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One-Pot Synthesis of the Metal-Free AD and BC Fragments of Vitamin B_{12}

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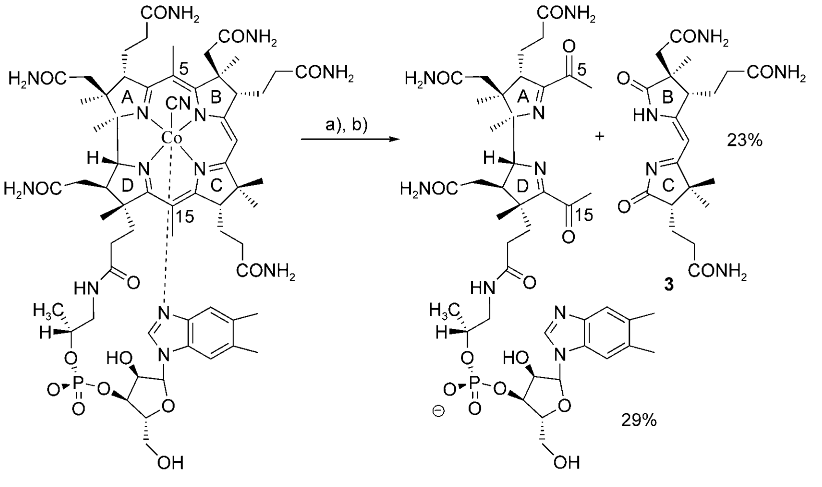
Cobalamins are essential cofactors for the metabolism in mammals.[1,2] Since fast proliferating cancer cells are strong consumers of vitamin B_{12} (**1**; abbreviated as B_{12}) the development of B_{12}-bioconjugates has recently attracted considerable attention.[3–10] On the other hand, the synthesis of vitamin B_{12} analogues with metals other than cobalt (met-balamins) is still an unsolved chemical challenge. Several attempts failed to remove the cobalt ion directly from B_{12}.[11,12] Although these potential antivitamins have been obtained from biotechnologically produced metal-free corrinoids followed by metal insertion,[13] thorough chemical and biological investigations of these metbalamin derivatives are still lacking.

A metal-templated macrocyclisation of suitable precursors in the form of the AD and BC fragments of the natural product would offer an attractive chemical alternative. This strategy is closely related to the first total synthesis of B_{12} achieved by the combined effort of Woodward et al. and Eschenmoser et al.[14–18] During the synthesis of the B_{12} precursor cobyric acid, a "western" AD fragment was successfully joined with an "eastern" BC fragment. The building blocks had to be synthesised in a linear sequence of 37 steps for the former and 17 steps for the latter fragment, respectively.[16,17] In our endeavour aimed at the development of metbalamins for biological studies, we explored an efficient one-pot strategy for the cleavage and demetallation of B_{12} into the related AD and BC fragments $\mathbf{2}^-$ and **3** (Scheme 1).



Scheme 1. Synthesis of the fragments $\mathbf{2}^-$ and **3** from B_{12} (**1**): a) $h\nu$, O_2, methylene blue, KCN, $C_2H_6O_2/CD_3CN$ (1:1), 20 °C, 5 d. Followed by b) 80 °C, 3 h.

Kräutler et al. and others investigated extensively the photo-oxidation of corrinoids[19–21] and demonstrated the efficient removal of the cobalt ion from oxidatively cleaved hydrophobic cobyric acid heptamethylester derivatives (abbreviated as cobester).[21–23] In contrast to the cobester, the synthesis and demetallation of the related natural counterpart, an oxidised bicyclic B_{12} derivative, has not yet been achieved. Considering the strong solvent dependence on the reactivity of singlet oxygen towards corrinoids,[21] we were able to efficiently photo-oxidise dicyano-B_{12} at the C-5 and C-15 *meso*-positions to the desired dicyano-tetraoxo-B_{12} derivative $\mathbf{5}^-$ in a 1:1 (v/v) mixture of [D$_3$]MeCN/[D$_3$]MeOD at room temperature (Scheme 2). After purification by preparative C18-reverse-phase HPLC, **5**-H was isolated in 46% yield.

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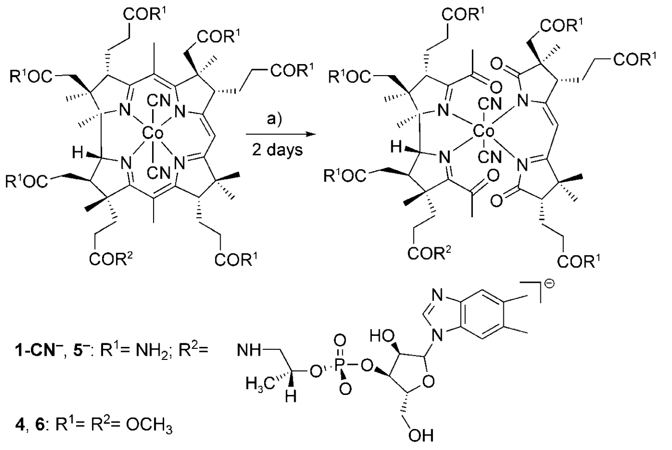


Scheme 2. Synthesis of **5**$^-$ and **6** from **1-CN**$^-$ and **4**: a) *hν*, O_2, methylene blue, CD_3OD/CD_3CN (1:1), 20 °C, 2d.

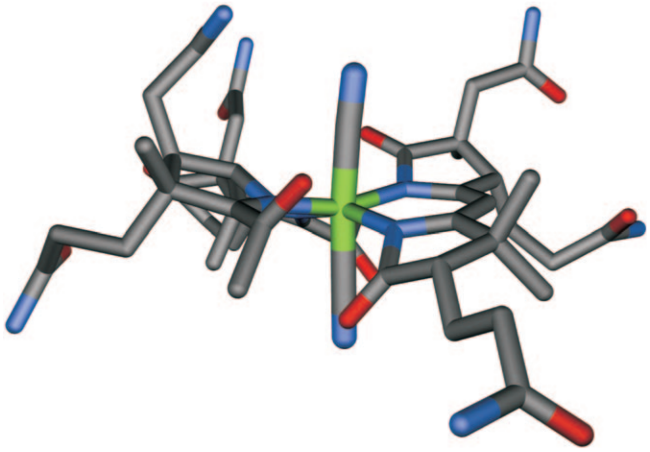


Figure 1. Energy minimised structure (Spartan 06, PM3 semi-empirical calculation) of a simplified model of **5**$^-$ (the hydrogen atoms have been removed for viewing clarity).

In the high resolution mass spectrum of **5**$^-$, an $[M]^-$ ion was observed at m/z 1444.5491 (m/z_{calcd} 1444.5507) consistent with a molecular formula of $C_{64}H_{88}CoN_{15}O_{18}P$.

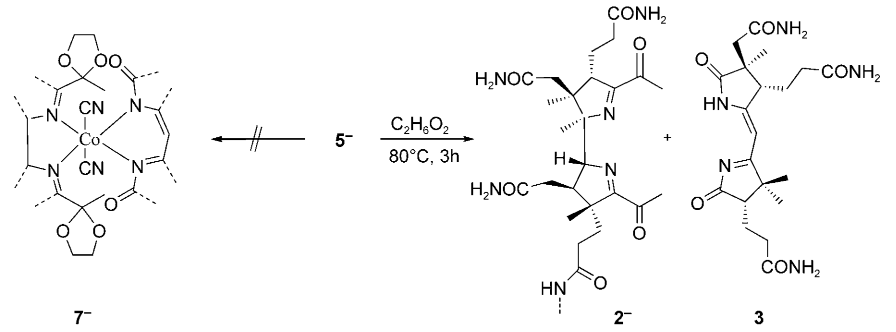
Further structural evidence was received by comparison of the UV/Vis and ^{1}H NMR spectra of **5**$^-$ with the bicyclic cobester derivative **6**.[21] Compound **6** was obtained from cobester **4** under the same reaction conditions as described for the preparation of **5**$^-$ (Scheme 2),[24] suggesting that the photo-oxidation occurred without further isomerisations at the periphery of the bicyclic macrocycle. Oxidised cobester derivatives have been demetallated with ethylenediaminetetraacetic acid (EDTA) after reduction with H_2 and Pt/C to the corresponding cobalt(II) complex.[21] However, this approach did not lead to success for the demetallation of the B_{12} derivative **5**$^-$.

We envisaged different strategies to synthesise the metal-free AD (**2**$^-$) and BC (**3**) fragments from **5**$^-$ and assumed that the key step in the decomplexation would still be the reduction of the robust cobalt(III) centre to the more labile cobalt(II) ion. To facilitate reduction, we intended to remove one cyanide with H^+ as well as Ag^+ ions, but observed only complex mixtures of **5**$^-$ with partially hydrolyzed amide side chains after running the reactions at elevated temperatures. These observations are contrary to the facile abstraction of cyanide from intact dicyano-corrinoids,[25,26] and could be explained by a stabilisation of the cobalt(III)–CN bonds due to a distortion of the octahedral coordination sphere. This assumption is supported by a semi-empirical quantum mechanical calculation (PM3, Spartan 06) carried out on a simplified model of **5**$^-$. These calculations suggest a helical distortion of the bicyclic macrocycle (Figure 1).

The two keto-functionalities of **5**$^-$ were critical towards reduction since they are susceptible to reduction to the corresponding alcohols. Therefore, we wanted to protect them as acetals prior to the decomplexation of the corresponding cobalt(II) or cobalt(I) ion. However, instead of acetal formation of **5**$^-$ to yield **7**$^-$, we observed removal of the cobalt ion and therefore, had developed a remarkable novel decomplexation method (Scheme 3). Stirring of crude **5**$^-$ in ethylene glycol at 80 °C for 3 h led to the formation of the desired fragments **2**$^-$ and **3**.

This suggests that the relative high effective concentration of the small bidentate ligand, ethylene glycol, favoured the decomplexation of cobalt from the sterically overcrowded macrocycle. Based on this observation, we developed a convenient one-pot synthesis of the desired metal-free fragments **2**$^-$ and **3** from **1**. The photo-oxidation of dicyano-B_{12} in a mixture of $[D_3]$MeCN/ethylene glycol followed by subsequent heating yielded **2**-H and **3** in 29 and 23 % isolated yields, respectively (Scheme 1).

The HRMS of **2**$^-$ and **3** showed an $[M]^-$ and $[M+Na]^+$ ion at m/z_{exp} 915.4016 (m/z_{calcd} 915.4023) and m/z_{exp} 442.2059 (m/z_{calcd} 442.2061) indicating the molecular formula to be $C_{42}H_{60}N_8O_{13}P$ for the former and $C_{20}H_{29}N_5NaO_5$ for the latter fragment.



Scheme 3. Attempted acetal formation (left) and observed decomplexation of **5**$^-$ in ethylene glycol (right).

Fragment $\mathbf{2}^-$ (Figure 2) and the slowly decomposing **3** showed absorption characteristics, chemical shifts and spin–spin splittings of their ^{1}H NMR spectra signals comparable to their hydrophobic bicyclic counterparts.[21,24] The acid–base properties of $\mathbf{2}^-$ and **3** were studied by UV/Vis pH titration and pK_a values of 5.5 and 6.2 were determined for **2**-H (Figure 3) and **3**-H$^+$, respectively. The pK_a value of the former fragment is comparable to that of α-ribazole with a protonation at N-3 of the dimethylbenzimidazole base (pK_a=5.6),[27,28] and similarities between the major changes in the absorption spectra between 250 and 300 nm of these two compounds suggest that the protonation site is the same for $\mathbf{2}^-$.

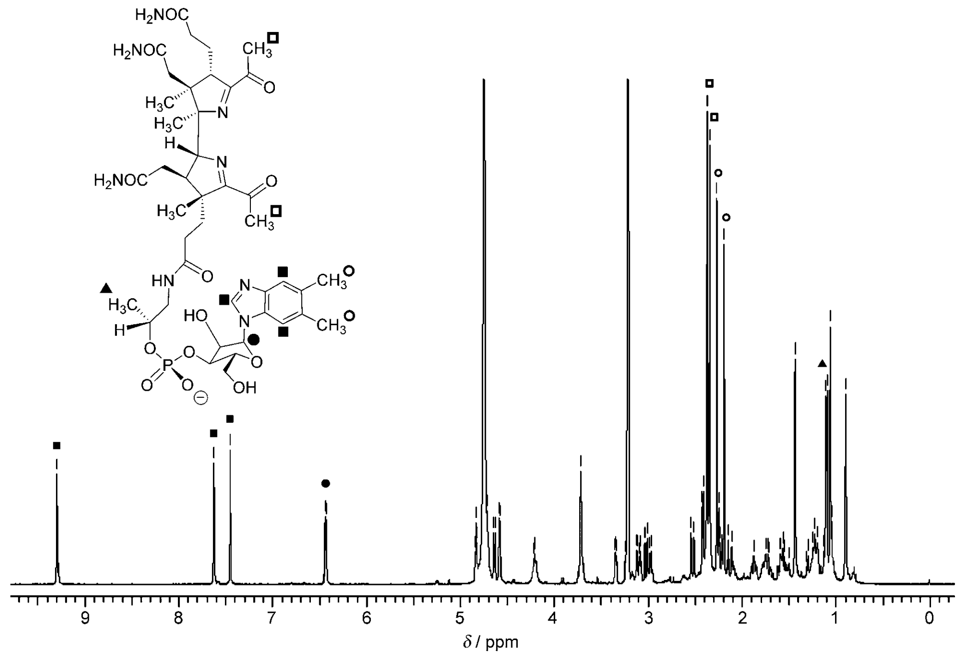


Figure 2. ^{1}H NMR spectra (500 MHz, D_2O, 300 K) of $\mathbf{2}^-$; some characteristic signals are indicated.

In summary, a one-pot synthesis of the AD and BC fragments of B_{12} that combines photo-oxidative scission with an unprecedented subsequent demetallation, is described. The fragments obtained are reminiscent of the western and eastern fragments described in the first total synthesis of B_{12} by Eschenmoser and Woodward. The readily accessible building blocks are important starting materials for the development of B_{12} derivatives with metals other than cobalt. Further investigations, as well as additional chemical transformations will be necessary on the way towards this challenging goal. Experiments towards a better understanding of the underlying demetallation reaction and an improvement of the isolated yields are currently under way.

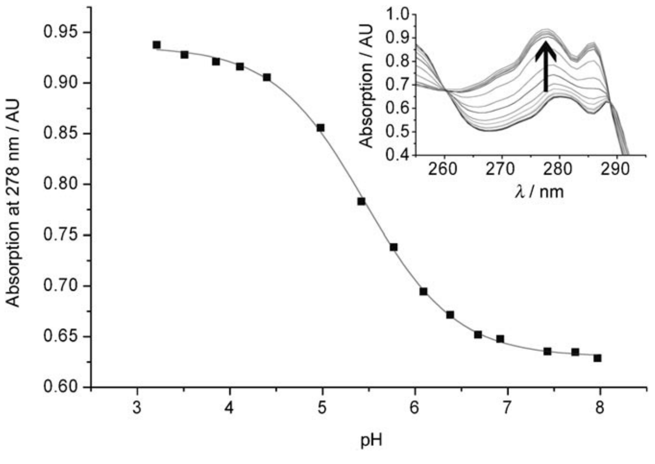


Figure 3. UV/Vis pH titration of a solution of $\mathbf{2}^-$ (278 nm). Inset: corresponding absorption spectra between 250 and 300 nm.

Experimental Section

Syntheses of $\mathbf{2}^-$, 3, $\mathbf{5}^-$, 6: The syntheses were performed by using a modified literature procedure[19] and the products were analyzed with UV/Vis, ^{1}H NMR spectroscopy, ESI-MS and ESI-HRMS (see the Supporting Information for details).

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Keywords: bioinorganic chemistry · coordination chemistry · corrinoids · demetalation · natural products

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