Electrochemical Synthesis and Structure Analysis of Neocoenzyme B_{12} – An Epimer of Coenzyme B_{12} with a Remarkably Flexible Organometallic Group

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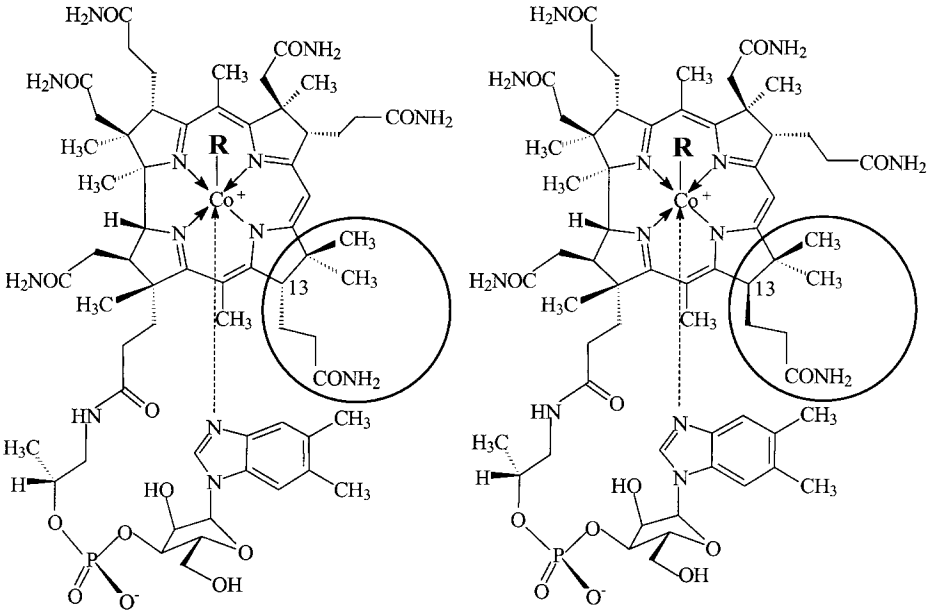
Dedicated to Prof. *Heinz Falk* on the occasion of his 60th birthday

In neocoenzyme B_{12} (=(5′-deoxy-5′-adenosyl)-13-epicob(III)alamin; **5**), an epimer of coenzyme B_{12} (**1**), the organometallic group and a propanamide side chain of the vitamin-B_{12} ligand compete for the same region in space. Interesting consequences for structure and organometallic reactivity of this isomer of **1** are to be expected. Neocoenzyme B_{12} (**5**; 89% yield) and methyl-13-epicobalamin (**6**; 88% yield) were prepared from neovitamin B_{12} (**4**) by electrochemical means (*Fig. 3*). The solution structure of the organometallic neovitamin-B_{12} derivative **5** was analyzed by homonuclear and heteronuclear NMR spectroscopy. Comparison of the structures of **1** and **5** informed on the structural consequences of the epimerization at C(13) and revealed a remarkable flexibility of the organometallic group in **5**. In **5**, both sterically interacting functionalities (organometallic group and propanamide side chain at C(13)) adapt their conformations dynamically to avoid significant mutual clashes. As one consequence of this structural adaptation, the major conformations of **5** feature counterclockwise and clockwise reorientations of the organometallic ligand with respect to its crystallographically determined position in coenzyme B_{12} (**1**). One of the dominant conformers of **5** exhibits an orientation of the organometallic functionality similar to that found in the crystal structure of the coenzyme-B_{12}-dependent methylmalonyl CoA mutase. The present NMR study also revealed the significant population of *syn*-conformers of the organometallic adenosine group, another remarkable feature of the solution structure of **5**.

1. Introduction. – By means of X-ray crystallography [1], coenzyme B_{12} (=(5′-deoxy-5′-adenosyl)cob(III)alamin; **1**) was discovered to be a unique organometallic derivative of vitamin B_{12} (=cyanocob(III)alamin, **2**) whose structure had similarly been elucidated about a decade earlier [1][2] (see *Fig. 1*). Coenzyme B_{12} (**1**) is the corrinoid cofactor in a series of enzymatic radical reactions [3], in which the role of **1** as cofactor has been associated closely with the reactivity of its organometallic bond [3][4]. Homolytic cleavage of enzyme-bound **1** into a 5′-deoxy-5′-adenosyl radical and the [Co^{II}(corrinato)] complex cob(II)alamin (**3**) induces the further reactions catalyzed by these enzymes [5]. Indeed, while **1** is highly susceptible towards homolysis of its organometallic bond, the homolysis has been estimated to be faster by *ca.* 10^{11} to 10^{12} in the enzyme than in homogeneous aqueous solution [4][6]. Apparently, the enzymic environment strongly labilizes coenzyme B_{12} (**1**) towards homolysis of its Co–C bond. In spite of extensive experimental and theoretical work, the factors that promote the homolysis reaction still are a matter of dispute, even though the rate enhancement is believed to result from 'steric' distortions rather than from 'electronic' effects [4][6][7].

X-Ray crystallography also established neovitamin B_{12} [8] as cyano-13-epicob(III)alamin (**4**) [9], in which the *e*-propanamide side chain is seen to be bound axially at



1 R = 5'-deoxy-5'adenosyl, coenzyme B_{12}
2 R = CN, vitamin B_{12}
3 R = e^-, cob(II)alamin
4 R = CN, neovitamin B_{12}
5 R = 5'-deoxy-5'-adenosyl, neocoenzyme B_{12}
6 R = Me, methyl-13-epicob(III)alamin
7 R = Me, methylcob(III)alamin

Fig. 1. *Structural formulae of vitamin-B_{12} derivatives.* Left: cobalamins; right: neocobalamins.

the 13-position and to extend from there into the upper hemisphere (see *Figs. 1* and *2*). Comparing the crystal structures of **4** and of coenzyme B_{12} (**1**) [1], *Hodgkin* suggested an interesting situation to arise with neocoenzyme B_{12} (=(5′-deoxy-5′-adenosyl)-13-epicob(III)alamin; **5**), in which the organometallic ligand and the *e*-propanamide chain would preferentially occupy the same region in space [9][10].

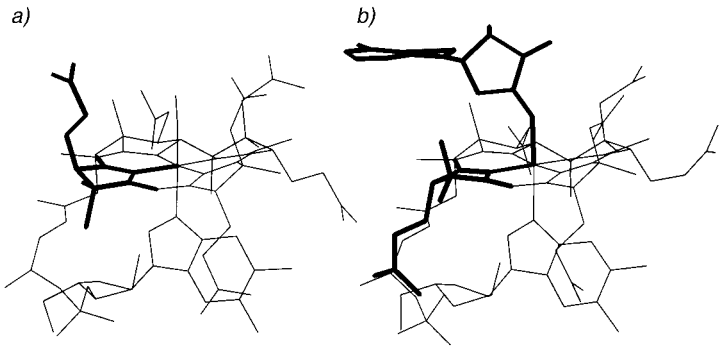


Fig. 2. *Stick model of the X-ray crystal structures* a) *of neovitamin B_{12}* (**4**) *and* b) *of coenzyme B_{12}* (**1**), *highlighting the organometallic group and the ring-C segment*

The present report details the preparation of **5** and of methyl-13-epicob(III)alamin (= methylneocob(III)alamin; **6**) by an electrochemical method [11], developed in our laboratories for improved synthesis of organometallic vitamin-B_{12} derivatives. Both, **5** and **6**, are organometallic derivatives of neovitamin B_{12} (**4**) that were prepared by *Hogenkamp* and co-workers in the seventies [12] to investigate their activity (as cofactors) in reactions of some coenzyme-B_{12}-dependent enzymes [13].

To test for effects of the expected steric interaction of the *e*-propanamide side chain and of the organometallic group of neocoenzyme B_{12} (**5**), we carried out a detailed investigation of the structure of **5** in aqueous solution, based on heteronuclear NMR spectroscopy and NMR-constrained molecular modeling. While our work was in progress and after it was published in preliminary form [11], *Brown*, *Cheng*, and *Marques* very recently published a study on the synthesis and on conformational properties of **5** [14]. The larger set of detailed experimental information obtained in our work supports important features of the dynamic solution structure of neocoenzyme B_{12} (**5**) not reported in [14], but relevant in the context of the reported cofactor activity [13] of **5**.

2. Results. – 2.1. *Electrochemical Syntheses.* The preparation of methyl-13-epicob(III)alamin (**6**) and neocoenzyme B_{12} (**5**) in *ca.* 30% yield each was first reported by *Hogenkamp* and coworkers [12]. *Brown*, *Cheng*, and *Marques* [14] prepared **5** in 85% yield from **4** and 5′-chloro-5′-deoxyadenosine using Zn wool as reducing agent. Here, we describe a highly efficient electrochemical method developed in our laboratory for the purpose of preparing reactive organometallic vitamin-B_{12} derivatives [15], which directly afforded the desired products **5** and **6** in chromatographically pure form. Electrochemical reduction of neovitamin B_{12} (**4**) to neocob(I)alamin and *in situ* alkylation of the latter with 5′-*O*-tosyladenosine or methyl iodide produced the organometallic compounds **5** and **6**, respectively, which were isolated after a simple workup in yields of nearly 90% (see *Fig. 3*). While in the earlier work [12], the UV/VIS and CD properties of **5** and **6** were used for their identification, further characterization by fast-atom-bombardment mass spectrometry (FAB-MS) and high-field NMR spectroscopy [14] was performed here.

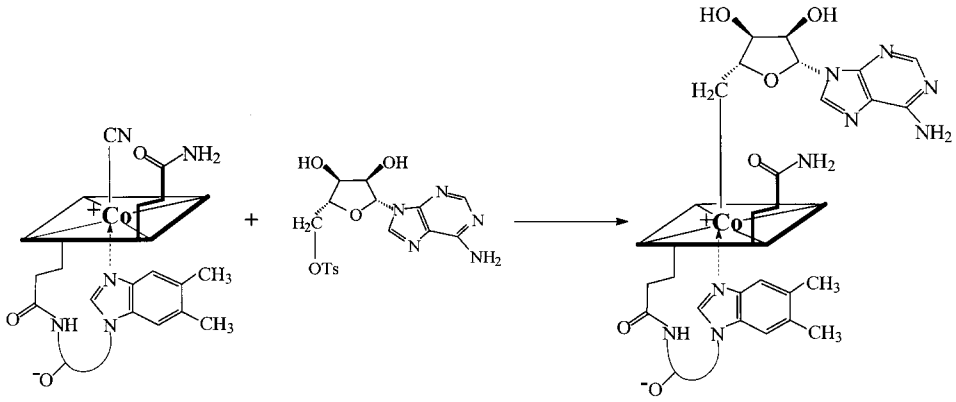


Fig. 3. *Electrochemical preparation of neocoenzyme B_{12}* (**5**) *from neovitamin B_{12}* (**4**) (see *Exper. Part* for details)

The standard positive-ion FAB-MS of **5** and **6** exhibit the signals for the molecular ion *M* at *m/z* 1580.2 and 1345.1, respectively, and of the [Co(corrinato)] fragment at *m/z* 1330.1 (loss of the organometallic groups), the typical pattern of positive-ion FAB-MS of organometallic vitamin-B_{12} derivatives.

In the ^{1}H-NMR spectrum of methylneocob(III)alamin (**6**) in D_2O, the diagnostic *singlet* at high field for the cobalt-bound Me group occurs at 0.13 ppm, downfield ($\Delta\delta = 0.18$ ppm) from that of methylcob(III)alamin (**7**) [10][16][17]. In contrast, slight upfield shifts are observed for two of the assigned resonances of the dimethylbenzimidazole H-atoms ($\Delta\delta = -0.09$ ppm and -0.10 ppm for H–C(4N) and H–C(7N), resp.). Methylneocob(III)alamin (**6**) was shown by *Hogenkamp et al.* to be more resistant than the cob(III)alamin **7** against acid-induced decoordination of the nucleotide base, with $pK_a(\mathbf{6}\cdot H^+) = 2.2$; $pK_a(\mathbf{7}\cdot H^+) = 2.7$ [12], indicating that **6** experiences a stronger stabilization by the nucleotide coordination than does **7**. The observed differences in chemical shifts indeed would not be inconsistent with a somewhat shortened Co–N bond of the axially coordinating nucleotide base in **6** as compared to **7** (see, *e.g.*, [18]).

2.2. *NMR-Spectroscopic Structure Analysis of Neocoenzyme B_{12}* (**5**). *Signal Assignments* (see *Fig. 4* for numbering scheme). From analysis of a solution of neocoenzyme B_{12} (**5**) in H_2O/D_2O 9:1, the ^{1}H-NMR signals of all H-atoms could be assigned on the basis of the NMR experiments described in the *Exper. Part*, except the signals of the exchange-labile OH groups of the ribose units (OH–C(5R), OH–C(2RL), OH–C(3RL)) (*Table 1*). Thus, all the amide protons were identified (see *Table 2*), as was the signal of OH–C(2R) (at 5.42 ppm).

The signals of all the amide protons and of the diastereotopic methylene protons at C(21), C(31), C(71), C(81), C(82), C(171), C(172), and C(5RL) could be assigned from analysis of the relative intensities of NOEs of these protons as well as from long-range couplings observed in HMBC spectra [18][19]. Likewise the resonances of all C-centers were found and assigned with the help of 2D–H,C-correlations from HSQC and HMBC spectra, as were those of the amide N-atoms (from an H,N-HSQC spectrum, see *Tables 1* and *2*). The assignment for the carboxamide atoms C(33) and C(133) are tentative and may be reversed (see *Table 1*). Aside from two relevant exceptions, the assignments agree with those given in [14] (a systematic high-field shift of *ca.* -0.12 ppm in our ^{1}H-NMR spectra is due to the different reference systems used). Configurational assignments of the diastereotopic protons of CH_2(21), CH_2(31), CH_2(71), CH_2(81), CH_2(82), CH_2(171), CH_2(172), and CH_2(5RL), were not reported in [14]. The assignments of the signals of CH_2(171) and CH_2(172) in the ^{1}H- and ^{13}C-NMR spectra according to [14] should be reversed, as established by our data and based on clear NOE contacts between H–C(2N) and H_R–C(172). The assignment of the signals of CH_2(171) and CH_2(172) is impaired by a frequent near-isochronicity of the C-signals. This problem could be resolved with the help of a high-resolution HMBC experiment in the case of methylcob(III)alamin [17].

Qualitative Conformational Analysis of **5** *Based on ^{13}C-NMR Chemical Shifts.* Comparison of the δ(C) of neocoenzyme B_{12} (**5**) (as reported here and in [14]) and of its 13-epimer coenzyme B_{12} (**1**) [20] reveals a striking lack of differences between the two sets of data, with the exception of the signals due to CH_3(12A) and CH_3(12B) at the center adjacent to the site of epimerization (see *Table 1*): CH_3(12A) and CH_3(12B)

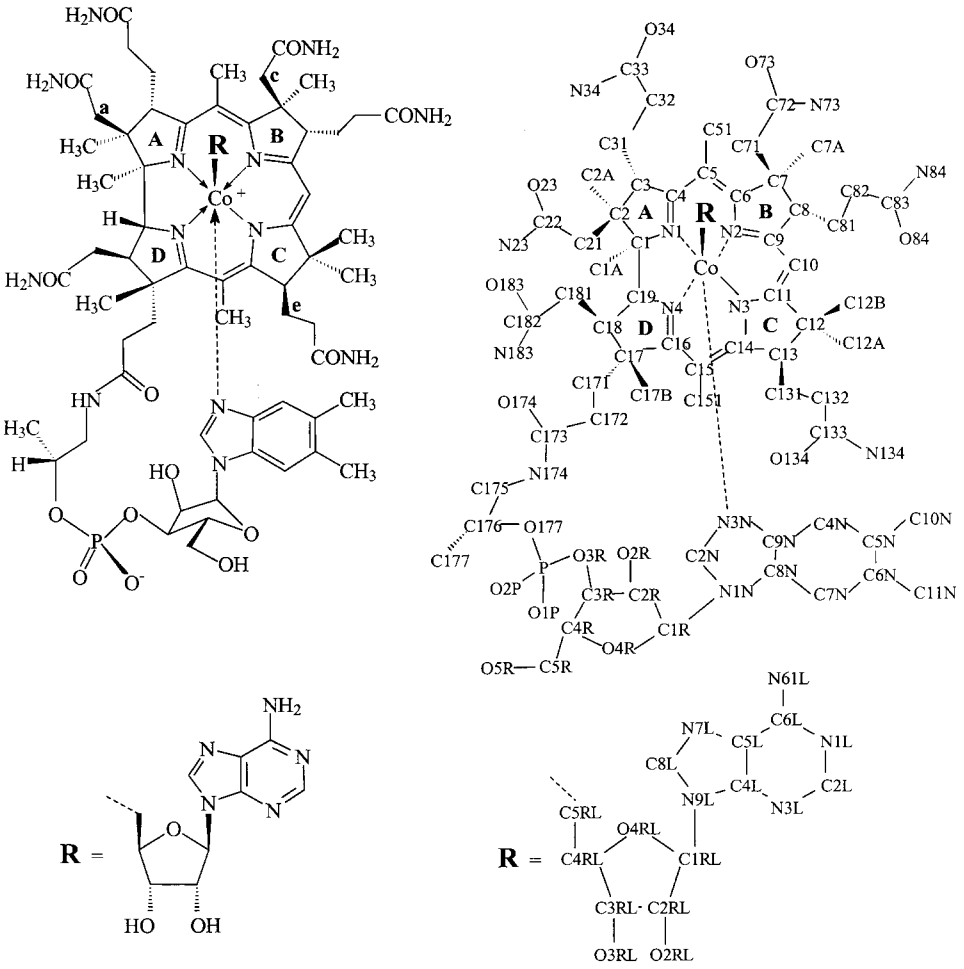


Fig. 4. *Structural formula of neocoenzyme B_{12}* (**5**) *together with the numbering scheme used.* The following letter labels are used (besides A and B): R for the ribose unit attached to the dimethylbenzimidazole moiety, N for the dimethylbenzimidazole moiety, RL for the ribose unit of the 5′-deoxy-5′-adenosyl ligand, and L for the adenine unit of the 5′-deoxy-5′-adenosyl ligand.

appear at 33.3 and 23.3 ppm, respectively, in the case of **5**, whereas their order is reversed in the case of **1** (CH_3(12A) at 23.9 and CH_3(12B) at 34.2 ppm [20]). A similar inversion of the position of the signals of CH_3(12A) and CH_3(12B) has also been observed in the spectrum of neovitamin B_{12} (**4**) [10] as compared to **2**. The $\Delta\delta$(C) of the signals of CH_3(12A) and CH_3(12B) in **1** and **2** is attributed to a strong conformational preference for the periphery of ring C: consistent with the observation of the steric γ-effect, the solution structures of the cob(III)alamins **1** and **2** adopt a conformation with CH_3(12A) and CH_3(12B) *syn*-clinal and *anti*-periplanar, respectively, with respect to the propanamide side chain at the adjacent C(13) [19]; this conformational pattern was first found in the crystal structures of **1** and **2** [1][2]. The reversed order of the (similar)

Table 1. *^{13}C- and ^{1}H-NMR Chemical Shifts* (in ppm) *of Neocoenzyme B_{12}* (**5**) *in H_2O/D_2O 9:1 at 26° and Comparison with Corresponding Chemical-Shift Values of Coenzyme B_{12}* (**1**)[a]. Coenzyme shifts are from [20].

	δ(C)	$\Delta\delta$(C)[b]	δ(H)	$\Delta\delta$(H)[b]
C(1)	89.0	0.5		
CH_3(1A)	23.1	−0.4	0.35	−0.12
C(2)	49.4	−0.1		
CH_3(2A)	19.5	−0.4	1.24	−0.12
CH_2(21)	45.8	−0.4	2.30 (*S*), 2.50 (*R*)	−0.09, −0.11
CH_2(22)	178.9	−0.4		
CH(3)	57.8	−0.7	4.02	−0.08
CH_2(31)	29.0	−0.2	1.89 (*R*), 1.99 (*S*)	−0.07, −0.07
CH_2(32)	37.5	−0.8	2.37[c]), 2.44[c])	−0.13, −0.06
C(33)[d])	181.5	0.4		
C(4)	178.4	−0.3		
C(5)	108.4	0.0		
CH_3(51)	17.6	−0.7	2.30	−0.15
C(6)	166.4	−0.2		
C(7)	52.9	−0.2		
CH_3(7A)	21.2	−0.6	1.54	−0.16
CH_2(71)	45.0	−0.3	1.41 (*R*), 1.86 (*S*)	−0.31, −0.33
C(72)	177.3	−0.6		
CH(8)	56.6	−0.9	3.09	−0.2
CH_2(81)	28.2	−1.5	0.69 (*S*), 1.69 (*R*)	−0.13, −0.06
CH_2(82)	34.5	0.3	0.69 (*R*), 1.59 (*S*)	−0.19, −0.14
C(83)	180.0	−0.3		
C(9)	172.5	−0.6		
CH(10)	97.2	−0.5	5.70	−0.23
C(11)	178.4	0.8		
C(12)	49.6	0.1		
CH_3(12A)	33.3	9.6	1.13	−0.19
CH_3(12B)	23.3	−10.9	1.28	0.41
CH(13)	55.2	−0.6	3.18	0.29
CH_2(131)	30.2	−0.1	1.60[c]), 1.66[c])	−0.40, −0.56
CH_2(132)	37.3	−0.8	2.34[c], 2.41[c])	−0.20, −0.13
C(133)[d])	180.0	−0.9		
C(14)	166.4	−0.8		
C(15)	107.3	0.4		
CH_3(151)	17.8	−1.1	2.37	−0.06
C(16)	179.3	0.6		
C(17)	60.5	−0.3		
CH_3(17B)	19.9	0.3	1.11	−0.25
CH_2(171)[e])	34.5	−0.1	1.68 (*S*), 2.38 (*R*)	−0.10, 0.60
CH_2(172)[e])	34.1	−0.3	2.04 (*R*), 2.32 (*S*)	−0.02, −0.13
C(173)	178.3	0.1		
CH_2(175)	48.5	0.7	2.96 (*S*), 3.45 (*R*)	−0.20, −0.09
CH(176)	76.7	0.7	4.26	−0.07
CH_3(177)	21.5	−0.2	1.13	−0.10
CH(18)	41.4	−1.1	2.50	−0.15
CH_2(181)	34.4	−0.4	2.50	−0.15
C(182)	178.9	−0.1		
CH(19)	75.8	−1.0	4.25	0.01
CH(1R)	88.6	−0.8	6.14	−0.10
CH(2R)[f])	72.4	0.4	4.25	0.02
CH(3R)	76.5	0.3	4.50	−0.22

Table 1 (continued)

	δ(C)	$\Delta\delta$(C)[b])	δ(H)	$\Delta\delta$(H)[b])
CH(4R)	83.9	– 0.7	3.99	– 0.11
CH_2(5R)	63.0	– 0.4	3.64[c]), 3.83[c])	– 0.10, – 0.05
CH(2N)	145.3	0.6	6.92	– 0.03
CH(4N)	121.0	– 0.4	6.10	– 0.14
C(5N)	134.7	0.2		
C(6N)	136.9	– 0.1		
CH(7N)	113.3	– 0.2	7.05	– 0.05
C(8N)	133.2	– 0.1		
C(9N)	141.0	0.0		
CH_3(10N)	22.2	– 0.3	2.10	– 0.09
CH_3(11N)	22.2	– 0.1	2.10	– 0.09
CH(1RL)	91.7	0.7	5.54	– 0.02
CH(2RL)	75.4	– 0.2	4.37	– 0.17
CH(3RL)	76.5	– 0.1	3.82	0.08
CH(4RL)	88.6	0.0	2.29	– 0.25
CH_2(5RL)	28.0	0.7	0.79 (*R*), 1.71 (*S*)	0.22, 0.16
CH(2L)	155.5	– 0.5	8.09	– 0.1
C(4L)	151.0	– 0.8		
C(5L)	122.1	0.3		
C(6L)	158.0	– 0.7		
CH(8L)	143.5	– 0.3	7.84	– 0.16

[a]) See *Fig. 4* for the numbering scheme used here; (*R*) and (*S*) denote *pro-R* and *pro-S* H-atoms, resp.; the following letter labels are used: R for the ribose unit attached to the dimethylbenzimidazole moiety, N for the dimethylbenzimidazole moiety, RL for the ribose unit of the 5′-deoxy-5′-adenosyl ligand, and L for the adenine unit of the 5′-deoxy-5′-adenosyl ligand. [b]) $\Delta\delta = \delta(\mathbf{5}) - \delta(\mathbf{1})$. [c]) Configurational assignments not available for these diastereotopic H-atoms. [d]) Assignment of C(33) and C(133) may be reversed. [e]) Original assignment [20] or signals for CH_2(171) and CH_2(172), corrected here based on the NOE data reported below. [f]) OH–C(2R) at 5.42 ppm.

Table 2. *^{15}N- and ^{1}H-NMR Chemical Shifts* (in ppm) *of the Amide Functions of Neocoenzyme B_{12}* (**5**) *in H_2O/D_2O 9:1 at 26°*

	δ(N)	δ(H)[a])		δ(N)	δ(H)[a])
NH_2(23)	110.5	7.72 (*trans*), 6.98 (*cis*)	NH_2(134)	104.5	7.52 (*trans*), 6.76 (*cis*)
NH_2(34)	104.2	7.48 (*trans*), 6.76 (*cis*)	NH(174)	111.7	8.11
NH_2(73)	109.0	7.33 (*trans*), 6.62 (*cis*)	NH_2(183)	106.0	7.72 (*trans*), 6.89 (*cis*)
NH_2(84)	101.5	6.16 (*trans*), 6.31 (*cis*)			

[a]) *trans*/*cis* with respect to the carbonyl O-atom.

δ(C) values for CH_3(12A) and CH_3(12B) of **5** as compared to **1** indicates that ring C undergoes a complete conformational inversion upon change of configuration at C(13). Thus, in **5** the propanamide side chain at C(13) adopts an axial and *anti*-periplanar position relative to CH_3(12A) and a *syn*-clinical position relative to CH_3(12B). This assignment is corroborated by the similarity of the δ(C) values for the ring-C atoms C(11) to C(14) and of the side-chain atoms C(131) and C(132) in **1** and **5** (see *Fig. 2*). The propanamide side chain of **5** seems to be conformationally flexible and to have a time-averaged structure with an outward pointing bond between C(131) and C(132).

Qualitative Conformational Analysis of **5** *Based on* ^{1}*H-NMR Chemical Shifts.* Comparison of the δ(H) of neocoenzyme B_{12} (**5**) and of its 13-epimer coenzyme B_{12} (**1**) (see *Table 1*) also reveals a striking inversion of the positions of the CH_3(12A) and CH_3(12B) signals. In this case, as in others to be discussed below, significant anisotropic (shielding and deshielding) effects by close-by unsaturated functionalities have to be considered, in addition to steric effects. Accordingly, the $\Delta\delta$(H) may be diagnostic for the position of the adenine heterocycle and in this way for the conformation of the organometallic group. Indeed, the particularly high-field shift CH_3(12B) (*vs.* that of CH_3(12A)) in the spectra of **1** may be largely due to the shielding effect of the close-by adenine group [15][20].

When comparing the ^{1}H-NMR spectra of **5** and of **1**, significant low-field shifts are only discernible for CH_3(12B) ($\Delta\delta = 0{,}41$ ppm) and for the adjacent H–C(13) ($\Delta\delta = 0.29$ ppm) of **5**. In contrast, the signals of CH_2(71) ($\Delta\delta = -0.33/-0.31$ ppm), CH_2(131) ($\Delta\delta = -0.56/-0.40$ ppm), and CH_3(17B) ($\Delta\delta = -0.25$ ppm) of **5** all occur at significantly higher field than in **1**. Clearly, these $\Delta\delta$(H) values consistently indicate a remarkable time-averaged redistribution of the organometallic group of **5**, compared to the situation in **1**. Apparently, in neocoenzyme B_{12} (**5**), the organometallic adenosyl moiety is bound in a flexible way and spends (part of) its time in conformations where the adenine moiety is not situated above ring C (the major orientation in the coenzyme **1**), but rather close to rings B and D.

2.3. *Conformational Analysis of the Nucleotide Loop.* In the 1D-^{1}H- and ^{13}C-NMR spectra of the 13-epicob(III)alamin **5**, the δ values for the nucleotide loop, as well as some associated vicinal-coupling constants, indicate a high degree of similarity of the loop conformations **5** and in cob(III)alamin **1**. Accordingly, the nucleotide-loop ribose units in both **1** and **5** adapt a 3′-*endo* conformation, as also observed for **1** in the crystal [1][2]. In particular, this is suggested by the size of the H,H coupling constants ^3J(1R,2R) = 3 Hz and ^3J(3R,4R) $\approx$ 9 Hz, indicating dihedral angles of *ca.* $-40°$ and $-170°$ according to a *Karplus* relationship for carbohydrates [21]. Such torsion angles are consistent with the ribose conformation in **1**, as determined from the crystal structure [1].

However, from analysis of some diagnostic cross-peaks in 2D-ROESY spectra, the NOE-derived distance information concerning the conformation of the 'loop bulge' is seen to be inconsistent with the picture given by the crystal structure of coenzyme **1**. As had similarly been noted in this laboratory for the solution spectrum of **1** [22a], of **7** [22b], and of a series of other organometallic cob(III)alamins [22a], strong NOE signals can be observed in the spectrum of **5** between the *f*-amide proton H–N(174) and the ribose protons at C(2R), C(4R), and O(2R) (see *Fig. 5,a*). This indicates that the H-bonded *f*-amide H-atom is oriented towards the inside of the nucleotide 'loop cavity' and towards the hydroxy group OH–C(2R) at the ribose. In the cob(III)alamins (and presumably in the neocob(III)alamin **5**), the deduced loop orientation appears to allow the formation in aqueous solution of a H-bonded H_2O bridge between H–N(174) and OH–C(2R) [22]. The specific configuration at the 13-position (at the periphery) of the corrinato ligand and the associated conformation of ring C only marginally influence the conformation of the nucleotide loop of organometallic base-on cobalamins, such as **1** and **5**.

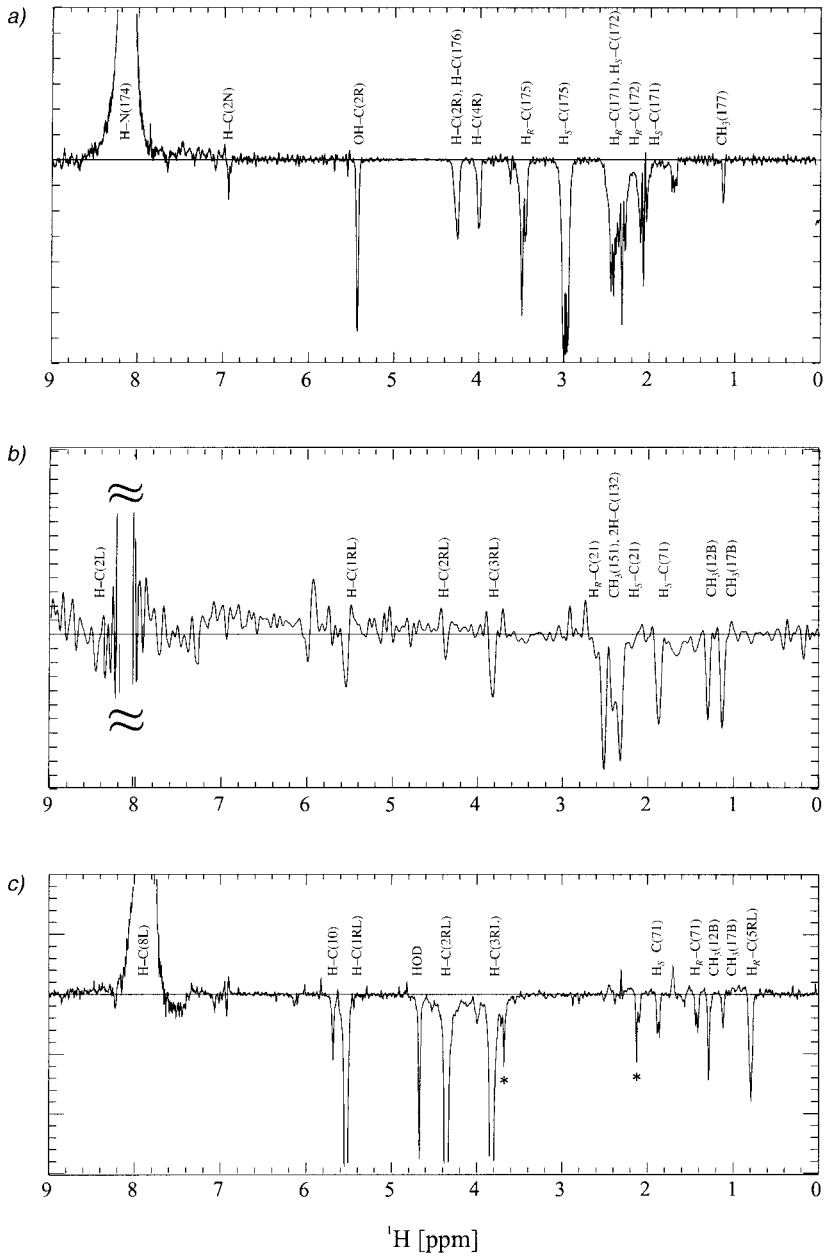


Fig. 5. *Selected one-dimensional cross sections of the 2D ROESY of neocoenzyme B_{12}* (**5**) *taken at the resonance position* a) *of the* f*-amide proton H–N(174)* (in H_2O), b) *of the adenine proton H–C(2L)* (in D_2O), *and* c) *of the adenine proton H–C(8L)* (in D_2O) *of* **5**. The NOE signals are labeled by their proton designations; H_R and H_S are short forms of $H_{pro\text{-}R}$ and $H_{pro\text{-}S}$, resp. The rows were taken in F_2 dimension (*a* and *c*) or in F_1 dimension (b). The stars (*) indicate T_1 noise artifacts.

2.4. *Conformation and Orientation of the Organometallic 5′-Deoxy-5′-adenosyl Moiety. Conformation of the Organometallic Moiety.* As noticed above, the ^{13}C-NMR chemical-shift values of the organometallic-ligand ribose moiety of 13-epicob(III)-alamin **5**, as well as some associated coupling constants ^3J(H,H), indicate the average ribose conformation of **5** to be similar to that in the cob(III)alamin **1**. The sizes of the H,H-coupling constants ^3J(1RL,2RL) = 3 Hz and ^3J(3RL,4RL) $\approx$ 9 Hz in the ribose unit are again consistent with a 3′-*endo* (^3E) conformation, as is also observed in the crystal structures of **1** [1][2] and of other 5′-deoxy-5′-adenosyl analogues of **1** [23][24]. In contrast, some δ(H) of the ribose protons in the spectra of **5** differ noticeably from the corresponding signals of the coenzyme **1**, pointing at significant differences of the conformation of the organometallic group in the stereoisomeric B_{12} derivatives **1** and **5**.

A qualitative description of the main conformation(s) of the nucleoside base of the organometallic group of **5** is derived from distance information, as provided by the 2D-ROESY experiments (see *Figs. 5–7*). The NOEs between protons of the adenine and ribose segments of the 5′-deoxy-5′-adenosyl moiety are particularly informative, but clearly are not compatible with a single major conformation. In the earlier work, the NOE pattern observed (several NOEs between ribose protons and H–C(8L), but none with H–C(2L)) was consistent with the *anti*-conformation of the 5′-deoxy-5′-adenosyl ligand [14], as was known from the structural work with coenzyme B_{12} [1][2][20]. However, in the spectra of **5**, an unusually intensive NOE between H–C(8L) and H–C(1RL) is observed, as well as further NOE contacts of H–C(2L) with H–C(2RL) and H–C(3RL) (see *Figs. 5,b* and *c*, *Fig. 6* and *Table 3*). These contacts are not consistent with the exclusive presence of the established *anti*-conformation, which would place H–C(2L) at a distance of *ca.* 7.5 Å from H–C(3RL) (see, *e.g.*, [1][2]). Together with the absence of an NOE of H–C(2L) with H–C(5RL), our data indicate a significant contribution of a (high) *syn*-conformation [25] of the organometallic ligand in **5**.

The signals of the two diastereotopic H-atoms at C(5RL) at 0.79 and at 1.71 ppm in the spectra of **5** can be assigned due to a strong NOE contact between one of them and H–C(8L). The signal at higher field (see *Fig. 6*) accordingly is assigned as that of $H_{pro\text{-}R}$–C(5RL) (= H_R–C(5RL)), a situation similar to that in the spectra of **1** [20]. This assignment is also supported by the relative size of the ^3J couplings to H–C(4RL) (10 Hz for the upfield and 5 Hz for the downfield resonance).

Orientation of the Organometallic 5′-Deoxy-5′-adenosyl Moiety with Respect to the Corrinato Ligand. Various NOE contacts between protons of the corrinato ligand and of the organometallic group (called 'interresidue' NOEs) help define its orientation (see *Table 4*). Analysis of the derived distance information reveals that the NOEs cannot be explained by a single orientation, a conclusion also reached in [14]. Such contacts with the ribose part of the organometallic unit most directly give information on the major orientation of the latter. NOE Contacts observable for the diastereotopic protons H_R–C(5RL) and H_S–C(5RL) of the methylene group directly attached to the Co-center provide major structural information (see *Fig. 6*): NOEs of H_R–C(5RL) with CH_2(21)(*w*) and CH_2(71)(*m*) are observed and of H_S–C(5RL) with CH_2(21)(*s*), CH_3(17B) (*m*) and H–C(19)(*s*); in contrast, NOEs of H_R–C(5RL) with H–C(19) and CH_3(17B) are absent or are insignificant. The orientation of the organometallic group

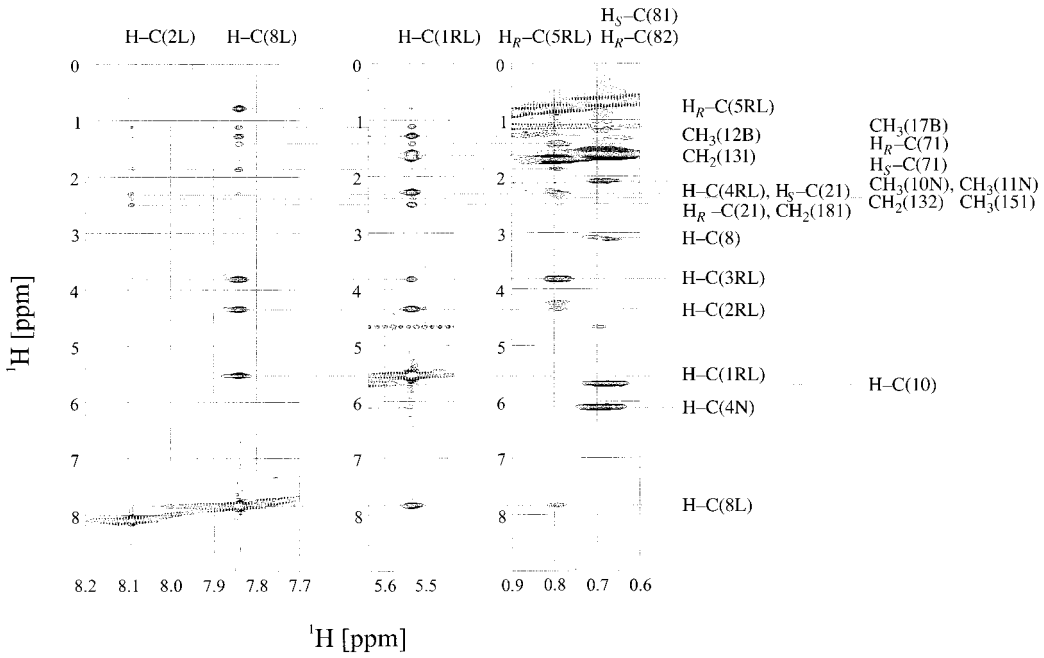


Fig. 6. *Selected sections of a 2D ROESY of neocoenzyme B_{12}* (**5**) *in D_2O solution highlighting various NOE contacts between protons of the deoxyadenosine moiety* (H–C(2L), H–C(8L), H–C(1RL), and H$_R$–C(5RL)) *and protons of the corrin system*. H$_R$ and H$_S$ are short forms of H$_{pro\text{-}R}$ and H$_{pro\text{-}S}$, resp.

can be described by the torsion angle around the organometallic bond (described by N(1)-Co-C(5RL)-C(4RL) [2]). In the major conformations, this torsion angle should not have values from *ca.* 30 to $-120°$. The strongly differentiating NOE contacts of the diastereotopic methylene protons at C(5RL) accordingly exclude major contributions of structures in which the organometallic group is in a northern orientation; this is in striking contrast to the conclusions reached in [14] which were heavily based on molecular-dynamics calculations. Consistent with this and with the other NOE contacts between ribose and corrin protons, the organometallic moiety of neoenzyme B_{12} (**5**) is sampling conformations in which the ribose unit is placed within a sector close to rings C and D and in which C(3RL) and the Co-center are approximately *anti*-periplanar (such as in **1** [1][2][26]).

As concerns the 'interresidue' NOE contacts of H–C(4RL), only those to CH$_3$(17B) are clearly detectable. NOE Contacts of H–C(4RL) with H–C(19) and CH$_2$(71) are likely, but cannot be clearly identified, due to signal overlap. On the other hand, NOE contacts clearly can be detected between H–C(3RL) or H–C(2RL) and several protons of the corrinato ligand. H–C(1RL) also gives rise to NOEs with CH$_2$(21), H$_R$–C(71), CH$_3$(12B), CH$_2$(131) and CH$_3$(17B), *i.e.*, with a complete set of β-substituents on all four rings of the corrinato ligand (see *Figs. 6* and *7*). Signal overlap and the presence of strong NOEs from H–C(1RL) to H–C(4RL) and H–C(2RL) impaired detection of other NOEs between protons of the ribose unit and of the corrinato ligand. Overall, the NOE pattern relating protons of the ribose unit and of the

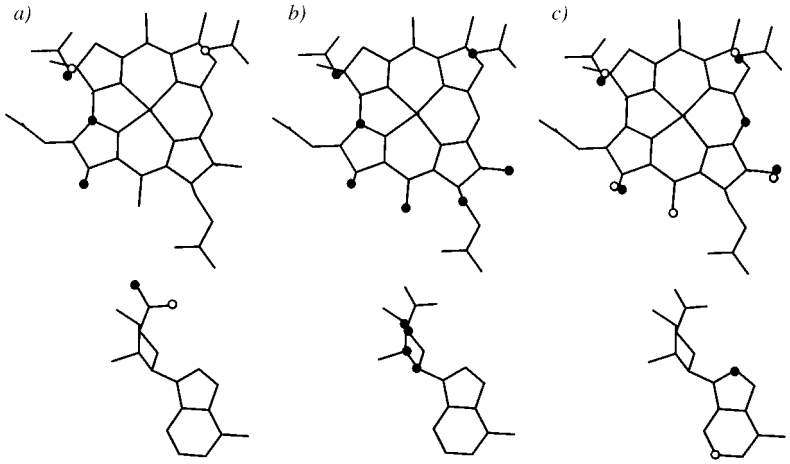


Fig. 7. *'Interresidue' NOE contacts between the upper face of the corrinato ligand and the organometallic deoxyadenosyl group of neocoenzyme B_{12}* (**5**): a) *NOE contacts with ribose protons $H_R-C(5RL)$* (●) *and $H_R-C(5RL)$* (○); b) *NOE contacts with ribose protons H–C(1RL), H–C(2RL), H–C(3LR), H–C(4RL)* (●); c) *NOE contacts with adenine protons H–C(8L)* (●) *and H–C(2L)* (○)

Table 3. *'Intraresidue' NOEs of the Deoxyadenosine Moiety*[a])

	Intensity[b])		Intensity[b])
H–C(1RL)/H–C(2RL)	*m*	H–C(2RL)/H–C(8L)	*m*
H–C(1RL)/H–C(3RL)	*w*	H–C(3RL)/H–C(4RL)	*m*
H–C(1RL)/H–C(4RL)	*m*	H–C(3RL)/H_S–C(5RL))	*m*
H–C(1RL)/H_R–C(5RL)	v*w*	H–C(3RL)/H_R–C(5RL)	*m*
H–C(1RL)/H–C(2L)	v*w*	H–C(3RL)/H–C(2L)	v*w*
H–C(1RL)/H–C(8L)	*w*	H–C(3RL)/H–C(8L)	*m*
H–C(2RL)/H–C(4RL)	*w*	H–C(4RL)/H_S–C(5RL)	*s*
H–C(2RL)/H_S–C(5RL)	*w*	H–C(4RL)/H_R–C(5RL)	*w*
H–C(2RL)/H_R–C(5RL)	*w*	H_R–C(5RL)/H–C(8L)	*w*
H–C(2RL)/H–C(2L)	v*w*		

[a]) H_R and H_S are short forms of $H_{pro\text{-}R}$ and $H_{pro\text{-}S}$, resp. [b]) *s.* 1.8–2.7 Å; *m:* 1.8–3.3 Å; *w:* 1.8–5.0 Å; v*w:* 1.8–6.0 Å.

corrinato ligand in the spectra of **5** (see *Figs. 6* and *7*) is inconsistent with a single major orientation of the organometallic group [14], but indicates a dynamic equilibrium between a restricted range of structures.

In accordance with the picture derived in the previous paragraph, 'interresidue' NOEs of H–C(8L) are clearly observed with H_S–C(21), CH_2(71), CH_3(12B), CH_2(131), and CH_3(17B). For H–C(2L), contacts to the corrin protons H_S–C(71), CH_3(12B), CH_2(132), or CH_3(151) and CH_3(17B) are significant, as are NOEs to both protons H_R–C(21) and H_S–C(71) at the *a*- and *c*-side chains (see *Figs. 5–7*). These NOE features are indicative of the existence of orientations of the organometallic deoxyadenosyl group in which the purine heterocycle is close to the three rings B, C, and D, but less so to ring A (see *Fig. 8*). In addition, they make likely the significant population of an eastern orientation of the organometallic group with a *syn*-

Table 4. *NOE Contacts between the Deoxyadenosine Moiety and the Corrin Ring System*[a])

	Intensity[b])		Intensity[b])
H–C(1RL)/CH_3(12B)	*w*	H_S–C(5RL)/H–C(19)	*s*
H–C(1RL)/CH_3(151)	v*w*[c])	H_S–C(5RL)/H_R–C(21)	*s*[c])
H–C(1RL)/CH_3(17B)	v*w*	H–C(2L)/CH_3(12B)	v*w*
H–C(1RL)/H_R–C(21)	*w*	H–C(2L)/CH_3(151)	v*w*[c])
H–C(1RL)/H_R–C(71)	v*w*	H–C(2L)/CH_3(17B)	v*w*[c])
H–C(1RL)/H_S–C(71)	v*w*	H–C(2L)/H_R–C(21)	v*w*
H–C(1RL)/CH_2(131)	*m*	H–C(2L)/H_S–C(21)	v*w*
H–C(2RL)/CH_3(17B)	v*w*[c])	H–C(2L)/H_S–C(71)	v*w*[c])
H–C(3RL)/H_R–C(71)	*w*	H–C(8L)/H–C(10)	v*w*
H–C(4RL)/CH_3(17B)	*w*	H–C(8L)/CH_3(12B)	v*w*
H–C(4RL)/H–C(19)	*s*[c])[d])	H–C(8L)/CH_3(17B)	v*w*
H_R–C(5RL)/H_R–C(21)	*w*	H–C(8L)/H_S–C(21)	v*w*
H_R–C(5RL)/H_R–C(71)	*w*	H–C(8L)/H_R–C(71)	v*w*
H_R–C(5RL)/H_S–C(71)	*w*	H–C(8L)/H_S–C(71)	*w*
H_S–C(5RL)/CH_3(17B)	*w*[c])		

[a]) H_R and H_S are short forms of $H_{pro\text{-}R}$ and $H_{pro\text{-}S}$, resp. [b]) *s:* 1.8–2.7 Å; *m:* 1.8–3.3 Å; *w:* 1.8–5.0 Å; v*w:* 1.8–6.0 Å. [c]) Not reported in [14]. [d]) Assignment uncertain due to overlap of signals.

conformation of the adenine heterocycle (to rationalize the NOE of H–C(2L) with CH_2(21)).

Force fields for corrinato cobalt complexes are still in an early stage of development [14][27]. From NMR-restrained molecular dynamics (MD) simulations, useful information on the dynamic properties of the solution structures of coenzyme B_{12} (**1**) and of vitamin B_{12} (**2**) has been deduced [28][29]. In conjunction with NMR analyses, MD simulations pointed to a very dynamic behavior of the organometallic ligand of coenzyme B_{12} (**1**) [28] at elevated temperatures and of neocoenzyme B_{12} (**5**) [14]. For these two adenosylcobamides, significantly populated solution conformations were suggested in which the organometallic group is close to one of every major section of the 'upper' hemisphere of the corrin ring system. While unconstrained MD calculations may provide good qualitative insights into the dynamics of vitamin-B_{12} derivatives, they appear to be only of limited use in structure prediction of cobalamins as there still exists a lack of good empirical force fields for organometallic vitamin-B_{12} derivatives [14][27]. The determination of the relevant conformations for organometallic vitamin-B_{12} derivatives, therefore, still depends crucially on the availability of an extensive set of reliable and detailed NMR-derived distance and angle constraints [22].

To describe the solution structure of neocoenzyme B_{12} (**5**), we made use of 127 experimentally based and NOE-derived relevant distance constraints in NMR-restrained MD/SA simulations (SA = simulated annealing), not counting 'trivial' NOEs between geminal groups. Importantly, twenty-nine of these NOEs represent contacts between specific protons of the organometallic and corrin moieties of **5** (see *Table 4*), and nineteen arise from contacts between protons of the adenine and ribose moieties of the organometallic ligand (see *Table 3*). Two sets of 36 starting conformations each were used (at ten-degree intervals), with sampling of all orientations of the deoxyadenosyl ligand with respect to the corrin. In the two sets of starting structures, the adenine heterocycle was either in the *anti*- or in the *syn*-

conformation. In addition, for defining better the orientation of the deoxyadenosine ligand, 'non-contacts' were also taken into account, *i.e.* the inability to observe some NOEs in the ROESY experiment was used to impose a lower limit of 4 Å on various internuclear distances. A total of eight such constraints (between H–C(3) and H–C(2L), H–C(8L), and H–C(1RL), between H–C(8) and H–C(2L) and H–C(8L), and between H–C(19) and H–C(2L), H–C(8L), and H–C(1RL)) were used as distance constraints in the calculations.

To account for the dynamic behavior and the high flexibility of the deoxyadenosine and to allow for multiple conformers, SA protocols using 'time-averaged distance restraints' were employed throughout [30]. This means applying distance restraints to the *time-averaged* position rather than to the *instantaneous* position of the two atoms involved in a distance restraint. This time average is defined over the MD trajectory by a 'retrospective' exponential correlation or 'memory' function. The annealing schedule consisted of 1 ps heating at 500 K and cooling to 100 K in steps of 100 K, followed by a final energy minimization. An integration step of 0.1 fs was used.

Following such a protocol, the starting structures annealed into a set of 72 structures with the organometallic moiety distributed between rings B and D with a preference for conformations in which the deoxyadenosyl ligand gets positioned between side chains (*i.e.*, between the *c*- and *e*-, or *e*- and *g*-chains). The major conformations (see *Fig. 8*) of the deoxyadenosine ligand in neocoenzyme B_{12} position the organometallic group into the section between the so called 'eastern' and 'southwestern' conformations. A 'western' conformation of the organometallic group has already been observed in the crystal structure of dideoxycoenzyme B_{12} [15], the 'eastern' conformation has been proposed to be a relevant contribution to the structure of coenzyme B_{12} (**1**) in aqueous solution [20]. The available simulations do not exclude contributions of a 'coenzyme-like' conformation of the organometallic group, *i.e.*, a 'southeastern' conformation (*Fig. 8*,*d*). Steric clashes between the *e*-chain and the adenine can be avoided because the conformational flexibility of the *e*-chain allows its *syn*-clinal conformation when sterically required.

Most remarkably, the adenine base of the organometallic adenosylcobamide undergoes *syn-anti* flips during the simulated annealing process, to produce *anti*- and *syn*-conformers of the nucleoside from starting structures either in the *syn*- or *anti*-conformation. In the majority of the resulting structures with the unprecedented *syn*-conformers, the organometallic moiety is oriented between rings B and C.

Fig. 8 shows four representative structures that were selected with respect to minimal energy and a minimum of NOE-constraint violations. The structural variability represented in *Fig. 8* and the range of 'interresidue' NOEs experienced between the corrinato ligand and the deoxyadenosyl group (see *Fig. 7*) gives an impression of the dynamics of the adenosyl ligand relative to the corrin moiety and of the conformations accessible to neocoenzyme B_{12} (**5**) under the empirical force field in combination with the experimental restraints. The four selected structures are representative from the bundle of 72 conformations generated by application of the protocol described above and using those NOEs only for final refinement that were compatible with the given conformer.

2.5. *Comparison with Previous Results Reported by* Brown, Chen, *and* Marques [14]. A large part of our results is in agreement with those of the previous study by

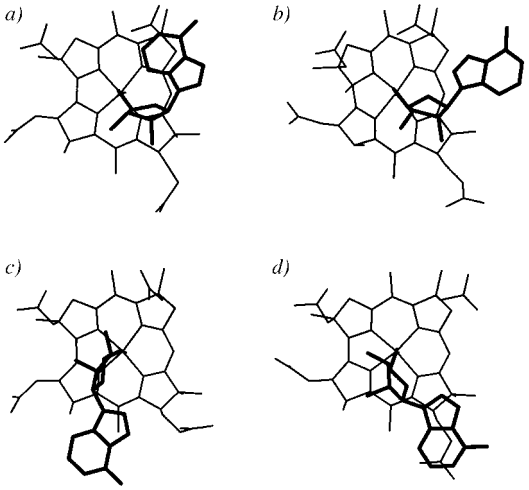


Fig. 8. *Representative models of the solution structure of neocoenzyme B_{12}* (**5**) *generated by NMR-restrained simulated annealing and energy minimization* (see *Exper. Part* for details; only the upper face of the cobamide and the adenine moiety are shown for clarity): a) '*eastern*' syn-*conformation*, b) '*eastern*' anti-*conformation*, c) '*southwestern*' anti-*conformation*; *and* d) *coenzyme-like* '*southeastern*' anti-*conformation of the adenine moiety*

Brown, *Chen*, and *Marques* [14]. Our larger set of experimental data (configurational assignment of diastereotopic methylene protons, additional relevant NOEs) provides a better basis for experimental constraints important in the structural refinement. A major discrepancy between our results and those reported in [14] arises from the existence of the organometallic nucleoside moiety with *syn*-conformation of the adenine base, proposed here and not reported in [14]. In turn, with the adenosine ligand in an 'eastern' *syn*-conformation, the 6-ring of the adenine heterocycle of the organometallic group can be situated close enough to ring A to be compatible with the observed NOE contacts of H–C(2L) with the diastereotopic protons CH_2(21) of the *a*-side chain. Our investigation accordingly does not provide any direct evidence for the presence of 'northern' and/or 'northwestern' conformations of the organometallic ligand, in contrast to what has been proposed in [14]. Such conformers would bring H_R–C(5RL) in close proximity to H–C(19) ($r < 3$ Å), rather than H_S–C(5RL). Nevertheless, corresponding NOEs between H_R–C(5RL) and H–C(19) were neither reported in [14], nor were they clearly observed by us. Furthermore, NOE contacts between H–C(3)/H–C(8) of the corrin and H–C(8L)/H–C(1RL) of the deoxyadenosine moiety could not be observed, but might be expected for most 'northern' conformations. In **5**, the 'western' orientation of the deoxyadenosine ligand in the *anti*-conformation [14] would bring H–C(1RL) and H–C(8L) in close proximity to H–C(19) and also H–C(1RL) close to H–C(3). Again, the corresponding NOE contacts cannot be experimentally observed [14]. Therefore, we conclude that the ribose moiety of the organometallic group should not be positioned close to ring A. Our data rather support the presence of relevant populations of 'southwestern', 'southeastern', and 'eastern' conformations, placing the ribose unit in a section close to rings C and D. Ambiguity in the assignment of a weak NOE between H–C(1RL) and

CH_2(132) does not provide sufficient evidence for suggesting a relevant contribution of the 'classic' 'southern' orientation of the organometallic group in **5**, a conclusion also reached by *Brown*, *Chen*, and *Marques* [14]. However, conformations which lead to positioning of the adenine moiety of the organometallic group close to ring C would be consistent with the high-field shifts observed for the methylene protons at the *e*-side chain (CH_2(131) and CH_2(132)).

3. Discussion. – Neocoenzyme B_{12} (**5**) has been suggested by *Hodgkin* and co-workers [1][9] to be a target of interest in the context of questions concerning the structural basis of the biologically important organometallic reactivity of coenzyme B_{12} (**1**) [4][6]. In more recent times, **5** [11][12][14] and its simple organometallic analogue methyl-13-epicob(III)alamin (**6**) [12] have been prepared by classic routes, and the effect of **5** as cofactor in two coenzyme-B_{12}-catalyzed enzymatic reactions was examined by *Hogenkamp* and co-workers. [13].

Assuming that the redox properties of 13-epicob(III)alamins (such as neovitamin B_{12} (**4**)) would not differ strongly from those of the cob(III)alamins (such as vitamin B_{12} (**2**)) [31], we applied an electrochemical method developed for the preparation of organocob(III)alamins [15] for the synthesis of neocoenzyme B_{12} (**5**) and methyl-13-epicob(III)alamin (**6**). Preparative controlled-potential reduction of the [Co^{III}(corrinato)] **4** gave the Co^{I} complex 13-epicob(I)alamin and alkylation of the latter with 5′-*O*-tosyl adenosine [11] or with MeI led to the formation of **5** in **6** in 89 and 88% yield, respectively, which were characterized by NMR, FAB-MS, UV/VIS, and CD spectra to confirm the proposed structures.

Information of the conformation and orientation of the 5′-deoxy-5′-adenosyl moiety of neocoenzyme B_{12} (**5**) is of specific interest. In this molecule, steric clashes between the adenine moiety and the *e*-chain would result if *i*) the position of the deoxyadenosyl ligand were the same as in coenzyme B_{12} (**1**), and *ii*) the *e*-chain were fully extended (as in neovitamin B_{12} [9][10]). As already pointed out by *Hodgkin* and coworkers [1][9], this scenario would make it likely that either the *e*-chain would not be fully extended in **5** or the adenosyl residue would be repositioned. This question induced us [11] and others [14] to study the structure of **5** in solution by extensive NMR analyses.

Having at hand samples of neocoenzyme B_{12} (**5**), we set out to examine the structural consequences of the configurational inversion at C(13), proposed to result in steric incompatibility of conformations of **5** with minimal strain of the organometallic functionality. As reported above, homonuclear and heteronuclear NMR techniques allowed us to establish a practically complete set of assigned signals for protons, C-atoms, and amide N-atoms of **5** in aqueous solution. In addition to the recently published assignments [14], we succeeded in identifying a large part of the diastereotopic methylene H-atoms present in neocoenzyme B_{12} (**5**), thus providing a sound basis for an extensive analysis of the solution structure of **5**. The latter task was performed by combining the information from chemical-shift data, vicinal coupling constants, and intramolecular NOE contacts by means of molecular-dynamics calculation (NMR-restrained MD/SA molecular modeling) [30][32][33]. The structural properties of neocoenzyme B_{12} (**5**) in aqueous solution derived from the spectral data agree in part with those proposed in [14], but differ remarkably with respect to the crucial conformational behavior of the organometallic adenosyl ligand of **5**.

Important differences between the structure of coenzyme B_{12} (**1**) in the crystal [1][2][26] and in solution [20][22][29] were revealed by NMR spectroscopy and concern the conformation of the nucleotide loop [22] and the mutual orientation of the corrinato ligand and the organometallic function [20][29]: a single major conformation was determined in the crystal [1][2][26], while 2D-NMR spectroscopy of an aqueous solution of **1** revealed the presence of the deoxyadenosyl group in two orientations, differing from each other by a (50°) rotation around the organometallic bond of **1** [20]. More recent work on **1** (in aqueous [22] and in DMSO solution [29]) and of other organometallic cob(III)alamins [10][14][22–24] confirmed the (restricted) conformational flexibility of the organometallic bond of these organocob(III)amides at room temperature. An investigation of the structure of **1** at 60° and in a DMSO solution indicated considerable torsional flexibility at that temperature [29].

As described above, the corrin part of neocoenzyme B_{12} (**5**) differs from that of coenzyme B_{12} (**1**) only in the structure of ring C and its substituents. Compatible with the information from crystal structures of neovitamin B_{12} (**4**) [9][10], the structure of ring C of **5** differs from that of **1** by the configuration at C(13), the center of epimerization, and by an inversion of the ring pucker, which places C(131) of the *e*-propanamide side chain axial and *anti*-periplanar to the CH_3(12A) group [11][14]. In contrast to the prevalence of the *e*-side chain for an extended conformation in neovitamin B_{12} (**4**), both in the crystal [9][10] and in solution [10], in **5** the *e*-propanamide function seems to adopt similar conformations as in the coenzyme **1** itself, including *syn*-clinal conformations with respect to the C–C bond connecting C(13) and C(131).

Various NOE contacts between the deoxyadenosyl moiety and the corrin ring system can be observed in aqueous solution, which help to determine the mutual position of these two major moieties of **5**. Analysis of the available NOE-derived distance constraints reveals that they cannot possibly be fulfilled *simultaneously* by a single conformation, as suggested also in [14]. Rather, a dynamic equilibrium of various conformers which are in fast mutual exchange (on the NMR time scale) has to be proposed. Clearly, the organometallic group of **5** is not adapting a single major orientation, but exists in a set of conformations that rapidly equilibrate. The remarkable lack of a major structural minimum is in contrast to the situation of coenzyme B_{12} (**1**), where one major orientation of the organometallic ligand (corresponding to that in the crystal [1][2]) predominates even in solution [20]. Using NMR-restrained MD simulations and starting from conformations with randomized orientation of the deoxyadenosyl ligand, a set of structures was generated, all of which place the deoxyadenosine group close to rings B, C, and D.

The 'interresidue' NOE contacts of the ribose protons to the corrinato ligand provide the crucial probes to describe the major orientations of the organometallic group (see *Figs. 6* and *8*). All of them are compatible only with conformations that place the ribose moiety in the 'southern' sectors close to rings C and D. One set of these structures is provided by conformations in which the organometallic group is rotated counterclockwise and by about one quadrant around the Co-C(5RL) bond from an orientation as found in the crystal structure of coenzyme B_{12} [1][2]. Such 'eastern' conformations can account for NOEs between protons of the adenine heterocycle and residues at rings B and C [14]. Another set of NOEs suggests relevant 'southern' and

'southwestern' orientations of the organometallic group, such as those between protons of the adenine heterocycle and residues at rings D (and C). In other minor conformations, the deoxyadenosyl moiety may be oriented 'southeastern', similarly to its situation in the crystal structure of coenzyme B_{12} [1][2]. In this case, the *e*-chain has to assume a *syn*-clinal conformation to avoid unfavorable interactions with the deoxyadenosine.

So far, the data mentioned for neocoenzyme B_{12} (**5**) support the prevalence of the well established *anti*-conformation of the deoxyadenosine moiety [14], also observed in coenzyme B_{12} (**1**) [1][2][20][26][29]. However, to provide an explanation for some of the remaining interresidue NOEs within the adenosine group, other conformations of the organometallic ligand have to be invoked, in which the adenine base assumes an unusual *syn*-conformation [25] (with respect to the glycosidic bond). The observed NOEs between H–C(2L) of the 6-ring of the adenine heterocycle and the methine protons at C(3RL) and C(2RL), in particular, are not likely to come about from the crystallographically established *anti*-conformation of the adenosine moiety [1][2][26] for which the distance between H–C(2L) and H–C(3RL) would be close to 8 Å. In the recently determined crystal structure of α-adenosylcob(III)alamin [24], the nucleoside was found to be remarkably disordered and to adopt (in its major conformation) a structure with the adenine ring nearly perpendicular to the corrin ring plane (*i.e.* an *anti*-periplanar conformation, according to [25]). NMR-Restrained MD/SA modeling for **5** indicates the *syn*-conformation of the adenosine group to be particularly likely for the 'eastern' orientation of the organometallic ligand. In conjunction with such an 'eastern' orientation, a high-*syn*-conformation of the adenosine ligand in neocoenzyme B_{12} (**5**) indeed positions the adenine 6-ring close enough to the ring-A sector of the corrinato ligand to be compatible also with the observation of all of the relevant NOEs between these two moieties of **5**.

The organometallic moiety of **5** exhibits an extraordinary conformational mobility, both internally and with respect to the corrin ring. The steric perturbation by the epimerization at C(13) of ring C appears to be significant enough to result in remarkable (clockwise or counterclockwise) conformational switches of the organometallic moiety, with major conformations placing the adenine base either close to ring D or to ring B of the corrinato ligand. The latter orientation of the adenosine group of the base-off/His-on form of enzyme-bound **1** has recently been observed crystallographically in an inactive form of methylmalonyl CoA mutase (Mcm) [34], a coenzyme-B_{12}-dependent enzyme [35]. In substrate-loaded Mcm, enzyme-bound coenzyme B_{12} (**1**) is known to be strongly activated towards homolysis of its Co–C bond; however, according to exploratory kinetic experiments, the tendency of **5** for homolytic dissociation of its Co–C bond appears to differ only insignificantly from that of the coenzyme **1** [36]. Accordingly, the counterclockwise rotation of the organometallic group towards ring B itself is not indicative of a significant weakening of the organometallic bond.

An intriguing structural feature of ring C of the naturally occurring vitamin-B_{12} derivatives is the lack of an acetic acid side chain in an 'upper' (or β) configuration, contrasting with the situation in the other three rings of the corrin ligand. Such a β-acetamide substituent would lead to similar steric interactions with the organometallic moiety in (5′-deoxy-5′ adenosyl) cobamide cofactors as seen here in neocoenzyme B_{12}

(**5**). The absence of a β-acetamide substituent at ring C, therefore, would be expected to have specific functional relevance in (5′-deoxy-5′-adenosyl)cobamides, such as coenzyme B_{12} (**1**). Indeed, this would correlate with the observed reduced cofactor activity of **5** in some (5′-deoxy-5′-adenosyl)cob(III)alamin-dependent enzymes [13].

Conclusions. – In this work, neocoenzyme B_{12} (**5**) and methyl-13-epicobalamin (**6**) were prepared in high yields by electrochemical means. The solution structure of **5** was specifically studied by extensive analyses with the aid of one- and two-dimensional heteronuclear and homonuclear NMR spectroscopy. The spectroscopic data revealed that the stereoisomer **5** of coenzyme B_{12} (**1**) exhibits an extraordinary conformational mobility of the organometallic group. This could be the consequence of the steric competition between the organometallic ligand and the *e*-propanamide substituent at C(13) whose position has been moved from the 'lower' α-side in coenzyme B_{12} (**1**) to the 'upper' β-side in the stereoisomer neocoenzyme B_{12} (**5**). The experiments indicate that the remarkable absence of sterically demanding β-positioned substituents at ring C of the naturally occurring vitamin-B_{12} derivatives might be a relevant structural property of (5′-deoxy-5′-adenosyl)cobamides, such as coenzyme B_{12} (**1**), and might contribute to the noted conformational preference of the organometallic ligand in **1**.

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Experimental Part

1. *Neovitamin B_{12} (=Cyano-13-epicob(III)alamin*; **4**). Neovitamin B_{12} was prepared from vitamin B_{12} (**2**) according to [8], purified by column chromatography (*RP-18*, MeCN/H_2O 1:1), and crystallized from H_2O (15.6% yield).

2. *Electrochemical Syntheses. General*. Electrolysis cell with two compartments separated by a medium-porosity glass frit; electrolyte soln.: 0.1M tetrabutylammonium hexafluorophosphate in MeOH; Hg-pool working electrode (WE), Pt-wire counter electrode, 0.1N calomel electrode (0.1N CE) as reference electrode; potentiostat *Amel 550*. The experiments were carried out in a glove box (*Mecaplex GB-80*, <10 ppm O_2) at r.t. under protection from light.

Methyl-13-epicob(III)alamin (**6**). A soln. of **4** (50 mg, 36.9 μmol) and benzoic acid (5.9 mg, 48 μmol) in electrolyte soln. (4 ml) was placed into the cathodic compartment of the electrolysis cell and reduced at -1.1 V *vs.* 0.1N CE. After *ca.* 5.5 h, when 6.2 C had been consumed, 6.8 μl (55.4 μmol) of MeI were added, and electrolysis was continued for *ca.* 2 more h (total: 7.15 C = 2.0 F/mol). With protection from light, the electrolysis soln. was poured into a separatory funnel containing CH_2Cl_2 (10 ml) and H_2O (5 ml). The aq. phase was extracted with CH_2Cl_2 (3×10 ml) and concentrated at r.t. to *ca.* 1 ml. Addition of acetone (10 ml) and storage overnight in a refrigerator resulted in a red precipitate that was dried under high vacuum: 43.8 mg (88%) of chromatographically pure **6**, analyzed as described below.

Neocoenzyme B_{12} (=(5′-Deoxy-5′-adenosyl)-13-epicob(III)alamin; **5**). As described for **6**, with **4** (50 mg; 36.9 μmol), benzoic acid (5.9 mg, 48 μmol), and electrolyte soln. (4 ml), and after *ca.* 5.5 h (consumption of 7.1 C) with 5′-*O*-tosyladenosine (23.3 mg, 55.4 μmol) (total: 7.75 C = 2.2 F/mol). The resulting red precipitate was dried under high vacuum: 52 mg (89%) of chromatographically pure **5**, analyzed as described below.

3. *Spectroscopy. General.* UV/VIS Spectra: *Hitachi-U2000:* MeOH soln.; λ_{max}(log ε). CD Spectra: *Jasco-J715*; λ_{max} ($\Delta\varepsilon$). ^{1}H- and ^{13}C-NMR Spectra: *Varian Unity 500 plus.* FAB-MS: *Finnigan MAT 95*; positive-ion mode; 3-nitrobenzyl alcohol matrix, Cs gun; *m*/*z* (rel. %).

Methyl-13-epicob(III)alamin (**6**). UV/VIS (MeOH): 529 (4.01), 378 (4.11), 341 (4.19), 318 (4.16), 281 (4.32), 266 (4.34). CD (H_2O): 550 (−16.0), 497 (+4.0), 453 (−10.0), 387 (+50.0), 344 (−52.0), 283 (+100.0), 230 (−50.0). ^{1}H-NMR (500 MHz, D_2O): 0.13 (*s*, 3 H); 0.40 (*s*, 3 H); 0.73–0.88 (*m*, 3 H); 1.09 (*s*, 3 H); 1.10 (*d*, 3 H); 1.25 (*s*, 6 H); 1.28 (*s*, 3 H); 1.32–1.58 (*m*, 3 H); 1.67 (*s*, 3 H); 1.60–1.78 (*m*, 5 H); 1.80–2.00 (*m*, 8 H);

2.11 (*s*, 3 H); 2.12 (*s*, 3 H); 2.02–2.08 (*m*, 2 H); 2.38 (*s*, 3 H); 2.36 (*s*, 3 H); 2.21–2.64 (*m*, 14 H); 2.98 (*m*, 1 H); 3.17 (*m*, 1 H); 3.32 (*s*, 1 H); 3.45 (*d*, 1 H); 3.54 (*m*, 1 H); 3.46 (*m*, 2 H); 3.91 (*d*, 2 H); 3.96–4.0 (*m*, 2 H); 4.24 (*m*, 2 H); 4.48–4.76 (*m*, 15 H); 5.68 (*s*, 1 H); 6.17 (*d*, 1 H); 6.20 (*s*, 1 H); 6.98 (*s*, 1 H); 7.09 (*s*, 1 H). FAB-MS: 1367.1 (15, $[M+Na]^+$), 1347.1 (22), 1346.2 (56), 1345.1 (100, $[M+H]^+$), 1332.1 (13), 1331.2 (47), 1330.1 (66, $[M+H^+-CH_3]^+$).

Neocoenzyme B_{12} (= 5′-Deoxy-5′-adenosyl)-13-epicob(III)alamin; **5**): UV/VIS (MeOH): 529 (3.84), 378 (3.82), 336 (3.92), 317 (3.90), 257 (4.38). CD (MeOH): 548 (– 17.1), 467 (+ 4.9), 455 (– 0.5), 385 (+ 30.5), 342 (– 34.2), 294 (+ 24.4), 267 (+ 100.0), 230 (– 50.0). FAB-MS: 1582.1 (16), 1581.1 (41), 1580.2 (59, $[M+H]^+$), 1332.1 (28), 1331.1 (55), 1330.12 (100, $[M+H-$adenosyl$]^+$), *etc.* ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz; both in D_2O or H_2O/D_2O 9 : 1): *Tables 1* and *2*.

4. *2D-NMR Experiments. General.* Abbreviations: RF, radiofrequency; CW, continuous wave; NOE, nuclear *Overhauser* effect; TOCSY, total correlation spectroscopy; ROESY, rotating-frame NOE spectroscopy; ge-HSQC, gradient-enhanced heteronuclear single-quantum correlation; ge-HMBC, gradient-enhanced heteronuclear multiple-bond correlation; GARP, globally optimized alternating-phase rectangular pulses. All spectra were performed on a *Varian Unity 500 plus* spectrometer (operating at 499.89 MHz for ^{1}H, 125.15 MHz for ^{13}C, and 50.60 MHz for ^{15}N), equipped with a 5 mm broad-band inverse detection probe and pulsed-field of gradient facilities with 10–20 mM solns. of **5** in either D_2O or H_2O at 26°. The δ(H) were referenced to residual HOD (δ 4.67) and the δ(C) to external $SiMe_4$ ($CDCl_3$: δ 0).

2D-TOCSY (D_2O or H_2O) [37]. Time-domain data size 256 × 1024 (complex); zero-filled to 512 × 2048; 16 scans per t_1 increment; mixing time 75 ms (MLEV-17 [38][39]; bracketed by 2-ms trim pulses, RF power 15 kHz); squared cosine bell functions in both t_1 and t_2 dimensions; *States* quadrature in F_1 [40].

2D ROESY (D_2O or H_2O) [41][42]. Time domain data size 256 × 1024 (complex); zero-filled to 512 × 2048; 16 scans per t_1 increment; mixing time 200 ms (CW spin lock, RF power 2.5 kHz); H_2O suppression with watergate echo [43]; squared cosine bell functions in both t_1 and t_2 dimensions; *States* quadrature in F_1 [40].

^{13}C 2D ge-HSQC [44][45]. Time domain size 256 × 1024 (complex); zero-filled to 512 × 2048; 16 scans per t_1 increment; a pair of *z*-gradients (20 G/cm 2 ms and 20 G/cm 0.5 ms) was used for coherence selection; GARP decoupling [46] during acquisition (RF power 3 kHz); squared shifted sine bell functions in both t_1 and t_2 dimensions.

^{13}C 2D HSQC-TOCSY (proton-relayed C,H-correlation spectroscopy) [47]. Time-domain size 128 × 1024 (complex); zero-filled to 512 × 2048; 32 scans per t_1 increment; mixing time 75 ms (MLEV-17 [38][39]; bracketed by 2-ms trim pulses, RF power 15 kHz); GARP decoupling during acquisition [46] (RF power 3 kHz); squared shifted sine bell functions in both t_1 and t_2 dimensions; *States* quadrature in F_1 [40].

^{13}C 2D ge-HMBC [44][48]. Time-domain size 256 × 1024; 256 scans per t_1 increment; magnitude mode spectra were obtained by a HMQC pulse sequence with an additional low-pass filter *z*-gradients (2 × 10 G/cm 2 ms and 5 G/cm 2 ms) were used for coherence selection and suppression of axial peaks; no decoupling during acquisition; squared sine bell functions in both t_1 and t_2 dimensions.

^{15}N 2D ge-HSQC (H_2O) [49]. Time-domain size 128 × 1024 (complex); zero-filled to 512 × 2048; 128 scans per t_1 increment; a pair of *z*-gradients (30 G/cm 2.5 ms and 15 G/cm 0.5 ms) was used for coherence selection and solvent suppression; GARP decoupling [46] (RF power 1 kHz) during acquisition; squared shifted sine bell functions in both t_1 and t_2 dimensions.

5. *Molecular Modeling and Structure Calculations.* Abbreviations: SA, simulated annealing; MD, molecular dynamics. Distance constraints were derived from 2D ROESY experiments with solns. of **5** in H_2O or D_2O. Due to internal mobility and RF offset effects, NOEs were conservatively classified as strong (*s*; 1.8–2.7 Å), medium (*m*; 1.8–3.3 Å), weak (*w*; 1.8–5 Å), and very weak (v*w*; 1.8–6 Å). Model building and exploratory molecular-mechanics calculations were performed with SYBYL software (Version 6.3A) (*Tripos* [32]) using the forcefield provided by *Tripos*. An initial model was built from the crystal-structure data of neovitamin B_{12} (**4**) [9] by replacement of the cyano ligand by a 5′-deoxy-5′-adenosyl group using a structure of the 5′-deoxyadenosine moiety and the Co–C bond length of the crystal structure of coenzyme B_{12} (**1**) [1][2].

More in-depth MD/SA simulations were performed with the program X-PLOR (Version 3.851) [33]. The necessary dictionary and parameter files for corrins were generated with the 'autodic' option of the xplo2d package [50]. To account for the conformational flexibility, especially of the 5′-deoxy-5′-adenosyl moiety, a MD refinement protocol with time-averaged distance restraints was used throughout [30]. In one run, 36 starting structures were generated by rotating the deoxyadenosyl group of the initial model around the Co–C(5RL) bond in increments of 10°. Then an analogous simulation experiment was carried out having a *syn*-conformation of the adenine as starting structure. The SA refinement protocol consisted of high-temp. dynamics at 500 K followed by slow cooling to 100 K and final energy minimization.

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