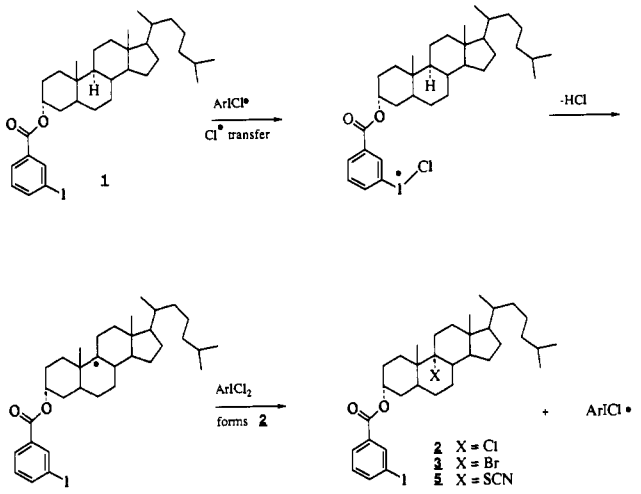
Scheme I

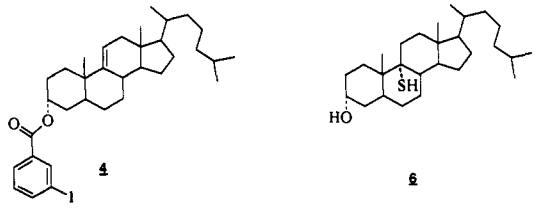


free chlorinating reagent such as $PhICl_2$, SO_2Cl_2, or Cl_2 to form a carbon–chlorine bond and regenerate a species that can transfer Cl• to a new template to continue the chain reaction.

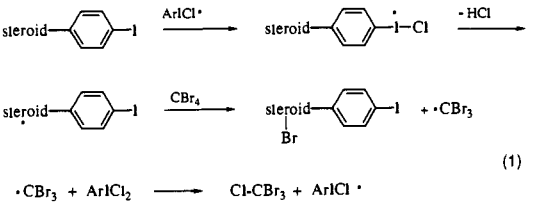
We wanted to generalize this chemistry. In some of the steroid examples[3,6] HCl elimination to generate a desired olefin (such as the 9(11) double bond needed for the synthesis of corticosteroids) is difficult, and Ag^+ was needed to promote it. If instead we had formed a C–Br bond it should eliminate more easily (as we demonstrate below). Furthermore, we wanted to modify our chemistry so as to produce a C–S bond. This could be useful in the synthesis of novel steroid derivatives, such as are used in immunochemistry.

We find that we cannot simply replace the two chlorine atoms in our reagents with bromines or SCN groups, for example. Apparently the Br• and •SCN radicals are too unreactive to be able to abstract hydrogen from unactivated C–H bonds as template complexes. Indeed, only a species as reactive as Cl• seems capable of such H-abstraction after it is stabilized by template complexing. However, we have been able to achieve the desired functionalization chemistry by introducing an additional radical chain reaction that runs in tandem, after the H-abstraction by complexed Cl•. With this scheme we can indeed prepare the desired C–Br and C–S derivatives in good yield, and with our normal geometric control directed by the template.

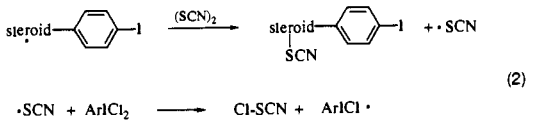
Photolysis of a 12.5 mM solution of substrate **1** with 1.5 equiv of p-NO_2PhICl_2 in CH_2Cl_2 solution affords the chlorosteroid **2** in >90% yield, as described previously.[1] However, when 20 equiv of CBr_4 is also included, the isolated products are instead the bromosteroid **3** in 32% yield and the 9(11) olefin[1] **4** in 51% yield. The bromosteroid was characterized by ^1H NMR (δ 0.670, 1.137 for C-18 and C-19). On silica chromatography, heating, or even standing it readily eliminated HBr to form the 9(11) olefin **4**. Under these conditions the chlorosteroid **2** is stable, so the bromosteroid is indeed a more useful intermediate for the mild formation of this olefin. The bromosteroid **3** was thus originally formed in 83% yield and partially converted to the olefin product on workup.



We propose a tandem radical sequence for this conversion (eq 1). No reaction occurs if the $ArICl_2$ is omitted, so we believe that the hydrogen abstraction step is the same as for chlorination. However, the resulting steroid radical can be trapped by the excess of CBr_4, and the CBr_3 radical[7] can then abstract chlorine from $ArICl_2$ to regenerate the ArICl• and continue the chain.



In another example, we ran the chlorination reaction of 5 mM **1** at 0 °C with $PhICl_2$ and addition of 11 equiv of thiocyanogen[8] ($(SCN)_2$). Now the product was the steroid thiocyanate **5**, isolated by silica chromatography in 64% yield (MS FAB M + 1676, ^1H NMR δ 0.720, 1.143, ^{13}C NMR δ 113.4, and IR 2137 cm^{-1} for SCN). Again no reaction occurred in the absence of the $ArICl_2$, so again we propose a tandem radical process (eq 2). On heating, **5** is converted again to olefin **4** so the SCN group is clearly located on C-9 of the steroid, as our mechanism requires. We have also reduced **5** to the thiol **6** (MS, NMR) with $LiAlH_4$.



These reactions succeed because higher concentrations of alternative reagents can divert the steroid radical away from chlorination, but the critical template-complexed chlorine atom is still produced after an additional atom transfer process. With the use of such tandem reactions, the scope of template-directed remote functionalization reactions has been considerably broadened.

Acknowledgment. This work has been supported by a grant from the National Institutes of Health.

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Magnetic Properties of *Pseudomonas stutzeri* Nitrous Oxide Reductase

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N_2O reductase is the terminal enzyme in the bacterial denitrification pathway.[5] This enzyme catalyzes the two-electron reduction of N_2O to N_2 in an energy-conserving process.[6] The structural gene (*nosZ*) for *Pseudomonas stutzeri* N_2O reductase has been sequenced[7] and corresponds to a protein of molecular weight 70 kD, whereas the purified enzyme has a molecular weight

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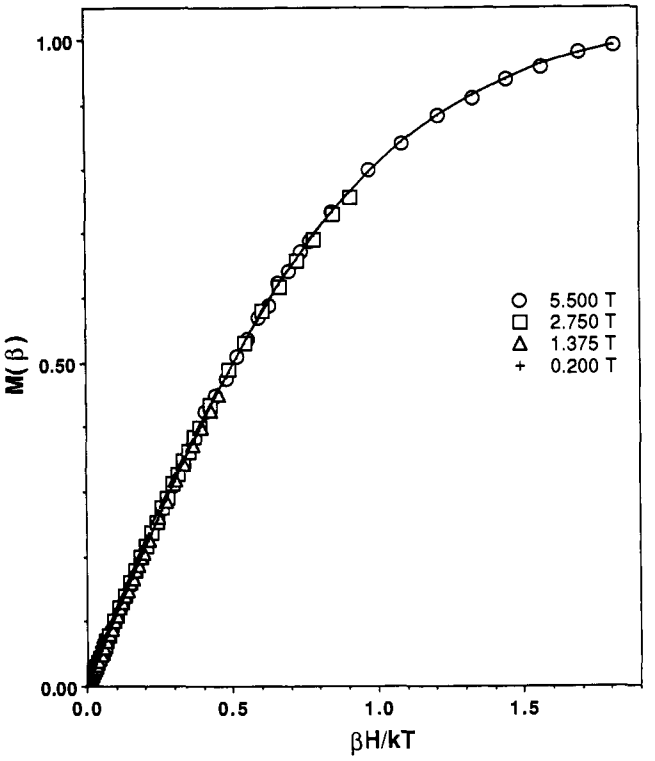

Figure 1. A plot of the saturation magnetization of oxidized *Pseudomonas stutzeri* N_2O reductase in units of Bohr magnetons (β) versus $\beta H/kT$ at the four indicated fields. This plot shows the low-temperature behavior of the data and the fit. The solid line was determined by least squares fitting theoretical magnetization curves, calculated from the Zeeman term for an $S = {}^1/_2$ paramagnet, to the data at all four fields. The vertical axis was scaled using the amount of $S = {}^1/_2$ paramagnetism obtained from the fit. The g values (2.18, 2.02, 2.02) used in the fit were taken from ref 13; for these g values the low temperature asymptote is 1.037 β.

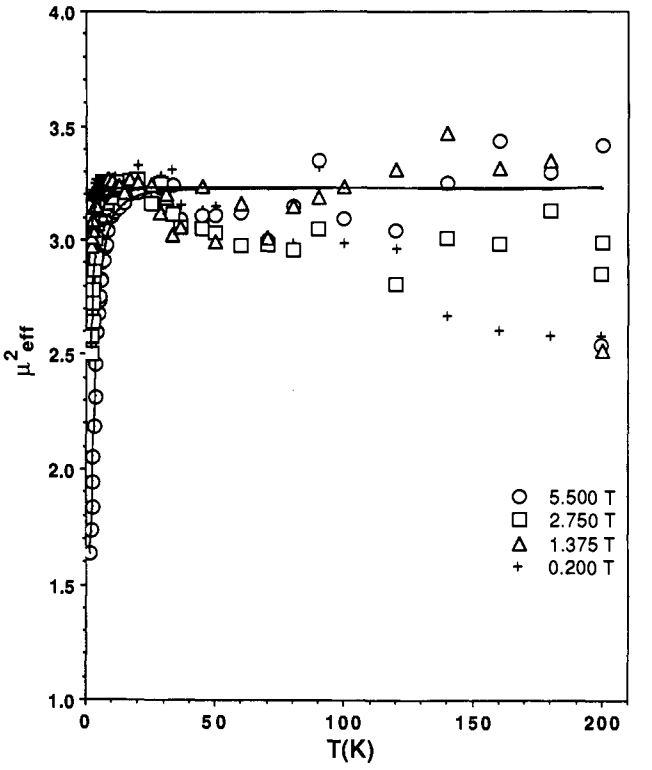

Figure 2. The data and fit shown in Figure 1 are replotted as μ_{eff}^2 versus temperature. This plot emphasizes the high-temperature behavior of the data and the fit. μ_{eff}^2 is 3.228 above 20 K for the g values used in the fit.

of 120–140 kD and a limiting stoichiometry of eight Cu/protein molecule.[8] We infer that the purified enzyme is a dimer composed of two identical subunits, each containing four copper ions. Recent work has shown that N_2O reductase contains a paramagnetic Cu(II) center that is (at least) closely similar to the Cu_A center in cytochrome *c* oxidase.[7,9-13] Relatively little is known about the structures or electronic properties of the other copper sites in N_2O reductase. Jin et al. have proposed that the copper ions in N_2O reductase are distributed in isolated Cu(II) sites, mixed-valent Cu(II)–Cu(I) sites, and antiferromagnetically coupled Cu(II) binuclear sites.[11] One problem hindering the characterization of the copper sites is the wide variation among independent determinations of the amount of EPR-detectable copper in oxidized N_2O reductase. For example, at pH 7.5 anywhere from 15 to 40% of the copper has been reported to be EPR-detectable in various high-activity preparations of the oxidized enzyme.[8,11,14] The goals of this study were to quantitate the paramagnetic species in oxidized N_2O reductase and check for magnetic interactions among the copper ions, using recently developed saturation magnetization techniques. In at least three cases these magnetization techniques have detected paramagnetism associated with Kramer's systems that had previously been missed by EPR spectroscopy.[15] We have measured the magnetization of oxidized N_2O reductase using a SQUID susceptometer over the temperature range 2–200 K at fields of 0.2, 1.375, 2.75, and 5.5 T. The data for two independently prepared samples[16] establish that 23 ± 3% of the copper is paramagnetic with $S = {}^1/_2$. The remainder must be present as Cu(I) or as strongly antiferromagnetically coupled ($\geq$200 cm^{-1} $S_1 \cdot S_2$) Cu(II).[17]

Magnetization data for oxidized N_2O reductase are displayed in Figure 1 as magnetization (in Bohr magnetons, β) versus $\beta H/kT$.[18] This plot emphasizes the low temperature saturation

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(16) Samples were exchanged into deuterated 25 mM Tris buffer, pD 7.5, under anaerobic conditions via several cycles of concentration and dilution in an Amicon stirred cell. Subsequently the sample was concentrated under Ar to 1.4 mM protein (sample 1, 6.9 Cu/protein) or 0.49 mM protein (sample 2, 8.0 Cu/protein). The first sample was loaded into a quartz bucket and frozen immediately following concentration. The magnetization of buffer collected from the final concentration step was measured to provide a background and subtracted from the protein data. Both the protein and the buffer from the concentration step of the second sample were placed under a gentle Ar purge for an additional $1^1/_2$ h prior to freezing. Copper contents of the samples were determined by flame atomic absorption spectroscopy, with a deuterium source used for background correction.
(17) The largest source of uncertainty in the determination of the paramagnetic/total Cu ratio is associated with the molecular weight determination of the protein. The gene sequence contains an open reading frame coding for a protein (presumably one subunit) of 638 amino acids with a predicted M_r of 70 822 (see ref 7). However, the NH_2 terminus probably contains an export signal sequence extending to 35, or perhaps 52, residues, consistent with a final M_r of 65 777–67 280. Estimates of the subunit M_r by SDS-polyacrylamide electrophoresis range from 62 to 74 kD. M_r for the intact protein has been measured (see ref 8) by gradient electrophoresis under nondenaturing conditions (130 kD) and HPLC (118 kD). The values reported here are referenced to an M_r of 130 kD for the oxidized enzyme. For the two samples we examined, the paramagnetic copper content is reduced to 20.2% and 22.0%, if the M_r is 140 kD (at the high end of the permissible range), or increased to 23.6% and 25.7%, if the M_r is 120 kD (at the low end).

behavior of the data. Note that the data at all four magnetic fields are superimposable, which indicates that the observed paramagnetism arises from $S = {}^1/_2$ centers. The solid line in Figure 1 represents the best fit using the EPR g values;[13] the amount of $S = {}^1/_2$ paramagnetism determined from this fit was 22% of the total copper in the sample. A similar result was obtained from a fit with the g values as a free parameter. Hence the vertical axis was scaled using the amount of $S = {}^1/_2$ paramagnetism determined from the first fit. Measurements on an independent sample gave essentially identical results, with the amount of $S = {}^1/_2$ paramagnetism equal to 24% of the total copper in this sample. Recent results[19] are beginning to converge on a figure near 25% for the amount of paramagnetic copper in samples of varying copper contents. Hence the data suggest that, at most, one copper out of four is paramagnetic in a subunit of the fully loaded enzyme.

The magnetization data of Figure 1 are presented in Figure 2 as μ_{eff}^2 ($g^2S(S + 1)$) versus temperature; the fit from Figure 1 is also shown as a solid line in Figure 2. This plot emphasizes the high-temperature, Curie law behavior of the data. The scattering of the data in Figure 2 about the horizontal theoretical line above 20 K provides strong evidence that no paramagnetic species is present other than the $S = {}^1/_2$ species assumed by the fit.[20] Hence we conclude (1) that the residual O_2 contribution has been satisfactorily removed from the protein data via subtraction of the buffer signal, and, more importantly (2), that no other paramagnetic state is significantly populated up to 200 K. Since the fits to the magnetization data from two independently prepared samples correspond to 23 ± 3% of the total copper, most of the copper in oxidized N_2O reductase is diamagnetic from 2 to 200 K. One possibility is that N_2O reductase contains antiferromagnetically coupled binuclear Cu(II) sites, similar to those found in hemocyanin or tyrosinase.[21] If this is the case, our data require that the splitting between the $S = 1$ state and the $S = 0$ ground state be 200 cm^{-1} or greater, which is consistent with the previous estimate from measurements of the EPR susceptibility.[11] Alternatively, some or, less likely, all of the diamagnetic copper may be Cu(I).

It is intriguing that nearly 25% of the copper in two independent preparations of oxidized N_2O reductase appears to be present as $S = {}^1/_2$ Cu(II). Recent EPR measurements on other N_2O reductases are consistent with this result.[22] A variety of spectroscopic evidence strongly indicates that N_2O reductase contains Cu_A-type sites,[10-13] which are paramagnetic and EPR detectable. Comparisons of the N_2O reductase sequence (inferred from translation of the structural gene) to cytochrome oxidase subunit II (cox II) sequences revealed significant homology between a 15-residue N_2O reductase sequence and the cox II sequences containing the putative Cu_A binding site.[7,10] Therefore it is highly probable that N_2O reductase contains two Cu_A-type sites out of a total of eight; in other words, all the paramagnetism may be accounted for by the Cu_A content. The remaining copper ions (*three per subunit*, if four are present in a fully loaded subunit) must be diamagnetic. Kroneck and co-workers have proposed that the Cu_A site is actually a binuclear, mixed-valent [Cu(II)···Cu(I)] site.[12,13,23] If so, the remaining copper could be present as antiferromagnetically coupled Cu(II) dimers. This is a plausible model for N_2O reductase. On the other hand, Chan and Malmström[24,25] have strongly challenged the binuclear model for Cu_A, arguing that a mononuclear $S = {}^1/_2$ model is more consistent with all the available data. In this case the diamagnetism of the "odd" copper could be rationalized in two ways. Each subunit might contain an isolated Cu(I) site, or a coupled binuclear site involving Cu(II) ions from each subunit might be formed. At this time it is not possible to exclude any of these models for the distribution of copper in oxidized N_2O reductase. Considered together with other data, the magnetization results establish that if antiferromagnetically coupled Cu(II) sites are present, the splitting between the singlet ground state and the triplet excited state ($E_{S=1} - E_{S=0}$) must be at least 200 cm^{-1}. This is similar to previous results obtained on other copper-containing oxidases that catalyze multielectron redox chemistry.[21]

Acknowledgment. This research was supported by the Cooperative State Research Service, U.S. Department of Agriculture, under Agreement 89-37280-4696 (to D.M.D.), the Deutsche Forschungsgemeinschaft and Fonds der Chemischen Industrie (W.G.Z.), and the National Institutes of Health (Grant GM-32394 to E.P.D.). We are very grateful to Professor Richard Frankel for his help and encouragement at the beginning of this study and to Dr. Chiou-Pirng Wang for her help in collecting and analyzing the data.

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(19) We independently determined the amount of EPR-detectable copper in the first sample of N_2O reductase and obtained a result that is fully consistent with the measurements reported here. The results reported in ref 11 suggest that 20-30% of the copper in resting *Pseudomonas stutzeri* N_2O reductase is EPR detectable; results reported in ref 22 are also consistent with an $S = {}^1/_2$ content of 20-25% for other N_2O reductases.

(20) For example, a similar fit to the raw data *before* subtracting the matched control gave a reasonable fit when viewed as shown in Figure 1 (with an 18% increase in the amount of "$S = {}^1/_2$" species), but when viewed as in Figure 2, decreased systematically with increasing temperature above 20 K, ending 100% below the theoretical line at 200 K. This dramatic and systematic deviation was caused by the presence of $S = 1$ oxygen that had not been subtracted from the data.

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Aerobic Conversion of Organic Halides to Alcohols. An Oxygenative Radical Cyclization

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Received July 1, 1991

Reductive cleavage of a carbon-halogen bond by a tin hydride reagent generates a carbon radical, and the subsequent synthetic sequence generally ends with the formation of a carbon-hydrogen bond.[1,2] We report a unique tin hydride mediated reaction that aerobically converts a carbon-halogen bond to a synthetically valuable carbon-oxygen bond. A striking *synergetic action of molecular oxygen and a tin hydride* at low temperatures (0-20 °C) effects an efficient conversion of an organic halide to the corresponding alcohol under neutral conditions through oxygenation of an intermediate radical[3] (Scheme I). The reaction tolerates a wide range of functional groups, thus complementing the classical conditions employed for this standard, yet sometimes nontrivial transformation.[4] The radical nature of the reaction

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