Electronic Supplementary Material (ESI) for ChemComm.

Depsipeptide synthesis using a late-stage Ag(I)-promoted macrolactonisation of peptide thioamides

Sadegh Shabani and Craig A. Hutton*

School of Chemistry and Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Victoria, Australia, 3010.

chutton@unimelb.edu.au

Contents

General Information

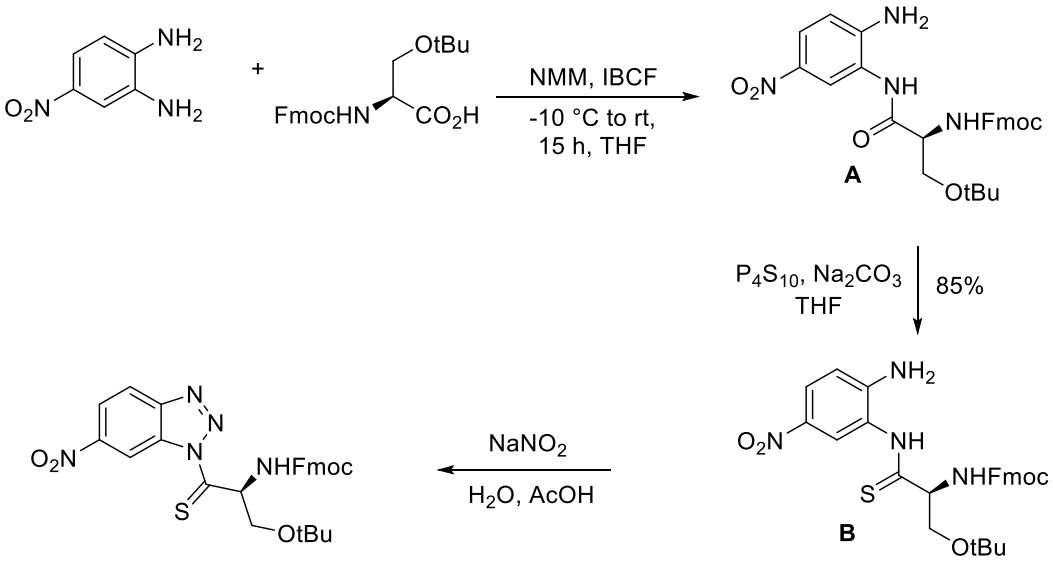
^{1}H NMR spectra were recorded using an Agilent 500 (500 MHz) or a Varian Unity Inova 400 (400 MHz) or a Varian Unity Inova 600 (600 MHz). Spectra were obtained in $CDCl_3$ (7.26), $(CD_3)_2CO$ (2.05), CD_3OD (3.31), or d_6 DMSO (2.50). The spectra are reported as: parts per million (ppm) downfield shift, relative to the residual solvent peak; relative integral, multiplicity (s = singlet, br = broad, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, dt = doublet of triplets, dq doublet of quartets, m = multiplet) and coupling constant (J in Hz). 13C NMR spectra were recorded using an Agilent 500 (125 MHz) or a Varian Unity Inova 600 (150 MHz). Chemical shifts (δ) are reported in parts per million (ppm) relative to the internal standard of the solvent peak; $CDCl_3$ (77.16), $(CD_3)_2CO$ (29.84, 206.26), CD_3OD (49.00), and DMSO-*d*$_6$ (39.52). All mass spectra were recorded on an MSFP OrbiTRAP infusion Mass Spectrometer. Most reagents were commercially available reagent grade chemicals and were used without further purification. Et2O, DMF, MeOH, EtOAc, Hex, ACN and CH_2Cl_2 were commercially available solvents and used without further drying. Analytical thin layer chromatography was performed with aluminium backed plates precoated with silica gel 60 F254 (0.2 mm), and visualisation was achieved by inspection under short-wave UV light followed by staining with phosphomolybdic acid dip [polyphosphomolybdic acid (12 g), ethanol (250mL)]. Flash chromatography was performed using silica gel (230–400 mesh); eluting solvents reported as % v/v mixtures. Analytical and preparative reverse phase HPLC (RP-HPLC) were performed using an Agilent 1200 series LC System. Analytical HPLC employed a Discovery C18 column (4.6 × 150 mm column, 5 μm particle size, flow rate of 1 mL min^{-1}). Preparative RP-HPLC employed a Phenomenex C18 column (21.2 × 150 mm, 5 μm particle size, flow rate 8 mL min^{-1}). The mobile phase consisted of eluents A (0.1% TFA in water) and B (0.1% TFA in acetonitrile). A linear gradient from 10–95% eluent B over 50

min was used for the preparative HPLC. A linear gradient from 0–100% eluent B over 30 min was used for the analytical HPLC. The results were analyzed on Agilent ChemStation version B.01.03 software.

General procedure for solid phase peptide synthesis:

The linear peptides were synthesised using standard Fmoc SPPS coupling methods on chlorotrityl resin. The coupling steps were performed using a CEM Liberty Blue microwave peptide synthesizer or within fritted syringes. All peptides were synthesised on 0.1 mmol scale using a 4- or 5-fold molar excess of Fmoc-protected amino acid (0.4 or 0.5 mmol for a 0.5mmol scale) that were activated using a 4- or 5-fold excess of HATU in the presence of *i*Pr$_2$NEt (4–8 equivalents). Fmoc deprotection was performed with 20% v/v piperidine in DMF.

Fmoc L-serine thiobenzotriazolide (S1)



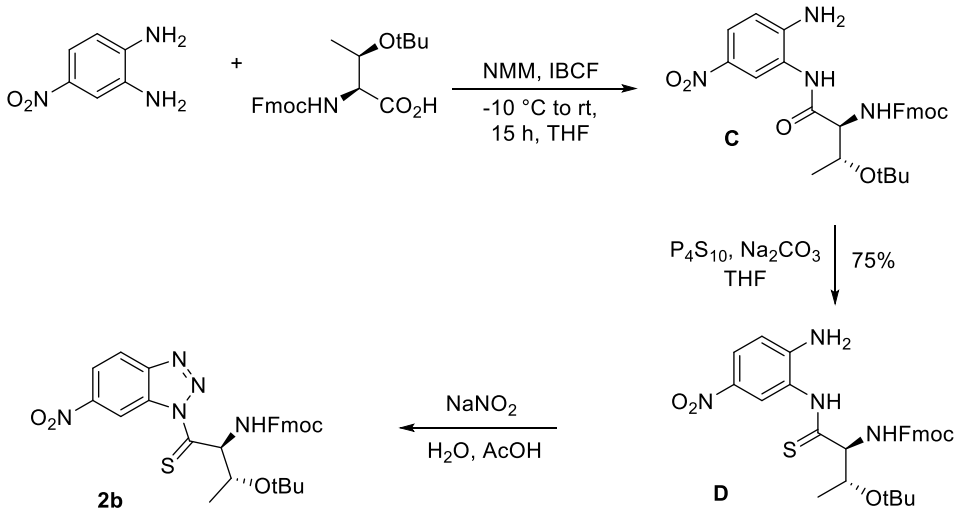
To a solution of Fmoc-Ser(*t*Bu)-OH (5.0 g, 13 mmol) in anhydrous THF (40 ml), N-methylmorpholine (1.42 ml, 13 mmol) and isobutylchloroformate (3.3 ml, 26 mmol) were added and stirred at –10 °C for 15 min. Diaminonitrobenzene (2.0 g, 13 mmol) was added and the mixture was warmed up to room temperature over 15 h. The solvent was evaporated and residue was dissolved in DMF (20 ml). Saturated KCl was added to the mixture and extracted with EtOAc (4 × 60 ml), and the combined organic layer was dried over magnesium sulfate. The solvent was evaporated and the crude amide **A** was used in the next step without further purification.

Anhydrous sodium carbonate (0.77 g, 7.24 mmol) and P_4S_{10} (3.2 g, 7.24 mmol) was dissolved in THF (60 ml) and stirred for 1 h at room temperature under argon atmosphere. Amide **A** (5.0 g, 9.65 mmol) was added and the mixture stirred for 5 h at room temperature. The solvent was evaporated and residue was dissolved in EtOAc (100 ml). The organic phase was washed with 5% $NaHCO_3$ (50 ml), brine (50 ml), and dried over magnesium sulfate. The solvent was evaporated and the residue was purified using column chromatography (EtOAc/Hex, 1:1) to afford thioamide **B** in 85% yield as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.62 (s, 1H), 8.26 (s, 1H), 8.05 (dd, *J*=9.0, 2.3 Hz, 1H), 7.77 (d, *J*=7.6 Hz, 2H), 7.62 (d, *J*=7.5 Hz, 2H), 7.47–7.38 (m, 2H), 7.36–7.26 (m, 2H), 6.76 (d, *J*=9.1 Hz, 1H), 6.57 (s, 1H), 4.56 (m, 1H), 4.52–4.41 (m, 2H), 4.33–4.21 (m, 3H), 1.35 (s, 9H), 1.18 (d, *J*=6.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 201.0, 155.7, 147.6, 143.6, 141.3, 141.3, 138.4, 127.8, 127.1, 125.1, 125.1, 124.4, 122.4, 120.0, 114.9, 69.1, 67.2, 63.7, 47.2, 46.3, 28.3, 14.6. MS (ESI) *m/z* 535 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{28}H_{31}N_4O_5S$ 535.2010, found 535.2012.

Thioamide **B** (4 g, 7.5 mmol) was dissolved in a solution of 5% water in glacial acetic acid (80 ml) at room temperature. The mixture was cooled down to 0 °C and $NaNO_2$ was added. The mixture was stirred for 45 min and cold water (300 ml) was added. The precipitates were filtered

and rinsed with cold water (200 ml) and dried under high vacuum. The resulting benzotriazole **S1** was used in SPPS without further purification. The compound is consistent with that reported.[1] Fmoc D-serine thiobenzotriazolide was also synthesised using the same procedure.

Fmoc threonine thiobenzotriazolide (S2)



To a solution of Fmoc-Thr(*t*Bu)-OH (5.1 g, 13 mmol) in anhydrous THF (40 ml), N-methylmorpholine (1.42 ml, 13 mmol) and isobutylchloroformate (3.3 ml, 26 mmol) were added and stirred at –10 °C for 15 min. Diaminonitrobenzene (2.0 g, 13 mmol) was added and the mixture was warmed up to room temperature over 15 h. The solvent was evaporated and residue was dissolved in DMF (20 ml). Saturated KCl was added to the mixture and extracted with EtOAc (4 × 60 ml), and the combined organic layer was dried over magnesium sulfate. The solvent was evaporated and the crude amide **C** was used in the next step without further purification.

Anhydrous sodium carbonate (0.77 g, 7.24 mmol) and P_4S_{10} (3.2 g, 7.24 mmol) were dissolved in THF (60 ml) and stirred for 1 h at room temperature under an atmosphere of argon. Amide **C** (5.1

g, 9.65 mmol) was added and the mixture stirred for 5 h at room temperature. The solvent was evaporated and the residue was dissolved in EtOAc (100 ml). The organic phase was washed with 5% $NaHCO_3$ (50 ml), brine (50 ml), and dried over magnesium sulfate. The solvent was evaporated and the residue was purified using column chromatography (EtOAc/Hex, 1:1) to afford thioamide **D** in 75% yield as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.27 (s, 1H), 8.07–7.98 (m, 2H), 7.79–7.71 (m, 3H), 7.63–7.50 (m, 3H), 7.44–7.37 (m, 2H), 7.35–7.24 (m, 3H), 6.67 (m, 1H), 4.72 (m, 1H), 4.54–4.37 (m, 2H), 4.22 (t, *J*=6.8 Hz, 1H), 3.62 (dd, *J*=8.8, 6.5 Hz, 1H), 1.22 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 203.1, 148.5, 143.5, 141.3, 127.9, 127.8, 127.1, 127.1, 125.7, 125.0, 120.1, 120.0, 120.0, 114.6, 74.8, 64.5, 61.9, 47.1, 46.3, 27.4. MS (ESI) *m/z* 549 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{29}H_{33}N_4O_5S$ 549.2166, found 549.2168.

Thioamide **D** (4.1 g, 7.5 mmol) was dissolved in a solution of 5% water in glacial acetic acid (80 ml) at room temperature. The mixture was cooled to 0 °C and $NaNO_2$ was added. The mixture was stirred for 45 min and cold water (300 ml) was added. The precipitates were filtered and rinsed with cold water (200 ml) and dried under high vacuum. The resulting benzotriazole **S2** was used in SPPS without further purification. Fmoc D-threonine thiobenzotriazolide was also synthesised using the same procedure.

Thioamide amino acid coupling

Resin (0.1 mmol) containing the appropriate peptide was transferred into a fritted syringe and washed with CH_2Cl_2 (× 5), then swelled in CH_2Cl_2 (10 ml). The required Fmoc-amino acid thiobenzotriazolide (0.15 mmol) and NEt_3 (20 µl, 0.15 mmol) were added and the mixture was shaken for 2 h. The resin was washed with DMF (× 5), CH_2Cl_2 (× 5), and DMF (× 5). The Fmoc

deprotection was performed using 20% v/v piperidine in DMF (15 ml) for 5 min. The resin was washed with DMF (× 5), CH_2Cl_2 (× 5), and DMF (× 5) to afford the N-terminal thiopeptide containing resin ready for the next coupling.

General procedure for coupling of amino acid to the N-terminus of thioamide residue

To a solution of Fmoc amino acid (0.4 mmol) in DMF (10 ml), HATU (0.15 g, 0.4 mmol) and *i*Pr_2NEt (0.05 ml, 0.4 mmol) were added and mixture was shaken for 5 min. The resin was swelled in DMF (5 ml) and the activated amino acid was added. The mixture was shaken for 2 h and discharged from the fritted syringe. The resin was washed with DMF (× 5), CH_2Cl_2 (× 5) and DMF (× 5) to furnish the protected linear peptide thioamide on resin.

Cleavage of linear peptide thioamide from resin

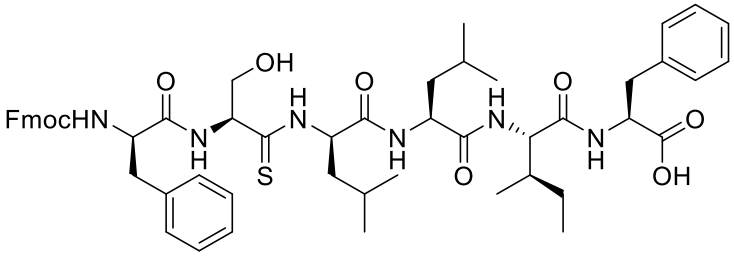
The resin with attached peptide thioamide (0.1 mmol) was treated with TFA in CH_2Cl_2 (2% v:v, 5 ml) for 15 min. The solvent was drained and this process was repeated a further two times. The filtrates were combined and solvent was evaporated under reduced pressure. The compound was redissolved in a solution of TFA in CH_2Cl_2 (75% v:v, 10 ml) and stirred for 1 h. The solvent was evaporated and the residue was dried under high vacuum then used directly in the next step.

General procedure for the macrolactonisation of peptide thioamides

The crude peptide thioamide (0.1 mmol) was dissolved in a solution of CH_3CN and CH_2Cl_2 (1:1, 12 ml, 8 mM) and Ag_2CO_3 (33 mg, 0.15 mmol) was added. The reaction mixture was stirred at

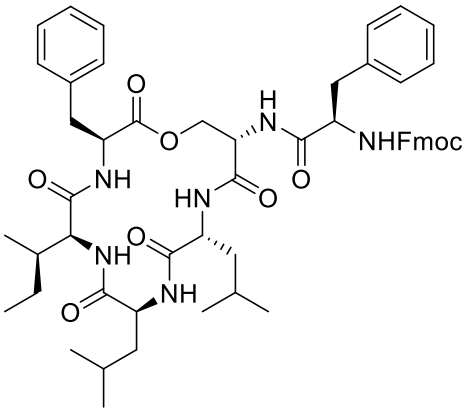
room temperature until cyclisation was complete. The mixture was centrifuged to remove the black Ag_2S precipitate, then the solvent was evaporated under a stream of nitrogen. The cyclic depsipeptide was purified using an Agilent RP-HPLC using gradient elution with buffer 20-85% over 45 min, monitoring at a wavelength of 214 nm.

Fmoc-DFS[S]DLLIF (1a)



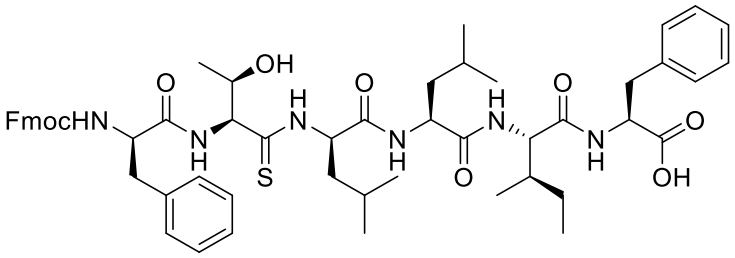
The linear Fmoc-DFS[S]DLLIF **1a** was synthesised using the general procedures for peptide thioamide synthesis (50 mg, 51%). MS (ESI) *m/z* 977 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{54}H_{69}N_6O_9S$ 977.4841, found 977.4847.

Macrocycle (2a)



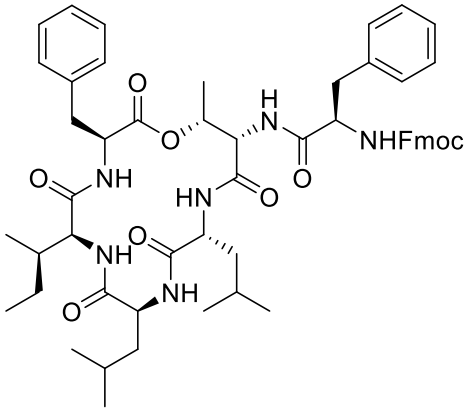
The linear Fmoc-DFS[S]DLLIF **1a** (50 mg, 0.051 mmol) was cyclised using the general procedure for peptide macrolactonisation. After 24 h the mixture was purified by preparative HPLC to give **2a** as a white foam (25 mg, 41% from linear peptide, 30% overall from starting resin). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.26–8.15 (m, 2H), 8.07 (d, *J*=8.5 Hz, 1H), 7.90–7.75 (m, 4H), 7.63–7.51 (m, 2H), 7.42–7.32 (m, 2H), 7.33–7.09 (m, 13H), 6.04 (d, *J*=9.4 Hz, 1H), 5.56 (d, *J*=12.5 Hz, 1H), 4.45–4.30 (m, 3H), 4.31–4.17 (m, 3H), 4.16–4.04 (m, 3H), 3.06–2.93 (m, 2H), 2.88 (dd, *J*=13.8, 8.7 Hz, 1H), 2.78 (t, *J*=12.2 Hz, 1H), 1.78 (m, 1H), 1.58–1.44 (m, 2H), 1.44–1.26 (m, 4H), 1.29–1.16 (m, 2H), 1.15–0.98 (m, 3H), 0.87–0.68 (m, 18H), 0.69–0.60 (m, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.3, 173.1, 172.4, 172.1, 171.9, 171.5, 171.2, 164.1, 156.3, 144.2, 144.1, 141.0, 138.3, 137.9, 135.4, 129.6, 129.5, 128.6, 128.5, 128.0, 127.5, 126.7, 125.7, 125.6, 120.5, 115.6, 105.0, 66.3, 58.4, 57.5, 56.2, 53.9, 53.7, 51.7, 51.0, 50.9, 46.9, 41.3, 37.4, 36.9, 36.5, 30.0, 24.9, 24.6, 24.3, 23.5, 23.3, 21.9, 21.7, 15.7. MS (ESI) *m/z* 943 [(M+H)$^+$, 100%]; HRMS (ESI, [M+H]$^+$) calcd. for $C_{54}H_{67}N_6O_9$ 943.4964, found 943.4966.

Fmoc-DFT[S]DLLIF (1b)



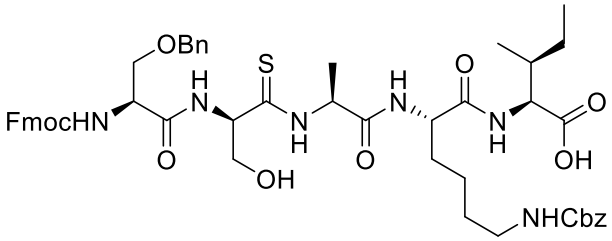
The linear Fmoc-DFT[S]DLLIF **1b** was synthesised using the general procedures for peptide thioamide synthesis (41 mg, 43%). MS (ESI) *m/z* 991 [(M+H)$^+$, 100%]; HRMS (ESI, [M+H]$^+$) calcd. for $C_{55}H_{71}N_6O_9S$ 991.4998, found 991.4999.

Macrocycle (2b)



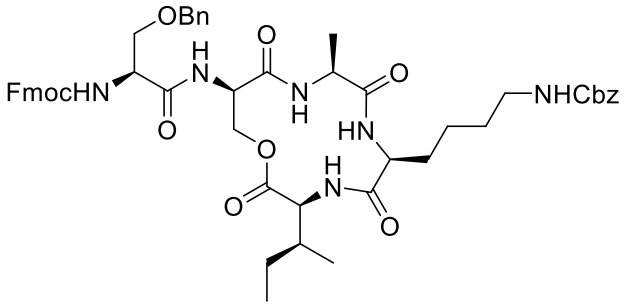
The linear Fmoc-DFT[S]DLLIF **1b** (41 mg, 0.04 mmol) was cyclised using the general procedure for macrolactonisation. After 24 h the mixture was purified by preparative HPLC to give **2b** as a white foam (23 mg, 58% from linear peptide, 25% overall from starting resin). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (dd, *J*=7.7, 4.5 Hz, 2H), 7.56–7.32 (m, 10H), 7.32–7.27 (m, 5H), 7.23–7.15 (m, 5H), 7.09 (d, *J*=8.6 Hz, 1H), 6.60 (d, *J*=7.6 Hz, 1H), 4.76–4.60 (m, 1H), 4.64–4.34 (m, 3H), 4.34–4.07 (m, 4H), 4.02 (d, *J*=5.6 Hz, 1H), 3.33 (m, 1H), 3.20 (m, 1H), 3.13 (m, 1H), 2.97 (dd, *J*=14.1, 9.1 Hz, 1H), 1.50–1.72 (m, 4H), 1.62 (d, *J*=7.3 Hz, 3H), 1.33–1.17 (m, 3H), 1.02–0.77 (m, 22H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.3, 173.2, 172.3, 165.5, 157.3, 143.4, 143.0, 141.4, 141.3, 137.2, 135.2, 129.3, 129.2, 128.9, 128.3, 127.9, 127.1, 127.0, 126.5, 124.7, 120.1, 67.3, 60.9, 58.1, 53.9, 53.3, 47.1, 39.4, 39.2, 37.1, 36.6, 35.7, 25.5, 25.1, 24.6, 23.2, 23.0, 21.1, 20.7, 15.5, 12.8, 11.6. MS (ESI) *m/z* 957 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{55}H_{69}N_6O_9$ 957.5121, found 957.5120.

Fmoc-S(Bn)DS[S]AK(Cbz)I (3a)



The linear Fmoc-S(Bn)DS[S]AK(Cbz)I **3a** was synthesised using the general procedures for peptide thioamide synthesis (60 mg, 62%). MS (ESI) *m/z* 967 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{51}H_{63}N_6O_{11}S$ 967.4270, found 967.4271.

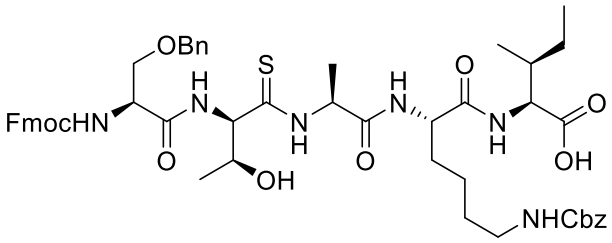
Macrocycle (4a)



The linear Fmoc-S(Bn)DS[S]AK(Cbz)I **3a** (60 mg, 0.06 mmol) was cyclised using the general procedure for macrolactonisation. After 48 h the mixture was purified by preparative HPLC to give **4a** as a white foam (20 mg, 35% from linear peptide, 22% overall from starting resin). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 (d, *J*=7.7 Hz, 3H), 7.57 (d, *J*=6.3 Hz, 2H), 7.43–7.19 (m, 15H), 5.15–4.94 (m, 3H), 4.75–4.43 (m, 5H), 4.43–4.05 (m, 5H), 3.90–3.57 (m, 2H), 3.25–2.96 (m, 2H), 2.07–1.57 (m, 4H), 1.58–1.08 (m, 6H), 1.02–0.72 (m, 7H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.8, 173.3, 172.6, 170.0, 163.9, 160.0, 156.7, 143.5, 141.3, 137.0, 136.3, 130.2, 128.9, 128.5, 128.5,

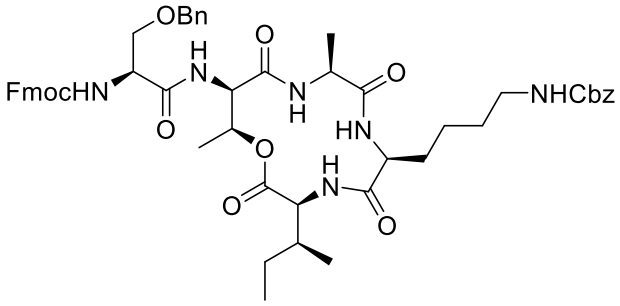
128.1, 128.1, 128.0, 127.8, 127.1, 125.0, 120.0, 73.5, 69.4, 69.1, 67.5, 67.3, 66.8, 57.3, 55.3, 53.8, 50.0, 47.0, 40.4, 36.8, 31.4, 29.2, 26.0, 25.0, 22.6, 17.8, 15.4, 11.4. MS (ESI) *m/z* 932 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{51}H_{61}N_6O_{11}$ 932.4320, found 932.4325.

Fmoc-S(Bn)DT[S]AK(Cbz)I 3b



The linear Fmoc-S(Bn)DT[S]AK(Cbz)I **3b** was synthesised using the general procedures for peptide thioamide synthesis (55 mg, 56%). MS (ESI) *m/z* 981 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{52}H_{65}N_6O_{11}S$ 981.4427, found 981.4427.

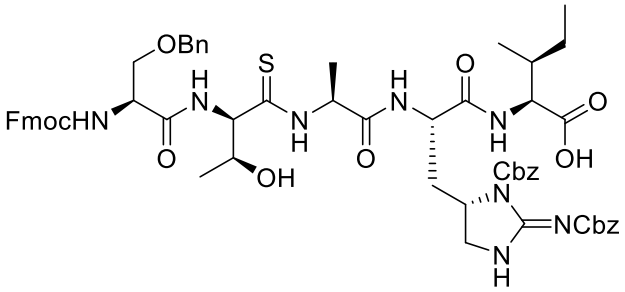
Macrocycle (4b)



The linear Fmoc-S(Bn)DT[S]AK(Cbz)I **3b** (55 mg, 0.056 mmol) was cyclised using the general procedure for macrolactonisation. After 48 h the mixture was purified by preparative HPLC to give **4b** as a white foam (18 mg, 34% from linear peptide, 20% overall from starting resin). ^1H

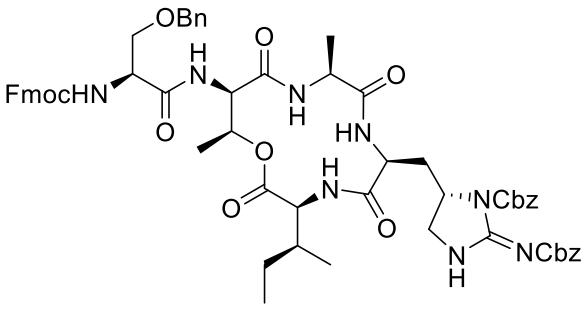
NMR (600 MHz, DMSO-d_6) δ 8.10 (d, *J*=7.5 Hz, 1H), 7.85 (d, *J*=7.5 Hz, 3H), 7.79 (m, 1H), 7.75–7.65 (m, 3H), 7.60 (d, *J*=8.4 Hz, 1H), 7.46 (d, *J*=7.0 Hz, 1H), 7.41–7.35 (m, 2H), 7.35–7.20 (m, 13H), 7.15 (d, *J*=6.0 Hz, 1H), 6.41 (q, *J*=7.0 Hz, 1H), 4.95 (d, *J*=3.7 Hz, 2H), 4.55–4.41 (m, 2H), 4.39–4.07 (m, 8H), 3.71–3.45 (m, 5H), 1.72 (m, 1H), 1.68–1.51 (m, 3H), 1.46 (m, 1H), 1.41–1.28 (m, 4H), 1.29–1.07 (m, 7H), 0.85–0.72 (m, 7H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.2, 172.4, 172.2, 171.9, 163.9, 156.5, 144.2, 141.1, 138.6, 138.4, 137.7, 128.8, 128.6, 128.6, 128.1, 128.1, 127.9, 127.9, 127.8, 127.5, 125.7, 125.7, 120.5, 72.7, 72.4, 66.3, 65.5, 56.6, 52.7, 49.0, 48.6, 47.0, 40.7, 36.8, 36.7, 32.1, 31.8, 29.6, 25.0, 23.0, 18.7, 18.2, 15.9, 13.5, 11.7. MS (ESI) *m/z* 947 [(M+H)$^+$, 100%]; HRMS (ESI, [M+H]$^+$) calcd. for $C_{52}H_{63}N_6O_{11}$ 947.4549, found 947.4547.

Fmoc-S(Bn)DT$^{[S]}$AEnd(Cbz)$_2$I (5)



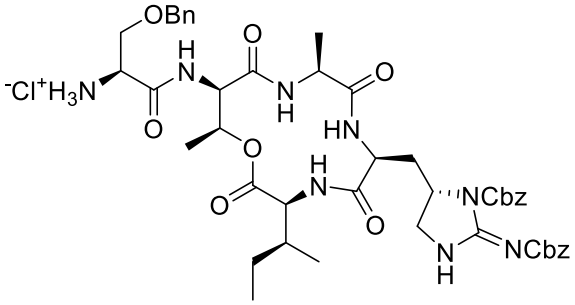
The linear Fmoc-S(Bn)DT$^{[S]}$AEnd(Cbz)$_2$I **5** was synthesised using the general procedures for peptide thioamide synthesis (38 mg, 33%). MS (ESI) *m/z* 1141 [(M+H)$^+$, 100%]; HRMS (ESI, [M+H]$^+$) calcd. for $C_{60}H_{69}N_8O_{13}S$ 1141.4699, found 1141.4716.

Macrocycle (6)



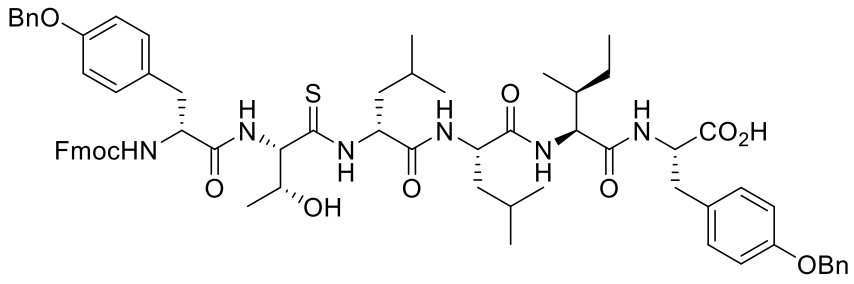
The linear Fmoc-S(Bn)DT[S]AE(Cbz)$_2$I **5** (38 mg, 0.033 mmol) was cyclised using the general procedure for macrolactonisation. After 48 h the mixture was purified by preparative HPLC to give the compound **6** as a white foam (10 mg, 27% starting from linear peptide, 10% overall starting from initial loaded resin). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.93 (s, 1H), 7.73 (d, *J*=7.6 Hz, 2H), 7.60–7.49 (m, 2H), 7.37 (t, *J*=7.5 Hz, 2H), 7.34–7.25 (m, 17H), 6.42 (s, 1H), 5.99–5.62 (m, 2H), 5.20–4.90 (m, 5H), 4.63–4.45 (m, 3H), 4.45–4.38 (m, 2H), 4.22–4.04 (m, 2H), 3.93–3.51 (m, 4H), 1.82 (s, 2H), 1.53 (s, 2H), 1.39–1.34 (m, 3H), 1.26–1.22 (m, 2H), 1.09 (s, 1H), 0.87–0.71 (m, 9H). MS (ESI) *m/z* 1107 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{60}H_{67}N_8O_{13}$ 1107.4822, found 1107.4825.

Macrocycle (7)



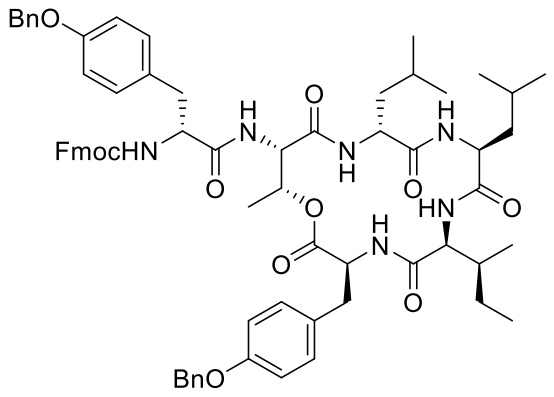
Fmoc protected peptide **6** (7 mg, 0.006 mmol) was treated with a solution of 20% of piperidine in DMF (1 ml) for 1 min. 1 N HCl (1 ml) was added and the solvent was evaporated under a stream of nitrogen. The compound was purified using HPLC to give **7** (2.7 mg, 50%) as a white foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.38 (s, 1H), 7.97 (s, 2H), 7.29 (d, *J*=13.3 Hz, 17H), 6.34 (s, 1H), 5.38–4.79 (m, 3H), 4.64–4.43 (m, 2H), 4.43–4.08 (m, 3H), 4.03–3.77 (m, 2H), 3.66 (s, 1H), 2.66 (s, 2H), 1.86 (d, *J*=15.8 Hz, 1H), 1.71 (d, *J*=14.8 Hz, 1H), 1.67–1.31 (m, 5H), 1.11 (s, 1H), 0.96–0.77 (m, 9H). MS (ESI) *m/z* 885 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{45}H_{57}N_8O_{11}$ 885.4141, found 885.4140.

Fmoc-DY(Bn)T$^{[S]}$DLLIY(Bn) (8)



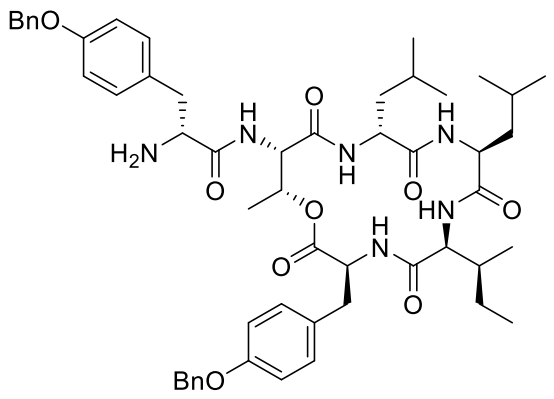
The linear Fmoc-DY(Bn)T$^{[S]}$DLLIY(Bn) **8** was synthesised using the general procedures for peptide thioamide synthesis (80 mg, 66%). MS (ESI) *m/z* 1203 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{69}H_{83}N_6O_{11}S$ 1203.5835, found 1203.5837.

Macrocycle (9)



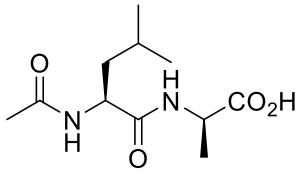
The linear Fmoc-DY(Bn)T[S]DLLIY(Bn) **8** (80 mg, 0.066 mmol) was cyclised using the general procedure for macrolactonisation. Ater 24 h the mixture was purified by preparative HPLC to give **9** as a white foam (23 mg, 30% starting from linear peptide, 20% overall from starting resin). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.07 (s, 1H), 7.75 (d, *J*=7.6 Hz, 2H), 7.51 (t, *J*=6.5 Hz, 2H), 7.45–7.25 (m, 16H), 7.14 (d, *J*=8.2 Hz, 2H), 7.07 (d, *J*=8.4 Hz, 2H), 7.01 (s, 1H), 6.91 (d, *J*=8.2 Hz, 2H), 6.88–6.81 (m, 2H), 6.65 (d, *J*=8.0 Hz, 1H), 5.53 (s, 1H), 4.99 (s, 2H), 4.96 (s, 2H), 4.43 (m, 3H), 4.38–4.19 (m, 4H), 4.16 (t, *J*=7.0 Hz, 1H), 3.24–3.08 (m, 2H), 3.07–2.89 (m, 2H), 2.04–1.75 (m, 3H), 1.75–1.65 (m, 2H), 1.65–1.54 (m, 3H), 1.51 (d, *J*=7.1 Hz, 3H), 1.42–1.20 (m, 2H), 1.11–0.99 (m, 1H), 0.94 (d, *J*=5.9 Hz, 3H), 0.89 (d, *J*=5.9 Hz, 3H), 0.84 (d, *J*=6.1 Hz, 3H), 0.82–0.66 (m, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.1, 172.8, 172.6, 171.9, 171.1, 165.4, 158.1, 157.8, 157.1, 143.5, 143.2, 141.3, 137.0, 136.9, 130., 130.2, 128.6, 128.5, 128.0, 128.0, 127.9, 127.9, 127.8, 127.5, 127.5, 127.4, 127.4, 127.1, 125.0, 124.9, 120.0, 115.2, 115.0, 115.0, 70.0, 67.6, 56.8, 55.0, 53.2, 52.7, 46.9, 40.2, 38.5, 36.4, 36.2, 35.9, 25.0, 24.7, 24.6, 23.0, 22.6, 21.9, 21.2, 15.2, 13.3, 11.1. MS (ESI) *m/z* 1169 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{69}H_{81}N_6O_{11}$ 1169.5958, found 1169.6027.

Macrocycle (S3)



Compound **9** (20 mg, 0.017 mmol) was treated with a solution of 20% piperidine in DMF (2 ml) for 1 min then 1 N HCl (10 ml) was added. The mixture was extracted with EtOAc (3 × 10 ml) and organic layers were washed with brine (10 ml), and dried over magnesium sulfate. The solvent was evaporated and the residue was purified using HPLC to afford the free amine **S3** (10 mg, 65%) as a white solid. ^{1}H NMR (500 MHz, CD_3OD) δ 8.19 (d, *J*=8.1 Hz, 1H), 7.86 (d, *J*=8.7 Hz, 1H), 7.44–7.40 (m, 4H), 7.40–7.34 (m, 5H), 7.33–7.28 (m, 2H), 7.24 (d, *J*=8.4 Hz, 2H), 7.17–7.12 (m, 2H), 6.98 (d, *J*=8.3 Hz, 2H), 6.91 (d, *J*=8.5 Hz, 2H), 6.26 (q, *J*=7.0 Hz, 1H), 5.08 (s, 2H), 5.03 (s, 3H), 4.61–4.54 (m, 1H), 4.48–4.32 (m, 2H), 4.28–4.19 (m, 2H), 3.26 (dd, *J*=14.2, 6.7 Hz, 1H), 3.13–3.03 (m, 2H), 2.96 (dd, *J*=14.0, 7.9 Hz, 1H), 1.93–1.80 (m, 1H), 1.67 (m, 3H), 1.58 (d, *J*=7.1 Hz, 3H), 1.51 (ddd, *J*=13.7, 7.6, 3.1 Hz, 1H), 1.21–1.07 (m, 1H), 1.03–0.79 (m, 23H). ^{13}C NMR (126 MHz, CD_3OD) δ 173.3, 173.0, 173, 172.0, 167.4, 166.3, 158.5, 157.8, 137.4, 137.2, 130.3, 130.0, 130.0, 129.5, 129.0, 128.1, 128.1, 127.5, 127.4, 127.1, 127.1, 126.1, 115.2, 114.5, 114.5, 69.5, 69.5, 57.6, 54.4, 54.0, 52.6, 51.9, 39.8, 39.6, 36.4, 36.2, 36.1, 24.7, 24.6, 24.4, 22.2, 21.8, 20.7, 20.3, 14.4, 12.1, 9.8. MS (ESI) *m/z* 947 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{54}H_{71}N_6O_9$ 947.5277, found 947.5277.

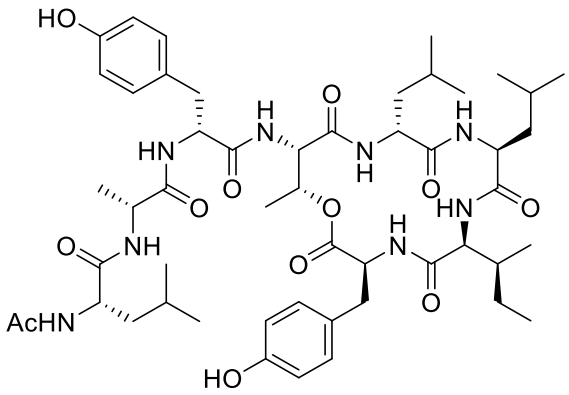
Ac-Leu-DAla-OH



To a mixture of Ac-Leu-OH (2.00 g, 11.5 mmol), EDC. Cl (2.10 g, 13.8 mmol), and HOBt (1.60 g, 13.8) in DMF (20 ml), D-Ala-OMe.HCl (1.90 g, 13.8) and NEt_3 (1.8 ml, 13.8 mmol) were added and the mixture was stirred overnight. Water (40 ml) was added to the mixture and extracted with EtOAc (3 × 50 ml). The combined organic layers were washed with saturated sodium bicarbonate (100 ml), 1N HCl (100 ml), brine (100 ml), and dried over magnesium sulfate. EtOAc was evaporated and the residue was subjected to the column chromatography (25% EtOAc/Hex) to give the protected dipeptide Ac-Leu-DAla-OMe (2.40 g, 80%) as a white solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.27 (d, *J*=7.5 Hz, 1H), 6.77 (d, *J*=8.4 Hz, 1H), 4.53 (m, 1H), 4.45 (m, 1H), 3.65 (s, 3H), 1.93 (s, 3H), 1.58 (m, 2H), 1.50 (m, 1H), 1.34 (d, *J*=7.2 Hz, 3H), 0.86 (m, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 173.1, 172.2, 170.4, 52.3, 51.4, 48.0, 41.1, 24.7, 22.9, 22.8, 22.1, 17.8. MS (ESI) *m/z* 259 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{12}H_{23}N_2O_4$ 259.1652, found 259.1654.

To a solution of Ac-Leu-DAla-OMe (0.26 g, 1 mmol) in dioxane (2 ml), lithium hydroxide (62 mg, 3 mmol) in water (0.2 ml) was added dropwise. The mixture was stirred for 3 h and the pH adjusted to 3 using 1N HCl. The mixture was extracted with EtOAc (3 × 5 ml) and the combined organic layers were washed with brine (10 ml). The organic layers were dried over magnesium sulfate and evaporated under reduced pressure to afford Ac-Leu-DAla-OH, which was used in the next step without further purification.

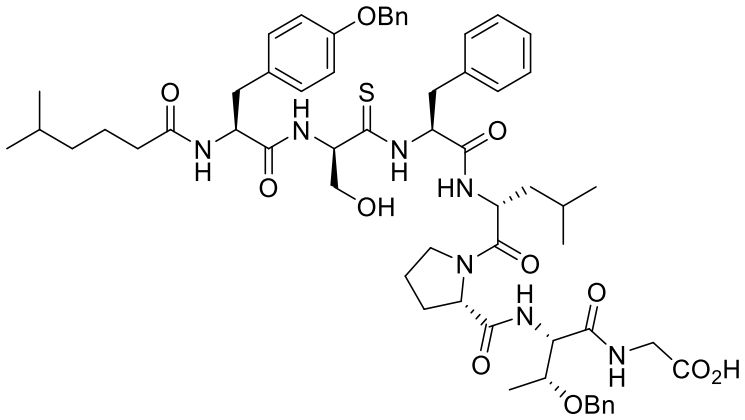
Seongsanamide E (10)



To the macrolactone **S3** (10 mg, 0.01 mmol) in DMF (1 ml), Ac-Leu-DAla-OH (2.5 mg, 0.011 mmol), EDC.Cl (2 mg, 0.011 mmol), HOBt (1.5 mg, 0.011 mmol), and NEt_3 (3 µl, 0.03 mmol) were added. The reaction was stirred overnight then water (5 ml) was added and the mixture was extracted with EtOAc (3 × 5 ml). Combined organic layers were washed with 1 N HCl (10 ml), saturated $NaHCO_3$ (10 ml), brine (10 ml) and dried over magnesium sulfate. The solvent was evaporated and residue was dissolved in a solution of MeOH/AcOH/1 N HCl (1: 0.2: 0.1, 5 ml). Pd/C (10% w, 1 mg) was added and the mixture was stirred for 12 h under hydrogen atmosphere at 200 psi. The mixture was filtered and the solvent was evaporated. The residue was purified using HPLC to provide seongsanamide E **10** (3.9 mg, 40%) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.20 (s, 1H), 9.13 (s, 1H), 8.48–8.39 (m, 2H), 8.11 (d, *J*=8.5 Hz, 1H), 8.08 (d, *J*=7.8 Hz, 1H), 7.99 (d, *J*=7.7 Hz, 1H), 7.94 (d, *J*=5.8 Hz, 1H), 7.70 (d, *J*=9.4 Hz, 1H), 7.44 (d, *J*=6.7 Hz, 1H), 7.02 (d, *J*=8.5 Hz, 2H), 6.93 (d, *J*=8.5 Hz, 2H), 6.64 (d, *J*=8.5 Hz, 2H), 6.61 (d, *J*=8.4 Hz, 2H), 5.11 (m, 1H), 4.60 (ddd, *J*=10.2, 8.5, 4.3 Hz, 1H), 4.38 (m, 1H), 4.34 (m, 1H), 4.30–4.18 (m, 4H), 4.02 (q, *J*=7.6 Hz, 1H), 3.92 (t, *J*=9.5 Hz, 1H), 2.98 (dd, *J*=13.6, 4.9 Hz, 1H), 2.92 (dd, *J*=14.0, 4.3 Hz, 1H), 2.85 (dd, *J*=13.6, 8.2 Hz, 1H), 2.63 (m, 1H), 1.79 (m, 4H), 1.65–1.46 (m, 7H), 1.41–1.38 (m, 2H), 1.35–1.33 (m, 1H), 1.04 (d, *J*=6.6 Hz, 3H), 0.97 (d, *J*=7.2 Hz, 3H), 0.90 (d, *J*=6.6

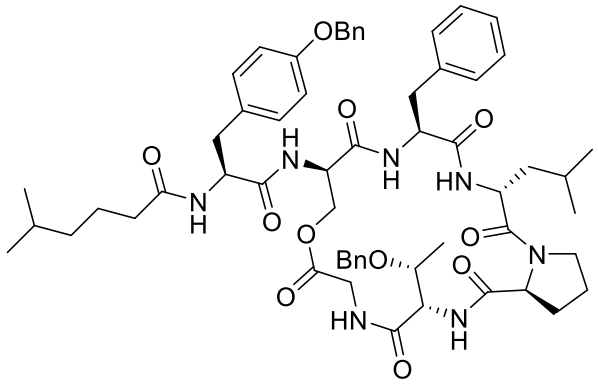
Hz, 4H), 0.87–0.79 (m, 23H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 172.8, 172.5, 170.2, 169.0, 156.4, 156.2, 130.4, 128.3, 127.2, 115.5, 115.3, 63.0, 57.2, 55.4, 54.5, 52.7, 52.0, 51.4, 48.5, 36.6, 36.2, 24.8, 24.6, 24.1, 23.5, 23.3, 23.0, 23.0, 22.8, 22.4, 22.2, 22.1, 21.2, 18.5, 15.9, 15.4, 12.2, 12.2. MS (ESI) *m/z* 947 [(M+H)$^+$, 100%]; HRMS (ESI, [M+H]$^+$) calcd. for $C_{51}H_{77}N_8O_{12}$ 993.5655, found 993.5654.

5-MeHex-Y(Bn)$_D$S$^{[S]}$F$_D$LPT(Bn)G (11)



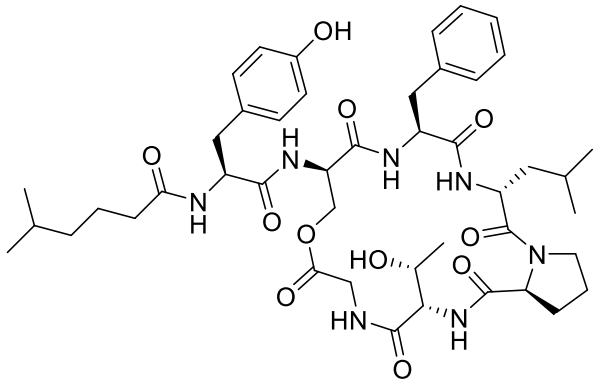
The linear 5-MeHex-Y(Bn)$_D$S$^{[S]}$F$_D$LPT(Bn)G **11** was synthesised using the general procedures for peptide thioamide synthesis (43 mg, 40%). MS (ESI) *m/z* 1092 [(M+H)$^+$, 100%]; HRMS (ESI, [M+H]$^+$) calcd. for $C_{59}H_{78}N_7O_{11}S$ 1092.5475, found 1092.5476.

Macrocycle (12)



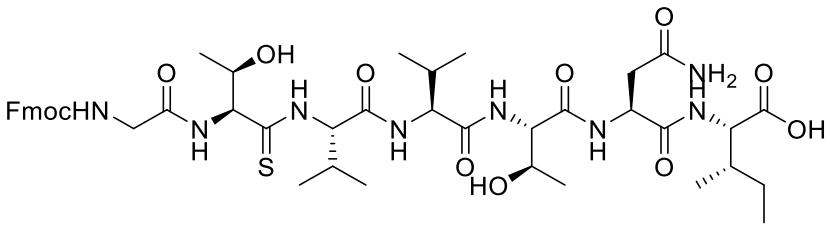
The linear 5-MeHex-Y(Bn)$_D$S$^{[S]}$F$_D$LPT(Bn)G **11** (43 mg, 0.04 mmol) was cyclised using the general procedure for macrolactonisation. After 96 h the mixture was purified by preparative HPLC to give **12** as a white foam (10 mg, 25% starting from resin, 10% overall starting from initial loaded resin). **^{1}H NMR** (500 MHz, DMSO-d_6) δ 8.58 (d, *J*=4.5 Hz, 1H), 8.19 (d, *J*=7.1 Hz, 1H), 8.07 (d, *J*=8.6 Hz, 1H), 7.96 (d, *J*=8.0 Hz, 1H), 7.76–7.62 (m, 2H), 7.47 (d, *J*=5.9 Hz, 1H), 7.40–7.35 (m, 5H), 7.23–7.13 (m, 5H), 7.10 (t, *J*=9.3 Hz, 4H), 6.87 (dt, *J*=8.9, 2.9 Hz, 2H), 5.01 (d, *J*=1.9 Hz, 2H), 4.88 (q, *J*=7.5 Hz, 1H), 4.70 (dd, *J*=11.8, 4.8 Hz, 1H), 4.62(m, 1H), 4.56–4.43 (m, 2H), 4.15–4.11 (m, 3H), 4.06–4.03 (m, 3H), 2.91–2.77 (m, 4H), 1.92–1.80 (m, 2H), 1.69 (s, 1H), 1.63 (m, 1H), 1.50–1.44 (m, 2H), 1.41 (m, 1H), 1.28–1.25 (m, 4H), 1.17–1.14 (m, 8H), 0.95 (d, *J*=7.7 Hz, 1H), 0.88–0.85 (m, 6H), 0.80–0.78 (m, 3H), 0.70 (d, *J*=5.8 Hz, 2H). MS (ESI) *m/z* 1058 [(M+H)$^+$, 100%]; HRMS (ESI, [M+H]$^+$) calcd. for $C_{59}H_{76}N_7O_{11}$ 1058.5597, found 1058.5608.

Kahalalide B (13)



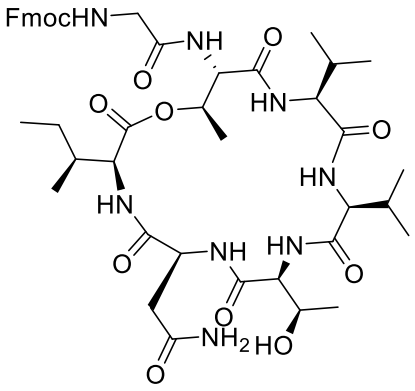
Benzyl protected peptide **12** (5 mg, 0.005 mmol) was dissolved in a solution of MeOH/AcOH/1 N HCl (1:0.2:0.1, 2 ml). Pd/C (10% w, 1 mg) was added and the mixture was stirred for 12 h under hydrogen atmosphere at 200 psi. The mixture was filtered and the solvent was evaporated under reduced pressure. The residue was purified using preparative HPLC to give kahalalide B **13** (2.5 mg, 65%) as a white foam. **^{1}H NMR** (500 MHz, DMSO-d_6) δ 9.13 (s, 1H), 8.70 (d, *J*=4.3 Hz, 1H), 7.96 (d, *J*=7.1 Hz, 1H), 7.92 (d, *J*=8.3 Hz, 1H), 7.80 (d, *J*=7.8 Hz, 1H), 7.59 (t, *J*=5.6 Hz, 1H), 7.27–7.19 (m, 3H), 7.19–7.10 (m, 3H), 7.05–6.99 (m, 2H), 6.65–6.50 (m, 2H), 4.74 (q, *J*=7.5, 6.9 Hz, 1H), 4.45–4.33 (m, 2H), 4.27 (m, 1H), 4.22 (m, 1H), 4.07 (dd, *J*=8.6, 3.6 Hz, 1H), 3.91–3.84 (m, 2H), 3.78 (d, *J*=5.1 Hz, 1H), 3.60 (d, *J*=6.1 Hz, 1H), 2.90 (dd, *J*=13.2, 5.8 Hz, 1H), 2.87–2.79 (m, 2H), 2.18 (m, 1H), 2.09 (m, 1H), 2.01–1.97 (m, 2H), 1.94 (d, *J*=4.4 Hz, 1H), 1.90 (d, *J*=4.1 Hz, 1H), 1.56–1.47 (m, 2H), 1.45–1.34 (m, 4H), 1.28–1.21 (m, 4H), 1.15 (dd, *J*=6.5, 4.0 Hz, 3H), 1.06–0.98 (m, 2H), 0.83 (dd, *J*=6.6, 2.1 Hz, 2H), 0.82–0.76 (m, 8H), 0.67 (d, *J*=5.5 Hz, 3H). MS (ESI) *m/z* 878 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{45}H_{46}N_7O_{11}$ 878.4658, found 878.4656.

Fmoc-GT[S]VVTNI (14)



The linear Fmoc-GT[S]VVTNI **14** was synthesised using the general procedures for peptide thiaomide synthesis (based on 0.01 mmol resin) (5 mg, 53%). MS (ESI) *m/z* 941 [(M+H)$^+$, 100%]; HRMS (ESI, [M+H]$^+$) calcd. for $C_{45}H_{65}N_8O_{12}S$ 941.4337, found 941.4341.

Macrocycle (16)



The linear Fmoc-GT[S]VVTNI **14** (5 mg, 0.005 mmol) was cyclised using the general procedure for macrolactonisation. After 96 h and the mixture was purified by preparative HPLC to give the macrocycle **16** as a white foam (1 mg, 22%). MS (ESI) *m/z* 907 [(M+H)$^+$, 100%]; HRMS (ESI, [M+H]$^+$) calcd. for $C_{45}H_{63}N_8O_{12}$ 907.4560, found 907.4559.

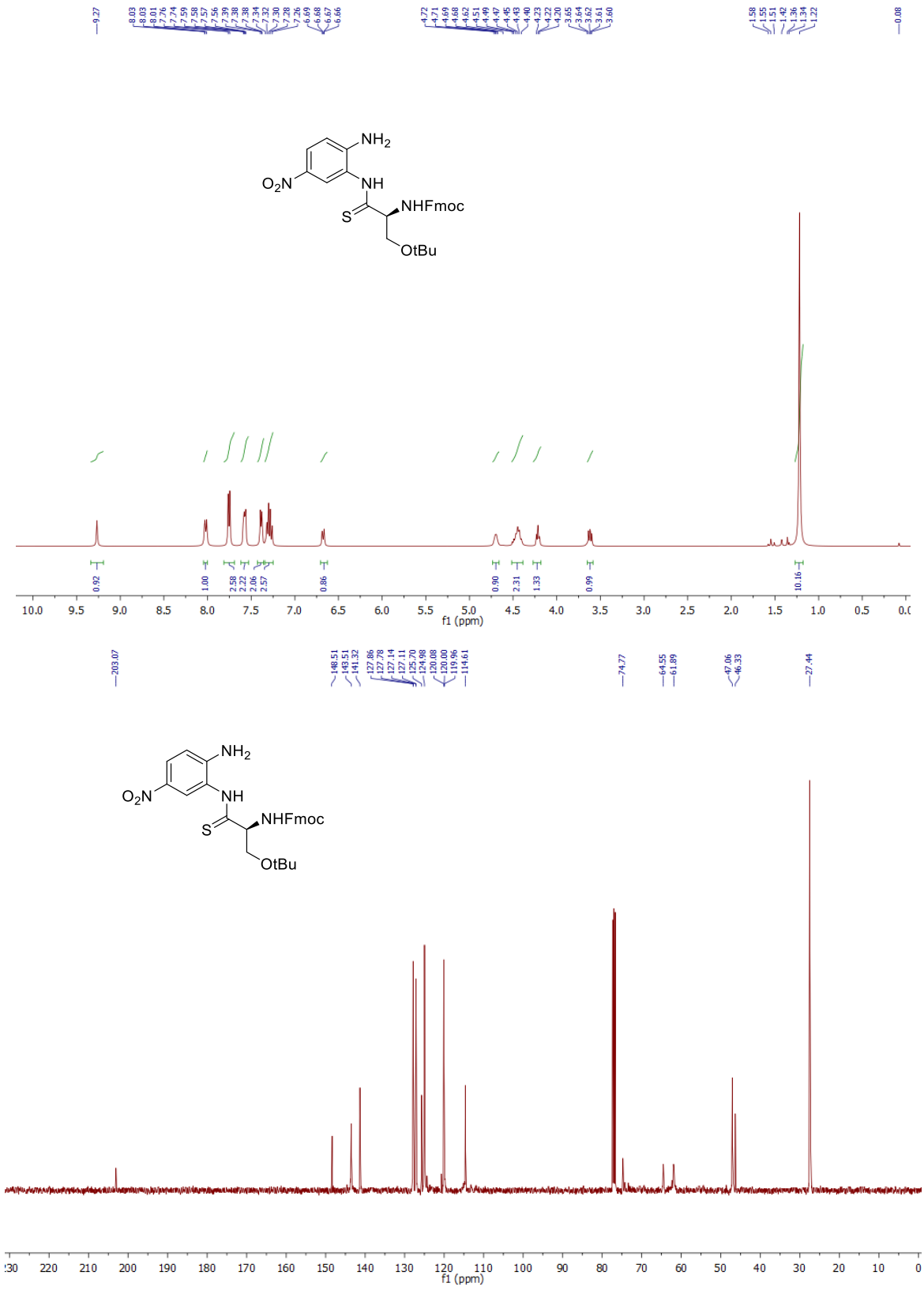


Figure S1. ^{1}H and ^{13}C NMR spectra of compound **B** (400/101 MHz, $CDCl_3$)

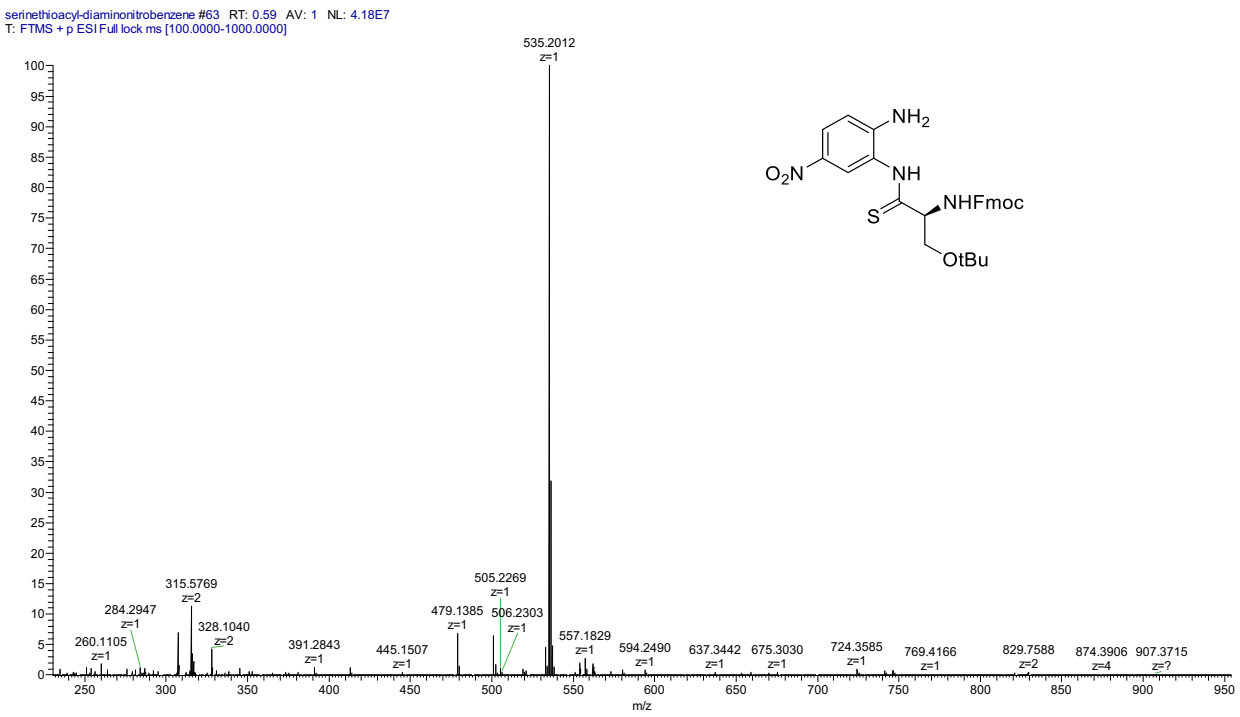


Figure S2. Mass spectrum of compound **B**.

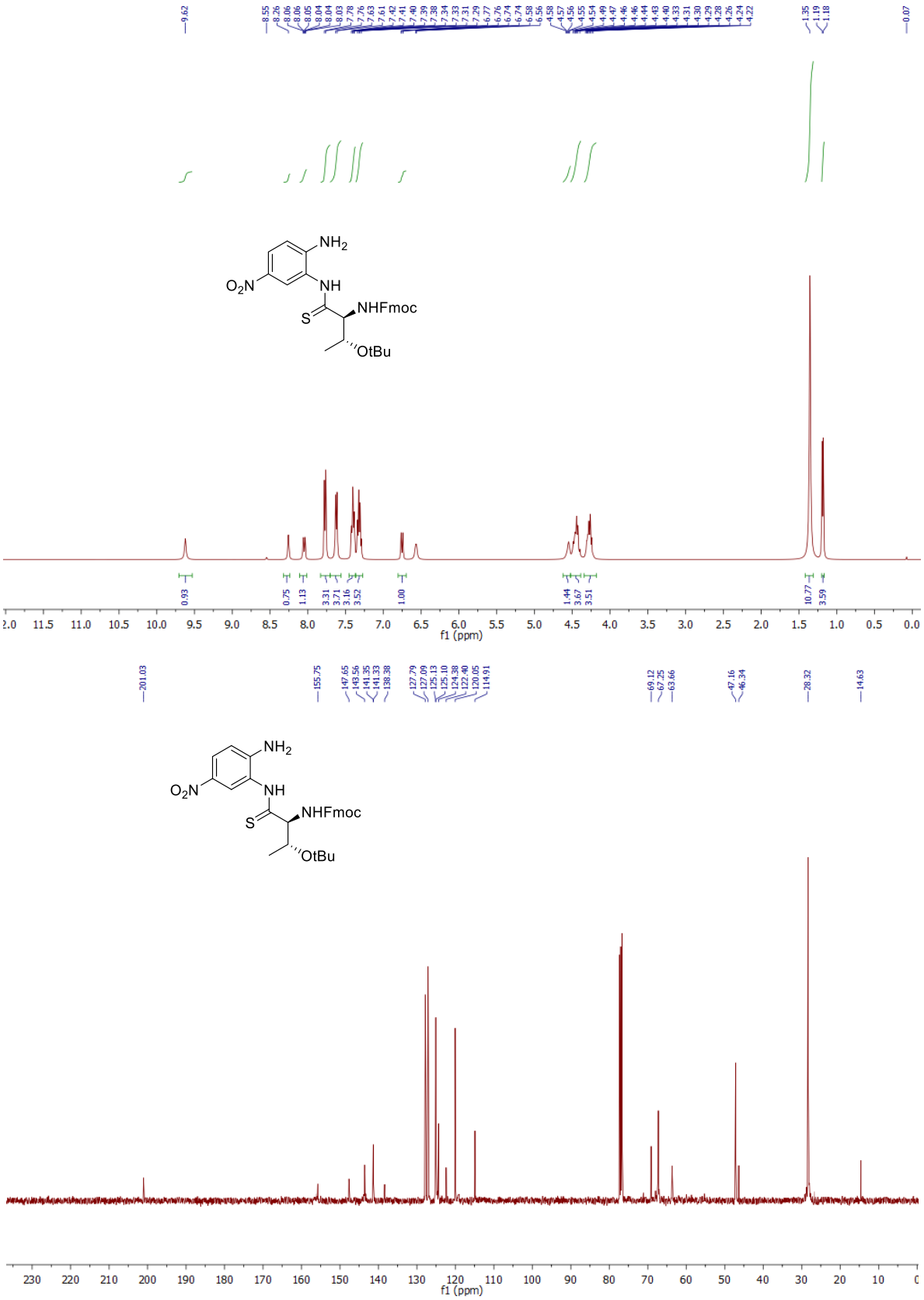


Figure S3. ^{1}H and ^{13}C NMR spectra of compound **D** (400/101 MHz, $CDCl_3$)

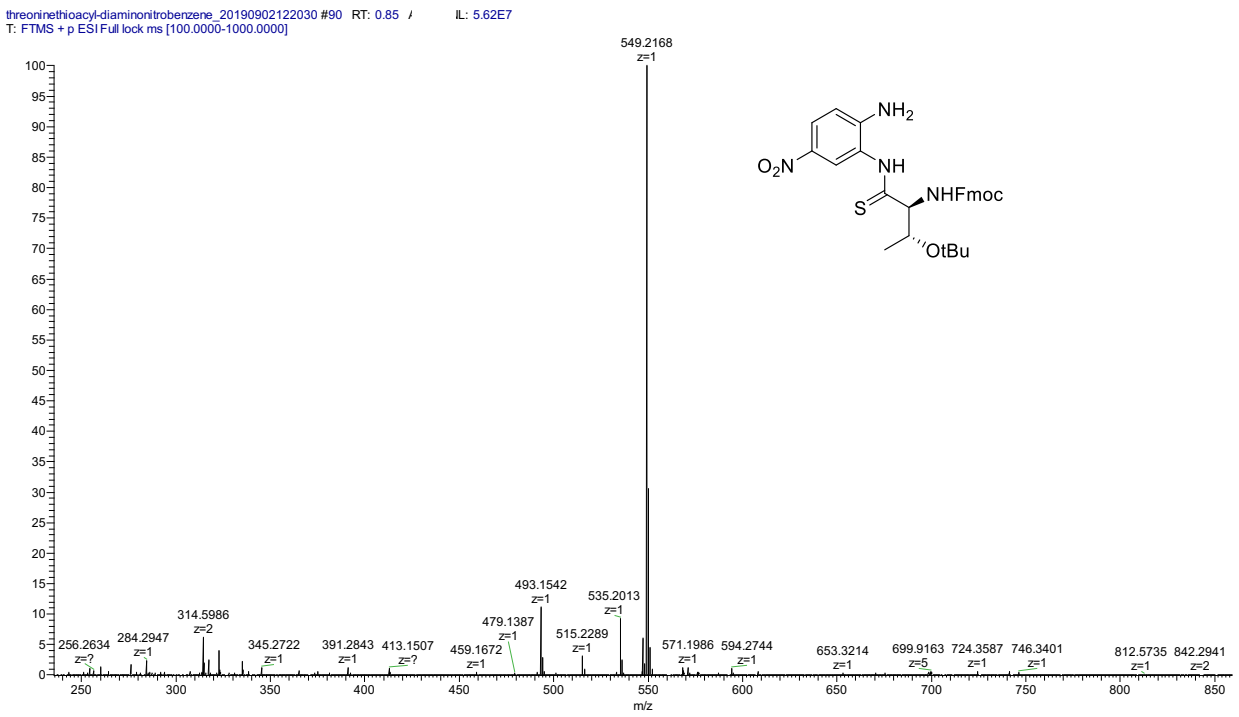


Figure S4. Mass spectrum of compound **D**.

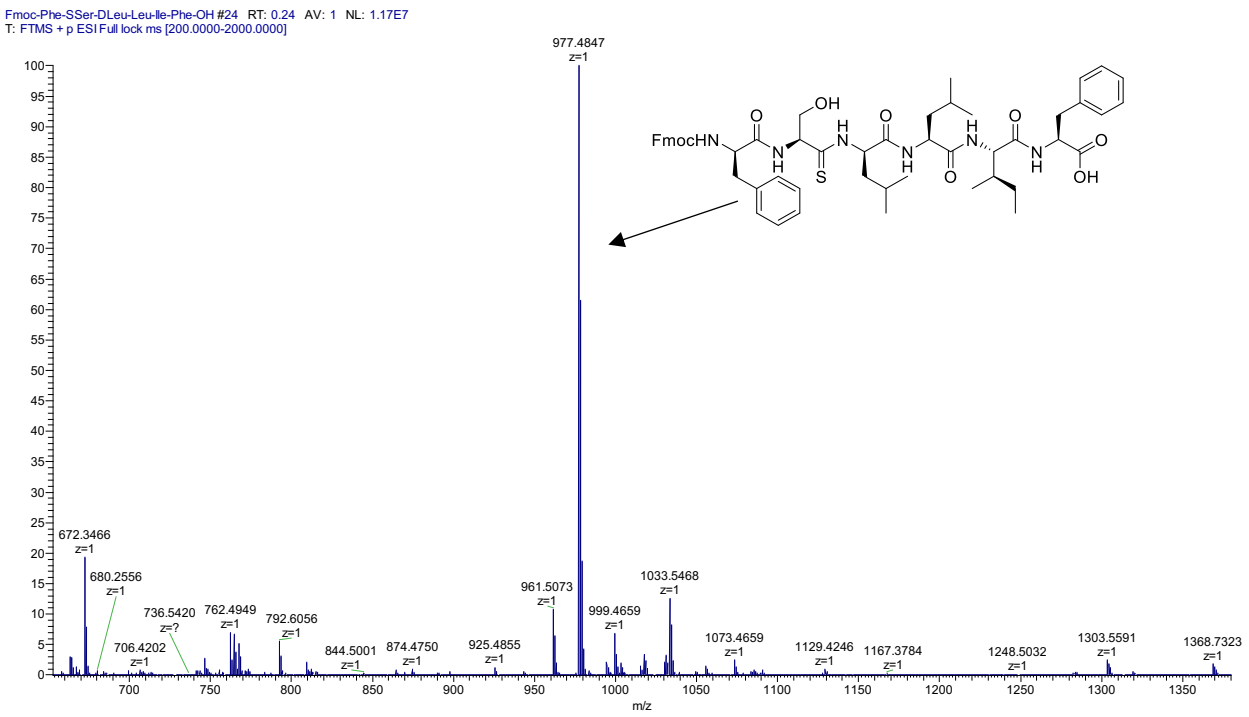


Figure S5. Mass spectrum of thiopeptide **1a**.

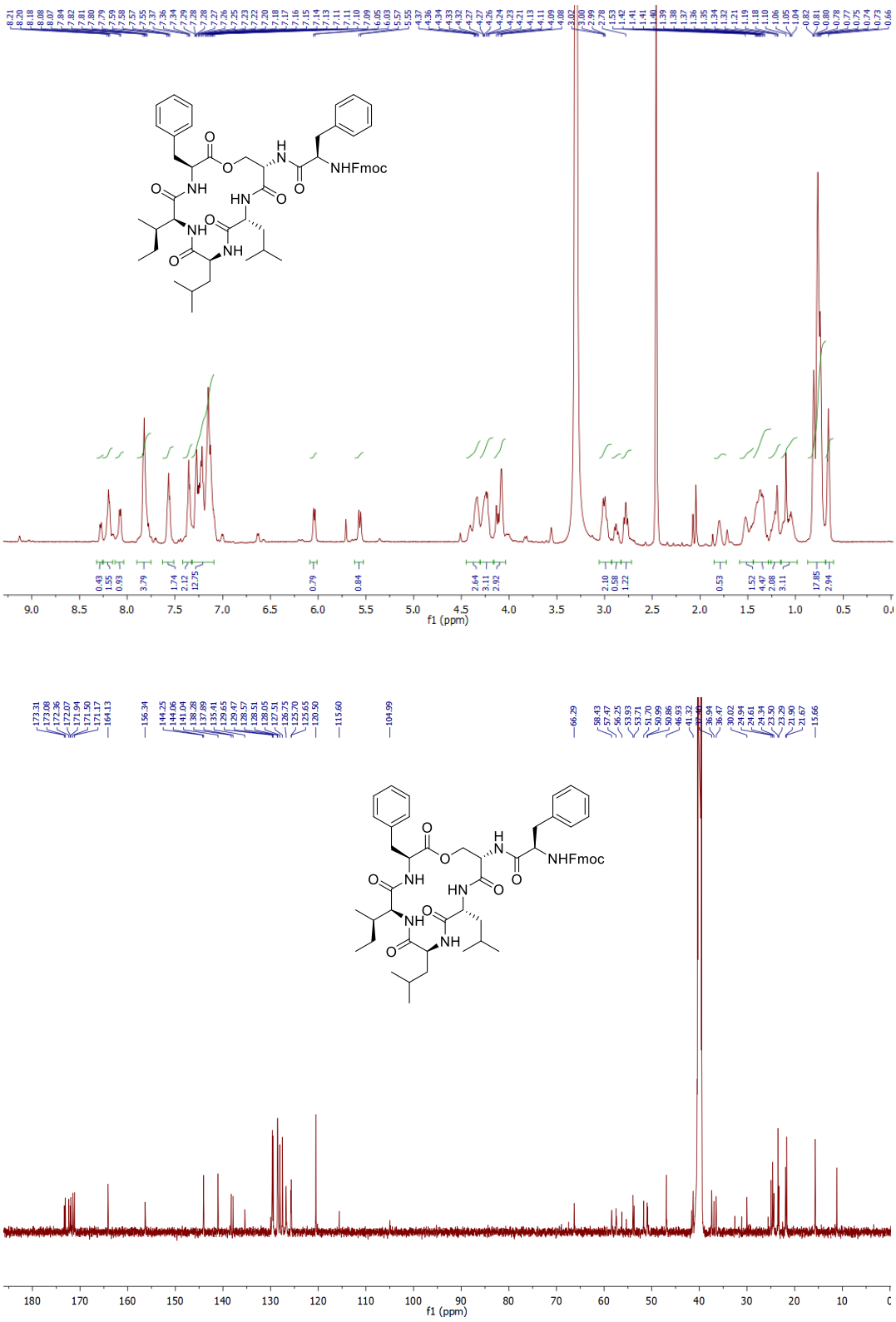


Figure S6. ^{1}H and ^{13}C NMR spectra of **2a** (600/151 MHz, $CDCl_3$)

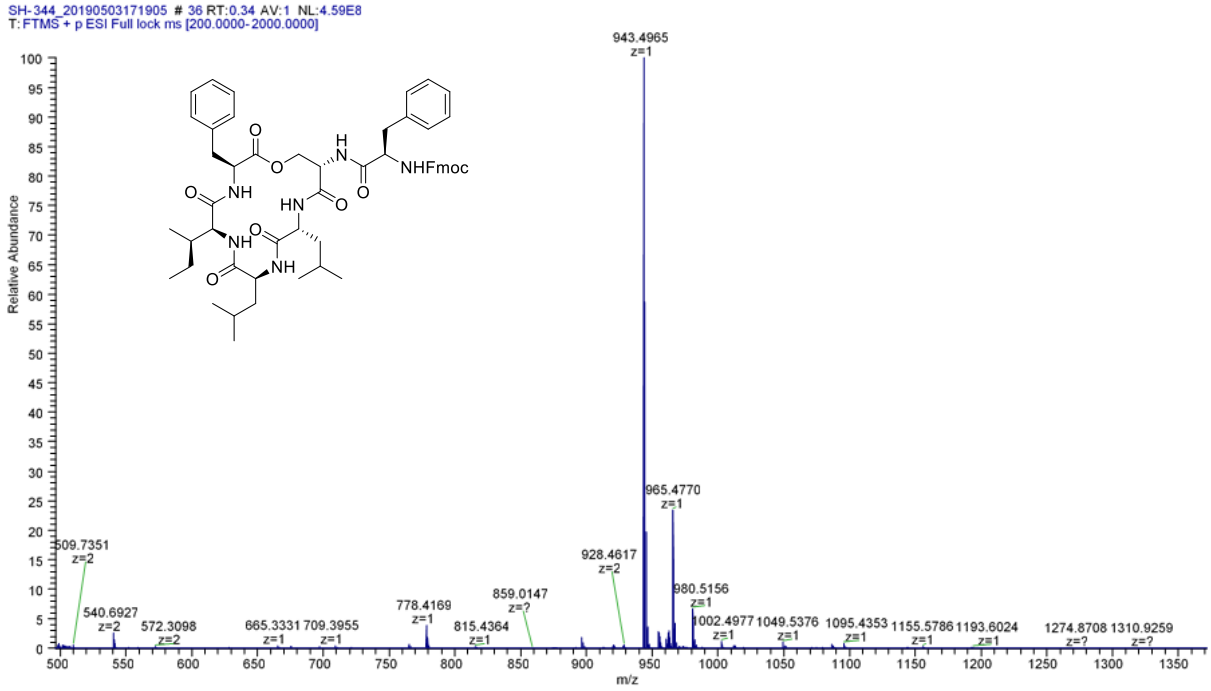


Figure S7. Mass spectrum of peptide **2a**.

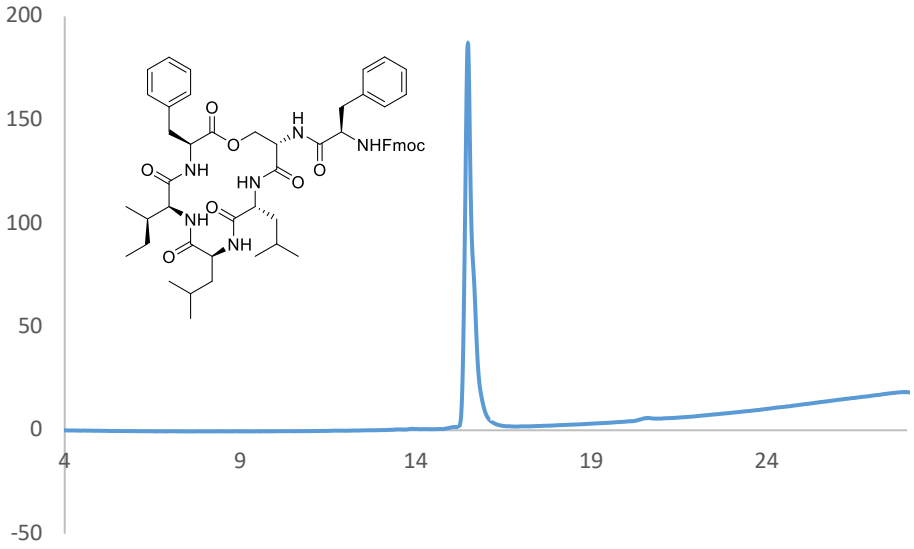


Figure S8. HPLC trace of peptide **2a**.

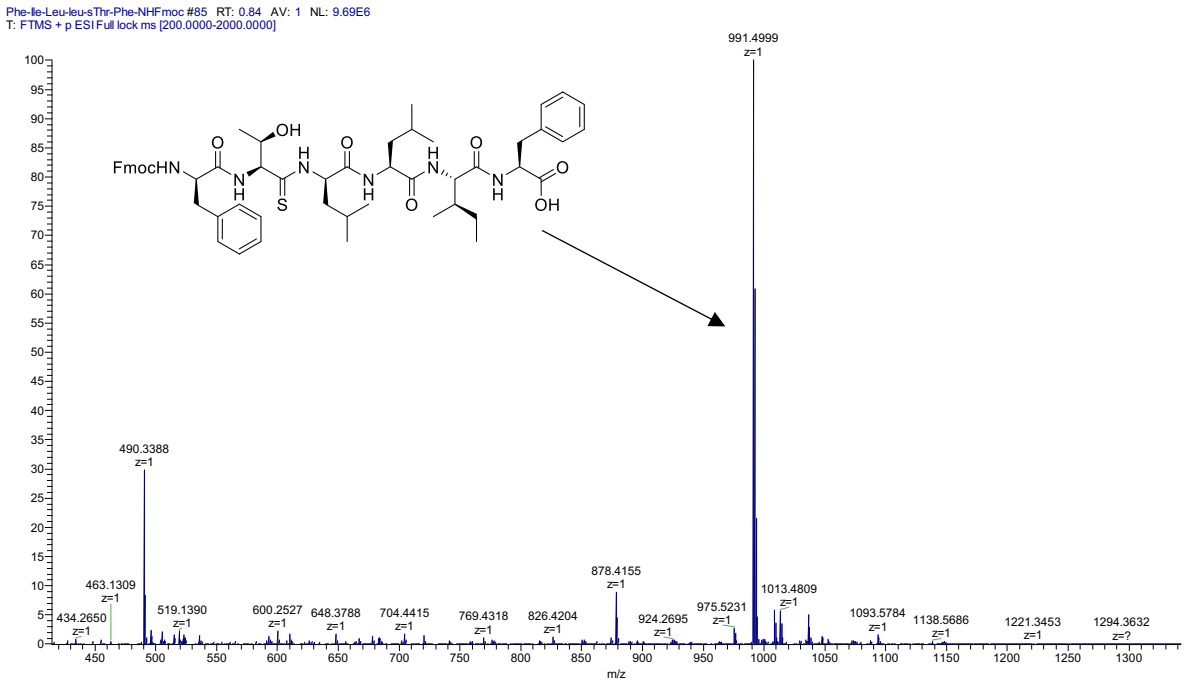


Figure S9. Mass spectrum of thiopeptide **1b**.

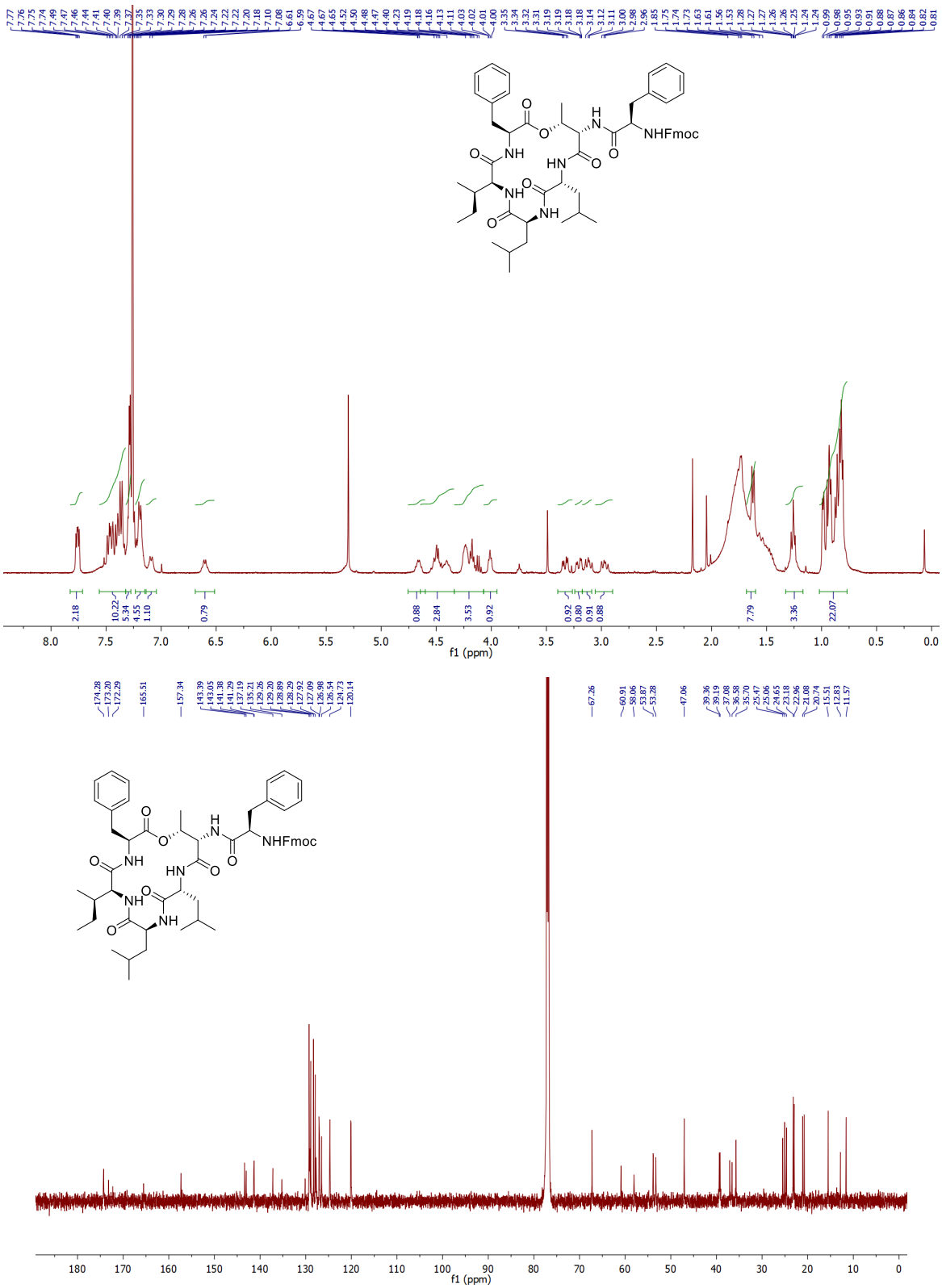


Figure S10. ^{1}H and ^{13}C NMR spectra of **2b** (400/126 MHz, $CDCl_3$)

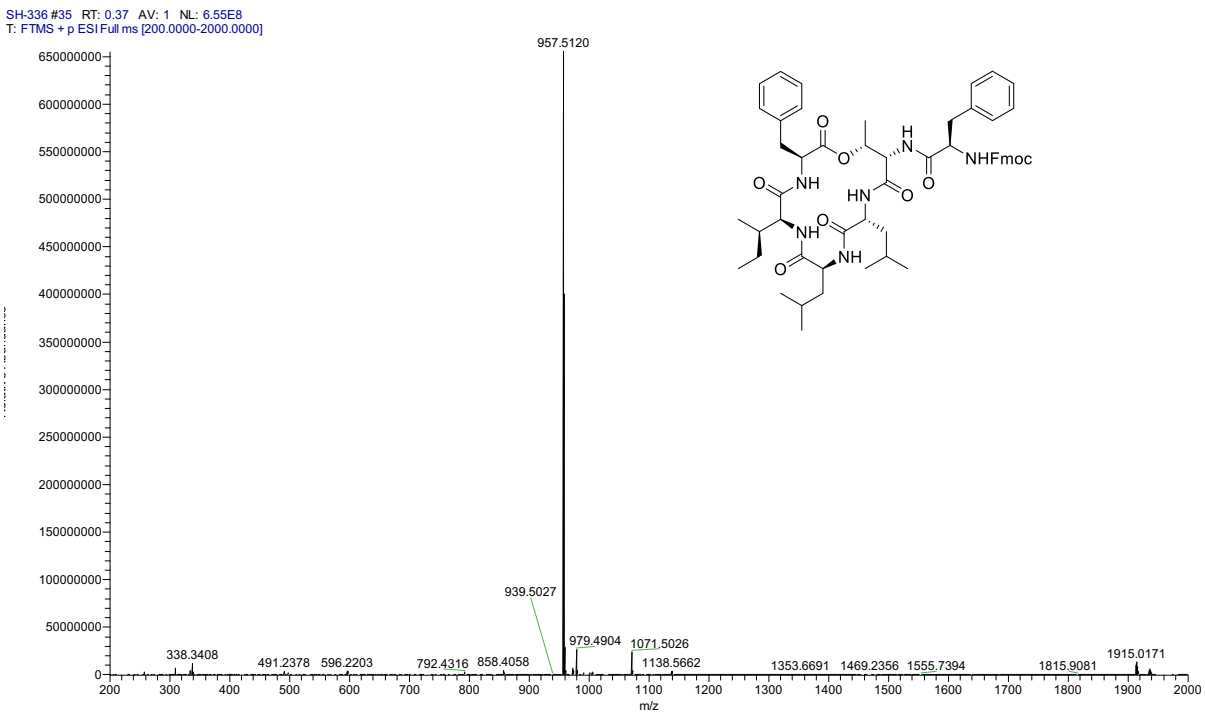


Figure S11. Mass spectrum of peptide **2b**.

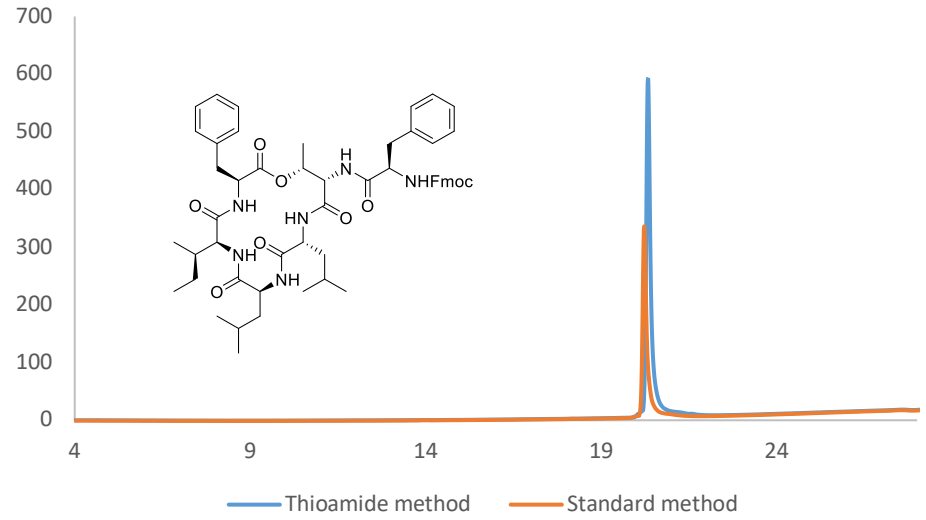


Figure S12. HPLC traces of **2b** from thioamide and standard macrolactonisation methods.

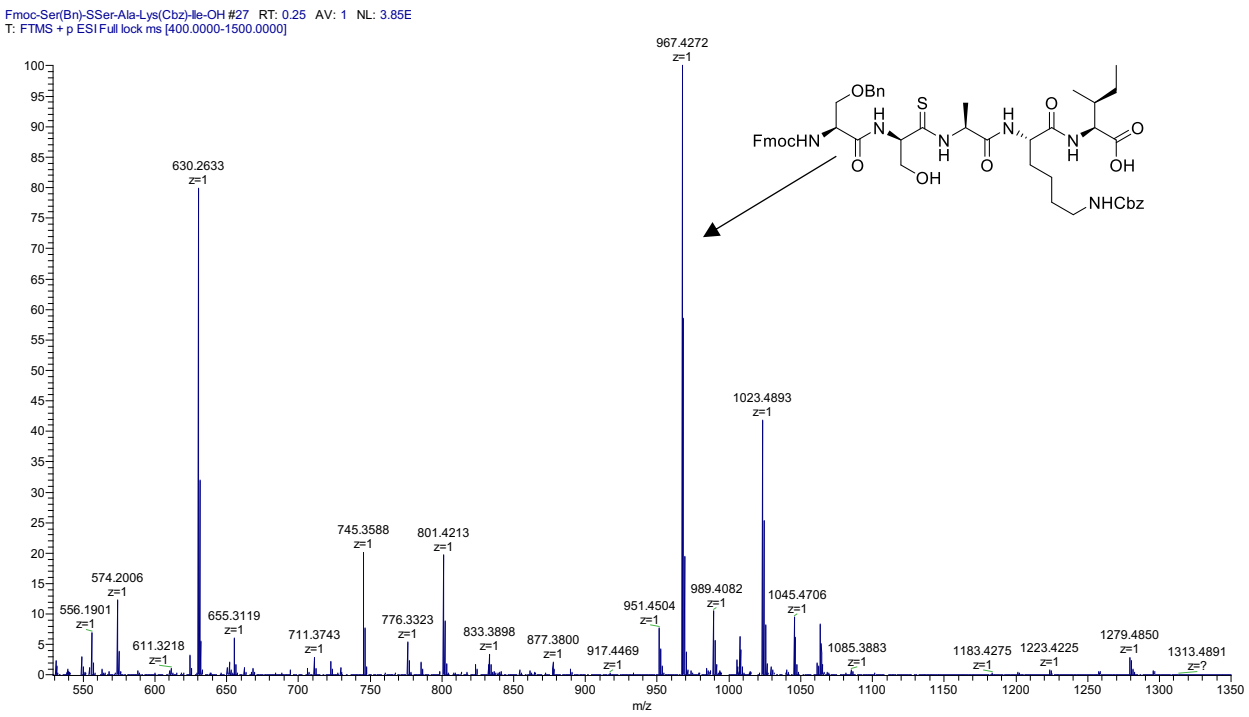


Figure S13. Mass spectrum of thiopeptide **3a**.

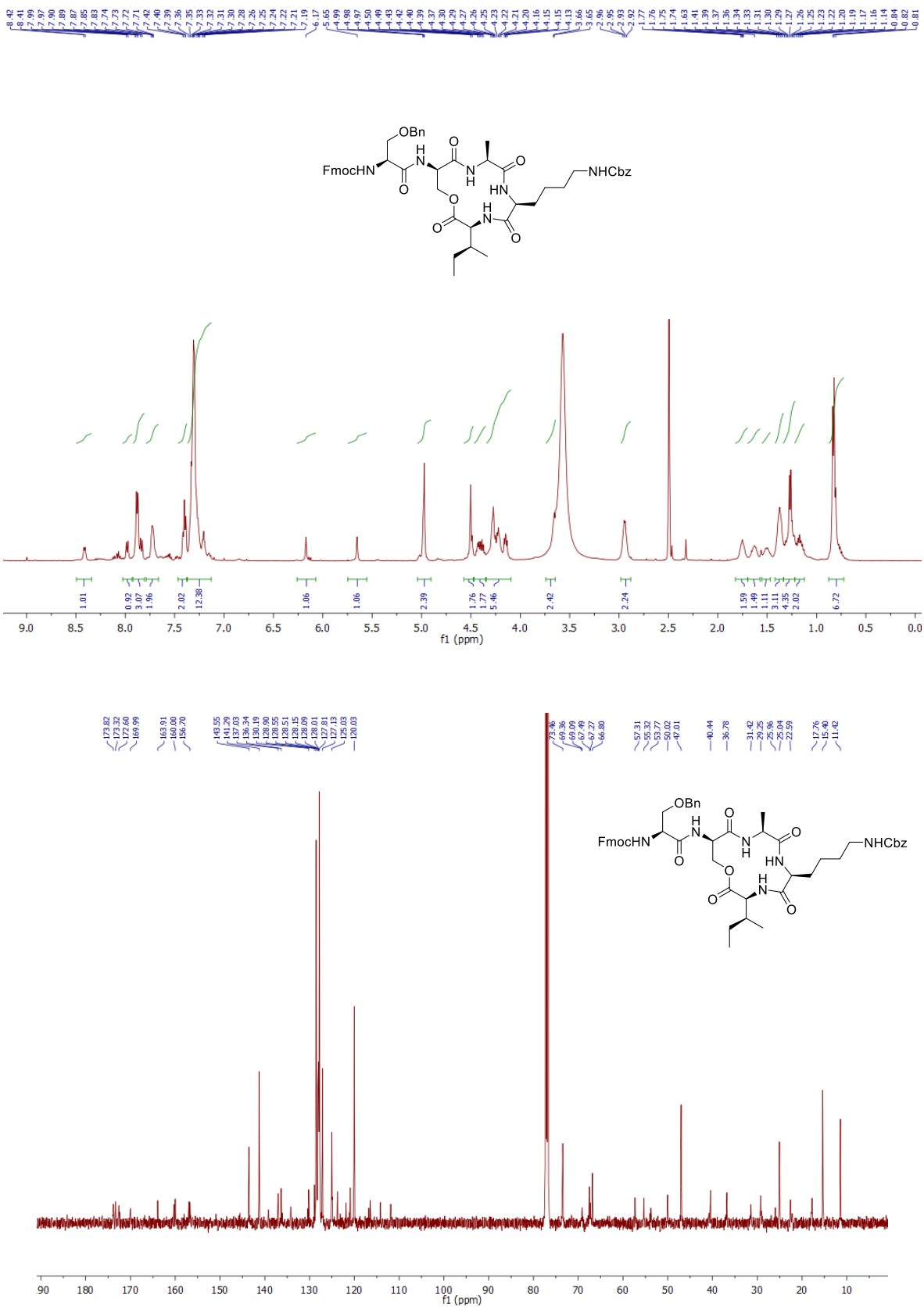


Figure S14. ^{1}H NMR and ^{13}C NMR spectra of peptide **4a** (400/126 MHz, $CDCl_3$)

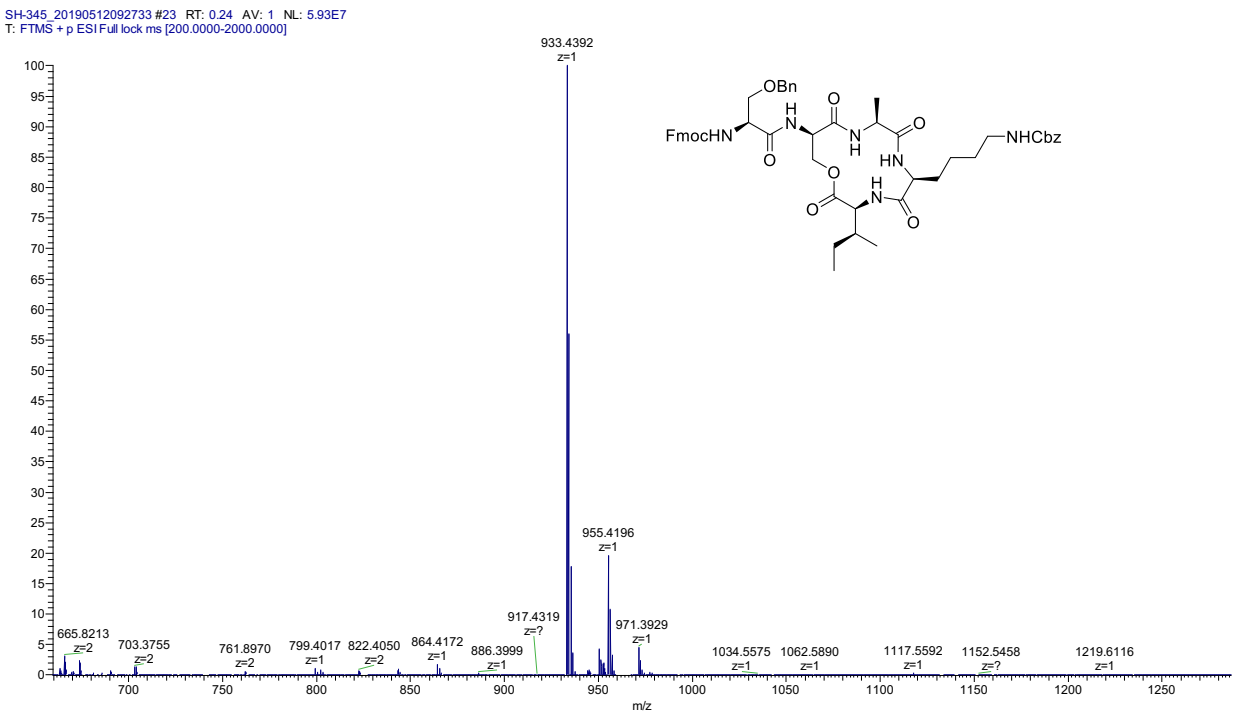


Figure S15. Mass spectrum of peptide **4a**.

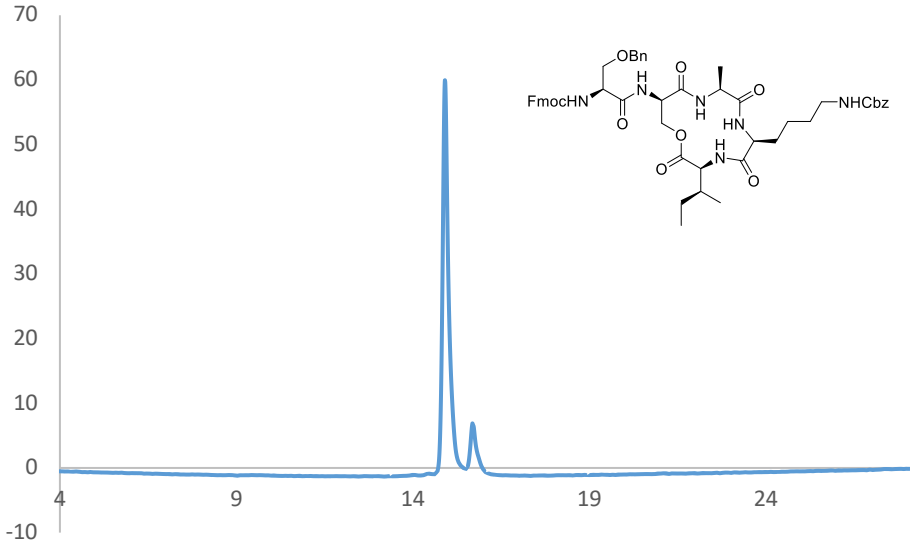


Figure S16. HPLC trace of peptide **4a**.

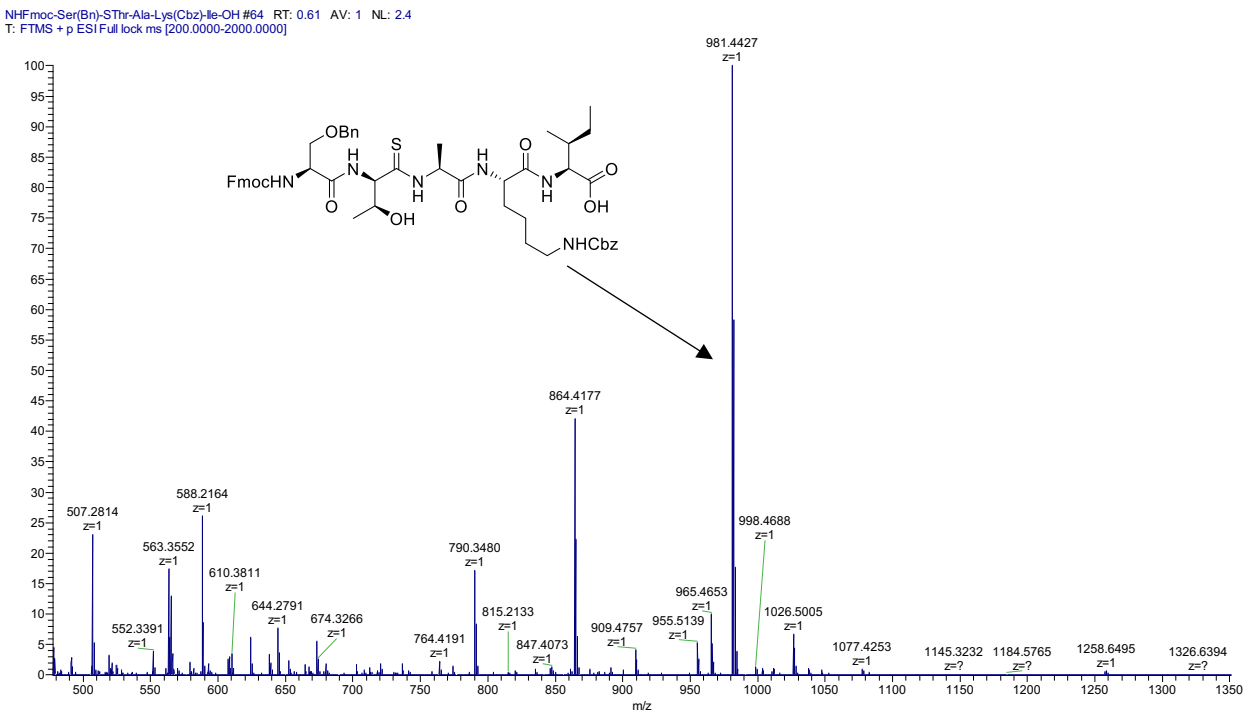


Figure S17. Mass spectrum of thiopeptide **3b**.

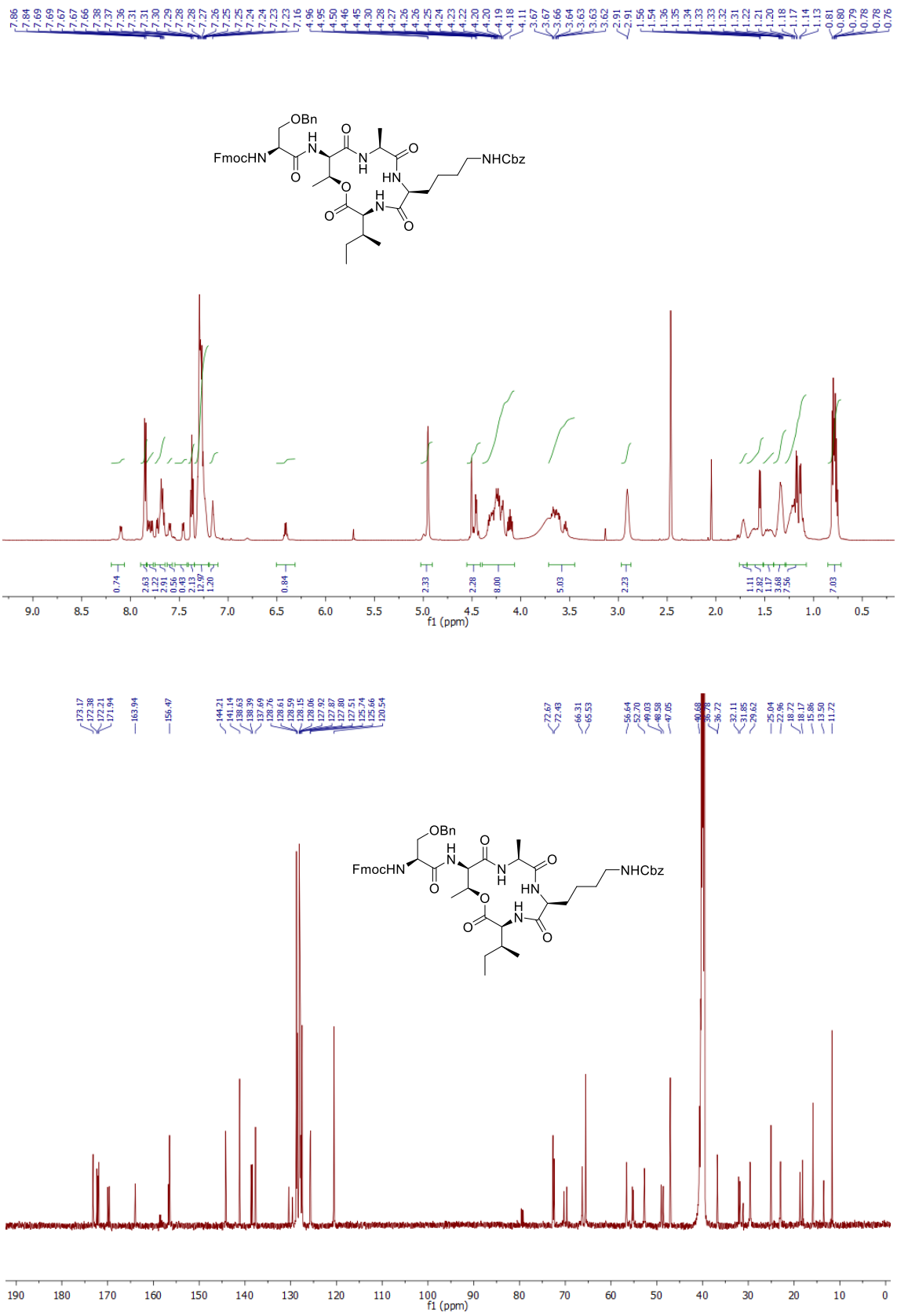


Figure S18. ^{1}H NMR and ^{13}C NMR spectra of peptide **4b** (500/151 MHz, DMSO-d_6)

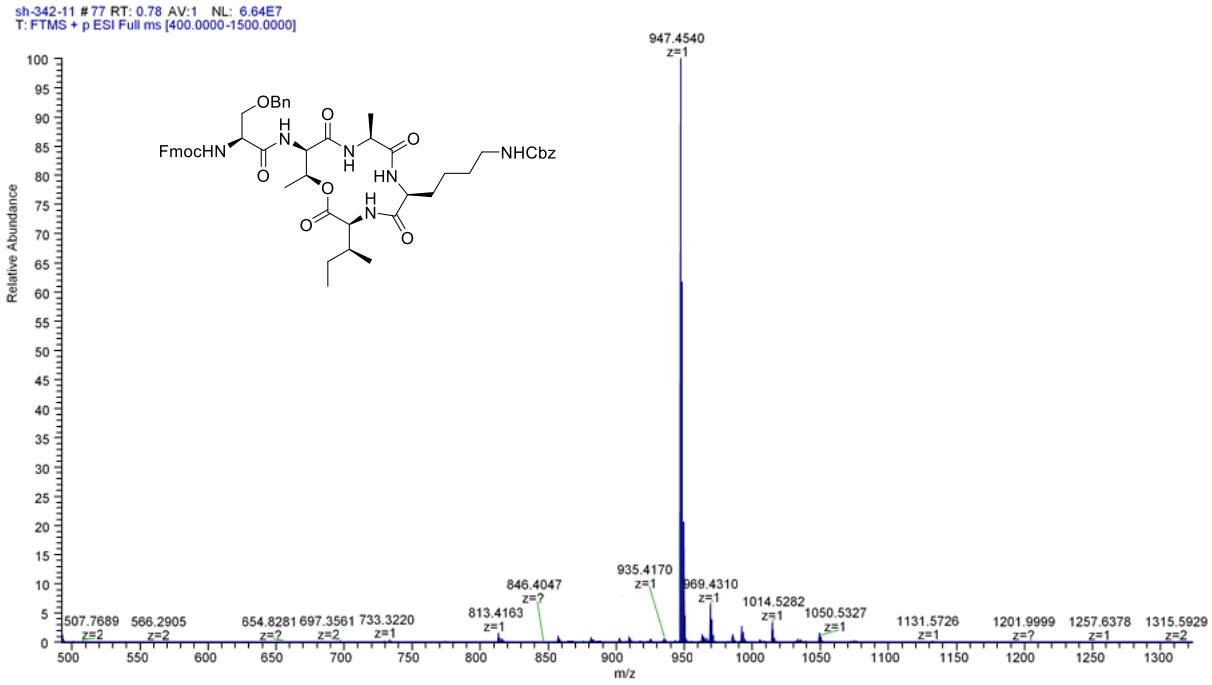


Figure S19. Mass spectrum of peptide **4b**.

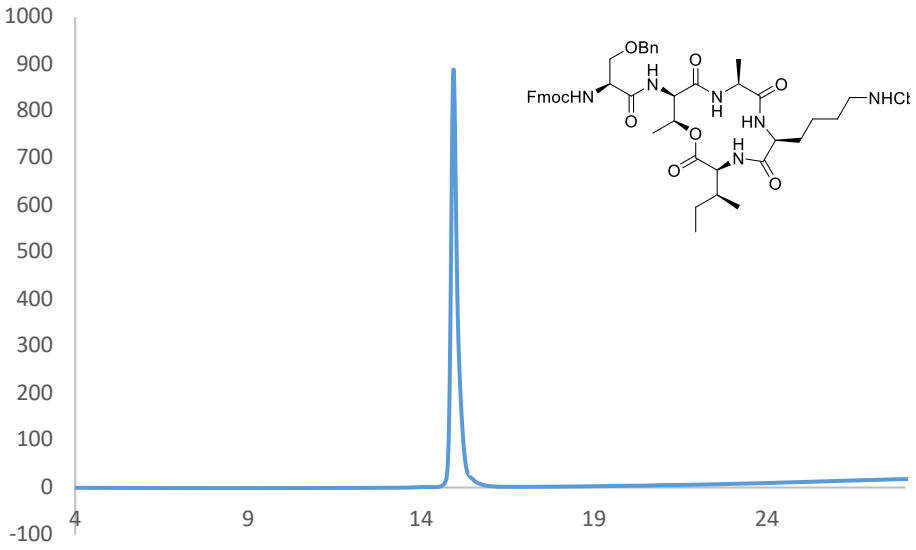


Figure S20. HPLC trace of peptide **4b**.

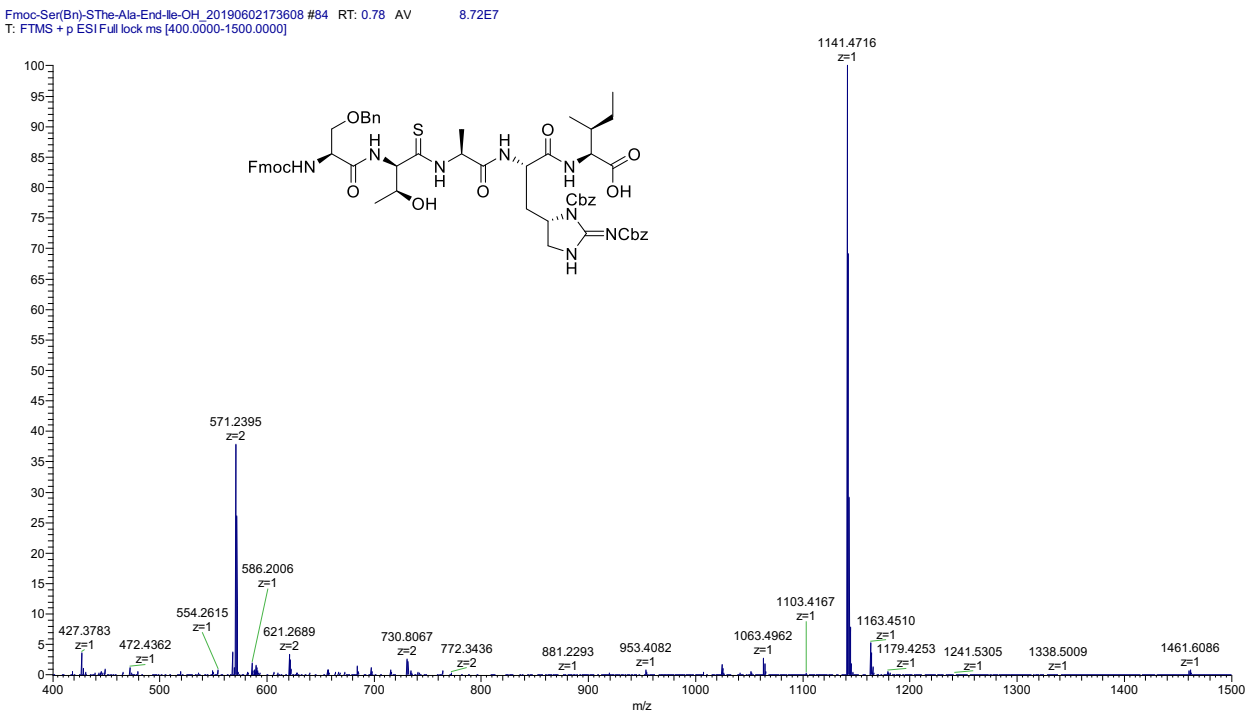


Figure S21. Mass spectrum of thiopeptide **5**.

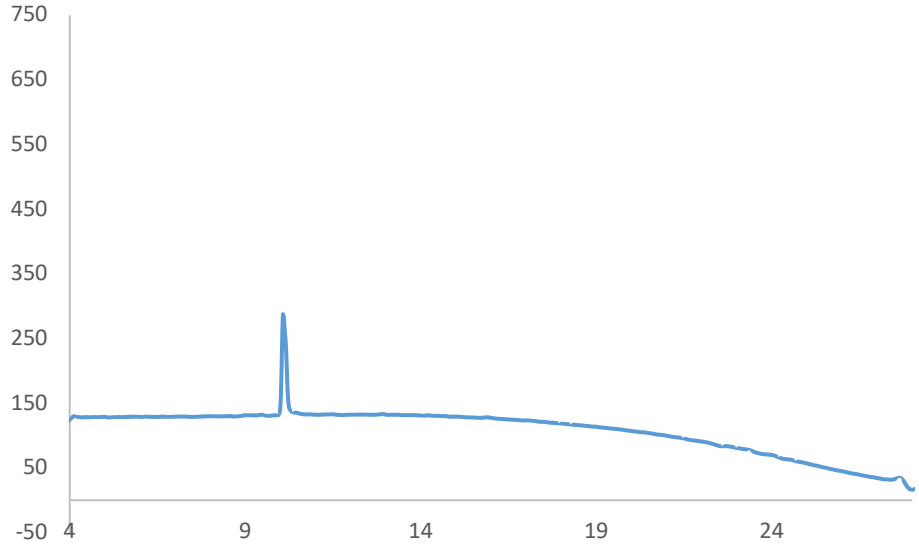


Figure S22. HPLC trace of peptide **5**.

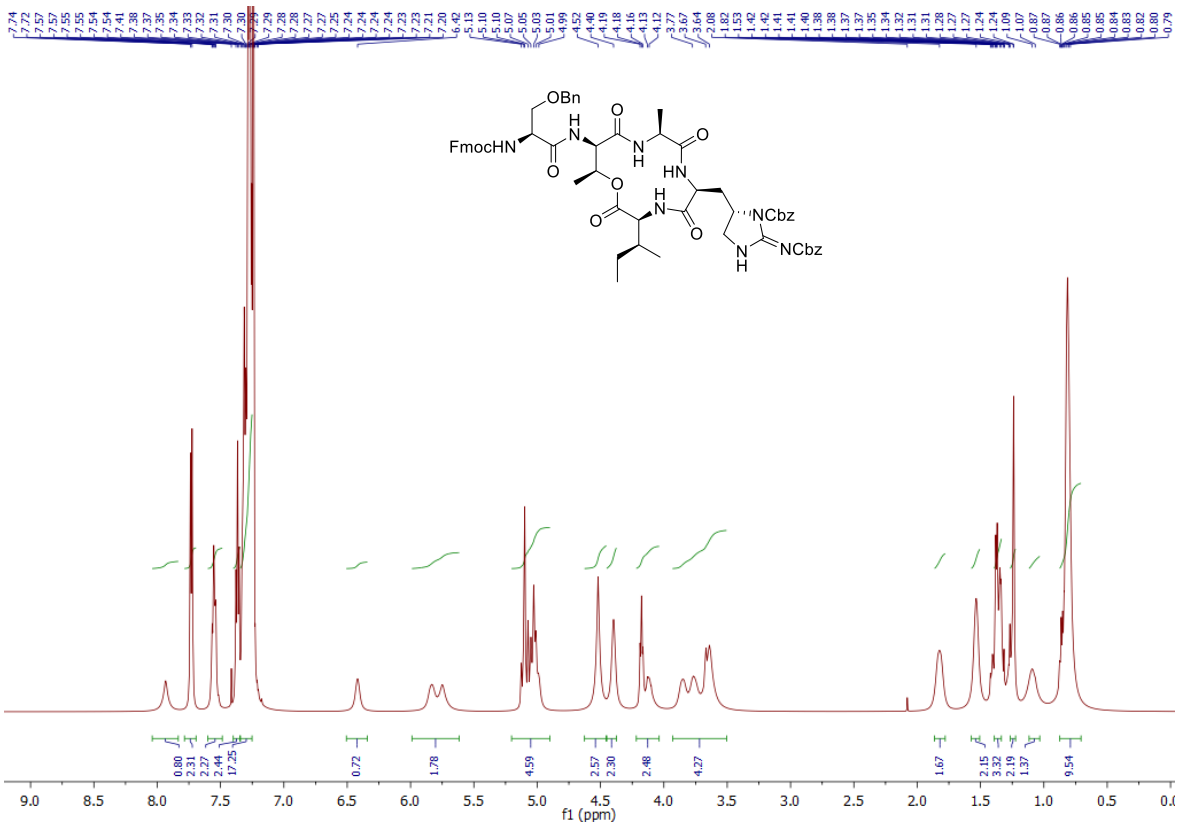


Figure S23. ^{1}H NMR spectrum of peptide **6** (600 MHz, $CDCl_3$)

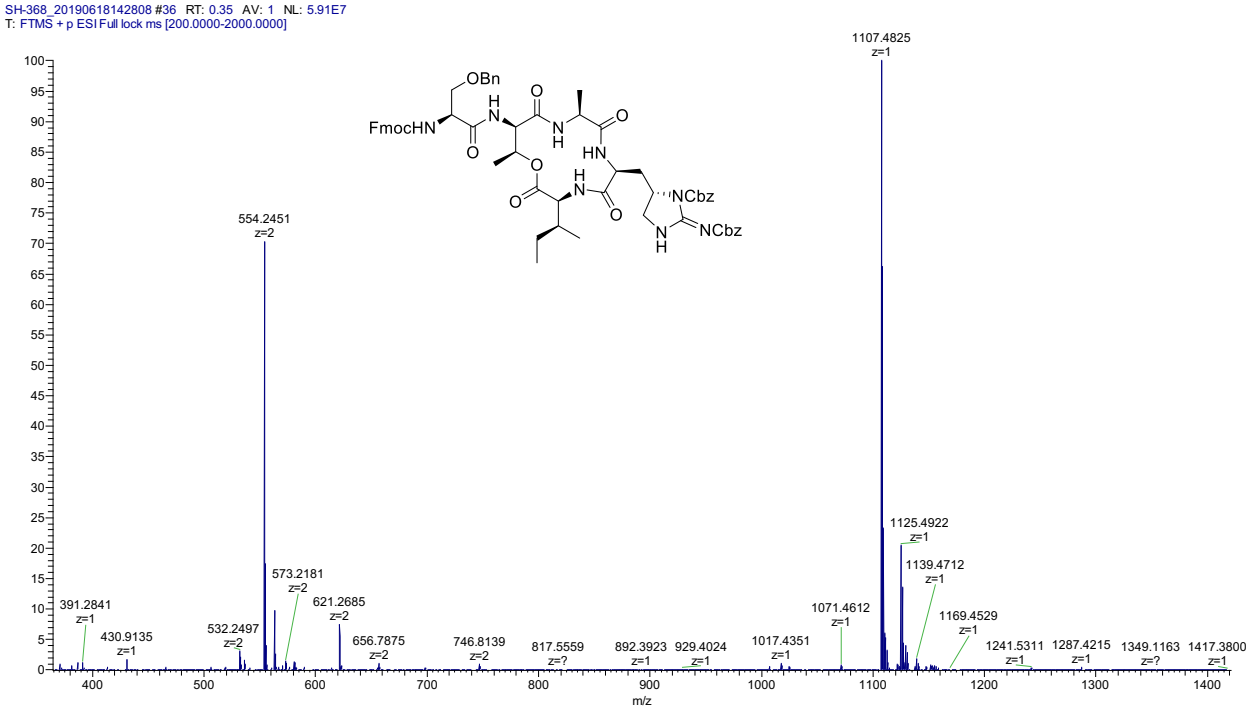


Figure S24. Mass spectrum of peptide **6**.

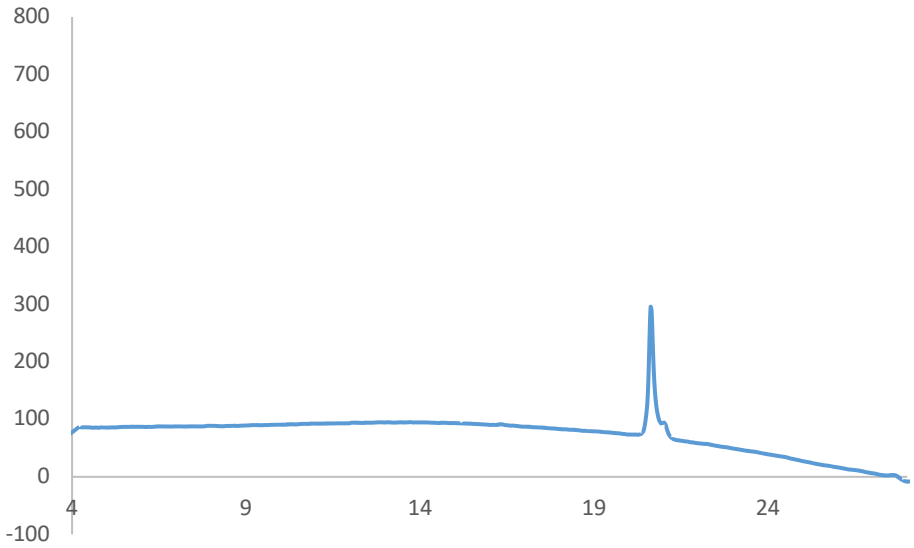


Figure S25. HPLC trace of peptide **6**.

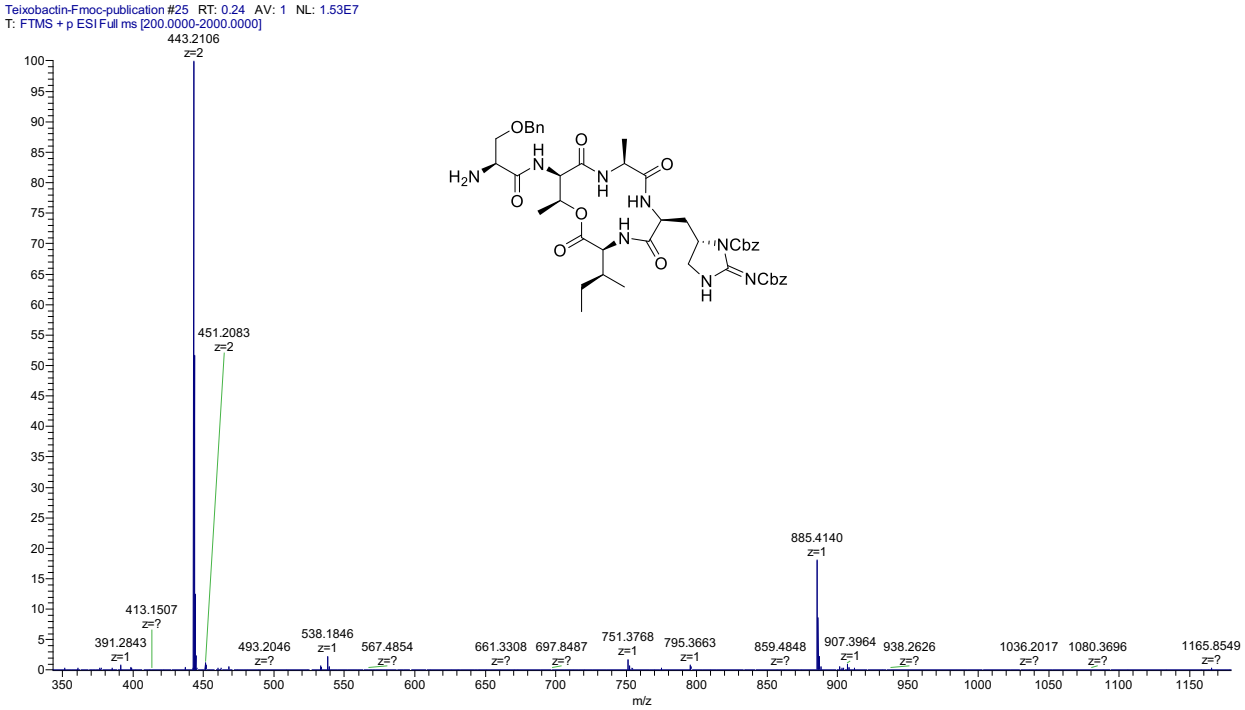


Figure S26. Mass spectrum of teixobactin macrocycle **7**.

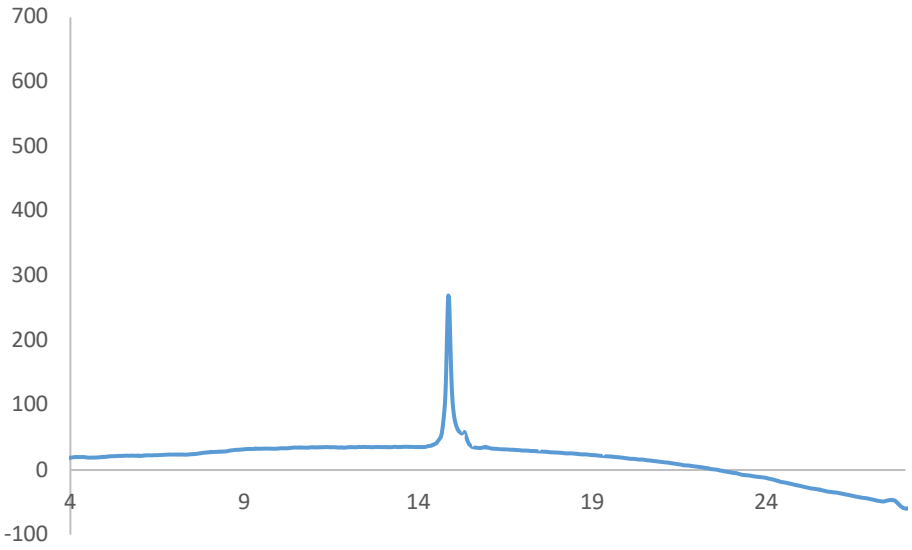


Figure S27. HPLC trace of teixobactin macrocycle **7**.

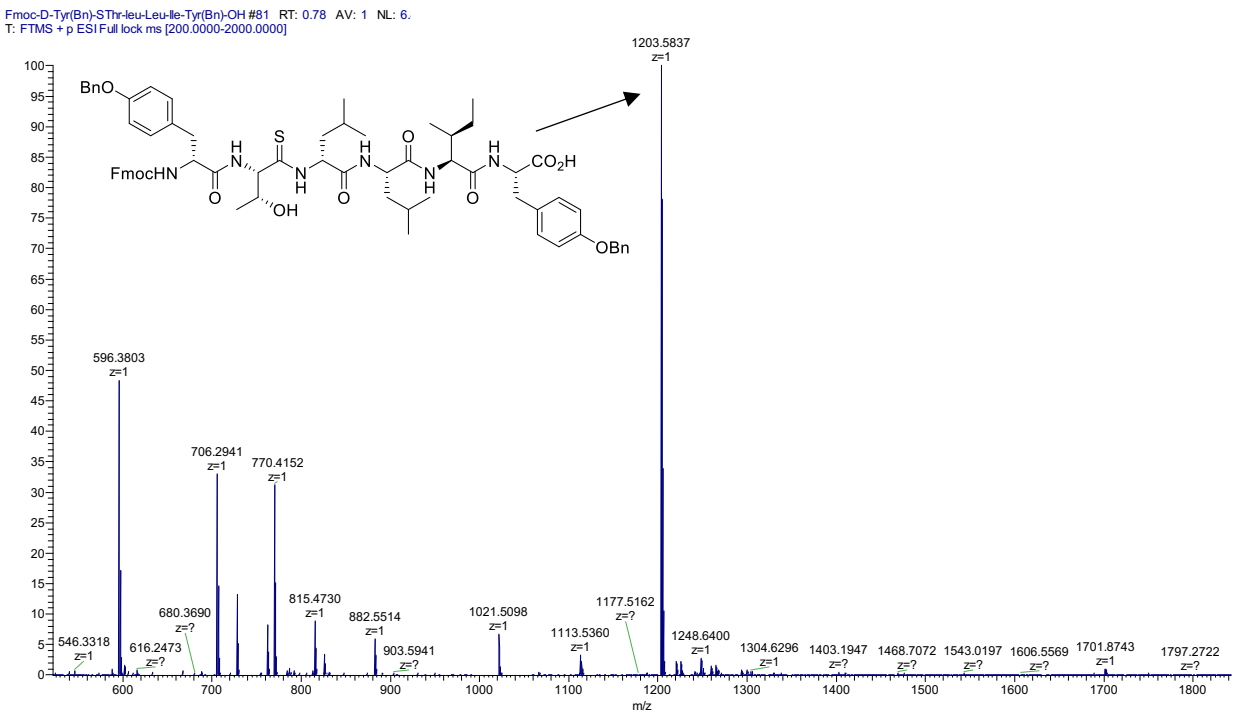


Figure S28. Mass spectrum of thiopeptide **8**.

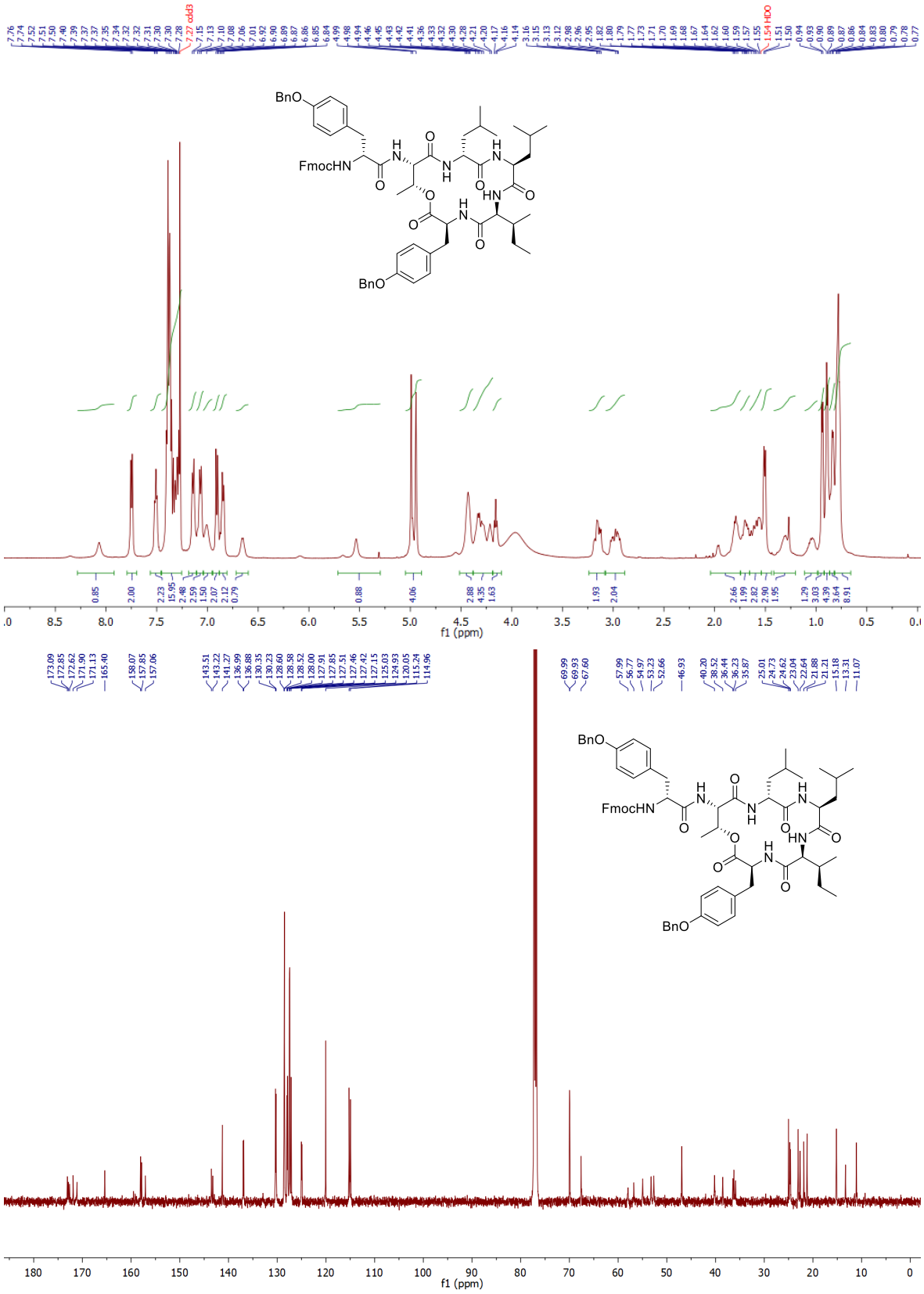


Figure S29. ^{1}H NMR and ^{13}C NMR spectrum of peptide **9** (500/151 MHz, $CDCl_3$)

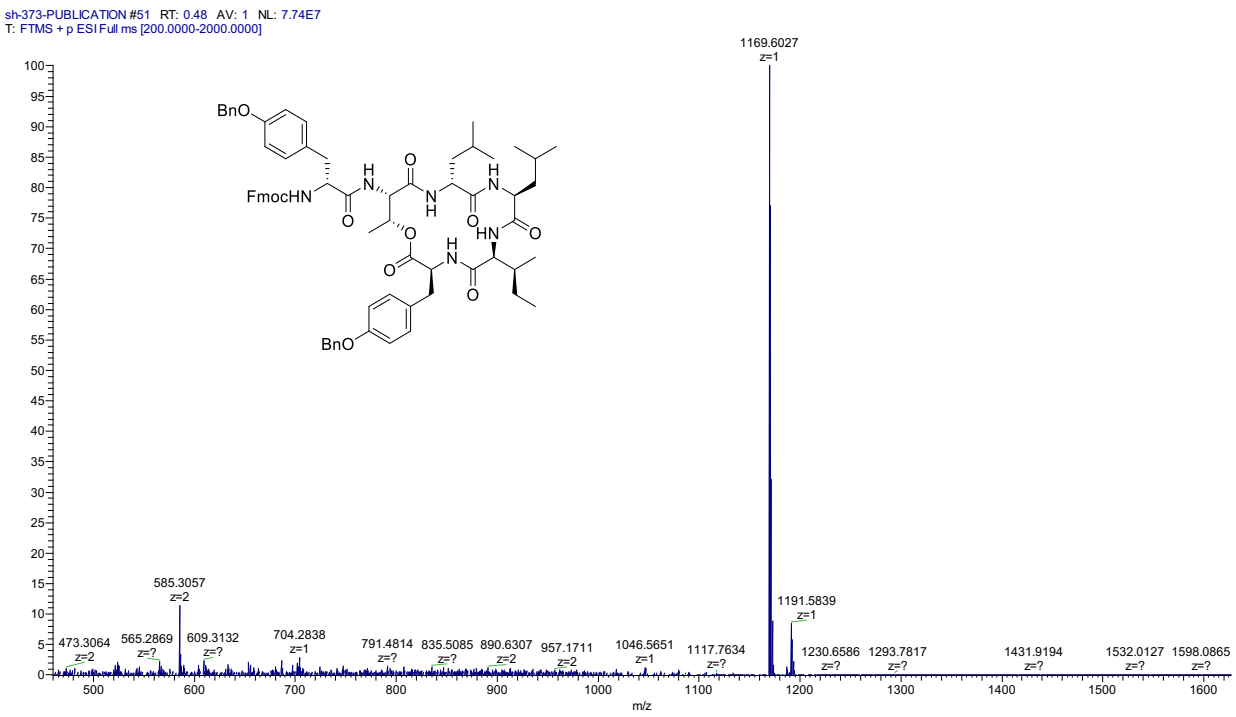


Figure S30. Mass spectrum of peptide **9**.

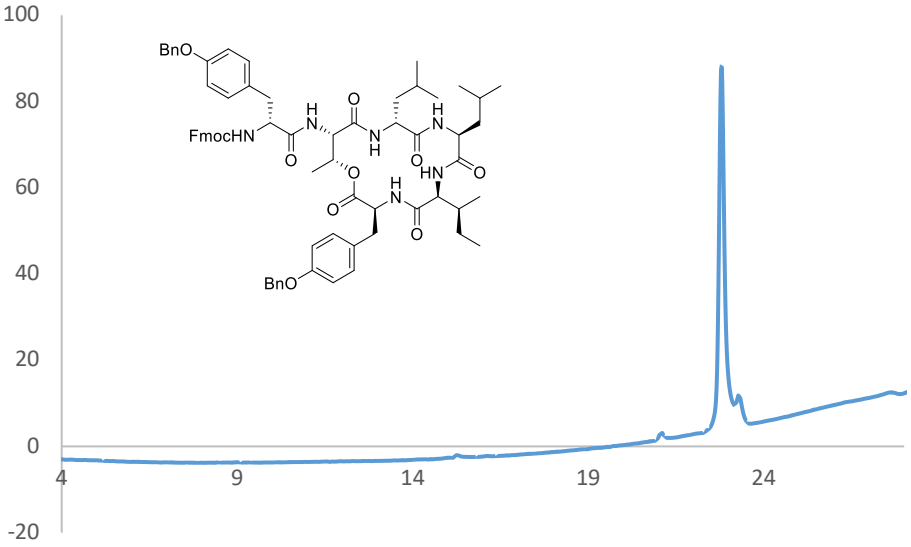


Figure S31. HPLC trace of peptide **9**.

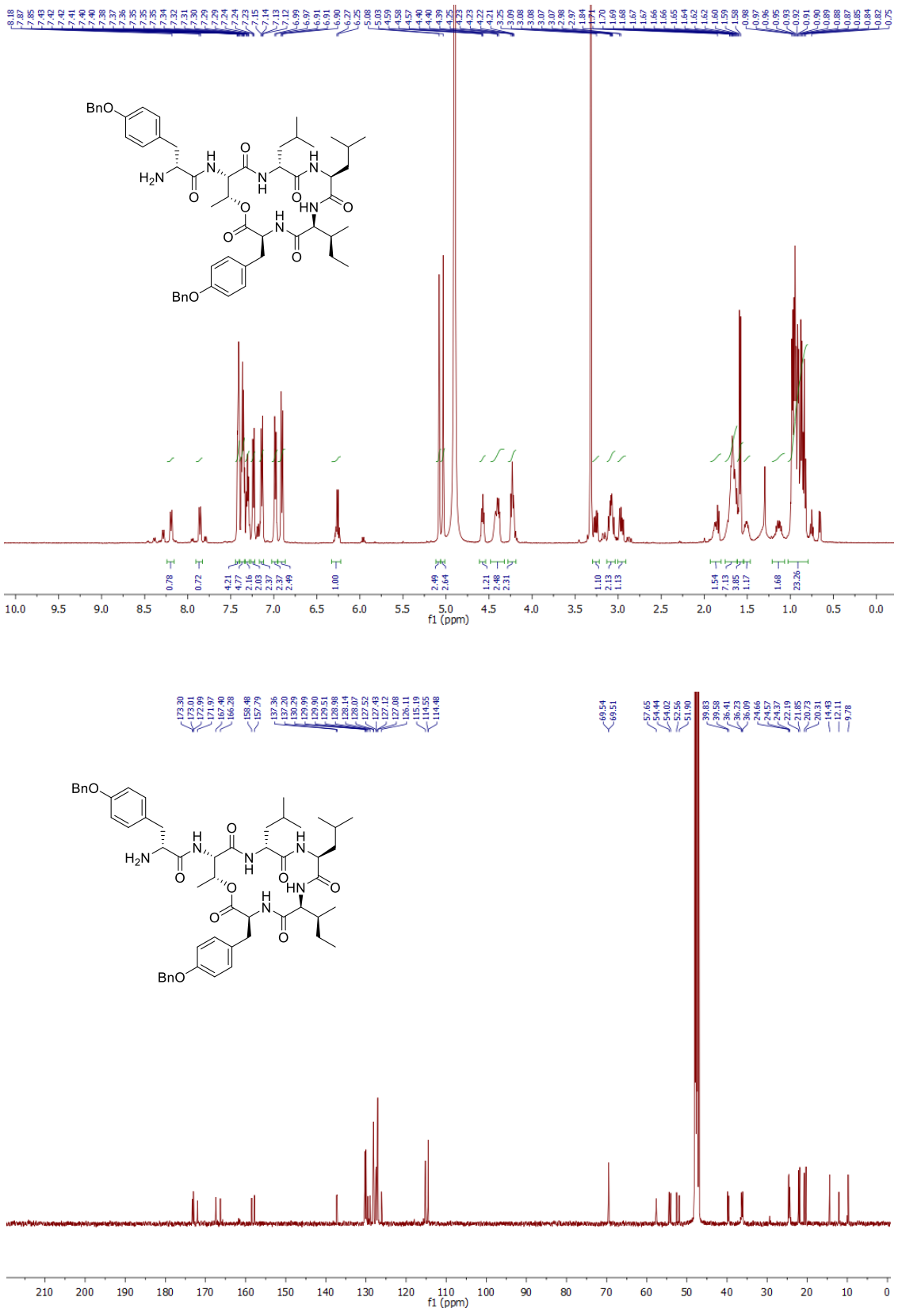


Figure S32. ^{1}H NMR and ^{13}C NMR spectra of peptide **S3** (500/151 MHz, CD_3OD)

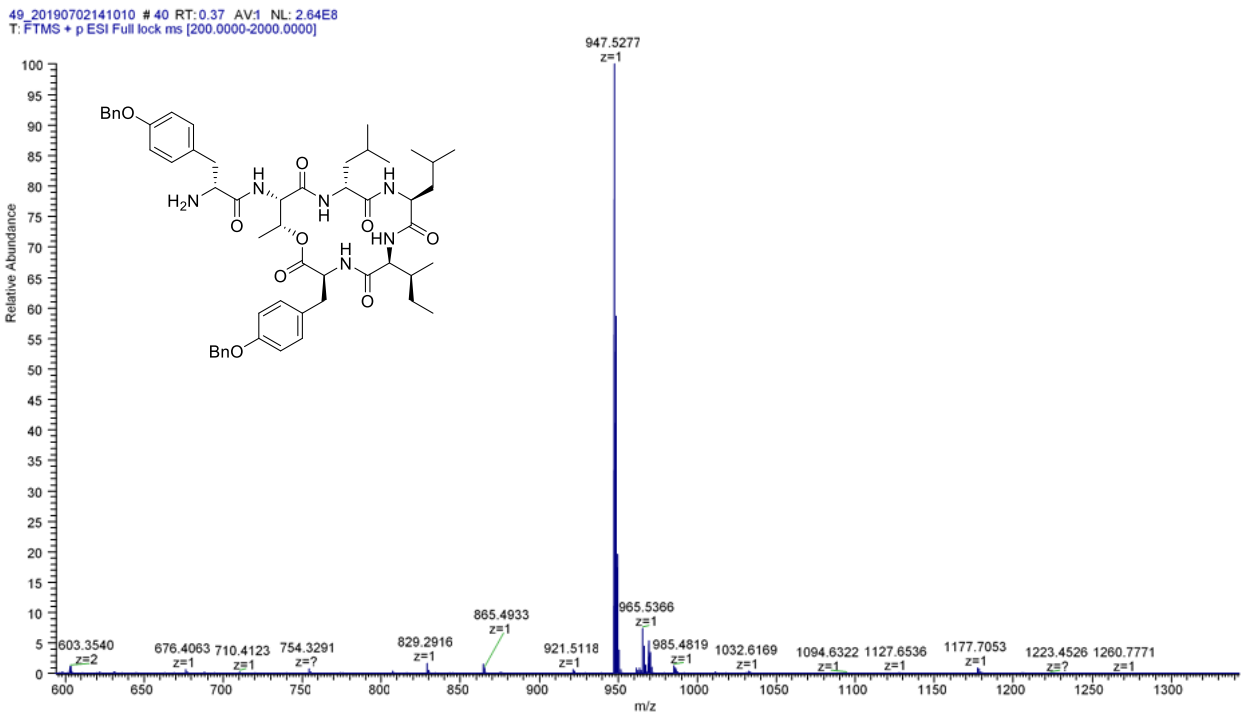


Figure S33. Mass spectrum of peptide **S3**.

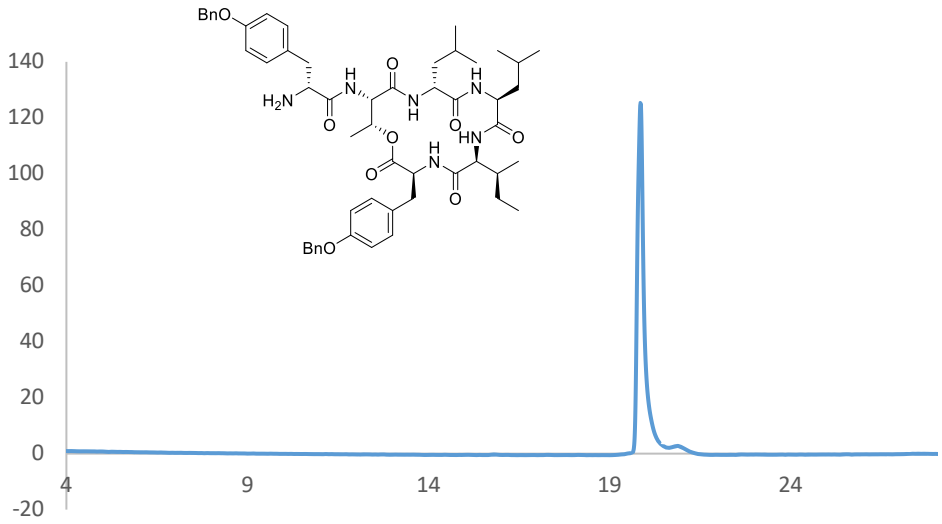


Figure S34. HPLC trace of peptide **S3**.

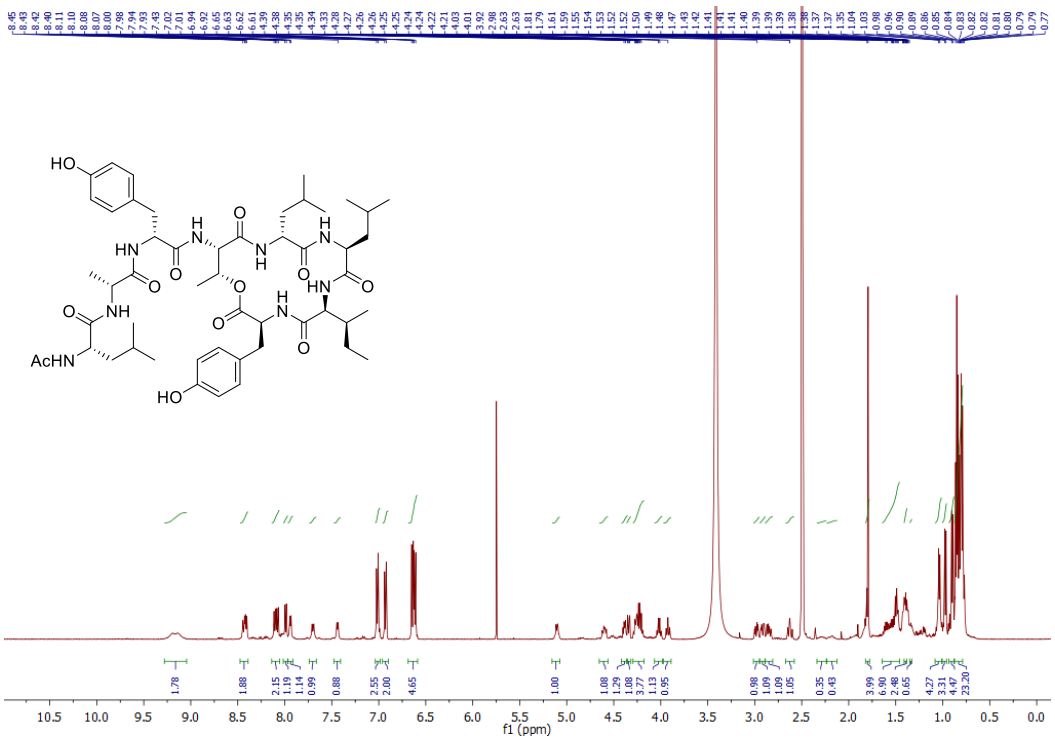


Figure S35. ^{1}H NMR spectrum of seongsanamide E **10** (500/151 MHz, DMSO-d_6)

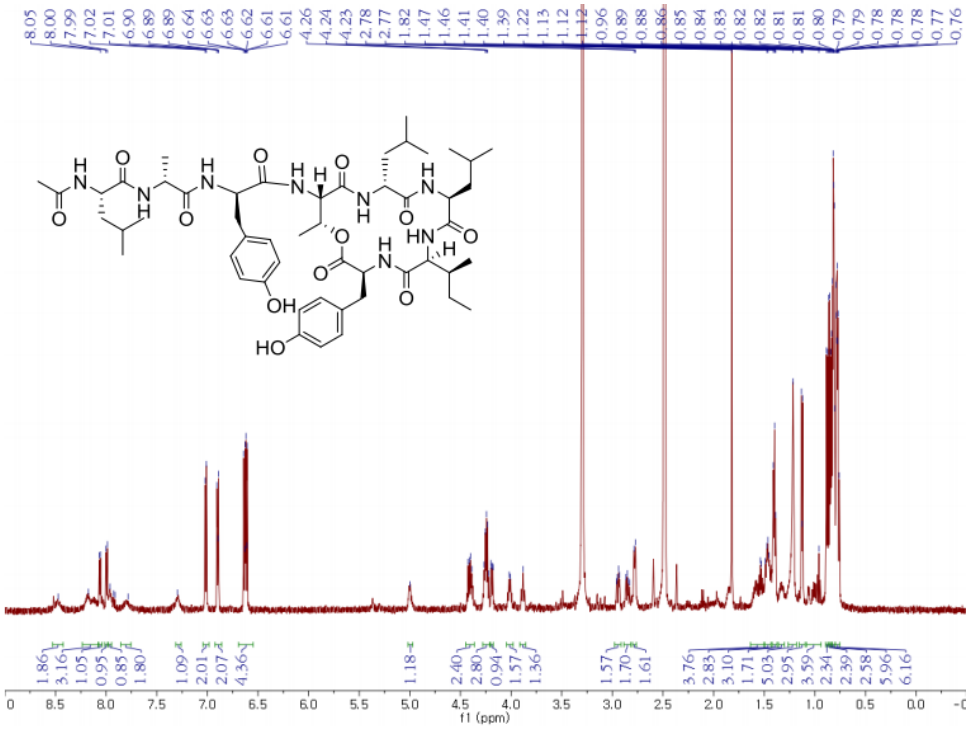


Figure S36. ^{1}H NMR spectrum of authentic natural product seongsanamide E **10** (from ref. 2) (500/151 MHz, DMSO-d_6).

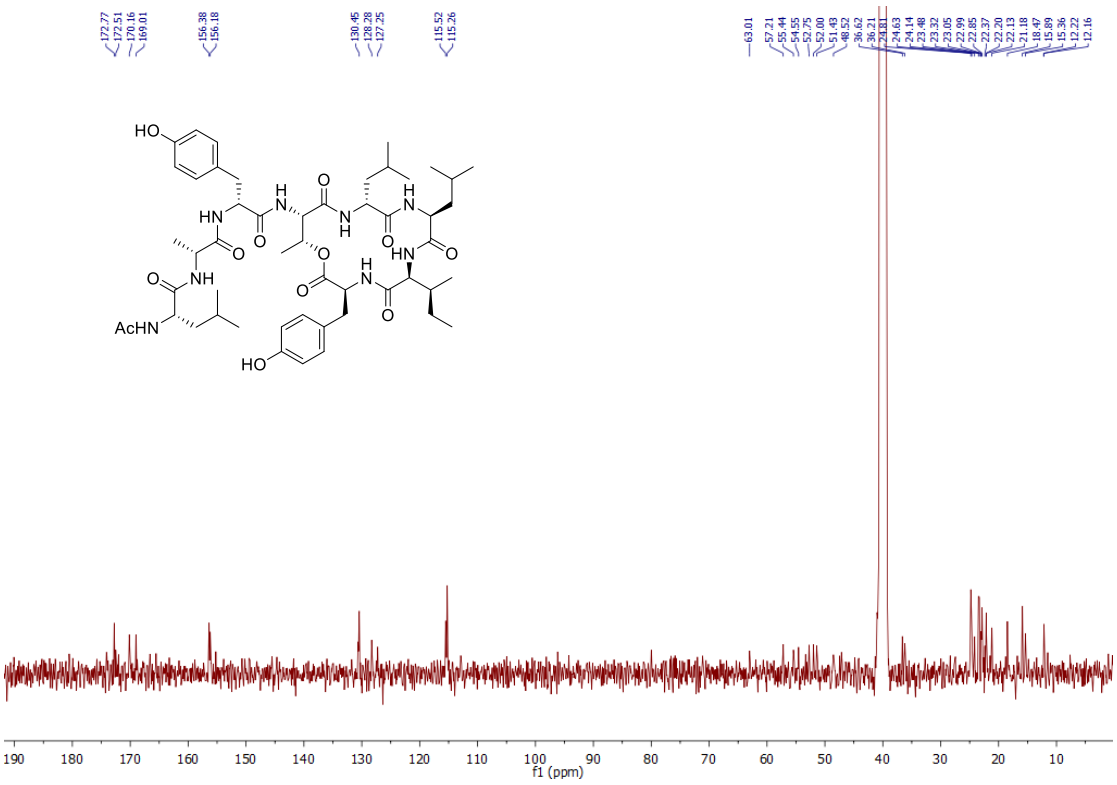


Figure S37. ^{13}C NMR spectrum of seongsanamide E **10** (500/151 MHz, DMSO-d_6)

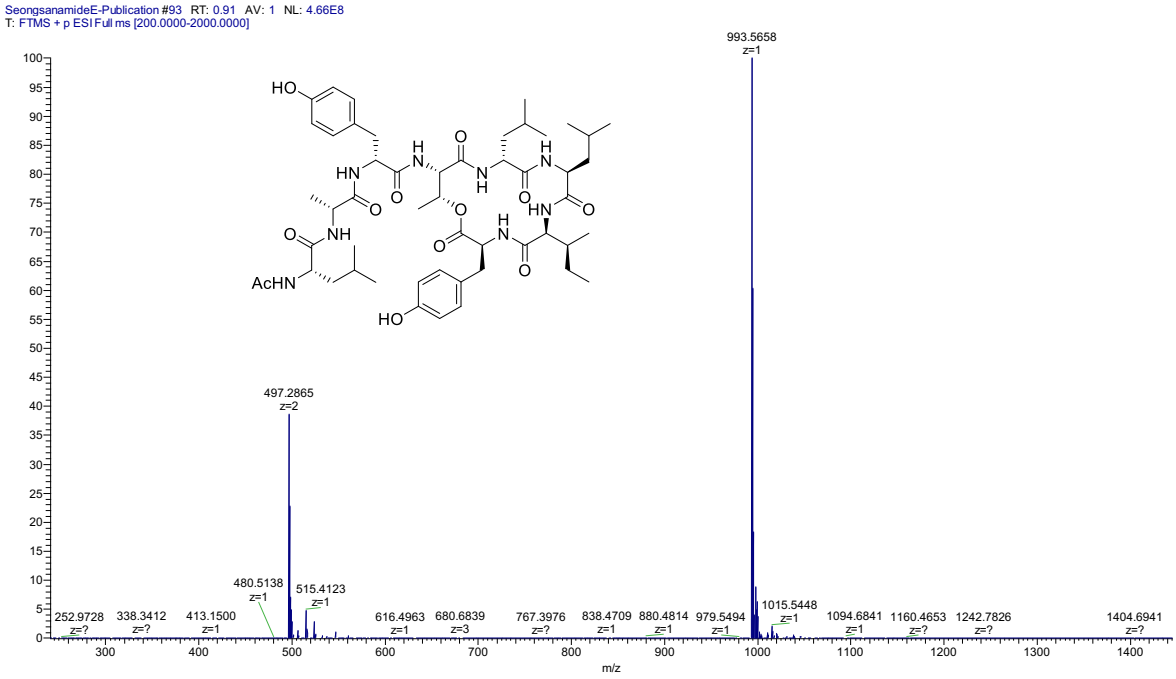


Figure S38. Mass spectrum of seongsanamide E **10**.

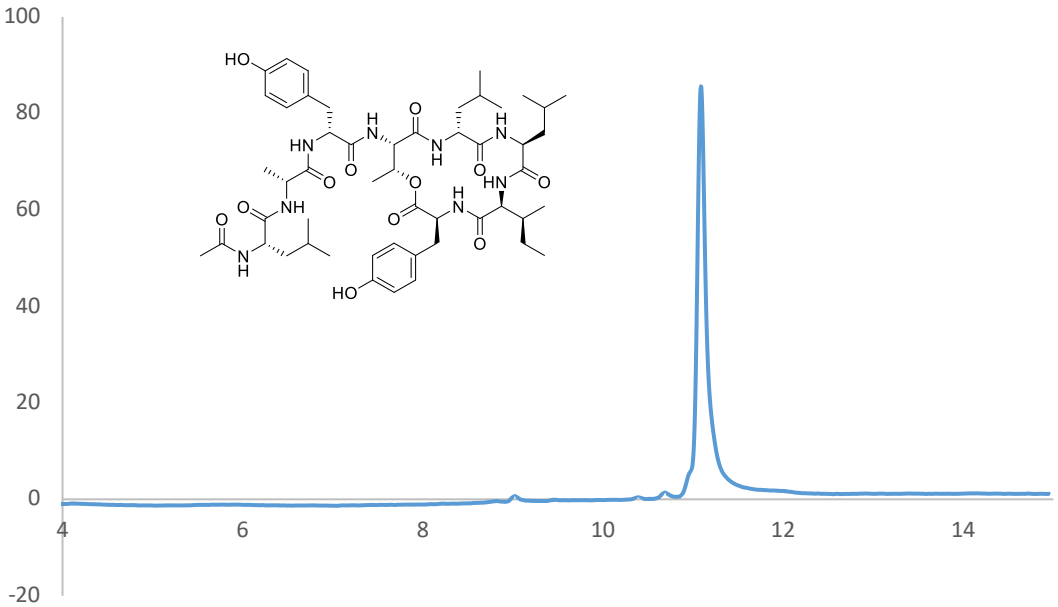


Figure S39. HPLC trace of seongsanamide E **10**.

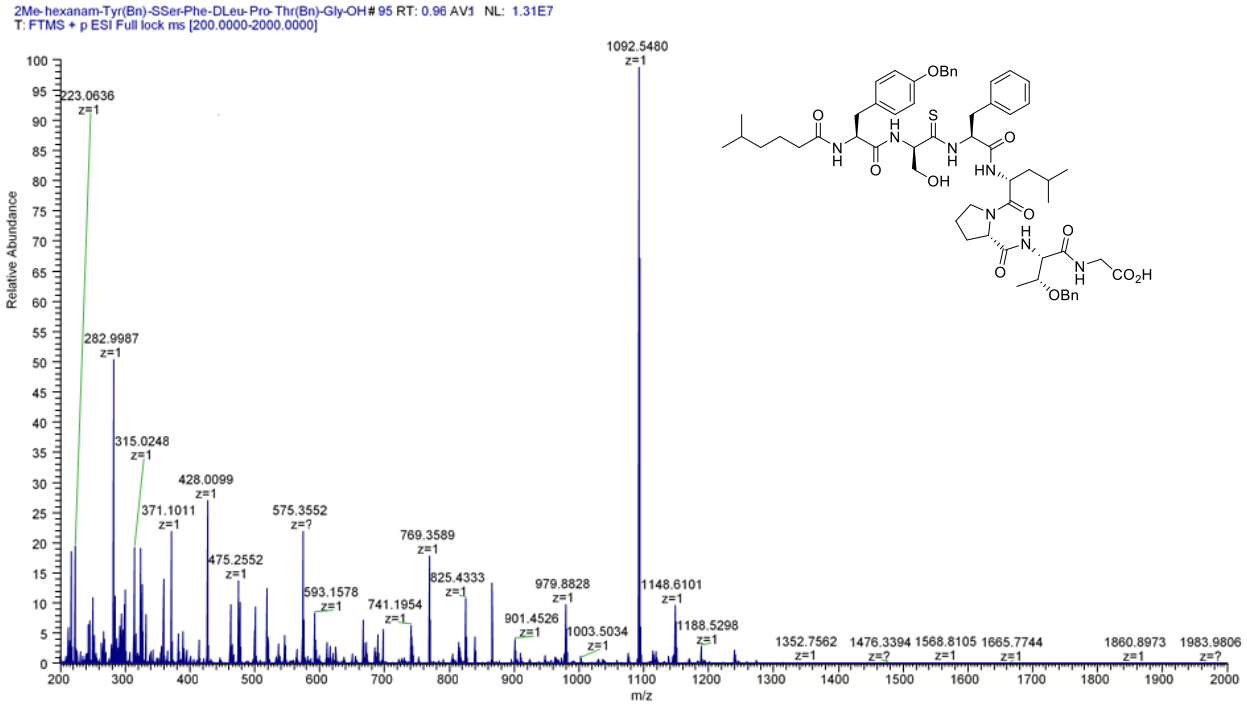


Figure S40. Mass spectrum of thiopeptide **11**.

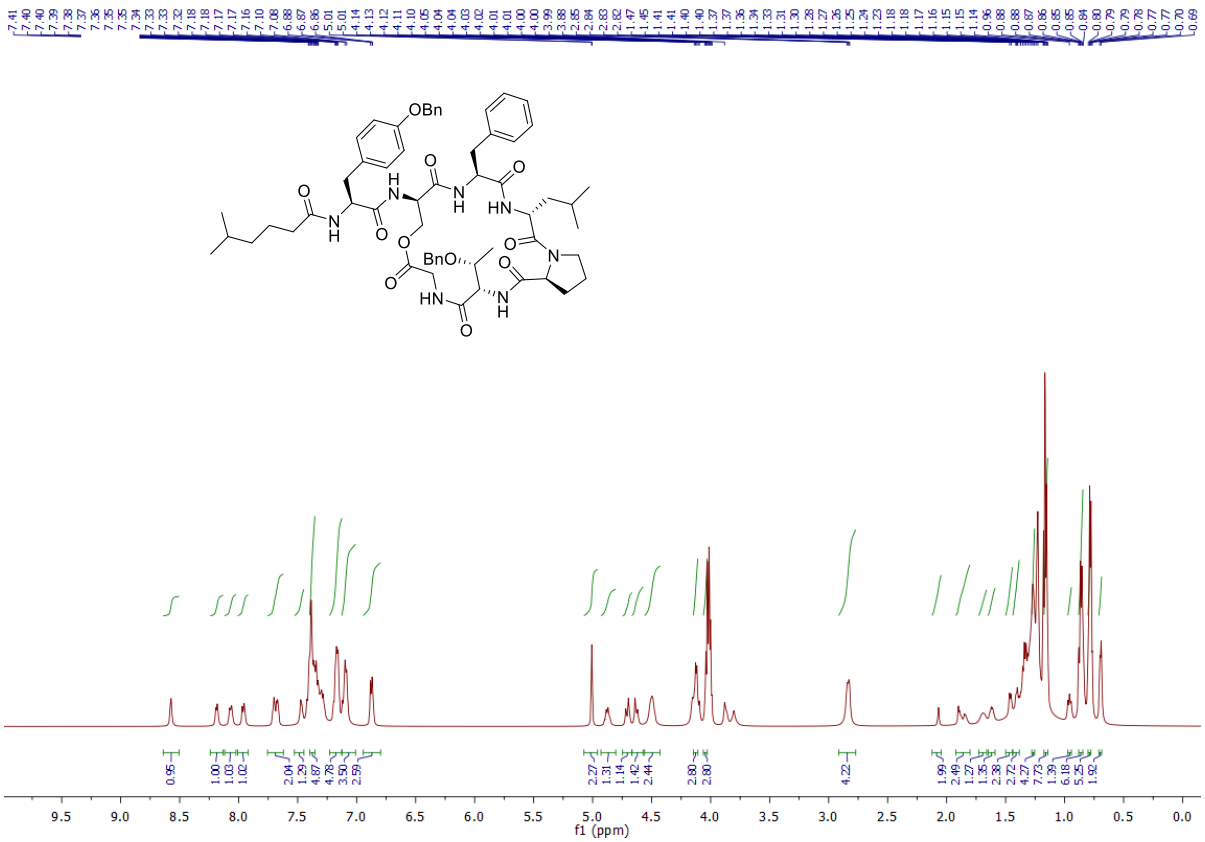


Figure S41. ^{1}H NMR spectrum of peptide **12** (500 MHz, DMSO-d_6)

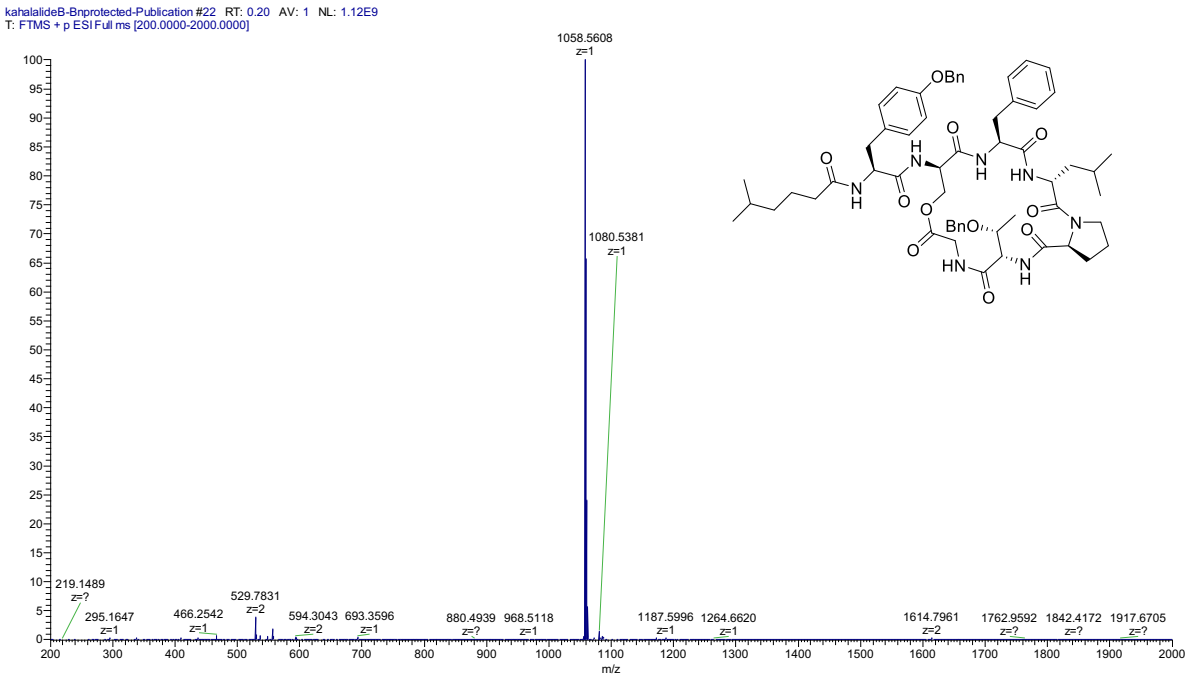


Figure S42. Mass spectrum of peptide **12**.

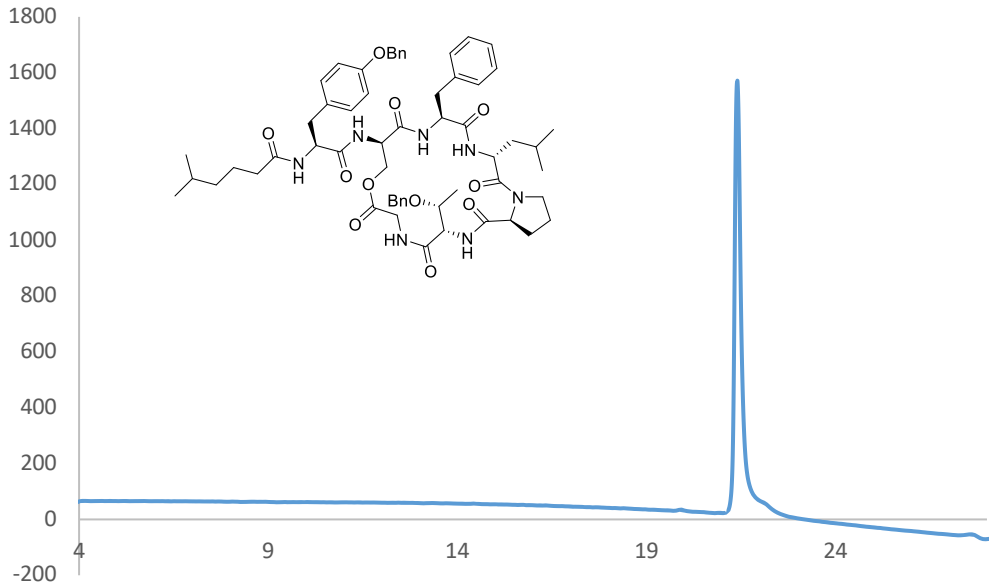


Figure S43. HPLC spectrum of peptide **12**.

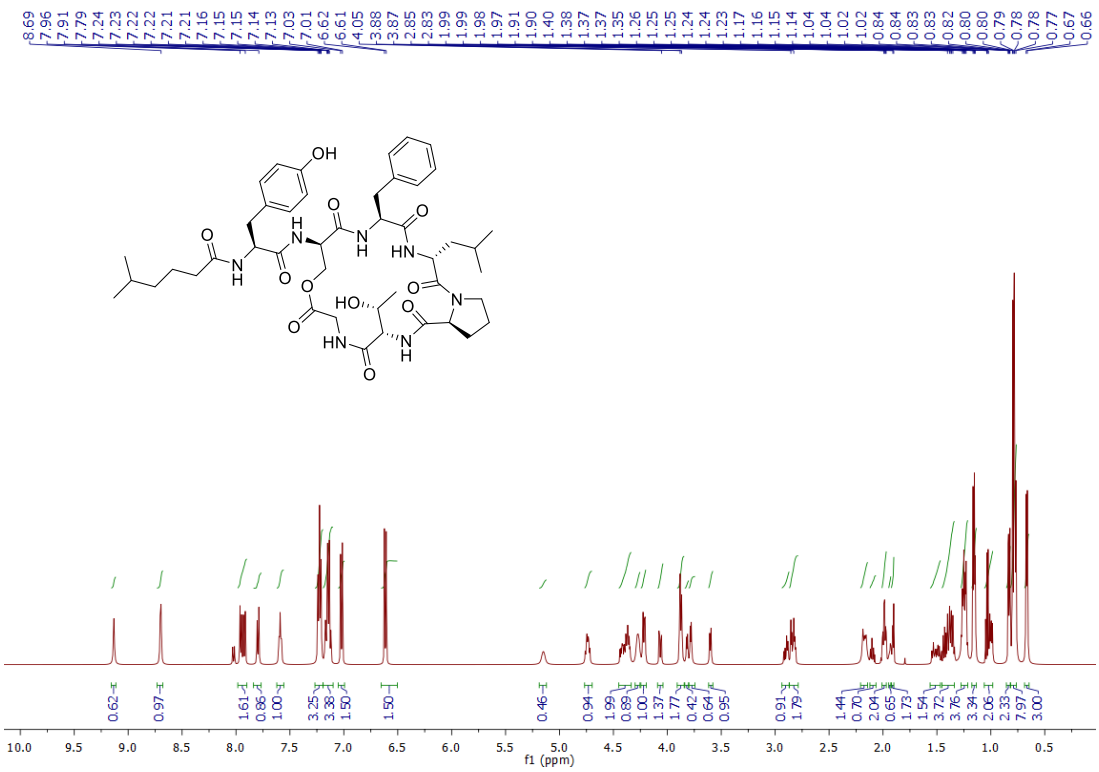


Figure S44. ^{1}H NMR spectrum of kahalalide B **13** (500 MHz, DMSO-d_6)

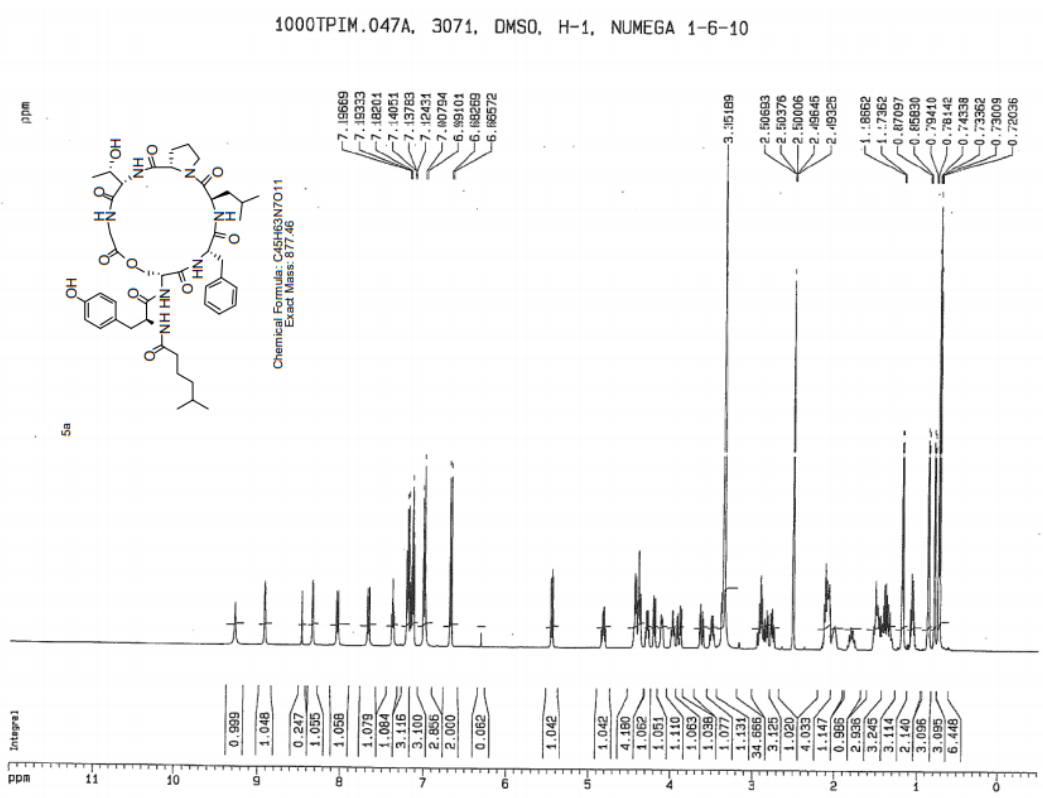


Figure S45. ^{1}H NMR spectrum of synthesized kahalalide B (from ref. 3).

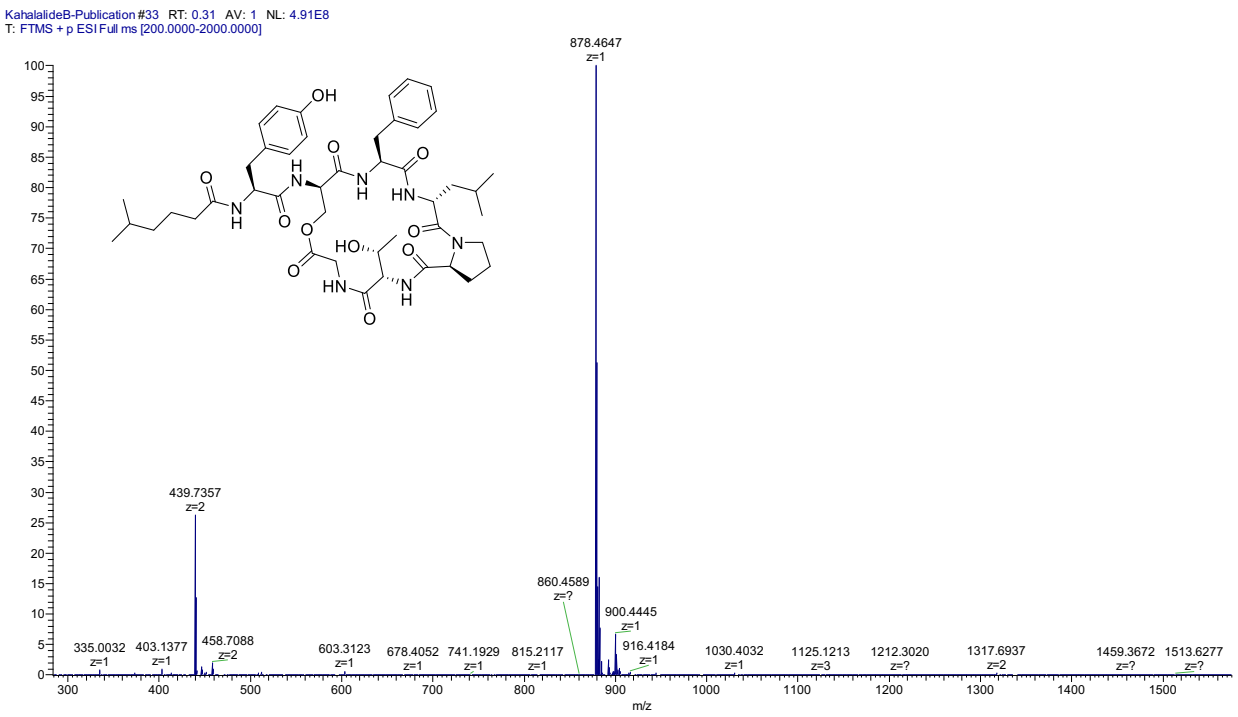


Figure S46. Mass spectrum of kahalalide B **13**.

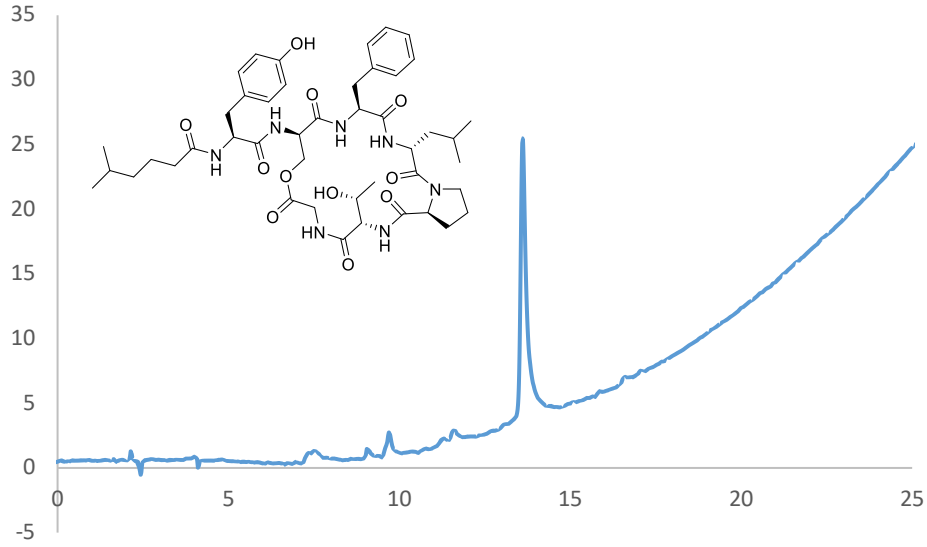


Figure S47. HPLC trace of kahalalide B **13**.

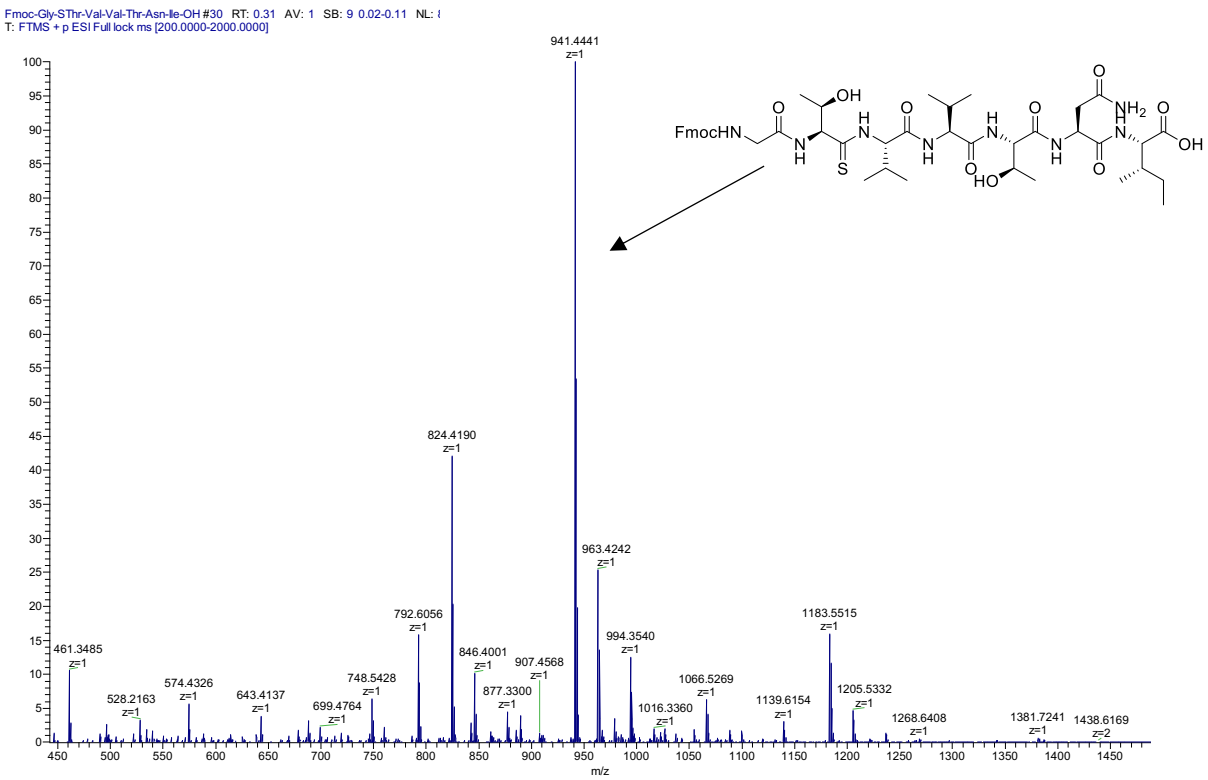


Figure S48. Mass spectrum of thiopeptide **14**.

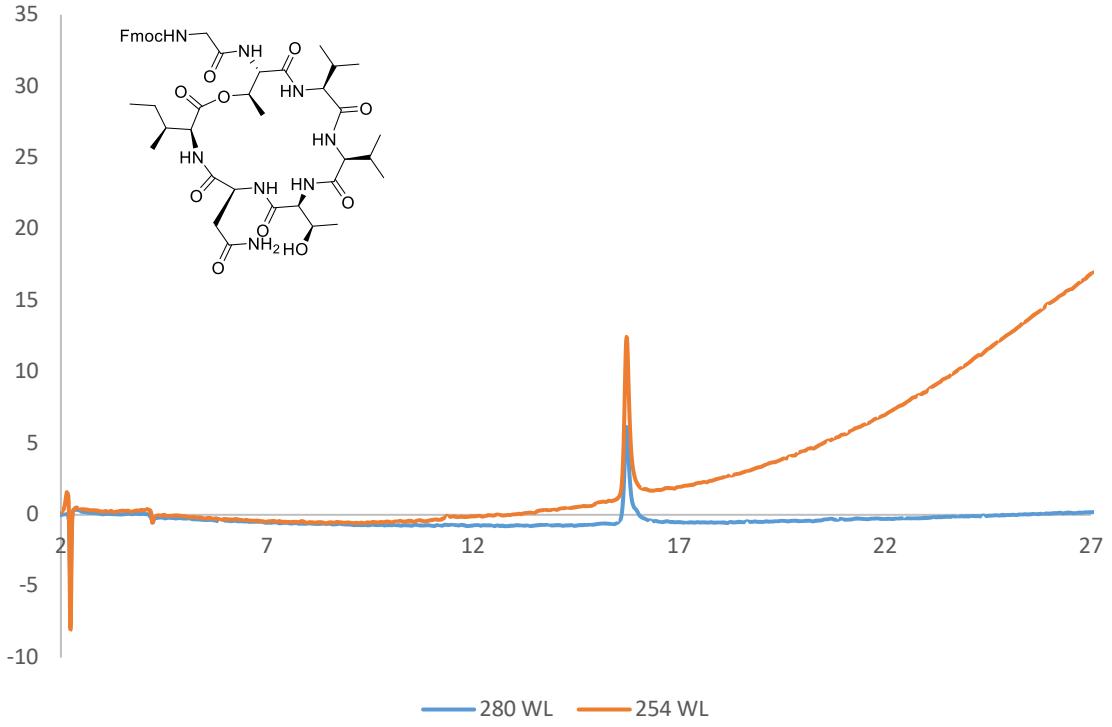


Figure S49. HPLC traces of peptide **16**.

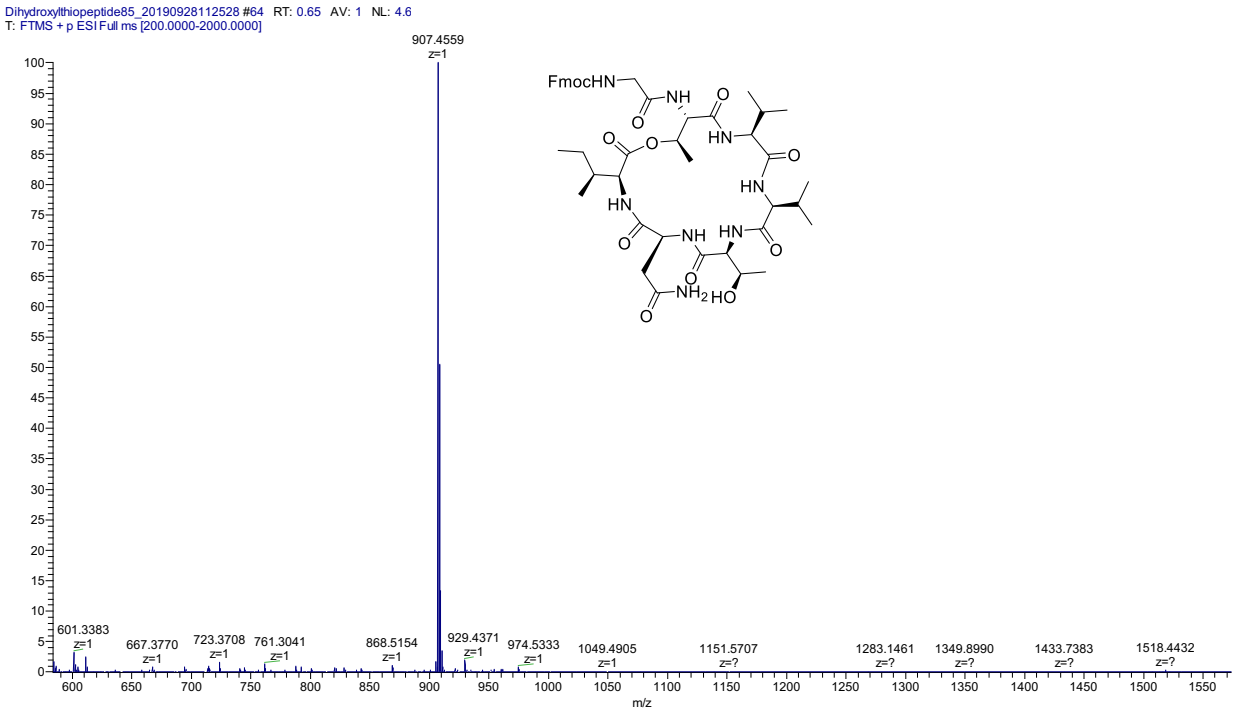


Figure S50. Mass spectrum of peptide **16**.

REFERENCES

1. Mukherjee, S.; Verma, H.; Chatterjee, J., Efficient site-specific incorporation of thioamides into peptides on a solid support. *Org. Lett.* **2015,** *17* (12), 3150-3153.
2. Kim, G. J.; Li, X.; Kim, S.-H.; Yang, I.; Hahn, D.; Chin, J.; Nam, S.-J.; Nam, J.-W.; Nam, D. H.; Oh, D.-C., Seongsanamides A–D: Antiallergic bicyclic peptides from Bacillus safensis KCTC 12796BP. *Org. Lett.* **2018,** *20* (23), 7539-7543.
3. Li, Y.; Giulionatti, M.; Houghten, R. A., Macrolactonization of Peptide Thioesters Catalyzed by Imidazole and Its Application in the Synthesis of Kahalalide B and Analogues. *Org. Lett.* **2010,** *12* (10), 2250-2253.