Supporting Information

Contents

Material and Methods:

Vitamin B_{12} was purchased from Sigma-Aldrich and a generous gift of B_{12} was obtained from DSM Nutritional Products. All other chemicals were obtained from Aldrich, Sigma, Fluka (Buchs, CH). The chemicals were of reagent grade and used without further purification. All reactions were performed under an inert atmosphere (N_2). Deuterated solvents were obtained from Armar Chemicals (Döttingen, Switzerland). 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. Coupling constants J are given in Hz. Multiplicity is described as s for singlet, d for doublet, t for triplet, m for multiplet, dd for doublet of doublet. The data processing was carried out with ACD/SpecManager (Advanced Chemistry Development). HPLC-ESI-MS spectra were measured on a Bruker HCT spectrometer equipped with an Aquinity UPLC (Waters) using a Macherey-Nagel EC 250/3 Nucleodur C18 Gravity (5 μm). HPLC solvents were 0.1% formic acid (solvent A) and methanol (solvent B). Preparative HPLC was performed on a Varian Pro Star system by using either a Macherey-Nagel VP 250/21 Nucleodur C18 Gravity (5 μm) or a Macherey-Nagel VP 250/40 Nucleosil 100–7 C18 column with a flow rate of 10 mL min^{-1} and 32 mL min^{-1} respectively. The solvents were 0.1% trifluoroacetic acid (solvent A) and methanol or acetonitrile (solvent B).

Mass spectra were recorded either in the positive or negative mode on an Esquire HCT from Bruker (Bremen, Germany).

High-resolution electrospray mass spectra were recorded on a Bruker maXis QTOF-MS instrument (Bruker Daltonics GmbH, Bremen, Germany). The samples were dissolved in MeOH and analyzed via continuous flow injection at 3 μL/min. The mass spectrometer was operated in positive ion mode with a capillary voltage of 4 kV, an endplate offset of –500 V, nebulizer pressure of 5.8 psig, and a drying gas flow rate of 4 L/min at 180°C. The instrument was calibrated with a sodium formate solution (500μl H_2O : 500μl iPrOH : 20ul HCOOH : 20μl 0.1M $NaOH_{aq}$). The resolution was optimized at 30’000 FWHM in the active focus mode. The accuracy was better than 2 ppm in a mass range between *m/z* 118 and 1600. All solvent used were purchased in best LC-MS qualities.

CV spectra were recorded with a 757 VA computrace electrochemical analyzer (Ω, Metrohm). General experimental condition: Derivatives **1** and **3** (3.3 μmol) and $K_3Fe(CN)_6$ (1.1 mg; 3.3 μmol) were dissolved in Tris-HCl buffer (5 mL, 0.2 M, pH 8.0) at 23°C. A standard three-elecrode setup was used in the experiments, including a glassy carbon working electrode, an Ag/AgCl reference electrode and Pt auxiliary electrodes. The first forward and reverse scans were recorded with a sweep rate of 5 mV/s.

For a better comparison the cathodic E_{pc} values of the cobalt(III) to cobalt(II) reduction have been expressed as E_{pc}* with $K_3Fe(CN)_6$ as an internal standard.

UV-Vis spectra were recorded on a Cary 50 spectrometer using quartz cells with a path length of 1 cm. The UV-Vis titration experiment for the $pK_{base\text{-}off}$ determination was performed as following. An aqueous solution of **3** (44 μM, 0.2 M KCl) was titrated stepwise at 20°C with an H_2SO_4 solution (96%). The UV-Vis absorption spectra and the corresponding pH values were measured after each step. 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 absorption at 550 +/- 1 nm.

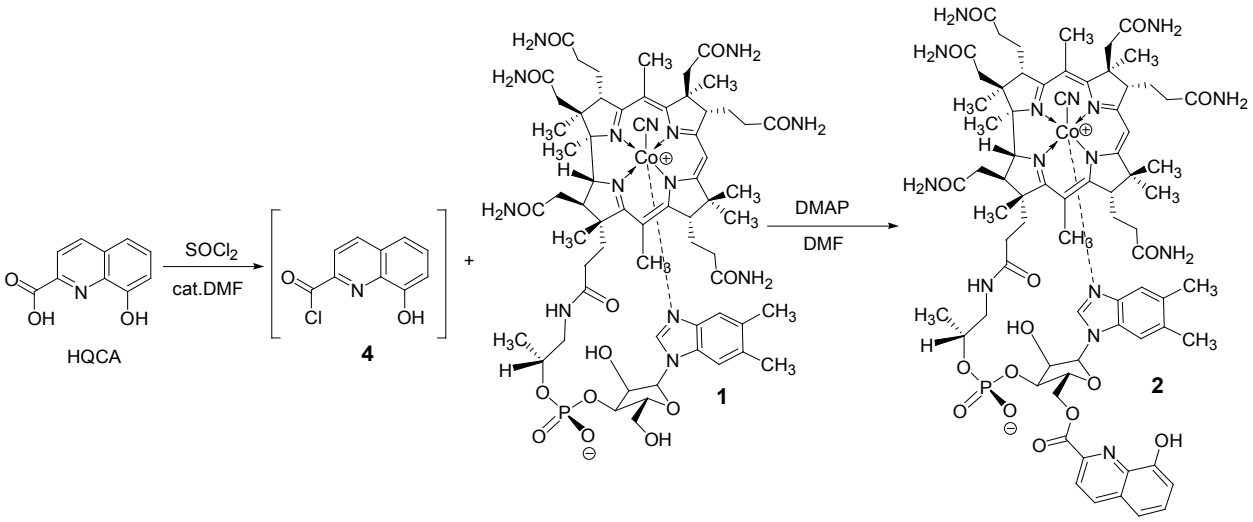
The cleavage experiments were performed as follows: Compound **2** (1.98 mg) or **3** (1.54 mg) was dissolved in 10 mL of the freshly prepared Lactobacillus medium (LB) at pH = 6.0. The final concentrations of **2** and **3** were 130 μM and 101 μM respectively. For metal triggered cleavage experiments, $ZnSO_4$ $7H_2O$ (4.67 mg or 3.62 mg) was added to 5 mL of freshly prepared solutions of **2** and **3** in the LB medium. The final concentration of Zn^{2+} ions was 25 fold higher (3.25 mM or 2.52 mM) than that of the B_{12} derivatives. The solutions were incubated at 25°C and samples were taken every 26 minutes and analyzed with HPLC-MS equipped with a DAD detector. The analyses were repeated under identical conditions to assure reproducibility.

B12 dependent *Lactobacillus delbrueckii* subsp. *lactis* was either a gift from Dr. Leo Meile (Institute of Food, Nutrition and Health, ETH Zurich, Switzerland) or purchased (as strain DSMZ 20355) from the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). For maintenance, stock cultures *Lactobacillus* were routinely grown overnight in "Difco™ B12 Inoculation Broth" (Becton Dickinson, Allschwil, Switzerland) in 10 ml screw cap test tubes at 30°C. To study effects of B12 and its derivatives, aliquots of 1 ml were transferred from the sock culture to sterile Eppendorf tubes, centrifuged for 2 min, washed

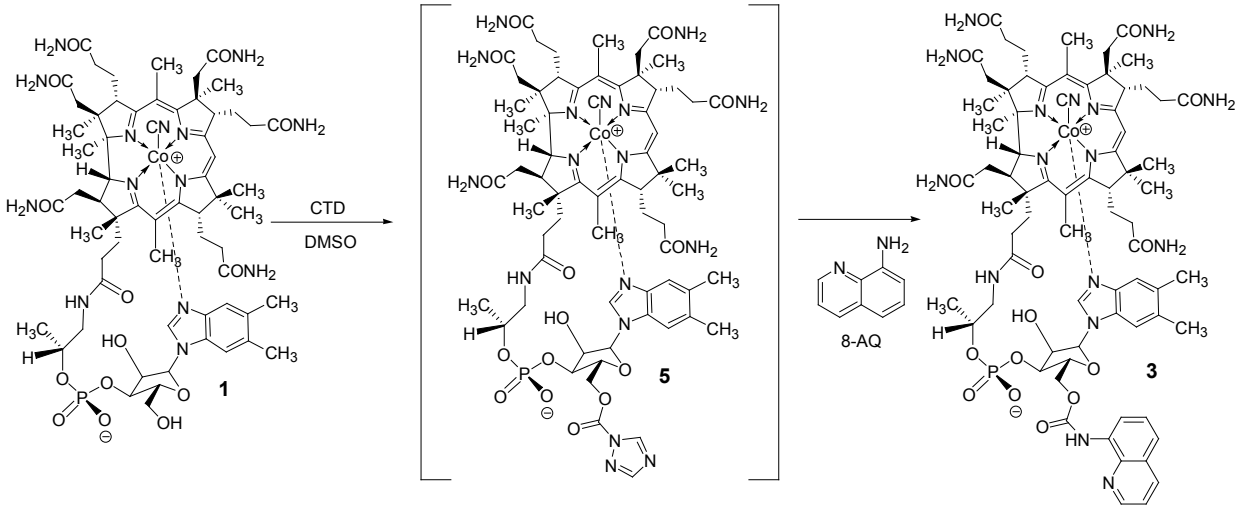
three times with sterile 0.9% NaCl solution and incubated for 45 min at 37°C to remove possible traces of B12 and to stabilize the inoculum.[1] Vitamin B12 Assay Medium (#82897; Fluka, Buchs, Switzerland) was prepared in 10 ml screw cap test tubes according to the supplier's instructions and sterilized (15 min at 121°C). Dilutions of B12 and its derivatives were added to the medium using sterile filters (0.22 µm) followed by the addition of a 200 µl inoculum. All growth experiments were always performed in triplicates at 37°C. Growth was monitored spectrophotometrically at 660 nm at time intervals of approximately 3 hours.[1]

Crystallographic data were collected at 183(2) K on an Oxford Diffraction Xcalibur system with a Ruby detector using Mo K_α radiation (λ = 0.710Å) that was graphite -monochromated. Suitable crystals were covered with oil (Infineum V8512, formerly known as Paratone N), mounted on top of a glass fibre and immediately transferred to the diffractometer. The program suite CrysAlis^{Pro} was used for data collection, multi-scan absorption correction and data reduction.[2] The structure was solved with direct methods using SIR97[3] refined by full-matrix least-squares methods against F^2 with SHELXL-97.[4] The structure was checked with help of the program Platon.[5] The structure was deposited at the CCDC center under the CSD number 820902.

Scheme S1: Synthesis of **2**:



Scheme S2: Synthesis of **3**:



Scheme S3: General Atom Numbering of B12 derivatives **2** and **3**:

Co$_\beta$-cyano-O5R-[(8-hydroxyquinoline)-2-carboxyl] cobalamin (**2**). 8-hydroxyquinoline 2-carboxylic acid (30 mg, 0.159 mmol) was dissolved in a mixture of DMF (20 µL) and $SOCl_2$ (1 mL). The mixture was stirred at 50°C under an inert atmosphere for 2 hours. The solvents were removed under high vacuum yielding crude **4** as a slightly yellow residue. B_{12} (**1**, 35 mg, 0.026 mmol) and DMAP (35 mg, 0.286 mmol) were dissolved in DMF (4 mL), and the violet solution was added to crude **4**. The reaction mixture was protected from light and was allowed to react for 2 days at room temperature. The crude product was precipitated with acetone (20 mL), filtered, dissolved in water (3 mL) and purified with preparative HPLC to afford ester **2** (6 mg, 15 %), as a red powder. ^{1}H-NMR (D_2O, see Table 1). UV/Vis (*c* = 29 µM, 0.2 M KCl): 550 nm (3.92), 520 nm (3.88), 407 nm (3.61), 361 nm (4.45), 320 nm (3.98), 306 nm (4.07), 278 nm (4.25), 256 nm (4.73). HPLC-MS (ESI): R_t = 12.8 min; m/z (%) 1526.7 (100) $[M + H]^+$, 763.9 (70) $[M + 2H]^{2+}$. Calcd. for $C_{73}H_{93}CoN_{15}O_{16}P$: 1525.6 (100). HR-MS: $[M]^{2+}$ calcd for $C_{73}H_{93}CoN_{15}Na_2O_{16}P$: 785.78894, found: 785.78821.

X-ray structure analysis: Crystals of **2** were grown from water/acetone at 4°C under protection from light. Crystal data of **2** are summarized in Table S2.

Co$_\beta$-cyano-O5R-(8-aminoquinoline)-8-carbamoyl) cobalamin (**3**). Derivative **3** was prepared according to published procedures with slight modifications [6, 7]. B_{12} (100 mg, 0.073 mmol) was dissolved in 2.0 mL of anhydrous DMSO and 1, 1`-carbonyl-di-(1,2,4-triazole) (CDT; 42 mg, 0.256 mmol) was added. The solution was stirred for 30 min, the intermediate Co$_\beta$-cyano-O5R-triazole cobalamin (**5**) was detected with HPLC-MS (R_t = 10.3 min; $[M+H]^+$ = 1450.7; $[M+H]^+_{theo.}$ = 1450.6). Excess of 8-amino quinoline (8-AQ; 100 mg, 0.694 mmol) was added. The mixture was shielded from light and stirred at 80°C for 2 days. The crude product was precipitated with acetone, filtered, dissolved in water (2 ml) and purified with preparative HPLC. Pure **3** was obtained as a red powder (45 mg, 40 %). ^{1}H-NMR (MeOD, see Table 1). UV/Vis (*c* = 44 µM, 0.2 M KCl): 549 nm (3.92), 520 nm (3.87), 408 nm (3.54), 361 nm (4.44), 304 nm (4.13), 279 nm (4.23). HPLC-MS (ESI): R_t = 12.4 min; m/z (%) 1525.7 (100) $[M + H]^+$, 763.4 (70) $[M + 2H]^{2+}$. Calcd. for $C_{73}H_{94}CoN_{16}O_{15}P$: 1524.6 (100). HR-MS: $[M]^{2+}$ calcd for $C_{73}H_{94}CoN_{16}Na_2O_{15}P$: 785.29663, found: 785.29657.

***Table S1**:* Comparison of the NMR chemical shift values of Co$_\beta$-cyano-O5R-acetyl cobalamin **(6)**,[8] **2** and **3**.[a]

	δ ^{1}H[ppm]		
	6[8]	**2**	**3**
C1A	0.47	0.41	0.49
C2A	1.41	1.36	1.39
C3	4.19	4.15	4.50
C7A	1.86	1.79	1.88
C8	3.42	3.33	3.59
C10	6.08	5.97	6.01
C12A	1.44	1.24	1.46
C12B	1.19.	1.07	1.19
C13	3.34	3.18	3.34
C17B	1.39	1.35	1.37
C18	2.76	2.72	2.90
C19	4.10	4.02	4.14
C21	2.41	2.36	2.37
C31	2.03	1.98	1.96
C32	2.50/2.56	2.44/2.50	2.61/2.75
C51	2.54	2.48	2.58
C71	2.18/2.57	2.11/2.52	2.06/2.53
C81	0.99/1.99	0.91/1.90	1.09/2.04
C82	1.00/1.82	0.86/1.69	1.19/1.67
C131	1.94/2.03	1.84/1.99	1.96/2.14
C132	2.66	2.39	2.46
C151	2.57	2.48	2.60
C171	1.84/2.63	1.79/2.56	1.84/2.58[b]
C172	2.11/2.54	2.11/2.53	2.22/2.56[b]
C175	3.01/3.60	2.96/3.49	2.91/3.66
C176	4.31	4.29	4.39
C177	1.24	1.13/1.14	1.24/1.25
C181	2.68/2.75	2.63/2.71	2.58/2.66[b]
C1R	6.37	6.42	6.38
C2R	4.31	4.33	4.26
C3R	4.84	4.96	4.99
C4R	4.25	4.44	4.36
C5R	4.21/4.56	4.63/4.94	4.41/4.89
C2N	7.11	7.11	7.19
C4N	6.50	6.45	6.57
C7N	7.26	7.21	7.28
C10N	2.26	2.19	2.27
C11N	2.26	2.16	2.25
C52L	2.18(Acetyl)		
C3L		8.06 d (^3J 8.28)	7.56 m
C4L		8.31 d (^3J 8.20)	8.29 dd ($^{3,4}J$ 8.33 and 1.66)
C5L		7.37 d (^3J 8.00)	7.56 m
C6L		7.46 t (dd) (^3J 7.80)	7.54 m
C7L		7.13 d (^3J 7.57)	8.33 dd ($^{3,4}J$ 7.03 and 1.96)
C2L			8.87 dd ($^{3,4}J$ 4.24 and 1.62)

[a]Assignment by ^{1}H-NMR and ^{13}C-HSQC correlation and comparison of **2** and **3** with **6** (Ref. 8). [b]Tentative assignement for the proton at C172/C171.

Table S2. *Crystal data and structure refinement for* ***2***$\cdot(C_3H_6O)_2\cdot(H_2O)_{10.5}$.

Empirical formula	$C_{79}H_{125}CoN_{15}O_{28.50}P$
Formula weight	1830.84
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
a [Å]	15.75386(17)
b [Å]	22.5570(3)
c [Å]	25.5975(3)
α [°]	90
β [°]	90
γ [°]	90
Volume [$Å^3$]	9096.32(19)
Z	4
Density (calculated) [Mg/m^3]	1.337
Absorption coefficient [mm^{-1}]	0.291
F(000)	3896
Crystal size [mm^3]	0.36 x 0.13 x 0.12
Crystal description	purple needle
Theta range for data collection [°]	2.71 to 26.37
Index ranges	-19<=h<=15, -28<=k<=18, -31<=l<=31
Reflections collected	39047
Independent reflections	18584 [R(int) = 0.0335]
Reflections observed	13327
Criterion for observation	>2sigma(I)
Completeness to theta	99.9 % to 26.37°
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.9659 and 0.8965
Data / restraints / parameters	18584 / 17 / 1162
Goodness-of-fit on F^2	0.940
Final R indices [I>2sigma(I)]	R1 = 0.0492, wR2 = 0.1085
R indices (all data)	R1 = 0.0707, wR2 = 0.1135
Absolute structure parameter	-0.001(11)
Largest diff. peak and hole [$e.Å^{-3}$]	0.494 and -0.302

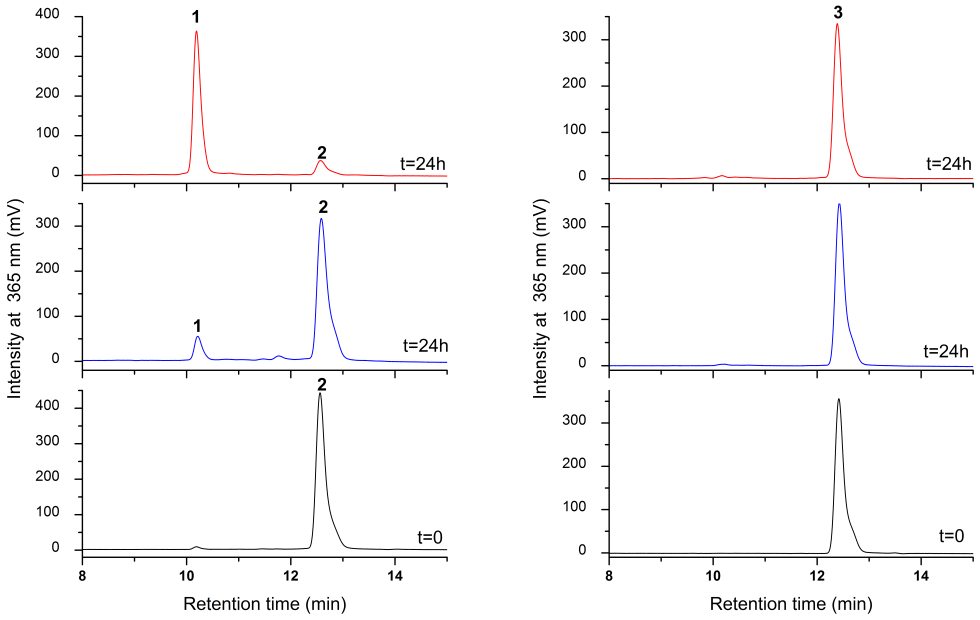


Figure S1. HPLC traces of background and metal-triggered hydrolysis of **2** (left, *c* = 130 μM) and **3** (right, *c* = 101 μM). The hydrolysis was monitored at 365 nm in the LB assay medium (pH 6.0) in the absence (blue) and presence of Zn^{2+}-ions (25 fold excess, *c* = 3.25 mM resp. 2.52 mM, red).

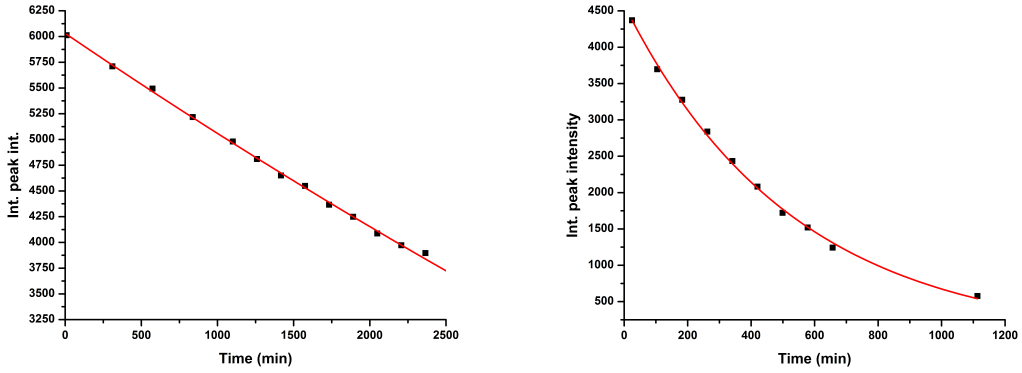


Figure S2. Corresponding plots of background hydrolysis (left, $t_{1/2}$ = 54.5 hours) and Zn^{2+}-triggered hydrolysis (25 folds excess, right, $t_{1/2}$ = 370 min) of **2** in LB assay medium (pH 6.0) at 25°C.

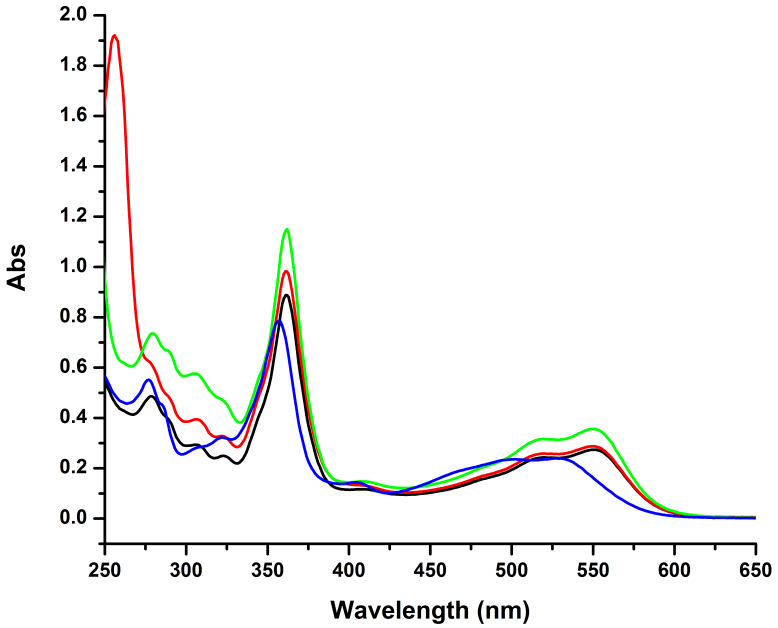


Figure S3: Comparison of the UV/Vis spectra of "base-on" **1** (black), **2** (red), **3** (green) and aquacyano-cobinamide (blue) in water (0.2 M KCl).

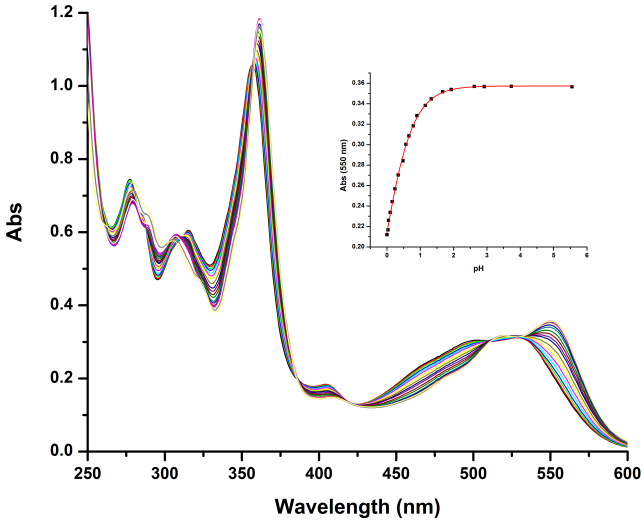


Figure S4. Absorption spectra of a solution of **3** in water (0.2 M KCl). Inset: corresponding UV-visible pH titration (550 nm).

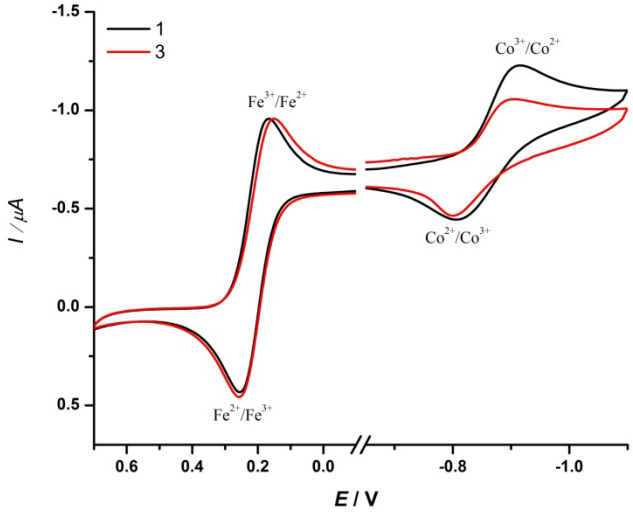


Figure S5. CV spectra of **1** and **3** ([Tris] = 0.2 M, ph 8.0) (reference electrode: Ag/AgCl, internal reference: $K_3Fe(CN)_6$).

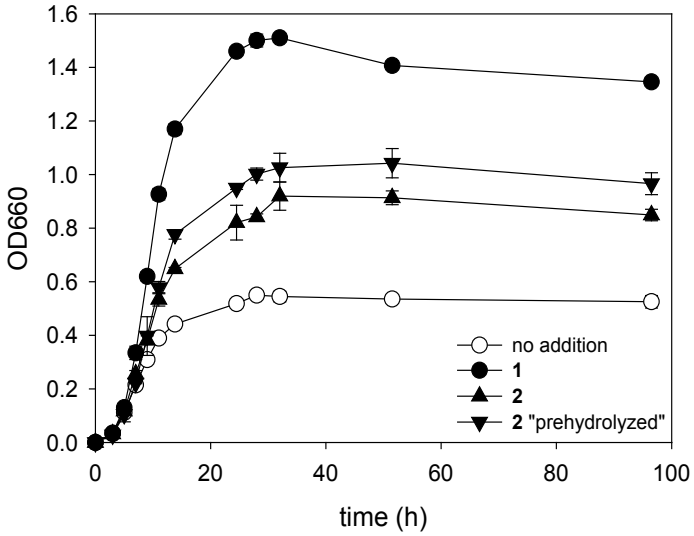


Figure S6. Growth of *L. delbrueckii* dependent on **1**, **2**, or prehydrolyzed **2** containing 30 % of **1** (total concentration is 250 pg/ml) at 37°C (n=3).

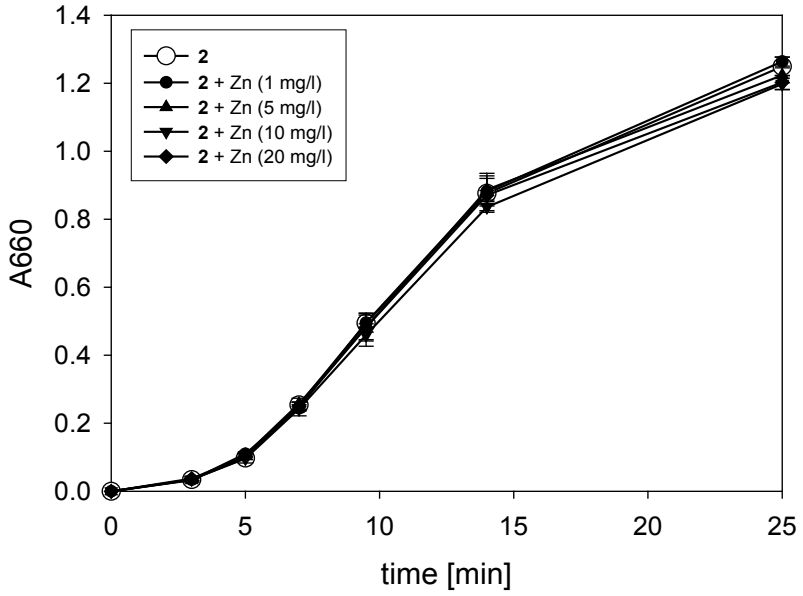


Figure S7. Representative examples of growth of *L. delbrueckii* with **2** (250 pg/ml) at 37°C (n=3) in the presence of different concentrations of zinc(II)-ions.

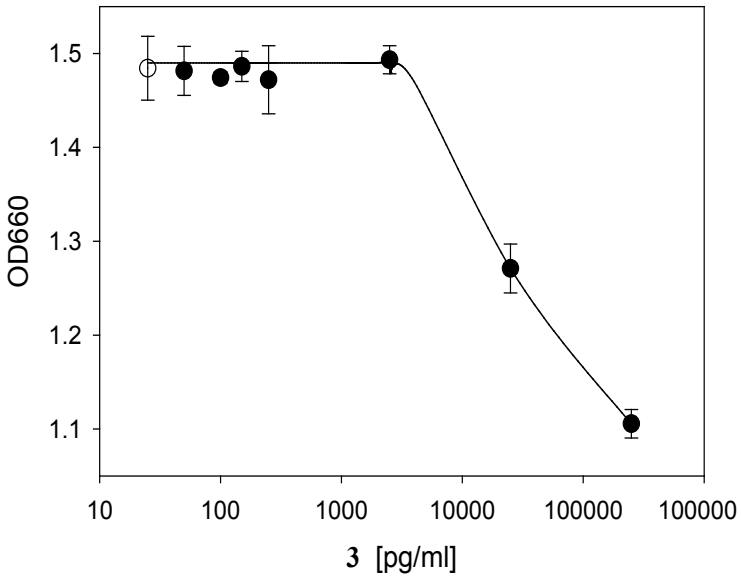


Figure S8. B12 dependent growth (**1;** final concentration 250 pg/ml) of *L. delbrueckii* after 24 h at 37°C as a function of different amounts of **3** (n=3).

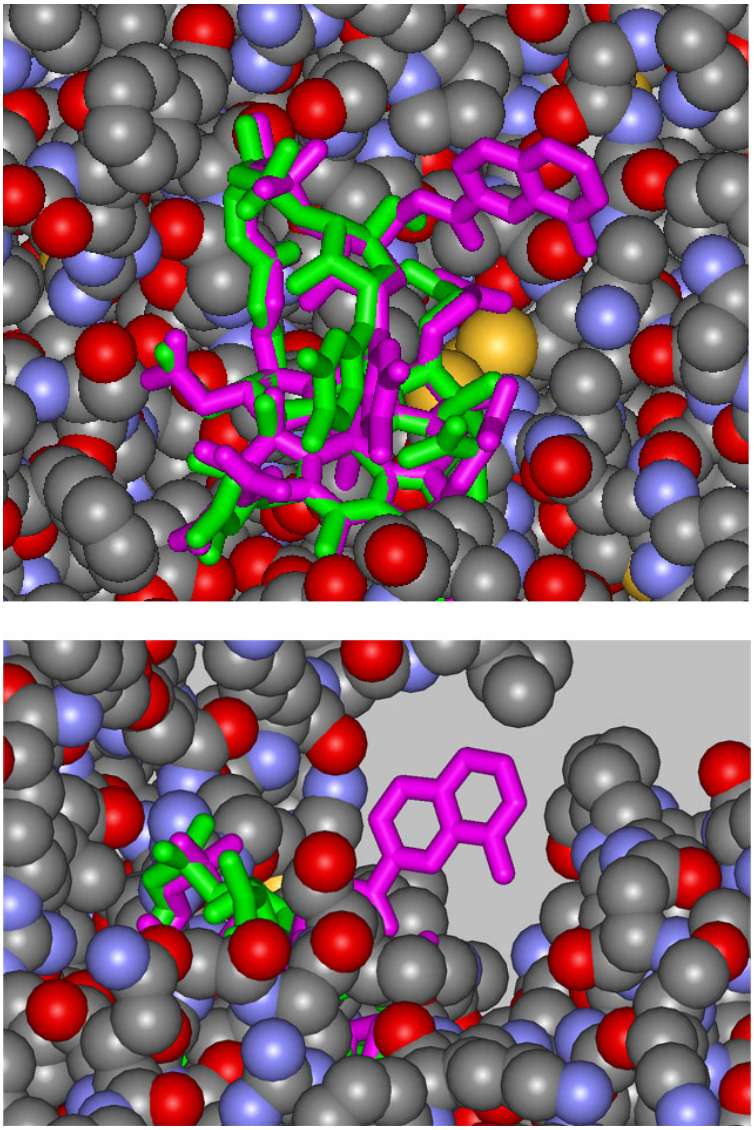


Figure S9. Superposition of **2** (violet) with AdoCbl (green) bound in the active site of RNR of *Thermotoga maritima*.[9] Different views show that the HQCA moiety attached to the 5`-OH moiety of B12 is not clashing with any of the amino acids of the protein.

References:

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