Inorg. Chem. **1999,** *38,* 1785−1794

Difluoromethylcobalamin: Structural Aspects of an Old Tree with a New Branch[†]

Trixie Wagner, Carol E. Afshar, H. L. Carrell, and Jenny P. Glusker*

The Institute for Cancer Research, Fox Chase Cancer Center, 7701 Burholme Avenue,
Philadelphia, Pennsylvania 19111

Ulli Englert

Institut für Anorganische Chemie, RWTH Aachen, Prof.-Pirlet-Strasse 1, 52074 Aachen, Germany

Harry P. C. Hogenkamp

Department of Biochemistry, University of Minnesota, Minneapolis, Minnesota 55455

Received June 30, 1998

Difluoromethylcobalamin (CF_2Cbl), a vitamin B_{12} analogue with CHF_2 replacing CN, can be synthesized in a two-step procedure from aquocobalamin and $CHClF_2$. Its crystal structure has been determined by X-ray diffraction. The compound crystallizes in the orthorhombic space group $P2_12_12_1$ with $Z = 4$ and 17 water molecules per formula unit. The unit cell dimensions are $a = 24.08(1)$ Å, $b = 21.143(3)$ Å, and $c = 15.981(3)$ Å. The refinement model was kept as simple as possible with no restraints, and with isotropic displacement parameters for all non-hydrogen atoms except for Co, P, and F. The agreement factors obtained this way are: $R_1 = 0.072$ for 5675 reflections with $F_o > 4\ \sigma(F_o)$ and $wR_2 = 0.194$ for all 11844 reflections. The packing motif of CF_2Cbl is very similar to that described for wet vitamin B_{12}, a distorted hexagonal close packing of the cobalamin, with channels of water running parallel to the crystallographic *c* axis through the crystal at $x = {}^1/_4$, $y = 0$, and $x = {}^3/_4$, $y = {}^1/_2$, respectively. An analysis of interactions involving water molecules and amide groups revealed a discontinuity between oxygen−oxygen distances (which are found to be less than 2.8 Å) and oxygen−nitrogen distances (which are found to be greater than 2.8 Å); this provides a useful criterion for distinguishing between oxygen and nitrogen atoms in amide groups. A superposition of the crystal structures of vitamin B_{12} and CF_2Cbl shows a significant change at the molecular level. In CF_2Cbl, the **c** side chain of ring **B** takes on a conformation that brings its terminal amide group near to the CHF_2 group. This results in both a relatively short contact (3.11 Å) between F2 and O39 of the **c** amide and a weak C1F−H1F···O39 interaction.

Introduction

Cobalamins (in the following abbreviated as Cbl's) have interested scientists of numerous disciplines ever since the structure of vitamin B_{12} (cyanocobalamin) was solved in the laboratory of Dorothy Hodgkin in the mid-1950s.[1,2] Their structural formulas and numbering systems are shown in Figure 1. In the equatorial plane the central six-coordinate cobalt(III) is surrounded by the four pyrrole nitrogen atoms of a corrin ligand (a heme system lacking one bridging CH group). One of the axial sites, the α-face below the macrocycle in the orientation in Figure 1, is occupied by a 5,6-dimethylbenzimidazole-α-D-ribofuranose-3′-phosphate (DMB). The second axial ligand position, on the *β*-face (above the equatorial plane), is occupied in the case of vitamin B_{12} by a cyanide group and in CF_2Cbl (this study) by a CHF_2 group. Corrinoids with the same structure as vitamin B_{12}, differing only in the nature of the upper ligand, are grouped together as cobalamins. Attached to the macrocyclic tetrapyrrole with rings **A** to **D** are seven side chains (**a**−**g**), each ending with amide groups. One of them, **f**, is substituted by the nucleotide containing the benzimidazole base. Of the seven side chains, only **c**, **d**, and **e** show conformational variability in numerous cobalamin structures, whereas the side chains **a**, **b**, **f**, and **g** are found, for steric reasons, to maintain their orientation.[3]

In vivo, vitamin B_{12} is enzymatically converted to adenosylcobalamin (AdoCbl, known as adenosylcobalamin or coenzyme B_{12}) or methylcobalamin (MeCbl) (formed in reactions involving adenosine triphosphate or *S*-adenosylmethionine, respectively).[4] These alkyl derivatives of the vitamin are the first known naturally occurring compounds with a biologically active carbon-metal bond. They are utilized as cofactors by a variety of enzymes[5] that catalyze rearrangement (AdoCbl) or methylation (MeCbl) reactions, both of which involve the cleavage of the cobalt carbon bond. In the case of AdoCbl the bond splitting is thought to be homolytic, yielding a pentacoordinated Co(II) and an adenosyl radical; in the case of MeCbl it seems to be heterolytic yielding a highly reactive Co(I) species

---

[†] Presented in part at the 1998 Annual Meeting of the German Crystallographic Association, Karlsruhe, Germany.

(1) Hodgkin, D. C.; Lindsey, J.; Sparks, R. A. *Proc. R. Soc.* **1962**, *A266*, 494−517.

(2) Brink-Shoemaker, C.; Cruickshank, D. W. J.; Hodgkin, D. C.; Kamper, M. J.; Pilling, D. *Proc. R. Soc.* **1964**, *A278*, 1−26.

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

(4) Cowan, J. A. *Inorganic Biochemistry: An Introduction*; Wiley-VCH: New York, 1997.

(5) Halpern, J. *Science* **1985**, *227*, 869−875.

10.1021/ic980751q CCC: $18.00

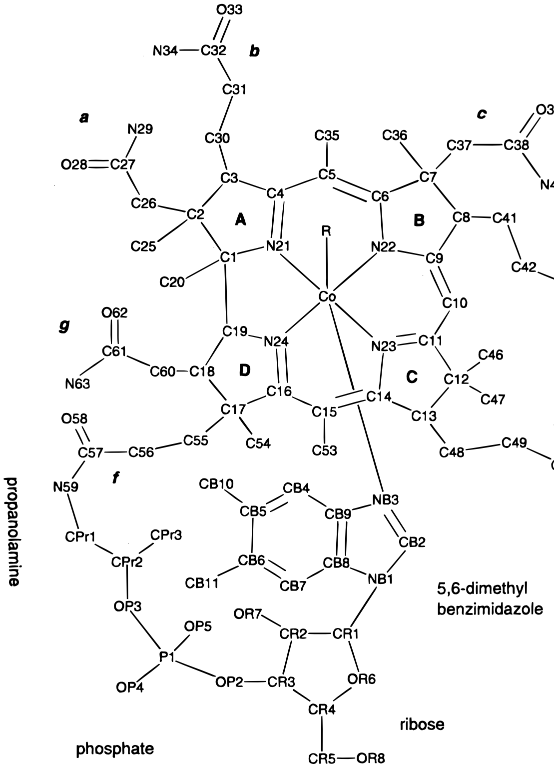

Figure 1. Structural formula and numbering system of cobalamins. R = CN, vitamin B_{12}; R = 5′-deoxyadenosyl, coenzyme B_{12}; R = methyl, methylcobalamin. In CF_2Cbl (this study) the sixth ligand is a difluoromethyl group (CHF_2). The three formal positive charges on the cobalt are balanced by negative charges on the axial ligand R, the corrin ring, and the phosphate group.

and a CH_3^+ fragment as the methylating agent.[6,7] Recent crystal structure determinations of methylmalonyl-coenzyme A mutase in complex with AdoCbl[6] and methionine synthase in complex with MeCbl[7] support the hypothesis that, upon binding of the cofactor by the enzyme, the DMB base is replaced by a histidine side chain from the protein.

The mechanism by which the cleavage of the Co−C bond occurs is not yet fully understood. There is, however, speculation that any lengthening of the Co−N bond will induce lengthening and hence, weakening, of the Co−C bond that is trans to it.[8−10] This “anomalous structural trans influence” has been the subject of much discussion for cobalamins and also for cobaloximes which can serve as model compounds for investigating factors controlling the reactivity of coenzyme B_{12}.[9,10] Also, the flexibility of the corrin macrocycle (allowing for transmission of steric effects between the α- and β-faces), is thought to play an important role in facilitating the dissociation of the adenosyl radical.[3,11] Both aspects will be discussed in our analysis of the difluoromethylcobalamin molecule and compared to the results for structurally related cobalamins.

Table 1. Crystal Data and Parameters of Structure Refinement for CF_2Cbl

empirical formula	$C_{63}H_{89}CoF_2N_{13}O_{14}P\cdot 17H_2O$	fw	1380.4 (w/o H_2O)
a	24.08(1) Å	space group (no.)	$P2_12_12_1$ (19)
b	21.143(3) Å	temperature	−153 °C
c	15.981(3) Å	λ	0.7107 Å
V	8135(4) Å^3	ρ_{calc}	1.35 g·cm^{-3}
Z	4	μ	3.2 cm^{-1}
$R_1(F_o)$[41a]	0.072	$wR_2(F_o^2)$[41b]	0.194

Experimental Section

(a) Synthesis and Crystallization. A 500 mg amount of aquocobalamin in 25 mL of deaerated water with 10 mg $CoCl_2$ in a 125 mL Erlenmeyer flask sealed with a serum stopper were reduced with a solution of 100 mg of $NaBH_4$ in 2 mL of 0.1 N NaOH. After the reduction was complete, as judged from the gray-green color, 4 mL of an 0.82 M ethanolic solution of CHF_2Cl (purchased from Union Carbide Company) was injected. After incubation in the dark for 2 h, the reaction mixture was desalted by phenol extraction and the concentrated aqueous solution was chromatographed on SP-Sephadex. The desired difluoromethylcobalamin was eluted with water, concentrated, and crystallized from aqueous acetone (yield 75%). The compound is fairly stable in the solid state but should be kept in the dark due to its light sensitivity, especially in dilute solution. The purity of the product was established by high-pressure liquid chromatography on a Browlee Labs Aquapore RP 300 C-8 column with the gradient elution method described by Jacobsen et al.[12] The ^{19}F NMR spectrum showed the expected doublet at 72.0 ppm ($J_{H-F} = 55.3$ Hz). The electronic spectra showed maxima at 518, 362, 332, 314, 276, and 260 nm. In 1 N HCl the “base-off” form has maxima at 454, 350, 320, 274, and 262 nm (pK_a for the “base on” → “base off” conversion: 2.2). The mass spectrum (electrospray ionization) shows the expected molecular peak at m/z 1380.9 $(M - H)^+$ and a fragment of m/z 1329.3, corresponding to $(M - H)^+ - {}^{\bullet}CHF_2$.

(b) X-ray Single-Crystal Structure Analysis. Crystals suitable for X-ray diffraction were grown from a mixture of acetone/water (1:1) by the vapor diffusion method. Intensity data were measured on an ENRAF-Nonius FAST area detector diffractometer (distance to crystal 50 mm) with graphite monochromatized Mo Kα radiation from a rotating anode generator at 50 kV and 50 mA. Crystal dimensions were $0.35 \times 0.08 \times 0.05$ mm^3. The data, measured to 0.9 Å resolution, were collected and processed using the software package MADNES.[13] The exposure time per frame (0.2° oscillation) was 25 s. In all, 11 844 out of a total of 23 140 measured reflections were unique ($R_{merge} = 0.165$). Unit cell dimensions were obtained using 250 intense reflections $[I/\sigma(I) > 20]$ with $11° < \theta < 21°$ over the full range of rotation for each of the crystal settings. The values were averaged, and esd values were obtained from the variance of these measurements. Crystal data, data collection parameters, and refinement results are compiled in Table 1.

(c) Structure Solution and Refinement. The structure was solved with direct methods using the structure solution routine SHELXS from the program package SHELX-97.[14] After completion and isotropic refinement of the model with the full matrix least-squares routine SHELXL from the same package, water sites were assigned to difference map peaks between 2.2 and 3.3 Å apart from each other or from polar atoms of the cobalamin moiety, with angles O,N···O···O,N between 68 and 150°.[15] Only 2 of the 17 water molecules refined to give large equivalent isotropic displacement parameters ($B_{iso} > 12$ Å^2); the occupancies at these two sites were set to 0.5. No hydrogen atoms were added at the water sites. The hydrogen atoms of the cobalamin were added in calculated positions and refined using a riding

---

(6) Mancia, F.; Keep, N. H.; Nakagawa, A.; Leadlay, P. F.; McSweeney, S.; Rasmussen, B.; Bösecke, P.; Diat, O.; Evans, P. R. *Structure* **1996**, *4*, 339−350.

(7) Drennan, C. L.; Matthews, R. G.; Ludwig, M. L. *Curr. Opin. Struct. Biol.* **1994**, *4*, 919−929.

(8) Gruber, K.; Jogl, G.; Klintschar, G.; Kratky, C. In *Vitamin B_{12} and B_{12} Proteins*; Kräutler, B., Arigoni, D., Golding, B. T., Eds.; Wiley-VCH: Weinheim, 1998; pp 335−347.

(9) Ridder, D. J. A. D.; Zangrando, E.; Bürgi, H.-B. *J. Mol. Struct.* **1996**, *374*, 63−83.

(10) Geremia, S.; Randaccio, L.; Zangrando, E. *Gaz. Chim. Ital.* **1992**, *122*, 229−232.

(11) Glusker, J. P. In *Vitamins and Hormones*; Litwack, G., Ed.; Academic Press: San Diego, 1995; pp 1−79.

(12) Jacobsen, D. N.; Green, R.; Brown, K. L. *Methods Enzymol.* **1986**, *123*, 14−22.

(13) Pflugrath, J. W.; Messerschmidt, A. *MADNES* (Munich Area Detector NE System); Cold Spring Harbor Laboratory, Cold Spring Harbor, MA, and Max Planck Institut für Biochemie, Munich, Germany, 1993.

(14) Sheldrick, G. M. *SHELX-97: A Program for Crystal Structure Determination*; University of Göttingen: Germany, 1997.

(15) Chiari, G.; Ferraris, G. *Acta Crystallogr.* **1982**, *B38*, 2331−2341.

Table 2. Comparison of Selected Bond Lengths in the Corrin Ring (Å) and Agreement Factors Obtained by Five Different Refinement Procedures (see also Experimental Section)[a]

bond	⟨ ⟩	I	II	III	IV	V
C1−C2	1.54	**1.592**(12)	**1.594**(14)	**1.592**(12)	1.566(7)	1.566(8)
C2−C3	1.54	1.563(11)	1.550(13)	1.570(11)	1.553(7)	1.554(7)
C3−C4	1.51	1.514(11)	1.510(13)	1.516(11)	1.516(7)	1.517(7)
C7−C37	1.54	**1.577**(11)	**1.577**(13)	**1.586**(11)	1.559(7)	1.560(7)
C4−N21	1.29	1.273(9)	1.281(11)	1.269(10)	1.287(7)	1.285(7)
C4−C5	1.41	**1.453**(11)	**1.451**(13)	**1.464**(11)	1.435(7)	1.438(7)
C5−C6	1.36	1.371(11)	1.348(13)	1.381(11)	1.379(7)	1.381(7)
C6−N22	1.36	**1.399**(10)	**1.403**(12)	1.389(10)	1.387(7)	1.387(7)
N22−C9	1.33	1.345(9)	1.338(11)	1.349(10)	1.342(7)	1.343(7)
C9−C10	1.38	1.358(11)	1.371(13)	1.361(11)	1.372(7)	1.375(7)
C10−C11	1.38	1.404(11)	1.410(13)	1.398(12)	1.392(7)	1.389(7)
C11−N23	1.33	1.344(10)	1.339(12)	1.355(10)	1.341(7)	1.341(7)
N23−C14	1.36	**1.408**(10)	**1.410**(11)	**1.407**(10)	1.390(7)	1.387(7)
C14−C15	1.36	1.375(11)	1.374(12)	1.370(11)	1.375(7)	1.373(7)
C15−C16	1.41	**1.445**(11)	**1.461**(13)	1.437(11)	1.436(7)	1.435(7)
C16−N24	1.29	1.303(10)	1.280(12)	1.308(10)	1.306(7)	1.307(7)
C16−C17	1.51	**1.561**(11)	**1.545**(13)	**1.569**(11)	**1.541**(7)	**1.543**(7)
C17−C18	1.54	**1.590**(11)	1.562(14)	**1.593**(12)	1.563(7)	1.565(8)
Co−NB3		2.187(7)	2.199(8)	2.187(7)	2.187(6)	2.183(6)
		1.949(8)	1.942(9)	1.950(8)	1.951(8)	1.948(8)
no. of parameters		476	476	926	476	946
no. of restraints		−	−	285	110	395
no. of observations		11844	5675	11844	11844	11844
wR_2		0.194	0.142	0.193	0.195	0.185
fold angle (deg)		15.4(2)	14.8(2)	15.5(2)	15.3(2)	15.3(2)

[a] ⟨ ⟩, expected values (see Figure 2); I, free isotropic refinement (Co, P, F anisotropic); II, same as I, data cutoff $4\sigma(F_o)$; III, anisotropic refinement (rigid bond model); IV, restrained isotropic refinement; V, restrained anisotropic refinement (rigid bond model). Bold numbers indicate deviations >0.03 Å. Numbers in parentheses are estimated standard deviations in the last significant digit.

model [where the displacement factor U_{iso}(H) was set at 1.5 times that of the atom to which it is attached, as set in SHELXL for the data collected at −153 °C]. Methyl (H−C−C−C) torsion angles were chosen to maximize the electron density at the three calculated H atom positions and were allowed to refine. An analogous procedure was applied to the hydrogen atoms on the ribose oxygen atoms OR7 and OR8 (torsion angle chosen to maximize electron density).

The complete structure was then refined against F^2, using the SHELXL routine. Anisotropic refinement required use of the DELU option provided by the refinement program; this applies a "rigid bond" model[16] that forces the components of the anisotropic displacement parameters in the direction of a bond to be equal to each other (procedure III in Table 2). The agreement factor obtained (wR_2 = 0.193), however, did not satisfy the Hamilton significance test[17] for the number of additional parameters. Therefore, the final choice made was to refine all non-hydrogen atoms (except Co, P, and F) isotropically. This refinement (procedure I in Table 2) converged to the agreement factors given in Table 1. The highest peak in the final difference Fourier map (0.90 Å away from OW14) map suggests a slight disorder around this solvent site. In view of the resolution of the data set, no attempt was made to resolve this disorder. The final coordinates of all non-hydrogen atoms and selected hydrogen atoms are given in Table 3.

(d) Restrained Refinement with SHELXL. The final model from procedure I was used as the starting model for a refinement applying 97 restraints on bond lengths (esd 0.01 Å) and 13 on the planarity (esd 0.005 Å) of the flat groups in the cobalamin [procedures IV (only Co, P, and F anisotropic) and V (all atoms in the cobalamin anisotropic, subject to a rigid body model, see Table 2). The target values for bond distances summarized in Figure 2 were obtained from a CSD database search[18] for the ribose (Refcodes ADPOSD, CYTIAC, CYTIAC01, GMPCUP, GUPCYT20, KURDMP, SURIDP, and URIDMP10) and the benzimidazole (Refcodes GEMMOA and GEMMUG) groups, from theoretical calculations for the resonance stabilized part of the corrin,[19] and from a collection of bond lengths in organic crystals[20] for all other bonds.

(e) Semiempirical and Force Field Calculations. The atomic coordinates from refinement procedure V were used as input for the semiempirical and force field calculations. Calculations were carried out with the CAChe program system[21] on a Power Macintosh 7200/90. The input structure was simplified by replacing all methyl groups and side chains **a**, **b**, **d**, **e**, and **g** with hydrogen atoms using the HFIX instruction from the SHELXL routine. Side chain **f** was replaced with a CH_2O^- group to maintain the negative charge of the phosphate group. The two methyl groups in the benzimidazole base were also replaced by hydrogen atoms. The number of atoms in the input file was thereby reduced to 74.

Results and Discussion

We present here the structure determination of a new member of the cobalamin family, with a difluoromethyl group occupying the upper axial position at the cobalt center. Crystal data and parameters of structure refinement are compiled in Table 1. A graphic representation of the crystal structure of the difluoromethylcobalamin molecule is shown in Figure 3. We address here different refinement strategies applied to this structural problem, some aspects of the molecular structure, the packing motif adopted by the cobalamin moiety and the interactions involving the amide groups and water in the crystal.

I. Structure Solution and the Choice of the Refinement Method. Due to their size and their high water content in the crystal, cobalamins are compounds at the borderline between proteins and small molecules. Thus, they provide ideal systems for testing the suitability of protein-based structure solution and refinement strategies for difficult "small" molecule structures.

We have applied the automated water divining routine SHELWAT from the program package SHELX-97[14] to our structure. Starting off with the complete structure, including one of the 17 water molecules we had found manually (OW1), the best agreement factors were achieved with 15 additional solvent positions. These were located in three cycles of refinement that included a maximum number of 10 peaks from a difference

---

(16) Hirshfeld, F. L. *Acta Crystallogr.* **1976**, *A32*, 239−244.

(17) Hamilton, W. C. *Acta Crystallogr.* **1965**, *18*, 502−510.

(18) Allen, F. H.; Bellard, S.; Brice, M. D.; Cartwright, B. A.; Doubleday, A.; Higgs, H.; Hummelink, T.; Hummelink-Peters, B. G.; Kennard, O.; Motherwell, W. D. S.; Rodgers, J. R.; Watson, D. G. *Acta Crystallogr.* **1979**, *B35*, 2331−2339.

(19) Venkatesan, K.; Dale, D.; Hodgkin, D. C.; Nockolds, C. E.; Moore, F. H.; O'Connor, B. H. *Proc. R. Soc.* **1971**, *A323*, 455−487.

(20) Allen, F. H.; Kennard, O.; Watson, D. G.; Brammer, L.; Orpen, A. G.; Taylor, R. *J. Chem. Soc., Perkin Trans II* **1987**, S1−S19.

(21) *CAChe*, Version 4.0 for Power Macintosh; Oxford Molecular: Oxford, United Kingdom, 1996.

Table 3. Fractional Coordinates *x, y, z* of Non-Hydrogen Atoms and Selected Hydrogen Atoms in CF_2Cbl; Numbers in Parentheses Are Estimated Standard Deviations in the Last Significant Digit

Co	0.03574(4)	0.12614(5)	−0.01827(7)
C1	−0.0122(3)	0.0456(4)	0.1066(5)
C2	0.0000(3)	0.0332(4)	0.2031(5)
C3	0.0050(3)	0.1024(4)	0.2368(5)
C4	0.0276(3)	0.1367(3)	0.1609(5)
C5	0.0542(3)	0.1983(4)	0.1651(5)
C6	0.0642(3)	0.2329(4)	0.0941(5)
C7	0.0881(3)	0.3005(4)	0.0873(5)
C8	0.0644(3)	0.3208(4)	0.0017(5)
C9	0.0597(3)	0.2576(4)	−0.0435(5)
C10	0.0650(3)	0.2498(4)	−0.1274(5)
C11	0.0622(3)	0.1926(4)	−0.1719(5)
C12	0.0790(3)	0.1841(4)	−0.2613(6)
C13	0.0484(3)	0.1226(4)	−0.2811(5)
C14	0.0441(3)	0.0916(4)	−0.1967(5)
C15	0.0327(3)	0.0284(4)	−0.1855(5)
C16	0.0214(3)	0.0047(4)	−0.1024(5)
C17	0.0108(3)	−0.0662(4)	−0.0801(5)
C18	−0.0115(3)	−0.0603(4)	0.0132(5)
C19	0.0162(3)	0.0011(4)	0.0418(5)
C20	−0.0732(3)	0.0511(4)	0.0870(5)
N21	0.0171(3)	0.1065(3)	0.0939(4)
N22	0.0537(2)	0.2110(3)	0.0130(4)
N23	0.0488(3)	0.1380(3)	−0.1339(4)
N24	0.0201(3)	0.0396(3)	−0.0352(4)
C25	−0.0451(3)	−0.0061(4)	0.2451(5)
C26	0.0573(3)	0.0017(4)	0.2155(5)
C27	0.0792(3)	−0.0006(4)	0.3029(6)
O28	0.0518(2)	−0.0232(3)	0.3627(4)
N29	0.1291(3)	0.0248(4)	0.3166(5)
C30	−0.0489(3)	0.1348(4)	0.2672(5)
C31	−0.0602(4)	0.1250(5)	0.3619(5)
C32	−0.1033(3)	0.1687(4)	0.3909(5)
O33	−0.0908(3)	0.2249(3)	0.4108(4)
N34	−0.1549(3)	0.1479(4)	0.3955(5)
C35	0.0707(3)	0.2195(4)	0.2535(5)
C36	0.0753(3)	0.3493(4)	0.1548(5)
C37	0.1531(3)	0.2959(4)	0.0758(6)
C38	0.1833(4)	0.2529(4)	0.1350(6)
O39	0.1945(2)	0.1977(3)	0.1121(4)
N40	0.1974(3)	0.2740(4)	0.2096(5)
H40A	0.2151	0.2491	0.2448
H40B	0.1892	0.3130	0.2243
C41	0.0065(3)	0.3530(4)	0.0077(5)
C42	−0.0246(3)	0.3563(4)	−0.0763(5)
C43	−0.0812(3)	0.3859(4)	−0.0767(6)
O44	−0.1119(2)	0.3791(3)	−0.1395(4)
N45	−0.0977(3)	0.4193(3)	−0.0104(5)
C46	0.0643(4)	0.2430(4)	−0.3165(6)
C47	0.1419(3)	0.1734(4)	−0.2652(6)
C48	−0.0114(3)	0.1260(4)	−0.3182(5)
C49	−0.0167(3)	0.1459(4)	−0.4100(5)
C50	0.0237(3)	0.1130(4)	−0.4689(6)
O51	0.0608(2)	0.1438(3)	−0.5016(4)
N52	0.0154(3)	0.0511(3)	−0.4808(5)
C53	0.0329(3)	−0.0154(4)	−0.2588(5)
C54	0.0675(3)	−0.0998(4)	−0.0800(6)
C55	−0.0303(3)	−0.1016(4)	−0.1358(5)
C56	−0.0847(3)	−0.0679(4)	−0.1561(5)
C57	−0.1122(3)	−0.0963(4)	−0.2315(5)
O58	−0.0913(2)	−0.0943(3)	−0.3018(4)
N59	−0.1606(3)	−0.1255(3)	−0.2179(4)
C60	−0.0031(3)	−0.1173(4)	0.0673(5)
C61	−0.0419(4)	−0.1723(4)	0.0490(5)
O62	−0.0919(2)	−0.1618(3)	0.0376(4)
N63	−0.0199(3)	−0.2286(3)	0.0463(4)
C1F	0.1136(3)	0.1061(4)	0.0018(5)
H1F	0.1233	0.1181	0.0605
F1	0.1296(2)	0.0471(2)	−0.0115(5)
F2	0.1470(2)	0.1401(3)	−0.0526(4)
P1	−0.28949(9)	−0.0208(1)	−0.2469(2)
OP2	−0.2307(2)	0.0076(3)	−0.2211(4)
OP3	−0.2722(2)	−0.0922(3)	−0.2599(4)
OP4	−0.3286(2)	−0.0217(3)	−0.1747(4)
OP5	−0.3079(2)	0.0103(3)	−0.3265(3)
NB1	−0.1234(3)	0.1787(3)	−0.1190(4)
CB2	−0.0743(3)	0.1469(4)	−0.1102(5)
NB3	−0.0485(3)	0.1605(3)	−0.0416(4)
CB4	−0.0793(3)	0.2330(4)	0.0795(5)
CB5	−0.1205(3)	0.2728(4)	0.1047(5)
CB6	−0.1667(4)	0.2846(4)	0.0550(6)
CB7	−0.1720(3)	0.2558(4)	−0.0216(5)
CB8	−0.1295(3)	0.2149(4)	−0.0471(5)
CB9	−0.0823(3)	0.2038(4)	0.0024(5)
CB10	−0.1148(4)	0.3060(5)	0.1887(6)
CB11	−0.2135(4)	0.3277(5)	0.0841(6)
CR1	−0.1683(3)	0.1588(4)	−0.1735(5)
CR2	−0.1988(3)	0.1015(4)	−0.1431(5)
CR3	−0.2193(3)	0.0744(4)	−0.2265(5)
CR4	−0.1710(3)	0.0868(4)	−0.2853(5)
CR5	−0.1869(4)	0.1010(4)	−0.3743(6)
OR6	−0.1435(2)	0.1432(3)	−0.2517(4)
OR7	−0.1605(2)	0.0606(3)	−0.1033(3)
OR8	−0.2199(3)	0.1561(3)	−0.3837(4)
CPR1	−0.1930(3)	−0.1547(4)	−0.2845(5)
CPR2	−0.2333(3)	−0.1104(4)	−0.3246(5)
CPR3	−0.2655(4)	−0.1404(4)	−0.3977(6)
OW1	0.4097(3)	0.2134(3)	0.2563(4)
OW2	0.1932(3)	0.3594(3)	0.5426(4)
OW3	0.2384(3)	0.2448(4)	0.5826(5)
OW4	0.3220(2)	0.5407(3)	0.5289(4)
OW5	0.3372(3)	0.1861(3)	0.5390(5)
OW6	0.1737(3)	0.1539(4)	0.4959(5)
OW7	0.1657(3)	0.3683(3)	0.3340(4)
OW8	0.2143(2)	0.4848(3)	0.4925(4)
OW9	0.4134(2)	0.5082(3)	0.4295(4)
OW10	0.2235(3)	0.1742(3)	0.3389(4)
OW11	0.3258(3)	0.2252(3)	0.3747(4)
OW12	0.2075(3)	0.0909(4)	0.2068(5)
OW13	0.3090(4)	0.0310(5)	0.1843(6)
OW14	0.1976(4)	−0.0010(5)	0.4705(7)
OW15	0.2894(5)	−0.0104(6)	0.3633(8)
OW16	0.3351(6)	0.0680(7)	0.6041(9)
OW17	0.2205(6)	0.0663(7)	0.5956(10)

Fourier synthesis as fully occupied solvent sites. The water molecules were rejected if their displacement parameter exceeded $U_{iso} = 0.20$ during three cycles of refinement. The water positions found were identical to the manually determined solvent sites OW2 through OW16. Only OW17 was not accessible in the automated routine. It could, however, be easily identified as the highest peak in the final $F_o - F_c$ map. Thus, the use of an automated procedure is not only appealing considering the time factor, it also permits for some objective, and therefore reproducible, criteria for the assignment of solvent sites, the randomness of which usually increases with the complexity of the structure.

As previously described, the molecular structure of CF_2Cbl is marked (as in other reports of X-ray studies of cobalamins) by a rather low precision as a result of the resolution (0.9 Å), the presence of a fairly heavy atom (Co), and the somewhat disordered water network. This is shown not only in bond lengths with large estimated standard deviations, but also in appreciable discrepancies between chemically equivalent bond lengths (see column II in Table 2). To study the results of restraining bond lengths and planar geometries on these problems we have carried out various refinement procedures, the details of which are given in the Experimental Section.

Table 2 shows that none of the different approaches "smooths out" the unrealistic geometric parameters completely. Both restrained refinements, however, create a noncrystallographic C_2 symmetry in the corrin ring about the Co−C10 axis, as already described for other B_{12} structures.[22,23] As expected, the application of restraints to bond lengths considerably reduces the overall discrepancies between observed and expected bond lengths as well as the esd's and thus creates an impression of higher reliability, without any significant improvement in the agreement factors. The idealized model therefore fits the experimental data just as well as the unrestrained model. This

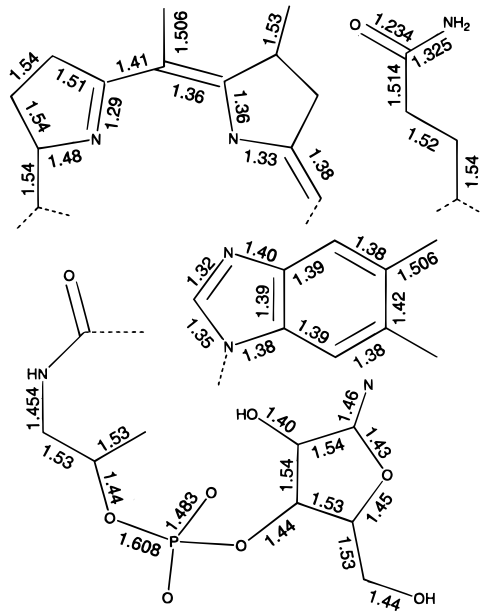

Figure 2. Target bond lengths (in Å) used in the restrained refinement. The values were obtained by the methods described in the Experimental Section, part d.

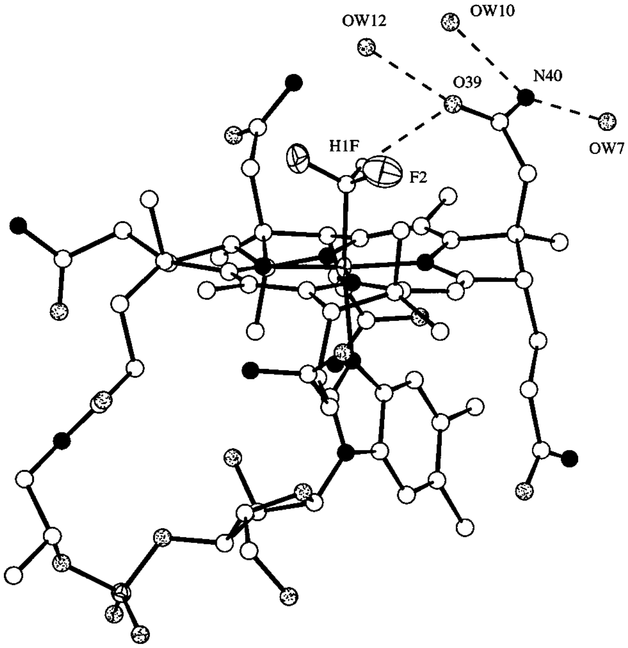

Figure 3. ICRVIEW[45] representation of CF_2Cbl, as observed at −153 °C in the crystal structure, showing the intramolecular contact between the carbonyl oxygen (O39) of the **c** side chain and H1F of the difluoromethyl substituent, and the hydrogen bonds involving this side chain as dotted lines. All atoms except for Co, P, and F (ellipsoids drawn at 50% probability) are displayed with an arbitrary radius of 0.2 (H1F: 0.1) Å. Carbon atoms are drawn as open, nitrogen atoms as full, and oxygen atoms as speckled circles. Hydrogen atoms except for H1F in the CHF_2 group have been omitted for clarity.

suggests that the *R* values are not only a function of uncertainties in the model or systematic errors, but also of the resolution of the data set in combination with the high number of weak reflections.

Table 4. Comparison of Different Cobalamins[a]

R	Co−NB3 (Å)	Co−R (Å)	fold angle (deg)	pK_a
OH_2[23]	1.925(2)	1.952(2)	18.7	−2.1
Cl[28]	1.990(4)	2.262(1)	17.8	no data available
N_3[28]	1.999(3)	1.985(3)	16.7	no data available
CN[43]	2.01(1)	1.86(1)	18.0	0.1
CHF_2 (this work)	2.187(7)	1.949(7)	15.6	2.2
CH_3[39]	2.19(2)	1.99(2)	15.8	2.9
adenosyl[44]	2.24(1)	2.00(1)	13.3	2.9

[a] The compounds are listed in order of increasing electron donor abilities of R. Numbers in parentheses are estimated standard deviations in the last significant digit. Additional data from ref 42.

As one would expect, the rejection of weak data has some cosmetic effect and yields a smaller crystallographic residual (see column II, Table 2), while the geometric parameters of interest (fold angle and metal centered bond distances) remain virtually the same for all five refinement strategies. The only procedure that introduced some bias into these values is the isotropic refinement with a data cutoff of $4\sigma(F_o)$, as shown in column II, Table 2. This result confirms that, especially in the case of weakly diffracting crystals, low intensities should be treated properly by applying an appropriate weighting scheme and not just be eliminated from the refinement procedure.[24,25]

Our final decision was to base our results reported here on the free refinement for the following reasons: (a) the molecular geometry has problems in minor details, but is reasonable on an overall scale, and therefore serves the purpose of this structure determination; (b) there is still considerable controversy about the weighting of supplementary equations in the refinement process,[26] which suggests that there is a need to avoid the introduction of too many restraints in order to obtain a reasonable structural model; (c) none of the geometrical parameters of interest, such as Co−N and Co−C bond distances or the upward folding angle, turned out to be dependent on the method of refinement. Therefore, we believe it is wisest to use the most direct approach, to manipulate the refinement process as little as possible, and to accept the geometric results from a straightforward refinement procedure.

II. Molecular Structure. Like all other cobalamins (for a recent list of available structural data see ref 22), difluoromethylcobalamin crystallizes with many water molecules per formula unit. Conclusions about the molecular structures of these compounds are therefore often hampered by the inherent inaccuracy caused by these (usually partially disordered) solvent molecules. In our structure, only 2 of the 17 water molecules were assigned high displacement parameters during refinement; this suggested half occupancy at these solvent sites (see also section II). Several bond lengths in the corrin ring are, however, larger (>0.04 Å) than the expected (target) values shown in Figure 2 (where esd's generally exceed 0.010 Å for the C−C bond distances). Therefore our discussion of the crystal structure of CF_2Cbl will be restricted to the Co-centered bonds and molecular features that are presumed to be unaffected by the artifacts just described (see Table 2).

Both the Co−carbon and the Co−NB3 bond distances do not differ significantly from the values found for MeCbl (see Table 4), even though chemical reasoning would predict a

---

(22) Kratky, C.; Färber, G.; Gruber, K.; Wilson, K.; Dauter, Z.; Nolting, H.-F.; Konrat, R.; Kräutler, B. *J. Am. Chem. Soc.* **1995**, *117*, 4654−4670.

(23) Glusker, J. P. In B_{12}; Dolphin, D., Ed.; Wiley: New York, 1982; pp 23−106.

(24) Hirshfeld, F. L.; Rabinovich, D. *Acta Crystallogr.* **1993**, *A29*, 510−513.

(25) Sheldrick, G. *SHELXL: A Program for the Refinement of Crystal Structures from Diffraction Data*; University of Göttingen: Germany, 1993.

(26) Watkin, D. *Acta Crystallogr.* **1994**, *A50*, 411−437.

Table 5. Hydrogen Bonding and Contacts <3.30 Å in CF_2Cbl; Bold Numbers Are Used in Figure 11

A		B	A···B [Å]	symmetry operation relating B to A	A		B	A···B [Å]	symmetry operation relating B to A
O28	–	N45	**2.87**	$-x, {}^1/_2 + y, {}^1/_2 - z$	OW6	–	O51	2.72	$x, y, z + 1$
	–	N52	**3.08**	$x, y, 1 + z$		–	OW3	2.83	x, y, z
N29	–	OW12	**2.93**	x, y, z		–	OW10	**2.81**	x, y, z
	–	OW14	**3.01**	x, y, z		–	OW17	**2.68**	x, y, z
O33	–	N63	**2.92**	$-x, {}^1/_2 + y, {}^1/_2 - z$	OW7	–	N40	2.91	x, y, z
	–	OW12	**2.68**	$-{}^1/_2 + x, {}^1/_2 - y, -z$		–	O62	2.78	$-x, {}^1/_2 + y, {}^1/_2 - z$
N34	–	OP3	3.26	$-{}^1/_2 + x, {}^1/_2 - y, -z$		–	OP5	2.64	${}^1/_2 + x, {}^1/_2 - y, -z$
	–	OP4	2.92	$-{}^1/_2 + x, {}^1/_2 - y, -z$		–	OR8	2.91	${}^1/_2 + x, {}^1/_2 - y, -z$
O39	–	F2	3.11	x, y, z	OW8	–	OP5	2.70	${}^1/_2 + x, {}^1/_2 - y, -z$
	–	OW12	**2.73**	x, y, z		–	OR7	2.71	$-x, {}^1/_2 + y, {}^1/_2 - z$
N40	–	OW7	**2.91**	x, y, z		–	OW2	2.81	x, y, z
	–	OW10	**3.01**	x, y, z		–	OW4	2.90	x, y, z
O44	–	OW1	**2.75**	$-{}^1/_2 + x, {}^1/_2 - y, -z$	OW9	–	N52	2.87	${}^1/_2 + x, {}^1/_2 - y, -z$
	–	OW13	**2.78**	$-{}^1/_2 + x, {}^1/_2 - y, -z$		–	O58	2.73	${}^1/_2 + x, {}^1/_2 - y, -z$
N45	–	O28	2.87	$-x, {}^1/_2 + y, {}^1/_2 - z$		–	OW4	2.79	x, y, z
	–	OW14	**3.00**	$-x, {}^1/_2 + y, {}^1/_2 - z$	OW10	–	N40	3.01	x, y, z
O51	–	N63	**2.95**	$-x, {}^1/_2 + y, {}^1/_2 - z$		–	OW12	2.77	x, y, z
	–	OW6	**2.72**	$x, y, z - 1$		–	OW6	2.81	x, y, z
N52	–	O28	3.08	$x, y, z - 1$		–	OW11	**2.74**	x, y, z
	–	OW9	**2.87**	$-{}^1/_2 + x, {}^1/_2 - y, -z$	OW11	–	OR8	2.74	${}^1/_2 + x, {}^1/_2 - y, -z$
O58	–	OW9	**2.73**	$-{}^1/_2 + x, {}^1/_2 - y, -z$		–	OW1	2.77	x, y, z
N59	–	OP2	3.28	x, y, z		–	OW5	**2.76**	x, y, z
	–	OP3	2.85	x, y, z		–	OW10	2.74	x, y, z
	–	OW2	**2.92**	$-x, {}^1/_2 + y, {}^1/_2 - z$	OW12	–	N29	2.93	x, y, z
O62	–	OW2	**2.79**	$-x, {}^1/_2 + y, {}^1/_2 - z$		–	O39	2.73	x, y, z
	–	OW7	**2.78**	$-x, {}^1/_2 + y, {}^1/_2 - z$		–	OW10	**2.77**	x, y, z
N63	–	O33	2.92	$-x, {}^1/_2 + y, {}^1/_2 - z$		–	OW13	**2.77**	x, y, z
	–	O51	2.95	$-x, {}^1/_2 + y, {}^1/_2 - z$	OW13	–	O44	2.78	${}^1/_2 + x, {}^1/_2 - y, -z$
OW1	–	O44	2.75	${}^1/_2 + x, {}^1/_2 - y, -z$		–	OW12	2.77	x, y, z
	–	OR6	3.29	${}^1/_2 + x, {}^1/_2 - y, -z$		–	OW15	**3.02**	x, y, z
	–	OW11	**2.77**	x, y, z		–	OW17	**2.59**	${}^1/_2 - x, -y, {}^1/_2 + z$
OW2	–	N59	2.92	$-x, {}^1/_2 + y, {}^1/_2 - z$	OW14	–	N29	3.01	x, y, z
	–	O62	2.79	$-x, {}^1/_2 + y, {}^1/_2 - z$			N45	3.00	$-x, {}^1/_2 + y, {}^1/_2 - z$
	–	OW3	**2.73**	x, y, z		–	OW15	**2.80**	x, y, z
	–	OW8	**2.81**	x, y, z		–	OW17	**2.51**	x, y, z
OW3	–	OW2	2.73	x, y, z	OW15	–	F1	2.89	${}^1/_2 - x, -y, {}^1/_2 + z$
	–	OW6	**2.83**	x, y, z		–	OW13	3.02	x, y, z
	–	OW12	**2.77**	x, y, z		–	OW14	2.80	x, y, z
OW4	–	OP4	2.68	$-x, {}^1/_2 + y, {}^1/_2 - z$	OW16	–	F1	3.17	${}^1/_2 - x, -y, {}^1/_2 + z$
	–	OW8	**2.90**	x, y, z		–	OW5	2.70	x, y, z
	–	OW9	**2.79**	x, y, z		–	OW17	**2.76**	x, y, z
OW5	–	O33	2.68	${}^1/_2 + x, {}^1/_2 - y, 1 - z$	OW17	–	OW6	2.68	x, y, z
	–	OW3	2.77	x, y, z		–	OW13	2.59	${}^1/_2 - x, -y, {}^1/_2 + z$
	–	OW11	2.76	x, y, z		–	OW14	2.51	x, y, z
	–	OW16	**2.70**	x, y, z		–	OW16	2.76	x, y, z

shortening of the Co−N bond along with decreasing donor abilities ($CHF_2 < CH_3$) of the ligand trans to it. The upward folding angle,[27] which is mainly influenced by the steric pressure placed on the corrin ring system by the axial substituents, is consequently also virtually the same in both structures (15.4° in CF_2Cbl, 15.8° in MeCbl), and lies within the range predicted by a scatterplot of corrin-fold angle versus Co−NB3 bond length.[8] On the other hand, the pK_a value for the protonation of the nucleotide base (2.2), which is another measure of the correlation between the strength of the DMB coordination and the nature of the upper ligand,[22] fits precisely within the range of values obtained if the cobalamins are listed in order of increasing donor abilities of the β-ligand, as seen in Table 4. In this series, the fold angle seems to be a simple function of the bulkiness of the ligand on the β-face. We note that the Co−C bond length in our structure does not differ significantly (difference only 0.001 Å) from that found in the corresponding imidazolylcobaloxime.[10]

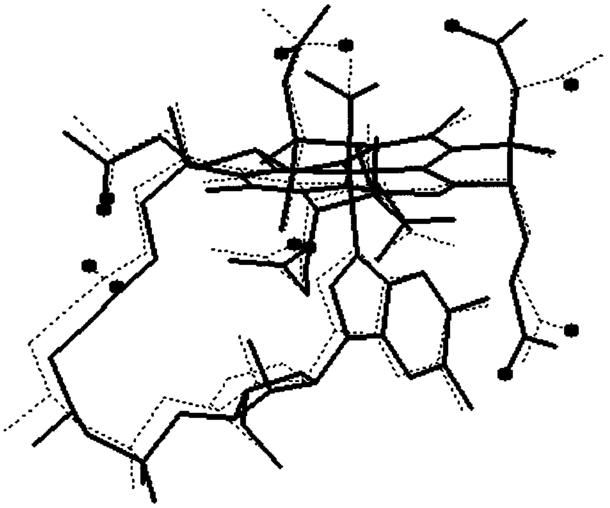

Figure 4. RasMol[46] superposition of the cobalamin molecules from CF_2Cbl (solid lines) and wet vitamin B_{12} (dashed lines). Oxygen atoms in the amide groups are represented as filled circles. The least-squares fit of Co, N21, N22, N23, and N24 was carried out with the program CHAIN.[47] Note the different conformations of the **c** side chain (top, far right).

As revealed by the superposition of the crystal structures of wet CNCbl and CF_2Cbl in Figure 4, the most interesting feature of the structure is the orientation of the amide group in side chain **c**. As already described for dry vitamin B_{12},[1] the aquocobalamin cation,[22] and chloro- and azidocobalamin,[28] which also form hydrogen bonds between the substituent at the β-face and the **c** amide group, this flexible side chain "reaches

---

(27) The upward folding angle is the angle between the least-squares planes through N21/C5/C6/C7/N22/C9/C10 and C10/C11/N23/C14/C15/N24.

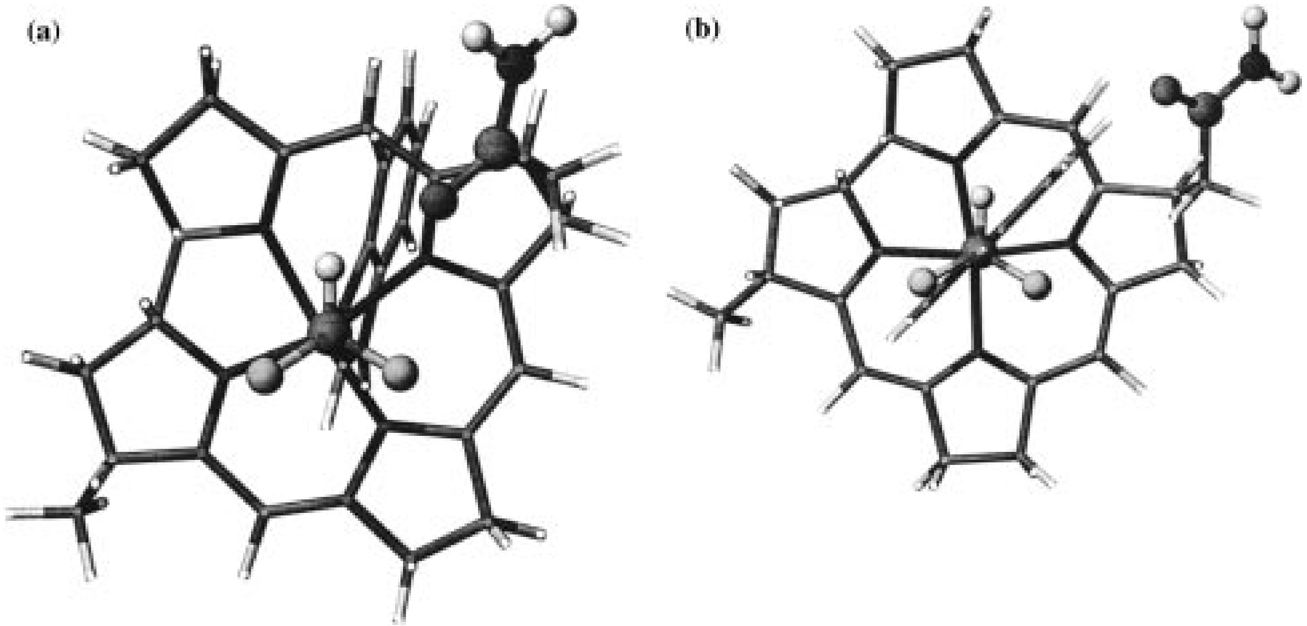

Figure 5. Optimized molecular geometries for a simplified model complex, the CHF$_2$ and amide side chain **c** moiety are represented as ball-and-stick, other atoms as wireframe. (a) Minimization of the crystallographically established geometry with ZINDO:[34] O$\cdots$F = 3.117 Å (X-ray, coordinates from refinement procedure V: 3.122 Å), O$\cdots$H = 2.477 (2.525) Å. (b) Molecular geometry after a MM2 force field[35] optimization: O$\cdots$F = 4.584 Å, O$\cdots$H = 4.196 Å. The difference in energy between the two conformations shown is only 3 kcal mol^{-1} (in favor of b).

upward” toward the CHF$_2$ group with the oxygen atom facing the fluorine F2 at a distance of 3.12 Å.

Even though this distance is slightly longer than the sum of their van der Waals radii (1.52 Å for oxygen, 1.47 Å for fluorine[29]), this is, at first sight, surprising result, since one would have expected the amide nitrogen atom to interact with the electronegative fluorine via one of its hydrogen atoms. A consideration of the energetic contributions not only from the fluorine−oxygen contact, but from the entire immediate environment of these two atoms, however, shows that this orientation of the amide group is the logical result of energetic aspects. Figure 3 shows the three hydrogen bonds to adjacent water molecules formed by O39 and N40 of the **c** amide group (see also Table 5). Each of these interactions contributes approximately 5 kcal mol^{-1} to the energy balance, which alone makes up for the loss of the fluorine−hydrogen interaction. The strength of this F$\cdots$H interaction is, even under optimal geometric conditions (distance 1.9 Å), only 2.4 kcal mol^{-1}, so that “if the hydrogen bond donor can find stabilization with an alternative acceptor to fluorine then it will do this”.[30]

Another aspect to take into account is the distance between the **c** amide oxygen atom, O39, and the carbon bound hydrogen atom, H1F, of the difluoromethyl group (2.54 Å); this distance is below the sum of their van der Waals radii (1.52 Å for oxygen, 1.20 Å for hydrogen[29]). On the basis of the study from Taylor and Kennard,[31] interactions of this type must be considered as C−H$\cdots$O=C hydrogen bonds[32] with directional properties and an energy in the range of 1−2 kcal mol^{-1}. Species with electron-withdrawing substituents such as fluorine in the donor group can be expected to lie at the high end or even beyond this range. The CHF$_2$ group contains an acidic hydrogen atom that is available as a weak hydrogen-bond donor [C1F−H1F$\cdots$O39].[33] The orientation of the CHF$_2$ group therefore appears to be a favorable compromise between F2$\cdots$O39 and H1F$\cdots$O39 interactions [see Figures 3 and 5a]. As shown in Figure 6, the hydrogen atom on C19 of the corrin ring is in contact with the other fluorine atom, F1, which is also in contact with water molecules.

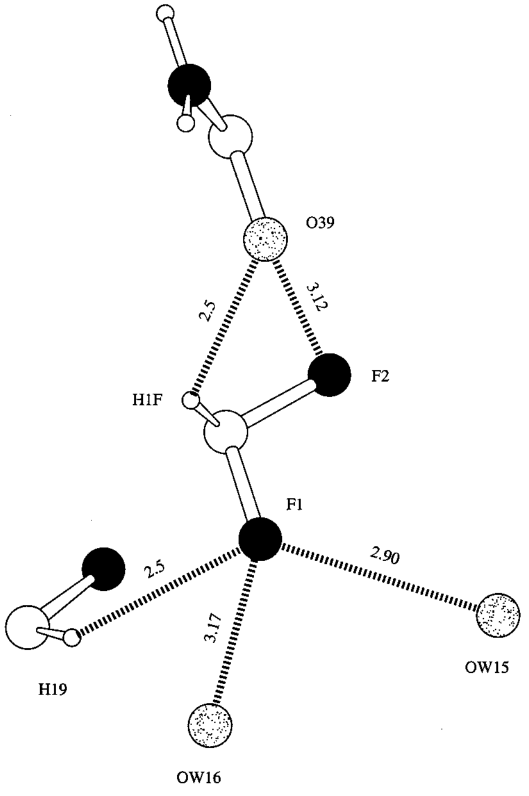

Figure 6. Surrounding of the CHF$_2$ group, with distances in Å.

These considerations are confirmed by semiempirical and force field calculations on a simplified model complex. Details

---

(28) Randaccio, L.; Furlan, M.; Geremia, S.; Slouf, M. *Inorg. Chem.* **1998**, *37*, 5390−5393.

(29) Bondi, A. *J. Phys. Chem.* **1964**, *68*, 441−451.

(30) Howard, J. A. K.; Hoy, V. J.; O’Hagan, D.; Smith, G. T. *Tetrahedron* **1996**, *52*, 12613−12622.

(31) Taylor, R.; Kennard, O. *J. Am. Chem. Soc.* **1982**, *104*, 5063−5070.

(32) Desiraju, G. R. *Acc. Chem. Res.* **1991**, *24*, 290−296.

(33) Steiner, T. *Crystallogr. Rev.* **1996**, *6*, 1−57.

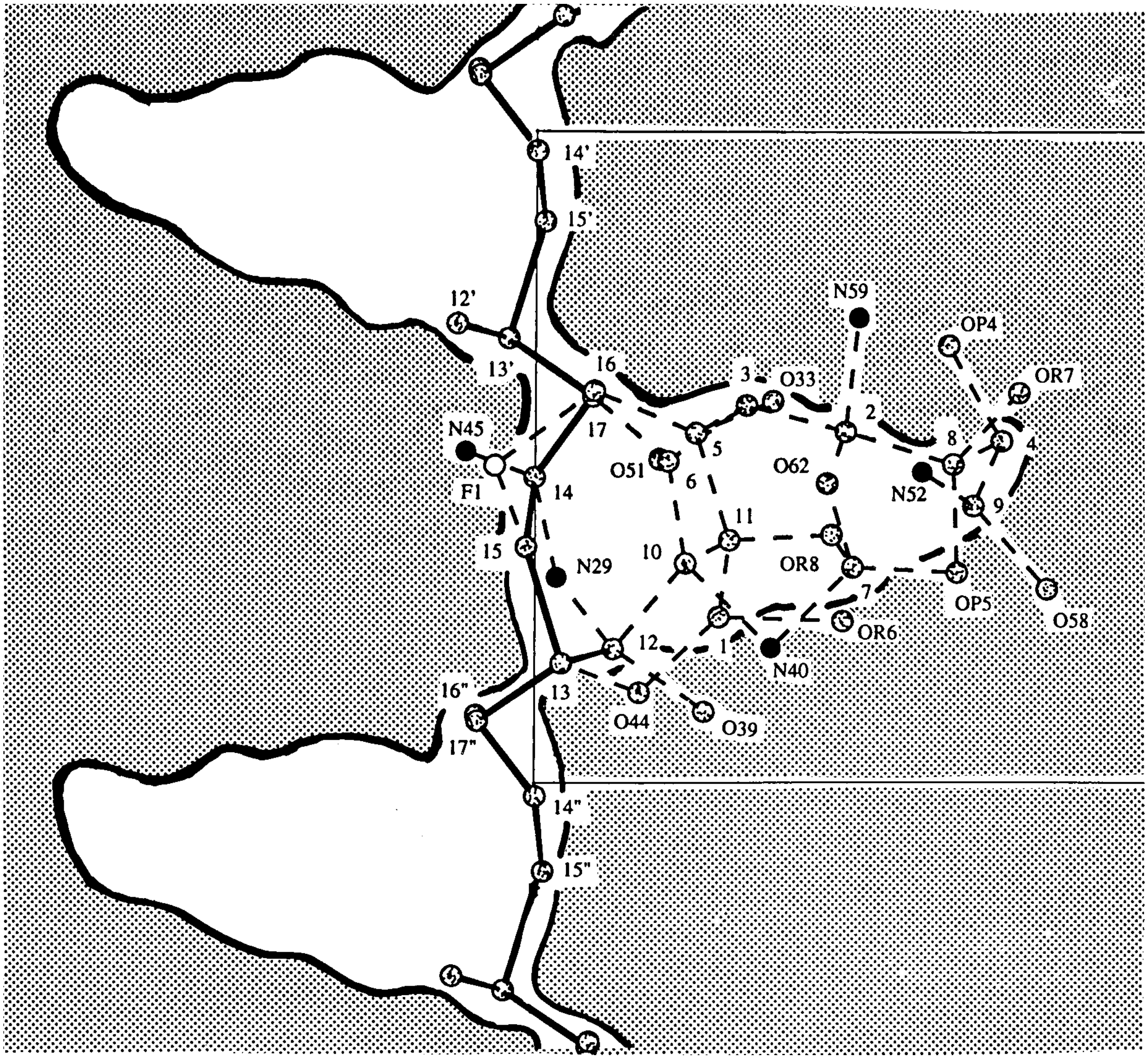

Figure 7. Interactions within the channel (solid lines) and pocket regions (dashed lines). View onto the *bc* plane (*b* horizontal, *c* vertical). Oxygen atoms are speckled, nitrogen atoms are solid, and fluorine atoms are open circles (ICRVIEW[45]).

of the procedure are given in the Experimental Section. Further minimization of the input structure with the semiempirical ZINDO program[34] shows that the molecular geometry based on the crystallographic results is very close to a reasonable local minimum for the isolated molecule (Figure 5a). Molecular dynamics calculations and geometry optimization with an extended MM2 force field[35,36] result in a strain-releasing displacement of the **c** side chain away from the center of the molecule (Figure 5b). The energy difference between the solid state and gas phase conformation amounts to only 3 kcal mol^{-1} in favor of the latter. Obviously, the rather compact geometry represented in Figure 5a is more appropriate in terms of packing efficiency in a crystal, where neighboring or solvent molecules can easily make up for this small energy difference.

The validity of the energetic considerations is supported by crystallographic evidence.

(a) Refinement of the structure, with O39 and N40 omitted (applying a hard restraint on the bond between C37 and C38), followed by a difference Fourier map yielded two peaks (4.1 e Å^{-3}, 1.19 Å away from C38; and 3.2 e Å^{-3}, 1.40 Å away from C38), identical in position to the omitted O39 and N40.

(b) Upon deletion of the hydrogen atoms bonded to nitrogen atoms and a switching of atom types in amide groups, the displacement parameters refined from $U_{\mathrm{iso}} = 0.028$ (when O) to 0.010 (when N) for "nitrogen" and from $U_{\mathrm{iso}} = 0.032$ (when N) to 0.055 (when O) for "oxygen", giving a clear indication that the "nitrogen" position is really an oxygen position.

(c) The distances to the adjacent water molecules (2.75 Å for O39···OW5, 2.91 Å for N40···OW7, and 3.01 Å for N40···OW10) positively differentiate the oxygen from the nitrogen (see also section II), since the lower limit for nitrogen–water contacts seems to be 2.80 Å.

(d) A CSD search[18] for high-quality data (R factor < 0.10) of compounds with intra- or intermolecular C–F···O distances below 3.2 Å provides more than 40 hits; the distance between the two atom types is found to be as low as 2.76 Å. Generally, there is no discussion of these contacts in the original papers, probably because the molecular structure and stereochemistry is the reason for X-ray characterization rather than details of the intermolecular network. It is, however, noteworthy in this context, that short contacts between fluorine and oxygen are more frequent than electronic reasoning might suggest.

III. Water Network. Cobalamins tend to incorporate large amounts of solvent (water, occasionally acetone) upon crystallization. A detailed description of the organization of solvent

---

(34) Zerner, M. C. *CAChe ZINDO Guide*; Oxford Molecular: Oxford, 1996, and the references therein.
(35) Allinger, N. L. *J. Am. Chem. Soc.* **1977**, *99*, 8127–8134.
(36) *CAChe Mechanics*; Oxford Molecular: Oxford, 1996.

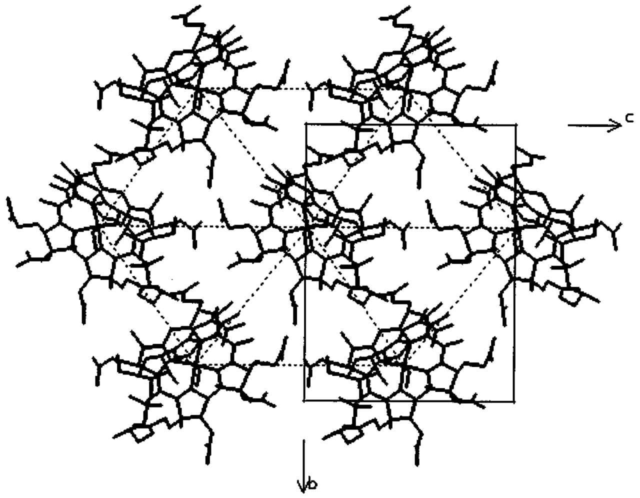

Figure 8. View down the *a* axis onto the *bc* plane, revealing the 2-dimensional packing CF_2Cbl. All molecules shown are centered at $x \approx 0$. The next layer runs parallel to this one at $x \approx {}^1/_2$, translated along [011]. According to the motif of hexagonal close packing, the third layer lies exactly above the one shown. Picture drawn with RasMol.[46]

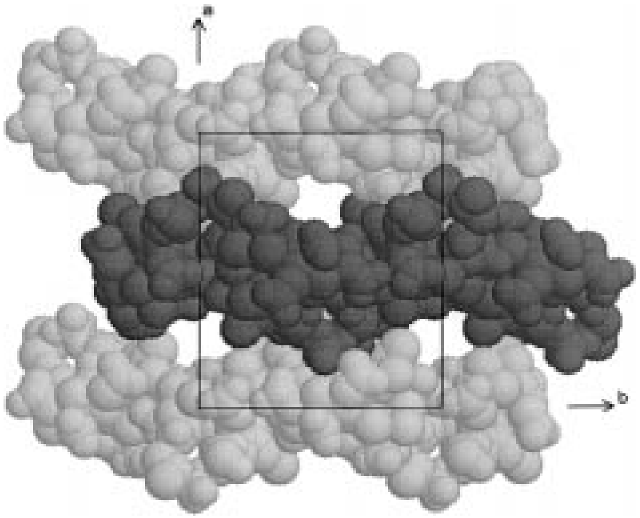

Figure 9. View onto the *ab* plane showing the water channels between three layers of CF_2Cbl molecules. The atoms are drawn at their van der Waals radius (RasMol[46]). The channels are located at $x = {}^1/_4$, $y = 0$, and $x = {}^3/_4$, $y = {}^1/_2$, respectively.

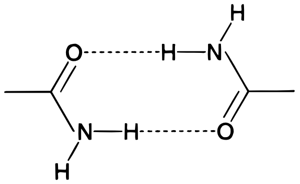

Figure 10. $R_2^2(8)$ hydrogen bonding motif (ring pattern of eight atoms with two donor and two acceptor hydrogen bonds), found in numerous small molecule compounds with amide groups present[48] but not in this structure.

in the structure of AdoCbl on the basis of a neutron diffraction experiment is given by Savage.[37] In CF_2Cbl we were able to locate 17 water molecules from $F_o - F_c$ maps, 14 of which are well ordered revealing equivalent isotropic displacement parameters below $B_{iso} = 11$ Å^2 and no residual electron density in their vicinity. For the purpose of this study of the water network, however, all water sites were treated as fully occupied.

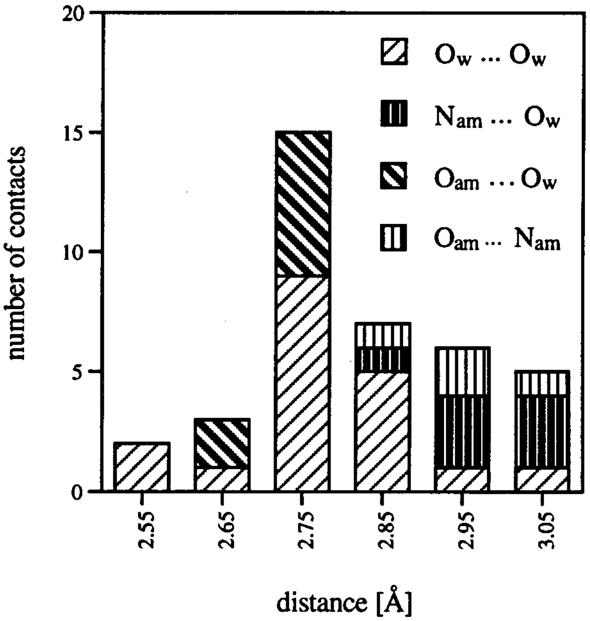

Figure 11. Distribution of water−water and water−amide interactions in the crystal structure of CF_2Cbl. The actual ranges are $O_w \cdots O_w$, 2.51−3.02 Å; $O_{am} \cdots O_w$, 2.68−2.79 Å; $N_{am} \cdots O_w$, 2.87−3.01 Å; $N_{am} \cdots O_{am}$, 2.87−3.08 Å. The cutoff distance for the search algorithm was set to 3.30 Å.[15]

As in other B_{12} structures,[37,38] one part of the solvent in CF_2Cbl crystals runs in distinct channels along the crystallographic *c* axis, whereas the other part is located in "pockets" extending from these channels between $0 < y < {}^1/_2$, and ${}^1/_4 < z < {}^1/_2$ (see Figure 7). This accumulation of water molecules, in combination with the presence of numerous asparagine- and glutamine-like side chains, allows for a more detailed study of water−water and water−amide interactions, which is of special importance regarding the biological significance of these interactions.

To understand the arrangement of the solvent channels, it is useful to have a brief look at the packing of the CF_2Cbl molecules. The majority of cobalamins (including CF_2Cbl) crystallize with a very similar two-dimensional arrangement of B_{12} molecules which resembles a close packing of spheres in a plane (see Figure 8). An exception is MeCbl,[39] which does not adopt any kind of regular packing motif and is one of the few cobalamins with different cell constants ($a = 17.887(6)$ Å, $b = 32.68(1)$ Å, $c = 17.447(5)$ Å) than the usually observed $a \approx 25$ Å, $b \approx 22$ Å, and $c \approx 16$ Å.

In the structure of CF_2Cbl the layers shown in Figure 8 are stacked in the sequence ABA, resulting in a (slightly distorted) hexagonal close packing. As shown in Figure 9, this manner of stacking leaves channels of empty space between the layers, which have to be filled with solvent molecules to achieve a reasonable packing efficiency. The interactions involving the water molecules and the polar atoms of the CF_2Cbl are summarized in Table 5. All water molecules form at least 3 contacts with other polar atoms within a hydrogen bonding distance (up to 3.30 Å[15]). Interestingly, there is no formation of the bidentate hydrogen bond motif $R_2^2(8)$ shown in Figure 10, which one would expect due to the presence of numerous suitable donor/acceptor amide groups.[40] In fact, among the cobalamins listed in Table 4, MeCbl is the only structure in which this kind of interaction has been observed.[39]

---

(37) Savage, H. *Biophys. J.* **1986**, *50*, 947−965.

(38) Savage, H. *Biophys. J.* **1986**, *50*, 967−980.

(39) Rossi, M.; Glusker, J. P.; Randaccio, L.; Summers, M. F.; Toscano, P. J.; Marzilli, L. G. *J. Am. Chem. Soc.* **1985**, *107*, 1729−1738.

The organization of the solvent contacts in the structure of CF_2Cbl is depicted in Figure 7, which shows the water channel at $x = {}^{1}/_{4}$, $y = 0$, extending into the same type of "pocket region" that Savage found in coenzyme B_{12}.[37] The water molecules in the pocket (OW1 through OW11, connected by broken lines) are more ordered than those in the channel (OW12 through OW17, connected by solid lines), as is revealed by their significantly smaller displacement parameters.

The hydrogen bond type interactions have been divided into four groups: water−water interactions ($O_w \cdots O_w$), water−amide-oxygen interactions ($O_w \cdots O_{am}$), water−amide-nitrogen interactions ($O_w \cdots N_{am}$), and amide-oxygen−amide-nitrogen interactions ($O_{am} \cdots N_{am}$). The distribution of distances at which these interactions occur show some regularities, as visualized in Figure 11. Whereas distances between water molecules cover a wide range from 2.51 to 3.02 Å, there is only a small variation in the distances for the other three groups. Nitrogen−oxygen interactions occur only between 2.87 and 3.08 Å, independent of the nature of the interaction partner (water or amide-oxygen). Interactions between amide-oxygens and water, on the other hand, tend to be significantly shorter (2.68−2.79 Å). This discontinuity at approximately 2.8 Å provides a useful criterion for distinguishing between nitrogen and oxygen atoms during structure solution which, for B_{12} data, is often not straightforward.

Acknowledgment. This work was supported by a Feodor Lynen Fellowship from the Alexander von Humboldt Foundation to T.W., NIH Grant CA-10925 to J.P.G., DOE Grant FGO 289ER14013 to H.P.C.H., NIH Grant CA-06927 to Fox Chase Cancer Center, and by an appropriation from the Commonwealth of Pennsylvania. The authors thank Ms. Amy Katz for collecting the diffraction data and Dr. Steve Seeholzer who provided the MS spectra.

Supporting Information Available: One X-ray crystallographic file, in CIF format, is available. This material is available free of charge via the Internet at http://pubs.acs.org.

IC980751Q

---

(40) Shimoni, L.; Glusker, J. P.; Bock, C. W. *J. Phys. Chem.* **1996**, *100*, 2957−2967.

(41) (a) $R_1 = \{\sum||F_o| - |F_c||/\sum|F_o|\}$ for 5675 reflections with $F_o > 4\sigma(F_o)$. (b) $wR_2 = \{\sum[w(F_o^2 - F_c^2)^2]/\sum[w(F_o^2)]\}^{1/2}$, $w = 1/[\sigma^2(F_o^2) + (0.0711P)^2]$.

(42) Brown, K. L.; Hakimi, J. M.; Jacobsen, D. W. *J. Am. Chem. Soc.* **1984**, *106*, 7894−7899.

(43) Kräutler, B.; Konrat, R.; Stupperich, E.; Färber, G.; Gruber, K.; Kratky, C. *Inorg. Chem.* **1994**, *33*, 4128−4139.

(44) Savage, H. F. J.; Lindley, P. F.; Finney, J. L. *Acta Crystallogr.* **1987**, *B43*, 280−295.

(45) Erlebacher, J.; Carrell, H. L. *ICRVIEW. Program from The Institute for Cancer Research*; Fox Chase Cancer Center: Philadelphia, PA, 1992.

(46) Sayle, R. *RasMol: A Molecular Visualisation Program*, Version 2.6; Glaxo Research and Development: Greenford, U.K., 1994.

(47) Sack, J. S. *J. Mol. Graphics* **1988**, *6*, 224−225.

(48) Etter, M. C. *Acc. Chem. Res.* **1990**, *23*, 120−126.