Direct Evidence for the Conformational Deformation of the Corrin Ring by the Nucleotide Base in Vitamin B_{12}: Synthesis and Solution Spectroscopic and Crystal Structure Analysis of *Coβ*-Cyanoimidazolylcobamide[1]

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Growing cells of *Propionibacterium shermanii*, when supplemented with imidazole, produced a "complete" corrinoid, as reported from a similar (but also cobinamide supplemented) setup by Müller and co-workers (Eberhard, G.; *et al. Biol. Chem. Hoppe-Seyler* **1988**, *369*, 1091). The corrinoid isolate from workup with added potassium cyanide was confirmed to consist mainly (84%) of the suggested *Coβ*-cyanoimidazolylcobamide (**1**), the vitamin B_{12} analogue, in which the cobalt-coordinating 5,6-dimethylbenzimidazole base of the vitamin is replaced by imidazole. An extensive NMR spectroscopic analysis of aqueous solutions of **1** was carried out: all ^1H and ^{13}C signals in the NMR spectra of **1** were assigned unambiguously, exploiting two-dimensional gradient enhanced homonuclear and heteronuclear experiments, including a new variant of the two-dimensional H-relayed X,H-Overhauser experiment at natural abundance, called 2D-HSQC-ROESY, which combines the merits of heteronuclear single quantum coherence (HSQC) and rotating frame Overhauser spectroscopy (ROESY). The structural analyses by NMR, UV, and CD spectroscopy of **1** in aqueous solution did not unravel any gross structural differences of the corrin ligand and of the nucleotide loop of **1** and of vitamin B_{12} (**2**). Cryo-temperature single-crystal structure analyses have been carried out for **1** and **2**, the latter one to obtain reference data more accurate than the ones available from Hodgkin's analysis (Brink-Shoemaker, C. *et al. Proc. R. Soc. London, A* **1964**, *278*, 1). The imidazolylcobamide **1** crystallized at room temperature from aqueous acetone in the orthorhombic space group $P2_12_12_1$, with $Z = 4$. Diffraction data were collected at 92 K (a = 15.335(16) Å, b = 21.974(9) Å, c = 25.501(11) Å, V = 8705(2) Å^3), the structure was solved by the Patterson method and refined to a final R value of 0.1017 for 6105 reflections with $F_o > 4\sigma(F_o)$. Vitamin B_{12} (**2**) was crystallized in the coldroom by vapor diffusion of acetone into a solution of **2** in water/ethylene glycol (20%). The crystal structure was determined at 88 K (orthorhombic, $P2_12_12_1$, $Z = 4$, a = 15.838(7) Å, b = 21.927(12) Å, c = 25.689(13) Å, V = 8921(8) Å^3) and refined to R = 0.0824 for 5638 significant reflections. In **1** the imidazole coordination at cobalt occurs from the lower face intramolecularly and with the "north/south" orientation also found for the benzimidazole base of vitamin B_{12} (**2**). Comparison of the X-ray data of **1** and of **2** indicates a considerably smaller "upward folding" of the corrin ring in **1** (11.3(0.2)°) than in **2** (18.0(0.3)°). A contribution to the "upward folding" of the corrin ligand of vitamin B_{12} accordingly is indicated here to arise from the bulky, Co-coordinated benzimidazole base of the cobalamins, in support of a hypothesis put forth earlier by Lenhert (Lenhert, P. G. *Proc. R. Soc. London, A* **1968**, *303*, 45), when describing the structure of coenzyme B_{12}.

Introduction

Many questions concerning the dependence of the biofunctionally important reactivities of the B_{12} coenzymes on their structural characteristics[2] are still open.[3-5] Coenzyme B_{12} (**3**, adenosylcobalamin) is known to act as a cofactor in a series of complex enzymatic reactions,[6,7] where its relevant reactivity is believed to be due to an enzyme activated homolysis of its organometallic bond to produce a 5′-deoxyadenosyl radical.[6-9] As pointed out by Halpern, the coenzyme **3** appears well adapted for its (hypothetical) biological function as a "reversible source of alkyl radicals".[7]

The rate of enzymatically accelerated homolytic cobalt–carbon bond cleavage of coenzyme B_{12} (**3**) has been suggested to exceed the rate observed in aqueous solution of **3** by a factor of about 10^{12}.[7,10] A widely discussed[3,4a,11-14,15b] explanation for this acceleration is based on the conformational distortion hypoth-

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esis,[7,15] which postulates a dependence of the rate of bond homolysis on steric deformations. Specifically, a "butterfly"-type "upward" deformation of the corrin-ring plane has been suggested to weaken the Co–C bond by "lifting" the adenosyl ligand by a "mechanochemical" mechanism.[3,15–17] This could indeed by an appealing mechanism by which binding of **3** to the apoprotein in the presence of substrate could trigger Co–C homolysis.

An outstanding structural feature of coenzyme B_{12} (**3**), methylcobalamin (**4**),[13] and other cobalamins,[3] is their metal-coordinating 5,6-dimethylbenzimidazole nucleotide function. This intramolecular nucleotide coordination may influence significantly by "electronic" and by "steric" effects the reactivities of the cobalamins in organometallic and other reactions.[4,15,17–19] Notably, it may be a factor influencing the conformation of the corrin ligand, as the spacial requirements of the bulky nucleotide base upon intramolecular coordination to the "lower" face of the corrin-bound cobalt-center may be met better by a nonplanar, "upward-bent" corrin ligand.[5,15] Originally this was pointed out by Lenhert in the course of the structural analysis of the coenzyme **3**.[2,3] Later, the corresponding "upward-flexing" of the corrin ligand was confirmed as the major discernible factor of conformational variability of natural corrins.[14]

Recently, Sagi and Chance[11] have invoked the strain between coordinated nucleotide base and equatorial corrin ligand of vitamin B_{12} (**2**) to explain a "remarkable and unexpectedly long" axial cobalt–nitrogen bond distance of 2.15(3) Å deduced from EXAFS-data from a frozen solution of **2**. The proposed long cobalt-nitrogen bond distance exceeds the one observed earlier by X-ray analysis[20] by almost 0.2 Å, and would indeed also be at variance with expectations based on an empirical correlation between Co–N_{ax} bondlength and nucleotide basicity.[2c,3b]

When supplemented with imidazole and cobinamide, a *Propionibacterium shermanii* culture has been suggested by Müller and co-workers[21] to produce imidazolylcobamides. These novel complete corrinoids would possess a less bulky imidazole base instead of the 5,6-dimethylbenzimidazole base present in coenzyme B_{12} and related cobalamins. Their structure analysis would provide an opportunity to test experimentally the effect of the steric bulk of the nucleotide base on the "upward-folding" of the corrin ligand in a vitamin B_{12} analogue. Here we report (a) on the preparation of *Coβ*-cyanoimidazolylcobamide (**1**) via "guided biosynthesis"[22] by an imidazole-supplemented culture of *P. shermanii*, (b) on the investigation of the spectroscopic and structural properties of **1** in solution, (c) on the determination of the crystal structure of **1**, and (d) on a redetermination of the crystal structure of vitamin B_{12} (**2**, *Coβ*-cyano-5′,6′-dimethylbenzimidazolylcobamide).[20] Both crystal structure analyses were carried out at cryotemperatures (≈100 K) in order to prevent radiation damage and to achieve optimum resolution.[23] Comparison of the high-resolution structural data thus obtained yields unprecedented insight into the effect of the steric bulk of the nucleotide base on the "folding" of the corrin ligand in vitamin B_{12}.[2,3] The spectroscopic and structural data of **1** also provide reference points for the analysis of cobamide binding proteins, in which coordination at the cobalt by the imidazole from a histidine residue is suspected.[24] The availability of imidazolylcobamides, such as **1**, also opens the way for kinetic and thermodynamic studies pertaining to the effect of the steric bulk of the nucleotide base by comparison of adenosyl and methyl derivatives of **1** with the B_{12} coenzymes **3** and **4**.

Experimental Part

General Data. Reagents and Solvents: imidazole, puriss, p.a.; CH_3OH, puriss, p.a.; acetone, puriss, p.a.; diethyl ether, puriss, p.a. (all from Fluka (Buchs, Switzerland, or Neu-Ulm, FRG)).

Water was deionized and passed through a "nanopore"-filter, Ultrafilter, Barnstead, USA; XAD-4 adsorbent resin, particle size 0.1–0.2 mm, research grade (Serva, Heidelberg, FRG), neutral aluminum oxide, type 507C, 100–125 mesh (Fluka, Neu-Ulm, FRG).

HPLC: stationary phase, nucleosil 120–10 C18 reversed phase (Macherey & Nagel, Düren, FRG) column (30 cm × 3.9 mm); mobile phase, methanol/0.017 M aqueous acetic acid (23:77 or 1:4) at a flow rate of 1 mL/min; detection at 254 and 540 nm.

UV–Vis: in H_2O; Perkin-Elmer PE 555, citation of λ_{max} (log ϵ) in nm; s denotes shoulder.

CD: in H_2O and in CH_3OH; Jobin-Yvon Mark III; wavelength of the extrema in nm (molar decadic circular dichroism [$\Delta\epsilon$]); s denotes shoulder.

IR: 0.3% in KBr, Perkin-Elmer PE 983, in cm^{-1}, relative intensities s, m, and w denote strong, medium, and weak, respectively.

FAB-MS: VG ZAB SEQ, nitrobenzyl alcohol (NOBA) matrix, argon bombardment at 8.4 keV, positive ion spectra with m/z [%] for signals at $m/z > 600$.

^{1}H-NMR: 400.13 MHz, Bruker AMX-400, in D_2O, $c \approx 2 \times 10^{-2}$ M, δ values (chemical shifts) in ppm, with δ(HDO) = 4.71 ppm; s, d, t, q, m denote singlet, doublet, etc.; coupling constants (J) in Hz.

^{13}C-NMR: 125.15 MHz, Varian UNITY plus 500, in D_2O, $c \approx 2 \times 10^{-2}$ M, chemical shifts (δ values) in ppm, with $\delta(TSP)_{ext}$ = 0 ppm; coupling constants $J(^{13}C-^{31}P)$ in Hz.

2D-NMR Experiments. 2D experiments were run on a Varian UNITY plus 500: 499.887 MHz (^{1}H)/125.15 MHz (^{13}C); 5 mm indirect detection probe equipped with gradient facilities; unbuffered solution of **1** in D_2O, ca. 2×10^{-2} M at 26 °C.

Gradient Enhanced Double-Quantum Filtered Correlation Spectroscopy (DQF-COSY).[25a] N-type coherence pathways were

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selected; spectra were obtained in the absolute value mode. Then 512 single scan FIDs of 1K complex points were obtained in 4 min. The applied pulsed field gradients had strengths of 9.9 and 19.8 G/cm (double-quantum filtered COSY), respectively, and durations of 2.5 ms each. Gaussian line broadening was used in both t_1 and t_2 dimensions. Zero filling in t_1 yielded spectra of 1K × 1K complex points. No base-line correction routines were applied.

Gradient-Enhanced Hetero-Single-Quantum Coherence Experiment (HSQC).[25b,c] Indirect detection of the low-γ carbon nuclei was achieved by means of the gradient enhanced HSQC-experiment.[25b,c] Two sets of spectra were recorded and processed according to the recipe by States et al.[25d] in order to yield a pure absorption spectrum with quadrature in F_1. The one-bond ^1H–^{13}C shift correlation spectrum, shown in the supplementary material, resulted from a 2 × 256 × 1024 data matrix size, with four scans per t_1 value and a delay time between scans of 0.6 s. The first gradient was applied with a strength of 19.75 G/cm and a duration of 2 ms, while the second gradient pulse of 19.48 G/cm was applied for 0.5 ms. Both gradients were rectangular and were applied along the z-axis. Decoupling (during acquisition) was achieved with the use of the GARP decoupling sequence,[25e] using a 3.8 kHz radio frequency field. Shifted squared sine bell windows were used for both t_1 and t_2.

Gradient-Enhanced ^1H-Detected Multiple-Bond Heteronuclear Multiple-Quantum Coherence Experiment (PFG-HMBC).[25c,f] Magnitude mode spectra were obtained using the standard gradient enhanced HMQC pulse sequence[25c] with an additional low-pass filter as in the original HMBC experiment.[25f] Values of δ_1 and δ_2 were 3.3 and 60 ms, respectively. The multiple-bond ^1H–^{13}C shift correlation spectrum resulted from a 512 × 2048 data matrix size, with 128 scans per t_1 value and a delay time between scans of 1 s. The first two gradients were applied with strengths of 9.87 G/cm each, while the third gradient pulse had a strength of 4.98 G/cm. The durations of the three gradient pulses were 2 ms. All gradients were rectangular and were applied along the z-axis. Shifted squared sine bell windows were used for both t_1 and t_2.

Spin-Locked NOE Spectroscopy (ROESY).[25g,h] The ROESY spectrum of **1** resulted from a 512 × 1024 before and 1K × 1K after zero-filling, with 32 scans per t_1 value. Predelay was 1.5 s, and the mixing time was 200 ms. A 1.8-kHz rf field strength was used. A cosine bell squared filter and additional line broadening were used in both t_1 and t_2 dimensions, in order to avoid truncation effects. Quadrature detection in F_1 was achieved by means of the recipe of States et al.[25d]

Total Correlation Spectroscopy (TOCSY)[25i] **or Homonuclear Hartmann–Hahn (HOHAHA) Spectroscopy.**[25j,k] The HOHAHA spectrum resulted from a 512 × 1024 data matrix with 32 scans per t_1 value. A MLEV-17 mixing sequence[25l] of 75 ms preceded by a 2.0-ms trim pulse was used. A 14.2-kHz rf field strength (corresponding to 17.6 μs 90° ^1H pulse width) was used. A cosine bell squared filter and additional line broadening were used in both t_1 and t_2 dimensions. Quadrature detection was done in F_1 by means of the recipe of States et al.[25d]

HSQC–TOCSY (Proton-Relayed X,H Correlation Spectroscopy).[25m,n] A 512 × 1024 data matrix with 128 scans per t_1 value was used, with a MLEV-17 mixing sequence[25l] of 0.75 ms preceded by 2.0-ms trim pulse, at 14.2-kHz rf field strength. Decoupling (during acquisition) was done with the GARP decoupling sequence,[25e] using a 3.6-kHz rf field. Shifted squared sine bell windows were used in t_1 and t_2. Quadrature detection was done in F_1 by means of the recipe of States et al.[25d] To suppress signals from protons bound to ^{13}C isotopes, BIRD nulling[25o] was used (delay 400 ms).

2D-HSQC–ROESY.[25p] The spectrum in Figure 4 resulted from a 512 × 1024 data matrix with 128 scans per t_1 value: mixing time 200 ms; 1.8-kHz rf field strength; other conditions same as for HSQC-TOCSY (above).

Preparation and Isolation of Crystalline *Coβ*-Cyanoimidazolylcobamide. *P. shermanii* fermentations as well as the extraction, purification and isolation of the corrinoids in their cyanide forms were performed by adaption of a procedure described elsewhere.[22b] The bacterium was grown anaerobically at 28 °C in 14 L of complex medium, which was supplemented with $CoCl_2$ hydrate (20 mg/L) and imidazole (100 mg/L).

About 480 g of wet cell material was harvested by centrifugation (15 min, 3000g, 4 °C) after 5 days of incubation. The cell paste was twice extracted in a boiling water bath with 4 L of 0.1 M sodium acetate buffer, pH 5.0, which contained 0.01 M KCN. The corrinoid solution (*ca.* 45 μmol) obtained after centrifugation (15 min, 3000g, 4 °C) was purified by column chromatography on XAD-4 (3 cm × 10 cm) and neutral aluminum oxide (2 cm × 7 cm). The corrinoids (*ca.* 39 μmol) were further purified by HPL-chromatography (C18-reversed phase, MeOH:AcOH = 23:77). A solution of the imidazolylcobamide eluted after 13.5 min (vitamin B_{12} after 18 min). After removal of the solvents with the help of a rotary evaporator (35 °C, 25 mbar) approximately 33 μmol of the imidazolylcobamide **1** were obtained as a chromatographically homogeneous fraction. About 90% of this sample was dissolved in *ca.* 0.5 mL of water. Addition of *ca.* 2 mL of acetone led to the formation of red needle-shaped crystals of the corrinoid **1**. The sample was dried (high vacuum, 38 h, room temperature) to yield 33.5 mg (*ca.* 22.3 μmol) of crystalline *Coβ*-cyanoimidazolylcobamide, to be used for the subsequent spectral analysis.

Spectroscopic Data for *Coβ*-Cyanoimidazolylcobamide (1). UV–vis (c = 5.17 × 10^{-5} M): 277 (4.01), 305 (3.90), 321 (3.85), 345 s (4.17), 361 (4.47), 410 (3.49), 485 s (3.73), 519 (3.91), 553 (3.95); min 262, 291, 315, 329, 398, 436, 534.

CD (c = 5.17 × 10^{-5} M, H_2O): 230 (9.1), 250 (–10.1), 286 (0.8), 307 (–9.5), 322 (–11.2), 347 (–9.1), 363 (–15.3), 388 s (2.1), 410 s (9.9), 428 (16.5), 479 (–1.0), 533 (1.4), 571 (1.2). CD (c = 8.0 × 10^{-5} M, CH_3OH): 253 (–10.4), 276 (–2.6), 308 (–7.8), 322 (–8.2), 347 (–6.9), 363 (–8.6), 386 s (2.0), 408 s (8.0), 427 (13.4), 500 (0.6), 530 (0.6), 554 (–1.0).

IR (3% in KBr): 2134 cm^{-1} (CN ligand), etc. FAB-MS: m/z (relative intensity in %): 1278.9 (21), 1277.9 (62), 1276.9 (100, M^+); 1252.1 (10), 1251.1 (19, M^+ – 26), 1250.1 (13), 1249.1 (17); etc.

^1H-NMR (400 MHz, see Figure 3): 0.67 (s, 3H, H_3(C20)); 1.19 (s, 3H, H_3(C46)); 1.25 (d, J = 5.3, 3H, H_3(CPr3)); 1.38 (s, 3H, H_3(C54)); 1.48 (s, H_3(C25), H_3(C47)), superimposed by m(H_a(C41)), in total 7H; 1.81 (s, H_3(C36)), superimposed by

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Table 1. ^{1}H- and ^{13}C-NMR Chemical Shifts[a] and Signal Assignments for Coβ-Cyanoimidazolylcobamide (1)

assignt	δ(^{13}C)[b]	δ(^{1}H)[c]	assignt	δ(^{13}C)[b]	δ(^{1}H)[c]
C53	17.4	2.50	CR5	62.8	3.86, 3.69
C35	18.0	2.38	CR2	72.2	4.24
C25	19.2	1.47	CR3	75.5[3]	4.61
C54	19.3	1.38	CPr2	75.7[4]	4.31
CPr3	21.6[1]	1.25	C19	77.6	4.04
C47	21.6	1.47	CR4	84.7[5]	3.99
C36	21.6	1.80	C1	87.1	
C20	23.3	0.67	CR1	91.5	5.97
C30	27.8	2.18, 2.01	C10	96.4	6.07
C41	28.4	2.16, 1.45	C15	105.6	
C48	30.5	1.83	C5	109.0	
C46	33.4	1.18	CB5	122.2	6.97
C60	34.3	2.77, 2.70	C64	126.2	
C55	34.6	2.56, 1.85	CB4	128.6	5.96
C56	34.7	2.56, 2.07	CB2	139.2	6.82
C42	34.9	2.04, 1.92	C6	165.9	
C49	36.9	2.55	C14	168.4	
C31	37.5	2.55	C9	175.9	
C18	41.6	2.81	C57	177.2	
C26	45.1	2.36, 2.30	C38	177.7	
C37	46.0	2.28, 2.24	C61	178.4	
CPr1	47.7[2]	3.59, 3.06	C27	178.5	
C2	49.6		C11	179.4	
C12	50.7		C32	180.6	
C7	53.3		C43	180.6	
C13	56.4	3.29	C50	180.6	
C8	58.2	3.47	C16	181.1	
C3	58.7	4.05	C4	181.6	
C17	61.6				

[a] ^{1}H-NMR: δ, with δ(HDO) = 4.71 ppm. ^{13}C-NMR: δ, with $\delta(TSP)_{expt}$ = 0 ppm. [b] ^{31}P-^{13}C couplings of (1) J_{CP} = 3.6 Hz, (2) J_{CP} = 4.3 Hz, (3) J_{CP} = 3.8 Hz, (4) J_{CP} = 6.6 Hz and (5) J_{CP} = 7.8 Hz. [c] See Experimental Part for detailed list of ^{1}H-NMR data.

m(H_2(C48), H_a(C55)), in total 6H; 1.93 (m, 1H, H_a(C42)); 2.0–2.2 (m, 5H, H_2(C30), H_b(C41), H_a(C56), H_b(C42)); 2.24/2.28 (AB-system, J_{AB} = 13.7, H_2(C37)); 2.34/2.39 (AB-system, J_{AB} = 13, H_2(C26)); 2.38, 2.50 (2s, H_3(C35), H_3(C53)), superimposed by 2.4–2.6 (m, H_2(C31), H_2(C49), H_b(C55), H_b(C56)), in total 16H; 2.6–2.9 (m, H(C18), H_2(C60)); 3.06 (dd, J = 14.5/8.6, 1H, H_a(CPr1)); 3.29 (t, $J \approx 6$, 1H, H(C13)); 3.47 (dd, J = 4.7/10.4, 1H, H(C8)); 3.59 (d, J = 14.5, 1H, H_b(CPr1)); 3.70 (dd, J = 4.2/12.5, 1H, H_a(CR5)) and 3.86 (dd, J = 2.5/12.5, 1H, H_b(CR5)); 3.99 (m, 1H, H(CR4)); 4.05 (d, 2H, H(C3), H(C19)); 4.25 (t, J = 3.6/4.5, 1H, H(CR2)); 4.31 (m, 1H, H(CPr2)); 4.61 (dt, J = 8.4/8.4/4.5, 1H, H(CR3)); 5.96 (s, 1H, H(CB4)); 5.97 (d, J = 4, 1H, H(CR1)); 6.06 (s, 1H, H(C10)); 6.82 (s, 1H, H(CB2)); 6.97 (s, 1H, H(CB5)).

^{13}C-NMR (125 MHz): see Table 1.

Crystallization and X-ray Single-Crystal Structure Analysis of Coβ-Cyanoimidazolylcobamide (1). Crystals were grown over a period of about 3 weeks at room temperature from a solution of **1** in aqueous acetone (acetone:water ≈ 2:1). A crystal of approximate dimensions 0.4 × 0.5 × 1.75 mm was removed from its mother liquor and immersed in a drop of hydrocarbon oil. With a tiny strip of filter paper, the surface of the crystal was dried from adhering mother liquor, the crystal was picked up with a glass fiber and shock-cooled by dumping into liquid propane.[23] Subsequently, the crystal was transferred to the cold-stream cryostate of the diffractometer with the help of a specially designed rapid-transfer device to prevent crystal damage by warming up.

Diffraction data were collected on a modified STOE diffractometer equipped with a locally constructed N_2-cold-stream low-temperature device using graphite-monochromated Mo Kα radiation (λ = 0.710 69 Å). Unit cell parameters were obtained by least-squares refinement against the setting angles of 37 reflections with 9° ≤ 2ϑ ≤ 11°. Crystals are orthorhombic, space group $P2_12_12_1$, with four formula units ($C_{57}H_{82}N_{14}O_{14}PCo \cdot 18H_2O.2(CH_3)_2CO$, formula weight 1717.7, 1277.3 for the cobalamin moiety plus 440.4 for solvent molecules) in the unit cell: a = 15.535(16) Å, b = 21.974(9) Å, c = 25.501(11) Å, V = 8705(2) Å^3, d_{calc} = 1.311 g/cm^3.

Intensity data were collected at 92(2) K using an ω step-scan technique with a fixed scan range of Δω = 2°. The scan speed was variable and depended on the result of a fast prescan. Stationary-crystal, stationary-counter backgrounds were measured at both ends of the scan, each for a quarter of the scan time. Three standard reflections from different regions of reciprocal space were periodically monitored (every 100 reflections). Their intensities showed no systematic trends during data collection.

Net intensities I were calculated as $I = S[C - 2(B_1 + B_2)]$ with C the total integrated peak count, B_1 and B_2 the two background counts, and S the scan rate. Standard deviations $\sigma(I)$ were obtained as $\sigma(I) = S[C + 4(B_1 + B_2)]^{1/2}$. Lorentz and polarization corrections were applied to I and $\sigma(I)$, but no absorption correction (μ = 0.30 mm^{-1}). All reflections for one octant of reciprocal space ($0 \le h \le 20$, $0 \le k \le 29$, $0 \le l \le 33$) with $(\sin \vartheta)/\lambda \le 0.66$ Å^{-1} were collected, leading to 11320 unique and 6111 significant ($I/\sigma(I) > 2$) reflections.

Structure solution of **1** involved locating the cobalt atom from a Patterson map and then determining the positions of the lighter atoms from subsequent electron density maps. Atomic coordinates, isotropic and anisotropic (see below) atomic displacement parameters (adp's) were refined with a full-matrix least-squares program[26] which minimized the quantity $\sum w(F_o^2 - F_c^2)^2$ with $w = 1/[\sigma^2(F_o^2) + (0.2P)^2 + 12P]$, $P = (\mathrm{Max}(F_o^2, 0) + 2F_c^2)/3$, using all reflections. Scattering factors were taken from ref 27.

Anisotropic adp's were refined for all non-hydrogen atoms of the cobalamin moiety, applying a "rigid-bond" restraint,[28] i.e. the components of the anisotropic displacement parameters in the direction of the bond were restrained to be equal within an effective standard deviation of 0.01 Å^2. The same restraint was applied to 1,3-distances. Hydrogen atom positions were calculated and refined as "riding" on their respective non-hydrogen atom. Methyl torsion angles were chosen to maximize the electron density at the three calculated H-atom positions and allowed to refine. An analogous procedure was applied to the two ribose–hydroxyl groups. The (isotropic) adp's for the hydrogen atoms were set to 1.5 times the equivalent isotropic adp of the adjacent non-H atom. Chemically equivalent bond lengths and 1,3-distances of the amide side chains were restrained to be equal, the atoms of the amide groups and of the imidazole base were restrained to be coplanar.

To model the solvent electron density, the following procedure was applied: the five best-ordered water molecules in the phosphate region were refined with anisotropic adp's and unit occupancy; the three acetone molecules were also refined anisotropically, but their structure was idealized by appropriate restraints on coordinates and thermal ellipsoids. For each acetone molecule, an occupation factor was also refined. The three occupation factors converged to 0.82(2), 0.55(3) and 0.81(4). The remaining peaks in the solvent region were modelled by including 27 "water" oxygen atoms with an isotropic adp and unit occupancy. Atoms which refined to adp values larger than 0.2 Å^2 were assigned partial occupancies in subsequent refinement cycles. This procedure eventually led to three fully occupied water sites plus 24 partially occupied sites. For the partial sites, a common isotropic adp was refined (which converged to 0.16(1) Å^2), together with an individual occupation factor for each site. The occupation factors refined to values between 0.2 and 0.8; the sum of all 24 occupation factors was 10.0 (0.2). Attempts to

(26) (a) Sheldrick, G. M. SHELXL-93, a program for the refinement of crystal structure from diffraction data. University of Göttingen, 1993. (b) SHELXTL-PC, Release 4.1. Siemens Crystallographic Research Systems, 1990.
(27) *International Tables for Crystallography*: Wilson, A. J. C., Ed.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1992; Vol. C.
(28) Hirshfeld, F. L. *Acta Crystallogr., Sect. A* **1976**, *32*, 239.

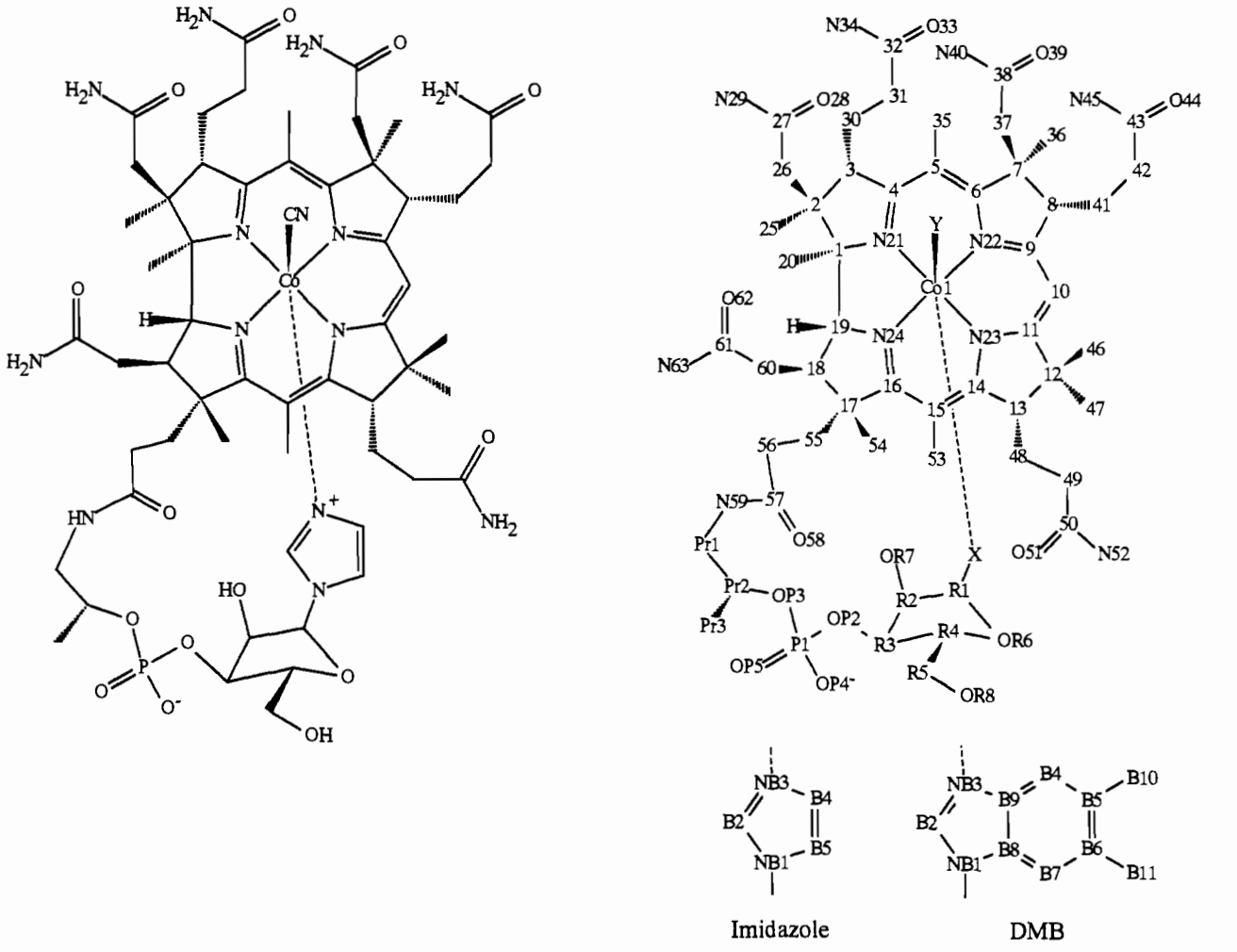

Figure 1. Left: structural formula for *Coβ*-cyanoimidazolylcobamide (**1**). Right: Numbering system adopted for the description of NMR and X-ray results. (**1**) *Coβ*-cyanoimidazolylcobamide (X = imidazol, Y = CN, with atom numbers 64 and 65 for C and N, respectively); (**2**) vitamin B_{12} (cyanocobalamin, X = DMB, Y = CN); (**3**) coenzyme B_{12} (*Coβ*-adenosylcobalamin, X = DMB, Y = 5′-desoxyadenosyl); (**4**) methylcobalamin (X = DMB, Y = CH_3).

interpret these peaks in terms of competing networks of hydrogen-bonded water molecules[29] were unsuccessful.

Refinement of 1065 parameters against 11320 intensity data and 508 restraints converged at the following values for the reliability indices: $wR_2 = [\sum[w(F_o^2 - F_c^2)^2/\sum[w(F_o^2)^2]]^{1/2} = 0.3241$ (for all 11320 reflections), $R_1 = \sum||F_o|-|F_c||/\sum|F_o| = 0.1017$ for 6105 reflections with $F_o > 4\sigma(F_o)$ and 0.1797 for all 11320 data, Goodness of fit $S = [\sum[w(F_o^2 - F_c^2)^2]/[n-p]]^{1/2} = 1.02$ (n = 11320, number of observations, p = 1065, number of parameters). The highest peak in a final difference electron density map was 1.2 e/Å^3; all difference peaks above 0.6 e/Å^3 were observed in the neighborhood (*ca.* 1 Å away) of the cobalt atom.

The atomic numbering used for the description of the structure is defined in Figure 1. Fractional coordinates of the fully-occupied non-hydrogen atoms are given together with their equivalent isotropic adp's in Table 2; anisotropic adp's of these atoms, fractional coordinates for partially occupied atoms and for hydrogen atoms, and tables describing the molecular geometry are given in the supplementary material.

Crystallization and X-ray Single-Crystal Structure Analysis of *Coβ*-Cyano-(5′-6′-dimethylbenzimidazolyl)cobamide (Vitamin B_{12}, 2). Procedure was analogous to the structure determination of **1**, unless explicitly stated otherwise. Crystals were grown in the coldroom by vapor-diffusing acetone into a solution of vitamin B_{12} ($c \approx 7.4$ mg/mL) in a mixture of water/ethylene glycol (20% v/v ethylene glycol). A crystal of dimensions 0.7 × 0.3 × 0.3 mm was investigated at 88 K with Zr-filtered Mo Kα radiation: orthorhombic, $P2_12_12_1$, Z = 4 for $C_{63}N_{88}N_{14}O_{14}PCo \cdot 20H_2O \cdot (CH_3)_2CO$, (formula weight 1773.8; 1355.4 B_{12}, 418.4 solvent), a = 15.838(7) Å, b = 21.927(12) Å, c = 25.689(13) Å, V = 8921(1) Å^3, d_{calc} = 1.32 g/cm^3, μ = 0.30 mm^{-1}, 95 reflections with $6° \le 2\vartheta \le 18°$ used for refinement of cell constants. Data collection ($\Delta\omega$ = 1.6°) for all reflections with $-1 \le h \le 17$, $-1 \le k \le 26$, $-1 \le l \le 30$ and $(\sin\vartheta)/\lambda \le 0.54$ Å^{-1}, yielding 8060 reflections, of which 7769 were unique and 5638 were significant.

Structure solution was performed with heavy atom techniques, refinement against F_o^2 with weights $w = 1/[\sigma^2(F_o^2) + (0.1P)^2 + 40P]$ (see above). The solvent density was modeled with 1 (fully occupied) acetone molecule, 12 fully and 17 partly occupied water sites (sum of partial occupancies 8.4(2)). Convergence (1001 parameter, 7769 data, 399 constraints) at wR_2 = 0.2315 (all data), R_1 = 0.0824 (5638 data with $F_o > 4\sigma(F_o)$ and R_1 = 0.1226 (all data), with S = 1.109. Highest peak in final ΔF Fourier synthesis: 0.77 e Å^{-3}.

Results

Preparation of *Coβ*-Cyanoimidazolylcobamide (1). About 480 g of wet cells were obtained from 14 L of *P. shermanii* fermentations, supplemented with ca. 100 mg of imidazole/L of

(29) (a) Savage, H. F. J.; Lindley, P. F.; Finney, J. L.; Timmins, P. A. *Acta Crystallogr., Sect. B* **1987**, *43*, 280. (b) Savage, H. F. J. *Biophys. J.* **1986**, *50*, 947. (c) Savage, H. F. J. *Biophys. J.* **1986**, *50*, 967. (d) Bouquiere, J. P.; Finney, J. L.; Lehmann, M. S.; Lindley, P. F.; Savage, H. F. J. *Acta Crystallogr., Sect. B* **1993**, *49*, 79. (e) Bouquiere, J. P. *Physica, B* **1992**, *180 & 181*, 745.

Table 2. Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Parameters ($Å^2 \times 10^3$) for the Non-Hydrogen Atoms of the Cobamide Moiety, the 3 Acetone Molecules, and the 10 Fully Occupied Water Molecules in the Crystal Structure of *Coβ*-cyanoimidazolylcobamide (**1**), Where *U*(eq) Is Defined as One-Third of the Trace of the Orthogonalized U_{ij} Tensor

	x/a	*y/b*	*z/c*	*U*(eq)		*x/a*	*y/b*	*z/c*	*U*(eq)
Co1	2668(1)	1038(1)	7411(1)	37(1)	C54	3355(8)	3133(5)	6938(4)	48(3)
C1	1503(6)	1902(4)	7870(4)	36(2)	C55	4038(6)	3250(4)	7822(3)	33(2)
C2	518(7)	2021(5)	7791(4)	42(2)	C56	4261(6)	2999(5)	8359(3)	39(2)
C3	155(7)	1381(5)	7805(4)	46(2)	C57	5048(7)	3306(4)	8574(3)	37(2)
C4	861(7)	985(5)	7613(4)	43(2)	O58	5734(5)	3276(3)	8328(3)	46(2)
C5	758(7)	399(5)	7390(5)	45(2)	N59	4961(5)	3592(3)	9015(3)	35(2)
C6	1437(7)	29(5)	7272(4)	41(2)	C60	1923(7)	3404(4)	7670(5)	50(2)
C7	1420(8)	-622(5)	7048(4)	44(2)	C61	3191(7)	3974(4)	7968(4)	48(2)
C8	2333(8)	-826(4)	7198(4)	46(2)	O62	2394(5)	3947(3)	8433(3)	54(2)
C9	2791(7)	-235(4)	7183(4)	40(2)	N63	2153(6)	4473(4)	7706(4)	51(2)
C10	3668(8)	-184(5)	7078(5)	50(3)	C64	2367(8)	1245(5)	6727(4)	44(2)
C11	4124(7)	348(5)	7086(4)	43(2)	N65	2175(8)	1403(5)	6320(4)	66(3)
C12	5025(7)	370(5)	6920(4)	43(2)	CPr1	5675(7)	3885(5)	9296(4)	40(2)
C13	5324(7)	981(5)	7179(4)	46(2)	CPr2	6005(7)	3472(5)	9732(4)	36(2)
C14	4462(7)	1323(5)	7237(3)	38(2)	CPr3	6674(7)	3792(5)	10044(4)	43(2)
C15	4394(6)	1911(5)	7327(3)	36(2)	P1	5182(2)	2642(1)	10252(1)	34(1)
C16	3600(6)	2188(4)	7458(3)	33(2)	OP2	5026(5)	2310(3)	9710(3)	39(2)
C17	3399(6)	2859(4)	7514(3)	36(2)	OP3	5306(5)	3319(3)	10060(2)	37(1)
C18	2519(6)	2864(4)	7761(4)	39(2)	OP4	4395(5)	2647(3)	10577(3)	44(2)
C19	2145(6)	2252(4)	7570(4)	39(2)	OP5	5955(5)	2385(3)	10501(3)	39(2)
C20	1733(7)	1870(4)	8445(4)	39(2)	CR1	4153(6)	863(4)	9319(4)	33(2)
N21	1598(6)	1270(4)	7640(3)	38(2)	CR2	4007(7)	1496(4)	9537(4)	34(2)
N22	2286(6)	228(4)	7292(3)	42(2)	CR3	4913(7)	1674(4)	9700(4)	35(2)
N23	3802(6)	884(4)	7229(3)	38(2)	CR4	5476(7)	1381(5)	9278(4)	40(2)
N24	2905(5)	1867(3)	7521(3)	37(2)	CR5	6309(7)	1187(5)	9473(5)	45(2)
C25	142(8)	2460(5)	8186(5)	47(2)	OR6	5006(4)	857(3)	9106(3)	39(2)
C26	390(7)	2267(6)	7231(4)	50(2)	OR7	3695(5)	1875(3)	9152(3)	41(2)
C27	-518(8)	2350(7)	7064(5)	68(3)	OR8	6259(5)	752(4)	9889(3)	50(2)
O28	-1150(6)	2235(6)	7325(5)	90(3)	NB1	3604(6)	712(4)	8889(3)	36(2)
N29	-626(9)	2655(10)	6633(6)	141(7)	CB2	3623(7)	953(4)	8414(4)	35(2)
C30	-184(6)	1119(4)	8328(4)	40(2)	NB3	2953(6)	787(4)	8131(3)	39(2)
C31	-1112(7)	1238(6)	8426(4)	61(3)	CB4	2477(6)	414(4)	8461(4)	36(2)
C32	-1440(7)	916(5)	8912(5)	54(3)	CB5	2877(7)	366(4)	8928(4)	38(2)
O33	-1775(7)	406(4)	8858(5)	93(4)	C1L[a]	2197(16)	987(9)	4961(9)	101(7)
N34	-1307(7)	1135(5)	9358(4)	59(2)	C2L[a]	3036(11)	301(7)	5569(8)	75(5)
C35	-147(7)	178(6)	7316(6)	58(3)	C3L[a]	2224(13)	397(8)	5255(6)	79(5)
C36	752(9)	-1044(5)	7250(5)	56(3)	O4L[a]	1700(9)	41(7)	5224(6)	94(5)
C37	1358(8)	-529(4)	6448(4)	46(2)	C5L[b]	3587(14)	4568(9)	6237(11)	71(8)
C38	1442(6)	-1111(4)	6152(4)	40(2)	C6L[b]	4819(20)	4434(17)	5605(9)	98(11)
O39	2161(5)	-1315(4)	6055(3)	52(2)	C7L[b]	4528(15)	4409(12)	6167(9)	80(9)
N40	741(6)	-1386(4)	6010(4)	47(2)	O8L[b]	4943(13)	4233(16)	6500(8)	105(10)
C41	2373(8)	-1140(5)	7735(4)	55(3)	C9L[c]	5171(17)	364(18)	4581(11)	151(14)
C42	3262(9)	-1313(8)	7899(5)	81(4)	C10L[c]	4769(27)	552(27)	3634(12)	259(29)
C43	3227(13)	-1674(8)	8424(6)	100(5)	C11L[c]	4466(21)	418(19)	4183(12)	171(19)
O44	2699(12)	-2067(7)	8485(5)	134(5)	O12L[c]	3768(15)	298(16)	4263(13)	211(16)
N45	3847(11)	-1541(9)	8729(5)	144(7)	O13L	2617(5)	2667(3)	9658(3)	45(2)
C46	5056(8)	461(7)	6304(5)	58(3)	O14L	1066(5)	3747(4)	9120(3)	50(2)
C47	5545(8)	-179(5)	7045(5)	52(3)	O15L	2717(5)	2477(3)	10739(3)	49(2)
C48	5683(6)	920(5)	7738(4)	41(2)	O16L	1679(6)	2786(4)	11580(4)	60(2)
C49	6605(6)	727(5)	7782(4)	46(2)	O17L	3288(6)	3786(4)	9442(4)	65(2)
C50	7205(7)	1053(5)	7414(5)	53(3)	O18L	1453(14)	4380(9)	6705(8)	151(6)
O51	7549(6)	774(4)	7053(3)	65(2)	O19L	2449(14)	4899(10)	5218(8)	149(6)
N52	7282(7)	1634(4)	7480(4)	57(2)	O20L	1010(17)	2269(10)	5869(9)	170(8)
C53	5181(7)	2314(5)	7277(4)	48(2)					

[a] Occupancy: 0.820(2). [b] Occupancy: 0.550(3). [c] Occupancy: 0.810(4).

medium. Workup as similarly described earlier,[21,22b] led to the isolation of ca. 39 μmol of a mixture of corrinoids, from which ca. 33 μmol of a chromatographically uniform "complete" corrinoid could be isolated after chromatographic purification by preparative HPLC.[22b] This noncrystalline sample exhibited the UV–vis properties suggested for the *Coβ*-cyanoimidazolylcobamide (**1**) by Müller et al.[21] and was separated from minor amounts of colorless impurities by crystallization from aqueous acetone. A sample of 33.5 mg (ca. 22.3 μmol) of bright red crystals of **1** was obtained in this way and was analyzed spectroscopically.

Spectroscopic Characterization of 1 in Aqueous Solution. An aqueous solution of **1** exhibited an UV–vis spectrum (Figure 2A) similar to that of vitamin B_{12} (**2**),[30a] indicating a "complete" corrinoid, with one cyanide ligand bound to the cobalt center. Compatible with a structurally modified nucleotide base, differences in the detailed structure in the near ultraviolet were observed in the spectra of **1**, as well as slight bathochromic shifts of the absorption maxima in the visible range (α-band, 547 → 551 nm; β-band, 513 → 517 nm, in methanol). The CD-spectrum of an aqueous solution of **1** (Figure 2B) turned out to differ more significantly from that of vitamin B_{12} (**2**):[30b] at long wavelengths $\Delta\epsilon$ is positive in the spectrum of **1**, but negative in that of **2**.[31] A

(30) (a) UV–vis spectra, reviewed in: Gianotti, C. In *B_{12}*; Dolphin, D., Ed.; Wiley: New York, 1982, Vol. 1, 393. (b) CD spectra: Bonnett, R.; Godfrey, J. M.; Math, V. B. *J. Chem. Soc., Perkin Trans 1* **1973**, 252. (c) FAB–MS spectra: Schiebel, H. M.; Shulten, H.-R. *Mass Spectrom. Rev.* **1986**, *5*, 249.

(31) As observed elsewhere for vitamin B_{12} (**2**) (see e.g. in ref 30b) the CD spectra show significant solvent dependence. A sign-inversion occurs for the long wavelength part of the CD spectrum of **1**, when going from an aqueous solution (positive $\Delta\epsilon$) to a methanolic solution (negative $\Delta\epsilon$ at 532 nm), see Experimental Part.

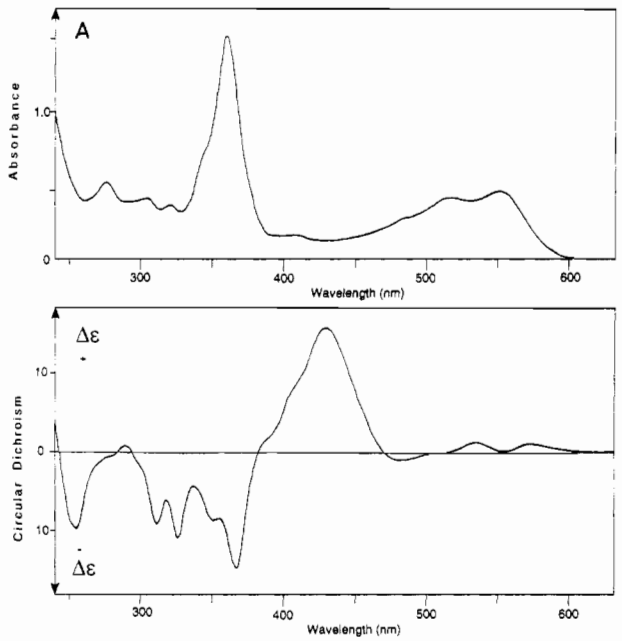

Figure 2. UV–vis absorption spectrum (A, top) and CD spectrum (B, bottom) of an aqueous solution of **1** (see text for details).

fast-atom bombardment (FAB)–MS[30c] exhibited signals due to the intact molecular ion (at m/z = 1277) and of the fragment arising from loss of the cyanide ligand (at m/z = 1251), confirming the molecular formula indicated ($C_{57}H_{82}CoN_{14}O_{14}P$) for this "complete" corrinoid.

More specific structural information was obtained from a series of one- and two-dimensional NMR-experiments:[25,32,33] In the ^{1}H-NMR spectra (at 400 MHz, see Figure 3) all the signals of nonexchangeable protons could be localized (see Table 1). Likewise in the ^{1}H-decoupled 125 MHz ^{13}C-NMR spectrum of **1** the signals of its 57 carbons were resolved into 50 singlets and 5 doublets (due to significant $^{2,3}J_{C,P}$ in the nucleotide loop); in addition a broad and weak signal due to carbon C64 of the cobalt-bound cyanide ligand is observed at 126.2 ppm (see Table 1).[34]

Complete assignment of both ^{1}H and ^{13}C signals of the NMR spectra of **1** was achieved by means of 2D gradient enhanced homonuclear (absolute value double quantum filtered correlation spectroscopy, PFG–DFQ–COSY)[25a] and heteronuclear experiments (heteronuclear single–quantum coherence, HSCQ,[25b,c] and gradient enhanced ^{1}H-detected multiple-bond heteronuclear multiple-quantum coherence, PFG–HMBC,[25c,f] experiments). In addition, conventional homonuclear techniques like total correlation spectroscopy (TOCSY)[25i] and rotating frame Overhauser spectroscopy (ROESY)[25g,h] have been performed. Because of severe overlap observed in the ^{1}H-NMR spectra, a proton-relayed correlation experiment (HSQC-TOCSY)[25m,n] was used to allow the observation of proton connectivities relayed at the greater dispersion afforded by the ^{13}C chemical shift.

From analysis of the COSY-spectra and of the chemical shifts of the (correlated) signals, the constitution and base-on nature of a *Coβ*-cyanoimidazolylcobamide (**1**) could be deduced clearly. A constitutionally and configurationally identical build-up of the corrin and nucleotide segments in the *Coβ*-cyano–Co(III)–corrin **1** and in vitamin B_{12} (**2**) was indicated by the close correspondence of the chemical shifts of most of their ^{1}H- and ^{13}C-NMR signals.[35,36] Notable exceptions from this correspondence in the ^{1}H-NMR spectra could be associated with the signals of protons that were found to the (constitutionally different) nucleotide heterocycles. In addition, in the spectrum of **1** the anomeric proton H(CR1) of the ribose experiences a smaller downfield shift, while the protons at the positions C20 and C41 (for the numbering scheme adopted here, see Figure 1) experience a smaller upfield shift in **1** than in **2** (obviously a consequence of the less powerful anisotropic effects of imidazole compared to benzimidazole). A comparison of the ^{13}C-NMR spectrum of **1** with that of **2** (that was assigned earlier)[36a] also shows similar chemical shifts ($\Delta\delta$ < 1 ppm) of signals due to constitutionally corresponding carbons, with the exception of the signal for CR1 ($\Delta\delta$ = 1.1 ppm (downfield) in **1**) and of the unsaturated carbons of the corrin ring ($\Delta\delta$ up to −2.2 ppm (upfield) in **1**).

To resolve multiplets in the ROESY spectrum, a new variant of proton-relayed X,H Overhauser experiment at natural abundance was developed, called 2D–HSQC–ROESY,[25p] which combines the relative mertis of HSQC and ROESY experiments. The HSQC part of the experiment simplifies the identification of the protons (the protons directly bound to the carbon), whereas the ROESY part establishes dipolar connectivities and thus provides information on spatial proximity. The unambiguous dipolar connectivities from the HSQC–ROESY completed the assignment and provided extra insight into conformational aspects of the solution structure of **1** (Figure 4). The conformational information was obtained from the ROESY and the HSQC–ROESY data by comparing the observed intensities with interproton distances from the crystal structure of **1** (a table with this comparison is supplied in the supplementary material). Using this approach, we arrived at several detailed conclusions about the solution conformation of *Coβ*-imidazolylcobamide. First, strong contacts were observed between the proton H(B2) (at 6.82 ppm) and the C48 methylene protons (1.83 ppm) as well as one of the protons at C56 (2.07 ppm). This is only consistent with the CB2→H(B2) vector pointing toward the "southern" part of the corrin ring. Weaker contacts of H(B2) to the methyl groups C20 and C53 support this conclusion. Second, cross-peaks between H(B4) and one of the protons at C41 (at 1.54 ppm) indicate a roughly identical conformation of the d side chain between solution and solid state. The scalar coupling constants for the protons H(C8) (3.47 ppm), H_a(C41) (1.45 ppm), and H_b(C41) (2.16 ppm) are consistent with a staggered conformation of the d side chain along the C8–C41 bond. In fact, the large vicinal coupling ($J_{H(C8),H_a(C41)} \approx 12$ Hz) and the NOE data help to identify H_a(C41) as H_{re}(C41), as the crystal structure shows H_{re}(C41) close to H(B4) of the imidazole ring. Thus, the solution conformation of nucleotide base and d side chain of **1** are compatible with the crystal structure.

Determination of the Crystal Structure of Vitamin B_{12} (2) at 88 K. Crystals of **2** were grown in the coldroom by vapor-diffusing acetone into vitamin B_{12} dissolved in a mixture of water/ethylene glycol (20% EG v/v). The structure determination was carried out on a shock-cooled specimen at 88 K.[37] The original structure determination on crystals of the "wet" vitamin by Hodgkin and co-workers[20b] involved intensity data determination[20] at room temperature on crystals grown from water. The differences in

(32) (a) Ernst, R. R. *Chimia* **1987**, *41*, 323. (b) Ernst, R. R.; Bodenhausen, G.; Wokaun, A. *Principles of Nuclear Magnetic Resonance in One and Two Dimensions*; Clarendon Press: Oxford, England, 1987.
(33) Kessler, H.; Gehrke, M.; Griesinger, C. *Angew. Chem.* **1988**, *100*, 507.
(34) The characteristic IR and UV–vis spectra confirm the presence of the cobalt-bound cyano ligand.
(35) (a) For a complete assignment of the ^{1}H and ^{13}C spectra of vitamin B_{12} (**2**), as well as of *Co*$_g$-cyano-5-hydroxybenzimidazolylcobamide, see ref 36a,b. The NMR data/assignments for **2**, reported in ref 36a, are inconsistent in two instances with our data (HSQC[25b,c] and HSQC–TOCSY[25n,o] recorded at 500 MHz (^{1}H)/125 MHz (^{13}C), unbuffered, ca. 0.02 M solution of **2** in D_2O, at 26 °C): $H_2(C_{41})$, 2 multiplets at 1.02 and 2.02 ppm; H_2(C55) and H_2(C56), pairwise at 1.82/2.66 and 2.12/2.51 ppm (or vice versa). (b) The 1H-NMR-spectrum of **2** has also been assigned earlier (however with several errors) by Kurumaya, K.; Kajiwara, M. *Chem. Pharm. Bull.* **1989**, *37*, 9.
(36) (a) Calafat, A. M.; Marzilli, L. G. *J. Am. Chem. Soc.* **1993**, *115*, 9182. (b) Eisenreich, W.; Bacher, A. J. *Biol. Chem.* **1991**, *266*, 23840. (c) Traylor, T. G.; Chang, C. K.; Geibel, J.; Berzinis, A.; Mincey, T.; Cannon, J. *J. Am. Chem. Soc.* **1979**, *101*, 6716. (d) Brown, K. L.; Hakimi, J. M. *J. Am. Chem. Soc.* **1986**, *108*, 496.
(37) Attempts to cool crystals grown from water led to destruction of the crystalline order, as indicated by a drastic increase of the mosaicity.[38]

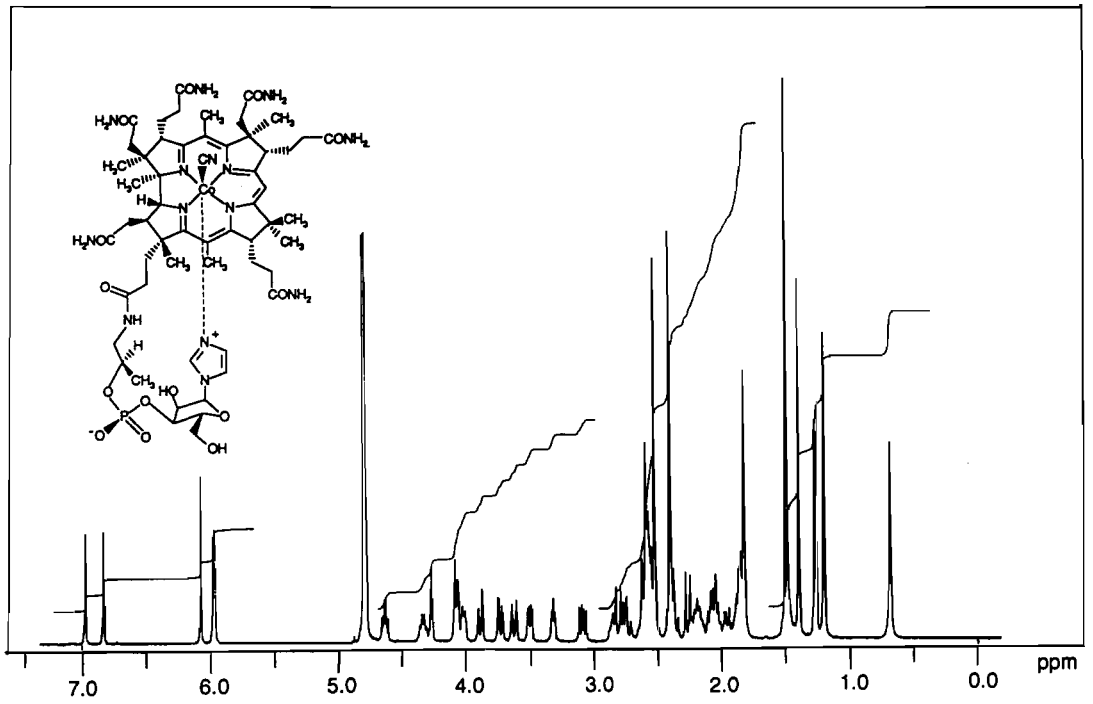

Figure 3. 400-MHz ^{1}H-NMR spectrum of a solution of **1** in D_2O (see text for details).

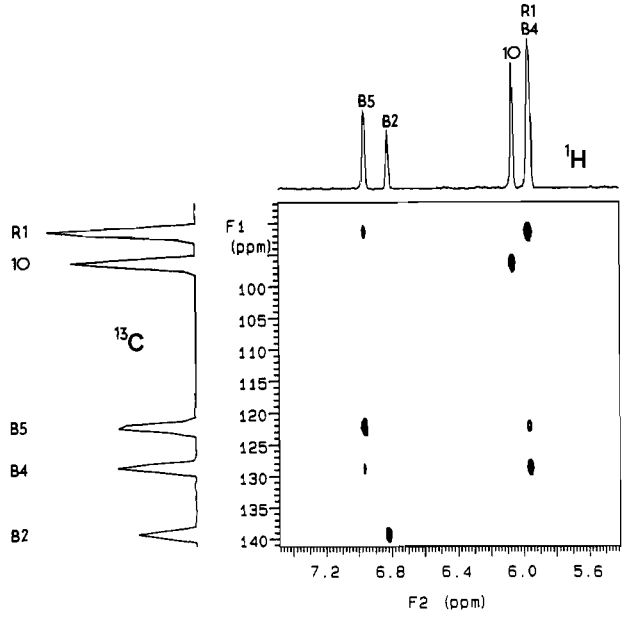

Figure 4. 2D–HSQC–ROESY[25p] of a solution of **1** in D_2O (low field section, see text for experimental details).

the conditions of crystallization, data collection, and structure refinement led to notable differences in the resulting crystal structures, mainly in the region of included solvent molecules.

Compared to the room temperature data, the 88 K structure shows by and large the same packing of B_{12} molecules in a unit cell with the same crystallographic symmetry (space group $P2_12_12_1$) but slightly different cell dimensions: a and b are decreased (by 0.5 and 1.8%, respectively), while c is increased (by 1.4%), leading in total to a decrease (by 0.9%) in the unit cell volume. There are significant differences in the structure of included solvent molecules: Hodgkin modeled the solvent region in the room-temperature crystal structure by including 8 whole and 30 half water molecules, while the solvent domain in the 88 K crystal structure consists of 1 acetone molecule (full occupancy) plus 12 fully and 21 partially occupied water sites (see experimental section). Each of Hodgkin's fully occupied water sites is within 0.5 Å from a fully occupied solvent site in the 88 K structure, one of them being close to the acetone molecule. Apart from these eight sites, there is no correspondence between the solvent domains in the two crystal structures. Similarities between the low-temperature crystal structure of **2** and Hodgkin's[20c] structure of "dry" B_{12} have been noted[38] and will be discussed elsewhere.

The molecular geometry of the B_{12}-molecule in the two structure determinations agrees within experimental error. Rms differences of 208 corresponding bond lengths amount to 0.08 Å, of 178 bond angles to 4.8°. Since the average least-squares esd's are 0.014 Å for bond length and 0.86° for bond angles in the 88 K crystal structure, is reasonable to blame inaccuracies in the room-temperature structure analysis for the better part of the above rms differences. A least-squares superposition of all B_{12}-atoms yields an rms deviation of 0.22 Å between corresponding atoms, significant deviations are observed for the ribose atoms C5r and C8r and for the conformation of the amide groups of the sidechains on ring A. Full documentation of the 88 K crystal structure analysis of vitamin B_{12}, including atomic coordinates and bonding parameters, plus a projection of the least-squares superposition of the B_{12} molecule from the two crystal structure determinations, is supplied in the supplementary material.

Determination of the Solid-State Structure of 1 by Single-Crystal X-ray Analysis. "Wet" crystals of **1** were grown at room temperature from aqueous acetone (acetone:water ≈ 2:1). A crystal structure analysis was carried out at 92 K, after shock-cooling the crystal by dumping it into liquid propane. **1** crystallizes in the orthorhombic space group $P2_12_12_1$ with four cobamide molecules in the unit cell. In addition, a considerable number of solvent molecules, some of them disordered, were observed in the crystal: judging from the sum of the crystallographically refined occupation factors, two acetone molecules were observed at three locations in the asymmetric unit, plus 18 water molecules distributed over 8 fully and 24 partly occupied sites.

A complete description of the crystal structure of **1**, based on the numbering system defined in Figure 1[3a] is given in Table 2 (atomic coordinates) and in the supplementary material. The cobalt atom is coordinated by the four equatorial corrin N-atoms, the axial cyano group from the β- ("upper") side and the imidazole from the ("lower") α-side. The intermolecularly coordinated

(38) Klintschar, G. Diplomarbeit, Universität Graz, 1993.

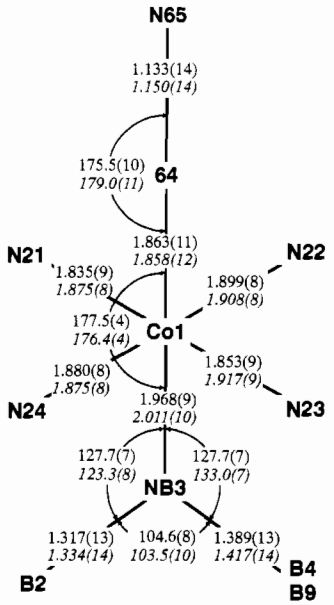

Figure 5. Selected bond lengths and bond angles around cobalt for the crystal structures of **1** (top numbers) and **2** (bottom numbers).

("base-on") imidazole group has the "north–south" orientation observed in all known cobalamin crystal structures.[2,3,39,40] Figure 5 shows a few selected bonding distances and bond angles pertaining to the cobalt coordination, together with the corresponding quantities from the crystal structure of vitamin B_{12} (**2**). Of particular interest is a decrease (relative to the vitamin B_{12} structure) in the Co–N_{ax} distance by 0.043(13) Å, as well as a change in the tilt[41] of the coordinated base, indicated by a large (9.7(1.1)°) difference between the two Co–N_{ax}–C bond angles in **2**.

Bond lengths and bond angles agree between **1** and the 88 K crystal structure of **2** within experimental error; the rms deviation of bond lengths between the two structures is 0.038 Å; that of bond angles, 1.9°. These values are compatible with the mean esd's from the two structure determinations: for bond lengths 0.026 (**1**) and 0.014 Å (**2**); for bond angles 1.5 (**1**) and 0.86° (**2**).

The deviations of the corrin atoms from the 4N-plane for the imidazolylcobamide (**1**) molecule and for the corresponding 5′-6′-dimethyl-benzimidazolyl derivative **2** (Figure 6) show that the corrin ring has a helical overall conformation as a result of the direct link between rings A and D via two tetrahedral carbon atoms. The nonplanarity of the conjugated part of the corrin ring can be characterized by the angle between the conjugated systems of the "northern" and the "southern" halves of the corrin ring. The planes consist of atoms N21, C4, C5, C6, N22, C9, and C10 (plane 1) and C10, C11, N23, C14, C15, C16, and N24 (plane 2), respectively. The angle between these two planes, which has been suggested[3,14] as a quantitative measure for the "upward folding" of the corrin ring, assumes values of 11.3 (0.2)° in **1** and 18.0 (0.3)° in **2**.

Distance matrix analysis[14] has been used as an alternative and complementary method to describe corrin ring deformations. To

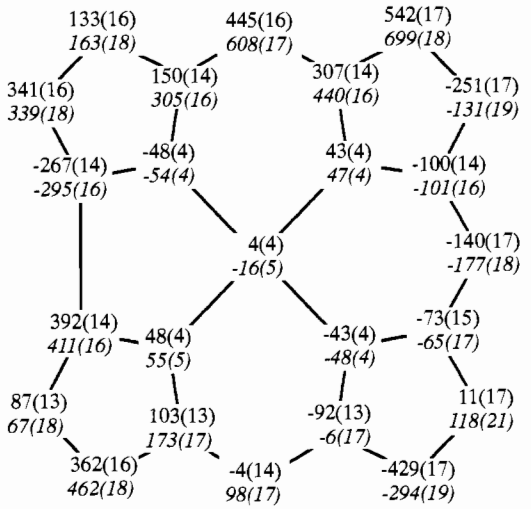

Figure 6. Deviations (Å × 1000) of corrin atoms from a least-squares plane through the four pyrrolic N-atoms. Top numbers: *Coβ*-cyanoimidazolylcobamide (**1**): bottom numbers: vitamin B_{12} (**2**).

X2	-6				
X3	-5	-12			
X4	6	-35	2		
X5	7	1	0	-6	
X6	1	-4	-8	-6	-2
	X1	X2	X3	X4	X5

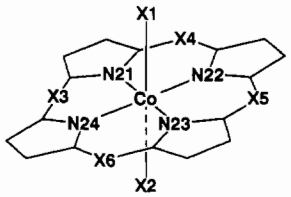

Figure 7. Matrix of the differences $d(X_i - X_j)^1 - d(X_i - X_j)^2$, with $d(X_i - X_j)^k$ being the distance between centroids X_i and X_j in structure **k**. The centroids were computed from the following atoms: X1, C64, N65; X2, NB1, CB2, NB3, CB4, CB5 (**1**); X2, NB1, CB2, NB3, CB8, CB9 (**2**); X3, C1, C19; X4, C4, C5, C6, C35; X5, C9, C10, C11; X6, C14, C15, C16, C53. Note that a negative entry in the matrix indicates that the corresponding distance is shorter in **1** than in **2**.

characterize the structural change induced by replacing the bulky 5,6-dimethylbenzimidazole ligand of vitamin B_{12} by the sterically less demanding imidazole group, we computed the (unweighted) centroids of the non-hydrogen atoms[42] for the β-axial cyano groups, the α-axial imidazole rings, the two atoms linking rings A and D, and the three methine bridges. Figure 7 gives the matrix of the differences of corresponding distances between structures **1** and **2**. The entries in Figure 7 show that the largest structural change on going from **2** to **1** is a decrease in the distance between the ("northern") C5-methine bridge and the center of the imidazole ring.

The crystal packing of **1** is very similar to the molecular arrangement observed in vitamin B_{12} and in the majority of cobalamin crystal structures determined so far.[38,43] The vacancy caused by the replacement of dimethylbenzimidazole with the less bulky imidazole is filled by two acetone molecules. Molecules related by the operation of the 2_1-screw axes along **a** are connected with their phosphate groups by the five "well-ordered" water molecules, leading to the formation of 1-dimensional chains running parallel to the crystallographic *a*-axis. The water molecules form an intricate network of hydrogen bonds, which also involves the amide heteroatoms of the b-, c-, e- and g-side chains of molecules belonging to neighboring chains. A table of the hydrogen bonds involving the atoms of the cobinamide moiety plus the (fully occupied) water molecules is given in the supplementary material.

(39) Kräutler, B.; Keller, W.; Kratky, C. *J. Am. Chem. Soc.* **1989**, *111*, 8936.
(40) Hohenester, E.; Kratky, C.; Kräutler, B. *J. Am. Chem. Soc.* **1991**, *113*, 4523.
(41) The term "tilt" follows Pratt's (ref 4a, p 359) description of a rotation of the nucleotide base relative to an axis running "east to west". The deformation, which involves an increase in the "northern" and a decrease of the "southern" Co–N_{ax}–C bond angle, is presumed to be caused by steric repulsion between the "northern" part of the DMB ligand and the corrin ring.[3,4a] As a quantitative measure for this deformation we suggest the "tilt angle" defined as half the difference between "northern" and "southern" Co–N_{ax}–C bond angles (i.e. (ϕ(Co1–NB3–CB9) – ϕ(Co1–NB3–CB2))/2 for **2** and (ϕ(Co1–NB3–CB4) – ϕ(Co1–NB3–CB2))/2 for **1**, with ϕ(A–B–C) denoting the bond angle between atoms A, B, and C).
(42) Note that the quantities computed for the distance matrix analysis by Pett et al.[14] are similar though not identical to the entries given in Figure 7.
(43) All fully reported cobalamin crystal structures are isostructural,[3,39,40,52] with the exception of adenosylcobalamin,[29] methylcobalamin,[13] and a monocarboxylic acid.[58]

Discussion

The elucidation of the effect(s) of the intramolecularly coordinating nucleotide function(s) of natural nucleotide containing (i.e. "complete") corrins on their reactivity in organometallic and other reactions is of considerable interest in view of the unique nature of these functions as well as of the constitutional diversity of their nucleotide bases.[22,44,50]

In the laboratories of Schrauzer,[15a,45] Pratt,[16] and others[12,17,18b] nucleotide coordinating organocobalamins were found to undergo spontaneous dealkylation in aqueous solutions considerably more readily, than their nucleotide-devoid organocobinamide or protonated "base-off" analogues. This led to the recognition of a "labilizing effect" of the axial nucleotide coordination on the transaxial organometallic Co–C bond. An appealing interpretation of the labilizing effect of the nucleotide coordination was offered by Schrauzer et al.,[15a,45] who suggested it to arise from the profound increase of the earlier noted[2a,3,22a] "upward-folding" of the corrin ligand upon intramolecular axial coordination of the bulky nucleotide base at the "lower" (α)-coordination site of the corrin-bound Co(III) center. They have described such effects "as 'mechanochemical', since they are induced by conformational motions of the corrin ligand."[45b]

The steric bulk of an axial ligand in organometallic B_{12}-model compounds has been suggested by Halpern also to be a factor that contributes kinetically to the activation of the trans organometallic bond toward homolytic cleavage.[5,46] On the basis of kinetic results for the thermally activated homolysis of the Co–C bond of coenzyme B_{12} (**3**, adenosylcobalamin) and of the analoguous nucleotide free *Coβ*-adenosylcobinamide, the Co–C bond dissociation energy (Co–CBDE) for the coenzyme has been estimated by Finke et al. to be smaller by ca. 4.5 ± 2.7 kcal/mol than that of the nucleotide-free *Coβ*-adenosylcobinamide.[17] On the other hand, studies of methyl transfer equilibria between methylcob(III)alamin (**4**), *Coβ*-methylcob(III)inamide, Co(II)–cobalamin and Co(II)–cobinamide revealed the CH_3–Co(III)–corrin **4** to be stabilized slightly by the nucleotide coordination against homolytic cleavage of the Co–C bond (by ca. 0.4 ± 0.1 kcal/mol), rather than to be labilized, compared to the nucleotide-free *Coβ*-methylcob(III)inamide.[18a] Also for the coenzyme **3** only a modest activating effect of the nucleotide coordination on the homolysis of the organometallic bond was estimated there.[18a] These data suggested only a minor, possibly even stabilizing "electronic" effect of the base coordination on the strength of the organometallic bond in organo–Co(III)–corrins; in Halpern's laboratory[5,46] in a series of organometallic "B_{12}-models" an increase of the Co–CBDE has also been noted with increasing basicity of the trans-axial ligand.

Extensive structural work with various "B_{12}-models", most notably with organometallic derivatives of Schrauzer's "cobaloximes"[47] and of Costa's "B_{12}-models"[48] by Marzilli, Randaccio and their co-workers[49,50] has shown significant deformation from planarity and steric response of the ligand in these noncorrinoid model compounds to the bulk of the axial ligands. Several of these structurally characterized model complexes had an axially coordinated DMB base,[50] consistently leading to considerable "upward" deformation of the equatorial ligand.

In line with this, the inherent nonplanarity and the presumed ease of deformation of the corrin ligand has been proposed by Geno and Halpern[5] as a rational for nature's preference of the corrin ligand over the "simpler" porphyrin ligand for the purpose of providing an alkyl radical reversibly from an organometallic precursor, which is the established biological function of the coenzyme B_{12}. Indeed, the "flexing" of the corrin ligand was revealed as a major factor of conformational variability of the corrin ligand in natural corrins.[14]

Coenzyme B_{12} (**3**) cocatalyzes a series of complex enzymatic reactions, indicated to be triggered by the homolysis of the organometallic bond of the protein-bound **3**.[6,7,51] However, the enzyme-catalyzed reactions occur at a rate exceeding by a factor of 10^{11-12} the rate of thermally induced homolysis of the coenzyme **3**.[7,10] The factors, which contribute to the indicated enzymatic labilization of **3** toward homolysis, are not yet established. A steric distortion of **3** by the binding to the apoprotein and in the presence of substrate is a widely accepted hypothetical basis for it (see e.g. refs 3, 4a, 6, 7, 10, 12, 15, and 39). Two major models of conformational flexibility have been recognized on the basis of Co(III)–corrin structures: the "folding" of the corrin ligand[3,14] and axial movements of the nucleotide base.[15b] Accordingly the hypothetic protein- and substrate-induced deformation of the bound coenzyme **3** has been suggested to come about by an "upward conformational distortion" of the corrin ring[7] or else by a movement of the benzimidazole base toward the corrin ring.[15b] An alternative third mechanism of activation of protein-bound coenzyme **3** toward homolysis of its cobalt–carbon bond was proposed based on an X-ray analytical determination of the corrinoid homolysis fragment from **3**, of Co(II)–cobalamin.[39] This analysis did not reveal any major structural differences between the cobalt–corrin part of the coenzyme and its corrinoid homolysis product, e.g. as concerns the "folding" of corrin ligands or the position of the nucleotide base with respect to the corrin ligand.[39] Accordingly, the earlier proposals[3,7,15] concerning the "conformational distortion" of the protein-bound coenzyme **3** did not receive support from this structure analysis. However, a "trivial" structural change results from the formation of two (separated) homolysis fragments from an intact molecule: therefore, a labilization of the organometallic bond of **3** may still result, if apoenzyme- and substrate-induced separation of the homolysis fragments (Co(II)–cobalamin and 5-adenosyl radical) is supported by stronger binding of the separated fragments to the protein than of the undistorted coenzyme.[39]

In order to gain insight into structural effects of the axial nucleotide coordination on the "upward-folding" of the corrin ligand we set out to test experimentally for the first time[52] in a "complete" corrin the suggested effect of the bulk of the nucleotide base. For the purpose of the envisaged structural analysis, the nonnatural vitamin B_{12} analog *Coβ*-cyanoimidazolylcobamide (**1**)[21] appeared well suited: replacement of DMB by imidazole should drastically reduce steric effects from the axial base, while leaving any "electronic" effects largely unchanged.[53] The

(44) (a) Friedrich, W. In *vitamin B_{12} und verwandte Corrinoide, Fermente, Hormone und Vitamine*; Ammon, R., Dirscherl, W., Eds., Georg Thieme Verlag: Stuttgart, Germany, 1975; p 25 ff. (b) Stupperich, E.; Eisinger, H. J.; Schurr, S. *FEMS Microbiol. Rev.* **1990**, *87*, 355.
(45) (a) Grate, J. H.; Schrauzer, G. N. *J. Am. Chem. Soc.* **1979**, *101*, 4601. (b) Schrauzer, G. N.; Grate, J. H. *J. Am. Chem. Soc.* **1981**, *103*, 541.
(46) Ng, F. T. T.; Rempel, G. L.; Mancuso, C.; Halpern, J. *Organometallics* **1990**, *9*, 2762.
(47) Schrauzer, G. N.; Kohnle, J. *Chem. Ber.* **1964**, *97*, 3056.
(48) Costa, G. *Pure Appl. Chem.* **1972**, *30*, 335.
(49) Randaccio, L.; Bresciani-Pahor, N.; Zangrando, E.; Marzilli, L. G. *Chem. Soc. Rev.* **1989**, *18*, 225.
(50) (a) Parker, W. O., Jr.; Zangrando, E.; Bresciani-Pahor, N.; Marzilli, P. A.; Randaccio, L.; Marcilli, L. G. *J. Am. Chem. Soc.* **1988**, *27*, 2170. (b) Charland, J.-P.; Zangrando, E.; Bresciani-Pahor, N.; Randaccio, L.; Marzilli, L. G. *Inorg. Chem.* **1993**, *32*, 4256.
(51) Rétey, J. *Angew. Chem.* **1990**, *102*, 373; *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 355.
(52) Kopf, J.; van Deuten, K.; Bieganowski, R.; Friedrich, W. *Z. Naturforsch* **1891**, *C36*, 506 report on an X-ray investigation of "factor A" (*Co*β-cyano-2′-methyladeninyl-cobamide), a "complete" corrinoid with 2′-methyladenine as nucleotide base. Compared to the structure of **2**, only small differences in the "folding" of the corrin ring about the Co–C10 line were noted. Using the published data, we computed a Co–N_{ax} distance of 2.12 Å and a fold angle[54] of 15.7° for "factor A".
(53) (a) From the respective pK_a values (7.0 for imidazole,[53b] 6.0 for dimethylbenzimidazole,[53c] and 5.6 for α-ribazole[36d]) it is evident that DMBz is a slightly weaker base toward proton than imidazole, and presumably also a weaker nucleophile toward cobalt. (b) Datta, S. P.; Grzybowski, A. K. *J. Chem. Soc. B* **1966**, *136*. (c) Davies, M. T.; Mamalis, P.; Petrow, V.; Sturgeon, B. *J. Pharm. Pharmacol.* **1951**, *3*, 420.

corrinoid **1** was synthesized by "guided biosynthesis"[22] with the help of a culture of *P. shermanii*, and it was isolated in crystalline form.

The spectroscopic examination of the "complete" corrinoid **1** in aqueous solution (by UV–vis, CD, IR, FAB–MS, and one- and two-dimensional ^{1}H- and ^{13}C-NMR spectroscopy) confirmed the structural identity of this complete corrinoid[21] as a *Coβ*-cyanoimidazolylcobamide. The spectra indicated a comparable mode of axial base coordination and did not reveal any significant differences in the buildup of the corrin ligand in **1** and in vitamin B_{12} (**2**).[3,30,36]

Comparison of the completely assigned ^{1}H- and ^{13}C-NMR spectra of **1** and **2** provides firm evidence for a similar solution structure of the two complete cobamides, more subtle differences being revealed by X-ray analysis (see below). Differences in chemical shifts in the ^{1}H-NMR spectra can be rationalized as a consequence of the different constitution of the nucleotide base (imidazole in **1**; 5,6-dimethylbenzimidazole in **2**) and, accordingly, less pronounced local (de)shielding effects of the base of **1**. A noteworthy and consistent upfield shift of the ^{13}C signals of the sp^2-hybridized carbon of the corrin chromophoric unit (up to 2.2 ppm at C6; average upfield shift 1.0 ppm) would be consistent with imidazole being a better donor ligand than 5,6-dimethylbenzimidazole,[50,53] i.e. an increased electron density at cobalt and in the corrin-π-system in **1** compared to the situation in **2**. A related correlation (but referring to the β-ligand) between ^{13}C-chemical shift and donor capacity of the axial ligand in a series of cobalamins has been noted and discussed earlier.[36a]

In the spectrum of **1**, the signals of H(B2), H(B4) and H(B5) of the cobalt-coordinated imidazole appear at 6.82, 5.96 and 6.97 ppm, i.e. at higher field by 0.81, 1.05, and 0.12 ppm, respectively, when compared to the spectrum of 1-(3-aminopropyl)imidazole.[36c] A similar situation can also be noted for the ^{1}H-NMR spectrum of **2**, where the signals of H(B2), H(B4), and H(B7) are shifted upfield by 1.31, 0.95, and 0.07 ppm, respectively, compared to the spectrum of the isolated nucleotide base of **2** (α-ribazole-phosphate).[36d] Such high-field shifts of the ^{1}H-signals are typical of the cobalt-coordinated nucleotide base in "complete" corrins and have been associated with a shielding-effect of the cobalt–corrin core.[36a,d]

In order to unambiguously assign dipolar connectivities and to circumvent problems caused by spectral overlap in bulk regions, the inclusion or addition of a, e.g., ^{13}C frequency domain has turned out to be extremely useful. Longitudinal experiments such as the proposed HMQC–NOESY[25q,r] are not applicable to molecules with a molecular weight similar to **1**, since the correlation time of its motion will have a critical magnitude (i.e. $\omega_0\tau_c \approx 1.1$).[25s] In this regime, different contributions to the dipolar cross-relaxation will be equal but opposite, so that a NOESY spectrum will hardly show any correlations, even for protons close in space. Information about dipolar connectivities can be obtained by measuring cross-relaxation rates in the rotating frame. However, two-dimensional rotating frame Overhauser spectroscopy (ROESY) may suffer from accidential overlap in the two-dimensional spectrum. The unambiguous identification of ROEs between protons in crowded regions can be simplified by spreading out the spectrum according to the chemical shift of the directly bonded ^{13}C nucleus.[25t]

Exploration of conformational aspects of the structure of **1** in aqueous solution based on distance constraints from 2D-HSQC-ROESY,[25p] (see the supplementary material for a table relating selected ROESY intensities with crystallographically observed interproton distances) support the picture of a "north–south" orientation of the nucleotide base, matching what is observed in the crystal. Likewise, from values of $^3J_{H,H}$ homonuclear couplings between the methine protons at C3, C8, and C13 and the adjacent methylene groups of the appended propionamide substituents a major conformation of the side chain in solution can be derived to be similar (d side chain) or at least compatible (b and e side chains) to the conformation present in the crystal. The $^3J_{H,H}$ couplings along the ribose unit (R1 and R5) support the solution conformation of the ribose ring in **1** to be compatible to the one in vitamin B_{12} (**2**).

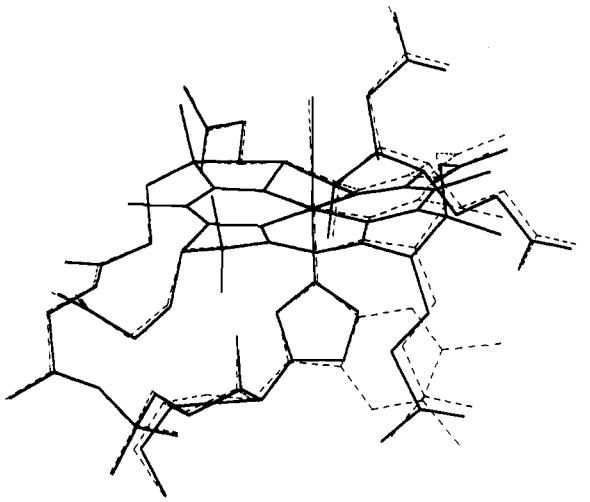

Figure 8. Superposition of the imidazolyl- and the 5,6-benzimidazolylcobamide molecules (**1**, solid line; **2**, broken line). The superposition involved least-squares minimization of the distance between atoms C10, C11, N23, C14, C15, C16, and N24, i.e. of the atoms constituting the "southern" half of the conjugated portion of the corrin ring.

The conformational similarity between **1** and **2** in solution is also manifest in the solid state. Crystals of the two compounds are isostructural, and the molecules assume similar overall conformations as shown in the superposition of the two molecules (Figure 8). However, a closer comparison of the two structures reveals a number of structural differences which can consistently be interpreted as indicating the relief of strain upon substitution of the 5,6-dimethylbenzimidazole α-ligand by imidazole. These structural differences include: (a) a change in the conformation of the corrin ring, evident from the deviations from a mean plane shown in Figure 6, leading to a a decrease in the "fold angle"[54] from 18.0 (**2**) to 11.3° (**1**); (b) a decrease in the Co–N_{ax} distance by 0.04(1) Å, from 2.01(1) Å in **2** to 1.97(1) Å in **1** (Figure 5); (c) absence in the imidazolylcobamide **1** of a "tilt"[41] of the nucleotide base, present in vitamin B_{12} (**2**), in all other cobalamins,[55] and in model complexes with an axial DMB ligand.[50] In these compounds, the "northern" Co–N_{ax}–C angle is typically larger by about 10° than the "southern" Co–N_{ax}–C angle (see Figure 5), resulting in a tilt[41] of the nucleotide base by about 5°. In contrast, the tilt angle in **1** is zero within experimental error.

As a consequence of these differences and as analyzed numerically by distance matrix analysis, there is one mayor geometric factor accompanying the substitution of DMB by imidazole, namely an approach (by 0.35 Å, Figure 7) of the C5-methine bridge and the imidazole ring. This is evident from the decrease in the distance between the corresponding centroids, which exceeds all other entries in the difference distance matrix (Figure 7) by a factor of at least 3. The conformational changes caused by the substitution of DMB by imidazole are summarized and illustrated in Figure 9, which shows a superposition of the "relevant" parts of the two molecules (**1** and **2**): for each molecule, the nucleotide base, the axial Co–N bond, and the two least-squares planes through the conjugated portions of the "northern" and "southern" halves of the corrin ring are drawn.[56]

The presented high-resolution structure analyses on **2** and **1**

(54) The "fold angle"[2c,3,14] has been used to describe the out-of-plane deformations of the corrin ring. It is defined as the angle between least-squares plane through the atoms N21, C4, C5, C6, N22, C9, C10 and C11, N23, C14, C15, C16, N24 (for a definition of atom numbers, see Figure 1.)

(55) Kratky, C.; Färber, G.; Gruber, K.; Wilson, K.; Dauter, Z.; Nolting, H.-F.; Kräutler, B. *J. Am. Chem. Soc.*, in preparation.

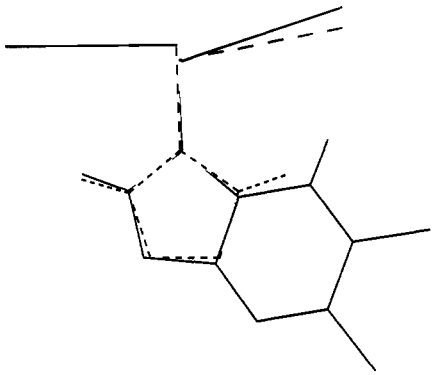

Figure 9. Illustration of the conformational effects induced by the exchange of the dimethylbenzimidazole base by imidazole. The figure shows a superposition of the molecules of **1** (dashed) and **2** (full). Each molecule is represented by its nucleotide base, the axial Co–N bond and the projection of the "northern" (N21, C4, C5, C6, N22, C9, C10) and "southern" (C10, C11, N23, C14, C15, C16, N24) least-squares planes.

are also relevant for an assessment EXAFS data presented recently by Sagi and Chance.[11] For the vitamin B_{12} (**2**), the EXAFS data yielded a Co–N_{ax} distance of 2.15(3) Å, exceeding by about 0.18 Å the Co–N_{ax} distance (1.97 Å) observed previously in the ("old") crystal structure of "wet" vitamin B_{12}.[20] The present work remarkably confirms Hodgkin's original Co–N_{ax} distance by modern cryotemperature crystallography (see Figure 5), excluding the possibility that the difference between EXAFS and X-ray results may be accounted to errors in the (old) X-ray structure analysis of **2**. In addition, knowledge of the crystal structure of the imidazolylcobamide **1** permits an assessment of the effect of the steric repulsion between the DMBz ligand and the corrin ring, which was suggested[11] as an explanation for the "unexpectedly" long Co–N_{ax} distance obtained from EXAFS. Comparison of the X-ray results on **2** and **1** (see Figure 5) yields a value of 0.042(13) Å for the elongation of the Co–N_{ax} bond. This represents an upper limit for the elongation of the axial Co–N bond by steric effects between nucleotide base and corrin ring, to which other factors may contribute, such as a change of the nucleophilicity of the nucleotide base.[53] This is in line with our own preliminary EXAFS data on vitamin B_{12},[57] with an EXAFS investigation on aquocobalamin,[55] and with recent NMR evidence,[36a] all of which yield no indication for structural differences between solution and the solid state for the two compounds. It follows that the conclusions presented by Sagi and Chance[11] based on their EXAFS data are likely to be in error.

(56) (a) The crystallographic evidence on **1** and **2** has revealed a considerable change in the corrin ring folding upon exchange of DMB by imidazole. However, these differences were still too small to be amenable to an analysis by NMR spectroscopy (at its present stage). It is therefore not surprising that a previous NMR investigation has yielded "no evidence for a change in corrin ring pucker on dissociation of the 5,6-dimethylbenzimidazole" ("base-off" form of coenzyme B_{12},[56b] "incomplete" 5′-deoxyadenosylcobinamide).[56c] (b) Bax, A.; Marzilli, L. G.; Summers, M. F. *J. Am. Chem. Soc.* **1987**, *109*, 566. (c) 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.

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Conclusions

Coβ-cyanoimidazolylcobamide (**1**) has been prepared and structurally characterized by modern NMR and X-ray crystallographic techniques. Compared to vitamin B_{12}, a number of structural differences are observed, permitting quantitative assessment of the effect of the steric bulk of the 5,6-dimethylbenzimidazole base. Exchange of imidazole by the more bulky 5,6-dimethylbenzimidazole (observed in the biologically relevant cobalamins) leads to a *stretch* of the Co–N_{ax} bond, an increased *fold* of the corring ring and a *tilt* of the cobalt-coordinated nucleotide base. Future chemical and enzymological studies using alkyl derivatives of **1** will establish the significance of the observed structural effects for the reactivity of the Co–C bond.

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Supplementary Material Available: HSQC spectrum and a table comparing ROESY intensities with crystallographically determined interproton distances for **1**, tables of crystallographic data, atomic coordinates for the solvent sites anisotropic atomic displacement parameters, hydrogen atom coordinates, and bonding geometry (bond lengths, bond angles, and torsion angles), hydrogen bonding distances for the crystal structures of *Coβ*-cyanoimidazolylcobamide (**1**) and vitamin B_{12} (**2**), and a figure showing the superposition of two structure determinations for vitamin B_{12} (**2**) (33 pages). Ordering information is given on any current masthead page.

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