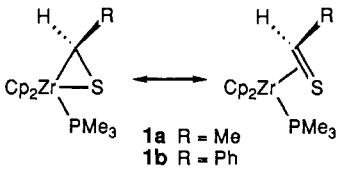


Figure 1.

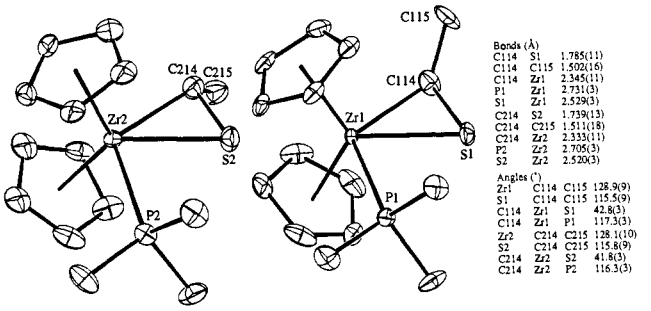
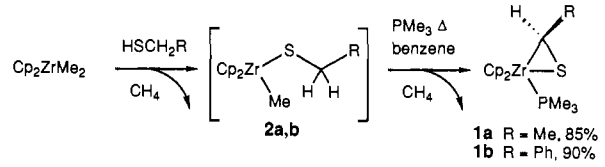
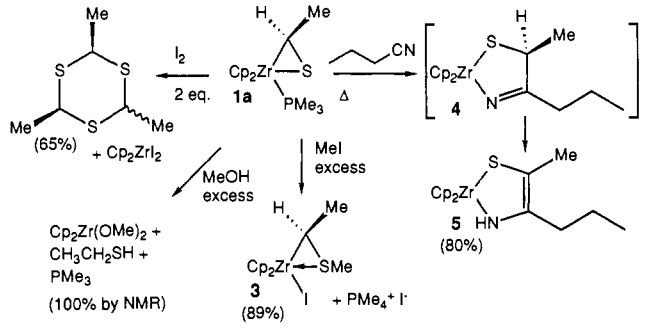


Figure 2. Molecular structure of **1a** with selected bond distances and angles.

Scheme I



Scheme II



zirconium(IV) metallathiirane, there is some contribution of the zirconium(II) π-thioaldehyde form.

Compounds **1a,b** are stable in benzene solution at 100 °C for an extended period of time, but they react with a variety of organic and inorganic compounds (Scheme II). For example, **1a** is oxidized by iodine to yield thioacetaldehyde trimer (2,4,6-trimethyl-1,3,5-trithiane) as a mixture of α and β isomers.[6] Protonation by methanol yields ethanethiol and trimethylphosphine (quantitatively by ^{1}H NMR), along with dimethoxyzirconocene.[7] Treatment of **1a** with excess methyl iodide yields the α-zirconocenyl thioether **3**. **3** appears to be η^2,[8] as evidenced by the downfield chemical shift of the thiomethyl protons. In addition, the failure of the zirconium to migrate to the neighboring primary carbon is in contrast to the behavior normally manifested by alkyl(halo)zirconocenes.[9] Butyronitrile reacts with **1a** to yield imine metallacycle **4** (observed by ^{1}H NMR), which tautomerizes under the reaction conditions to cleanly give enamine metallacycle **5**.[10]

We are currently investigating the reactivity of zirconocene thioaldehyde complexes toward other substrates, the use of ligands other than trimethylphosphine, and the application of our methodology for the synthesis of thioaldehyde complexes of other early transition metal systems.

Acknowledgment. We are grateful for support from the donors of the Petroleum Research Fund, administered by the American Chemical Society, Dr. Alfred Bader, and Firmenich, S.A. S.L.B. is the recipient of a Distinguished New Faculty Grant from the Camille & Henry Dreyfus Foundation, Inc. R.B.N. is the recipient of a National Science Foundation Predoctoral Fellowship, which is gratefully acknowledged. We also thank the Biomedical Research Support Shared Instrumentation Grant Program, Division of Research Resources, for funds to purchase the X-ray diffraction equipment (NIH Grant S10 RR02243).

Supplementary Material Available: Experimental section containing the preparation and spectroscopic characterization of compounds, along with crystallographic data and procedures, ORTEP diagrams of **1a**, tables of bond distances and angles, and table of final positional and thermal parameters (6 pages); table of structure factors (14 pages). Ordering information is given on any current masthead page.

Site-Selective Observation of Nuclear Overhauser Effects in Proteins via Isotopic Labeling

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Received September 2, 1986

The ^{1}H–^{1}H nuclear Overhauser effect (NOE) plays a central role in NMR studies of the conformation and dynamics of small proteins. Griffey, Redfield, and co-workers have described several powerful, one-dimensional techniques for observing specific NOEs in biological macromolecules which have been isotopically labeled at selected sites.[1,2] These experiments employ conventional NOE difference methods which are modified to make use of heteronuclear decoupling to edit the resulting one-dimensional spectra. In this paper we present a very simple, alternative method for obtaining isotopic label-edited NOE difference spectra that has several practical advantages over the previously proposed techniques.

The basic pulse sequence is shown in Figure 1a. The principle of the experiment is to invert selectively the Zeeman order of the protons directly bonded to a heteronuclear label (referred to as the labeled protons) and subsequently to allow cross-relaxation with the unlabeled protons to occur during a mixing period τ_m. The selective population inversion is achieved through the use of the bilinear rotation sequence (BIRD).[3-5] This experiment is fundamentally equivalent to the well-known transient NOE ex-

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(10) All new compounds were characterized by ^{1}H NMR, ^{13}C NMR, IR, and high-resolution mass spectrometry. Details are available in the supplementary material.

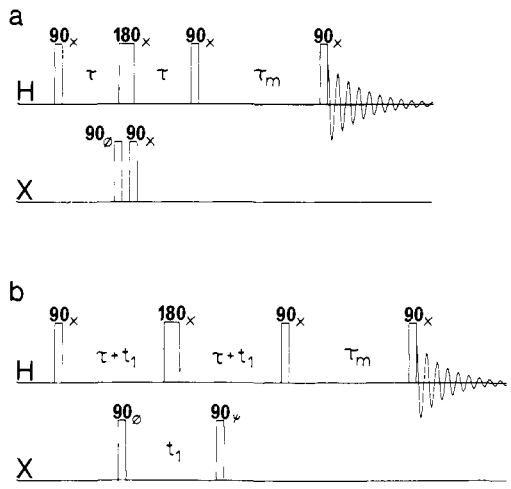

Figure 1. Pulse schemes of the site-selective NOE experiment. (a) For 1D spectra: the phase $\phi = \pm X$ with alternate addition and subtraction of the resulting FIDs to select only that part of the signal originating from labeled protons. τ is tuned to $1/(2J_{XH})$, and τ_m is the NOE mixing period. (b) For 2D X–H correlated spectra: the phase ψ is cycled in steps of 90° in successive experiments to achieve quadrature detection in ω_1.[11]

periment,[6,7] except that the frequency-selective 180° pulse employed in the latter technique is replaced with the label-selective BIRD sequence in the present experiment. A reference spectrum is obtained by omitting the heteronuclear 180° pulse, which decouples the labeled protons from the heteronuclei so that all proton populations are at thermal equilibrium at the start of the mixing period (ignoring homonuclear coupling effects and relaxation during 2τ). The difference between the spectra acquired with and without the heteronuclear pulse yields a spectrum in which NOE peaks are observed only for those protons which cross-relax with the labeled protons (in the absence of spin diffusion).

The advantages of the proposed technique are as follows: (i) it is more easily implemented than the heteronuclear decoupling techniques,[1,2] since no heteronuclear decoupling hardware is required and the exact resonance frequencies of the labeled protons or the heteronucleus need not be predetermined; (ii) it is not necessary to preirradiate the labeled proton resonance; and (iii) the experiment can be easily implemented in a two-dimensional mode as shown in Figure 1b. The first 90°_ϕ(X) pulse converts proton single quantum coherence to heteronuclear two-spin coherence which is subsequently converted back to proton single quantum coherence after an evolution period t_1.[8-10] After a refocusing period, the second 90°_x(H) pulse creates a nonequilibrium Zeeman order for the labeled protons. The first heteronuclear pulse is phase-cycled to eliminate contributions from unlabeled protons. Two-dimensional Fourier transformation yields heteronuclear shift information in the ω_1 dimension and proton shifts in ω_2. Thus, a slice parallel to the ω_2 axis of the 2D matrix at the chemical shift of the heteronuclear label will contain resonances at the frequencies of the directly bonded protons and the corresponding NOE peaks. If multiple heteronuclear labels have been employed, the 2D experiment allows proton NOE subspectra to be obtained for each of the labeled sites.

We demonstrate the application of the isotope-edited NOE experiment to [1-^{13}C]ethyl isocyanide labeled leghemoglobin (MW ~16 000) in Figure 2. A 1D isotope-edited NOE spectrum is shown in Figure 2b. The largest peaks arise from the diastereotopic methylene protons directly bonded to the ^{13}C label and

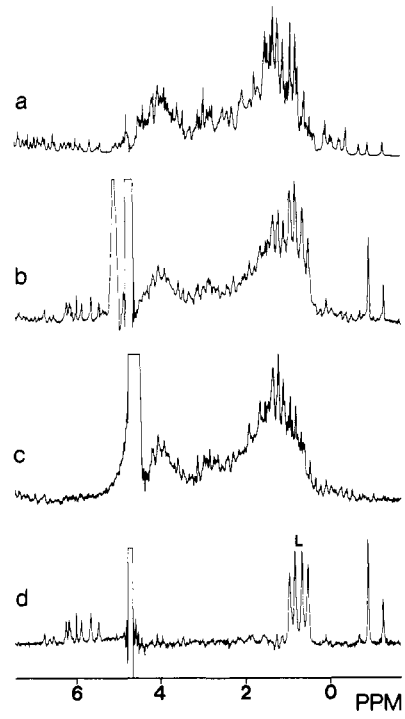

Figure 2. (a) Part of the 500-MHz ^{1}H NMR spectrum of [1-^{13}C]ethyl isocyanide labeled leghemoglobin. (b) 1D site-selective NOE spectrum of the sample of a, obtained by using the sequence shown in Figure 1a with 4096 scans, τ = 3.55 ms, and τ_m = 150 ms. (c) Spectrum of leghemoglobin with unlabeled ethyl isocyanide ligand, obtained under the same conditions as b, showing the background signals arising from protons attached to the natural abundance ^{13}C. (d) Slice taken from a 2D site-selective NOE spectrum of the labeled leghemoglobin at the ω_1 frequency of the ^{13}C label of the ethyl isocyanide ligand. The spectrum was obtained with the sequence of Figure 1b; 448 scans were recorded for each of 128 t_1 values. The ω_1 spectral width was 2000 Hz. Samples were prepared by addition of [1-^{13}C]ethyl isocyanide (synthesized by literature methods[12]) to soybean deoxyleghemoglobin in phosphate buffer (3.5 mM, 6.9 pH) in ^2H_2O under argon in a 5-mm NMR tube. The NMR experiments were performed at 30 °C on a modified Bruker AM-500 spectrometer. Details of the modifications will be supplied on request. Assignments were obtained from COSY and NOESY spectra.

appear as doublets due to the heteronuclear coupling, J_{CH} = 140 Hz. Large NOEs are observed to the methyl resonance of the ethyl isocyanide ligand at −0.88 ppm and one of the $C^\delta H_3$ groups of the distal Leu 65 (E11) at −1.24 ppm. Other NOEs to aromatic resonances between 5 and 7 ppm are observed, including the C2H and C4H (6.17/6.02 ppm) of the distal histidine, His 61 (E7).

Unambiguous identification of the NOEs involving the labeled protons is difficult due to an intense background of peaks arising from protons attached to natural abundance ^{13}C nuclei. The background spectrum can be measured directly by repeating the experiment with a similar sample in which the ethyl isocyanide is unlabeled (Figure 2c). In cases where the background signal poses a problem, the 2D version of the experiment becomes most useful: since the natural abundance ^{13}C signals are spread over a range of chemical shifts, the undesired signals are dispersed in the ω_1 domain such that their contribution to any particular subspectrum in most cases will not be observable. The dramatic improvement in the site-selective NOE spectrum is demonstrated in Figure 2d.

In the complex proton spectra of biopolymers, where direct selective excitation is not possible, the presence of a heteronucleus provides a means of selecting specific proton resonances through coherent and incoherent magnetization transfer processes. The label-edited NOE experiment effectively uses the heteronucleus as a site-specific label from which information about the local structure of the molecule can be obtained.

Acknowledgment. We thank Linda Tennant, Claudio Dalvit, and Walter Chazin for assistance and discussions, Dennis Hare

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for software, and Bruker GmbH for their cooperation in the modification of our spectrometer. This work was supported in part by the NSF (Grant DMB-8517959) and by the NIH (Grant AM 34909). B.A.M. acknowledges receipt of a Herbert Johnson travel grant and a Commonwealth Postgraduate Research Award.

Coenzyme B_{12} Co–C Bond Homolysis: Insights from Qualitative Molecular Orbital Theory

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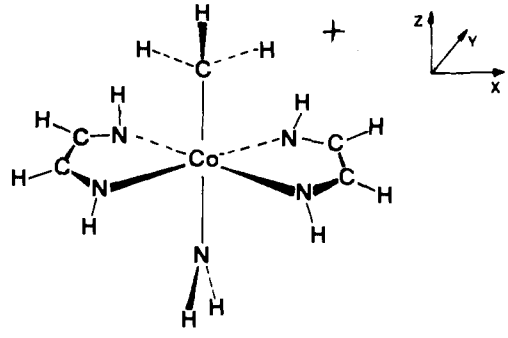
From extensive investigations into the factors which influence Co–C bond cleavage in coenzyme B_{12},[1] a few features are now well established: (i) the enzymatic cleavage is homolytic; (ii) the measured dissociation energy is ca. 25-30 kcal/mol;[1b,d] (iii) this energy increases with increasing basicity of the trans ligand in relevant organocobalt compounds.[1b] Many structures of such compounds with different substituents at both the axial carbon and nitrogen ligating atoms have shown that in some cases the Co–C and Co–N bonds can elongate by >0.1 Å from unstrained values (Co–C ≈ 2.00, Co–N ≈ 2.10 Å).[2] This elongation is evidence for the dependence of bond length on steric effects and on the trans influence. Approximate ab initio studies of geometrical deformations introduced in a model system did not reveal the existence of any major electronic effect.[3]

Herein we outline some insights, based on qualitative MO and perturbation theories,[4] into the Co–C dissociation process. The shortcomings of EHMO[5] in providing correct bond distances do not obscure the essential effects which our study illustrates. The model employed, **1**, contains simplified ligands, especially for the corrin.



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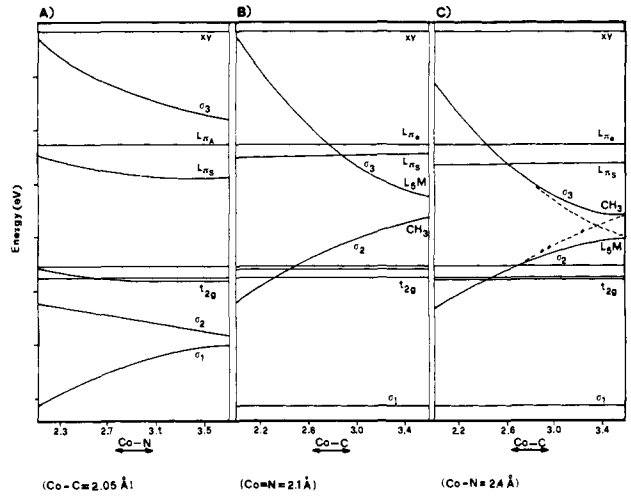
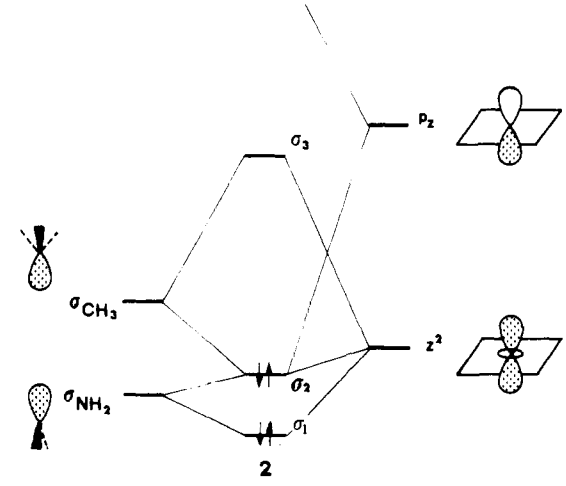


Figure 1. Evolution of MOs for the model $[(NH_2)Co(HN{=}CH{-}CH{=}NH)_2Co(CH_3)]^+$: (A) the Co—N (amido group) bond is elongated with Co—C fixed at 2.05 Å; (B) the Co—C bond is elongated with Co—N fixed at 2.1 Å; (C) the Co—C bond is elongated with Co—N fixed at 2.4 Å.

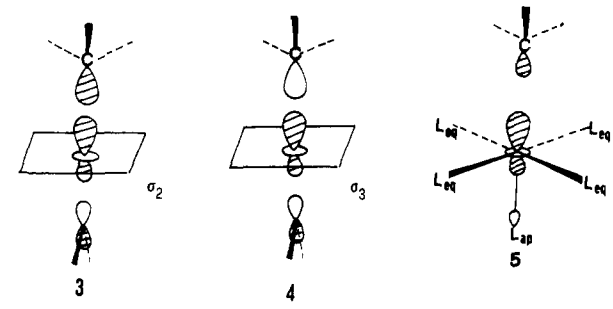
However, our interest focuses on the axial interactions along N–Co–C as illustrated in **2**.[7] The metal atom in the square plane



2

contributes with two fundamental levels: i.e., almost pure z^2 and p_z orbitals. The groups NH_2 and CH_3 each utilize a σ hybrid. For electronegativity reasons, $\sigma(NH_2)$ lies below $\sigma(CH_3)$, which is closer to z^2.

Of the four MOs, the two lower ones (σ_1 and σ_2) are populated. An oversimplified view of the nature of σ_1 and σ_2 is to equate them with the bonding combinations z^2–$\sigma(NH_2)$ and p_z–$\sigma(CH_3)$, respectively. Actually, second-order perturbations complicate the composition of σ_2, **3**. Both the metal z^2 and $\sigma(NH_2)$ levels are



3 **4** **5**

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(6) In B_{12} the coordination environment of the metal is not perfectly octahedral and the four equatorial ligands are not all equivalent. The NH_2 group simulates the two-electron σ-donor function of benzimidazole as well as some of its π-donor character.

(7) As found by others,[8] we observe that the important axial MOs are perturbed by p combinations of the conjugated equatorial ligands (the corrinoid ring in coenzyme B_{12}). However, the perturbation does not alter other important features and may be disregarded as a first approximation.

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