 ml) of complex **2b** (0.822 g., 0.760mmol) and stirred the reaction mixture for 8 hrs.. The mixture was filtered over celite and solvent was evaporated upto dryness under vacuum. The bluish-green coloured microcrystalline compound was obtained by slow cooling of acetonitrile solution at -20 ^{0}C in 55% yield. Anal. Calcd for $C_{28}H_{46}N_7BCo$: C, 61.09; H, 8.42; N, 17.81; Found: C, 60.65; H, 8.24; N, 17.52. IR (KBr, cm^{-1}) ν(BH) 2535, ν(CN) 2045. FD-MS((m\z) = 550.

Chapter-4

Synthesis of 3-tert-butyl-5-isopropylpyrazole [3-tBu-5-iPrpzH] (4a)

A solution of pinacolin (136.50 g., 1.28 mol) was added dropwise to a suspension of lithium amide (50.0 g., 2.17 mol) in 250 ml diethyl ether over 1 hour with continous stirring. Then ethylisobutyrate (196.54 g., 1.69 mol) was added dropwise to the resulting mixture over 90 minutes. The whole reaction mixture was refluxed for 7 hrs. and the thick sludge was hydrolyzed by dilute HCl solution. The compound was extracted with diethyl ether (3 x 100 ml). The ether layer was treated with a saturated NaCl aqueous solution three times and dried over $MgSO_4$. After drying, the diethyl ether was removed by evaporation. The resulting solution was distilled under reduced pressure at 70 °C afforded 139.3 g. 2,2-dimethyl-6-methyl-3,5-heptandione.

Hydrazine monohydrate (69.65 g., 1.39 mol) was added dropwise to a solution of 2,2-dimethyl-6-methyl-3,5-heptandione (139.3 g., 0.81 mol) in 100 ml ethanol. After 10 hrs. refluxing, the solution was treated with 100 ml saturated NaCl aqueous solution and was extracted with diethyl ether (3 x 100 ml). After drying over $MgSO_4$ for 2 hrs., the solvent

was evaporated to dryness. The resulting white solid was dissolved in acetonitrile and allowed to stand overnight at –20 °C. The white needles in 74% yield were filtered off and dried under vacuum. ^{1}H NMR ($CDCl_3$, 270 MHz) δ, 1.26 (d, J = 7Hz, 6H, CH*Me*$_2$), 1.31 (s, 9H, C*Me*$_3$), 2.97 (sept, J = 7 Hz, 1H, C*H*Me$_2$), 5.88 (s 1H, Pz), 9.07 (br, 1H, NH). 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, ν(CH) 2930, ν(CH) 2905.

Synthesis of Potassium hydrotris(3-tert-butyl-5-isopropyl-1-pyrazolyl)borate [(KHB(3-tBu-5-iPrpz)$_3$)] (4b)

A mixture of 3-tert-butyl-5-isopropylpyrazole (35.6 g., 0.21 mol) and KBH_4 (3.85 g., 0.071 mol) was heated in an oil bath with continous stirring. The temperature was elevated gradually up to 260 °C and the heating was continued at the same temperature until no hydrogen evolution was observed. The mixture was allowed to cool to room temperature and the resulting solid was dissolved in dichloromethane, 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 °C. Then it was filtered off and dried under vacuum (yield 64%). ^{1}H NMR (C_6D_6, 270 MHz) δ 1.00 (d, J = 7Hz, 18H, CH*Me*$_2$), 1.16 (s, 27H, C*Me*$_3$), 2.02 (s, 3H, *Me*CN), 3.09 (sept, J = 7 Hz, 3H, CH*Me*$_2$), 5.77 (s, 3H, Pz). Anal. Calcd for $C_{30}H_{52}N_6BK$: C, 65.90; H, 9.58; N, 15.37; Found: C, 65.45; H, 9.42; N, 15.44. IR (KBr, cm^{-1}) ν(CH) 2962, ν(BH) 2476.

[(HB(3-tBu-5-iPrpz)$_3$)CdCl] (4c)

$CdCl_2$ (0.183 g., 1.0 mmol) and KHB(3-tBu-5-iPrpz)$_3$ (0.547 g., 1.0 mmol) was stirred in a mixture of dichloromethane and methanol (4:1 ratio) for 24 hrs.. The mixture was filtered over celite and dried under vacuum. The colourless microcrystalline was obtained from

acetonitrile solution at –20 °C in 76% yield. Anal. Calcd for $C_{30}H_{52}N_6BClCd$: C, 54.97; H, 7.99; N, 12.82; Found: C, 54.06; H, 7.90; N, 12.10. IR (KBr, cm^{-1}) ν(BH) 2570. FD-MS((m\z) = 655

[(HB(3,5-iPr_2pz)$_3$)NiCl] (4d)

(0.237 g., 1.0 mmol) $NiCl_2.6H_2O$ and (0.504 g., 1.0 mmol) KHB(3,5-iPr_2pz)$_3$ were stirred in a mixtutre of dichloromethane and methanol in 5:1 ratio for 5 hrs.. The mixture was filtered over celite and solvent was evaporated to dryness under vacuum. The compound was dissolved in 5 ml toluene and orange-red crystals were obtained at –20 °C in 74% yield. Anal. Calcd for $C_{27}H_{46}N_6BClNi$: C, 57.95; H, 8.28; N, 15.02; Cl, 6.33; Found: C, 58.22; H, 8.37; N, 14.90; Cl, 5.96. IR (KBr, cm^{-1}) ν(BH) 2545.

[(HB(3-tBu-5-iPrpz)$_3$)NiCl] (4e)

$NiCl_2.6H_2O$ (0.237 g., 1.0 mmol) and KHB(3-Bu^t-5-Pr^ipz)$_3$ (0.5468 g., 1.0 mmol) were stirred in a mixture of dichloromethane and methanol in 4:1 ratio for 5 hrs.. The mixture was filtered over celite and solvent was evacuated under vacuum. The purple coloured microcrystalline solid was obtained from the acetonitrile solution at –20 °C in 75% yield. Anal. Calcd for $C_{30}H_{52}N_6BClNi$: C, 59.88; H, 8.71; N, 13.97; Cl, 5.89; Found: C, 59.18; H, 9.03; N, 13.63; Cl, 6.28. IR (KBr, cm^{-1}) ν(BH) 2567.

[HB(3,5-iPr_2pz)$_3$Ni)$_2$(OH)$_2$] (4f)

20 ml aqueous solution of NaOH (1N) was added to a toluene solution (20 ml) of complex **4d** (0.488 g., 0.874 mmol). The mixture was stirred at room temperature for 1 hour. Sodium hydroxide layer was removed and to the toluene layer, sodium sulphate anhydrous (~2.0 g.) was added and again stirred 1hr.. The sodium sulphate was removed by filtration

over celite and the filtrate was evaporated under vacuum, green coloured microcrystalline compound in 36.69% yield was obtained at –20 °C. Anal. Calcd for $C_{54}H_{94}N_{12}O_2B_2Ni_2$; C, 59.92; H, 8.75; N, 15.53; Found: C, 59.85; H, 8.65; N, 15.38. IR (KBr, cm^{-1}) ν(OH) 3702, ν(BH) 2544.

[(HB(3-tBu-5-iPrpz)$_3$)NiOH] (4g)

10 ml aqueous solution of NaOH (1N) was added to dichloromethane solution (20 ml) of complex **4e** (0.478 g., 0.794 mmol). The mixture was stirred at room temperature for 30 minutes and the aqueous layer was removed. To dichloromethane layer, sodium sulphate anhydrous ~ 1.0 g. was added and again stirred for 1hour. The sodium sulphate was removed by filtration and the filtrate was evaporated under the vacuum. Purple microcrystalline compounds was obtained from pentane solution at –20 °C in 68.64% yield. Anal. Calcd for $C_{30}H_{53}ON_6BNi$: C, 61.78; H, 9.16; N, 14.41; Found: C, 61.96; H, 8.97; N, 13.99. IR (KBr, cm^{-1}) ν(OH) 3676, ν(BH) 2544.

[(HB(3-tBu-5-iPrpz)$_3$)CdOH] (4h)

10 ml aqueous solution of 1N NaOH was added to dichloromethane solution (20 ml) of complex **4c** (0.520g., 0.794 mmol). The mixture was stirred at room temperature for 30 minutes and the aqueous layer was removed. To dichloromethane layer, sodium sulphate anhydrous ~ 1.0 g. was added and again stirred for 1hour. The sodium sulphate was removed by filtration over the celite and the filtrate was evaporated under the vacuum. The colourless microcrystalline compounds in 70% yield was obtained from acetonitrile solution at –20 °C. Anal. Calcd for $C_{30}H_{53}ON_6BCd$: C, 56.57; H, 8.39; N, 13.19; Found: C, 56.96; H, 8.15; N, 13.89. IR (KBr, cm^{-1}) ν(OH) 3674, ν(BH) 2542. FD-MS(m\z) = 637

Kinetic Experiments for Ester Cleavage.

Kinetic measurements for the hydrolysis of different esters were performed at 25 °C with a Lambda 35 Perkin Elmer UV-Vis Spectrophotometer by monitoring either the rate of appearance of p-nitrophenol / p-nitrophenolate. Spectra were recorded immediately after mixing the solution and then followed until formation of p-nitrophenol / p-nitrophenolate ion completed and no change in the spectra was observed [10]. The observed rate constant K_{obs} (S^{-1}) was calculated from the decay slope [11, 12].

Reference:

1. Perrin, D. D., Armarego, W. L. and Perrin, D. R., "Purification of laboratory chemicals", 2nd ed., Pergamon, New York (1980).
2. Figgis, B. N. and Lewis, J., "Modern coordination chemistry", Ed. By Lewis, J. and Wilkins, R. G., Interscience, New York (1960).
3. Higashi, T., Program for absorption correction, Rigaku Corp., Tokyo, Japan (1995).
4. TeXsan; Crystal structure analysis package, Ver. I:II; Rigaku Corp., Tokyo, Japan (2000).
5. International tables for X-ray crystallography, Kynoch Press: Birmingham, Vol. 4 (1975).
6. (a) Sheldrick, G. M. SHELXS-86: Program for crystal structure determination; University of Göttingen: Göttingen, Germany (1986). (b) Sheldrick, G. M. SHELXS-97: Program for crystal structure determination; University of Göttingen: Göttingen, Germany (1997).

7. Beurskens, P. T., Admiral, G., Beurskens, G., Bosman, W. P., Garcia-Grands, S., Gould, R. O., Smits, J. M. M., Smykalla, C., The DIRDIF program system, Technical report of the crystallography laboratory, University of Nimegen, Nimegen, The Netherland (1992).

8. Sheldrick, G. M., Acta Cryst., A, **46**, 467 (1990).

9. Sheldrick, G. M., SHELXTL – NT 2000 version 6.12, reference manual, University of Göttingen: Göttingen, Germany.

10. Brown, R. S., Zamkanei, M. and Cocho, J. L., "Tris(imadazole)-containing phosphine: M^{2+} complexes as biomimetic catalysts. Importance of a L:M^{2+}-OH^- in the catalyzed biomolecular hydrolysis of p-nitrophenylpicolinate", J. Am. Chem. Soc., **106**, 18 (1984).

11. Ichikawa, K., Khabir Uddin, M. and Nakata, K., "Zinc complexes of artificial histidine-containing dipeptides as catalysts of hydrolyses of p-nitrophenyl phosphates", Chem. Lett., 115 (1999).

12. Ito, M., Sakai, K., Tsubomura, T. and Takita, Y-S., "Transesterification by (hydroxo)nickel (II) complex in the presence of external alcohol", Bull. Chem. Soc. Jpn., **72**, 239 (1999).