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Identification of Diastereomeric Cyano–Aqua Cobinamides with a Backbone-Modified Vitamin B12 Derivative and with ^{1}H NMR Spectroscopy

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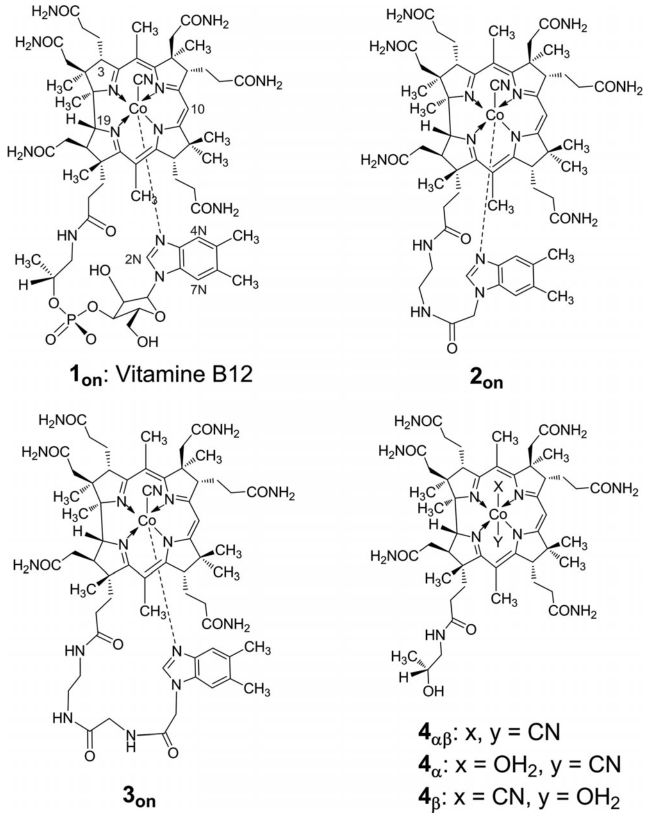
A new backbone-modified vitamin B12 derivative with an unusual configuration at the cobalt center has been used for the identification of the two axial diastereomers of cyano–aqua cobinamides (Cbi) by using ^{1}H NMR spectroscopy.

Introduction

Cobinamides (Cbi) are "incomplete" corrinoids lacking the dimethylbenzimidazole (Dmbz) -containing α-ribazole-3′-phosphate linker of natural cobalamins (Cbl).[1,2] These biologically important vitamin B12 (B12) precursors represent useful starting materials for the preparation of chemically modified analogues.[3,4] Recently, "incomplete" corrinoids have regained considerable attention as biologically inspired catalysts[5–7] as well as chemosensors for the straightforward detection of cyanide (Figure 1).[8–10]

Enormous effort has been devoted by Friedrich et al. to characterize axial diastereomers of cyano–aqua Cbi.[11–13] In other important studies, Brown et al. assigned, by heteronuclear NMR spectroscopy, the α- (lower side) and β- (upper side) positions of various Cbi with the help of chemically enriched axial ligands and the corresponding Cbl analogues.[14,15] For mixtures of diastereomeric cyano–aqua Cbi, the ^{13}C NMR signals of the axially coordinated cyano ligands partially overlap.[16] This behavior seems disadvantageous for the unequivocal assignment of the isolated isomers. For applications such as cyanide detection, only diastereomeric mixtures of "incomplete" cyano–aqua corrinoids have been employed,[17–20] whereas initial kinetic studies suggest differences in cyanide coordination.[20] The identification and evaluation of well-defined isomers are important with regard to future improvements of corrin-based chemosensors. In this communication, we report the identification of diastereomeric cyano–aqua Cbi with a backbone-modified vitamin B12 derivative and with ^{1}H NMR spectroscopy.



$\mathbf{1}_{on}$: Vitamine B12

$\mathbf{2}_{on}$

$\mathbf{3}_{on}$

$\mathbf{4}_{\alpha\beta}$: x, y = CN

$\mathbf{4}_{\alpha}$: x = OH_2, y = CN

$\mathbf{4}_{\beta}$: x = CN, y = OH_2

Figure 1. "Complete" cobalamines $\mathbf{1}_{on}$–$\mathbf{3}_{on}$ and "incomplete" Cobinamides $\mathbf{4}_{\alpha\beta}$, $\mathbf{4}_{\alpha}$, and $\mathbf{4}_{\beta}$. The subscript "on" refers to the "base on" constitution. The subscripts "α" and "β" indicate the position of the cyano ligand at the lower and upper side of the macrocycle, respectively (charges have been omitted).

Results and Discussion

Diastereomeric mixtures of "incomplete" cyano–aqua corrinoids are generally synthesized by non-selective displacement of one cyanide from the corresponding dicyano species under acidic conditions.[20] For instance, α-aqua, β-cyano- ($\mathbf{4}_{\beta}$) and α-cyano, β-aqua- ($\mathbf{4}_{\alpha}$) cobinamide are derived from $\mathbf{4}_{\alpha\beta}$ (Scheme 1, I) and separated by preparative reverse-phase C18 chromatography. Differences in their ^{1}H NMR spectra (Figure 3, bottom) can easily be identified in a clearly arranged region between 3.9 and 6.6 ppm for the

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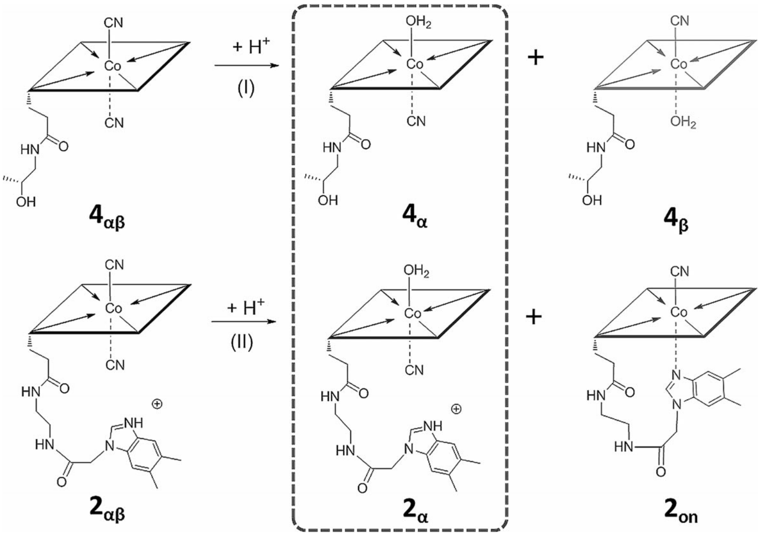


Scheme 1. Synthesis of $\mathbf{4}_\alpha$ and $\mathbf{4}_\beta$ from $\mathbf{4}_{\alpha\beta}$ (I) and of $\mathbf{2}_\alpha$ and $\mathbf{2}_{on}$ from $\mathbf{2}_{\alpha\beta}$ (II). $\mathbf{4}_\alpha$ and its structural analogue $\mathbf{2}_\alpha$ are depicted in a frame.

characteristic signals of H3, H10, and H19 of the corrin ring (Table 1, Entries 1 and 2; Figure 3, bottom). These differences are not conclusive with regard to the configuration at the cobalt center. In order to structurally identify these isomers, we planned to design a backbone-modified B12 derivative that mimics the structural and spectroscopic features of cyano–aqua Cbi in solution. We envisaged that the removal of one cyano ligand from a "complete" "base off" dicyano-Cbl derivative ($\text{Cbl}_{\alpha\beta}$) may lead to a mixture of Cbl_α and Cbl_{on} (see Supporting Information). "Base off" Cbl_α with a well-defined configuration at the metal center would represent a perfect surrogate of its "incomplete" counterpart and has comparable ^{1}H NMR signals for the protons at the corrin macrocycle.

Table 1. ^{1}H NMR data for 4_α, 4_β, 1_{on}, 2_{on} and $(2_{on} + 2_\alpha)$.[a,b]

Entry	Corrinoid	Configuration	H3[c]	H10	H19[d]	H2N	H4N	H7N
1	4_α	α-cyano, β-aqua	4.00	6.45	4.08			
2	4_β	α-aqua, β-cyano	4.11	6.52	4.25			
3	2_{on}	α-Dmbz, β-cyano	4.23	6.15	4.16	6.99	6.46	7.24
4a	$(2_{on} + 2_\alpha)$	α-Dmbz, β-cyano	4.24	6.17	4.18	7.01	6.48	7.25
4b	$(2_{on} + 2_\alpha)$	α-cyano, β-aqua	4.00	6.46	4.06	9.15	7.57	7.71
5	1_{on}	α-Dmbz, β-cyano	4.22	6.11	4.12	7.12	6.54	7.31

[a] 500 MHz, 300 K, $[D_2]D_2O$. [b] δ, ppm. [c] J = 8 Hz. [d] J = 11 Hz.

In a first experiment, dicyano-B12 ($1_{\alpha\beta}$) was treated with trifluoroacetic acid at pH 2 (see Supporting Information). Cyanide removal was indicated by a color change from violet to red ($\Delta\lambda_{max}$ = 30 nm), and the reaction products were subsequently isolated by using solid-phase extraction (SPE) techniques as described in the Experimental Section. The UV/Vis spectrum of the reaction solution coincide with that of $\mathbf{1}_{on}$ (Figure 2, top) and suggests the rapid formation of "base on" B12.

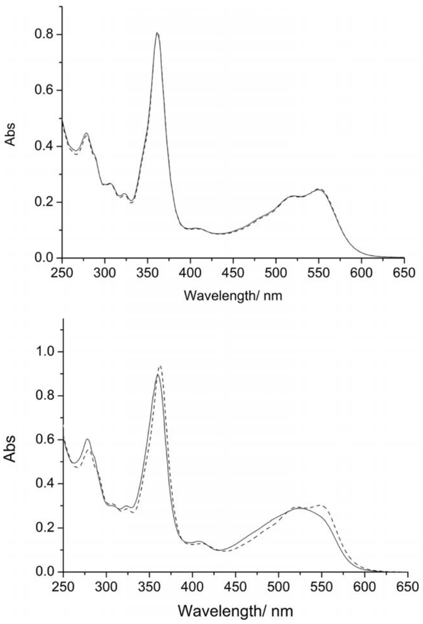
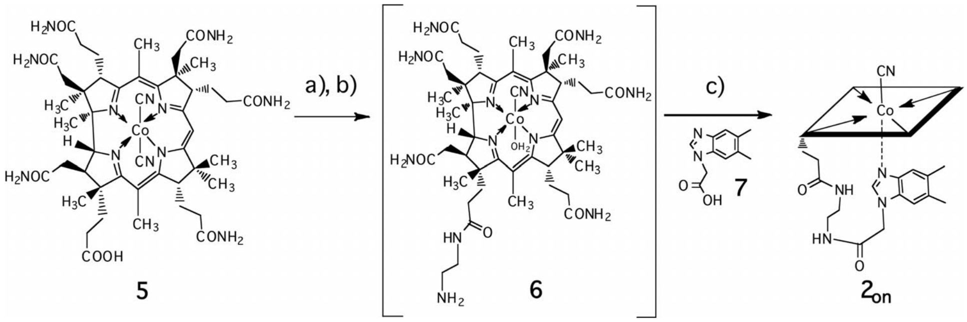


Figure 2. (bottom) UV/Vis spectra of ($\mathbf{2}_{on}$ + $\mathbf{2}_\alpha$) (solid line) derived from $\mathbf{2}_{\alpha\beta}$ (Scheme 1, II) in comparison with $\mathbf{2}_{on}$ (dashed line); (top) UV/Vis spectra of $\mathbf{1}_{on}$ (solid line) derived from $\mathbf{1}_{\alpha\beta}$ (see Supporting Information) in comparison with $\mathbf{1}_{on}$ (dashed line).

^{1}H NMR spectroscopy supports this observation and indicates the formation of more than 85% of $\mathbf{1}_{on}$ after SPE. Additionally, the formation of a minor product (<15%) was observed that further converted to $\mathbf{1}_{on}$. The signals of the corrin moiety of this intermediate overlap with the signals of $\mathbf{1}_{on}$. These drawbacks impede the analysis of the reaction mixture and preclude potential applications for the identification of diastereomeric Cbi. Apparently, cyanide removal from the lower side (α side) of the macrocycle followed by simultaneous coordination of the Dmbz base to the cobalt center as well as isomerization to $\mathbf{1}_{\text{on}}$ are strongly favored. This behavior can be explained by the strong intramolecular coordination of the Dmbz base to the metal center, which is expressed by a low $pK_{\text{base-off}}$ value of 0.1[2] for $\mathbf{1}_{\text{on}}$. We assumed that the synthesis of "base off" isomers of cyano–aqua Cbl would be more favorable, if intramolecular coordination of the Dmbz base to the metal center would be weaker than that in $\mathbf{1}_{\text{on}}$. Peptide B12, a recently introduced class of B12 mimics, possesses such tunable coordination properties.[21] These B12 derivatives with a corrin macrocycle and a peptide backbone instead of the α-ribofuranotide moiety of B12 have the same number of atoms between the metal center and the nitrogen-anchoring group of the Dmbz base.

For this project, we decided to synthesize a novel derivative $\mathbf{2}_{\text{on}}$ with a 3-atom shorter backbone than the linker of the previously described prototype $\mathbf{3}_{\text{on}}$.[21] We speculated that this structural deviation would lead to a destabilization of the coordinated "base on" form. A "shortened linker" is also advantageous with regard to ^{1}H NMR analysis, since it contains fewer protons that potentially interfere with the protons of the corrin ring. The B12 derivative $\mathbf{2}_{\text{on}}$ was synthesized from dicyano-cobyric acid (**5**) through an intermediate **6** and then coupled with **7** as reported earlier for $\mathbf{3}_{\text{on}}$ (Scheme 2).[21] The high-resolution mass spectrum of $\mathbf{2}_{\text{on}}$ indicates a $[M]^+$ ion peak at 1186.57087 ($m/z_{\text{calcd.}}$ = 1186.57191), which suggests a molecular formula of $C_{59}H_{81}CoN_{15}O_8$. The UV/Vis spectrum (Figure 2, bottom in dashed line) and ^{1}H NMR signals of the corrin ring and the Dmbz base of $\mathbf{2}_{\text{on}}$ were in accordance to those of $\mathbf{1}_{\text{on}}$ (Table 1, Entries 3 vs. 5) and other peptide B12 derivatives in its "base on" forms.[21] Spectrophotometric pH titration reveals that the "base on" stability of $\mathbf{2}_{\text{on}}$ ($pK_{\text{base-off}}$ = 2.58) is indeed 16 and 302 times lower than that of $\mathbf{3}_{\text{on}}$ ($pK_{\text{base-off}}$ = 1.38[21]) and $\mathbf{1}_{\text{on}}$ ($pK_{\text{base-off}}$ = 0.1[2]), respectively. For the synthesis of axial diastereomers of **2** (Scheme 1, II), we followed the same strategy as that described for the experiments with $\mathbf{1}_{\alpha\beta}$.

The UV/Vis spectrum of the reaction product differs this time significantly from that of the corresponding "base on" form ($\mathbf{2}_{\text{on}}$) and suggests rather the existence of a mixture of "base on" and "base off" compounds (Figure 2, bottom). The ^{1}H NMR spectrum shows clearly two sets of signals for the characteristic signals of the corrin ring at H3, H10, and H19 [Figure 3, top ($\mathbf{2}_{\text{on}}$ + $\mathbf{2}_{\alpha}$)] and of the aromatic Dmbz base (Table 1, Entries 4a and 4b) in a ratio of approximately 1:1. This information indicates univocally the existence of only two forms of backbone-modified B12 derivatives in solution. The chemical shifts and the coupling constants of one set of signals are identical to those of $\mathbf{2}_{\text{on}}$ (Table 1, Entries 3 vs. 4a). Cobalt-coordinated cyanide at the lower side (α side) of $\mathbf{2}_{\alpha\beta}$ was substituted by the Dmbz base during cyanide removal to form $\mathbf{2}_{\text{on}}$ (Scheme 1, II). The other set of signals in the mixture show an indicative downfield shift for the protonated Dmbz base between 0.46–2.14 ppm (Table 1, Entries 4a vs. 4b).[22] This suggests the existence of $\mathbf{2}_{\alpha}$ with a cyano ligand at the lower side of the macrocycle derived from the abstraction of the β-cyano ligand from $\mathbf{2}_{\alpha\beta}$. A "complete" cyano–aqua Cbl with a cyano ligand at the α position has not yet been reported under neutral conditions, to the best of our knowledge.[23] This behavior can be explained by the 300-times higher preference of the "base off" form of $\mathbf{2}_{\text{on}}$ relative to that of $\mathbf{1}_{\text{on}}$. The mixture of ($\mathbf{2}_{\text{on}}$ + $\mathbf{2}_{\alpha}$) synthesized from $\mathbf{2}_{\alpha\beta}$ with two separated sets of indicative ^{1}H NMR signals (H3, H10 and H19; Table 1, Entries 4a and 4b) serve now for the identification of $\mathbf{4}_{\alpha}$ and $\mathbf{4}_{\beta}$. Subtraction of the characteristic set of signals of pure $\mathbf{2}_{\text{on}}$ (Table 1, Entry 3) from that of ($\mathbf{2}_{\text{on}}$ + $\mathbf{2}_{\alpha}$) (Table 1, Entries 4a + 4b) leads to signals (almost) that are identical to those of $\mathbf{4}_{\alpha}$ (Table 1, Entry 1; Figure 3, top). Friedrich et al. reported earlier on "incomplete" cyano–aqua corrinoids such as cyano–aqua cobinamide and cyano–aqua cobyric acid. They assumed that the slower migrating derivatives shared the same configuration at the cobalt center and assigned it to the α- (lower side) cyano form on a tentative basis (Figure 4, inset).[12,13] Later, Firth et al. showed that a freshly dissolved sample of crystallographically characterized α-cyano, β-aqua cobyric acid was indeed the slower migrating derivative.[24] The UV/Vis spectra of $\mathbf{4}_{\alpha}$ was slightly blueshifted relative to that of $\mathbf{4}_{\beta}$ (Figure 4). This is in agreement with earlier reports and confirms now unequivocally Friedrich's earlier assumptions on the configuration at the metal center.[11–13]



Scheme 2. Synthesis of $\mathbf{2}_{\text{on}}$ from **5**: (a) NEt_3, ethyl chloroformate, N-*Boc*-ethylenediamine, DMF, 5 min, 25 °C. (b) HCl (1 M), 1 h, 25 °C. (c) EDC·HCl, DMAP, DMF, 4 h, 0–25 °C.

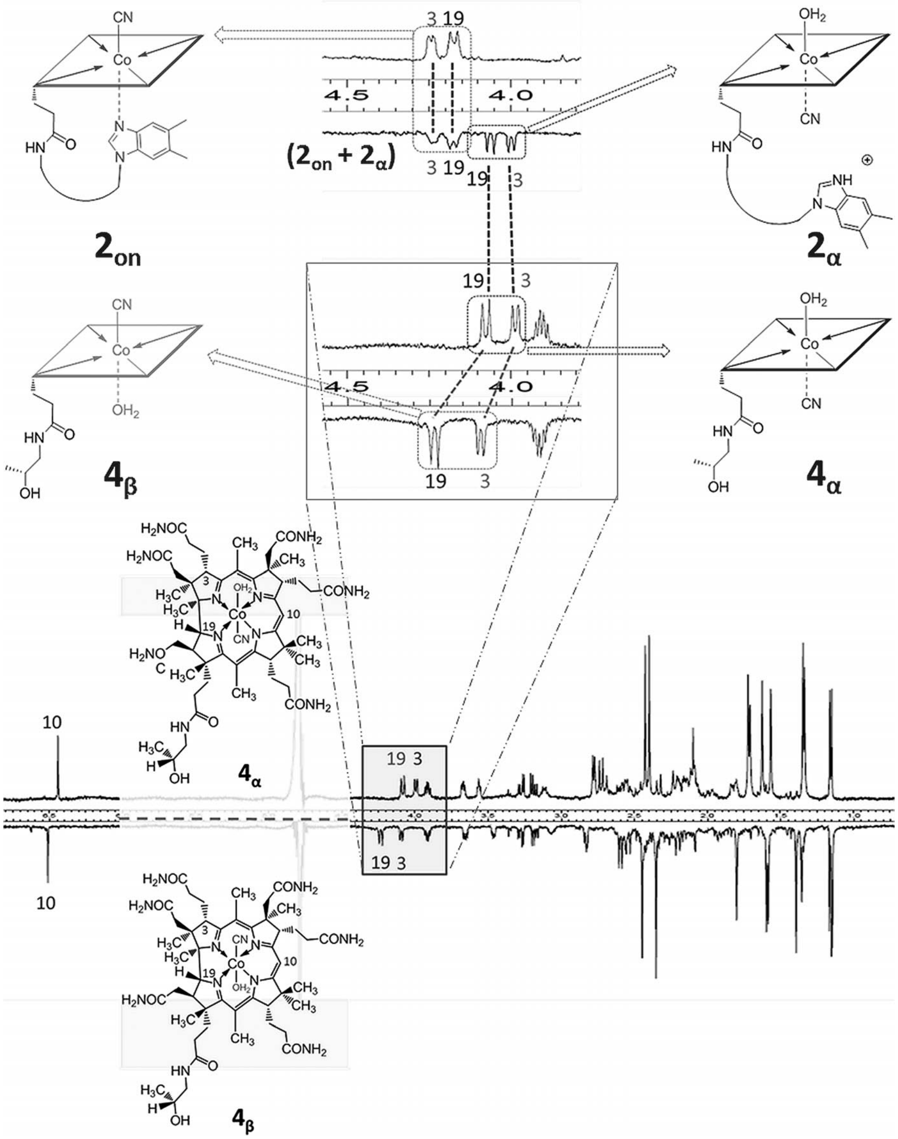
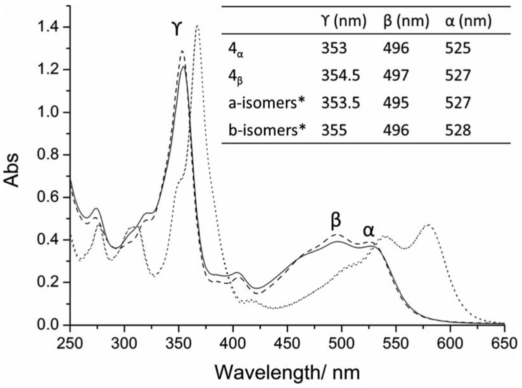


Figure 3. (Bottom) ^{1}H NMR spectra of $\mathbf{4}_{\alpha}$ (above) compared to $\mathbf{4}_{\beta}$ (below). Characteristic signals are indicated. (Top) Identification of $\mathbf{4}_{\alpha}$ by analysis of the chemical shifts of H3 and H19: signals ($\mathbf{4}_{\alpha}$) = signals [($\mathbf{2}_{on}$ + $\mathbf{2}_{\alpha}$) – $\mathbf{2}_{on}$] (500 MHz, 300 K, D_2O).



	ϒ (nm)	β (nm)	α (nm)
4_{α}	353	496	525
4_{β}	354.5	497	527
a-isomers*	353.5	495	527
b-isomers*	355	496	528

Figure 4. UV/Vis spectra of $\mathbf{4}_{\alpha}$ (α-cyano,β-aqua-cobinamide: dashed line) and $\mathbf{4}_{\beta}$ (α-aqua,β-cyano-cobinamide, solid line). [$\mathbf{4}_{\alpha}$] = [$\mathbf{4}_{\beta}$] = 46 μM. The dotted lines over layer and indicate $\mathbf{4}_{\alpha\beta}$ from the reaction of $\mathbf{4}_{\alpha}$ and $\mathbf{4}_{\beta}$ with excess KCN* (ref.[11–13]).

Conclusions

We present a new backbone-modified vitamin B12 derivative with an unusual configuration at the cobalt center. This compound has been used for the identification of the two axial diastereomers of cyano–aqua cobinamides (Cbi) by using ^{1}H NMR spectroscopy. The results are in agreement with earlier tentative assignments. We envisage that backbone-modified B12 derivatives with adjustable coordination properties will find further interesting chemical, analytical and biological applications.

Experimental Section

Synthesis of $\mathbf{4}_{\alpha}$ and $\mathbf{4}_{\beta}$: Compound $\mathbf{4}_{\alpha\beta}$ (20.8 mg, 20 mmol) was dissolved in 0.1% aqueous TFA (10 mL), and the reaction solution was stirred for 10 min. Compound $\mathbf{4}_{\alpha}$ (10.1 mg, 8.8 mmol, 44%) and $\mathbf{4}_{\beta}$ (10.3 mg, 9.0 mmol, 45%) were separated by preparative

HPLC as the corresponding TFA salts. ^{1}H NMR (500 MHz, [D$_2$]D$_2$O): see Supporting Information. MS (ESI-MS): *m*/*z* (%) = 1015.6 (100) [M – H$_2$O]$^+$. HPLC-UV/Vis: R_t = 8.8 min ($\mathbf{4}_\alpha$), R_t = 13.5 min ($\mathbf{4}_\beta$).

Synthesis of $\mathbf{2_{on}}$: Intermediate **6** was synthesized from dicyanocobyric acid (4.9 mg, 5.0 µmol) as described in previous work.[21] It was dissolved in DMF (1 mL) and cooled to 0 °C, after which DMAP (1.0 mg, 8 µmol) and **7**[21] (4.5 mg, 15 µmol) were added. After 10 min, EDC·HCl (3.0 mg, 15 µmol) was added. The solution was warmed up to room temperature. After 4 h, the solvent was removed under reduced pressure. The residue was washed with acetone and further purified with preparative HPLC to afford $\mathbf{2}_{on}$ (3.8 mg, 2.9 µmol, 58 %) as the corresponding TFA salt. ^{1}H NMR (500 MHz, [D$_2$]D$_2$O): see Supporting Information. HR-MS: calcd. for $C_{59}H_{81}CoN_{15}O_8$ [M]$^+$ 1186.57191; found 1186.57087. UV/Vis (*c* = 30 µM, 0.2 M KCl, pH = 5.98): 548 nm (3.96), 521 nm (3.94), 413 nm (3.72), 361 nm (4.43), 279 nm (4.27), HPLC-UV/Vis: R_t = 14.2 min; p$K_{\text{base-off}}$ = 2.58.

Synthesis of ($\mathbf{2_{on}} + \mathbf{2_\alpha}$): Compound $\mathbf{2}_{on}$ (1.3 mg, 1.0 µmol) and KCN (1.3 mg, 20 µmol) were dissolved in water (5 mL) to obtain a violet solution of $\mathbf{2}_{\alpha\beta}$. The pH value of the solution was adjusted to 2 with trifluoroacetic acid. After purging the solution with N_2 for 20 min to remove HCN, the reaction solution was adsorbed on a reverse-phase Chromafix® C18ec cartridge. It was washed with water (30 mL) and then eluted with MeOH (3 mL). The solvent was removed under vacuum to yield $\mathbf{2}_{on}$ and $\mathbf{2}_\alpha$ as a mixture of the corresponding TFA salts (1.3 mg, 100 %). ESI-MS: *m*/*z* (%) = 1186.6 (100) [M]$^+$ for $\mathbf{2}_{on}$ or [M – H$_2$O]$^+$ for $\mathbf{2}_\alpha$.

Synthesis of ($\mathbf{1_{on}} + \mathbf{1_\alpha}$): The synthesis and separation was performed as described for the synthesis of ($\mathbf{2}_{on}$ + $\mathbf{2}_\alpha$) starting from $\mathbf{1}_{\alpha\beta}$. The UV/Vis spectrum of ($\mathbf{1}_{on}$ + $\mathbf{1}_\alpha$) is shown in Figure 2 (top).

Supporting Information (see footnote on the first page of this article): Materials and methods, the general atom numbering of B12 derivatives, the synthesis of Cbl$_\alpha$ and Cbl$_{on}$, and NMR chemical shifts for $\mathbf{2}_{on}$, $\mathbf{4}_\alpha$, and $\mathbf{4}_\beta$.

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