Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Immobilised Vitamin B12 as Biomimetic Model for base-off/ histidine-on Coordination

Christine Männel-Croisé and Felix Zelder*

Institute of Inorganic Chemistry, University of Zürich, Winterthurerstrasse 190, 8057 Zürich, Switzerland

zelder@aci.uzh.ch

Contents

Material and Methods

Vitamin B_{12} (**3**), hydroxocobalamin hydrochloride (**2**), adenosine, ammonium chloride, zinc, DL-histidine, methyl iodide, potassium cyanide, silica, HCl and KOH were obtained from Fluka or Sigma Aldrich. Adenosylcobalamin (**1**) was synthesized according to literature.[S1]

The pH values of solutions were adjusted by addition of HCl (6N or conc.) or KOH (2N or conc.) with the help of a Metrohm 827 pH lab. Chromabond C18ec polypropylene columns (100 mg), Chromafix C18ec cartridges (270 mg) and a Chromabond adapter PP were obtained from Machery-Nagel AG Switzerland.

Methanol (0.5 ml) and water (10 ml) were pressed with the help of a syringe through the columns (cartridges) in order to condition the *C18ec silica* material before further use. Afterwards an aqueous solution of **1**-**3** (1 ml, 20 µM) was passed through the *C18ec silica* material and the cobalamins were adsorbed as colored rings on the top of the white solid material.

UV-vis spectra were measured at T = 21 ±1°C with a Cary 50 spectrometer using quartz cells with a path length of 1 cm.

DRUV-vis spectra were recorded on a Perkin-Elmar Lambda 50 spectrometer equipped with an integrating sphere setup (diameter 110 mm) using quartz object holder and pure $MgSO_4$ as reference material.

NMR spectra were recorded on a Bruker *AV-500* spectrometer (Karlsruhe, Germany). The chemical shifts are given in ppm relative to the signal from the deuterated solvent. The data processing was carried out with ACD/SpecManager (*Advanced Chemistry Development*). Mass spectra were recorded either in the positive or negative mode on an Esquire HCT from Bruker (Bremen, Germany).

LC-MS was performed using a Waters Acquity Ultra Performance LC with a Macherey Nagel column C-18ec RP (5 µm particle size, 100 Å pore size, 250 x 3 mm. Flow rate: 0.3 ml min^{-1}); solvent system: 0.1% formic acetic acid in water (solvent A); MeOH (solvent B); gradient: 25% B for 5 min, then 100% B in 25 min and 100% B for 10 min.

HPLC was performed using a Merck Hitachi system equipped with an Interface 7000, a Diode Array Detector L-7455, a pump L-7100 and Macherey Nagel Nucleosil C-18ec RP columns (5 µm particle size, 100 Å pore size, 250 x 3 mm. Flow rate: 0.5 ml min^{-1}). The solvent system and the gradient was the same as for LC-MS.

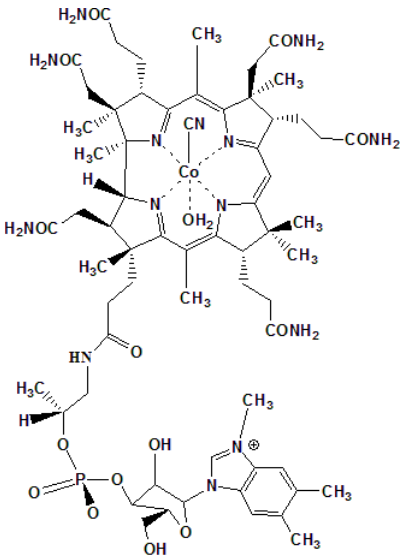
Preparative HPLC purification was performed using a Merck RP 18 LiChroCART® 250-10 column (5 µm particle size, 100 Å pore size, 250 x 10 mm. Flow rate: 0.5 ml min^{-1}). The solvent system and the gradient was the same as for LC-MS.

Scanning electron microscopy images were acquired on a JEOL JSM-6060.

pH dependent measurements. A Chromabond C18ec column that is connected to a 5 ml syringe via a Chromabond adapter PP (Macherey-Nagel) was loaded with aqueous solutions of the desired Cbl (1 ml, 20 µM). pH dependent measurements were performed by passing solutions with the desired pH value (5 ml) through the column and allow to equilibrate for 2 h. The remaining solution was removed afterward from the solid-phase and the pH value was rechecked.

The silica C18ec material was pressed out of the column with a second syringe connected to the lower side of the column. The colored part of the C18ec material with the immobilised Cbl was used for DRUV-vis measurements. Investigations with unmodified silica were performed in the same way.

The $pK_{base\text{-}off}$ values were obtained from the analysis of a Boltzmann function: $y = A_2 + (A_1 - A_2) / (1 + \exp((x - x_0) / dx))$ fitting the shift of the γ-band for all Cbls but **1** (α/β-band).



Compound 4^+. A Chromafix C18ec cartridge (270 mg) was loaded with vitamin B12 (10^{-4} M, 1 ml, 2% MeOH). Compound $\mathbf{4}^+$ was prepared by pressing 2 ml of methyl iodide through the cartridge. The upper and the lower sides of the cartridge were closed by syringes. After 4 days, excess of methyl iodide was removed from the cartridge and the orange product was subsequently eluted with methanol. The solvents were removed under reduced pressure. The residue was purified by preparative HPLC to afford **4** ($\mathbf{4^+CF_3COO^-}$) (yield of 4 cartridges: 1.4 mg, 0.9 µmol, 38 %).

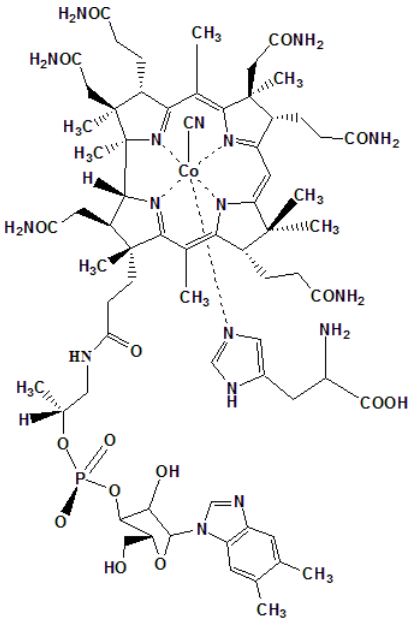
DRUV-Vis: (see Table S1)

^{1}H-NMR (500 MHz, D_2O): (see Table S2).

MS (ESI-MS): m/z (%): 1370.1 (100) $[M + H]^+$.

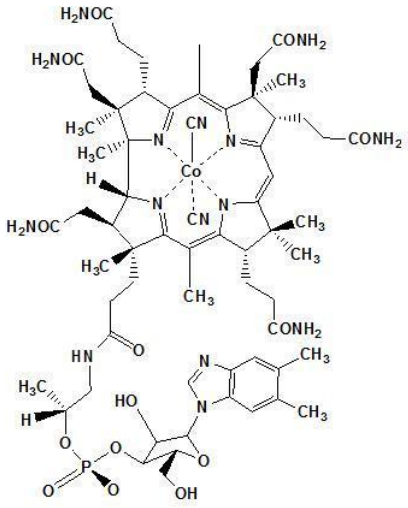
LC-MS: R_t = 20.1 min

A control experiment was carried out in solution. An aqueous solution of vitamin B12 (1 mM; 2 ml) and methyl iodide (2 ml) were stirred for 7 days at room at 30 °C. The reaction was periodically tested with LCMS, but compound **4**$^{+}$ was not observed.



Compound 5. A Chromabond C18ec column was loaded with vitamin B12 (1 ml, 40 µM). An aqueous solution of DL-histidine (1.8 mM; 7 ml) was pressed through the cartridge and it was allowed to react for 24 hours at room temperature. The preparation for DRUV-vis measurements was carried out as described in the section of pH depending investigations.

DRUV-Vis: (see Table S1)



Compound 6^{-}. A Chromabond C18ec column was loaded with vitamin B12 (1 ml, 20 µM). An aqueous solution of KCN (5 ml; 1.04 mg/ l; [Hepes] = 20 mM; pH 7.5) was passed through the column (1 ml/ s). Compound **6**$^{-}_{SP}$ was indicated by a color change from red to violet. The preparation for DRUV-vis measurements was carried out as described in the section of pH depending investigations.

DRUV-Vis: (see Table S1)

^{1}H-NMR (500 MHz, MeOD): (see Table S2).

Table S1. Selected diffuse reflectance data for Cbls $\mathbf{1}_{SP}$ $\mathbf{-6^-}_{SP}$:

Cbl	Diffuse reflectance spectroscopy				
	pH	λ_{max} (nm)			
$\mathbf{1}_{SP}$	7.0	-	-	375	457
$\mathbf{2}_{SP}$	0.2	352	405	496	520
	9.0	355	417	511	533
$\mathbf{3}_{SP}$	0.1	355	403	497	527
	7.0	358	403	-	528
$\mathbf{4^+}_{SP}$	7.0	355	403	497	527
$\mathbf{5}_{SP}$	7.0	361	410	520	551
$\mathbf{6^-}_{SP}$	7.0	367	419	543	578

Table S2. Selected ^{1}H NMR spectroscopy data for Cbls **3**, $\mathbf{4^+}$:

cobalamin	^{1}H NMR (**3**, $\mathbf{4^+}$) chemical shift (ppm)				
	B2	B4	B7	R1	H10
3	7.04	6.46	7.23	6.30	6.03
$\mathbf{4^+}$	9.18	7.51	7.52	6.45	6.38

The assignment was performed by comparison to B12 (**3**) (S[2]).

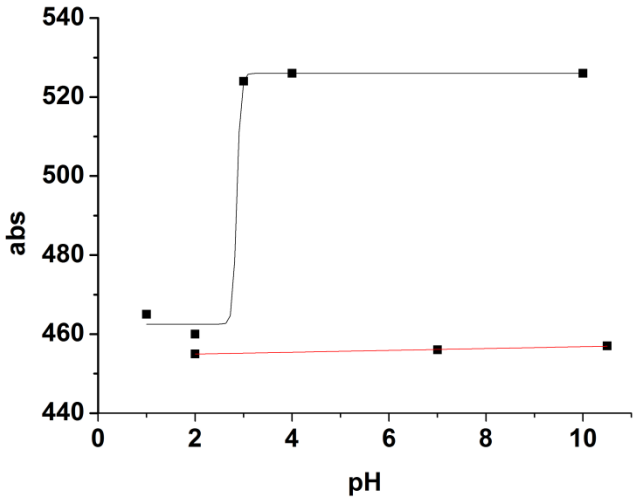


Figure S1. pH-dependence of the absorption maxima of the α/β band of Ado-Cbl (**1**) immobilised on *silica C18ec* (**1_{SP}**; *red line*) or on unmodified *silica* (**1_{Silica}**; *black line*; $pK_{base\text{-}off}$ = 2.9).

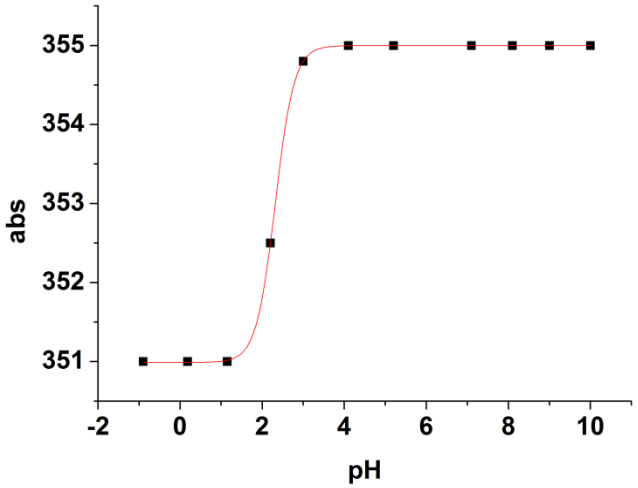


Figure S2. pH-dependence of the absorption maxima of the γ-band of aqua-Cbl **2^+** immobilised on *silica C18ec* (**2^+_{SP}**; $pK_{base\text{-}off}$ = 2.3)**.**

References

S[1] K. L. Brown, S. Cheng, X. Zou, J. Li, G. D. Chen, E. J. Valente, J. D. Zubkowski, H. M. Marques, *Biochemistry* **1998**, *37*, 9704.

S[2] P. Butler, M. O. Ebert, A. Lyskowski, K. Gruber, C. Kratky, B. Kräutler, *Angewandte Chemie-International Edition* **2006**, *45*, 989.