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Supporting Information

69451 Weinheim, Germany

Access to Organometallic Arylcobaltcorrins through Radical Synthesis: 4-Ethylphenylcobalamin, a Potential "Antivitamin B_{12}"**

*Markus Ruetz, Carmen Gherasim, Karl Gruber, Sergey Fedosov, Ruma Banerjee, and Bernhard Kräutler**

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Supporting Information

General.

Materials. Aquocobalamin chloride *Roussel UCLAF;* water was purified using Epure, Barnstead Co; 4-ethylaniline (*Merck, for synthesis*) was distilled prior to use; acetone *puriss. p.a.*, sodium formate *puriss p.a*., sodium nitrite, *puriss,* tertrafluoroboric acid 50% *purum* were from *Fluka*, acetonitrile (MeCN) and methanol (MeOH) HPLC-grade were from *BDH prolabo*, 1 g Sep-Pak-C18 Cartridges were from *Waters Associates.* LiChroprep RP-18 (25 40μm) was from *Merck.*

Spectroscopy. *UV/Vis Spectra*: Hitachi-U3000; λ_{max} (log ε) in nm. *CD Spectra*: Jasco J715; λ_{max} or λ_{min} (Δ ε) in nm. *^{1}H and ^{13}C-NMR spectra:* Bruker AM 300 (for the diazonium salt) and 500 MHz Varian Unity Inova (for **EtPhCbl**), equipped with 5 mm triple-resonance probe with z-gradients; δ(HDO) = 4.77 ppm; HSQC: 2k x 512 complex data points. SW2 11 ppm centered around the residual water signal, SW1 -10-200 ppm. 64 scans per increment. HMBC: 2k x 512 data points (real). SW2 11 ppm centred around a residual water signal, SW1 -10-200 ppm. 64 scans per increment. ROESY:2k x 512 complex data points, 32 scans per t1 increment, mixing time 250 ms. *EI- and ESI-MS*: Finnigan MAT95-S*,* positive-ion mode, spray voltage 1.4 kV, solvent MeOH.

HPLC: Hitachi HPLC system with manual sampler, L-2130 pump, online degasser and diode array detector L2130 , reverse phase C18 column (Phenomenex Hyperclone 250 x 4.6 mm); Solvent A: 10 mM potassium phosphate (pH 7.0), solvent B: MeOH; standard solvent compositions: (A/B) as function of time (0 - 20 min): 80/20 to 20/80.

Binding and dissociation experiments with the Cbl-specific proteins. All reactions were performed in 0.2 M Na-phosphate buffer, pH 7.5, 22°C.

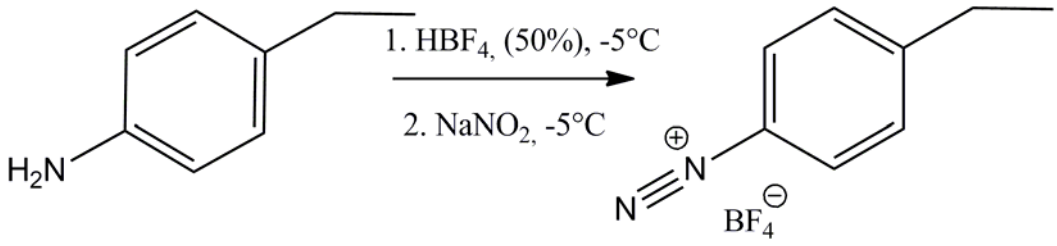
The binding kinetics was monitored on the stopped-flow spectrofluorometer DX.17 MV (Applied Biophysics, UK) using the fluorescent conjugate **CBC** (see ref. [S1]), excitation 525 nm, emission > 550 nm, slit 1.5 mm, voltage 380 – 400, band pass of 18.6 nm, light path of 1 cm. Binding reactions were started by rapid injections of **CBC** or **CBC** + **XCbl** mixture to the protein (E). All final concentrations were equal to 0.5 μM. The records corresponded to averaging of 4 – 6 individual curves. Current fluorescence F was recalculated to the concentration, $[E{\cdot}\mathbf{CBC}] = [E]_0 \cdot (F - F_{max}) / (F_0 - F_{max})$.
Dissociation was monitored on Varian Eclipse fluorometer (Varian, Australia). The binding protein E (1.0 μM) was loaded with a test B_{12}-derivative **XCbl** (1.3 μM) and incubated over 1 – 2 hours at room temperature. The dissociation was initiated by **CBC** (1.3 μM), whereupon changes in fluorescence were recorded (excitation 525 nm, emission 550 nm, slits 5 nm, photomultiplier PMT = 600 V, averaging time 1 s). Averaging of two independent records was done in each case. Background drift of fluorescence for free CBC was subtracted. Current fluorescence F was recalculated to the concentration, $[E{\cdot}\mathbf{CBC}] = [E]_0 \cdot (F - F_{CBC}) / (F_{E{\cdot}CBC} - F_{CBC})$.

Processing of EtPhCbl and MeCbl by CblC. Dealkylation of EtPhCbl and MeCbl was followed in 100 mM HEPES pH 8.0, 150 mM KCl, 10% glycerol buffer at 25°C and monitored in the dark and under aerobic conditions. The reactions were initiated by the addition of 1mM glutathione (GSH) to a mixture containing 50 μM CblC and 40-45 μM EtPhCbl or MeCbl in a final volume of 200 μl. Formation of aquocobalamin (H_2OCbl) was followed at 355 nm.

Binding of EtPhCbl to CblC. Titrations were performed on a VP-ITC microcalorimeter (1.44 ml cell volume) (Microcal Inc., Northampton, MA), equipped with a ThermoVac sample degasser and a 300-μl syringe. The buffer, protein and ligand solutions were filtered and degassed prior to running the experiments. In all experiments, the ligand loaded in the syringe was added to the protein in the sample cell. CblC (5-10 μM) was titrated with twenty-nine 10 μl aliquots from a 70-125 μM solution of EtPhCbl in 100 mM HEPES pH 8.0, 150 mM KCl, 10% glycerol buffer at 25°C. The calorimetric signals were integrated, and the data from three independent experiments were analyzed with Microcal ORIGIN software using a single-site binding model to determine the equilibrium association constant, K_A.

Synthetic Procedures

Synthesis of 4-ethylphenyldiazonium tetrafluoroborate (procedure modified from that described for the synthesis of 4-tert.-butylphenyldiazonium tetrafluoroborate [S2])



In a 100 ml 3-necked round bottom flask, 2.0 ml of 4-ethylaniline (1.87 g, 15.8 mmol) was mixed with 20 ml of distilled water at room temperature. 8.9 ml 50 % aqueous HBF_4 (71.1 mmol) were slowly added and the resulting solution was cooled to -5°C. A saturated aqueous solution of 2.19 g (32.2 mmol) $NaNO_2$ was added drop wise within 15 min (white crystals formed). Stirring was continued for additional 15 min at -5°C, then the off-white crystals were filtered off using a Büchner funnel, washed with a small amount of ice-cold water and dried under high vacuum. In this way 2.14 g (9.7 mmol, 61.8 % yield) of crystalline 4-ethylphenyl-diazonium tetrafluoroborate were obtained, with the following spectral data: **UV/Vis:** (c = 2.3 x 10^{-5} M, H_2O): λ_{max} (log ε) = 280 (4.31) nm. **^{1}H-NMR:** (300 MHz, D_2O, 298 K, c = 21 mM): 1.29 (*t*, J = 7.6 Hz, 3H, -CH_2-C**H_3**), 2.93 (*q*, J = 7.6 Hz, 2H, -C**H_2**-CH_3) 7.81 (*d*, J = 8.7 Hz, 2H, m-ArH), 8.46 (*d*, J = 8.7 Hz, 2H, o-ArH).(see Figure S1) **^{13}C-NMR:** (75 MHz, D_2O, 298 K, c = 21 mM): 13.7, 29.7, 109.9, 131.5, 132.4, 162.1. **MS:** Electron impact (EI): 124.09 (32, $[4\text{-EtPhF}]^+$), 109.06 (100, $[4\text{-EtPhF-H}_3C]^+$).

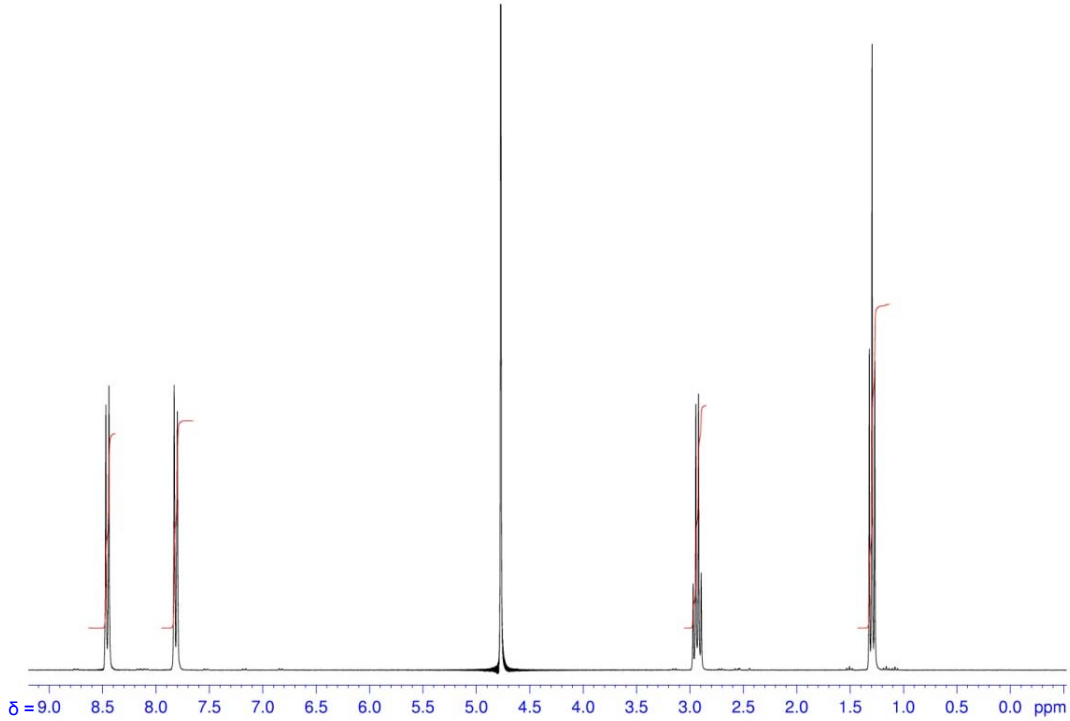
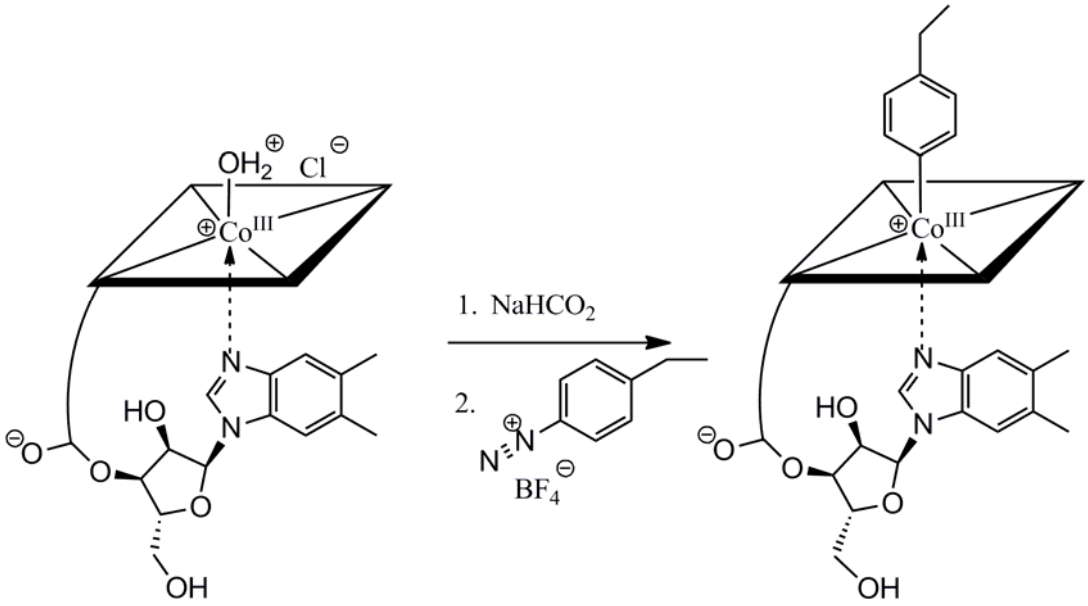


Figure S1. 300 MHz ^{1}H-NMR-spectrum of **4-ethylphenyl-diazonium tetrafluoroborate** in D_2O (298 K, c = 21 mM)

Synthesis of Co$_\beta$-(4-ethylphenyl)-cobalamin (EtPhCbl)



H_2OCbl **EtPhCbl**

In a 10 ml two necked round bottom flask, 50.0 mg of aquocobalamin chloride (**H_2OCbl**) (36.2 μmol) were dissolved in 2 ml of distilled water and the mixture was degassed with argon for 15 min. To this solution 43.5 mg sodium formate (0.65 mmol) was added under argon and the solution was stirred for 30 min at RT. To the now brown solution 12.2 mg 4-ethylphenyldiazonium tetrafluoroborate (55.5 μmol) were added under argon and the mixture was stirred for another hour. All further operations were done under protection from light. The reaction mixture was poured in 15 ml of acetone to precipitate the raw product. The mother liquor was removed and the orange-red precipitate was dried under high vacuum for 2 hours. The raw product was purified by RP-18 column chromatography using MeCN/phosphate buffer pH7 (50mM), solvent system (5 - 30% MeCN, in 2% steps). Aquocobalamin **H_2OCbl** was eluted first at 12 % MeCN followed, at 16 % MeCN, by the red fraction of the product **EtPhCbl**. The product fraction was collected and the solvents were evaporated on rotary evaporator at room temperature. Raw orange-red corrin **EtPhCbl** was dissolved in water (l ml). This solution was applied to a 1 g RP-18 cartridge, which was first washed with water (20 ml), followed by elution of a red substance with MeOH (5-7 ml). Solvents were evaporated using a rotary evaporator, and the red residue was dissolved in water (0.3 ml) and precipitated with 3 ml of acetone. The mother liquor was removed and the precipitate (of **EtPhCbl**) was dissolved in 0.4 ml of water and crystallised after addition of 5 ml of acetone. The crystals of **EtPhCbl** were first washed with (1:9) mixture of water: acetone, then with acetone. The sample of **EtPhCbl** was dried under high vacuum over night, to give 29.1 mg (20.3 μmol, 56% yield) of red crystalline solid, which was characterized as follows: **TLC:** RP-18 Silica gel 60, MeCN/H_2O (3/7): R_f = 0.39. **UV/Vis:** (c = 3.31 x 10^{-5} M, 50 mM phosphate buffer pH 7): λ_{max} (log ε) = 518 (3.80), 473 (3.65), 374 (3.88), 343(4.04), 281(4.16), 268 (4.18) nm (see Figure S2). **CD:** (c = 3.31 x 10^{-5} M, 50 mM phosphate buffer pH 7): λ_{max}, λ_{min}; 545 (–6.2), 467 (5.7), 403 (-1.7), 384.5 (0.1), 361.5 (–3.4), 335 (2.8), 291 (–3.1), 267 (-5.0), 231 (4.6) nm (mol^{-1} cm^3 cm^{-1}); λ_0: 494, 419, 388, 382, 354, 310, 245, 241, 220 nm (see Figure S3). **^{1}H-NMR:** (500 MHz, 298 K, D_2O, c = 4.2 mM) δ = 0.49 (*s*, 3H, H_3C-1A), 0.90 (*s*, 3H, H_3C-12B), 0.94-1.05 (*m*, 4H, HC-81) superimposed by 1.00 (*t*, 3H, J =

7.3 Hz, H_3C-8L)), 1.17 (*s*, 3H, H_3C-17B), 1.18-1.25 (*m*, 4H, HC-82) superimposed by 1.22 (*d*, J = 6.5 Hz, 3H, H_3C-177), 1.27 (*s*, 3H, H_3C-2A), 1.42 (*s*, 3H, H_3C-12A), 1.60-2.03 (*m*, 13H, H_2C-21, 171, HC-31, 71, 81, 82, 131, 171) superimposed by 1.87 (*s*, 3H, H_3C-7A), 2.03-2.55 (*m*, 18H, H_2C-32, 172, 181 HC-31, 131) superimposed by 2.23 (*s*, 3H, H_3C-11N), 2.30 (*s*, 3H, H_3C-10N) and 2.34 (*q*, 2H, J = 7.3 Hz, H_3C-7L), 2.55-2.81 (*m*, 12H, HC-18, 71, H_2C-132, 181) superimposed by 2.65 (*s*, 3H, H_3C-151) and 2.75 (*s*, 3H, H_3C-51), 2.98 (*dd*, 1H, J = 8.7, 14.5 Hz, H_aC-175), 3.34-3.40 (*m*, 2H, HC-13,19), 3.43 (*dd*, J = 5.0, 9.6 Hz, 1H, HC-8), 3.55 (*d*, J = 14.5 Hz, 1H, H_bC-175), 3.75 (*dd*, J = 3.0, 12.6, Hz, 1H, H_aC-5R), 3.93 (*dd*, J = 2.0, 12.6 Hz, 1H, H_bC-5R), 4.13 (*m*, J = 8.5 Hz, 1H, HC-4R), 4.22-4.27 (*m*, 2H, HC-3, 2R), 4.33 (*dd*, J = 6.4, 13.8 Hz, 1H, HC-176), 4.77 (*ddd*, 1H, HC-3R, superimposed by water peak), 5.75 (*d*, J = 7.8 Hz, 2H, HC-2L, 6L), 5.99 (*s,* 1H, HC-10), 6.26 (*d*, J = 2.8 Hz, 1H, HC-1R), 6.61 (*s*, 1H, HC-4N), 6.66 (*d*, J = 7.8 Hz, 2H, HC-3L, 5L), 7.18 (*s*, 1H, HC-7N), 7.24 (*s*, 1H, HC-2N) (see Figure S4 and Table S1). **MS:** ESI pos, MeOH: m/z (%) = 1458.7 (14), 1457.7 (36), 1456.7 (40, $[M+Na]^+$); 1436.7 (41), 1435.7 (84), 1434.7 (100, $[M+H]^+$).

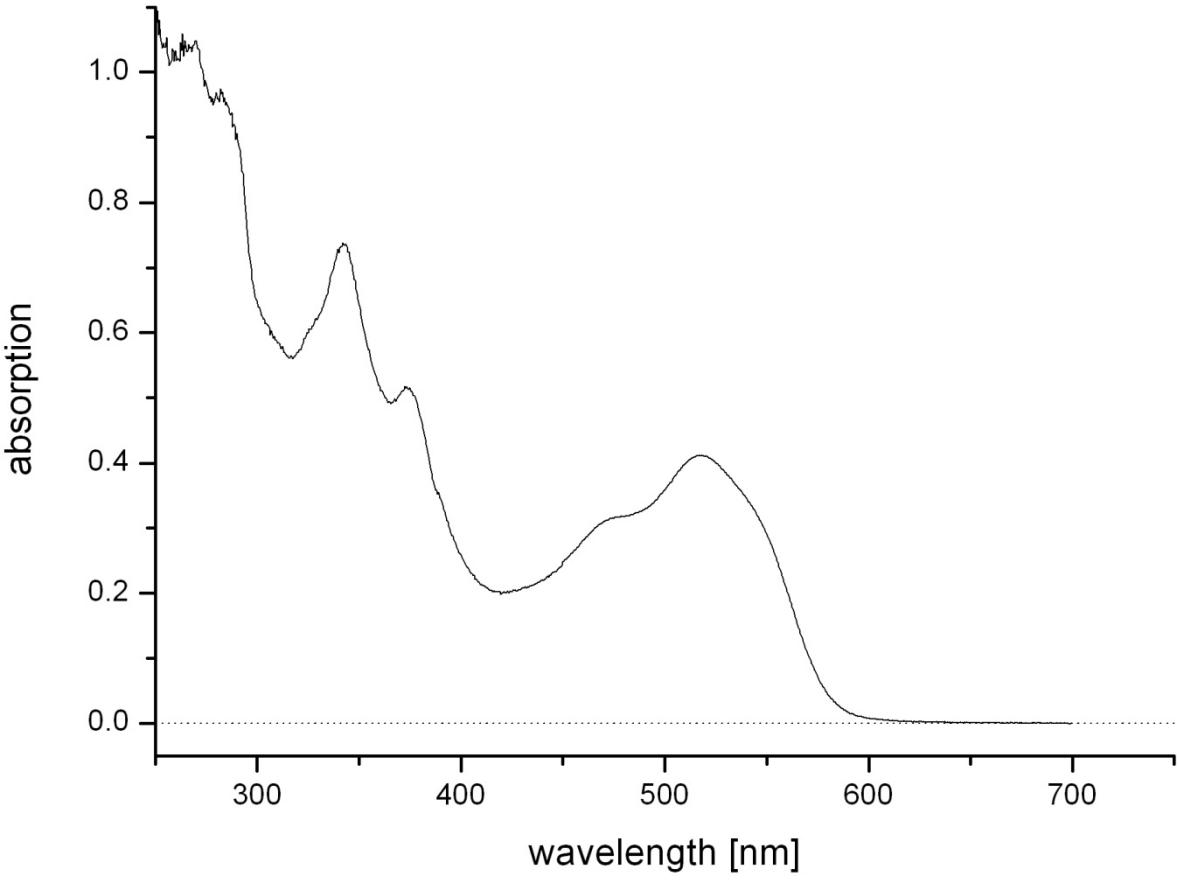


Figure S2. UV/Vis spectrum of **EtPhCbl** (c = 3.31 x 10^{-5} M, in 50 mM aquous phosphate buffer pH7)

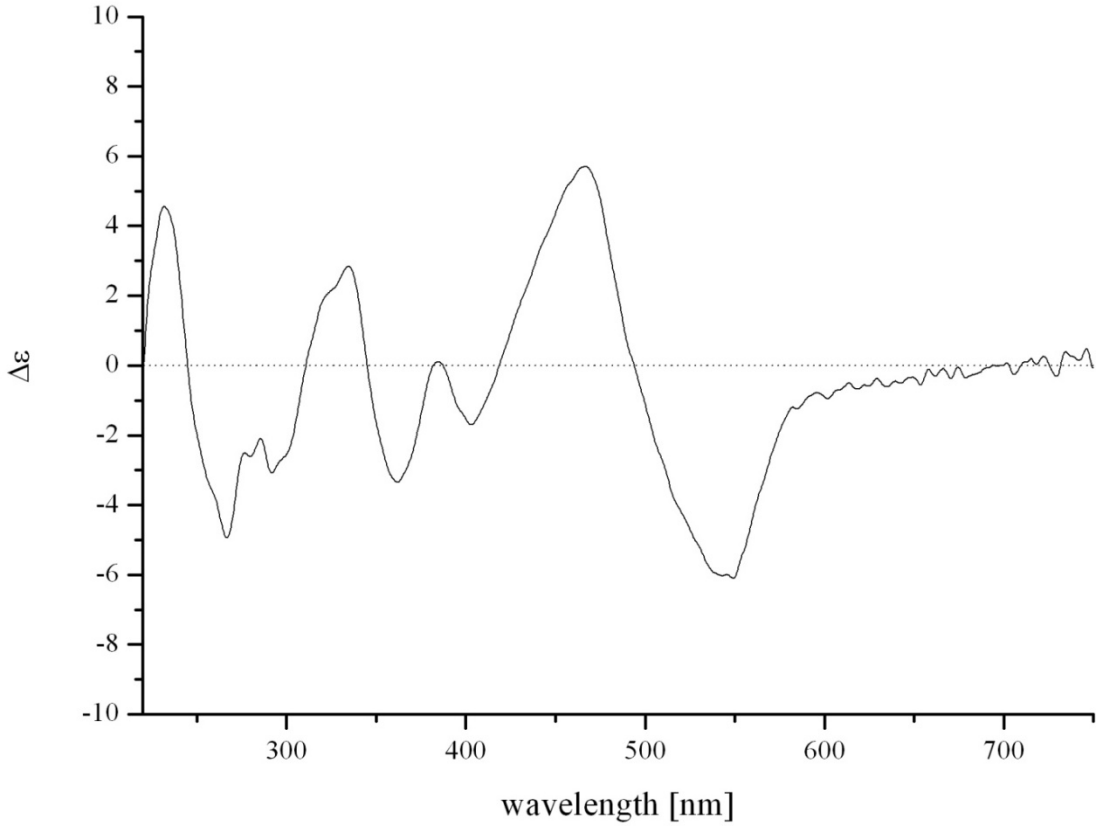


Figure S3. CD spectrum of **EtPhCbl** (c =3.31 x 10^{-5} M, in 50 mM aqueous phosphate buffer, pH7)

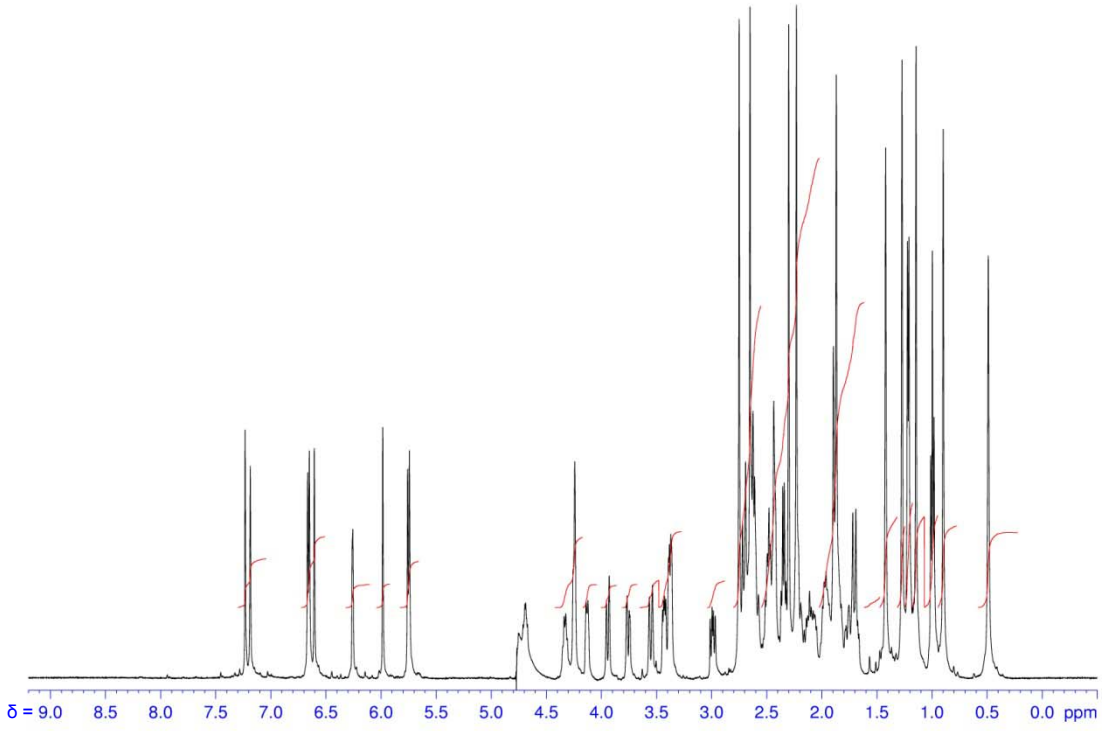


Figure. S4 500 MHz ^{1}H-NMR of **EtPhCbl** in D_2O (298 K, c = 4.2 mM)

Table S1 **Assignments of signals** / chemical shift values of ^{1}H- and ^{13}C-NMR-spectra of **EtPhCbl** in D_2O (500 MHz Varian Unity Inova spectrometer, 298K, c = 4.2 mM)

Assignment	δ(^{13}C) [ppm]	δ(^{1}H) [ppm]	Assignment	δ(^{13}C) [ppm]	δ(^{1}H) [ppm]
C1	88.1	-	C16	178.4	-
C1A	23.2	0.49	C17	61.4	-
C2	48.6	-	C17B	19.1	1.17
C2A	18.5	1.27	C171	34.9	1.76/2.01
C21	44.1	1.89	C172	34.6	2.07/2.45
C22	178.6	-	C173	177.6	-
C3	57.8	4.25	C175	47.5	2.98/3.55
C31	27.6	1.97/2.07	C176	74.8	4.33
C32	37.2	2.49	C177	20.9	1.22
C33	180.5	-	C18	40.7	2.70
C4	178.4	-	C181	34.2	2.42
C5	109.7	-	C182	178.3	-
C51	18.2	2.75	C19	77.4	3.38
C6	166.1	-	C1R	89.0	6.26
C7	52.2	-	C2R	71.2	4.24
C7A	21.2	1.87	C3R	75.1	4.70
C71	44.5	1.71/2.71	C4R	83.9	4.13
C72	177.8	-	C5R	62.6	3.77/3.93
C8	56.4	3.43	C2N	144.7	7.24
C81	28.8	0.99/1.85	C4N	121.2	6.61
C82	34.1	1.20/1.70	C5N	134.4	-
C83	180.0	-	C6N	136.1	-
C9	173.8	-	C7N	112.8	7.18
C10	96.6	5.99	C8N	132.6	-
C11	177.7	-	C9N	140.2	-
C12	49.8	-	C10N	21.7	2.30
C12A	21.9	1.42	C11N	27.7	2.23
C12B	32.6	0.90	C1L	140.1	-
C13	55.9	3.37	C2L	135.6	5.75
C131	30.5	1.98/2.13	C3L	129.5	6.66
C132	37.0	2.63	C4L	143.5	-
C133	180.8	-	C5L	129.5	6.66
C14	166.6	-	C6L	135.6	5.75
C15	107.5	-	C7L	28.8	2.34
C151	17.3	2.65	C8L	16.9	1.00

For atom numbering, see Figure S5

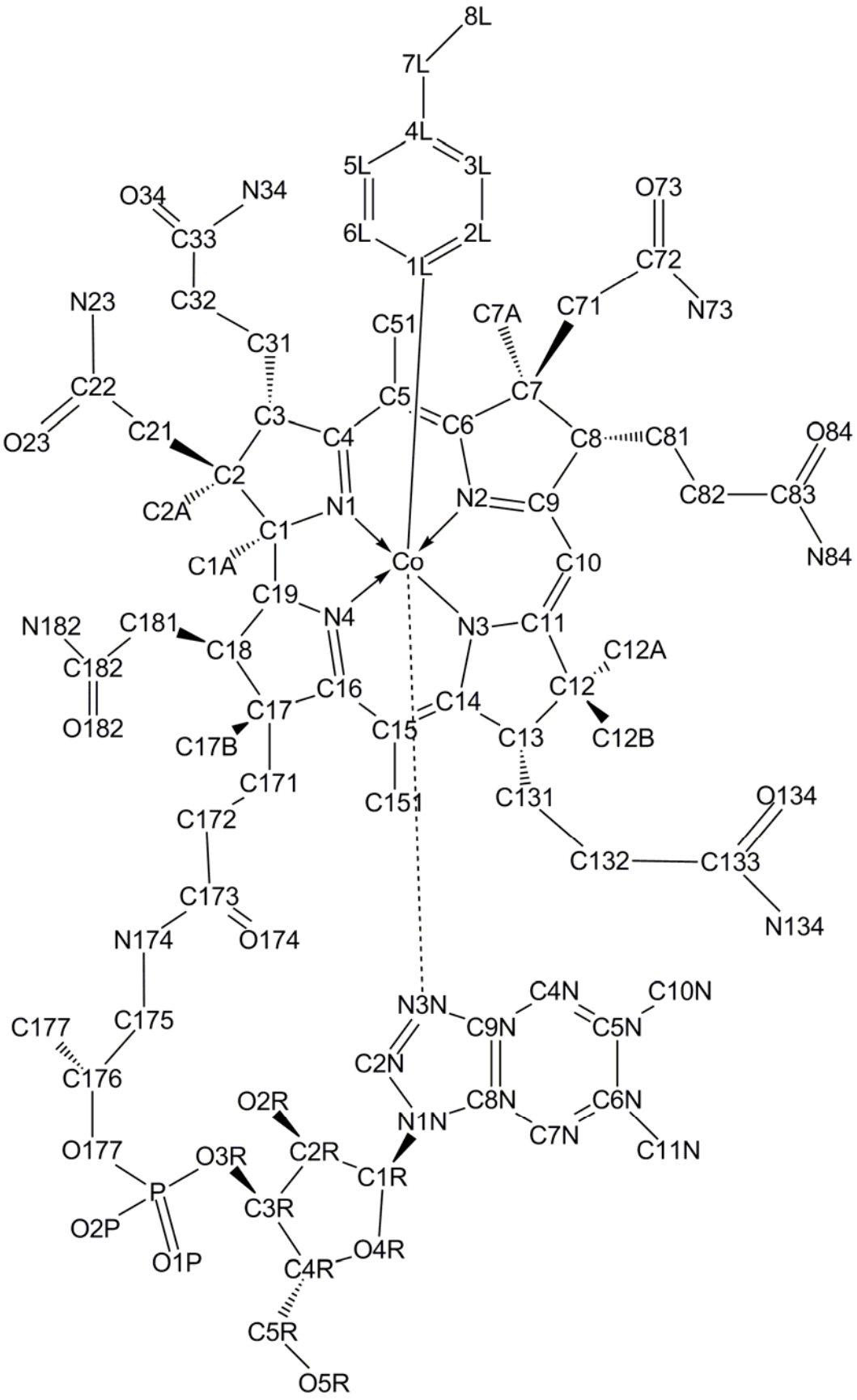


Figure S5. Atom numbering used for **EtPhCbl**

Structure determination of Co_β-(4-ethylphenyl)-cobalamin (EtPhCbl)

Crystals of **EtPhCbl** were grown from water/acetone. A crystal specimen was immersed into hydrocarbon oil, picked up with a rayon loop, and flash-cooled with liquid nitrogen. Diffraction experiments were carried out at 100 K on the beam line ID23-1 at the European Synchrotron Radiation Facility (ESRF) in Grenoble (France). Diffraction data extended to a resolution of 0.8 Å. The asymmetric unit of the orthorhombic crystal contained one B_{12} molecule, one acetone and 18 partially disordered water molecules.

Indexing of diffraction images, intensity integration, and data scaling were performed with the program XDS.[S3] The crystal was orthorhombic (space group $P2_12_12_1$) with unit cell constants a=15.650(3) Å, b=23.180(5) Å, and c=24.780(5) Å. The structure was solved by direct methods and refined against F^2-values using the program SHELXL-97.[S4] Full matrix least-squares anisotropic refinement converged at $R1$=0.0587 for all data. No absorption correction was applied to the data. The solvent region was modeled using one acetone and 18 water molecules with anisotropic atomic displacement parameters (adp). Restraints for adp's - DELU and SIMU as well as ISOR for solvent atoms – were applied. H-Atom positions were calculated and refined as 'riding' on their respective non-H-atom. For methyl- and hydroxyl-groups the torsion angle around the C-C or C-O bond was also refined. The isotropic adp for each H-atom was set to 1.5 times (for methyl- and hydroxyl-groups) and 1.2 times (for all other hydrogen atoms) the equivalent isotropic atomic displacement parameters of the adjacent non-H-atom. Data pertaining to diffraction data collection and structure refinement are summarized in Table S2.

The geometry of the inner coordination sphere of the cobalt is not unusual. Bond distances in the equatorial plane are 1.878(4) Å (Co-N1), 1.923(4) Å (Co-N2), 1.915(4) Å (Co-N3) and 1.886(4) Å (Co-N4). The distances to the axial ligands are 1.981(5) Å (Co-Cβ) and 2.230(4) Å (Co-Nα). The Co-atom is slightly out of the plane of the four corrin nitrogen atoms and is shifted by 0.029(2) Å towards the upper 4-ethyl-phenyl-ligand. The phenyl ring of the β-ligand is oriented north-south relative to the corrin ring (torsion angle C5-Co-C1β-C2β: 0.6°) and is tilted by only 3° with the dimethylbenzimidazole ligand. The fold angle of the corrin ring is 7.6(1)°. The ethyl group of the upper ligand is disordered over two alternate conformations (see main text, Figure 1)

Out of the 18 water molecules in the final structure only 7 are very well defined with atomic displacement parameters comparable to those of the B_{12} molecule. These water molecules are mostly interacting with the phosphate group of the nucleotide loop. The acetone molecule is located underneath C10 and is sequestered between the dimethylbenzimidazole moiety and the amide side chains D and E. Its carbonyl group is hydrogen bonded to the D-propionamide group of B_{12}.

CCDC 848663 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

Table S2: Crystallographic data for Co$_\beta$-(4-ethylphenyl)-cobalamin (**EtPhCbl**).

Empirical formula	$C_{70}H_{97}N_{13}O_{14}PCo$
Formula weight	1434.5
acetone sites	1
H_2O sites	18
Crystal system	orthorhombic
space group	$P2_12_12_1$
Unit cell dimensions	
a [Å]	15.650(3)
b [Å]	23.180(5)
c [Å]	24.780(5)
V [Å^3]	8989(3)
Z	4
Crystal size [mm^3]	0.2 x 0.2 x 0.1
θ_{max} for data collection [°]	26.8 (0.8 Å resol.)
Wavelength [Å]	0.7241
Reflections collected	134976
Independent Reflections	9889
R(int)	0.059
Completeness to θ = 26.8° (0.8 Å)	99.7%
Data/restraints/parameters	9889/967/1117
Final R indices (all data)	
R_1	0.0587 [0.0583 for 9768 reflections with I>2σ(I)]
wR_2	0.1613
Largest diff. peak/hole (e Å^{-3})	1.06/-0.80

Determination of pK_a of (Co_β-(4-ethylphenyl)-cobalamin-H^+)

A stock solution of 2.2 mg **EtPhCbl** in 3 ml MeOH was prepared. 300 µl of the stock solution were transferred into a flask, then the solvent was removed and the residue was re-dissolved in 2 ml of the corresponding buffer solution. The UV/Vis spectra of these solutions (see Figure S5) show isosbestic points at 495, 389 and 341 nm, respectively. Maximum spectral changes in absorption occurred at 518, 455, 374 and 302 nm. The spectral changes for these wavelengths (see Table S3) were analyzed according to Equation S1 by the method of least square. The results of these fits are given in Table S4.

Equation S1: $pH_x = pK_a + \log (|A_x - A_{AH}|/|A_A^- - A_x|)$

Table S3 pH-dependence of absorption data of **EtPhCbl** at wavelengths at maximal spectral changes; buffer solutions (100 mM, 1.0 M total ion strength with NaCl)

buffer system	pH	absorbance at			
		518 nm	455 nm	374 nm	302 nm
HCl	0.64	0.182	0.453	0.395	0.967
H_3PO_4/NaH_2PO_4	1.74	0.181	0.449	0.399	0.962
H_3PO_4/NaH_2PO_4	2.34	0.176	0.407	0.370	0,874
H_3PO_4/NaH_2PO_4	2.65	0.190	0.413	0.385	0.890
CH_3CO_2H/CH_3CO_2Na	3.41	0.242	0.377	0.415	0.831
CH_3CO_2H/CH_3CO_2Na	3.83	0.285	0.346	0.443	0.777
CH_3CO_2H/CH_3CO_2Na	4.31	0.355	0.305	0.483	0.705
CH_3CO_2H/CH_3CO_2Na	4.87	0.386	0.278	0.498	0.652
NaH_2PO_4/Na_2HPO_4	5.90	0.403	0.262	0.508	0.624
NaH_2PO_4/Na_2HPO_4	7.99	0.401	0.259	0.504	0.617

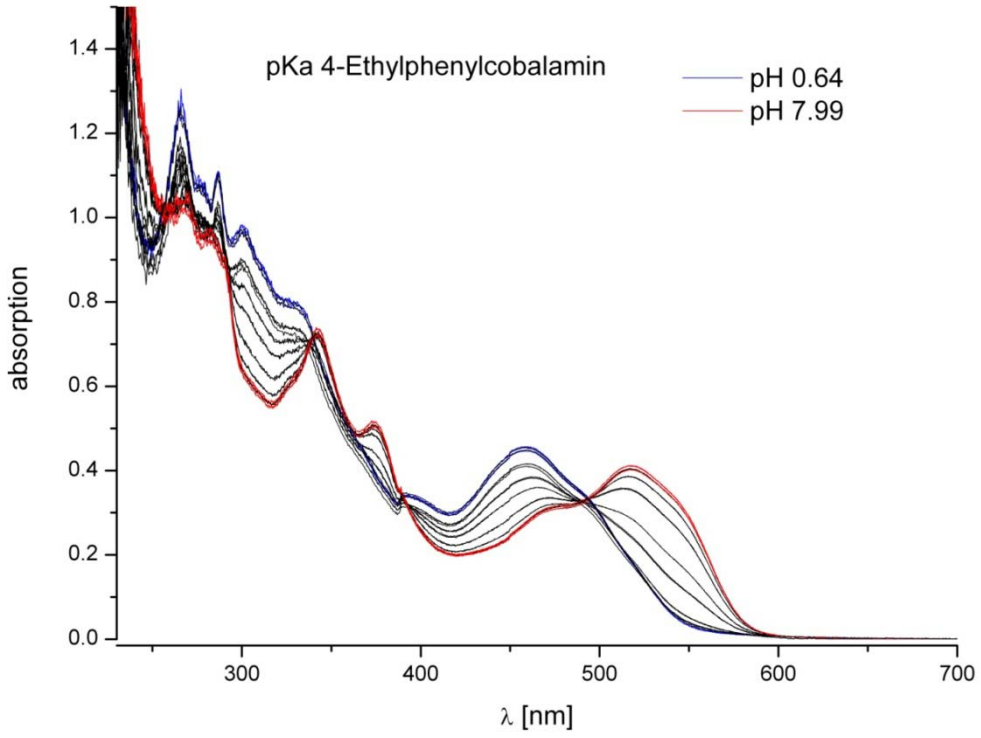


Figure S6. Dependence of UV/Vis spectra of **EtPhCbl** in aqueous buffers at pH-values from 7.15 (red line) to 0.64 (blue line).

Table S4 Mean value of calculated pK_a by least squares analysis of spectral data given in Table S3

wavelength (nm)	Intercept (pK_a)	gradient	R^2
518	3.86	0.95	0.9951
455	3.57	1.31	0.9650
374	3.73	1.22	0.9275
302	3.60	1.28	0.9399

Calculated **pK_a (EtPhCbl-H^+) = 3.7 ± 0.1**

Experiments testing the stability of EtPhCbl:

Hydrolytic conditions at pH 2:
1.2 mg (0.83 µmol) of **EtPhCbl** were dissolved in 600 µl 10 mM phosphate buffer pH 2 /D_2O (9/1) and transferred into a NMR tube. The sample was stored under air at RT in the dark for 11 days. ^{1}H-NMR spectra were recorded at regular time intervals. Only insignificant changes in the ^{1}H-NMR spectrum were observed (see Figure S7 trace A and B). Subsequently, the sample in the NMR tube was heated to 95°C for 6 hours and a ^{1}H-NMR spectrum again showed insignificant changes (see Figure S7, trace C). TLC analysis of the mixture indicated about 20% of a less polar hydrolysis product, but decomposition to aquocobalamin could not be detected.

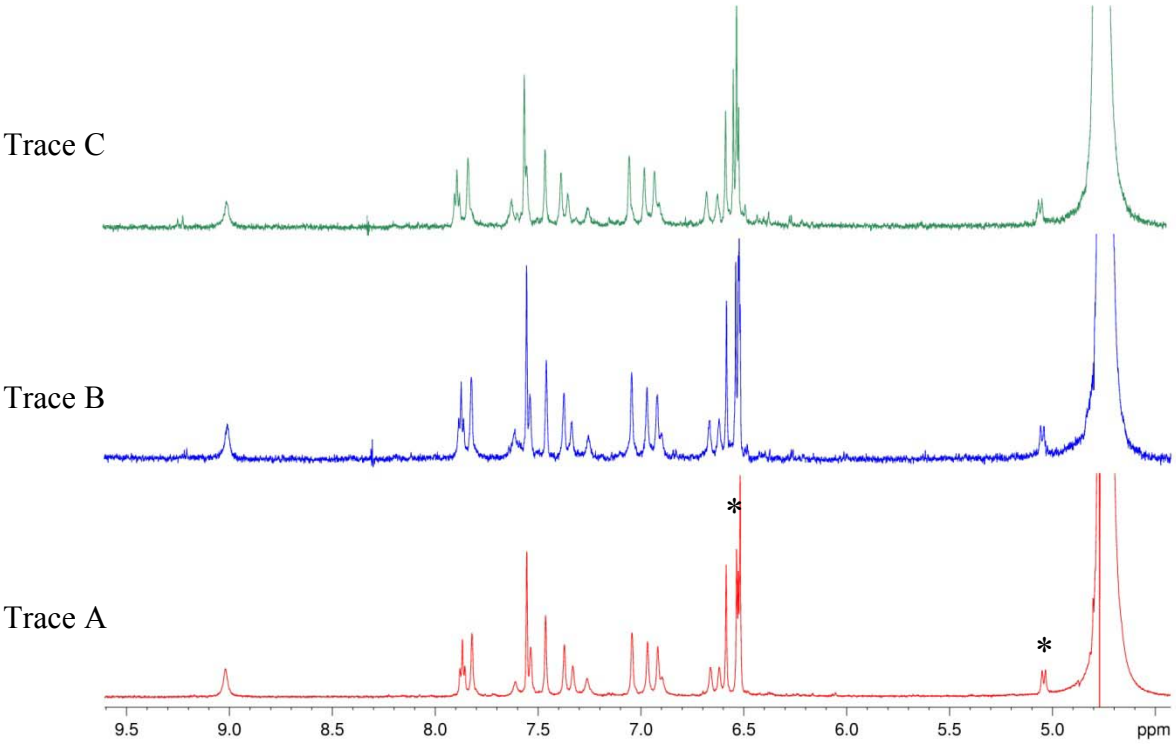


Figure S7. Low field region of the 500 MHz ^{1}H-NMR spectrum of **EtPhCbl** in pH2/D_2O (9/1) at 25°C, immediately after preparation of the solution (trace A), after 11 days at 25°C, (trace B) and after 6 hours heating to 95°C (trace C). Signals of 'aromatic' H-atoms at the organometallic 4-ethylphenyl ligand are marked with asterisks.

Thermolytic conditions at about 100 °C:
In a 10 ml two necked flask fitted with a reflux condenser 1.0 mg (0.70 µmol) of **EtPhCbl** were dissolved in 1.0 ml 10 mM phosphate buffer pH 7 (50 mM) and heated to reflux for 24 h under protection from light. UV/Vis spectra were recorded at regular time intervals, but, after 24 hours, only insignificant absorbance changes were detected (see Figure S8). TLC analysis indicated ~10 % of a slightly less polar, orange decomposition product, but aquo-cobalamin (**H_2OCbl**) was not detected.

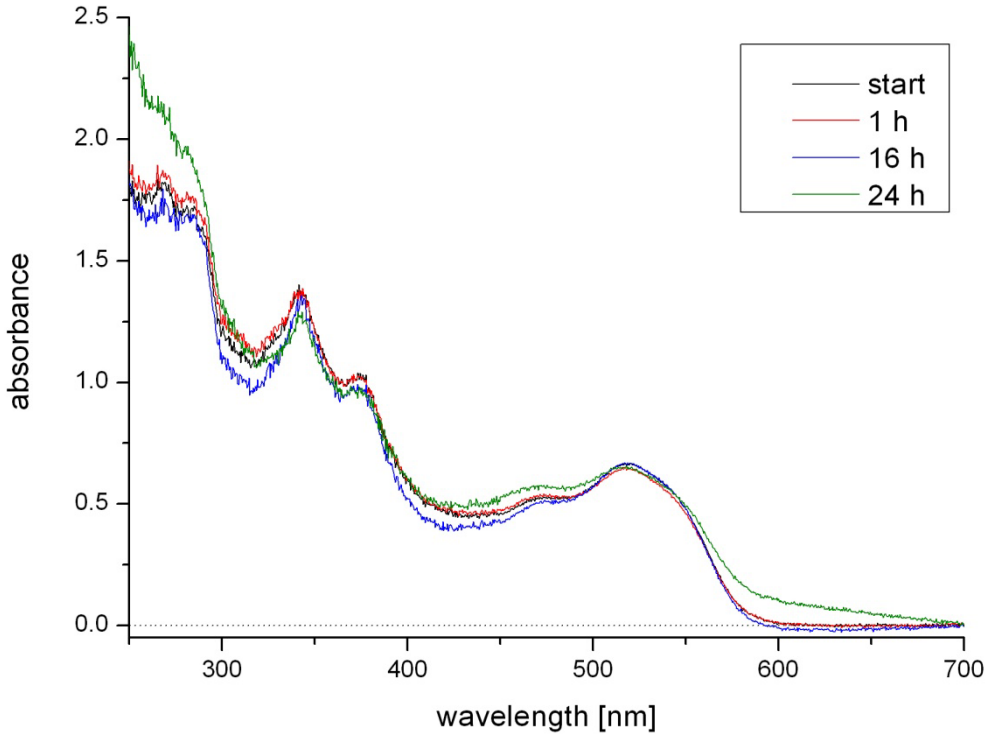


Figure S8 UV/Vis spectra of **EtPhCbl** in 50 mM aerated phosphate buffer pH7 before (black line), after heating to 100°C for 1 h (red line), 16 hours (blue line) and 24 hours (green line).

Photolytic conditions:
In a UV/Vis cell 0.16 mg of **EtPhCbl** were dissolved in 3 ml phosphate buffer pH7 (50 mM) and exposed to daylight. UV/Vis spectra were recorded at regular intervals for 24 h. After 24 h the UV/Vis spectrum was similar to that of **H_2OCbl** (see Figure S11). HPLC analysis showed clean partial decomposition to **H_2OCbl** (**EtPhCbl** still present).

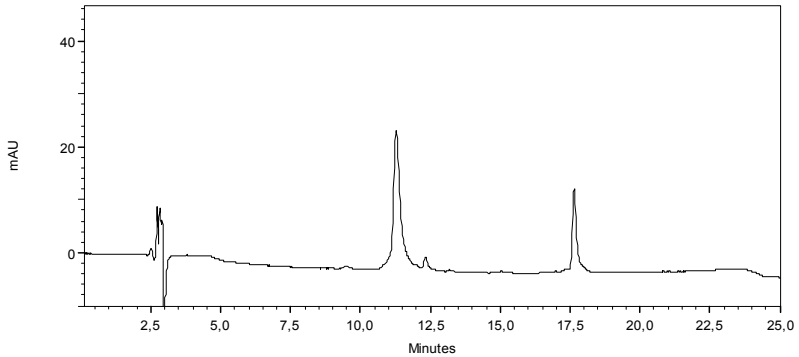


Figure S9 HPLC-analysis of photolysis of an aerated solution of **EtPhCbl** with daylight. HPLC-trace of reaction mixture after 24 hours exposure to day light (detection at 500 nm).

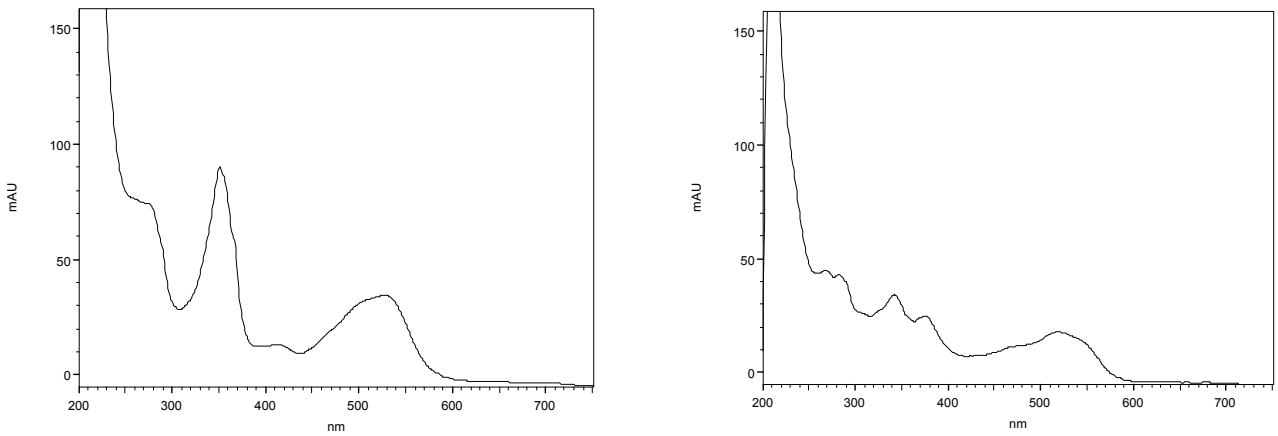


Figure S10 On-line UV/Vis spectra of red fraction (**H_2OCbl**) at R_t = 10.2 min (left) and of **EtPhCbl** at R_t = 17.5 min (right)

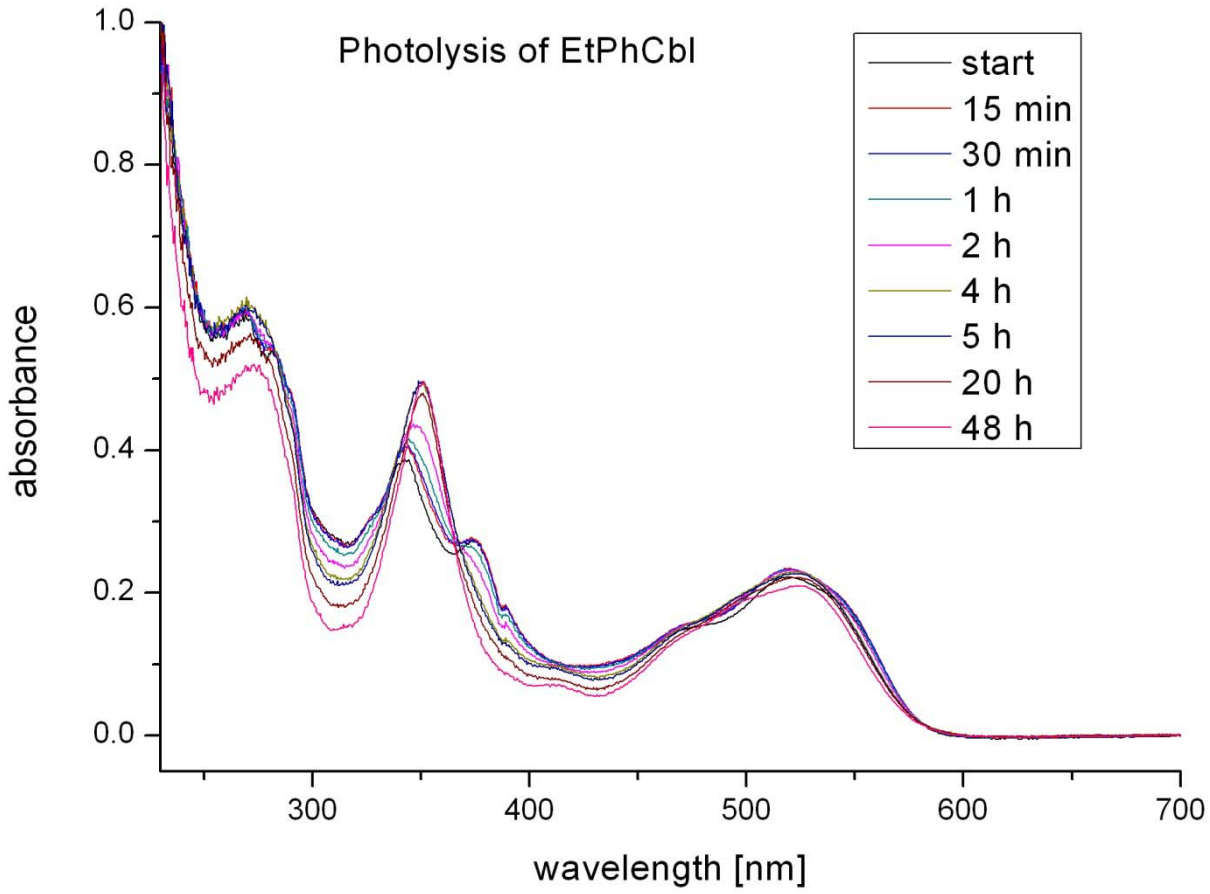


Figure S11. Changes in the UV/Vis spectra of an aerated aqueous solution (phosphate buffer pH 7) of **EtPhCbl** during irradiation with daylight

Reactivity of EtPhCbl toward iodide as nucleophile
In a 5 ml flask 1.3 mg (0.91 µmol) of **EtPhCbl** were dissolved in 1.0 ml phosphate buffer pH7 under protection from light. Then 15.1 mg (91 µmol) of potassium iodide were added and the mixture was stirred under air at RT with protection from light. UV/Vis-spectra of the reaction mixture were recorded. Even after 72 hours no decomposition was observed.

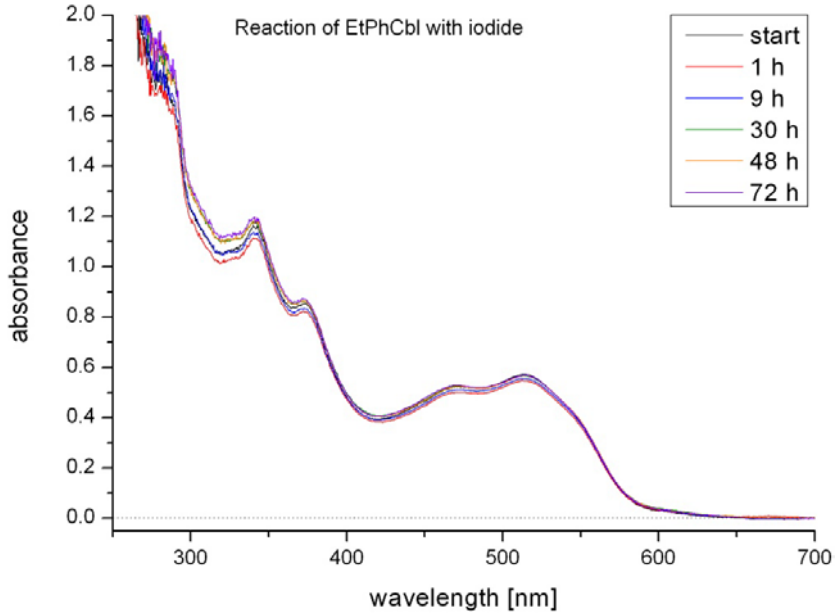


Figure S12. Changes in the UV/Vis spectra of an aerated aqueous solution (phosphate buffer pH 7) of **EtPhCbl** and of potassium iodide (91 µM)

Control experiment with MeCbl and potassium iodide
In a 5 ml flask 1.6 mg (1.19 µmol) of **MeCbl** were dissolved in 2.0 ml phosphate buffer pH7 under protection from light. Then 22.7 mg (139 µmol) of potassium iodide were dissolved. the mixture was stirred under air at RT. 300 µl of the solution were transferred into a 1 mm quartz cell, which was closed by a stopper; the reaction was monitored by UV/Vis spectra. Gradual decomposition of **MeCbl** to cob(II)alamin (mainly) was observed.

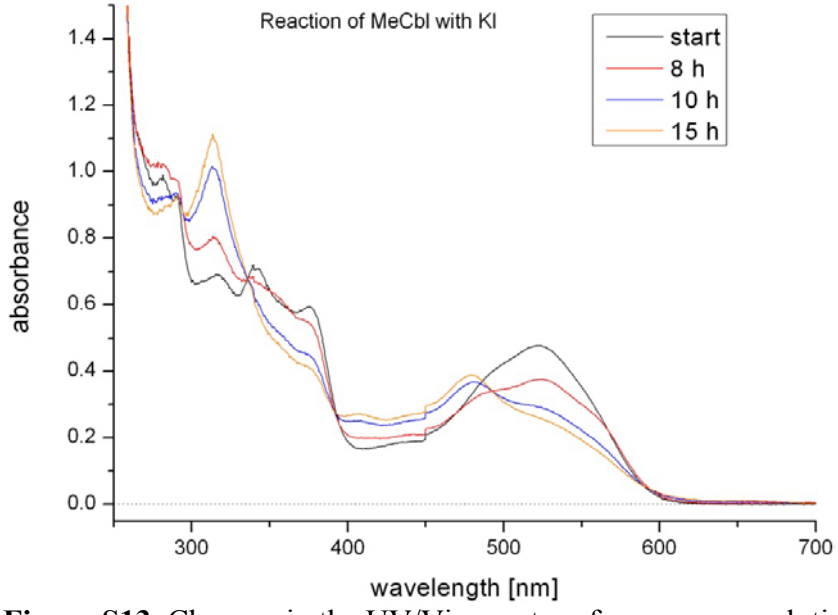


Figure S13. Changes in the UV/Vis spectra of an aqueous solution (phosphate buffer pH 7) of **MeCbl** and of potassium iodide (70 µM).

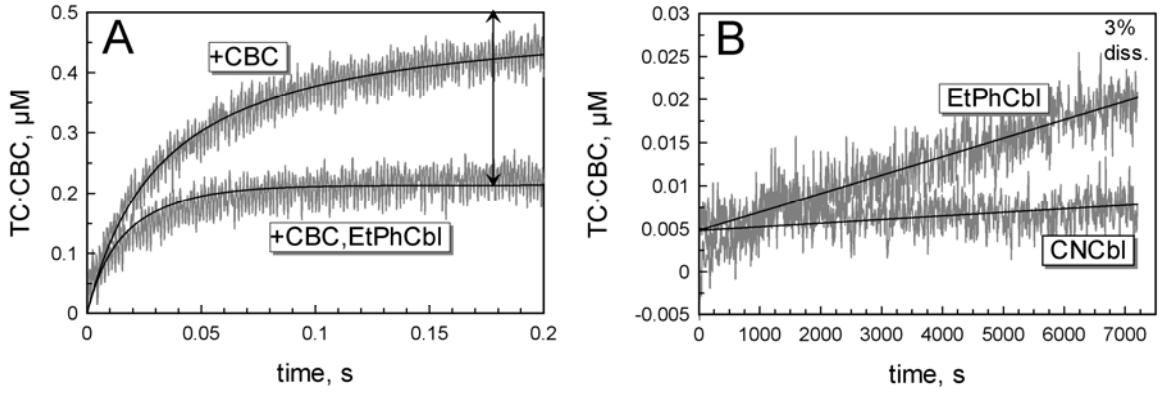


Figure S14. Interaction of **EtPhCbl** with human Cbl-binding protein transcobalamin (TC). (**A**) Binding of **EtPhCbl** to TC was measured via competition with the fluorescent vitamin B_{12}-derivative **CBC** ($k_+ = 61 \cdot 10^6$ $M^{-1}s^{-1}$). TC was rapidly mixed with either the fluorescent B_{12}-derivative **CBC** or the solution containing **CBC** and **EtPhCbl**. Attachment of **CBC** to TC was monitored by increasing fluorescent signal recalculated to the concentration of TC·**CBC** complex. The final concentrations of all reactants were equal to 0.5 µM. Arrow indicates the effect of competition consistent with $k_{+EtPhCbl} = 93 \cdot 10^6$ $M^{-1}s^{-1}$. (**B**) Dissociation of **EtPhCbl** and **CNCbl** from TC. The pre-incubated complex of TC (1.0 µM) and either **EtPhCbl** or **CNCbl** (1.3 µM) was mixed with the chasing fluorescent B_{12}-derivative **CBC** (1.3 µM). Concentration of the accumulating IF·**CBC** complex was calculated from the fluorescence. The initial slopes corresponded to $k_{–EtPhCbl} \approx 2.1 \cdot 10^{-6}$ s^{-1} and $k_{–CNCbl} \approx 4 \cdot 10^{-7}$ s^{-1}.

Table S5. Binding characteristics of **EtPhCb**l with intrinsic factor (IF) and with transcobalamin (TC) in comparison to coenzyme B_{12} (**AdoCbl**) and vitamin B_{12} (**CNCbl**).

Ligand	TC rate constants		IF rate constants	
	k_+ , $M^{-1}s^{-1}$	$k_–$, s^{-1}	k_+ , $M^{-1}s^{-1}$	$k_–$, s^{-1}
CNCbl	$68 \cdot 10^6$ [a]	3 [a] / $4 \cdot 10^{-7}$	$74 \cdot 10^6$	4 [a] / $6 \cdot 10^{-7}$
AdoCbl [a]	$52 \cdot 10^6$	$5 \cdot 10^{-7}$	$65 \cdot 10^6$	$5 \cdot 10^{-7}$
EtPhCbl	$93 \cdot 10^6$	$2.1 \cdot 10^{-6}$	$79 \cdot 10^6$	$1.5 \cdot 10^{-6}$

[a] Data from ref.[S4]

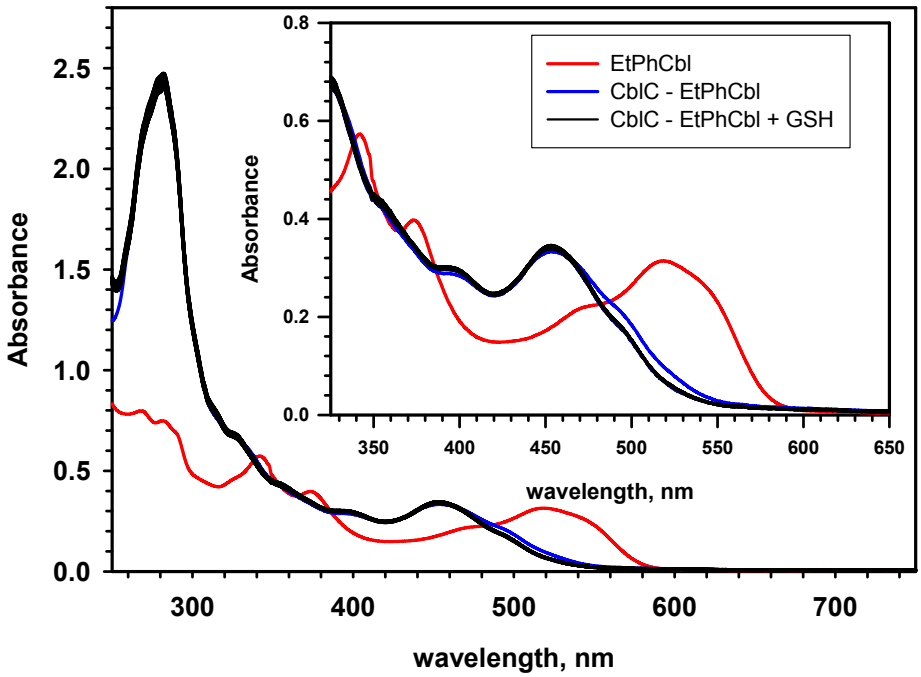


Figure S15. Spectral changes following binding of **EtPhCbl** to CblC. Addition of **EtPhCbl** to a CblC solution (50 µM in 100 mM HEPES pH 8.0, 150 mM KCl, 10% glycerol) is accompanied by a large blue shift of the **EtPhCbl** spectra (blue trace) compared to the **EtPhCbl** alone (red trace). Effect of GSH (1mM) on the **EtPhCbl**·CblC complex was monitored every minute over 3 h at 25°C (black traces).

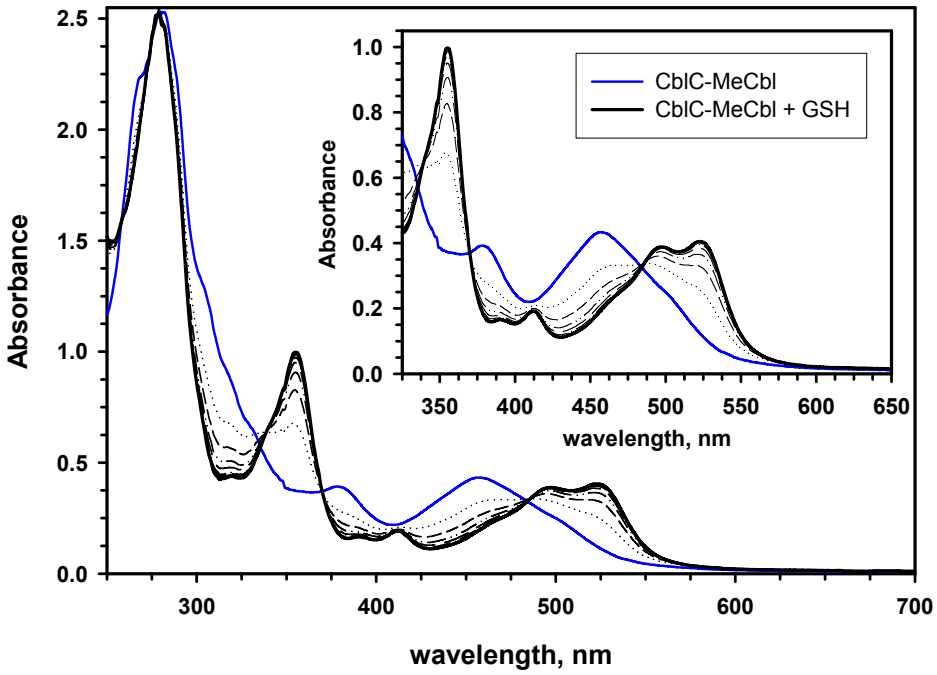


Figure S16. Binding and dealkylation of **MeCbl** by CblC. A **MeCbl**·CblC solution (50:45 µM; blue trace) in 100 mM HEPES pH 8, 150 mM KCl, 10% glycerol was treated with GSH (1mM) under aerobic conditions. The time-dependent conversion of bound **MeCbl** to **H_2OCbl** was recorded every minute over 10 minutes at 25°C (black traces).

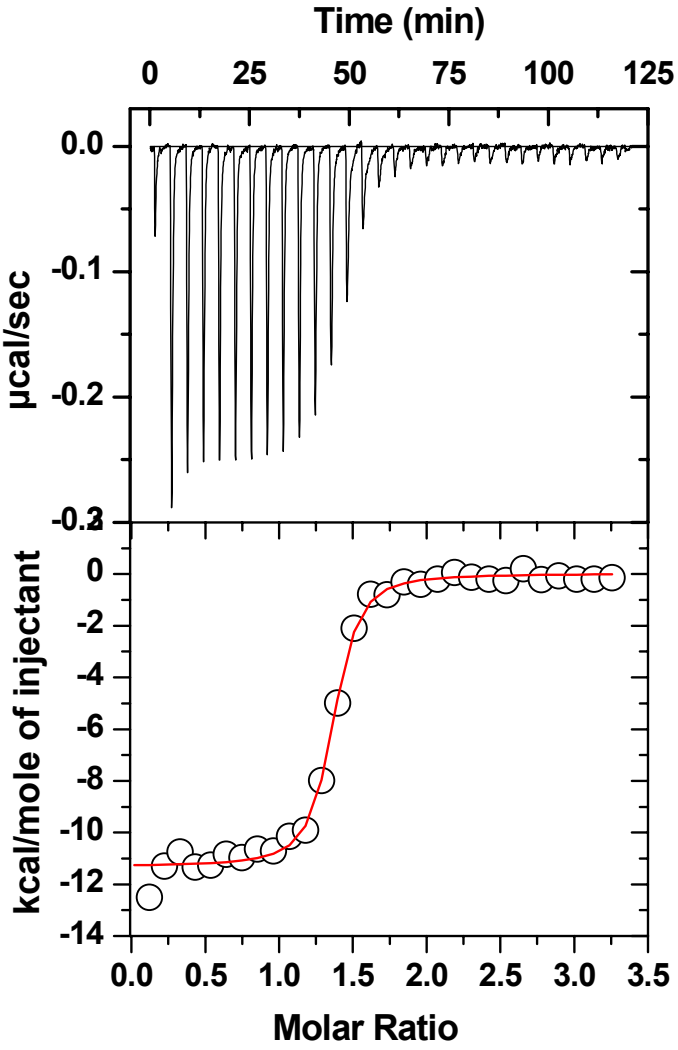


Figure S17. Binding of **EtPhCbl** to CblC. Isothermal titration calorimetry was used to monitor binding of **EtPhCbl** to CblC as described under Supplementary Information. The area under each spike (upper panel) is proportional to the heat produced with each injection of **EtPhCbl** into CblC (5 µM). The integrated areas normalized to the number of moles of **EtPhCbl** added with each injection (lower panel) were best fitted to a single-site binding model yielding a K_D=29.7±7.8 nM (average of three independent experiments).

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[S2] J.M. Englert, C. Dotzer, G. Yang, M. Schmid, C. Papp, J.M. Gottfried, H.P. Steinrück, E. Spieker, F. Hauke, A. Hirsch, *Nat. Chem*. **3**, 279-286 (2011)

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