Supporting Information

# Discovery and Characterization of Natural Product-Based Disruptors of Glycosomal Function in the Protozoan Parasite *Trypanosoma brucei*

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**General Techniques**

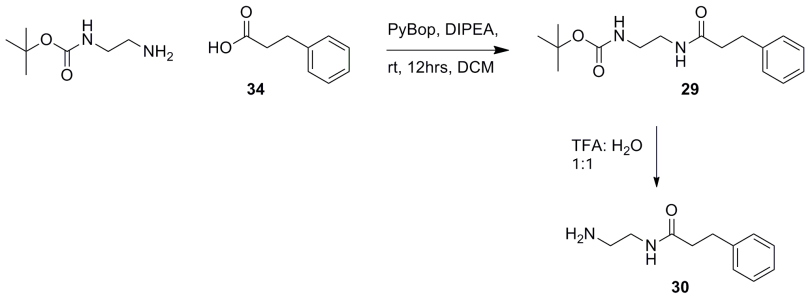
All commercially available reagents were used without further purification. Dry DCM, and THF were purified by using a solvent purification system. Diisopropylethylamine was distilled over CaH using a dried short path distillation apparatus. The following abbreviations were used were appropriate diisopropylethylamine (DIPEA) and dichloromethane (DCM). All standard amino acid abbreviations are used.

The structures of synthetic substrates were confirmed by cleavage using standard peptide chemistry methods, and subsequent LCMS analysis utilizing an Agilent 1200 series/6130 quadrupole LCMS, equipped with a Phenomenex Jupiter $C_{18}$ (4.6 x 250 mm, 5μm) RP-HPLC column. All solvents were HPLC grade and were used without further purification. Gradient method for all LC runs is as follows: 40% to 100% MeOH with $H_2O$/ 0.002% formic acid over 20 min, 1 mL/min.

NMR spectra were acquired on inverse 500 and 600 MHz spectrometers, equipped with 5mm broadband probe and 5mm HCN triple resonance cryoprobe respectively, and referenced to residual solvent proton and carbon signals ($\delta_H$ 1.94, $\delta_C$ 1.4 for $CD_3CN$ and $\delta_H$3.31, $\delta_C$49.15 for $CD_3OD$ and $\delta_H$ 7.26, $\delta_C$ 77.1 for $CDCl_3$).

**Experimental Procedure**

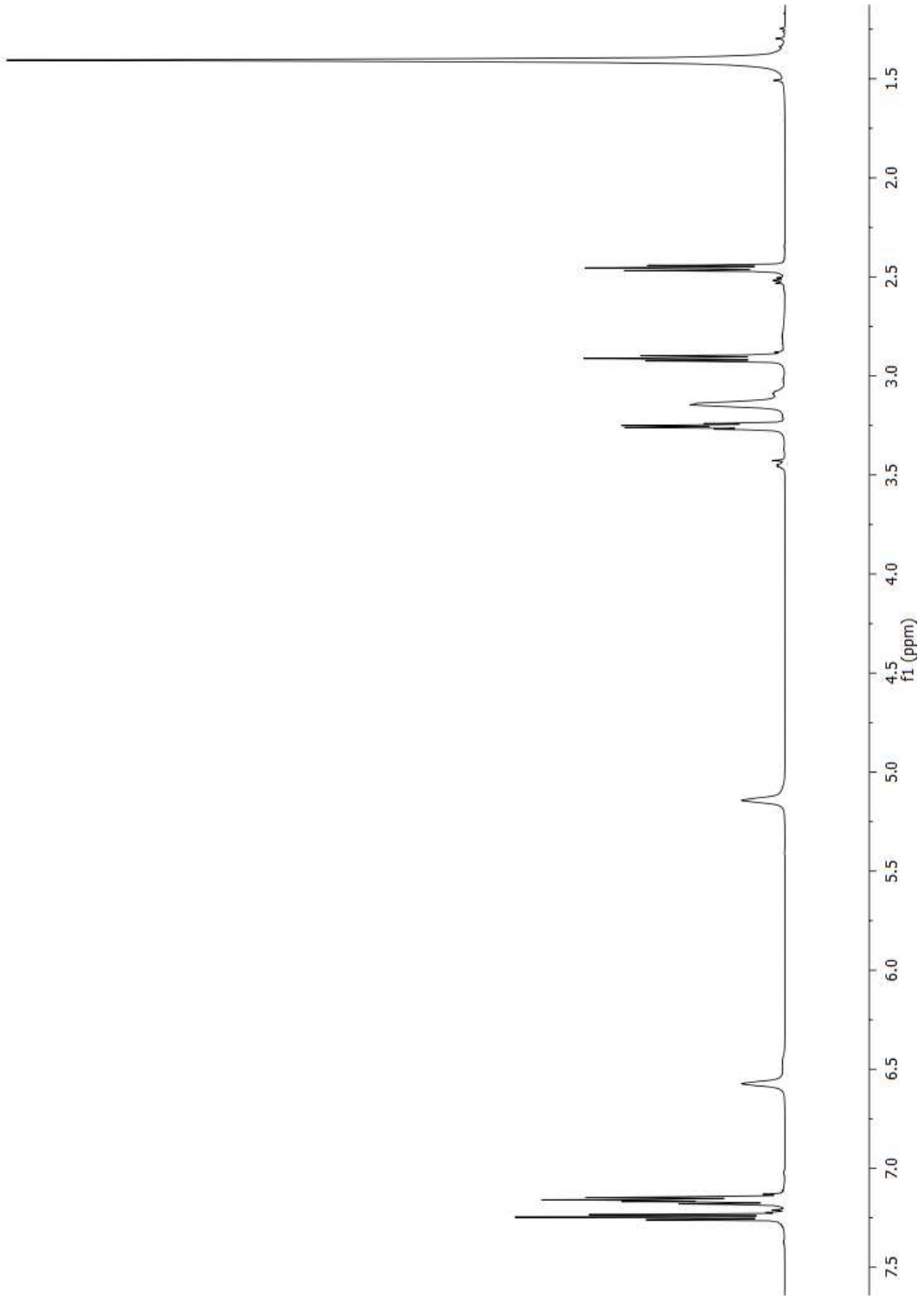
**Control 30 Synthesis**



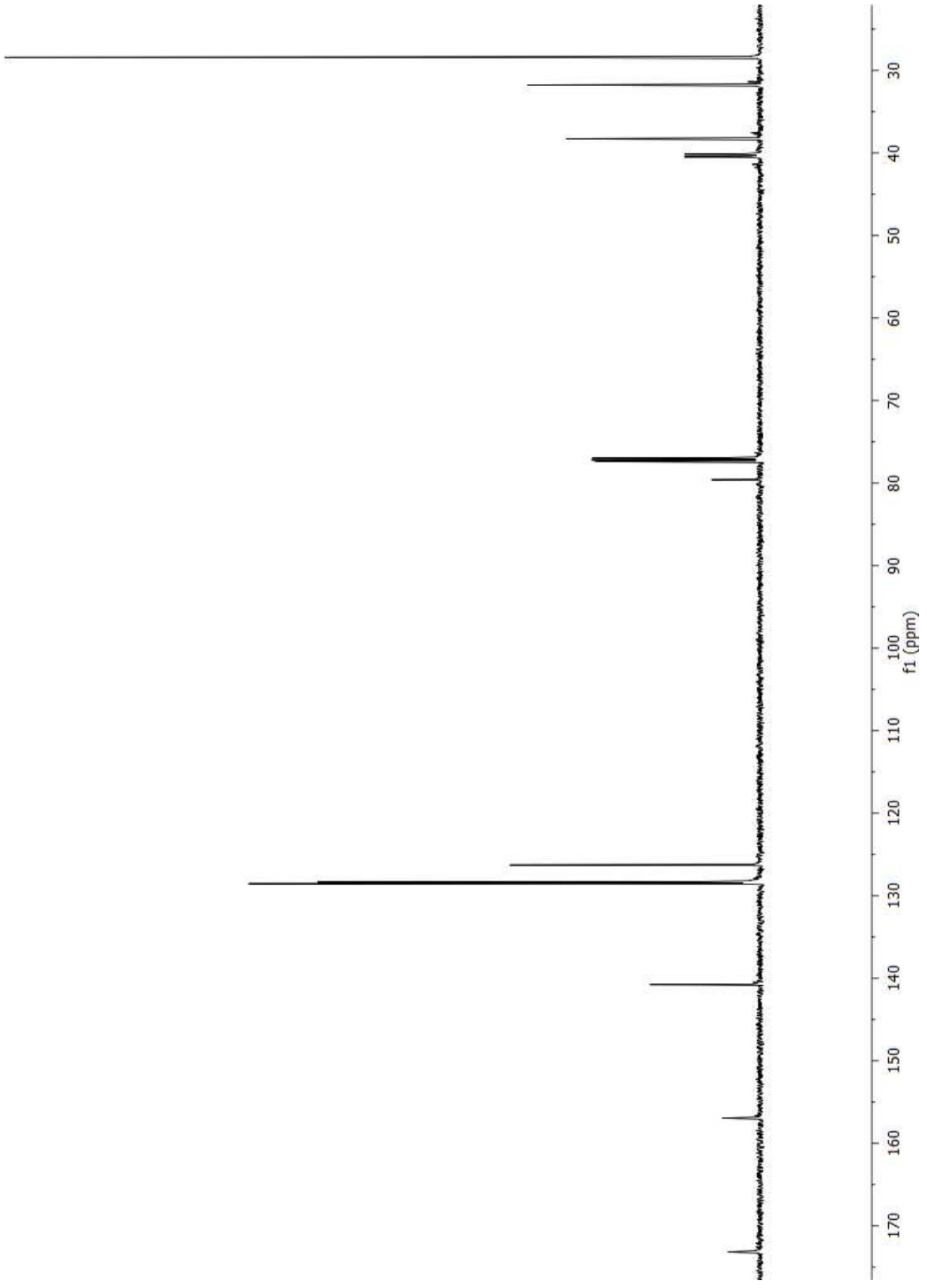

Commercially available **34** (0.100 g, 0.67 mmol) was dissolved in a minimal amount of DMF. The resulting solution was stirred and DIPEA (927uL, 5.61 mmol) added dropwise. Commercially available *N*-Boc-ethylenediamine (0.112 g, 0.70 mmol) was dissolved in a minimum amount of DMF and added dropwise to the stirred solution. Lastly, PyBOP (0.270 g, 0.52 mmol) was added to the mixture and allowed to stir for 2hrs. The reaction was quenched by removing the solvent *in vacuo*. The resulting crude reaction product was subjected to flash column chromatography (1:10 methanol/DCM, $R_f$ = 0.2). The product was then further purified using reverse-phase HPLC (65% MeOH, 35% $H_2O$ + 0.02% HCOOH, Phenomenex Synergi $C_{18}$ 250 x 10 mm column, 10 μm, 2 mL/min, 210 nm), yielding 0.072 g of white solid **29** (37% yield).

**29:** white solid; UV (MeOH) $\lambda_{max}$ (log ε):209 nm (3.73), 257 nm (2.04); $^1$H NMR ($CDCl_3$, 600 MHz) δ 7.25 (t,*J* = 7.8 Hz), 7.18-7.15 (m, 3H), 6.57 (s, 1H), 5.14 (s, 1H), 3.25 (q, *J* = 12 Hz, 6 Hz, 2H), 3.14 (s, 2H), 2.91 (t, *J* = 7.8 Hz, 2H), 2.45 (t, *J* = 7.8 Hz, 2H), 1.40 (s, 9H); $^{13}$C NMR ($CDCl_3$, 150 MHz) δ 28.4, 31.8, 38.3, 40.2, 40.5, 79.6, 126.3, 128.4, 128.6, 140.8, 157.0, 173.2; HRESIFTMS *m/z*$[M+H]^+$293.1890 (calcd for $C_{16}H_{25}N_2O_3$, 293.1890).

$^{1}H$ spectrum of **29** recorded in $CDCl_3$ at 600 MHz.



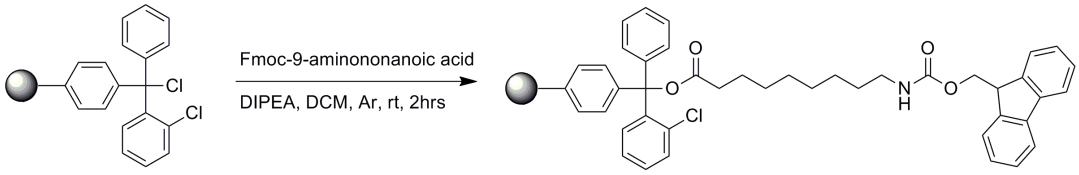
$^{13}C$ spectrum of **29** recorded in $CDCl_3$ at 150 MHz.



For further reactions, the Boc protecting group was removed by stirring a desired amount of **29** in 1:1 TFA/$H_2O$ solution and monitoring the reaction progress by TLC. After the reaction was complete by TLC, the reaction was quenched by removing the solvent *in vacuo*. The resulting yellow oil **30** was used immediately without further purification.[1]

**30:** yellow oil; UV (MeOH) $\lambda_{max}$ (log ε): 216 nm (3.34), 259 nm (1.82); $^1$H NMR ($CDCl_3$, 600 MHz) δ 2.03 (bs, 1H), 2.44 (t, *J* = 7.2 Hz, 2H), 2.82 (t, *J* = 7.2 Hz, 2H), 2.96 (s, 2H), 3.37 (s, 2H), 7.09 (d, *J* = 7.8 Hz, 2H), 7.13 (t, *J* = 7.2 Hz, 1H), 7.20 (t, *J* = 7.2 Hz, 2H), 7.98 (bs, 2H); $^{13}$C NMR ($CDCl_3$, 150 MHz) δ 31.5, 37.6, 37.7, 40.3, 126.6, 128.4, 128.7, 140.3, 175.3; HRESIFTMS *m/z* $[M+H]^+$ 193.1335 (calcd for $C_{11}H_{17}N_2O$, 193.1335).

**Methyl Ester Linker (22) Synthesis**



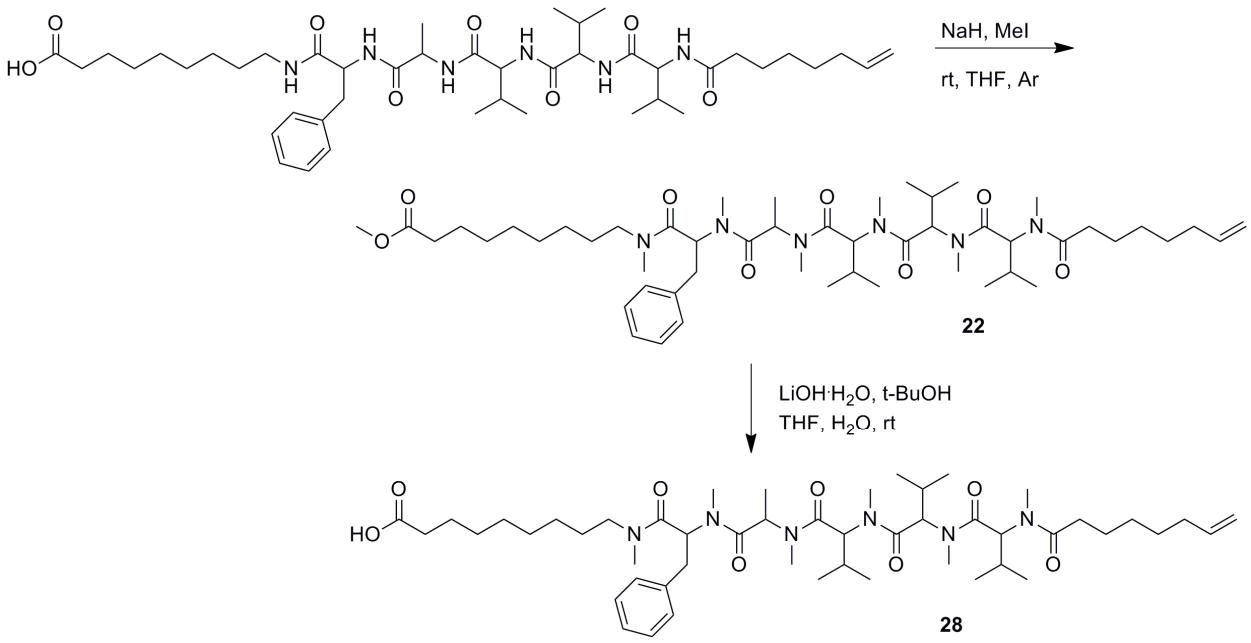

Fmoc-9-aminononanoic acid (1.49 g, 3.92mmol) and DIPEA (1.29 mL, 7.83mmol) were added to a stirred suspension of 2-chlorotrityl chloride resin (0.955 g; 200-400 mesh; 1% DBV) in DCM (20 mL) under argon, and the resulting suspension stirred at room temperature for two hours. The solution was transferred to a polypropylene vessel containing a fritted disk and fitted with a Teflon stopcock, drained, and washed (3 x 5 mL DCM, 3 x 5 mL DMF, 3 x 5 mL DCM). The resulting pale yellow resin was dried for 24 hours, and analyzed to determine the loading value of the 9-aminononanoic acid substrate as outlined below.

Two aliquots (8.82 mg and 7.01 mg) of the loaded resin were transferred to Eppendorf tubes, dissolved in 1% DBU in DMF (500 μL), and agitated for 1 hour. 50 μL aliquots of these solutions were diluted with DMF (2 mL), and absorbance measured at $\lambda = 301$ nm to determine the loading value of the Fmoc-9-aminononanoic acid as 0.443mmol per gram of resin.

Subsequent amino acids were added using a standard protocol. The dried resin was allowed to swell for 5 minutes in DCM (5 mL), drained, and washed with DMF (5 mL). The washed resin was deprotected using 20% piperidine in DMF (5 mL) with agitation for 20 minutes. After deprotection, the resin was drained, and washed (3 x 5 mL DMF, 3 x 5 mL DCM, 3x 5 mL DMF). To this washed resin was added a solution of HBTU (0.786 g, 2.07mmol), the appropriate amino acid (2.11mmol), and DIPEA (0.700 mL, 4.23mmol). The resulting suspension was gently agitated for 1 hour, drained, and washed, as described above. This process

was repeated with successive amino acids to form resin-bound 9-aminononane-Phe-Ala-Val-Val-Val-Fmoc.

From this peptide precursor, the loaded resin (0.995 g) was deprotected by treatment with 20% piperidine in DMF (5 mL) with agitation for 20 minutes. 7-Octenoic acid (241 μL, 1.76 mmol), HBTU (0.625 g, 1.72 mmol), and DIPEA (559 μL, 3.53 mmol) were added and the resulting suspension agitated for 1 hour, drained, and washed as previously described. The resulting linear lipopeptide was cleaved from the resin by treatment with 1% TFA in DCM for 20 minutes and the solvent removed by evaporation under a stream of $N_2$ gas. The resulting glossy white solid was triturated with *t*-butyl methyl ether and centrifuged at 1000 rpm for 20 minutes. Removal of the *t*-butyl methyl ether afforded the lipopeptide as a white solid (432 mg), which was carried to the next step without further purification.

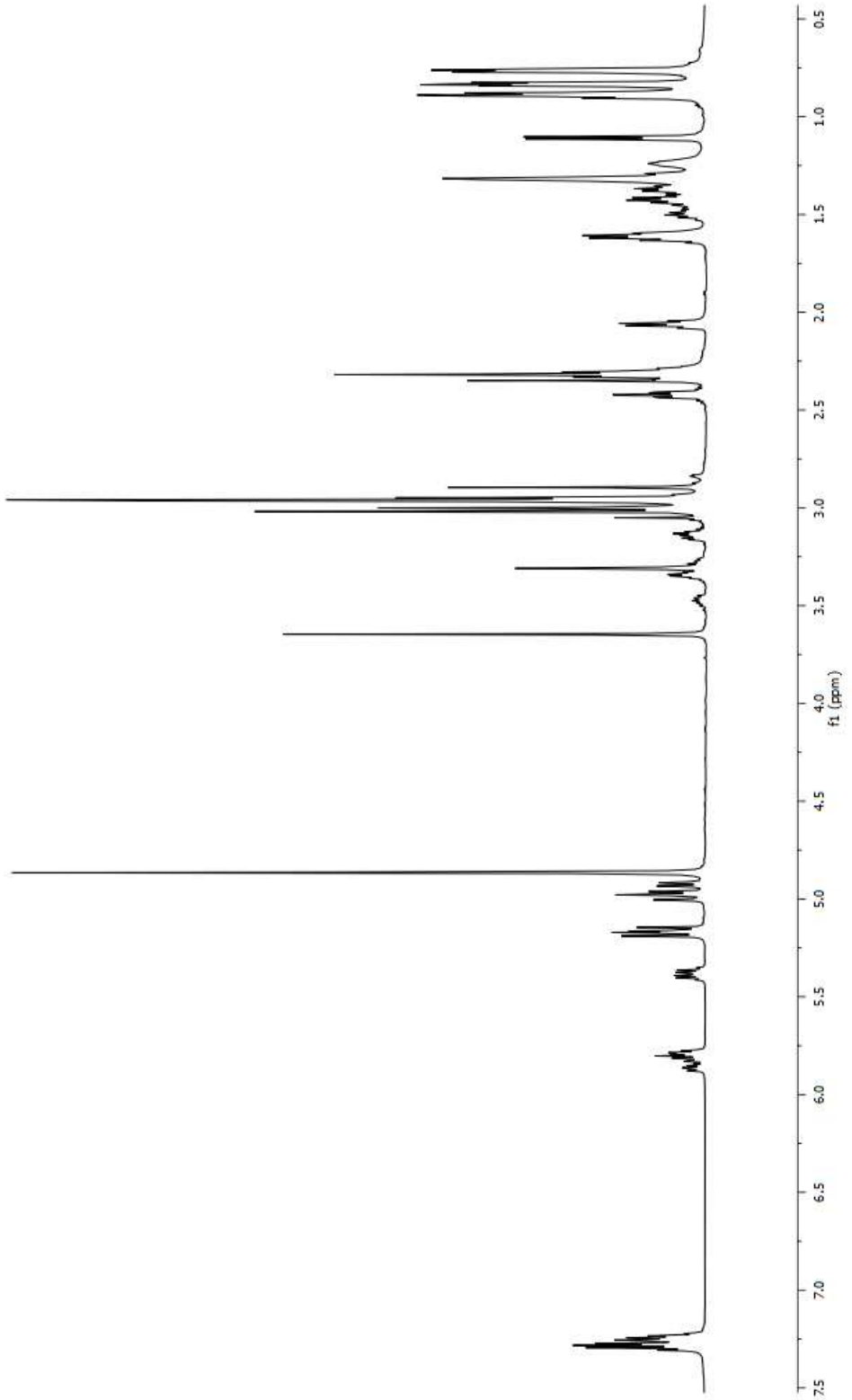



To a solution of this lipopeptide (66.2 mg, 0.083 mmol) in THF (10 mL) under argon at -78°C was added 0.745 g NaH (60% dispersed in mineral oil, 0.745 g, 18.6 mmol). After stirring for 20 minutes, MeI (1.42 mL, 22.7 mmol) was added dropwise over two minutes, and the

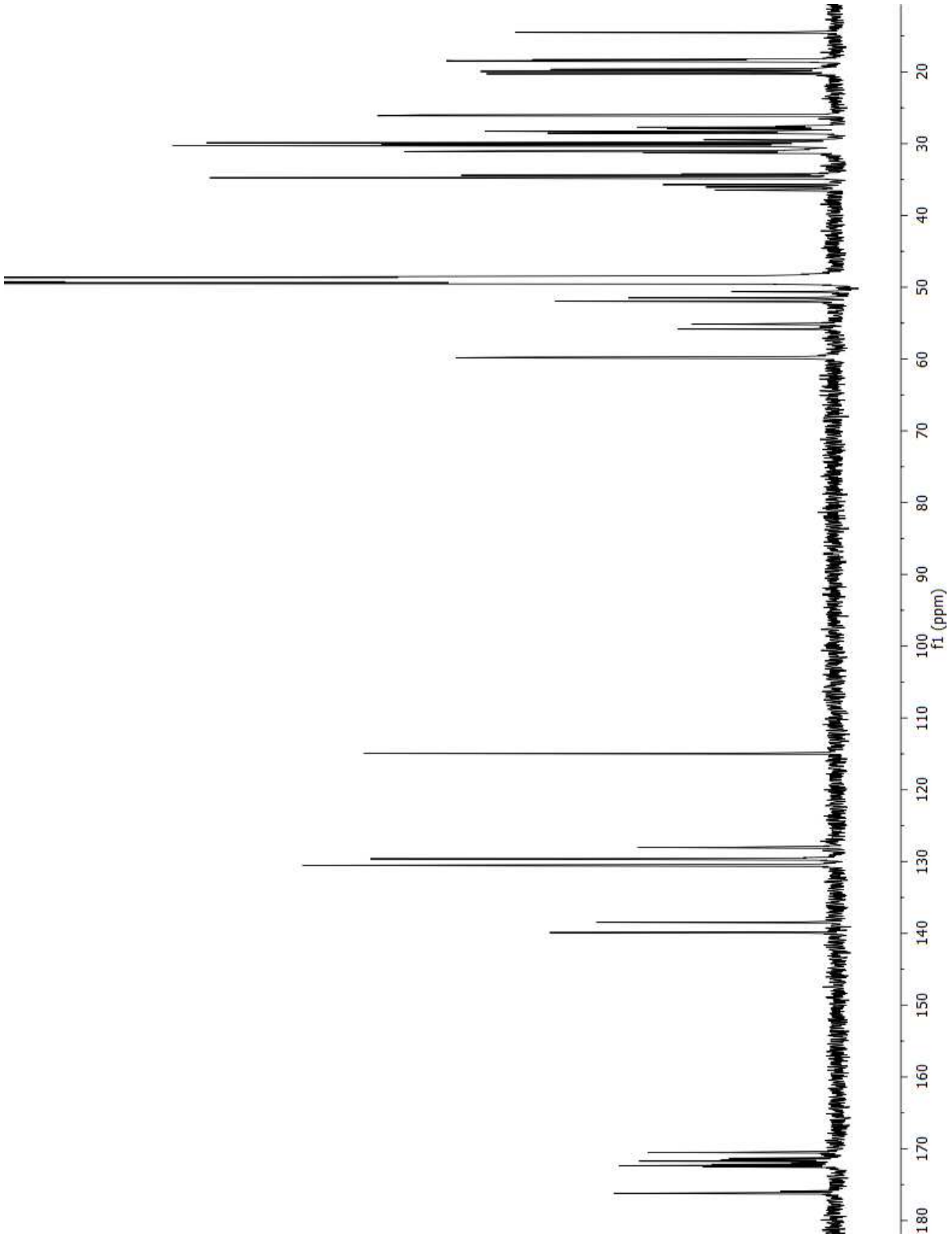
reaction mixture allowed to warm to room temperature with stirring over 4 hours. Water was added dropwise until effervescence subsided, and the resulting suspension concentrated to dryness *in vacuo*. The resulting slurry was diluted with water (50 mL) and acidified to pH 1 using 1N HCl. The acidified mixture was partitioned against EtOAc (50 mL), the phases separated, and the aqueous phase washed with EtOAc (2 x 50 mL). The combined organics were concentrated to dryness *in vacuo* and the resulting yellow oil partitioned between MeOH (50 mL) and pentane (3 x 100 mL) to remove residual mineral oil. The MeOH phase was concentrated to dryness *in vacuo* to give a yellow solid, and purified by $C_{18}$ RP-HPLC Phenomenex Jupiter $C_{18}$ (4.6 x 250 mm, 5 μm), 81% MeOH/19% $H_2O$ (acidified with 0.002% formic acid), 1 mL/min, $t_R$= 22.1 min to give **22** as an amorphous white solid (4.28 mg, 5.6%).

**22:** white solid; $[\alpha]_D^{20}$ -149 (*c* 0.075, MeOH); UV (MeOH) $\lambda_{max}$ (log ε): 223 nm (3.95); $^1$H NMR ($CD_3OD$, 600 MHz) δ7.30-7.23 (m, 5H), 5.83-5.77 (m, 1H), 5.18 (d, *J* = 10.8 Hz, 1H), 5.15 (d, *J* =10.8 Hz, 1H), 5.00-4.96 (m, 2H), 4.92 (d, *J* = 10.2 Hz, 1H), 3.64 (s, 3H), 3.01 (s, 3H), 3.00 (s, 3H), 2.96 (s, 3H), 2.95 (s, 3H), 2.94 (s, 3H), 2.42 (dt, *J* = 2.4 Hz, 7.2 Hz, 2H), 2.34 (s, 3H), 2.32-2.30 (m, 6H), 2.06 (q, *J* = 7.2 Hz, 2H), 1.61 (q, *J* = 7.8 Hz, 4H), 1.50 (p, *J* = 7.2 Hz, 2H), 1.41 (p, *J* = 7.2 Hz, 2H), 1.38-1.22 (m, 12H), 1.10 (d, *J* = 6.6 Hz, 3H), 0.90-0.87 (m, 8H), 0.84-0.82 (m, 7H), 0.77-0.76 (m, 7H); $^{13}$C NMR ($CD_3OD$, 150 MHz) δ 14.6, 18.4, 18.5, 18.6, 19.7, 20.0, 20.4, 26.1, 26.2, 27.7, 27.8, 28.0, 28.4, 28.5, 28.6, 30.2, 30.4, 31.1, 31.2, 31.4, 34.3, 34.5, 34.9, 35.8, 36.2, 36.6, 50.7, 51.6, 51.6, 52.1, 55.2, 55.9, 59.8, 59.9, 60.0, 115.0, 128.1, 128.2, 129.7, 129.8, 130.6, 138.6, 140.0, 170.6, 171.4, 171.7, 171.8, 172.3, 172.5, 172.6, 176.3; HRESIMS *m/z* $[M+Na]^+$ 933.6422 (calcd for $C_{51}H_{86}N_6O_8Na$, 933.6399).

$^{1}$H spectrum of **22** recorded in $CD_3OD$ at 600 MHz.



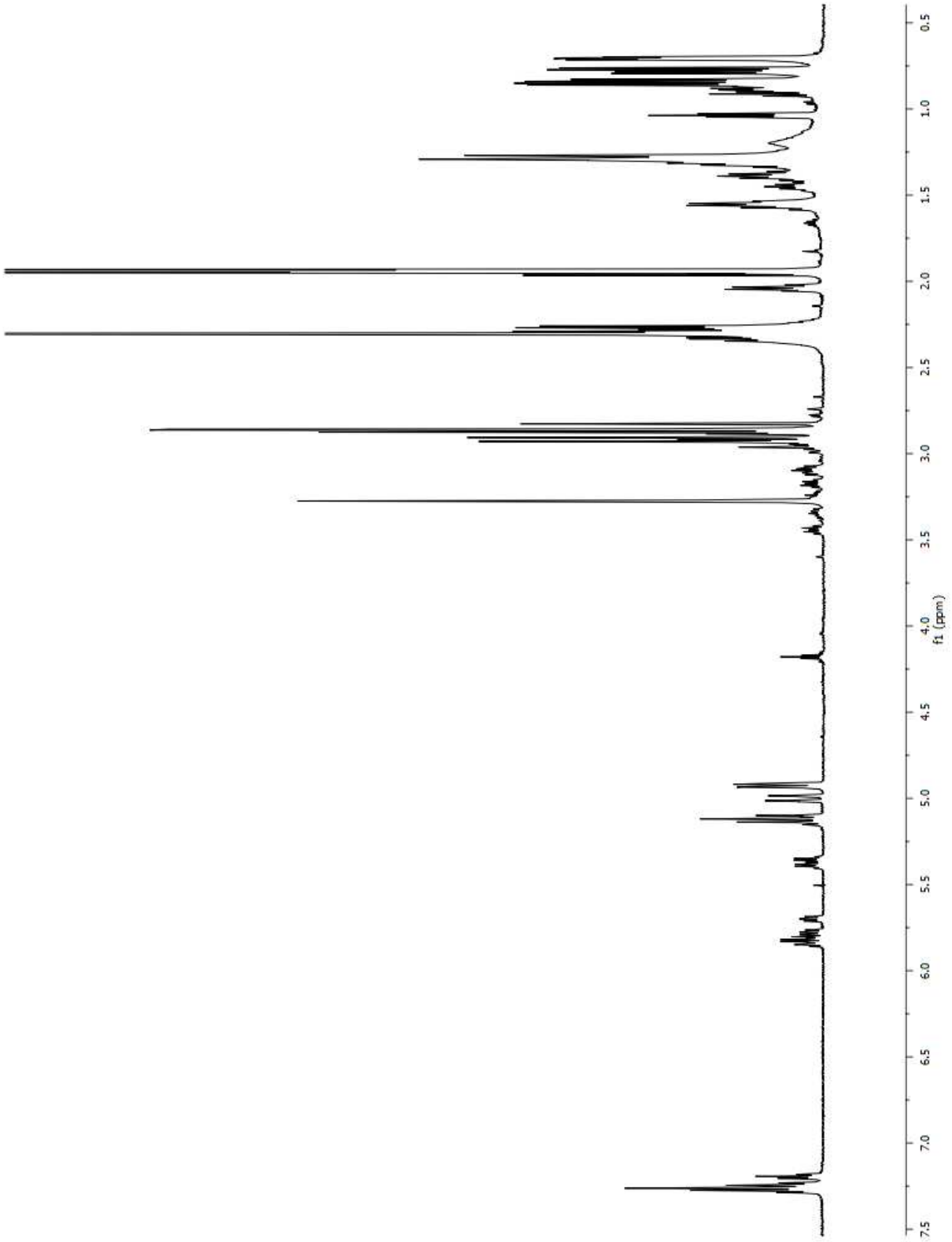
$^{13}C$ spectrum of **22** recorded in $CD_3OD$ at 150 MHz.



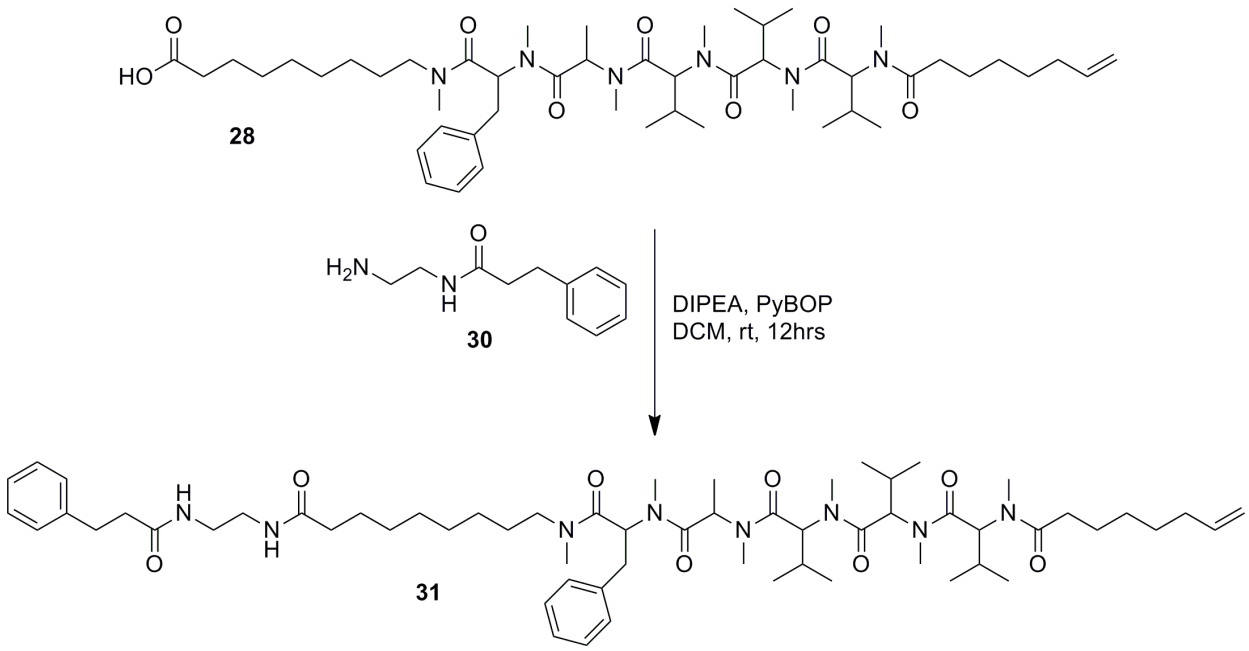
For further reactions, the methyl ester of **22** was hydrolyzed to give the free acid. Compound **22** (3.75 mg, 4.11 μmol) was added to 250 μL of THF and allowed to stir with *t*-BuOH (250 μL, 2.63 mmol) and $H_2O$ (250 μL, 13.8 mmol). Then LiOH monohydrate (1.73 mg, 41.2 μmol) was added to the reaction and allowed to stir overnight at room temperature. The mixture was transferred to a separatory funnel and 5 mL of $H_2O$ was added. The water layer was washed with DCM (2 x 5 mL) and acidified to pH 1 using 1N HCl. The acidified mixture was partitioned against EtOAc (5 mL), the phases separated, and the aqueous phase washed with EtOAc (2 x 5 mL). The combined organics were concentrated to dryness *in vacuo* to give a white solid **28** (3.32 mg, 89.9% yield).

**28:** white solid; $[\alpha]_D^{20}$ -159 (*c* 0.070, MeOH); UV (MeOH) $\lambda_{max}$ (log ε): 218 nm (4.15); $^1$H NMR ($CD_3CN$, 600 MHz) (major rotamer) δ 11.06 (bs, 1H), 7.28-7.23 (m, 4H), 7.19 (t, *J* = 7.2 Hz, 1H), 5.82 (dddd, *J* = 6.6 Hz, 6.6 Hz, 10.2 Hz, 16.8 Hz, 1H), 5.69 (dd, *J* = 6.6 Hz, 9 Hz, 1H), 5.35 (q, *J* = 6.6 Hz, 1H), 5.15-5.10 (m, 2H), 4.92 (d, *J* = 10.2, 1H), 2.91 (s, 3H), 2.90 (s, 3H), 2.87 (s, 3H), 2.86 (s, 3H), 2.85 (s, 3H), 2.34-2.32 (m, 2H), 2.30 (s, 3H), 2.29-2.25 (m, 6H), 2.04 (q, *J* = 7.2 Hz, 2H), 1.56 (p, *J* = 7.2 Hz, 4H), 1.43 (p, *J* = 7.8 Hz, 2H), 1.38 (p, *J* = 7.2 Hz, 4H), 1.32-1.16 (m, 10H), 1.03 (d, *J* = 6.6 Hz, 3H), 0.90-0.88 (m, 4H), 0.85 (d, *J* = 6.6 Hz, 3H), 0.83 (d, *J* = 6.6 Hz, 3H), 0.78 (d, *J* = 6.6 Hz, 3H), 0.76 (d, *J* = 6.6 Hz, 3H), 0.70 (d, *J* = 6.6 Hz, 3H), 0.70 (d, *J* = 6.6 Hz, 3H); HRESIMS *m/z* $[M+H]^+$ 897.6446 (calcd for $C_{50}H_{85}N_6O_8$, 897.6423).

$^{1}$H spectrum of **28** recorded in $CD_3CN$ at 600 MHz.



**Almiramide Control (31) Synthesis**

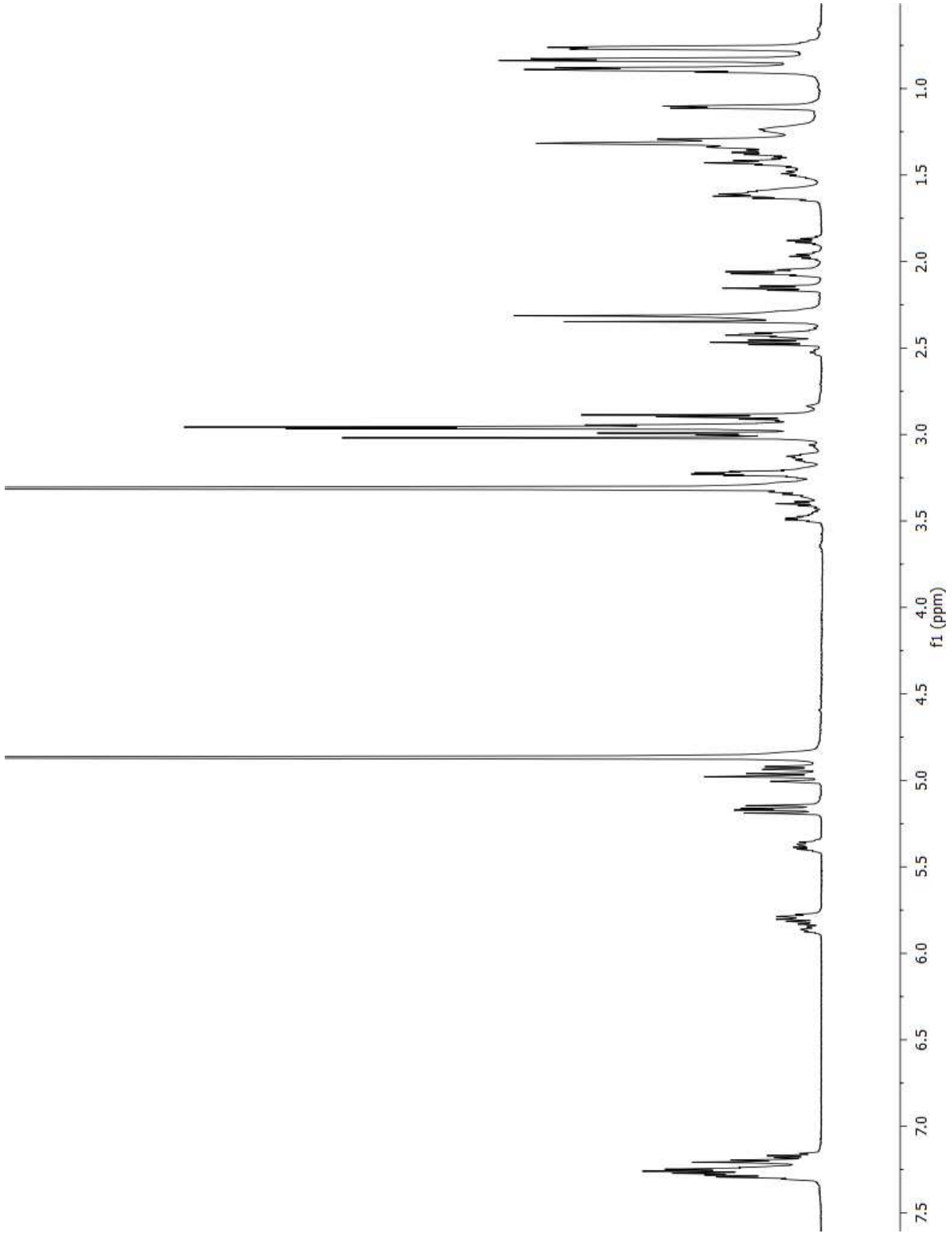



To a dissolved solution of **28** (3.32 mg, 3.70 μmol) in DCM (450 μL) solutions of PyBOP (2.88 μmol) dissolved in DCM and DIPEA (29.6 μmol) dissolved in DCM were added to the reaction and allowed to stir for 20 mins. Then, a dissolved solution of **30** (3.85 μmol) in DCM was added to the reaction. The reaction was allowed to stir overnight at room temperature and verified reaction completion with TLC. The reaction was quenched by removing solvent *in vacuo* and immediately purified by $C_{18}$ RP-HPLC Phenomenex Jupiter $C_{18}$ (4.6 x 250 mm, 5 μm), 83% MeOH/ 17% $H_2O$ (acidified with 0.002% formic acid), 1 mL/min, $t_R$= 18.01 min to give **31** as an amorphous white solid (0.51 mg, 12.8%).

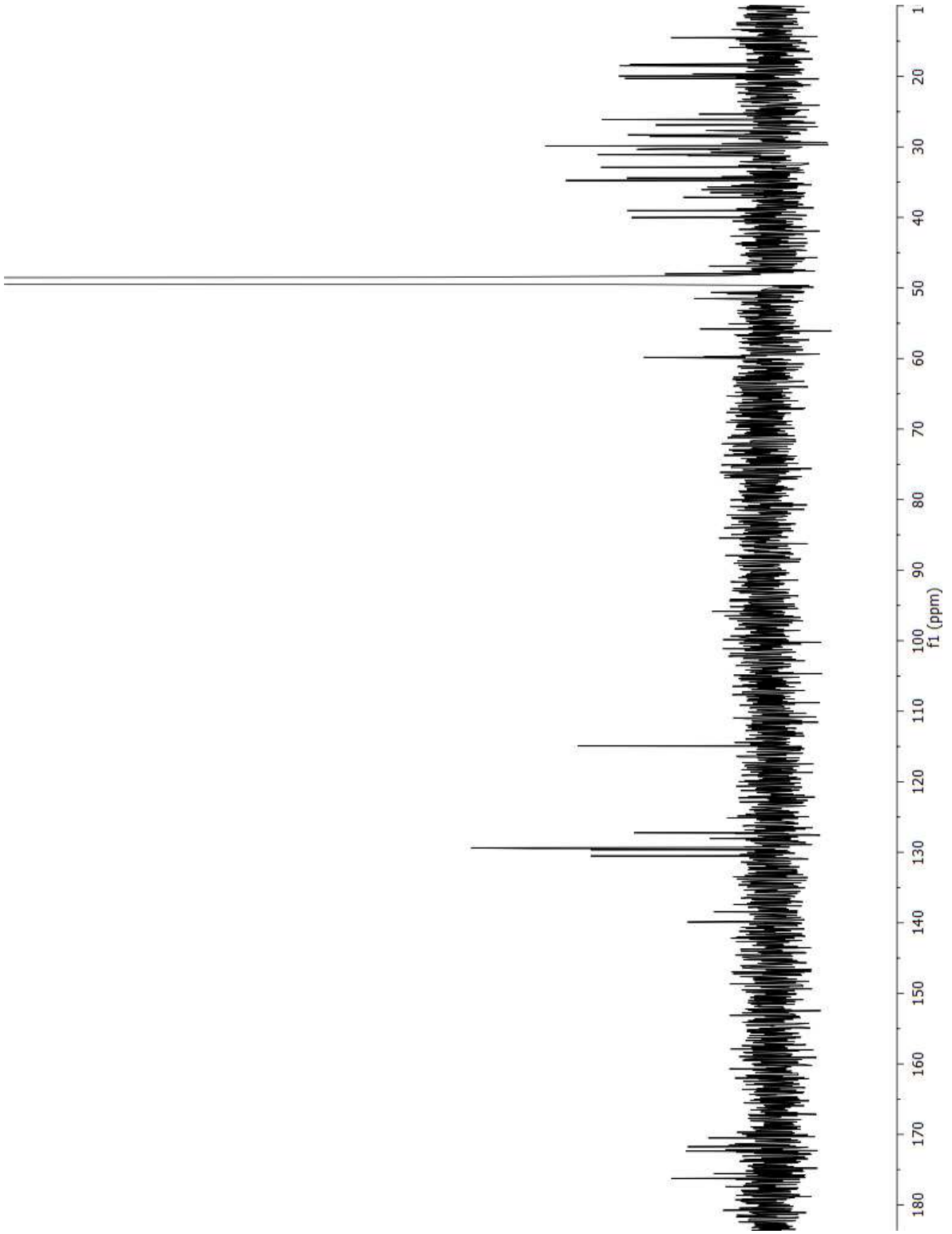
**31:** white solid; $[\alpha]_D^{20}$ -126 (*c* 0.043, MeOH); UV (MeOH) $\lambda_{max}$ (log ε): 216 nm (4.11); $^1$H NMR ($CD_3OD$, 600 MHz) δ 7.30-7.15 (m, 10H), 5.39-5.35 (m, 1H), 5.18 (d, *J* = 10.8 Hz, 1H), 5.15 (d, *J* = 10.8 Hz, 1H), 4.96 (d, *J* = 10.2 Hz, 1H), 4.92 (d, *J* = 10.8 Hz, 1H), 3.50-3.47 (m, 1H), 3.40 (t, *J* = 7.2 Hz, 1H), 3.22 (dt, *J* = 5.4 Hz, 9.6 Hz, 3H), 3.15-3.12 (m, 1H), 3.01 (s, 3H), 2.99 (s, 3H), 2.96 (s, 3H), 2.95 (s, 3H), 2.88 (s, 3H), 2.46 (t, *J* = 7.8 Hz, 2H), 2.42 (dt, *J* = 3 Hz, 7.2

Hz, 2H), 2.34 (s, 3H), 2.15 (t, *J* = 7.8 Hz, 1H), 2.06 (q, *J* = 7.2 Hz, 2H), 1.64-1.60 (m, 4H), 1.49 (p, *J* = 7.2 Hz, 15 Hz, 2H), 1.43 (p, *J* = 7.2 Hz, 15 Hz, 3H), 1.35-1.35 (m, 3H), 1.33-1.29 (m, 9H), 1.25-1.21 (m, 2H), 1.10 (d, *J* = 6.6 Hz, 3H), 0.90-0.87 (m, 7H), 0.83 (d, *J* = 6.6 Hz, 6H), 0.76 (d, *J* = 6 Hz, 6H); $^{13}$C NMR ($CD_3OD$, 150 MHz) δ 14.6, 18.4, 18.5, 18.6, 19.7, 20.0, 20.4, 25.5, 26.2, 27.0, 27.8, 28.4, 28.5, 29.6, 30.0, 30.0, 30.3, 30.4, 30.7, 30.8, 31.2, 33.0, 34.3, 34.5, 34.8, 35.4, 35.6, 36.2, 37.2, 39.1, 40.1, 40.2, 51.6, 55.9, 59.8, 59.9, 60.0, 115.0, 127.3, 128.1, 128.2, 129.5, 129.6, 129.7, 129.8, 130.6, 138.6, 140.0, 170.6, 171.7, 171.8, 172.3, 172.5, 175.6, 176.3, 177.5; HRESIMS *m/z* $[M+H]^+$1071.7596 (calcd for $C_{61}H_{99}N_8O_8$, 1071.7580).

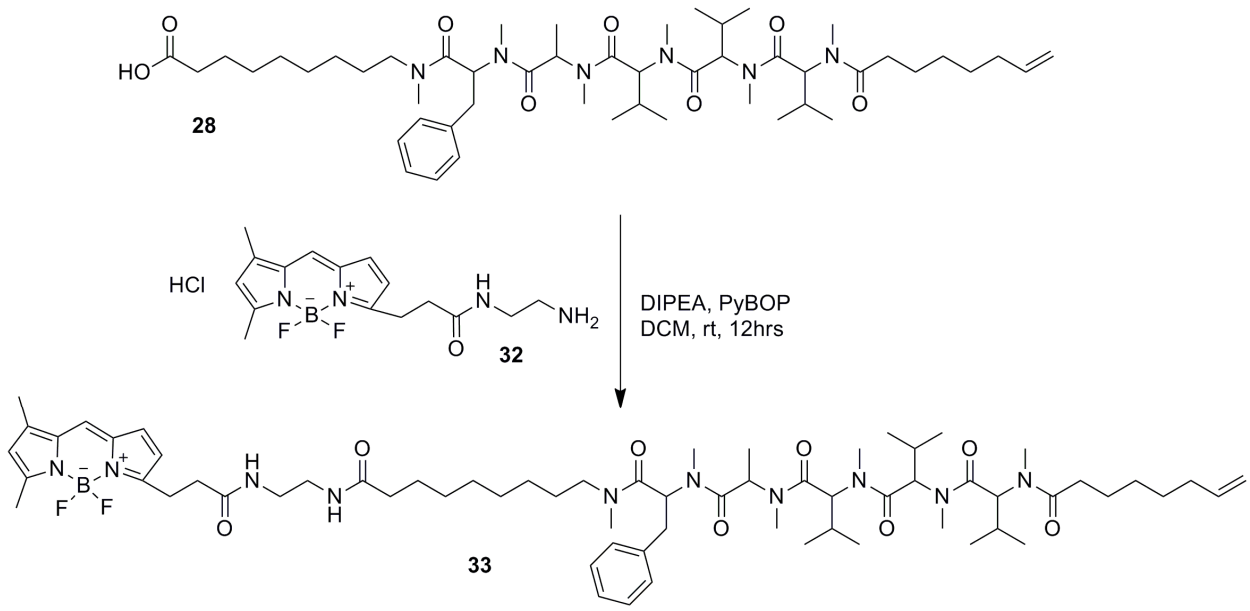
$^{1}$H spectrum of **31** recorded in $CD_3OD$ at 600 MHz.



$^{13}C$ spectrum of **31** recorded in $CD_3OD$ at 150 MHz.



**Almiramide BoDIPY (33) Synthesis**

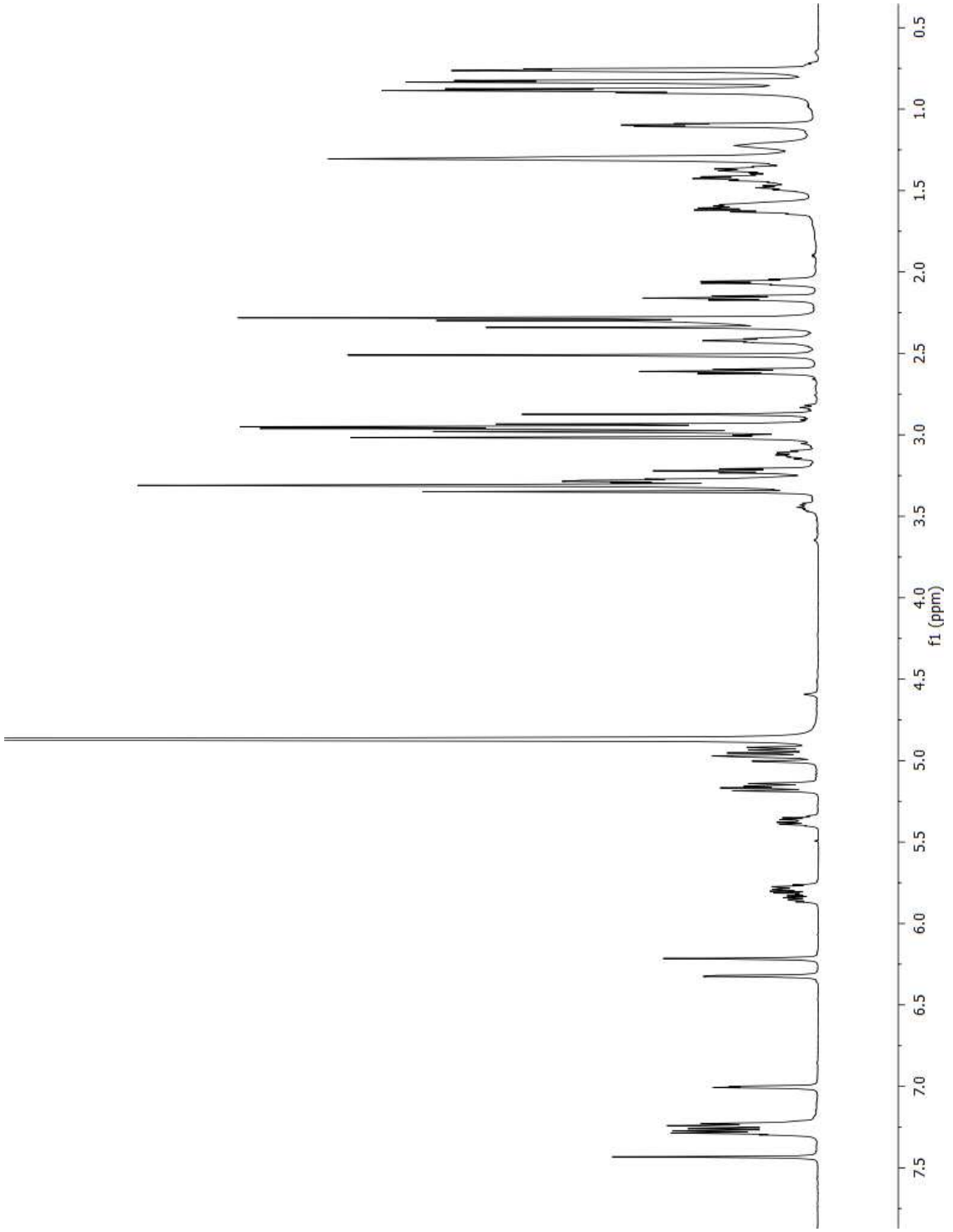



To a solution of **28** (3.82 mg, 4.26 μmol) in DCM (450 μL) were added solutions of PyBOP (3.31 μmol) dissolved in DCM and DIPEA (34.1 μmol) dissolved in DCM and the resulting solution allowed to stir for 20 mins. A dissolved solution of commercially available BODIPY® FL EDA **32** (4.02 μmol) in DCM was added to the reaction. The reaction was allowed to stir overnight at room temperature. The reaction was quenched by removing solvent *in vacuo* and immediately purified by $C_{18}$ RP-HPLC Phenomenex Jupiter $C_{18}$ (4.6 x 250 mm, 5 μm), 83% MeOH/ 17% $H_2O$ (acidified with 0.002% formic acid), 1 mL/min, $t_R$ = 22.7 min to give **33** as an dark red solid (2.05 mg, 42.1%).

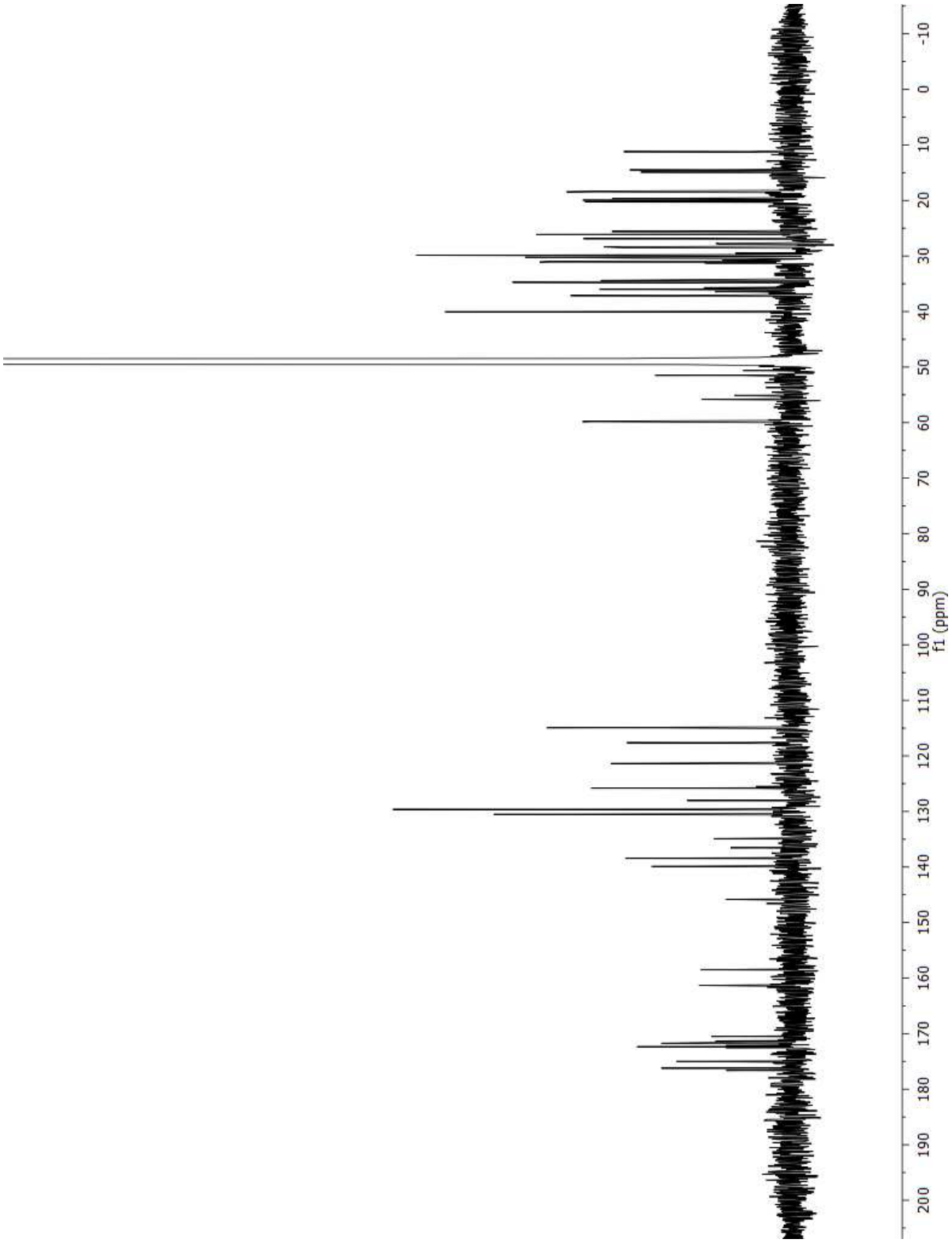
**33:** dark red solid; $[\alpha]_D^{20}$ -79.1 (*c* 0.140, MeOH); UV (MeOH) $\lambda_{max}$ (log ε): 225 nm (4.11), 356 nm (3.45), 504 nm (4.40); $^1$H NMR ($CD_3OD$, 600 MHz) δ 7.43 (s, 1H), 7.29-7.22 (m, 5H), 7.00 (d, *J* = 4.2 Hz, 1H), 6.32 (d, *J* = 4.2 Hz, 1H), 6.21 (s, 1H), 5.86-5.76 (m, 2H), 5.40-5.35 (m, 1H), 5.17 (d, *J* = 10.8 Hz, 1H), 5.14 (d, *J* = 10.8 Hz, 1H), 5.00 (s, 1H), 4.96 (d, *J* = 10.8 Hz, 1H), 4.92

(d, $J$ = 10.8 Hz, 1H), 3.34 (s, 2H), 3.29-3.27 (m, 4H), 3.22 (t, $J$ = 7.2 Hz, 2H), 3.14-3.09 (m, 1H), 3.01 (s, 3H), 2.98 (s, 3H), 2.95 (s, 3H), 2.95 (s, 6H), 2.93 (s, 1H), 2.87 (s, 1H), 2.41 (dt, $J$ = 3 Hz, 7.2 Hz, 2H), 2.34 (s, 2H), 2.16 (t, $J$ = 7.2 Hz, 2H), 2.06 (q, $J$ = 6 Hz, 2H), 1.63-1.58 (m, 4H), 1.48 (p, $J$ = 7.2 Hz, 15 Hz, 2H), 1.42 (p, $J$ = 7.2 Hz, 15 Hz, 2H), 1.37-1.35 (m, 3H), 1.30 (s, 9H), 1.25-1.20 (m, 2H), 1.09 (q, $J$ = 4.2 Hz, 3H), 0.88 (t, $J$ = 7.2 Hz, 7H), 0.82 (d, $J$ = 6 Hz, 6H), 0.75 (d, $J$ = 6 Hz, 6H); $^{13}$C NMR ($CD_3OD$, 150 MHz) δ 11.3, 14.6, 15.0, 18.4, 18.5, 18.6, 19.7, 20.0, 20.4, 25.7, 26.2, 27.0, 27.8, 27.9, 28.0, 28.4, 28.5, 28.6, 30.0, 30.1, 30.3, 30.4, 31.1, 31.2, 34.3, 34.5, 34.8, 35.8, 36.1, 36.5, 37.2, 40.1, 51.6, 55.2, 55.9, 59.8, 59.9, 60.0, 115.0, 117.7, 121.5, 125.9, 128.1, 128.2, 129.7, 129.8, 130.6, 130.6, 135.0, 136.6, 138.6, 140.0, 145.9, 158.6, 161.4, 170.6, 171.4, 171.6, 171.8, 172.2, 172.5, 172.6, 175.1, 176.3, 176.7; HRESIMS *m/z* $[M+Na]^+$ 1235.7928 (calcd for $C_{66}H_{103}BF_2N_{10}O_8Na$, 1235.7914).

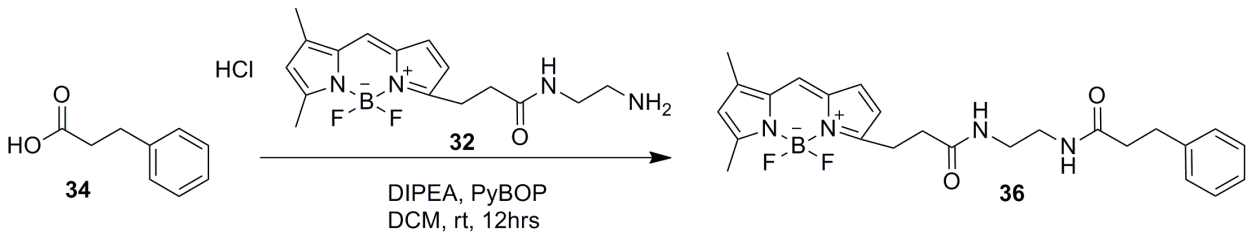
$^{1}$H spectrum of **33** recorded in $CD_3OD$ at 600 MHz.



$^{13}C$ spectrum of **33** recorded in $CD_3OD$ at 150 MHz.



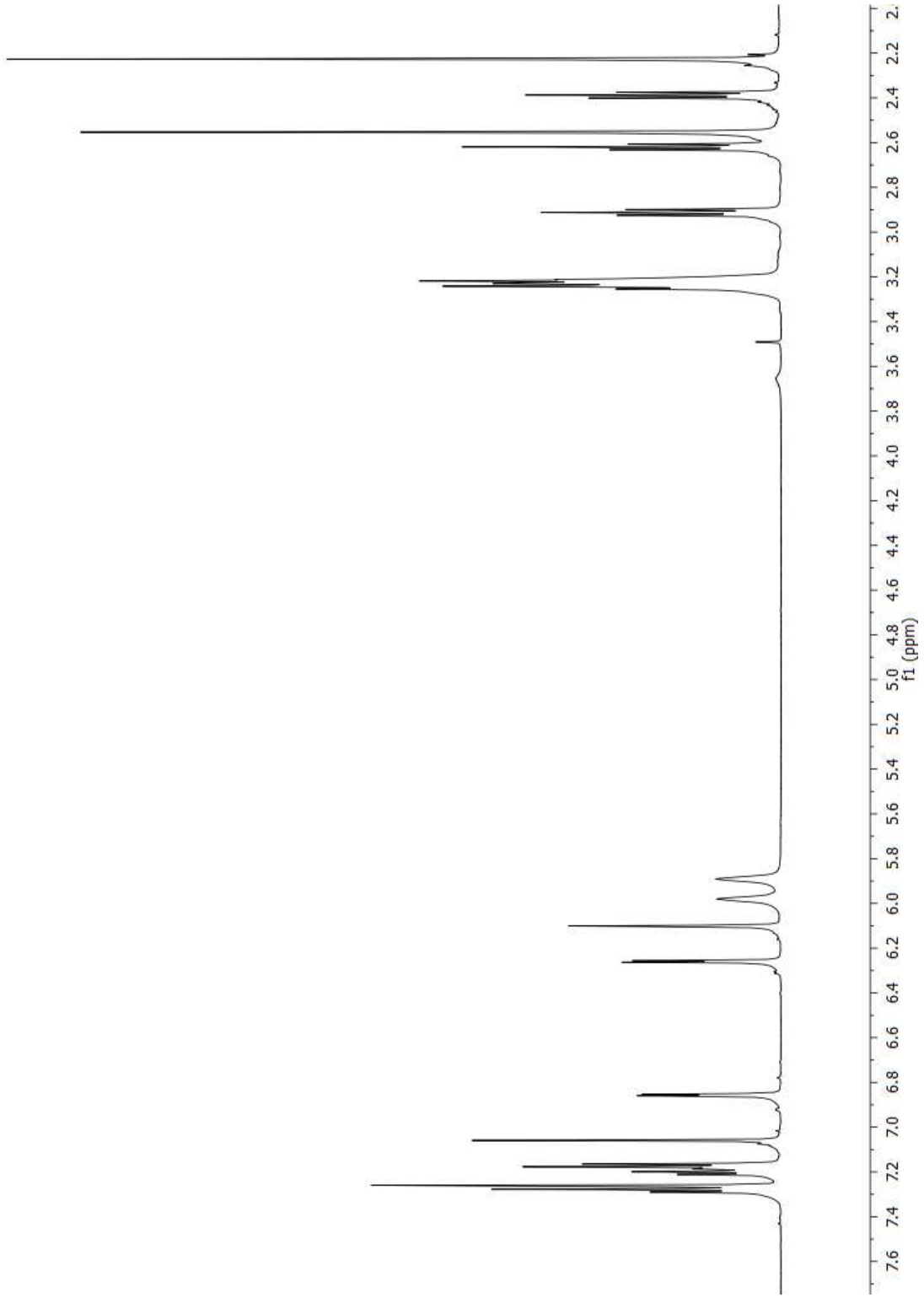
**Bodipy Control (36) Synthesis**



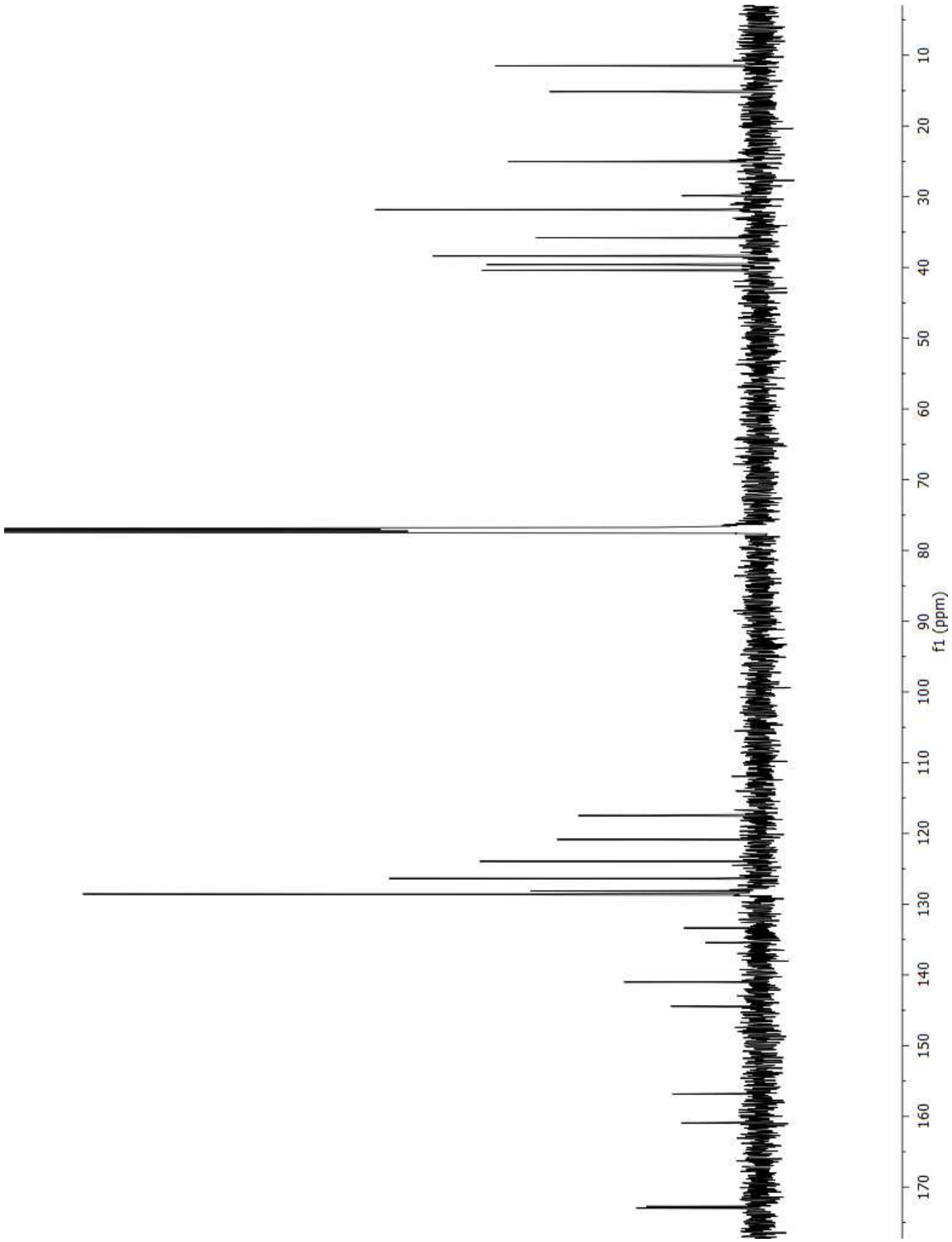

To a solution of **34** (1.12 mg, 7.46 μmol) in DCM (450 μL) were added solutions of PyBOP (2.53 μmol) dissolved in DCM and DIPEA (47.8 μmol) dissolved in DCM and the reaction allowed to stir for 20 mins. A dissolved solution of commercially available BODIPY® FL EDA **32** (2.70 μmol) in DCM was added and the reaction allowed to stir overnight at room temperature. The reaction was quenched by removing solvent *in vacuo* and immediately purified by $C_{18}$ RP-HPLC Phenomenex Jupiter $C_{18}$ (4.6 x 250 mm, 5 μm), 60% MeOH/ 40% $H_2O$ (acidified with 0.002% formic acid), 1 mL/min, $t_R$ = 14.3 min to give **36** as an dark red solid (0.75 mg, 60.0%).

**36:** red solid; UV (MeOH) $\lambda_{max}$ (log ε): 227 nm (3.38), 328 nm (3.18), 504 nm (3.82); $^1$H NMR ($CDCl_3$, 600 MHz) δ 7.27-7.24 (m, 2H), 7.19-7.14 (m, 3H), 7.03 (s, 1H), 6.83 (d, *J* = 4.2 Hz, 1H), 6.24 (d, *J* = 4.2 Hz, 1H), 6.08 (s, 1H), 5.96 (bs, 1H), 5.87 (bs, 1H), 3.23-3.19 (m, 6H), 2.89 (t, *J* = 7.8 Hz, 2H), 2.59 (t, *J* = 7.8 Hz, 2H), 2.53 (s, 3H), 2.36 (t, *J* = 7.8 Hz, 2H), 2.20 (s, 3H); $^{13}$C NMR ($CDCl_3$, 150 MHz) δ 11.5, 15.2, 25.1, 29.9, 31.8, 35.8, 38.4, 39.6, 40.4, 117.5, 120.9, 124.0, 126.4, 128.2, 128.6, 128.7, 133.4, 135.4, 141.0, 141.0, 144.5, 156.8, 161.0, 172.7, 173.0; HRESIMS *m/z* $[M+H]^+$ 467.2430 (calcd for $C_{25}H_{30}BF_2N_4O_2$, 467.2424).

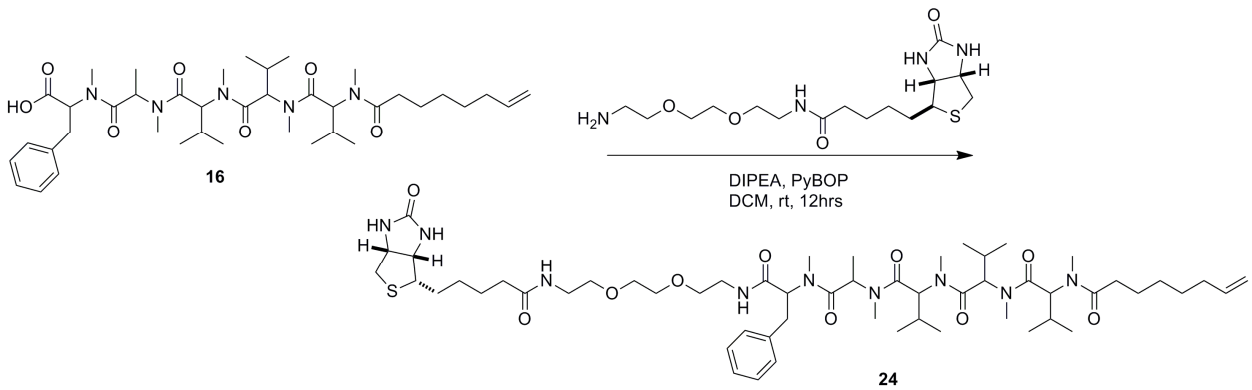
$^{1}H$ spectrum of **36** recorded in $CDCl_3$ at 600 MHz.



$^{13}C$ spectrum of **36** recorded in $CDCl_3$ at 150 MHz.



**Biotinylated Almiramide (24) Synthesis**

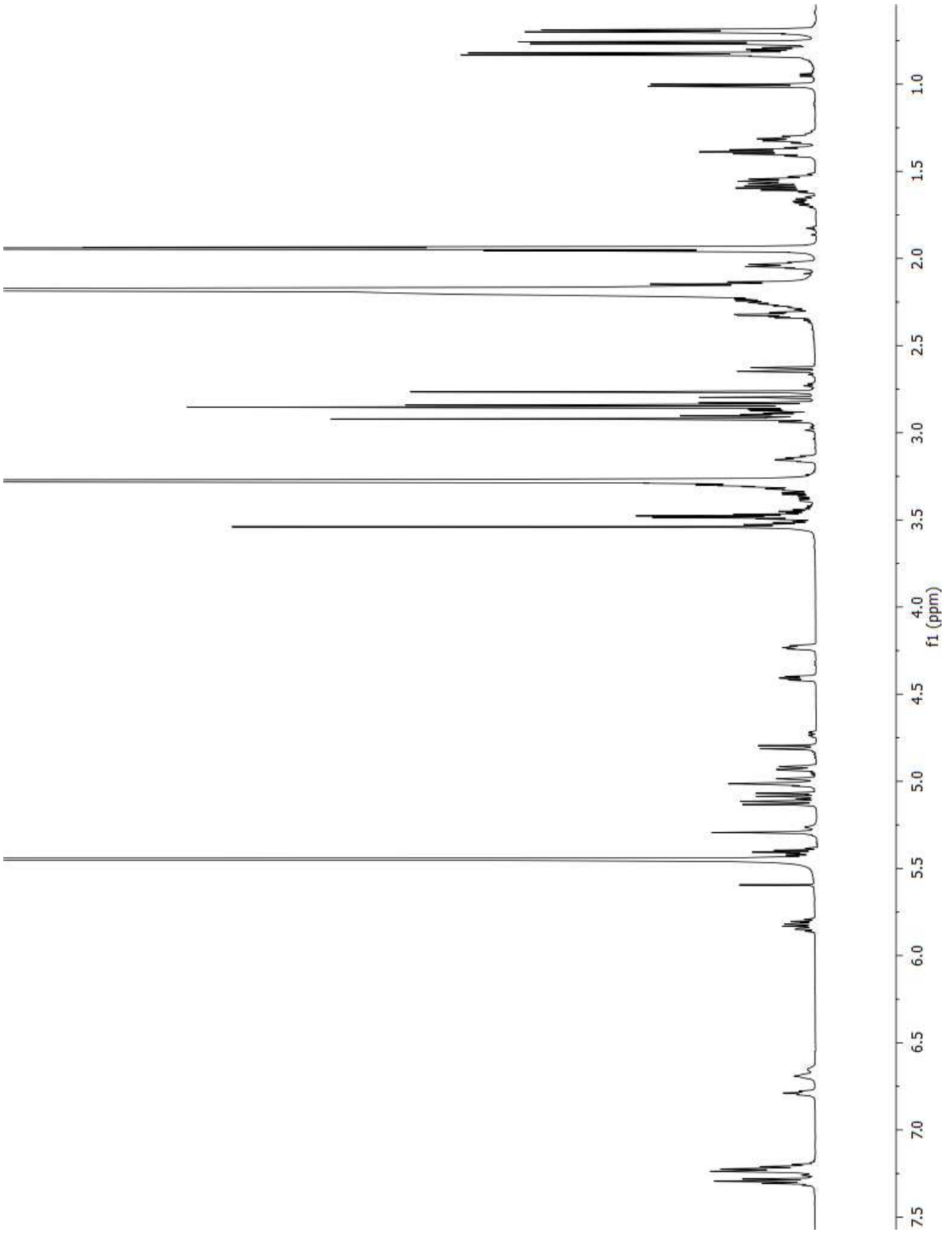



To a dissolved solution of lipopentapeptide **16** (10.0 mg, 13.7 μmol; prepared as previously described[2]) in DCM (1 mL) were added solutions of PyBOP (10.7 μmol) dissolved in DCM and DIPEA (0.22 mmol) dissolved in DCM and the reaction allowed to stir for 20 mins. A solution of commercially available EZ-Link Biotin PEO-Amine (14.4 μmol) in DCM was added and the reaction allowed to stir overnight at room temperature. The reaction was quenched by removing solvent *in vacuo* and immediately purified by $C_{18}$ RP-HPLC (Phenomenex Synergi $C_{18}$ 250 x 10 mm column, 10 μm), 80%MeOH/ 20% $H_2O$ (acidified with 0.002% formic acid), 2 mL/min, $t_R$ = 28.1 min to give **24** as a white solid (2.55 mg, 17.1%).

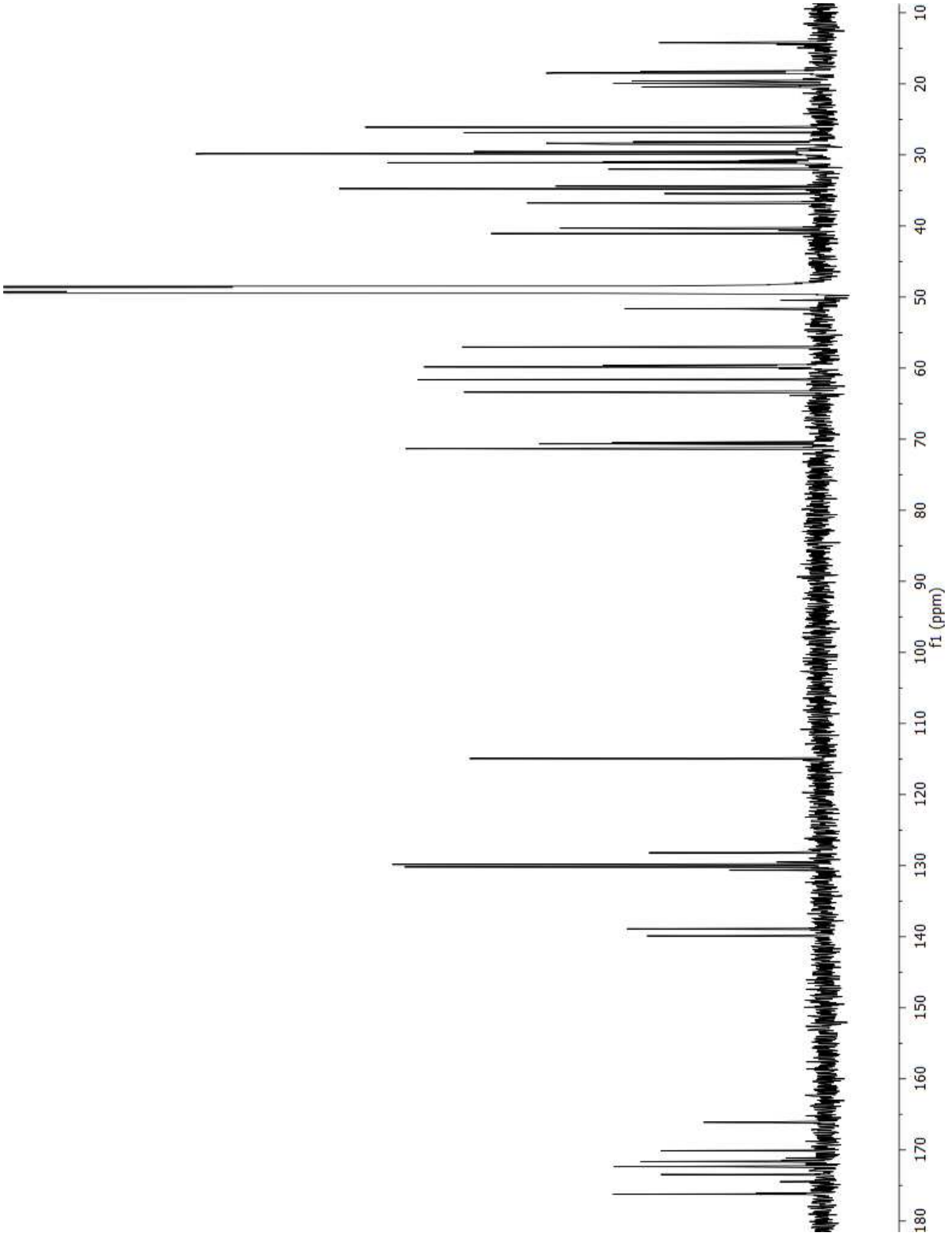
**24:** white solid; $[\alpha]_D^{20}$ -136 (*c* 0.171, MeOH); UV (MeOH) $\lambda_{max}$ (log ε): 218 nm (4.09); $^1$H NMR ($CD_3CN$, 600 MHz) δ 7.30 (t, *J* = 7.2 Hz, 2H), 7.26-7.19 (m, 3H), 6.77 (t, *J* = 5.4 Hz, 1H), 6.65 (t, *J* = 5.4 Hz, 1H), 5.82 (dddd, *J* = 6.6, 6.6, 10.2, 16.8 Hz, 1H) 5.43-5.38 (m, 2H), 5.28 (s, 1H), 5.12 (d, *J* = 10.8 Hz, 1H), 5.07 (d, *J* = 10.2 Hz, 1H), 5.02 (d, *J* = 7.8 Hz, 1H), 4.98 (s, 1H), 4.92 (d, *J* = 10.2 Hz, 1H), 4.40 (t, *J* = 6 Hz, 1H), 4.23 (t, *J* = 6 Hz, 1H), 3.53-3.50 (m, 5H), 3.48 (q, *J* = 5.4 Hz, 3H), 3.46-3.44 (m, 1H), 3.38-3.27 (m, 6H), 3.14 (q, *J* = 7.8 Hz, 1H), 2.92 (s, 3H), 2.90-2.86 (m, 3H), 2.85 (s, 6H), 2.84 (s, 3H), 2.76 (s, 3H), 2.32 (dt, *J* = 4.2 Hz, 7.2 Hz, 2H), 2.28-2.21 (m, 4H), 2.03 (q, *J* = 6 Hz, 3H), 1.95 (s, 3H), 1.67 (s, *J* = 7.2 Hz, 1H), 1.59 (p, *J* = 7.8 Hz, 2H),

1.55 (t, $J$ = 7.2 Hz, 3H), 1.38 (p, $J$ = 7.2 Hz, 15 Hz, 5H), 1.33-1.31 (m, 2H), 1.00 (d, $J$ = 6.6 Hz, 2H), 0.82 (d, $J$ = 6.6 Hz, 6H), 0.80 (dd, $J$ = 4.8 Hz, 6.6 Hz, 2H), 0.76 (d, $J$ = 6.6 Hz, 6H), 0.69 (d, $J$ = 6.6 Hz, 6H); $^{13}$C NMR ($CD_3OD$, 150 MHz) δ 14.3, 18.4, 18.5, 18.6, 19.7, 20.0, 20.5, 26.2, 27.0, 28.3, 28.5, 28.6, 29.6, 29.9, 30.0, 30.1, 30.9, 31.0, 31.2, 32.1, 34.5, 34.8, 35.5, 36.9, 40.4, 40.5, 41.2, 51.8, 57.1, 59.7, 59.8, 59.9, 61.7, 63.5, 70.5, 70.6, 70.8, 71.4, 71.4, 115.0, 128.3, 129.9, 130.0, 130.3, 130.8, 139.0, 140.0, 166.2, 170.2, 171.7, 172.4, 172.5, 173.6, 176.2, 176.3; HRESIMS *m/z* $[M+H]^+$ 1084.6853 (calcd for $C_{56}H_{94}N_9O_{10}S$, 1084.6839).

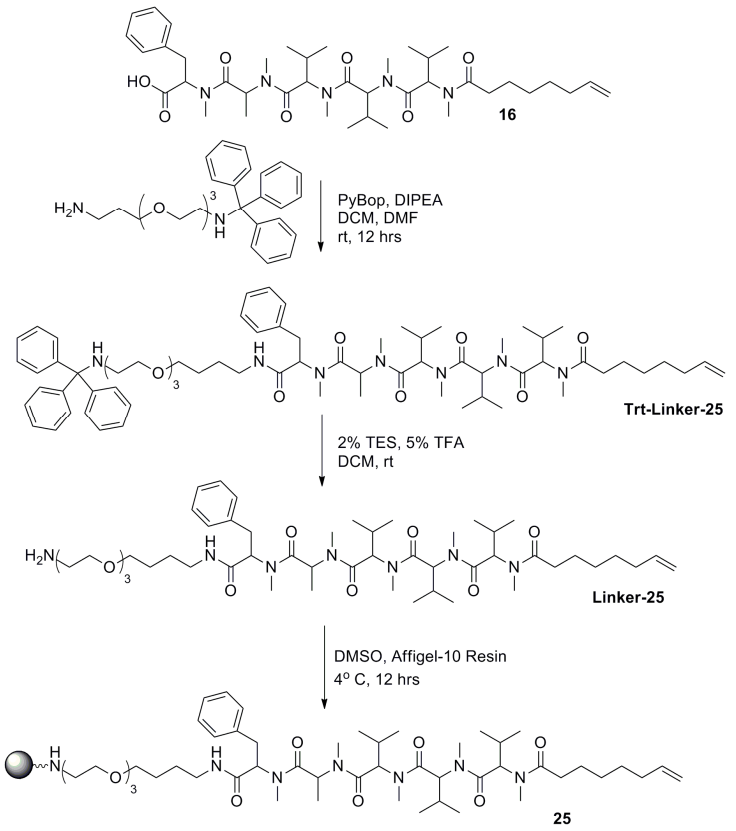
$^{1}$H spectrum of **24** recorded in $CD_3CN$ at 600 MHz.



$^{13}C$ spectrum of **24** recorded in $CD_3OD$ at 150 MHz.



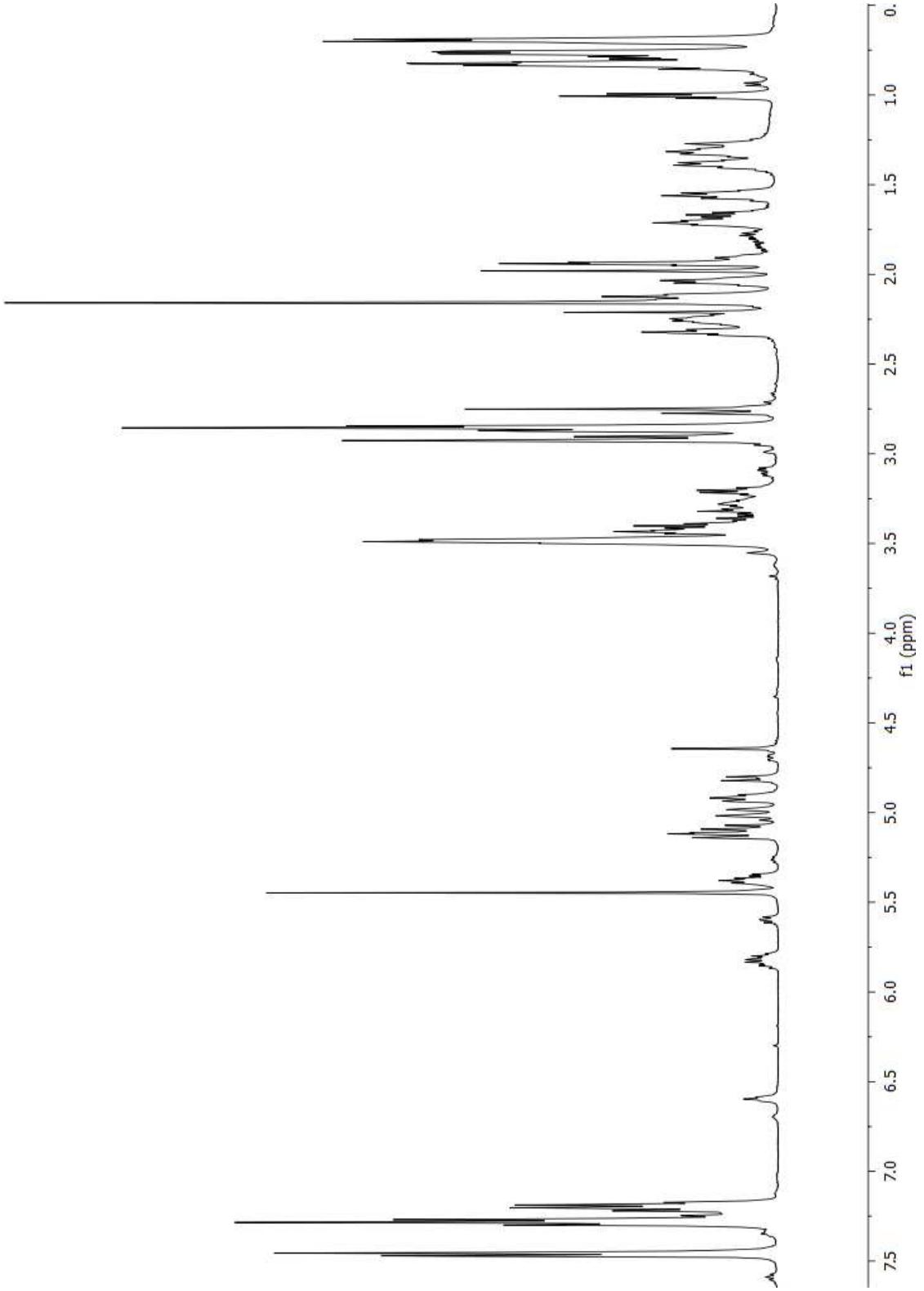
**Affinity Gel Almiramide (25) Synthesis**




To a dissolved solution of lipopentapeptide **16** (21.4 mg, 29.40 μmol) in DCM (1 mL) were added solutions of PyBOP (49.97 μmol) dissolved in DCM and DIPEA (0.93 mmol) dissolved in DCM and the reaction allowed to stir for 20 mins. A solution of commercially available Trt-NH-$PEG_2$-$NH_2$ (73.27 μmol) in DCM was added and the reaction allowed to stir overnight at room temperature. The reaction was quenched by removing solvent *in vacuo* and the resulting slurry basified to pH 8 using 0.1 M NaOH. The basified mixture was partitioned against EtOAc (20 mL), the phases separated, and the aqueous phase washed with EtOAc (2 x 20 mL). The combined organics were concentrated to dryness *in vacuo* and the resulting yellow powder purified by flash column chromatography (2:100 MeOH/ DCM, $R_f$ = 0.2) to give **Trt-Linker-25** as a yellow solid (21.23 mg, 61.58%).

**Trt-Linker-25:** yellow solid; $^1$H NMR ($CD_3CN$, 500 MHz) δ 7.46 (d, *J* = 7.5 Hz, 6H), 7.28 (t, *J* = 7.5 Hz, 8H), 7.22-7.17 (m, 6H), 5.86 (dddd, *J* = 6.6, 6.6, 10.2, 16.8 Hz, 1H), 5.60 (dd, *J* = 6 Hz, 9.5 Hz, 1H), 5.39-5.34 (m, 2H), 5.14-5.07 (m, 2H), 5.02-4.98 (m, 1H), 4.93-4.90 (m, 1H), 4.81 (d, *J* = 11 Hz, 1H), 4.64 (s, 1H), 3.49-3.48 (m, 8H), 3.44-3.40 (m, 4H), 3.30-3.25 (m, 2H), 3.21 (q, *J* = 6.5 Hz, 2H), 2.92 (s, 3H), 2.87 (s, 3H), 2.85 (s, 3H), 2.84 (s, 3H), 2.75 (s, 3H), 2.33-2.21 (m, 6H), 2.04-2.03 (m, 2H), 1.98 (s, 1H), 1.71-1.65 (m, 2H), 1.56 (p, *J* = 7.5 Hz, 2H), 1.39 (p, *J* = 7.5 Hz, 2H), 1.31-1.27 (m, 2H), 1.00 (t, *J* = 6 Hz, 3H), 0.85-0.79 (m, 8H), 0.76-0.75 (m, 6H), 0.70-0.68 (m, 6H).

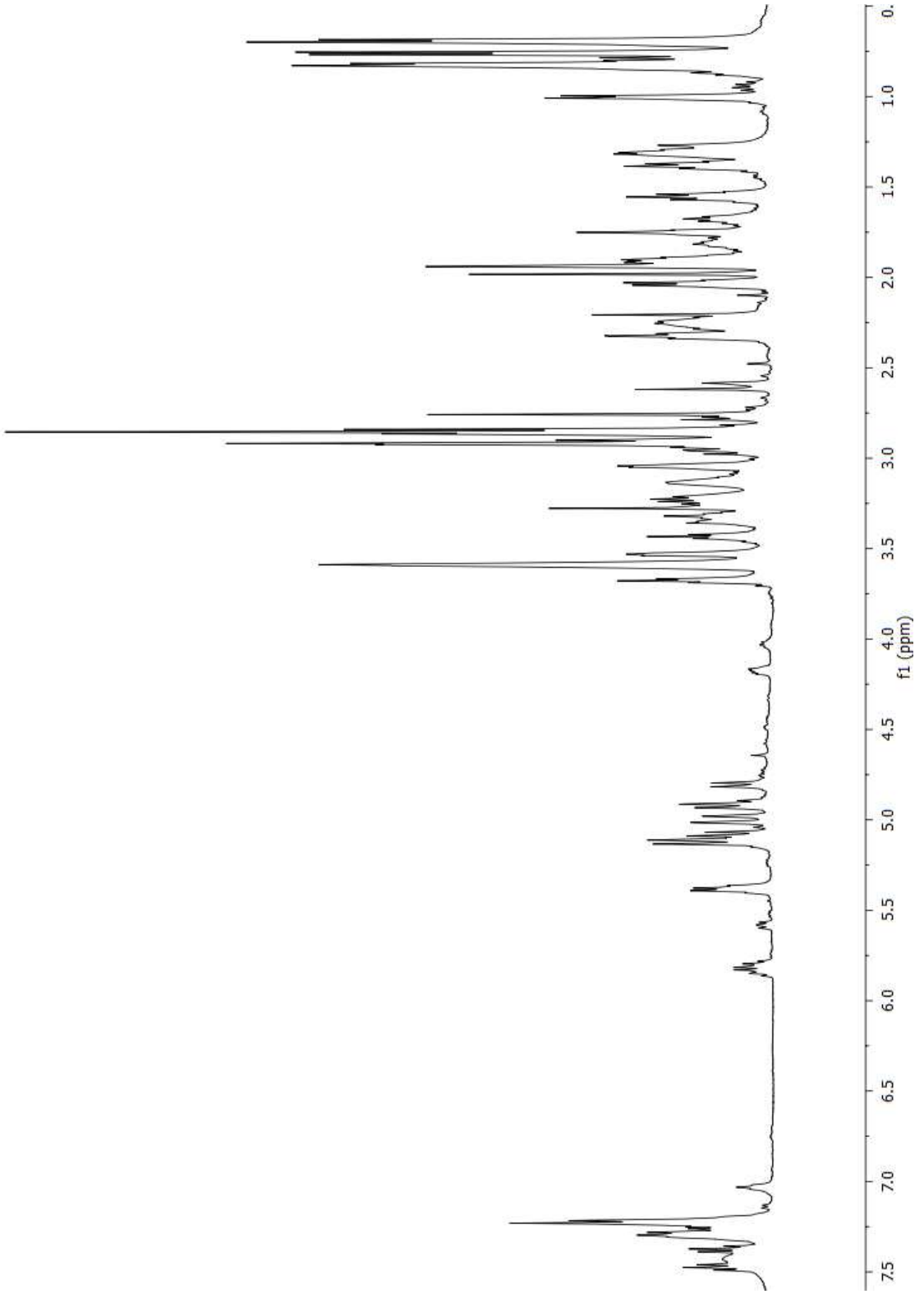
$^{1}$H spectrum of **Trt-Linker-25** recorded in $CD_3CN$ at 500 MHz.



For further reactions, the Trt protecting group was removed by stirring a desired amount of **Trt-Linker-25** in solution of 2% TES and 5% TFA in DCM and monitoring the reaction progress by observing the color of the solution. After the reaction was complete, the reaction was quenched by removing the solvent *in vacuo*. The resulting slurry was purified by flash column chromatography (2:100 MeOH/ DCM) and **Linker-25** was eluted from the column with 100% MeOH to give **Linker-25** as a white solid (100%).

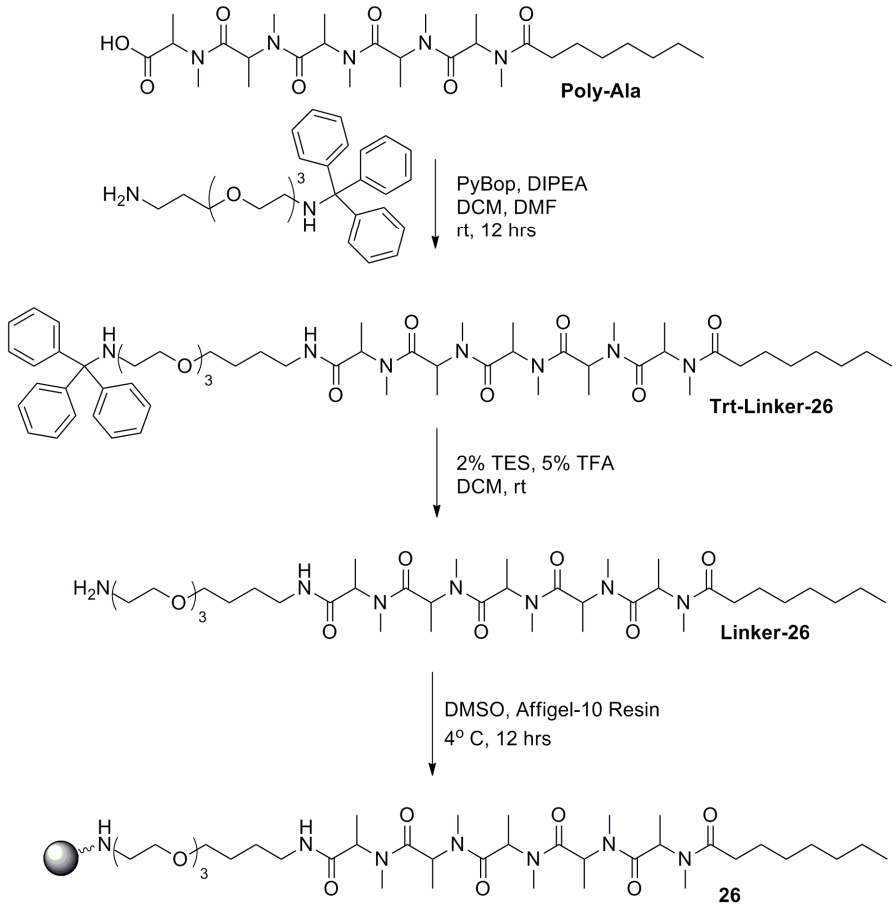
**Linker-25:** yellow solid; $^{1}$H NMR ($CD_3CN$, 500 MHz) δ 7.47 (t, *J* = 7.5 Hz, 1H), 7.37 (t, *J* = 7.5 Hz, 2H), 7.22 (d, *J* = 7.5 Hz, 2H), 5.82 (dddd, *J* = 6.6, 6.6, 10.2, 16.8 Hz, 1H), 5.38 (q, *J* = 6.5 Hz, 1H), 5.13-5.06 (m, 3H), 5.01-4.98 (m, 1H), 4.93-4.89 (m, 1H), 4.80 (d, *J* = 10.5 Hz, 1H), 3.67 (t, *J* = 5 Hz, 2H), 3.59-3.58 (m, 4H), 3.43 (t, *J* = 5.5 Hz, 2H), 3.35-3.30 (m, 2H), 3.27-3.21 (m, 4H), 3.14-3.13 (m, 2H), 3.05-3.04 (m, 2H), 2.91 (s, 3H), 2.86 (s, 3H), 2.85 (s, 3H), 2.84 (s, 3H), 2.75 (s, 3H), 2.62 (s, 1H), 2.28-2.24 (m, 4H), 2.04-2.03 (m, 2H), 1.98 (s, 1H), 1.82-1.75 (m, 2H), 1.67 (p, *J* = 6 Hz, 2H), 1.55 (p, *J* = 7.5 Hz, 2H), 1.38 (p, *J* = 6 Hz, 2H), 1.32-1.26 (m, 2H), 1.00-0.99 (m, 3H), 0.82-0.81 (m, 8H), 0.75 (d, *J* = 7 Hz, 6H), 0.69 (d, *J* = 6.5 Hz, 6H); HRFTESIMS *m/z* $[M+H]^+$ 930.6643 (calcd for $C_{50}H_{88}N_7O_9$, 930.6638).

$^{1}$H spectrum of **Linker-25** recorded in $CD_3CN$ at 500 MHz.



Compound **Linker-25** was immobilized on a solid support for use in affinity chromatography experiments. Before use in **Linker-25-**immoblization, 7 mL of Affigel-10 resin (3.5 mL settled resin) was rinsed in five volumes of isopropanol (2x). Dried **Linker-25** was dissolved in 1 mL of DMSO and added to the washed Affigel resin. The suspension was allowed to rock at 4 ºC overnight. The beads were then centrifuged at 1,300 *g* for 10 min to remove the DMSO, and the beds were then washed in HEPES buffer (50 mM HEPES, pH 8.5) (3x). The resulting resin was stored at 4 ºC.

**Affinity Control (26) Synthesis**




Fmoc-Alanine (2.48 g, 7.97 mmol) and DIPEA (2.64 mL, 15.97 mmol) were added to a stirred suspension of 2-chlorotrityl chloride resin (1.00 g; 200-400 mesh; 1% DBV) in DCM (40 mL)

under argon. The resulting suspension stirred at room temperature for two hours. The solution was transferred to a polypropylene vessel containing a fritted disk and fitted with a Teflon stopcock, drained, and washed (3 x 5 mL DCM, 3 x 5 mL DMF, 3 x 5 mL DCM). The resulting pale yellow resin was dried for 24 hours, and analyzed to determine the loading value of the phenylalanine substrate as outlined below.

Two aliquots (6.64 mg and 6.87 mg) of the loaded resin were transferred to Eppendorf tubes, dissolved in 1% DBU in DMF (500 μL), and agitated for 1 hour. 50 μL aliquots of these solutions were diluted with DMF (2 mL), and absorbance measured at $\lambda = 301$ nm to determine the loading value of the Fmoc-alanine as 0.256 mmol per gram of resin.

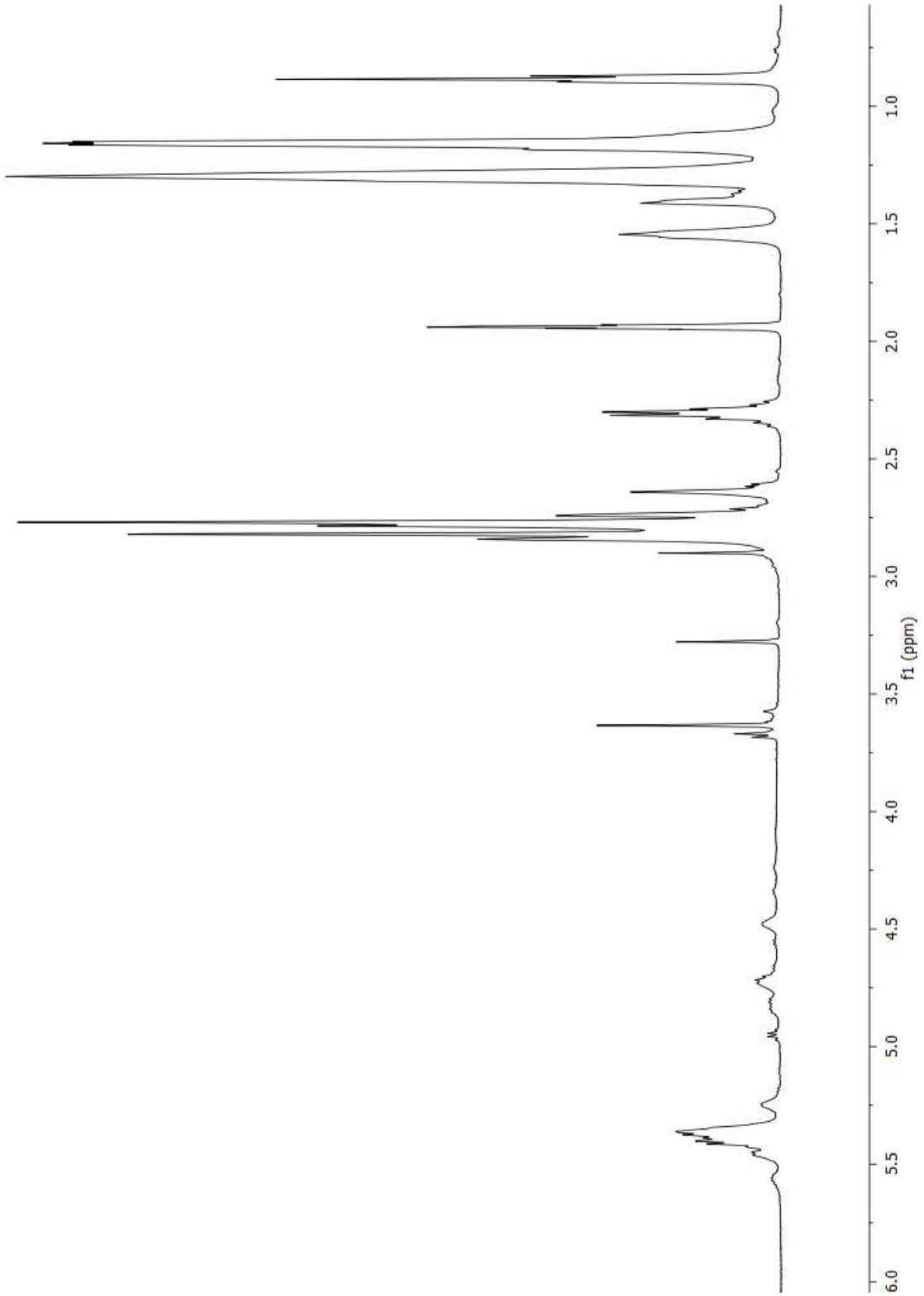
Subsequent amino acids were added using a standard protocol. The dried resin was allowed to swell for 5 minutes in DCM (5 mL), drained, and washed with DMF (5 mL). The washed resin was deprotected using 20% piperidine in DMF (5 mL) with agitation for 20 minutes. After deprotection, the resin was drained, and washed (3 x 5 mL DMF, 3 x 5 mL DCM, 3x 5 mL DMF). To this washed resin was added a solution of HBTU (0.55 g, 1.46mmol), the appropriate amino acid (1.50 mmol), and DIPEA (0.495 mL, 3.00mmol). The resulting suspension was gently agitated for 1 hour, drained, and washed, as described above. This process was repeated with successive amino acids to form resin-bound Ala-Ala-Ala-Ala-Ala-Fmoc.

From this peptide precursor, the loaded resin (0.995 g) was deprotected by treatment with 20% piperidine in DMF (5 mL) with agitation for 20 minutes. 7-Octenoic acid (240 μL, 1.50 mmol), HBTU (0.557 g, 1.47 mmol), and DIPEA (495μL, 3.00 mmol) were added and the resulting suspension agitated for 1 hour, drained, and washed as previously described. The resulting linear lipopeptide was cleaved from the resin by treatment with 1% TFA in DCM for 20 minutes and the solvent removed by evaporation under a stream of $N_2$ gas. The resulting glossy

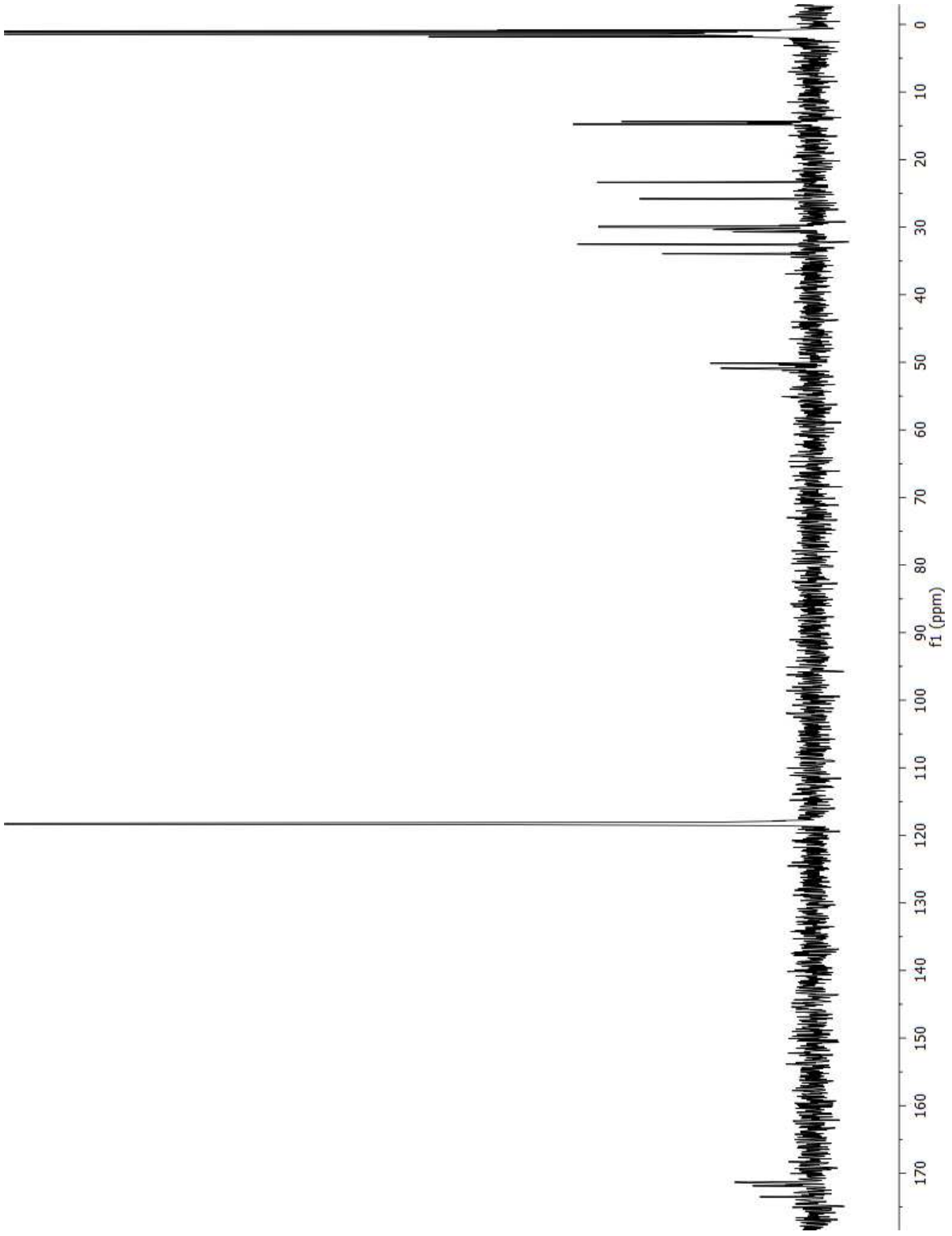
white solid was triturated with *t*-butyl methyl ether and centrifuged at 1000 rpm for 20 minutes. Removal of the *t*-butyl methyl ether afforded the lipopeptide as a white solid (811 mg), which was carried to the next step without further purification.

**Poly-Ala:** white solid; $^{1}$H NMR ($CD_3CN$, 500 MHz) δ 5.45-5.36 (m, 5H), 2.84 (s, 3H), 2.82 (s, 3H), 2.78 (s, 3H), 2.77 (s, 3H), 2.74 (s, 3H), 2.33-2.28 (m, 2H), 1.55-1.53 (m, 2H), 1.41-1.40 (m, 2H), 1.31-1.29 (m, 11H), 1.18-1.15 (m, 10H), 0.88 (t, $J$ = 7.2 Hz, 3H); $^{13}$C NMR ($CD_3CN$, 125 MHz) δ 14.3, 14.5, 14.7, 23.3, 25.7, 29.8, 30.0, 30.2, 30.3, 30.6, 32.5, 33.9, 50.1, 50.3, 50.8, 51.0, 51.2, 171.3, 171.4, 171.8, 173.4; HRFTESIMS *m/z* $[M+Na]^+$ 592.3679 (calcd for $C_{28}H_{51}N_5O_7Na$, 592.3681).

$^{1}$H spectrum of **Poly-Ala** recorded in $CD_3CN$ at 500 MHz.



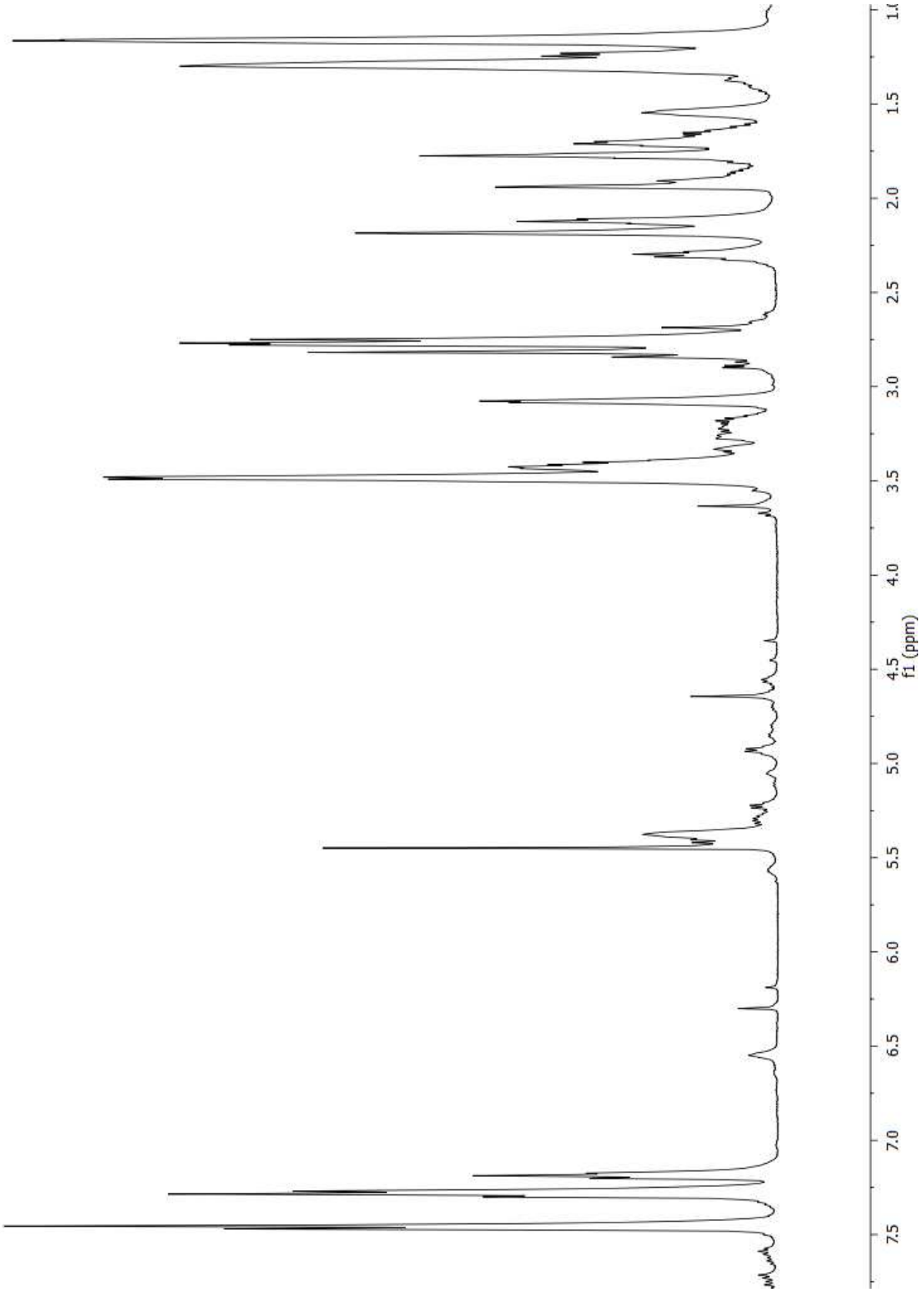
$^{13}C$ spectrum of **Poly-Ala** recorded in $CD_3CN$ at 125 MHz.



To a dissolved solution of per-methylated lipopentapeptide prepared as previously described[2] (20.4 mg, 35.88 μmol) in DCM (1 mL) were added solutions of PyBOP (60.9 μmol) dissolved in DCM and DIPEA (1.14 mmol) dissolved in DCM and the reaction and allowed to stir for 20 mins. A solution of commercially available Trt-NH-$PEG_2$-$NH_2$ (89.7 μmol) in DCM was added and the reaction allowed to stir overnight at room temperature. The reaction was quenched by removing solvent *in vacuo* and the resulting slurry was basified to pH 8 using 0.1 M NaOH. The basified mixture was partitioned against EtOAc (20 mL), the phases separated, and the aqueous phase washed with EtOAc (2 x 20 mL). The combined organics were concentrated to dryness *in vacuo* and the resulting yellow powderwas purified by flash column chromatography (2:100 MeOH/ DCM, $R_f$ = 0.2) to give **Trt-Linker-26** as a yellow solid (22.02 mg, 60.5%).

**Trt-Linker-26:** yellow solid; $^1$H NMR ($CD_3CN$, 500 MHz) δ 7.46 (d, *J* = 7.5 Hz, 6H), 7.28 (t, *J* = 7.5 Hz, 6H), 7.18 (t, *J* = 7.5 Hz, 3H), 5.44-5.36 (m, 5H), 3.49-3.40 (m, 12H), 3.18-3.16 (m, 2H), 2.84 (s, 3H), 2.81 (s, 3H), 2.77 (s, 3H), 2.76 (s, 3H), 2.74 (s, 3H), 2.32-2.28 (m, 2H), 1.78-1.70 (m, 8H), 1.53-1.51 (m, 2H), 1.29-1.15 (m, 21H), 0.88-0.87 (m, 3H).

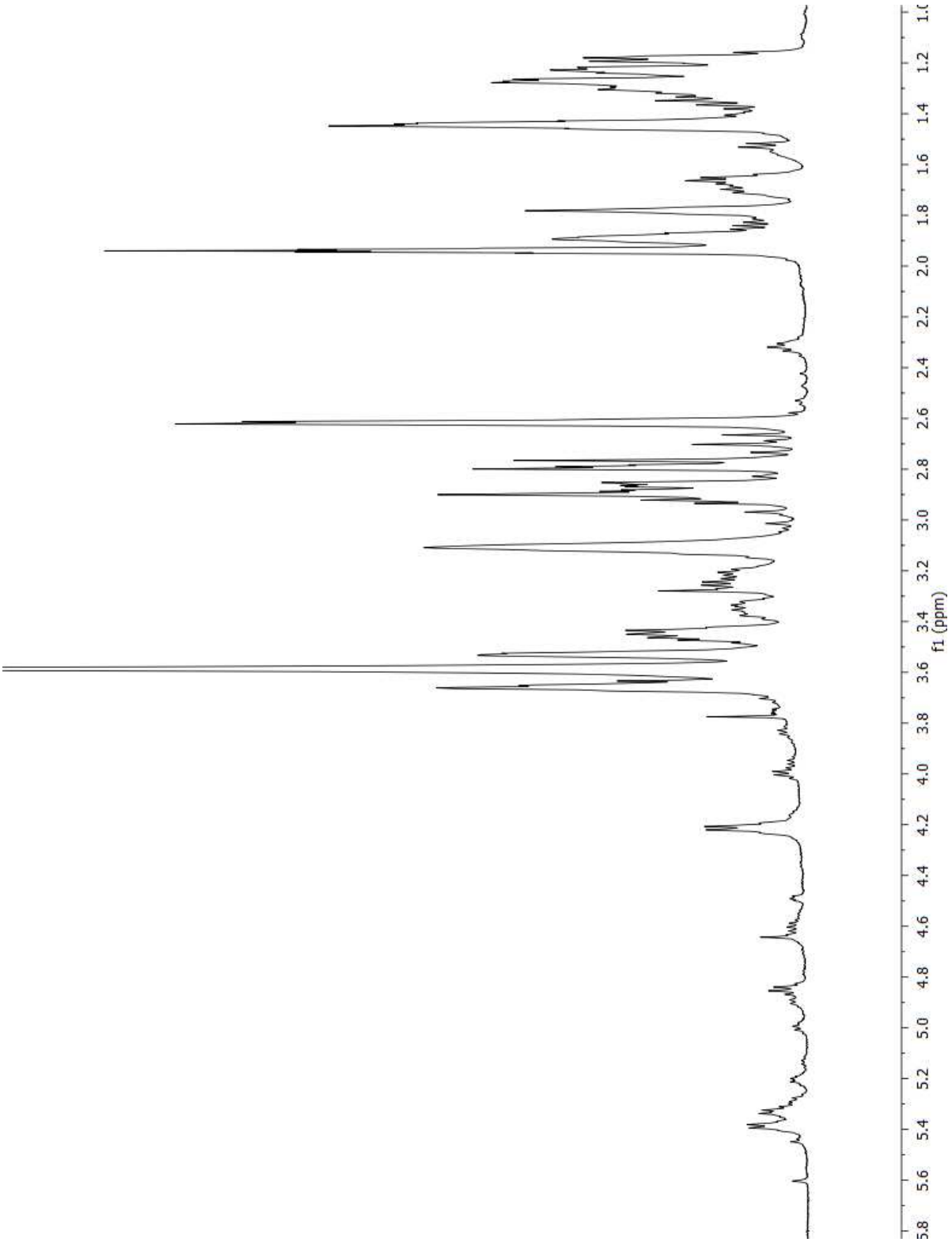
$^{1}$H spectrum of **Trt-Linker-26** recorded in $CD_3CN$ at 500 MHz.



For further reactions, the Trt protecting group was removed by stirring a desired amount of **Trt-Linker-26** in solution of 2% TES and 5% TFA in DCM and monitoring the reaction progress by observing the color of the solution. After the reaction was complete, the reaction was quenched by removing the solvent *in vacuo*. The resulting slurry was purified by flash column chromatography (2:100 MeOH/ DCM) and **Linker-26** was eluted from the column with 100% MeOH to give **Linker-26** as a white solid (100%).

**Linker-26:** yellow solid; $^{1}$H NMR ($CD_3CN$, 500 MHz) δ 5.40-5.31 (m, 5H), 4.21 (q, $J$ = 5 Hz, 2H), 3.66-3.65 (m, 2H), 3.64-3.58 (m, 4H), 3.55-3.53 (m, 4H), 3.46-3.43 (m, 2H), 3.38-3.32 (m, 2H), 3.27-3.20 (m, 4H), 3.10 (bs, 3H), 2.90 (s, 3H), 2.87 (s, 3H), 2.79 (s, 3H), 2.62 (s, 3H), 1.89-1.87 (m, 4H), 1.71-1.66 (m, 4H), 1.56-1.51 (m, 4H), 1.44-1.42 (m, 6H), 1.36-1.30 (m, 6H), 1.27-1.17 (m, 6H).

$^{1}$H spectrum of **Linker-26** recorded in $CD_3CN$ at 500 MHz.



Compound **Linker-26** was immobilized on a solid support for use in affinity chromatography experiments. Before use in **Linker-26-**immobilization, 5 mL of Affigel-10 resin (2.5 mL settled resin) was rinsed in five volumes of isopropanol (2x). Dried **Linker-26** was dissolved in 1 mL of DMSO and added to the washed Affigel resin. The suspension was allowed to rock at 4 ºC overnight. The beads were then centrifuged at 1,300 *g* for 10 min to remove the DMSO, and the beds were then washed in HEPES buffer (50 mM HEPES, pH 8.5) (3x). The resulting resin is stored at 4 ºC.

**Biotinylated Photoreactive ABPP Almiramide Probe (27)**

Fmoc-4-benzoyl-phenylalanine (0.982 g, 2.00 mmol) and DIPEA (0.66 mL, 4.00 mmol) were added to a stirred suspension of 2-chlorotrityl chloride resin (0.25 g; 200-400 mesh; 1% DBV) in DCM (20 mL) under argon. The resulting suspension stirred at room temperature for two hours. The solution was transferred to a polypropylene vessel containing a fritted disk and fitted with a Teflon stopcock, drained, and washed (3 x 5 mL DCM, 3 x 5 mL DMF, 3 x 5 mL DCM). The resulting pale yellow resin was dried for 24 hours, and analyzed to determine the loading value of the phenylalanine substrate as outlined below.

Two aliquots (7.60 mg and 8.40 mg) of the loaded resin were transferred to Eppendorf tubes, dissolved in 1% DBU in DMF (500 µL), and agitated for 1 hour. 50 µL aliquots of these solutions were diluted with DMF (2 mL), and absorbance measured at $\lambda$ = 301 nm to determine the loading value of the Fmoc-4-benzoyl-phenylalanine as 0.723 mmol per gram of resin.

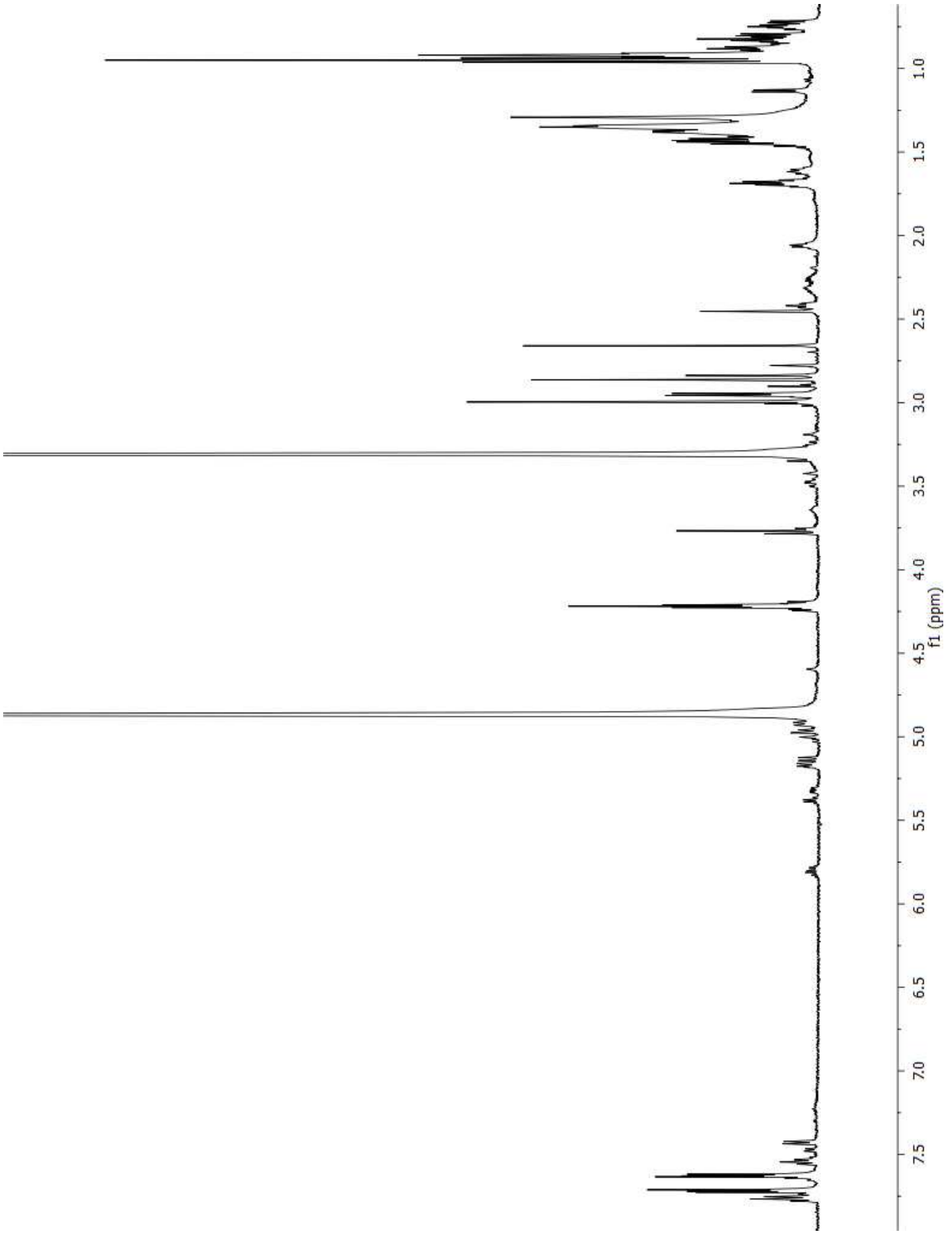
Subsequent amino acids were added using a standard protocol. The dried resin was allowed to swell for 5 minutes in DCM (5 mL), drained, and washed with DMF (5 mL). The washed resin was deprotected using 20% piperidine in DMF (5 mL) with agitation for 20

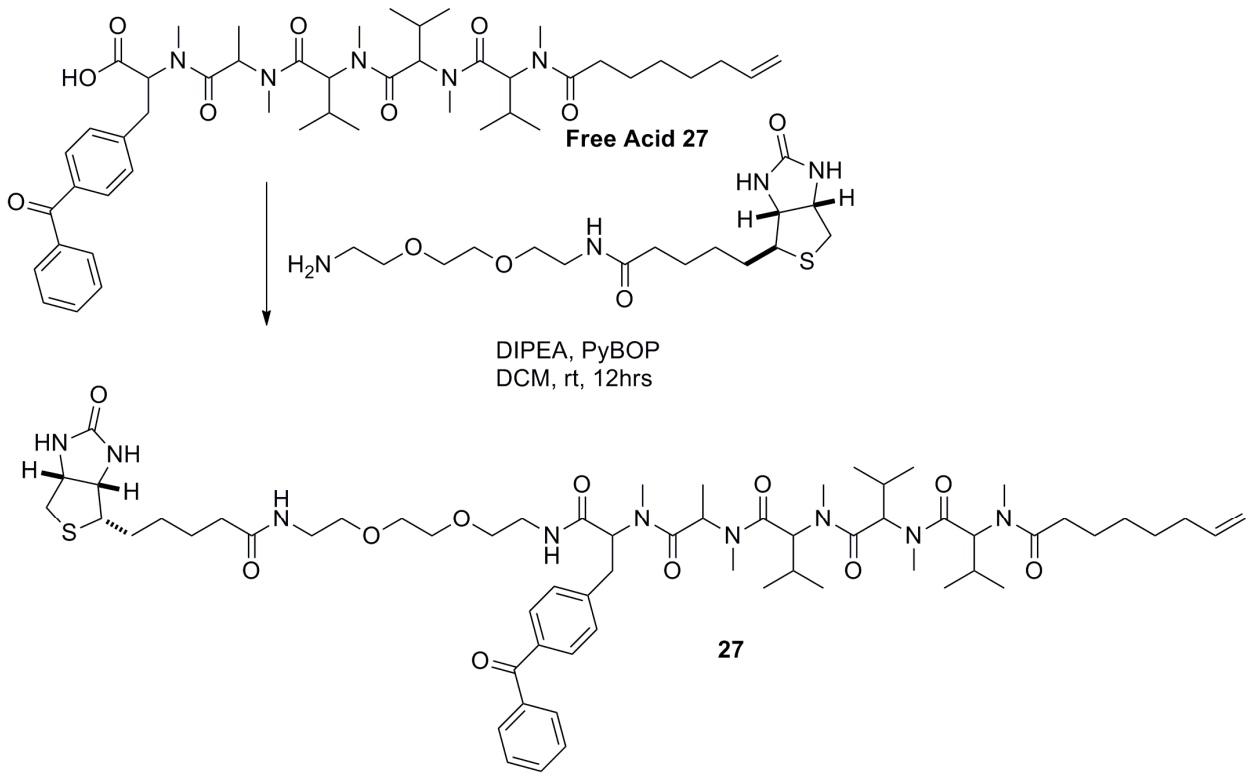
minutes. After deprotection, the resin was drained, and washed (3 x 5 mL DMF, 3 x 5 mL DCM, 3x 5 mL DMF). To this washed resin was added a solution of HBTU (0.336 g, 0.88 mmol), the appropriate amino acid (0.90 mmol), and DIPEA (0.298 mL, 1.80 mmol). The resulting suspension was gently agitated for 1 hour, drained, and washed, as described above. This process was repeated with successive amino acids to form resin-bound 4-benzoyl-Phe-Ala-Val-Val-Val-Fmoc.

From this peptide precursor, the loaded resin (0.250 g) was deprotected by treatment with 20% piperidine in DMF (5 mL) with agitation for 20 minutes. 7-Octenoic acid (142 μL, 1.0 mmol), HBTU (0.33 g, 0.88mmol), and DIPEA (298μL, 1.80 mmol) were added and the resulting suspension agitated for 1 hour, drained, and washed as previously described. The resulting linear lipopeptide was cleaved from the resin by treatment with 1% TFA in DCM for 20 minutes and the solvent removed by evaporation under a stream of $N_2$ gas. The resulting glossy white solid was triturated with *t*-butyl methyl ether and centrifuged at 1000 rpm for 20 minutes. Removal of the *t*-butyl methyl ether afforded the lipopeptide as a white solid (18.9 mg), which was carried to the next step without further purification.

**Methyl Ester 27:** white solid; $^1$H NMR ($CD_3OD$, 600 MHz) δ 7.77-7.75 (m, 4H), 7.54 (t, $J$ = 7.8 Hz, 2H), 7.42 (d, $J$ = 7.8 Hz, 2H), 5.80 (dddd, $J$ = 6.6, 6.6, 10.2, 16.8 Hz, 1H), 5.38 (q, $J$ = 7.2 Hz, 1H), 5.31 (dd, $J$ = 4.8 Hz, 12 Hz, 1H), 5.16 (dd, $J$ = 4.2, 11.4, 1H), 5.14 (d, $J$ = 10.8 Hz, 1H), 5.03-4.97 (m, 2H), 4.93-4.91 (m, 1H), 3.76 (s, 3H), 3.00 (s, 3H), 2.95 (s, 3H), 2.94 (s, 3H), 2.86 (s, 3H), 2.66 (s, 3H), 2.45 (s, 2H), 2.41 (td, $J$ = 3 Hz, 7.8 Hz, 7.8 Hz, 2H), 2.06 (q, $J$ = 7.8 Hz, 2H), 1.62 (p, $J$ = 6 Hz, 2H), 1.46-1.40 (m, 10H), 1.13 (d, $J$ = 6.6 Hz, 3H), 0.89-0.87 (m, 8H), 0.84-0.79 (m, 6H), 0.76-0.71 (m, 6H); HRFTESIMS *m/z* $[M+Na]^+$ 868.5221 (calcd for $C_{48}H_{71}N_5O_8Na$, 868.5195).

$^{1}$H spectrum of **Methyl Ester 27** recorded in $CD_3OD$ at 600 MHz.



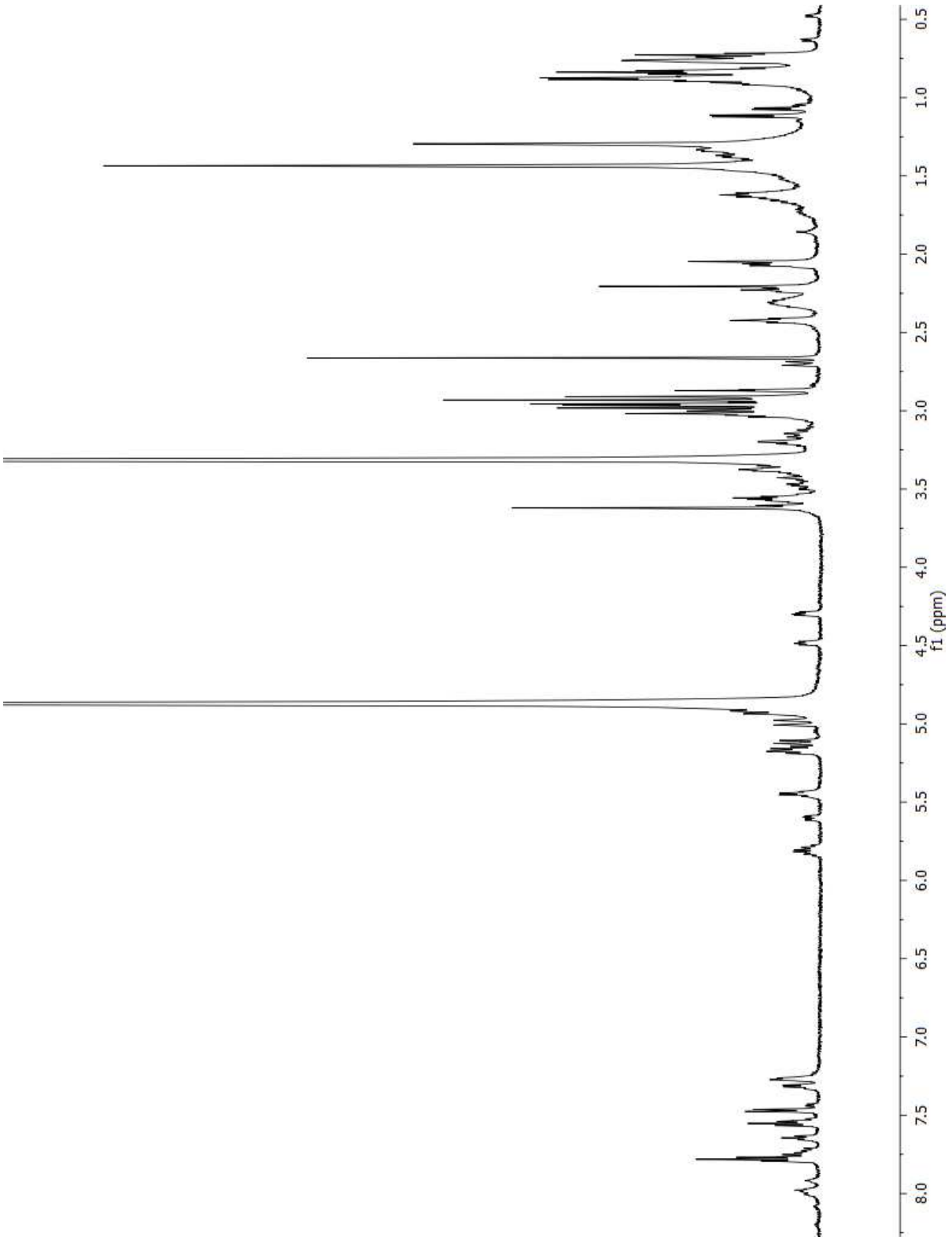



To a dissolved solution of lipopentapeptide **Free acid 27** prepared as previously described[2] (2.8 mg, 3.37 μmol) in DCM (0.10 mL) were added solutions of PyBOP (5.71 μmol) dissolved in DCM and DIPEA (0.10mmol) dissolved in DCM and the reaction allowed to stir for 20 mins. A solution of commercially available EZ-Link Biotin PEO-Amine(8.41 μmol) in DCM was added and the reaction allowed to stir overnight at room temperature. The reaction was quenched by removing solvent *in vacuo* and immediately purified by $C_{18}$ RP-HPLC Phenomenex Jupiter $C_{18}$ (4.6 x 250 mm, 5 μm), 80% MeOH/ 20% $H_2O$ (acidified with 0.02% formic acid), 1 mL/min, $t_R$ = 11.6 min to give **27** as a white solid (0.44 mg, 11.0%).

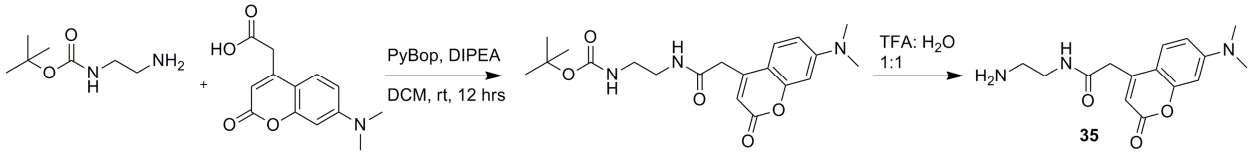
**27:** white solid; $^1$H NMR ($CD_3OD$, 600 MHz) δ 7.77 (t, *J* = 7.2 Hz, 2H), 7.64 (t, *J* = 7.8 Hz, 1H), 7.56-7.53 (m, 2H), 7.46 (d, *J* = 8.4 Hz, 2H), 7.30 (d, *J* = 6.6 Hz, 1H), 7.26 (d, *J* = 6.6 Hz, 1H),

5.80 (dddd, $J$ = 6.6, 6.6, 10.2, 16.8 Hz, 1H), 5.60 (dd, $J$ = 4.2 Hz, 12 Hz, 1H), 5.44 (q, $J$ = 7.2 Hz, 1H), 5.18-5.10 (m, 3H), 4.98 (m, 2H), 4.49-4.47 (m, 1H), 4.30-4.29 (m, 1H), 3.61 (s, 2H), 3.57-3.53 (m, 4H), 3.49-3.42 (m, 2H), 3.21-3.14 (m, 2H), 3.01 (s, 3H), 2.97 (s, 3H), 2.95 (s, 3H), 2.86 (s, 3H), 2.66 (s, 3H), 2.43-2.40 (m, 2H), 2.33-2.27 (m, 4H), 2.23-2.20 (m, 4H), 2.07-2.04 (m, 4H), 1.66-1.60 (m, 4H), 1.49-1.39 (m, 8H), 1.36-1.29 (m, 8H), 1.11 (d, $J$ = 6.6 Hz, 3H), 0.90-0.85 (m, 8H), 0.84-0.81 (m, 6H), 0.81-0.72 (m, 6H); HRFTESIMS *m/z* $[M+Na]^+$ 1210.6925 (calcd for $C_{63}H_{97}N_9O_{11}SNa$, 1210.6920).

$^{1}$H spectrum of **27** recorded in $CD_3OD$ at 600 MHz.



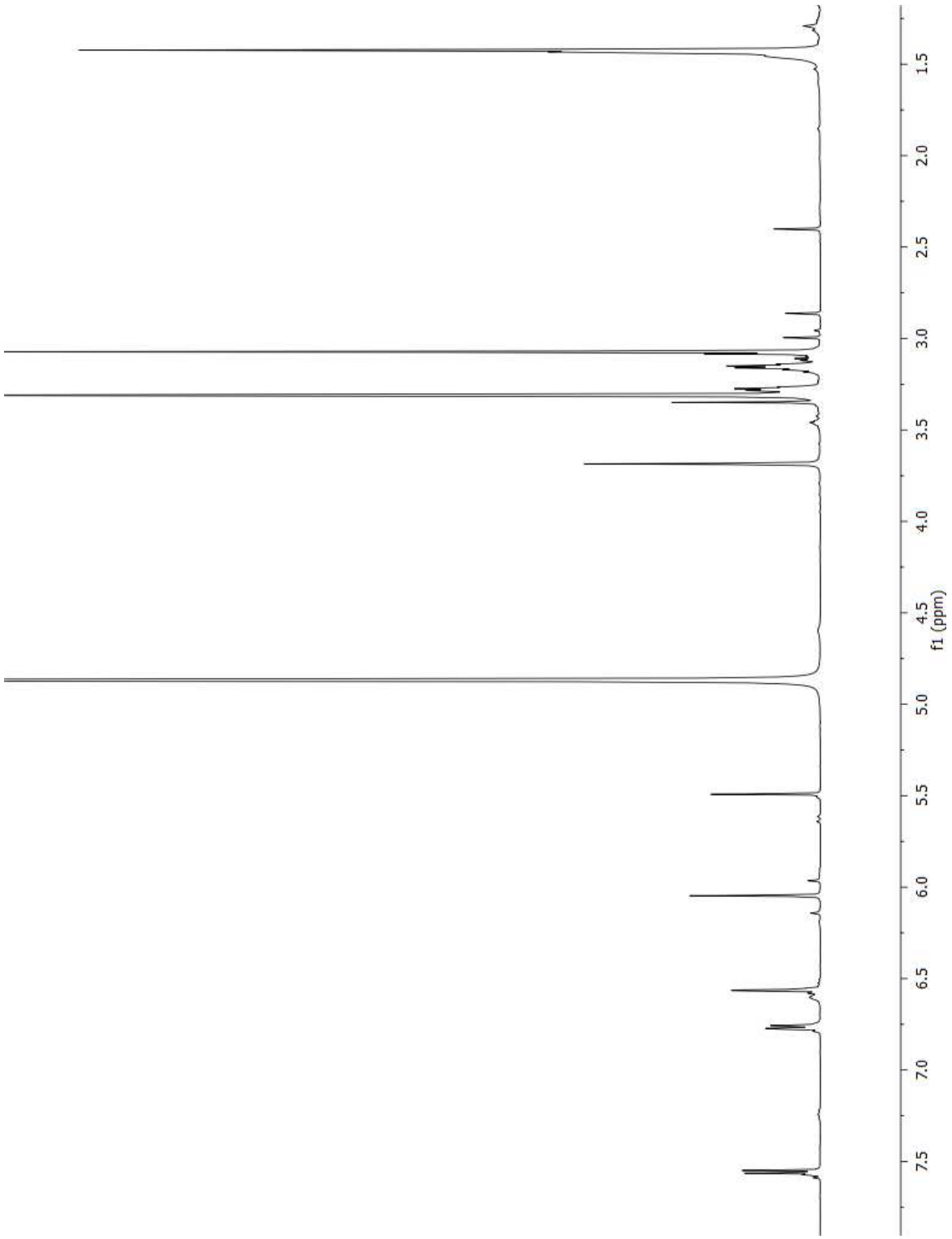
**Coumarin Synthesis**



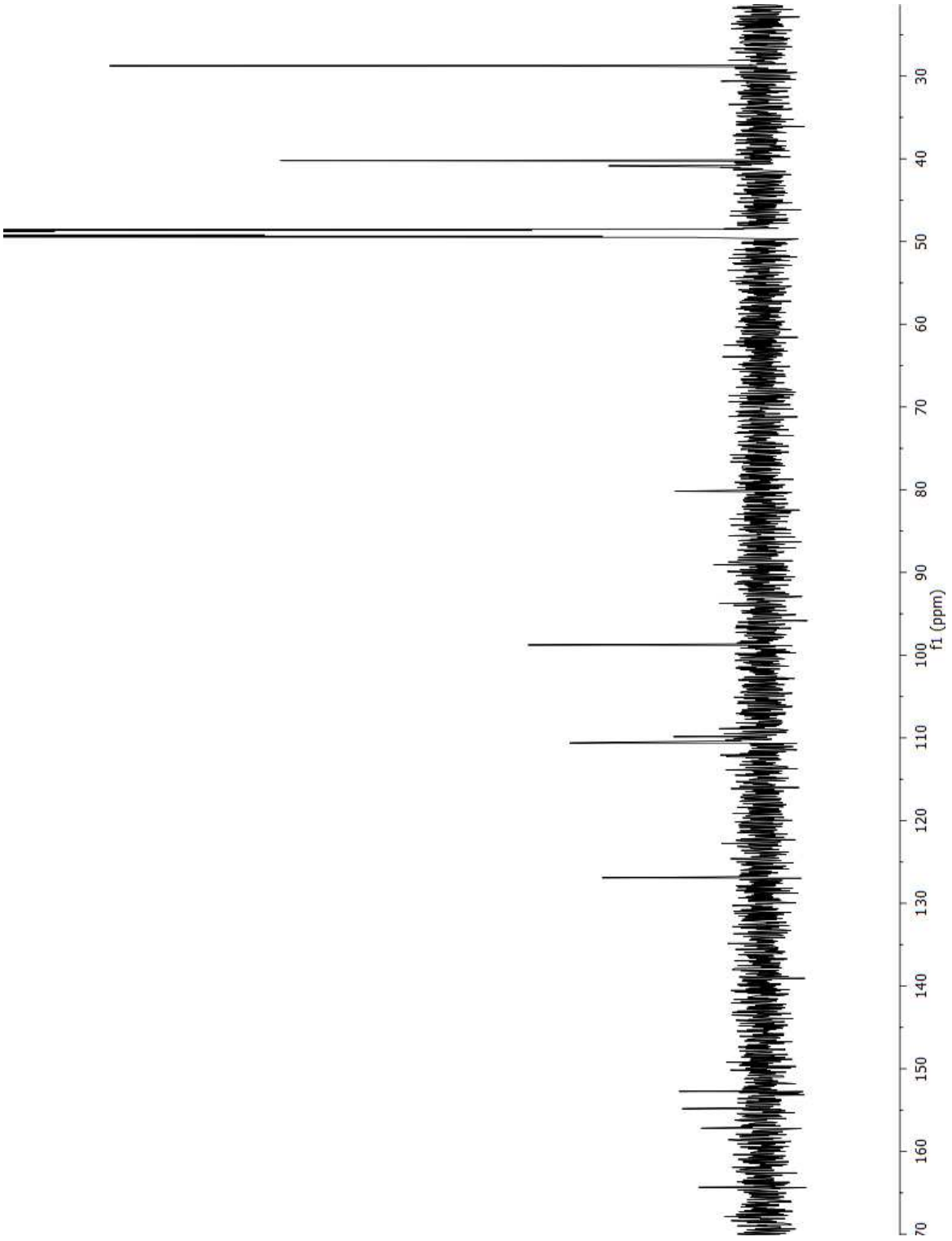

7-Dimethylaminocoumarin-4-acetic acid (0.0127 g, 0.051 mmol) was dissolved in a minimal amount of DCM.and DIPEA (67 µL, .411 mmol) added dropwise to the solution. Commercially available N-Boc-ethylenediamine (8.64 mg, 0.054 mmol) was dissolved in a minimum amount of DMF and added to the stirred solution. Lastly, PyBOP (0.0208 g, 0.040mmol) was added and the solution allowed to stir for 12hrs. The reaction was quenched by removing the solvent *in vacuo*. The crude product was then purified using reverse-phase HPLC (60% MeOH, 40% $H_2O$ + 0.02% formic acid, Phenomenex Synergi $C_{18}$ 250 x 10 mm column, 10 µm, 2 mL/min, 461 nm), yielding 0.0119 g of yellow solid (60% yield).

***N*-Boc-Ethylenediamine-7-dimethylaminocoumarin:** yellow solid; UV (MeOH) $\lambda_{max}$ (log ε): 209 nm (4.30), 247 nm (4.02), 375 nm (4.14); $^1$H NMR ($CD_3OD$, 600 MHz) δ 7.55 (d, $J$ = 9 Hz, 1H), 6.76 (d, $J$ = 9 Hz, 1H), 6.56 (d, $J$ = 2.4 Hz, 1H), 6.04 (s, 1H), 3.68 (s, 2H), 3.34 (s, 1H), 3.07 (m, 8H), 1.42 (s, 9H) ; $^{13}$C NMR ($CD_3OD$, 150 MHz) δ 28.8, 40.3, 40.9, 40.9, 80.3, 98.9, 110.0, 110.6, 110.7, 127.0, 152.8, 154.9, 157.3, 164.4; HRESIMS *m/z* $[M+Na]^+$ 412.1847 (calcd for $C_{20}H_{27}N_3O_5Na$, 412.1848).

$^1$H spectrum of ***N*-Boc-Ethylenediamine-7-dimethylaminocoumarin** recorded in $CD_3OD$ at 600 MHz.



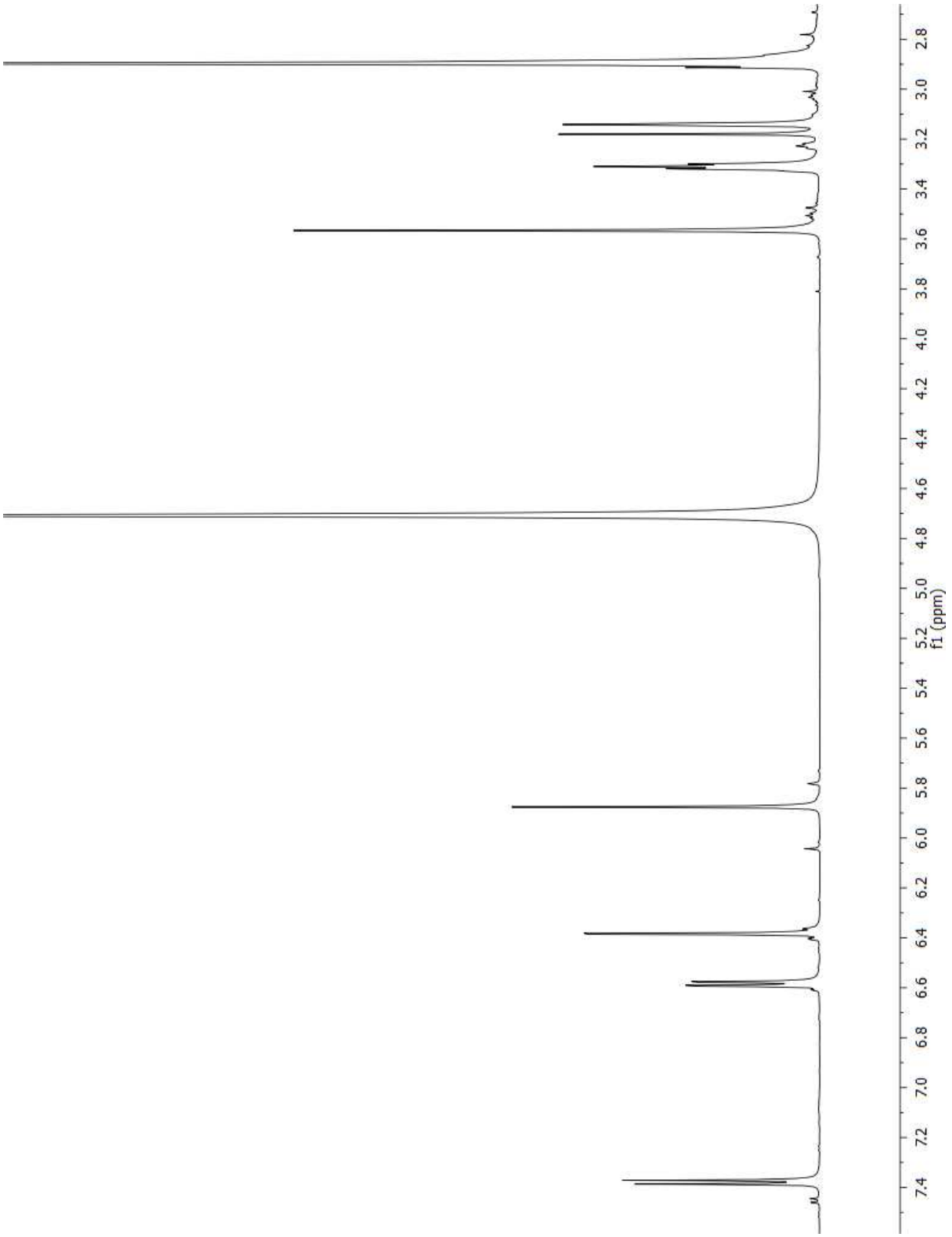
$^{13}C$ spectrum of ***N*-Boc-Ethylenediamine-7-dimethylaminocoumarin** recorded in $CD_3OD$ at 150 MHz.



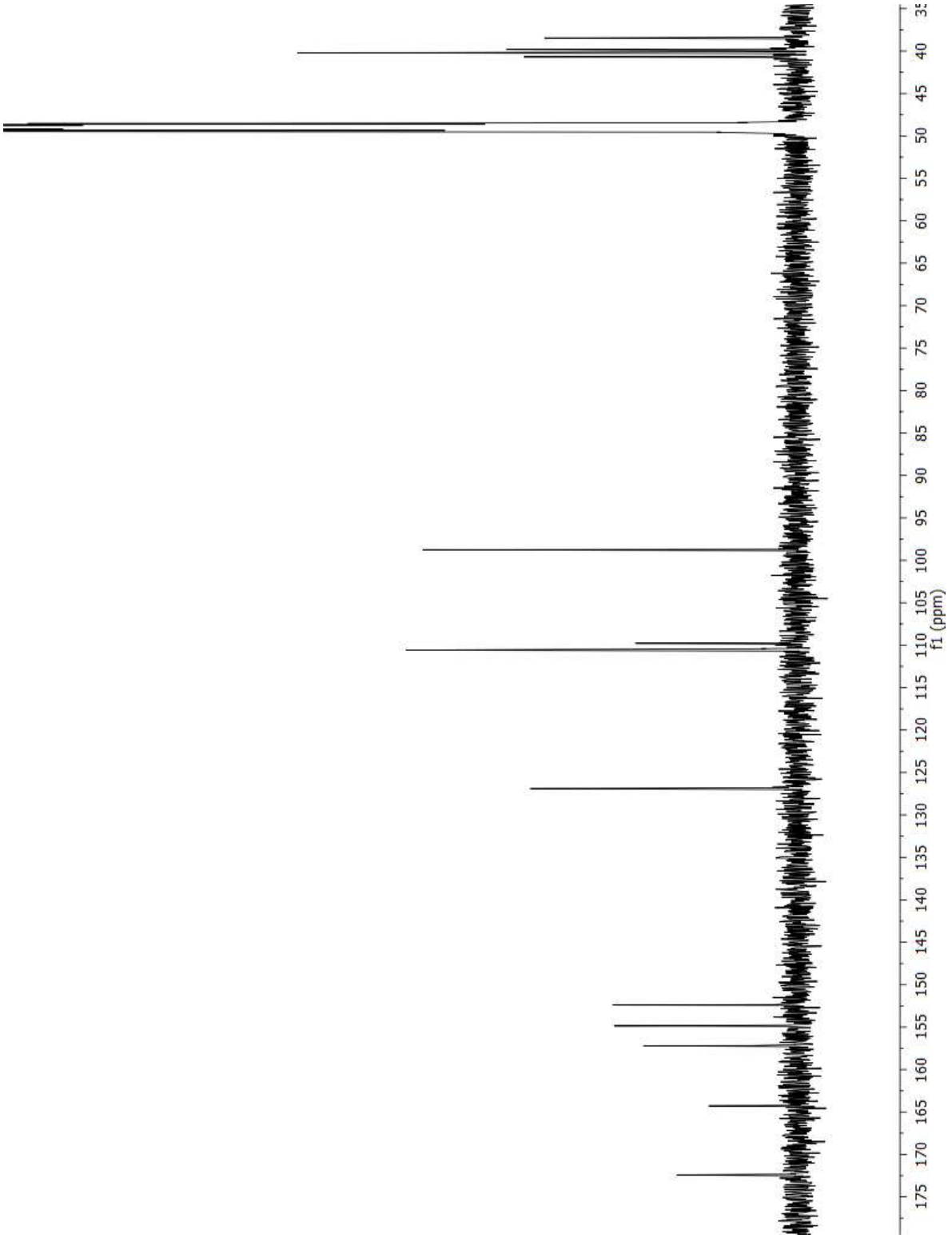
For further reactions, the Boc protecting group was removed by stirring a desired amount of ***N*-Boc-Ethylenediamine-7-dimethylaminocoumarin** in 1:1 TFA/$H_2O$ solution and monitoring the reaction progress by TLC. After the reaction was complete the reaction was quenched by removing the solvent *in vacuo*. The resulting yellow oil **35** was used immediately with no further purification.

**35:** yellow solid; UV (MeOH) $\lambda_{max}$ (log ε): 208 nm (4.10), 246 nm (3.81), 375 nm (3.94); $^1$H NMR ($CD_3OD$, 600 MHz) δ 7.54 (d, *J* = 9 Hz, 1H), 6.75 (dd, *J* = 9 Hz, 2.4 Hz, 1H), 6.55 (d, *J* = 2.4 Hz, 1H), 6.04 (s, 1H), 3.73 (s, 2H), 3.47 (t, *J* = 5.4 Hz, 2H), 3.06 (m, 8H) ; $^{13}$C NMR ($CD_3OD$,150 MHz) δ 38.6, 39.9, 40.3, 40.8, 98.9, 109.9, 110.6, 110.7, 127.0, 152.5, 155.0, 157.3, 164.4, 172.5; HRESIMS *m/z* $[M+H]^+$ 290.1503 (calcd for $C_{15}H_{20}N_3O_3$, 290.1504).

$^{1}$H spectrum of **35** recorded in $CD_3OD$ at 600 MHz.



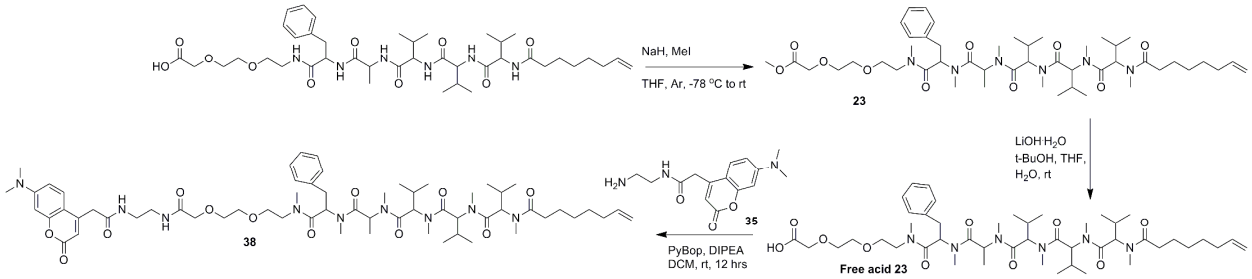
$^{13}C$ spectrum of **35** recorded in $CD_3OD$ at 150 MHz.



**Coumarin Control**

Coumarin control **37** was prepared as previously described.[3]

**Coumarin Almiramide (38) Synthesis**

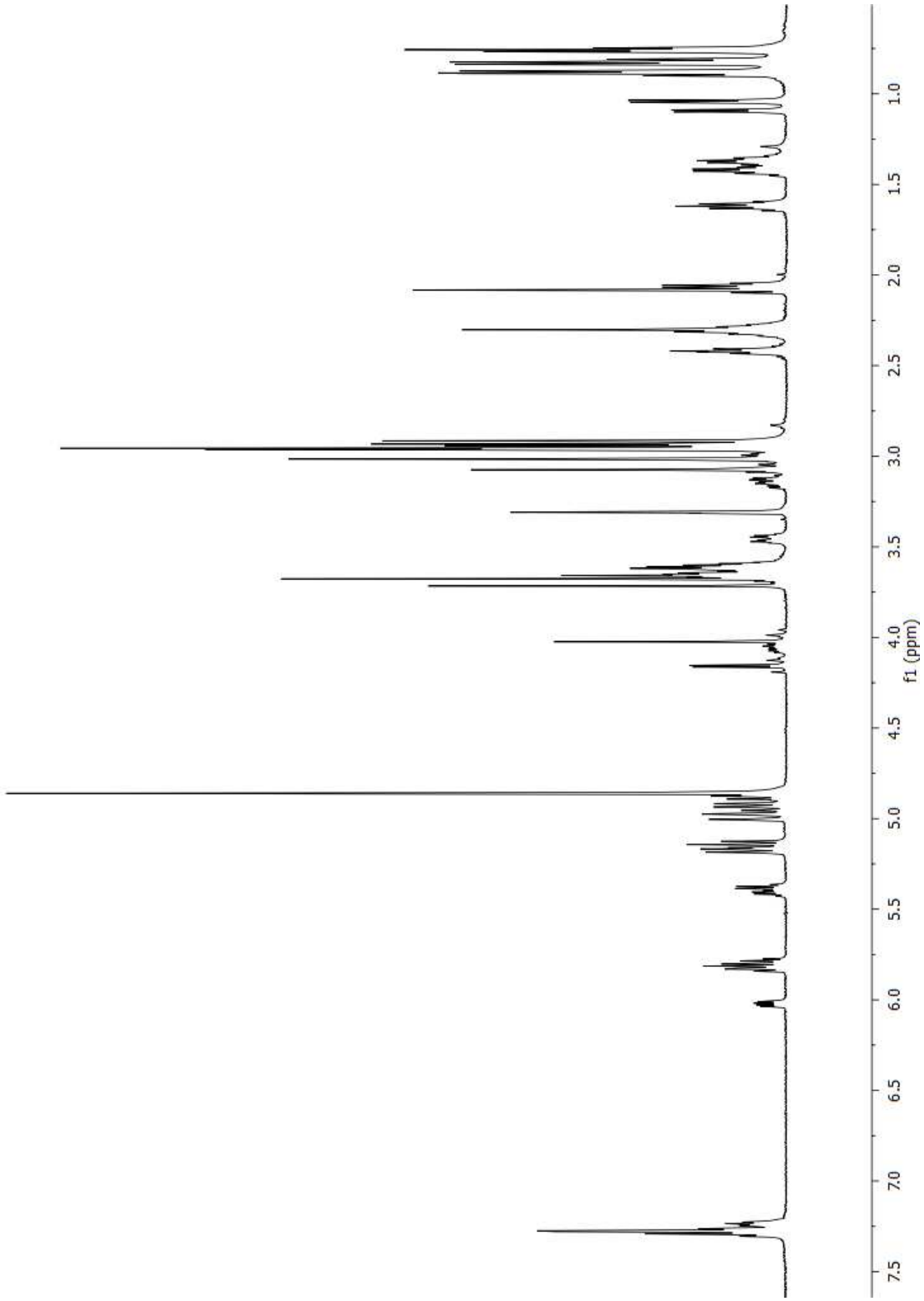



Commercially available Fmoc-8-Amino-3,6-dioxaoctanoic acid was loaded on 2-chlorotrityl chloride resin as previously described.[2] The per-methylated lipopentapeptide (**23**) was prepared and purified as described above, and the resulting free acid (**Free acid 23**) reacted with ethylenediamine-7-dimethylaminocoumarin. To a dissolved solution of **Free acid 23** (2.95 mg, 3.33 μmol) in DCM (450 μL) were added solutions of PyBOP (2.59 μmol) dissolved in DCM and DIPEA (0.026 mmol) dissolved in DCM and the reaction allowed to stir for 20 mins. A solution of ethylenediamine-7-dimethylaminocoumarin (3.49 μmol) in DCM was added and the reaction allowed to stir overnight at room temperature. The reaction was quenched by removing solvent *in vacuo* and immediately purified by $C_{18}$ RP-HPLC Phenomenex Jupiter $C_{18}$ (4.6 x 250 mm, 5μm), 75% MeOH/ 25% $H_2O$ (acidified with 0.002% formic acid), 1 mL/min, $t_R$ = 30.4 min to give the **38** as a yellow solid (0.69 mg, 17.9%).

**23:** white solid; $[\alpha]_D^{20}$ -196 (*c*0.063, MeOH); UV (MeOH) $\lambda_{max}$ (log ε): 216 nm (4.11); $^1$H NMR ($CD_3OD$, 600 MHz) δ7.30-7.22 (m, 5H), 5.80 (dddd, *J* = 16.8, 10.2, 6.6, 6.6 Hz, 1H), 5.42-5.37 (m, 2H), 5.17 (d, *J* = 10.8 Hz, 1H), 5.13 (d, *J* = 10.2 Hz, 1H), 5.01-4.97 (m, 1H), 4.92 (d, *J* = 10.2 Hz, 1H), 4.88 (d, *J* = 10.8 Hz, 1H), 3.67-3.59 (m, 8H), 3.07 (s, 2H), 3.01 (s, 3H), 2.96 (s,

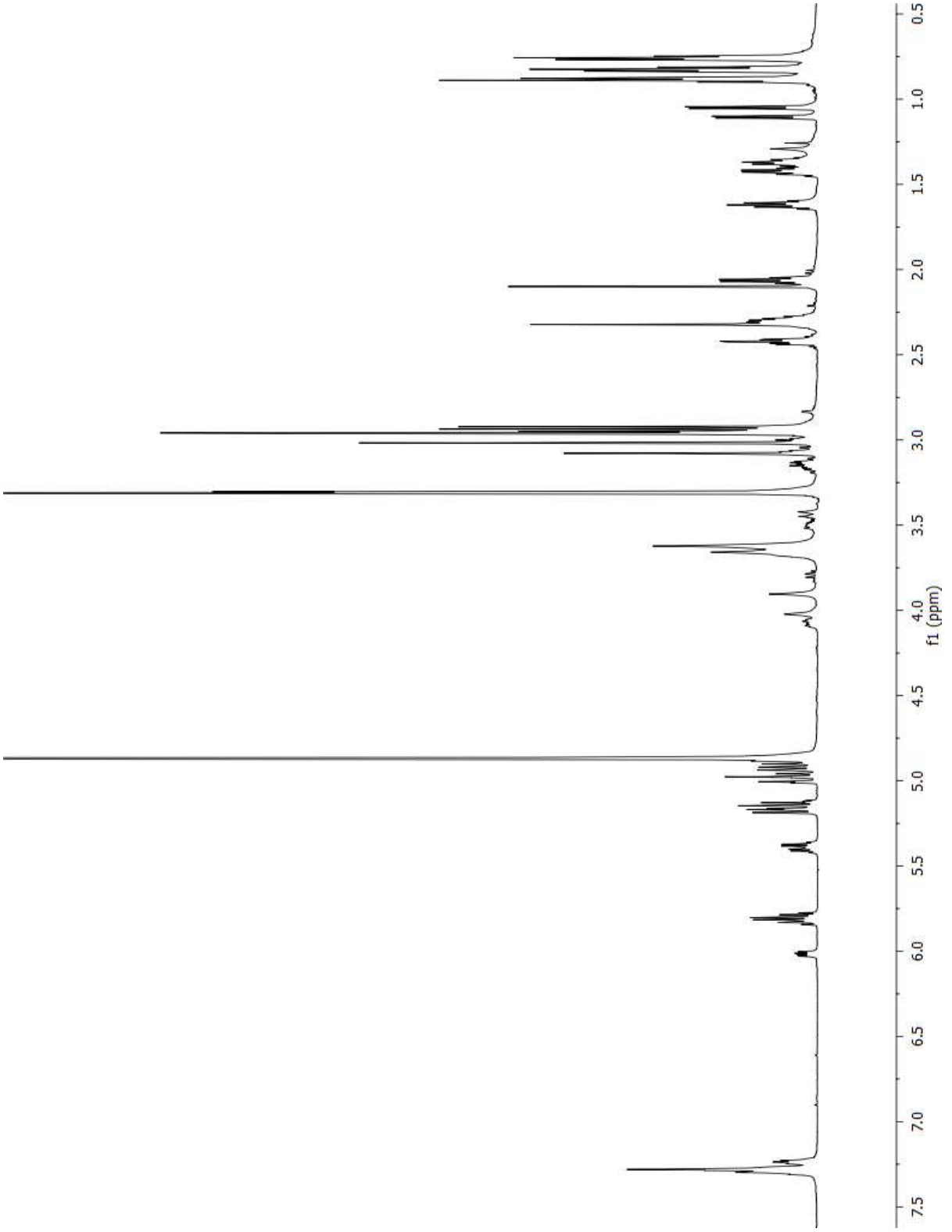
3H), 2.95 (s, 3H), 2.93 (s, 3H), 2.91 (s, 3H), 2.42 (dt, *J* = 7.8, 2.4 Hz, 2H), 2.30 (s, 3H), 2.07 (p, *J* = 7.2 Hz, 2H), 1.64 (p, *J* = 7.8 Hz, 2H), 1.45-1.41 (m, 2H), 1.39-1.35 (m, 2H), 1.04 (d, *J* = 6.6 Hz, 3H), 0.90-0.87 (m, 6H), 0.83-0.81 (m, 6H), 0.77-0.74 (m, 6H); HRESIMS *m/z* $[M+Na]^+$ 923.5821 (calcd for $C_{48}H_{80}N_6O_{10}Na$, 923.5828).

$^{1}$H spectrum of **23** recorded in $CD_3OD$ at 600 MHz.



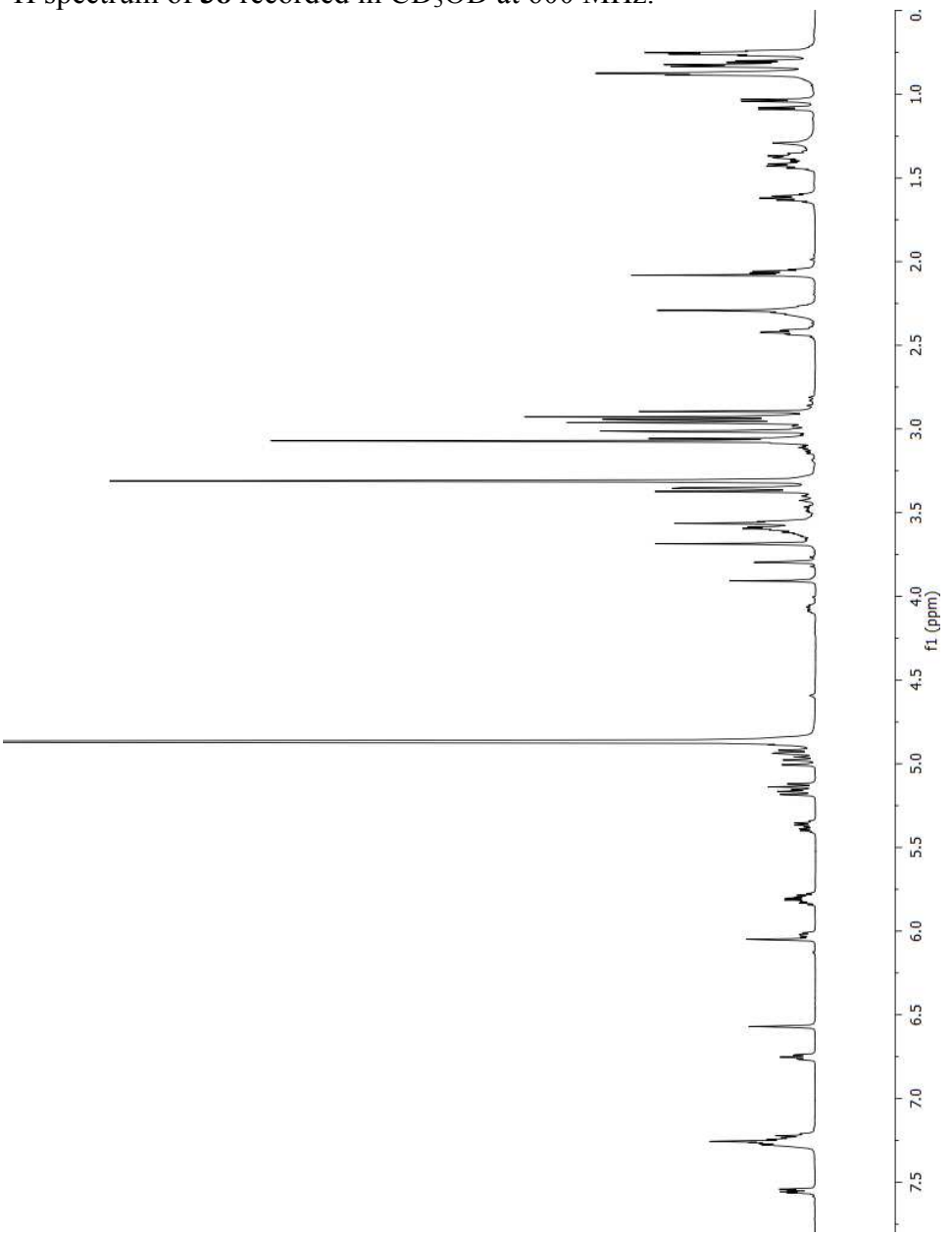
**Free acid 23:** white solid; $[\alpha]_D^{20}$ -183 (*c* 0.053, MeOH); UV (MeOH) $\lambda_{max}$ (log ε): 216 nm (4.18); $^1$H NMR ($CD_3OD$, 600 MHz) δ 7.29-7.22 (m, 5H), 6.01 (dd, *J* = 4.8, 10.2 Hz, 1H), 5.80 (dddd, *J* = 6.6, 6.6, 10.2, 16.8 Hz, 1H), 5.39 (q, *J* = 6.6 Hz, 1H), 5.18 (d, *J* = 10.8 Hz, 1H), 5.13 (d, *J* = 10.8 Hz, 1H), 5.01-4.90 (m, 3H), 3.01 (s, 3H), 2.96 (s, 3H), 2.94 (s, 3H), 2.93 (s, 3H), 2.92 (s, 3H), 2.42 (dt, *J* = 3.6, 7.2 Hz, 2H), 2.32 (s, 3H), 2.06 (q, *J* = 7.2 Hz, 2H), 1.62 (p, *J* = 7.8 Hz, 2H), 1.44-1.35 (m, 4H), 1.07 (d, *J* = 6.6 Hz, 3H), 0.90-0.87 (m, 6H), 0.83-0.81 (m, 6H), 0.76-0.74 (m, 6H); HRESIMS *m/z* $[M+H]^+$ 887.5869 (calcd for $C_{47}H_{79}N_6O_{10}$).

$^{1}$H spectrum of **Free acid 23** recorded in $CD_3OD$ at 600 MHz.

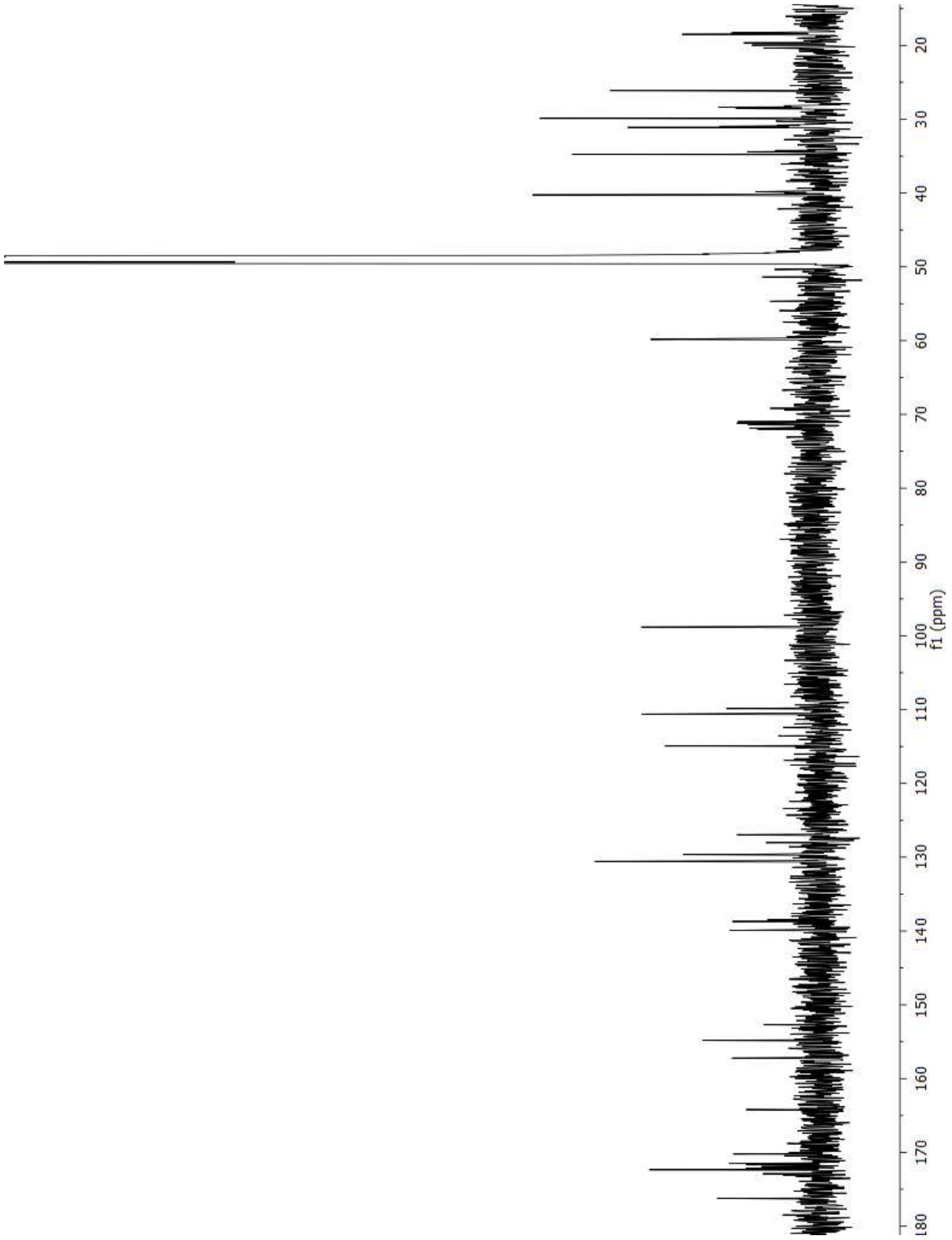


**38:** yellow solid; $[\alpha]_D^{20}$ -133 (*c* 0.047, MeOH); UV (MeOH) $\lambda_{max}$ (log ε): 207 nm (4.64), 240 nm (4.08), 375 nm (4.08); $^1$H NMR ($CD_3OD$, 600 MHz) δ 7.55 (dd, *J* = 3.6, 9 Hz, 1H), 7.27-7.22 (m, 5H), 6.76-6.73 (m, 1H), 6.57 (s, 1H), 6.04 (s, 1H), 5.80 (dddd, *J* = 6.6, 6.6, 10.2, 16.8 Hz, 1H), 5.41-5.34 (m, 1H), 5.17 (d, *J* =10.8 Hz, 1H), 5.12 (d, *J* = 10.2 Hz, 1H), 5.00-4.91 (m, 3H), 3.68 (s, 2H), 3.61-3.55 (m, 6H), 3.37-3.34 (m, 4H), 3.07 (s, 6H), 3.01 (s, 3H), 2.96 (s, 3H), 2.94 (s, 3H), 2.92 (s, 3H), 2.89 (s, 3H) 2.42 (dt, *J* = 2.4, 7.2 Hz, 2H), 2.29 (s, 3H), 2.08-2.04 (m, 3H), 1.62 (p, *J* = 7.2 Hz, 2H), 1.39 (p, *J* = 7.8 Hz, 2H), 1.36 (p, *J* = 7.8 Hz, 2H), 1.03 (d, *J* = 6.6 Hz, 3H), 0.88-0.86 (m, 6H), 0.82-0.76 (m, 6H), 0.76-0.74 (m, 6H); $^{13}$C NMR ($CD_3OD$, 150 MHz) δ 18.3, 18.4, 18.5, 18.6, 19.7, 19.8, 20.0, 20.1, 20.4, 26.2, 28.3, 28.4, 28.5, 28.6, 30.0, 31.0, 31.1, 31.2, 31.3, 34.3, 34.5, 34.8, 39.9, 40.3, 40.3, 51.5, 54.7, 59.8, 59.9, 71.1, 71.3, 71.5, 72.0, 72.1, 98.9, 110.0, 110.6, 110.7, 110.8, 115.0, 127.1, 128.1, 129.8, 130.7, 138.8, 140.0, 152.8, 152.8, 154.9, 157.3, 164.3, 170.3, 171.6, 171.7, 171.8, 171.9, 172.5, 173.0, 176.3; HRESIMS *m/z* $[M+Na]^+$ 1180.6986 (calcd for $C_{62}H_{95}N_9O_{12}Na$, 1180.6992).

$^1$H spectrum of **38** recorded in $CD_3OD$ at 600 MHz.



$^{13}C$ spectrum of **38** recorded in $CD_3OD$ at 600 MHz.



(1) Choi, S.-W.; Elmaleh, D. R.; Hanson, R. N.; Fischman, A. J. *J. Med. Chem.***1999**, *42*, 3647-3656.

(2) Sanchez, L. M.; Lopez, D.; Vesely, B. A.; Della Togna, G.; Gerwick, W. H.; Kyle, D. E.; Linington, R. G. *J. Med. Chem.* **2010**, *53*, 4187-4197.

(3) Navarro, G.; Chokpaiboon, S.; De Muylder, G.; Bray, W. M.; Nisam, S. C.; McKerrow, J. H.; Pudhom, K.; Linington, R. G. *PLoS ONE* **2012**, *7*, e46172.