X-ray Crystallographic and Two-Dimensional NMR Investigations of a Coenzyme B_{12} Analogue with 5′-Deoxyadenosine Replaced by 9-$(CH_2)_3$-Adenine

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Abstract: The structure of (adeninylpropyl)cobalamin (AdePrCbl), a coenzyme B_{12} ((5′-deoxyadenosyl)cobalamin) analogue in which the ribose moiety of the adenosyl group has been replaced by a propylene chain, has been determined by X-ray diffraction methods. AdePrCbl crystallizes in the orthorhombic space group $P2_12_12_1$, with $Z = 4$, $a = 23.868$ (9) Å, $b = 21.024$ (7) Å, $c = 16.047$ (4) Å, and $V = 8053.07$ Å^3. The final R value is 0.100 based on 6621 observed reflections. The general conformations of the corrin ring, benzimidazole, phosphate, and ribose in AdePrCbl are very similar to those of (5′-deoxyadenosyl)cobalamin and methylcobalamin except for the amide side chains, which show some variability in the orientations of their amide groups. The adenine ring in AdePrCbl lies over the D ring of the corrin system, rotated about 120° clockwise from its position in coenzyme B_{12}. The ten water molecules in the crystal structure of AdePrCbl are well located and show no evidence of disorder. Complete ^{1}H and ^{13}C NMR assignments of AdePrCbl have been made by using the following two-dimensional NMR methods: homonuclear Hartmann–Hahn spectroscopy (HOHAHA), rotating frame Overhauser enhancement spectroscopy (ROESY), ^{1}H-detected heteronuclear multiple-quantum-coherence (HMQC) spectroscopy, and ^{1}H-detected multiple-bond heteronuclear multiple-quantum-coherence spectroscopy (HMBC). In addition to the adenine orientation found in the crystal structure, a second orientation, in which the adenine lies over the B ring of the corrin, is suggested by ^{1}H NOEs and by a comparison of the ^{1}H and ^{13}C shifts of AdePrCbl to those of coenzyme B_{12}. Our results suggest that alkyladenine groups in cobalamins may have a highly fluxional character permitting several orientations of the adenine. Previous studies have shown that binding to the B_{12}-dependent enzymes ribonucleotide reductase and diol dehydrase is tighter for (adeninylpentyl)cobalamin than for coenzyme B_{12} and the other (adeninylalkyl)cobalamins. On the basis of our studies, we conclude that the flexibility of the alkyl chain, exhibited by the fluxional character of the alkyladenine group, and the orientation of the adenine ring could be responsible for the increased affinity of this analogue for the enzyme. Differences in the orientation of the adenine and the fluxional character of the alkyladenine group, in addition to corrin ring flexibility, may also be useful in explaining the changes in the circular dichroism spectra of (adeninylalkyl)cobalamins upon binding to ethanolamine ammonia-lyase.

Introduction

(Adeninylalkyl)cobalamins[1] (Ade$(-CH_2-)_n$Cbls) are coenzyme B_{12} ((5′-deoxyadenosyl)cobalamin, AdoCbl) analogues in which an adenine residue is attached to the cobalt by a methylene chain from two to six carbons in length. These analogues have been used to gain insight into the homolytic cleavage of the Co–C bond in AdoCbl by B_{12}-dependent enzymes; homolysis of this bond is an essential step in the catalytic cycle.[2–5] While inactive, the analogues are very good inhibitors of the extensively studied B_{12}-dependent enzymes, ribonucleotide reductase,[1,6] diol dehydrase,[7] and ethanolamine ammonia-lyase.[8,9] The binding of the Ade$(-CH_2-)_n$Cbls to all three enzymes was found to be strong. Therefore, factors other than binding affinity are probably responsible for the inactivity.

It has been proposed that the B_{12}-dependent enzymes promote homolysis of the Co–C bond by the application of a stretching force on the Co–C bond by interaction with the adenosyl residue and the corrin ring.[10] The energy for this distorting force may originate from the energy released when the coenzyme binds to the enzyme. This proposal was based on the results of photolysis[8–10] and CD (circular dichroism) studies[8,9] of the Ade$(-CH_2-)_n$Cbls with ethanolamine ammonia-lyase. The CD spectra of the longer chain analogues (number of methylene groups = 4–6) changed on binding to the enzyme, suggesting a change in the conformation of the corrin ring.[8,9] Such a change was not observed with AdoCbl or (adeninylethyl)cobalamin (AdeEtCbl).[8] (Adeninylpropyl)cobalamin (AdePrCbl) showed less change in its CD spectrum upon binding to the enzyme than did the longer chain analogues.[8,9] It was suggested that binding of the cobalamins to the active site activates forces which tend to induce a change in the conformation of the enzyme and also an adjustment in the corrin ring.[8] The lack of change in the CD spectra of AdoCbl and AdeEtCbl upon binding to the enzyme was explained by steric constraints imposed on the corrin ring by the β-alkyl groups. (The β side of the corrin is the side on which the 5′-deoxyadenosyl group is attached in AdoCbl, while the α side is where the 5,6-dimethylbenzimidazole moiety is attached.) It was suggested that these constraints prevent the enzyme from assuming its energetically favored conformation and that, in the enzymatic cycle, AdoCbl Co–C bond homolysis is required for the enzyme to assume its favored conformation.[8]

For the longer chain analogues no photolysis of the Co–C bond of the enzyme-bound analogues was observed.[9,10] The Co–C bond in enzyme-bound AdoCbl is readily photolyzed. These photolysis results provide additional support for the hypothesis that the flexibility of the longer chain Ade$(-CH_2-)_n$Cbls allows the enzyme to assume its favored conformation without homolysis occurring. Since the longer chain analogues allow the enzyme to adopt its favored conformation, there is no conformational restriction to prevent light cleaved fragments from recombining.[10] These analogues do undergo photolysis in aqueous solution in the absence of enzyme.[8]

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(1) Sando, G. N.; Blakely, R. L.; Hogenkamp, H. P. C.; Hoffmann, P. J. *J. Biol. Chem.* **1975**, *250*, 8774–8779.
(2) *B_{12}*: Dolphin, D., Ed.; Wiley: New York, 1982.
(3) Halpern, J. *Science (Washington, D.C.)* **1985**, *227*, 869–875.
(4) Bresciani-Pahor, N.; Forcolin, M.; Marzilli, L. G.; Randaccio, L.; Summers, M. F.; Toscano, P. J. *Coord. Chem. Rev.* **1985**, *63*, 1–125.
(5) Finke, R. G.; Schiraldi, D. A.; Mayer, B. J. *Coord. Chem. Rev.* **1984**, *54*, 1–22.
(6) Sando, G. N.; Grant, M. E.; Hogenkamp, H. P. C. *Biochim. Biophys. Acta* **1976**, *428*, 228–232.
(7) Toraya, T.; Ushio, K.; Fukui, S.; Hogenkamp, H. P. C. *J. Biol. Chem.* **1977**, *252*, 963–970.
(8) Krouwer, J. S.; Holmquist, B.; Kipnes, R. S.; Babior, B. M. *Biochim. Biophys. Acta* **1980**, *612*, 153–159.
(9) Babior, B. M. In *Proceedings of the Third European Symposium on Vitamin B_{12} and Intrinsic Factor*; Zagalak, B., Friedrich, W., Eds.; Walter de Gruyter & Co.: Berlin, 1979; pp 461–470.
(10) Babior, B. M.; Krouwer, J. S. *CRC Crit. Rev. Biochem.* **1979**, *6*, 35–102.

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The inhibition constants, K_i, for the longer chain Ade($-CH_2-)_n$Cbls can be compared to K_m, the Michaelis constant, for the AdoCbl/enzyme complex. In the ribonucleotide reductase system K_i is 16.1, 0.24, and 4.5 μM for the n = 4, 5, and 6 analogues, respectively, compared to a K_m of 4.1 μM with AdoCbl.[6] In the diol dehydrase system K_i is 0.47 and 0.27 μM for the n = 4 and 5 analogues, respectively, compared to a K_m of 0.8 μM with AdoCbl.[7] These K_i values indicate that the longer chain Ade($-CH_2-)_n$Cbls bind to the enzyme nearly as tightly or, in some cases, even more tightly than AdoCbl. The explanation for the higher affinity appears to rest in the requirement for less energy for the analogue/enzyme system to adopt its favored conformation compared to the AdoCbl/enzyme system.[10] This lower energy requirement translates into a more stable adduct. On the basis of the finding that (adeninylpentyl)cobalamin (AdePeCbl) binds more tightly to ribonucleotide reductase[1,6] and diol dehydrase[7] than the other Ade($-CH_2-)_n$Cbls, it was suggested that the five-carbon chain of AdePeCbl is best able to mimic A11, the ring oxygen, A14, and A15 of the ribose moiety of AdoCbl.[1] Since fewer adjustments of the five-carbon chain are required than for chains of other lengths, the energy saved may be reflected as an optimal enzyme–cobalamin interaction.

Corey–Pauling–Koltun models of the longer chain analogues have been used to demonstrate that four-, five-, and six-carbon chains allow the positioning of the adenine moiety over ring C of the corrin nucleus[6] as has been found in the crystal structure of AdoCbl.[11] Two-dimensional (2D) NMR studies of AdoCbl[12,13] have suggested that there is a fluxional character to the adenosyl group. It is possible that the trends observed for binding and CD spectral changes in Ade($-CH_2-)_n$Cbls reflect the need for either a limited fluxional character of the β ligand or a particular orientation of the adenine ring rather than corrin ring flexibility. The differences observed between the CD spectra of AdeEtCbl and the longer chain analogues may result from an electronic or steric effect of the alkyl group on dimethylbenzimidazole binding. The proximity of the adenine moiety to the carbon bound to cobalt may have a large inductive effect on the binding of the trans ligand.

The electronic and solution structural properties of Ade($-CH_2-)_n$Cbls can be determined through NMR spectroscopy. The complexity of these molecules requires that 2D NMR methods be used. 2D NOE experiments can give structural information about these analogues,[12-14] possibly determining the orientation of the adenine moiety relative to the corrin ring in solution. Assignment of the ^{13}C NMR spectrum will allow a comparison of the electronic and structural properties of the analogues with coenzyme B_{12}. Modern 2D NMR techniques, which were used previously to assign completely the ^{1}H and ^{13}C NMR spectra of coenzyme B_{12},[12,13] can be used to make complete assignments of the ^{1}H and ^{13}C NMR spectra of Ade($-CH_2-)_n$Cbls. In this paper we discuss the assignments by modern 2D NMR techniques[15-18] of the ^{1}H and ^{13}C NMR spectra of one of the Ade($-CH_2-)_n$Cbls, AdePrCbl. This is only the second alkylcobalamin for which such methods have been used.

X-ray crystallography is currently the best method to provide structural information for cobalamins. Since there are often not enough NOE cross peaks in the 2D NMR spectra of cobalamins to obtain a definitive structure, more information about the orientation of the adenine moiety in Ade($-CH_2-)_n$Cbls can be provided by X-ray crystallography than by 2D NMR. X-ray crystal structures of the Ade($-CH_2-)_n$Cbls can establish the orientation of the adenine moiety relative to the corrin ring in the crystalline state. Also, X-ray crystal structures of the analogues may reveal differences between the corrin rings of the analogues and of AdoCbl. To date, only four crystal structures of cobalamins containing a Co–C (alkyl) bond, including AdoCbl, have been reported.[20-22] We report the structure of AdePrCbl, only the second cobalamin containing the important adenine ring. Since we have fully assigned the ^{1}H and ^{13}C NMR spectra and obtained an X-ray crystal structure of AdePrCbl, we can compare the structural and electronic properties of AdePrCbl and AdoCbl.

Experimental Section

Reagents. All reagents were purchased from Aldrich, except for hydroxocobalamin which was purchased from Roussel Corporation (Englewood, NJ) and used without further purification.

Preparations. 9-(3-Chloropropyl)adenine was prepared by a procedure similar to that reported by Carraway et al.[19]

(Adeninylpropyl)cobalamin. A solution of $NaBH_4$ (0.13 g, 3.3 mmol in 5 mL of H_2O) was added to a solution of hydroxocobalamin (1 g, 0.75 mmol) in H_2O (50 mL) under N_2, followed by a solution of 9-(3-chloropropyl)adenine (1.6 g, 7.5 mmol, dissolved in 200 mL of ethanol with heat and N_2 purging). After the solution was stirred for 30 min, the excess $NaBH_4$ was quenched with acetone, and the N_2 purging was stopped. The solution was concentrated on a rotary evaporator, and the excess of 9-(3-chloropropyl)adenine was removed by filtration. The solution was desalted on an Amberlite XAD-2 column. The product was purified with a SP-Sephadex column by elution with H_2O. The eluent was concentrated with a rotary evaporator, and the product was precipitated by addition of acetone. Crystals were grown by acetone diffusion into an H_2O/methanol (90% H_2O) solution of the product.

Other Ade($-CH_2-)_n$Cbls. The other Ade($-CH_2-)_n$Cbls (n = 2, 4, and 5) were prepared from the corresponding 9-(ω-chloroalkyl)adenine[19] compounds by using the same procedure described above for AdePrCbl.

2D NMR Spectroscopy. All of the 2D NMR experiments were performed on a sample containing 7.2 mg of AdePrCbl in 0.5 mL of D_2O and having a pH of 8.3. All of the 2D NMR experiments were performed on a General Electric GN-500 spectrometer at room temperature (20 °C), except the ROESY (rotating frame Overhauser enhancement spectroscopy) experiment which was performed at 5 °C, without sample spinning. Proton and carbon chemical shifts were referenced to internal TSP. All 2D spectra were processed by using the FTNMR program (Hare Research, Inc., Woodinville, WA).

HOHAHA (Homonuclear Hartmann–Hahn) Spectroscopy.[15] The HOHAHA spectra resulted from a 256 × 2048 data matrix size with 16 scans per t_1 value. Delay time between scans was 1.0 s. An MLEV-17 mixing sequence of 67.3 or 10.6 ms preceded and followed by 2.0-ms trim pulses was used. Six watts of power provided a 51-μs 90° ^{1}H pulse width. A Gaussian function with a line broadening of −1 Hz and a Gaussian coefficient of 0.1 was used prior to Fourier transformation in the t_2 dimension. A cosine bell squared filter was used prior to Fourier transformation in the t_1 dimension.

Spin-Locked NOE Spectroscopy (ROESY).[16] The ROESY spectrum resulted from a 400 × 2048 data matrix size with 32 scans per t_1 value. The delay time between scans was 2.1 s, and the mixing time was 200 ms. A 4.9 kHz rf field strength (51-μs 90° pulse width) was used. A Gaussian function with a line broadening of −1 Hz and a Gaussian coefficient of 0.1 was used prior to Fourier transformation in the t_2 dimension. A cosine bell squared filter was used prior to Fourier transformation in the t_1 dimension.

HMQC (^{1}H-Detected Heteronuclear Multiple-Quantum-Coherence) Spectroscopy.[17] The one-bond ^{1}H–^{13}C shift correlation spectra resulted from a 256 × 1024 data matrix size with 448 scans per t_1 value (preceded by four dummy scans). Delay time between scans was 0.8 s. Forty-one watts of ^{13}C rf power and a 38-μs 90° pulse width were used. A combination of a Gaussian and a sine bell function was used prior to Fourier transformation in the t_2 dimension. A sine bell filter function was used prior to Fourier transformation in the t_1 dimension.

(11) Glusker, J. P. In *B_{12}*; Dolphin, D., Ed.; Wiley: New York, 1982; Vol. 1, Chapter 3, pp 23–106, and references cited therein.
(12) Summers, M. F.; Marzilli, L. G.; Bax, A. *J. Am. Chem. Soc.* **1986**, *108*, 4285–4294.
(13) Bax, A.; Marzilli, L. G.; Summers, M. F. *J. Am. Chem. Soc.* **1987**, *109*, 566–574.
(14) Pagano, T. G.; Yohannes, P. G.; Hay, B. P.; Scott, J. R.; Finke, R. G.; Marzilli, L. G. *J. Am. Chem. Soc.* **1989**, *111*, 1484–1491.
(15) Braunschweiler, L.; Ernst, R. R. *J. Magn. Reson.* **1983**, *53*, 521–528. Davis, D. G.; Bax, A. *J. Am. Chem. Soc.* **1985**, *107*, 2820–2821. Bax, A.; Davis, D. G. *J. Magn. Reson.* **1985**, *65*, 355–360.
(16) Bothner-By, A. A.; Stephens, R. L.; Lee, J.; Warren, C. D.; Jeanloz, R. W. *J. Am. Chem. Soc.* **1984**, *106*, 811–813. Bax, A.; Davis, D. G. *J. Magn. Reson.* **1985**, *63*, 207–213.
(17) Müller, L. *J. Am. Chem. Soc.* **1979**, *101*, 4481–4484. Bax, A.; Subramanian, S. *J. Magn. Reson.* **1986**, *67*, 565–569.
(18) Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093–2094.
(19) Carraway, K. L.; Huang, P. 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, pp 3–5.
(20) Lenhert, P. G.; Hodgkin, D. C. *Nature (London)* **1962**, *192*, 937.
(21) Rossi, M.; Glusker, J. P.; Randaccio, L.; Summers, M. F.; Toscano, P. J.; Marzilli, L. G. *J. Am. Chem. Soc.* **1985**, *107*, 1729–1738.
(22) Alcock, N. W.; Dixon, R. M.; Golding, B. T. *J. Chem. Soc., Chem. Commun.* **1985**, 603–605.

Table I. Crystal Data for (Adeninylpropyl)cobalamin

formula	$C_{70}H_{98}N_{18}O_{14}PCo{\cdot}10H_2O$
formula weight	1685.76 (including H_2O)
$F(000)$	3196
space group	$P2_12_12_1$
Z	4
a, Å	23.868 (9)
b, Å	21.024 (7)
c, Å	16.047 (4)
V, Å^3	8053.07
D_x, g cm^{-3}	1.243
λ (Mo Kα), Å	0.70926
μ (Mo Kα), cm^{-1}	2.620
crystal size, mm^3	0.12 × 0.12 × 0.2
temperature, °C	−110

HMBC (¹H-Detected Multiple-Bond Heteronuclear Multiple-Quantum-Coherence) Spectroscopy.[18] The multiple-bond ¹H–¹³C shift correlation spectra resulted from a 256 × 1024 data matrix size with 256 scans (preceded by four dummy scans) per t_1 value and a delay time between scans of 1.0 s. Forty-one watts of power and a 38-μs 90° ¹³C pulse width were used. Values of Δ_1 (the delay between the first 90° proton pulse and the first 90° ¹³C pulse) and Δ_2 (the delay between the first and second 90° ¹³C pulses) were 3.3. and 50 ms, respectively. In the t_2 and t_1 dimensions, a sine bell filter was used prior to Fourier transformation.

Crystal Data Collection. A red crystal of AdePrCbl of dimensions 0.12 × 0.12 × 0.2 mm was mounted with silicon grease at the end of a glass fiber; the low temperatures subsequently used served to seal the crystal to the fiber. Cell parameters (Table I) were obtained from 25 well-centered reflections. Data were collected at low temperature, −110 °C, on a Nicolet P2₁ four-circle diffractometer with Mo Kα radiation and a graphite monochromator. The ω scan technique (bisecting mode) was used to measure 7119 unique reflections to a 2θ limit of 55°, at variable scan rates. The scan/background time ratio was 0.4, and 6621 reflections with $I > 2\sigma(I)$ (where $\sigma(I)$ was determined from counting statistics) were considered observed and included in further calculations. There was no decay in intensity of the check reflections. Values of $\sigma(F)$ were calculated as $\sigma(F) = (F/2)[\sigma^2(I)/(I)^2 + \delta^2]^{1/2}$, where δ (= 0.038) is an instrumental uncertainty determined from the variation in the intensity of the check reflections. A correction for anomalous scattering and the empirical absorption correction of Walker and Stuart[23] were applied. The data were corrected for Lorentz and polarization effects and put on an absolute scale with a Wilson plot.

Structure Determination and Refinement. The structure of AdePrCbl was solved by locating the cobalt atom from the Patterson map and then locating the lighter atoms in subsequent electron density maps. After five cycles of calculations of electron density maps, all of the 114 non-hydrogen atoms (including ten water oxygen atoms) were located. The positions of hydrogen atoms were calculated but not refined.

The atomic positions and anisotropic thermal parameters of all non-hydrogen atoms were refined by a full-matrix least-squares computer program. The quantity minimized was $\sum w(||F_o| - |F_c||)^2$ where the weights, w, were $1/\sigma^2(F)$. Atomic scattering factors for non-hydrogen atoms were those listed in the *International Tables for X-ray Crystallography*[24] and for hydrogen atoms those of Stewart, Davidson, and Simpson.[25] The final value of $R = \sum||F_o| - |F_c||/\sum|F_o|$ is 0.100, and the weighted R value, $wR = (\sum w(||F_o| - |F_c||)^2/\sum w(|F_o|)^2)^{1/2}$, is 0.079. The maximum shift in the atomic parameters is less than one standard deviation. The highest peak in the final difference Fourier map is 0.6 e Å^{-3}. Fractional coordinates of non-hydrogen atoms are listed in Table II; temperature factors of these atoms and fractional coordinates for hydrogen atoms are given in supplementary material Tables A and B, respectively. Observed and calculated structure factors are reported in supplementary material Table C.

Results

X-ray Structural Results. The molecular structure of AdePrCbl is illustrated in Figure 1, which also shows the numbering system adopted. A list of bond distances is given in supplementary material Table D. The arrangement of atoms attached to the cobalt atom forms a distorted octahedron because of the direct linkage of rings A and D. The distortions appear in the angles N(21)–Co–N(22), N(22)–Co–N(23), N(23)–Co–N(24), and N(24)–Co–N(21), which are 90.8°, 96.5°, 89.9°, and 83.0°, respectively.

(23) Walker, N.; Stuart, D. *Acta Crystallogr.* **1983**, *A39*, 158–166.
(24) Vol. IV, Revised and Supplementary Tables to Volumes II and III. In *International Tables for X-ray Crystallography*; Ibers, J. A., Hamilton, W. C., Eds.; The Kynoch Press: Birmingham, 1974; pp 71–98.

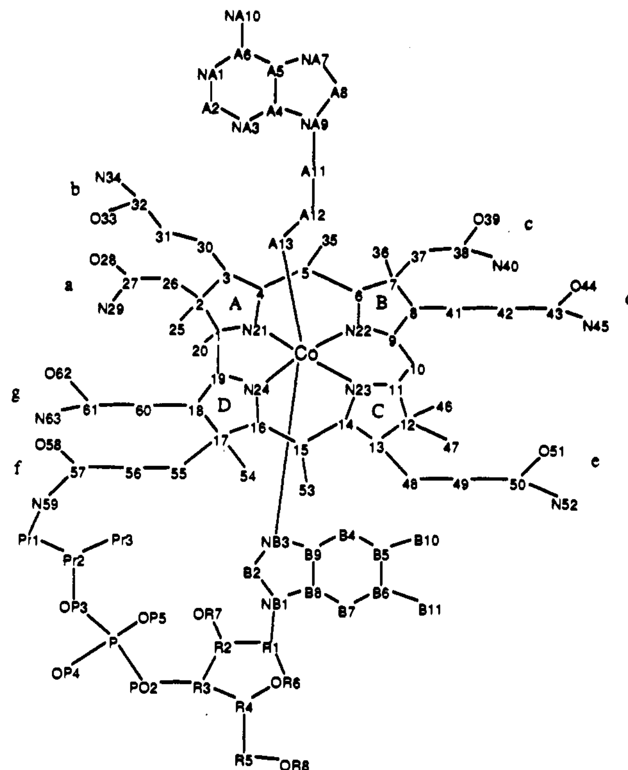

Figure 1. Molecular structure of adeninylpropylcobalamin showing numbering system adopted.

A list of torsion angles of the side chains in AdePrCbl, AdoCbl,[26] methylcobalamin (MeCbl)[11], wet B_{12},[11] and dry B_{12}[11] is presented in Table III. Side chain c is the most variable, and side chains b and e show variability in only one of the three torsion angles reported for each of them. However, there seem to be mainly two types of conformation for the b, c, and e side chains in all the five structures analyzed here.

The adenine ring in the upper axial substituent of AdePrCbl lies almost parallel to the corrin ring. The plane of the adenine ring makes an angle of 6.3° with the best plane through the four nitrogen atoms in the corrin system. In AdePrCbl, the adenine ring is rotated about 120° clockwise in this parallel plane from the position in the adenosyl coenzyme and lies mainly over the D ring of the corrin. The distances from C(19) to C(A2), N(A3), C(A4), and N(A9) are within the interval 4.61–5.00 Å; from C(16) to C(A8) and N(A9) are 4.89 and 4.85 Å, respectively; from N(24) to C(A4) and N(A9) are 4.67 and 4.71 Å, respectively; and from C(17) to C(A5) is 4.97 Å.

Unlike other B_{12} crystal structures, the crystal structure of AdePrCbl shows no evidence of disorder in the ten water molecules; they are well-located. The hydrogen-bonding network is described in Table IV. Most of the nitrogen and oxygen atoms of the amide side chains of AdePrCbl are involved in hydrogen bonding to water molecules or to neighboring AdePrCbl molecules. There are no water molecules that are solely hydrogen bonded to other water molecules; this may be one of the reasons that the crystal structure is so well-ordered.

The AdePrCbl molecules are closely packed together, with the main planar groups of neighboring molecules making angles of 45° to one another. The corrin ring plane is almost parallel to the *bc* plane of the crystal, and the axial Co–N(Bzm) and Co–C bonds lie approximately parallel to the *a* axis.

Assignment of the ¹H NMR Spectrum. Most of the proton signals of AdePrCbl were assigned by using two-dimensional HOHAHA and ROESY spectra. We designate the ¹H nuclei by the carbon atom to which they are attached. For nonequivalent geminal protons, H′ and H″ refer to the protons with the downfield and upfield signals, respectively. We designate the ¹³C nuclei by

Table II. Atomic Coordinates and Their esd's

atom	x/a	y/b	z/c	atom	x/a	y/b	z/c
Co	−0.0466 (1)	−0.1018 (1)	−0.0137 (1)	C(57)	0.1248 (5)	0.1128 (5)	−0.2163 (6)
C(1)	0.0084 (4)	−0.0245 (4)	0.1109 (5)	O(58)	0.1019 (3)	0.1153 (3)	−0.2881 (4)
C(2)	−0.0038 (4)	−0.0112 (4)	0.2077 (5)	N(59)	0.1743 (4)	0.1377 (4)	−0.2003 (5)
C(3)	−0.0110 (4)	−0.0813 (4)	0.2395 (6)	C(60)	0.0080 (5)	0.1383 (4)	0.0718 (6)
C(4)	−0.0372 (4)	−0.1145 (4)	0.1640 (5)	C(61)	0.0484 (5)	0.1912 (4)	0.0492 (6)
C(5)	−0.0668 (4)	−0.1734 (4)	0.1674 (5)	O(62)	0.0992 (3)	0.1782 (3)	0.0382 (4)
C(6)	−0.0768 (4)	−0.2090 (4)	0.0970 (6)	N(63)	0.0300 (4)	0.2501 (4)	0.0450 (5)
C(7)	−0.1022 (4)	−0.2772 (4)	0.0902 (6)	P	0.2900 (1)	0.0143 (1)	−0.2442 (2)
C(8)	−0.0816 (4)	−0.2970 (4)	0.0023 (5)	O(P2)	0.2275 (3)	−0.0081 (3)	−0.2226 (5)
C(9)	−0.0787 (4)	−0.2318 (4)	−0.0387 (5)	O(P3)	0.2788 (3)	0.0880 (3)	−0.2515 (4)
C(10)	−0.0848 (5)	−0.2232 (5)	−0.1240 (6)	O(P4)	0.3079 (3)	−0.0147 (3)	−0.3245 (4)
C(11)	−0.0781 (4)	−0.1670 (4)	−0.1669 (6)	O(P5)	0.3275 (3)	0.0065 (3)	−0.1719 (4)
C(12)	−0.0905 (5)	−0.1577 (4)	−0.2594 (6)	C(PR1)	0.2085 (5)	0.1638 (5)	−0.2670 (7)
C(13)	−0.0559 (4)	−0.0972 (4)	−0.2775 (5)	C(PR2)	0.2417 (4)	0.1133 (5)	−0.3140 (7)
C(14)	−0.0504 (4)	−0.0662 (4)	−0.1912 (5)	C(PR3)	0.2752 (5)	0.1405 (6)	−0.3848 (7)
C(15)	−0.0337 (4)	−0.0043 (4)	−0.1795 (5)	N(B1)	0.1129 (3)	−0.1701 (4)	−0.1131 (5)
C(16)	−0.0218 (4)	0.0186 (4)	−0.0967 (5)	C(B2)	0.0664 (4)	−0.1339 (4)	−0.1062 (5)
C(17)	−0.0076 (4)	0.0883 (4)	−0.0761 (6)	N(B3)	0.0371 (3)	−0.1434 (3)	−0.0367 (4)
C(18)	0.0144 (4)	0.0809 (4)	0.0155 (6)	C(B4)	0.0640 (4)	−0.2157 (4)	0.0862 (6)
C(19)	−0.0182 (4)	0.0222 (4)	0.0456 (5)	C(B5)	0.1028 (4)	−0.2585 (4)	0.1162 (6)
C(20)	0.0684 (4)	−0.0366 (4)	0.0918 (5)	C(B6)	0.1503 (4)	−0.2748 (4)	0.0658 (7)
N(21)	−0.0263 (3)	−0.0838 (3)	0.0952 (4)	C(B7)	0.1569 (4)	−0.2465 (4)	−0.0124 (7)
N(22)	−0.0686 (3)	−0.1846 (3)	0.0172 (5)	C(B8)	0.1174 (4)	−0.2039 (4)	−0.0410 (5)
N(23)	−0.0597 (3)	−0.1113 (3)	−0.1289 (4)	C(B9)	0.0708 (4)	−0.1873 (4)	0.0071 (6)
N(24)	−0.0258 (3)	−0.0181 (3)	−0.0310 (4)	C(B10)	0.0948 (5)	−0.2885 (5)	0.2005 (6)
C(25)	0.0424 (4)	0.0247 (4)	0.2505 (6)	C(B11)	0.1923 (5)	−0.3219 (5)	0.0978 (8)
C(26)	−0.0601 (4)	0.0229 (4)	0.2206 (6)	C(R1)	0.1588 (4)	−0.1559 (5)	−0.1687 (5)
C(27)	−0.0809 (5)	0.0231 (5)	0.3112 (6)	C(R2)	0.1935 (4)	−0.0974 (4)	−0.1414 (5)
O(28)	−0.0532 (3)	0.0487 (4)	0.3659 (4)	C(R3)	0.2131 (4)	−0.0739 (4)	−0.2238 (6)
N(29)	−0.1293 (4)	−0.0055 (5)	0.3271 (5)	C(R4)	0.1639 (4)	−0.0852 (4)	−0.2803 (6)
C(30)	0.0404 (4)	−0.1184 (4)	0.2739 (5)	C(R5)	0.1755 (5)	−0.0958 (5)	−0.3720 (7)
C(31)	0.0528 (5)	−0.1045 (5)	0.3632 (6)	O(R6)	0.1364 (3)	−0.1427 (3)	−0.2480 (4)
C(32)	0.0947 (5)	−0.1505 (6)	0.3988 (6)	O(R7)	0.1576 (3)	−0.0532 (3)	−0.1042 (4)
O(33)	0.0827 (3)	−0.2041 (4)	0.4198 (5)	O(R8)	0.2111 (4)	−0.1488 (4)	−0.3838 (5)
N(34)	0.1446 (4)	−0.1280 (5)	0.4035 (6)	N(A1)	−0.1846 (6)	0.1574 (5)	0.1305 (8)
C(35)	−0.0830 (5)	−0.1947 (5)	0.2535 (7)	C(A2)	−0.1911 (6)	0.0976 (7)	0.1586 (10)
C(36)	−0.0832 (5)	−0.3256 (5)	0.1542 (6)	N(A3)	−0.2048 (5)	0.0438 (5)	0.1134 (7)
C(37)	−0.1670 (5)	−0.2745 (5)	0.0830 (7)	C(A4)	−0.2103 (5)	0.0620 (6)	0.0318 (8)
C(38)	−0.2021 (4)	−0.2336 (5)	0.1433 (7)	C(A5)	−0.2077 (5)	0.1207 (5)	−0.0002 (8)
O(39)	−0.2165 (4)	−0.1803 (4)	0.1265 (5)	C(A6)	−0.1897 (7)	0.1709 (8)	0.0493 (10)
N(40)	−0.2169 (4)	−0.2625 (4)	0.2160 (6)	N(A7)	−0.2159 (4)	0.1178 (4)	−0.0864 (7)
C(41)	−0.0232 (4)	−0.3299 (4)	0.0024 (6)	C(A8)	−0.2241 (6)	0.0561 (7)	−0.1011 (8)
C(42)	0.0077 (5)	−0.3292 (5)	−0.0818 (6)	N(A9)	−0.2206 (4)	0.0186 (4)	−0.0343 (6)
C(43)	0.0643 (5)	−0.3593 (4)	−0.0846 (7)	N(A10)	−0.1743 (8)	0.2267 (5)	0.0212 (9)
O(44)	0.0952 (3)	−0.3504 (3)	−0.1466 (4)	C(A11)	−0.2262 (5)	−0.0493 (7)	−0.0344 (9)
N(45)	0.0807 (4)	−0.3938 (4)	−0.0214 (5)	C(A12)	−0.1674 (5)	−0.0815 (5)	−0.0527 (8)
C(46)	−0.1543 (5)	−0.1427 (5)	−0.2699 (6)	C(A13)	−0.1225 (4)	−0.0718 (4)	0.0107 (6)
C(47)	−0.0768 (5)	−0.2168 (5)	−0.3090 (6)	O(W1)	0.2169 (3)	0.0132 (4)	0.0092 (5)
C(48)	0.0039 (4)	−0.1083 (4)	−0.3099 (5)	O(W2)	0.0874 (3)	0.0275 (3)	−0.4203 (5)
C(49)	0.0103 (4)	−0.1278 (4)	−0.4044 (6)	O(W3)	−0.3448 (3)	0.0204 (4)	0.0062 (5)
C(50)	−0.0291 (4)	−0.0919 (4)	−0.4581 (6)	O(W4)	0.2120 (4)	0.1457 (4)	−0.0224 (5)
O(51)	−0.0706 (3)	−0.1169 (3)	−0.4920 (4)	O(W5)	0.1793 (3)	0.0536 (3)	0.4766 (4)
N(52)	−0.0147 (4)	−0.0314 (4)	−0.4731 (5)	O(W6)	−0.2125 (4)	−0.0653 (5)	0.2137 (5)
C(53)	−0.0285 (4)	0.0379 (4)	−0.2547 (6)	O(W7)	−0.0806 (4)	0.2344 (4)	−0.2243 (6)
C(54)	−0.0632 (4)	0.1264 (4)	−0.0764 (6)	O(W8)	−0.1596 (4)	−0.3625 (4)	0.3183 (5)
C(55)	0.0364 (5)	0.1210 (4)	−0.1297 (5)	O(W9)	−0.3308 (4)	−0.0450 (5)	0.1610 (6)
C(56)	0.0913 (4)	0.0853 (4)	−0.1458 (6)	O(W10)	0.1904 (5)	−0.2730 (4)	−0.3305 (7)

the numbers of Figure 1 with the numbers preceded by the following designations: C for carbons of the corrin ring and its side chains, A for carbons of the propyladenine moiety, B for carbons of the 5,6-dimethylbenzimidazole moiety, R for carbons of the ribose moiety, and Pr for carbons of the propanolamine group.

We will first discuss the HOHAHA spectra (Figures 2, S1, S2, and S3) that were used to group the proton signals into *J* coupled networks and assign the protons within the networks. Two HOHAHA experiments were performed, one with a short mixing time and one with a longer mixing time. Since it is sometimes extremely difficult to distinguish between direct and relayed connectivities in the HOHAHA experiment,[12] the experiment with a short mixing time (10.6 ms) was used to determine the direct connectivities that were indistinguishable from the relayed connectivities in the HOHAHA experiment with a longer mixing time (67.3 ms). From the short mixing time HOHAHA spectrum (Figure S3) the ribose protons are easily assigned starting with the anomeric ribose proton, R1H, identified as the only doublet in the downfield region of the ^{1}H NMR spectrum. R1H shows a correlation to R2H which shows connectivity to R3H. R3H shows a correlation to R4H which shows connectivities to R5H′ and R5H″ completing the assignment of the ribose protons.

The propanolamine protons were also assigned from the short mixing time HOHAHA spectrum (Figure S3) starting with $Pr3H_3$. The doublet at 1.21 ppm corresponding to three protons is identified as $Pr3H_3$ since it is the only methyl group in the molecule attached to a protonated carbon. $Pr3H_3$ shows connectivity to Pr2H which shows correlations to Pr1H′ and Pr1H″, completing the assignment of the propanolamine protons.

From the longer mixing time HOHAHA spectrum (Figure 2) the methylene proton signals of the b, d, e, f, and g side chains were identified. These assignments were based on the assignments (from the ROESY spectrum, see below) of the methine protons to the corrin ring which are coupled to the methylene protons of

Table III. Side-Chain Torsion Angles (deg) and Data on (Adeninylpropyl)cobalamin (AdePrCbl, This Work), Adenosylcobalamin (AdoCbl[37]), Methylcobalamin (MeCbl[21]), Wet B_{12} (Cyanocobalamin[11]), and Dry B_{12} (Cyanocobalamin[11])

bond	side chain																	
	a		b			c		d			e			f			g	
(see below)	1	2	3	4	5[a]	6[a]	7[a]	8	9	10	11	12	13[a]	14	15	16	17	18
AdePrCbl	166	58	86	−157	168	−49	−157	−160	−48	179	77	−169	40	−48	66	165	170	−72
AdoCbl	166	59	82	−161	59	−180	71	−169	−58	−174	83	−167	−145	−47	68	166	156	−84
MeCbl	180	66	98	−147	179	−178	71	−170	−59	−167	82	−164	62	−51	65	−179	161	−78
wet B_{12}	171	54	89	−150	178	−177	78	−162	−54	−168	81	−171	49	−34	80	167	160	−83
dry B_{12}	161	55	85	−158	176	−64	−165	−168	−57	176	74	−171	46	−47	75	170	179	−66

bond	atoms	bond	atoms	bond	atoms
1	C(1)–C(2)–C(26)–C(27)	7	C(8)–C(7)–C(37)–C(38)	13	C(13)–C(48)–C(49)–C(50)
2	C(3)–C(2)–C(26)–C(27)	8	C(7)–C(8)–C(41)–C(42)	14	C(16)–C(17)–C(55)–C(56)
3	C(2)–C(3)–C(30)–C(31)	9	C(9)–C(8)–C(41)–C(42)	15	C(18)–C(17)–C(55)–C(56)
4	C(4)–C(3)–C(30)–C(31)	10	C(8)–C(41)–C(42)–C(43)	16	C(17)–C(55)–C(56)–C(57)
5	C(3)–C(30)–C(31)–C(32)	11	C(12)–C(13)–C(48)–C(49)	17	C(19)–C(18)–C(60)–C(61)
6	C(6)–C(7)–C(37)–C(38)	12	C(14)–C(13)–C(48)–C(49)	18	C(17)–C(18)–C(60)–C(61)

[a] Torsion angles that exhibit variability.

Table IV. Hydrogen Bonding Involving (Adeninylpropyl)cobalamin and Water Molecules

A	B	A···B, Å	symmetry operation relating B to A
N(29) → H	O(W3)	2.956 (1)	$-1/2 - x, -y, 1/2 + z$
N(29) → H	O(W6)	2.972 (1)	x, y, z
N(34) → H	O(P5)	2.906 (1)	$1/2 - x, -y, 1/2 + z$
N(40) → H	O(W8)	2.997 (1)	x, y, z
N(40) → H	O(W10)	2.971 (1)	$-1/2 + x, -1/2 - y, -z$
N(45) → H	O(28)	2.848 (1)	$-x, -1/2 + y, 1/2 - z$
N(52) → H	O(W2)	2.863 (1)	x, y, z
N(52) → H	N(45)	3.294 (1)	$-x, 1/2 + y, -1/2 - z$
N(59) → H	O(W4)	2.998 (1)	x, y, z
N(63) → H	O(33)	2.913 (1)	$-x, 1/2 + y, 1/2 - z$
N(63) → H	O(51)	3.078 (1)	$-x, 1/2 + y, -1/2 - z$
O(R7) → H	O(W1)	2.695 (1)	x, y, z
O(R8) → H	O(W10)	2.792 (1)	x, y, z
N(A10) → H	O(33)	2.792 (2)	$-x, 1/2 + y, 1/2 - z$
O(W1) → H	O(W4)	2.833 (1)	x, y, z
O(W1) → H	O(P4)	2.734 (1)	$1/2 - x, -y, 1/2 + z$
O(W2) → H	O(W5)	2.801 (1)	$x, y, z - 1$
O(W2) → H	O(58)	2.834 (1)	x, y, z
O(W3) → H	O(51)	2.862 (1)	$-1/2 - x, -y, 1/2 + z$
O(W3) → H	O(W9)	2.860 (1)	x, y, z
O(W4) → H	O(62)	2.942 (1)	x, y, z
O(W4) → H	O(R8)	2.885 (1)	$1/2 - x, -y, 1/2 + z$
O(W5) → H	O(P5)	2.702 (1)	$1/2 - x, -y, 1/2 + z$
O(W5) → H	O(W1)	2.896 (1)	$1/2 - x, -y, 1/2 + z$
O(W6) → H	O(39)	2.795 (1)	x, y, z
O(W6) → H	N(A3)	2.809 (1)	x, y, z
O(W7) → H	O(44)	2.756 (1)	$-x, 1/2 + y, -1/2 - z$
O(W7) → H	O(R6)	2.940 (1)	$-x, 1/2 + y, -1/2 - z$
O(W8) → H	O(62)	2.848 (1)	$-x, -1/2 + y, 1/2 - z$
O(W8) → H	O(P4)	2.698 (1)	$-1/2 + x, -1/2 - y, -z$
O(W9) → H	O(44)	2.831 (1)	$-1/2 + x, -1/2 - y, -z$
O(W9) → H	O(W6)	2.977 (1)	x, y, z
O(W10) → H	N(A7)	2.723 (1)	$-x, -1/2 + y, -1/2 - z$
O(W10) → H	O(W7)	2.769 (2)	$-x, -1/2 + y, -1/2 - z$

the b, d, e, and g side chains. The C30H_2, C41H_2, and C48H_2 signals were assigned from correlations to C3H, C8H, and C13H, respectively, in the short mixing time HOHAHA spectrum (Figure S3). A correlation between C18H and C19H in the short mixing time spectrum was used to distinguish between C60H_2 and C18H, both of which show correlations to C19H in the longer mixing time spectrum. Since the f side chain is the only propionamide side chain attached to the corrin ring at a nonprotonated carbon, three signals (at 2.42, 2.04, and 1.79 ppm) which show correlations to each other in the HOHAHA spectra were identified as belonging to the f side chain. More specific assignments of these three signals were not possible from the HOHAHA spectra since all the connectivities to these protons are direct connectivities. Other experiments (see below) were needed for more specific assignments.

The HOHAHA spectra also show the connectivities between the methylene protons of a given side chain (Figure S2), including

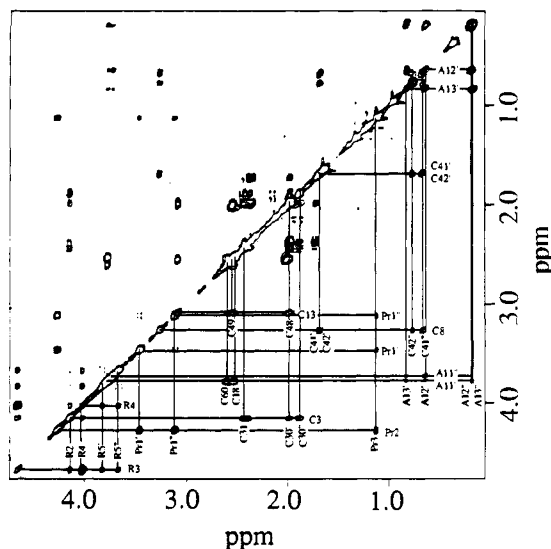

Figure 2. Part of the longer mixing time (67.3 ms) 2D HOHAHA spectrum of AdePrCbl. The connectivity patterns for the ribose ring and the propanolamine ^{1}H signals are indicated by drawn lines. Connectivity between the corrin ring methine signals and the methylene signals of the corresponding side chains are also indicated by drawn lines.

the a and c acetamide side chains which are attached to the corrin ring at nonprotonated carbons. At least one of the two inequivalent methylene protons from the a and c side chains were assigned from the ROESY spectrum (see below), and the assignment of the other methylene proton was made or confirmed from the HOHAHA spectra.

The methylene protons of the propylene chain were identified from the longer mixing time HOHAHA spectrum (Figure 2). Since the only set of three methylene groups coupled to each other in the molecule belongs to the propyladenine moiety, the six signals at 0.24, 0.27, 0.71, 0.90, 3.78, and 3.84 ppm, which all show correlations to each other, must be the A11, A12, and A13 protons. The two signals at 3.78 and 3.84 ppm were assigned to the A11 methylene protons. A11 is attached to a nitrogen which would cause these protons to be shifted downfield relative to the other protons of the methylene chain. Furthermore, the ROESY spectrum (see below) shows an NOE cross peak between these two protons and A8H, indicating that these protons are close in space to A8H, as would be expected for the A11 protons. The HMQC spectrum (see below) shows that the signals at 3.78 and 3.84 ppm are attached to the same carbon. In the short mixing time HOHAHA spectrum the A11 protons show correlations only to the signals at 0.71 and 0.27 ppm assigning them to A12H' and A12H'', which in turn show correlations to A13H' and A13H'' completing the assignment of the propyl chain protons of the propyladenine moiety.

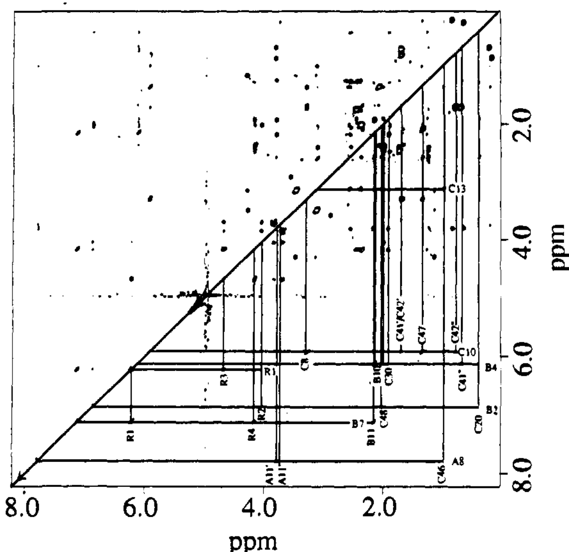

Figure 3. 2D ROESY spectrum of AdePrCbl obtained with a 200 ms spin lock period. Drawn lines indicate the NOE connectivity of the downfield signals.

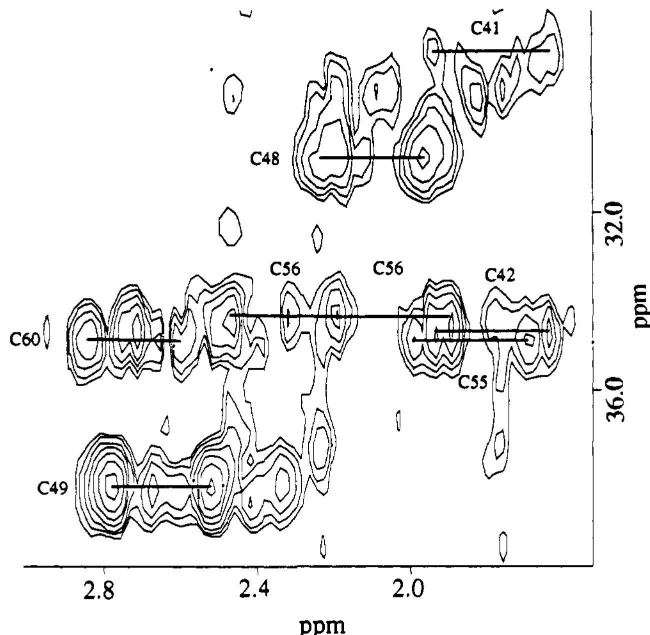

Figure 4. Part of the HMQC spectrum showing the ^{13}C assignments of some of the methylene groups. Each ^1H signal is split into a pair of cross peaks by $^1H-^{13}C$ coupling constant. Pairs are connected by a drawn line that has a length in Hertz corresponding to the coupling constant. The center of the line corresponds to the ^1H chemical shift.

The ROESY spectrum (Figures 3 and S4) was used to establish through-space connectivity between the networks of coupled protons and to complete the assignment of most of the ^1H spectrum. The experiment was performed at 5 °C to increase NOE intensities and possibly decrease the rate of rotation of the propyladenine moiety about the Co–C bond.

The ROESY spectrum interpretation began with the unassigned downfield proton signals (Figure 3). Three of the signals must be for B2H, B4H, and B7H. Only two of the downfield signals (at 6.92 and 7.17 ppm) show NOEs to R1H and must be B2H and B7H. A third proton signal at 6.17 ppm shows scalar connectivity to a dimethylbenzimidazole methyl group signal ($B10H_3$) at 2.21 ppm in the longer mixing time HOHAHA spectrum (Figure S1) but no NOEs to any of the ribose protons. This peak is assigned to B4H, the only single proton in the benzimidazole moiety that is not close to the ribose ring. The signal at 7.17 ppm shows NOE cross peaks to R1H, R2H, and a methyl group at 2.22 ppm ($B11H_3$) is assigned to B7H. Scalar connectivity between B7H and $B11H_3$ can also be seen in the longer mixing time HOHAHA spectrum (Figure S1). The remaining benzimidazole signal at 6.92 ppm shows NOEs to R1H, R4H, and some of the corrin protons (i.e., $C48H_2$ and $C20H_3$) and is assigned to B2H.

Two of the remaining unassigned downfield proton signals belong to the adenine moiety. The signal at 7.83 ppm shows NOE connectivities to $A11H_2$ and A13H″ and is assigned to A8H. This signal also shows a cross peak to the C46 methyl group. On the basis of the HMBC spectrum (see below) and the absence of NOEs to the propyl group or any of the corrin protons, the signal at 8.12 ppm was assigned to A2H. The remaining downfield peak (at 5.98 ppm) is assigned to C10H, the only remaining uncoupled single proton at a conjugated site.

C10H is the starting point from which most of the corrin protons were assigned from the ROESY spectrum (Figure 3). C10H shows five correlations in the ROESY spectrum, two of which are to the methylene protons of the d side chain. One of the correlations is assigned to the methine proton C8H. The remaining two correlations are to methyl groups. The two methyl groups close to C10H are $C46H_3$ and $C47H_3$. The more upfield of the two signals was assigned to $C46H_3$ based on the HMBC spectrum (see below) and the NOE cross peak between this signal and A8H. $C46H_3$ projects up from the β face of the corrin ring (see X-ray structure). From this starting point most of the corrin resonances were assigned by following the NOE connectivities around the corrin ring as described previously.[12,14]

At this point all of the ^1H NMR signals of AdePrCbl were assigned unambiguously except for the C55 and C56 methylene protons. There is no way to assign the three signals, identified as belonging to the f side chain from the HOHAHA spectra, to their specific methylene groups from any of the homonuclear two-dimensional experiments used. Unlike the other propionamide side chains, there is no distinguishing correlation to the C55 methylene protons in the short mixing time HOHAHA experiment since there is no proton attached to C17, the carbon to which the f side chain is attached to the corrin ring. However, the assignment of the C55 and C56 methylene protons can be made from $^1H-^{13}C$ correlation experiments.

Assignment of the ^{13}C NMR Spectrum. The signals of the protonated carbons were assigned with the HMQC spectrum (Figures 4 and S5), which gives one-bond $^1H-^{13}C$ correlations. From the HMQC spectrum (Figure 4) it was found that the proton signal at 1.75 ppm has correlations to two carbon signals at 28.4 and 34.7 ppm, identified below as C41 and C42, indicating the overlap of two proton signals (i.e., C41H′ and C42H′). In the HOHAHA spectra the peaks at 1.75, 0.83, and 0.74 ppm were identified as belonging to the d side chain. From the short mixing time HOHAHA spectrum the peaks at 1.75 and 0.83 ppm were identified as C41H′ and C41H″. The peak at 0.74 ppm was then assigned to $C42H_2$. The carbon at 28.4 ppm was assigned to C41 from its correlation with C41H″. Since no proton signals besides those of the d side chain were found to be at 1.75 ppm, the second correlation to this signal at 34.7 ppm in the HMQC spectrum must be between C42H′ and C42.

The proton assignments of the propylene chain were confirmed from the HMQC spectrum. Peaks assigned from the HOHAHA spectra as belonging to the same methylene group also show correlations to the same carbon in the HMQC spectrum (Table V). The carbon chemical shifts provide additional evidence for assigning the most downfield of the proton signals of the propylene group to $A11H_2$. Since it is attached to a nitrogen, A11 would be expected to have the most downfield shift of the propylene group carbons. Also, since it is bound to Co, A13 would be expected to have the most upfield shift of the propyl group carbons.

Assignments of the nonprotonated carbons were made from the HMBC spectrum (Figures 5, S6, and S7), which displays two- and three-bond $^1H-^{13}C$ connectivities. The nonprotonated adenine carbons were readily assigned, and the assignment of A2H was confirmed from the HMBC spectrum (Figure 5). A5 shows a correlation to A8H only, A6 shows a correlation to A2H only, and A4 shows a correlation to both. Since A4 can only show

Table V. ^1H and ^{13}C NMR Chemical Shifts and Signal Assignments for (Adeninylpropyl)cobalamin (AdePrCbl) at pH 8.3 and Comparisons to the Corresponding Signals in (5'-Deoxyadenosyl)cobalamin (AdoCbl, Coenzyme B_{12}) at pH 7.0[a]

	^{13}C NMR		^1H NMR			^{13}C NMR		^1H NMR	
assignment	AdePrCbl	AdoCbl[b]	AdePrCbl	AdoCbl[b]	assignment	AdePrCbl	AdoCbl[b]	AdePrCbl	AdoCbl[b]
C35	18.1	18.3	2.42	2.45	R2	71.8	72.0	4.22	4.23
C53	18.6	18.8	2.45	2.43	Pr2	75.8	76.0	4.34	4.33
C54	19.3	19.6	1.31	1.36	R3	75.9	76.2	4.74	4.72
C25	19.7	19.9	1.34	1.36	C19	76.5	76.8	3.86	4.24
C36	21.6	21.7	1.73	1.70	R4	84.3	84.6	4.10	4.10
Pr3	21.7	21.7	1.21	1.21	C1	87.8	88.5		
B11	22.4	22.3	2.22	2.19	R1	89.1	89.4	6.27	6.26
B10	22.4	22.5	2.21	2.19	C10	97.3	97.7	5.98	5.93
C20	23.5	23.5	0.46	0.47	C15	106.6	106.9		
C47	23.4	23.9	1.42	1.32	C5	108.1	108.4		
A13	25.2	*c*	0.90, 0.24	*c*	B7	113.2	113.5	7.17	7.16
C41	28.4	28.7	1.75, 0.83	1.75, 0.81	A5	120.9	121.8		
C30	29.4	29.2	2.04, 1.95	2.06, 1.96	B4	121.0	121.4	6.17	6.24
C48	30.8	30.3	2.05	2.22, 2.00	B8	133.0	133.3		
A12	33.8	*c*	0.71, 0.27	*c*	B5	134.1	134.5		
C56	34.3	34.4[d]	2.42, 2.04	2.45, 2.06[d]	B6	136.5	136.8		
C46	34.6	34.2	1.06	0.87	B9	140.6	141.0		
C42	34.7	34.8	1.75, 0.74	1.73, 0.88	B2	144.3	144.7	6.92	6.95
C60	34.8	34.8	2.66	2.65	A8	145.1	143.8	7.83	8.00
C55	34.9	34.6[d]	1.79	1.78[d]	A4	151.2	151.8		
C49	38.1	38.1	2.62	2.54	A2	154.8	156.0	8.12	8.19
C31	38.3	38.3	2.50, 2.46	2.50	A6	158.0	158.7		
C18	42.6	42.5	2.59	2.65	C6	165.8	166.6		
C37	45.2	45.3	2.19, 1.51	2.19, 1.72	C14	166.6	167.2		
C26	45.8	46.2	2.23, 1.98	2.41	C9	172.3	173.1		
A11	45.9	*c*	3.84, 3.78	*c*	C11	176.4	177.6		
Pr1	47.8	47.8	3.54, 3.20	3.54, 3.16	C38	176.8	177.9		
C2	49.4	49.5			C16	177.3	178.7		
C12	49.5	49.5			C57	177.7	178.2		
C7	52.9	53.1			C4	178.0	178.7		
C13	55.9	55.8	3.17	2.89	C27	178.3	179.3		
C8	57.1	57.5	3.35	3.29	C61	178.4	179.0		
C3	58.0	58.5	4.22	4.10	C43	179.9	180.3		
C17	60.5	60.8			C32	180.5	181.1		
R5	63.2	63.4	3.91, 3.75	3.88, 3.74	C50	180.8	181.1		

[a] Shifts in ppm relative to internal TSP (sodium 3-(trimethylsilyl)propionate-2,2,3,3-d_4). [b] From ref 12. [c] A11, A12, and A13 are propyl group carbons of AdePrCbl; there are no corresponding carbons in AdoCbl. [d] We tentatively switch the assignments of the C55 and C56 carbons and protons of AdoCbl to agree with our assignments. For AdePrCbl, the shift difference between the C55 and C56 carbons is greater than for AdoCbl, which has a poorly resolved HMBC spectrum in this region.

connectivity to A8H and A2H, the assignment of the proton signal at 8.12 ppm to A2H is confirmed. Connectivity around the corrin ring can be established from the HMBC spectrum as was described previously,[12] assigning all of the nonprotonated carbons of the corrin ring. The nonprotonated benzimidazole carbons were also assigned from the HMBC spectrum as described previously.[12,27]

The tentative assignments of $C46H_3$ and $C47H_3$ can be confirmed by consideration of the intensity of the correlations of C13H to C46 and C47 in the HMBC spectra (Figure S7).[12] The correlation between C13H and C46 is much more intense than the one between C13H and C47, indicating that C13H has a larger coupling with C46 than with C47. The larger coupling between C13H and C46 is expected since there is a cis configuration between C13H and C46 and a gauche configuration between C13H and C47. C46 and C47 were assigned from their correlations with $C46H_3$ and $C47H_3$, confirming the assignments of the proton signals.

The HMQC and HMBC spectra enabled the C55 and C56 methylene protons to be assigned. From the HMBC spectrum the ^{13}C resonance at 34.9 ppm which shows a correlation to $C54H_3$ is assigned to C55. $C54H_3$ is too many bonds away from C56 to show connectivity between the two in the HMBC spectrum. In the HMQC spectrum the ^1H signal at 1.79 ppm shows a correlation to the carbon signal at 34.9 ppm, assigning the signal to $C55H_2$. The other two peaks identified, from the HOHAHA

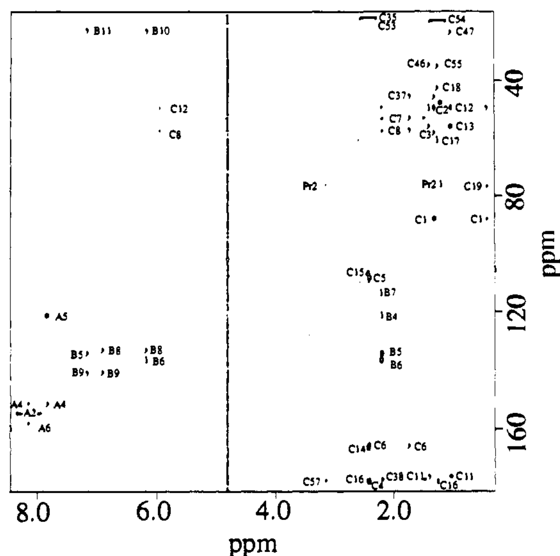

Figure 5. ^1H-detected ^1H-^{13}C multiple-bond shift-correlation (HMBC) spectrum of AdePrCbl. Pairs of cross peaks connected by a drawn line (A2, C35, C53, and C54) are incompletely suppressed one-bond correlations.

spectra, as belonging to the f side chain showed connectivity to a carbon signal at 34.3 ppm, assigning the proton signals at 2.42 and 2.04 ppm to C56H' and C56H'', respectively.

The ^1H and ^{13}C assignments of AdePrCbl are now complete. The ^1H and ^{13}C assignments of AdePrCbl are shown in Table V

(25) Stewart, R. F.; Davidson, E. R.; Simpson, W. T. *J. Chem. Phys.* **1965**, *42*, 3175.
(26) Savage, H. F. J.; Lindley, P. F.; Finney, J. L.; Timmins, P. A. *Acta Crystallogr.* **1987**, *B43*, 280–295.
(27) Pagano, T. G.; Marzilli, L. G. *Biochemistry* **1989**, *28*, 7213–7223.

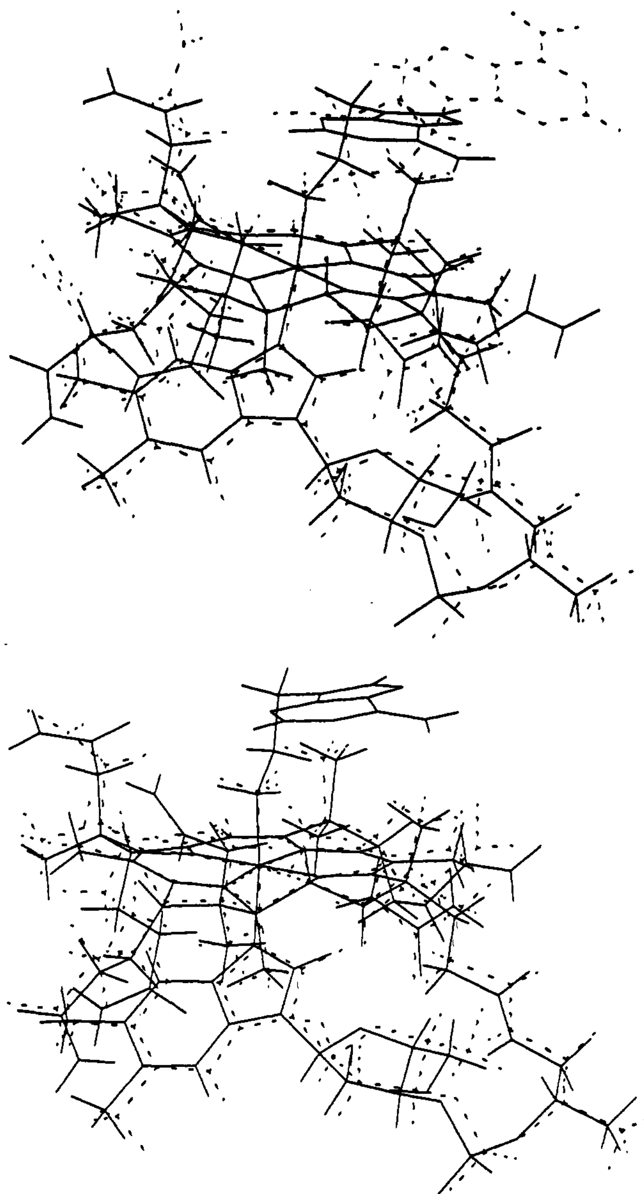

Figure 6. Comparison of the conformation of three alkylcobalamins: (a) (adeninylpropyl)cobalamin (solid line) and adenosylcobalamin (short dashes) and (b) (adeninylpropyl)cobalamin (solid line) and methylcobalamin (short dashes).

along with the corresponding resonances of coenzyme B_{12}.

Discussion

The general conformation of the molecule of AdePrCbl is very similar to the conformations of both B_{12} coenzymes (AdoCbl[26] and MeCbl[21]). A comparison of these structures is given in Figures 6a,b and Table VI. The orientation of the benzimidazole group and the general disposition of the ribose, phosphate, and side chains are the same in each structure. The side chains on rings B and C exhibit more variability, which is in accordance with a previous study of several corrin systems.[11,28]

The corrin ring is not perfectly planar. The direct link between rings A and D (the C1–C19 bond) is formed between two tetrahedral carbon atoms, and this causes the interior of the corrin ring to be slightly helical. One method that has been used to describe the conformation of the corrin ring in cobalamins involves using the fold angle between the planes of the conjugated system of double bonds. The planes are defined as follows: atoms N21, C4, C5, C6, N22, C9, and C10 in plane 1 and atoms N24, C16, C15, C14, N23, C11, and C10 in plane 2 (Table VI). This fold angle is a function of the bulkiness of the axial substituents above and below the corrin ring system. Those cobalamin derivatives with small substituents on the upper side, as in MeCbl, have large fold angles, reflecting the bulkiness of the dimethylbenzimidazole on the underside. In AdePrCbl, the fold angle is 10.9°, whereas in MeCbl it is 15.8°, and in the coenzyme it is 13.3°.

The upper and lower axial ligands are not linearly disposed but slightly bent, so that the C–Co–N(Bzm) angle is 175.3°. The corresponding value for AdoCbl is 172.7°, and for MeCbl is 171.1°. The axial Co–N(Bzm) bond (2.21 Å) is longer than any of the equatorial Co–N bonds (1.85–1.89 Å), and the axial Co–C bond is 1.96 Å in length. These distances from the cobalt atom to its two axial ligands may give information on the trans effect. The axial Co–C–C bond angle is 119.0°, smaller than the value in AdoCbl (124.4°) but considerably larger than the tetrahedral angle of 109.5°. From the bond distances shown in Figure 7 it can be seen that the system of six double bonds of the corrin ring is highly delocalized.

The most obvious difference between the crystal structures of AdoCbl and AdePrCbl is the orientation of the adenine ring in the two compounds. When viewed from the β side, the adenine ring in AdePrCbl is over the corrin D ring, rotated about 120°

Table VI. Comparison of the Crystallographic Structures of Three Alkylcobalamins: (Adeninylpropyl)cobalamin (AdePrCbl, This Work), Adenosylcobalamin (AdoCbl[26]), and Methylcobalamin (MeCbl[21])

	AdePrCbl	AdoCbl	MeCbl
Bond Distances to Co (Å)			
Co–N(21)	1.853 (7)	1.873	1.881
Co–N(22)	1.885 (7)	1.906	1.972
Co–N(23)	1.886 (7)	1.905	1.928
Co–N(24)	1.850 (7)	1.886	1.892
Co–C	1.959 (10)	1.999	1.990
Co–N(Bzm)	2.212 (8)	2.237	2.195
av length of the equatorial bonds	1.868	1.893	1.918
Bond Angles to Co (deg)			
N(21)–Co–N(22)	90.8 (3)	90.1	93.0
N(22)–Co–N(23)	96.5 (3)	96.7	95.3
N(23)–Co–N(24)	89.9 (3)	90.4	90.6
N(24)–Co–N(21)	83.0 (3)	83.0	81.3
C–Co–N(Bzm)	175.3 (3)	172.7	171.1
Fold Angle of the Corrin (deg)			
angle between planes 1 and 2[a]	10.9	13.3	15.8
rms deviation, Å			
plane 1	0.07	0.08	0.05
plane 2	0.03	0.03	0.02

[a] Plane 1 is defined as the best plane through atoms N(21), C(4), C(5), C(6), N(22), C(9), and C(10). Plane 2 is defined as the best plane through atoms N(24), C(16), C(15), C(14), N(23), C(11), and C(10).

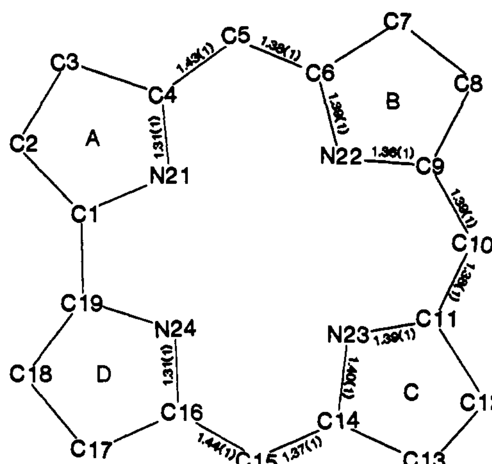

Figure 7. Bond distances for the conjugated system of six double bonds in the corrin ring of AdePrCbl.

(28) Pett, V. B.; Liebman, M. N.; Murray-Rust, P.; Prasad, K.; Glusker, J. P. J. Am. Chem. Soc. 1987, 109, 3207–3215.

clockwise from its position in AdoCbl.

The shifts of some of the corrin protons and the adenine carbons and the NOE cross peaks in the ROESY spectrum provide information on the orientation of the adenine ring in AdePrCbl in solution. A comparison of the shifts of the corrin ring protons and the adenine carbons between AdePrCbl and AdoCbl indicates that the adenine moiety is in different positions in the two molecules. The C13H and $C46H_3$ signals are shifted significantly downfield in AdePrCbl relative to AdoCbl (0.28 and 0.19 ppm, respectively). In the X-ray crystal structure of AdoCbl[11] the adenine ring is over these C ring protons. A change in position of the adenine ring would remove or decrease the shielding effect of the adenine ring.[29]

A comparison of the shifts of C13H and $C46H_3$ in MeCbl with those in AdoCbl and AdePrCbl suggests that a combination of removing the adenine shielding effect and the addition of a deshielding effect is responsible for the differences between AdoCbl and AdePrCbl. C13H in MeCbl has a shift of 3.05 ppm, a value between the shifts of 2.89 and 3.17 ppm for AdoCbl and AdePrCbl, respectively. The removal of the adenine shielding effect in AdoCbl could give the shift found in MeCbl, and the addition of a deshielding effect from the adenine ring in AdePrCbl could give the relatively downfield shift found for this analogue. Support for the presence of this deshielding ring current effect can be found in the shifts of $C46H_3$, which is close to C13H, in MeCbl, AdoCbl, and AdePrCbl. The shifts of $C46H_3$ in MeCbl and AdoCbl are quite similar (0.92 and 0.87 ppm, respectively) indicating that the downfield shift of $C46H_3$ found in AdePrCbl (1.06 ppm) is due to the presence of a deshielding ring current effect and not the removal of a shielding effect.

C19H is shifted significantly upfield in AdePrCbl relative to AdoCbl (0.38 ppm). This upfield shift may be caused in part by the presence of the adenine ring over C19 in AdePrCbl as found in the crystal structure (Figure 8). However, the shift of C19H in MeCbl (3.94 ppm) is closer to that in AdePrCbl (3.86 ppm) than in AdoCbl (4.24 ppm). A downfield value for C19H in AdoCbl must reside primarily in some structural feature of coenzyme B_{12}, e.g., a steric effect from the ribose.[30] The small additional upfield shift of C19H in AdePrCbl could then be explained by the presence of the adenine ring over this proton, as shown by the X-ray crystal structure.

The only NOE cross peak observed between an adenine proton and a corrin proton in the ROESY spectrum is the fairly weak A8H–$C46H_3$ cross peak. This correlation is also observed for AdoCbl in which the adenine ring is known to be over ring C of the corrin nucleus.[11] However, the volume of the A8H–$C46H_3$ cross peak is an order of magnitude lower in AdePrCbl than in AdoCbl relative to the volume of the B2H–R1H cross peak. The volume of this cross peak was selected for comparison since no major change in the conformation of the nucleotide loop is indicated in the X-ray crystal structures. In the crystal structure of AdePrCbl, A8H is not close enough to $C46H_3$ to show a correlation in the ROESY spectrum. The presence of this NOE cross peak suggests a fluxional character of the adenine moiety of AdePrCbl. In AdoCbl it was proposed,[13] based on NOE intensities, that the adenosyl exists part of the time in a conformation similar to that observed in the crystal structure and part of the time with the adenosyl rotated counterclockwise about 50° about the Co–C bond. In this proposed conformation the five-membered ring of the adenine would be over ring B of the corrin. Since the NOE intensities used as a basis for this proposed conformation involved only the ribose protons, it is difficult to compare this orientation of the adenine in AdoCbl with the possible orientations of the adenine in AdePrCbl.

The ^{13}C shifts of the adenine moiety also indicate different orientations of the adenine ring in AdePrCbl and AdoCbl. With the exception of A8, all the adenine ^{13}C shifts in AdePrCbl are

(29) Griessner-Prettre, C.; Pullman, B.; Borer, P. N.; Kan, L.-S.; Ts'o, P. O. P. *Biopolymers* **1976**, *15*, 2277–2286.
(30) Santos, R. A.; Tang, P.; Harbison, G. S. *Biochemistry* **1989**, *28*, 9372–9378. Grant, D. M.; Cheney, B. V. *J. Am. Chem. Soc.* **1967**, *89*, 5315–5318.

Table VII. ^1H Chemical Shifts of the Downfield Resonances of AdoCbl and the (Adeninylalkyl)cobalamins[a]

compd	A2H	A8H	B2H	B4H	B7H	R1H	C10H
AdoCbl[b]	8.19	8.00	6.95	6.24	7.16	6.26	5.93
AdePeCbl	8.20	7.96	6.97	6.20	7.15	6.22	5.98
AdeBuCbl	8.15	7.96	6.88	6.21	7.15	6.22	5.98
AdePrCbl	8.12	7.83	6.92	6.17	7.17	6.27	5.98
AdeEtCbl	8.05	7.69	6.97	6.29	7.15	6.29	6.09

[a] Shifts in ppm relative to internal TSP. [b] From ref 12.

significantly upfield of their positions in AdoCbl. One explanation for these upfield shifts is that the adenine moiety can be closer to the anisotropic corrin ring when attached by a propylene chain than by a ribose moiety. Additional evidence for the adenine moiety being closer to the corrin ring can be found by comparing the shifts of A2H and A8H across the series of Ade($-CH_2-$)$_n$Cbls (Table VII). It can be seen that, as the length of the chain decreases, A2H and A8H shift upfield. These shifts suggest that as the chain decreases in length the adenine moiety is closer to the corrin ring.

The shifts of A2H and A8H in AdePeCbl are quite similar to those in AdoCbl, supporting the arguments that the five-carbon chain best mimics the ribose moiety of AdoCbl[1] and allows for a similar positioning of the adenine ring over the corrin.[6] The shift of $C46H_3$ in AdePeCbl (0.79 ppm) is closer to the shift in AdoCbl (0.87 ppm) than any of the other Ade($-CH_2-$)$_n$Cbls. As the chain length decreases, $C46H_3$ shifts downfield (from 0.99 to 1.06 to 1.02 ppm for n = 4, 3, and 2, respectively). A possible explanation for these downfield shifts is that as the chain length decreases the adenine moiety is becoming less parallel to the plane of the corrin ring and in AdeEtCbl the adenine ring is thought to be perpendicular to the corrin ring.[6] As the edge of the adenine ring points more toward $C46H_3$ with decreasing chain length, the deshielding region of the ring current would have more of an effect on the shift of these protons, since the shorter chains could allow the adenine ring to be closer to this methyl group.

NOE cross peaks between the propylene protons and some of the corrin protons give an indication of the position of the propylene chain and provide additional evidence for the fluxional character of the propyladenine moiety. Strong NOEs are observed between C19H and both A12H′ and A13H′. A12H′ also shows a weak correlation to $C54H_3$ in the ROESY spectrum. Weak NOE cross peaks are also observed between A13H″ and $C46H_3$ and A12H″ and C37H″. In the X-ray crystal structure the closest A12 proton is 4.20 Å away from C19H, too far to show a detectable NOE cross peak. Also, the closest A13 proton to $C46H_3$ is 4.95 Å away. The A13H′–C19H and A12H″–C37H″ cross peaks are consistent with the crystal structure which shows these pairs of protons to be 2.07 and 3.03 Å apart, respectively.

The A12H′–C19H, A12H′–$C54H_3$, A13H″–$C46H_3$, and A8H–$C46H_3$ cross peaks can be accounted for by a second orientation of the propyladenine moiety in which there is an approximately 70° clockwise rotation about the Co–C bond, a clockwise rotation of 100–110° about the A11–A12 bond, and a 160–180° clockwise rotation about the A11–N9 bond (Figure 8). This orientation, in addition to giving reasonable distances (approximately 2.1 Å for A12H′–C19H, 2.4 Å for A12H′–$C54H_3$, 3.8 Å for A13H″–$C46H_3$, and 3.5 Å for A8H–$C46H_3$) for these NOE cross peaks to be observed, would also explain the downfield shifts of C13H and $C46H_3$ mentioned above. In this orientation the adenine plane is tilted relative to the corrin ring with the imidazole ring closer to the corrin ring than the pyrimidine ring. A8H points toward ring C, causing the deshielding region of the adenine ring current to affect $C46H_3$ and C13H and also allowing A8H to be close enough to $C46H_3$ for the NOE cross peak to be observed.

An indicator of the electronic environment of Co in cobalamins is the ^{13}C NMR shift of the 5,6-dimethylbenzimidazole moiety. There are only slight upfield shifts in the ^{13}C signals of the benzimidazole moiety in AdePrCbl relative to AdoCbl and MeCbl (Table VIII). These shifts indicate that the three alkyl groups are qualitatively very similar electronically.[32,33]

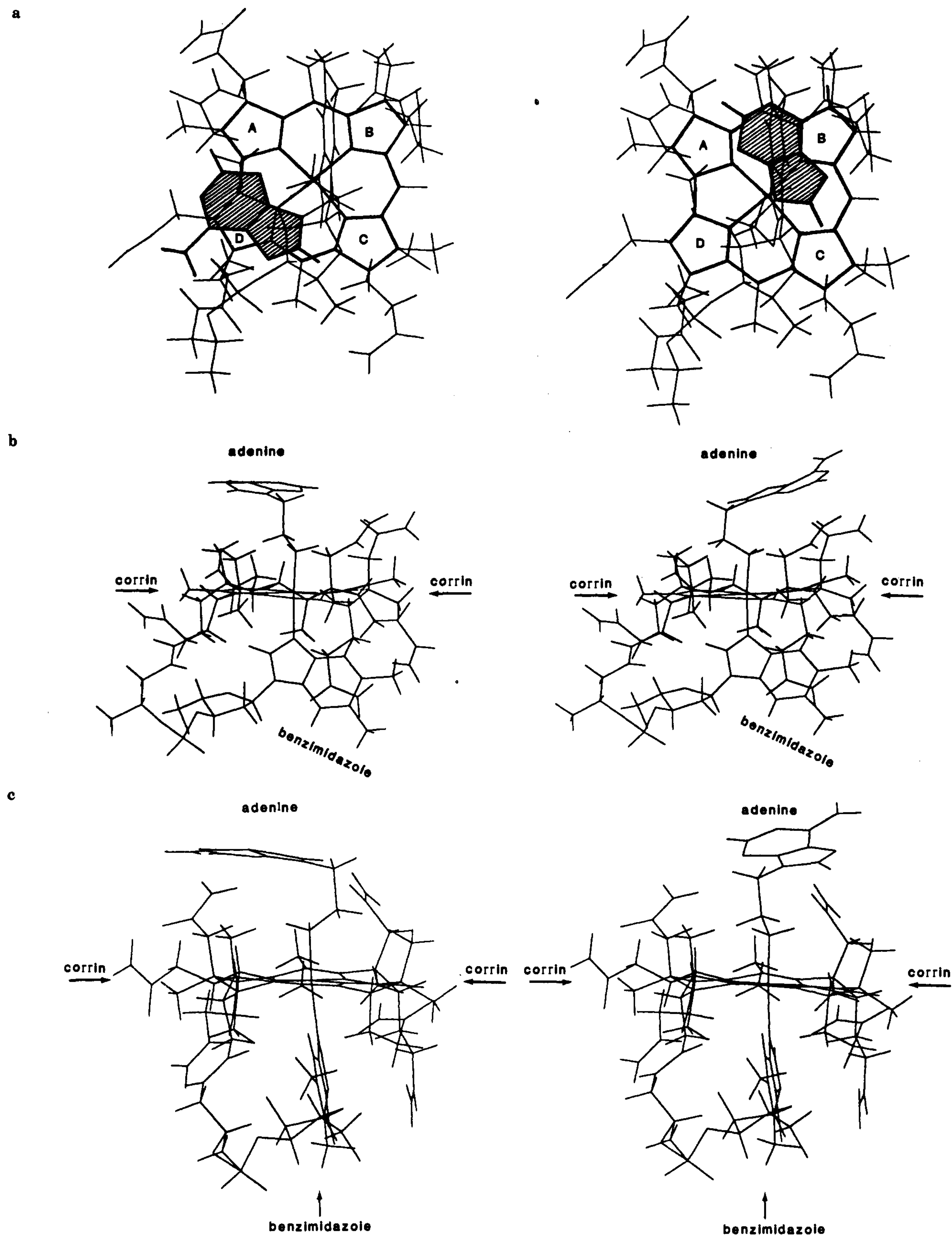

Figure 8. X-ray crystal structure of AdePrCbl (left) and possible second orientation of the adenine ring of AdePrCbl in solution (right) looking down the (a) A13–Co bond, (b) C10–Co bond, and (c) C15–Co bond. All figures were generated using the MACROMODEL program (Version 2.5, C. Still, Columbia University Modelling System, 1989, New York, NY).

However, the shifts are influenced by both through-bond and through-space (anisotropy of the Co and corrin) effects,[32-35] and both factors must be considered in assessing the donor ability of the alkyl ligand. From such an analysis,[35] the order of increasing

(31) Bratt, G. T.; Hogenkamp, H. P. C. *Biochemistry* **1984**, *23*, 5653–5659.

(32) Parker, W. O., Jr.; Zangrando, E.; Bresciani-Pahor, N.; Marzilli, P. A.; Randaccio, L.; Marzilli, L. G. *Inorg. Chem.* **1988**, *27*, 2170–2180, and references cited therein.

Table VIII. ^{13}C Chemical Shift of Selected Nonprotonated Corrin Carbons and the Benzimidazole Carbons of CNCbl, AdoCbl, MeCbl, and AdePrCbl[a]

carbon	CNCbl[b]	AdoCbl[c]	MeCbl[d]	AdePrCbl
C4	182.8	178.7	178.1	178.0
C16	181.7	178.7	177.7	177.3
C11	179.7	177.6	176.1	176.4
C9	176.4	173.1	172.5	172.3
C14	168.8	167.2	166.4	166.6
C6	168.1	166.6	165.9	165.8
B2	144.7	144.7	144.7	144.3
B9	139.5	141.0	141.0	140.6
B6	137.9	136.8	136.5[e]	136.5
B5	135.8	134.5	134.3[e]	134.1
B8	132.8	133.3	133.2	133.0
B4	119.3	121.4	121.1	121.0
B7	114.3	113.5	113.3	113.2
C5	110.3	108.4	108.0	108.1
C15	106.9	106.9	106.2	106.6
C10	97.7	97.7	96.7	97.3
C1	87.9	88.5	87.9	87.8

[a] Shifts in ppm relative to internal TSP. [b] From ref 27. [c] From ref 12. [d] Shifts have been referenced to TSP by adding 2.8 ppm to the values given in ref 31, in which neat Me_4Si was used as the reference. [e] The assignments of B5 and B6 have been switched from those made by Bratt and Hogenkamp[31] in light of their misassignments of these two carbon signals in AdoCbl[12] and CNCbl.[27]

electron donor ability for the alkyl groups is AdePr < Me < Ado, although there is not much difference in their electron donor ability. The greater electron donor ability of the Ado ligand, if real, could be due to favorable interactions of the sugar with the amide side chains.

In studies of model compounds it was found that the Co–N bond distance of the trans axial ligand increases as the electron donor ability of the alkyl group increases.[32,36] The very similar Co–N (dimethylbenzimidazole) bond lengths of AdePrCbl and MeCbl[21] (2.21 Å and 2.19 Å, respectively) suggest there is little difference in the electron donor ability of the two alkyl groups. The Co–N bond distance for AdoCbl[11] is slightly longer (2.24 Å) suggesting, in agreement with the ^{13}C trends, that the adenosyl group is a better electron donor than both propyladenine and methyl.

Significant upfield shifts are found for the nonprotonated corrin carbons C1, C4, C6, C9, C11, C14, and C16 in AdePrCbl relative to their shifts in AdoCbl. These shifts may be attributed to differences in the electronic environments or conformations of the corrin ring in the two compounds. As can be seen in Table VIII there is a large upfield shift of the C1, C4, C6, C9, C11, C14, and C16 signals of the alkylcobalamins relative to cyanocobalamin (CNCbl). Since cyanide is known to be a much worse electron donor than alkyl groups, the shifts of these carbons may be an indicator of the electron donor ability of an alkyl group, assuming no contributions from conformational changes. The shifts of these carbons in MeCbl and AdePrCbl are similar (Table VIII), indicating similar electron donor ability.

The shifts of C1, C9, and C16 in AdePrCbl are more upfield than would be expected based solely on the electron donor ability of the alkyl group. The crystal structure of AdePrCbl indicates that the adenine ring is over C16, and the adenine ring current could cause the additional upfield shift observed for this carbon signal. Even though the adenine ring is not over C1 in the crystal structure of AdePrCbl, a slight clockwise rotation of the adenine ring would put it over C1, causing the additional upfield shift. In support of this argument, the carbons and protons of the nearby acetamide side chain are shifted upfield in AdePrCbl relative to AdoCbl. The upfield shift of C9, like the A8H–C46H_3 NOE cross peak, cannot be explained by the position of the adenine ring in the crystal structure. However, the upfield shift of C9 can be explained by the tilted orientation of the adenine ring mentioned above, since for this orientation the adenine ring is over C9.

Our studies of AdePrCbl can be used to interpret the results of CD and binding studies of the Ade($-CH_2-)_n$Cbls. The CD spectra of the longer chain analogues (n = 4–6) showed a definite change on binding of the analogues to the enzyme ethanolamine ammonia–lyase.[8,9] The CD spectrum of AdePrCbl showed less change on binding, and the spectra of AdoCbl and AdeEtCbl showed no change. Binding studies of the Ade($-CH_2-)_n$Cbls to the enzymes ribonucleotide reductase[6] and diol dehydrase[7] revealed that AdePeCbl binds more tightly to the enzyme than the other Ade($-CH_2-)_n$Cbls and even than AdoCbl.

The possible factors affecting these observed trends are as follows: electronic or steric effects of the alkyl group on dimethylbenzimidazole binding, the fluxional character of the β ligand, the orientation of the adenine, and corrin ring flexibility. On the basis of the Co–N (benzimidazole) bond lengths and ^{13}C shifts of the benzimidazole moiety, the propyladenine group is only a slightly worse electron donor than adenosyl and this factor can be dismissed.

The trends observed in the binding constants of the Ade($-CH_2-)_n$Cbls to the enzymes ribonucleotide reductase[6] and diol dehydrase[7] may be explained by the flexibility of the alkyl chains, evidenced by the fluxional character of the β ligand, and the orientation of the adenine ring. There appears to be a greater fluxional character of the adenine ring in AdePrCbl than in AdoCbl. The two orientations of the adenine ring in AdoCbl differ only by a 50° rotation about the Co–C bond, while the two major orientations found for the adenine ring in AdePrCbl differ by a 70° rotation about the Co–C bond plus a 180° rotation about the A11–N9 bond. The flexibility of the alkyl chains in the Ade($-CH_2-)_n$Cbls allows changes in the orientation of the adenine ring to occur more easily than in AdoCbl, leading to a greater fluxional character of the β ligand and, in the case of AdePeCbl, to a higher affinity of the analogue for the enzyme than AdoCbl. The ^1H shifts of A2H, A8H, and C46H_3 are quite similar in AdePeCbl and AdoCbl, suggesting a similar positioning of the adenine ring over the corrin in both molecules. As the chain lengths of the Ade($-CH_2-)_n$Cbls decrease, the shifts of A2H, A8H, and C46H_3 differ more from AdoCbl and the binding constants decrease. These results seem to indicate that a specific orientation of the adenine is required for optimum binding to the enzyme. The analogue in which the orientation of the adenine most closely resembles the orientation in AdoCbl, i.e., AdePeCbl, binds more tightly to the enzyme than the others.

It was suggested that the CD spectral changes observed on binding of the Ade($-CH_2-)_n$Cbls to the enzyme were caused by a change in the conformation of the corrin ring.[8] The corrin ring flexibility of these analogues is not immediately obvious. The X-ray crystal structures of AdePrCbl and AdoCbl indicate that there is little difference in the corrin ring conformation in these two compounds. Furthermore, the X-ray crystal stucture of cob(II)alamin[37] (the Co(II) form of cobalamins produced upon homolysis of the Co–C bond) shows that the corrin ring conformation in this species is very similar to the conformation found in MeCbl and AdoCbl. If the adenosyl group was preventing the corrin ring from assuming its favored conformation, there should be a significant difference between the conformations in AdoCbl and cob(II)alamin, in which there is no group in the position occupied by the alkyl group in cobalamins to prevent an upward folding of the corrin ring. Also, the absence of the other axial ligand, 5,6-dimethylbenzimidazole, appears to have no effect on the corrin ring conformation. On the basis of NMR data, particularly the C18H–C19H coupling constants, the corrin ring conformations of base-off AdoCbl[13] and (5'-deoxyadenosyl)cobinamide,[14] in which the dimethylbenzimidazole nucleotide of

(33) Brown, K. L.; Hakimi, J. M. *J. Am. Chem. Soc.* **1986**, *108*, 496–503.
(34) McConnell, H. M. *J. Chem. Phys.* **1957**, *27*, 226–229. Pople, J. A. *Proc. Roy. Soc. London, Ser. A* **1957**, *239*, 550–556.
(35) For this regression analysis, we used the programs described as the dual substituent parameter program: Marzilli, L. G.; Bayo, F.; Summers, M. F.; Thomas, L. B.; Zangrando, E.; Bresciani-Pahor, N.; Mari, M.; Randaccio, L. *J. Am. Chem. Soc.* **1987**, *109*, 6045–6052.
(36) Zangrando, E.; Bresciani-Pahor, N.; Randaccio, L.; Charland, J. P.; Marzilli, L. G. *Organometallics* **1986**, *5*, 1938–1944.
(37) Kräutler, B.; Keller, W.; Kratky, C. *J. Am. Chem. Soc.* **1989**, *111*, 8936–8938.

AdoCbl has been chemically cleaved, are not significantly different from the corrin ring conformation of base-on AdoCbl. Therefore, any change in the conformation of the corrin ring occurs only when the molecule is bound to the enzyme. To create such a large change in the conformation of the corrin ring the enzyme must be creating a large upward conformational distortion of the corrin. It has been suggested that, in AdoCbl and AdeEtCbl, the steric effect of the alkyl group prevents this upward conformational distortion from occurring without homolysis of the Co–C bond,[9] whereas it is possible with the less bulky Ade($-CH_2-$)$_n$Cbls. We believe it is possible that the adenine orientation must be correct for the distortion to occur and that this orientation is not correct for AdeEtCbl.

In conclusion, the trends observed in the enzyme binding and CD spectral changes in Ade($-CH_2-$)$_n$Cbls may not be simply a function of the bulk differences in the alkyladenine group. In addition, the orientation of the adenine ring and the fluxional character of the alkyladenine groupings probably play a role. The nucleotide loop does not appear to be affected by alkyladenine bulk or electronic properties. Although corrin ring flexibility remains a viable explanation, the X-ray and NMR results continue to point to only small changes in the corrin ring conformation in the absence of enzyme binding.

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Registry No. AdePrCbl, 34502-77-7; AdeEtCbl, 59209-78-8; AdeBuCbl, 21806-90-6; AdePeCbl, 56226-23-4; hydroxocobalamin, 13422-51-0; 9-(3-chloropropyl)adenine, 19255-49-3; 9-(2-chloroethyl)adenine, 19255-48-2; 9-(4-chlorobutyl)adenine, 69293-19-2; 9-(ω-chloropentyl)-adenine, 53359-09-4.

Supplementary Material Available: NMR figures of the longer mixing time HOHAHA spectrum, part of the longer mixing time HOHAHA spectrum showing connectivities between the methylene protons, the short mixing time HOHAHA spectrum, part of the ROESY spectrum showing the NOE connectivities of the upfield protons, the HMQC spectrum, part of the HMBC spectrum showing the methyl proton region, and part of the HMBC spectrum showing the methine proton region, tables of anisotropic thermal parameters for all non-hydrogen atoms, fractional coordinates of hydrogen atoms, and bond distances between adjacent non-hydrogen atoms and their esd's, and an ORTEP diagram of AdePrCbl (17 pages); table of observed and calculated structure factors (41 pages). Ordering information is given on any current masthead page.

Combined X-ray Crystallographic, Single-Crystal EPR, and Theoretical Study of Metal-Centered Radicals of the Type $\{\eta^5\text{-}C_5R_5Cr(CO)_2L\}$ (R = H, Me; L = CO, Tertiary Phosphine)

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Abstract: The X-ray crystal structures of the compounds $\eta^5\text{-}C_5Me_5Mn(CO)_3$, $\{\eta^5\text{-}C_5Me_5Cr(CO)_2(PMe_3)\}$, and $\eta^5\text{-}C_5Me_5Mn(CO)_2(PMe_3)$, the latter two new, have been determined: $\eta^5\text{-}C_5Me_5Mn(CO)_3$ crystallizes in the centric space group $P2_1/m$, $\eta^5\text{-}C_5Me_5Mn(CO)_2(PMe_3)$ in *Pbca*, and $\{\eta^5\text{-}C_5Me_5Cr(CO)_2(PMe_3)\}$ in *Pmnb*. All three compounds assume essentially "piano-stool-like" structures, the manganese compounds with OC–Mn–CO and OC–Mn–P bond angles of $\simeq 92°$, normal for this type of 18-electron compound. In contrast, the 17-electron chromium compound exhibits a closed-in OC–Cr–CO bond angle of only 79.9 (1)°, comparable with that of the only other such compound previously reported, $\{\eta^5\text{-}C_5H_5Cr(CO)_2(PPh_3)\}$ (80.9 (1)°). Single crystals containing about 1% of $\{\eta^5\text{-}C_5Me_5Cr(CO)_3\}$ and $\{\eta^5\text{-}C_5Me_5Cr(CO)_2(PMe_3)\}$ doped into their manganese analogues have been studied by EPR spectroscopy at 20 K, and the g and ^{31}P hyperfine tensors have been assembled in the crystal-axis systems of the hosts. Although interpretation of the data for $\{\eta^5\text{-}C_5Me_5Cr(CO)_3\}$ is not unambiguous, $^2A''$ ground states seem likely for both it and $\{\eta^5\text{-}C_5Me_5Cr(CO)_2(PMe_3)\}$, as was observed previously for $\{\eta^5\text{-}C_5H_5Cr(CO)_2(PPh_3)\}$. Complementing the above, LCAO-HFS calculations for $\{\eta^5\text{-}C_5H_5Cr(CO)_3\}$ and $\{\eta^5\text{-}C_5H_5Cr(CO)_2(PH_3)\}$ have also been carried out, the nature and geometry-optimized structures of the ground states being determined. While $\{\eta^5\text{-}C_5H_5Cr(CO)_3\}$ is predicted to assume a $^2A'$ ground state, the first excited state, of $^2A''$ character, lies only some 7 kJ mol^{-1} higher; thus both states should be populated in the gas phase, and the relative ordering in condensed media may well be sensitive to the environment. Interestingly, $\{\eta^5\text{-}C_5H_5Cr(CO)_2(PH_3)\}$ is predicted to assume a $^2A''$ ground state with an optimized geometry in which the OC–Cr–CO angle is $\simeq 80°$, in agreement with the EPR data and the X-ray crystal structures of both $\{\eta^5\text{-}C_5Me_5Cr(CO)_2(PMe_3)\}$ and $\{\eta^5\text{-}C_5H_5Cr(CO)_2(PPh_3)\}$.

Considerable interest has developed in recent years in 17-electron, organotransition-metal complexes, in large part because very little is known as yet of the chemistry and of the electronic structures of this unusual class of compounds.[1] Relevant to the present study, aspects of the chemistry of the 17-electron com-

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(1) Baird, M. C. *Chem. Rev.* **1988**, *88*, 1217.

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