Supporting Information

Solution-Phase Parallel Synthesis of a Pharmacophore Library of HUN-7293 Analogues: A General Chemical Mutagenesis Approach to Defining Structure–Function Properties of Naturally Occurring Cyclic (Depsi)peptides

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General Methods. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AC-250, AMX-400, DRX-500, or DRX-600 spectrometer at ambient temperature. FAB–HRMS mass spectra were performed on a VG ZAB–VSE double focusing high resolution mass spectrometer and HRMALDI–FTMS mass spectra were obtained on IonSpec FTMS mass spectrometer as noted. Infrared spectra were recorded on a Perkin–Elmer 1600 Series FTIR spectrometer. Optical rotation was taken on AUTOPOL® III automatic polarimeter at ambient temperature.

The analysis and separation of amino acids were performed on CHIRALCEL® OD™ columns which were purchased from CHIRA TECHNOLOGIES, INC.

Peptide analysis was carried out on NovaPak® C18 (3.9 × 300 mm) HPLC column, 1.0 mL/min, and peptide separation was performed on *Prep*LC™ 25 mm Module, 10 mL/min, detected at 220 nm with a Waters 996 photodiode array detector. HPLC solvent systems were indicated with each specific compound.

Separation of linear heptadepsipeptides: MeOH–H_2O was used as solvent with a flow rate of 10 mL/min. Linear gradients of 86:14 to 89:11 at 15 min, then 89:11 to 100:0 at 35 min were applied.

Separation of cyclic depsipeptides: MeOH–H_2O was used as solvent with a flow rate of 10 mL/min. Linear gradients of 82:18 to 88:12 at 15 min, then 88:12 to 100:0 at 35 min were applied.

General procedure for *N*-Boc deprotection reactions using HCl–EtOAc: the substrates (neat or dissolved in EtOAc) were treated with 4.0 or 4.1 M HCl–EtOAc at 0 °C for the time period indicated, until no starting materials were detected by TLC. The volatiles were removed by a stream of N_2, and the sample was further dried under high vacuum.

General procedure for *N*-Boc deprotection reactions using (1) HCO_2H, (2) $NaHCO_3$: the substrate was treated with HCO_2H (0.1 M) at 25 °C for the time period indicated, until no starting material was detected by TLC. Most of the volatiles were removed in vacuo, and the residue was treated with saturated aqueous $NaHCO_3$ until pH = 7–8. The aqueous solution was extracted with EtOAc (5 mL × 2). The organic layers were combined, dried ($MgSO_4$), and filtered. Removal of the solvent afforded the free amines.

General procedure for basic hydrolysis of methyl esters to carboxylic acids: the methyl ester was dissolved in *tert*-BuOH–H_2O (2:1, 0.1 M), and the reaction mixture was cooled to 0 °C, 2 equiv of $LiOH·H_2O$ powder was added, and stirring was continued for

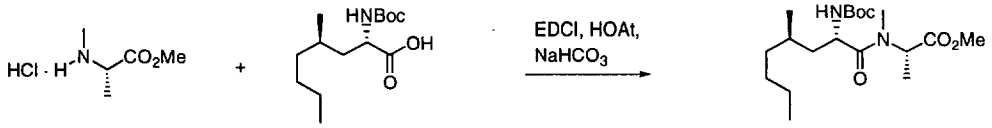
the time period indicated until no starting material was detected by TLC. The reaction was quenched with the addition of 1 M aqueous HCl until pH = 1–2. The aqueous layer was extracted with EtOAc (5 mL × 3), and the combined organic layers were dried ($MgSO_4$), and filtered. Removal of the solvent afforded the free carboxylic acid.

General procedure for peptide coupling reactions: amino acids or peptides were dissolved in the designated solvents, and at the indicated temperatures, coupling reagents (including base, if necessary) were added. After stirring the reaction mixture for indicated period of time, the reaction was quenched by the addition of 1 M aqueous HCl (5–10 mL) and the organic layer was diluted with EtOAc (10–20 mL). After the separation of the aqueous layer, the organic layer was washed with 1 M aqueous HCl (5–10 mL), saturated aqueous $NaHCO_3$ (5–10 mL × 2), saturated aqueous NaCl (5–10 mL × 2), dried ($MgSO_4$), and filtered. Removal of the solvent gave the indicated products.

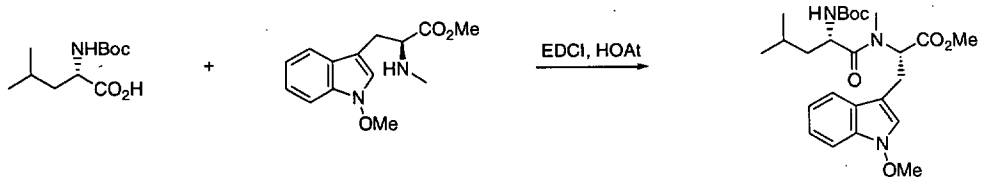
General procedure for cyclization reactions: the linear depsipeptide was treated with TFA–CH_2Cl_2 (1:5, 5 mM) in the presence of anisole (a drop or two) at 25 °C for 2 h. The volatiles were removed by a stream of N_2, and the sample was further dried under high vacuum. This residue at 0 °C was treated with HCl–EtOAc (4.0 M, 5 mM) which was removed by a stream of N_2 after 1 min forming the amine HCl salt. The sample was further dried under high vacuum for 15–30 min. The cyclization reactions are detailed for the different substrates. The reaction was quenched with the addition of aqueous 1 M HCl (5 mL) and the aqueous layer was extracted with EtOAc (5 mL × 5). The combined organic layers were dried ($MgSO_4$), filtered, and the solvent was removed in vacuo.

Compound numbering system: precursors of the cyclic hepta(depsi)peptides were numbered beginning with the number of the final cyclized compounds, followed by capitalized letter(s) indicating features of that specific compound, e.g., D stands for dipeptide, T stands for tripeptide or tetrapeptide, H represents hepta(depsi)peptide and N means the *N*-version of the indicated compounds. For example, **5D** is the dipeptide in the fragment that differs from that of natural product and ultimately leads to cyclized compound **5**. Additional number(s) or letter(s) were added, if necessary, to clarify or distinguish multiple precursors.

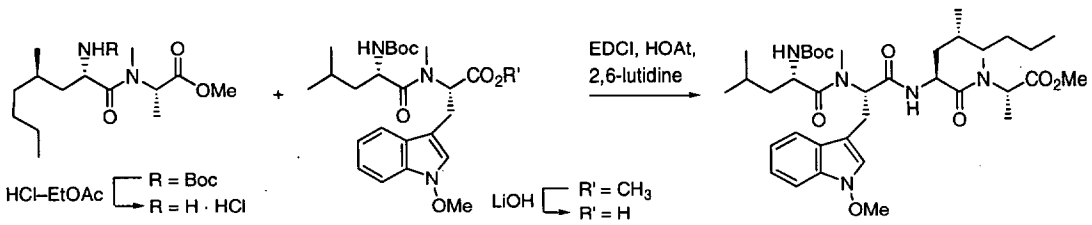
Preparation of HUN-7293 (1) from precursors purified by liquid–liquid extraction:



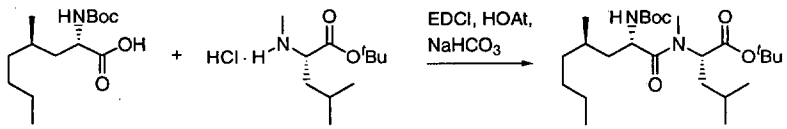
***N*-[(2*S*,4*R*)-2-[*N*-(*tert*-Butoxycarbonyl)amino]-4-methyloctanoyl]-*N*-methyl-L-alanine methyl ester (45)**: A CH_2Cl_2–DMF (5:1, 1 mL, 0.1 M) solution of (2*S*,4*R*)-2-[*N*-(*tert*-butoxycarbonyl)amino]-4-methyloctanoic acid (**35**, 27.3 mg, 0.10 mmol) and *N*-methyl-L-alanine methyl ester (**44**, 17.6 mg, 0.15 mmol) at 0 °C was treated with EDCI (38.3 mg, 0.2 mmol), HOAt (20.4 mg, 0.15 mmol) and $NaHCO_3$ (8.4 mg, 0.10 mmol), and the reaction mixture was allowed to warm to 25 °C and was stirred for a total of 16 h. Work-up of the reaction mixture as described and removal of solvent in vacuo afforded **45** (31.4 mg, 84% yield) as a colorless thick oil which exhibited the same ^1H NMR spectrum as the authentic sample.[8] HPLC analysis (MeOH/H_2O, 80/20) of this crude product showed one major peak (R_t = 5.6 min, 91%) which is identical to an authentic sample.[8]



***N*-[*N*-(*tert*-Butoxycarbonyl)-L-leucinyl]-*N*$^{1'}$-methoxy-*N*-methyl-L-tryptophan methyl ester (42)**: A CH_2Cl_2–DMF (5:1, 1 mL, 0.1 M) solution of *N*-(*tert*-butoxycarbonyl)-L-leucine monohydrate (**40**, 28.9 mg, 0.11 mmol) and *N*$^{1'}$-methoxy-*N*-methyl-L-tryptophan methyl ester (**41**, 24.9 mg, 0.10 mmol) at 0 °C was treated with EDCI (38.3 mg, 0.2 mmol) and HOAt (15.0 mg, 0.11 mmol), and the reaction mixture was allowed to warm to 25°C and was stirred for a total of 21 h. Work-up of the reaction mixture as described and removal of solvent in vacuo afforded **42** (47.0 mg, 99% yield) as a light yellow foamy solid which exhibited the same ^{1}H NMR spectrum as the authentic sample.[8] HPLC analysis (MeOH/H_2O, 80/20) of this crude product showed one major peak (R_t = 5.8 min, 89%) which is identical to an authentic sample.[8]

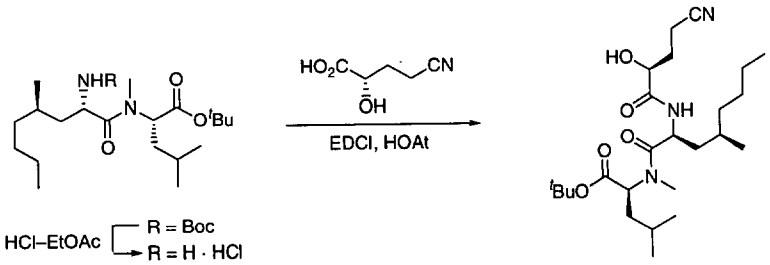


***N*-[(2*S*,4*R*)-2-[[*N*-[*N*-(*tert*-Butoxycarbonyl)-L-leucinyl]-*N*$^{1'}$-methoxy-*N*-methyl-L-tryptophanyl]amino]-4-methyloctanoyl]-*N*-methyl-L-alanine methyl ester (47)**: A HCl–EtOAc solution (4.0 M, 1.0 mL) was added to **45** (82.0 mg, 220 μmol) at 0 °C, and the stirring was continued for 1 h, until no starting material was detected by TLC. The volatiles were removed by a stream of N_2. To this residue, **43** (crude product from saponification reaction of its methyl ester **42**, 95.1 mg, 200 μmol, 0 °C, 6 h) was added, followed by CH_2Cl_2–DMF (5:1, 2.0 mL, 0.1 M). The resulting solution at –30 °C was treated with EDCI (76.7 mg, 400 μmol), HOAt (32.7 mg, 240 μmol) and 2,6-lutidine (25.6 μL, 220 μmol), and the reaction mixture was stirred at –30 °C for 6 h. Work-up of the reaction mixture as described and removal of solvent in vacuo afforded **47** (140.7 mg, 98% yield) as a yellow foamy solid. HPLC analysis (MeOH/H_2O, 80/20) of this crude product showed one major peak (R_t = 15.9 min, 85%) which is identical to an authentic sample.[8] From HPLC, the ratio of two diastereomers was 32:1.

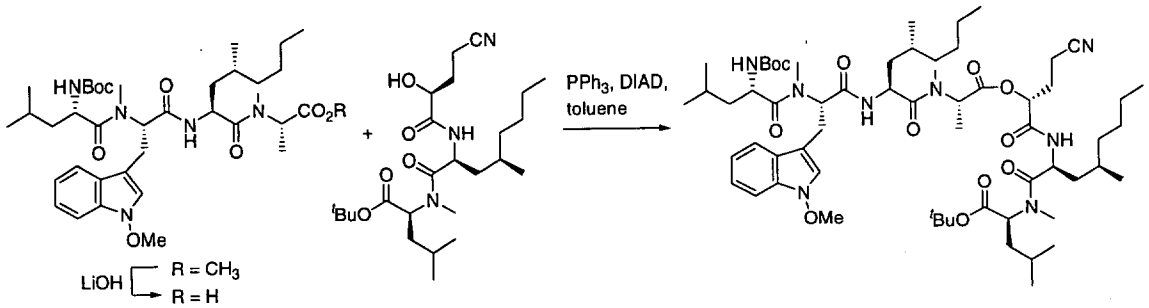


***N*-[(2*S*,4*R*)-2-[*N*-(*tert*-Butoxycarbonyl)amino]-4-methyloctanoyl]-*N*-methyl-L-leucine *tert*-butyl ester (37)**: A CH_2Cl_2–DMF (5:1, 3 mL, 0.1 M) solution of (2*S*,4*R*)-2-[*N*-(*tert*-

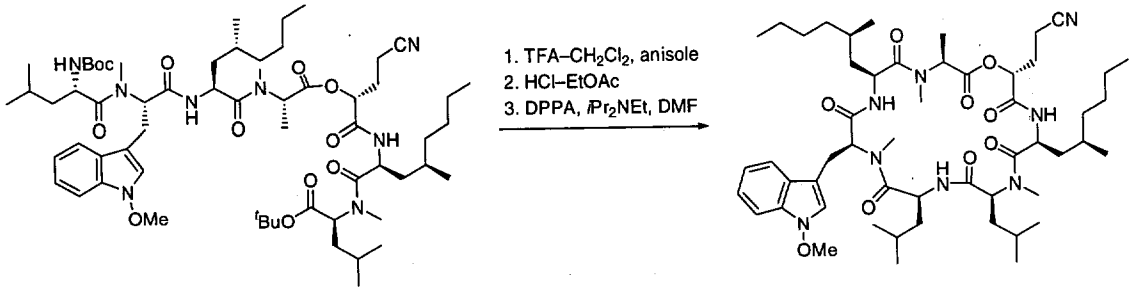
butoxycarbonyl)amino]-4-methyloctanoic acid (**35**, 82.0 mg, 0.30 mmol) and *N*-methyl-L-leucine *tert*-butyl ester hydrochloride (**36**, 107.0 mg, 0.45 mmol) at 0 °C was treated with EDCI (81.7 mg, 0.6 mmol), HOAt (63.3 mg, 0.33 mmol) and $NaHCO_3$ (37.8 mg, 0.45 mmol), and the reaction mixture was allowed to warm to 25 °C and was stirred for a total of 17 h. Work-up of the reaction mixture as described and removal of solvent in vacuo afforded **37** (123.1 mg, 90% yield) as a colorless oil which exhibited the same ^{1}H NMR spectrum as an authentic sample.[8] HPLC analysis (MeOH/H_2O, 80/20) of this crude product showed one major peak (R_t = 20.2 min, 84%) which is identical to an authentic sample.[8]



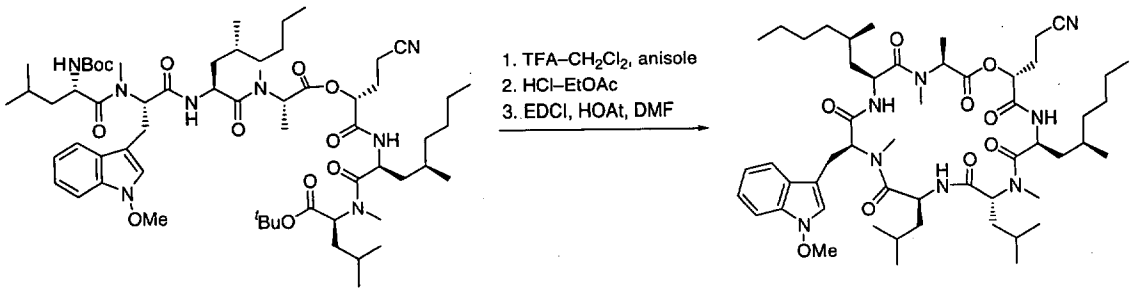
***N*-[(2*S*,4*R*)-2-[*N*-[(*S*)-4-Cyano-2-hydroxybutanoyl]amino]-4-methyloctanoyl]-*N*-methyl-L-leucine *tert*-butyl ester (3)**: An EtOAc (1.0 mL) solution of **37** (90.4 mg, 198 μmol) was treated with HCl–EtOAc (4.0 M, 1.0 mL) at 0 °C for 3 h, until no starting material was detected from TLC. After removing the volatiles by a stream of N_2, a CH_2Cl_2–DMF (5:1, 2.0 mL, 0.1 M) solution of (*S*)-4-cyano-2-hydroxybutanoic acid (**39**, 25.6 mg, 198 μmol) was added to the residue. This reaction mixture at –30 °C was treated with EDCI (75.9 mg, 0.4 mmol), HOAt (28.3 mg, 0.2 mmol) and 2,6-lutidine (23.1 mg, 0.2 mmol), and the stirring was continued for 4 h at –30 °C. Work-up of the reaction mixture as described and removal of solvent in vacuo afforded **3** (66.2 mg, 71% yield) as a thick light yellow oil. HPLC analysis (MeOH/H_2O, 80/20) of this crude product showed one major peak (R_t = 6.4 min, 84%) which is identical to an authentic sample.[8] ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 7.25 (br d, J = 8.8 Hz, 1H), 5.09 (dd, J = 10.3, 5.1 Hz, 1H), 4.96 (d, J = 5.5 Hz, 1H), 4.93 (ddd, J = 10.8, 8.8, 2.4 Hz, 1H), 4.15–4.18 (m, 1H), 2.97 (s, 3H), 2.49 (ddd, J = 16.7, 9.0, 7.5 Hz, 1H), 2.41 (ddd, J = 16.7, 8.6, 5.7 Hz, 1H), 2.13–2.20 (m, 1H), 1.90–1.99 (m, 1H), 1.53–1.66 (m, 4H), 1.20–1.47 (m, partially overlapped, 8H), 1.42 (s, 9H), 0.96 (d, J = 5.9 Hz, 3H), 0.90 (d, J = 6.6 Hz, 3H), 0.83–0.87 (m, 6H).



For **48**: A toluene (1.3 mL, 0.05 M) solution of **3** (30.6 mg, 65.4 μmol), **2** (crude product from saponification reaction of its methyl ester **47**, 45.9 mg, 65.4 μmol) and PPh_3 (51.5 mg, 196 μmol) at 0 °C was treated dropwise with DIAD (97% purity, 39.9 μL, 196 μmol), and the mixture was stirred at 0 °C for 18 h. The solvent was removed by a stream of N_2, and the residue was dissolved in MeOH, and was subjected to reverse-phase HPLC chromatography. Compound **48** (R_t = 27 min, 35.6 mg, 47% yield) was obtained as a foamy white solid, which was identical to an authentic sample[8] by both ^{1}H NMR and HPLC analysis.



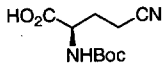
HUN-7293 (**1**): Linear heptadepsipeptide **48** (1.6 mg, 1.4 μmol) was deprotected following the standard procedure, and the residue was dissolved in DMF (1.4 mL, 1 mM) at 0 °C. DPPA (0.6 μL, 2.8 μmol) and iPr$_2$NEt (0.5 μL, 2.8 μmol) were added, and the mixture was stirred at 0 °C for 64 h. After work-up of the reaction mixture as described, the residue was subjected to HPLC chromatography and gave **1** (R_t = 21 min, 0.9 mg, 64% yield) as a white solid, which is identical to an authentic sample of the natural product (obtained from Novartis) both by ^{1}H NMR and HPLC analysis.



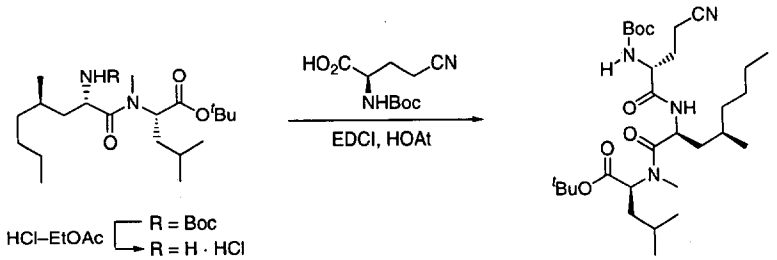
For **C$_2^3$-*epi*-1**: Linear heptadepsipeptide **48** (1.7 mg, 1.5 μmol) was deprotected following the standard procedure, and the residue was dissolved in DMF (1.5 mL, 1 mM) at 0 °C. EDCI (0.6 mg, 3.0 μmol) and HOAt (0.4 mg, 3.0 μmol) were added, and the mixture was stirred at 0 °C for 23 h. After work-up of the reaction mixture as described, the residue was subjected to HPLC chromatography and gave **C$_2^3$-*epi*-1** (R_t = 22 min, 0.6 mg, 43% yield) as a white solid. HUN-7293 (**1**) was not detected. ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 7.97 (br d, J = 9.9 Hz, 1H), 7.72 (d, J = 7.8 Hz, 1H), 7.58 (br d, J = 8.2 Hz, 1H), 7.38 (d, J = 7.8 Hz, 1H), 7.21 (dd, J = 7.8, 7.8 Hz, 1H), 7.09 (dd, J = 7.8, 7.8 Hz, 1H), 7.04 (s, 1H), 6.32 (br d, J = 9.2 Hz, 1H), 5.33 (dd, J = 12.5, 4.4 Hz, 1H), 5.12–5.13 (m, 1H), 4.99 (ddd, J = 10.0, 9.9, 3.9 Hz, 1H), 4.93 (dd, J = 10.8, 5.3 Hz, 1H), 4.56 (ddd, J = 12.1, 8.2, 4.0 Hz, 1H), 4.54–4.62 (m, 2H), 4.02 (s, 3H), 3.61 (q, J = 6.6 Hz, 1H), 3.21 (s, 3H), 3.19–3.21 (m, partially overlapped, 2H), 2.98 (s, 3H), 2.57 (s, 3H),

2.41–2.45 (m, 1H), 2.22–2.27 (m, 1H), 2.06–2.17 (m, 2H), 1.10–1.75 (m, 25H), 0.83–0.97 (m, 18H), 0.54 (d, J = 6.6 Hz, 3H), 0.06 (d, J = 6.6 Hz, 3H), –0.31 (ddd, J = 14.3, 10.5, 3.7 Hz, 1H); IR (neat) ν_{max} 3279, 2956, 2928, 2246, 1753, 1731, 1686, 1677, 1660, 1637, 1531, 1462, 1208 cm^{-1}; HRMALDI–FTMS *m/z* 999.6214 ([M + Na]$^+$, $C_{53}H_{84}N_8O_9Na$ requires 999.6253).

Preparation of aza-HUN-7293 (4) and C_2^3-*epi*-4:

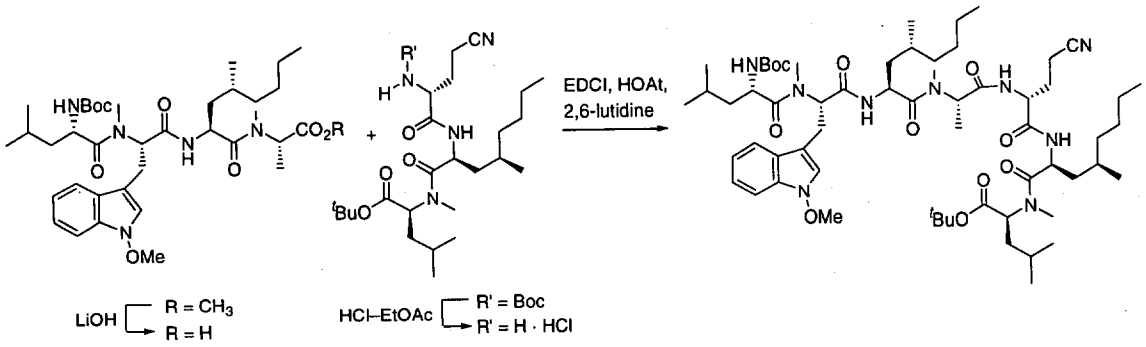


(*R*)-2-[*N*-(*tert*-Butoxycarbonyl)amino]-4-cyanobutanoic acid: An anhydrous acetone (8.0 mL, 0.25 M) solution of *N*-(*tert*-butoxycarbonyl)-D-glutamine (492.5 mg, 2.0 mmol) at 25 °C was treated with pyridine (2.0 mL, 1.0 M) and DCC (453.9 mg, 2.2 mmol), and a white suspension was obtained. After 2 h, the reaction mixture was filtered, and the solid was washed with acetone (5 mL × 3). The filtrates were collected and concentrated. Water (5 mL), and 1 M aqueous HCl were added to this concentrated filtrate until pH ≈ 4. The aqueous solution was extracted with EtOAc (10 mL × 3), and the organic layers were combined, dried ($MgSO_4$), filtered. A light yellow oil was obtained after the removal of the solvent in vacuo, and was further purified by silica gel chromatography (CH_2Cl_2 : MeOH : AcOH = 10:1:0.1) to give the title compound (R_f = 0.1, 328 mg, 72% yield) as a thick colorless oil: $[\alpha]_D^{23}$ –13 (*c* 3.7, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, two rotamers) δ 7.22 (br s, 0.6H), 5.24 (br s, 0.4H), 4.38 (br s, 0.4H), 4.29–4.30 (br d, J = 5.4 Hz, 0.6H), 2.41–2.54 (m, 2H), 2.20–2.29 (m, 1H), 2.08–2.13 (m, 1H), 1.43, 1.46 (s, s, 9H); ^{13}C NMR (62.5 MHz, $CDCl_3$, two rotamers) δ 174.9, 174.1, 157.0, 155.6, 118.7, 83.1, 81.0, 53.0, 52.3, 28.9, 28.2, 13.6, 13.5; IR (neat) ν_{max} 3733–2610 (br), 3342, 2979, 2249, 1714, 1698, 1519, 1369, 1162 cm^{-1}; HRMALDI–FTMS *m/z* 251.0998 ([M + Na]$^+$, $C_{10}H_{16}N_2O_4Na$ requires 251.1008).

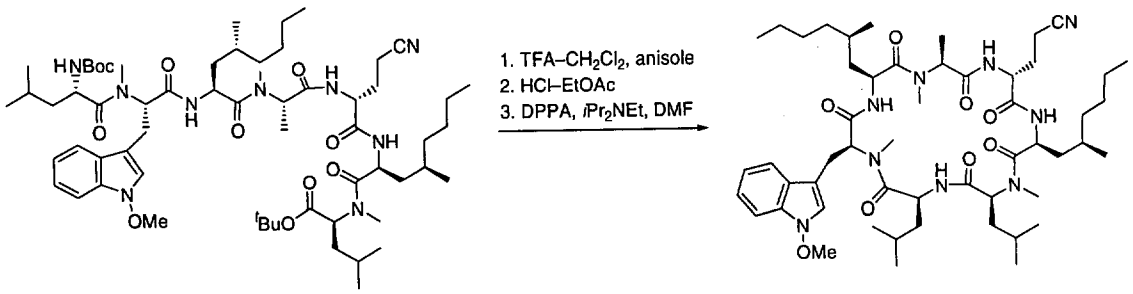


***N*-[(2*S*,4*R*)-2-[*N*-[(*R*)-2-(*tert*-Butoxycarbonyl)amino-4-cyanobutanoyl]amino]-4-methyloctanoyl]-*N*-methyl-L-leucine *tert*-butyl ester (3N)**: An EtOAc (0.5 mL) solution of **37** (40.0 mg, 87.6 μmol) was treated with HCl–EtOAc (4.1 M, 0.5 mL) at 0 °C for 5 h, until no starting material was detected by TLC. After removing the volatiles by a stream of N_2, a CH_2Cl_2–DMF (5:1, 0.9 mL, 0.1 M) solution of (*R*)-2-*N*-(*tert*-butoxycarbonyl)amino-4-cyanobutanoic acid (19.0 mg, 83.2 μmol) was added to the residue. The reaction mixture at 0 °C was treated with EDCI (33.6 mg, 0.18 mmol), HOAt (11.9 mg, 87.6 μmol) and $NaHCO_3$ (7.4 mg, 87.6 μmol), and stirring was continued for 3 h at 0 °C. Work-up of the reaction mixture as described and removal of solvent in vacuo afforded **3N** (44.1 mg, 89% yield) as a thick colorless oil. HPLC analysis (MeOH/H_2O, 80/20) of this crude product showed one major peak (R_t = 13 min,

62%): ^{1}H NMR (400 MHz, $CDCl_3$, major rotamer) δ 6.80 (d, J = 8.1 Hz, 1H), 5.14 (dd, J = 10.5, 5.1 Hz, 1H), 4.93 (br dd, J = 9.6, 9.6 Hz, 1H), 4.25 (br s, 1H), 2.92 (s, 3H), 2.34–2.50 (m, 2H), 2.21 (br s, 1H), 1.87–1.96 (m, 1H), 1.42, 1.43 (s, s, partially overlapped, 18H), 1.42–1.71 (m, partially overlapped, 5H), 1.22–1.23 (m, 7H), 0.97 (d, J = 5.9 Hz, 3H), 0.90 (d, J = 6.8 Hz, 3H), 0.84–0.87 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$, major rotamer) δ 172.8, 170.5, 170.2, 155.2, 118.9, 81.6, 80.5, 55.3, 53.3, 47.7, 40.1, 37.3, 37.0, 30.8, 29.6, 29.2, 29.1, 28.2, 28.0, 24.9, 23.2, 22.8, 21.5, 19.1, 14.0, 13.8; IR (neat) ν_{max} 3294, 2958, 2930, 2871, 2247, 1731, 1715, 1657, 1651, 1634, 1531, 1519, 1488, 1251, 1165 cm^{-1}; HRMALDI–FTMS *m/z* 589.3946 ([M + Na]$^+$, $C_{30}H_{54}N_4O_6Na$ requires 589.3941).

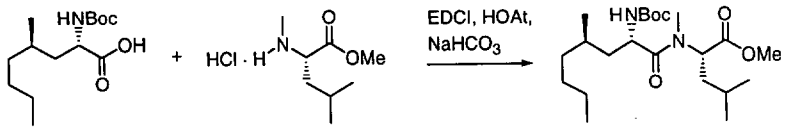


***N*-[(2*S*,4*R*)-2-[*N*-[(*S*)-2-[*N*-[[(2*S*,4*R*)-2-[[*N*-[*N*-(*tert*-Butoxycarbonyl)-L-leucinyl]-*N*$^{1'}$-methoxy-*N*-methyl-L-tryptophanyl]amino]-4-methyloctanoyl]-*N*-methyl-L-alanyl]-4-cyanobutanoyl]amino]-4-methyloctanoyl]-*N*-methyl-L-leucine *tert*-butyl ester (48N)**: A solution of HCl–EtOAc (4.1 M, 0.2 mL) was added to an EtOAc (0.2 mL) solution of **3N** (6.2 mg, 10.9 μmol) at 0 °C, and the reaction mixture was stirred at 0 °C for 2.5 h, until no starting material was detected by TLC. The volatiles were removed by a stream of N_2. To this residue, **2** (crude product from saponification reaction of its methyl ester **47**, 7.2 mg, 10.1 μmol, 0 °C, 2.5 h) was added, followed by CH_2Cl_2–DMF (5:1, 0.1 mL, 0.1 M). The resulting solution at –30 °C was treated with EDCI (5.8 mg, 30.2 μmol), HOAt (1.6 mg, 12.1 μmol) and 2,6-lutidine (1.3 μL, 11.1 μmol), and the reaction mixture was stirred at –30 °C for 5 h. The reaction was worked-up as described, and **48N** (9.9 mg, 86% yield) was obtained as a light yellow solid. HPLC analysis (MeOH/H_2O, 90/10) of this crude product showed one major peak (R_t = 17.5 min, 33%). A small amount of this sample was further purified by HPLC to give pure compound for characterization: ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 8.56 (br d, J = 6.2 Hz, 1H), 8.29 (br d, J = 7.3 Hz, 1H), 7.60 (dd, J = 8.1, 8.1 Hz, 1H), 7.34–7.37 (m, 1H), 7.19–7.23 (m, 1H), 7.03–7.12 (m, 1H), 6.97 (s, 1H), 6.70 (br d, J = 5.5 Hz, 1H), 5.09–5.20 (m, 2H), 4.92–4.95 (m, 1H), 4.75–4.79 (m, 2H), 4.44–4.53 (m, 2H), 4.00 (s, 3H), 3.26 (dd, J = 17.1, 6.8 Hz, 1H), 3.06–3.10 (m, 1H), 2.92 (s, 3H), 2.90 (s, 3H), 2.77 (s, 3H), 2.15–2.57 (m, 4H), 1.22–1.81 (m, partially overlapped, 26H), 1.40 (s, partially overlapped, 9H), 1.38 (s, partially overlapped, 9H), 0.78–0.95 (m, 18H), 0.37 (d, J = 6.6 Hz, 3H), 0.00 (d, J = 6.6 Hz, 3H), –0.56 (dd, J = 10.3, 10.3 Hz, 1H); IR (neat) ν_{max} 3295, 2957, 2929, 2871, 2246, 1732, 1713, 1682, 1651, 1634, 1531, 1455, 1387, 1252, 1166 cm^{-1}; HRMALDI–FTMS *m/z* 1172.7723 ([M + Na]$^+$, $C_{62}H_{103}N_9O_{11}Na$ requires 1172.7674).

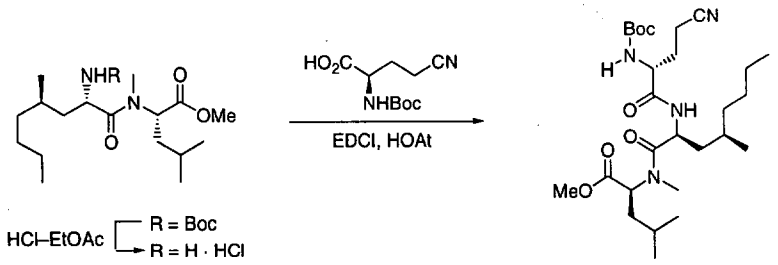


Aza-HUN-7293 (4): Linear heptadepsipeptide **48N** (2.6 mg, 2.3 μmol) was deprotected following the standard procedure, and the residue was dissolved in DMF (0.5 mL, 5 mM) at 0 °C. DPPA (1.0 μL, 4.5 μmol) and iPr$_2$NEt (0.8 μL, 4.5 μmol) were added, and the mixture was stirred at 0 °C for 43 h. After work-up of the reaction mixture as described, a yellowish solid was obtained. PTLC (toluene:MeOH = 9:1) of the crude product gave **4** and $\mathbf{C_2^3}$***-epi*-4** (R_f = 0.3, 0.6 mg, 27% combined yield) as a white solid in a ratio of 16:1 from HPLC integration. Pure product **4** was obtained by HPLC separation (MeOH–H_2O, gradient 86:14 to 90:10): $[\alpha]_D^{23}$ –109 (*c* 0.08, $CHCl_3$); ^{1}H NMR (500 MHz, CD_3OD, major rotamer) δ 7.65 (d, *J* = 8.1 Hz, 1H), 7.59 (d, *J* = 7.7 Hz, 1H), 7.22–7.25 (m, 2H), 7.09–7.12 (m, 1H), 5.05 (dd, *J* = 9.2, 5.1 Hz, 1H), 4.94 (dd, *J* = 10.6, 4.0 Hz, 1H), 4.82 (m, overlapped with water peak, 1H), 4.46 (m, 2H), 4.36–4.40 (m, 2H), 4.07 (s, 3H), 3.22–3.32 (m, overlapped with solvent peak, 2H), 3.27 (s, partially overlapped, 3H), 2.93 (s, 3H), 2.59 (s, 3H), 2.16–2.21 (m, 1H), 1.94–1.99 (m, 1H), 1.82–1.84 (m, 1H), 1.10–1.75 (m, 27H), 0.88–1.05 (m, 18H), 0.46 (d, *J* = 6.6 Hz, 3H), 0.00 (d, *J* = 6.6 Hz, 3H), –0.59 (ddd, *J* = 12.8, 11.0, 1.8 Hz, 1H); IR (neat) ν_{max} 3296, 2923, 2246, 1732, 1682, 1661, 1634, 1615, 1538, 1463, 1385, 1204 cm^{-1}; HRMALDI–FTMS *m/z* 998.6455 ([M + Na]$^+$, $C_{53}H_{85}N_9O_8Na$ requires 998.6413).

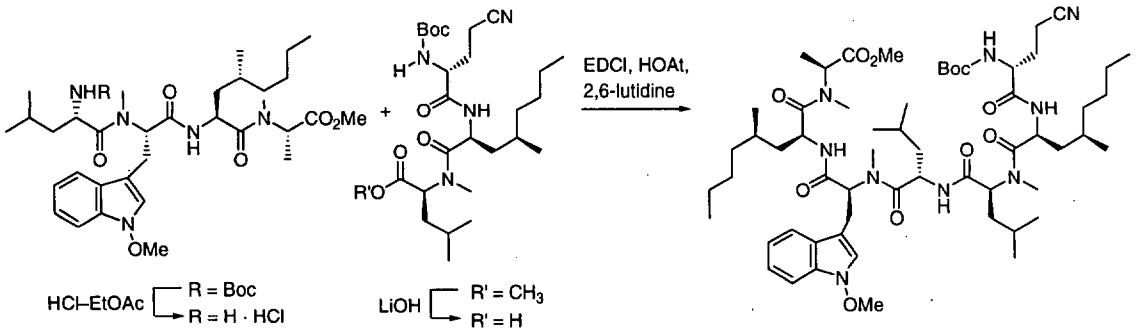
Alternative route for the synthesis of aza-HUN-7293 (4):



***N*-[(2*S*,4*R*)-2-[*N*-(*tert*-Butoxycarbonyl)amino]-4-methyloctanoyl]-*N*-methyl-L-leucine methyl ester (37Me)**: A CH_2Cl_2–DMF (5:1, 1 mL, 0.1 M) solution of **35** (27.3 mg, 0.10 mmol) and *N*-methyl-L-leucine methyl ester hydrochloride (29.3 mg, 0.15 mmol) at 0 °C was treated with EDCI (38.3 mg, 0.2 mmol), HOAt (14.3 mg, 0.105 mmol) and $NaHCO_3$ (12.6 mg, 0.15 mmol), and the solution was allowed to warm to 25 °C for a total of 11 h. The reaction mixture was worked-up as described and afforded **37Me** (37.8 mg, 91% yield) as a light yellow oil. HPLC analysis (MeOH/H_2O, 80/20) of this crude product showed one major peak (R_t = 9.7 min, 77%): ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 5.30 (dd, *J* = 9.5, 6.2 Hz, 1H), 5.17 (br d, *J* = 9.2 Hz, 1H), 4.59–4.64 (m, 1H), 3.66 (s, 3H), 2.95 (s, 3H), 1.62–1.72 (m, 2H), 1.31–1.54 (m, partially overlapped, 2H), 1.38 (s, partially overlapped, 9H), 1.19–1.25 (m, 8H), 0.84–0.95 (m, 12H); IR (neat) ν_{max} 3319, 2957, 2929, 2871, 1744, 1708, 1648, 1497, 1458, 1366, 1251, 1174 cm^{-1}; HRMALDI–FTMS *m/z* 437.2998 ([M + Na]$^+$, $C_{22}H_{42}N_2O_5Na$ requires 437.2986).

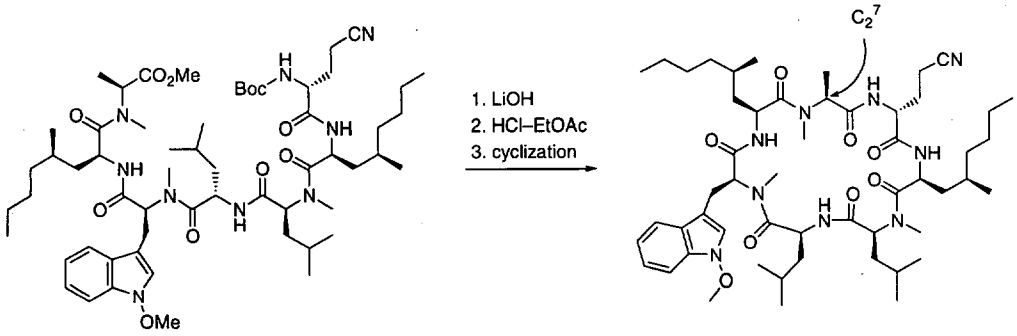


***N*-[(2*S*,4*R*)-2-[*N*-[(*R*)-2-(*tert*-Butoxycarbonyl)amino-4-cyanobutanoyl]amino]-4-methyloctanoyl]-*N*-methyl-L-leucine methyl ester (3NMe)**: An EtOAc (0.5 mL) solution of **37Me** (37.8 mg, 91.2 μmol) was treated with HCl–EtOAc (4.0 M, 0.5 mL) at 0 °C for 2 h, until no starting material was detected by TLC. After removing the volatiles by a stream of N_2, a CH_2Cl_2–DMF (5:1, 0.8 mL, 0.1 M) solution of (*R*)-2-*N*-(butoxycarbonyl)amino-4-cyanobutanoic acid (18.9 mg, 82.9 μmol) was added to the residue, and the mixture was cooled to 0 °C. EDCI (31.8 mg, 165.8 mmol), HOAt (11.8 mg, 87.0 μmol) and $NaHCO_3$ (7.0 mg, 82.9 μmol) were added, and the solution was stirred for 4 h at 0 °C. The reaction mixture was worked-up as described and afforded **3NMe** (33.0 mg, 76% yield) as a thick colorless oil. HPLC analysis (MeOH/H_2O, 80/20) of this crude product showed one major peak (R_t = 6.2 min, 71%): ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 6.94 (br d, *J* = 6.2 Hz, 1H), 5.25 (dd, *J* = 10.6, 5.5 Hz, 1H), 5.21 (br s, 1H), 4.91–4.95 (m, 1H), 4.25 (br s, 1H), 3.67 (s, 3H), 2.96 (s, 3H), 2.35–2.42 (m, 2H), 2.19 (br s, 1H), 1.87–1.96 (m, 1H), 1.57–1.74 (m, 3H), 1.41 (s, partially overlapped, 9H), 1.41–1.47 (m, partially overlapped, 2H), 1.22–1.25 (m, 7H), 0.84–0.97 (m, 12H); IR (neat) ν_{max} 3296, 2957, 2930, 2246, 1744, 1718, 1636, 1508, 1458, 1367, 1248, 1171 cm^{-1}; HRMALDI–FTMS *m/z* 547.3474 ([M + Na]$^+$, $C_{27}H_{48}N_4O_6Na$ requires 547.3466).

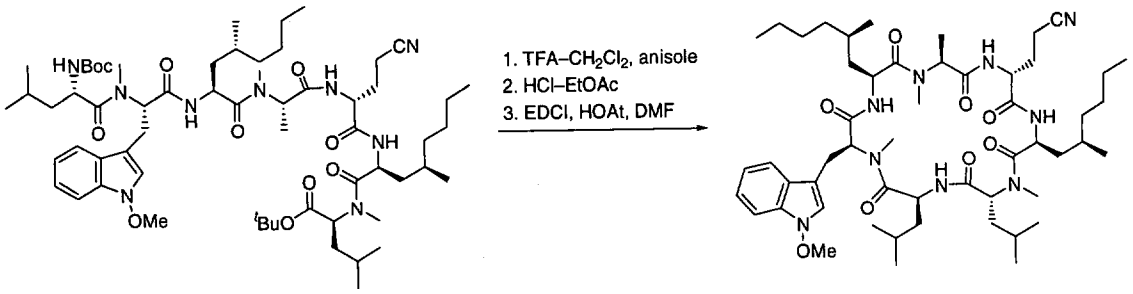


***N*-[*N*-[(2*S*,4*R*)-2-[*N*-[*N*-[*N*-[(2*S*,4*R*)-2-[*N*-[(*R*)-2-(*tert*-Butoxycarbonyl)amino-4-cyanobutanoyl]amino]-4-methyloctanoyl]-*N*-methyl-L-leucinyl]amino-L-leucinyl]amino-*N*$^{1'}$-methoxy-*N*-methyl-L-tryptophanyl]amino-4-methyloctanoyl]-*N*-methyl-L-alanine methyl ester (48NA)**: A mixture of **47** (47.3 mg, 66.0 μmol) and anisole (2 drops) at 0 °C was treated with 0.3 mL of HCl–EtOAc (4.0 M), and the solution was stirred at 0 °C for 3 h, until no starting material was detected by TLC. Following removal of the volatiles by a stream of N_2, compound **3NMe**-OH (crude product from saponification reaction of its methyl ester **3NMe**, 33.0 mg, 62.9 μmol, 0 °C, 2.5 h) was added, followed by CH_2Cl_2–DMF (5:1, 0.6 mL, 0.1 M). The resulting solution at –30 °C was treated with EDCI (24.1 mg, 125.8 μmol), HOAt (9.0 mg, 66.0 μmol) and 2,6-lutidine (7.7 μL, 66.0 μmol), and the reaction mixture was stirred at –30 °C for 4 h.

The reaction was worked-up as described, and **48NA** (51.2 mg, 73% yield) was obtained as a light yellow solid. HPLC analysis (MeOH/H_2O, 90/10) of this crude product showed one major peak (R_t = 6.5 min, 46%). A small amount of this sample was further purified by HPLC to give pure compound for characterization: ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 8.38 (br d, J = 7.7 Hz, 1H), 7.50 (br d, J = 8.1 Hz, 1H), 7.36 (br d, J = 8.4 Hz, 1H), 7.18–7.22 (m, 1H), 7.05–7.12 (m, 1H), 6.97 (s, 1H), 6.78 (br d, J = 6.2 Hz, 1H), 5.39 (q, J = 7.2 Hz, 1H), 5.20 (dd, J = 13.6, 6.6 Hz, 1H), 4.78–5.01 (m, 3H), 4.27–4.29 (m, 1H), 4.14–4.15 (m, 1H), 4.00 (s, 3H), 3.70 (s, 3H), 3.32 (dd, J = 15.2, 3.9 Hz, 1H), 3.07–3.13 (m, partially overlapped, 1H), 3.09 (s, partially overlapped, 3H), 2.94 (s, 3H), 2.92 (s, 3H), 2.35–2.41 (m, 2H), 2.21 (br s, 1H), 1.10–1.91 (m, partially overlapped, 24H), 1.41 (s, partially overlapped, 9H), 0.82–0.97 (m, 21H), 0.39 (d, J = 6.2 Hz, 3H), –0.10 (d, J = 6.6 Hz, 3H), –0.48 (br dd, J = 12.7, 12.3 Hz, 1H); IR (neat) ν_{max} 3293, 2956, 2929, 2246, 1744, 1717, 1701, 1654, 1636, 1540, 1457, 1384, 1250, 1170, 1097 cm^{-1}; HRMALDI–FTMS *m/z* 1130.7238 ([M + Na]$^+$, $C_{59}H_{97}N_9O_{11}Na$ requires 1130.7265).



For **4**: A *tert*-BuOH–H_2O (2:1) solution of linear heptapeptide **48NA** (2.6 mg, 2.3 μmol) at 0 °C was treated with LiOH·H_2O (0.2 mg, 4.7 μmol) powder, and the mixture was stirred at 0 °C for 1 h, until no starting material was detected by TLC. After work-up as described, the free carboxylic acid was obtained as a pale yellow solid. Anisole (1 drop) and 0.3 mL of HCl–EtOAc (4.0 M) was added to this solid at 0 °C, and the mixture was kept at 0 °C for 1 h. The volatiles were removed by a stream of N_2, and the sample was further dried under high vacuum for 30 min. CH_2Cl_2–DMF (5:1, 0.5 mL, 5 mM) was added, and the solution was cooled to 0 °C, followed by the addition of EDCI (0.9 mg, 4.7 μmol), HOAt (0.4 mg, 2.8 μmol) and 2,6-lutidine (0.5 μL, 4.7 μmol). The reaction mixture was stirred at 0 °C for 5 h, and was quenched with the addition of 2 mL of aqueous 1 M HCl. The aqueous layer was extracted with EtOAc (2 mL × 3), and the organic layers were combined, and dried ($MgSO_4$). After removing the solvent in vacuo, the crude product was obtained as a light yellow solid. Chromatography (PTLC, SiO_2) of the crude product provided **4** and its C_2^7-epimer (R_f = 0.3, 0.7 mg, 30% combined yield) as a white solid in a 2:1 ratio by HPLC (MeOH/H_2O, 88/12, R_t = 9.3, 10.5 min). Pure **4** was obtained by HPLC separation.

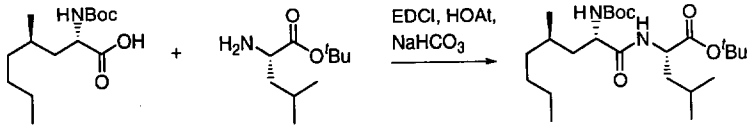


For C_2^3-*epi*-**4**: Linear heptapeptide **48N** (3.9 mg, 3.4 μmol) was deprotected as described. DMF (0.3 mL, 10 mM) was added to the resulting white solid, and the solution was cooled to 0 °C. EDCI (6.5 mg, 34 μmol) and HOAt (4.6 mg, 34 μmol) were added, and after 1 h, the reaction was allowed to warm up to 25 °C and stirred for another 17 h. The reaction was worked-up as described, and the solvent was removed in vacuo providing crude product as white solid (C_2^3-*epi*-**4**:**4** = 4.6:1). A major peak from semi-preparative HPLC was collected (0.8 mg, 24% yield) as a white solid.

Purified linear heptapeptide **48N** (1.0 mg, 0.9 μmol), after deprotection as described, was treated with EDCI (1.7 mg, 8.7 μmol) and HOAt (1.2 mg, 8.7 μmol) in DMF (0.2 mL, 5 mM), and provided C_2^3-*epi*-**4** and **4** (0.5 mg) in 85% combined yield: C_2^3-*epi*-**4**:**4** = 32.2:1.

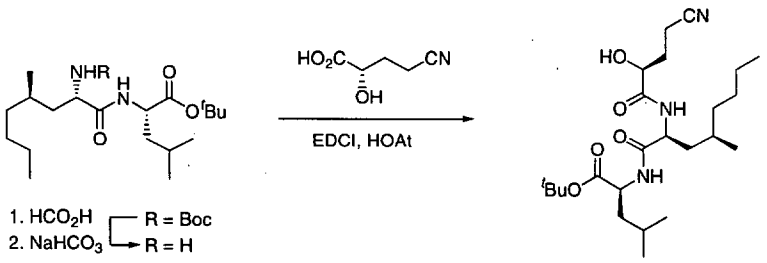
For C_2^3-*epi*-**4**: ^{1}H NMR (500 MHz, CD_3OD, major rotamer) δ 8.24 (br d, J = 8.1 Hz, 1H), 7.63 (br d, J = 8.1 Hz, 1H), 7.57 (br d, J = 7.6 Hz, 1H), 7.21–7.24 (m, 2H), 7.10 (dd, J = 7.6, 7.6 Hz, 1H), 5.19 (dd, J = 12.3, 3.9 Hz, 1H), 5.05 (dd, J = 7.2, 7.2 Hz, 1H), 4.96 (dd, J = 9.5, 4.4 Hz, 1H), ~4.84 (overlapped with water peak, 1H), 4.52–4.54 (m, 2H), 4.45 (dd, J = 8.3, 5.3 Hz, 1H), 4.06 (s, 3H), 3.20–3.21 (m, 2H), 3.12 (br s, 3H), 2.94 (s, 3H), 2.78 (s, 3H), 2.52 (br dd, J = 7.0, 7.0 Hz, 2H), 2.17–2.21 (m, 1H), 1.98–2.03 (m, 1H), 1.81 (br dd, J = 13.2, 11.7 Hz, 1H), 1.69–1.74 (m, 2H), 1.59–1.63 (m, 1H), 1.20–1.41 (m, 28H), 0.85–0.96 (m, 12H), 0.52 (d, J = 6.6 Hz, 3H), 0.05 (d, J = 6.6 Hz, 3H), –0.38 (br dd, J = 13.2, 11.7 Hz, 1H); IR (neat) ν_{max} 3290, 2956, 2927, 2871, 2246, 1745, 1721, 1698, 1678, 1651, 1634, 1531, 1463, 1410, 1385, 1248, 1173, 1098 cm^{-1}; HRMALDI–FTMS *m/z* 998.6422 ([M + Na]$^+$, $C_{53}H_{85}N_9O_8Na$ requires 998.6413).

_N_-Methyl deletion analogues:

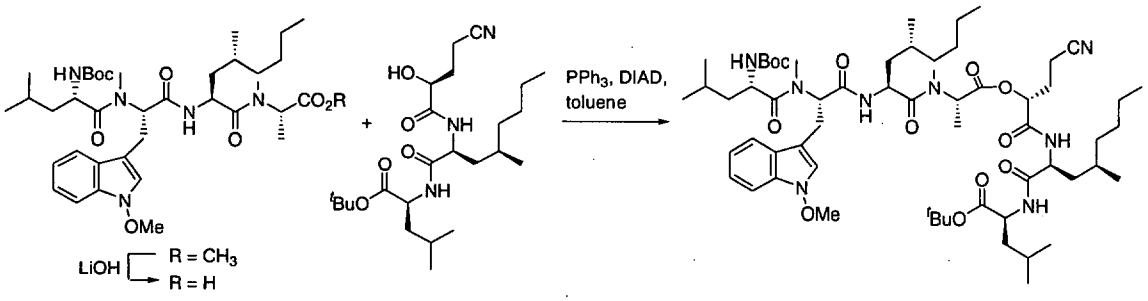


***N*-[(2*S*,4*R*)-2-[*N*-(*tert*-Butoxycarbonyl)amino]-4-methyloctanoyl]-L-leucine *tert*-butyl ester (5D)**: A CH_2Cl_2–DMF (5:1, 1 mL, 0.1 M) solution of **35** (27.3 mg, 0.10 mmol) and L-leucine *tert*-butyl ester hydrochloride (33.6 mg, 0.15 mmol) at 0 °C was treated with EDCI (38.3 mg, 0.2 mmol), HOAt (15.0 mg, 0.11 mmol) and $NaHCO_3$ (12.6 mg, 0.15 mmol), and the reaction mixture was allowed to warm to 25 °C and stirred for a total of 16 h. After work-up as described and removal of the solvent in vacuo, **5D** (40.3 mg, 91% yield) was obtained as a light yellow oil. HPLC analysis (MeOH/H_2O, 80/20) of this crude product showed one major peak (R_t = 12.1 min, 78%): ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 6.37 (br d, J = 8.4 Hz, 1H), 4.84 (br d, J = 7.7 Hz, 1H), 4.42–4.47 (m,

1H), 4.08 (br s, 1H), 1.44–1.65 (m, 2H), 1.42 (s, 9H), 1.41 (s, 9H), 1.41–1.29 (m, 10H), 0.88–0.90 (m, 9H), 0.85 (br t, J = 6.6 Hz, 3H); IR (neat) ν_{max} 3327, 3285, 2960, 1745, 1686, 1651, 1547, 1391, 1153 cm^{-1}; HRMALDI–FTMS m/z 465.3292 ([M + Na]$^+$, $C_{24}H_{46}N_2O_5Na$ requires 465.3304).

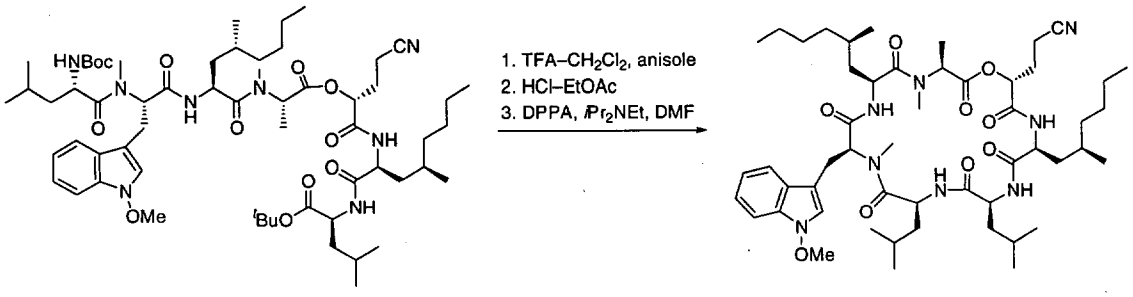


***N*-[(2*S*,4*R*)-2-[*N*-[(*S*)-4-Cyano-2-hydroxybutanoyl]amino]-4-methyloctanoyl]-L-leucine *tert*-butyl ester (5T)**: A HCO_2H (0.5 mL) solution of **5D** (22.1 mg, 50.0 μmol) was stirred at 25 °C for 2 h, until no starting material was detected by TLC. Work-up the reaction mixture as described and removal of the solvent in vacuo afforded light yellow thick oil. A CH_2Cl_2–DMF (5:1, 0.5 mL, 0.1 M) solution of **39** (6.5 mg, 50.0 μmol) was added at –30 °C to this amine. EDCI (19.2 mg, 0.1 mmol) and HOAt (7.1 mg, 52.5 μmol) were added, and the reaction was stirred for 3 h at –30 °C. The reaction mixture was worked-up as described providing **5T** (16.7 mg, 74% yield) as a thick light yellow oil. HPLC analysis (MeOH/H_2O, 80/20) of this crude product showed one major peak (R_t = 5.4 min, 62%): ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 7.16 (br d, J = 8.4 Hz, 1H), 6.47 (br d, J = 8.2 Hz, 1H), 4.44–4.49 (m, 1H), 4.37 (ddd, J = 8.2, 8.2, 5.5 Hz, 1H), 4.20 (dd, J = 8.1, 4.0 Hz, 1H), 2.45–2.54 (m, 2H), 2.13–2.21 (m, 1H), 1.89–1.98 (m, 1H), 1.69 (ddd, J = 14.0, 10.2, 4.3 Hz, 1H), 1.42–1.65 (m, partially overlapped, 5H), 1.44 (s, partially overlapped, 9H), 1.20–1.31 (m, 6H), 0.86–0.92 (m, 12H); IR (neat) ν_{max} 3297, 2958, 2246, 1737, 1650, 1547, 1368, 1152 cm^{-1}; HRMALDI–FTMS m/z 476.3076 ([M + Na]$^+$, $C_{24}H_{43}N_3O_5Na$ requires 476.3095).

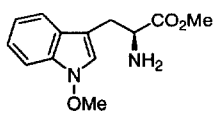


***N*-[(2*S*,4*R*)-2-[*N*-[(*R*)-2-[*N*-[[(2*S*,4*R*)-2-[[*N*-[*N*-(*tert*-Butoxycarbonyl)-L-leucinyl]-*N*$^{1'}$-methoxy-*N*-methyl-L-tryptophanyl]amino]-4-methyloctanoyl]-*N*-methyl-L-alanyloxy]-4-cyanobutanoyl]amino]-4-methyloctanoyl]-L-leucine *tert*-butyl ester (5H)**: A toluene (348 μL, 0.05 M) solution of **5T** (7.9 mg, 17.4 μmol), **2** (12.2 mg, 17.4 μmol) and PPh_3 (22.8 mg, 87.1 μmol) at 0 °C was treated dropwise with DIAD (95% purity, 18.0 μL, 87.1 μmol), and the mixture was allowed to warm to 25 °C and stirred for a total of 19 h. The solvent was removed by a stream of N_2, and the residue was

dissolved in MeOH, and was subjected to reverse-phase HPLC chromatography. Compound **5H** (R_t = 36 min, 4.1 mg, 21% yield) was obtained as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 7.54–7.55 (m, 2H), 7.49 (br d, J = 7.7 Hz, 1H), 7.34 (d, J = 7.8 Hz, 1H), 7.18 (dd, J = 7.8, 7.8 Hz, 1H), 7.10 (s, 1H), 7.06 (dd, J = 7.8, 7.8 Hz, 1H), 6.16 (br d, J = 8.1 Hz, 1H), 5.49 (dd, J = 9.5, 6.6 Hz, 1H), 5.33 (br d, J = 9.2 Hz, 1H), 5.16 (dd, J = 7.7, 4.0 Hz, 1H), 4.98–5.01 (m, 1H), 4.78–4.81 (m, 1H), 4.48–4.56 (m, 2H), 4.39–4.43 (m, 1H), 3.98 (s, 3H), 3.27 (dd, J = 15.6, 6.4 Hz, 1H), 3.14–3.20 (m, 1H), 3.13 (s, 3H), 2.99 (s, 3H), 2.26–2,35 (m, 2H), 2.11–2.15 (m, 1H), 1.90–2.04 (m, 2H), 1.08–1.74 (m, partially overlapped, 26H), 1.44 (s, 9H), 1.40 (s, 9H), 0.84–0.95 (m, 18H), 0.79 (d, J = 6.2 Hz, 3H), 0.72 (d, J = 6.2 Hz, 3H); IR (neat) ν_{max} 3294, 2957, 2246, 1749, 1733, 1682, 1651, 1631, 1558, 1457, 1368, 1162 cm^{-1}; HRMALDI–FTMS *m/z* 1160.7339 ([M + Na]$^+$, $C_{61}H_{100}N_8O_{12}Na$ requires 1159.7353).

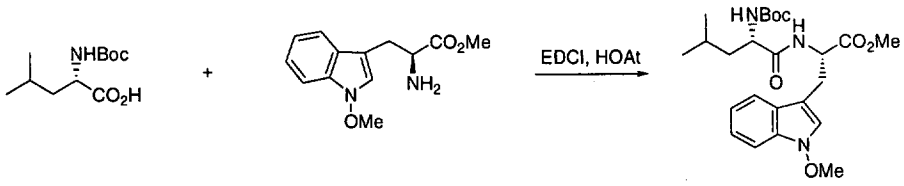


For **5**: Linear heptadepsipeptide **5H** (1.9 mg, 1.7 μmol) was deprotected following the standard procedure, and the residue was dissolved in DMF (1.7 mL, 1 mM) at 0 °C. DPPA (0.7 μL, 3.3 μmol) and iPr$_2$NEt (0.6 μL, 3.3 μmol) were added, and the mixture was stirred at 0 °C for 63 h. After work-up of the reaction mixture as described, the residue was subjected to HPLC chromatography and gave **5** (R_t = 22 min, 0.4 mg, 25% yield) as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 8.07 (br d, J = 9.5 Hz, 1H), 8.00 (br d, J = 8.1 Hz, 1H), 7.71 (d, J = 7.7 Hz, 1H), 7.38 (d, J = 7.7 Hz, 1H), 7.20–7.24 (m, overlapped with solvent peak, 1H), 7.10 (dd, J = 7.7, 7.7 Hz, 1H), 7.00 (s, 1H), 5.99 (br d, J = 8.4 Hz, 1H), 5.26 (dd, J = 4.6, 4.6 Hz, 1H), 4.96–5.04 (m, 2H), 4.39–4.44 (m, 1H), 4.29–4.33 (m, 1H), 4.15–4.20 (m, 1H), 4.02 (s, 3H), 3.77 (q, J = 7.0 Hz, 1H), 3.31 (s, 3H), 3.16–3.21 (m, 2H), 2.92 (s, 3H), 2.39–2.43 (m, 1H), 2.27–2.34 (m, 2H), 2.12–2.17 (m, 1H), 1.84–1.89 (m, 1H), 1.09–1.64 (m, 25H), 0.93 (d, J = 6.6 Hz, 3H), 0.82–0.89 (m, 15H), 0.53 (d, J = 6.6 Hz, 3H), –0.10 (d, J = 6.6 Hz, 3H), –0.28 (ddd, J = 13.9, 10.6, 3.3 Hz, 1H); IR (neat) ν_{max} 3282, 2924, 2246, 1752, 1725, 1677, 1658, 1641, 1630, 1546, 1467, 1215, 1096 cm^{-1}; HRMALDI–FTMS *m/z* 985.6085 ([M + Na]$^+$, $C_{52}H_{82}N_8O_9Na$ requires 985.6097).

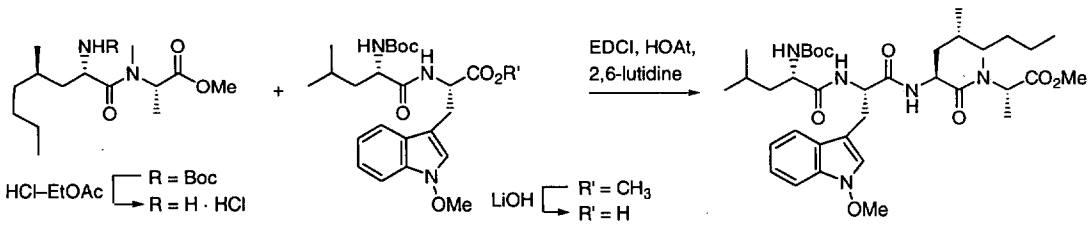


N^1-Methoxy-L-tryptophan methyl ester: Adapting the procedure of making N^1-methoxy-*N*-methyl-L-tryptophan methyl ester,[8] starting from *N*-(9-fluorenylmethoxycarbonyl)-L-tryptophan (853.0 mg, 2.0 mmol), N^1-methoxy-L-tryptophan methyl ester (132.7 mg, 27% overall yield) was obtained as a very light

yellow thick oil: $[\alpha]_D^{23}$ +58 (*c* 1.7, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.68 (br d, *J* = 7.6 Hz, 1H), 7.50 (br d, *J* = 7.6 Hz, 1H), 7.34 (dd, *J* = 7.6, 7.6 Hz, 1H), 7.20–7.24 (m, 4H), 4.14 (s, 3H), 3.89 (br s, 1H), 3.80 (s, 3H), 3.32 (dd, *J* = 14.3, 4.2 Hz, 1H), 3.11 (dd, *J* = 14.3, 7.5 Hz, 1H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 175.5, 132.3, 123.8, 122.4, 121.8, 119.6, 118.9, 108.2, 107.0, 65.6, 54.9, 51.9, 30.5; IR (neat) ν_{max} 3374, 3313, 3120, 3054, 2937, 1735, 1676, 1596, 1438, 1384, 1202 cm^{-1}; LRMS (ESI) *m/z* 271 ([M + Na]$^+$), FABHRMS *m/z* 217.0978 ([M – OMe]$^+$, $C_{12}H_{13}N_2O_2$ requires 217.0977).

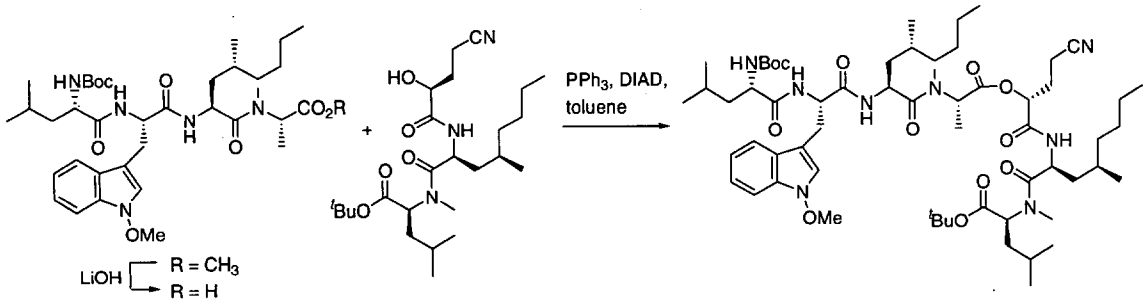


***N*-[*N*-(*tert*-Butoxycarbonyl)-L-leucinyl]-*N*^{1}-methoxy-L-tryptophan methyl ester (6D)**: A CH_2Cl_2–DMF (5:1, 1 mL, 0.1 M) solution of *N*^{1}-methoxy-L-tryptophan methyl ester (27.3 mg, 0.11 mmol) and *N*-(*tert*-butoxycarbonyl)-L-leucine hydrate (**40**, 24.9 mg, 0.1 mmol) at 0 °C was treated with EDCI (38.3 mg, 0.2 mmol) and HOAt (14.3 mg, 0.105 mmol), and the reaction mixture was allowed to warm to 25 °C for a total of 13 h. The reaction mixture was worked-up as described and afforded **6D** (39.9 mg, 86% yield) as a very light yellow foamy solid. HPLC analysis (MeOH/H_2O, 80/20) of this crude product showed one major peak (R_t = 4.6 min, 96%): ^1H NMR (500 MHz, $CDCl_3$) δ 7.47 (br d, *J* = 7.7 Hz, 1H), 7.37 (br d, *J* = 8.1 Hz, 1H), 7.20 (br dd, *J* = 8.0, 7.3 Hz, 1H), 7.14 (br s, 1H), 7.08 (ddd, *J* = 7.7, 7.3, 0.7 Hz, 1H), 6.58 (br d, *J* = 7.7 Hz, 1H), 4.84–4.87 (m, 1H). 4.80 (br d, *J* = 5.9 Hz, 1H), 4.07 (br s, 1H), 4.03 (s, 3H), 3.64 (s, 3H), 3.26 (d, *J* = 5.1 Hz, 2H), 1.59–1.65 (m, 2H), 1.40 (br s, 10H), 0.88 (d, *J* = 5.9 Hz, partially overlapped, 3H), 0.87 (d, *J* = 5.9 Hz, partially overlapped, 3H); IR (neat) ν_{max} 3309, 3117, 3056, 2956, 1744, 1692, 1661, 1520, 1439, 1367, 1169 cm^{-1}; HRMALDI–FTMS *m/z* 484.2419 ([M + Na]$^+$, $C_{24}H_{35}N_3O_6Na$ requires 484.2418).

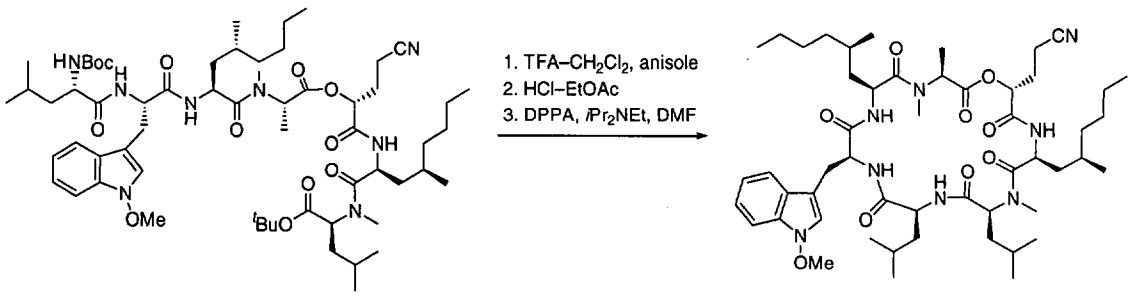


***N*-[(2*S*,4*R*)-2-[[*N*-[*N*-(*tert*-Butoxycarbonyl)-L-leucinyl]-*N*^{1}-methoxy-L-tryptophanyl]amino]-4-methyloctanoyl]-*N*-methyl-L-alanine methyl ester (6T)**: A HCl–EtOAc solution (4.1 M, 0.4 mL) was added to **45** (35.4 mg, 95.1 μmol) at 0 °C, and the stirring was continued for 1 h, until no starting material was detected by TLC. The volatiles were removed by a stream of N_2. **6D**-OH (crude product from saponification reaction of its methyl ester **6D**, 39.9 mg, 86.4 μmol, 0 °C, 2 h) was added, followed by CH_2Cl_2–DMF (5:1, 0.9 mL, 0.1 M). The resulting solution at –30 °C was treated with EDCI (33.1 mg, 172.9 μmol), HOAt (12.4 mg, 90.8 μmol) and 2,6-lutidine (11.1 μL, 95.1 μmol), and the reaction mixture was stirred at –30 °C for 3 h. The reaction mixture was worked-up as described and afforded **6T** (58.1 mg, 96% yield) as a light yellow foamy

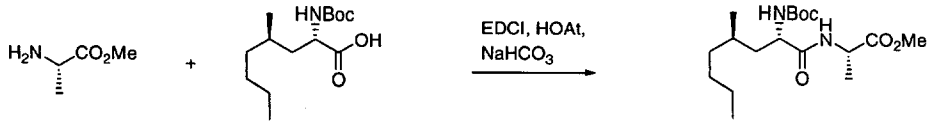
solid. HPLC analysis (MeOH/H_2O, 80/20) of this crude product showed one major peak (R_t = 12.5 min, 57%): ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 7.62 (br d, J = 8.1 Hz, 1H), 7.34 (d, J = 7.9 Hz, 1H), 7.16–7.19 (m, 2H), 7.05 (dd, J = 7.9, 7.3 Hz, 1H), 6.82 (br d, J = 7.7 Hz, 1H), 6.53 (br d, J = 7.7 Hz, 1H), 5.12–5.16 (m, 1H), 4.80–4.86 (m, 2H), 4.65–4.69 (m, 1H), 4.08 (br s, 1H), 4.01 (s, 3H), 3.66 (s, 3H), 3.21 (dd, J = 13.4, 5.0 Hz, 1H), 3.08 (dd, J = 14.5, 7.5 Hz, 1H), 2.89 (s, 3H), 1.59–1.62 (m, 2H), 1.30–1.48 (m, partially overlapped, 4H), 1.37 (s, partially overlapped, 9H), 1.07–1.26 (m, 9H), 0.80–0.89 (m, 12H); IR (neat) ν_{max} 3285, 3059, 2955, 2870, 1745, 1692, 1636, 1522, 1453, 1385, 1245, 1169 cm^{-1}; HRMALDI–FTMS *m/z* 724.4230 ([M + Na]$^+$, $C_{37}H_{59}N_5O_8Na$ requires 724.4261).



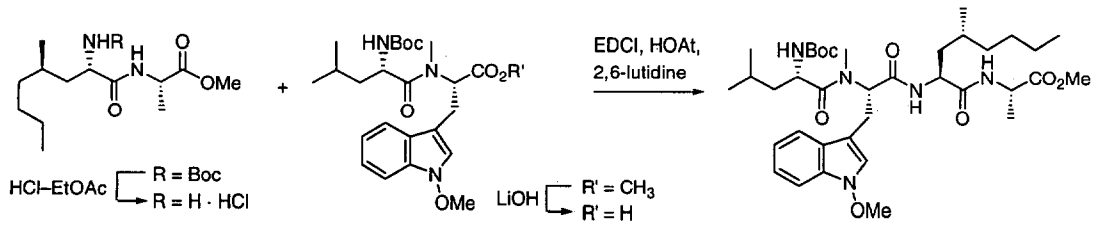
***N*-[(2*S*,4*R*)-2-[*N*-[(*R*)-2-[*N*-[[(2*S*,4*R*)-2-[[*N*-[*N*-(*tert*-Butoxycarbonyl)-L-leucinyl]-*N*^{1}-methoxy-L-tryptophanyl]amino]-4-methyloctanoyl]-*N*-methyl-L-alanyloxy]-4-cyanobutanoyl]amino]-4-methyloctanoyl]-*N*-methyl-L-leucine *tert*-butyl ester (6H)**: A toluene (0.4 mL, 0.05 M) solution of **3** (9.4 mg, 20.0 μmol), **6T**-OH (crude product from saponification reaction of its methyl ester **6T**, 14.0 mg, 20.0 μmol, 0 °C, 2 h) and PPh_3 (26.2 mg, 100 μmol) at 0 °C was treated dropwise with DIAD (95% purity, 20.7 μL, 100 μmol), and the mixture was allowed to warm to 25 °C for a total of 17 h. The solvent was removed by a stream of N_2, and the residue was dissolved in MeOH, and was subjected to reverse-phase HPLC chromatography. Compound **6H** (R_t = 28 min, 3.9 mg, 17% yield) was obtained as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 8.44 (br d, J = 8.9 Hz, 1H), 7.85 (br d, J = 8.4 Hz, 1H), 7.40 (d, J = 7.3 Hz, 1H), 7.35 (d, J = 7.3 Hz, 1H), 7.16–7.19 (m, 2H), 7.02 (dd, J = 7.3, 7.3 Hz, 1H), 6.67 (br d, J = 7.3 Hz, 1H), 5.28 (dd, J = 10.5, 5.3 Hz, 1H), 5.22 (dd, J = 9.5, 2.6 Hz, 1H), 5.11–5.12 (m, 2H), 4.97 (ddd, J = 11.2, 9.1, 2.5 Hz, 1H), 4.83–4.84 (m, 1H), 4.73 (br s, 1H), 4.03 (s, 3H), 3.92 (q, J = 6.6 Hz, 1H), 3.28–3.32 (m, partially overlapped, 1H), 3.29 (s, 3H), 2.96–2.98 (m, partially overlapped, 1H), 2.96 (s, 3H), 2.05–2.10 (m, 3H), 1.22–1.74 (m, partially overlapped, 28H), 1.36 (s, 9H), 1.43 (s, 9H), 0.93 (d, J = 6.6 Hz, 3H), 0.83–0.89 (m, 21H); IR (neat) ν_{max} 3302, 2957, 2246, 1754, 1723, 1672, 1632, 1544, 1384, 1369, 1164 cm^{-1}; HRMALDI–FTMS *m/z* 1159.7357 ([M + Na]$^+$, $C_{61}H_{100}N_8O_{12}Na$ requires 1159.7353).



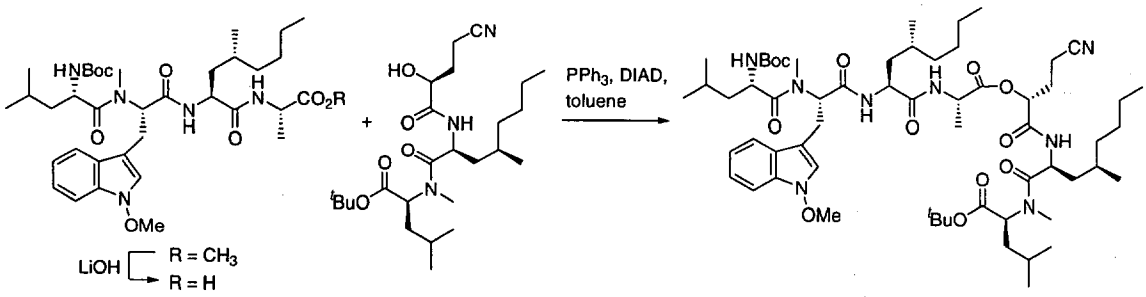
For **6**: Linear heptadepsipeptide **6H** (1.9 mg, 1.7 μmol) was deprotected following the standard procedure, and the residue was dissolved in DMF (1.7 mL, 1 mM) at 0 °C. DPPA (0.7 μL, 3.3 μmol) and *i*Pr_2NEt (0.6 μL, 3.3 μmol) were added, and the mixture was stirred at 0 °C for 61 h. After work-up as described, the residue was subjected to HPLC chromatography and gave **6** (R_t = 28 min, 0.7 mg, 44% yield) as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 8.29 (br d, *J* = 6.6 Hz, 1H), 7.64 (d, *J* = 7.5 Hz, 1H), 7.62 (br d, *J* = 8.8 Hz, 1H), 7.48 (br d, *J* = 9.5 Hz, 1H), 7.45 (s, 1H), 7.32 (d, *J* = 7.5 Hz, 1H), 7.16 (dd, *J* = 7.5, 7.5 Hz, 1H), 7.04 (dd, *J* = 7.5, 7.5 Hz, 1H), 6.82 (br d, *J* = 6.6 Hz, 1H), 5.30 (dd, *J* = 8.8, 3.3 Hz, 1H), 5.00–5.05 (m, 1H), 4.95 (ddd, *J* = 8.8, 7.1, 4.5 Hz, 1H), 4.61 (ddd, *J* = 10.9, 6.6, 3.9 Hz, 1H), 4.01 (s, 3H), 3.68 (q, *J* = 6.6 Hz, 1H), 3.45–3.49 (m, 2H), 3.34 (s, 3H), 3.32 (s, 3H), 3.23–3.32 (m, 2H), 2.40 (ddd, *J* = 17.4, 7.1, 3.9 Hz, 1H), 2.29 (ddd, *J* = 17.4, 9.8, 3.9 Hz, 1H), 2.18–2.22 (m, 1H), 1.99–2.11 (m, 2H), 1.88 (ddd, *J* = 14.2, 9.8, 4.5 Hz, 1H), 1.22–1.58 (m, 25H), 0.99 (d, *J* = 6.6 Hz, 3H), 0.95 (d, *J* = 6.6 Hz, 3H), 0.82–0.90 (m, 12H), 0.78 (d, *J* = 6.6 Hz, 3H), 0.59 (d, *J* = 6.6 Hz, 3H); IR (neat) ν_{max} 3327, 2926, 2249, 1751, 1736, 1666, 1650, 1629, 1540, 1459, 1289, 1095 cm^{-1}; HRMALDI–FTMS *m/z* 985.6102 ([M + Na]$^+$, $C_{52}H_{82}N_8O_9Na$ requires 985.6097).



***N*-[(2*S*,4*R*)-2-[*N*-(*tert*-butoxycarbonyl)amino]-4-methyloctanoyl]-L-alanine methyl ester (7D)**: A CH_2Cl_2–DMF (5:1, 1 mL, 0.1 M) solution of **35** (27.3 mg, 0.10 mmol) and L-alanine methyl ester hydrochloride (20.9 mg, 0.15 mmol) at 0 °C was treated with EDCI (38.3 mg, 0.2 mmol), HOAt (15.0 mg, 0.11 mmol) and $NaHCO_3$ (12.6 mg, 0.15 mmol), and the reaction mixture was allowed to warm to 25 °C for a total of 13 h. The reaction mixture was worked-up as described and afforded **7D** (32.5 mg, 91% yield) as a colorless thick oil. HPLC analysis (MeOH/H_2O, 80/20) of this crude product showed one major peak (R_t = 5.0 min, 66%): ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 6.68 (br s, 1H), 4.91 (br d, *J* = 8.1 Hz, 1H), 4.50–4.55 (m, 1H), 4.12 (br s, 1H), 3.70 (s, 3H), 1.44–1.58 (m, 3H), 1.41 (s, 9H), 1.36 (d, *J* = 7.3 Hz, 3H), 1.12–1.29 (m, 6H), 0.88 (d, *J* = 6.2 Hz, 3H), 0.85 (br t, *J* = 6.6 Hz, 3H); IR (neat) ν_{max} 3301, 2956, 1749, 1682, 1659, 1539, 1457, 1366, 1168 cm^{-1}; HRMALDI–FTMS *m/z* 381.2370 ([M + Na]$^+$, $C_{18}H_{34}N_2O_5Na$ requires 381.2365).

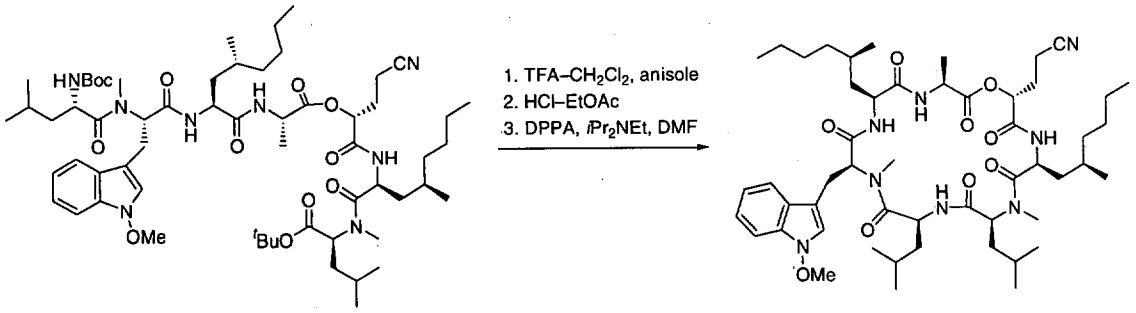


***N*-[(2*S*,4*R*)-2-[[*N*-[*N*-(*tert*-Butoxycarbonyl)-L-leucinyl]-*N*$^{1'}$-methoxy-*N*-methyl-L-tryptophanyl]amino-4-methyloctanoyl]-L-alanine methyl ester (7T)**: A HCl–EtOAc solution (4.0 M, 1.0 mL) was added to **7D** (32.5 mg, 90.7 μmol) at 0 °C, and the stirring was continued for 1 h, until no starting material was detected by TLC. The volatiles were removed by a stream of N_2. A CH_2Cl_2–DMF solution (5:1, 0.8 mL, 0.1 M) of **2** (crude product from saponification reaction of its methyl ester **47**, 39.2 mg, 82.4 μmol, 0 °C, 2 h) was added to the residue at –30 °C. The resulting solution was treated with EDCI (31.6 mg, 164.8 μmol), HOAt (11.8 mg, 86.5 μmol) and 2,6-lutidine (10.6 μL, 90.7 μmol), and the reaction mixture was stirred at –30 °C for 3 h. The reaction mixture was worked-up as described and afforded **7T** (56.6 mg, 98% yield) as a light yellow foamy solid. HPLC analysis (MeOH/H_2O, 80/20) of this crude product showed one major peak (R_t = 12.1 min, 76%): ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 7.95 (br d, J = 8.4 Hz, 1H), 7.60 (d, J = 8.0 Hz, 1H), 7.38 (d, J = 8.0 Hz, 1H), 7.22 (dd, J = 8.0, 7.3 Hz, 1H), 7.09 (dd, J = 8.0, 7.3 Hz, 1H), 7.00 (s, 1H), 4.92 (br d, J = 7.0 Hz, 1H), 4.65 (dd, J = 10.6, 3.3 Hz, 1H), 4.49–4.66 (m, 2H), 4.13–4.17 (m, 1H), 4.00 (s 3H), 3.69 (s, 3H), 3.35 (dd, J = 15.1, 7.5 Hz, 1H), 3.16 (dd, J = 15.1, 7.3 Hz, 1H), 2.92 (s, 3H), 1.64–1.74 (m, 2H), 1.16–1.44 (m, partially overlapped, 12H), 1.36 (s, partially overlapped, 9H), 0.80–0.91 (m, 6H), 0.34 (d, J = 6.6 Hz, 3H), 0.09 (d, J = 6.2 Hz, 3H), –0.45 (br dd, J = 11.4, 11.4 Hz, 1H); IR (neat) ν_{max} 3300, 3057, 2956, 1746, 1687, 1852, 1539, 1456, 1166 cm^{-1}; HRMALDI–FTMS *m/z* 724.4284 ([M + Na]$^+$, $C_{37}H_{59}N_5O_8Na$ requires 724.4261).



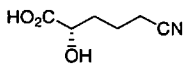
***N*-[(2*S*,4*R*)-2-[*N*-[(*R*)-2-[*N*-[[(2*S*,4*R*)-2-[[*N*-[*N*-(*tert*-Butoxycarbonyl)-L-leucinyl]-*N*$^{1'}$-methoxy-*N*-methyl-L-tryptophanyl]amino]-4-methyloctanoyl]-L-alanyloxy]-4-cyanobutanoyl]amino]-4-methyloctanoyl]-*N*-methyl-L-leucine *tert*-butyl ester (7H)**: A toluene (200 μL, 0.05 M) solution of **3** (4.7 mg, 10.0 μmol), **7T**-OH (6.9 mg, 10.0 μmol, crude product from saponification reaction of its methyl ester **7T**, 0 °C, 2 h) and PPh_3 (7.9 mg, 30.0 μmol) at 0 °C was treated dropwise with DIAD (97% purity, 6.1 μL, 30.0 μmol), and the mixture was stirred at 0 °C for 26 h. The solvent was removed by a stream of N_2, and the residue was dissolved in MeOH, and was subjected to reverse-phase HPLC chromatography. Compound **7H** (R_t = 24 min, 2.2 mg, 19% yield) was obtained as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 7.74 (br d, J = 8.4 Hz,

1H), 7.59–7.62 (m, 1H), 7.36–7.40 (m, 2H), 7.20–7.24 (m, 1H), 7.09–7.14 (m, 3H), 5.18–5.21 (m, 2H), 4.88 (br d, J = 7.7 Hz, 1H), 4.79 (ddd, J = 10.9, 7.7, 3.0 Hz, 1H), 4.56–4.61 (m, 1H), 4.42–4.46 (m, 1H), 4.10–4.14 (m, 1H), 4.00 (s, 3H), 3.81–3.86 (m, 1H), 3.41 (dd, J = 14.5, 8.4 Hz, 1H), 3.12 (dd, J = 14.5, 7.7 Hz, 1H), 2.92 (s, 6H), 2.07–2.50 (m, 6H), 1.21–1.85 (m, partially overlapped, 24H), 1.43 (s, 9H), 1.41 (s, 9H), 0.78–0.98 (m, 18H), 0.35 (d, J = 6.6 Hz, 3H), 0.13 (d, J = 6.6 Hz, 3H), –0.39 (ddd, J = 14.2, 11.5, 3.0 Hz, 1H); IR (neat) ν_{max} 3301, 2929, 2254, 1733, 1640, 1532, 1456, 1164 cm^{-1}; HRMALDI–FTMS *m/z* 1159.7401 ([M + Na]$^+$, $C_{61}H_{100}N_8O_{12}Na$ requires 1159.7353).



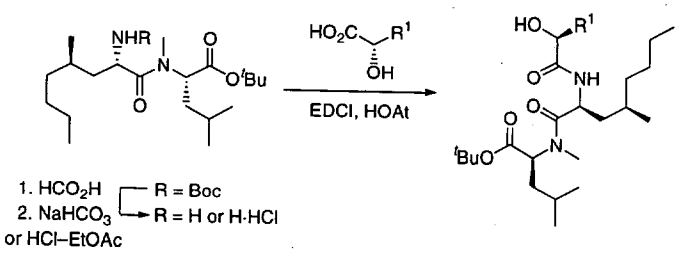
For **7**: Linear heptadepsipeptide **7H** (1.9 mg, 1.7 μmol) was deprotected following the standard procedure, and the residue was dissolved in DMF (1.7 mL, 1 mM) at 0 °C. DPPA (0.7 μL, 3.3 μmol) and iPr$_2$NEt (0.6 μL, 3.3 μmol) were added, and the mixture was stirred at 0 °C for 64 h. After work-up as described, the residue was subjected to HPLC chromatography and gave **7** (R_t = 21 min, 0.2 mg, 13% yield) as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 8.32 (br d, J = 10.3 Hz, 1H), 7.69 (d, J = 8.1 Hz, 1H), 7.53–7.57 (m, 2H), 7.39–7.41 (m, 1H), 7.07–7.20 (m, 1H), 7.03 (s, 1H), 5.93 (br d, J = 5.9 Hz, 1H), 5.72 (br d, J = 9.2 Hz, 1H), 5.53 (dd, J = 4.6, 4.6 Hz, 1H), 5.33 (dd, J = 9.0, 3.5 Hz, 1H), 4.50–4.87 (m, 4H), 4.02 (s, 3H), 3.96 (q, J = 6.8 Hz, 1H), 3.43 (dd, J = 14.9, 4.8 Hz, 1H), 3.11 (dd, J = 14.9, 10.1 Hz, 1H), 2.87 (s, 3H), 2.53 (s, 3H), 1.91–2.33 (m, 4H), 1.78–1.83 (m, 2H), 1.11–1.60 (m, 24H), 0.73–0.97 (m, 18H), 0.50 (d, J = 6.6 Hz, 3H), –0.19 (d, J = 6.6 Hz, 3H), –0.50 (ddd, J = 13.7, 10.7, 3.0 Hz, 1H); IR (neat) ν_{max} 3282, 2923, 2851, 2246, 1744, 1656, 1641, 1528, 1451, 1380, 1246, 1097 cm^{-1}; HRMALDI–FTMS *m/z* 985.6115 ([M + Na]$^+$, $C_{52}H_{82}N_8O_9Na$ requires 985.6097).

Analogues with variations in the DGCN[1] side-chain:



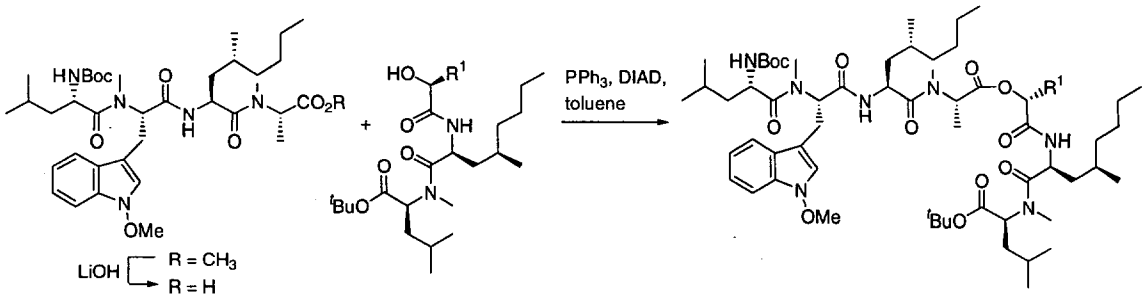
(*S*)-5-Cyano-2-hydroxypentanoic acid: The title compound was synthesized (Knapp, S.; Hale, J. J.; Bastos, M.; Molina, A.; Chen, K. Y. *J. Org. Chem.* **1992**, *57*, 6239–6256.) from (*S*)-2-amino-5-cyanopentanoic acid[32] which was prepared adapting the literature procedure for the synthesis of (*S*)-2-amino-4-cyanobutanoic acid,[8] starting from commercially available L-homoglutamine. A HOAc–H_2O (1:5, 0.23 mL, 0.1 M) solution of (*S*)-2-amino-5-cyanopentanoic acid (3.2 mg, 22.5 mmol) at 0 °C was treated with $NaNO_2$ (3.1 mg, 45.0 mmol) and the solution was allowed to warm to 25 °C and was stirred for 19 h. The solvent was removed in vacuo and the crude product was used

immediately for the synthesis of **9T** without further purification. ^{1}H NMR (400 MHz, D_2O) δ 4.09 (dd, J = 3.8, 3.8 Hz, 1H), 2.51 (t, J = 6.5 Hz, 2H), 1.67–1.88 (m, 4H).



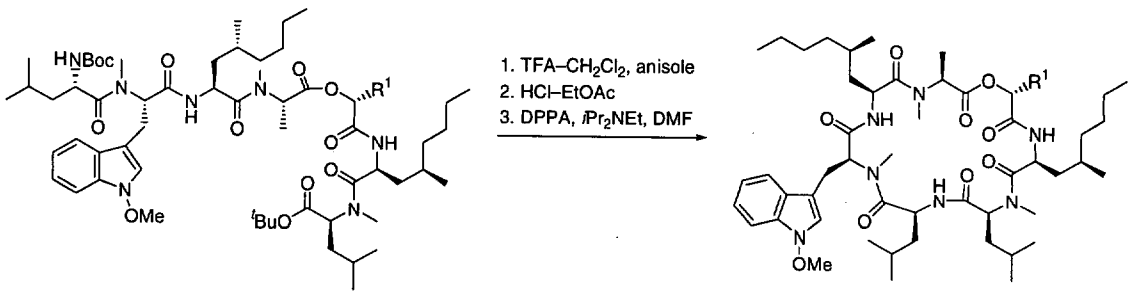
***N*-[(2*S*,4*R*)-2-[(*S*)-2-Hydroxypropanoyl]amino-4-methyloctanoyl]-*N*-methyl-L-leucine *tert*-butyl ester (8T**, R^1 = CH_3): A HCO_2H (0.5 mL) solution of **37** (22.8 mg, 50.0 μmol) was stirred at 25 °C for 2 h, until no starting material was detected by TLC. The reaction mixture was worked-up as described and afforded a light yellow solid. A CH_2Cl_2–DMF (5:1, 0.9 mL, 0.1 M) solution of L-(+)-lactic acid (5.0 mg, 50.0 μmol) was added to this crude amine at –30 °C. This solution was treated with EDCI (19.2 mg, 0.1 mmol), HOAt (7.1 mg, 52.5 μmol) and the stirring was continued for 2 h at –30 °C. The reaction mixture was worked-up as described and afforded **8T** (14.3 mg, 67% yield) as a thick light yellow oil. HPLC analysis (MeOH/H_2O, 80/20) of this crude product showed one major peak (R_t = 7.3 min, 73%): ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 7.09 (br d, J = 8.7 Hz, 1H), 5.13 (dd, J = 10.6, 5.1 Hz, 1H), 4.96 (ddd, J = 10.6, 8.7, 2.6 Hz, 1H), 4.20 (q, J = 6.6 Hz, 1H), 2.96 (s, 3H), 1.64–1.71 (m, 1H), 1.55–1.62 (m, 1H), 1.38–1.50 (m, 13H), 1.42 (s, partially overlapped, 9H), 0.97 (d, J = 6.6 Hz, 3H), 0.90 (d, J = 6.6 Hz, 3H), 0.84–0.86 (m, 6H); IR (neat) ν_{max} 3393, 3303, 2958, 2930, 1733, 1645, 1520, 1456, 1368, 1273 cm^{-1}; HRMALDI–FTMS *m/z* 451.3159 ([M + Na]$^+$, $C_{23}H_{44}N_2O_5Na$ requires 451.3142).

***N*-[(2*S*,4*R*)-2-[*N*-[(*S*)-5-Cyano-2-hydroxypentanoyl]amino]-4-methyloctanoyl]-*N*-methyl-L-leucine *tert*-butyl ester (9T**, R^1 = $(CH_2)_3CN$): An EtOAc (0.1 mL) solution of **37** (6.9 mg, 15.0 μmol) was treated with HCl–EtOAc (4.0 M, 0.1 mL) at 0 °C for 3 h, until no starting material was detected by TLC. After removing the volatiles by a stream of N_2, a CH_2Cl_2–DMF (5:1, 150 μmmol, 0.1 M) solution of (*S*)-5-cyano-2-hydroxypentanoic acid (3.2 mg, 22.5 μmol) was added to the residue at –30 °C. EDCI (5.8 mg, 30.0 μmol), HOAt (2.1 mg, 15.8 μmol), and 2,6-lutidine (3.5 μL, 30.0 μmol) were added, and the solution was stirred for 3 h at –30 °C. The reaction mixture was worked-up as described and provided **9T** (5.7 mg, 79% yield) as a thick light yellow oil. HPLC analysis (MeOH/H_2O, 80/20) of this crude product showed one major peak (R_t = 6.3 min, 96%): ^{1}H NMR (400 MHz, $CDCl_3$, major rotamer) δ 7.03 (br d, J = 8.8 Hz, 1H), 5.13 (dd, J = 10.4, 5.1 Hz, 1H), 4.95 (ddd, J = 11.3, 8.8, 2.6 Hz, 1H), 4.14–4.16 (m, 1H), 2.96 (s, 3H), 2.38 (br dd, J = 7.0, 7.0 Hz, 2H), 1.84–1.91 (m, 1H), 1.23–1.84 (m, partially overlapped, 15H), 1.42 (s, 9H), 0.98 (d, J = 6.2 Hz, 3H), 0.91 (d, J = 6.7 Hz, 3H), 0.85–0.87 (m, 6H); IR (neat) ν_{max} 3382, 3323, 2957, 2246, 1732, 1651, 1645, 1633, 1519, 1470, 1368, 1159 cm^{-1}; HRMALDI–FTMS *m/z* 504.3422 ([M + Na]$^+$, $C_{26}H_{47}N_3O_5Na$ requires 504.3408).



***N*-[(2*S*,4*R*)2-[*N*-[(*R*)-2-[*N*-[(2*S*,4*R*)-2-[[*N*-[*N*-(*tert*-Butoxycarbonyl)-L-leucinyl]-*N*$^{1'}$-methoxy-*N*-methyl-L-tryptophanyl]amino]-4-methyloctanoyl]-*N*-methyl-L-alanyloxy]propanoyl]amino]-4-methyloctanoyl]-*N*-methyl-L-leucine *tert*-butyl ester** (**8H**, $R^1 = CH_3$): A toluene (0.2 mL, 0.05 M) solution of **8T** (4.3 mg, 10 μmol), **2** (7.0 mg, 10 μmol) and PPh_3 (7.9 mg, 30 μmol) at 0 °C was treated dropwise with DIAD (97% purity, 6.1 μL, 30 μmol), and the mixture was stirred at 0 °C for 20 h. The solvent was removed by a stream of N_2, and the residue was dissolved in MeOH, and was subjected to reverse-phase HPLC chromatography. Compound **8H** (R_t = 28 min, 3.1 mg, 28% yield) was obtained as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 7.51 (br d, *J* = 7.7 Hz, 1H), 7.37 (d, *J* = 8.1 Hz, 1H), 7.33 (d, *J* = 8.1 Hz, 1H), 7.16–7.21 (m, 1H), 7.06–7.12 (m, 2H), 5.47 (dd, *J* = 8.6, 7.2 Hz, 1H), 5.09–5.23 (m, 3H), 4.93–5.01 (m, 1H), 4.52–4.58 (m, 2H), 3.95–3.40 (m, partially overlapped, 1H), 3.98 (s, partially overlapped, 3H), 3.27 (dd, *J* = 15.4, 7.0 Hz, 1H), 3.10–3.16 (m, 1H), 3.07 (s, 3H), 3.01 (s, 3H), 2.91 (s, 3H), 1.08–1.78 (m, partially overlapped, 26H), 1.42 (s, partially overlapped, 18H), 0.72–1.00 (m, 21H), 0.43 (d, *J* = 6.6 Hz, 3H), 0.04 (d, *J* = 7.0 Hz, 3H), –0.36 (br dd, *J* = 10.5, 10.5 Hz, 1H); IR (neat) ν_{max} 3302, 2957, 2929, 1731, 1708, 1833, 1530, 1467, 1453, 1409, 1367, 1165 cm^{-1}; HRMALDI–FTMS *m/z* 1134.7391 ([M + Na]$^+$, $C_{60}H_{101}N_7O_{12}Na$ requires 1134.7400).

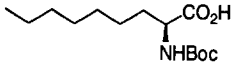
***N*-[(2*S*,4*R*)-2-[*N*-[(*R*)-2-[*N*-[[(2*S*,4*R*)-2-[[*N*-[*N*-(*tert*-Butoxycarbonyl)-L-leucinyl]-*N*$^{1'}$-methoxy-*N*-methyl-L-tryptophanyl]amino]-4-methyloctanoyl]-*N*-methyl-L-alanyloxy]-5-cyanopentanoyl]amino]-4-methyloctanoyl]-*N*-methyl-L-leucine *tert*-butyl ester** (**9H**, $R^1 = (CH_2)_3CN$): A toluene (0.2 mL, 0.05 M) solution of **9T** (4.8 mg, 10 μmol), **2** (7.2 mg, 10.2 μmol) and PPh_3 (7.9 mg, 30 μmol) at 0 °C was treated dropwise with DIAD (97% purity, 6.1 μL, 30 μmol), and the mixture was stirred at 0 °C for 16.5 h. The solvent was removed by a stream of N_2, and the residue was dissolved in MeOH, and was subjected to reverse-phase HPLC chromatography. Compound **9H** (R_t = 27 min, 2.2 mg, 19% yield) was obtained as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 7.55 (br d, *J* = 8.8 Hz, 1H), 7.52 (d, *J* = 8.1 Hz, 1H), 7.32–7.38 (m, 1H), 7.19 (dd, *J* = 7.2, 7.2 Hz, 1H), 7.07–7.10 (m, 2H), 5.47 (dd, *J* = 8.1, 8.1 Hz, 1H), 5.30 (dd, *J* = 11.0, 5.1 Hz, 1H), 5.22 (br d, *J* = 8.8 Hz, 1H), 5.13 (dd, *J* = 5.7, 5.7 Hz, 1H), 5.00 (dd, *J* = 16.5, 7.3 Hz, 1H), 4.52–4.56 (m, 1H), 4.25 (q, *J* = 7.3 Hz, 1H), 3.98 (s, 3H), 3.19–3.22 (m, 2H), 3.12 (s, 3H), 3.00 (s, 3H), 2.92 (s, 3H), 2.30–2.36 (m, 1H), 2.11 (dd, *J* = 7.3, 7.3 Hz, 2H), 1.82–1.91 (m, 3H), 1.11–1.72 (m, partially overlapped, 28H), 1.43 (s, 9H), 1.40 (s, 9H), 0.84–0.94 (m, 18H), 0.80 (d, *J* = 6.6 Hz, 3H), 0.68 (d, *J* = 6.2 Hz, 3H); IR (neat) ν_{max} 3306, 2957, 2931, 2246, 1749, 1732, 1717, 1656, 1651, 1634, 1538, 1456, 1367, 1166 cm^{-1}; HRMALDI–FTMS *m/z* 1187.7698 ([M + Na]$^+$, $C_{63}H_{104}N_8O_{12}Na$ requires 1187.7665).



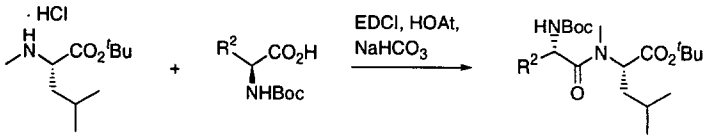
For **8** ($R^1 = CH_3$): Linear heptadepsipeptide **8H** (2.5 mg, 2.2 μmol) was deprotected the following standard procedure, and the residue was dissolved in DMF (2.2 mL, 1 mM) at 0 °C. DPPA (1.0 μL, 4.5 μmol) and *i*Pr_2NEt (0.8 μL, 4.5 μmol) were added, and the mixture was stirred at 0 °C for 64 h. After work-up as described, the residue was subjected to HPLC chromatography and gave **8** (R_t = 22 min, 0.7 mg, 33% yield) as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 8.68 (br d, *J* = 9.9 Hz, 1H), 7.76 (br d, *J* = 10.5 Hz, 1H), 7.36–7.45 (m, 2H), 7.18–7.28 (m, 1H), 7.07 (dd, *J* = 7.0, 7.0 Hz, 1H), 7.00 (s, 1H), 5.98 (br d, *J* = 6.2 Hz, 1H), 5.05 (q, *J* = 7.2 Hz, 1H), 4.95–5.00 (m, 2H), 4.84–4.88 (m, 1H), 4.50 (dd, *J* = 11.2, 3.9 Hz, 1H), 4.12 (ddd, *J* = 10.5, 6.7, 3.8 Hz, 1H), 4.03 (s, 3H), 3.53–3.56 (m, 1H), 3.48 (q, *J* = 7.0 Hz, 1H), 3.04–3.09 (m, partially overlapped, 1H), 3.08 (s, 3H), 2.90 (s, 3H), 2.51 (s, 3H), 1.94–1.99 (m, 1H), 1.78–1.81 (m, 1H), 1.08–1.63 (m, 27H), 1.03 (d, 3H), 0.83–0.96 (m, 15H), 0.61 (d, *J* = 6.6 Hz, 3H), 0.06 (d, *J* = 6.6 Hz, 3H), –0.01 (ddd, *J* = 14.2, 10.4, 3.8 Hz, 1H); IR (neat) ν_{max} 3288, 2954, 2927, 1749, 1682, 1667, 1661, 1651, 1634, 1539, 1456, 1205, 1092 cm^{-1}; HRMALDI–FTMS *m/z* 960.6163 ([M + Na]$^+$, $C_{51}H_{83}N_7O_9Na$ requires 960.6144).

For **9** ($R^1 = (CH_2)_3CN$): Linear heptadepsipeptide **9H** (1.3 mg, 1.1 μmol) was deprotected following the standard procedure, and the residue was dissolved in DMF (1.1 mL, 1 mM) at 0 °C. DPPA (0.5 μL, 2.2 μmol) and *i*Pr_2NEt (0.4 μL, 2.2 μmol) were added, and the mixture was stirred at 0 °C for 65 h. After work-up as described, the residue was subjected to HPLC chromatography and gave **9** (R_t = 20 min, 0.7 mg, 64% yield) as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 8.60 (br d, *J* = 10.3 Hz, 1H), 7.96 (br d, *J* = 9.9 Hz, 1H), 7.58 (d, *J* = 8.0 Hz, 1H), 7.39 (d, *J* = 8.0 Hz, 1H), 7.21–7.24 (m, 1H), 7.10 (dd, *J* = 7.0, 7.0 Hz, 1H), 7.03 (s, 1H), 5.96 (br d, *J* = 7.0 Hz, 1H), 5.03 (dd, *J* = 7.9, 4.2 Hz, 1H), 4.97–5.00 (m, 1H), 4.94 (dd, *J* = 9.7, 2.4 Hz, 1H), 4.84–4.87 (m, 1H), 4.40 (dd, *J* = 11.2, 4.2 Hz, 1H), 4.26–4.30 (m, 1H), 4.02 (s, 3H), 3.54 (q, *J* = 7.0 Hz, 1H), 3.29 (dd, *J* = 15.2, 4.4 Hz, 1H), 3.22 (s, 3H), 3.14 (dd, *J* = 15.2, 10.3 Hz, 1H), 2.98 (s, 3H), 2.50 (s, 3H), 2.20–2.22 (m, 2H), 1.89–2.05 (m, 2H), 1.24–1.81 (m, 28H), 1.04 (d, *J* = 6.2 Hz, 3H), 0.82–9.96 (m, 15H), 0.55 (d, *J* = 6.6 Hz, 3H), 0.11 (d, *J* = 6.2 Hz, 3H), –0.30 (ddd, *J* = 14.2, 10.7, 3.6 Hz, 1H); IR (neat) ν_{max} 3281, 2923, 2851, 2241, 1748, 1677, 1662, 1651, 1634, 1539, 1456, 1200, 1092 cm^{-1}; HRMALDI–FTMS *m/z* 1013.6416 ([M + Na]$^+$, $C_{54}H_{86}N_8O_9Na$ requires 1013.6441).

Analogues with variations in the PrLEU^2 side-chain:



(*S*)-2-(*N*-*tert*-Butoxycarbonyl)aminooctanoic acid: A H_2O solution (8.0 mL) of DL-2-(*N*-acetylamino)octanoic acid (402 mg, 2.0 mmol) which also contains 1 mM $CoCl_2$ was adjusted to pH = 7–8 by careful addition of $LiOH{\cdot}H_2O$. The solution was deoxygenated by a stream of N_2, and Acylase I (from *Aspergillus*, 33.2 mg, 8.3 U) was added. The mixture was kept at 35–40 °C for 39 h and was worked-up following a literature procedure.[32] (*S*)-2-Aminooctanoic acid was obtained as colorless shiny thin flakes (112.7 mg, 35% yield). The solid was then mixed with 1,4-dioxane (7.1 mL, 0.1 M), saturated aqueous $NaHCO_3$ (7.1 mL, 0.1 M) and Boc_2O (464 mg, 2.1 mmol), and the mixture was stirred at 25 °C for 5 h. The reaction mixture was acidified to pH = 1–2 by 1 M HCl (aq), and the aqueous layer was extracted with EtOAc (15 mL × 3). The combined organic layer was dried ($MgSO_4$), filtered, and the solvent was removed in vacuo. Silica gel chromatography of the crude product (CH_2Cl_2 : MeOH = 10:1) gave the title compound (R_f = 0.3, 123 mg, 67% yield) as a colorless oil: $[\alpha]_D^{23}$ +29 (*c* 0.31, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, two rotamers:) δ 5.78 (br s, 0.3H), 4.94 (br d, *J* = 7.6 Hz, 0.7 H), 4.26–4.28 (m, 0.7H), 4.11 (br s, 0.3H), 1.83 (br s, 1H), 1.59–1.68 (m, 1H), 1.43 (s, 9H), 1.26–1.35 (m, 8H), 0.86 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 177.7, 177.4, 157.0, 155.6, 81.6, 80.0, 54.6, 53.3, 32.4, 31.5, 28.8, 28.2, 25.1, 22.4, 13.9; IR (neat) ν_{max} 3337 (br), 2929, 1705, 1367, 1167 cm^{-1}; FABHRMS (NBA/NaI) *m/z* 282.1687 ([M + Na]$^+$, $C_{13}H_{25}NO_4Na$ requires 282.1681). The optical purity of the product was established to be >99% *ee*, which was examined by HPLC (CHIRALCEL OD), after conversion to methyl (*S*)-2-(benzyloxycarbonyl)aminooctanoate by standard procedures [(a) TFA–CH_2Cl_2; (b) CbzCl, Na_2CO_3; (c) $TMSCHN_2$] and was compared with the corresponding racemic compound. The racemic compound showed two distinct peaks (retention time: 10.01 and 13.63 min) by using 5% isopropanol–hexanes, with a flow rate of 1 mL/min, and with 254 nm as the detection wavelength. Under the same conditions, the derivative of the title compound appeared as a single peak matching the former peak (retention time: 10.01 min).

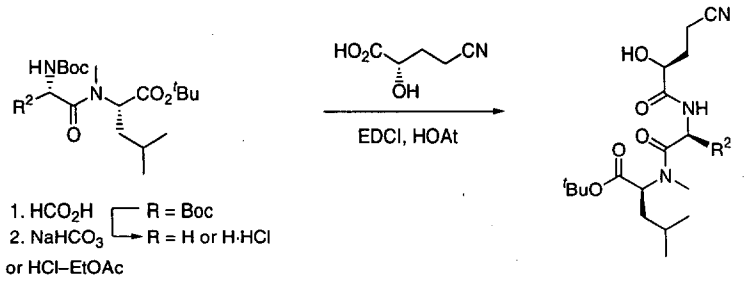


***N*-[*N*-(*tert*-Butoxycarbonyl)-L-alanyl]-*N*-methyl-L-leucine *tert*-butyl ester (10D**, R^2 = CH_3): A CH_2Cl_2–DMF (5:1, 1 mL, 0.1 M) solution of *N*-(*tert*-butoxycarbonyl)-L-alanine (18.9 mg, 0.10 mmol) and **36** (35.7 mg, 0.15 mmol) at 0 °C was treated with EDCI (38.3 mg, 0.2 mmol), HOAt (15.0 mg, 0.11 mmol) and $NaHCO_3$ (12.6 mg, 0.15 mmol), and the reaction mixture was allowed to warm to 25 °C and was stirred for a total of 21 h. The reaction mixture was worked-up as described and afforded **10D** (36.2 mg, 97% yield) as a colorless thick oil. HPLC analysis (MeOH/H_2O, 80/20) of this crude product showed one major peak (R_t = 5.3 min, 88%): ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 5.42 (br d, *J* = 7.7 Hz, 1H), 5.19 (dd, *J* = 10.8, 5.0 Hz, 1H), 4.56–4.62 (m, 1H), 2.91 (s, 3H), 1.57–1.70 (m, 2H), 1.41–1.49 (m, partially overlapped, 1H), 1.41 (br s, partially overlapped, 18H), 1.31 (d, *J* = 6.6 Hz, 3H), 0.91 (d, *J* = 6.6 Hz, 3H), 0.88 (d, *J* = 6.6 Hz, 3H); IR (neat) ν_{max} 3327, 2976, 2871, 1731, 1708, 1650, 1484, 1367, 1166 cm^{-1}; HRMALDI–FTMS *m/z* 395.2501 ([M + Na]$^+$, $C_{19}H_{36}N_2O_5Na$ requires 395.2516).

***N*-[(2*S*)-2-[*N*-(*tert*-Butoxycarbonyl)amino]octanoyl]-*N*-methyl-L-leucine *tert*-butyl ester (11D**, R^2 = $(CH_2)_5CH_3$): A CH_2Cl_2–DMF (5:1, 1 mL, 0.1 M) solution of (2*S*)-*N*-(*tert*-

butoxycarbonyl)aminooctanoic acid (25.9 mg, 0.10 mmol) and **36** (35.7 mg, 0.15 mmol) at 0 °C was treated with EDCI (38.3 mg, 0.2 mmol), HOAt (15.0 mg, 0.11 mmol) and $NaHCO_3$ (12.6 mg, 0.15 mmol), and the reaction mixture was allowed to warm to 25 °C and was stirred for a total of 21 h. The reaction mixture was worked-up as described and afforded **11D** (41.8 mg, 94% yield) as a colorless oil. HPLC analysis ($MeOH/H_2O$, 80/20) of this crude product showed one major peak (R_t = 14.2 min, 90%): ^1H NMR (500 MHz, $CDCl_3$, major rotamer) δ 5.24 (br d, J = 8.8 Hz, 1H), 5.18 (dd, J = 10.6, 5.1 Hz, 1H), 4.53–4.57 (m, 1H), 2.93 (s, 3H), 1.56–1.72 (m, 2H), 1.24–1.51 (m, partially overlapped, 11H), 1.41 (s, partially overlapped, 9H), 1.40 (s, partially overlapped, 9H), 0.90 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H), 0.84 (t, J = 6.8 Hz, 3H); IR (neat) ν_{max} 3319, 2957, 2870, 1731, 1710, 1648, 1492, 1367, 1168 cm^{-1}; HRMALDI–FTMS *m/z* 465.3301 ($[M + Na]^+$, $C_{24}H_{46}N_2O_5Na$ requires 465.3304).

***N*-[*N*-(*tert*-Butoxycarbonyl)-L-leucinyl]-*N*-methyl-L-leucine *tert*-butyl ester (12D**, R^2 = $CH_2CH(CH_3)_2$): A CH_2Cl_2–DMF (5:1, 1 mL, 0.1 M) solution of *N*-(*tert*-butoxycarbonyl)-L-leucine monohydrate (**40**, 24.9 mg, 0.10 mmol) and **36** (35.7 mg, 0.15 mmol) at 0 °C was treated with EDCI (38.3 mg, 0.2 mmol), HOAt (15.0 mg, 0.11 mmol) and $NaHCO_3$ (12.6 mg, 0.15 mmol), and the reaction mixture was allowed to warm to 25 °C and was stirred for a total of 21 h. The reaction mixture was worked-up as described and afforded **12D** (40.2 mg, 97% yield) as a white solid. HPLC analysis ($MeOH/H_2O$, 80/20) of this crude product showed one major peak (R_t = 9.0 min, 76%): ^1H NMR (500 MHz, $CDCl_3$, major rotamer) δ 5.20 (dd, J = 10.6, 5.1 Hz, 1H), 5.15 (br d, J = 9.2 Hz, 1H), 4.59–4.64 (m, 1H), 2.95 (s, 3H), 1.57–1.76 (m, 4H), 1.38–1.48 (m, partially overlapped, 2H), 1.42 (s, partially overlapped, 9H), 1.40 (s, partially overlapped, 9H), 0.98 (d, J = 6.6 Hz, 3H), 0.92 (d, J = 6.6 Hz, 3H), 0.91 (d, J = 6.6 Hz, 3H), 0.88 (d, J = 6.6 Hz, 3H); IR (neat) ν_{max} 3315, 2957, 2870, 1731, 1710, 1649, 1470, 1367, 1168 cm^{-1}; HRMALDI–FTMS *m/z* 437.3004 ($[M + Na]^+$, $C_{22}H_{42}N_2O_5Na$ requires 437.2986).

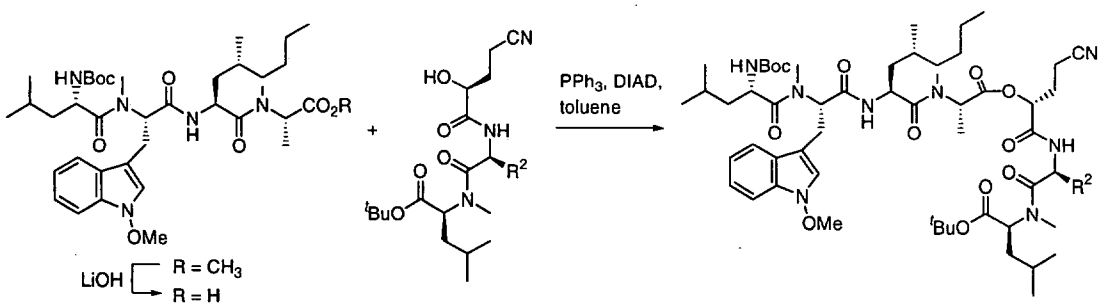


***N*-[*N*-[(*S*)-4-Cyano-2-hydroxybutanoyl]-L-alanyl]-*N*-methyl-L-leucine *tert*-butyl ester (10T**, R^2 = CH_3): A HCO_2H (0.5 mL) solution of **10D** (18.6 mg, 50.0 μmol) was stirred at 25 °C for 2 h, until no starting material was detected by TLC. The reaction was worked-up as described and gave a light yellow thick oil. A CH_2Cl_2–DMF (5:1, 0.5 mL, 0.1 M) solution of **39** (6.5 mg, 50.0 μmol) was added to this crude amine at –30 °C. The solution was treated with EDCI (19.2 mg, 0.1 mmol) and HOAt (7.1 mg, 52.5 μmol) and was stirred for 3 h at –30 °C. The reaction mixture was worked-up as described and afforded **10T** (7.9 mg, 41% yield) as a thick light yellow oil. HPLC analysis ($MeOH/H_2O$, 80/20) of this crude product showed one major peak (R_t = 2.9 min, 79%): ^1H NMR (500 MHz, $CDCl_3$, major rotamer) δ 7.38 (br d, J = 7.2 Hz, 1H), 5.12 (dd, J = 10.6, 5.1 Hz, 1H), 4.86 (qd, J = 7.2, 7.2 Hz, 1H), 4.19 (dd, J = 7.9, 3.9 Hz, 1H), 2.95 (s, 3H), 2.43–2.56 (m, 2H),

2.13–2.18 (m, 1H), 1.92–2.00 (m, 1H), 1.67–1.73 (m, 1H), 1.61 (ddd, J = 14.4, 10.5, 4.3 Hz, 1H), 1.39–1.46 (m, partially overlapped, 1H), 1.42 (s, 9H), 1.37 (d, J = 7.2 Hz, 3H), 0.92 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 6.2 Hz, 3H); IR (neat) ν_{max} 3388, 2959, 2862, 2247, 1731, 1643, 1462, 1369, 1158 cm^{-1}; HRMALDI–FTMS *m/z* 406.2319 ([M + Na]$^+$, $C_{19}H_{33}N_3O_5Na$ requires 406.2312).

***N*-[*N*-[(*S*)-4-Cyano-2-hydroxybutanoyl]amino]octanoyl]-*N*-methyl-L-leucine *tert*-butyl ester** (**11T**, R^2 = $(CH_2)_5CH_3$): A HCO_2H (0.2 mL) solution of **11D** (8.9 mg, 20.0 μmol) was stirred at 25 °C for 2 h, until no starting material was detected by TLC. The reaction was worked-up as described and afforded a light yellow thick oil. A CH_2Cl_2–DMF (5:1, 0.2 mL, 0.1 M) solution of **39** (2.6 mg, 20.0 μmol) was added to the crude amine at –30 °C. The solution was treated with EDCI (7.7 mg, 40 μmol) and HOAt (2.9 mg, 21.0 μmol) and was stirred for 3 h at –30 °C. The reaction mixture was worked-up as described and afforded **11T** (7.2 mg, 79% yield) as a thick light yellow oil. HPLC analysis (MeOH/H_2O, 80/20) of this crude product showed one major peak (R_t = 5.7 min, 55%): ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 7.17 (br d, J = 8.4 Hz, 1H), 5.12 (dd, J = 10.5, 5.3 Hz, 1H), 4.86 (ddd, J = 8.4, 8.4, 4.4 Hz, 1H), 4.19 (dd, J = 7.7, 3.7 Hz, 1H), 2.98 (s, 3H), 2.42–2.57 (m, 2H), 2.14–2.21 (m, 1H), 1.90–2.00 (m, 1H), 1.55–1.81 (m, 4H), 1.41–1.46 (m, partially overlapped, 1H), 1.43 (s, 9H), 1.23–1.36 (m, 8H), 0.91 (d, J = 6.6 Hz, 3H), 0.84–0.89 (m, 6H); IR (neat) ν_{max} 3387, 2957, 2871, 2247, 1790, 1732, 1641, 1524, 1468, 1368, 1275, 1159 cm^{-1}; HRMALDI–FTMS *m/z* 476.3085 ([M + Na]$^+$, $C_{24}H_{43}N_3O_5Na$ requires 476.3095).

***N*-[*N*-[(*S*)-4-Cyano-2-hydroxybutanoyl]-L-leucinyl]-*N*-methyl-L-leucine *tert*-butyl ester** (**12T**, R^2 = $CH_2CH(CH_3)_2$): A HCO_2H (0.5 mL) solution of **12D** (20.7 mg, 50.0 μmol) was stirred at 25 °C for 2 h, until no starting material was detected by TLC. The reaction was worked-up as described and gave an off-white solid. A CH_2Cl_2–DMF (5:1, 0.5 mL, 0.1 M) solution of **39** (6.5 mg, 50.0 μmol) was added to the crude amine at –30 °C. The solution was treated with EDCI (19.2 mg, 0.1 mmol) and HOAt (7.1 mg, 52.5 μmol) and was stirred for 3 h at –30 °C. The reaction mixture was worked-up as described and afforded **12T** (18.3 mg, 86% yield) as a thick light yellow oil. HPLC analysis (MeOH/H_2O, 80/20) of this crude product showed one major peak (R_t = 3.8 min, 76%): ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 7.22 (br d, J = 8.8 Hz, 1H), 5.10 (dd, J = 10.6, 5.1 Hz, 1H), 4.91 (ddd, J = 9.6, 8.8, 3.8 Hz, 1H), 4.17 (dd, J = 8.1, 4.0 Hz, 1H), 2.98 (s, 3H), 2.40–2.55 (m, 2H), 2.14–2.21 (m, 1H), 1.91–1.99 (m, 1H), 1.50–1.72 (m, 4H), 1.40–1.46 (m, partially overlapped, 2H), 1.42 (s, 9H), 0.98 (d, J = 6.6 Hz, 3H), 0.94 (d, J = 6.6 Hz, 3H), 0.91 (d, J = 6.6 Hz, 3H), 0.85 (d, J = 6.6 Hz, 3H); IR (neat) ν_{max} 3331, 2959, 2871, 2248, 1732, 1645, 1524, 1470, 1369, 1159 cm^{-1}; HRMALDI–FTMS *m/z* 448.2799 ([M + Na]$^+$, $C_{22}H_{39}N_3O_5Na$ requires 448.2782).

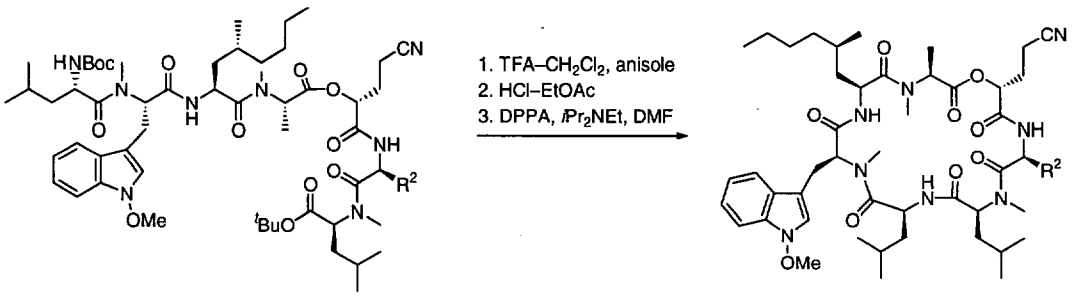


***N*-[*N*-[(*R*)-2-[*N*-[(2*S*,4*R*)-2-[[*N*-[*N*-(*tert*-Butoxycarbonyl)-L-leucinyl]-*N*$^{1'}$-methoxy-*N*-methyl-L-tryptophanyl]amino]-4-methyloctanoyl]-*N*-methyl-L-alanyloxy]-4-cyanobutanoyl]-L-alanyl]-*N*-methyl-L-leucine *tert*-butyl ester (10H**, $R^2 = CH_3$): A toluene (0.4 mL, 0.05 M) solution of **10T** (7.7 mg, 20 μmol), **2** (14.0 mg, 20 μmol) and PPh_3 (26.2 mg, 100 μmol) at 0 °C was treated dropwise with DIAD (95% purity, 20.7 μL, 100 μmol), and the mixture was allowed to warm to 25 °C for a total of 17 h. The solvent was removed by a stream of N_2, and the residue was dissolved in MeOH, and was subjected to reverse-phase HPLC chromatography. Compound **10H** (R_t = 28 min, 3.5 mg, 16% yield) was obtained as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 8.19 (br d, *J* = 7.7 Hz, 1H), 7.48–7.58 (m, 2H), 7.35 (d, *J* = 7.8 Hz, 1H), 7.19 (dd, *J* = 7.8, 7.8 Hz, 1H), 7.01–7.12 (m, 2H), 5.43 (dd, *J* = 7.3, 7.3 Hz, 1H), 5.26 (dd, *J* = 11.0, 5.1 Hz, 1H), 5.17–5.20 (m, 2H), 4.95–4.99 (m, 1H), 4.74–4.80 (m, 1H), 4.53–4.57 (m, 1H), 4.39 (q, *J* = 6.8 Hz, 1H), 3.99 (s, 3H), 3.29 (dd, *J* = 15.4, 6.6 Hz, 1H), 3.12–3.18 (m, 1H), 3.08 (s, 3H), 3.00 (s, 3H), 2.91 (s, 3H), 2.42–2.46 (m, 1H), 2.30–2.35 (m, 2H), 2.04–2.23 (m, 3H), 1.11–1.73 (m, partially overlapped, 19H), 1.42 (s, 9H), 1.40 (s, 9H), 0.82–0.96 (m, 15H), 0.72 (d, *J* = 6.6 Hz, 3H); IR (neat) ν_{max} 3311, 2958, 2252, 1731, 1700, 1669, 1639, 1539, 1456, 1164, 1097 cm^{-1}; HRMALDI–FTMS *m/z* 1089.6556 ([M + Na]$^+$, $C_{56}H_{90}N_8O_{12}Na$ requires 1089.6570).

***N*-[(2*S*)-*N*-[[(*R*)-2-[*N*-[(2*S*,4*R*)-2-[*N*-[*N*-(*tert*-Butoxycarbonyl)-L-leucinyl]-*N*$^{1'}$-methoxy-*N*-methyl-L-tryptophanyl]amino-4-methyloctanoyl]-*N*-methyl-L-alanyloxy]-4-cyanobutanoyl]amino]octanoyl]-*N*-methyl-L-leucine *tert*-butyl ester (11H**, $R^2 = (CH_2)_5CH_3$): A toluene (0.4 mL, 0.05 M) solution of **11T** (9.1 mg, 20 μmol), **2** (14.0 mg, 20 μmol) and PPh_3 (26.2 mg, 100 μmol) at 0 °C was treated dropwise with DIAD (95% purity, 20.7 μL, 100 μmol), and the mixture was allowed to warm to 25 °C for a total of 18 h. The solvent was removed by a stream of N_2, and the residue was dissolved in MeOH, and was subjected to reverse-phase HPLC chromatography. Compound **11H** (R_t = 26 min, 2.9 mg, 13% yield) was obtained as white solid: ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 7.53 (d, *J* = 8.4 Hz, 1H), 7.45 (br d, *J* = 8.5 Hz, 1H), 7.33–7.38 (m, 2H), 7.19 (dd, *J* = 7.5, 7.5 Hz, 1H), 7.04–7.17 (m, 2H), 5.46 (dd, *J* = 9.2, 6.6 Hz, 1H), 5.26 (dd, *J* = 10.6, 5.1 Hz, 1H), 5.17–5.23 (m, 2H), 4.97–5.04 (m, 1H), 4.75–4.77 (m, 1H), 4.54 (br dd, *J* = 8.6, 8.6 Hz, 1H), 4.28 (q, *J* = 7.3 Hz, 1H), 3.98 (s, 3H), 3.27 (dd, *J* = 15.8, 6.2 Hz, 1H), 3.15–3.21 (m, 1H), 3.12 (s, 3H), 2.99 (s, 3H), 2.92 (s, 3H), 2.36–2.44 (m, 1H), 2.26–2.35 (m, 2H), 2.00–2.08 (m, 1H), 1.22–1.75 (m, partially overlapped, 27H), 1.43 (s, 18H), 0.83–0.95 (m, 12H), 0.78 (d, *J* = 6.6 Hz, 3H), 0.43 (d, *J* = 6.6 Hz, 3H), 0.08 (d, *J* = 6.6 Hz, 3H), –0.34 (br dd, *J* = 13.9, 13.9 Hz, 1H); IR (neat) ν_{max} 3301, 2956, 2870, 2246, 1749, 1733, 1718, 1635, 1539, 1457, 1368, 1164 cm^{-1}; HRMALDI–FTMS *m/z* 1159.7369 ([M + Na]$^+$, $C_{61}H_{100}N_8O_{12}Na$ requires 1159.7353).

***N*-[*N*-[(*R*)-2-[*N*-[(2*S*,4*R*)-2-[[*N*-[*N*-(*tert*-Butoxycarbonyl)-L-leucinyl]-*N*$^{1'}$-methoxy-*N*-methyl-L-tryptophanyl]amino]-4-methyloctanoyl]-*N*-methyl-L-alanyloxy]-4-cyanobutanoyl]-L-leucinyl]-*N*-methyl-L-leucine *tert*-butyl ester (12H**, $R^2 = CH_2CH(CH_3)_2$): A toluene (300 μL, 0.05 M) solution of **12T** (6.4 mg, 15.0 μmol), **2** (10.5 mg, 15.0 μmol) and PPh_3 (11.8 mg, 45.0 μmol) at 0 °C was treated dropwise with DIAD (97% purity, 9.1 μL, 45.0 μmol), and the mixture was stirred at 0 °C for 15 h. The solvent was removed by a stream of N_2, and the residue was dissolved in MeOH, and was subjected to reverse-phase HPLC chromatography. Compound **12H** (R_t = 22 min, 7.4 mg, 45% yield) was obtained as white solid: ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 7.62 (br d, *J* = 8.8 Hz, 1H), 7.54 (d, *J* = 7.6 Hz, 1H), 7.51 (br d, *J* = 7.6 Hz, 1H), 7.34 (d, *J* = 8.1 Hz, 1H), 7.19 (dd, *J* = 7.6, 7.6 Hz, 1H), 7.06–7.08 (m, 2H), 5.70 (dd, *J* = 9.5,

5.9 Hz, 1H), 5.40 (dd, J = 9.2, 6.6 Hz, 1H), 5.23 (br d, J = 8.1 Hz, 1H), 5.16–5.20 (m, 1H), 4.96–5.01 (m, 1H), 4.79–4.83 (m, 1H), 4.54–4.55 (m, 1H), 4.14 (q, J = 6.8 Hz, 1H), 3.98 (s, 3H), 3.29 (dd, J = 15.6, 6.4 Hz, 1H), 3.12–3.16 (m, 1H), 3.09 (s, 3H), 3.01 (s, 3H), 2.92 (s, 3H), 2.25–2.31 (m, 2H), 1.98–2.13 (m, 2H), 1.24–1.88 (m, partially overlapped, 39H), 0.84–0.94 (m, 21H), 0.78 (d, J = 6.6 Hz, 3H); IR (neat) ν_{max} 3307, 2957, 2250, 1752, 1732, 1716, 1634, 1540, 1456, 1368, 1165, 1097 cm^{-1}; HRMALDI–FTMS *m/z* 1131.7050 ([M + Na]$^+$, $C_{59}H_{96}N_8O_{12}Na$ requires 1131.7039).

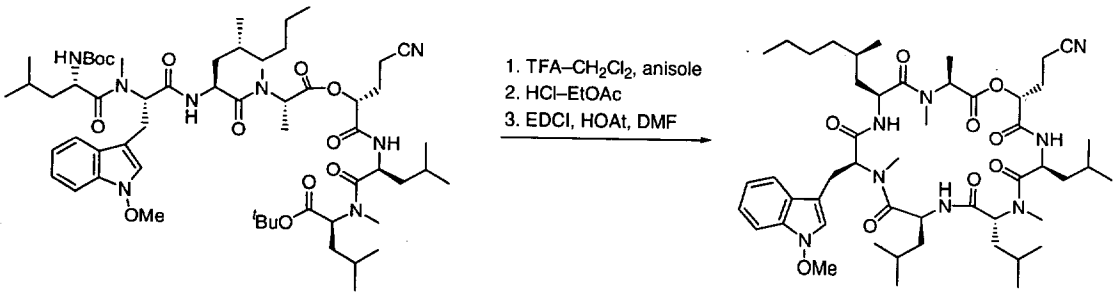


For **10** (R^2 = CH_3): Linear heptadepsipeptide **10H** (1.8 mg, 1.7 μmol) was deprotected following the standard procedure, and the residue was dissolved in DMF (1.7 mL, 1 mM) at 0 °C. DPPA (0.7 μL, 3.4 μmol) and *i*Pr_2NEt (0.6 μL, 3.4 μmol) were added, and the mixture was stirred at 0 °C for 69 h. After work-up as described, the residue was subjected to HPLC chromatography and gave **10** (R_t = 9.6 min, 0.5 mg, 33% yield) as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 8.52 (br d, J = 9.9 Hz, 1H), 8.13 (br d, J = 9.5 Hz, 1H), 7.66 (d, J = 7.6 Hz, 1H), 7.40 (d, J = 7.6 Hz, 1H), 7.21–7.24 (m, overlapped with solvent peak, 1H), 7.10 (dd, J = 7.6, 7.6 Hz, 1H), 7.01 (s, 1H), 6.02 (br d, J = 7.7 Hz, 1H), 5.11 (dd, J = 8.8, 3.7 Hz, 1H), 4.99–5.04 (m, 1H), 4.87–4.93 (m, 2H), 4.30 (dd, J = 11.0, 4.4 Hz, 1H), 4.17–4.20 (m, 1H), 4.03 (s, 3H), 3.66 (q, J = 6.8 Hz, 1H), 3.25–3.29 (m, partially overlapped, 1H), 3.26 (s, 3H), 3.17 (dd, J = 15.0, 11.0 Hz, 1H), 2.91 (s, 3H), 2.52 (s, 3H), 2.24–2.29 (m, 1H), 2.10–2.19 (m, 1H), 1.91–1.97 (m, 1H), 1.76 (ddd, J = 13.1, 10.9, 3.8 Hz, 1H), 1.09–1.61 (m, 20H), 0.99 (d, J = 6.2 Hz, 3H), 0.84–0.95 (m, 9H), 0.53 (d, J = 6.6 Hz, 3H), –0.10 (d, J = 6.6 Hz, 3H), –0.42 (ddd, J = 14.1, 10.8, 3.5 Hz, 1H); IR (neat) ν_{max} 3280, 2923, 2246, 1750, 1700, 1683, 1662, 1652, 1635, 1558, 1539, 1457, 1386, 1054 cm^{-1}; HRMALDI–FTMS *m/z* 915.5309 ([M + Na]$^+$, $C_{47}H_{72}N_8O_9Na$ requires 915.5314).

For **11** (R^2 = $(CH_2)_5CH_3$): Linear heptadepsipeptide **11H** (2.3 mg, 2.0 μmol) was deprotected following the standard procedure, and the residue was dissolved in DMF (2.0 mL, 1 mM) at 0 °C. DPPA (0.9 μL, 4.0 μmol) and *i*Pr_2NEt (0.7 μL, 4.0 μmol) were added, and the mixture was stirred at 0 °C for 60 h. After work-up as described, the residue was subjected to HPLC chromatography and gave **11** (R_t = 18 min, 0.9 mg, 47% yield) as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 8.45 (br d, J = 9.9 Hz, 1H), 8.05 (br d, J = 9.5 Hz, 1H), 7.65 (d, J = 7.7 Hz, 1H), 7.40 (d, J = 7.7 Hz, 1H), 7.20–7.25 (m, overlapped with solvent peak, 1H), 7.09 (dd, J = 7.7, 7.7 Hz, 1H), 7.01 (s, 1H), 5.99 (br d, J = 7.5 Hz, 1H), 5.18 (dd, J = 8.4, 3.3 Hz, 1H), 4.99–5.04 (m, 1H), 4.92 (dd, J = 10.8, 3.9 Hz, 1H), 4.72–4.77 (m, 1H), 4.37 (dd, J = 11.0, 4.0 Hz, 1H), 4.18 (ddd, J = 11.4, 7.5, 3.5 Hz, 1H), 4.03 (s, 3H), 3.66 (q, J = 6.8 Hz, 1H), 3.25–3.29 (m, partially overlapped, 1H), 3.25 (s, 3H), 3.16–3.19 (m, 1H), 2.91 (s, 3H), 2.51 (s, 3H), 2.22–2.28 (m, 1H), 2.09–2.18 (m, 1H), 1.91–1.99 (m, 1H), 1.73–1.78 (m, 1H), 1.11–1.67 (m, 27H),

1.02 (d, J = 6.6 Hz, 3H), 0.82–0.96 (m, 12H), 0.54 (d, J = 6.6 Hz, 3H), –0.097 (d, J = 6.6 Hz, 3H), –0.40 (ddd, J = 14.5, 11.2, 3.5 Hz, 1H); IR (neat) ν_{max} 3364, 3291, 2954, 2862, 2256, 1749, 1733, 1683, 1662, 1653, 1635, 1558, 1539, 1457, 1385, 1275, 1073 cm^{-1}; HRMALDI–FTMS *m/z* 985.6118 ([M + Na]$^+$, $C_{52}H_{82}N_8O_9Na$ requires 985.6097).

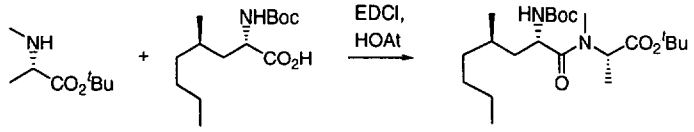
For **12** (R^2 = $CH_2CH(CH_3)_2$): Linear heptadepsipeptide **12H** (4.3 mg, 3.9 μmol) was deprotected following the standard procedure, and the residue was dissolved in DMF (3.9 mL, 1 mM) at 0 °C. DPPA (1.7 μL, 7.8 μmol) and iPr$_2$NEt (1.4 μL, 7.8 μmol) were added, and the mixture was stirred at 0 °C for 60 h. After work-up as described, the residue was subjected to HPLC chromatography and gave **12** (R_t = 13 min, 1.7 mg, 47% yield) as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 8.45 (br d, J = 9.9 Hz, 1H), 8.03 (br d, J = 9.9 Hz, 1H), 7.65 (d, J = 8.1 Hz, 1H), 7.40 (d, J = 8.1 Hz, 1H), 7.19–7.24 (m, overlapped with solvent peak, 1H), 7.09 (dd, J = 7.3, 7.3 Hz, 1H), 7.01 (s, 1H), 6.00 (br d, J = 7.7 Hz, 1H), 5.18 (dd, J = 8.3, 3.5 Hz, 1H), 4.99–5.04 (m, 1H), 4.92 (dd, J = 10.6, 4.0 Hz, 1H), 4.82–4.87 (m, 1H), 4.43 (dd, J = 11.0, 3.7 Hz, 1H), 4.17 (ddd, J = 11.7, 7.6, 3.5 Hz, 1H), 4.03 (s, 3H), 3.66 (q, J = 6.8 Hz, 1H), 3.23–3.29 (m, partially overlapped, 1H), 3.25 (s, 3H), 3.14–3.19 (m, 1H), 2.91 (s, 3H), 2.51 (s, 3H), 2.23–2.28 (m, 1H), 2.09–2.18 (m, 1H), 1.92–1.99 (m, 1H), 1.74–1.80 (m, 1H), 1.09–1.64 (m, 20H), 1.03 (d, J = 6.2 Hz, 3H), 0.86–0.96 (m, 15H), 0.54 (d, J = 6.6 Hz, 3H), –0.09 (d, J = 6.6 Hz, 3H), –0.39 (ddd, J = 14.6, 11.1, 3.6 Hz, 1H); IR (neat) ν_{max} 3281, 3128, 2956, 2246, 1749, 1687, 1658, 1641, 1632, 1547, 1462, 1402, 1205, 1092 cm^{-1}; HRMALDI–FTMS *m/z* 957.5776 ([M + Na]$^+$, $C_{50}H_{78}N_8O_9Na$ requires 957.5784).



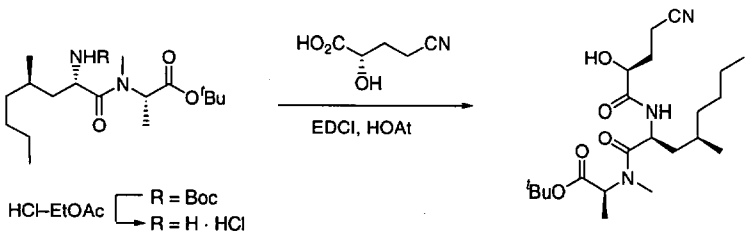
For C_2^3-***epi*-12**: Linear heptadepsipeptide **12H** (4.4 mg, 4.0 μmol) was deprotected following the standard procedure, and the residue was dissolved in DMF (4.0 mL, 1 mM) at 0 °C. EDCI (1.5 mg, 7.9 μmol) and HOAt (1.1 mg, 7.9 μmol) were added, and the mixture was stirred at 0 °C for 15 h. After work-up as described, the residue was subjected to HPLC chromatography and gave C_2^3-***epi*-12** (R_t = 15 min, 1.5 mg, 44% yield) as a white solid. Compound **12** was detected in the crude reaction mixture and the ratio C_2^3-***epi*-12** : **12** = 5.1:1. For C_2^3-***epi*-12**: ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 7.97 (br d, J = 9.9 Hz, 1H), 7.72 (d, J = 7.6 Hz, 1H), 7.56 (br d, J = 10.3 Hz, 1H), 7.45 (d, J = 7.6 Hz, 1H), 7.21 (dd, J = 7.6, 7.6 Hz, 1H), 7.09 (dd, J = 7.6, 7.6 Hz, 1H), 7.04 (s, 1H), 5.90 (br d, J = 8.8 Hz, 1H), 5.31 (dd, J = 12.5, 4.4 Hz, 1H), 5.13 (dd, J = 4.6, 4.6 Hz, 1H), 4.89–4.99 (m, 3H), 4.54–4.59 (m, 1H), 4.02 (s, 3H), 3.62 (q, J = 6.6 Hz, 1H), 3.21 (s, 3H), 3.18–3.23 (m, partially overlapped, 2H), 2.98 (s, 3H), 2.57 (s, 3H), 2.40–2.44 (m, 1H), 2.25–2.28 (m, 1H), 2.08–2.16 (m, 2H), 1.88 (dd, J = 9.2, 9.2 Hz, 1H), 1.71–1.76 (m, 1H), 1.11–1.63 (m, 18H), 0.85–0.97 (m, 18H), 0.55 (d, J = 6.6 Hz, 3H), 0.06 (d, J = 6.6 Hz, 3H), –0.30 (ddd, J = 14.0, 10.5, 3.8 Hz, 1H); IR (neat) ν_{max} 3285, 2957, 2862, 2246,

1754, 1677, 1667, 1651, 1637, 1615, 1532, 1471, 1204 cm^{-1}; HRMALDI–FTMS *m/z* 957.5777 ([M + Na]$^+$, $C_{50}H_{78}N_8O_9Na$ requires 957.5784).

Analogues with variations in the MLEU[3] side-chain:

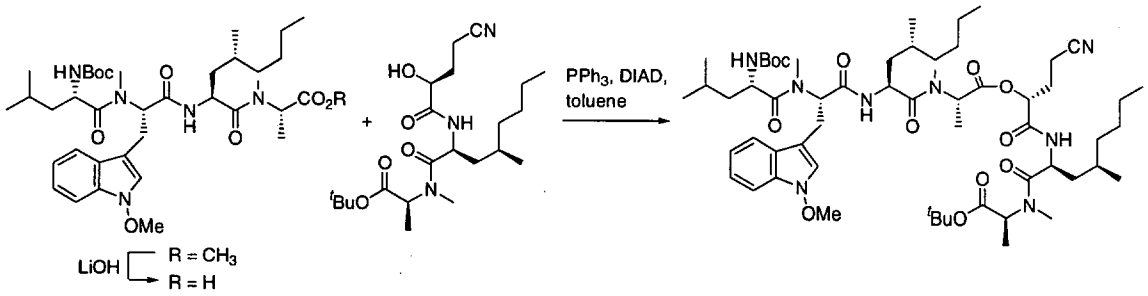


***N*-[(2*S*,4*R*)-2-[*N*-(*tert*-Butoxycarbonyl)amino]-4-methyloctanoyl]-*N*-methyl-L-alanine *tert*-butyl ester (13D)**: A CH_2Cl_2–DMF (5:1, 1 mL, 0.1 M) solution of **35** (27.3 mg, 0.10 mmol) and *N*-methyl-L-alanine *tert*-butyl ester (31.8 mg, 0.2 mmol) at 0 °C was treated with EDCI (38.3 mg, 0.2 mmol), HOAt (14.3 mg, 0.105 mmol) and $NaHCO_3$ (12.6 mg, 0.15 mmol), and the reaction mixture was allowed to warm to 25 °C and stirred for a total of 13 h. The reaction mixture was worked-up as described and afforded **13D** (32.0 mg, 77% yield) as a colorless oil. HPLC analysis (MeOH/H_2O, 80/20) of this crude product showed one major peak (R_t = 8.9 min, 82%): ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 5.21 (br d, *J* = 9.2 Hz, 1H), 5.13 (q, *J* = 7.3 Hz, 1H), 4.60–4.65 (m, 1H), 2.93 (s, 3H), 1.50 (ddd, *J* = 14.2, 10.9, 3.4 Hz, 1H), 1.40 (s, 9H), 1.39 (s, 9H), 1.31 (d, *J* = 7.3 Hz, 3H), 1.18–1.1.28 (m, 8H), 0.97 (d, *J* = 6.6 Hz, 3H), 0.84 (br t, *J* = 6.6 Hz, 3H); IR (neat) ν_{max} 3313, 2929, 1734, 1715, 1646, 1521, 1457, 1406, 1367, 1249, 1168 cm^{-1}; HRMALDI–FTMS *m/z* 437.2966 ([M + Na]$^+$, $C_{22}H_{42}N_2O_5Na$ requires 437.2986).

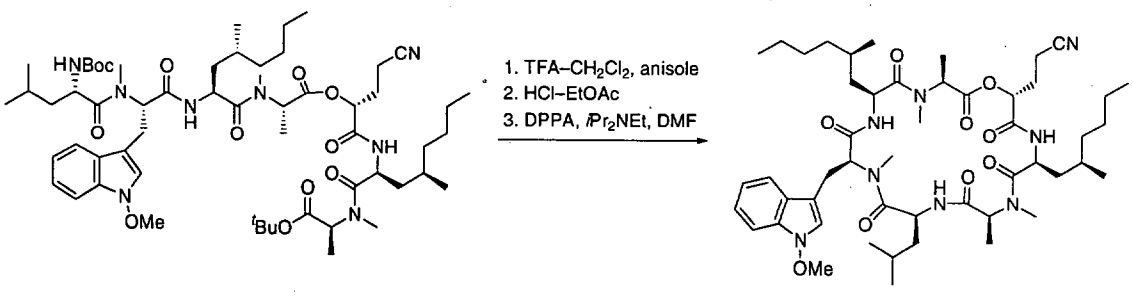


***N*-[(2*S*,4*R*)-2-[*N*-[(*S*)-4-Cyano-2-hydroxybutanoyl]amino]-4-methyloctanoyl]-*N*-methyl-L-alanine *tert*-butyl ester (13T)**: An EtOAc (0.4 mL) solution of **13D** (35.1 mg, 84.7 μmol) was treated with HCl–EtOAc (4.0 M, 0.4 mL) at 0 °C for 2 h, until no starting material was detected by TLC. The volatiles were removed by a stream of N_2 and a CH_2Cl_2–DMF (5:1, 0.8 mL, 0.1 M) solution of **39** (10.9 mg, 84.7 μmol) was added to the residue at 0 °C. The solution was treated with EDCI (32.5 mg, 0.17 mmol), HOAt (12.1 mg, 88.9 μmol) and 2,6-lutidine (9.9 μL, 84.7 μmol), and was stirred for 3 h at –30 °C. The reaction mixture was worked-up as described and afforded **13T** (25.6 mg, 65% yield) as a thick colorless oil. HPLC analysis (MeOH/H_2O, 80/20) of this crude product showed one major peak (R_t = 4.2 min, 55%): ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 7.09 (br d, *J* = 8.8 Hz, 1H), 5.10 (q, *J* = 5.7 Hz, 1H), 4.94–5.00 (m, 1H), 4.19–4.22 (m, 1H), 3.69 (d, *J* = 5.1 Hz, 1H), 2.98 (s, 3H), 2.43–2.58 (m, 2H), 2.13–2.20 (m, 1H), 1.93–1.98 (m, 1H), 1.23–1.64 (m, partially overlapped, 12H), 1.43 (s, 9H), 0.98–1.00 (m, 3H), 0.86–0.88 (m, 3H); IR (neat) ν_{max} 3382, 3315, 2930, 2245, 1735, 1637, 1160, 1090 cm^{-1};

HRMALDI–FTMS *m/z* 392.2161 ([M – (*tert*-Bu) + H + Na]$^+$, $C_{18}H_{31}N_3O_5Na$ requires 392.2156).



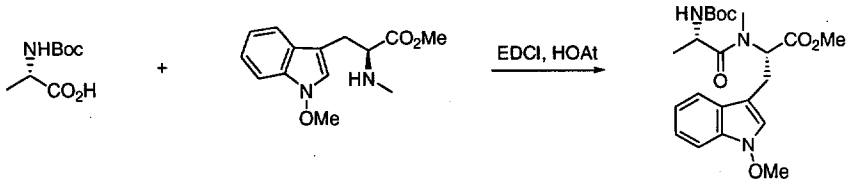
***N*-[(2*S*,4*R*)-2-[*N*-[(*R*)-2-[*N*-[[(2*S*,4*R*)-2-[[*N*-[*N*-(*tert*-Butoxycarbonyl)-L-leucinyl]-*N*$^{1'}$-methoxy-*N*-methyl-L-tryptophanyl]amino]-4-methyloctanoyl]-*N*-methyl-L-alanyloxy]-4-cyanobutanoyl]amino]-4-methyloctanoyl]-*N*-methyl-L-alanine *tert*-butyl ester (13H)**: A toluene (179 μL, 0.05 M) solution of **13T** (3.8 mg, 8.9 μmol), **2** (6.3 mg, 8.9 μmol) and PPh_3 (7.0 mg, 26.8 μmol) at 0 °C was treated dropwise with DIAD (97% purity, 5.4 μL, 26.8 μmol), and the mixture was stirred at 0 °C for 18 h. The solvent was removed by a stream of N_2, and the residue was dissolved in MeOH, and was subjected to reverse-phase HPLC chromatography. Compound **13H** (R_t = 25 min, 1.2 mg, 12% yield) was obtained as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 8.15 (br d, *J* = 9.2 Hz, 1H), 7.77 (br d, *J* = 8.8 Hz, 1H), 7.45 (d, *J* = 7.9 Hz, 1H), 7.33 (d, *J* = 7.9 Hz, 1H), 7.17 (dd, *J* = 7.9, 7.9 Hz, 1H), 7.03–7.06 (m, 2H), 5.57 (dd, *J* = 8.8, 7.0 Hz, 1H), 5.31 (q, *J* = 7.2 Hz, 1H), 5.21 (dd, *J* = 8.4, 3.7 Hz, 1H), 5.18 (br d, *J* = 8.8 Hz, 1H), 5.05–5.10 (m, 1H), 4.94 (ddd, *J* = 11.6, 8.8, 2.8 Hz, 1H), 4.52–4.55 (m, 1H), 3.97 (s, 3H), 3.90 (q, *J* = 7.0 Hz, 1H), 3.17–3.28 (m, partially overlapped, 2H), 3.26 (s, 3H), 3.04 (s, 3H), 2.86 (s, 3H), 2.25–2.32 (m, 2H), 2.12–2.15 (m, 1H), 1.98 (br dd, *J* = 11.6, 11.6 Hz, 1H), 1.85–1.92 (m, 1H), 1.69–1.73 (m, 1H), 1.18–1.58 (m, partially overlapped, 25H), 1.43 (s, 9H), 1.39 (s, 9H), 0.82–1.00 (m, 18H); IR (neat) ν_{max} 3301, 2928, 2251, 1754, 1734, 1715, 1671, 1636, 1538, 1458, 1368, 1166, 1096 cm^{-1}; HRMALDI–FTMS *m/z* 1131.7025 ([M + Na]$^+$, $C_{59}H_{96}N_8O_{12}Na$ requires 1131.7040).



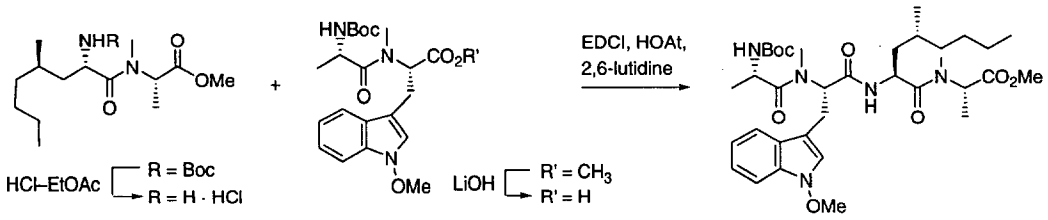
For **13**: Linear heptadepsipeptide **13H** (2.5 mg, 2.3 μmol) was deprotected following the standard procedure, and the residue was dissolved in DMF (2.3 mL, 1 mM) at 0 °C. DPPA (1.0 μL, 4.5 μmol) and *i*Pr_2NEt (0.8 μL, 4.5 μmol) were added, and the mixture was stirred at 0 °C for 59 h. After work-up as described, the residue was subjected to HPLC chromatography and gave **13** (R_t = 16 min, 0.8 mg, 38% yield) as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 8.51 (br d, *J* = 9.2 Hz, 1H), 8.03 (br d, *J* = 9.5

Hz, 1H), 7.65 (d, J = 7.7 Hz, 1H), 7.38–7.41 (m, 1H), 7.20–7.23 (m, 1H), 7.07–7.11 (m, 1H), 7.01 (s, 1H), 7.03 (br d, J = 7.6 Hz, 1H), 5.15–5.18 (m, 1H), 4.92–5.03 (m, 2H), 4.76–4.84 (m, 1H), 4.47–4.49 (m, 1H), 4.15 (ddd, J = 11.9, 7.6, 4.0 Hz, 1H), 4.03 (s, 3H), 3.67 (q, J = 7.0 Hz, 1H), 3.14–3.29 (m, partially overlapped, 2H), 3.25 (s, 3H), 2.91 (s, 3H), 2.55 (s, 3H), 2.24–2.26 (m, 2H), 2.12–2.18 (m, 1H), 2.05 (br dd, J = 12.1, 12.1 Hz, 1H), 1.89–1.96 (m, 1H), 1.80 (ddd, J = 13.8, 8.8, 4.8 Hz, 1H), 1.07–1.68 (m, 24H), 0.93 (d, J = 6.2 Hz, 3H), 0.83–0.89 (m, 9H), 0.54 (d, J = 6.6 Hz, 3H), –0.11 (d, J = 6.6 Hz, 3H), –0.39 (ddd, J = 14.2, 10.9, 3.4 Hz, 1H); IR (neat) ν_{max} 3272, 2926, 2256, 1749, 1672, 1662, 1646, 1639, 1533, 1456, 1097 cm^{-1}; HRMALDI–FTMS *m/z* 957.5796 ([M + Na]$^+$, $C_{50}H_{78}N_8O_9Na$ requires 957.5784).

Analogues with variations in the LEU^4 side-chain:

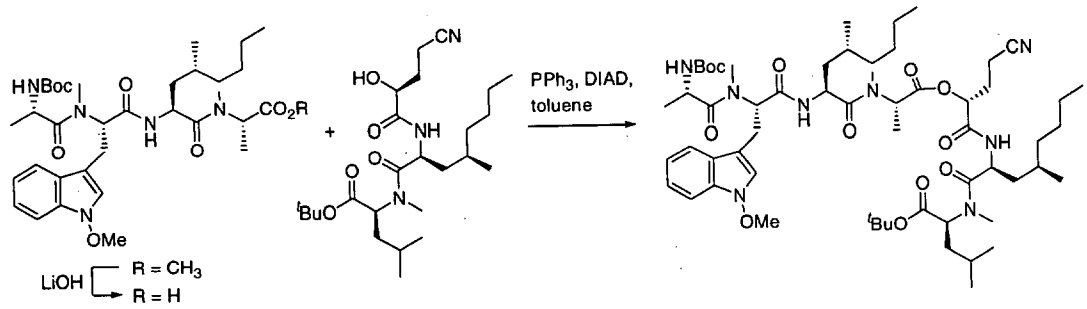


***N*-[*N*-(*tert*-Butoxycarbonyl)-L-alanyl]-*N*$^{1'}$-methoxy-*N*-methyl-L-tryptophan methyl ester (14D)**: A CH_2Cl_2–DMF (5:1, 1 mL, 0.1 M) solution of *N*-(*tert*-butoxycarbonyl)-L-alanine (18.9 mg, 0.10 mmol) and **41** (28.9 mg, 0.11 mmol) at 0 °C was treated with EDCI (38.3 mg, 0.2 mmol) and HOAt (14.3 mg, 0.105 mmol), and the reaction mixture was allowed to warm to 25 °C where it was stirred for a total of 13 h. Work-up of the reaction mixture as described and removal of the solvent in vacuo afforded **14D** (42.5 mg, 98% yield) as a light yellow foamy solid. HPLC analysis (MeOH/H_2O, 80/20) of this crude product showed one major peak (R_t = 4.2 min, 92%): ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 7.55 (d, J = 7.9 Hz, 1H), 7.37 (d, J = 7.9 Hz, 1H), 7.22 (dd, J = 7.9, 7.9 Hz, 1H), 7.05–7.11 (m, 2H), 5.36 (br d, J = 8.1 Hz, 1H), 5.26 (dd, J = 10.1, 5.3 Hz, 1H), 4.49–4.55 (m, 1H), 4.00 (s, 3H), 3.72 (s, 3H), 3.42 (dd, J = 15.4, 5.3 Hz, 1H), 3.21 (dd, J = 15.4, 10.1 Hz, 1H), 2.87 (s, 3H), 1.40 (s, 9H), 1.27 (d, J = 7.0 Hz, 3H); IR (neat) ν_{max} 3324, 2977, 2936, 1740, 1706, 1652, 1558, 1506, 1456, 1366, 1170 cm^{-1}; HRMALDI–FTMS *m/z* 456.2118 ([M + Na]$^+$, $C_{22}H_{31}N_3O_5Na$ requires 456.2110).

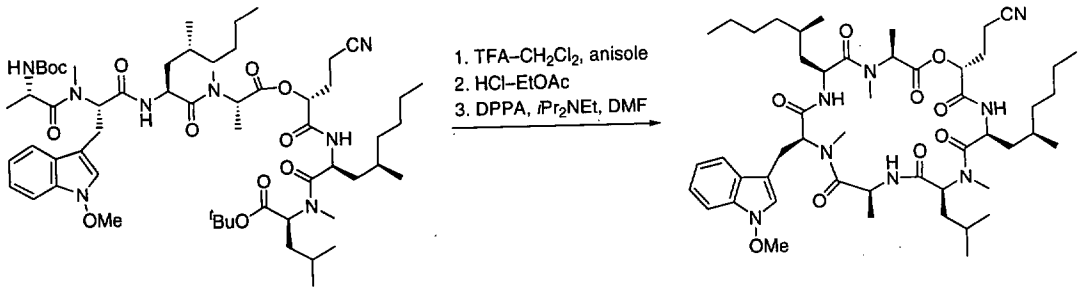


***N*-[(2*S*,4*R*)-2-[[*N*-[*N*-(*tert*-Butoxycarbonyl)-L-alanyl]-*N*$^{1'}$-methoxy-*N*-methyl-L-tryptophanyl]amino-4-methyloctanoyl]-*N*-methyl-L-alanine methyl ester (14T)**: A HCl–EtOAc solution (4.0 M, 1.0 mL) was added to **45** (40.2 mg, 107.8 μmol) at 0 °C, and the mixture was stirred for 1 h, until no starting material was detected by TLC. The volatiles were removed by a stream of N_2. **14D**-OH (crude product from saponification reaction of its methyl ester **14D**, 42.5 mg, 98.0 μmol, 0 °C, 1.5 h) was added, followed by

CH_2Cl_2–DMF (5:1, 1.0 mL, 0.1 M). The resulting solution was cooled to –30 °C and EDCI (37.6 mg, 196.1 μmol), HOAt (14.0 mg, 102.9 μmol) and 2,6-lutidine (12.6 μL, 107.8 μmol) were added, and the reaction mixture was stirred at –30 °C for 3 h. Work-up of the reaction mixture as described and removal of the solvent in vacuo afforded **14T** (61.6 mg, 93% yield) as a yellow foamy solid. HPLC analysis (MeOH/H_2O, 80/20) of this crude product showed one major peak (R_t = 8.7 min, 73%): ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 8.24 (d, J = 8.4 Hz, 1H), 7.57 (d, J = 8.1 Hz, 1H), 7.35 (d, J = 8.1 Hz, 1H), 7.18–7.22 (m, 1H), 7.06–7.11 (m, 2H), 5.35–5.43 (m, 2H), 5.18 (dd, J = 14.7, 7.3 Hz, 1H), 4.90–4.97 (m, 1H), 4.76 (br d, J = 6.2 Hz, 1H), 4.50–4.54 (m, 1H), 3.99 (s, 3H), 3.65 (s, 3H), 3.30 (dd, J = 15.5, 3.9 Hz, 1H), 3.13 (dd, J = 15.5, 8.8 Hz, 1H), 2.95 (s, 3H), 2.93 (s, 3H), 1.44–1.51 (m, 2H), 1.40 (s, 9H), 1.07–1.28 (m, 7H), 0.81–0.98 (m, 9H), 0.10 (d, J = 6.6 Hz, 3H); IR (neat) ν_{max} 3303, 2930, 2871, 1744, 1700, 1653, 1558, 1539, 1457, 1167 cm^{-1}; HRMALDI–FTMS *m/z* 696.3950 ([M + Na]$^+$, $C_{35}H_{55}N_5O_8Na$ requires 696.3948).

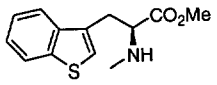


***N*-[(2*S*,4*R*)-2-[*N*-[(*R*)-2-[*N*-[[(2*S*,4*R*)-2-[[*N*-[*N*-(*tert*-Butoxycarbonyl)-L-alanyl]-*N*$^{1'}$-methoxy-*N*-methyl-L-tryptophanyl]amino]-4-methyloctanoyl]-*N*-methyl-L-alanyloxy]-4-cyanobutanoyl]amino]-4-methyloctanoyl]-*N*-methyl-L-leucine *tert*-butyl ester (14H)**: A toluene (264 μL, 0.05 M) solution of **14T** (6.2 mg, 13.2 μmol), **2** (crude product from saponification reaction of its methyl ester, 8.9 mg, 13.2 μmol, 0 °C, 1 h) and PPh_3 (6.9 mg, 26.4 μmol) at 0 °C was treated dropwise with DIAD (97% purity, 5.4 μL, 26.4 μmol), and the mixture was stirred at 0 °C for 13 h. The solvent was removed by a stream of N_2, and the residue was dissolved in MeOH, and was subjected to reverse-phase HPLC chromatography. Compound **14H** (R_t = 23 min, 3.7 mg, 25% yield) was obtained as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 7.79 (br d, J = 8.8 Hz, 1H), 7.61 (br d, J = 7.0 Hz, 1H), 7.53 (d, J = 7.3 Hz, 1H), 7.34 (d, J = 7.3 Hz, 1H), 7.19 (dd, J = 7.3, 7.3 Hz, 1H), 7.04–7.11 (m, 2H), 5.44–5.47 (m, 2H), 5.28 (dd, J = 10.6, 5.1 Hz, 1H), 5.17 (dd, J = 7.3, 4.0 Hz, 1H), 5.02–5.05 (m, 1H), 4.87 (ddd, J = 10.2, 8.0, 2.1 Hz, 1H), 4.48–5.00 (m, 1H), 3.96–4.01 (m, partially overlapped, 1H), 3.98 (s, 3H), 3.27–3.32 (m, 1H), 3.13–3.20 (m, 1H), 3.13 (s, 3H), 2.98 (s, 3H), 2.91 (s, 3H), 2.23–2.27 (m, 2H), 2.07–2.13 (m, 1H), 1.94–1.99 (m, 1H), 1.24–1.73 (m, partially overlapped, 27H), 1.43 (s, 9H), 1.40 (s, 9H), 0.83–0.97 (m, 12H), 0.75 (d, J = 6.6 Hz, 3H), 0.61 (d, J = 6.6 Hz, 3H); IR (neat) ν_{max} 3301, 2954, 2246, 1754, 1728, 1708, 1633, 1539, 1455, 1384, 1163 cm^{-1}; HRMALDI–FTMS *m/z* 1131.7019 ([M + Na]$^+$, $C_{59}H_{96}N_8O_{12}Na$ requires 1131.7045).



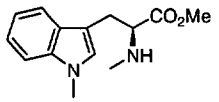
For **14**: Linear heptadepsipeptide **14H** (2.2 mg, 2.0 μmol) was deprotected following the standard procedures, and the residue was dissolved in DMF (2.0 mL, 1 mM) at 0 °C. DPPA (0.9 μL, 4.0 μmol) and iPr$_2$NEt (0.7 μL, 4.0 μmol) were added, and the mixture was stirred at 0 °C for 61 h. After work-up as described, the residue was subjected to HPLC chromatography and gave **14** (R_t = 17 min, 1.6 mg, 84% yield) as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 8.35 (br d, J = 10.3 Hz, 1H), 8.0 (br d, J = 9.9 Hz, 1H), 7.60 (d, J = 7.7 Hz, 1H), 7.40 (d, J = 8.4 Hz, 1H), 7.20–7.24 (m, overlapped with solvent peak, 1H), 7.05–7.10 (m, 2H), 5.95 (br d, J = 7.0 Hz, 1H), 5.17 (dd, J = 8.4, 3.7 Hz, 1H), 5.00–5.09 (m, 2H), 4.82–4.86 (m, 1H), 4.46 (dd, J = 11.4, 3.7 Hz, 1H), 4.06–4.10 (m, 1H), 4.02 (s, 3H), 3.65 (q, J = 6.8 Hz, 1H), 3.30 (dd, J = 14.9, 4.6 Hz, 1H), 3.24 (s, 3H), 3.15 (dd, J = 14.9, 10.3 Hz, 1H), 2.87 (s, 3H), 2.50 (s, 3H), 2.33 (ddd, J = 17.2, 8.1, 3.5 Hz, 1H), 2.15–2.27 (m, 2H), 1.91–1.98 (m, 1H), 1.73–1.83 (m, 2H), 1.12–1.59 (m, 22H), 1.03 (d, J = 6.6 Hz, 3H), 0.80–0.95 (m, 15H), 0.33 (d, J = 6.6 Hz, 3H); IR (neat) ν_{max} 3279, 2929, 2246, 1744, 1677, 1667, 1651, 1634, 1539, 1456, 1200, 1097 cm^{-1}; HRMALDI–FTMS m/z 957.5757 ([M + Na]$^+$, $C_{50}H_{78}N_8O_9Na$ requires 957.5784).

Analogues with variations in the MTO[5] side-chain:

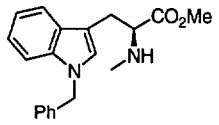


***N*-Methyl-3-(3-benzothienyl)-L-alanine methyl ester**: A THF solution of *N*-(*tert*-butoxycarbonyl)-3-(3-benzothienyl)-L-alanine (300 mg, 0.93 mmol) at 0 °C was treated with MeI (465 μL, 7.5 mmol), and NaH (60% dispersion in mineral oil, 112 mg, 2.8 mmol). The resulting solution was stirred at 0 °C for 3 h and was then allowed to warm to 25 °C for another 14 h. The reaction was quenched by the addition of H_2O and the mixture was acidified with the addition of 1 M aqueous HCl to pH = 2. The solvents were removed in vacuo and afforded a yellow foam. Toluene (10 mL) and CH_3OH (2 mL) were added and the solution at 0 °C was treated dropwise with $TMSCHN_2$ (2.0 M in hexanes, 0.52 mL, 1.03 mmol). The yellowish solution was allowed to warm to 25 °C, and was stirred for 30 min. The reaction was quenched by dropwise addition of HOAc, until the yellow color disappeared. The volatiles were removed in vacuo, and a thick colorless oil was obtained. Anisole (101 μL, 0.93 mmol) and HCl–EtOAc (4.0 M, 4.6 mL) were added at 0 °C and the solution was stirred for 1 h. The volatiles were removed in vacuo. Saturated aqueous $NaHCO_3$ solution was added to the residue, extracted with EtOAc (3 × 30 mL), washed with saturated aqueous NaCl, dried ($MgSO_4$), filtered and

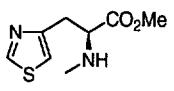
concentrated in vacuo. Silica gel chromatography (50% EtOAc–hexanes) provided the title compound (278 mg, 76%) as a colorless oil: $[\alpha]_D^{23}$ +33 (*c* 1.1, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$, major rotamer) δ 7.88 (d, *J* = 7.7 Hz, 1H), 7.81 (d, *J* = 7.7 Hz, 1H), 7.35–7.46 (m, 2H), 7.24 (s, 1H), 3.68 (s, 3H), 3.63 (t, *J* = 6.2 Hz, 1H), 3.19–3.33 (m, 2H), 2.43 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$, major rotamer) δ 174.7, 140.4, 138.8, 131.8, 124.3, 124.0, 123.5, 122.9, 121.5, 63.1, 51.7, 34.7, 32.2; IR (neat) ν_{max} 3332, 2948, 2852, 2799, 1732, 1428, 1349, 1258, 1197, 1173, 1117, 1020 cm^{-1}; HRMALDI–FTMS *m/z* 250.0896 ($[M + H]^+$, $C_{13}H_{15}NO_2S$ requires 250.0893). The *ee* of the product was established to be > 99%, which was examined by HPLC analysis (CHIRACEL® OD™, 0.46 × 25 cm) using 10% isopropanol–hexanes (retention time: 9.84 min).



***N,N′*-Dimethyl-L-tryptophan methyl ester**: A 1,4-dioxane/H_2O (1/1, 14 mL, 0.1 M) solution of 1-methyl-L-tryptophan (300 mg, 1.37 mmol) was treated with $NaHCO_3$ (242 mg, 2.89 mmol), and Boc_2O (329 mg, 1.51 mmol), and the suspension was stirred at 25 °C for 2 h. Water (30 mL) was added to quench the reaction and the aqueous layer was extracted with EtOAc (2 × 15 mL). The organic layers were discarded, and the aqueous layer was acidified to pH = 1–2 with the addition of 1 M HCl (aq), and extracted with EtOAc (3 × 20 mL). The organic layers were combined, dried ($MgSO_4$), and the solvent was removed in vacuo to provide a light purple oil. Anhydrous THF (14 mL) was added and the solution was treated with MeI (0.68 ml, 11.0 mmol), NaH (60% dispersion in mineral oil, 164 mg, 4.11 mmol) at 0 °C. The reaction mixture was allowed to warm to 25 °C and was stirred for 23 h. The reaction was worked-up as described above and afforded a light yellow oil. Toluene (5 mL) and MeOH (1 mL) were added and the solution was treated with $TMSCHN_2$ (2.0 M in hexanes, 0.63 mL, 1.26 mmol) at 0 °C. The yellowish solution was allowed to warm to 25 °C and was stirred for another 30 min. Acetic acid was added dropwise to quench the reaction until the solution became colorless. The volatiles were removed in vacuo and a light yellow oil was obtained. Anisole (148 μL, 1.37 mmol) and HCl–EtOAc (4.0 M, 3.4 mL) were added at 0 °C and the solution was stirred for 1 h. The volatiles were removed in vacuo. Saturated aqueous $NaHCO_3$ was added to the residue, extracted with EtOAc (3 × 50 mL), washed with saturated aqueous NaCl, dried ($MgSO_4$), filtered and concentrated in vacuo. Silica gel chromatography (50% EtOAc–hexanes) provided the title compound (256 mg, 76%) as a colorless oil: $[\alpha]_D^{23}$ +100 (*c* 0.9, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$, major rotamer) δ 7.58 (d, *J* = 7.8 Hz, 1H), 7.28 (d, *J* = 8.1 Hz, 1H), 7.22 (dt, *J* = 8.1, 1.1 Hz, 1H), 7.10 (dt, *J* = 7.8, 1.1 Hz, 1H), 6.91 (s, 1H), 3.74 (s, 3H), 3.67 (s, 3H), 3.53 (dd, *J* = 7.2, 1.1 Hz, 1H), 3.15–3.20 (m, 1H), 3.06–3.11 (m, 1H); 2.37 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$, major rotamer) δ 175.3, 137.2, 128.1, 127.8, 121.9, 119.1, 119.0, 109.8, 109.4, 64.1, 52.0, 35.1, 32.9, 29.2; IR (neat) ν_{max} 3426, 2947, 1733, 1652, 1475, 1377, 1326, 1251, 1202, 1173, 1012 cm^{-1}; HRMALDI–FTMS *m/z* 247.1447 ($[M + H]^+$, $C_{14}H_{18}N_2O_2$ requires 247.1441). The *ee* of the product was established to be = 95%, which was examined by HPLC analysis (CHIRACEL® OD™, 0.46 × 25 cm) using 10% isopropanol–hexanes (retention time: 12.9 min).

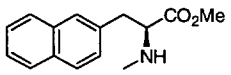


$N^{1'}$-Benzyl-*N*-methyl-L-tryptophan methyl ester: A 1,4-dioxane/H_2O (1/1, 6 mL, 0.1 M) solution of $N^{1'}$-benzyl-L-tryptophan (185 mg, 0.63 mmol) was treated with $NaHCO_3$ (111 mg, 1.32 mmol), and Boc_2O (151 mg, 0.69 mmol), and the suspension was stirred at 25 °C for 3 h. Water (20 mL) was added to quench the reaction and the aqueous layer was extracted with EtOAc (2 × 15 mL). The organic layers were discarded, and the aqueous layer was acidified to pH = 1–2 with the addition of 1 M HCl (aq), and extracted with EtOAc (3 × 20 mL). The organic layers were combined, dried ($MgSO_4$), and the solvent was removed in vacuo to provide a light brown oil. Anhydrous THF (6 mL) was added and the solution was treated with MeI (0.32 mL, 5.1 mmol), NaH (60% dispersion in mineral oil, 76.1 mg, 1.90 mmol) at 0 °C. The reaction mixture was allowed to warm to 25 °C and was stirred for 20 h. The reaction was worked-up as described above and afforded a light yellow oil. Toluene (5 mL) and MeOH (1 mL) were added and the solution was treated with $TMSCHN_2$ (2.0 M in hexanes, 0.35 mL, 0.69 mmol) at 0 °C. The yellowish solution was allowed to warm to 25 °C and was stirred for another 30 min. Acetic acid was added dropwise to quench the reaction until the solution became colorless. The volatiles were removed in vacuo and a light yellow oil was obtained. Anisole (68 μL, 1.37 mmol) and HCl–EtOAc (4.0 M, 1.7 mL) were added at 0 °C and the solution was stirred for 1 h. The volatiles were removed in vacuo. Saturated aqueous $NaHCO_3$ was added to the residue, extracted with EtOAc (3 × 30 mL), washed with saturated aqueous NaCl, dried ($MgSO_4$), filtered and concentrated in vacuo. Silica gel chromatography (50% EtOAc–hexanes) provided the title compound (127 mg, 63%) as a colorless oil: $[\alpha]_D^{23}$ +7.6 (*c* 1.4, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 7.62 (d, *J* = 7.7 Hz, 1H), 7.23–7.31 (m, 6H), 7.17 (dt, *J* = 7.0, 1.1 Hz, 1H), 7.12 (dt, *J* = 8.1, 1.1 Hz, 1H), 6.98 (s, 1H), 5.28 (s, 2H), 3.61 (s, 3H), 3.56 (t, *J* = 6.6 Hz, 1H), 3.11–3.22 (m, 2H), 2.39 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$, major rotamer) δ 174.9, 137.5, 136.6, 128.7, 128.1, 127.6, 126.8, 126.6, 121.9, 119.2, 118.9, 110.3, 109.7, 63.9, 51.6, 49.9, 34.7, 29.0; IR (neat) ν_{max} 3447, 2948, 1732, 1653, 1558, 1466, 1355, 1259, 1198, 1173, 1014 cm^{-1}; HRMALDI–FTMS *m/z* 323.1752 ([M + H]$^+$, $C_{20}H_{22}N_2O_2$ requires 323.1754). The *ee* of the product was established to be > 99%, which was examined by HPLC analysis (CHIRACEL® OD™, 0.46 × 25 cm) using 50% isopropanol–hexanes (retention time: 9.35 min).



***N*-Methyl-3-(4-thiazolyl)-L-alanine methyl ester**: A THF solution of *N*-(*tert*-butoxycarbonyl)-3-(4-thiazolyl)-L-alanine (136.2 mg, 0.5 mmol) at 0 °C was treated with MeI (249 μL, 4.0 mmol), and NaH (60% dispersion in mineral oil, 60.0 mg, 1.5 mmol). The resulting white cloudy solution was stirred at 0 °C for 4 h and was then allowed to warm to 25 °C for another 9 h. The reaction was quenched by the addition of H_2O and

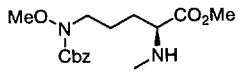
the mixture was acidified with the addition of 1 M aqueous HCl to pH = 2. The solvents were removed in vacuo and afforded a yellowish solid. Toluene (1.5 mL) and CH_3OH (0.3 mL) were added and the solution at 0 °C was treated dropwise with $TMSCHN_2$ (2.0 M in hexanes, 0.4 mL, 0.75 mmol). The yellowish solution was allowed to warm to 25 °C, and was stirred for 30 min. The reaction was quenched by dropwise addition of HOAc, until the yellow color disappeared. The volatiles were removed in vacuo, and a thick colorless oil was obtained. Anisole (65 μL, 0.6 mmol) and CH_2Cl_2–TFA (5:1, 4 mL) were added and the solution was stirred at 25 °C for 1 h. The volatiles were removed in vacuo. Silica gel chromatography (EtOAc : MeOH = 3:1) of the crude product provided the TFA salt of the title compound (R_f = 0.2 <CH_2Cl_2 : MeOH = 9:1>, 80.2 mg, 64% yield) as a white solid: $[\alpha]_D^{23}$ +8.2 (*c* 1.1, CH_3OH); ^1H NMR (500 MHz, CD_3OD) δ 8.98 (d, *J* = 1.8 Hz, 1H), 7.44 (br d, *J* = 1.8 Hz, 1H), 4.29 (dd, *J* = 5.6, 5.6 Hz, 1H), 3.77 (s, 3H), 3.46 (br d, *J* = 5.6 Hz, 1H), 2.72 (s, 3H); ^{13}C NMR (62.5 MHz, CD_3OD) δ 171.2, 156.0, 151.9, 118.4, 62.2, 53.6, 33.2, 31.7; IR (neat) ν_{max} 3432, 3106, 2961, 1749, 1682, 1517, 1437, 1207, 1135, 839 cm^{-1}; FABHRMS (NBA/NaI) *m/z* 201.0700 ([M + H]$^+$, $C_8H_{13}N_2O_2S$ requires 201.0698). The solid product was further treated with saturated aqueous $NaHCO_3$ until pH = 7–8, and the aqueous solution was extracted with EtOAc. The combined organic layers were dried ($MgSO_4$), and filtered. The solvent was removed in vacuo, and the title compound was obtained as a light yellow thick oil: $[\alpha]_D^{23}$ +15 (*c* 0.8, CH_3OH); ^1H NMR (400 MHz, CD_3OD) δ 8.93 (d, *J* = 2.1 Hz, 1H), 7.32 (br d, *J* = 2.1 Hz, 1H), 3.74 (dd, *J* = 6.6, 6.6 Hz, 1H), 3.67 (s, 3H), 3.22 (d, *J* = 6.6 Hz, 2H), 2.41 (s, 3H); ^{13}C NMR (62.5 MHz, CD_3OD) δ 174.2, 155.3, 153.6, 117.3, 63.5, 52.5, 34.0.



Methyl (*S*)-2-(*N*-methylamino)-3-(2-naphthalenyl)propanoate: A 1,4-dioxane (5 mL, 0.1 M) solution of 3-(2-naphthalenyl)-L-alanine (107.6 mg, 0.5 mmol) was treated with saturated aqueous $NaHCO_3$ (5 mL, 0.1 M), and Boc_2O (327 mg, 1.5 mmol), and the solution was stirred at 25 °C for 3 h. Water (10 mL) was added to quench the reaction and the aqueous layer (pH ~8) was extracted with EtOAc (8 mL × 2). The organic layers were discarded, and the aqueous layer was acidified to pH = 1–2 with the addition of 1 M HCl (aq), and extracted with EtOAc (8 mL × 3). The organic layers were combined, dried ($MgSO_4$), and the solvent was removed in vacuo to provide a thick light yellow oil. Anhydrous THF (5 mL) was added and the solution was treated with MeI (249 μL, 4.0 mmol), and NaH (60% dispersion in mineral oil, 60 mg, 1.5 mmol) at 0 °C. The reaction mixture was allowed to warm to 25 °C and was stirred for 21 h. The reaction was worked-up as described above and afforded a foamy light yellow solid. Toluene (1 mL) and MeOH (0.2 mL) were added and the solution was treated with $TMSCHN_2$ (2.0 M in hexanes, 0.2 mL, 0.5 mmol) at 0 °C. The yellowish solution was allowed to warm to 25 °C and was stirred for another 30 min. Acetic acid was added dropwise to quench the reaction until the solution became colorless. The volatiles were removed in vacuo and a light yellow oil was obtained. Anisole (51 μL, 0.45 mmol) and CH_2Cl_2–TFA (5:1, 3 mL) were added, and the reaction mixture was stirred at 25 °C for 1.5 h. The volatiles were removed in vacuo and a light yellow solid was obtained. Further purification of the crude product (trituration from EtOAc–hexanes) provided the TFA salt of the title compound (50.4 mg, 28% yield from L-3-(2-naphthalenyl)alanine) as an off-white solid: $[\alpha]_D^{23}$ +65 (*c* 1.1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.74–7.79 (m, 3H), 7.66 (br s, 1H), 7.43–

7.46 (m, 2H), 7.27 (dd, J = 8.3, 1.7 Hz, 1H), 4.12 (dd, J = 8.2, 6.1 Hz, 1H), 3.64 (s, 3H), 3.50 (dd, J = 14.0, 6.1 Hz, 1H), 3.39 (dd, J = 14.0, 8.2 Hz, 1H), 2.66 (s, 3H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 168.5, 133.4, 132.7, 131.1, 128.9, 128.3, 127.7, 126.52, 126.50, 126.2, 62.2, 53.0, 36.1, 32.0; IR (neat) ν_{max} 3409, 3053, 1747, 1676, 1439, 1202, 1134 cm^{-1}; HRMALDI–FTMS *m/z* 244.1337 ([M + H]$^+$, $C_{15}H_{18}NO_2$ requires 244.1330). The solid product could be further treated with saturated aqueous $NaHCO_3$ until pH = 7–8, and the aqueous solution was extracted with EtOAc (10 mL × 3). The combined organic layers were dried ($MgSO_4$), and filtered. The solvent was removed in vacuo to provide a light yellow thick oil: $[\alpha]_D^{23}$ +35 (*c* 1.6, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75–7.80 (m, 3H), 7.61 (br s, 1H), 7.40–7.46 (m, 2H), 7.29 (dd, J = 8.4, 1.8 Hz, 1H), 3.64 (s, 3H), 3.53 (dd, J = 6.8, 6.8 Hz, 1H), 3.12 (dd, J = 13.6, 6.8 Hz, 1H), 3.08 (dd, J = 13.6, 6.8 Hz, 1H), 2.36 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.8, 134.7, 133.4, 132.3, 128.1, 127.7. 127.6, 127.5, 127.3, 126.0, 125.5, 64.6, 51.7, 39.7, 34.8.

The *ee* of the product was established to be >99%, which was examined by HPLC (CHIRALCEL® OD™, 0.46 × 25 cm) and was compared with the racemic compound [prepared in three steps: (a) Boc_2O, $NaHCO_3$, 1,4-dioxane–H_2O, 25 °C, 22 h; (b) MeI, NaH, DMF, 25 °C, 24 h; (c) TFA–CH_2Cl_2, anisole, 25 °C, 1 h]. The racemic compound showed two peaks (retention time: 7.39 and 7.70 min) by using 10% isopropanol–hexanes, with a flow rate of 1 mL/min, and with 254 nm as the detection wavelength. Under the same conditions, title compound appeared as a single peak matching the latter peak (retention time: 7.71 min).

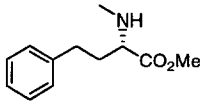


***tert*-Butyl (*S*)-2-[*N*-(*tert*-butoxycarbonyl)amino]-5-[*N*-(benzyloxycarbonyl)-*N*-methoxyamino]pentanoate**: A THF (8.3 mL, 0.06 M) solution of *tert*-butyl (*S*)-2-[*N*-(*tert*-butoxycarbonyl)amino]-5-hydroxypentanoate[35] (1.01 g, 3.5 mmol), PPh_3 (1.2 g, 4.6 mmol) and *O*-benzyl-*N*-methoxycarbamate[36] (634 mg, 3.5 mmol) at 25 °C was treated with dropwise addition of DEAD (0.73 mL, 4.4 mmol) over 2 h. This yellow clear solution was stirred at 25 °C for another 5 h. The solvent was removed in vacuo and silica gel chromatography (hexanes : EtOAc = 1:1) gave the title compound (R_f = 0.7, 900 mg, 57% yield) as a colorless oil: $[\alpha]_D^{23}$ +15 (*c* 1.7, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27–7.35 (m, 5H), 5.16 (s, 2H), 5.02 (br d, J = 8.1 Hz, 1H), 4.12–4.18 (m, 1H), 3.66 (s, 3H), 3.50 (t, J = 6.6 Hz, 2H), 1.54–1.82 (m, 4H), 1.42 (s, 9H), 1.41 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6, 156.8, 155.3, 136.0, 128.5, 128.2, 128.0, 81.9, 79.6, 67.6, 62.4, 53.5, 48.4, 30.1, 28.3, 27.9, 22.9; IR (neat) ν_{max} 3362, 2977, 1714, 1498, 1367, 1154 cm^{-1}; FABHRMS (NBA/NaI) *m/z* 453.2616 ([M + H]$^+$, $C_{23}H_{37}N_2O_7$ requires 453.2602).

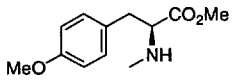
tert-Butyl (*S*)-2-[*N*-(*tert*-Butoxycarbonyl)amino]-5-[*N*-(benzyloxycarbonyl)-*N*-methoxyamino]-pentanoic Acid: A mixture of *tert*-butyl (*S*)-2-[*N*-(*tert*-butoxycarbonyl)-amino]-5-[*N*-(benzyloxycarbonyl)-*N*-methoxyamino]pentanoate (676 mg, 1.5 mmol), anisole (340 μL, 3.1 mmol) and TFA–CH_2Cl_2 (1:5, 14.9 mL, 0.1 M) was stirred at 25 °C for 1 h. The volatiles were removed in vacuo, and a colorless oil was obtained. The oily product was dissolved in 1,4-dioxane (14.9 mL, 0.1 M), saturated aqueous $NaHCO_3$ (14.9 mL, 0.1 M) and Boc_2O (977 mg, 4.5 mmol) were added and the white cloudy solution was stirred at 25 °C for 2 h. The reaction mixture was acidified to pH = 1–2 with the addition of 1 M HCl (aq), and the aqueous layer was extracted with EtOAc (15 mL × 3). The combined organic layers were dried ($MgSO_4$), filtered, and the

solvent was removed in vacuo. Silica gel chromatography (hexanes : EtOAc = 10:1) gave the title compound (R_f = 0.2, 185 mg, 31% yield) as a white solid: $[\alpha]_D^{23}$ +16 (*c* 0.55, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, two rotamers) δ 7.29–7.35 (m, 5H), 5.84 (br s, 0.1H), 5.17 (s, 2H), 5.02 (br d, *J* = 7.84 Hz, 0.9H), 4.29–4.31 (m, 0.9H), 4.15 (br s, 0.1H), 3.67 (s, 3H), 3.48–3.58 (m, 2H), 1.83–1.88 (m, 1H), 1.64-1.74 (m, 3H), 1.42 (s, 9H); ^{13}C NMR (62.5 MHz, $CDCl_3$, two rotamers) δ 176.3, 176.2, 157.0, 155.8, 135.8, 128.6, 128.3, 128.1, 82.5, 80.5, 67.9, 66.0, 65.9, 62.3, 61.1, 54.2, 52.9, 48.0, 29.5, 28.2, 27.9, 24.4, 23.1; IR (neat) ν_{max} 3341, 2976, 1711, 1499, 1367, 1161, 1056 cm^{-1}; FABHRMS (NBA/NaI) *m/z* 419.1784 ([M + Na]$^+$, $C_{19}H_{28}N_2O_7Na$ requires 419.1794).

Methyl (*S*)-2-(*N*-methylamino)-5-[*N*-(benzyloxycarbonyl)-*N*-methoxyamino]-pentanoate: A DMF (0.5 mL) solution of (*S*)-2-[*N*-(*tert*-butoxy-carbonyl)amino]-5-[*N*-(benzyloxycarbonyl)-*N*-methoxyamino]pentanoic acid (9.8 mg, 0.025 mmol) was treated with Ag_2O (28.6 mg, 0.12 mmol) and MeI (15.4 μL, 0.2 mmol), and the reaction mixture was allowed to warm to 45 °C and stirred for 4 h at 45 °C and another 13 h at 25 °C. The yellowish cloudy solution was passed through a silica gel pad, and washed with EtOAc (10 mL × 3). The combined filtrate was washed with saturated aqueous NaCl (10 mL × 5), dried ($MgSO_4$), and filtered. The solvent was removed in vacuo and a oily product was obtained. Anisole (4 μL, 0.04 mmol) was added, followed by TFA–CH_2Cl_2 (1:5, 0.2 mL, 0.1 M). The reaction mixture was stirred at 25 °C for 2 h, and the volatiles were removed in vacuo. Saturated aqueous $NaHCO_3$ was added until pH ~8 and the aqueous layer was extracted with EtOAc (10 mL × 3). The organic layers were combined, dried ($MgSO_4$), and filtered. Silica gel chromatography (CH_2Cl_2 : MeOH = 10 :1) of the crude product provided the TFA salt of the title compound (R_f = 0.6, 7.9 mg, 99% yield) as an off white solid: $[\alpha]_D^{23}$ +34 (*c* 0.74, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29–7.35 (m, 5H), 5.16 (s, 2H), 3.77–3.81 (m, 1H, partially overlapped), 3.77 (s, 3H, partially overlapped), 3.64 (s, 3H), 3.52 (t, *J* = 6.3 Hz, 2H), 2.66 (s, 3H), 1.96 (br s, 2H), 1.78–1.83 (m, 1H), 1.60–1.65 (m, 1H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 168.6, 156.8, 135.9, 128.6, 128.3, 128.1, 67.9, 62.3, 60.2, 53.2, 47.4, 31.3, 25.9, 22.4; IR (neat) ν_{max} 3334, 3032, 2937, 1733, 1456, 1169 cm^{-1}; FABHRMS (NBA/NaI) *m/z* 325.1755 ([M + H]$^+$, $C_{16}H_{25}N_2O_5$ requires 325.1765). The solid product could be further treated with saturated aqueous $NaHCO_3$ until pH = 7–8, and the aqueous solution was extracted with EtOAc. The combined organic layers were dried ($MgSO_4$), and filtered. The solvent was removed in vacuo to provide a colorless thick oil: $[\alpha]_D^{23}$ +36 (*c* 1.8, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28–7.30 (m, 5H), 5.16 (s, 2H), 3.69 (s, 3H), 3.66 (s, 3H), 3.49 (br t, *J* = 6.7 Hz, 2H), 3.14 (br t, *J* = 6.3 Hz, 1H), 2.33 (s, 3H), 1.86 (br s, 1H), 1.54–1.73 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.2, 156.8, 136.1, 128.5, 128.2, 128.0, 67.6, 62.7, 62.4, 51.7, 48.5, 34.6, 30.1, 23.5. The optical purity of the product was established to be >99% *ee*, which was examined by HPLC (CHIRALCEL® OD™, 0.46 × 25 cm), compared with the racemic compound (prepared by the *N*-methylation of corresponding *N*-Boc compound in DMF in the presence of NaH at 45 °C for 19 h, followed by Boc deprotection with TFA). The racemic compound showed two distinct peaks (retention time: 8.53 and 9.03 min) by using 10% isopropanol–hexanes, with a flow rate of 1 mL/min, and with 254 nm as the detection wavelength. Under the same conditions, title compound appeared as single peak matching the latter peak (retention time: 9.01 min).

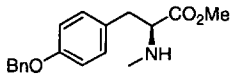


***N*-Methyl-L-homophenylalanine methyl ester**: A THF solution of *N*-(*tert*-butoxycarbonyl)-L-homophenylalanine (260 mg, 0.93 mmol) at 0 °C was treated with MeI (0.46 mL, 7.44 mmol) and NaH (60% dispersion in mineral oil, 111 mg, 2.79 mmol). The resulting suspension was stirred for 3 h at 0 °C and was then allowed to warm up to 25 °C and stirred for another 12 h. The reaction was quenched by the addition of H_2O and the mixture was acidified with 1 M aqueous HCl to pH 1–2, and extracted with EtOAc. The solvents were removed in vacuo to afford a yellow oil. Toluene (5 mL) and MeOH (1 mL) were added and the solution was treated dropwise at 0 °C with $TMSCHN_2$ (2.0 M in hexanes, 0.51 mL, 1.02 mmol). The yellowish solution was allowed to warm to 25 °C, and was stirred for 20 min. The reaction was quenched by dropwise addition of HOAc, until the yellow color disappeared. The volatiles were removed in vacuo and a yellow oil was obtained. Anisole (30 μL, 0.28 mmol) and HCl–EtOAc (4.0 M, 4.0 mL) were added at 0 °C and the solution was stirred for 1 h. The volatiles were removed in vacuo. Saturated aqueous $NaHCO_3$ solution was added to the residue, extracted with EtOAc (3 × 20 mL), washed with saturated aqueous NaCl, dried ($MgSO_4$), filtered and concentrated in vacuo. Silica gel chromatography (hexanes : EtOAc = 1/1) provided the title compound (150 mg, 78%) as a colorless oil: $[\alpha]_D^{23}$ +19 (*c* 1.6, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$, major rotamer) δ 7.32–7.37 (m, 2H), 7.23–7.28 (m, 3H), 3.79 (s, 3H), 3.25 (t, *J* = 6.5 Hz, 2H), 2.71–2.82 (m, 2H), 2.44 (s, 3H), 2.01–2.10 (m, 1H), 1.91–2.10 (m, 2H); ^{13}C NMR (62.5 MHz, $CDCl_3$, major rotamer) 175.6, 141.2, 128.4, 128.2, 125.9, 76.6, 62.5, 51.6, 34.8, 32.0; IR (neat) ν_{max} 3339, 2949, 1728, 1454, 1173, 1030 cm^{-1}; HRMALDI–FTMS *m/z* 208.1333 ($[M + H]^+$, $C_{12}H_{18}NO_2$ requires 208.1332). The *ee* of the product was established to be >99%, which was examined by HPLC analysis (CHIRACEL® OD™, 0.46 × 25 cm) using 10% isopropanol–hexanes (retention time: 6.54 min).

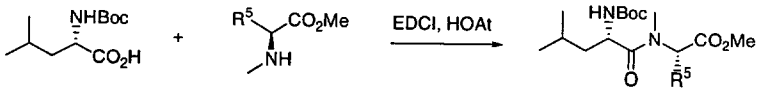


***N,O*-Dimethyl-L-tyrosine methyl ester**: A THF solution of *N*-(*tert*-butoxycarbonyl), *O*-methyl-L-tyrosine (200 mg, 0.68 mmol) at 0 °C was treated with MeI (0.34 mL, 5.4 mmol) and NaH (60% dispersion in mineral oil, 81 mg, 2.03 mmol). The resulting suspension was stirred for 2 h at 0 °C and was then allowed to warm to 25 °C and stirred for another 14 h. The reaction was quenched by the addition of H_2O and the mixture was acidified with 1 M aqueous HCl to pH 1–2, and extracted with EtOAc. The solvents were removed in vacuo to afford a yellow oil. Toluene (5 mL) and MeOH (1 mL) were added and the solution was treated dropwise at 0 °C with $TMSCHN_2$ (2.0 M in hexanes, 0.32 mL, 0.64 mmol). The yellowish solution was allowed to warm to 25 °C, and was stirred for 20 min. The reaction was quenched by dropwise addition of HOAc, until the yellow color disappeared. The volatiles were removed in vacuo and a yellow oil was obtained. Anisole (63 μL, 0.58 mmol) and HCl–EtOAc (4.0 M, 1.2 mL) were added at 0 °C and the solution was stirred for 1 h. The volatiles were removed in vacuo. Saturated aqueous $NaHCO_3$ was added to the residue, extracted with EtOAc (3 × 20 mL), washed with saturated aqueous NaCl, dried ($MgSO_4$), filtered and concentrated in vacuo. Silica gel chromatography (hexanes : EtOAc = 1/1) provided the title compound (124 mg, 82%) as a colorless oil: $[\alpha]_D^{23}$ +24 (*c* 0.65, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$, major rotamer) δ 7.08 (d, *J* = 8.5 Hz, 2H), 6.84 (d, *J* = 8.5 Hz, 2H), 3.78 (s, 3H), 3.67 (s, 3H), 3.36–3.45 (m, 1H), 2.87–2.98 (m, 2H), 2.37 (s, 3H); ^{13}C NMR (62.5 MHz, $CDCl_3$, major rotamer)

174.8, 158.4, 130.0, 129.1, 113.9, 64.8, 55.2, 51.6, 38.6, 34.7; IR (neat) ν_{max} 3422, 2949, 1734, 1612, 1513, 1438, 1248, 1176, 1034 cm^{-1}; HRMALDI–FTMS *m/z* 224.1281 ([M + H]$^+$, $C_{12}H_{17}NO_3$ requires 224.1281). The *ee* of the product was established to be >99 %, which was examined by HPLC analysis (CHIRACEL® OD™, 0.46 × 25 cm) using 10% isopropanol–hexanes (retention time: 5.98 min).



***O*-Benzyl-*N*-methyl-L-tyrosine methyl ester**: A THF solution of *O*-benzyl-*N*-(*tert*-butoxycarbonyl)-L-tyrosine (140 mg, 0.38 mmol) at 0 °C was treated with MeI (0.19 mL, 3.0 mmol) and NaH (60% dispersion in mineral oil, 46 mg, 1.14 mmol). The resulting suspension was stirred for 3 h at 0 °C and was then allowed to warm to 25 °C and stirred for another 12 h. The reaction was quenched by the addition of H_2O and the mixture was acidified with 1 M aqueous HCl to pH 1–2, and extracted with EtOAc. The solvents were removed in vacuo to afford a yellow foam. Toluene (3 mL) and MeOH (0.6 mL) were added and the solution was treated dropwise at 0 °C with $TMSCHN_2$ (2.0 M in hexanes, 0.20 mL, 0.40 mmol). The yellowish solution was allowed to warm to 25 °C, and was stirred for 20 min. The reaction was quenched by dropwise addition of HOAc, until the yellow color disappeared. The volatiles were removed in vacuo and a yellow oil was obtained. Anisole (39 μL, 0.36 mmol) and HCl–EtOAc (4.0 M, 3.6 mL) were added at 0 °C and the solution was stirred for 1 h. The volatiles were removed in vacuo. Saturated aqueous $NaHCO_3$ was added to the residue, extracted with EtOAc (3 × 20 mL), washed with saturated aqueous NaCl, dried ($MgSO_4$), filtered and concentrated in vacuo. Silica gel chromatography (hexanes : EtOAc = 1/1) provided the title compound (93 mg, 81%) as a colorless oil: $[\alpha]_D^{23}$ +20 (*c* 1.0, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$, major rotamer) δ 7.25–7.50 (m, 5H), 7.10 (d, *J* = 8.5 Hz, 2H), 6.92 (d, *J* = 8.5 Hz, 2H), 5.06 (s, 2H), 3.69 (s, 3H), 3.39–3.48 (m, 1H), 2.88–2.96 (m, 2H), 2.38 (s, 3H); ^{13}C NMR (62.5 MHz, $CDCl_3$, major rotamer) 174.8, 157.6, 137.0, 130.0, 129.4, 128.6, 127.9, 127.5, 114.8, 69.9, 64.8, 51.6, 38.6, 34.7; IR (neat) ν_{max} 3403, 2942, 1735, 1610, 1511, 1452, 1240, 1132, 1021 cm^{-1}; HRMALDI–FTMS *m/z* 300.1582 ([M + H]$^+$, $C_{18}H_{21}NO_3$ requires 300.1594). The *ee* of the product was established to be >99%, which was examined by HPLC analysis (CHIRACEL® OD™, 0.46 × 25 cm) using 10% isopropanol–hexanes (retention time: 10.6 min).



***N*-[*N*-(*tert*-Butoxycarbonyl)-L-leucinyl]-*N*-methyl-L-alanine methyl ester (15D**, R^5 = CH_3): A CH_2Cl_2–DMF (5:1, 1 mL, 0.1 M) solution of *N*-methyl-L-alanine methyl ester (23.4 mg, 0.2 mmol) and *N*-(*tert*-butoxycarbonyl)-L-leucine hydrate (**40**, 24.9 mg, 0.1 mmol) at 0 °C was treated with EDCI (38.3 mg, 0.2 mmol) and HOAt (16.3 mg, 0.12 mmol), and the reaction mixture was allowed to warm to 25 °C and stirred for a total of 13 h. The reaction mixture was worked-up as described and afforded **15D** (22.4 mg, 68% yield) as a colorless thick oil: ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 5.19–5.25 (m, 2H), 4.60–4.65 (m, 1H), 3.68 (s, 3H), 2.97 (s, 3H), 1.63–1.84 (m, 2H), 1.37–1.48 (m, partially overlapped, 4H), 1.39 (s, partially overlapped, 9H), 0.98 (d, *J* = 6.5 Hz, 3H),

0.91 (d, J = 6.7 Hz, 3H); IR (neat) ν_{max} 3296, 2957, 2870, 1746, 1721, 1711, 1503, 1366, 1170 cm^{-1}; HRMALDI–FTMS *m/z* 353.2052 ([M + Na]$^+$, $C_{16}H_{30}N_2O_5Na$ requires 353.2062).

***N*-[*N*-(*tert*-Butoxycarbonyl)-L-leucinyl]-*N*-methyl-3-(3-benzothienyl)-L-alanine methyl ester** (**16bD**, R^5 = CH_2(benzothien-3-yl)): A CH_2Cl_2–DMF (5:1, 3 mL, 0.1 M) solution of *N*-(*tert*-butoxycarbonyl)-L-leucine monohydrate (**40**, 80.0 mg, 0.32 mmol) and *N*-methyl-3-(3-benzothienyl)-L-alanine methyl ester (80.0 mg, 0.32 mmol) at 0 °C was treated with EDCI (73.8 mg, 0.38 mmol) and HOAt (52.4 mg, 0.38 mmol), and the reaction mixture was allowed to warm to 25 °C and was stirred for a total of 24 h. Work-up of the reaction mixture as described and removal of solvent in vacuo afforded **16bD** (112 mg, 73%) as a colorless foamy solid. HPLC analysis (MeOH/H_2O, 80/20) of this crude product showed one major peak (R_t = 8.47 min, 91%): ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 7.83 (d, J = 8.1 Hz, 1H), 7.77 (d, J = 8.1 Hz, 1H), 7.31–7.45 (m, 2H), 7.17 (s, 1H), 5.34 (dd, J = 9.9, 5.2 Hz, 1H), 5.09 (d, J = 9.2 Hz, 1H), 4.56 (td, J = 9.5, 3.6 Hz, 1H), 3.74 (s, 3H), 3.54 (dd, J = 15.8, 5.5 Hz, 1H), 3.35 (dd, J = 15.5, 10.3 Hz, 1H), 2.91 (s, 3H), 1.65–1.79 (m, 1H), 1.43 (s, 9H), 1.24–1.60 (m, 2H), 0.93 (d, J = 6.2 Hz, 3H), 0.92 (d, J = 6.6 Hz, 3H); IR (neat) ν_{max} 3307, 2956, 1744, 1714, 1652, 1506, 1456, 1365, 1250, 1169, 1107, 1046, 1020 cm^{-1}; HRMALDI–FTMS *m/z* 485.2081 ([M + Na]$^+$, $C_{24}H_{34}N_2O_5SNa$ requires 485.2081).

***N*-[*N*-(*tert*-Butoxycarbonyl)-L-leucinyl]-*N*,*N*′-dimethyl-L-tryptophan methyl ester** (**17D**, R^5 = CH_2(*N*′-methyl-tryptophan-3-yl)): A CH_2Cl_2–DMF (5:1, 3.2 mL, 0.1 M) solution of *N*-(*tert*-butoxycarbonyl)-L-leucine monohydrate (**40**, 80.0 mg, 0.32 mmol) and *N*,*N*′-dimethyl-L-tryptophan methyl ester (80.1 mg, 0.32 mmol) at 0 °C was treated with EDCI (74.5 mg, 0.39 mmol) and HOAt (52.9 mg, 0.39 mmol), and the reaction mixture was allowed to warm to 25 °C and was stirred for a total of 19 h. Work-up of the reaction mixture as described and removal of solvent in vacuo afforded **17D** (98 mg, 66%) as a light yellow foamy solid. HPLC analysis (MeOH/H_2O, 80/20) of this crude product showed one major peak (R_t = 7.36 min, 84%): ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer) δ 7.58 (d, J = 6.6 Hz, 1H), 7.19–7.28 (m, 2H), 7.09–7.14 (m, 1H), 6.87 (s, 1H), 5.37 (dd, J = 8.5, 5.0 Hz, 1H), 5.16 (d, J = 7.4 Hz, 1H), 4.54–4.60 (m, 1H), 3.73 (s, 3H), 3.72 (s, 3H), 3.46 (dd, J = 12.5, 4.5 Hz, 1H), 3.22 (dd, J = 12.5, 9.0 Hz, 1H), 2.92 (s, 3H), 1.70–1.75 (m, 1H), 1.42 (s, 9H), 1.23–1.35 (m, 2H), 0.97 (d, J = 5.2 Hz, 3H), 0.93 (d, J = 5.6 Hz, 3H); IR (neat) ν_{max} 3304, 2956, 2340, 1751, 1690, 1645, 1472, 1366, 1251, 1169, 1106 cm^{-1}; HRMALDI–FTMS *m/z* 482.2622 ([M + Na]$^+$, $C_{25}H_{37}N_3O_5Na$ requires 482.2625).

***N*-[*N*-(*tert*-Butoxycarbonyl)-L-leucinyl]-*N*′-benzyl-*N*-methyl-L-tryptophan methyl ester** (**18D**, R^5 = CH_2(*N*′-benzyl-tryptophan-3-yl)): A CH_2Cl_2–DMF (5:1, 1.8 mL, 0.1 M) solution of *N*-(*tert*-butoxycarbonyl)-L-leucine monohydrate (**40**, 45.4 mg, 0.18 mmol) and *N*′-benzyl-*N*-methyl-L-tryptophan methyl ester (48.0 mg, 0.18 mmol) at 0 °C was treated with EDCI (42.3 mg, 0.22 mmol) and HOAt (30.1 mg, 0.22 mmol), and the reaction mixture was allowed to warm to 25 °C and was stirred for a total of 16 h. Work-up of the reaction mixture as described and removal of solvent in vacuo afforded **18D** (63 mg, 64%) as a light yellow foamy solid. HPLC analysis (MeOH/H_2O, 80/20) of this crude product showed one major peak (R_t = 13.1 min, 86%): ^{1}H NMR (600 MHz, $CDCl_3$, major rotamer) δ 7.61 (d, J = 7.8 Hz, 1H), 7.21–7.31 (m, 5H), 7.16 (br t, J = 7.4 Hz, 1H), 6.97 (s, 1H), 5.30 (s, 2H), 5.22–5.30 (m, 1H), 5.14 (d, J = 8.3 Hz, 1H), 4.52–4.61 (m, 1H),