EurJIC European Journal of Inorganic Chemistry

ChemPubSoc Europe

FULL PAPER

DOI:10.1002/ejic.201402254

A Nickel-Containing Model System of Acireductone Dioxygenases that Utilizes a C(1)-H Acireductone Substrate

CLUSTER ISSUE

Caleb J. Allpress[a] and Lisa M. Berreau*[a]

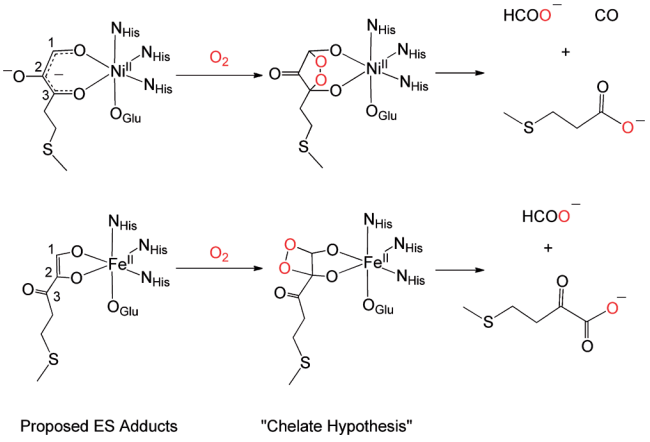
Keywords: Enzyme mimics / Metalloenzymes / Nickel / Oxygen / Cleavage reactions / Regioselectivity

A mononuclear Ni^{II} complex bearing the monoanion of 1-acetoxy-3-phenylpropane-2,3-dione (**4**) as a ligand has been prepared {[(6-Ph_2TPA)Ni{PhC(O)C(O)CHOC(O)CH_3)]ClO_4, **5**; 6-Ph_2TPA = *N*,*N*-bis[(6-phenyl-2-pyridyl)methyl]-*N*-(2-pyridylmethyl)amine}. This complex was characterized by ^{1}H NMR, UV/Vis and IR spectroscopy, mass spectrometry, and elemental analysis. Exposure of solutions of **5** to O_2 did not result in any reaction over the course of hours. Deprotection of **5** by the addition of $NaOCH_3$ in methanol generated a Ni^{II} species (**6**) that contains a coordinated dianionic C(1)-H acireductone. Exposure of **6** to O_2 led to regioselective oxidative cleavage reactivity akin to that found for the Ni^{II}-containing acireductone dioxygenase enzyme. The strategy outlined herein is the first synthetic approach that enables examination of the oxidative reactivity of a synthetic Ni^{II} species containing a dianionic C(1)-H acireductone ligand.

Introduction

Methionine is an essential amino acid that plays an important role in protein structure, biosynthetic pathways, and is the start codon for translation in eukaryotic cells.[1] Its function in biosynthetic pathways is dominated by its enzymatic derivatization by adenosine triphosphate (ATP) to form *S*-adenosylmethionine (SAM).[2,3] SAM has numerous important biological functions due in part to its sulfonium cation with three S–C bonds,[3,4] including 1) as a co-substrate for methyltransferases, which leads to the loss of the methyl group and the formation of *S*-adenosylhomomethionine,[5] 2) as a co-factor in radical SAM enzymes,[6] and 3) as a source of *n*-propylamine during polyamine biosynthesis, leading to the formation of methylthioadenosine (MTA).[7] Polyamines such as spermine and spermidine are associated with cell growth, and defects in polyamine biosynthesis regulation are associated with oncogenesis.[8] Therefore metabolic pathways that regulate polyamine synthesis are a potentially therapeutically important area of study. One such pathway is the methionine salvage pathway (MSP), a ubiquitous enzymatic pathway in which the methylthio unit is recycled into methionine after SAM has been converted into MTA during polyamine biosynthesis.[7,9]

A pair of enzymes known as acireductone dioxygenases (ARD and ARD′) catalyze the dioxygenolytic cleavage of an acireductone intermediate within the MSP.[10–12] These enzymes are found at the only known branch point within this pathway and thus are of current interest. In *Klebsiella pneumonia*, the on-pathway reaction is catalyzed by the iron-containing enzyme Fe-ARD′, whereas the off-pathway reaction is catalyzed by the nickel-containing enzyme Ni-ARD.[13–15] These enzymes cleave the C–C bond(s) of their substrate with differing regiospecificity (Scheme 1) and are particularly interesting from a chemical standpoint as the only constitutive difference between the enzymes is the identity of the metal ion at the active site (Fe^{II} or Ni^{II}).[15] The reaction catalyzed by Ni-ARD also produces CO, a well-known cellular signaling molecule with therapeutic potential,[16] thereby combining a regulatory junction with the production of a cellular signal. Efforts to gain an understanding of the chemical factors that control the regiospec-



Scheme 1. Carbon–carbon bond-cleavage reactions of nickel- and iron-containing acireductone dioxygenases.

[a] Department of Chemistry and Biochemistry, Utah State University,
0300 Old Main Hill, Logan, UT 84322-0300, USA
E-mail: lisa.berreau@usu.edu
http://lisaberreau.org/

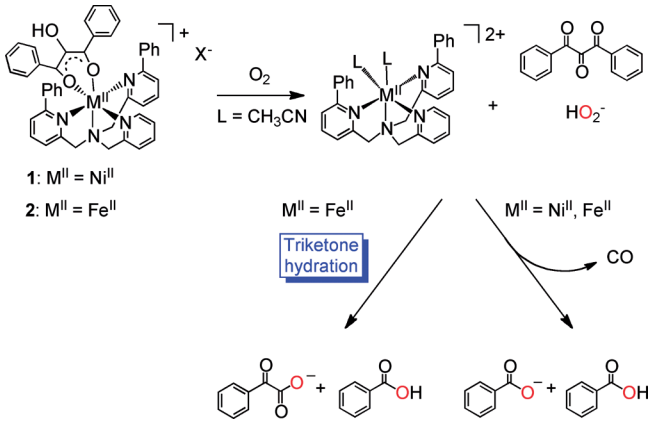
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejic.201402254.

ificity of the acireductone dioxygenase cleavage reactions are therefore warranted.

The original explanation for the difference in regiospecificity exhibited by Fe-ARD′ and Ni-ARD inferred that changes in the binding mode of the acireductone to the metal center, from a five-membered chelate ring in Fe-ARD′ to a six-membered chelate ring in Ni-ARD, would respectively activate the C(1) and C(2) or C(1) and C(3) carbon atoms towards reaction with dioxygen (Scheme 1).[17] Subsequent collapse of the resulting dioxetane rings would lead to the observed regiospecificity of the products. This "chelate hypothesis" was supported by NMR studies of the tertiary structures of the enzymes, wherein it was found that the position of a tryptophan residue in the Ni-ARD active site could favor coordination of the six-membered chelate binding mode of the substrate.[18] However, direct spectral analyses of the enzyme–substrate (ES) adducts, including by UV/Vis spectroscopy and XAS, did not give conclusive evidence of a change in binding mode between Fe-ARD′ and Ni-ARD.[19] In addition, a recent mixed QM/DMD study of the acireductone dioxygenases found that there does not appear to be a difference in binding mode between the two enzymes.[20] Rather, it is proposed that the lower occupation of the Fe^{II} d orbitals (by two electrons compared with Ni^{II}) and the greater redox flexibility of Fe^{II} facilitates a key O–O bond-cleavage step that is not accessible to Ni-ARD.

As an alternative to enzymatic studies as a method for investigating the mechanistic details of the acireductone dioxygenases, we synthesized functional small molecular model complexes of the proposed enzyme–substrate adducts of Ni-ARD and Fe-ARD′ (**1** and **2**, respectively, Scheme 2).[21,22] Upon exposure to O_2, these synthetic complexes undergo dioxygenolytic carbon–carbon bond cleavage via an intermediate trione/hydroperoxide pair.[22,23] It was found that the regioselectivity of the reaction of **1** and **2** with O_2 was identical in dry solvent, consistent with the chelate hypothesis. However, in the presence of H_2O a change in regioselectivity was observed in the reaction of **2**, but not of **1**, which suggests that the chelate hypothesis is not sufficient to explain the chemistry in these model systems.[22] We therefore proposed a new reaction pathway for **2** in which the ferrous center promotes hydration of the triketone intermediate, thereby facilitating the change in regioselectivity (Scheme 2).

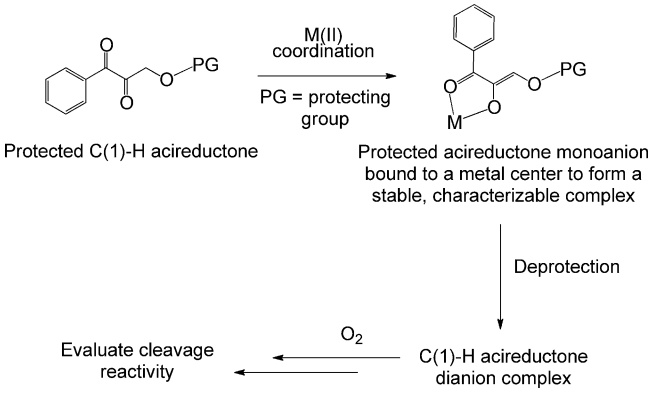
Unfortunately, this model system is not truly biomimetic as it uses an acireductone that is not a substrate for the acireductone dioxygenase enzymes and involves O_2 reactivity at the monoprotonation level of the coordinated model substrate.[10] Computational studies have additionally suggested significant differences in the electronic structures and reactivities of acireductones that have a phenyl group or a hydrogen atom at the C(1) position.[23,24] To provide better insight into the acireductone dioxygenase reaction pathways, we endeavored to use a C(1)-H acireductone that is a substrate for the native enzyme. To date there are no reported non-enzymatic syntheses of the native substrate of the acireductone dioxygenases (1,2-dihydroxy-3-oxo-*S*-



Scheme 2. Reactivity of **1** and **2** with O_2.

methylthiopentene), presumably due to the presence of the methylthio unit.[25] As a target we have chosen 3-oxo-3-phenylpropene-1,2-diol (phenyl acireductone), previously reported as a substrate for the enzyme, which has UV/Vis properties that are amenable for spectroscopic studies (λ_{max} = 320 nm as a monoanion).[19] Previously reported synthetic routes to phenyl acireductone focused on generating a phosphorylated precursor and subsequently using the E1 enolase/phosphatase enzyme from the MSP to dephosphorylate this precursor.[25] The resulting acireductone monoanion was then employed in situ for enzymatic studies. The difficulty in isolating phenyl acireductone is a result of its propensity to oxidize under the alkaline conditions used in its synthesis.

To generate model systems of enhanced relevance to the acireductone dioxygenases, our goal was to generate nickel and iron complexes containing a coordinated dianionic phenyl acireductone moiety. Our strategy is outlined in Scheme 3 and involves the generation of a protected acireductone. We hypothesized that the binding of this protected acireductone to a metal center would allow us to generate a well-defined complex. Subsequent deprotection and exposure to O_2 would then enable us to investigate the role that the metal center plays in directing the regioselectivity of aci-



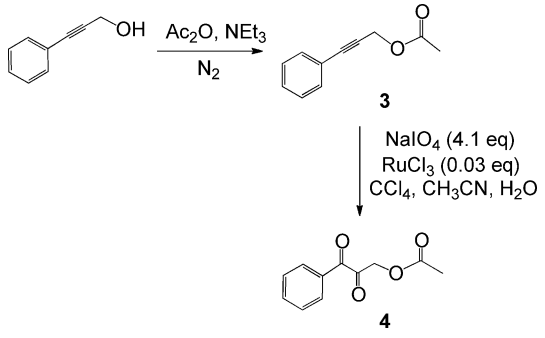
Scheme 3. Strategy for generating a protected C(1)-H acireductone complex and its subsequent deprotection to investigate the reactivity of a coordinated dianionic acireductone with O_2.

reductone cleavage. The results of this approach for Ni^{II} are described herein.

Results

Synthesis of Protected Phenyl Acireductone

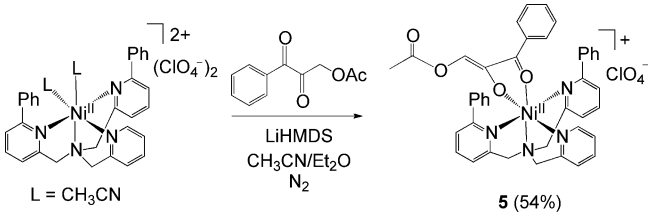
3-Phenyl-2-propyn-1-ol was acylated using acetic anhydride to generate 3-phenyl-2-propynyl acetate (**3**; Scheme 4; see Figures S1 and S2 in the Supporting Information). Oxidation of the alkyne **3** with $NaIO_4$ using $RuCl_3$ as a catalyst generated the diketone **4** (Scheme 4; see Figures S3 and S4). Careful monitoring of this reaction by TLC was necessary due to the propensity of **4** to over-oxidize to give carboxylic acids, presumably by an initial hydrolytic cleavage of the acetyl protecting group. Owing to the formation of byproducts, purification by column chromatography was required. The diketone tautomeric form of **4** was confirmed by the presence of two ketone ^{13}C NMR signals (Figure S4), an integral of two protons for the methylene group by ^1H NMR, and by a UV absorption band at 255 nm.



Scheme 4. Synthesis of 1-acetoxy-3-phenylpropane-2,3-dione (**4**), a protected acireductone.

Synthesis and Characterization of the Ni^{II} Complex

Deprotonation of **4** by LiHMDS in Et_2O led to the formation of a pale-yellow slurry. Combination of this with $[(6\text{-}Ph_2TPA)Ni(CH_3CN)_2](ClO_4)_2$[23] {6-$Ph_2$TPA = *N*,*N*-bis[(6-phenyl-2-pyridyl)methyl]-*N*-(2-pyridylmethyl)amine} led to the formation of $[(6\text{-}Ph_2TPA)Ni\{PhC(O)C(O)CHOC(O)CH_3\}]ClO_4$ (**5**), which was isolated in moderate yield as a powder (Scheme 5). Attempts to produce X-ray-quality single crystals of **5** have thus far not proven successful. Elemental analysis of the powdered sample of **5** revealed the presence of 0.5 equiv. of CH_2Cl_2, which was also confirmed by a ^1H NMR spectrum of the elemental analysis sample. This solvent was retained despite trituration and extensive drying of the solid. We note that other transition-metal complexes of the 6-Ph_2TPA chelate ligand similarly exhibit a strong affinity for CH_2Cl_2.[26,27]



Scheme 5. Synthesis of **5**.

Complex **5** was analyzed by HRMS and exhibits a $[M - ClO_4]^+$ molecular ion, in excellent agreement with the calculated exact mass and isotopic distribution (see Figure S5 in the Supporting Information). The UV/Vis absorption spectrum of **5** contains an absorption band arising from the coordinated enolate with a maximum at 350 nm. Consistent with a proposed five-membered-ring coordination motif, the FTIR spectrum of **5** contains a carbonyl band at 1749 cm^{-1}, attributable to the acetyl ester group, and a band at 1600 cm^{-1}, due to the non-enolized ketone carbonyl group vicinal to the phenyl ring. The ^1H NMR spectrum of **5** (see Figure S6), collected using paramagnetic parameters, exhibits features consistent with those exhibited by other Ni^{II}–enolate complexes containing 6-Ph_2TPA as an ancillary ligand.[21,28]

Anaerobic Reactivity

Addition of 5 equiv. of $NaOCH_3$ to a solution of **5** in MeOH under anaerobic conditions led to the decay of the 350 nm absorption with concomitant growth of a new absorption band centered at 390 nm (ε = 5400 $M^{-1}cm^{-1}$, assuming that conversion is to a single absorbing species; Figure 1). This spectral change is consistent with nucleophilic substitution of the acetyl group to generate methyl acetate and a new compound **6** containing a coordinated dianionic acireductone (Scheme 6). In contrast, addition of a strong, non-nucleophilic base, such as LiHMDS in CH_3CN, did not lead to any change in the position of the 350 nm absorption maximum. We note that the conversion of **5** into **6** does not proceed with an isosbestic point, which suggests

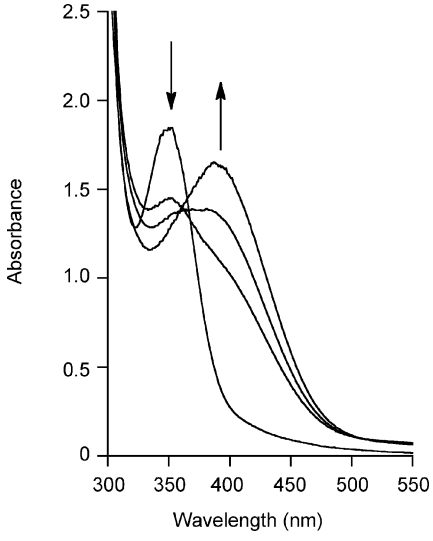
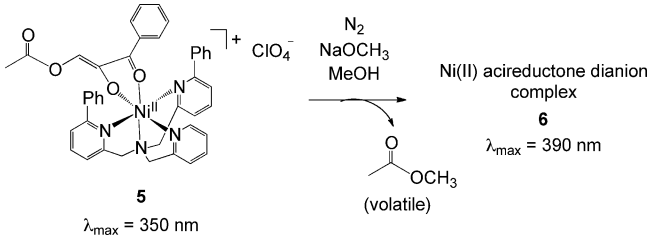


Figure 1. UV/Vis spectra showing the effect of the addition of $NaOCH_3$ to a methanolic solution of **5** under anaerobic conditions.

that this conversion may involve the formation of an intermediate that is spectroscopically similar to **5** and **6**. Removal of the solvent from the reaction of **5** with $NaOCH_3$ under reduced pressure and subsequent analysis of the product by solid-state FTIR spectroscopy in a KBr pellet revealed the disappearance of the band at 1749 cm^{-1}, consistent with removal of the acetyl group and its subsequent volatilization as methyl acetate. When the reaction was carried out at higher concentrations, a white precipitate of $NaClO_4$ was also formed as the reaction proceeds.

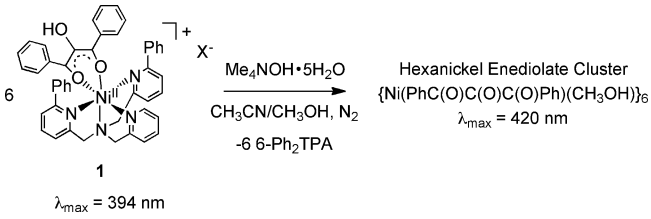


Scheme 6. Reaction of **5** with excess $NaOCH_3$ under anaerobic conditions.

Evidence that **6** contains a dianionic form of phenyl acireductone comes from a comparison of the observed spectral features with those reported in previous studies of this acireductone.[19] Specifically, the 40 nm bathochromic shift observed upon treatment of **5** with $NaOCH_3$ is exactly the same magnitude of shift as was previously found for the conversion of the free phenyl acireductone mononanion (λ_{max} = 320 nm) into the dianion (λ_{max} = 360 nm) in phosphate buffer under anaerobic conditions.[19] An identical 40 nm redshift is also observed when the phenyl acireductone monoanion is exposed to the Ni-ARD enzyme under anaerobic conditions, which was taken as evidence of a coordinated acireductone dianion in the enzyme–substrate complex. The observed difference in absorption maxima between **6** and the ES complex of Ni-ARD (390 vs. 360 nm, respectively) is likely due to the differences in the primary coordination environment of the Ni^{II} center.

^{1}H NMR Studies

We previously reported that the treatment of **1** (Scheme 2) with additional base under anaerobic conditions resulted in the loss of the 6-Ph_2TPA chelate ligand and the formation of a hexanickel enediolate cluster species (Scheme 7).[29] We followed up our anaerobic UV/Vis studies of the present system with ^{1}H NMR investigations to evaluate the chelate ligand binding properties of the C(1)-H-containing phenyl acireductone dianion species **6**. Treatment of **5** with 5 equiv. of $NaOCH_3$ under anaerobic conditions in CD_3OD led to the initial growth of a new species with resonances at δ = 49.2 and 46.2 ppm, followed by the gradual disappearance of all of the paramagnetically shifted peaks associated with the methylene and pyridyl ring protons of the 6-Ph_2TPA ligand (see Figure S7 in the Supporting Information). Analysis of the diamagnetic region of the spectrum recorded at the end of the reaction showed resonances consistent with the presence of the free 6-Ph_2TPA ligand (see Figure S8), albeit broadened due to the presence of a paramagnetic species in solution. These results suggest the initial formation of a new acireductone dianion complex in which the 6-Ph_2TPA ligand is coordinated to the metal center, followed by its subsequent demetalation. The involvement of a transient intermediate containing the coordinated 6-Ph_2TPA ligand is consistent with our anaerobic UV/Vis studies, which suggest the formation of an intermediate in the conversion of **5** into **6**. Overall, these studies suggest that **6** is a Ni^{II}(enediolate) species that could reasonably be either a mononickel solvate species or a polynuclear cluster, as has been observed previously.[29] The displacement of the ancillary ligand in both systems is assumed to be due to the poor affinity of the phenylpyridyl donors of 6-Ph_2TPA for Ni^{II} relative to the affinity of the enolate moieties of the acireductone dianion. Notably, paramagnetically shifted chelate ligand resonances were not observed when **5** was deprotected under O_2 (see Figure S9). This suggests that if a 6-Ph_2TPA-ligated Ni^{II}–acireductone dianion complex is initially formed, it is rapidly intercepted by O_2.



Scheme 7. Previously reported reactivity of **1** with excess base resulting in the formation of a hexanickel enediolate cluster species.

Additional efforts to characterize **6** were made by using ESI-MS. Analysis of an anaerobic CH_3OH solution of **5** within 10 min following the addition of $NaOCH_3$ (5 equiv.) did not give a molecular ion consistent with either the deprotected dianion-bound form of **5** (expected $[M]^+$: m/z = 663) or with the formation of Ni^{II}(enediolate) cluster species that could be identified. Instead, only isotope clusters consistent with the presence of the starting material **5** (m/z = 705), $[H(6\text{-}Ph_2TPA)]^+$ and $[Na(6\text{-}Ph_2TPA)]^+$ species, and oxidation products (see below) were identified.

Aerobic Reactivity

Exposure of CH_3CN or MeOH solutions of **5** to O_2 did not lead to the decomposition of the complex over the course of 24 hours, as revealed by UV/Vis and ^{1}H NMR spectroscopy. This is the expected result because the coordinated phenyl acireductone should not react with O_2 due to the presence of the acetyl protecting group. However, once deprotected, this complex should react rapidly with O_2. In this regard, a methanolic solution of **5** was deprotected by the addition of 5 equiv. of $NaOCH_3$ under anaerobic conditions to form **6**. Once this reaction was complete, as monitored by UV/Vis spectroscopy, the resulting solution was purged with O_2. This led to the rapid decay of the absorp-

tion at 390 nm (Figure 2, left), consistent with oxidative cleavage of the acireductone dianion. As an alternative approach to examining O_2 reactivity, a methanolic solution of **5** was prepared and purged with O_2. This solution was then mixed with 5 equiv. of $NaOCH_3$ and the subsequent decay monitored by UV/Vis spectroscopy (Figure 2, right). The observed decay of the absorption at 350 nm, without any significant growth of the peak at 390 nm, suggests a rapid oxidative cleavage of the acireductone dianion as it is formed.

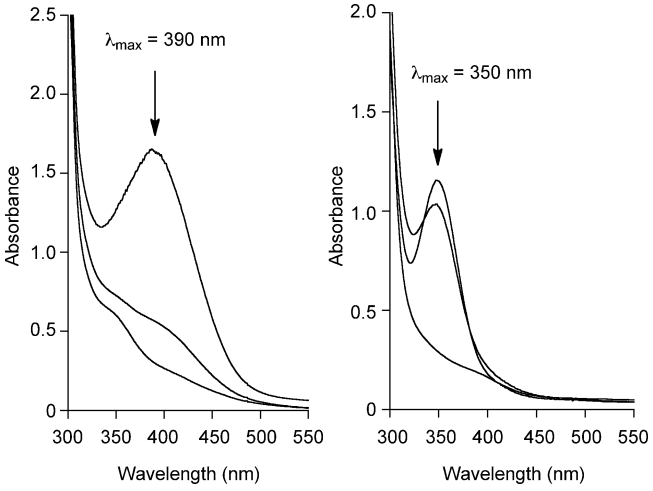
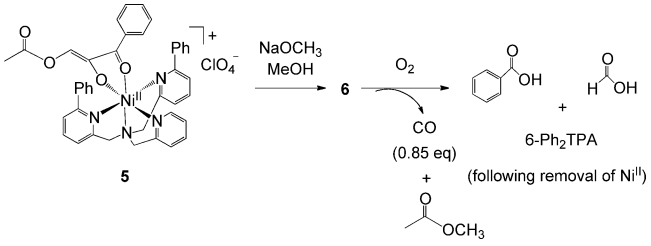


Figure 2. Left: Spectral change produced upon the introduction of O_2 into a solution of **5** in MeOH that had previously been treated with 5 equiv. of $NaOCH_3$; a decrease in the band at 390 nm is observed. Right: Spectral change produced upon treatment of an O_2-purged solution of **5** with 5 equiv. of $NaOCH_3$; a decrease in the band at is observed.

In the presence of O_2, the hexanickel enediolate cluster species produced upon treatment of **1** with base underwent reaction to yield a Ni^{II}–benzoate species and CO. The former exists as a coordination complex with the 6-Ph_2TPA chelate ligand, [(6-Ph_2TPA)Ni(O_2CPh)$_2$].[21] After extended stirring under oxygen, methanol solutions of **6** were analyzed by ESI-MS and found to contain isotope clusters consistent with the formation of [(6-Ph_2TPA)Ni(O_2CH)]$^+$ and [(6-Ph_2TPA)Ni(O_2CPh)]$^+$ species (see Figures S10 and S11 in the Supporting Information), along with [H(6-Ph_2TPA)]$^+$. Divalent nickel formate and benzoate complexes containing these cationic species have previously been prepared and comprehensively characterized in our laboratory.[30] ^{1}H NMR analysis of the reaction product mixture provided additional evidence for the presence of these carboxylate species with characteristic paramagnetically shifted resonances in the range of 10–50 ppm (Figure S12). Importantly, the observation of these oxidative cleavage products suggests Ni^{II}-ARD-type regioselective C(1)–C(2) and C(2)–C(3) cleavage with release of CO.

Organic Product Analysis

Analysis of the head-space gas once the reaction of **5** with 5 equiv. of $NaOCH_3$ in MeOH in the presence of O_2 had proceeded to completion showed that 0.85 equiv. of CO had been produced in the reaction (Scheme 8). CO is the expected product if both the C(1)–C(2) and C(2)–C(3) bonds of the phenyl acireductone substrate are cleaved. The yield of CO gas suggests that this cleavage reaction is the major reaction pathway, which is consistent with the formation of the [(6-Ph_2TPA)Ni(O_2CH)]$^+$and [(6-Ph_2TPA)Ni(O_2CPh)]$^+$ species as the major products, as were detected by ESI-MS and ^{1}H NMR spectroscopy. Additional evidence for the formation of the carboxylic acid products was obtained following removal of the Ni^{II} ion from the reaction mixture and analysis of the organic products. Formate was detected by using a previously reported method for the detection of low-molecular-weight carboxylates involving derivatization with 4′-phenylphenacyl bromide[13] and benzoic acid was detected by GC–MS. Notably, no evidence was found by GC–MS of a tricarbonyl-type intermediate (benzoylglyoxal) akin to that involved in reaction the reaction of complex **1** bearing the bulky acireductone 2-hydroxy-1,3-diphenylpropane-1,3-dione as ligand (Scheme 2).[22,23] In addition, no evidence was found for the formation of benzoylformic acid, which would be the expected product if only C(1)–C(2) cleavage (Fe-ARD′-type) reaction occurred.



Scheme 8. Products of the oxidative cleavage reaction resulting from treatment of **5** with $NaOCH_3$ in the presence of O_2.

Discussion

At the outset of this study, our goal was to develop an approach for generating a mononuclear Ni^{II} complex of a protected C(1)-H acireductone that upon deprotection would enable reactivity studies of relevance to the enzymatic chemistry of Ni-ARD. In this regard, we have succeeded in developing a high-yielding, simple synthetic route to a protected C(1)-H acireductone precursor and the Ni^{II} complex (**5**) of its monoanion. We note that this methodology should be extendable to the synthesis of a variety of protected C(1)-H acireductones and thus represents a significant step forward in the synthetic modeling of acireductone dioxygenases.

We found that the treatment of **5** with $NaOCH_3$ to deprotect the acireductone moiety, followed by exposure of a solution of the complex to O_2, results in the formation of the same products as a Ni-ARD-type reaction (CO, formate, benzoate). This is significant as it is the first example of a model system that undergoes oxidative cleavage of a C(1)-H acireductone substrate to give products akin to

those produced by the Ni^{II}-containing acireductone dioxygenase enzyme. Notably, in the absence of a metal ion, the dianionic form of phenyl acireductone undergoes oxidative cleavage to give the products of C(1)–C(2) cleavage (formate and benzoylformic acid).[19] The fact that no benzoylformic acid is found in the product mixture starting from **5** indicates that the Ni^{II} center in this system influences the regioselectivity of the reaction. These results distinguish this chemistry from our prior studies of a bulky acireductone that was predisposed to C(1)–C(2) and C(2)–C(3) (Ni-ARD-type) cleavage chemistry. In our former studies, it was only under wet conditions in the presence of Fe^{II} that we found evidence for metal-dependent regioselective carbon–carbon bond cleavage. This led us to propose a mechanistic pathway in which the Fe^{II} center had an enhanced ability to promote the hydration of a vicinyl triketone intermediate, leading to a differentiation in regioselectivity.[22] In the current system, in the absence of water, the Ni^{II} center has a directing effect on the chemistry, which makes this model system particularly relevant to Ni^{II}-ARD.

In terms of the reaction pathway leading to carbon–carbon bond cleavage, we note that the lack of an observed tricarbonyl intermediate (benzoylglyoxal) does not explicitly rule out the involvement of such a species. However, it may be difficult to detect this possible intermediate due to the lack of a facile benzoyl migration that would produce a stable side-product (phenylglyoxal) (see Scheme S1 in the Supporting Information).

A complicating challenge in the chemistry of synthetic Ni^{II}–acireductone dianion species is supporting chelate ligand loss, which in a prior system resulted in the formation of a hexanickel–enediolate cluster species that reacted with O_2.[29] Upon deprotection of **5** under anaerobic conditions, we found preliminary evidence in terms of paramagnetically shifted ^1H NMR chelate ligand resonances to suggest that the formation of **6** may initially involve the generation of a 6-Ph_2TPA-ligated acireductone dianion complex, which subsequently undergoes loss of the chelate ligand, likely to give a Ni^{II}(enediolate) cluster species. Elucidation of the structural features of this 6-Ph_2TPA-ligated complex will depend upon our ability to stabilize it relative to loss of the chelate ligand. Experiments directed towards addressing this issue are underway in our laboratory. In addition, the nature of the cluster species that may form and play a role in the reactivity of **6** towards O_2 is also under investigation. Although the characterization of **6** is a key ongoing challenge, the results presented herein conclusively demonstrate the feasibility of a new synthetic approach that will enable examination of the oxidative chemistry of C(1)-H acireductone dianion complexes.

Experimental Section

General Methods: All reagents were obtained from commercial sources and used without additional purification unless stated otherwise. Solvents were dried according to published procedures and purified by distillation under N_2 prior to use.[31] Air-sensitive reactions were performed in an MBraun Unilab glovebox under N_2 or by using standard Schlenk techniques. The 6-Ph_2TPA {*N*,*N*-bis[(6-phenyl-2-pyridyl)methyl]-*N*-(2-pyridylmethyl)amine} ligand was synthesized according to a previously published procedure.[32]

Physical Methods: ^1H and ^{13}C NMR spectra of organic compounds were recorded by using a Bruker ARX-400 spectrometer; chemical shifts are referenced to the residual solvent peak in CD_2HCN (^1H NMR: 1.94 ppm, quintet; ^{13}C: 1.39 ppm, quintet). ^1H NMR spectra of paramagnetic complexes were collected by using the above spectrometer and parameters as described previously.[28] UV/Vis data were collected by using a Hewlett-Packard HP8453A spectrometer at ambient temperature. IR spectra were recorded with a Shimadzu FTIR-8400 spectrometer as neat oils or KBr pellets. GC–MS data was obtained with a Shimadzu GC/MS-QP5000 gas chromatograph/mass spectrometer with a GC-17A gas chromatograph, using an Alltech EC5 30 m × 25 mm × 25 μm thin-film capillary column and the following temperature program: $T_{initial}$: 70 °C (5 min); temperature gradient: 23 °C min^{-1}; T_{Final}: 250 °C (10 min). GC-TCD data of the reaction head-space gas were collected by using an Agilent 3000A Micro gas chromatograph. Mass spectroscopic data were collected by the Mass Spectrometry Facility, University of California, Riverside. Elemental analyses of compounds **3**–**5** were performed by Atlantic Microlabs Inc., Norcross, GA.

3-Phenyl-2-propynyl Acetate (3): 3-Phenyl-2-propyn-1-ol (1.00 g, 7.57 mmol) and NEt_3 (1 mL) were dissolved in Ac_2O (5 mL) and stirred for 16 h under N_2. The resulting orange solution was diluted with EtOAc (150 mL) and washed repeatedly with 100 mL portions of H_2O. The organic layer was passed through an activated charcoal filter and dried with anhydrous Na_2SO_4. The solvent was then removed under reduced pressure to yield a yellow oil (1.10 g, 83%). ^1H NMR (400 MHz, CD_3CN): δ = 7.46–7.35 (m, 5 H), 4.87 (s, 2 H), 2.07 (s, 3 H) ppm. ^{13}C NMR (100 MHz, CD_3CN): δ = 171.01, 132.54, 129.94, 129.58, 122.90, 86.47, 84.54, 53.23, 20.88 ppm. FTIR (neat): $\tilde{\nu}$ = 2234 (ν_{CC}), 1734 (ν_{CO}) cm^{-1}. $C_{11}H_{10}O_2$ (174.20): calcd. C 75.84, H 5.79; found C 75.77, H 5.83.

1-Acetoxy-3-phenylpropane-2,3-dione (4): $NaIO_4$ (1.07 g, 5.00 mmol) was dissolved in H_2O (15 mL) and combined with $RuCl_3$ (0.037 mmol) to form a yellow solution. 3-Phenyl-2-propynyl acetate (**3**; 0.215 g, 1.23 mmol) dissolved in a mixture of CCl_4 (10 mL) and CH_3CN (10 mL) was added to the aqueous solution, and the resulting slurry stirred for 15 min, monitoring carefully by TLC. The slurry was then diluted with CH_2Cl_2 (150 mL) and filtered. The filtrate was cooled in an ice bath and then carefully mixed with Na_2SO_3 (100 mL, 1.0 M). The resulting suspension was placed in a separatory funnel and the aqueous layer extracted with CH_2Cl_2 (3 × 100 mL). The organic layers were combined, dried with anhydrous Na_2SO_4, and the solvent removed under reduced pressure. The crude yellow product mixture was purified by column chromatography using a silica gel solid phase and eluting with 4:1 hexanes/EtOAc (180 mg, 71%). ^1H NMR (300 MHz, CD_3CN): δ = 8.01 [d, ^3J(H,H) = 8.2 Hz, 2 H], 7.73 [t, ^3J(H,H) = 7.2 Hz, 1 H], 7.57 [t, ^3J(H,H) = 7.9 Hz, 2 H], 5.21 (s, 2 H), 2.13 (s, 3 H) ppm. ^{13}C NMR (100 MHz, CD_3CN): δ = 196.23, 191.26, 171.24, 135.95, 132.94, 131.07, 129.92, 67.19, 20.49 ppm. FTIR (neat): $\tilde{\nu}$ = 1753 (ν_{CO}), 1730 (ν_{CO}), 1669 (ν_{CO}) cm^{-1}. UV/Vis (Et_2O): λ_{max} (ε) = 255 nm (12000 M^{-1} cm^{-1}). $C_{11}H_{10}O_4$ (206.20): calcd. C 64.07, H 4.89; found C 64.19, H 5.27.

Caution! *Perchlorate salts of metal complexes with organic ligands are potentially very explosive. Only small amounts of material should be prepared, and these should be handled with extreme caution.*[33]

[(6-Ph_2TPA)Ni{PhC(O)C(O)CHOC(O)CH_3}]ClO_4 (5): A CH_3CN (5 mL) solution of [(6-Ph_2TPA)Ni($CH_3CN)_2$]($ClO_4)_2$ (0.078 mmol) was stirred with 1-acetoxy-3-phenylpropane-2,3-dione (16 mg,

0.078 mmol) under N_2 until it was completely dissolved. This solution was then added to LiHMDS (14 mg, 0.082 mmol) in Et_2O (1 mL) and stirred overnight. The solvent was then removed under reduced pressure and the solid redissolved in CH_2Cl_2 and the solution filtered through a glass wool/Celite plug to remove insoluble $LiClO_4$. The CH_2Cl_2 solution was concentrated under reduced pressure and then layered with hexanes to yield a pale-yellow precipitate. The precipitate was collected, triturated with Et_2O, and then dried under vacuum for 48 h (36 mg, 54%). FTIR (KBr): $\tilde{\nu}$ = 1749 (ν_{CO}), 1609 (ν_{CO}), 1451, 1355, 1094 (ν_{ClO4}), 765, 623 (ν_{ClO4}) cm^{-1}. UV/Vis (MeOH): λ_{max} (ε) = 350 (6100 $M^{-1} cm^{-1}$). $C_{41}H_{35}ClN_4NiO_8 \cdot 0.5CH_2Cl_2$: calcd. C 58.74, H 4.28, N 6.61; found C 59.17, H 4.67, N 7.09. HRMS (ESI): calcd. for $[C_{41}H_{35}N_4NiO_4]^+$ 705.2012 $[M - ClO_4]^+$; found 705.2010.

Treatment of 5 with $NaOCH_3$: For UV/Vis experiments, an approximate 0.2 mM MeOH stock solution of **5** was prepared. A 2.4 mL aliquot of this solution (ca. 0.48 μmol) was placed in a quartz UV/Vis cell and then combined with a 200 μL aliquot of a 12 mM solution of $NaOCH_3$ (2.4 μmol). For NMR experiments, **5** (ca. 2.5 mg, 3 μmol) was dissolved in CD_3OD (0.75 mL) and $NaOCH_3$ (15 μmol) also dissolved in CD_3OD (0.25 mL). The two solutions were then combined.

Treatment of 5 with LiHMDS: An approximate 0.2 mM CH_3CN stock solution of **5** was prepared. A 2.4 mL aliquot of this solution (ca. 0.48 μmol) was placed in a quartz UV/Vis cell. This solution was then combined with a 200 μL aliquot of a 12 mM Et_2O solution of LiHMDS (2.4 μmol).

O_2 Reactivity: Solutions for monitoring by UV/Vis or ^1H NMR were prepared as described above. Oxygen was introduced by purging the solutions with dry O_2 gas for 30 s, and then the reaction vessels were sealed. In an alternative set of experiments, solutions of **5** were purged with O_2 and then subsequently treated with 5 equiv. of $NaOCH_3$.

Identification of Organic Products: To analyze the products of the O_2 reaction, a MeOH solution containing **5** (2 mg/mL, ca. 2.5 mM) was prepared. The solution was then purged with O_2 for 30 s. The aerated solution was then combined with solid $NaOCH_3$ (0.6 mg per mL of solution) to give a $NaOCH_3$ concentration of ca. 12.5 mM (5 equiv.). The solution was then repurged with O_2 for 30 s, sealed with a rubber septum, and stirred for 12 h. The solvent was then removed under reduced pressure and the products analyzed as described below.

Detection of Formate: The crude reaction mixture was redissolved in a mixture of CH_3CN (10 mL) and benzene (10 mL). 4′-Phenylphenacyl bromide (10 equiv.) and 18-crown-6 (5 equiv.) were then added to the reaction mixture. The resulting slurry was heated at reflux under nitrogen for 18 h. The solvent was then removed under reduced pressure and the solid extracted with CH_2Cl_2. The CH_2Cl_2 solution was then passed through a short plug of silica and analyzed by GC–MS. 4′-Phenylphenacyl formate was detected in the reaction mixture.

Detection of Benzoic Acid: The crude reaction mixture was redissolved in a small amount of CH_3CN and passed through a short silica column, eluting with ethyl acetate. The organic products were then analyzed by GC–MS and benzoic acid was identified by comparison of the molecular ion, fragmentation pattern, and retention time with those of an authentic sample.

Analysis of Gaseous Products: Complex **5** (0.01 mmol) was dissolved in MeOH (1.0 mL) and the solution placed in a 50 mL round-bottomed flask equipped with a stirring bar. The flask was purged with O_2 and sealed with a rubber septum. A solution of $NaOCH_3$ (0.05 mmol) in MeOH (1.0 mL) was injected into the round-bottomed flask by using a gas-tight syringe and the resulting solution stirred for 12 h. The head-space gas (10 mL) was removed by means of a gas-tight syringe and analyzed by GC-TCD. The yield of CO generated in the reaction was determined from a calibration curve generated by using gas mixtures of O_2 and CO.

Supporting Information (see footnote on the first page of this article): ^1H and ^{13}C NMR and mass spectra; schematic description of a possible reaction pathway for benzoylglyoxal.

Acknowledgments

This research was supported by the National Science Foundation (NSF) (grant numbers CHE-0848858 and CHE-1301092 to L. M. B.). C. A. and L. M. B. thank Jay Riley Argue and Sushma Saraf for technical assistance.

[1] a) J. D. Finkelstein, *J. Nutr. Biochem.* **1990**, *1*, 228–237; b) C. C. Valley, A. Cembran, J. D. Perlmutter, A. K. Lewis, N. P. Labello, J. Gao, J. N. Sachs, *J. Biol. Chem.* **2012**, *287*, 34979–34991; c) P. Cavuoto, M. F. Fenech, *Cancer Treat. Rev.* **2012**, *38*, 726–736; d) T. Nakamoto, *Gene* **2009**, *432*, 1–6.
[2] M. A. Pajares, G. D. Markham, in: *Advances in Enzymology and Related Areas of Molecular Biology* (Ed.: E. J. Toone), Wiley, Hoboken, NJ, **2011**, vol. 78, p. 449–521.
[3] M. H. Stipanuk, *Ann. Rev. Nutr.* **1986**, *6*, 179–209.
[4] W. A. M. Loenen, *Biochem. Soc. Trans.* **2006**, *34*, 330–333.
[5] a) H. L. Schubert, R. M. Blumenthal, X. Cheng, *Trends Biochem. Sci.* **2003**, *28*, 329–335; b) A.-W. Struck, M. L. Thompson, L. S. Wong, J. Micklefield, *ChemBioChem* **2012**, *13*, 2642–2655.
[6] a) P. A. Frey, A. D. Hegeman, F. J. Ruzicka, *Crit. Rev. Biochem. Mol. Biol.* **2008**, *43*, 63–88; b) K. A. Shisler, J. B. Broderick, *Curr. Opin. Struct. Biol.* **2012**, *22*, 701–710.
[7] M. Sauter, B. Moffatt, M. C. Saechao, R. Hell, M. Wirtz, *Biochem. J.* **2013**, *451*, 145–154.
[8] a) M. E. Martinez, T. G. O'Brien, K. E. Fultz, N. Babbar, H. Yerushalmi, N. Qu, Y. Guo, D. Boorman, J. Einspahr, D. S. Alberts, E. W. Gerner, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 7859–7864; b) R. M. Pascale, M. M. Simile, M. R. De Miglio, F. Feo, *Alcohol* **2002**, *27*, 193–198.
[9] a) E. Albers, *IUBMB Life* **2009**, *61*, 1132–1142; b) A. Sekowska, V. Dénervaud, H. Ashida, K. Michoud, D. Haas, A. Yokota, A. Danchin, *BMC Microbiol.* **2004**, *4*, 9; c) M. A. Avila, E. R. Garćia-Trevijano, S. C. Lu, F. J. Corrales, J. M. Mato, *Int. J. Biochem. Cell Biol.* **2004**, *36*, 2125–2130.
[10] T. C. Pochapsky, T. Ju, M. Dang, R. Beaulieu, G. M. Pagani, B. OuYang, in: *Metal Ions in Life Sciences* (Eds.: A. Sigel, H. Sigel, R. K. O. Sigel), Wiley-VCH, Weinheim, Germany, **2007**, vol. 2, p. 473–500.
[11] M. J. Maroney, S. Ciurli, *Chem. Rev.* **2014**, *114*, 4206–4228.
[12] C. J. Allpress, L. M. Berreau, *Coord. Chem. Rev.* **2013**, *257*, 3005–3029.
[13] J. W. Wray, R. H. Abeles, *J. Biol. Chem.* **1995**, *270*, 3147–3153.
[14] J. W. Wray, R. H. Abeles, *J. Biol. Chem.* **1993**, *268*, 21466–21469.
[15] Y. Day, P. C. Wensink, R. H. Abeles, *J. Biol. Chem.* **1999**, *274*, 1193–1195.
[16] R. Motterlini, L. E. Otterbein, *Nat. Rev. Drug Discovery* **2010**, *9*, 728–743.
[17] T. C. Pochapsky, S. S. Pochapsky, T. Ju, H. Mo, F. Al-Mjeni, M. J. Maroney, *Nat. Struct. Biol.* **2002**, *9*, 966–972.
[18] a) T. Ju, R. B. Goldsmith, S. C. Chai, M. J. Maroney, S. S. Pochapsky, T. C. Pochapsky, *J. Mol. Biol.* **2006**, *363*, 823–834; b) T. C. Pochapsky, S. S. Pochapsky, T. Ju, C. Hoefler, J. Liang, *J. Biomol. NMR Spectroscopy* **2005**, *34*, 117–127; c) S. S. Pochap-

sky, J. C. Sunshine, T. C. Pochapsky, *J. Am. Chem. Soc.* **2008**, *130*, 2156–2157.
[19] a) Y. Dai, T. C. Pochapsky, R. H. Abeles, *Biochemistry* **2001**, *40*, 6379–6387; b) S. C. Chai, T. Ju, M. Dang, R. B. Goldsmith, M. J. Maroney, T. C. Pochapsky, *Biochemistry* **2008**, *47*, 2428–2438.
[20] M. Sparta, C. E. Valdez, A. N. Alexandrova, *J. Mol. Biol.* **2013**, *425*, 3007–3018.
[21] E. Szajna, A. M. Arif, L. M. Berreau, *J. Am. Chem. Soc.* **2005**, *127*, 17186–17187.
[22] C. J. Allpress, K. Grubel, E. Szajna-Fuller, A. M. Arif, L. M. Berreau, *J. Am. Chem. Soc.* **2013**, *135*, 659–668.
[23] L. M. Berreau, T. Borowski, K. Grubel, C. J. Allpress, J. P. Wikstrom, M. E. Germain, E. V. Rybak-Akimova, D. L. Tierney, *Inorg. Chem.* **2011**, *50*, 1047–1057.
[24] T. Borowski, A. Bassan, P. E. M. Siegbahn, *THEOCHEM* **2006**, *772*, 89–92.
[25] a) R. W. Myers, J. W. Wray, S. Fish, R. H. Abeles, *J. Biol. Chem.* **1993**, *268*, 24785–24791; b) Y. Zhang, M. H. Heinsen, M. Kostic, G. M. Pagani, T. V. Riera, I. Perovic, L. Hedstrom, B. B. Snider, T. C. Pochapsky, *Bioorg. Med. Chem.* **2004**, *12*, 3847–3855.
[26] a) C. J. Allpress, A. M. Arif, D. T. Houghton, L. M. Berreau, *Chem. Eur. J.* **2011**, *17*, 14962–14973; b) K. Grubel, K. Rudzka, A. M. Arif, K. L. Klotz, J. A. Halfen, L. M. Berreau, *Inorg. Chem.* **2010**, *49*, 82–96.
[27] E. Szajna-Fuller, K. Rudzka, A. M. Arif, L. M. Berreau, *Inorg. Chem.* **2007**, *46*, 5499–5507.
[28] E. Szajna, P. Dobrowolski, A. L. Fuller, A. M. Arif, L. M. Berreau, *Inorg. Chem.* **2004**, *43*, 3988–3997.
[29] K. Rudzka, K. Grubel, A. M. Arif, L. M. Berreau, *Inorg. Chem.* **2010**, *49*, 7623–7625.
[30] E. Szajna-Fuller, B. M. Chambers, A. M. Arif, L. M. Berreau, *Inorg. Chem.* **2007**, *46*, 5486–5498.
[31] W. L. F. Armarego, D. D. Perrin, *Purification of Laboratory Chemicals*, 4th ed., Butterworth–Heinemann, Boston, **1996**.
[32] M. M. Makowska-Grzyska, E. Szajna, C. Shipley, A. M. Arif, M. H. Mitchell, J. A. Halfen, L. M. Berreau, *Inorg. Chem.* **2003**, *42*, 7472–7488.
[33] W. C. Wolsey, *J. Chem. Educ.* **1973**, *50*, A335–A337.

Received: March 31, 2014
Published Online: August 25, 2014

