

Electronic Supplementary Information

for

**Synthesis of New Hydrophilic and Hydrophobic Cobinamides as NO-Independent sGC
Activators**

**Keith ó Proinsias,^a Maciej Giedyk,^a Iraida G. Sharina,^b Emil Martin^{*b} and Dorota
Gryko^{*a}**

^aInstitute of Organic Chemistry PAS, Kasprzaka 44/52, 01-224 Warsaw, Poland

*^bDepartment of Internal Medicine, Division of Cardiology, University of Texas Health
Science Center in Houston, 1941 East Road, The University of Texas, Houston, Texas 77054*

Table of Contents

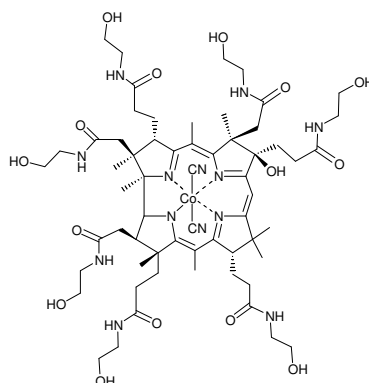
| | |
|--|-----|
| General Information | S3 |
| (CN) ₂ Cob(III)(<i>c</i> -hydroxy)heptakis(2-hydroxyethylamide) 1 | S4 |
| (CN) ₂ Cob(III)(<i>c</i> -hydroxy)heptakis(<i>n</i> -butylamide) 2 | S5 |
| (CN) ₂ Cob(III)heptakis(2-hydroxyethylamide) 4 | S6 |
| Attempted synthesis of hydrophobic heptaamide 5 from heptamethylester 3 | S7 |
| (CN) ₂ Cob(III)hexakis(<i>n</i> -butylamide)(<i>c</i> -lactone) 9 | S8 |
| (CN) ₂ Cob(III)hexakis(<i>n</i> -butylamide)(<i>c</i> -acid) 10 | S9 |
| (CN) ₂ Cob(III)heptakis(<i>n</i> -butylamide) 5 | S10 |
| Preparation and in vitro assay of recombinant human sGC enzyme. | S11 |
| ¹ H and ¹³ C NMR spectra of 1 | S12 |
| ¹ H and ¹³ C NMR spectra of 2 | S14 |
| ¹ H and ¹³ C NMR spectra of 4 | S16 |
| ¹ H and ¹³ C NMR spectra of 9 | S18 |
| ¹ H NMR spectrum of 10 | S20 |
| ¹ H and ¹³ C NMR spectra of 5 | S21 |

General Information

Analytical grade solvents were used as received. ^1H and ^{13}C NMR spectra were recorded at RT on Bruker 500 MHz or Varian 500 MHz with TMS as an internal standard. DCVC (dry column vacuum chromatography) was performed using Merck Silica Gel (200-300 mesh). Thin layer chromatography (TLC) was performed using Merck Silica Gel GF254, 0.20 mm thickness. High resolution ESI mass spectra were recorded on a Mariner spectrometer. UV/Vis absorption spectra were recorded in DCM on a Perkin Elmer λ -25.

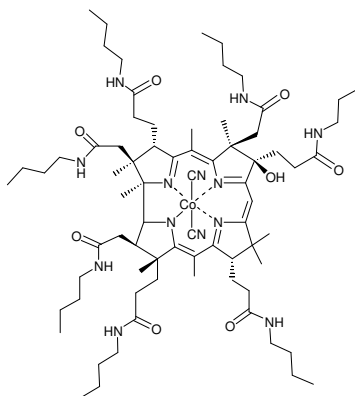
Note: In the case of compounds **1**, **2** and **4** due to overlapping of DMSO and EtOH or pentane peaks in the spectra the number of assigned protons is lower than the value stated in the molecular formula.

(CN)₂Cob(III)(*c*-hydroxy)heptakis(2-hydroxyethylamide) **1**



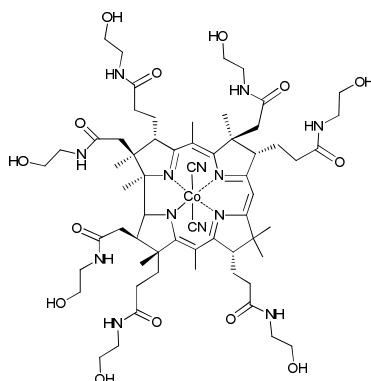
c-Lactone **7** (15 mg, 0.014 mmol) was dissolved in CCl₄ (1.0 mL) in a Schlenk tube under an argon atmosphere. Ethanolamine (0.1 mL, 1.6 mmol) and Bu₄N⁺CN⁻ (15 mg, 0.047 mmol) were then added. The mixture was stirred at 50°C for 24 h, after which it was diluted with water (10 mL) and washed with DCM (3 x 50 mL). The aqueous layer was concentrated *in vacuo* giving a purple residue, which was triturated with Et₂O/CHCl₃ (50 mL, 1:1) overnight. The crude product was purified using DCVC, 20-50% MeOH in DCM. Cobinamide **1** was redissolved in isopropanol, filtered through glass wool and the filtrate concentrated *in vacuo*. It was recrystallized from EtOH/Et₂O giving a purple solid (11 mg, 61%). M.p. 224-231°C. R_f 0.42, 50% MeOH in DCM. Anal. calcd. for C₆₁H₉₄CoN₁₃O₁₅ + EtOH + 2H₂O: C 54.42, H 7.54, N 13.10; found: C 54.35, H 7.58, N 13.14. HRMS ESI (m/z) calcd. for C₆₀H₉₄CoN₁₂O₁₅ [M-CN]⁺ 1281.6209; found 1281.6267. UV/Vis EtOH, λ_{max}, ε (L·mol⁻¹·cm⁻¹): 586 (8.43x10³), 546 (6.62x10³), 422 (2.22x10³), 369 (2.33x10⁴), 316 (8.05x10³), 308 (7.77x10³), 280 (8.04x10³). ¹H NMR (500 MHz, DMSO-d₆) δ (ppm): 8.24 (t, *J* = 5.3 Hz, 1H), 8.20 (t, *J* = 5.4 Hz, 1H), 7.96 (m, 2H), 7.89 (t, *J* = 5.5 Hz, 1H), 7.60 (t, *J* = 5.6 Hz, 1H), 7.37 (t, *J* = 5.4 Hz, 1H), 5.71 (s, 1H), 4.79 (t, *J* = 6.2 Hz, 1H), 4.76 (t, *J* = 6.2 Hz, 1H), 4.68 (m, 3H), 4.59 (t, *J* = 5.5 Hz, 2H), 4.52 (t, *J* = 5.5 Hz, 1H), 4.34 (d, *J* = 9.1 Hz, 1H), 3.63 (d, *J* = 10.3 Hz, 1H), 3.49 (t, *J* = 5.6 Hz, 1H), 3.46-3.44 (m, 4H), 3.40-3.90 (m, 6H), 3.27-3.24 (m, 4H), 3.15-3.06 (m, 8H), 3.03-3.00 (m, 4H), 2.93-2.87 (m, 1H), 2.76 (m, 1H), 2.36-2.31 (m, 3H), 2.24-2.21 (m, 3H), 2.19 (m, 4H), 2.16-2.13 (m, 3H), 2.09 (s, 3H), 2.04-1.97 (m, 6H), 1.80-1.71 (m, 4H), 1.49 (s, 3H), 1.43 (m, 2H), 1.34 (s, 3H), 1.29 (s, 3H), 1.22 (s, 3H), 1.15 (s, 3H), 1.07 (s, 3H). ¹³C NMR (125 MHz, DMSO-d₆) δ (ppm): 179.6, 178.9, 178.2, 176.1, 175.9, 174.7, 173.9, 165.4, 165.2, 105.5, 104.7, 92.3, 88.2, 85.5, 77.8, 68.2, 63.1, 63.09, 63.07, 63.04, 63.03, 62.9, 62.8, 62.7, 61.3, 58.1, 56.5, 55.9, 49.4, 49.1, 49.0, 45.0, 44.9, 44.8, 44.7, 41.9, 38.7, 36.1, 35.1, 34.9, 34.6, 34.4, 34.1, 28.9, 28.8, 24.7, 22.7, 20.8, 20.7, 19.4, 18.6, 18.4, 18.2, 18.1.

(CN)₂Cob(III)(c-hydroxy)heptakis(*n*-butylamide) 2



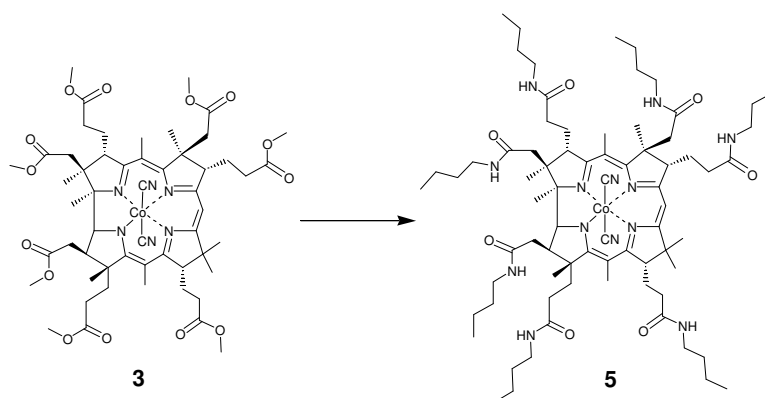
c-Lactone **7** (70 mg, 0.065 mmol) was dissolved in CCl₄ (5.0 mL) in a Schlenk tube under an argon atmosphere. *n*-Butylamine (5.0 mL, 37 mmol) and NaN₃ (75 mg, 1.2 mmol) were then added. The mixture was stirred at 50°C for 72 h, after which it was diluted with water (20 mL) and washed with DCM (3 x 100 mL). The aqueous layer was concentrated *in vacuo* giving a purple residue, which was triturated with Et₂O/CHCl₃ (100 mL, 1:1) overnight. Cobinamide **2** was purified using DCVC, 2-10% EtOH in DCM. The product was recrystallized from AcOEt/pentane giving a purple solid (66 mg, 73 %). M.p. 145-148 °C. R_f 0.42, 5% EtOH in DCM. Anal. calcd. for C₇₅H₁₂₂CoN₁₃O₈ + 3H₂O: C 62.26, H 8.92, N 12.59; found: C 62.21, H 8.87, N 12.45. HRMS ESI (*m/z*) calcd. for C₇₅H₁₂₂CoN₁₃O₈Na [M+Na]⁺ 1414.8767; found 1414.8763. UV/Vis EtOH, λ_{max}, ε (L·mol⁻¹·cm⁻¹): 583 (5.39x10³), 544 (5.96x10³), 419 (2.68x10³), 368 (1.77x10⁴), 318 (7.79x10³), 280 (8.03x10³). ¹H NMR (500 MHz, DMSO-*d*₆) δ (ppm): 8.17 (t, *J* = 4.8 Hz, 1H), 8.13 (t, *J* = 5.3 Hz, 1H), 7.94 (t, *J* = 5.3 Hz, 1H), 7.82 (t, *J* = 5.7 Hz, 1H), 7.54 (t, *J* = 5.3 Hz, 2H), 7.33 (t, *J* = 4.4 Hz, 1H), 6.65 (s, 1H), 5.64 (s, 1H), 4.49 (d, *J* = 8.8 Hz, 0.8H), 4.40 (d, *J* = 4.4 Hz, 0.2H), 3.62 (m, 1H), 3.16-2.91 (m, 16H), 2.80-2.76 (m, 2H), 2.36-2.32 (m, 2H), 2.20 (m, 2H), 2.10-1.96 (m, 9H), 1.88-1.83 (m, 3H), 1.75-1.68 (m, 4H), 1.68 (m, 4H), 1.55-1.50 (m, 5H), 1.44-1.35 (m, 10H), 1.22-1.16 (m, 12H), 1.08 (s, 4H), 0.99 (d, *J* = 6.6 Hz, 1H), 0.90-0.83 (m, 16H), 0.81-0.78 (m, 5H). ¹³C NMR (125 MHz, DMSO-*d*₆) δ (ppm): 176.1, 175.7, 175.3, 172.3, 172.1, 171.6, 171.0, 170.7, 170.3, 170.2, 170.1, 162.1, 157.9, 105.8, 102.2, 88.6, 84.7, 82.4, 74.6, 67.2, 67.1, 58.3, 54.9, 54.8, 52.9, 46.0, 45.9, 38.8, 38.6, 38.3, 38.2, 38.18, 38.15, 31.3, 31.2, 31.18, 31.12, 31.0, 30.9, 30.8, 30.5, 19.9, 19.8, 19.7, 19.6, 19.56, 19.50, 19.4, 19.3, 17.4, 15.9, 15.8, 15.0, 14.6, 13.7, 13.6, 13.57, 13.56.

(CN)₂Cob(III)heptakis(2-hydroxyethylamide) 4



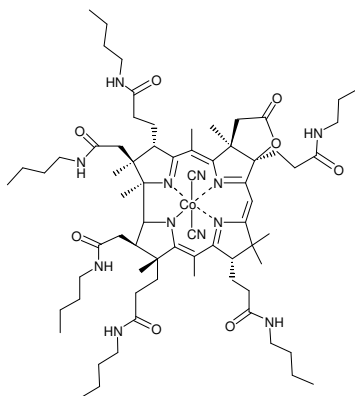
Heptamethylester **3** (15 mg, 0.014 mmol) was dissolved in toluene (1.0 mL) in a Schlenk tube under an argon atmosphere. Ethanolamine (1.0 mL, 1.6 mmol) and Bu₄N⁺CN⁻ (15 mg, 0.047 mmol) were then added. The mixture was stirred at 50°C for 24 h, after which it was diluted with water (10 mL) and washed with DCM (3 x 50 mL). The aqueous layer was concentrated *in vacuo* giving a purple residue, which was triturated with Et₂O/chloroform (50 mL, 1:1) overnight. The crude product was purified using DCVC, 20-50% MeOH in DCM. Cobinamide **4** was redissolved in isopropanol, filtered through glass wool and the filtrate concentrated *in vacuo*. Cobinamide **4** was then recrystallized from EtOH/Et₂O and dried under vacuum giving a purple solid (11 mg, 60%). M.p. 182-192°C. R_f 0.48, 50% MeOH in DCM. Anal. calcd. for C₆₁H₉₄CoN₁₃O₁₄ + EtOH + 2H₂O: C 55.05, H 7.63, N 13.25; found: C 54.82, H 7.60, N 13.19. HRMS ESI (m/z) calcd. for C₆₁H₉₄CoN₁₃O₁₄Na [M+Na]⁺ 1314.6267; found 1314.6209. UV/Vis CH₃OH, λ_{max}, ε (L·mol⁻¹·cm⁻¹): 278 (5.93x10³), 317 (6.37x10³), 367 (8.74x10³), 473 (4.23x10³), 541 (3.19x10³), 583 (3.02x10³). ¹H NMR (500 MHz, DMSO-d₆) δ (ppm): 8.35 (t, *J* = 5.1 Hz, 1H), 8.31 (t, *J* = 5.3 Hz, 1H), 7.99 (m, 2H), 7.91 (t, *J* = 5.3 Hz, 1H), 7.67 (t, *J* = 5.3 Hz, 1H), 7.62 (t, *J* = 5.5 Hz, 1H), 5.64 (s, 1H), 4.83 (s(br), 2H), 4.69 (s(br), 2H), 4.61 (m(br), 3H), 4.41 (d, *J* = 9.3 Hz, 1H), 3.59 (m, 2H), 3.45-3.41 (m, 4H), 3.33 (m, 11H), 3.26-3.22 (m, 1H), 3.16-3.09 (m, 8H), 3.04-3.03 (m, 6H), 2.78 (s(br), 1H), 2.63-2.56 (m, 1H), 2.46-2.37 (m, 3H), 2.30-2.25 (m, 1H), 2.18 (s, 5H), 2.14 (s, 3H), 2.09-1.99 (m, 6H), 1.93-1.72 (m, 6H), 1.51 (s, 3H), 1.33 (s, s, 6H), 1.22 (s, 3H), 1.14 (s, 3H), 1.07 (m, 3H). ¹³C NMR (125 MHz, DMSO-d₆) δ (ppm): 175.8, 175.7, 175.3, 172.5, 172.1, 171.6, 171.4, 171.3, 170.7, 170.5, 169.2, 162.8, 162.3, 103.5, 101.6, 90.0, 82.4, 74.7, 64.7, 59.8, 59.79, 59.77, 59.71, 59.5, 59.1, 54.8, 54.3, 52.8, 50.4, 48.6, 46.0, 45.7, 43.6, 41.7, 41.6, 41.5, 41.4, 38.5, 35.3, 32.7, 32.1, 32.0, 31.9, 31.2, 30.8, 27.4, 25.6, 25.2, 21.5, 19.4, 18.2, 17.5, 16.1, 15.2, 15.1, 14.9.

Attempted synthesis of hydrophobic cobinamide **5** from heptamethylester **3**



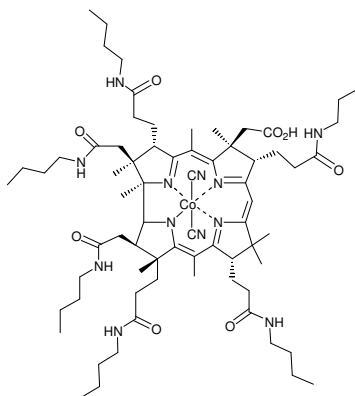
Heptamethylester **3** (15 mg, 0.014 mmol) was dissolved in an appropriate solvent (1.0 mL). A catalyst and *n*-butylamine (1.0 mL) were then added and the mixture was stirred at an appropriate temperature for the given time. All reactions were worked-up as described in the synthesis of cobinamide **2**.

| Solvent | Catalyst [eq.] | Time [h] | Temperature [°C] | Yield [%] of 5 |
|------------------------|---|----------|------------------|-----------------------|
| Toluene | Bu ₄ N ⁺ CN ⁻ ; 0,5eq. | 24 | 50 | - |
| Toluene | Bu ₄ N ⁺ CN ⁻ ; 0,5eq. | 72 | 50 | - |
| CCl₄ | Bu ₄ N ⁺ CN ⁻ ; 0,5eq. | 24 | RT | - |
| CCl₄ | Bu ₄ N ⁺ CN ⁻ ; 0,5eq. | 24 | 50 | - |
| CCl₄ | NaN ₃ | 72 | 50 | - |
| CCl₄ | NaN ₃ and NaCN | 72 | 50 | - |
| EtOH | NaCN, excess | 24 | RT | - |
| EtOH | Bu ₄ N ⁺ CN ⁻ ; 0,5eq. | 24 | RT | - |
| EtOH | Bu ₄ N ⁺ CN ⁻ ; 0,5eq. | 24 | 50 | - |
| EtOH | Bu ₄ N ⁺ CN ⁻ ; 0,5eq. | 72 | 50 | - |
| EtOH | NaN ₃ , excess | 72 | 50 | - |
| MeOH | Bu ₄ N ⁺ CN ⁻ ; 0,5eq. | 24 | 50 | - |
| MeOH | Bu ₄ N ⁺ CN ⁻ ; 0,5eq. | 48 | 50 | - |
| H₂O | NaCN, excess | 24 | 50 | - |
| Brine | Bu ₄ N ⁺ CN ⁻ ; 0,5eq. | 72 | 50 | - |



Cobinamide **2** (80 mg, 0.057 mmol) was dissolved in DCM (3.0 mL) and treated with TFA (3.0 mL, 39 mmol). The mixture was stirred at RT for 1 h, after which the acid was neutralized using NaHCO₃. The aqueous layer was washed with DCM and the combined organic layers were washed with aq. NaCN, dried over Na₂SO₄, filtrated and concentrated *in vacuo*. The crude product was purified using DCVC, 2-5 % EtOH in DCM. *c*-Lactone **9** was recrystallized from AcOEt/pentane and dried under vacuum giving a purple solid (55 mg, 56%). M.p. 147-150°C. *R*_f 0.31, 4% EtOH in DCM. Anal. calcd. for C₇₁H₁₁₁CoN₁₂O₈ + 2H₂O: C 62.90, H 8.55, N 12.40; found: C 62.81, H 8.56, N 12.29. HRMS ESI (*m/z*) calcd. for C₇₀H₁₁₁CoN₁₁O₈ [M-CN]⁺ 1292.7944; found 1292.7944. UV/Vis CH₃OH, λ_{max}, ε (L·mol⁻¹·cm⁻¹): 280 (9.56x10³), 305 (7.64x10³), 317 (8.71x10³), 366 (2.82x10⁴), 419 (2.47x10³), 547 (8.11x10³), 856 (9.52x10³). ¹H NMR (500 MHz, DMSO-*d*₆) δ (ppm): 8.13 (t, *J* = 5.4 Hz, 2H), 7.92 (t, *J* = 5.4 Hz, 1H), 7.85 (t, *J* = 5.4 Hz, 1H), 7.58 (t, *J* = 5.5 Hz, 1H), 7.49 (t, *J* = 5.5 Hz, 1H), 5.60 (s, 1H), 4.40 (d, *J* = 8.7 Hz, 1H), 3.64-3.60 (m, 1H), 3.29 (d, *J* = 6.3 Hz, 1H), 3.25 (s, 1H), 3.21-3.14 (m, 1H), 3.09-2.91 (m, 12H), 2.82 (s(br), 1H), 2.61 (d, *J* = 18.7 Hz, 1H), 2.38-2.35 (m, 1H), 2.26-2.16 (m, 12H), 2.07-1.92 (m, 6H), 1.88-1.67 (m(br), 4H), 1.60 (s, 3H), 1.43-1.20 (m, 35H), 1.13 (s, 3H), 1.06 (s, 3H), 0.89-0.84 (m, 12H), 0.82-0.79 (m, 6H). ¹³C NMR (125 MHz, DMSO-*d*₆) δ (ppm): 177.7, 176.5, 175.8, 173.3, 171.8, 170.9, 170.6, 170.5, 170.3, 170.1, 164.5, 162.0, 159.5, 103.9, 103.4, 94.5, 87.0, 82.6, 75.3, 58.4, 55.2, 52.9, 49.8, 46.8, 45.8, 41.7, 38.6, 38.4, 38.3, 38.2, 38.1, 35.5, 32.6, 32.2, 32.0, 31.2, 31.17, 31.5, 31.1, 30.9, 30.4, 30.0, 29.5, 26.2, 25.2, 21.6, 19.7, 19.6, 19.5, 19.3, 18.8, 17.8, 16.1, 16.0, 15.0, 13.7, 13.63, 13.60, 13.58, 13.56.

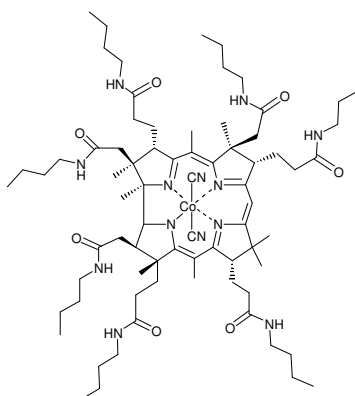
(CN)₂Cob(III)hexakis(*n*-butylamide)(*c*-acid) 10



c-Lactone **9** (15 mg, 0.011 mmol) was dissolved in a degassed 15 % AcOH/toluene solution (1.0 mL). Activated zinc (22 mg, 0.34 mmol) was added and the mixture was vigorously stirred at RT for 30 min. The solution was neutralized using phosphate buffer (5.0 mL) and washed with DCM. The combined organic layers were washed with aq. NaCN, dried over Na₂SO₄, filtrated and concentrated *in vacuo*. *c*-Acid **10** was crudely purified using DCVC, 5-10% MeOH in DCM, in which the most intense band was isolated. *c*-Acid **10** was recrystallized from AcOEt/pentane and dried under vacuum giving a purple solid. *R_f* 0.47, 10% EtOH in DCM. HRMS ESI (*m/z*) calcd. for C₇₀H₁₁₃CoN₁₁O₈ [M-CN]⁺ 1294.8100; found 1294.8048.

c-Acid **10** was used without further purification. The broadening of peaks in the ¹H NMR spectrum made it impossible to decipher and consequently high resolution ¹³C spectra could not be obtained. This was caused by the presence of the acid group.

(CN)₂Cob(III)heptakis(*n*-butylamide) 5



Cobinamide **10** (20 mg, 0.015 mmol) was dissolved in DMF (1.5 mL) under an argon atmosphere. *n*-Butylamine (12 μ L), DIPEA (18 μ L, 0.11 mmol) and DEPC (15 μ L, 0.11 mmol) were then added and the mixture was stirred at RT for 24 h. The mixture was then diluted with DCM and washed with water. The organic layer was dried over Na₂SO₄, filtered and concentrated *in vacuo* giving a purple residue. The crude product was purified using DCVC, 2-10% EtOH in DCM. Cobinamide **5** was recrystallized using AcOEt/pentane and dried under vacuum giving a purple solid (13 mg, 62%). M.p. 142-146 °C. R_f 0.44, 5% EtOH in DCM. Anal. calcd. for C₇₅H₁₂₂CoN₁₃O₇ + 2H₂O: C 63.76, H 8.99, N 12.89; found: C 63.68, H 9.07, N 12.81. HRMS ESI (m/z) calcd. for C₇₄H₁₂₂CoN₁₂O₇ [M-CN]⁺ 1398.8814; found 1398.8815. UV/Vis CH₃OH, λ_{max} , ϵ (L·mol⁻¹·cm⁻¹): 279 (1.06x10⁴), 315 (9.48x10³), 368 (2.78x10⁴), 419 (2.75x10⁴), 544 (8.32x10³), 581 (9.43x10³), 940 (7.08x10³). ¹H NMR (500 MHz, DMSO-d₆) δ (ppm): 8.14-8.10 (m, 2H), 7.94 (t, *J* = 5.4 Hz, 1H), 7.84 (t, *J* = 5.4 Hz, 1H), 7.80 (t, *J* = 5.4 Hz, 1H), 7.55 (t, *J* = 5.4 Hz, 1H), 7.50 (t, *J* = 5.5 Hz, 1H), 5.65 (s, 1H), 4.45 (d, *J* = 9.0 Hz, 1H), 3.60 (d, *J* = 10.1 Hz, 1H), 3.47 (t, *J* = 5.5 Hz, 1H), 3.30 (s, 1H), 3.22-3.15 (m, 1H), 3.10-2.90 (m, 14H), 2.79 (s(br), 1H), 2.36-2.28 (m, 3H), 2.24-2.12 (m, 9H), 2.05-1.85 (m, 8H), 1.79-1.67 (m, 4H), 1.57-1.52 (m, 4H), 1.46-1.19 (m, 39H), 1.14 (s, 3H), 1.07 (s, 3H), 0.90-0.80 (m, 21H). ¹³C NMR (125 MHz, DMSO-d₆) δ (ppm): 175.9, 175.7, 175.4, 172.1, 171.7, 171.6, 171.0, 170.8, 170.4, 170.2, 168.8, 162.5, 162.3, 103.8, 101.7, 82.4, 74.6, 58.2, 55.0, 48.9, 46.0, 45.8, 40.0, 39.84, 39.76, 39.7, 39.6, 39.5, 39.3, 39.2, 39.0, 38.6, 38.5, 38.33, 38.27, 38.18, 38.16, 38.1, 31.9, 31.8, 31.3, 31.18, 31.17, 31.15, 31.1, 31.0, 30.9, 21.6, 19.7, 19.59, 19.56, 19.53, 19.50, 19.4, 18.2, 17.6, 16.0, 15.0, 14.9, 13.66, 13.55, 13.62, 13.56, 13.54, 13.48

Preparation and in vitro assay of recombinant human sGC enzyme.

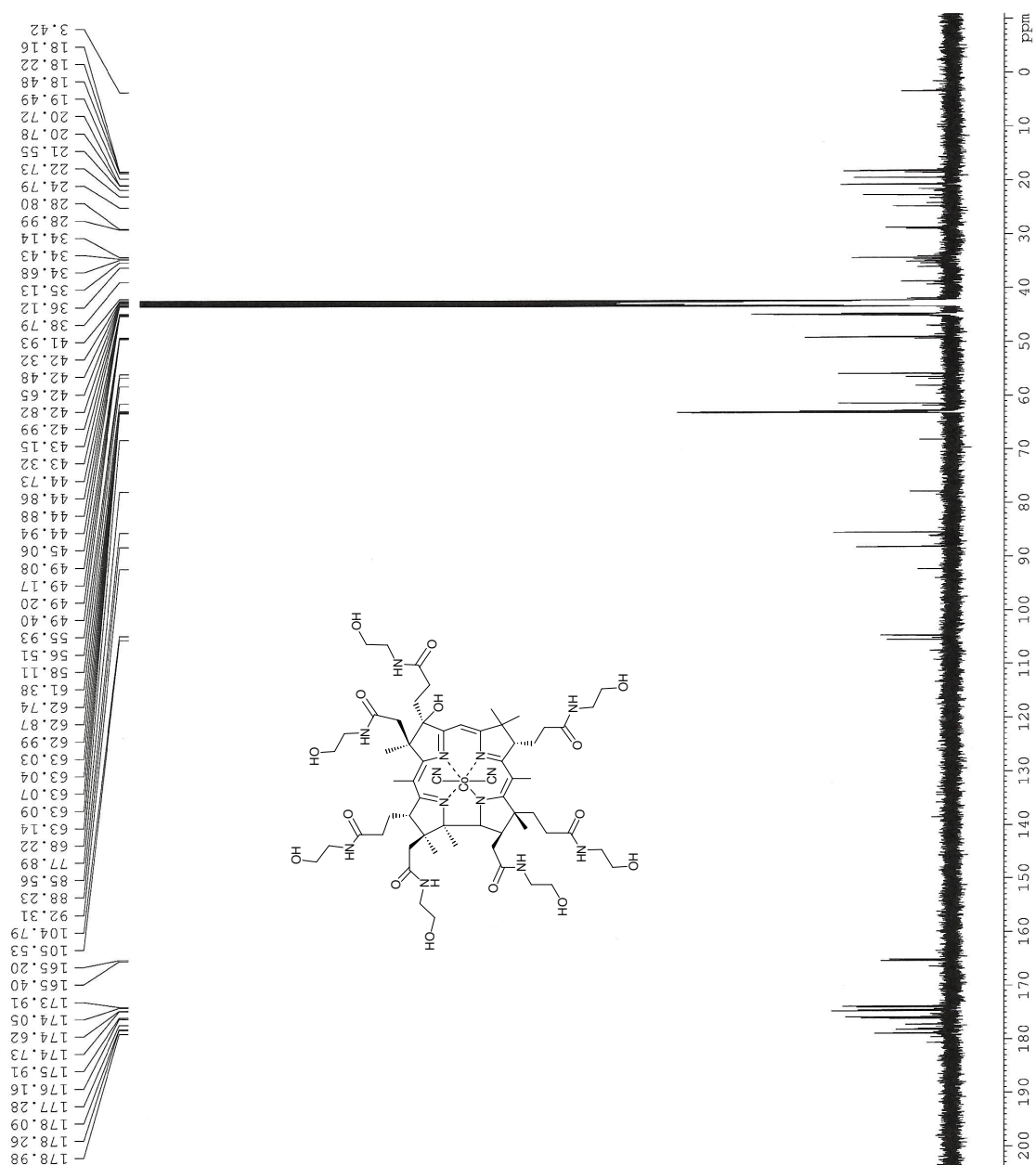
Full-length sGC was purified from Sf9 cells as described previously.¹ Enzymatic activity was assayed using [α -³²P]GTP to [³²P] cGMP conversion assay. To evaluate the effect of (CN2)Cbi or its derivatives on sGC activity, 0.5 μ g sGC was preincubated for 10 min at room temperature with indicated concentration of the tested compound in 25 mM TEA, pH 7.5, 1 mg/ml BSA, 1 mM 3-isobutyl-1-methylxanthine (IBMX), 1 mM DTT, 1 mM cGMP, 3 mM MgCl₂, 0.05 mg/ml creatine phosphokinase and 5 mM creatine phosphate. To assay the cGMP-forming activity of treated sGC the reaction was initiated by adding 1 mM GTP/ [α -³²P]GTP (~ 150000 cpm) and incubated at 37°C for 10 minutes. The reaction was stopped by 400 μ l of 100 mM zinc acetate followed by 500 μ l of 120 mM sodium carbonate. Unreacted GTP was precipitated by centrifugation and the supernatant containing cGMP was loaded onto a 2 ml Al₂O₃ column. cGMP was eluted with 10 ml of 50 mM Tris pH 7.5 and the amount of generated [³²P] cGMP was calculated based on the Cherenkov counts in a beta scintillation counter.

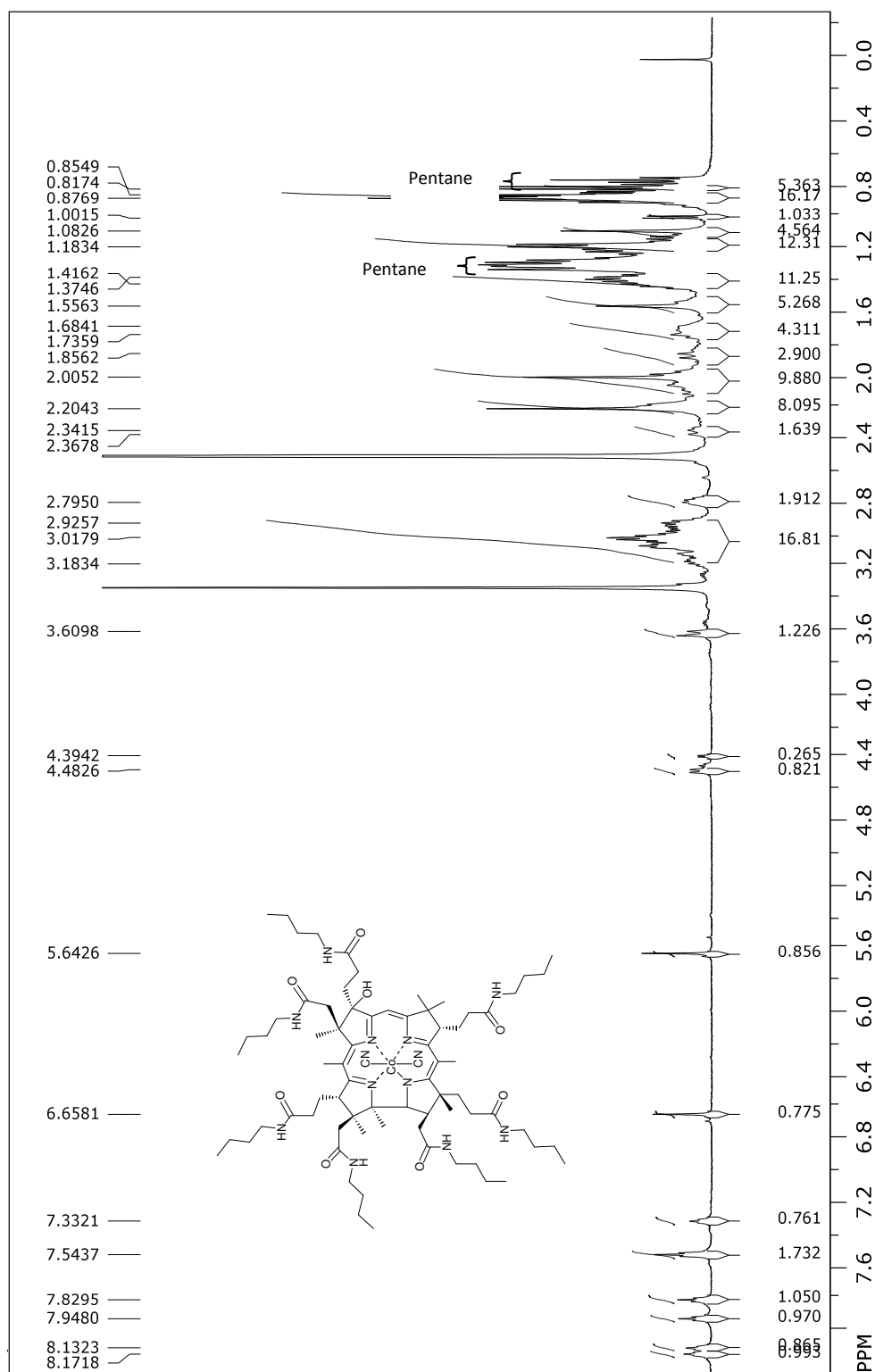
¹Martin, E.; Berka, V.; Tsai, A.L.; Murad, F. *Methods Enzymol.*, 2005, **396**, 478-492.

1

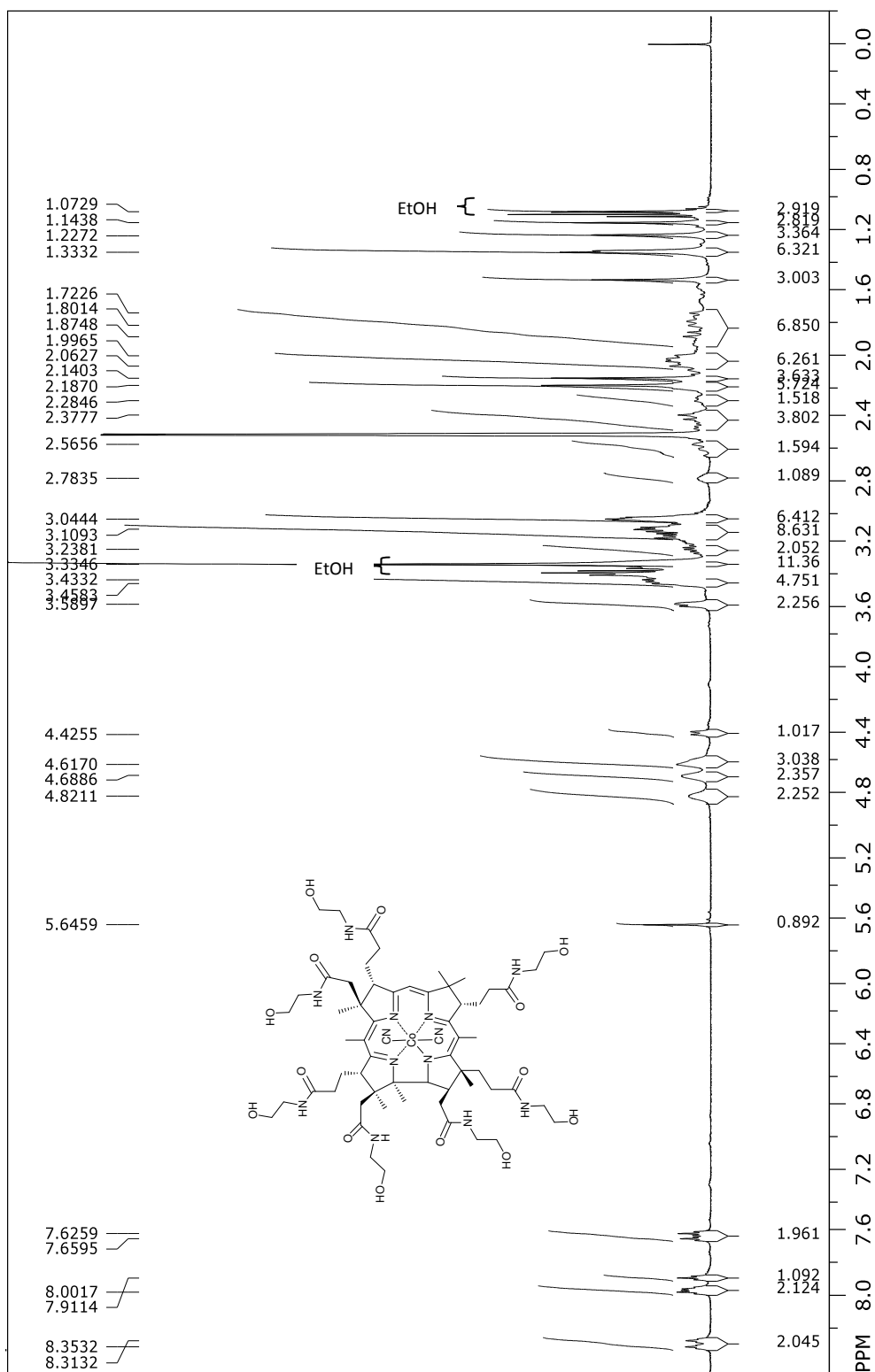


(CN)₂Cob(III)(*c*-hydroxy)heptakis(2-hydroxyethylamide) 1

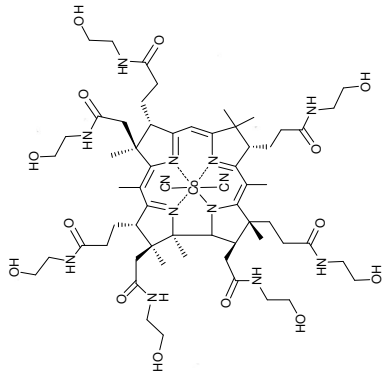


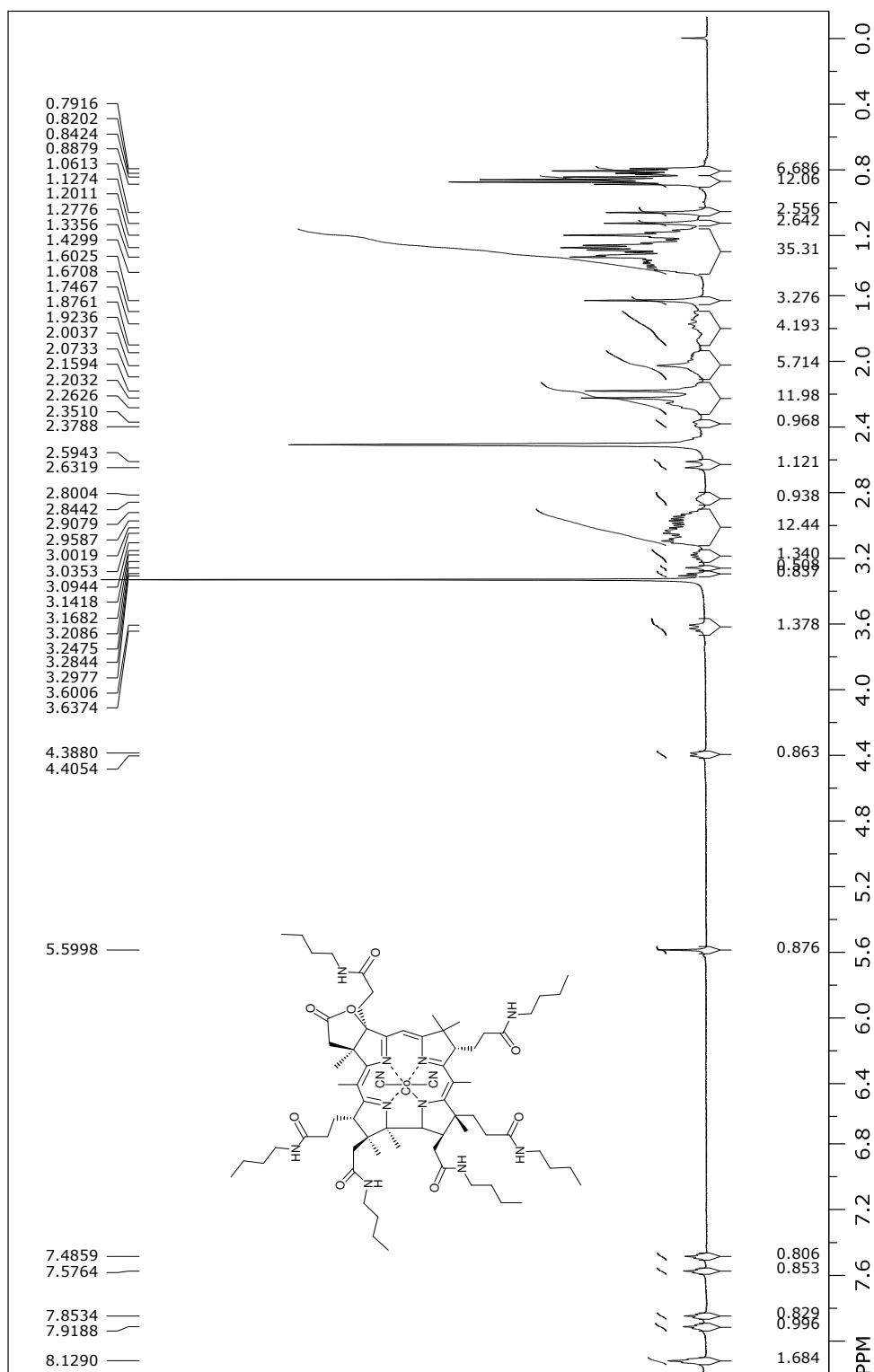


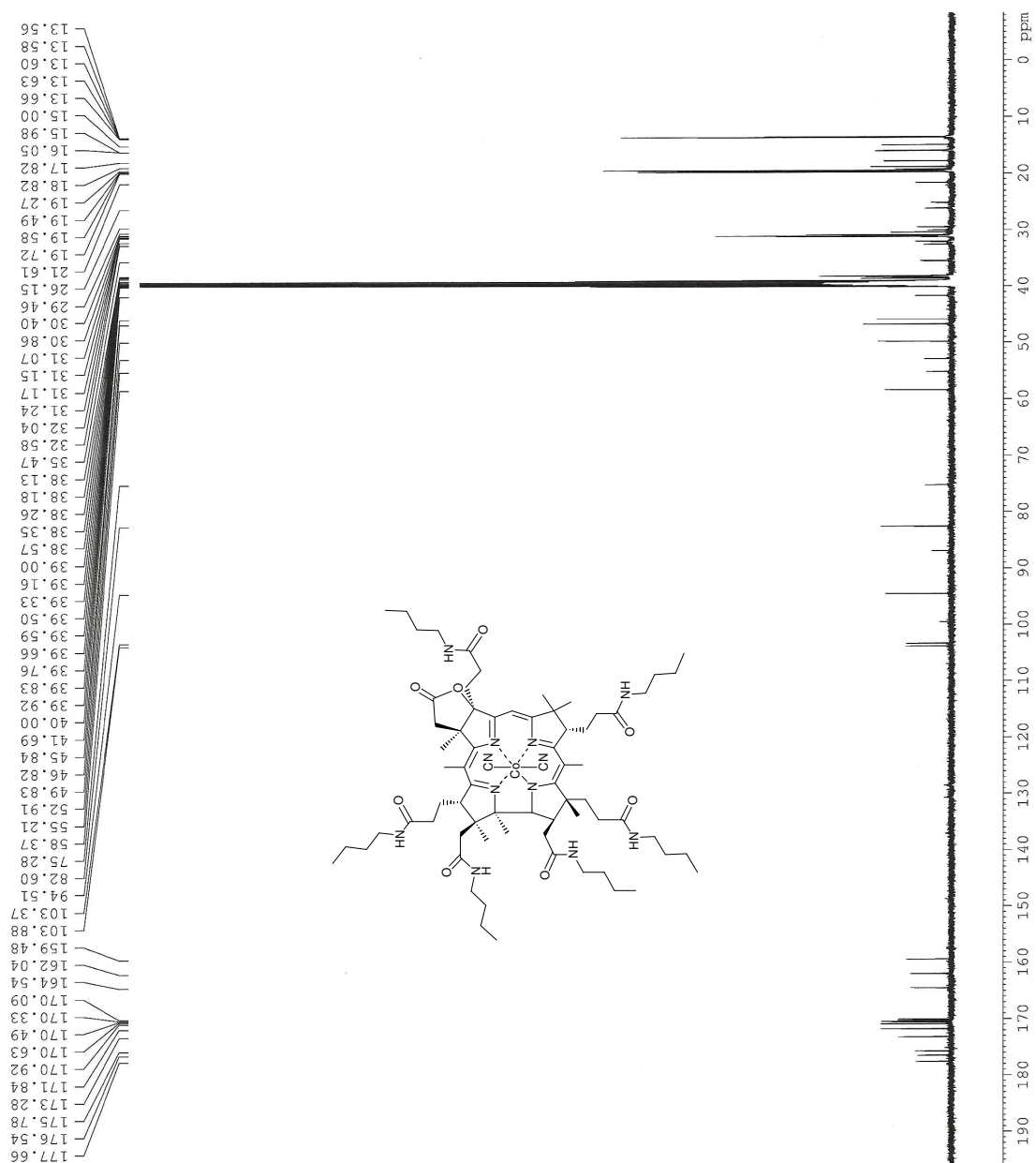
(CN)₂Cob(III)heptakis(2-hydroxyethylamide) **4**

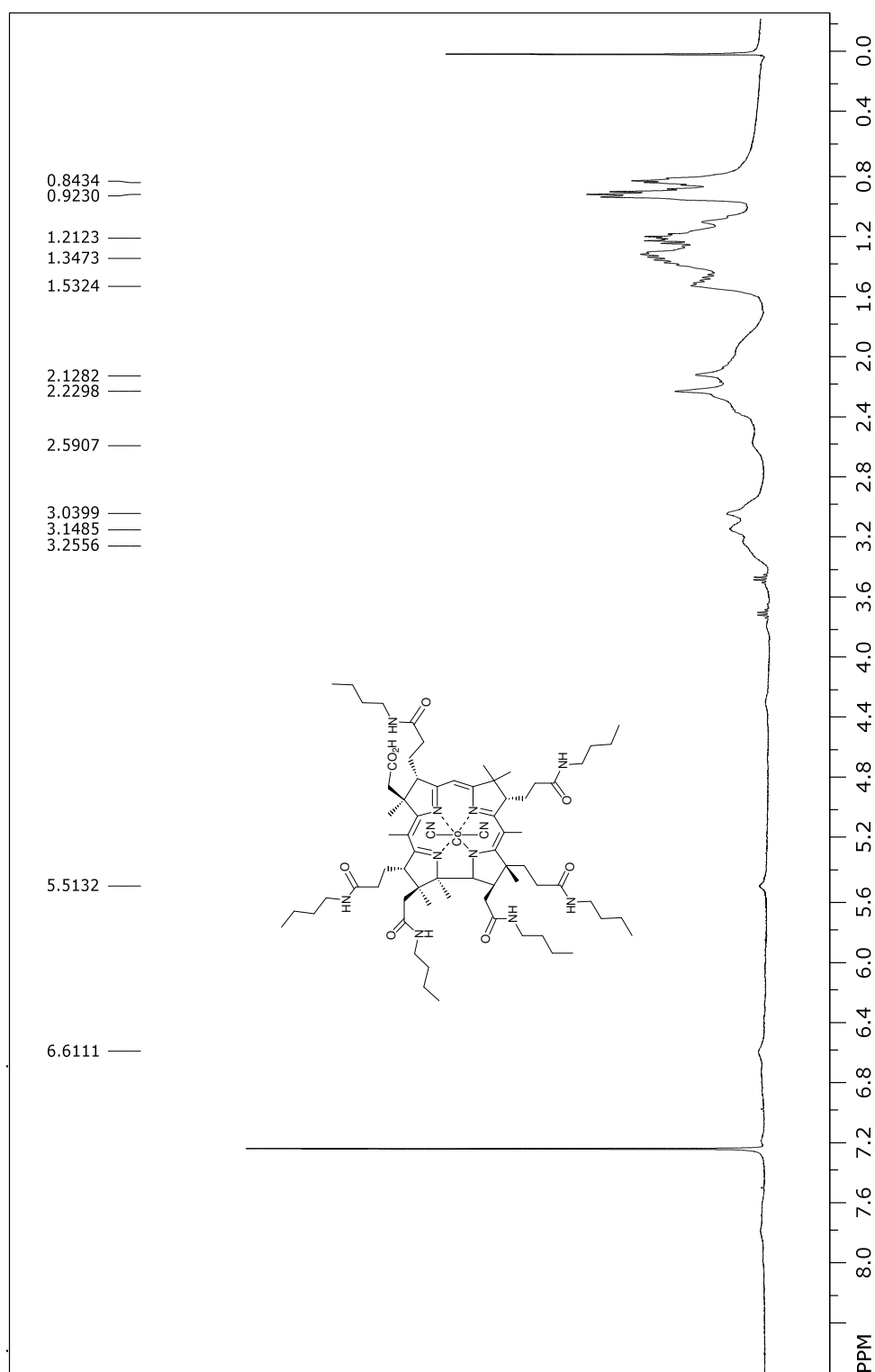


4





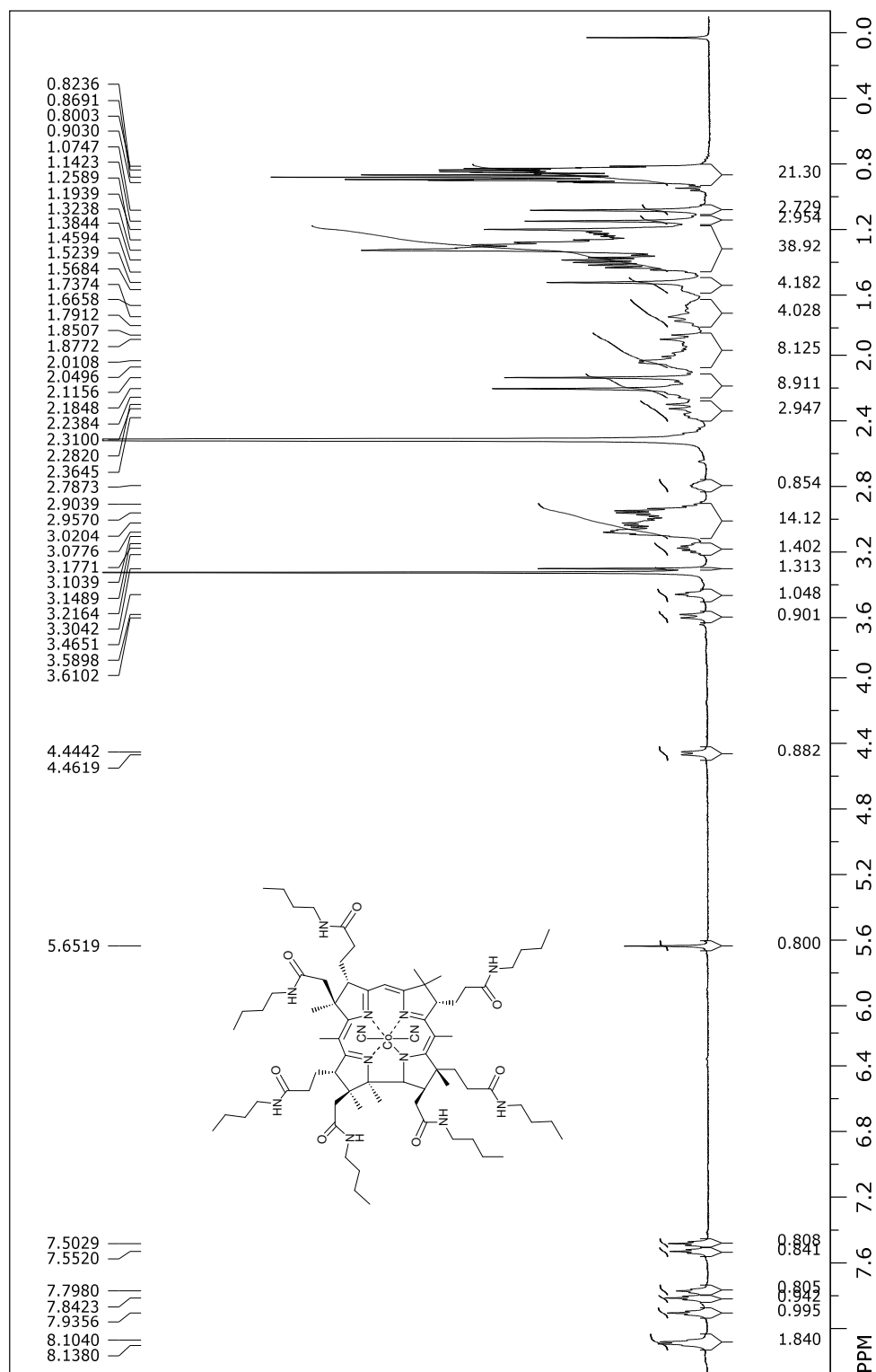




Note: The broadening of peaks in the ¹H NMR spectrum made it impossible to decipher and consequently high resolution ¹³C spectra could not be obtained. This was caused by the presence of the *c*-acid group.

Varian 400MHz, DMSO-d₆ at 25°C

(CN)₂Cob(III)heptakis(*n*-butylamide) 5



(CN)₂Cob(III)heptakis(*n*-butylamide) 5

