Supplementary Note
Compound synthesis protocols and characterization

Riboglow: A multicolor riboswitch-based platform for live cell imaging of mRNA and small non-coding RNA in mammalian cells

1. General Information

Commercially available reagents and solvents were used as received. 6-FAM alkyne and sulfo-Cyanine5 alkyne were purchased from Lumiprobe and ATTO propargylamides were obtained from ATTO-TEC. The structure of ATTO 633 alkyne was not provided by the producer, hence it is not included on schemes.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker 500 MHz or Varian 500 MHz spectrometer with the residual solvent peak used as an internal standard. Data are reported as follows: chemical shift [ppm], multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant and integration. HRMS spectra were recorded on a spectrometer with TOF mass analyzer.

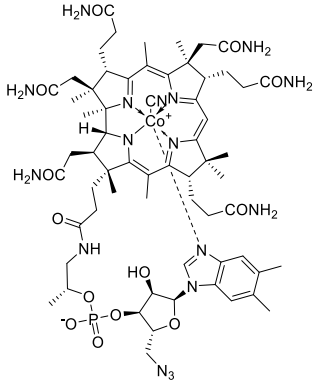
The scale of the reactions with ATTO and Cyanine dyes did not provide sufficient amount of products for NMR analyses thus the HPLC and MS analyses were performed to characterize those compounds. During the synthesis of Cbl conjugates, the conversion of a substrate to a product was estimated based on a dye as the vitamin B_{12} derivative was used in excess and was calculated using the integration of a signal coming from the dye in RP-HPLC analysis. All reactions described in Section 3 proceeded with the conversion >99% (on the HPLC chromatograms only signals corresponding to the desired conjugate and the remaining azide were observed. For Cbl-1xPEG-FAM, Cbl-2xPEG-FAM and Cbl-3xPEG-FAM signals in ^{1}H NMR spectra recorded in CD_3OD were much broader comparing to Cbl-FAM and Cbl-C6-FAM and subtle structure of multipletes or integrations could not be fully distinguished.

Preparative chromatography was performed using LiChroprep® RP-18 gel (Merck) with redistilled water and HPLC grade MeCN as eluents. Progress of the reactions was monitored using RP-HPLC techniques. HPLC measurement conditions: column, Eurospher II 100-5, C18, 250 mm × 4.6 mm with a precolumn or Kromasil C18 5 µm 250 mm × 4.0 mm; detection, UV/vis; pressure, 10 MPa; temperature, 30°C; flow rate, 1mL/min; wavelengths and HPLC methods are listed for each compound.

Abbreviations: CDT – 1,1′-Carbonyl-di-(1,2,4-triazole); RP HPLC – Reverse-phase high-performance liquid chromatography; TBTA – Tris[(1-benzyl-1H-1,2,3-triazol-4 yl)methyl]amine; TEA – Triethylamine

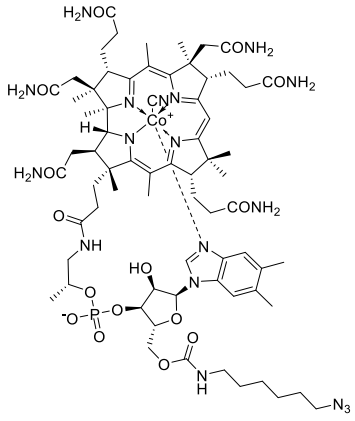
2. Synthesis of cobalamin azide

2.1 Cbl-N_3



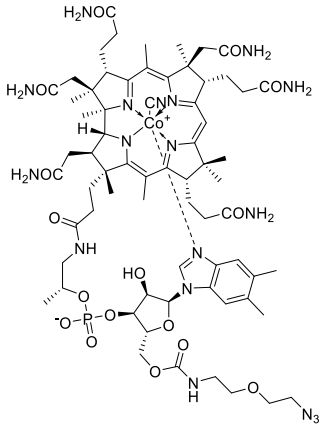
Compound was synthesized according to the procedure described in *Chem. Eur. J.*, **19**, 5141 – 5148 (2013). All spectra matched that reported in the literature.

2.2 Cbl-C6-N_3



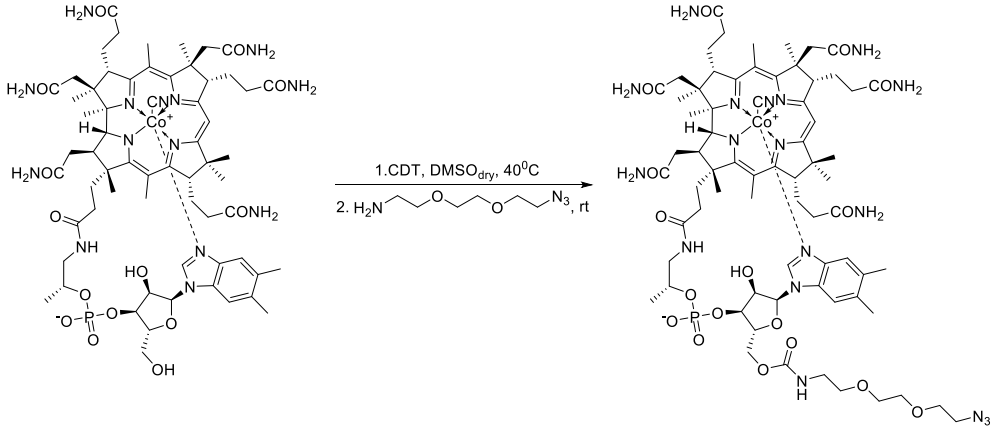
Compound was synthesized according to the procedure described in *J. Porphyrins Phthalocyanines*, **17**, 110-117 (2013). All spectra matched those reported in the literature.

2.3 Cbl-1xPEG-N_3



Compound was synthesized according to the procedure described in *J. Porphyrins Phthalocyanines*, **17**, 110-117 (2013). All spectra matched those reported in the literature.

2.4 Cbl-2xPEG-N_3

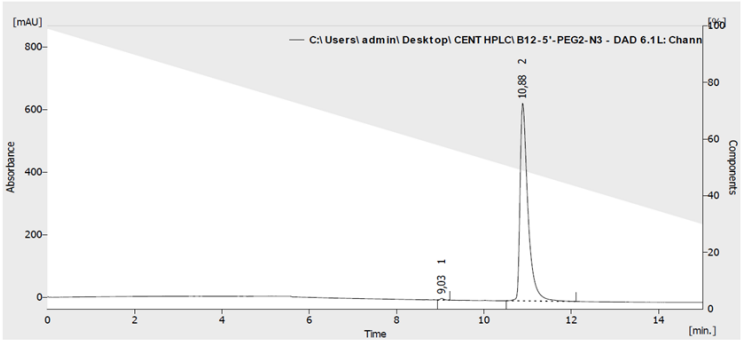


Cbl-2xPEG-N_3: Cobalamin (0.075 mmol, 100 mg) was dissolved in dry DMSO (2.5 mL) at 40 °C under an argon atmosphere. To a stirring solution under argon solid CDT (50 mg, 0.30 mmol) was added. When full consumption of the substrate (monitored by the RP HPLC) was observed (approx. 1.5 h), heating bath was removed and 2-[2-(2-azidoethoxy)ethoxy]-

ethanamine (100 µL) was added in one portion. The resulting solution was stirred overnight, then the reaction mixture was poured into AcOEt (50 mL), and centrifuged. The precipitate was washed twice with Et_2O (2 x 15mL). After drying it was dissolved in water and purified by RP column chromatography (80 mL) with a mixture of MeCN and H_2O as eluents (10% v/v). The desired compound was obtained as a red powder; yield: 66% (0.0495 mmol, 77 mg). ^{1}H NMR (500 MHz, CD_3OD) δ 7.25 (s, 1H), 7.15 (s, 1H), 6.58 (s, 1H), 6.23 (d, *J* = 2.6 Hz, 1H), 6.04 (s, 1H) 4.66 (d, *J* = 9.9 Hz, 1H), 4.51 (d, *J* = 8.2 Hz, 1H), 4.40 – 4.32 (m, 1H), 4.24 – 4.20 (m, 2H), 4.17 (dd, *J* = 12.2, 2.4 Hz, 1H), 4.13 (d, *J* = 11.5 Hz, 1H), 3.60 – 3.67 (m, 7H), 3.54 (t, *J* = 5.6 Hz, 2H), 3.36 (t, *J* = 5.6 Hz, 2H), 2.93 – 2.85 (m, 2H), 2.59 (s, 3H), 2.58 (s, 3H), 2.67 – 2.42 (m, 12H), 2.41 – 2.34 (m, 2H), 2.29 (s, 3H), 2.28 (s, 3H), 2.21 – 2.14 (m, 1H), 2.12 – 1.96 (m, 4H), 1.94 – 1.82 (m, 3H), 1.89 (s, 3H), 1.77 – 1.70 (m, 1H), 1.47 (m, 3H), 1.39 (s, 3H), 1.39 – 1.37 (m, 2H), 1.37 (s, 3H), 1.30 –1.26 (m, 1H), 1.25 (d, *J* = 6.3 Hz, 3H), 1.19 (s, 3H), 1.16 – 1.08 (m, 1H), 0.47 (s, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ 180.1, 178.7, 176.1, 176.0, 175.9, 175.1, 174.1, 174.1, 173.8, 173.2, 172.6, 165.7, 165.5, 157.2, 141.9, 136.8, 134.2, 132.5, 129.9, 116.5, 111.0, 107.3, 103.8, 94.2, 86.8, 85.0, 79.9, 74.9, 73.7, 72.0, 72.0, 70.1, 70.0, 69.7, 69.6, 69.1, 62.8, 58.9, 56.2, 55.5, 53.6, 51.1, 50.3, 45.2, 42.5, 41.6, 40.4, 38.7, 34.8, 33.7, 31.8, 31.5, 31.2, 30.9, 30.9, 28.1, 26.0, 25.9, 19.5, 19.1, 19.0, 18.9, 18.7, 18.7, 18.5, 16.1, 15.7, 14.9, 14.7. UV/vis (H_2O) λ_{max} (nm) (ε, L mol^{-1} cm^{-1}) 551 (7.8 × 10^3), 522 (6.8 × 10^3), 361 (2.4 × 10^4), 278 (1.3 × 10^4), 222 (4.2 × 10^4). HRMS (ESI) *m/z* [M + Na]$^+$ calcd for $C_{70}H_{100}N_{18}O_{17}PCoNa$ 1577.6481, found 1577.6455. Anal. calcd for $C_{70}H_{100}N_{18}O_{17}PCo \cdot 6H_2O$: C, 50.54; H, 6.79; N, 15.15. Found: C, 50.62; H, 7.03; N, 14.95.

HPLC Method:

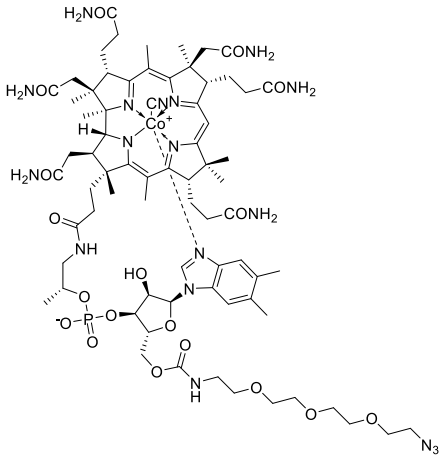
Time [min]	H_2O+0.5‰TFA [%]	MeCN [%]	λ [nm]	R_t [min]
Initial	99	1	361	10.88
15	30	70		



Result Table (Uncal - C:\Users\admin\Desktop\CENT HPLC\B12-5'-PEG2-N3 - DAD 6.1L: Channel 2)

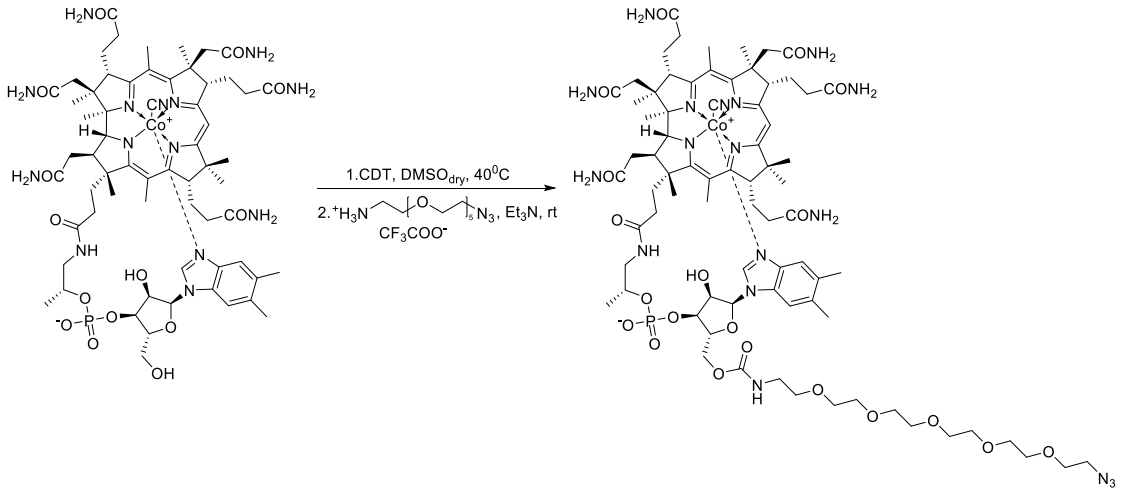
	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	9,033	31,715	5,208	0,4	0,8	0,12	779
2	10,883	7950,937	631,293	99,6	99,2	0,18	573
	Total	7982,652	636,501	100,0	100,0		

2.5 Cbl-3xPEG-N_3



Compound was synthesized according to the procedure described in *J. Porphyrins Phthalocyanines*, **17**, 110-117 (2013). All spectra matched those reported in the literature.

2.6 Cbl-5xPEG-N_3

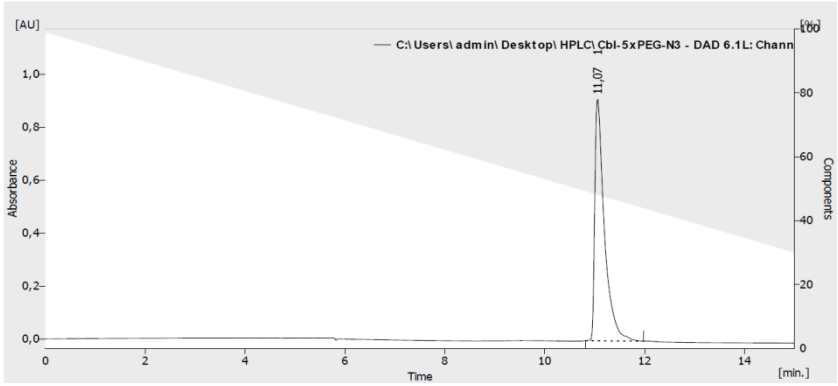


Cbl-5xPEG-N_3: Cobalamin (0.146 mmol, 200 mg) was dissolved in dry DMSO (5 mL) at 40 °C under an argon atmosphere. To a stirring solution under argon solid CDT (100 mg, 0.609 mmol) was added. When full consumption of the substrate (monitored by the RP HPLC) was observed (approx. 1.5 h), heating bath was removed and 2-[2-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]-ethanamine in the form of TFA salt (100 mg) was added in one portion. Subsequently TEA (80 µL) was added and the resulting solution was stirred overnight. Then the reaction mixture was poured into AcOEt (50 mL) and centrifuged. The precipitate was then washed twice with Et_2O (2 x 15 mL). After drying it was dissolved in water and purified by RP column chromatography (80 mL) with a mixture of MeCN and H_2O as eluents (gradually from 10 to 15% v/v). The desired compound was obtained as a red powder; yield: 43% (0.063 mmol, 106 mg). ^{1}H NMR (500 MHz, CD_3OD) δ 7.24 (s, 1H), 7.14 (s, 1H), 6.57 (s, 1H), 6.22 (d, *J* = 2.6 Hz, 1H), 6.03 (s, 1H), 4.92 (m, 1H), 4.65 (d, *J* = 10.5 Hz, 1H), 4.49 (d, *J* = 8.8 Hz, 1H), 4.41 – 4.30 (m, 1H), 4.26 – 4.08 (m, 4H), 3.67 – 3.56 (m, 23H), 3.52 (t, *J* = 5.4, 2H), 3.37 – 3.32 (m, 2H), 3.27 (m, 1H), 2.95 – 2.81 (m, 2H), 2.70 – 2.31 (m, 8H), 2.58 (s, 3H), 2.57 (s, 3H), 2.28 (s, 3H), 2.27 (s, 3H), 2.22 – 1.93 (m, 6H), 1.92 – 1.80 (m, 3H), 1.88 (s, 3H), 1.80 – 1.57 (m, 1H), 1.46 (s, 3H), 1.38 (s, 3H), 1.36 (s, 3H), 1.31 – 1.20 (m,

2H), 1.24 (d, *J* = 6.3 Hz, 3H), 1.18 (s, 3H), 1.14 – 1.05 (m, 1H), 0.46 (s, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ 181.6, 180.2, 177.6, 177.426, 177.4, 176.6, 176.4, 175.6, 175.5, 175.3, 174.6, 167.2, 166.9, 143.4, 138.3, 135.7, 134.0, 131.4, 117.9, 112.5, 108.8, 105.3, 95.7, 88.3, 86.4, 76.4, 73.4, 71.6, 71.6, 71.6, 71.3, 71.1, 71.0, 70.6, 60.3, 57.7, 57.0, 55.1, 52.6, 51.8, 49.6, 48.4, 46.6, 44.0, 43.0, 41.8, 40.1, 36.2, 35.1, 33.3, 33.0, 32.6, 32.4, 32.3, 29.5, 27.4, 27.4, 20.3, 20.6, 20.5, 20.3, 20.2, 20.0, 17.5, 17.1, 16.4, 16.2. UV/vis (H_2O) λ_{max} (nm) (ε, L mol^{-1} cm^{-1}) 549 (5.8 × 10^3), 520 (5.2 × 10^4), 361 (1.8 × 10^4), 277 (1.1 × 10^4), 220 (3.3 × 10^4). HRMS (ESI) *m/z* [M + Na]$^+$ calcd for $C_{76}H_{112}N_{18}O_{20}PCoNa$ 1709.7268, found 1709.7219. Anal. calcd for $C_{76}H_{112}N_{18}O_{20}PCo \cdot 7H_2O$: C, 50.33; H, 7.00; N, 13.90. Found: C, 50.22; H, 6.76; N, 14.22.

HPLC Method:

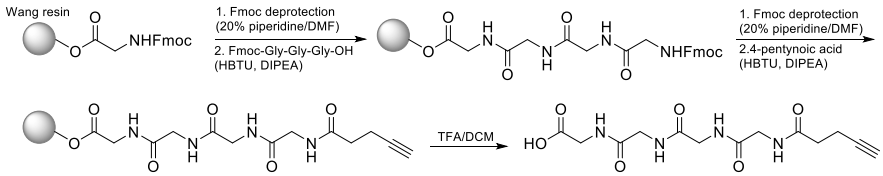
Time [min]	H_2O+0.5‰TFA [%]	MeCN [%]	λ [nm]	R_t [min]
Initial	99	1	361	11.07
15	30	70		



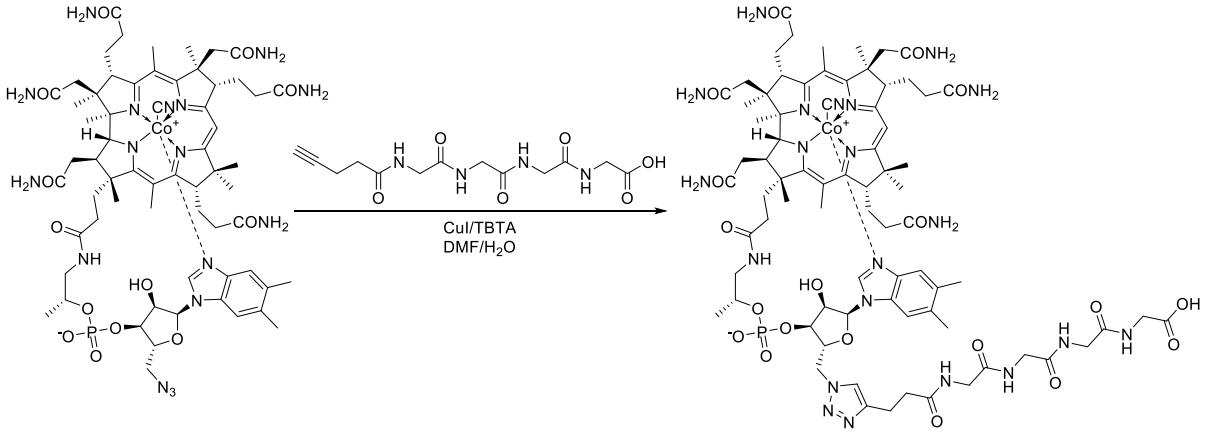
Result Table (Uncal - C:\Users\admin\Desktop\HPLC\Cbl-5xPEG-N3 - DAD 6.1L: Channel 2)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	11,067	12246,454	911,440	100,0	100,0	0,20	616
	Total	12246,454	911,440	100,0	100,0		

2.7 Cbl-5xGly-N_3



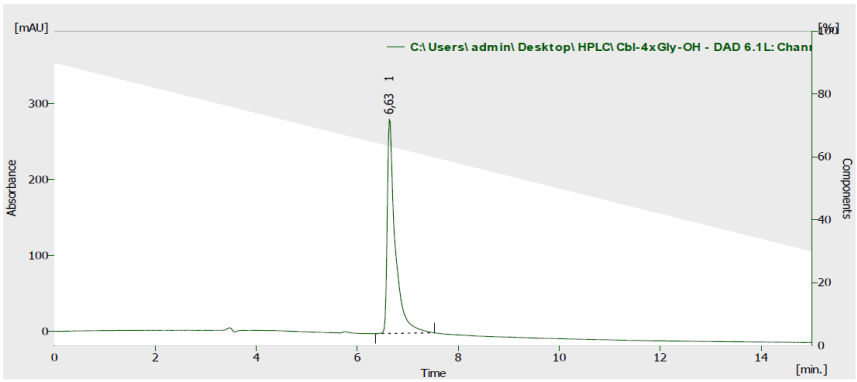
STEP 1: HO-4xGly-alkyne was synthesized manually by Fmoc chemistry on a 0.124 mmol scale of Fmoc-Gly attached to the Wang resin (Fmoc-Gly-Wang resin), 4-fold molar excess of the Fmoc-Gly-Gly-Gly-OH and 5-fold molar excess of 4-pentynoic acid. Fmoc deprotection was performed with 20% piperidine in DMF (1.5 mL, 1-2 h) and coupling with the use of HBTU (6 equiv.) and DIPEA (6 equiv.) in DMF (2 mL). After final coupling the resin was washed with DMF (5 x 1 mL), DCM (5 x 1 mL) and dried. Cleavage from the resin was carried with the use of a TFA/DCM (25%, v/v) with the catalytic amount of anisole for 2.5 h. Obtained product was precipitated with Et_2O and centrifuged. LRMS (ESI) *m/z* [M + Na]$^+$ calcd for $C_{13}H_{18}N_4O_6Na$ 349.12, found 349.20.



STEP 2: Cbl-N_3 (0.068 mmol, 94 mg) and HO-4xGly-alkyne (0.062 mmol, 20 mg) was dissolved in DMF/H_2O mixture (5:3 v/v, Σ = 4 mL). Catalyst – CuI (0.032 mmol, 6 mg) and TBTA (0.057 mmol, 30 mg) mixed in 2 mL of DMF for 20 min – was added and the resulting solution was stirred for 16 h at 40°C. The reaction mixture was diluted with MeOH (5 mL) and poured into Et_2O (60 mL). The resulting precipitate was filtered through a cotton wool, washed with AcOEt (2 x 10 mL) and Et_2O (2 x 10 mL). After drying, the resulting solid was dissolved in MeOH and concentrated in vacuo. The crude was dissolved in water and purified by RP column chromatography with a mixture of MeCN/H_2O (10% v/v) as an eluent. The desired compound was obtained as a red powder, yield: 59% (0.073 mmol, 124 mg). ^{1}H NMR spectra were recorded for D_2O at rt and at 80°C but the signals were very broad and subtle structure of multipletes or integrations could not be fully distinguished (see part NMR spectra). ^{13}C NMR (126 MHz, D_2O) δ 182.5, 181.4, 180.2, 180.0, 179.4, 179.4, 178.2, 178.1, 177.5, 177.2, 176.0, 175.1, 174.6, 174.2, 168.4, 167.8, 144.3, 139.0, 137.7, 135.6, 132.4, 119.0, 113.8, 110.0, 106.7, 97.4, 89.0, 87.6, 81.3, 77.3, 76.8, 75.6, 71.1, 61.6, 58.7, 58.2, 56.2, 53.9, 50.5, 49.7, 47.6, 45.4, 45.2, 45.1, 41.8, 41.5, 37.4, 37.0, 36.6, 34.4, 34.3, 34.7, 34.0, 33.8, 30.1, 28.9, 28.5, 28.4, 27.1, 26.9, 22.3, 21.9, 21.8, 21.7, 21.5, 21.5, 19.3, 18.3, 17.8, 17.7. UV/vis (H_2O) λ_{max} (nm) (ε, L mol^{-1} cm^{-1}) 549 (5.8 × 10^3), 520 (5.2 × 10^3), 361 (1.8 × 10^4), 276 (1.0 × 10^4), 222 (3.5 × 10^4). HRMS (ESI) *m/z* $[M + Na]^+$ calcd for $C_{76}H_{105}N_{21}O_{19}PCoNa$ 1728.6863, found 1728.6897.

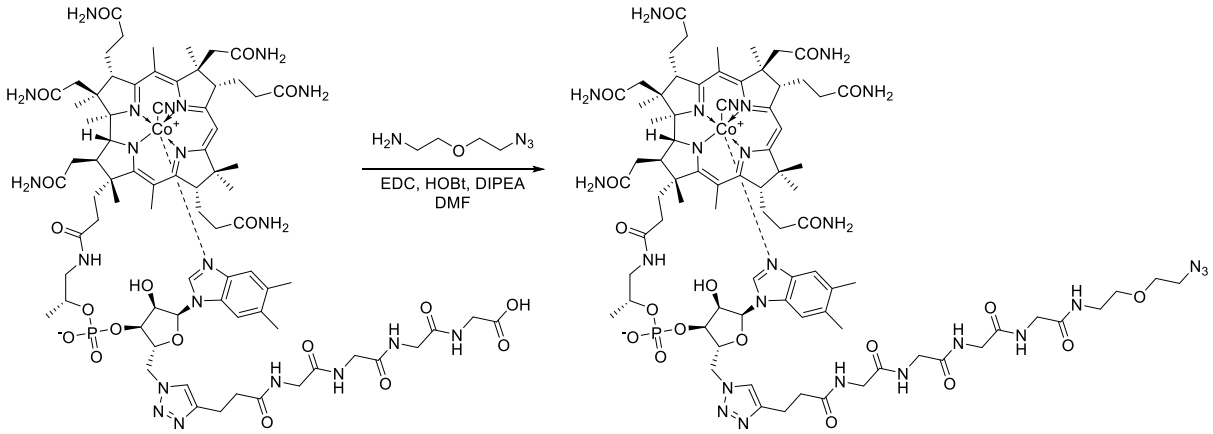
HPLC Method:

Time [min]	H_2O+0.2‰TFA [%]	MeCN [%]	λ [nm]	R_t [min]
Initial	90	10	361	6.63
15	30	70		



Result Table (Uncal - C:\Users\admin\Desktop\HPLC\Cbl-4xGly-OH - DAD 6.1L: Channel 2)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	6,633	3089,950	282,314	100,0	100,0	0,15	823
	Total	3089,950	282,314	100,0	100,0		

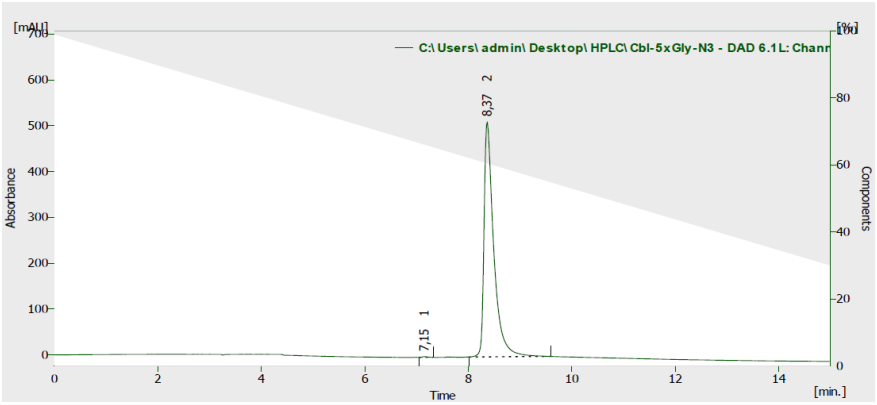


STEP 3: Cbl-4xGly-OH (80 mg, 0.047 mmol) and 2-(2-azidoethoxy)ethanamine (300 µL) was dissolved in DMF (5 mL). EDC (90 mg, 0.470 mmol), HOBt (127 mg, 0.940 mmol) and DIPEA (0.470, 82 µL) were added. The resulting solution was stirred at rt for 1 h. Desired product, the product lacking CN ligand and the unreacted substrate were present in the reaction mixture in the approx. ratio 2.3 : 2.5 : 1 (according to RP-HPLC). The reaction mixture was diluted with MeOH (5 mL) and poured into Et_2O (60 mL). The resulting precipitate was filtered through a cotton wool, washed with AcOEt (2 x 10 mL) and Et_2O (2 x 10 mL). After drying, the resulting solid was dissolved in MeOH and concentrated in vacuo. Desired compound was purified by RP column chromatography with a mixture of MeCN/H_2O (gradually from 8% to 40% v/v). Order of elution: substrate, desired product, product lacking CN ligand. The solvent was concentrated in vacuo and the product was obtained as a red solid. Yield of desired product (with CN ligand): 41% (0.019 mmol, 35 mg), yield of product without CN ligand (L=H_2O): 14% (0.007 mmol, 12 mg). ^{1}H NMR (500 MHz, CD_3OD) δ 8.18 (s, 1H), 7.19 (s, 1H), 7.15 (s, 1H), 6.56 (s, 1H), 6.06 (s, 1H), 5.90 (d, J = 2.9 Hz, 1H), 5.02 (dd, J = 14.7, 3.5 Hz, 1H), 4.86 – 4.82 (m, 1H), 4.54 (td, J = 8.4, 4.0 Hz, 1H), 4.49 (d, J = 8.7 Hz, 1H), 4.44 – 4.33 (m, 2H), 4.13 (d, J = 11.3 Hz, 1H), 4.09 – 4.04 (m, 1H), 4.02 – 3.81 (m, 8H), 3.69 (d, J = 13.9 Hz, 1H), 3.66 – 3.60 (m, 3H), 3.55 (t, J = 5.7 Hz, 2H), 3.38 – 3.34 (m, 4H), 3.25 (d, J = 10.5 Hz, 1H), 3.09 – 3.06 (m, 2H), 2.90 – 2.85 (m, 2H), 2.73 – 2.31 (m, 19H), 2.27 (s, 3H), 2.24 (s, 3H), 2.22 – 1.97 (m, 5H), 1.96 – 1.80 (m, 3H), 1.89 (s, 3H), 1.77 – 1.68 (m, 1H), 1.49 (s, 3H), 1.380 (s, 3H), 1.375 (s, 3H), 1.32 – 1.24 (m, 1H), 1.28 (d, J = 6.2 Hz, 3H), 1.20 (s, 3H), 1.15 – 1.07 (m, 1H), 0.44 (s, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ 181.6, 180.1, 177.5, 177.3, 177.2, 176.6, 176.0, 175.6, 175.5, 175.3, 174.6, 174.2, 173.0, 172.7, 172.2, 171.7, 167.8, 166.9, 147.3, 143.3, 138.2, 135.8, 134.0, 131.3, 126.0, 117.8, 112.6, 108.8, 105.2, 95.8, 87.9, 86.4, 80.9, 80.7, 76.4, 75.0, 73.7, 73.6, 70.9, 70.4, 70.3, 66.9, 60.3, 57.7, 57.0, 55.5, 52.6, 51.8, 50.6, 46.8, 44.3, 44.0,

43.9, 43.5, 43.1, 40.4, 40.1, 36.2, 35.9, 35.4, 33.4, 32.9, 32.8, 32.6, 32.4, 32.3, 29.5, 27.4, 22.4, 20.9, 20.5, 20.4, 20.3, 19.9, 17.5, 17.1, 16.4 16.1, 15.4. UV/vis (H_2O) λ_{max} (nm) (ε, L mol^{-1} cm^{-1}) 548 (8.0×10^3), 520 (7.0×10^3), 361 (2.5×10^4), 279 (1.3×10^4), 222 (4.5×10^4). HRMS (ESI) *m/z* $[M + Na]^+$ calcd for $C_{80}H_{113}N_{25}O_{19}PCoNa$ 1840.7612, found 1840.7611.

HPLC Method:

Time [min]	H_2O+0.2‰TFA [%]	MeCN [%]	λ [nm]	R_t [min]
Initial	90	10	361	8.37
15	30	70		

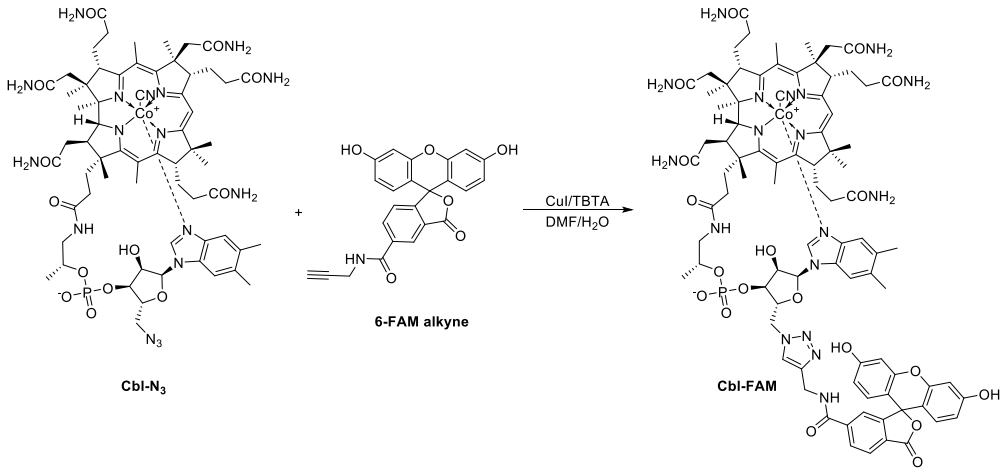


Result Table (Uncal - C:\Users\admin\Desktop\HPLC\Cbl-5xGly-N3 - DAD 6.1L: Channel 2)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	7,150	11,134	1,720	0,2	0,3	0,12	912
2	8,367	6698,033	512,670	99,8	99,7	0,20	563
	Total	6709,167	514,390	100,0	100,0		

3. Synthesis of Cbl conjugates with various dyes

3.1 Cbl-FAM

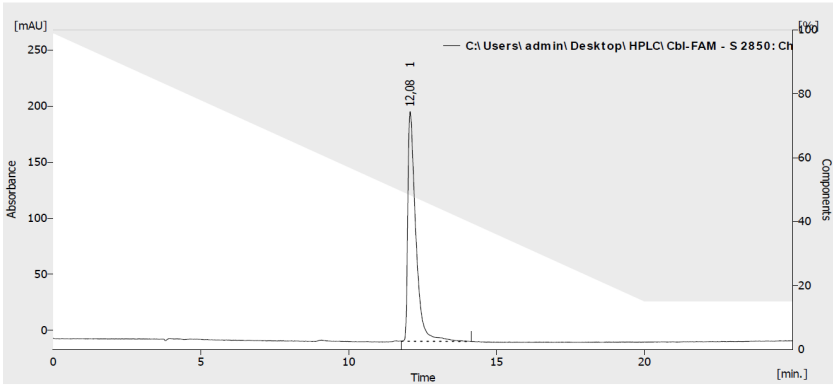


Cbl-FAM: CuI (1 mg, 5 μmol) and TBTA (5 mg, 10 μmol) were dissolved in DMF/H_2O (1 mL, 3:1 v/v) and stirred for 20 min. Subsequently Cbl-N_3 (24 mg, 17.4 μmol) and 6-FAM alkyne (6 mg, 14.5 μmol) were added and the reaction mixture was stirred overnight. Then it was diluted with DMF (1 mL), poured into AcOEt (15 mL) and the precipitate was centrifuged and dried. The crude solid was dissolved in MeOH (1 mL), precipitated with Et_2O (15 mL), and then centrifuged. The dried solid was then dissolved in H_2O, loaded onto RP column (30 mL) and purified gradually with MeCN/H_2O from 10 to 20% v/v yielding orange solid. ^{1}H NMR (500 MHz,

CD_3OD) δ 8.21 (s, 1H), 8.20 (s, 1H), 8.11 (d, *J* = 7.6 Hz, 1H), 7.74 (s, 1H), 7.14 (s, 1H), 7.10 (s, 1H), 6.75 (bs, 2H), 6.71 – 6.64 (m, 2H), 6.59 (m, 2H), 6.56 (s, 1H), 6.03 (s, 1H), 5.95 (d, *J* = 2.3, 1H), 4.98 (d, *J* = 13.0 Hz, 1H), 4.62 (bs, 2H), 4.53 (bs, 1H), 4.49 (d, *J* = 8.6 Hz, 1H), 4.41 – 4.35 (m, 1H), 4.31 (bs, 1H), 4.13 (d, *J* = 11.4 Hz, 1H), 4.06 (m, 1H), 3. 63 (dd, *J* = 5.1, 10.7 Hz, 1H), 3.57 (d, *J* = 13.8 Hz, 1H), 3.21 (d, *J* = 10.1 Hz, 1H), 2.88 – 2.81 (m, 1H), 2.78 (dd, *J* = 9.2, 13.7 Hz, 1H), 2.68 – 2.43 (m, 8H), 2.58 (s, 3H), 2.55 (s, 3H), 2.41 – 2.31 (m, 2H), 2.27 (s, 3H), 2.21 (s, 3H), 2.14 – 1.95 (m, 6H), 1.95 – 1.83 (m, 2H), 1.89 (s, 3H), 1.82 – 1.65 (m, 2H), 1.42 (s, 3H), 1.38 (s, 3H), 1.35 (s, 3H), 1.30 – 1.20 (m, 2H), 1.23 (d, *J* = 5.7 Hz, 3H), 1.20 – 1.07 (m, 1H), 1.18 (s, 3H), 0.42 (s, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ 181.6, 180.1, 177.6, 177.35, 177.2, 176.5, 175.6, 175.5, 175.3, 174.6, 174.2, 167.2, 166.9, 143.2, 138.2, 135.8, 134.0, 131.3, 130.6, 117.9, 114.5, 112.4, 108.8, 105.1, 103.6, 95.7, 87.9, 86.4, 81.2, 76.3, 75.8, 70.4, 60.3, 57.7, 57.0, 55.4, 52.6, 43.9, 43.1, 40.1, 36.2, 35.4, 33.5, 33.1, 32.9, 32.4, 32.3, 29.3, 27.4, 20.9, 20.5, 20.30, 20.26, 20.2, 19.9, 17.5, 17.1, 16.4, 16.2. HRMS (ESI) m/z $[M + 2Na]^{2+}$ calcd for $C_{87}H_{102}CoN_{18}O_{19}PNa_2$, 919.3211; found, 919.3182.

HPLC Method:

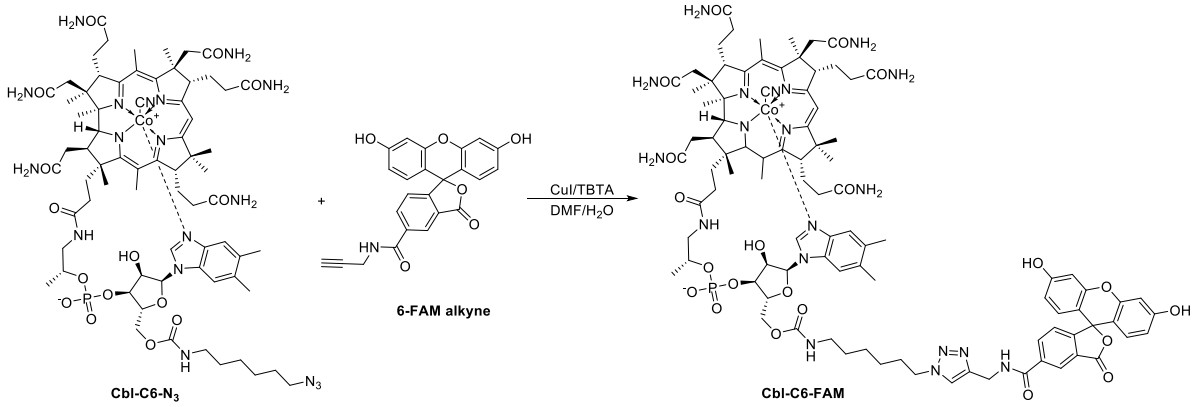
Time [min]	H_2O+0.5‰TFA [%]	MeCN [%]	λ [nm]	R_t [min]
Initial	99	1	361	12.08
20	15	85		
40	15	85		



Result Table (Uncal - C:\Users\admin\Desktop\HPLC\Cbl-FAM - S 2850: Ch 2)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	12,083	3867,965	204,936	100,0	100,0	0,28	913
	Total	3867,965	204,936	100,0	100,0		

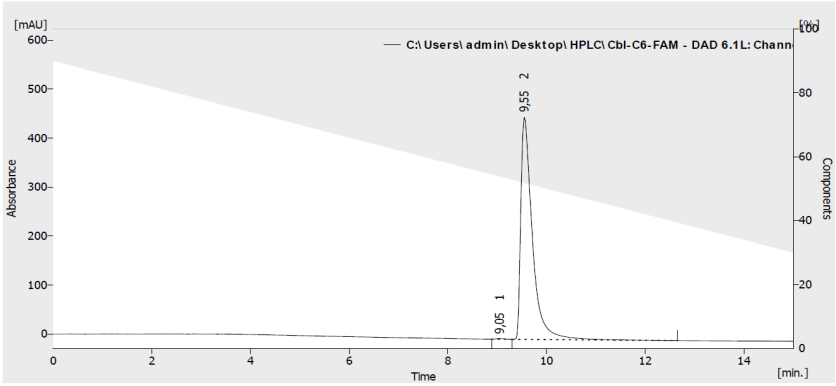
3.2 Cbl-C6-FAM



Cbl-C6-FAM: CuI (1 mg, 5 µmol) and TBTA (5 mg, 10 µmol) were dissolved in DMF/H_2O (1 mL, 3:1 v/v) and stirred for 20 min. Subsequently Cbl-C6-N_3 (27 mg, 17.7 µmol) and 6-FAM alkyne (6 mg, 14.5 µmol) were added and the reaction mixture was stirred overnight. Then it was diluted with DMF (1 mL), poured into AcOEt (15 mL) and the precipitate was centrifuged and dried. The crude solid was dissolved in MeOH (1 mL), precipitated with Et_2O (15 mL) and then centrifuged. The dried solid was then dissolved in H_2O (small amount of MeOH was added for better dissolution), loaded onto RP column (30 mL) and purified gradually with MeCN/H_2O from 15 to 30% v/v yielding orange solid. ^{1}H NMR (500 MHz, CD_3OD) δ 8.13 (s, 2H), 7.88 (s, 1H), 7.68 (s, 1H), 7.18 (s, 1H), 7.13 (s, 1H), 6.79 (bs, 2H), 6.68 (s, 2H), 6.61 – 6.49 (m, 3H), 6.18 (bs, 1H), 6.03 (s, 1H), 4.92 (m, 1H), 4.66 – 4.47 (m, 6H), 4.42 – 4.28 (m, 1H), 4.33 (t, *J* = 6.8 Hz, 2H), 4.20 (bs, 2H), 4.16 – 4.07 (m, 2H), 3.67 – 3.57 (m, 2H), 3.29 (m, 1H), 3.06 – 2.92 (m, 2H), 2.94 – 2.82 (m, 2H), 2.68 – 2.42 (m, 8H), 2.58 (s, 6H), 2.41 – 2.31 (m, 2H), 2.28 (s, 3H), 2.23 (s, 3H), 2.20 – 1.78 (m, 8H), 1.88 (s, 3H), 1.77 – 1.68 (m, 1H), 1.48 – 1.40 (m, 2H), 1.44 (s, 3H), 1.38 (s, 3H), 1.36 (s, 3H), 1.33 – 1.20 (m, 8H), 1.24 (d, *J* = 6.1 Hz, 3H), 1.18 (s, 3H), 1.16 – 1.07 (m, 1H), 0.46 (s, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ 181.6, 180.1, 177.6, 177.5, 177.4, 176.7, 175.5, 175.3, 174.6, 174.1, 168.3, 167.2, 166.9, 143.3, 140.1, 138.2, 136.8, 135.6, 133.9, 131.3, 131.2, 131.1, 130.1, 117.9, 112.4, 108.7, 105.2, 103.8, 95.6, 88.2, 86.4, 81.4, 76.4, 75.2, 73.5, 70.5, 64.2, 60.3, 57.7, 56.9, 55.0, 52.6, 51.3, 49.9, 46.6, 43.9, 43.0, 41.6, 40.1, 36.3, 36.2, 35.2, 33.3, 33.1, 32.7, 32.4, 32.3, 31.0, 30.8, 30.6, 29.5, 27.4, 27.0, 20.9, 20.5, 20.4, 20.2, 19.9, 17.5, 17.1, 16.4, 16.2. HRMS (ESI) m/z $[M + Na]^{+\cdot}$ calcd for $C_{94}H_{116}CoN_{19}O_{21}P$, 1959.7560; found, 1959.7555.

HPLC Method:

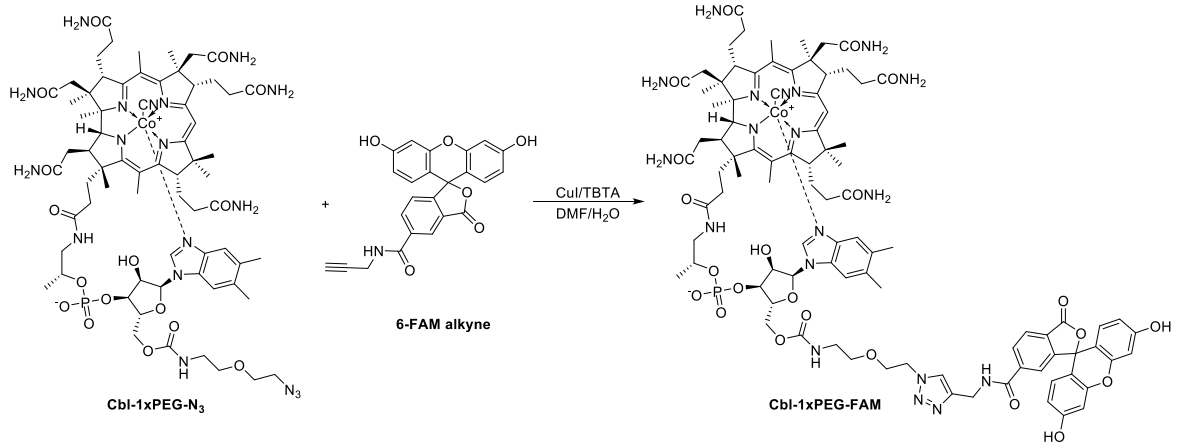
Time [min]	H_2O+0.5‰TFA [%]	MeCN [%]	λ [nm]	R_t [min]
Initial	90	10	361	9.55
15	30	70		



Result Table (Uncal - C:\Users\admin\Desktop\HPLC\Cbl-C6-FAM - DAD 6.1L: Channel 2)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	9,050	14,792	1,208	0,2	0,3	0,22	999
2	9,550	7407,348	453,759	99,8	99,7	0,25	460
	Total	7422,140	454,967	100,0	100,0		

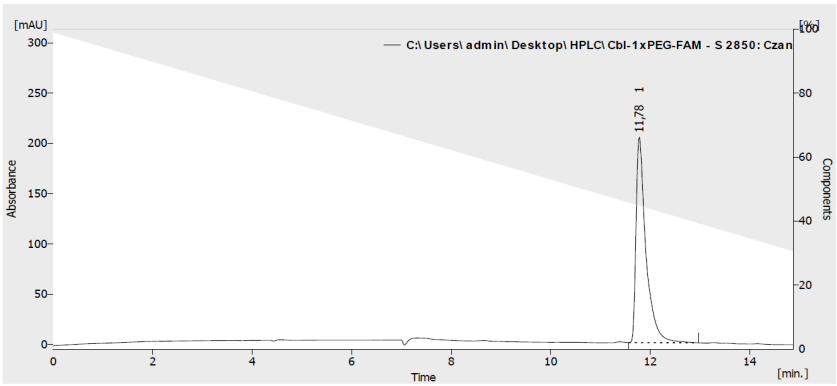
3.3 Cbl-1xPEG-FAM



Cbl-1xPEG-FAM: : CuI (1 mg, 5 µmol) and TBTA (5 mg, 10 µmol) were dissolved in DMF/H_2O (1 mL, 3:1 v/v) and stirred for 20 min. Subsequently Cbl-1xPEG-N_3 (27 mg, 17.9 µmol) and 6-FAM alkyne (6 mg, 14.5 µmol) were added and the reaction mixture was stirred overnight. Then it was diluted with DMF (1 mL), poured into AcOEt (15 mL) and the precipitate was centrifuged and dried. The crude solid was dissolved in MeOH (1 mL), precipitated with Et_2O (15 mL) and then centrifuged. The dried solid was then dissolved in H_2O, loaded onto RP column (30 mL) and purified gradually with MeCN/H_2O from 10 to 20% v/v yielding orange solid. ^{1}H NMR (500 MHz, CD_3OD) δ 8.22 (bs, 1H), 8.19 – 8.13 (m, 2H), 7.76 (bs, 1H), 7.11 (s, 1H), 7.13 (s, 1H), 6.87 (d, *J* = 4.0 Hz, 2H), 6.84 – 6.77 (m, 2H), 6.72 (s, 1H), 6.70 (s, 1H), 6.57 (s, 1H), 6.28 (bs, 1H), 6.02 (s, 1H), 4.98 (bs, 1H), 4.60 (bs, 1H), 4.55 – 4.44 (m, 3H), 4.40 (bs, 1H), 4.28 (bs, 1H), 4.22 (bs, 1H), 4.14 (d, *J* = 11.2 Hz, 1H), 4.05 (bs, 1H), 3.78 (bs, 2H), 3.71 – 3.58 (m, 2H), 3.46 – 3.36 (m, 2H), 3.27 (d, *J* = 10.7 Hz, 1H), 3.13 – 3.02 (m, 2H), 2.92 – 2.82 (m, 2H), 2.72 – 2.44 (m, 10H), 2.58 (s, 6H), 2.43 – 2.32 (m, 3H), 2.27 (s, 3H), 2.19 (s, 3H), 2.15-1.67 (m, 8H), 1.89 (s, 3H), 1,40 (s, 3H), 1.38 (s, 3H), 1.37 (s, 3H), 1.33 – 1.22 (m, 2H), 1.25 (bs, 3H), 1.17 (s, 3H), 1.20 – 1.08 (m, 1H), 0.45 (s, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ 181.6, 180.1, 177.6, 177.4, 176.7, 175.6, 175.5, 175.4, 174.6, 174.3, 167.2, 166.9, 158.3, 143.3, 138.3, 135.7, 134.0, 131.3, 131.2, 130.7, 117.9, 115.7, 112.5, 108.7, 105.2, 103.7, 95.6, 88.3, 86.4, 81.3, 76.3, 75.4, 74.7, 70.7, 70.6, 69.9, 64.1, 60.3, 57.7, 57.0, 55.2, 52.5, 46.6, 43.0, 41.6, 40.1, 36.2, 33.5, 33.3, 33.0, 32.3, 29.5, 27.5, 27.4, 20.9, 20.53, 20.45, 20.3, 20.1, 19.9, 17.5, 17.1, 16.4, 16.1. HRMS (ESI) m/z [M + H + Na]$^{2+}$ calcd for $C_{92}H_{112}CoN_{19}O_{22}PNa$, 973.8593; found, 973.8585.

HPLC Method:

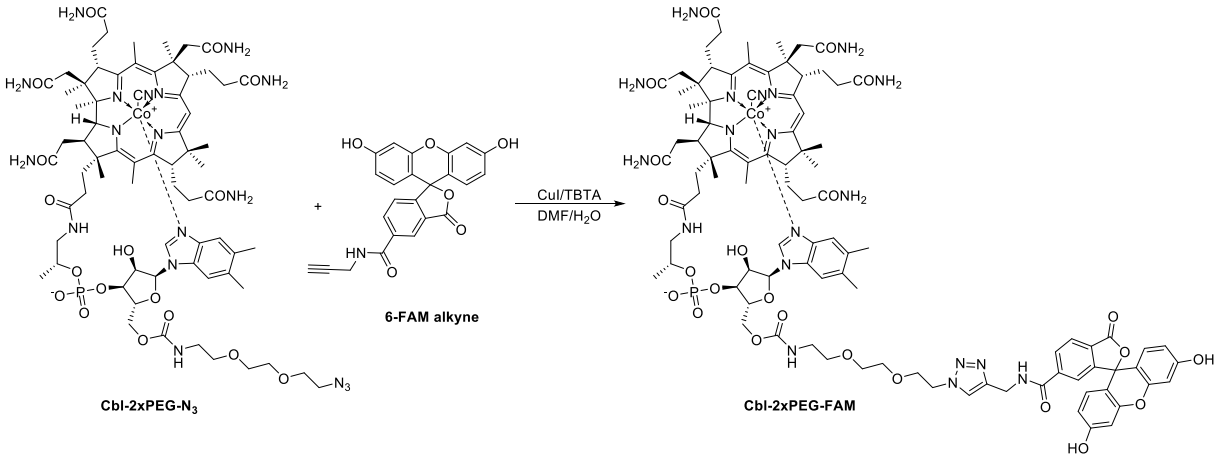
Time [min]	H_2O+0.5‰TFA [%]	MeCN [%]	λ [nm]	R_t [min]
Initial	99	1	361	11.78
15	30	70		



Result Table (Uncal - C:|Users|admin|Desktop|HPLC|Cbl-1xPEG-FAM - S 2850: Czanel 1)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	11,783	2832,624	204,294	100,0	100,0	0,20	664
	Total	2832,624	204,294	100,0	100,0		

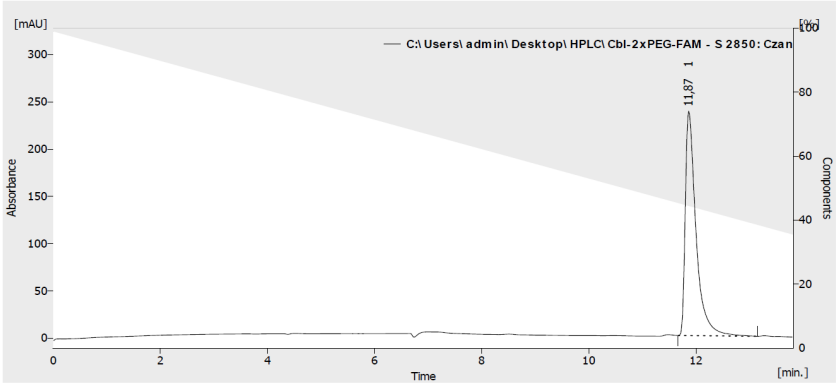
3.4 Cbl-2xPEG-FAM



Cbl-2xPEG-FAM: CuI (1 mg, 5 µmol) and TBTA (5 mg, 10 µmol) were dissolved in DMF/H_2O (1 mL, 3:1 v/v) and stirred for 20 min. Subsequently Cbl-2xPEG-N_3 (27 mg, 17.4 µmol) and 6-FAM alkyne (6 mg, 14.5 µmol) were added and the reaction mixture was stirred overnight. Then it was diluted with DMF (1 mL), poured into AcOEt (15 mL) and the precipitate was centrifuged and dried. The crude solid was dissolved in MeOH (1 mL), precipitated with Et_2O (15 mL), and then centrifuged. The dried solid was then dissolved in H_2O, loaded onto RP column (30 mL) and purified gradually with MeCN/H_2O from 10 to 20% v/v yielding orange solid. ^{1}H NMR (500 MHz, CD_3OD) δ 8.20 (bs, 1H), 8.19 – 8.12 (m, 2H), 7.72 (bs, 1H), 7.18 (s, 1H), 7.13 (s, 1H), 6.84 (s, 2H), 6.82 – 6.76 (m, 2H), 6.71 – 6.64 (m, 2H), 6.56 (s, 1H), 6.24 (bs, 1H), 6.02 (s, 1H), 4.93 (bs, 1H), 4.68 – 4.55 (m, 2H), 4.55 – 4.44 (m 3H), 4.41 (bs, 1H), 4.23 (bs, 2H), 4.13 (d, *J* = 11.1 Hz, 2H), 3.82 (s, 2H), 3.71 – 3.57 (m, 2H), 3.50 (bs, 2H), 3.43 (bs, 2H), 3.34 – 3.29 (m, 4H), 3.27 (d, *J* = 10.7, 1H), 3.10 (bs, 2H), 2.93 – 2.78 (m, 2H), 2.69 – 2.44 (m, 9H), 2.58 (s, 6H), 2.42 – 2.31(m, 3H), 2.27 (s, 3H), 2.21 (s, 3H), 2.16 – 1.70 (m, 6H), 1.89 (s, 3H), 1.41 (s, 3H), 1.38 (s, 3H), 1.36 (s, 3H), 1.33 – 1.21 (m, 2H), 1.26 (bs, 3H), 1.17 (s, 3H), 1.19 – 1.06 (m, 1H), 0.45 (s, 3H). ^{13}C NMR (126 MHz, CD_3OD) 181.6, 180.1, 177.6, 177.4, 176.7, 175.6, 175.5, 175.4, 174.6, 174.3, 169.8, 167.2, 166.9, 143.3, 138.3, 135.7, 134.1, 131.3, 131.1, 130.6, 117.9, 115.3, 112.4, 112.2, 108.7, 105.2, 103.6, 95.6, 88.3, 86.4, 81.3, 76.3, 75.4, 74.6, 71.3, 71.2, 70.8, 70.5, 70.2, 64.1, 60.3, 57.7, 57.0, 55.2, 52.5, 51.7, 46.6, 44.0, 43.0, 41.8, 40.1, 36.2, 33.5, 33.2, 33.0, 32.3, 29.5, 27.5, 27.4, 20.9, 20.53, 20.45, 20.3, 20.0, 19.9, 17.5, 17.0, 16.4, 16.1. HRMS (ESI) m/z $[M + 2Na]^{2+}$ calcd for $C_{94}H_{115}CoN_{19}O_{23}PNa_2$, 1006.8634; found, 1006.8627.

HPLC Method:

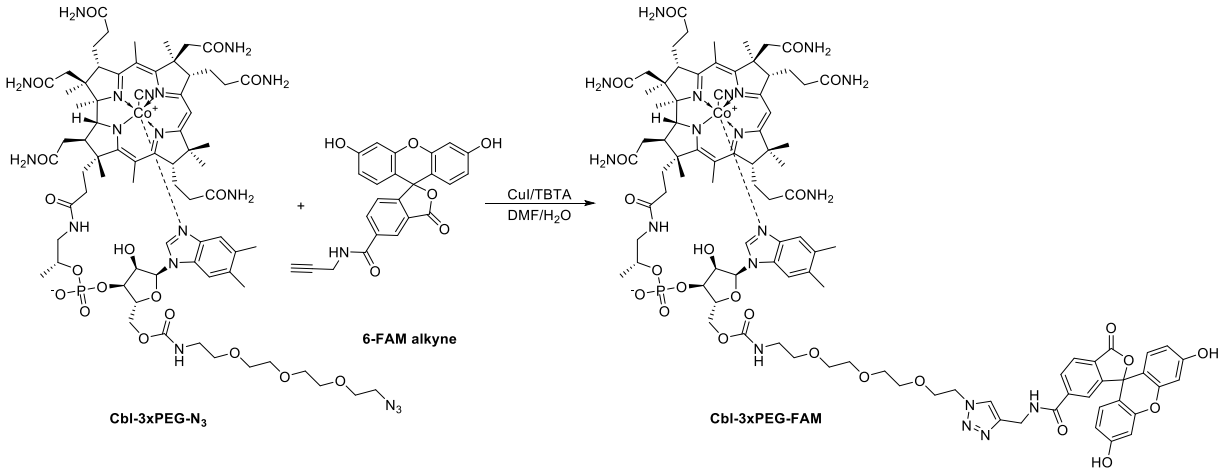
Time [min]	H_2O+0.5‰TFA [%]	MeCN [%]	λ [nm]	R_t [min]
Initial	99	1	361	11.87
15	30	70		



Result Table (Uncal - C:\Users\admin\Desktop\HPLC\Cbl-2xPEG-FAM - S 2850: Czanel 1)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	11,867	3308,287	237,538	100,0	100,0	0,22	951
	Total	3308,287	237,538	100,0	100,0		

3.5 Cbl-3xPEG-FAM

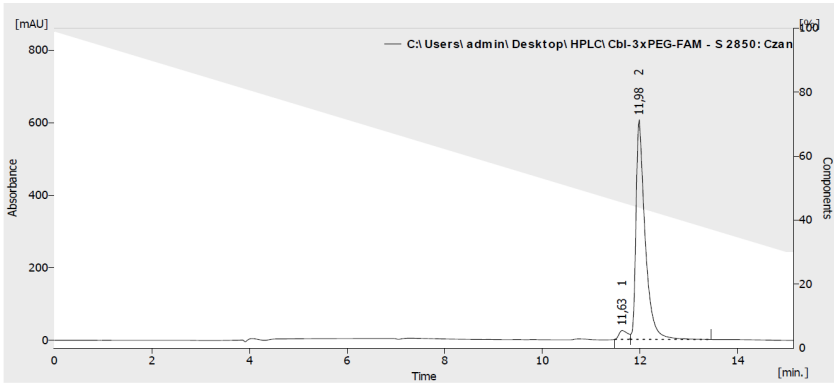


Cbl-3xPEG-FAM: CuI (1 mg, 5 µmol) and TBTA (5 mg, 10 µmol) were dissolved in DMF/H_2O (1 mL, 3:1 v/v) and stirred for 20 min. Subsequently Cbl-3xPEG-N_3 (28 mg, 17.5 µmol) and 6-FAM alkyne (6 mg, 14.5 µmol) were added and the reaction mixture was stirred overnight. Then it was diluted with DMF (1 mL), poured into AcOEt (15 mL) and the precipitate was centrifuged and dried. The crude solid was dissolved in MeOH (1 mL), precipitated with Et_2O (15 mL), and then centrifuged. The dried solid was then dissolved in H_2O, loaded onto RP column (30 mL) and purified gradually with MeCN/H_2O from 10 to 20% v/v yielding orange solid. ^{1}H NMR (500 MHz, CD_3OD) δ 8.25 (bs, 1H), 8.11 (s, 1H), 7.75 (bs, 2H), 7.20(s, 1H), 7.14 (s, 1H), 6.70 (s, 2H), 6.61 (s, 2H), 6.58 – 6.45 (m, 3H), 6.23 (bs, 1H), 6.02 (s, 1H), 4.65 – 4.44 (m, 5H), 4.32 – 4.17 (m, 3H), 4.17 – 4.06 (m, 2H), 3.87 (bs, 2H), 3.70 – 3.65 (m, 2H), 3.65 – 3.58 (m, 2H), 3.52 – 3.36 (m, 12H), 3.28 (m, 1H), 3.25 – 3.14 (m, 2H), 2.95 – 2.82 (m, 2H), 2.71 – 2.41(m, 8H), 2.58 (s, 6H), 2.42 – 2.32 (m, 2H), 2.28 (s, 3H), 2.22 (s, 3H), 2.17 – 1.79 (m, 6H), 1.88 (s, 3H), 1.79 – 1.70 (m, 1H), 1.42(s, 3H), 1.38 (s, 3H), 1.36 (s, 3H), 1.33 – 1.20 (m, 5H), 1.17 (s, 3H), 1.14-1.05 (m, 1H), 0.46 (s, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ 181.6, 180.2, 177.6, 177.4, 176.6, 175.2, 175.3, 167.2, 166.9, 154.0, 143.4, 138.3, 135.7, 134.0,

131.4, 130.3, 117.9, 112.5, 110.9, 108.7, 105.2, 103.7, 95.6, 86.4, 76.4, 71.4, 71.37, 71.32, 71.2, 71.1, 70.9, 60.3, 57.7, 56.9, 55.2, 52.5, 51.9, 43.0, 41.8, 40.1, 33.3, 32.3, 29.5, 27.5, 27.4, 20.9, 20.6, 20.6, 20.3, 19.9, 17.5, 17.1, 16.4, 16.1. HRMS (ESI) m/z $[M + H + Na]^{2+}$ calcd for $C_{96}H_{120}CoN_{19}O_{24}PNa$, 1017.8855; found, 1017.8862.

HPLC Method:

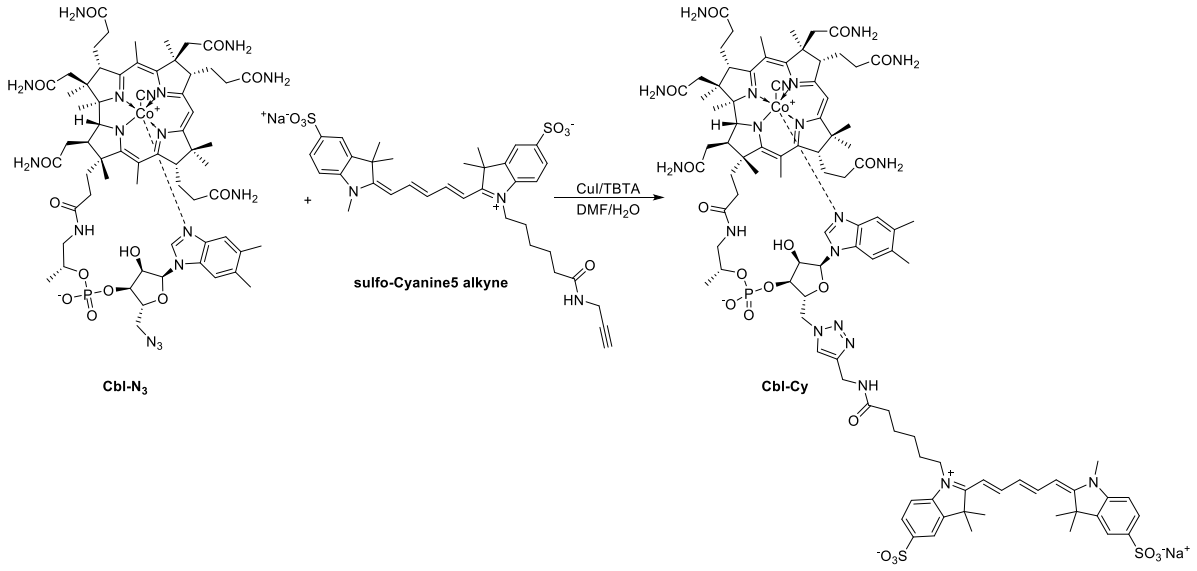
Time [min]	H_2O+0.5‰TFA [%]	MeCN [%]	λ [nm]	R_t [min]
Initial	99	1	361	11.98
15	30	70		



Result Table (Uncal - C:\Users\admin\Desktop\HPLC\Cbl-3xPEG-FAM - S 2850: Czanel 1)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	11,633	290,747	24,181	3,6	3,8	0,25	568
2	11,983	7871,066	605,778	96,4	96,2	0,20	568
	Total	8161,813	629,959	100,0	100,0		

3.6 Cbl-Cy5

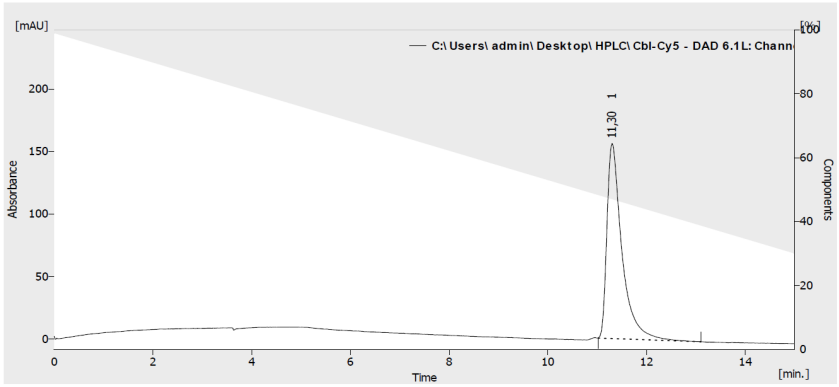


Cbl-Cy5: Preparation of a catalyst solution: CuI (1 mg, 5 μmol) and TBTA (5 mg, 10 μmol) were dissolved in DMF (2 mL) and stirred for 20 min. Cbl-N_3 (3 mg, 2.20 μmol) and sulfo-Cyanine5 alkyne (0.5 mg, 0.72 μmol) were dissolved in DMF/H_2O (200 μL, 1:1, v/v) and subsequently freshly prepared catalyst solution (300 μL) was added and the reaction mixture was stirred overnight. Then it was diluted with DMF (1 mL), poured into AcOEt (10 mL) and the precipitate was centrifuged and dried. The crude solid was dissolved in MeOH (1 mL), precipitated with Et_2O (10 mL), and then centrifuged. The dried solid was then dissolved in H_2O, loaded onto RP column (10 mL) and purified gradually with MeCN/H_2O from 10 to 20%

v/v yielding blue solid. HRMS (ESI) m/z [M + 2Na]$^{2+}$ calcd for $C_{98}H_{127}CoN_{20}O_{20}PS_2Na_3$, 1063.3864; found, 1063.3871.

HPLC Method:

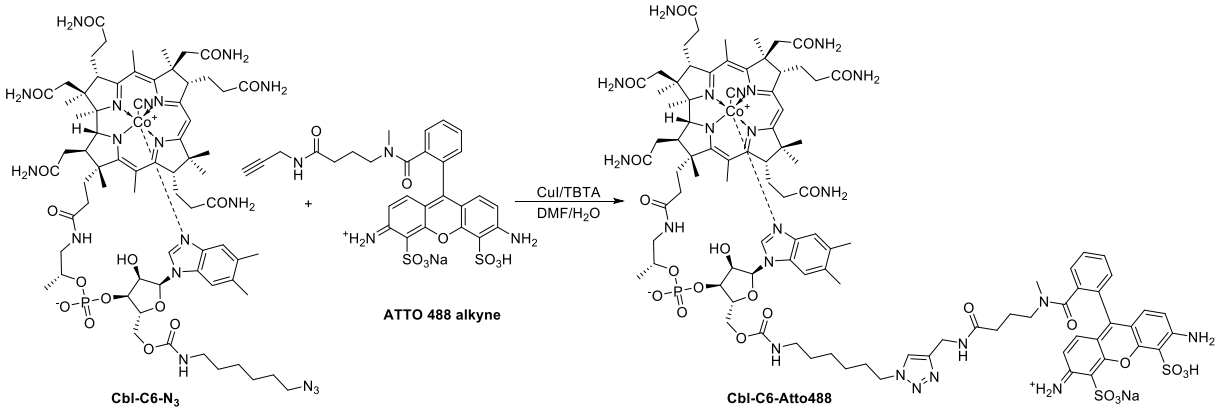
Time [min]	H_2O+0.5‰TFA [%]	MeCN [%]	λ [nm]	R_t [min]
Initial	99	1	646	11.30
15	30	70		



Result Table (Uncal - C:\Users\admin\Desktop\HPLC\Cbl-Cy5 - DAD 6.1L: Channel 3)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	11,300	3283,150	155,931	100,0	100,0	0,30	613
	Total	3283,150	155,931	100,0	100,0		

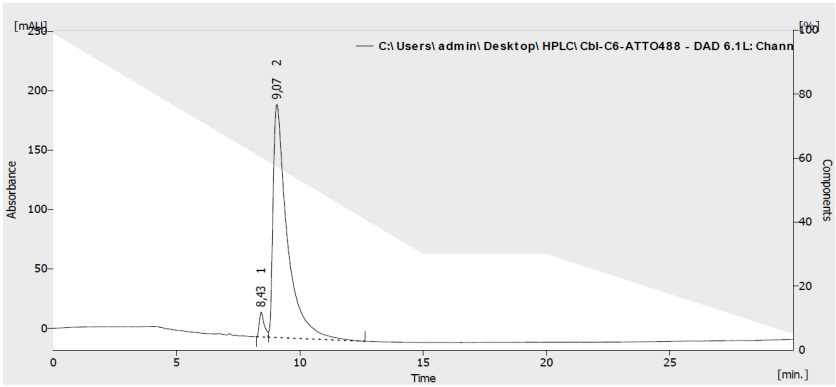
3.7 Cbl-C6-ATTO 488



Cbl-C6-ATTO 488: Preparation of a catalyst solution: CuI (1 mg, 5 µmol) and TBTA (5 mg, 10 µmol) were dissolved in DMF (2 mL) and stirred for 20 min. Cbl-C6-N_3 (3 mg, 1.97 µmol) and ATTO 488 alkyne (0.5 mg, 0.68 µmol) were dissolved in DMF/H_2O (200 µL, 1:1, v/v) and subsequently freshly prepared catalyst solution (300 µL) was added and the reaction mixture was stirred overnight. Then it was diluted with DMF (1 mL), poured into AcOEt (10 mL) and the precipitate was centrifuged and dried. The crude solid was dissolved in MeOH (1 mL), precipitated with Et_2O (10 mL), and then centrifuged. The dried solid was then dissolved in H_2O, loaded onto RP column (10 mL) and purified gradually with MeCN/H_2O from 10 to 20% v/v yielding orange solid. HRMS (ESI) m/z [M + Na]$^{2+}$ calcd for $C_{98}H_{126}CoN_{22}O_{24}PS_2Na_2^+$, 1097.3805; found, 1097.3818.

HPLC Method:

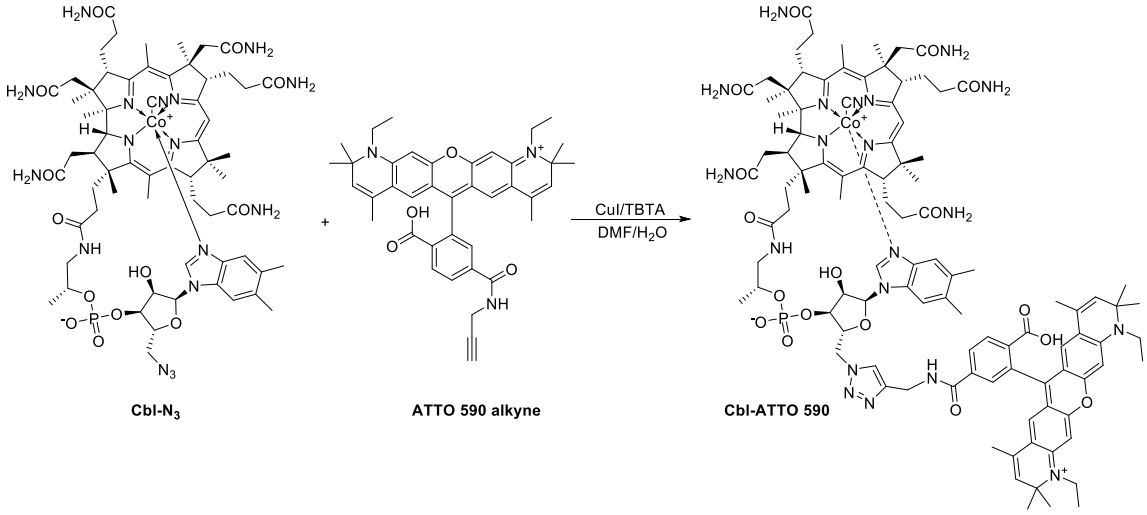
Time [min]	H_2O+0.2‰TFA [%]	MeCN [%]	λ [nm]	R_t [min]
Initial	99	1	488	9.07
15	30	70		
20	30	70		
30	5	95		



Result Table (Uncal - C:\Users\admin\Desktop\HPLC\Cbl-C6-ATTO488 - DAD 6.1L: Channel 3)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	8,433	287,697	20,925	3,5	9,6	0,22	729
2	9,067	7857,586	196,321	96,5	90,4	0,55	480
	Total	8145,283	217,246	100,0	100,0		

3.8 Cbl-ATTO 590

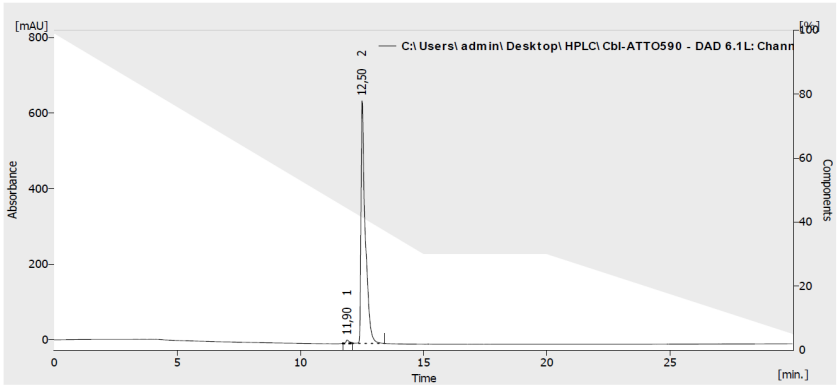


Cbl-ATTO 590: Preparation of a catalyst solution: CuI (1 mg, 5 µmol) and TBTA (5 mg, 10 µmol) were dissolved in DMF (2 mL) and stirred for 20 min. Cbl-N_3 (3 mg, 2.20 µmol) and ATTO 590 alkyne (0.5 mg, 0.68 µmol) were dissolved in DMF/H_2O (200 µL, 1:1, v/v) and subsequently freshly prepared catalyst solution (300 µL) was added and the reaction mixture was stirred overnight. Then it was diluted with DMF (1 mL), poured into AcOEt (10 mL) and the precipitate was centrifuged and dried. The crude solid was dissolved in MeOH (1 mL), precipitated with Et_2O (10 mL), and then centrifuged. The dried solid was then dissolved in H_2O, loaded onto RP column (10 mL) and purified gradually with MeCN/H_2O from 15 to 30%

v/v yielding violet solid. HRMS (ESI) m/z $[M + H]^{2+}$ calcd for $C_{103}H_{130}CoN_{20}O_{17}P^+$, 1004.4491; found, 1004.4499.

HPLC Method:

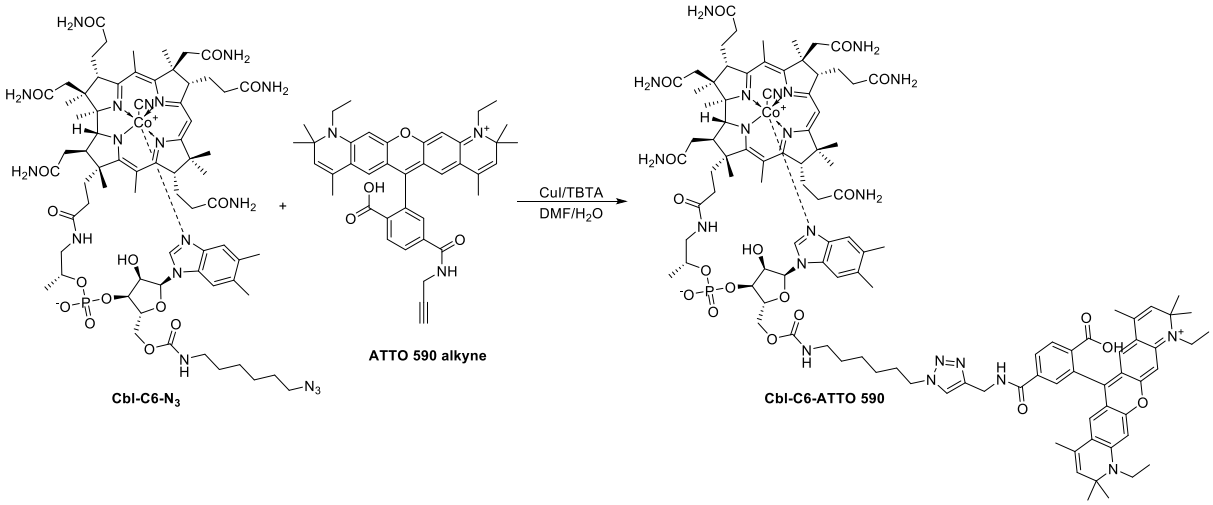
Time [min]	H_2O+0.2‰TFA [%]	MeCN [%]	λ [nm]	R_t [min]
Initial	99	1	590	12.50
15	30	70		
20	30	70		
30	5	95		



Result Table (Uncal - C:\Users\admin\Desktop\HPLC\Cbl-ATTO590 - DAD 6.1L: Channel 3)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	11,900	88,896	9,427	1,1	1,4	0,15	989
2	12,500	7728,290	641,767	98,9	98,6	0,17	413
	Total	7817,186	651,194	100,0	100,0		

3.9 Cbl-C6-ATTO 590

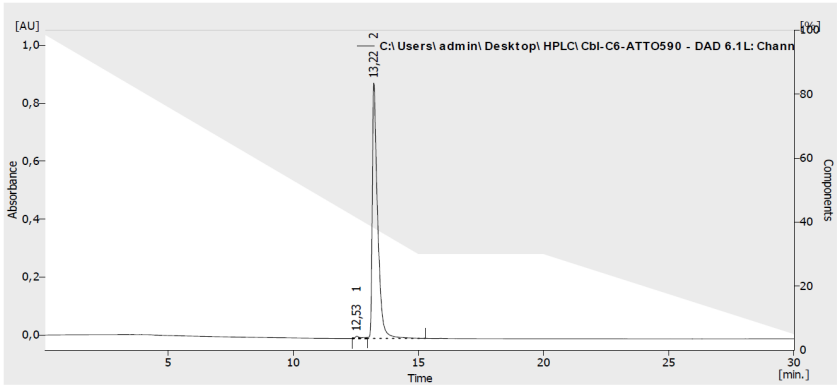


Cbl-C6-ATTO 590: Preparation of a catalyst solution: CuI (1 mg, 5 µmol) and TBTA (5 mg, 10 µmol) were dissolved in DMF (2 mL) and stirred for 20 min. Cbl-C6-N_3 (3 mg, 1.97 µmol) and ATTO 590 alkyne (0.5 mg, 0.68 µmol) were dissolved in DMF/H_2O (200 µL, 1:1, v/v) and subsequently freshly prepared catalyst solution (300 µL) was added and the reaction mixture was stirred overnight. Then it was diluted with DMF (1 mL), poured into AcOEt (10 mL) and the precipitate was centrifuged and dried. The crude solid was dissolved in MeOH (1 mL), precipitated with Et_2O (10 mL), and then centrifuged. The dried solid was then dissolved in H_2O, loaded onto RP column (10 mL) and purified gradually with MeCN/H_2O from 15 to 40%

v/v yielding violet solid. HRMS (ESI) m/z $[M + H]^{2+}$ calcd for $C_{110}H_{143}CoN_{21}O_{19}P^+$, 1075.9964; found, 1075.9967.

HPLC Method:

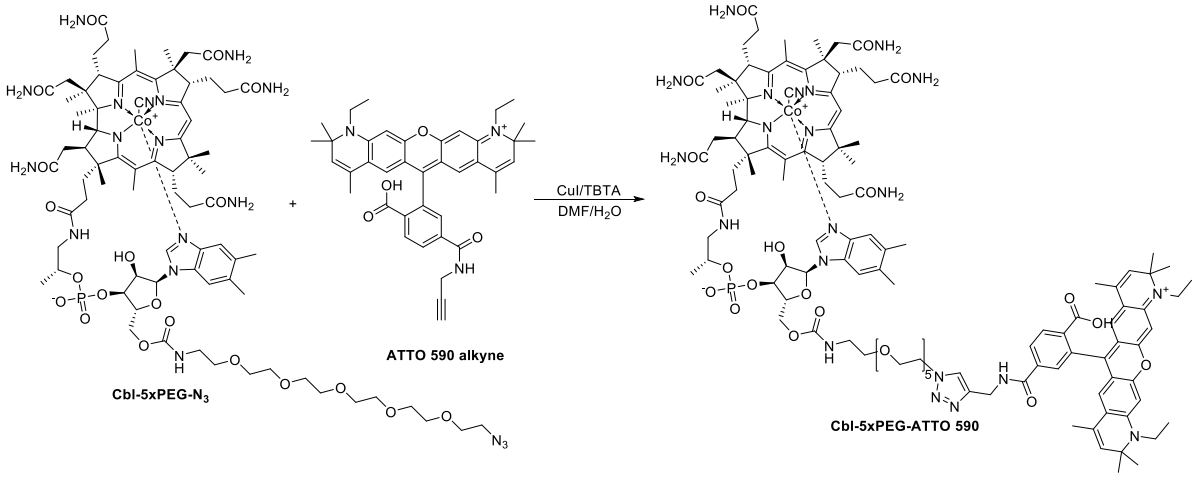
Time [min]	H_2O+0.2‰TFA [%]	MeCN [%]	λ [nm]	R_t [min]
Initial	99	1	590	13.22
15	30	70		
20	30	70		
30	5	95		



Result Table (Uncal - C:\Users\admin\Desktop\HPLC\Cbl-C6-ATTO590 - DAD 6.1L: Channel 3)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	12,533	113,565	7,654	0,9	0,9	0,18	994
2	13,217	13048,298	881,392	99,1	99,1	0,22	152
	Total	13161,864	889,046	100,0	100,0		

3.10 Cbl-5xPEG-ATTO 590

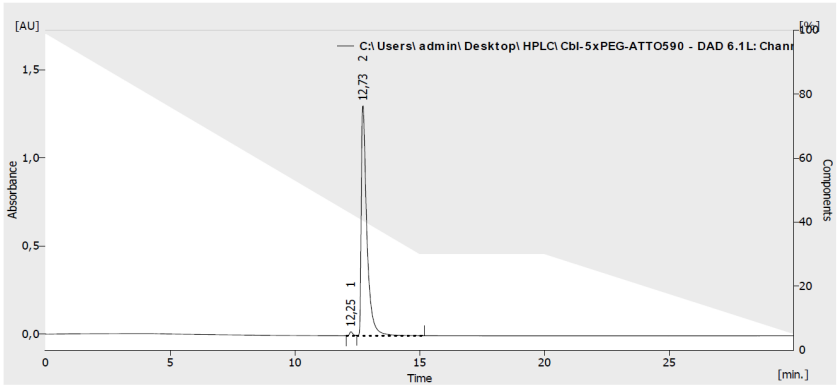


Cbl-5xPEG-ATTO 590: Preparation of a catalyst solution: CuI (1 mg, 5 µmol) and TBTA (5 mg, 10 µmol) were dissolved in DMF (2 mL) and stirred for 20 min. Cbl-5xPEG-N_3 (3 mg, 1.78 µmol) and ATTO 590 alkyne (0.5 mg, 0.68 µmol) were dissolved in DMF/H_2O (200 µL, 1:1, v/v) and subsequently freshly prepared catalyst solution (300 µL) was added and the reaction mixture was stirred overnight. Then it was diluted with DMF (1 mL), poured into AcOEt (10 mL) and the precipitate was centrifuged and dried. The crude solid was dissolved in MeOH (1 mL), precipitated with Et_2O (10 mL), and then centrifuged. The dried solid was then dissolved in H_2O, loaded onto RP column (10 mL) and purified gradually with MeCN/H_2O from 15 to 30%

v/v yielding violet solid. HRMS (ESI) m/z [M + Na]$^{2+}$ calcd for $C_{116}H_{154}CoN_{21}O_{24}PNa^{+}$, 1169.0216; found, 1169.0219.

HPLC Method:

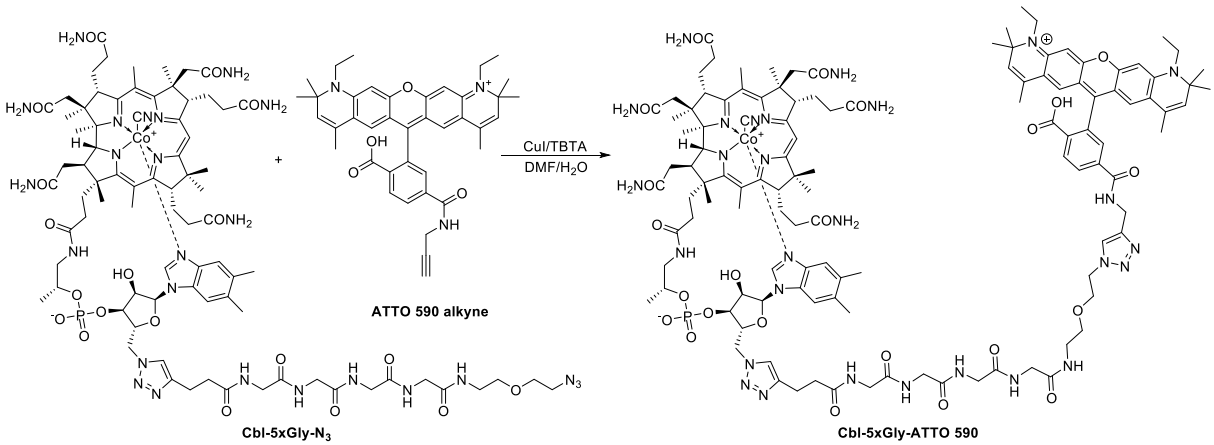
Time [min]	H_2O+0.5‰TFA [%]	MeCN [%]	λ [nm]	R_t [min]
Initial	99	1	590	12.73
15	30	70		
20	30	70		
30	5	95		



Result Table (Uncal - C:\Users\admin\Desktop\HPLC\Cbl-5xPEG-ATTO590 - DAD 6.1L: Channel 3)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	12,250	207,748	23,311	1,0	1,8	0,15	899
2	12,733	20468,553	1304,040	99,0	98,2	0,23	346
	Total	20676,302	1327,351	100,0	100,0		

3.11 Cbl-5xGly-ATTO 590

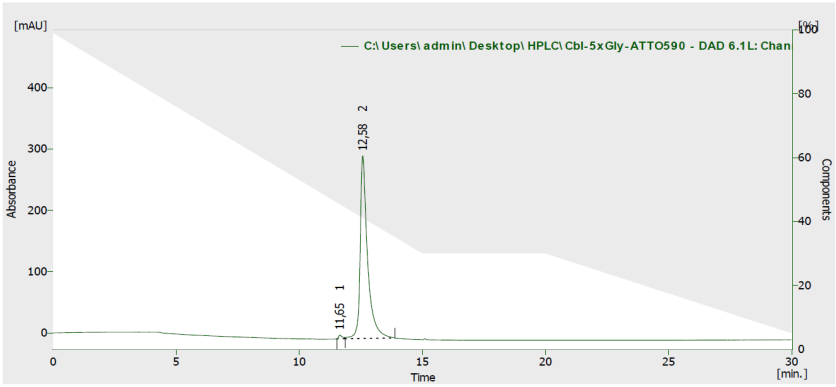


Cbl-5xGly-ATTO 590: Preparation of a catalyst solution: CuI (1 mg, 5 µmol) and TBTA (5 mg, 10 µmol) were dissolved in DMF (2 mL) and stirred for 20 min. Cbl-5xGly-N_3 (4 mg, 2.20 µmol) and ATTO 590 alkyne (0.5 mg, 0.68 µmol) were dissolved in DMF/H_2O (400 µL, 3:1, v/v) and subsequently freshly prepared catalyst solution (300 µL) was added and the reaction mixture was stirred overnight. Then it was diluted with DMF (1 mL), poured into AcOEt (10 mL) and the precipitate was centrifuged and dried. The crude solid was dissolved in MeOH (1 mL), precipitated with Et_2O (10 mL), and then centrifuged. The dried solid was then dissolved in

H_2O, loaded onto RP column (10 mL) and purified gradually with MeCN/H_2O from 15 to 40% v/v yielding violet solid. HRMS (ESI) m/z $[M + H]^{2+}$ calcd for $C_{120}H_{156}CoN_{28}O_{23}P^+$, 2447.0957; found, 2447.0932.

HPLC Method:

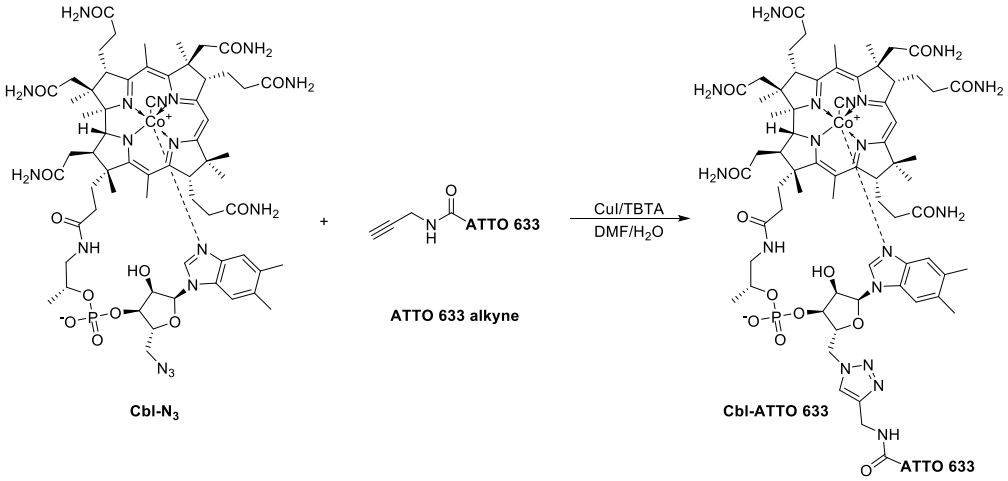
Time [min]	H_2O+0.5‰TFA [%]	MeCN [%]	λ [nm]	R_t [min]
Initial	99	1	590	12.58
15	30	70		
20	30	70		
30	5	95		



Result Table (Uncal - C:\Users\admin\Desktop\HPLC\Cbl-5xGly-ATTO590 - DAD 6.1L: Channel 3)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	11,650	61,377	6,124	1,0	2,0	0,17	998
2	12,583	6236,030	297,715	99,0	98,0	0,28	345
	Total	6297,407	303,838	100,0	100,0		

3.12 Cbl-ATTO 633

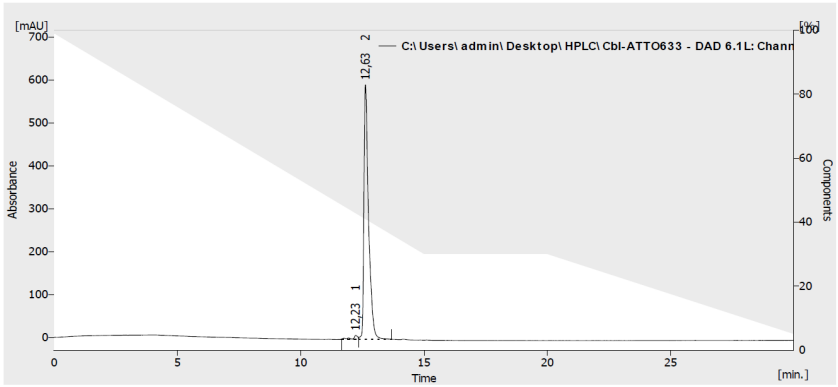


Cbl-ATTO 633: Preparation of a catalyst solution: CuI (1 mg, 5 µmol) and TBTA (5 mg, 10 µmol) were dissolved in DMF (2 mL) and stirred for 20 min. Cbl-N_3 (3 mg, 2.20 µmol) and ATTO 633 alkyne (0.5 mg, 0.72 µmol) were dissolved in DMF/H_2O (200 µL, 1:1, v/v) and and subsequently freshly prepared catalyst solution (300 µL) was added and the reaction mixture was stirred overnight. Then it was diluted with DMF (1 mL), poured into AcOEt (10 mL) and the precipitate was centrifuged and dried. The crude solid was dissolved in MeOH (1 mL), precipitated with Et_2O (10 mL), and then centrifuged. The dried solid was then dissolved in

H_2O, loaded onto RP column (10 mL) and purified by RP column chromatography gradually with MeCN/H_2O from 15 to 40% v/v yielding blue solid. LRMS (ESI) m/z $[M + Na + H]^{2+}$ found, 995.96.

HPLC Method:

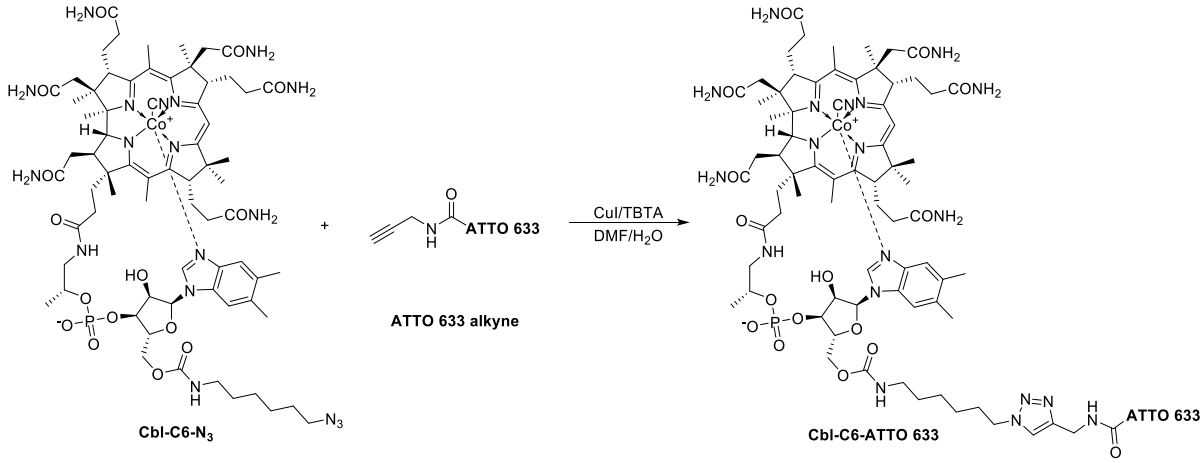
Time [min]	H_2O+0.2‰TFA [%]	MeCN [%]	λ [nm]	R_t [min]
Initial	99	1	633	12.63
15	30	70		
20	30	70		
30	5	95		



Result Table (Uncal - C:\Users\admin\Desktop\HPLC\Cbl-ATTO633 - DAD 6.1L: Channel 3)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	12,233	139,633	9,482	1,8	1,6	0,18	855
2	12,633	7783,171	592,556	98,2	98,4	0,20	508
	Total	7922,804	602,037	100,0	100,0		

3.13 Cbl-C6-ATTO 633

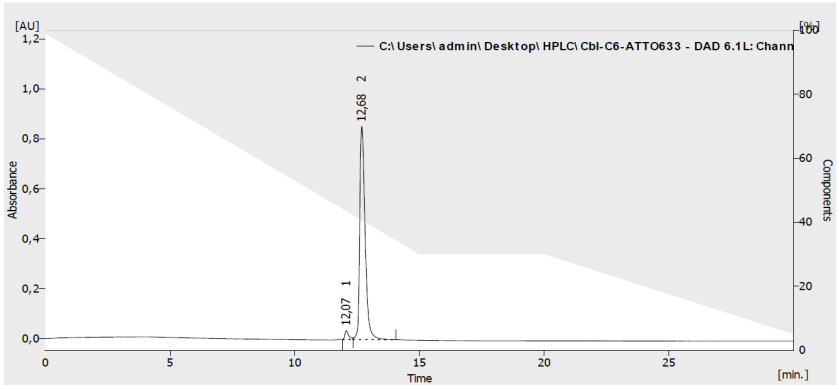


Cbl-C6-ATTO 633: Preparation of a catalyst solution: CuI (1 mg, 5 µmol) and TBTA (5 mg, 10 µmol) were dissolved in DMF (2 mL) and stirred for 20 min. Cbl-C6-N_3 (3 mg, 1.97 µmol) and ATTO 633 alkyne (0.5 mg, 0.72 µmol) were dissolved in DMF/H_2O (200 µL, 1:1, v/v) and subsequently freshly prepared catalyst solution (300 µL) was added and the reaction mixture was stirred overnight. Then it was diluted with DMF (1 mL), poured into AcOEt (10 mL) and the precipitate was centrifuged and dried. The crude solid was dissolved in MeOH (1 mL), precipitated with Et_2O (10 mL), and then centrifuged. The dried solid was then dissolved in H_2O, loaded onto RP column (10 mL) and purified crude product was purified by RP column

chromatography gradually with MeCN/H_2O from 20 to 50% v/v yielding blue solid. LRMS (ESI) m/z $[M + Na + H]^{2+}$ found, 1067.51.

HPLC Method:

Time [min]	H_2O+0.2‰TFA [%]	MeCN[%]	λ [nm]	R_t [min]
Initial	99	1	633	12.68
15	30	70		
20	30	70		
30	5	95		

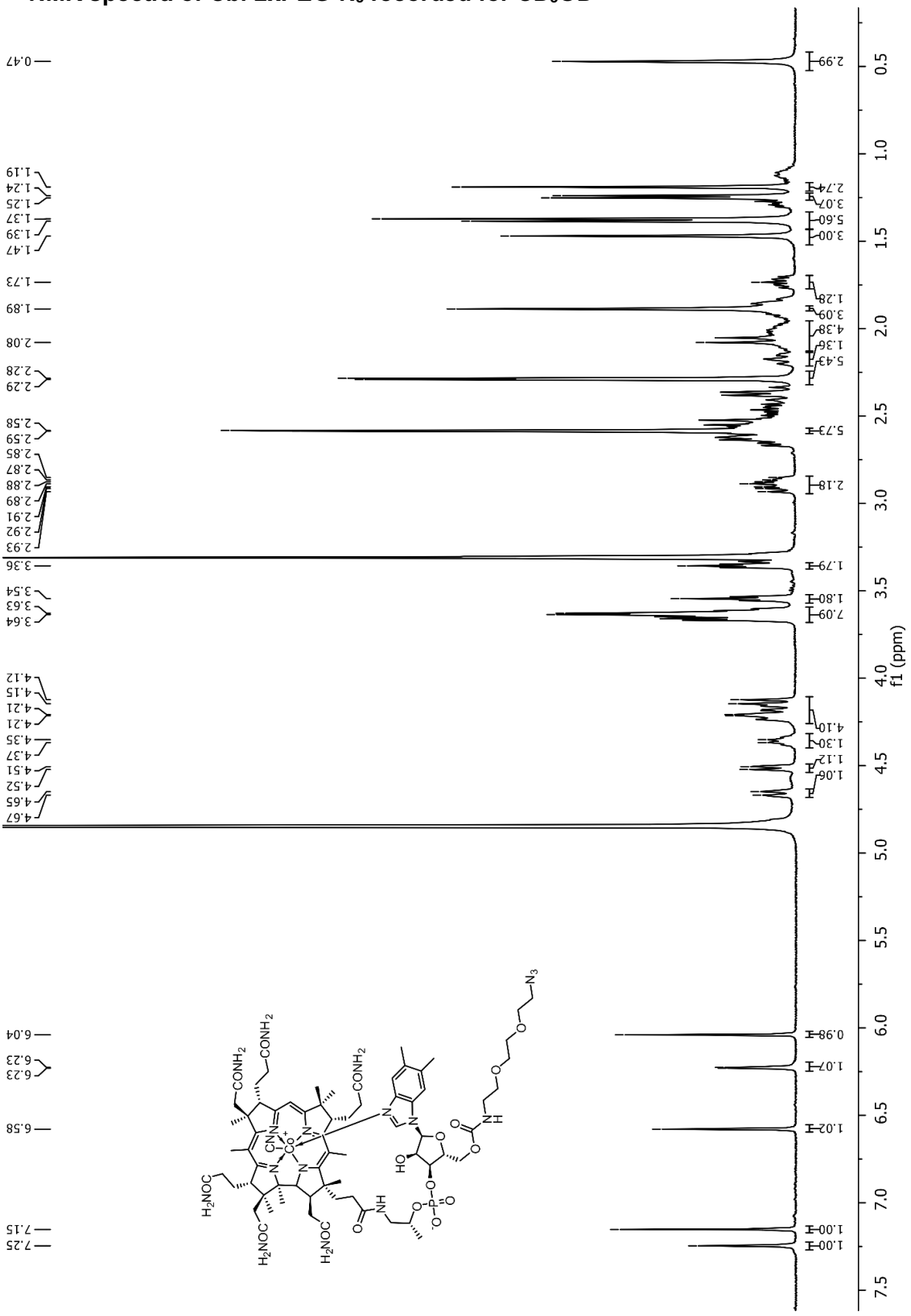


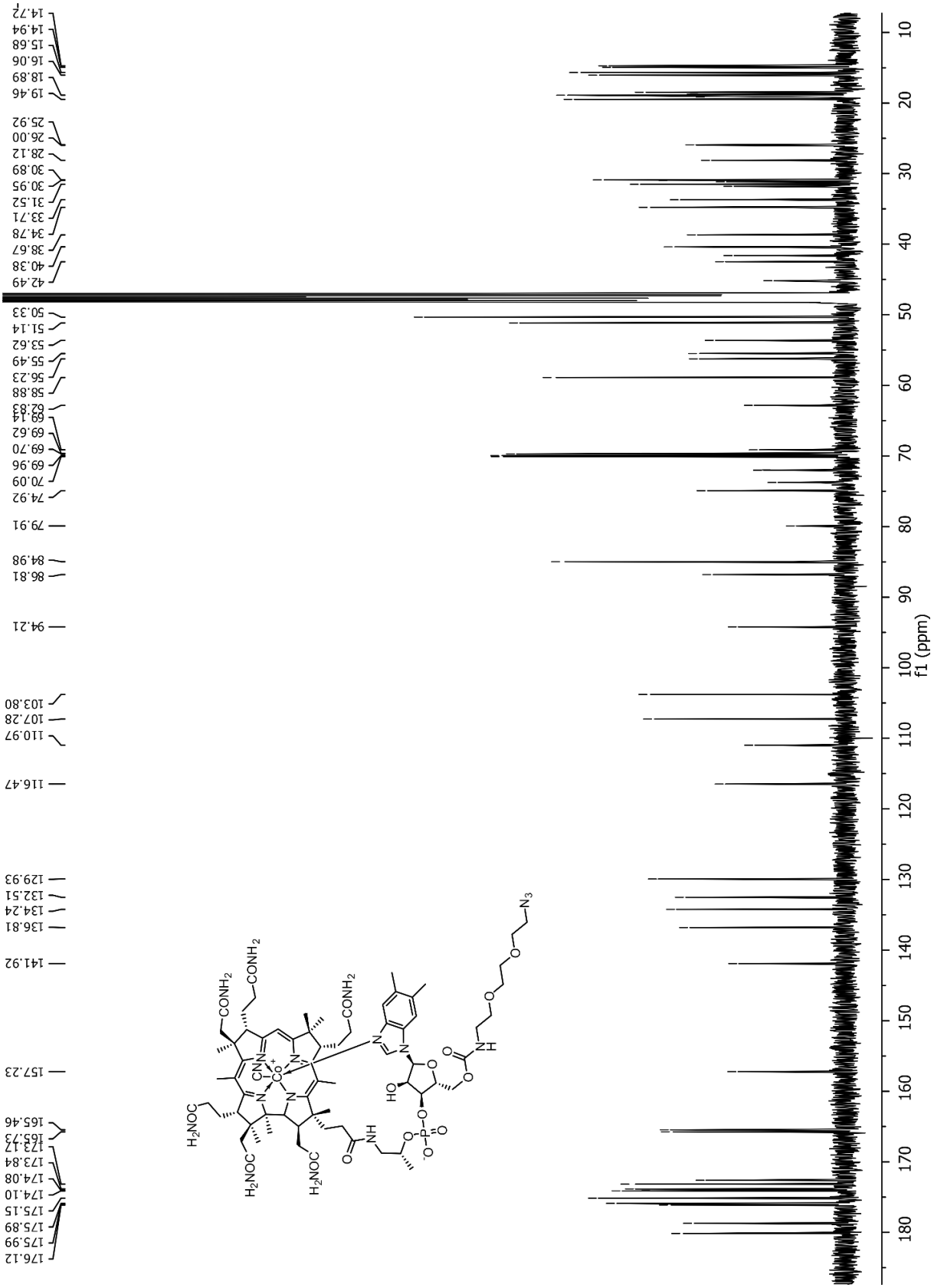
Result Table (Uncal - C:\Users\admin\Desktop\HPLC\Cbl-C6-ATTO633 - DAD 6.1L: Channel 3)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	12,067	387,217	36,352	2,9	4,1	0,17	873
2	12,683	12923,830	854,169	97,1	95,9	0,25	518
	Total	13311,047	890,520	100,0	100,0		

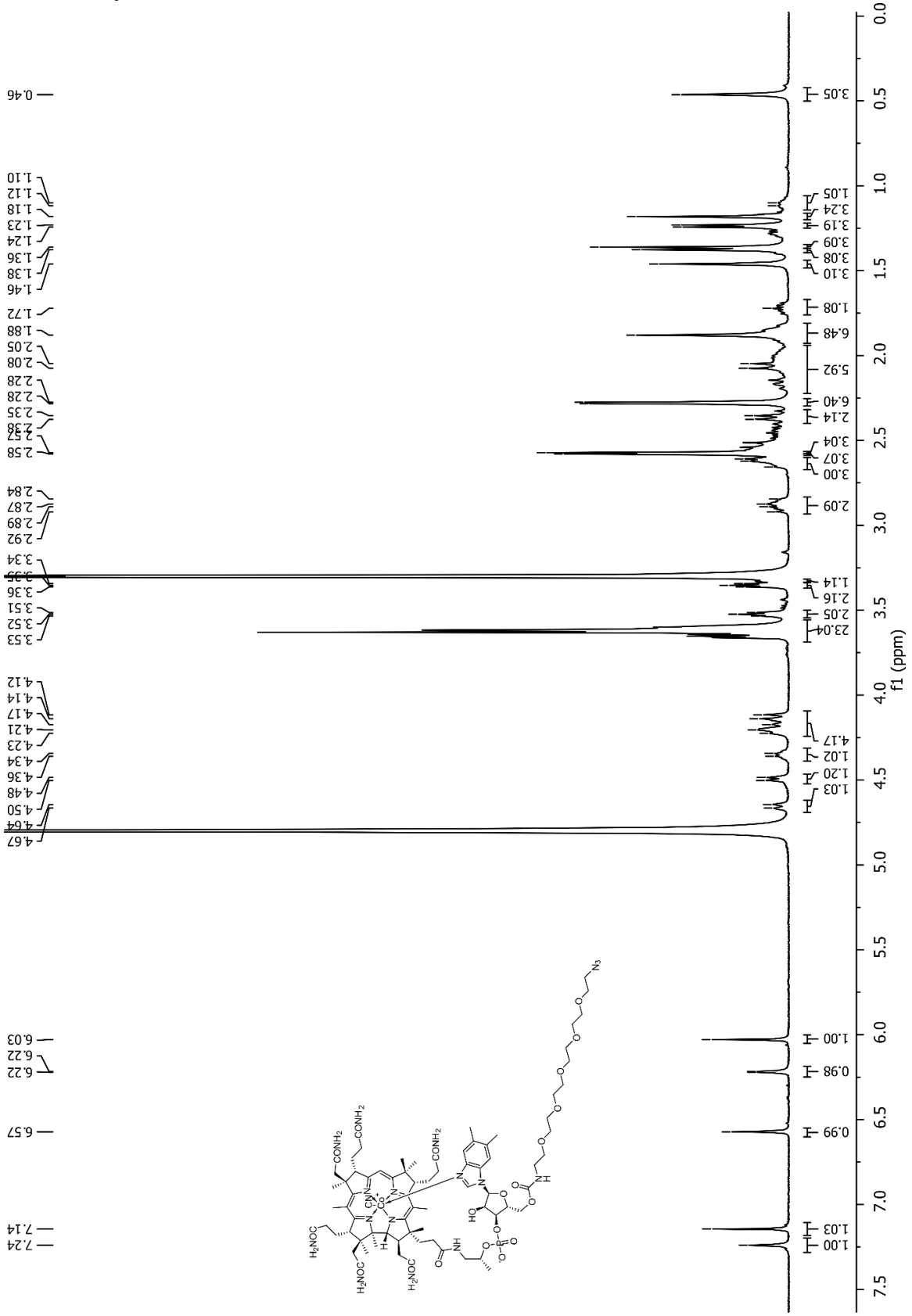
4. NMR spectra

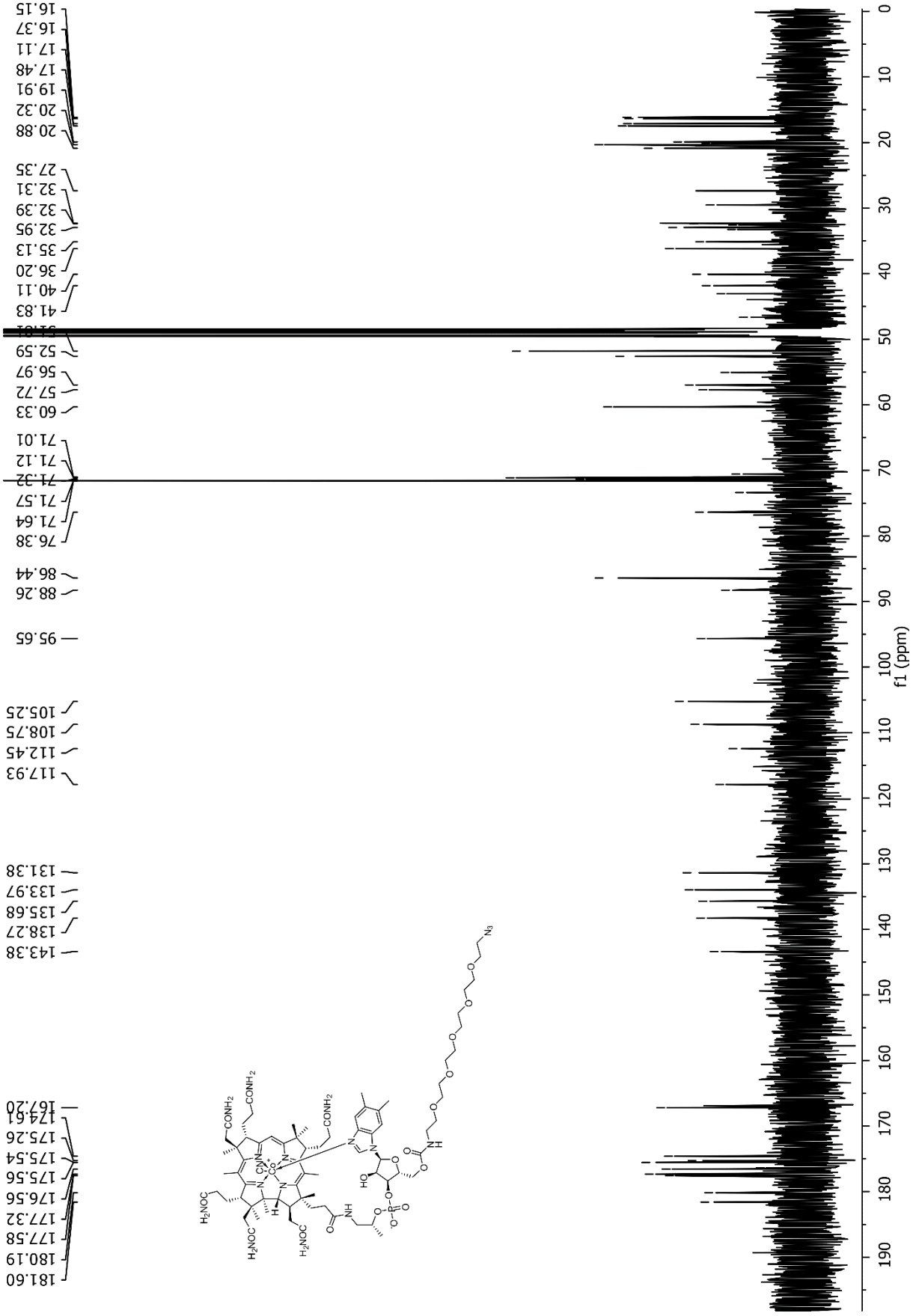
4.1 NMR spectra of CbI-2xPEG-N_3 recorded for CD_3OD




14.72
14.94
15.68
16.06
18.89
19.46
25.92
26.00
28.12
30.89
30.95
31.52
33.71
34.78
38.67
40.38
42.49
50.33
51.14
53.62
55.49
56.23
58.88
62.83
69.14
69.62
69.70
69.96
70.09
74.92
79.91
84.98
86.81
94.21
103.80
107.28
110.97
116.47
129.93
132.51
134.24
136.81
141.92
157.23
165.46
165.73
173.17
173.84
174.08
174.10
175.15
175.89
175.99
176.12
f1 (ppm)

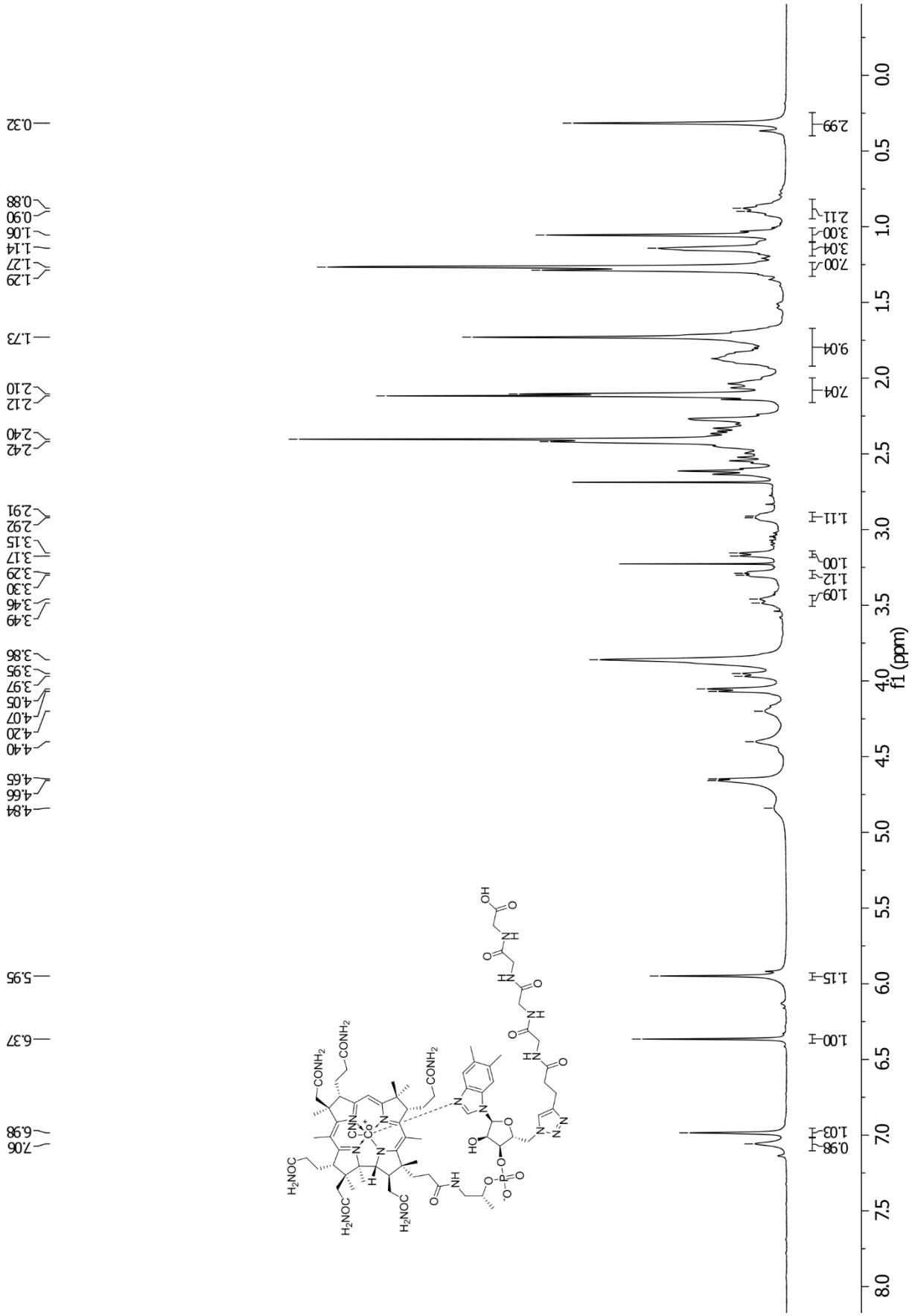
4.2 NMR spectra of Cbl-5xPEG-N_3 recorded for CD_3OD



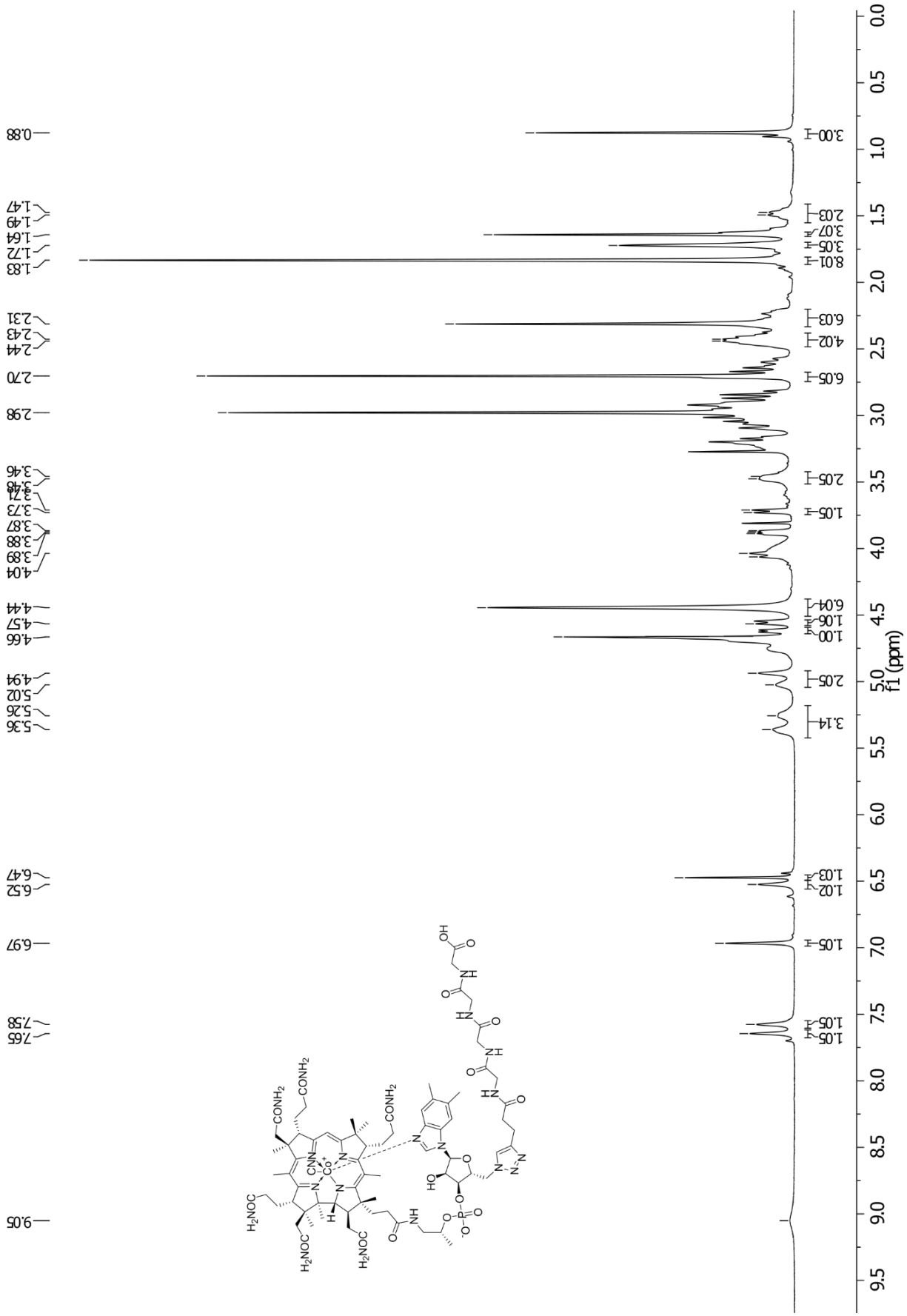

181.60
180.19
177.58
177.32
176.56
175.56
175.54
175.26
174.61
167.20
143.38
138.27
135.68
133.97
131.38
117.93
112.45
108.75
105.25
95.65
88.26
86.44
76.38
71.64
71.57
71.32
71.12
71.01
60.33
57.72
56.97
52.59
51.81
41.83
40.11
36.20
35.13
32.95
32.39
32.31
27.35
20.88
20.32
19.91
17.48
17.11
16.37
16.15
f1 (ppm)

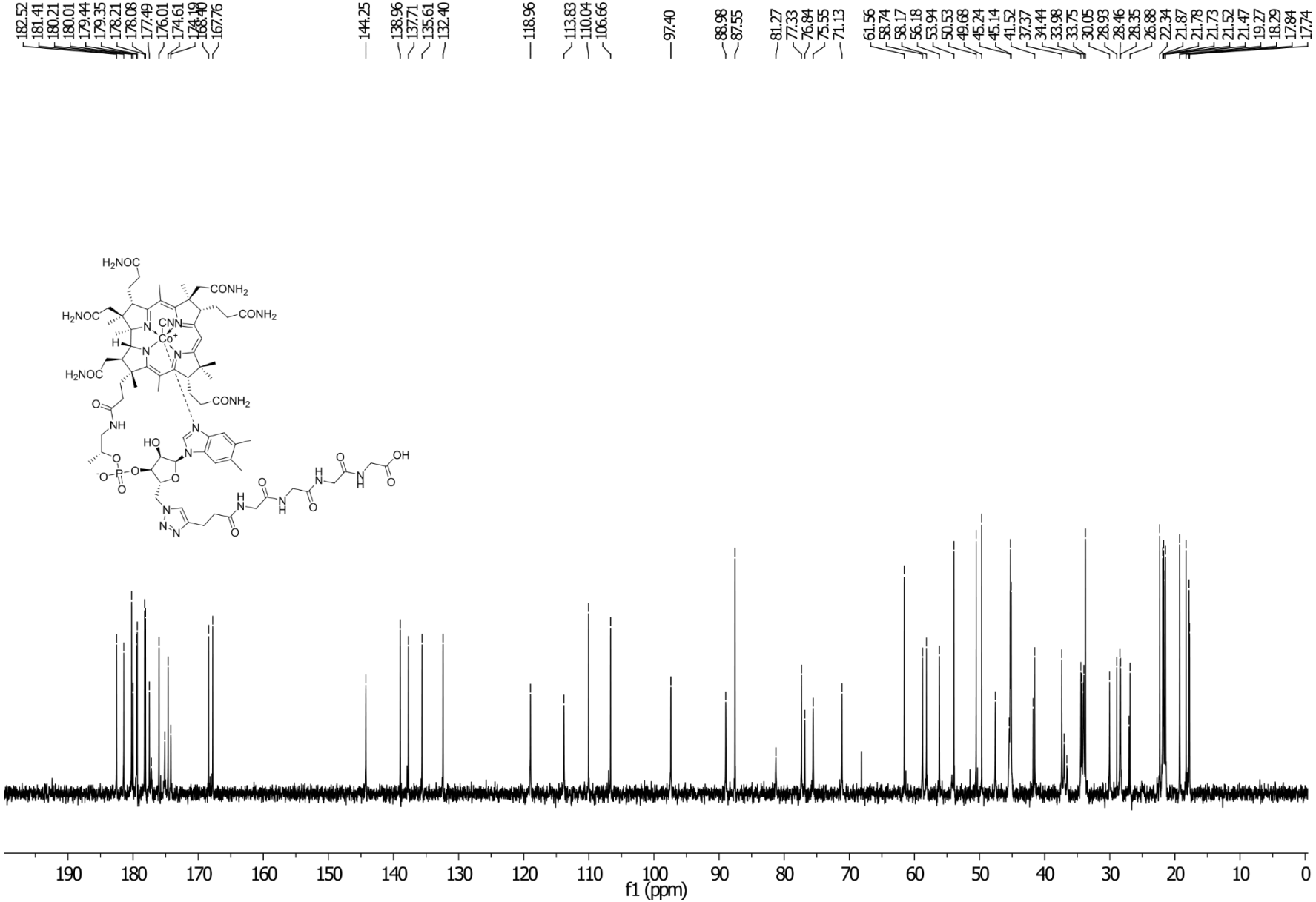
4.3 NMR spectra of Cbl-4xGly-OH recorded for D_2O

Note: Water signal was suppressed using presaturation (presat)

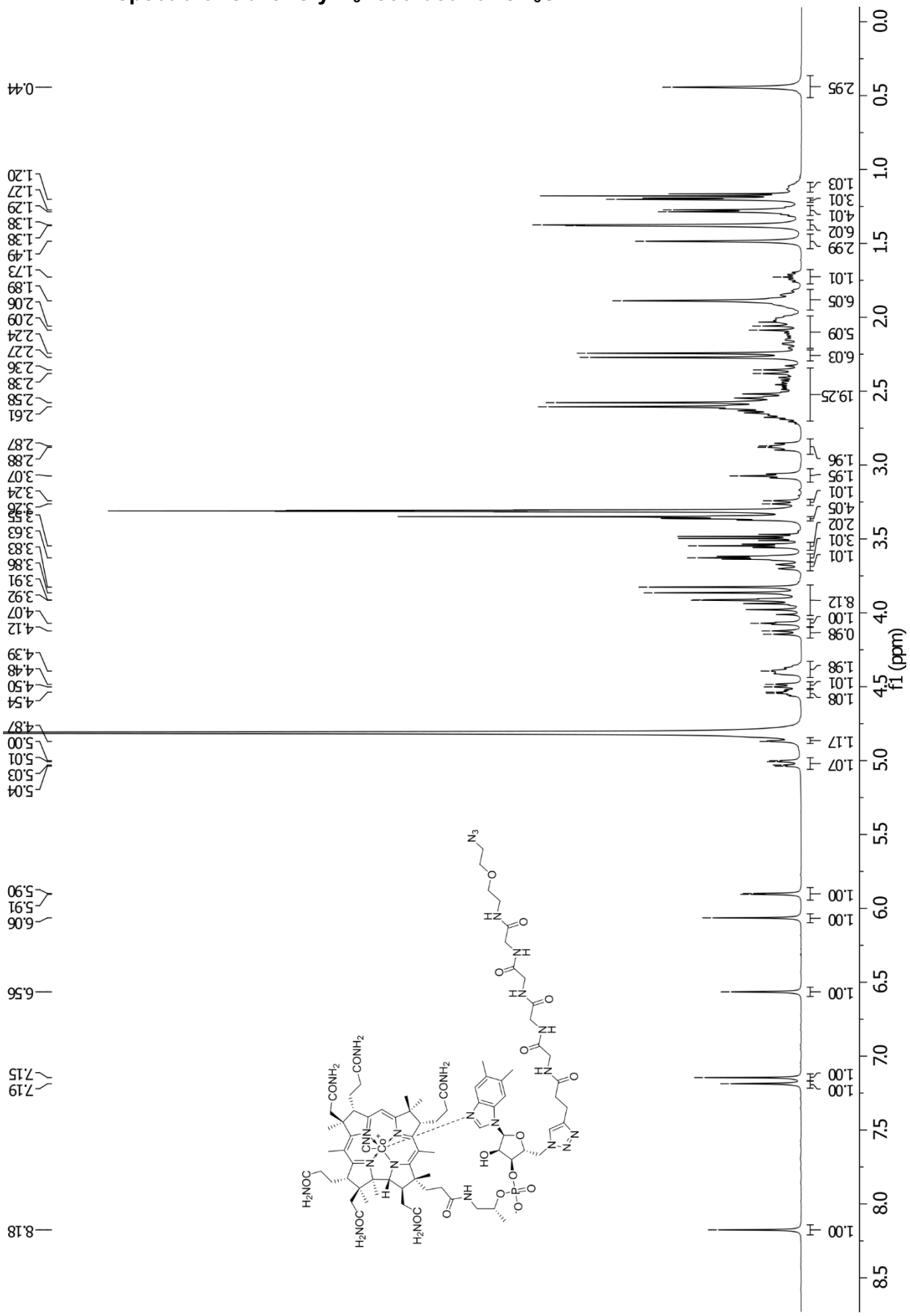


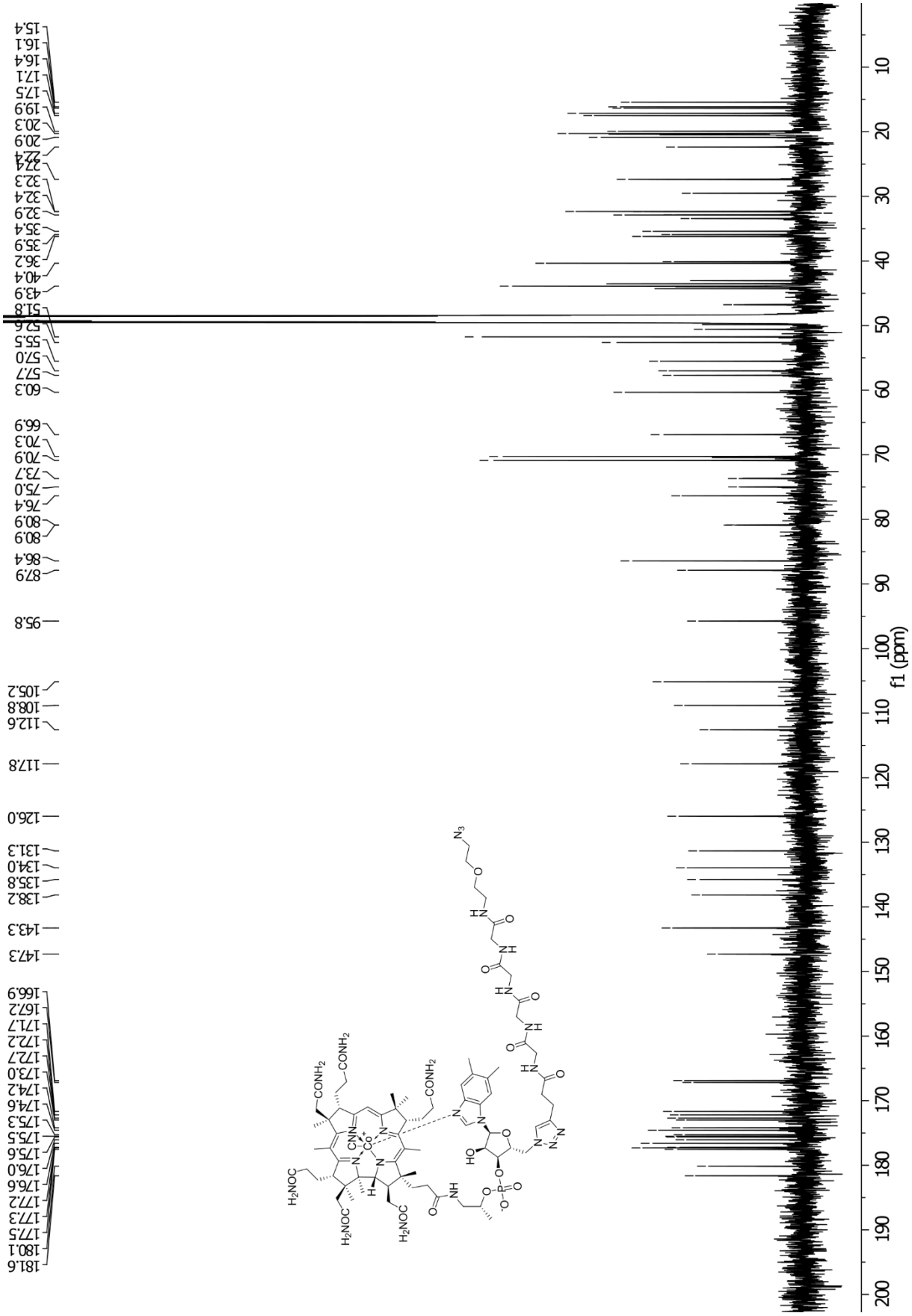
Note: Water signal was suppressed using presaturation, the spectrum was recorded at 80°C



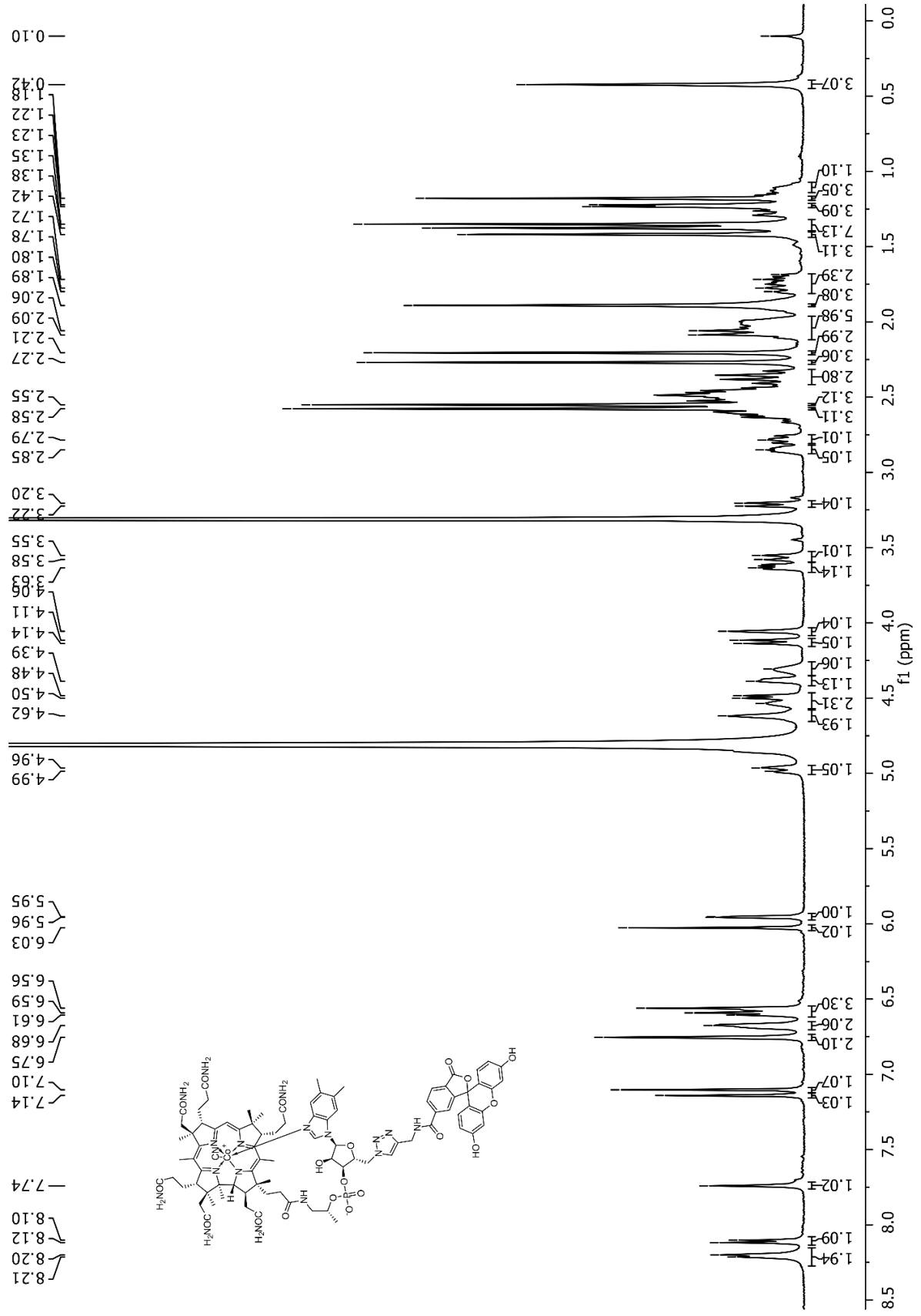

182.52
181.41
180.21
180.01
179.44
179.35
178.21
178.08
177.49
176.01
174.61
144.25
138.96
137.71
135.61
132.40
118.96
113.83
110.04
106.66
97.40
88.98
87.55
81.27
77.33
76.84
75.55
71.13
61.56
58.74
58.17
56.18
53.94
50.53
49.68
45.24
45.14
41.52
37.37
34.44
33.98
33.75
30.05
28.93
28.46
28.35
26.88
22.34
21.87
21.78
21.73
21.52
21.47
19.27
18.29
17.84
17.74
H2NOC
CONH2
CNN
Co+
HO
OH
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

4.4 NMR spectra of Cbl-5xGly-N_3 recorded for CD_3OD



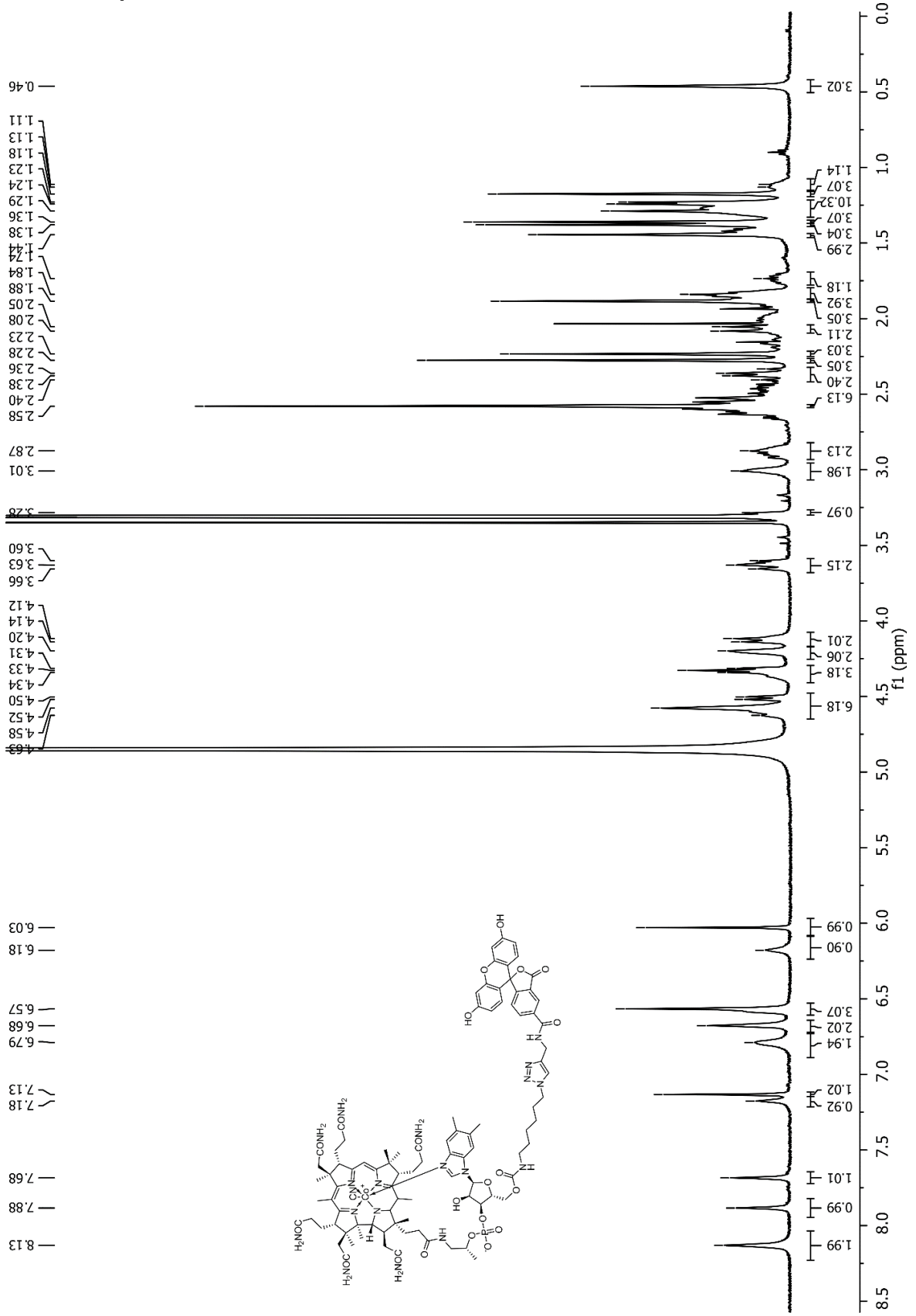

181.6
180.1
177.5
177.3
177.2
176.6
176.0
175.6
175.5
175.3
174.6
174.2
173.0
172.7
172.2
171.7
167.2
166.9
147.3
143.3
138.2
135.8
134.0
131.3
126.0
117.8
112.6
108.8
105.2
95.8
87.9
86.4
80.9
80.9
76.4
75.0
73.7
70.9
70.3
66.9
60.3
57.7
57.0
55.5
52.6
51.8
43.9
40.4
36.2
35.9
35.4
32.9
32.4
32.3
22.4
20.9
20.3
19.9
17.5
17.1
16.4
16.1
15.4
f1 (ppm)

4.5 NMR spectra of Cbl-FAM recorded for CD_3OD



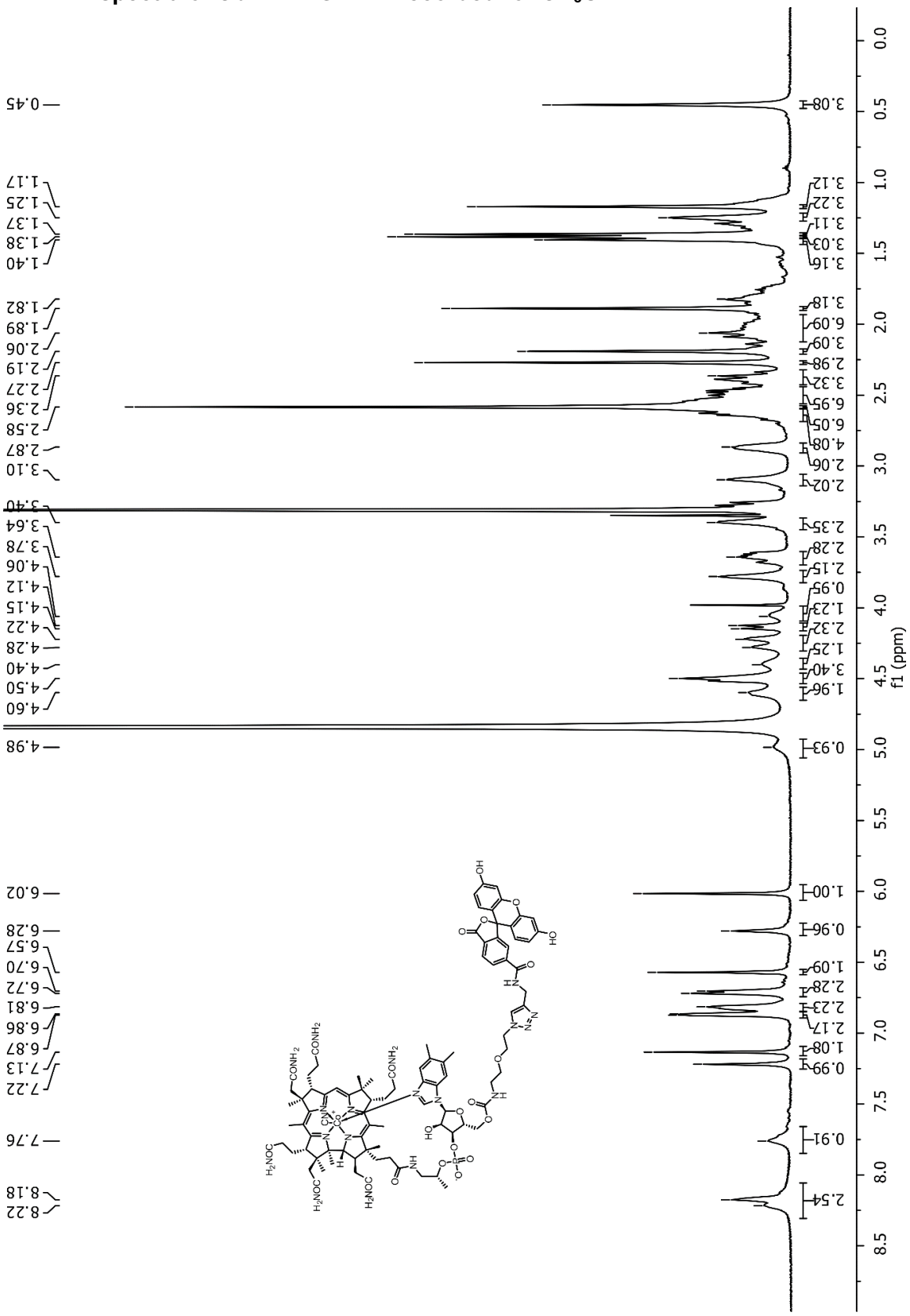
16.19
16.38
17.06
17.48
19.91
20.26
20.87
27.37
29.27
32.29
32.38
32.93
33.54
35.42
36.17
52.62
55.41
56.97
57.65
60.32
70.38
74.09
75.83
76.33
81.18
86.44
87.88
95.71
103.63
103.66
105.13
108.80
112.44
114.46
117.86
130.63
131.30
134.00
135.79
138.18
143.17
166.91
167.18
174.60
175.29
175.50
175.64
176.55
177.20
177.35
177.60
181.64
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
f1 (ppm)

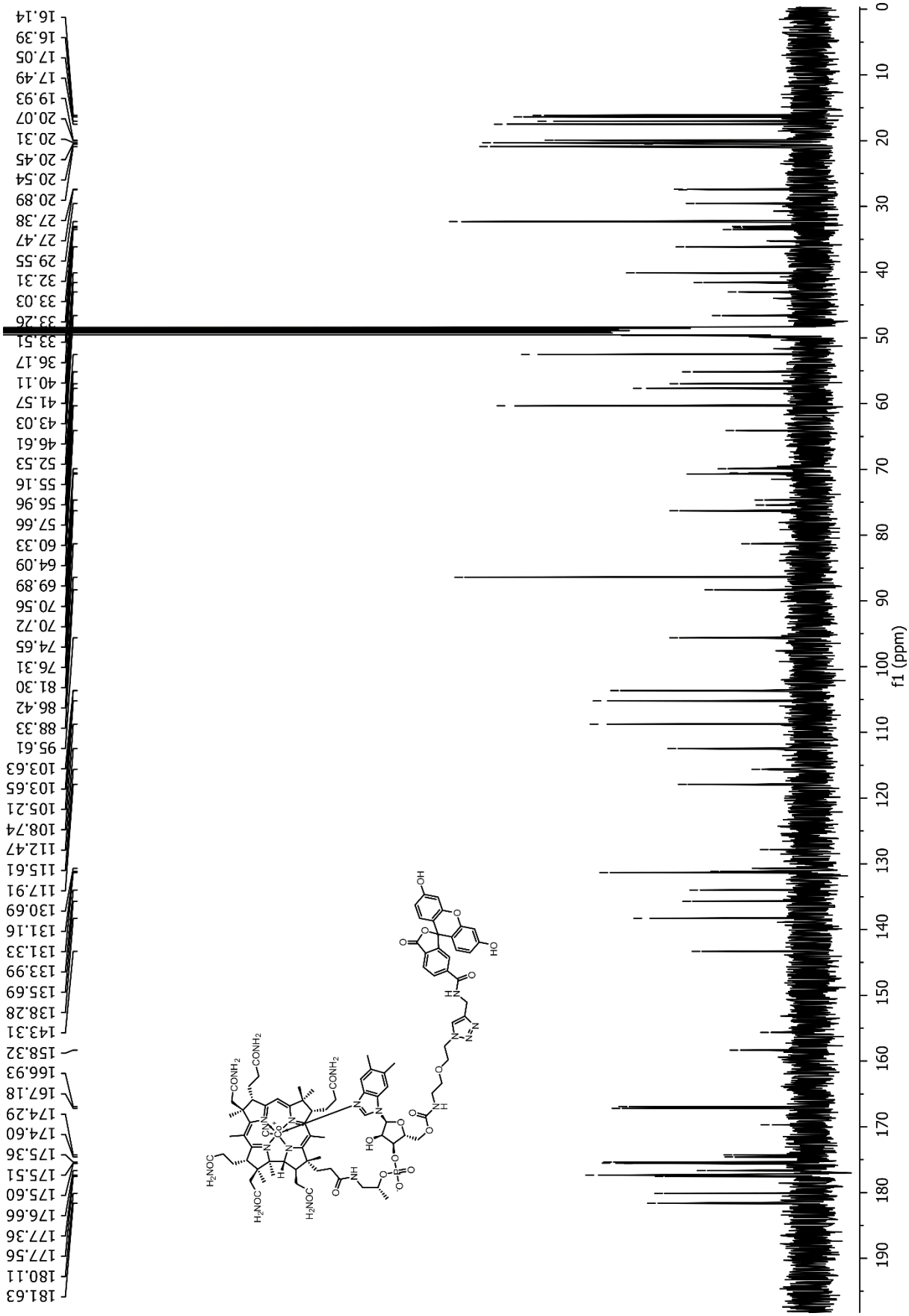
4.6 NMR spectra of Cbl-C6-FAM recorded for CD_3OD



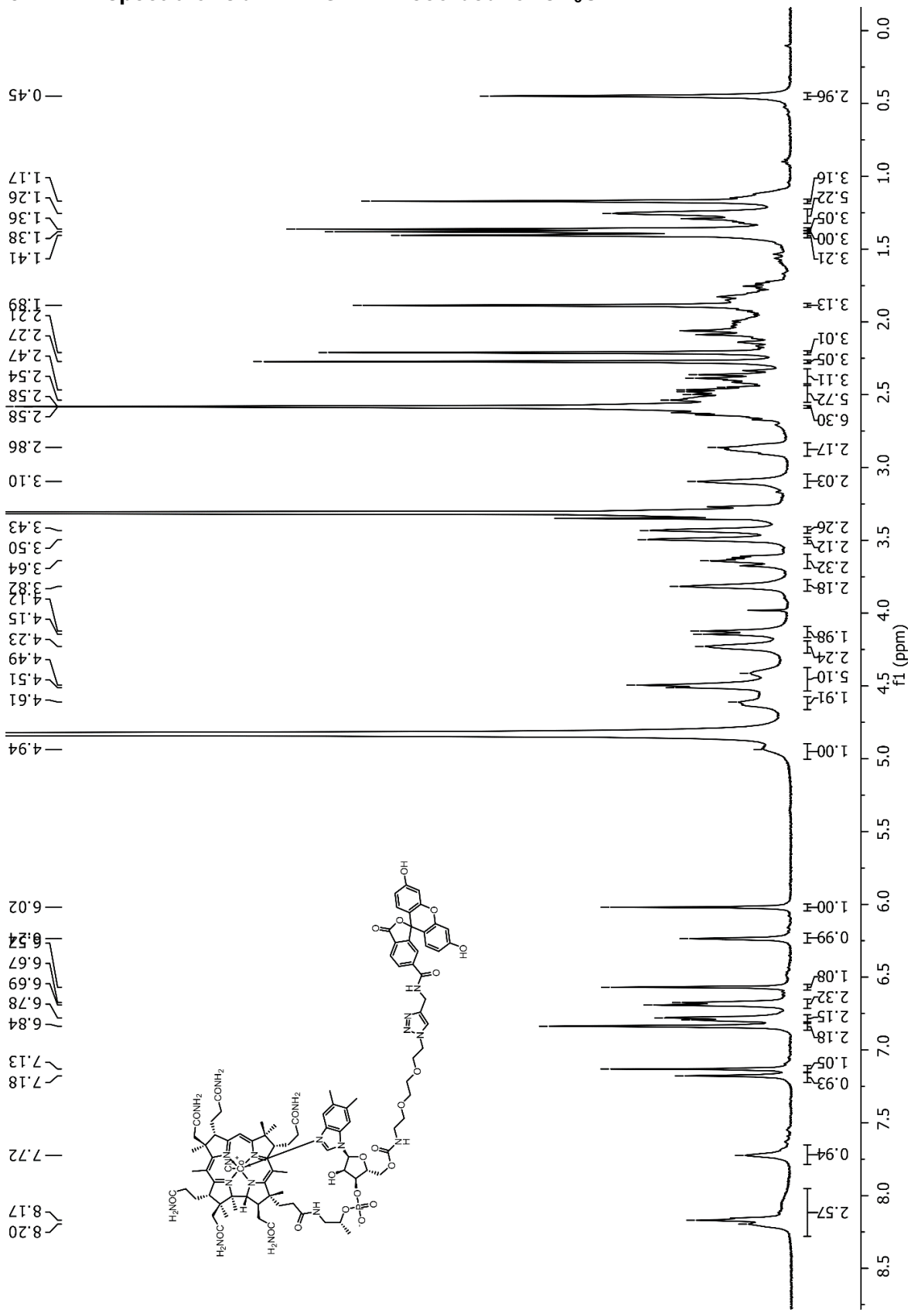
16.15
16.38
17.11
17.48
19.92
20.17
20.35
20.54
20.91
26.97
27.02
27.42
29.53
30.57
30.77
30.99
32.32
32.38
32.66
33.07
33.28
35.15
36.20
36.28
40.09
41.57
43.03
43.94
46.62
49.85
51.25
52.56
55.02
56.92
57.65
60.32
70.51
76.35
81.41
86.41
88.22
95.64
103.83
105.21
108.71
112.40
117.88
131.34
133.91
135.63
138.24
143.34
166.88
167.17
174.07
174.61
175.30
175.50
175.54
176.66
177.35
177.56
180.12
181.56
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
f1 (ppm)

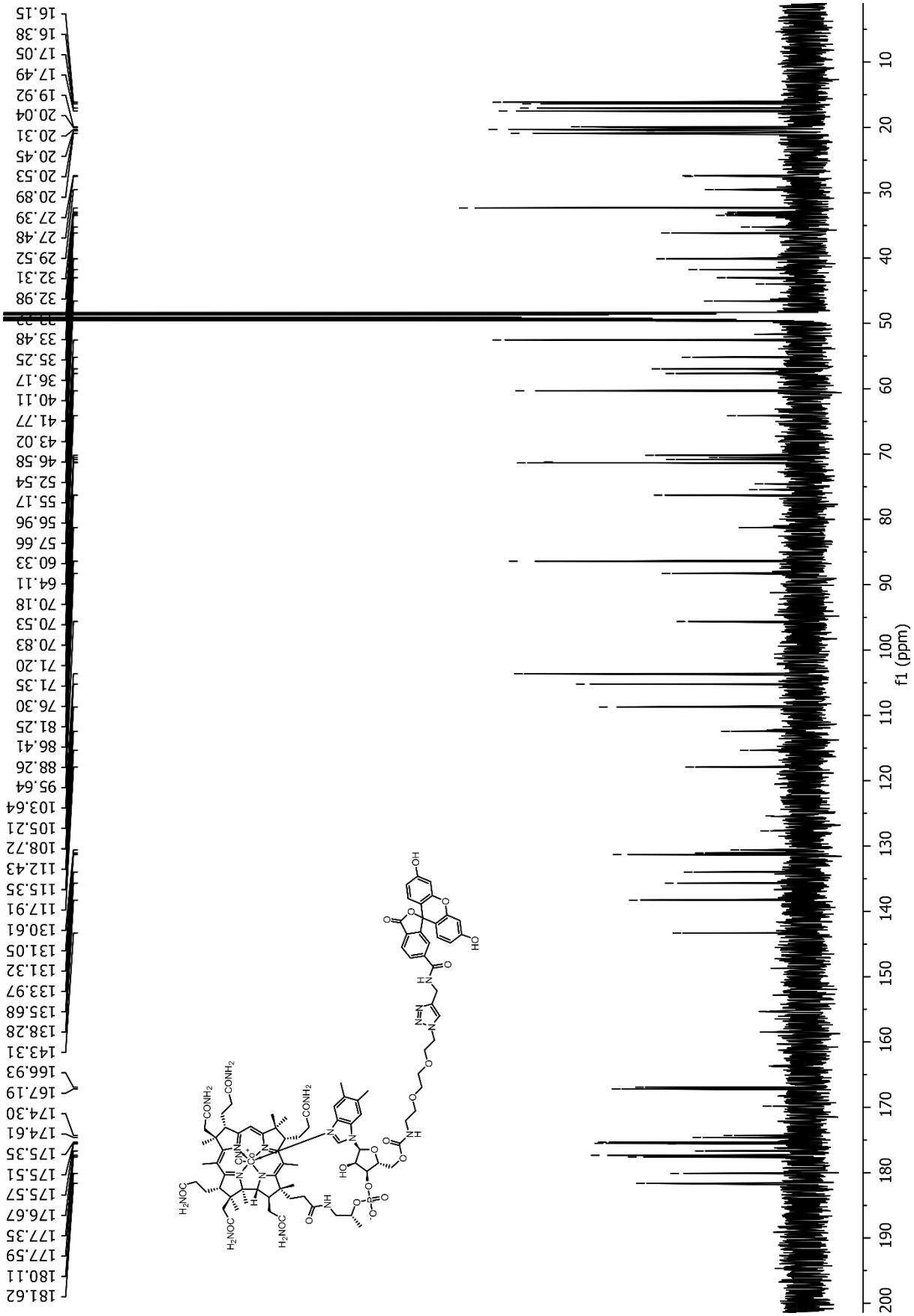
4.7 NMR spectra of Cbl-1xPEG-FAM recorded for CD_3OD



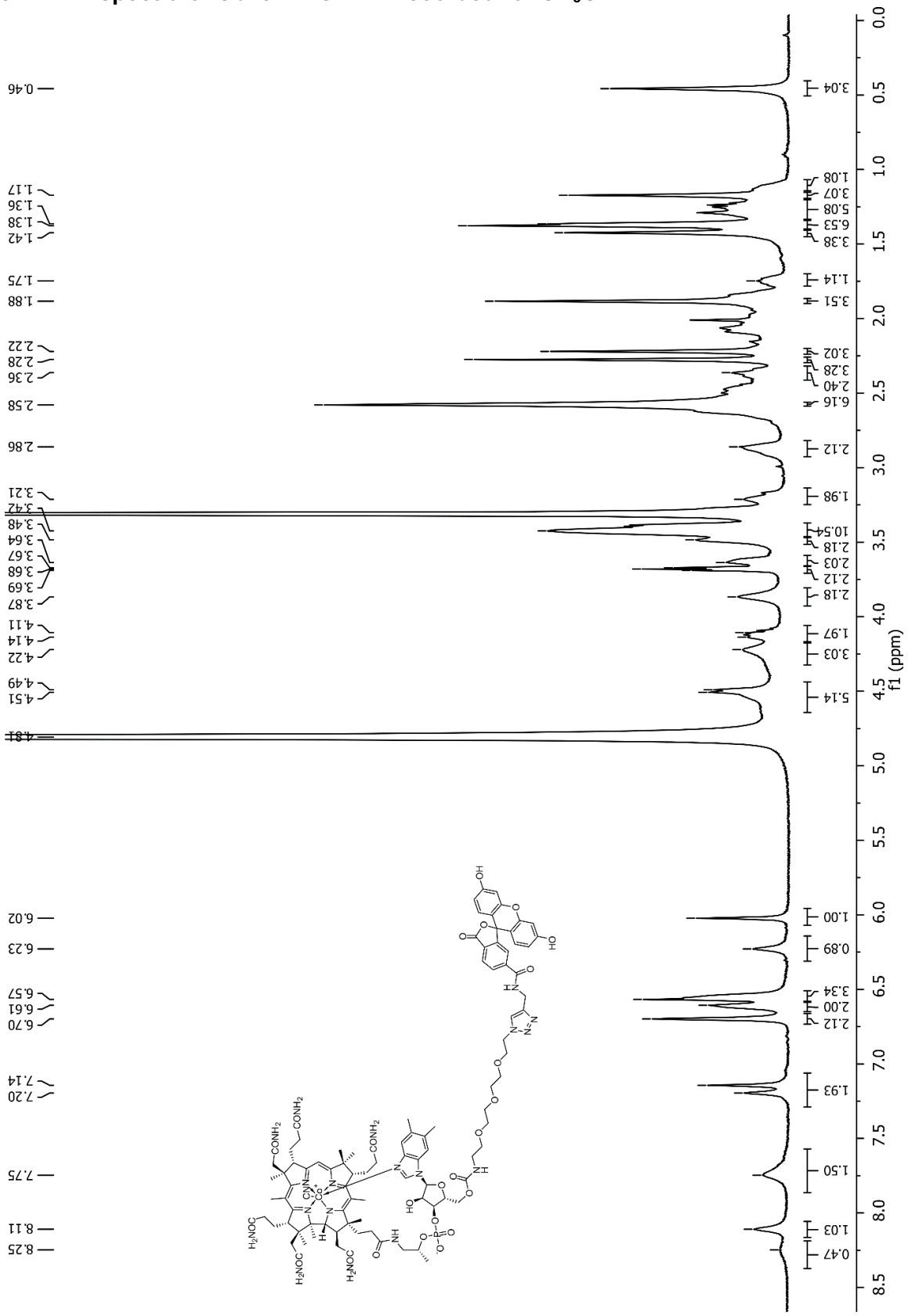

181.63
180.11
177.56
177.36
176.66
175.60
175.51
175.36
174.60
174.29
167.18
166.93
158.32
143.31
138.28
135.69
133.99
131.33
131.16
130.69
117.91
115.61
112.47
108.74
105.21
103.65
103.63
95.61
88.33
86.42
81.30
76.31
74.65
70.72
70.56
69.89
64.09
60.33
57.66
56.96
55.16
52.53
46.61
43.03
41.57
40.11
36.17
33.51
33.26
33.03
32.31
29.55
27.47
27.38
20.89
20.54
20.45
20.31
20.07
19.93
17.49
17.05
16.39
16.14
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
f1 (ppm)

4.8 NMR spectra of Cbl-2xPEG-FAM recorded for CD_3OD




181.62
180.11
177.59
177.35
176.67
175.57
175.51
175.35
174.61
174.30
167.19
166.93
143.31
138.28
135.68
133.97
131.32
131.05
130.61
117.91
115.35
112.43
108.72
105.21
103.64
95.64
88.26
86.41
81.25
76.30
71.35
71.20
70.83
70.53
70.18
64.11
60.33
57.66
56.96
55.17
52.54
46.58
43.02
41.77
40.11
36.17
35.25
33.48
32.98
32.31
29.52
27.48
27.39
20.89
20.53
20.45
20.31
20.04
19.92
17.49
17.05
16.38
16.15
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

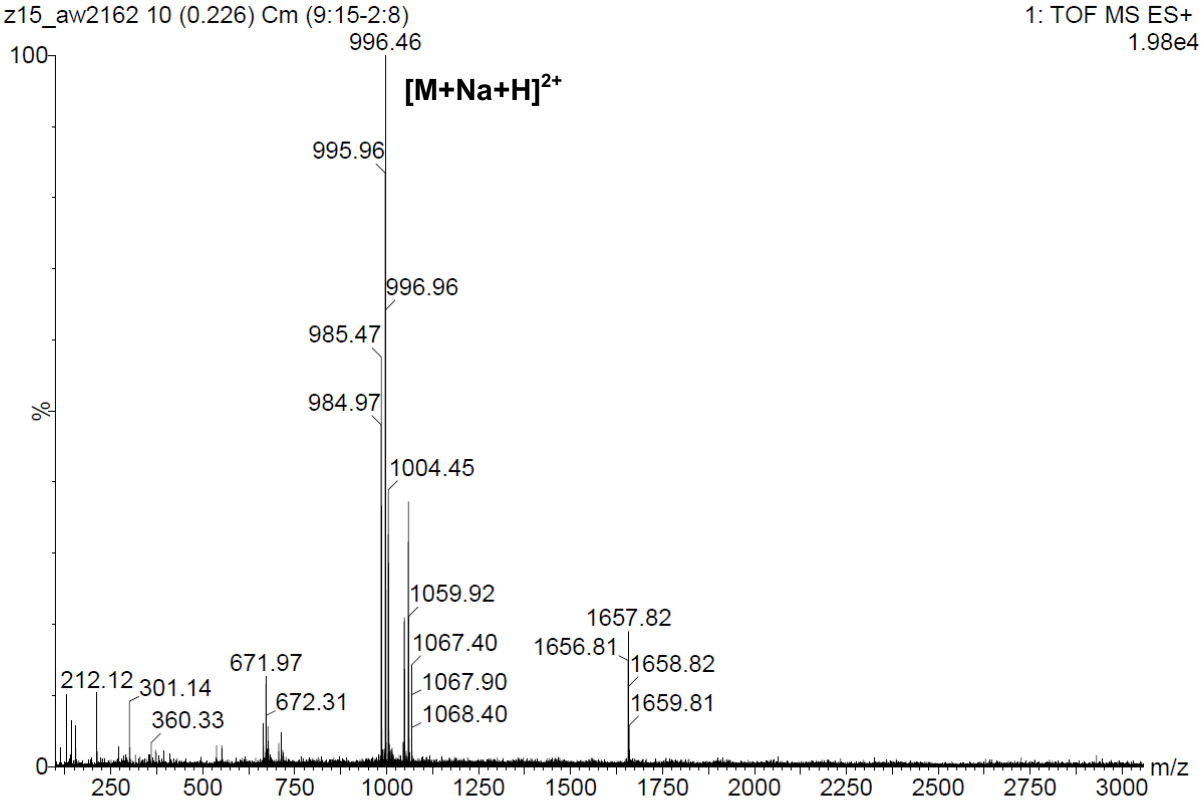
4.9 NMR spectra of Cbl-3xPEG-FAM recorded for CD_3OD

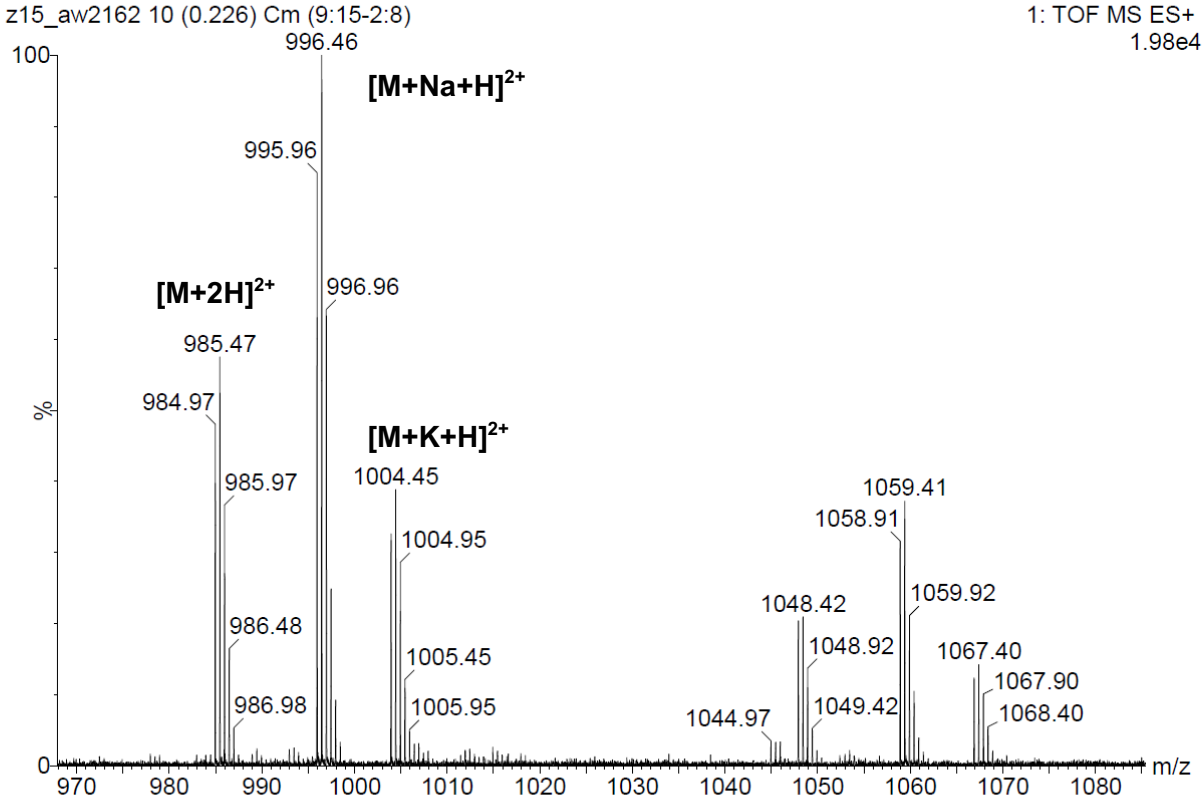


16.14
16.39
17.11
17.49
19.92
20.32
20.89
27.41
27.47
29.48
32.33
40.10
41.82
43.05
51.85
55.20
56.94
57.70
60.30
71.07
71.23
71.33
71.37
71.44
76.35
86.43
88.27
95.64
103.70
105.20
108.72
110.91
112.47
113.82
117.91
130.32
131.37
133.97
135.68
138.26
143.38
154.01
166.94
167.18
175.31
175.52
175.59
176.64
177.35
177.59
180.15
181.62
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
f1 (ppm)

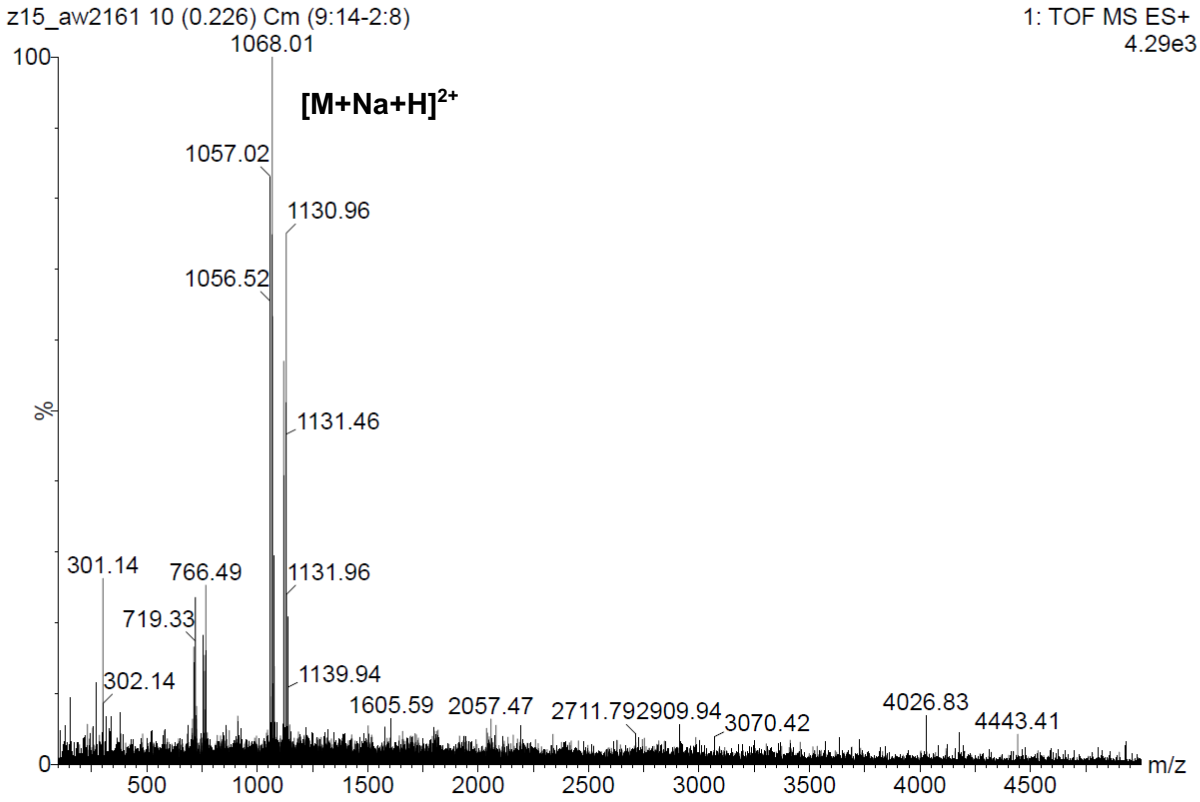
5. MS spectra

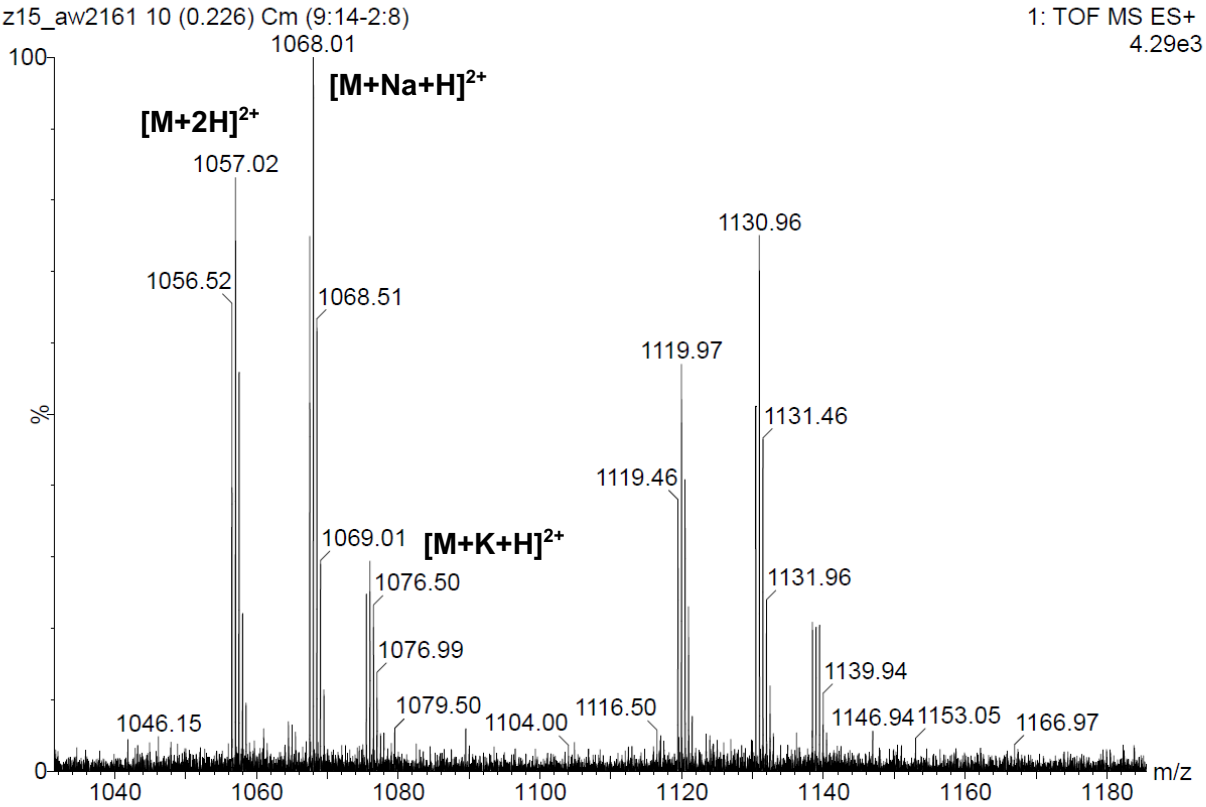
5.1 MS spectrum of Cbl-ATTO633

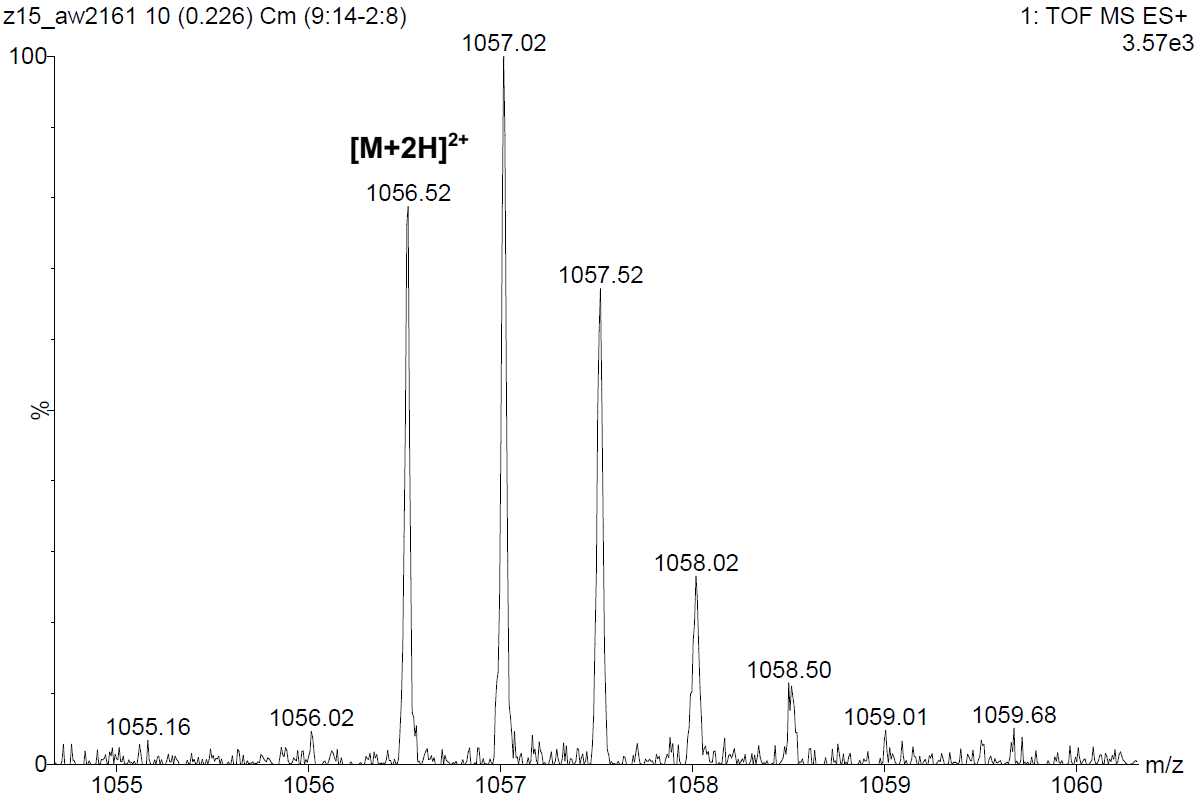




5.2 MS spectrum of Cbl-C6-ATTO633






z15_aw2161 10 (0.226) Cm (9:14-2:8)
1: TOF MS ES+
3.57e3
100
%
0
[M+2H]2+
1057.02
1056.52
1057.52
1058.02
1058.50
1055.16
1056.02
1059.01
1059.68
m/z
1055
1056
1057
1058
1059
1060