Supporting Information

Systematic Chemical Mutagenesis Identifies a Potent Novel Apratoxin A/E Hybrid with Improved In Vivo Antitumor Activity

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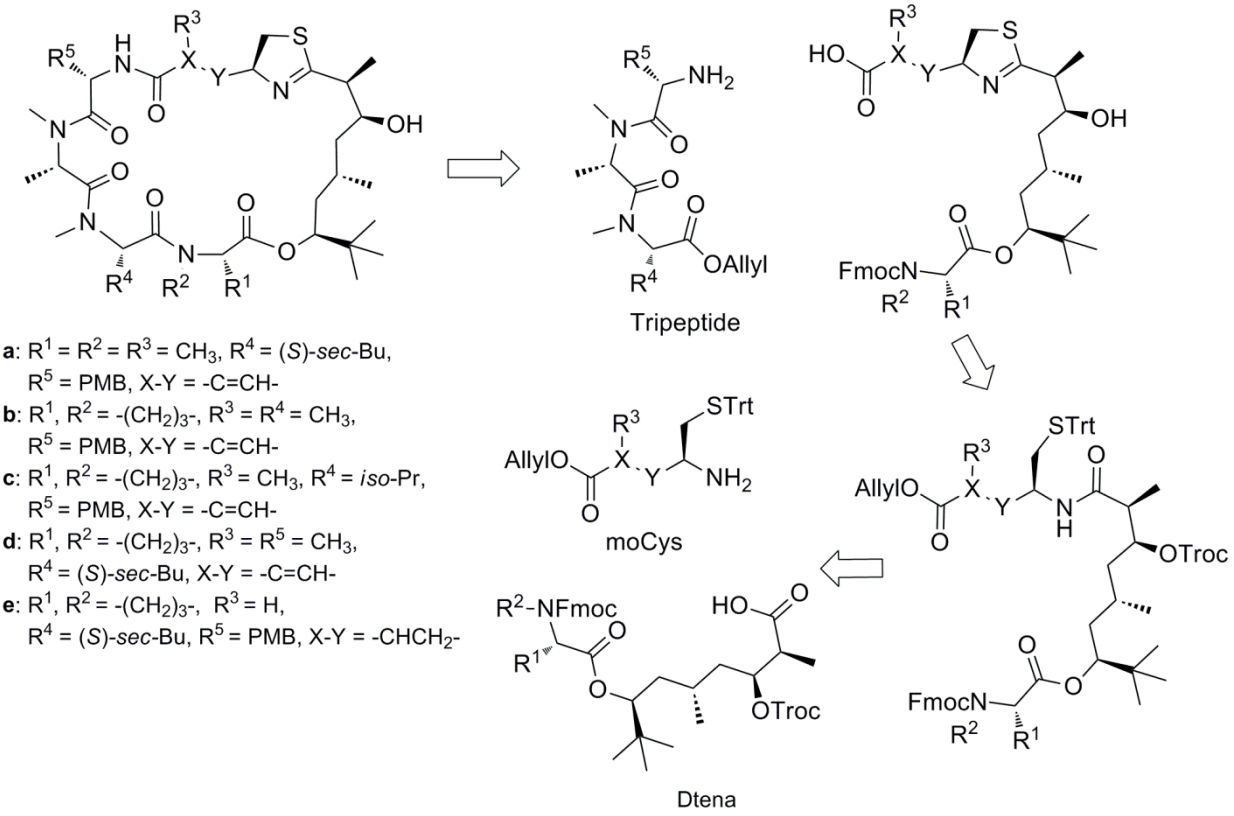
General Chemistry Experimental Procedures

Solvents were purified according to the guidelines in *Purification of Laboratory Chemicals*.[1] Tetrahydrofuran (THF) and diethyl ether were distilled from sodium chips in the presence of small amount of benzophenone; CH_2Cl_2 and toluene were distilled from CaH_2; MeCN, *N*,*N*-dimethylformamide (DMF) were dried with 4Å molecular sieves (MS) and MeOH dried with 3Å MS; 4 M Hydrochloric acid (HCl) solution in ethyl acetate was prepared by dissolving HCl gas (liberated by dropping aqueous hydrochloric acid (34%) to concentrated sulfuric acid (98%)) in ethyl acetate. Roush's crotylborate was prepared according to published procedures.[2] All other reagents were purchased from Aldrich-Sigma company and used without further purification. Thin layer chromatography was performed on EMD silica gel 60Å F_{254} glass plates and preparative thin layer chromatography was performed on Whatman silica gel 60Å F_{254} glass plates (layer thick 1000 μm). Flash column chromatography was performed with Fisher 170-400 mesh silica gel. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian Mercury

400 MHz, Bruker Avance 500 MHz or Bruker Avance 600 MHz spectrometer as indicated in the data list. Chemical shifts for proton nuclear magnetic resonance (^{1}H NMR) spectra are reported in parts per million relative to the signal residual $CDCl_3$ at 7.26 ppm; Chemicals shifts for carbon nuclear magnetic resonance (^{13}C NMR) spectra are reported in parts per million relative to the center line of the $CDCl_3$ triplet at 77.16 ppm; The abbreviations s, d, dd, ddd, dddd, t, q, br and m stand for the resonance multiplicity singlet, doublet, doublet of doublets, doublet of doublet of doublets, doublet of doublet of doublet of doublets, triplet, quartet, broad and multiplet, respectively. Optical rotation was measured on a Perkin-Elmer 341 polarimeter. High resolution mass spectra (HRMS) data were obtained using an Agilent-LC-TOF mass spectrometer with an APCI/ESI multimode ion source detector.

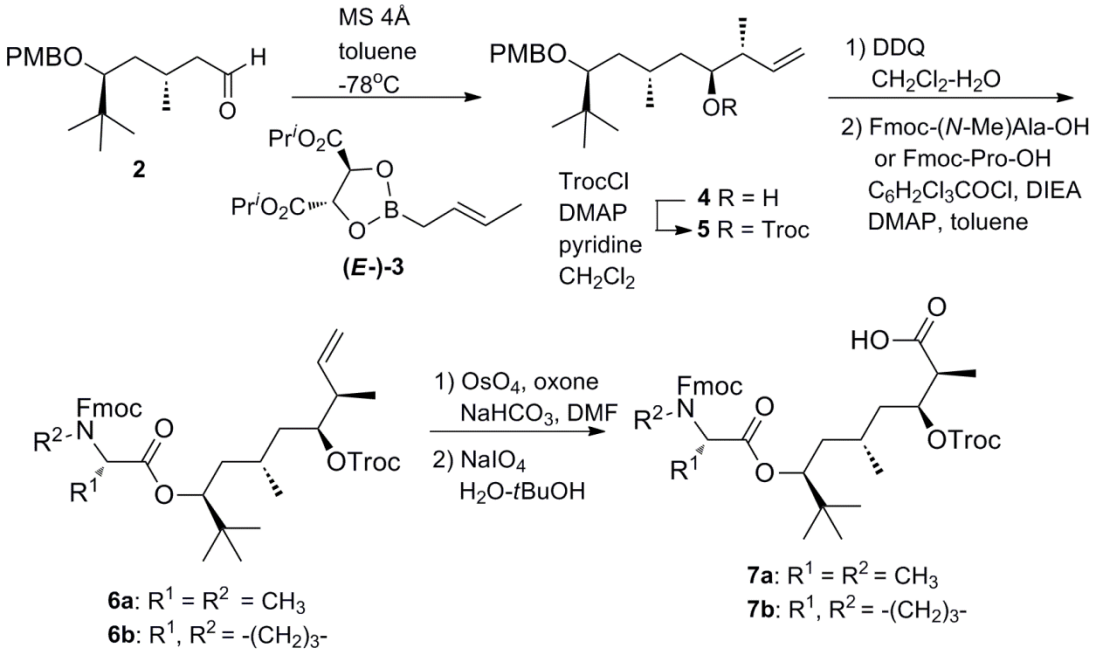
Retrosynthetic Analysis of Analogues 1a-1e of Apratoxin A.[3]



1a: $R^1 = R^2 = R^3 = CH_3$, R^4 = (*S*)-*sec*-Bu, R^5 = PMB, X-Y = -C=CH-
1b: R^1, R^2 = $-(CH_2)_3-$, $R^3 = R^4 = CH_3$, R^5 = PMB, X-Y = -C=CH-
1c: R^1, R^2 = $-(CH_2)_3-$, $R^3 = CH_3$, R^4 = *iso*-Pr, R^5 = PMB, X-Y = -C=CH-
1d: R^1, R^2 = $-(CH_2)_3-$, $R^3 = R^5 = CH_3$, R^4 = (*S*)-*sec*-Bu, X-Y = -C=CH-
1e: R^1, R^2 = $-(CH_2)_3-$, R^3 = H, R^4 = (*S*)-*sec*-Bu, R^5 = PMB, X-Y = $-CHCH_2-$

Scheme S1. Retrosynthetic analysis of analogues **1a**-**1e** of apratoxin A.

Synthesis of Amino Acid Coupled Dihydroxylated Carboxylic Acid Moieties 7a-7b (Dtena)



Scheme S2. Synthesis of *3,7*-dihydroxy-*2,5-8,8*-tetramethylnonanoic acid (Dtena) moiety **7a-7b**.

Aldehyde **2**[3a] and crotylborate (*E*-)-**3**[2] were prepared according to published procedures. NMR spectra of prepared products were identical to those published.[2,3a]

(3*R*,4*S*,6*S*,8*S*)-8-(4-methoxybenzyloxy)-3,6,9,9-tetramethyldec-1-en-4-ol (**4**).[3b] 4Å molecular sieves (140 mg) were dried under 300°C in vacuo for 15 min and then cooled down to room temperature. To a suspended solution of the above dried 4Å molecular sieves and crotylborate (*E*-)-**3** (1M in toluene, 2.2 ml, 2.190 mmol) in toluene (4.0 ml) was added aldehyde **2** (256 mg, 0.876 mmol) dropwise in toluene (1.0 ml) over 15 min at -78 °C. After being stirred at the same temperature for 6 h, this reaction was quenched with aqueous NaOH (2 M, 4.0 ml), stirred at 0°C for 30 min, filtered through a pad of celite and extracted with diethyl ether (10 ml × 4). The combined organic layer was washed with aqueous HCl (1M), saturated $NaHCO_3$, brine, dried with $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography on silica gel (4.5% ethyl acetate in hexane) to give product **4** (275 mg, 90%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.31(d, J = 8.4 Hz, 2H), 6.86 (d, J = 8.4 Hz, 2H), 5.80-5.71(m, 1H), 5.15-5.11(m, 2H), 4.64 (d, J = 10.8 Hz, 1H), 4.51 (d, J = 10.8 Hz, 1H), 3.79 (s, 3H), 3.50 (m, 1H), 3.11 (dd, J = 9.3, 2.9 Hz), 2.17 (m ,1H), 1.98 (br m, 1H), 1.58 (ddd, J = 13.2, 10.5, 2.7 Hz, 1H), 1.48 (ddd, J =

14.1, 8.9, 3.8 Hz, 1H), 1.36 (ddd, *J* = 14.1, 9.4, 2.5 Hz, 1H), 1.13 (ddd, *J* = 13.7, 9.2, 2.5 Hz, 1H), 1.04 (d, *J* = 6.8 Hz, 3H), 0.97 (d, *J* = 6.8 Hz, 3H), 0.94 (s, 9H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.0, 140.8, 131.7, 129.3, 116.4, 113.8, 85.3, 74.3, 72.4, 55.4, 45.4, 40.9, 39.9, 36.2, 26.7, 26.7, 21.1, 16.3 ppm.

(3*R*,4*S*,6*S*,8*S*)-8-(4-methoxybenzyloxy)-3,6,9,9-tetramethyldec-1-en-4-yl 2,2,2-trichloroethyl carbonate (**5**).[3b] To the solution of **4** (163.3 mg, 0.469 mmol) and pyridine (114 µl, 1.407 mmol) in CH_2Cl_2 (2.0 ml) was added *2*,*2*,*2*-trichloroethoxylcarbonyl chloride (Troc-Cl) (119.2 mg/77.5 µl, 0.563 mmol) and 4-dimethylaminopyridine (DMAP)(2.86 mg, 23.4 µmol) at 0^{o}C. After being stirred at the same temperature for 1 h, the reaction was quenched with aqueous HCl (1 M, 3 ml). The water layer was extracted with ethyl acetate (5 ml × 4). The combined organic layer was washed with saturated $NaHCO_3$, brine, dried with $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography on silica gel (4.5% ethyl acetate in hexane) to give **5** (256 mg, 100%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.29 (d, *J* = 8.8 Hz, 2H), 6.86 (d, *J* = 8.4 Hz, 2H), 5.80-5.71 (m, 1H), 5.14-5.07 (m, 2H), 4.91-4.86 (m, 2H), 4.78 (d, *J* = 11.6 Hz, 1H), 4.58 (d, *J* = 10.8 Hz, 1H), 4.51 (d, *J* = 10.4 Hz, 1H), 4.50 (d, *J* = 12 Hz, 1H), 3.79 (s, 3H), 3.07 (dd, *J* = 9.4, 2.4 Hz, 1H), 2.51-2.46 (m, 1H), 1.91 (ddd, *J* = 14.2, 11.3, 2.4 Hz, 1H), 1.79 (br m, 1H), 1.48 (ddd, *J* = 14.3, 9.4, 3.9 Hz, 1H), 1.36 (ddd, *J* = 14.2, 9.7, 2.5, 1H), 1.18 (ddd, *J* = 14.3, 9.4, 2.1 Hz, 1H), 1.08 (d, *J* = 6.8 Hz, 3H), 1.00 (d, *J* = 6.8 Hz, 3H), 0.93 (s, 9H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.0, 154.3, 139.0, 131.6, 129.0, 116.5, 113.8, 94.8, 85.2, 80.8, 77.4, 76.6, 74.6, 55.4, 42.8, 39.8, 37.7, 36.2, 26.6, 26.4, 21.0, 15.8 ppm.

Synthesis of *N*-Fmoc amino acid ester derivatives 6a-6b. To a solution of **5** (255.5 mg, 0.49 mmol) in the mixture of CH_2Cl_2 (3.0 ml) and H_2O (0.3 ml) was added *2*,*3*-dichloro-*5*,*6*-dibenzoquinone (DDQ) (133.3 mg, 0.59 mmol) at 0°C. The reaction mixture was stirred at the same temperature for 1h, quenched with saturated aqueous $NaHCO_3$, and filtered in vacuo. The organic layer was separated and water layer was extracted with CH_2Cl_2 (15 ml × 3). The organic phase was combined and washed with brine (15 ml), dried with anhydrous $MgSO_4$, filtered and concentrated in vacuo. This residue was used for the next reaction without further purification.

To the suspended solution of Fmoc-(*N*-Me)Ala-OH or Fmoc-Pro-OH (0.99 mmol) in toluene (3.0 ml) was added *N*,*N*-diisopropylethylamine (DIEA) (0.26 ml, 1.49 mmol), *2*,*4*,*6*-trichlorobenzoyl chloride (0.23 ml, 1.49 mmol) at room temperature under argon, and stirred at

the same temperature for 10 min. Then the crude alcohol in toluene (3.0 ml) and DMAP (212.2 mg, 1.74 mmol) were added to the above mixture, respectively. After being stirred at room temperature for 4 h, the reaction mixture was quenched with water and the aqueous phase was extracted with diethyl ether (10 ml × 3). The combined organic layer was washed with saturated NH_4Cl, saturated $NaHCO_3$, brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel column chromatography (eluted by 10% ethyl acetate in hexane) to give ester **6**.

(*S*)-[(3*R*,4*S*,6*S*,8*S*)-8-*tert*-butyl-4-(2,2,2-trichloroethoxycarbonyloxy)-3,6-dimethyloct-1-en-8-yl]-2-[(9*H*-fluoren-9-ylmethoxy)carbonyl(methyl)amino]propanoate (6a) (100%). $[\alpha]^{25}_{D}$: -21.5 (c 0.2, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers): δ 7.78-7.75 (m, 2H), 7.63-7.55 (m, 2H), 7.40 (dd, *J* = 7.2, 7.6 Hz, 2H), 7.31 (dd, *J* = 7.2, 7.6 Hz, 2H), 5.78-5.68 (m, 1H), 5.12-5.05 (m, 2H), 5.01-4.70 (m, 5H), 4.51-4.22 (m, 3H), 2.97-2.90 (m, 3H), 2.50-2.43 (m, 1H), 1.95-1.81 (m, 1H), 1.59-1.38 (m, 5H), 1.23-1.12 (m, 2H), 1.07-1.04 (m, 3H), 0.94-0.91 (m, 3H), 0.88-0.85 (m, 9H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, mixture of rotamers): δ 171.8,156.6, 156.2, 154.2, 144.2, 144.2, 144.0, 143.9, 141.4, 141.4, 138.9, 127.8, 127.2, 127.1, 125.2, 125.2, 120.1, 116.4, 116.4, 80.5, 80.4, 80.3, 79.6, 79.4, 78.8, 76.7, 76.7, 68.0, 67.9, 67.8, 54.6, 54.3, 54.1, 47.3, 42.6, 42.5, 37.6, 37.5, 37.4, 36.8, 36.0, 36.0, 35.0, 34.9, 30.6, 30.5, 30.3, 29.8, 26.5, 26.0, 25.9, 25.8, 20.3, 20.3, 15.7, 15.6, 15.3, 15.1 ppm. HRMS (ESI) *m/z* calcd for $C_{36}H_{46}NO_7Cl_3$ $(M+Na)^+$ 732.2232, found 732.2237.

Pyrrolidine-1,2-dicarboxylic acid-(2*S*)-2-[(3*R*,4*S*,6*S*,8*S*)-8-*tert*-butyl-4-(2,2,2-trichloroethoxycarbonyloxy)-3,6-dimethyloct-1-en-8-yl]ester 1-(9*H*-fluoren-9-ylmethyl)ester (6b) [3b] (91%). ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers): δ 7.78-7.72 (m,2 H), 7.67-7.59 (m, 2H), 7.42-7.38 (m, 2H), 7.34-7.29 (m, 2H), 5.77-5.66 (m, 1H), 5.11-5.02 (m, 2H), 4.86-4.69 (m, 4H), 4.51 (dd, *J* = 8.4, 2.4 Hz, 0.5H), 4.46 (dd, *J* = 8.4, 2.4 Hz, 0.5H), 4.44-4.11 (m, 3H), 3.67-3.49 (m, 2H), 2.49 (m, 0.5H), 2.42 (m, 0.5H), 2.35-2.07 (m, 2H), 2.04-1.93 (m, 2H), 1.86-1.80 (m, 1H), 1.60-1.34 (m, 3H), 1.26 (ddd, *J* = 14.2, 10.2, 2.8 Hz, 0.5 H), 1.12 (ddd, *J* = 13.2, 10.4, 2.1 Hz, 0.5H), 1.04 (d, *J* = 7.2 Hz, 1.5H), 1.03 (d, *J* = 6.8 Hz, 1.5H), 0.96 (d, *J* = 6.4 Hz, 1.5 H), 0.88 (s, 4.5 H), 0.87 (s, 4.5 H), 0.74 (d, *J* = 6.4 Hz, 1.5 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, mixture of rotamers): δ 172.6, 1724, 154.8, 154.4, 154.3, 154.1, 144.3, 144.0, 143.9, 141.4, 141.4, 141.3, 141.3, 139.0, 138.9, 127.8, 127.8, 127.2, 127.1, 127.1, 127.1,

125.5, 125.4, 125.3, 125.2, 120.1, 120.0, 116.4, 80.6, 80.4, 79.6, 79.4, 76.7, 67.9, 67.5, 59.9, 59.5, 47.3, 47.3, 47.1, 46.5, 42.7, 42.5, 38.0, 37.8, 37.2, 37.0, 35.0, 34.8, 31.4, 30.1, 26.6, 26.5, 25.9, 25.9, 24.5, 23.5, 20.4, 20.4, 15.6, 15.6 ppm.

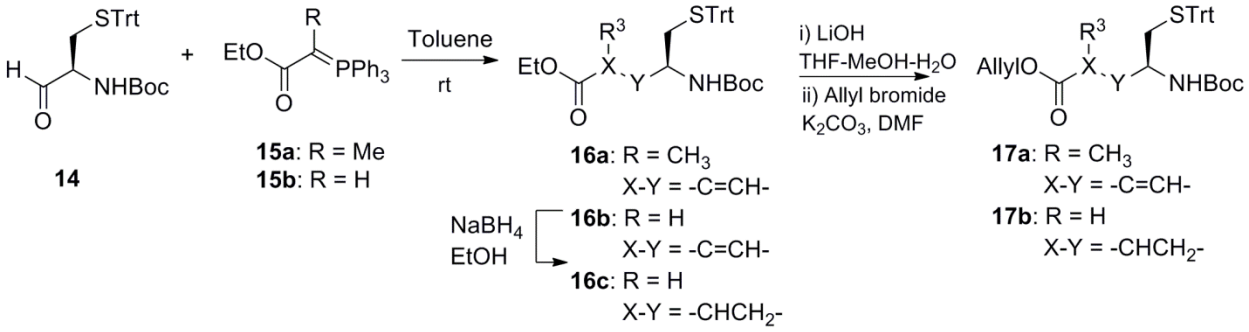
Synthesis of carboxylic acid 7 by oxidation of ester 6. To the solution of **6** (0.42 mmol) in DMF (4.0 ml) was added Oxone (1.04 g, 1.70 mmol), $NaHCO_3$ (143.0 mg, 1.70 mmol) and OsO_4 (2.5 % solution in *tert*-BuOH) (0.053 ml, 0.004 mmol) at room temperature. After being stirred at the same temperature for 15 h, the reaction mixture was diluted with water (2.5 ml) and *tert*-BuOH (5.0 ml), and then $NaIO_4$ (181.7 mg, 0.85 mmol) was added. The reaction mixture was stirred at room temperature for additional 5 h and poured into aqueous HCl (1M, 10 ml) and CH_2Cl_2 (10 ml). The water layer was extracted with CH_2Cl_2 (10 ml × 3). The combined CH_2Cl_2 layer was washed with 10wt % $Na_2S_2O_3$ (10 ml × 3), brine (10 ml × 1), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (eluted by ethyl acetate-hexane (1:5, v/v)) to give product **7**.

(*S*)-[(1*S*,3*S*,5*S*,6*S*)- 1-*tert*-butyl-6-carboxy-5-(2,2,2-trichloroethoxycarbonyloxy)-3-methylhep-1-yl]-2-[(9*H*-fluoren-9-ylmethoxy)carbonyl(methyl)amino]propanoate (7a) (57%). $[\alpha]^{25}_{D}$: -12.5 (c 0.04, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers): δ 7.77-7.74 (m, 2H), 7.62-7.53 (m, 2H), 7.41-7.37 (m, 2H), 7.32-7.29 (m, 2H), 5.23-5.14 (m, 1H), 5.00-4.70 (m, 4H), 4.52-4.32 (m, 2H), 4.29-4.22 (m, 1H), 2.95-2.89 (m, 3H), 2.03-1.89 (m ,1H), 1.60-1.39 (m, 6H), 1.26-1.11 (m, 6H), 0.98-0.95 (m, 3H), 0.92-0.84 (m, 9H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, mixture of rotamers): δ 178.5, 178.2, 171.9, 171.8, 171.7, 171.5, 156.8, 156.7, 156.2, 156.2, 153.8, 144.2, 144.1, 144.0, 143.9, 141.4, 127.8, 127.2, 125.2, 125.2, 120.1, 79.6, 79.3, 79.0, 78.7, 77.6, 77.3, 77.3, 76.8, 68.0, 67.9, 67.9, 54.7, 54.3, 54.1, 53.9, 47.3, 43.3, 37.6, 37.4, 37.3, 36.2, 35.5, 35.0, 34.8, 30.5, 30.4, 30.3, 29.8, 26.2, 26.1, 26.0, 25.9, 25.8, 25.6, 20.3, 20.2, 20.1, 15.7, 15.6, 15.1, 12.0, 11.7 ppm. HRMS (ESI) *m/z* calcd for $C_{35}H_{44}NO_9Cl_3$ $(M+Na)^+$ 750.1974, found 750.1974.

Pyrrolidine-1,2-dicarboxylic acid (2*S*)-2-[(1*S*,3*S*,5*S*,6*S*)-1-*tert*-butyl-6-carboxy-5-(2,2,2-trichloroethoxycarbonyloxy)-3-methylhept-1-yl]ester 1-(9*H*-fluoren-9-ylmethyl)ester (7b) [3b] (76%). ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers): δ 7.77-7.74 (m, 2H), 7.66-7.59 (m, 2H), 7.41-7.38 (m, 2H), 7.34-7.29 (m, 2H), 5.18-5.09 (m, 1H), 4.88-4.66 (m, 3H), 4.52-4.15 (m, 4H), 3.67-3.47 (m, 2H), 2.94 (dq, *J* = 7.2, 7.2 Hz, 0.6H), 2.86 (dq, *J* = 7.2, 7.2 Hz, 0.4H), 2.36-

2.07 (m, 2H), 2.00-1.82 (m, 3H), 1.62 (m, 0.6H), 1.54-1.49 (m, 1.4H), 1.43-1.22 (m, 2H), 1.21 (d, J = 7.2 Hz, 3H), 0.99 (d, J = 6.8 Hz, 1.6 H), 0.88 (s, 1.5H), 0.86 (s, 1.5H), 0.76 (d, J = 6.4 Hz, 1.4H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, mixture of rotamers): δ 178.5, 178.1, 172.7, 172.3, 154.9, 154.5, 153.8, 153.7, 144.2, 144.1, 143.9, 143.8, 141.3, 141.3, 141.3, 127.8, 127.8, 127.2, 127.1, 127.1, 125.5, 125.4, 125.2, 125.2, 120.0, 120.0, 120.0, 79.6, 79.2, 77.9, 77.4, 76.9, 76.8, 67.9, 67.6, 59.8, 59.5, 47.3, 47.1, 46.5, 43.6, 43.2, 38.0, 37.6, 36.7, 36.3, 35.0, 34.7, 31.3, 30.1, 29.8, 26.2, 26.1, 25.9, 24.4, 23.4, 20.4, 20.2, 12.2, 12.1 ppm.

The Synthesis of *N*-Boc Derivative of D-moCys 8: 17a-17b



Scheme S3. The preparation of **17:** *N*-Boc derivative of D-moCys **8**.

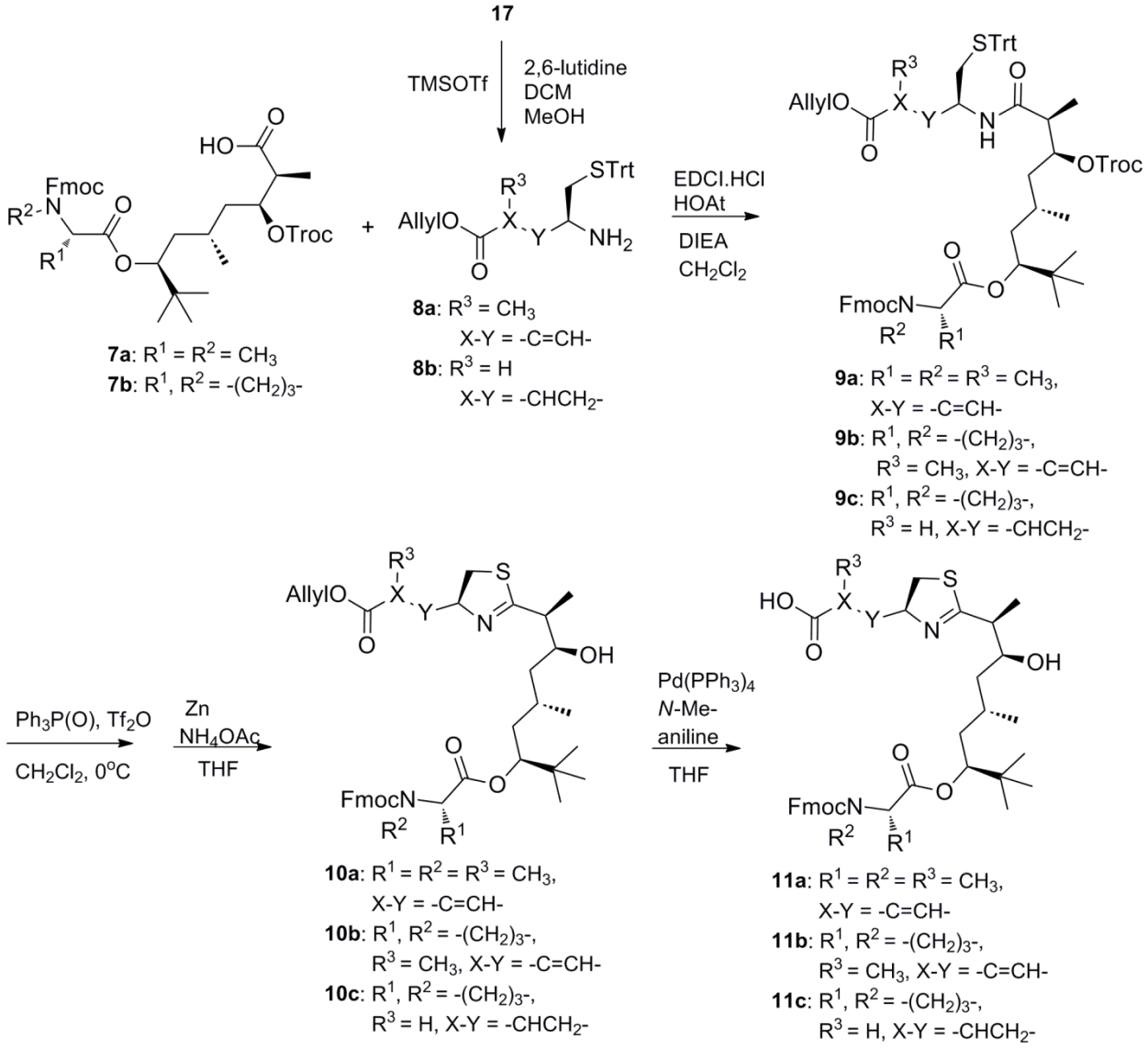
16a[3], **16b**, **16c**[5] were prepared according to published procedures. NMR spectra of prepared products were identical to those published.[3,5]

Synthesis of 17a[3] **and 17b**: To the solution of **16a** or **16c** (0.43 mmol) in 95% ethanol (2 ml) was added NaOH (1 M, 1.3 ml) at room temperature. After being stirred for 2 h at the same temperature, the reaction mixture was diluted with water (6 ml), acidified with 0.5 M HCl to pH 4-5, and extracted with diethyl ether (5 ml × 2). The combined organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was used in the next step without further purification. To the above crude acid solution in dimethyl sulfoxide (DMSO) (2.5 ml) were added K_2CO_3 (120 mg, 0.86 mmol) and allyl bromide (60 µl, 0.70 mmol) at room temperature. After being stirred at the same temperature overnight, the reaction was quenched with water (15 ml) and extracted with ethyl acetate (15 ml × 3). The combined organic layer was washed with brine, filtered, concentrated in vacuo and the residue was purified by column chromatography on silica gel (eluted by 10% ethyl acetate in hexane).

(**2*E*)-(4*S*)-4-*N*-*tert*-Butoxycarbonylamino-2-methyl-5-(triphenylmethylthio)-2-pentenoic acid allyl ester** (**17a**)[3b] (86% in 2 steps). (^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40 (m, 6H), 7.25-7.30 (m, 6H), 7.22 (m, 3H), 6.43 (dq, *J* = 9.3, 1.5 Hz, 1H), 5.93 (m, 1H), 5.32 (dd, *J* = 17.0, 1.5 Hz, 1H), 5.22 (dd, *J* = 14.8, 1.5 Hz, 1H), 4.62 (d, *J* = 6.0 Hz, 2H), 4.56 (m, 1H), 4.38 (m,1H), 2.42 (dd, *J* = 12.3, 7.2 Hz, 1H), 2.34 (dd, *J* = 12.2, 5.9 Hz, 1H), 3.64 (br m, 1H), 1.78 (d, *J* = 1.5 Hz, 3H), 1.42 (s, 9H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.3, 155.0, 144.6, 140.5, 132.5, 129.7, 128.1, 126.9, 118.2, 79.7, 77.4, 67.1, 65.5, 48.4, 36.5, 28.5, 13.0 ppm.

(4*S*)-4-*N*-*tert*-Butoxycarbonylamino-5-(triphenylmethylthio)-pentanoic acid allyl ester (**17b**) (95% in 2 steps). $[\alpha]^{25}_{D}$: -11.6 (c 0.181, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40 (d, *J* = 7.6 Hz, 6H), 7.28 (dd, *J* = 7.6, 8.0 Hz, 3H), 7.22 (d, *J* = 7.6 Hz, 6H), 5.94-5.84 (m, 1H), 5.29 (dd, *J* = 16, 1.2 Hz, 1H), 5.22 (dd, *J* = 10, 1.2 Hz, 1H), 4.54 (d, *J* = 6.0 Hz, 2H), 4.46 (d, *J* = 9.2 Hz, 1H), 3.64 (br m, 1H), 2.33 (br m, 2H), 2.25 (t, *J* = 8.0 Hz, 2H), 1.75-1.58 (m, 2H), 1.42 (s, 9H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.9, 155.3, 144.7, 132.3, 129.7, 128.1, 126.9, 118.4, 79.5, 77.4, 66.8, 65.3, 49.5, 37.3, 31.1, 29.8, 29.7, 28.5 ppm. HRMS (ESI) *m/z* calcd for $C_{32}H_{37}NO_4S$ $(M+Na)^+$ 554.2336, found 554.2346.

The Synthesis of *N*-Fmoc Amino Acid-Dtena-moCys Thiazoline Carboxylic Acids 11a-11c



Scheme S4. Synthesis of *N*-Fmoc amino acid-Dtena-moCys thiazoline carboxylic acid **11**.

Coupling of 7 and 8 to prepare 9: To the solution of **17** (0.195 mmol) and *2,6*-lutidine (257.2 mg, 1.20 mmol) in CH_2Cl_2 (3.0 ml) was added trimethylsilyl trifluoromethanesulfonate (TMSOTf) (0.22 ml, 1.20 mmol) dropwise at room temperature under argon. After being stirred at the same temperature for 3 h, the reaction mixture was quenched with MeOH (6 ml) and water (6 ml), and extracted with $CHCl_3$ (15 ml × 4). The combined organic layer was washed with

brine, dried over $MgSO_4$, evaporated in vacuo to give the crude product **8**, which was used in the next step without further purification.

To the solution of the crude **8** in CH_2Cl_2 (2 ml) was added **7** (0.150 mmol), HOAt (24.5 mg, 0.180 mmol), EDCI•HCl (34.5 mg, 0.180 mmol), and *N*,*N*- diisopropylethylamine (DIEA) (0.10 ml, 0.60 mmol) at 0°C. After being stirred at the same temperature for 8 h, the reaction mixture was diluted with ethyl acetate (5 ml), quenched with pre-cooled 1M aq. HCl (5 ml), and the organic layer was extracted with ethyl acetate (5 ml × 3). The combined organic layer was washed with 1M aq. HCl, saturated $NaHCO_3$ and brine, dried with $MgSO_4$, concentrated in vacuo, and purified by column chromatography on silica gel (eluted by ethyl acetate/hexane 1:2, v/v) to give product **9**.

(*S*)-{(1*S*,3*R*,5*S*,6*S*)-6-[(2*E*)-(1*R*)-3-allyloxycarbonyl-3-methyl-1-triphenylmethylthiomethyl-2-propenylcarbamoyl]-1-*tert*-butyl-5-(2,2,2-trichloroethoxycarbonyloxy)-3-methylhept-1-yl]} 2-(9*H*-fluoren-9-ylmethoxy)carbonyl(methyl)amino]propanoate (9a) (87% in 2 steps). $[\alpha]^{25}_D$: -18.0 (c 0.05, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers): δ 7.77 (d, J = 7.2 Hz, 2H), 7.62-7.55 (m, 2H), 7.42-7.20 (m, 19H),6.42-6.32 (m, 1H), 6.10 (d, J = 8.4 Hz, 0.16H), 6.08 (d, J = 8.0 Hz, 0.34H), 5.97-5.85 (m, 1H), 5.69 (d, J = 7.6 Hz, 0.25H), 5.60 (d, J = 8.0 Hz, 0.08H), 5.54 (d, J = 8.4 Hz, 0.17H), 5.34-5.19 (m, 2H), 5.08-4.93 (m, 2H), 4.88-4.72 (m, 2H), 4.69-4.48 (m, 4H), 4.46-4.34 (m, 2H), 4.28-4.22 (m, 1H), 2.95-2.88 (m, 3H), 2.59-2.32 (m, 3H), 2.06-1.69 (m, 4H), 1.66-1.44 (m, 5H), 1.38-1.22 (m, 2H), 1.15-1.09 (m, 3H), 0.91-0.86 (m, 12H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, mixture of rotamers): δ 172.1, 172.0, 171.8, 171.7, 167.2, 167.2, 156.7, 156.6, 156.2, 153.9, 153.7, 144.5, 144.5, 144.2, 144.1, 144.0, 141.4, 139.6, 139.5, 139.4, 139.3, 132.3, 130.4, 130.3, 130.2, 129.7, 129.6, 128.1, 128.0, 127.8, 127.2, 127.1, 127.0, 127.0, 125.2, 125.1, 120.1, 118.4, 118.3, 118.2, 78.9, 78.8, 78.6, 78.5, 78.5, 77.3, 76.7, 76.6, 68.0, 67.8, 67.3, 67.2, 65.6, 65.5, 65.4, 54.6, 54.3, 54.2, 53.9, 47.4, 47.3, 47.2, 45.2, 45.1, 37.5, 37.3, 36.6, 36.4, 36.0, 35.8, 35.3, 35.0, 34.8, 31.7, 30.5, 29.8, 26.0, 25.9, 25.4, 22.8, 20.2, 15.8, 15.1, 14.2, 13.3, 13.2, 13.0, 12.7, 12.4 ppm. HRMS (ESI) *m/z* calcd for $C_{63}H_{71}Cl_3N_2O_{10}S$ $(M+Na)^+$ 1175.3767, found 1175.3787.

Pyrrolidine-1,2-dicarboxylic acid (2*S*)-2-{(1*S*,3*R*,5*S*,6*S*)-6-[(2*E*)-(1*R*)-3-allyloxycarbonyl-3-methyl-1-triphenylmethylthiomethyl-2-propenylcarbamoyl]-1-*tert*-butyl-5-(2,2,2-trichloro ethoxycarbonyloxy)-3-methylhept-1-yl}ester 1-(9*H*-fluoren-9-ylmethyl)ester (9b) [3b] (81% in

2 steps). ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers): δ 7.77-7.75 (m, 2H), 7.69 (d, *J* = 7.69 Hz, 0.4H), 7.60-7.57 (m, 1.6H), 7.41-7.18 (m, 19H), 6.40-6.35 (m, 1.6H), 5.50 (d, *J* = 7.6 Hz, 0.4H), 5.32 (dd, *J* = 17.5, 1.6 Hz, 0.4 H), 5.25 (dd, *J* = 17.6, 1.5 Hz, 0.6H), 5.23 (dd, *J* = 10.3, 1.5 Hz, 0.4H), 5.17 (dd, *J* = 10.6, 1.5 Hz, 0.6H), 4.97 (m, 1H), 4.84 (dd, *J* = 8.3, 3.8 Hz, 0.6H), 4.81 (d, *J* = 12.3 Hz, 0.6H), 4.80 (dd, *J* = 10.2, 5.7 Hz, 0.4H), 4.71 (d, *J* = 12.3, 0.4H), 4.49-4.67 (m, 5H), 4.42-4.36 (m, 1H), 4.30-4.19 (m, 2H), 3.66-3.46 (m, 2H), 2.55-2.08 (m, 5H), 2.02-1.83 (m, 3H), 1.73 (br s, 3H), 1.58-1.62 (m, 1H), 1.22-1.49 (m, 3H), 1.09 (d, *J* = 6.8 Hz, 1.8H), 1.07 (d, *J* = 7.3 Hz, 1.2H), 0.92 (d, *J* = 6.8 Hz, 1.8H), 0.87 (s, 3.6H), 0.85 (s, 5.4H), 0.73 (d, *J* = 6.4 Hz, 1.2H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, mixture of rotamers): δ 172.6, 172.2, 171.9, 167.2, 154.9, 154.5, 153.9, 153.8, 144.6, 144.5, 144.1, 143.9, 143.8, 141.4, 141.3, 139.7, 139.5, 132.3, 130.4, 130.2, 129.7, 129.6, 128.1, 128.0, 127.8, 127.2, 127.0, 126.9, 125.7, 125.4, 125.2, 120.0, 118.3, 118.2, 79.3, 79.1, 78.7, 78.5, 76.7, 76.6, 67.9, 67.6, 67.3, 67.1, 65.6, 65.5, 59.8, 59.5, 47.3, 47.2, 47.1, 46.4, 45.3, 44.7, 38.1, 37.4, 36.8, 36.4, 36.0, 35.8, 35.1, 34.8, 31.7, 31.4, 30.1, 26.1, 26.0, 25.9, 24.4, 23.5, 20.4, 19.9, 14.2, 13.5, 13.0, 12.9 ppm.

Pyrrolidine-1,2-dicarboxylic acid (2*S*)-2-{(1*S*,3*R*,5*S*,6*S*)-6-[(2*R*)-4-allyloxycarbonyl-1-triphenylmethylthio-2-butylcarbamoyl]-1-*tert*-butyl-5-(2,2,2-trichloroethoxycarbonyloxy)-3-methylhept-1-yl}ester 1-(9*H*-fluoren-9-ylmethyl)ester (9c) (65% in 2 steps). $[\alpha]^{25}_{D}$: -46.4 (c 0.11, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers): δ 7.77-7.74 (m, 2H), 7.68 (d, *J* = 7.2 Hz, 0.4H), 7.68 (d, *J* = 7.2 Hz, 1.6H), 7.41-7.36 (m, 8H), 7.33-7.26 (m, 8H), 7.21-7.18 (m, 3H), 6.15 (d, *J* = 8.0 Hz, 0.6H), 5.91-5.78 (m, 1H), 5.43 (d, *J* = 8.8 Hz, 0.4H), 5.30-5.17 (m, 2H), 5.0 (br m, 0.6H), 4.86-4.83 (m, 0.4H), 4.78 (d, *J* = 12.4 Hz, 1.2H), 4.72 (d, *J* = 12.4 Hz, 0.4H), 4.62 (d, *J* = 12.4 H, 0.4H), 4.52-4.47 (m, 4H), 4.42-4.38 (m, 1H), 4.30-4.18 (m, 2H), 3.87-3.78 (m, 1H), 3.68-3.61 (m, 1H), 3.59-3.47 (m, 1H), 2.58-2.51 (m, 0.2H), 2.44-2.38 (m, 0.2H), 2.35-2.26 (m, 3H), 2.19-1.12 (m, 4H), 2.00-1.78 (m, 2.6H), 1.74-1.56 (m, 3H), 1.37-1.26 (m, 1H), 1.24-1.18 (m, 1H), 1.12 (d, *J* = 6.4 Hz, 1.8H), 1.11 (d, *J* = 6.0 Hz, 1.2H), 0.94 (d, *J* = 6.4 Hz, 1.8H),0.88 (s, 3.6H), 0.86 (s, 5.4H), 0.75 (d, *J* = 6.4 Hz, 1.2H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, mixture of rotamers): δ 172.9, 172.8, 172.6, 172.2, 172.1, 154.8, 154.5, 153.9, 153.9, 144.7, 144.6, 144.4, 144.2, 144.0, 143.8, 141.4, 141.3, 132.2, 132.2, 129.7, 129.7, 129.6, 128.1, 128.1, 127.8, 127.3, 127.2, 127.1, 126.9, 126.9, 125.7, 125.4, 125.2, 120.1, 118.5, 118.4, 94.9, 94.9, 79.4, 79.1, 78.9, 78.5, 77.4, 76.7, 76.6, 67.9, 67.6, 66.9, 66.8, 65.3, 65.3, 59.9, 59.5, 48.5, 48.4, 47.3, 47.1, 46.5, 45.5, 45.0, 38.2, 37.6, 36.8, 36.6, 36.4, 35.1, 34.8, 31.7, 31.4, 30.9,

30.1, 29.2, 26.2, 26.1, 26.0, 25.9,24.4, 23.5, 22.8, 20.4, 20.0, 13.7, 13.1 ppm. HRMS (ESI) *m/z* calcd for $C_{63}H_{71}Cl_3N_2O_{10}S$ $(M+Na)^+$ 1175.3787, found 1175.3787.

Thiazoline ring formation- synthesis of 10a-10c: To a solution triphenylphosphine oxide (263 mg, 0.945 mmol) in CH_2Cl_2 (2.5 ml) was added dropwise trifluoromethanesulfonic anhydride (Tf_2O) (0.08 ml, 0.472 mmol) at 0^oC under argon. After being stirred at the same temperature for 10 min, **9** (0.118 mmol) in CH_2Cl_2 (1 ml) was added at 0^oC. The reaction mixture was stirred at the same temperature for 30 min and quenched with saturated $NaHCO_3$ (6 ml) at 0^oC. The aqueous layer was extracted with ethyl acetate (10 ml × 5), washed with brine (10 ml × 2), dried with $MgSO_4$, filtered and concentrated in vacuo to give Troc protected thiazoline intermediate. The residue was used in the next step immediately without further purification.

The above residue was dissolved in THF (6 ml), and then aqeous NH_4OAc (1 M, 1.5 ml), and zinc powder (freshly activated with 1 M aqeous HCl) (150 mg) were added at room temperature. After being stirred at the same temperature for 30 min, ethyl acetate (9 ml), brine (6 ml) was added. The aqueous layer was extracted with ethyl acetate (15 ml × 5). The combined organic layer was dried with $MgSO_4$, filtered, concentrated in vacuo, and purified by column chromatography on silica gel (eluted by ethyl acetate/hexane 1:2, v/v) to give thiazoline ring product **10**.

(*S*)-{(1*S*,3*S*,5*S*,6*S*)-6-[5-[(1*E*)-2-allyloxycarbonyl-1-propenyl]-(*S*)-4,5-dihydro-thiazol-2-yl]-1-*tert*-butyl-5-hydroxy-3-methylhept-1-yl} 2-[(9*H*-fluoren-9-ylmethoxy)carbonyl(methyl) amino]propanoate (10a) (65% in 2 steps). $[\alpha]^{25}_D$: -16.0 (c 0.1, CH_2Cl_2). ^1H NMR (400 MHz, $CDCl_3$, mixture of rotamers): δ 7.77-7.75 (m, 2H), 7.63-7.53 (m, 2H), 7.41-7.37 (m, 2H), 7.32-7.29 (m, 2H), 6.81 (d, J = 8.8 Hz, 0.8H), 6.79 (d, J = 7.2 Hz, 0.2H), 6.00-5.87 (m, 0.5H), 5.34-5.14 (m, 2.5H), 4.88-4.79 (m, 2H), 4.65-4.62 (m, 2H), 4.47-4.22 (m, 3H), 3.72 (m, 1H), 3.47-3.36 (m, 1H), 3.02-2.90 (m, 4H), 2.74-2.61 (m, 1H), 1.98-1.94 (m, 3H), 1.75-1.61 (m, 2H), 1.58-1.49 (m, 1H), 1.46-1.43 (m, 3H), 1.01 (m, 1H), 0.94-0.90 (m, 3H), 0.89-0.85 (m, 9H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, mixture of rotamers): δ 176.4, 172.5, 172.4, 172.2, 167.4, 156.9, 156.6, 156.2, 144.3, 144.1, 144.0, 141.4, 140.7, 140.5, 140.4, 140.3, 140.1, 132.4, 130.0, 129.7,129.6, 127.8, 127.2, 127.1, 125.3, 125.2, 120.1, 118.3, 79.9, 79.5, 79.3, 79.1, 79.0, 77.4, 74.2, 74.0, 73.9, 71.7, 68.0, 67.9, 65.6, 54.7, 54.6, 54.5, 54.3, 54.0, 47.3, 46.0, 45.9, 45.8, 45.6, 45.5, 45.4, 45.3, 40.3, 40.1, 39.9, 39.8, 37.8, 37.2, 37.7, 37.6, 34.8, 34.7, 30.9, 30.4, 30.4, 29.8, 26.1, 26.1,

25.7, 25.5, 20.8, 20.7, 16.5, 16.4, 16.2, 16.1, 15.9, 15.7, 15.5, 13.2 ppm. HRMS (ESI) *m/z* calcd for $C_{41}H_{54}N_2O_7S$ $(M+H)^+$ 719.3725, found 719.3726.

Pyrrolidine-1,2-dicarboxylic acid (2*S*)-2-{(1*S*,3*S*,5*S*,6*S*)-6-[5-[(1*E*)-2-allyloxycarbonyl-1-propenyl]-(*S*)-4,5-dihydro-thiazol-2-yl]-1-*tert*-butyl-5-hydroxy-3-methylhept-1-yl}ester 1-(9*H*-fluoren-9-ylmethyl)ester (10b) [3b] (54% in 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers): δ 7.75 (d, *J* = 7.3 Hz, 2H), 7.63 (dd, *J* = 6.8, 7.3 Hz, 2H), 7.39 (dd, *J* = 7.3, 7.3 Hz, 2H), 7.32-7.28 (m, 2H), 6.82-6.78 (m, 1H), 6.00-5.84 (m, 1H), 5.35-5.16 (m, 3H), 4.90 (dd, *J* = 11.6, 1.9 Hz, 0.8H), 4.82 (dd, *J* = 10.6, 1.4 Hz, 0.2H), 4.66-4.59 (m, 2H), 4.53-4.33 (m, 3H), 4.31-4.19 (m, 1H), 3.80 (m, 0.8H), 3.69 (m, 0.2H), 3.62 (m ,1H), 3.52 (m, 1H), 3.43 (dd, *J* = 11.1, 8.7 Hz, 0.2H), 3.31 (dd, *J* = 11.2, 8.8 Hz, 0.8H), 3.02-2.92 (m, 1H), 2.72-2.65 (m, 1H), 2.25 (m, 1H), 2.10-1.98 (m, 3H), 1.97 (d, *J* = 1.5 Hz, 0.6H), 1.95 (d, *J* = 1.0 Hz, 2.4H), 1.74-1.38 (m ,4H), 1.25 (d, *J* = 7.3 Hz, 0.6H), 1.21 (d, *J* = 6.8 Hz, 2.4H), 0.99 (m,1H), 0.95 (d, *J* = 6.8 Hz, 2.4H), 0.88 (s, 9H), 0.79 (d, *J* = 6.4 Hz, 0.6H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, mixture of rotamers): δ 172.5, 167.3, 154.9, 154.4, 144.3, 144.1, 143.9, 141.4, 141.3, 140.3, 132.3, 127.7, 127.0, 125.5, 125.4, 125.3, 119.9, 118.3, 118.1, 79.4, 78.4, 74.1, 71.4, 67.9, 67.8, 65.6, 65.5, 59.6, 47.3, 47.2, 47.0, 46.5, 45.9, 45.3, 40.2, 39.1, 37.9, 37.6, 37.5, 34.8, 34.6, 31.6, 31.2, 30.0, 29.8, 26.1, 25.6, 25.0, 24.6, 23.3, 22.7, 20.6, 20.4, 16.2, 15.6, 14.2, 13.1 ppm.

Pyrrolidine-1,2-dicarboxylic acid (2*S*)-2-{(1*S*,3*S*,5*S*,6*S*)-6-[5-(2-allyloxycarbonylethane)-(*S*)-4,5-dihydro-thiazol-2-yl]-1-*tert*-butyl-5-hydroxy-3-methylhept-1-yl}ester 1-(9*H*-fluoren-9-ylmethyl)ester (10c) (78% in 2 steps). $[\alpha]^{25}_D$: -40.5 (c 0.15 , CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers): δ 7.75 (d, *J* = 7.6 Hz, 2H), 7.64 (dd, *J* = 6.8, 6.8 Hz, 1.7 H), 7.57 (d, *J* = 7.2 Hz, 0.3H), 7.39 (dd, *J* = 6.8, 7.2 Hz, 2H), 7.30 (dd, *J* = 6.8, 7.2 Hz, 2H), 5.95-5.84 (m, 1H), 5.31-5.19 (m, 2H), 4.88 (d, *J* = 11.2 Hz, 0.7H), 4.82 (d, *J* = 10.4 Hz, 0.3H), 4.58-4.19 (m, 7H), 3.77 (br m, 1H), 3.67-3.61 (m, 1H), 3.58-3.48 (m, 1H), 3.35-3.21 (m, 1H), 2.90-2.82 (m, 1H), 2.67-2.49 (m, 2H), 2.30-2.19 (m, 1H), 2.10-1.93 (m, 5H), 1.82 (m, 1H), 1.71-1.58 (m, 2.3H), 1.50-1.42 (m, 0.7H), 1.38-1.25 (m, 2H), 1.22 (d, *J* = 6.8 Hz, 0.9H), 1.18 (d, *J* = 6.4 Hz, 2.1H), 0.96 (d, *J* = 6.4 Hz, 2.1H), 0.88 (s, 9H), 0.80 (d, *J* = 6.4 Hz, 0.9H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, mixture of rotamers): δ 173.1, 173.0, 172.8, 172.5, 155.0, 155.0, 154.4, 144.4, 144.3, 144.1, 143.9, 141.4, 141.4, 141.3, 132.3, 132.3, 127.7, 127.7, 127.1, 125.5, 125.4, 125.3, 120.0, 118.4, 118.3, 79.4, 78.5, 76.0, 75.7, 71.6, 71.5, 67.9, 67.7, 65.3, 65.2, 59.7, 47.3, 47.2,

47.1, 46.6, 45.7, 45.1, 40.4, 39.8, 39.3, 38.0, 37.8, 37.4, 37.2, 34.8, 34.7, 31.6, 31.4, 31.3, 30.4, 30.3, 30.0, 29.8, 26.1, 26.1, 25.7, 25.2, 24.6, 23.4, 20.6, 20.5, 16.4, 15.7 ppm. HRMS (ESI) *m/z* calcd for $C_{41}H_{54}N_2O_7S$ $(M+H)^+$ 719.3725, found 719.3731.

Synthesis of 11a-11c: To a solution of **10** (0.062 mmol) in THF (2 ml) were added $Pd(PPh_3)_4$ (7.1 mg, 0.0062 mmol) and *N*-methyl aniline (0.017 ml, 0.154 mmol) at room temperature under argon. This reaction was protected with aluminum foil. After being stirred at the same temperature for 1 h, the reaction mixture was concentrated in vacuo and purified by preparative TLC (20 cm × 20 cm plate) to give acid **11**.

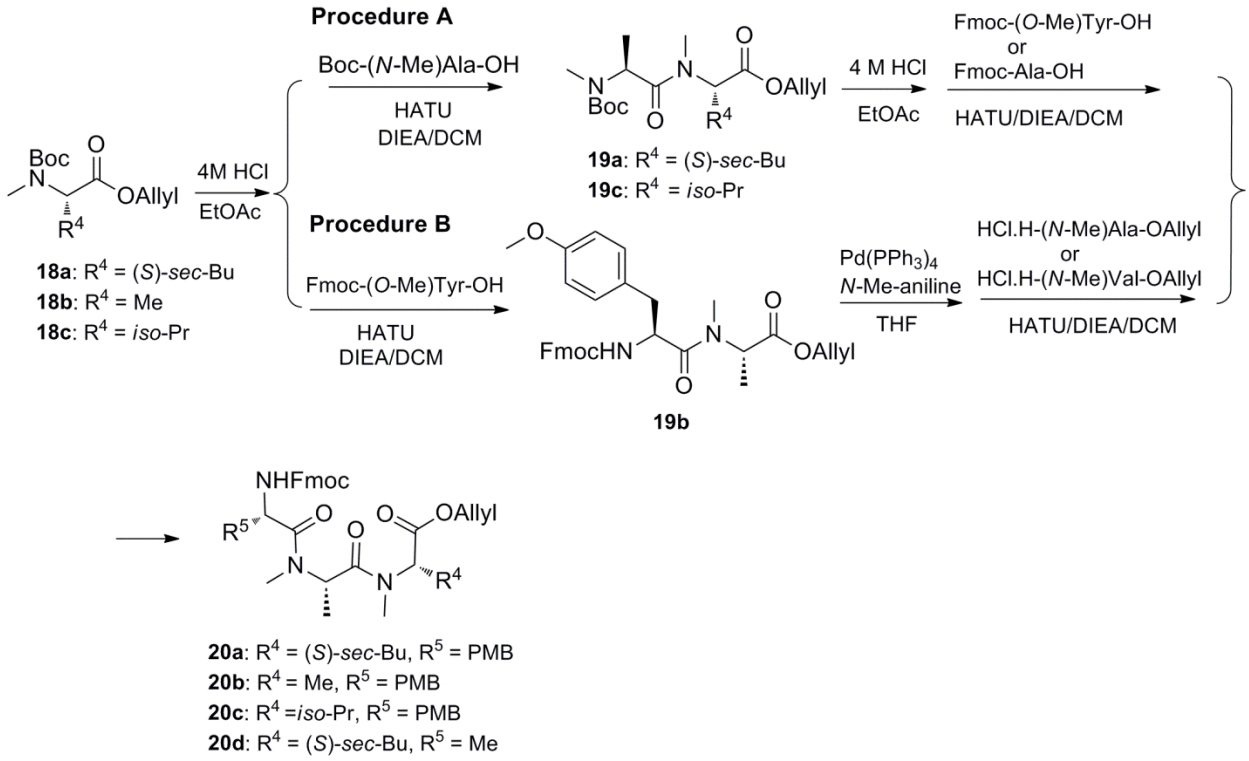
(*S*)-{(1*S*,3*S*,5*S*,6*S*)-6-[5-[(1*E*)-2-carbonyl-1-propenyl]-(*S*)-4,5-dihydro-thiazol-2-yl]- 1-*tert*-butyl-5-hydroxy-3-methylhept-1-yl} 2-[(9*H*-fluoren-9-ylmethoxy)carbonyl(methyl) amino]propanoate (11a) (97%). $[\alpha]^{25}_D$: -20.0 (c 0.03, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers): δ 7.77-7.74 (m, 2H), 7.63-7.55 (m, 2H), 7.41-7.37 (m, 2H), 7.32-7.29 (m, 2H), 6.88-6.84 (m, 1H), 5.31-5.18 (m, 1H), 4.85-4.79 (m, 2H), 4.48-4.23 (m, 3H), 3.84-3.65 (m, 1H), 3.47-3.36 (m, 1H), 3.03-2.97 (m, 1H), 2.94-2.90 (m, 3H), 2.74-2.64 (m, 1H), 1.94 (s, 0.9H), 1.92 (s, 0.1H), 1.72-1.60 (m, 1H), 1.58-1.48 (m, 1H), 1.46-1.43 (m, 3H), 1.41-1.30 (m, 1H), 1.28-1.21 (m, 3H), 1.11-0.97 (m, 1H), 0.94-0.90 (m, 3H), 0.90-0.83 (m, 9H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, mixture of rotamers): δ 177.4, 177.8, 172.6, 172.4, 172.3, 172.0, 157.0, 156.8, 156.2, 144.2, 144.1, 144.0, 142.2, 141.9, 141.8, 141.4, 132.3, 132.2, 132.2, 132.1, 129.3, 129.1, 128.7, 128.6, 127.8, 127.2, 125.3, 125.2, 120.1, 79.5, 79.4, 79.1, 77.3, 74.0, 73.9, 73.8, 71.7, 70.8, 70.7, 68.1, 68.0, 54.8, 54.6, 54.6, 54.4, 47.3, 45.6, 45.4, 45.3, 40.4, 39.9, 39.7, 37.8, 37.7, 37.6, 34.8, 34.7, 31.0, 30.4, 30.4, 29.8, 29.5, 29.2, 26.1, 26.0, 25.7, 25.5, 16.6, 16.2, 16.1, 15.7, 15.5, 15.0,12.9 ppm. HRMS (ESI) *m/z* calcd for $C_{38}H_{50}N_2O_7S$ $(M+H)^+$ 679.3412, found 679.3426.

Pyrrolidine-1,2-dicarboxylic acid (2*S*)-2-{(1*S*,3*S*,5*S*,6*S*)-6-[5-[(1*E*)-2-carbonyl-1-propenyl]-(*S*)-4,5-dihydro-thiazol-2-yl]-1-*tert*-butyl-5-hydroxy-3-methylhep-1-yl}ester 1-(9*H*-fluoren-9-ylmethyl)ester (11b) [3b] (100%). ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers): δ 7.75 (d, *J* = 7.3 Hz, 2H), 7.63 (m, 2H), 7.38 (m, 2H), 7.30 (m, 2H), 6.89-6.84 (m, 1H), 5.28-5.14 (m, 1H), 4.90 (dd, *J* = 11.7, 1.5 Hz, 0.7H), 4.82 (br d, *J* = 10.3 Hz, 0.3H), 4.53-4.18 (m, 4H), 3.81-3.39 (m, 3H), 3.36 (dd, *J* = 10.8, 7.8 Hz, 0.3H), 3.30 (dd, *J* = 10.8, 8.8 Hz, 0.7H), 2.97 (m, 1H), 2.72 (m, 1H), 2.23 (m, 1H), 2.07-1.94 (m, 3H), 1.94 (br s, 0.9H), 1.92 (d, *J* = 7.1 Hz, 2.1H), 1.84 (m,

1H), 1.78-1.29 (m, 4H), 1.24 (d, *J* = 7.3 Hz, 0.9H), 1.21 (d, *J* = 7.3 Hz, 2.1H), 0.96 (d, *J* = 6.4 Hz, 2.1H), 0.89 (s, 2.7H), 0.87 (s, 6.3H), 0.78 (d, *J* = 6.8 Hz, 0.9H), ppm. ^{13}C NMR (100 MHz, $CDCl_3$, mixture of rotamers): δ 173.0, 172.5, 172.0, 155.1, 155.0, 154.4, 144.3, 144.2, 144.1, 144.0, 143.8, 142.3, 141.4, 141.3, 141.2, 127.7, 127.6, 127.1, 125.5, 125.3, 119.9, 79.5, 78.6, 78.4, 71.5, 70.9, 67.9, 67.8, 59.6, 47.3, 47.2, 47.1, 47.0, 46.5, 46.0, 45.3, 40.1, 39.4, 39.0, 37.9, 37.6, 37.4, 34.8, 34.6, 31.2, 30.0, 26.0, 25.5, 25.1, 24.9, 24.6, 23.4, 20.5, 20.3, 16.2, 14.5, 12.9, 12.8 ppm.

Pyrrolidine-1,2-dicarboxylic acid (2*S*)-2-{(1*S*,3*S*,5*S*,6*S*)-6-[5-(2-carbonylethane)-(*S*)-4,5-dihydro-thiazol-2-yl]-1-*tert*-butyl-5-hydroxy-3-methylhept-1-yl}ester 1-(9*H*-fluoren-9-ylmethyl)ester (11c) (100%). $[\alpha]^{25}_{D}$: -45.5 (c 0.11 , CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers): δ 7.75 (d, *J* = 7.6 Hz, 2H), 7.64-7.58 (m, 2H), 7.39 (dd, *J* = 7.6, 7.4 Hz, 2H), 7.30 (dd, *J* = 7.6, 7.4 Hz, 2H),5.90 (br, 1H), 4.91-4.79 (m, 1H), 4.52-4.17 (m, 5H), 3.81-3.47 (m, 3H), 3.36-3.14 (m, 1H), 2.92-2.81 (m, 1H), 2.68-2.50 (m, 2H), 2.31-2.20 (m, 1H), 2.06-1.88 (m, 5H), 1.82 (br m, 1H), 1.71-1.60 (m, 2.3H), 1.50-1.42 (m, 0.7H), 1.38-1.25 (m, 2H), 1.21 (d, *J* = 6.8 Hz, 0.9H), 1.17 (d, *J* = 6.4 Hz, 2.1H), 0.95 (d, *J* = 6.4 Hz, 2.1H), 0.87 (s, 9H), 0.75 (d, *J* = 6.4 Hz, 0.9H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, mixture of rotamers): δ 177.7, 175.9, 172.6, 155.2, 155.1, 144.2, 144.1, 143.8, 141.4, 141.4, 127.8, 127.1, 125.5, 125.4, 125.3, 120.0, 79.6, 78.7, 78.6, 77.4, 75.8, 75.3, 71.5, 70.9, 67.9, 67.9, 59.6, 47.3, 47.1, 46.6, 46.0, 45.8, 45.1, 40.4, 39.4, 39.1, 38.0, 37.7, 37.6, 37.5, 34.8, 34.6, 32.8, 31.7, 33.0, 30.2, 30.0, 29.9, 29.8, 26.1, 25.4, 25.1, 24.9, 24.6, 23.4, 22.8, 20.8, 20.5, 20.4, 16.5, 15.7, 14.4, 14.2 ppm. HRMS (ESI) *m/z* calcd for $C_{38}H_{50}N_2O_7S$ $(M+H)^+$ 679.3412, found 679.3423.

Synthesis of 20: *N*-Fmoc Derivative of Tripeptide 12



Scheme S5. Synthesis of **20**: *N*-Fmoc derivative of tripeptide **12**.

The synthesis of dipeptides 19a-19c: *N*-Boc-(*N*-Me)-OAllyl amino acid **18** (3.426 mmol) was treated with 4 M HCl in ethyl acetate (10 ml) at 0^{o}C for 30 min. After being stirred at room temperature for 1 h, the reaction mixture was concentrated in vacuo. The residue was azeotroped with diethyl ether three times, dried under reduced pressure for 2 h, and then dissolved in CH_2Cl_2 (15 ml). To the above solution was added DIEA (1.24 ml, 7.126 mmol), *N*-protected amino acid (*N*-Boc-(*N*-Me)Ala-OH or *N*-Fmoc-Tyr(*O*-Me)-OH) (2.912 mmol), HATU (1.524 g, 4.009 mmol) at 0^{o}C. After being stirred at room temperature for 5 h, the reaction mixture was concentrated in vacuo and purified by column chromatography on silica gel (eluted by ethyl acetate-hexane 1:7, v/v) to give dipeptide **19**.

***N*-Boc-(*N*-Me)Ala-(*N*-Me)Ile-OAllyl (19a)**[3]**.** NMR spectra of the prepared product were identical to those published.[3]

***N*-Fmoc-Tyr(*O*-Me)-(*N*-Me)Ala-OAllyl** (**19b**) (99%). $[\alpha]^{25}_{D}$: -19.5 (c 0.44 , CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers): δ 7.75 (d, J = 7.6 Hz, 2H), 7.75 (d, J = 7.6 Hz, 2H),

7.61-7.56 (m, 2H), 7.39 (dd, *J* = 7.4, 7.2 Hz, 2H), 7.33-7.28 (m, 2H), 7.16-7.12 (m, 2H), 6.83-6.80 (m, 2H), 5.99-5.75 (m, 2H), 5.32 (dd, *J* = 16.8, 1.2 Hz, 1H), 5.24 (dd, *J* = 10.4, 1.5 Hz, 1H), 5.19 (q, *J* = 7.6 Hz, 1H), 4.92 (ddd, *J* = 8.4, 6.4 Hz, 6.2 Hz, 1H), 4.63-4.53 (m, 2H), 4.40 (dd, *J* = 10.4, 7.6 Hz, 1H), 4.27 (dd, *J* = 10.2, 7.2 Hz, 1H), 4.19-4.16 (m, 1H), 3.73 (s, 3H), 3.11-3.06 (m, 1H), 3.02-2.94 (m, 1H), 2.91 (s, 2.6H), 2.80 (m ,0.4H), 1.40 (d, *J* = 7.2 Hz, 2.6H), 1.03 (d, *J* = 7.2 Hz, 0.4H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, mixture of rotamers): δ 171.8, 171.6, 170.9, 170.2, 158.6, 158.5, 155.6, 155.4, 143.8, 143.8, 141.2, 131.7,131.3, 130.6, 130.4, 128.0, 127.8, 127.6, 127.0, 125.1, 125.1, 119.9, 119.9, 118.6, 114.0, 113.7, 67.0, 66.9, 66.0, 65.7, 55.2, 55.0, 54.9, 52.8, 52.2, 52.0, 47.0, 39.4, 37.8, 31.5, 14.1 ppm. HRMS (ESI) *m/z* calcd for $C_{32}H_{34}N_2O_6$ $(M+H)^+$ 543.2490, found 543.2491.

***N*-Boc-(*N*-Me)Ala-(*N*-Me)Val-OAllyl (19c)** (72%). $[\alpha]^{25}_D$: -148.2 (c 0.5, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers): δ 5.86-5.80 (m, 1H), 5.26-5.14 (m, 2H), 5.1-5.02 (m, 0.75H), 4.88-4.80 (m, 0.25H), 4.79 (d, *J* = 10.4 Hz, 0.75H), 4.61-4.47 (m, 2H), 4.18 (d, *J* = 10.0 Hz, 0.15H), 4.01 (d, *J* = 10.4 Hz, 0.10H), 2.94 (s, 2H), 2.88 (s, 0.4H), 2.77 (s, 0.6H), 2.69 (s, 2H), 2.58 (s, 0.6H), 2.52 (s, 0.4H), 2.20-2.12 (br m, 0.85H), 2.05 (br s, 0.15H), 1.43 (s, 1.2H), 1.40 (s, 1.8H), 1.39 (s, 6H), 1.21 (d, *J* = 6.8 Hz, 2H), 1.19 (d, *J* = 6.8 Hz, 1H), 0.95 (d, *J* = 6.4 Hz, 3H), 0.83 (d, *J* = 7.2 Hz, 1.5H), 0.79 (d, *J* = 7.2 Hz, 1.5H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, mixture of rotamers): δ 172.6, 172.0, 171.7, 171.1, 170.9, 170.7, 170.0, 155.5, 155.1, 154.7, 153.8, 131.8, 131.7, 119.6, 119.4, 118.6, 118.5, 80.4, 80.0, 66.1, 65.9, 65.3, 64.9, 64.5, 62.1, 52.3, 52.1, 50.6, 31.1, 30.9, 29.6, 29.5, 29.2, 29.0, 28.7, 28.5, 28.4, 28.3, 28.3, 27.8, 27.6, 27.4, 19.9, 19.9, 19.8, 19.6,19.4, 19.3, 19.0, 18.6, 14.9, 14.8, 14.5 ppm. HRMS (ESI) *m/z* calcd for $C_{18}H_{32}N_2O_5$ $(M+Na)^+$ 379.2203, found 379.2211.

The synthesis of tripeptides 20a-20d: (**20c** was synthesized by **procedure A** and **procedure B**, respectively)

For **20a**[3]**, 20c** and **20d**: (**Procedure A**) *N*-Boc-OAllyl dipeptide **19a** or **19d** (0.802 g, 2.165 mmol) was treated with 4 M HCl in ethyl acetate (5 ml) at 0^{o}C for 30 min. After being stirred at room temperature for 1 h, the reaction mixture was concentrated in vacuo. The residue was azeotroped with diethyl ether three times, dried under reduced pressure for 2 h, then dissolved in CH_2Cl_2 (8 ml). To the above solution were added DIEA (1.03 ml, 5.910 mmol), *N*-protected amino acid (*N*-Fmoc-(*N*-Me)Ala-OH or *N*-Fmoc-Tyr(*O*-Me)-OH) (1.97 mmol) and HATU

(1.123 g, 2.952 mmol) at 0°C. After being stirred at room temperature for 5 h, the reaction mixture was concentrated in vacuo and purified by column chromatography on silica gel (eluted by ethyl acetate-hexane 1:1, v/v) to give tripeptides **20a, 20c** and **20d**.

For **20b** and **20c:** (**Procedure B**) To the solution of **19b** *N*-Fmoc-Tyr(*O*-Me)-(*N*-Me)Ala-OAllyl (95.0 mg, 0.184 mmol) (0.062 mmol) in THF (5 ml) were added $Pd(PPh_3)_4$ (21.3 mg, 0.018 mmol), and *N*-methylaniline (50 µl, 0.461 mmol) at room temperature under argon. This reaction was protected with aluminum foil. After being stirred at the same temperature for 2 h, the reaction mixture was concentrated in vacuo and purified by column chromatography on silica gel (eluted by 5% MeOH in CH_2Cl_2) to give *N*-Fmoc-Tyr(*O*-Me)-(*N*-Me)Ala-OH (93 mg, 90%).

N-Boc-(*N*-Me)Ala-OAllyl or *N*-Boc-(*N*-Me)Val-OAllyl (0.167 mmol) was treated with 4 M HCl in ethyl acetate (1 ml) at 0°C for 30 min. After being stirred at room temperature for 1 h, the reaction mixture was concentrated in vacuo. The residue was azeotroped with diethyl ether three times, dried under reduced pressure for 2 h, and then dissolved in CH_2Cl_2 (1 ml). To the above solution was added DIEA (0.08 ml, 0.456 mmol), *N*-protect amino acid *N*-Fmoc-Tyr(*O*-Me)-(*N*-Me)Ala-OH (76.5 mg, 0.152 mmol), HATU (86.5 mg, 0.227 mmol) at room temperature. After being stirred at same temperature overnight, the reaction mixture was concentrated in vacuo and purified by column chromatography on silica gel (eluted by ethyl acetate-hexane 1:1, v/v) to give tripeptides **20b** and **20c**.

***N*-Fmoc-Tyr(*O*-Me)-(*N*-Me)Ala-(*N*-Me)Ile-OAllyl (20a)**[3] (85%). NMR spectra of the prepared product were identical to those published.[3]

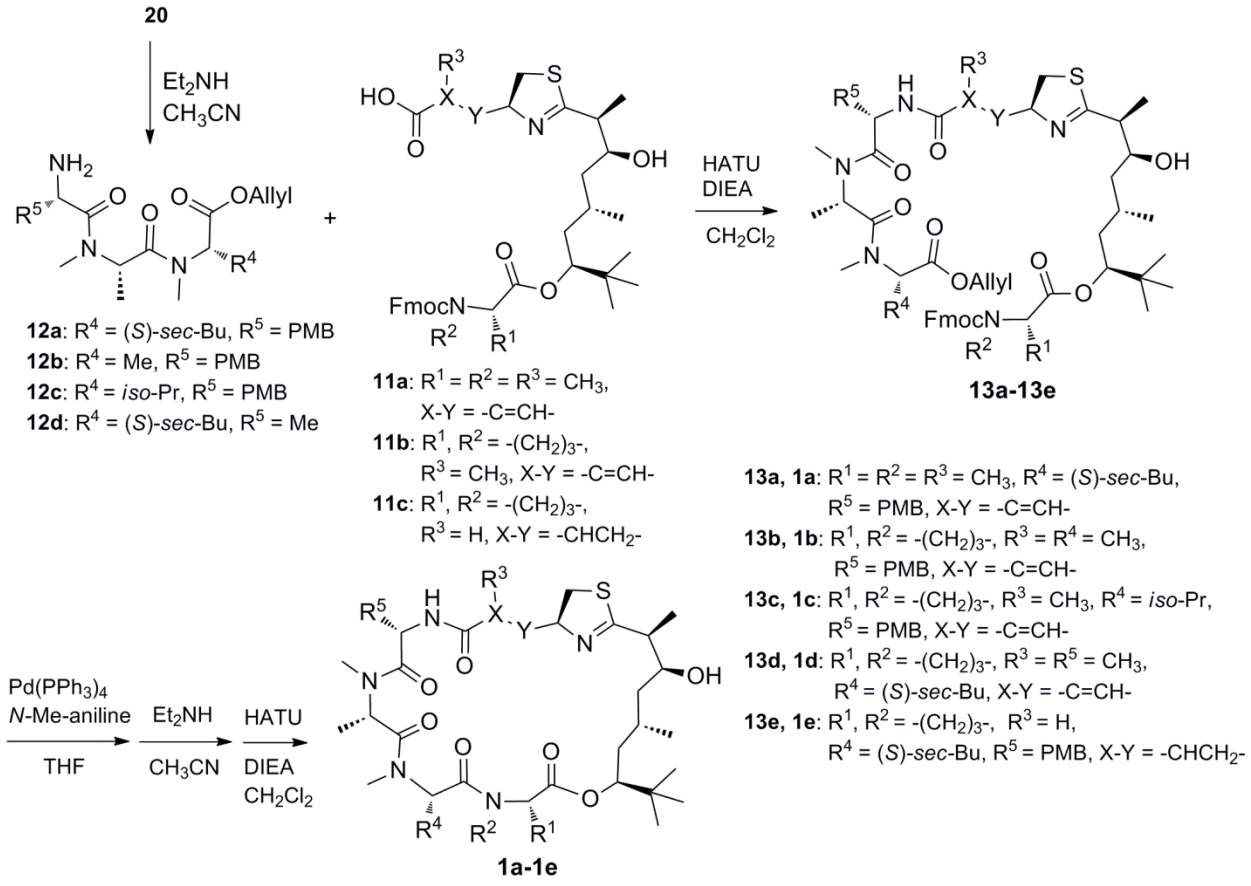
***N*-Fmoc-Tyr(*O*-Me)-(*N*-Me)Ala-(*N*-Me)Ala-OAllyl (20b)** (82%). $[\alpha]^{25}_{D}$: -16.7(c 0.03, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers): δ 7.76 (d, J = 7.6 Hz, 2H), 7.56 (m, 2H), 7.40 (dd, J = 7.6, 7.2 Hz, 2H), 7.31 (dd, J = 7.4, 7.2 Hz, 2H), 7.10 (d, J = 8.4 Hz, 2H), 5.94-5.84 (m, 1H), 5.44 (q, J = 6.8 Hz, 1H), 5.33-5.23 (m, 2H), 5.02 (q, J = 7.2 Hz, 1H), 4.94-4.88 (m, 1H), 4.70-4.50 (m, 2H), 4.41-4.27 (m, 2H), 4.22-4.16 (m, 1H), 3.74 (s, 2.7 H), 3.72 (s, 0.3H), 3.06-3.00 (m, 1H), 2,.99 (s, 0.3H), 2.96 (s, 2.7H), 2.90-2.85 (m, 1H), 2.83 (s, 0.3H), 2.76 (s, 2.7H), 1.49 (d, J = 7.2 Hz, 0.6H), 1.38 (d, J = 7.2 Hz, 2.4H), 1.30 (d, J = 6.8 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.5, 171.5, 171.3, 158.9, 155.9, 144.0, 141.5, 132.0, 130.7, 128.1, 128.0, 127.3, 125.4, 125.3, 120.2, 118.9, 114.2, 114.1, 77.5, 67.2, 66.0, 55.4, 53.3, 52.4, 49.7,

47.4, 38.3, 31.7, 30.6, 14.6, 14.5 ppm. HRMS (ESI) *m/z* calcd for $C_{36}H_{41}N_3O_7$ $(M+Na)^+$ 650.2837, found 650.2825.

***N*-Fmoc-Tyr(*O*-Me)-(*N*-Me)Ala-(*N*-Me)Val-OAllyl (20c)** (Prepared according to **procedure A** and **B**; procedure A 15% yield in 2 steps, procedure B 80% yield in 2 steps). $[\alpha]^{25}_D$: -89.1 (c 0.32, CH_2Cl_2) ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers): δ 7.75 (d, J = 7.6 Hz, 2H), 7.55 (m, 2H), 7.39 (dd, J = 6.8, 7.6 Hz, 2H), 7.30 (dd, J = 7.2, 7.4 Hz, 2H), 7.11 (d, J = 8.0 Hz, 2H), 6.78 (d, J = 8.4 Hz, 2H), 5.93-5.81 (m, 1H), 5.61 (d, J = 9.2 Hz, 0.83H), 5.50 (d, J = 9.2 Hz, 0.17H), 5.42 (q, J = 6.4 Hz, 1H), 5.32-5.22 (m, 2H), 4.92 (q, J = 6.8 Hz, 1H), 4.82 (d, J = 10.4 Hz, 1H), 4.6 (d, J = 5.6 Hz, 2H), 4.40-4.10 (m, 3H), 3.74 (s, 2.5H), 3.71 (s, 0.5H), 3.05-3.00 (m, 1H), 2.97 (s, 2.5H), 2.88-2.83 (m, 1H), 2.76 (s, 2.5H), 2.50 (s, 0.5H), 2.36 (s, 0.5H), 2.19-2.10 (m, 1H), 1.28 (d, J = 7.2 Hz, 3H), 1.07 (d, J = 6.0 Hz, 0.5H), 1.00 (d, J = 6.4 Hz, 2.5H), 0.93 (d, J = 6.4 Hz, 0.5H), 0.75 (d, J = 6.8 Hz, 2.5H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, mixture of rotamers): δ 171.9, 171.6, 170.6, 158.7, 155.8, 143.9, 141.4, 131.8, 130.6, 128.0, 127.8, 127.1, 125.2, 125.2, 120.1, 118.8, 114.0, 67.1, 65.5, 62.0, 55.2, 52.3, 49.7, 47.2, 38.1, 31.1, 30.7, 27.4, 19.9, 19.1, 14.5 ppm. HRMS (ESI) *m/z* calcd for $C_{38}H_{45}N_3O_7$ $(M+Na)^+$ 678.3150, found 678.3157.

***N*-Fmoc-Ala-(*N*-Me)Ala-(*N*-Me)Ile-OAllyl (20d)** (86%). $[\alpha]^{25}_D$: -73.1 (c 0.42, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers): δ 7.74 (d, J = 7.6 Hz, 2H), 7.59 (dd, J = 8.0, 2.8 Hz, 2H), 7.38 (dd, J = 7.6, 7.6 Hz, 2H), 7.29 (dd, J = 7.6, 7.6 Hz, 2H), 5.93-5.82 (m, 1.8H), 5.70 (d, J = 8.0 Hz, 0.2H), 5.52 (q, J = 7.2 Hz, 1H), 5.30 (dd, J = 17.2, 1.2 Hz, 1H), 5.22 (dd, J = 10.4, 1.2 Hz, 1H), 4.93 (d, J = 10.4 Hz, 1H), 4.71-4.52 (m, 3H), 4.35 (d, J = 6.8 Hz, 2H), 4.21-4.18 (m, 0.8H), 4.15-4.11 (m, 0.2H), 3.00 (s, 2.4H), 2.98 (s, 2.4H), 2.93 (s, 0.6H), 2.82 (s, 0.6H), 2.09-2.01 (m, 1H), 1.33-1.21 (m, 7H), 1.11-0.94 (m, 4H), 0.90-0.82 (m, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, major of the mixture of rotamers): δ 172.6, 172.1, 171.9, 171.2, 170.7, 169.7, 155.6, 144.0, 143.9, 143.8, 141.3, 131.7, 131.4, 127.7, 127.1, 125.2, 120.0, 119.9, 118.7, 67.0, 67.0, 66.0, 65.5, 64.4, 60.6, 49.3, 49.1, 47.3, 47.2, 34.5, 33.2, 31.1, 30.3, 30.1, 25.1, 25.0, 22.7, 18.8, 18.6, 16.1, 15.8, 14.9, 14.3, 14.2, 11.7, 10.5 ppm. HRMS (ESI) *m/z* calcd for $C_{32}H_{41}N_3O_6$ $(M+Na)^+$ 586.2888, found 586.2891.

Synthesis of Final Target Analogues 1a-1e



Scheme S6. Synthesis of targets **1a-1e**.

The synthesis of cyclic precursors 13a-13e: To a solution of Fmoc protected tripeptide **20** (0.064 mmol) in MeCN (2.4 ml) was added diethylamine (1.2 ml) at room temperature. After being stirred at the same temperature for 30 min, the reaction mixture was evaporated in vacuo, then azeotroped with toluene and CH_2Cl_2 two times, respectively, and dried under reduced pressure for 1 h to give the free amine **12**, which was used in the next coupling step without further purification.

The above crude amine **12** was dissolved in CH_2Cl_2 (1.5 ml). To this solution was added acid **11** (0.032 mmol) in CH_2Cl_2 (0.5 ml), HATU (26.1 mg, 0.069 mmol), DIEA (0.025 ml, 0.144 mmol) at room temperature. After being stirred at the same temperature for 10 h, the reaction mixture was concentrated in vacuo and purified by preparative TLC plate (delevoped by acetone/hexane (2:3, v/v)) to give the precursor **13**.

Cyclic precursor (**13a**) (76% in 2 steps). $[\alpha]^{25}_{D}$: -46.5 (c 0.20, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$, mixture of rotamers): δ 7.76 (d, *J* = 7.6 Hz, 2H), 7.63-7.53 (m, 2H), 7.39 (dd, *J* = 7.2, 7.4 Hz, 2H), 7.30 (dd, *J* = 7.2, 7.2 Hz, 2H), 7.08 (d, *J* = 8.4 Hz, 2H), 6.78 (d, *J* = 7.6 Hz, 2H), 6.58-6.51 (m, 1H), 6.32-6.27 (m, 1H), 5.93-5.83 (m, 1H), 5.42-5.36 (m, 1H), 5.32-5.07 (m, 4H), 4.93 (d, *J* = 10.0 Hz, 1H), 4.86-4.81 (m, 2H), 4.59 (d, *J* = 6.0 Hz, 2H), 4.70-4.24 (m, 3H), 3.75 (s, 3H), 3.73 (br m, 1H), 3.67-3.64 (m, 1H), 3.43-3.33 (m, 1H), 3.10-3.02 (m, 1H), 2.98-2.84 (m, 9H), 2.74-2.56 (m, 4H), 2.02-1.80 (m, 5H), 1.74-1.63 (m, 1H), 1.56-1.42 (m, 4H), 1.38-1.17 (m, 9H), 1.02-0.84 (m, 19H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, mixture of rotamers): δ 172.6, 172.4, 172.0, 171.6, 171.5, 170.8, 168.2, 158.8, 155.2, 144.3, 144.1, 141.5, 131.9, 130.6, 127.8, 127.2, 125.2, 120.1, 118.8, 114.1, 77.9, 74.2, 71.7, 67.8, 65.5, 60.6, 55.3, 54.7, 50.7, 49.8, 47.4, 45.5, 40.0, 38.0, 37.9, 37.7, 34.9, 34.8, 33.5, 32.1, 31.1, 30.7, 30.5, 30.1, 29.5, 26.2, 26.1, 25.7, 25.2, 22.8, 20.8, 20.7, 16.3, 15.9, 15.0, 14.5, 14.3, 13.6, 10.8 ppm. HRMS (ESI) *m/z* calcd for $C_{62}H_{85}N_5O_{11}S$ $(M+H)^+$ 1108.6039, found 1108.6037.

Cyclic precursor (**13b**) (70% in 2 steps). $[\alpha]^{25}_{D}$: -59.0 (c 0.20, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers): δ 7.75 (d, *J* = 7.6 Hz, 2H), 7.66-7.60 (m, 2H), 7.39 (dd, *J* = 7.2, 7.2 Hz, 2H), 7.30 (dd, *J* = 7.2, 7.4 Hz, 2H), 7.10-7.06 (m, 2H), 6.78-6.76 (m, 2H), 6.57 (br m, 1H), 6.32-6.27 (m, 1H), 5.93-5.83 (m, 1H), 5.42-5.35 (m, 1H), 5.32-5.25 (m, 2H), 5.22-4.98 (m, 3H), 4.92-4.89 (m, 1H), 4.59 (d, *J* = 5.6 Hz, 2H), 4.52-4.19 (m, 5H), 3.76-3.75 (br m, 1H), 3.75 (s, 3H), 3.69-3.61 (m, 1H), 3.52 (br m, 1H), 3.43-3.26 (m, 1H), 3.06-2.98 (m, 2H), 2.96-2.80 (m, 5H), 2.74-2.69 (m, 3H), 2.25 (br m, 1H), 2.08-1.60 (m, 9H), 1.47 (d, *J* = 6.4 Hz, 1H), 1.37 (d, *J* = 7.2 Hz, 3H), 1.30-1.22 (m, 6H), 1.14-1.07 (m, 1H), 0.97-0.94 (m, 3H), 0.88 (s, 9H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, mixture of rotamers): δ 172.6, 171.4, 171.3, 171.2, 168.2, 158.7, 155.2, 144.3, 144.1, 144.0, 141.4, 141.3, 131.8, 130.6, 128.0, 127.8, 127.1, 125.4, 125.3, 120.0, 118.8, 114.0, 78.6, 78.5, 71.6, 70.8, 67.9, 65.9, 59.7, 55.3, 53.2, 50.7, 49.6, 47.3, 47.0, 46.6,46.1, 45.9, 40.1, 39.7, 39.1, 37.8, 37.6, 37.5, 34.8, 34.6, 31.7, 31.6, 30.5, 30.0, 29.8, 26.1, 26.1, 25.2, 25.1, 24.6, 23.4, 22.8, 20.6, 20.5, 16.1, 15.6, 14.5, 14.3, 13.5 ppm. HRMS (ESI) *m/z* calcd for $C_{60}H_{79}N_5O_{11}S$ $(M+H)^+$ 1078.5570, found 1078.5579.

Cyclic precursor (**13c**) (88% in 2 steps). $[\alpha]^{25}_{D}$: -62.5 (c 0.12, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers): δ 7.75 (d, *J* = 7.6 Hz, 2H), 7.66-7.56 (m, 2H), 7.38 (dd, *J* = 7.2, 7.4 Hz, 2H), 7.29 (dd, *J* = 7.6, 7.6 Hz, 2H), 7.10-7.06 (m, 2H), 6.77-6.75 (m, 2H), 6.54 (d, *J* =

7.6 Hz, 1H), 6.31-6.27 (m, 1H), 5.92-5.83 (m, 1H), 5.40-5.34 (m, 1H), 5.31-5.08 (m, 4H), 4.93-4.88 (m, 1H), 4.81 (d, *J* = 10.4 Hz, 1H), 4.59 (d, *J* = 6.0 Hz, 2H), 4.52-4.21 (m, 4H), 3.75 (s, 3H), 3.75-3.22 (m, 5H), 3.05-2.79 (m, 6H), 2.74-2.58 (m, 4H), 2.30-1.82 (m, 8H), 1.78-1.59 (m, 2H), 1.52-1.48 (m, 1H), 1.39-1.18 (m, 8H), 1.00-0.73 (m, 18H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, mixture of rotamers): δ 176.3, 172.5, 171.9, 171.6, 170.6, 168.3, 158.7, 155.1, 155.0, 154.4, 144.3, 144.1, 141.4, 135.0, 134.7, 132.8, 131.8, 131.4, 130.5, 130.4, 128.0, 127.7, 127.1, 125.5, 125.4, 125.3, 120.0, 118.8, 114.0, 78.5, 77.4, 74.1, 71.6, 70.8, 67.8, 65.5, 62.0, 59.7, 55.2, 50.6, 49.7, 47.3, 46.6, 46.0, 39.8, 39.0, 37.9, 37.8, 37.7, 34.8, 34.7, 31.7, 31.3, 31.1, 30.7, 30.0, 27.4, 26.1, 25.2, 25.1, 24.6, 23.4, 22.8, 20.6, 20.5, 19.9, 19.1, 15.5, 14.5, 13.5 ppm. HRMS (ESI) *m/z* calcd for $C_{62}H_{83}N_5O_{11}S$ $(M+H)^+$ 1106.5883, found 1106.5885.

Cyclic precursor (**13d**) (73% in 2 steps). $[\alpha]^{25}_D$: -130.0 (c 0.20, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$, mixture of rotamers): δ 7.75 (d, *J* = 7.6 Hz, 2H), 7.63 (m, 2H), 7.38 (dd, *J* = 7.2, 7.4 Hz, 2H), 7.30 (dd, *J* = 7.6, 7.6 Hz, 2H), 6.86-6.83 (m, 1H), 6.42-6.32 (m, 1H), 5.94-5.84 (m, 1H), 5.54-5.47 (m, 1H), 5.32-5.22 (m, 2H), 5.19-5.06 (m, 1H), 4.93-4.86 (m, 3H), 4.67-4.17 (m, 7H), 3.77 (br m, 1H), 3.69-3.60 (m, 1H), 3.56-3.46 (m, 1H), 3.43-3.26 (m, 1H), 3.01-2.99 (m, 3H), 2.97-2.95 (m, 3H), 2.95-2.90 (m, 1H), 2.70-2.58 (m, 1H), 2.30-2.17 (m, 1H), 2.06-1.90 (m, 7H), 1.83 (br m, 1H), 1.78-1.59 (m, 2H), 1.51-1.41 (m, 1H), 1.35-1.25 (m, 10H), 1.21-1.19 (m, 3H), 1.07-0.95 (m, 5H), 0.87-0.83 (m, 12H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, mixture of rotamers): δ 177.1, 176.1, 172.7, 172.6, 171.9, 170.8, 169.8, 168.0, 155.1, 155.0, 154.4, 144.4, 144.1, 141.5, 141.4, 135.0, 134.7, 132.9, 131.9, 127.8, 127.7, 127.1, 125.4, 125.3, 120.0, 118.8, 78.7, 78.5, 74.2, 73.7, 71.7, 70.9, 67.9, 67.8, 66.1, 65.6, 60.7, 59.7, 49.3, 47.4, 47.3, 47.1, 46.6, 46.2, 46.0, 39.8, 39.1, 37.9, 37.7, 37.6, 34.9, 34.8, 34.7, 33.4, 31.2, 30.3, 30.0, 29.8, 29.5, 26.1,26.1, 25.3, 25.1, 24.6, 23.4, 22.8, 20.6, 20.5, 18.6, 18.5, 16.2, 15.9, 15.6, 15.0, 14.5, 14.4, 14.2, 13.5, 11.8, 10.6 ppm. HRMS (ESI) *m/z* calcd for $C_{56}H_{80}N_5O_{10}S$ $(M+H)^+$ 1014.5620, found 1014.5641.

Cyclic precursor (**13e**) (65% in 2 steps). $[\alpha]^{25}_D$: -92.2 (c 0.09, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers): δ 7.75 (d, *J* = 7.2 Hz, 2H), 7.64-7.56 (m, 2H), 7.38 (dd, *J* = 7.2, 7.4 Hz, 2H), 7.29 (dd, *J* = 7.2, 7.4 Hz, 2H), 7.09 (d, *J* = 8.4 Hz, 2H), 6.76 (d, *J* = 8.4 Hz, 2H), 5.92-5.82 (m, 1H), 5.39-5.35 (m, 1H), 5.27-5.13 (m, 3H), 4.73-4.80 (m, 2H), 4.58-4.51 (m, 2H), 4.45-4.18 (m, 5H), 3.80-3.74 (br m, 1H), 3.74 (s, 3H), 3.63 (br m, 1H), 3.52 (br m, 1H), 3.27-3.11 (m, 1H), 3.03-2.89 (m, 4H), 2.82-2.62 (m, 6H), 2.39-2.20 (m, 3H), 2.07-1.58 (m, 8H), 1.50-

1.41 (m, 1H), 1.36-1.17 (m, 8H), 1.03-0.92 (m, 8H), 0.87-0.82 (m, 12H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, mixture of rotamers): δ 173.2, 173.0, 172.7, 172.3, 172.3, 172.2, 171.9, 171.9, 171.7, 171.6, 171.4, 170.8, 158.6, 155.1, 155.0, 154.6, 144.6, 144.3, 144.1, 144.0, 143.9, 141.4, 141.3, 131.8, 130.5, 130.5, 128.4, 128.3, 127.7, 127.1, 125.5, 125.4, 125.3, 120.0, 118.8, 114.0, 79.8, 79.4, 78.7, 78.7, 77.4, 71.6, 70.8, 70.6, 68.0, 67.8, 66.1, 65.5, 63.8, 60.6, 59.7, 55.3, 50.4, 49.8, 49.8, 47.3, 47.3, 47.2, 47.0, 46.6, 45.8, 45.1, 40.5, 39.7, 39.5, 38.1, 37.8, 37.8, 37.6, 37.5, 37.4, 37.2, 34.8, 34.7, 33.6, 33.5, 33.4, 31.3, 31.0, 30.6, 30.0, 29.8, 26.1, 25.3, 25.2, 25.1, 24.6, 23.4, 22.8, 20.6, 16.7, 16.2, 16.0, 15.9, 14.6, 14.4, 14.2, 11.7, 10.6 ppm. HRMS (ESI) *m/z* calcd for $C_{62}H_{85}N_5O_{11}S$ $(M+H)^+$ 1108.6039, found 1108.6041.

Synthesis of final targets 1a-1e: To a solution of cyclic precursor **13** (25.2 µmol) in THF (1.0 ml) were added $Pd(PPh_3)_4$ (2.9 mg, 2.5 µmol), and *N*-methylaniline (8.2 µl, 75.5 µmol) at room temperature under argon. This reaction was protected with aluminum foil. After being stirred at the same temperature for 1h, the reaction mixture was concentrated in vacuo and purified by preparative TLC plate (developed with MeOH/CH_2Cl_2 1:9, v/v) to give the free acid cyclic precursor. To the solution of free acid cyclic precursor in CH_2Cl_2 (40 ml) were added DIEA (45 µl, 0.525 mmol), and HATU (29 mg, 75.5 µmol) at 0^{o}C. After being stirred at 0^{o}C for 30 min, the reaction was allowed to warm up to room temperature and stirred for additional 20 h. Then the reaction was concentrated in vacuo and purified by semipreparative reversed-phase HPLC (Phenomenex Ultracarb, ODS 250 × 10 mm, 5 µm, 3.0 mL/min, UV detection at 200/220 nm) using an isocratic system of 80% aqueous MeCN for 30 min, 80-100% MeCN for 30-40 min, and 100% MeCN for 40-60 min to afford **1a-1e**.

Apratoxin F (1a)[6] (45% in 3 steps). $[\alpha]^{25}_D$: -141.2 (c 0.131, CH_2Cl_2) (Lit[6] $[\alpha]_D$: -250 (c 0.33, CH_3CN)). t_R = 21.5 min. ^{1}H NMR (500 MHz, $CDCl_3$, major of the mixture of rotamers, major rotamer > 90%): δ 7.16 (d, *J* = 8.5 Hz, 2H), 6.80 (d, *J* = 8.5 Hz, 2H), 6.35 (d, *J* = 9.5, 1.0 Hz, 1H), 6.01 (d, *J* = 9.5 Hz, 1H), 5.48 (d, *J* = 11.5 Hz, 1H), 5.24 (ddd, *J* = 9.0, 9.0, 4.5 Hz, 1H), 5.05 (ddd, *J* = 10.5, 9.5, 4.5 Hz, 1H), 4.89 (dd, *J* = 12.5, 2.0 Hz, 1H), 4.48 (q, *J* = 8.0, 1H), 4.47 (d, *J* = 11.0 Hz, 1H), 3.78 (s, 3H), 3.56 (dddd, *J* = 11.0, 10.5, 10.5, 3.0, 1H), 3.46 (dd, *J* = 11.0, 9.0 Hz, 1H), 3.29 (br m, 1H), 3.27 (s, 3H), 3.17-3.06 (m, 2H), 2.87 (dd, *J* = 12.0, 5.0 Hz, 1H), 2.80 (s, 3H), 2.69 (s, 3H), 2.66-2.61 (m, 1H), 2.27 (br m, 1H), 2.13 (br m, 1H), 1.96 (s, 3H), 1.79 (ddd, *J* = 14.0, 13.0, 3.5 Hz, 1H), 1.50-1.48 (m, 1H), 1.44 (d, *J* = 7.5 Hz, 3H), 1.37-1.26 (m, 1H),

1.22 (d, *J* = 7.0 Hz, 3H), 1.14-1.08 (m, 1H), 1.06 (d, *J* = 6.5 Hz, 3H), 1.00 (d, *J* = 6.5 Hz, 3H), 0.97-0.95 (m, 1H), 0.92 (d, *J* = 6.9 Hz, 3H), 0.92 (d, *J* = 6.5 Hz, 3H), 0.88 (s, 9H) ppm. ^{13}C NMR (100 Hz, $CDCl_3$, major of the mixture of rotamers, major rotamer > 90%): δ177.4, 173.8,173.2, 170.6, 170.1, 169.7, 158.8, 136.4, 130.8, 130.5, 128.4, 114.3, 114.2, 114.0, 77.5, 72.7, 71.8, 60.8, 57.4, 55.4, 55.0, 54.5, 49.0, 38.5, 37.9, 37.7, 37.4, 36.8, 35.2, 35.1, 31.8, 31.2, 30.5, 29.9, 26.3, 26.2, 24.8, 24.5, 20.0, 16.8, 14.9, 14.3, 14.1, 13.5, 9.0 ppm. HRMS (ESI) *m/z* calcd for $C_{44}H_{69}N_5O_8S$ $(M+H)^+$ 828.4940, found 828.4950.

Apratoxin S1 (1b) (18% in 3 steps). $[\alpha]^{25}_D$: -148.1 (c 0.133, CH_2Cl_2). t_R = 10.1 min. ^{1}H NMR (500 MHz, $CDCl_3$, major of the mixture of rotamers, major rotamer > 90%): *δ* 7.14 (d, *J* = 8.5 Hz, 2H), 6.81 (d, *J* = 8.5 Hz, 2H), 6.42 (d, *J* = 9.0 Hz, 1H), 6.25 (d, *J* = 8.5 Hz, 1H), 5.52 (ddd, *J* = 7.0, 7.0, 7.0 Hz, 1H), 5.23 (ddd, *J* = 8.5, 8.5, 5.0 Hz, 1H), 4.99 (q, *J* = 8.0 Hz, 1H), 4.92 (dd, *J* = 12.5, 2.0 Hz, 1H), 4.48 (d, *J* = 11.0 Hz, 1H), 4.23 (t, *J* = 6.5 Hz, 1H), 4.03-3.98 (m, 1H), 3.78 (s, 3H), 3.73-3.68 (m, 1H), 3.55 (dddd, *J* = 11.0, 10.5, 10.5, 3.0 Hz, 1H), 3.48 (dd, *J* = 11.0, 9.0 Hz, 1H), 3.33 (br m, 1H), 3.15 (dd, *J* = 11.0, 5.0 Hz, 1H), 2.96 (d, *J* = 8.0 Hz, 2H), 2.69 (s, 3H), 2.67 (m, 1H) ,2.64 (s, 3H), 2.24-2.19 (m, 1H), 2.11 (br m, 1H), 2.06-2.04 (m, 1H), 1.97 (s, 3H), 1.91-1.85 (m, 2H), 1.79-1.74 (m, 1H), 1.62-1.56 (m, 1H), 1.31-1.28 (m, 1H), 1.21 (d, *J* = 6.5 Hz, 3H), 1.15 (d, *J* = 7.0 Hz, 3H), 1.11 (m, 1H), 1.06 (d, *J* = 7.0 Hz, 3H), 0.99 (d, *J* = 6.5 Hz, 3H), 0.87 (s, 9H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, major of the mixture of rotamers, major rotamer > 90%): *δ* 177.2, 172.2, 171.0, 170.4, 169.9, 169.1, 158.9, 136.7, 131.2, 130.8, 128.1, 114.1, 77.6, 72.8, 71.8, 59.7, 55.4, 50.8, 50.0, 49.2, 47.5, 38.5, 38.1, 37.8, 37.7, 36.4, 35.0, 30.4, 30.1, 29.8, 29.2, 26.2, 25.5, 24.4, 20.0, 16.7, 15.2, 14.0, 13.3 ppm. HRMS (ESI) *m/z* calcd for $C_{42}H_{63}N_5O_8S$ $(M+Na)^+$ 820.4290, found 820.4290.

Apratoxin S2 (1c) (36% in 3 steps). $[\alpha]^{25}_D$: -189.5 (c 0.246, CH_2Cl_2). t_R = 18.0 min. ^{1}H NMR (500 MHz, $CDCl_3$, major of the mixture of rotamers, major rotamer > 90%): *δ* 7.14 (d, *J* = 8.5 Hz, 2H), 6.80 (d, *J* = 9.0 Hz, 2H), 6.43 (dd, *J* = 10.0, 1.0 Hz, 1H), 6.09 (d, *J* = 9.0 Hz, 1H), 5.27 (ddd, *J* = 10.0, 8.8, 3.5 Hz, 1H), 5.06 (ddd, *J* = 11.0, 9.5, 5.0 Hz, 1H), 5.01 (d, *J* = 11.5 Hz, 1H), 4.97 (dd, *J* = 12.8, 2.5 Hz, 1H), 4.67 (d, *J* = 10.5 Hz, 1H), 4.29-4.25 (m, 1H), 4.19 (t, *J* = 8.0 Hz, 1H), 3.77 (s, 3H), 3.69-3.63 (m, 1H), 3.54 (dddd, *J* = 11.0, 10.5, 10.5, 3.5 Hz, 1H), 3.46 (dd, *J* = 11.0, 9.0 Hz, 1H), 3.27 (br m, 1H), 3.16 (dd, *J* = 11.0, 3.5 Hz, 1H), 3.08 (dd, *J* = 12.5, 12.5 Hz, 1H), 2.87 (dd, *J* = 13.0, 5.0 Hz, 1H), 2.81 (s, 3H), 2.69 (s, 3H), 2.67-2.60 (m, 1H), 2.27-2.20 (m,

2H), 2.16 (br m, 1H), 2.05 (br m, 1H), 1.97 (s, 3H), 1.93-1.84 (m, 2H), 1.79 (ddd, *J* = 14.0, 13.0, 3.5 Hz, 1H), 1.60-1.55 (m, 1H), 1.29-1.23 (m, 1H), 1.18 (d, *J* = 6.5 Hz, 3H), 1.13-1.09 (m, 1H), 1.07 (d, *J* = 7.0 Hz, 3H), 0.99 (d, *J* = 6.0 Hz, 3H), 0.99 (d, *J* = 6.0 Hz, 3H), 0.87 (s, 9H), 0.73 (d, *J* = 6.0 Hz, 3H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, major of the mixture of rotamers, major rotamer > 90%): δ 177.7, 172.9, 170.9, 170.6, 170.3, 169.5, 158.8, 136.6, 130.8, 130.3, 114.0, 77.6, 72.6, 71.7, 60.8, 59.9, 59.6, 55.4, 50.6, 49.3, 47.8, 38.3, 37.8, 37.7, 37.3, 36.9, 35.0, 30.6, 29.4, 27.4, 26.1, 25.8, 24.4, 19.9, 19.9, 19.0, 16.8, 14.0, 13.3 ppm. HRMS (ESI) *m/z* calcd for $C_{44}H_{67}N_5O_8S$ $(M+H)^+$ 826.4783, found 826.4789.

Apratoxin S3 (1d) (18% in 3 steps). $[\alpha]^{25}_D$: -217.9 (c 0.145, CH_2Cl_2). t_R = 15.8 min. ^{1}H NMR (500 MHz, $CDCl_3$, major of the mixture of rotamers, major rotamer > 90%): *δ* 6.30 (dd, *J* = 9.0, 1.0 Hz, 1H), 5.77 (d, *J* = 9.5 Hz, 1H), 5.20 (ddd, *J* = 9.0, 9.0, 6.0 Hz, 1H), 5.13 (d, *J* = 11.5 Hz, 1H), 5.03-4.96 (m, 2H), 4.75 (d, *J* = 11.0 Hz, 1H), 4.31-4.27 (m, 1H), 4.22 (t, *J* = 8.0 Hz, 1H), 3.68-3.63 (m, 1H), 3.58 (dddd, *J* = 11.0, 10.5, 10.5, 3.0 Hz, 1H), 3.50 (q, *J* = 7.0 Hz, 1H),3.47 (dd, *J* = 10.5, 9.0 Hz, 1H), 3.20 (s, 3H), 3.09 (dd, *J* = 11.0, 6.0 Hz, 1H), 2.84 (s, 3H), 2.63 (dq, *J* = 10.0, 7.0, Hz, 1H), 2.31-2.23 (m, 2H), 2.16 (br, 1H), 2.01-1.96 (m, 1H),1.94 (s, 3H), 1.91-1.85 (m,1H), 1.80 (ddd, *J* = 14.0, 13.0, 3.0 Hz, 1H), 1.60-1.55 (m, 1H), 1.42 (d, *J* = 7.0 Hz, 3H), 1.31 (d, *J* = 6.5 Hz, 3H), 1.29-1.27 (m, 1H), 1.14-1.12 (m, 1H), 1.09-1.08 (m, 1H), 1.06 (d, *J* = 7.0 Hz, 3H), 1.00 (d, *J* = 7.5 Hz, 3H), 0.97 (d, *J* = 7.0 Hz, 3H), 0.96-0.93 (m, 1H), 0.90 (t, *J* = 7.5 Hz, 3H), 0.88 (s, 9H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, major of the mixture of rotamers, major rotamer > 90%): *δ* 177.3, 172.7, 170.8, 170.7, 170.5, 169.6, 137.8, 136.5, 131.7,128.5, 77.8, 77.5, 73.0, 72.6, 72.2, 71.9, 60.4, 59.9, 59.2, 58.0, 57.0, 49.3, 48.4, 47.8, 44.8, 44.0, 38.6, 38.3, 37.8, 37.7, 37.3, 37.1, 36.9, 35.2, 35.1, 33.6, 32.5, 31.0, 30.3, 29.9, 29.6, 29.4, 29.3, 26.3, 26.2, 25.8, 25.4, 25.1, 25.1, 24.9, 24.5, 20.3, 20.0, 19.3, 17.3, 17.0, 16.9, 16.6, 14.2, 14.1, 14.1, 13.6, 12.8, 9.8, 9.3 ppm. HRMS (ESI) *m/z* calcd for $C_{38}H_{63}N_5O_7S$ $(M+Na)^+$ 756.4640, found 756.4341.

Apratoxin S4 (1e) (52% in 3 steps). $[\alpha]^{25}_D$: -69.1 (c 0.109, CH_2Cl_2). t_R = 17.9 min. ^{1}H NMR (600 MHz, $CDCl_3$, mixture of rotamers, major and minor (7/3)): *δ* 7.13 (d, *J* = 8.4 Hz, 1.4H), 7.12 (d, *J* = 8.4 Hz, 0.6H), 6.80 (d, *J* = 8.4 Hz, 0.6H), 6.78 (d, *J* = 8.4 Hz, 1.4H), 6.17 (d, *J* = 9.0 Hz, 0.3H), 5.80 (d, *J* = 9.6 Hz, 0.7H), 5.27 (d, *J* = 11.4 Hz, 0.7H), 5.14 (ddd, *J* = 10.2, 10.2, 4.8 Hz, 1H), 4.96 (dd, *J* = 12.6, 2.4 Hz, 0.7H), 4.89 (d, *J* = 11.4 Hz, 0.3H), 4.87 (dd, *J* = 12.6, 2.4

Hz, 0.3H), 4.62 (q, *J* = 6.6 Hz, 0.3H), 4.52 (d, *J* = 10.8 Hz, 0.7H), 4.36-4.32 (m, 0.7H), 4.31-4.27 (m, 0.7H), 4.21 (t, *J* = 7.8 Hz, 1H), 4.20-4.17 (m, 0.3H), 4.10-4.07 (m, 0.3H), 3.81 (d, *J* = 10.8 Hz, 0.3H), 3.76 (s, 2.1H), 3.76 (s, 0.9H), 3.70-3.66 (m, 0.7H), 3.65-3.61 (m, 0.3H), 3.60-3.53 (m, 1H), 3.31 (dd, *J* = 10.8, 8.4 Hz, 0.7H), 3.29 (q, *J* = 6.6 Hz, 0.7H), 3.23 (dd, *J* = 10.8, 8.4 Hz, 0.3H), 3.09 (dd, *J* = 12.0, 11.4 Hz, 1H), 3.01 (dd, *J* = 10.8, 4.8 Hz, 1H), 2.96 (dd, *J* = 12.6, 4.2 Hz, 0.3H), 2.88 (s, 0.9H), 2.80 (s, 2.1H), 2.77 (dd, *J* = 12.6, 4.8 Hz, 0.7H), 2.72 (s, 2.1H), 2.64 (dq, *J* = 9.9, 6.6 Hz, 0.3H), 2.61 (s, 0.9H), 2.59 (dq, *J* = 9.9, 6.6 Hz, 0.7H), 2.48 (ddd, *J* = 14.7, 12.9, 3.6 Hz, 0.7H), 2.39-2.34 (m, 1H), 2.30-2.21 (m, 2H), 2.13 (br m, 1H), 2.08-2.04 (m, 0.7H), 1.94-1.84 (m, 3.3H), 1.82-1.74 (m, 3H), 1.57-1.50 (m, 1.4H), 1.42-1.37 (m, 0.3H), 1.30-1.24 (m, 1H), 1.22 (d, *J* = 6.6 Hz, 2.1 H), 1.20-1.18 (m, 0.3H), 1.12-1.09 (m, 0.3H), 1.07 (d, *J* = 7.2 H, 0.9H), 1.06 (d, *J* = 7.2 Hz, 0.9H), 1.03 (d, *J* = 7.2 Hz, 2.1H), 1.03 (t, *J* = 7.2 Hz, 2.1H), 1.00 (d, *J* = 6.6 Hz, 2.1H), 0.98 (d, *J* = 6.6 Hz, 2.1H), 0.97 (d, *J* = 7.2 Hz, 0.9H), 0.95-0.90 (m, 0.7H), 0.87 (s, 9H), 0.84 (t, *J* = 7.2 Hz, 0.9H), 0.54 (d, *J* = 6.6 Hz, 0.9H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, mixture of rotamers, major and minor): δ 176.1, 175.2, 172.6, 172.1, 172.0, 171.2, 170.6, 170.4, 170.2, 169.9, 158.9, 158.7, 130.7, 130.6, 128.7, 128.5, 114.2, 114.0, 78.0, 77.5, 75.6, 75.2, 72.5, 71.7, 60.7, 59.8, 59.3, 57.9, 57.1, 55.5, 55.4, 53.8, 51.0, 49.8, 49.0, 47.9, 39.9, 39.0, 38.0, 37.8, 37.5, 37.5, 37.4, 36.9, 35.9, 35.1, 35.0, 34.6, 34.0, 33.7, 33.7, 32.6, 31.5, 30.7, 30.7, 30.7, 29.8, 29.3, 29.3, 28.9, 26.3, 26.2, 25.7, 25.5, 25.2, 25.1, 24.5, 20.7, 20.0, 16.6, 16.5, 15.0, 14.3, 14.1, 14.1, 9.9, 9.7 ppm. HRMS (ESI) *m/z* calcd for $C_{44}H_{69}N_5O_8S$ $(M+H)^+$ 828.4940, found 828.4951.

Apratoxin S5: *2-epi*-**Apratoxin S4 (***2-epi*-**1e**) (14% in 3 steps). $[\alpha]^{25}_D$:-61.6 (c 0.134, CH_2Cl_2). t_R = 19.7 min. ^{1}H NMR (500 MHz, $CDCl_3$, major of the mixture of rotamers, major rotamer > 90%): δ 7.14 (d, *J* = 8.5 Hz, 2H), 6.80 (d, *J* = 9.0 Hz, 2H), 5.34 (q, *J* = 6.5 Hz, 1H), 5.21 (ddd, *J* = 9.0, 7.0, 7.0 Hz, 1H), 5.17 (d, *J* = 11.0 Hz, 1H), 4.78 (dd, *J* = 7.2, 3.5 Hz, 1H), 4.52 (dd, *J* = 8.8, 3.0 Hz, 1H), 4.16 (tdd, *J* = 11.4, 8.2, 3.0 Hz, 1H), 4.06 (d, *J* = 11.1 Hz, 1H), 4.00-3.96 (m, 1H), 3.77 (s, 3H), 3.57 (dt, *J* = 10, 7.5 Hz, 1H), 3.50 (ddd, *J* = 11.5, 11.2, 11.0 Hz, 1H), 3.33 (dd, *J* = 10.5, 8.0 Hz, 1H), 3.01-2.97 (m, 1H), 2.93 (dd, *J* = 11.0, 11.0 Hz, 1H), 2.88 (s, 3H), 2.86-2.82 (m, 2H), 2.77 (s, 3H), 2.54 (ddd, *J* = 11.8, 9.5, 2.0 Hz, 2H), 2.27-2.16 (m, 3H), 2.13-1.92 (m, 4H), 1.84-1.72 (m, 2H), 1.52-1.48 (m, 1H), 1.38-1.33 (m, 1H), 1.22-1.20 (m, 1H), 1.18 (d, *J* = 7.0 Hz, 3H), 1.12 (d, *J* = 7.0 Hz, 3H), 1.02 (d, *J* = 6.5 Hz, 3H), 1.00-0.90 (m, 1H), 0.88-0.86 (m, 15H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, major of the mixture of rotamers, major rotamer > 90%): δ 176.9, 173.2, 171.9, 171.3, 170.4, 168.3, 158.8, 130.6, 128.2, 113.9, 79.9, 77.7, 75.3,

59.5, 58.1, 55.3, 52.1, 50.7, 47.5, 47.4, 44.5, 40.6, 38.3, 36.2, 35.5, 32.1, 31.3, 30.5, 30.2, 29.9, 29.3, 26.1, 25.0, 24.5, 21.2, 17.1, 15.3, 15.0, 10.3 ppm. HRMS (ESI) *m/z* calcd for $C_{44}H_{69}N_5O_8S$ $(M+H)^+$ 828.4940, found 828.4927.

Apratoxin S6: *34-epi*-**Apratoxin S4** (*34-epi*-**1e**) (14% in 3 steps). $[\alpha]^{25}_D$: -106.3 (c 0.143, CH_2Cl_2). t_R = 23.5 min. ^{1}H NMR (500 MHz, $CDCl_3$, major of the mixture of rotamers, major rotamer > 90%): δ 7.12 (d, *J* = 8.5 Hz, 2H), 6.80 (d, *J* = 9.0 Hz, 2H), 6.17 (d, *J* = 8.5 Hz, 1H), 5.17 (ddd, *J* = 10.2, 9.0, 4.5 Hz, 1H), 4.89 (dd, *J* = 12.2, 3.0 Hz, 1H), 4.88 (d, *J* = 11.0 Hz, 1H), 4.61 (q, *J* = 6.5 Hz, 1H), 4.45-4.39 (m, 1H), 4.31 (dd, *J* = 8.2, 7.5 Hz, 1H), 4.13-4.08 (m, 1H), 4.02 (dddd, *J* = 9.8, 9.5, 2.5, 2.5 Hz,1H), 3.94 (dd, *J* = 9.5, 1.8 Hz, 1H), 3.77 (s, 3H), 3.66-3.58 (m, 1H), 3.31 (dd, *J* = 11.0, 8.0 Hz, 1H), 3.08 (dd, *J* = 12.5, 10.5 Hz, 1H), 3.02 (dd, *J* = 10.8, 4.0 Hz, 1H), 2.93 (dd, *J* = 12.5, 4.0 Hz, 1H), 2.88 (s, 3H), 2.59 (s, 3H), 2.51 (q, *J* = 6.5 Hz, 1H), 2.40-2.25 (m, 3H), 2.10-2.02 (m, 1H), 2.01-1.86 (m, 4H), 1.84-1.68 (m, 4H), 1.30-1.25 (m, 2H), 1.17 (d, *J* = 7.0 Hz, 3H), 1.09 (d, *J* = 6.5 Hz, 3H), 1.00 (d, *J* = 6.5 Hz, 3H), 0.99-0.92 (m, 2H), 0.87 (s, 9H), 0.83 (t, *J* = 7.5 Hz, 3H), 0.51 (d, *J* = 6.5 Hz, 3H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, major of the mixture of rotamers, major rotamer > 90%): δ 175.0, 172.2, 171.8, 170.9, 170.5, 170.2, 158.8, 130.5, 128.7, 114.2, 78.0, 76.4, 70.8, 59.4, 58.0, 55.5, 54.3, 50.8, 47.9, 45.6, 40.3, 39.9, 37.4, 37.0, 35.2, 34.1, 32.1, 30.6, 30.4, 29.9, 29.5, 29.3, 28.9, 26.3, 25.9, 25.7, 25.4, 22.8, 21.1, 15.0, 14.3, 14.1, 10.0, 9.9 ppm. HRMS (ESI) *m/z* calcd for $C_{44}H_{69}N_5O_8S$ $(M+H)^+$ 828.4940, found 828.4931.

Configuration Analysis *2-epi*-1e and *34-epi*-1e

Along with the isolation of **1e** (t_R = 17.9 min), two side products, *2-epi*-**1e** and *34-epi*-**1e**, (t_R = 19.7 and 23.5 min, respectively) were isolated (**Figure S1**). Target **1e** is the major product. The side products *2-epi*-**1e** and *34-epi*-**1e** were identified and characterized by LC-MS, and ^{1}H NMR analysis, and chiral HPLC-MS of degradation products.

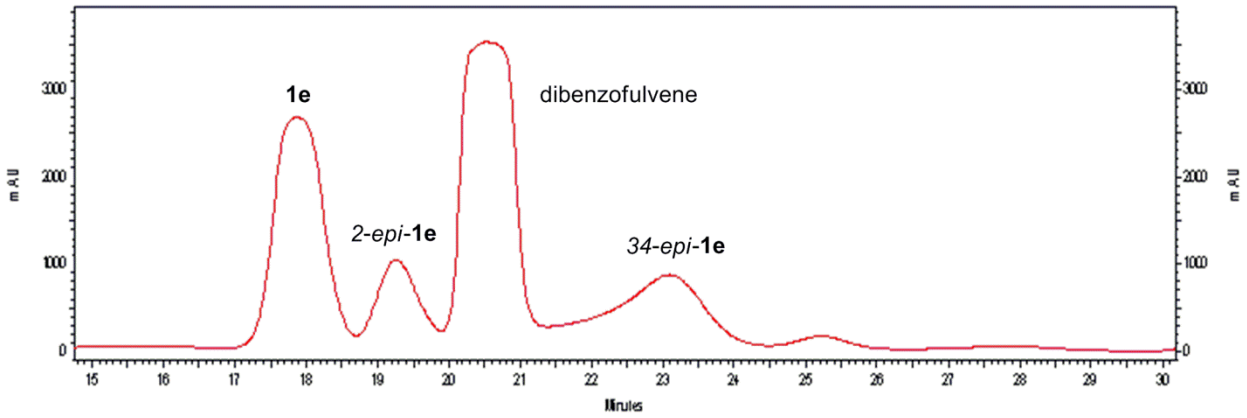


Figure S1. The reversed-phase HPLC profile of **1e** isolation from reaction mixture of preparation **1e** (UV dectection at 220nm).

Acid Hydrolysis and Chiral Amino Acid Analysis by LC-MS and HPLC

Samples of **1e**, *2-epi*-**1e** or *34-epi*-**1e** (50 µg each) were treated with 6 N HCl (0.5 ml) at 110 °C for 24 h. The hydrolysates were concentrated to dryness, reconstituted in H_2O (100 µl), and then analyzed by chiral HPLC [column, Chirobiotic TAG (4.6 × 250 mm), Supelco, solvent, MeOH-10 mM NH_4OAc (40:60, pH 5.28); flow rate 0.5 ml/min; detection by ESIMS in positive ion mode (MRM scan)]. The retention times (t_R/min; MRM ion pair, parent→product) of the authentic amino acids for every sample are depicted in **Table S1**. The gas source parameters were as follows: CUR 35, CAD medium, IS 4500, TEM 750, GS1 65, GS2 65.

Table S1. The retention times of the authentic amino acids corresponding to each samples and their MRM parameters.

	Pro 116→70		*N*-Me-Ala 104→58		*N*-Me-Ile 146→100				Tyr(OMe) 196→137		Tyr 182→91	
	L-	D-	L-	D-	L-	L-allo-	D-	D-allo-	L-	D-	L-	D-
t_R/min	13.5	35.9	11.5	75.0	12.6	15.2	50.0	51.0	14.0	21.0	10.2	15.0
DP	45		35		30				25		20	
EP	4.0		4.0		4.0				3.8		7.0	
CEP	8.0		7.5		5.0				10.0		20.0	
CE	25		17		20				20		30	
CXP	3.0		3.0		4.0				13.0		18.0	

The retention times corresponding to every amino acid of three hydrolysates are listed in **Table S2**.

Table S2. The retention times corresponding to every amino acid of three hydrolysates**.**

	Pro	*N*-Me-Ala	*N*-Me-Ile	Tyr(OMe)	Tyr
1e	13.5→ L-	11.5→ L-	12.6→ L-	14.0→ L-	10.2→ L-
2-epi-**1e**	35.9→ D-	11.5→ L-	12.6→ L-	14.0→ L-	10.2→ L-
34-epi-**1e**	13.5→ L-	11.5→ L-	12.6→ L-	14.0→ L-	10.2→ L-

Based on the retention times (**Table S1** and **S2**), it was concluded that the hydrolysates of **1e** and *34-epi*-**1e** contained L-Pro, *N*-Me-L-Ala, *N*-Me-L-Ile and L-Tyr(OMe), and that *2-epi*-**1e** is comprised of D-Pro, *N*-Me-L-Ala, *N*-Me-L-Ile and L-Tyr(OMe).

In order determine the difference between *34-epi*-**1e** and **1e**, ^{1}HNMR analysis was performed. The ^{1}H NMR signal of H-34 in **1e** was a doublet of quartets (dq), like in apratoxin A[7] were H-34 and H-35 are *anti* (large coupling, **Figure S2a**). In contrast, a broad quartet (br q) in *34-epi*-**1e** the coupling between H-34 and H-35 was small, suggesting *gauche* conformation (**Figure S2b**) due to epimerization of C-34. Consequently, we propose that the absolute configuration of C-34 for **1e** is *S*, for *34-epi*-**1e** is *R*.

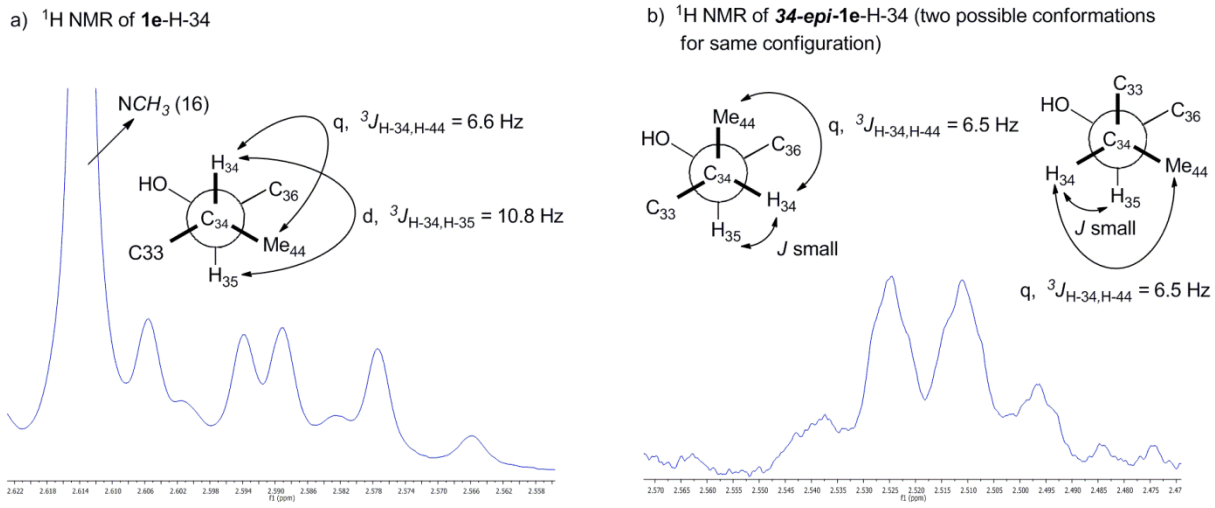


Figure S2. Comparison of ^{1}H NMR signal for H-34 in **1e** (a) and *34-epi*-**1e** (b).

Biological Material and Methods

Cell Culture

Human colon adenocarcinoma HCT116 cells were purchased from ATCC (Manassas, VA) and cultured in Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (HyClone, Logan, UT) at 37 ºC humidified air and 5% CO_2.

Cell Viability Assay (MTT)

HCT116 cells were seeded at a density of 1×10^4 cells per well in 96-well clear bottom plate, and 24 h later the cells were treated with various concentrations of the apratoxins (1 pM–10 µM) or solvent control (EtOH). After 48 h of incubation, cell viability was detected using MTT according to the manufacturer's instructions (Promega, Madison, WI).

Measurement of VEGF-A Secretion

HCT116 (1×10^4 cells per well) were seeded in 96-well clear bottom plates. Cells were treated with various concentrations of apratoxins (1 pM–10 μM) or solvent control (EtOH). After 12 h incubation, culture supernatants were collected for detection of VEGF-A by using alphaLISA kit (PerkinElmer, Waltham, MA) following the manufacturer's instruction. Briefly, acceptor bead and anti-VEGF-A antibody were incubated with the supernatants for 60 min firstly, donor beads were added later and incubated for another 30 min, and VEGF-A values were detected using Envision (PerkinElmer).

Cell Cycle Analysis

HCT116 cells were incubated with apratoxin S4 (**1e**) at various concentrations (0, 0.32, 3.2, 10 nM) for 24 h. Cells were pelleted by centrifugation and fixed in ice-cold 70% ethanol. DNA was stained with 10 μg/ml propidium iodide (Invitrogen) in a reaction solution containing 100 μg/mL RNase A (Sigma-Aldrich, St. Louis, MO). Fluorescence emitted from the propidium iodide-DNA complex was quantified using FACScan (Becton Dickinson Medical Systems, Sharon, MA).

Immunoblot Analysis

HCT116 cells were seeded in 60-mm dishes at a density of 4×10^5 cells the day before the treatment. The next day, cells were given the same treatment of apratoxin A or apratoxin A analogues (0.32 nM–10 μM) or solvent control (EtOH). 24 h later, whole cell lysates were collected using PhosphoSafe buffer (EMD Chemicals, Inc, Gibbstown, NJ). Protein concentrations were measured with the BCA Protein Assay kit (Thermo Fisher Scientific, Rockford, IL). Lysates containing equal amounts of protein were separated by SDS polyacrylamide gel electrophoresis (4–12%), transferred to polyvinylidene difluoride membranes, probed with primary and secondary antibodies, and detected with the SuperSignal West Femto Maximum Sensitivity Substrate (Thermo Fisher Scientific). Anti-PDGFR-β antibody was obtained from Santa Cruz Biotechnology, Inc (Santa Cruz, CA). Anti-VEGFR2, Met and secondary anti-mouse and rabbit antibodies were from Cell Signaling Technology, Inc (Danvers, MA).

In Vitro Translation

The translation reactions containing 17.5 µL of nuclease-treated rabbit reticulocyte lysate (Promega), 0.5 µL of amino acid mix (minus methionine, 1 mM), 2.0 µL of canine pancreatic microsomal membranes (Promega), 1.0 µL of RNA substrate in nuclease-free water (β-lactamase or α-factor mRNA at 0.1 µg/µL), 1 µL mixture (0.875 µL water, 0.125 µL of 2 µM, 6.4 µM, 20 µM, 64 µM, 200 µM, 640 µM, 2 mM apratoxin S4 (**1e**) or solvent control), 1.5–2.0 µL of [^{35}S] methionine (PerkinElmer, Waltham, MA) and nuclease-free water to a final volume of 25 µL were incubated at 30 °C for 60 min. One reaction without canine pancreatic microsomal membranes was included. 5 µL of the reaction was used for analyzing the results of translation and processing by SDS-PAGE/autoradiography.

In Vitro Transcription/Translation

Human PDGFR-β cDNA plasmid was obtained from Origene Technologies (Rockville, MD). *In vitro* transcription/translation was carried out by using TNT T7 quick coupled transcription/translation systems (Promega). The reactions containing 20 µL of T7 TNT quick master mix, 1 µL of plasmid DNA (1 µg/µL), 1.5 µL canine pancreatic microsomal membranes (Promega), 1 µL mixture (0.875 µL water, 0.125 µL of 2 µM, 6.4 µM, 20 µM, 64 µM, 200 µM, 640 µM, 2 mM apratoxin S4 (**1e**) or solvent control), 1.5–2.0 µL of [^{35}S] methionine (PerkinElmer) and nuclease-free water to a final volume of 25 µL were incubated at 30 °C for 90 min. One reaction without canine pancreatic microsomal membranes also was included. 5 µL of the reaction was used for analyzing the results of transcription/translation and processing by SDS-PAGE/autoradiography.

In Vivo Studies

3–5 Weeks old female nude mice (*nu/nu*) were obtained from Charles River Laboratory (Wilmington, MA). Tumors were established by subcutaneous injection of 1×10^6 HCT116 cells on the left rear flank of a nude mouse in a volume of 100 µl of sterile saline. Tumor dimensions were measured using calipers every day and tumor volumes were calculated using the formula $W^2 \times L \times 0.5$, where width (W) ≤ length (L). Mice were injected intraperitoneally with optimized dose of 0.25 mg/kg or DMSO solvent control every day until the control tumor size in one dimension reached 15 mm and tumor tissue was harvested on the following day. Apratoxin

treatment was continued for several more days. 50 mg of tumor tissue was sonicated in PhosphoSafe lysis (EMD Chemicals, Inc) buffer and used for immunoblot analysis described as the above.

All studies were carried out under the protocol approved by the Institutional Animal Care and Use Committee at the University of Florida.

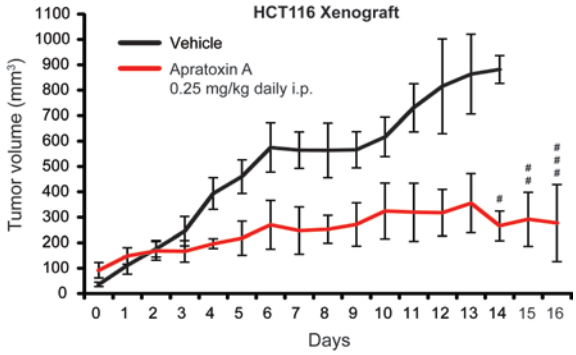


Figure S3. Efficacy studies with apratoxin A using a HCT116 xenograft mouse model. Subcutaneous tumor-bearing mice were injected (daily i.p.) with **1e** (*n* = 6) or DMSO vehicle (*n* = 8) and tumor volumes monitored over time. Error bars indicate S.E.M. Each # indicates a death occurring, which started after 2 weeks of treatment.

References:

[1] W. L. F. Armarego, Christina L. L. Chai, *Purification of Laboratory Chemicals, Fifth Edition*. Butterworth-Heinemann: Oxford, 2003.

[2] W. R. Roush, K. Ando, D. B. Powers, A. D. Palkowitz, R. L. Halterman, *J. Am. Chem. Soc*. **1990**, *112*, 6339-6348.

[3] a) T. Doi, Y. Numajiri, A. Munakata, T. Takahashi, *Org. Lett*. **2006**, *8*, 531-534; b) Y. Numajiri, T. Takahashi, T. Doi, *Chem. Asian J*. **2009**, *4*, 111-125.

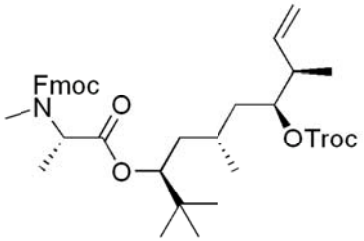
[4] Z. S. Xu, Z. Y. Chen, T. Ye, *Tetrahedron: Asymmetry* **2004**, *15*, 355-363.

[5] C. Claperon, R. Rozenfeld, X. Iturrioz, N. Inguimbert, M. Okada, B. Roques, B. Maigret, C. Llorens-Cortes, *Biochem. J.* **2008**, *416*, 37-46.

[6] K. Tidgewell, N. Engene, T. Byrum, J. Media, T. Doi, F. Valeriote, W. H. Gerwick, *ChemBioChem* **2010**, *11*, 1458-1466.

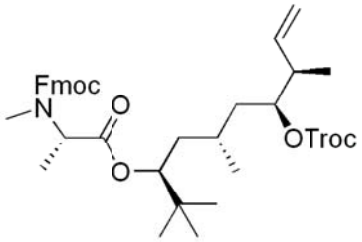
[7] H. Luesch, W. Y. Yoshida, R. E. Moore, V. J. Paul, T. H. Corbett, *J. Am. Chem. Soc.* **2001**, *123*, 5418-5423.

^{1}H NMR Spectrum of **6a** in $CDCl_3$ (400 MHz) at 25°C



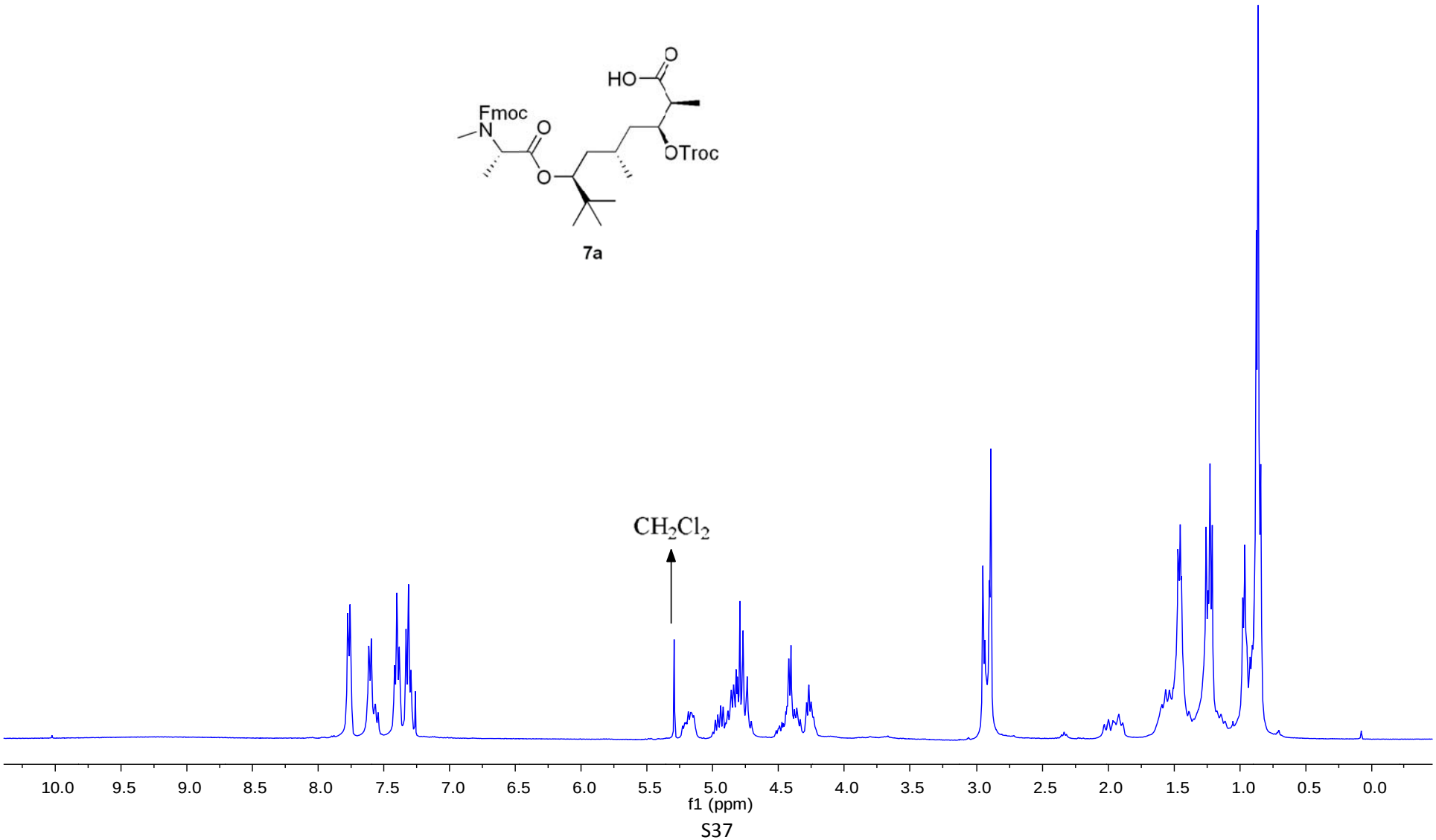
6a

^{13}C NMR Spectrum of **6a** in $CDCl_3$ (100 MHz) at 25°C

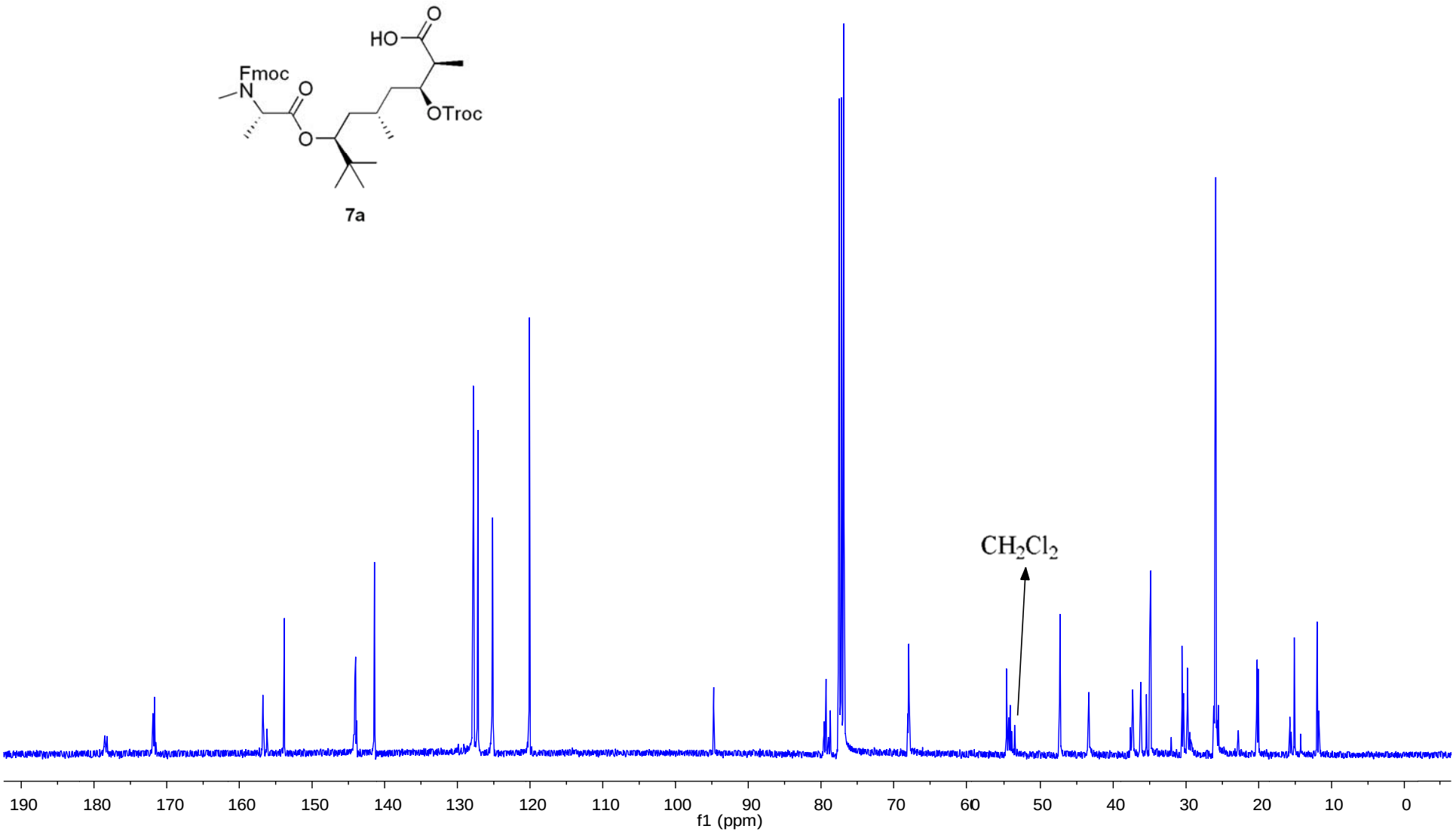


6a

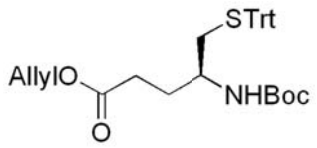
^{1}H NMR Spectrum of **7a** in $CDCl_3$ (400 MHz) at 25°C



^{13}C NMR Spectrum of **7a** in $CDCl_3$ (100 MHz) at 25°C

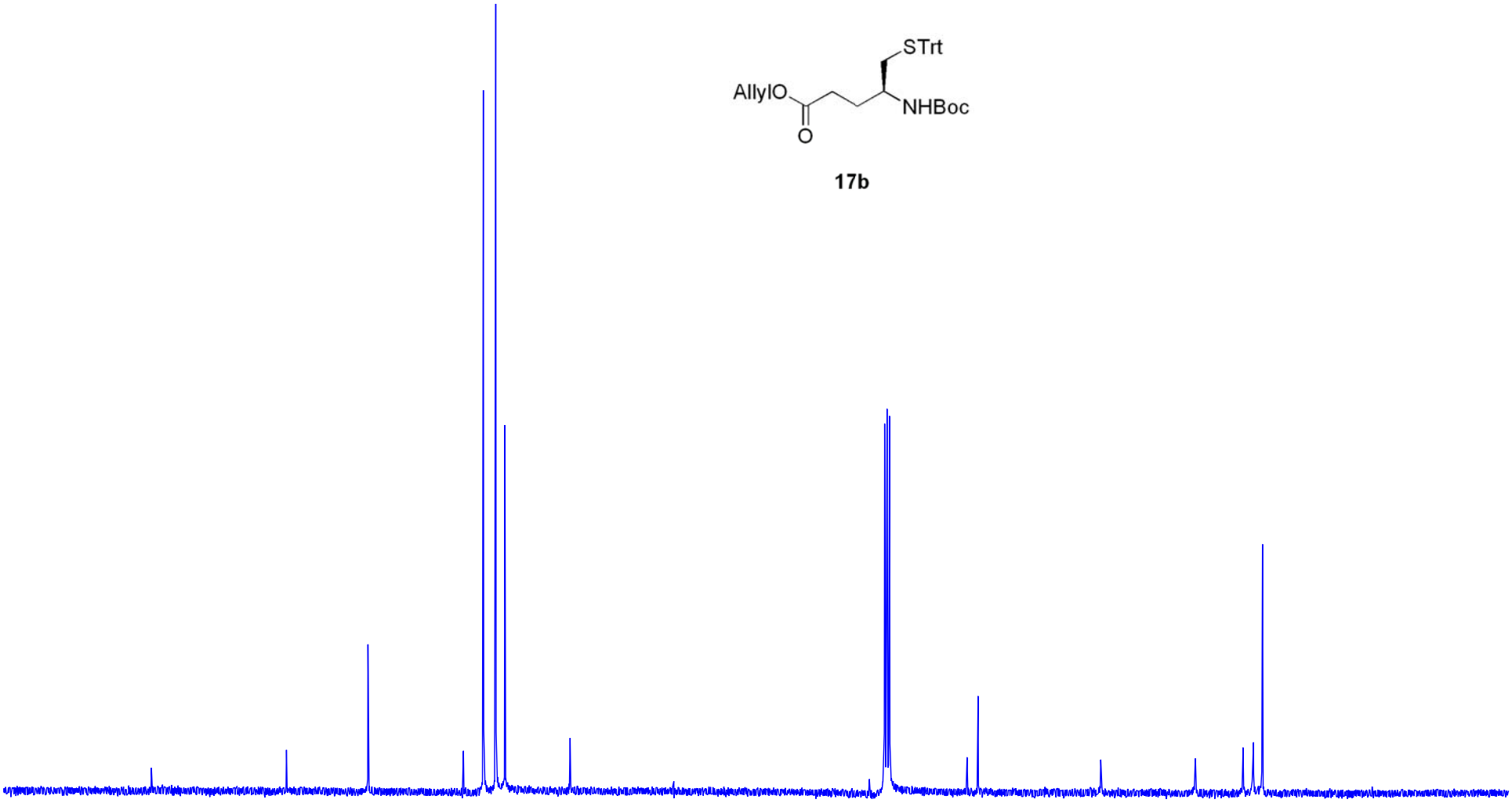


^{1}H NMR Spectrum of **17b** in $CDCl_3$ (400 MHz) at 25°C

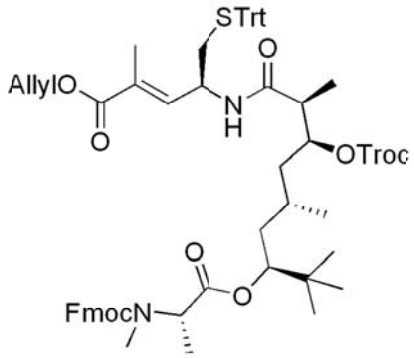


17b

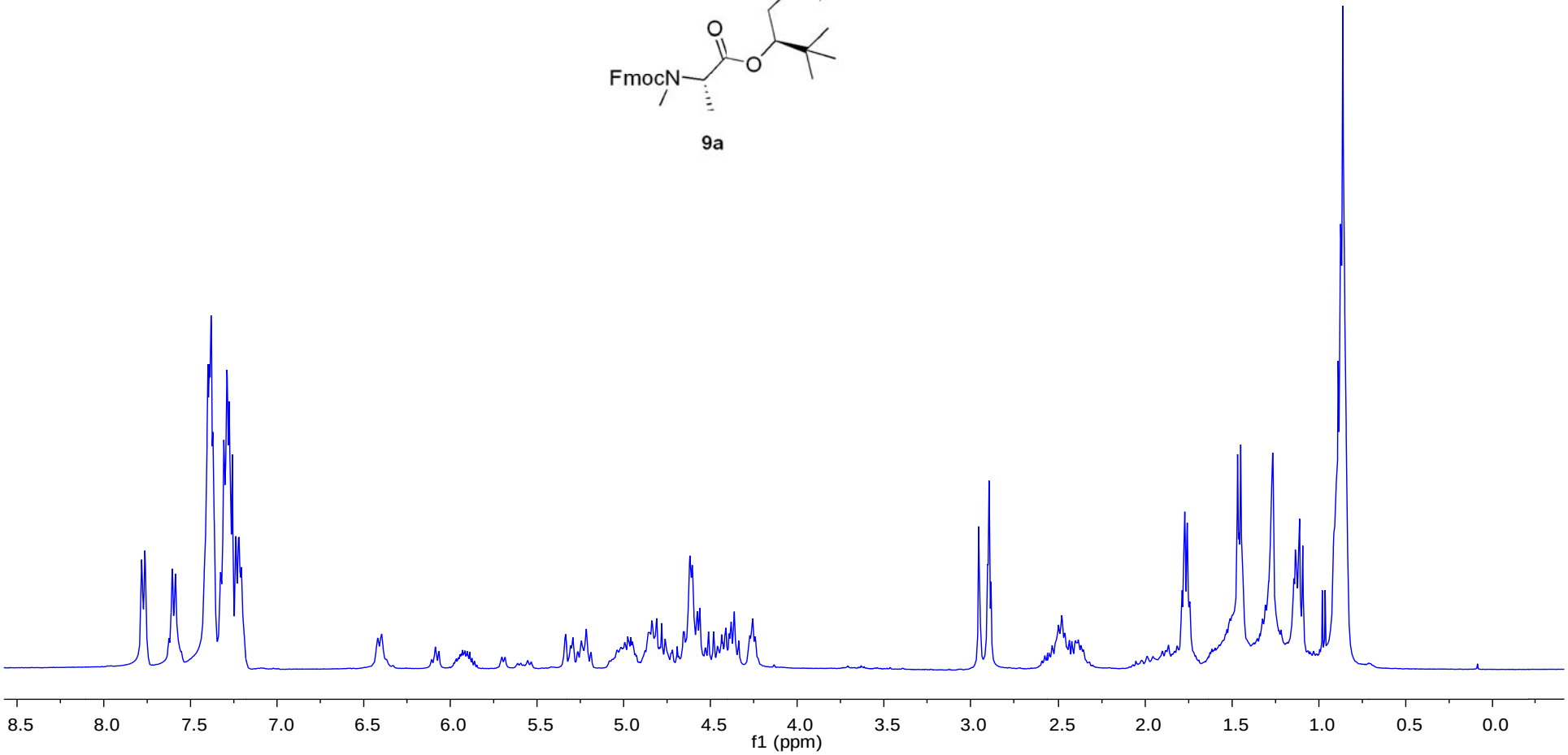
^{13}C NMR Spectrum of **17b** in $CDCl_3$ (100 MHz) at 25°C



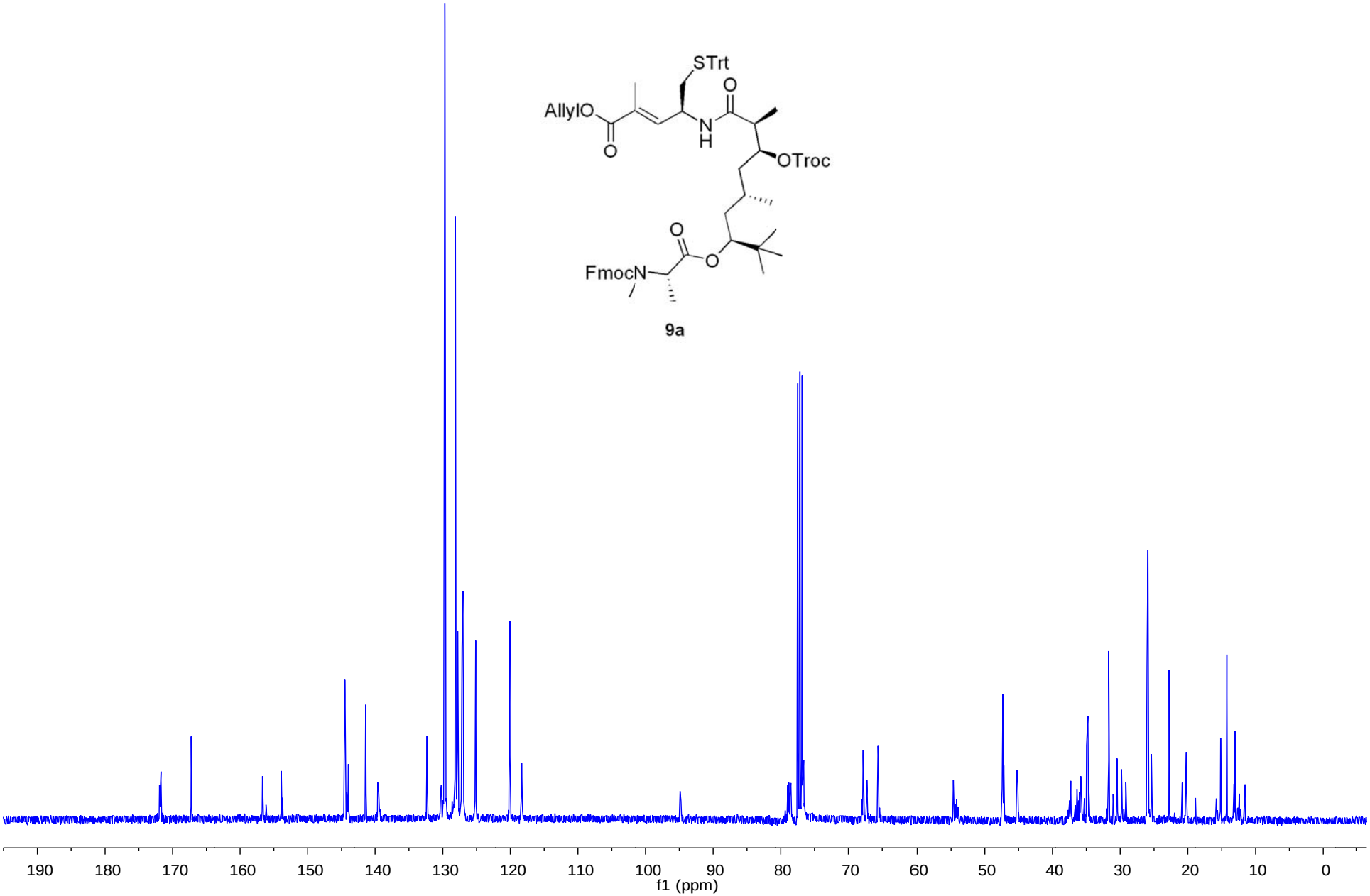
^{1}H NMR Spectrum of **9a** in $CDCl_3$ (400 MHz) at 25°C



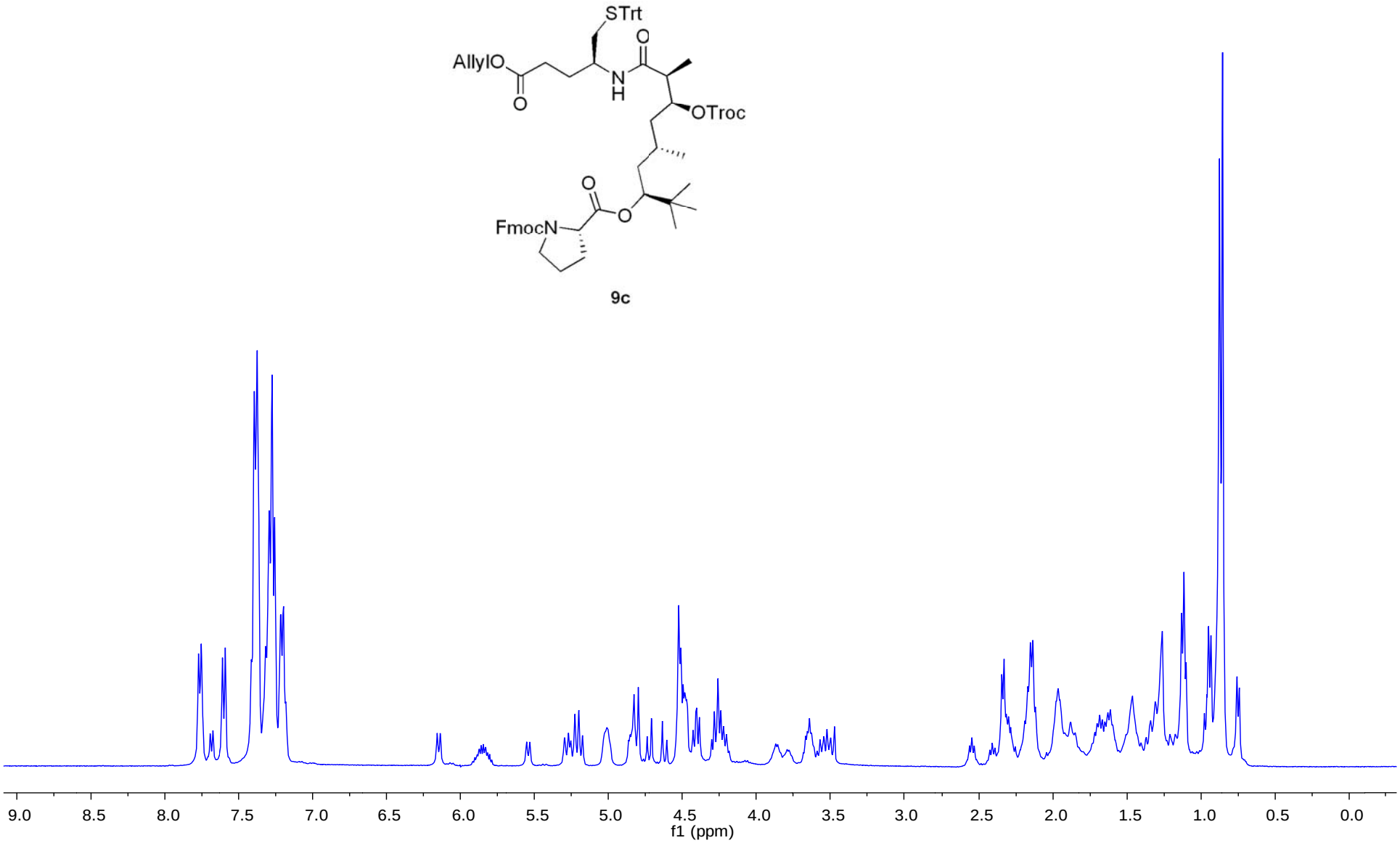
9a



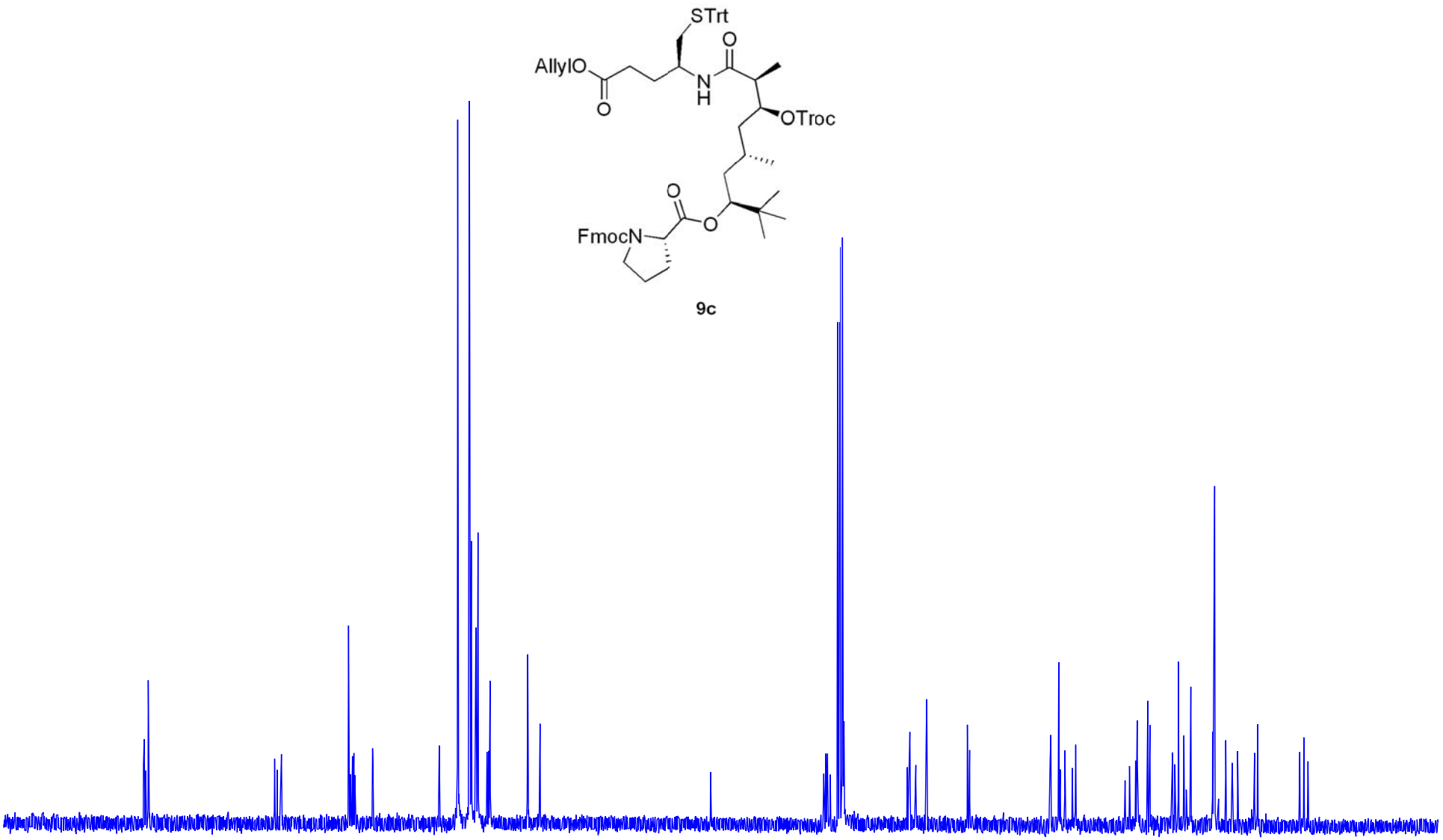
^{13}C NMR Spectrum of **9a** in $CDCl_3$ (100 MHz) at 25°C



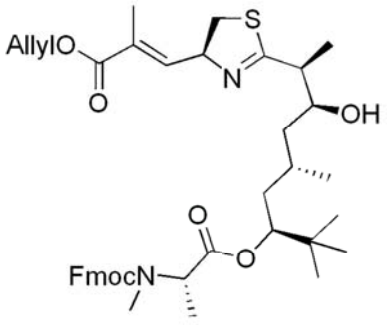
^{1}H NMR Spectrum of **9c** in $CDCl_3$ (400 MHz) at 25°C



^{13}C NMR Spectrum of **9c** in $CDCl_3$ (100 MHz) at 25°C

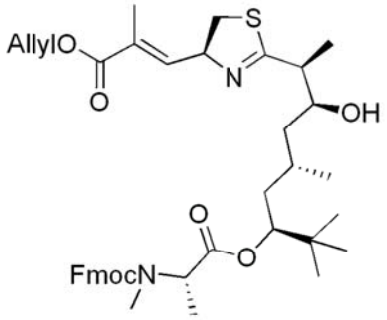


^{1}H NMR Spectrum of **10a** in $CDCl_3$ (400 MHz) at 25°C



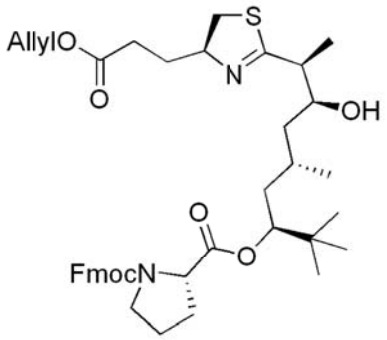
10a

^{13}C NMR Spectrum of **10a** in $CDCl_3$ (100 MHz) at 25°C



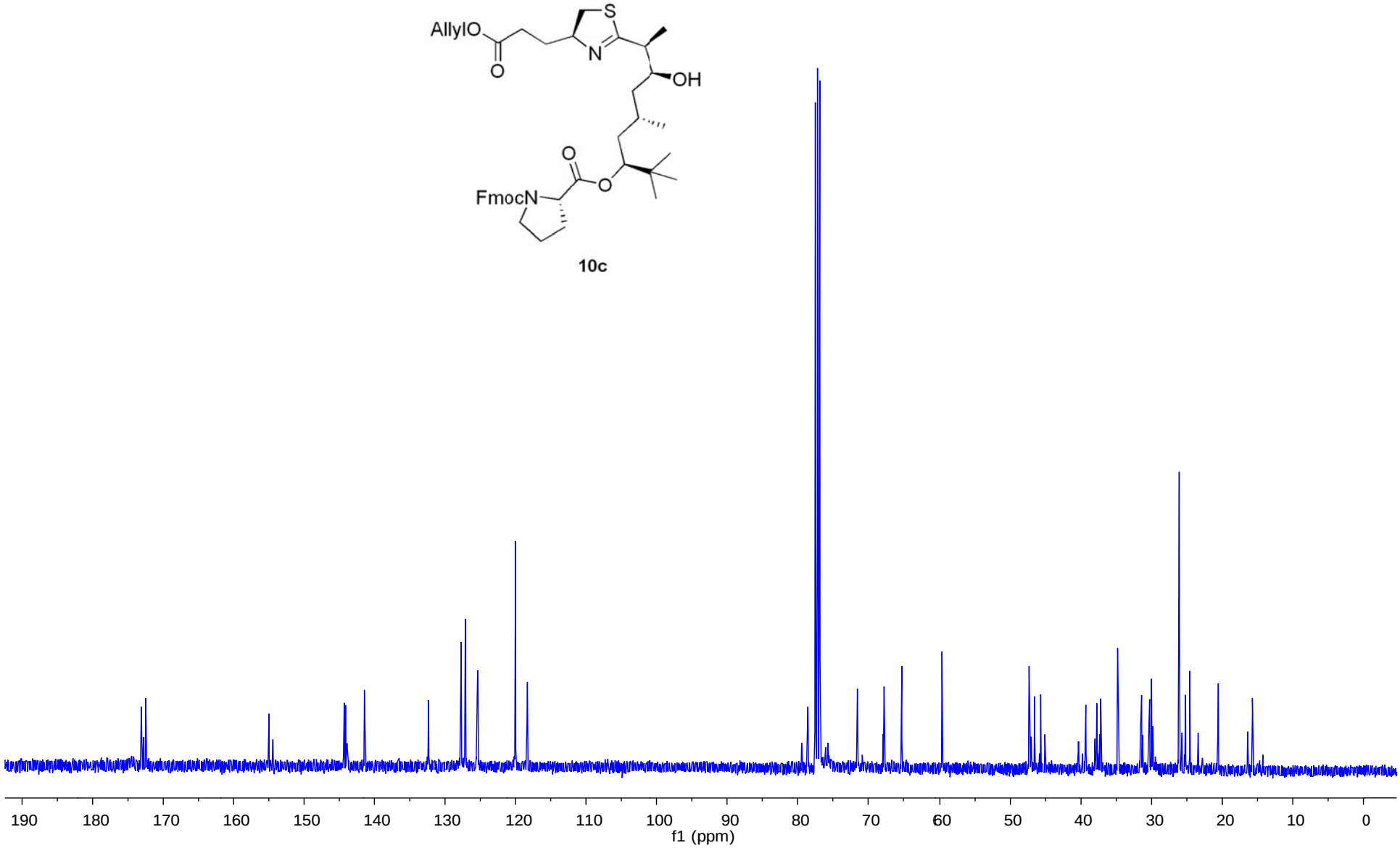
10a

^{1}H NMR Spectrum of **10c** in $CDCl_3$ (400 MHz) at 25°C

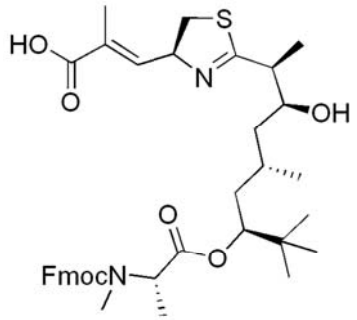


10c

^{13}C NMR Spectrum of **10c** in $CDCl_3$ (100 MHz) at 25°C

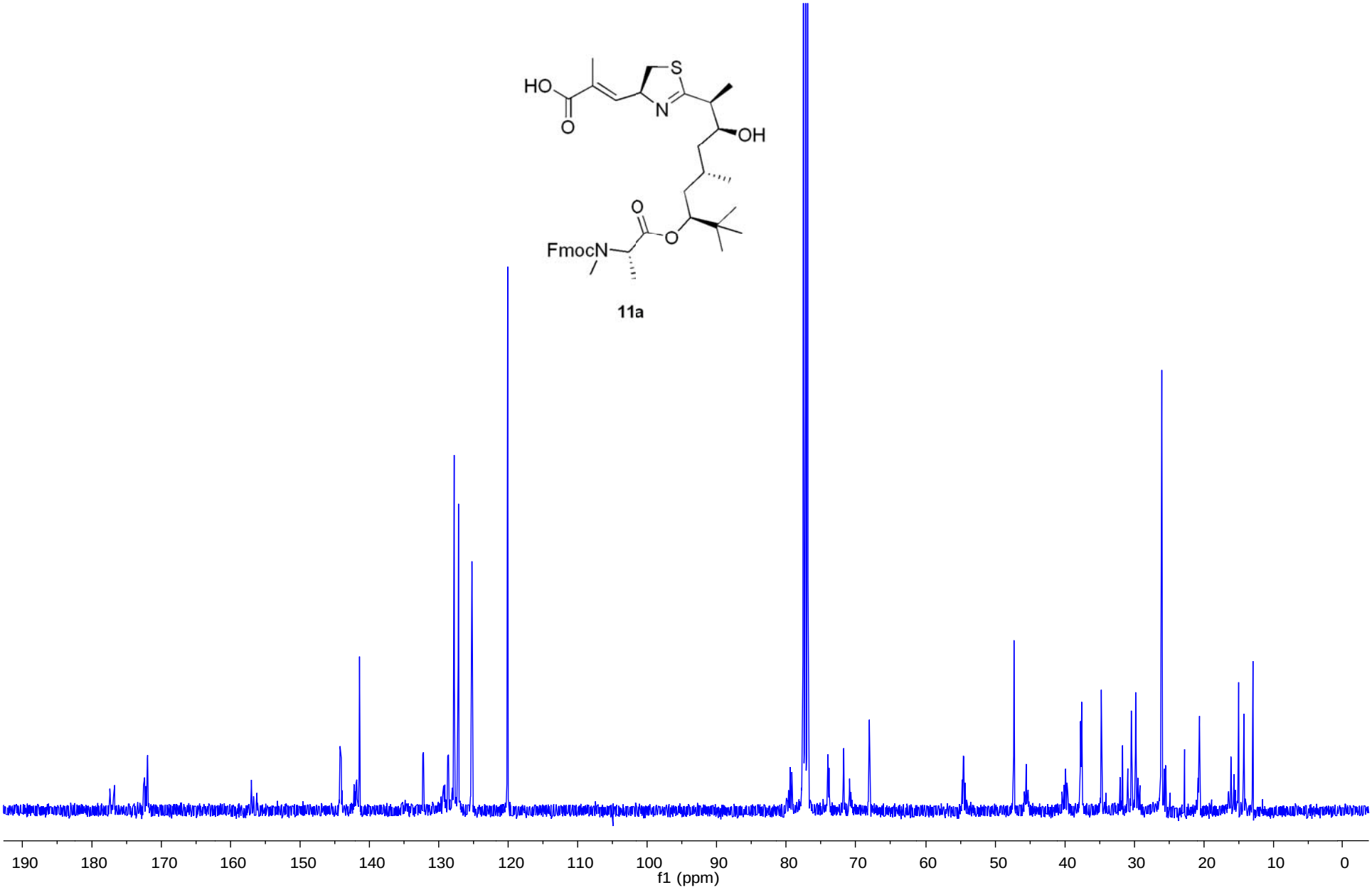


^{1}H NMR Spectrum of **11a** in $CDCl_3$ (400 MHz) at 25°C

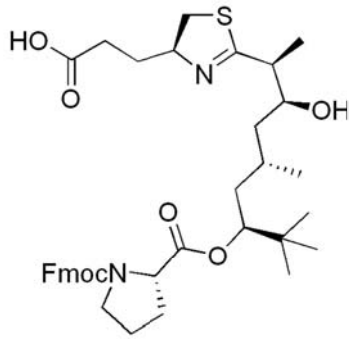


11a

^{13}C NMR Spectrum of **11a** in $CDCl_3$ (100 MHz) at 25°C

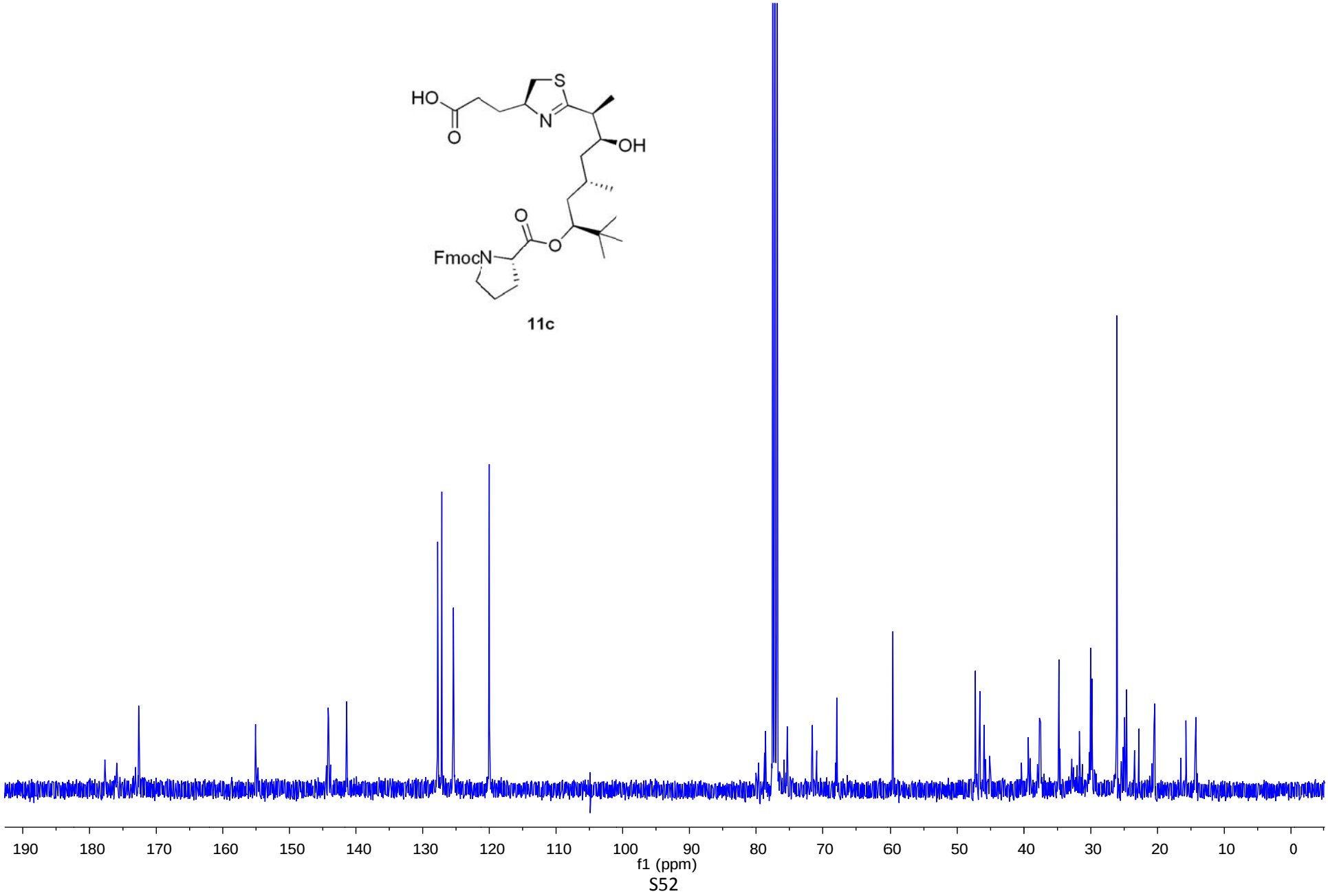


^{1}H NMR Spectrum of **11c** in $CDCl_3$ (400 MHz) at 25°C

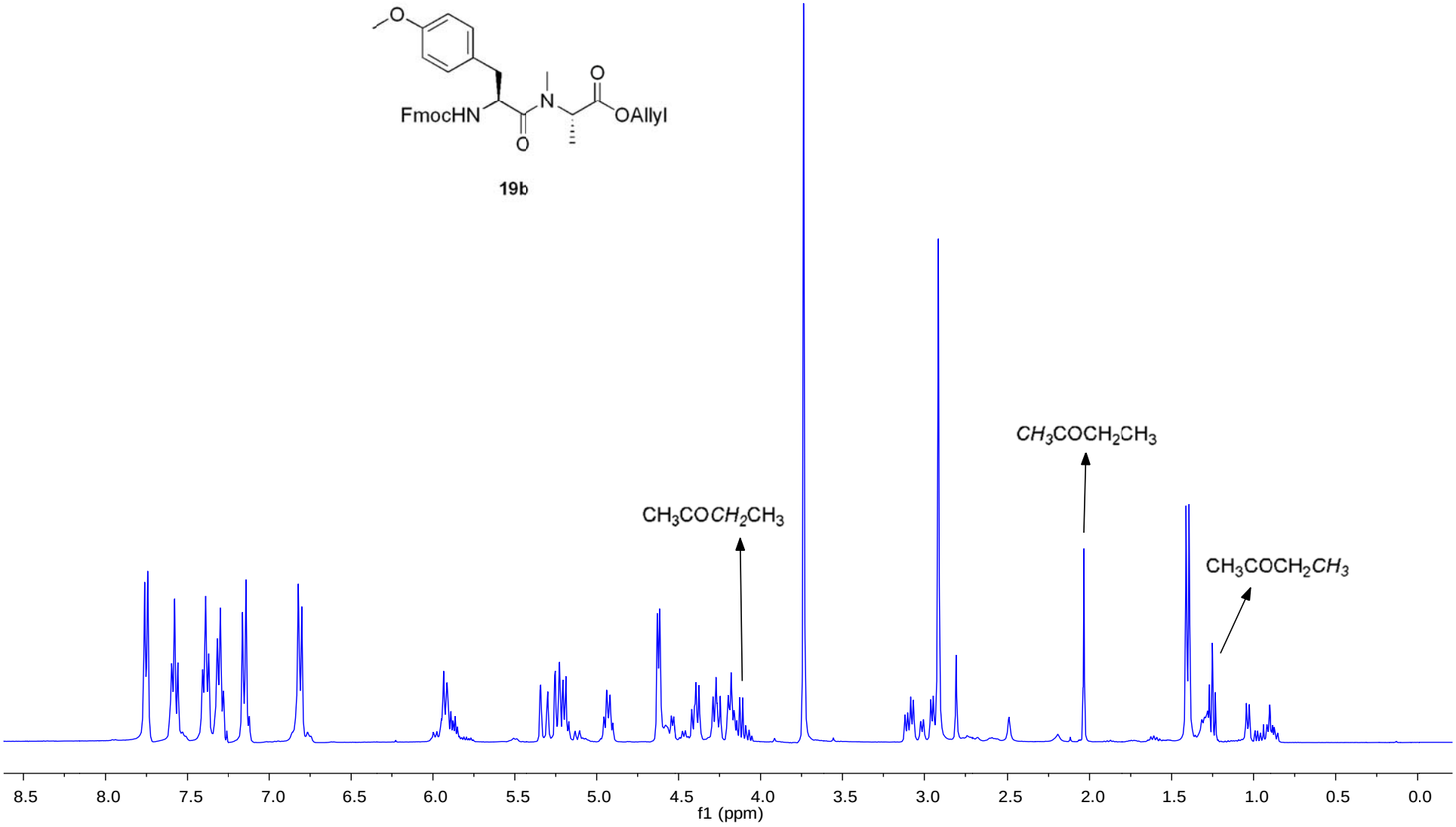


11c

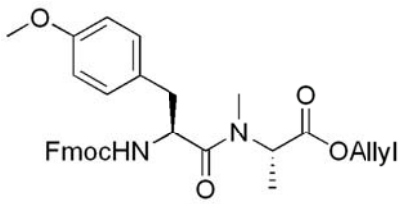
^{13}C NMR Spectrum of **11c** in $CDCl_3$ (100 MHz) at 25°C



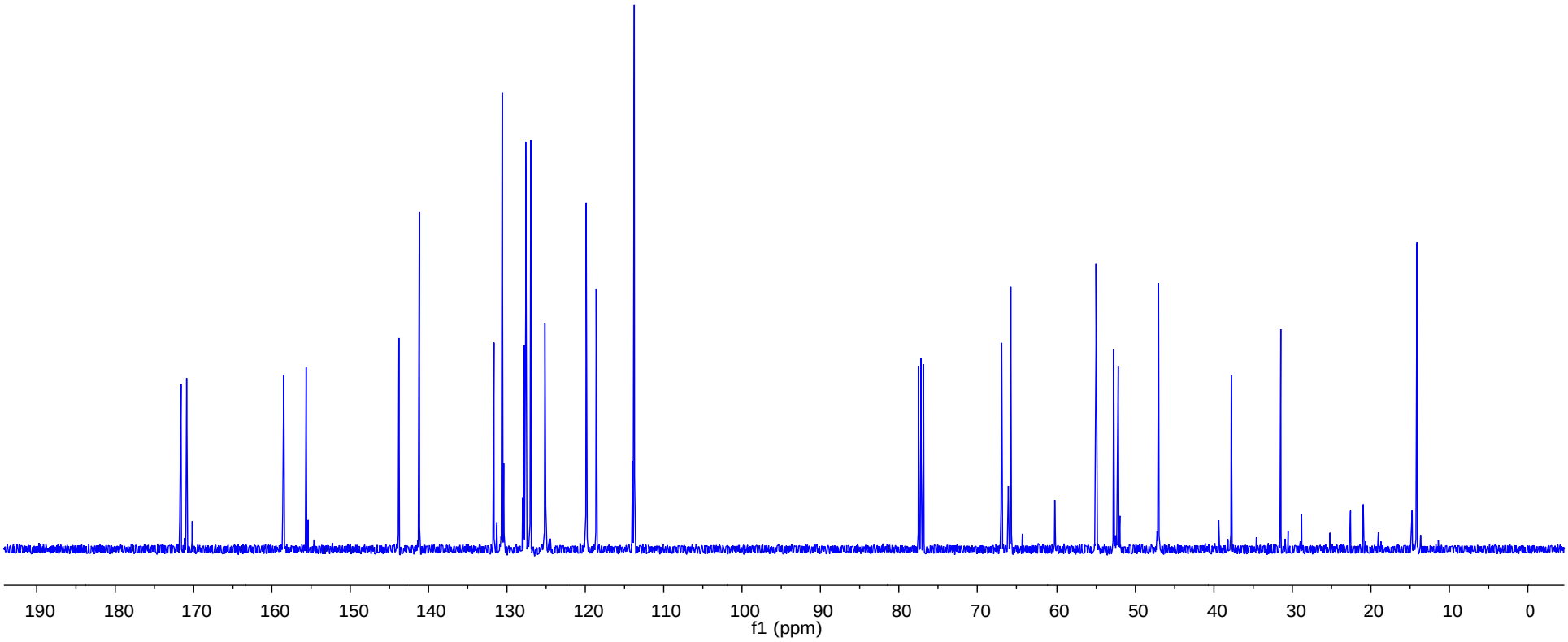
^{1}H NMR Spectrum of **19b** in $CDCl_3$ (400 MHz) at 25°C



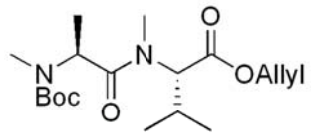
^{13}C NMR Spectrum of **19b** in $CDCl_3$ (100 MHz) at 25°C



19b

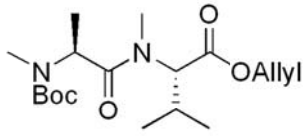


^{1}H NMR Spectrum of **19c** in $CDCl_3$ (400 MHz) at 25°C



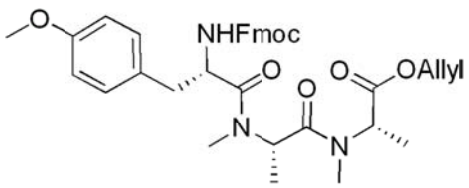
19c

^{13}C NMR Spectrum of **19c** in $CDCl_3$ (100 MHz) at 25°C

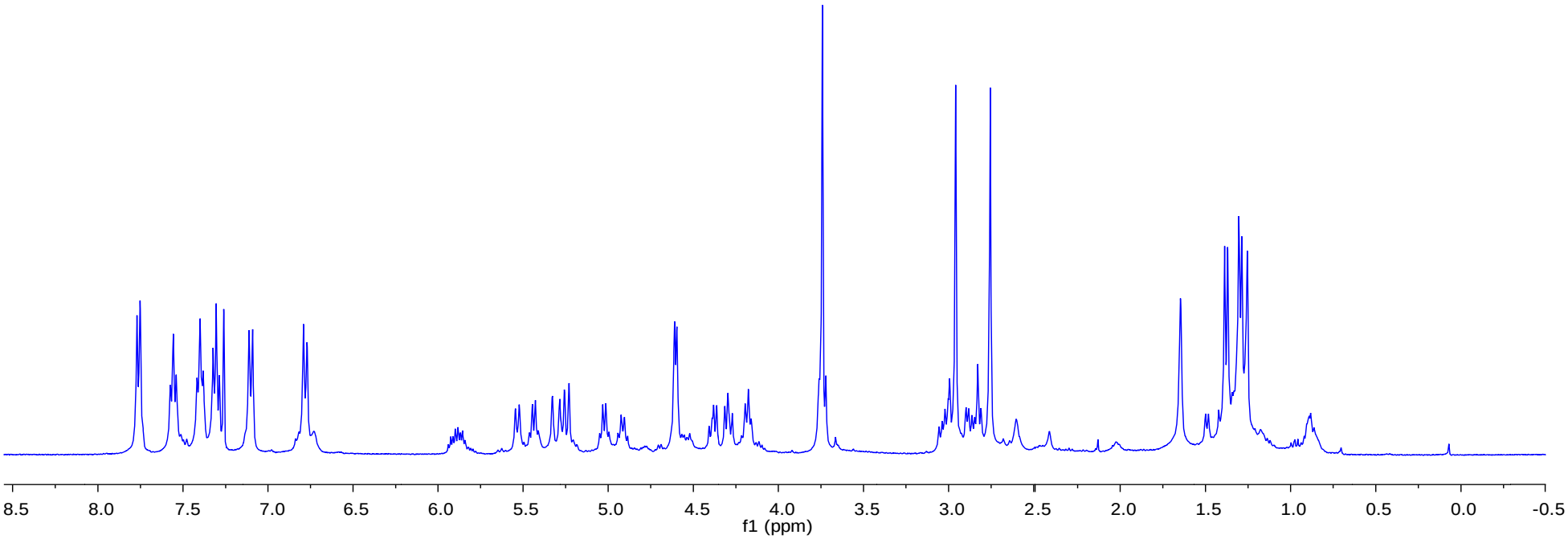


19c

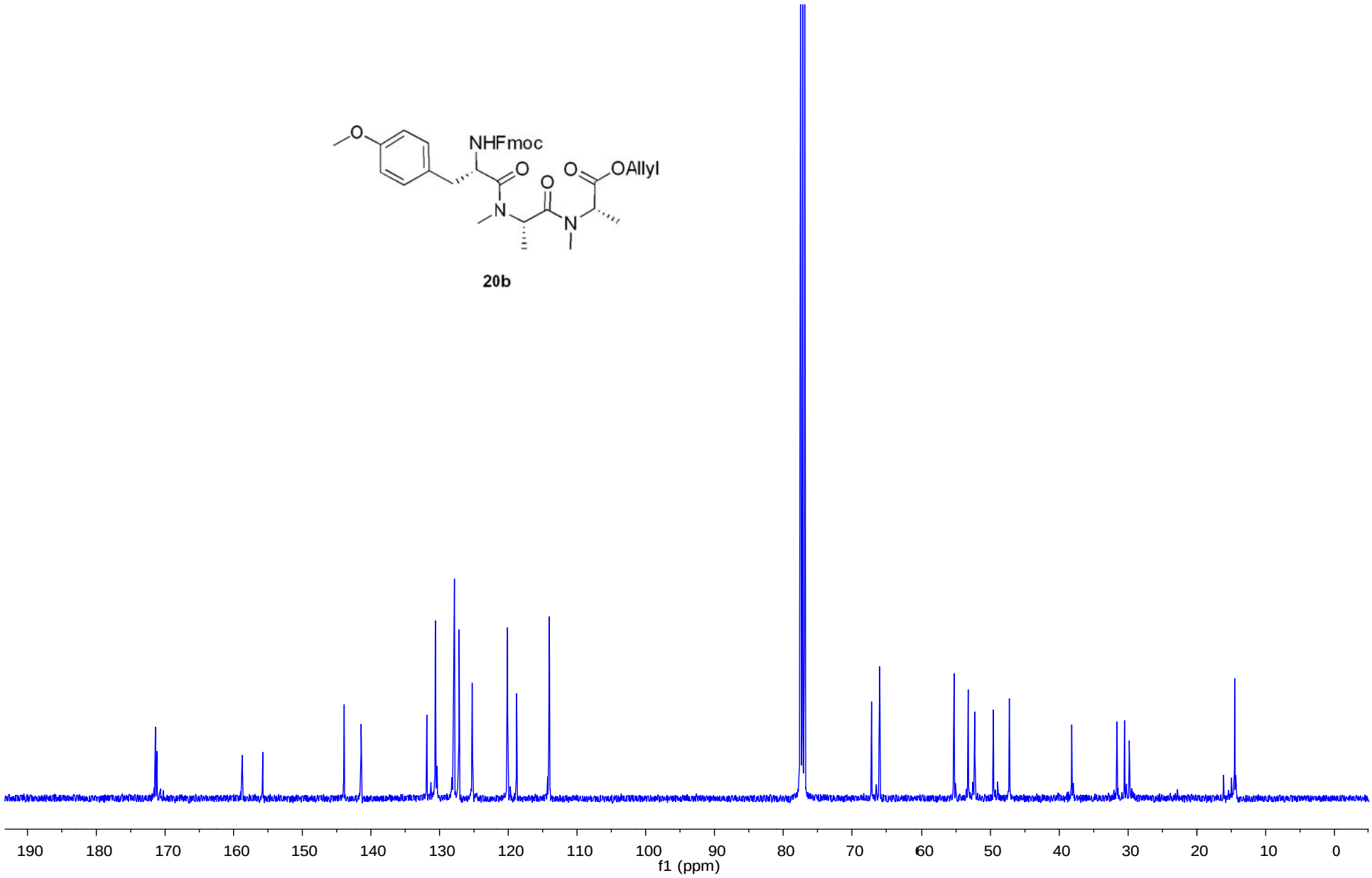
^{1}H NMR Spectrum of **20b** in $CDCl_3$ (400 MHz) at 25°C



20b



^{13}C NMR Spectrum of **20b** in $CDCl_3$ (100 MHz) at 25°C



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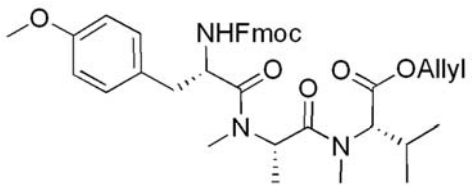
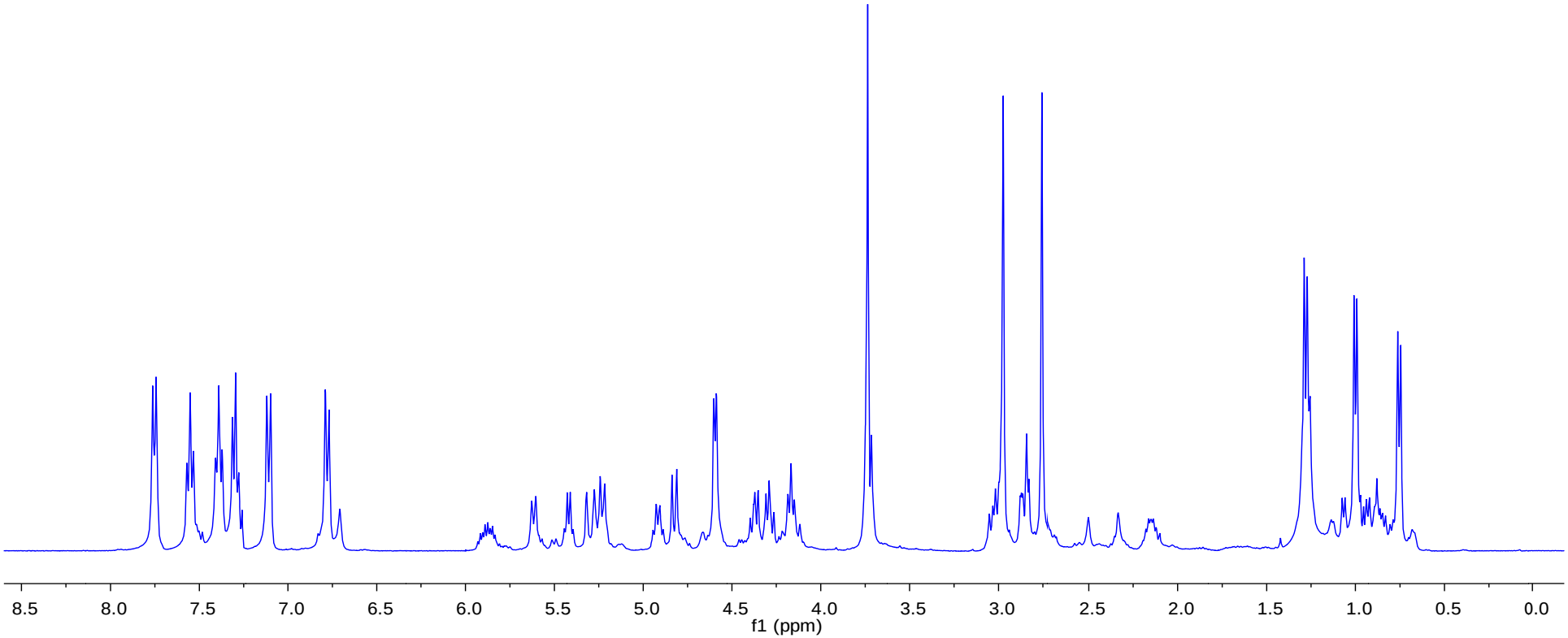
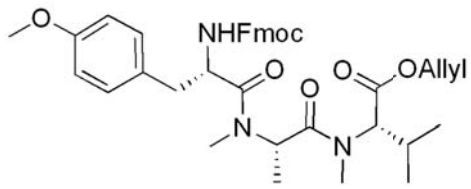


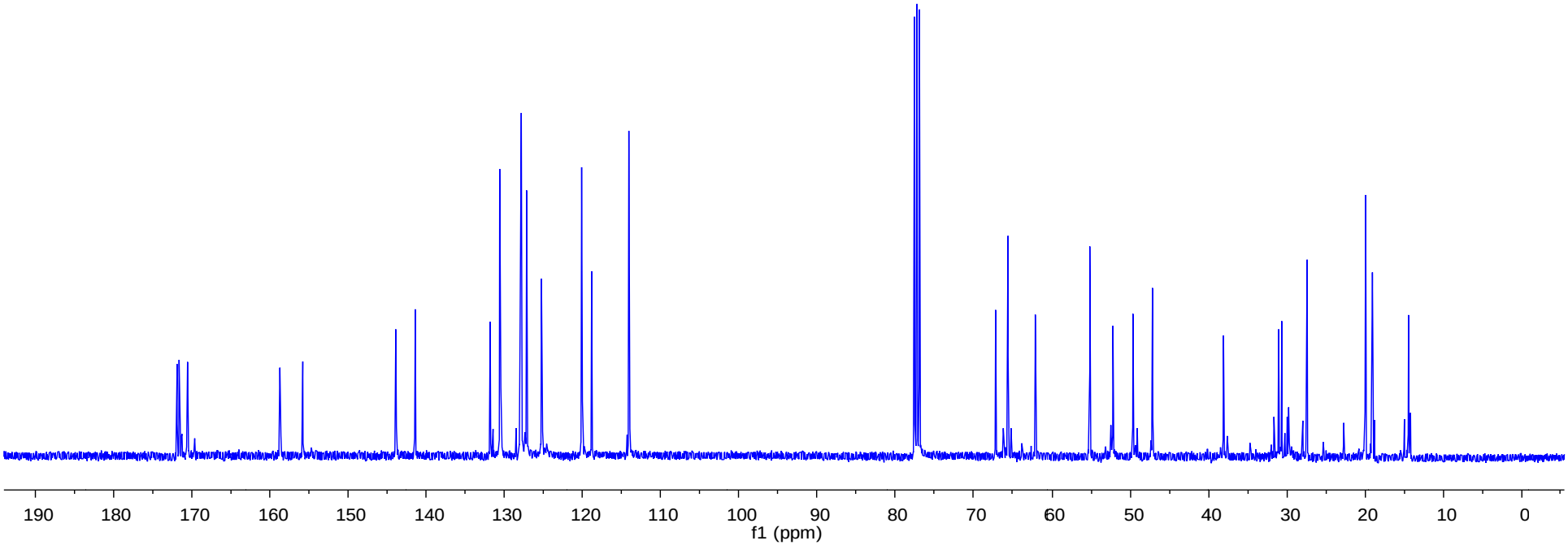
20c



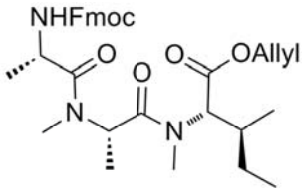
^{13}C NMR Spectrum of **20c** in $CDCl_3$ (100 MHz) at 25°C



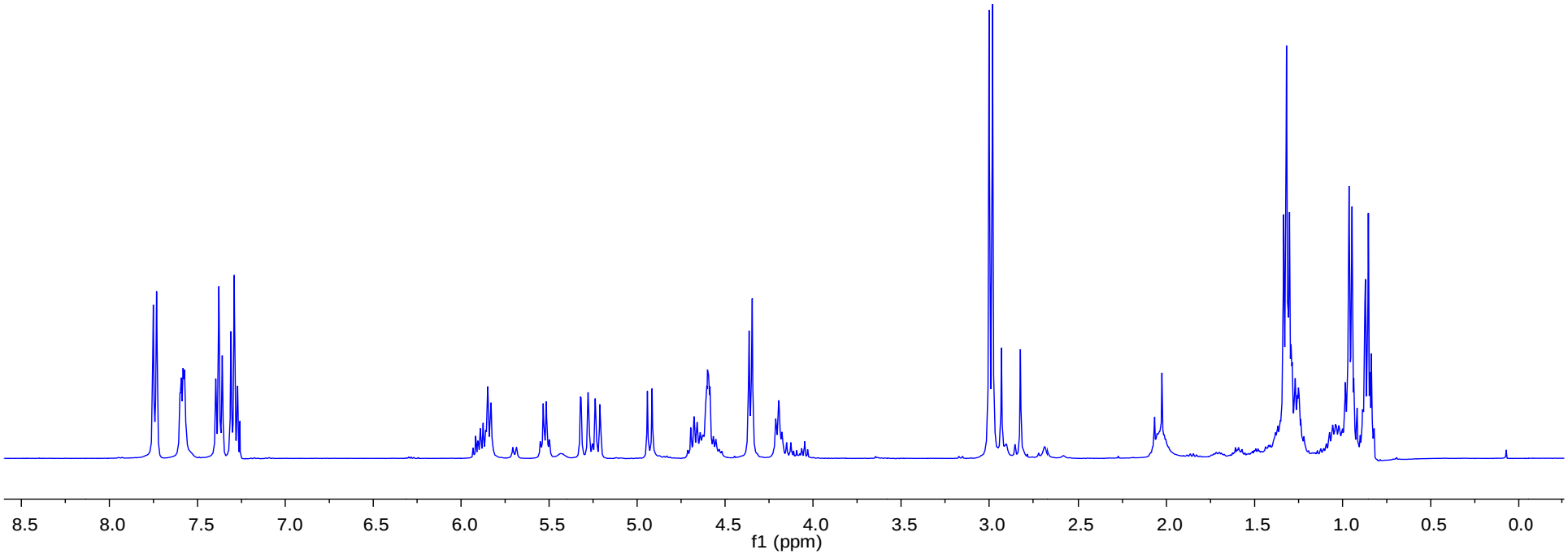
20c



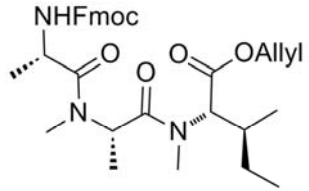
^{1}H NMR Spectrum of **20d** in $CDCl_3$ (400 MHz) at 25°C



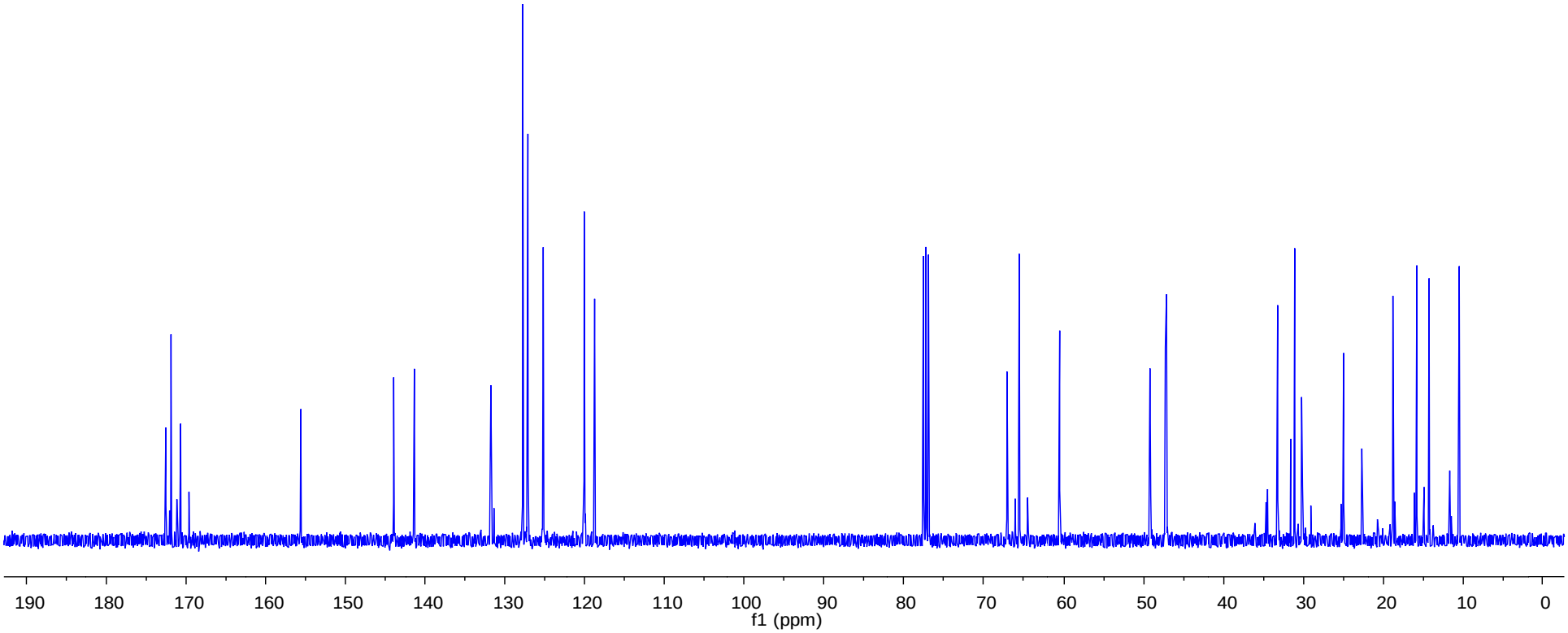
20d



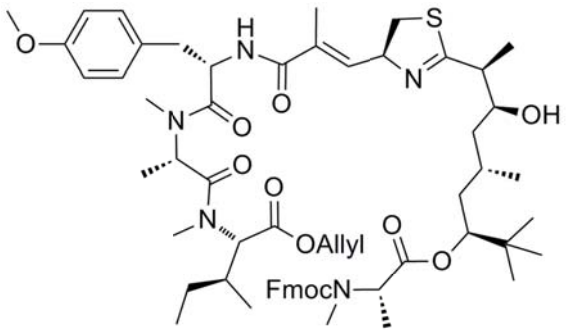
^{13}C NMR Spectrum of **20d** in $CDCl_3$ (100 MHz) at 25°C



20d

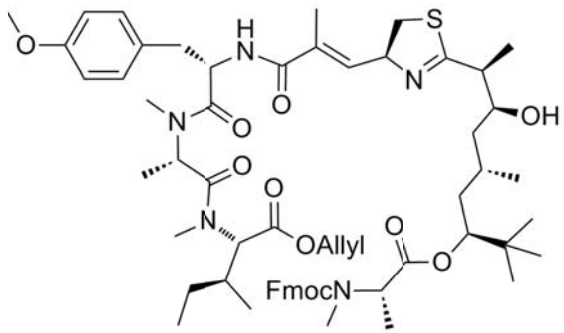


^{1}H NMR Spectrum of **13a** in $CDCl_3$ (500 MHz) at 25°C



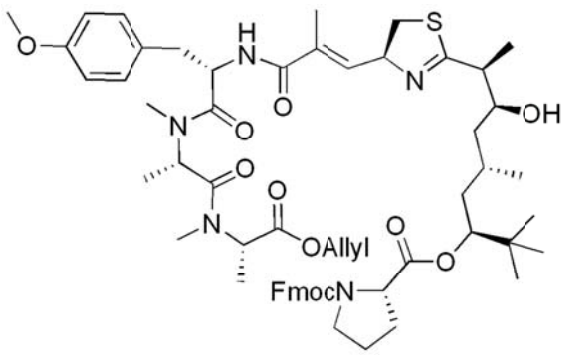
13a

^{13}C NMR Spectrum of **13a** in $CDCl_3$ (125 MHz) at 25°C



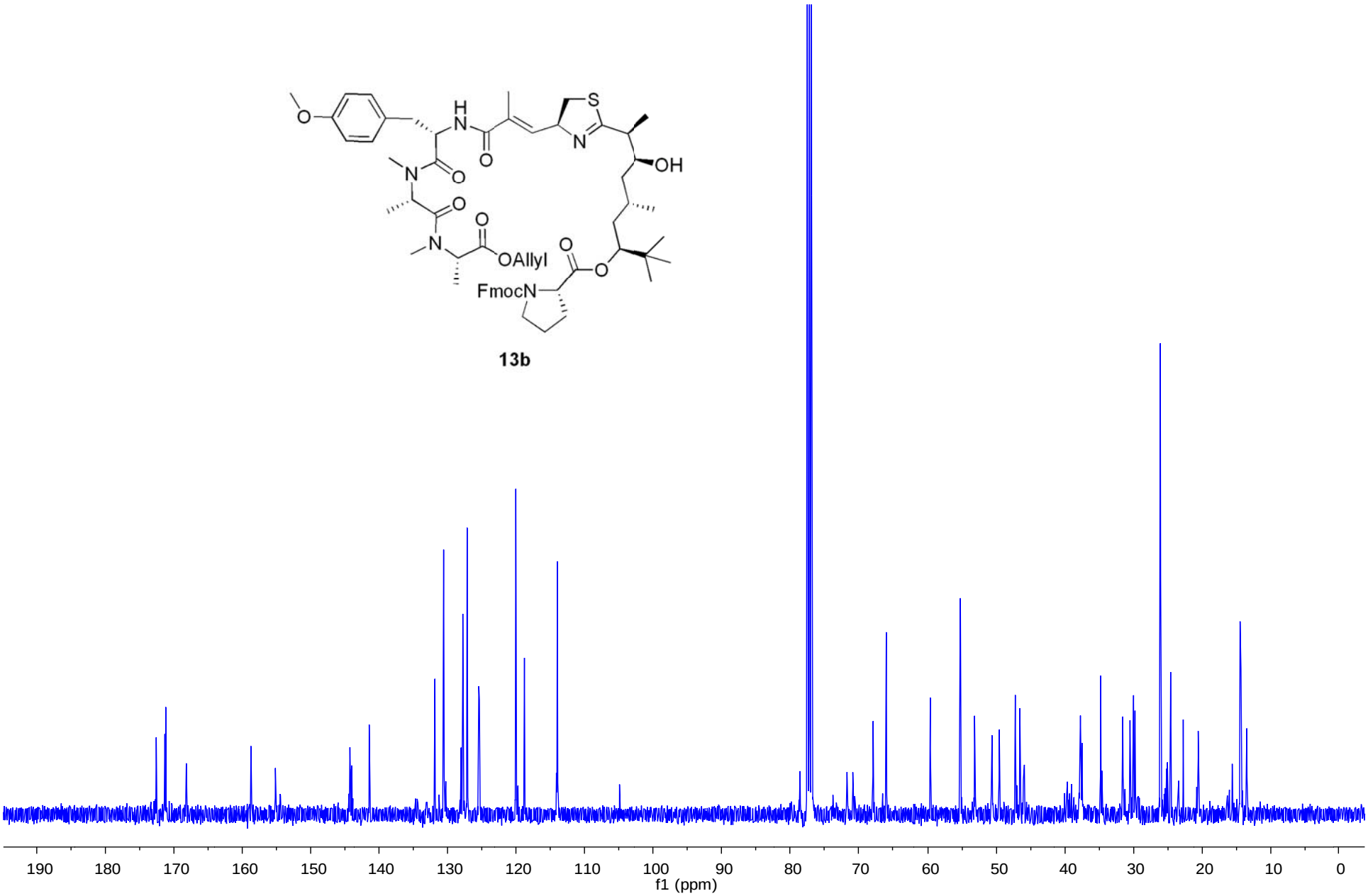
13a

^{1}H NMR Spectrum of **13b** in $CDCl_3$ (400 MHz) at 25°C



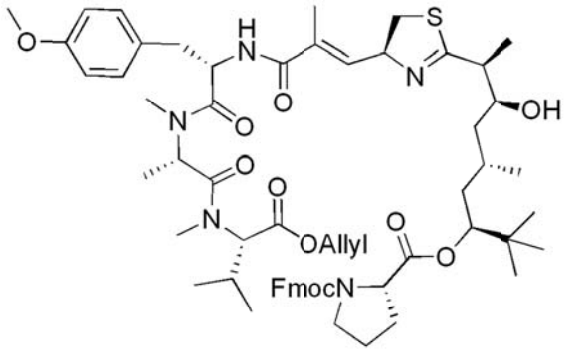
13b

^{13}C NMR Spectrum of **13b** in $CDCl_3$ (100 MHz) at 25°C



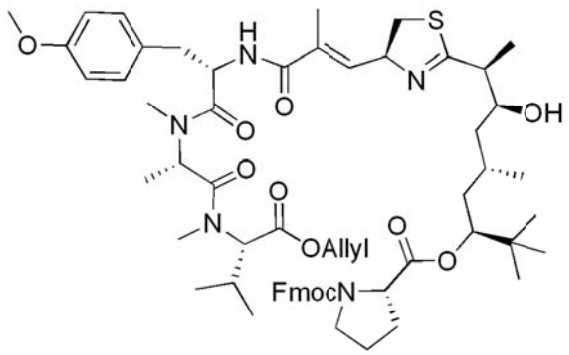
13b

^{1}H NMR Spectrum of **13c** in $CDCl_3$ (400 MHz) at 25°C



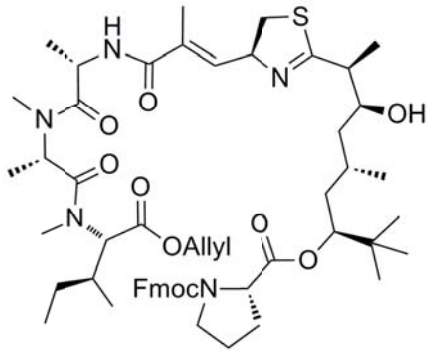
13c

^{13}C NMR Spectrum of **13c** in $CDCl_3$ (100 MHz) at 25°C



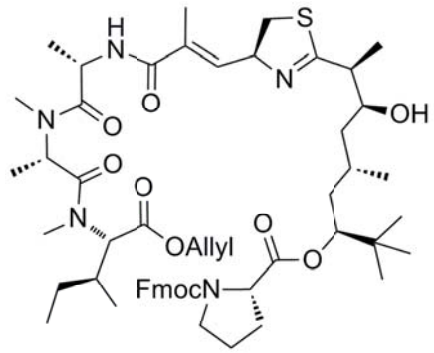
13c

^{1}H NMR Spectrum of **13d** in $CDCl_3$ (500 MHz) at 27°C



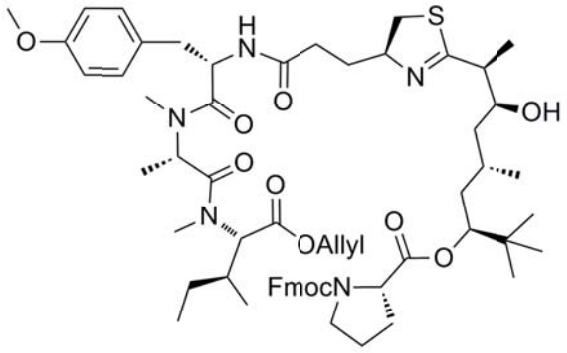
13d

^{13}C NMR Spectrum of **13d** in $CDCl_3$ (125 MHz) at 27°C



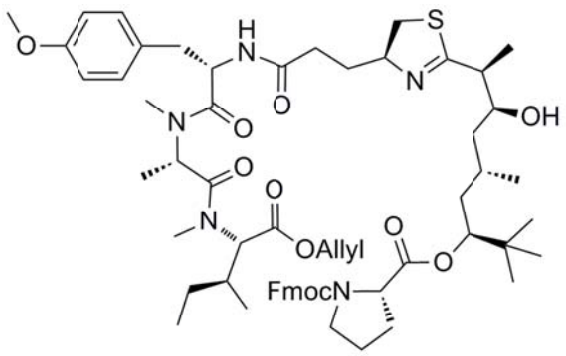
13d

^{1}H NMR Spectrum of **13e** in $CDCl_3$ (400 MHz) at 25°C



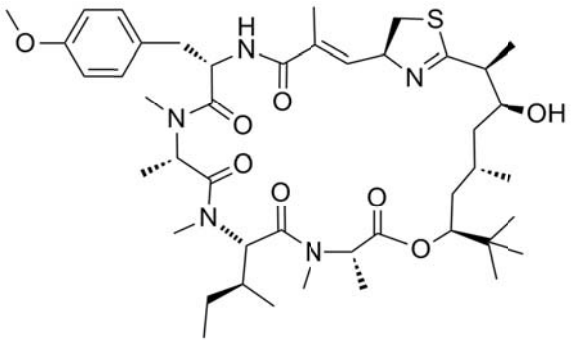
13e

^{13}C NMR Spectrum of **13e** in $CDCl_3$ (100 MHz) at 25°C



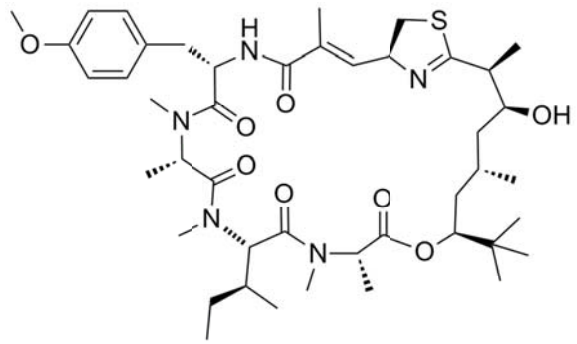
13e

^{1}H NMR Spectrum of **1a** in $CDCl_3$ (500 MHz) at 27°C



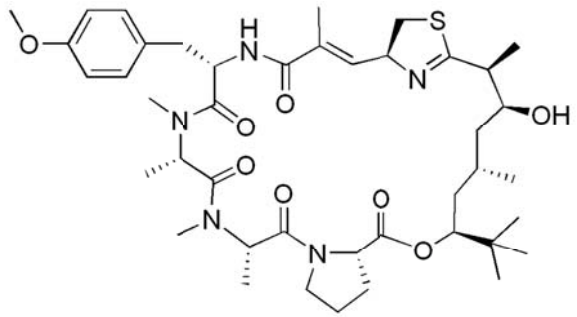
1a

^{13}C NMR Spectrum of **1a** in $CDCl_3$ (125 MHz) at 27°C



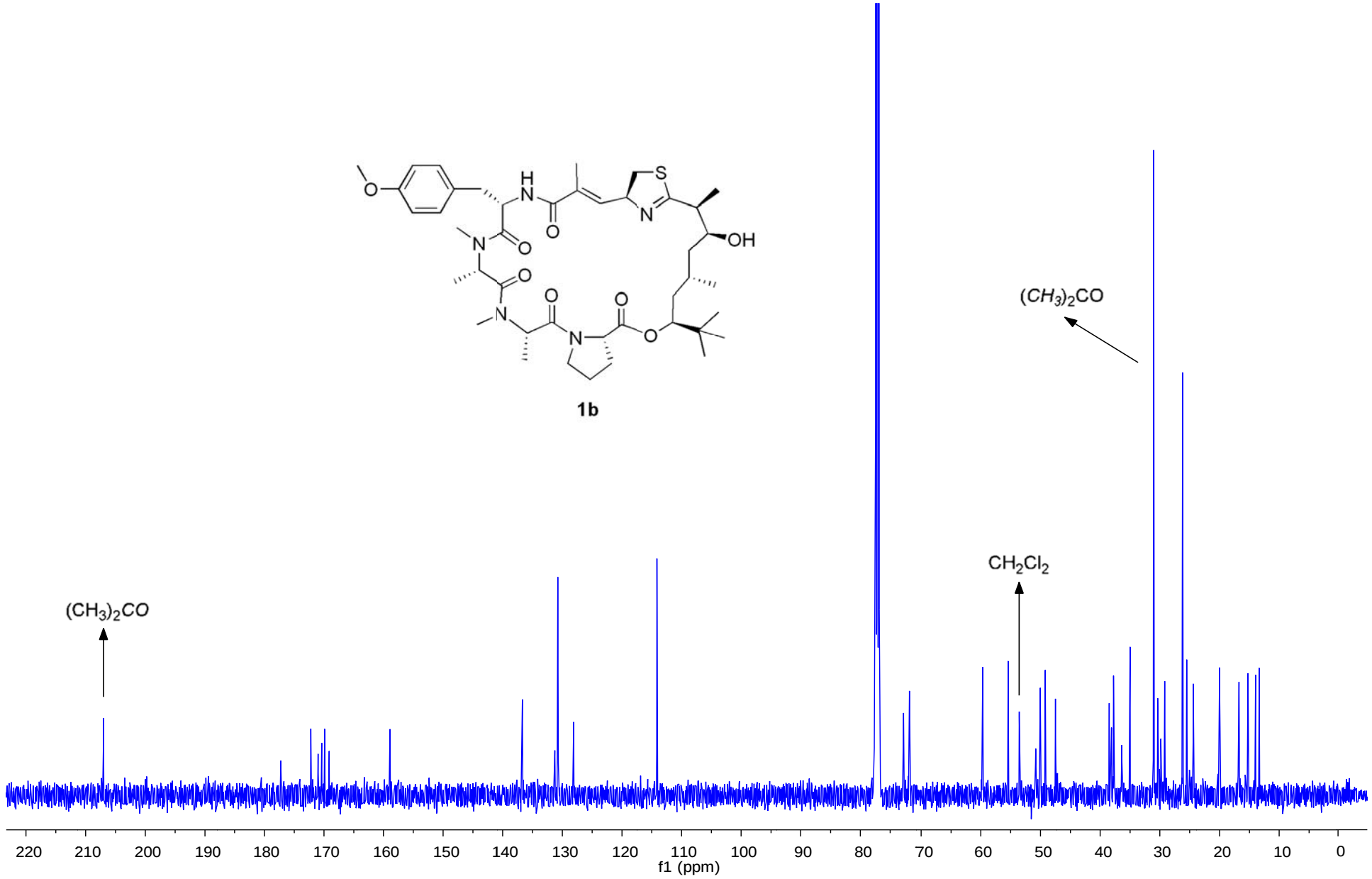
1a

^{1}H NMR Spectrum of **1b** in $CDCl_3$ (500 MHz) at 27°C



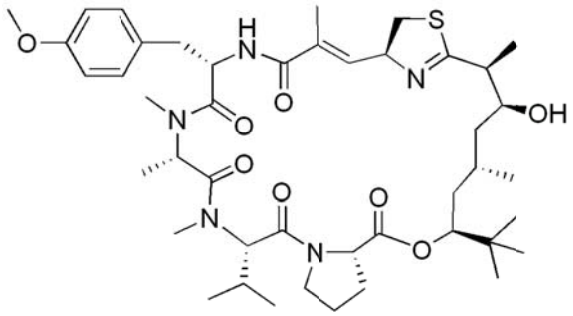
1b

^{13}C NMR Spectrum of **1b** in $CDCl_3$ (125 MHz) at 27°C



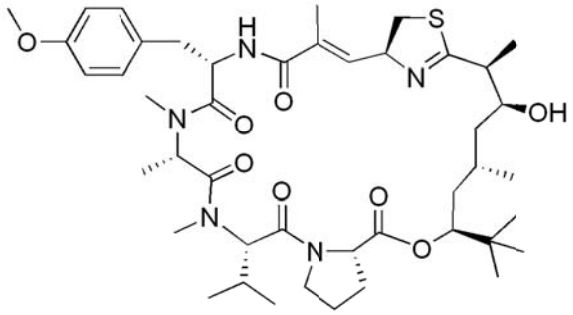
1b

^{1}H NMR Spectrum of **1c** in $CDCl_3$ (500 MHz) at 27°C



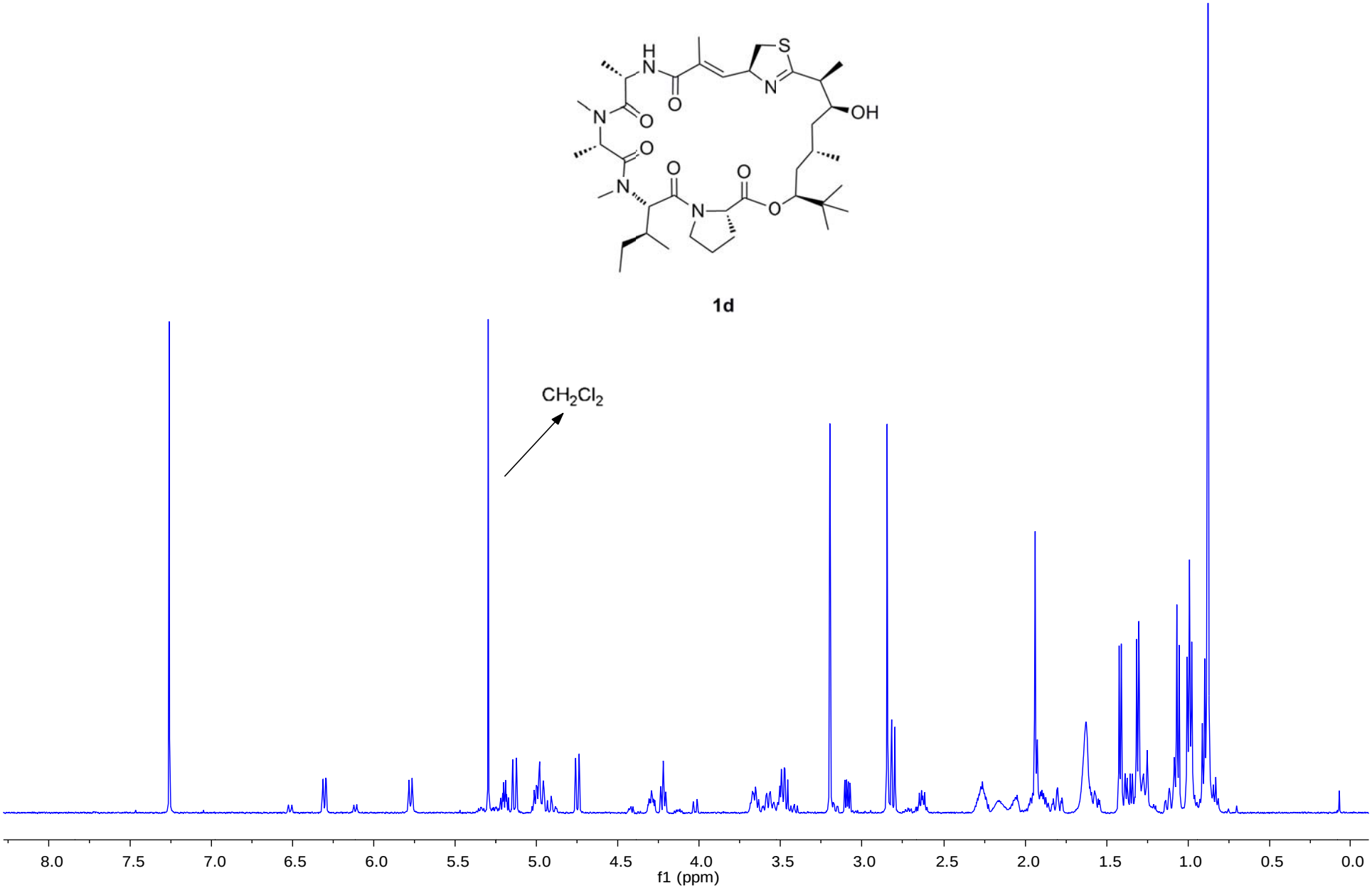
1c

^{13}C NMR Spectrum of **1c** in $CDCl_3$ (125 MHz) at 27°C

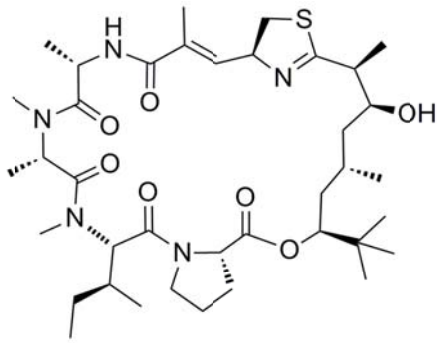


1c

^{1}H NMR Spectrum of **1d** in $CDCl_3$ (500 MHz) at 27°C

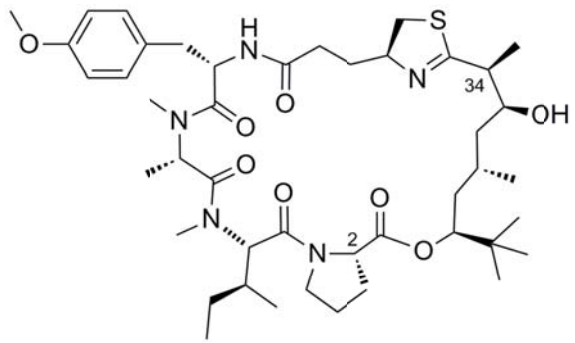


^{13}C NMR Spectrum of **1d** in $CDCl_3$ (125 MHz) at 27°C



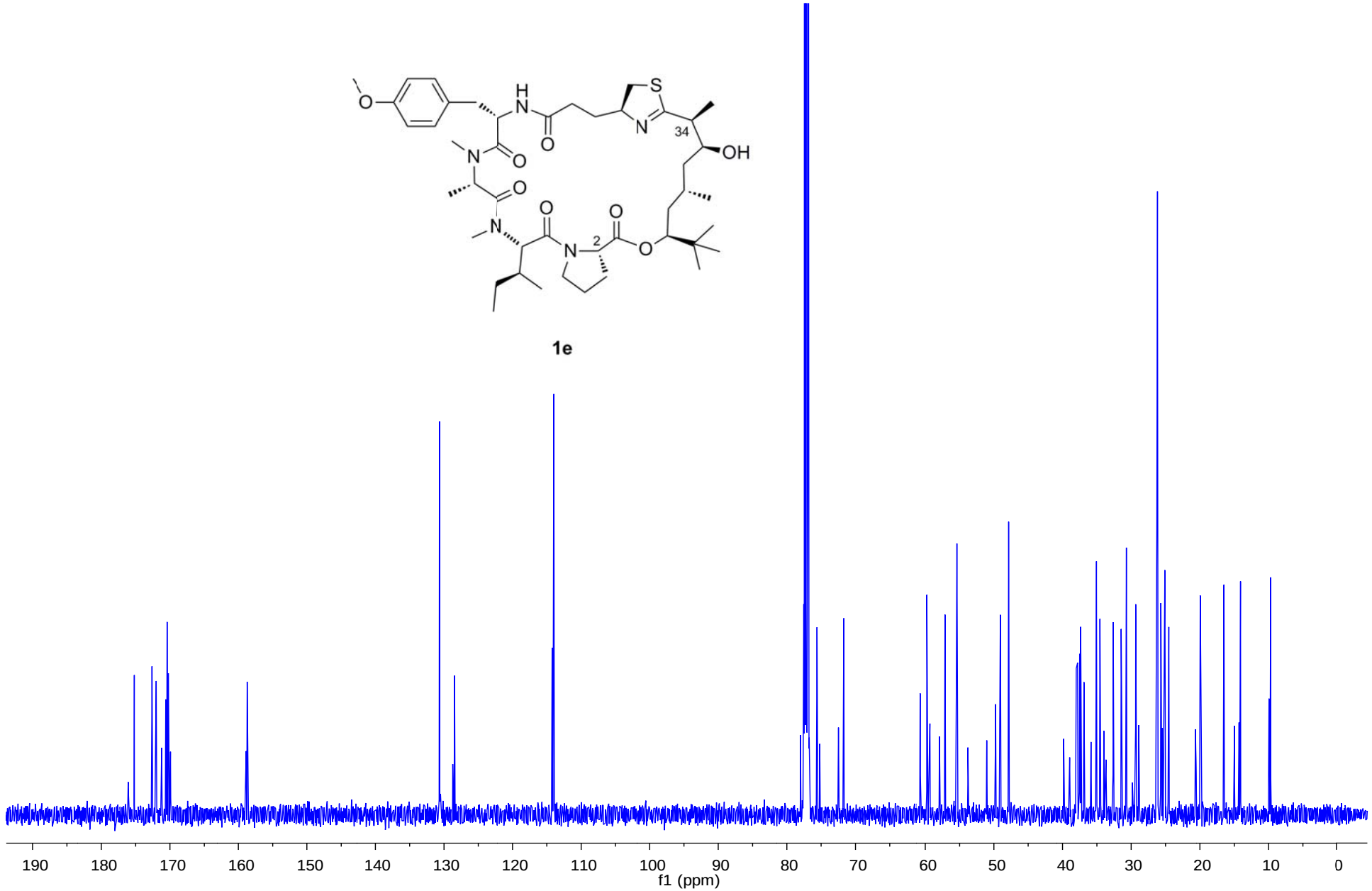
1d

^{1}H NMR Spectrum of **1e** in $CDCl_3$ (600 MHz) at 27°C

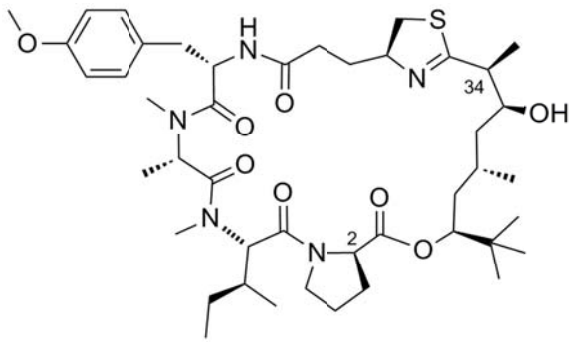


1e

^{13}C NMR Spectrum of **1e** in $CDCl_3$ (125 MHz) at 27°C

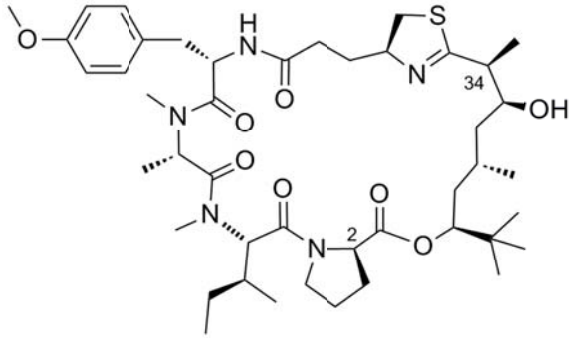


^{1}H NMR Spectrum of ***2-epi*-1e** in $CDCl_3$ (500 MHz) at 27°C



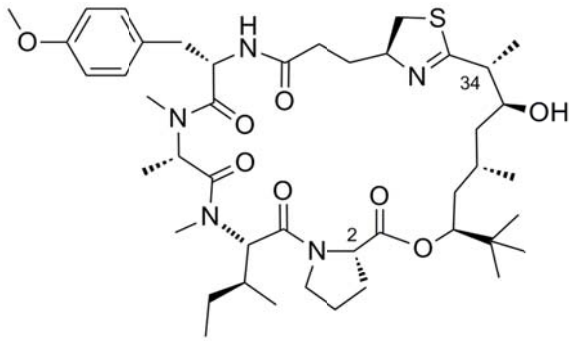
***2-epi*-1e**

^{13}C NMR Spectrum of ***2-epi*-1e** in $CDCl_3$ (125 MHz) at 27°C



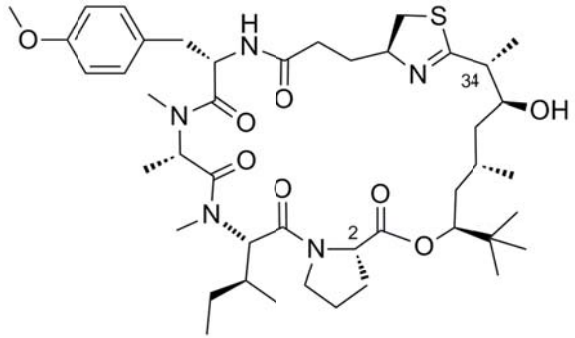
***2-epi*-1e**

^{1}H NMR Spectrum of ***34-epi*-1e** in $CDCl_3$ (500 MHz) at 27°C



***34-epi*-1e**

^{13}C NMR Spectrum of ***34-epi*-1e** in $CDCl_3$ (125 MHz) at 27°C



***34-epi*-1e**