capable of Watson-Crick pairing with T. In fact, incubation of primed template 2 (containing T in place of d-*iso*-C) with d-*iso*-GTP, dCTP, and TTP did yield full-length product (lane 5, Figure 3). Comparison with lane 6 shows that incorporation of d-*iso*-G accounts for full-length product formation. In an incubation of primed template 3 (containing dC in place of d-*iso*-C) carried out in the same way as with primed template 2, no full-length product was detected (lane 7, Figure 3). Therefore, while d-*iso*-GTP showed undesired pairing with T, no incorporation of d-*iso*-G was observed opposite dC.

T7 RNA polymerase was also shown to accept the new base pair. Thus, template 4 possessing the T7 consensus promoter yielded 75% more full-length product in the presence of *iso*-GTP than in its absence (compare lanes 2 and 3, Figure 4).[12] The "read-through" in the absence of *iso*-GTP observed in this case is consistent with the lower fidelity of RNA polymerases relative to DNA polymerases.[13] Sequencing of the product RNA transcript using a standard protocol[14] positively established incorporation of *iso*-G at the expected position.

These experiments demonstrate for the first time that both a DNA polymerase (Klenow enzyme) and an RNA polymerase (T7) will incorporate into a growing oligonucleotide a nucleotide with a novel pattern of hydrogen-bonding groups, under the direction of its intended partner in a template. We are currently extending this work to include other base pairs with novel pairing schemes.

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Ligand Oxidation in a Nickel Thiolate Complex: A Model for the Deactivation of Hydrogenase by O_2

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Many hydrogenases (H_2ases) possess Ni centers with S-donor ligands that have unusual redox properties.[1] Upon exposure to O_2, these enzymes are deactivated but may be reductively activated in a multistep process to yield active enzyme.[1] We have recently described oxidations of the dimeric complex **1** involving one-half and two electrons per Ni that lead to an EPR-active dimeric radical and a monomeric disulfide complex, respectively.[2] These oxidation products support a redox role for thiolate ligands in the H_2ase active site. Herein we report a four-electron oxidation of a related Ni complex upon exposure to molecular oxygen. The resulting Ni(II) sulfinato complex is a rare example of a structurally characterized product of thiolate oxidation employing molecular oxygen and provides a plausible chemical model for the deactivation of H_2ase by O_2.

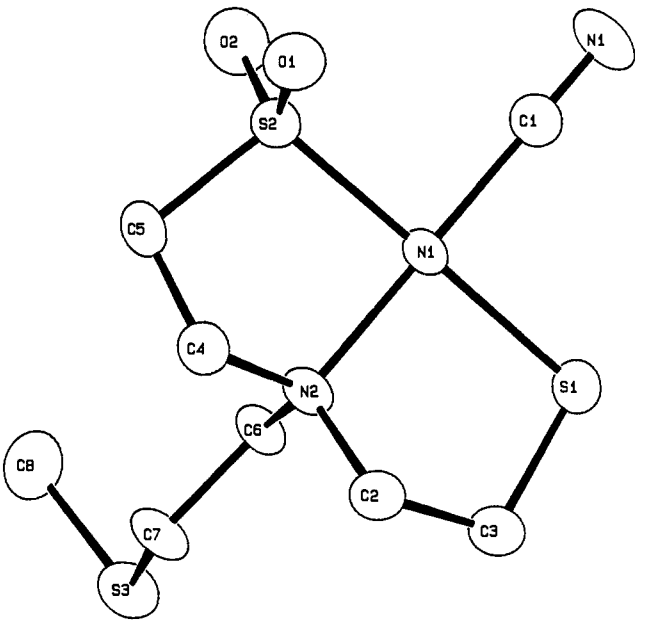
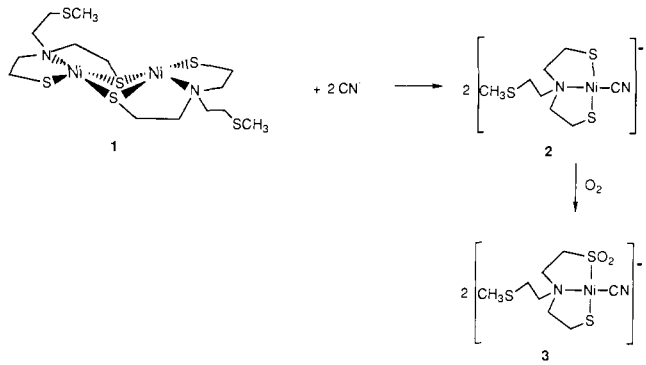


Figure 1. ORTEP plot of **2** with thermal ellipsoids at the 30% probability level. Selected bond distances in Å are as follows: Ni-S1, 2.175 (3); Ni-S2, 2.146 (3); Ni-N2, 1.973 (7); Ni-C1, 1.875 (10); S2-O1, 1.488 (8); S2-O2, 1.419 (7). Selected bond angles in degrees are as follows: S1-Ni-N2, 89.6 (2); S1-Ni-C1, 90.5 (3); S2-Ni-N2, 89.0 (2); S2-Ni-C1, 91.0 (3); S1-Ni-S2, 176.2 (1); N2-Ni-C1, 180.0 (8); Ni-S2-O1, 111.2 (3); Ni-S2-O2, 118.3 (4); O1-S2-O2, 114.4 (4); O1-S2-C5, 103.6 (5); O2-S2-C5, 105.6 (5).

Scheme I



Reaction of **1** with 2 equiv of $Et_4N(CN)$ in DMF[3] (Scheme I) leads to the formation of a structurally characterized square-planar complex (**2**),[4] in analogy with a similar system employing thiophenolate as the fourth ligand.[5] Upon exposure to air or an oxygen atmosphere, **2** undergoes oxidation to the sulfinato complex **3** (Figure 1).[6] This novel diamagnetic Ni(II) complex, a four-electron-oxidation product of **2** that features one thiolate ligand and one sulfinate ligand, can be isolated in 84% yield upon addition of toluene to a DMF solution of **2** stirred under O_2 overnight.[7]

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(3) **CAUTION**: Solutions of $Et_4N(CN)$ are extremely toxic by skin absorption.
(4) Under oxygen-deficient conditions, cocrystals of **2** and **3** that are isomorphous with pure **3** and contain ca. 63% **2** are obtained from DMF/toluene. Lattice constants: a = 12.762 (9) Å, b = 12.740 (7) Å, c = 14.797 (8) Å, β = 117.56 (4)°. Details of this structure will be reported elsewhere.
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(6) X-ray analysis on orange crystals obtained from slow aerobic evaporation of a DMF/MeOH solution of **2**: monoclinic space group $P2_1/c$, a = 12.784 (4) Å, b = 12.757 (2) Å, c = 14.847 (4) Å, β = 115.00 (3)°, V = 2194.5 Å^3, Z = 4. The present values of R = 0.056 and R_w = 0.068 are based on anisotropic unit-weighted refinement of non-hydrogen atoms. Full details will be published elsewhere.

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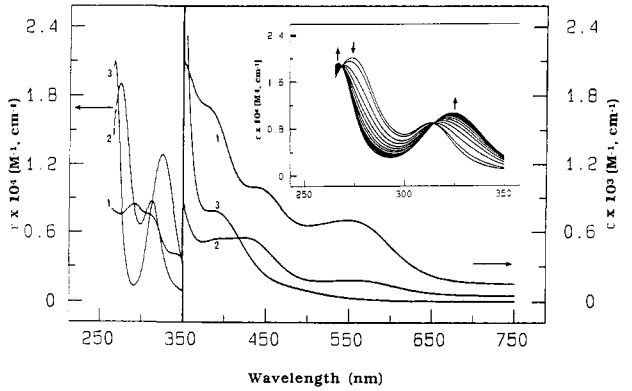

Figure 2. Electronic absorption spectra in DMF. Spectrum 1 is 0.30 mM **1**. Spectrum 2 is 0.30 mM dimer +2 equiv of $Et_4N(CN)$ under N_2 (0.60 mM **2**). Spectrum 3 is a 0.60 mM solution of **3**. The inset describes the reaction of **2** with O_2 at 30 °C at times t = 7, 30, 90, 150, 210, 270, 330, 390, 450, 510, 570, 630, 720, and 750 min. Extinction coefficients are for a constant [Ni] = 0.60 mM.

Although several examples of S-coordinated sulfinato complexes have been structurally characterized,[8] few examples of thiol oxidation by O_2 to give sulfinato complexes are found in the literature.[8a,9] More common routes to the synthesis of sulfinato complexes involve the use of sulfinate salts,[8c,10,11] the insertion of SO_2 into a M–R bond,[10,12] and the reaction of thiolate complexes with peroxides.[10,13-18] The latter reaction has been studied in detail by employing kinetically inert Co(III) complexes in aqueous media.[19-22] These reactions require 2 equiv of peroxide and proceed through the formation of isolable sulfenato intermediates.[18,21] Each two-electron oxidation follows second-order kinetics (first order in [thiolate] and [peroxide]), with a characteristic dependence on $[H^+]$. The oxidation of the Ni(II) thiolate complex described here differs from these studies in that dry, aprotic solvents are employed, Ni(II) complexes are kinetically labile, and O_2 is employed as an oxidant. Despite these differences, a similar rate law is observed. Kinetic investigations (DMF solvent, at 30 °C, monitoring ΔA_{325}) using the initial-rate method[23] reveal that the oxidation is first order in both [Ni] and $[O_2]$[24] with a pseudo-first-order rate constant measured under 1 atm of O_2 of 3.0 × 10^{-3} s^{-1}. Reactions carried out by using $^{18}O_2$ demonstrate that O_2 is the source of the sulfinato oxygen atoms. The infrared spectrum of **3** displays absorptions near 1173 cm^{-1} (ν_a SO_2) and 1047 cm^{-1} (ν_s SO_2) that shift to 1131 cm^{-1} and 1000 cm^{-1}, respectively, in the ^{18}O-labeled product.[10] FAB mass spectral results reveal that, within the limits of purity of the $^{18}O_2$ gas used (97–98%), only the dilabeled product is formed. These results rule out oxygen transfer or exchange involving DMF or trace water. Manometric O_2 uptake experiments at ambient temperature reveal that 1 equiv of O_2 is taken up in several hours, but the total uptake approaches 2 equiv upon prolonged exposure (days). The product of prolonged oxidation has novel spectroscopic features and an elemental analysis consistent with the analogous disulfinato complex,[25] but has not yet been crystallized. Apparently, oxidation of the second thiolate ligand occurs, but at a much slower rate.

The electronic absorption spectral changes that are associated with the formation of **2** and **3** are summarized in Figure 2. The spectrum of **2** is unchanged after several hours under an N_2 atmosphere, but slowly changes to that of **3** in the presence of O_2. Spectra obtained during the oxidation of **2** to **3** reveal isosbestic points, indicating that no stable sulfenyl intermediate is formed in the conversion. Since known coordinated sulfenates are stable to oxidation by O_2,[18] if a sulfenyl intermediate is involved it is likely to be a much more reactive free sulfenate.[19,26] The nucleophilic attack of an uncoordinated thiolate on a transiently coordinated O_2 molecule is a likely mechanism that is consistent with the nucleophilicity of thiolates[19,20,27] and the potent oxidizing power of Ni–O_2 adducts.[28]

The studies reported here demonstrate for the first time that Ni thiolate complexes can undergo oxidation to sulfinato complexes under biological conditions (i.e., air oxidation) and raise the possibility that a similar oxidation of a Ni cysteinate ligand may be responsible for the deactivation of Ni-containing H_2ases upon exposure to O_2. The oxidation of cysteine residues in proteins and the role of metal catalysis in these oxidations have been reviewed.[26,29,30] In several cases, evidence supporting the oxidation of active site cysteine sulfhydryl groups to sulfenic acids has accumulated. These sulfenyl groups are readily reduced to reactivate the enzymes. Studies of the oxidative deactivation of *Desulfovibrio gigas* hydrogenase reveal that oxidation of the enzyme in the presence of O_2, or exposure of anaerobically oxidized enzyme to O_2, leads to an inactive form.[1d,31-33] Active enzyme may be regenerated from this "unready state" through a multistep process involving extensive incubation with H_2 or exposure to strong reductants.[31,32] These facts, as well as the generally high tolerance of the Ni H_2ases to air (half-life of several hours to weeks),[31] are observations that are consistent with oxidation of a Ni cysteinate ligand in the deactivation process. Sulfur K-edge X-ray absorption spectroscopic experiments are in progress to

(7) In a representative synthesis, the dimer **1** (165 mg, 0.31 mmol) was dissolved in 5 mL of dry, anaerobic DMF under an N_2 atmosphere in a 50-mL Schlenk tube. A solution of $Et_4N(CN)$ (97mg, 0.62 mmol) similarly prepared in 5 mL of DMF was added via syringe, causing a color change from dark red to green. This solution was bubbled with O_2 for 15 min and then allowed to stand under an O_2 atmosphere overnight. Toluene (30 mL) was layered on the now red-orange solution. After 2 days, orange crystals of **3** were isolated in 84% yield via suction filtration. Anal. ($NiC_{16}H_{35}N_3S_3O_2$), C, H, N. The ^{18}O-labeled derivative was prepared in the same yield by substituting $^{18}O_2$ in the preparation.

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address this possibility in the enzyme.

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Supplementary Material Available: Tables of positional parameters and their estimated standard deviations and of general displacement parameter expressions (4 pages). Ordering information is given on any current masthead page.

Tertiary Hydroxylation Using Fluorine: Activation of the C–H Bond

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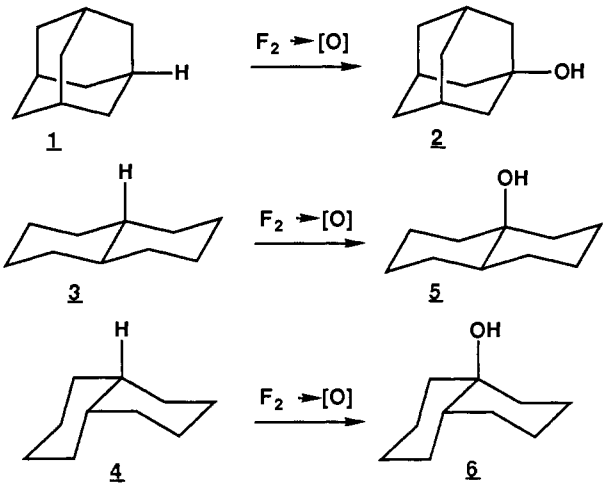
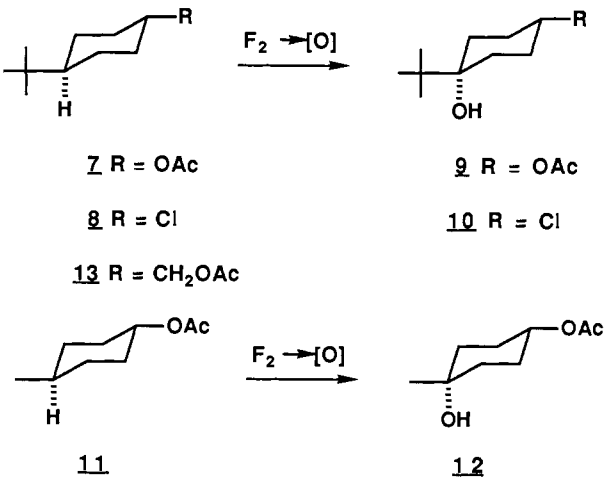
The use of F_2 as a fluorinating agent has been steadily increasing in the last decade.[1] It is clear, however, that the potential of this halogen as a synthetic tool reaches far beyond the field of fluoroorganics. Recently we have been able to demonstrate the ability of this very reactive element to take part in the synthesis of some difficult to obtain fluorine free compounds via the in situ generation of active intermediary agents. Such agents either are strongly polarized in an unusual way or create highly energetic intermediates with organic substrates thus performing some unexpected reactions.[2] We report now yet another unusual reaction involving fluorine, acetonitrile, and water whose outcome is selective hydroxylation of remote unactivated CH bonds.

Activation of paraffinic and other CH bonds far away from any functional group is a subject of many recent projects. The most conspicuous approaches are homogeneous catalysis with organometallic complexes and oxygenation processes involving the peroxide bond, including the use of O_3 or H_2O_2 with or without metal cations.[3] Regio- and stereoselective activation is of course a much more demanding process, and the two most successful approaches involve Breslow's[4] remote radical activation and electrophilic substitution of tertiary hydrogens by F_2.[5]

When fluorine is passed through wet acetonitrile at 0 °C, an oxidizing solution is formed.[6] We have already reported that this oxidizing solution is able to epoxidize olefins with unprecedented efficiency in an electrophilic mode.[7] When no olefin is present, the bonds most susceptible to electrophilic attack are the ones with the highest p-orbital contribution. These are usually the tertiary C–H bonds situated far away from any electron-withdrawing moiety. Furthermore, such an attack should inevitably take place with full retention of configuration.[5,8] Thus, when adamantane (**1**) is treated with the oxidative solution,[9] only a tertiary hydrogen is substituted, resulting in an 80% yield of 1-adamantanol (**2**). Other paraffins were also similarly hydroxylated, including *trans*- and *cis*-decalin (**3** and **4**), which gave respectively *trans*- and *cis*-9-decalol (**5** and **6**) in yields higher than 80%.



The ability to activate paraffins, either in a radical or in an ionic mode, is common to most of the few existing hydroxylation reagents. Similar reactions on heteroatom-containing derivatives are extremely rare however, since the heteroatom represses a chain reaction in a radical process or lowers the electron density of the CH bonds to such an extent that they are practically immune to attack by the known peroxy reagents. Although our oxidative agent is also somewhat affected by the proximity of a deactivating heteroatom group, it still can substitute a tertiary hydrogen in such compounds. Thus, when *trans*-acetoxy- or *trans*-chloro-4-*tert*-butylcyclohexane (**7** or **8**) is reacted, the simple but previously inaccessible trans 4-hydroxy derivatives are obtained in 50% and 30% yields, respectively.[10]



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(6) It is reasonable to assume that first a fast reaction between F_2 and water takes place to form HOF (Appelman, E. H.; Jache, A. W. *J. Am. Chem. Soc.* **1987**, *109*, 1754.) The next step is either a stabilization of the HOF by the acetonitrile or a reaction between the two compounds to form yet another oxidizing material. We will publish later our ongoing studies, in collaboration with Appelman's group, which already indicate that the oxidant contains fluorine and does not have a peroxy moiety.

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