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Isovitamin B_{12}: A Vitamin B_{12} Derivative That Flips Its Tail

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Dedicated to Professor Karl Grubmayr on the occasion of his 60th birthday

Vitamin B_{12} (cyanocobalamin, **1**) features a cobalt corrin and a nucleotide functionality in the stable, "base-on" form,[1–3] a hallmark of Nature's "most beautiful" cofactors.[4] The intramolecular coordination of the nucleotide appendage to the corrin-bound cobalt center gives a unique three-dimensional structure[1] and modulates the reactivity of the cobalt center.[5] The complexity of **1** has been considered an impressive challenge for the (current understanding on the) evolution of the catalytic moieties of essential cofactors. According to Eschenmoser, the natural corrinoids may represent a further evolved form of the hypothetical B_{12} progenitor "protocobyrinic acid" and the seemingly specific nucleotide loop of **1** may have arisen from adaptation and (non-enzymatic) self-constitution.[6] This loop is a specific structural selection element for recognition by proteins involved in controlled B_{12} uptake[7] or by the protein parts of some B_{12}-dependent enzymes.[8]

We report on isovitamin B_{12} (**2**, Co_{β}-cyano-5″,6″-dimethylbenzimidazolyl-176-isocobamide, see Figure 1),[9] an isomer of vitamin B_{12}, in which *n*-propanolamine constitutes the linker, rather than (*R*)-isopropanolamine. Our studies were induced by the discovery of nor-pseudovitamin B_{12}, the natural "complete" B_{12} cofactor (with an ethanolamine linker) of perchloroethylene reductase from *Sulfurospirillum multivorans*.[10] The corresponding cobalamin analogue, norvitamin B_{12} (**3**, see Scheme 1), was synthesized in more recent studies in this area,[11] and turned out to be a natural corrinoid also, which was detected in *Salmonella sp.*[12]

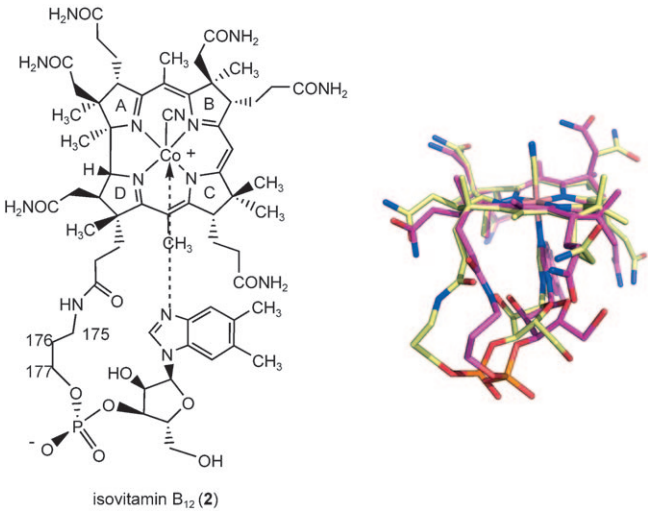

Figure 1. Isovitamin B_{12} (**2**). Left: structural formula. Right: superposition of the structures of two conformers of **2** in the crystal (stick models) magenta: inward conformer **2a**, yellow: outward conformer **2b**.

Isovitamin B_{12} (**2**) was prepared by the method[9,13] outlined for the partial synthesis of norvitamin B_{12} (**3**).[11] It was obtained in 65% yield by condensation of cobyric acid (**4**)[9,14] and (3-aminopropyl)-3′-α-ribazolyl-diphosphate (**5**) (see Scheme 1, Experimental Section and Supporting Information) and recrystallization from aqueous acetone. The chromatographic behavior of **2** was similar to that of vitamin B_{12} (**1**), and the UV/Vis spectra of **1** and of its isomer **2** were practically indistinguishable.[15] FAB-mass spectra exhibited a pseudo-molecular ion at m/z 1355, that is, at the same value as that of **1**. UV/Vis and ^{1}H NMR spectra, as

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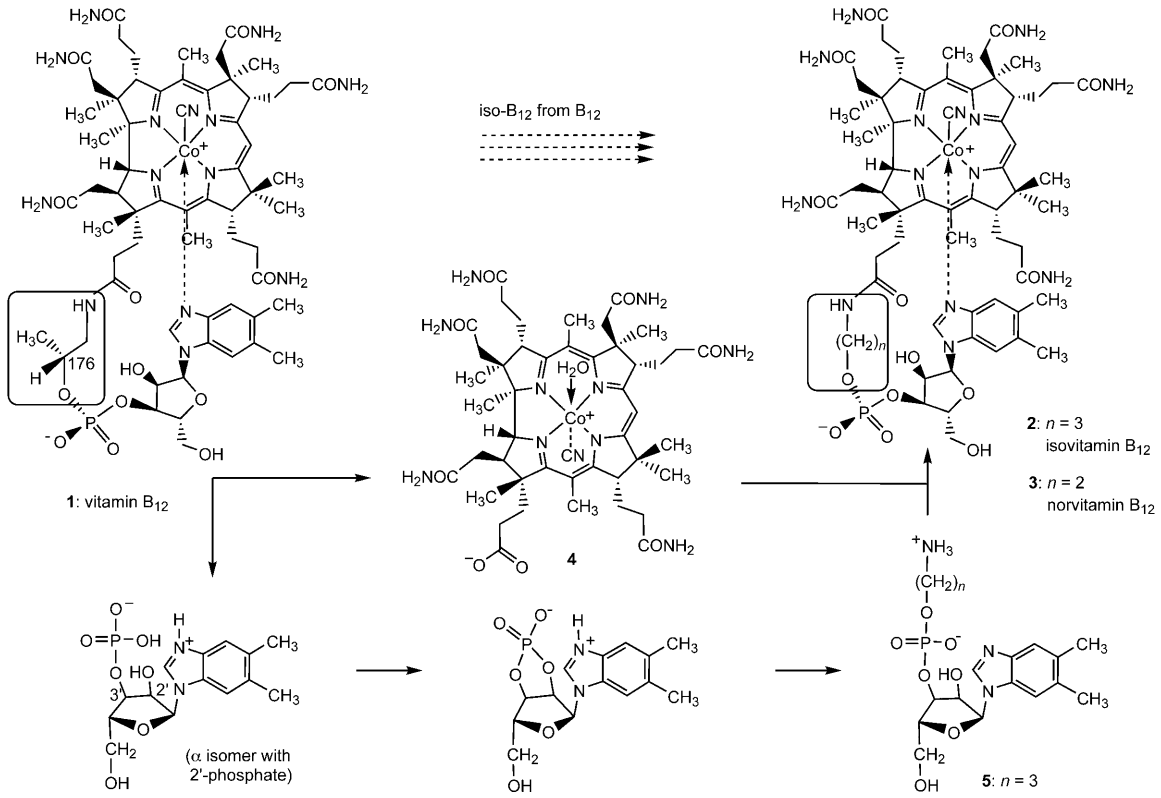


Scheme 1. Outline of the synthesis of isovitamin B_{12} (**2**, Co_β-cyano-5″,6″-dimethylbenzimidazolyl-176-isocobamide) from vitamin B_{12} (**1**, Co_β-cyano-5″,6″-dimethylbenzimidazolylcobamide) via cobyric acid (**4**) (see Supporting Information for further details).

well as ^{13}C NMR data all supported a base-on structure of **2** in aqueous solution.

Crystals of isovitamin B_{12} (**2**) were obtained from aqueous acetone. The structure of **2** in the crystal was determined at 0.9 Å resolution using synchrotron radiation. The asymmetric unit of the monoclinic crystal contained two independent B_{12} molecules, which showed significant conformational differences (see Figure 1). In one of these isomers, called the "inward" conformer (**2a**) of isovitamin B_{12}, the nucleotide linker was strongly displaced towards the corrin moiety (see Figure 2). In the other, the "outward" conformer (**2b**) of isovitamin B_{12}, the side chain connecting with the nucleotide is bent away from the corrin moiety, adapting a similar overall conformation in the crystal, as seen in vitamin B_{12} (see Figure 2 and Supporting Information, Figure S5).[16,17] However, the inner coordination sphere of the cobalt center (geometry and bond lengths) of **2a** and **2b** is similar and is not significantly different to that of **1**[16,17] (see Experimental Section).

The inward conformer **2a** features two remarkable intramolecular H-bonds of the two ribose OH groups (between 2′-OH and 5′-OH and the f- and e-side chain amides, respectively, see Figure 3, left). Such direct intramolecular H-bonds have not been observed previously in vitamin B_{12} (**1**) and other cobalamins.[16–19] They could, however, help lay out structural models for the critical "precyclic" states of vitamin B_{12} on the way to its selective chemical reconstitution,[6] as well as for the suggested, but elusive "tuck-in" structure of "base-off" forms of cobalamins.[20] The new loop conformation in **2a** is accompanied by an unusually strong increase in the corrin fold angle from 18.0(0.3)° in **1** to 29.6-(0.2)° in **2a**. This restructuring of the corrin ring in **2a** is also accompanied by a complete loss of puckering in corrin ring C section, and an increase of the puckering of ring B.

In isomer **2b** and in other B_{12} structures, the two ribose OH groups undergo intermolecular H-bonds with water (see Figure 3, right). Indeed, the structure of the "outward" conformer (**2b**) of isovitamin B_{12} (see Figure 2 and Supporting Information Figure S5) is more similar to that of vitamin B_{12} (**1**) with respect to both, the conformational features of the nucleotide loop and of the corrin ring (e.g., fold angle of 18.2(0.2)° in **2b** compared to 18.0(0.3)° in **1**).[16,17]

The UV/Vis and CD spectra of **2** in aqueous solution were nearly temperature independent between 5 and 55 °C and were all consistent with a "base-on" form. Comparison of the NMR data of **1**,[21–23] with those of **2** not only showed the expected local substituent effects (on chemical shift values and coupling networks), but also suggested significant conformational differences of the loop portion. Homo- and heteronuclear NMR spectra indicated a global loop conformation rather similar to that of the "inward" conformer **2a** in the crystal. Specifically, the ^1H chemical shift values for HO-R2 (at 6.21 ppm) and of HN174 (at 7.75 pm) were consistent with the existence of a direct H-bond between the

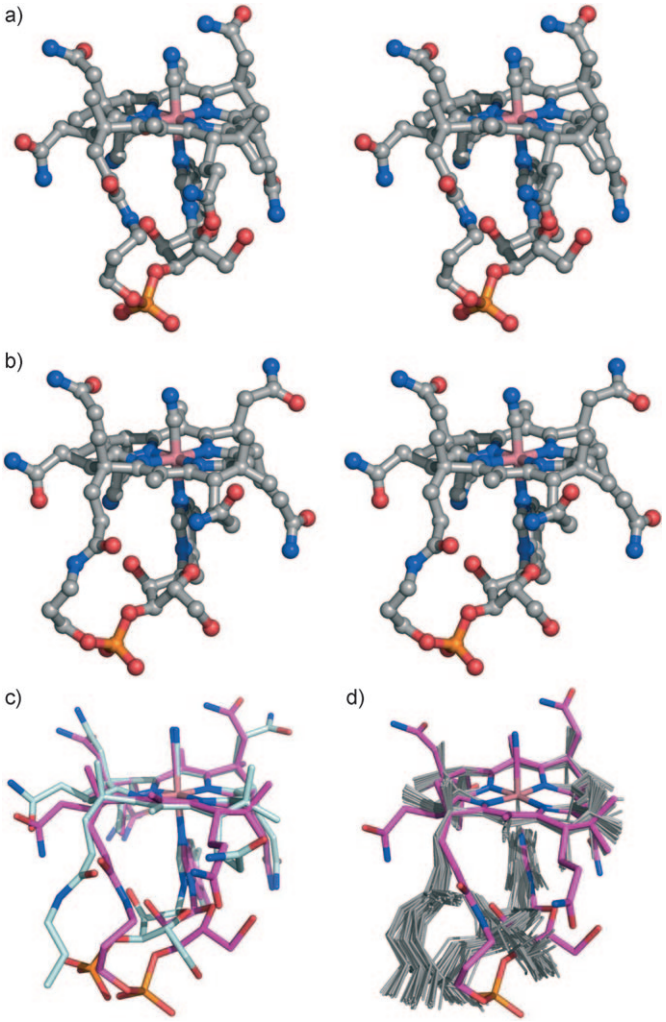


Figure 2. Top: Stereo figures of the two conformers of isovitamin B_{12}: a) "inward" form (**2a**), b) "outward" form (**2b**), ball and stick models, with C, N, O, P, Co atoms colored grey, blue, red, orange and pink, respectively. Bottom: Superpositions of stick models (generated using the program PyMOL (http://www.pymol.org)) of 3D-crystal structures of c) vitamin B_{12} (**1**, cyan) with the "inward" form of isovitamin B_{12} (**2a**, magenta), d) **2a** (magenta) and of all crystal structures of cobalamins deposited in the CSD (grey lines, 59 structures with 62 independent cobalamins). For clarity, acet- and propionamide side chains were truncated to the first carbon atom in the structures extracted from the CSD.

amide group of the f-side chain and the ribose 2-OH, as displayed in the crystal structure for **2a** (see Figure 3, left). A short distance between these two groups was also indicated by the observation of a significant H,H NOE between HN174 and HR4 (see Supporting Information). The dominant loop conformation of **2** in aqueous solution was thus deduced to be similar that of **2a** in the crystal. This conclusion was supported further by NOE signal between H175a (assigned as $H_{pro\text{-}R}$) and HR4, as well as between HN174 and H177. The signals in the ^{1}H NMR and ^{13}C NMR spectra of **2** exhibited small-temperature dependences only in the range between 5 and 55 °C. The most significant shifts occurred for the signals of the H-atoms at C175, at the d-side chain and at the ribose unit, as well as for the C-atom C-10.

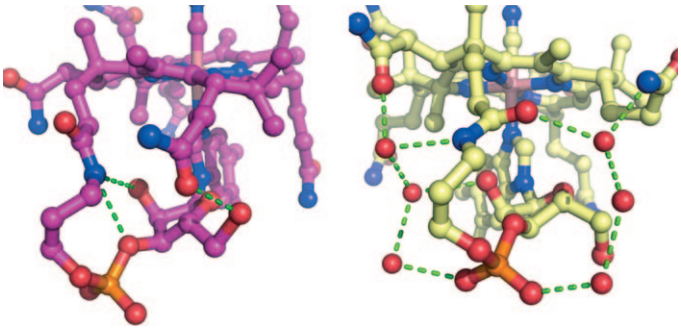


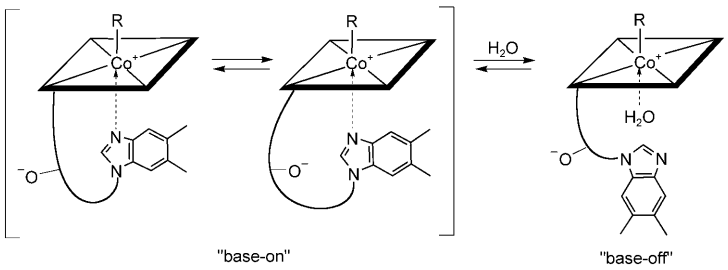
Figure 3. Section from the crystal structures of conformers **2a** (left, magenta) and **2b** (right, yellow) of isovitamin B_{12}, highlighting H-bonds (green dashed lines), and water molecules (right, red spheres) that were identified in the crystal in an H-bonding network in the vicinity of the nucleotide loop of **2b**.

Comparison of the ^{13}C NMR spectra of **1** and **2** showed significant shift differences only for the loop carbon atoms C171 to C177.

Tinkering with the structure of the nucleotide loop of vitamin B_{12} (**1**), as done here in isovitamin B_{12} (**2**), induced a distinct distortion from the well known structures of the "base-on" form of the cobalamins (see Figure 2 and Figure S5, Supporting Information). The unique stereostructural fit of the 19-membered nucleotide loop of **1**, and the corresponding misfit of its 20-membered analogue in **2**, result in strongly dissimilar mechanochemical and steric effects, as deduced from structural comparison of **1** and **2a**. However, as shown here, in recent studies on norvitamin B_{12} (**3**),[11] and on cobamides with an oligomethylene-linked dimethylbenzimidazole base,[24] the propensity of complete cobalt–corrins to give the "base-on" structure by intramolecular cobalt coordination of the dimethylbenzimidazole base is remarkably tolerant with respect to the precise nature of the linker. These findings also fall in line with the documented variability of natural "complete corrinoids" with respect to the nature of the nucleotide heterocycle.[5,25]

"Complete" cobamides, such as the cobalamins, may exist either in their "base-on" form, or, alternatively, as their "base-off" constitutional isomers (see Scheme 2).[26,27] The precise structure of the "base-on" form of the cobalamins is a strict selection criterion for B_{12} uptake in humans (and other higher organisms).[28] In contrast, not all B_{12}-dependent enzymes bind corrinoid cofactors "base-on". B_{12}-dependent methyl transferases,[26,29] and carbon-skeleton mutases,[30,31] use the bound B_{12} cofactor in a "base-off" form. Studies with isocobalamins may reveal the relevance of the natural B_{12} loop structures in binding interactions with B_{12}-binding proteins and B_{12}-dependent enzymes. As close structural mimics of the genuine cobalamins, isocobalamin cofactors are thus expected to reveal subtle positional aspects in catalysis, such as considered relevant for the much discussed role of the corrinoid moiety as "conductor" in B_{12}-dependent radical reactions.[32]

"Complete" corrinoid cofactors (organometallic cobalamins, see Scheme 2: R = organic ligand) are thus molecular



Scheme 2. Schematic representation of "base-on" and "base-off" forms of cobalamin cofactors in aqueous solution.

switches,[33] that are tunable by their (macromolecular) binding partners.[27] This topological feature of B_{12} derivatives has a remarkable structural complement in B_{12} riboswitches.[34] These sections of un-translated mRNA regulate the expression of proteins by a conformational switch that is induced by direct binding of natural corrinoids. The *BtuB* riboswitch (of the B_{12}-transporter *BtuB*) of *Escherichia coli* displays an intriguing tolerance with respect to the structure of the B_{12} derivatives, and is recognized and switched by corrinoids that are bound "base-on" or "base-off".[35]

In the last step of the biosynthesis of coenzyme B_{12}, the entire guanosyl-nucleotide moiety of a natural "base-off" B_{12}-precursor is replaced by the dimethylbenzimidazolyl nucleotide.[36,37] Thus the genetically programmed path to coenzyme B_{12} in microorganisms exhibits a strategy, which may reflect the nature of both its two prominent moieties as exemplary molecular fossils.[6] The remarkable propensity of vitamin B_{12} to reconstitute from its two natural moieties, in turn, is a corner stone of the chemical rationalization of its structure.[6] Indeed, the assembly of the two moieties of the B_{12} cofactors in their unique and stable "base-on" form depends critically on the specific stereo-structural fit of the linker, as underscored here.

Experimental Section

Selected spectroscopic data of iso-vitamin B_{12} (2): UV/Vis ($c = 4.43 \times 10^{-5}$ M, H_2O): λ_{max} (logε) = 548 (3.83), 518(3.78), 408(3.47), 360(4.33), 322-(3.80), 306 (3.86), 278 nm (4.08); FAB-MS: m/z: 1357.4(16), 1356.4 (29), 1355.4 (42) $[M+H]^+$, 1332.4 (21), 1331.4 (58), 1330.4 (99) 1329.4 (100) ($C_{62}H_{88}N_{13}O_{14}PCo$) $[M+H-CN]^+$, 1328.4 (27), 1327.4 (22); for details see Supporting Information.

Structure determination of isovitamin B_{12} (2): Crystals were grown from water/acetone; diffraction data (monoclinic space group $P2_1$, $a = 15.805$, $b = 24.336$, $c = 22.112$ Å, $\beta = 89.97(3)°$, $R_{sym} = 0.043$) to a maximum resolution of 0.9 Å were collected at 103 K using synchrotron radiation ($\lambda = 0.8081$ Å) on beam line X13 at EMBL/DESY in Hamburg. Refinement on F^2 (11887 unique reflections, 2074 parameters and 2143 restraints) converged at crystallographic residuals of $R1 = 0.0597$ and $wR2 = 0.1513$ for all reflections.

Selected bond lengths around cobalt center (averaged over the two independent molecules): cobalt to corrin nitrogen atoms: Co–N1 1.870(6), Co–N2 1.891(6), Co–N3 1.903(6) and Co–N4 1.883(6) Å; cobalt to axial ligands: Co–C_β 1.884(8), Co–N_α 2.045(6) Å; discrete disorder was observed for amide side chains a and d in one of the two B_{12} molecules (**2b**) and for the ribose hydroxymethylene group in the other (**2a**); the solvent region was modeled using 3 acetone and 30 water molecules; further details are given in the Supporting Information.

CCDC 765917 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif

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Keywords: bioinorganic chemistry · conformation analysis · crystal structure · molecular switch · vitamins

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