Electronic Supplementary Material (ESI) for ChemComm.

Electronic Supplementary Information

for

Amphiphilic, catalytically active, vitamin B_{12} derivative

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General Information

All solvents and chemicals used in the syntheses were of reagent grade and were used without further purification. Tested compounds were greater than 95% chemical purity as measured by elemental analysis. A microvave-assisted synthesis was performed using microwave oven CEM Discover. UV-vis absorption spectra were measured on Jenway 7315 spectrometer and Perkin Elmer λ-25 at room temperature. High resolution ESI mass spectra were recorded on a Mariner and SYNAPT spectrometer. ^{1}H and ^{13}CNMR spectra were recorded at rt on Bruker 500 and Varian 500 MHz instruments with TMS as an internal standard. DCVC (dry column vacuum chromatography)[1] was performed using Merck Silica Gel (200-300 mesh) and Silica Gel 90 C18 (Fluka). Thin layer chromatography (TLC) was performed using Merck Silica Gel GF254, 0.20 mm thickness.

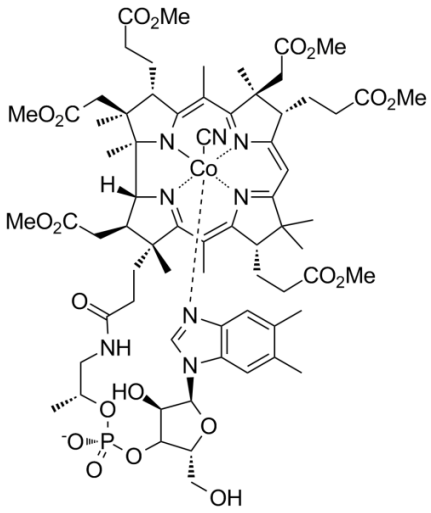
Partition *n*-octanol/water coefficient P_{OW} was determined using standard shake-flask method.[2] Concentrations were determined using UV-Vis spectroscopy.

HPLC Measurement conditions: Column: Eurospher II 100-5 C18 250 mmx4.6 mm (Knauer) with a precolumn; detection: UV-Vis, wavelength: λ=361 nm; flow rate: 1ml/min; pressure: 10 Mpa, Temperature: 30 °C. HPLC method:

Time [min]	H_2O [%]	MeCN [%]
initial	99	1
15	30	70
15	30	70

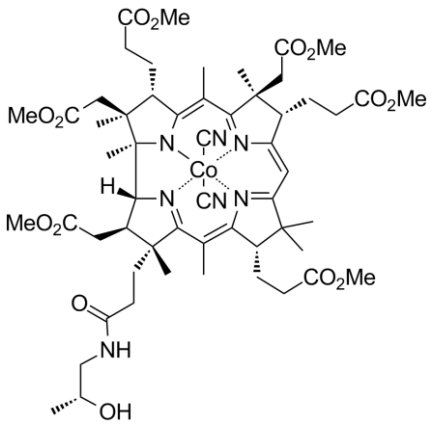
Diffractions experiments were performed on Bruker APEX-II CCD apparatus and the crystal structure was solved using SHELXL-97 software.

Cobalester (3) synthesis procedure



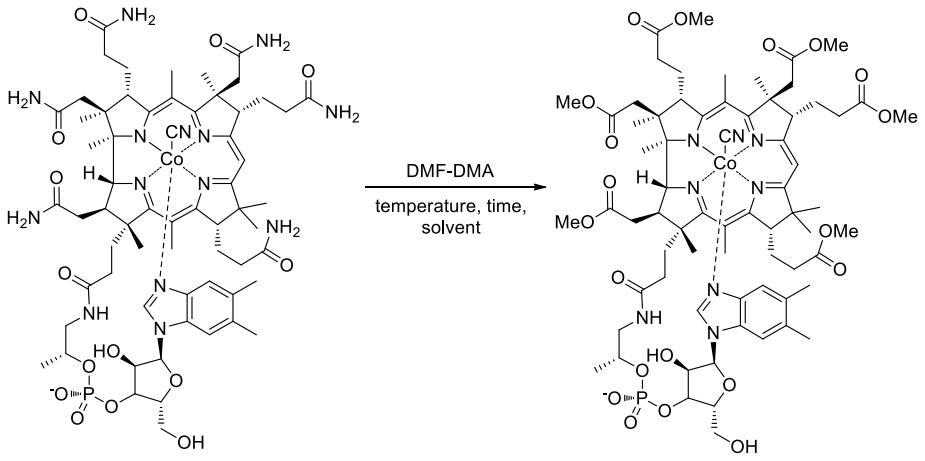
Cyanocobalamin (**1**) (15.0 mg, 0.011 mmol) was dissolved in a degased mixture of HFIP/MeOH (0.5 ml; 1:1), in a pressure tube equipped with a stirring bar. DMF-DMA (88 µl, 0.662 mmol) was added. The reaction mixture was vigorously stirred at 60 °C for 48 h in anaerobic conditions. It was then diluted with DCM (30 ml) and washed with brine (5 ml). The organic layer was concentrated in vacuo giving a red residue. It was dissolved in Et_2O (5 ml) and precipitated with hexane (20 ml. The crude product was purified using reverse phase column chromatography (gradually from 2.5 to 15% MeOH in water). Recrystalization from $CHCl_3$/hexane gave a red solid (11 mg; 72%). R_f 0.32, 20% MeOH in DCM/Toluene (1:1). logP_{OW} = 0.70. HRMS ESI (m/z) calcd for $C_{69}H_{95}CoN_8O_{20}P$ $[M+H]^+$ 1445.5732, found 1445.5707. UV/Vis (H_2O): λ_{max} (ε) = 278 ($1.44x10^4$), 361 ($2.54x10^4$), 549 ($8.23x10^3$). UV/Vis (DMSO): λ_{max} (ε) = 277 ($1.89x10^4$), 362 ($2.66x10^4$), 547 ($9.42x10^3$). UV/Vis (DCM): λ_{max} (ε) = 277 ($1.51x10^4$), 361 ($2.52x10^4$), 547 ($8.65x10^3$). UV/Vis (1-octanol): λ_{max} (ε) = 276 ($1.48x10^4$), 362 ($2.58x10^4$), 518 ($7.52x10^3$), 550 ($8.63x10^3$) Anal. calcd. for $C_{69}H_{94}CoN_8O_{20}P + 3H_2O$: C 54.54, H 6.96, N 7.17; found: C 54.17, H 7.35, N 7.17. ^{1}H NMR (500 MHz, [D4]MeOH): δ = 7.29 (s, 1H), 7.15 (s, 1H), 6.53 (s, 1H), 6.30 (d, J = 3.1 Hz, 1H), 6.01 (s, 1H), 4.77-4.71 (m, 1H), 4.37-4.30 (m, 1H), 4.18 (t, J = 3.3 Hz, 1H), 4.15-4.10 (m, 2H), 4.06-4.03 (m, 1H), 3.86-3.81 (m, 1H), 3.78-3.70 (m, 14H), 3.69 (s, 3H), 3.65 (s(br), 1H), 3.51-3.48 (m, 1H), 3.45 (s, 3H), 2.91-2.87 (m, 1H), 2.82-2.45 (m, 19H), 2.31-2.26 (m, 7H), 2.21-2.06 (m, 3H), 1.98-1.84 (m, 7H), 1.77-1.70 (m, 1H), 1.40 (s, 3H), 1.35 (s, 3H), 1.29-1.23 (m, 8H), 1.19-1.11 (m, 4H), 0.46 (s, 3H) ppm. ^{13}C NMR (125 MHz, [D4]MeOH): δ = 181.2, 180.1, 177.9, 175.1, 174.9, 174.4, 173.92, 173.90, 173.7, 173.1, 172.1, 167.12, 167.07, 143.5, 138.2, 135.6, 134.0, 131.5, 117.6, 112.6, 108.2, 105.5, 95.5, 88.1, 86.1, 83.6, 76.5, 70.7, 61.8, 60.5, 58.0, 56.1, 55.2, 52.9, 52.6, 52.4, 52.37, 52.3, 51.9, 51.7, 46.8, 43.1, 42.4, 40.6, 34.2, 33.8, 33.1, 32.7, 32.1, 31.7, 31.6, 28.7, 26.9, 26.5, 20.8, 20.6, 20.4, 20.3, 20.09, 20.06, 17.8, 17.0, 16.4, 16.1 ppm.

Cobinester (5) synthesis procedure



Cobinamide (**4**) (15.0 mg, 0.014 mmol) was dissolved in a degased mixture of HFIP/MeOH (0.5 ml; 1:1), in a pressure tube equipped with a stirring bar. DMF-DMA (88 µl, 0.662 mmol) was added. The reaction mixture was vigorously stirred at 60 °C for 48 h in anaerobic conditions. It was then diluted with DCM (30 ml) and washed with brine (5 ml). The organic layer was concentrated *in vacuo* giving a red residue. It was dissolved in Et_2O (5 ml) and precipitated with hexane (20 ml. The crude product was dissolved in DCM, washed with $NaCN_{aq}$ and purified using DCVC (gradually from 2.5 to 10% EtOH in DCM). Recrystalization from AcOEt/hexane gave a purple solid (9.1 mg; 56%). R_f 0.35, 5% EtOH in DCM. HRMS ESI (*m/z*) calcd for $C_{55}H_{78}CoN_6O_{14}$ $[M–CN]^+$ 1105.4908, found: 1105.4904; UV/Vis (DMSO): λ_{max} (ε) = 316 (1.13×10^4), 371 (3.07×10^4), 550 (1.01×10^4), 589 (1.23×10^4); Anal. calcd for $C_{56}H_{78}CoN_7O_{14}+H_2O$: C 58.48, H 7.01, N 8.52, found: C 58.13, H 7.00, N 8.43. 1H NMR (500 MHz, [D6]Acetone): δ = 7.33 (t, J = 4.0 Hz, 1H), 5.73 (s, 1H), 3.92-3.87 (m, 1H), 3.82-3.76 (m, 1H), 3.75-3.64 (m, 14H), 3.61 (s, 3H), 3.56 (s, 3H), 3.42-3.39 (m, 1H), 3.27-3.22 (m, 1H), 3.19-3.17 (m, 1H), 3.11-3.06 (m, 1H), 2.91-2.81 (m, 2H), 2.71-2.62 (m, 2H), 2.56-2.40 (m, 7H), 2.36-2.22 (m, 11H), 2.17-2.08 (m, 2H), 1.86-1.66 (m, 3H), 1.63 (s, 3H), 1.50 (s, 3H), 1.44 (s, 3H), 1.41 (s, 3H), 1.28 (s, 3H), 1.16 (s, 3H), 1.07 (d, J = 6.2 Hz, 3H) ppm. ^{13}C NMR (125 MHz, [D6]Acetone): δ = 176.84, 176.83, 176.2, 174.6, 173.9, 173.3, 173.0, 172.7, 172.5, 172.4, 172.0, 171.5, 164.3, 163.5, 104.5, 103.6, 91.4, 83.6, 75.6, 67.3, 67.2, 59.2, 57.6, 55.0, 54.2, 52.4, 52.0, 51.9, 51.8, 51.7, 51.6, 49.3, 47.94, 47.91, 47.8, 47.4, 46.7, 42.8, 42.0, 40.5, 32.1, 33.6, 32.3, 32.2, 31.9, 31.89, 31.6, 31.5, 31.4, 27.2, 26.5, 25.6, 23.3, 22.7, 21.3 ppm.

Optimization of cobalester synthesis

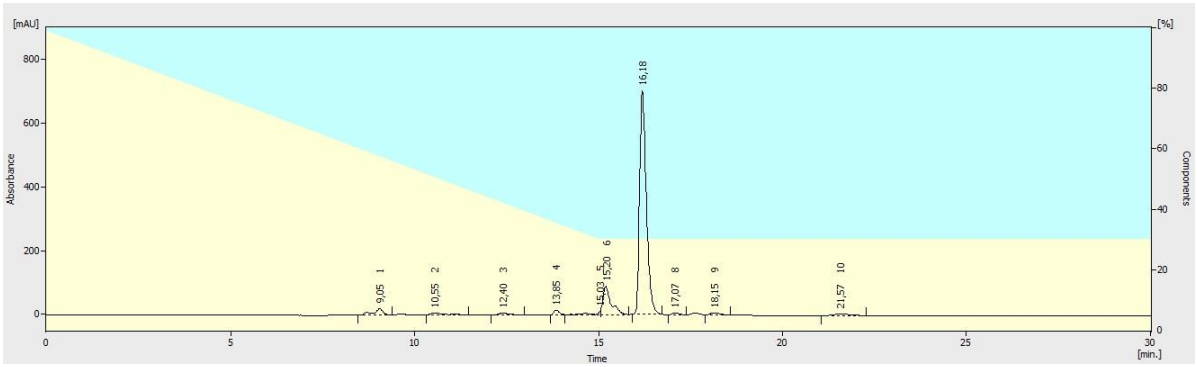


entry	T [°C]	time [h]	solvent	c [M]	DMF-DMA [eq.]**	conversion [%]	yield [%]
1	rt	1	MeOH	0,022	3	11	0
2	45	1	MeOH	0,022	3	66	0
3	45	25	MeOH	0,022	3	100	0
4	45	91	MeOH	0,022	3	100	0
5	60	1	MeOH	0,022	3	100	0
6	60	20	MeOH	0,022	3	100	0
7	60	44	MeOH	0,022	3	100	1
8	60	68	MeOH	0,022	3	100	0
9	60	44	MeOH	0,022	10	100	0
10	60	68	MeOH	0,022	10	100	0
11	60	25	MeOH/DMF*	0,022	10	100	9
12	60	25	MeOH/*i*PrOH*	0,022	10	100	traces
13	60	25	MeOH/C_6F_6*	0,022	10	100	0
14	60	25	MeOH/$C_5H_3F_8OH$*	0,022	10	100	0
15	60	25	MeOH/HFIP*	0,022	10	100	61
16	60	25	MeOH/CH_3CN*	0,022	10	100	0
17	60	25	MeOH/DMSO*	0,022	10	100	0
18	60	25	MeOH/H_2O*	0,022	10	0	0
19	60	48	MeOH/DMF*	0,022	10	100	0
20	60	48	MeOH/HFIP*	0,022	10	100	72
21	60	72	MeOH/HFIP*	0,022	10	100	65
22	60	25	MeOH/HFIP*	0,089	10	100	5
23	60	25	MeOH/HFIP*	0,011	10	100	38
24	60	48	MeOH/HFIP*	0,089	10	100	0

25	60	48	MeOH/HFIP*	0,011	10	100	54
26	60	48	MeOH/HFIP***	0,022	10	100	1
27	60	48	MeOH/HFIP****	0,022	10	100	25

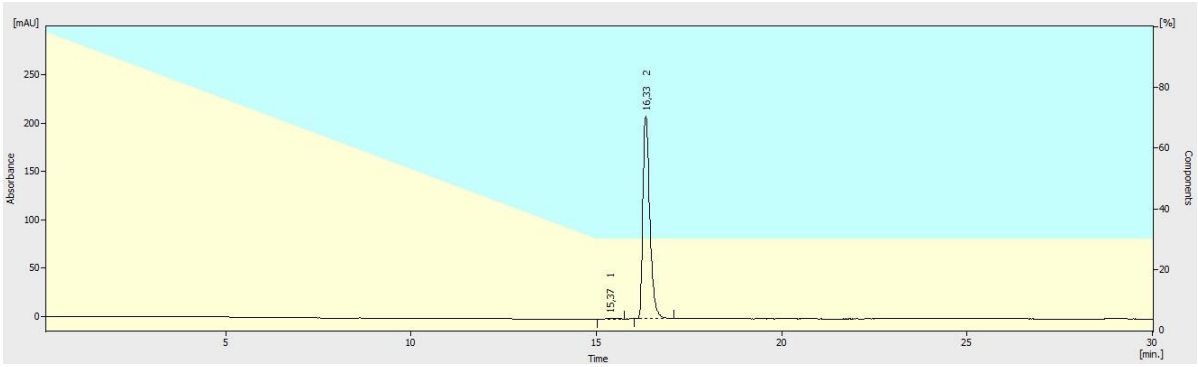
* 1:1 (MeOH:solvent) solvent ratio was used
** equivalents per one amide group
*** 5:1 (MeOH:HFIP) solvent ratio was used
**** 1:5 (MeOH:HFIP) solvent ratio was used

Copy of chromatogram of crude reaction mixture in optimal conditions (entry 20):



	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]
1	9,050	349,661	19,543	3,0	2,3	0,22	992
2	10,550	143,427	5,966	1,2	0,7	0,28	995
3	12,400	129,575	6,207	1,1	0,7	0,32	993
4	13,850	173,528	16,144	1,5	1,9	0,18	991
5	15,033	192,671	9,913	1,7	1,1	0,08	992
6	15,200	1393,044	89,108	12,0	10,3	0,22	990
7	**16,183**	**8865,269**	**702,957**	**76,3**	**81,1**	**0,22**	**960**
8	17,067	79,079	5,154	0,7	0,6	0,27	993
9	18,150	124,440	6,965	1,1	0,8	0,30	995
10	21,567	173,621	5,198	1,5	0,6	0,57	987
	Total	11624,315	867,156	100,0	100,0		

Copy of chromatogram of pure cobalester **3**:



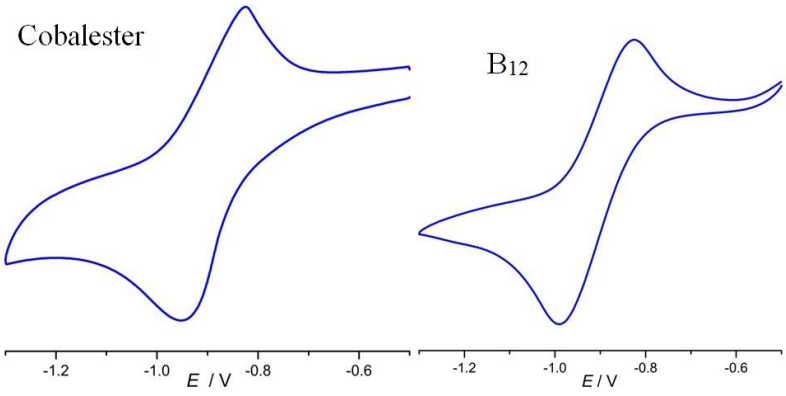
	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]
1	15,367	4,064	0,240	0,2	0,1	0,28	999
2	16,333	2695,988	209,654	99,8	99,9	0,22	884
	Total	2700,052	209,894	100,0	100,0		

Cyclic voltammetry measurements

Cyclic voltammograms of vitamin B_{12} (**1**) and cobalester (**3**) were recorded in deoxygenated 0.2 M solutions of tris buffer in deionized water (C_{B12} = 1.7 mM, $C_{cobalester}$ = 0.7 mM). A three-electrode setup was used in both experiments, including glassy carbon working electrode, Ag/AgCl reference electrode and auxiliary platinum foil (scan rate v = 10 mVs^{-1}, Ar, 20 °C). For a better comparison, E values of the cobalt(III) to cobalt(II) reduction have been expressed as E^* versus $K_3Fe(CN)_6$ used as a standard in the same experimental conditions ($E_{1/2}$ = 216 mV).

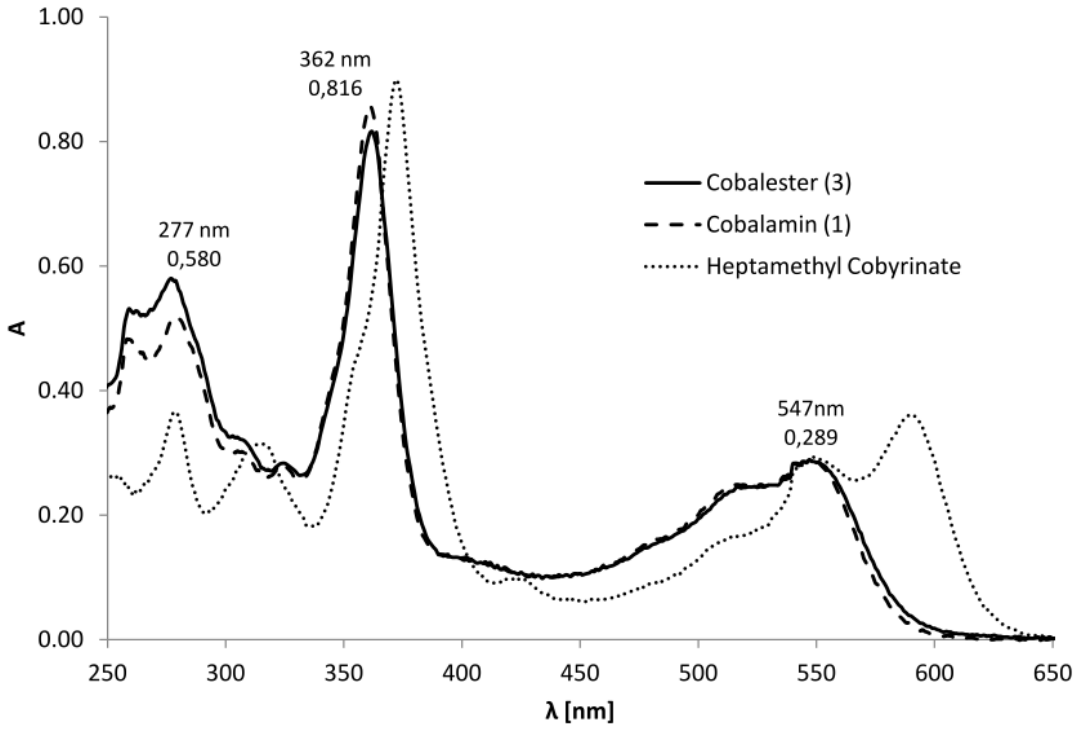
vitamin B_{12} (**1**)			cobalester (**3**)		
E_{pc}	E_{pa}	$E_{1/2}$	E_{pc}	E_{pa}	$E_{1/2}$
-0.826	-0.990	-0.908	-0.824	-0.953	-0.888
E_{pc}^*	E_{pa}^*	$E_{1/2}^*$	E_{pc}^*	E_{pa}^*	$E_{1/2}^*$
-1.042	**-1.206**	**-1.124**	**-1.040**	**-1.169**	**-1.104**

$K_3Fe(CN)_6$
$E_{1/2}$ [V]
0.216



Cyclic voltammogram of cobalester (**3**) and cobalamin (**1**).

UV/Vis spectra of cobalamin (1), cobalester (3), and heptamethyl cobyrinate in DMSO

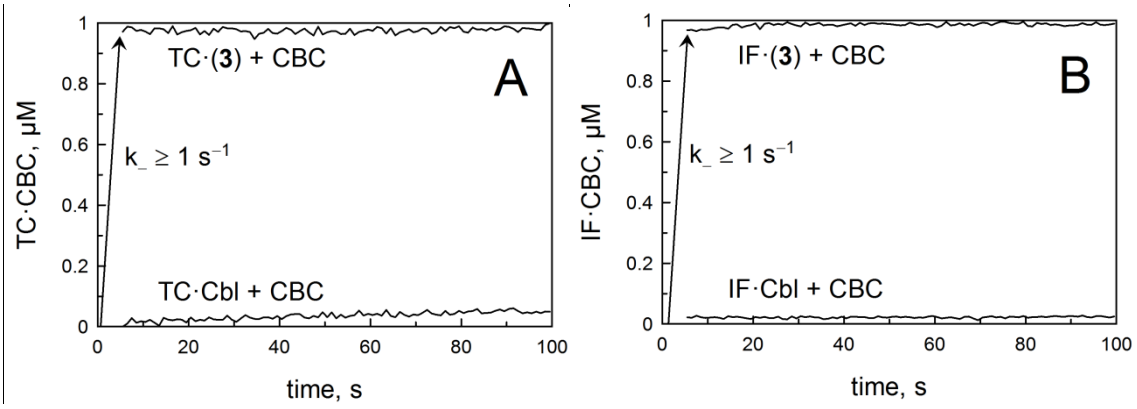


Binding of cobalester 3 to IF and TC

The potential biological properties of this new derivative **3** were examined. We tested its interactions with essential cobalamin transport proteins in an established competition assay, where the fluorescent B_{12} conjugate served as a tracer.[3] Because the peripheral primary amide groups are essential for binding to transport proteins, the biological properties of cobalester **3** were dubious. Not surprisingly, compound **3** displayed no affinity for either intrinsic factor (IF) or transcobalamin (TC). This result directly showed that the amide groups are important in the recognition process. However, these alterations do not preclude cobalester (**3**) from becoming a powerful chemical catalyst.

Dissociation experiments were conducted as follows. 1 μM binding protein (TC or IF) was incubated with 1.1 μM ligand (cobalester (**3**) or CNCbl (**1**)) for 10-20 min, whereupon 1.1 μM CBC was added. Increase in fluorescence upon formation of TC·CBC or IF·CBC was followed. Span between the fluorescence of free CBC in phosphate buffer with 0.05 mg/mL albumin and the signal of TC·CBC or IF·CBC corresponded to 100% (or 1 μM of the fluorescing complex). No trace of protein-ligand complex was found after 5 s of incubation, i.e. all protein was converted to the complex with CBC after this time. The dissociation rate constants are of above 1 s^{-1}.

The equilibrium dissociation constants can be assessed as above 10 μM.

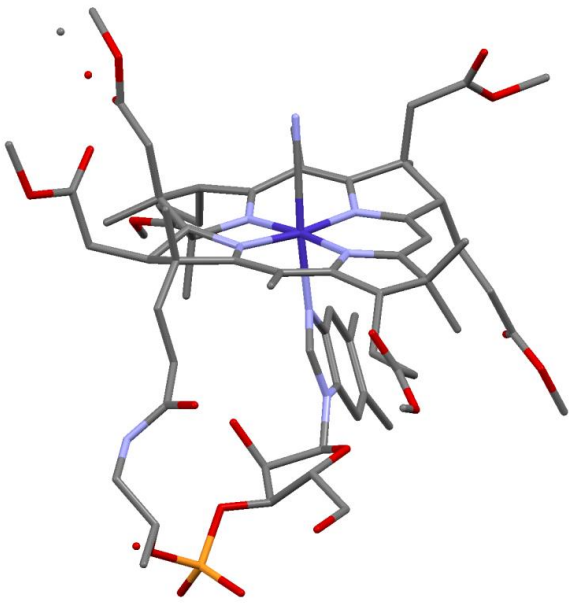


Binding cobalester (**3**) to TC (Fig. A) and IF (Fig.B).

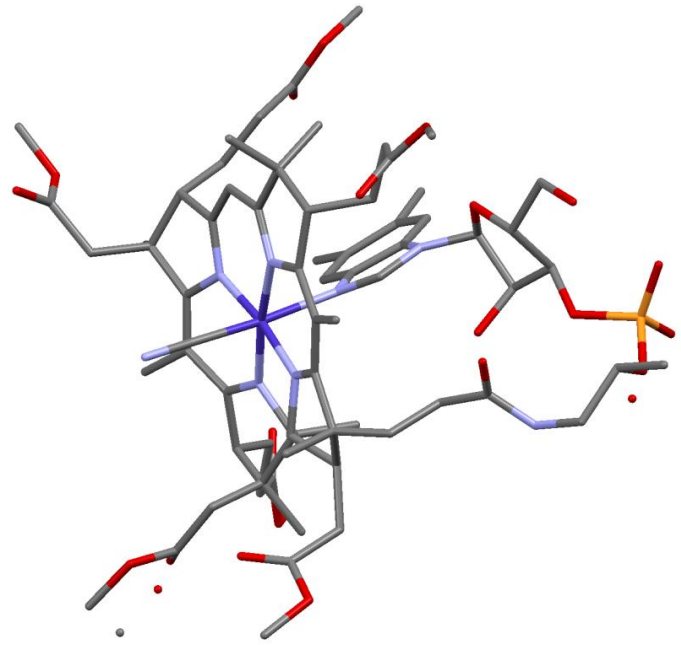
Crystallographic data and crystal structure of cobalester (3)

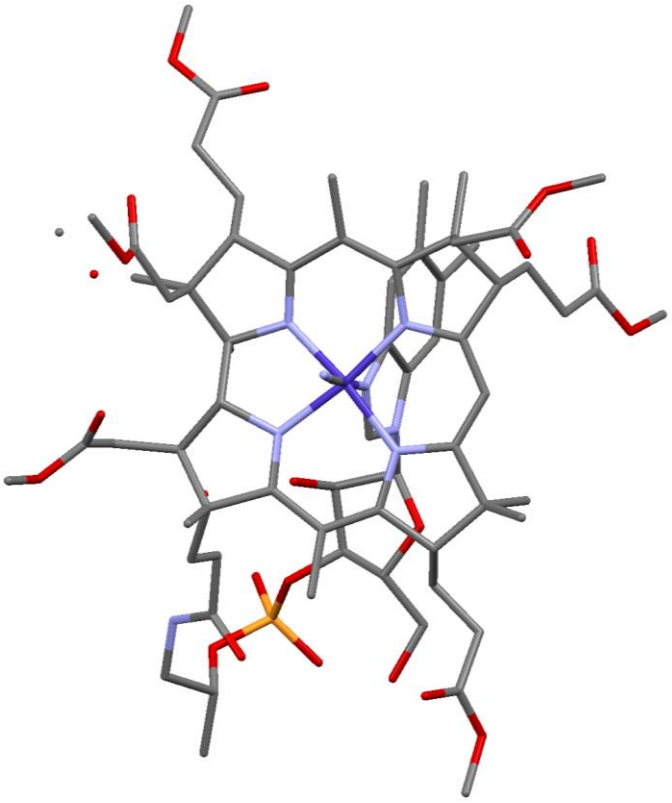
Formula	$C_{207}H_{282}Co_3N_{24}O_{61}P_3$
Space group	R3
Cell Lengths (Å)	**a** 39.7853(10) **b** 39.7853(10) **c** 14.0608(4)
Cell Angles (°)	**α** 90 **β** 90 **γ** 120
Cell Volume (Å)	19274.6
Z, Z’	Z: 3 Z': 0
R factor (%)	5.7
CCDC deposition number	981457

Cobalester (**3**) crystallized in the trigonal space group, R3. This was unusual for vitamin B_{12} analogs, which typically form orthorhombic crystals.[4–7] The axial Co-CN bond length in cobalester (**3**) (1.872 Å) is not significantly different from that in cobalamin (1) (1.858 Å).[4] In contrast, the axial Co-N bond to the purine base (2.048 Å) is almost 0.04 Å longer than that of cobalamin (1) (2.011 Å). The distances from the Co atom to the N atoms of the A and D rings are shorter than those to the N atoms of the C and B rings (Co-N_A 1.885 Å, Co-N_B 1.921 Å, Co-N_C 1.942 Å, Co-N_D 1.900 Å).



Crystal structure of cobalester (**3**) (hydrogen atoms omitted for clarity).



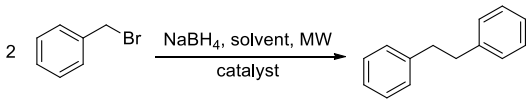


Stability test

The solubility of cobalester (**3**) exhibits features of both (CN)Cbl (**1**) and $(CN)_2Cby(OMe)_7$. It dissolves well in both polar (water, MeOH, DMSO) and nonpolar (DCM, $CHCl_3$) solvents. In buffered solutions, the compound was stable for 3 h between pH 7 and 2, but at pH = 10, it rapidly decomposed to carboxylic salts. pH Stability was performed by incubating solutions of cobalester (**3**) in buffers of pH ranging from 2 to 12 ($7x10^{-4}$ M) and conversion was controlled using HPLC.

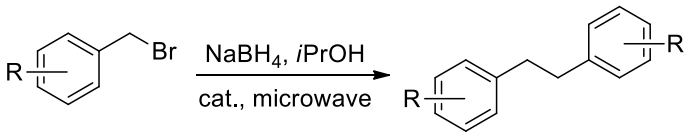
entry	pH	temperature [°C]	time [h]	conversion [%]
1	2	rt	1	0
2	2	rt	5	4
3	4	rt	1	0
4	4	rt	5	0
5	7	rt	1	0
6	7	rt	5	0
7	7	rt – UV lamp irradiation	1	0
8	7	rt – UV lamp irradiation	5	0
11	7	90	1	11
12	7	90	5	87
13	10	rt	1	80
14	10	rt	5	100
15	12	rt	1	99
16	12	rt	5	100

Optimization of benzylbromides homocoupling



entry	benzyl bromide [mmol]	solvent	solvent volume [ml]	catalyst loading [%]	$NaBH_4$ [eq.]	temperature [°C]	time [min]	yield [%]
1	0.25	iPrOH	1	1.5	2.0	120	10	19
2	0.25	iPrOH	1	1.5	2.0	120	15	84
3	0.25	Dioxane	1	1.5	2.0	120	15	traces
4	0.25	THF	1	1.5	2.0	120	15	-
5	0.25	n-BuOH	1	1.5	2.0	120	15	80
6	0.25	iPrOH	1	1	2.0	120	15	84
7	0.25	iPrOH	1	0.5	2.0	120	15	86
8	0.25	iPrOH	1	0.25	2.0	120	15	42
9	0.25	iPrOH	0.25	0.5	2.0	120	15	37
10	0.25	iPrOH	1	0.5	1.0	120	15	54
11	0.25	iPrOH	0.25	0.5	0.5	120	15	traces
12	0.25	iPrOH	0.25	0.5	1.0	120	15	traces
13	0.25	iPrOH	0.5	0.5	1.0	120	15	traces
14	0.25	iPrOH	1	0.5	2.0	90	15	84
15	0.25	iPrOH	1	0.5	2.0	60	15	10
16	0.25	iPrOH	1	-	2.0	90	15	-
17	0.25	iPrOH	1	0.5	2.0	reflux (oil bath)	15	traces
18	0.25	iPrOH	1	0.5	2.0	reflux (oil bath)	60	58

General bibenzyl synthesis procedure



A MW reaction vessel equipped with a stirring bar was charged with cobalester (**3**) (1.8 mg, 0.5 mol%) and $NaBH_4$ (19 mg, 0.5 mmol). Degased isopropanol (1.0 ml) and subsequently appropriate benzyl bromide (0.25 mmol) were added under anaerobic atmosphere. The reaction mixture was heated to 90 °C in a microwave reactor for 15 minutes (maximum power 300 W; ramping time not included). After cooling down to rt it was filtered through a celite pad and the filtrate concentrated in vacuo. The crude product was purified using column chromatography.

References

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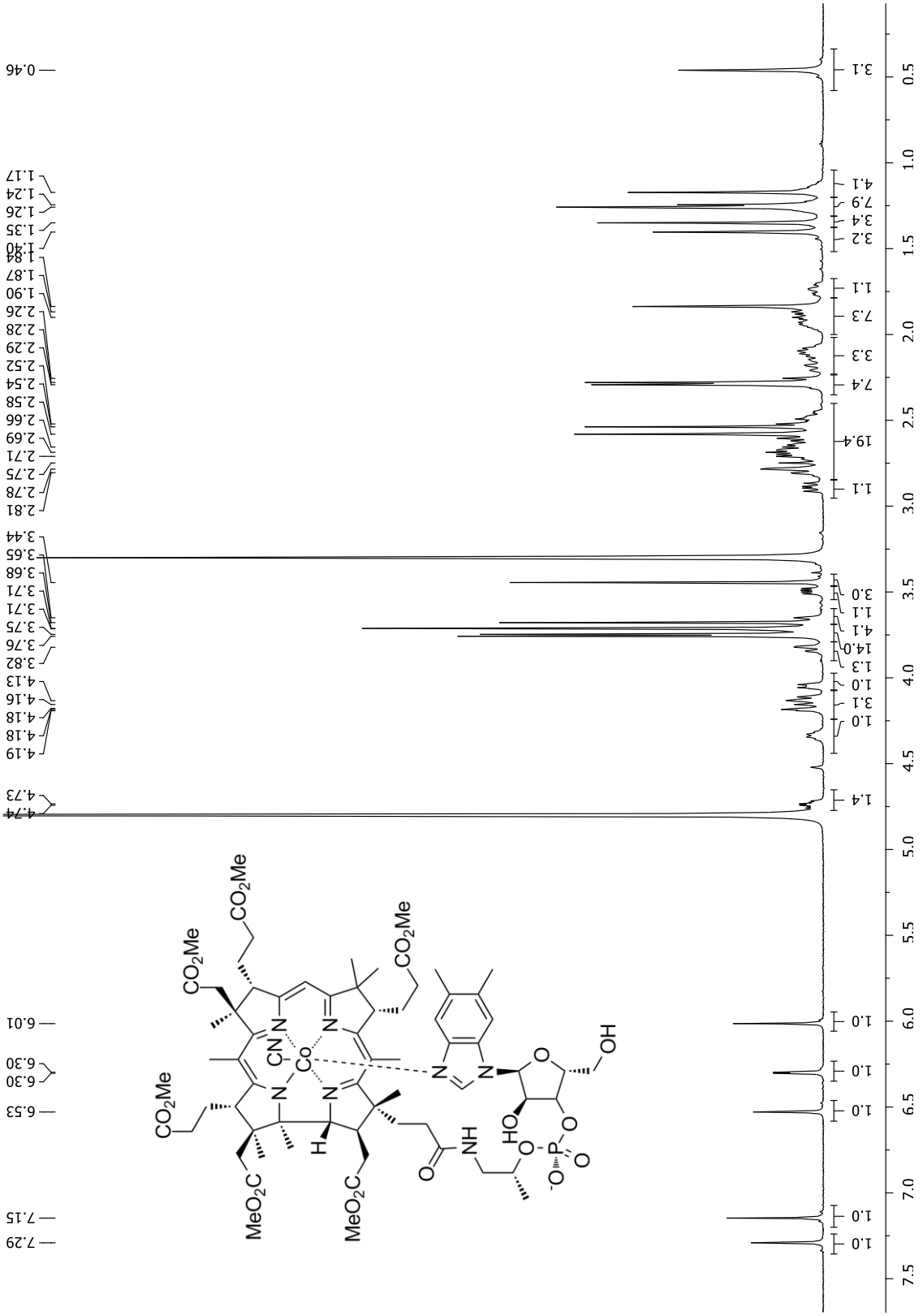
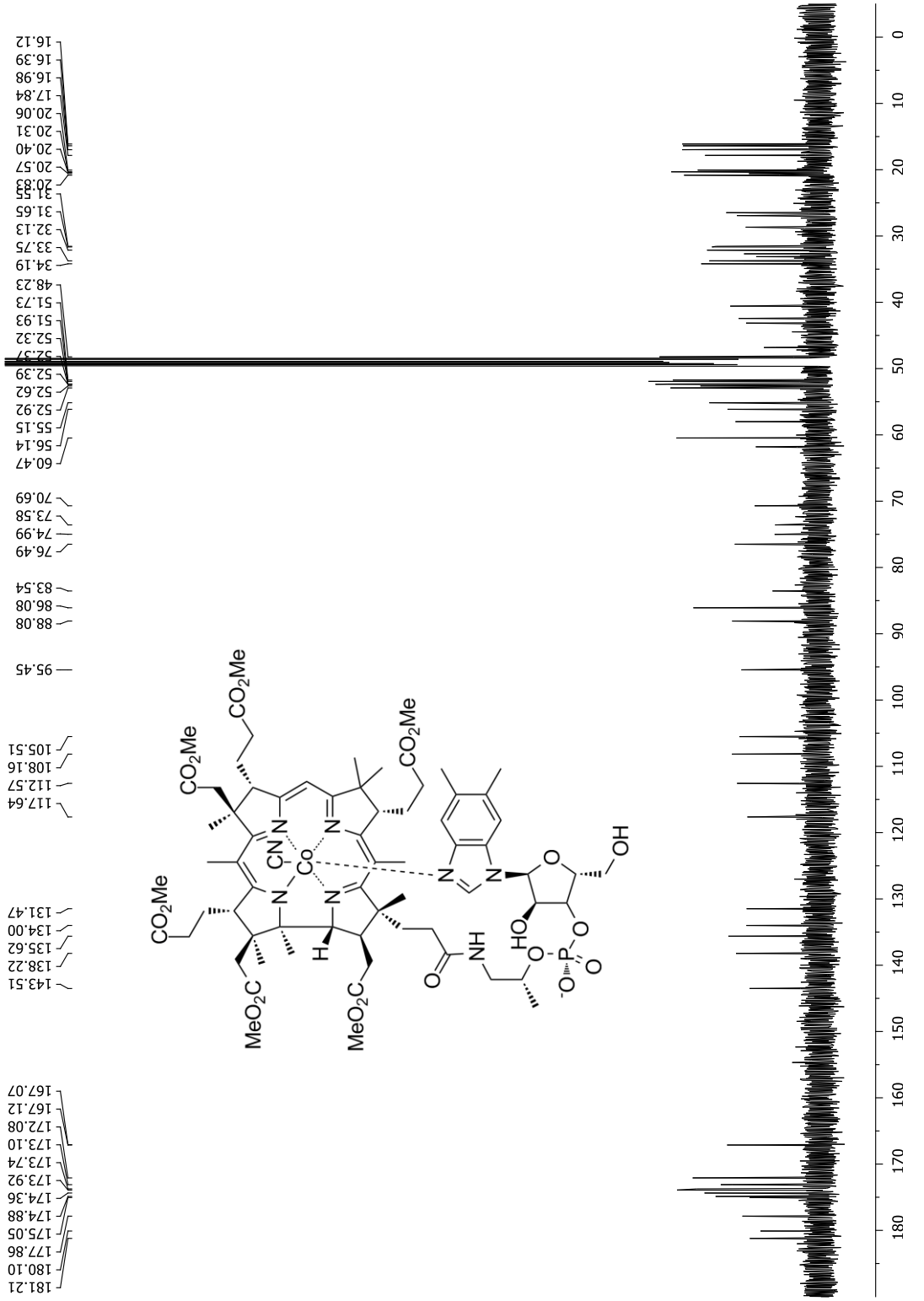
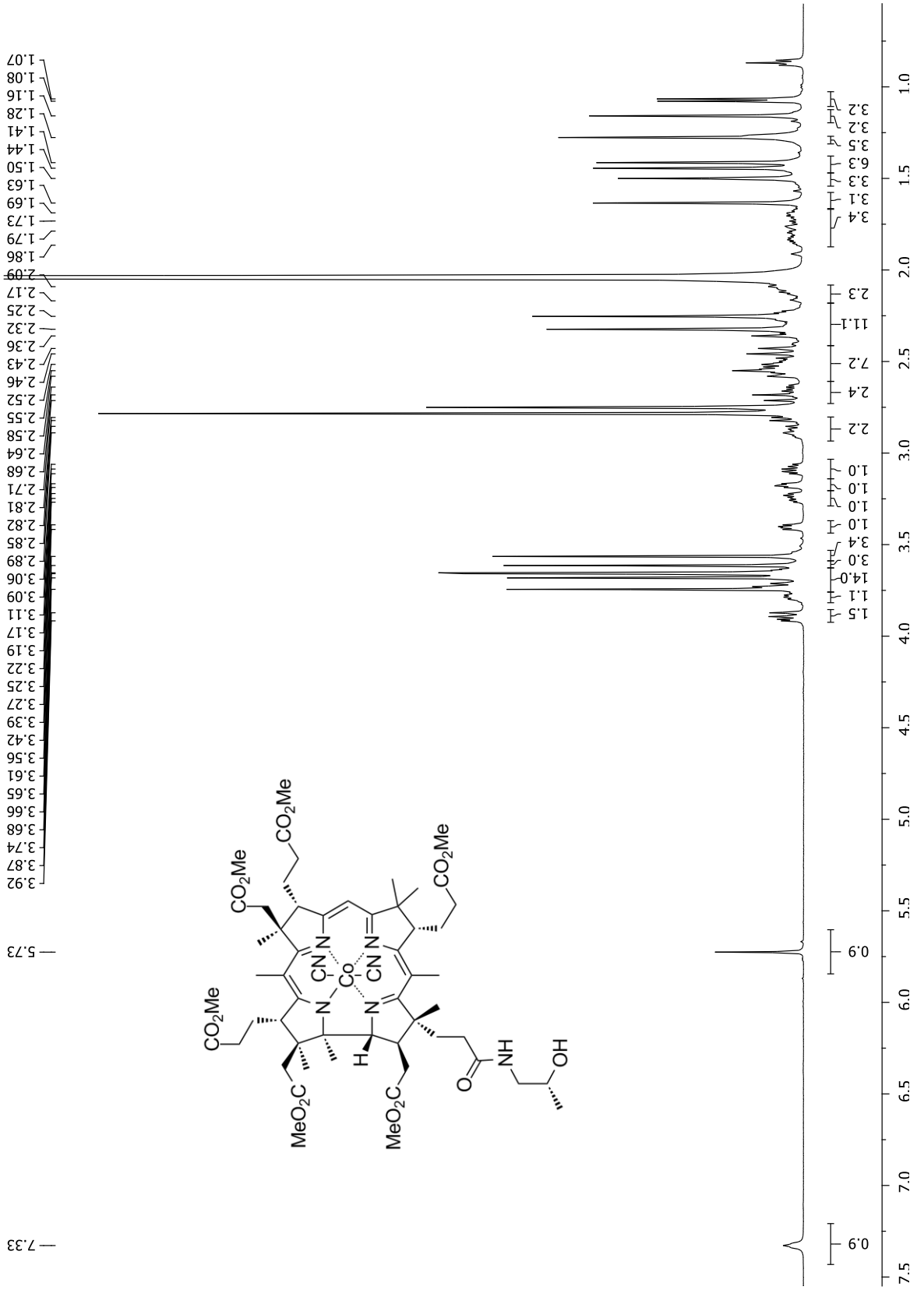
Cobalester (3) - ^{1}H NMR



Cobalester (3) – ^{13}C NMR



Cobinester (5) – ^{1}H NMR



Cobinester (5) – ^{13}C NMR