SUPPORTING INFORMATION

SYNTHESIS OF THE SOUTHERN TRIPEPTIDE (C₁-N₁₂) OF SANGLIFEHRINS USING ASYMMETRIC ORGANOCATALYSIS

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Table of Contents	Page
Experimental section	S2-12
NMR Spectra	S13-30
HPLC Chromatograms	S31-34
HRMS reports	S35-45

EXPERIMENTAL SECTION AND CHARACTERIZATION DATA

(*S*)-Di-*tert*-butyl-1-(1-(3-(benzyloxy)phenyl)-3-hydroxypropan-2-yl)hydrazine-1,2-dicarboxylate **8a**

To a solution of 3-(3-(benzyloxy)phenyl) propanal **6** (1 g, 4.1 mmol) in CH₃CN (30 mL), di-*tert*-butyl azodicarboxylate (0.64 g, 2.7 mmol) and (*R*)-proline (28 mg, 0.24 mmol, 10 mol%) were added at 0 °C and stirred at the same temperature for 12 h. After the disappearance of yellow color, ethanol (30 mL) was added to the reaction mixture. At 0 °C, NaBH₄ (79 mg, 2.08 mmol) was added and stirred at the same temperature for 30 min. Reaction mixture was quenched with 10% aqueous citric acid (10 mL) and extracted with ethyl acetate (2 x 20 mL). The organic layer was dried over Na₂SO₄, concentrated in *vacuo* and the residue was purified through column chromatography to give compound **8a** as a solid (1.84 g, 94%, 93% *ee*). Enantiomeric excess was determined by chiral HPLC (CHIRAL PAK-IA; 250 X 4.6 mm, 5U, Mobile phase: 20% IPA in hexane, Flow rate: 1 mL/min, Detection: 210 nm; major *t*_R 6.696 min, minor *t*_R 17.432 min. M.P: 139-140 °C. [α]_D²⁰: -25.35 (*c* = 1.0, CHCl₃). IR (neat): $\tilde{\nu}_{\text{max}}$ = 3424, 3218, 2925, 1715, 1279, 1163, 1026, 698 cm⁻¹. ¹H NMR (CDCl₃, 500 MHz): δ 7.47-7.29 (m, 5H), 7.19 (t, *J* = 7.9 Hz, 1H), 6.87-6.70 (m, 3H), 6.07-5.99 (br, 1H), 5.04 (s, 2H), 4.64-4.44 (br, 1H), 3.66-3.39 (m, 2H), 2.75-2.44 (m, 2H), 1.50 (s, 9H), 1.41-1.20 (m, 9H). ¹³C NMR (CDCl₃, 125 MHz): δ 158.9, 155.4, 139.4, 136.8, 129.6, 128.5, 127.9, 127.4, 121.4, 115.9, 112.2, 82.7, 81.8, 77.5, 69.9, 62.2, 34.6, 28.0, 27.9. HRMS (ESI): calcd. for C₂₆H₃₆N₂NaO₆ [M+Na]⁺ 495.2466; found 495.2462.

(S)-Di-*tert*-butyl-1-(3-(3-(benzyloxy)phenyl)-1-methoxy-1-oxopropan-2-yl)hydrazine-1,2-di carboxylate 9

To a solution of 3-(3-(benzyloxy)phenyl)propanal **6** (2 g, 8.3 mmol) in CH₃CN (60 mL), di-*tert*-butyl azodicarboxylate (1.28 g, 5.5 mmol) and (*R*)-proline (57 mg, 0.49 mmol, 10 mol%) were added at 0 °C and stirred at the same temperature for 12 h. After the disappearance of yellow color of the reaction mixture, reaction was quenched with 10% aqueous citric acid (20 mL) and extracted with ethyl acetate (2 x 30 mL). The organic layer was dried over Na₂SO₄ and concentrated in *vacuo* to give α -hydrazino aldehyde, which was taken for next step without purification. To the solution of α -hydrazino aldehyde (3.91 g, 8.3 mmol) in *tert*-butanol and water (1:1, 40 mL), were added NaClO₂ (1.12 g, 12.4 mmol) and NaH₂PO₄·2H₂O (2.59 g, 16.6 mmol) at 0 °C. After stirring at room temperature for 3 h, *tert*-butanol was evaporated. The residue was washed with brine (20 mL) and extracted with ethyl acetate (2 x 30 mL). The combined extract was dried over Na₂SO₄, evaporated to give the intermediate acid and the residue was taken for next step without purification. To the stirred solution of acid residue (4.04 g, 8.3 mmol) in ether, ethereal solution of diazomethane (2.57 g, 24.9 mmol) was added slowly at 0 °C. After 30 min, aqueous NH₄Cl solution (20 mL) was added and extracted with ethyl acetate (3 x 20 mL). The combined organic layer was dried over Na₂SO₄ and concentrated in *vacuo*. Residue was purified using silica gel column chromatography (10-15% ethyl acetate/hexanes) to give corresponding ester **9** (3.7 g, 90%, from compound **6**) as colorless oil. $[\alpha]_D^{20}$: - 20.8 (*c* = 1, CHCl₃). IR (neat): $\tilde{\nu}_{\max}$ = 2977, 2931, 1716, 1369, 1250, 1157, 758, 697 cm⁻¹. ¹H NMR (CDCl₃, 500 MHz): δ 7.45-7.41 (m, 2H), 7.40-7.35 (m, 2H), 7.34-7.30 (m, 1H), 7.23-7.18 (m, 1H), 7.03-6.81 (m, 3H), 5.07-5.02 (br s, 3H), 3.68 (s, 3H), 3.18-3.10 (br, 2H), 1.50-1.33 (m, 18H) ppm. ¹³C NMR (CDCl₃, 125 MHz) : δ 171.5, 158.8, 155.4, 155.1, 139.1,

137.1, 129.3, 128.4, 127.8, 127.4, 121.6, 115.4, 113.1, 82.0, 81.1, 77.2, 69.8, 52.2, 35.1, 28.0 ppm. HRMS (ESI): calcd for $C_{27}H_{36}N_2NaO_7$ $[M+Na]^+$ 523.2415; found 523.2438.

(S)-Di-*tert*-butyl-1-(3-(3-hydroxyphenyl)-1-methoxy-1-oxo-propan-2-yl)hydrazine-1,2-dicarboxylate 10

To a solution of compound **9** (1.5 g, 3 mmol) in MeOH was added 10% Pd/C at room temperature. The reaction mixture was stirred under hydrogen atmosphere for 8 h. The reaction mixture was diluted with $CHCl_3$ (20 mL), filtered through a small pad of celite and concentrated under reduced pressure. Residue was purified by silica gel column chromatography (30% ethyl acetate/hexane) to obtain debenzylated compound **10** (1.05 g, 86% yield) as a colorless oil. $[\alpha]^{20}_D$: -19.7 ($c = 1$, $CHCl_3$). IR (neat): $\tilde{\nu}_{max} = 3382, 2979, 1714, 1368, 1249, 1155, 756\text{ cm}^{-1}$. 1H NMR ($CDCl_3$, 500 MHz): δ 7.12 (t, $J = 7.7\text{ Hz}$, 1H), 6.98-6.82 (br, 1H), 6.77 (d, $J = 7.5\text{ Hz}$, 1H), 6.70 (d, $J = 7.7\text{ Hz}$, 1H), 6.58-6.47 (br, 1H), 5.22-4.72 (m, 1H), 3.68 (s, 3H), 3.20-3.00 (m, 2H), 1.58-1.20 (m, 18H) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ 171.7, 156.2, 155.2, 155.1, 138.9, 129.4, 120.7, 116.1, 113.7, 82.4, 81.5, 77.1, 52.2, 34.8, 28.0, 27.9 ppm. HRMS (ESI): calcd. for $C_{20}H_{30}N_2NaO_7$ $[M+Na]^+$ 433.1945; found 433.1969.

(S)-Methyl-2-(*tert*-butoxycarbonylamino)-3-(3-hydroxyphenyl)propanoate 11

To a solution of debenzylated ester **10** (1 g, 2.4 mmol) in dichloromethane (3 mL) was added trifluoroacetic acid (1.1 mL, 14.6 mmol) slowly at 0 °C. After 3 h, reaction mixture was concentrated and washed with toluene (to remove the traces of TFA) to give Boc deprotected hydrazine. To the residue (0.5 g, 2.3 mmol) in MeOH (5 mL), activated W-2 Raney-Nickel (30 mg, pre-washed with anhydrous MeOH) and acetic acid (3 drops) were added. The reaction mixture was degassed and saturated with hydrogen (60 psi) for two times. The suspension was

stirred at room temperature for 20 h, filtered through celite and the residue was washed thoroughly with MeOH (20 mL) and CH₂Cl₂ (10 mL). The combined filtrate was concentrated to afford the corresponding amine. To the stirred solution of the crude amine in MeOH (6.0 mL) were added (Boc)₂O (0.8 g, 3.5 mmol) and NaHCO₃ (until pH becomes basic). Reaction mixture was stirred at room temperature for 8 h. Solvent was evaporated under reduced pressure and the residue was purified by column chromatography (20% ethyl acetate/hexane) to furnish Boc-protected amine **11** as a white solid (0.48 g, 67% over three steps). M.P: 61-62 °C. $[\alpha]_D^{20}$: +37.1 ($c = 1.0$, CHCl₃). IR (KBr): $\tilde{\nu}_{\max} = 3376, 2980, 1744, 1684, 1593, 1515, 1368, 1163, 1059, 772, 698 \text{ cm}^{-1}$. ¹H NMR (CDCl₃, 500 MHz): δ 7.15 (t, $J = 7.7 \text{ Hz}$, 1H), 6.74-6.70 (dd, $J = 7.7, 1.9 \text{ Hz}$, 1H), 6.67 (d, $J = 7.7 \text{ Hz}$, 1H), 6.64-6.62 (m, 1H), 5.02 (d, $J = 7.7 \text{ Hz}$, 1H), 4.61-4.54 (m, 1H), 3.71 (s, 3H), 3.09-3.02 (dd, $J = 13.8, 5.6 \text{ Hz}$, 1H), 3.01-2.94 (dd, $J = 13.5, 6.7 \text{ Hz}$, 1H), 1.42 (s, 9H) ppm. ¹³C NMR (CDCl₃, 75 MHz): δ 172.5, 156.2, 155.4, 137.4, 129.6, 121.0, 116.1, 114.1, 80.3, 54.3, 52.2, 38.0, 28.2 ppm. HRMS (ESI): calcd. for C₁₅H₂₁NO₅Na [M+Na]⁺ 318.1311; found 318.1307.

(S)-Methyl-3-(3-(benzyloxy)phenyl)-2-(tert-butoxycarbonylamino)propanoate **3**^[14]

To a solution of compound **11** (0.45 g, 1.5 mmol) in acetone (10 mL) were added K₂CO₃ (0.320 g, 2.28 mmol) and benzyl bromide (0.23 mL, 1.83 mmol) and refluxed for 12 h. Reaction mixture was filtered, concentrated under *vacuo* and the residue was purified through silica gel column chromatography (20% ethyl acetate/hexane) to afford compound **3** as a solid (0.57 g, 98%). M.P: 72-73 °C. $[\alpha]_D^{20}$: + 30.4 ($c = 1$, CHCl₃). IR (neat): $\tilde{\nu}_{\max} = 3435, 3372, 2964, 2927, 1744, 1714, 1496, 1219, 1025, 772 \text{ cm}^{-1}$. ¹H NMR (CDCl₃, 500 MHz): δ 7.45-7.31 (m, 5H), 7.21 (t, $J = 7.7 \text{ Hz}$, 1H), 6.88-6.85 (dd, $J = 8.2, 2.1 \text{ Hz}$, 1H), 6.78-6.71 (m, 2H), 5.04 (s, 2H), 4.62-4.55 (m, 1H), 3.70 (s, 3H), 3.13-2.99 (m, 2H), 1.43 (s, 9H) ppm. ¹³C NMR (CDCl₃, 75 MHz): δ

172.2, 158.9, 155.0, 137.5, 136.9, 129.5, 128.5, 127.9, 127.4, 121.8, 115.9, 113.3, 79.9, 69.9, 54.3, 52.1, 38.3, 28.2 ppm. HRMS (ESI): calcd for C₂₂H₂₇O₅NNa [M+Na]⁺ 408.1781; found 408.1757.

(S)-Di-*tert*-butyl-1-(5-bromo-1-hydroxypentan-2-yl)hydrazine-1,2-dicarboxylate **12**^[15]

Di-*tert*-butyl azodicarboxylate (2.61 g, 11.3 mmol) and (*R*)-proline (116 mg, 1.01 mmol, 10 mol%) were added to 5-bromopentanal **7** (3 g, 16.9 mmol) in acetonitrile (80 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 24 h. After complete consumption of starting material, mixture was treated with ethanol (40 mL) and NaBH₄ (960 mg, 23.2 mmol) and was stirred for 30 min at 0 °C. The reaction was quenched with aqueous citric acid (30 mL) and extracted with ethyl acetate (3 x 20 mL). The combined organic layer was dried over Na₂SO₄ and concentrated in *vacuo*. Residue was purified by column chromatography on silica gel (30-40% ethyl acetate/hexane) to afford alcohol **12** as a white solid (3.45 g, 81% *ee*, 52% yield). M.P: 110-111 °C. [α]_D²⁰: + 9.1 (*c* = 1.1, CHCl₃). IR (KBr): $\tilde{\nu}_{\text{max}}$ = 3289, 2978, 2933, 1709, 1394, 1368, 1283, 1254, 1156, 1056, 763 cm⁻¹. ¹H NMR (CDCl₃, 500 MHz): δ 6.58-6.41 (br, 1H), 4.46-4.12 (m, 1H), 3.54-3.26 (m, 3H), 1.90-1.69 (m, 2H), 1.52-1.29 (m, 20H) ppm. ¹³C NMR (CDCl₃, 75 MHz): δ 155.7, 82.0, 81.3, 62.0, 57.3, 33.5, 28.9, 28.0, 27.9, 26.3 ppm. HRMS (ESI): calcd. for C₁₅H₂₉BrN₂NaO₅ 419.1152; found 419.1174. Enantiomeric excess was determined for its benzoyl derivative (**12a**) of compound (**12**) by chiral HPLC (CHIRALCEL-OD-H; 250 X 4.6 mm, 5U, Mobile phase: 5% IPA in hexane, Flow rate: 0.75 mL/min, minor *t*_R 7.467 min, major *t*_R 8.291 min.

(S)-Di-*tert*-butyl-1-(1-(benzoyloxy)-5-bromopentan-2-yl)hydrazine-1,2-dicarboxylate **12a**

To a solution of compound **12** (300 mg, 0.75 mmol) in CH₂Cl₂ (10 mL) were added

pyridine (0.15 mL, 1.89 mmol) and benzoyl chloride (0.1 mL, 0.98 mmol) at 0 °C and stirred at room temperature for 3 h. Reaction was quenched with saturated CuSO₄ (10 mL), extracted with CH₂Cl₂ (2 x 10 mL) and the residue was purified through column chromatography (10% ethyl acetate/hexane) to give benzoyl derivative **12a** as a liquid (367 mg, 97% yield). $[\alpha]^{20}_{\text{D}}: +0.83$ ($c = 0.8$, CHCl₃). IR (KBr): $\tilde{\nu}_{\text{max}} = 2926, 1716, 1220, 772, 712 \text{ cm}^{-1}$. ¹H NMR (CDCl₃, 500 MHz): δ 8.01 (d, $J = 7.4 \text{ Hz}$, 2H), 7.55 (t, $J = 7.4 \text{ Hz}$, 1H), 7.42 (t, $J = 7.4 \text{ Hz}$, 2H), 4.59 (br, 2H), 4.24 (br, 1H), 3.61-3.38 (m, 2H), 2.34-2.19 (m, 1H), 2.09-1.97 (m, 1H), 1.62 (br, 2H), 1.47 (s, 9H), 1.35-1.21 (m, 9H) ppm. ¹³C NMR (CDCl₃, 125 MHz): δ 171.1, 155.5, 155.2, 133.2, 129.7, 128.2, 81.2, 80.8, 67.9, 60.3, 29.6, 28.1, 27.8, 25.5, 20.9 ppm. HRMS (ESI): calcd. for C₂₂H₃₃BrN₂NaO₆ [M+Na]⁺ 523.1414; found 523.1432.

(S)-Di-*tert*-butyl-3-((*ter*-butyldimethylsilyloxy)methyl)piperazine-1,2-dicarboxylate 13

To a solution of alcohol **12** (3.3 g, 8.3 mmol) and 2,6-lutidine (2.96 mL, 24.9 mmol) in dichloromethane (35 mL) at 0 °C was added TBSOTf (2.28 mL, 9.9 mmol). The reaction mixture was stirred for 30 min at the same temperature and quenched with aqueous ammonium chloride (20 mL). The aqueous layer was extracted with dichloromethane (2 x 20 mL), and the combined organic layer was dried over sodium sulfate. After evaporation of the solvent under reduced pressure, the crude product was purified by silica gel column chromatography (5% ethyl acetate/hexane) to afford TBS protected compound (4.25 g, 98%) as colorless oil. To a solution of NaH (60%, 280 mg, 7.6 mmol) in dry DMF (10 mL), solution of TBS ether (2 g, 3.8 mmol) in DMF (25 mL) was added slowly at 0 °C. Reaction mixture was stirred at same temperature for 40 min, quenched with 10% citric acid (20 mL) and extracted with ethyl acetate (30 x 2 mL). The combined extract was dried over sodium sulfate and concentrated in *vacuo*. The residue was purified by silica gel column chromatography (10% ethyl acetate/hexane) to give cyclized

compound **13** (1.56 g, 95%) as a colorless oil. $[\alpha]_D^{20}$: - 24.5 (c = 1.0, CHCl_3). IR (KBr): $\tilde{\nu}_{\text{max}}$ = 2955, 2932, 1705, 1391, 1367, 1254, 1172, 1083, 838, 774 cm^{-1} . ^1H NMR (CDCl_3 , 300 MHz): δ 4.35-4.25 (br, 1H), 4.08- 4.02 (dt, J = 12.4, 4.8 Hz, 1H), 3.76-3.70 (dd, J = 9.7, 5.0 Hz, 1H), 3.54 (t, J = 9.7 Hz, 1H), 2.90-2.82 (m, 1H), 1.86-1.70 (m, 2H), 1.68-1.54 (m, 2H), 1.49-1.39 (m, 18H), 0.86 (s, 9H), 0.03 (s, 6H) ppm. ^{13}C NMR (CDCl_3 , 75 MHz): δ 154.3, 154.2, 80.4, 80.1, 60.4, 53.0, 43.2, 28.1, 25.7, 22.1, 18.7, 18.0, -5.5, -5.6 ppm. HRMS (ESI): calcd. for $\text{C}_{21}\text{H}_{43}\text{N}_2\text{O}_5\text{Si}$ $[\text{M}+\text{H}]^+$ 431.2936; found 431.2965.

(S)-Di-tert-butyl-3-(hydroxymethyl)piperazine-1,2-dicarboxylate **14**

To a stirred solution of compound **13** (2.5 g, 5.8 mmol) in THF (30 mL) was added 1M TBAF at 0 °C (11.6 mL, 11.6 mmol) and stirred at 0 °C for 2 h. The reaction was quenched with brine and extracted with ethyl acetate. The combined extracts were dried over sodium sulfate and concentrated in *vacuo*. The residue was purified by silica gel column chromatography (20% ethyl acetate/hexane) to give alcohol **14** in 96% (1.75 g) yield as colorless oil. $[\alpha]_D^{20}$: - 0.5 (c = 1.0, CHCl_3). IR (KBr): $\tilde{\nu}_{\text{max}}$ = 3470, 2977, 1703, 1397, 1368, 1165, 1073, 771 cm^{-1} . ^1H NMR (CDCl_3 , 300 MHz): δ 4.49-4.25 (br, 1H), 4.10- 3.88 (m, 1H), 3.75-3.53 (m, 1H), 3.50-3.38 (br, 1H), 3.14-2.83 (m, 1H), 2.53-2.36, 1.78-1.61 (m, 2H), 1.49-1.42 (m, 20 H) ppm. ^{13}C NMR (CDCl_3 , 75 MHz): δ 153.8, 81.5, 59.8, 53.4, 45.1, 28.0, 22.4, 19.2 ppm (rotamers are observed). HRMS (ESI): calcd. for $\text{C}_{15}\text{H}_{28}\text{N}_2\text{O}_5\text{Na}$ $[\text{M}+\text{Na}]^+$ 339.1890; found 339.1882.

(S)-1,2-Di-tert-butyl-3-methyl-piperazine-1,2,3-tricarboxylate **5**

To a stirred solution of alcohol **14** (1.3 g, 4.1 mmol) in $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (1:1, 20 mL), were added BAIB (2.64 g, 8.2 mmol) and TEMPO (130 mg, 0.8 mmol) at 0 °C. After stirring at room temperature for 4 h, the reaction mixture was quenched with saturated solution of hypo (50 mL)

and extracted with ethyl acetate (3 x 20 mL). The combined extracts were dried over Na₂SO₄ and concentrated in *vacuo* to give the intermediate acid. Crude diBoc protected piperazic acid was dissolved in ether (20 mL) and treated with ethereal solution of diazomethane (1.27 g, 12.3 mmol) at 0 °C. After 30 min, aqueous NH₄Cl (10 mL) was added and extracted with ethyl acetate (20 X 2 mL). The combined organic layer was dried over Na₂SO₄ and concentrated in *vacuo*. Purification of the residue by column chromatography (10% ethyl acetate/hexane) resulted the pure diBoc protected ester **5** (1.23 g, 87% yield over two steps). $[\alpha]^{20}_{\text{D}} = -34.4$ ($c = 1.0$, CHCl₃). IR (KBr): $\tilde{\nu}_{\text{max}} = 2977, 2932, 1731, 1704, 1395, 1367, 1167, 1086, 771 \text{ cm}^{-1}$. ¹H NMR (CDCl₃, 300 MHz): δ 5.01-4.96 (br, 1H), 4.16-4.07 (m, 1H), 3.72 (s, 3H), 2.87-2.77 (m, 1H), 2.11-2.04 (m, 1H), 1.96-1.80 (m, 1H), 1.79-1.64 (m, 2H), 1.50-1.42 (m, 18H) ppm. ¹³C NMR (CDCl₃, 75 MHz): δ 170.4, 154.5, 153.9, 81.6, 80.2, 54.4, 51.9, 42.6, 28.2, 28.1, 24.2, 20.0 ppm. HRMS (ESI): calcd. for C₁₆H₂₈N₂O₆Na [M+Na]⁺ 367.184; found 367.1863.

(S)-Methylpiperazine-3-carboxylate **15^[6n]**

To a solution of compound **5** (0.5 g, 1.45 mmol) in CH₂Cl₂ (5 mL) was added TFA (5 mL) slowly at 0 °C and allowed to stir at room temperature for 3 h. Solvent was evaporated and the residue was azeotroped with toluene (to remove acid traces) to afford compound **15** (0.182 g, 87%) as yellow oily compound which was used for next step without purification. $[\alpha]^{20}_{\text{D}} = +2.1$ ($c = 1$, CHCl₃). IR (KBr): $\tilde{\nu}_{\text{max}} = 3360, 2955, 2927, 1703, 1440, 1157, 771 \text{ cm}^{-1}$. ¹H NMR (CDCl₃, 500 MHz): δ 7.83-6.70 (br, 2H), 4.05-3.94 (br, 1H), 3.74 (s, 3H), 3.44-3.14 (m, 2H), 2.23-2.10 (br, 1H), 2.02-1.77 (m, 3H) ppm. ¹³C NMR (CDCl₃, 75 MHz): δ 171.2, 55.9, 52.6, 44.9, 24.8, 19.9 ppm. HRMS (ESI): calcd. for C₆H₁₃N₂O₂ [M+H]⁺ 145.0972; found 145.0982.

**(S)-Methyl-2-((S)-2-(tert-butoxycarbonylamino)-3-methylbutanamido)-hydroxyphenyl
propanoate 16**

Compound **3** (0.22 g, 0.57 mmol) was treated with TFA (0.08 mL, 1.14 mmol) in dichloromethane (1 mL) at 0 °C for 3 h under nitrogen atmosphere to give the TFA salt of *m*-tyrosine methyl ester, which was basified using DIPEA (0.29 mL, 1.71 mmol) at 0 °C in dry DMF (2 mL) and stirred for 10 min. To a stirred solution of *N*-Boc-(*S*)-valine **4** (0.108 g, 0.49 mmol) in dry DCM (3 mL), were added sequentially HOBt (0.073 g, 0.54 mmol) and EDC (0.190 g, 0.99 mmol) at 0 °C. After 15 min, for the same, was added basified solution of *m*-tyrosine amine (0.141 g, 0.49 mmol) at 0 °C and stirred for 12 h at room temperature. The reaction mixture was diluted with CH₂Cl₂ (15 mL), washed with saturated NH₄Cl solution (15 mL), aqueous NaHCO₃ (15mL) and brine (15 mL). The combined organic layer was dried over Na₂SO₄, concentrated in *vacuo* and the major diastereomer of the dipeptide **16** was separated by silica gel column chromatography (30% ethyl acetate/hexane) as a solid (0.213 g, 89% yield). M.P: 91-92 °C. $[\alpha]_D^{20}$: +28.9 (*c* = 1.08, CHCl₃). IR (neat): $\tilde{\nu}_{\max}$ = 3328, 2965, 1745, 1653, 1525, 1298, 1169, 1044, 696 cm⁻¹. ¹H NMR (CDCl₃, 500 MHz): δ 7.46-7.29 (m, 5H), 7.20 (t, *J* = 7.9 Hz, 1H), 6.89- 6.83 (dd, *J* = 8.1, 2.0 Hz, 1H), 6.79-6.68 (m, 2H), 6.34 (d, *J* = 6.9 Hz, 1H), 5.04 (s, 2H), 4.91- 4.83 (m, 1H), 3.95-3.86 (m, 1H), 3.69 (s, 3H), 3.09 (d, *J* = 5.6 Hz, 2H), 2.16-2.01 (m, 1H), 1.43 (s, 3H), 0.93 (d, *J* = 6.7 Hz, 3H), 0.87 (d, *J* = 6.7 Hz, 3H) ppm. ¹³C NMR (CDCl₃, 125 MHz): δ 171.6, 171.2, 158.9, 155.6, 137.2, 136.9, 129.6, 128.5, 127.8, 127.4, 121.7, 115.6, 113.6, 79.8, 69.8, 59.8, 52.9, 52.2, 38.0, 30.8, 28.2, 19.1, 17.6 ppm. HRMS (ESI): calcd. for C₂₇H₃₆N₂NaO₆ [M+Na]⁺ 507.2466; found 507.2452.

**(S)-3-(3-(Benzyloxy)phenyl)-2-(S)-2-(tert-butoxycarbonyl-amino)-3-methylbutanamido)
propanoic acid 17**

To a solution of compound **16** (0.150 g, 0.3 mmol) in THF: H₂O (7:3; 5 mL), LiOH.H₂O (40 mg, 0.95 mmol) was added at 0 °C. Reaction mixture was stirred at room temperature for 3 h, acidified with NaHSO₄ and extracted with ethyl acetate (2 x 20 mL). Organic layer was concentrated under *vacuo* to afford acid **17** (0.126 g, 87%) as yellow liquid. $[\alpha]^{20}_D$: + 41.0 (*c* = 0.8, CHCl₃). IR (KBr): $\tilde{\nu}_{\max}$ = 3321, 2974, 2929, 1720, 1662, 1517, 1257, 1162, 1022, 697 cm⁻¹. ¹H NMR (CDCl₃, 300 MHz): δ 7.43-7.29 (m, 5H), 7.15 (t, *J* = 8.6 Hz, 1H), 6.85- 6.71 (m, 3H), 5.19 (d, *J* = 8.6 Hz, 1H), 4.99 (s, 2H), 4.89-4.78 (m, 1H), 4.00-3.89 (m, 1H), 3.79-3.70 (m, 1H), 3.19-3.06 (dd, *J* = 13.7, 4.7 Hz, 1H), 3.03-2.92 (dd, *J* = 13.7, 6.4 Hz, 1H), 1.89-1.81 (m, 1H), 1.40 (s, 9H), 0.91-0.79 (m, 6H) ppm. ¹³C NMR (CDCl₃, 75 MHz): δ 173.4, 171.6, 158.8, 156.0, 137.4, 136.9, 129.5, 128.4, 127.8, 127.5, 122.0, 115.9, 113.4, 80.2, 69.8, 59.8, 53.0, 37.5, 30.8, 28.2, 19.1, 17.7 ppm. HRMS (ESI): calcd. for C₂₆H₃₄N₂NaO₆ [M+Na]⁺ 493.2309; found 493.2341.

Characterization data of other isomer of tripeptide 2

$[\alpha]^{20}_D$ = -54.1 (*c* = 0.4, MeOH). IR (KBr): $\tilde{\nu}_{\max}$ = 3429, 2960, 2927, 2253, 1655, 1220, 825, 767 cm⁻¹. ¹H NMR (CDCl₃, 700 MHz): δ 7.46-7.43 (m, 2H), 7.41-7.37 (m, 2H), 7.34-7.31 (m, 1H) 7.18 (t, *J* = 7.6 Hz, 1H), 6.86-6.82 (m, 2H), 6.78-6.74 (m, 1H), 6.61 (d, *J* = 7.7 Hz, 1H), 5.68-5.62 (m, 1H), 5.10-5.05 (m, 1H), 5.04 (s, 2H), 4.48-4.43 (m, 1H), 4.02-3.96 (m, 1H), 3.71 (s, 3H), 3.61-3.55 (m, 1H), 3.07 (d, *J* = 11.8 Hz, 1H), 3.00-2.95 (dd, *J* = 13.3, 7.1 Hz, 1H), 2.94-2.89 (m, 1H), 2.42-2.48 (m, 1H), 2.13-1.98 (m, 2H), 1.83-1.61 (m, 2H), 1.43 (s, 9H), 0.88 (d, *J* = 6.8 Hz, 3H), 0.79 (d, *J* = 6.8 Hz, 3H) ppm. ¹³C NMR (CDCl₃, 175 MHz): δ 172.2, 171.7, 170.8,

158.7, 155.6, 138.0, 137.0, 129.2, 128.5, 127.8, 127.5, 121.9, 115.7, 113.5, 79.6, 69.8, 59.4, 58.3, 52.1, 49.9, 41.8, 39.4, 31.2, 28.2, 22.8, 19.1, 17.2 ppm. HRMS (ESI): calcd. for $C_{32}H_{45}N_4O_7$ $[M+H]^+$ 597.3283; found 597.3296.

COPIES OF NMR SPECTRA

^1H and ^{13}C NMR Spectra of compound **8a**

^1H and ^{13}C NMR Spectra of compound **9**

¹H and ¹³C NMR Spectra of compound **10**

^1H and ^{13}C NMR Spectra of compound **11a**

¹H and ¹³C NMR Spectra of compound **3**

^1H and ^{13}C NMR Spectra of compound **12**

¹H and ¹³C NMR Spectra of compound **12a**

^1H and ^{13}C NMR Spectra of compound **13**

¹H and ¹³C NMR Spectra of compound **14**

^1H and ^{13}C NMR Spectra of compound **5**

^1H and ^{13}C NMR Spectra of compound **15**

¹H and ¹³C NMR Spectra of compound **16**

^1H and ^{13}C NMR Spectra of compound **17**

^1H NMR Spectrum of compound **2** in DMSO

^{13}C NMR Spectrum of compound **2** in DMSO

^1H and ^{13}C NMR Spectra of Compound **2** in CDCl₃

^1H NMR Spectrum of other isomer of tripeptide 2 in CDCl_3

^{13}C NMR Spectrum of other isomer of tripeptide **2** in CDCl_3

COPIES OF HPLC CHROMATOGRAMS

PeakTable

Peak#	Ret. Time	Area	Height	Area %
1	6.767	20233220	1035191	55.669
2	18.380	16112486	170719	44.331
Total		36345706	1205910	100.000

PeakTable

Peak#	Ret. Time	Area	Height	Area %
1	6.696	32873172	1481201	96.575
2	17.432	1165765	12348	3.425
Total		34038937	1493548	100.000

HPLC report of compound 8a

PeakTable

Peak#	Ret. Time	Area	Height	Area %
1	7.591	3847648	251075	52.725
2	8.566	3449881	187068	47.275
Total		7297530	438143	100.000

PeakTable

Peak#	Ret. Time	Area	Height	Area %
1	7.467	1438320	101274	9.605
2	8.291	13536344	805858	90.395
Total		14974664	907131	100.000

HPLC report of compound **12a**

=====

Acq. Operator : B.RAMESH
 Acq. Instrument : Instrument 1 Location : Vial 31
 Injection Date : 10/17/2013 10:38:32 AM Inj Volume : 20 µl

Acq. Method : C:\Chem32\1\METHODS\lcm.m
 Last changed : 10/17/2013 10:36:25 AM by B.RAMESH
 Analysis Method : C:\Chem32\1\METHODS\onlymass.m
 Last changed : 10/17/2013 12:40:20 PM by B.RAMESH
 Method Info : COLUMN: ATLANTIS dC18, 150X4.6, 5U
 MOBILE PHASE: 60% ACN IN H2O(0.1% FA)
 DETECTION: 210NM
 FLOW RATE: 1.0ML/MIN

Sample Info : ATLANTIS dC18, 60% ACN IN WATER(0.1% FA)

=====
 Area Percent Report
 =====

Sorted By : Signal
 Multiplier : 1.0000
 Dilution : 1.0000
 Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=210,8 Ref=360,5

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.233	MM	0.2638	920.04071	58.13396	80.7835
2	9.714	MM	0.2624	218.85667	13.90254	19.2165

Totals : 1138.89738 72.03649

Display Report - Selected Window Selected

Analysis Name: 151013000025.d

Instrument: LC-MSD-Trap-SL

Print Date: 10/17/2 12:47:25

Method: lsm.m

Operator: ramesh

Acq. Date: 10/17/2011

Sample Name: RADHIKA

10:38:40 AM

Analysis Info: ATLANTIS dc18, 60% ACN IN WATER(0.1% FA)

Agilent Technologies

COPIES OF HRMS REPORTS

HRMS of Compound **8a**

Sample Name	Radhika-500	Position	Vial 11	Instrument Name	Instrument 1	User Name	
Inj Vol	1	InjPosition		SampleType	Sample	IRM Calibration Status	Success
Data Filename	19082013 Radhika-500	ACQ Method		Comment		Acquired Time	8/19/2013 4:53:46 PM

HRMS of Compound 9

Sample Name Radhika-410
Inj Vol 1
Data Filename 19082013 Radhika-410

Position Vial 11
InjPosition
ACQ Method

Instrument Name Instrument 1
SampleType Sample
Comment

User Name
IRM Calibration Status
Acquired Time

Success
8/19/2013 5:12:43 PM

HRMS of Compound 10

SCS-LR-M-TYR-295 #6-35 RT: 0.02-0.12 AV: 30 NL: 1.29E7
T: FTMS (1,1) + p ESI Full ms [100.00-2000.00]

HRMS of Compound **11a**

Sample Name	RAD-BEZ-500	Position	Vial 11	Instrument Name	Instrument 1	User Name	
Inj Vol	10	InjPosition		SampleType	Sample	IRM Calibration Status	Success
Data Filename	30092013 RAD-BEZ-500	ACQ Method		Comment		Acquired Time	9/30/2013 4:53:40 PM

HRMS of Compound **12a**

Sample Name	SCS-RADIKA-430	Position	Vial 1	Instrument Name	Instrument 1	User Name	
Inj Vol	1	InjPosition		SampleType	Sample	IRM Calibration Status	Success
Data Filename	22 112012 SCS-RADIKA	ACQ Method		Comment		Acquired Time	11/22/2012 11:55:33 AM

HRMS of Compound **13**

Sample Name	RADHIKA-484	Position	Vial 11	Instrument Name	Instrument 1	User Name	
Inj Vol	1	InjPosition		SampleType	Sample	IRM Calibration Status	Success
Data Filename	21082013 RADHIKA-484	ACQ Method		Comment		Acquired Time	8/21/2013 4:38:12 PM

HRMS of Compound 14

Sample Name	RADHIKA-484	Position	Vial 11	Instrument Name	Instrument 1	User Name	
Inj Vol	1	InjPosition		SampleType	Sample	IRM Calibration Status	Success
Data Filename	21082013 RADHIKA-484	ACQ Method		Comment		Acquired Time	8/21/2013 4:38:12 PM

HRMS of Compound **16**

Sample Name	RADHIKA-ACID-470	Position	Vial 11	Instrument Name	Instrument 1	User Name	
Inj Vol	10	InjPosition		SampleType	Sample	IRM Calibration Status	Success
Data Filename	30092013 RADHIKA-ACI	ACQ Method		Comment		Acquired Time	9/30/2013 5:23:28 PM

HRMS of Compound **17**

SCS-LR-MIMER-596 #3-34 RT: 0.02-0.13 AV: 32 NL: 1.37E7
T: FTMS {1,1} + p ESI Full ms [100.00-2000.00]

HRMS of Compound 2 (Tripeptide major)

Sample Name	radhika-596	Position	Vial 1	Instrument Name	Instrument 1	User Name	
Inj Vol	1	InjPosition		SampleType	Sample	IRM Calibration Status	Success
Data Filename	16122013 radhika-596	ACQ Method		Comment		Acquired Time	12/16/2013 3:08:33 PM

HRMS of other isomer of tripeptide **2**