Heteronuclear NMR Studies of Cobalt Corrinoids. 18. Correlation of Structure and Magnetic Resonance Parameters in Base-On Cobalamins[1]

Kenneth L. Brown,*,† Daniel R. Evans,† Jeffrey D. Zubkowski,‡ and Edward J. Valente§

Departments of Chemistry, Mississippi State University, Mississippi State, Mississippi 39762, Jackson State University, Jackson, Mississippi 39217, and Mississippi College, Clinton, Mississippi 39085

Received July 28, 1995⊗

Recent X-ray crystal structure determinations (including a new X-ray determination of the structure of cyano-13-epicobalamin reported herein) create a series of seven base-on cobalamins structurally characterized by modern crystallographic techniques in which the intramolecular equilibrium constant for coordination of the axial benzimidazole ligand (Bzm) varies from 76.6 to 4.90 × 10^7. For the five normal, unepimerized cobalamins, the free energy change for this equilibrium correlates linearly with the axial Co−N bond length ($r^2 = 0.99$). Absolute assignment of the ^{1}H and ^{13}C NMR spectra of two of these structurally characterized cobalamins (CH_3Cbl and CN-13-epiCbl) together with literature assignments for the other complexes now provides reliable ^{13}C NMR assignments and chemical shifts for all seven complexes. The magnetic anisotropies of the central cobalt atom of all seven complexes, estimated by a method described earlier, are well correlated with the axial Co−N bond distance ($r^2 = 0.97$) and the free energy of coordination of the Bzm ligand ($r^2 = 0.95$). The ^{31}P NMR chemical shift of the phosphodiester moiety of the nucleotide loop is excellently correlated to the axial Co−N bond length ($r^2 = 0.996$) of the unepimerized cobalamins and provides a reliable method of estimating this bond length. The ^{15}N chemical shifts of the axially coordinated Bzm nitrogen vary strongly with the axial Co−N bond distance and correlate linearly with this structural parameter ($r^2 = 0.991$) except for the case of H_2OCbl^+, which deviates substantially. However, there is a good linear correlation ($r^2 = 0.98$) of this ^{15}N chemical shift with the free energy of Bzm coordination for the five unepimerized cobalamins. Attempts to correlate ^{13}C NMR chemical shifts with structural, thermodynamic, and corrin ring conformational parameters are discussed.

Introduction

There is much interest in the relationship between NMR properties and structure and conformation in cobalt corrinoids (Figure 1). The ability to predict structure and conformation, in either an absolute or a relative sense, in such molecules from measured NMR parameters would represent a very powerful tool inasmuch as the absolute assignment of the ^{1}H and ^{13}C NMR spectra of such complexes has now become routine, and a number of such assignments have been made.[2−17] Such an understanding of the relationship between NMR properties and structure would be particularly advantageous for important derivatives, such as the base-off Cbls and the cobinamides (which lack an axial nucleotide), that have so far resisted crystallization.

In recent years, interest in the structural basis of the chemistry and biochemistry of base-on cobalamins led to the determination of a number of high-resolution crystal structures of such complexes. These include a redetermination[17] of the original Hodgkin structures[18] of CNCbl, neutron diffraction structures[19] of AdoCbl (replacing the original Lenhert structure[20]), and structures of CH_3Cbl,[21] and AdePrCbl,[10] an AdoCbl analog in which the ribose moiety of the 5′-deoxyadenosyl ligand is replaced by a propylene group. These structures provide a series of base-on Cbls for which the intrinsic intramolecular

---

* To whom correspondence should be addressed.

† Mississippi State University.

‡ Jackson State University.

§ Mississippi College.

⊗ Abstract published in *Advance ACS Abstracts,* December 15, 1995.

(1) Standard IUPAC−IUB nomenclature is used throughout. Abbreviations: CNCbl, cyanocobalamin (vitamin B_{12}); AdoCbl, 5′-deoxyadenosylcobalamin (coenzyme B_{12}); AdePrCbl, adeninylpropylcobalamin; CH_3Cbl, methylcobalamin; H_2OCbl^+, aquocobalamin; CN-13-epiCbl, cyano-13-epicobalamin; CN-8-epiCbl, cyano-8-epicobalamin; Bzm, 5,6-dimethylbenzimidazole.

(2) Ernst, L. *Liebigs Ann. Chem.* **1981**, 376.

(3) Battersby, A. R.; Edington, C.; Fookes, C. J. R.; Hook, J. M. *J. Chem. Soc., Perkin Trans. 1* **1982**, 2265.

(4) Summers, M. F.; Marzilli, L. G.; Bax, A. *J. Am. Chem. Soc.* **1986**, *108*, 4285.

(5) Bax, A.; Marzilli, L. G.; Summers, M. F. *J. Am. Chem. Soc.* **1987**, *109*, 566.

(6) Pagano, T. G.; Yohannes, P. G.; Hay, B. P.; Scott, J. R.; Finke, R. G.; Marzilli, L. G. *J. Am. Chem. Soc.* **1989**, *111*, 1484.

(7) Pagano, T. G.; Marzilli, L. G. *Biochemistry* **1989**, *28*, 7213.

(8) Brown, K. L.; Brooks, H. B.; Gupta, B. D.; Victor, M.; Marques, H. M.; Scooby, D. C.; Goux, W. J.; Timkovich, R. *Inorg. Chem.* **1991**, *30*, 3430.

(9) Alelyunus, Y. W.; Fleming, P. E.; Finke, R. G.; Pagano, T. G.; Marzilli, L. G. *J. Am. Chem. Soc.* **1991**, *113*, 3781.

(10) Pagano, T. G.; Marzilli, L. G.; Flocco, M. M.; Tsai, C.; Carrell, H. L.; Glusker, J. P. *J. Am. Chem. Soc.* **1991**, *113*, 531.

(11) Eisenreich, W.; Bacher, A. *J. Biol. Chem.* **1992**, *266*, 23840.

(12) Brown, K. L.; Zou, X. *J. Am. Chem. Soc.* **1992**, *114*, 9643.

(13) Calafat, A. M.; Marzilli, L. G. *J. Am. Chem. Soc.* **1993**, *115*, 9182.

(14) Brown, K. L.; Zou, X.; Savon, S. R.; Jacobsen, D. W. *Biochemistry* **1993**, *32*, 8421.

(15) Brown, K. L.; Zou, X.; Wu, G.-Z.; Zubkowski, J. D.; Valente, E. J. *Polyhedron* **1995**, *14*, 1621.

(16) Brown, K. L.; Evans, D. R. *Polyhedron*, in press.

(17) Kräutler, B.; Konrat, R.; Stupperich, E.; Fäberm G.; Gruber, K.; Kratky, C. *Inorg. Chem.* **1994**, *33*, 4128.

(18) (a) Hodgkin, D. C.; Lindsey, J.; Sparks, R. A.; Trueblood, K. N.; White, J. G.; *Proc. R. Soc. London* **1962**, *A266*, 494. (b) Brink-Shoemaker, C.; Cruickshank, D. W. J.; Hodgkin, D. C.; Kamper, M. J. *Proc. R. Soc. London* **1964**, *A278*, 1.

(19) (a) Savage, H. F. J.; Lindley, P. F.; Finney, J. L.; Timmins, P. A. *Acta Crystallogr.* **1987**, *B43*, 280. (b) Savage, H. *Biophys. J.* **1989**, *50*, 947. (c) Bouquiere, J. P.; Finney, J. L.; Lehman, M. S.; Lindley, P. F.; Savage, H. F. J. *Acta Crystallogr..* **1993**, *B49*, 79. (d) Bouquiere, J. P.; Finney, J. L.; Savage, H. F. J. *Acta Crystallogr..* **1994**, *B50*, 566.

(20) Lenhert, P. G. *Proc. R. Soc. London* **1968**, *A303*, 45.

(21) Rossi, M.; Glusker, J. P.; Randaccio, L.; Summers, M. F.; Toscano, P. J.; Marzilli, L. G. *J. Am. Chem. Soc.* **1985**, *107*, 1729.

0020-1669/96/1335-0415$12.00/0

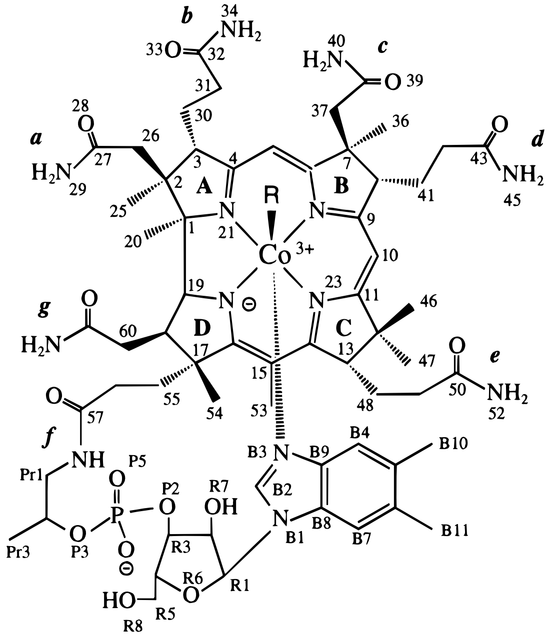
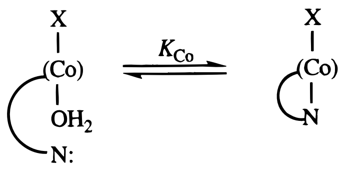


Figure 1. Numbering scheme for cobalamins. For the cyanocobalamins, the cyanide carbon is C64 and the cyanide nitrogen is N65. In CN-13-epiCbl, R = CN and epimerization at C13 brings the *e* propionamide side chain into an "upwardly" axial position.

base-off/base-on equilibrium constant, K_{Co} (eq 1), varies from



(1)

76.6[22] to 2.88 × 10^5.[23] Absolute assignments of the ^{13}C NMR spectra of these complexes are also available,[4,7,8,10] except for CH_3Cbl, for which only assignments by analogy to other RCbls have been made.[13] In order to extend this series to its most extreme member, H_2OCbl^+, for which K_{Co} is 4.90 × 10^7,[22] we determined the X-ray crystal structure of the perchlorate salt of H_2OCbl^+. While this report was in preparation, however, a paper[24] appeared describing this structure. As our structure is virtually identical to the one reported by Kratky et al.,[24] it will not be described in this report. Nonetheless, the absolute assignment of the ^{1}H and ^{13}C NMR spectra of CH_3Cbl and the determination of K_{Co} for AdePrCbl reported here complete the construction of a set of five base-on normal Cbls for which the crystal structure and absolute ^{13}C NMR assignments are known and in which the free energy of axial Bzm coordination varies from −2.57 to −10.48 kcal mol^{-1}.

In addition, the epicorrinoids CN-13-epiCbl,[25,26] in which the *e* propionamide side chain is epimerized into an upwardly pseudoaxial position, and CN-8-epiCbl,[15,27] in which the *d* propionamide side chain is epimerized into a pseudoequatorial position, would seem to represent an ideal opportunity to study the relationship between ^{13}C NMR chemical shifts and corrinoid conformation since the inner sphere ligands are identical to those in CNCbl. To the extent to which the inner sphere geometry is the same for these three complexes, electronic and anisotropic effects on ^{13}C chemical shifts should also be the same for all three. Thus, the relationship between chemical shift differences which are not due to the diastereomerism itself and conformational differences can be assessed. We recently reported the X-ray crystal structure and absolute NMR assignments of CN-8-epiCbl.[15] While the X-ray crystal structure of CN-13-epiCbl was originally reported by Hodgkin, et al.,[28] this 23-year-old structure is not sufficiently accurate for our purposes. We have consequently redetermined the crystal structure of CN-13-epiCbl and assigned its ^{1}H and ^{13}C NMR spectra by rigorous 2D NMR techniques.

Experimental Section

Aquocobalamin acetate was from Roussell, and CN-13-epiCbl was obtained by a modification[26] of the method of Bonnett et al.[25b] 9-(3-Chloropropyl)adenine was prepared by the method of Carraway et al.[29] and used to reductively alkylate H_2OCbl^+ to give AdePrCbl.

The pK_a for the base-on/base-off reaction of AdePrCbl was determined spectrophotometrically at 528 nm and 25.0 °C on a Cary 219 spectrophotometer as described previously.[30] The value obtained (3.31 ± 0.02) was used to calculate K_{Co} (177 ± 8) as described previously.[31]

^{31}P NMR measurements were made at 25 °C on a GE QE 300 NMR spectrometer operating at 121.71 MHz. Samples were ca. 10 mM in D_2O, and chemical shifts were determined relative to external 85% H_3PO_4. ^{13}C chemical shifts for all seven Cbls were determined relative to internal TSP at 25 °C from 1D spectra taken on the same instrument operating at 75.61 MHz. The ^{15}N NMR chemical shifts of the axial nucleotide of AdePrCbl were determined on a Bruker AMX 300 NMR spectrometer at 27 °C via the inverse-detected ^{1}H, ^{15}N HMQC experiment as previously described.[32] Chemical shifts were externally referenced to CH_3NO_2 but are reported relative to NH_3(l) using $\delta_{CH_3NO_2} = 380.32$ ppm.[33] Two-dimensional homonuclear and heteronuclear NMR experiments on CH_3Cbl and CN-13-epiCbl were carried out as described previously[12,14−16] on a Bruker AMX 600 NMR spectrometer.

X-ray diffraction measurements were carried out on a Siemans R3m/V diffractometer using Mo *K*α radiation (0.710 73 Å). A 0.5 × 0.5 × 0.3 mm crystal of CN-13-epiCbl was sealed in a tapered glass capillary containing saturated aqueous liquor with silicone grease. Accurate centering of 25 reflections for which $2\theta > 25°$ gave the orthorhombic cell constants $a = 15.337(8)$, $b = 21.592(10)$, $c = 24.176(12)$ Å. A total of 12 630 reflections were observed, including 12 595 unique reflections, of which 8553 had $I > 4\sigma_I$. Corrections were applied for Lorentz and polarization effects but not for deterioration or decomposition. An empirical absorption correction was calculated and applied.[34] The locations of the cobalt and phosphorus atoms were discovered by application of the Patterson function in SHELXS.[35] Several cycles of Fourier calculations revealed all the non-H atoms in the complex and 12 ordered water oxygens. Four other disordered water oxygens were found in a channel between cobalamin molecules. The positions and anisotropic vibrational factors for all non-H atoms were refined by blocked and full-matrix least-squares refinement using

---

(22) Brown, K. L.; Hakimi, J. M.; Jacobsen, D. W. *J. Am. Chem. Soc.* **1984**, *106*, 7894.

(23) Brown, K. L.; Wu, G.-Z. *Inorg. Chem.* **1994**, *33*, 4122.

(24) Kratky, C.; Färber, G.; Gruber, K.; Wilson, K.; Dauter, Z.; Nolting, H.-F.; Konrat, R.; Kräutler, B. *J. Am. Chem. Soc.* **1995**, *117*, 4654.

(25) (a) Bonnett, R.; Godfrey, J. M.; Math, V. B.; Edmond, E.; Evans, H.; Hodder, O. J. R. *Nature*, **1971**, *229*, 473. (b) Bonnett, R.; Godfrey, J. M.; Math, V. B. *J. Chem. Soc. C* **1971**, 3736.

(26) Brown, K. L.; Zou, X.; Evans, D. R. *Inorg. Chem.* **1994**, *33*, 5713.

(27) Rapp, P.; Oltersdorf, U. *Hoppe-Seyler's Z. Physiol. Chem.* **1973**, *354*, 32.

(28) Stoeckli-Evans, H.; Edmond, E.; Hodgkin, D. C. *J. Chem. Soc., Perkin Trans. 2* **1972**, 605.

(29) Carraway, K. L.; Huang, D. C.; Scott, T. G. In *Synthetic Procedures in Nucleic Acid Chemistry*; Zorbach, W. W., Tipson, R. S., Eds.; John Wiley & Sons, Inc.: New York, 1968; Vol. 1, p 1.

(30) Brown, K. L.; Hakimi, J. M.; Nuss, D. M.; Montejano, Y. D.; Jacobsen, D. W. *Inorg. Chem.* **1984**, *23*, 1463.

(31) Brown, K. L.; Peck-Siler, S. *Inorg. Chem.* **1988**, *27*, 3548.

(32) Brown, K. L.; Evans, D. R.; Zou, X.; Wu, G.-Z. *Inorg. Chem.* **1993**, *32*, 4487.

(33) Srinivasen, P. R.; Lichter, R. L. *J. Magn. Reson.* **1977**, *28*, 277.

(34) SHELXA-90 ver.1, Siemans Analytical X-Ray Instruments, Inc., Madison, WI.

(35) Sheldrick, G. M. *Acta Crystallogr.* **1990**, *A46*, 467.

Table 1. Inner Sphere Bond Distances and Bond Angles for CNCbl and the CN-epiCbls

	bond angle, deg				bond distance, Å		
atoms	CNCbl[a]	CN-8-epiCbl[b]	CN-13-epiCbl[c]	atoms	CNCbl[a]	CN-8-epiCbl[b]	CN-13-epiCbl[c]
N65-C64-Co	179.0	178.3	175.4	C64-N65	1.15	1.13	1.13
C64−Co−B3	174.6	177.5	174.0	Co−C64	1.86	1.91	1.87
N21-Co−N22	90.7	89.4	90.1	Co-B3	2.01	2.02	2.02
N22-Co−N23	96.8	96.1	95.7	Co−N21	1.88	1.85	1.91
N23-Co−N24	89.3	91.1	90.8	Co−N22	1.91	1.89	1.91
N24-Co−N21	83.8	83.5	83.5	Co−N23	1.92	1.90	1.92
C64-Co−N21	91.7	88.9	92.3	Co−N24	1.88	1.87	1.89
C64-Co−N23	90.5	92.9	88.5				
C64-Co−N24	94.8	87.8	89.1				
C64-Co−N22	87.9	87.9	88.0				
N21−Co−B3	90.7	91.5	93.0				
N23−Co−B3	87.5	86.9	86.5				
N24−Co−B3	94.8	94.7	94.4				
N22−Co−B3	89.4	89.6	89.1				

[a] Reference 17. [b] Reference 15. [c] This work.

Table 2. Structural, Thermodynamic, and Magnetic Resonance Parameters for the Base-On Cobalamins

	AdoCbl	AdePrCbl	CH_3Cbl	CN-13-epi	CN-8-epi	CNCbl	H_2OCbl^+
$d_{Co-N_{Bzm}}$, Å	2.24[a]	2.21[b]	2.19[c]	2.02[d]	2.02[e]	2.01[f]	1.93[g]
K_{Co}	7.66×10^{h}	1.77×10^{2} [d]	4.52×10^{2} [i]	2.88×10^{6} [j]	1.14×10^{5} [j]	3.35×10^{5} [j]	4.90×10^{7} [h]
ΔG_{Co}, kcal mol^{-1}	−2.57	−3.07	−3.62	−8.81	−6.89	−7.53	−10.48
fold angle,[k] deg (Glusker)	14.6	10.9	15.8	23.7	23.8	18.0	18.7
fold angle,[d,l] deg	12.3	10.4	15.3	23.4	23.3	17.6	17.5
twist angle,[d,l] deg	4.7	2.9	3.9	3.3	3.9	3.5	6.0
$10^{29}\Delta\chi$,[d] cm^3 molecule^{-1}	−16.0	−16.7	−12.0	−2.02	−3.16	−3.26	1.04
$1 - \alpha$[m]	0.091	0.070	0.125	0.450	0.510	0.410	0.600
$\delta_{^{31}P}$,[n] ppm	−0.58	−0.51[d]	−0.44	0.13[d]	0.11[d]	−0.02	0.12
$\delta_{^{15}N}$,[o] ppm	229.9[p]	224.3[d]	224.9	187.2	188.4	188.3	143.1

[a] Reference 19a. [b] Reference 10. [c] Reference 21. [d] This work. [e] Reference 15. [f] Reference 17. [g] Reference 24. [h] Reference 22. [i] Reference 31. [j] Reference 23. [k] Defined as the angle between the normals to the least-squares planes through N21, C4, C5, C6, N22, C9, C10 and through N24, C16, C15, C14, N23, C11, C10.[38] [l] See text. [m] Apparent "partial protonation" of Bzm due to coordination to the Co center. [n] Reference 22, except as noted. [o] For the axially coordinated nitrogen, B3; ref 32, except as noted. [p] Reference 39.

SHELXL-932b.[36] H atom positions and isotropic vibrational factors were included in the refinement, the positions being calculated from the molecular geometry and confirmed by examination of the difference fourier maps. The model converged with $R_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o| = 0.0541$, $R_{w2} = \{\Sigma[w(F_o^2 - F_c^2)^2]/\Sigma[w(F_o^2)^2]\}^{1/2} = 0.1005$ for all 12 595 data. Flack's analysis[37] supports the absolute configuration of the model.

Results and Discussion

X-ray Crystal Structure of CN-13-epiCbl. Details of the crystal data and structure refinement, atomic coordinates, anisotropic temperature factors, bond angles and bond distances between adjacent non-hydrogen atoms, calculated hydrogen atom coordinates, and intermolecular contacts are available as Supporting Information. The principal, gross structural differences between CN-13-epiCbl and CNCbl revealed by the original X-ray structure of the former[28] are largely confirmed by the new structure. These include the change in pucker of the C pyrrole ring, the upwardly pseudoaxial orientation of the *e* propionamide side chain at C13, and the change in orientation of the *gem* dimethyls at C12 such that C46 becomes equatorial and C47 becomes downwardly axial.

However, the new structure corrects several anomalies seen in the older structure. The impossibly short Co−C bond distance from the original structure (1.74 Å) now has the more reasonable value of 1.87 Å, and the suspiciously long axial Co−N_{Bzm} bond distance (2.06 Å) is reduced to 2.02 Å. These corrections show that the inner sphere geometry of CN-13-epiCbl is much closer to that of CNCbl than the older X-ray structure suggested. Table 1 shows a comparison of the inner sphere geometries of CNCbl, CN-13-epiCbl, and CN-8-epiCbl. The only significant differences are a slight elongation (ca. 0.04 Å) of the Co−C bond in CN-8-epiCbl, a similarly slight shortening of the Co−N_{Bzm} bond in this complex, a slight decrease (ca. 3−4°) of the N65−C64−Co, C64−Co−B3, and C64−Co−N23 bond angles in CN-13-epiCbl, a similarly slight decrease of the C64-Co−N21 bond angle in CN-8-epiCbl, and a slightly more pronounced decrease (5−7°) of the C64-Co−N24 bond angle in both of the epicobalamins. Hence, in contrast to the impression given by the older X-ray crystal structure of CN-13-epiCbl, the new structure shows that the inner spheres of CNCbl and the two cyanoepicobalamins are quite similar.

As is the case for the other base-on Cbls, the corrin ring of CN-13-epiCbl is folded upward about the Co···C10 axis. Glusker[38] has attempted to quantitate this fold as the angle between the normals to a "northern" plane, defined by N21, C4, C5, C6, N22, C9, and C10, and a "southern" plane, defined by N24, C16, C15, C14, N23, C11, and C10. The Glusker fold angle for CN-13-epiCbl is 23.7°, almost identical to that for CN-8-epiCbl (23.8°)[15] but significantly larger than those for CNCbl (18.0°) and H_2OCbl^+ (18.7°) (Table 2). However, there is also a significant twist between the two Glusker planes, roughly about the C15···C5 line. This is best seen in the structure of $H_2OCbl \cdot ClO_4$, shown in Figure 2 as a corrin ring edge-on view from the "southern" perspective along the C15···Co axis. Here it can be clearly seen that the D−C ring ensemble (in the foreground) slopes downward from left to right while the A−B ring ensemble (in the background) slopes upward from left to right. In order to deconvolute the actual fold of the

---

(36) Sheldrick, G. M. *J. Appl. Crystallogr.*, in press.
(37) Flack, H. D. *Acta Crystallogr.* **1983**, *A39*, 876.
(38) Glusker, J. P. in *B₁₂*; Dolphin, D. D., Ed.; Wiley-Interscience: New York, 1982; Vol. 1 p 23.
(39) Brown, K. L.; Evans, D. R. *Inorg. Chem.* **1994**, *33*, 525.

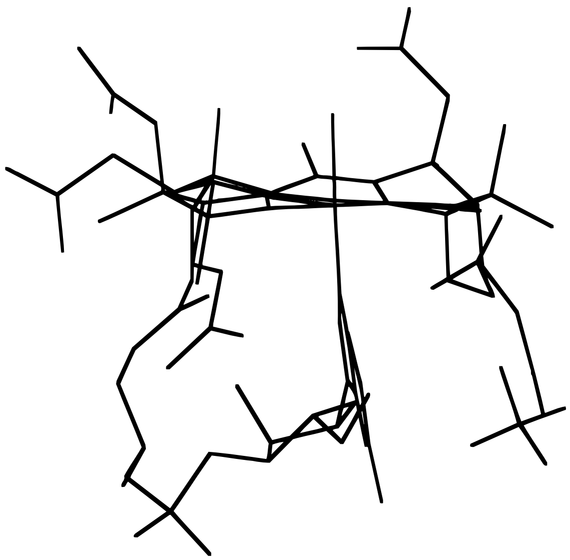


Figure 2. Edge-on view of $H_2OCbl{\cdot}ClO_4$ viewed from the "southern" perspective (roughly along the C15···Co axis), showing the twist in the corrin ring.

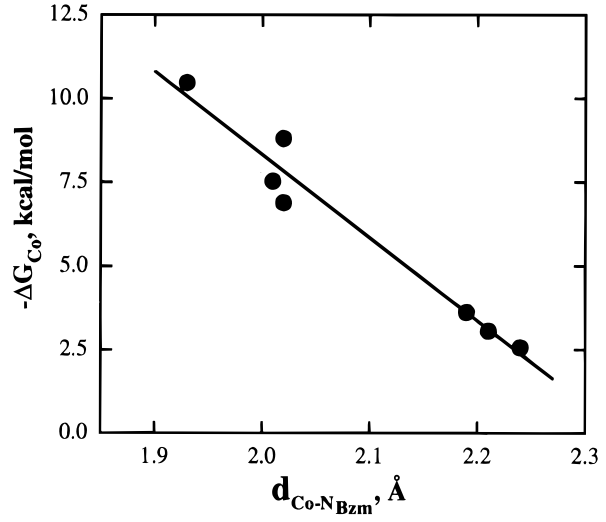


Figure 3. Plot of $-\Delta G_{Co}$ for the formation of the base-on species of the RCbls vs the axial Co−N bond length. The solid line is a linear regression omitting the points for CN-8- and CN-13-epiCbl; $N = 5$, slope = −24.8 ± 1.4 kcal mol^{-1} $Å^{-1}$, intercept = 57.9 ± 3.0 kcal mol^{-1}, $r^2 = 0.990$.

Glusker planes about the Co···C10 axis from this twist, we have recalculated the fold angle as the angle between the projections of the normals to the Glusker planes onto a plane perpendicular to the least-squares plane through the four corrin nitrogens and bisecting the N21···N22 and N23···N24 axes. This results in fold angles of 17.5° for $H_2OCbl{\cdot}ClO_4$ and 23.4° for CN-13-epiCbl. Similarly, the twist angle has been estimated as the angle between the projections of the normals to the Glusker planes onto a plane perpendicular to the least-squares plane through the four corrin nitrogens and bisecting the N21···N24 and N22···N23 axes. This results in a twist angle of 6.0° for $H_2OCbl{\cdot}ClO_4$. Similar calculations have been carried out for the other six base-on Cbls, and the resulting values of the deconvoluted fold and twist angles are listed in Table 2.

Relationship of ΔG_{Co} to the Axial Co−N_{Bzm} Bond Length. The availability of values for the equilibrium constant, K_{Co} (eq 1), for the intramolecular base-off/base-on reaction of the seven structurally characterized Cbls listed in Table 2 now permits an assessment of the relationship between the free energy for Bzm coordination and the axial Co−N_{Bzm} bond length in base-on Cbls in a data set in which ΔG_{Co} varies by almost 8 kcal mol^{-1} and $d_{Co-N_{Bzm}}$ varies by 0.31 Å. The correlation is shown in Figure 3, where it can be seen that the ΔG_{Co} values for the epicobalamins deviate badly from the correlation (solid line)

Table 3. ^1H and ^{13}C NMR Assignments for CH_3Cbl and CN-13-epiCbl[a]

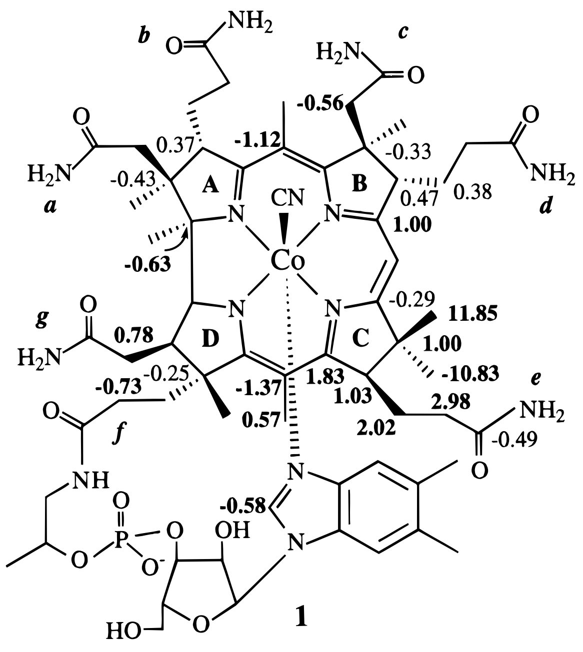
	CH_3Cbl		CN-13-epiCbl	
atom	$\delta_{^{13}C}$, ppm	$\delta_{^1H}$, ppm	$\delta_{^{13}C}$, ppm	$\delta_{^1H}$, ppm
Co−CH_3	9.60	-0.09		
C53	18.16	2.45	17.38	2.55
C35	18.12	2.49	17.79	2.47
C54	19.30	1.33	18.49	1.35
C25	19.44	1.35	19.42	1.37
Pr3	21.42[b]	1.23	21.56[c]	1.20
C36	21.57	1.79	21.68	1.76
B11	21.93	2.22	21.97	2.24
B10	22.25	2.21	22.42	2.24
C47	22.42	1.39	32.68	1.16
C20	22.91	0.47	21.61	0.45
C41	28.53	0.97, 1.85	28.14	1.02, 1.93
C30	28.76	1.93, 2.01	28.70	1.99, 2.07
C48	30.38	1.98, 2.05	28.57	1.80, 2.11
C42	34.36	1.03, 1.73	33.94	0.78, 1.83
C56	34.55	1.80	34.77	1.83, 2.46
C46	34.55	0.93	22.01	1.36
C55	34.65	2.07, 2.45	34.84	2.13, 2.56
C60	34.65	2.63, 2.68	34.03	2.62, 2.72
C49	37.52	2.58	34.18	1.95, 2.00
C31	37.81	2.42, 2.48	37.44	2.45, 2.54
C18	41.70	2.60	41.41	2.67
C37	45.53	1.85, 2.34	45.88	2.13, 2.52
C26	45.85	2.23, 2.31	45.48	2.38, 2.47
Pr1	47.50	3.09, 3.55	48.00	2.95, 3.60
C2	48.94		50.22	
C12	49.23		49.70	
C7	52.74		54.36	
C13	55.81	3.05	55.20	3.63
C8	57.06	3.40	57.81	3.42
C3	57.98	4.14	58.48	4.21
C17	60.44		62.02	
R5	62.98	3.75, 3.89	62.94	3.76, 3.96
R2	71.58	4.23	71.36	4.32
Pr2	75.56[d]	4.33	75.39[e]	4.34
R3	75.73[f]	4.71	75.55[g]	4.60
C19	76.36	3.94	77.65	4.18
R4	84.09[h]	4.08	84.35[i]	4.04
C1	87.69		88.30	
R1	89.00	6.27	89.11	6.34
C10	96.56	5.91	96.73	5.85
C15	106.03		108.02	
C5	107.84		111.22	
B7	113.12	7.18	114.04	7.28
B4	120.91	6.28	118.96	6.49
B8	133.06		132.54	
B5	134.16		135.64	
B6	136.37		137.85	
B9	140.75		139.49	
B2	144.48	6.97	144.97	7.15
C6	165.73		167.83	
C14	166.20		166.75	
C9	172.30		139.49	
C11	175.95		179.73	
C16	177.48		181.64	
C38	177.60		177.64	
C57	177.81		177.29	
C4	177.92		182.57	
C61	178.78		178.18	
C27	178.94		178.35	
C43	180.04		179.40	
C32	180.71		180.35	
C50	180.88		181.15	

[a] In D_2O, chemical shifts relative to internal TSP. [b] $^3J_{PC} = 3.6$ Hz. [c] $^3J_{PC} = 2.5$ Hz. [d] $^2J_{PC} = 5.9$ Hz. [e] $^2J_{PC} = 6.5$ Hz. [f] $^2J_{PC} = 4.9$ Hz. [g] $^2J_{PC} = 3.6$ Hz. [h] $^3J_{PC} = 8.5$ Hz. [i] $^3J_{PC} = 8.0$ Hz.

for the normal cobalamins. This is, in fact, not unexpected, since the values of K_{Co} for both epicobalamins have previously been shown to be anomalous with respect to the values for the normal Cbls.[23] Given the high degree of similarity of the inner sphere geometries of the three isomeric cyanocobalamins, these anomalies cannot be due to differential electronic effects. It now seems most likely that, in the base-on normal Cbls, contact between the downwardly projecting *e* propionamide side chain and the Bzm weakens the coordination, so that epimerization of the *e* side chain to the upper face increases K_{Co}.[23] In the case of the 8-epiCbls, the *d* propionamide side chain is known to form a hydrogen bond to the Bzm B1 nitrogen in the base-on species of the normal Cbls.[40] Evidently, this hydrogen bond stabilizes the base-on species of the normal Cbls so that removal of the *d* side chain from the lower corrin face in the 8-epiCbls results in a decrease in K_{Co}. For the five remaining normal Cbls, the correlation between $-\Delta G_{Co}$ and $d_{Co-N_{Bzm}}$ (Figure 3) is excellent ($r^2 = 0.990$). Thus, ΔG_{Co} is an accurate indicator of the axial $Co-N_{Bzm}$ bond length, and differences in this bond length between normal Cbls are in fact reflected in differences in the strength of axial coordination.

^{1}H and ^{13}C NMR Assignments for CN-13-epiCbl and CH_3Cbl. In order to make the ^{13}C NMR comparisons described below, absolute NMR assignments were needed for CN-13-epiCbl and for CH_3Cbl, the ^{13}C NMR spectrum of which had previously been assigned[21] only by analogy. This was accomplished using the usual battery of 2D homonuclear (COSY, HOHAHA, NOESY, and ROESY) and heteronuclear (HMQC and HMBC) experiments and the now familiar strategies for assignments in the cobalt corrinoids.[4−16] Briefly, the direct and relayed connectivities in the COSY and HOHAHA spectra were used to assign the protons in the various spin systems (R1−R5, Pr1−Pr3, C19−C18−C60, C3−C30−C31, C8−C41−C42, C13−C48−C49, and C55−C56). Through-space nOe interactions in the NOESY and ROESY spectra were used to assign protons not spin-coupled to other protons and to confirm the assignments of the various spin systems. Carbon assignments were then made from the HMQC and HMBC spectra for the protonated and unprotonated carbons, respectively. The latter spectrum was also used to confirm the assignments of spin-coupled protons by their 2- and 3-bond connectivities to assigned carbon resonances. Complete correlation tables for both complexes are available as Supporting Information, and the ^{1}H and ^{13}C NMR assignments for CN-13-epiCbl and CH_3Cbl are given in Table 3. A comparison of the ^{13}C chemical shifts of CN-13-epiCbl and CNCbl is shown in structure **1**, which gives all significant chemical shift differences ($\Delta\delta > 0.25$ ppm) with large chemical shift differences ($\Delta\delta > 0.5$ ppm) in boldface. This comparison shows that, unlike CN-8-epiCbl,[15] where chemical shift differences are almost completely confined to the region near the site of epimerization, CN-13-epiCbl has significant chemical shift differences quite remote from the site of epimerization. This gives rise to the conjecture that these remote chemical shift differences must be due to differences in conformation between CNCbl and CN-13-epiCbl since the similarity of the inner spheres of these two complexes makes it unlikely that either electronic or anisotropic differences contribute to the chemical shift differentials significantly. This issue is addressed below.

Absolute assignment of the ^{13}C NMR spectrum of CN-13-epiCbl provides an interesting perspective on the chemical shifts of the *gem* dimethyl substituents (C46 and C47) on C12. In the normal Cbls, these two ^{13}C resonances appear near 34.0 and 22.3 ppm. The more downfield resonance has been assigned to the upwardly axial C46 methyl on the basis of its more intense HMBC correlation to C13H due to larger coupling resulting from the *cis* configuration between C13H and C46 vs the *gauche* configuration between C13H and the equatorial C47. In addition, the equatorial C47 methyl displays nOe correlations to the hydrogens on the downwardly projecting *e* propionamide side chain, and in AdoCbl and AdePrCbl, the axially upward C46 methyl has nOes to adenosyl protons.[4,10] However, in CN-13-epiCbl, in which the C46 methyl is equatorial, and hence *gauche* to C13H, and the C47 methyl is downwardly axial, and *cis* to C13H, an HMBC cross peak to C13H is seen only for C47. In addition, the more downfield methyl resonance has nOe correlations with B2H, R4H, R5H′, and R5H″, while the upfield methyl has an nOe to C48H’. Thus, in CN-13-epiCbl, the C46 and C47 assignments are reversed, C46 being the upfield resonance (22.01 ppm) and C47 the downfield resonance (32.68 ppm). The relative positions of the C46 and C47 resonances in the normal Cbls and the 13-epiCbl are evidently the result of significant steric effects on the ^{13}C chemical shifts.[41−43] In the normal Cbls, the equatorial C47 is crowded by the pseudoaxially downward *e* propionamide, while in the 13-epiCbls, the equatorial C46 is crowded by the now upwardly pseudoaxial *e* propionamide.



1

$\delta_{CNCbl} - \delta_{CN\text{-}13\text{-}epiCbl}$

Finally, in the crystal structure of $H_2OCbl{\cdot}ClO_4$,[24] the cobalt-coordinated water molecule is hydrogen-bonded to the carbonyl oxygen (O39) of the *c* side chain acetamide ($d_{O{\cdot\cdot}O} = 2.66$ Å, O−H··O angle 158°), and Kratky et al.[24] have provided ^{1}H NMR evidence that this hydrogen bond persists in water. The ^{13}C NMR spectrum of H_2OCbl^+ provides confirming evidence, in the form of anomalous chemical shifts for the *c* side chain of this complex. Thus, for C37, the average chemical shift for five of the other Cbls (excluding CN-8-epiCbl, since the epimerization affects all of the chemical shifts in this region) is 45.31 ± 0.41 ppm, but in H_2OCbl^+, the C37 shift is 48.21 ppm. A similar effect is seen at C36, where the average chemical

---

(40) Brown, K. L.; Evans, D. R. *Inorg. Chem.* **1993**, *32*, 2544.

(41) (a) Grant, D. M.; Cheney, B. V. *J. Am. Chem. Soc.* **1967**, *89*, 5315. (b) Cheney, B. V.; Grant, D. M. *J. Am. Chem. Soc.* **1967**, *89*, 5319.

(42) Schneider, H.-J.; Price, R.; Keller, T. *Angew. Chem., Int. Ed. Engl.* **1971**, *10*, 730.

(43) Breitmaier, E.; Voelter, W. *Carbon-13 NMR Spectroscopy*; VCH: Weinheim, Germany, 1989.

shift (again excluding CN-8-epiCbl) is 21.48 ± 0.18 ppm, but the shift in H_2OCbl^+ is 23.81 ppm. The effect is smaller for the C38 carbonyl carbon ($\delta_{av} = 177.63 \pm 0.15$ ppm vs 178.12 ppm in H_2OCbl^+) but nonetheless significant. The persistence of this hydrogen bond in solution has significant implications for the energetics of ligand substitution in H_2OCbl^+, since such substitution reactions are known to be dissociatively dominated.[44−46] An intramolecular hydrogen bond to the coordinated water must be expected to significantly influence the rate of dissociation of this ligand, an effect which we are currently attempting to demonstrate.

Magnetic Anisotropy of the Central Cobalt Atom. In earlier work,[47] we devised a method for estimating the magnetic anisotropy, $\Delta\chi$, for the central Co atom of base-on Cbls of known structure, using the ^{13}C chemical shifts of the coordinated Bzm ligand. Briefly, Bzm ^{13}C chemical shifts were assumed to be dominated by electronic effects owing to coordination and dipolar shielding due to the cobalt quadrupole. The former were accounted for using the known chemical shifts of the protonated and free base forms of the detached nucleoside (α-ribazole) and a fitting parameter, 1 − α, which considers the electronic effect of coordination to be approximated as “partial protonation”. The anisotropic shielding was accounted for using McConnell’s equation[48] and the known geometry of the base-on Cbl. The resulting equation in two unknowns ($\Delta\chi$ and α) was solved by 21 pairwise calculations using the chemical shifts of the seven carbons of the Bzm nucleus. The results for the three base-on Cbls whose X-ray structures were then available (AdoCbl, CH_3-Cbl, and CNCbl) gave values of 1 − α which increased as the upper axial ligand became a poorer donor and negative values for $\Delta\chi$ which became smaller in the same order.[49] The rather steep trend of the latter parameter suggested that $\Delta\chi$ might, in fact, be positive for H_2OCbl^+.

The availability of the geometry for $H_2OCbl\cdot ClO_4$[24] now permits the estimation of $\Delta\chi$ by this method, and the value obtained (1.04×10^{-29} cm^3 molecule^{-1}) shows that its sign is, indeed, inverted. Moreover, estimation of $\Delta\chi$ for the remaining complexes (Table 2) by the same method shows that this parameter is linearly correlated to $d_{\mathrm{Co-N_{Bzm}}}$ (Figure 4A, $r^2 = 0.971$). In addition, because of the relationship between ΔG_{Co} and $d_{\mathrm{Co-N_{Bzm}}}$ (Figure 3), $\Delta\chi$ also correlates well ($r^2 = 0.947$) with $-\Delta G_{\mathrm{Co}}$ (Figure 4B). This means that reasonably accurate estimates of $\Delta\chi$ may now be obtained for base-on Cbls whose structure is not known, simply from the readily measured values of K_{Co}. We also note that the values of $\Delta\chi$ for CNCbl and CN-8-epiCbl are nearly identical, although the value for CN-13-epiCbl is slightly smaller. Thus, the dipolar shielding due to the Co quadrupole will be very similar in these three isomers.

Finally, 1 − α, the apparent degree of partial protonation of the coordinated Bzm, increases as expected when the upper axial ligand becomes a poorer donor (Table 2) and is well correlated (not shown) with both $d_{\mathrm{Co-N_{Bzm}}}$ ($N = 7$, $r^2 = 0.97$) and $-\Delta G_{\mathrm{Co}}$ ($N = 5$, omitting the epiCbls, $r^2 = 0.992$).

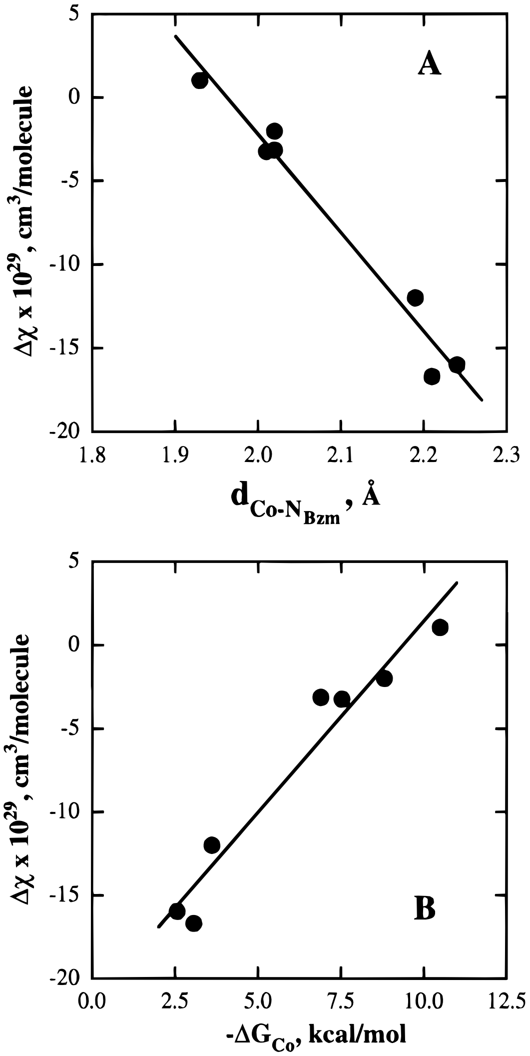


Figure 4. (A) Plot of $\Delta\chi$, the magnetic anisotropy of the cobalt atom, vs the axial Co−N bond length for the base-on RCbls. The solid line is a linear regression; $N = 7$, slope = −58.5 ± 4.6 cm^3 molecule^{-1} Å^{-1}, intercept = 114.8 ± 9.5 cm^3 molecule^{-1}, $r^2 = 0.971$. (B) Plot of $\Delta\chi$, the magnetic anisotropy of the cobalt atom, vs $-\Delta G_{\mathrm{Co}}$ for the formation of the base-on species of the RCbls. The solid line is a linear regression, $N = 7$, slope = 2.29 ± 0.24 cm^3 mol molecule^{-1} kcal^{-1}, intercept = −21.5 ± 1.6 cm^3 molecule^{-1}, $r^2 = 0.947$.

^{31}P NMR. In earlier work,[22] we showed that the ^{31}P NMR chemical shift of the nucleotide loop phosphodiester of base-on Cbls is linearly related to the strength of the axial Co−N$_{\mathrm{Bzm}}$ bond as measured by ΔG_{Co}. Similarly, there was an apparently linear relationship between $\delta_{^{31}\mathrm{P}}$ and $d_{\mathrm{Co-N_{Bzm}}}$, although only three values of the axial Co−N$_{\mathrm{Bzm}}$ bond length were available at that time. These effects were attributed to the high sensitivity of the ^{31}P NMR shifts of phosphate compounds to phosphate conformation (i.e., O−P−O bond angles)[50−53] and the induction of conformational strain in the nucleotide loop phosphodiester as the axial Co−N$_{\mathrm{Bzm}}$ bond length changes.[22] The current data (Table 2) allow a more accurate assessment of the dependence of $\delta_{^{31}\mathrm{P}}$ on $d_{\mathrm{Co-N_{Bzm}}}$. In fact, an excellent linear correlation ($r^2 = 0.996$) is obtained (Figure 5) for the five normal base-on Cbls. As the range of complexes now available probably comes close to spanning the maximum possible variation in this bond length, the ^{31}P chemical shift of base-on Cbls emerges as an

---

(44) Marques, H. M.; Breet, E. L. J.; Prinsloo, F. F. *J. Chem. Soc., Dalton Trans.* **1991**, 2941.

(45) Marques, H. M.; Bradley, J. C.; Campbell, L. A. *J. Chem. Soc., Dalton Trans.* **1992**, 2019.

(46) Marques, H. M.; Munro, O. Q.; Cumming, B. M.; de Nysschen, C. *J. Chem. Soc., Dalton Trans.* **1994**, 297.

(47) Brown, K. L.; Hakimi, J. M. *J. Am. Chem. Soc.* **1986**, *108*, 496.

(48) McConnell, H. M. *J. Chem. Phys.* **1957**, *27*, 226.

(49) In our original work on the Co anisotropy of the base-on Cbls,[47] the older, less precise X-ray structures for AdoCbl and CNCbl were used, and the assignments of the ^{13}C resonances of the B5 and B6 carbons were interchanged. The values of $\Delta\chi$ listed in Table 2 were obtained using the new geometries and correct ^{13}C assignments.

(50) Gorenstein, D. G. *J. Am. Chem. Soc.* **1975**, *97*, 898.

(51) Gorenstein, D. G.; Kar, D. *Biochem. Biophys. Res. Commun.* **175**, *65*, 1073.

(52) Gorenstein, D. G. *J. Am. Chem. Soc.* **1977**, *99*, 2254.

(53) Gorenstein, D. G. in *Phosphorus-31 NMR*; Gorenstein, D. G., Ed.; Academic Press: Orlando, FL, 1984; p 7.

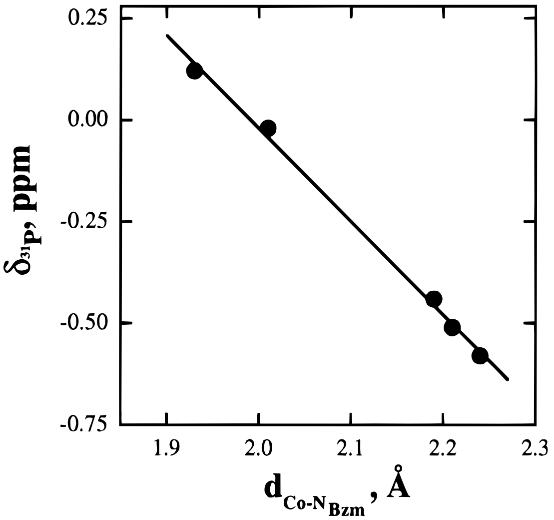


Figure 5. Plot of the ^{31}P NMR chemical shift of the five normal base-on RCbls vs the axial Co$-$N$_{Bzm}$ bond length. The solid line is a linear regression; $N = 5$, slope $= -2.28 \pm 0.08$ ppm Å^{-1}, intercept $= 4.54 \pm 0.17$ ppm, r^2 0.996.

excellent indicator of axial Co$-$N$_{Bzm}$ bond length. The sole drawback to the use of this chemical shift to predict Co$-$N$_{Bzm}$ bond lengths is its relatively small variation (0.7 ppm) across the series of complexes.

^{15}N NMR of the Axially Coordinated Nitrogen (B3). The ^{15}N NMR resonance of the coordinated nitrogen of the Bzm ligand does not suffer from this shortcoming. We recently demonstrated[32] that this resonance can be observed in samples of Cbls at natural abundance using the inverse-detected ^{1}H, ^{15}N HMQC experiment optimized for the B2H$-$B3 coupling (ca. 7 Hz[54]). Chemical shifts for this nitrogen have now been determined for 16 base-on Cbls,[32,39] and these vary by 86 ppm. Figure 6A shows, however, that the excellent ($r^2 = 0.991$) linear correlation between $\delta_{^{15}\mathrm{N}}$ and $d_{\mathrm{Co-N_{Bzm}}}$ for six of the seven base-on Cbls in Table 2 does not extend to H$_2$OCbl$^+$, the chemical shift of which falls 28.4 ppm below the value predicted by the correlation for the other six complexes. However, Figure 6B shows that there is a very good correlation ($r^2 = 0.976$) between the B3 ^{15}N chemical shift and ΔG_{Co} for the five normal base-on Cbls. Thus, while this chemical shift may not be ideal as an indicator of the absolute Co$-$N$_{Bzm}$ bond length, its exquisite sensitivity to this parameter makes it an excellent indicator of changes in axial Co$-$N$_{Bzm}$ bond length which might, for instance, accompany complexation of Cbls to proteins.

^{13}C NMR: Electronic Effects on the Corrin Ring Carbons. In order to make precise comparisons of the ^{13}C chemical shifts among the seven structurally characterized Cbls, chemical shifts for all seven complexes were determined on the same instrument under identical conditions. The values for CH$_3$Cbl and CN-13-epiCbl are given in Table 3, while those for the other five Cbls are given in the Supporting Information.

Trends in the ^{13}C chemical shifts of the corrin ring carbons must be expected to be dominated by differences in electronic effects at the central metal atom and differences in dipolar shielding due to trends in the magnetic anisotropy of the metal, although conformational effects may also be significant. Calculations based on the current values of $\Delta\chi$ using McConnell's equation[48] show that differential magnetic anisotropy of the Co atom across the series of complexes can affect corrin ring ^{13}C shifts by as much as 2.4 ppm. Since $\Delta\chi$ has now been shown to correlate well with both the axial Co$-$N$_{Bzm}$ bond length and the free energy of Bzm coordination, and both of these parameters are sensitive to electronic effects at the metal center, we have attempted to correlate corrin ring ^{13}C shifts with $d_{\mathrm{Co-N_{Bzm}}}$ and ΔG_{Co}. In all such correlations, the epiCbls have been omitted from correlations of ring carbons close to the epimerized centers. For correlations with $d_{\mathrm{Co-N_{Bzm}}}$ (data not shown), seven ring carbons (C2, C4, C9, C12, C13, C16, and C19) had reasonably good correlations ($r^2 = 0.91-0.94$) and five additional carbons (C3, C5, C11, C14, and C17) had somewhat poorer correlations ($r^2 = 0.81-0.86$). Similar results were obtained for correlations of $\delta_{^{13}\mathrm{C}}$ with ΔG_{Co}. However, introduction of a conformational parameter, the Glusker fold angle, which allows for the possibility of significant conformational effects on the ^{13}C shifts, significantly improved such correlations. Here, the ring carbon chemical shifts were correlated to both $d_{\mathrm{Co-N_{Bzm}}}$ and the composite fold angle according to eq 2. Using this dual parameter approach, 10

$$\delta_{^{13}\mathrm{C}} = A(d_{\mathrm{Co-NBzm}}) + B(\text{fold angle}) + C \qquad (2)$$

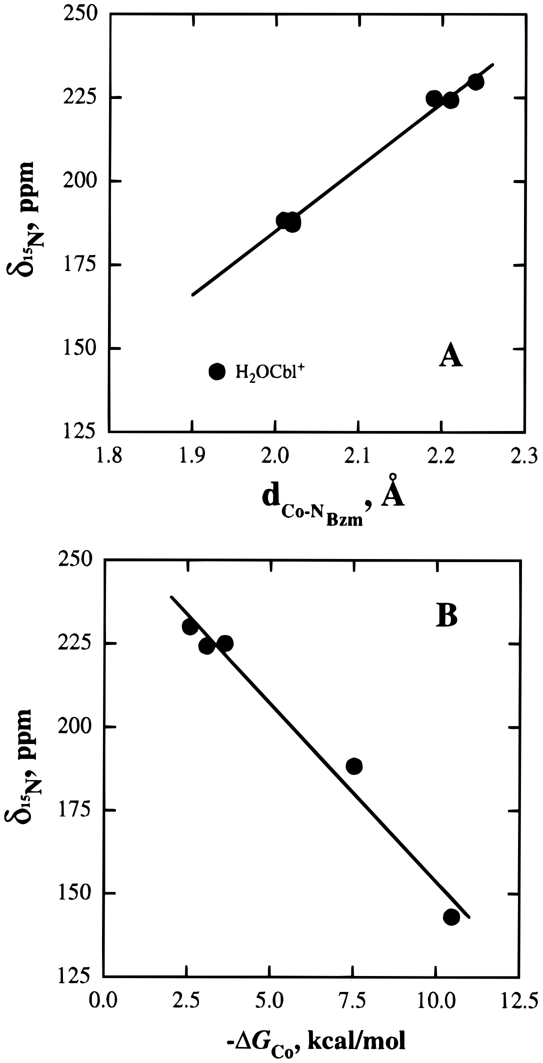


Figure 6. (A) Plot of the ^{15}N NMR chemical shifts of the coordinated nitrogen (B3) of the Bzm ligand vs the axial Co$-$N$_{Bzm}$ bond distance. The solid line is a linear regression; $N = 6$ (omitting the point for H$_2$OCbl$^+$), slope $= 192 \pm 9$ ppm Å^{-1}, intercept $= -198 \pm 20$ ppm, $r^2 = 0.991$. (B) Plot of the ^{15}N NMR chemical shift of the coordinated nitrogen (B3) of the Bzm ligand for the normal Cbls vs $-\Delta G_{\mathrm{Co}}$ for the formation of the base-on species of the RCbls. The solid line is a linear regression; $N = 5$, slope $= -10.6 \pm 1.0$ ppm mol kcal^{-1}, intercept $= 260 \pm 6$ ppm, $r^2 = 0.976$.

---

(54) Brown, K. L.; Brooks, H. B.; Zou, X.; Victor, M.; Ray, A.; Timkovich, R. *Inorg. Chem.* **1990**, *29*, 4841.

Table 4. Results of the Correlations of the Corrin Ring Carbon Chemical Shifts with the Axial Co$-$N$_{\mathrm{Bzm}}$ Bond Length and Glusker's Fold Angle[a]

carbon	A	B	C	N	r^2
C2	-3.59	0.036	56.5	7	0.94
C3	-5.96	-0.041	71.7	7	0.85
C4	-20.0	-0.007	222.7	7	0.96
C5	-7.02	0.145	121.6	7	0.94
C7	-1.35	0.163	53.5	7	0.86
C8	-10.7	-0.092	81.9	6[b]	0.87
C9	-18.0	-0.108	213.9	7	0.95
C11	-15.1	0.033	209.7	7	0.90
C12	-5.55	0.055	60.7	6[b]	0.97
C13	-2.90	-0.001	62.0	6[b]	0.96
C14	-6.76	0.052	180.9	6[b]	0.87
C16	-19.4	-0.003	220.8	7	0.94
C17	-3.83	0.076	67.9	7	0.96
C19	-4.20	0.043	85.1	7	0.98

[a] Equation 2. [b] The epiCbls have been omitted from the correlations of corrin ring carbons near the site of epimerization.

carbons (C2, C4, C5, C9, C11, C12, C13, C16, C17, and C19) gave correlations with $r^2 = 0.90-0.98$, and four additional carbons (C3, C7, C8, and C14) gave correlations with $r^2 = 0.85-0.88$ (Table 4). Even though the dependence on the Glusker fold angle is quite small, inclusion of this parameter improved the correlations significantly in virtually every case. Again, very similar results were obtained when $-\Delta G_{\mathrm{Co}}$ was substituted for $d_{\mathrm{Co-N_{Bzm}}}$ in eq 2. These correlations suggest that, for at least some corrin ring carbons, trends in the chemical shifts due to electronic and dipolar shielding effects can indeed be correlated with a structural ($d_{\mathrm{Co-N_{Bzm}}}$) or thermodynamic (ΔG_{Co}) parameter, although it seems as if conformational effects may also be important for these shifts.

^{13}C NMR and Conformation: The Epicobalamins. Carbon chemical shifts are largely determined by the paramagnetic shielding term, which is dominated by the hybridization of the carbon atom and its bonding partners.[43,55−58] Consequently, carbon chemical shifts are extremely sensitive to conformation.[59−62] Wilcox and Gleiter's correlation[58] of the ^{13}C chemical shifts of the bridge carbons of bicyclic hydrocarbons with changes in the calculated 2s orbital populations gives a linear dependence with a slope of 1115.6 ppm. Thus, changes in 2s orbital populations of just 1%, which would be associated with bond angle changes of just a few degrees, can have significant effects on carbon chemical shifts.

The cyanoepicobalamins and the isomeric CNCbl are an ideal system in which to look for evidence of conformational effects on Cbl chemical shifts since they all have the same inner sphere ligands, their inner sphere geometries are nearly identical, and the magnetic anisotropies of their Co atoms are very similar. As a result, differential electronic effects and differential dipolar shieldings of the carbon atoms in these complexes are expected to be very small.

For such comparisons, carbon atoms close to the site of epimerization must be avoided since the change in configuration will alter the magnetic environments in which these carbons find themselves. For CN-8-epiCbl, almost all of the carbons with chemical shifts significantly different from those in CNCbl occur at or near the site of epimerization.[15] However, 15 carbons remote from the epimerization site in CN-13-epiCbl (**1**) have chemical shifts significantly different from those of their counterparts in CNCbl. For all 15 of these carbons, calculations using McConnell's equation[48] show that the small differential magnetic anisotropy of the Co atom in CNCbl and CN-13-epiCbl (Table 2) makes only a minor contribution to the differences in chemical shift. In order to assess the significance of any conformational differences found for these carbons, we note that since the maximum esd for a bond length in these two structures is 0.012 Å, bond length differences of >0.017 Å are significant. Similar considerations suggest that bond angle differences >1.4° and torsion angle differences >2.1° are significant.

A complete analysis of the bond lengths, bond angles, and torsion angles for these 15 carbons (Supporting Information) shows that each has significant differences in one or more of these three conformational indicators. For example, for C7, for which the chemical shift differential is −0.33 ppm (and the differential dipolar shielding is only 0.07 ppm), three of seven bond angles and seven of nine torsion angles are significantly different between CNCbl and CN-13-epiCbl. For C5, one of three bond angles, four of eight torsion angles, and one of three bond distances are significantly different. We conclude that a detailed analysis of the ^{13}C chemical shifts and conformations of CNCbl and CN-13-epiCbl at carbons remote from the site of epimerization supports the idea that the chemical shifts of Cbl carbons are significantly affected by conformational differences.

Table 5. Results of the Correlations of Peripheral Carbon Chemical Shifts with the Deconvoluted Fold and Twist Angles[a]

carbon	A	B	C	N[b]	r^2
C20	-0.128	0.104	24.2	7	0.88
C25	-1.82	0.298	18.3	7	0.82
C37	0.076	0.009	44.2	5	0.86
C36	0.034	0.039	20.8	5	0.87
C53	-0.056	0.133	18.4	7	0.85
C49	-0.058	-0.035	38.5	6	0.81
C38	0.024	0.175	176.6	7	0.88
C57	-0.056	-0.081	178.8	7	0.81

[a] Equation 3. [b] The epiCbls have been omitted from the correlations of corrin ring carbons near the site of epimerization, and H$_2$OCbl$^+$, has been omitted from the correlations of C36 and C37.

^{13}C NMR and Conformation: The Peripheral Carbons. In recent work,[16] we attempted to use the differential ^{13}C chemical shifts of Cbl peripheral carbons as indicators of changes in corrin ring conformation (i.e., differential corrin fold angles). The current, more extensive data now permit an assessment of the extent to which ^{13}C chemical shifts of peripheral carbons depend on corrin ring fold and twist angles. Single-parameter correlations, using either Glusker's fold angle or our deconvoluted fold angle, gave very poor results in general. However, dual-parameter correlations using the deconvoluted fold and twist angles (eq 3) provided a substantial improvement.

$$\delta_{^{13}\mathrm{C}} = A(\text{fold angle}) + B(\text{twist angle}) + C \qquad (3)$$

For these correlations, the epiCbls were omitted from the correlations of carbons near the epimerized centers, and H$_2$OCbl$^+$ was omitted from the correlations of C36 and C37 because of the effect of the hydrogen bond to the coordinated water on these chemical shifts. As shown in Table 5, only eight of the 26 peripheral carbons gave statistically significant correlations, and for all of these correlations, $r^2 < 0.9$. We conclude that it will probably not be possible to use peripheral carbon chemical shifts as indicators of differential corrin ring folding.

---

(55) Farmun, D. G. *Adv. Phys. Org. Chem.* **1975**, *11*, 123.
(56) Criak, D. J.; Higgins, K. A. *Annu. Rep. NMR Spectrosc.* **1990**, *22*, 61.
(57) Yamanobe, T.; Ando, I. *J. Chem. Phys.* **1985**, *83*, 3154.
(58) Wilcox, C. F., Jr.; Gleiter, R. *J. Org. Chem.* **1989**, *54*, 2688.
(59) Chesnut, D. B. *Annu. Rep. NMR Spectrosc.* **1989**, *21*, 51.
(60) Chesnut, D. B.; Foley, C. K. *J. Chem. Phys.* **1986**, *84*, 852.
(61) Saito, H.; Ando, I. *Annu. Rep. NMR Spectrosc.* **1989**, *21*, 209.
(62) Ando, I.; Yamanobe, T.; Karosu, H.; Webb, G. *Annu. Rep. NMR Spectrosc.* **1990**, *22*, 205.

Acknowledgment. This research was supported by the National Institute of General Medical Sciences (Grant GM 48858), the National Science Foundation EPSCoR Program (Grant EHR 9108767), the State of Mississippi, and Mississippi State University.

Supporting Information Available: Tables of crystal data and structure refinement details, atomic coordinates, anisotropic temperature factors, bond angles and bond distances between adjacent non-hydrogen atoms, calculated hydrogen atom coordinates, and intermolecular contacts for the crystal structure of CN-13-epiCbl, 2D NMR correlations for CH_3Cbl and CN-13-epiCbl, observed ^{13}C chemical shifts for all seven base-on Cbls, and a comparison of bond angles, torsion angles, and bond lengths in CNCbl and CN-13-epiCbl for the 15 carbon atoms remote from the site of epimerization but showing significant differences in ^{13}C chemical shift (48 pages). Ordering information is given on any current masthead page.

IC950951R