Potent Integrin Antagonists from a Small Library of RGD-Including Cyclic Pseudopeptides

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Supporting Information

Experimental Section. Synthesis.

Reagents and solvents: SASRIN resin (200-400 mesh, 1.02 mmol/g) was purchased from Bachem. All the solvents used for the solid-phase synthesis were of HPLC quality or Analytical Reagent grade and were dried over molecular sieves before use. Flash chromatography: silica gel (Kieselgel 60, 230-400 mesh). TLC: silica plates (60 F_{254}, 0.25 mm, Merck). NMR: Bruker AC-200, AC-300 and Avance-400 (200 MHz, 300 MHz and 400 MHz for ^{1}H, 50.3 MHz, 75.4 MHz and 100.5 MHz for ^{13}C). Optical rotations: Perkin Elmer 241 polarimeter. Mass spectrometry: VG 7070 EQ-HF and PE-SCIEX API-100. Elemental analysis: Perkin Elmer 240. All solid-phase reaction were carried out on a wrist shaker.

Abbreviations: DCM: dichloromethane, DIC: N,N'-diisopropylcarbodiimide, HOAt: 1-hydroxy-7-azabenzotriazole, HOBt: 1-hydroxybenzotriazole, HATU: O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium exafluorophosphate, TNBS: 2,4,6-trinitrobenzenesulfonic acid.

TNBS test was performed following this procedure: a few resin beads were sampled and washed several times with ethanol. The sample was then placed in a vial and 1 drop of a 10 % solution of DIPEA in DMF and 1 drop of 1 % 2,4,6-trinitrobenzenesulfonic acid (TNBS) in DMF were added. The sample was then observed and colour changes were noted. The TNBS test is considered to be positive (presence of free amino groups) when the resin beads turn orange or red within 1 min and negative (no free amino groups) when the beads remain colourless.

General Procedure 1. Synthesis of *N*-Fmoc-Temp-OH (1-8). To a solution of the starting *N*-Boc-Temp-OtBu (0.62 mmol) in dichloromethane (4.8 ml) was added, under N_2, trifluoroacetic acid (4.8 ml) and the resulting mixture was stirred at room temperature for 1 h. The solvents were then evaporated under reduced pressure, the crude residue was dissolved in THF (0.32 ml) and 10 % Na_2CO_3 (0.77 ml) was added. After 15 min the solution was cooled to 0 °C, a solution of Fmoc-ONSu (95 mg) in THF (1.4 ml) was added and the resulting mixture was stirred at room temperature for 3 h (TLC $CHCl_3$/MeOH/AcOH 75:25:5). THF was then evaporated under reduced pressure, the aqueous phase was washed with AcOEt, conc. HCl was added to pH 3-4 and the solution extracted with AcOEt (3 x 5 ml). The combined organic layers were dried with Na_2SO_4 and evaporated under reduced pressure to afford the crude product as a white foam, which was used without further purification.

(3S,6S,9S)-1-aza-9-carboxy-3-(9'-fluorenylmetoxycarbonylamino)-2-oxo-bicyclo[4.3.0]nonane (1). Was prepared in quantitative overall yield following general procedure 1. ^{1}H NMR (200 MHz, C_6D_6, 323 °K) δ = 1.1-2.0 (m, 8 H, 4 C*H*$_2$), 2.9 (m, 1 H, C*H*-N), 4.12 (dd, $J_1 = J_2$ = 6.5 Hz, 1 H, C*H*-CH_2O), 4.20-4.50 (m, 4 H, C*H*-NHFmoc, C*H*CO_2H, C*H*$_2O$), 6.30 (d, J = 7 Hz, 1 H, N*H*), 7.10-7.30 (m, 4 H, aromatic), 7.45-7.65 (m, 4 H, aromatic), 11.2 (bs, 1 H, CO_2*H*). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ = 26.5, 26.9, 28.8, 31.9, 47.0, 50.2, 56.9, 58.5, 67.1, 119.8, 125.2, 127.1, 127.6, 141.2, 143.7, 143.9, 156.6, 170.3, 173.2, 174.0. MS (FAB$^+$): 421 (M^++1).

(3R,6S,9S)-1-aza-9-carboxy-3-(9'-fluorenylmetoxycarbonylamino)-2-oxo-bicyclo[4.3.0]nonane (2). Was prepared in quantitative overall yield following general procedure 1. ^{1}H NMR (200 MHz, C_6D_6, 323 °K) δ = 1.1-2.0 (m, 8 H, 4 C*H*$_2$), 3.0 (m, 1 H, C*H*-N), 3.9 (m, 1 H, C*H*-NHFmoc), 4.12 (dd, $J_1 = J_2$ = 6.5 Hz, 1 H, C*H*-CH_2O), 4.25-4.55 (m, 3 H, C*H*CO_2H, C*H*$_2O$), 6.02 (bs, 1 H, N*H*), 7.10-7.25 (m, 4 H, aromatic), 7.45-7.60 (m, 4 H, aromatic), 7.70 (bs, 1 H, CO_2*H*). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ = 27.7, 27.8, 28.1, 31.3, 47.0, 51.8, 58.6, 60.5, 67.0, 119.8, 125.1, 127.0, 127.6, 141.1, 143.8, 156.5, 170.0, 173.2, 174.3. MS (FAB$^+$): 421 (M^++1).

(3S,6R,9S)-1-aza-9-carboxy-3-(9'-fluorenylmetoxycarbonylamino)-2-oxo-bicyclo[4.3.0]nonane (3). Was prepared in quantitative overall yield following general procedure 1. ^{1}H NMR (200 MHz, C_6D_6, 323 °K) δ = 1.1-2.0 (m, 8 H, 4 C*H*$_2$), 3.0-3.2 (m, 1 H, C*H*-N), 4.20 (dd, $J_1 = J_2$ = 7 Hz, 1 H, C*H*-CH_2O), 4.25-4.50 (m, 4 H, C*H*-NHFmoc, C*H*CO_2H, C*H*$_2O$), 6.43 (d, J = 7 Hz, 1 H, N*H*), 7.10-7.30 (m, 4 H, aromatic), 7.40-7.80 (m, 4 H, aromatic), 10.80 (bs, 1 H, CO_2*H*). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ = 27.3, 27.4, 28.0, 32.5, 47.1, 51.9, 58.9, 60.2, 67.1, 119.8, 125.2, 127.0, 127.6, 141.2, 143.8, 143.9, 156.8, 169.5, 173.8. MS (FAB$^+$): 421 (M^++1).

(3R,6R,9S)-1-aza-9-carboxy-3-(9'-fluorenylmetoxycarbonylamino)-2-oxo-bicyclo[4.3.0]nonane (4). Was prepared in quantitative overall yield following general procedure 1. ^{1}H NMR (200 MHz,

C_6D_6, 323 °K) δ = 0.8-1.9 (m, 8 H, 4 C*H*$_2$), 3.15 (m, 1 H, C*H*-N), 4.10 (dd, $J_1 = J_2 = 6$ Hz, 1 H, C*H*-CH_2O), 4.30-4.60 (m, 4 H, C*H*-NHFmoc, C*H*CO_2H, C*H*$_2$O), 6.20 (bs, 1 H, N*H*), 7.10-7.30 (m, 4 H, aromatic), 7.50-7.60 (m, 4 H, aromatic), 9.80 (bs, 1 H, CO_2*H*). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ = 25.3, 26.6, 27.5, 32.1, 46.9, 49.9, 57.7, 58.8, 67.2, 119.8, 125.1, 127.0, 127.6, 141.1, 143.7, 143.8, 156.6, 173.1, 174.2. MS (FAB$^+$): 421 (M^++1).

(3S, 7S, 10S)-1-aza-10-carboxy-3-(9'-fluorenylmetoxycarbonylamino)-2-oxo-bicyclo[5.3.0] decane (5). Was prepared in quantitative overall yield following general procedure 1. ^{1}H NMR (200 MHz, $CDCl_3$) δ = 1.2-2.4 (m, 10 H, 5 C*H*$_2$), 2.72 (s, 1 H, C*H*-CH_2O), 3.90 (m, 1 H, C*H*-N), 4.25 (m, 1 H, C*H*-CO_2H), 4.4 (m, 2 H, C*H*$_2$O), 4.75 (m, 1 H, C*H*-NHFmoc), 6.35 (d, J = 5 Hz, 1 H, N*H*), 7.3-7.9 (m, 8 H, aromatic), 9.6 (s, CO_2*H*). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ = 25.36, 27.15, 27.39, 31.34, 32.97, 34.00, 47.20, 54.75, 59.39, 60.67, 67.08, 119.84, 125.07, 127.02, 127.59, 141.23, 143.84, 143.92, 155.78, 172.04, 174.67. MS (FAB$^+$): 435 (M^++1).

(3R, 7S, 10S)-1-aza-10-carboxy-3-(9'-fluorenylmetoxycarbonylamino)-2-oxo-bicyclo[5.3.0] decane (6). Was prepared in quantitative overall yield following general procedure 1. ^{1}H NMR (200 MHz, $CDCl_3$) δ = 1.6-2.3 (m, 10 H, 5 C*H*$_2$), 2.65 (s, 1 H, C*H*-CH_2O), 3.98 (m, 1 H, C*H*-N), 4.22 (m, 1 H, C*H*-CO_2H), 4.5 (m, 3 H, C*H*-NHFmoc, C*H*$_2$O), 6.3 (bs, 1 H, N*H*), 7.28-7.85 (m, 8 H, aromatic), 9.6 (bs, 1 H, CO_2*H*). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ = 22.02, 25.25, 26.63, 32.96, 46.92, 58.54, 60.60, 61.76, 68.74, 119.91, 124.77, 124.82, 127.00, 127.71. MS (FAB$^+$): 435 (M^++1).

(3S, 7R, 10S)-1-aza-10-carboxy-3-(9'-fluorenylmetoxycarbonylamino)-2-oxo-bicyclo[5.3.0] decane (7). Was prepared in quantitative overall yield following general procedure 1. ^{1}H NMR (200 MHz, $CDCl_3$) δ = 1.70-2.35 (m, 10 H, 5 C*H*$_2$), 2.72 (m, 1 H, C*H*-CH_2O), 3.85 (m, 1 H, C*H*-N), 4.26 (m, 1 H, C*H*-NHFmoc), 4.40 (m, 2 H, C*H*$_2$O), 4.70 (m, 1 H, C*H*-CO_2H), 6.05 (bs, 1 H, N*H*), 7.10-7.80 (8 H, aromatic). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ = 24.9, 26.4, 29.8, 33.1, 34.5, 47.3, 55.0, 59.1, 61.2, 67.6, 119.6, 119.9, 123.6, 125.4, 127.0, 127.5, 128.0, 131.4. MS (FAB$^+$): 435 (M^++1).

(3R, 7R, 10S)-1-aza-10-carboxy-3-(9'-fluorenylmetoxycarbonylamino)-2-oxo-bicyclo[5.3.0] decane (8). Was prepared in quantitative overall yield following general procedure 1. ^{1}H NMR (200 MHz, $CDCl_3$) δ = 1.70-2.35 (m, 10 H, 5 C*H*$_2$), 2.70 (m, 1 H, C*H*-CH_2O), 4.05 (m, 1 H, C*H*-N), 4.25 (m, 1 H, C*H*-NHFmoc), 4.40 (m, 2 H, C*H*$_2$O), 4.65 (m, 1 H, C*H*-CO_2H), 6.15 (bs, 1 H, N*H*), 7.10-7.80 (8 H, aromatic). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ = 25.3, 26.9, 29.6, 32.1, 34.0, 47.0, 54.7, 59.4, 60.4, 67.1, 119.8, 119.9, 124.6, 125.1, 127.0, 127.5, 127.6, 131.1. MS (FAB$^+$): 435 (M^++1).

General Procedure 2. Synthesis of *N*-Fmoc-Gly-O-SASRIN Resin. In a solid phase reaction vessel SASRIN resin (500 mg, 0.51 mmol) was suspended in a solution of *N*-Fmoc-Gly-OH (455 mg, 1.53 mmol), HOBt (206 mg, 1.53 mmol), DIC (0.24 ml, 1.53 mmol) and DMAP (19 mg, 0.15 mmol) in DMF (10 ml) for 15 h. The solution was drained and the resin was washed with DMF (3 x 10 ml) and DCM (3 x 10 ml). The possibly unreacted hydroxy groups present were capped by treatment with a solution of acetic anhydride (0.096 ml, 1 mmol) and DMAP (57 mg, 0.51 mmol) in DMF (12 ml) for 2 h. The solution was drained and the resin washed with DMF (3 x 10 ml) and DCM (3 x 10 ml).

General Procedure 3. Synthesis of *N*-Fmoc-Arg(Pmc)-Gly-O-SASRIN Resin. In a solid phase reaction vessel *N*-Fmoc-Gly-O-SASRIN resin (0.51 mmol) was treated with a 20 % piperidine/DMF solution (10 ml, 1 x 3 min, 2 x 17 min). The solution was drained and the resin was washed with DMF (3 x 10 ml), MeOH (2 x 10 ml) and DCM (3 x 10 ml). The deprotection was assessed by performing a TNBS test. *N*-Fmoc-Arg(Pmc)-OH (1.014 g, 1.53 mmol) and HOAt (208 mg, 1.53 mmol) were dissolved in DCM/DMF 2:1 (10 ml). At 0 °C, DIC (0.24 ml, 1.53 mmol) was added dropwise to this solution. The resulting mixture was stirred for 10 min at this temperature and for a further 10 min at room temperature, then added to the resin. This mixture was shaken at room temperature for 2.5 h. The solution was drained and the resin washed with DMF (3 x 10 ml) and DCM (3 x 10 ml). The success of the coupling was assessed by performing a TNBS test. The unreacted amino groups possibly present were capped by treatment with a solution of acetylimidazole (560 mg, 5.1 mmol) in DCM (12 ml) for 2 h. The solution was drained and the resin washed with DCM (3 x 10 ml).

General Procedure 4. Synthesis of *N*-Fmoc-Temp-Arg(Pmc)-Gly-O-SASRIN Resin. In a solid phase reaction vessel *N*-Fmoc-Arg(Pmc)-Gly-O-SASRIN resin (0.51 mmol) was treated with a 20 % piperidine/DMF solution (10 ml, 1 x 3 min, 2 x 17 min). The solution was drained and the resin was washed with DMF (3 x 10 ml), MeOH (2 x 10 ml) and DCM (3 x 10 ml). The deprotection was assessed by performing a TNBS test. The resin was suspended in a solution of *N*-Fmoc-Temp-OH (0.54 mmol), HATU (387 mg, 1.02 mmol), HOAt (139 mg, 1.02 mmol) and 2,4,6-collidine (0.135 ml, 1.02 mmol) in DMF/DCM 3:1 (13 ml) for 15 h. The solution was drained and the resin was washed with DMF (3 x 10 ml), MeOH (2 x 10 ml) and DCM (3 x 10 ml). The success of the coupling was assessed by performing a TNBS test. The unreacted amino groups possibly present were capped by treatment with a solution of acetylimidazole (560 mg, 5.1 mmol) in DCM (12 ml) for 2 h. The solution was drained and the resin was washed with DCM (3 x 10 ml).

General Procedure 5. Synthesis of *N*-Fmoc-Asp(tBu)-Temp-Arg(Pmc)-Gly-O-SASRIN Resin. In a solid phase reaction vessel *N*-Fmoc-Temp-Arg(Pmc)-Gly-O-SASRIN resin (0.51 mmol) was treated with a 20 % piperidine/DMF solution (10 ml, 1 x 3 min, 2 x 17 min). The solution was drained and the resin was washed with DMF (3 x 10 ml), MeOH (2 x 10 ml) and DCM (3 x 10 ml). The deprotection was assessed by performing a TNBS test. The resin was suspended in a solution of *N*-Fmoc-Asp(tBu)-OH (840 mg, 2.04 mmol), HATU (776 mg, 2.04mmol), HOAt (278 mg, 2.04 mmol) and 2,4,6-collidine (0.27 ml, 2.04 mmol) in DMF/DCM 3:1 (13 ml) for 15 h. The solution was drained and the resin was washed with DMF (3 x 10 ml), MeOH (2 x 10 ml) and DCM (3 x 10 ml). The success of the coupling was assessed by performing a TNBS test. The unreacted amino groups possibly present were capped by treatment with a solution of acetylimidazole (560 mg, 5.1 mmol) in DCM (12 ml) for 2 h. The solution was drained and the resin was washed with DCM (3 x 10 ml).

General Procedure 6. Cleavage of H_2N-Asp(tBu)-Temp-Arg(Pmc)-Gly-OH (9-16) from the Resin. In a solid phase reaction vessel *N*-Fmoc-Asp(tBu)-Temp-Arg(Pmc)-Gly-O-SASRIN resin (739 mg) was treated with a 20 % piperidine/DMF solution (10 ml, 1 x 3 min, 2 x 17 min). The solution was drained and the resin was washed with DMF (3 x 10 ml), MeOH (2 x 10 ml) and DCM (3 x 10 ml). The deprotection was assessed by performing a TNBS test. The resin was treated with 1 % TFA/DCM solution (7.4 ml, x 3 min). The filtrates were immediately neutralized with a 18 % pyridine/MeOH solution (0.89 ml). The fractions containing the product (TLC DCM/MeOH 8:2) were combined and concentrated under reduced pressure to yield a residue, which was purified from the pyridinium salts by size-exclusion chromatography (AMBERLITE XAD-2 resin, H_2O then MeOH). Evaporation of the combined MeOH fractions containing the product afforded a yellow residue which was used in the successive reaction without further purification.

H_2N-Asp(tBu)-Temp1-Arg(Pmc)-Gly-OH (9). Was prepared from the corresponding template in 40 % overall yield following general procedures 2-6. MS (FAB^+): 849 (M^++1).

H_2N-Asp(tBu)-Temp2-Arg(Pmc)-Gly-OH (10). Was prepared from the corresponding template in 40 % overall yield following general procedures 2-6. MS (FAB^+): 849 (M^++1).

H_2N-Asp(tBu)-Temp3-Arg(Pmc)-Gly-OH (11). Was prepared from the corresponding template in 55 % overall yield following general procedures 2-6. MS (FAB^+): 849 (M^++1).

H_2N-Asp(tBu)-Temp4-Arg(Pmc)-Gly-OH (12). Was prepared from the corresponding template in 30 % overall yield following general procedures 2-6. MS (FAB^+): 849 (M^++1).

H_2N-Asp(tBu)-Temp5-Arg(Pmc)-Gly-OH (13). Was prepared from the corresponding template in 67 % overall yield following general procedures 2-6. MS (FAB^+): 863 (M^++1).

H_2N-Asp(tBu)-Temp6-Arg(Pmc)-Gly-OH (14). Was prepared from the corresponding template in 54 % overall yield following general procedures 2-6. MS (FAB^+): 863 (M^++1).

H_2N-Asp(tBu)-Temp7-Arg(Pmc)-Gly-OH (15). Was prepared from the corresponding template in 63 % overall yield following general procedures 2-6. MS (FAB^+): 863 (M^++1).

H_2N-Asp(tBu)-Temp8-Arg(Pmc)-Gly-OH (16). Was prepared from the corresponding template in 66 % overall yield following general procedures 2-6. MS (FAB^+): 863 (M^++1).

General Procedure 7. Synthesis of Cyclo[-Temp-Arg(Pmc)-Gly-Asp(tBu)-] (17-23). The linear peptide (0.18 mmol) was dissolved in DMF (45 ml) under N_2. HATU (205 mg, 0.54 mmol), HOAt (73 mg, 0.54 mmol) and 2,4,6-collidine (0.072 ml, 0.54 mmol) were added and the resulting mixture was stirred for 24 h at room temperature. The solvent was evaporated under reduced pressure and the residue was dissolved in AcOEt. The organic phase was washed twice with 5 % $NaHCO_3$, dried with Na_2SO_4 and evaporated under reduced pressure. The crude residue was purified by flash chromatography on silica gel (DCM/MeOH from 95:5 to 9:1) to afford side-chain protected cyclopeptide as a yellow foam.

Cyclo[-Temp1-Arg(Pmc)-Gly-Asp(tBu)-] (17). Was prepared in 70 % yield following general procedure 7. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 1.25, 1.3 [2 s, 6 H, $(CH_3)_2$C-O], 1.46 [s, 9 H, $(CH_3)_3$CO], 1.5 (m, 2 H, H-C_γ Arg), 1.6 (m, 2 H, H-C_4 Temp), 1.8 (m, 4 H, C$H_2$$CH_2$Ar, H-$C_5$ Temp), 2.0 (m, 2 H, H-C_β Arg), 2.1 (s, 3 H, CH_3Ar), 2.2 (m, 4 H, H-C_7 Temp, H-C_8 Temp), 2.52, 2.56 (2 s, 6 H, CH_3Ar), 2.6 (m, 3 H, CH_2CH_2Ar, H-C_β Asp), 3.1 (dd, *J* = 5, 17.6, 1 H, H-C_β Asp), 3.25 (m, 2 H, H-C_δ Arg), 3.55 (m, 1 H, H-C_6 Temp), 3.65 (dd, *J* = 6, 13.6, 1 H, H-C_α Gly), 3.85 (dd, *J* = 4, 13.6, 1 H, H-C_α Gly), 4.22 (dd, *J* = 0, 9, 1 H, H-C_9 Temp), 4.45 (m, 1 H, H-C_3 Temp), 4.54 (m, 1 H, H-C_α Arg), 4.68 (m, 1 H, H-C_α Asp), 6.3 (s, 3 H, H-N_ε Arg, $HNSO_2$, =NH), 6.8 (d, *J* = 6, 1 H, NH Temp), 7.61 (d, *J* = 9, 1 H, NH Asp), 7.8 (d, *J* = 8, 1 H, NH Arg), 8.9 (bs, 1 H, NH Gly). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ = 12.1, 17.4, 18.5, 21.4, 25.2, 26.2, 26.8, 27.5, 28.0, 29.6, 31.4, 32.2, 32.9, 36.4, 40.2, 46.0, 47.7, 50.3, 51.9, 60.6, 62.1, 73.5, 81.8, 117.8, 123.8, 134.8, 134.9, 135.5, 153.3, 156.5, 168.0, 169.8, 170.2, 170.9, 173.6, 175.0. $[\alpha]_D^{20}$ = -36.1 (*c* = 1.0, $CHCl_3$). MS (FAB^+): 830 (M^+), 853 (M^++Na).

Cyclo[-Temp3-Arg(Pmc)-Gly-Asp(tBu)-] (18). Was prepared in 60 % yield following general procedure 7. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.3 [2 s, 6 H, $(CH_3)_2$C-O], 1.45 [s, 9 H, $(CH_3)_3$CO],

1.5 (m, 4 H, H-C_γ Arg, H-C_7 Temp), 1.7 (m, 1 H, H-C_β Arg), 1.8 (m, 3 H, C*H*$_2$CH$_2$Ar, H-C_8 Temp), 1.9 (m, 1 H, H-C_4 Temp), 2.0 (m, 1 H, H-C_β Arg), 2.05 (s, 3 H, CH_3Ar), 2.2 (m, 3 H, H-C_4 Temp, H-C_5 Temp), 2.50 (m, 2 H, H-C_β Asp, H-C_8 Temp), 2.52, 2.56 (2 s, 6 H, CH_3Ar), 2.6 (m, 2 H, CH$_2$C*H*$_2$Ar), 2.80 (dd, *J* = 8, 16, 1 H, H-C_β Asp), 3.25 (m, 2 H, H-C_δ Arg), 3.45 (dd, *J* = 5, 16, 1 H, H-C_α Gly), 3.80 (m, 1 H, H-C_6 Temp), 4.10 (m, 1 H, H-C_3 Temp), 4.15 (m, 1 H, H-C_α Gly), 4.35 (dd, *J* = 10, 10, 1 H, H-C_9 Temp), 4.5 (m, 1 H, H-C_α Arg), 4.70 (m, 1 H, H-C_α Asp), 6.22 (bs, 3 H, H-N_ε Arg, $HNSO_2$, =NH), 7.1 (d, *J* = 8, 1 H, NH Arg), 7.20 (d, *J* = 8, 1 H, NH Asp), 7.70 (d, *J* = 8, 1 H, NH Temp), 8.1 (bs, 1 H, NH Gly). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ = 12.0, 17.4, 19.4, 21.3, 25.3, 26.7, 27.4, 27.9, 28.6, 29.6, 32.8, 36.7, 40.4, 45.1, 50.2, 50.7, 51.9, 60.7, 61.6, 73.5, 81.6, 117.8, 123.8, 133.7, 134.7, 135.3, 153.3, 156.3, 168.7, 169.8, 170.7, 171.4, 173.3. $[\alpha]_D^{20}$ = -57.1 (*c* = 1.0, $CHCl_3$). MS (FAB^+): 832 (M^++2).

Cyclo[-Temp4-Arg(Pmc)-Gly-Asp(tBu)-] (19). Was prepared in 40 % yield following general procedure 7. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.3 [2 s, 6 H, $(CH_3)_2$C-O], 1.4 (m, 1 H, H-C_5 Temp), 1.45 [s, 9 H, $(CH_3)_3$CO], 1.5-1.6 (m, 4 H, H-C_γ Arg, H-C_4 Temp, H-C_8 Temp), 1.8 (m, 2 H, C*H*$_2$CH$_2$Ar), 1.97 (m, 1 H, H-C_8 Temp), 2.0 (m, 4 H, H-C_β Arg, H-C_4 Temp, H-C_5 Temp), 2.15 (s, 3 H, CH_3Ar), 2.17, 2.43 (m, 2 H, H-C_7 Temp), 2.5 (m, 1 H, H-C_β Asp), 2.60 (s, 3 H, CH_3Ar), 2.60 (m, 2 H, CH$_2$C*H*$_2$Ar), 2.62 (s, 3 H, CH_3Ar), 2.9 (dd, *J* = 7, 17, 1 H, H-C_β Asp), 3.2 (m, 2 H, H-C_δ Arg), 3.55 (dd, *J* = 0, 12, 1 H, H-C_α Gly), 4.05 (m, 1 H, H-C_9 Temp), 4.1 (m, 1 H, H-C_α Gly), 4.2 (m, 1 H, H-C_6 Temp), 4.3 (m, 1 H, H-C_3 Temp), 4.6 (m, 1 H, H-C_α Arg), 4.65 (m, 1 H, H-C_α Asp), 6.2-6.4 (bs, 3 H, H-N_ε Arg, $HNSO_2$, =NH), 7.3 (bs, 1 H, NH Temp), 7.45 (bs, 1 H, NH Arg), 7.90 (bs, 1 H, NH Gly), 8.0 (bs, 1 H, NH Asp). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ = 12.0, 17.4, 18.4, 21.3, 22.0, 25.6, 26.2, 26.7, 27.9, 30.1, 32.7, 34.0, 35.0, 50.9, 51.0, 51.8, 56.5, 62.5, 73.5, 81.2, 95.0, 117.8, 123.9, 133.3, 134.7, 135.3, 153.4, 156.2, 170.4, 170.7, 171.9, 172.5, 173.3. $[\alpha]_D^{20}$ = -71.0 (*c* = 0.7, $CHCl_3$). MS (FAB^+): 832 (M^++2).

Cyclo[-Temp5-Arg(Pmc)-Gly-Asp(tBu)-] (20). Was prepared in 35 % yield following general procedure 7. ^{1}H NMR (300 MHz, DMSO-D6) δ = 1.25, 1.38 [2 s, 6 H, $(CH_3)_2$C-O], 1.31 (m, 2 H, H-C_γ Arg), 1.38 [s, 9 H, $(CH_3)_3$CO], 1.8 (m, 2 H, C*H*$_2$CH$_2$Ar), 2.05 (s, 3 H, CH_3Ar), 2.05 (m, 1 H, H-C_9 Temp), 2.15 (m, 2 H, H-C_β Arg), 2.35 (dd, *J* = 6.8, 17, 1 H, H-C_β Asp), 2.50, 2,52 (2 s, 6 H, 3 CH_3Ar), 2.5 (m, 1 H, H-C_9 Temp), 2.6 (m, 2 H, C*H*$_2$CH$_2$Ar), 2.8 (dd, *J* = 8.6, 17, 1 H, H-C_β Asp), 3.1 (m, 2 H, H-C_δ Arg), 3.61 (d, *J* = 9.8, 1 H, H-C_α Gly), 3.98 (d, *J* = 9.8, 1 H, H-C_α Gly), 4.0 (m, 2 H, H-C_α Arg, H-C_7 Temp), 4.31 (m, 2 H, H-C_3 Temp, H-C_{10} Temp), 4.55 (m, 1 H, H-C_α Asp), 6.48 (bs, 2 H, H-N_ε Arg, $HNSO_2$), 6.78 (bs, 1 H, =NH), 7.68 (d, *J* = 5.1, 1 H, NH Temp), 7.84 (bd, 1 H,

NH Asp), 8.22 (m, 1 H, NH Arg), 8.5 (bt, 1 H, NH Gly). ^{13}C NMR (50.3 MHz, DMSO-D6) δ = 11.9, 17.1, 18.2, 26.4, 27.7, 20.8, 25.3, 27.0, 30.6, 32.2, 32.5, 36.3, 38.7, 40.3, 42.4, 49.6, 53.1, 58.8, 62.0, 73.5, 80.3. $[\alpha]_D^{20}$ = -36.7 (*c* = 1, $CHCl_3$). MS (FAB^+): 844 (M+).

Cyclo[-Temp6-Arg(Pmc)-Gly-Asp(tBu)-] (21). Was prepared in 26 % yield following general procedure 7. ^{1}H NMR (300 MHz, DMSO-D6) δ = 1.3 [s, 3 H, $(CH_3)_2$C-O], 1.31 (m, 2 H, H-C_γ Arg), 1.38 [s, 9 H, $(CH_3)_3$CO], 1.4 [s, 3 H, $(CH_3)_2$C-O], 1.8-1.95 (m, 6 H, C*H*$_2$$CH_2$Ar, H-$C_9$ Temp, H-C_β Arg), 2.05 (s, 3 H, CH_3Ar), 2.39 (dd, *J* = 4.3, 10.6, 1 H, H-C_β Asp), 2.50, 2,52 (2 s, 6 H, 3 CH_3Ar), 2.6 (m, 2 H, C*H*$_2$$CH_2$Ar), 2.9 (dd, *J* = 4.3, 10.6, 1 H, H-C_β Asp), 3.1 (m, 2 H, H-C_δ Arg), 3.80 (m, 3 H, H-C_α Gly, H-C_α Arg), 4.3 (m, 1 H, H-C_3 Temp), 4.35 (m, 1 H, H-C_{10} Temp), 4.48 (m, 1 H, H-C_α Asp), 6.45 (bs, 2 H, H-N_ε Arg, $HNSO_2$), 6.60 (bs, 1 H, =NH), 7.5 (bd, 1 H, NH Asp), 7.51 (bd, 1 H, NH Temp), 8.55 (m, 1 H, NH Arg), 8.75 (bt, 1 H, NH Gly). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ = 12.1, 14.3, 17.5, 18.6, 21.4, 23.5, 26.8, 28.1, 29.7, 31.7, 32.8, 33.6, 49.7, 60.8, 73.6, 117.9, 124.0, 134.8, 135.5, 153.6, 171.5. $[\alpha]_D^{20}$ = -72.9 (*c* = 1, $CHCl_3$). MS (FAB^+): 844 (M+).

Cyclo[-Temp7-Arg(Pmc)-Gly-Asp(tBu)-] (22). Was prepared in 50 % yield following general procedure 7. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.30 [s, 6 H, $(CH_3)_2$C-O], 1.41 [s, 9 H, $(CH_3)_3$CO], 1.49 (m, 1 H, H-C_γ Arg), 1.50-1.90 (10 H, C*H*$_2$$CH_2$Ar, H-$C_5$ Temp, H-C_6 Temp, H-C_8 Temp), 1.51 (m, 2 H, H-C_4 Temp), 1.60 (m, 1 H, H-C_γ Arg), 1.90 (m, 2 H, H-C_β Arg), 1.98 (m, 1 H, H-C_9 Temp), 2.15 (s, 3 H, CH_3Ar), 2.53 (s, 3 H, CH_3Ar), 2.58 (s, 3 H, CH_3Ar), 2.32 (m, 1 H, H-C_9 Temp), 2.51 (m, 1 H, H-C_β Asp), 2.85 (m,1 H, H-C_β Asp), 3.20 (m, 2 H, H-C_δ Arg), 3.51 (bd, 1 H, H-C_α Gly), 4.12 (m, 1 H, H-C_7 Temp), 4.18 (m, 1 H, H-C_α Gly), 4.32 (m, 1 H, H-C_{10} Temp), 4.5 (m, 1 H, H-C_3 Temp), 4.58 (m, 1 H, H-C_α Arg), 4.80 (m, 1 H, H-C_α Asp), 6.3 (bs, 3 H, H-N_ε Arg, $HNSO_2$, =NH), 7.2 (bd, 1 H, NH Arg), 7.65 (bd, 1 H, NH Temp), 7.80 (bt, 1 H, NH Gly), 7.95 (bd, 1 H, NH Asp); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ = 12.0, 17.3, 18.4, 21.3, 25.3, 26.7, 27.4, 28.6, 28.9, 30.7, 32.8, 33.0, 34.9, 40.6, 44.4, 49.9, 51.9, 53.3, 54.1, 56.5, 59.3, 63.2, 63.8, 73.5, 81.3, 117.8, 123.9, 133.4, 134.7, 135.3, 153.5, 156.3, 170.4, 172.5, 172.7. $[\alpha]_D^{20}$ = -72.3 (*c* = 0.004, $CHCl_3$). MS (FAB^+): 867 (M^++Na).

Cyclo[-Temp8-Arg(Pmc)-Gly-Asp(tBu)-] (23). Was prepared in 56 % yield following general procedure 7. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.27, 1.31 [2 s, 6 H, $(CH_3)_2$C-O], 1.44 [s, 9 H, $(CH_3)_3$CO], 1.50 (m, 3 H, H-C_γ Arg, H-C_6 Temp), 1.60 (m, 2 H, H-C_β Arg, H-C_γ Arg), 1.70 (m, 2 H, H-C_8 Temp), 1.8 (m, 2 H, C*H*$_2$$CH_2$Ar), 1.85 (m, 1 H, H-$C_4$ Temp), 1.95 (m, 2 H, H-C_β Arg, H-C_4 Temp), 1.98 (m, 2 H, H-C_5 Temp), 2.11 (s, 3 H, CH_3Ar), 2.32 (m, 1 H, H-C_9 Temp), 2.56 (s, 3 H, CH_3Ar), 2.57 (dd, *J* = 7.4, 16.7, 1 H, H-C_β Asp), 2.58 (s, 3 H, CH_3Ar), 2.65 (m, 2 H,

C*H*$_2$CH$_2$Ar), 2.87 (dd, *J* = 7.4, 16.7, 1 H, H-C$_\beta$ Asp), 3.20 (m, 2 H, H-C$_\delta$ Arg), 3.54 (bd, 1 H, H-C$_\alpha$ Gly), 4.18 (m, 1 H, H-C$_\alpha$ Gly), 4.22 (m, 1 H, H-C$_7$ Temp), 4.36 (m, 1 H, H-C$_{10}$ Temp), 4.55 (m, 1 H, H-C$_3$ Temp), 4.6 (m, 1 H, H-C$_\alpha$ Arg), 4.83 (m, 1 H, H-C$_\alpha$ Asp), 6.33 (bs, 3 H, H-N$_\varepsilon$ Arg, HNSO$_2$, =NH), 7.49 (bd, 1 H, NH Arg), 7.71 (bt, 1 H, NH Gly), 7.80 (bd, 1 H, NH Temp), 7.95 (bd, 1 H, NH Asp). ^{13}C NMR (50.3 MHz, CDCl$_3$) δ = 12.0, 17.4, 18.4, 26.7, 27.4, 36.4, 21.4, 25.3, 28.5, 29.6, 30.8, 33.0, 34.9, 40.6, 44.3, 49.9, 51.9, 54.1, 59.3, 63.2, 73.5, 81.3, 117.8, 123.9, 133.4, 134.7, 135.3, 153.5, 156.3, 170.3, 170.5, 172.3, 172.6. $[\alpha]_D^{20}$ = -54 (*c* = 0.05, CHCl$_3$). MS (FAB$^+$): 844 (M+).

General Procedure 8. Synthesis of Cyclo(-Temp-Arg-Gly-Asp-) (24-30). Side-chain protected cyclopeptide (0.1 mmol) was treated with TFA/thioanisole/1,2-ethanedithiol/anisole 90:5:3:2 (35 ml) for 2 h. The reaction mixture was evaporated under reduced pressure and the residue was dissolved in H$_2$O. The aqueous phase was washed twice with iPr$_2$O and evaporated under reduced pressure to afford side-chain deprotected cyclopeptide as a white foam. Trifluoroacetate ion was substituted with chloride by ion-exchange chromatography (AMBERLITE IRA-93 resin, chloride form). The purity of cyclic RGD final compounds was checked by HPLC on a W01521Q002 4x250 mm Waters column, employing a gradient of 0-70 % of MeCN/0.1 % CF$_3$COOH in H$_2$O/0.1 % CF$_3$COOH at 214 nm. Purity was greater than 95 % for every compound, as judged by peak area at 214 nm (PDA detector).

Cyclo(-Temp1-Arg-Gly-Asp-) (24). Was prepared in quantitative yield following general procedure 8. ^{1}H NMR (400 MHz, D$_2$O) δ = 1.6-1.75 (m, 2 H, H-C$_\gamma$ Arg), 1.65-1.95 (m, 6 H, H-C$_4$ Temp, H-C$_5$ Temp, H-C$_7$ Temp), 2.2 (m, 2 H, H-C$_\beta$ Arg), 2.3-2.45 (m, 2 H, H-C$_8$ Temp), 2.73 (dd, *J* = 0, 6, 2 H, H-C$_\beta$ Asp), 3.25-3.50 (m, 2 H, H-C$_\delta$ Arg), 3.8-3.95 (m, 1 H, H-C$_6$ Temp), 3.82 (d, *J* = 13.5, 1 H, H-C$_\alpha$ Gly), 4.25 (d, *J* = 13.5, 1 H, H-C$_\alpha$ Gly), 4.50 (dd, *J* = 0, 10, 1 H, H-C$_9$ Temp), 4.55 (dd, *J* = 0, 8, 1 H, H-C$_\alpha$ Arg), 4.58 (m, 1 H, H-C$_3$ Temp), 4.75 (m, 1 H, H-C$_\alpha$ Asp). ^{13}C NMR (75.4 MHz, D$_2$O) δ = 25.0, 26.4, 28.2, 29.8, 30.8, 32.2, 39.0, 41.3, 45.8, 48.3, 52.7, 53.1, 61.7, 62.6, 157.7, 170.1, 172.6, 174.0, 175.4, 175.7, 178.4. $[\alpha]_D^{20}$ = -52.6 (*c* = 0.88, H$_2$O). MS (FAB$^+$): 509 (M$^+$+1).

Cyclo(-Temp3-Arg-Gly-Asp-) (25). Was prepared in quantitative yield following general procedure 8. ^{1}H NMR (400 MHz, D$_2$O) δ = 1.5 (m, 2 H, H-C$_5$ Temp), 1.6 (m, 2 H, H-C$_\beta$ Arg), 1.8 (m, 2 H, H-C$_4$ Temp), 1.9 (m, 2 H, H-C$_\gamma$ Arg), 2.2 (m, 2 H, H-C$_7$ Temp), 2.42-2.52 (m, 2 H, H-C$_8$ Temp), 2.7-2.85 (m, 2 H, H-C$_\beta$ Asp), 3.15-3.30 (m, 2 H, H-C$_\delta$ Arg), 3.55 (d, *J* = 14, 1 H, H-C$_\alpha$

Gly), 3.83-3.95 (m, 1 H, H-C_6 Temp), 4.10 (d, *J* = 14, 1 H, H-C_α Gly), 4.28 (m, 1 H, H-C_3 Temp), 4.37 (dd, *J* = 0, 9, 1 H, H-C_8 Temp), 4.45 (dd, *J* = 5, 10, 1 H, H-C_α Arg), 4.65 (m, 1 H, H-C_α Asp). ^{13}C NMR (50.3 MHz, D_2O) δ = 27.1, 29.3, 30.4, 31.4, 31.8, 35.1, 38.1, 43.3, 47.2, 52.8, 53.5, 54.8, 64.0, 64.5, 159.6, 166.1, 172.5, 174.8, 175.2, 176.4, 176.6, 177.9. $[\alpha]_D^{20}$ = -94.6 (*c* = 1.32, H_2O). MS (FAB^+): 508 (M+).

Cyclo(-Temp4-Arg-Gly-Asp-) (26). Was prepared in quantitative yield following general procedure 8. ^{1}H NMR (400 MHz, D_2O) δ = 1.6-1.7 (m, 2 H, H-C_4 Temp), 1.7 (m, 2 H, H-C_γ Arg), 2.0 (m, 2 H, H-C_7 Temp), 2.2 (m, 2 H, H-C_β Arg), 2.4 (m, 2 H, H-C_5 Temp), 2.6 (m, 2 H, H-C_8 Temp), 2.70 (dd, *J* = 7, 17, 1 H, H-C_β Asp), 3.05 (dd, *J* = 7, 17, 1 H, H-C_β Asp), 3.15-3.25 (m, 2 H, H-C_δ Arg), 3.52 (d, *J* = 15, 1 H, H-C_α Gly), 4.05 (m, 1 H, H-C_6 Temp), 4.28 (d, *J* = 15, 1 H, H-C_α Gly), 4.3 (m, 1 H, H-C_3 Temp), 4.35 (m, 1 H, H-C_{10} Temp), 4.53 (dd, *J* = 7, 7, H-C_α Asp), 4.6 (m, 1 H, H-C_α Arg). ^{13}C NMR (50.3 MHz, D_2O) δ = 24.6, 25.3, 25.9, 26.5, 27.8, 27.9, 28.9, 29.5, 31.1, 32.9, 33.7, 35.0, 38.2, 41.3, 41.4, 43.3, 43.7, 49.2, 51.7, 52.0, 52.5, 52.7, 54.5, 57.9, 60.1, 61.2, 63.1, 157.6, 167.9, 171.3, 172.2, 172.7, 173.9, 174.5, 174.8, 175.2, 175.7, 176.7. $[\alpha]_D^{20}$ = -63.7 (*c* = 0.95, H_2O). MS (FAB^+): 508 (M+).

Cyclo(-Temp5-Arg-Gly-Asp-) (27). Was prepared in quantitative yield following general procedure 8. ^{1}H NMR (400 MHz, D_2O) δ = 1.5-1.8 (m, 2 H, H-C_γ Arg), 1.7-2.0 (m, 2 H, H-C_β Arg), 2.8 (m, 2 H, H-C_β Asp), 3.22 (m, 2 H, H-C_δ Arg), 4.0 (m, 2 H, H-C_α Gly, H-C_7 Temp), 4.3 (dd, *J* = 7, 7, 1 H, H-C_α Arg), 4.5-4.6 (m, 1 H, H-C_3 Temp, H-C_{10} Temp), 4.68 (m, 1 H, H-C_α Asp). ^{13}C NMR (50.3 MHz, D_2O) δ = 27.2, 29.8, 30.1, 31.0, 33.6, 35.3, 36.2, 39.0, 43.3, 45.7, 53.8, 56.3, 62.8, 65.2, 159.5, 174.3, 174.4, 175.6, 176.4, 178.5. $[\alpha]_D^{20}$ = -87.4 (*c* = 1.2, H_2O). MS (FAB^+): 522 (M+).

Cyclo(-Temp6-Arg-Gly-Asp-) (28). Was prepared in quantitative yield following general procedure 8. ^{1}H NMR (400 MHz, D_2O) δ = 1,5-1.8 (m, 2 H, H-C_6 Temp), 1.6 (m, 2 H, H-C_γ Arg), 1.75-1.9 (m, 2 H, H-C_β Arg), 1.8-1.95 (m, 2 H, H-C_4 Temp), 2.15 (m, 4 H, H-C_8 Temp, H-C_9 Temp), 2.65-2.8 (m, 2 H, H-C_β Asp), 3.2 (m, 2 H, H-C_δ Arg), 3.82 (d, *J* = 17, 1 H, H-C_α Gly), 4.05 (d, *J* = 17, 1 H, H-C_α Gly), 4.1 (m, 1 H, H-C_7 Temp), 4.37 (dd, *J* = 0, 7, 1 H, H-C_{10} Temp), 4.42 (dd, *J* = 0, 10, 1 H, H-C_3 Temp), 4.52 (dd, *J* = 5, 10, 1 H, H-C_α Arg), 4.70 (m, 1 H, H-C_α Asp). ^{13}C NMR (75.4 MHz, D_2O) δ = 22.3, 25.0, 25.9, 28.7, 30.4, 33.7, 34.2, 37.7, 41.4, 43.2, 51.5, 53.3, 57.5, 59.1, 63.6, 157.6, 171.6, 173.7, 174.2, 175.0, 176.9. $[\alpha]_D^{20}$ = -47.9 (*c* = 0.71, H_2O). MS (FAB^+): 522 (M+).

Cyclo(-Temp7-Arg-Gly-Asp-) (29). Was prepared in quantitative yield following general procedure 8. ^{1}H NMR (400 MHz, D_2O) δ = 1.6-1.9 (m, 6 H, -CH_2-), 1.61 (m, 2 H, H-C_γ Arg), 1.77 (m, 2 H, H-C_β Arg), 1.78 (m, 1 H, H-C_4 Temp), 2.3 (m, 3 H, H-C_4 Temp, H-C_8 Temp), 2.95 (m, 2 H, H-C_β Asp), 3.2 (m, 2 H, H-C_δ Arg), 3.62 (d, *J* = 14.3, 1 H, H-C_α Gly), 3.96 (d, *J* = 14.3, 1 H, H-C_α Gly), 4.18 (m, 1 H, H-C_7 Temp), 4.3 (dd, *J* = 7, 11, 1 H, H-C_3 Temp), 4.44 (dd, *J* = 6, 10, 1 H, H-C_α Arg), 4.51 (dd, *J* = 2, 13, 1 H, H-C_{10} Temp), 4.73 (dd, *J* = 7, 7, 2 H, H-C_α Asp). ^{13}C NMR (50.3 MHz, D_2O) δ = 24.9, 27.4, 29.4, 30.1, 30.8, 32.2, 33.5, 36.6, 37.4, 43.4, 47.4, 53.4, 54.6, 55.9, 62.7, 68.2, 173.8, 174.9, 175.9, 177.0, 177.7, 178.5. $[\alpha]_D^{20}$ = -58.1 (*c* = 0.97, H_2O). MS (FAB^+): 523 (M^++1).

Cyclo(-Temp8-Arg-Gly-Asp-) (30). Was prepared in quantitative yield following general procedure 8. ^{1}H NMR (400 MHz, D_2O) δ = 1.4 (m, 3 H, H-C_5 Temp, H-C_8 Temp), 1.55-1.7 (m, 2 H, H-C_γ Arg), 1.8 (m, 4 H, H-C_4 Temp, H-C_8 Temp, H-C_9 Temp), 2.0 (m, 2 H, H-C_β Arg), 2.26 (m, 2 H, H-C_6 Temp), 2.38 (m, 1 H, H-C_9 Temp), 2.68 (dd, *J* = 7, 18, 1 H, H-C_β Asp), 2.98 (dd, *J* = 7, 18, 1 H, H-C_β Asp), 3.2 (m, 2 H, H-C_δ Arg), 3.5 (d, *J* = 15, 1 H, H-C_α Gly), 4.2 (d, *J* = 15, 1 H, H-C_α Gly), 4.2 (m, 1 H, H-C_7 Temp), 4.38 (m, 1 H, H-C_{10} Temp), 4.48 (dd, *J* = 5, 11, 1 H, H-C_α Arg), 4.53 (dd, *J* = 0, 11, 1 H, H-C_3 Temp), 4.63 (dd, *J* = 7, 7, 2 H, H-C_α Asp). ^{13}C NMR (50.3 MHz, D_2O) δ = 27.4, 29.3, 30.2, 30.6, 33.0, 35.1, 36.2, 43.3, 46.1, 53.8, 54.9, 56.8, 62.8, 66.1, 159.5, 174.2, 174.8, 175.9, 176.4, 177.6. $[\alpha]_D^{20}$ = -38.1 (*c* = 1.2, H_2O). MS (FAB^+): 522 (M^+).

Receptor binding assay

The receptor binding assay was performed as described by Orlando and Cheresh (*Arginine-Glycine-Aspartic Acid Binding Leading to Molecular Stabilization between Integrin* $\alpha_v\beta_3$ *and Its Ligand*, J. Biol. Chem. 266: 19543-19550, 1991). Integrin $\alpha_v\beta_3$ purified protein from human placenta was purchased from CHEMICON International Inc. $\alpha_v\beta_3$ was diluted at 500 ng/ml in coating buffer (20 mM Tris, pH 7.4, 150 mM NaCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM $MnCl_2$) and an aliquot of 100 µl/well was added to a 96-well microtiter plate and incubated overnight at 4°C. The plate was washed once with blocking/binding buffer (50 mM Tris, pH 7.4, 100 mM NaCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 1% bovine serum albumin), and incubated an additional 2 h at room temperature. The plate was rinsed twice with the same buffer and incubated with radiolabelled ligand at the indicated concentrations. In particular, saturation binding isotherms of ^{125}I-echistatin

binding to $\alpha_v\beta_3$ receptor were determined in a solid-phase receptor binding. In particular, integrin $\alpha_v\beta_3$ was coated and incubated with various concentrations (0.02-5 nM) of ^{125}I-echistatin. To prevent no specific binding to $\alpha_v\beta_3$ all experiments were performed in presences of 10^{-6} M cold echistatin.

In competition binding assay ^{125}I-echistatin was added to the wells to a final concentration of 0.05 nM the presence of competing ligand. Cold unlabelled echistatin and peptides were dissolved in binding buffer at concentrations ranging between 10^{-4} to 10^{-9} M.

After additional three washes, ^{125}I-echistatin solubilized with boiling 2N NaOH and subjected to γ-counting. Each data point is an average of triplicate measurements, and was analysed by non-linear regression analysis with Allfit program.

^{125}I-Echistatin labeled by the lactoperoxidase method to a specific activity of 2000 Ci/mmole was purchased from Amersham Pharmacia Biotech. This method of radiolabeling (Kumar C.C., Nie H., Armstrong L., Zhang R., Vijay-Kumar S., Tsarbopoulos A., FEBS Lett, 1998, Jun 16, 429 (3):239-248) did not alter disintegrins' biological activity, and echistatin may bind to integrin $\alpha_v\beta_3$ receptor in the same fashion of native echistatin (high affinity and in a non-dissociable manner).