

Supporting Information

Syntheses and Cellular Investigations of 17³-, 15²- and 13¹-Amino Acid Derivatives of Chlorin e₆

*R. G. Waruna Jinadasa, Xiaoke Hu, M. Graça H. Vicente and Kevin M. Smith**

Louisiana State University, Department of Chemistry, Baton Rouge LA, 70803, USA

Table of Contents:

Cover Page	S1
Normalized absorption spectra (3 , 11 , and 13) (Figure S1)	S2
Energy minimized conformations in water (3 , 7b , and 13) (Figure S2)	S3
Time-dependent uptake of conjugates (Figure S3)	S4
Microscopy figures (Figures S4-S10)	S4-S11
¹ H NMR spectra	S12-S32
¹³ C NMR shifts	S33
¹³ C NMR spectra	S41

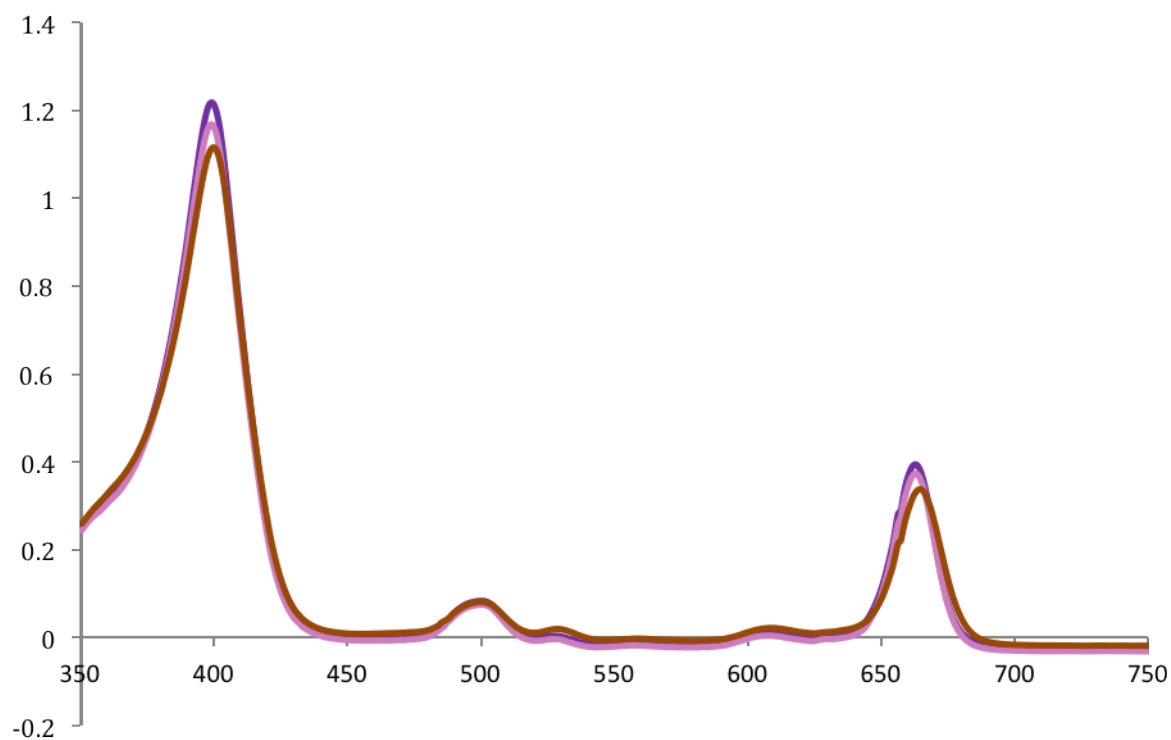


Figure S1. Normalized absorbance spectra of 17³-LysCe₆TME (**3**, brown line), 13¹-β-AlaAspCe₆DME (**11**, purple line) and 13¹-EDLysCe₆DME (**13**, pink line) in acetone.

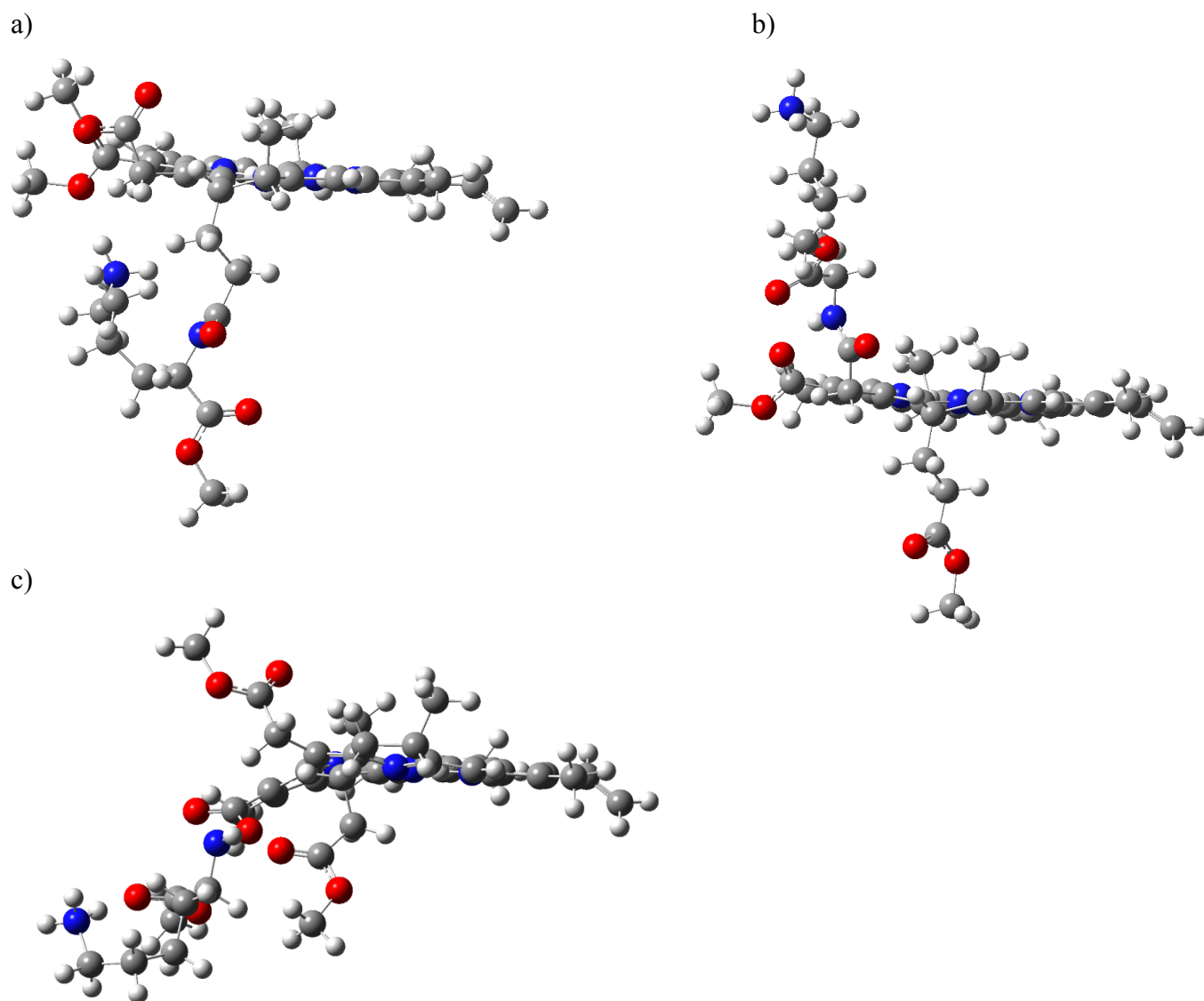


Figure S2. Energy minimized conformations in water phase for chlorin e_6 derivatives (a) **3**, (b) **7b** and (c) **13**. Optimization by energy was carried out at HF/6-31G level.

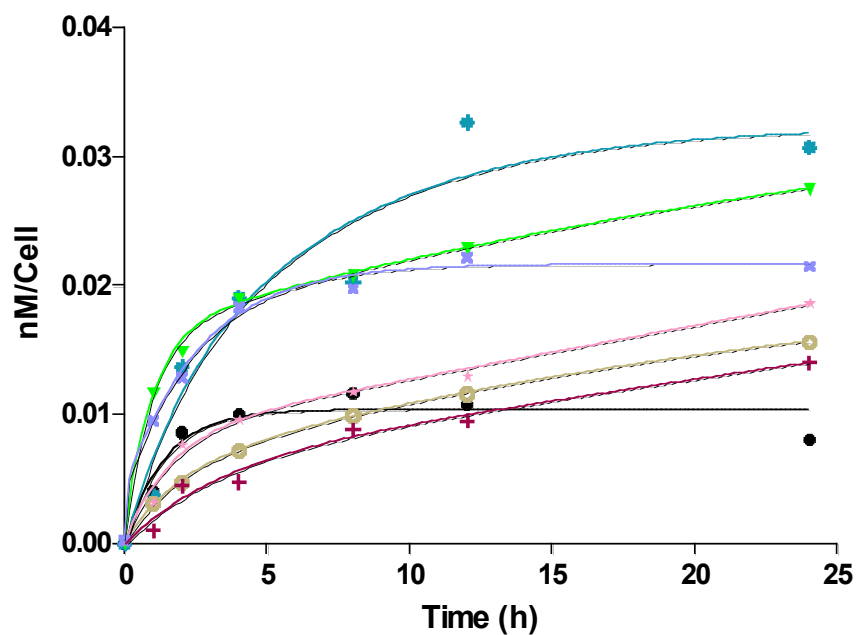


Figure S3. Time-dependent uptake of chlorin e_6 (**5**, black line) and its derivatives 17³-LysChlorin e_6 TME (**3**, brown line), 15²-AspChlorin e_6 DME (**7a**, light blue line), 15²-AspPdChlorin e_6 DME (**8a**, green line), 13¹-AspChlorin e_6 DME (**9**, maroon line), 13¹-βAlaAspChlorin e_6 DME (**11**, purple line) and 13¹-EDLysChlorin e_6 DME (**13**, pink line) at 10 μ M by HEp2 cells.

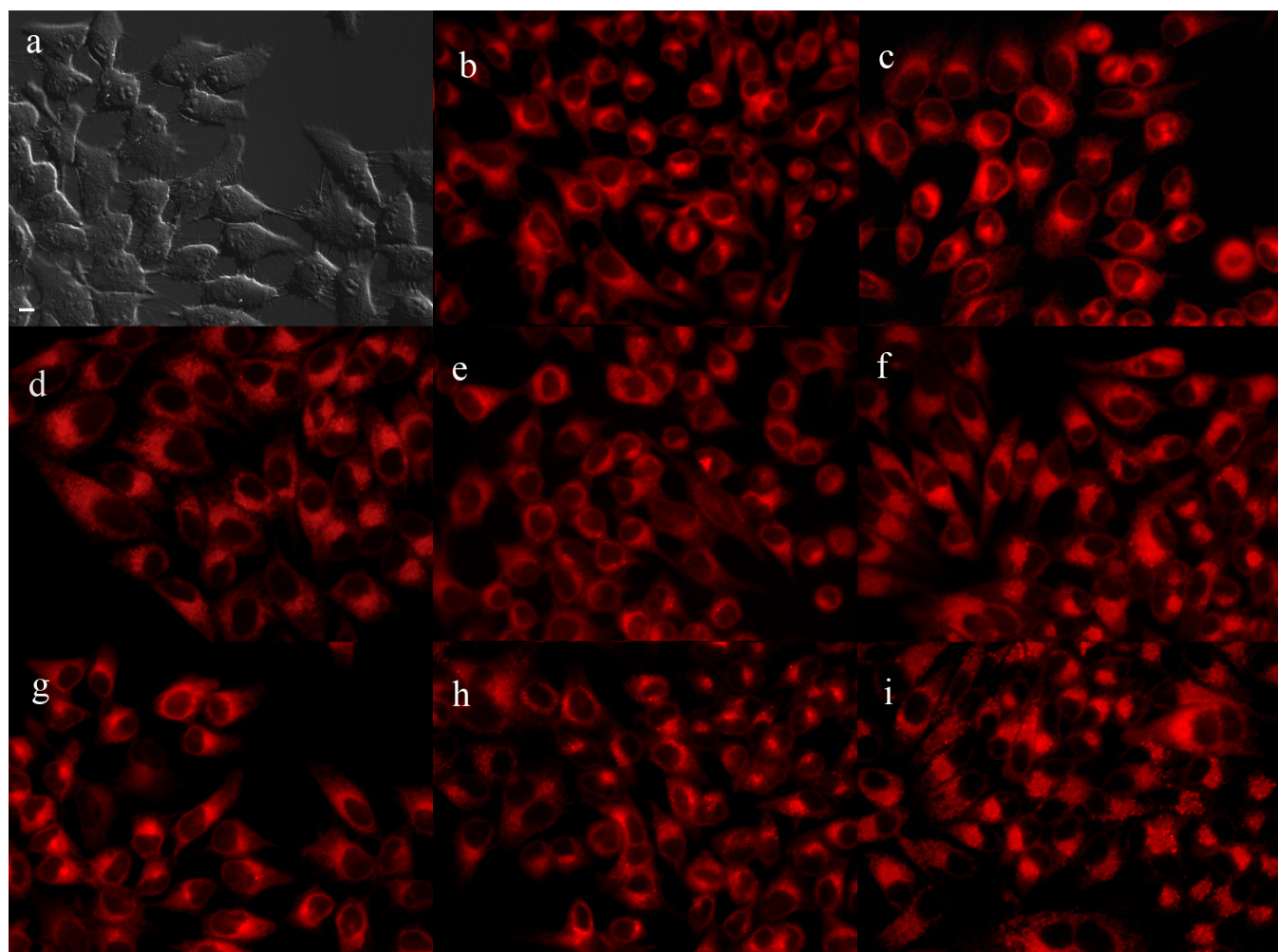


Figure S4. Subcellular fluorescence of chlorin e_6 derivatives in HEp2 cells at 10 μM for 18 h. (a)

Phase contrast, (b) **3**, (c) **7a**, (d) **7b**, (e) **8a**, (f) **8b**, (g) **9**, (h) **11**, (i) **13**. Scale bar: 10 μm .

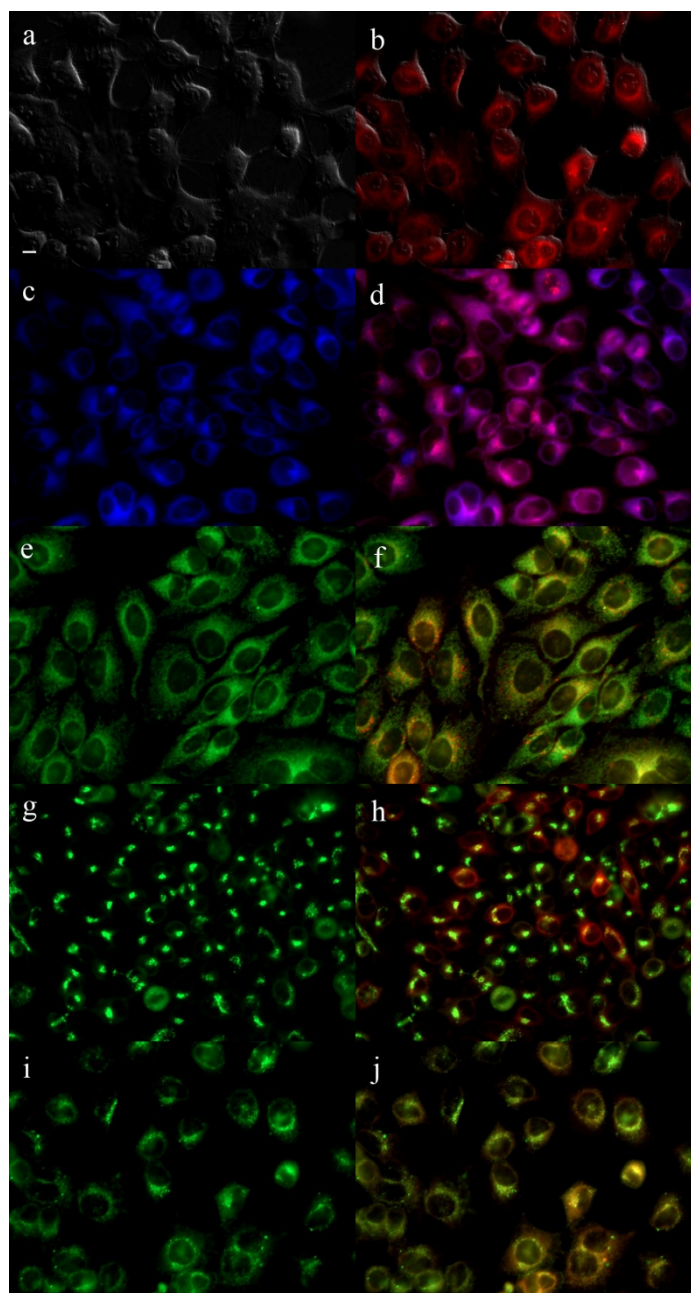


Figure S5. Subcellular localization of chlorin-e₆ in HEP-2 cells at 10 μ M for 18 h (a) phase contrast, (b) overlay of chlorin-e₆ and phase contrast, (c) ER tracker Blue/White fluorescence (e) MitoTracker Green fluorescence, (g) BoDIPY Ceramide, (i) LysoSensor Green fluorescence, and (d, f, h, j) overlays of organelle tracers with compound fluorescence. Scale bar: 10 μ m.

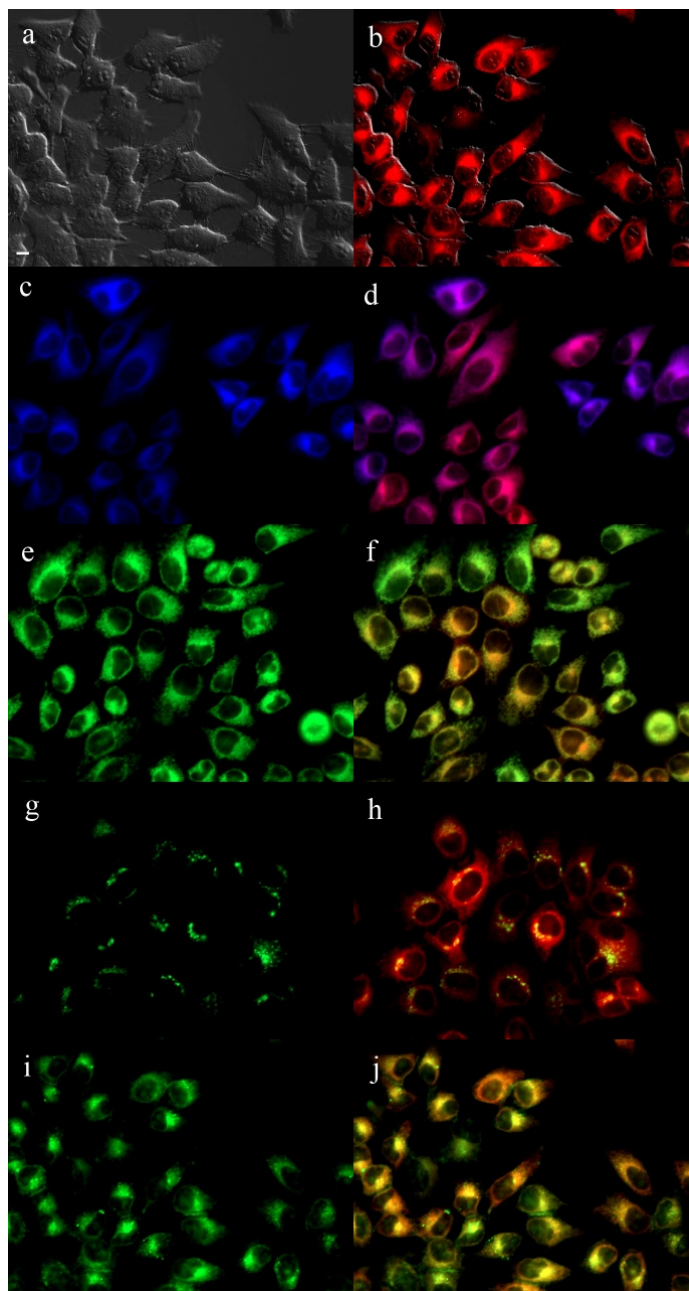


Figure S6. Subcellular localization of conjugate 15^2 -AspCe₆DME (**7a**) in HEp-2 cells at 10 μ M for 18 h (a) phase contrast, (b) overlay of conjugate **7a** and phase contrast, (c) ER tracker Blue/White fluorescence (e) MitoTracker Green fluorescence, (g) BoDIPY Ceramide, (i) LysoSensor Green fluorescence, and (d, f, h, j) overlays of organelle tracers with compound fluorescence. Scale bar: 10 μ m.

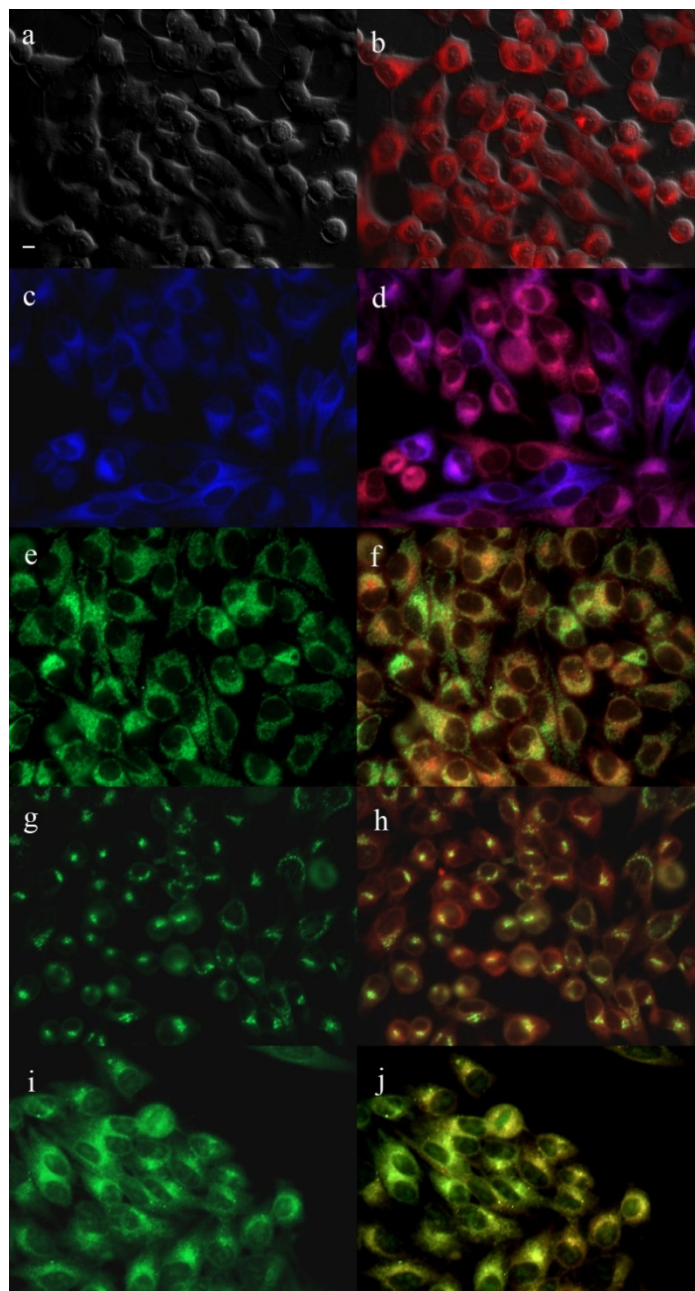


Figure S7. Subcellular localization of conjugate $15^2\text{-PdAspCe}_6\text{DME}$ (**8a**) in HEp-2 cells at 10 μM for 18 h (a) phase contrast, (b) overlay of conjugate **8a** and phase contrast, (c) ER tracker Blue/White fluorescence (e) MitoTracker Green fluorescence, (g) BoDIPY Ceramide, (i) LysoSensor Green fluorescence, and (d, f, h, j) overlays of organelle tracers with compound fluorescence. Scale bar: 10 μm .

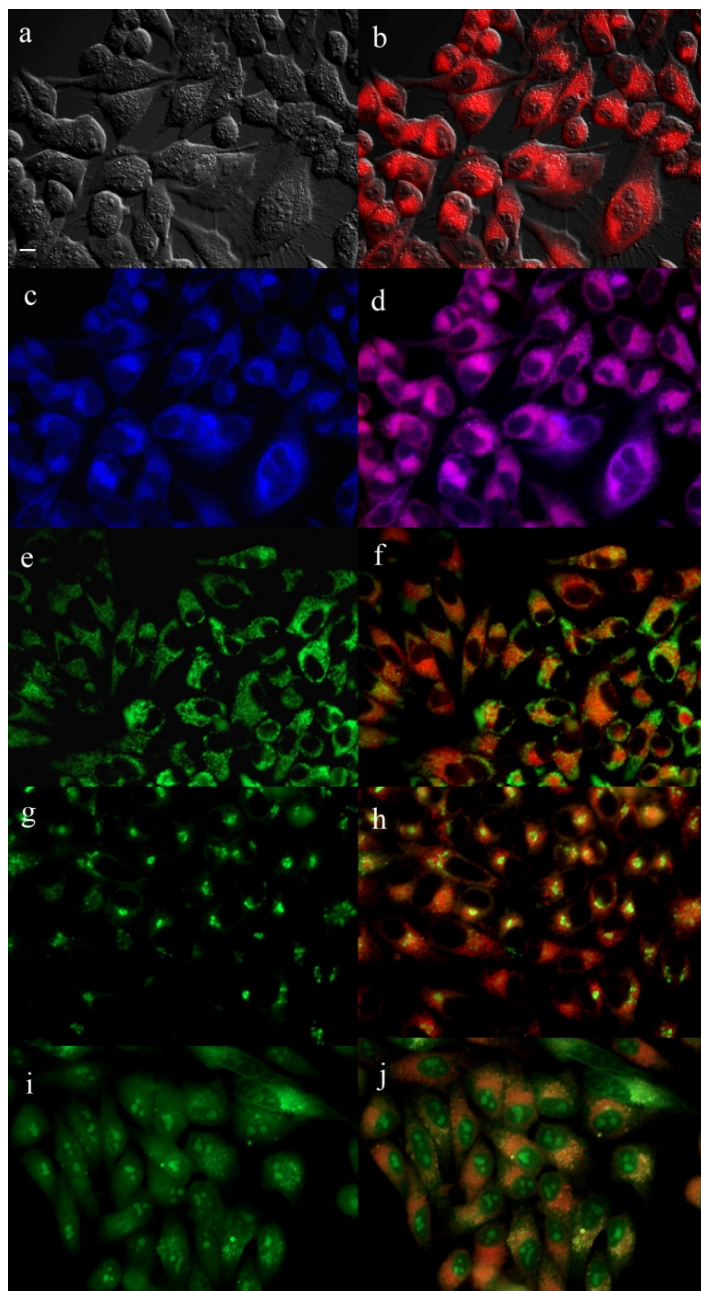


Figure S8. Subcellular localization of conjugate 15^2 -LysCe₆TME (**7b**) in Hep-2 cells at 10 μ M for 18 h (a) phase contrast, (b) overlay of conjugate **7b** and phase contrast, (c) ER tracker Blue/White fluorescence (e) MitoTracker Green fluorescence, (g) BoDIPY Ceramide, (i) LysoSensor Green fluorescence, and (d, f, h, j) overlays of organelle tracers with compound fluorescence. Scale bar: 10 μ m.

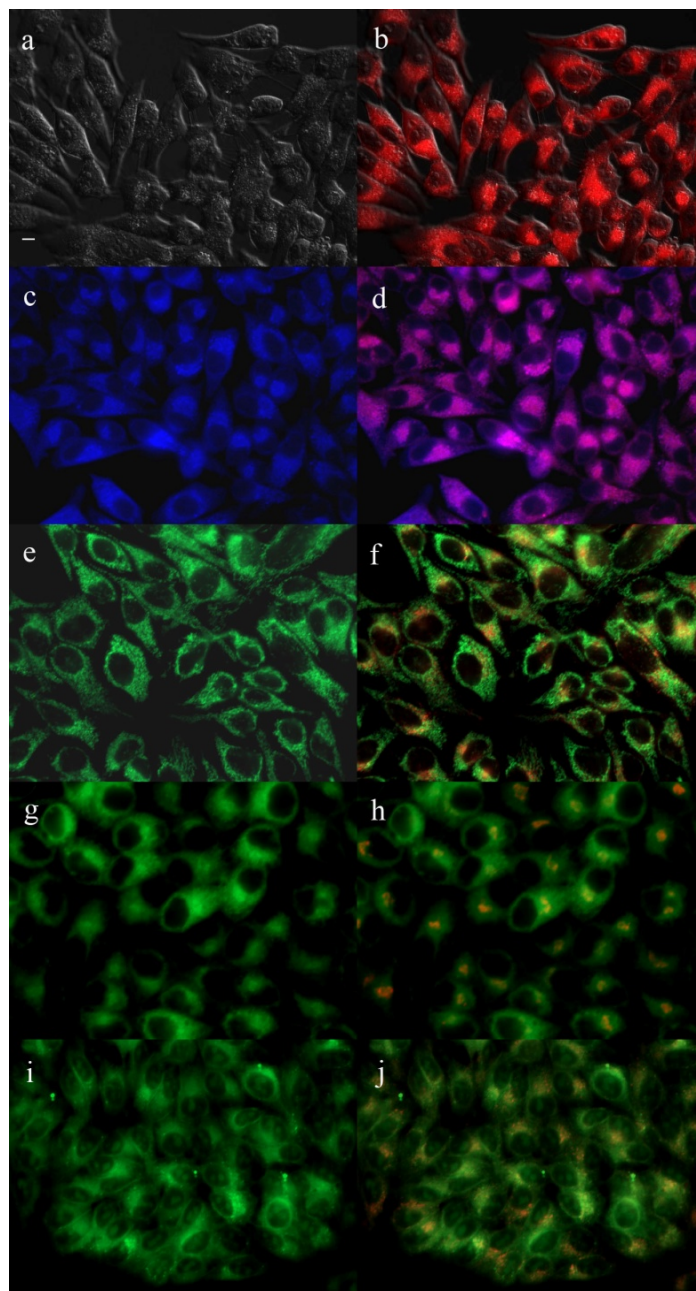


Figure S9. Subcellular localization of conjugate 15^2 -PdLysCe₆TME (**8b**) in HEp-2 cells at 10 μ M for 18 h (a) phase contrast, (b) overlay of conjugate **8b** and phase contrast, (c) ER tracker Blue/White fluorescence (e) MitoTracker Green fluorescence, (g) BoDIPY Ceramide, (i) LysoSensor Green fluorescence, and (d, f, h, j) overlays of organelle tracers with compound fluorescence. Scale bar: 10 μ m.

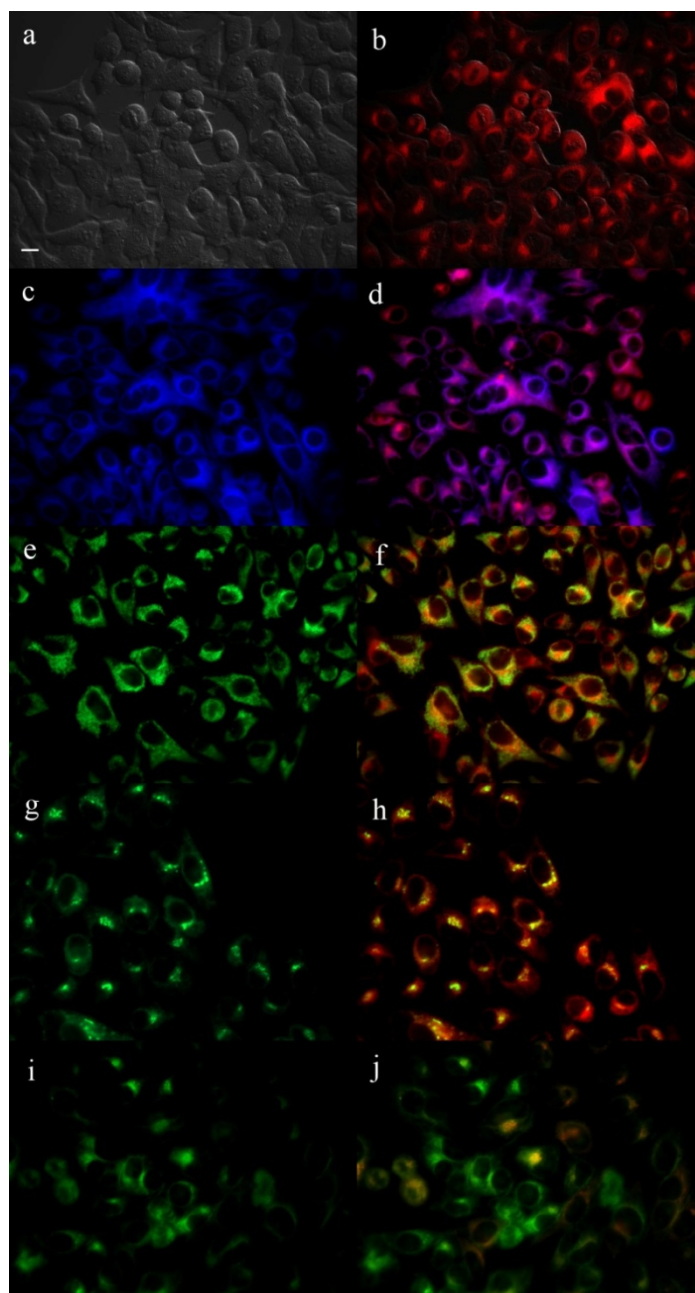
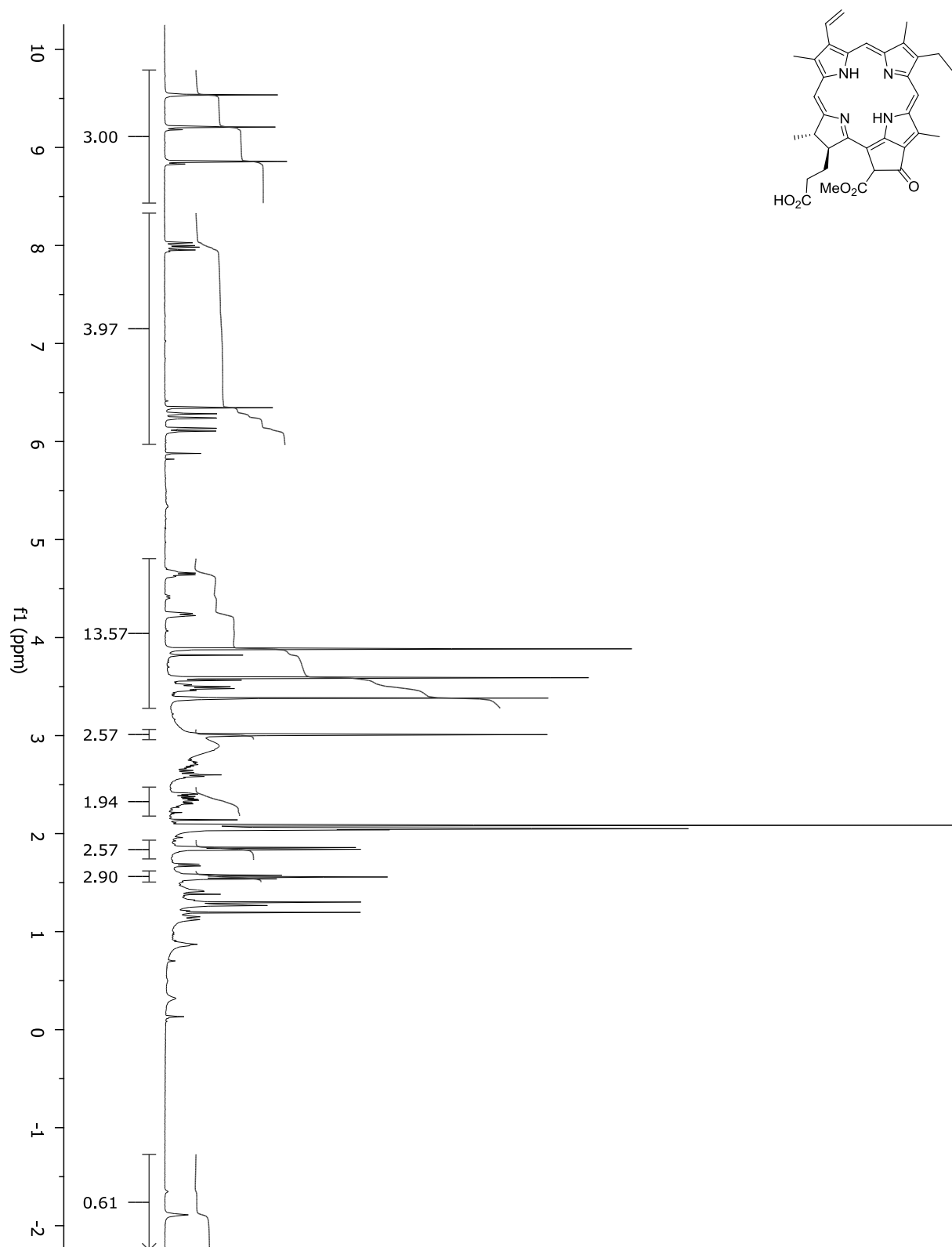
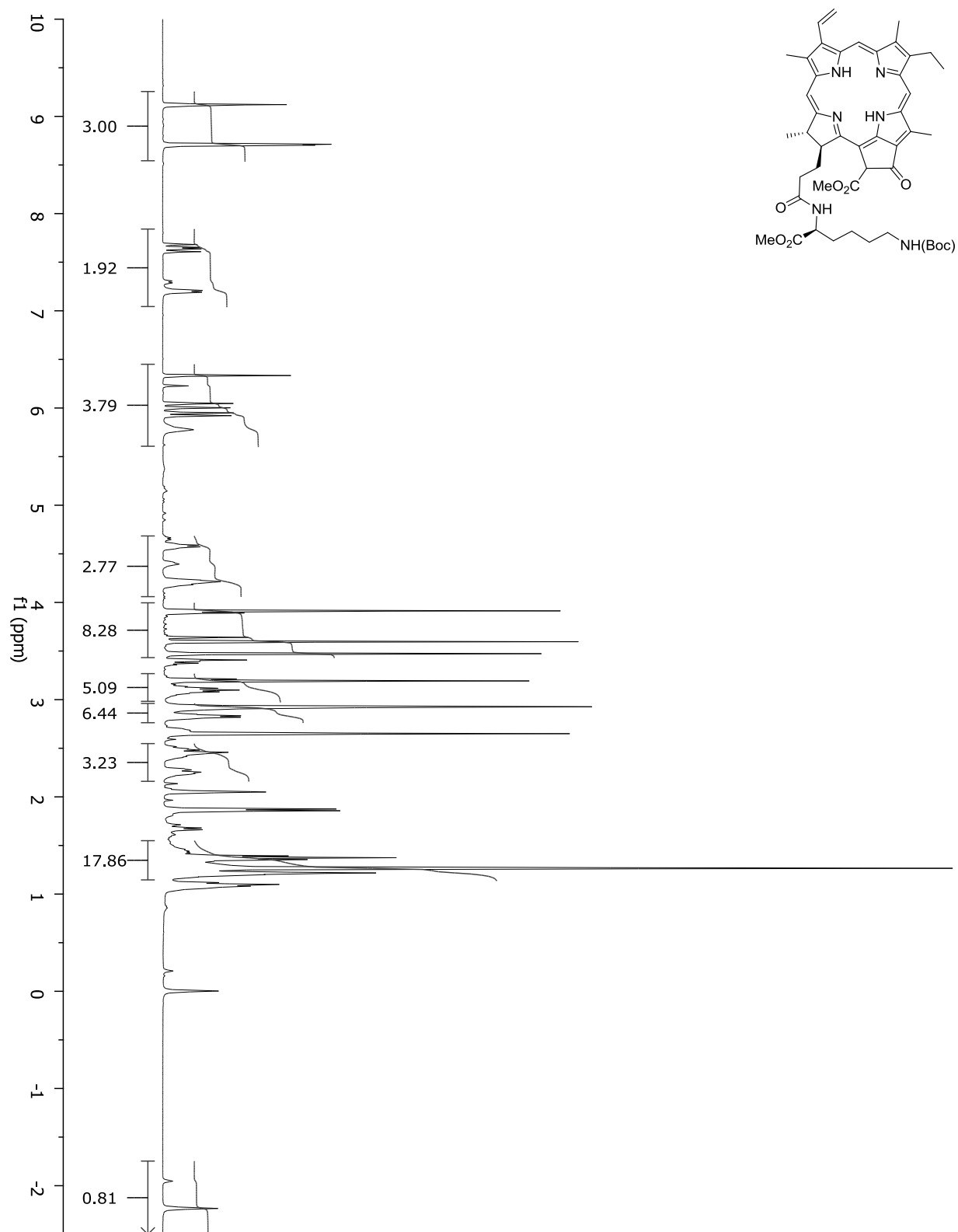


Figure S10. Subcellular localization of conjugate $17^3\text{-LysCe}_6\text{TME}$ (**3**) in HEp-2 cells at $10\ \mu\text{M}$ for 18 h (a) phase contrast, (b) overlay of conjugate **3** and phase contrast, (c) ER tracker Blue/White fluorescence (e) MitoTracker Green fluorescence, (g) BoDIPY Ceramide, (i) LysoSensor Green fluorescence, and (d, f, h, j) overlays of organelle tracers with compound fluorescence. Scale bar: $10\ \mu\text{m}$.

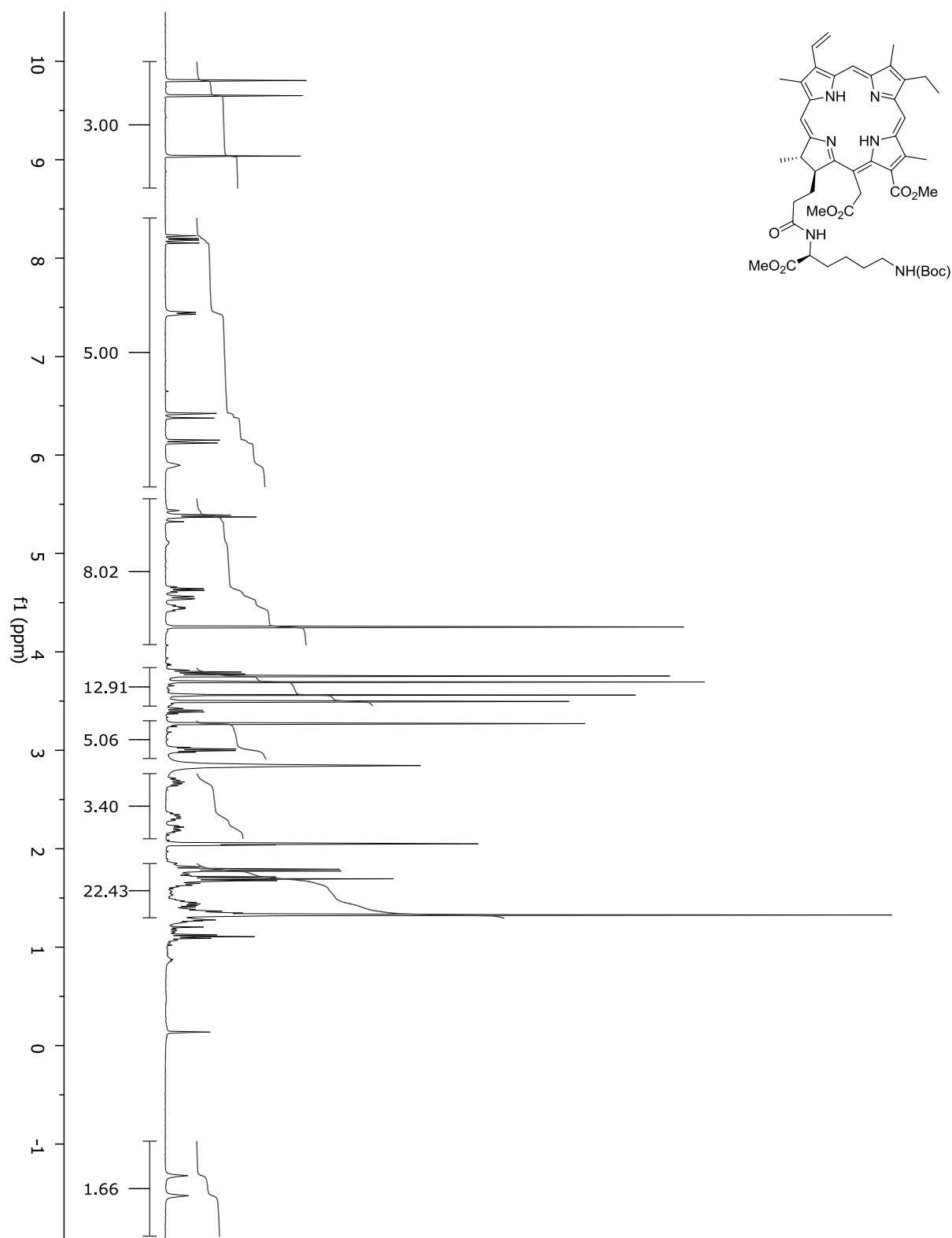
^1H NMR of Pheophorbide-a in $(\text{CD}_3)_2\text{CO}$ at 400 MHz



^1H NMR of Lys(Boc)-OMe pheophorbide-a (Compound 2) in $(\text{CD}_3)_2\text{CO}$ at 400 MHz

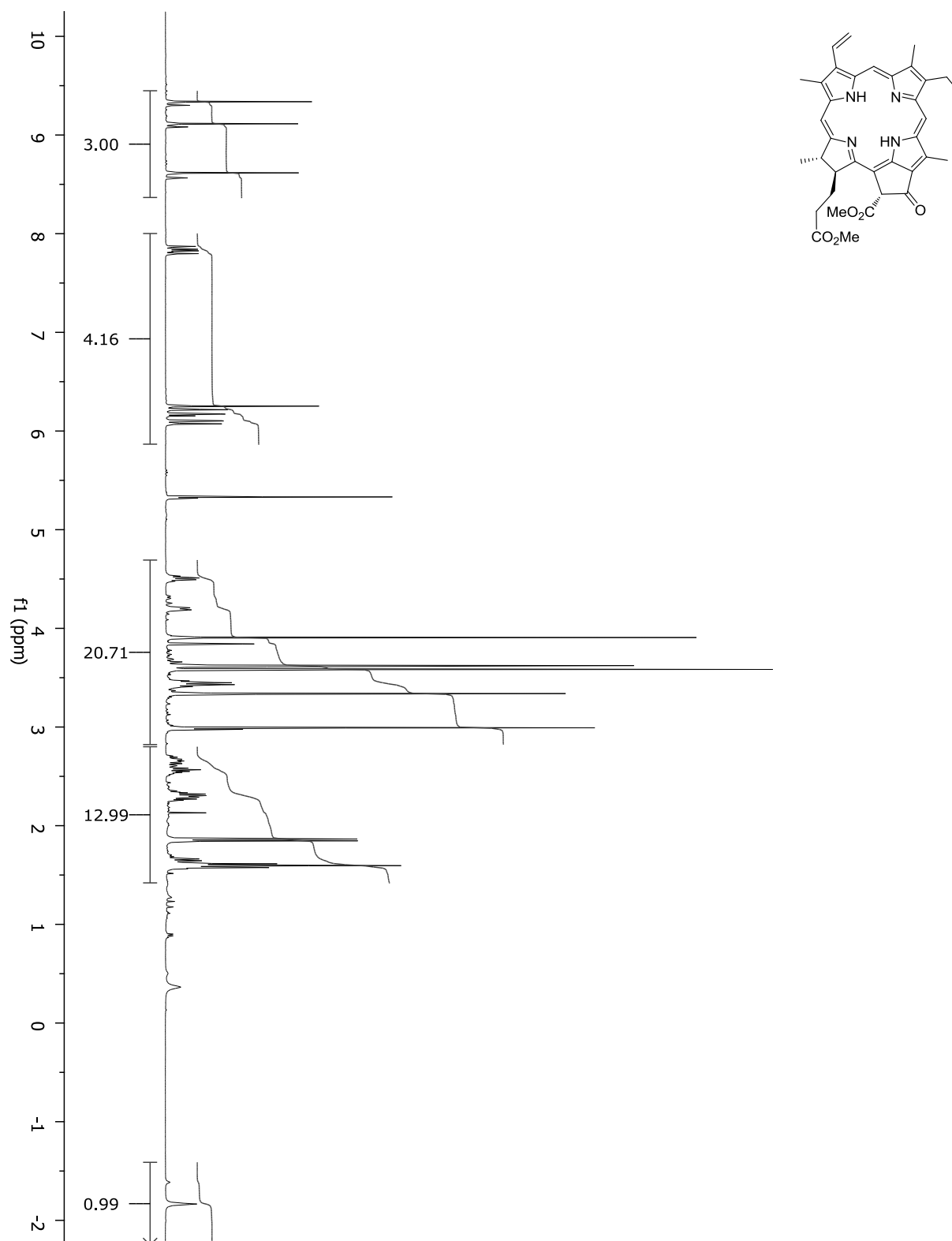


^1H NMR of 17^3 Lys(Boc)Ce₆TME in (CD₃)₂CO at 400 MHz

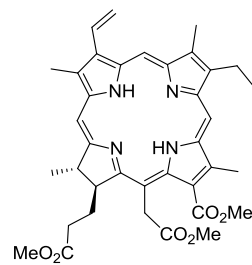
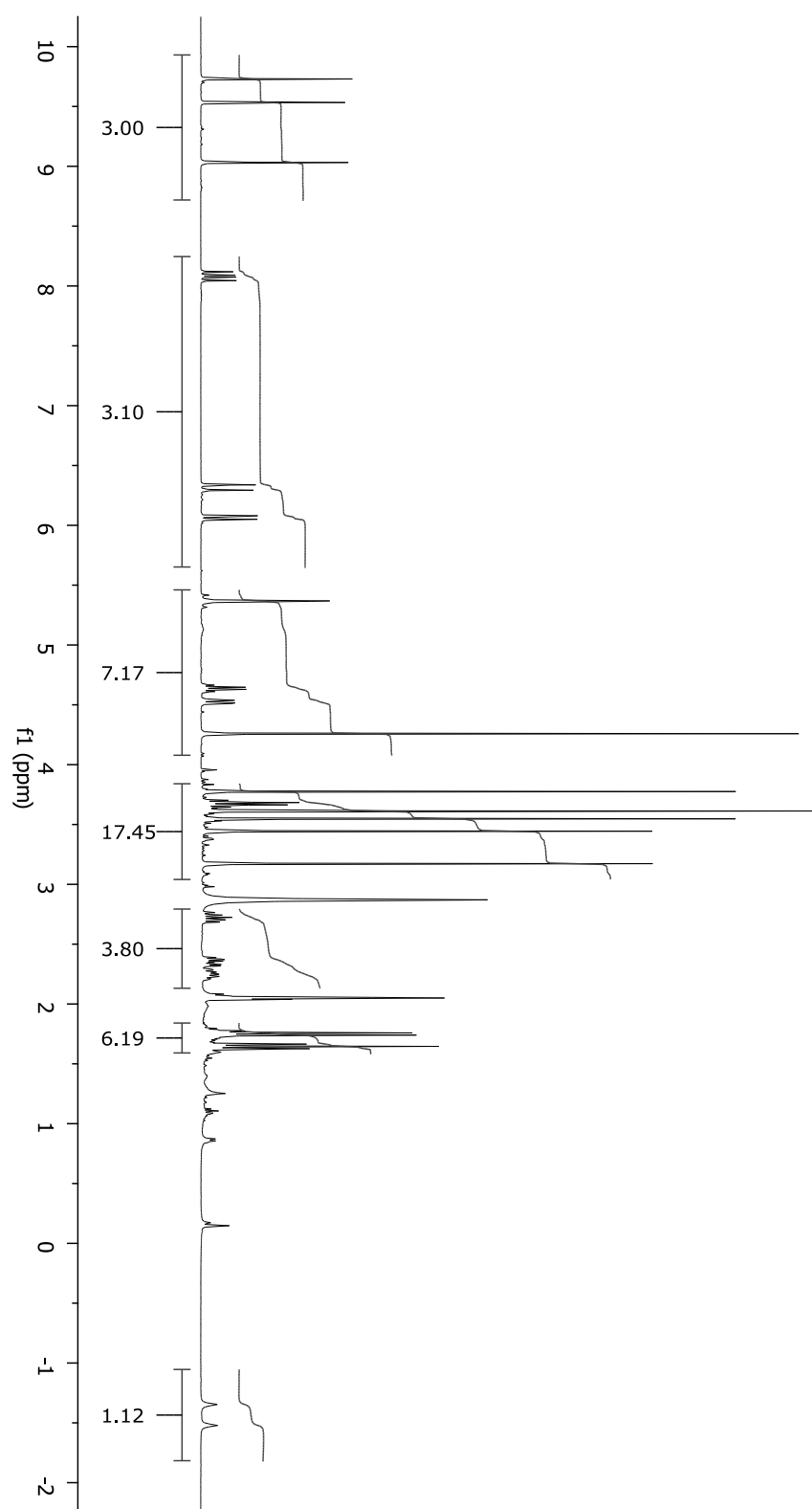


[illegible]

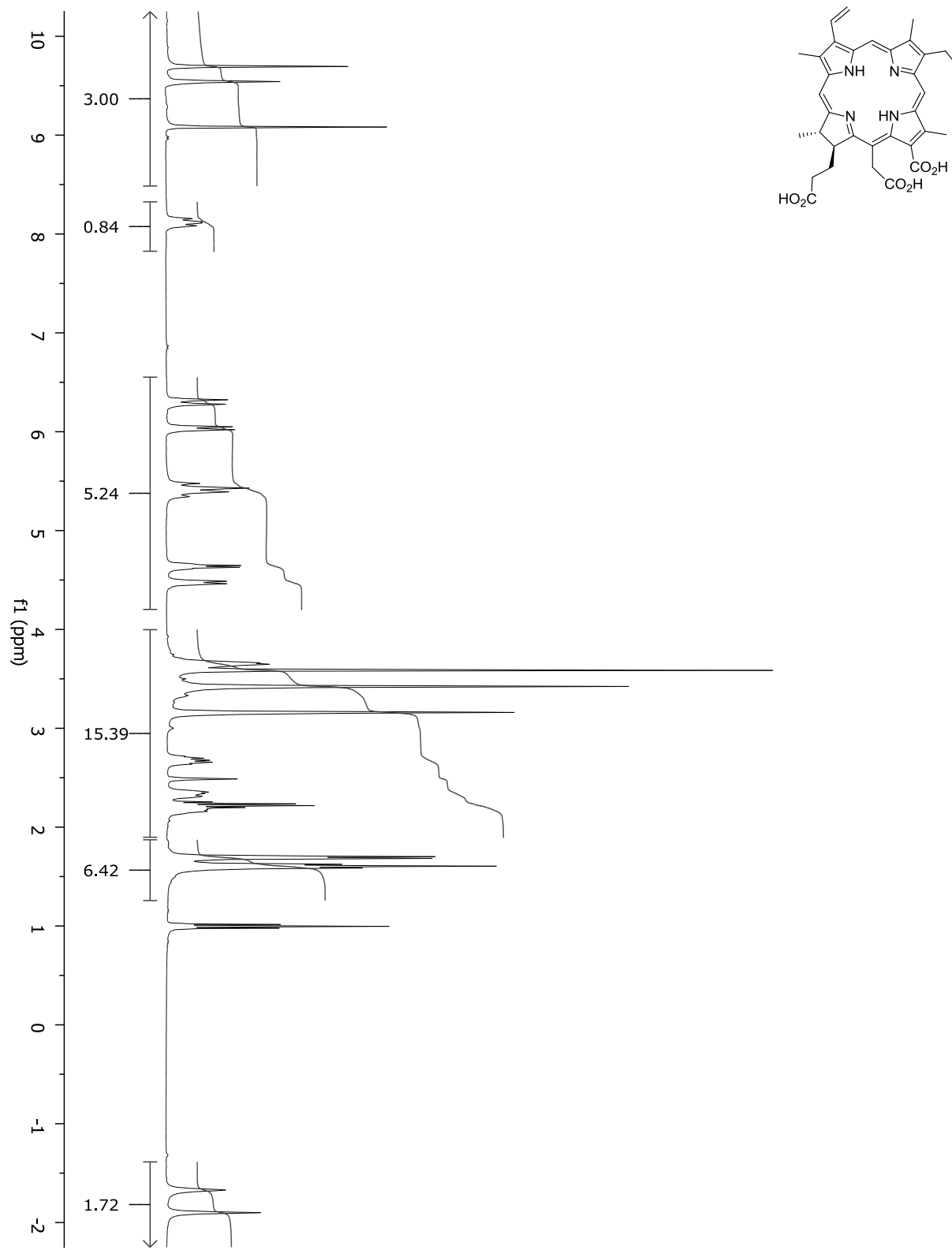
^1H NMR of Methyl Pheophorbide-a (Compound 4) in $(\text{CD}_3)_2\text{CO}$ at 400 Mhz



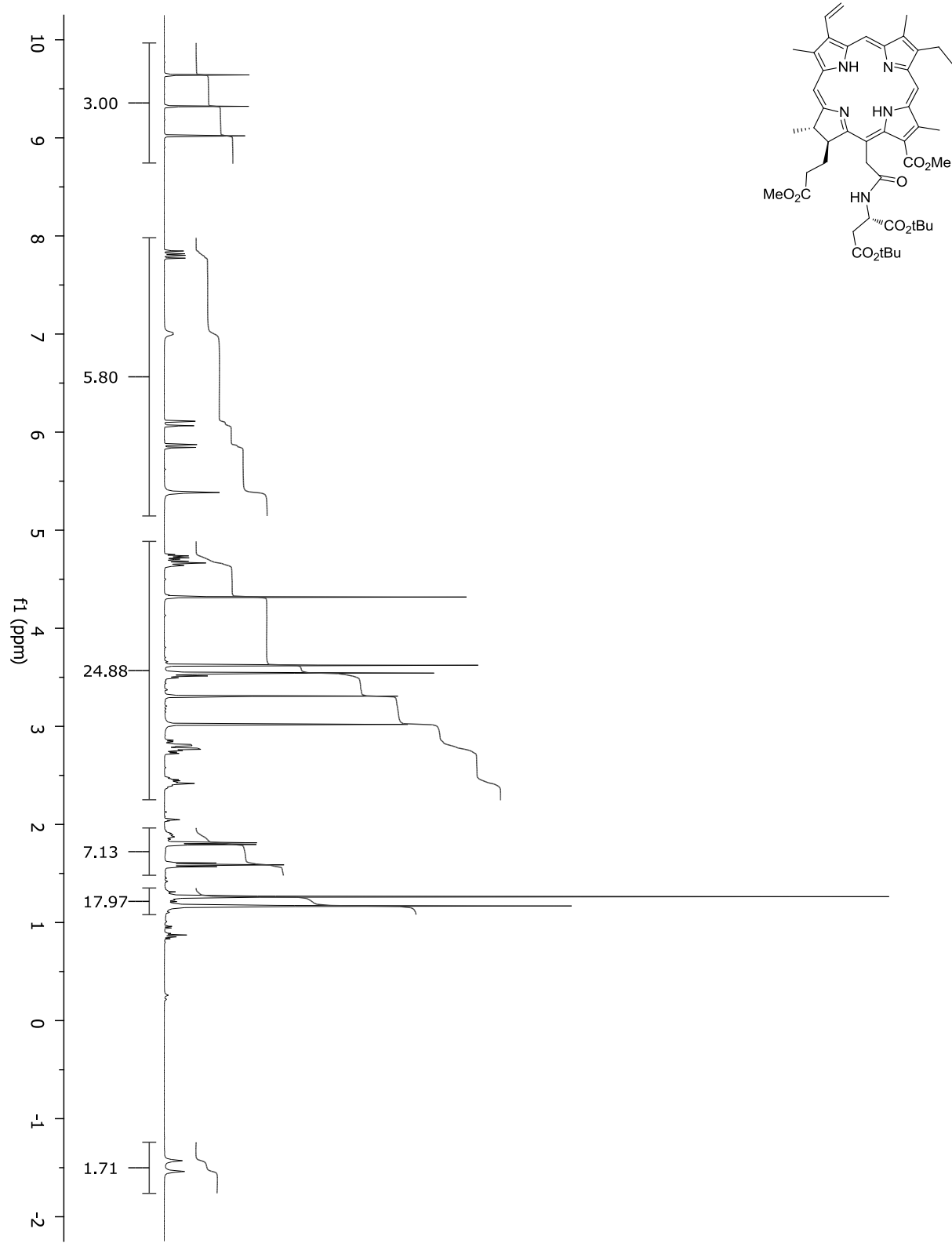
^1H NMR of Ce₆TME in (CD₃)₂CO at 400 MHz



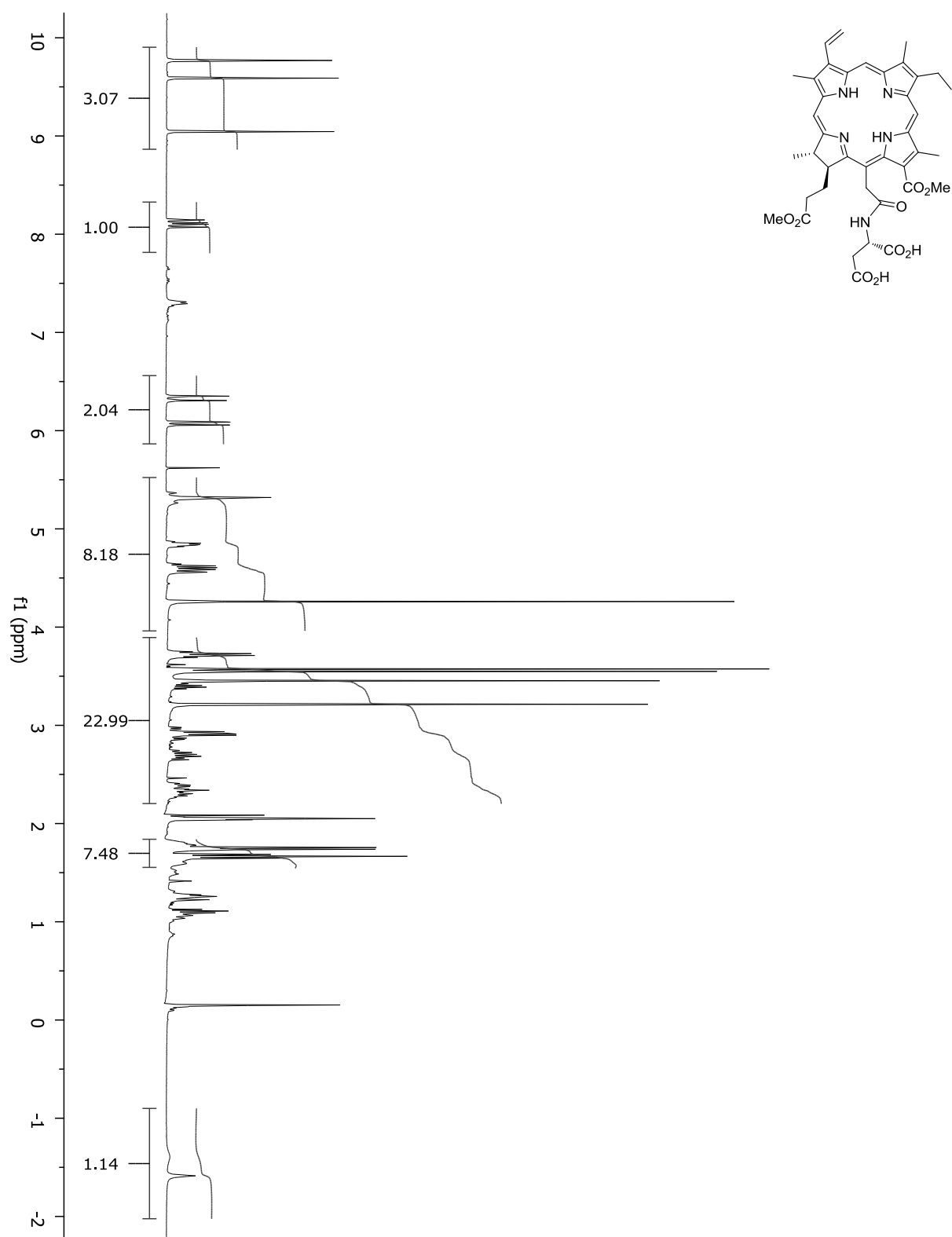
^1H NMR of Ce_6 (Compound 5) in d-DMSO at 400 MHz

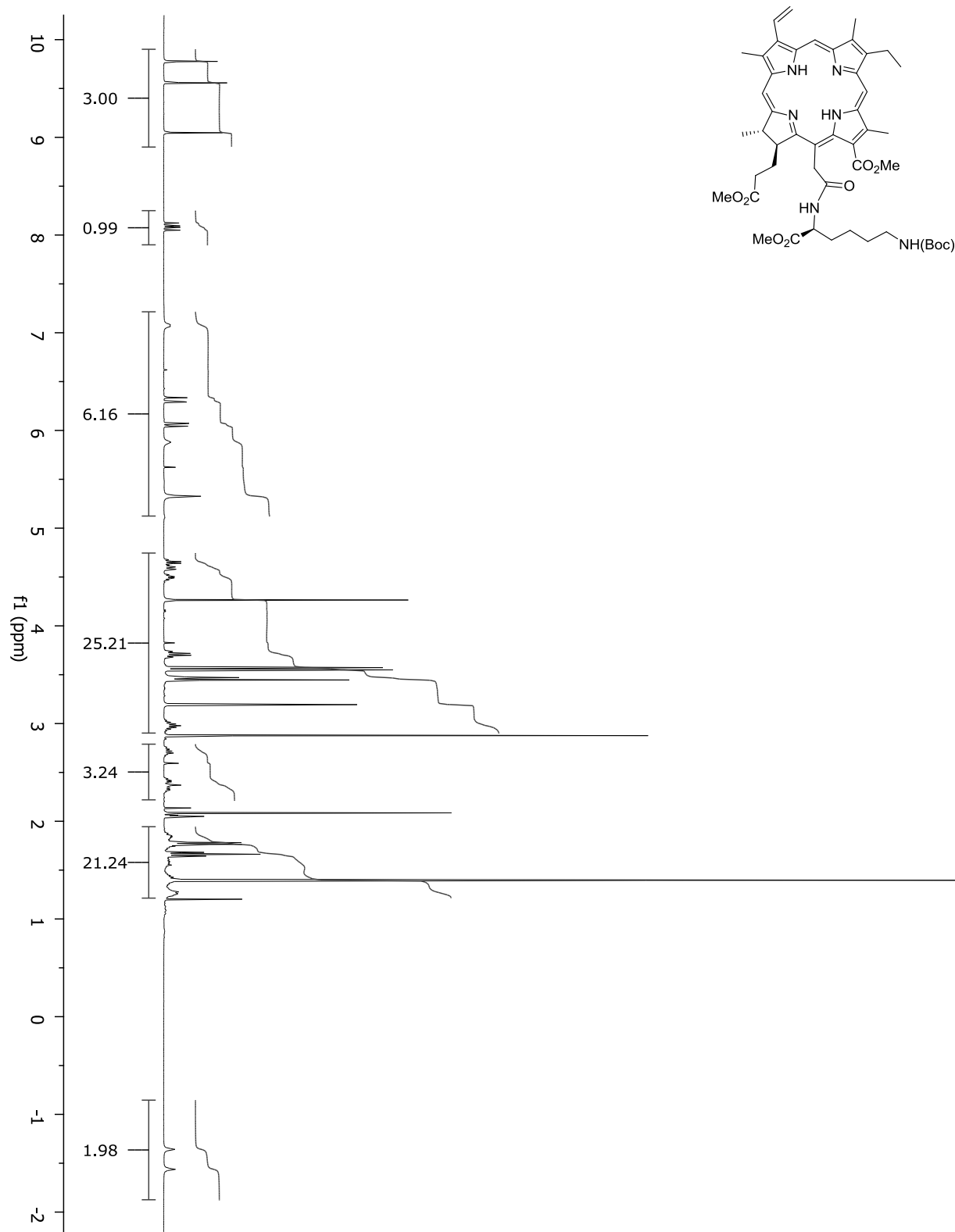


^1H NMR of 15^2 Aspdi(tBu)Ce₆DME in (CD₃)₂CO at 400 MHz



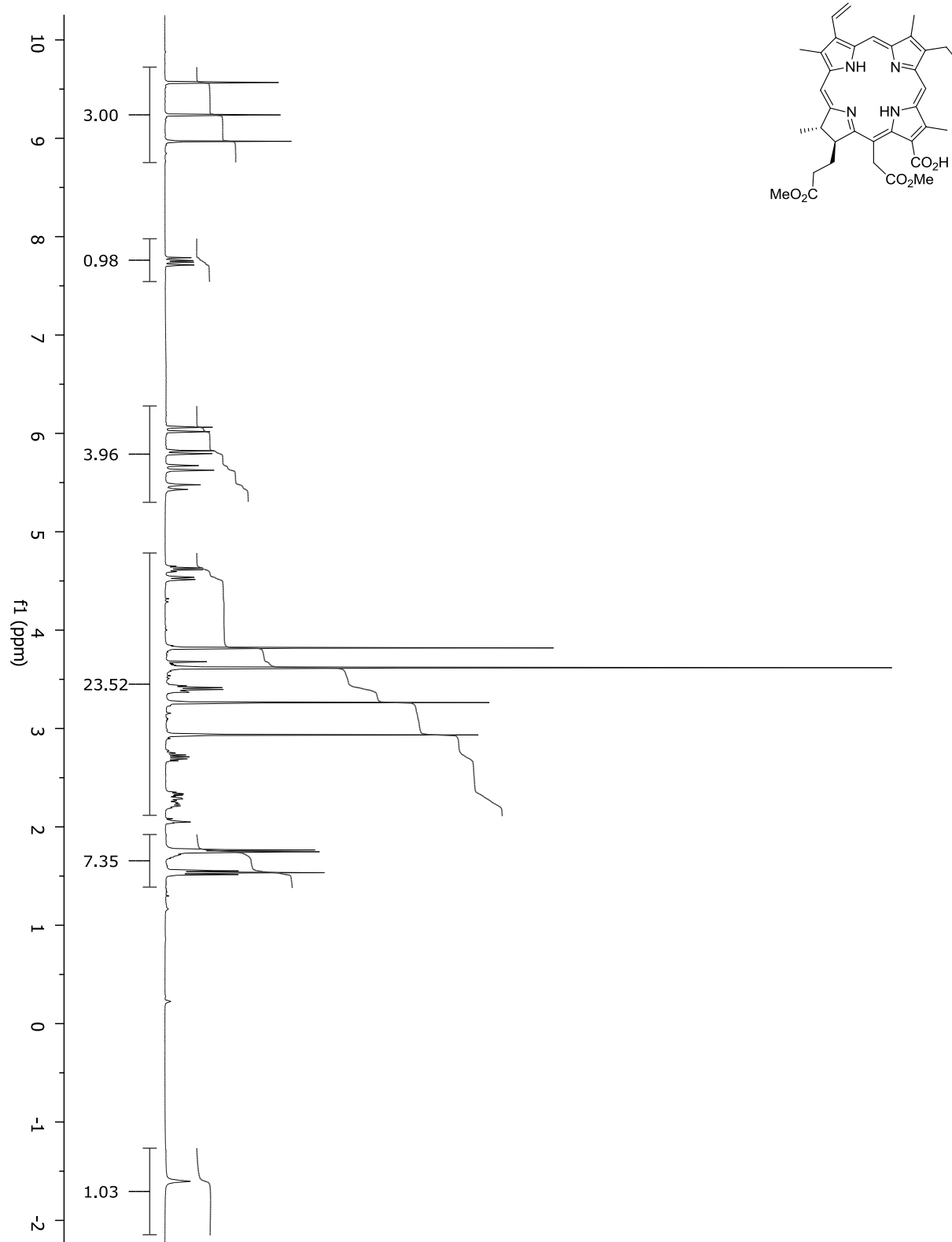
^1H NMR of 15^2 AspCe₆DME (Compound 7a) in $(\text{CD}_3)_2\text{CO}$ at 400 MHz



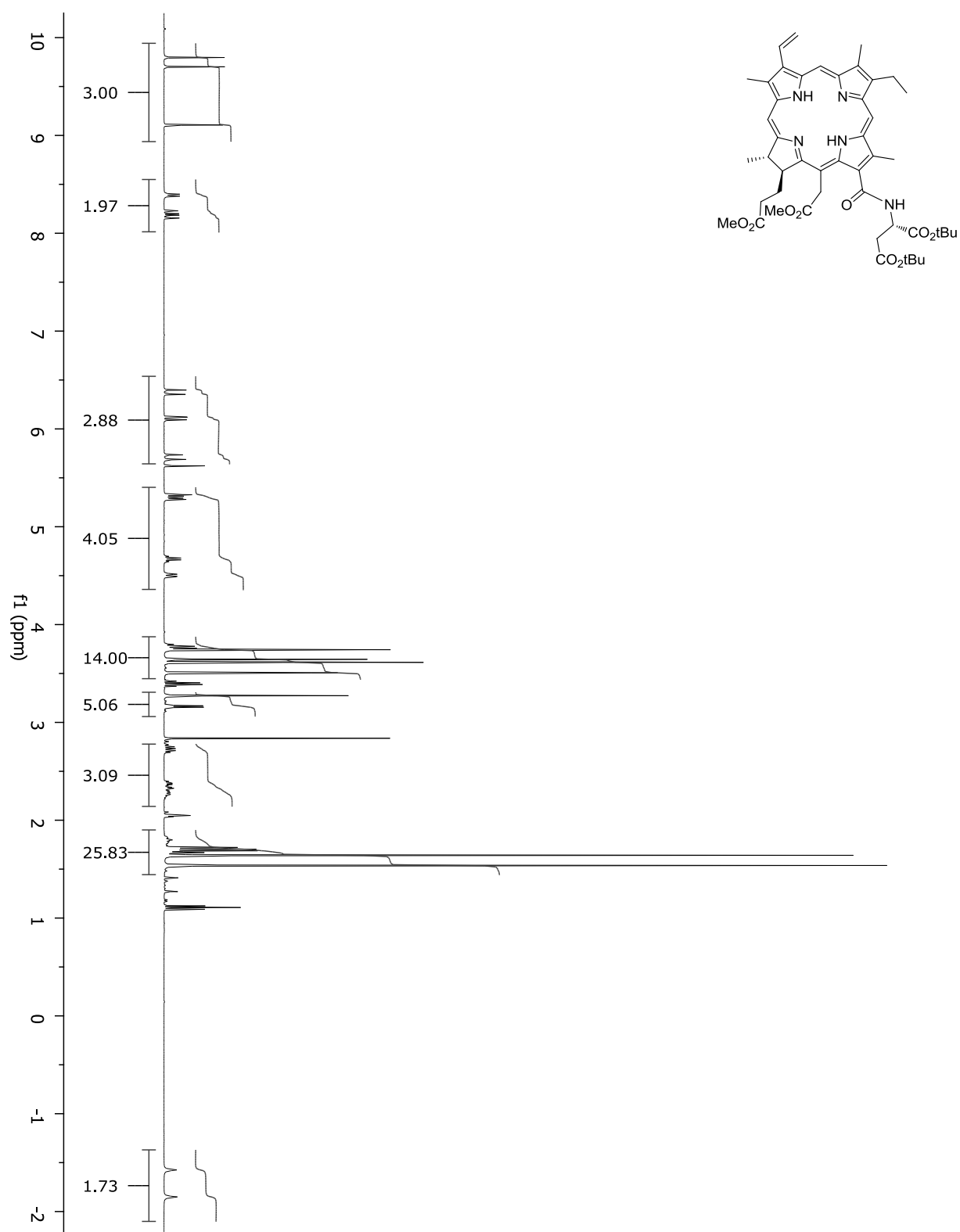
^1H NMR of 15^2 Lys(Boc)Ce₆TME in (CD₃)₂CO at 400MHz

Chemical structure of compound 10 is shown in the top right corner. The structure is a complex macrocyclic molecule with multiple nitrogen atoms and various functional groups, including a carboxylate group (CO₂Me) and a long alkyl chain (NH(CH₂)₄CH₂CO₂Me).

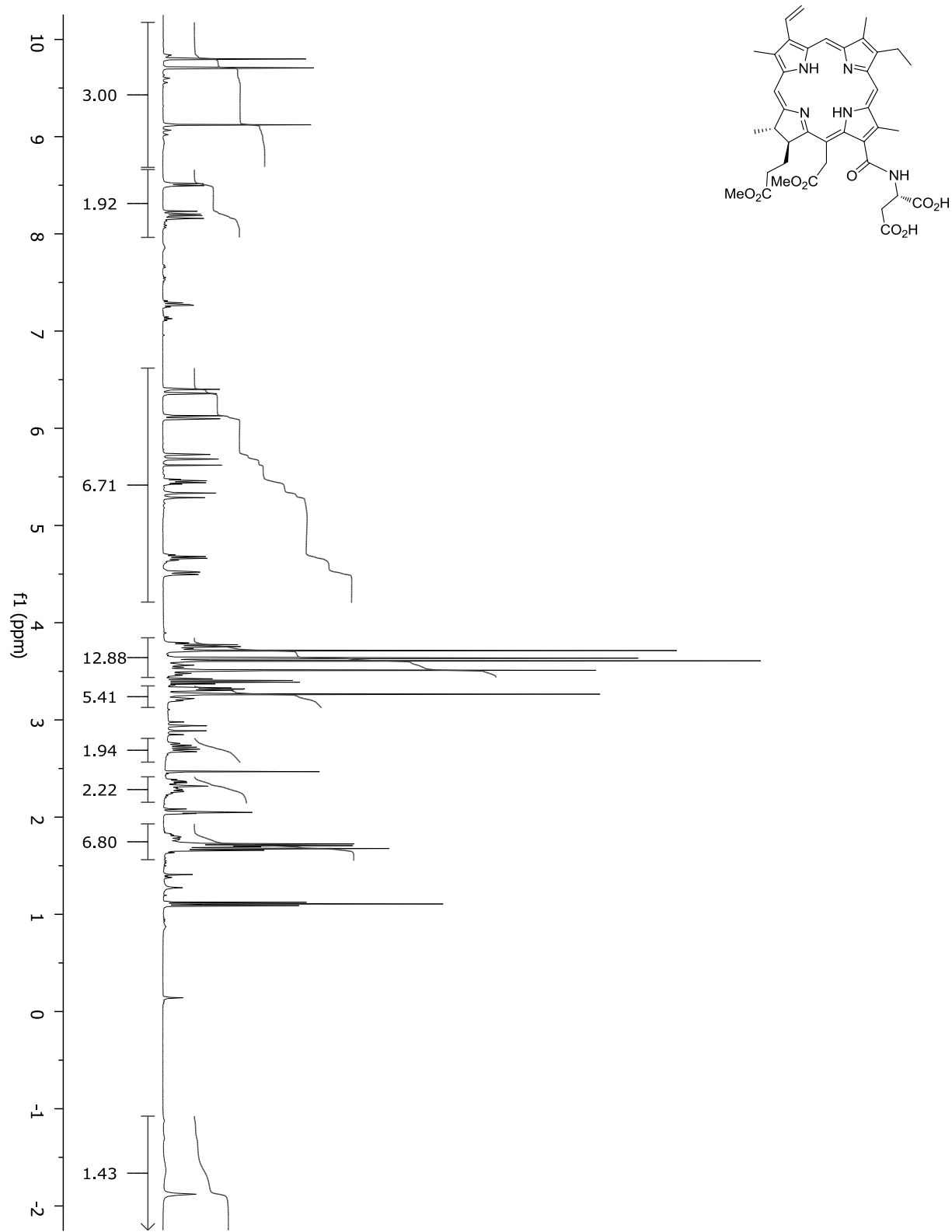
^1H NMR of Ce₆DME (Compound 6) in (CD₃)₂CO at 400 MHz



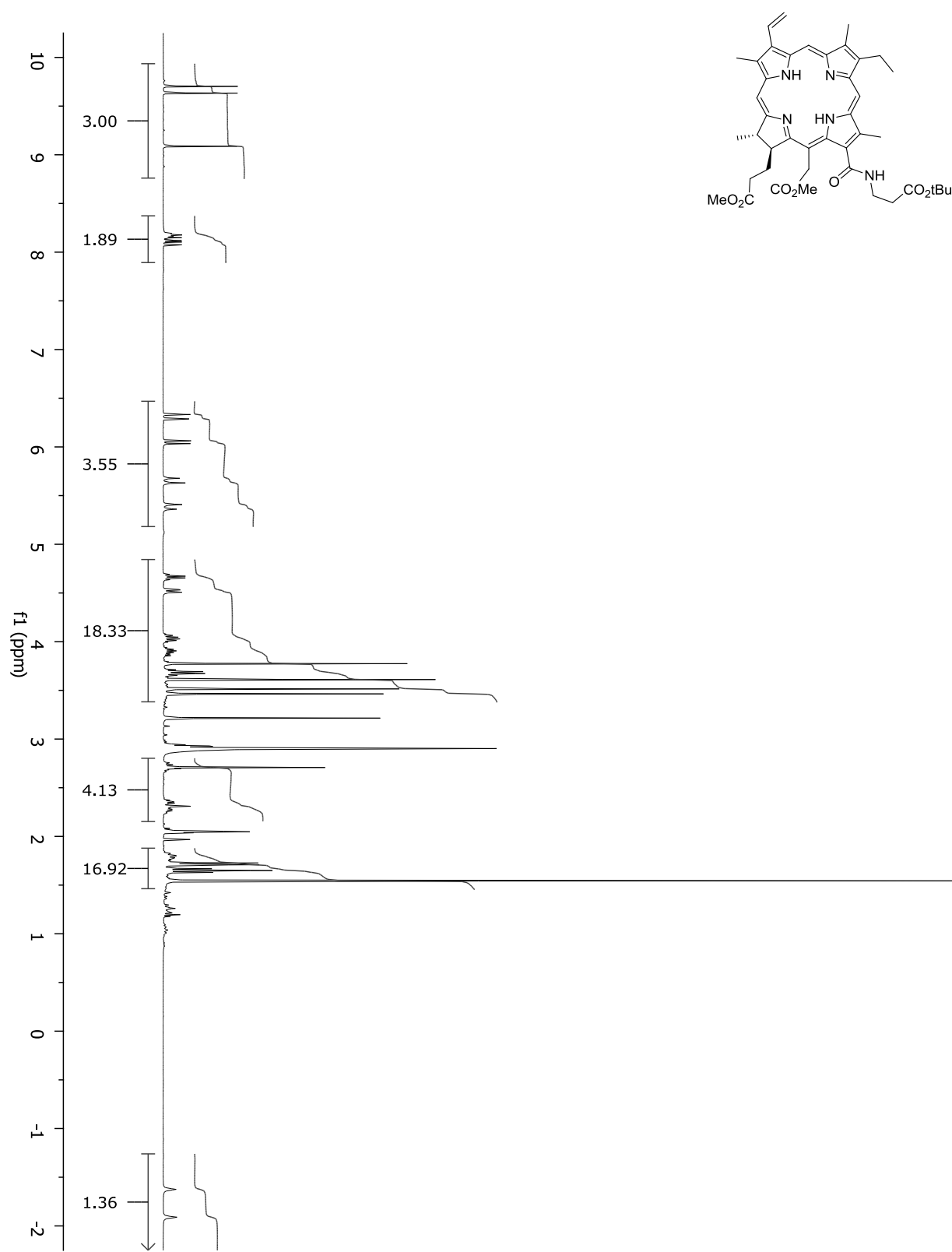
^1H NMR of 13^1 Aspdi(tBu)Ce₆DME in (CD₃)₂CO at 400 MHz



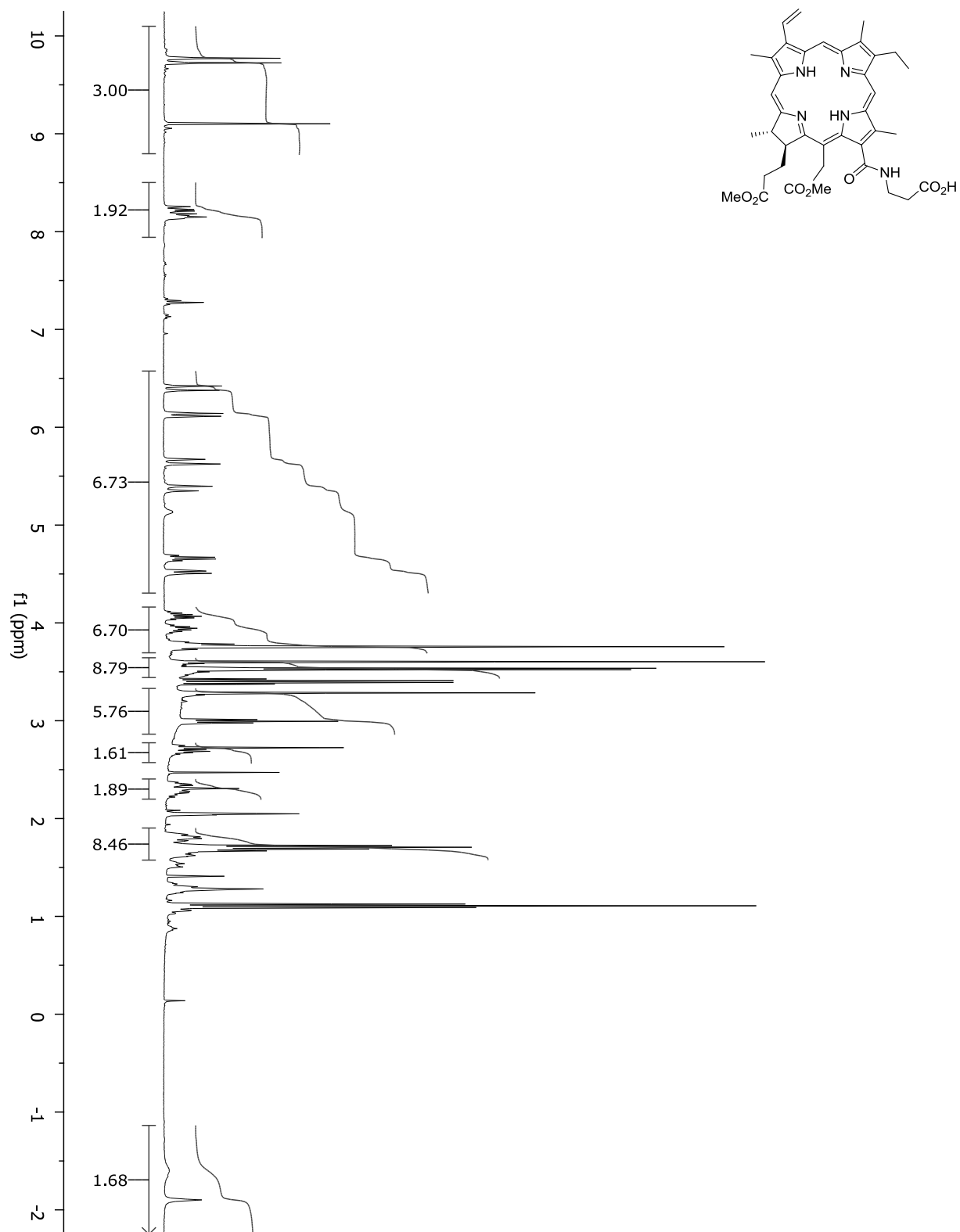
^1H NMR of ^{13}C AspCe₆DME (Compound 9) in $(\text{CD}_3)_2\text{CO}$ at 400 MHz



^1H NMR of ^{13}C b-Ala(tBu)Ce₆DME in (CD₃)₂CO at 400 MHz



^1H NMR of 13^1 b-AlaCe₆DME (Compound 10) in $(\text{CD}_3)_2\text{CO}$ at 400 MHz

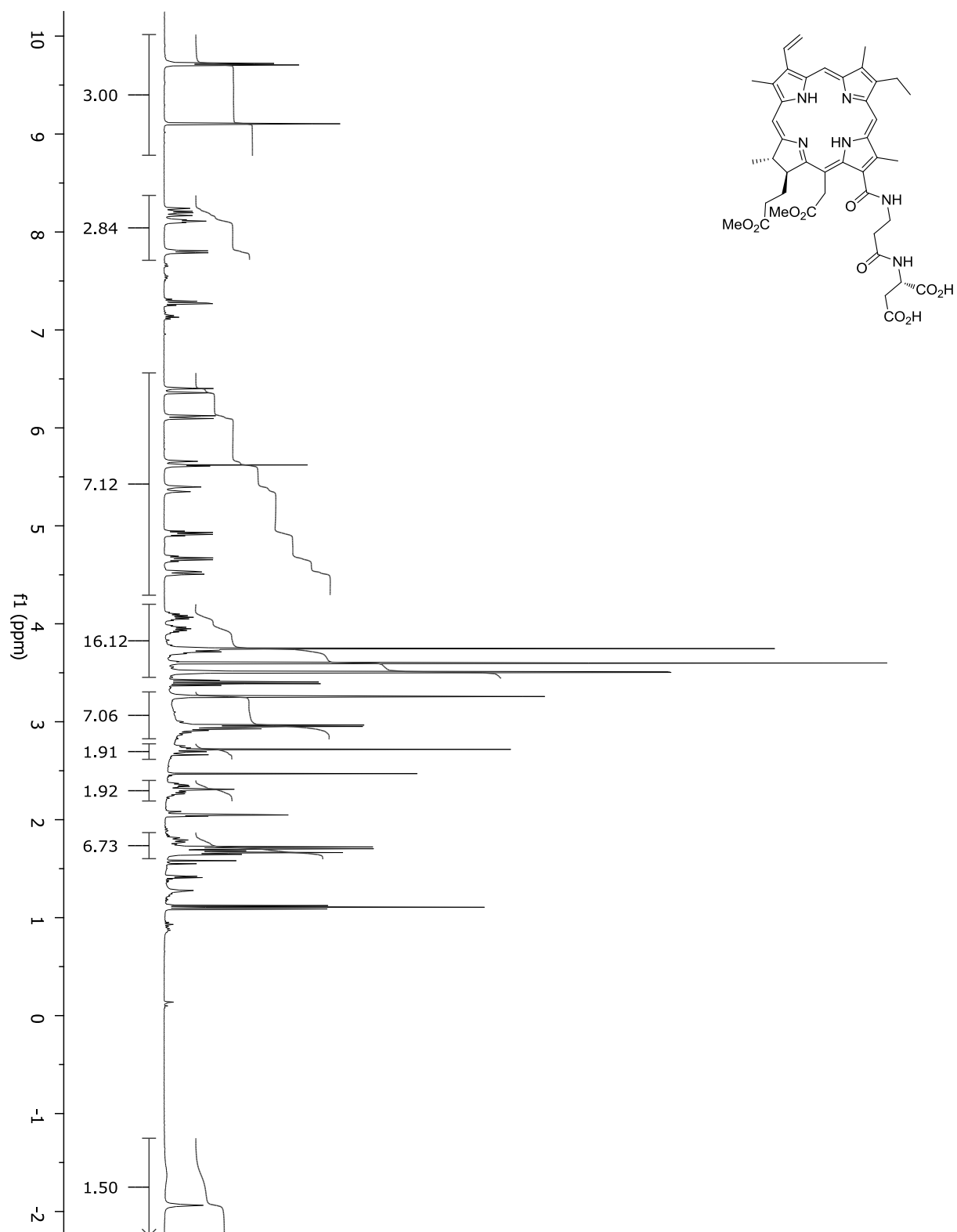


¹H NMR spectrum of compound 10 in CDCl₃. The x-axis represents chemical shift (ppm) from -2 to 10. The spectrum shows several peaks with corresponding integration values:

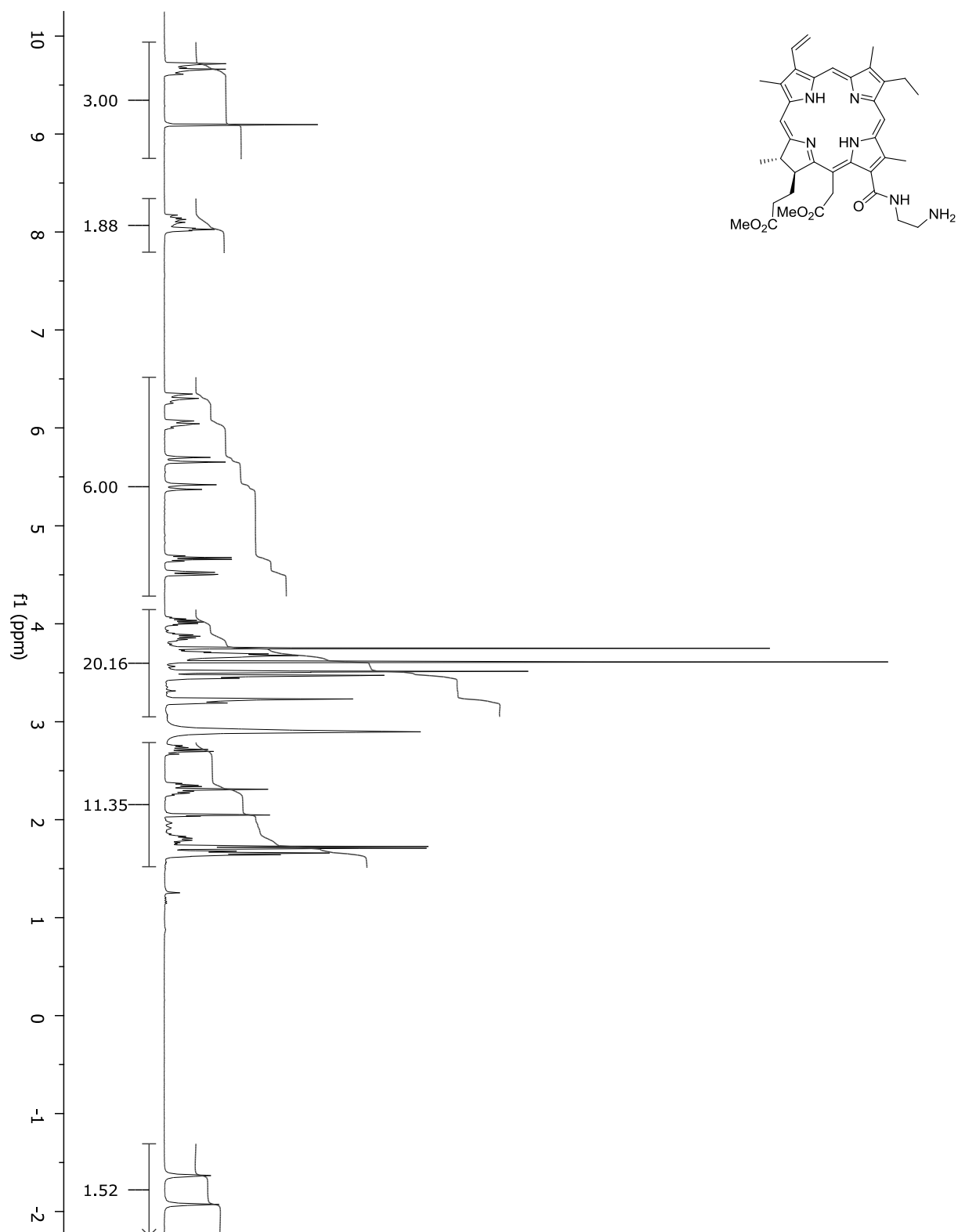
- 18.57 (NH, integration 18.57)
- 7.01 (aromatic protons, integration 7.01)
- 4.31 (CH₂ protons, integration 4.31)
- 3.00 (CH₃ protons, integration 3.00)
- 2.99 (CH₂ protons, integration 2.99)
- 2.27 (CH₃ protons, integration 2.27)
- 2.02 (CH₂ protons, integration 2.02)
- 1.99 (CH₃ protons, integration 1.99)
- 1.73 (CH₃ protons, integration 1.73)

The chemical structure of compound 10 is shown in the top right corner. It is a complex molecule featuring a central macrocyclic core with multiple nitrogen atoms and a side chain containing a carboxylate group and a chiral center.

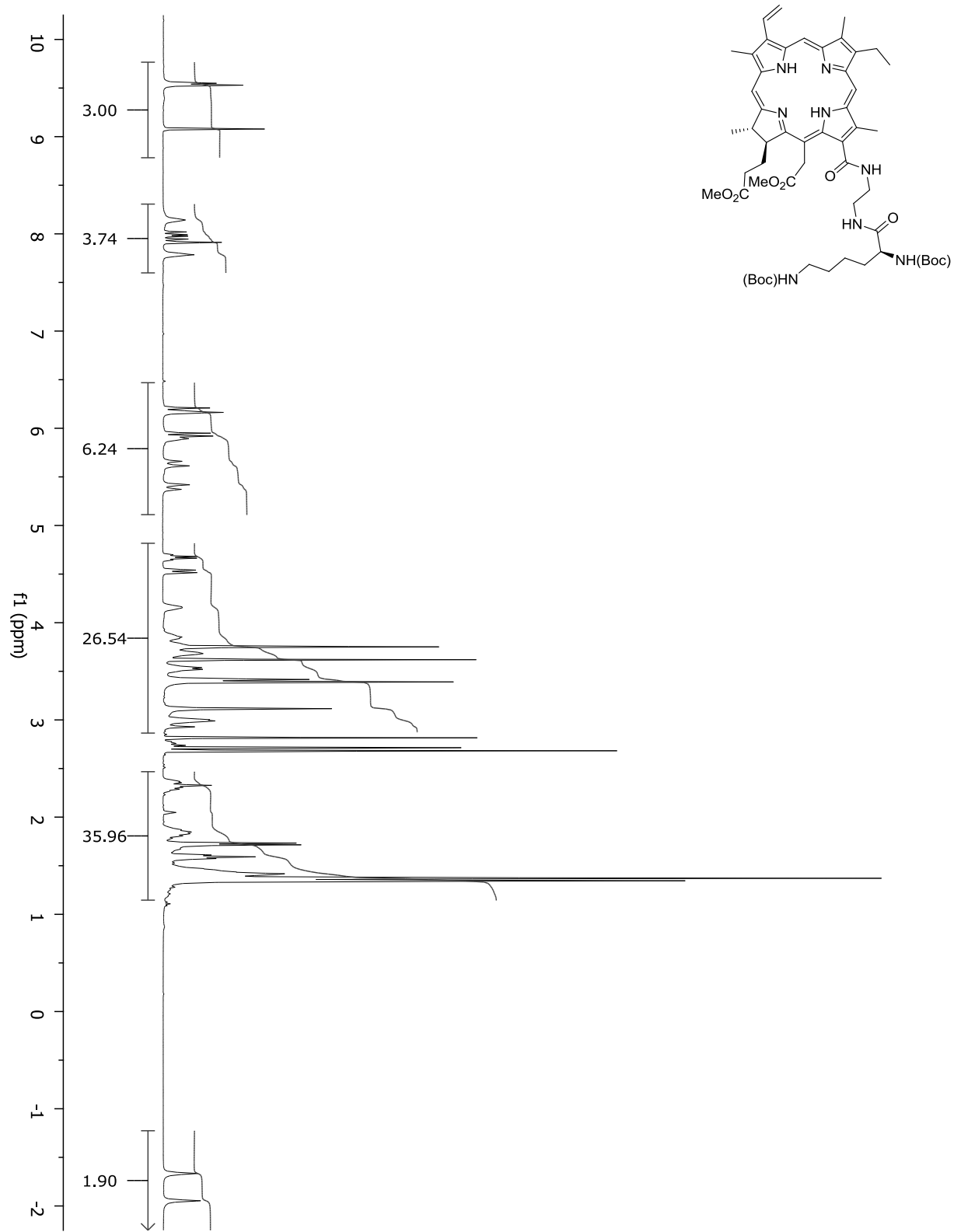
^1H NMR of ^{13}C b-Ala-AspCe₆DME (Compound 11) in $(\text{CD}_3)_2\text{CO}$ at 400 MHz



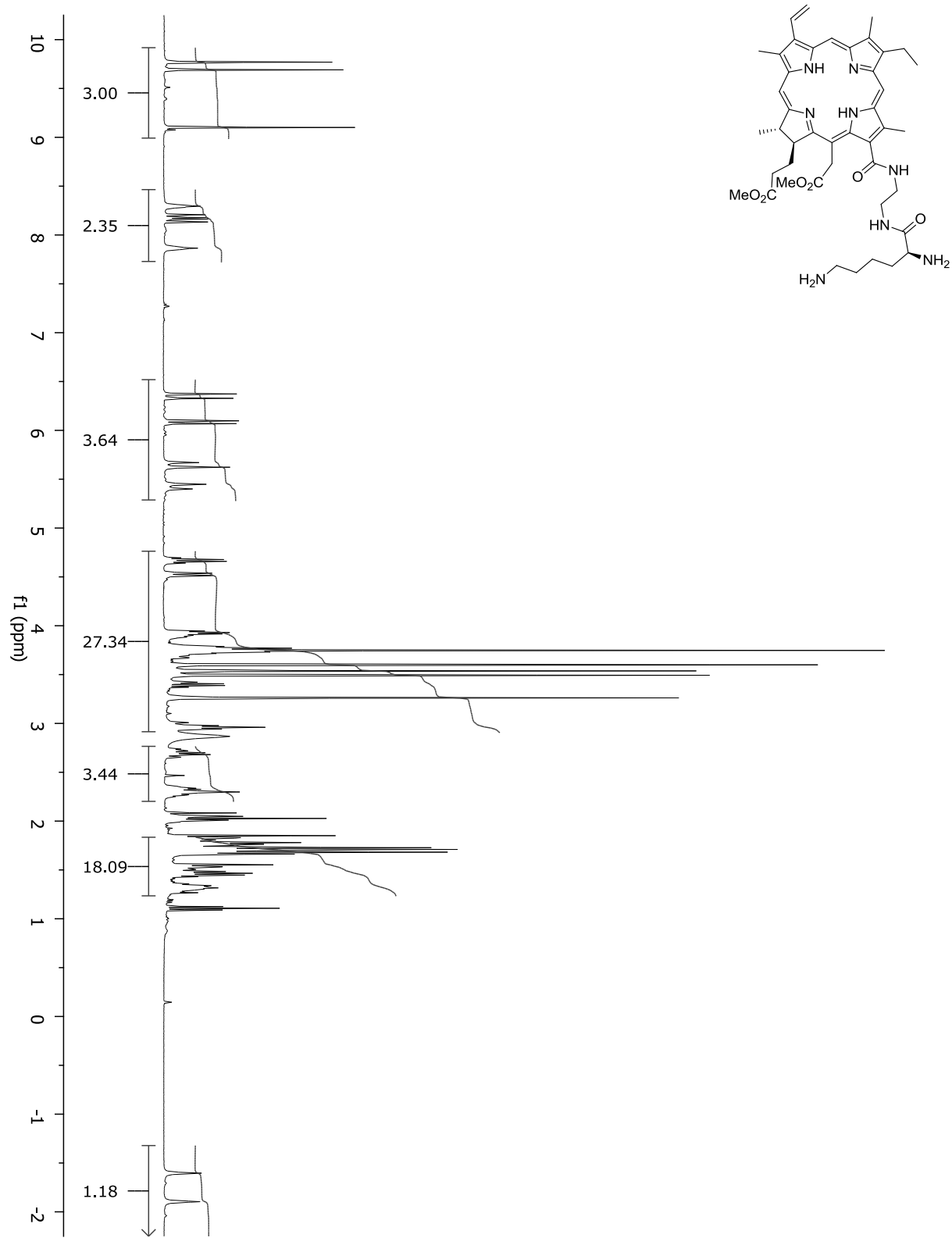
^1H NMR of ^{13}C ED-Ce₆DME (Compound 12) in $(\text{CD}_3)_2\text{CO}$ at 400 Mhz



^1H NMR of ^{13}C ED-(Boc)Lys(Boc)Ce₆DME in $(\text{CD}_3)_2\text{CO}$ at 400 MHz



^1H NMR of 13^1 ED-LysCe₆DME (Compound 13) in $(\text{CD}_3)_2\text{CO}$ at 400 MHz



CARBON-13 NMR SHIFTS OF NEW COMPOUNDS

¹⁷³-Lysyl-chlorin e₆ TME (3):

¹³C NMR (CD₂Cl₂, 100 MHz) δ 173.8, 173.3, 172.6, 170.9, 169.9, 167.9, 155.3, 149.4, 145.6, 140.0, 136.8, 136.6, 135.9, 135.7, 135.2, 131.4, 129.7, 129.6, 124.1, 122.1, 103.1, 102.6, 99.0, 94.2, 53.5, 53.4, 52.5, 50.0, 41.7, 38.9, 34.4, 32.8, 32.2, 31.1, 30.8, 23.4, 22.9, 20.0, 18.0, 12.6, 12.4, 11.5 ppm.

¹⁵²-Aspartylchlorin e₆ DME (7a):

¹³C NMR [(CD₃)₂CO, 100 MHz]: δ 174.5, 173.5, 173.2, 172.6, 171.7, 170.4, 169.4, 155.8, 150.3, 146.3, 140.4, 137.4, 136.6, 136.3, 135.8, 132.0, 130.6, 126.0, 122.6, 104.2, 103.1, 99.7, 95.4, 54.2, 54.0, 52.2, 50.4, 41.2, 37.8, 32.1, 31.0, 26.1, 23.8, 20.3, 18.4, 12.6, 11.6 ppm.

¹⁵²-Lysylchlorin e₆ TME (7b):

¹³C NMR (CD₂Cl₂, 100 MHz) δ 173.0, 172.7, 170.7, 170.0, 168.2, 167.8, 155.5, 149.5, 145.7, 140.2, 136.9, 136.8, 136.0, 135.5, 135.4, 131.5, 129.7, 129.6, 124.3, 122.2, 102.8, 102.5, 99.2, 94.4, 53.3, 52.4, 52.3, 52.0, 49.7, 40.8, 40.2, 34.4, 32.5, 31.4, 29.8, 28.2, 23.3, 22.5, 20.0, 18.0, 12.5, 12.4, 11.5 ppm.

¹³¹-Aspartylchlorin e₆ DME (9):

¹³C NMR [(CD₃)₂CO, 100 MHz] δ 174.0, 173.8, 172.6, 170.3, 169.1, 168.9, 154.5, 149.9, 145.4, 139.2, 137.0, 136.6, 136.4, 135.1, 131.0, 130.7, 130.3, 129.7, 122.0, 104.2, 101.8, 99.4, 95.0, 54.1, 52.3, 51.8, 50.4, 49.7, 43.2, 38.4, 36.6, 31.7, 30.7, 23.5, 20.0, 18.1, 12.3, 12.0, 11.3 ppm.

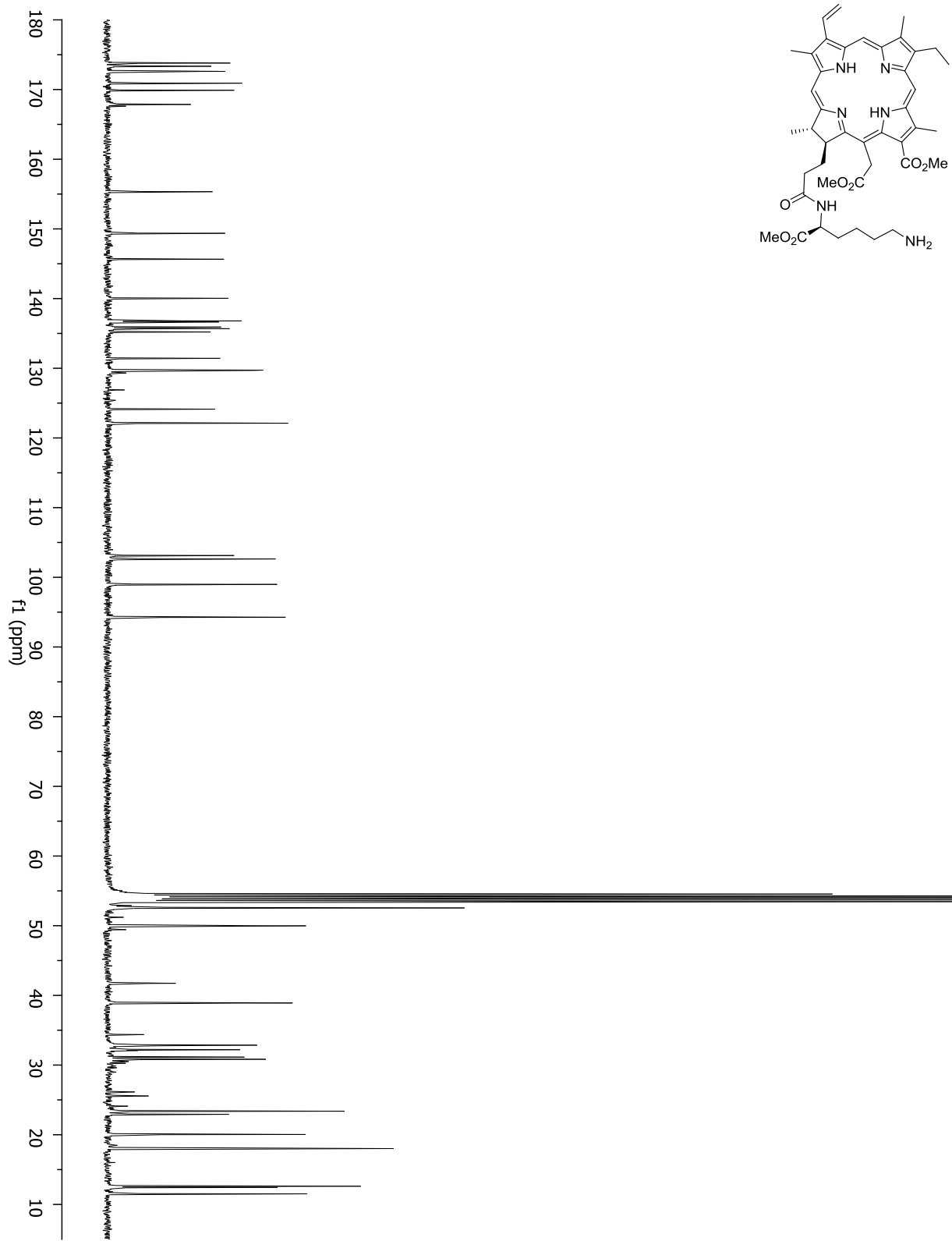
¹³¹-β-Alanyl-aspartylchlorin e₆ DME (11):

¹³C NMR [(CD₃)₂CO, 100 MHz] δ 174.1, 173.9, 173.7, 173.4, 171.6, 170.5, 169.0, 168.4, 143.5, 139.8, 135.3, 134.8, 134.2, 130.7, 130.4, 129.5, 129.0, 128.7, 126.8, 125.2, 123.6, 122.3, 103.5, 100.5, 98.0, 95.0, 53.4, 52.5, 51.9, 49.4, 49.0, 38.8, 37.5, 36.6, 34.7, 31.2, 29.7, 23.3, 19.0, 17.0, 12.1, 11.5, 10.8 ppm.

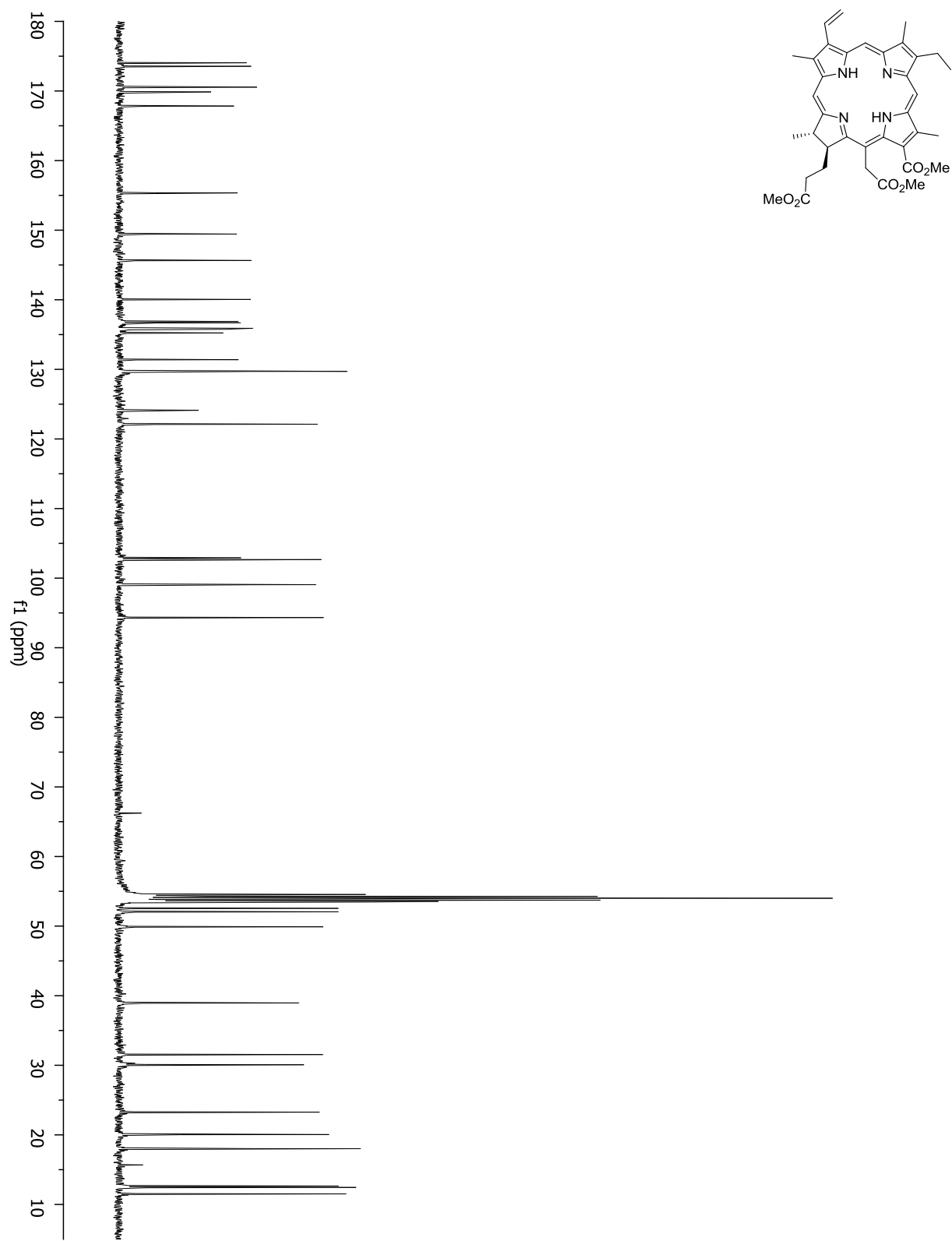
¹³¹-Ethylenediaminyl-lysylchlorin e₆ DME (13):

¹³C NMR (CD₂Cl₂, 100 MHz) δ 176.6, 174.2, 174.0, 169.9, 169.7, 167.7, 154.5, 149.5, 145.3, 139.3, 136.8, 135.4, 135.2, 134.9, 130.9, 130.2, 129.8, 129.7, 129.0, 122.2, 102.9, 101.8, 99.2, 94.4, 55.4, 52.6, 52.0, 49.7, 41.4, 41.1, 39.6, 38.2, 35.0, 31.5, 31.1, 30.2, 23.3, 23.0, 20.0, 18.0, 12.4, 12.2, 11.6, 11.5 ppm.

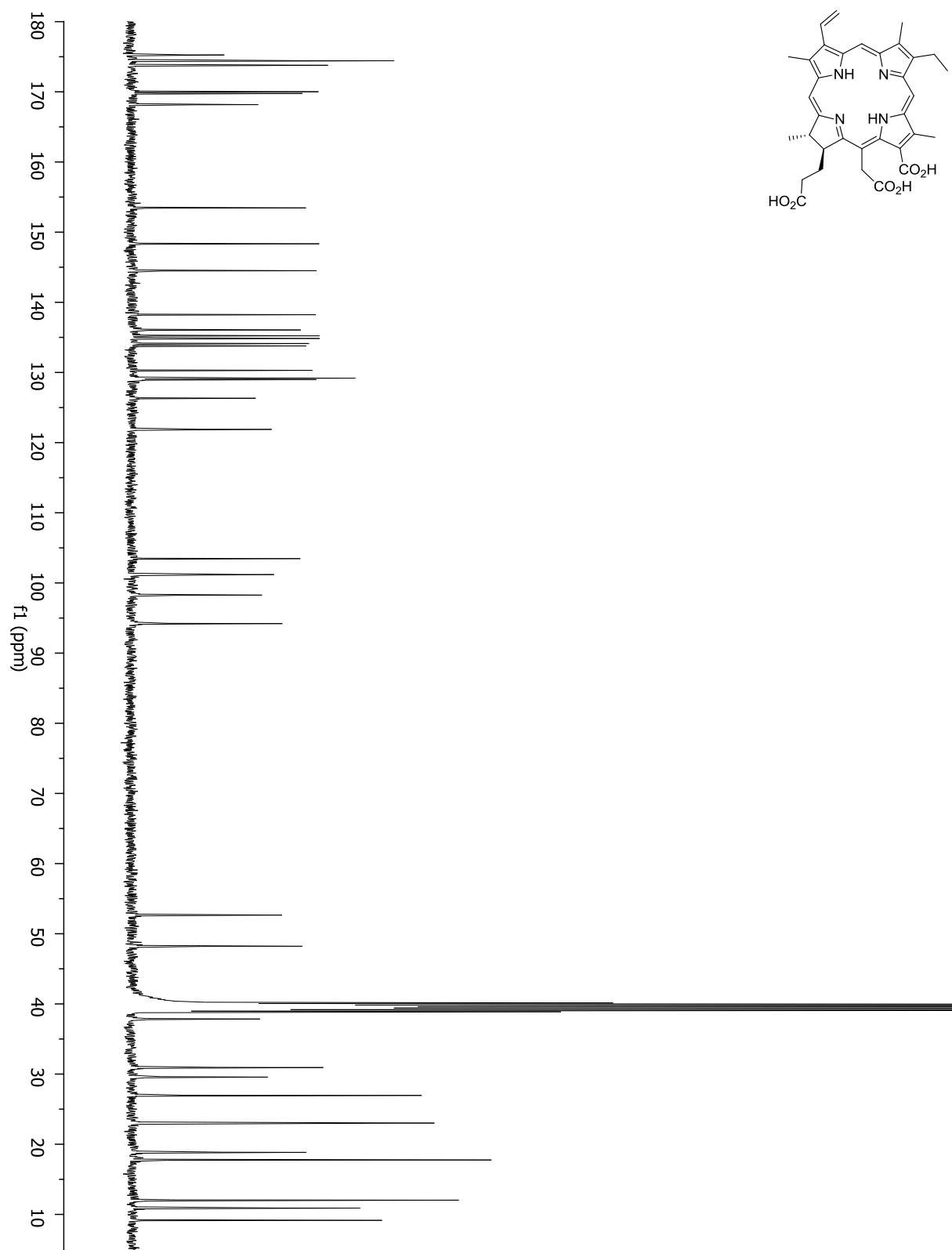
¹³C NMR of 17³ LysCe₆TME (Compound 3) in CD₂Cl₂ at 100 MHz



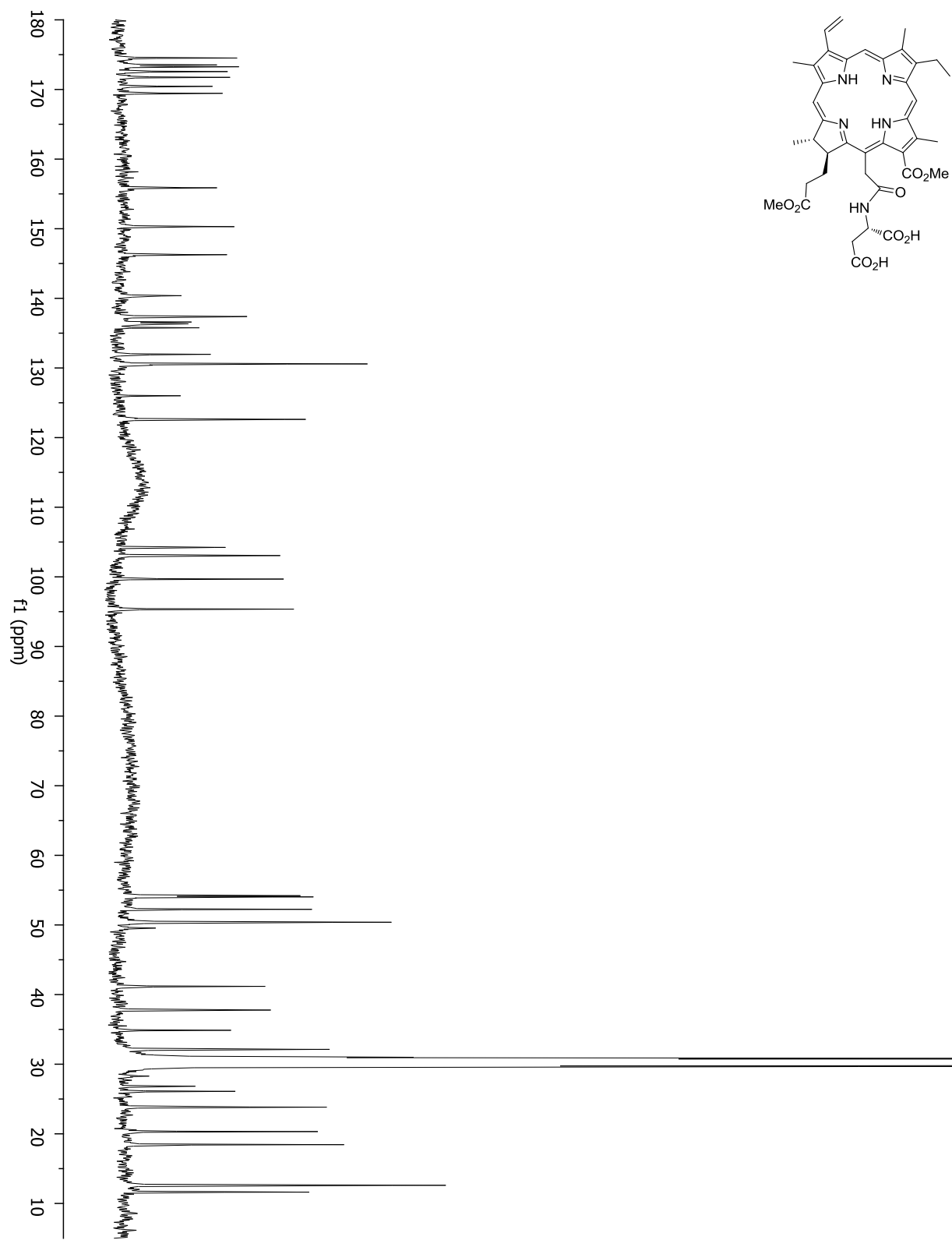
^{13}C NMR of Ce₆TME in CD₂Cl₂ at 100 MHz



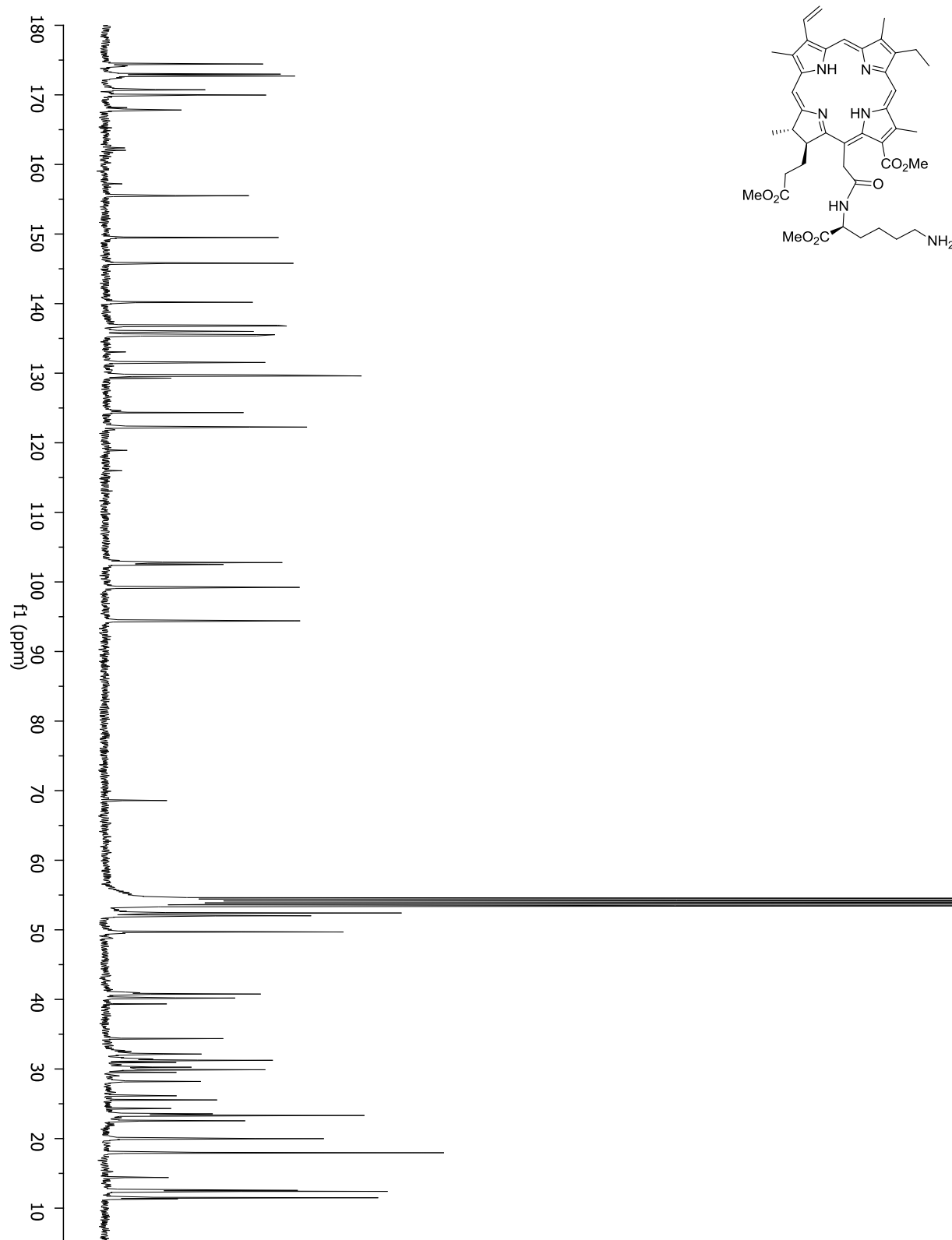
^{13}C NMR of Ce₆ (Compound 5) in DMSO at 100 MHz



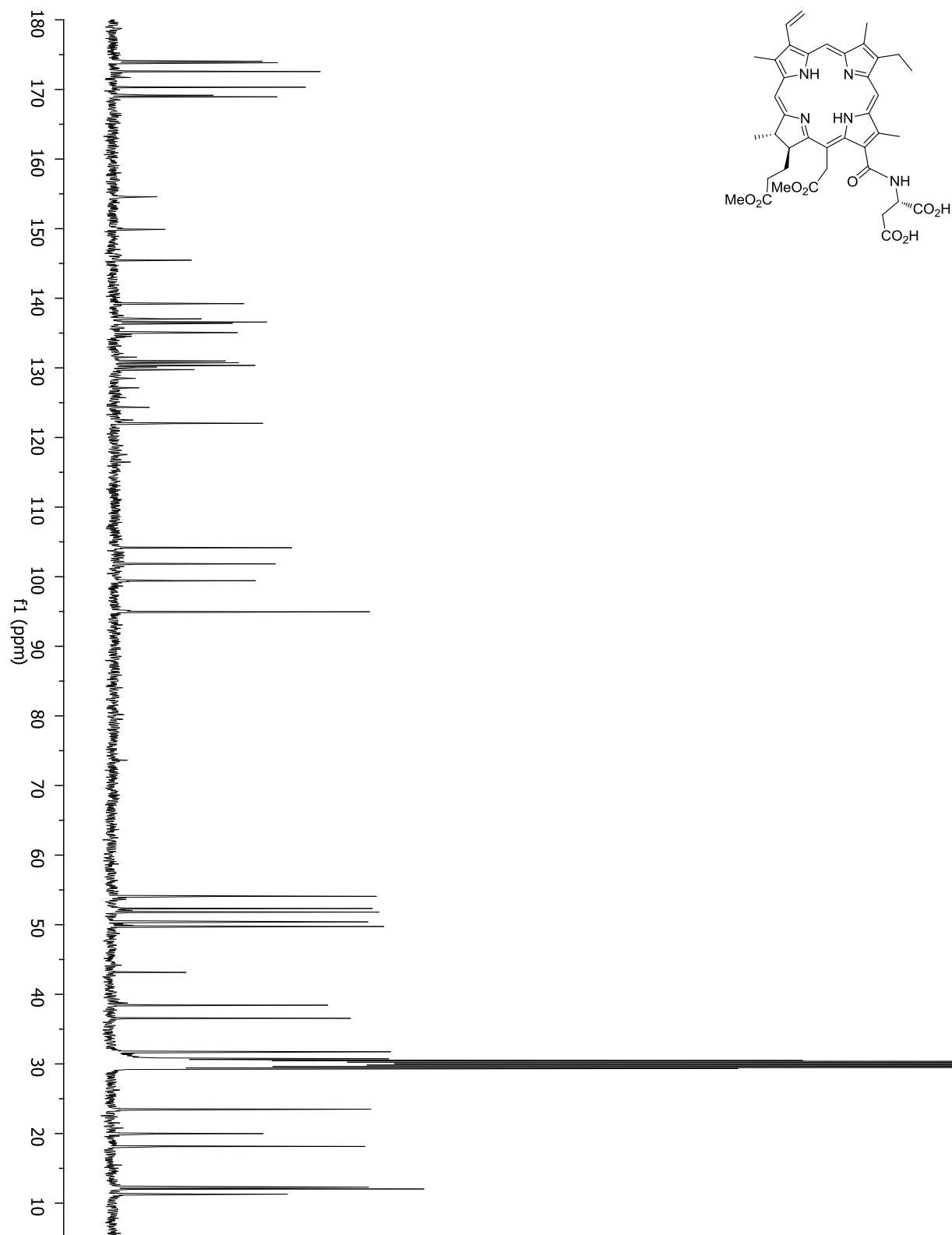
^{13}C NMR of 15^2 AspCe₆DME (Compound 7a) in (CD₃)₂CO at 100 MHz



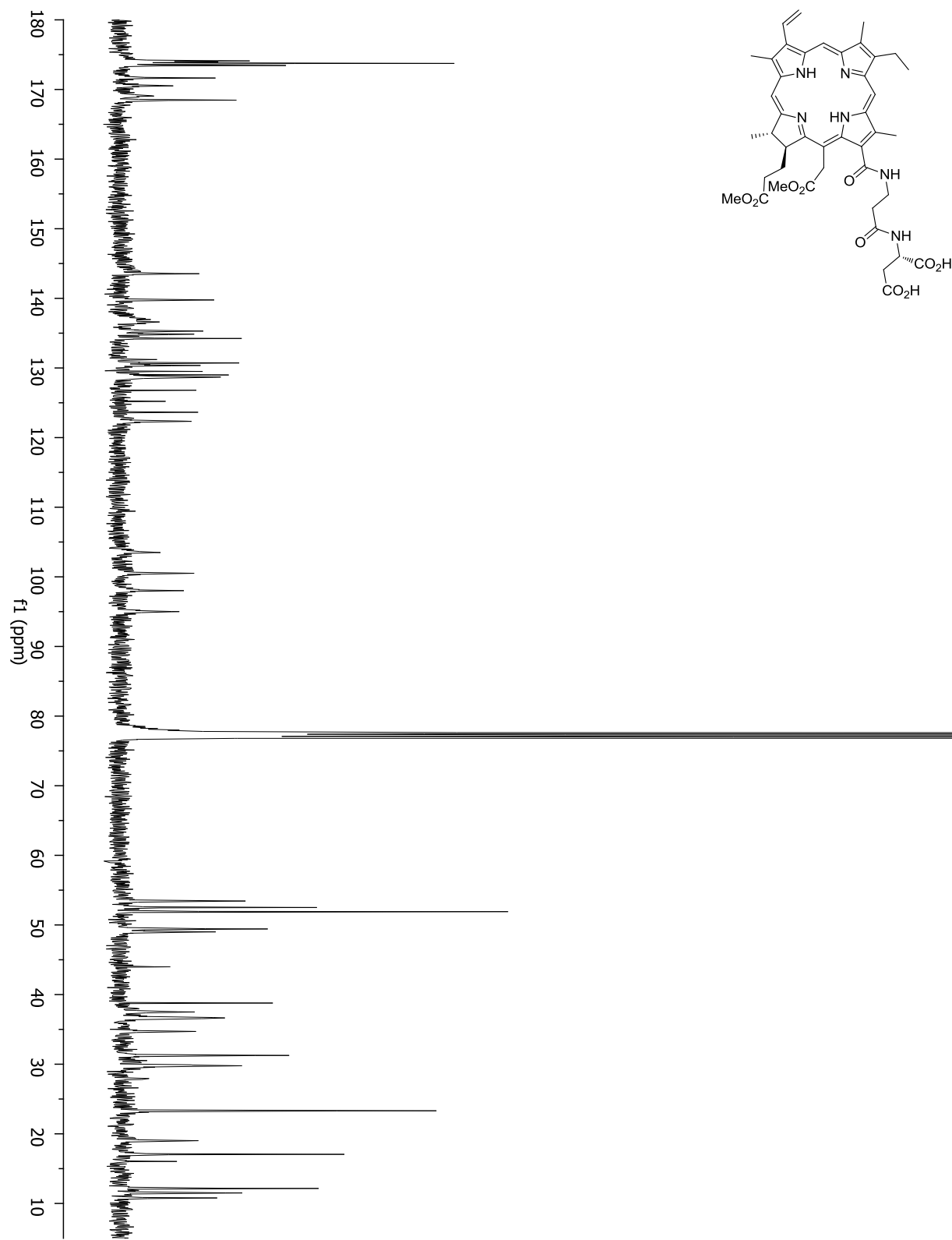
^{13}C NMR of 15^2 LysCe₆TME (Compound 7b) in CD_2Cl_2 at 100 MHz



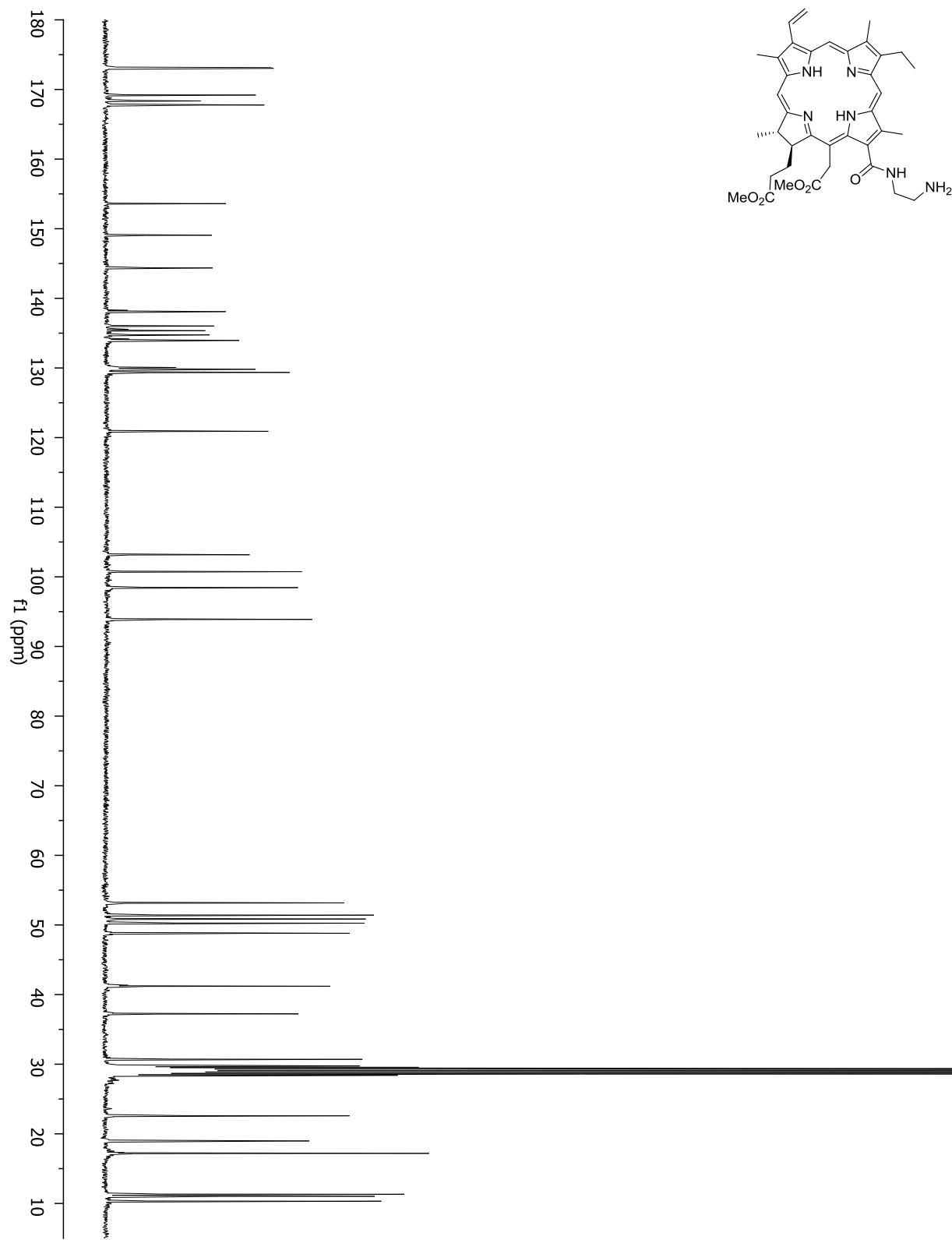
^{13}C NMR of $^{13}\text{C}_1$ AspCe₆DME (Compound 9) in $(\text{CD}_3)_2\text{CO}$ at 100 MHz



^{13}C NMR of ^{13}C b-Ala-AspCe₆DME (Compound 11) in CDCl_3 at 100 MHz



^{13}C NMR of ^{13}C ED-Ce₆DME (Compound 12) in (CD₃)₂CO at 100 MHz



¹³C NMR of ¹³¹ ED-LysCe₆DME (Compound 13) in CD₂Cl₂ at 100 MHz

