Supplementary Information

Development of novel epoxyketone-based proteasome inhibitors as a strategy to overcome cancer resistance to carfilzomib and bortezomib

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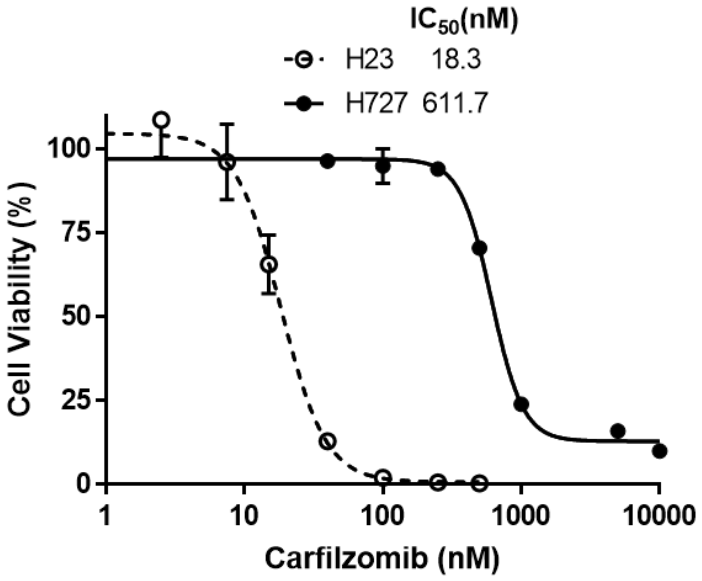
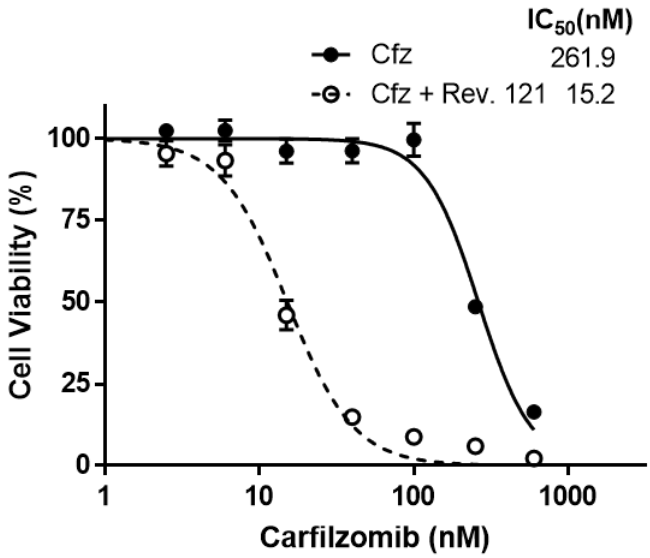
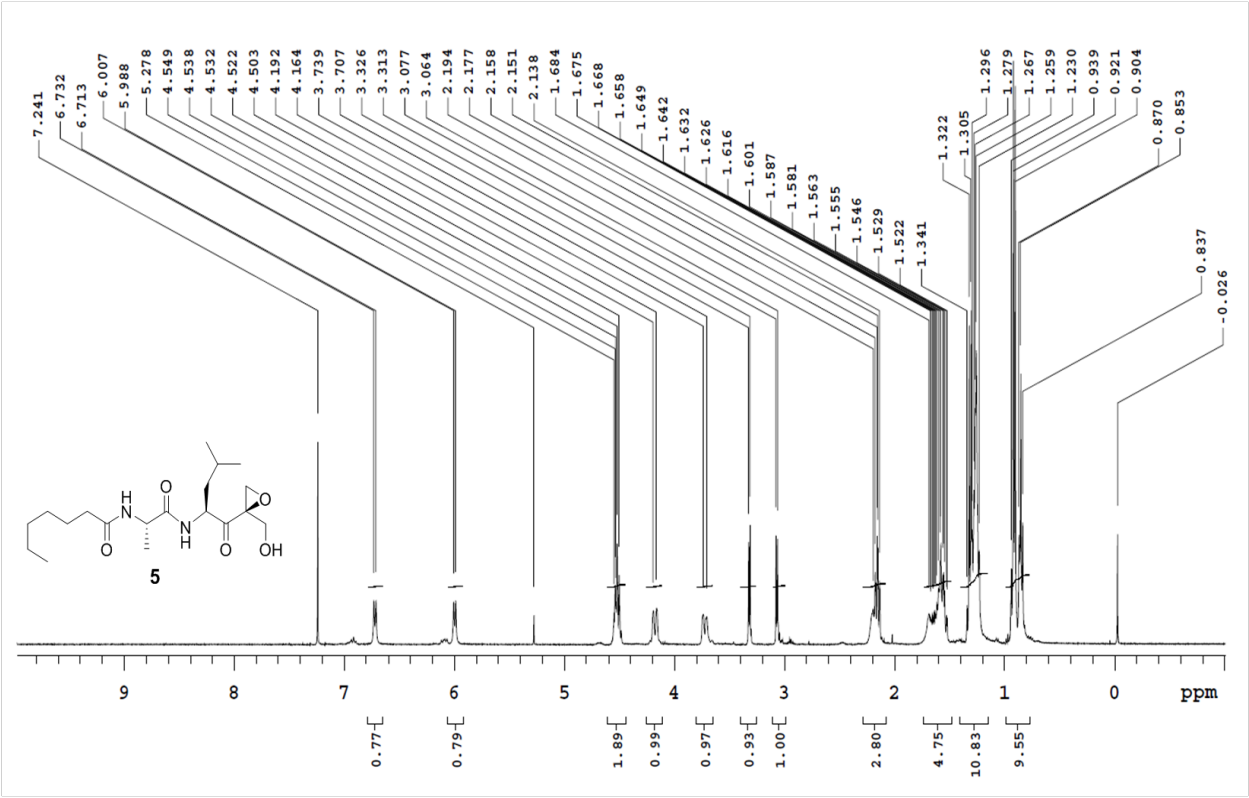
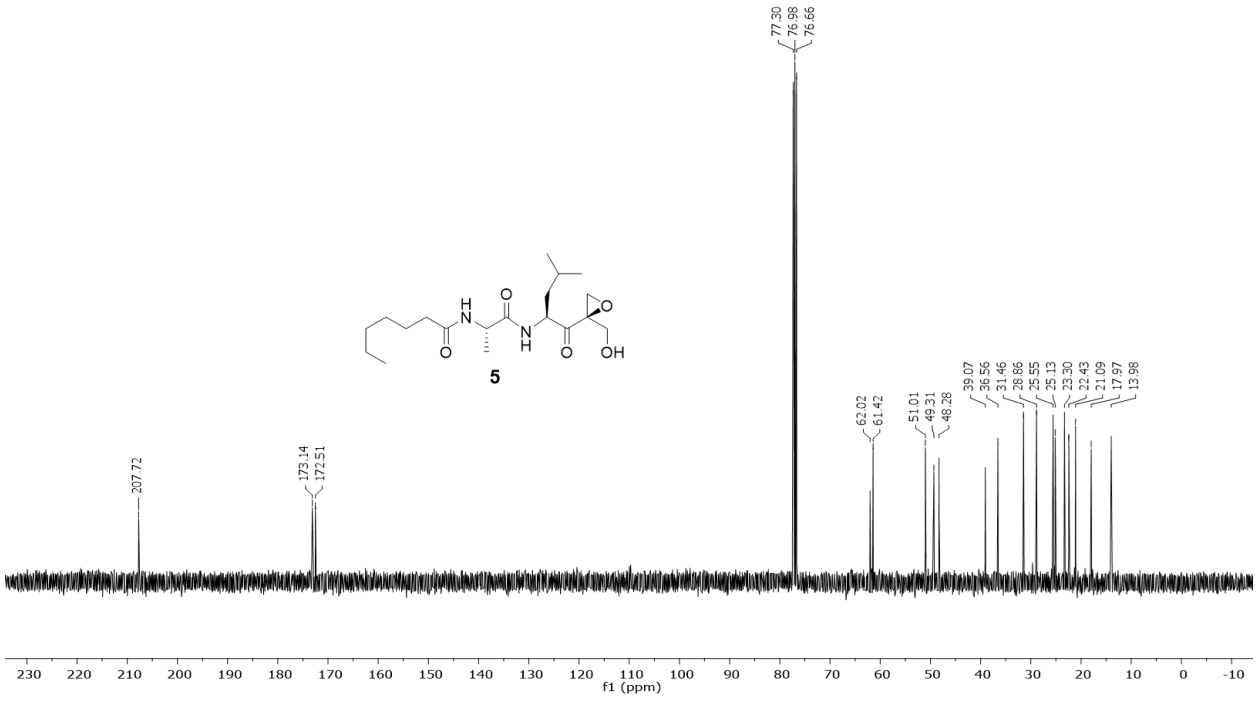


Supplemental Figure 1. Cell viability of H727 and H23 cells for Cfz. Data shown are representative of biological triplicate experiments.

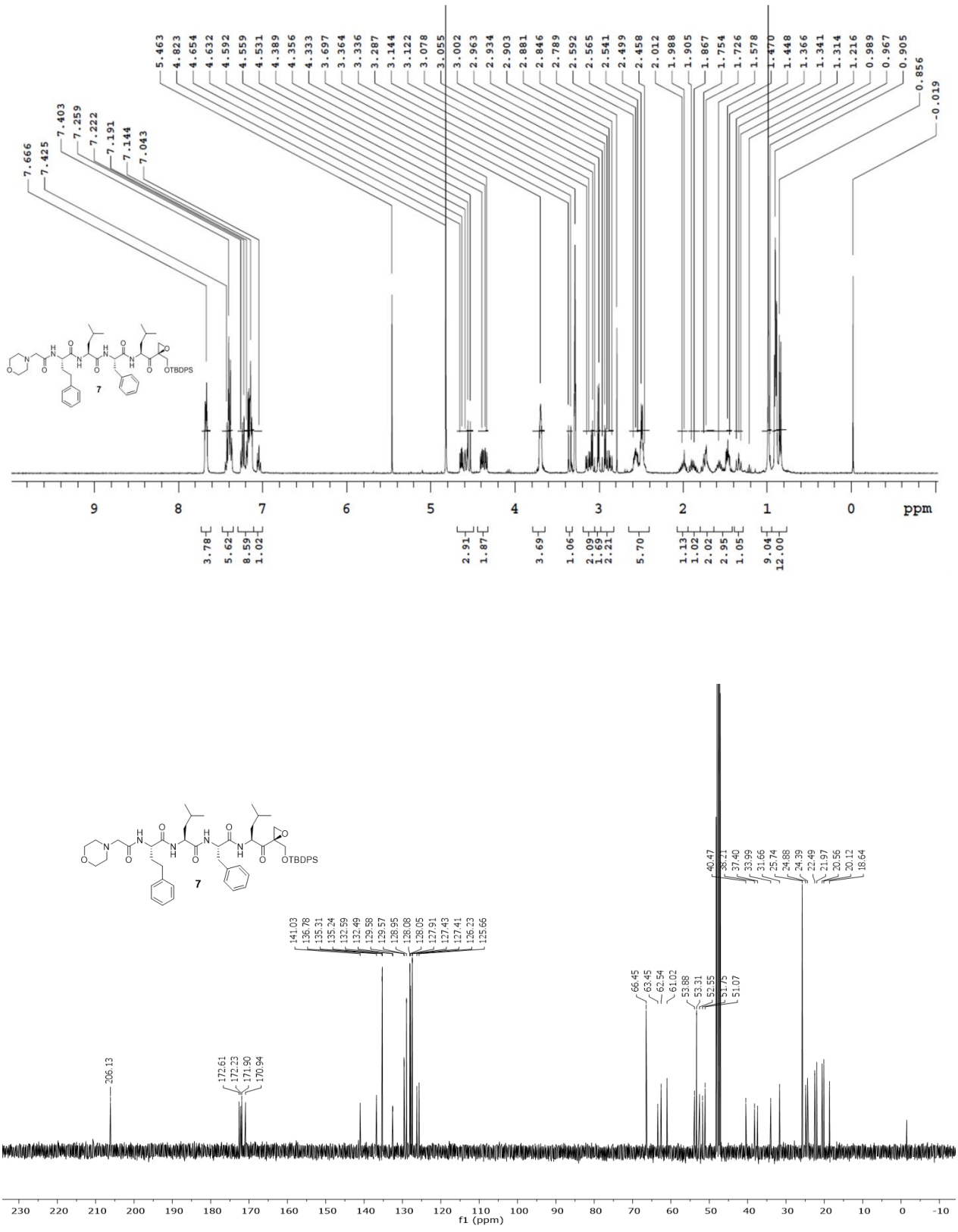


Supplemental Figure 2. Effect of reversin-121 co-treatment on carfilzomib sensitivity of RPMI 8226/CfzR cells. Co-treatment of RPMI 8226/CfzR cells with reversin-121 (7.5 μM, P-gp inhibitor) sensitized RPMI8226/CfzR cells to Cfz. Data shown are representative of biological triplicate experiments.

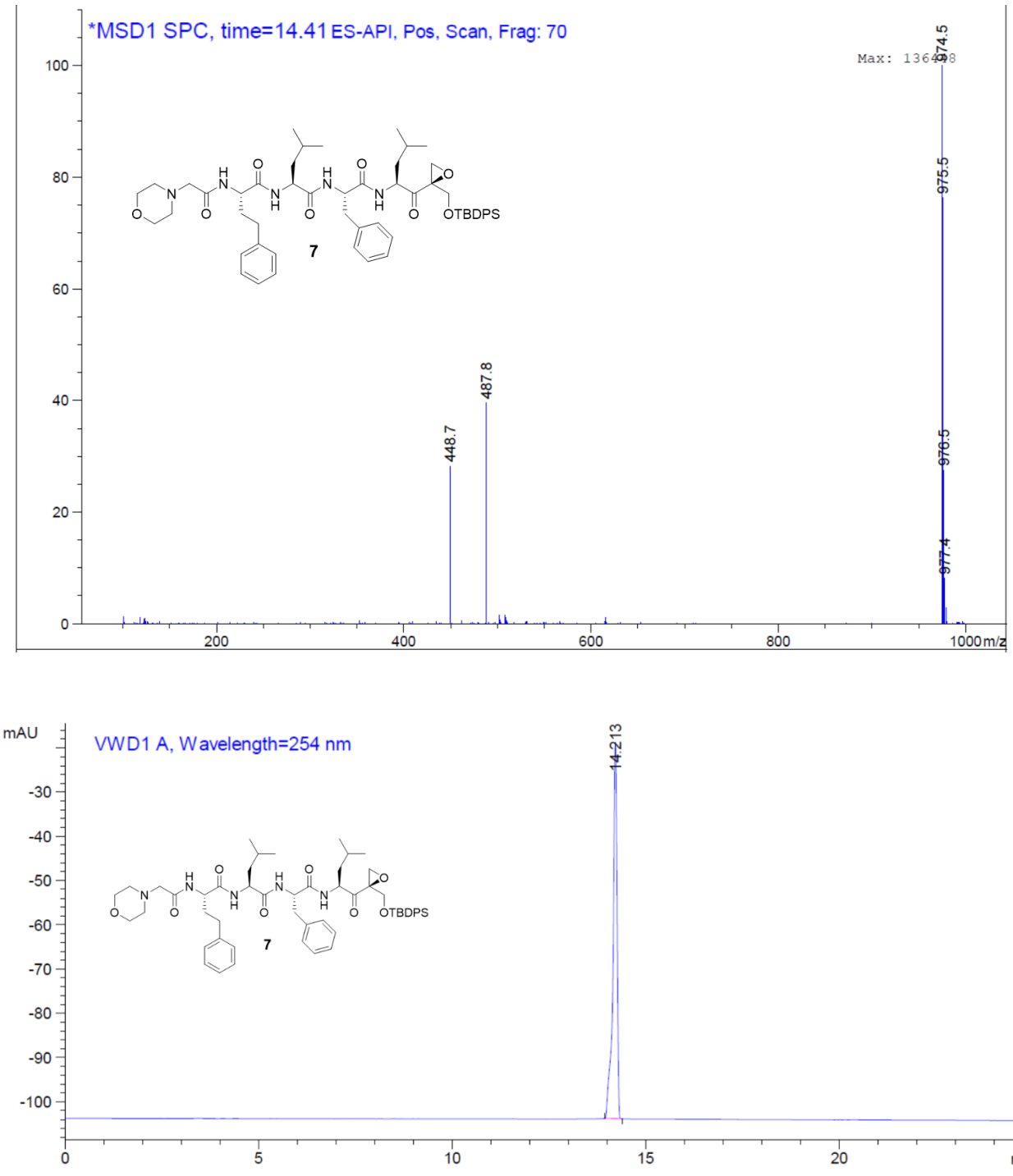




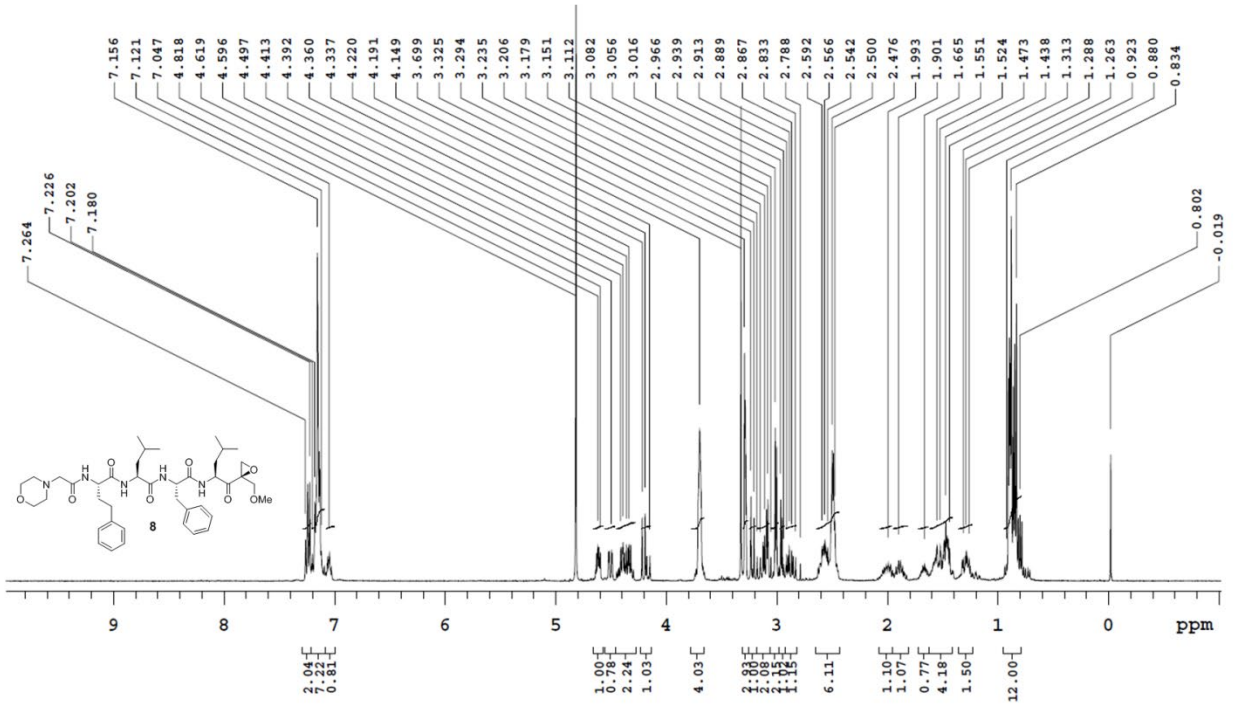
^{1}H NMR, and ^{13}C NMR spectra of **5**

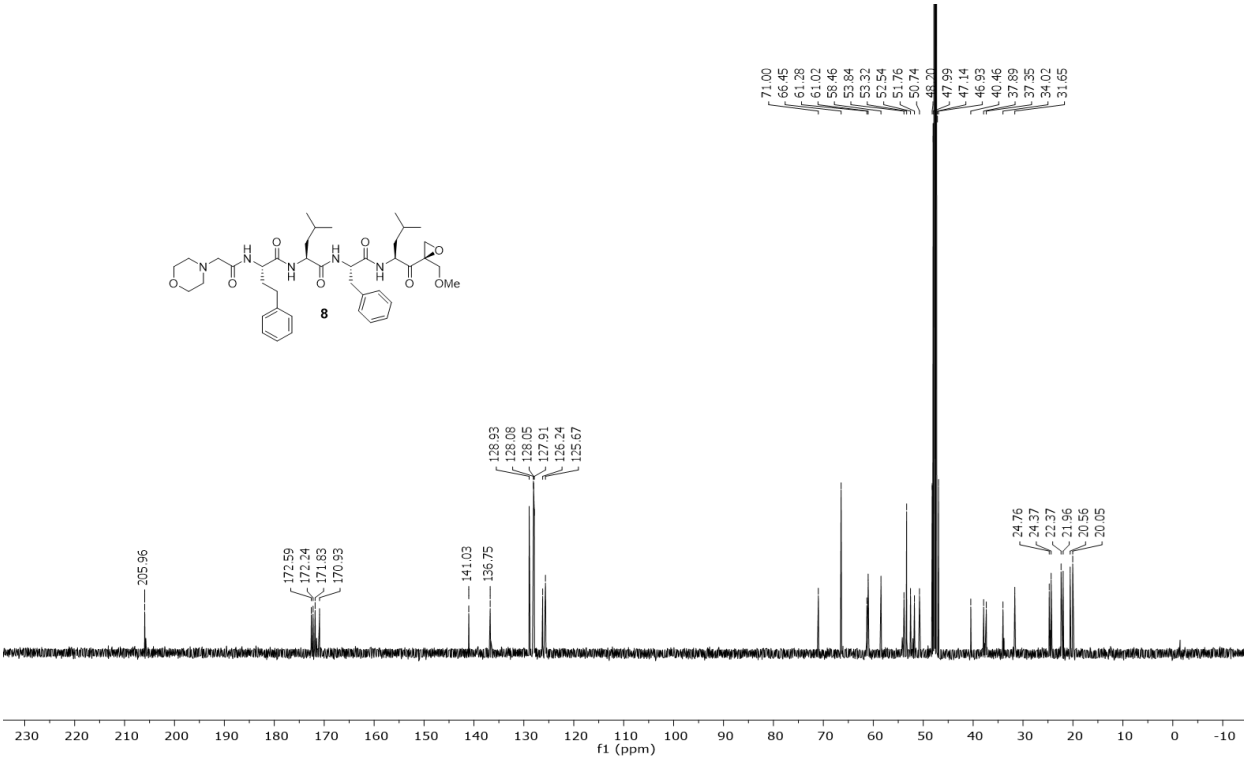


^{1}H NMR, and ^{13}C NMR spectra of **7**

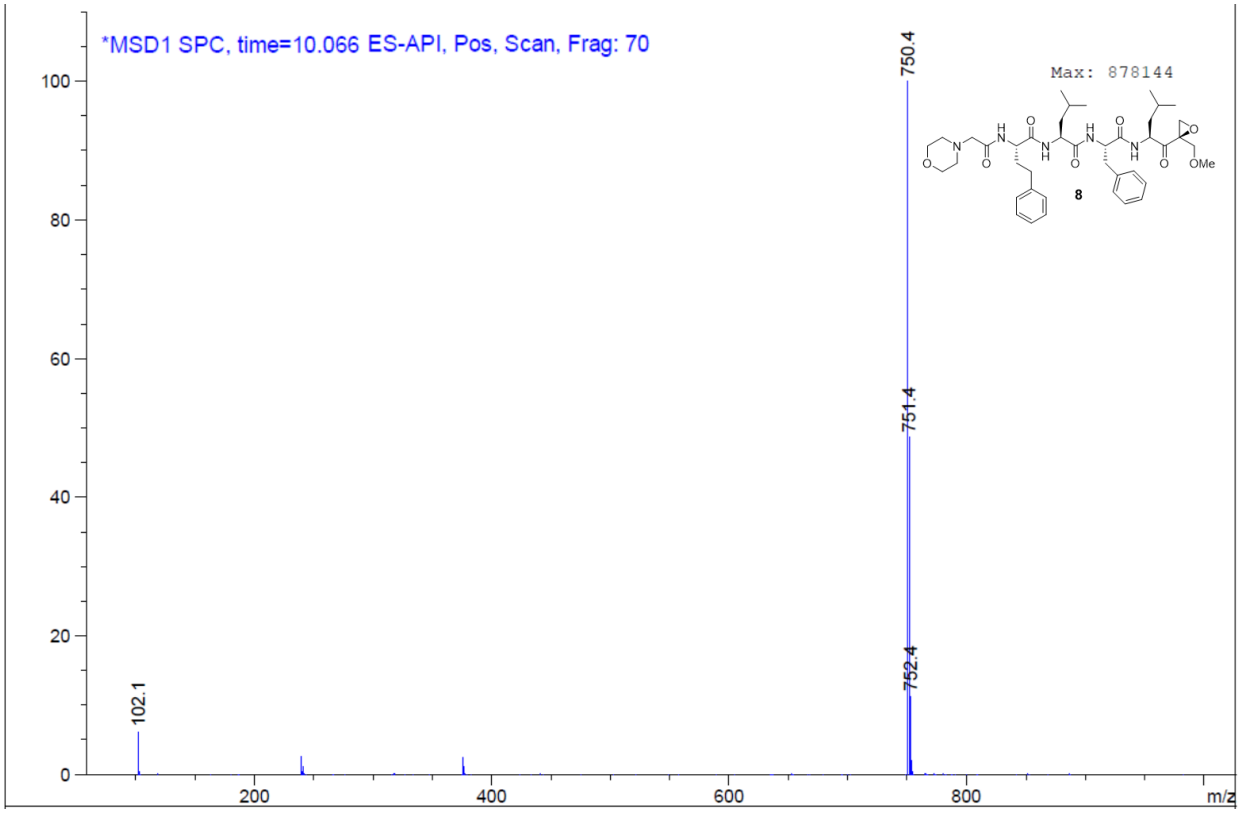


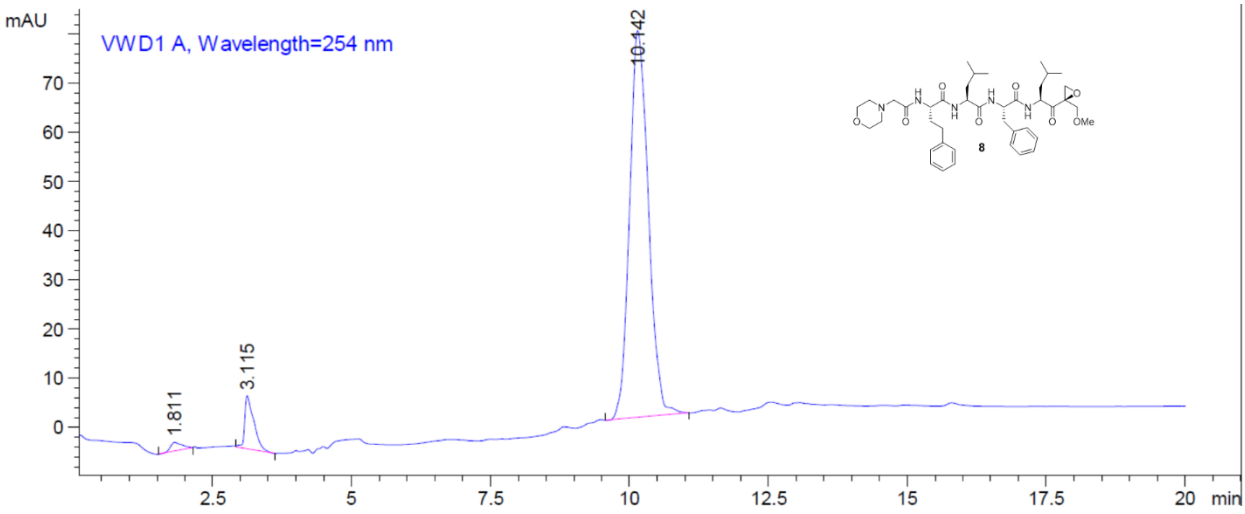
LC/MS traces and elution profile of **7**





^{1}H NMR, and ^{13}C NMR spectra of **8**





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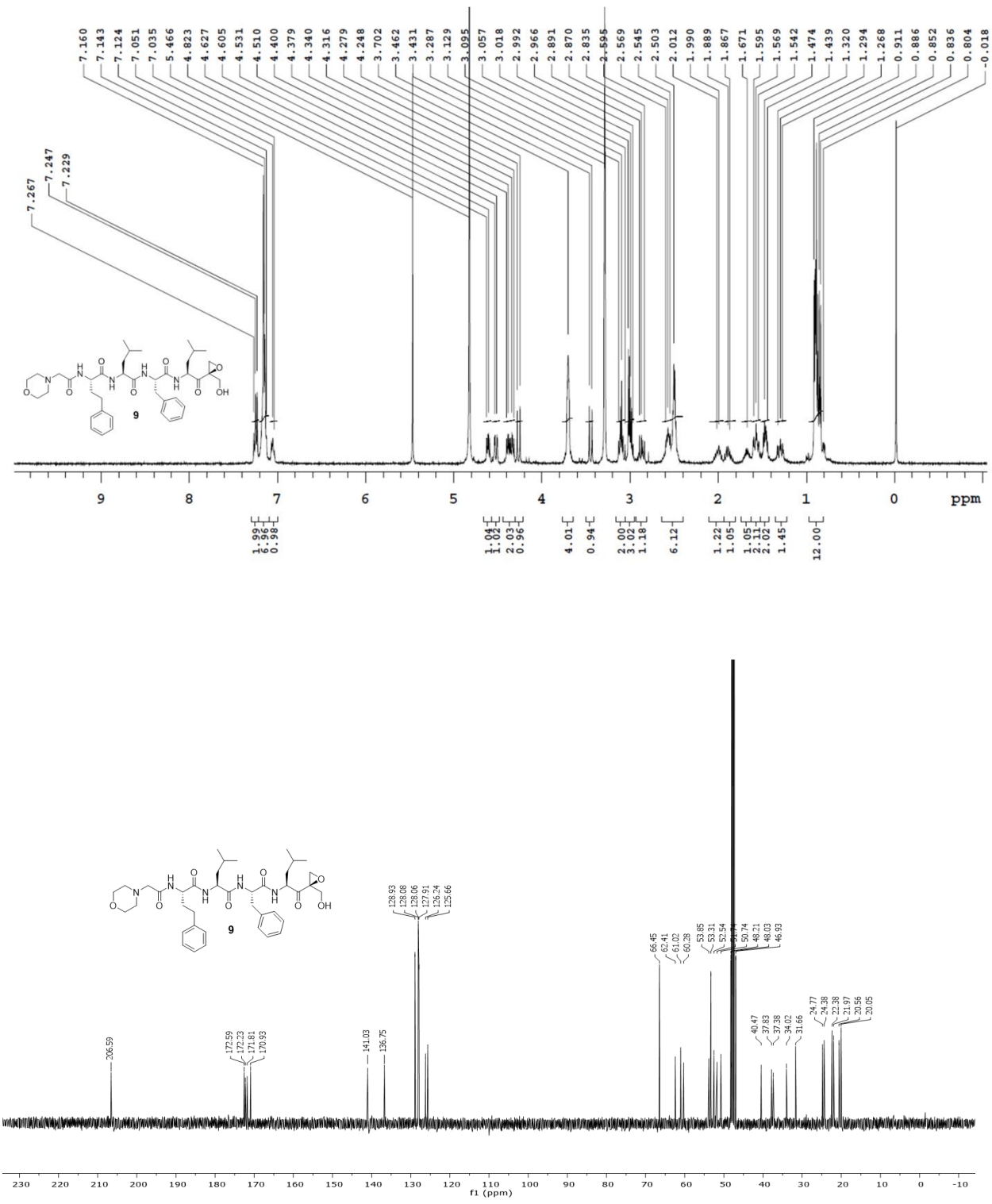
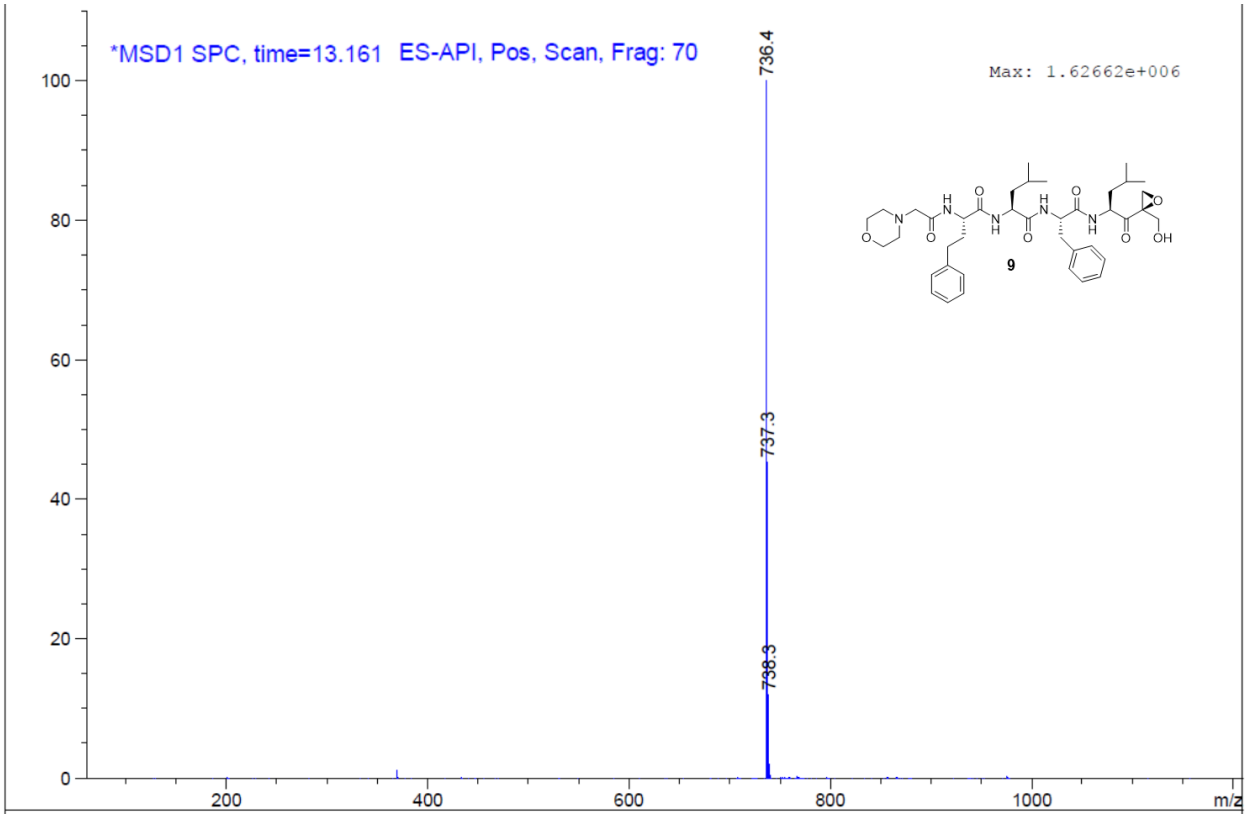
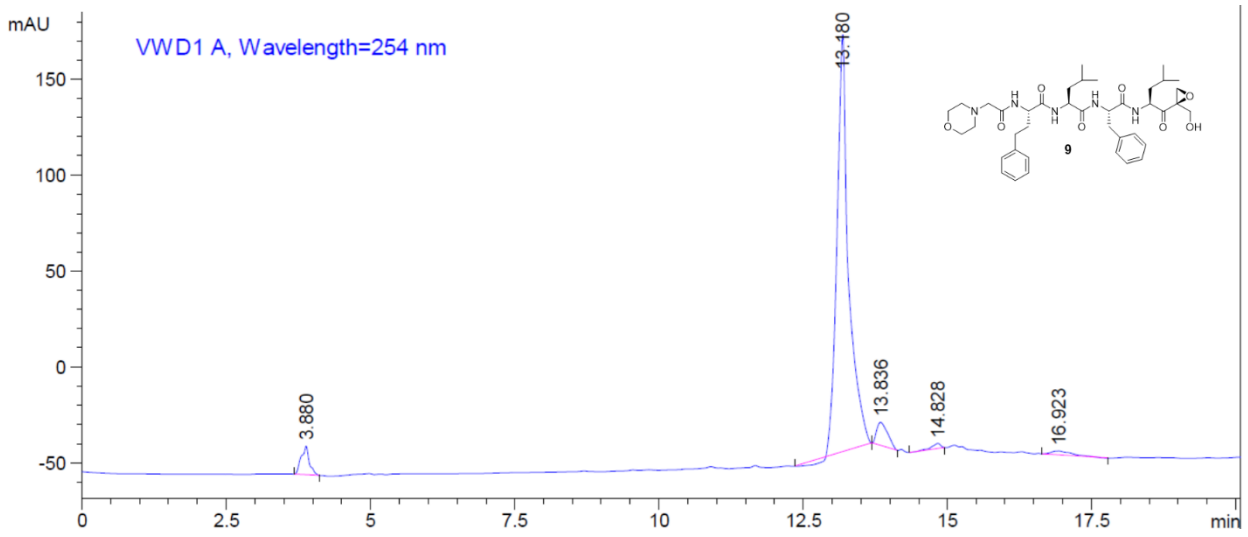


^{1}H NMR, and ^{13}C NMR spectra of **9**





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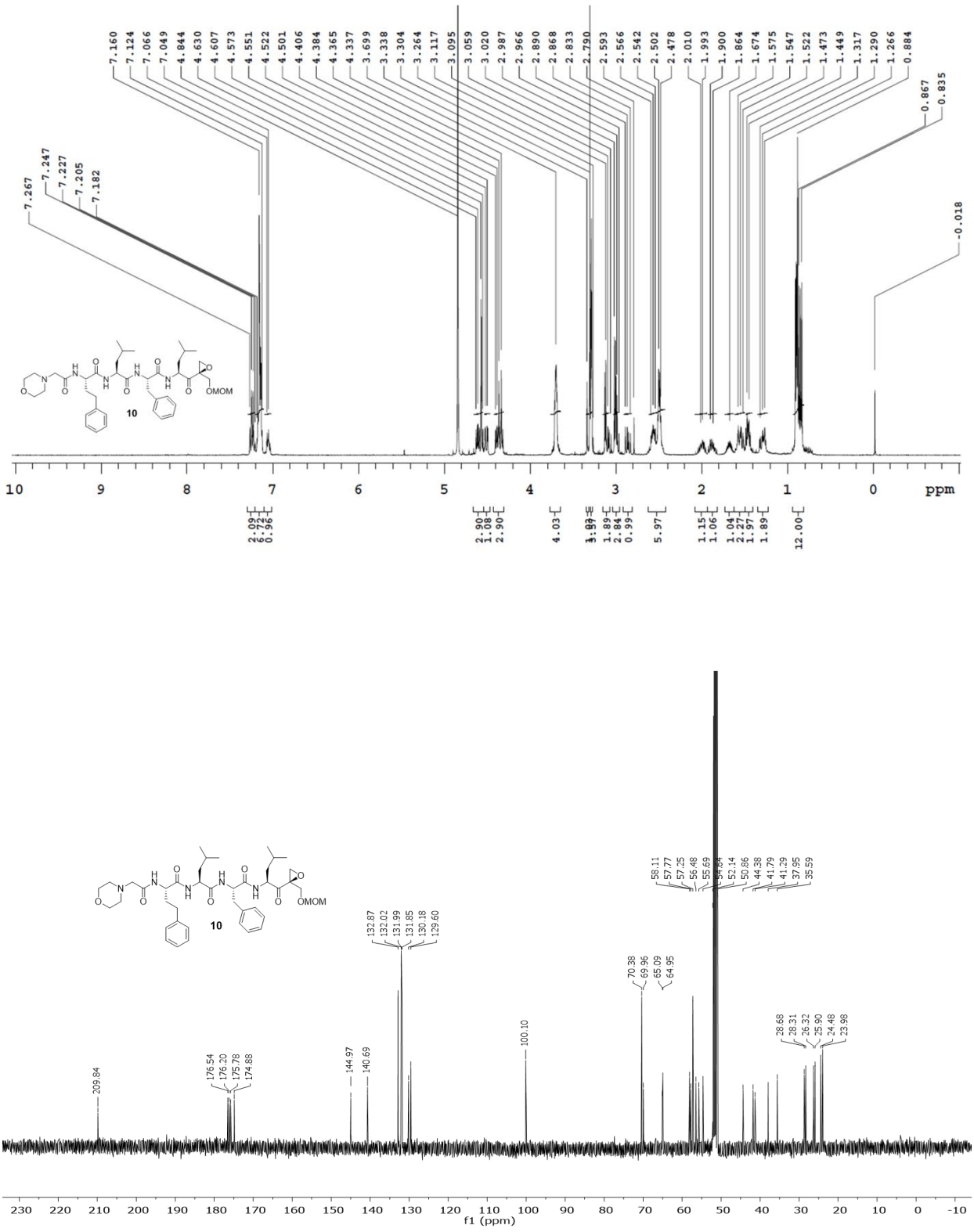
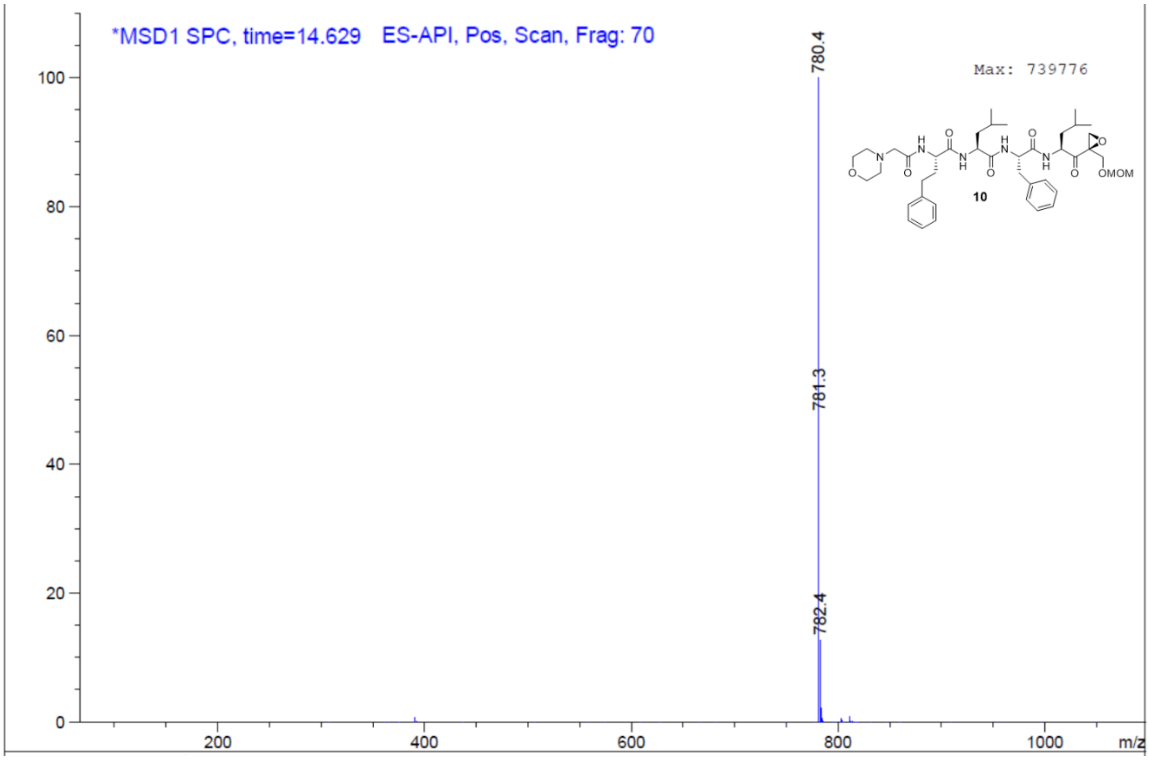
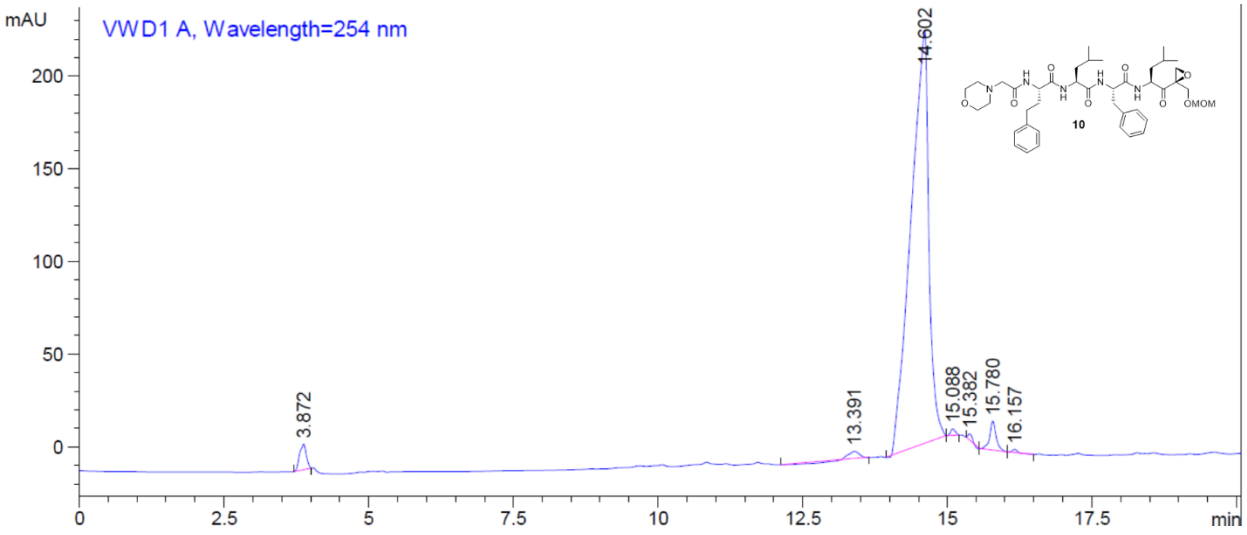
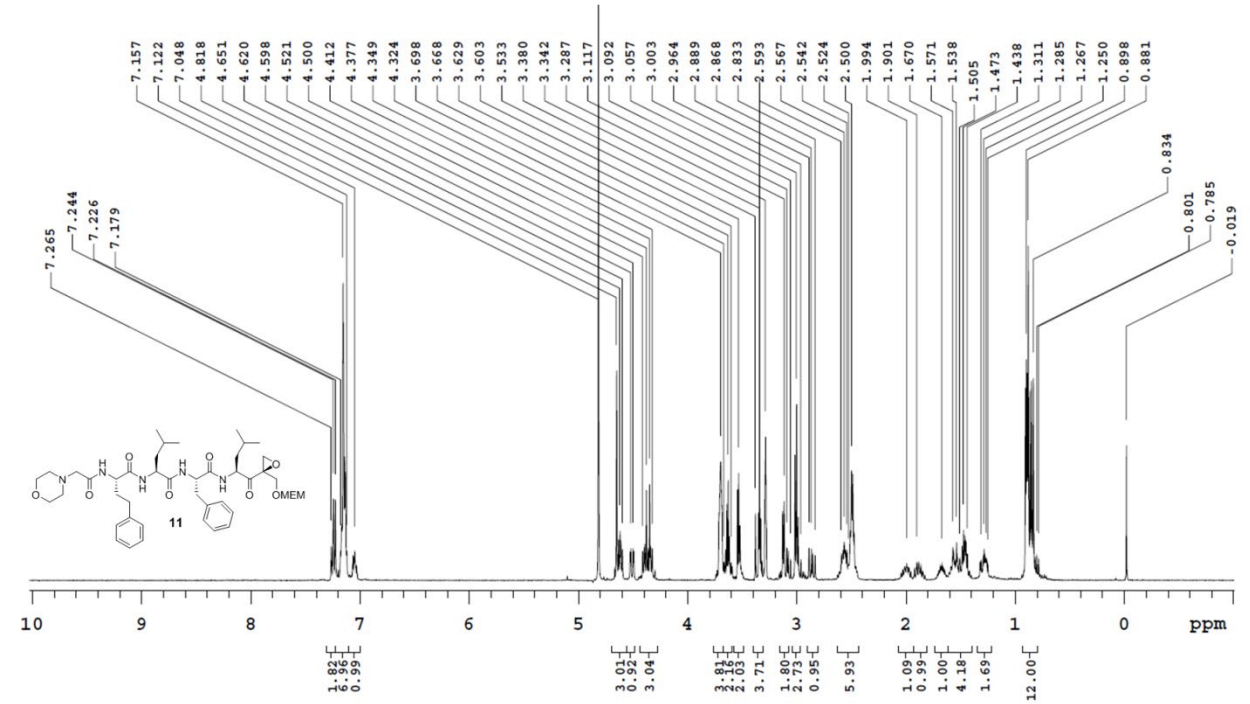


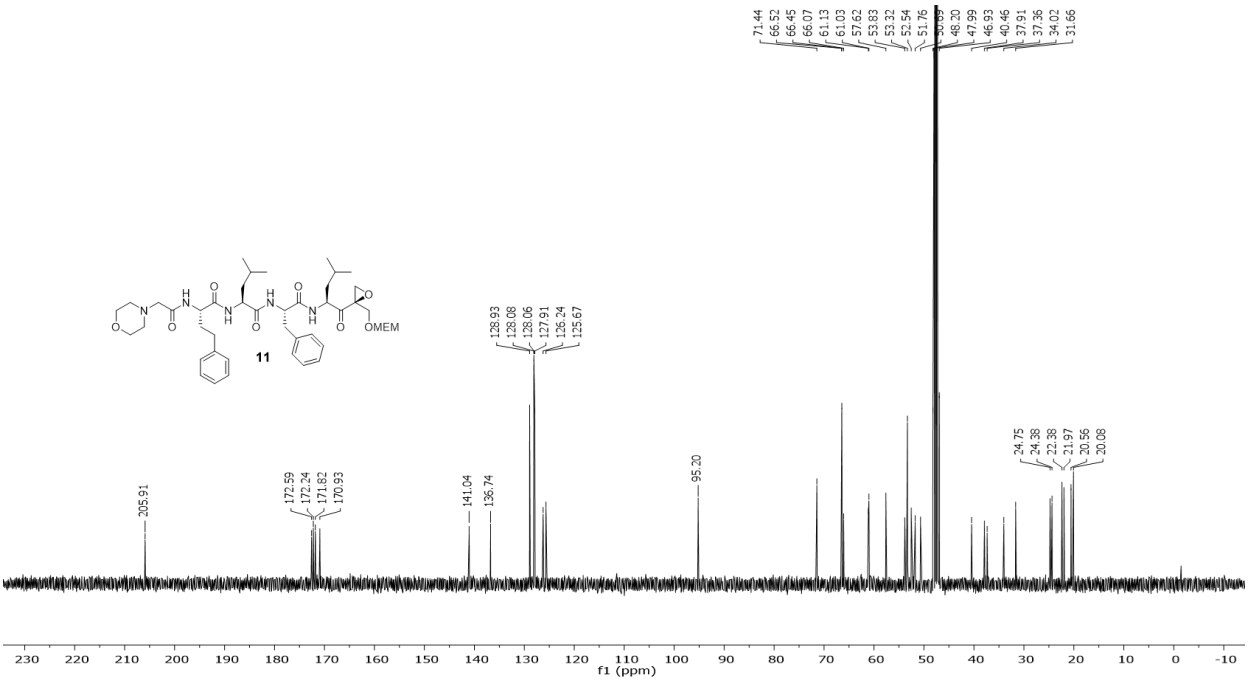
^{1}H NMR, and ^{13}C NMR spectra of **10**



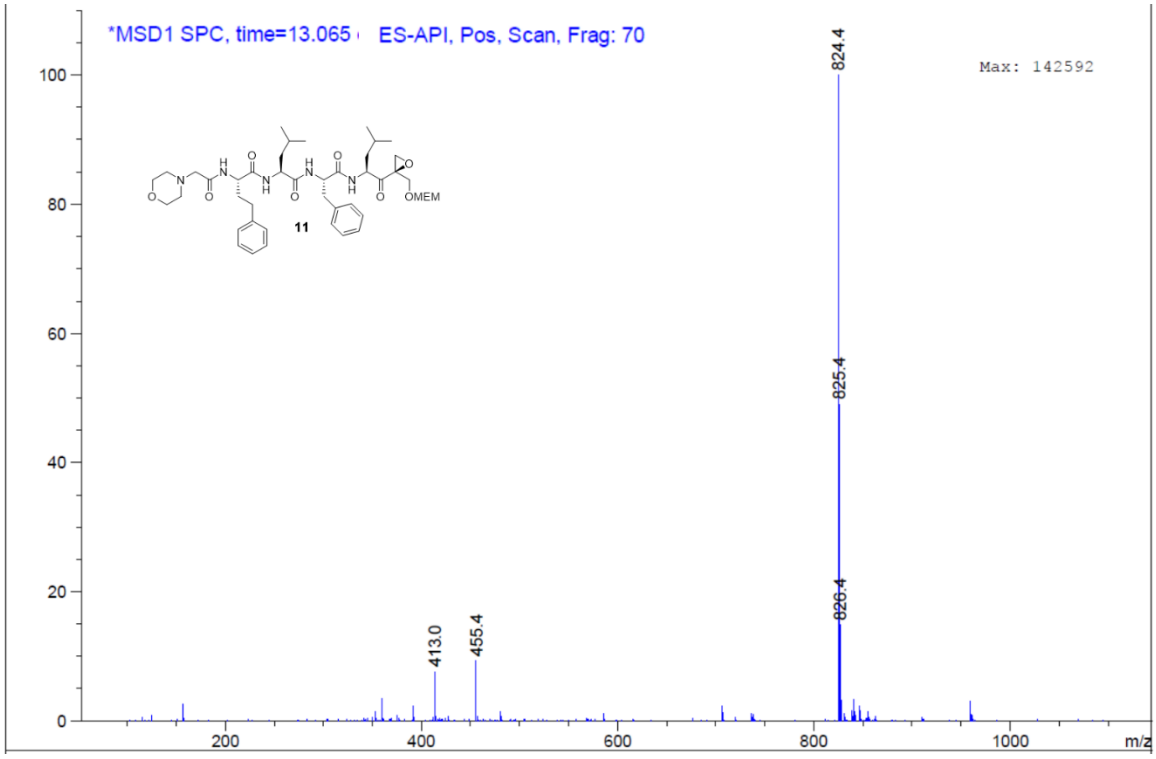


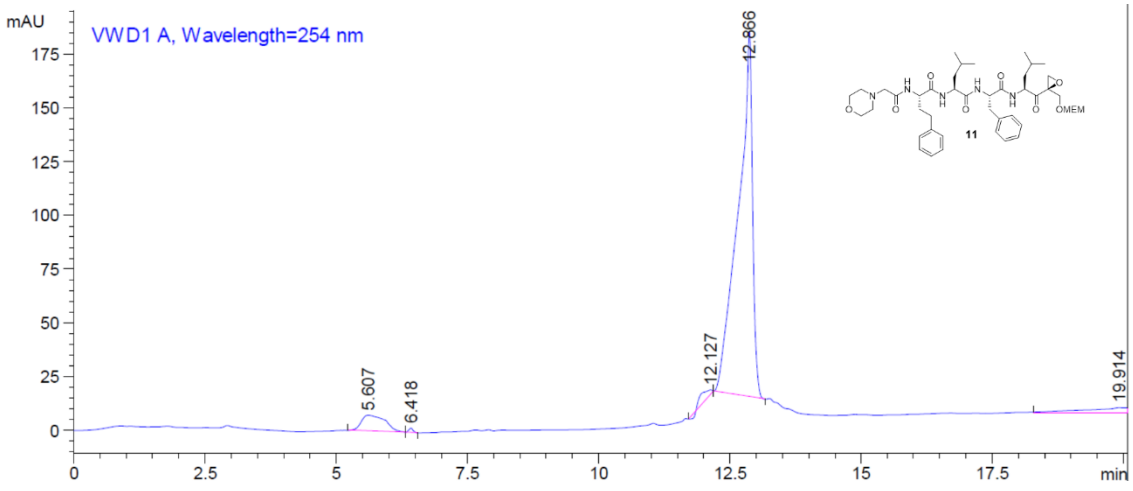
LC/MS traces and elution profile of **10**



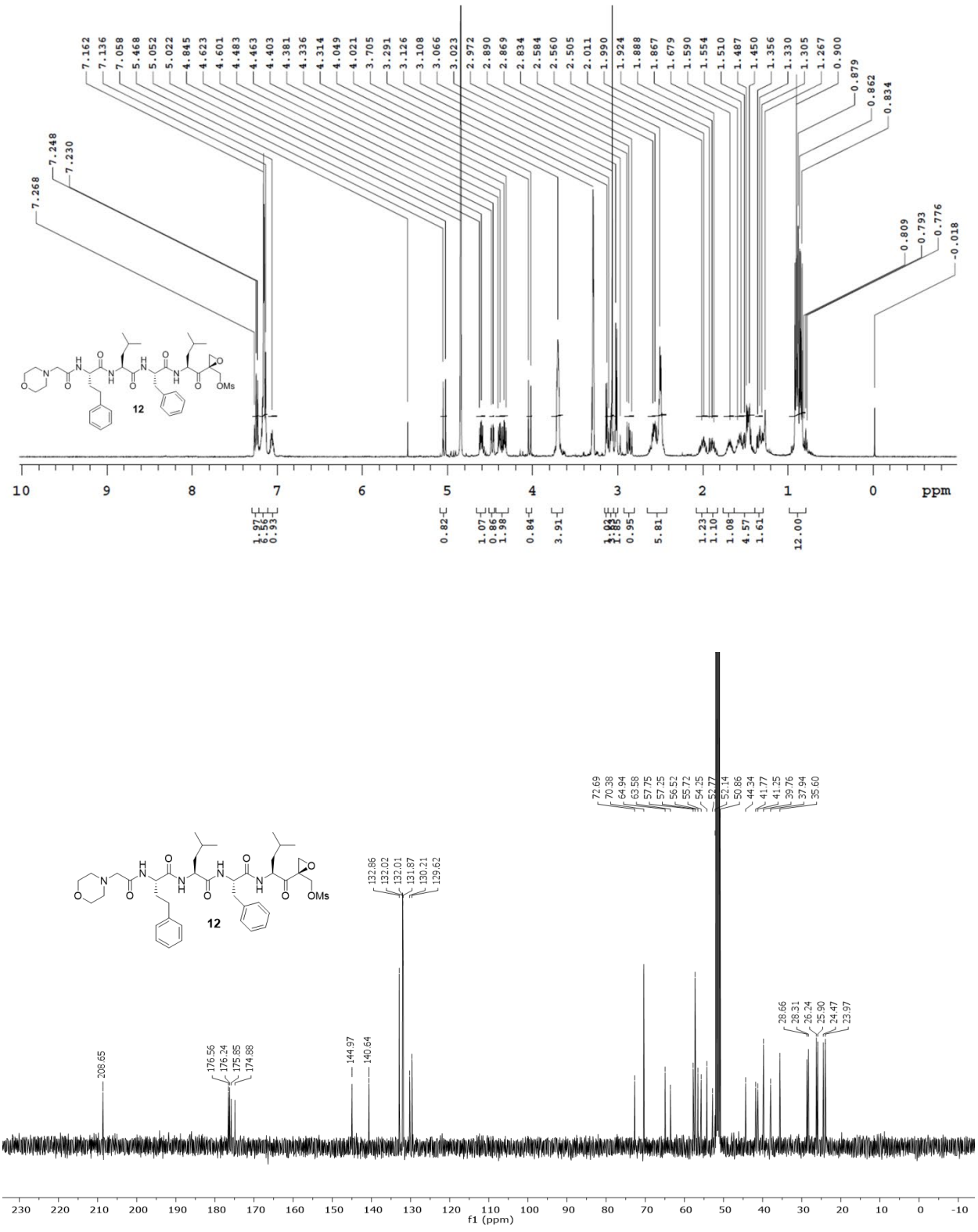


^{1}H NMR, and ^{13}C NMR spectra of **11**

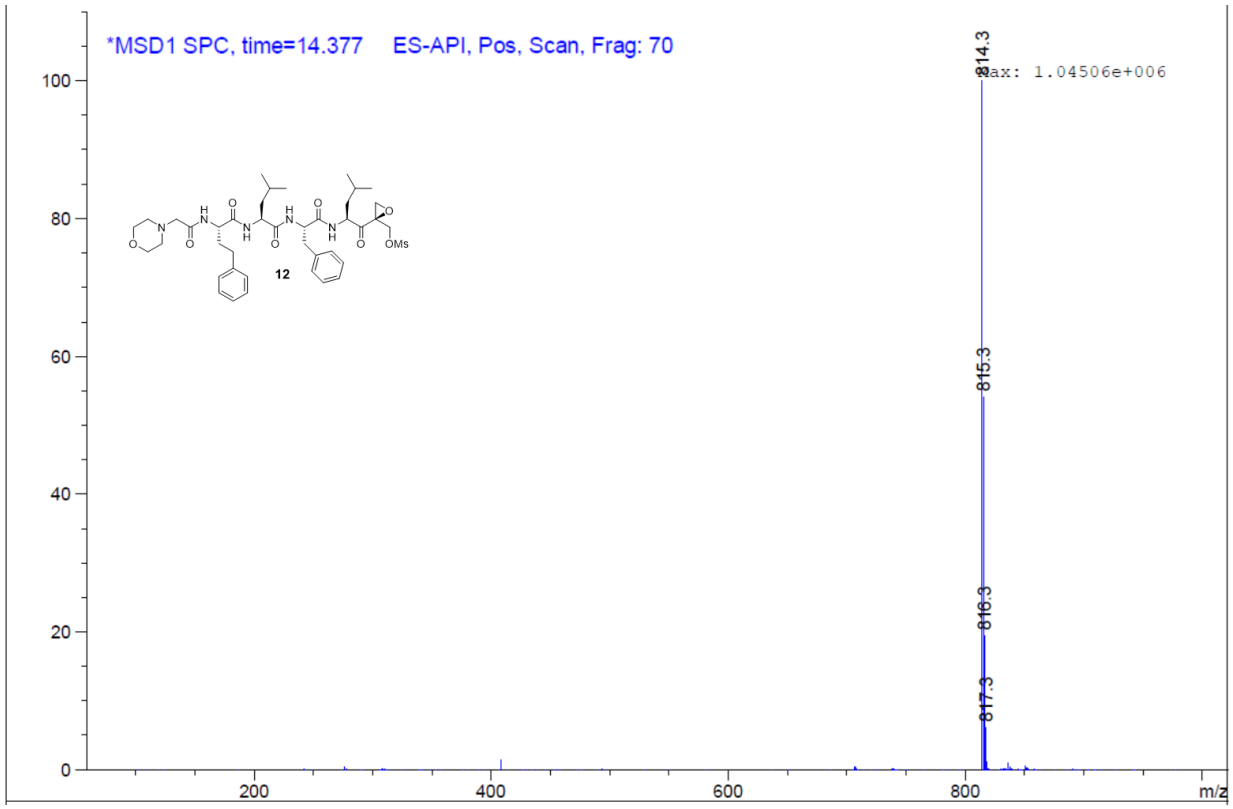


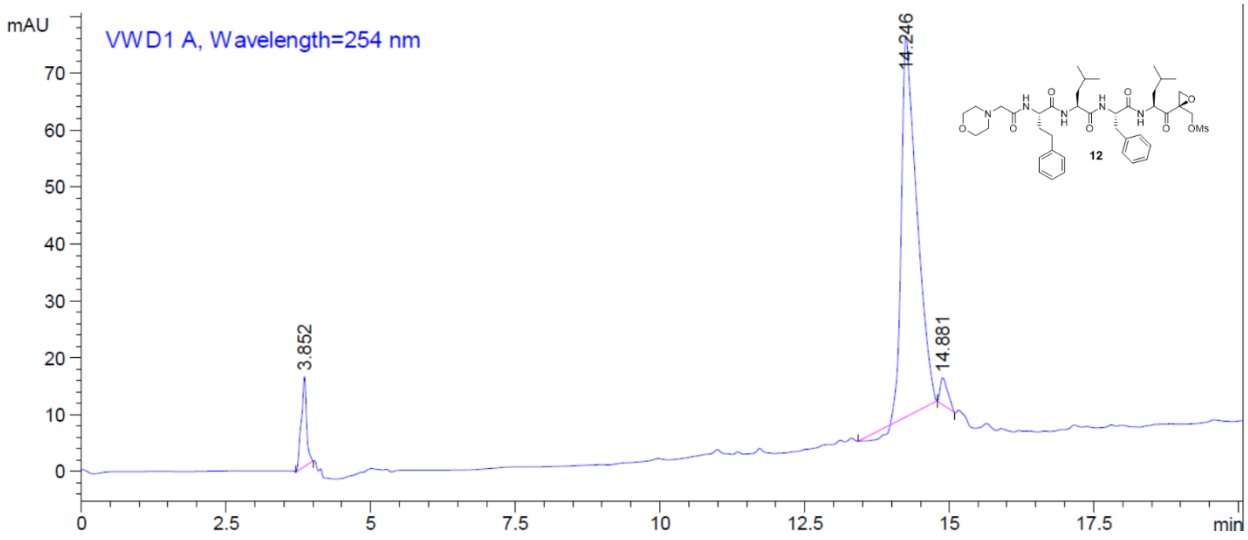


LC/MS traces and elution profile of **11**



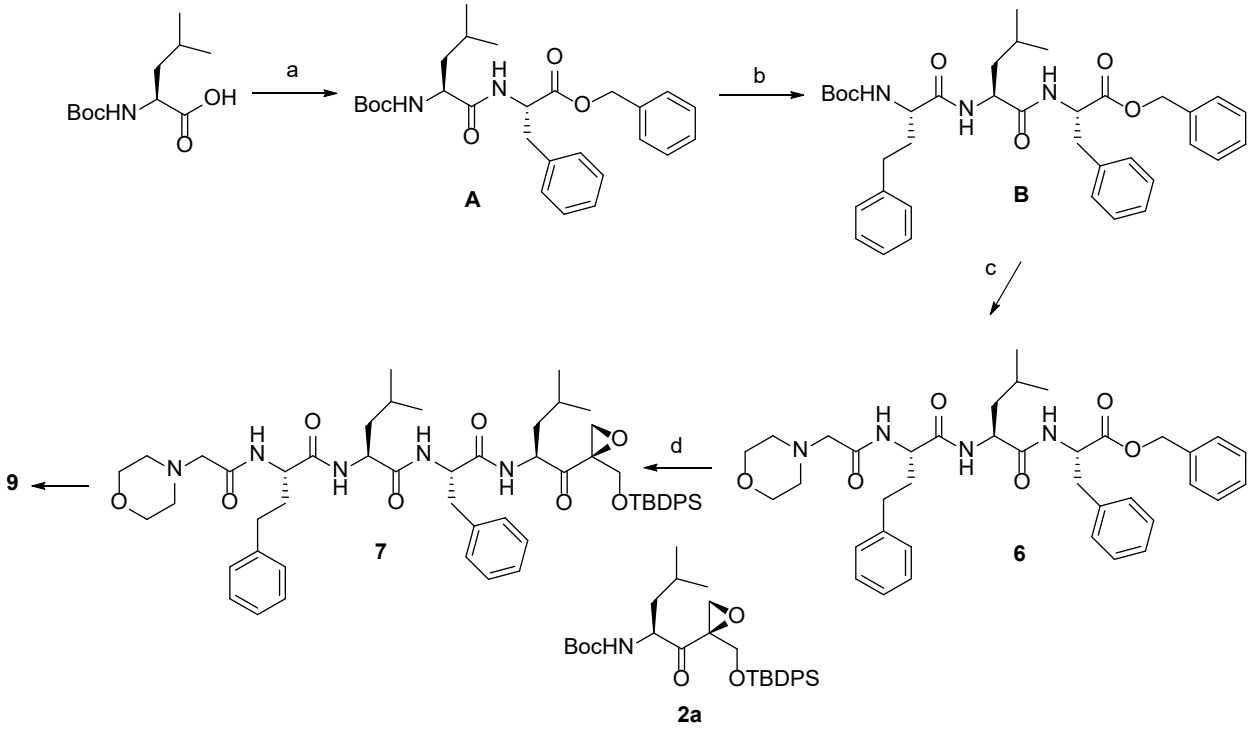
^{1}H NMR, and ^{13}C NMR spectra of **12**





LC/MS traces and elution profile of **12**

Scheme S1: Synthesis of compound 9



Reagent and condition: (a) Phenylalanine benzyl ester hydrochloride, HBTU, HOBt, DIEA, DCM, rt, 18 h, 81%; (b) TFA, DCM, rt, 1 h then evaporation and drying, Boc-homoPhe-OH, HBTU, HOBt, DIEA, DCM, rt, 18 h, 79%; (c) TFA, DCM, rt, 1 h then evaporate and dried, Morpholin-4-yl-acetic acid hydrochloride, HBTU, HOBt, DIEA, DCM, rt, 18 h, 65%; (d) i) H_2/Pd, C, methanol, 1 h; ii) boc deprotected **2a**, HBTU, HOBt, DIEA, DCM, rt, 18 h, 45%; (c) TBAF, DCM, 2 h, 64%.

(S)-benzyl 2-((S)-2-(*tert*-butoxycarbonylamino)-4-methylpentanamido)-3-phenylpropanoate (A)

To the stirred solution of phenylalanine benzyl ester hydrochloride (2.12 g, 4.97 mmol) and Boc-leucine (1.15 g, 4.97 mmol) in DCM, HBTU (3.72 g, 9.94 mmol), HOBt (1.34 g, 9.94 mmol) and DIEA (3.47 mL, 19.88 mmol) were added at room temperature and the resulting reaction mixture was stirred at the same temperature overnight. Solvent was removed under reduced pressure and residue was purified by flash column chromatography using 25% ethyl acetate in Hexane. Pure product was collected as white solid (1.89 g, 81%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63-7.31 (m, 5H), 5.95 (s, 1H), 5.16 (t, *J* = 4.6 Hz, 2H), 4.72 - 4.56 (m, 1H), 2.18 (td, *J* = 2.8, 8.0 Hz, 2H), 1.59 (d, *J* = 9.0 Hz, 2H), 1.46 - 1.12 (m, 9H), 0.86 (t, *J* = 8.6 Hz, 3H).

(S)-benzyl 2-((S)-2-((S)-2-(tert-butoxycarbonylamino)-4-phenylbutanamido)-4-methylpentanamido)-3-phenylpropanoate (B)

To the solution of A (1.89 g, 4.03 mmol) in DCM, excess of TFA was added and the mixture was stirred at room temperature for 2 h. Solvent was evaporated and the residue was dried under vacuum and used without further purification. To the solution of boc deprotected A in DCM, Boc-homoPhe-OH (1.12 g, 4.03 mmol), HBTU (3.02 g, 8.06 mmol), HOBt (1.09 g, 8.06 mmol) and DIEA (2.81 mL, 16.12 mmol) were added at room temperature and the resulting reaction mixture was stirred at the same temperature overnight. Solvent was removed under reduced pressure and residue was purified by flash column chromatography using 2% DCM in methanol. Pure product was collected as white solid (2.0 g, 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (dd, *J* = 4.8, 1.8 Hz, 2H), 7.29 - 7.22 (m, 5H), 7.21 – 7.12 (m, 5H), 7.01 - 6.94 (m, 2H), 6.43 (d, *J* = 7.5 Hz, 1H), 6.37 (d, *J* = 8.0 Hz, 1H), 5.20 – 5.00 (m, 2H), 4.97 - 4.77 (m, 2H), 4.36 (td, *J* = 8.7, 4.9 Hz, 1H), 3.99 (d, *J* = 7.4 Hz, 1H), 3.07 (q, *J* = 7.9, 7.1 Hz, 2H), 2.62 (t, *J* = 7.9 Hz, 2H), 2.06 (dt, *J* = 14.2, 7.0 Hz, 1H), 1.85 (dd, *J* = 14.1, 7.6 Hz, 1H), 1.57 (s, 3H), 1.42 (s, 9H), 0.84 (t, *J* = 5.9 Hz, 6H).

(S)-benzyl 2-((S)-4-methyl-2-((S)-2-(2-morpholinoacetamido)-4-phenylbutanamido) pentan-emido)-3-phenylpropanoate (6)

To the solution of B (1.0 g, 1.58 mmol) in DCM, excess of TFA was added and the mixture was stirred at room temperature for 2 h. Solvent was evaporated and the residue was dried under vacuum and used without further purification. To the solution of boc deprotected B in DCM, morpholin-4-yl-acetic acid hydrochloride (0.23 g, 1.58 mmol), HBTU (1.18 g, 3.16 mmol), HOBt (0.43 g, 3.16 mmol) and DIEA (1.1 mL, 6.32 mmol) were added at room temperature and the resulting reaction mixture was stirred at the same temperature overnight. Solvent was removed under reduced pressure and residue was purified by flash column chromatography using 2% DCM in methanol. Pure product was collected as white solid (0.68 g, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (s, 2H), 7.56 (d, *J* = 8.4 Hz, 1H), 7.34 - 7.27 (m, 2H), 7.26 - 7.18 (m, 3H), 7.17 - 7.08 (m, 5H), 6.97 (dd, *J* = 7.2, 2.3 Hz, 2H), 6.84 (d, *J* = 7.8 Hz, 1H), 6.66 (d, *J* = 7.7 Hz, 1H), 5.14 - 4.98 (m, 2H), 4.83 (dt, *J* = 7.6, 6.0 Hz, 1H), 4.44 (td, *J* = 8.1, 5.8 Hz, 1H), 4.35 (ddd, *J* = 9.0, 7.8, 5.3 Hz, 1H), 3.74 - 3.63 (m, 4H), 3.12 - 2.97 (m, 2H), 2.94 (d, *J* = 5.9 Hz, 2H), 2.57 (dq, *J* = 8.8, 3.1 Hz, 2H), 2.46 (q, *J* = 4.5 Hz, 4H), 2.17 - 2.03 (m, 1H), 1.97 - 1.84 (m, 1H), 1.62 - 1.39 (m, 3H), 0.81 (dd, *J* = 9.5, 6.1 Hz, 6H).

(S)-*N*-((S)-1-((S)-1-((S)-2-((*tert*-Butyldiphenylsilyloxy)methyl)oxiran-2-yl)-4-methyl-1-oxopentan-2-ylamino)-1-oxo-3-phenylpropan-2-yl)-4-methyl-2-((S)-2-(2-morpholinoacetamido)-4-phenylbutanamido)pentanamide (7)

The solution of intermediate **6** in methanol with Pd/C was stirred at room temperature under hydrogen gas for 1 h. Benzyl deprotected acid solution was filtered through celite and concentrated as white solid.

2a was dissolved in DCM and excess of TFA was added to it. The reaction mixture was stirred at room temperature for 1 h. Solvent was removed under reduced pressure and residue was dried under vacuum.

The above prepared benzyl deprotected acid C (0.5 g, 0.85 mmol) was dissolved in DCM at room temperature and boc deprotected **2a** (0.46 g, 0.85 mmol), HBTU (0.64 g, 1.7 mmol), HOBt (0.23 g, 1.7 mmol) and DIEA (0.6 mL, 3.4 mmol) were added at room temperature. The reaction solution was stirred at room temperature overnight. Solvent was removed under reduced pressure and residue was purified by flash column chromatography using 5% DCM in methanol. Pure product was collected as white solid (0.38 g, 45%). melting point, 87-91 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.69-7.66 (m, 4H), 7.47 - 7.34 (m, 6H), 7.29 - 7.09 (m, 9H), 7.09 - 6.98 (m, 1H), 4.69 - 4.50 (m, 3H), 4.41- 4.33 (m, 2H), 3.69 (m, 4H), 3.35 (d, *J* = 11.4 Hz, 1H), 3.18 - 3.04 (m, 2H), 3.04 - 2.97 (m, 2H), 2.96 - 2.83 (m, 2H), 2.62 - 2.42 (m, 6H), 2.01 - 1.86 (m, 2H), 1.72 - 1.72 (m, 2H), 1.57-1.44 (m, 3H), 1.34 (t, *J* = 10.5 Hz, 1H), 0.99 (d, *J* = 0.7 Hz, 9H), 0.94 – 0.78 (m, 12H).^{13}C NMR (101 MHz, CD_3OD) δ 135.31, 135.24, 129.58, 129.57, 128.95, 128.08, 128.05, 127.91, 127.43, 127.41, 126.23, 125.66, 66.45, 63.45, 61.02, 53.88, 53.31, 52.55, 51.07, 48.20, 47.99, 47.84, 47.77, 47.56, 47.35, 47.13, 46.92, 40.47, 38.21, 37.40, 33.99, 31.66, 25.74, 24.88, 24.39, 22.49, 21.97, 20.56, 20.12; LCMS (ES+) m/z calcd for $C_{56}H_{76}N_5O_8Si$ $[M+H]^+$ 974.5, found: 974.5; Purity: ≥ 98% and retention time is 14.21 min by HPLC analysis.

(S)-*N*-((S)-1-((S)-1-((R)-2-(Hydroxymethyl)oxiran-2-yl)-4-methyl-1-oxopentan-2-ylamino)-1-oxo-3-phenylpropan-2-yl)-4-methyl-2-((S)-2-(2-morpholinoacetamido)-4-phenylbutanamido)pentanamide (9)

To the solution of compound **7** (0.36 g, 0.37 mmol) in DCM, TBAF (0.81 mL, 0.81 mmol) was added at room temperature and the reaction mass was stirred at the same temperature for 30 min. Solvent was removed under reduced pressure and the resulting residue was purified by flash column chromatography using 5% methanol in DCM to yield pure product as white solid (0.17 g, 64%); melting point, 118-122 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.30 - 7.21 (m, 2H), 7.21 - 7.09 (m, 7H), 7.09 - 7.01 (m, 1H), 4.61 (dd, *J* = 5.4, 8.7 Hz, 1H), 4.52 (dd, *J* = 2.9, 11.0 Hz, 1H), 4.36 (ddd, *J* = 5.6, 8.8, 19.5 Hz, 2H), 4.26 (d, *J* = 12.6 Hz, 1H), 3.79 – 3.66 (m, 4H), 3.45 (d, *J* = 12.6 Hz, 1H), 3.14 – 3.05 (m, 2H), 3.00 (dd, *J* = 5.0, 10.1 Hz, 3H), 2.86 (dd, *J* = 8.7, 14.0 Hz, 1H), 2.63 - 2.42 (m, 6H), 2.06 - 1.83 (m, 2H), 1.72 - 1.42 (m, 5H), 1.29 (ddd, *J* = 3.9, 10.9, 14.5 Hz, 1H), 0.97 - 0.77 (m, 12H).^{13}C NMR (101 MHz, CD_3OD) δ 206.57, 172.59, 172.26, 171.80, 170.95, 141.03, 136.74, 128.92, 128.07, 128.05, 127.90, 126.23, 125.66, 66.45, 62.40, 61.02, 60.28, 53.85, 53.31, 52.55, 51.76, 50.74, 48.19, 48.05, 47.98, 47.84, 47.76, 47.55, 47.34, 47.12, 46.91, 40.43, 37.82, 37.35, 33.98, 31.64, 24.76, 24.37, 22.36, 21.94, 20.53, 20.02; LCMS (ES+) m/z calcd for $C_{40}H_{58}N_5O_8$ $[M+H]^+$ 736.4, found: 736.4; Purity: ≥ 95% and retention time is 13.18 min by HPLC analysis.