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The Cofactor of Tetrachloroethene Reductive Dehalogenase of *Dehalospirillum multivorans* Is Norpseudo-B_{12}, a New Type of a Natural Corrinoid

by **Bernhard Kräutler***, **Wolfgang Fieber**[1]), **Sigrid Ostermann**, **Mario Fasching**[2]), and **Karl-Hans Ongania**

Institute of Organic Chemistry, University of Innsbruck, Innrain 52a, A-6020 Innsbruck
(phone: 0043-512-507-5200; e-mail: bernhard.kraeutler@uibk.ac.at)

and

Karl Gruber, **Christoph Kratky***, and **Christian Mikl**

Institute of Chemistry, University of Graz, Heinrichstrasse 28, A-8010 Graz

and

Anke Siebert and **Gabriele Diekert***

Institute of Microbiology, Department of Applied & Environmental Microbiology, University of Jena, Philosophenweg 12, D-07743 Jena

Dedicated to Professor *Duilio Arigoni* on the occasion of his 75th birthday

The corrinoid cofactor of the tetrachloroethene reductive dehalogenase of *Dehalospirillum multivorans* was isolated in its Coβ-cyano form. This cofactor represents the main corrinoid found in *D. multivorans* cells. Analysis of the isolated cyano-corrinoid by a combination of HPLC and UV/VIS-absorbance spectroscopy revealed it to be nonidentical to a variety of known natural B_{12} derivatives. From high-resolution mass-spectrometric analysis, the molecular formula of the corrinoid isolated from *D. multivorans* could be deduced as $C_{58}H_{81}CoN_{17}O_{14}P$. The sample of the novel corrinoid from *D. multivorans* was further analyzed by UV/VIS, CD, and one- and two-dimensional ^{1}H-, ^{13}C-, and ^{15}N-NMR spectroscopy, which indicated its structure to be closely related to that of pseudovitamin B_{12} (Coβ-cyano-7″-adeninylcobamide). By the same means, the corrinoid could be shown to differ from pseudovitamin B_{12} only by the lack of the methyl group attached to carbon 176, and, therefore, it was named norpseudovitamin B_{12} (or, more precisely, 176-norpseudovitamin B_{12}). Norpseudovitamin B_{12} represents the first example of a 'complete' B_{12}-cofactor that lacks one of the methyl groups of the cobamide moiety, indicating that the B_{12}-biosynthetic pathway in *D. multivorans* differs from that of other organisms. X-Ray crystal-structures were determined for norpseudovitamin B_{12} from *D. multivorans* and the analogues pseudovitamin B_{12} and factor A (Coβ-cyano-7″-[2-methyl]adeninylcobamide). These first accurate crystal structures of complete corrinoids with an adeninyl pseudonucleotide confirmed the expected coordination properties around Co and corroborated the close conformational similarity of the nucleotide moieties of norpseudovitamin B_{12} and its two homologues.

1. Introduction. – The build-up of the corrin moiety of the naturally occurring corrinoids known so far [1][2] is characterized by complete structural integrity and conservation, a feature of the catalytic moieties of essential cofactors [3]. However, the natural corrinoid cofactors differ from vitamin B_{12} (**1**; cyanocobalamin) in the nature of the Co-bound (organometallic) axial ligand, which depends on the particular cofactor function. In addition, the natural 'complete' corrinoids may also vary the pseudonu-

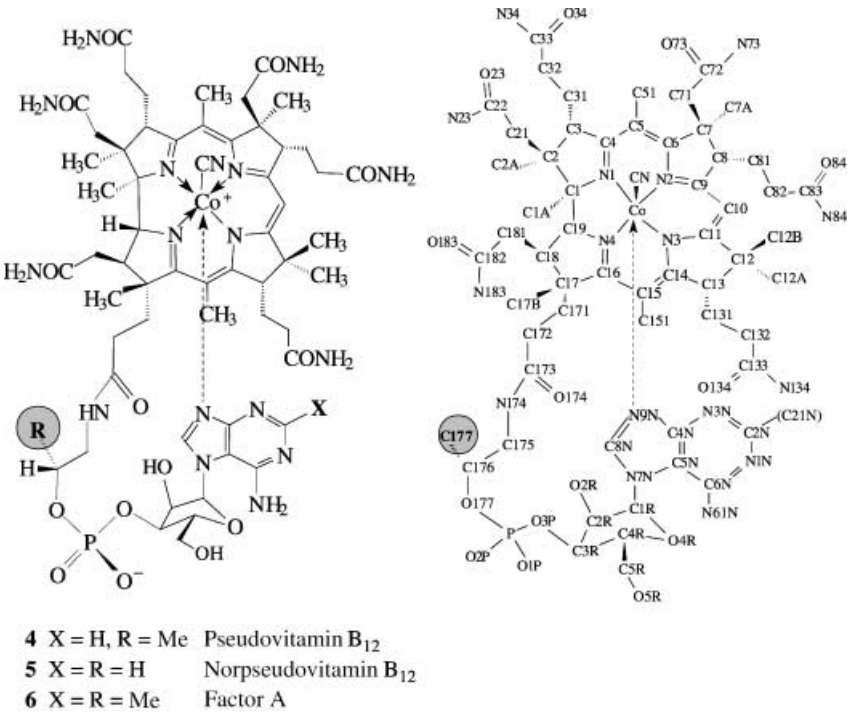
[1]) Present address: Institute of Molecular Biology, University of Copenhagen, Denmark
[2]) Present address: *Lenzing AG*, Lenzing, Austria

cleotide 'base', *e.g.*, an adenine in pseudocoenzyme B_{12} (**2**; Coβ-5′-deoxy-5′-adenosyl-7″-adeninylcobamide), but a 5,6-dimethylbenzimidazole in coenzyme B_{12} (**3**; Coβ-5′-deoxy-5′-adenosylcobalamin) [1][2]. The respective and limited biosynthetic capacities of the microorganisms from which the corrinoids originate have been suggested as the main source of this structural variety [4]. However, the structural differences may also have a functional component, as the pseudonucleotide base may have either the coordinative capacity to give 'base-on' forms of these 'complete' corrinoids in solution (*e.g.*, in coenzyme B_{12} (**3**)) or to result in 'base-off' forms otherwise, as in pseudocoenzyme B_{12} (**2**) or in the 'complete' corrinoids from the acetogen *Sporomusa ovata* [1][4]. The recent discovery of 'base-off' and 'base-on' forms of protein-bound corrinoid cofactors [5] has added flavor to the natural availability of the 'complete' corrinoids in both 'base-on' and 'base-off' forms.

In recent years, several anaerobic bacteria that couple the reductive dehalogenation of tetrachloroethene and other chlorinated substrates to energy conservation (dehalorespiration), thereby offering interesting biological means to detoxify ubiquitous environmental halo(hydro)carbon pollutants, have been isolated [6–8]. Almost all of the reductive dehalogenases that mediate dehalogenation reactions contain a corrinoid as cofactor, as well as two Fe–S clusters. The tetrachloroethene reductive dehalogenase (PCE dehalogenase) of *Dehalospirillum multivorans* has been isolated and characterized [9]. This enzyme uses a corrinoid cofactor to catalyze the reductive dehalogenation of tetrachloroethene and trichloroethene to (*Z*)-1,2-dichloroethene with exceptionally high specific activities [9] and is also able to dechlorinate chlorinated propenes [10]. It has been indicated that the reduction of tetrachloroethene with reduced corrinoids occurs by a radical reaction involving redox changes to the B_{12} derivative [7][11][12]. The protein-free corrinoid cofactor from PCE dehalogenase is also able to abiotically dehalogenate a variety of halogenated compounds such as CCl_3COOH (TCA) with reduced methyl viologen (and titanium(III) citrate) as artificial electron donors [10]. The rate of this abiotic dechlorination differs among structurally diverse, complete corrinoids. Dechlorination of trichloroacetate by the corrinoid cofactor of the PCE dehalogenase of *D. multivorans* is *ca.* 50 times faster than the corresponding reaction with vitamin B_{12}, and *ca.* three times faster than with pseudovitamin B_{12} (**4**; Coβ-cyano-7″-adeninylcobamide) [10]. The cyano form of the B_{12} cofactor of PCE dehalogenase showed UV/VIS-spectroscopic properties of a 'complete' corrinoid, but had chromatographic properties differing from those of the known B_{12} derivatives [13].

We report here the isolation and the structural properties of the cyano-Co^{III} form of the main corrinoid from *D. multivorans*, a homologue of **4** named *norpseudovitamin* B_{12} (**5**; Coβ-cyano-7″-adeninyl-176-norcobamide or 176-norpseudovitamin B_{12}; see *Fig. 1*). This novel corrinoid is the first example of a naturally occurring 'complete' B_{12} cofactor lacking a characteristic peripheral Me group of the cobamide ligand.

2. Results. – *Isolation of Norpseudovitamin* B_{12}. HPLC Analysis of cyanide extracts obtained from cell extracts of *D. multivorans* revealed the presence of four corrinoid fractions at retention times of 22.5, 25, 28, and 30 min, under the experimental conditions described in the *Exper. Part*. According to the peak areas at 360 and 545 nm, *ca.* two-thirds of the total corrinoids eluted at 22.5 min. This fraction was identified by



4 X = H, R = Me Pseudovitamin B_{12}
5 X = R = H Norpseudovitamin B_{12}
6 X = R = Me Factor A

Fig. 1. *Structural formulae* (left) *of the vitamin B_{12} derivatives pseudovitamin B_{12}* (**4**; Coβ-cyano-7″-adeninylcobamide), *norpseudovitamin B_{12}* (**5**; Coβ-cyano-7″-adeninyl-176-norcobamide), *and factor A* (**6**; Coβ-cyano-7″-[2-methyl]adeninylcobamide), *with atom numbering* (right) [14].

reversed-phase high-performance liquid chromatography (RP-HPLC) with the cofactor extracted from the purified PCE reductive dehalogenase [13]. Moreover, the abiotic trichloroacetate dechlorination rate [10] was the same as that for cofactor isolated from the purified enzyme. The major corrinoid from *D. multivorans* (from the fraction eluting at a retention time of 22.5 min) thus represents the corrinoid cofactor of the PCE reductive dehalogenase.

Absorption Spectra, ε, Mass, Midpoint Potentials. The UV/VIS and CD spectra of an aqueous solution of the oxidized Coβ-cyano form of the PCE dehalogenase cofactor, recorded at room temperature, differed only insignificantly from the corresponding spectra of **4** [15]. However, comparison of the chromatographic retention behavior by HPLC of **4** and of the corrinoid from *D. multivorans* indicated the latter to be more polar [13]. The ε values were determined by measuring the absorptions at 360, 518, and 548 nm of cofactor solutions, for which the Co content was determined as described in the *Exper. Part*. From the linear regression of the data depicted in *Fig. 2*, extinction coefficients (mM^{-1} cm^{-1}) of 23.21 (361 nm), 6.66 (518 nm), and 6.76 (548 nm) were calculated.

The electrochemical midpoint potentials of the CN-Co^{III}/Co^{II} and Co^{II}/Co^{I} redox couples of the corrinoid from *D. multivorans* were determined by UV/VIS potentiometry (see *Fig. 3*) and were compared with those obtained under the same conditions

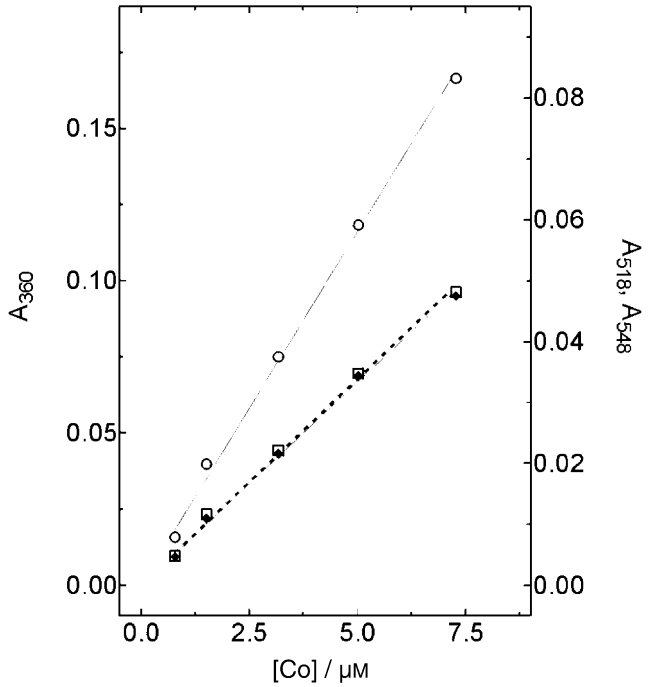


Fig. 2. *Determination of the molar extinction coefficients* (ε_{360}: ○, ε_{518}: ◆, and ε_{548}: □) *of aqueous solutions* (pH 7, 23°) *of norpseudovitamin* B_{12}. The Co concentrations were determined by ICP-AES as described in the *Exper. Part*.

for solutions of **4**. At pH 7, the midpoint potentials for the Co^{II}/Co^{I} couples of **5** and of **4** were estimated to be -0.49 V and -0.48 (± 0.01) V (*vs.* standard hydrogen electrode (SHE)), respectively. Under the same experimental conditions, the effective midpoint potentials for the CN-Co^{III}/Co^{II} couples of **5** and of **4** were calculated to be -0.14 V and -0.01 V (*vs.* SHE), respectively. The concentration of CN^- ions was not specified by the experimental conditions, but can be estimated from the original concentration of the cyano-corrinoid (35 μM) and the pK_a of 9.31 for HCN [16] to be roughly 0.1 μM at 50% reduction. At this concentration of CN^- ions, the electrochemical half-wave potential for the redox pair cyano-cob(III)alamin/cob(II)alamin would be more negative, *ca.* -0.23 V [17][18]. The UV/VIS absorption spectrum of the partially reduced solution (equilibrated at -0.273 V *vs.* SHE) confirmed the presence of the Co^{II} corrinoid at this potential. Comparison with the corresponding pH-dependent absorption spectra of cob(II)alamin [17] indicates the 7′-adeninyl-176-norcob(II)-amide exists predominantly in the 'base-off' form in buffered aqueous solution, at pH 7.0 (see *Fig. 4*).

A fast-atom-bombardment (FAB) mass spectrum of the corrinoid isolated from *D. multivorans* exhibited a base peak at *m*/*z* 1330.5, due to the intact pseudo-molecular ion $[M+H]^+$, and signals of a fragment ion at *m*/*z* 1304.4 due to the loss of the CN ligand. High-resolution mass analysis of the $[M+H]^+$ ion gave *m*/*z* 1330.5092, corresponding to $C_{58}H_{82}CoN_{17}O_{14}P$ (calc. 1330.5296). These data established the molecular formula

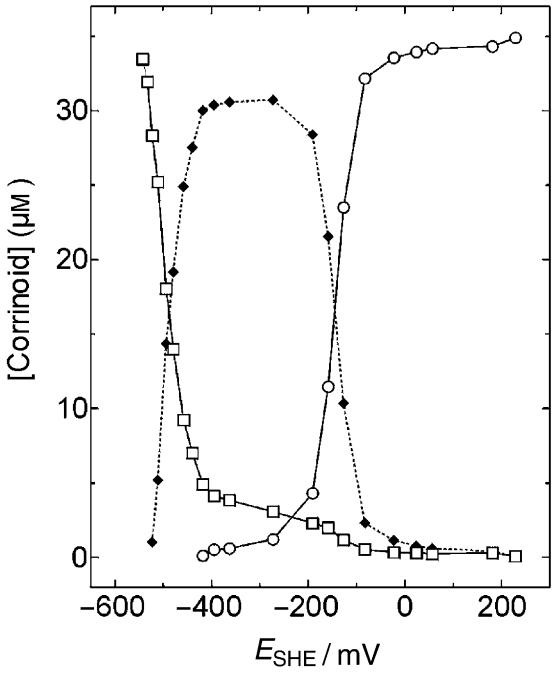


Fig. 3. *Determination of the midpoint potentials at pH 7 of the Co^{III}/Co^{II} and Co^{II}/Co^{I} redox couples of norpseudovitamin B_{12}* (**5**). The total corrinoid content was 35 μM in 100 mM Tris-HCl buffer. Reductive titration was performed by stepwise addition of titanium(III) citrate solution. The potentials are given *vs.* SHE. After each addition of titanium(III) citrate, the absorption spectrum was recorded. The corrinoid concentrations were calculated from the absorption at 360 nm (○) for the Co^{III} form, 473 nm (◆) for the Co^{II} form, and at 386 nm (□) for the Co^{I} form (see *Fig. 2*).

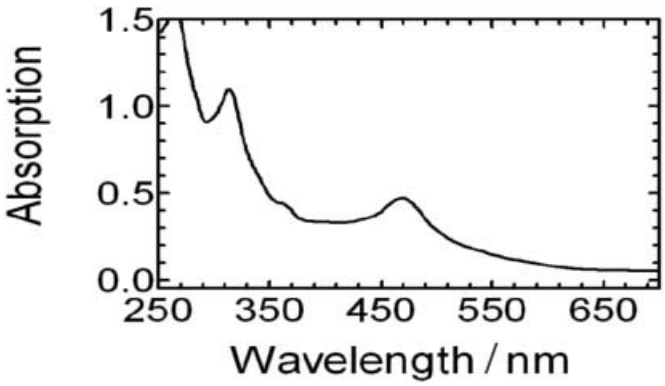


Fig. 4. *Absorption spectrum of the norpseudovitamin B_{12}* (**5**) *Co^{II} form at a redox potential of −273 mV and pH 7.0* (experimental conditions as described in *Fig. 3*)

$C_{58}H_{81}CoN_{17}O_{14}P$ and indicated the mass-spectrometric signal obtained earlier by MALDI-TOF mass spectrometry (at *m/z* 1305.5) [13] to correspond to a fragment ion from loss of the CN ligand. Therefore, a molecule of the corrinoid from *D. multivorans* was indicated to contain one C- and two H-atoms fewer than pseudovitamin B_{12} (**4**).

Small crystals of the CN form of the B_{12} cofactor from *D. multivorans* were obtained by slow crystallization from H_2O by addition of acetone. Under application of modern 1D and 2D homo- and heteronuclear NMR experiments and techniques for H_2O suppression [19], the corrinoid sample from *D. multivorans* could be identified as 176-norpseudovitamin B_{12} (*i.e.*, as Coβ-cyano-7″-adeninyl-176-norcobamide; **5**). Signals of all H-atoms, except for those of the exchange-labile OH groups of the ribose unit (HO−C(2R), HO−C(5R), see *Fig. 1*) and NH_2 groups of the adenine moiety (H_2N(61N)) could be detected. The signals of all the amide H-atoms and of all diastereotopic CH_2 H-atoms, respectively, except for those at C(176) and C(5R), could be individually assigned from analysis of NOE-derived distance constraints, and homo- and heteronuclear correlations. Complete assignment of ^1H and ^{13}C signals was obtained (except for C(3R) and C(4N), because of spectral overlap or weak signal intensity; see *Tables 1* and *2*) from 2D gradient-enhanced heteronuclear experiments (PFG-HSQC [20][21], PFG-HMBC [20][22]), and homonuclear experiments, such as total correlation spectroscopy (WATERGATE-TOCSY [23][24]) and rotating-frame *Overhauser*-enhancement spectroscopy (WATERGATE-ROESY [25][26]). ^1H,^{15}N-PFG-HSQC Experiments [20][21] were carried out to achieve complete assignment of the amide H-atoms and to obtain the chemical-shift data of the amide N-atoms.

With the exception of resonances originating from the constitutionally different side-chain section, the ^1H and ^{13}C chemical shifts of corrinoid **5**, from *D. multivorans* and of **4** differed only slightly. In the ^1H-NMR spectra of the two corrinoids, the signals of all Me H-atoms were found at nearly identical positions. However, in the spectrum of **5**, a new signal of a CH_2 H-atom CH(176) appeared at lower field, while the *doublet* of Me(177) group of **4** was missing (see *Fig. 5*). In comparison with the NMR spectra of **4**, the largest ^1H-chemical-shift differences for the corrinoid from *D. multivorans* were observed for some of the H-atoms bound to N(174), C(175), and C(176), with $\Delta\delta =$ 0.10, 0.31, and 0.33 ppm, respectively. Smaller shift differences were observed for H-atoms bound to C(171), C(172), C(3R), C(4R), and C(8N), all other signals matching to better than 0.04 ppm. The largest ^{13}C-chemical-shift differences, likewise, were observed for C(175) and C(176), with $\Delta\delta = 5.1$ and 7.6 ppm, respectively; a smaller shift difference ($\Delta\delta = 1.2$ ppm) was found for C(8N), while all other ^{13}C signals matched better than 0.5 ppm. The ^1H,^{15}N-PFG-HSQC spectra of **5** in combination with WATERGATE-ROESY spectra allowed assignment of all seven side-chain amide N-atoms, together with their directly bonded amide H-atoms (see *Table 2*).

The low-field ^1H signal of each amide group was assigned to H_E, based on the ROESY cross-peaks displayed between the amide H-atoms and side-chain CH_2 H-atoms (as observed earlier [19][27]). Analysis of the chemical shifts of the ^1H, ^{15}N, and ^{13}C signals clearly confirmed the constitution and 'base-on' nature of norpseudovitamin B_{12} (**5**).

Analysis of the ROESY spectra corroborated these findings. In the spectrum of **5**, all NOE contacts observed in the spectrum of **4** could also be found, except for those between HC(175), HC(176), and Me(177). Instead of these, a set of new NOEs with

Table 1. *Signal Assignments in ^{1}H- and ^{13}C-NMR Spectra of Norpseudovitamin B_{12}* (**5**) *and Comparison of the Chemical-Shift Data with Those of Pseudovitamin B_{12}* (**4**). See *Fig. 1* for numbering. δ in ppm.

	Norpseudovitamin B_{12} (**5**)[a])		Pseudovitamin B_{12} (**4**)[a])[b])	
	^{1}H	^{13}C	^{1}H	^{13}C
C(1)		87.9		87.9
Me(1A)	0.44	22.4	0.42	22.2
C(2)		50.4		50.4
2 H–C(21)	2.36	45.9	2.36	45.9
C(22)		179.4		179.5
Me(2A)	1.38	19.9	1.38	19.8
H–C(3)	4.03	59.3	4.03	59.5
2 H–C(31)	1.96 (H_S), 2.08 (H_R)	28.5	1.95 (H_S), 2.07 (H_R)	28.5
2 H–C(32)	2.49	38.1	2.49	38.2
C(33)		182.0		182.1
C(4)		183.5		183.2
C(5)		111.8		111.7
Me(51)	2.41	18.9	2.40	18.9
C(6)		166.9		167.6
C(7)		53.9		53.8
2 H–C(71)	2.16 (H_R), 2.52 (H_S)	46.4	2.17 (H_R), 2.53 (H_S)	46.5
C(72)		178.7		179.4
Me(7A)	1.75	22.1	1.75	22.1
H–C(8)	3.35	59.3	3.34	59.5
2 H–C(81)	0.94 (H_R), 1.88 (H_S)	29.0	0.96 (H_R), 1.87 (H_S)	29.1
2 H–C(82)	1.31 (H_R), 1.80 (H_S)	35.1	1.31 (H_R), 1.81 (H_S)	35.1
C(83)		182.1		181.9
C(9)		176.8		176.9
H–C(10)	6.04	97.3	6.03	97.3
C(11)		178.9		179.3
C(12)		51.1		51.0
Me(12A)	1.47	22.3	1.47	22.0
Me(12B)	1.14	34.4	1.15	34.4
H–C(13)	3.29	57.1	3.29	57.1
2 H–C(131)	1.96	31.2	1.96	31.3
2 H–C(132)	2.63	37.8	2.63	37.8
C(133)		181.3		182.1
C(14)		169.6		169.6
C(15)		105.9		105.9
Me(151)	2.52	18.1	2.53	18.1
C(16)		181.7		181.8
C(17)		62.5		62.4
Me(17B)	1.35	19.4	1.34	19.6
H–C(18)	2.72	42.0	2.73	42.0
2 H–C(181)	2.72, 2.66	34.9	2.71, 2.66	34.9
C(182)		179.1		179.5
H–C(19)	4.06	78.3	4.06	78.4
2 H–C(171)	2.58 (H_R), 1.81 (H_S)	35.4	2.60 (H_R), 1.77 (H_S)	36.0
2 H–C(172)	2.06 (H_R), 2.43 (H_S)	35.7	2.01 (H_R), 2.46 (H_S)	35.7
C(173)		178.0		178.3
2 H–C(175)	3.18 (H_S), 3.64 (H_R)	43.6	2.87 (H_S), 3.57 (H_R)	48.7
(2) H–C(176)	3.92 (H_a), 4.00 (H_b)	68.5	4.25	76.1
Me(177)			1.14	22.0
H–C(1R)	6.47	91.9	6.49	92.2
H–C(2R)	4.27	73.4	4.23	73.3

Table 1 (cont.)

	Norpseudovitamin B_{12} (**5**)[a])		Pseudovitamin B_{12} (**4**)[a])[b])	
	^1H	^{13}C	^1H	^{13}C
H–C(3R)	4.70		4.66	75.7
H–C(4R)	4.06	85.6	4.00	85.8
2 H–C(5R)	3.74, 3.91	63.3	3.71, 3.90	63.3
H–C(2N)	8.09	156.0	8.09	156.6
C(4N)				113.1
C(5N)		160.0		160.7
C(6N)		154.9		155.1
H–C(8N)	7.20	146.5	7.15	147.7

[a]) ^1H and ^{13}C signals were referenced on external 2,2-dimethyl-2-silapentane-5-sulfonate sodium salt (DSS). H_R and H_S denote pro-*R* and pro-*S* H-atoms, respectively. [b]) Data from *Hoffmann et al.* [15].

Table 2. *^1H and ^{15}N Chemical Shifts of Norpseudovitamin B_{12}* (**5**) *and Pseudovitamin B_{12}* (**4**). See *Fig. 1* for numbering. δ in ppm.

	Norpseudovitamin B_{12} (**5**)[a])		Pseudovitamin B_{12} (**4**)[a])[b])	
	^1H	^{15}N	^1H	^{15}N
N(23)	7.05 (H_E), 7.75 (H_Z)	111.1	7.05 (H_E), 7.75 (H_Z)	110.8
N(34)	6.88 (H_E), 7.57 (H_Z)	104.9	6.88 (H_E), 7.56 (H_Z)	104.7
N(73)	6.93 (H_E), 7.36 (H_Z)	109.7	6.93 (H_E), 7.36 (H_Z)	109.5
N(84)	6.46 (H_E), 6.96 (H_Z)	102.9	6.45 (H_E), 6.94 (H_Z)	102.6
N(134)	6.96 (H_E), 7.69 (H_Z)	105.4	6.96 (H_E), 7.68 (H_Z)	105.3
N(174)	8.04	112.5	8.14	113.4
N(183)	7.01 (H_E), 7.87 (H_Z)	106.4	7.01 (H_E), 7.87 (H_Z)	106.3

[a]) ^1H-NMR Signals referenced to external DSS. ^{15}N Chemical shifts are rel. to the shift of external liquid NH_3 (= 0.0 ppm). H_E and H_Z denote pro-*E* and pro-*Z* H-atoms, respectively. [b]) Data from *Hoffmann et al.* [15].

signals at 3.18, 3.64, 3.92, and 4.00 ppm appeared. These protons also gave rise to mutual through-bond coupling in the TOCSY spectrum and were shown by the ^{13}C-HSQC spectra to be part of two CH_2 groups: the signals at 3.18 ppm/3.64 ppm (assigned to H_S– and H_R–C(175)), and at 3.92/4.00 ppm (assigned to CH_2(176)) exhibited pairwise heteronuclear $^1J(^{13}C, ^1H)$ coupling to one C-atom. The location of these two CH_2 groups was confirmed by an NOE between HN(174) and H_SC(175) to be in the nucleotide loop of **5**. HN(174), on the other hand, showed intrachain NOEs to H_RC(171) and H_SC(172). These data define an ethylene moiety as the link between the propanamide N-atom of the corrin ligand and the phosphate O-atom of the nucleotide function in **5**. In contrast, an isopropylene group is the corresponding link in pseudovitamin B_{12} and in all other natural 'complete' corrinoids [1][2].

X-Ray crystallography was used to determine the accurate solid-state structures (*Fig. 6*) of **5**, and factor A (**6**, Coβ-cyano-7″-[2-methyl]adeninylcobamide) with crystals grown from aqueous solution after addition of acetone. For all three compounds, the structures were refined against data collected at a synchrotron X-ray source, and the

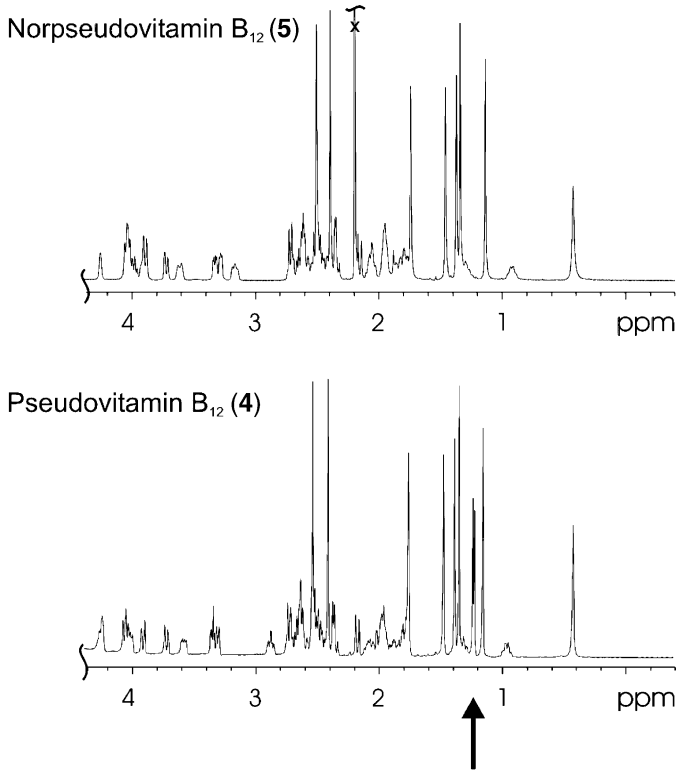


Fig. 5. *^{1}H-NMR Spectra* (500 MHz) *of aqueous solutions of norpseudovitamin B_{12}* (**5**, top, crystallized from H_2O, acetone) *and pseudovitamin B_{12}* (**4**, bottom). The arrow in the spectrum of pseudovitamin B_{12} points to the *doublet* signal due to the Me(177) group that is missing in the spectrum of norpseudovitamin B_{12}. The signal of acetone is marked with 'x'.

structural data are correspondingly among the most-accurate crystal structures of isolated B_{12} cofactors [28][29].

3. Discussion. – The bacterium *D. multivorans*, which is strictly anaerobic, is able to gain metabolic energy from the reductive dechlorination of tetrachloroethene and trichloroethene *via* a respiratory process [6][7]. The key enzyme in tetrachloroethene respiration is PCE dehalogenase, which mediates the reductive dechlorination of tetrachloroethene *via* trichloroethene to (*Z*)-1,2-dichloroethene. It contains a corrinoid and two Fe–S clusters as cofactors [7], and the reduction of the corrinoid cofactor in its Co^{III} oxidation state is believed to constitute the energy-conserving step in dehalorespiration. The corrinoid cofactor of the PCE dehalogenase from *D. multivorans*, the major corrinoid from this organism, was isolated in its Coβ-cyano form and was shown to differ from a group of known bacterial corrinoids [10][13]. It was characterized with the help of UV/VIS, FAB-MS, and homo- and heteronuclear NMR-spectroscopic analyses, which showed the corrinoid from *D. multivorans* to carry an adenine nucleotide base and to differ from pseudovitamin B_{12} (**4**) [15] only by the lack of the

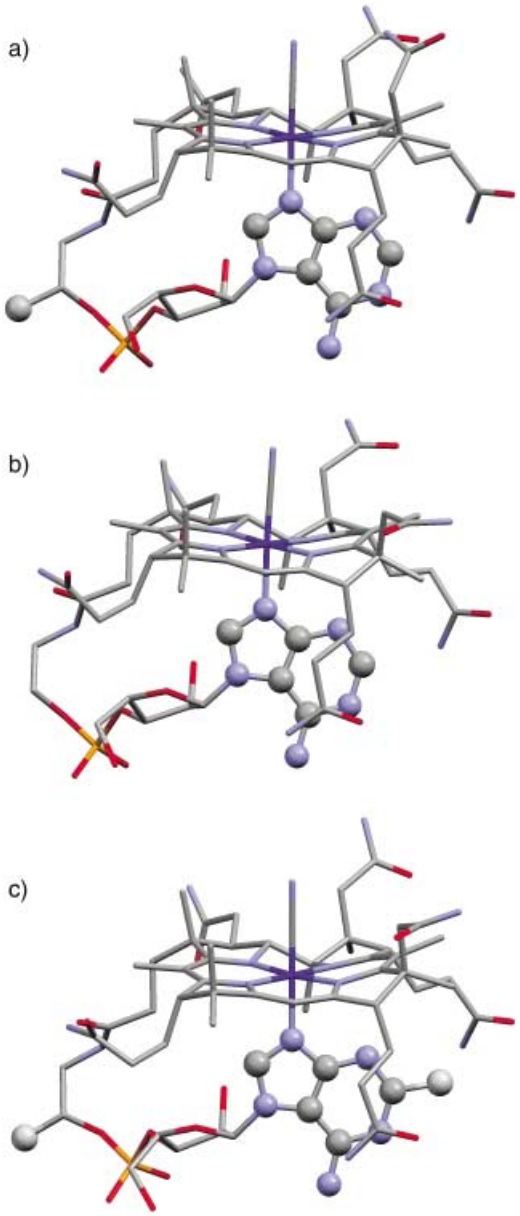


Fig. 6. *Ball and stick 3D models of the structures of Coβ-cyano-Coα-adeninyl-corrinoids* a) *pseudovitamin* B_{12} (**4**), b) *norpseudovitamin* B_{12} (**5**), *and* c) *factor A* (**6**). The nucleotide base and the Me(177) group are highlighted.

Me(177) group. Accordingly, the corrinoid isolated from *D. multivorans* was identified as Coβ-cyano-7″-adeninyl-176-norcobamide (**5**). This new corrinoid, designated 'norpseudovitamin B_{12}' actually represents (the cyano-Co^{III} form of) the first known

natural B_{12} cofactor that lacks a carbon center at the periphery of the cobamide moiety, a feature completely conserved in all other known 'complete' corrinoid cofactors [1].

In **5**, the 1-aminopropan-2-ol group of **4** and of other cobamides is replaced by an 2-aminoethanol linker. This finding points to a different biosynthetic origin of the alkanol-amine linker in the cobamides and in **5**. The (2*R*)-1-aminopropan-2-ol unit of vitamin B_{12} (**1**) or of adeninyl-cobamides [33][34] has been shown to be biosynthetically derived from threonine *via* L-threonine phosphate [35]. However, a *Pseudomonas denitrificans* mutant deficient in B_{12} biosynthesis identified by *Thibaut et al.* [35] accepted L-threonine phosphate as its natural substrate, but could also incorporate L-serine phosphate (a product of an enzymatic phosphorylation of serine [36]) into the nor-analogue of **1**.

The fascinating problem of the biosynthetic origin of the peripheral Me groups in natural corrinoids and their effect on the structure and reactivity of vitamin B_{12} derivatives has attracted considerable interest (see, *e.g.*, [3][37–39]). The particular role of the Me(177) group was first studied by *Friedrich et al.*, who prepared vitamin B_{12} and the related cyano-Co^{III}-corrinoids Coβ-cyano-3″-(5,6-dimethyl)benzimidazolyl-176-norcoba mide ('norvitamin B_{12}') and 176-*epi*-vitamin B_{12} by partial chemical synthesis [2][40]. The UV/VIS-spectroscopic properties of these B_{12} derivatives indicated only minor influences on the intramolecular Co coordination of the 5,6-dimethylbenzimidazole base by the lack of the Me(177) group in norvitamin B_{12} or by the change of the configuration from (176*R*) to (176*S*) in 176-*epi*-vitamin B_{12}.

Eschenmoser and co-workers [3][41] have carried out a qualitative theoretical analysis of the conformational properties of the nucleotide loop of 'base-off' cobalamins, *i.e.*, in which the nucleotide base is not coordinated intramolecularly to the corrin-bound Co center. According to these studies, the Me(177) group would induce local steric effects in 'base-off' cobalamins, affecting the dihedral angles (N(174)–C(175)–C(176)–O(177)) and (C(175)–C(176)–O(177)–P), in particular. Thus, the Me(177) group was suggested to be a key element in control of the relevant conformations of the 'base-off' constitutions by assisting in the preformation of a 'quasi-cyclic' conformation, as needed eventually to achieve unstrained Co coordination of the nucleotide base. These sections feature synclinal and nearly anticlinal (!) conformations around the C(175)–C(176) and the C(176)–O(177) bonds, respectively [3][41]. When attached with (*R*)-configuration at C(176), as observed in **1**, Me(177) would, thus, contribute to the formation of the cyclic 'base-on' constitution of 'complete' corrinoids. The Me(177) group is, thus, expected to influence chemical equilibria of complete corrinoids, which involve coordination/decoordination of the nucleotide base [3]. In the cobamides, a remarkable long-distance effect of Me(177), thus, arises at the Co center, *i.e.*, some eleven atoms away, since organometallic reactivity and redox properties differ significantly in the alternative 'base-off' or 'base-on' constitutions [1][18].

Analysis of the ^{1}H- and ^{13}C-NMR spectra of **5** and **4** confirmed their 'base-on' constitutions and revealed only minor differences in the conformations of the nucleotide segment of the two adeninyl-cobamides. Only the signals of the nuclei directly involved experienced significant chemical-shift differences. The rather small ^{1}H- and ^{13}C-chemical-shift differences observed for other positions (see *Table 1*) indicated only minor averaged conformational differences elsewhere in the nucleotide

moieties of **5** and **4**. In these two cyano-Co^{III}-adeninyl-cobamides, the Me(177) group exerts significant effect neither on the coordination geometry around the Co center nor on the conformational properties of the nucleotide loop.

The crystallographic data (*Fig. 6*) for pseudovitamin B_{12} (**4**), norpseudovitamin B_{12} (**5**), and factor A (**6**) provided the first high-resolution structural characterization of complete B_{12} cofactors with an adenine replacing the usually observed 5,6-dimethylbenzimidazole base and confirmed the NMR-based assignment of the structure of **5**. Previously reported crystallographic results of **6** [31] were rather inaccurate, as reflected by a high crystallographic residual ($R = 14.8\%$) and provided a chemically unreasonable $Co-N_{ax}$ distance. In view of this, we re-determined the crystal structure and include its results in the present publication. Besides factor A, a third obvious reference compound for structural comparison is cyanocobalamin (**1**; vitamin B_{12}), whose original crystal-structure analysis [42] has been repeated [30] to improve the crystallographic reliability ($R = 0.0824$ for 5638 reflections).

The pertinent molecular geometric parameters for the above four compounds, the distances between the Co center and its two axial ligands, the corrin 'fold angle' [43] and the 'base tilt' [30][31] (listed in *Table 3* and defined in the caption) provide particular information on the interaction of the corrin and nucleotide moieties of complete corrinoids [29]. The corrin 'fold angle' consists of an 'upward' folding of the corrin about a line running 'east' to 'west' and describes the main mode of structural variability of the corrin ring observed experimentally in crystal structures of B_{12} derivatives [28][29][32]. The upward folding deformation in $CN-Co^{III}$ corrins was traced back to steric interactions between corrin ring and coordinated nucleotide base, leading to a stretch of the $Co-N_{ax}$ bond and an increased fold of the corrin ring [28][29]. The base 'tilt' refers to a deformation involving an increase in the 'northern' and a decrease in the 'southern' $Co-N_{ax}-C$ bond angles presumed to be caused similarly by steric repulsion between the 'northern' part of the base and the corrin ring [30][31]. The data presented for **4**, **5**, and **6** show that the structural consequences of a replacement of 5,6-dimethylbenzimidazole by adenine or 2-methyladenine are hardly significant: while the $Co-N_{ax}$ distance increases by 0.01 – 0.02 Å, the base tilt decreases by 1.1° (2.0°) for 2-methyladenine and increases by 1.6° (decreases by 2.4°) for adenine, reflecting only minor differences between 5,6-dimethylbenzimidazole, adenine, and 2-methyladenine in steric bulk and coordinative capacity.

The crystallographic evidence corroborated the high degree of conformational similarity between **4** and **5** derived from NMR. The only major difference concerns the flexible carbamoylmethyl side chain extending from C(7). *Fig. 7* shows a superposition of the two crystal structures, in which the non-H-atoms of corrin ring and nucleotide loop segments superimpose with a remarkably small rms deviation of < 0.1 Å. As in the other known 'complete' corrinoids in their 'base-on' forms, the Me(177) group in **4** and **6** occupies position that is antiplanar to C(175)–C(176) with respect to N(174). The key torsion angles N(174)–C(175)–C(176)–O(177) and C(175)–C(176)–O(177)–P are similar in all three structures and do not deviate significantly from that in vitamin B_{12} (in which they are −66° and 134°, respectively [30][42]).

For the cofactor functions of the corrinoids, clearly, the $CN-Co^{III}$ forms investigated here are hardly considered directly relevant. Indeed, the absorption

Table 3. *Selected Molecular Geometry Parameters for Norpseudovitamin B_{12}* (**5**) *and Several of Its Analogues*

	d(Co–N_{ax}) [Å]	d(Co–C_{ax}) [Å]	Fold angle [°][a])	Base tilt [°][b])	Ref.
Pseudovitamin B_{12} (**4**)	2.021	1.832	19.6	2.5	This work
Norpseudovitamin B_{12} (**5**)	2.035	1.845	19.6	3.9	This work
Vitamin B_{12} (**1**)	2.011	1.858	18.0	4.9	[30]
Factor A (**6**)	2.118	1.861	15.7	5.5	[31]
Factor A (**6**)	2.026	1.840	16.9	2.9	This work

[a]) The fold angle has been used to describe the out-of-plane deformations of the corrin ring [32]. It is defined as the angle between least-squares plane defined by the atoms N(1), C(4), C(5), C(6), N(2), C(9), C(10) and the plane defined by C(10), C(11), N(3), C(14), C(15), C(16), N(4) (see *Fig. 1* for atom-labeling scheme). [b]) The base tilt describes a deformation involving an increase in the 'northern' and a decrease of the 'southern' Co–N_{ax}–C bond angle, presumed to be caused by steric repulsion between the 'northern' part of the base and the corrin ring [32]. It is defined as half the difference between 'northern' Co–N(9N)–C(4N) and 'southern' Co–N(9N)–C(8N) bond angles.

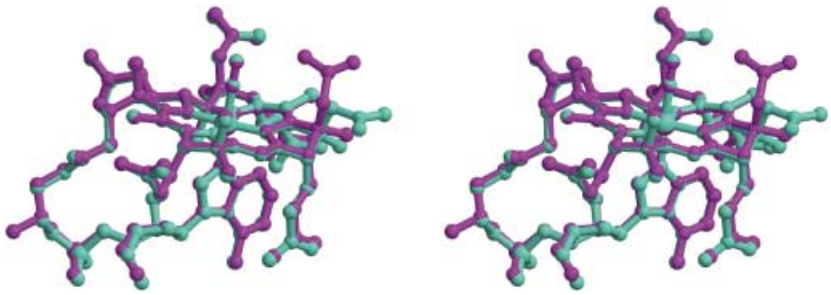


Fig. 7. *Stereodiagram of a superposition of pseudovitamin B_{12}* (**4**, violet) *and nor-pseudovitamin B_{12}* (**5**, cyan) *as observed in their respective crystal structures*

spectrum of isolated PCE dehalogenase was consistent with the presence of the Co^{II} form of the bound corrinoid, and the reduction of the bound corrinoid to its Co^{I} form is considered crucial for its activity [7]. For the native enzyme, the redox potential of the Co^{II}/Co^{I} couple was determined by EPR to be *ca.* −0.38 V at pH 7.5 (data not shown). Redox titrations of **4** and **5** revealed redox potentials of the Co^{II}/Co^{I} couple at pH 7 of −0.49 V (−0.48 V) *vs.* SHE. This compares well with the electrochemical half-wave potential of the protonated 'base-off' redox couple H^{+}-cob(II)alamin/H-cob(I)alamin at −0.50 V [18]. Both these midpoint potentials are about 0.11 V more positive than that of the redox couple cob(II)alamin/cob(I)alamin determined at −0.61 V *vs.* SHE [18]. In neutral aqueous solution, cob(II)alamin is present in its 'base-on' form and the Co^{II} to Co^{I} reduction is indicated to be accompanied by decoordination of the nucleotide base [1][18]. In contrast, the redox potentials and the UV/VIS spectra of the Co^{II} forms of norpseudovitamin B_{12} (see *Figs. 3* and *4*) and pseudovitamin B_{12} were both consistent with a 'base-off' nature of the two Co^{II}-adeninyl-cobamides at neutral pH. The lower tendency of the adenine moiety to coordinate to the corrin-bound Co^{II} center, compared to dimethylbenzimidazole of cob(II)alamin, parallels the finding of

the predominant 'base-off' structure of pseudocoenzyme B_{12} in aqueous solution [44][45].

The rather positive midpoint potential for the Co^{II}/Co^{I} redox step of PCE dehalogenase indicates a significant shift of the Co^{II}/Co^{I} redox properties of the bound corrinoid, as would be expected from weakened axial coordination of the Co^{II} form in the protein environment [18][46]. The EPR spectrum of the isolated PCE dehalogenase from *D. multivorans* is also consistent with 'base-off' binding of the corrinoid cofactor, while the lack of a B_{12}-binding motif [47] points to the absence of a Co-coordinating histidine. Indeed, EPR spectroscopy also showed that both the PCE dehalogenase from *Dehalobacter restrictus* [48] and the *o*-chlorophenol dehalogenase from *Desulfitobacterium dehalogenans* [49] bind their corrinoid cofactors 'base-off'.

The binding of a B_{12} cofactor in a 'base-off' form in PCE dehalogenase may be enhanced by a high tendency of the corrinoid to be 'base-off' in aqueous solution [50][51]. In this case, the absence of the Me(177) group in the norpseudo-B_{12} cofactors, which is suggested to contribute slightly to the formation of 'base-off' components in 'base-off/base-on' equilibria, would have indirectly a beneficial effect on binding. However, other interactions with the protein are likely to override weak conformational effects resulting from a Me group at the periphery of a cobamide cofactor. The use of norpseudo-B_{12} as cofactor in PCE dehalogenase is likely to be the consequence of the biosynthetic capacity of *D. multivorans*, rather than to have a significant functional explanation. In *D. multivorans,* the availability of both threonine and serine implies a new and selective biosynthetic path (presumably involving serine as indirect precursor) for the ethylene linker in the nucleotide loop of norpseudo-B_{12} cofactors. For the reductive dehalogenases of *D. restrictus* [48] and of *D. dehalogenans* [49], Co^{II}/Co^{I} redox potentials of -0.35 and -0.37 V, respectively, have been reported, although it is not known for these organisms what type of corrinoid is the enzymatic cofactor. In the dechlorinating *Gram*-positive *Desulfitobacterium* strain PCE-S, *Desulfitobacterium hafniense*, *Desulfitobacterium frappieri* TCE-1, and *Desulfitobacterium dehalogenans*, norpseudo-B_{12} could not be detected (data not shown), indicating that the presence of norpseudo-B_{12} might be restricted to *Gram*-negative dechlorinating microorganisms.

4. Conclusions and Outlook. – The vitamin B_{12} derivative described here as the title compound, the 176-norcobamide norpseudovitamin B_{12} (**5**), is the first known natural 'complete' corrinoid that lacks a characteristic Me group of the cobamide structure. The observed structural simplification appears to be of special interest in the context of the hypothesis that the basic structural elements of corrinoid and related tetrapyrrolic cofactors may have an origin of low structural complexity [3]. These findings, thus, bring up intriguing questions once more concerning the diverse structural and functional roles of the characteristic peripheral Me groups of the corrin ligand of the natural corrinoids. In tetrachloroethene reductive dehalogenase of the anaerobe *D. multivorans*, the B_{12} cofactor acts as an active redox center and has a function different from that of the well known organometallic corrinoid cofactors. The discovery of a norpseudo-B_{12} as corrinoid from *D. multivorans* is also reminiscent of the structural variability of the 'complete' corrinoids and suggests further diversity in the biosynthesis of B_{12} derivatives.

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Experimental Part

General. All chemicals were of the highest available purity from *Fluka* (Neu-Ulm, Germany), *Merck* (Darmstadt, Germany), *Aldrich* (Steinheim, Germany), *Roth* (Karlsruhe, Germany), and *Sigma* (Deisendorf, Germany). Gases (CO_2 grade 4.5, N_2 grade 5.0, and N_2/H_2 (95%:5%)) were supplied by *Linde* (Leuna, Germany). Chromatography materials were purchased from *Merck* (Darmstadt, Germany) and *Macherey-Nagel* (Düren, Germany). XAD-4 and Al_2O_3 were purchased from *Sigma*.

UV/VIS Spectra: *Hitachi U-3000*, H_2O solns., λ_{max} (log ε) in nm (molar extinction coefficients ε were determined with a *Varian Cary-100* spectrophotometer (*Varian*, Germany)). CD: *Jasco J715* spectropolarimeter, H_2O solns., λ_{max} or λ_{min} ($\Delta\varepsilon$) in nm. NMR: *Varian Unity-500-plus* spectrometer, pulse-field gradient, 5 mm indirect-detection probe, 5-mm broadband direct-detection probes, and a triple-resonance probe with actively shielded z gradients; ^{1}H: 499.876 MHz, ^{13}C: 125.706 MHz, ^{15}N: 50.658 MHz. Redox-titration: *Pharmacia Ultraspec-2000*, *Pharmacia Biotech*, Freiburg, Germany. FAB-MS Spectra: *Finnigan MAT95* spectrometer, nitrobenzyl alcohol (NOBA) matrix, Cs^+ bombardment, *m*/z (% rel. int.).

Strains and Sources of Organisms. D. multivorans was isolated as described earlier [52] and is available at the *Deutsche Sammlung von Mikroorganismen und Zellkulturen* (DSM 12446; Braunschweig, Germany).

Cultivation of D. multivorans. *D. multivorans* was grown anaerobically at 25° in mineral medium containing 0.2% yeast extract and 80 nM cyanocobalamin as described earlier [53]. The cells were grown in four 100-l batches, starting from 100-ml precultures routinely grown on 40 mM formate, 5 mM acetate, and 200 μM PCE. The precultures were used to inoculate a 1-l culture containing 40 mM each pyruvate and fumarate (no significant decrease of the dechlorination activity was observed under these conditions, since the PCE-dehalogenase appears to be a constitutive enzyme [52][53]). The fermentation was scaled up stepwise from six 1-l cultures to a 35-l culture, which was used to inoculate two 50-l carboys with pyruvate/fumarate medium. Cells were harvested aerobically by centrifugation and were stored at −20°.

Isolation and Purification of the Corrinoids from D. multivorans. For the isolation of corrinoids, 765 g of cells were used. The corrinoids were extracted with cyanide from *D. multivorans* as described earlier [7]. The isolation and analysis of the corrinoids from *D. multivorans* in the cyano form was carried out essentially as described [54]. The final corrinoid extract (150 ml) was subjected to chromatography on a *Lichroprep RP-18* column (25–40 μm mesh, 400 mm i.d. × 900 mm). The enriched corrinoid was eluted with 20% MeOH. The corrinoid containing fractions were flash-evaporated to a final vol. of 3 ml. Further purification was carried out on a *RP-18* column (*Nucleosil 100-7 C18*, 250 mm i.d. × 1000 mm length). The corrinoid was eluted with a flow rate set to 5 ml min^{-1}, starting with 50 ml of 15% MeOH in 0.1% AcOH and increasing the content of MeOH, 75 ml with 25% MeOH, 50 ml with 50% MeOH. Then, 5-ml fractions were collected while the absorbance at 280 nm was monitored to detect the corrinoid. *Fractions 23* to *25* of the sample of corrinoids from *D. multivorans* were found to contain a homogeneous B_{12} derivative, while two other corrinoids were contained in *Fr. 28/29* and *32*. The fractions were flash-evaporated to dryness, dissolved in H_2O, applied to a *Chromabond® C18 ec* (*Machery-Nagel GmbH*, Düren, Germany) column to remove traces of AcOH, and eluted with MeOH. *Fr. 24*, which contained *ca.* 2 mg (corresponding to 1.5 μmol) of the novel corrinoid **5**, was further purified by crystallization from H_2O with acetone and was analyzed by UV/VIS-, CD-, NMR spectroscopy and mass spectrometry.

NMR Experiments. A sample of *ca.* 2 mg of **5** was dissolved in 0.55 ml of H_2O/D_2O 9:1. All spectra were recorded at 26° with modern water-suppression techniques [15][16]. For subsequent processing and analysis, nmrPipe [55] and NMRView [56] software were used. Data were doubled by linear prediction in the indirect dimensions and processed with squared sine bells before zero-filling and *Fourier* transformation. 2D TOCSY [23]: time-domain data size 204 × 1984 (complex); 32 scans per t_1 increment; mixing time 70 ms (MLEV-17

[57][58], bracketed by 2 ms trim pulses, RF power 4.5 kHz); H_2O suppression with WATERGATE echo [24]. 2D ROESY [25][26]: time-domain data size 256×1984 (complex); 32 scans per t_1 increment; mixing time 250 ms (CW spin lock, RF power 2.3 kHz); H_2O suppression with WATERGATE echo [24]. $^1H,^{13}C$-PFG-HSQC [20][21]: time-domain data size 52×1024 (complex); 128 scans per t_1 increment; a pair of z-gradients (18.3 G/cm 1.8 ms and -18.1 G/cm 0.45 ms) was used for coherence selection; GARP decoupling [59] during acquisition (RF power 2.8 kHz). High-resolution $^1H,^{13}C$-PFG-HSQC [20][21]: time-domain data size 109×1024 (complex); 128 scans per t_1 increment; a pair of z-gradients (18.3 G/cm 1.8 ms and -18.1 G/cm 0.45 ms) was used for coherence selection, sweep width (*F1*) 2000 Hz. $^1H,^{15}N$-PFG-HSQC [25][26]: time-domain data size 64×1024 (complex); 512 scans per t_1 increment; a pair of z-gradients (27.5 G/cm 2.5 ms and -13.7 G/cm 0.5 ms) was used for coherence selection; GARP decoupling [59] during acquisition (RF power 1 kHz). $^1H,^{13}C$-PFG-HMBC [20][22]: Magnitude mode spectra were obtained with the standard HMQC pulse sequence with an additional delay for the evolution of long-range heteronuclear coupling; time-domain data size 128×1984 (real); 512 scans per t_1 increment; z-gradients (2×9.2 G/cm 2 ms and 4.6 G/cm 2 ms) used for coherence selection and suppression of axial peaks; no decoupling during acquisition.

Determination of ε. The molar extinction coefficients of **5** were determined by measuring the absorption spectra and Co content of samples from a dilution series (see *Fig. 2*). Co was determined by inductively coupled plasma atomic-emission spectroscopy (ICP-AES) with a *Liberty 150* device (*Varian*, Mulgrave, Australia). Gas flow was 15 l min^{-1} Ar; the sample flow rate was 2 ml min^{-1}; 228.616 and 238.892 nm; calculations were based on the measurements at 238.892 nm and did not significantly differ from the data obtained when measuring at 228.616 nm. H_2O from the same source as that used for the dilution series served as a blank. The analysis was kindly performed by *K. Voigt* in the laboratory of Prof. Dr. *H. Bergmann* (Institute for Nutritional Sciences, University of Jena, Germany).

Data of Norpseudovitamin B_{12} (**5**): UV/VIS (H_2O, 23°): 547 (3.83), 516 (3.80), 408 (3.46), 359 (4.38), 320 (3.82), 305 (3.85), 275 (4.19). CD (H_2O, 20°): 577 (2.0), 488 (−5.2), 429 (13.0), 363 (−10.1), 324 (−5.9), 296 (0.05), 269 (−3.4), 248 (−5.5), 236 (2.0). NMR: 2.2 mM aq. solns.; for 500 MHz ^1H-NMR spectra of **4** and **5**, see *Fig. 5*; a complete listing of assigned ^1H, ^{13}C, and ^{15}N signals is given in *Tables 1* and *2* (for the atom-numbering of B_{12} derivatives, see *Fig. 1* [14]). FAB-MS: 1353.5 (21), 1352.5 (18, $[M + Na]^+$) 1332.5 (20), 1331.5 (68),1330.5 (100, $[M + H]^+$), 1305.4 (23), 1304.4 (16, $[M + H - CN]^+$), 1303.4 (16), 1302.4 (20). HR-MS: 1330.5092 ($C_{58}H_{82}CoN_{17}O_{14}P$; calc. 1330.5296); 1331.5212 ($^{12}C_{57}{}^{13}CH_{82}CoN_{17}O_{14}P$; calc. 1331.5329).

Determination of Electrochemical Midpoint Potentials. Midpoint potentials of the Co^{III}/Co^{II} and Co^{II}/Co^{I} redox couples of **5** and **4** were determined by UV/VIS potentiometry at pH 7.0 [60]. The electron donor titanium(III) citrate [61] was added stepwise to corrinoid solns. in 100 mM *Tris*-HCl buffer pH 7 (corrinoid content *ca.* 30–50 μM). After equilibrium was reached, the UV/VIS-absorption spectrum was recorded and the redox potential of the soln. was simultaneously determined with a Pt/Ag/AgCl electrode (*Mettler Toledo*, Steinbach, Germany). For calculation of the potential *vs.* SHE, 207 mV was added to the recorded potential. Redox standard solns. at +220 mV and +468 mV purchased from *Mettler Toledo* (Steinbach, Germany) as well as methyl viologen soln. (50 μM in 100 mM *Tris*-HCl buffer pH 7; $E^{\circ\prime} = -449$ mV) served as controls. The contents of the three redox forms of the corrinoids were determined at 360 nm and 548 nm for Co^{III}, at 473 nm for Co^{II}, and at 386 nm for Co^{I}. The corrinoid concentration could be calculated from the ε_{360} determined for **5** or from the reported ε_{473} (cob(II)alamin) and ε_{386} (cob(I)alamin) [2]. The dilution caused by the addition of the titanium(III) citrate soln. was taken into account. The midpoint potential was calculated *via* the *Nernst* equation with a one-electron transfer assumed for each reduction step.

X-Ray Crystal-Structure Analyses. Crystals of **5**, **4**, and of factor A (**6**) were grown from H_2O/acetone. A crystal specimen was immersed in a drop of hydrocarbon oil, picked up with a rayon loop, and quickly cooled to cryotemperature by immersing in liquid N_2. All diffraction experiments were carried out on the EMBL beamline X13 at DESY in Hamburg (Germany), which was equipped with a *MARCCD* detector (wavelength $\lambda = 0.80150$ Å) and a gas-stream low-temperature (103(2) K) device. Data pertaining to the collection and refinement of diffraction data for the two compounds are collected in *Table 4*.

Indexing of diffraction images, intensity integration, and data scaling were performed with programs Denzo/Scalepack [62], Mosflm [63] and Scala [64]. The structures were solved by direct methods to yield the Co-atoms plus most remaining atoms of the structure. Missing atoms (mostly in the solvent region) were located in subsequent electron-density maps. Full-matrix least-squares refinement on F^2 was performed with program SHELXL-97 [65]. No absorption correction was applied to the data. Scattering factors including real and imaginary dispersion corrections were taken from the 'International Tables of Crystallography'. Anisotropic atomic displacement parameters (adp) were refined for all non-H-atoms, with application of a 'rigid-bond' restraint [66] (effective standard deviation $\sigma = 0.01$ Å^2) for all 1,2- and 1,3-distances involving C-, N-, and O-

Table 4. *Crystal Data and Structure Refinement for Norpseudovitamin B_{12}* (**5**), *Pseudovitamin B_{12}* (**4**), *and Factor A* (2-methyladeninyl-cyanocobamide, **6**)

	Norpseudovitamin B_{12} (**5**)	Pseudovitamin B_{12} (**4**)	Factor A (**6**)
Empirical formula	$C_{58}H_{81}CoN_{17}O_{14}$ P	$C_{59}H_{83}CoN_{17}O_{14}P$	$C_{60}H_{85}CoN_{17}O_{14}P$
H_2O sites	23	21	23
Sum of partial occupancies	19	16	18
Acetone sites	1	1	2
Formula weight	1730.7	1690.6	1798.8
Crystal system	orthorhombic	orthorhombic	orthorhombic
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Unit-cell dimensions			
a [Å]	15.730(3)	16.211(3)	15.767(3)
b [Å]	21.320(4)	20.485(4)	21.584(4)
c [Å]	24.130(5)	24.768(5)	26.213(5)
V [Å^3]	8092	8225	8920
Z	4	4	4
D_{calc} [g cm^{-3}]	1.421	1.365	1.339
μ [mm^{-1}]	0.33	0.32	0.30
$F(000)$	3696	3608	3848
Crystal size [mm^3]	$0.4 \times 0.2 \times 0.2$	$0.5 \times 0.2 \times 0.2$	$0.5 \times 0.2 \times 0.2$
θ Range for data collection [°]	1.74 – 26.40	1.69 – 26.40	1.7 – 26.40
Wavelength [Å]	0.801	0.801	0.801
Reflections collected	48073	44882	47488
Data reduction programs	Mosflm/Scala	Denzo/Scalepack	Denzo/Scalepack
Independent reflections	6322	6421	6957
R(int)	0.0360	0.0340	0.039
Completeness to $\theta = 26.40°$	99.2%	99.1%	98.4%
Data/restraints/parameters	6322/366/1076	6421/404/1050	6957/348/1080
Final R indices (all data)			
R_1	0.0535	0.0536	0.0538
wR_2	0.1471	0.1483	0.1470
Largest diff. peak/hole [e Å^{-3}]	0.59/ – 0.56	0.61/ – 0.43	0.61/ – 0.46

atoms. H-Atom positions were calculated and refined as 'riding' on their respective non-H-atom. Me-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 two ribose OH groups. The isotropic adp for each H-atom was set to 1.5 times the equiv. isotropic adp of the adjacent non-H-atom. The solvent electron density was modeled with acetone and H_2O molecules included (see *Table 4*). Crystallographic residuals at the close of the refinement are also given in *Table 4*.

Crystallographic data (excluding structure factors) have been deposited with the *Cambridge Crystallographic Data Centre* as supplementary publication numbers CCDC-217273 (**4**), CCDC-217274 (**5**), and CCDC-217275 (**6**). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).

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