Supporting Information

Blocking the heat shock response and depleting HSF-1 levels through heat shock protein 90 (hsp90) inhibition: a significant advance on current hsp90 chemotherapies.

Yen Chin Koay, Jeanette R. McConnell, Yao Wang, Shelli R. McAlpine

School of Chemistry, University of New South Wales, Sydney, NSW 2052 Australia

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Chemistry: General solid phase peptide synthesis

General Remarks
All chemicals were purchased from commercial suppliers (Chem-Impex International, Peptide International and GL-Biochem) and used without further purification. All moisture sensitive reactions were performed under nitrogen gas and was monitored by thin-layer chromatography (TLC) and liquid-chromatography mass spectrometry (LC/MS). TLC was performed on aluminium silica gel sheets 250 µm Whatman® (4861-820) using UV light (λ = 254 nm) as visualizing method. The developing agents for TLC include potassium permanganate (general purpose) and ninhydrin (for amine group detection).

SiliCycle SiliaFlash silica gel (60 Å, particle size 40-63 µm) were used for flash chromatography. ¹H and ¹³C NMR spectra were obtained and recorded at 25ºC on Bruker Avance III 500 MHz and 600 MHz. Multiplicity of NMR signals were represented by the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet, br = broad, dd = doublet of doublet. Assignment of resonances for each amino acid residue was done using ¹H, ¹³C, HMOC, HMBQ and COSY. Each residue was assigned using ¹H chemical shifts and confirmed by COSY and HMOC crosspeaks. The final cyclized structure was characterized by considering long range HMBC correlations from both the α-protons and NH protons to the adjacent carbonyl carbons and the adjacent amino acid residues.

High-resolution mass spectrometry (HRMS) analyses were recorded on a Thermo LTQ Orbitrap XL ESI/APCI with UPLC system at the Bioanalytical Mass Spectrometry Facility in Mark Wainwright Analytical Centre at the University of New South Wales.

LC/MS analyses were performed on Shimadzu Prominence High performance LCMS 2010EV system (Water Symmetry® C18 column, 3.5 µm, 4.65x75 mm) connected to a Shimadzu LCMS 2010EV mass spectrometer. The mobile phase consist of DDI water with 0.1% (v/v) formic acid (solvent A), and HPLC grade acetonitrile with 0.1% (v/v) formic acid (solvent B) at a flow rate of 0.5 mL/min, starting at 70% solvent A, 30% solvent B.

Semi-preparative HPLC for purification was performed on Shimadzu Prominence High performance LCMS 2010EV system (Phenomenex® Jupiter C18 column, 4µm, 250x10 mm). The mobile phase was prepared by DDI water with 0.1% (v/v) formic acid (solvent A), and HPLC grade acetonitrile with 0.1% (v/v) formic acid (solvent B). The gradient elution were as follow: flow rate 2 mL/min; initial 70% solvent A, 30% solvent B hold for 35 min; at 35 min 100% solvent B hold for 18 min; at 53 min 70% solvent A, 30% solvent B hold for 7 min.

Synthesis: Experimental Procedures
Synthesis of N-methylated peptides:

ο-NBS Protection
A solution of o-NBS-Cl (4 equivalents) and collidine (10 equivalents) in CH₂Cl₂ was added to the resin-bound free amine peptide in a round bottom flask and stirred for 4 hours at room temperature. The resin was then washed with DCM (5x). Completion of the reaction was monitored and analyzed by LCMS by treatment of a small amount resin with TFE:CH₂Cl₂ (1:1) to cleave the ο-NBS-peptides.

N-Methylation under Mitsunobu conditions
A solution of triphenylphosphine (5 equivalents) and MeOH (10 equivalents) in dry THF was added to the resin-bound free amine peptide in a round bottom flask and stirred for 4 hours at room temperature. The resin was then washed with DCM (5x). Completion of the reaction was monitored and analyzed by LCMS by treatment of a small amount resin with TFE:CH₂Cl₂ (1:1) to cleave the ο-NBS-peptides.

ο-NBS deprotection
For ο-NBS deprotection, the resin-bound N-Methyl-ο-NBS-peptides was treated with a solution of mercaptoethanol (10 equivalents) and DBU (5 equivalents) in DMF for 2 hours. The deprotection procedure was repeated one more time and the resin was washed with DMF (5x).

Benoiton method
The Benoiton method to generate N-methylated amino acid was done by adding iodometane (10 equivalents) as methylating agent in portion wise to a stirred solution of Boc protected amino acid and strong base sodium hydride (60% dispersion in mineral oil, 10 equivalents) in anhydrous THF (0.30 M) to 0 ºC. The mixture was allowed to
stir at room temperature for 24 hours under nitrogen. The completion of the reaction was monitored by TLC. The reaction mixture was then dried in vacuo and diluted with ethyl acetate. The organic layer was washed with 10% (v/v) HCl(aq), dried over Na₂SO₄, filtered, concentrated in vacuo and subjected to the next reaction without purification.
Experimental procedures

Following “General peptide coupling” procedure for SPPS and the published procedure for SPPS ( Koay, Y. C.; McConnell, J. R.; Wang, Y.; Kim, S. J.; Buckton, L. K.; Mansour, F.; McAlpine, S. R. ACS Med. Chem. Lett. 2014, 5, 771-776), linear pentapeptide of resin-O-Leu-N-Me-Val-D-Leu-D-His(Boc)-3,3-Diphenyl-D-Ala-NH2 was synthesized by using 1.00 g (0.5 mmol, 1.0 equivalent) of resin-O-Leu-NH2 and the subsequent peptide coupling in the sequence as mentioned below: 0.53 g (1.5 mmol, 3.0 equivalents) of Fmoc-N-Me-Val-OH, 0.53 g (1.5 mmol, 3.0 equivalents) of Fmoc-D-Leu-OH, 0.72 g (1.5 mmol, 3.0 equivalents) of Fmoc-D-His(Boc)-OH and 0.70 g (1.5 mmol, 3.0 equivalents) of Fmoc-3,3-Diphenyl-D-Ala-OH. Each peptide coupling was done in the presence of 0.20 g of HOAt (1.5 mmol, 3.0 equivalents) or 0.20 g of HOBt (1.5 mmol, 3.0 equivalents), 0.47 mL of DIC (3.0 mmol, 6.0 equivalents) and 2.5 mL of DMF to generate a concentration of 0.20 M. Each coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained to afford the Fmoc-protected resin-bound pentapeptide and the Fmoc protecting group was removed following the “General Fmoc removal” after the completion of each coupling reaction.

The double deprotected linear pentapeptide (DDLP) HO-Leu-N-Me-Val-D-Leu-D-His(Boc)-3,3-Diphenyl-D-Ala-NH2 was generated following the “Resin cleavage of linear peptide” procedure for SPPS. The linear pentapeptide was cleaved from the resin using a mixed solution of 6 mL of TFE and 6 mL of CH2Cl2. The resin containing solution was filtered and dried in vacuo to yield the DDLP as a white solid (286 mg, overall 70%).

LC/MS (ESI): m/z called for C44H63N7O8 (M+1) = 818.47, found 818.10.

Cyclo Leu-N-Me-Val-D-Leu-D-His(Boc)-3,3-Diphe-D-Ala (7) was synthesized using 0.29 g of the DDLP generated (0.35 mmol, 1.0 equivalent), 0.09 g of TBTU (0.28 mmol, 0.80 equivalent), 0.11 g of HATU (0.28 mmol, 0.80 equivalent), 0.08 g of DMTMM (0.28 mmol, 0.80 equivalent), 0.49 mL of DIPEA (2.8 mmol, 8.0 equivalents) in anhydrous CH2Cl2 (355 mL, 0.001M) following “Macrocyclization procedure (syringe pump)” procedure. The reaction was then stirred overnight and the reaction was monitored via LC/MS. Upon completion, the reaction mixture was subjected to acid-base wash, which was extracted twice with 10% (v/v) HCl (aq). The organic layer was then re-extracted with a saturated of NaHCO3 aqueous solution. The combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo. The resulting crude product was purified via flash column chromatography on silica gel using an ethyl acetate-hexane gradient system, followed by purification via HPLC to yield compound 7 as white solid (58 mg, 21%).

Rf: 0.58 (EtOAc:MeOH = 0.95:0.05)

LC/MS (ESI): m/z called for C44H61N7O7Na1 (M+Na+) = 822.45, found 822.00.

HRMS (ESI-TOF): M+Na+, found 822.4524 C44H61N7O7Na1 requires 822.4530.

1H NMR (600 MHz, DMSO): δ 8.26 (d, J = 8.9 Hz, NH), 8.20 (s, D-His), 7.89 (d, J = 7.6 Hz, NH), 7.77 (d, J = 9.8 Hz, NH), 7.42 (br, NH), 7.34 (m, 3H, D-His, D-Biphe), 4.45 (m, 1H, CHα D-His), 4.35 (d, J = 12.2 Hz, 1H, CHβ D-Biphe), 4.29 (m, 1H, CHα D-Leu), 3.38 (q, J = 11.0 Hz, 1H, CHα Leu), 2.61 (m, 1H, CH2β2 D-Leu), 2.57 (m, 4H, CH2β1 D-His, NCH3), 2.13 (m, 1H, CHβ Val), 1.59 (s, 9H, OC(CH3)3), 1.54 (m, 1H, CH2β2 Leu), 1.48 (m, 1H, CH2β2 D-Leu), 1.41 (m, 1H, CH2β1 Leu), 1.29 (m, 2H, CHβ2 D-Leu, CHβ Leu), 1.17 (m, 1H, CHγ D-Leu), 0.79-0.67 (m, 18H, CH2CH(CH3)2, CHCH(CH3)2).

13C NMR (150 MHz, DMSO): δ 170.72, 170.00, 169.68, 169.08, 168.56, 147.07, 141.61, 141.30, 139.07, 137.41, 128.66, 128.56, 128.51, 128.48, 127.02, 126.87, 114.88, 85.70, 63.05, 57.01, 53.80, 51.80, 47.90, 41.18, 40.53, 37.97, 30.18, 27.87, 25.33, 24.50, 24.25, 23.53, 22.94, 22.53, 22.51, 19.57, 18.80.

The Boc protecting group of compound 7 was removed following “Boc removal” procedure, utilizing a mixture of TFA/CH2Cl2 (1:4, 0.1 M) and anisole (2.0 equivalents) to generate compound 8.

Rf: 0.50 (EtOAc:MeOH = 1:1)

LC/MS: m/z called for C39H53N7O5 (M+1) = 700.41, found 700.00.

HRMS (ESI-TOF): M+1, found 700.4179 C39H53N7O5 requires 699.4108.

1H NMR (600 MHz, DMSO): δ 7.00, 170.00, 169.68, 169.08, 168.56, 147.07, 141.61, 141.30, 139.07, 137.41, 128.66, 128.56, 128.51, 128.48, 127.02, 126.87, 114.88, 85.70, 63.05, 57.01, 53.80, 51.80, 47.90, 41.18, 40.53, 37.97, 30.18, 27.87, 25.33, 24.50, 24.25, 23.53, 22.94, 22.53, 22.51, 19.57, 18.80.
5.24 (dd, J = 10.1, 12.1 Hz, 1H, CHα D-Biphe), 4.56 (d, J = 11.3 Hz, 1H, CHα Val), 4.48 (m, 2H, CHβ D-Biphe, CHα D-Leu), 4.26 (m, 1H, CHα D-His), 3.27 (q, J = 7.2 Hz, 1H, CHα Leu), 2.72 (br, 2H, CHβ D-His), 2.56 (s, 3H, NCH3), 2.11 (m, 1H, CHβ Val), 1.54 (m, 2H, CHβ2 Leu, CHβ2 D-Leu), 1.40 (m, 1H, CHβ D-Leu), 1.27 (m, 3H, CHβ1 Leu, CHγ Leu, CHγ D-Leu), 0.81-0.65 (m, 18H, CH2CH(CH3)2, CHCH(CH3)2).

13C NMR (150 MHz, DMSO): δ 170.70, 170.30, 169.56, 169.24, 168.57, 141.69, 141.30, 135.13, 134.94, 128.78, 128.71, 128.52, 128.38, 127.12, 126.78, 116.03, 62.69, 57.16, 54.54, 53.67, 52.06, 47.77, 41.24, 40.53, 37.85, 30.09, 25.35, 24.47, 24.35, 23.49, 22.97, 22.78, 22.53, 19.61, 18.75.

The tBu protecting group of compound 9 was removed following “Tert-butyl group (tBu) Removal” procedure, utilizing a mixture of TFA/CH2Cl2 (2:4, 0.1 M) and anisole (2.0 equivalents) to generate compound 10. The free acid was taken to the subsequent methyl ester formation without purification.

Rf: 0.20 (EtOAc:MeOH = 0.80:0.20)
LC/MS: m/z called for C38H53N5O7 (M+1) = 692.39, found 692.05.
HRMS (ESI-TOF): M+Na+, found 714.3834 C38H53N5O7Na1 requires 714.3843.

1H NMR (600 MHz, DMSO): δ 8.10 (br, NH), 7.99 (d, J = 8.9 Hz, NH), 7.83 (d, J = 7.4 Hz, NH), 7.49 (d, J = 7.3 Hz, NH), 7.37-7.12 (m, 10H, D-Biphe), 5.23 (dd, J = 10.0, 11.9 Hz, 1H, CHα D-Biphe), 4.59 (m, 2H, CHα Val, CHα D-Glu), 4.32 (d, J = 12.1 Hz, 1H, CHβ D-Biphe), 3.76 (m, 1H, CHα D-Leu), 3.50 (q, J = 7.4 Hz, 1H, CHα Leu), 2.64 (s, 3H, NCH3), 2.16 (m, 1H, CHβ Val), 1.69 (m, 4H, CHβ D-Glu, CHβ D-Leu), 1.48 (m, 3H, CHγ Leu, CHγ D-Leu), 1.39 (m, 2H, CHβ D-Glu), 1.25 (m, 1H, CHγ D-Leu), 0.88-0.63 (m, 18H, CH2CH(CH3)2, CHCH(CH3)2).

13C NMR (150 MHz, DMSO): δ 174.20, 171.07, 170.81, 170.07, 169.65, 168.85, 141.69, 141.13, 128.91, 128.76, 128.58, 128.44, 128.30, 127.29, 127.41, 126.82, 63.22, 56.98, 55.52, 53.63, 51.75, 47.59, 41.46, 37.80, 30.11, 29.73, 26.76, 25.29, 24.77, 24.55, 23.33, 22.91, 22.88, 22.51, 19.62, 18.90.

The free acid of compound 10 was converted to the methyl ester compound 11 using the methylating agent Trimethylsilyl diazomethane (TMSD). Compound of 10 was dissolved in a mixture of anhydrous benzene and methanol (3:1) in a round bottom flask to make a 0.1 M solution. The methylating agent TMSD (2.0 M in diethyl ether) was added drop-wise into the reaction mixture became slightly yellow. The reaction was stirred under nitrogen and monitored by thin layer chromatography (TLC). Upon completion, the solvent was evaporated in vacuo and resulting compound 11.

Rf: 0.48 (Hex:EtOAc = 0.25:0.75)
LC/MS: m/z called for C39H48N6O7 (M+1) = 706.41, found 706.05.
HRMS (ESI-TOF): M+Na+, found 728.3996 C39H55N5O7Na1 requires 728.3999.

1H NMR (600 MHz, DMSO): δ 8.10 (d, J = 7.7 Hz, NH), 8.00 (d, J = 9.0 Hz, NH), 7.83 (d, J = 7.6 Hz, NH), 7.49 (d, J = 8.5 Hz, NH), 7.36-7.12 (m, 10H, D-Biphe), 5.24 (dd, J = 9.9, 11.9 Hz, 1H, CHα D-Biphe), 4.59 (m, 2H, CHα Val, CHα D-Glu), 4.32 (d, J = 12.1 Hz, 1H, CHβ D-Biphe), 3.76 (m, 1H, CHα D-Leu), 3.56 (s, 3H, OCH3), 3.50 (q, J = 7.4 Hz, 1H, CHα Leu), 2.64 (s, 3H, NCH3), 2.16 (m, 1H, CHβ Val), 1.69 (m, 4H, CHβ D-Glu, CHβ D-Leu), 1.48 (m, 3H, CHγ Leu, CHγ D-Leu), 1.39 (m, 2H, CHβ D-Glu), 1.25 (m, 1H, CHγ D-Leu), 0.88-0.63 (m, 18H, CH2CH(CH3)2, CHCH(CH3)2).

13C NMR (150 MHz, DMSO): δ 172.90, 170.95, 170.82, 170.22, 169.68, 168.89, 141.59, 141.14, 128.91, 128.76, 128.58, 128.44, 128.30, 127.29, 127.14, 126.82, 63.22, 56.88, 55.43, 53.59, 51.72, 51.69, 47.62, 41.42, 37.79, 30.14, 29.60, 26.51, 25.30, 24.78, 24.56, 23.31, 22.90, 22.86, 22.50, 19.60, 18.91.
Following “General peptide coupling” procedure for SPPS and the published procedure for SPPS (Koay, Y. C.; McConnell, J. R.; Wang, Y.; Kim, S. J.; Buckton, L. K.; Mansour, F.; McAlpine, S. R. ACS Med. Chem. Lett. 2014, 5, 771-776), linear pentapeptide of resin-O-Leu-N-Me-Val-3-(2’-Pyr)-D-Ala-D-Phe-3,3-Diphe-D-Ala-NH₂ was synthesized by using 1.00 g (0.5 mmol, 1.0 equivalent) of resin-O-Leu-NH₂ and the subsequent peptide coupling in the sequence as mentioned below: 0.53 g (1.5 mmol, 3.0 equivalents) of Fmoc-N-Me-Val-OH, 0.58 g (1.5 mmol, 3.0 equivalents) of Fmoc-D-3-(2’-Pyr)Ala-OH, 0.58 g (1.5 mmol, 3.0 equivalents) of Fmoc-D-Phe-OH and 0.70 g (1.5 mmol, 3.0 equivalents) of Fmoc-3,3-Diphenyl-D-Ala-OH. Each peptide coupling was done in the presence of 0.20 g of HOAt (1.5 mmol, 3.0 equivalents) or 0.20 g of HOBT (1.5 mmol, 3.0 equivalents), 0.47 mL of DIC (3.0 mmol, 6.0 equivalents) and 2.5 mL of DMF to generate a concentration of 0.20 M. Each coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained to afford the Fmoc-protected resin-bound pentapeptide and the Fmoc protecting group was removed following the “General Fmoc removal” after the completion of each coupling reaction.

The double deprotected linear pentapeptide (DDLP) HO-Leu-N-Me-Val-3-(2’-Pyr)-D-Ala-D-Phe-3,3-Diphe-D-Ala-NH₂ was generated following the “Resin cleavage of linear peptide” procedure for SPPS. The linear pentapeptide was cleaved from the resin using a mixed solution of 6 mL of TFE and 6 mL of CH₂Cl₂. The resin containing solution was filtered and dried in vacuo to yield the DDLP as a white solid (192 mg, overall 50%).

Cyclo Leu-N-Me-Val-3-(2’-Pyr)-D-Ala-D-Phe-3,3-Diphe-D-Ala (12) was synthesized using 0.05 g of the DDLP generated (0.07 mmol, 1.0 equivalent), 0.02 g of TBTU (0.05 mmol, 0.80 equivalent), 0.02 g of HATU (0.05 mmol, 0.80 equivalent), 0.01 g of DMTMM (0.05 mmol, 0.80 equivalent), 0.09 mL of DIPEA (0.52 mmol, 8.0 equivalents) or HOBt (1.5 mmol, 3.0 equivalents), 0.20 g of DIC (3.0 mmol, 6.0 equivalents) and 2.5 mL of DMF to generate a concentration of 0.20 M. Each coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained to afford the Fmoc-protected resin-bound pentapeptide and the Fmoc protecting group was removed following the “General Fmoc removal” after the completion of each coupling reaction.

*Note: Due to the limited amount of compound, HSQC and HMBC spectra were used for the complete assignment of all the carbon peaks.

**Rf:** 0.39 (Hex:EtOAc = 0.25:0.75)

**LC/MS: m/z** called for C₄₄H₅₂N₆O₅S (M+1) = 745.40, found 745.35.

**HRMS (ESI-TOF):** M+1, found 745.4065 C₄₄H₅₂N₆O₅ requires 745.3999.

**[1]H NMR (600 MHz, CDCl₃):** δ 8.36 (br, D-Pyr), 8.01 (br, NH), 7.54 (br, NH), 7.24-7.08 (m, 17H, D-BiPhe, D-Phe, D-Val), 3.20 (dd, J = 4.6, 14.3 Hz, 1H, CH₂-D-Phe), 4.84 (dd, J = 6.9, 10.6 Hz, 1H, CH₂-D-Phe), 3.35 (m, 1H, CH₂-D-Pyr), 4.11 (q, J = 8.1 Hz, 1H, CH₂-D-Pyr), 3.80 (m, 1H, CH₂-D-Ala), 3.27 (dd, J = 8.3, 14.5 Hz, 1H, CH₂-Leu), 1.37 (m, 1H, CH₂₁ Lue), 0.85-0.66 (m, 13H, CH₃ Leu, CHCH(CH₃)₂, CH₂CH(CH₃)₂).


Following “General peptide coupling” procedure for SPPS and the published procedure for SPPS (Koay, Y. C.; McConnell, J. R.; Wang, Y.; Kim, S. J.; Buckton, L. K.; Mansour, F.; McAlpine, S. R. ACS Med. Chem. Lett. 2014, 5, 771-776), linear pentapeptide of resin-O-Leu-N-Me-Val-3-(3-pyridyl)-D-Ala-D-Phe-3,3-Diphe-D-Ala-NH₂ was synthesized by using (0.5 mmol, 1.0 equivalent) of resin-O-Leu-NH₂ and the subsequent peptide coupling in the sequence as mentioned below: 0.16 g (0.5 mmol, 1.0 equivalent) of HOAt (1.5 mmol, 3.0 equivalents) or HOBT (1.5 mmol, 3.0 equivalents), 0.20 g of DIC (3.0 mmol, 6.0 equivalents) and DMF to generate a concentration of 0.20 M based on the amino acid. Each coupling
reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained to afford the Fmoc-protected resin-bound pentapeptide and the Fmoc protecting group was removed following the “General Fmoc removal” after the completion of each coupling reaction.

The double deprotected linear pentapeptide (DDLP) HO-Leu-N-Me-Val-3-(3-pyridyl)-D-Ala-D-Phe-3,3-DiPhe-D-Ala-NH₂ was generated following the “Resin cleavage of linear peptide” procedure for SPPS. The linear pentapeptide was cleaved from the resin using a mixed solution of 6 mL of TFE and 6 mL of CH₂Cl₂. The resin containing solution was filtered and dried in vacuo to yield the DDLP as a white solid (Yield 68%).

LC/MS (ESI): m/z called for C₄₄H₅₂N₆O₅ (M+1) = 745.40, found 763.40.

**Cyclo Leu-N-Me-Val-3-(3-pyridyl)-D-Ala-D-Phe-3,3-DiPhe-D-Ala (13)** was synthesized using the DDLP (1.0 equivalent), TBTU (0.80 equivalent), HATU (0.80 equivalent), DMTMM (0.80 equivalent), DIPEA (8.0 equivalents) in anhydrous CH₂Cl₂ (0.001M) following “Macrocyclization procedure (syringe pump)” procedure.

The reaction was then stirred overnight and the reaction was monitored via LC/MS. Upon completion, the reaction mixture was subjected to acid-base wash, which was extracted twice with 10% (v/v) HCl (aq). The organic layer was then re-extracted with a saturated of NaHCO₃ aqueous solution. The combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo. The resulting crude product was purified via flash column chromatography on silica gel using an ethyl acetate-hexane gradient system, followed by purification via HPLC to yield compound 13 as white solid (Yield 60%).

Rₚ 0.25 (Hex:EtOAc = 0.25:0.75)

LC/MS: m/z called for C₄₄H₅₂N₆O₅ (M+1) = 745.40, found 745.00.

**General peptide coupling** procedure for SPPS and the published procedure for SPPS (Koay, Y. C.; McConnell, J. R.; Wang, Y.; Kim, S. J.; Buckton, L. K.; Mansour, F.; McAlpine, S. R. ACS Med. Chem. Lett. 2014, 5, 771-776), linear pentapeptide of resin-O-Leu-N-Me-Val-3-(4-pyridyl)-D-Ala-D-Phe-3,3-DiPhe-D-Ala-NH₂ was synthesized by using (0.5 mmol, 1.0 equivalent) of resin-O-Leu-NH₂ and the subsequent peptide coupling in the sequence using 1.5 mmol (3.0 equivalents) of each mentioned below: Fmoc-O-Leu-OH, Fmoc-3-(4-pyridyl)-D-Ala-OH, Fmoc-D-Phe-OH, and Fmoc-3,3-Diphenyl-D-Ala-OH. Each peptide coupling was done in the presence of HOAt (1.5 mmol, 3.0 equivalents) or HOBT (1.5 mmol, 3.0 equivalents), DIC (3.0 mmol, 6.0 equivalents) and DMF to generate a concentration of 0.20 M based on the amino acid. Each coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained to afford the Fmoc-protected resin-bound pentapeptide and the Fmoc protecting group was removed following the “General Fmoc removal” after the completion of each coupling reaction.

The double deprotected linear pentapeptide (DDLP) HO-Leu-N-Me-Val-3-(3-pyridyl)-D-Ala-D-Phe-3,3-DiPhe-D-Ala-NH₂ was generated following the “Resin cleavage of linear peptide” procedure for SPPS. The linear pentapeptide was cleaved from the resin using a mixed solution of 6 mL of TFE and 6 mL of CH₂Cl₂. The resin containing solution was filtered and dried in vacuo to yield the DDLP as a white solid (Yield 78%).

LC/MS (ESI): m/z called for C₄₄H₅₂N₆O₅ (M+1) = 763.40, found 763.00.

**Cyclo Leu-N-Me-Val-3-(4-pyridyl)-D-Ala-D-Phe-3,3-DiPhe-D-Ala (14)** was synthesized using the DDLP (1.0 equivalent), TBTU (0.80 equivalent), HATU (0.80 equivalent), DMTMM (0.80 equivalent), DIPEA (8.0 equivalents) in anhydrous CH₂Cl₂ (0.001M) following “Macrocyclization procedure (syringe pump)” procedure. The reaction was then stirred overnight and the reaction was monitored via LC/MS. Upon completion, the reaction

\[ \text{Following “General peptide coupling” procedure for SPPS and the published procedure for SPPS (Koay, Y. C.; McConnell, J. R.; Wang, Y.; Kim, S. J.; Buckton, L. K.; Mansour, F.; McAlpine, S. R. ACS Med. Chem. Lett. 2014, 5, 771-776), linear pentapeptide of resin-O-Leu-N-Me-Val-3-(4-pyridyl)-D-Ala-D-Phe-3,3-DiPhe-D-Ala-NH₂ was synthesized by using (0.5 mmol, 1.0 equivalent) of resin-O-Leu-NH₂ and the subsequent peptide coupling in the sequence using 1.5 mmol (3.0 equivalents) of each mentioned below: Fmoc-O-Leu-OH, Fmoc-3-(4-pyridyl)-D-Ala-OH, Fmoc-D-Phe-OH, and Fmoc-3,3-Diphenyl-D-Ala-OH. Each peptide coupling was done in the presence of HOAt (1.5 mmol, 3.0 equivalents) or HOBT (1.5 mmol, 3.0 equivalents), DIC (3.0 mmol, 6.0 equivalents) and DMF to generate a concentration of 0.20 M based on the amino acid. Each coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained to afford the Fmoc-protected resin-bound pentapeptide and the Fmoc protecting group was removed following the “General Fmoc removal” after the completion of each coupling reaction.} \]
mixture was subjected to acid-base wash, which was extracted twice with 10% (v/v) HCl_{aq}. The organic layer was then re-extracted with a saturated NaHCO₃ aqueous solution. The combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo. The resulting crude product was purified via flash column chromatography on silica gel using an ethyl acetate-hexane gradient system, followed by purification via HPLC to yield compound 14 as white solid (Yield 38%).

Following the “General peptide coupling” procedure for SPPS and the published procedure for SPPS (Koay, Y. C.; McConnell, J. R.; Wang, Y.; Kim, S. J.; Buckton, L. K.; Mansour, F.; McAlpine, S. R. ACS Med. Chem. Lett. 2014, 5, 771-776), linear pentapeptide of resin-O-Leu-N-Me-Val-D-Tyr(OMe)-D-Phe-3,3-Diphe-D-Ala-NH₂ was synthesized by using 1.00 g (0.5 mmol, 1.0 equivalent) of resin-O-Leu-NH₂ and the subsequent peptide coupling in the sequence as mentioned below: 0.53 g (1.5 mmol, 3.0 equivalents) of Fmoc-N-Me-Val-OH, 0.63 g (1.5 mmol, 3.0 equivalents) of Fmoc-D-Tyrosine-OMe, 0.58 g (1.5 mmol, 3.0 equivalents) of Fmoc-D-Phe-OH and 0.70 g (1.5 mmol, 3.0 equivalents) of Fmoc-3,3-Diphenyl-D-Ala-OH. Each peptide coupling was done in the presence of 0.20 g of HOAt (1.5 mmol, 3.0 equivalents) or 0.20 g of HOBt (1.5 mmol, 3.0 equivalents), 0.47 mL of DIC (3.0 mmol, 6.0 equivalents) and 2.5 mL of DMF to generate a concentration of 0.20 M. Each coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained to afford the Fmoc-protected resin-bound pentapeptide and the Fmoc protecting group was removed following the “General Fmoc removal” after the completion of each coupling reaction.

The double deprotected linear pentapeptide (DDLП) HO-Leu-N-Me-Val-D-Tyr(OMe)-D-Phe-3,3-Diphe-D-Ala-NH₂ was generated following the “Resin cleavage of linear peptide” procedure for SPPS. The linear pentapeptide was cleaved from the resin using a mixed solution of 6 mL of TFE and 6 mL of CH₂Cl₂. The resin containing solution was filtered and dried in vacuo to yield the DDDП as a white solid (352 mg, overall 89%).

LC/MS (ESI): m/z called for C₄₆H₅₅N₅O₆ (M+1) = 792.43, found 792.50.

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Cyclo Leu-N-Me-Val-D-Tyr(OMe)-D-Phe-3,3-Diphe-D-Ala (16) was synthesized using 0.35 g of the DDDLП generated (0.44 mmol, 1.0 equivalent), 0.11 g of TBTU (0.35 mmol, 0.80 equivalent), 0.13 g of HATU (0.35 mmol, 0.80 equivalent), 0.10 g of DMTMM (0.35 mmol, 0.80 equivalent), 0.62 mL of DIPEA (3.5 mmol, 8.0 equivalents) in anhydrous CH₂Cl₂ (442 mL, 0.001M) following “Macrocyclization procedure (syringe pump)” procedure. The reaction was then stirred overnight and the reaction was monitored via LC/MS. Upon completion, the reaction mixture was subjected to acid-base wash, which was extracted twice with 10% (v/v) HCl_{aq}. The organic layer was dried over sodium sulfate, filtered, and concentrated in vacuo. The resulting crude product was purified via flash column chromatography on silica gel using an ethyl acetate-hexane gradient system, followed by purification via HPLC to yield compound 16 as white solid (120 mg, 27%).

R_f: 0.51 (EtOAc:Hex = 0.65:0.35)

LC/MS: m/z called for C₄₆H₅₇N₅O₇ (M+1) = 792.48, found 792.43.
Following “General peptide coupling” procedure for SPPS and the published procedure for SPPS (Koay, Y. C.; McConnell, J. R.; Wang, Y.; Kim, S. J.; Buckton, L. K.; Mansour, F.; McAlpine, S. R. ACS Med. Chem. Lett. 2014, 5, 771-776), linear pentapeptide of resin-O-Leu-N-Me-Val-N-Boc-D-Trp-D-Phe-3,3-Diphe-D-Ala-NH₂ was synthesized by using 1.00 g (0.5 mmol, 1.0 equivalent) of resin-O-Leu-NH₂ and the subsequent peptide coupling in the sequence as mentioned below: 0.53 g (1.5 mmol, 3.0 equivalents) of Fmoc-N-Me-Val-OH, 0.79 g (1.5 mmol, 3.0 equivalents) of Fmoc-N-Boc-D-Tryptophan-OH, 0.58 g (1.5 mmol, 3.0 equivalents) of Fmoc-D-Phe-OH and 0.70 g (1.5 mmol, 3.0 equivalents) of Fmoc-3,3-Diphenyl-D-Ala-OH. Each peptide coupling was done in the presence of 0.20 g of HOAt (1.5 mmol, 3.0 equivalents) or 0.20 g of HOBt (1.5 mmol, 3.0 equivalents), 0.47 mL of DIC (3.0 mmol, 6.0 equivalents) and 2.5 mL of DMF to generate a concentration of 0.20 M. Each coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained to afford the Fmoc-protected resin-bound pentapeptide and the Fmoc protecting group was removed following the “General Fmoc removal” after the completion of each coupling reaction.

The double deprotected linear pentapeptide (DDLP) HO-Leu-N-Me-Val-N-Boc-D-Trp-D-Phe-3,3-Diphe-D-Ala-NH₂ was generated following the “Resin cleavage of linear peptide” procedure for SPPS. The linear pentapeptide was cleaved from the resin using a mixed solution of 6 mL of TFE and 6 mL of CH₂Cl₂. The resin containing solution was filtered and dried in vacuo to yield the DDLP as a white solid (315 mg, overall 70%).

**Cyclo Leu-N-Me-Val-N-Boc-D-Trp-D-Phe-3,3-Diphe-D-Ala** was synthesized using 0.32 g of the DDLP generated (0.35 mmol, 1.0 equivalent), 0.09 g of TBTU (0.28 mmol, 0.80 equivalent), 0.11 g of HATU (0.28 mmol, 0.80 equivalent), 0.08 g of DMTMM (0.28 mmol, 0.80 equivalent), 0.49 mL of DIPEA (2.8 mmol, 8.0 equivalents) in anhydrous CH₂Cl₂ (350 mL, 0.001M) following “Macrocyclization procedure (syringe pump)” procedure. The reaction was then stirred overnight and the reaction was monitored via LC/MS. Upon completion, the reaction mixture was subjected to acid-base wash, which was extracted twice with 10% (v/v) HCl (aq). The organic layer was then re-extracted with a saturated of NaHCO₃ aqueous solution. The combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo. The resulting crude product was purified via flash column chromatography on silica gel using an ethyl acetate-hexane gradient system, followed by purification via HPLC. The Boc protecting group was removed following “Boc removal” procedure, utilizing a mixture of TFA/CH₂Cl₂ (1:4, 0.1 M) and anisole (2.0 equivalents) to yield compound 17 as white solid (45 mg, 15%).

Rf: 0.38 (EtOAc:Hex = 0.65:0.35)
LC/MS: m/z called for C₄₇H₅₄N₆O₅ (M+1) = 783.42, found 782.95.
HRMS (ESI-TOF): M+Na+, found 805.4037 C₄₇H₅₄N₆O₅Na₁ requires 805.4156.

**1H NMR (600 MHz, DMSO):** δ 10.77 (s, NH, D-Trp), 8.10 (t, J = 7.5 Hz, NH), 7.79 (d, J = 7.4 Hz, NH), 7.57 (d, J = 7.9 Hz, NH), 7.51 (d, J = 9.6 Hz, NH), 7.30-6.97 (m, 19H, D-BiPhe, D-Phe, D-Trp), 6.86 (m, 1H, D-Trp), 5.18 (dd, J = 9.7, 11.8 Hz, 1H, CHα D-BiPhe), 4.84 (m, 1H, CHα D-Phe), 4.52 (d, J = 11.3 Hz, 1H, CHα Val), 4.30 (d, J = 11.9 Hz, 1H, CHβ D-BiPhe), 4.05 (m, 1H, CHα D-Trp), 3.47 (q, J = 7.4 Hz, 1H, CHβ Leu), 3.24 (m, 1H, CHβ₂ D-Phe), 2.87 (m, 1H, CHβ₂ D-Phe), 2.70 (m, 1H, CHβ₂ D-Trp), 2.62 (m, 1H, CHβ₁ D-Trp), 2.55 (s, 3H, NCH₃), 2.05 (m, 1H, CHβ Val), 1.46 (m, 1H, CHβ₂ Leu), 1.39 (m, 1H, CHβ₁ Leu), 1.22 (m, 1H, CHγ Leu), 0.79-0.52 (m, 12H, CHCH(CH₃)₂, CH₂CH(CH₃)₂).

**13C NMR (150 MHz, DMSO):** δ 170.84, 170.63, 170.00, 169.89, 169.08, 141.70, 141.07, 137.52, 136.40, 129.32, 128.87, 128.81, 128.75, 128.56, 128.44, 128.15, 127.15, 126.95, 126.82, 123.74, 121.30, 118.88, 118.67, 111.70, 110.76, 63.36, 57.23, 53.73, 52.00, 50.44, 49.06, 40.51, 38.12, 37.50, 30.20, 24.51, 22.87, 22.54, 22.07, 19.67, 18.91.
Following “General peptide coupling” procedure for SPPS and the published procedure for SPPS (Koay, Y. C.; McConnell, J. R.; Wang, Y.; Kim, S. J.; Buckton, L. K.; Mansour, F.; McAlpine, S. R. *ACS Med. Chem. Lett.* 2014, 5, 771-776), linear pentapeptide of resin-O-Leu-N-Me-Val-D-Leu-D-His(Boc)-3,3-Diphenyl-D-Ala-NH₂ was synthesized by using 1.00 g (0.5 mmol, 1.0 equivalent) of resin-O-Leu-NH₂ and the subsequent peptide coupling in the sequence as mentioned below: 0.53 g (1.5 mmol, 3.0 equivalents) of Fmoc-N-Me-Val-OH, 0.72 g (1.5 mmol, 3.0 equivalents) of Fmoc-D-His(Boc)-OH, 0.58 g (1.5 mmol, 3.0 equivalents) of Fmoc-D-Phe-OH and 0.70 g (1.5 mmol, 3.0 equivalents) of Fmoc-3,3-Diphenyl-D-Ala-OH. Each peptide coupling was done in the presence of 0.20 g of HOBt (1.5 mmol, 3.0 equivalents) or 0.20 g of HOAt (1.5 mmol, 3.0 equivalents), 0.47 mL of DIC (3.0 mmol, 6.0 equivalents) and 2.5 mL of DMF to generate a concentration of 0.20 M. Each coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained to afford the Fmoc-protected resin-bound pentapeptide and the Fmoc protecting group was removed following the “General Fmoc removal” after the completion of each coupling reaction.

The double deprotected linear pentapeptide (DDLP) HO-Leu-N-Me-Val-D-His(Boc)-D-Phe-3,3-Diphe-D-Ala-NH₂ was generated following the “Resin cleavage of linear peptide” procedure for SPPS. The linear pentapeptide was cleaved from the resin using a mixed solution of 6 mL of TFE and 6 mL of CH₂Cl₂. The resin containing solution was filtered and dried in vacuo to yield the DDLP as a white solid (264 mg, overall 62%).

**Cyclo Leu-N-Me-Val-D-His(Boc)-D-Phe-3,3-Diphe-D-Ala** (**18**) was synthesized using 0.26 g of the DDLP generated (0.31 mmol, 1.0 equivalent), 0.08 g of TBTU (0.25 mmol, 0.80 equivalent), 0.09 g of HATU (0.25 mmol, 0.80 equivalent), 0.43 mL of DIPEA (2.5 mmol, 8.0 equivalents) and 2.5 mL of DMF to generate a concentration of 0.20 M. Each coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained to afford the Fmoc-protected resin-bound pentapeptide and the Fmoc protecting group was removed following the “General Fmoc removal” after the completion of each coupling reaction.

The Boc protecting group of compound **18** was removed following “Boc removal” procedure, utilizing a mixture of TFA/CH₂Cl₂ (1:4, 0.1 M) and anisole (2.0 equivalents) in anhydrous CH₂Cl₂ (310 mL, 0.001M) following the “Resin cleavage of linear peptide” procedure for SPPS. The organic layer was then re-extracted with a saturated NaHCO₃ aqueous solution. The combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo to yield the DDLP as a white solid (264 mg, overall 62%).

**LC/MS (ESI):** m/z called for C₄₇H₅₉N₇O₇ (M+Na⁺) = 856.45, found 856.44.

**HRMS (ESI-TOF):** M⁺, found 834.4559 C₄₇H₅₉N₇O₇ requires 833.4476.

**1H NMR (600 MHz, DMSO):** δ 8.12 (s, D -His), 8.07 (d, J = 7.5 Hz, NH), 7.54 (d, J = 9.4 Hz, NH), 7.27-7.16 (m, 14H, D -BiPhe, D -Phe, D -His), 6.84 (m, 2H, D -Phe), 5.17 (dd, J = 9.6, 11.6 Hz, 1H, CHα D-Biphe), 4.82 (q, J = 7.6 Hz, 1H, CHα D-Phe), 4.55 (d, J = 11.5 Hz, 1H, CHα Val), 4.31 (d, J = 11.8 Hz, 1H, CHβ D-Phe) 4.03 (m, J = 7.5 Hz, 1H CHα D-His), 3.53 (q, J = 7.4 Hz, 1H, CHα Leu), 3.02 (m, 1H, CHβ(D-Phe), 2.77 (m, 1H, CHβ D-Phe), 2.69 (m, 1H, CHβ D-His), 2.64 (s, 3H, NCH₃), 2.55 (m, 1H, CHβ D-His), 2.15 (m, 1H, CHβ Val), 1.53 (s, 9H, t-Bu D-His), 1.47 (m, 1H, CHβ₂ Leu), 1.39 (m, 1H, CHβ₁ Leu), 1.23 (m, 1H, CHγ Leu), 0.82-0.63 (m, 12H, CH₂CH(CH₃)₂, CHCH(CH₃)₂).

**13C NMR (150 MHz, DMSO):** δ 170.38, 170.28, 170.00, 169.95, 168.89, 147.17, 141.71, 141.01, 140.28, 137.51, 136.74, 129.18, 129.02, 128.84, 128.59, 128.59, 127.14, 126.84, 126.81, 114.31, 85.46, 63.41, 57.20, 56.84, 53.69, 51.88, 49.26, 40.52, 38.11, 37.67, 30.29, 27.82, 25.25, 24.53, 22.81, 22.57, 19.69, 18.79.

The Boc protecting group of compound **18** was removed following “Boc removal” procedure, utilizing a mixture of TFA/CH₂Cl₂ (1:4, 0.1 M) and anisole (2.0 equivalents) to generate compound **19**. The free amine was taken to the subsequent biotinylation reaction without purification.

**Rf:** 0.43 (EtOAc:MeOH = 9:1)

**LC/MS (ESI):** m/z called for C₄₂H₄₉N₇O₅ (M+Na⁺) = 734.40, found 734.40.

**HRMS (ESI-TOF):** M⁺, found 734.4023 C₄₂H₄₉N₇O₅ requires 733.3952.

**1H NMR (600 MHz, DMSO):** δ 8.83 (s, D -His), 8.25 (d, J = 8.5 Hz, NH), 8.08 (d, J = 8.3 Hz, NH), 7.90 (d, J = 7.7 Hz, NH), 7.63 (d, J = 9.5 Hz, NH), 7.26-7.16 (m, 14H, D-Biphe, D-Phe, D-His), 6.85 (m, 2H, D-Phe), 5.17 (t, J = 10.7 Hz, 1H, CHα D-Biphe), 4.81 (q, J = 7.5 Hz, 1H, CHα D-Phe), 4.58 (d, J = 11.6 Hz, 1H, CHα Val), 4.33 (d, J =
Following the “General peptide coupling" procedure for SPPS and the published procedure for SPPS (Koay, Y. C.; McConnell, J. R.; Wang, Y.; Kim, S. J.; Buckton, L. K.; Mansour, F.; McAlpine, S. R. ACS Med. Chem. Lett. 2014, 5, 771-776), linear pentapeptide of resin-O-Leu-N-Me-Val-D-Glu(OtBu)-D-Phe-3,3-Diphe-D-Ala-NH2 was synthesized by using 1.00 g (0.5 mmol, 1.0 equivalent) of resin-O-Leu-NH2 and the subsequent peptide coupling in the sequence as mentioned below: 0.53 g (1.5 mmol, 3.0 equivalents) of Fmoc-N-Me-Val-OH, 0.64 g (1.5 mmol, 3.0 equivalents) of Fmoc-D-Glu-γ-OtBu, 0.58 g (1.5 mmol, 3.0 equivalents) of Fmoc-D-Phe-OH and 0.70 g (1.5 mmol, 3.0 equivalents) of Fmoc-3,3-Diphenyl-D-Ala-OH. Each peptide coupling was done in the presence of 0.20 g of HOAt (1.5 mmol, 3.0 equivalents) or 0.20 g of HOAT (1.5 mmol, 3.0 equivalents), 0.47 mL of DIC (3.0 mmol, 6.0 equivalents) and 2.5 mL of DMF to generate a concentration of 0.20 M. Each coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained to afford the Fmoc-protected resin-bound pentapeptide and the Fmoc protecting group was removed following the “General Fmoc removal" after the completion of each coupling reaction.

The double deprotected linear pentapeptide (DDLP) HO-Leu-N-Me-Val-D-Glu(OtBu)-D-Phe-3,3-Diphe-D-Ala-NH2 was generated following the “Resin cleavage of linear peptide" procedure for SPPS. The linear pentapeptide was cleaved from the resin using a mixed solution of 6 mL of TFE and 6 mL of CH2Cl2. The resin containing solution was filtered and dried in vacuo to yield the DDLP as a white solid (260 mg, overall 65%).

LC/MS (ESI): m/z called for C45H59N5O7 (M+1) = 800.45, found 800.35.

Cyclo Leu-N-Me-Val-D-Glu(OtBu)-D-Phe-3,3-Diphe-D-Ala (20) was synthesized using 0.26 g of the DDLP generated (0.33 mmol, 1.0 equivalent), 0.08 g of TBTU (0.26 mmol, 0.80 equivalent), 0.10 g of HATU (0.26 mmol, 0.80 equivalent), 0.07 g of DMTMM (0.26 mmol, 0.80 equivalent), 0.45 mL of DPEA (2.6 mmol, 8.0 equivalents) in anhydrous CH2Cl2 (325 mL, 0.001M) following “Macrocyclization procedure (syringe pump)" procedure. The reaction was then stirred overnight and the reaction was monitored via LC/MS. Upon completion, the reaction mixture was subjected to acid-base wash, which was extracted twice with 10% (v/v) HCl (aq). The combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo. The resulting crude product was purified via flash column chromatography on silica gel using an ethyl acetate-hexane gradient system, followed by purification via HPLC to yield compound 20 as white solid (60 mg, 24%).

Rf: 0.63 (Hex:EtOAc = 0.25:0.75)
LC/MS: m/z called for C45H59N5O7 (M+1) = 800.44, found 800.15.

HRMS (ESI-TOF): M+Na+, found 804.4309 C46H59N5O7Na1 requires 804.4414.

1H NMR (600 MHz, DMSO): δ 8.01 (br, 2N H), 7.88 (d, J = 7.4 Hz, NH), 7.55 (d, J = 9.3 Hz, NH), 7.31-7.15 (m, 13H, D-Biphe, D-Phe), 6.92 (m, 2H, D-Phe), 5.19 (t, J = 10.7 Hz, 1H, CHα D-Biphe), 4.59 (d, J = 11.5 Hz, 1H, CHα Val), 4.44 (q, J = 7.3 Hz, 1H, CHβ D-Glu), 4.31 (d, J = 11.9 Hz, 1H, CHβ D-Biphe), 4.16 (m, 1H, CHγ D-Phe), 3.55 (q, J = 7.4 Hz, 1H, CHβ Leu), 2.75 (m, 2H, CHβ D-Phe), 2.59 (s, 3H, NCH3), 2.20 (m, 1H, CHβ Val), 2.03 (t, J = 7.6 Hz, 2H, CHγ D-Glu), 1.90 (m, 1H, CHβ D-Glu), 1.70 (m, 1H, CHβ D-Glu), 1.41 (m, 11H, CHβ Leu, OC(CH3)3), 1.22 (m, 1H, CHγ Leu), 0.83-0.62 (m, 12H, CH2D(CH3)2, CHCH(CH3)2).

13C NMR (150 MHz, DMSO): δ 172.34, 172.21, 170.25, 170.13, 169.77, 168.77, 141.72, 141.09, 137.20, 129.35, 128.80, 128.70, 128.60, 128.45, 128.10, 127.17, 127.03, 126.83, 79.95, 63.39, 57.27, 56.29, 53.75, 51.68, 49.20, 38.11, 37.85, 31.32, 30.16, 28.24, 27.28, 25.18, 24.49, 22.82, 22.55, 19.66, 18.07.
The tBu protecting group of compound 20 was removed following “Tert-butyl group (tBu) Removal” procedure, utilizing a mixture of TFA/CH₂Cl₂ (2:4, 0.1 M) and anisole (2.0 equivalents) to generate compound 21. The free acid was taken to the subsequent methyl ester formation without purification.

Rf: 0.30 (100% EtOAc)

LC/MS: m/z called for C₄₁H₅₁N₅O₇ (M+1) = 726.38, found 726.10.

HRMS (ESI-TOF): M+Na+, found 748.3679 C₄₁H₅₁N₅O₇Na requires 748.3788.

1H NMR (600 MHz, DMSO): δ 8.08 (br, 2N H), 7.93 (br, N H), 7.62 (br, N H), 7.30 - 7.13 (m, 13H, D-Biphe, D-Phe), 6.91 (m, 2H, D-Phe), 5.17 (t, J = 10.8 Hz, 1H, CHα D-Biphe), 4.59 (d, J = 11.4 Hz, 1H, CHα Val), 4.49 (m, 1H, CHα D-Glu), 4.31 (d, J = 11.9 Hz, 1H, CHβ D-Biphe), 4.16 (q, J = 7.5 Hz, 1H, CHβ D-Phe), 3.48 (m, 1H, CHα Leu), 2.76 (m, 1H, CHβ₁ D-Phe), 2.65 (m, 1H, CHβ₂ D-Phe), 2.59 (s, 3H, NCH₃), 2.15 (m, 1H, CHβ Val), 2.09 (m, 2H, CHγ D-Glu), 1.95 (m, 1H, CHβ D-Glu), 1.76 (m, 1H, CHβ₂ D-Glu), 1.47 (m, 1H, CHβ₁ Leu), 1.39 (m, 1H, CHβ₂ Leu), 1.23 (m, 1H, CHγ Leu), 0.83 - 0.63 (m, 12H, CH₂CH(CH₃)₂, CHCH(CH₃)₂).

13C NMR (150 MHz, DMSO): δ 174.96, 171.78, 171.07, 170.54, 169.93, 169.78, 168.79, 141.76, 141.07, 137.37, 129.28, 128.84, 128.80, 128.57, 128.39, 128.09, 127.13, 126.97, 126.78, 63.20, 57.35, 56.39, 53.77, 51.81, 49.34, 38.00, 37.94, 30.16, 29.77, 27.42, 25.22, 24.50, 22.87, 22.53, 19.70, 18.82.

The free acid of compound 21 was converted to the methyl ester compound 22 using the methylating agent Trimethylsilyl diazomethane (TMSD) in a mixture of anhydrous benzene and methanol (3:1) in a round bottom flask to make a 0.1 M solution. The methylating agent TMSD (2.0 M in diethyl ether) was added drop-wise into the reaction mixture became slightly yellow. The reaction was stirred under nitrogen and monitored by thin layer chromatography (TLC). Upon completion, the solvent was evaporated in vacuo and resulting compound 22.

Rf: 0.33 (Hex:EtOAc = 0.25:0.75)

LC/MS: m/z called for C₄₂H₅₃N₅O₇ (M+1) = 740.39, found 740.00.

HRMS (ESI-TOF): M+Na+, found 762.3831 C₄₂H₅₃N₅O₇Na requires 762.3945.

1H NMR (600 MHz, DMSO): δ 8.33 (br, N H), 8.22 (br, N H), 7.93 (br, 2N H), 7.29 - 7.14 (m, 13H, D-Biphe, D-Phe), 6.93 (m, 2H, D-Phe), 5.15 (t, J = 10.7 Hz, 1H, CHα D-Biphe), 4.57 (d, J = 11.4 Hz, 1H, CHα Val), 4.45 (q, J = 7.3 Hz, 1H, CHα D-Glu), 4.33 (d, J = 11.9 Hz, 1H, CHβ D-Biphe), 4.18 (q, J = 7.4 Hz, 1H, CHα D-Phe), 3.59 (s, 3H, OCH₃), 3.44 (m, 1H, CHα Leu), 2.77 (m, 1H, CHβ₂ D-Phe), 2.66 (m, 1H, CHβ₁ D-Phe), 2.55 (s, 3H, NCH₃), 2.14 (m, 3H, CHβ Val, CHγ D-Glu), 1.92 (m, 1H, CHβ₂ D-Glu), 1.73 (m, 1H, CHβ₁ D-Glu), 1.45 (m, 1H, CHβ₂ Leu), 1.40 (m, 1H, CHβ₁ Leu), 1.22 (m, 1H, CHγ Leu), 0.82-0.61 (m, 12H, CH₂CH(CH₃)₂, CHCH(CH₃)₂).

13C NMR (150 MHz, DMSO): δ 173.54, 170.62, 170.22, 169.89, 169.73, 168.63, 141.80, 141.09, 137.39, 129.29, 128.81, 128.77, 128.57, 128.44, 128.33, 127.07, 126.93, 126.73, 63.14, 57.54, 56.26, 53.79, 51.84, 51.72, 49.20, 38.01, 37.91, 30.23, 30.12, 27.11, 25.18, 24.49, 22.86, 22.54, 19.72, 18.71.

Dipeptide resin-O-Leu-3-(4-Thia)Ala-Fmoc was synthesized following “General peptide coupling” procedure, by using 1.00 g (0.5 mmol, 1.0 equivalent) of resin-O-Leu-NH₂, 0.59 g (1.5 mmol, 3.0 equivalents) of Fmoc-3-(4-Thiazoyl)Ala-OH, 0.20 g of HOBt (1.5 mmol, 3.0 equivalents), 0.47 mL of DIC (3.0 mmol, 6.0 equivalents) and 2.5 mL of DMF to generate a concentration of 0.20 M and the Fmoc group was removed following the “General Fmoc removal” procedure for SPPS.

o-NBS protected resin-bound dipeptide was synthesized following the “o-NBS Protection” procedure, by using 0.44 g of o-NBS-Cl (2.0 mmol, 4 equivalents) and 0.65 mL of collidine (5.0 mmol, 10 equivalents) and 17 mL of CH₂Cl₂ to generate a concentration of 0.03 M.

LC/MS (ESI): m/z called for C₁₈H₂₃N₄O₇S₂ (M+1) = 471.09, found 471.55.
A solution of 0.66 g of triphenylphosphine (2.5 mmol, 5 equivalents) and 0.20 mL of MeOH (10 equivalents) in dry THF was added to the resin bound o-NBS-protected peptides and stirred for 10 mins. A solution of 0.49 mL DIAD (5 equivalents) in dry THF was then added portion by portion to the reaction mixture and stirred for 4 hours at room temperature. The resin was filtered off, and washed with DMF (5x). N-Methyl-N-o-NBS-peptides were then cleaved from resin by treatment of a small amount of resin with TFE:CH₂Cl₂ (1:1) and analyzed by LCMS to monitor the reaction completion to generate resin-O-Leu-3-(4-Thia)Ala-NCH₃-o-NBS.

LC/MS (ESI): m/z called for C₁₀H₂₅N₄O₇S₂ (M+1) = 485.11, found 485.60.

For o-NBS deprotection, the resin-bound N-Methyl-N-o-NBS-peptides was treated with a solution of 0.35 mL mercaptoethanol (10 equivalents) and 0.37 mL DBU (5 equivalents) in DMF for 2 hours. The deprotection procedure was repeated one more time and the resin was washed with DMF (5x) to generate resin-O-Leu-3-(4-Thia)Ala-NH-CH₃.

LC/MS (ESI): m/z called for C₁₃H₂₁N₃O₃S (M+1) = 300.13, found 300.20.

Following “General peptide coupling” and published procedure for SPPS (Koay, Y. C.; McConnell, J. R.; Wang, Y.; Kim, S. J.; Buckton, L. K.; Mansour, F.; McAlpine, S. R. ACS Med. Chem. Lett. 2014, 5, 771-776), linear pentapeptide of resin-O-Leu-N-Me-3-(4-Thia)Ala-D-Leu-D-Phe-3,3-Diphe-D-Ala-NH₂ was synthesized by using resin-O-Leu-3-(4-Thia)Ala-NH-CH₃ and the subsequent peptide coupling in the sequence as mentioned below: 0.53 g (1.5 mmol, 3.0 equivalents) of Fmoc-D-Leu-OH, 0.58 g (1.5 mmol, 3.0 equivalents) of Fmoc-D-Leu-ONH₂, 0.50 g (1.5 mmol, 3.0 equivalents) of Fmoc-D-Phe-ONH₂, 0.50 g (1.5 mmol, 3.0 equivalents) of Fmoc-3,3-Diphe-D-Ala-ONH₂ and the subsequent peptide coupling was done in the presence of 0.20 g of HOAt (1.5 mmol, 3.0 equivalents) or 0.20 g of HOBt (1.5 mmol, 3.0 equivalents), 0.47 mL of DIC (3.0 mmol, 6.0 equivalents) and 2.5 mL of DMF to generate a concentration of 0.20 M. Each coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained to afford the Fmoc-protected resin-bound pentapeptide and the Fmoc protecting group was removed following the “General Fmoc removal” after the completion of each coupling reaction.

The double deprotected linear pentapeptide (DDLP) HO-Leu-N-Me-3-(4-Thia)Ala-D-Leu-D-Phe-3,3-Diphe-D-Ala-NH₂ was generated following the “Resin cleavage of linear peptide” procedure for SPPS. The linear pentapeptide was cleaved from the resin using a mixed solution of 6 mL of TFE and 6 mL of CH₂Cl₂. The resin containing solution was filtered and dried in vacuo to yield the DDLP as a white solid (301 mg, overall 77%).

LC/MS (ESI): m/z called for C₄₃H₅₄N₆O₆S (M+1) = 783.39, found 783.45.

Cyclo Leu-N-Me-3-(4-Thia)Ala-D-Leu-D-Phe-3,3-Diphe-D-Ala (23) was synthesized using 0.30 g of the DDLP generated (0.38 mmol, 1.0 equivalent), 0.10 g of TBTU (0.31 mmol, 0.8 equivalent), 0.12 g of HATU (0.31 mmol, 0.8 equivalent), 0.09 g of DMTMM (0.31 mmol, 0.80 equivalent), 0.55 mL of DIPEA (3.13 mmol, 8.0 equivalents) in anhydrous CH₂Cl₂ (391 mL, 0.001M) following “Macrocyclization procedure (syringe pump)” procedure. The reaction was then stirred overnight and the reaction was monitored via LC/MS. Upon completion, the reaction mixture was subjected to acid-base wash, which was extracted twice with 10% (v/v) HClₐ (aq). The organic layer was then re-extracted with a saturated of NaHCO₃ aqueous solution. The combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo. The resulting crude product was purified via flash column chromatography on silica gel using an ethyl acetate-hexane gradient system, followed by purification via HPLC to yield compound 23 as white solid (95 mg, 32%).

Rₜ: 0.28 (100% EtOAc)
LC/MS: m/z called for C₄₃H₅₂N₆O₅S (M+1) = 765.37, found 765.00.
HRMS (ESI-TOF): M+Na⁺, found 787.3616 C₄₃H₅₂N₆O₅SNa₁ requires 787.3618.
Following “General Fmoc removal” procedure for SPPS and the published procedure for SPPS (Koay, Y. C.; McConnell, J. R.; Wang, Y.; Kim, S. J.; Buckton, L. K.; Mansour, F.; McAlpine, S. R. *ACS Med. Chem. Lett.* 2014, *5*, 771-776), linear pentapeptide of Resin-O-D-Phe-3,3-DiPhe-D-Ala-3-(2-pyridyl)-Ala-N-Me-Val-D-Leu-NH₂ was synthesized by using (1.0 equivalent) of resin-O-D-Phe-NH₂ and the subsequent peptide coupling in the sequence as mentioned below: Fmoc-3,3-Diphenyl-D-Ala-OH (1.5 mmol, 3.0 equivalents), Fmoc-3-(2-pyridyl)-Ala-OH, Fmoc-N-Me-Val-OH (1.5 mmol, 3.0 equivalents), and Fmoc-D-Leu-OH (1.5 mmol, 3.0 equivalents). Each peptide coupling was done in the presence of HOAt (1.5 mmol, 3.0 equivalents) or HOBT (1.5 mmol, 3.0 equivalents), DIC (3.0 mmol, 6.0 equivalents) and DMF to generate a concentration of 0.20 M based on the amino acid. Each coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained in vacuo to yield the DDLP as a white solid (Yield 93%).

The double deprotected linear pentapeptide (DDLP) HO-D-Phe-3,3-DiPhe-D-Ala-3-(2-pyridyl)-Ala-N-Me-Val-D-Leu-NH₂ was generated following the “Resin cleavage of linear peptide” procedure for SPPS. The linear pentapeptide was cleaved from the resin using a mixed solution of 6 mL of TFE and 6 mL of CH₂Cl₂. The resin containing solution was filtered and dried in vacuo to yield the DDLP as a white solid (Yield 63%).

**Cyclo D-Phe-3,3-DiPhe-D-Ala-3-(2-pyridyl)-Ala-N-Me-Val-D-Leu** (24) was synthesized using the DDLP (1.0 equivalent), TBTU (0.80 equivalent), HATU (0.80 equivalent), DMTMM (0.80 equivalent), DIPEA (8.0 equivalents) in anhydrous CH₂Cl₂ (0.001 M) following “Macrocyclization procedure (syringe pump)” procedure. The reaction was then stirred overnight and the reaction was monitored via LC/MS. Upon completion, the reaction mixture was subjected to acid-base wash, which was extracted twice with 10% (v/v) HCl (aq). The organic layer was re-extracted with a saturated of NaHCO₃ aqueous solution. The combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo. The resulting crude product was purified via flash column chromatography on silica gel using an ethyl acetate-hexane gradient system, followed by purification via HPLC to yield compound 24 as white solid (Yield 63%).

Rc: 0.43 (Hex:EtOAc = 0.25:0.75)

LC/MS (ESI): m/z called for C₄₄H₅₄N₆O₆ (M+1) = 763.00, found 763.00 and (½ M+1) 382.00
Following “General peptide coupling” procedure for SPPS and the published procedure for SPPS (Koay, Y. C.; McConnell, J. R.; Wang, Y.; Kim, S. J.; Buckton, L. K.; Mansour, F.; McAlpine, S. R. ACS Med. Chem. Lett. 2014, 5, 771-776), linear pentapeptide of Resin-O-D-Phe-3,3-Diphe-D-Ala-3-(3-pyridyl)-Ala-N-Me-Val-D-Leu-NH₂ was synthesized by using (1.0 equivalent) of resin-O-D-Phe-NH₂ and the subsequent peptide coupling in the sequence as mentioned below: Fmoc-3,3-Diphenyl-D-Ala-OH (1.5 mmol, 3.0 equivalents), Fmoc-3-(3-pyridyl)-Ala OH, Fmoc-N-Me-Val-OH (1.5 mmol, 3.0 equivalents), and Fmoc-D-Leu-OH (1.5 mmol, 3.0 equivalents). Each coupling peptide was done in the presence of HOAt (1.5 mmol, 3.0 equivalents) or HOBT (1.5 mmol, 3.0 equivalents), DIC (3.0 mmol, 6.0 equivalents) and DMF to generate a concentration of 0.20 M based on the amino acid. Each coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained to afford the Fmoc-protected resin-bound pentapeptide and the Fmoc protecting group was removed following the “General Fmoc removal” after the completion of each coupling reaction.

The double deprotected linear pentapeptide (DDLP) HO-D-Phe-3,3-Diphe-D-Ala-3-(3-pyridyl)-Ala-N-Me-Val-D-Leu-NH₂ was generated following the “Resin cleavage of linear peptide” procedure for SPPS. The linear pentapeptide was cleaved from the resin using a mixed solution of 6 mL of TFE and 6 mL of CH₂Cl₂. The resin mixture was subjected to acid-base wash, which was extracted twice with 10% (v/v) HCl (aq). The organic layer was then re-extracted with a saturated of NaHCO₃ aqueous solution. The combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo to yield the DDLP as a white solid (Yield 79%).

LC/MS (ESI): m/z called for C₄₄H₅₂N₆O₅Na (M+1) = 767.3893 C₄₄H₅₂N₆O₅ requires 767.3897.

HRMS (ESI-TOF): M+Na+, found 767.3893 C₄₄H₅₂N₆O₅Na₁ requires 767.3897.

The double deprotected linear pentapeptide (DDLP) HO-D-Phe-3,3-Diphe-D-Ala-3-(3-pyridyl)-Ala-N-Me-Val-D-Leu-NH₂ was generated following the “Macrocyclization procedure (syringe pump)” procedure. The reaction was then stirred overnight and the reaction was monitored via LC/MS. Upon completion, the reaction mixture was subjected to acid-base wash, which was extracted twice with 10% (v/v) HCl (aq). The organic layer was then re-extracted with a saturated of NaHCO₃ aqueous solution. The combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo. The resulting crude product was purified via flash column chromatography on silica gel using an ethyl acetate-hexane gradient system, followed by purification via HPLC to yield compound 25 as white solid (Yield 32%).

Rf: 0.25 (Hex:EtOAc = 0.25:0.75)
LC/MS (ESI): m/z called for C₄₄H₅₂N₆O₅ (M+1) = 767.40, found 767.00 and (½ M+1) 382.00

Cyclo D-Phe-3,3-DiPhe-D-Ala-3-(3-pyridyl)-Ala-N-Me-Val-D-Leu (25) was synthesized using the DDLP (1.0 equivalent), TBTU (0.80 equivalent), HATU (0.80 equivalent), DMTrMM (0.80 equivalent), DIPEA (8.0 equivalents) in anhydrous CH₂Cl₂ (0.001M) following “General peptide coupling” procedure. The reaction mixture was subjected to acid-base wash, which was extracted twice with 10% (v/v) HCl (aq). The organic layer was then re-extracted with a saturated of NaHCO₃ aqueous solution. The combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo. The resulting crude product was purified via flash column chromatography on silica gel using an ethyl acetate-hexane gradient system, followed by purification via HPLC to yield compound 25 as white solid (Yield 32%).

Following “General peptide coupling” procedure for SPPS and the published procedure for SPPS (Koay, Y. C.; McConnell, J. R.; Wang, Y.; Kim, S. J.; Buckton, L. K.; Mansour, F.; McAlpine, S. R. ACS Med. Chem. Lett. 2014, 5, 771-776), linear pentapeptide of Resin-O-D-Phe-3,3-Diphe-D-Ala-3-(3-pyridyl)-Ala-N-Me-Val-D-Leu-NH₂ was synthesized by using (1.0 equivalent) of resin-O-D-Phe-NH₂ and the subsequent peptide coupling in the sequence as mentioned below: Fmoc-3,3-Diphenyl-D-Ala-OH (1.5 mmol, 3.0 equivalents), Fmoc-3-(3-pyridyl)-Ala OH, Fmoc-N-Me-Val-OH (1.5 mmol, 3.0 equivalents), and Fmoc-D-Leu-OH (1.5 mmol, 3.0 equivalents). Each coupling peptide was done in the presence of HOAt (1.5 mmol, 3.0 equivalents) or HOBT (1.5 mmol, 3.0 equivalents), DIC (3.0 mmol, 6.0 equivalents) and DMF to generate a concentration of 0.20 M based on the amino acid. Each coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained to afford the Fmoc-protected resin-bound pentapeptide and the Fmoc protecting group was removed following the “General Fmoc removal” after the completion of each coupling reaction.

The double deprotected linear pentapeptide (DDLP) HO-D-Phe-3,3-Diphe-D-Ala-3-(3-pyridyl)-Ala-N-Me-Val-D-Leu-NH₂ was generated following the “Resin cleavage of linear peptide” procedure for SPPS. The linear pentapeptide was cleaved from the resin using a mixed solution of 6 mL of TFE and 6 mL of CH₂Cl₂. The resin containing solution was filtered and dried in vacuo to yield the DDLP as a white solid (Yield 79%).

LC/MS (ESI): m/z called for C₄₄H₅₂N₆O₅ (M+1) = 767.3893 C₄₄H₅₂N₆O₅ requires 767.3897.

HRMS (ESI-TOF): M+Na+, found 767.3893 C₄₄H₅₂N₆O₅Na₁ requires 767.3897.

1H NMR (600 MHz, DMSO): δ 8.37 (m, 2H, Pyridyl), 8.01 (m, 1H, NH), 7.93 (d, 1H, J= 7.45, Pyridyl), 7.57 (m, 1H, Pyridyl) 7.38-7.10 (m, 15H, D-BiPhe, D-Phe, NH), 6.89 (m, 3H, NH), 5.21 (m, 1H, CH₂ D-BiPhe), 4.49 (m, 1H, CH₂ Pyridyl) 4.30 (d, J = 11.68, 1H, CHβ D-BiPhe) 4.17 (d, J = 10.76, 1H, CHα Val), 4.11 (m, 1H, CHα D-Phe), 3.71 (m, 1H, CHα Leu), 2.98 (m, 1H, CHβ Pyridyl), 2.88 (m, 1H, CHβ Pyridyl), 2.74 (m, 1H, CHβ Phe), 2.65 (s, 3H, NCH₃), 2.59 (m, 1H, CH₂ D-Phe), 2.08 (m, 1H, CHβ Leu), 1.92 (m, 0.5H, CHβ Val), 1.60 (m, 1H, CHβ Leu), 1.48 (m, 0.5H, CHβ Val), 1.35 (m, 1H, CHβ₂ Leu), 0.91-0.44 (m, 12H, CHCH(CH₃)₂, CH₂CH(CH₃)₂).

13C NMR (125 MHz, DMSO): δ 171.39, 171.18, 170.19, 140.80,140.13, 136.80, 129.03, 128.88, 128.71, 128.53, 128.32, 128.24, 128.09, 127.95, 127.04, 126.65, 126.53, 60.40, 57.65, 55.06, 50.80, 48.02, 40.68, 32.72, 30.62, 29.69, 26.10, 25.85, 24.85, 24.65, 22.81, 22.62, 22.46, 21.05, 19.39, 18.08, 18.28, 17.83.
was draped to afford the Fmoc-protected resin-bound pentapeptide and the Fmoc protecting group was removed following the “General Fmoc removal” after the completion of each coupling reaction.

The double deprotected linear pentapeptide (DDLPL) HO-D-Phe-3,3-DiPhe-D-Ala-3-(4-pyridyl)-Ala-N-Me-Val-D-Leu-NH₂ was generated following the “Resin cleavage of linear peptide” procedure for SPPS. The linear pentapeptide was cleaved from the resin using a mixed solution of 6 mL of TFE and 6 mL of CH₂Cl₂. The resin containing solution was filtered and dried in vacuo to yield the DLP as a white solid (Yield 89%).

LC/MS (ESI): m/z called for C₄₄H₅₂N₆O₆ (M+1) = 769.37, found 769.10.

**Cyclo D-Phe-3,3-DiPhe-D-Ala-3-(4-pyridyl)-Ala-N-Me-Val-D-Leu (26)** was synthesized using the DSDL (1.0 equivalent), TBTU (0.80 equivalent), HATU (0.80 equivalent), DMTMM (0.80 equivalent), DIPEA (8.0 equivalents) in anhydrous CH₂Cl₂ (0.001M) following “Macrocyclization procedure (syringe pump)” procedure. The reaction was then stirred overnight and the reaction was monitored via LC/MS. Upon completion, the reaction mixture was subjected to acid-base wash, which was extracted twice with 10% (v/v) HCl (aq). The organic layer was then re-extracted with a saturated of NaHCO₃ aqueous solution. The combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo. The resulting crude product was purified via flash column chromatography on silica gel using an ethyl acetate-hexane gradient system, followed by purification via HPLC to yield compound 26 as white solid (Yield 60%).

Following “General peptide coupling” procedure for SPPS and the published procedure for SPPS (Koay, Y. C.; McConnell, J. R.; Wang, Y.; Kim, S. J.; Buckton, L. K.; Mansour, F.; McAlpine, S. R. ACS Med. Chem. Lett. 2014, 5, 771-776), linear pentapeptide of resin-O-D-Leu-D-Phe-3,3-Diphe-D-Ala-3-(4-Thia)Ala-N-Me-Val-NH₂ was synthesized by using 1.00 g (0.53 mmol, 1.0 equivalent) of resin-O-D-Leu-NH₂ and the subsequent peptide coupling in the sequence as mentioned below: 0.62 g of Fmoc-3,3-Diphenyl-D-Ala-OH and 0.56 g (1.6 mmol, 3.0 equivalents) of Fmoc-3-(4-Thiazoyl)Ala-OH and 0.56 g (1.6 mmol, 3.0 equivalents) of Fmoc-N-Me-Val-OH. Each peptide coupling was done in the presence of 0.22 g of HOAt (1.6 mmol, 3.0 equivalents) or 0.21 g of HOBT (1.6 mmol, 3.0 equivalents), 0.50 mL of DIC (3.2 mmol, 6.0 equivalents) and 2.65 mL of DMF to generate a concentration of 0.20 M. Each coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained to afford the Fmoc-protected resin-bound pentapeptide and the Fmoc protecting group was removed following the “General Fmoc removal” after the completion of each coupling reaction.

The double deprotected linear pentapeptide (DDLPL) HO-D-Leu-D-Phe-3,3-DiPhe-D-Ala-3-(4-Thia)Ala-N-Me-Val-NH₂ was generated following the “Resin cleavage of linear peptide” procedure for SPPS. The linear pentapeptide was cleaved from the resin using a mixed solution of 6 mL of TFE and 6 mL of CH₂Cl₂. The resin containing solution was filtered and dried in vacuo to yield the DLP as a white solid (204 mg, overall 50%).

LC/MS (ESI): m/z called for C₄₄H₅₂N₆O₆ (M+1) = 769.37, found 769.10.

**Cyclo D-Leu-D-Phe-3,3-Diphe-D-Ala-3-(4-Thia)Ala-N-Me-Val (27)** was synthesized using 0.20 g of the DDDL generated (0.27 mmol, 1.0 equivalent), 0.07 g of TBTU (0.21 mmol, 0.80 equivalent), 0.08 g of HATU (0.21 mmol, 0.80 equivalent), 0.06 g of DMTMM (0.21 mmol, 0.80 equivalent), 0.37 mL of DIPEA (2.1 mmol, 8.0 equivalents) in anhydrous CH₂Cl₂ (265 mL, 0.001M) following “Macrocyclization procedure (syringe pump)” procedure. The reaction was then stirred overnight and the reaction was monitored via LC/MS. Upon completion, the reaction mixture was subjected to acid-base wash, which was extracted twice with 10% (v/v) HCl (aq). The organic layer was then re-extracted with a saturated of NaHCO₃ aqueous solution. The combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo. The resulting crude product was purified via
Following “General peptide coupling” procedure for SPPS and the published procedure for SPPS (Koay, Y. C.; McConnell, J. R.; Wang, Y.; Kim, S. J.; Buckton, L. K.; Mansour, F.; McAlpine, S. R. ACS Med. Chem. Lett. 2014, 5, 771-776), linear pentapeptide of resin-O-Leu-N-Me-Val-3-(4-Thia)-D Ala-3-(4-Thia)-D Ala-3,3-Diphe-D-Ala-NH₂ was synthesized by using 1.00 g (0.5 mmol, 1.0 equivalent) of resin-O-Leu-NH₂ and the subsequent peptide coupling in the sequence as mentioned below: 0.53 g (1.5 mmol, 3.0 equivalents) of Fmoc-N-Me-Val-OH, 0.59 g (1.5 mmol, 3.0 equivalents) of Fmoc-D-3-(4-Thiazoyl)Ala-OH, 0.59 g (1.5 mmol, 3.0 equivalents) of Fmoc-D-3-(4-Thiazoyl)Ala-OH and 0.70 g (1.5 mmol, 3.0 equivalents) of Fmoc-3,3-Diphenyl-D Ala-OH. Each peptide coupling was done in the presence of 0.20 g of HOAt (1.5 mmol, 3.0 equivalents) or 0.20 g of HOBt (1.5 mmol, 3.0 equivalents), 0.47 mL of DIC (3.0 mmol, 6.0 equivalents) and 2.5 mL of DMF to generate a concentration of 0.20 M. Each coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained to afford the Fmoc-protected resin-bound pentapeptide and the Fmoc protecting group was removed following the “General Fmoc removal” after the completion of each coupling reaction.

The double deprotected linear pentapeptide (DLP) HO-Leu-N-Me-Val-3-(4-Thia)-D Ala-3-(4-Thia)-D Ala-3,3-Diphe-D-Ala-NH₂ was generated following the “Resin cleavage of linear peptide” procedure for SPPS. The linear pentapeptide was cleaved from the resin using a mixed solution of 6 mL of TFE and 6 mL of CH₂Cl₂. The resin containing solution was filtered and dried in vacuo to yield the DLP as a white solid (310 mg, overall 80%).

LC/MS (ESI): 

**Cyclo Leu-N-Me-Val-3-(4-Thia)-D Ala-3-(4-Thia)-D Ala-3,3-Diphe-D-Ala (28)** was synthesized using 0.31 g of the DLP generated (0.52 mmol, 1.0 equivalent), 0.13 g of TBTU (0.42 mmol, 0.80 equivalent), 0.12 g of DMTMM (0.42 mmol, 0.80 equivalent), 0.72 mL of DIPEA (4.2 mmol, 8.0 equivalents) in anhydrous CH₂Cl₂ (520 mL, 0.001M) following “Macrocyclization procedure (syringe pump)” procedure. The reaction was then stirred overnight and the reaction was monitored via LC/MS. Upon completion, the reaction mixture was subjected to acid-base wash, which was extracted twice with 10% (v/v) HCl (aq). The organic layer was then re-extracted with a saturated of NaHCO₃ aqueous solution. The combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo. The resulting crude product was purified via flash column chromatography on silica gel using an ethyl acetate-hexane gradient system, followed by purification via HPLC to yield compound 28 as white solid (140 mg, 36%).

Rc: 0.53 (EtOAc:MeOH = 0.975:0.025)

LC/MS: m/z called for C₃₉H₄₇N₇O₅S₂ (M+1) = 776.32, found 775.95.

**HRMS (ESI-TOF):** 

M+Na⁺, found 780.2971 requires 780.3080.

**1H NMR (600 MHz, DMSO):** δ 9.02 (s, 1H, D-Thia2), 8.94 (s, 1H, D-Thia1), 8.41 (br, NH), 8.21 (br, NH), 7.87 (d, J = 7.1 Hz, NH), 7.82 (br, NH), 7.36-7.12 (m, 11H, D-BiPhe, D-Thia2), 6.88 (s, 1H, D-Thia1), 5.22 (t, J = 10.9 Hz, 1H, CHβ D-BiPhe), 4.99 (q, J = 7.7 Hz, 1H, CHα D-Thia2), 4.52 (d, J = 11.3 Hz, 1H, CHα Val), 4.36 (d, J = 12.1 Hz, 1H, CHβ D-BiPhe), 4.19 (m, 1H, CHα D-Thia1), 3.20 (m, 1H, CHα Leu), 2.90-2.79 (m, 4H, CHβ D-
The double deprotected linear pentapeptide (DDLPC) HO-Leu-N-Me-Val-D-Tyr(OMe)-3-(4-Thia)-D-Ala-3,3-Diphe-D-Ala-NH₂ was synthesized following the “Resin cleavage of linear peptide” procedure for SPPS. The linear pentapeptide was cleaved from the resin using a mixed solution of 6 mL of TFE and 6 mL of CH₂Cl₂. The resin containing solution was filtered and dried in vacuo to yield the DDLPC as a white solid (320 mg, overall 80%).

LC/MS (ESI): m/z called for C₄₃H₅₄N₆O₇S (M+1) = 799.38, found 798.95.

Cyclo Leu-N-Me-Val-D-Tyr(OMe)-3-(4-Thia)-D-Ala-3,3-Diphe-D-Ala (29) was synthesized using 0.32 g of the DDLPC generated (0.40 mmol, 1.0 equivalent), 0.10 g of TBTU (0.32 mmol, 0.80 equivalent), 0.12 g of HATU (0.32 mmol, 0.80 equivalent), 0.09 g of DMTMM (0.32 mmol, 0.80 equivalent), 0.56 mL of DIPEA (3.2 mmol, 8.0 equivalents) in anhydrous CH₂Cl₂ (400 mL, 0.001M) following “Macrocyclization procedure (syringe pump)” procedure. The reaction was then stirred overnight and the reaction was monitored via LC/MS. Upon completion, the reaction mixture was subjected to acid-base wash, which was extracted twice with 10% (v/v) HCl (aq). The organic layer was then re-extracted with a saturated of NaHCO₃ aqueous solution. The combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo. The resulting crude product was purified via flash column chromatography on silica gel using an ethyl acetate-hexane gradient system, followed by purification via HPLC to yield compound 29 as white solid (110 mg, 35%).

Rf: 0.56 (100% EtOAc)

LC/MS: m/z called for C₄₃H₅₂N₆O₆S (M+1) = 781.37, found 781.05.

HRMS (ESI-TOF): M+Na⁺, found 803.3560 C₄₃H₅₂N₆O₆SNa requires 803.3669.

1H NMR (600 MHz, DMSO): δ 9.02 (s, 1H, D-Thia), 8.42 (br, NH), 8.15 (d, J= 8.6 Hz, NH), 7.31-7.05 (m, 12H, D-BiPhe), 6.81 (d, J= 8.6 Hz, 1H, D-Thia), 6.76 (d, J= 8.3 Hz, 2H, O-Me-D-Tyr), 6.61 (d, J= 8.6 Hz, 1H, D-Thia), 6.76 (d, J= 8.3 Hz, 2H, O-Me-D-Tyr), 5.21 (t, J= 10.9 Hz, 1H, CH₂D-BiPhe), 4.71 (q, J= 7.5 Hz, 1H, CH₃D-Thia), 4.52 (d, J= 11.3 Hz, 1H, CH₂ Val), 4.36 (d, J= 12.1 Hz, 1H, CHβ D-BiPhe), 4.15 (m, 1H, CHα O-Me-D-Tyr), 3.68 (s, 3H, NCH₃), 3.01 (m, 1H, CHα Leu), 2.83 (m, 2H, CHβ D-BiPhe), 2.59 (m, 2H, CHβ D-Thia), 2.56 (s, 3H, NCH₃), 2.08 (1H, CHβ Val), 1.49 (m, 1H, CHβ₂ Leu), 1.40 (m, 1H, CHβ₁ Leu), 1.26 (m, 1H, CHγ Leu), 0.81-0.58 (m, 12H, CHCH(CH₃)₂), 2.56 (s, 3H, NCH₃).

13C NMR (150 MHz, DMSO): δ 170.46, 170.32, 169.85, 169.68, 168.77, 158.06, 154.92, 153.50, 141.76, 141.26, 131.19, 129.46, 129.41, 128.92, 128.87, 127.87, 127.80, 126.62, 114.37, 114.34, 62.90, 56.20, 55.00, 54.10, 53.50, 52.10, 50.60, 37.70, 37.00, 33.30, 29.40, 25.40, 24.90, 24.12, 23.27, 19.20, 18.44.

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Following “General peptide coupling” procedure for SPPS and the published procedure for SPPS (Koay, Y. C.; McConnell, J. R.; Wang, Y.; Kim, S. J.; Buckton, L. K.; Mansour, F.; McAlpine, S. R. *ACS Med. Chem. Lett.* *2014*, *5*, 771-776), linear pentapeptide of resin-O-Leu-N-Me-Val-D-Glu(OtBu)-3-(4-Thia)-D-Ala-3,3-Diphenyl-D-Ala-NH$_2$ was synthesized by using 1.00 g (0.5 mmol, 1.0 equivalent) of resin-O-Leu-NH$_2$ and the subsequent peptide coupling in the sequence as mentioned below: 0.53 g (1.5 mmol, 3.0 equivalents) of Fmoc-N-Me-Val-OH, 0.64 g (1.5 mmol, 3.0 equivalents) of Fmoc-D-Glu-g-OtBu, 0.59 g (1.5 mmol, 3.0 equivalents) of Fmoc-D-3-(4-Thiazoyl)Ala-OH and 0.70 g (1.5 mmol, 3.0 equivalents) of Fmoc-3,3-Diphenyl-D-Ala-OH. Each peptide coupling was done in the presence of 0.20 g of HOAt (1.5 mmol, 3.0 equivalents) or 0.20 g of HOBT (1.5 mmol, 3.0 equivalents), 0.47 mL of DIC (3.0 mmol, 6.0 equivalents) and 2.5 mL of DMF to generate a concentration of 0.20 M. Each coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained to afford the Fmoc-protected resin-bound pentapeptide and the Fmoc protecting group was removed following the “General Fmoc removal” after the completion of each coupling reaction.

The double deprotected linear pentapeptide (DDLP) HO-Leu-N-Me-Val-D-Glu(OtBu)-3-(4-Thia)-D-Ala-3,3-Diphenyl-D-Ala-NH$_2$ was generated following the “Resin cleavage of linear peptide” procedure for SPPS. The linear pentapeptide was cleaved from the resin using a mixed solution of 6 mL of TFE and 6 mL of CH$_2$Cl$_2$. The resin containing solution was filtered and dried in vacuo to yield the DDLP as a white solid (278 mg, overall 69%).

**Cyclo Leu-N-Me-Val-D-Glu(OtBu)-3-(4-Thia)-D-Ala-3,3-Diphe-D-Ala-NH$_2$ (30)** was synthesized using 0.28 g of the DDLP generated (0.34 mmol, 1.0 equivalent), 0.09 g of TBTU (0.28 mmol, 0.80 equivalent), 0.08 g of DMTMM (0.28 mmol, 0.80 equivalent), 0.48 mL of DIPEA (2.8 mmol, 8.0 equivalents) in anhydrous CH$_2$Cl$_2$ (383 mL, 0.001M) following "Macrocyclization procedure (syringe pump)" procedure. The reaction was then stirred overnight and the reaction was monitored via LC/MS. Upon completion, the reaction mixture was subjected to acid-base wash, which was extracted twice with 10% (v/v) HCl(aq). The organic layer was then re-extracted with a saturated of NaHCO$_3$ aqueous solution. The combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo to yield the DDLP as a white solid (278 mg, overall 69%).

LC/MS (ESI): m/z called for C$_{42}$H$_{58}$N$_6$O$_7$S (M+1) = 789.40, found 789.10.

HRMS (ESI-TOF): M+Na+, found 789.10, requires 789.10.

**Rf:** 0.30 (100% EtOAc).

**1H NMR (600 MHz, DMSO):** δ 9.14 (d, J = 1.9 Hz, 1H, D-Thia), 8.29 (d, J = 8.6 Hz, NH), 7.91 (m, 2NH), 7.71 (d, J = 9.8 Hz, NH), 7.37-7.12 (m, 11H, D-Biphe), 7.08 (s, 1H, D-Thia), 7.06 (d, J = 11.0 Hz, 1H, CH$_2$D-Biphe), 6.99 (d, J = 11.5 Hz, 1H, CH$_2$Val), 4.48 (q, J = 7.6 Hz, 1H, CH$_2$D-Glu), 4.33 (m, 2H, CH$_2$B-D-Biphe, CH$_2$A-D-Thia), 3.42 (q, J = 7.3 Hz, 1H, CH$_2$Leu), 2.87 (d, J = 6.2 Hz, 2H, CH$_2$B-D-Thia), 2.56 (s, 3H, NCH$_3$), 1.75 (m, 2H, CH$_2$Val, CH$_2$D-Glu), 1.98 (m, 2H, CH$_2$Yi, D-Glu, CH$_2$B-D-Glu), 1.83 (m, 1H, CH$_2$B-D-Glu), 1.53 (m, 2H, CH$_2$B Leu), 1.40 (s, 9H, OC(CH$_3$)$_3$), 1.31 (m, 1H, CH$_7$Leu), 0.82-0.65 (m, 12H, CH$_2$CH(CH$_3$)$_2$, CHCH(CH$_3$)$_2$).

**13C NMR (150 MHz, DMSO):** δ 172.34, 170.28, 170.24, 169.68, 169.37, 168.57, 154.63, 152.73, 141.59, 141.30, 130.90, 128.58, 128.55, 128.49, 127.24, 126.87, 116.35, 79.88, 63.05, 56.90, 54.56, 53.88, 51.72, 48.26, 37.99, 32.90, 31.66, 30.06, 28.27, 27.52, 25.31, 24.49, 22.94, 22.51, 19.55, 18.77.

The tBu protecting group of compound 30 was removed following “Tert-butyl group (tBu) Removal” procedure, utilizing a mixture of TFA/CH$_2$Cl$_2$ (2:4, 0.1 M) and anisole (2.0 equivalents) to generate compound 31. The free acid was taken to the subsequent methyl ester formation without purification.

**Rf:** 0.30 (100% EtOAc).

LC/MS: m/z called for C$_{43}$H$_{56}$N$_6$O$_7$S (M+1) = 807.40, found 807.05.

HRMS (ESI-TOF): M+Na+, found 807.32, requires 807.33.

**1H NMR (600 MHz, DMSO):** δ 8.20 (d, J = 1.9 Hz, 1H, D-Thia), 7.89 (d, J = 8.6 Hz, NH), 7.91 (m, 2NH), 7.71 (d, J = 9.8 Hz, NH), 7.37-7.12 (m, 11H, D-Biphe), 7.08 (s, 1H, D-Thia), 7.06 (d, J = 11.0 Hz, 1H, CH$_2$D-Biphe), 6.99 (d, J = 11.5 Hz, 1H, CH$_2$Val), 4.48 (q, J = 7.6 Hz, 1H, CH$_2$D-Glu), 4.33 (m, 2H, CH$_2$B-D-Biphe, CH$_2$A-D-Thia), 3.42 (q, J = 7.3 Hz, 1H, CH$_2$Leu), 2.87 (d, J = 6.2 Hz, 2H, CH$_2$B-D-Thia), 2.56 (s, 3H, NCH$_3$), 1.75 (m, 2H, CH$_2$Val, CH$_2$D-Glu), 1.98 (m, 2H, CH$_2$Yi, D-Glu, CH$_2$B-D-Glu), 1.83 (m, 1H, CH$_2$B-D-Glu), 1.53 (m, 2H, CH$_2$B Leu), 1.40 (s, 9H, OC(CH$_3$)$_3$), 1.31 (m, 1H, CH$_7$Leu), 0.82-0.65 (m, 12H, CH$_2$CH(CH$_3$)$_2$, CHCH(CH$_3$)$_2$).
(dd, J = 10.0, 14.1 Hz, 1H, CHα Leu), 2.87 (m, 2H, CHβ D-Thia), 2.56 (s, 3H, NCH3), 2.18 (m, 1H, CHβ Val), 2.01 (m, 2H, CHγ D-Glu), 1.84 (m, 1H, CHββ D-Thia), 1.56 (m, 1H, CHββ D-Glu), 1.39 (m, 2H, CHββ Leu), 1.29 (m, 1H, CHγ Leu), 0.82-0.65 (m, 12H, CH2CH(CH3)2, CHCH(CH3)2).

13C NMR (150 MHz, DMSO): δ 174.63, 170.40, 170.32, 169.68, 169.38, 168.62, 154.64, 152.72, 141.60, 141.29, 128.91, 128.60, 128.53, 128.49, 127.24, 126.87, 116.35, 63.06, 56.91, 54.64, 53.85, 51.70, 49.03, 38.00, 32.95, 30.40, 30.10, 27.47, 25.32, 24.48, 22.92, 22.51, 19.56, 18.81.

The free acid of compound 31 was converted to the methyl ester compound 32 using the methylating agent Trimethylsilyl diazomethane (TMSD), which was dissolved in a mixture of anhydrous benzene and methanol (3:1) in a round bottom flask to make a 0.1 M solution and methylating agent TMSD (2.0 M in diethyl ether) was added dropwise into the reaction mixture. The reaction was stirred under nitrogen and monitored by thin layer chromatography (TLC). Upon completion, the solvent was evaporated in vacuo and resulting compound 32, Rf: 0.51 (EtOAc:MeOH = 0.95:0.05).

LC/MS: m/z called for C39H50N6O7S (M+1) = 747.35, found 747.05. HRMS (ESI-TOF): M+Na+, found 769.3346 C39H50N6O7SNa requires 769.3462.

1H NMR (600 MHz, DMSO): δ 9.09 (s, 1H, D-Thia), 8.40 (br, NH), 8.05 (br, NH), 7.99 (d, J = 7.2 Hz, NH), 7.89 (br, NH), 7.34-7.15 (m, 10H, D-Biphe), 7.06 (s, 1H, D-Thia), 5.26 (t, J = 11.2 Hz, 1H, CHα D-Biphe), 4.56 (d, J = 11.4 Hz, 1H, CHα Val), 4.49 (q, J = 7.5 Hz, 1H, CHα D-Glu), 4.35 (m, 2H, CHβ D-Biphe, CHα D-Thia), 3.60 (s, 3H, OCH3), 3.22 (m, 1H, CHα Leu), 2.87 (m, 1H, CHβ Val), 2.60 (m, 1H, CHββ D-Thia), 2.53 (s, 3H, NCH3), 2.09 (m, 3H, CHβ Val, CHγ D-Glu), 1.88 (m, 1H, CHββ D-Glu), 1.60 (m, 1H, CHββ1 D-Thia), 1.50 (m, 1H, CHββ2 Leu), 1.41 (m, 1H, CHββ1 Leu), 1.29 (m, 1H, CHγ Leu), 0.81-0.65 (m, 12H, CH2CH(CH3)2, CHCH(CH3)2).

13C NMR (150 MHz, DMSO): δ 173.56, 170.47, 170.26, 169.69, 169.36, 168.50, 154.56, 152.78, 141.65, 141.28, 128.89, 128.68, 128.53, 128.41, 126.85, 126.80, 116.28, 62.89, 57.06, 54.45, 53.83, 51.79, 51.70, 48.92, 37.85, 33.09, 30.04, 29.95, 27.34, 25.31, 24.49, 22.97, 22.49, 19.59, 18.72.

Following “General peptide coupling” procedure for SPPS and the published procedure for SPPS (Koay, Y. C.; McConnell, J. R.; Wang, Y.; Kim, S. J.; Buckton, L. K.; Mansour, F.; McAlpine, S. R. ACS Med. Chem. Lett. 2014, 5, 771-776), linear pentapeptide of resin-O-Leu-N-Me-Val-(4-Methoxy)-D-Phe-(4-Methoxy)-D-Phe-3,3-Diphe-D-Ala-NH2 was synthesized by using (0.5 mmol, 1.0 equivalent) of resin-O-Leu-NH2 and the subsequent peptide coupling in the sequence using 1.5 mmol (3.0 equivalents) of each mentioned below: Fmoc-N-Me-Val-OH, Fmoc-(4-Methoxy)-D-Phe-OH, Fmoc-(4-Methoxy)-D-Phe-OH, Fmoc-D-Phe-OH, and Fmoc-3,3-Diphenyl-D-Ala-OH. Each peptide coupling was done in the presence of HOAt (1.5 mmol, 3.0 equivalents) or HOBt (1.5 mmol, 3.0 equivalents), DIC (3.0 mmol, 6.0 equivalents) and DMF to generate a concentration of 0.20 M based on the amino acid. Each coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained to afford the Fmoc-protected resin-bound pentapeptide and the Fmoc protecting group was removed following the “General Fmoc removal” after the completion of each coupling reaction.

The double deprotected linear pentapeptide (DDLP) HO-Leu-N-Me-Val-3-(3-pyridyl)-D-Ala-D-Phe-3,3-Diphe-D-Ala-NH2 was generated following the “Resin cleavage of linear peptide” procedure for SPPS. The linear pentapeptide was cleaved from the resin using a mixed solution of 6 mL of TFE and 6 mL of CH2Cl2. The resin containing solution was filtered and dried in vacuo to yield the DDLP as a white solid (Quantitative yield) LC/MS (ESI): m/z called for C47H59N5O8 (M+1) = 822.42, found 822.00.

Cyclo Leu-N-Me-Val-(4-Methoxy)-D-Phe-(4-Methoxy)-D-Phe-3,3-Diphe-D-Ala (33) was synthesized using the DDLP (1.0 equivalent), TBTU (0.80 equivalent), HATU (0.80 equivalent), DMTMM (0.80 equivalent), DIPEA (8.0 equivalents) in anhydrous CH2Cl2 (0.001M) following “Macrocyclization procedure (syringe pump)” procedure. The reaction was then stirred overnight and the reaction was monitored via LC/MS. Upon completion, the reaction mixture was subjected to acid-base wash, which was extracted twice with 10% (v/v) HCl (aq). The organic layer was then re-extracted with a saturated of NaHCO3 aqueous solution. The combined organic layers

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were dried over sodium sulfate, filtered, and concentrated in vacuo. The resulting crude product was purified via flash column chromatography on silica gel using an ethyl acetate-hexane gradient system, followed by purification via HPLC to yield compound 33 as white solid (Yield 16%).

HRMS (ESI-TOF): M+Na+, found 826.4149 C47H57N5O7Na1 requires 826.4156.

The double deprotected linear pentapeptide (DDLP) HO-Leu-N-Me-Val-3-(4-Thia)-D-Ala-(4-Methoxy)-D-Phe-3,3-Diphe-D-Ala-NH2 was synthesized using (0.5 mmol, 1.0 equivalent) of resin-O-Leu-NH2 and the subsequent peptide coupling in the sequence using 1.5 mmol (3.0 equivalents) of each mentioned below: Fmoc-N-Me-Val-OH, Fmoc-3-(4-Thia)-D-Ala-OH, Fmoc-(4-Methoxy)-D-Phe-OH, Fmoc-D-Phe-OH, and Fmoc-3,3-Diphenyl-D-Ala-OH. Each peptide coupling was done in the presence of HOAt (1.5 mmol, 3.0 equivalents) or HOBt (1.5 mmol, 3.0 equivalents), DIC (3.0 mmol, 6.0 equivalents) and DMF to generate a concentration of 0.20 M based on the amino acid. Each coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained to afford the Fmoc-protected resin-bound pentapeptide and the Fmoc protecting group was removed following the “General Fmoc removal” after the completion of each coupling reaction.

The double deprotected linear pentapeptide (DDLP) HO-Leu-N-Me-Val-3-(3-pyridyl)-D-Ala-D-Phe-3,3-Diphe-D-Ala-NH2 was generated following the “Resin cleavage of linear peptide” procedure for SPPS. The linear pentapeptide was cleaved from the resin using a mixed solution of 6 mL of TFE and 6 mL of CH2Cl2. The resin containing solution was filtered and dried in vacuo to yield the DDLP as a white solid (Quantitative yield). The resulting crude product was purified via flash column chromatography on silica gel using an ethyl acetate-hexane gradient system, followed by purification via HPLC to yield compound 34 as white solid (Yield 26%).

HRMS (ESI-TOF): M+Na+, found 799.37, found 799.00.

Cyclo Leu-N-Me-Val-3-(4-Thia)-D-Ala-(4-Methoxy)-D-Phe-3,3-Diphe-D-Ala (34) was synthesized using the DDLP (1.0 equivalent), TBTU (0.80 equivalent), HATU (0.80 equivalent), DMTrMM (0.80 equivalent), DIPEA (8.0 equivalents) in anhydrous CH2Cl2 (0.001M) following “Macrocyclization procedure (syringe pump)” procedure. The reaction mixture was subjected to acid-base wash, followed by purification via HPLC to yield compound 34 as white solid (Yield 26%).


Following “General peptide coupling” procedure for SPPS and the published procedure for SPPS (Koay, Y. C.; McConnell, J. R.; Wang, Y.; Kim, S. J.; Buckton, L. K.; Mansour, F.; McAlpine, S. R. ACS Med. Chem. Lett. 2014, 5, 771-776), linear pentapeptide of resin-O-Leu-N-Me-Val-3-(4-Thia)-D-Ala-(4-Methoxy)-D-Phe-3,3-Diphe-D-Ala-NH2 was synthesized by using (0.5 mmol, 1.0 equivalent) of resin-O-Leu-NH2 and the subsequent peptide coupling in the sequence using 1.5 mmol (3.0 equivalents) of each mentioned below: Fmoc-N-Me-Val-OH, Fmoc-3-(4-Thia)-D-Ala-OH, Fmoc-(4-Methoxy)-D-Phe-OH, Fmoc-D-Phe-OH, and Fmoc-3,3-Diphenyl-D-Ala-OH. Each peptide coupling was done in the presence of HOAt (1.5 mmol, 3.0 equivalents) or HOBt (1.5 mmol, 3.0 equivalents), DIC (3.0 mmol, 6.0 equivalents) and DMF to generate a concentration of 0.20 M based on the amino acid. Each coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained to afford the Fmoc-protected resin-bound pentapeptide and the Fmoc protecting group was removed following the “General Fmoc removal” after the completion of each coupling reaction.
Following “General peptide coupling” procedure for SPPS and the published procedure for SPPS (Koay, Y. C.; McConnell, J. R.; Wang, Y.; Kim, S. J.; Buckton, L. K.; Mansour, F.; McAlpine, S. R. *ACS Med. Chem. Lett.* 2014, 5, 771-776), linear pentapeptide of Resin-O-D-Leu-3-(4-Thia)-D-Ala-3,3-Diphe-D-Ala-3-(4'-Pyr)Ala-N-Me-Val-NH₂ was synthesized by using 1.00 g (0.53 mmol, 1.0 equivalent) of resin-O-D-Leu-NH₂ and the subsequent peptide coupling in the sequence as mentioned below: 0.63 g (1.6 mmol, 3.0 equivalents) of Fmoc-3-(4-Thiazoyl)Ala-OH, 0.74 g (1.6 mmol, 3.0 equivalents) of Fmoc-3-(4'-Pyridyl)Ala-OH and 0.56 g (1.6 mmol, 3.0 equivalents) of Fmoc-N-Me-Val-OH. Each peptide coupling was done in the presence of 0.22 g of HOAt (1.6 mmol, 3.0 equivalents) or 0.21 g of HOBt (1.6 mmol, 3.0 equivalents), 0.50 mL of DIC (3.2 mmol, 6.0 equivalents) and 2.65 mL of DMF to generate a concentration of 0.20 M. Each coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained to afford the Fmoc-protected resin-bound pentapeptide and the Fmoc protecting group was removed following the “General Fmoc removal” after the completion of each coupling reaction.

The double deprotected linear pentapeptide (DDLP) HO-D-Leu-3-(4-Thia)-D-Ala-3,3-Diphe-D-Ala-3-(4'-Pyr)Ala-N-Me-Val-NH₂ was generated following the “Resin cleavage of linear peptide” procedure for SPPS. The linear pentapeptide was cleaved from the resin using a mixed solution of 6 mL of TFE and 6 mL of CH₂Cl₂. The resin containing solution was filtered and dried in vacuo to yield the DDLP as a white solid (196 mg, overall 48%).

**Cyclo D-Leu-3-(4-Thia)-D-Ala-3,3-Diphe-D-Ala-3-(4'-Pyr)Ala-N-Me-Val (35)** was synthesized using 0.20 g of the DDLP generated (0.26 mmol, 1.0 equivalent), 0.07 g of TBTU (0.21 mmol, 0.80 equivalent), 0.08 g of HATU (0.21 mmol, 0.80 equivalent), 0.06 g of DMTMM (0.21 mmol, 0.80 equivalent), 0.36 mL of DIPEA (2.1 mmol, 8.0 equivalents) in anhydrous CH₂Cl₂ (260 mL, 0.001M) following the “Macrocyclization procedure (syringe pump)” procedure. The reaction was then stirred overnight and the reaction was monitored via LC/MS. Upon completion, the reaction mixture was subjected to acid-base wash, which was extracted twice with 10% (v/v) HCl (aq). The organic layer was then re-extracted with a saturated of NaHCO₃ aqueous solution. The combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo. The resulting crude product was purified via flash column chromatography on silica gel using an ethyl acetate-hexane gradient system, followed by purification via HPLC to yield compound 35 as white solid (6 mg, 3%).

*Note: Cyclization at the bulky residue of N-methyl has resulted a poor reaction and thus HPLC yield. Due to the limited amount of compound, HSQC and HMBC spectra were used for the complete assignment of all the carbon peaks.

Rf: 0.63 (100% EtOAc)

LC/MS: m/z called for C₄₁H₅₁N₇O₆S (M+1) = 770.36, found 770.10.

HRMS (ESI-TOF): M+Na+, found 774.3414 C₄₁H₄₉N₇O₅S₁Na₁ requires 774.3399.

1H NMR (600 MHz, CDCl₃): δ 9.32 (br, D-Thia), 9.09 (br, NH), 8.68 (m, 2H, Pyr), 7.67 (m, NH, D-Thia), 7.47-7.00 (m, 12H, D-BiPhe, Pyr, 2NH), 5.09 (m, 2H, CHα D-BiPhe, CHβ D-BiPhe), 4.89 (m, 1H, CHα Pyr), 4.79 (m, 2H, CHα Leu, CHα D-Thia), 4.59 (d, J = 11.3 Hz, 1H, CHβ Val), 3.44 (m, 1H, CHβ₂ Pyr), 2.82 (m, 2H, CHβ₂ D-Thia, CHβ₁ Pyr), 2.68 (m, 4H, CHβ₁ D-Thia, NCH₃), 2.12 (m, 1H, CHβ Val), 1.28 (m, 3H, CHβ Leu, CHγ Leu), 0.84-0.76 (m, 12H, CH(CH₃)₂, CH₂CH(CH₃)₂).

13C NMR (150 MHz, DMSO): δ 175.55, 173.17, 172.17, 171.26, 169.25, 153.73, 152.77, 149.55, 145.96, 140.33, 139.32, 138.33, 136.25, 129.91, 128.84, 128.37, 128.06, 127.91, 127.35, 124.32, 115.05, 62.40, 57.48, 52.54, 52.31, 51.75, 48.30, 40.45, 37.37, 31.71, 29.07, 27.16, 25.47, 24.52, 22.54, 19.16, 18.13.
3.0 equivalents) or HOBT (1.5 mmol, 3.0 equivalents) and DIPEA (3.0 mmol, 6.0 equivalents) and DMF to generate a concentration of 0.20 M based on the amino acid. Each coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained to afford the Fmoc-protected resin-bound pentapeptide and the Fmoc protecting group was removed following the “General Fmoc removal” after the completion of each coupling reaction.

The double deprotected linear pentapeptide (DDLP) HO-D-Phe-3,3-Diphe-D-Ala-3-(2-pyridyl)-Ala-N-Me-Val-3-(4-Thia)-D-Ala-NH₂ was generated following the “Resin cleavage of linear peptide” procedure for SPPS. The linear pentapeptide was cleaved from the resin using a mixed solution of 6 mL of TFE and 6 mL of CH₂Cl₂. The resin containing solution was filtered and dried in vacuo to yield the DDLP as a white solid (Yield 48%).

LC/MS (ESI): m/z called for C₄₄H₄₇N₇O₅S (M⁺1) = 786.34, found 786.00.

Cyclo D-Phe-3,3-Diphe-D-Ala-3-(2-pyridyl)-Ala-N-Me-Val-3-(4-Thia)-D-Ala (36) was synthesized using the DDLP (1.0 equivalent), TBTU (0.80 equivalent), HATU (0.80 equivalent), DMTMM (0.80 equivalent), DIPEA (8.0 equivalents) in anhydrous CH₂Cl₂ (0.001M) following “Macrocyclization procedure (syringe pump)” procedure. The reaction was then stirred overnight and the reaction was monitored via LC/MS. Upon completion, the reaction mixture was subjected to acid-base wash, which was extracted twice with 10% (v/v) HCl (aq). The organic layer was then re-extracted with a saturated of NaHCO₃ aqueous solution. The combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo to yield compound 36 as white solid (Yield 48%).

Rₖ: 0.13 (Hex:EtOAc = 0.25:0.75).

LC/MS: m/z called for C₄₄H₄₇N₇O₅S (M⁺1) = 786.34, found 786.00.

HRMS (ESI-TOF): M⁺Na⁺, found 786.3434 C₄₄H₄₇N₇O₅S requires 786.3359.

1H NMR (600 MHz, DMSO): δ 8.98 (d, J = 1.9 Hz, 1H, D-Thia), 8.65 (s, 1H, NH), 8.39 (m, 1H, Pyridyl), 8.30 (s, 1H, NH), 7.94 (s, 1H, NH), 7.60 (m, 1H, NH) 7.40-7.07 (m, 15H, D-BiPhe, D-Phe), 7.00 (d, , , J = 7.79 Hz, Pyridyl), 6.71 (d, J = 7.05 2H, Pyridyl), 5.21 (m, 2H, CH₃ D-BiPhe, CH₃ D-Thia), 4.61 (2, J = 12.02 Hz, 1H, CHβ D-BiPhe), 4.45 (m, 1H, CH₂ D-Phe), 4.23 (d, J = 11.17 Hz, 1H, CH₃ Val), 3.82 (m, 1H, CHα Pyridyl), 3.33 (m, 1H, CHββ D-Thia), 2.98 (dd, J = 14.05 Hz 1H, CH3β D-Thia), 2.90-2.73 (m, 2H, CHββ Pyridyl), 2.64 (s, 3H, NCH₃), 2.34 (m, 1H, CHββ D-Phe), 2.17 (dd, 1H, J = 13.82 Hz, CHββ D-Phe), 1.83 (m, 1H, CH Val), 0.43 (d, J = 6.56 , 3H, CH₃ Val), 0.32 (d, J = 6.42 1H, CH₃ Val)

Following “General peptide coupling” procedure for SPPS and the published procedure for SPPS (Koay, Y. C.; McConnell, J. R.; Wang, Y.; Kim, S. J.; Buckton, L. K.; Mansour, F.; McAlpine, S. R. ACS Med. Chem. Lett. 2014, 5, 771-776), linear pentapeptide of resin-O-Leu-Val-3-(4-Thia)-D-Ala-N-Me-D-Phe-3,3-Diphenyl-D-Ala-NH₂ was synthesized by using 1.00 g (0.5 mmol, 1.0 equivalent) of resin-O-Leu-NH₂ and the subsequent peptide coupling in the sequence as mentioned below: 0.51 g (1.5 mmol, 3.0 equivalents) of Fmoc-Val-OH, 0.59 g (1.5 mmol, 3.0 equivalents) of Fmoc-D-3-(4-Thiazoyl)Ala-OH, 0.60 g (1.5 mmol, 3.0 equivalents) of Fmoc-N-Me-D-Phe-OH and 0.70 g (1.5 mmol, 3.0 equivalents) of Fmoc-3,3-Diphenyl-D-Ala-OH. Each peptide coupling was done in the presence of 0.20 g of HOBt (1.5 mmol, 3.0 equivalents) or 0.20 g of HOAt (1.5 mmol, 3.0 equivalents), 0.47 mL of DIC (3.0 mmol, 6.0 equivalents) and 2.5 mL of DMF to generate a concentration of 0.20 M. Each coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained to afford the Fmoc-protected resin-bound pentapeptide and the Fmoc protecting group was removed following the “General Fmoc removal” after the completion of each coupling reaction.

The double deprotected linear pentapeptide (DDLDP) HO-Leu-Val-3-(4-Thia)-D-Ala-N-Me-D-Phe-3,3-Diphenyl-D-Ala-NH₂ was generated following the “Resin cleavage of linear peptide” procedure for SPPS. The linear pentapeptide was cleaved from the resin using a mixed solution of 6 mL of TFE and 6 mL of CH₂Cl₂. The resin containing solution was filtered and dried in vacuo to yield the DDDLDP as a white solid (311 mg, overall 81%).

LC/MS (ESI): m/z called for C₄₄H₄₇N₇O₅S (M⁺1) = 769.50.

Cyclo Leu-Val-3-(4-Thia)-D-Ala-N-Me-D-Phe-3,3-Diphe-D-Ala (37) was synthesized using 0.31 g of the DDDLDP generated (0.40 mmol, 1.0 equivalent), 0.10 g of TBTU (0.32 mmol, 0.80 equivalent), 0.12 g of HATU (0.32 mmol, 0.80 equivalent), 0.09 g of DMTMM (0.32 mmol, 0.80 equivalent), 0.56 mL of DIPEA (3.2 mmol, 8.0 equivalents) in anhydrous CH₂Cl₂ (0.001M) following “Macro cyclicization procedure (syringe pump)” procedure. The reaction was then stirred overnight and the reaction was monitored via LC/MS. Upon completion, the reaction mixture was subjected to acid-base wash, which was extracted twice with 10% (v/v) HCl (aq). The organic layer was then re-extracted with a saturated of NaHCO₃ aqueous solution. The combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo to yield compound 37 as white solid (Yield 45%).

Rₖ: 0.13 (Hex:EtOAc = 0.25:0.75).

LC/MS: m/z called for C₄₄H₄₇N₇O₅S (M⁺1) = 769.37, found 769.50.
equivalents) in anhydrous CH₂Cl₂ (404 mL, 0.001M) following “Macrocyclization procedure (syringe pump)” procedure. The reaction was then stirred overnight and the reaction was monitored via LC/MS. Upon completion, the reaction mixture was subjected to acid-base wash, which was extracted twice with 10% (v/v) HCl (aq). The organic layer was then re-extracted with a saturated of NaHCO₃ aqueous solution. The combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo. The resulting crude product was purified via flash column chromatography on silica gel using an ethyl acetate-hexane gradient system, followed by purification via HPLC to yield compound 37 as white solid (155 mg, 51%).

Rf: 0.30 (100% EtOAc)

LC/MS (ESI-TOF): m/z called for C₄₂H₅₀N₆O₅S (M+1) = 751.36, found 751.45.

HRMS (ESI-TOF): M+Na+, found 773.3450 C₄₂H₅₀N₆O₅SNa requires 773.3461.

a) Major conformer

¹H NMR (600 MHz, DMSO): δ 9.05 (d, J = 1.9 Hz, 1H), 9.01 (dd, J = 3.8, 5.7 Hz, 2NH), 8.08 (dd, J = 8.1, 12.8 Hz, NH), 8.01 (d, J = 8.6 Hz, NH), 7.43-7.06 (m, 13H, D-BiPhe, D-Phe), 6.96 (m, 2H, D-Phe), 6.35 (m, 1H), 5.42 (dd, J = 9.8, 4.0 Hz, 1H, CHαD-Phe), 5.26 (t, J = 10.4 Hz, 1H, CHαD-BiPhe), 4.49 (q, J = 7.3 Hz, 1H, CHαD-Thia), 4.38 (d, J = 11.2 Hz, 1H, CHβD-BiPhe), 4.10 (m, 1H, CHαD-Leu), 3.71 (t, J = 9.0 Hz, 1H, CHαVal), 3.48 (d, J = 3.8, 14.3 Hz, 1H, CH2β2D-Phe), 3.19 (dd, J = 6.7, 14.4 Hz, 1H, CHβ2D-Thia), 2.80 (dd, J = 9.9, 13.9 Hz, 1H, CHβ1D-Phe), 2.50 (s, 3H, NCH₃), 1.89 (m, 1H, CHβVal), 1.27 (m, 1H, CHβ2Leu), 0.89 (m, 2H, CHγLeu), 0.82-0.56 (m, 12H, CH2CH(CH₃)₂, CHCH(CH₃)₂).

¹³C NMR (150 MHz, DMSO): δ 172.34, 172.21, 170.25, 170.13, 169.77, 168.77, 141.72, 141.09, 137.20, 129.35, 128.80, 128.70, 128.60, 128.45, 128.10, 127.17, 127.03, 126.83, 79.95, 63.39, 57.27, 56.29, 53.75, 51.68, 49.20, 38.11, 37.85, 31.82, 30.16, 28.24, 27.28, 25.18, 24.99, 22.82, 22.55, 19.66, 18.07.

b) Minor conformer

¹H NMR (600 MHz, DMSO): δ 9.01 (m, NH, 1H), 8.27 (d, J = 6.5 Hz, NH, 1H), 8.01 (d, J = 8.6, NH), 7.43-7.24 (m, 13H, D-BiPhe, D-Phe), 6.96 (m, 2H, D-Phe), 6.76 (m, 1H), 5.51 (dd, J = 8.4, 11.5 Hz, 1H, CHαD-Phe), 5.26 (t, J = 10.4 Hz, 1H, CHαD-BiPhe), 4.63 (m, 1H, CHαD-Thia), 4.44 (d, J = 11.6 Hz, 1H, CHβD-BiPhe), 4.10 (m, 1H, CHαLeu), 3.75 (m, 1H, CHαVal), 3.28 (m, 1H, CHβ2D-Phe), 3.10 (m, 1H, CHβ2D-Thia), 2.92 (dd, J = 9.9, 14.3 Hz, 1H, CHβ1D-Phe), 2.92 (s, 3H, NCH₃), 2.67 (m, 1H, CHβ1D-Thia), 1.94 (m, 1H, CHβVal), 1.48 (m, 1H, CHβ2Leu), 1.30 (m, 1H, CHγLeu), 1.26 (m, 1H, CHβ1Leu), 0.71-0.66 (m, 12H, CH₂CH(CH₃)₂, CHCH(CH₃)₂).

Dipeptide resin-O-Val-3-(4-Thia)-D-Ala-Fmoc was synthesized following “General peptide coupling” procedure, by using 1.00 g (0.74 mmol, 1.0 equivalent) of resin-O-Val-NH₂, 0.88 g (2.22 mmol, 3.0 equivalents) of Fmoc-D-3-(4-Thiazoyl)Ala-OH, 0.30 g of HOBt (2.22 mmol, 3.0 equivalents), 0.70 mL of DIC (4.44 mmol, 6.0 equivalents) and 3.7 mL of DMF to generate a concentration of 0.20 M and the Fmoc group was removed following the “General Fmoc removal” procedure for SPPS. o-NBS protected resin-bound dipeptide was synthesized following the “o-NBS Protection” procedure, by using 0.66 g of o-NBS-Cl (2.96 mmol, 4 equivalents) and 0.98 mL of collidine (7.4 mmol, 10 equivalents) and 25 mL of CH₂Cl₂ to generate a concentration of 0.03 M.

A solution of 0.97 g of triphenylphosphine (3.7 mmol, 5 equivalents) and 0.30 mL of MeOH (10 equivalents) in dry THF was added to the resin bound o-NBS-protected peptides and stirred for 10 mins. A solution of 0.73 mL DIAD (5 equivalents) in dry THF was then added portion by portion to the reaction mixture and stirred for 4 hours at room temperature. The resin was filtered off, and washed with DMF (5x). N-Methyl-N-o-NBS-peptides were then cleaved from resin by treatment of a small amount of resin with TFE:CH₂Cl₂ (1:1) and analysed by LCMS to monitor the reaction completion.

LC/MS (ESI): m/z called for C₁₇H₂₀N₄O₇S₂ (M+1) = 456.08, found 456.80.

A solution of 0.97 g of triphenylphosphine (3.7 mmol, 5 equivalents) and 0.30 mL of MeOH (10 equivalents) in dry THF was added to the resin bound o-NBS-protected peptides and stirred for 4 hours at room temperature. The resin was filtered off, and washed with DMF (5x). N-Methyl-N-o-NBS-peptides were then cleaved from resin by treatment of a small amount of resin with TFE:CH₂Cl₂ (1:1) and analysed by LCMS to monitor the reaction completion.

LC/MS (ESI): m/z called for C₁₃H₂₂N₄O₅S₂ (M+1) = 471.09, found 470.85.
For o-NBS deprotection, the resin-bound N-Methyl-N-o-NBS-peptides was treated with a solution of 0.52 mL mercaptoethanol (10 equivalents) and 0.55 mL DBU (5 equivalents) in DMF for 2 hours. The deprotection procedure was repeated one more time and the resin was washed with DMF (5x).

LC/MS (ESI): m/z called for C_{12}H_{19}N_{3}O_{3}S (M+1) = 286.11, found 285.80.

Following “General peptide coupling” and published procedure for SPPS (Koay, Y. C.; McConnell, J. R.; Wang, Y.; Kim, S. J.; Buckton, L. K.; Mansour, F.; McAlpine, S. R. ACS Med. Chem. Lett. 2014, 5, 771-776), linear pentapeptide of resin-O-Val-N-Me-3-(4-Thia)-D-Ala-D-Phe-3,3-Dipe-D-Ala-Leu-NH₂ was synthesized by using resin-O-Val-3-(4-Thia)-D-Ala-NH-CH₃ and the subsequent peptide coupling in the sequence as mentioned below: 0.86 g (2.2 mmol, 3.0 equivalents) of Fmoc-D-Phe-OH, 1.03 g (2.2 mmol, 3.0 equivalents) of Fmoc-3,3-Diphe-D-Ala-OH and 0.78 g (2.2 mmol, 3.0 equivalents) of Fmoc-Leu-OH. Each peptide coupling was done in the presence of 0.30 g of HOAt (2.2 mmol, 3.0 equivalents) or 0.30 g of HOBt (2.2 mmol, 3.0 equivalents), 0.70 mL of DIC (4.4 mmol, 6.0 equivalents) and 3.7 mL of DMF to generate a concentration of 0.20 M. Each coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained to afford the Fmoc-protected resin-bound pentapeptide and the Fmoc protecting group was removed following the “General Fmoc removal” after the completion of each coupling reaction.

The double deprotected linear pentapeptide (DDLP) HO-Val-N-Me-3-(4-Thia)-D-Ala-D-Phe-3,3-Diphe-D-Ala-Leu-NH₂ was generated following the “Resin cleavage of linear peptide” procedure for SPPS. The linear pentapeptide was cleaved from the resin using a mixed solution of 6 mL of TFE and 6 mL of CH₂Cl₂. The resin containing solution was filtered and dried in vacuo to yield the DDLP as a white solid (284 mg, overall 50%).

LC/MS (ESI): m/z called for C_{42}H_{52}N_{6}O_{6} (M+1) = 769.37, found 769.40.

Cyclo Val-N-Me-3-(4-Thia)-D-Ala-D-Phe-3,3-Diphe-D-Ala-Leu (38) was synthesized using 0.15 g of the DDLP generated (0.20 mmol, 1.0 equivalent), 0.05 g of TBTU (0.16 mmol, 0.80 equivalent), 0.07 g of HATU (0.20 mmol, 1.0 equivalent), 0.03 g of DMTMM (0.12 mmol, 0.60 equivalent), 0.27 mL of DIPEA (1.6 mmol, 8.0 equivalents) in anhydrous CH₂Cl₂ (195 mL, 0.001M) following “Macrocyclization procedure (syringe pump)” procedure. The reaction was then stirred overnight and the reaction was monitored via LC/MS. Upon completion, the reaction mixture was subjected to acid-base wash, which was extracted twice with 10% (v/v) HCl (aq). The organic layer was then re-extracted with a saturated of NaHCO₃ aqueous solution. The combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo. The resulting crude product was purified via flash column chromatography on silica gel using an ethyl acetate-hexane gradient system, followed by purification via HPLC to yield compound 38 as white solid (40 mg, 27%).

R⁺: 0.28 (100% EtOAc)
LC/MS: m/z called for C_{42}H_{50}N_{6}O_{5}S (M+1) = 751.36, found 750.95.

HRMS (ESI-TOF): M+1, found 751.3636 C_{42}H_{50}N_{6}O_{5}S requires 751.3636.

1H NMR (600 MHz, DMSO): δ 9.02 (d, J = 1.9 Hz, 1H, D-Thia), 8.46 (d, J = 9.1 Hz, NH), 7.84 (d, J = 6.7 Hz, NH), 7.67 (d, J = 7.3 Hz, NH), 7.48 (d, J = 10.0 Hz, NH), 7.37-7.15 (m, 13H, D-BiPhe, D-Phe), 5.03 (dd, J = 9.1, 11.7 Hz, 1H, CH₂ D-Thia), 4.67 (m, 1H, CH₂ α D-Phe), 4.41 (d, J = 11.7 Hz, 1H, CHβ D-BiPhe), 3.99 (m, 2H, CHα Val, CHα D-Phe), 3.87 (m, 1H, CHβ Leu), 3.43 (dd, J = 3.8, 14.9 Hz, 1H, CHβ2 D-Phe), 3.21 (dd, J = 9.5, 14.9 Hz, 1H, CHβ2 D-Phe), 2.69 (m, 1H, CHβ2 D-Thia), 2.59 (s, 3H, NCH₃), 2.56 (d, J = 6.4 Hz, 1H, CHβ3 D-Thia), 1.76 (m, 1H, CHβ Val), 1.50 (m, 1H, CHβ2 Leu), 1.22 (m, 1H, CHβ2 Leu), 1.14 (m, 1H, CHγ Leu), 0.79 (t, J = 6.6 Hz, 6H, CH(CH₃)₂), 0.67 (d, J = 6.5 Hz, 3H, CH₂CH(CH₃)₂), 0.56 (d, J = 6.5 Hz, 3H, CH₂CH(CH₃)₂).

13C NMR (150 MHz, DMSO): δ 170.61, 170.22, 170.16, 169.92, 168.17, 155.06, 154.02, 142.28, 141.14, 137.14, 129.26, 128.97, 128.63, 128.55, 128.35, 127.06, 126.86, 126.79, 115.57, 65.09, 61.01, 58.52, 52.73, 52.22, 50.77, 40.53, 39.13, 38.68, 31.74, 29.63, 25.14, 23.39, 21.81, 19.66, 19.53.
Following “General peptide coupling” procedure for SPPS and the published procedure for SPPS (Koay, Y. C.; McConnell, J. R.; Wang, Y.; Kim, S. J.; Buckton, L. K.; Mansour, F.; McAlpine, S. R. ACS Med. Chem. Lett. 2014, 5, 771-776), linear pentapeptide of resin-O-D-Phe-3,3-Diphe-D-Ala-N-Me-D-Leu-Val-3-(4-Thia)-D-Ala-NH₂ was synthesized by using 1.00 g (0.72 mmol, 1.0 equivalent) of resin-O-D-Phe-NH₂ and the subsequent peptide coupling in the sequence as mentioned below: 1.00 g (2.2 mmol, 3.0 equivalents) of Fmoc-3,3-Diphe-D-Ala-OH, 0.79 g (2.2 mmol, 3.0 equivalents) of Fmoc-N-Me-D-Leu-OH, 0.73 g (2.2 mmol, 3.0 equivalents) of Fmoc-Val-OH and 0.85 g (2.2 mmol, 3.0 equivalents) of Fmoc-D-3-(4-Thiazoyl)Ala-OH. Each peptide coupling was done in the presence of 0.29 g of HOAt (2.2 mmol, 3.0 equivalents) or 0.29 g of HOBt (2.2 mmol, 3.0 equivalents), 0.68 mL of DIC (4.3 mmol, 6.0 equivalents) and 3.6 mL of DMF to generate a concentration of 0.20 M. Each coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained to afford the Fmoc-protected resin-bound pentapeptide and the Fmoc protecting group was removed following the “General Fmoc removal” after the completion of each coupling reaction.

The double deprotected linear pentapeptide (DDLP) HO-D-Phe-3,3-Diphe-D-Ala-N-Me-D-Leu-Val-3-(4-Thia)-D-Ala-NH₂ was generated following the “Resin cleavage of linear peptide” procedure for SPPS. The linear pentapeptide was cleaved from the resin using a mixed solution of 6 mL of TFE and 6 mL of CH₂Cl₂. The resin containing solution was filtered and dried in vacuo to yield the DDLP as a white solid (393 mg, overall 71%).

LC/MS (ESI): m/z called for C₄₂H₅₂N₆O₆S (M+1) = 769.37, found 769.00.

Cyclo D-Phe-3,3-Diphe-D-Ala-N-Me-D-Leu-Val-3-(4-Thia)-D-Ala (39) was synthesized using 0.39 g of the DDLP generated (0.51 mmol, 1.0 equivalent), 0.13 g of TBTU (0.41 mmol, 0.80 equivalent), 0.16 g of HATU (0.41 mmol, 0.80 equivalent), 0.11 g of DMTMM (0.41 mmol, 0.80 equivalent), 0.71 mL of DIPEA (4.1 mmol, 8.0 equivalents) in anhydrous CH₂Cl₂ (511 mL, 0.001M) following “Macrocyclization procedure (syringe pump)” procedure. The reaction was then stirred overnight and the reaction was monitored via LC/MS. Upon completion, the reaction mixture was subjected to acid-base wash, which was extracted twice with 10% (v/v) HCl (aq). The organic layer was then re-extracted with a saturated of NaHCO₃ aqueous solution. The combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo. The resulting crude product was purified via flash column chromatography on silica gel using an ethyl acetate-hexane gradient system, followed by purification via HPLC to yield compound 39 as white solid (63 mg, 16%).

Rf: 0.30 (100% EtOAc)
LC/MS: m/z called for C₄₂H₅₀N₆O₅S (M+1) = 751.36, found 751.00.
HRMS (ESI-TOF): M+Na+, found 773.3455 C₄₂H₅₀N₆O₅S₁Na₁ requires 773.3461.

1H NMR (600 MHz, DMSO): δ 9.10 (d, J = 9.1 Hz, 1H), 9.02 (m, 2NH), 8.34 (br, NH), 7.68 (br, NH), 7.41-7.15 (m, 15H, D-BiPhe, D-Phe), 6.88 (d, J = 9.1 Hz, 1H), 5.19 (t, J = 10.9 Hz, 1H, CHα D-BiPhe), 4.78 (q, J = 7.4 Hz, 1H, CHα D-Phe), 4.56 (br, 1H, CHα Leu), 4.50 (d, J = 12.3 Hz, 1H, CHβ D-BiPhe), 4.28 (t, J = 9.1 Hz, 1H, CHα Val), 3.82 (q, J = 7.5 Hz, 1H, CHα D-Thia), 3.15 (dd, J = 7.3, 14.2 Hz, 1H, CHβ D-BiPhe), 3.08 (dd, J = 6.9, 14.0 Hz, 1H, CHβ D-Thia), 2.90 (m, 2H, CHβ D-Thia), 2.74 (s, 3H, NCH₃), 2.02 (m, 1H, CHβ Val), 0.99 (m, 1H, CHβ2 Leu), 0.85-0.56 (m, 14H, CH2γ Leu, CH2β1 Leu, CH2CH(CH₃)₂, CHCH(CH₃)₂).

13C NMR (150 MHz, DMSO): δ 171.51, 171.14, 170.61, 170.57, 169.62, 153.83, 152.75, 141.80, 141.10, 138.00, 129.09, 129.03, 128.82, 128.77, 128.61, 128.13, 127.19, 126.97, 126.73, 116.19, 59.11, 57.11, 57.00, 54.43, 52.32, 52.26, 39.43, 36.51, 34.87, 30.00, 29.96, 24.20, 23.15, 22.63, 20.02, 18.50.

Following “Benoiton method” procedure for N-methylation, Boc-N-Me-3,3-Diphe-D-Ala-OH was synthesized using 1.00 g (2.93 mmol, 1 equivalent) of Boc-3,3-Diphe-D-Ala-NH₂, 1.17 g of NaH (29.3 mmol, 10 equivalents) and 4.16 g of CH₃I (29.3 mmol, 10 equivalents) in anhydrous CH₂Cl₂ (325 mL, 0.001M). The completion of the reaction was monitored by TLC. The reaction mixture was then dried in vacuo and diluted with ethyl acetate. The organic layer was washed with 10% (v/v) HCl (aq), dried over Na₂SO₄, filtered, concentrated in vacuo and subjected to the next reaction without purification.

LC/MS (ESI): m/z called for C₂₃H₂₆NO₄Na (M+ Na⁺) = 378.1681, found 378.00.
Following “General peptide coupling” procedure for SPPS and the published procedure for SPPS (Koay, Y. C.; McConnell, J. R.; Wang, Y.; Kim, S. J.; Buckton, L. K.; Mansour, F.; McAlpine, S. R. ACS Med. Chem. Lett. 2014, 5, 771-776), linear pentapeptide of resin-O-Leu-Val-3-(4-Thia)-D-Ala-D-Phe-3,3-Diphenyl-D-Ala-NH(CH3) was synthesized by using 1.00 g (0.5 mmol, 1.0 equivalent) of resin-O-Leu-NH2 and the subsequent peptide coupling in the sequence as mentioned below: 0.51 g (1.5 mmol, 3.0 equivalents) of Fmoc-Val-OH, 0.59 g (1.5 mmol, 3.0 equivalents) of Fmoc-D-3-(4-Thiazolyl)Ala-OH, 0.58 g (1.5 mmol, 3.0 equivalents) of Fmoc-D-Phe-OH and 0.53 g (1.5 mmol, 3.0 equivalents) of Boc-N-Me-3,3-Diphenyl-D-Ala-OH. Each peptide coupling was done in the presence of 0.20 g of HOAt (1.5 mmol, 3.0 equivalents) or 0.20 g of HOBt (1.5 mmol, 3.0 equivalents), 0.47 mL of DIC (3.0 mmol, 6.0 equivalents) and 2.5 mL of DMF to generate a concentration of 0.20 M. Each coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained after the completion of each coupling reaction. The mixture was dried to afford the Fmoc-protected resin-bound pentapeptide and the Fmoc protecting group was removed following the “General Fmoc removal” after the completion of each coupling reaction.

The double deprotected linear pentapeptide (DDLP) HO-Leu-Val-3-(4-Thia)-D-Ala-D-Phe-3,3-Diphenyl-D-Ala-NH(CH3) was generated following the “Resin cleavage of linear peptide” procedure for SPPS. The linear pentapeptide was cleaved from the resin using a mixed solution of 6 mL of TFA and 6 mL of CH2Cl2. The resin containing solution was filtered and dried in vacuo to yield the DDLP as a white solid (261 mg, overall 68%).

**Cyclo Leu-Val-3-(4-Thia)-D-Ala-D-Phe-N-Me-3,3-Diphe-D-Ala (40)** was synthesized using 0.25 g of the DDLP generated (0.33 mmol, 1.0 equivalent), 0.06 g of TBTU (0.20 mmol, 0.60 equivalent), 0.15 g of HATU (0.39 mmol, 1.20 equivalent), 0.05 g of DMTMM (0.20 mmol, 0.60 equivalent), 0.45 mL of DIPEA (2.6 mmol, 8.0 equivalents) in anhydrous CH2Cl2 (325 mL, 0.001M) following “Macrocyclization procedure (syringe pump)” procedure. The reaction was then stirred overnight and the reaction was monitored via LC/MS. Upon completion, the reaction mixture was subjected to acid-base wash, which was extracted twice with 10% (v/v) HCl (aq). The organic layer was then re-extracted with a saturated of NaHCO3 aqueous solution. The combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo containing solution was filtered and dried in vacuo to yield the DDLP as a white solid (27 mg, 11%).

**HRMS (ESI-TOF):** M+1, found 769.37, found 769.40.

**LC/MS (ESI):** m/z called for C42H50N6O5S (M+1) = 751.36, found 751.30.

**LC/MS (ESI):** m/z called for C42H52N6O6 (M+1) = 769.37, found 769.40.

**a) Major conformer**

1H NMR (600 MHz, DMSO): δ 9.06 (d, J = 1.9 Hz, 1H, D-Thia), 8.55 (d, J = 7.6 Hz, NH), 8.49 (d, J = 6.1 Hz, NH), 7.73 (m, NH), 7.47-7.01 (m, 15H, D-BiPhe, D-Phe, NH), 6.67 (m, 1H, D-Thia), 5.87 (d, J = 11.6 Hz, 1H, CHα-D-BiPhe), 4.71 (m, 1H, CHα-D-Phe), 4.55 (d, J = 11.5 Hz, 1H, CHβ-D-BiPhe), 4.41 (ddd, J = 3.8, 11.4, 11.4 Hz, 1H, CHα-D-Thia), 3.92 (m, 1H, CHα-Leu), 3.81 (dd, J = 5.2, 7.6 Hz, 1H, CHβ Val), 3.24 (dd, J = 8.7, 14.3 Hz, 1H, CHβ2-D-Phe), 3.12 (m, 1H, CHβ1-D-Phe), 2.99 (m, 1H, CHβ2-D-Thia), 2.64 (dd, J = 11.2, 13.6 Hz, 1H, CHβ1-D-Thia), 2.54 (s, 3H, NCH3), 2.06 (m, 1H, CHβ Val), 1.44 (m, 1H, CHβ2-Leu), 0.81-0.60 (m, 14H, CHβ1-Leu, CHγ-Leu, CHCH(CH3)2), CH2CH(CH3)2).

13C NMR (150 MHz, DMSO): δ 173.42, 172.70, 170.10, 169.34, 168.35, 154.12, 152.60, 142.54, 141.06, 137.63, 129.52, 129.24, 129.07, 128.81, 128.69, 128.08, 127.18, 126.73, 126.09, 116.16, 61.47, 59.96, 58.58, 54.24, 53.67, 49.03, 47.24, 42.21, 39.62, 29.68, 28.94, 23.50, 22.42, 19.58, 17.74.

**b) Minor conformer**

1H NMR (600 MHz, DMSO): δ 9.01 (d, J = 1.9 Hz, 1H, D-Thia), 8.22 (d, J = 6.8 Hz, NH), 8.02 (d, J = 7.7 Hz, NH), 7.71 (br, NH), 7.64 (d, J = 7.9 Hz, NH), 7.47-7.01 (m, 15H, D-BiPhe, D-Phe, 6.74 (m, 1H, D-Thia), 5.82 (d, J = 11.6 Hz, 1H, CHα-D-BiPhe), 4.71 (m, 1H, CHβ-D-BiPhe), 4.64 (m, 1H, CHα-D-Phe), 4.34 (m, 1H, CHβ-Leu), 3.64 (m, 1H, CHα Val), 3.45 (m, 1H, CHα-D-Thia), 3.12 (m, 1H, CHβ2-D-Phe), 2.99 (m, 3H, CHβ2-D-Phe, CHβ2-D-Thia, CHβ1-D-Thia), 2.70 (s, 3H, NCH3), 1.85 (m, 1H, CHβ Val), 1.67 (1H, CHβ2-Leu), 1.36 (m, 1H, CHγ-Leu), 1.10 (m, 1H, CHβ1-Leu), 0.81-0.60 (m, 12H, CHCH(CH3)2), CH2CH(CH3)2).
Following “General peptide coupling” procedure for SPPS and the published procedure for SPPS (Koay, Y. C.; McConnell, J. R.; Wang, Y.; Kim, S. J.; Buckton, L. K.; Mansour, F.; McAlpine, S. R. ACS Med. Chem. Lett. 2014, 5, 771-776), linear pentapeptide of resin-O-Leu-Val-3-(4-Thia)-D-Ala-D-Phe-3,3-Diphenyl-D-Ala-NH₂ was synthesized using 1.00 g (0.5 mmol, 1.0 equivalent) of Fmoc-Val-OH, 0.59 g (1.5 mmol, 3.0 equivalents) of Fmoc-D-Phe-OH and 0.70 g (1.5 mmol, 3.0 equivalents) of Fmoc-3,3-Diphenyl-D-Ala-OH. Each coupling peptide was done in the presence of 0.20 g of HOAt (1.5 mmol, 3.0 equivalents) or 0.20 g of HOBT (1.5 mmol, 3.0 equivalents), 0.47 mL of DIC (3.0 mmol, 6.0 equivalents) and 2.5 mL of DMF to generate a concentration of 0.20 M. Each coupling reaction was run for 3 hours and a negative ninhydrin test was used to confirm the reaction completion. The reaction mixture was drained to afford the Fmoc-protected resin-bound pentapeptide and the Fmoc protecting group was removed following the “General Fmoc removal” after the completion of each coupling reaction.

The double deprotected linear pentapeptide (DDLP) HO-Leu-Val-3-(4-Thia)-D-Ala-D-Phe-3,3-Diphenyl-D-Ala-NH₂ was generated following the “Resin cleavage of linear peptide” procedure for SPPS. The linear pentapeptide was cleaved from the resin using a mixed solution of 6 mL of TFE and 6 mL of CH₂Cl₂. The resin containing solution was filtered and dried in vacuo to yield the DDLP as a white solid (271 mg, overall 72%).

LC/MS (ESI): m/z called for C₄₁H₄₈N₆O₅S (M+1) = 737.34, found 737.00.

a) Major conformer

1H NMR (500 MHz, DMSO): δ 8.98 (d, J = 1.9 Hz, 1H, D-Thia), 8.60 (br, NH), 8.33 (br, NH), 8.13 (m, 2NH), 7.60 (br, NH), 7.38-7.12 (m, 15H, 1H, D-BiPhe, D-Phe), 6.76 (m, 1H, D-Thia), 5.14 (t, J = 10.8 Hz, 1H, CHα D-BiPhe), 4.55 (m, 2H, CHβ D-Phe, CHα Val), 3.88 (m, 3H, CHβ D-BiPhe, CHα Leu, CHα D-Thia), 3.22 (dd, J = 7.6, 14.5 Hz, 1H, CHβ2 D-Phe), 3.03 (dd, J = 7.2, 14.5 Hz, 1H, CHβ1 D-Phe), 2.81 (dd, J = 7.6, 13.7 Hz, 1H, CHβ2 D-Thia), 2.62 (dd, J = 6.9, 13.7 Hz, 1H, CHβ1 D-Thia), 1.81 (m, 1H, CHβ Val), 1.19 (m, 2H, CHβ Leu), 0.84 (m, 1H, CHα Leu), 0.73-0.59 (m, 12H, CHCH(CH₃)₂, CH₂CH(CH₃)₂).

13C NMR (125 MHz, DMSO): δ 171.73, 171.39, 170.85, 170.62, 170.27, 153.88, 153.73, 141.80, 141.48, 137.79, 129.17, 128.90, 128.86, 128.73, 128.51, 128.46, 127.17, 126.73, 126.68, 115.33, 61.25, 59.16, 55.39, 53.34, 52.91, 51.59, 37.26, 32.84, 29.55, 24.24, 23.04, 22.20, 21.60, 19.34, 19.13.

b) Minor conformer

1H NMR (500 MHz, DMSO): δ 8.98 (d, J = 1.9 Hz, 1H, D-Thia), 8.60 (br, NH), 8.33 (br, NH), 8.27 (m, J = 6.7 Hz, NH), 7.95 (d, J = 8.6 Hz, NH), 7.43 (d, J = 7.4 Hz, NH), 7.38-7.12 (m, 15H, D-BiPhe, D-Phe), 6.94 (d, J = 6.8 Hz, 1H, D-Thia), 5.06 (m, 1H, CHα D-BiPhe), 4.38 (m, 3H, CHα D-Phe), 4.33 (m, J = 11.8 Hz, 1H, CHα Val), 3.98 (m, 1H, CHα D-Thia), 3.88 (m, 1H, CHβ D-BiPhe), 3.73 (m, J = 8.5 Hz, 1H, CHα Leu), 3.16 (m, 1H, CHβ D-Phe), 2.98 (m, 2H, CHβ2 D-Phe, CHβ1 D-Thia), 2.44 (dd, J = 6.3, 13.7 Hz, 1H, CHβ2 D-Thia), 1.44 (m, 1H, CHβ Val), 1.16 (m, 2H, CHβ Leu), 0.78 (m, 1H, CHα Leu), 0.73-0.59 (m, 12H, CHCH(CH₃)₂, CH₂CH(CH₃)₂).

S27
Compound 7: LCMS of DDLP HO-Leu-N-Me-Val-D-Leu-D-His(Boc)-3,3-Diphe-D-Ala-NH₂

==== Shimadzu LCMSsolution Analysis Report ====
S29

Compound 7: LCMS of cyclo Leu-N-Me-Val-D-Leu-D-His(Boc)-3,3-Diphe-D-Ala

===== Shimadzu LCMSsolution Analysis Report =====
Compound 7: HRMS of cyclo Leu-N-Me-Val-D-Leu-D-His(Boc)-3,3-Diphe-D-Ala

MS Data from Orbitrap

Full spectrum
Compound 7: $^1$HNMR of cyclo Leu-N-Me-Val-D-Leu-D-His(Boc)-3,3-Diphe-D-Ala

Compound 7: $^{13}$CNMR of cyclo Leu-N-Me-Val-D-Leu-D-His(Boc)-3,3-Diphe-D-Ala
Compound 7: $^1$H-$^{13}$C HSQC of cyclo Leu-N-Me-Val-D-Leu-D-His(Boc)-3,3-Diphe-D-Ala

Compound 7: $^1$H-$^{13}$C HMBC of cyclo Leu-N-Me-Val-D-Leu-D-His(Boc)-3,3-Diphe-D-Ala
Compound 7: $^1$H-$^1$H COSY of cyclo-Leu-N-Me-Val-D-Leu-D-His(Boc)-3,3-Diphe-D-Ala
Compound 8: LCMS of cyclo Leu-N-Me-Val-D-Leu-D-His-3,3-Diphe-D-Ala

== Shima Rossi LCMS solution Analysis Report ==
Compound 8: HRMS of cyclo Leu-N-Me-Val-D-Leu-D-His-3,3-Diphe-D-Ala

MS Data from Orbitrap

Full spectrum
Compound 8: $^1$HNMR of cyclo Leu-N-Me-Val-D-Leu-D-His-3,3-Diphe-D-Ala

Compound 8: $^{13}$CNMR of cyclo Leu-N-Me-Val-D-Leu-D-His-3,3-Diphe-D-Ala
Compound 9: LCMS of DDLP HO-Leu-N-Me-Val-D-Leu-D-Glu(OtBu)-3,3-Diphe-D-Ala-NH₂

==== Shimadzu LCMSsolution Analysis Report ====

Chromatogram
LPP282 C:\LabSolutions\Data\McAlpine\kay\2014\140618\LPP282.lcd

MS Spectrum Graph
Ret Time: 3.900 (Scan#229)
BG Mode: Off
Mass Peaks: 1692, Base Peak: 766.60 [69663400]; Polarity: Pos, Segment 1 - Event1

S37
Compound 9: LCMS of cyclo Leu-N-Me-Val-D-Leu-D-Glu(OtBu)-3,3-Diphe-D-Ala

==== Shimadzu LCMSsolution Analysis Report ====
Compound 9: HRMS of cyclo Leu-N-Me-Val-D-Leu-D-Glu(OtBu)-3,3-Diphe-D-Ala

MS Data from Orbitrap

Full spectrum:
S30312_11 Full #8 RT: 0.41 AV: 1 NL: 23457
T: FTMS + c NSI Full ms [50:0.05-2000.00]
Compound 9: $^1$HNMR of cyclo Leu-N-Me-Val-D-Leu-D-Glu(OtBu)-3,3-Diphe-D-Ala

![HNMR Spectrum](image1)

**Compound 9: $^{13}$CNMR of cyclo Leu-N-Me-Val-D-Leu-D-Glu(OtBu)-3,3-Diphe-D-Ala**

![CNMR Spectrum](image2)
Compound 10: LCMS of cyclo Leu-N-Me-Val-D-Leu-D-Glu-3,3-Diphe-D-Ala

Shimadzu LCMSsolution Analysis Report

Chromatogram

MS Spectra Graph

Retention Time: 2.207 (Scan: 511)
BG Mode: 3
Mass Peaks: 1513 Base Peak 692.05 (11533500) Polarity: Pos Segment 1 - Event 1
Compound 10: HRMS of cyclo Leu-N-Me-Val-D-Leu-D-Glu-3,3-Diphe-D-Ala

MS Data from Orbitrap

Full spectrum
Compound 10: $^1$HNMR of cyclo Leu-N-Me-Val-D-Leu-D-Glu-3,3-Diphe-D-Ala

Compound 10: $^{13}$CNMR of cyclo Leu-N-Me-Val-D-Leu-D-Glu-3,3-Diphe-D-Ala
Compound 11: LCMS of cyclo Leu-N-Me-Val-D-Leu-D-Glu(OMe)-3,3-Diphe-D-Ala

==== Shimadzu LCMSsolution Analysis Report====

Chromatogram

MS Spectrum Graph

Ret Time: 5.700 (Scan#337)
BG Mode: 2
Mass Peaks: 1490 Base Peak: 728.00 (14019388) Polarity: Pos Segment1 - Event1

S44
Compound 11: HRMS of cyclo Leu-N-Me-Val-D-Leu-D-Glu(OMe)-3,3-Dipe-D-Ala

MS Data from Orbitrap

Full spectrum
Compound 11: $^1$H-NMR of cyclo Leu-N-Me-Val-D-Leu-D-Glu(OMe)-3,3-Diphe-D-Ala

Compound 11: $^{13}$C-NMR of cyclo Leu-N-Me-Val-D-Leu-D-Glu(OMe)-3,3-Diphe-D-Ala
Compound 12: LCMS of DDLP HO-Leu-N-Me-Val-3-(2’-Pyr)-D-Ala-D-Phe-3,3-Diphe-D-Ala-NH₂
Compound **12**: LCMS of cyclo Leu-N-Me-Val-3-(2’-Pyr)-D-Ala-D-Phe-3,3-Diphe-D-Ala
Compound 12: HRMS of cyclo Leu-N-Me-Val-3-(2’-Pyr)-D-Ala-D-Phe-3,3-Diphe-D-Ala
Compound 12: $^1$HNMR of cyclo Leu-N-Me-Val-3-(2’-Pyr)-D-Ala-D-Phe-3,3-Diphe-D-Ala

S50
Compound 12: $^1$H-$^{13}$C HSQC of cyclo Leu-N-Me-Val-3-(2'-Pyr)-D-Ala-D-Phe-3,3-Diphe-D-Ala

Compound 12: $^1$H-$^{13}$C HMBC of cyclo Leu-N-Me-Val-3-(2'-Pyr)-D-Ala-D-Phe-3,3-Diphe-D-Ala
Compound 12: $^1$H-$^1$H COSY of cyclo Leu-N-Me-Val-3-(2'-Pyr)-D-Ala-D-Phe-3,3-DiPhe-D-Ala

Compound 13: LCMS of DDLP HO-Leu-N-Me-Val-3-(3-pyridyl)-D-Ala-D-Phe-3,3-DiPhe-D-Ala-NH$_2$
Compound 13: LCMS of Leu-N-Me-Val-3-(3-pyridyl)-D-Ala-D-Phe-3,3-DiPhe-D-Ala

S53
Compound 13: HRMS of cyclo Leu-N-Me-Val-3-(3-pyridyl)-D-Ala-D-Phe-3,3-DiPhe-D-Ala

[Graph of mass spectra with peaks at m/z values and relative abundances.]
Compound 13: $^1$HNMR of cyclo Leu-N-Me-Val-3-(3-pyridyl)-D-Ala-D-Phe-3,3-DiPhe-D-Ala

Compound 13: $^{13}$C NMR of cyclo Leu-N-Me-Val-3-(3-pyridyl)-D-Ala-D-Phe-3,3-DiPhe-D-Ala
Compound 13: $^1$H-$^{13}$C HSQC of cyclo Leu-N-Me-Val-3-(3-pyridyl)-D-Ala-D-Phe-3-3-DiPhe-D-Ala

Compound 13: $^1$H-$^{13}$C HMBC of cyclo Leu-N-Me-Val-3-(3-pyridyl)-D-Ala-D-Phe-3-3-DiPhe-D-Ala
Compound 14: LCMS of DDLP HO-Leu-N-Me-Val-3-(4-pyridyl)-D-Ala-D-Phe-3,3-DiPhe-D-Ala-NH₂

==== Shimadzu LCMSsolution Analysis Report ====

Chromatogram

MS Spectrum Graph

Retention Time: 2.700 (Scan #: 127)
Mass Peak: 1578, 1825, 2163 (Scan #: 104)
Parent: Pos, Segment 1 - Event 1

m/z
Compound 14: LCMS of Leu-N-Me-Val-3-(4-pyridyl)-D-Ala-D-Phe-3,3-DiPhe-D-Ala
Compound 14: HRMS of cyclo Leu-N-Me-Val-3-(4-pyridyl)-D-Ala-D-Phe-3,3-DiPhe-D-Ala

2_SM257 #2-17  RT: 0.04-0.47  AV: 16  NL: 4.01E8
T: FTMS + c NSI Fullscan [150.00-2000.00]
Compound 14: $^1$HNMR of cyclo Leu-N-Me-Val-3-(4-pyridyl)-D-Ala-D-Phe-3,3-DiPhe-D-Ala

Compound 14: $^1$H-13C HSQC of cyclo Leu-N-Me-Val-3-(4-pyridyl)-D-Ala-D-Phe-3,3-DiPhe-D-Ala
Compound 14: $^1$H-$^{13}$C HMBC of cyclo Leu-N-Me-Val-3-(4-pyridyl)-D-Ala-D-Phe-3,3-DiPhe-D-Ala
Compound 15: LCMS of DDLP HO-Leu-N-Me-Val-3-(4-Thia)-D-Ala-D-Phe-3,3-Diphe-D-Ala-NH₂
Compound 15: LCMS of cyclo Leu-N-Me-Val-3-(4-Thia)-D-Ala-D-Phe-3,3-Diphe-D-Ala
Compound 15: HRMS of cyclo Leu-N-Me-Val-3-(4-Thia)-D-Ala-D-Phe-3,3-Diphe-D-Ala

MS Data from Orbitrap

Full spectrum

SM758_Positve #6  RT: 0.28  AV: 1  NL: 2.53E8
T: FTMS + p NSI Full ms [160.00-200.00]
Compound 15: $^1$HNMR of cyclo Leu-N-Me-Val-3-(4-Thia)-D-Ala-D-Phe-3,3-Diphe-D-Ala

Compound 15: $^{13}$CNMR of cyclo Leu-N-Me-Val-3-(4-Thia)-D-Ala-D-Phe-3,3-Diphe-D-Ala
Compound 15: $^1$H-$^{13}$C HSQC of cyclo Leu-N-Me-Val-3-(4-Thia)-D-Ala-D-Phe-3,3-Diphe-D-Ala

Compound 15: $^1$H-$^{13}$C HMBC of cyclo Leu-N-Me-Val-3-(4-Thia)-D-Ala-D-Phe-3,3-Diphe-D-Ala
Compound 15: $^1$H-$^1$H COSY of cyclo Leu-N-Me-Val-3-(4-Thia)-D-Ala-D-Phe-3,3-Diphe-D-Ala
Compound 16: LCMS of DDLP HO-Leu-N-Me-Val-D-Tyr(OMe)-D-Phe-3,3-Diphe-D-Ala-NH₂
Compound 16: LCMS of cyclo Leu-N-Me-Val-D-Tyr(OMe)-D-Phe-3,3-Diphe-D-Ala
Compound 16: HRMS of cyclo Leu-N-Me-Val-D-Tyr(OMe)-D-Phe-3,3-Diphe-D-Ala

MS Data from Orbitrap
Compound 16: $^1$HNMR of cyclo Leu-N-Me-Val-D-Tyr(OMe)-D-Phe-3,3-Diphe-D-Ala

Compound 16: $^{13}$CNMR of cyclo Leu-N-Me-Val-D-Tyr(OMe)-D-Phe-3,3-Diphe-D-Ala
Compound 16: $^1$H-$^{13}$C HSQC of cyclo Leu-N-Me-Val-D-Tyr(OMe)-D-Phe-3,3-Diphe-D-Ala

Compound 16: $^1$H-$^{13}$C HMBC of cyclo Leu-N-Me-Val-D-Tyr(OMe)-D-Phe-3,3-Diphe-D-Ala
Compound 16: $^1$H-$^1$H COSY of cyclo Leu-N-Me-Val-D-Tyr(OMe)-D-Phe-3,3-Diphe-D-Ala
Compound 17: LCMS of DDLP HO-Leu-N-Me-Val-D-Trp(Boc)-D-Phe-3,3-Diphe-D-Ala-NH₂

== Shimadzu LCMSsolution Analysis Report ==
Compound 17: LCMS of cyclo Leu-N-Me-Val-D-Trp(Boc)-D-Phe-3,3-Diphe-D-Ala

Shimadzu LCMSsolution Analysis Report

Chromatogram

MS Spectrum Graph
Compound 17: LCMS of cyclo Leu-N-Me-Val-D-Trp-D-Phe-3,3-Diphe-D-Ala

==== Shimadzu LCMSsolution Analysis Report ==== 
Compound 17: HRMS of cyclo Leu-N-Me-Val-D-Trp-D-Phe-3,3-Diphe-D-Ala

**MS Data from Orbitrap**

**Full spectrum**

```
SM289_Positive_*#1-5 RT: 0.91-0.23 AV: 5 NL: 4.78E7
T: FTMS + p MS/MS [1000.2000.00]
```

Compound 17: $^1$HNMR of cyclo Leu-N-Me-Val-D-Trp-D-Phe-3,3-Diphe-D-Ala
Compound 17: $^{13}$CNMR of cyclo Leu-N-Me-Val-D-Trp-D-Phe-3,3-Diphe-D-Ala

Compound 17: $^1$H-$^{13}$C HSQC of cyclo Leu-N-Me-Val-D-Trp-D-Phe-3,3-Diphe-D-Ala
Compound 17: $^1$H-$^{13}$C HMBC of cyclo Leu-N-Me-Val-D-Trp-D-Phe-3,3-Diphe-D-Ala
Compound 17: $^1$H-$^1$H COSY of cyclo Leu-N-Me-Val-D-Trp-D-Phe-3,3-Diphe-D-Ala
Compound 18: LCMS of DDLP HO-Leu-\textit{\textbf{N}}-Me-Val-D-His(Boc)-D-Phe-3,3-Diphe-D-Ala-NH\textsubscript{2}

\textbf{Shimadzu LCMSsolution Analysis Report}

![LCMS Chromatogram]

![MS Spectrum Graph 1]

![MS Spectrum Graph 2]
Compound 18: LCMS of cyclo Leu-N-Me-Val-D-His(Boc)-D-Phe-3,3-Diphe-D-Ala

==== Shimadzu LCMSsolution Analysis Report ====
Compound 18: HRMS of cyclo Leu-N-Me-Val-D-His(Boc)-D-Phe-3,3-Diphe-D-Ala

**MS Data from Orbitrap**

Sample: SM291

Full spectrum:

SM291_Full #3: RT 0.13 AV 1 NL: 2.9TE7 T: FTMS + e NSI Full ms [100.00-2000.00]
Compound 18: $^1$HNMR of cyclo Leu-N-Me-Val-D-His(Boc)-D-Phe-3,3-Diphe-D-Ala

Compound 18: $^{13}$CNMR of cyclo Leu-N-Me-Val-D-His(Boc)-D-Phe-3,3-Diphe-D-Ala
Compound 18: $^1$H-$^{13}$C HSQC of cyclo Leu-N-Me-Val-D-His(Boc)-D-Phe-3,3-Diphe-D-Ala

Compound 18: $^1$H-$^{13}$C HMBC of cyclo Leu-N-Me-Val-D-His(Boc)-D-Phe-3,3-Diphe-D-Ala
Compound 18: $^1$H-$^1$H COSY of cyclo Leu-N-Me-Val-D-His(Boc)-D-Phe-3,3-Diphe-D-Ala
Compound 19: LCMS of cyclo Leu-N-Me-Val-D-His-D-Phe-3,3-Diphe-D-Ala

Shimadzu LCMS solution Analysis Report

Chromatogram
CPP291_withoutBoc C:\LabSolutions\Data\McAlpine\2014-140624\CPP291_withoutBoc.lcd

MS Spectrum Graph

Rat Time: 3.567 (Scans: 209)
BG Mode 2
Mass Peaks: 1591 Base Peak 754.80 (51883482) Polarity: Pos Segment 1 - Event 1

S89
Compound 19: HRMS of cyclo Leu-N-Me-Val-D-His-D-Phe-3,3-Diphe-D-Ala

MS Data from Orbitrap

Sample: SM291
Full spectrum:

[Graph showing mass spectrum data]
Compound 19: $^1$HNMR of cyclo Leu-N-Me-Val-D-His-D-Phe-3,3-Diphe-D-Ala

Compound 19: $^{13}$CNMR of cyclo Leu-N-Me-Val-D-His-D-Phe-3,3-Diphe-D-Ala

Compound 20: LCMS of DDLP HO-Leu-N-Me-Val-D-Glu(OtBu)-D-Phe-3,3-Diphe-D-Ala-NH$_2$
Compound 20: LCMS of cyclo Leu-N-Me-Val-D-Glu(OtBu)-D-Phe-3,3-Diphe-D-Ala

S92
Compound 20: HRMS of cyclo Leu-N-Me-Val-D-Glu(OtBu)-D-Phe-3,3-Diphe-D-Ala

S93
Compound 20: $^1$HNMR of cyclo Leu-N-Me-Val-D-Glu(OtBu)-D-Phe-3,3-Diphe-D-Ala

S94
Compound 20: $^{13}$CNMR of cyclo Leu-N-Me-Val-D-Glu(OtBu)-D-Phe-3,3-Diphe-D-Ala

Compound 21: LCMS of cyclo Leu-N-Me-Val-D-Glu-D-Phe-3,3-Diphe-D-Ala
Compound 21: HRMS of cyclo Leu-N-Me-Val-D-Glu-D-Phe-3,3-Diphe-D-Ala

S96
Compound 21: $^1$HNMR of cyclo Leu-N-Me-Val-D-Glu-D-Phe-3,3-Diphe-D-Ala
Compound 21: $^{13}$CNMR of cyclo Leu-N-Me-Val-D-Glu-D-Phe-3,3-Diphe-D-Ala

Compound 22: LCMS of cyclo Leu-N-Me-Val-D-Glu(OMe)-D-Phe-3,3-Diphe-D-Ala
Compound 22: HRMS of cyclo Leu-N-Me-Val-D-Glu(OMe)-D-Phe-3,3-Diphe-D-Ala
Compound 22: $^1$HNMR of cyclo Leu-N-Me-Val-D-Glu(OMe)-D-Phe-3,3-Diphe-D-Ala

S100
Compound 22: $^{13}$CNMR of cyclo Leu-N-Me-Val-D-Glu(OMe)-D-Phe-3,3-Diphe-D-Ala

Compound 23: LCMS of HO-Leu-3-(4-Thia)Ala-NH-o-NBS
Compound 23: LCMS of HO-Leu-3-(4-Thia)Ala-NCH₃-ο-NBS

S102
Compound 23: LCMS of HO-Leu-3-(4-Thia)Ala-NH(CH₃)
Compound 23: LCMS of DDLP HO-Leu-N-Me-3-(4-Thia)Ala-D-Leu-D-Phe-3,3-Diphe-D-Ala-NH₂

Compound 23: LCMS of cyclo Leu-N-Me-3-(4-Thia)Ala-D-Leu-D-Phe-3,3-Diphe-D-Ala

S105
Compound 23: HRMS of cyclo Leu-N-Me-3-(4-Thia)Ala-D-Leu-D-Phe-3,3-Diphe-D-Ala
MS Data from Orbitrap

Full spectrum

S107
Compound 23: $^1$HNMR of cyclo Leu-N-Me-3-(4-Thia)Ala-D-Leu-D-Phe-3,3-Diphe-D-Ala

Compound 23: $^{13}$CNMR of cyclo Leu-N-Me-3-(4-Thia)Ala-D-Leu-D-Phe-3,3-Diphe-D-Ala
Compound 23: $^1$H-$^{13}$C HSQC of cyclo Leu-N-Me-Val-3-(4-Thia)-D-Ala-D-Phe-3,3-Diphe-D-Ala

Compound 23: $^1$H-$^{13}$C HMBC of cyclo Leu-N-Me-Val-3-(4-Thia)-D-Ala-D-Phe-3,3-Diphe-D-Ala
Compound 23: $^1$H-$^1$H COSY of cyclo Leu-N-Me-Val-3-(4-Thia)-D-Ala-D-Phe-3,3-Diphe-D-Ala
Compound 24: LCMS of DDLP HO-D-Phe-3,3-DiPhe-D-Ala-3-(2-pyridyl)-Ala-N-Me-Val-D-Leu-NH₂
Compound 24: LCMS cyclo D-Phe-3,3-DiPhe-D-Ala-3-(2-pyridyl)-Ala-N-Me-Val-D-Leu
Compound 24: HRMS of cyclo D-Phe-3,3-DiPhe-D-Ala-3-(2-pyridyl)-Ala-N-Me-Val-D-Leu

3_SM259 #1-17 RT: 0.00-0.46 AV: 17 NL: 7.58E8
T: FTMS + c NSI Full ms [150.00-2000.00]

m/z

Relative Abundance

201.0884 353.2659 413.2658 659.4489 825.3471 1169.0688 1512.7904 1816.2429 201.0884 353.2659 413.2658 659.4489 825.3471 1169.0688 1512.7904 1816.2429
Compound 24: $^1$HNMR of *cyclo* D-Phe-3,3-DiPhe-D-Ala-3-(2-pyridyl)-Ala-N-Me-Val-D-Leu

![HNMR spectrum of Compound 24](image1)

Compound 24: $^1$H-$^{13}$C HSQC of *cyclo* D-Phe-3,3-DiPhe-D-Ala-3-(2-pyridyl)-Ala-N-Me-Val-D-Leu

![HSQC spectrum of Compound 24](image2)
Compound 24: $^1$H-$^{13}$C HSQC of cyclo D-Phe-3,3-DiPhe-D-Ala-3-(2-pyridyl)-Ala-$N$-Me-Val-D-Leu

Compound 24: $^1$H-$^{13}$C HMBC of cyclo D-Phe-3,3-DiPhe-D-Ala-3-(2-pyridyl)-Ala-$N$-Me-Val-D-Leu
Compound 25: LCMS of DDLP HO-D-Phe-3,3-DiPhe-D-Ala-3-(3-pyridyl)-Ala-N-Me-Val-D-Leu-NH₂
Compound 25: LCMS cyclo D-Phe-3,3-DiPhe-D-Ala-3-(3-pyridyl)-Ala-N-Me-Val-D-Leu

--- Shimadzu LCMS solution Analysis Report ---

Chromatogram

MS250 17 C:\LabSolutions\Data\McAlpine\JRM\140915\SM250 17.licd

Ret Time: 4.100(Scan#241)
REG Mode:
Mass Peaks: 1314 Base Peak: 745.05 (1970753) Polarity:Pos Segment 1: Event1

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Compound 25: HRMS of cyclo D-Phe-3,3-DiPhe-D-Ala-3-(3-pyridyl)-Ala-N-Me-Val-D-Leu
Compound 25: $^1$HNMR of cyclo D-Phe-3,3-DiPhe-D-Ala-3-(3-pyridyl)-Ala-N-Me-Val-D-Leu

Compound 25: $^{13}$C NMR of cyclo D-Phe-3,3-DiPhe-D-Ala-3-(3-pyridyl)-Ala-N-Me-Val-D-Leu
Compound 25: $^1$H-$^{13}$C HSQC of cyclo D-Phe-3,3-Diphe-D-Ala-3-(3-pyridyl)-Ala-$N$-Me-Val-D-Leu

Compound 25: $^1$H-$^{13}$C HMBC of cyclo D-Phe-3,3-Diphe-D-Ala-3-(3-pyridyl)-Ala-$N$-Me-Val-D-Leu
Compound 26: LCMS of DDLP HO-D-Phe-3,3-DiPhe-D-Ala-3-(4-pyridyl)-Ala-N-Me-Val-D-Leu-NH₂

Shimadzu LCMSsolution Analysis Report

Chromatogram

Ref. (time:2.3005Scan#:127)
BG Mode=5
Mass Peaks:1634 Base Peak:382.25/187/070/03 Polarity:Pos Segment1 - Event1

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Compound 26: LCMS cyclo D-Phe-3,3-DiPhe-D-Ala-3-(4-pyridyl)-Ala-N-Me-Val-D-Leu
Compound 26: HRMS of cyclo D-Phe-3,3-DiPhe-D-Ala-3-(4-pyridyl)-Ala-N-Me-Val-D-Leu

5_SMD261 #1-17  RT: 0.01-0.47  AV: 17  NL: 1.09E8
T: FTMS + c NSI Full ms [150.00-2000.00]

201.0884  745.4070
241.0681  393.2972  301.1408
301.1408  685.4352  825.3476
417.2608  1816.2135  1816.2135
610.5267  1057.5949
768.3920  1605.7443
825.3476  1972.6717
1057.5949  1408.8566
1200.7750  1605.7443
1408.8566  1816.2135
1605.7443  1972.6717
1816.2135  1972.6717
1972.6717  2000.0000
Compound 26: $^1$HNMR of cyclo D-Phe-3,3-DiPhe-D-Ala-3-(4-pyridyl)-Ala-N-Me-Val-D-Leu

Compound 26: $^1$H-$^{13}$C HSQC of cyclo D-Phe-3,3-DiPhe-D-Ala-3-(4-pyridyl)-Ala-N-Me-Val-D-Leu
Compound 26: $^1$H-$^{13}$C HMBC of cyclo D-Phe-3,3-DiPhe-D-Ala-3-(4-pyridyl)-Ala-N-Me-Val-D-Leu
Compound 27: LCMS of DDLP HO-D-Leu-D-Phe-3,3-Diphe-D-Ala-3-(4-Thia)Ala-Val-NH(CH₃)

==== Shimadzu LCMSsolution Analysis Report ====

--- Chromatogram ---

--- MS Spectrum Graph ---

--- Data ---
Compound 27: LCMS of cyclo D-Leu-D-Phe-3,3-Diphe-D-Ala-3-(4-Thia)Ala-N-Me-Val

==== Shimadzu LCMSsolution Analysis Report ====
Compound 27: HRMS of cyclo D-Leu-D-Phe-3,3-Diphe-D-Ala-3-(4-Thia)Ala-N-Me-Val

**MS Data from Orbitrap**

Full spectrum
Compound 27: $^1$HNMR of cyclo D-Leu-D-Phe-3,3-Diphe-D-Ala-3-(4-Thia)Ala-N-Me-Val

Compound 27: $^1$H-$^{13}$C HSQC of cyclo D-Leu-D-Phe-3,3-Diphe-D-Ala-3-(4-Thia)Ala-N-Me-Val
Compound 27: $^1$H-$^{13}$C HMBC of cyclo D-Leu-D-Phe-3,3-Diphe-D-Ala-3-(4-Thia)Ala-$N$-Me-Val

Compound 27: $^1$H-$^1$H COSY of cyclo D-Leu-D-Phe-3,3-Diphe-D-Ala-3-(4-Thia)Ala-$N$-Me-Val
Compound 28: LCMS of DDLP HO-Leu-N-Me-Val-3-(4-Thia)-D-Ala-3-(4-Thia)-D-Ala-3,3-Diphe-D-Ala-NH₂

==== Shimadzu LCMSsolution Analysis Report ====

Chromatogram

MS Spectrum Graph

MS Spectrum Graph

S131
Compound **28**: LCMS of cyclo Leu-N-Me-Val-3-(4-Thia)-D-Ala-3-(4-Thia)-D-Ala-3,3-Diphe-D-Ala
Compound 28: HRMS of cyclo Leu-N-Me-Val-3-(4-Thia)-D-Ala-3-(4-Thia)-D-Ala-3,3-Diphe-D-Ala

S133
Compound 28: $^1$HNMR of cyclo Leu-N-Me-Val-3-(4-Thia)-D-Ala-3-(4-Thia)-D-Ala-3,3-Diphe-D-Ala
Compound 28: $^{13}$C NMR of cyclo Leu-N-Me-Val-3-(4-Thia)-D-Ala-3-(4-Thia)-D-Ala-3,3-Diphe-D-Ala

Compound 28: $^1$H-$^{13}$C HSQC of cyclo Leu-N-Me-Val-3-(4-Thia)-D-Ala-3-(4-Thia)-D-Ala-3,3-Diphe-D-Ala

S135
Compound 28: $^1$H, $^{13}$C HMBC of cyclo Leu-N-Me-Val-3-(4-Thia)-D-Ala-3-(4-Thia)-D-Ala-3,3-Diphe-D-Ala

Compound 28: $^1$H-$^1$H COSY of cyclo Leu-N-Me-Val-3-(4-Thia)-D-Ala-3-(4-Thia)-D-Ala-3,3-Diphe-D-Ala
Compound 29: LCMS of DDLP HO-Leu-N-Me-Val-D-Tyr(OMe)-3-(4-Thia)-D-Ala-3,3-Diphe-D-Ala-NH₂
Compound 29: LCMS of cyclo Leu-N-Me-Val-D-Tyr(OMe)-3-(4-Thia)-D-Ala-3,3-Diphe-D-Ala

S138
Shimadzu LCMS solution Analysis Report

Chromatogram
HPLC1a_CFF272 C:\LabSolutions\Data\McAlpine\key\2014\140228\HPLC1a_CFF272.lic

MS Spectrum Graph

Rat. Time: 7.467 (Scan #: 323)
BG Mode: 7
Mass Peaks: 1393 Base Peak: 781.55 (947035) Polarity: Pos Segment 1 - Event 1
Compound 29: HRMS of cyclo Leu-\textit{N}-Me-Val-D-Tyr(OMe)-3-(4-Thia)-D-Ala-3,3-Diphe-D-Ala

**MS Data from Orbitrap**

Full spectrum
Compound 29: $^1$HNMR of cyclo Leu-N-Me-Val-D-Tyr(OMe)-3-(4-Thia)-D-Ala-3,3-Diphe-D-Ala

Compound 29: $^{13}$CNMR of cyclo Leu-N-Me-Val-D-Tyr(OMe)-3-(4-Thia)-D-Ala-3,3-Diphe-D-Ala
Compound 29: $^1$H-$^{13}$C HSQC of cyclo Leu-N-Me-Val-D-Tyr(OMe)-3-(4-Thia)-D-Ala-3,3-Diphe-D-Ala
Compound 29: $^1$H-$^{13}$C HMBC of cyclo Leu-N-Me-Val-D-Tyr(OMe)-3-(4-Thia)-D-Ala-3,3-Diphe-D-Ala

Compound 29: $^1$H-$^1$H COSY of cyclo Leu-N-Me-Val-D-Tyr(OMe)-3-(4-Thia)-D-Ala-3,3-Diphe-D-Ala
Compound 30: LCMS of DDLP HO-Leu-N-Me-Val-D-Glu(OtBu)-3-(4-Thia)-D-Ala-3,3-Diphe-D-Ala-NH₂
Compound 30: LCMS of cyclo Leu-N-Me-Val-D-Glu(OtBu)-3-(4-Thia)-D-Ala-3,3-Diphe-D-Ala
Compound 30: HRMS of cyclo Leu-N-Me-Val-D-Glu(OtBu)-3-(4-Thia)-D-Ala-3,3-Diphe-D-Ala
Compound 30: $^1$HNMR of cyclo Leu-N-Me-Val-D-Glu(OtBu)-3-(4-Thia)-D-Ala-3,3-Diphe-D-Ala
Compound 30: $^{13}$CNMR of cyclo Leu-N-Me-Val-D-Glu(OtBu)-3-(4-Thia)-D-Ala-3,3-Diphe-D-Ala

Compound 31: LCMS of cyclo Leu-N-Me-Val-D-Glu-3-(4-Thia)-D-Ala-3,3-Diphe-D-Ala
Compound 31: HRMS of cyclo Leu-N-Me-Val-D-Glu-3-(4-Thia)-D-Ala-3,3-Diphe-D-Ala

S149
Compound 31: $^1$HNMR of cyclo Leu-N-Me-Val-D-Glu-3-(4-Thia)-D-Ala-3,3-Diphe-D-Ala

S150
Compound 31: $^{13}$CNMR of cyclo Leu-N-Me-Val-D-Glu-3-(4-Thia)-D-Ala-3,3-Diphe-D-Ala

Compound 32: LCMS of cyclo Leu-N-Me-Val-D-Glu(OMe)-3-(4-Thia)-D-Ala-3,3-Diphe-D-Ala
Compound 32: HRMS of cyclo Leu-\text{-\textup{N}}\text{-Me-Val-D-Glu(OMe)-3-(4-Thia)-D-Ala-3,3-Diphe-D-Ala}
Compound 32: $^1$HNMR of cyclo Leu-N-Me-Val-D-Glu(OMe)-3-(4-Thia)-D-Ala-3,3-Diphe-D-Ala

S153
Compound 32: $^{13}$CNMR of cyclo Leu-N-Me-Val-D-Glu(OMe)-3-(4-Thia)-D-Ala-3,3-Diphe-D-Ala
Compound 33: LCMS of DDLP HO-Leu-N-Me-Val-(4-Methoxy)-D-Phe-(4-Methoxy)-D-Phe-3,3-Diphe-D-Ala-NH₂
Compound 33: LCMS of Cyclo Leu-N-Me-Val-(4-Methoxy)-D-Phe-(4-Methoxy)-D-Phe-3,3-Diphe-D-Ala
Compound 33: HRMS of Cyclo Leu-N-Me-Val-(4-Methoxy)-D-Phe-(4-Methoxy)-D-Phe-3,3-Diphe-D-Ala
Compound 33: $^1$HNMR of Cyclo Leu-N-Me-Val-(4-Methoxy)-D-Phe-(4-Methoxy)-D-Phe-3,3-Diphe-D-Ala

Compound 33: $^1$H-$^{13}$C HSQC of Cyclo Leu-N-Me-Val-(4-Methoxy)-D-Phe-(4-Methoxy)-D-Phe-3,3-Diphe-D-Ala
Compound 33: $^1$H-$^{13}$C HMBC of Cyclo Leu-N-Me-Val-(4-Methoxy)-D-Phe-(4-Methoxy)-D-Phe-3,3-Dipe-D-Ala
Compound 34: LCMS of DDLP HO-Leu-N-Me-Val-3-(4-Thia)-D-Ala-(4-Methoxy)-D-Phe-3,3-Diphe-D-Ala-NH$_2$
Compound 34: LCMS of Cyclo Leu-N-Me-Val-3-(4-Thia)-D-Ala-(4-Methoxy)-D-Phe-3,3-Diphe-D-Ala

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==== Shimadzu LCMSsolution Analysis Report ====  

[Graph showing chromatogram and mass spectrum]
Compound 34: HRMS of cyclo Leu-N-Me-Val-3-(4-Thia)-D-Ala-(4-Methoxy)-D-Phe-3,3-Diphe-D-Ala
Compound 34: $^1$H NMR of cyclo Leu-$\text{N}$-Me-Val-3-(4-Thia)-D-Ala-(4-Methoxy)-D-Phe-3,3-Diphe-D-Ala

![NMR Spectrum]

Compound 34: $^1$H-$^{13}$C HSQC of cyclo Leu-$\text{N}$-Me-Val-3-(4-Thia)-D-Ala-(4-Methoxy)-D-Phe-3,3-Diphe-D-Ala

![HSQC Spectrum]
Compound 34: $^1$H-$^{13}$C HMBC of cyclo Leu-N-Me-Val-3-(4-Thia)-D-Ala-(4-Methoxy)-D-Phe-3,3-Diphe-D-Ala
Compound 35: LCMS of DDLP HO-D-Leu-3-(4-Thia)-D-Ala-3,3-Dipe-D-Ala-3-(4'-Pyr)Ala-Val-NH(CH₃)

===== Shimadzu LCMSsolution Analysis Report =====

Chromatogram

MS Spectrum Graph

Retention Time: 5.33 (Scan#: 147)
BG Mode: None
Mass Peaks: 3139 Base Peak: 770.00 [13873532] Purity: Pos Segment 1 - Event 1

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S165
Compound 35: LCMS of cyclo D-Leu-3-(4-Thia)-D-Ala-3,3-Diphe-D-Ala-3-(4’-Pyr)Ala-N-Me-Val

==== Shimadzu LCMSsolution Analysis Report ====

Chromatogram

MS Spectrum Graph

Retention Time: 4.667 (Scan #: 221)
BG Mode: 0
Mass Peaks: 1321 Base Peak: 752.50 (56524463) Polarity: Pos Segment1: Event1

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Compound 35: HRMS of cyclo D-Leu-3-(4-Thia)-D-Ala-3,3-Diphe-D-Ala-3-(4’-Pyr)Ala-N-Me-Val

MS Data from Orbitrap

Full spectrum
Compound 35: $^1$HNMR of cyclo D-Leu-3-(4-Thia)-D-Ala-3,3-Diphe-D-Ala-3-(4'-Pyr)Ala-N-Me-Val

Compound 35: $^1$H-$^13$C HSQC of cyclo D-Leu-3-(4-Thia)-D-Ala-3,3-Diphe-D-Ala-3-(4'-Pyr)Ala-N-Me-Val
Compound 35: $^1$H-$^1$C HMBC of cyclo D-Leu-3-(4-Thia)-D-Ala-3,3-Diphe-D-Ala-3-(4'-Pyr)Ala-N-Me-Val

Compound 35: $^1$H-$^1$H COSY of cyclo D-Leu-3-(4-Thia)-D-Ala-3,3-Diphe-D-Ala-3-(4'-Pyr)Ala-N-Me-Val
Compound 36: LCMS of DDLP HO-D-Phe-3,3-DipheD-Ala-3-(2-pyridyl)-Ala-N-Me-Val-3-(4-Thia)-D-Ala
Compound 36: LCMS of Cyclo D-Phe-3,3-Diphe-D-Ala-3-(2-pyridyl)-Ala-N-Me-Val-3-(4-Thia)-D-Ala

==== Shimadzu LCMSsolution Analysis Report====
Compound 36: HRMS of Cyclo D-Phe-3,3-Diphe-D-Ala-3-(2-pyridyl)-Ala-N-Me-Val-3-(4-Thia)-D-Ala
Compound 36: $^1$HNMR of cyclo D-Phe-3,3-Diphe-D-Ala-3-(2-pyridyl)-Ala-N-Me-Val-3-(4-Thia)-D-Ala

![HNMR spectrum](image1)

Compound 36: $^1$H-$^{13}$C HSQC of cyclo D-Phe-3,3-Diphe-D-Ala-3-(2-pyridyl)-Ala-N-Me-Val-3-(4-Thia)-D-Ala

![HSQC spectrum](image2)
Compound 36: $^1$H-$^{13}$C HMBC of cyclo D-Phe-3,3-DiPhe-D-Ala-3-(2-pyridyl)-Ala-N'-Me-Val-3-(4-Thia)-D-Ala
Compound 37: LCMS of DDLP HO-Leu-Val-3-(4-Thia)-D-Ala-V-Me-D-Phe-3,3-Diphe-D-Ala-NH₂

==== Shimadzu LCMSsolution Analysis Report ====

[Chromatogram image with data]

[MS Spectrum Graph image with data]
Compound 37: LCMS of cyclo Leu-Val-3-(4-Thia)-D-Ala-N-Me-D-Phe-3,3-Diphe-D-Ala

== Shimadzu LCMSsolution Analysis Report ==

Chromatogram

MS Spectrum Graph

Ret Time: 5.767 (Scan# 341)
BG Mode: 5
Mass Peaks: 1464 Base Peak: 750.95 (1485165) Polarity: Pos Segment 1 - Event 1

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Compound 37: HRMS of cyclo Leu-Val-3-(4-Thia)-D-Ala-N-Me-D-Phe-3,3-Diphe-D-Ala

MS Data from Orbitrap

Full spectrum
Compound 37: $^1$HNMR of cyclo Leu-Val-3-(4-Thia)-D-Ala-N-Me-D-Phe-3,3-Diphe-D-Ala

Compound 37: $^{13}$CNMR of cyclo Leu-Val-3-(4-Thia)-D-Ala-N-Me-D-Phe-3,3-Diphe-D-Ala
Compound 37: $^1$H$-^{13}$C HSQC of cyclo Leu-Val-3-(4-Thia)-D-Ala-$N$-Me-D-Phe-3,3-Diphe-D-Ala

Compound 37: $^1$H$-^{13}$C HMBC of cyclo Leu-Val-3-(4-Thia)-D-Ala-$N$-Me-D-Phe-3,3-Diphe-D-Ala
Compound 37: $^1$H-$^1$H COSY of cyclo Leu-Val-3-(4-Thia)-D-Ala-N-Me-D-Phe-3,3-Diphe-D-Ala

Compound 37: $^1$H-$^1$H ROESY of cyclo Leu-Val-3-(4-Thia)-D-Ala-N-Me-D-Phe-3,3-Diphe-D-Ala
Compound 37: Variant temperature $^1$HNMR of cyclo Leu-N-Me-Val-D-His-D-Phe-3,3-Diphe-D-Ala
Compound 38: LCMS of HO-Val-3-(4-Thia)-D-Ala-NH-\textit{o}-NBS
Compound 38: LCMS of HO-Val-3-(4-Thia)-D-Ala-NCH₃-o-NBS

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Compound 38: LCMS of HO-Val-3-(4-Thia)-D-Ala-NH(CH₃)
Compound 38: LCMS of DDLP HO-Val-N-Me-3-(4-Thia)-D-Ala-D-Phe-3,3-Diphe-D-Ala-Leu-NH₂
Compound 38: LCMS of cyclo Val-N-Me-3-(4-Thia)-D-Ala-D-Phe-3,3-Diphe-D-Ala-Leu
Shimadzu LCMS solution Analysis Report

Chromatogram
CPP_SM310_final C:\LabSolutions\Data\McAlpin\key\2014140910\CPP_SM310_final_lcd

MS Spectrum Graph
Compound 38: HRMS of cyclo Val-N-Me-3-(4-Thia)-D-Ala-D-Phe-3,3-Diphe-D-Ala-Leu
Compound 38: $^1$HNMR of cyclo Val-N-Me-3-(4-Thia)-D-Ala-D-Phe-3,3-Diphe-D-Ala-Leu

Compound 38: $^{13}$CNMR of cyclo Val-N-Me-3-(4-Thia)-D-Ala-D-Phe-3,3-Diphe-D-Ala-Leu
Compound 38: 1H-1H COSY of cyclo Val-N-Me-3-(4-Thia)-D-Ala-D-Phe-3,3-Dipe-D-Ala-Leu
Compound **39**: LCMS of DDLP HO-D-Phe-3,3-Diphe-D-Ala-N-Me-Leu-Val-3-(4-Thia)-D-Ala-NH₂
Compound 39: LCMS of cyclo D-Phe-3,3-Diphe-D-Ala-\(N\)-Me-Leu-Val-3-(4-Thia)-D-Ala
Compound 39: HRMS of cyclo D-Phe-3,3-Diphe-D-Ala-N-Me-Leu-Val-3-(4-Thia)-D-Ala
Compound 39: $^1$HNMR of cyclo D-Phe-3,3-Diphe-D-Ala-$N$-Me-Leu-Val-3-(4-Thia)-D-Ala

Compound 39: $^{13}$CNMR of cyclo D-Phe-3,3-Diphe-D-Ala-$N$-Me-Leu-Val-3-(4-Thia)-D-Ala
Compound 39: $^1$H-$^{13}$C HSQC of cyclo D-Phe-3,3-Diphe-D-Ala-$N$-Me-Leu-Val-3-(4-Thia)-D-Ala

Compound 39: $^1$H-$^{13}$C HMBC of cyclo D-Phe-3,3-Diphe-D-Ala-$N$-Me-Leu-Val-3-(4-Thia)-D-Ala
Compound 39: $^1$H-$^1$H COSY of cyclo D-Phe-3,3-Diphe-D-Ala-N-Me-Leu-Val-3-(4-Thia)-D-Ala
Compound 40: LCMS of DDLP Boc-N-Me-3,3-Diphe-D-Ala-OH
Compound 40: LCMS of DDLP HO-Leu-Val-3-(4-Thia)-D-Ala-D-Phe-3,3-Diphe-D-Ala-NH(CH₃)
Compound 40: LCMS of cyclo Leu-Val-3-(4-Thia)-D-Ala-D-Phe-N-Me-3,3-Diphe-D-Ala

==== Shimadzu LCMSsolution Analysis Report ====

Chromatogram

MS Spectrum Graph

Retention Time: 1.400 (Scan #: 319)

Mass Peaks: 1494, 751.30 (33538122) Polarity: Pos Segment1 - Event1
Compound 40: HRMS of cyclo Leu-Val-3-(4-Thia)-D-Ala-D-Phe-N-Me-3,3-Diphe-D-Ala

MS Data from Orbitrap

Full spectrum
Compound 40: $^1$HNMR of cyclo Leu-Val-3-(4-Thia)-D-Ala-D-Phe-$N$-Me-3,3-Diphe-D-Ala

Compound 40: $^{13}$CNMR of cyclo Leu-Val-3-(4-Thia)-D-Ala-D-Phe-$N$-Me-3,3-Diphe-D-Ala
Compound 40: $^1$H-$^{13}$C HSQC of cyclo Leu-Val-3-(4-Thia)-D-Ala-D-Phe-N-Me-3,3-Diphe-D-Ala

Compound 40: $^1$H-$^{13}$C HMBC of cyclo Leu-Val-3-(4-Thia)-D-Ala-D-Phe-N-Me-3,3-Diphe-D-Ala
Compound 40: $^1$H-$^1$H COSY of cyclo Leu-Val-3-(4-Thia)-D-Ala-D-Phe-N-Me-3,3-Diphe-D-Ala

Compound 40: $^1$H-$^1$H ROESY of cyclo Leu-Val-3-(4-Thia)-D-Ala-D-Phe-N-Me-3,3-Diphe-D-Ala
Compound 40: Variant temperature $^1$HNMR of cyclo Leu-Val-3-(4-Thia)-D-Ala-D-Phe-$N$-Me-3,3-Diphe-D-Ala
Compound 41: LCMS of DDLP HO-Leu-Val-3-(4-Thia)-D-Ala-D-Phe-3,3-Diphe-D-Ala-NH₂
Compound 41: LCMS of cyclo Leu-Val-3-(4-Thia)-D-Ala-D-Phe-3,3-Diphe-D-Ala
Compound 41: HRMS of cyclo Leu-Val-3-(4-Thia)-D-Ala-D-Phe-3,3-Diphe-D-Ala
Compound 41: $^1$HNMR of cyclo Leu-Val-3-(4-Thia)-D-Ala-D-Phe-3,3-Diphe-D-Ala

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Compound 41: $^{13}$CNMR of cyclo Leu-Val-3-(4-Thia)-D-Ala-D-Phe-3,3-Diphe-D-Ala
Compound 41: $^1$H-$^{13}$C HSQC of cyclo Leu-Val-3-(4-Thia)-D-Ala-D-Phe-3,3-Diphe-D-Ala

Compound 41: $^1$H-$^{13}$C HMBC of cyclo Leu-Val-3-(4-Thia)-D-Ala-D-Phe-3,3-Diphe-D-Ala
Compound 41: $^1$H-$^1$H COSY of cyclo Leu-Val-3-(4-Thia)-D-Ala-D-Phe-3,3-Diphe-D-Ala
Biology: Results
Cytotoxicity
Compound 1 HCT116
IC$_{50}$ = 18.05 ± 0.43 µM

Compound 2 HCT116
IC$_{50}$ = 20.75 ± 2.52 µM

Compound 3 HCT116
IC$_{50}$ = 15.72 ± 2.93 µM

Compound 4 (SM253) HCT116
IC$_{50}$ = 5.03 ± 0.53 µM

Compound 5 HCT116
IC$_{50}$ = 3.9 ± 0.2 µM

Compound 6 HCT116
IC$_{50}$ = 5.14 ± 0.64 µM

Compound 7 HCT116
IC$_{50}$ = 15.72 ± 2.93 µM

Compound 8 HCT116
IC$_{50}$ = 3.0 ± 1.4 µM

Compound 9 HCT116
IC$_{50}$ = 3.0 ± 1.4 µM

Compound 10 HCT116
IC$_{50}$ = 9.6 ± 1.5 µM

Compound 11 HCT116
IC$_{50}$ = 9.6 ± 1.5 µM

Compound 12 HCT116
IC$_{50}$ = 6.0 ± 0.2 µM

Compound 13 HCT116
IC$_{50}$ = 8.8 ± 1.0 µM

Compound 14 HCT116
IC$_{50}$ = 5.3 ± 0.9 µM

Compound 15 HCT116
IC$_{50}$ = 3.0 ± 1.4 µM

Compound 16 HCT116
IC$_{50}$ = 5.3 ± 0.9 µM

Compound 17 HCT116
IC$_{50}$ = 6.0 ± 0.2 µM

Compound 18 HCT116
IC$_{50}$ = 3.0 ± 1.4 µM
Supplemental Figure S1: Series I-IV (GI50) IC50 results HCT-116

Compound 19 HCT116
IC50 = 30 µM

Compound 22 HCT116
IC50 = 5.9 ± 0.3 µM

Compound 25 HCT116
IC50 = 15.4 ± 0.8 µM

Compound 20 HCT116
IC50 ≥ 30 µM

Compound 23 HCT116
IC50 = 6.2 ± 0.5 µM

Compound 26 HCT116
IC50 = 22.2 ± 3.6 µM

Compound 21 HCT116
IC50 ≥ 30 µM

Compound 24 HCT116
IC50 = 6.3 ± 0.1 µM

Compound 27 HCT116
IC50 > 30 µM

Compound 1 MiaPaCa-2
IC50 = 23.57 ± 2.45 µM

Compound 4 (SM253) MiaPaCa-2
IC50 = 5.53 ± 0.22 µM

Compound 7 MiaPaCa-2
IC50 > 30 µM

Compound 8 MiaPaCa-2
IC50 > 30 µM

Compound 9 MiaPaCa-2
IC50 = 5.0 ± 1.8 µM
Supplemental Figure S2: Series I-IV (GI50) IC50 results MiaPaCa-2
Supplemental Figure S3: Series V-VII (GI50) IC50 results HCT-116

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50</th>
<th>Cell Line</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound 28</td>
<td>≥30 µM</td>
<td>HCT116</td>
</tr>
<tr>
<td>Compound 31</td>
<td>≥30 µM</td>
<td>HCT116</td>
</tr>
<tr>
<td>Compound 34</td>
<td>10.4 ± 2.8 µM</td>
<td>HCT116</td>
</tr>
<tr>
<td>Compound 29</td>
<td>8.4 ± 0.5 µM</td>
<td>HCT116</td>
</tr>
<tr>
<td>Compound 32</td>
<td>≥30 µM</td>
<td>HCT116</td>
</tr>
<tr>
<td>Compound 33</td>
<td>5.2 ± 2.2 µM</td>
<td>HCT116</td>
</tr>
</tbody>
</table>

Supplemental Figure S4: Series V-VII (GI50) IC50 results MiaPaCa-2

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50</th>
<th>Cell Line</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound 28</td>
<td>20.2 ± 4.3 µM</td>
<td>MiaPaCa-2</td>
</tr>
<tr>
<td>Compound 29</td>
<td>8.8 ± 0.4 µM</td>
<td>MiaPaCa-2</td>
</tr>
<tr>
<td>Compound 30</td>
<td>9.2 ± 1.0 µM</td>
<td>MiaPaCa-2</td>
</tr>
<tr>
<td>Compound 31</td>
<td>≥30 µM</td>
<td>MiaPaCa-2</td>
</tr>
<tr>
<td>Compound 32</td>
<td>8.7 ± 1.0 µM</td>
<td>MiaPaCa-2</td>
</tr>
<tr>
<td>Compound 33</td>
<td>7.5 ± 0.9 µM</td>
<td>MiaPaCa-2</td>
</tr>
<tr>
<td>Compound 34</td>
<td>7.5 ± 0.9 µM</td>
<td>MiaPaCa-2</td>
</tr>
<tr>
<td>Compound 35</td>
<td>≥30 µM</td>
<td>MiaPaCa-2</td>
</tr>
<tr>
<td>Compound 36</td>
<td>20.9 ± 3.8 µM</td>
<td>MiaPaCa-2</td>
</tr>
</tbody>
</table>
Supplemental Figure S5: N-methyl analogues (GI50) IC50 results HCT-116

Supplemental Figure S6: N-methyl analogues (GI50) IC50 results MiaPaCa-2

Supplemental Figure S7: Representative western blots of protein levels after treatment with Compound 9, 15, and AUY922.