Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supplementary Information

Improved Total Synthesis of the Antibiotic A54145B†

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General methods

All commercially available materials were used without further purification. All solvents were reagent grade or HPLC grade (RCI or DUKSAN). Anhydrous solvents were either prepared from AR grade solvents via standard methods (DCM, THF), or purchased in anhydrous form (DMF). Solution-phase reactions were carried out under an atmosphere of dry nitrogen or argon, unless otherwise specified.

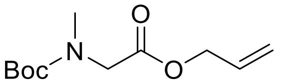
Flash column chromatography was performed using Grace Davisil LC60A silica 43-60μm eluting with gradients as specified. Analytical thin layer chromatography (TLC) was performed on commercially prepared silica plates (Merck Silica Gel 60 F_{254}) and visualized under UV light (254 nm) or by staining with ninhydrin.

^{1}H NMR, ^{13}C NMR spectra were recorded at 298K using a Bruker AVANCE DRX400 or ASCEND 500 spectrometers. Chemical shifts are reported in parts per million (ppm) and are referenced to solvent residual signals: $CDCl_3$ δ 7.26 [^{1}H] and δ 77.16 [^{13}C]. ^{1}H NMR data are reported as chemical shifts, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, ddd = doublet of doublet of doublets, m = multiplet, br = broad), coupling constant *J* (Hz).

High-resolution ESI$^+$ mass spectra were measured on a Waters Micromass Q-TOF Premier Mass Spectrometer. Optical rotations were measured at ambient temperature (293 K) on a Bellingham & Stanley ADP440+ polarimeter with a path length of 5 cm, and the concentrations are reported in g/100 mL.

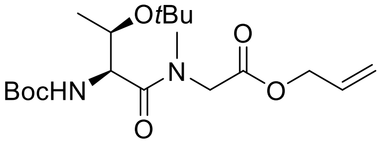
Preparative HPLC separations involving a mobile phase of 0.1% TFA (v/v) in acetonitrile (Solvent A) and 0.1% TFA (v/v) in water (Solvent B) were performed on a Waters HPLC system equipped with a quaternary pump (Waters 2545) and a UV/Vis detector (Waters 2489) using a Grace Vydac 218TP^{TM} C18 column (10 μm, 300 Å , 22 × 250 mm) at a flow rate of 10 mL/min. All analytical reversed-phase HPLC separations involving a mobile phase of 0.1% TFA (v/v) in acetonitrile (Solvent A) and 0.1% TFA (v/v) in water (Solvent B) were performed on a Waters UPLC H-class system equipped with an ACQUITY UPLC photodiode array detector and a Waters SQ Detector 2 mass spectrometer using a Waters ACQUITY BEH C18 column (1.7 μm, 130 Å, 2.1 x 50 mm) at a flow rate of 0.4 mL/min.

Allyl *N*-(*tert*-Butoxycarbonyl)-*N*-methylglycinate (5).



To a 250 mL round bottom flask were added Boc-Sar-OH **4** (5.104 g, 26.98 mmol, 1.0 equiv) and DMAP (330 mg, 0.270 mmol, 0.1 equiv). After the flask was charged with argon, anhydrous DCM (35 mL) was added and the mixture was cooled to 0°C. Allyl alcohol (3.6 mL, 52.81 mmol, 2.0 equiv) and EDCI (9.9 g, 51.6 mmol, 2.0 equiv) were added sequentially. The mixture was stirred at rt for 4 h. The reaction mixture was diluted with ethyl acetate (200 mL) and washed with 1 N HCl (3 × 100 mL), brine (1 × 100 mL), dried with Na_2SO_4 and concentrated *in vacuo.* The residue was purified by flash column chromatography on silica gel (hexane/EtOAc, 4:1) to give **5** (5.207 g, 84 %) as colorless oil. NMR spectra of compound **5** showed rotational isomers. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 5.98 – 5.85 (m, 1H), 5.33 (d, *J* = 2.0 Hz,1H), 5.25 (d, *J* = 1.0 Hz, 1H), 4.64 (d, *J* = 1.0 Hz, 2H), 4.00 (s, 1H), 3.92 (s, 1H), 2.93 (s, 3H), 1.46 (d, *J* = 1.5Hz, 9H).; **^{13}C NMR** (100 MHz, $CDCl_3$) δ 169.69, 156.18, 155.51, 131.87, 131.81, 118.76, 118.58, 80.28, 80.24, 65.63, 51.15, 50.38, 35.68, 28.43; **HRMS (ESI$^+$)** calcd. for $C_{11}H_{19}NO_4$ $[M+H]^+$ *m/z*: 230.1387; found: 230.1377.

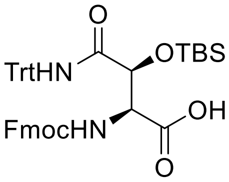
Allyl *N*-(*N*-(*tert*-butoxycarbonyl)-*O*-(tert-butyl)-L-threonyl)-*N*-methylglycinate (6).



To a 25 mL round bottom flask was added **5** (881.8 mg, 3.84 mmol, 1.0 equiv), 95% TFA (3 mL) was then added and the reaction mixture was stirred for 1 h. Then TFA was removed under a stream of nitrogen. The crude product was used for the next step without purification. Boc-Thr(*t*Bu)-OH (2.11 g, 7.66 mmol, 2.0 equiv), HATU (2.89 g, 7.60 mmol, 2.0 equiv) and DIEA (2.68 mL, 15.38 mmol, 4.0 equiv) were dissolved in DMF (20 mL) and this mixture was added to the crude product and stirred at rt for 2 h. The reaction mixture was diluted with ethyl acetate (100 mL) and washed with 1 N HCl (3 × 50 mL), brine (1 × 50 mL), dried with Na_2SO_4 and concentrated *in vacuo.* The residue was purified by flash column chromatography on silica gel (hexane/EtOAc, 4:1) to give **6** (1.41 g, 95 %) as colorless oil. NMR spectra of compound **6** showed rotational isomers. **$[\alpha]_D^{20}$**= +53.9 (c = 0.51, DCM); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.96 5.86 (m, 1H), 5.57 (d, *J* = 7.4 Hz, 0.3 H), 5.46 (d, *J* = 8.1 Hz, 1H), 5.35 (s, 1H), 5.30 (s, 1H), 5.25 (d, *J* = 10.4 Hz, 1H), 4.78 (d, *J* = 18.5 Hz, 0.46 H), 4.65 (dd, *J* = 11.9, 4.8 Hz, 4H), 4.44 (dd, *J* = 7.6, 4.0 Hz, 0.33H), 4.16 (d, *J* = 2.8 Hz, 2H), 4.11 (s, 0.25H), 3.93 – 3.81

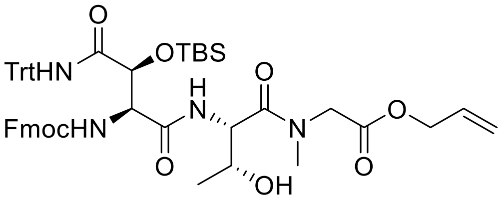
(m, 1H), 3.24 (s, 3H), 3.00 (s, 1H), 1.44 (d, *J* = 3.1 Hz, 14H), 1.23 (d, *J* = 4.8 Hz, 14H), 1.13 (d, *J* = 6.3 Hz, 4H), 1.06 (d, *J* = 6.3 Hz, 1H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 171.34, 169.70, 155.42, 138.23, 131.71, 118.83, 79.52, 74.58, 68.97, 65.84, 51.16, 49.94, 37.36, 35.39, 28.48, 28.30, 28.25, 19.08; **HRMS (ESI$^+$)** calcd. for $C_{19}H_{34}N_2O_6$ $[M+H]^+$ *m/z*: 387.2490; found: 387.2472.

(2*S*,3*S*)-2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-3-((*tert*-butyldimethylsilyl)oxy)-4-oxo-4-(tritylamino)butanoic acid (7)



Compound **7** was synthesized according to the literature.[1]

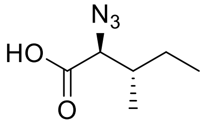
Allyl *N*-(((2*S*,3*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-((*tert*-butyldimethylsilyl)oxy)-4-oxo-4-(tritylamino)butanoyl)-L-threonyl)-*N*-methylglycinate (8).



To a 100 mL round bottom flask were added **6** (850 mg, 1.93 mmol, 1.0 equiv) and 4 N HCl in dioxane (10 mL). The reaction mixture was stirred for 3 h. Then HCl was removed under a stream of nitrogen. The crude product was used directly for the next step without purification. The crude product was dissolved in dry DCM (10 mL) and stirred. Then, a mixture of **7** (700 mg, 0.964 mmol, 0.5 equiv), HATU (733 mg, 1.93 mmol, 1.0 equiv) and DIEA (0.797 mL, 4.82 mmol, 2.5 equiv) in 5 mL DCM were added. The mixture was stirred at rt for 2 h. The reaction mixture was concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc, 2:1) to give **8** (900 mg, 96 %) as white solid. NMR spectra of compound **8** showed rotational isomers. **$[\alpha]_D^{20}$**= -36.2 (c = 1.47, DCM); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.09 (s, 1H), 8.01 (s, 0.39H), 7.73 (d, *J* = 7.5 Hz, 3H), 7.59 (t, *J* = 8.0 Hz, 3H), 7.52 (d, *J* = 8.8 Hz, 1H), 7.36 (td, *J* = 7.2, 3.4 Hz, 4H), 7.34 – 7.20 (m, 19H), 7.17 – 7.09 (m, 10H), 6.48 – 6.42 (m, 1H), 6.43 – 6.36 (m, 1H), 5.94 – 5.83 (m, 2H), 5.38 – 5.32 (m, 1H), 5.32 – 5.24 (m, 2H), 5.04 (dd, *J* = 8.8, 2.3 Hz, 1H), 4.99 (s, 0.25H), 4.65 – 4.58 (m, 4H), 4.57 (d, *J* = 4.2 Hz, 0.32H), 4.51 (d, *J* = 8.4 Hz, 0.46H), 4.43 (d, *J* = 3.7 Hz, 1H), 4.41 – 4.32 (m, 2H), 4.30 – 4.22 (m, 2H), 4.14 (ddd, *J* = 12.2, 9.2, 4.7

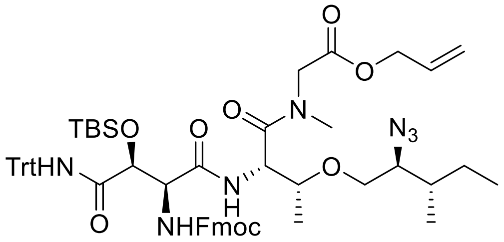
Hz, 4H), 3.44 (d, J = 18.6 Hz, 0.42H), 2.98 (s, 3H), 2.87 (d, J = 17.3 Hz, 1H), 2.43 (s, 1H), 2.17 (s, 1H), 1.35 – 1.16 (m, 34H), 0.98 – 0.77 (m, 29H), 0.27 (d, J = 4.4 Hz, 4H), 0.09 (d, J = 2.9 Hz, 5H).; **^{13}C NMR** (100 MHz, $CDCl_3$) δ 207.12, 172.89, 171.26, 169.43, 169.24, 168.87, 168.77, 168.32, 156.27, 144.74, 144.64, 144.15, 144.08, 143.92, 141.36, 141.29, 132.26, 131.56, 131.33, 128.97, 128.18, 128.12, 127.72, 127.69, 127.39, 127.29, 127.22, 125.63, 125.58, 125.47, 119.96, 119.70, 119.14, 77.48, 77.36, 77.16, 76.84, 73.03, 72.67, 70.89, 70.74, 67.63, 67.46, 66.47, 66.15, 57.30, 53.22, 52.06, 51.91, 49.74, 47.17, 37.14, 34.82, 33.84, 32.05, 31.04, 30.28, 30.16, 29.83, 29.78, 29.48, 25.71, 25.66, 22.82, 18.99, 17.95, 17.92, 14.30, 14.24, -4.76, -4.94, -5.07; **HRMS (ESI$^+$)** calcd. for $C_{54}H_{62}N_4O_9Si$ $[M+H]^+$ *m/z*: 939.4359; found: 939.4320.

N_3-Ile-OH



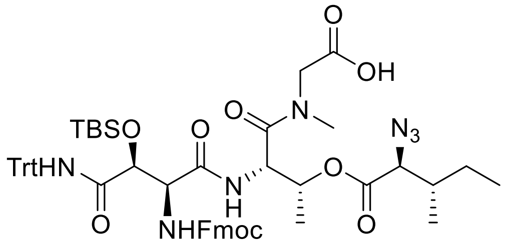
This compound was synthesized according to the literature.[2]

(*E*)-Prop-1-en-1-yl (5*S*,6*S*,9*S*)-6-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-9-((*R*)-1-(((2*S*,3*S*)-2-azido-3-methylpentanoyl)oxy)ethyl)-2,2,3,3,11-pentamethyl-7,10-dioxo-5-(tritylcarbamoyl)-4-oxa-8,11-diaza-3-silatridecan-13-oate (9).



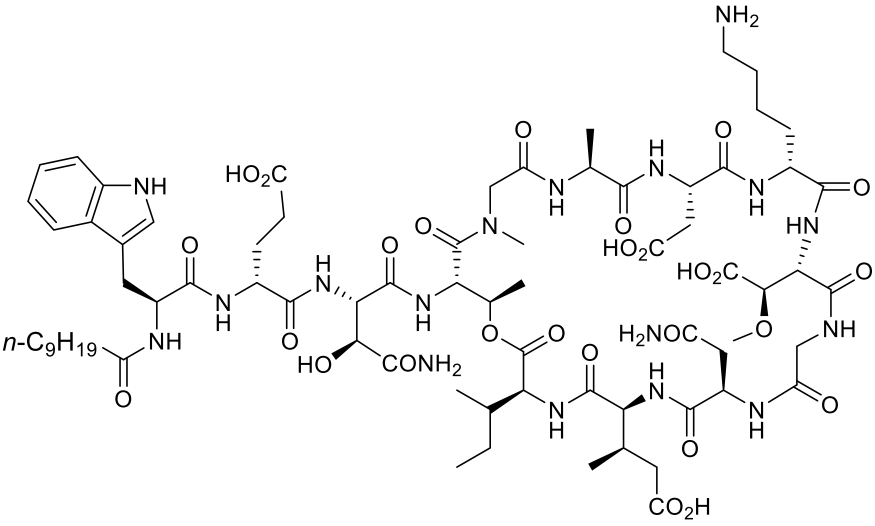
To a 100 mL round bottom flask were added **8** (900 mg, 0.931 mmol, 1.0 equiv) and DMAP (23 mg, 0.186 mmol, 0.2 equiv). After the flask was charged with argon, anhydrous DCM (14 mL) was added and the mixture was cooled to 0°C. N_3-Ile-OH (292 mg, 1.86 mmol, 2.0 equiv) and DIC (0.288 mL, 1.86 mmol, 2.0 equiv) were then added sequentially. The mixture was stirred at rt for 2 h. The reaction mixture was filtered by celite and concentrated *in vacuo.* The residue was purified by flash column chromatography on silica gel (hexane/EtOAc, 2:1) to give **9** (800 mg, 80 %) as colorless oil. NMR spectra of compound **9** showed rotational isomers. **$[\alpha]_D^{20}$**= -16.3 (c = 0.55, DCM); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.15 (s, 1H), 7.72 (dd, J = 14.3, 8.1 Hz, 3H), 7.60 (t, J = 7.5 Hz, 2H), 7.37 (t, J = 7.2 Hz, 2H), 7.34 – 7.21 (m, 13H), 7.15 – 7.05 (m, 6H), 6.34 (d, J = 9.5 Hz, 1H), 5.88 (dq, J = 10.8, 5.6 Hz, 1H), 5.41 (dd, J = 6.2, 4.2 Hz, 1H), 5.29 (dd, J = 25.0, 13.0 Hz, 2H), 5.17 (dd, J = 8.4, 4.0 Hz, 1H), 4.70 – 4.57 (m, 2H), 4.52 (dd, *J* = 13.3, 5.6 Hz, 1H), 4.42 (d, *J* = 3.4 Hz, 1H), 4.36 (dd, *J* = 10.1, 7.2 Hz, 1H), 4.30 – 4.22 (m, 1H), 4.20 – 4.06 (m, 2H), 3.82 (d, *J* = 5.1 Hz, 1H), 2.92 (s, 3H), 2.83 (d, *J* = 17.3 Hz, 1H), 1.62 (s, 6H), 1.39 – 1.11 (m, 10H), 1.07 (d, *J* = 6.9 Hz, 1H), 1.01 – 0.76 (m, 23H), 0.29 (d, *J* = 6.8 Hz, 4H), 0.09 (d, *J* = 14.5 Hz, 4H), -0.00 (s, 2H).; **^{13}C NMR** (100 MHz, $CDCl_3$) δ 169.67, 169.29, 168.35, 168.27, 168.05, 156.27, 144.78, 144.19, 143.91, 141.36, 141.30, 131.91, 128.93, 128.78, 128.18, 128.14, 127.73, 127.69, 127.37, 127.21, 125.67, 125.50, 119.97, 118.62, 77.48, 77.16, 76.84, 73.29, 70.67, 67.60, 67.26, 65.92, 57.39, 52.60, 49.63, 47.17, 38.76, 37.17, 37.03, 29.82, 25.80, 25.04, 24.62, 18.01, 16.95, 16.30, 16.14, 11.51, -5.05.; **HRMS (ESI$^+$)** calcd. for $C_{60}H_{71}N_7O_{10}Si$ [M+H]$^+$ *m/z*: 1078.5104; found: 1078.5044.

***N*-(*N*-((2*S*,3*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-((*tert*-butyldimethylsilyl)oxy)-4-oxo-4-(tritylamino)butanoyl)-*O*-((2*S*,3*S*)-2-azido-3-methylpentanoyl)-L-threonyl)-*N*-methylglycine (3)**.



To a 100 mL round bottom flask were added **9** (800 mg, 0.742 mmol, 1.0 equiv) and $Pd(PPh_3)_4$ (168 mg, 0.148 mmol, 0.2 equiv). After the flask was charged with argon, anhydrous DCM (14 mL) and $PhSiH_3$ (0.224 mL, 1.85 mmol, 2.5 equiv) were added. The mixture was stirred at rt for 2 h. The reaction mixture was concentrated *in vacuo.* The residue was purified by flash column chromatography on silica gel (hexane/EtOAc/AcOH, 2:1:0.03) to give **3** (711 mg, 91 %) as off-white solid. NMR spectra of compound **3** showed rotational isomers. **$[\alpha]_D^{20}$**= -17.0 (c = 0.44, DCM); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.12 (s, 1H), 7.73 (d, *J* = 7.6 Hz, 3H), 7.68 – 7.53 (m, 8H), 7.47 (td, *J* = 7.7, 2.9 Hz, 3H), 7.37 (td, *J* = 7.4, 3.3 Hz, 3H), 7.32 – 7.21 (m, 18H), 7.16 (s, 1H), 7.09 (t, *J* = 8.4 Hz, 8H), 6.34 (d, *J* = 9.5 Hz, 1H), 5.40 – 5.33 (m, 1H), 5.20 (dd, *J* = 8.5, 4.4 Hz, 1H), 4.59 (dd, *J* = 9.5, 3.4 Hz, 1H), 4.42 (d, *J* = 3.5 Hz, 2H), 4.36 (dd, *J* = 10.3, 7.3 Hz, 2H), 4.25 (dd, *J* = 10.2, 7.9 Hz, 2H), 4.14 (ddd, *J* = 18.0, 14.5, 7.7 Hz, 5H), 3.75 (d, *J* = 5.8 Hz, 1H), 2.91 (s, 4H), 2.04 (s, 4H), 1.32 (d, *J* = 6.4 Hz, 4H), 1.26 (t, *J* = 7.1 Hz, 6H), 1.02 – 0.91 (m, 15H), 0.91 – 0.76 (m, 9H), 0.28 (s, 3H), 0.10 (s, 4H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 171.64, 169.85, 169.39, 168.67, 168.39, 156.27, 144.61, 144.12, 143.86, 141.33, 141.26, 132.31, 132.21, 128.86, 128.78, 128.66, 128.15, 127.72, 127.69, 127.37, 127.19, 125.61, 125.45, 119.95, 77.48, 77.16, 76.84, 73.20, 71.19, 70.69, 67.61, 66.95, 57.37, 52.47, 49.14, 47.13, 37.14, 36.78, 32.03, 29.80, 29.76, 29.46, 25.78, 24.86, 22.80, 17.97, 16.93, 16.07, 14.23, 11.37, -5.09.; **HRMS (ESI$^+$)** calcd. for $C_{57}H_{67}N_7O_{10}Si$ $[M+H]^+$ *m/z*: 1038.4791; found: 1038.4741.

A54145B (**1**):



The 2-chlorotrityl chloride (2-CTC) resin (47.4 mg, loading: 0.5mmol/g) was swollen in 2 mL dry DCM for 20 min in a 5 mL disposable vessel (TORIVQ) equipped with a porous polypropylene disc at the bottom. A solution of Fmoc-D-Lys(Boc)-OH (2.0 equiv) and DIEA (4.0 equiv) in DCM was added and the reaction vessel was shaken on the vortex at room temperature for 1 h. The resin was washed with DMF (5 × 3 mL) followed by a solution of DCM/MeOH/DIEA (17:2:1, v/v/v, 3 mL) for 20 min and washed with DMF (5 × 3 mL). The resin was subsequently submitted to iterative peptide assembly via the standard Fmoc-SPPS protocol. The Fmoc deprotection was carried out using 3 mL of 20% piperidine in DMF at room temperature for 20 min. The resin was then washed with DMF (5 × 3 mL). For standard coupling procedure, a solution of the Fmoc-amino acid or decanoic (4 equiv), HATU (4 equiv), DIEA (8 equiv) in DMF was added to the resin and gently agitated on a vortex at room temperature for 1 h. The resin was then washed with DMF (5 × 3 mL). After obtaining resin bound tripeptide **10**, a solution of **3** (2 equiv), HATU (2 equiv) and DIEA (4 equiv) in DMF was added to the resin and gently agitated on a vortex at room temperature for 2 h to generate **11**. Then standard Fmoc-SPPS procedure was followed to obtain **12**. The resin was washed with DMF (3 × 3 mL), DCM (2 × 3 mL) and THF (2 × 3 mL). Then a solution of TBAF/AcOH (1:1, m/m) in 2 mL THF (6 equiv) was added to the resin and gently agitated on vortex at room temperature for 1 h to yield **14**. The resin was then washed with THF (5 × 3 mL) and DMF (5 × 3 mL). The resin was washed with DCM (3 × 3 mL), and dry in vacuum for 30 min. The plastic syringe with resin in it was charged with argon. A solution of tin(II) chloride (10 equiv), thiophenol (40 equiv) and triethylamine (30 equiv) in dry DMF with a total concentration of 0.1 M tin(II) chloride were added to the resin to produce **16**. The resin was shaken at rt for 1 h. Afterwards, the resin washed with DMF (3 × 3 mL), DCM (2 × 3 mL) and DMF (2 × 3 mL). A solution of Fmoc-3*R*-MeGlu(OtBu)-OH[1] (2 equiv), HATU (2 equiv) and DIEA (4 equiv) in DMF was then added to the resin and agitated on vortex for 2 h. Afterwards, standard Fmoc-SPPS conditions were employed to produce **2**. The resin was washed with DCM (5 × 3 mL) and was subjected to 5 mL of mild acidic cleavage cocktail of DCM/AcOH/trifluoroethanol (8/1/1, v/v/v) for 1 h and this step was repeated for another 30 min. Following filtration, the resulting cleavage solutions were combined and concentrated *in vacuo* to give the crude side-chain protected peptide **17**.

For the macrolactamization, to a solution of the crude peptide **17** (1 equiv) in DCM: DMF=3:1 (5 mM) was added PyBOP (5 equiv), Oxyma Pure (5 equiv) and DIEA (2 equiv). The solution was stirred at room temperature for 16 h. The solvent was removed in *vacuo* afterwards. Then, a solution of 10 mL cleavage cocktail (TFA: TIPS: H_2O= 95: 2.5: 2.5, v/v/v) was added and the solution was stirred at room temperature for 2 h. The solution was concentrated under a stream of nitrogen and the crude product was precipitated by cold diethyl ether. Diethyl ether was discarded after centrifugation and the crude product was washed with diethyl ether for 3 times. The washed crude product was dried under vacuum to remove residual diethyl ether and the white solid was purified by RP-HPLC with a gradient of 30-60% Solvent A over 35 mins to give A54145B (7.5 mg, 19%) as a white solid.

LC-MS (ESI$^+$): calcd. for $C_{73}H_{111}N_{17}O_{27}$ $[M+H]^+$ *m/z:*1659.77; found: 1659.99, $[M+2H]^{2+}$ *m/z:* 830.39; found: 830.23. **$[\alpha]_D^{20}$**= -6.0 (c = 0.20, H_2O). **^{1}H NMR** (500 MHz, D_2O) δ 7.54 (d, *J* = 8.0 Hz, 1H), 7.39 (d, *J* = 7.9 Hz, 1H), 7.18 – 7.09 (m, 2H), 7.05 (t, *J* = 7.4 Hz, 1H), 5.34 (t, *J* = 5.6 Hz, 1H), 5.08 (d, *J* = 4.5 Hz, 1H), 4.89 (d, *J* = 3.1 Hz, 1H), 4.18 – 4.11 (m, 2H), 4.09 – 3.95 (m, 2H), 3.93 – 3.75 (m, 2H), 3.30 – 3.23 (m, 2H), 3.23 – 3.15 (br, 1H), 3.15 – 3.03 (m, 3H), 2.94 – 2.84 (m, 2H), 2.84 (s, 1H), 2.73 (dd, *J* = 15.1, 6.5 Hz, 1H), 2.65 (dd, *J* = 15.0, 7.9 Hz, 1H), 2.57 (d, *J* = 6.9 Hz, 2H), 2.42 – 2.29 (br, 1H), 2.21 – 2.06 (m, 3H), 1.97 (dd, *J* = 14.3, 9.6 Hz, 1H), 1.83 – 1.67 (m, 5H), 1.67 – 1.49 (m, 5H), 1.39 – 1.22 (m, 9H), 1.18 (t, *J* = 6.7 Hz, 4H), 1.16 – 1.10 (m, 7H), 1.10 – 1.01 (m, 6H), 0.95 (d, *J* = 9.2 Hz, 3H), 0.84 (d, *J* = 6.7 Hz, 3H), 0.80 – 0.69 (m, 9H).

^{1}H and ^{13}C NMR Spectra

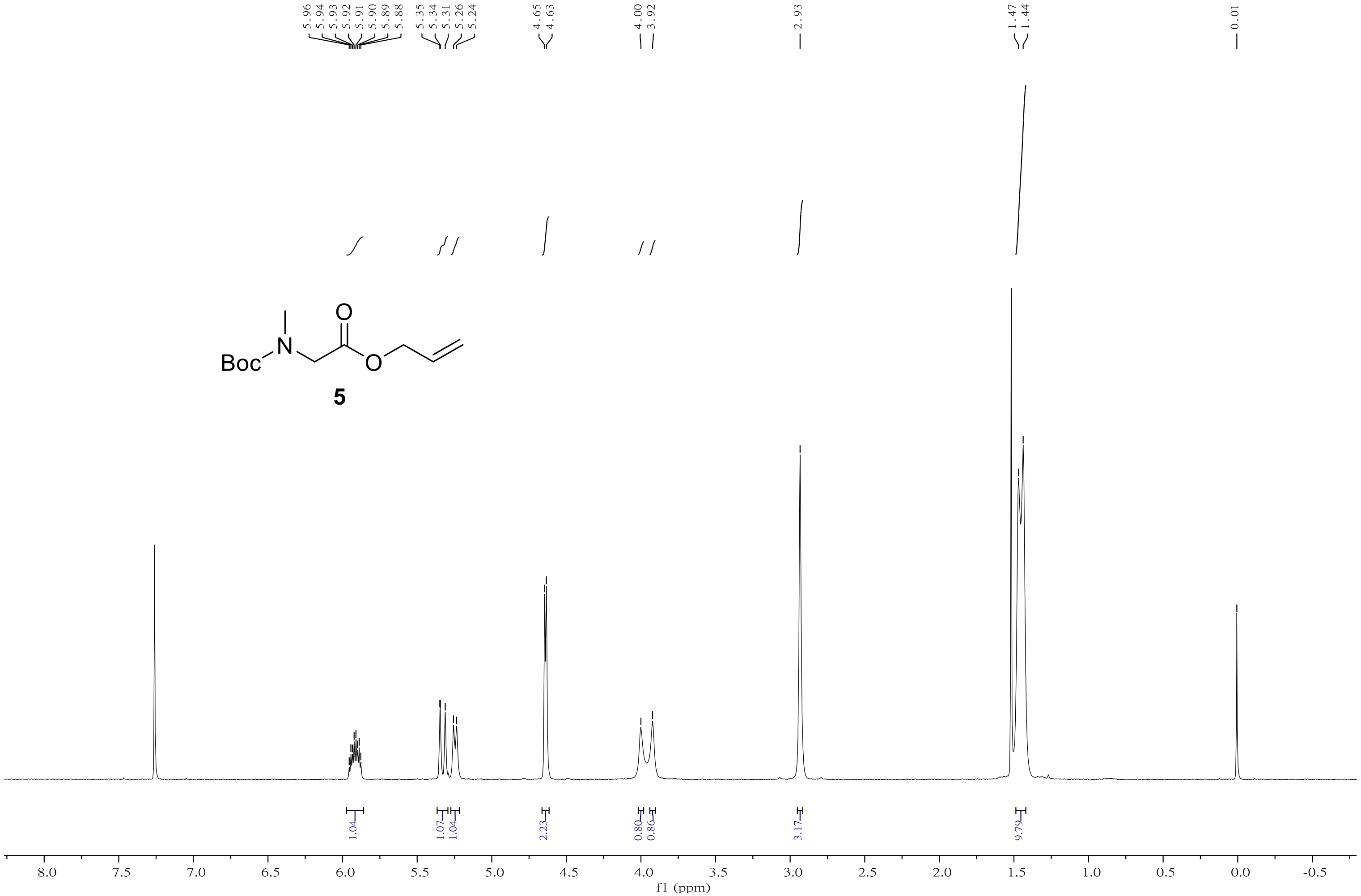


Figure S1. ^{1}H NMR spectrum of compound **5**.

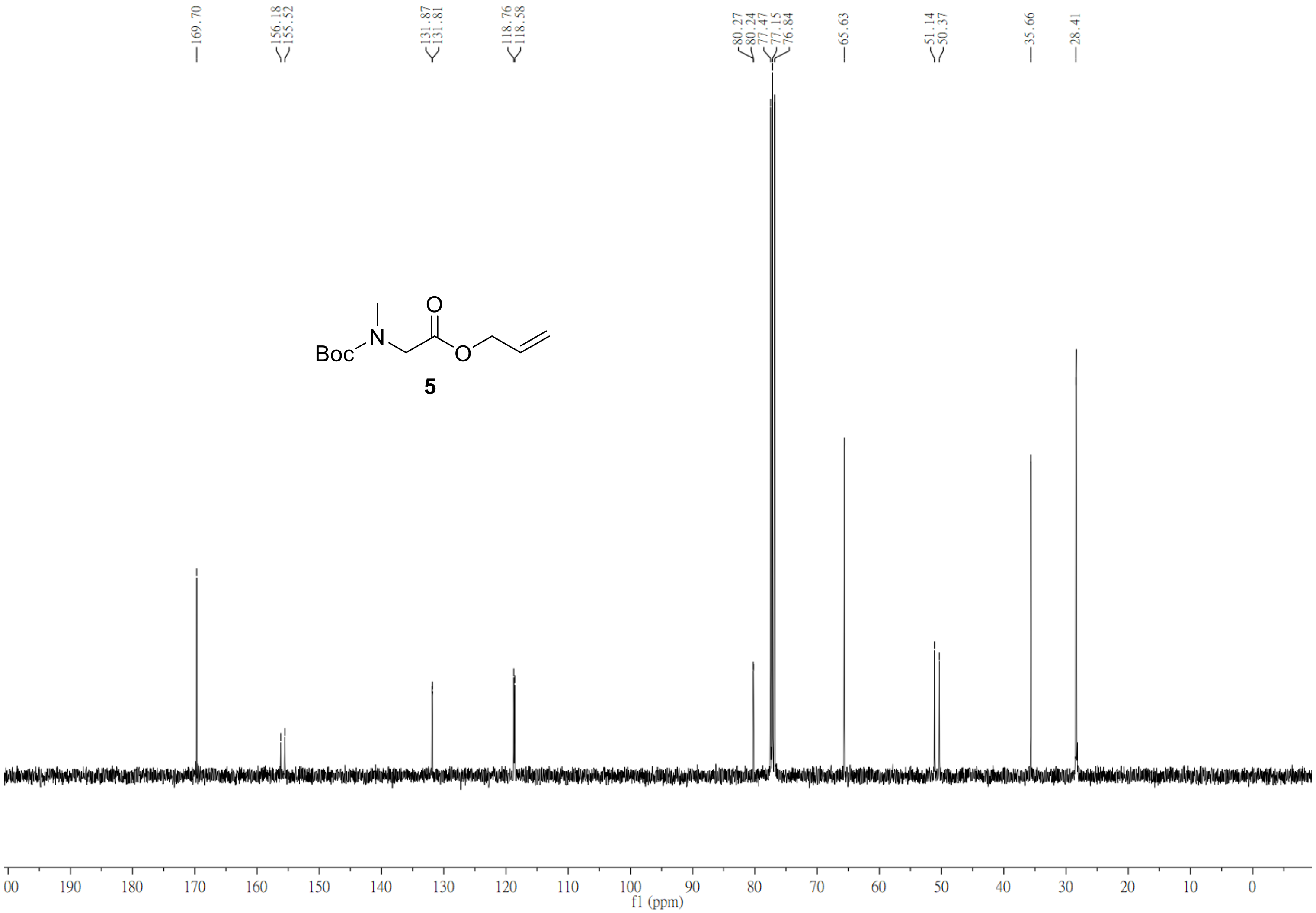


Figure S2. ^{13}C NMR spectrum of compound **5.**

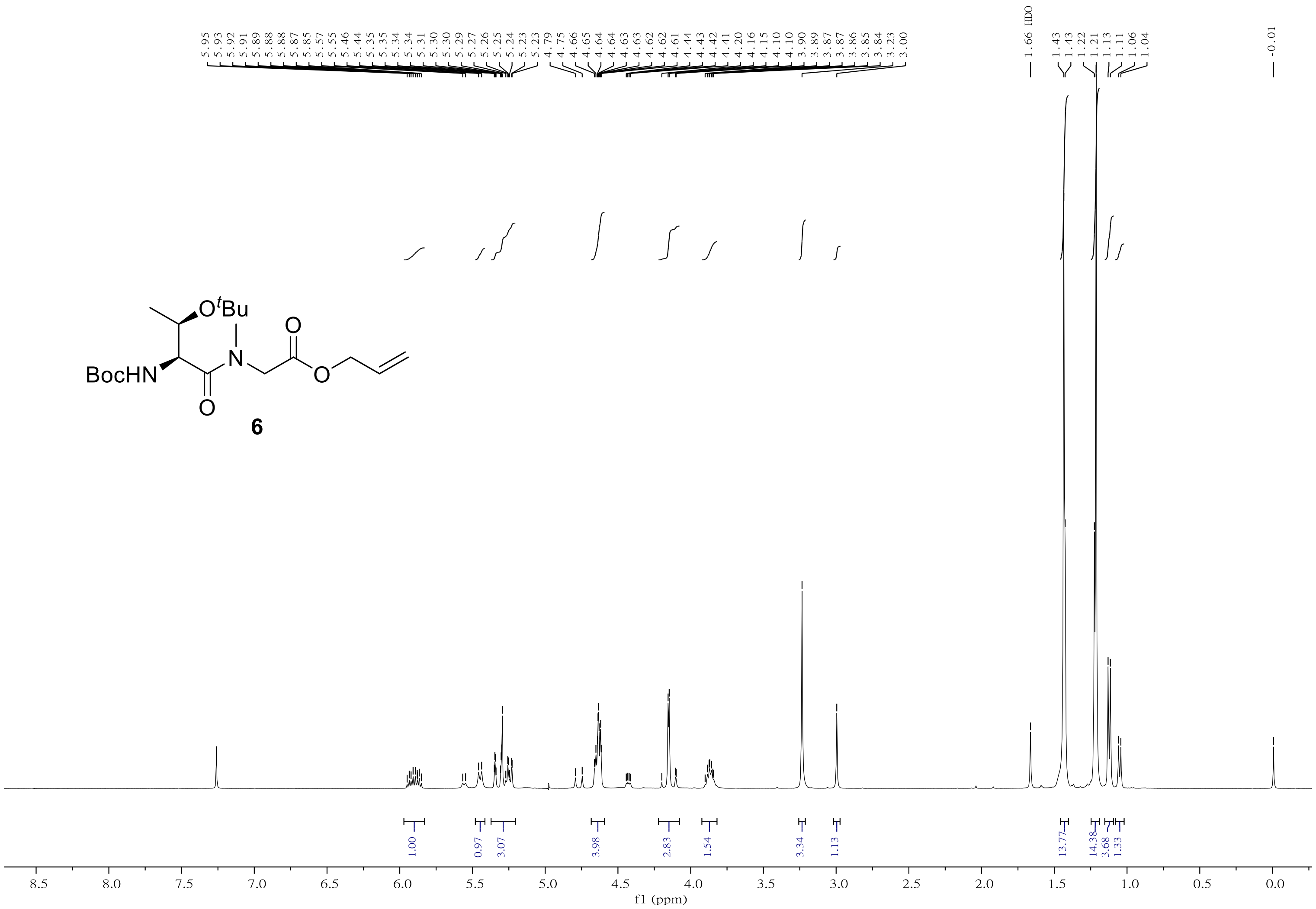


Figure S3. ^{1}H NMR spectrum of compound **6.**

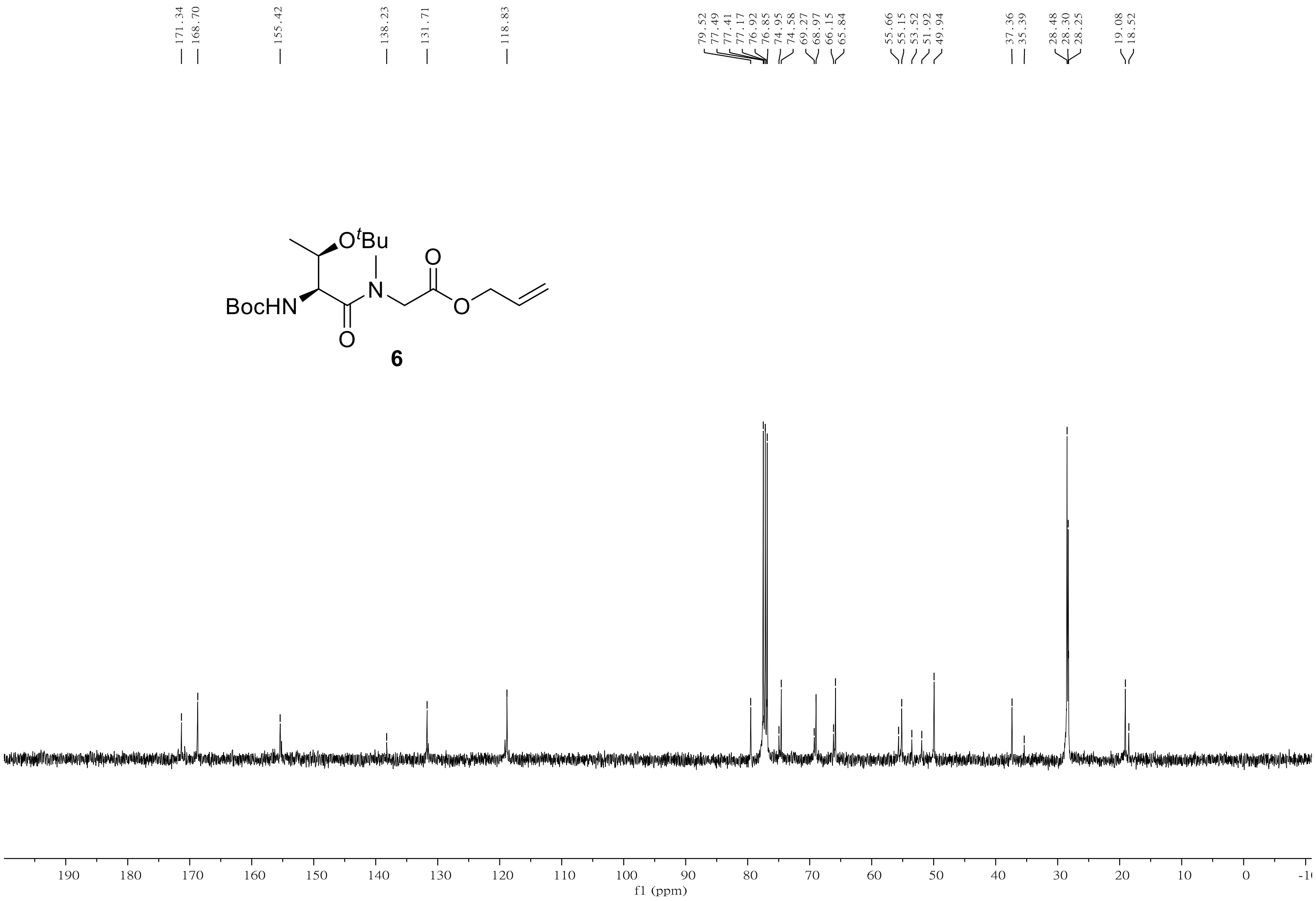


Figure S4. ^{13}C NMR spectrum of compound **6.**

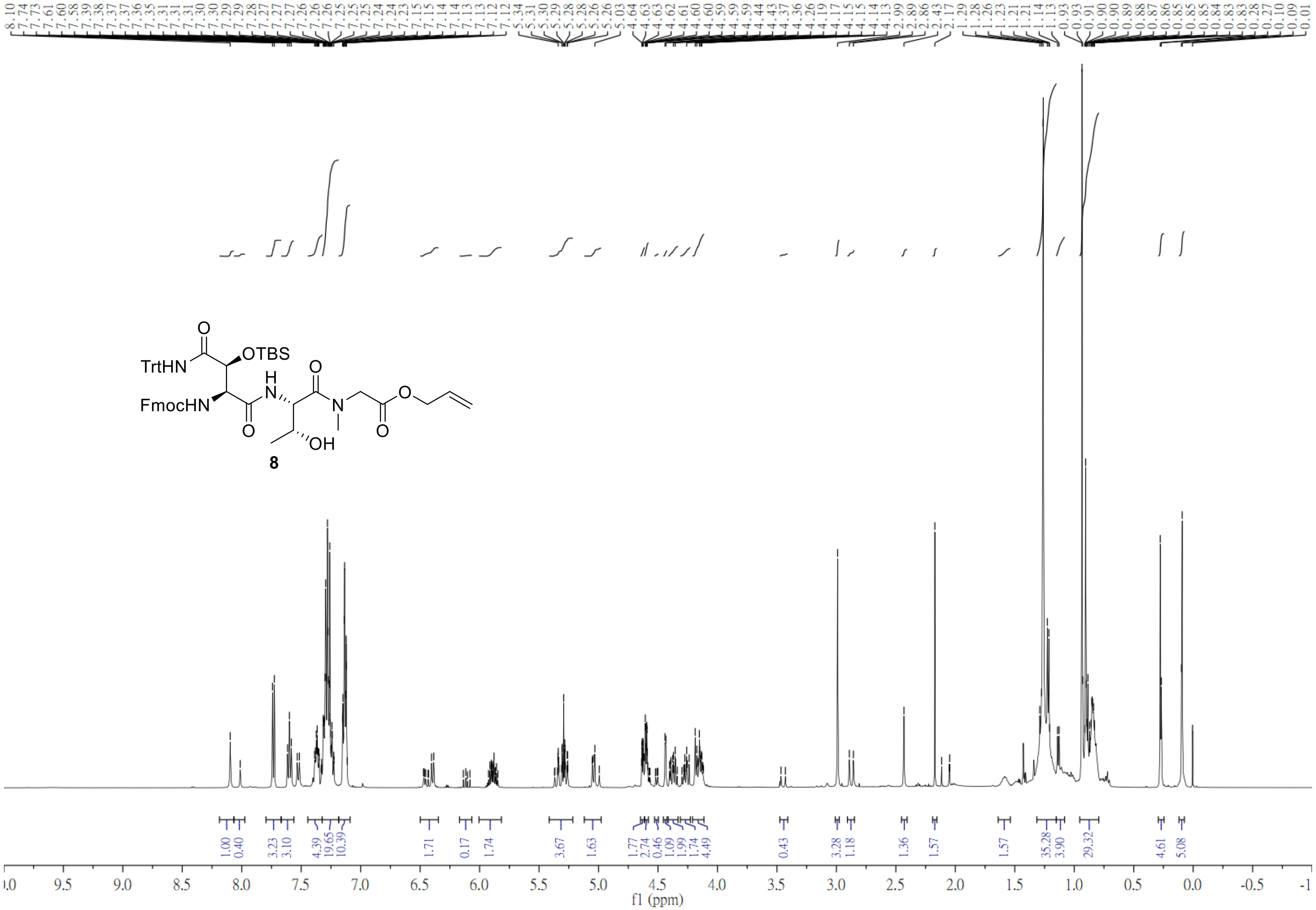


Figure S5. ^{1}H NMR spectrum of compound **8.**

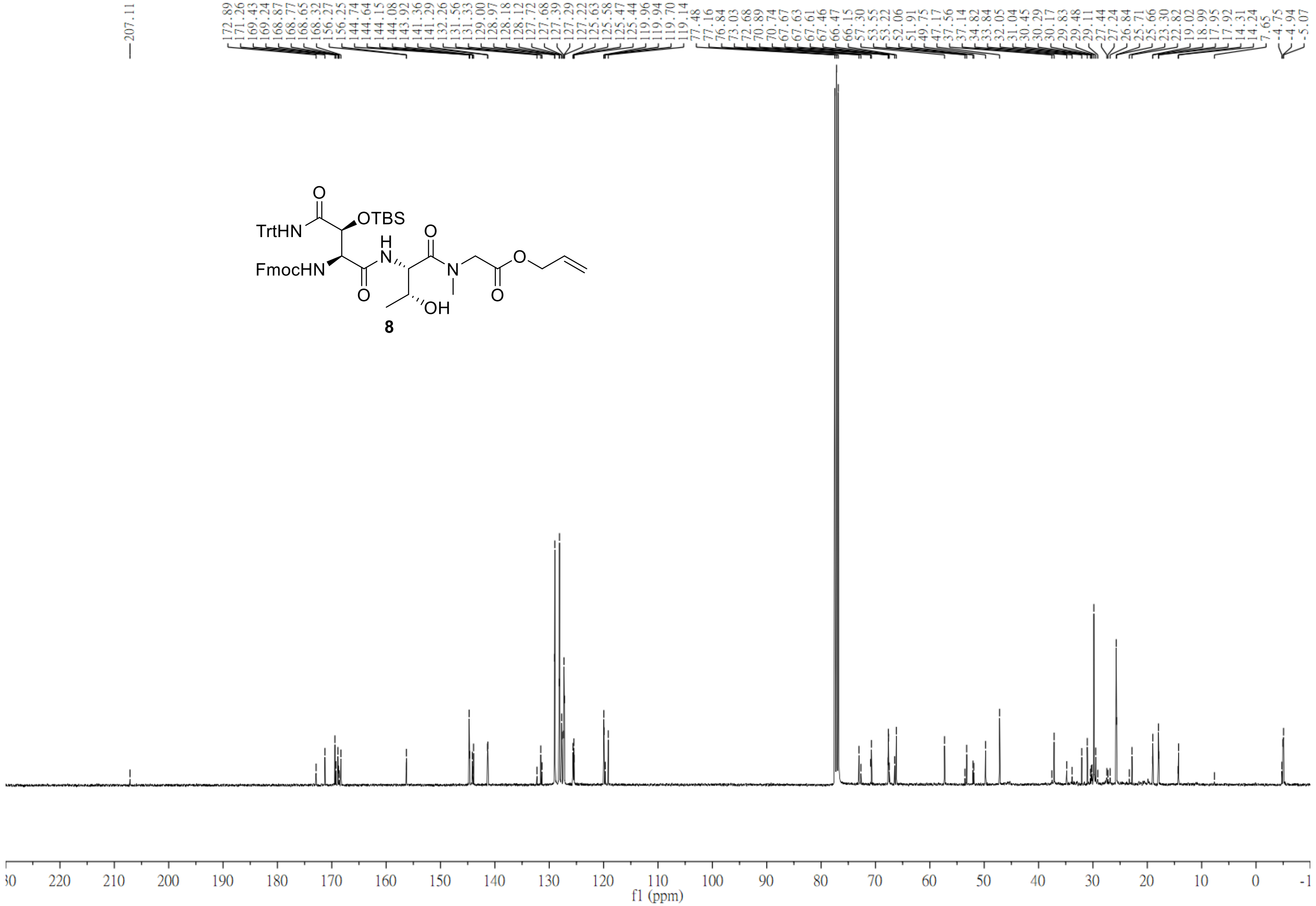


Figure S6. ^{13}C NMR spectrum of compound **8.**

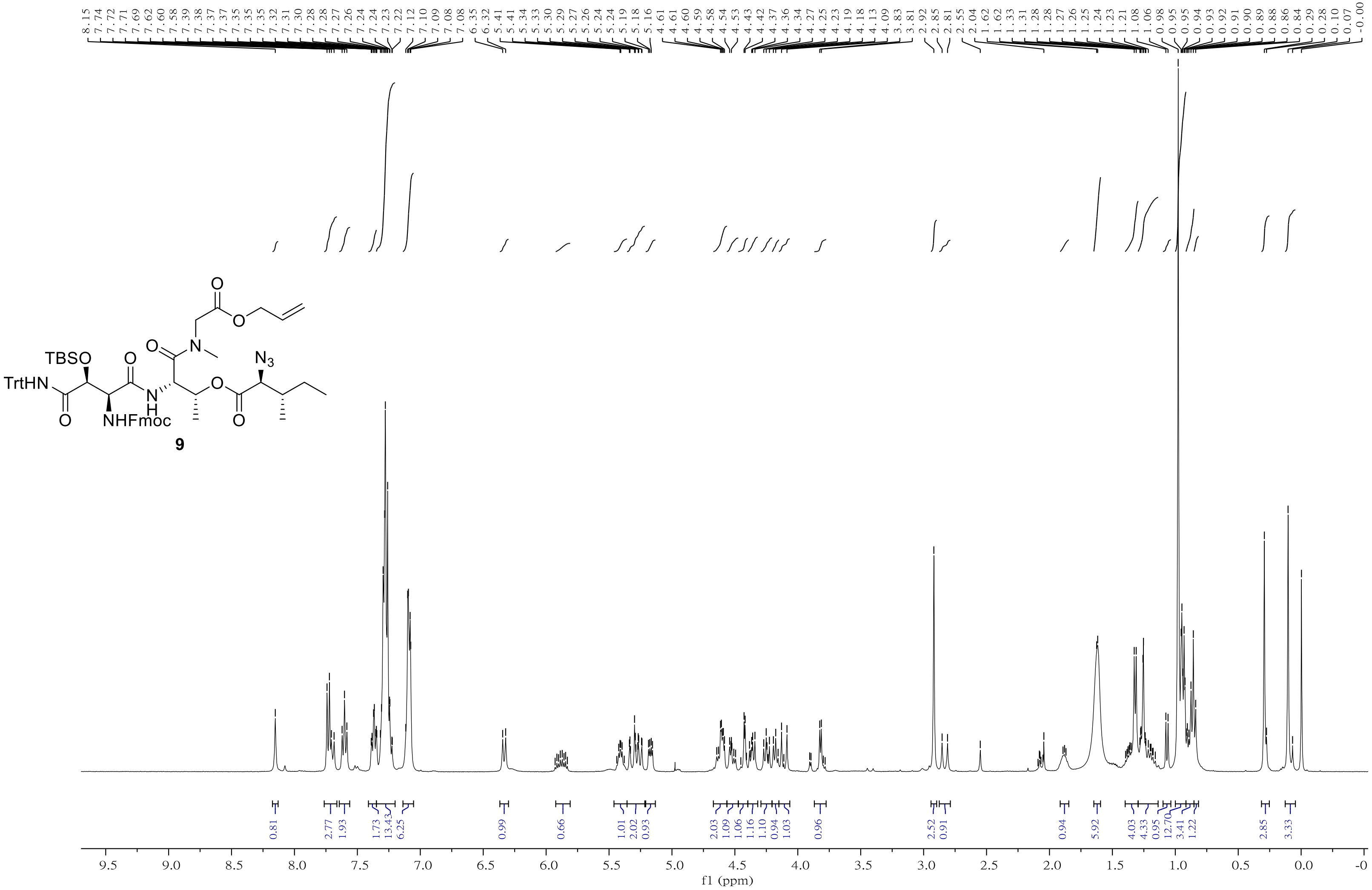


Figure S7. ^{1}H NMR spectrum of compound **9.**

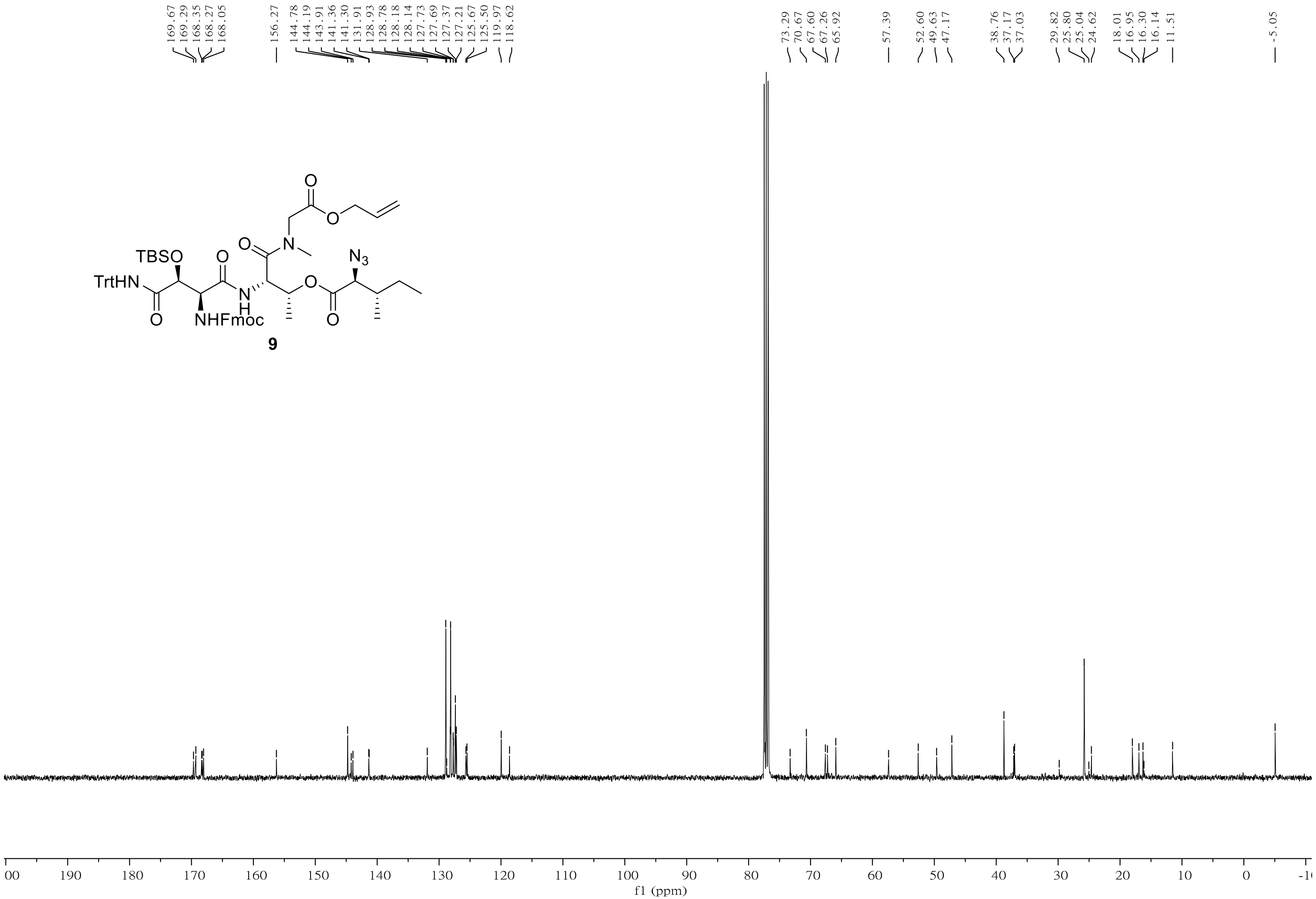
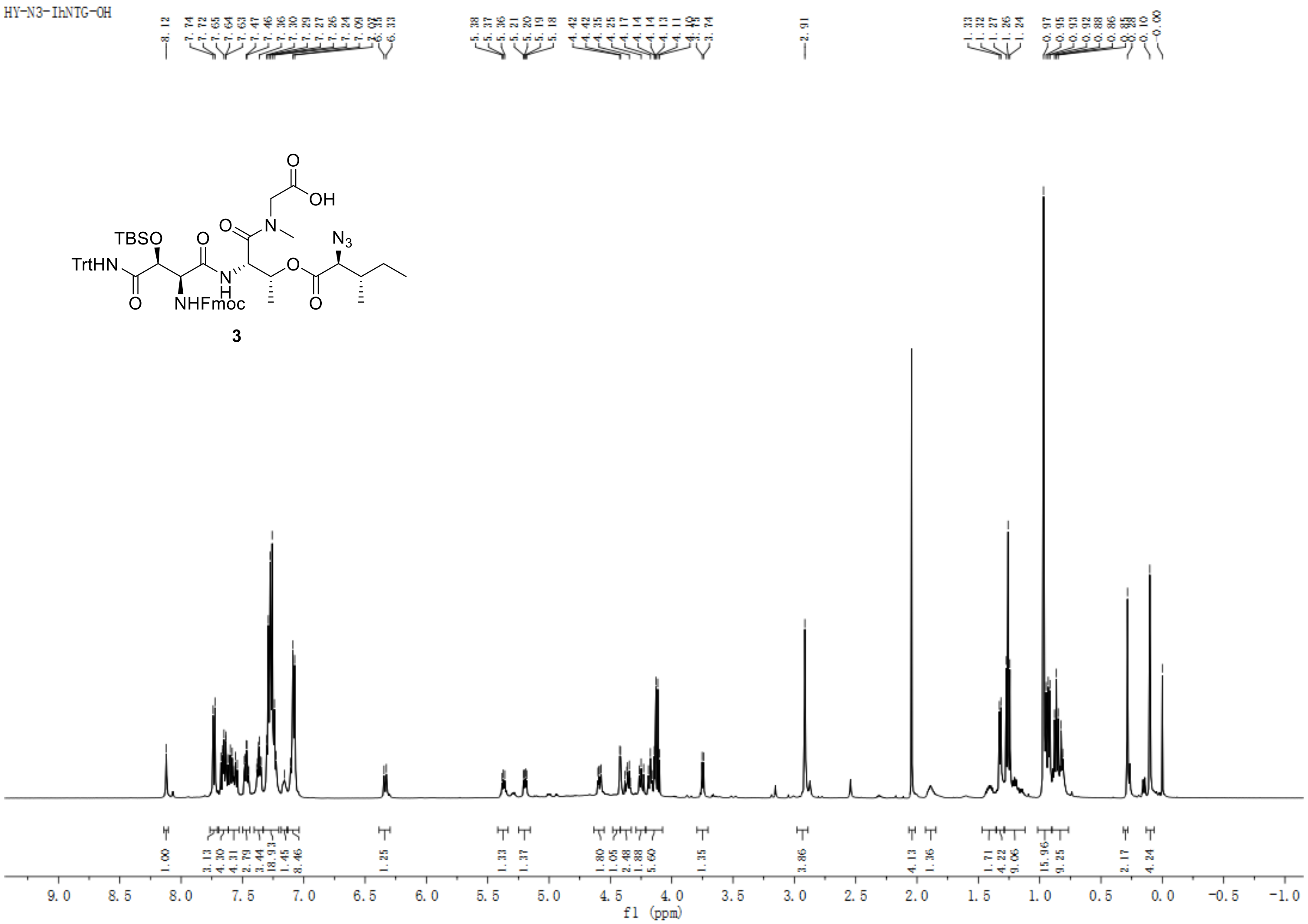


Figure S8. ^{13}C NMR spectrum of compound **9.**



3

Figure S9. ^{1}H NMR spectrum of compound **3.**

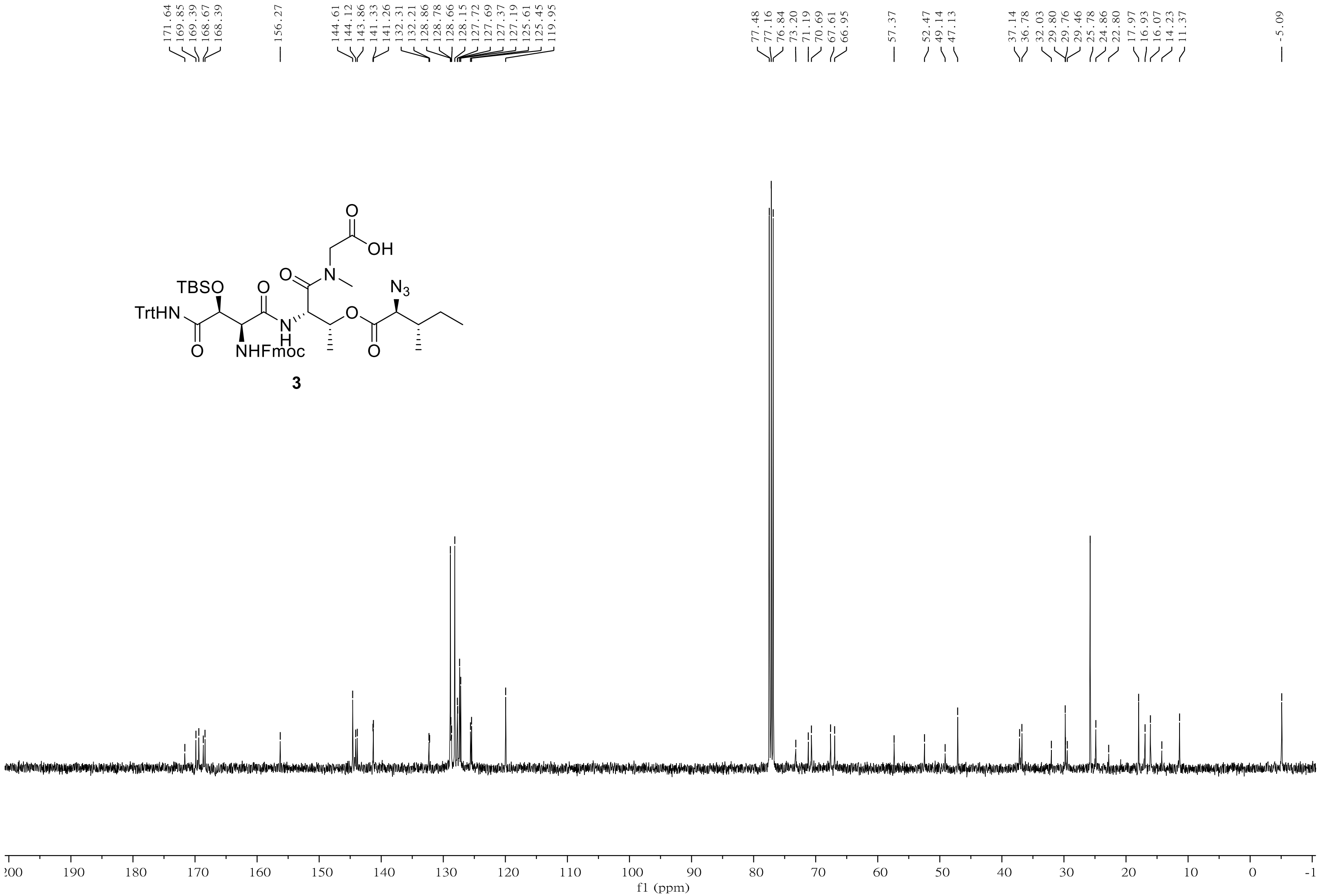


Figure S10. ^{13}C NMR spectrum of compound **3.**

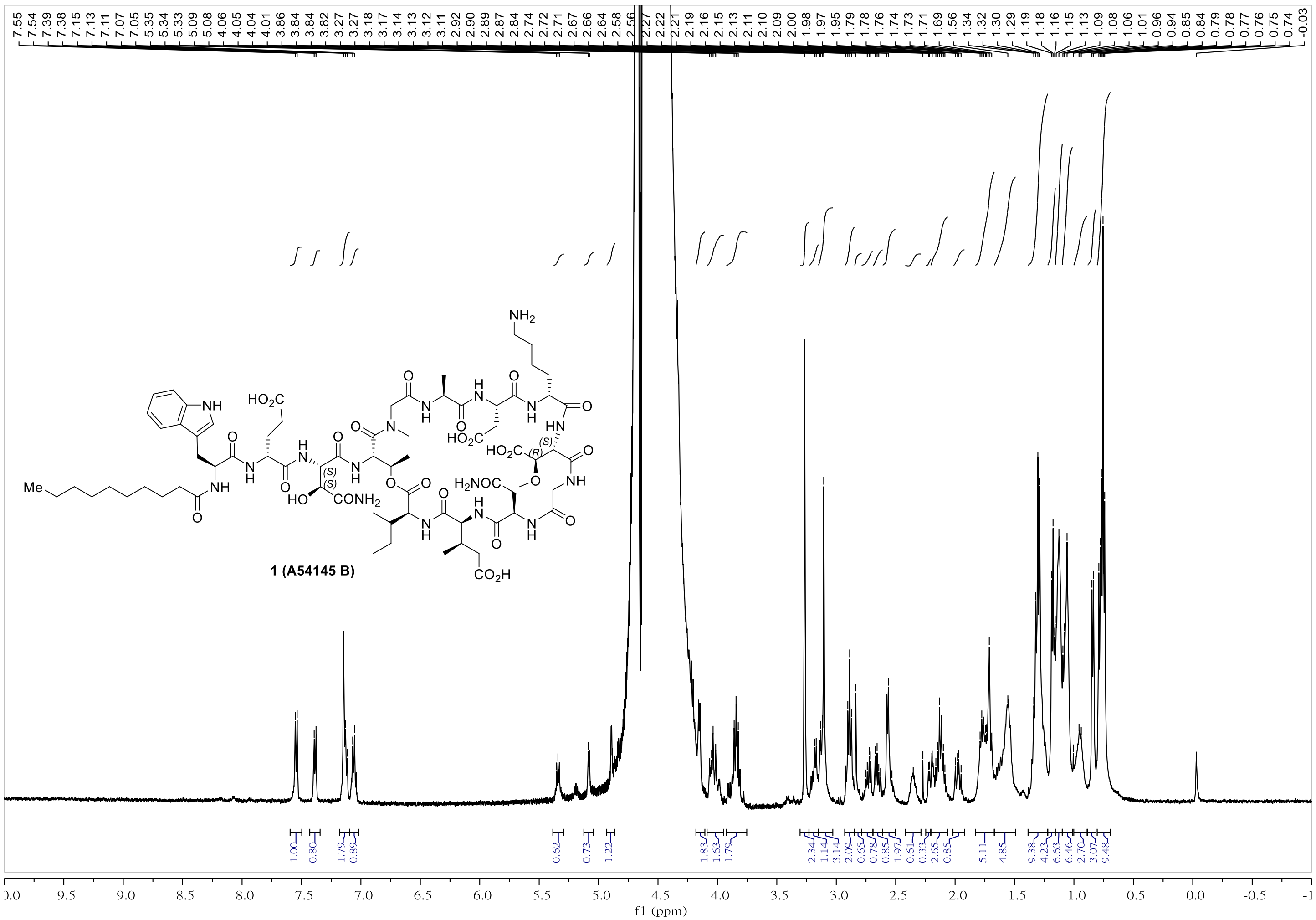


Figure S11. ^{1}H NMR spectrum of compound **1** (A54145B) dissolved in PBS buffer pH 7.4 in D_2O with a concentration ~1mg/mL. The NMR spectrum was consistent with the previous published spectrum using our original synthetic route.[1]

LC-MS traces

A54145B (Compound **1,** with D-Lys)

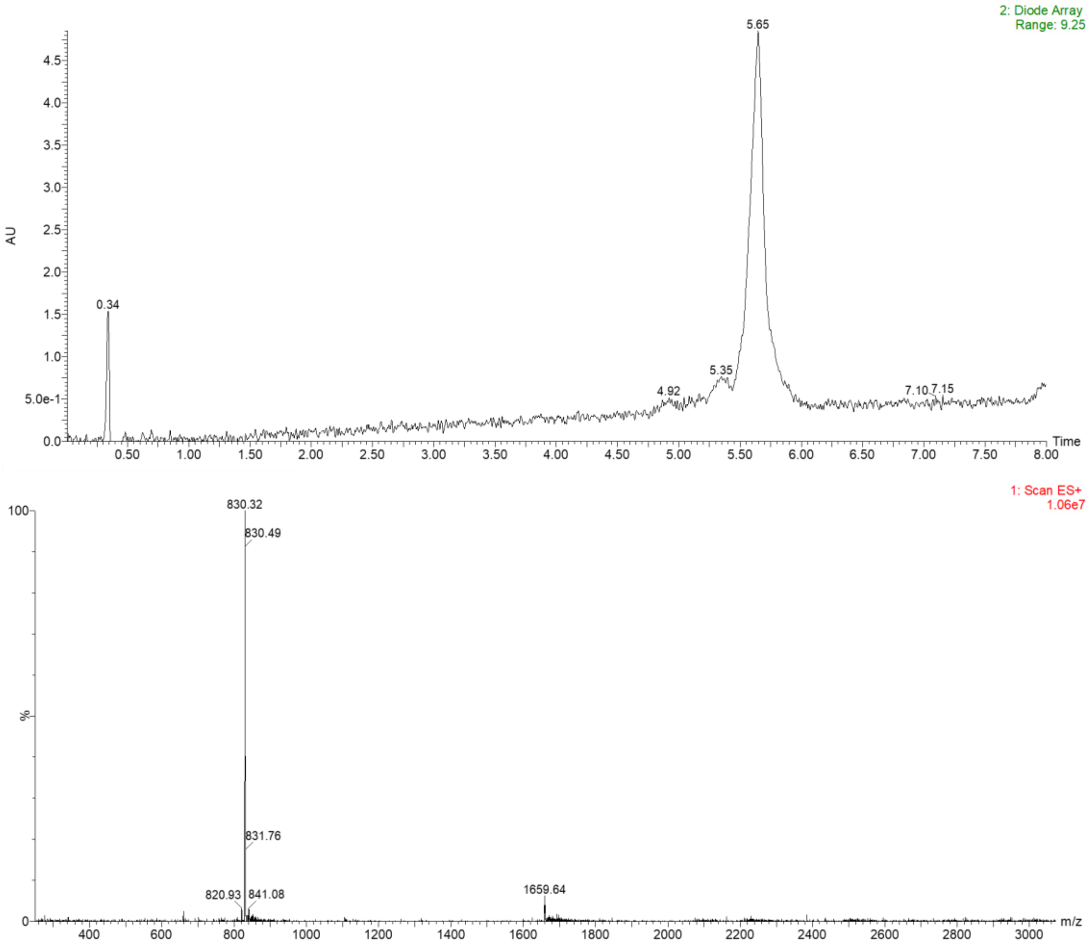


Figure S12. Analytical UPLC analysis UV trace and ESI-MS of purified **1**. Gradient: 30–45 % solvent A/solvent B over 8 min at a flow rate of 0.4 mL/min. MS (ESI$^+$) calcd. for $C_{73}H_{111}N_{17}O_{27}$ $[M+H]^+$ *m/z:* 1659.77; found: 1659.64; $[M+2H]^{2+}$ *m/z:* 830.39; found: 830.32.

A54145B epimer (with L-Lys)

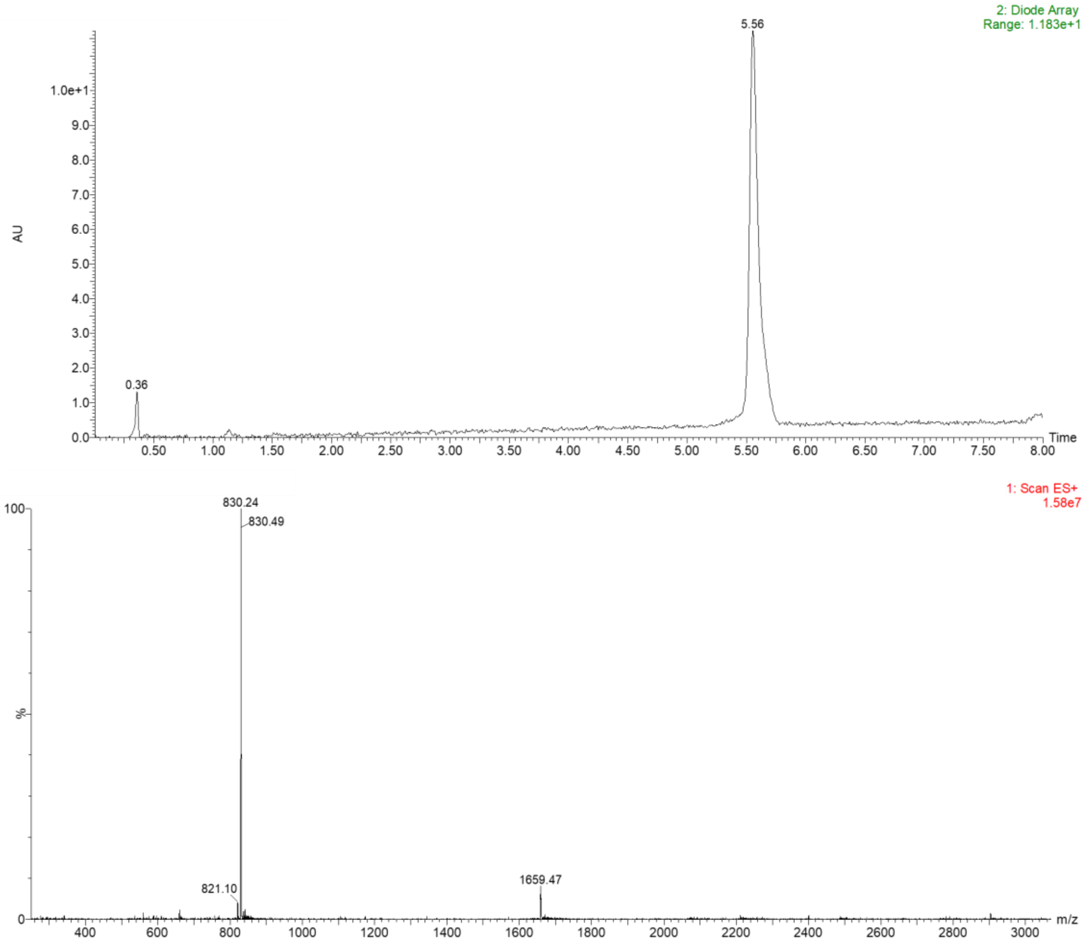


Figure S13. Analytical UPLC analysis UV trace and ESI-MS of purified A54145B epimer. Gradient: 30–45 % solvent A/solvent B over 8 min at a flow rate of 0.4 mL/min. MS (ESI$^+$) calcd. for $C_{73}H_{111}N_{17}O_{27}$ $[M+H]^+$ *m/z:* 1659.77; found: 1659. 47; $[M+2H]^{2+}$ *m/z:* 830.39; found: 830.24

References

1. D. Chen, H. Y. Chow, K. H. L. Po, W. Ma, E. L. Y. Leung, Z. Sun, M. Liu, S. Chen and X. Li, *Org. Lett.*, 2019, **21**, 5639-5644.
2. J. T. Lundquist IV and J. C. Pelletier, *Org. Lett.*, 2001, **3**, 781-783.