Biomimetics

DOI: 10.1002/anie.201001928

Vitamin B_{12} Mimics Having a Peptide Backbone and Tuneable Coordination and Redox Properties**

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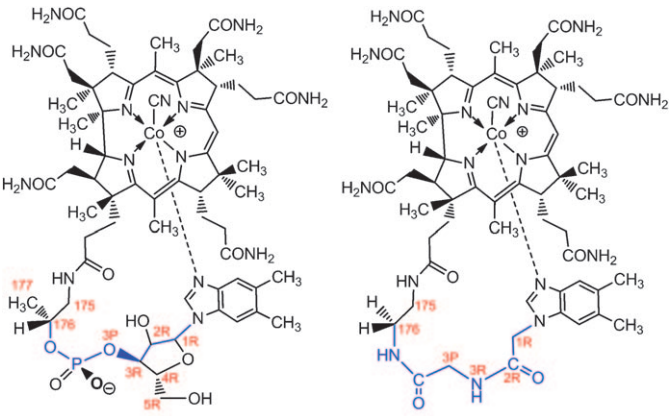
Cobalamines (Cbls) are corrinoids that can adopt different constitutional states, in which the dimethylbenzimidazole base (Dmbz) is either bound (base on) or has been displaced (base off) from the Co center of the macrocycle.[1,2] This equilibrium plays an important role for the delivery, transformation, and reactivity of vitamin B_{12} (**1**, CNCbl) and its organometallic analogues.[3] Small amounts of base-on vitamin B_{12} (10 μg per day) are channeled through a sophisticated and highly selective pathway into the cells before it is converted to the cofactors adenosylcobalamin (AdoCbl) and methylcobalamin (MeCbl).[4]

Only recently, the reductive decyanation of base-on CNCbl to a base-off Co^{II} species catalyzed by the trafficking chaperone *MMACHC* was reported.[5] Difficulties in this reduction are related to mutations of the corresponding *MMACHC* genes,[4] and medical studies suggest some therapeutic effect for Cbl derivatives that are easier to reduce than **1** to the intermediate Co^{II} state.[6,7]

Detailed studies have been reported for the inorganic chemistry of Cbls with different ligands at the β side (upper side),[8–10] but rather little is known how structural changes at the α side (lower side) of the molecule influence the coordination behavior of the Dmbz base[11–13] and the corresponding redox properties of the metal ion. Herein, we report on the coordination chemistry and accompanying electrochemical properties of a new class of vitamin B_{12} mimics in which a peptide linker tethers the corrin macrocycle to the Dmbz base.

Kräutler et al. demonstrated in an elegant study that a single methyl group quite distant from the coordination site stabilizes the base-on form of natural Cbls, as had been predicted earlier by Eschenmoser et al.[11,14] In another pioneering study, Toraya and Ishida replaced the α-ribofuranotide moiety in vitamin B_{12} with "methylene bridges" of different length.[12]

With a view toward future biological applications, we are interested in the development of artificial derivatives of vitamin B_{12} with tuneable coordination and electrochemical properties. We decided to investigate the replacement of the ribose phosphodiester moiety of vitamin B_{12} with peptide structures containing the same number of atoms between the corrin macrocycle and the nitrogen donor of the Dmbz base (Scheme 1), because amides have already been excellent mimics of phospodiesters in other natural products leading to derivatives with interesting novel physico-chemical properties and biological functions.[17–20]



Scheme 1. Structural formula of vitamin B_{12} (**1**) and the peptide B_{12} prototype $\mathbf{2}^+$. The natural linker in **1** and the peptide mimic in $\mathbf{2}^+$ contain the same number of atoms (shown in blue; the complete atom numbering is given in the Supporting Information).

An energy-minimized structure of $\mathbf{2}^+$ (Scheme 2, top right) was obtained from semiempirical quantum chemical calculations (PM3, Spartan '06 software) in which the Dmbz base is in a similar position as in its natural counterpart **1**. The artificial linker **8** was synthesized in six steps and coupled with dimethylaminopyridine and *N*′-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride to dicyanocobyric acid **7**[15] to yield **2** in 62% yield (Scheme 2).[16]

The high-resolution mass spectrum of **2** displays the signal of a $[M]^+$ ion at m/z 1243.59334 (m/z_{calc} 1243.59337) consistent with the molecular formula $C_{61}H_{84}CoN_{16}O_9$. Compound **2** was further characterized by UV/Vis spectroscopy, RP-HPLC, ^{1}H NMR analysis, and ^{1}H,^{13}C-HSQC correlations.[16] The UV/Vis spectra of base-on vitamin B_{12} (**1**) and **2** are identical, but differ significantly ($\Delta\lambda_{max} = 29$ nm) from the absorption spectra of aquocyano-cobinamide indicating that **2** occurs in its base-on form.[16] The corresponding ^{1}H NMR spectra show only minor shifts for the signals of the corrin macrocycle as well as the Dmbz base, but differ substantially for the connecting linker (Figure 1).[16]

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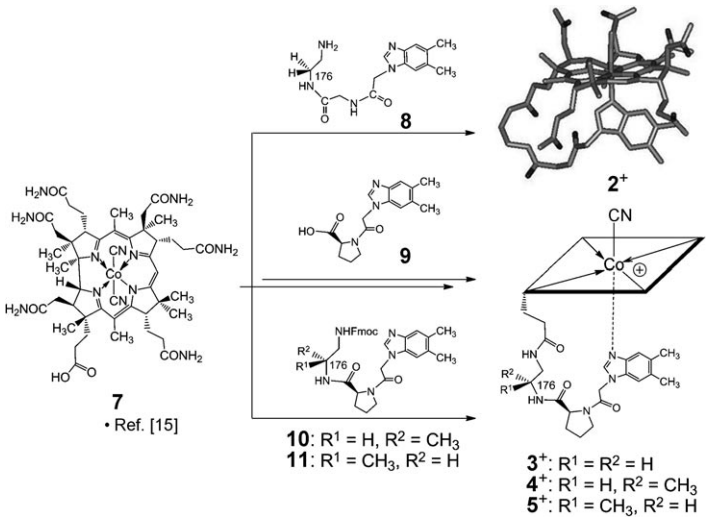
[**] We thank R. Alberto, A. Eschenmoser, T. Fox, and O. Zerbe for helpful discussions and L. Bigler for recording the HR-ESI-MS spectra. A generous gift of vitamin B_{12} from DSM Nutritional Products AG (Basel/Switzerland) is acknowledged. This work was supported by the Swiss National Science Foundation (grant no. 200021-117822).

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201001928.



Scheme 2. Synthesis of peptide B_{12} derivatives $\mathbf{2}^+$–$\mathbf{5}^+$ from dicyanocobyric acid (**7**)[15]and peptide linkers **8**–**11**.[16] Compounds $\mathbf{2}^+$–$\mathbf{5}^+$ were isolated as $\mathbf{2}^+\cdot CF_3COO^-$–$\mathbf{5}^+\cdot CF_3COO^-$ (**2**–**5**). Derivative $\mathbf{2}^+$(top right) is shown as the energy-minimized structure (Spartan '06, PM3 semiempirical calculation; H atoms not shown). Fmoc = 9-fluorenylmethoxycarbonyl.

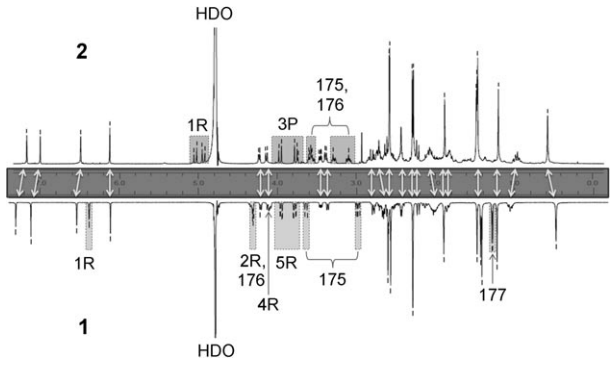


Figure 1. ^{1}H NMR spectrum of **2** (top) and **1** (bottom) in D_2O. The arrows indicate corresponding positions of signals arising from the corrin ring and the Dmbz base. The assignments refer to the protons of the linker (Scheme 1).

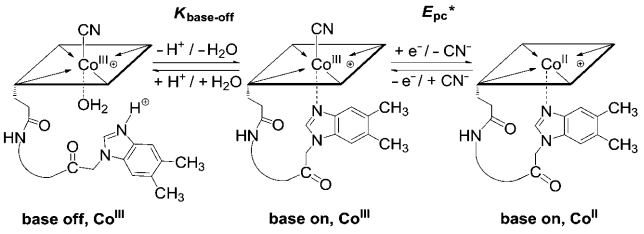
The intramolecular dissociation of the Dmbz base (Table 1, top left) of **2** was studied by spectrophotometric pH titration. The stability of the coordinated base-on form of **2** ($pK_{base\text{-}off} = 1.38$) is 19 times lower ($K^*$; Table 1 entry 2 vs. 1) than that of the natural counterpart, which displays strong intramolecular coordination and a low $pK_{base\text{-}off}$ value of 0.1.[2] On the other hand, the value indicates that the preference of **2** for the base-on constitution is 25 times greater than that of another artificial vitamin B_{12} analogue, **6**,[12,21] in which the linker contains a phosphodiester (Table 1 entry 6). The altered coordination properties of **2** affect the reduction of the octahedral cobalt(III) to the square-pyramidal cobalt(II) complex with loss of the β-cyano group (Table 1, top right) and was investigated with cyclic voltammetry (CV) in water ([Tris] = 0.2 M, pH 8.0; Tris = tris(hydroxymethyl)aminomethane). The CV trace of **2** displays a cathodic E_{pc}^* value at −1.056 V, a value 70 mV more positive than in **1**. The facilitated reduction of cobalt(III) in **2** can be explained by a lower electron density at the metal center resulting from the weaker coordination of the Dmbz base.

We envisaged that an enhanced rigidity of the linker structure might lead to a tighter intramolecular coordination which would make the reduction from cobalt(III) to cobalt(II) more difficult. First, we decided to replace the glycine unit in the linker of **2** with (L)-proline to yield **3** (Scheme 2). We thought that this cyclic building block might act as a turn mimic; it would mimic the α-ribose moiety of natural cobalamines and thus stabilize the base-on form. Compound **3** (Scheme 2) was synthesized as described in the Supporting Information. The stability of the base-on form of **3** ($pK_{base\text{-}off} = 0.97$) increased by a factor of 2.6 compared to the prototype **2**, and electrochemical studies showed that **3** is—as expected—more difficult to reduce to Co^{II} than **2** ($\Delta V = -21$ mV; Table 1, entry 3).

In natural cobalamines the methyl group at C176 of *R* configuration plays an important role in the stabilization of the base-on form,[11] and we envisaged a comparable effect for the peptide B_{12} mimics. To test this hypothesis, derivative **4**, which has an *R*-configured methyl group at C176, was synthesized from **10** and **7** (Scheme 2).[16] The base-on form of **4** ($pK_{base\text{-}off} = 0.62$) was favored by a factor of two, and the reduction from Co^{III} to Co^{II} was more difficult than in **3** ($\Delta V = -19$ mV; Table 1 entry 4 vs. 3), which lacks this modification. The influence of the remote methyl group on the base-on/base-off equilibrium in **4** is almost identical to that observed for natural cobalamines;[11] this underscores the utility of peptide structures as artificial linkers in vitamin B_{12} analogues.

In earlier studies Eschenmoser et al. claimed that a change in configuration at C176 from *R* to *S* may destabilize the base-on constitution of vitamin B_{12}.[14] This behavior can be explained by an additional *gauche* effect in the base-on form.[16] Encouraged by our findings with **4**, we synthesized and characterized its epimer **5** (Scheme 2). This derivative, in

Table 1: Base-on/base-off (left) and Co^{III}/Co^{II} equilibria (right).



Entry	Compound	$pK_{base\text{-}off}$	K^*[a]	E_{pc}^* [V][b]
1	**1**	0.1[c]	19	−1.126
2	**2**	1.38	1	−1.056
3	**3**	0.97	2.6	−1.077
4	**4**	0.62	5.8	−1.096
5	**5**	1.64	0.55	−1.039
6	**6**[d]	2.8[d]	0.04	n.r.[e]

[a] $K^* = K_{base-off(x)}/K_{base-off(2)}$. [b] $E_{pc}^* = E_{pc} - E_0$' (see the Supporting Information). [c] Ref. [2]. [d] Refs. [12, 21]. [e] n.r. = not reported.

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which the methyl group at C176 has an *S* configuration, displays a $pK_{base\text{-}off}$ of 1.64, an tremendous tenfold destabilization of the base-on form compared to that of **4**. It is remarkable that the energetically unfavorable conformation of the cyclic base-on structure of **5** outweighs the entropic gain obtained from the preorganization of the linker structure; this is reflected in a higher $pK_{base\text{-}off}$ value of **5** compared to that of the prototype **2** (Table 1 entry 5 vs. 2). The switch of configuration at C176 also has electrochemical consequences: derivative **5** is roughly 60 mV easier to reduce than its epimer **4**.

The peptide B_{12} mimics **2**–**5** show a linear correlation between the cathodic E_{pc}* values of the Co^{III}/Co^{II} reduction and their $pK_{base\text{-}off}$ values ($\Delta V = 57$ mV, $\Delta pK_{base\text{-}off} = 1.02$; Figure 2). Since the same β-cyano group is released from the cobalt center during reduction from cobalt(III) to

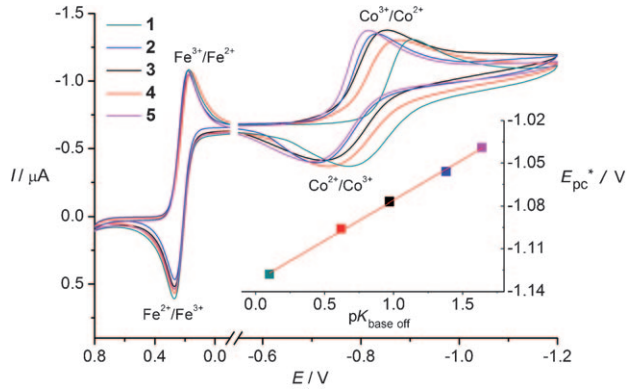

Figure 2. CV spectra of **1**–**5** in water ([Tris] = 0.2 M, pH 8.0) (reference electrode: Ag/AgCl, internal reference: $K_3Fe(CN)_6$). Inset: Plot of $pK_{base\text{-}off}$ vs. E_{pc}*. The value $pK_{base\text{-}off(1)} = 0.1$ is from reference [2].

cobalt(II) (Table 1, top right), the entropy of the reaction must be comparable and thus the reduction potentials are directly related to the enthalpy of the Co–CN bond. A lower $pK_{base\text{-}off}$ value corresponds to a stronger coordination of the Dmbz base and increasing back-donation from the Co ion to the ß-cyano group. This may subsequently hamper reduction and shift the redox potential to a more negative value.

In summary, we have introduced a new class of vitamin B_{12} mimics in which a peptide linker tethers the corrin macrocycle to the Dmbz base. Studies with four different peptide B_{12} derivatives demonstrated that through the appropriate design of the peptide backbone both the coordination and the accompanying redox properties at the Co center can be adjusted. This implies that it might be possible to fine-tunereactivity in cofactor-catalyzed reactions in which an intermediate base-on cobalt(II) species is the catalytically active species. The development of organometallic peptide B_{12} analogues for biological applications as well as further modifications of the linker for physico-chemical studies are subjects of current research.

Received: March 31, 2010
Published online: June 22, 2010

Keywords: bioinorganic chemistry · coordination chemistry · electrochemistry · peptides · vitamins

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