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Glyoxalase I-type Hemithioacetal Isomerization Reactivity of a Mononuclear Ni(II) Deprotonated Amide Complex

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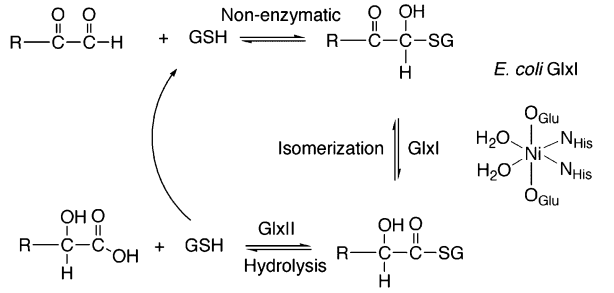
Abstract: The synthesis, characterization, and hemithioacetal isomerization reactivity of a mononuclear Ni(II) deprotonated amide complex, [(bppppa$^-$)Ni]ClO_4·CH_3OH (**1**, bppppa$^-$ = monoanion of *N*,*N*-bis-[(6-phenyl-2-pyridyl)methyl]-*N*-[(6-pivaloylamido-2-pyridyl)methyl]amine), are reported. Complex **1** was characterized by X-ray crystallography, ^{1}H NMR, UV−vis, FTIR, and elemental analysis. Treatment of **1** with an equimolar amount of the hemithioacetal PhC(O)CH(OH)SCD_3 in dry acetonitrile results in the production of the thioester PhCH(OH)C(O)SCD_3 in ∼60% yield. This reaction is conveniently monitored via ^{2}H NMR spectroscopy. A protonated analogue of **1**, [(bppppa)Ni]$(ClO_4)_2$ (**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)SCD_3, which indicates that the pK_a value for the PhC*H*(OH)C(O)SCD_3 proton of the thioester must be significantly higher than the pK_a value of the C−*H* proton of the hemithioacetal (PhC(O)C*H*(OH)SCD_3). Complex **1** is the first well-characterized Ni(II) coordination complex to exhibit reactivity relevant to Ni(II)-containing *E. coli* glyoxalase I. Treatment of $NiBr_2$·$2H_2O$ with PhC(O)CH(OH)SCD_3 in the presence of 1-methylpyrrolidine also yields thioester product, albeit the reaction is slower and involves the formation of multiple −SCD_3 labeled species, as detected by ^{2}H NMR spectroscopy. 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, $CH_3C(O)C(O)H$) 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 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.[2,3] 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, $[(N_{His})_2(O_{Glu})_2Ni(OH_2)_2]$ (Scheme 1).[6] The role of the Ni(II) center in the *E. coli* GlxI-mediated hemithioacetal isomerization reaction is not yet defined.[7,8] 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.[7,8] Specifically, the Ni(II)−OH is proposed to abstract a proton from the hemithioacetal (Scheme 2). In this reaction, an anion is produced that is subsequently protonated at the former carbonyl carbon to yield the thioester product.[9] It is unclear whether the intermediate anion in this reaction transiently interacts with the Ni(II) center. X-ray

Scheme 1



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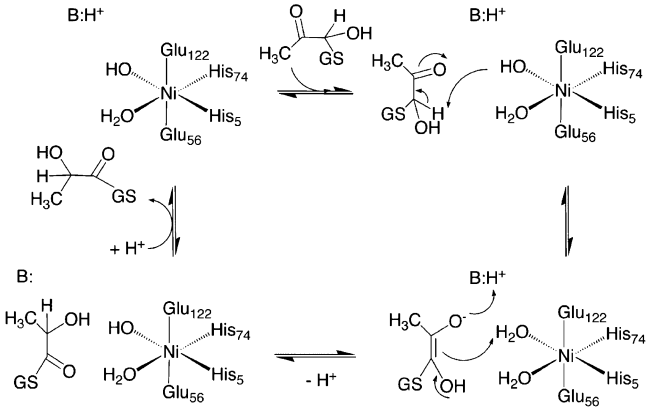
Scheme 2



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 2.

To date, only three structurally characterized mononuclear Ni(II)−OH complexes have been reported in the literature.[10] Neither these complexes nor 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 $NiBr_2 \cdot 2H_2O$ 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 $NiBr_2 \cdot 2H_2O$/1-methylpyrrolidine in DMF.[11] Using a deuterium-labeled hemithioacetal and ^2H NMR spectroscopy, 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 species that are detectable by ^2H NMR spectroscopy. For the reaction involving the deprotonated amide coordination complex, no evidence was found for Ni(II)-coordinated species, whereas in the $NiBr_2 \cdot 2H_2O$-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,

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

(10) (a) Kieber-Emmons, M. T.; Schenker, R.; Yap, G. P. A.; Brunold, T. C.; Riordan, C. G. *Angew. Chem., Int. Ed.* **2004**, *43*, 6716−6718. (b) Cámpora, J.; Palma, P.; del Río, D.; Alvarez, E. *Organometallics* **2004**, *23*, 1652−1655. (c) Cámpora, J.; Matas, I.; Palma, P.; Graiff, C.; Tiripicchio, A. *Organometallics* **2005**, *24*, 2827−2830.

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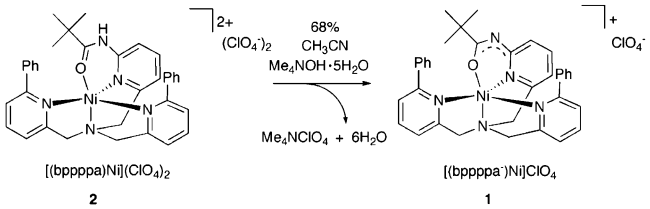
Scheme 3



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 [(bpppp$^-$)Ni]ClO_4 (1). Chelate ligands containing secondary amide appendages have been shown to stabilize a variety of mononuclear metal hydroxide complexes.[12−16] 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 [(bpppp)Ni]$(ClO_4)_2$ (**2**, Scheme 3).[17] Treatment of this complex with 1 equiv of $Me_4NOH \cdot 5H_2O$ in CH_3CN resulted in the formation of an orange/brown solution. Following workup and recrystallization from $CH_3CN/CH_3OH/Et_2O$, [(bpppp$^-$)Ni]$ClO_4 \cdot CH_3OH$ (**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 1 equiv of base resulted in the formation of a deprotonated amide complex.

An ORTEP drawing of the cationic portion of **1** is shown in Figure 1. Details of the X-ray data collection and refinement are given in Table 1. Selected bond distances in **1** and its parent complex [(bpppp)Ni]$(ClO_4)_2$[17] (**2**) are provided in Figure 1. Additional bond distances and angles for **1** are given in Table 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 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 anionic charge into the pyridyl ring. ^1H NMR spectroscopic evidence for such delocalization has been previously reported for a zinc analogue complex.[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_{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

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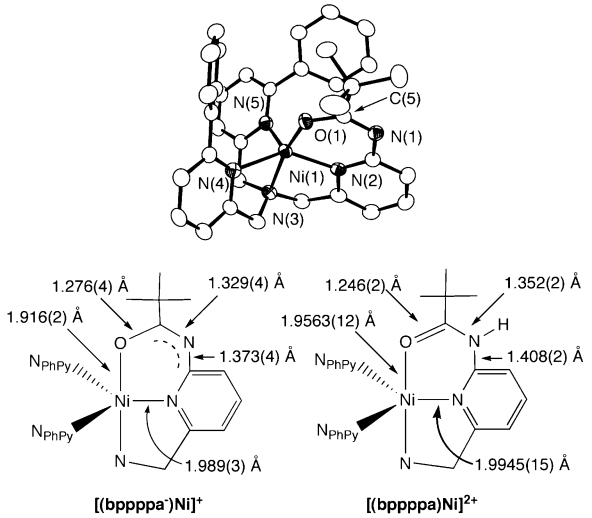

Figure 1. Top: ORTEP drawing of the cationic portion of **1**. Ellipsoids are drawn at the 50% probability level. Hydrogen atoms are omitted for clarity. Bottom: Comparison of bond distances within the cationic portions of **1** and **2**.

Table 1. Summary of X-ray Data Collection and Refinement[a]

	1
empirical formula	$C_{36}H_{38}ClN_5NiO_6$
formula weight	730.87
crystal system	orthorhombic
space group	$Pb2_1a$
a (Å)	13.0931(3)
b (Å)	13.8192(2)
c (Å)	18.4276(4)
α (deg)	90
β (deg)	90
γ (deg)	90
V (Å^3)	3334.22(12)
Z	4
density (calcd), Mg m^{-3}	1.456
temp (K)	150(1)
color	brown
crystal habit	plate
crystal size (mm)	0.33 × 0.20 × 0.08
diffractometer	Nonius KappaCCD
abs. coeff. (mm^{-1})	0.717
2θ max (deg)	54.96
completeness to $\theta = 27.48°$	99.8%
reflections collected	7306
indep. reflections	7306 [R(int) = 0.0000]
variable parameters	476
R1/wR2[b]	0.0437/0.0926
GOF (F^2)	1.102
largest diff. (e Å^{-3})	0.460/−0.581

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

a geometry that is intermediate between trigonal bipyramidal and square pyramidal (**1**, $\tau = 0.44$; **2**, $\tau = 0.54$).[19]

A region of the ^{1}H NMR spectra of **1** and **2** is shown in Figure 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$_2$TPA ligand, these resonances are assigned to the β/β'-H's of the pyridyl rings.[20] Complex **1** is orange when dissolved in acetonitrile, with an absorption feature at 440 nm

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Table 2. Selected Bond Distances (Å) and Angles (deg)[a]

	1
Ni−O(1)	1.916(2)
Ni−N(2)	1.989(3)
Ni−N(3)	2.056(3)
Ni−N(4)	2.148(3)
Ni−N(5)	2.076(3)
O(1)−Ni−N(2)	89.94(10)
O(1)−Ni−N(3)	163.44(11)
N(2)−Ni−N(3)	83.72(11)
O(1)−Ni−N(5)	115.51(10)
N(2)−Ni−N(5)	110.17(11)
N(3)−Ni−N(5)	81.05(11)
O(1)−Ni−N(4)	95.63(10)
N(2)−Ni−N(4)	136.87(10)
N(3)−Ni−N(4)	78.97(10)
N(5)−Ni−N(4)	105.72(10)

[a] Estimated standard deviations indicated in parentheses.

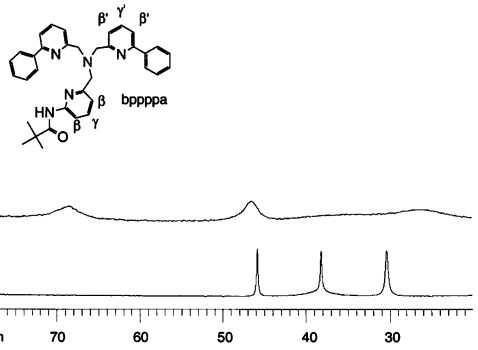


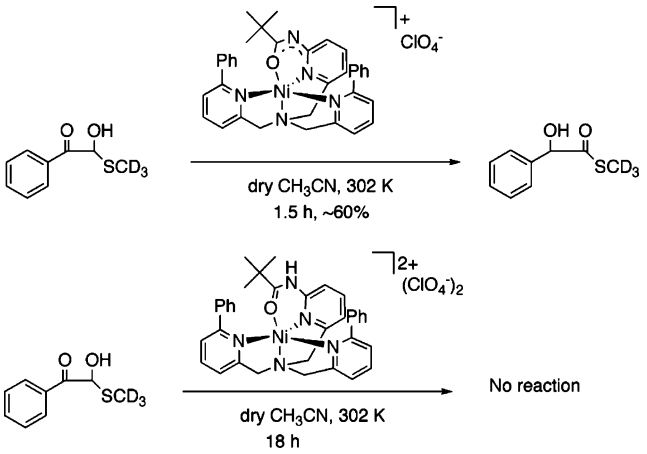
Figure 2. A region of the ^{1}H NMR spectra of **1** (a) and **2** (b). Both spectra were acquired in CD_3CN solution at 302 K.

($\epsilon = 320$ M^{-1} cm^{-1}). This feature is not present in the UV−vis spectrum of **2**.[17]

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* GlxI, 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$_3$[21] in dry CH_3CN 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). As shown in Figure 3, this GlxI-type reaction can be conveniently monitored via ^{2}H NMR spectroscopy, 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_6D_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

(20) Szajna, E.; Dobrowolski, P.; Fuller, A. L.; Arif, A. M.; Berreau, L. M. *Inorg. Chem.* **2004**, *43*, 3988−3997.
(21) Russell, G. A.; Mikol, G. J. *J. Am. Chem. Soc.* **1966**, *88*, 5498−5504.
(22) In the ^{2}H NMR spectra acquired in CH_3CN, the natural abundance ^{2}H NMR signal for the solvent methyl group is positioned under the signal associated with the −SCD$_3$ group of the hemithioacetal.

Scheme 4



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 $Me_4NOH{\cdot}5H_2O$. Notably, no change in the ^2H 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 $Me_4NOH{\cdot}5H_2O$ in acetonitrile in this time period.

The ^2H 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**. Specifically, the chemical shifts of the −SCD_3 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 spectroscopy was also used to monitor the reaction of **1** with $PhC(O)CH(OH)SCH_3$ in CD_3CN (Figure 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 spectrum 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 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 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 $[(bppppa)Ni](ClO_4)_2$ (**2**) with $PhC(O)CH(OH)SCD_3$ (Scheme 4) resulted in no reaction after 18 h as determined via ^2H NMR spectroscopy, 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

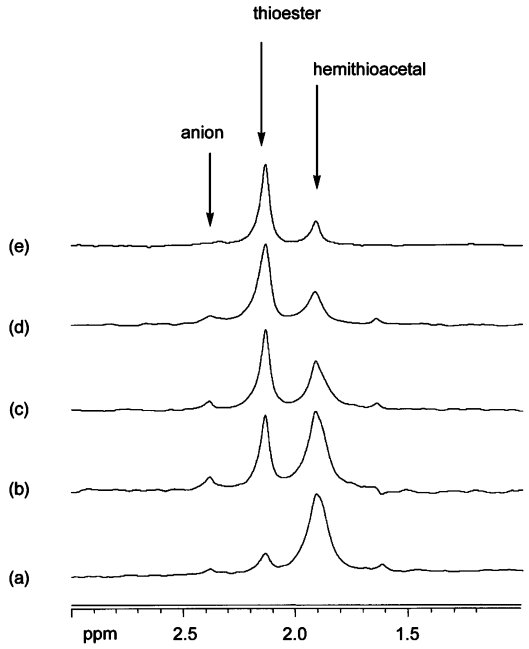


Figure 3. ^2H NMR spectra of a reaction mixture of **1** and $PhC(O)CH(OH)SCD_3$ in dry CH_3CN at various times after mixing. The first spectrum (a), which was obtained within 5 min of mixing of the reagents, is taken as $t = 0$ min; (b) 19 min; (c) 43 min; (d) 83 min; and (e) 23 h 18 min. The integrated intensity of the combined −SCD_3 signals remains constant for (a)−(d) relative to the integrated intensity of a C_6D_6 internal standard. However, for (e) the integrated intensity of the combined −SCD_3 species is reduced by ∼30% relative to that 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.

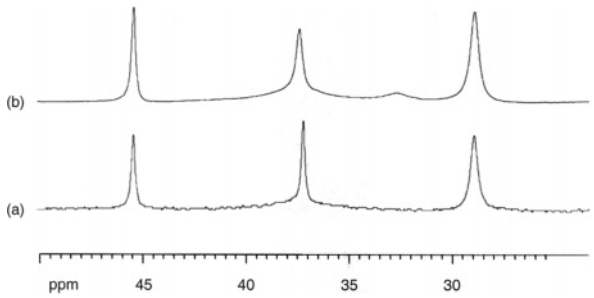


Figure 4. (a) A region of the ^1H NMR spectrum of **1**. (b) The same region for compound **1** in the presence of an equimolar amount of $PhC(O)CH(OH)SCD_3$. Both spectra were acquired in CD_3CN at 302 K.

a time period of ∼40 h at 298 K, thus indicating the requirement of the Ni(II) center 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 the nitrogen. Because the bound amide oxygen atom is protected by the hydrophobic phenyl groups of the chelate ligand, as well as by the bulky *tert*-butyl

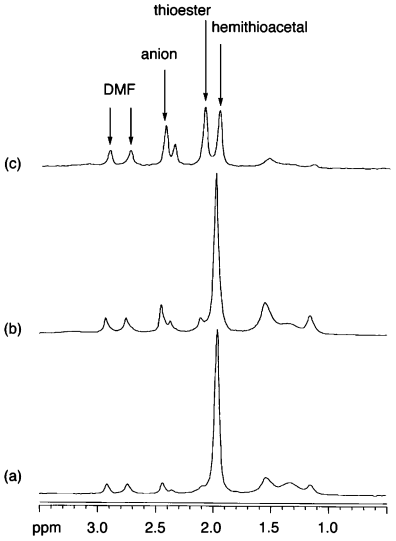


Figure 5. ^{2}H NMR spectra of a reaction mixture of $NiBr_2 \cdot 2H_2O$, 1-methylpyrrolidine, and $PhC(O)CH(OH)SCD_3$ in dry DMF at various times after mixing. The first spectrum (a), which was obtained within 10 min of mixing of the reagents, is taken as $t = 0$ min; (b) 1.5 h; and (c) 23 h. The signals marked DMF are the natural abundance ^{2}H NMR signals for protio DMF.[22]

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 $PhC(O)CH(OH)SCD_3$ in a 1:5 molar ratio and C_6D_6 (internal standard). Spectroscopic monitoring of this reaction mixture using ^{2}H NMR spectroscopy over the course of 66 h revealed the formation of ~1.5 equiv of the thioester $PhCH(OH)C(O)SCD_3$. Overall, the spectra for this mixture were very similar to those shown in Figure 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 $-SCD_3$ 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 $NiBr_2 \cdot 2H_2O$/1-Methylpyrrolidine in DMF Using $PhC(O)CH(OH)SCD_3$ and ^{2}H NMR Spectroscopy. There is one previous report in the literature of hemithioacetal isomerization promoted by $NiBr_2 \cdot 2H_2O$ 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 ^{2}H-labeled hemithioacetal and ^{2}H NMR spectroscopy. Solutions were prepared containing an equimolar mixture of $NiBr_2 \cdot 2H_2O$, $PhC(O)CH(OH)SCD_3$, and C_6D_6 (internal standard) in dry DMF. Addition of 1 equiv of 1-methylpyrrolidine to this mixture produced a color change from yellow-green to red-brown. ^{2}H NMR spectra collected at various times following addition of the base are shown in Figure 5. Unlike the relatively simple spectra found for hemithioacetal isomerization promoted by **1** (Figure 3), several broad resonances in the ^{2}H NMR spectra of the $NiBr_2 \cdot 2H_2O$/1-methylpyrrolidine indicate the presence of multiple $-SCD_3$ containing species. For example, at least three new resonances are present in the region of 1.0−1.6 ppm.[23] Another new resonance is also present at ~2.35 ppm. We hypothesize that the more exposed Ni(II) center in $NiBr_2 \cdot 2H_2O$, versus that 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 ~60% yield of thioester in the hemithioacetal isomerization reaction involving **1**, only a trace amount of thioester is produced in the $NiBr_2 \cdot 2H_2O$/1-methylpyrrolidine reaction (Figure 5b). Similar to the reaction involving **1**, after ~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 5c), the amounts 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 1) is ubiquitous in biological systems, and the role of the metal center in the hemithioacetal isomerization catalyzed by GlxI remains to be fully defined. In 1970, Hall and Poet demonstrated that the conversion of model hemithioacetals to the corresponding α-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 GlxI enzymes. The studies reported herein, although not of direct structural relevance to *E. coli* GlxI, 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.[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)

(23) Control experiments confirm that the $-SCD_3$ derived resonances in the region of 1.0−1.6 ppm were only produced when $NiBr_2 \cdot 2H_2O$, 1-methylpyrrolidine, and $PhC(O)CH(OH)SCD_3$ were all present in the reaction mixture.

(24) Hall, S. S.; Doweyko, A. M.; Jordan, F. *J. Am. Chem. Soc.* **1978**, *100*, 5934−5939.

complex is paramagnetic, monitoring of this reaction was performed using a deuterium-labeled hemithioacetal and ^{2}H NMR spectroscopy. This novel approach is also applicable to monitoring hemithioacetal isomerization reactions involving simple Ni(II) salts, as was outlined for the reaction involving $NiBr_2 \cdot 2H_2O$ and 1-methylpyrrolidine. Importantly, comparison of the reactions promoted by **1** and $NiBr_2 \cdot 2H_2O$/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 $NiBr_2 \cdot 2H_2O$ in DMF has multiple available coordination positions for interaction with hemithioacetal-derived species. In terms of *E. coli* GlxI, 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 $NiBr_2 \cdot 2H_2O$/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 $-SCD_3$ species in each reaction, this solid contains hemithioacetal-derived species. Ongoing 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 N_2 prior to use.[25] Water-sensitive reactions were performed in an MBraun Unilab glovebox under an atmosphere of purified N_2. The organic compounds bppppa (*N*,*N*-bis[(6-phenyl-2-pyridyl)methyl]-*N*-[(6-pivaloylamido-2-pyridyl)methyl]amine),[17] $PhC(O)CH(OH)SCD_3$ (hemithioacetal),[21] and $PhCH(OH)C(O)SCD_3$ (thioester)[11] were prepared according to literature procedures.

Physical Methods. ^{1}H and ^{2}H NMR spectra were collected as previously described.[20] 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*.[26]

[(bppppa$^-$)Ni]$ClO_4 \cdot CH_3OH$ (1). [(bppppa)Ni]$(ClO_4)_2$[17] (26 mg, 0.032 mmol) dissolved in CH_3CN (∼2 mL) was added to a slurry of $Me_4NOH \cdot 5H_2O$ (5.8 mg, 0.032 mmol) in CH_3CN (∼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 CH_2Cl_2, and the solution was filtered through a glass wool/Celite plug. The CH_2Cl_2 was removed under reduced pressure. The remaining orange solid was recrystallized by Et_2O diffusion into a CH_3CN/CH_3OH (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]$(ClO_4)_2$. Anal. Calcd for $C_{35}H_{34}N_5O_5ClNiCH_3OH$: C, 59.24; H, 5.25; N, 9.60. Found: C, 58.79; H, 5.09; N, 9.64. UV−vis (CH_3CN), nm (λ_{max}, M^{-1} cm^{-1}): 440 (320). FTIR (KBr, cm^{-1}): 1088 (ν_{ClO_4}), 628 (ν_{ClO_4}).

Monitoring Stoichiometric Hemithioacetal Isomerization via ^{2}H NMR Spectroscopy. A NMR tube containing equimolar amounts of **1** and $PhC(O)CH(OH)SCD_3$ in dry protio acetonitrile (700 μL) was prepared under a nitrogen atmosphere. An internal standard (C_6D_6, 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 ^{2}H NMR spectrum was referenced to the chemical shift of the C_6D_6 standard. The C_6D_6 signal was also used as an internal integration standard.

Na(bppppa). The bppppa ligand (27 mg, 4.9×10^{-5} mol) was treated with NaH (1.2 mg, 5.1×10^{-5} mol) in dry THF. After the mixture was stirred for 1.5 h at ambient temperature, the solvent was removed under reduced pressure, and a ^{1}H NMR spectrum of the remaining pasty solid was obtained. ^{1}H NMR (CD_3CN, 400 MHz): δ 7.83−7.77 (br m, 7H), 7.64 (br d, J = 7.8 Hz, 3H), 7.47−7.45 (m, 6H), 7.37−7.31 (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 bppppa.[17] Addition of water to Na(bppppa) in acetonitrile yields the neutral bppppa ligand, as determined by ^{1}H NMR spectroscopy.

Treatment of Na(bppppa) with $PhC(O)CH(OH)SCD_3$ in Protio Acetonitrile. This reaction was set up and monitored via ^{2}H NMR spectroscopy in a fashion identical 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 $NiBr_2 \cdot 2H_2O$ and 1-Methylpyrrolidine. A solution of $NiBr_2 \cdot 2H_2O$ (10 mg, 3.9×10^{-5} mol) in DMF (700 μL) was prepared. Stirring of this solution for ∼30 min at room temperature was required for the $NiBr_2 \cdot 2H_2O$ to fully dissolve. To this solution were added the internal standard C_6D_6 (1 μL), the hemithioacetal $PhC(O)CH(OH)SCD_3$ (7.3 mg, 3.9×10^{-5} mol), and 1-methylpyrrolidine (4.5 μL, 3.9×10^{-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_6D_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.

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Supporting Information Available: X-ray crystallographic file for **1** (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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