Organometallic B_{12} Chemistry

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Phenylethynylcobalamin: A Light-Stable and Thermolysis-Resistant Organometallic Vitamin B_{12} Derivative Prepared by Radical Synthesis**

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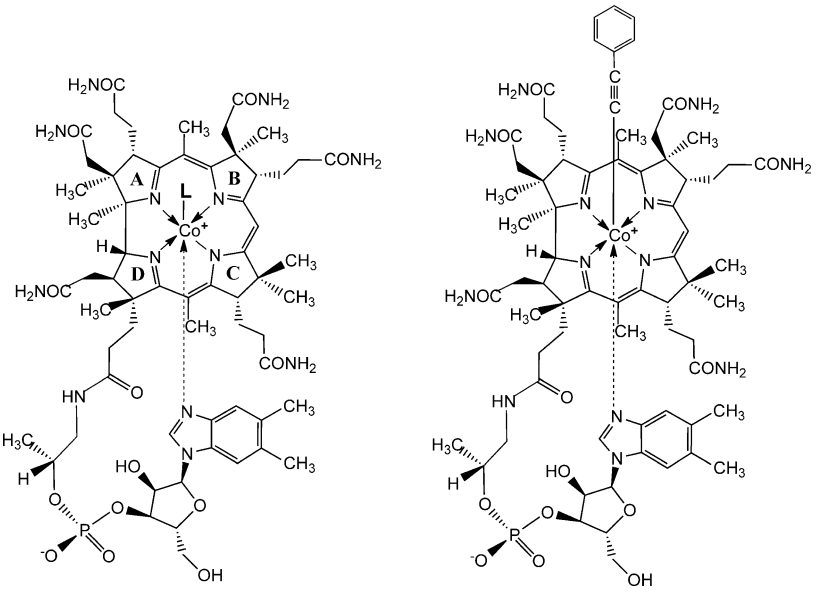
Dedicated to Professor Helmut Schwarz on the occasion of his 70th birthday

The biologically important roles of the B_{12} cofactor coenzyme B_{12} (**1**; 5′-deoxyadenosylcobalamin) and methylcobalamin (**2**) are associated with their organometallic and redox chemistry.[1,2] Enzyme-controlled organometallic reactions, homolysis of the weak Co–C bond of coenzyme B_{12} (**1**), and nucleophile-induced heterolytic abstraction of the cobalt-bound methyl group of methylcobalamin (**2**), as well as processes in the (formal) reverse sense, are the known key steps in B_{12}-dependent enzymes.[3–6] To explore possible biological roles of B_{12} cofactors, such as **1** and **2**, significant and extensive efforts have been put into studies of the chemical features of organometallic B_{12} derivatives.[1,7] Efficient and selective homolytic cleavage of the Co–C bond of organometallic B_{12} derivatives upon thermolysis or exposure to visible light appears to be their characteristic.[8,9] Organometallic aryl cobalamins deviate, in part, from this rule and exhibit thermolysis-resistant Co–C_{sp^2} bonds. They are a new line of designed B_{12} antimetabolites and are potential antivitamins B_{12}.[10] In this regard, organometallic alkynyl B_{12} derivatives with a Co–C_{sp} bond may also be of particular interest. Remarkably, there is a surprising lack of pertinent information on alkynyl corrins,[1,7] and from the older work on alkynyl cobalamins[11–13] only largely preliminary analytical and structural information is available. However, organometallic B_{12} derivatives with a directly cobalt-bound alkyne group exhibit exceptional structural properties and chemical reactivities, as reported here for Co_β-2-phenylethynylcobalamin (**3**; see Scheme 1).



Scheme 1. Left: General structural formula of important cobalamins: coenzyme B_{12} (**1**, 5′-deoxyadenosylcobalamin, L = 5′-deoxyadenosyl), methylcobalamin (**2**, L = methyl), cob(II)alamin (**4**, L = e^-), vitamin B_{12} (**5**, cyanocobalamin, L = CN), and aquocobalamin chloride (**6**, L = H_2O^+ [chloride]); Right: Structural formula of Co_β-2-phenylethynyl-cobalamin (**3**).

Co_β-2-phenylethynylcobalamin (**3**) was prepared in a one-pot reaction in deoxygenated aqueous solution, starting with aquocobalamin chloride (**6**), 2-phenylethynyl iodide, and triethylammonium formate (Scheme 2). Formate reduction of **6** furnished cob(II)alamin (**4**),[10] which reacted with 2-phenylethynyl iodide presumably by a radical reaction. We suggest the formation of **3** to result from recombination of a 2-phenylethynyl radical with cob(II)alamin (**4**) generated in situ (Scheme 3). The organocobalamin **3** was obtained in 68% yield after crystallization of the raw red isolate from aqueous acetone.

The UV/Vis spectrum of **3** (Supporting Information, Figure S1) indicated a base-on cobalamin and was similar to that of vitamin B_{12} (**5**). However, the spectrum of **3** differed significantly from the spectra of the organometallic B_{12} cofactors **1** and **2**. In a MALDI-TOF mass spectrum of **3**, a pseudo molecular ion at $m/z = 1430.7$ was observed, as well as a base peak at $m/z = 1329.6$ (which is due to loss of the organometallic phenylethynyl ligand). An absorbance at

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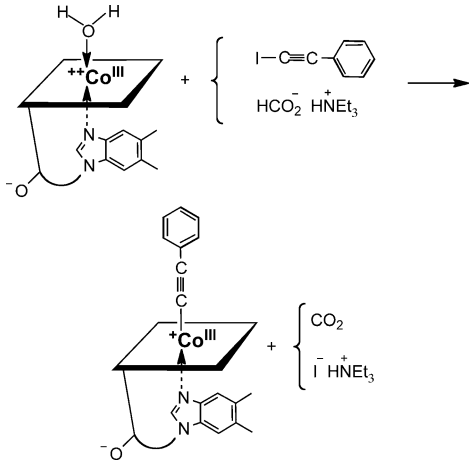
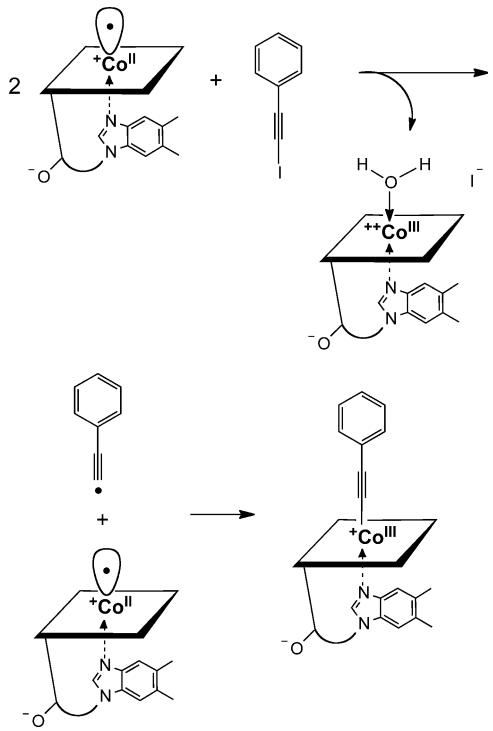


Scheme 2. Outline of the preparation of Co_{β}-2-phenylethynylcobalamin (**3**) from aquocobalamin chloride (**6**) and 2-phenylethynyl iodide (see Scheme 3 for a suggested mechanism and the Supporting Information for further experimental details).



Scheme 3. Suggested mechanism for the formation of Co_{β}-2-phenylethynylcobalamin (**3**). A hypothetical one-electron reduction of 2-phenylethynyl iodide by cob(II)alamin (**4**) gives a 2-phenylethynyl radical, which is efficiently trapped by the Co^{II}-corrin **4**.

2117 cm^{-1} in the IR spectrum of **3** (Supporting Information, Figure S3) was assigned to the cobalt-bound alkyne function. Homonuclear and heteronuclear ^{1}H NMR and ^{13}C NMR spectroscopic studies of **3** supported attachment of the phenylethynyl ligand at the "upper" β-face of the corrin-bound Co^{III} center, and established its structure as "base-on" Co_{β}-2-phenylethynylcobalamin. With the exception of the directly cobalt-bound carbon, the signals of the other nuclei of the organometallic group could be assigned (Supporting Information, Table S1 and Figure S4). The common signal of the two *ortho*-hydrogen atoms (d at 6.85 ppm) of the 2-phenylethynyl moiety indicated the effective equivalence of these protons, which is due to (rapid) rotation of the phenyl ring.

Orthorhombic crystals of Co_{β}-2-phenylethynylcobalamin (**3**) were grown from aqueous acetone and were used for an X-ray analysis. The structure of **3** was refined to 0.84 Å, revealing a base-on cobalamin with an "upper" 2-phenylethynyl ligand (see Figure 1).[28]

The inner coordination sphere (geometry and bond distances) of the cobalt-center of **3** is similar to that of vitamin B_{12} (**5**).[14–16] The axial Co–C_{β} bond has a length of only 1.861(3) Å, and the Co–N_{α} bond to the dimethylbenzimidazole (DMB) base is 2.084(2) Å long (compared to 1.858 and 2.011 Å, respectively, in **5**).[14,16] The bond distances in the equatorial plane are 1.881(2) Å (Co–N1), 1.921(2) Å (Co–N2), 1.914(2) Å (Co–N3) and 1.890(2) Å (Co–N4). The fold angle of the corrin ligand of **3** is 15.26(10)°, and the Co atom is situated slightly out of the plane of the four corrin nitrogen

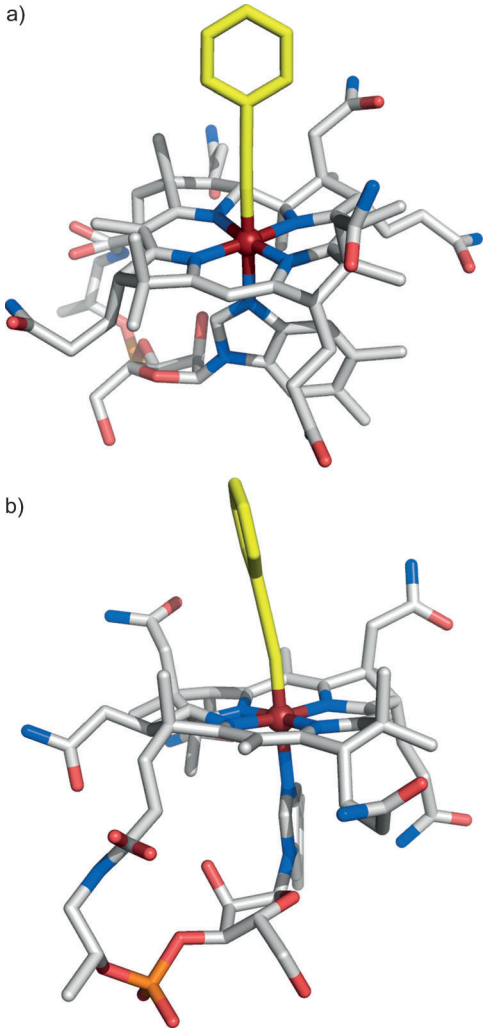


Figure 1. Two projections (a, b) of the crystal structure of Co_{β}-2-phenylethynylcobalamin (**3**); C gray, N blue, O red, P orange, Co dark red. The organometallic phenyethynyl group is colored bright yellow.

atoms and is shifted by 0.031(1) Å towards the α-DMB ligand, two structural features similar to those of vitamin B_{12} (**5**). The orientation of the phenyl group (in the crystal) is in "north–south", and the plane of the phenyl group has a twist of 15.36(10)° relative to the DMB ligand. The angle of the triple bond to the aromatic carbon is almost linear (C1L-C2L-C3L = 179.2(4)°) whereas the Co-C1L-C2L angle (172.1(3)°) is slightly bent. The triple bond C1L–C2L is longer (1.209(5) Å) than in organic alkynes (mean value of 1.18 Å[17,18]). The bond C2L–C3L (from the alkyne to the phenyl group) has a length of 1.444(5) Å, and is in the typical range of sp^2–sp bonds (mean value of 1.43 Å[17]).

Not unexpectedly, the alkyne group of **3** is bound with a short Co–C_{sp} bond (1.861(3) Å), which is slightly shorter than that reported for a model alkynyl Co^{III} complex.[19] The length of the Co–C_β bond in **3** also falls below the range found in all other known organometallic B_{12} derivatives.[14,15] Similarly, the *trans*-axial Co–N_α bond of **3** (2.084(2) Å) is also shorter than in other organometallic B_{12} derivatives (Figure 2). The correlated shortening (or lengthening) of the two axial bonds is a qualitative feature of organocorrinoids and has been described as the inverse "structural" *trans* effect.[14,15]

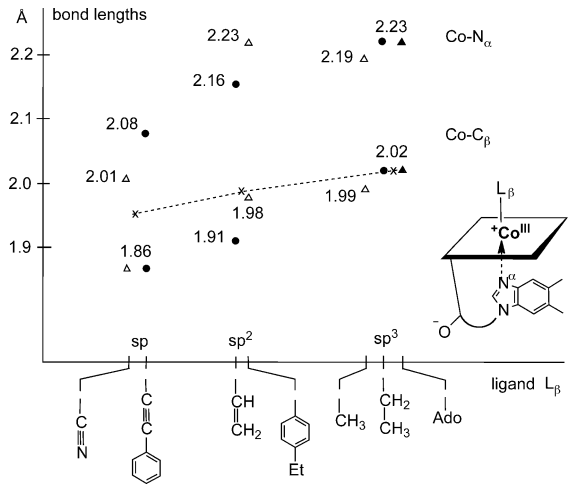


Figure 2. Lengths of axial bonds in selected organometallic B_{12} derivatives (see text). The C_β–Co bond lengths of organocobalamins correlate with the s character of the Co-bound carbon; that is, C_{sp}–Co < C_{sp2}–Co < C_{sp3}–Co. However, the organometallic bonds of the ethynylcobalamin **3**, of vinylcobalamin, and of vitamin B_{12} (**5**) are shorter than expected on the basis of the sum of the corresponding radii of covalent single bonds of C and of an (octahedral) Co^{III} center (see marks "x" on the dotted line).[20] Ado = 5′-deoxyadenosyl.

As shown in Figure 2, the lengths of the Co–C bonds of model organocobalamins correlate qualitatively with the hybridization of the directly bound carbon atom.[18] In fact, the length of the Co–C_β bonds in coenzyme B_{12} (**1**), methylcobalamin (**2**),[14,15] and ethylcobalamin[21] roughly matches the sum of the covalent radii of an sp^3 C (r = 0.76 Å) and of a low-spin Co^{III} ion (r = 1.26 Å).[20] However, in **3** the Co–C_β bond (1.861 Å) is strikingly shorter by about 0.09 Å than the sum of the covalent radii of an sp C (r = 0.69 Å) and the Co^{III} ion (see Figure 2). The length of the Co–C_β bond of 4-ethylphenyl cobalamin (1.98 Å)[10] roughly matches the sum of the covalent radii of an sp^2 C (r = 0.73 Å) and of the Co^{III} ion, whereas in vinylcobalamin it is considerably shorter (by about 0.08 Å).[22] The structural data may thus suggest a higher effective bond order of the Co–C_β bonds to the unsaturated carbon of ethynylcobalamin **3** (and of vinylcobalamin). A similar situation would also hold for **5**[16] (Figure 2).

The acetylide ligand of 2-phenylethynylcobalamin (**3**) exerts a thermodynamic *trans* effect that is roughly similar to that of the cyano group in **5**. A similar observation was made with ethynylcobalamin[11] and ethynylaquocobinamide.[23] The *trans* effect of alkynyl groups falls outside of the range known for alkyl and aryl groups in corresponding organocobalamins.[13] Thus, protonation of the DMB moiety of **3** is insignificant even at pH 1.6, indicating a tightly cobalt-bound DMB base. However, proteolytic detachment of the phenylethynyl moiety occurred readily at this pH ($t_{1/2}$ of about 15 min at room temperature), furnishing aquocobalamin (**6**) and phenylacetylene (Supporting Information, Figures S6 and S8). In the pH range from pH 2 to pH 5 the rate of decomposition of **3** at room temperature decreased linearly with the proton concentration, and **3** was extrapolated to be effectively rather stable at pH 7 and room temperature ($t_{1/2}$ ca. 350 days; Supporting Information, Figure S7). In contrast to the acidolytic removal of the organometallic ligand of **3** observed here, ethynylcobalamin was reported to decompose in acidic aqueous solution to acetylcobalamin by an acid-induced addition of water to the ethynyl group.[11] In both cases, the organometallic group presumably undergoes protonation at one of the sp-hybridized carbon atoms with intermediate formation of a stabilized, metal-coordinated vinyl cation. By this path, ethynylcobalamin is indicated to be protonated at the terminal carbon, whereas 2-phenylethynylcobalamin (**3**) appears to be protonated at the directly cobalt-bound carbon.

As expected, 2-phenylethynylcobalamin (**3**) displayed a high intrinsic resistance to thermolytic cleavage of its Co–C bond: a deoxygenated solution of **3** in dry DMSO could be heated to 100 °C for 5 days without significant decomposition (see the Supporting Information for details). An oxygen-saturated solution of **3** in [D_6]DMSO decomposed slowly at 100 °C ($t_{1/2}$ > 72 h), giving **6** and other Co-corrins (Supporting Information, Figures S11 and S12). At 120 °C **3** eventually decomposed in [D_6]DMSO (with a half-life of about 30 h) to a mixture of Co-corrins and some phenylacetylene. The decomposition products formed at this temperature were largely indicative of the cleavage of the Co–C bond.

In further contrast to other organocobalamins, an aerated aqueous solution of **3** did not undergo significant spectral changes when exposed to daylight for 75 h (Supporting Information, Figure S15). However, this observation is consistent with early findings with ethynylcobalamin,[13] and it is in line with the remarkable photostability of vitamin B_{12} (**5**).[24]

To explore the potential use of Co_β-2-phenylethynylcobalamin (**3**) in studies with mammals, binding of **3** to the

essential human B_{12} transporter intrinsic factor (IF) and transcobalamin (TC) was studied in an established competition assay with a fluorescent B_{12} conjugate.[10,25] As assumed on the basis of the known crystal structures of these two B_{12}-binding proteins,[7] the organometallic B_{12} derivative **3** had high affinities for them, similar as vitamin B_{12} (**5**): **3** attached with similar binding rates to IF ($k_{on} = 93\ \mu L\,mol^{-1}s^{-1}$) and to TC ($k_{on} = 129\ \mu L\,mol^{-1}s^{-1}$), and **3** also detached with a k_{off} rate ($1.5 \times 10^{-7}\ s^{-1}$), similar to **5** (Supporting Information, Figures S16 and S17). Therefore, **3** is likely to undergo uptake in humans and mammals with similar efficiency as vitamin B_{12} and as other natural cobalamins.

Co_β-2-phenylethynylcobalamin (**3**) is an organometallic B_{12} derivative that features a short and exceptionally strong $Co–C_{sp}$ bond. As a consequence, **3** is remarkably resistant to thermolytic cleavage of its organometallic bond. This alkynylcobalamin is also strikingly inert to visible light. However, **3** is prone to acid-induced decomposition. It may thus be of interest, to consider designing related, substituted alkynylcobalamins that would be expected to be more inert against proteolysis. Indeed, the procedure developed here for the synthesis of **3** uses the unique radical trapping capacity of cob(II)alamin (**4**),[10,26] and promises to provide a path for the efficient preparation of a range of alkynylcobalamins.

The alkynylcobalamin **3** is bound well by proteins of B_{12} uptake and transport. In analogy to the recently developed, light-sensitive 4-ethylphenylcobalamin,[10] but in contrast to vitamin B_{12} (**5**), the strong covalent Co–C bonds of organometallic ethynylcobalamins (such as **3**) may resist cleavage by the enzyme CblC[27] and further metabolic transformations to the typical biologically active B_{12} derivatives. In this case, suitable ethynylcobalamins are likely to function as alternative, light-stable B_{12} antimetabolites. Such compounds are now developed and may become useful (as light-stable "antivitamins B_{12}") in studies of induced "functional" B_{12} deficiency in laboratory animals.

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[28] CCDC 939826 (**3**, without structure factors) 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.

