Synthetic Complexes of Relevance to Ni(II)-Containing Enzymes

Katarzyna Rudzka
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_I dedicate this dissertation to my mom._

_Kasia_
ABSTRACT

Synthetic Complexes of Relevance to Ni(II)-Containing Enzymes

by

Katarzyna Rudzka, Doctor of Philosophy

Utah State University, 2008

Major Professor: Dr. Lisa M. Berreau
Department: Chemistry and Biochemistry

The work outlined herein presents an investigation of synthetic model complexes of relevance to the active sites of Ni(II)-containing enzymes, particularly urease, glyoxalase I, and acireductone dioxygenase. The research focuses on studying the structural and reactivity features of nickel complexes with biologically relevant substrates.

The anion of acetohydroxamic acid is a well-known inhibitor of urease enzymes, including those isolated from Klebsiella aerogenes and Bacillus pasteurii. A precursor to the acetohydroxamate coordination in ureases is proposed to be an interaction between Ni(II) and acetohydroxamic acid. By using a novel supporting chelate ligand capable of secondary hydrogen bonding interactions a novel pseudo-octahedral, Ni(II) acetohydroxamic acid complex has been isolated and characterized. Detailed analysis of the structural features and acetohydroxamic displacement reactivity of this complex has provided fundamental chemical insight toward understanding of the inhibition mechanism in urease enzymes.
Glyoxalase I (Glx I) catalyzes one step of the cellular detoxification pathway for α-ketoaldehydes (e.g. methylglyoxal) in humans and bacteria. The GlxI enzyme from *E. coli* is a Ni(II)-containing enzyme that catalyzes the isomerization of a hemithioacetal to produce a thioester. Of relevance to this enzyme, the first example of a Ni(II) complex that promotes a hemithioacetal isomerization is reported herein. In order to monitor this type of reaction a new approach involving a deuterium-labeled hemithioacetal (PhC(O)CH(OH)SCD₃) and ²H NMR was employed.

Acireductone dioxygenases (ARDs) catalyze aliphatic oxidative C-C bond cleavage of an acireductone (1,2-dihydroxy-3-oxo-5-(methylthio)pent-1-ene) intermediate in the methionine salvage pathway. A unique aspect of these enzymes is that the regioselectivity of the dioxygenase reaction depends on the metal ion bound in the active site. Outlined herein are descriptions of the synthesis, characterization, and O₂ reactivity of a novel trinuclear Ni(II) enediolate complex of relevance to the proposed enzyme/substrate adduct in Ni(II)-ARD.

Efforts have also been made toward the preparation of C(1)-H acireductone compounds using a combined synthetic/enzymatic approach. A phenyl appended-C(1)-H acireductone was isolated and introduced to a Ni(II) precursor complex. This reaction produced spectroscopic changes consistent with the formation of a new Ni(II) acireductone complex. Preliminary studies of the O₂ reactivity of this complex are reported.
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CHAPTER 1
INTRODUCTION

The Coordination Chemistry of Nickel(II)

The coordination chemistry of nickel spans a variety of geometries, coordination numbers, and oxidation states. Nickel complexes are known with oxidation states ranging from -1 to +4. However, the most common oxidation state is Ni(II) ([Ar]3d8). The majority of early coordination chemistry work focused on Ni(II) complexes, although recent interest toward understanding the nickel centers in redox active enzymes has shifted attention to less common oxidation states (-1, 0, +1, +3 and +4). Ni(II) forms a large number of complexes with a coordination number of 4, 5 or 6, and their geometries include all major structural types. With regard to Lewis acidity, Ni(II) is considered to be a borderline metal ion. This is because it binds to both soft and hard ligands and sometimes, albeit rarely, to both in the same complex. Table 1-1 summarizes the information on the oxidation states and geometries that are common for Ni(II) complexes as well as examples from the literature.

Table 1-1. Oxidation States and Stereochemistry of Nickel(II) complexes.

<table>
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<th>Oxidation State</th>
<th>Coordination number</th>
<th>Geometry</th>
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<td>Ni(II), d8</td>
<td>4</td>
<td>Square planar</td>
<td>NiBr₂(PEt₃)₂, [Ni(CN)₄]²⁻</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tetrahedral</td>
<td>[NiCl₄]²⁻, NiCl₂(PPh₃)₂</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Square pyramidal (sp)</td>
<td>[Ni(CN)₅]³⁻, [Ni₂Cl₈]⁴⁺</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trigonal bipyramidal (tbp)</td>
<td>[Ni(CN)₅]³⁻, Ni(SiCl₃)₂(CO)₃</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Octahedral</td>
<td>Ni(NH₃)₆²⁺, [Ni(bipy)₃]²⁻</td>
</tr>
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Nickel in Biological Systems

Nickel is found in the active site of eight metalloenzymes. Of this group, nickel is redox active in carbon monoxide dehydrogenase, acetyl-CoA synthase, iron-nickel hydrogenase, superoxide dismutase and methyl coenzyme M reductase. For the other three enzymes (urease, glyoxalase I, and acireductone dioxygenase) the oxidation state of the nickel center is +2 and does not change during catalysis. Instead the nickel center(s) in these enzymes is/are proposed to be a Lewis acid that coordinates and facilitates deprotonation of a substrate (or inhibitor), or lowers the pKₐ of water to produce a Ni(II)-OH species. The following sections provide additional details about the current state of knowledge of these enzymes and relevant model studies.

Ni(II)-containing Enzymes with no Redox Activity

The role of nickel ions in the catalytic mechanism of urease

The isolation and crystallization of urease was the culmination of nearly a century of lasting endeavor to purify the enzyme. It was the very first enzyme to be isolated and crystallized in 1926 in research performed by the Nobel Prize winner James B. Sumner. However, the enzyme was not shown to contain nickel in its active site until fifty years later. Before this discovery, nickel was considered to be a toxic element with no specific biological role.

Urease is produced by plants, algae, fungi, and bacteria, and catalyzes the hydrolytic decomposition of urea. It is important to note that urea spontaneously decomposes in water producing cyanic acid and ammonia, however at 38 °C this reaction proceeds with a half life of ~3.6 years (Scheme 1-1, (A)). The unusually high hydrolytic
Scheme 1-1. (A) Reaction scheme for uncatalyzed urea hydrolysis; (B) Reaction scheme for the urease-catalyzed urea hydrolysis.

stability of urea is presumably caused by its high resonance stabilization energy (ca. 30-40 kcal/mol).\(^5\) This energy can be significantly reduced upon binding of urea to a nickel center of the urease enzyme. The enzymatic reaction results in the formation of ammonia and carbamic acid, the latter of which spontaneously decomposes at physiological pH to give a second molecule of ammonia and bicarbonate (Scheme 1-1, (B)). Urease is a highly efficient enzyme, with a \(k_{\text{cat}}/K_m\) value approximately \(10^{14}\) times higher than the rate of the uncatalyzed reaction.\(^6\) Other alternative substrates, such as acetamide, \(N\)-methylurea, or semicarbazide can also be utilized by urease, although with lower rates of hydrolysis (\(k_{\text{cat}}\) values \(-10^2\)-\(-10^3\)-fold lower).\(^4\)

Several X-ray structures of ureases have been deposited in the Protein Data Bank (PDB). There are structures of the native proteins isolated from a variety of bacterial organisms, as well as urease mutants and ureases complexed with small molecules.\(^7\) The active site of the enzyme (Figure 1-1) contains two nickel ions bridged by a carbamylated lysine and a hydroxide ion. It has been confirmed that carbon dioxide is
required for the binding of nickel to urease. Upon the reaction with carbon dioxide, the lysine residue is converted into the carbamate, which captures the nickel ions in the active site. Each nickel additionally coordinates two histidine residues and a water molecule. The coordination sphere of Ni(2) is filled by an additional terminally bound aspartate resulting in a pseudo-octahedral environment. The other nickel ion (Ni(1)) has a vacant coordination site and a distorted square pyramidal geometry.

Due to its nickel content, urease is a unique example among the hydrolytic enzymes, which typically contain zinc as the essential cofactor. In this context, the question of the role of nickel in the catalytic mechanism remains intriguing for the bioinorganic community. An approach toward addressing this issue has involved attempts to substitute nickel with other divalent metal ions, such as Zn$^{2+}$, Co$^{2+}$, and Mn$^{2+}$. Removal of both Ni$^{2+}$ ions by treating the enzyme with EDTA at low pH causes irreversible denaturation of the protein. Removal of a single Ni$^{2+}$ ion by dialysis in the presence of citrate was reported, indicating that the two metal ions are bound with

![Figure 1-1. Schematic representation of the active site of urease.](image-url)
different affinities. These findings can be interpreted on the basis of the active site structure in which Ni(2) is six-coordinate while Ni(1) is ligated only by five residues. The more labile metal ion can be substituted with Zn$^{2+}$ or Co$^{2+}$ using long term dialysis. The mixed-metal derivatives, as well as the single-Ni$^{2+}$ protein, are catalytically inactive, indicating that the presence of both nickel ions is essential for the hydrolysis reaction.

High-resolution X-ray structures of native ureases (PDB entries: 1FWJ and 2UBP) and the structures of enzymes complexed with either a substrate analog (1S3T) or inhibitor (1IE7), have enabled researchers to propose a mechanistic pathway for the enzymatic hydrolysis of urea. In the proposed mechanism, the carbonyl oxygen of urea binds to the coordinatively unsaturated Ni(1) with displacement of a water molecule (Scheme 1-2). Nucleophilic attack by the Ni(2)-terminal hydroxide at the sp$^2$ carbon atom of urea yields a tetrahedral transition state. Protonation of the leaving group is the driving force for the urea C-N bond breakage and the release of ammonia.

Many ureolytic microorganisms contribute to the development of various human disorders, such as the formation of urinary stones and gastric ulceration. In addition, a high activity of soil ureolytic microorganisms can have negative effects in agriculture decreasing the efficiency of urea fertilizers. Due to its harmful role in medicine and agriculture, urease is a target for inhibitory studies. Several classes of molecules have been tested as competitive inhibitors for these enzymes, including acetohydroxamic acid (AHA). It has been suggested that AHA initially binds to the active site of urease (B. pasteurii and K. aerogenes) as a neutral molecule resulting in formation of a weak
Scheme 1-2. The proposed catalytic mechanism for the enzymatic hydrolysis of urea based on X-ray crystallographic data.\textsuperscript{15}

enzyme/inhibitor (E-I) complex (Scheme 1-3).\textsuperscript{13,16,17} The coordination of the neutral inhibitor to Ni(1) may be assisted by a hydrogen bonding interaction between the bridging hydroxide and the hydroxyl group of AHA. This E-I complex is then proposed to slowly convert to a more stable E-I* structure in which the negatively charged hydroxamate O\textsubscript{B} oxygen bridges the two Ni ions. The carbonyl oxygen is bound to Ni(1) to form a five-membered chelate ring. X-ray structures of ureases complexed with acetohydroxamate (AH\textsuperscript{−}) isolated from \textit{B. pasteurii} \textsuperscript{16} (4UBP) and \textit{K. aerogenes} \textsuperscript{17} (1FWE) have been reported.
Synthetic models for the active site of urease

Many aspects of the catalytic pathway of urease are still under debate. Recent synthetic modeling studies employ well-designed nickel complexes to address questions about the mode of urea binding, the exact identity of the nucleophile and the mechanism of the urease-catalyzed reaction. Despite the report of several model complexes for urease, none of them is capable of mediating the direct hydrolytic degradation of urea. The only synthetic compound with urease-like activity promotes the slow ethanolysis of urea to give four equivalents of ethyl carbamate after 12 h at 80 °C (Figure 1-2). However a fully functional model complex for urease is not yet available.

In efforts to elucidate the inhibition process in the urease system, a number of Ni(II) complexes of acetohydroxamate derivatives have been reported. One of the synthetic models that reveals the coordination of AH− to the dinickel site is presented in Figure 1-3. The acetohydroxamate moiety features a bridging coordination mode akin to the acetohydroxamate-inhibited urease.

Scheme 1-3. The conversion of a weak enzyme/inhibitor complex (E-I) into a stable complex of urease and hydroxamate (E-I').
Glyoxalase I and its contribution to cellular detoxification

Glyoxalase I catalyzes a key step of the cellular detoxification pathway for α-ketoaldehydes in bacteria, animals and humans. The first step of this pathway is a non-enzymatic reaction of the α-ketoaldehyde (e.g. methylglyoxal (MG)) and glutathione (GSH) which results in the formation of a hemithioacetal (Scheme 1-4). Glyoxalase I (GlxI) catalyzes the isomerization of the hemithioacetal to a thioester product. Subsequently, the glyoxalase II enzyme (GlxII) promotes the hydrolysis of the thioester,
producing the corresponding α-hydroxy acid and GSH. These two enzymes are referred to as the glyoxalase system.

Due to their electrophilic nature, α-ketoaldehydes tend to form adducts with nucleic acids as well as certain protein residues, leading to deleterious effects in cells. The consequences of MG cellular accumulation include lipid modification, oxidative damage, the inhibition of protein and RNA synthesis, and increased apoptosis.\textsuperscript{24,25}

Additionally, methylglyoxal as well as other 1,2-dicarbonyl compounds are often responsible for non-enzymatic, irreversible glycation of amino acids.\textsuperscript{26} The glycation reaction, followed by a number of rearrangements, dehydration and redox reactions, leads to the formation of advanced glycation end products (AGEs). AGEs are associated with the chronic complications of diabetes mellitus and have been reported to play an important role in the pathogenesis of Alzheimer’s disease.\textsuperscript{27} Inhibition of GlxI results in elevated levels of MG that can be lethal for cells. Notably, this toxicity can be utilized in the design of anticancer and antimalarial agents with a number of small molecule inhibitors of human glyoxalase I having been described in the literature.\textsuperscript{28-30}

\textbf{Scheme 1-4}. The reactions catalyzed by the glyoxalase system.
Two distinct classes of GlxI enzymes have been identified to date. A zinc-dependent glyoxalase I (e.g. human and rat liver GlxI) is a prevalent form of the enzyme and has been extensively studied. However more recent studies revealed that various bacterial GlxI enzymes exhibit maximal activity in the presence of Ni\(^{2+}\) (E. coli\(^{32,33}\), as well as Y. pestis, P. aeruginosa, and N. meningitis\(^3\)). GlxI from the human parasite L. major was also found to be Ni-dependant.

X-ray crystallographic studies have shown that the active site of the Ni\(^{2+}\)-containing GlxI enzyme from E. coli possesses a distorted octahedral geometry, with the metal ion being ligated by two histidine residues, two glutamates, and two water (hydroxide) molecules (Figure 1-4).\(^{35,36}\) Interestingly, the zinc-substituted form of the enzyme was found to have a different geometry around the metal center, specifically the five-coordinate zinc has all of the amino acid ligands found in Ni\(^{2+}\) GlxI but only one water (hydroxide) ligand.\(^{23}\) A correlation between catalytic activity and the coordination number at the metal binding site has been found.\(^{36}\) The E. coli enzyme containing Zn\(^{2+}\) as the metal cofactor is inactive, therefore it has been concluded that an octahedral geometry is a prerequisite for activity in that enzyme.

![Figure 1-4](image_url) (Left) Drawing of the active site of E. coli glyoxalase I, (right) drawing of the active site of Zn-dependent GlxI.
The proposed mechanisms for Ni(II)-dependent glyoxalase I-catalyzed hemithioacetal isomerization

Based on spectroscopic studies of product- and inhibitor bound forms of the *E. coli* enzyme, the catalytic reaction is proposed to involve the formation of a nickel hydroxide species. This nickel hydroxide can serve as a general base to promote a proton abstraction from the hemithioacetal substrate (Scheme 1-5). Extended X-ray absorption fine structure (EXAFS) and X-ray absorption near-edge spectroscopy (XANES) studies of the Ni$^{2+}$ enzyme showed that the first coordination sphere of the metal center remains unchanged upon addition of substrate.$^{37}$ Hence the enediolate intermediate generated by the deprotonation of the hemithioacetal presumably is not coordinated to the Ni(II) center in the course of the reaction. The enediolate anion is subsequently protonated at the former carbonyl carbon to yield the thioester product.

Another interesting mechanistic hypothesis has been formulated following the inspection of the human Zn$^{2+}$ glyoxalase co-crystallized with S-(N-hydroxy-N-p-iodophenylcarbamoyl)gluthatione (HIPC-GSH, Figure 1-5).$^{38}$ HIPC-GSH is a transition state analogue. In the X-ray structure, the hydroxycarbamoyl function is directly coordinated to the active site zinc ion. X-ray crystallography also revealed the displacement of one of the glutamate residues from the metal center; hence this residue was postulated to play the role of the general base in the mechanistic pathway.

Based on EXAFS studies, a similar displacement was suggested for the *E. coli* Ni$^{2+}$ enzyme complexed with a acetohydroxamate derivative of gluthiatione (Figure 1-6).$^{39}$ However, the differences in metal binding affinities of hydroxamate derivatives and the enediolate intermediate might question the idea that hydroxamates are accurate
Scheme 1-5. Proposed mechanism for *E. coli* glyoxalase I.\(^{37}\)

models for enediolate binding. As a consequence, caution is required in interpreting these studies.\(^{23}\)

Given the lack of a clearly defined general base in the GlxI reaction pathway, the synthetic modeling studies come to play an important role in elucidation of mechanistic questions. Due to the fact that mononuclear Ni(II)-OH complexes remain scarce in the
literature, these investigations are rather challenging. To date, only one mononuclear Ni(II)-OH has been characterized, although it has not been shown to promote the isomerization of hemithioacetal of relevance to the GlxI reaction.40

![Figure 1-5](image)

**Figure 1-5.** (Left) $S$-($N$-hydroxy-$N$-$p$-iodophenylcarbamoyl)glutathione (HIPC-GSH); (Right) Drawing of the active site of human GlxI complexed with HIPC-GSH.

![Figure 1-6](image)

**Figure 1-6.** $L$-glutamyl-$N$-methyl-$L$-glutaminglecine.
Acireductone dioxygenase (ARD) enzymes are associated with the methionine salvage pathway (MSP), a ubiquitous biological cycle. ARDs catalyze oxygen-dependent aliphatic C-C bond cleavage in 1,2-dihydroxy-3-oxo-5-(methylthio)pent-1-ene (acireductone). Over-expression of the ARD gene in *E. coli* produces two ARD enzymes, one containing Fe$^{2+}$ and a second containing Ni$^{2+}$ as the metal cofactor. A unique feature of the reactions catalyzed by these enzymes is that the regioselectivity of the carbon-carbon bond cleavage depends on the metal ion bound in the active site. While Ni$^{2+}$-ARD catalyzes a reaction that is a shunt out of the methionine salvage pathway, Fe$^{2+}$-ARD operates on-pathway enabling the recovery of methionine (Scheme 1-6). The Ni$^{2+}$-ARD reaction gives as products methylthiopropionic acid, formate and carbon monoxide. None of these products is a precursor for methionine, and more importantly methylthiopropionic acid is cytotoxic. Alternatively, carbon monoxide has been found to play a role as a neurotransmitter in mammals. It is proposed that the activity of Ni$^{2+}$-containing ARD might aid in the regulation of methionine levels, however the precise function of this enzyme is still unclear. Fe-ARD converts the acireductone intermediate to an α-ketoacid that is a precursor for methionine, and formate. Interestingly, the oxidative breakdown of an acireductone proceeds also under non-enzymatic conditions, resulting in the formation of the same products as the reaction catalyzed by iron-containing ARD.

Methionine, apart from being an essential amino acid in the human diet, is also an important intermediate in biosynthetic pathways. *S*-Adenosyl methionine (SAM), a
coenzyme involved in methyl group transfer reactions, is formed enzymatically from adenosine triphosphate (ATP) and methionine, and plays a critical role in numerous metabolic methylations (e.g. DNA methylation). SAM is also a source of the aminopropyl group for the syntheses of polyamines (spermidine and spermine) which are associated with the cell cycle and cell growth. The byproduct of aminopropyl transfer is methylthioadenosine (MTA), a potent regulatory metabolite that affects both polyamine synthesis and transmethylation reactions. A subsequent breakdown of the MTA molecule proceeds via multiple steps of methionine salvage pathway and provides the carbon skeleton as well as the thiomethyl group for methionine regeneration. The MTA breakdown leads to the formation of an acireductone intermediate that is the substrate for both nickel- and iron-containing ARD enzymes and represents a branch point in the
Ni\textsuperscript{2+}- and Fe\textsuperscript{2+}-containing acireductone dioxygenases are monomeric enzymes that have an identical amino acid sequence. Hence, the only chemical difference between them is the nature of the metal cofactor.\textsuperscript{50} The fact that nickel- and iron-containing ARDs can be separated using hydrophobic interaction and strong anion exchange chromatography columns suggests that significant structural differences exist between these two isoforms. These two enzymes can be interconverted by metal removal and reconstitution with the other metal ion. The ARD protein binds Ni\textsuperscript{2+} more tightly than Fe\textsuperscript{2+}, as demonstrated by the fact that Fe\textsuperscript{2+} can be removed from the folded enzyme by dialysis against EDTA, whereas removal of Ni\textsuperscript{2+} requires the protein to be unfolded.\textsuperscript{42} Furthermore, addition of apo-ARD to an equimolar mixture of Fe\textsuperscript{2+} and Ni\textsuperscript{2+} produced >80% of the Ni-containing form within one minute, indicating a substantial preference for Ni\textsuperscript{2+} binding over Fe\textsuperscript{2+}.\textsuperscript{42} Attempts to reconstitute ARD with other metal ions have been also reported.\textsuperscript{51} Ni\textsuperscript{2+}-type reactivity is exhibited by Co\textsuperscript{2+} or Mn\textsuperscript{2+} containing ARD analogs, and Mg\textsuperscript{2+}-ARD exhibits reduced Fe\textsuperscript{2+}-type activity.

**Structural features of Ni\textsuperscript{2+}-ARD**

To date, the only crystallographic structure of an ARD enzyme deposited in the Protein Data Bank does not provide information about the identity of the metal ion in the active site (ARD mouse homolog, PDB entry 1VR3).\textsuperscript{52} Therefore, the proposed structural features of nickel-containing ARD are based on X-ray absorption spectroscopy (XANES and EXAFS), NMR studies (PDB entries 1M40 and 1ZRR) and homology modeling.\textsuperscript{41} XAS experiments revealed a pseudo octahedral geometry for the Ni\textsuperscript{2+} ion in the ARD.
active site, with six N/O donor ligands, at least three of which are histidine imidazole nitrogens. Due to the paramagnetic nature of Ni\textsuperscript{2+}, the \textsuperscript{1}H NMR resonances of residues within 9 Å of the metal center are broad and significantly shifted (45-75 ppm) region. These signals are assigned to imidazole protons of the histidine ligands. NMR studies together with conserved domain homology modeling (based on the structure of jack bean canavalin) were used to predict a structure of the Ni\textsuperscript{2+}-ARD active site. Figure 1-7 (left) shows the primary coordination environment of the nickel-containing ARD. The Ni\textsuperscript{2+} center is ligated by three histidine residues (His 96, 98, and 140) and one glutamate (Glu 102). Two water molecules complete the Ni\textsuperscript{2+} coordination sphere. The secondary environment (Figure 1-7 (right)) consists of hydrophobic (Phe142, Phe92, Trp162) as well as hydrogen-bond donor residues (Arg104, Arg154). It is proposed that these secondary residues influence the positioning of the substrate in the active site.
Mechanistic studies of the reaction catalyzed by Ni\textsuperscript{2+}-containing ARD

The acireductone substrate (1,2-dihydroxy-3-oxo-5-(methylthio)pent-1-ene, (Figure 1-8 (A)) belongs to a class of compounds called reductones. These compounds contain an enediol functionality stabilized by conjugation and hydrogen bonding with an adjacent carbonyl group (RC(OH)=C(OH)C(=O)R). They are strong reducing agents and fairly strong acids (e.g. ascorbic acid).\textsuperscript{55} To date, a synthetic route for the native ARD substrate has not been reported. However, straightforward syntheses of analogs have been described in the literature (Figure 1-8, (B)-(D)).\textsuperscript{56} These analogs have been proven to serve as alternative substrates for the ARD enzymes. Due to their acidic nature, acireductones exist as singly deprotonated species in aqueous solutions at physiological pH.\textsuperscript{50}

Structural, spectroscopic, and mechanistic studies reported to date have enabled the formulation of a proposed mechanistic pathway for the reaction catalyzed by nickel-containing ARD (Scheme 1-7).\textsuperscript{50} Based on the identification of a UV-vis spectral shift upon substrate binding to the active site, it was concluded that the substrate coordinates to Ni\textsuperscript{2+} as a dianion in the anaerobic ES complex. The binding of the substrate results in the loss of two-coordinated ligands (either the two water molecules (Scheme 1-7) or one water molecule and His98 (as previously proposed\textsuperscript{47})). In the anaerobic ES complex, the acireductone is believed to bind in 1,3-coordination mode.

Coordination of the substrate to Ni\textsuperscript{2+} is followed by electron transfer from the doubly deprotonated acireductone to O\textsubscript{2} and subsequent binding of peroxide at the C-1
Figure 1-8. (A) 1,2-dihydroxy-3-oxo-5-(methylthio)pent-1-ene (acireductone, native substrate for ARD enzymes); (B)-(D) Synthetic acireductone analogs (proven to serve as substrates for ARD enzymes).

carbon of the substrate. Rearrangement of this species gives rise to a five-membered cyclic peroxide intermediate, which breaks down to produce formate, carbon monoxide and methythiopropionate. In order to confirm this mechanistic proposal, $^{18}$O$_2$ labeling experiments were performed. This revealed the incorporation of one oxygen atom into each carboxylate product (Scheme 1-7, bold face oxygen atoms represent the result of isotopic labeling studies). In the case of iron-containing ARD, acireductone is proposed to bind to the metal center and interact with O$_2$ to produce a four-membered peroxide ring. As a result, the products of the oxidative C-C bond cleavage reaction in Fe$^{2+}$-ARD differ from those of the Ni$^{2+}$-catalyzed reaction. Specifically, in the iron-catalyzed reaction C(1)-C(2) bond cleavage occurs, versus C(1)-C(2) and C(2)-C(3) in the Ni$^{2+}$ catalyzed reaction.
Scheme 1-7. A mechanistic proposal for the Ni$^{2+}$-ARD reaction.$^{50}$

Scheme 1-8. Proposed mechanism for iron-dependent ARD reaction.$^{54}$
Synthetic models for the active site of nickel-containing ARD

As mentioned above, the coordination mode of the acireductone molecule in the active site of ARD appears to trigger the distinct chemical reactions of these two enzymes. To investigate the possible coordination motifs of acireductone, studies of mononuclear Ni^{2+} complex with the acireductone substrate analog (2-hydroxy-1,3-diphenylpropan-1,3-dione) were performed.\textsuperscript{57} This molecule is a sterically hindered model of the ARD substrate and can be conveniently prepared according to a literature procedure.\textsuperscript{58} Combining equimolar amounts of the 6-Ph\textsubscript{2}TPA ligand,\textsuperscript{59} Ni(ClO\textsubscript{4})\textsubscript{2}·6H\textsubscript{2}O, Me\textsubscript{4}NOH·5H\textsubscript{2}O, and 2-hydroxy-1,3-diphenylpropan-1,3-dione under an inert atmosphere, gave a mononuclear Ni^{2+} complex, [(6-Ph\textsubscript{2}TPA)Ni(PhC(O)C(OH)C(O)Ph)]ClO\textsubscript{4} (Scheme 1-9). Single crystals suitable for X-ray analysis were obtained by pentane diffusion into a CH\textsubscript{2}Cl\textsubscript{2} solution of complex. Based on X-ray crystallographic studies, this Ni^{2+} complex has a distorted octahedral geometry with the acireductone monoanion bound in 1,3-coordination mode (Figure 1-9). This Ni^{2+} complex has been fully characterized, including by \textsuperscript{1}H NMR, and electronic absorption spectroscopy. The \textsuperscript{1}H NMR spectrum contains a number of relatively broad resonances characteristic of

Scheme 1-9. Synthesis of [(6-Ph\textsubscript{2}TPA)Ni(PhC(O)C(OH)C(O)Ph)]ClO\textsubscript{4} complex.
Figure 1-9. (Left) ORTEP drawing of \([(6-\text{Ph}_2\text{TPA})\text{Ni}(\text{PhC(O)C(OH)C(O)Ph})]\text{ClO}_4\) (Right) ORTEP drawing of the Ni(II) coordination environment in the cationic portion of \([(6-\text{Ph}_2\text{TPA})\text{Ni}(\text{PhC(O)C(OH)C(O)Ph})]\text{ClO}_4\). Ellipsoids are depicted at the 50% probability level. All hydrogen atoms except the hydroxyl proton have been omitted for clarity. Reprinted with permission from *J. Am. Chem. Soc.* 2005, 127, 17186. Copyright 2005 American Chemical Society.

6-Ph$_2$TPA-Ni$^{2+}$ complexes. The resonances in a range of 30-60 ppm have been assigned to $\beta$-protons of the pyridyl rings. The electronic absorption spectrum of \([(6-\text{Ph}_2\text{TPA})\text{Ni}(\text{PhC(O)C(OH)C(O)Ph})]\text{ClO}_4\) has a distinctive feature at 399 nm ($\varepsilon = 2400$ M$^{-1}$cm$^{-1}$).

This complex undergoes reaction with O$_2$ to give a Ni$^{2+}$ benzoate complex ([(6-Ph$_2$TPA)Ni(O$_2$CPh)]ClO$_4$), benzil (PhC(O)C(O)Ph), carbon monoxide and other yet unidentified Ph-containing species (Scheme 1-10). The oxidative aliphatic C-C bond cleavage within the acireductone ligand and the formation of carbon monoxide are
Scheme 1-10. O\textsubscript{2} reactivity of [(6-Ph\textsubscript{2}TPA)Ni(PhC(O)C(OH)C(O)Ph)]ClO\textsubscript{4}. Bold face oxygen atoms represent the result of isotopic labeling experiments.

relevant to the reaction catalyzed by the Ni\textsuperscript{2+}-containing ARD enzyme. In the absence of a general acid in this synthetic model system, the benzoate product remains coordinated to the Ni\textsuperscript{2+} center, which does not happen in the enzymatic system wherein the carboxylates are released from the active site. However, upon addition of acid, benzoate can be released from the synthetic complex.\textsuperscript{60} The Ni\textsuperscript{2+} benzoate complex ([6-Ph\textsubscript{2}TPA)Ni(O\textsubscript{2}CPh)]ClO\textsubscript{4}) has been identified through independent synthesis and was characterized by X-ray crystallography (Figure 1-10), as well as by \textsuperscript{1}H NMR, FAB-MS, FTIR and elemental analysis. Results of isotopic labeling experiments performed using \textsuperscript{18}O\textsubscript{2} confirmed the incorporation of one oxygen atom into the Ni\textsuperscript{2+}-coordinated benzoate product (Scheme 1-10).\textsuperscript{60} The level of \textsuperscript{18}O incorporation was found to be \textasciitilde50\%, as determined by mass spectrometry. Additionally, analysis of the headspace gas of the reaction shown in Scheme 1-10 using the PdCl\textsubscript{2} method indicated the formation of CO.\textsuperscript{61}

This mononuclear Ni\textsuperscript{2+}-acireductone complex, supported by the 6-Ph\textsubscript{2}TPA ligand, represents an attempt to model the structural, spectroscopic and reactivity features of the Ni\textsuperscript{2+}-ARD enzyme. There is still very little known about the precise function of ARD
enzymes and the mechanism of Ni$^{2+}$-ARD catalyzed reaction. Despite the fact that the nickel- and iron-containing ARD enzymes share the same amino acid sequence, they catalyze distinct chemical reactions. This metal content-dependant type reactivity has not been identified in any other enzymatic system. Moreover, nickel-containing acireductone dioxygenase is the only known example of nickel-containing dioxygenase.$^{47}$ Accordingly, these features make the Ni$^{2+}$-ARD enzyme an interesting candidate for synthetic modeling studies.

**Figure 1-10.** ORTEP representation of the cationic portion of [(6-Ph$_2$TPA)Ni(O$_2$CPh)]ClO$_4$. Ellipsoids are drawn at the 50% probability level. All hydrogen atoms have been omitted for clarity.$^{60}$ Reprinted with permission from *Inorg. Chem.* 2007, 46, 5486. Copyright 2007 American Chemical Society.
Summary

The redox inactive Ni(II)-containing enzymes described in this introductory chapter have in common a pseudo octahedral geometry of the active site metal center(s). In the dinuclear active site of urease, as well as in the mononuclear active site Ni(II) centers of glyoxalase I and acireductone dioxygenase, each nickel center has at least one open coordination site which is available for binding of a substrate or inhibitor. Several open questions remain regarding substrate/inhibitor binding and the subsequent reactivity for these metalloenzymes. For urease, the mechanism of inhibition involving acetohydroxamic acid (Figure 1-11(A)) is suggested to involve the formation of a weak enzyme/inhibitor complex, with coordination of acetohydroxamic acid to one of the two Ni(II) centers. This complex (designated $E$-$I$) is then proposed to undergo dehydration to form the hydroxamate inhibited species $E$-$I^*$ (Scheme 1-3). Our hypothesis concerning coordination of acetohydroxamic acid to a transition metal center was that hydrogen bonding is essential for the stabilization of such an adduct. Prior to the results presented herein, there were no examples of acetohydroxamic acid coordination to any transition metal center, thus our work, as outlined in Chapters 2 and 3, has provided the first structural precedent for this type of coordination.

Figure 1-11. (a) Acetohydroxamic acid, (b) hemithioacetal, (c) acireductone.
The glyoxalase system is a very intriguing target for mechanistic investigations. The hypothesis that we put forward is that a Ni(II)-OH or other deprotonated species is required for hemithioacetal isomerization. We further hypothesize that the Ni(II) center is involved in stabilizing a deprotonated form of the hemithioacetal substrate (Figure 1-11(B)). As outlined in Chapter 4 we have found that a Ni(II) coordination complex having a deprotonated chelate ligand can promote hemithioacetal isomerization. This reaction proceeds more quickly and cleanly than a reaction involving a simple hydrated Ni(II) salt and base. Spectroscopic monitoring of these reactions has suggested possible deprotonated hemithioacetal coordination in the reaction involving the hydrated Ni(II) salt but not in the reaction promoted by the Ni(II) coordination complex.

With regard to the Ni(II)-dependent acireductone dioxygenase, several mechanistic questions remain to be addressed. UV-vis spectroscopic investigations of the enzyme/substrate complex suggest that the acireductone (Figure 1-11(C)) coordinates to the Ni(II) center as a doubly deprotonated molecule. However, the coordination mode of the acireductone is unknown. We decided to test the hypothesis that the coordination mode of the acireductone influences its reactivity with O₂. A synthetic modeling approach, wherein Ni(II) acireductone dianion complexes are prepared, characterized, and studied in terms of O₂ reactivity, offers the opportunity to determine structural, spectroscopic, and reactivity relationships. As outlined in Chapter 5 we have succeeded in the preparation of such a complex using 2-hydroxy-1,3-diphenylpropan-1,3-dione to isolate a novel Ni(II) enediolate complex. Structural, spectroscopic and reactivity studies of this complex are presented. Additionally, in Chapter 6 efforts to prepare a Ni(II) complexes of acireductones relevant to the native ARD substrate are outlined.
Overall, the work outlined in this dissertation focuses on studying the interactions of small organic molecules (Figure 1-11) with redox-inactive Ni(II) centers in synthetic complexes. The results obtained provide chemical precedent on which biological reactions may be evaluated.

References


CHAPTER 2
NEUTRAL ACETOHYDROXAMIC ACID COORDINATION TO
A MONONUCLEAR NI (II) CENTER STABILIZED BY AN
INTRAMOLECULAR HYDROGEN-BONDING INTERACTION\(^1\)

Abstract

Treatment of a new chelate ligand having both amide- and phenyl-appended pyridyl moieties with Ni(ClO\(_4\))\(_2\)•6H\(_2\)O and acetohydroxamic acid in methanol solution results in the production of a novel pseudo-octahedral Ni(II) complex having a neutral acetohydroxamic acid ligand stabilized by a hydrogen-bonding interaction.

Introduction

The acetohydroxamato monoanion (AHA\(^-\)) is a well-known inhibitor of several metalloenzymes, including urease enzymes from plants and bacteria, which contain a binuclear Ni(II) center within the active site.\(^1\)-\(^5\) AHA\(^-\) inhibition of *Klebsiella aerogenes* and *Bacillus pasteurii* ureases has been suggested to involve initial formation of a weak enzyme/inhibitor complex (E-I, Scheme 2-1) having coordination of a neutral acetohydroxamic acid (AHA) molecule at a single Ni(II) ion.\(^6\)-\(^8\)

Stabilization of the E-I species may involve formation of a hydrogen-bonding interaction involving the bridging hydroxyl group.\(^8\) This E-I complex is then proposed to slowly convert to a more stable E-I* structure (Scheme 2-1), which exhibits a bridging

Scheme 2-1. Formation of a weak enzyme/inhibitor complex with acetoxy-hydroxamic acid in *Klebsiella aerogenes* and *Bacillus pasteurii* ureases.

coordination mode for the hydroxamate monoanion, a structural motif that has also been identified in model systems.\(^9,10\) In regard to the *E*-I species, to our knowledge, a structurally characterized complex having neutral acetoxy-hydroxamic acid coordination has not previously been reported for *any* transition metal ion.\(^11\) Herein we report the preparation and characterization of a novel synthetic mononuclear Ni(II) complex having a coordinated neutral acetoxy-hydroxamic acid ligand, the hydroxyl proton of which forms a strong hydrogen-bonding interaction with a noncoordinated pyridyl nitrogen of the supporting chelate ligand.

A new chelate ligand, \(N,N\)-bis[(6-phenyl-2-pyridyl)methyl]\(N\)-[(6-pivaoylamido)-2-pyridyl]methyl]amine (bppppa) having both amide\(^{-12}\) and aryl-substituted pyridyl moieties was assembled as shown in Scheme 2-2. Admixture of this ligand with equimolar amounts of \(\text{Ni(ClO}_4)_2\cdot6\text{H}_2\text{O}\) and acetoxy-hydroxamic acid in CH\(_3\)OH solution, followed by recrystallization via Et\(_2\)O diffusion into a CH\(_3\)CN : CH\(_3\)OH solution of the complex at ambient temperature resulted in the deposition of purple block crystals of \([(\text{bppppa})\text{Ni(CH}_3\text{C(O)NH(OH)})\text{](ClO}_4)_2\) (1) in 72% yield. Complex 1 has been characterized by X-ray crystallography, elemental analysis, FTIR, UV-vis, and a solution
magnetic moment measurement. X-ray crystallographic analysis of 1 revealed a mononuclear pseudo-octahedral Ni(II) center and a tetradentate N₃O-donor coordination mode for the bppppa chelate ligand with one phenyl-appended pyridyl moiety noncoordinated (Figure 2-1). The N-H and O-H hydrogen atoms of the acetohydroxamic acid ligand, as well as the amide N-H hydrogen of the bppppa ligand, were located and refined independently. Importantly, the noncoordinated pyridyl nitrogen acts as a hydrogen bond acceptor for the Ni(II)-coordinated hydroxyl group of the acid. This hydrogen bond may be classified as moderate based on the short heteroatom distance (O(2)-H(2)...N(5) 2.518(3) Å) and somewhat acute bond angle (158(5)°). A similar

Scheme 2-2. Synthesis of [(bppppa)Ni(CH₂C(O)NH(OH)))(ClO₄)₂ (1) supported by the (N,N-bis((6-phenyl-2-pyridyl)methyl)-N-((6-pivaloylamido)-2-pyridyl)methyl)amine) ligand.
Figure 2-1. ORTEP representation of the cationic portion of 1. All ellipsoids are drawn at the 35% probability level. All hydrogen atoms except the N-H and O-H protons not shown for clarity. Selected bond lengths (Å) and angles (°): Ni(1)-N(2) 2.027(3), Ni(1)-N(3) 2.130(3), Ni(1)-N(4) 2.128(3), Ni(1)-O(1) 2.040(2), Ni(1)-O(2) 2.091(2), Ni(1)-O(3) 2.037(2), O(2)-Ni(1)-O(3) 79.71(9).

hydrogen-bonding interaction has been reported involving a Mn(II)-coordinated methanol ligand and a noncoordinated phenyl-appended pyridyl moiety in [(6-Ph$_2$TPA)Mn(CH$_3$OH)$_3$](ClO$_4$)$_2$ {6-Ph$_2$TPA = N,N-bis((6-phenyl-2-pyridyl)methyl)-N-((2-pyridyl)methyl)amine}. The Ni-O(H) distance involving the hydroxamic acid ligand is elongated in 1 by ~0.072 Å relative to that found in a structurally related pseudo-octahedral Ni(II) hydroxamato (AHA$^-$) complex of the 6-Ph$_2$TPA ligand [(6 Ph$_2$TPA)Ni(ONHC(O)CH$_3$)]ClO$_4$, [2, Figure 2-2(a), Ni(1)-O(2) 2.0203(15) Å]. The Ni-O bond distance involving the acetohydroxamic acid carbonyl oxygen in 1 [Ni(1)-O(3) 2.037(2) Å] is also slightly longer than the analogous bond in 2 [1.9964(14) Å]. Within the hydroxamic acid/AHA$^-$ units in 1 and 2, the C-O and C-N bond distances are very
Figure 2-2. (a) Structure of [(6-Ph2TPA)Ni(ONHC(O)CH3)]ClO4 (2). (b) Comparison of core structural features of 1 and 2.

similar, with the largest difference outside experimental error being ~0.005 Å in the C-N bond. Overall, these combined structural parameters indicate that the neutral hydroxamic acid binds more weakly to the mononuclear Ni(II) center in 1 than does the monoanionic acetohydroxamato ligand (AHA⁻) in 2. Finally, we note that the average Ni-O bond distance for 1 (2.06 Å) is slightly longer than that of 2 (2.01 Å) and the symmetric Ni-O distance (2.0 Å) in acetohydroxamato-inhibited urease from Bacillus pasteurii.⁸,¹⁴

In regard to the structural features of 1 versus 2, it is also worth noting that the shorter Ni-N_{PhPy} distance in 1 [2.128(3) Å] as compared to those found in 2 [2.2630(17)/2.2292(17) Å] suggests the presence of a more Lewis acidic Ni(II) center in 1, consistent with the coordination of the neutral acetohydroxamic ligand.
The solid state infrared spectra of 1 and 2 differ in several ways. For example, as shown in Figure 2-3 the region of 3600-3000 cm\(^{-1}\) for 1 contains a broader, more intense feature than is found for 2 under identical conditions. This is consistent with the presence of the additional hydroxamic acid –OH and amide NH (within the bppppa ligand) moieties in 1, both of which participate in hydrogen-bonding interactions. In Figure 2-4 a \(\nu_{C=O}\) vibration can be identified at 1656 cm\(^{-1}\) for the Ni(II)-coordinated bppppa amide carbonyl group. The \(\nu_{(C=O)}\) vibration for the acetohydroxamic acid/AHA\(^{-}\) carbonyl

Figure 2-3. 3800-2600 cm\(^{-1}\) region of the infrared spectra of 1 and 2.

Figure 2-4. 1700-1200 cm\(^{-1}\) region of the infrared spectra of 1 and 2.
groups in 1 and 2 should be present near 1600 cm\(^{-1}\). However, this region in both complexes is complicated by a pyridyl ring vibration, which precludes conclusive assignments.

The energy of the \(3\text{A}_2g \rightarrow 3\text{T}_1g(F)\) and \(3\text{A}_2g \rightarrow 3\text{T}_2g(F)\) transitions differ in the electronic absorption spectra of 1 and 2 (Figure 2-5) in dry acetonitrile solution.\(^{15}\) The former transition is shifted to slightly higher energy in 1 (570 nm vs. 585 nm in 2). In addition, whereas in 1 the latter transition is found at \(\sim 920\) cm\(^{-1}\), in 2 this feature is shifted into the near-IR region.

**Experimental Section**

**General and physical methods.** Performed as previously described.\(^{14}\)

**Synthesis of ligand precursors.** The organic precursors 2-(pivalolylamido)-6-(bromomethyl)pyridine,\(^{12}\) 6-phenyl-2-pyridinecarboxyaldehyde,\(^{16}\) 6-phenyl-2-pyridinemethanol\(^{14}\) were prepared as previously reported.

**Figure 2-5.** Electronic absorption spectra of 1 and 2 in dry CH\(_3\)CN. The feature for 2 that extends into the UV range is a shoulder that is centered at \(\sim 372\) nm (\(\varepsilon \sim 330\) M\(^{-1}\) cm\(^{-1}\)).
(6-phenyl-2-pyridyl)methyl amine. A multistep synthetic procedure for the preparation of this compound from 6-phenyl-2-pyridinemethanol has been previously reported by Chuang et al.\textsuperscript{16} This literature procedure was followed for conversion of 6-phenyl-2-pyridinemethanol to 2-(chloromethyl)-6-phenylpyridine hydrochloride and conversion of this halide derivative to 2-(phthalimidomethyl)-6-phenyl pyridine. However, removal of the phthalimido protecting group was achieved via treatment of 2-(phthalimidomethyl)-6-phenyl pyridine (2.1 g, 6.5 mmol) with hydrazine monohydrate (0.42 g, 8.4 mmol) in ethanol (65 mL) solution. This reaction mixture was heated at reflux under a nitrogen atmosphere for 2 h. After cooling the solution to room temperature, water (45 mL) and 1M HCl were added until a pH~2 was attained. The resulting cloudy solution was warmed gently for 2 h. The reaction mixture was then allowed to cool to ambient temperature and was filtered. The yellow filtrate was treated with 1M NaOH until the pH~12. Extraction with CH\textsubscript{2}Cl\textsubscript{2} (3 x 100 mL), followed by drying of the combined organic solutions over Na\textsubscript{2}SO\textsubscript{4}, filtration, and removal of the solvent under reduced pressure yielded a dark yellow oil (1.17 g, 98%). The \textsuperscript{1}H NMR properties of the material in CDCl\textsubscript{3} matched those previously reported.\textsuperscript{16}

\textit{N,N-bis(6-phenyl-2-pyridyl)methyl}-\textit{N}-(\textit{N}-(6-pivaloylamido)-2-pyridyl)methyl)amine (bpppppa). \textbf{Step 1}. To an ethanol solution (~5 mL) of (6-phenyl-2-pyridyl)methyl amine (0.72 g, 3.9 mmol) was added an ethanol solution (~5 mL) of 6-phenyl-2-pyridinecarboxyaldehyde (0.72 g, 3.9 mmol). The resulting yellow mixture was stirred at 45(1) °C for ~50 min. The solution was then cooled to room temperature and solid NaBH\textsubscript{4} (0.16 g, 4.7 mmol) was added at which point the solution color became
orange. This mixture was stirred at ambient temperature for 24 h. To this solution was added 1 M HCl (~20 ml) until a pH~2 was reached. Following removal of all volatiles under reduced pressure, the resulting yellow semisolid was dissolved in CH₂Cl₂ (10 mL) and the solution was treated with 1 M NaOH until the pH > 11. The organic portion was removed and the aqueous fraction was extracted with Et₂O (3 x 50 mL). The combined organic fractions were dried over excess Na₂SO₄, filtered, and evaporated to dryness yielding a brown oil (1.35 g). The major product in this oil has ¹H NMR properties consistent with the formation of the reductive amination product ⁴N,⁴N-bis(((6-phenyl)-2-pyridyl)methyl)amine (¹H NMR (CD₃CN, 400 MHz) δ 8.09-8.06 (m, 4H), 7.78 (t, J = 7.7 Hz, 2H), 7.71 (d, J = 8.1 Hz, 2H), 7.47-7.38 (m, 6H), 7.34 (d, J = 7.7 Hz, 2H) 4.03 (s, 4H); a N-H resonance was not identified) However, an impurity with a benzylic resonance at 4.71 ppm and aromatic resonances that partially overlap with the signals outlined above is consistently present in the product isolated using this reaction pathway. Thus far we have been unable to find reaction and/or column conditions that are suitable for the clean isolation of ⁴N,⁴N-bis(((6-phenyl)-2-pyridyl)methyl)amine. However, the secondary amine product can be used in its crude form in a reaction to generate the bppppa ligand as described below.

**Step 2.** To a CH₃CN (50 mL) solution of 2-(pivaloylamido)-6-(bromomethyl)pyridine (0.33 g, 1.2 mmol) was added crude ⁴N,⁴N-bis(((6-phenyl)-2-pyridyl)methyl)amine (0.42 g, 1.2 mmol), Na₂CO₃ (0.50 g, 4.7 mmol) and ~5 mg of tetrabutylammonium bromide. The resulting mixture was heated at reflux under nitrogen for ~14 h. At this point, the reaction mixture was cooled to room temperature and 1 M NaOH (~50 mL) was added. The organic/aqueous mixture was extracted with CH₂Cl₂ (3
x 50 mL). The combined organic fractions were dried over Na₂SO₄, filtered, and the organic solvent was removed under reduced pressure yielding a thick brown oil. This crude product was purified via column chromatography on silica gel (240-400 mesh, CH₂Cl₂:CH₃OH 10:1, Rᶠ = 0.62; impurity with Rᶠ = 0.65 could not be separated). The final sample was isolated as a pale yellow oil (0.33 g, 73%) and contains ~15% impurity (¹H NMR properties suggest a structure for this impurity involving the (6-phenyl-2-pyridyl)methyl fragment). Attempts to purify the bppppa ligand further using various column conditions have thus proven unsuccessful. However use of this impure ligand did not cause problems in metal complexation reactions (see preparation of 1 below). The bppppa ligand can be isolated free of the impurity via treatment of crystalline 1 with NaCN in methanol/water to remove the Ni(II) ion (66% recovery yield). Characterization data was recorded for clean bppppa obtained from the metal complexation/removal strategy: ¹H NMR (CD₃CN, 400 MHz) δ 8.15 (br, N-H), 8.05-8.02 (m, 4H), 7.97 (d, J = 7.9 Hz, 1H), 7.71-7.76 (m, 3H), 7.71-7.67 (m, 3H), 7.54 (d, J = 7.5 Hz, 2H), 7.48-7.38 (m, 7H), 3.97 (s, 4H), 3.87 (s, 2H), 1.24 (s, 9H); ¹³C{¹H} NMR (CD₃CN, 100 MHz) δ 178.0, 160.5, 159.5, 157.0, 152.3, 140.4, 139.6, 138.4, 129.9, 129.7, 127.8, 122.7, 119.6, 119.5, 112.7, 61.1, 60.9, 40.5, 27.6 (19 signals expected and observed); FTIR (neat, cm⁻¹): 1690 (νc=O); LRFAF-MS (CH₂Cl₂:NBA) m/z (relative intensity): 542 ([M+H]⁺, 100%). Anal. Calcd. For C₃₅H₃₅N₅O•1.75 H₂O: C 73.34; H, 6.77, N, 12.22. Found: C, 73.34; H, 6.39; N, 11.73. The presence of water in the sample was confirmed by ¹H NMR.

*Caution! Perchlorate salts containing organic ligands are potentially explosive. These materials should be handled on a small scale and handled with great care.*
[(bpppa)Ni(HONC(O)CH₃)](ClO₄)₂ (1). To a methanol solution (~2 mL) of bpppa (41 mg, 0.075 mmol) was added a methanol solution (~2 mL) of Ni(ClO₄)₂•6H₂O (27.6 mg, 0.075 mmol). The resulting mixture was stirred for 40 min at room temperature at which point a methanol solution (~1 mL) of acetohydroxamic acid (5.7 mg, 0.075 mmol) was added. The pale blue solution was then stirred for overnight at room temperature. At this point, the solvent was removed under reduced pressure. Recrystallization of the residue via Et₂O diffusion into a CH₃OH:CH₃CN (1:2.5) of the complex yielded purple crystals suitable for single crystal X-ray diffraction (48 mg, 72%). UV-vis (CH₃OH) [λₘₐₓ, nm (ε, M⁻¹cm⁻¹)]: 570 (15), 920 (20); FTIR (KBr, cm⁻¹) 1656 (ν_c=O, bpppa amide); μₑᶠₕ = 3.3 μ_B (298K); Anal. Calcd for C₃₇H₄₀N₆O₁₁Cl₂Ni: C, 50.91; H, 4.62; N, 9.63. Found: C, 50.51; H, 4.87; N, 9.49.

Conclusions

In summary, we have found that a novel mononuclear Ni(II) complex having neutral acetohydroxamic acid coordination may be isolated using a chelate ligand that provides an internal hydrogen bond acceptor. The structural and spectroscopic properties of 1 are notably different from those of a structurally-related Ni(II) complex of the acetohydroxamato anion (2). This work provides the first chemical precedent upon which to evaluate acetohydroxamic acid versus acetohydroxamato anion coordination to a Ni(II) center, a topic that is important toward fully understanding the inhibition properties of urease enzymes.
Notes and references

† Crystal data: for 1: C_{38}H_{44}Cl_{2}N_{6}NiO_{12}, M = 906.40, orthorhombic, space group Pbca, a = 19.2023(5), b = 34.9478(9), c = 12.2308(2) Å, V = 8207.8(3) Å³, Z = 8, μ = 0.672 mm⁻¹. Using Mo-Kα radiation (0.71073 Å), a total of 16542 reflections were collected (4.84 < 2θ < 54.96) of which 9172 were independent. Refinement converged to R₁ = 0.0555, wR₂ = 0.1229 (I >2σI) and R₁ = 0.0962, wR₂ = 0.1440 (all data). Complex 1 crystallized with one molecule of uncoordinated methanol per formula unit.

11. A search of the Cambridge Crystallographic Database (v. 5.25, July 2004) revealed no examples of hydroxamic acid coordination to transition metal ion.


CHAPTER 3

CHEMISTRY OF A Ni(II) ACETOHYDROXAMIC ACID COMPLEX: FORMATION, REACTIVITY WITH WATER, AND ATTEMPTED PREPARATION OF ZINC AND COBALT ANALOGUES

Abstract

Mononuclear Ni(II), Co(II), and Zn(II) complexes of the bppppa (N,N-bis[(6-phenyl-2-pyridyl)methyl]-N-[(6-pivaloylamido-2-pyridyl)methyl]amine) ligand have been synthesized and characterized by X-ray crystallography, $^1$H NMR, UV-vis (Ni(II) and Co(II)), and infrared spectroscopy, and elemental analysis. Each complex has the empirical formula [(bppppa)M](ClO$_4$)$_2$ (M = Ni(II): 2; Zn(II): 3; Co(II): 4) and in the solid state exhibits a metal center having a coordination number of five, albeit the cation of 2 also has a sixth weak interaction involving a perchlorate anion. Treatment of [(bppppa)Ni](ClO$_4$)$_2$ (2) with one equivalent of acetohydroxamic acid results in the formation of [(bppppa)Ni(HONHC(O)CH$_3$)](ClO$_4$)$_2$ (1) a novel Ni(II) complex having a coordinated neutral acetohydroxamic acid ligand. In 1, one phenyl-appended pyridyl donor of the bppppa chelate ligand is dissociated from the metal center and acts as a hydrogen bond acceptor for the hydroxyl group of the bound acetohydroxamic acid ligand. Treatment of 1 with excess water results in the formation of 2 and free acetohydroxamic acid. We hypothesize that this reaction occurs due to disruption of the intramolecular hydrogen bonding interaction involving the bound acid. In this series of

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reactions, the bppppa ligand exhibits behavior reminiscent of a Type III hemilabile ligand in terms of one phenyl-pyridyl donor. Treatment of 3 or 4 with acethydroxamic acid results in no reaction, indicating that the bppppa-ligated Ni(II) derivative 2 exhibits unique coordination chemistry with respect to reaction with acethydroxamic acid within this series of complexes. We attribute this reactivity to the ability of the bppppa-ligated Ni(II) center to adopt a pseudo-octahedral geometry, whereas the Zn(II) and Co(II) complexes retain five coordinate metal centers.

**Introduction**

Recently we reported the synthesis and characterization of a novel mononuclear Ni(II) complex containing a neutral bidentate acethydroxamic acid ligand, [(bppppa)Ni(HONHC(O)CH₃)](ClO₄)₂ (1, Figure 3-1).¹ A phenyl-appended pyridyl moiety of the supporting chelate ligand (bppppa, N,N-bis[(6-phenyl-2-pyridyl)methyl]-N-[6-pivaloylamido-2-pyridyl)methyl]amine) in 1 accepts a hydrogen bond from the hydroxyl group of the Ni(II)-coordinated acid. Complex 1 is interesting in that while structurally characterized examples of d-block metal complexes of the acethydroxamate anion (AHA⁻) are common, particularly for Ni(II)²⁻⁵, Zn(II)⁵⁻⁷, and Co(II)⁵⁻⁸, complex 1 represents the first example of a synthetic complex wherein neutral acethydroxamic acid (AHA) ligation has been identified.

Notably, neutral acethydroxamic acid coordination at a single Ni(II) center has been suggested for a weak enzyme/inhibitor complex (E-I) formed within the active site of urease enzymes obtained from *Klebsiella aerogenes* and *Bacillus pasteurii* (Figure 3-2).⁹⁻¹¹ Stabilization of this E-I species may involve a hydrogen-bonding interaction with
Figure 3-1. Drawing of [(bpppa)Ni(HONHC(O)CH3)](ClO4)2 (1).

Figure 3-2. E-I species proposed to form in urease enzymes from *Klebsiella aerogenes* and *Bacillus pasteurii*.

In the experiments reported herein, we have examined the formation of 1 from a Ni(II) precursor complex, [(bpppa)Ni](ClO4)2 (2). Paramagnetic 1H NMR and UV-vis measurements indicate that quantitative formation of 1 from 2 occurs in the presence of only a single equivalent of acetohydroxamic acid. However, the acid ligand of 1 can be displaced in the presence of water to regenerate 2 and free acetohydroxamic acid. Spectroscopic investigation of the reactivity of Zn(II) and Co(II) analogs of 2 with
acetohydroxamic acid revealed no evidence for formation of neutral acid-bound complexes of these metals.

**Experimental Section**

**General Methods.** All reagents and solvents were obtained from commercial sources and used as received without further purification. The bpppppa (N,N-bis[(6-phenyl-2-pyridyl)methyl]-N-[(6-pivaloylamido-2-pyridyl)methyl]amine) ligand was prepared according to the literature procedure.¹

**Physical Methods.** Diamagnetic ¹H and ¹³C NMR spectra were collected using a Bruker ARX-400 spectrometer. Paramagnetic ¹H NMR spectra were recorded as previously described.¹² UV-vis spectra of 1, 2, and 4 were recorded at ambient temperature using a Hewlett Packard 8453 diode array spectrophotometer. Solid-state infrared spectra were recorded on a Shimadzu FTIR-8400 spectrometer as KBr pellets. Elemental analyses were performed by Atlantic Microlabs of Norcross, GA.

*Caution!* Perchlorate salts of metal complexes with organic ligands are potentially explosive. Only small amounts of material should be prepared and these should be handled with great care.¹³

**General Method for Preparation of 2-4.** Stirring of a methanol solution of the bpppppa ligand (~ 0.06 mmol in ~4 mL methanol) with a molar equivalent amount of the appropriate metal perchlorate salt (M(ClO₄)₂•6H₂O, M = Ni, Zn, Co), followed by removal of the solvent under reduced pressure, and recrystallization via Et₂O diffusion into a CH₃CN/CH₃OH solution of the residue yielded the perchlorate complexes in >70% yield as single crystals suitable for X-ray crystallographic analysis.
[(bppppa)Ni](ClO₄)₂ (2). Yield: 72%. UV-vis, nm (ε, M⁻¹ cm⁻¹) 572 (20), 890 (26); FTIR (KBr, cm⁻¹) 3354 (v₅N-H), 1620, 1099 (v₅ClO₄), 621 (v₅ClO₄). Anal. Calcd for C₃₅H₃₅N₅O₉Cl₂Ni: C, 52.69; H, 4.43; N, 8.78. Found: C, 52.62; H, 4.22; N, 8.74.

[(bppppa)Zn](ClO₄)₂ (3). Yield: 82%. ¹H NMR (CD₃CN, 400 MHz): δ 8.82 (br s, 1H), 8.22-8.13 (m, 3H), 7.82-7.78 (m, 2H), 7.64-7.60 (m, 3H), 7.47-7.42 (m, 2H), 7.37-7.33 (m, 4H), 7.25 (d, J = 7.4 Hz, 4H), 4.58-4.43 (m, 6H), 0.54 (s, 9H); ¹³C{¹H} NMR (CD₃CN, 100 MHz): δ 185.4, 160.5, 157.6, 153.5, 152.7, 144.8, 143.3, 139.2, 131.5, 130.6, 129.0, 127.2, 124.6, 122.7, 117.7, 57.1, 56.7, 42.1, 26.7 (19 signals expected and observed); FTIR (KBr, cm⁻¹) 3352 (v₅N-H), 1622, 1105 (v₅ClO₄), 621 (v₅ClO₄). Anal. Calcd for C₃₅H₃₅N₅O₉Cl₂Zn: C, 52.16; H, 4.38; N, 8.69. Found: C, 52.29; H, 4.32; N, 8.68.

[(bppppa)Co](ClO₄)₂ (4). Yield: 76%. UV-vis, nm (ε, M⁻¹ cm⁻¹) 470 (110), 590 (50), 725 (30); FTIR (KBr, cm⁻¹) 3433 (v₅N-H), 1618, 1105 (v₅ClO₄), 623 (v₅ClO₄). Anal. Calcd for C₃₅H₃₅N₅O₉Cl₂Co: C, 52.62; H, 4.42; N, 8.77. Found: C, 52.63; H, 4.38; N, 8.67.

**X-ray Crystallography.** Crystals of 2-4 were each mounted on a glass fiber with viscous oil and then transferred to a Nonius KappaCCD diffractometer with Mo Kα radiation (λ = 0.71073 Å) for data collection at 150(1) K. The methodology used for determination of final unit cell constants has been previously reported.¹² For the data collected for 2-4, each reflection was indexed, integrated, and corrected for Lorentz, polarization, and absorption effects using DENZO-SMN and SCALEPACK.¹⁴ All of the nonhydrogen atoms of 2-4 were refined with anisotropic displacement coefficients.
**Structure Solution and Refinement.** Complexes 2-4 all crystallize in the space group \( P-1 \). For 2 and 4, all hydrogen atoms were located and refined independently using SHELXL97.\(^{15} \) For 3, all hydrogens except the amide proton were assigned isotropic displacement coefficients (\( U(H) = 1.2U(C) \) or \( 1.5U(C_{methyl}) \)), and their coordinates were allowed to ride on their attached carbons using SHELXL97. The amide proton of 3 was located and refined independently.

**Results**

**Synthesis.** In order to more fully investigate coordination chemistry related to the formation and reactivity of the novel acetohydroxamic acid complex

\[ ([\text{bppppa})\text{Ni}(\text{HONHC(O)CH}_3)](\text{ClO}_4)_2 \]  (1), a Ni(II) complex of the bppppa (\( N,N\)-bis(6-phenyl-2-pyridyl)methyl]-\( N\)-[6-pivaloylamido-2-pyridyl]methyl]amine) chelate ligand,

\[ ([\text{bppppa})\text{Ni}](\text{ClO}_4)_2 \]  (2), was prepared as shown in Scheme 3-1. For complementary reactivity studies, Zn(II) (\( [(\text{bppppa})\text{Zn}](\text{ClO}_4)_2 \), 3) and Co(II) (\( [(\text{bppppa})\text{Co}](\text{ClO}_4)_2 \), 4) analogs were generated in a similar fashion. Complexes 2-4 were isolated as crystalline solids via recrystallization from \( \text{CH}_3\text{CN/CH}_3\text{OH/Et}_2\text{O} \) solutions in yields that consistently exceeded 70%.

**X-ray Crystallography. Part I.** Parameters associated with the data collection and refinement of the X-ray structures of 2-4 are given in Table 3-1. Selected bond distances and angles are given in Table 3-2.

\[ ([\text{bppppa})\text{Ni}](\text{ClO}_4)_2 \]  (2). An ORTEP representation of the cationic portion of 2, along with one \( \text{ClO}_4^- \) anion, is shown in Figure 3-3(a). The Ni(II) ion has a coordination geometry that may be described as intermediate between square pyramidal and trigonal...
bipyramidal ($\tau = 0.54$).\footnote{Reference number} Notably, the largest angular distortion from 120° within the pseudo equatorial plane is found in the N(2)-Ni(1)-N(5) bond angle (137.77(6)°). This expanded angle is bisected by a weak interaction involving a perchlorate oxygen atom (Ni(1)…O(7) 2.975 Å). The Ni-O(amide) bond is ~0.08 Å shorter than the corresponding bond in 1.\footnote{Reference number} In addition, the average Ni-N_{PhPy} distance in 2 (2.086 Å) is shorter than the Ni-N_{PhPy} interaction in 1 (Ni(1)-N(4) 2.128(3) Å),\footnote{Reference number} and notably shorter than the Ni-N_{PhPy} distances in [(6-Ph$_2$TPA)Ni(ONHC(O)CH$_3$)]ClO$_4$ (2.2630(17) and 2.2292(17) Å, Figure 3-4(a)) and [(6-Ph$_2$TPA)Ni(CH$_3$CN)(CH$_3$OH)](ClO$_4$)$_2$•CH$_3$OH (2.200(5) and 2.218(5) Å, Figure 3-4(b)).\footnote{Reference number} Following a similar trend, the bonding interactions involving the Ni(II) center of 2 and the tertiary amine nitrogen and amide-appended pyridyl donor are both

**Scheme 3-1.** Synthetic route for preparation of complexes 2-4.
contracted relative to the same interactions in 1. In sum, these differences indicate an enhanced Lewis acidity for the five-coordinate Ni(II) center of 2 versus the six coordinate Ni(II) centers found in 1, [(6-Ph2TPA)Ni(ONHC(O)CH3)]ClO4 (Figure 3-4(a)) and [(6-Ph2TPA)Ni(CH3CN)(CH3OH)](ClO4)2•CH3OH (Figure 3-4(b)).1,5

In the solid-state structure of 2, a t-butyl methyl group is positioned to form weak CH/π interactions with the phenyl appendages of the bppppa ligand (C(3)…arene centroid 3.95, 3.80 Å).17 While a similar interaction is not present in 1, where the shortest C(methyl)…arene centroid distance is 4.26 Å, CH/π interactions involving the methyl group of a Ni(II)-bound CH3CN ligand have been identified in [(6-Ph2TPA)Ni(CH3CN)(CH3OH)](ClO4)2•CH3OH (C(methyl)…arene centroid, 3.66 and 3.63 Å; Figure 3-4(b)).

Solution Characterization of [(bppppa)Ni(HONHC(O)CH3)](ClO4)2 (1) and [(bppppa)Ni](ClO4)2 (2). (a) Paramagnetic 1H NMR. Recently we reported the solution characterization of a number of pseudo octahedral 6-Ph2TPA and TPA-ligated Ni(II) complexes using paramagnetic 1H NMR.12 In that work, we used as variety of deuterated ligand derivatives, as well as 2H NMR and two-dimensional 1H-1H COSY spectra, to assign the 1H NMR resonances of the supporting chelate ligands. As shown in Figure 3-5(a), we were able to clearly assign the β-H resonances (protons on meta positions) of the pyridyl rings in [(6-Ph2TPA)Ni(CH3CN)CH3OH)](ClO4)2 in the region of ~39-60 ppm. The β and β’ designations differentiate the pyridyl (β) and phenylpyridyl (β’) donors in 1. Notably, a similar, albeit more complex set of resonances is observed for 1 under identical conditions (Figure 3-5(b)). We assign the resonances at 63.7, 53.9, 38.7, and 34.9 ppm in 2 as β or β’-H’s on the basis of similarity in chemical shift to the β/β’-
Figure 3-3. ORTEP representations of the cationic portions of 2-4. For all, ellipsoids are plotted at the 50% probability level and hydrogen atoms, except the amide N-H proton, have been omitted for clarity.
Figure 3-4. Drawings of 6-Ph₂TPA, (a) [(6-Ph₂TPA)Ni(ONHC(O)CH₃)]ClO₄, and (b) [(6-Ph₂TPA)Ni(CH₃CN)(CH₃OH)](ClO₄)₂·CH₃OH.
# Table 3-1. Summary of X-ray Data Collection and Refinement

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$^a$Radiation used: Mo Kα (λ = 0.71073 Å).

$^b$R1 = Σ ||F$_o$| - |F$_c$|| / Σ |F$_o$|;

wR2 = [Σ[w(F$_o^2$ - F$_c^2$)²]/Σ(F$_o^2$)]$^{1/2}$

where w = 1/[σ(F$_o^2$) + (aP$^2$ + bP)]

# Table 3-2. Selected Bond Distances (Å) and Angles (°)

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H’s of [(6-Ph2TPA)Ni(CH3CN)(CH3OH)](ClO4)2·CH3OH, as well as other Ni(II)
derivatives of the 6-Ph2TPA and TPA ligands.12 However, unlike the β and β’-H
resonances of [(6-Ph2TPA)Ni(CH3CN)(CH3OH)](ClO4)2·CH3OH, which appear in a
1:2:1:2 relative intensity ratio, the four sharpest signals assigned as β/β’-H resonances for
1 appear with a 1:1:1:1 integrated relative intensity. This can be explained by the fact that
all three pyridyl rings are inequivalent for 1 and that these four sharp signals represent the
β/β’-H protons of only two of the pyridyl rings. The β-H resonances of the third pyridyl
ring remain to be identified. However, we hypothesize that this missing set of β-H resonances for 1 is for the noncoordinated phenylpyridyl donor that is involved in the
hydrogen-bonding interaction with the bound acetohydroxamic acid. Because these
protons are positioned further from the nickel center than the other pyridyl β/β’-H’s, their
1H NMR signals are likely in or near the diamagnetic region, which is obscured by
additional ligand and solvent resonances. For 2, which has a five-coordinate Ni(II) center
in the solid state (with a weak sixth perchlorate oxygen interaction), the paramagnetic 1H
NMR features of the complex in dry CD3CN are strikingly different from those of 1 and
other pseudo octahedral Ni(II) complexes that we have investigated.12 First, there are
only two cleanly identifiable β-type resonances at 68.0 and 46.3 ppm, respectively. These
signals exhibit a 1:1 integrated intensity ratio. Several additional broad features are
present in the range of ~0-40 ppm, which have not been assigned. Notably, both 1 and 2
exhibit a resonance upfield of 0 ppm (Figure 3-5(b) and(c)). Based on the fact that these
signals disappear in the presence of D2O, they are assigned as the amide N-H protons in
these complexes. Addition of a large excess of D2O (~600 eq) to a CD3CN solution of the
acetohydroxamic acid complex 1 also produces additional changes consistent with the
release of the acid ligand (vide infra). Importantly, these preliminary paramagnetic $^1$H NMR studies indicate that 1 and 2 exhibit unique paramagnetic $^1$H NMR signatures that may be used, in conjunction with complementary electronic absorption spectroscopic

![Chemical Structures](image)

**Figure 3-5.** Paramagnetic $^1$H NMR spectra of (a) [(6-Ph$_2$TPA)Ni(CH$_3$CN)(CH$_3$OH)](ClO$_4$)$_2$, (b) 1, and (c) 2. All spectra were collected in dry CD$_3$CN solution at 25(1) °C.
Figure 3-6. Electronic absorption spectroscopic features of 1 and 2.

Experiments (vide infra), to monitor the solution reactivity 2 with acetohydroxamic acid, a reaction that results in the formation of 1.

(b) Electronic Absorption Spectroscopy. The electronic absorption spectroscopic properties of 2 are subtly different from those of 1, as shown in Figure 3-6, thus giving a secondary technique with which to monitor reactions involving these complexes. In particular, the $^3\text{A}_{2g} \rightarrow ^3\text{T}_{2g}(\text{F})$ transition at $\sim 890$ nm in 2, is shifted into the near-IR region in 1.$^{18}$

Reactivity of 2 with Acetohydroxamic Acid Results in the Formation of 1.

Evaluation by Paramagnetic $^1$H NMR and Electronic Absorption Spectroscopy. Treatment of [(bppppa)Ni(ClO$_4$)$_2$, 2, with one equivalent of acetohydroxamic acid in CD$_3$CN solution results in spectral changes in the paramagnetic $^1$H NMR consistent with the formation of the acetohydroxamic acid complex, 1 (Scheme 3-2). As shown in Figure 3-7, the formation of [(bppppa)Ni(HONHC(O)CH$_3$)](ClO$_4$)$_2$ (1, Figure 3-7(b)) is
indicated by the appearance of four $\beta$-H resonances of equal intensity in the region of 30-70 ppm. The chemical shifts of these resonances exactly match those observed for analytically pure 1 under identical conditions (Figure 3-7(d)). Formation of 1 appears to be complete upon the addition of one equivalent of acetohydroxamic acid to 2 (Scheme 2), as the addition of a second equivalent of acetohydroxamic acid results in no additional changes in the observed $^1$H NMR features (Figure 3-7(c)). As shown in Figure 3-8, electronic absorption spectroscopic changes produced upon treatment of 2 with one equivalent of acetohydroxamic acid also indicate the clean formation of 1.

**Displacement of Acetohydroxamic Acid from 1 Occurs in the Presence of Water to Yield 2.** Treatment of 1 with excess equivalents of water results in $^1$H NMR spectroscopic changes (Figure 3-9(a)-(d)) that indicate the displacement of the acetohydroxamic acid ligand from 1 and the formation of 2. As shown in Figure 3-9(b),

Scheme 3-2. Formation of [(bpppa)Ni(HONHC(O)CH$_3$)](ClO$_4$)$_2$ (1) in dry CH$_3$CN and displacement of acetohydroxamic acid upon addition of H$_2$O.
Figure 3-7. $\beta$-H region of the paramagnetic $^1$H NMR spectra of (a) 2, (b) 2 in the presence of one equivalent of acetohydroxamic acid, (c) 2 in the presence of two equivalents of acetohydroxamic acid, and (d) analytically pure 1. All spectra were recorded at 25(1) °C in CD$_3$CN.

Addition of 100 eq. of D$_2$O to a CD$_3$CN solution of 1 yields subtle spectral changes in the $\beta$-H region as compared to those observed for analytically pure [(bppppa)Ni(HONHC(O)CH$_3$)](ClO$_4$)$_2$ (1) under identical conditions (Figure 3-9(a)). Addition of increasing equivalents of water, up to 600 equivalents total (Figures 3-9(c) and 3-9(d)) results in the generation of a paramagnetic $^1$H NMR spectrum identical to that exhibited by analytically pure 2 under identical conditions. (Figure 3-9(e)) Examination of the diamagnetic region of these same spectra (Figure 3-10) supports a similar interpretation. This conclusion is further supported by the fact that precipitation of analytically pure 1 from a CH$_3$CN/acetone/water (~20%/~10%/70%) solution, followed by recrystallization of the precipitated solid from CH$_3$CN/Et$_2$O, yields crystalline 2. The
identification of this crystalline solid as 2 was confirmed by X-ray crystallographic
determination of the unit cell parameters, which matched those previously determined for
2. As shown in Scheme 3-2, these results indicate hemilabile behavior for the supporting
bppppa ligand when coordinated to Ni(II). In the presence of acetohydroxamic acid,
the five-coordinate Ni(II) complex 2 undergoes dissociation of one of the phenyl-
appended pyridyl donors resulting in stabilization of the acetohydroxamic acid complex 1
via an intramolecular hydrogen-bonding interaction. However, in the presence of excess
water, the acetohydroxamic acid ligand is displaced and complex 2 is regenerated.

**Figure 3-8.** Electronic absorption spectroscopic changes produced upon treatment of 2
with one equivalent of acetohydroxamic acid. These changes produce an absorption
spectrum (dashed line) that is indistinguishable from that observed for analytically pure 1
in CH$_3$CN solution (dashed line with diamond-shaped markers).
Will Acetohydroxamic Acid Complexes Form with Other d-Block Metals?

Evaluation of the Reactivity of Zn(II) and Co(II) Analogs of 2 with Acetohydroxamic Acid. To our knowledge, 2 is the first d-block metal complex to be reported that reacts with acetohydroxamic acid to produce an isolable metal complex having a neutral, coordinated acetohydroxamic acid ligand.1 To test whether this reactivity will be common in other d-block metal complexes of the bppppa chelate ligand, we have generated Zn(II) ([((bppppa)Zn](ClO₄)₂, 3) and Co(II) ([((bppppa)Co](ClO₄)₂, 4), analogs of 2. These metals were chosen due to the propensity of acetohydroxamate complexes known of these d-block metals.5-8

![Figure 3-9](image)

**Figure 3-9.** Spectrum (a) shows the β-H region of the paramagnetic ¹H NMR spectrum of 1 in CD₃CN at 25(1) °C. Spectra (b)-(d) show this solution plus (b) 100 eq, (c) 350 eq, and (d) 600 eq D₂O. Spectrum (e) is for analytically pure 2 in CD₃CN containing 600 eq D₂O.
**X-ray Crystallography. Part II.** The cationic portions of 3 and 4 are shown in Figure 3-3(b) and (c). As with the Ni(II) derivative 2, the metal centers of 3 and 4 both exhibit an overall coordination number of five. Within each cation, there are weak CH/π interactions akin to those found for 2.\(^{17}\) The geometry of the cations of 3 and 4 is best described as distorted trigonal bipyramidal (3: \(\tau = 0.63;\) 4: \(\tau = 0.67\)).\(^{16}\) As previously noted, the Ni(II) center of 2 is intermediate between square pyramidal and trigonal bipyramidal (\(\tau = 0.54\)). Key structural differences for the zinc and cobalt cations, versus the nickel analog 2 are: (a) a subtle decrease in the linearity of the O(1)-M-N(3) interaction, (b) a more acute equatorial N\(_{\text{AmPy}}\)-M-N\(_{\text{PhPy}}\) angle (e.g. 2: N(2)-Ni-N(5) 137.77°; 3: N(2)-Zn-N(5) 126.03°), and (c) more open N(2)-M-N(4) (~110°) and N(4)-M-N(5) (~112°) bond angles in the equatorial plane for the cations of 3 and 4. In addition, for the Zn(II) and Co(II) complexes, no interaction is found between the cation and either of the perchlorate anions. Thus, the nickel derivative 2 is unique within this subset of complexes in having a weak sixth interaction involving a perchlorate anion. This interaction, which bisects the N(2)-Ni-N(5) angle, provides a rationale for the differences in the equatorial bond angles of 3 and 4, versus those of 2, as described above.

**Solution Characterization of [(bppppa)Zn](ClO\(_4\))\(_2\) (3) and [(bppppa)Co](ClO\(_4\))\(_2\) (4) in the Absence and Presence of Acetohydroxamic Acid.**

The \(^1\)H NMR features of 3 in CD\(_3\)CN solution at 25(1) °C are given in the experimental section. The most notable \(^1\)H NMR feature of this complex is an upfield shift of the \(t\)-butyl methyl protons of the supporting bppppa chelate ligand (3: 0.56 ppm) relative to chemical shift position of this signal for the free ligand (1.24 ppm). This shielding effect
may be attributed to the weak CH/π interaction between the t-butyl methyl protons and
the phenyl appendages of the bppppa ligand, as noted in the solid-state structure of 3.17

Addition of excess acetohydroxamic acid (10 eq) to a CD₃CN solution of 3 results
in the appearance of new signals at ~9.1, 7.6, and 1.8 ppm. These resonances are at
chemical shifts consistent with the presence of free acetohydroxamic acid in CD₃CN
solution. No changes were found in terms of the multiplicity or chemical shifts of the
resonances of 3 in the presence of the acid. This indicates that no reaction takes
place between the zinc complex and acetohydroxamic acid.

The Co(II) complex 4 exhibits three electronic transitions (470 (110), ~590 (50),
725 (30) nm; Figure 3-11), with extinction coefficients consistent with a five-coordinate
Co(II) center for 4 in CH₃CN solution.18 Measurement of the absorption spectrum of 4 in
the presence of ten equivalents of acetohydroxamic acid in CH₃CN produced no change
in the intensity or wavelength of the electronic transitions, suggesting that no reaction
occurs.

Paramagnetic ¹H NMR can also be used to probe the solution reactivity of Co(II)
compounds.21 Shown in the (a) portions of Figure 3-12 are the features found in two
different regions of the paramagnetic ¹H NMR spectrum of 4. Assignment of selected
resonances has been performed on the basis of integrated intensity and comparison of
spectral features to assigned resonances of structurally-related mononuclear Co(II)
complexes.21 Two sharp signals at 74.9 and 35.2 ppm, respectively, are assigned as the β-
H’s of the phenyl-appended pyridyl rings. A ¹H-¹H COSY spectrum of 4 (data not
shown) indicates that these signals are coupled to a γ-H (para to the pyridyl nitrogen)
resonance at 0.26 ppm. The t-butyl methyl resonance is present at 13.8 ppm. The signal at
Figure 3-10. Spectrum (a) shows the diamagnetic region of the paramagnetic $^1\text{H}$ NMR spectrum of 1 in CD$_3$CN at 25(1) °C. Spectra (b)-(d) show this solution plus (b) 100 eq, (c) 350 eq, and (d) 600 eq D$_2$O. Spectrum (e) is for analytically pure 2 in CD$_3$CN containing 600 eq D$_2$O.

-15.6 ppm disappears in the presence of D$_2$O and is assigned as the amide N-H resonance for 4. In the (b) and (c) portions of Figure 3-12 are the NMR spectra produced upon treatment of 4 with 10 and 20 equivalents of acetohydroxamic acid, respectively. Similar to the electronic absorption spectroscopic results, the NMR data indicates that no reaction occurs between 4 and the acid.
**Discussion**

We recently reported the isolation and structural characterization of \( [(bpppa)\text{Ni(HONHC(O)CH}_3)](\text{ClO}_4)_2 \), 1.\(^1\) This complex has biological relevance, as neutral acetohydroxamic acid coordination has been suggested for a weak enzyme/inhibitor (E-I) complex involving the active site nickel cluster of urease.\(^9\)-\(^11\)

From a coordination chemistry perspective, 1 is unique in being the first reported example of a \( d \)-block metal complex having a coordinated neutral acetohydroxamic acid ligand.\(^{22}\) In the work described herein, we have performed experiments to probe the formation and reactivity of 1 in acetonitrile solution. Formation of 1 from a five-coordinate Ni(II) precursor complex, \([(bpppa)\text{Ni(\text{ClO}_4})_2 \) (2), and one equivalent of acetohydroxamic acid occurs readily at ambient temperature. The paramagnetic \(^1\)H NMR and electronic absorption spectroscopic features of 1 and 2 differ sufficiently so that Ni(II) coordination of the acetohydroxamic ligand is easily identifiable.

**Figure 3-11.** The solid line is the electronic absorption spectrum of 4 in \( \text{CH}_3\text{CN} \) at 25(1) \( ^\circ \)C. The dashed line is the absorption spectrum of 4 in the presence of ten equivalents of acetohydroxamic acid.
Potentially tetradeutate tris((pyridyl)methyl)amine-type ligands with an aryl or alkyl appendage in the α position of a pyridyl ring, have been previously shown to exhibit tridentate coordination modes in Fe(II) and Fe(III) complexes, with an α-functionalized pyridyl donor dissociated from the metal center. In 1, a noncoordinated phenyl-appended pyridyl donor acts as a hydrogen bond acceptor for the coordinated acetohydroxamic acid ligand. The structural parameters of this hydrogen-bonding interaction indicate that it may be classified as moderate and involves an energy of 4-14 kcal/mol. A similar hydrogen-bonding interaction was identified in a Mn(II) complex.

**Figure 3-12.** Spectra labeled (a) show features of the paramagnetic $^1$H NMR spectrum of 4 in CD$_3$CN at 25(1) °C. Spectra labeled as (b) and (c) show (a) with 10 and 20 eq of acetohydroxamic acid added, respectively. The resonances labeled with (*) indicate the presence of free acetohydroxamic acid. The solvent residual proton resonance is found at 1.94 ppm.
complex of the 6-Ph:TPA ligand, wherein a noncoordinated phenyl-appended pyridyl nitrogen atom acts as a hydrogen bond acceptor for a metal-coordinated methanol ligand.\textsuperscript{5} Treatment of 1 with excess water results in the release of acetohydroxamic acid and formation of 2, a complex that does not have a coordinated water ligand. Our hypothesis is that the presence of water disrupts the intramolecular hydrogen bonding interaction involving the coordinated acetohydroxamic acid ligand. This disruption could produce a structure in which coordination of the hydroxamic acid is thermodynamically disfavored with respect to release of the neutral acid and formation of 2.

The binding of the acid to 2 to form 1 involves behavior of the bppppa ligand that is reminiscent of a Type III hemilabile ligand, as defined by Braunstein and Naud.\textsuperscript{20} Specifically, type III hemilabile behavior (Figure 3-13(top)) involves coordination of an external reagent that results in the breaking of a labile metal-ligand bond.\textsuperscript{20} Typical Type III hemilabile systems involve CO, or another gaseous reactant (e.g. CO\textsubscript{2}, SO\textsubscript{2}) as the external reagent, and the reversibility of the reaction results simply from a change in the partial pressure of the gas. In the reaction to produce 1 from 2, the dissociated ligand appendage is a phenyl-appended pyridyl moiety that accepts a hydrogen bond from the bound acid (Figure 3-13(bottom)). The introduction of water results in release of the acid. As discussed above, we hypothesize that this involves water acting to disrupt the intramolecular hydrogen bonding interaction. These results indicate that coordination of a neutral acetohydroxamic acid ligand to a Ni(II) center, even when stabilized via an intramolecular hydrogen bonding interaction, is susceptible to displacement in the presence of water. Thus, a neutral acetohydroxamic acid adduct in a biological system, such as is proposed to occur in the weak enzyme/inhibitor $E$-$I$ complex of urease, could
be disrupted in the presence of water.

As outlined above, Zn(II) and Co(II) analogs of 2 do not form complexes having a neutral acetohydroxamic acid ligand, even in the presence of a large excess of the acid. We rationalize this lack of reactivity in terms of coordination number preferences for the bppppa-ligated metal centers. Specifically, whereas the Ni(II) complex 2 forms a sixth weak interaction with a perchlorate anion in the solid state, and exhibits a geometry approximately half-way between square pyramidal and trigonal bipyramidal, the Zn(II) and Co(II) complexes (3 and 4) have strictly five-coordinate distorted trigonal bipyramidal metal centers. Based on this data, it appears that the key to forming the acetohydroxamic acid complex, 1, is the ability of the nickel center to form a sixth interaction and adopt a pseudo-octahedral geometry.

Figure 3-13. (top) Type III hemilabile ligand behavior as defined by Braunstein and Naud.20 (bottom) Reactivity properties of 2 and 1.
References


22. A search of the Cambridge Crystallographic Database (v. 5.26, with update Feb. 2005) found no examples of neutral acetohydroxamic acid coordination to a d-block metal center.
CHAPTER 4

GLYOXALASE I-TYPE HEMITHIOACETAL ISOMERIZATION REACTIVITY OF A MONONUCLEAR Ni(II) DEPROTONATED AMIDE COMPLEX

Abstract

The synthesis, characterization, and hemithioacetal isomerization reactivity of a mononuclear Ni(II) deprotonated amide complex, [(bpppa⁻)Ni]ClO₄·CH₃OH (1, bpppa⁻ = monoanion of N,N-bis-[6-phenyl-2-pyridyl]methyl-\textit{N}-[(6-pivaloylamido-2-pyridyl)methyl]amine) is reported. Complex 1 was characterized by X-ray crystallography, \textsuperscript{1}H NMR, UV-vis, FTIR, and elemental analysis. Treatment of 1 with an equimolar amount of the hemithioacetal PhC(O)CH(OH)SCD₃ in dry acetonitrile results in the production of the thioester PhCH(OH)C(O)SCD₃ in ~60% yield. This reaction is conveniently monitored by \textsuperscript{2}H NMR. A protonated analog of 1, [(bpppa)Ni](ClO₄)₂ (2), is unreactive with the hemithioacetal, thus indicating the requirement of the anionic chelate ligand in 1 for hemithioacetal isomerization reactivity. Complex 1 is unreactive with the thioester product, PhCH(OH)C(O)SCD₃, which indicates that the pKₐ value for the PhCH(OH)C(O)SCD₃ proton of the thioester must be significantly higher than the pKₐ value of the C-H proton of the hemithioacetal (PhC(O)CH(OH)SCD₃). Complex 1 is the first well-characterized Ni(II) coordination complex to exhibit reactivity relevant to Ni(II)-containing \textit{E. coli} glyoxalase I. Treatment of NiBr₂·2H₂O with PhC(O)CH(OH)SCD₃ in the presence of 1-methylpyrrolidine also yields thioester.

product, albeit the reaction is slower and involves the formation of multiple –SCD₃ labeled species, as detected by ²H NMR. The results of this study provide the first insight into hemithioacetal isomerization promoted by a synthetic Ni(II) coordination complex versus a simple Ni(II) ion.

**Introduction**

The glyoxalase system catalyzes the detoxification of cytotoxic 2-oxoaldehydes (e.g., methyl glyoxal, CH₃C(O)C(O)H) by a two-step reaction pathway.¹ In the first step, glyoxalase I (GlxI) catalyzes the isomerization of a hemithioacetal to produce a thioester (Scheme 4-1). Glyoxalase II (GlxII) then catalyzes the hydrolysis of the thioester to produce free glutathione and a 2-hydroxy acid. The GlxI enzyme from *Escherichia coli* contains a mononuclear Ni(II) center within the enzyme active site.²³ Bacterial GlxI enzymes from *Y. pestis, P. aeruginosa*, and *N. meningitidis* also exhibit maximal activity in the presence of Ni(II).⁴ Recently, the GlxI from the human parasite *Leishmania major* was also found to be a Ni(II)-dependent enzyme.⁵ An X-ray crystal structure of the *E. coli* GlxI enzyme revealed a distorted octahedral Ni(II) center having a mixture of nitrogen and oxygen donor ligands, [(N₉His)₂(OGlu)₂Ni(OH₂)₂] (Scheme 4-1).⁶

**Scheme 4-1.** The reaction pathway for the glyoxalase system.
Scheme 4-2. The proposed mechanism for *E. coli* glyoxalase I reaction.

The role of the Ni(II) center in the *E. coli* GlxI-mediated hemithioacetal isomerization reaction is not yet defined. On the basis of X-ray absorption spectroscopic studies of product- and inhibitor-bound *E. coli* GlxI, a mechanistic pathway is favored in which the role of Ni(II) ion is to generate a nickel hydroxide moiety that can serve as a general base for the isomerization reaction. Specifically, the Ni(II)-OH is proposed to abstract a proton from the hemithioacetal (Scheme 4-2). In this reaction, an anion is produced that is subsequently protonated at the former carbonyl carbon to yield the thioester product. It is unclear whether the intermediate anion in this reaction transiently interacts with the Ni(II) center. X-ray absorption studies of the enzyme-product complex of *E. coli* GlxI show no evidence of Ni(II)-product interactions, leading previous researchers to favor the proton transfer mechanism shown in Scheme 4-2.

To date, only three structurally characterized mononuclear Ni(II)-OH complexes have been reported in the literature. Neither these complexes or any other mononuclear
Ni(II) complex has been shown to promote the isomerization of a hemithioacetal in a fashion akin to that proposed for *E. coli* GlxI. In 1970, a single brief report appeared in the literature in which the use of NiBr$_2$·2H$_2$O and the base 1-methylpyrrolidine was indicated to promote the isomerization of a hemithioacetal to produce a thioester product in DMF.$^{11}$ No experimental details or yield of the thioester product were reported for this reaction.

In the work described herein, we have explored the hemithioacetal isomerization reactivity of mononuclear Ni(II) complex supported by a chelate ligand containing an oxygen-coordinated deprotonated amide ligand. This complex, which was produced during attempts to generate a mononuclear Ni(II)-OH complex, promotes the isomerization of a hemithioacetal to produce a thioester product. Importantly, this is the first synthetic Ni(II) complex to be reported that exhibits glyoxalase I type reactivity. Control studies indicate that the presence of the deprotonated amide in the supporting chelate ligand is required for hemithioacetal isomerization reactivity. Additionally, we have reexamined the previously reported hemithioacetal isomerization reaction promoted by NiBr$_2$·2H$_2$O/1-methylpyrrolidine in DMF.$^{11}$ Using a deuterium labeled hemithioacetal and $^2$H NMR, we have found that while thioester formation does occur, this reaction is significantly slower than the reaction involving the deprotonated amide complex. These hemithioacetal isomerization reactions also differ in the nature of intermediate species that are detectable by $^2$H NMR. For the reaction involving the deprotonated amide coordination complex, no evidence was found for Ni(II)-coordinated intermediates whereas in the NiBr$_2$·2H$_2$O-promoted reaction several spectroscopically identifiable new species are present in the reaction mixture, some of which may involve coordination
between the hemithioacetal and Ni(II). Overall, the results of this study provide the first insight into hemithioacetal isomerization promoted by a well-characterized synthetic Ni(II) complex versus simple Ni(II) ion.

Results and Discussion

Synthesis and Characterization of [(bpppap)NiClO₄](1). Chelate ligands containing secondary amide appendages have been shown to stabilize a variety of mononuclear metal hydroxide complexes. With this in mind, we attempted to prepare a new mononuclear Ni(II)-OH complex starting from the X-ray crystallographically characterized mononuclear Ni(II) complex [(bpqpqpa)Ni](ClO₄)₂ (2, Scheme 4-3). Treatment of this complex with one equivalent of Me₄NOH·5H₂O in CH₃CN resulted in the formation of an orange/brown solution. Following work-up and recrystallization from CH₃CN/CH₃OH/Et₂O, [(bpqpqpa–)Ni]ClO₄·CH₃OH (1) was isolated in 68% yield as orange-brown crystals. Complex 1 has been characterized by X-ray crystallography, ¹H NMR, UV-vis, FTIR, and elemental analysis. These combined characterization methods indicate that instead of formation of the desired mononuclear Ni(II)-OH complex, treatment of 2 with one equivalent of base resulted in the formation of a deprotonated amide complex.

Scheme 4-3. The synthesis of a Ni(II) deprotonated amide complex.
Figure 4-1. Top: ORTEP drawing of the cationic portion of 1. Ellipsoids are drawn at the 50% probability level. Hydrogen atoms are omitted for clarity. Bottom: Comparison of bond distances within the cationic portions of 1 and 2.

An ORTEP drawing of the cationic portion of 1 is shown in Figure 4-1. Details of the X-ray data collection and refinement are given in Table 4-1. Selected bond distances in 1 and its parent complex [(bppppa)Ni](ClO₄)₂ (2) are provided in Figure 4-1. Additional bond distances and angles for 1 are given in Table 4-2. As expected, the presence of the deprotonated amide in 1 results in a slight contraction of the C(5)-N(1) bond and elongation of the amide C(5)-O(1) bond (Figure 4-1 (bottom)) relative to the distances found in the structurally similar 2 wherein a protonated amide is present. The shorter C(6)-N(1) distance in 1 (1.373(4) Å) may be attributed to delocalization of the
Table 4-1. Summary of X-ray Data Collection and Refinement

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$^a$Radiation used: Mo Kα ($\lambda = 0.71073$ Å).

$^bR1 = \sum| |F_o| - |F_c| | / \sum |F_o|;$

$wR2 = [\sum(w(F_o^2-F_c^2)^2)/[\sum(F_o^2)^2]]^{1/2}$

where $w = 1/\left[\sigma^2(F_o^2) + (aP)^2 + bP\right]$.  


Table 4-2. Selected Bond Distances (Å) and Angles (°)\textsuperscript{a}

<table>
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<tr>
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</tr>
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<td>136.87(10)</td>
</tr>
<tr>
<td>N(3)-Ni-N(4)</td>
<td>78.97(10)</td>
</tr>
<tr>
<td>N(5)-Ni-N(4)</td>
<td>105.72(10)</td>
</tr>
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</table>

\textsuperscript{a}Estimated standard deviations indicated in parentheses.

An anionic charge into the pyridyl ring. \textsuperscript{1}H NMR spectroscopic evidence for such delocalization has been previously reported for a zinc analog complex.\textsuperscript{18} This delocalization, along with the presence of the Ni(II) ion, stabilizes the deprotonated amide moiety in 1. Notably, the average Ni(1)-N\textsubscript{PhPy} distance increases slightly in 1 (2.11 Å) relative to that found in 2 (2.09 Å). This is an indication of a less Lewis acidic Ni(II) center in 1. The Ni(II) center in both complexes exhibits a geometry that is intermediate between trigonal bipyramidal and square pyramidal (1: \(\tau = 0.44\); 2: \(\tau = 0.54\)).\textsuperscript{19}

A region of the \textsuperscript{1}H NMR spectra of 1 and 2 is shown in Figure 4-2. Three sharp resonances are present in the spectrum of 1 in the range of 30-50 ppm that clearly differentiate the spectrum of this complex from that of its protonated parent complex. Based on studies of a series of mononuclear Ni(II) complexes of the 6-Ph\textsubscript{2}TPA ligand, these resonances are assigned to the \(\beta/\beta’\)-H’s of the pyridyl rings.\textsuperscript{20} Complex 1 is orange when dissolved in acetonitrile, with an absorption feature at 440 nm (\(\epsilon = 320\) M\textsuperscript{-1}cm\textsuperscript{-1}).
Figure 4-2. A region of the $^1$H NMR spectra of 1 (a) and 2 (b). Both spectra were acquired in CD$_3$CN solution at 302 K.

This feature is not present in the UV-vis spectrum of 2.

**Hemithioacetal Isomerization Reactivity of 1.** Our initial goal was to generate a new mononuclear Ni(II)-OH complex and examine its reactivity with a hemithioacetal. Although 1 does not contain a Ni(II)-OH moiety, it does contain a Ni(II)-bound anionic ligand in the form of a deprotonated amide. While this functional group is not directly relevant to the chemistry proposed for *E. coli* Glx I, we decided to initially continue our studies with this complex as to date no synthetic Ni(II) coordination complex has been shown to promote the isomerization of a hemithioacetal. Treatment of 1 with a stoichiometric amount of the hemithioacetal PhC(O)CH(OH)SCD$_3$\textsuperscript{21} in dry CH$_3$CN at 302 K results in hemithioacetal isomerization over the course of ~1.5 h to produce
the thioester PhCH(OH)C(O)SCD$_3$ in ~60\% yield (Scheme 4-4).

As shown in Figure 4-3, this GlxI-type reaction can be conveniently monitored by $^2$H NMR, where PhC(O)CH(OH)SCD$_3$ exhibits a $^2$H NMR signal at 1.88 ppm, and the product PhCH(OH)C(O)SCD$_3$ has a signal at 2.13 ppm when referenced to an internal C$_6$D$_6$ standard (7.37 ppm). A third minor resonance at 2.38 ppm has been tentatively identified as indicating the presence of a non-coordinated anionic hemithioacetal-derived species in solution (*vida infra*). After 24 h, some unreacted hemithioacetal remains and the combined integrated intensity of the –SCD$_3$ species has decreased by ~30\%. This decrease appears to correlate with the formation of a dark orange-brown precipitate in the NMR tube. The chemical composition of this solid remains under investigation. It is insoluble in common organic solvents, thus precluding its characterization by solution spectroscopic methods.

The anionic species (-SCD$_3$, 2.38 ppm) can also be produced in dry acetonitrile by treatment of PhC(O)CH(OH)SCD$_3$ with an equimolar amount of Me$_4$NOH·5H$_2$O.
Notably, no change in the $^2$H NMR spectral features of this mixture is identifiable after ~18 h at room temperature, thus indicating that hemithioacetal isomerization to produce thioester does not occur in the presence of only Me$_4$NOH⋅5H$_2$O in acetonitrile in this time period. The $^2$H NMR studies of the reaction of 1 with PhC(O)CH(OH)SCD$_3$ suggest that the hemithioacetal, thioester, and the spectroscopically observable anionic species do not directly coordinate to the Ni(II) center in 1.

The integrated intensity of the combined -SCD$_3$ signals remains constant for (a)-(d) relative to the integrated intensity of a C$_6$D$_6$ internal standard. However, for (e) the integrated intensity of the combined -SCD$_3$ species is reduced by ~30% relative to that

![Figure 4-3. $^2$H NMR spectra of a reaction mixture of 1 and PhC(O)CH(OH)SCD$_3$ in dry CH$_3$CN at various times after mixing. The first spectrum (a), which was obtained within 5 minutes of mixing of the reagents, is taken as t = 0 min; (b) 19 min; (c) 43 min; (d) 83 min; (e) 23 h 18 min.](image)
found for (a)-(d). This appears to correlate with the appearance of a dark orange-brown precipitate in the reaction mixture. All spectra were recorded at 302 K. Opening of the spectral window to a chemical shift range of -180 to -20 ppm did not reveal any additional signals. Specifically, the chemical shifts of the –SCD₃ resonances of these molecules are identical to those found for the individual molecules in the absence of the paramagnetic Ni(II) complex. However, this is not conclusive evidence and does not rule out the possibility of transient interactions. ¹H NMR was also used to monitor the reaction of 1 with PhC(O)CH(OH)SCH₃ in CD₃CN (Figure 4-4). During the time period required for the isomerization reaction at 302 K (~1.5 h as determined by the ²H NMR studies described above) the ¹H NMR looks generally similar to that of analytically pure 1, with only subtle broadening of selected resonances of 1. One new broad signal is present at ~32 ppm. This signal is not indicative of the presence of protonated amide complex 2, as it does not have a distinct resonance at this chemical shift (Figure 4-2). Opening of the chemical shift window to examine a range of 180 to -20 ppm, did not reveal any additional new resonances. Overall, the results of these NMR combined experiments suggest that the majority of the Ni(II) complex present in the hemithioacetal isomerization reaction mixture is unaltered 1.

**Control Reactions.** Treatment of the protonated amide complex [(bppppa)Ni](ClO₄)₂ (2) with PhC(O)CH(OH)SCD₃ (Scheme 4-4) resulted in no reaction after 18 h as determined by ²H NMR, thus indicating the requirement of the anionic chelate ligand in 1 for hemithioacetal isomerization reactivity. A deprotonated form of the ligand, Na(bppppa), does not promote hemithioacetal isomerization or deprotonation over a time period of ~40 h at 298 K, thus indicating the requirement of the Ni(II) center
Figure 4-4. (a) A region of the $^1$H NMR spectrum of 1. (b) The same region for compound 1 in the presence of an equimolar amount of PhC(O)CH(OH)SCD$_3$. Both spectra were acquired in CD$_3$CN at 302 K.

for hemithioacetal isomerization reactivity. Treatment of 1 with an equimolar amount of the thioester PhCH(OH)C(O)SCD$_3$ in dry acetonitrile at 302 K resulted in no reaction after 16 h. This suggests that the $pK_a$ value for the PhCH(OH)C(O)SCD$_3$ proton of the thioester product must be significantly higher than the $pK_a$ value of the PhC(O)CH(OH)SCD$_3$ proton of the hemithioacetal. Overall, the results of these combined studies suggest that 1 may serve as a general base for the hemithioacetal isomerization reaction. The basic site of 1 that accepts and subsequently distributes the proton may be either the amide oxygen or nitrogen. Because the bound amide oxygen atom is protected by the hydrophobic phenyl groups of the chelate ligand, as well as by the bulky $t$-butyl substituent, we favor a reaction pathway wherein the deprotonated amide nitrogen atom is involved in proton transfer reactivity.

If 1 is acting as a general base for the hemithioacetal isomerization reaction, the reaction should be catalytic under appropriate conditions. To evaluate this possibility, an
NMR tube was prepared containing 1 and PhC(O)CH(OH)SCD₃ in a 1:5 molar ratio and C₆D₆ (internal standard). Spectroscopic monitoring of this reaction mixture using ^2H NMR over the course of 66 h revealed the formation of ~1.5 equivalents of the thioester PhC(OH)C(O)SCD₃. Overall, the spectra for this mixture were very similar to those shown in Figure 4-3, albeit a larger amount of the anionic 2.38 ppm species was present. Similar to the stoichiometric reaction, the combined integrated intensity of the –SCD₃ species decreased by ~37% after 66 h and a deep orange-brown precipitate deposited in the NMR tube.

**Evaluation of the Hemithioacetal Isomerization Reactivity of NiBr₂·2H₂O/1-methylpyrrolidine in DMF using PhC(O)CH(OH)SCD₃ and ^2H NMR.** There is one previous report in the literature of hemithioacetal isomerization promoted by NiBr₂·2H₂O and 1-methylpyrrolidine in DMF.¹¹ No experimental details or yield were reported for this reaction. To gain insight into this reaction, we again used the ^2H-labeled hemithioacetal and ^2H NMR. Solutions were prepared containing an equimolar mixture of NiBr₂·2H₂O, PhC(O)CH(OH)SCD₃ and C₆D₆ (internal standard) in dry DMF. Addition of one equivalent of 1-methylpyrrolidine to this mixture produced a color change from yellow-green to red-brown. ^2H NMR spectra collected at various times following addition of the base are shown in Figure 4-5. Unlike the relatively simple spectra found for hemithioacetal isomerization promoted by 1 (Figure 4-3), several broad resonances in the ^2H NMR spectra of the NiBr₂·2H₂O/1-methylpyrrolidine indicate the presence of multiple –SD₃ containing species. For example, at least three new resonances are present in the region of 1.0-1.6 ppm.²² Another new resonance is also present at ~2.35 ppm. We hypothesize that the more exposed Ni(II) center in NiBr₂·2H₂O, versus that
Figure 4-5. $^2$H NMR spectra of a reaction mixture of NiBr$_2$·2H$_2$O, 1-methylpyrrolidine and PhC(O)CH(OH)SCD$_3$ in dry DMF at various times after mixing. The first spectrum (a), which was obtained within 10 minutes of mixing of the reagents, is taken as $t = 0$ min; (b) 1.5 h; and (c) 23 h. The signals marked DMF are the natural abundance $^2$H NMR signals for protio DMF.$^{23}$

found in 1, allows for the formation of Ni(II) complexes with hemithioacetal-derived species. Over the course of 1.5 h, which is the time required for $\sim$60% yield of thioester in the hemithioacetal isomerization reaction involving 1, only a trace amount of thioester is produced in the NiBr$_2$·2H$_2$O/1-methylpyrrolidine reaction (Figure 4-5(b)).

Similar to the reaction involving 1, after $\sim$1.5 h a heavy red-brown precipitate began to deposit in the NMR tube. At this point, the overall integrated intensity of the combined –SCD$_3$ species had decreased by 15%. After 24 h at 25 °C (Figure 4-5(c)), the amount of
thioester and hemithioacetal present in solution are similar, but the overall integrated intensity for all –SCD$_3$ species has declined ~50%.

**Conclusions**

The glyoxalase pathway (Scheme 4-1) is ubiquitous in biological systems and the role of the metal center in the hemithioacetal isomerization catalyzed by Glx I remains to be fully defined. In 1970, Hall and Poet demonstrated that the conversion of model hemithioacetals to the corresponding $\alpha$-hydroxy thioesters was accelerated in the presence of a divalent metal ion (e.g. Mg(II)).$^{11}$ Specifically, the rate of thioester formation from a hemithioacetal was found to increase by 30-fold in a DMF solution containing Mg(NO$_3$)$_2$ and sodium acetate (or tertiary amines), versus a DMF solution containing only the base. The role of the Mg(II) center was proposed to be stabilization of an enediol(ate) intermediate. Similar reactivity was indicated to occur using other divalent metal ions (including Ni(II)) and bases, albeit no experimental details or yields were provided.$^{11}$ Notably, in a later study involving an aqueous environment and using imidazole as the base, the rate of hemithioacetal isomerization was accelerated only by a factor of 1.8 in the presence of Mg(II).$^{24}$ Under these conditions, water and imidazole likely compete as ligands for the Mg(II) center, thus limiting interactions between the metal and any anionic enediol(ate) intermediate. Overall, these studies represent the entirety of what has been previously reported in terms of metal-containing model studies relevant to the chemistry of Glx I enzymes. The studies reported herein, although not of direct structural relevance to *E. coli* Glx I, provide interesting new insight into the chemistry of metal ion promoted hemithioacetal isomerization.
During attempts to prepare a mononuclear Ni(II)-OH complex of the amide-appended bppppa ligand, we found that a deprotonated amide complex is produced. This type of reactivity has been recently identified in a structurally similar amide-appended Zn(II) complex.\textsuperscript{18} Stabilization of the deprotonated amide moiety occurs both through amide oxygen coordination to the divalent metal center and via delocalization of the anionic charge into the pyridyl ring. While exploration of the acid/base properties of 1 and related compounds will be the subject of a future study, we hypothesize that the stabilization factors noted above produce an amide linkage having enhanced acidity.

The reactivity of 1 with a hemithioacetal provides the first example of a well-characterized Ni(II) complex which promotes a hemithioacetal isomerization reaction. Because the Ni(II) complex is paramagnetic, monitoring of this reaction was performed using a deuterium-labeled hemithioacetal and \textsuperscript{2}H NMR. This novel approach is also applicable to monitoring hemithioacetal isomerization reactions involving simple Ni(II) salts, as was outlined for the reaction involving NiBr\textsubscript{2}·2H\textsubscript{2}O and 1-methylpyrrolidine. Importantly, comparison of the reactions promoted by 1 and NiBr\textsubscript{2}·2H\textsubscript{2}O/1-methylpyrrolidine provides clear evidence that the reaction involving the coordination complex is faster and involves fewer spectroscopically identifiable species. This is likely a consequence of the fact that while five coordination positions in 1 are occupied by the chelate ligand, the Ni(II) ion derived from NiBr\textsubscript{2}·2H\textsubscript{2}O in DMF has multiple available coordination positions for interaction with hemithioacetal-derived species. In terms of \textit{E. coli} Glx I, the presence of four amino acid ligands to the Ni(II) center likely limits interaction with the hemithioacetal or anionic species derived from deprotonation of this substrate.
A problem encountered in using either 1 or NiBr₂·2H₂O/1-methylpyrrolidine is the precipitation of a red-brown solid in the reaction mixture after several hours. Based on the integrated intensity of the combined –SCD₃ species in each reaction, this solid contains hemithioacetal-derived species. On-going efforts are focused on determining the chemical composition of this solid and its relevance to hemithioacetal isomerization reactivity.

Experimental Section

General Methods. All reagents were obtained from commercial sources and were used as received without further purification. Solvents were dried according to published procedures and were distilled under N₂ prior to use. Water-sensitive reactions were performed in an MBraun Unilab glovebox under an atmosphere of purified N₂. The organic compounds bpppa (N,N-bis[[6-phenyl-2-pyridyl)methyl]-N-[(6-pivaloylamido-2-pyridyl)methyl]amine), PhC(O)CH(OH)SCD₃ (hemithioacetal), and PhCH(OH)C(O)SCD₃ (thioester) were prepared according to literature procedures.

Physical Methods. ¹H and ²H NMR spectra were collected as previously described. UV-vis spectra were recorded at ambient temperature using a Hewlett Packard 8453 diode array spectrophotometer. Solid-state IR spectra were recorded using a Shimadzu FTIR-8400 spectrometer as KBr pellets. Elemental analyses were performed by Atlantic Microlabs of Norcross, GA.

CAUTION! Perchlorate salts of metal complexes with organic ligands are potentially explosive. Only small amounts of material should be prepared, and these should be handled with great care.
[(bppppa)Ni]ClO4·CH3OH (1). [(bppppa)Ni](ClO4)2 (26 mg, 0.032 mmol) dissolved in CH3CN (~2 mL) was added to a slurry of Me4NOH·5H2O (5.8 mg, 0.032 mmol) in CH3CN (~2 mL). The resulting mixture was stirred under a dry nitrogen atmosphere for 4 h. The solvent was then removed under reduced pressure. The orange residue was dissolved in CH2Cl2 and the solution filtered through a glass wool/Celite plug. The CH2Cl2 was removed under reduced pressure. The remaining orange solid was recrystallized by Et2O diffusion into a CH3CN/CH3OH (2:1) solution to give deep orange-brown crystals (15 mg, 68%). This reaction may be safely performed on a larger scale, starting with ~60 mg of [(bppppa)Ni](ClO4)2. Anal. Calcd for C35H34N5O5ClNiCH3OH: C, 59.24; H, 5.25; N, 9.60. Found: C, 58.79; H, 5.09; N, 9.64. UV-vis (CH3CN), nm (λmax, M⁻¹cm⁻¹): 440 (320); FTIR (KBr, cm⁻¹): 1088 (νClO4), 628 (νClO4).

Monitoring Stoichiometric Hemithioacetal Isomerization by ²H NMR. A NMR tube containing equimolar amounts of 1 and PhC(O)CH(OH)SCD₃ in dry protio acetonitrile (700 µL) was prepared under a nitrogen atmosphere. An internal standard (C₆D₆, 1 µL) was added and the tube was sealed using a stopcock. The tube was then quickly transferred to the NMR system that had been previously optimized for data collection. Each ²H NMR spectrum was referenced to the chemical shift of the C₆D₆ standard. The C₆D₆ signal was also used as an internal integration standard.

Na(bppppa). The bppppa ligand (27 mg, 4.9 x 10⁻⁵ mol) was treated with NaH (1.2 mg, 5.1 x 10⁻⁵ mol) in dry THF. After stirring for 1.5 h at ambient temperature, the solvent was removed under reduced pressure and a ¹H NMR spectrum of the remaining pasty solid was obtained. ¹H (CD₃CN, 300 MHz) δ 8.00-7.75 (br m, 7H), 7.64 (br d, J =
7.8 Hz, 3H), 7.60-7.45 (br m, 6H), 7.50-7.30 (br m, 2H), ~6.6 (br, 1H), 3.79 (s, 4H), 3.65 (s, 2H), 1.03 (s, 9H). These resonances are shifted relative to those found for neutral bppppp. Addition of water to Na(bpppp) in acetonitrile yields the neutral bppppp ligand, as determined by $^1$H NMR.

**Treatment of Na(bppppp) with PhC(O)CH(OH)SCD$_3$ in Protio Acetonitrile.**

This reaction was set up and monitored by $^2$H NMR in an identical fashion to that employed for 1. No evidence for hemithioacetal isomerization or deprotonation was found over a time period of ~40 h at 298 K.

**Hemithioacetal Reactions involving NiBr$_2$·2H$_2$O and 1-methylpyrrolidine.** A solution of NiBr$_2$·2H$_2$O (10 mg, 3.9 x 10$^{-5}$ mol) in DMF (700 µL) was prepared. Stirring of this solution for ~30 minutes at room temperature was required for the NiBr$_2$·2H$_2$O to fully dissolve. To this solution was added the internal standard C$_6$D$_6$ (1 µL), the hemithioacetal PhC(O)CH(OH)SCD$_3$ (7.3 mg, 3.9 x 10$^{-5}$ mol) and 1-methylpyrrolidine (4.5 µL, 3.9 x 10$^{-5}$ mol). The resulting red-brown mixture was transferred to an NMR tube that was sealed using a stopcock. This tube was transferred within 10 min to the NMR instrument that had been previously optimized for data collection. $^2$H NMR spectra were collected at timed intervals and were referenced to the chemical shift of the C$_6$D$_6$ internal standard (7.37 ppm). Reactions of this type were monitored for a minimum of 24 h at 298 K. After approximately 40 min, the color of the solution had intensified and a red-brown precipitate began to appear. Subtle loss of color and an increasing amount of precipitate are apparent over the course of the reaction. The integrated intensity of all –SCD$_3$ labeled species in the reaction mixture decreases by ~50% after 24 h. As a prelude to evaluating the results of these experiments, individual $^2$H NMR spectra of the
hemithioacetal PhC(O)CH(OH)SCD$_3$ (1.97 ppm) and thioester PhC(OH)CH(O)SCD$_3$
(2.11 ppm) were collected in DMF.

References


9. Proton transfer reactions involving the oxygen atoms of the hemithioacetal are also required for production of the thioester product.


22. Control experiments confirm that the –SCD₃ derived resonances in the region of 1.0-1.6 ppm were only produced when NiBr₂·2H₂O, 1-methylpyrrolidine, and PhC(O)CH(OH)SCD₃ were all present in the reaction mixture.

23. In the ²H NMR spectra acquired in CH₃CN, the natural abundance ²H NMR signal for the solvent methyl group is positioned under the signal associated with the –SCD₃ group of the hemithioacetal.


CHAPTER 5

A TRINUCLEAR Ni(II) ENEDIOLATE COMPLEX: SYNTHESIS, CHARACTERIZATION, AND DIOXYGEN REACTIVITY

Abstract

Using a new N₄-donor chelate ligand having a mixture of hydrophobic phenyl and hydrogen bond donor appendages, a trinuclear Ni(II) complex of the doubly deprotonated form of 2-hydroxy-1,3-diphenylpropane-1,3-dione was isolated, characterized (X-ray crystallography, elemental analysis, UV-vis, ¹H NMR, FTIR, magnetic moment measurement), and evaluated for O₂ reactivity. This complex, [(6-NA-6-Ph₂TPANi)₂(µ-PhC(O)C(O)C(O)Ph)₂Ni](ClO₄)₂ (4), has two terminal pseudo octahedral Ni(II) centers supported by the tetradentate chelate ligand, and a central square planar Ni(II) ion ligated by oxygen atoms of two bridging enediolate ligands. In CH₃CN, 4 exhibits a deep orange/brown color and λ_max = 463 nm (ε = 16,000 M⁻¹cm⁻¹). The room temperature magnetic moment of 4, determined by Evans method, is µ_eff = 5.3(2) µ_B. This is consistent with the presence of two non-interacting high-spin Ni(II) centers, a diamagnetic central Ni(II) ion, and an overall quintet ground state. Exposure of a CH₃CN solution of 4 to O₂ results in the rapid loss of the orange/brown color to give a green solution. The products identified from this reaction are [(κ³-6-NA-6-Ph₂TPA)Ni(O₂Ph)(H₂O)]ClO₄ (5), benzil (PhC(O)C(O)Ph), and CO. Identification of 5 was achieved via its independent synthesis and comparison of its ¹H NMR and mass spectral features with those of the 6-NA-6-

Ph$_2$TPA-containing product generated upon reaction of 4 with O$_2$. The independently prepared sample of 5 was characterized by X-ray crystallography, elemental analysis, UV-vis, mass spectrometry, and FTIR. The O$_2$ reactivity of 4 has relevance to the active site chemistry of Ni(II)-containing acireductone dioxygenase (Ni(II)-ARD).

**Introduction**

Acireductone dioxygenase (ARD) enzymes are found in variety of species, including humans.$^{1-7}$ They are associated with the methionine salvage pathway wherein they catalyze O$_2$-dependent oxidative aliphatic carbon-carbon bond cleavage reactions involving an acireductone intermediate.$^1$ The enzymes Ni(II)-ARD and Fe(II)-ARD’, which have the same protein component and differ only in the active site metal ion, have been shown to catalyze different reactions of the acireductone intermediate in *Klebsiella oxytoca* ATCC 8724. As shown in Scheme 5-1, the reaction catalyzed by Ni(II)-ARD is a shunt out of the methionine salvage pathway and gives carboxylic acids and CO as products. Carbon monoxide production via a similar reaction has also been identified in *Bacillus subtilis* and *E. coli*.8,9 The reaction catalyzed by Fe(II)-ARD’ gives a α-ketoacid product that is a precursor to methionine in an “on-pathway” type reaction. It is important to note that the activities of Ni(II)-ARD and Fe(II)-ARD’ can be interconverted by exchange of the metal ion.$^{10}$

A recently deposited X-ray structure of a putative ARD from mouse (MmARD, PDB 1VR3)$^{11}$, combined with NMR structural studies (PDB 1ZRR), conserved domain homology modelling, and X-ray absorption spectroscopic studies of *K. oxytoca* Ni(II)-ARD, indicate that the Ni(II) center in this enzyme is ligated by a facial array of three histidine residues and a glutamate ligand from the protein.$^{12-14}$ The two remaining
coordination positions of the Ni(II) center are occupied by water/hydroxide ligands in the resting state. A histidine residue may be displaced from the Ni(II) complex in the ES complex. The structural studies also revealed several important secondary residues that are located near the active site Ni(II) center. These include Phe92, Phe142, Arg104, and Arg154 in *K. oxytoca* Ni(II)-ARD. The phenylalanine residues have been proposed to orient the substrate for reaction with O₂ and are close enough to exhibit paramagnetically shifted resonances. Arg104 and Arg154 have been suggested to play a role in stabilizing substrate coordination via hydrogen bonding and/or may donate or accept a proton. In Ni(II)-ARD a 1,3-coordination motif is proposed for the acireductone dianion (Scheme 5-1) in the enzyme/substrate complex. In this binding mode, Lewis acid activation of the C(1)-O(1) and C(3)-O(3) units is proposed to favor the formation of a five-membered cyclic peroxide upon reaction with oxygen. Breakdown of this peroxide ring would then lead to the formation of the carboxylic acids and CO (Scheme 5-1).

**Scheme 5-1.** Oxidative cleavage of acireductone catalyzed by acireductone dioxygenase enzymes (Ni(II)-ARD and Fe(II)-ARD’).
There is currently very little known about the coordination chemistry of acireductones and the O$_2$ reactivity of M(II)-acireductone species. As shown in Figure 5-1(a), neutral acireductones have been proposed to adopt structures involving a six-membered ring.\textsuperscript{18} However, the X-ray structure of the rubidium salt and neutral form of triose reductone (R = R' = H) revealed that all three oxygen atoms are positioned on one side of the carbon chain (Figure 5-1(b)).\textsuperscript{19,20} Prior to 2005, one transition metal compound having a coordinated acyclic acireductone ligand had been characterized by X-ray crystallography. This compound, [([Ru(bipy)$_2$](µ-C$_4$H$_4$O$_3$))(PF$_6$)$_2$ (Figure 5-1(c)), was generated as a byproduct in reactions of [Ru(bipy)$_2$Cl$_2$·2H$_2$O] in ethylene glycol in
the presence of \( \text{NH}_4\text{PF}_6 \). Similar to the triose reductone structures, the acireductone ligand in \([\{(\text{Ru(bipy})_2\}_2(\mu-\text{C}_4\text{H}_4\text{O}_3)\}_2(\text{PF}_6)_2\] is coordinated with all three oxygen atoms of the enediolate on the same side of the carbon chain. Similar backbone carbon-carbon distances, and C(1)-O and C(3)-O bonds (1.29 and 1.30 Å, respectively) that are shorter than the C(2)-O bond (1.39 Å), indicate a delocalized enolate anion along the carbon backbone. No \( \text{O}_2 \) reactivity studies have been reported for this complex.

Of relevance to \( \text{Ni(II)} \)-ARD, we generated a \( \text{Ni(II)} \) \( \text{cis} \)-\( \beta \)-keto-enolate complex, \([\{(6-\text{Ph}_2\text{TPA})\text{Ni(PhC(O)C(OH)C(O)Ph)}\}_2\text{ClO}_4 \) (1, Scheme 5-2) which can be isolated and characterized. Treatment of 1 with one equivalent of base followed by the introduction of \( \text{O}_2 \) results in the formation of a \( \text{Ni(II)} \) carboxylate complex, \([\{(\kappa^3-6-\text{Ph}_2\text{TPA})\text{Ni(O}_2\text{CPh)}_2(\text{H}_2\text{O})\}_2\text{ClO}_4 \) (2), and CO, as well as benzil (\( \text{PhC(O)C(O)Ph} \)). The formation of carboxylates and CO, as well as \( ^{18}\text{O} \) incorporation into one oxygen atom of each carboxylate ligand, is consistent with an ARD-type pathway. However, the formation of benzil suggests that an alternative reaction pathway is also operative. Complex 1 undergoes reaction with \( \text{O}_2 \) in the absence of excess base to give a \( \text{Ni(II)} \) monobenzoate complex \([\{(6-\text{Ph}_2\text{TPA})\text{Ni(O}_2\text{CPh)}\}_2\text{ClO}_4 \) (3), CO, benzil (\( \text{PhC(O)C(O)Ph} \)), and other phenyl-containing organic byproducts. From these combined studies we found that the \( \text{O}_2 \) reaction is cleaner, with a higher level of \( ^{18}\text{O} \) incorporation in the benzoate product, under conditions wherein the acireductone ligand can access a dianionic form as is proposed to occur in the \( \text{Ni(II)} \)-ARD catalyzed reaction. Our initial approach toward modeling the active site chemistry of \( \text{Ni(II)} \)-ARD, as outlined in Scheme 5-2, was to use a tetradentate nitrogen donor ligand having two hydrophobic phenyl appendages (6-\( \text{Ph}_2\text{TPA} \)). The phenyl groups were included to create a
hydrophobic microenvironment that could have relevance to the possible role of active site phenylalanine side chains in Ni(II)-ARD. To model the acireductone ligand we have used the deprotonated form of 2-hydroxy-1,3-diphenylpropan-1,3-dione.

In the research outlined herein we present the Ni(II) coordination chemistry of a new chelate ligand wherein a secondary amine hydrogen bond donor has been incorporated to create a mixed hydrophobic/hydrogen bond donor microenvironment. This ligand, 6-NA-6-Ph$_2$TPA (Figure 5-2), is the reduced form a previously reported amide-containing ligand.$^{24}$ Having a rigidly fixed hydrogen bond donor, the

Scheme 5-2. O$_2$-dependent chemistry of [(6-Ph$_2$TPA)Ni(PhC(O)C(OH)C(O)Ph)]ClO$_4$.

Figure 5-2. The 6-NA-6-Ph$_2$TPA chelate ligand.
6-NA-6-Ph₂TPA ligand is appropriate for investigating the influence of a single hydrogen bond donor on the chemistry of Ni(II) enolate or enediolate complexes. This investigation is relevant to evaluating the possible influence of hydrogen bonding involving an arginine side chain with a Ni(II)-coordinated acireductone in the Ni(II)-ARD enzyme/substrate complex. Our results outlined herein demonstrate that use of the 6-NA-6-Ph₂TPA ligand enables the isolation and characterization of a trinuclear Ni(II) complex, \([(6-NA-6-Ph₂TPANi)₂(\mu-PhC(O)C(O)C(O)Ph)₂Ni]ClO₄\)₂ (4), wherein two enediolate ligands derived from 2-hydroxy-1,3-diphenylpropan-1,3-dione bridge between terminal pseudo octahedral Ni(II) centers and a central square planar Ni(II) ion (Scheme 5-3). Complex 4, which is the first structurally characterized example of a Ni(II)-enediolate complex of relevance to Ni(II)-ARD, reacts with O₂ to yield a 6-NA-6-Ph₂TPA-ligated Ni(II) benzoate complex, CO, benzil (PhC(O)C(O)Ph), and a nickel benzoate species.

Scheme 5-3. Synthesis of \([(6-NA-6-Ph₂TPANi)₂(\mu-PhC(O)C(O)C(O)Ph)₂Ni]ClO₄\)₂ (4).
Experimental Section

General. All reagents and solvents were purchased from commercial sources and used as received unless otherwise noted. Acetonitrile and diethyl ether were dried according to published procedures.\textsuperscript{25} Air sensitive reactions were performed in a MBrAun Unilab glovebox or a Vacuum Atomospheres MO-20 glovebox under an atmosphere of purified N\textsubscript{2}. \textsuperscript{18}O\textsubscript{2} was purchased from Icon Services, Summit, NJ. The ligand precursors 2-(pivaloylamido)-6-(aminomethyl)pyridine, 2-(chloromethyl)-6-phenylpyridine hydrochloride, and 2-hydroxy-1,3-diphenylpropan-1,3-dione were prepared according to literature procedures.\textsuperscript{26-28} Qualitative CO detection was performed using the PdCl\textsubscript{2} method.\textsuperscript{29}

Physical Methods. \textsuperscript{1}H NMR spectra were recorded on a Bruker ARX 400 spectrometer with the spectra for 4 and 5 being recorded under conditions as previously described.\textsuperscript{30} Chemical shifts (\(\delta\)) are referenced to the residual solvent peak in CD\textsubscript{2}HCN (\textsuperscript{1}H: 1.94 ppm). UV-vis spectra were recorded on a Hewlett-Packard 8453 diode array spectrophotometer. FTIR spectra were recorded on a Shimadzu FTIR-8400 spectrometer. Mass spectrometry experiments were performed at the University of California, Riverside. Room temperature magnetic susceptibilities were determined by the Evans method.\textsuperscript{31} Elemental analyses were performed by Canadian Microanalytical, Service, Inc., British Columbia or Atlantic Microlabs, Inc., Norcross, GA.

Caution! Perchlorate salts of metal complexes supported by organic ligands are potentially explosive.\textsuperscript{32}

2-(neopentylamino)-6-(aminomethyl) pyridine. LiAlH\textsubscript{4} (1.02 g, 0.0268 mol) was added to 50 mL of freshly distilled Et\textsubscript{2}O and the resulting slurry was stirred under N\textsubscript{2}
for ~10 min. This mixture was then added dropwise to 2-(pivaloylamido)-6-
(aminomethyl)pyridine (0.69 g, 0.0033 mol). The resulting mixture was stirred at room
temperature for 24 hours. Distilled water (75 mL) was then added, with the first 10 mL
being added dropwise. The solution was then filtered and the filtrate was extracted with
ethyl acetate (3x50 mL), the combined organic fractions were dried over Na₂SO₄, and the
solvent was removed under reduced pressure, yielding a pale yellow oil (0.58, 90%). ¹H
NMR (CD₃CN, 400 MHz) δ 7.32 (t, J = 7.7 Hz, 1H), 6.46 (d, J = 7.2 Hz, 1H), 6.31 (d, J
= 8.3 Hz, 1H), 5.02 (br, 2H), 3.61 (s, 2H), 3.15 (d, J = 6.4 Hz, 2H), 0.94 (s, 9H) ppm.

6-NA-6-Ph₂TPA. 2-(neopentylamino)-6-(aminomethyl)pyridine (0.58 g, 3.0 x 10⁻³ mol),
2-(chloromethyl)-6-phenylpyridine hydrochloride (1.44 g, 6.00 x 10⁻³ mol),
Na₂CO₃ (3.18 g, 3.01 x 10⁻² mol), and a catalytic amount of TBABr were combined in
CH₃CN (150 mL). This mixture was refluxed under a N₂ atmosphere for 20 h. After
cooling to room temperature, the reaction mixture was added to an equal volume of 1 M
NaOH. Extraction with CH₂Cl₂ (3 x 80 mL) followed by drying of the combined organic
fractions over Na₂SO₄ and removal of the solvent under reduced pressure yielded a
yellow-brown oil. Purification by column chromatography (silica gel, 230-400 mesh;
ethyl acetate/hexanes 1:1) gave the product as a yellow oil (1.2 g, 74%). ¹H NMR
(CD₃CN, 400 MHz) δ 8.05 (d, J = 8.5 Hz, 4H), 7.78 (t, J = 8Hz, 2H), 7.69 (d, J = 7.7 Hz,
2H), 7.63 (d, J = 7.7 Hz, 2H), 7.49-7.41 (m, 6H), 7.36 (m, 1H), 6.78 (d, J = 7.2 Hz, 1H),
6.35 (d, J = 8.3 Hz, 1H), 5.02 (br, 1H), 3.98 (s, 4H), 3.73 (s, 2H), 3.18 (d, J = 6.4 Hz,
2H), 0.94 (s, 9H) ppm; ¹³C{¹H} NMR (CD₃CN, 100 MHz) δ 160.9, 160.4, 158.5, 157.0,
140.5, 138.4, 129.7, 127.8, 122.5, 119.5, 111.8, 106.7, 61.0, 60.9, 53.5, 33.0, 27.9 (19
signals expected, 18 observed; overlap of two resonances in aromatic region); FTIR (KBr, cm⁻¹) 3400 (v_{N-H}).

\[(6-\text{NA}-6-\text{Ph}_2\text{TPA})\text{Ni}\{(\mu-\text{PhC(O)C(O)C(O)Ph})_2\text{Ni}(\text{ClO}_4)_2\} (4)\]

To a solution of 6-NA-6-Ph₂TPA (0.042 g, 0.080 mmol) in CH₃CN (2 mL) was added a CH₃CN solution (2 mL) of Ni(ClO₄)₂·6H₂O (0.044 g, 0.12 mmol) and PhC(O)CH(OH)C(O)Ph (0.019 g, 0.080 mmol). After 15 min of stirring, this mixture was transferred to a new vessel containing Me₄NOH·5H₂O (0.029 g, 0.16 mmol). The solution was stirred for 3 h at room temperature under a nitrogen atmosphere after which the solvent was removed under reduced pressure and the residue was redissolved in CH₂Cl₂, filtered, and the filtrate reduced to ~1 mL. Addition of excess Et₂O (~15 mL) resulted in the precipitation of an orange-brown solid that was collected and dried under vacuum. Long needles were obtained by Et₂O diffusion into a CH₂Cl₂ solution of 4 (29 mg, 38%). Crystals suitable for X-ray crystallography were obtained by Et₂O diffusion into an acetonitrile/methanol solution of 1. Anal calcd. for C₁₀₀H₉₄N₁₀O₁₄Ni₃Cl₂·0.₆₆CH₂Cl₂: C, 61.58; H, 4.89; N, 7.13. Found: C, 61.50; H, 4.83; N, 6.75 (The presence of methylene chloride in the sample was confirmed by ¹H NMR). UV-vis, nm (ε, M⁻¹cm⁻¹) 463 (16,000); μ_{eff} = 5.3(2) μΒ; FTIR (KBr, cm⁻¹) 3411 (br, v_{N-H}), 1096 (v_{ClO₄}), 624 (v_{ClO₄}).

Independent synthesis of [(6-NA-6-Ph₂TPA)Ni(O₂CPh)(H₂O)]ClO₄ (5). A solution of 6-NA-6-Ph₂TPA (0.028 g, 0.052 mmol) in CH₂Cl₂ was added to solid Ni(ClO₄)₂·6H₂O (0.019 g, 0.052 mmol). To this slurry was added a methanol solution of sodium benzoate (0.0075 g, 0.052 mmol). The resulting green solution was stirred for 1.5 h at room temperature. The solvent was then removed under reduced pressure. The green residue was redissolved in CH₂Cl₂, filtered, and the filtrate was brought to dryness under
vacuum. The solid was dissolved in methanol and this solution was added to an equal volume of water. Slow evaporation of this mixture produced green crystals (0.029 g, 68%). Anal Calcd. for C_{42}H_{44}N_{5}O_{7}NiCl: C, 61.22; H, 5.39; N, 8.50. Found: C, 60.89; H, 5.44; N, 8.36. UV-vis, nm (ε, M^{-1} cm^{-1}) 600 (19), 1004 (18); FTIR (KBr, cm^{-1}) 3360 (ν_{N-H}), 1092 (ν_{ClO_4}), 621 (ν_{ClO_4}); FAB-MS MeOH:NBA, m/z (relative intensity) 706 ([M-ClO_4-H_2O]^+, 100%).

Product isolation and identification following the reaction of 4 with $^{18}$O$_2$.

Compound 4 (57 mg, 0.030 mmol) was dissolved in CH$_3$CN (10 mL). It was degassed by two freeze/pump/thaw cycles and was then exposed to $^{18}$O$_2$ and stirred for 12 h at room temperature. This resulted in the formation of a pale green reaction mixture. After removal of the solvent under reduced pressure, to the remaining solids was added hexanes:ethyl acetate (4:1 mixture) and then CH$_3$CN dropwise until all solids had dissolved. The mixture was then transferred to a silica gel column and fractions were collected. Using hexanes:ethyl acetate (4:1) benzil (PhC(O)C(O)Ph) was eluted from the column. This product was identified by $^1$H NMR and GC-MS. The yield of benzil from this reaction was consistently ~20% based on the formulation that one benzil molecule can form from each enediolate ligand of 4. A control reaction indicated that an independent sample of benzil was recovered in ~85% yield using the column conditions outlined above.

Switching of the solvent to pure acetonitrile resulted in the elution of a pale green band. Removal of the solvent from this fraction and analysis by $^1$H NMR indicated the presence of [(6-NA-6-Ph$_2$TPA)Ni(O$_2$CPh)(H$_2$O)]ClO$_4$ (5) as the signals of this product in the 30-70 ppm region exactly matched those obtained for the independently prepared 5.
The isolated yield of this product from the column was variable (~50-75% based on producing two equivalents of 5 from each equivalent of 4). A control reaction indicated that an independent sample of 5 was recovered in ~75% yield using the column conditions outlined above. Thus a portion of 5 is lost during the chromatographic separation. These combined results suggest that 5 is produced in nearly quantitative yield in the reaction of 4 with O₂, which is consistent with ¹H NMR spectra of the reaction mixture (Figure 5-5(b)). Switching of the column eluent to MeOH resulted in the elution of a yellow material. Following removal of the solvent under reduced pressure the pale yellow material was evaluated using ¹H NMR and FTIR. The ¹H NMR spectrum did not contain any resonances in the paramagnetically-shifted region beyond 10 ppm, only broad peaks overlapping a few sharp resonances in the 5-8 ppm region. The infrared spectrum of the solid yellow material shows carboxylate vibrations at 1554 (νₐs(COO⁻)) and 1405 cm⁻¹ (νₛ(COO⁻)), respectively, and phenyl group vibrations at 1598 cm⁻¹ and 717 cm⁻¹. These combined results are consistent with the yellow compound being a Ni(II) benzoate species, such as Ni(O₂CPh)₂·nCH₃OH. Attempts were made to characterize this yellow compound using mass spectrometry methods (FAB and MALDI). Unfortunately, this material, which has limited solubility in organic solvents, did not give clear spectral evidence for a particular formulation. The data from both methods exhibited only low intensity m/z peak envelopes (e.g. <20% intensity relative to the peaks present for the nitrobenzylacohol matrix in the FAB-MS spectrum) that could not be conclusively identified.

**X-ray Crystallography.** Single crystals of 4·2Et₂O·3CH₃CN and 5·CH₃OH were each mounted on a glass fiber with traces of viscous oil and then transferred to a
Nonius KappaCCD diffractometer (Mo Kα, \( \lambda = 0.71073 \) Å). Ten frames of data were collected with an oscillation range of 1 deg/frame and an exposure time of 20 sec/frame. Indexing and unit cell refinement based on all observed reflections from those ten frames indicated a monoclinic \( C \) lattice for \( 4\cdot2\text{Et}_2\text{O}\cdot3\text{CH}_3\text{CN} \) and a monoclinic \( P \) lattice for \( 5\cdot\text{CH}_3\text{OH} \). A total of 17358 reflections for \( 4\cdot2\text{Et}_2\text{O}\cdot3\text{CH}_3\text{CN} \) and 17258 reflections for \( 5\cdot\text{CH}_3\text{OH} \) were indexed, integrated and corrected for Lorentz, polarization, and absorption effects using DENZO-SMN and SCALEPAC.\(^{33} \) The structure was solved by a combination of direct methods and heavy atom using SIR97.\(^{34} \) All of the non-hydrogen atoms of \( 4\cdot2\text{Et}_2\text{O}\cdot3\text{CH}_3\text{CN} \) and \( 5\cdot\text{CH}_3\text{OH} \) were refined with anisotropic displacement coefficients. The N(1)-H in \( 4\cdot2\text{Et}_2\text{O}\cdot3\text{CH}_3\text{CN} \) was located and refined independently. All other hydrogen atoms were assigned isotropic displacement coefficients U(H) = 1.2U(C) or 1.5U(Cmethyl) and their coordinates were allowed to ride on their respective carbons using SHELXL97.\(^{35} \) One perchlorate anion and the \( t \)-butyl methyl group exhibit disorder in \( 4\cdot2\text{Et}_2\text{O}\cdot3\text{CH}_3\text{CN} \). The perchlorate anion in \( 5\cdot\text{CH}_3\text{OH} \) exhibits disorder.

**Results and Discussion**

Acireductone dioxygenases are fascinating metalloenzymes. The Ni(II)-ARD and Fe(II)-ARD’ enzymes represent the only example in biology wherein the simple change of a metal ion results in a different chemical reaction. The key issue that is proposed to determine ARD vs. ARD’ reactivity is the coordination mode of the acireductone on the divalent metal center. Metal-centered reactivity with \( \text{O}_2 \) is not proposed in these systems. Instead, direct reaction between the coordinated acireductone and \( \text{O}_2 \) is proposed to result in the formation of a 4- or 5-membered peroxide species involving the acireductone from which the ARD and ARD’ products are generated, respectively.
Synthesis. A goal of our work is to prepare and characterize examples of divalent metal complexes of acireductone-type ligands and examine their reactivity with O₂. A recent Cambridge Crystallographic Database search (v. 5.29 updated Jan. 2008) revealed that structurally characterized examples of transition metal complexes having an acyclic acireductone-type ligand remain rare, with the only examples to our knowledge being [(Ru(bipy)₂)(μ-C₄H₄O₃)](PF₆)₂ (Figure 5-1(c)) and [(6-Ph₂TPA)Ni(PhC(O)C(OH)C(O)Ph)]ClO₄ (1, Scheme 5-2). In the diruthenium complex the acireductone is coordinated as a dianion in a coordination motif akin to that found in the neutral and rubidium salt forms of triose reductone (Figure 5-1). Complex 1 contains a cis-β-keto enolate-type ligand of the monodeprotonated form of 2-hydroxy-1,3-diphenylpropan-1,3-dione (Scheme 5-2). The different coordination modes of these acireductone-type ligands could be due to their structural differences and/or the protonation level. Our investigations with the 6-NA-6-Ph₂TPA ligand started with an attempt to prepare an analog of 1 wherein one oxygen atom of the coordinated acireductone-type ligand could form a hydrogen bonding interaction with the neopentyl substituent of the chelate ligand. From a reaction mixture comprised of a 1:1:1:1 stoichiometry (6-NA-6-Ph₂TPA, Ni(ClO₄)₂⋅6H₂O, Me₄NOH⋅6H₂O, 2-hydroxy-1,3-diphenylpropane-1,3-dione) of reagents we obtained a few small orange-brown needle-type crystals. This material was identified by single crystal X-ray crystallography as the trinuclear complex [(6-NA-6-Ph₂TPANi)₂(μ-PhC(O)C(O)C(O)Ph)₂Ni](ClO₄)₂ (4). The production of 4 under these conditions is interesting as it suggests that the presence of the internal hydrogen bond donor may be important toward promoting dianion coordination. Adjustment of the reaction stoichiometry to that required for the formation of the
trinuclear 4 (Scheme 5-3) gave an orange-brown crystalline solid in 38% overall yield. This relatively low yield appears to be due in part to the presence of at least one additional product in this reaction mixture as indicated by $^1$H NMR.

**X-ray Crystallography.** A drawing of the cationic portion of $4\cdot2\text{Et}_2\text{O}\cdot3\text{CH}_3\text{CN}$ is shown in Figure 5-3(a). The two terminal pseudo octahedral Ni(II) centers are equivalent via a $C_2$ rotation. A drawing of the trinuclear core and metal coordinated atoms in $4\cdot2\text{Et}_2\text{O}\cdot3\text{CH}_3\text{CN}$ are shown in Figure 5-3(b), with the full ligand environments of the pseudo octahedral and square planar Ni(II) centers being shown in Figure 5-3(c) and 5-3(d). Details of the X-ray data collection and refinement are given in Table 5-1. Selected bond distances and angles are given in Table 5-2.

The Ni(1) center exhibits a pseudo octahedral coordination geometry. The Ni-N bonds vary in length over a range of ~0.3 Å, with the shortest Ni-N distance involving the tertiary amine donor of the chelate ligand (Ni(1)-N(3) 2.039(3) Å). The longest Ni-N distance is found for one of the two phenyl-appended pyridyl donors (Ni(1)-N(4) 2.341(3) Å). A survey of the Ni(II) complexes reported to date of chelate ligands containing an aryl-appended pyridyl donor indicates that a range of Ni-N$_{PhPy}$ distances (2.15-2.36 Å) are possible depending on the other ligands present on the metal center.\cite{22,23,27,30,37,38} The Ni(1)…Ni(2) distance is 3.73 Å.

The enediolate ligand in $4\cdot2\text{Et}_2\text{O}\cdot3\text{CH}_3\text{CN}$ is coordinated as a *cis* bridging ligand between Ni(1) and Ni(2), forming two five-membered chelate rings. This coordination motif is similar to that found in the rubidium salt and neutral form of triose
Figure 5-3. Thermal ellipsoid drawings (50% probability) of the cationic portion of 4·2Et₂O·3CH₃CN. (a) Full cation; (b) trinuclear metal core; (c) the pseudo octahedral Ni(1) center; and (d) the square planar Ni(2) center. Hydrogen atoms except the neopentylamine proton have been omitted for clarity.

reductone (Figure 5-1) and in [(Ru(bipy)₂(μ-C₄H₄O₃))(PF₆)₂].¹⁹⁻²¹ The Ni(1)-O distances differ only slightly, with the axial Ni(1)-O(1) distance being ~0.07 Å shorter than the bridging Ni(1)-O(2) bond length. The square planar Ni(II) center has shorter Ni-O bonds (~1.86 Å). The five-membered chelate ring of the enediolate results in a O(2)-Ni(2)-O(3) bond angle (85.77(11)°) that is acute relative to the O(3)#1-Ni(2)-O(2) angle (94.20(11)°). The C(36)-O(1) (1.287(5) Å) and C(38)-O(3) (1.310(4) Å) bond distances
are shorter than the C(37)-O(2) (1.380(5) Å) bond indicating more single bond character in the latter. This is consistent with a localized anion at O(2) and a delocalized enolate anion in the O(1)-C(36)-C(37)-C(38)-O(3) backbone. Finally, at the Ni(1) site, O(1) accepts a hydrogen bond from the neopentylamine moiety of the chelate ligand ((N(1)...O(1) 2.838(5) Å; N(1)-H(1)...O(1) 163(5)°). These parameters are consistent with the presence of a moderate hydrogen bonding interaction.39

Overall, the isolation and structural characterization of 4 reveals that the enediolate form of the coordinated acireductone-type ligand can be stabilized via the recruitment of an additional Ni(II) center, with a contribution from a hydrogen bonding interaction. Stabilization of a Ni(II)-coordinated acireductone dianion species in the active site of Ni(II)-ARD is proposed to involve hydrogen bonding interactions with one or more arginine residues.1

Spectroscopic Properties. The solid-state infrared spectrum of 4 in KBr contains a broad, intense feature at ~3420 cm⁻¹ for the neopentylamine N-H moiety. Vibrations at 1096 and 623 cm⁻¹ are consistent with the presence of the perchlorate anions.

The UV-vis spectrum of 4 in CH₂Cl₂ (Figure 5-4) contains a broad absorption band at 463 nm ($\varepsilon = 16,000$ M⁻¹cm⁻¹; Figure 5-4). This spectrum is notably distinct from that exhibited by 1 ($\lambda_{\text{max}} = 399$ nm ($\varepsilon = 6800$ M⁻¹cm⁻¹) in CH₂Cl₂).23 The intensity of the absorption band in 4 is consistent with a charge transfer type transition.

The $^1$H NMR spectrum of 4 contains several distinct paramagnetically shifted resonances in the region of 20-70 ppm (Figure 5-5(a)). Assignments of individual resonances in this region have not been made, as the method typically employed to
conclusively identify such signals (2D-COSY)\textsuperscript{30} is not feasible due to the broad nature of several of the signals.

Pseudo octahedral high-spin Ni(II) centers each have two unpaired spins and the central Ni(II) is diamagnetic. The observed magnetic moment is slightly higher than the spin-only value predicted for this electronic structure ($\mu_{so} = 4.9 \mu_B$), which could be due to orbital contributions to the magnetic moment.

**O$_2$ Reactivity of 4. Product Identification.** Exposure of an acetonitrile solution of 4 to O$_2$ results in the rapid loss of the orange-brown color to give a pale green solution. The overall reaction that takes place is shown in Scheme 5-4. The methods used for product identification and/or isolation are outlined below.

Analysis of the products generated in the reaction of 4 with O$_2$ by $^1$H NMR revealed the presence of several new resonances in the region of 20-70 ppm (Figure 5-6(b)). Mass spectroscopic analysis of the mixture revealed a parent ion at $m/z$ 706. The complex associated with this $^1$H NMR spectrum and parent ion was identified via the independent synthesis as [(6-NA-6-Ph$_2$TPA)Ni(O$_2$CPh)(H$_2$O)]ClO$_4$ (5). This compound was prepared via admixture of equimolar amounts of 6-NA-6-Ph$_2$TPA, Ni(ClO$_4$)$_2$·6H$_2$O, and sodium benzoate in wet CH$_2$Cl$_2$/MeOH. Recrystallization of the product from MeOH/water yielded 5 as green crystals in 68% yield. The independently synthesized complex has been characterized by X-ray crystallography, elemental analysis, FTIR, mass spectrometry, and UV-vis. Compound 5 can be isolated from the reaction mixture of 4 with O$_2$ in \textasciitilde50-75% yield by column chromatography. A less than stoichiometric amount is isolated due to the conditions employed (column chromatography) for separation of 4 from the other reaction products.
Table 5-1. Summary of X-ray data collection and refinement for 4·2Et2O·3CH3CN and 5·CH3OH

<table>
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<th>5·CH3OH</th>
</tr>
</thead>
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<tr>
<td>empirical formula</td>
<td>C114H123Ni15O16Ni3Cl2</td>
<td>C43H48N3O3NiCl</td>
</tr>
<tr>
<td>formula weight</td>
<td>2178.28</td>
<td>857.02</td>
</tr>
<tr>
<td>crystal system</td>
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<td>Monoclinic</td>
</tr>
<tr>
<td>space group</td>
<td>C2/c</td>
<td>P21/n</td>
</tr>
<tr>
<td>a (Å)</td>
<td>24.4466(8)</td>
<td>11.6202(3)</td>
</tr>
<tr>
<td>b (Å)</td>
<td>18.6303(7)</td>
<td>23.7494(4)</td>
</tr>
<tr>
<td>c (Å)</td>
<td>23.8127(7)</td>
<td>15.1919(4)</td>
</tr>
<tr>
<td>a (deg)</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>b (deg)</td>
<td>96.160(2)</td>
<td>95.9420(11)</td>
</tr>
<tr>
<td>g (deg)</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>V (Å³)</td>
<td>10782.8(6)</td>
<td>4170.03(17)</td>
</tr>
<tr>
<td>Z</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>(d_{calc.}) Mg m⁻³</td>
<td>1.342</td>
<td>1.365</td>
</tr>
<tr>
<td>temp (K)</td>
<td>150(1)</td>
<td>150(1)</td>
</tr>
<tr>
<td>crystal size (mm³)</td>
<td>0.23 x 0.13 x 0.08</td>
<td>0.30 x 0.25 x</td>
</tr>
<tr>
<td>diffractometer</td>
<td>Nonius KappaCCD</td>
<td>Nonius KappaCC</td>
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<tr>
<td>abs. coeff. (mm⁻¹)</td>
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<td>0.588</td>
</tr>
<tr>
<td>2θ max (deg)</td>
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<td>reflections</td>
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<td>17258</td>
</tr>
<tr>
<td>indep. reflections</td>
<td>9891</td>
<td>9480</td>
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<tr>
<td>variable parameters</td>
<td>721</td>
<td>685</td>
</tr>
<tr>
<td>R1 / wR2ᵇ</td>
<td>0.0582/0.1174</td>
<td>0.0643/0.1487</td>
</tr>
<tr>
<td>goodness-of-fit (Fr²)</td>
<td>1.043</td>
<td>1.042</td>
</tr>
<tr>
<td>largest diff. (e Å³)</td>
<td>0.609, -0.638</td>
<td>1.304, -1.026</td>
</tr>
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</table>

[b]Radiation used: Mo Kα (\(\lambda = 0.71073\) Å). [b]R1 = \(\sum |F_o| - |F_c| / \sum |F_o|\), wR2 = \(\sum [w(F_o^2 - F_c^2)^2]/\sum(F_o^2)]^{1/2}\) where \(w = 1/[σ(F_o^2)] + (aP)^2 + bP). 

Table 5-2. Selected bond distances (Å) and angles (°) in the cationic portion of 4·2Et2O·3CH3CNᵃ

<table>
<thead>
<tr>
<th>Bond</th>
<th>Distance (Å)</th>
<th>Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ni(1)-O(1)</td>
<td>1.988(3)</td>
<td></td>
</tr>
<tr>
<td>Ni(1)-O(2)</td>
<td>2.057(3)</td>
<td></td>
</tr>
<tr>
<td>Ni(1)-N(2)</td>
<td>2.119(3)</td>
<td></td>
</tr>
<tr>
<td>Ni(1)-N(3)</td>
<td>2.039(3)</td>
<td></td>
</tr>
<tr>
<td>Ni(1)-N(4)</td>
<td>2.341(3)</td>
<td></td>
</tr>
<tr>
<td>Ni(1)-N(5)</td>
<td>2.188(3)</td>
<td></td>
</tr>
<tr>
<td>Ni(2)-O(2)</td>
<td>1.864(2)</td>
<td></td>
</tr>
<tr>
<td>Ni(2)-O(3)</td>
<td>1.856(3)</td>
<td></td>
</tr>
<tr>
<td>Ni(1)-Ni(1)</td>
<td>1.97(1)</td>
<td></td>
</tr>
<tr>
<td>Ni(1)-Ni(2)</td>
<td>1.97(1)</td>
<td></td>
</tr>
<tr>
<td>Ni(2)-O(2)</td>
<td>1.856(3)</td>
<td></td>
</tr>
<tr>
<td>Ni(2)-O(3)</td>
<td>1.856(3)</td>
<td></td>
</tr>
</tbody>
</table>

ᵃEstimated standard deviations in the last significant figure are given in parentheses.
Figure 5-4. UV-vis spectrum of 4 in CH$_2$Cl$_2$ under a N$_2$ atmosphere.

Scheme 5-4. Exposure of an acetonitrile solution of 4 to O$_2$.

Figure 5-5. $^1$H NMR spectra of: (a) analytically pure 4; (b) the reaction mixture produced from treatment of 4 with O$_2$; and (c) independently synthesized, analytically pure 5.
A drawing of the cationic portion of 5 is shown in Figure 5-6. Selected bond distances and angles of this cation are given in Table 5-3. Complex 5 exhibits κ³-coordination of the 6-NA-6-Ph₂TPA ligand, with the noncoordinated phenyl-appended pyridyl moiety serving as a hydrogen bond acceptor for a metal-bound water molecule. This hydrogen bonding interaction involves a N(5)…O(3) distance of 2.68 Å and a N(5)…H-O(3) angle of 172°. A similar hydrogen bonding interaction, involving a noncoordinated phenyl-appended phenyl nitrogen, was identified in a Ni(II) acetohydroxamic acid complex wherein the hydrogen bond donor is the N-H moiety of the Ni(II) bound acid.24 The Ni(1)-O(3) (water) distance in 5 is 2.061(3) Å, which is ~0.03 Å shorter than the average of the other five metal ligand bond distances. The Ni(1)-N(4) distance (2.137(3) Å) is on the short end of the range of bond lengths previously identified for a phenyl-appended pyridyl donor to a pseudo octahedral Ni(II) center (2.15-2.36 Å).22,23,27,30,37,38 The benzoate ligand is coordinated in a bidentate fashion, with the O(1) atom of the benzoate ligand accepting a hydrogen bond from neopentylamine moiety of the supporting chelate ligand.

In dry CD₃CN 5 exhibits at least seven resonances in the range of 20-70 ppm (Figure 5-5(c)). As all three pyridyl donors are inequivalent in this complex, at least six resonances can be expected in this range for the β/β’-H’s (Figure 5-2). We note that methylene –CH₂- resonances are also typically found in this region in mononuclear Ni(II) complexes of tripodal pyridyl ligands.30 In the reaction mixture produced upon treatment of 4 with O₂ two additional products have been identified (Scheme 5-4). Exposure of a sample of the headspace gas of the reaction to an aqueous PdCl₂ solution resulted in the deposition of Pd(0) which is consistent with presence of CO according to
Figure 5-6. Thermal ellipsoid drawing (50% probability) of the cationic portion of 5. Hydrogen atoms except the neopentylamine proton and the water protons have been omitted for clarity.

Table 5-3. Selected bond distances (Å) and angles (°) in the cationic portion of 5-CH₃OH

<table>
<thead>
<tr>
<th>Bond</th>
<th>Distance (Å)</th>
<th>Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ni(1)-O(1)</td>
<td>2.075(3)</td>
<td>167.90(12)</td>
</tr>
<tr>
<td>Ni(1)-O(2)</td>
<td>2.163(3)</td>
<td>95.29(11)</td>
</tr>
<tr>
<td>Ni(1)-O(3)</td>
<td>2.061(3)</td>
<td>79.62(12)</td>
</tr>
<tr>
<td>Ni(1)-N(2)</td>
<td>2.055(3)</td>
<td>170.31(11)</td>
</tr>
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<td>Ni(1)-N(3)</td>
<td>2.099(3)</td>
<td>86.15(11)</td>
</tr>
<tr>
<td>Ni(1)-N(4)</td>
<td>2.137(3)</td>
<td>62.50(10)</td>
</tr>
<tr>
<td>O(1)-N(1)</td>
<td>88.24(12)</td>
<td>83.73(11)</td>
</tr>
<tr>
<td>N(2)-Ni(1)</td>
<td>109.23(11)</td>
<td></td>
</tr>
<tr>
<td>O(3)-Ni(1)</td>
<td>85.86(11)</td>
<td></td>
</tr>
<tr>
<td>N(2)-Ni(1)</td>
<td>81.30(12)</td>
<td></td>
</tr>
<tr>
<td>O(3)-Ni(1)</td>
<td>97.14(12)</td>
<td></td>
</tr>
<tr>
<td>O(1)-N(1)</td>
<td>169.20(11)</td>
<td></td>
</tr>
<tr>
<td>N(2)-Ni(1)</td>
<td>102.68(12)</td>
<td></td>
</tr>
</tbody>
</table>

*Estimated standard deviations in the last significant figure are given in parentheses.*
the equation:\textsuperscript{29}:

\[
PdCl_2 + CO + H_2O \rightarrow Pd^0 + CO_2 + 2 \text{HCl}
\]

A second product, benzil (PhC(O)C(O)Ph) was isolated from the reaction mixture via column chromatography on silica gel with hexanes/ethyl acetate (4:1) containing trace CH\textsubscript{3}CN as the eluent. This organic product has also been identified in the reactions of 1 as outlined in Scheme 5-2. The yield of benzil obtained in repeat reactions of 4 with O\textsubscript{2} is \textasciitilde20\%.

The column chromatography separation method used to isolate benzil also enables the separation of 5 from another fraction that is yellow in color. Pure acetonitrile is used to elute 5 after which point the addition of methanol results in the elution of the yellow product. When dissolved in CD\textsubscript{3}CN the yellow material exhibits several broad \textsuperscript{1}H NMR resonances in the region of 5-8 ppm. In the portion of the spectrum from 7-8 ppm the broad resonances are overlapped with at least two smaller, sharper signals (\textasciitilde7.5 and 8.0 ppm). The broad nature of the most intense resonances is consistent with a Ni(II)-containing species. Infrared spectroscopic studies indicate that this material does not contain the 6-NA-6-Ph\textsubscript{2}TPA ligand or a perchlorate anion, but has vibrations indicating the presence of one or more benzoate anions. Specifically, carboxylate vibrations are present at 1554 (\textit{v\textsubscript{as(COO\textsubscript{2})}}) and 1405 cm\textsuperscript{-1} (\textit{v\textsubscript{s(COO\textsubscript{2})}}), respectively, and phenyl group vibrations at 1598 cm\textsuperscript{-1} and 717 cm\textsuperscript{-1}. These vibrations are similar to the major vibrations of sodium benzoate (1598, 1550, 1414, 706, and 681 cm\textsuperscript{-1}). Additional vibrations for the reaction product are found at \textasciitilde3400 and \textasciitilde2950 cm\textsuperscript{-1}. We propose that these vibrations
may be due to the presence of methanol, suggesting a formulation such as

\[ \text{Ni(O}_2\text{CPh)}\text{H}_2\text{O)}\text{CH}_3\text{OH}. \]

A Cambridge Crystallographic Database search revealed that a series of nickel benzoate complexes having monodentate carboxylate ligands and four coordinated water molecules of the empirical formula \([\text{Ni(O}_2\text{CAr)}\text{H}_2\text{O)}\text{nH}_2\text{O}}\ (\text{Ar} = 3,5\text{-dihydroxyphenyl (n = 3); 3,5\text{-dinitrophenyl (n = 4); or 4\text{-formylphenyl (n=4)) have been structurally characterized.}^{40-42} \)

However, X-ray quality crystals of the yellow product have not yet been obtained.

Treatment of a CH\text{3CN} (ACROS extra dry, <10 ppm water) solution of \(4 \) with \(^{18}\text{O}_2\) results in \(~86\%\) \(^{18}\text{O}\) labelling of one oxygen atom of the benzoate ligand of \(5\).

Reactions of this type performed using \(^{16}\text{O}_2\), extra dry CH\text{3CN}, and \(\text{H}_2^{18}\text{O}\) did not result in \(^{18}\text{O}\) incorporation into \(5\). Thus, the \(~14\%\) of \(5\) that contains unlabeled benzoate must be generated from adventitious \(^{16}\text{O}_2\) in the reaction mixture or via an alternative reaction pathway.

**Comments on the \(\text{O}_2\) Reactivity of \(1\) and \(4\).** In the absence of added base \(1\) reacts with \(\text{O}_2\) to give a nickel benzoate complex, CO, benzil, and other phenyl-containing organic products.\(^{23}\) This reaction occurs with only a modest level of \(^{18}\text{O}\) incorporation (~50%) into one oxygen atom position of the benzoate ligand of \([\text{(6-Ph}_2\text{TPA)}\text{Ni(O}_2\text{CPh)}\text{H}_2\text{O)}\text{ClO}_4)}\ (3). Under conditions wherein the dianionic enediolate form of 2-hydroxy-1,3-diphenylpropane-1,3-dione is present, for example \(1\) upon treatment with base or in \(4\), the reaction is cleaner, with the only organic byproduct being benzil. These reactions also exhibit a higher level of \(^{18}\text{O}\) incorporation into one of the oxygen atom position in the product benzoate complex, \([\text{6-Ph}_2\text{TPA)}\text{Ni(O}_2\text{CPh)}\text{H}_2\text{O)}\] \((2, 67\%)\) or \([\text{6-NA-6-Ph}_2\text{TPA)}\text{Ni(O}_2\text{CPh)}\text{(H}_2\text{O)}\text{ClO}_4)}\ (5, 86\%), respectively. We note that these levels
of $^{18}$O incorporation are near that reported for the Ni(II)-ARD catalyzed reaction (77.5%).

**Conclusions**

The presence of a secondary hydrogen bond donor in the 6-NA-6-Ph$_2$TPA ligand produces chemistry involving Ni(II) and 2-hydroxy-1,3-diphenylpropane-1,3-dione in the presence of base that is distinctly different from that found for the 6-Ph$_2$TPA chelate ligand. Specifically, use of the 6-NA-6-Ph$_2$TPA ligand has enabled the formation and isolation of the trinuclear $^4$ which contains a coordinated enediolate form of 2-hydroxy-1,3-diphenylpropane-1,3-dione. This indicates that the Ni(II) coordination chemistry of this acireductone-type ligand is strongly influenced by the nature of the supporting chelate ligand and secondary environment surrounding the metal center. As the key factor in determining Ni(II)-ARD versus Fe(II)-ARD’ type reactivity has been proposed to be the coordination mode of the acireductone, which is likely influenced by the secondary environment of the metal center, additional investigations of ligand secondary environment modifications are clearly warranted.

Current efforts in our laboratory are also focused on kinetic and mechanistic studies of the O$_2$ reactions involving $^1$ and $^4$. One avenue of investigation is directed at determining the pathway by which benzil is generated. We hypothesize that benzil may be formed via a reaction wherein the enolate or endiolate moiety of $^1$ or $^4$ is oxidized by O$_2$ to give 1,3-diphenylpropanetrione (PhC(O)C(O)C(O)Ph) and hydroperoxide/peroxide anion.$^{44}$ A phenyl migration reaction involving this triketone could yield benzil and CO.$^{45}$ As triketones have also been shown to undergo reaction with H$_2$O$_2$ to yield carboxylates
and CO, we hypothesize that the products generated in the reactions of 1 and 4 with O₂ could be generated via a “triketone” pathway or via a mechanism involving a Ni(II)-coordinated cyclic peroxide species, as has been proposed for Ni(II)-ARD. Efforts are underway to determine if one or both of these types of reaction pathways are occurring upon treatment of 1 and 4 with O₂.

References


5. Gotoh, I.; Uekita, T.; Seiki, M. Genes Cells 2007, 12, 105-117.


36. Several structurally characterized metal complexes of ascorbic acid, which contains the 1-oxo-2-ene-2,3-diol (acireductone) structural unit within a cyclic structure, have been reported. See Zümreoglu-Karan, B. Coord. Chem. Rev. 2006, 250, 2295-2307; Davies, M. B. Polyhedron 1992, 11, 285-321.


CHAPTER 6

A STRATEGY TOWARD PRODUCING A Ni(II) COMPLEX
HAVING A C(1)-H ACIREDUCTONE LIGAND

Abstract

In order to investigate chemistry of relevance to a Ni(II)-containing acireductone dioxygenase (ARD) enzyme, a new Ni(II) complex having a C(1)-H acireductone ligand has been prepared. The acireductone (2,3-dihydroxy-1-phenyl-propenone) used in this study is an analog of the native ARD substrate and can be obtained via a previously reported combined synthetic/enzymatic route. This method involves the convenient synthesis of the phosphate-protected precursor followed by an enolase/phosphatase (E1) catalyzed reaction. A His-tagged version of the E1 enzyme was used in this study, which significantly simplified the protein purification process. A monosodium salt of the C(1)-H acireductone was generated via extraction of the enzymatic reaction mixture at low pH followed by a treatment of the isolated acireductone with NaOH. Introduction of this acireductone salt into a CH$_3$CN solution of [(6-Ph$_2$TPA)Ni](ClO$_4$)$_2$ resulted in the formation of a new O$_2$ sensitive complex which is formulated as [(6-Ph$_2$TPA)Ni(PhC(O)C(OH)C(O)H)]ClO$_4$ based on $^1$H NMR and UV-visible spectroscopic analyses. This complex reacts with O$_2$ to produce a Ni(II) benzoate complex, [(6-Ph$_2$TPA)Ni(O$_2$CPh)]ClO$_4$. The preliminary results presented in this chapter indicate that Ni(II) coordination of an acireductone influences the regioselectivity of the oxidative carbon-carbon bond cleavage reaction within the acireductone ligand.

Introduction
Compounds containing an acireductone functionality (α-keto enediol, RC(OH)=C(OH)C(=O)R') are strong reducing agents and fairly strong acids (e.g. ascorbic acid).\footnote{1} Furthermore, their anionic forms are electron rich and are susceptible to electrophilic attack by dioxygen.\footnote{2} An acireductone (2,1,2-dihydroxy-3-oxo-5-(methylthio)pent-1-ene) was identified as an advanced intermediate in the methionine salvage pathway (MSP) (Scheme 6-1).\footnote{3} This ubiquitous pathway recycles the methylthio-portion of S-adenosylmethionine (SAM) to produce methionine.\footnote{4} A breakdown of the ribose ring of methylthioadenosine (MTA) provides a carbon skeleton for the formation of the intermediate 1 (1-phosphonoxy-2,2-dihydroxy-3-oxo-5-methylhiopentane). Compound 1 is subsequently converted into acireductone 2 via the E1 enolase/phosphatase catalyzed reaction.

\textbf{Scheme 6-1.} An outline of the methionine salvage pathway (MSP).
To date, there has not been a synthetic route reported for the native -SMe containing acireductone substrate (2, Scheme 6-1). This is likely due to the presence of the -SMe substituent at the C(5) position, as this moiety is susceptible to oxidation and $\beta$-elimination reactions. This problem was addressed by the Abeles group with the preparation of desthio analogs of the naturally occurring acireductone substrate (3 and 4, Figure 6-1). These compounds were shown to be alternative substrates for the ARD enzymes. However, the synthetic pathway reported by Abeles et al. gave relatively low overall yields and required the use of explosive diazomethane. In 2004, a combined synthetic/enzymatic approach for preparing 3 and 4 was reported by Pochapsky et al. This method involves a convenient 3-step synthesis of the phosphate esters (Scheme 6-2) and their subsequent E1-catalyzed enolization/dephosphorylation reaction, which results in the formation of the desired acireductone.

Our laboratory has previously reported the preparation of a Ni(II) complex of a mono-deprotonated form of 2-hydroxy-1,3-diphenylpropan-1,3-dione (5, Figure 6-2). This molecule contains an acireductone-type motif albeit it has not been shown to be an alternative substrate for Ni(II)- or Fe(II)-ARD. The Ni(II) complex reacts with $O_2$ to produce carboxylates and CO, hence this reaction is relevant to the chemistry of Ni(II)-ARD. However, additional organic side products (e.g. benzil, PhC(O)C(O)Ph)
are generated in the O$_2$-dependent reaction, suggesting that multiple reaction pathways may be operative in this system. Specifically, we hypothesize that the phenyl group at the C(1) position of the coordinated enolate enables a pathway involving triketone formation that is not relevant to the Ni(II)-ARD catalyzed reaction.

In order to prepare a more accurate model complex for the enzyme/substrate adduct in Ni(II)-ARD, we have used the combined synthetic/enzymatic approach reported by Pochapsky to prepare the C(1)-H acireductone 3. Admixture of the monosodium salt of this acireductone with a Ni(II) complex of the 6-Ph$_2$TPA ligand has resulted in the formation of a new Ni(II) acireductone complex 8. Its $^1$H NMR and UV-visible spectroscopic properties are consistent with the formulation [(6-Ph$_2$TPA)Ni(PhC(O)C(OH)C(O)H)]ClO$_4$. This complex reacts with O$_2$ to produce 9 [(6-Ph$_2$TPA)Ni(O$_2$CPh)]ClO$_4$ in a reaction that is relevant to Ni(II)-ARD enzyme reaction. Thus, a new reactive model system for Ni(II)-ARD involving a C(1)-H containing acireductone has been developed.

**Figure 6-2.** Ni(II) complex of a deprotonated form of 2-hydroxy-1,3-diphenylpropan-1,3-dione.
Experimental Section

**General Methods.** All reagents and solvents for synthetic procedures were obtained from commercial sources and were used as received without further purification unless otherwise noted. Solvents were dried according to published methods and distilled under N\textsubscript{2} atmosphere prior to use.\textsuperscript{7} Water- and oxygen-sensitive reactions were performed in a M-20 Vacuum Atmosphere glovebox under an atmosphere of purified N\textsubscript{2}. The E1 enolase/phosphatase coding plasmid (pDioxHIS1) was obtained from Prof. Wilson Francisco (Arizona State University) and was stored at -80 °C.

**Physical Methods.** Diamagnetic \textsuperscript{1}H and \textsuperscript{13}C spectra were collected using a Bruker ARX-400 spectrometer. Paramagnetic \textsuperscript{1}H NMR spectra were recorded as previously described.\textsuperscript{8} UV-vis spectra of buffered solutions of 3 and a acetonitrile solution of 8 were recorded at ambient temperature using a Hewlett Packard 8453 diode array spectrophotometer.

*Caution!* Perchlorate salts of metal complexes with organic ligands are potentially explosive. Only small amounts of material should be prepared and these should be handled with great care.\textsuperscript{9}

**Synthesis of a Phosphate Ester Protected E1 Substrate Analog (6).** The ester compound 1-phosphonoxy-2,2-dihydroxy-3-phenylpropane 6 was prepared according to the literature procedure.\textsuperscript{6} Its acidic, aqueous solutions were stored at -20 °C.

**Overexpression and Purification of a His-tagged E1 Enolase/phosphatase Enzyme.** Single colonies of *E. coli* (BL21) transformed with the pDioxHIS10 plasmid were selected from LB-agar plates supplemented with kanamycin and placed in kanamycin-supplemented LB media. Protein expression was induced upon addition of
isopropyl β-D-thioglucopuranoside (IPTG). Once cell density reached ~0.6 at 600 nm
cells were harvested by centrifugation (10,000 x g, 10 min), the supernatant was
discarded, and the remaining protein pellets stored at -20°C until purified.

**Purification Procedure.** Cell pellets were resuspended in 30 mL of 50 mM Tris
buffer (pH 7.6) containing 500 mM NaCl. To this mixture 30 µL of a 1 mM iPrOH
solution of phenylmethylsulfonyl fluoride (PMSF) was added in order to inhibit protease
activity. A catalytic amount of DNases was then added and the cells were lysed by
passing through a French Press. The lysate was cleared by centrifugation (10,000 x g, 10
min) and the resulting pellets discarded. The E1 enzyme present in the supernatant was
then purified using a Ni-affinity column. Following the introduction of a protein sample,
the column was washed with ~30 mL of 50 mM Tris buffer (pH 7.6). The E1-containing
fraction was eluted from the column using 15 mL of Tris buffer containing 500 mM
imidazole. The E1 fractions were dialyzed overnight against 50 mM HEPES buffer
containing 0.5 mM MgCl₂ (replaced with fresh buffer solution twice). The purity of the
E1 enzyme was confirmed by Coomassie-stained SDS-PAGE and was estimated to be
~95 %. The enzyme was assayed for enolase/phosphatase activity according to the
literature procedure.⁶ Enzyme assays were performed under N₂ atmosphere. The obtained
E1 enzyme exhibited ~ 53 % of reported previously activity level. A buffered E1 enzyme
solution was stored at 8 °C.

**Sodium 2-hydroxy-3-oxo-3-phenyl-propen-1-olate (7).** An aqueous solution of
the phosphate ester-protected acireductone precursor 1-phosphonooxy-2,2-dihydroxy-3-
phenylpropane (6, ~200 mg) was brought to pH 7-8 using 1 M NaOH solution. This
solution was then diluted to ~200 ml with 50 mM HEPES buffer, containing 0.5 mM
MgCl₂ (pH 7.4) and degassed. It was combined with 20 ml of E1/HEPES buffer solution (50 mM, pH 7.4) under an inert atmosphere. The reaction to produce acireductone was monitored by UV-visible spectroscopy by placing 1 drop of the reaction mixture into 2 ml of 50 mM HEPES buffer at pH 7.4 in an anaerobic cuvette. Completion of the reaction was indicated by a single, absorption band at 320 nm. The enzyme solution was then brought to pH ~1 using 6 M HCl (~5 mL) and was extracted with CH₂Cl₂ (4 x 30 ml) under a N₂ atmosphere. The organic fractions were combined and solvent was removed under vacuum. The presence of the acireductone product in the solid residue was confirmed by UV-visible spectroscopy by dissolving a minimal amount of the solid (~1 mg) in 2 ml of 50 mM HEPES buffer at pH 7.4 under anaerobic conditions. The purity of the extracted acireductone sample (~90%) was established using ¹H NMR. (3) ¹H NMR (CD₂CN, 400 MHz) δ 7.58-7.52 (m, 3 H), δ 7.47-7.43 (m, 2 H), δ 7.11 (br, 1 H). The remaining solid product was then dissolved in distilled H₂O under a N₂ atmosphere and the pH of the solution was adjusted to ~8-9 using 0.1 M NaOH. Removal of water under vacuum yielded a yellow-orange material (0.055g, 36% yield). (7) ¹H NMR (CD₃OD, 400 MHz) δ 8.07 (s, 1H), δ 7.45-7.43 (m, 2 H), δ 7.38-7.33 (m, 3 H). UV-vis (7.4 HEPES buffer), nm (λ_max, M⁻¹cm⁻¹): 320 (8000).

[(6-Ph₂TPA)Ni(PhC(O)C(OH)C(O)H)]ClO₄ (8). Equimolar amounts of 6-Ph₂TPA (0.0313 g, 0.0714 mmol) and Ni(ClO₄)₂•6H₂O (0.0261 g, 0.0714 mmol) were dissolved in ~ 2 mL of CH₃CN under a N₂ atmosphere. This mixture was then combined with a methanol solution of sodium 2-hydroxy-3-oxo-3-phenyl-propen-1-olate 7 (0.0133 g, 0.0714 mmol) and the resulting orange solution was stirred under a N₂ atmosphere for ~40 min. Analysis of the reaction mixture using UV-visible spectroscopy (in CH₃CN)
indicated the formation of new O₂ sensitive species with λ_{max} = 378 nm. An aliquot of the reaction mixture was then dried under reduced pressure and was subsequently dissolved in CD₃CN for a ¹H NMR experiment. This analysis revealed the formation of one major Ni(II) complex product. This compound has β/β' pyridyl ring proton resonances at 46.6, 41.4, 43.2, and 33.4 ppm. The overall mixture was then brought to dryness under vacuum and the remaining orange/brown residue was dissolved in CH₂Cl₂, filtered, and the solvent was again removed under reduced pressure. Attempts to crystallize the product have not yet yielded crystals suitable for single crystal X-ray diffraction.

O₂ Reactivity of [(Ph₂TPA)Ni(PhC(O)C(OH)C(O)H)]ClO₄ (8). Isolation and Identification of the Major Ni(II)-containing Product (9). The proposed Ni(II) acicreductone complex [(6-Ph₂TPA)Ni(PhC(O)C(OH)C(O)H)]ClO₄ 8 (~10 mg) was dissolved in CH₃CN (~2 ml) resulting in the formation of a deep orange solution. Upon exposure of this solution to air, bleaching of the orange color occurred and the resulting yellow mixture was stirred for several hours at ambient temperature. Removal of the solvent under reduced pressure yielded a yellow solid material. This crude product was recrystallized via Et₂O diffusion into a CH₃CN/MeOH solution to give a blue crystalline material. ¹H NMR analysis of this product revealed the formation of 9 [(6-Ph₂TPA)Ni(O₂CPh)]ClO₄ as indicated by a distinct set of β/β’ pyridyl-ring proton resonances in the range of 30-50 ppm. This compound has been previously isolated and fully characterized.¹⁰ The proposal of [(6-Ph₂TPA)Ni(O₂CPh)]ClO₄ as the reaction product is further supported by mass spectrometry analysis of the sample which confirmed the presence of the [(6-Ph₂TPA)Ni(O₂CPh)]⁺ ion.
Results and Discussion

Synthesis of an E1 Substrate Analog (6). The phosphate ester protected precursor 6 was synthesized according to the literature procedure. The synthetic pathway, as well as percent yields is shown in Scheme 6-2. Compound 6 was isolated from the reaction mixture under acidic conditions and was stored as an aqueous solution at -20 °C. This compound was then used as a substrate analog for the E1 (enolase/phosphatase) enzyme (Scheme 6-3).

Scheme 6-2. A synthetic pathway for the enolase/phosphatase (E1) substrate analog 6.
Scheme 6-3. Enzymatic activity of enolase/phosphatase (E1) with the substrate analog 6.

Overexpression and Purification of a His-tagged E1 Enolase/phosphatase Enzyme.

The E1 enolase/phosphatase encoding plasmid (pDioxHIS1) was kindly provided to us by Professor Wilson Francisco (Arizona State University). This plasmid was modified by incorporating a new NdeI site into the start codon for the E1 gene to improve expression. The resulting construct was reinserted into pEt30a to give the plasmid pDioxHIS10. Overexpression of this His-tagged vector in *E. coli* (BL21) resulted in production of the desired E1 enolase/phosphatase enzyme. This enzyme was purified using a Ni-affinity column and showed activity with the Ph-containing E1 substrate analog 1 (Scheme 6-3).

An enzymatic activity assay of the E1 enolase/phosphatase enzyme was performed anaerobically following the literature procedure. This reaction is conveniently monitored using UV-visible spectroscopy, as the acireductone product has a characteristic absorption maximum at 320 nm. This reaction proceeds with a transient build-up of an enol-phosphate intermediate that can be observed spectroscopically at 278 nm (Scheme 6-3). Although, the observed level of E1 activity was only ~50% of that reported for the non-His tagged enzyme this level of activity appears to be sufficient for our synthetic
purposes. Under these conditions (HEPES buffer, pH 7.4) the C(1)-H acireductone is produced as a monoanion which is highly O$_2$ sensitive. As shown in Figure 6-3, spontaneous decomposition of this monoanion in the presence of O$_2$ results in the disappearance of its characteristic absorption band at 320 nm and subsequent formation of a band at 252 nm, which is assigned to the $\alpha$-keto acid product.

**Isolation of the Phenyl Acireductone Monoanion Salt Sodium 2,3-Dihydroxy-1-phenyl-propenoate (7).** This procedure initially involves monitoring the E1 reaction mixture to determine whether the conversion of 1 to acireductone is complete (Scheme 6-3). Acidification of the reaction mixture to pH ~1 under N$_2$, followed by extraction with CH$_2$Cl$_2$ enabled the isolation of the acireductone product. Treatment of this product with NaOH solution resulted in its conversion into the monosodium salt.

![Diagram](image_url)

**Figure 6-3.** Reaction that takes place between the 3 and O$_2$ at pH 7.4 (top). Disappearance of the absorption band at 320 nm for 3 and appearance of an absorption feature at 252 nm for the $\alpha$-keto acid product (bottom).
This overall procedure enables the routine isolation of ~50 mg of the acireductone salt. Additional efforts to optimize this procedure are in progress.

**Synthesis of (6-Ph₂TPA)Ni(PhC(O)C(OH)C(O)H)ClO₄ (8).** The 6-Ph₂TPA supporting chelate has been used previously to produce a Ni(II) cis-β-keto-enolate complex, [(6-Ph₂TPA)Ni-(PhC(O)C(OH)C(O)Ph)]ClO₄ (5, Figure 6-2). This complex is relevant to the enzyme/substrate adduct in Ni(II)-ARD.¹¹ The work outlined herein describes the preparation of a new Ni(II)-acireductone complex using this supporting chelate ligand and acireductone 3. Under a nitrogen atmosphere, equimolar amounts of 6-Ph₂TPA and Ni(ClO₄)₂·6 H₂O in acetonitrile were combined with a methanol solution of sodium 2-hydroxy-3-oxo-3-phenyl-propen-1-olate (7). ¹H NMR and UV-vis analyses of obtained orange solution indicated the formation of a new 6-Ph₂TPA-supported Ni(II) acireductone complex (8, Scheme 6-5). As shown in Figure 6-4, the 30-60 ppm, region of the ¹H NMR spectrum of this complex is similar to that exhibited by [(6-Ph₂TPA)Ni(PhC(O)C(OH)C(O)Ph)]ClO₄ (5). These paramagnetically shifted resonances are assigned to β/β’ pyridyl ring protons of the chelate ligand. The resemblance of the observed patterns indicates similar overall mirror plane symmetry (equivalent phenyl pyridyl appendages). The UV-vis spectrum of the new complex 8 contains a broad absorption band at 378 nm (CH₃CN solution; Figure 6-5). This spectrum is notably distinct from that exhibited by a non-coordinated 2-hydroxy-3-oxo-3-phenyl-propen-1-olate monoanion (λ_max = 320 nm, pH = 7.4 (HEPES buffer)).⁶ On the basis of these initial results, we formulate 8 as [(6-Ph₂TPA)Ni(PhC(O)C(OH)C(O)H)]ClO₄. Additional experiments (FTIR, EA) are planned to further characterize this complex. We are also
attempting to recrystallize 8 and efforts thus far have yielded crystalline materials, albeit
not X-ray quality crystals.

Exposure of a CH$_3$CN solution of 8 to the air results in a gradual loss of the 378
nm absorption band (Figure 6-5). Examination of the reaction products by $^1$H NMR and
mass spectrometry revealed the production of a single Ni(II) compound, the benzoate
derivative [(6-Ph$_2$TPA)Ni(O$_2$CPh)]ClO$_4$ (9). This compound has been previously isolated
and fully characterized.$^{10}$ A comparison of the $^1$H NMR features of the reaction product

\[
\begin{align*}
\text{Acireductone} & \quad \overset{H^+}{\underset{pH \sim 1}{\longrightarrow}} \quad \text{Acireductone} \\
& \quad \overset{\text{Extracted from aqueous reaction mixture using CH$_2$Cl$_2$}}{\underset{pH \sim 7-8}{\longrightarrow}} \quad \text{Acireductone sodium salt}
\end{align*}
\]

$\lambda_{max} = 320 \text{ nm}$

**Scheme 6-4.** The isolation of C(1)-H acireductone from the E1 reaction mixture followed
by the conversion of the acireductone product into its sodium salt.

\[
\begin{align*}
6-\text{Ph$_2$TPA} & \quad + \quad \text{Ni(ClO$_4$)$_2$ \cdot 6 H$_2$O} & \quad \overset{\text{CH$_3$CN, MeOH}}{\underset{\text{Under N$_2$ atmosphere}}{\longrightarrow}} & \quad \text{Formation of a new Ni(II)-containing $O_2$ sensitive complex}
\end{align*}
\]

**Scheme 6-5.** Synthetic procedure for the preparation of 8.
Figure 6-4. Comparison of a region of the $^1$H NMR spectra of the 6-Ph$_2$TPA-supported Ni(II) acireductone complexes [(6-Ph$_2$TPA)Ni(PhC(O)C(OH)C(O)Ph)]ClO$_4$ 5 (top) and 8 (bottom).

Figure 6-5. Electronic absorption spectra of 8 [(6-Ph$_2$TPA)Ni(PhC(O)C(OH)C(O)H)]ClO$_4$]. Disappearance of the absorption maximum at 378 nm represents the oxygen-dependent reaction.
Figure 6-6. Paramagnetically shifted $^1$H NMR resonances of [(6-Ph$_2$TPA)Ni(O$_2$CPh)]ClO$_4$; region of 20-70 ppm (top). $^1$H NMR spectrum of the product, resulting from the oxidative decomposition of [(6-Ph$_2$TPA)Ni(PhC(O)C(OH)C(O)H)]ClO$_4$ (9) (bottom).

of 8 upon treatment with O$_2$ and those of 5 is shown in Figure 6-6. The formation of 9 is notable, as it indicates that the reaction of 8 with O$_2$ gives different products than the reaction of the acireductone monoanion 3 with O$_2$ (Figure 6-3). This means that coordination to the metal center affects the regioselectivity of the oxidative carbon-carbon bond cleavage reaction. Further studies to characterize the reaction of 8 with O$_2$ are in progress. These include efforts to identify formic acid and CO as products of the reaction.

Conclusions

A new synthetic methodology has been investigated for the preparation of Ni(II) acireductone complexes using an acireductone that can serve as an alternative substrate for Ni(II)-ARD. Initial indications are that we will be able to isolate and characterize a new acireductone complex and explore in detail its reactivity with O$_2$. 
References


CHAPTER 7

CONCLUSIONS

The work presented in this dissertation summarizes our efforts to investigate the structural, spectroscopic, and reactivity properties of Ni(II) complexes with biologically relevant compounds. These complexes are model systems of relevance to the properties of redox inactive Ni(II)-containing enzymes. Among nickel-containing metalloenzymes there are only three examples known to date in which nickel exhibits no redox activity, namely urease, glyoxalase I, and acireductone dioxygenase. The active sites of these enzymes contain Ni(II) ion(s) ligated by N/O-donor amino acid residues and water molecules in a common pseudo octahedral geometry.

The anion of acetohydroxamic acid (Figure 7-1, (A)) is a well known competitive inhibitor of urease enzymes. The mechanism of inhibition is proposed to involve the initial formation of a weak enzyme/inhibitor complex with acetohydroxamic acid to one of the two Ni(II) centers. This complex is then suggested to undergo dehydration to form the acetohydroxamate inhibited species. However, this hypothesis is based solely on kinetic investigations of the inhibition reaction and there was no structural evidence to support this proposal.

![Chemical structures](image)

Figure 7-1. Acetohydroxamic acid (A); hemithioacetal (B); acireductone (C)
In order to more fully understand the inhibition mechanism in urease, we investigated the chemistry of a mononuclear Ni(II) complex supported by a N/O-donor chelate ligand. Treatment of this complex with an equimolar amount of acetohydroxamic acid (AHA) resulted in the formation of a novel pseudo-octahedral Ni(II)-AHA complex. Interestingly, binding of AHA to the nickel center was supported by a hydrogen-bonding interaction with a hemi-labile pyridyl arm of the chelate ligand. Prior to our report of this unique structure, there were no examples of this type of neutral AHA coordination to any transition metal complex. We also demonstrated that the observed hydrogen-bonding interaction was essential in stabilizing AHA coordination. Addition of water to a solution of the AHA-containing Ni(II) complex disrupted the H-bonding thereby causing the release of this ligand from the metal center. This chemical model provides precedent for the feasibility of a weak enzyme/inhibitor complex of AHA in urease.

Nickel-containing glyoxalase I is another example of an enzyme in which a Ni(II) center is redox inactive. Glyoxalase I (GlxI) is involved in a cellular detoxification process and catalyzes the isomerization of a hemithioacetal to a thioester (the general structure of a hemithioacetal is shown in Figure 7-1 (B)). The precise function of a nickel center in this catalytic reaction is still under debate, although one of the proposed mechanisms suggests the formation of a Ni(II)-OH species. This hydroxide species is suggested to play the role of a general base in the catalytic process.

Our goal was to create a synthetic system that could promote hemithioacetal isomerization. Although, we did not succeed in the synthesis of a hydroxide-containing Ni(II) complex, we were able to prepare a Ni(II) complex having an anionic chelate ligand. This complex was shown to promote a hemithioacetal isomerization reaction in a
fashion akin to that proposed in the enzymatic system. Prior to our report there was no precedent in the literature of a well-defined coordination compound that is able to promote the isomerization of a hemithioacetal. Spectroscopic monitoring of this reaction led us to propose that the enediolate intermediate that is generated by proton abstraction likely does not interact with Ni(II) center.

The role of Ni(II) in the glyoxalase I-catalyzed isomerization is also intriguing for another reason. Recent studies revealed that the Ni(II)-containing form of GlxI is significantly less abundant than its Zn(II)-activated analog. Specifically, there are very few examples of bacterial GlxI enzymes that are activated by Ni(II) ions. However, the Ni(II)-containing GlxI enzyme isolated from *E. coli* displays an interesting propensity toward Ni(II) activation and lose activity when the nickel ion is replaced by Zn(II) in the active site. Investigation of the Ni(II) role in GlxI might serve as a basis to address questions concerning how protein structure might influence selectivity for a particular metal ion versus another in the same catalytic reaction. This question can also be probed through synthetic modeling studies. The most intuitive approach would be to synthesize a Zn(II) analog of our previously reported functional Ni(II) model complex and study its reactivity toward a hemithioacetal.

Acireductone dioxygenase (ARD) is a less studied example of a Ni(II)-containing redox inactive enzyme. However, the role of Ni(II) in its catalytic reaction is very interesting. ARD enzymes (Ni(II)- and Fe(II)-containing) are associated with the methionine salvage pathway (MSP), a ubiquitous biological cycle. These enzymes catalyze aliphatic oxidative C-C bond cleavage in an acireductone (1,2-dihydroxy-3-oxo-5-(methylthio)pent-1-ene) intermediate. A unique feature of these enzymes is that the
regioselectivity of the dioxygenase reaction depends on the metal ion bound in the active site. A mechanistic pathway for the reactions catalyzed by these enzymes have been proposed, but not yet rigorously evaluated.

Our approach toward evaluating the Ni(II)-ARD mechanism involves the synthesis of Ni(II) complexes having acireductone ligands coordinated to the metal center, reminiscent of the anaerobic enzyme/substrate complex in ARD. Scheme 7-1 (C) shows the general structure of an acireductone. Using an appropriate N₄-donor chelate ligand we have prepared and fully characterized a functional model complex relevant to Ni(II)-containing ARD. This novel trinuclear complex, formulated as [(6-NA-6-Ph₂TPANi)₂(μ-PhC(O)C(O)C(O)Ph)₂Ni](ClO₄)₂, contains a doubly deprotonated form of the acireductone coordinated to the metal center. Two of the Ni(II) centers are pseudo octahedral and they are supported by the chelate ligand whereas the third square planar Ni(II) ion (central) is ligated by two acireductone molecules. This complex has been proven to react with O₂ resulting in the formation of carboxylate products and CO, which is relevant to the reaction catalyzed by Ni(II)-containing ARD. Analysis of the reaction mixture revealed also the presence of benzil (PhC(O)C(O)Ph), indicating that an alternative reaction pathways may be operative in this system.

In efforts to further elucidate the oxygen reactivity of Ni(II)-coordinated acireductones, we have attempted the preparation of a new Ni(II) complex having a C(1)-H acireductone ligand, an analog of the native Ni(II)-ARD substrate. ¹H NMR and UV-visible spectroscopic properties of this new species are consistent with the formulation of [(6-Ph₂TPA)Ni(PhC(O)C(OH)C(O)H)]ClO₄. This complex reacts with O₂ to produce [(6-Ph₂TPA)Ni(O₂CPh)]ClO₄, which was confirmed spectroscopically. This new functional
model complex requires further characterization, specifically X-ray crystallographic and elemental analyses in order to confirm the proposed formulation. The formation of the carboxylate-containing Ni(II) complex upon the reaction with oxygen is indicative of the an ARD-relevant pathway. However, additional experiments need to be performed in order to prove our hypothesis, such as CO detection and analysis of the organic byproducts in the reaction mixture. Furthermore, if this model system is proven to be truly functional, the synthesis of its iron analog would be very advantageous. Analysis of the structural properties and the oxygen reactivity of the iron analog might provide insight into the chemistry of ARD system, wherein Ni(II)-ARD and Fe(II)-ARD catalyze distinct oxidation reactions.
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Education

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Graduate Student, Department of Chemistry and Biochemistry,
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Publications


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1. 235th ACS National Meeting, New Orleans, LA, Apr. 6-10, 2008


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