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Electronic Supporting Information

Reduction-free synthesis of stable acetylide cobalamins

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General information: Commercial vitamin B_{12} and other reagents and solvents were used as received. Preparative chromatography was performed using Silica Gel 90 C18 (Fluka) and Silica Gel 90 C2 (Fluka). For preparative chromatography redistilled water and HPLC grade acetonitrile was used.

^{1}H and ^{13}C NMR spectra were recorded at RT on Varian 600 MHz or Varian 500 MHz spectrometers with residual solvent peak as an internal standard.

UV- Vis spectra were recorded on Jenway 7315 spectrometer.

HRMS spectra were recorded SYNAPT G2-S HDMS (Waters) spectrometer

All reactions and product purity were monitored using HPLC method.

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
30	30	70

pH stability was performed by incubating solutions of compounds **2**, **3**, and **4** in buffers of pH ranging from 1 to 8 and ($5x10^{-5}$ M) and recording UV/Vis spectra.

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

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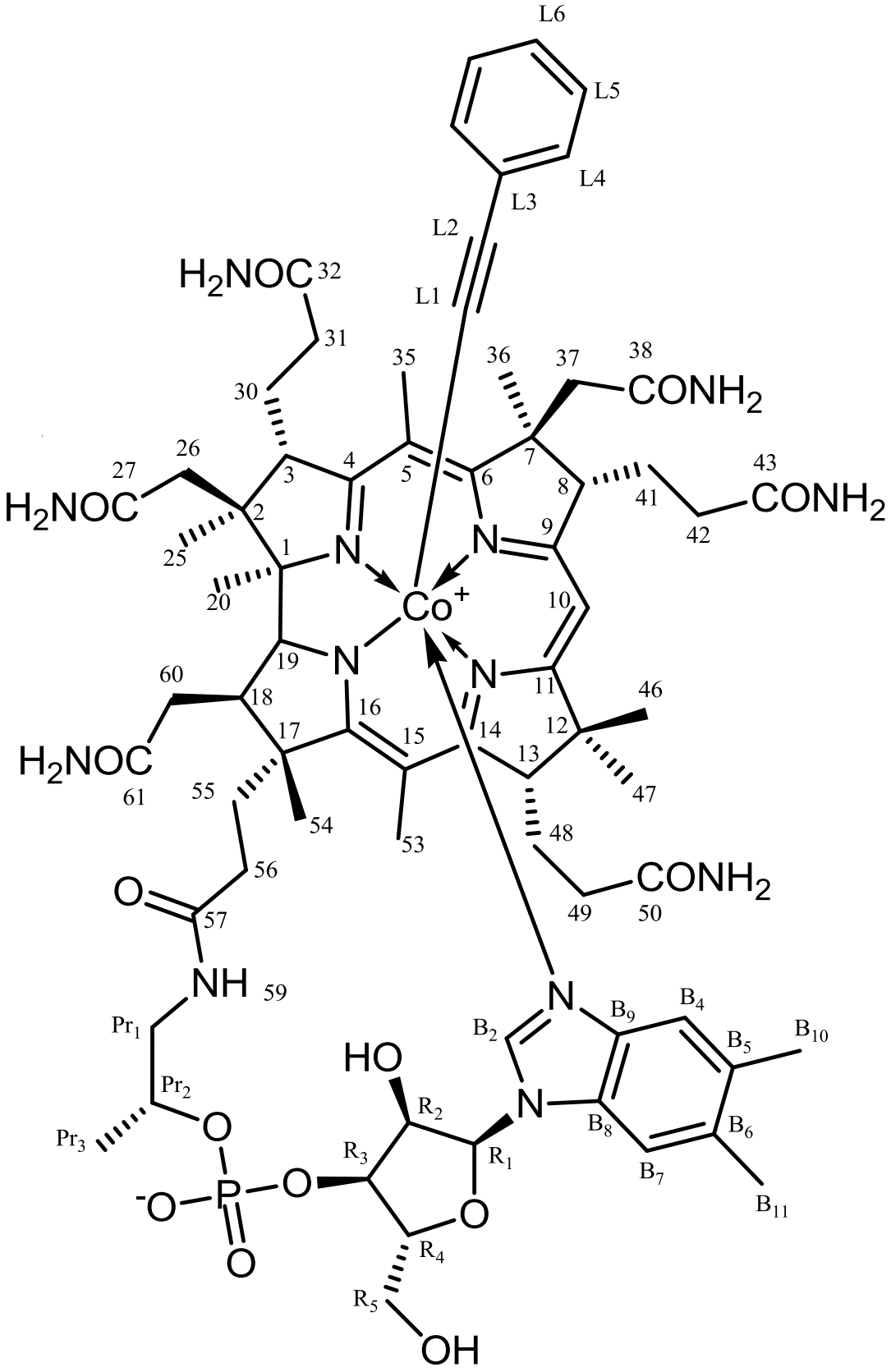
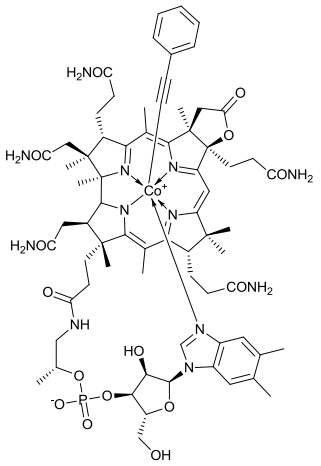
Numbering of alkynylated B_{12} derivatives



Preparation of compound 1.



Vitamin B_{12} (67 mg, 0.05 mmol), copper (I) acetylide (82 mg, 0.50 mmol) and Ph_3P (130 mg, 0,50 mmol) were dissolved under argon in anhydrous DMF (5 ml). Then molecular sieves 4Å (500 mg) were added. The reaction was placed in an oil bath, slowly heated to 60 °C and after reaching the set temperature stirred for 20 h. The reaction mixture was then filtered through a pad of Celite, the filtrate was precipitated with Et_2O and centrifuged. The precipitate was washed twice with Et_2O, centrifuged and dried under reduced pressure. The residue was dissolved in water, charged on RP C2 column and eluted with H_2O/MeCN (10-70%). The collected fractions were immediately evaporated under reduced pressure without heating. Fractions containing pure product (HPLC analysis) were dissolved in MeOH, precipitated with Et_2O, centrifuged and dried under reduced pressure. **1** was obtained as red crystals. Yield 15 mg (22%).

HRMS(ESI) m/z: calculated for $C_{70}H_{91}N_{12}O_{15}PCo[M+H]^+$ 1429.5796, found 1429.5784.

^{1}H NMR (600 MHz, DMSO-[d_6]) δ: 7.74 (s, 1H), 7.59 (s, 2H), 7.52 (s, 1H), 7.34 (s, 1H), 7.30 (s, 1H), 7.12 (s, 1H), 7.09 (s, 1H), 7.06 (s, 1H), 7.05-7.01 (m, 2H), 7.00-6.95 (m, 1H), 6.99-6.97 (m, 2H), 6.89 (s, 1H), 6.75 (s, 1H), 6.70 (m, 2H), 6.65 (s, 1H), 6.64 (s, 1H), 6.38 (s, 1H), 6.25 (s, 1H), 6.08 (s, 1H), 5.83 (s, 1H), 4.56 (s, *J* = 5.7 Hz, 1H), 4.51 (bs, 1H), 4.17 (d, *J* = 11.2 Hz, 1H), 4.10 (bs, 1H), 3.94 (s, 1H), 3.90-3.83 (m, 1H), 3.60-3.47 (m, 3H), 3.26 (d, *J* = 18.5 Hz, 1H), 3.07 (d, *J* = 11.1 Hz, 1H), 2.79-2.67 (m, 3H), 2.64 (m, 2H), 2.45-2.32 (m, 4H), 2.44 (s, 3H), 2.39 (s, 3H), 2.30-2.16 (m, 3H), 2.16-1.99 (m, 5H), 2.14 (s, 6H), 1.77 (s, 3H), 1.74-1.54 (m, 5H), 1.36 (s, 3H), 1.15 (s, 6H), 1.04 (d, *J* = 5.7 Hz, 3H), 0.93 (s, 3H), 0.33 (s, 3H) ppm.

^{13}C NMR (150 MHz, DMSO-[d_6]) δ: 178.5, 177.7, 176.8, 174.0, 173.7, 173.6, 173.1, 173.0, 172.5, 171.4, 166.2, 164.1, 160.9, 143.1, 137.3, 132.8 , 131.6, 130.7, 130.3, 128.6, 126.8, 126.1, 116.9, 111.9, 105.9, 104.9, 100.4, 94.9, 90.5, 85.9, 84.9, 81.8, 75.4, 74.8, 71.3, 69.7, 62.0, 58.8, 55.2, 53.2, 51.5, 48.2, 47.0, 45.7, 41.9, 40.9, 38.3, 35.7, 34.1, 32.2, 31.7, 31.3, 30.3, 29.7, 29.0, 27.7, 25.9, 20.7, 20.4, 20.3, 19.7, 17.0, 16.9, 16.7, 15.5 ppm.

HPLC: τ = 12.52 min.

^{1}H and ^{13}C NMR assignments of lactone **1**

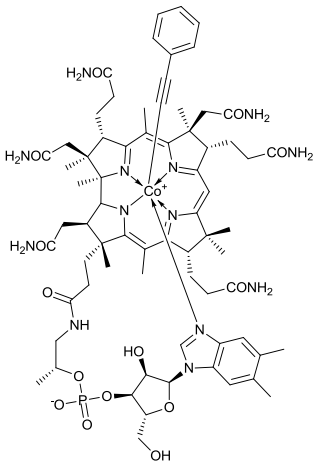
Assignment	^{13}C NMR [ppm]	Observed correlations	
		HQSC [ppm]	HMBC
C4	178.5		C35
C16	177.7		C53, C54
C11	176.8		C10, C13, C46, C47
	174.0		
	173.70		
C27, C32, C38, C43, C50, C57, C61[b]	173.65 173.05 173.02		
	172.5		
	171.4		
C9	166.2		C10
C14	164.1		C13, C53
C6	160.9		C35, C36, C37
B2	143.1	7.09	
B9	137.3		B2
B6	132.8		B4, B10
B5	131.6		B11
L4	130.7	6.86	L6
B8	130.3		B2, B4
L5	128.6	7.04	
L3	126.8		L5
L6	126.1	6.98	L4
B4	116.9	6.37	B11
B7	111.9	7.30	B10
C5	105.9		C35
C15	104.9		C53
L2	100.4		L4
C8	94.9		C10, C 36, C37, C41, C42
C10	90.5	5.83	
R1	85.9	6.25	
C1	84.9		C3, C19, C20, C25
R4	81.8	3.88	
C19	75.4	4.18	C20
R3	74.8	4.51	
Pr2	71.3	4.10	Pr3
R2	69.7	3.94	
R5	62.0	3.56	
C17	58.8		C53, C54
C3	55.2	4.56	C25
C13	53.2	3.07	C46, C47
C7	51.5		C36, C37
C12	48.2		C13, C46, C47
C2	47.0		C20, C25
Pr1	45.7	3.53, 2.71	Pr3
C26	41.9	2.12	C25
C37	40.9	3.25, 2.64	C36
C18	38.3	2.72	C54
C31	35.7	2.20	
C49	34.1	2.36, 2.27	C13
C60	32.2	2.46	
C55	31.7	1.73	C54
C46	31.3	0.93	C13, C47
C56	30.3	2.63, 2.03	
C41	29.7	1.61	C42
C42	29.0	2.09, 1.94	C41
C48	27.7	1.92, 1.57	C13
C30	25.9	1.70	C31
Pr3	20.7	1.04	
B10/B11	20.4	2.14	B4/B7
C20	20.3	0.33	
C47	20.3	1.35	
C36	19.7	1.77	C37, C41, C42
C25	17.0	1.15	
C35	16.9	2.43	
C54	16.7	1.15	
C53	15.5	2.39	

[a] Spectra were recorded on Varian 600 MHz spectrometer, residual solvent peaks (2.50 ppm and 39.52 ppm) were used as internal standards. [b] Amide carbonyl groups, cross peaks in HMBC unclear or overlap.

General procedure for the preparation of compounds 2, 3, 4 and 5.

Vitamin B_{12} (135 mg, 0.1 mmol), CuAcO (12 mg, 0.1 mmol) were dissolved in DMA (1.5 ml) followed by addition of the respective alkyne (1 mmol) and solution of DBU (76 µl, 0.5 mmol) in DMA (0.5 ml). The reaction mixture was vigorously stirred at room temperature for 4 hours and monitored by HPLC. When the reaction reached almost full conversion of the starting material, the reaction mixture was precipitated with Et_2O (15 ml) and centrifuged. The resulting precipitate was washed twice with Et_2O (2 x 15 ml) followed by centrifugation and drying on air. The resulting solid was then dissolved in distilled water (30 ml), centrifuged and the solution over the yellowish precipitate was collected and concentrated *in vacuo*. The solid was charged on RP C18 column and eluted with H_2O/MeCN (10-70%). The second fraction containing the desired product was collected and concentrated *in vacuo*. The obtained red solid was dissolved in MeOH (2 ml) , precipitated with Et_2O, centrifuged and dried under reduced pressure at 50 °C.

Compound 2



Yield: 114 mg, 85%.

HRMS(ESI) m/z: calculated for $C_{70}H_{93}N_{13}O_{14}PCoNa$ $[M+Na]^+$ 1452.5932, found 1452.5931.

IR (KBr) ν = 3333, 3189, 2967, 2937, 2123, 1666, 1574, 1496, 1217, 1151, 1068, 995, 847, 759 cm^{-1}.

UV/Vis (H_2O): λ_{max}, (ε)= 551 ($7.5x10^3$), 522 ($6.5x10^3$), 363 ($1.3x10^4$), 259, 218 nm ($L \cdot mol^{-1} \cdot cm^{-1}$).

^{1}H NMR (600 MHz, DMSO-[d_6]) δ: 7.69 (s, 1H), 7.57 (s, 2H), 7.48 (s, 2H), 7.30 (s, 1H), 7.26 (s, 1H), 7.11 (s, 1H), 7.08 (s, 1H), 7.05-6.99 (m, 3H), 6.98-6.94 (m, 1H), 6.93 (s, 1H), 6.88 (s, 1H), 6.74 (s, 1H), 6.74-6.70 (m, 1H), 6.63 (s, 1H), 6.50 (s, 1H), 6.48 (s, 1H), 6.33-6.27 (m, 1H), 6.25 (s, 1H), 6.06 (bs, 1H), 5.83 (s, 1H), 4.58 (d, *J* = 6.2 Hz, 1H), 4.51-4.41 (m, 1H), 4.20 (d, *J* = 11.0 Hz, 1H), 4.11-4.04 (m, 1H) 3.92-3.38 (m, 2H), 3.75 (dd, *J* = 5.5, *J* = 9.6 Hz, 1H), 3.60-3.49 (m, 3H), 3.03 (d, *J* = 11.1 Hz, 1H), 2.74-2.56 (m, 3H), 2.46-2.31 (m, 5H), 2.44 (s, 3H), 2.36 (s, 3H), 2.28-2.08 (m, 4H), 2.14 (s, 3H), 2.13 (s, 3H), 2.08-1.99 (m, 1H), 1.94-1.82 (m, 4H), 1.80-1.66 (m, 4H), 1.64 (s, 3H), 1.60-1.49 (m, 2H), 1.32 (s, 3H), 1.25-1.17 (m, 1H), 1.16 (s, 3H), 1.14 (s, 3H), 1.01 (d, *J* = 5.9 Hz, 3H), 0.95 (s, 4H), 0.29 (s, 3H) ppm.

^{13}C NMR (150 MHz, DMSO-[d_6]) δ: 178.3, 176.9, 174.1, 173.99, 173.74, 173.35, 173.23, 173.10, 172.7, 171.80, 171.5, 164.9, 164.3, 143.1, 137.3, 132.3, 131.1, 130.7, 130.2, 128.5, 127.3, 125.7, 117.4, 111.5, 105.6, 103.1, 100.3, 93.6, 85.2, 84.7, 81.7, 75.1, 74.8, 71.1, 69.7, 62.3, 58.7, 55.1, 53.9,

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53.4, 50.4, 47.6, 46.8, 45.9, 42.2, 42.0, 38.3, 35.7, 34.2, 32.5, 32.4, 31.8, 31.6, 30.3, 27.7, 26.4, 26.0, 20.7, 20.34, 20.32, 20.2, 19.2, 16.9, 16.8, 16.1, 15.4 ppm.

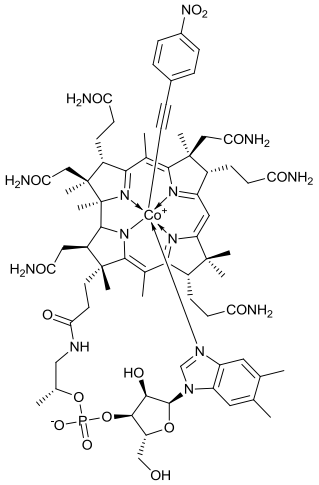
HPLC: τ = 11.72 min.

^{1}H and ^{13}C NMR assignments of compound **2**

Assignment	^{13}C NMR [ppm]	Observed correlations	
		HQSC [ppm]	HMBC
C4	178.3		C35
C16	176.9		C13, C54, C53
C11	174.1		C10, C13, C46, C47
C27, C32, C38, C43, C50, C57, C61[b]	173.7 173.5 173.3 173.2 173.1		
C9	172.7		C8, C10
C27, C32, C38, C43, C50, C57, C61[b]	171.8 171.5		
C6	164.9		C8, C35
C14	164.3		C13, C53
B2	143.1	7.07	
B9	137.3		B7, B2
B6	132.3		B4, B10
B5	131.1		B7, B11
L4	130.7	6.72	L6
B8	130.2		B2, B4
L5	128.5	7.03	
L3	127.3		L5
L4	125.7	6.95	L4
B4	117.4	6.48	B11
B7	111.5	7.26	B10
C5	105.6		C35
C15	103.1		C53
L2	100.3		L4
C10	93.6	5.85	
R1	85.24	6.24	
C1	84.7		C3, C19, C20, C25
R4	81.7	3.87	
R3	75.1	4.46	R2, R5
C19	74.8	4.21	C20
Pr2	71.1	4.08	Pr3
R2	69.7	3.88	
R5	62.3	3.55	
C7	58.7		C54, C60
C3	55.1	4.58	C25, C26
C8	53.9	3.74	C10, C36. C37
C13	53.4	3.04	C46, C47, C49
C7	50.3		C8, C36, C37,
C12	47.6		C10, C46, C47
C2	46.8		C20, C25
Pr1	45.9	3.53, 2.44	Pr3
C37	42.2	2.37, 1.73	C36
C26	42.0	2.13	C25
C18	38.3	2.70	C54
C31	35.7	2.22	
C49	34.2	2.35, 2.26	
C60	32.5	2.44	C55
C42	32.4	1.55, 1.21	
C55	31.8	2.38, 1.72	C54
C46	31.6	0.94	C47
C56	30.3	2.63, 2.04	
C48	27.7	1.89, 1.54	C13, C49
C41	26.4	1.70, 0.94	C8, C42
C30	26.0	1.70, 1.64	C3, C31
Pr3	20.7	1.01	
B11	20.34	2.13	B7
B10	20.32	2.14	B4
C20	20.2	0.29	
C36	19.2	1.64	C41
C54	16.9	1.16	
C25	16.8	1.14	
C35	16.1	2.43	
C53	15.4	2.36	

[a] Spectra were recorded on Varian 600 MHz spectrometer, residual solvent peaks (2.50 ppm and 39.52 ppm) were used as internal standards. [b] Amide carbonyl groups, cross peaks in HMBC unclear or overlap.

Compound 3



Yield: 132 mg, 90%.

HRMS(ESI) m/z: calculated for $C_{70}H_{92}N_{14}O_{16}PCoNa$ $[M+Na]^+$ 1497.5783, found 1497.5770.

IR (KBr) ν = 3319, 3189, 2968, 2938, 2119, 1668, 1573, 1499, 1402, 1337, 1214, 1146, 1107, 1065, 996, 856, 750 cm^{-1}.

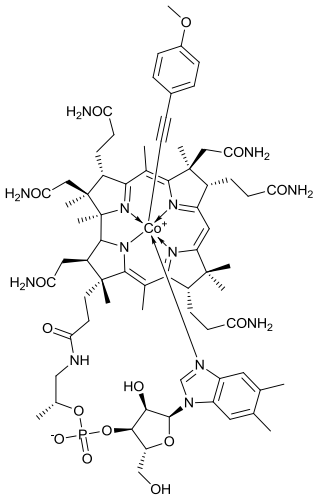
UV/Vis (H_2O): λ_{max}, (ε)= 549 ($6.8x10^3$), 522 ($5.3x10^3$), 363 ($1.7x10^4$), 263, 216 nm ($L \cdot mol^{-1} \cdot cm^{-1}$).

^1H NMR (500 MHz, DMSO-[d_6]) δ: 7.97 (s, 1H), 7.95 (s, 1H), 7.68 (s, 1H), 7.58 (s, 1H), 7.54 (s, 1H), 7.50 (s, 1H), 7.32 (s, 1H), 7.30 (s, 1H), 7.14 (s, 1H), 7.09 (s, 1H), 7.05 (s, 1H), 6.96 (s, 1H), 6.93 (s, 1H), 6.98 (s, 1H), 6.76 (s, 1H), 6.66 (s, 1H), 6.52 (s, 1H), 6.49 (s, 1H), 6.37-6.29 (m, 1H), 6.27 (s, 1H), 6.07 (s, 1H), 5.87 (s, 1H), 4.56 (d, *J* = 6.8 Hz, 1H), 4.51-4.44 (m, 1H), 4.18-4.03 (m, 2H), 3.95-3.83 (m, 2H), 3.78 (dd, *J* = 5.3, *J* = 9.6 Hz, 1H), 3.64-3.49 (m, 3H), 3.07 (d, *J* = 10.8 Hz, 1H), 2.77-2.56 (m, 3H), 2.47-2.32 (m, 5H), 2.46 (s, 3H), 2.39 (s, 3H), 2.31-2.22 (m, 4H), 2.18 (s, 3H), 2.16 (s, 3H), 2.12-1.98 (m, 4H), 1.95-1.69 (m, 6H), 1.66 (s, 2H), 1.61-1.51 (m, 2H), 1.34 (s, 3H), 1.26-1.19 (m, 2H), 1.18 (s, 3H), 1.17 (s, 3H), 1.04 (d, *J* = 6.1 Hz, 3H), 0.97 (s, 4H), 0.31 (s, 3H) ppm.

^{13}C NMR (125 MHz, DMSO-[d_6]) δ: 178.6, 177.3, 174.4, 174.0, 173.7, 173.3, 173.09, 173.1, 171.7, 171.4, 165.2, 164.4, 144.9, 143.1, 137.2, 133.9, 132.5, 131.5, 131.2, 130.2, 124.1, 117.3, 111.6, 105.6, 103.2, 100.9, 93.7, 85.7, 84.7, 81.8, 75.1, 74.8, 71.1, 69.7, 62.3, 58.8, 55.2, 54.0, 53.4, 50.3, 49.0, 47.7, 46.8, 45.9, 42.1, 38.4, 35.7, 34.2, 32.5, 32.4, 31.7, 30.4, 27.7, 26.4, 26.0, 20.7, 20.4, 20.3, 20.14, 19.2, 19.0, 16.9, 16.1, 15.4 ppm.

HPLC: τ = 12.08 min.

Compound 4



Yield: 116 mg, 80%.

HRMS(ESI) m/z: calculated for $C_{71}H_{95}N_{13}O_{15}PCoNa$ $[M+Na]^+$ 1482.6032, found 1482.6035.

IR (KBr) ν = 3333, 3193, 2968, 2940, 2123, 1667, 1573, 1504, 1402, 1350, 1281, 1240, 1213, 1147, 1104, 1068, 997, 927, 903, 868, 834, 812, 729 cm^{-1}.

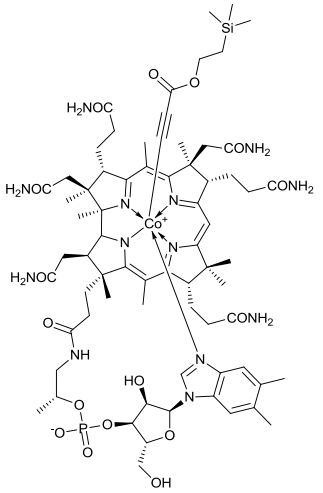
UV/Vis (H_2O): λ_{max}, (ε)= 550 ($5.8x10^3$), 522 ($4.4x10^3$), 364 ($1.2x10^4$), 262, 218 nm ($L \cdot mol^{-1} \cdot cm^{-1}$).

^1H NMR (500 MHz, DMSO-[d_6]) δ: 7.65 (s, 1H), 7.60 (s, 1H), 1.50 (s, 3H), 7.32 (s, 1H), 1.29 (s, 1H), 7.16 (s, 1H), 7.10 (s, 1H), 7.05 (s, 1H), 6.96 (s, 1H), 6.86 (s, 1H), 6.76 (s, 1H), 6.70-6.60 (m, 5H), 6.52 (s, 1H), 6.50 (s, 1H), 6.39-6.32 (m, 1H), 6.26 (s, 1H), 6.05 (s, 1H), 5.85 (s, 1H), 4.59 (d, *J* = 8.1 Hz, 1H), 4.54-4.42 (m, 1H), 4.21 (d, *J* = 11.0 Hz, 1H), 4.17-4.05 (m, 1H), 3.39-3.85 (m, 2H), 3.75 (dd, *J* = 5.3, *J* = 9.9 Hz, 1H), 3.63 (s, 3H), 3.61-3.52 (m, 3H), 3.05 (d, *J* = 11.5 Hz, 1H), 2.72-2.57 (m, 3H), 2.49-2.35 (m, 5H), 2.46 (s, 3H), 2.39 (s, 3H), 2.32-2.20 (m, 6H), 2.18 (s, 3H), 2.16, (s, 3H), 2.12-2.01 (m, 1H), 1.95-1.82 (m, 1H), 1.83-1.50 (m, 6H), 1.67 (s, 3H), 1.35 (s, 3H), 1.29-1.20 (m, 1H), 1.18 (s, 3H), 1.17 (s, 3H), 1.06 (d, *J* = 6.3 Hz, 3H), 0.97 (s, 4H), 0.31 (s, 3H) ppm.

^{13}C NMR (125 MHz, DMSO-[d_6]) δ: 178.2, 176.8, 174.0, 173.8, 173.4, 173.2, 173.1, 172.7, 171.9, 171.5, 164.8, 164.2, 157.5, 143.2, 137.3, 132.3, 131.9, 131.1, 130.2, 119.8, 117.4, 114.1, 110.0, 105.6, 103.1, 99.5, 93.5, 85.7, 84.7, 75.1, 74.7, 69.7, 62.3, 58.7, 55.5, 55.0, 54.0, 53.4, 50.4, 47.6, 46.7, 45.9, 42.3, 35.7, 34.2, 32.5, 32.4, 31.6, 26.4, 26.0, 20.7, 20.3, 20.2, 20.1, 19.3, 16.9, 16.8, 16.2, 15.4 ppm.

HPLC: τ = 11.68 min.

Compound 5



Yield: 127 mg, 85%.

HRMS(ESI) m/z: calculated for $C_{70}H_{102}N_{13}O_{16}PCoSi$ $[M+H]^+$ 1498.6406, found 1498.6396.

UV/Vis (H_2O): λ_{max}, (ε)= 547 ($5.3x10^3$), 522 ($4.3x10^3$), 367 ($9.6x10^3$), 269, 215 nm ($L \cdot mol^{-1} \cdot cm^{-1}$).

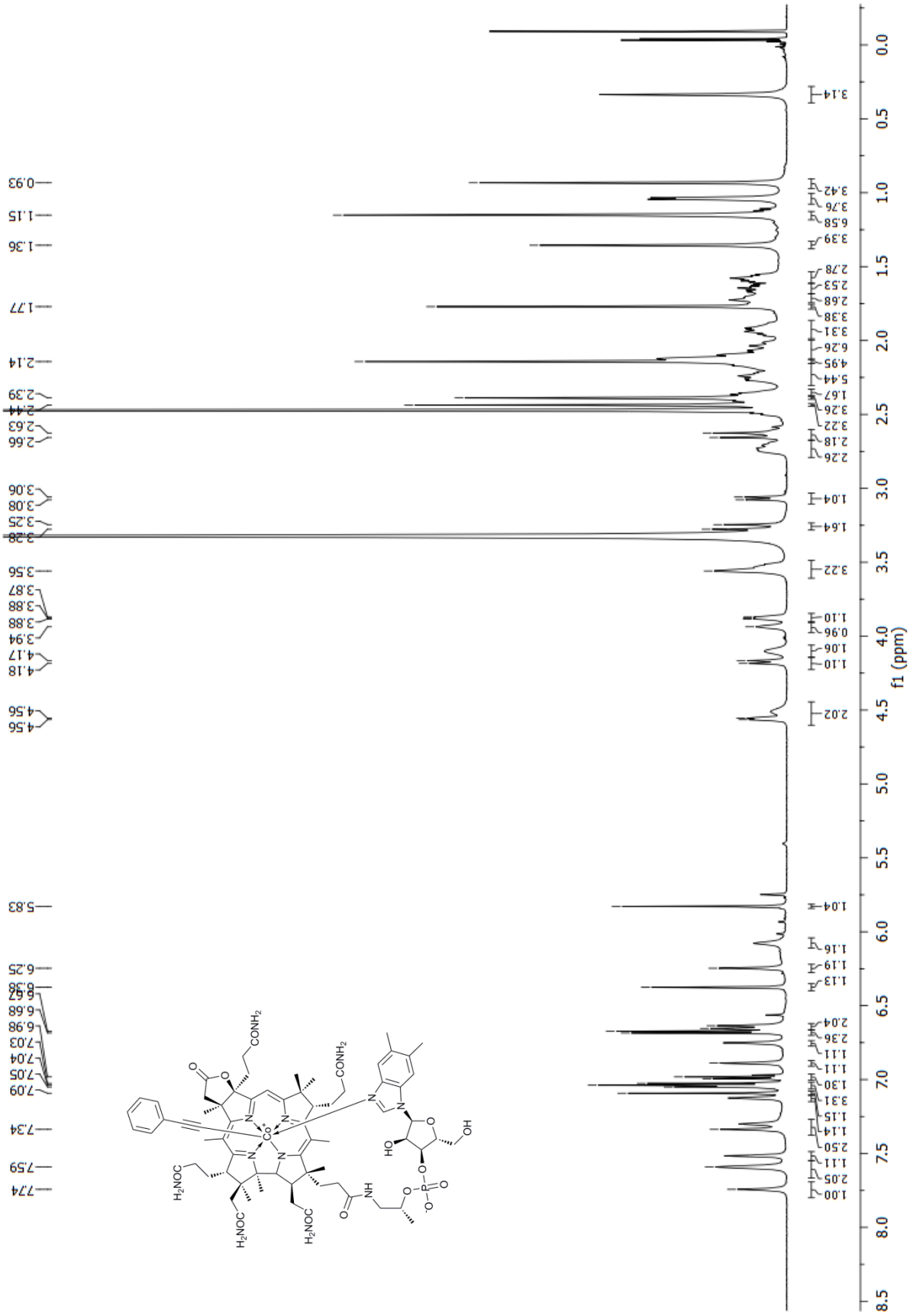
IR (KBr) ν = 3348, 3195, 2952, 2121, 1667, 1573, 1497, 1402, 1223, 1146, 1068, 1000, 841, 756 cm^{-1}.

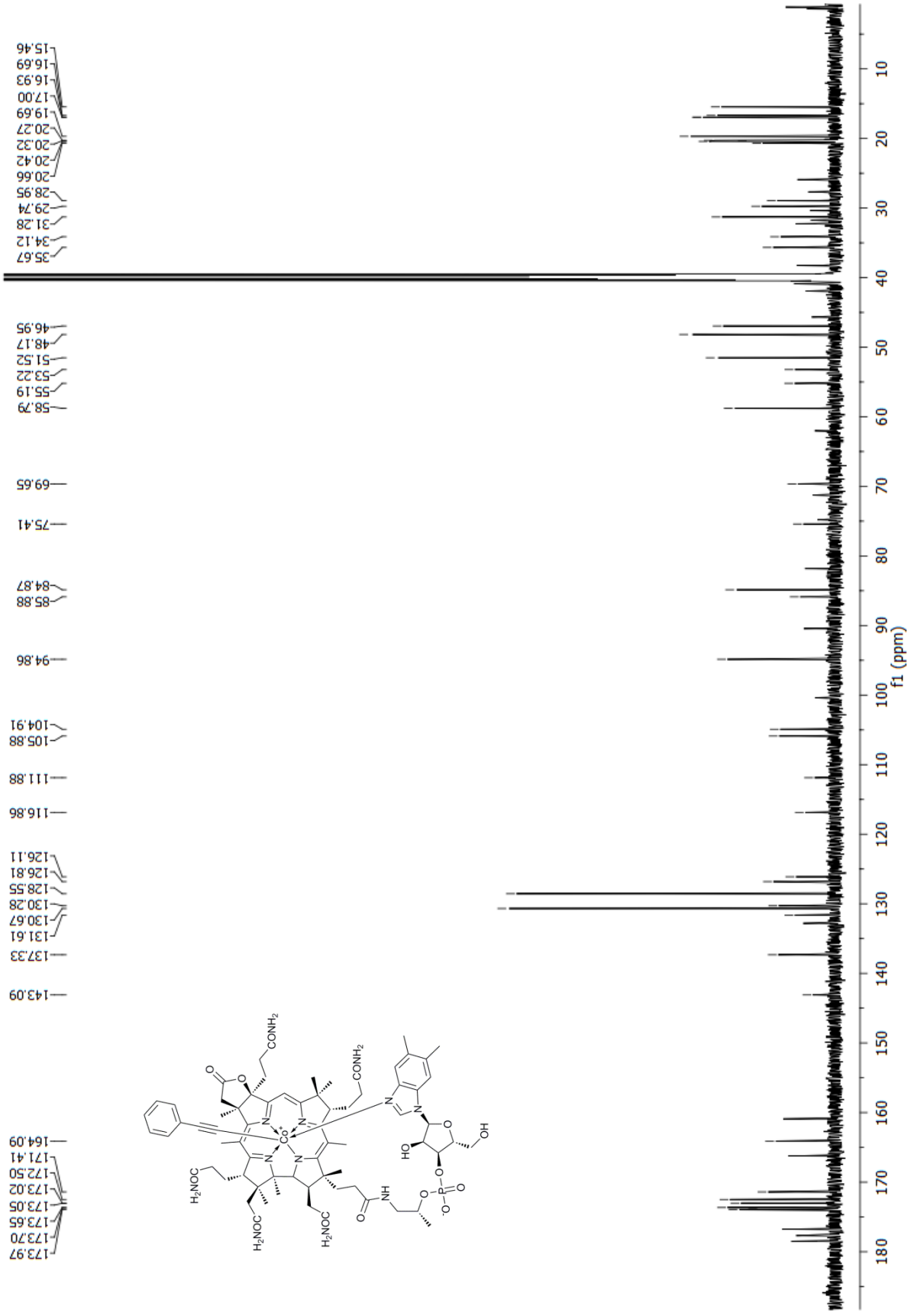
^{1}H NMR (500 MHz, DMSO-[d_6]) δ: 7.62 (s, 1H), 7.59 (s, 1H), 7.54 (s, 2H), 7.50 (s, 1H), 7.30 (s, 2H), 7.14 (s, 1H), 7.10 (s, 1H), 7.06 (s, 1H), 6.99 (s, 1H), 6.75 (s, 1H), 6.63 (s, 1H), 6.50 (s, 1H), 6.46 (s, 1H), 6.40-6.32 (m, 1H), 6.28 (s, 1H), 6.09 (s, 1H), 5.85 (s, 1H), 4.59 (d, *J* = 6.7 Hz, 1H), 4.52-4.44 (m, 1H), 4.15-4.05 (m, 1H), 4.03 (d, *J* = 11.1 Hz, 1H), 3.94-3.84 (m, 4H), 3.80 (dd, *J* = 5.5, *J* = 9.8 Hz, 1H), 3.66-3.48 (m, 3H), 3.08 (d, *J* = 11.1 Hz, 1H), 2.76-2.57 (m, 3H), 2.48-2.35 (m, 4H), 2.47 (s, 3H), 2.39 (s, 3H), 2.31-1.97 (m, 5H), 2.18 (s, 3H), 2.16 (s, 3H), 1.97-1.84 (m, 1H), 1.82-1.70 (m, 4H), 1.67 (s, 3H), 1.64-1.46 (m, 6H), 1.33 (s, 3H), 1.25-1.14 (m, 1H), 1.18 (s, 3H), 1.16 (s, 3H), 1.06 (d, *J* = 6.2 Hz, 3H), 1.01 (s, 3H), 0.97-0.85 (m, 1H), 0.78-0.67 (m, 2H), 0.29 (s, 3H), -0.08 (s, 9H) ppm.

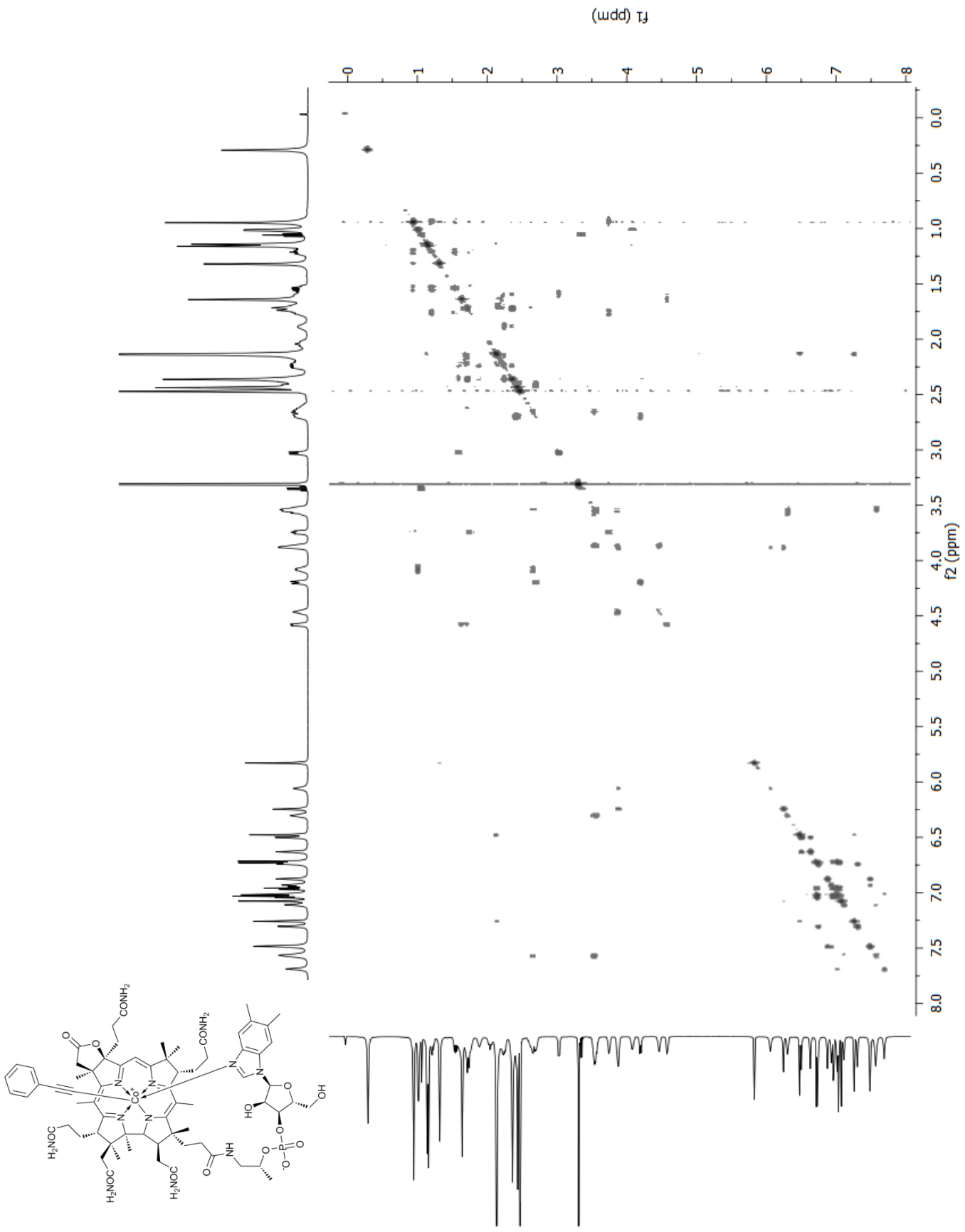
^{13}C NMR (125 MHz, DMSO-[d_6]) δ: 178.3, 177.1, 174.2, 173.4, 173.2, 172.9, 172.79, 172.7, 172.5, 171.3, 170.7, 164.9, 164.1, 151.4, 142.6, 136.6, 132.1, 130.8, 129.7, 116.7, 111.2, 105.2, 102.7, 93.2, 85.3, 84.3, 81.3, 74.7, 74.3, 70.6, 69.2, 62.0, 61.7, 58.3, 54.7, 53.3, 52.9, 49.9, 47.2, 46.3, 45.4, 41.7, 37.8, 35.1, 33.7, 31.9, 31.3, 29.8, 27.1, 26.0, 25.5, 20.3, 19.9, 19.8, 19.6, 18.7, 16.4, 16.2, 15.6, 14.9, -1.5 ppm.

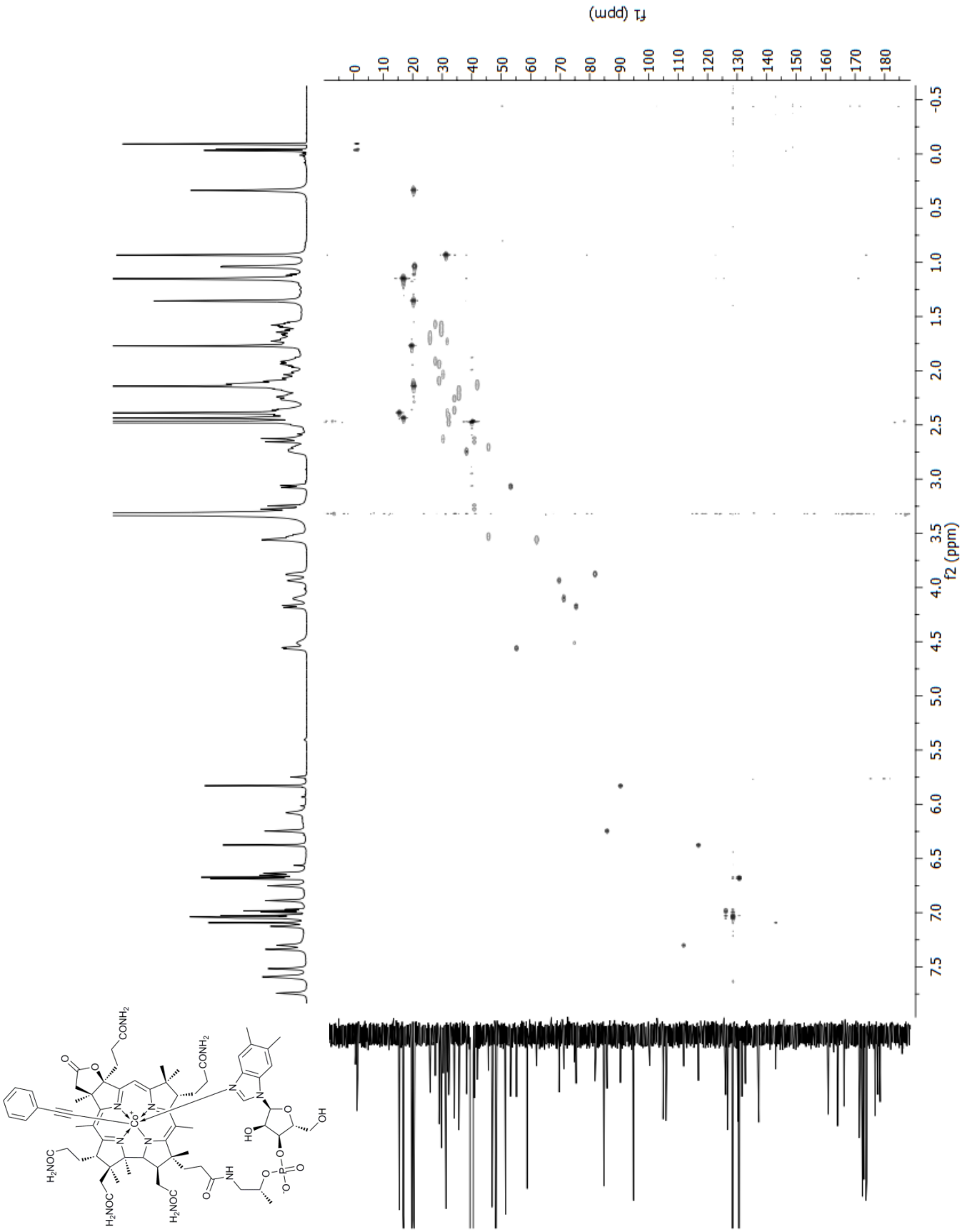
HPLC: τ = 13.25 min.

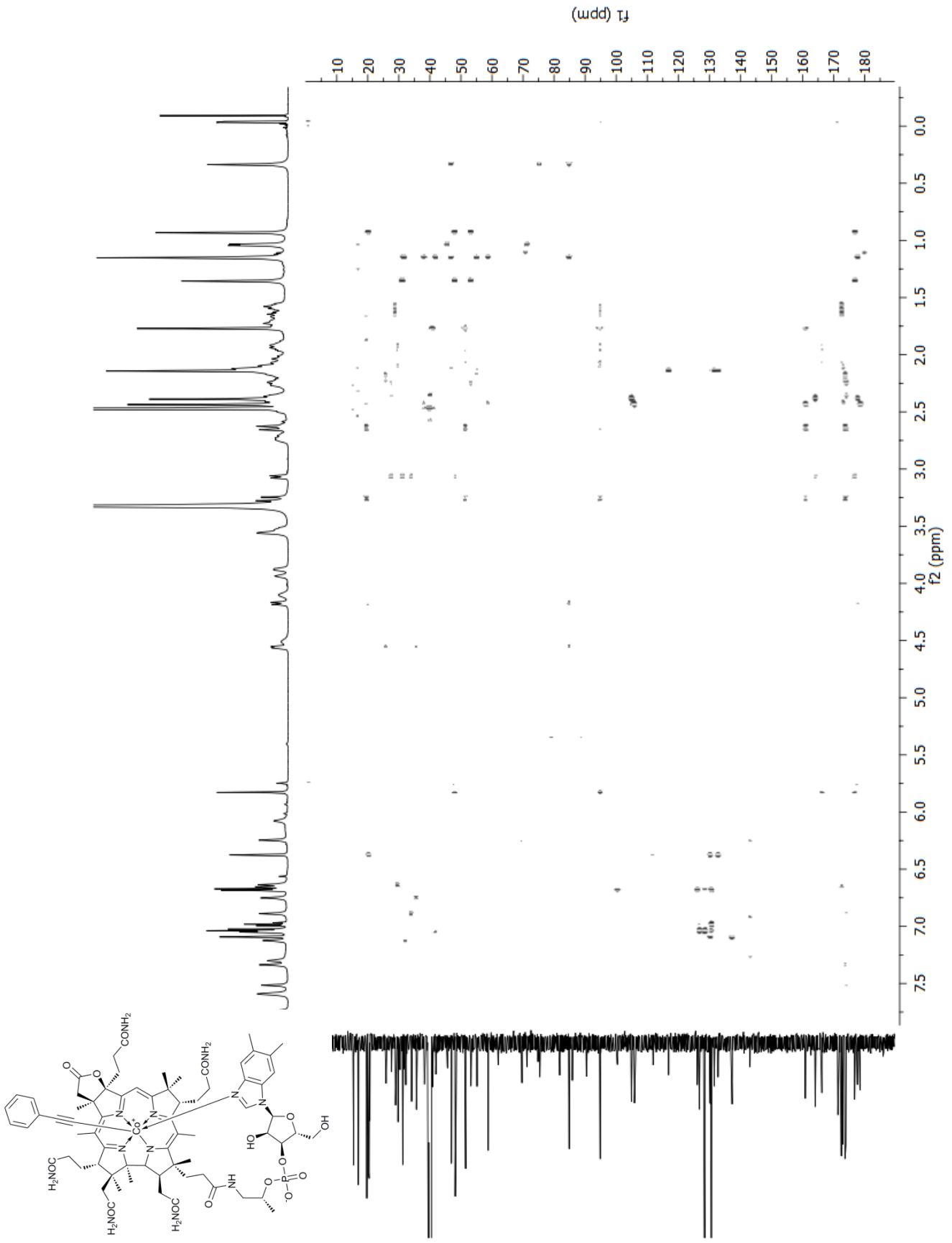
Copies of NMR spectra of compound **1**



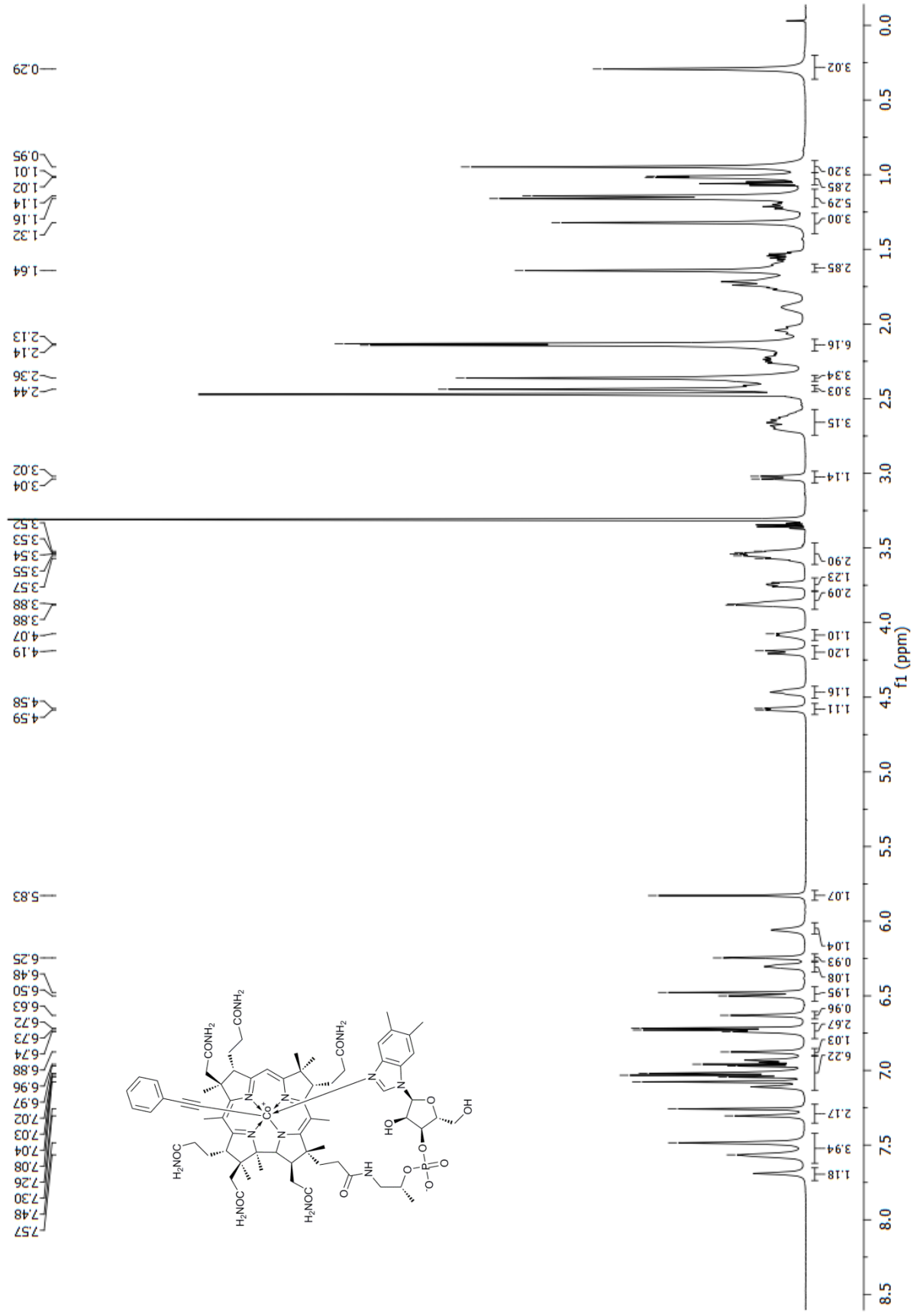

15.46
16.69
16.93
17.00
19.69
20.27
20.32
20.42
20.66
28.95
29.74
31.28
34.12
35.67
46.95
48.17
51.52
53.22
55.19
58.79
69.65
75.41
84.87
85.88
94.86
104.91
105.88
111.88
116.86
126.11
126.81
128.55
130.28
130.67
131.61
137.33
143.09
164.09
171.41
172.50
173.02
173.05
173.65
173.70
173.97
f1 (ppm)

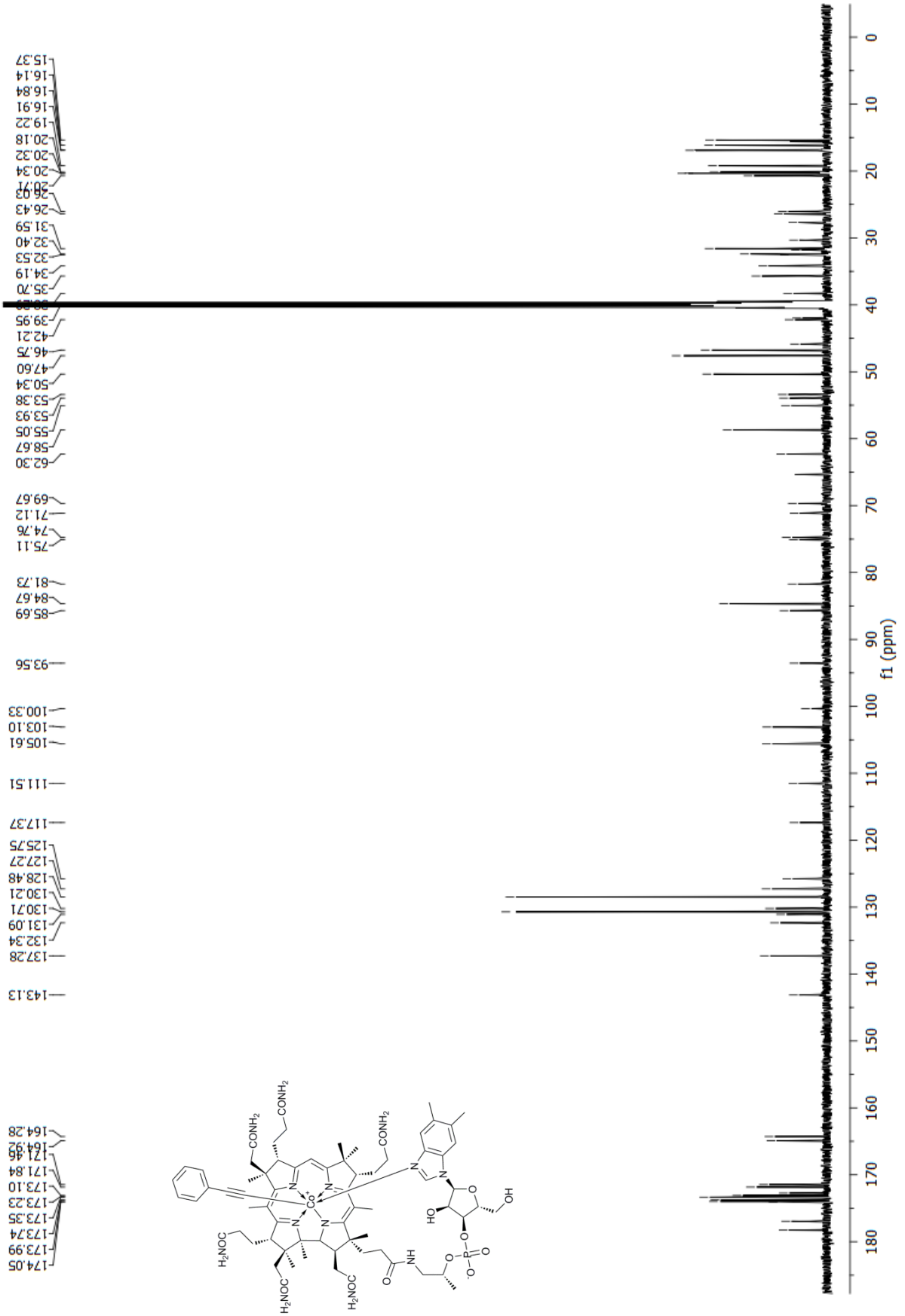

f1 (ppm)
f2 (ppm)
CONH2
H2NOC
NH
HO
OH
O
N
Co


f1 (ppm)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
-0.5
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
f2 (ppm)
CONH2
H2NOC
OH
HO
NH
O
P
N


f1 (ppm)
f2 (ppm)

Copies of NMR spectra of compound **2**




15.37
16.14
16.84
16.91
19.22
20.18
20.32
20.34
20.71
26.03
26.43
31.59
32.40
32.53
34.19
35.70
39.29
39.95
42.21
46.75
47.60
50.34
53.38
53.93
55.05
58.67
62.30
69.67
71.12
74.76
75.11
81.73
84.67
85.69
93.56
100.33
103.10
105.61
111.51
117.37
125.75
127.27
128.48
130.21
130.71
131.09
132.34
137.28
143.13
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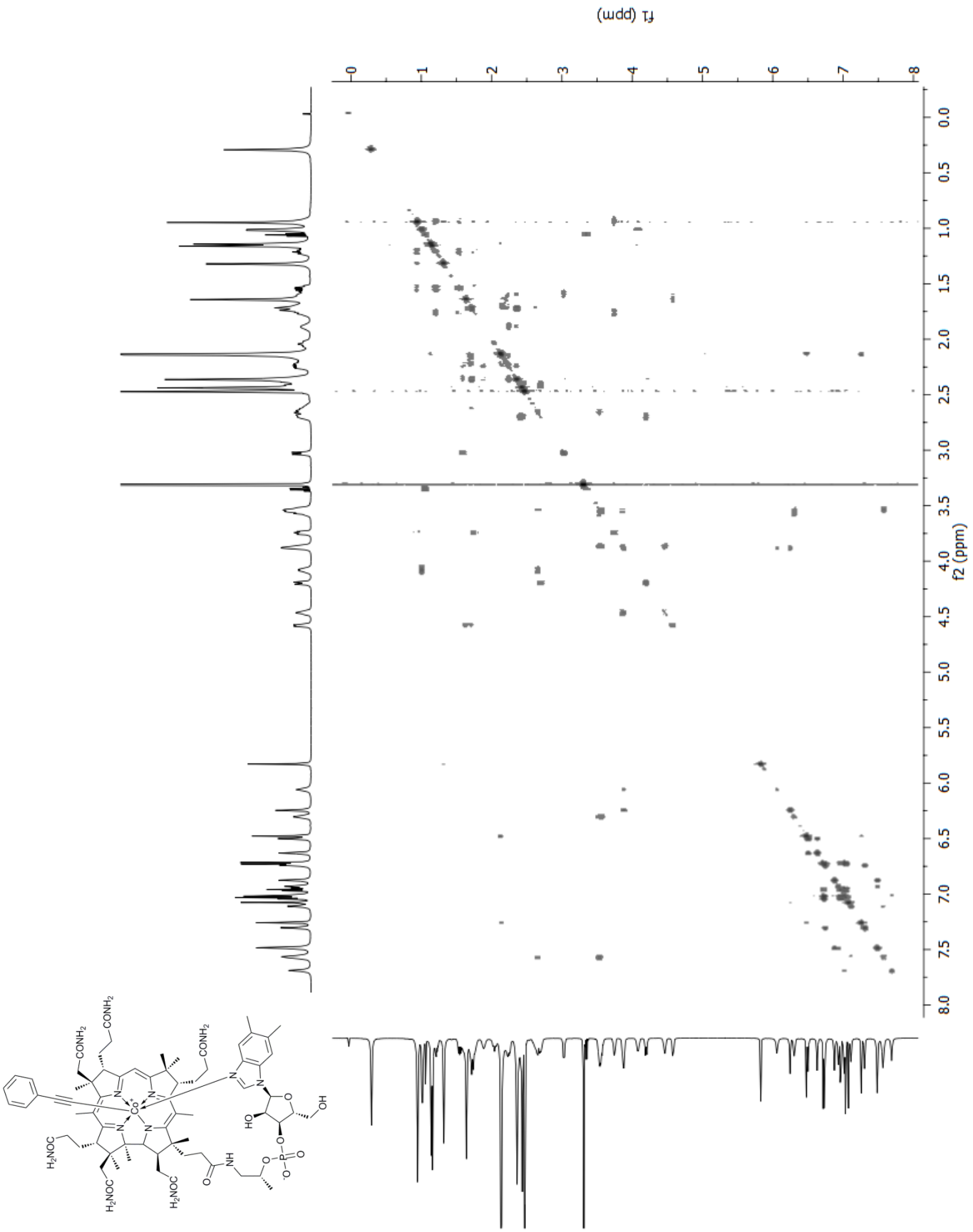
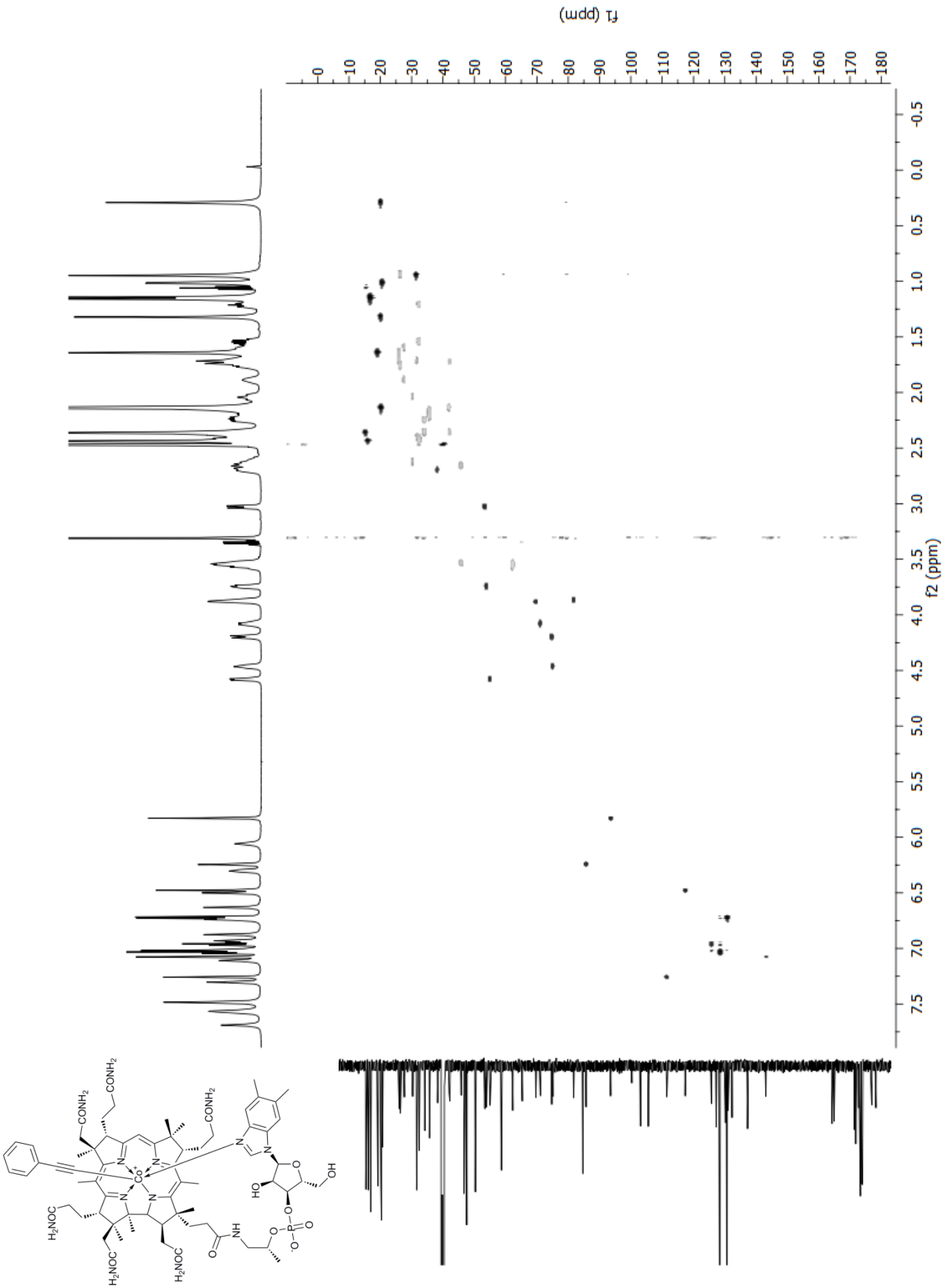
173.35
173.74
173.99
174.05
f1 (ppm)


f1 (ppm)
f2 (ppm)
CONH2
H2NOC
HO
NH
O
P
Co


f1 (ppm)
f2 (ppm)
CONH2
H2NOC
HO
OH
NH
O
P
N
Co

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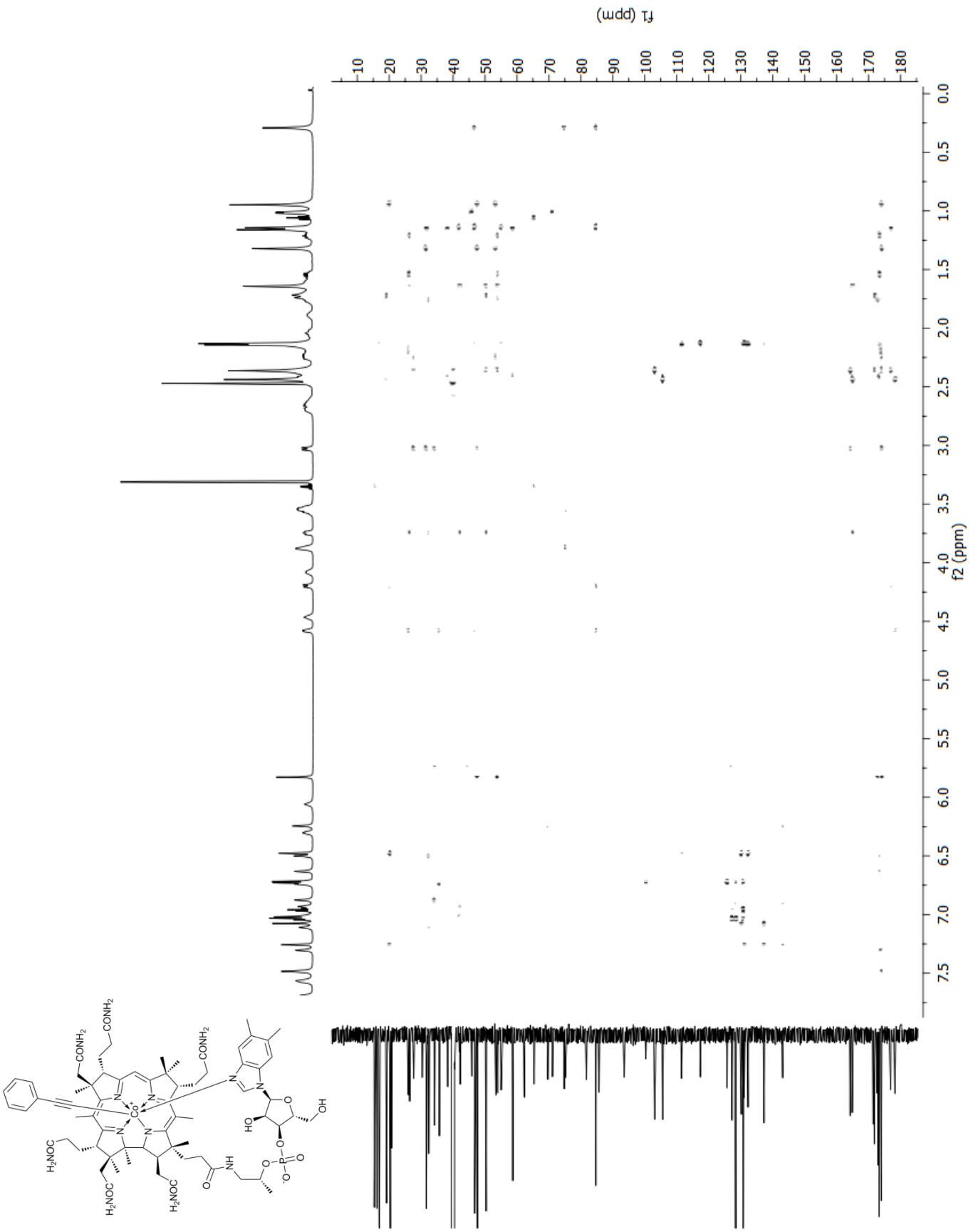
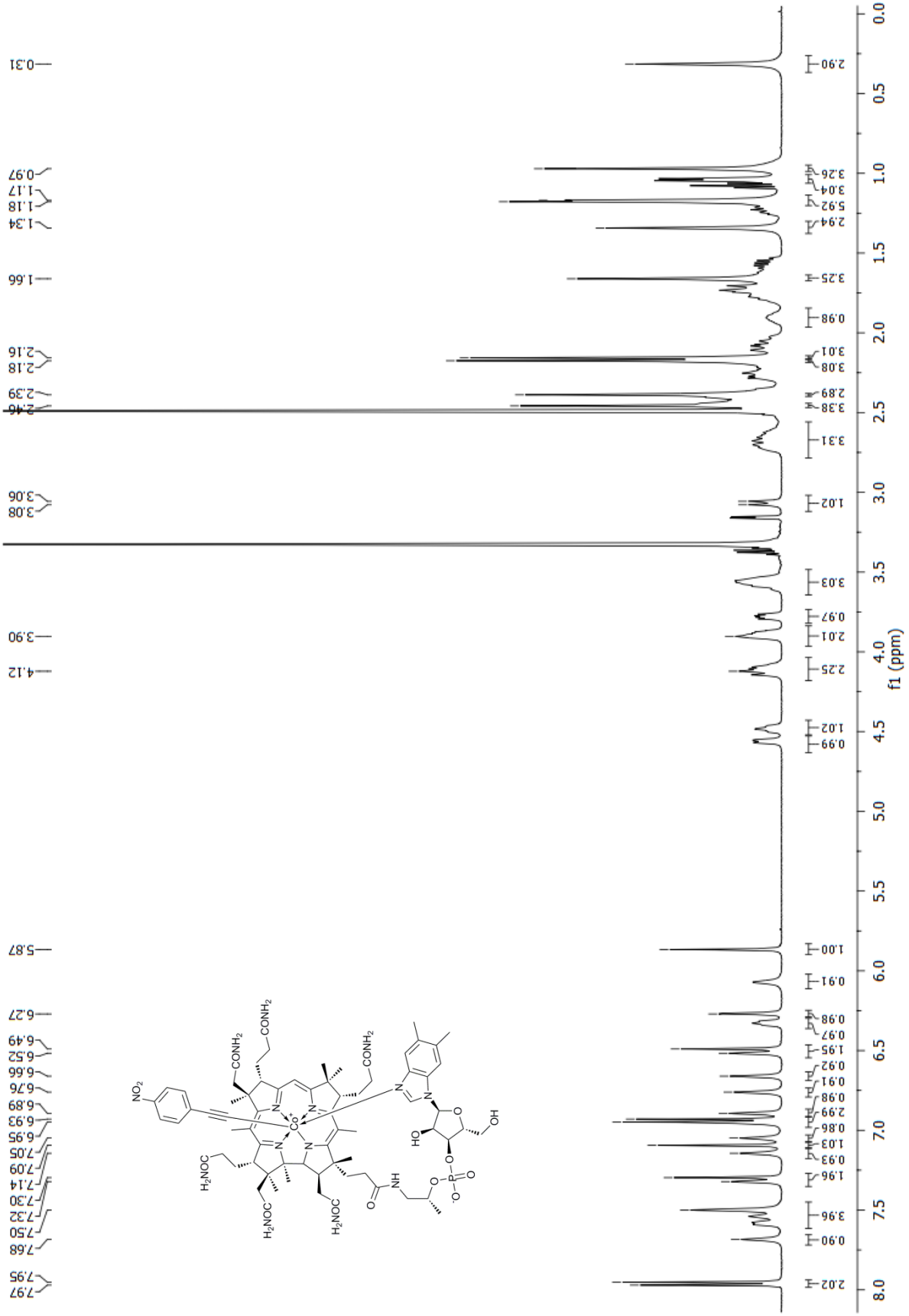
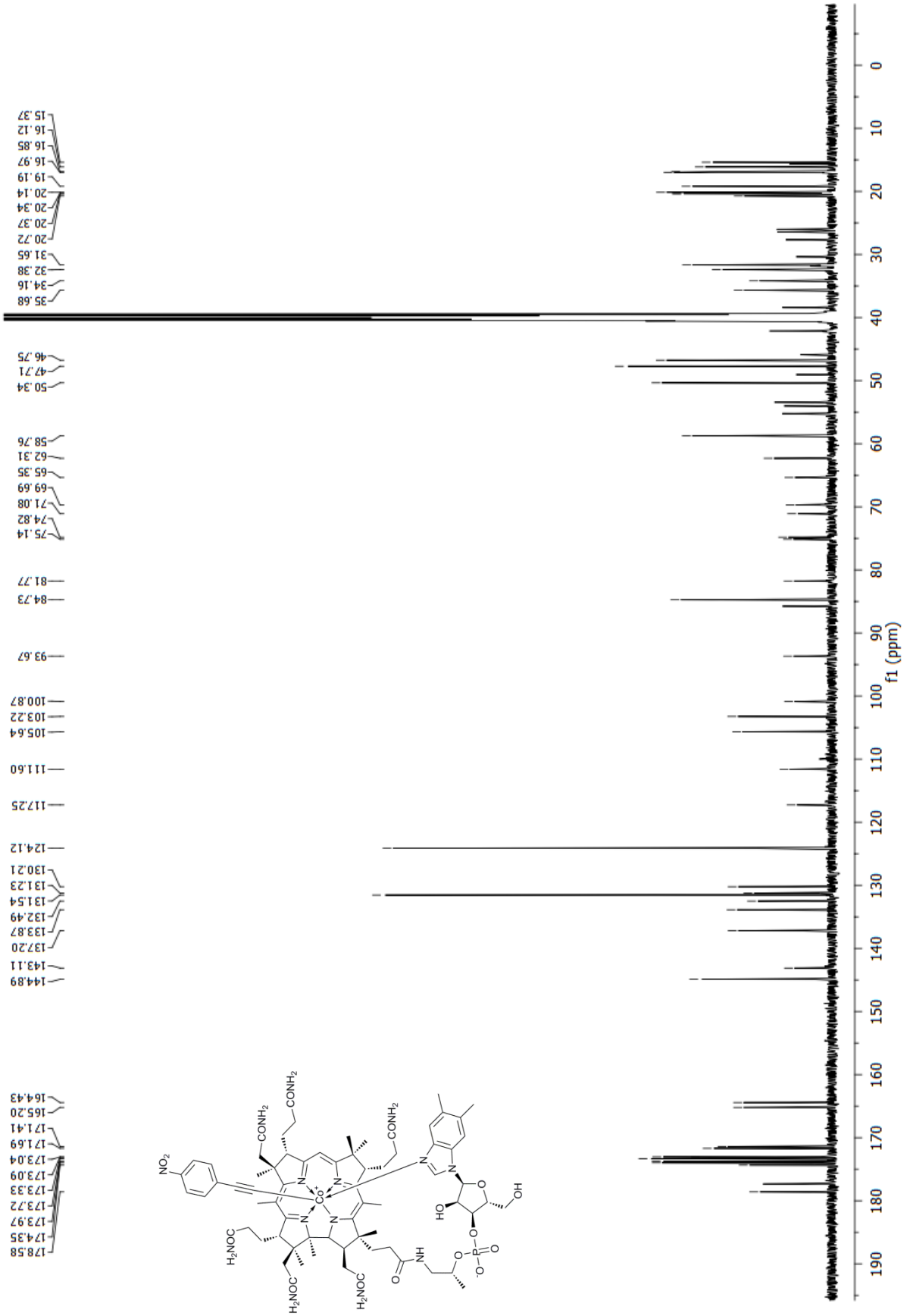
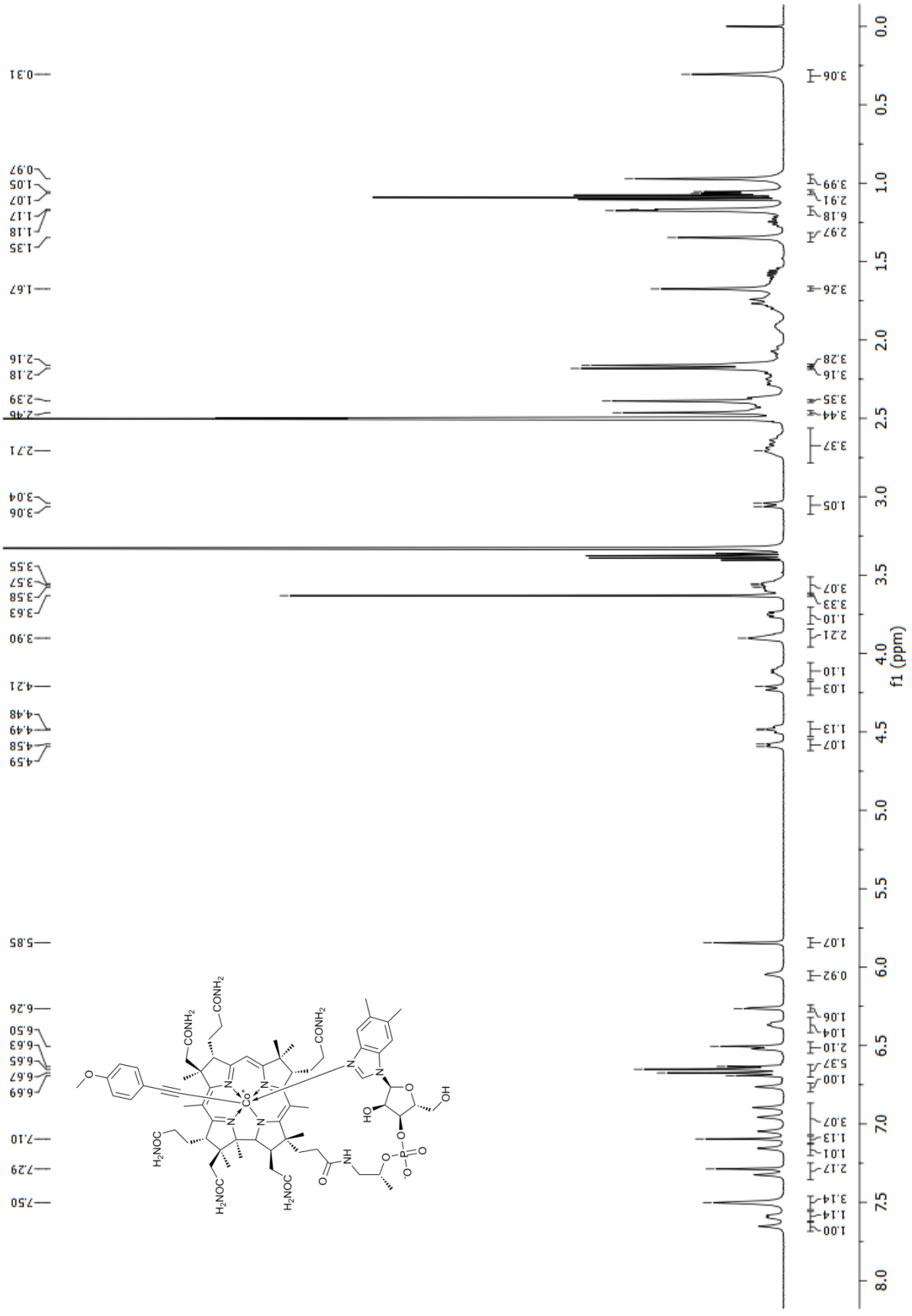


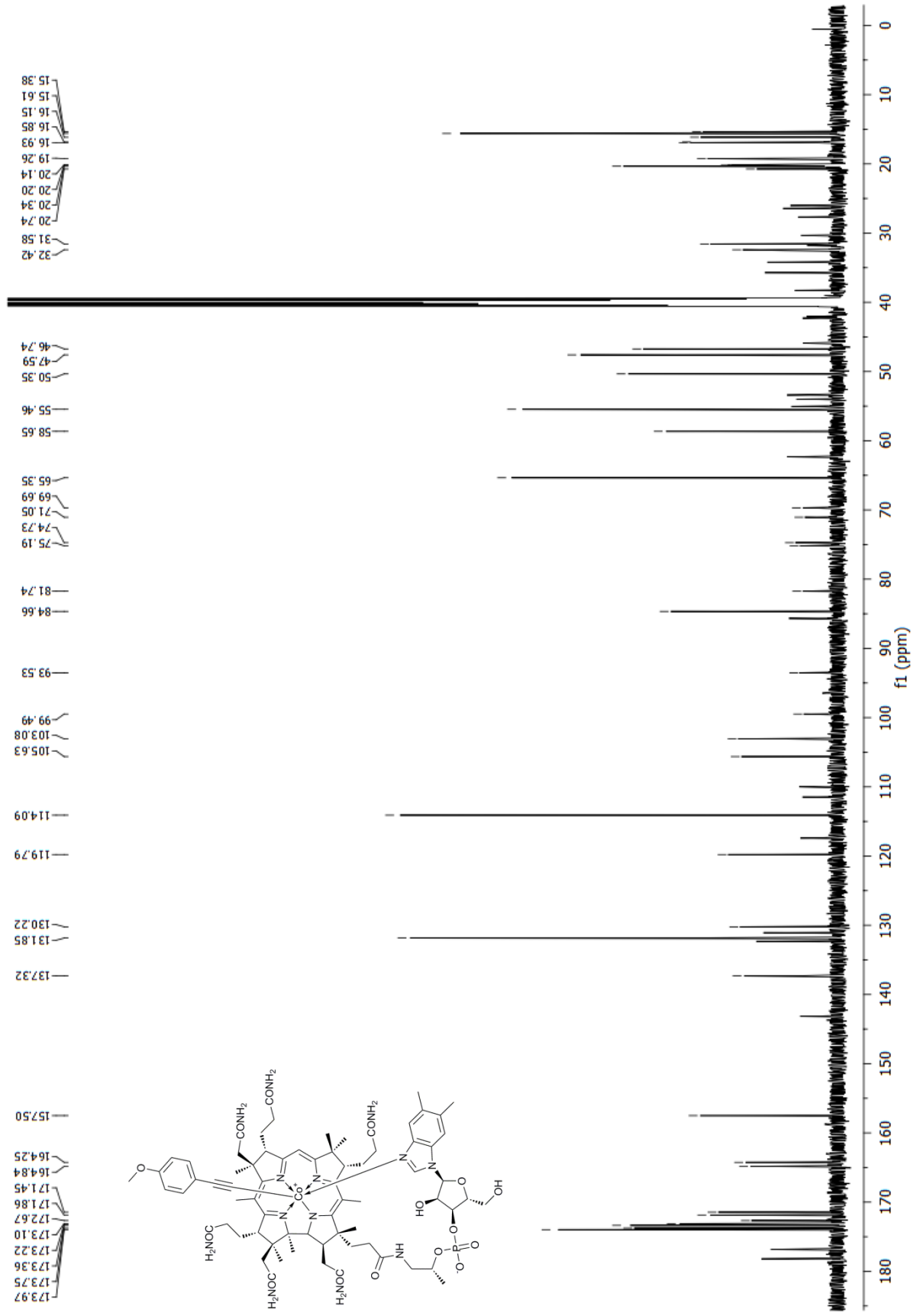
Copies of NMR spectra of compound **3**




15.37
16.12
16.85
16.97
19.19
20.14
20.34
20.37
20.72
31.65
32.38
34.16
35.68
46.75
47.71
50.34
58.76
62.31
65.35
69.69
71.08
74.82
75.14
81.77
84.73
93.67
100.87
103.22
105.64
111.60
117.25
124.12
130.21
131.23
131.54
132.49
133.87
137.20
143.11
144.89
164.43
165.20
171.41
171.69
173.04
173.09
173.33
173.72
173.97
174.35
178.58
f1 (ppm)

Copies of NMR spectra of compound **4**




15.38
15.61
16.15
16.85
16.93
19.26
20.14
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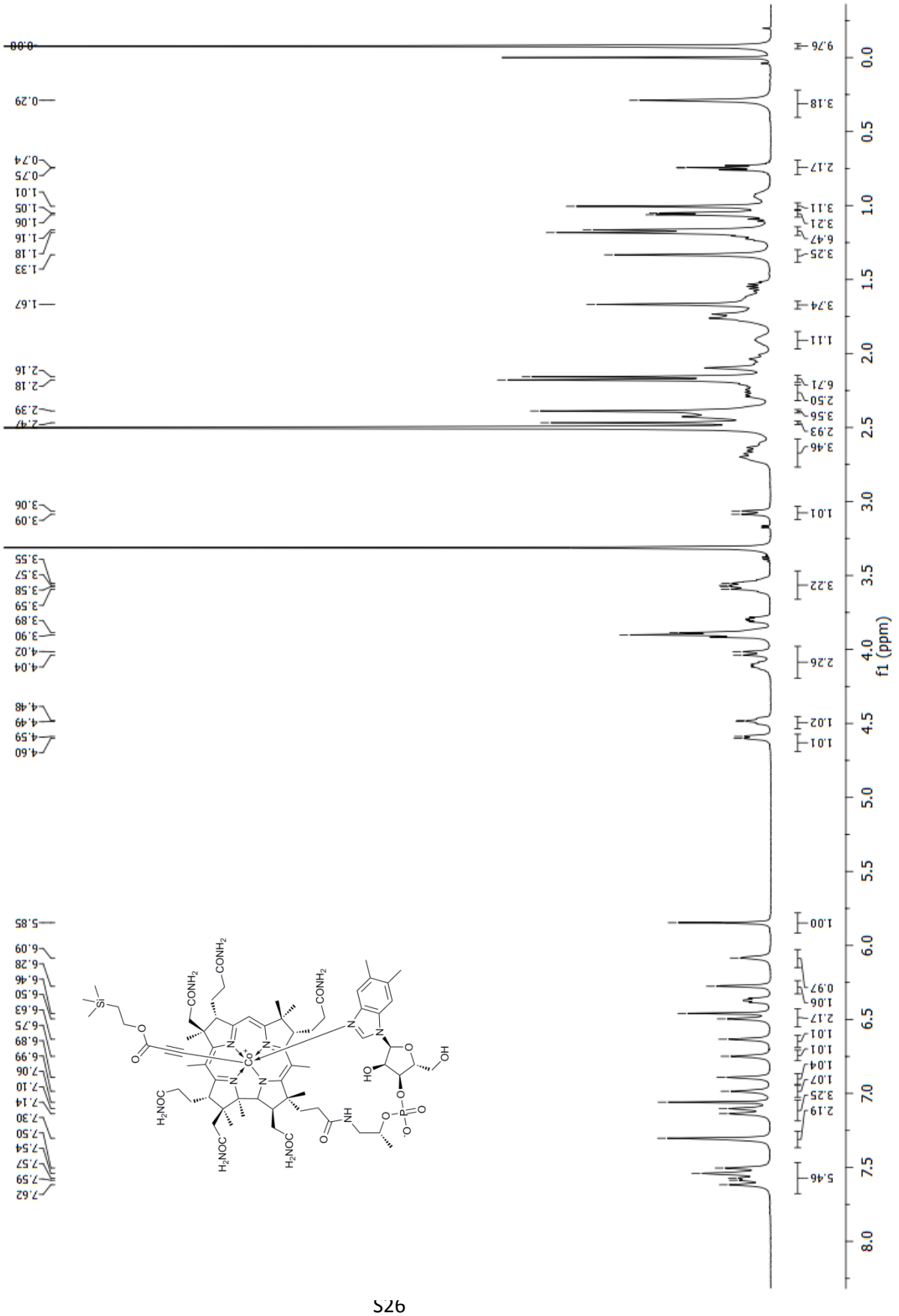
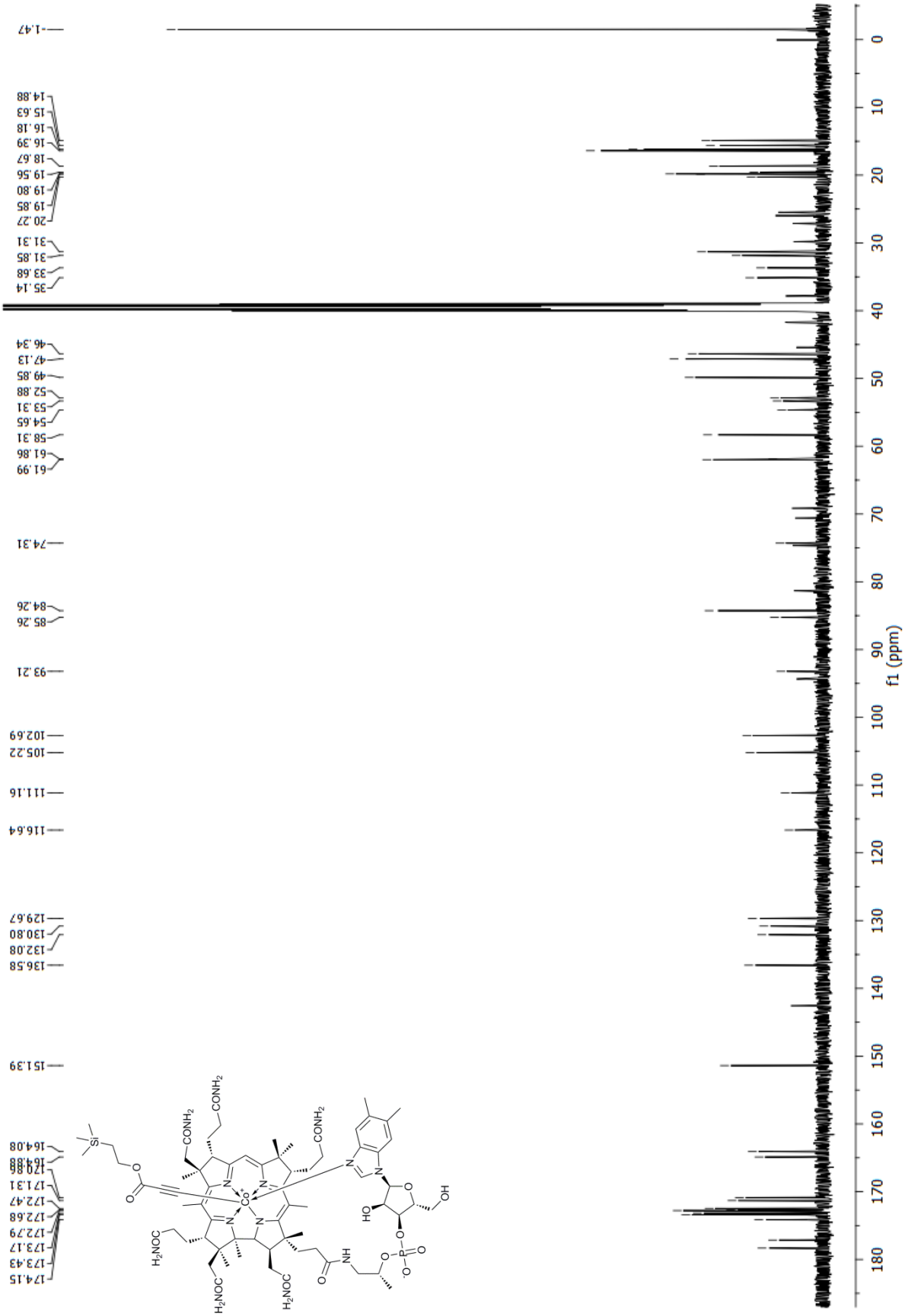
20.34
20.74
31.58
32.42
46.74
47.59
50.35
55.46
58.65
65.35
69.69
71.05
74.73
75.19
81.74
84.66
93.53
99.49
103.08
105.63
114.09
119.79
130.22
131.85
137.32
157.50
164.25
164.84
171.45
171.86
172.67
173.10
173.22
173.36
173.75
173.97
f1 (ppm)

Copies of NMR spectra of compound **5**




-1.47
14.88
15.63
16.18
16.39
18.67
19.56
19.80
19.85
20.27
31.31
31.85
33.68
35.14
46.34
47.13
49.85
52.88
53.31
54.65
58.31
61.86
61.99
74.31
84.26
85.26
93.21
102.69
105.22
111.16
116.64
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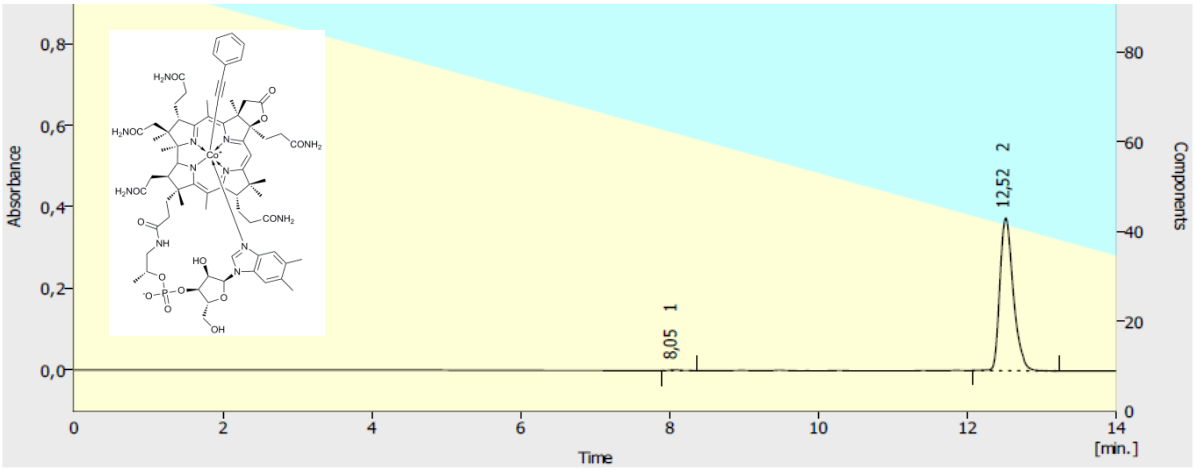
130.80
132.08
136.58
151.39
164.08
170.86
171.31
172.47
172.68
172.79
173.17
173.43
174.15
f1 (ppm)

Copies of chromatograms of compounds **1-5**

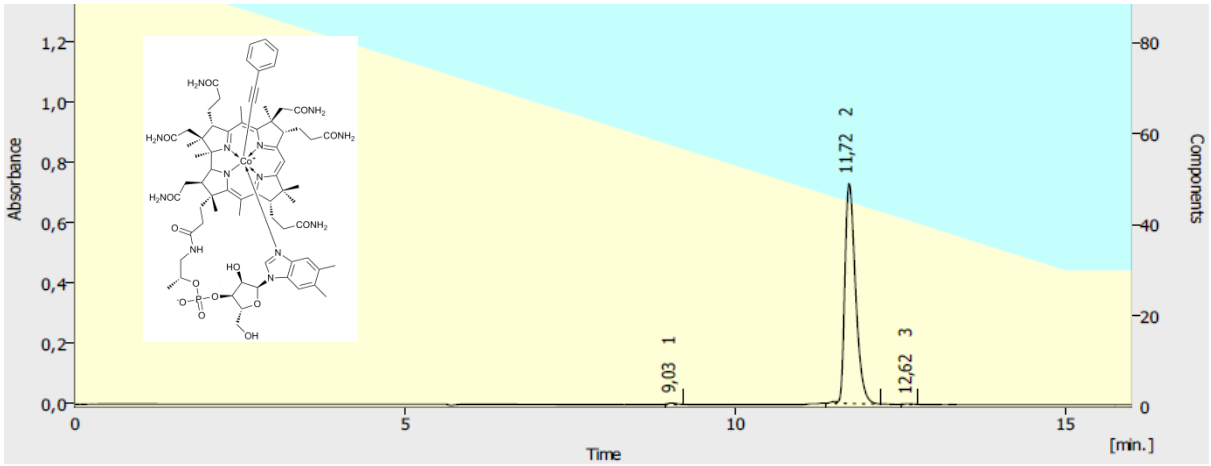
Compound **1**



Result Table (Uncal - C:\Users\użytkownik\Desktop\mikołaj - chromatogramy\MIK 565\mik 565 po liofilizacji - S 2850: Channel 2)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]
1	8,050	18,256	1,899	0,4	0,5	0,17	739
2	12,517	4700,334	374,689	99,6	99,5	0,22	890
	Total	4718,590	376,587	100,0	100,0		

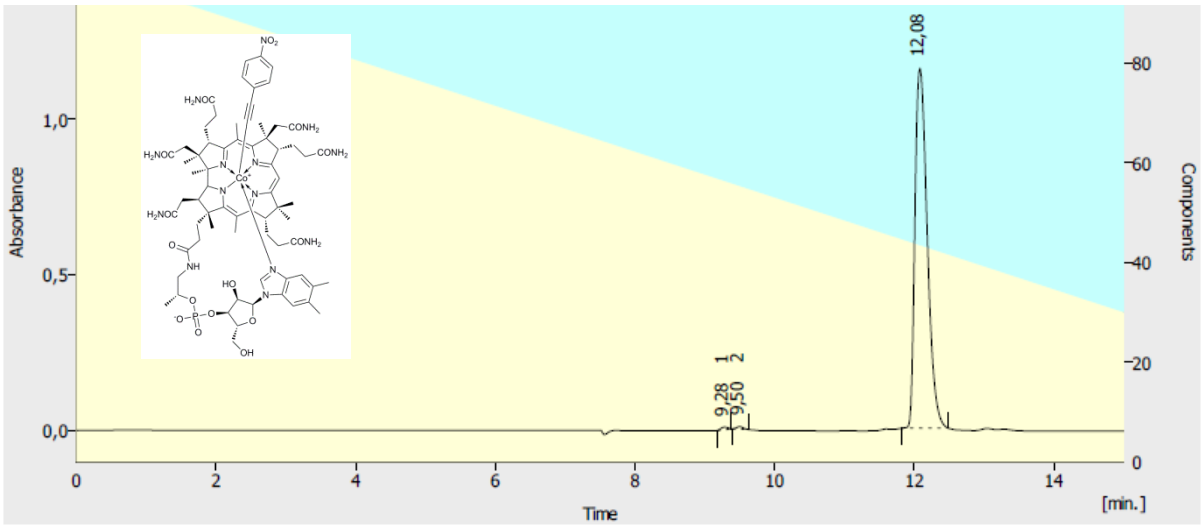
Compound **2**



Result Table (Uncal - C:\Users\użytkownik\Desktop\Agnieszka Lewalska\hplc\hplc ładne\AL 6'19-lip-2013 AL 6' Ph - S 2650: Channel 2)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]
1	9,033	36,926	4,500	0,4	0,6	0,15	993
2	11,717	8201,345	730,168	99,3	99,1	0,18	893
3	12,617	19,743	2,270	0,2	0,3	0,17	999
	Total	8258,015	736,938	100,0	100,0		

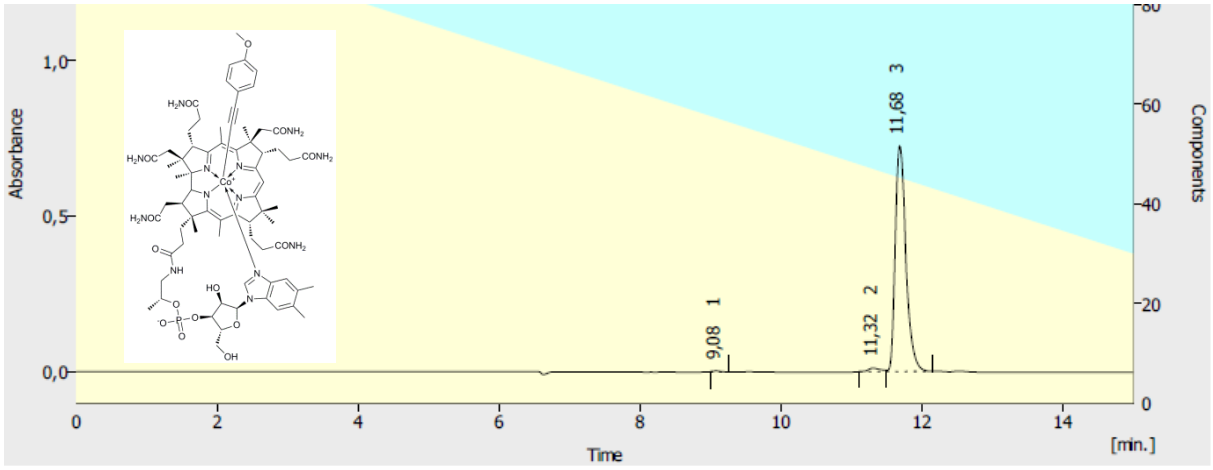
Compound **3**



Result Table (Uncal - C:\Users\użytkownik\Desktop\Agnieszka Lewalska\hplc\hplc ładne\MIK 556 4-NO219-lip-2013 MIK 556 4-NO2 - S 2850: Channel 2)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]
1	9,283	55,683	8,509	0,4	0,7	0,15	989
2	9,500	54,988	8,333	0,4	0,7	0,13	997
3	12,083	14088,805	1154,510	99,2	98,6	0,20	919
	Total	14199,475	1171,352	100,0	100,0		

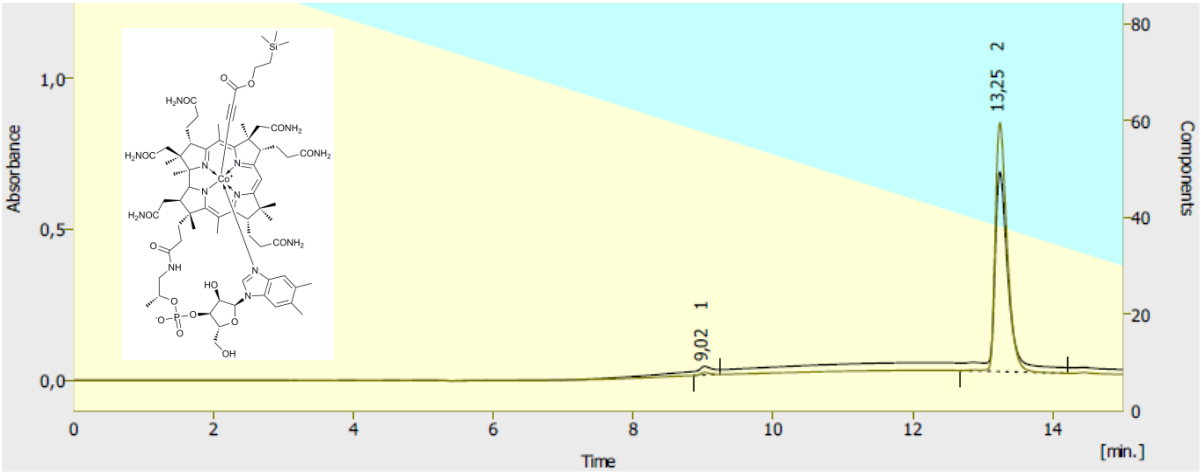
Compound **4**



Result Table (Uncal - C:\Users\użytkownik\Desktop\Agnieszka Lewalska\hplc\hplc ładne\AL 557 4-OMe19-lip-2013 AL 557 4-OMe - S 2850: Channel 2)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]
1	9,083	21,959	2,851	0,3	0,4	0,13	982
2	11,317	129,873	11,265	1,7	1,5	0,22	994
3	11,683	7626,036	724,173	98,0	98,1	0,17	889
	Total	7777,868	738,288	100,0	100,0		

Compound **5**



Result Table (Uncal - C:\USERS\UŻYTKOWNIK\DESKTOP\AGNIESZKA LEWALSKA\HPLC\AL60\AL60 PO 2 KOLUMNIE08_3013-SIE-2013 - S 2850: Channel 1)

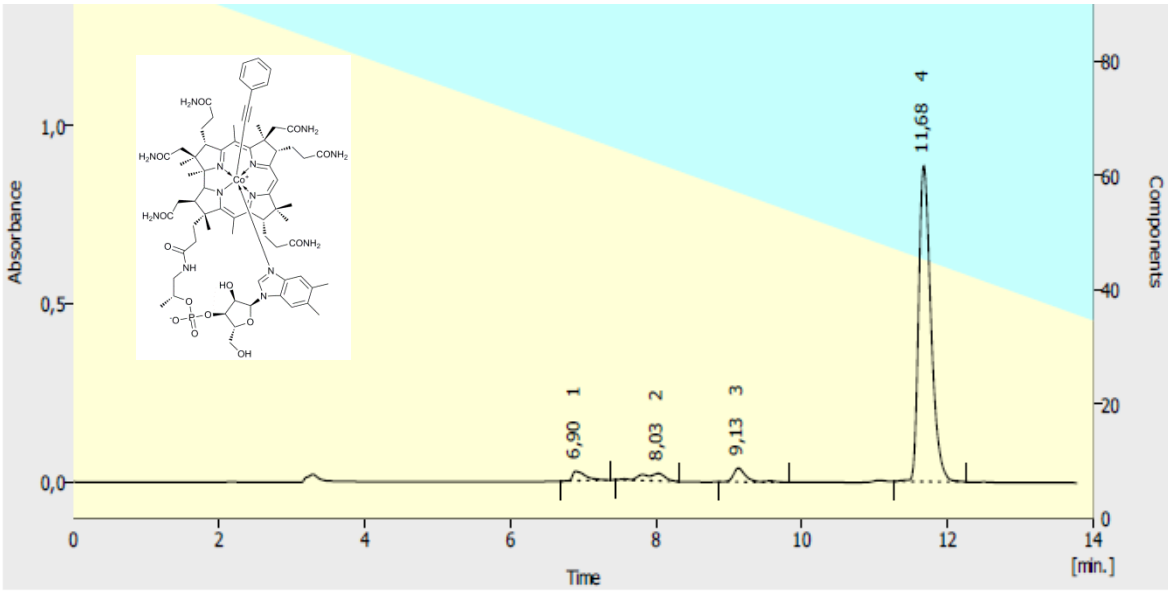
	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]
1	9,017	75,049	8,732	0,8	1,0	0,15	997
2	13,250	9692,535	823,263	99,2	99,0	0,20	937
	Total	9767.585	831.995	100.0	100.0		

Stability tests

Thermostability:

Samples of ~ 2 mg of compounds **2**, **3** and **4**, were dissolved in of phosphate buffer (pH = 7, 0.5 ml) and transferred into a tube equipped with Rotaflo screw cap. Tubes were placed in the preheated oil bath (100 °C) for 24h. Then mixtures were cooled down to room temperature and samples were analyzed using HPLC. For compound **4** the first analysis was performed after 2 h of heating, showing a high degree of decomposition.

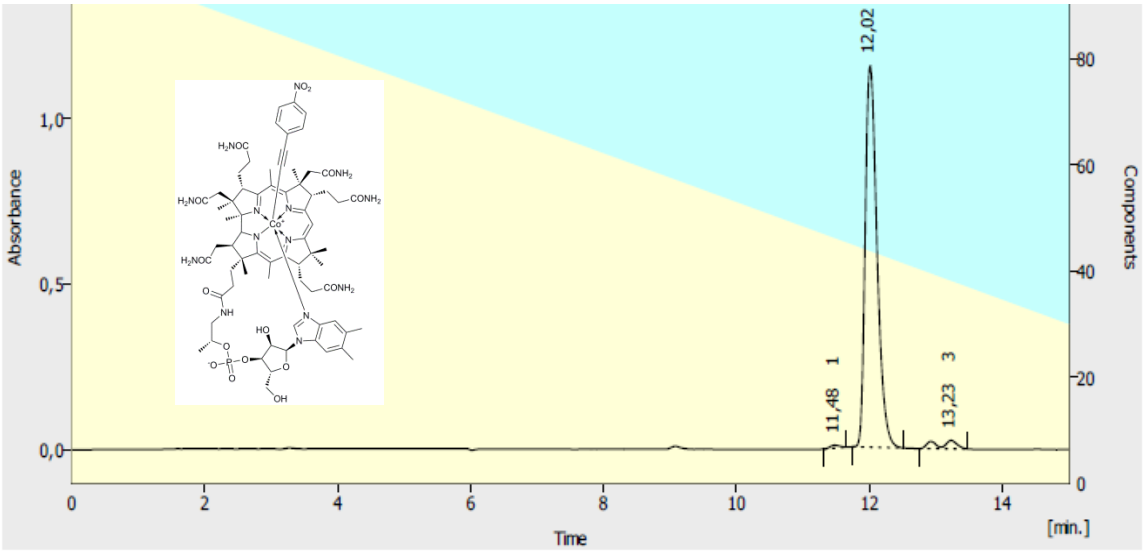
HPLC chromatogram of **2** after 24 h of heating at 100 °C



Result Table (Uncal - C:\Users\użytkownik\Desktop\mikolaj - chromatogramy\thermo - stability\thermo stability - wrzesien\Ph, 20h @ 10007_1916-wrz-2013 - S 2850: Channel 2)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]
1	6,900	328,998	26,989	2,8	2,8	0,20	969
2	8,033	461,194	21,437	4,0	2,2	0,40	993
3	9,133	461,251	38,666	4,0	4,0	0,18	979
4	11,683	10388,656	885,515	89,2	91,0	0,18	838
	Total	11640,098	972,606	100,0	100,0		

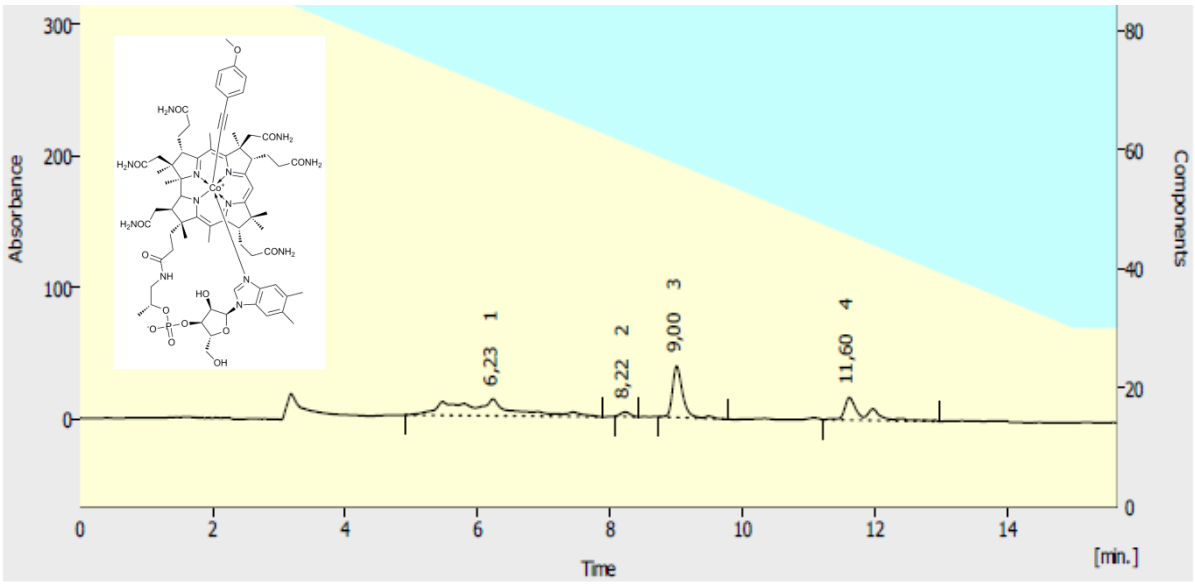
HPLC chromatogram of **3** after 24 h of heating at 100 °C



Result Table (Uncal - C:|Users|użytkownik|Desktop|mikołaj - chromatogramy|thermo - stability|thermo stability - wrzesien|4-NO216_5215-wrz-2013 - S 2850: Channel 2)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]
1	11,483	84,288	8,976	0,6	0,8	0,20	851
2	12,017	14193,265	1150,636	96,0	97,1	0,22	892
3	13,233	511,422	25,570	3,5	2,2	0,18	872
	Total	14788,976	1185,182	100,0	100,0		

HPLC chromatogram of **4** after 2 h of heating at 100 °C



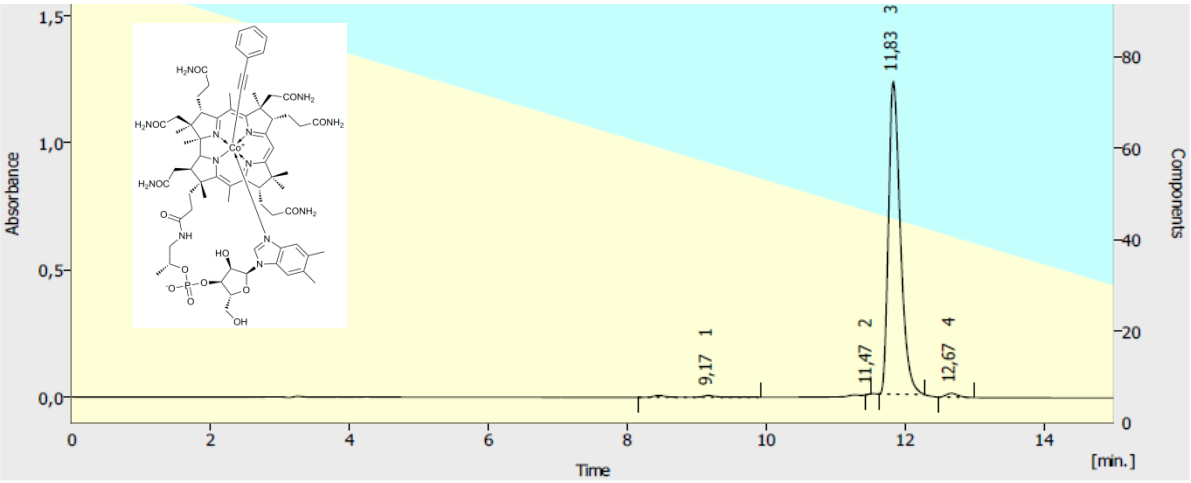
Result Table (Uncal - C:|Users|użytkownik|Desktop|mikołaj - chromatogramy|thermo - stability|thermo stability - wrzesien|4-OMe, 20h @ 10006_5216-wrz-2013 - S 2850: Channel 2)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]
1	6,233	773,325	12,785	49,2	17,7	0,35	996
2	8,217	34,219	3,441	2,2	4,8	0,18	995
3	9,000	424,138	39,068	27,0	54,1	0,17	955
4	11,600	341,092	16,987	21,7	23,5	0,18	529
	Total	1572,775	72,282	100,0	100,0		

B. Light stability

Samples of ~ 2 mg of compounds **2**, **3** and **4**, were dissolved in of phosphate buffer (pH = 7, 0.5 ml) and exposed to intensive light. After 6 hours samples were analyzed using HPLC.

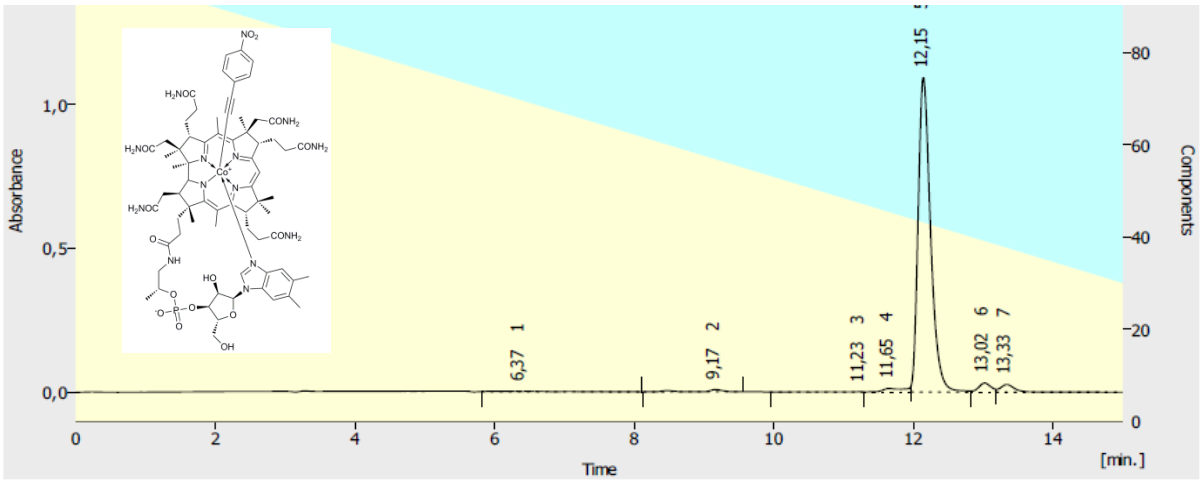
HPLC chromatogram of **2** after 6 h of light exposure.



Result Table (Uncal - C:\Users\użytkownik\Desktop\mikołaj - chromatogramy\fotostabilność II\pheny\16_1916-wrz-2013 - S 2850: Channel 2)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]
1	9,167	203,947	7,290	1,4	0,6	0,18	936
2	11,467	1,688	0,135	0,0	0,0	0,05	992
3	11,833	14596,347	1231,022	97,4	98,2	0,20	901
4	12,667	179,041	15,594	1,2	1,2	0,20	680
	Total	14981,022	1254,041	100,0	100,0		

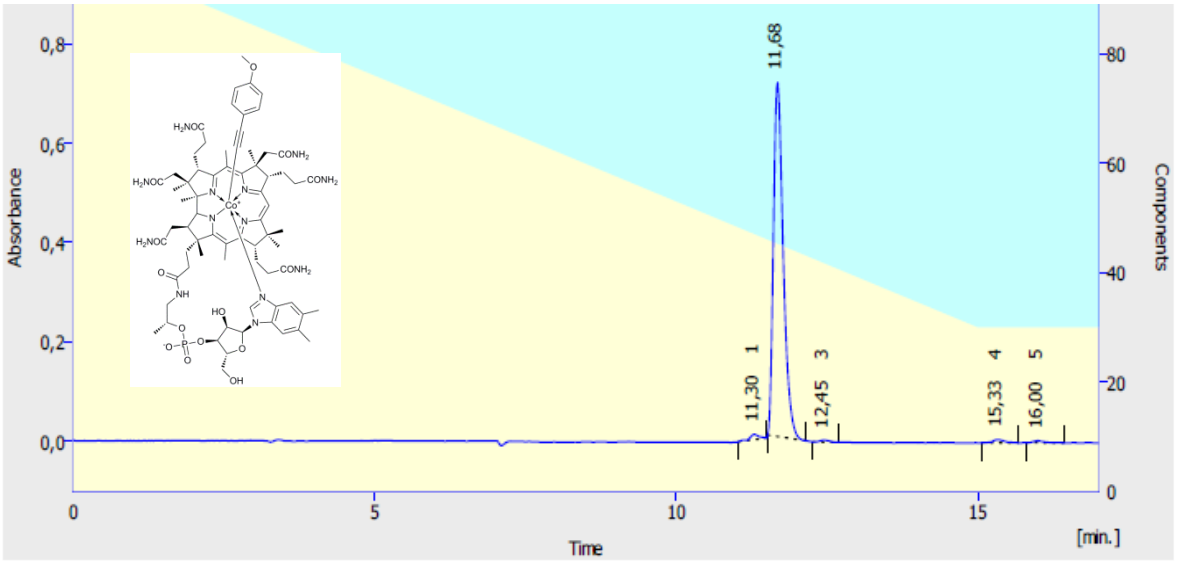
HPLC chromatogram of **3** after 6 h of light exposure.



Result Table (Uncal - C:|Users|użytkownik|Desktop|mikolaj - chromatogramy|fotostabilność II|4 - NO@15_5116-wrz-2013 - S 2850: Channel 2)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]
1	6,367	91,990	1,237	0,6	0,1	0,10	991
2	9,167	134,794	7,533	0,9	0,6	0,18	975
3	11,233	43,532	0,923	0,3	0,1	1,17	946
4	11,650	295,411	12,703	2,0	1,1	0,42	899
5	12,150	13522,973	1091,224	90,7	93,1	0,20	675
6	13,017	387,654	31,883	2,6	2,7	0,22	713
7	13,333	428,013	26,514	2,9	2,3	0,22	819
	Total	14904,367	1172,015	100,0	100,0		

HPLC chromatogram of **4** after 6 h of light exposure.



Result Table (Uncal - C:|Users|użytkownik|Desktop|mikolaj - chromatogramy|thermo - stability|thermo stability - wrzesien|4-OMe16_2915-wrz-2013 - S 2850: Channel 2)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]
1	11,300	103,565	10,314	1,2	1,4	0,18	990
2	11,683	8013,846	711,843	96,7	96,7	0,18	922
3	12,450	38,321	3,702	0,5	0,5	0,18	959
4	15,333	85,690	6,452	1,0	0,9	0,22	961
5	16,000	48,035	4,027	0,6	0,5	0,20	960
	Total	8289,458	736,338	100,0	100,0		

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Crystallographic data for **2**,**3** and **4**

	Compound		
	2	**3**	**4**
Formula	$C_{70}H_{93}N_{13}O_{14}PCo$	$C_{70}H_{92}N_{14}O_{16}PCo$	$C_{71}H_{95}N_{13}O_{15}PCo$
Space group	P 2_1 2_1 2_1	P 2_1 2_1 2_1	P 2_1 2_1 2_1
Cell Lengths (Å)	a 15.8504(3) b 21.6765(4) c 26.2587(5)	a 12.8063(3) b 22.7406(6) c 29.1298(7)	a 15.8339(4) b 21.8117(6) c 25.9778(7)
Cell Angles (°)	α 90.00 β 90.00 γ 90.00	α 90.00 β 90.00 γ 90.00	α 90.00 β 90.00 γ 90.00
Cell Volume (Å^3)	9022	8483.27	8971.8
Z, Z’	Z: 4 Z': 0	Z: 4 Z': 0	Z: 4 Z': 0
R factor (%)	4.22	5.22	5.83
CCDC deposition number	961230	961228	961229

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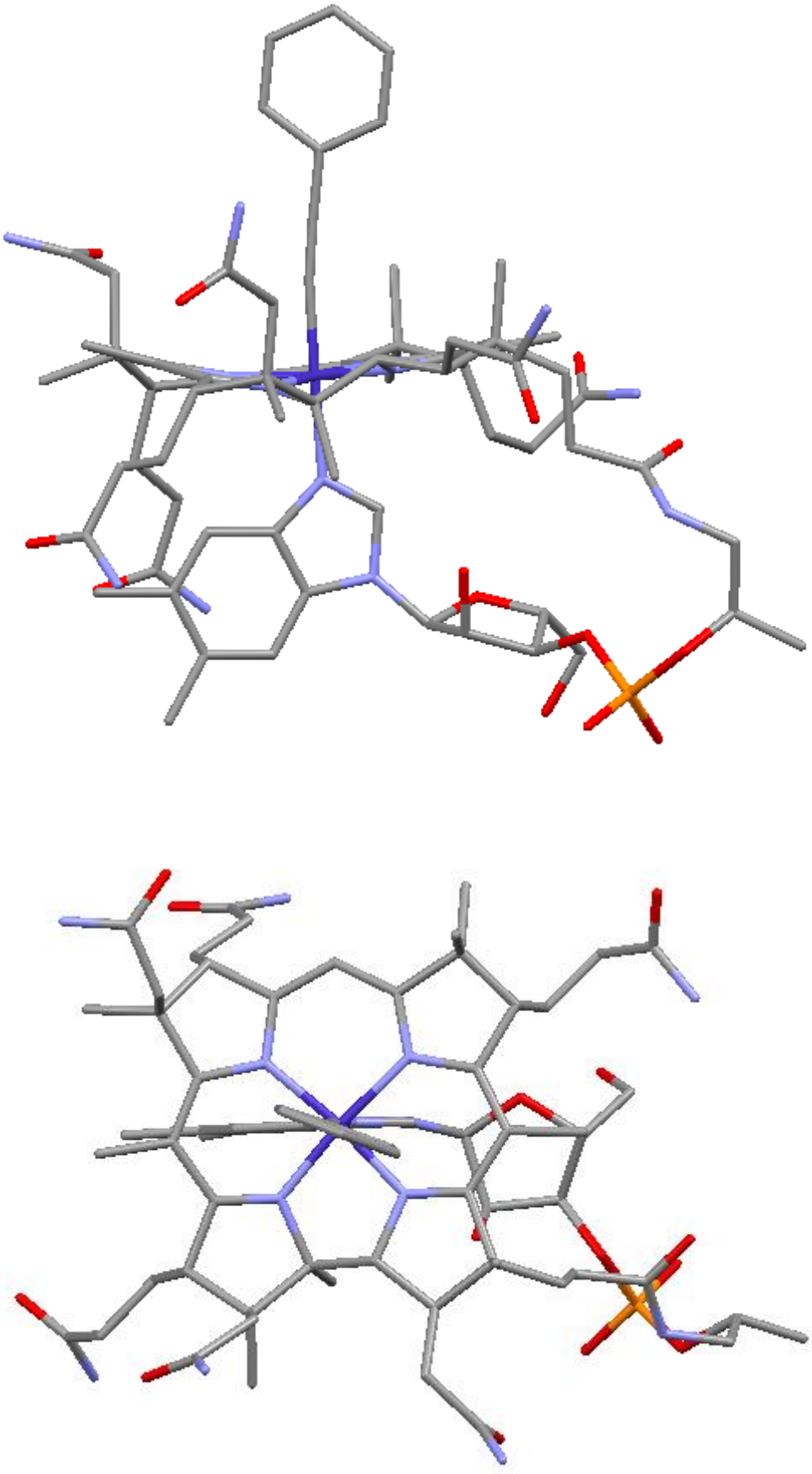
Crystal structure of **2** (hydrogen atoms omitted for clarity)



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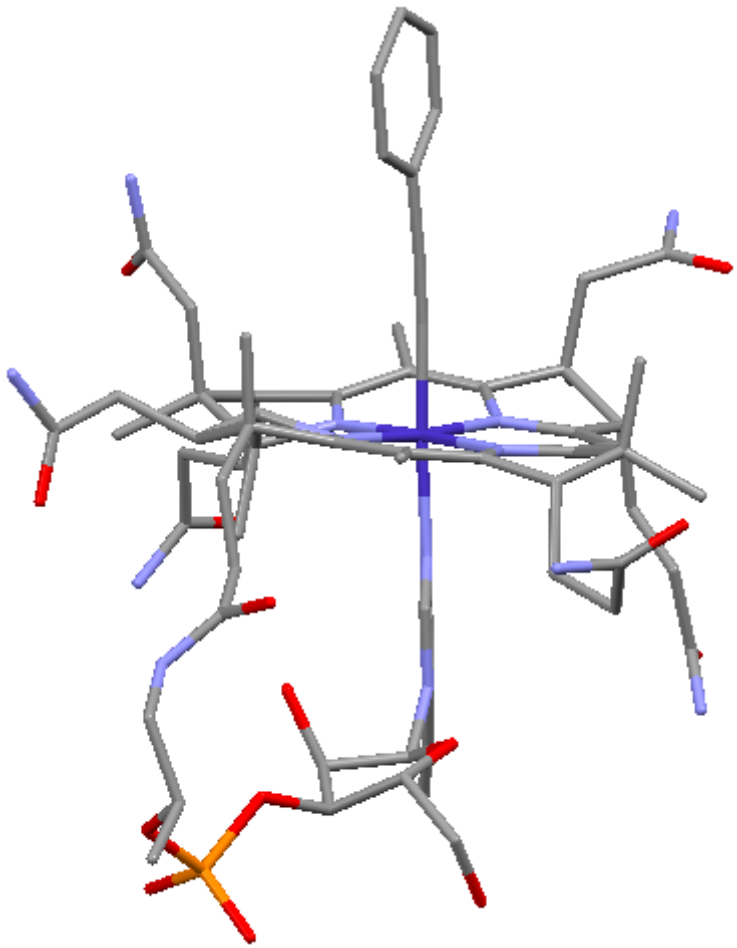

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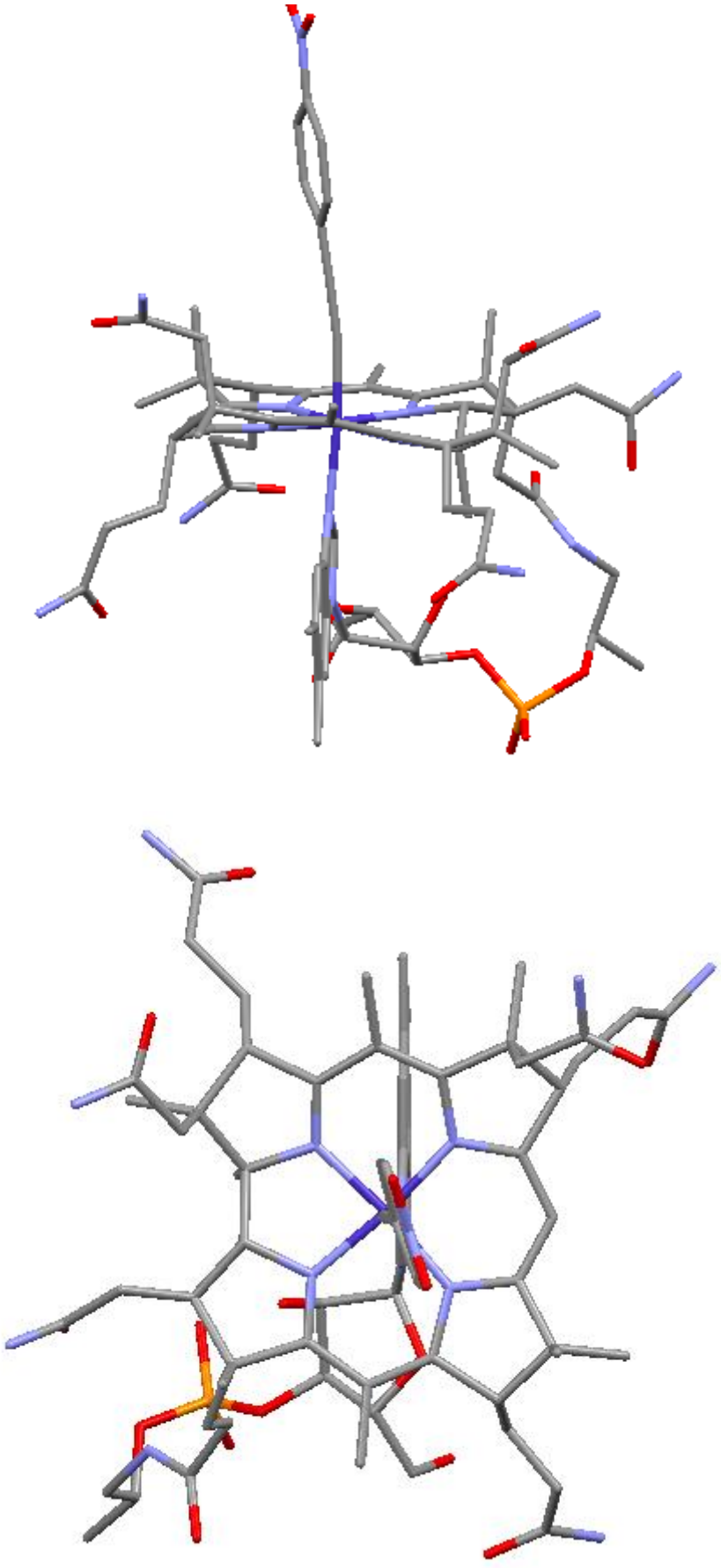
Crystal structure of **3** (hydrogen atoms omitted for clarity)



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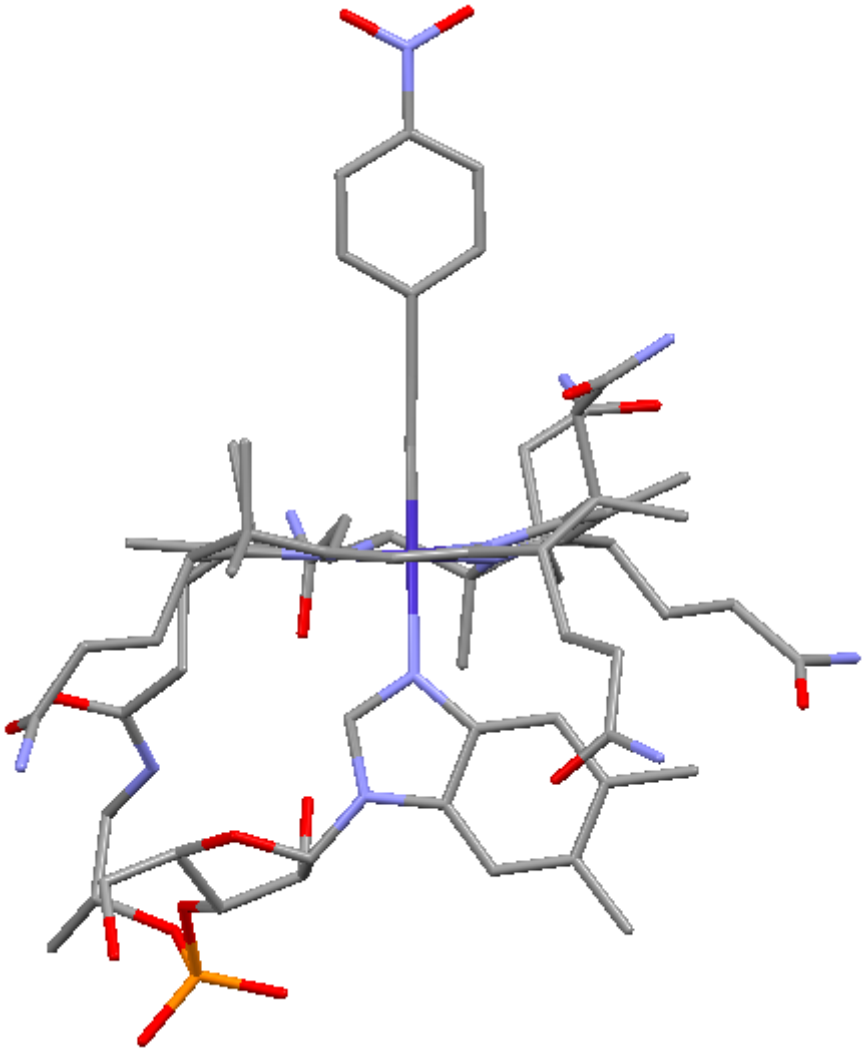
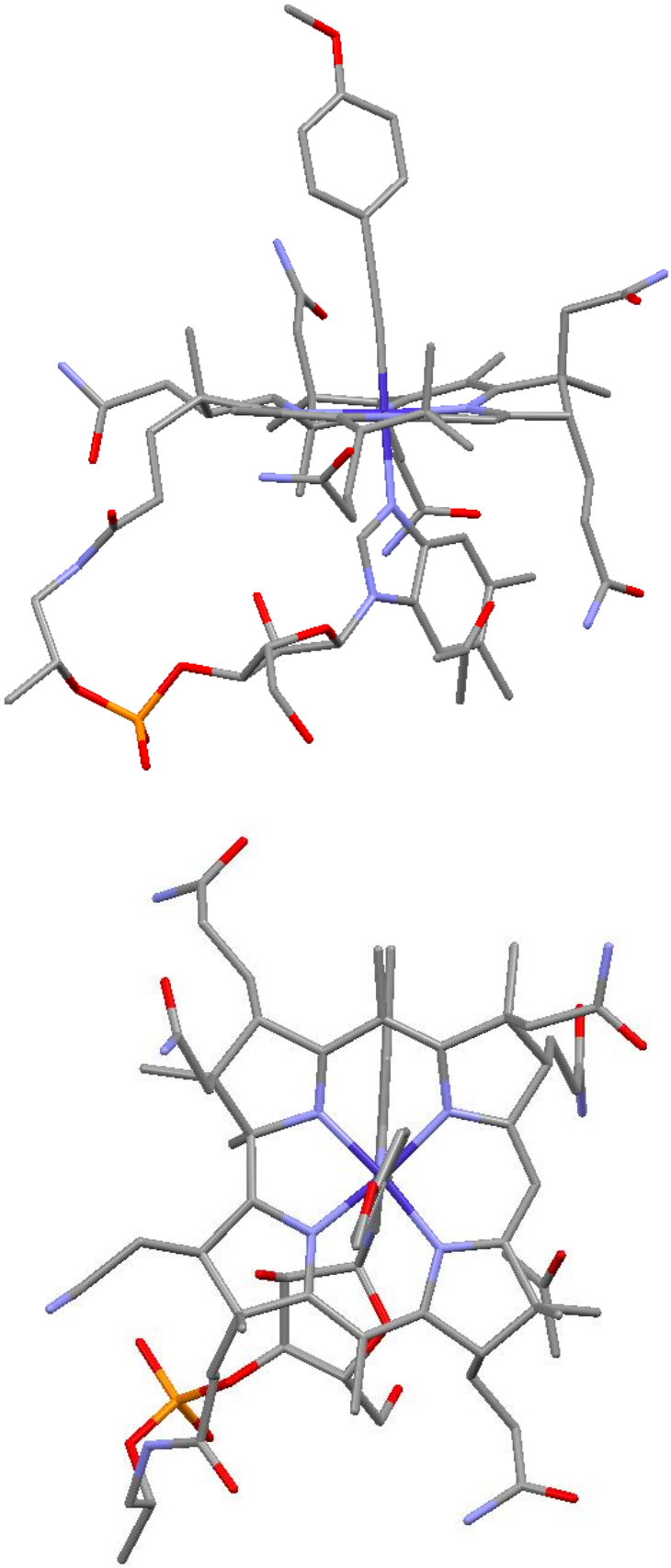


Crystal structure of **4** (hydrogen atoms omitted for clarity)



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