Investigations of B_{12} Derivatives with Inorganic Ligands Using 2D NMR Spectroscopy. Ligand-Responsive Shifts Suggest That the Deoxyadenosyl Moiety in Coenzyme B_{12} Has a Steric Trans Influence

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Abstract: The ^1H and ^{13}C NMR spectra of aquocobalamin (B_{12a}, H_2OCbl) and azidocobalamin (N_3Cbl) have been assigned unambiguously by modern homonuclear and heteronuclear 2D NMR techniques. In addition, ^1H-detected multiple-bond heteronuclear multiple quantum coherence spectroscopy (HMBC) has been used to assign the ^1H spectra and to evaluate past ^{13}C spectral assignments of hydroxocobalamin (B_{12b}, OHCbl), as well as to evaluate the ^1H and ^{13}C assignments of vitamin B_{12} (cyanocobalamin, CNCbl). Ligand-responsive trends in ^1H, ^{13}C, and ^{31}P NMR spectra for a series of cobalamins (Cbls) with R or X axial ligands were compared with analogous trends available for B_{12} model compounds, such as the recently reported lariat-type (C_1py) organocobalt derivatives. Moreover, the ligand-responsive spectroscopic results for Cbls were compared to electronic and steric parameters for the axial ligand R (or X). In general, the pattern of ^{13}C shifts of most of the 5,6-dimethylbenzimidazole (DMBz) and ribose carbons and some of the corrin ring carbons can be understood if the ^{13}C NMR shifts are primarily influenced by inductive electronic effects. ^{31}P shifts have been reported to reflect changes in phosphodiester conformation as the axial ligand R (or X) changes. Even though the ^{31}P, ^{13}C, and ^1H NMR shift changes probably are influenced by different factors (e.g., inductive vs conformational effects), good correlations are observed between the ^{31}P shifts and many of the Cbl ^{13}C shifts, suggesting that these signals respond to the trans influence of the axial ligand. The chemical shifts of equatorial C's, DMBz C's, and ^{31}P of coenzyme B_{12} (5′-deoxyadenosylcobalamin, AdoCbl) are downfield, similar, and upfield, respectively, to shifts of methylcobalamin (CH_3Cbl, the other biologically important B_{12} coenzyme). The most reasonable explanation of this shift pattern rests on a trans steric influence of the Ado moiety (which lengthens the Co–N(DMBz) bond) and on a smaller electron-donating ability of Ado compared to CH_3. The weaker electron-donating Ado moiety explains the relative downfield shifts of the corrin C's. Likewise, the longer bond between DMBz and the Co decreases electron donation from DMBz to Co to the level found in CH_3Cbl, explaining the similar shifts of DMBz C's for both coenzymes. The longer Co–DMBz bond in AdoCbl compared to that in CH_3Cbl explains the upfield shift of ^{31}P in AdoCbl. Thus, the shift patterns provide strong evidence for a steric trans influence of Ado in coenzyme B_{12}. The net lower electron density at Co in AdoCbl vs CH_3Cbl indicated by electrochemical studies is consistent with the steric trans influence. Steric lengthening of both the Co–C and Co–N axial bonds will together facilitate Co–C bond homolysis, a key step in holoenzyme function.

Introduction

An enzyme-induced distortion of 5′-deoxyadenosylcobalamin (coenzyme B_{12}, AdoCbl) is believed to lead to homolysis of the Co–C bond, the essential first reaction step in the catalytic cycle of B_{12}-dependent enzymes.[1-6] There may be an interplay between the trans and steric influences of the axial Ado and 5,6-dimethylbenzimidazole (DMBz) ligands.[2-7]

To assess such effects, one must first understand the relative donor ability of the Ado ligand vs other axial ligands. While electrochemical measurements suggest that Ado is a poor donor, ^{31}P NMR shifts of the phosphodiester group of Cbls place Ado among the better electron donors. The solid-state structure of AdoCbl reveals one of the longest Co–N bonds known.[5,8] However, in the solid state, relevant structural information on Cbls useful for comparison is sparse, lattice effects influence the conformation of the flexible corrin ring, and the large amount of solvent of crystallization limits structural precision. Therefore, methods of assessment of solution structure and properties are necessary.[9,10]

Investigations of simple structurally well-defined B_{12} model compounds have identified important relationships between structure, reactivity, and spectroscopic patterns.[5,6,11-18] In particular, the axial Co–N bond length from X-ray structures

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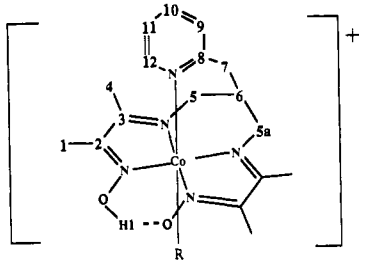
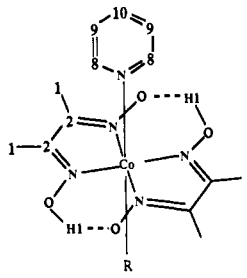
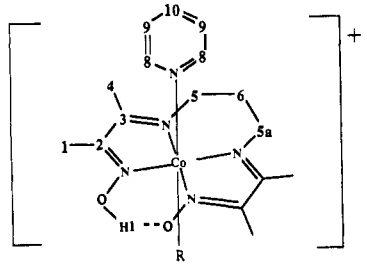
Scheme I. Structures of C_1py, (DO)(DOH)pn, and $(DH)_2$ (Cobaloximes) Derivatives with the Atom-Numbering Scheme



C_1py



$(DH)_2$



(DO)(DOH)pn

correlates very well with some ^{13}C shifts of the axial N-heterocyclic ligand.[6] Inorganic complexes (i.e., lacking a Co–C bond) have played an important role in elucidating the chemistry of the simpler organocobalt compounds.[6,16,17] The inorganic ligands have weak trans influences, and the lengths of the trans axial Co–N bonds are shorter than in related organometallic complexes. Similar comparative structural and spectroscopic information on Cbls lacking Co–C bonds will likewise facilitate assessment of the properties of the Co center in Cbls. Understanding such properties will eventually lead to a better understanding of Co–C bond cleavage in coenzyme B_{12} and the role that enzymes play in promoting the Co–C bond cleavage. However, relatively little information is available on B_{12} derivatives with traditional inorganic ligands. Recent EXAFS studies on aquocobalamin (B_{12a} or H_2OCbl) and vitamin B_{12} (CNCbl) suggest unexpectedly long Co–N(DMBz) bonds.[7] The long bonds in solution were attributed to steric effects between the DMBz and the corrin ring. Past X-ray studies of such inorganic B_{12} compounds have shown short bonds, including three different crystalline forms of CNCbl.[5,19] Recently, the azidocobalamin (N_3Cbl) structure has been under study.[20] Therefore, an NMR investigation of Cbls with inorganic ligands is particularly timely.

The most extensively studied model systems (Scheme I) include the cobaloximes $[R(or\ X)Co(DH)_2L]$[6,21,22] and Costa type derivatives $\{[R(or\ X)Co((DO)(DOH)pn)L]^+\}$[11-15] (L = neutral base, two-electron donor; R (or X) = alkyl or mononegative electron donor; DH = monoanion of dimethylglyoxime; (DO)(DOH)pn = $N^2,N^{2'}$-propanediylbis(2,3-butanedione 2-imine 3-oxime). Recently we have reported studies of a new model, the lariat-type (C_1py) compounds[16,17] $\{[R(or\ X)Co(C_1py)]^+$; C_1py = 2,3,9,10-tetramethyl-6-[(2-pyridyl)methyl]-1,4,8,11-tetraazaundeca-1,3,8,10-tetraene-1,11-diolato(1-)$\}$.

The E_{pc} ($E_{1/2}$) ($E_{1/2} = (E_{pc}+E_{pa})/2$; $E_{1/2}$, experimental approximation of the standard potential of a redox couple; E_{pa} and E_{pc}, anodic and cathodic peak potential, respectively) values for the Co^{III}/Co^{II} redox couple of C_1py derivatives correlate well with those of analogous B_{12} compounds.[16] Moreover, we recently reported that E_{pc} values reflect, at least in large part, the electronic properties of the Co center in C_1py derivatives.[17] These data suggest that there is a relationship between the electrochemical behavior and electron density of the Co center in Cbls. However, electrochemical studies of alkyl-Cbls (RCbls) (R = CH_3, CH_2CH_3, $CH_2CH_2CH_3$, $CH_2CH(CH_3)_2$, and $CH_2C(CH_3)_3$) suggested that the bulkiness of R may have a significant influence on $E_{1/2}$ values.[23] $E_{1/2}$ for the couple $RCo + e^- = RCo^{\bullet-}$ decreases, starting with CH_3Cbl (an important coenzyme) and continuing through the alkylcobalamin series to $(CH_3)_3CCH_2$Cbl. This decrease may reflect changes in the Co–R distance which modulate the energy of the lowest σ type MO (LUMO) of these complexes.[23] The energy of the σ LUMO (orbital in which the electron added to RCo must enter) increases when the Co–R distance decreases.[23] Thus, since CH_3Cbl should have the shortest Co–R bond, by this reasoning it should be the most difficult to reduce.[23] Although methyl is the smallest alkyl group, it is usually less electron donating than ethyl, *n*-propyl, and neopentyl.[21] NMR spectroscopy has the advantage that effects throughout the molecule can be explored (e.g., effects in the axial ligands can be compared to those in the equatorial ligand). However, the redox properties give a net assessment of the electronic properties of the species under study. In the C_1py system, our analysis of the NMR spectral changes[17] led us to offer a new interpretation. We concluded that whereas the bulkier alkyls were somewhat better donors than methyl, the trans pyridyl group was a relatively much weaker donor. The *net* result was weaker *total* donation by the two axial groups.

Therefore, it is interesting to determine if ligand-responsive spectroscopic patterns would reveal whether the relative electron donation by alkyl groups in Cbls was the same as in C_1py models. If so, our new explanation could be confirmed for Cbls and the relationships we found between $E_{1/2}$ (E_{pc}) for Cbls and C_1py compounds could be understood.[16]

Due to the complexity of Cbls, 2D NMR methods are required to assess the electronic and solution structural properties by NMR spectroscopy. We report the complete assignment by modern 2D NMR techniques of the ^1H and ^{13}C spectra of three Cbls with relatively innocent, poor-electron-donor, nonalkyl ligands, H_2OCbl, hydroxocobalamin (OHCbl), and N_3Cbl, and of the ^1H NMR spectra of CNCbl. It should be noted that CNCbl is vitamin B_{12} and that aquocobalamin may be the precursor of coenzyme B_{12}.[24] The ^1H and ^{13}C spectra of these two important species have previously been assigned by analogy[25-27] and by homonuclear 2D methods,[28] but not by heteronuclear 2D NMR methods. The recent ^1H NMR assignment of CNCbl by

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homonuclear 2D NMR methods[28] disagrees in several assignments with the more accurate assignments reported here.

Experimental Section

Reagents. NaN_3 from Fisher, CNCbl from Sigma, and H_2OCbl ("hydroxocobalamin acetate") from Roussel were used without further purification.

Solutions of N_3Cbl prepared from NaN_3 (15 μL, 0.2 M) and H_2OCbl (4 mg), CNCbl (2.8 mg), OHCbl (11.8 mg, pD = 8.9, adjusted with NaOD), and H_2OCbl (11.9 mg) were lyophilized and dissolved in 0.5 mL of 99.96% D_2O (MSD Isotopes). After lyophilization, all the solutions, except for OHCbl, had pD ~7. No pD adjustment was carried out.

The 1D NMR spectral measurements were made with Nicolet NB-360 (360 MHz, ^1H), GE QE-300 (75.48 MHz, ^{13}C), and IBM WP200-SY (81.01 MHz, ^{31}P) spectrometers. ^1H and ^{13}C chemical shifts were referenced internally to TSP (sodium 3-(trimethylsilyl)tetradeuteriopropionate), and ^{31}P chemical shifts were referenced externally to TMP (trimethyl phosphate). The 2D NMR experiments were performed on GE GN-500 and Omega GN-600 spectrometers at room temperature (20 °C, N_3Cbl), 25 °C (OHCbl), and 30 °C (H_2OCbl, CNCbl) without sample spinning. The 2D spectra were processed by using the FTNMR and Felix programs (Hare Research Inc.). The NMR tubes were wrapped to minimize exposure to light.

HOHAHA Spectroscopy.[29] The HOHAHA spectrum of H_2OCbl resulted from a 512 × 1024 data matrix size with 32 scans per t_1 value. Predelay was 1.0 s. An MLEV-17 mixing sequence of 67.3 ms preceded and followed by 2.0-ms trim pulses was used. A 51-μs 90° ^1H pulse width (pw) was provided by 0.2 W (65 dB) of power. The HOHAHA spectrum of N_3Cbl resulted from a 1024 × 2048 data matrix size with 16 scans per t_1 value. Predelay was 100 μs. An MLEV-17 mixing sequence of 40 ms preceded and followed by 2.0-ms trim pulses was used. A 9.25-μs 90° ^1H pw was provided by 82 dB of power. A Gaussian function with a line broadening (lb) of −1 Hz and with a Gaussian coefficient of 0.1 and a cosine bell squared filter were used prior to Fourier transformation in the t_2 and t_1 dimensions, respectively.

Phase-Sensitive COSY Spectroscopy.[30] The phase-sensitive COSY spectrum of H_2OCbl resulted from a 512 × 1024 data matrix size with 16 scans per t_1 value. Predelay was 1.0 s. Presaturation of the residual HOD peak was used. A cosine bell squared filter and a Gaussian function with lb = −1 Hz and with a Gaussian coefficient of 0.1 were used prior to Fourier transformation in the t_2 and t_1 dimensions, respectively.

COSY Spectroscopy.[31] The COSY spectrum of N_3Cbl resulted from a 1024 × 2048 data matrix size with 48 scans per t_1 value, with presaturation of the residual HOD peak. A sine bell squared filter was used before Fourier transformation in the t_2 and t_1 dimensions.

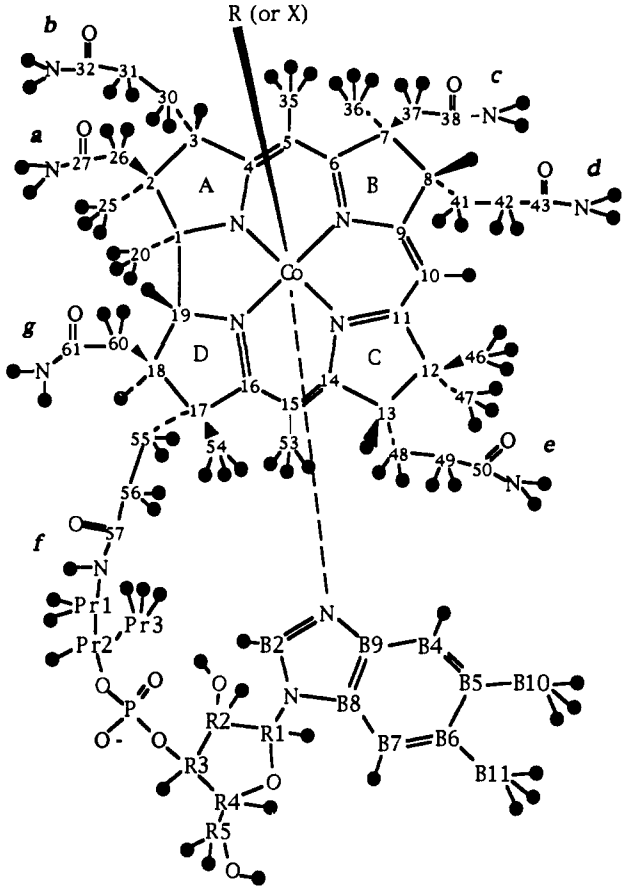
Phase-Sensitive NOE Spectroscopy.[32] The phase-sensitive NOE spectrum of N_3Cbl resulted from a 1024 × 2048 data matrix size with 64 scans per t_1 value. The mixing time was 500 ms. Presaturation of the residual HOD peak was used. A sine bell squared filter and a Gaussian function with lb = −1 Hz and with a Gaussian coefficient of 0.1 were used prior to Fourier transformation in the t_2 and t_1 dimensions, respectively.

Spin-Locked NOE Spectroscopy (ROESY).[33] The ROESY spectrum of H_2OCbl resulted from a 512 × 1024 data matrix size with 32 scans per t_1 value. Predelay was 2.1 s, and the mixing time was 200 ms. A 4.8-kHz rf field strength (52-μs 90° pulse width) was used. A sine bell squared filter and a Gaussian function with lb = −1 Hz and with a Gaussian coefficient of 0.1 were used prior to Fourier transformation in the t_2 and t_1 dimensions, respectively.

^1H-Detected Heteronuclear Multiple Quantum Coherence Spectroscopy (HMQC).[34] The one-bond ^1H–^{13}C shift correlation spectra of H_2OCbl and N_3Cbl resulted from a 512 × 1024 data matrix size with 512 scans (preceded by four dummy scans) per t_1 value. Predelay was 1 s. A 38-μs 90° ^{13}C pulse width and 41 W (63 dB) of ^{13}C rf power were used. A sine bell squared filter was used prior to Fourier transformation in both the t_2 and t_1 dimensions.

^1H-Detected Multiple-Bond Heteronuclear Multiple Quantum Coherence Spectroscopy (HMBC).[35] The multiple-bond ^1H–^{13}C shift correlation spectra resulted from a 1024 × 2048 data matrix size with 464, 288, 320, and 104 scans (preceded by four dummy scans) per t_1 value (for H_2OCbl, N_3Cbl, CNCbl, and OHCbl, respectively). Predelay was 1.6 s, except for OHCbl (2.75 s). A 31-μs 90° ^{13}C pulse width and 66 W (71 dB) of power were used. Values of Δ_1 and Δ_2 were 3.3 and 53.3 ms, respectively. A sine bell squared filter was used prior to Fourier transformation in both t_1 and t_2 dimensions.

Scheme II. R (or X) = N_3 (N_3Cbl); H_2O (H_2OCbl); OH (OHCbl); CN (Vitamin B_{12})



Results

Assignment of the ^1H NMR Spectra. Most of the ^1H NMR signals of both H_2OCbl and N_3Cbl were assigned by using 2D homonuclear experiments (e.g., COSY, HOHAHA, and NOESY or ROESY) and strategies described previously.[26,36-40] The ^1H nuclei are designated by the carbon atom to which they are attached. For nonequivalent geminal signals, H′ and H″ refer to the downfield and upfield signals, respectively. The ^{13}C nuclei are designated in Scheme II with numbers, following B for carbons of the DMBz moiety, C for carbons of the corrin ring and side

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chains, and Pr for carbons of the propanolamine group. Assignments are reported in Tables I and II. Literature ^{1}H NMR shift values for selected resonances in RCbls (Table II) were used in some correlations.[41]

The HMBC, HOHAHA, COSY, and NOESY (ROESY) connectivities for H_2OCbl and N_3Cbl (supplementary material) were used for ^{1}H and ^{13}C assignments. The propanolamine signals were assigned from the COSY spectra, starting with Pr3H_3, the only methyl doublet. Similarly, the ribose signals were assigned starting with R1H, the only downfield doublet.

Some correlations between corrin protons and corrin and methylene protons were also observed in the COSY spectra. For instance, C19H shows a correlation with C18H, which shows connectivities to C60H′ for both H_2OCbl and N_3Cbl, as well as to C60H″ for N_3Cbl. C13H shows a connectivity with C48H_2 (N_3Cbl) and C48H″ (H_2OCbl). C3H is correlated to C30H″ of H_2OCbl and N_3Cbl, as well as to C30H′ of N_3Cbl. C8H shows correlations to C41H″ of H_2OCbl and N_3Cbl, and to C41H′ of N_3Cbl.

The connectivity networks for the corrin side chains, as well as other connectivities already observed in the COSY spectra, can be seen with HOHAHA spectroscopy. The relayed connectivities allow assignment of the protons to a specific propionamide side chain (i.e., b, d, e, and f) (supplementary material). C31H_2, C42H_2, and C49H_2 were assigned from correlations to C3H and C30H_2, C8H and C41H_2, and C13H and C48H_2, respectively. Since f is the only propionamide side chain attached by a nonprotonated carbon, four signals, which show correlations only to each other, were identified as C55H_2 and C56H_2 for both H_2OCbl and N_3Cbl.

Either a ROESY (H_2OCbl) (Figure 1 and supplementary material) or a NOESY (N_3Cbl) spectrum was used to establish through-space connectivity between the networks of coupled protons as well as to complete the assignments of most of the ^{1}H spectra. The most downfield signals must correspond to B7H, B4H, B2H, C10H, and R1H. For both Cbls, the most downfield signal, which shows NOEs to R1H and B11H_3, as well as a correlation to B11H_3 in the HOHAHA spectra, was assigned to B7H. A second signal around 6.45 ppm, which is correlated to B10H_3 in both NOESY (or ROESY) and HOHAHA spectra, corresponds to B4H. A signal showing NOEs to C8H and C47H_3 must be C10H. The remaining downfield singlet is assigned to B2H.

From C10H, most of the remaining corrin and methylene signals can be assigned as described previously.[26,36,37,39] The ^{1}H homonuclear experiments allowed us to identify the four signals of the C55 and C56 methylene protons. Ambiguities in the assignment of these signals can be resolved with HMBC spectra. For H_2OCbl, two ^{1}H signals show correlations to C18 in the HMBC spectrum. From C18 there are three bonds to C55 protons, but *four* to C56 protons. Therefore, these ^{1}H signals were assigned to the C55 protons. Furthermore, C56H′ correlates to C55 and, therefore, cannot be a C55 proton. For Cbls, the C55 signal can be assigned from the C54H_3 signal. For N_3Cbl, both C56 protons show two bond connectivities to C55.

Assignment of the ^{13}C Spectra. All the methyl and protonated corrin carbons of H_2OCbl and N_3Cbl were assigned from the HMQC spectra (supplementary material). Assignment of the methylene carbons was difficult using HMQC, since the signals overlap in the ^{1}H dimension and are sometimes in a crowded region in the ^{13}C dimension as well. HMBC spectra, which display two- and three-bond ^{1}H–^{13}C correlations, were used to confirm the HMQC-based assignments and to assign the nonprotonated carbons. HMBC was used to assign the ^{1}H signals and confirm previous ^{13}C assignments of CNCbl[26-28] (Figure 2) and OHCbl[25] (supplementary material).

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Discussion

Background. The axial-ligand-responsive chemical shift differences ($\Delta\delta_{LR}$) in Cbls resulting from a change in the axial ligand trans to DMBz could be influenced by several factors. The most important include (1) through-space anisotropic effects of both the cobalt ($\Delta\chi$) and the axial and equatorial ligands;[12,14,42,43] (2) through-bond inductive effects;[6] and (3) conformational strain effects, particularly in the loop which links the DMBz to the corrin.[17,41,44]

As the electron-donor ability of the R (or X) axial group decreases, the ligand-responsive changes in $\Delta\chi$ ($\Delta(\Delta\chi)_{LR}$) normally induce downfield shifts of the equatorial ligand signals and upfield shifts of the L axial ligand signals. From McConnell's equation ($\Delta\sigma = \Delta\chi \times (1-3\cos^2\theta)/3r^3$),[45] the anisotropic shielding effect ($\Delta\sigma$) is inversely proportional to r^3 (r = distance between Co and the observed nucleus) and independent of the observed nucleus. In cobaloximes, ^{1}H NMR shift changes are known to reflect this heavy-atom effect of cobalt.[6,43] The inductive effects on ^{1}H $\Delta\delta_{LR}$ are relatively small, in most cases. In contrast, inductive effects on ^{13}C $\Delta\delta_{LR}$ are usually large.[17] The observed ^{13}C $\Delta\delta_{LR}$ are relatively large compared to ^{1}H $\Delta\delta_{LR}$. Therefore, the contribution of $\Delta(\Delta\chi)_{LR}$ effects to ^{13}C $\Delta\delta_{LR}$ is not evident except for carbons close to the Co center.[6] It has been found that ^{13}C $\Delta\delta_{LR}$ reflects trends in electron donation by axial ligands to the cobalt. Indeed, previous studies with model systems such as cobaloximes {R(or X)Co(DH)$_2$L},[6] Costa type derivatives {[R(or X)Co((DO)(DOH)pn)L]$^+$},[11,12,15] and C_1py compounds {[R(or X)Co(C_1py)]$^+$}[17] indicated that the ^{13}C $\Delta\delta_{LR}$ of *both* axial and equatorial ligands moved downfield as the electron-donating ability of the axial ligand R (or X) diminished.

For cobaloximes, it was suggested that the anisotropy of the DH ligand was not an important contributor to the ^{1}H chemical shift trend, unless large distortions in the planar moiety occurred.[46] However, comparisons of C_1py and (DO)(DOH)pn compounds[17] have demonstrated that the (DO)(DOH)pn equatorial ligand has an appreciable anisotropic upfield shifting effect on the close-in nuclei such as the α-H of pyridine.[11,14] These equatorial ligand effects are mostly manifest via comparisons of different model systems, but axial-ligand-responsive modulation of equatorial ligand anisotropy has not been clearly identified. In Cbls, the magnitude of the equatorial ligand anisotropic effect has not been clearly assessed.

The clearest demonstrations of strain effects have involved the loop region. In C_1py compounds, the $\Delta\delta_{LR}$ for C6 (see Scheme I) can be explained neither by Co anisotropy nor by inductive effects.[17] Therefore, the $\Delta\delta_{LR}$ was attributed to strain in the link induced by changes in Co–N axial bond distance as the trans influence of the X or R axial ligand changed.[17] Likewise, the ^{31}P $\Delta\delta_{LR}$ in Cbls have been attributed to conformational changes in the nucleotide loop.[41,44]

^{13}C NMR Shift Dependence on the Axial Ligand. Corrin ^{13}C Signals. The corrin C signals can be divided into those from the inner periphery (1, 4–6, 9–11, 14–16, and 19), the outer periphery (2, 3, 7, 8, 12, 13, 17, and 18), and all other C's designated by C(number) in Scheme II. (All C's designated as Pr, R, and B are in the nucleotide loop.) We have compared the $\Delta\delta_{LR}$ of all these C's with each other, with shifts in models, with ^{31}P shifts, and with electronic (EP[21]) and Taft steric parameters (E_s[47]). EP for an R (or X) group, developed from ^{13}C NMR data for cobaloxime compounds, ranks the R (or X) group by relative electron-donating ability.[21] E_s, determined from the rate of

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Table I. NMR Chemical Shifts and Signal Assignments for Cyanocobalamin, Hydroxocobalamin, Aquocobalamin, and Azidocobalamin (^{1}H and ^{13}C) and for 5′-Deoxyadenosylcobalamin and Methylcobalamin (^{13}C)[a]

	^{13}C NMR[b]						^{1}H NMR[c]			
assignment	H_2OCbl	OHCbl	CNCbl	N_3Cbl[d]	AdoCbl[e]	MeCbl[f]	H_2OCbl	OHCbl	N_3Cbl	CNCbl
C35	18.2	18.3	18.2	18.2	18.2	18.2	2.60	2.57	2.54	2.54
C53	18.3	18.4	18.0	18.0	18.6	18.3	2.68	2.64	2.60	2.57
C54	19.5	19.6	18.8	19.8	19.5	19.5	1.37	1.40	1.44	1.39
C25	20.6	20.4	19.6	20.0	19.7	19.6	1.46	1.47	1.45	1.40
C36	24.0	23.1	21.9	21.9	21.7	21.9	1.91	1.89	1.89	1.87
Pr3	22.0	21.9	21.8	21.9	21.6	21.6	1.24	1.26	1.26	1.26
B11	22.1	22.1	22.1	22.0	22.1	22.1	2.20	2.22	2.23	2.26
B10	22.8	22.7	22.8	22.6	22.4	22.4	2.26	2.27	2.26	2.27
C20	22.7	22.9	22.2	22.5	23.4	22.5	0.50	0.45	0.47	0.45
C47	22.5	22.7	22.1	22.5	23.8	23.1	1.49	1.47	1.51	1.45
C41	29.3	29.1	28.8	28.8	28.7	28.8	2.09, 0.98	2.01	2.09, 1.00	2.02
C30	29.3	29.3	28.8	29.0	29.2	28.9	2.17, 2.07	1.89	2.01, 1.98	1.95
C48	30.9	30.7	30.8	30.9	30.2	30.6	2.03	2.09	2.01	1.98
C46	35.1	34.6	34.1	35.3	34.0	34.6	1.34	1.30	1.36	1.20
C55	35.5	35.5	35.3	35.5	34.4	34.7	2.73, 1.87	2.88, 2.51	2.59, 2.16	2.51, 1.82
C56	35.8	34.7	34.3	34.5	34.6	34.8	2.64, 2.12	2.17	2.70, 1.87	2.66, 2.12
C60	34.3	34.6	35.0	34.5	34.8	34.8	2.76, 2.72	2.78, 2.70	2.78, 2.73	2.75, 2.68
C43	34.3	35.5	34.6	34.5	34.7	34.8	1.81, 0.95	1.83, 1.01	1.86, 0.96	1.85, 1.02
C49	37.6	37.8	37.5	37.5	38.0	37.7	2.65	2.66	2.67	2.63
C31	37.8	38.0	37.8	37.7	38.2	38.1	2.63, 2.55	2.59, 2.54	2.59, 2.50	2.58, 2.50
C18	42.5	42.3	41.9	42.1	42.5	41.9	2.99	2.68	2.93	2.76
C37	48.4	47.4	45.9	46.0	45.3	45.8	2.72, 2.60	2.59, 2.53	2.56, 2.21	2.59, 2.20
C26	46.3	45.8	45.6	45.6	46.1	46.1	2.42	2.67, 2.33	2.41, 2.34	2.40
Pr1	48.3	48.2	48.3	47.6	47.8	47.8	3.61, 2.89	2.96	3.62, 2.96	3.61, 2.96
C2	50.6	49.9	50.1	49.9	49.3	49.1				
C12	51.1	50.4	50.9	50.7	49.3	49.4				
C7	53.8	53.3	54.2	54.0	52.9	52.8				
C13	56.7	56.5	56.5	56.5	55.7	56.0	3.54	3.40	3.44	3.33
C8	60.2	59.2	58.5	58.6	57.3	57.4	3.45	3.43	3.55	3.42
C3	60.0	59.6	59.2	59.2	58.4	58.3	4.24	3.99	4.16	4.17
C17	62.0	61.4	62.0	61.9	60.7	60.6				
R5	63.3	63.2	63.3	63.2	63.3	63.3	3.88, 3.71	3.93	3.93, 3.74	3.93, 3.75
R2	71.6	71.6	71.7	71.4	72.0	71.9	4.23	4.23	4.23	4.28
A12					75.4					
Pr2	75.7	75.7	75.8	75.7	75.8	75.8	4.27	3.84, 3.63	4.30	4.30
R3	75.8	75.8	75.9	75.7	76.0	75.9	4.68	4.72	4.71	4.73
A13					76.4					
C19	78.0	77.1	77.7	77.2	76.7	76.5	4.26	4.25	4.18	4.11
R4	85.1	84.9	84.9	84.9	84.4	84.3	4.00	4.05	4.05	4.04
C1	87.9	88.0	87.9	88.1	88.3	87.9				
A14					88.4					
R1	90.3	89.9	89.8	89.9	89.2	89.2	6.22	6.25	6.27	6.36
A11					90.8					
C10	97.7	96.1	97.7	97.3	97.5	96.7	6.26	6.07	6.19	6.09
C15	107.1	106.3	106.9	105.5	106.9	106.2				
C5	110.8	109.5	110.3	109.9	108.4	108.0				
B7	114.8	114.1	114.3	114.2	113.3	113.3	7.16	7.17	7.19	7.28
B4	118.4	119.6	119.3	119.3	121.3	121.1	6.44	6.50	6.45	6.51
A5					121.8					
B8	132.1	132.4	132.8	132.2	133.3	133.2				
B5	136.7	135.7	135.8	135.8	134.5	134.3				
B6	138.8	137.9	137.9	138.0	136.6	136.5				
B9	139.0	139.6	139.5	138.5	141.0	141.0				
A8					143.7					
B2	144.4	145.0	144.7	145.2	144.5	144.7	6.51	6.74	6.84	7.10
A4					151.7					
A2					155.9					
A6					158.7					
C6	167.1	166.8	168.1	166.9	166.5	165.9				
C14	168.8	168.0	168.8	167.6	167.0	166.4				
C9	177.5	175.8	176.4	176.3	173.0	172.5				
C11	181.8	179.8	179.7	180.4	177.5	176.1				
C38	178.5	178.2	177.9	177.6	177.9	177.9				
C57	177.5	177.8	177.5	178.0	178.1	178.0				
C16	184.1	181.2	181.7	182.1	178.7	177.7				
C4	184.3	181.9	182.8	182.5	178.7	178.1				
C61	178.5	178.8	178.5	178.8	179.0	179.0				
C27	178.8	179.2	178.6	178.6	179.3	179.2				
C43	179.8	180.1	180.0	179.9	180.3	180.4				
C32	180.6	180.8	180.7	180.6	181.1	181.0				
C50	180.9	181.1	181.0	180.9	181.1	181.2				

[a] Shifts (in ppm) relative to internal TSP. [b] All the ^{13}C shifts, except for N_3Cbl, are from ref 25 and referenced to TSP by adding 2.8 to the reported values (relative to TMS). We corrected the wrong assignments based on the 2D NMR data. [c] ^{1}H shifts were obtained from 2D data. [d] ^{13}C shifts were obtained from 300-MHz 1D data. [e] Assignment from ref 36. [f] No 2D data. Assigned by analogy to the other Cbls.

Table II. ^{13}C, ^{31}P, and ^{1}H NMR Chemical Shifts for Some Cobalamins and Cobalamin Model Compounds[a]

	L-Cbl												L-C_1py[b]			
L	R1	R2	B6	B7	B5	B4	B8	B9	B2	B4H	B2H	^{31}P[c]	C9	EP[d]	E_s[e]	$E_{1/2}$[f]
H_2O[g,h]	90.1	71.4	138.6	114.6	136.5	118.3	131.9	138.8	144.2	6.46	6.53	−3.66	130.0			−0.07
OH[i]	89.9	71.6	137.9	114.1	135.7	119.6	132.4	139.6	145.0	6.49	6.70	−3.80			−0.55	−0.90[j]
N_3	89.9	71.4	138.0	114.2	135.8	119.3	132.2	138.5	145.2	6.45	6.84	−3.76	129.6	−1.43		
CN[g,h]	89.7	71.5	137.7	114.1	135.7	119.1	132.6	139.3	144.5	6.50	7.09	−3.77	129.5	−1.58	−0.51	1.00[k]
CF_3[g]	89.6	71.5	137.1	113.6	134.9	120.3	132.7	140.0	144.6			−3.90[l]		−0.96[m]	−2.4	−1.27[n]
CHF_2[g]	89.3	71.7	136.8	113.4	134.6	120.8	133.0	140.5	144.7			−4.07[l]			−1.91	
CH_2CN[g,h]	89.5	71.6	137.0	113.6	134.7	120.5	132.8	140.2	144.8	6.36	7.04	−4.06		−0.75	−2.38	
CH_2CF_3[g,h]	89.3	71.7	136.8	113.4	134.4	120.8	132.9	140.4	144.4	6.33	7.00	−4.16	128.7	−0.55		
Ado[g,h]	89.1	71.8	136.4	113.2	134.1	121.1	133.1	140.8	144.4	6.24	6.94	−4.34				−1.35
CH_3[g,h]	89.1	71.8	136.4	113.2	134.2	121.0	133.1	140.8	144.6	6.30	7.00	−4.20	128.3	0.00	−1.24	−1.60
CH_2CH_3[h]										6.26	7.00	−4.36		0.12	−1.31	−1.54
$(CH_2)_3CN$[g]	89.1	71.8	136.5	113.2	134.2	121.1	133.1	140.8	144.4			−4.29[l]			−1.74	
$(CH_2)_2CH_3$[g,h]	89.0	72.0	136.2	113.2	134.0	121.3	133.3	141.4	144.4	6.28	7.00	−4.39		0.18	−1.60	−1.55
$CH_2CH(CH_3)_2$[h]										6.34	7.07	−4.29		0.20	−2.17	−1.48

[a] ^{1}H and ^{13}C shifts (in ppm) relative to TSP. [b] From ref 17. [c] Shifts (in ppm) relative to trimethyl phosphate (TMP).[41] [d] Electronic parameter.[21] [e] Taft steric parameter.[47] [f] In volts relative to SCE. Unless indicated, in 1:1 DMF:H_2O.[23] [g] ^{13}C shifts referenced to TSP by adding 0.16 ppm to the values of ref 44. [h] ^{31}P and ^{1}H shifts, from ref 41. [i] ^{13}C shifts from ref 25. [j] In H_2O (Lexa, D.; Savéant, J.-M. *Acc. Chem. Res.* **1983**, *16*, 235–243). [k] In 4:5 DMSO:1-propanol (Lexa, D.; Savéant, J.-M.; Zickler, J. *J. Am. Chem. Soc.* **1980**, *102*, 2654–2663). [l] From ref 53 by adding −0.75 ppm to the reported shifts. [m] Calculated as indicated in ref 21 from the ^{13}C NMR spectrum of [pyCo(DH)$_2$CF$_3$] (Toscano, P. J.; Konieczny, L.; Liu, S.; Zubieta, J. *Inorg. Chim. Acta* **1989**, *166*, 163–165). [n] In H_2O (Swetik, P. G.; Brown, D. G. *Electroanal. Chem.* **1974**, *51*, 433–439).

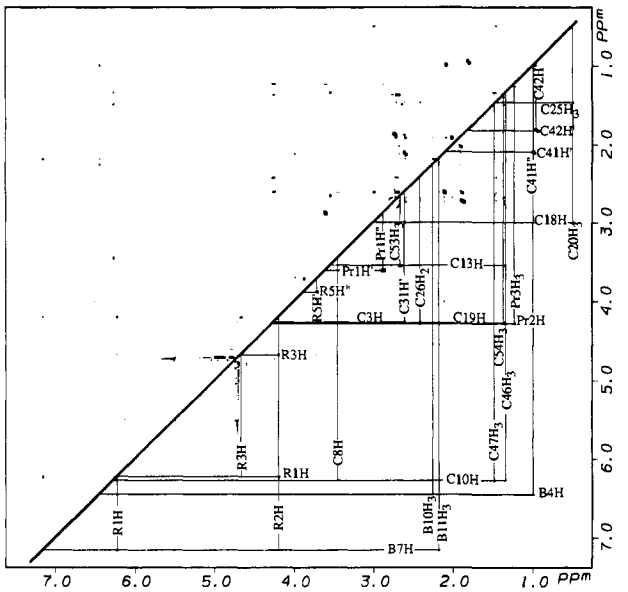

Figure 1. ROESY spectrum (after symmetrization) of H_2OCbl. Only positive contours (direct NOEs) are shown.

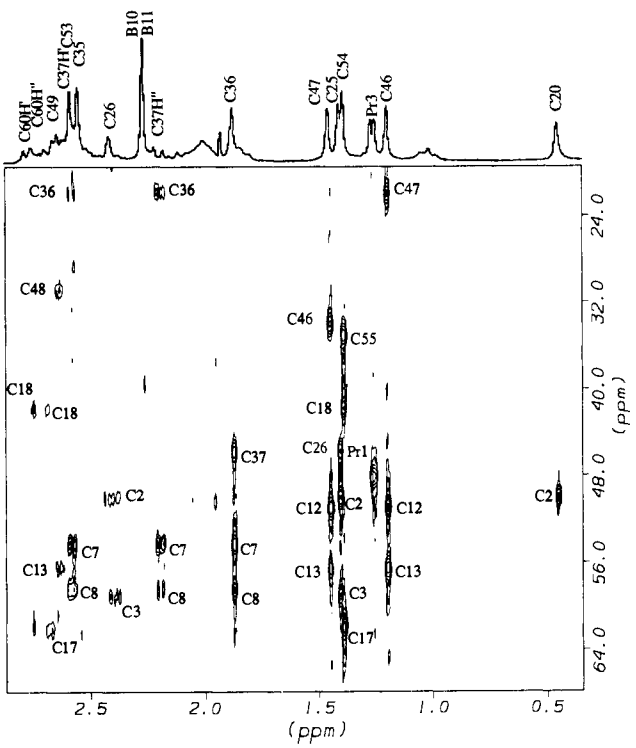

Figure 2. Expanded region of the HMBC spectrum of CNCbl.

hydrolysis of esters of the type R(or X)COOR′, is a near-quantitative measure of the total steric effects associated with R(or X).[47] We focus on the clear trends observed. There are several cases in which the $\Delta\delta_{LR}$ is small for a particular signal and therefore correlations are poor. However, we note when a particular derivative has an unusual shift.

Inner Peripheral C's. This group can be divided into methine C's (C5, C10, and C15), conjugated pyrrole α-C's (4, 6, 9, 11, 14, 16), and nonconjugated pyrrole α-C's (1, 19).

Methine C's. The ^{13}C NMR chemical shifts of the methine carbons have been correlated with the energy of the β-band (first vibrational fine structure of the first π–π^* electronic transition) at ~460–580 nm.[48] Furthermore, the ^{13}C shifts of the methine bridge carbons (C5, C10, and C15) have been related to the electronic environment of the corrin ring.[39,48] Downfield shifts of these resonances upon substitution of a good-electron-donor axial ligand by a poor one may be attributed[48] to an increased demand by the cobalt center for electron density from the corrin ring; this effect would decrease the charge density at these methine bridge carbons.

C5 shows significant downfield shift changes (up to 2.7 ppm) on going from RCbls to H_2OCbl (Table I). Correlations of C5 with C4 (Figure 3), C9, C11, C16, and C19 are good. Some unusual shifts were found. The shift of C10 in N_3Cbl and OHCbl is more upfield than in AdoCbl, even though azido and hydroxo are worse electron donors than Ado. Furthermore, the chemical shift of C10 or C15 is similar for H_2OCbl, CNCbl, and AdoCbl, whereas more upfield values are found in OHCbl and CH_3Cbl. Factors other than the electronic environment, e.g., corrin ring conformation, could be influencing the chemical shifts of C10 and C15.

Conjugated Pyrrole α-C's. The ^{13}C signals of most of these α-C's move downfield (Table I) when going from RCbls to H_2OCbl. Significant $\Delta\delta_{LR}$ were found for C4, C9, C11, C14, and C16. C11 and C16 correlate reasonably well both with each other (supplementary material) and with C3 and C4. A similar

(48) Hensens, O. D.; Hill, H. A. O.; McClelland, C. E.; Williams, R. J. P. In B_{12}; Dolphin, D., Ed.; Wiley: New York, 1982; Vol. 1, Chapter 13, pp 463–500.

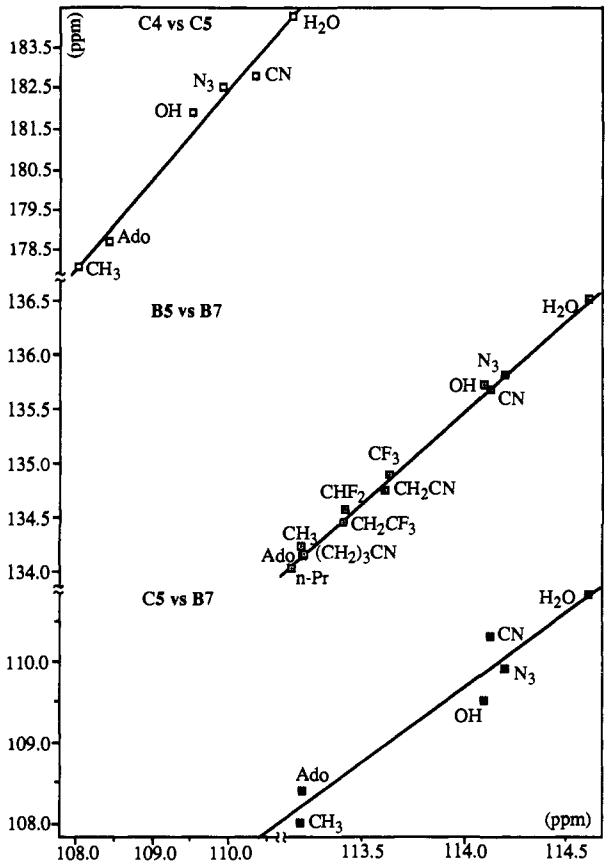

Figure 3. Plot of the ^{13}C NMR shifts of C4 vs C5 (for six points, s = 2.23, r = 0.983), of C5 vs B7 (for six points, s = 1.84, r = 0.938), and of B5 vs B7 (for 12 points, s = 1.68, r = 0.996) for R(or X)Cbl.

trend, but with a much smaller downfield shift, was observed for C6 (the C6 shift of CNCbl is anomalously downfield), and correlations are poor.

Nonconjugated Pyrrole α-C's. The shift of C1 in general is not sensitive to inductive or anisotropic effects. Shift changes of C1 may result from conformational changes of the corrin ring,[37,49] perhaps explaining the downfield shift of C1 in AdoCbl (88.3 ppm) compared to the other Cbls (~87.9 ppm). A fairly good correlation between C19 and conjugated C14 was observed. As for C1, these shifts are slightly downfield for AdoCbl compared to CH_3Cbl.

Outer Peripheral and Other C's. The signals of the outer periphery and other C's generally show small shift ranges. However, significant downfield shifts are observed for C8, C36, and C37 in H_2OCbl and OHCbl relative to other Cbls.

In summary, for the corrin C's, the signals with the most significant $\Delta\delta_{LR}$ trends are C4, C5, C9, C11, C14, and C16. Unusual shifts were found for C1, C6, C8, C36, and C37.

Comparisons to the C_1py Models. The non-methyl corrin C shifts were compared with those of the equatorial C's (C1-C6) of C_1py compounds (supplementary material). The C_1py C1, C2, C3, and C4 signals give good correlations (positive slope (s)) with C3, C11, and C16 and somewhat poorer correlations with C4, C5 and C8. Correlations of C5 and C6 of C_1py derivatives were also good, but with negative s.

Since C1 to C4 in the C_1py model reflect the average electron density on Co,[17] their correlations with C3, C4, C5, C8, C11, and C16 for Cbls suggest that the $\Delta\delta_{LR}$ for these Cbl carbons are sensitive to the electron richness of the Co center. Comparing the R = CH_3 and Ado Cbls, the $\Delta\delta_{LR}$ suggest that the total donation in CH_3Cbl is greater. In particular, the H_2OCbl signals for C4, C11, and C16 are very downfield compared to those of the two coenzymes; these signals for AdoCbl are an average of ~1 ppm downfield compared to CH_3Cbl.

The $\Delta\delta_{LR}$ for C6 (the saturated equatorial carbon in the middle of the propylene bridge) in C_1py compounds is influenced by strain in the linker arm caused by ligand-induced changes in the Co–N axial bond lengths.[17] This strain reflects the trans influence, and the correlations of C4, C5, C11, and C16 with C6 for C_1py complexes suggest that the $\Delta\delta_{LR}$ for all these carbons respond to the trans influence. In summary, the $\Delta\delta_{LR}$ for C3, C8, and C16 in Cbls are particularly sensitive to through-bond inductive effects.

Nucleotide Loop C's. The ligand-responsive trends in ^{13}C shifts for the C's of the nucleotide loop of base-on Cbls have been attributed to (a) inductive effects, related to the increasing electron-density donation from the DMBz to the Co as the electron-donor ability of R (or X) diminishes;[44] (b) magnetic anisotropy of the Co;[44] and (c) changes in the nucleotide loop conformation.[44] However, as shown by structural studies of AdoCbl,[8,50,51] CH_3Cbl,[41] and CNCbl,[19,51] the DMBz moiety does not undergo large positional changes. Thus, a and b are probably more important than c. For ^{31}P shifts, however, a and b are less important and c is relatively more important.

The DMBz ^{13}C $\Delta\delta_{LR}$, with the exception of B2, B10, and B11, show significant trends with decreasing electron-donor ability of the axial ligand. The ^{13}C resonances of B5, B6, and B7 move downfield, while B8, B9, and B4 move upfield. B2 changes erratically, as reported previously,[44] and B2 correlations are always poor. BN3 protonation of the DMBz in the α-ribazole nucleotide induces ^{13}C upfield shifts of B2, B4, B8, and B9 and downfield shifts for the ^{13}C resonances of B5, B6, B7, B10, B11, and R1.[44] This pattern for protonation is similar to that calculated for coordination of α-ribazole to Cbls,[44] suggesting that there is a significant through-bond inductive effect on the DMBz C's and R1 due to an increased electron donation from the DMBz to the Co as the electron-donor ability of R (or X) diminishes.

The ribose carbon $\Delta\delta_{LR}$ are not large except for R1;[52] the ^{13}C resonance shifts downfield as the electron-donor ability of the R (or X) group diminishes. Previously,[25,44] the R2 signal was misassigned as Pr2; the signal moves upfield with decreasing electron-donor axial ligands, suggesting a slight conformational change. However, the trend is somewhat erratic.

For the twelve Cbls in the B5 vs B7 plot in Figure 3, the correlations of B6, B7, B5, and R1 with each other are excellent. Good correlations, but with negative s, are found for B4 with B6, B7, B5, and R1. B8 and B9 correlate reasonably well with each other. The correlations of B8 and B9 with the other nucleotide loop C's are good and have negative s (except for B4).

Comparisons to Corrin C's, EP, and Models. Comparing the ^{13}C shifts of R1 and the DMBz C's with those of the corrin C's (supplementary material), we find for R (or X) = H_2O, OH, N_3,

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(51) Glusker, J. P. In *B_{12}*; Dolphin, D., Ed.; Wiley: New York, 1982; Vol. 1, Chapter 3, pp 23–106 and references cited therein.

(52) In previous studies, comparison of the ribose carbon chemical shifts (particularly R2 and R4) of some base-on Cbls with those of the detached nucleotide suggested that there was a noticeable difference in the ribose ring conformation in the two.[40,44] It is known that, in the solid state, the conformation of the ribose moiety of the nucleotide loop in AdoCbl is R3-endo, whereas in both "dry" and "wet" CNCbl it is R2-exo.[51] Solid-state ^{13}C NMR has been used to determine the DNA sugar ring pucker: Santos, R. A.; Tang, P.; Harbison, G. S. *Biochemistry* **1989**, *28*, 9372–9378. Significant chemical shift differences were observed among the C3′-endo, C3′-exo, and C2′-endo conformers. For R(or X)Cbl (R (or X) = H_2O, N_3, CN, CH_3, and Ado) no relevant differences are observed in the ribose ^{13}C chemical shifts throughout the series, suggesting that the ribose conformation in solution is practically the same in all cases. However, very large differences are not expected since the R2-exo and R3-endo conformers are very similar, with R3 displaced to the side of the ribose ring to which R5 lies, although R2 is more displaced than R3 for the R2-exo conformer. The R2 and R4 resonances in AdoCbl and CH_3Cbl are slightly different from those in H_2OCbl, OHCbl, CNCbl, and N_3Cbl. Although these differences are very small, they could indicate that in solution the ribose ring conformation of CH_3Cbl is closer to that in AdoCbl than those in the inorganic Cbls.

CN, CH_3, and Ado (a) very good correlations with positive s between B5, B6, B7, and R1 and C3, C4, C5, C11, and C16 (C5 vs B7 in Figure 3); (b) good correlations with negative s for B4 with C3, C4, C5, C11, and C16; (c) good to fairly good correlations with negative s for C9 with B8 and B9; and (d) poor correlations for B2.

Since ^{13}C NMR shifts of the DMBz moiety could reflect the electronic environment of the Co, we compared the shifts to the EP[21] for the R (or X) group. Good linear relationships between EP and the ^{13}C resonances of B5, B6, B7, R1 (all negative s), and B4 (positive s) were found (supplementary material). These good correlations for loop C's remote from Co suggest that these $\Delta\delta_{LR}$ are mainly influenced by inductive effects. In contrast, the correlations of close-in nuclei with EP are either not very good (B8 and B9, $r \sim 0.850$, positive s) or poor (B2). These shifts may be influenced by Co anisotropy [B2, B8, and B9 have the highest values of the geometric term ($GT = (1 - 3\cos^2\theta)/3r^3$ in McConnell's equation) of any loop carbons].[44] Moreover, GT for C9 is comparable to that of B8. The good correlations between B8 and B9 with each other and with C9 suggest that all these shifts must be responding to several effects (anisotropy, inductive effects, and strain) which are difficult to deconvolute.

There are good linear relationships between the ^{13}C shifts of C9 (β-C) of the C_1py derivatives[17] and those of B4, B5, B6, B7, and R1 (Table II, supplementary material). In similar comparisons with C10 (γ-C) and C11 (β-C) shifts of C_1py derivatives, the best correlations were found for B7, but they are not as good as that of B7 with C9 (β-C). B4, B8, and B9 give correlations with these three C_1py C's with negative s. Since the $\Delta\delta_{LR}$ for the β-C's (C9 and C11) and γ-C (C10) in C_1py derivatives respond mainly to the inductive nature of the axial ligand, these results support the suggestion that the shifts of the DMBz carbons (B4-B7) and R1 also reflect through-bond inductive effects.

Summary. The ^{13}C $\Delta\delta_{LR}$ of some corrin (C3, C4, C5, C11, C16) and loop (B4, B5, B6, B7, R1) signals reflect the electronic environment of the Co, which depends on the nature of the axial R (or X) ligand. In addition, C4, C5, and C11 may also be affected by steric effects, whereas B2, B8, B9, and C9 respond to several effects (anisotropy, inductive effects, and strain).

^{31}P NMR Shift Dependence on the Axial Ligand. The ^{31}P NMR resonance of Cbls provides an additional spectroscopic probe. X-ray studies show that the ^{31}P is too far from the Co or corrin ring to be much influenced by anisotropy.[41] Since the phosphorus atom is at least eight bonds away from the cobalt center, it is not likely that an inductive effect resulting from changing the axial ligand will greatly affect the ^{31}P shifts. B4H correlates fairly well with the ^{31}P NMR shifts. Therefore, the ^{31}P $\Delta\delta_{LR}$ for the linker arm of Cbls is most likely influenced by strain caused by ligand-induced changes in Co–N axial bond lengths.[41,53] The observed B4H $\Delta\delta_{LR}$ trends may reflect slight alterations in B4H position. We believe that the corrin ring anisotropy exerts a large upfield shifting effect on the B2H and B4H signals.[38]

Comparisons of ^{31}P shifts and ^{13}C shifts are instructive. In general, correlations of ^{31}P with R1 and the DMBz C's are better than those with the corrin C's. A good correlation with negative s was found for the ^{31}P shifts with B4. However, the H_2O point seemed to deviate from the correlation, probably due to its different charge. The correlation without the H_2O point is shown in Figure 4. Good correlations, but with positive s, were found for B5, B6, B7, and R1. ^{31}P shifts give fairly good linear relationships with C4 and C5 (supplementary material). The correlations of B4, B5, B6, B7, C4, and C5 with ^{31}P shifts support the conclusion, reached above, that the ^{13}C $\Delta\delta_{LR}$ for these C's are related to the electronic environment of the Co; the B4, C4 and C5 shifts are also modulated by steric effects (e.g., nucleotide loop conformational changes).

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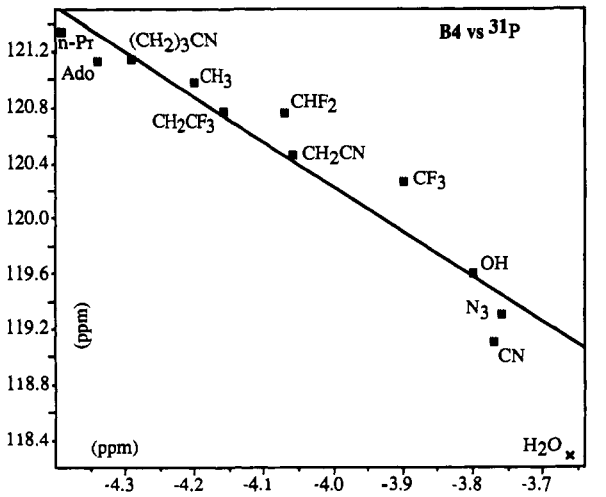

Figure 4. Plot of the B4 ^{13}C NMR shifts vs the ^{31}P NMR shifts for R (or X)Cbl (for 11 points, H_2O excluded, $s = -3.25$, $r = 0.924$).

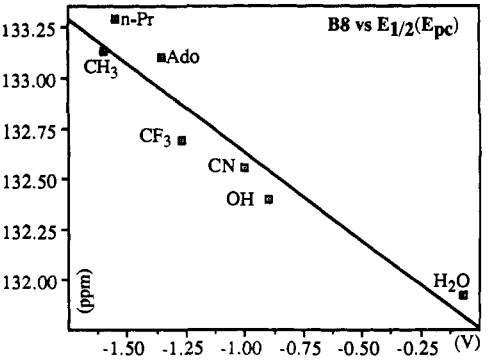

Figure 5. Plot of the B8 ^{13}C NMR shifts for R(or X)Cbl vs E_{pc} ($E_{1/2}$) (for seven points, $s = -0.88$, $r = 0.910$).

Electrochemical Correlations. We recently reported that E_{pc} ($E_{1/2}$) values reflect, at least in large part, the electronic properties of the Co center in C_1py model compounds.[17] In addition, the E_{pc} values for the Co^{III}/Co^{II} redox couple of C_1py derivatives were shown to correlate well with those of analogous B_{12} compounds.[16] Previously, the increasingly less negative $E_{1/2}$ values for RCbls (R = CH_3, CH_2CH_3, $CH_2CH_2CH_3$, $CH_2CH(CH_3)_2$, and $CH_2C(CH_3)_3$) were interpreted to suggest that the bulkiness of the alkyl group significantly reduced alkyl group electron donation since the redox properties of RCbl correlated with E_s.[23] It is interesting to compare $E_{1/2}$ (E_{pc}) with ^{13}C and ^{31}P NMR shifts.

For the seven Cbls in Figure 5, $E_{1/2}$ (E_{pc}) correlates fairly well with the ^{13}C NMR shifts of B5, B6, B7, and B8 (supplementary material). $E_{1/2}$ (E_{pc}) gives good correlations (supplementary material) with some corrin carbons (C8, C11, and C16). The CN point is normally out of the correlations. If the CN point is excluded, in general, the correlations improve. These correlations suggest that the electronic properties of the Co center, reflected in these ^{13}C shifts (B5, B6, B7, C8, C11, and C16), are also reflected in E_{pc}, as previously found for C_1py derivatives.[17] Although the best correlation of $E_{1/2}$ (E_{pc}) with the corrin C's is with C9, this signal is responding to several effects (anisotropy, inductive effects, and strain) and is difficult to assess.

Likewise, the correlation between $E_{1/2}$ and the ^{31}P NMR shifts was not very good (supplementary material). Since the ^{31}P $\Delta\delta_{LR}$ are related to loop conformational strain, this result suggests that $E_{1/2}$ (E_{pc}) is not particularly sensitive to such effects.

The reported good linear relationship between $E_{1/2}$ and E_s for RCbls[23] is lost if X = OH and CN points are added. Perhaps steric effects, which influence the $E_{1/2}$ values in RCbls, have a somewhat different effect on XCbls. More likely, the observed steric effect may be a small modulating factor, secondary to the

electronic effect. If the electronic effects of the alkyl groups were similar, then the steric effect could dominate, explaining the reported $E_{1/2}$ correlation with E_s.

Other Correlations with E_s. For R (or X) = CN, OH, CH_2CN, $(CH_2)_3CN$, CHF_2, CF_3, CH_3, and $CH_2CH_2CH_3$, the correlations between E_s and the DMBz C's[44] and R1 are poor, primarily because the points for CN and OH lie well off the line. The anomalous effect of the CN ligand on ^{13}C spectra has been observed for cobaloximes[22] and C_1py[17] compounds. The participation of CN^- in π-bonding in cobaloximes has been reported recently.[18] A number of authors have concluded that E_s sometimes contains substantial electronic contributions, either direct or hyperconjugative.[47] If these CN and OH points are excluded, all the correlations improve, especially for B6 and B7. However, E_s, by itself, does not appear to be generally useful.

Axial Ligand Donation in the B_{12} Cofactors. In structural studies with model compounds it was found that the Co–N(axial) bond distance increases as the electron-donor ability of the axial ligand increases.[11,21] The Co–N(DMBz) bond distances differ slightly for the two B_{12} cofactors; that for AdoCbl[8,51] is slightly longer (2.24 ± 0.02 Å) than for CH_3Cbl[41] (2.19 ± 0.02 Å). This difference suggests that the 5′-deoxyadenosyl group is a better electron donor than methyl or that the bond is lengthened by steric effects. However, the corrin ^{13}C resonances in AdoCbl are downfield-shifted relative to CH_3Cbl, and CH_3Cbl is more difficult to reduce. The ^{31}P shift for AdoCbl (–4.34 ppm) is upfield to that of CH_3Cbl(–4.2 ppm), consistent with the longer Co–N(DMBz) bond in AdoCbl.

The best rationale for these results begins with the two assumptions that Ado is a weaker donor than CH_3 but that its trans steric influence lengthens the Co–N(DMBz) bond. The corrin ^{13}C signals reflect net axial donation, which is lower for AdoCbl than for CH_3Cbl. This accounts for the greater ease of reduction of AdoCbl. The longer Co–N(DMBz) bond leads to torsion angles at P that are consistent with the upfield shift. However, the greater electron deficiency of the Co center and the longer Co–N(DMBz) bond in AdoCbl vs CH_3Cbl lead to similar net electron donation to Co by the DMBz heterocycle in the two cofactors. Consequently, the DMBz ^{13}C shifts are similar in the two cofactors. The two assumptions appear to be justified.

Conclusions

We have shown that ligand-responsive shifts are useful for probing in a "directional" manner the effect on the metal center of the ligand being varied. This NMR method has advantages over techniques such as electrochemistry, which reflect the net effect of all ligands. The most important conclusions of such analyses are that steric effects prevent the Ado ligand in coenzyme B_{12} from being as good a donor as it might otherwise be and that a steric trans influence of the Ado ligand increases the Co–N bond length. The long Co–N bond makes the DMBz ligand not as good a donor as might be expected in the absence of the steric influence. The net effect on the Cbl moiety of AdoCbl produces a conformation similar to that of CH_3Cbl.[41] This conformational similarity is reflected in the X-ray structures of these two coenzymes. Our analysis of the NMR data for H_2OCbl and CNCbl does not support the recent conclusions of EXAFS studies[7] that the Co–N(DMBz) bonds for these two species in solution are longer than the expected lengths and longer than the value found crystallographically for CNCbl.[5] Our NMR results show that the Co–N(DMBz) bond of N_3Cbl must be short in solution, and a recent X-ray structrure determination confirms this conclusion giving a Co–N value of 2.02 ± 0.02 Å.[20]

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Supplementary Material Available: Tables of the observed HMBC, HOHAHA, COSY, and NOESY (ROESY) connectivities for H_2OCbl and N_3Cbl, tables of r from the comparisons between the ^{13}C shifts of Cbls and the other ^{13}C shifts, the ^{31}P shifts, $E_{1/2}$, EP, E_s, and the ^{13}C shifts of the C_1py models, plot of the B6 ^{13}C and ^{31}P NMR shifts for R(or X)Cbl vs EP for the R (or X) ligand, plot of the C9 ^{13}C NMR shifts of [R(or X)Co(C_1py)]ClO_4 vs B6 of R(or X)Cbl, HMBC and upfield regions of the HOHAHA and ROESY spectra of H_2OCbl, HMBC spectrum of OHCbl, and COSY and part of the HMQC spectra of N_3Cbl (17 pages). Ordering information is given on any current masthead page.