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Synthesis of Bicyclic Alkene/Alkane-Bridged Nisin Mimics by Ring-Closing Metathesis and Their Biochemical Evaluation as Lipid II Binders: Toward the Design of Potential Novel Antibiotics

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Experimental section

General: Analytical HPLC runs were performed on a Shimadzu automated HPLC system equipped with an evaporative light scattering detector (PL-ELS 1000, Polymer Laboratories) and a UV/VIS detector operating at 220 nm. Preparative HPLC runs were performed on a Gilson HPLC workstation. Liquid chromatography electrospray ionization mass spectrometry (ES-MS) was measured on a Shimadzu LCMS-QP8000 single quadrupole bench-top mass spectrometer operating in a positive ionization mode. MALDI-TOF analysis was performed on a Kratos Axima CFR apparatus, with bradykinin(1-7) as external reference and α-cyano-4-hydroxycinnamic acid as matrix. MS/MS spectra were analyzed on a Micromass Quattro Ultima or a Micromass Q-TOF mass spectrometer. ^{1}H NMR spectra were recorded on a Varian G-300 (300 MHz) spectrometer or on a Varian INOVA-500 (500 MHz) spectrometer and the chemical shifts are given in ppm (δ) relative to TMS. R_f values were determined by thin layer chromatography (TLC) on Merck precoated silicagel 60F_{254} plates. Spots were visualized by UV-quenching, ninhydrin or Cl_2/TDM.[1] The numbering of the amino acids listed in the NMR-characterizations is from the N- to C-terminus. When appropriate, for clarity this numbering is indicated as superscripts in the compound name.

Chemicals and reagents: ArgoGel™ resin with a free hydroxyl moiety was used in all the syntheses. The coupling reagents 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU)[2] and benzotriazol-1-yl-oxy-tris-(dimethylamino)phosphonium hexafluorophosphate (BOP)[3] were obtained from Richelieu Biotechnologies Inc. *N*-hydroxybenzotriazole (HOBt) and N$^{\alpha}$-9-fluorenylmethyloxycarbonyl (Fmoc) amino acids were obtained from Advanced ChemTech. Peptide grade dichloromethane (DCM), *N*,*N*-dimethylformamide (DMF), *tert*-butyl methylether (MTBE), trifluoroacetic acid (TFA) and HPLC grade acetronitrile were purchased from Biosolve. Piperidine and *N*,*N*-diisopropylethylamine (DIPEA) were obtained from Acros Organics. Triisopropylsilane (TIS) and HPLC grade TFA were obtained from Merck. Second generation Grubbs' catalyst (RuII) was obtained from Aldrich.

Solid-phase peptide synthesis: Peptides were synthesized manually on a 0.25 mmol scale on ArgoGel™ resin. Each synthetic cycle consisted of N$^{\alpha}$-Fmoc removal by treatment with 20% piperidine in DMF (3 × 10 mL, 8 min), a washing step (DMF: 3 × 10 mL, 2 min; DCM: 3 × 10 mL, 2 min and DMF: 3 × 10 mL, 2 min) a coupling step

(60 min) with 1.0 mmol of preactivated Fmoc amino acid in the presence of 2 equiv DIPEA in DMF (10 mL) and a final washing step (DMF: 3 × 10 mL, 2 min; DCM: 3 × 10 mL, 2 min and DMF: 3 × 10 mL, 2 min). N^{α}-Fmoc amino acids (1 mmol) were activated in situ with BOP (1 mmol) in the presence of DIPEA (2 mmol). Fmoc-removal and coupling reactions were monitored by the Kaiser test.[4] The peptides were cleaved from the resin by treatment with a catalytic amount of KCN in MeOH (15 mL) during 16 h. The resin was filtered and washed with MeOH (3 × 10 mL), the filtrate was concentrated in vacuo to yield the crude peptide.

Solution-phase peptide synthesis: *Coupling reaction:* The carboxylic acid moiety (1 equiv) was coupled to the amine derivative (or its TFA-salt, 1 equiv) in the presence of BOP (1 equiv) and DIPEA (2 equiv, when the amine was protonated 3 equiv were used) as coupling reagents in DCM (10 mL per mmol) as solvent. Coupling time was 16 h. After completion of the reaction, DCM was removed under reduced pressure and the residue was dissolved in EtOAc (25 mL per mmol). The EtOAc solution was washed with 1N $KHSO_4$ (3 × 25 mL), 10% Na_2CO_3 (3 × 25 mL) and brine (1 × 25 mL), dried (Na_2SO_4), filtrated and evaporated in vacuo. The obtained crude product was analyzed by TLC, ^{1}H NMR and ESI-MS and in general pure enough to be used in the next synthesis steps.

Boc-removal: A Boc-protected intermediate was dissolved in TFA/DCM 1:1 *v*/*v* (4 mL per mmol) and stirred for 2 h. Then, the solvents were removed under reduced pressure and the residue was coevaporated with toluene (2 × 25 mL), CH_3CN (2 × 25 mL) and DCM (2 × 25 mL) to remove any residual TFA. The obtained TFA-salt was used without further purification in the next synthesis steps.

Peptide purification: The crude lyophilized peptides (30-60 mg) were dissolved in a minimum amount of 0.1% TFA in CH_3CN/H_2O 8:2 *v*/*v* and loaded onto an Adsorbosphere XL C8 HPLC column (90Å pore size, 10 μm particle size, 2.2 × 25 cm). The peptides were eluted with a flow rate of 10.0 mL/min using a linear gradient of buffer B (100% in 60 min) from 100% buffer A (buffer A: 0.1% TFA in H_2O, buffer B: 0.1% TFA in CH_3CN/H_2O 95:5 *v*/*v*). The peptide purity and retention times (R_t) were evaluated by analytical HPLC on an Adsorbosphere XL C8 column (90Å pore size, 5 μm particle size, 0.46 × 25 cm) at a flow rate of 1 mL/min using a linear gradient of buffer B (100% in 30 min) from 100% buffer A (buffer A: 0.1% TFA in H_2O; buffer B: 0.1% TFA in CH_3CN/H_2O 95:5 *v*/*v*).

Peptide characterization: The peptides were characterized by mass spectrometry (ES-MS, MALDI-TOF and LC-MS/MS) and ^{1}H NMR (300 or 500 MHz). The mass of each analogue was measured and the observed monoisotopic $(M + H)^+$ values were correlated with the calculated $(M + H)^+$ values using MacBioSpec (Perkin Elmer Sciex Instruments, Thornhill, Ontario, Canada). Peak assingments were based on ^{1}H NMR COSY, TOCSY and/or NOESY spectra.

Fmoc-Alg-O-ArgoGel™ resin (8): The loading of the first amino acid was carried out according to the procedures of Sieber. In a typical experiment: plain ArgoGel™ resin (1 g, 0.23 mmol) and Fmoc-Alg-OH (337 mg, 1.0 mmol, 4 equiv) were dried over P_2O_5 in a vacuum dessicator for 16 h. Then

Boc-Alg-Ile-Ala-Leu-Alg-OMe (solid phase) (9): The crude solid phase cleavage product was purified by silicagel column chromatography (DCM/MeOH 97.5:2.5 $\rightarrow$ DCM/MeOH 95:5 *v*/*v*). Yield: 150 mg (80%); R_t: 17.5 min; R_f(DCM/MeOH 9:1 *v*/*v*): 0.55; ES-MS: calcd for $C_{31}H_{54}N_5O_8$: 624.4, found: *m*/*z*: $[M + H]^+$ 624.7, $[M + Na]^+$ 646.7, $[(M - C_4H_8) + H]^+$ 568.6, $[(M - C_5H_8O_2) + H]^+$ 524.7.

Boc-Alg^1-Ile^2-Ala^3-Leu^4-Alg^5-OMe (solution phase) (9): Coupling and Boc-removal were carried out as described in the general procedure solution-phase peptide synthesis.

Boc-Leu-Alg-OMe: R_f(DCM/MeOH 9:1 *v*/*v*): 0.78; ^{1}H NMR ($CDCl_3$, 300 MHz) Leu: δ 4.91 (d, 1H, NH), 4.62 (m, 1H, CαH), 1.71-1.63 (m, 2H, CβH), 1.45 (m, 1H, CγH), 1.45 (s, 9H, Boc), 0.96-0.92 (m, 6H, Cδ'H/CδH); Alg: 6.62 (d, 1H, NH), 5.63 (m, 1H, CγH), 5.10 (m, 2H, CδH), 4.12 (m, 1H, CαH), 3.74 (s, 3H, OCH_3) 2.50 (m, 2H, CβH); ES-MS: calcd for $C_{17}H_{31}N_2O_5$: 343.2, found: *m*/*z*: $[M + H]^+$ 343.3, $[M + Na]^+$ 365.2, $[(M - C_4H_8) + H]^+$ 287.05, $[(M - C_5H_8O_2) + H]^+$ 243.2.

Boc-Ala-Leu-Alg-OMe: R_f(DCM/MeOH 9:1 *v*/*v*): 0.57; ^{1}H NMR ($CDCl_3$, 300 MHz) Ala: δ 5.07 (d, 1H, NH), 4.18 (m, 1H, CαH), 1.34 (d, 3H, CβH); Leu: 6.72 (d, 1H, NH), 4.44 (m, 1H, CαH), 1.70-1.55 (m, 2H, CβH), 1.55 (m, 1H, CγH), 1.45 (s, 9H, Boc), 0.94-0.90 (m, 6H, Cδ'H/CδH); Alg: 6.72 (d, 1H, NH), 5.62 (m, 1H, CγH), 5.07 (m, 2H, CδH), 4.63 (m, 1H, CαH), 3.74 (s, 3H, OCH_3), 2.64-2.49 (m, 2H, CβH); ES-MS: calcd for $C_{20}H_{36}N_3O_6$: 414.3, found: *m*/*z*: $[M + H]^+$ 414.5, $[M + Na]^+$ 436.35, $[(M - C_4H_8) + H]^+$ 385.3, $[(M - C_5H_8O_2) + H]^+$ 314.0.

Boc-Ile-Ala-Leu-Alg-OMe: Coupling of Boc-Ile-OH with TFA.H-Ala-Leu-Alg-OMe was carried out in DMF. The tetrapeptide was isolated by trituration with EtOAc. R_f (DCM/MeOH 9:1 *v*/*v*): 0.56; ^{1}H NMR ($CDCl_3$, 300 MHz) Ile: δ 5.75 (d, 1H, NH), 4.20 (m, 1H, CαH), 1.80 (m, 1H, CβH), 1.77 (m, 1H, CγH), 1.54 (m, 1H, CγH), 1.43 (s, 9H, Boc), 0.91-0.85 (m, 6H, Cδ'H/CδH); Ala: 7.69 (d, 1H, NH), 4.72 (m, 1H, CαH), 1.33 (d, 3H, CβH); Leu: 7.69 (d, 1H, NH), 4.72 (m, 1H, CαH), 1.70-1.60 (m, 2H, CβH), 1.04 (m, 1H, CγH), 0.91-0.85 (m, 6H, Cδ'H/CδH); Alg: 7.69 (d, 1H, NH), 5.74 (m, 1H, CγH), 5.01 (m, 2H, CδH), 4.72 (m, 1H, CαH), 3.74 (s, 3H, OCH_3), 2.56-2.49 (m, 2H, CβH); ES-MS: calcd for $C_{26}H_{47}N_4O_7$: 527.3, found: *m*/*z*: $[M + H]^+$ 527.5, $[M + Na]^+$ 549.5, $[(M - C_4H_8) + H]^+$ 471.4, $[(M - C_5H_8O_2) + H]^+$ 427.55.

Boc-Alg^1-Ile^2-Ala^3-Leu^4-Alg^5-OMe: Coupling of Boc-Alg-OH with TFA.H-Ile-Ala-Leu-Alg-OMe was carried out in DMF. Pentapeptide **2** was isolated by trituration with EtOAc. Yield: 1.56 g (70% over 7 steps); R_t: 17.5 min; R_f(DCM/MeOH 9:1 *v*/*v*): 0.55; ^{1}H NMR ($CDCl_3$/CD_3OH 14.5:1 *v*/*v*, 500 MHz) Alg1: δ 6.05 (d, 1H, NH), 5.68 (m, 1H, CγH), 5.01 (m, 2H, CδH), 4.51 (m, 1H, CαH), 2.39 (m, 2H, CβH), 1.44 (s, 9H, Boc); Ile2: 7.34 (d, 1H, NH), 4.66 (m, 1H, CαH), 1.77 (m, 1H, CγH), 1.54 (m, 1H, CγH), 1.06 (m, 1H, CβH), 0.84 (m, 6H, Cγ'H/CδH); Ala3: 8.45 (d, 1H, NH), 4.93 (m, 1H, CαH), 1.32 (d, 3H, CβH); Leu4: 8.19 (d, 1H, NH), 4.68 (m, 1H, CαH), 1.68 (m, 1H, CγH), 1.60 (m, 2H, CβH), 0.84 (m, 6H, Cδ'H/CδH); Alg5: 8.04 (d, 1H, NH), 5.68 (m, 1H, CγH), 5.01 (m, 2H, CδH), 4.68 (m, 1H, CαH), 3.74 (s, 3H, OCH_3), 2.39 (m, 2H, CβH), ES-MS: calcd for $C_{31}H_{54}N_5O_8$: 624.4, found: *m*/*z*: $[M + H]^+$ 624.7, $[M + Na]^+$ 646.7, $[(M - C_4H_8) + H]^+$ 568.6, $[(M - C_5H_8O_2) + H]^+$ 524.7.

Boc-*cyclo*[Alg^1-Ile^2-Ala^3-Leu^4-Alg^5]-OMe (10) Linear pentapeptide **9** (410 mg, 0.66 mmol) was dissolved in DCM (250 mL) and the solution was flushed with N_2 for 30 min. The solution was heated to reflux and a solution of second generation Grubbs' (RuII) catalyst (56 mg, 0.066 mmol) in DCM (1 mL) was added. The obtained mixture was refluxed overnight under a nitrogen atmosphere. After evaporation of the solvent, the product was purified by column chromatography (DCM/MeOH 97.5:2.5 → DCM/MeOH 95:5 *v*/*v*). The *E*/*Z* isomers could be partially separated during purification. Cyclic pentapeptide **10** was obtained as a brownish foam. Yield: 296 mg (76%); R_t: 16.4 min (*Z* isomer) and 20.8 min (*E* isomer); R_f(DCM/MeOH 9:1 *v*/*v*): 0.52 and 0.49; ^{1}H NMR ($CDCl_3$/CD_3OH 14.5:1 *v*/*v*, 500 MHz) *E* isomer: Alg1: δ 5.72 (d, 1H, NH), 5.36 (m, 1H, CγH), 4.14 (m, 1H, CαH), 2.39 (m, 2H, CβH), 1.45 (s, 9H, Boc);

Ile2: 7.81 (d, 1H, NH), 4.12 (m, 1H, CαH), 1.88 (m, 1H, CγH), 1.57 (m, 1H, CγH), 1.15 (m, 1H, CβH), 0.92 (m, 6H, Cγ'H/CδH); Ala3: 7.46 (d, 1H, NH), 4.24 (m, 1H, CαH), 1.49 (d, 3H, CβH); Leu4: 8.15 (d, 1H, NH), 4.11 (m, 1H, CαH), 1.88 (m, 1H, CγH), 1.75 (m, 2H, CβH), 0.92 (m, 6H, Cδ'H/CδH); Alg5: 7.28 (d, 1H, NH), 5.36 (m, 1H, CγH), 4.50 (m, 1H, CαH), 3.75 (s, 3H, OCH_3), 2.39 (m, 2H, CβH); *E* isomer: Alg1: δ 5.88 (d, 1H, NH), 5.25 (m, 1H, CγH), 4.69 (m, 1H, CαH), 2.37 (m, 2H, CβH), 1.48 (s, 9H, Boc); Ile2: 9.05 (br, 1H, NH), 4.96 (m, 1H, CαH), 1.88 (m, 1H, CβH), 1.62 (m, 2H, CγH), 0.84 (m, 6H, Cγ'H/CδH); Ala3: 8.96 (br, 1H, NH), 4.79 (m, 1H, CαH), 1.31 (d, 3H, CβH); Leu4: 7.91 (br, 1H, NH), 4.96 (m, 1H, CαH), 1.62 (m, 3H, CγH/CβH), 0.84 (m, 6H, Cδ'H/CδH); Alg5: 8.04 (br, 1H, NH), 5.25 (m, 1H, CγH), 4.96 (m, 1H, CαH), 3.80 (s, 3H, OCH_3), 2.69/2.16 (m, 2H, CβH); ES-MS: calcd for $C_{29}H_{50}N_5O_8$: 596.4, found: *m*/*z*: $[M + H]^+$ 596.7, $[M + Na]^+$ 618.7, $[(M - C_4H_8) + H]^+$ 540.5, $[(M - C_5H_8O_2) + H]^+$ 496.4.

Boc-Ile^{1}-Ala^{2}-Alg^{3}-Ile^{4}-Ala^{5}-Leu^{6}-Alg^{7}-OMe (solid phase) (11): Yield: 121 mg (60%); R_t: 18.4 min; R_f(DCM/MeOH 9:1 *v*/*v*): 0.39; ^{1}H NMR ($CDCl_3$/CD_3OH 14.5:1 *v*/*v*; 500 MHz) δ 7.65-7.31 (m, 6H, (NH's Ala, Alg, Ile, Ala, Leu, Alg); Ile1: 5.71 (1H, NH), 3.91 (m, 1H, CαH), 1.80 (m, 2H, CγH), 1.49/1.19 (m, 1H, CβH), 1.47 (s, 9H, Boc), 0.84 (m, 6H, Cγ'H/CδH); Ala2/Ala5: 4.36/4.24 (m, 2H, CαH), 1.40/1.37 (2 (d, 6H, CβH); Alg3/Alg7: 5.75-5.68 (m, 2H, CγH), 5.13-5.07 (m, 4H, CδH), 4.53/4.40 (m, 2H, CαH), 3.74 (s, 3H, OCH_3), 2.59-2.47 (m, 4H, CβH) Ile4: 4.18 (m, 1H, CαH), 1.91 (m, 2H, CγH), 1.49/1.19 (m, 1H, CβH), 0.89 (m, 6H, Cγ'H/CδH); Leu6: 4.41 (m, 1H, CαH), 1.68 (m, 1H, CγH), 1.60 (m, 2H, CβH), 0.89 (m, 6H, Cδ'H/CδH); ES-MS: calcd for $C_{40}H_{70}N_7O_{10}$: 808.5, found: *m*/*z*: $[M + H]^+$ 808.6, $[M + Na]^+$ 830.6, $[(M – C_4H_8) + H]^+$ 752.5, $[(M – C_5H_8O_2) + H]^+$ 708.9.

Boc-Ile-Ala-OH: Boc-Ile-Ala-OMe was synthesized as described in the general procedure solution-phase peptide synthesis. Yield: 594 mg (94%); R_f (CH_2Cl_2/MeOH 9:1 *v*/*v*): 0.67; ^{1}H NMR ($CDCl_3$, 300 MHz) Ile: δ 5.00 (d, 1H, NH), 3.86 (m, 1H, CαH), 1.81 (m, 1H, CβH), 1.45 (s, 3H, Boc), 1.16-1.00 (m, 2H, CγH), 0.98-0.82 (m, 3H, Cγ'H/CδH); Ala: 6.37 (d, 1H, NH), 4.46 (m, 1H, CαH), 3.68 (s, 3H, OCH_3), 1.37-1.16 (m, 3H, CβH); ES-MS: calcd for $C_{15}H_{28}N_2O_5Na$: 339.0, found: *m*/*z*: $[M + Na]^+$ 339.1. The dipeptide methyl ester (300 mg, 0.94 mmol) was saponified with 0.2N LiOH/H_2O (8.5 mL) in THF (8.5 mL) at 0 °C for 2 h. Then, the reaction mixture was concentrated and

the residual aqueous solution was acidified with 1N $KHSO_4$ and subsequently extracted with EtOAc (3 × 10 mL). The combined extracts were washed with brine (1 × 10 mL), dried (Na_2SO_4), filtered and concentrated in vacuo. Boc-Ile-Ala-OH was obtained as a white solid in 81% yield (230 mg). R_f(DCM/MeOH 9:1 *v*/*v*): 0.41. ^{1}H NMR ($CDCl_3$, 300 MHz) Ile: δ 5.23 (d, 1H, NH), 3.91 (m, 1H, CαH), 1.76 (m, 1H, CβH), 1.36 (s, 9H, Boc), 1.18-1.06 (m, 2H, CγH), 0.86-0.80 (m, 3H, Cγ'H/CδH); Ala: 6.80 (d, 1H, NH), 4.47 (m, 1H, CαH), 1.39-1.31 (m, 3H, CβH); ES-MS: calcd for $C_{14}H_{26}N_2O_5Na$: 325.2, found: *m*/*z*: $[M + Na]^+$ 325.25.

Boc-Ile^1-Ala^2-*cyclo*[Alg^3-Ile^4-Ala^5-Leu^6-Alg^7]-OMe (12): Cyclic peptide **10** (269 mg, 0.45 mmol) was treated with TFA to remove the Boc-group as described in the standard procedure. The obtained TFA-salt was dissolved in DMF (6 mL) and Boc-Ile-Ala-OH (200 mg, 0.6 mmol) followed by DIPEA (262 µL, 1.46 mmol) and BOP (228 mg, 0.6 mmol) were added. After stirring for 16 h the solvent is evaporated and the residue is triturated with EtOAc. On TLC two spots were visible corresponding to the two isomers (*E*/*Z*). Yield: 284 mg (80%). R_t: 16.8 min and 20.2 min. R_f (DCM/MeOH 9:1 v/v): 0.42 and 0.39. ^{1}H NMR ($CDCl_3$/CD_3OH 14.5:1 *v*/*v*, 500 MHz) Ile1: δ 5.80 (d, 1H, NH), 3.89 (m, 1H, CαH), 1.80 (m, 1H, CβH), 1.50/1.16 (m, 2H, CγH), 1.46 (s, 9H, Boc), 0.92 (m, 6H, Cγ'H/CδH); Ala2: 8.10 (d, 1H, NH), 4.28 (m, 1H, CαH), 1.71 (d, 3H, CβH); Alg3: 7.71 (d, 1H, NH), 5.33 (m, 1H, CγH), 4.38 (m, 1H, CαH), 2.55-2.45 (m, 2H, CβH); Ile4: 7.61 (d, 1H, NH), 4.08 (m, 1H, CαH), 1.92 (m, 1H, CβH), 1.53/1.22 (m, 1H, CγH), 0.92 (m, 6H, Cγ'H/CδH); Ala5: 7.80 (d, 1H, NH), 4.22 (m, 1H, CαH), 1.41 (d, 3H, CβH); Leu6: 7.65 (d, 1H, NH), 4.34 (m, 1H, CαH), 1.68 (m, 2H, CβH), 1.35 (m, 1H, CγH), 0.92 (m, 6H, Cδ'H/CδH); Alg7: 7.70 (d, 1H, NH), 5.33 (m, 1H, CγH), 4.61 (m, 1H, CαH), 3.76 (s, 3H, OCH_3), 2.55-2.45 (m, 2H, CβH); ES-MS: calcd for $C_{38}H_{66}N_7O_{10}$: 780.5, found: *m*/*z*: $[M + H]^+$ 780.8, $[M + Na]^+$ 803.0, $[(M - C_4H_8) + H]^+$ 725.2.

Fmoc-Lys(Boc)-O-ArgoGel™ resin (13): The loading of the first amino acid was carried out according to the procedures of Sieber.

Fmoc-Alg-Pro-Gly-Alg-Lys(Boc)-OMe (solid phase) (14): This peptide was synthesized on Fmoc-Lys(Boc)-O-ArgoGel™ resin (885 mg, 0.32 mmol). After cleavage from the resin with KCN/MeOH two products were isolated: Fmoc-Alg-Pro-Gly-Alg-Lys(Boc)-OMe (**14**, 34.2 mg, 0.04 mmol, 13%) and H-Alg-Pro-Gly-Alg-Lys(Boc)-OMe (52 mg, 0.08 mmol, 25%).

Fmoc-Alg^{1}-Pro^{2}-Gly^{3}-Alg^{4}-Lys^{5}(Boc)-OMe (solution phase) (14): Fmoc-Alg-Lys(Boc)-OMe (**17**, 600 mg, 1.03 mmol) was dissolved in a solution of Et_2NH in THF (4 mL, 1:1 *v*/*v*) to remove the Fmoc group. After stirring for 1 h, the solvent was removed and the residue was coevaporated with toluene (2×5 mL) and $CHCl_3$ (2×5 mL) to remove any residual diethylamine. The free amine (**18**) was dissolved in DMF (15 mL) and tripeptide acid **16** (490 mg, 1 mmol) followed by BOP (442 mg, 1 mmol) and DIPEA (261 μL, 148 mmol) were added and the mixture was stirred overnight. The reaction mixture was concentrated in vacuo and the residue was dissolved in EtOAc (20 mL) and this solution was washed with 1N $KHSO_4$ (3×5 mL), 10% Na_2CO_3 (3×5 mL) and brine (1×5 mL), dried (Na_2SO_4), filtrated and evaporated in vacuo. Pentapeptide **14** was purified by column chromatography (DCM/MeOH 97.5:2.5 → DCM/MeOH 95:5 *v*/*v*) and obtained in 36% yield (309 mg). R_t: 18.4 min; R_f(DCM/MeOH 9:1 *v*/*v*): 0.47. ^{1}H NMR ($CDCl_3$, 500 MHz) Alg1: δ 7.77-7.30 (m, 8H, Fmoc), 6.50/6.13 (d, 1H, NH), 5.83-5.76 (m, 1H, Cγ*H*), 5.23-5.16 (m, 2H, Cδ*H*), 4.58 (m, 1H, Cα*H*), 4.44-4.28 (m, 3H, Fmoc), 2.63-2.46 (m, 2H, Cβ*H*); Pro2: 4.44 (m, 1H, Cα*H*), 3.81-3.66 (m, 2H, Cδ*H*), 2.17-2.10 (m, 2H, Cβ*H*), 2.11-1.93 (m, 2H, Cγ*H*); Gly3: 8.84/7.87 (d, 1H, NH), 4.22/4.19 and 3.80/3.77 (m, 2H, Cα*H*); Alg4: 7.66/7.61 (d, 1H, NH), 5.83-5.76 (m, 1H, Cγ*H*), 5.23-5.16 (m, 2H, Cδ*H*), 4.61 (m, 1H, Cα*H*), 2.63-2.46(m, 2H, Cβ*H*); Lys5: 7.52/7.22 (d, 1H, α*NH*), 5.92/5.08 (m, 1H, ε*NH*), 4.70/4.58 (m, 1H, Cα*H*), 3.69 (s, 3H, OCH_3), 3.14/3.05 (m, 2H, Cε*H*), 1.83-1.72 (m, 2H, Cβ*H*), 1.54 (m, 2H, Cγ*H*), 1.42 (s, 9H, Boc), 1.31 (m, 2H, Cδ*H*); ES-MS: calcd for $C_{44}H_{59}N_6O_{10}$: 831.4 found: *m*/*z*: $[M + H]^+$ 831.7, $[M + Na]^+$ 854.6, $[(M - C_4H_8) + H]^+$ 775.8, $[(M - C_5H_8O_2) + H]^+$ 731.7.

Fmoc-*cyclo*[Alg^{1}-Pro^{2}-Gly^{3}-Alg^{4}]-Lys^{5}(Boc)-OMe (15): Ring-closing metathesis reaction was carried out as described for **10**. Cyclic pentapeptide **15** was purified by silica gel column chromatography (DCM/MeOH 97.5:2.5 → DCM/MeOH 95:5 *v*/*v*) and was obtained as a brownish foam in 85% yield (252 mg). It was not possible to separate the *E*/*Z* isomers. R_t: 18.2 min; R_f(DCM/MeOH 9:1 *v*/*v*): 0.43; ^{1}H NMR ($CDCl_3$, 500 MHz) Alg1: δ 7.77-7.32 (m, 8H, Fmoc), 6.22/5.91 (d, 1H, NH), 5.53 (m, 1H, Cγ*H*), 4.76 (m, 1H, Cα*H*), 4.54-4.38 (m, 3H, Fmoc), 2.69-2.47 (m, 2H, Cβ*H*); Pro2: 4.54/4.41 (m, 1H, Cα*H*), 3.69-3.66 (m, 2H, Cδ*H*), 2.27/1.96 (m, 2H, Cβ*H*), 2.11-1.93 (m, 2H, Cγ*H*); Gly3: 7.93/9.34 (d, 1H, NH), 3.62-3.59/4.27-4.24 (dd, 2H, Cα*H*); Alg4: 7.44/7.26 (d, 1H, NH), 5.53 (m, 1H, Cγ*H*), 4.91 (m, 1H, Cα*H*), 2.69-2.47 (m, 2H,

CβH); Lys5: 7.46/7.06 (d, 1H, αNH), 5.78/4.71 (m, 1H, εNH), 4.41/4.38 (m, 1H, CαH), 3.71 (s, 3H, OCH_3), 3.20/3.03 (m, 3H, CεH), 1.89-1.66 (m, 2H, CβH), 1.57 (m, 2H, CγH), 1.43 (s, 9H, Boc), 1.33 (m, 2H, CδH); ES-MS: calcd for $C_{42}H_{54}N_6O_{10}$: 803.4, found: *m*/*z*: $[M + H]^+$ 803.9, $[M + Na]^+$ 825.7, $[(M - C_4H_8) + H]^+$ 747.6, $[(M - C_5H_8O_2) + H]^+$ 703.7.

Fmoc-Alg-Pro-Gly-OH (16) Boc-Pro-OH (646 mg, 3.0 mmol) and HCl.H-Gly-OMe (377 mg, 3.0 mmol) were coupled by BOP (1.3 g, 3.0 mmol) in the presence of DIPEA (1.2 mL) in DCM (20 mL) as described in the general procedure solution-phase peptide synthesis.

Boc-Pro-Gly-OMe: Yield: 768 mg (90%); R_f(DCM/MeOH 9:1 *v*/*v*): 0.48; ^{1}H NMR ($CDCl_3$, 300 MHz) Pro: δ 4.31 (m, 1H, CαH), 3.49 (m, 2H, CδH), 2.20-2.14 (m, 2H, CβH), 1.98-1.86 (m, 2H, CγH), 1.46 (s, 9H, Boc); Gly: 7.39/7.01 (d, 1H, NH), 4.04 (d, 2H, CαH), 3.74 (s, 3H, OCH_3); ES-MS: calcd for $C_{13}H_{22}N_2O_5Na$: 309.1, found: *m*/*z*: $[M + Na]^+$ 309.2, $[M + Na + CH_3CN]^+$ 350.2, $[(M - C_4H_8) + H]^+$ 228.1, $[(M - C_5H_8O_2) + H]^+$ 187.1.

Fmoc-Alg-Pro-Gly-OMe: Yield: 801 mg (94%); R_f(DCM/MeOH 9:1 *v*/*v*): 0.59; ^{1}H NMR ($CDCl_3$, 300 MHz) Alg: δ 7.74 (d, 2H, Fmoc), 7.57 (d, 2H, Fmoc), 7.25 (m, 4H, Fmoc), 6.01 (d, 1H, NH), 5.18 (m, 1H, CγH), 5.10 (m, 2H, CδH), 4.57 (m, 1H, CαH), 4.38-4.19 (m, 3H, Fmoc), 2.53-2.42 (m, 2H, CβH); Pro: 4.57 (m, 1H, CαH), 3.58 (m, 2H, CδH), 2.28-2.02 (m, 2H, CβH), 1.99-1.89 (m, 2H, CγH); Gly: 7.25 (d, 1H, NH), 3.97 (d, 2H, CαH), 3.69 (s, 3H, OCH_3); ES-MS: calcd for $C_{28}H_{32}N_3O_6$: 506.2, found: *m*/*z*: $[M + H]^+$ 506.4, $[M + Na]^+$ 528.35.

Fmoc-Alg-Pro-Gly-OH: Fmoc-Alg-Pro-Gly-OMe (500 mg, 0.99 mmol) was dissolved in THF (5 mL) and the methyl ester was saponified with 0.2 N $LiOH/H_2O$ (5 mL) for 2 h.[34] Then, THF was partially removed in vacuo and the aqueous solution was acidified with 1N $KHSO_4$ and subsequently extracted with EtOAc (3 × 20 mL). The combined EtOAc layers were washed with brine (1 × 15 mL), dried (Na_2SO_4), filtered and concentrated in vacuo. Fmoc-Alg-Pro-Gly-OH (**16**) was obtained in quantitative yield (492 mg). R_f(DCM/MeOH 9:1 *v*/*v*): 0.13; Alg: δ 7.74 (d, 2H, Fmoc), 7.58 (d, 2H, Fmoc), 7.24 (m, 4H, Fmoc), 6.08 (d, 1H, NH), 5.74 (m, 1H, CγH), 5.11 (m, 2H, CδH), 4.57 (m, 1H, CαH), 4.38-4.20 (m, 3H, Fmoc), 2.46-2.42 (m, 2H, CβH); Pro: 4.57 (m, 1H, CαH), 3.81-3.63 (m, 2H, CδH), 2.30-2.07 (m, 2H, CβH), 2.13-1.90 (m, 2H, CγH);

Gly: 7.26 (d, 1H, NH), 4.08-4.00 (dd, 2H, CαH); ES-MS: calcd for $C_{27}H_{30}N_3O_6$: 492.2, found: *m*/*z*: $[M + H]^+$ 492.5, $[M + Na]^+$ 514.4.

Fmoc-Alg-Lys(Boc)-OMe (17): Dipeptide **17** was obtained in 78% yield (820 mg). R_f(DCM/MeOH 9:1 *v*/*v*): 0.65. ^{1}H NMR ($CDCl_3$, 300 MHz) Alg: δ 7.73 (d, 2H, Fmoc), 7.57 (d, 2H, Fmoc), 7.29 (m, 4H, Fmoc), 5.74 (d, 1H, NH), 5.74 (m, 1H, CγH), 5.12 (m, 2H, CδH), 4.41 (m, 1H, CαH), 4.53-4.18 (m, 3H, Fmoc), 2.59-2.49 (m, 2H, CβH); Lys: 6.98 (d, 1H, αNH), 4.88 (m, 1H, εNH), 4.56 (m, 1H, CαH), 3.70 (s, 3H, OCH_3), 3.05-3.03 (m, 3H, CεH), 1.84-1.65 (m, 2H, CβH), 1.34-1.29 (m, 2H, CγH), 1.37-1.29 (m, 2H, CδH); ES-MS: calcd for $C_{32}H_{41}N_3O_7Na$: 602.3, found: *m*/*z*: $[M + Na]^+$ 602.6, $[(M - C_5H_8O_2) + H]_+$ 480.5.

H-Alg-Lys(Boc)-OMe (18): This compound was synthesized in situ as described in the solution phase synthesis of compound **14**.

Boc-Alg-Gly-Ala-D-Leu-Nle-Gly-Alg-OMe (solid phase) (19): This peptide was synthesized on Fmoc-Alg-O-ArgoGel™ (0.36 mmol/g) and obtained in 37% yield.

Boc-Alg^1-Gly^2-Ala^3-D-Leu^4-Nle^5-Gly^6-Alg^7-OMe (solution phase) (19): Pentapeptide **22** (1.05 g, 1.8 mmol) was dissolved in TFA/DCM 1:1 *v*/*v* (20 mL) to remove the Boc group and worked up as described. The obtained TFA-salt was dissolved in DMF (20 mL) and to this solution was added: Boc-Alg-Gly-OH (550 mg, 2.0 mmol) and HOBt.H_2O (307 mg, 2.0 mmol). The obtained mixture was cooled to -15 °C and EDCI (382 mg, 2.0 mmol) followed by DIPEA (784 µL, 4.4 mmol) were added. After stirring for 16 h, DMF was removed under reduced pressure and peptide **19** was purified by trituration with EtOAc. Yield: 1.18 g (89%); R_f(DCM/MeOH 9:1 *v*/*v*): 0.46; R_t: 16.6 min; ^{1}H NMR ($CDCl_3$/CD_3OH 14.5:1 *v*/*v*, 500 MHz) Alg1: δ 5.67 (d, 1H, NH), 5.74-5,72 (m, 1H, CγH), 5.20-5.11 (m, 2H, CδH), 4.08 (m, 1H, CαH), 2.57-2.42 (m, 2H, CβH), 1.43 (s, 9H, Boc); Gly2/6: 8.06/7.92 (d, 1H, NH), 4.08/3.65 (m, 2H, CαH); Ala3: 7.82 (d, 1H, NH), 4.29 (m, 1H, CαH), 1.33 (d, 3H, CβH); D-Leu4: 7.92 (d, 1H, NH), 4.37 (m, 1H, CαH), 1.76 (m, 2H, CβH), 1.59 (m, 1H, CγH), 0.91 (m, 6H, Cδ'H/CδH); Nle5: 7.76 (d, 1H, NH), 4.29 (m, 1H, CαH), 1.89 (m, 2H, CβH), 1.69 (m, 2H, CγH), 1.33 (m, 2H, CδH), 0.91 (m, 3H, CεH); Alg6: 7.61 (d, 1H, NH), 5.72 (m, 1H, CγH), 5.20-5.11 (m, 2H, CδH), 4.58 (m, 1H, CαH), 3.74 (s, 3H, OCH_3), 2.57-2.42 (m, 2H, CβH); ES-MS: calcd for $C_{35}H_{60}N_7O_{10}$: 738.4, found: *m*/*z*: $[M + H]^+$ 738.8, $[M + Na]^+$ 760.8, $[(M - C_4H_8) + H]^+$ 682.8, $[(M - C_5H_8O_2) + H]^+$ 638.8.

Boc-Alg-Gly-OH: Coupling was carried out as described in the general procedure solution-phase peptide synthesis.

Boc-Alg-Gly-OMe: Yield 91% (601 mg); R_f(DCM/MeOH (9:1 *v*/*v*): 0.68; ^{1}H NMR ($CDCl_3$, 300 MHz) Alg: δ 5.52 (m, 1H, NH), 5.77 (m, 1H, CγH), 5.18-5.11 (m, 2H, CδH), 4.29 (m, 1H, CαH), 2.54-2.44 (m, 2H, CβH), 1.43 (s, 9H, Boc); Gly: 7.38 (d, 1H, NH), 4.02 (d, 2H, CαH), 3.74 (s, 3H, OCH_3); ES-MS: calcd for $C_{13}H_{22}N_2O_5Na$: 309.1, found: *m*/*z*: $[M + Na]^+$ 309.2, $[M + Na + CH_3CN]^+$ 350.2,$[(M - C_4H_8) + H]^+$ 228.1, $[(M - C_5H_8O_2) + H]^+$ 187.1.

Boc-Alg-Gly-OH: Boc-Alg-Gly-OMe(570 mg, 2.0 mmol) was dissolved in THF (20 mL) and the methyl ester was saponified with 0.2 N $LiOH/H_2O$ (20 mL) during 3 h. Then, THF was partially removed in vacuo and the aqueous solution was acidified with 1 N $KHSO_4$ and subsequently extracted with EtOAc (3 × 20 mL). The combined EtOAc layers were washed with brine (1 × 15 mL), dried (Na_2SO_4), filtered and concentrated in vacuo. Yield: 550 mg (quant); R_f (DCM/MeOH 9:1 v/v): 0.22; ^{1}H NMR ($CDCl_3$, 300 MHz) Alg: δ 5.55 (m, 1H, NH), 5.80-5.69 (m, 1H, CγH), 5.18-5.12 (m, 2H, CδH), 4.35 (m, 1H, CαH), 2.51-2.42 (m, 2H, CβH), 1.43 (s, 9H, Boc); Gly: 10.06 (s, 1H, OH), 7.31 (d, 1H, NH), 4.03 (d, 2H, CαH). ES-MS: calcd for $C_{12}H_{20}N_2O_5Na$: 295.1, found: *m*/*z*: $[M + Na]^+$ 295.3, $[M + Na + CH_3CN]^+$ 336.3.

Boc-*cyclo*[Alg-Gly-Ala-D-Leu-Nle-Gly-Alg]-OMe (20): A solution of the fully protected linear heptapeptide **19** (200 mg, 0.27 mmol) was suspended in TCE, purged with nitrogen for 30 min at 60 °C, and RuII catalyst (66 mg, 0.08 mmol) was added followed by addition after 3 h of an extra amount of catalyst (23 mg, 0.03 mmol) and the reaction was allowed to react overnight at 60 °C under a weak flow of N_2. After concentrating the reaction mixture in vacuo, the cyclic product was purified by silicagel column chromatography (DCM/MeOH 97.5:2.5 → DCM/MeOH 95:5 → DCM/MeOH 9:1 *v*/*v*). Yield: 97 mg (50%); R_f(DCM/MeOH 9:1 *v*/*v*): 0.33. R_t: 15.4 min; ES-MS: calcd for $C_{33}H_{56}N_7O_{10}$: 710.4, found: *m*/*z*: $[M + H]^+$ 711.0, $[M + Na]^+$ 732.9, $[(M - C_4H_8) + H]^+$ 654.7, $[(M - C_5H_8O_2) + H]^+$ 610.7.

Boc-Ala-D-Leu-Nle-Gly-OH (21): Coupling and Boc-removal were carried out as was described in the general procedure solution-phase peptide synthesis.

Boc-Nle-Gly-OMe: R_f(DCM/MeOH 9:1 *v*/*v*): 0.67. ^{1}H NMR ($CDCl_3$, 300 MHz) Nle: δ 5.32 (d, 1H, NH), 4.18 (m, 1H, CαH), 1.87-1.80 (m, 1H, CβH), 1.65-1.55 (m, 1H,

CβH), 1.44 (s, 9H, Boc), 1.37-1.26 (m, 4H, CγH/CδH), 0.92-0.87 (m, 3H, CεH); Gly: 7.12 (d, 1H, NH), 4.02 (d, 2H, CαH) 3.74 (s, 3H, OCH_3).

Boc-D-Leu-Nle-Gly-OMe: R_f(DCM/MeOH 9:1 *v/v*): 0.71; ^{1}H NMR ($CDCl_3$, 300 MHz) D-Leu: δ 7.38 (d, 1H, NH), 4.08 (m, 1H, CαH), 1.76 (m, 1H, CγH), 1.63-1.52 (m, 2H, CβH), 1.42 (s, 9H, Boc), 0.98-0.86 (m, 6H, Cδ'H/CδH); Nle: 7.12 (d, 1H, NH), 4.52 (m, 1H, CαH), 1.67-1.61 (m, 1H, CβH), 1.63-1.52 (m, 1H, CβH), 1.47-1.42 (m, 2H, CγH), 1.32-1.26 (m, 2H, CδH), 0.98-0.86 (m, 3H, CεH); Gly: 7.54 (d, 1H, NH), 4.04-3.96 (dd, 2H, CαH), 3.72 (s, 3H, OCH_3); ES-MS: calcd for $C_{20}H_{38}N_3O_6$: 416.3, found: *m/z*: $[M + H]^+$ 416.5, $[M + Na]^+$ 438.5, $[(M - C_4H_8) + H]^+$ 360.3, $[(M - C_5H_8O_2) + H]^+$ 316.2.

Boc-Ala-D-Leu-Nle-Gly-OMe: The fully protected tetrapeptide was obtained in 84% yield (1.48 g) after purification by silicagel column chromatography (DCM/MeOH 97.5:2.5 *v/v*). R_f(DCM/MeOH 9:1 v/v): 0.43; ^{1}H NMR ($CDCl_3$, 300 MHz) Ala: δ 5.79 (d, 1H, NH), 4.50-4.45 (m, 1H, CαH), 1.41 (s, 9H, Boc), 1.34 (d, 3H, CβH); D-Leu: 7.81 (d, 1H, NH), 4.63-4.60 (m, 1H, CαH), 1.89-1.84 (m, 1H, CγH), 1.67-1.55 (m, 2H, CβH), 0.96-0.86 (m, 6H, Cδ'H/CδH); Nle: 7.55 (d, 1H, NH), 4.63-4.60 (m, 1H, CαH), 1.67-1.55 (m, 2H, CβH), 1.34-1.26 (m, 4H, CγH/CδH), 0.96-0.86 (m, 3H, CεH); Gly: 7.84 (d, 1H, NH), 4.08 (m, 1H, CαH), 3.70 (s, 3H, OCH_3), 3.65 (m, 1H, CαH); ES-MS: calcd for $C_{23}H_{43}N_4O_7$: 487.3, found: *m/z*: $[M + H]^+$ 487.6, $[M + Na]^+$ 509.6, $[(M - C_4H_8) + H]^+$ 431.5, $[(M - C_5H_8O_2) + H]_+$ 387.4.

Boc-Ala-D-Leu-Nle-Gly-OH: Boc-Ala-D-Leu-Nle-Gly-OMe (1.05 g, 2.15 mmol) was dissolved in THF (15 mL) and the methyl ester saponified with 0.2 N LiOH/H_2O (15 mL) during 2 h. Then, THF was partially removed in vacuo and the aqueous solution was acidified with 1 N $KHSO_4$ and subsequently extracted with EtOAc (3 × 20 mL). The combined EtOAc layers were washed with brine (1 × 15 mL), dried (Na_2SO_4), filtered and concentrated in vacuo. Tetrapeptide acid **21** was obtained in quantitative yield (1.01 g). R_f (DCM/MeOH 9:1 *v/v*): 0.09; ^{1}H NMR ($CDCl_3$, 300 MHz) Ala: δ 5.94 (d, 1H, NH), 4.50-4.45 (m, 1H, CαH), 1.41 (s, 9H, Boc), 1.34 (d, 3H, CβH); D-Leu: 7.81 (d, 1H, NH), 4.63-4.60 (m, 1H, CαH), 1.89-1.84 (m, 1H, CγH), 1.67-1.55 (m, 2H, CβH), 0.96-0.86 (m, 6H, Cδ'H/CδH); Nle: 7.57 (d, 1H, NH), 4.49 (m, 1H, CαH), 1.67-1.55 (m, 2H, CβH), 1.34-1.26 (m, 4H, CγH/CδH), 0.96-0.86 (m, 3H, CεH); Gly: 7.73

(d, 1H, NH), 4.09 (m, 1H, CαH), 3.88 (m, 1H, CαH); ES-MS: calcd for $C_{22}H_{41}N_4O_7$: 473.3, found: *m*/*z*: $[M + H]^+$ 473.3, $[M + Na]^+$ 495.5.

Boc-Ala-D-Leu-Nle-Gly-Alg-OMe (22): To a solution of tetrapeptide acid **21** (959 mg, 2.03 mmol) in DCM (25 mL) were added HOBt.H_2O (311 mg, 2.03 mmol) and HCl.H-Alg-OMe (338 mg, 3.03 mmol) followed by DIPEA (814 µL). The mixture was cooled to -15 °C and EDCI (390 mg, 2.03 mmol) was added. The obtained reaction mixture was stirred for 16 h. Then, the reaction mixture was worked up using the standard procedures as described above. Yield: 1.05 g (89%); R_f (DCM/MeOH 9:1 *v*/*v*): 0.47; ^{1}H NMR ($CDCl_3$, 300 MHz) Ala: δ 5.83 (d, 1H, NH), 4.30 (m, 1H, CαH), 1.41 (s, 9H, Boc), 1.33 (d, 3H, CβH); D-Leu: 8.00 (d, 1H, NH), 4.75 (m, 1H, CαH), 1.76 (m, 1H, CγH), 1.63 (m, 2H, CβH), 0.94-0.88 (m, 6H, Cδ'H/CδH); Nle: 7.94 (d, 1H, NH), 4.75 (m, 1H, CαH), 1.82/1.63 (m, 2H, CβH), 1.47-1.42 (m, 2H, CγH), 1.32-1.26 (m, 2H, CδH), 0.94-0.88 (m, 3H, CεH); Gly: 7.94 (d, 1H, NH), 4.05-3.98 (dd, 2H, CαH); Alg: 7.94 (d, 1H, NH), 5.80-5.68 (m, 1H, CγH), 5.12-5.06 (m, 2H, CδH), 4.68 (m, 1H, CαH), 3.72 (s, 3H, OCH_3), 2.50 (m, 2H, CβH). ES-MS: calcd for $C_{28}H_{50}N_5O_8$: 484.4, found: *m*/*z*: $[M + H]^+$ 484.5, $[M + Na]^+$ 606.6, $[(M - C_5H_8O_2) + H]^+$ 484.4.

Boc-Ile-Ala-cyclo[Alg-Ile-Ala-Leu-Alg]-OH (23): This compound was synthesized in situ as described in the synthesis of compound **25**.

H-cyclo[Alg-Pro-Gly-Alg]-Lys(Boc)-OMe (24): This compound was synthesized in situ as described in the synthesis of compound **25**.

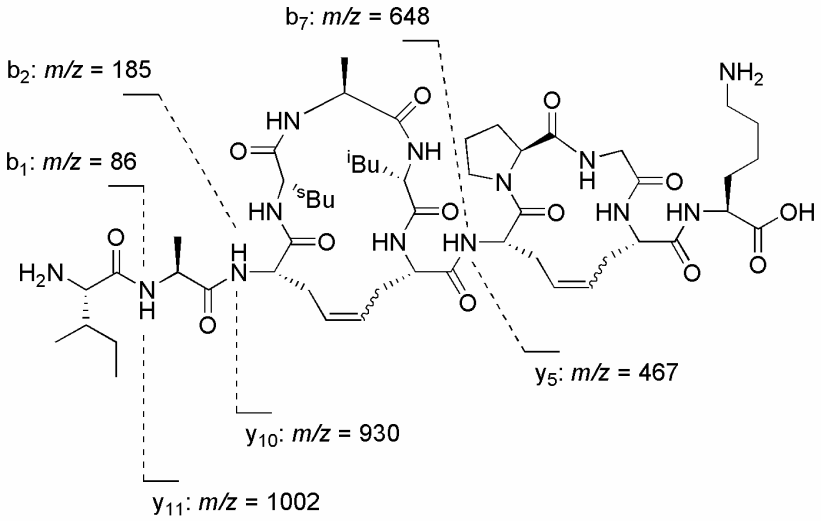
Boc-Ile-Ala-*cyclo*[Alg-Ile-Ala-Leu-Alg]-*cyclo*[Alg-Pro-Gly-Alg]-Lys(Boc)-OMe (25): *N-terminal deprotection of peptide **15**:* Fmoc-protected cyclic peptide **15** (200 mg, 0.25 mmol) was dissolved in THF (3 mL) and a solution of Me_2NH in water (40 wt%, 3 mL) was added and the reaction mixture was stirred for 30 min. Next, the volatiles were removed in vacuo and the residue was coevaporated with toluene (2 × 5 mL), $CHCl_3$ (2 × 5 mL) and DCM (2 × 5 mL) to remove any dimethylamine. The obtained free amine **24** was directly used in the next synthesis step.

*C-terminal deprotection of peptide **12**:* Cyclic peptide **12** (200 mg, 0.26 mmol) was suspended in THF (3 mL) and the suspension cooled on ice. Then, a solution of 0.2 N LiOH/H_2O (2.3 mL) was added dropwise. During the addition of the LiOH solution the reaction mixture became clear. After stirring for 4 h, the saponification was complete according to TLC analysis and the reaction mixture was concentrated at reduced

pressure. The aqueous solution was neutralized with 1M HCl after which compound **23** precipitated. Yield: 160 mg (81%); ES-MS: calcd for $C_{37}H_{64}N_7O_{10}$: 766.5, found: *m/z*: $[M + H]^+$ 766.8, $[M + Na]^+$ 788.6, $[(M - C_4H_8) + H]^+$ 710.6, $[(M - C_5H_8O_2) + H]^+$ 666.6.

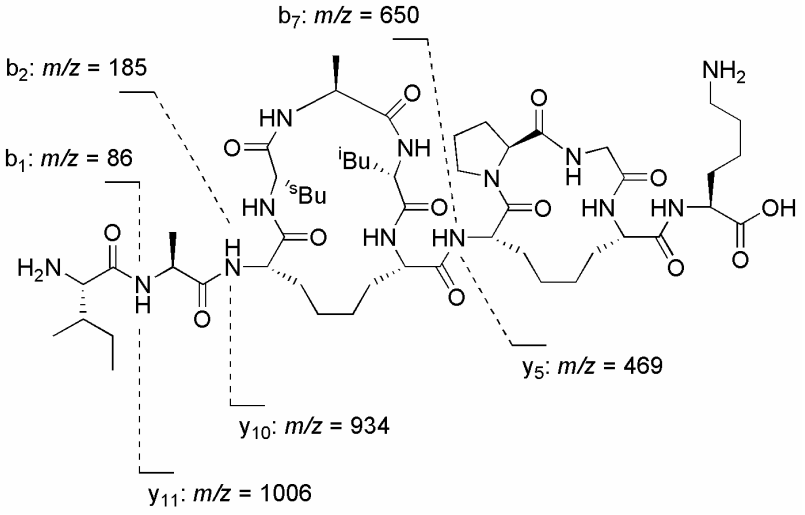
Amine **24** (0.25 mmol) was dissolved in DMF (10 mL) and peptide acid **23** (151 mg, 0.20 mmol) followed by HOBt.H_2O (31 mg, 0.20 mmol) were added and the solution was cooled to -15°C followed by the addition of EDCI (39 mg, 0.20 mmol). The obtained reaction mixture was stirred for 16 h. Then, DMF was evaporated in vacuo and the residue was triturated with EtOAc to isolate compound **25** in 65% yield (171 mg). ES-MS: calcd for $C_{64}H_{106}N_{13}O_{17}$: 1328.8, found: *m/z*: $[M + H]^+$ 1329.0, $[M + Na]^+$ 1351.5.

2TFA.H-Ile-Ala-*cyclo*[Alg-Ile-Ala-Leu-Alg]-*cyclo*[Alg-Pro-Gly-Alg]-Lys-OH (1): Peptide **25** (40 mg, 0.03 mmol) was saponified by 0.2N LiOH/H_2O in THF, neutralized and lyophilized. After treatment with TFA/TIS/H_2O (2 mL, 95:2.5:2.5 *v/v/v*) for 2 h the TFA solution was diluted with MTBE/hexane (10 mL, 1:1 *v/v*) and the fully unprotected peptide was precipitated. The peptide was collected by centrifugation and lyophilized from CH_3CN/H_2O (1:1 *v/v*). The peptide was purified by preparative HPLC and analyzed by mass spectrometry. Yield: 8.2 mg (24%); R_t: 14.2 min; ES-MS: calcd for $C_{53}H_{88}N_{13}O_{13}$: 1114.7, found: *m/z*: $[M + H]^+$ 1114.75; MALDI-TOF: $[M + H]^+$ 1114.97, $[M + Na]^+$ 1136.92; MS/MS analysis:



Bicyclic alkane-bridged compound 2: Bicyclic alkene-bridged compound **1** was dissolved in *tert*-butanol/H_2O 1:1 *v/v* (2 mL) and a catalytic amount of Pd (10%)/carbon was added. The reaction mixture was shaken in a Parr apparatus for 16 h in a H_2

atmosphere. The catalyst was removed by filtration over Hyflo and compound **2** was obtained in 42% yield. R_t: 14.7 min; ES-MS: calcd for $C_{53}H_{92}N_{13}O_{13}$: 1118.7, found: *m/z*: $[M + H]^+$ 1118.8, $[M + 2H]^{2+}$ 560.1; MALDI-TOF: $[M + H]^+$ 1119.0, $[M + Na]^+$ 1141.0; MS/MS analysis:



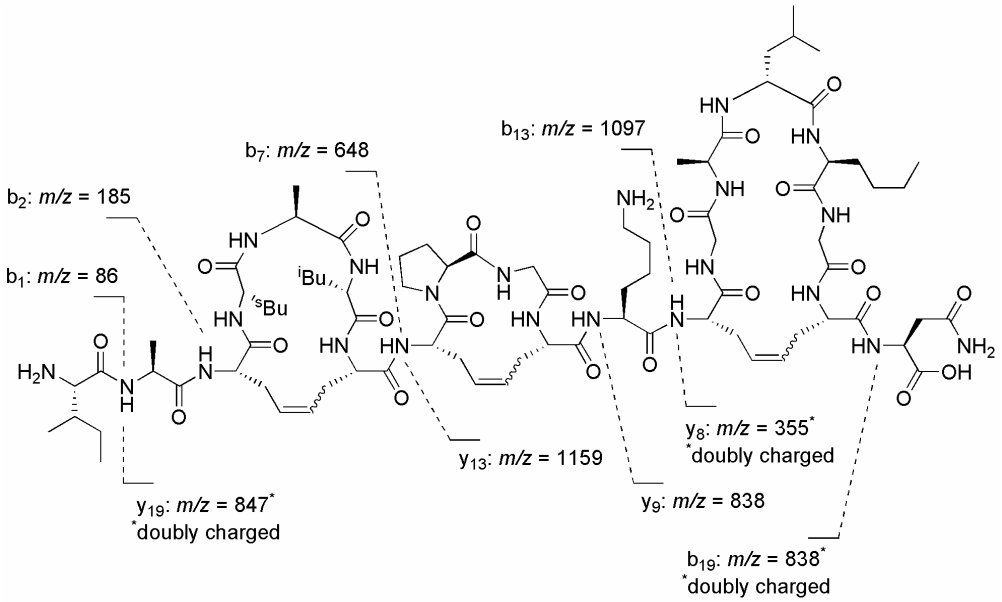
TFA.H-*cyclo*[Alg^{1}-Gly^{2}-Ala^{3}-D-Leu^{4}-Nle^{5}-Gly^{6}-Alg^{7}]-Asn-OMe (26) Cyclic peptide methyl ester **20** (101 mg, 0.14 mmol) was saponified by 0.2N LiOH/H_2O in THF as described. The corresponding peptide acid was isolated quantitatively. The acid was dissolved in DMF (5 mL) and H-Asn(Trt)-OMe (71 mg, 0.18 mmol) followed by HOBt•H_2O (21 mg, 0.14 mmol) were added and the solution was cooled to -15 °C, followed by the addition of EDCI (27 mg, 0.14 mmol). After stirring for 16 h the solvent was removed in vacuo and the residue was purified by column chromatography (DCM/MeOH 97.5:2.5 *v/v*) and Boc-*cyclo*[Alg^{1}-Gly^{2}-Ala^{3}-D-Leu^{4}-Nle^{5}-Gly^{6}-Alg^{7}]-Asn(Trt)-OMe was isolated in 65% yield (97 mg). R_f(DCM/MeOH 9:1 *v/v*): 0.33; R_t: 18.2 min; ^{1}H NMR ($CDCl_3$/CD_3OH 14.5:1 *v/v*, 500 MHz) Alg1: δ 5.74 (d, 1H, NH), 5.47-5.43 (m, 1H, CγH), 4.06 (m, 1H, CαH), 2.51/2.27 (m, 2H, CβH), 1.43 (s, 9H, Boc); Gly2: 8.10/7.88 (d, 1H, NH), 3.95-3.74 (m, 2H, CαH); Ala3: 7.80 (d, 1H, NH), 4.44 (m, 1H, CαH), 1.34 (d, 3H, CβH) D-Leu4: 7.58 (d, 1H, NH), 4.25 (m, 1H, CαH), 1.73 (m, 1H, CγH), 1.55 (m, 2H, CβH), 0.94-0.88 (m, 6H, Cδ'H/CδH); Nle5: 7.58 (d, 1H, NH), 4.44 (m, 1H, CαH), 1.77 (m, 2H, CβH), 1.59 (m, 2H, CγH), 1.28 (m, 2H, CδH), 0.94-0.88 (m, 3H, CεH); Alg6: 7.37 (d, 1H, NH), 5.47-5.43 (m, 1H, CγH), 4.44 (m, 1H, CαH), 2.37 (m, 2H, CβH); Asn(Trt): 7.69 (d, 1H, δNH), 7.58 (s, 1H, NH), 7.32-7.16 (m, 15H, Trt), 4.75 (m, 1H, CαH), 3.68 (s, 3H, OCH_3), 3.40 (d (*J* = 21 Hz), 1H, CβH), 2.81 (d (*J* = 21

Hz), 1H, CβH); ES-MS: calcd for $C_{56}H_{76}N_9O_{12}$: 1066.6, found: *m*/*z*: $[M + H]^+$ 1066.9, $[M + Na]^+$ 1089.0.

Boc-*cyclo*[Alg-Gly-Ala-D-Leu-Nle-Gly-Alg]-Asn(Trt)-OMe (80 mg, 80 (mol) was dissolved in TFA/TIS/H_2O (2 mL, 95:2.5:2.5 *v*/*v*/*v*) and stirred for 2 h. The TFA solution was diluted with MTBE/hexane (1:1 *v*/*v*) to precipitate the peptide. The crude peptide was isolated by centrifugation and finally lyophilized from CH_3CN/H_2O 1:1 *v*/*v*. Product **26** was used without further purification. Yield: 75 mg (94%); ES-MS: calcd for $C_{32}H_{54}N_9O_{10}$: 724.4, found: *m*/*z*: $[M + H]^+$ 724.8, $[M + Na]^+$ 747.6.

2HCl•H-Ile-Ala-*cyclo*[Alg-Ile-Ala-Leu-Alg]-*cyclo*[Alg-Pro-Gly-Alg]-Lys-*cyclo*[Alg-Gly-Ala-D-Leu-Nle-Gly-Alg]-Asn-OH (3): *C-terminal deprotection of peptide **25**:* Peptide **25** (125 mg, 0.09 mmol) was saponified by 0.2 N LiOH/H_2O in THF, subsequently neutralized and lyophilized. The reaction was monitored by ES-MS. Due to the low solubility, the obtained protected peptide acid was used without further purification. Yield: 118 mg (quant); ES-MS: calcd for $C_{63}H_{104}N_{13}O_{17}$: 1314.8, found: *m*/*z*: $[M + H]^+$ 1315.2, $[M + Li]^+$ 1320.7, $[(M - C_5H_8O_2) + H]^+$ 1215.2.

To a solution of the protected peptide acid (118 mg, 0.09 mmol) in DMF (5 mL) were added peptide amine **26** (75 mg), followed by HOBt.H_2O (14 mg, 90 μmol) and DIPEA (18 μL, 0.1 mmol), and the solution was cooled to -15 °C, subsequently followed by the addition of EDCI (17 mg, 90 μmol). After stirring for 16 h, the solvent was removed by evaporation and the residue was triturated with EtOAc. The crude peptide was isolated by centrifugation and subsequently dissolved in TFA/TIS/H_2O (2 mL, 95:2.5:2.5 *v*/*v*/*v*) to remove both Boc groups. After stirring for 2 h the TFA solution was diluted with MTBE/hexane (1:1 *v*/*v*) to precipitate product which was dissolved in CH_3CN/H_2O 1:1 *v*/*v* and lyophilized. This product was redissolved in 1M HCl/CH_3CN (1:3 *v*/*v*) and stirred until the acid hydrolysis of the methyl ester was complete according to ES-MS. After lyophilization the crude peptide **3** was purified by preparative HPLC. Yield: 7 mg (5%); R_t: 14.7 min; calcd for $C_{84}H_{137}N_{22}O_{22}$: 1807.1, found: *m*/*z*: MALDI-TOF: $[M + H]^+$ 1807.13, $[M + Na]^+$ 1829.1; MS/MS analysis:



Boc-D-Alg-Ile-Ala-Leu-Alg-OMe (solution phase) (27): The synthesis of this pentapeptide was performed in solution starting from HCl.H-Alg-OMe in 84% overall yield (over 7 reaction steps). R_f(DCM/MeOH 9:1 *v/v*): 0.58; R_t: 17.6 min; ^{1}H NMR (500 MHz, $CDCl_3/CD_3OH$ 14.5:1 *v/v*) D-Alg: *d* = 5.78 (m, 1H, CγH), 5.73 (br s, 1H, NH), 5.13-5.10 (m, 2H, CδH), 4.13 (m, 1H, CαH), 2.57-2.48 (m, 2H, CβH), 1.44 (s, 9H, Boc); Ile: 7.76 (d, 1H, NH), 4.45 (m, 1H, CαH), 1.87 (m, 1H, CβH), 1.65 (m, 2H, CγH), 0.84 (m, 6H, Cγ'H/CδH); Ala: 7.42 (br s, 1H, NH), 4.23 (m, 1H, CαH), 1.38 (br s, 3H, CβH); Leu: 7.60 (d, 1H, NH), 4.45 (m, 1H, CαH), 1.74 (m, 1H, CγH), 1.65 (m, 2H, CβH), 0.84 (m, 6H, CδH/Cδ'H); Alg: 7.55 (br s, 1H, NH), 5.78 (m, 1H, CγH), 5.13-5.09 (m, 2H, CδH), 4.13 (m, 1H, CαH), 3.74 (s, 3H, OCH_3), 2.57-2.43 (m, 2H, CβH); ES-MS: calcd for $C_{31}H_{54}N_5O_8$: 624.4, found: *m/z*: $[M + H]^+$ 624.4, $[M + Na]^+$ 646.7, $[(M - C_4H_8) + H]^+$ 568.6, $[(M - C_5H_8O_2) + H]^+$ 524.7.

Boc-*cyclo*[D-Alg-Ile-Ala-Leu-Alg]-OMe (28): The linear pentapeptide **27** (380 mg, 0.60 mmol) was dissolved in DCE (250 mL) and the solution was flushed with N_2 while it was heated to 60°C. After 30 min, a first portion of 2nd generation Grubbs' catalyst (48 mg, 0.06 mmol) was added followed by a second portion (16 mg, 0.02 mmol) after 3 h. Subsequently, the reaction mixture was stirred for an additional 2 h. Then, the solvent was removed by evaporation and the residue was purified by column chromatography on silica gel (eluens: DCM/MeOH 98/2 → DCM/MeOH 95:5 *v/v*). It was not possible to separate the *E* and *Z* isomer during the purification. Cyclic peptide **28** was obtained as a brownish foam in 90% yield (330 mg). R_f (DCM/MeOH

9:1 *v*/*v*): 0.50 and 0.51; R_t: 16.3 min and 19.5 min; ES-MS: calcd for $C_{29}H_{50}N_5O_8$: 596.4, found: *m*/*z*: $[M + H]^+$ 596.7, $[M + Na]^+$ 618.6, $[(M - C_4H_8) + H]^+$ 540.5, $[(M - C_5H_8O_2) + H]^+$ 496.6.

Boc-Ile^1-Ala^2-*cyclo*[D-Alg^3-Ile^4-Ala^5-Leu^6-Alg^7]-OMe (29): Cyclic peptide **28** (220 mg, 0.37 mmol) was treated with TFA to remove the Boc functionality as described in the general procedure. In the next step, the TFA salt was dissolved in DMF (5 mL) and the dipeptide acid Boc-Ile-Ala-OH (121 mg, 0.36 mmol) followed by HOBt•H_2O (62 mg, 0.41 mmol) were added. The obtained solution was cooled to -15°C and DIPEA (106 µL, 0.60 mmol) followed by EDCI (77 mg, 0.40 mmol) were added. This reaction mixture was stirred for 1 h at -15°C followed by 16 h at room temperature. Then, the solvent was removed at reduced presssure and the residue was triturated with EtOAc. Peptide **29** was obtained as a white solid in 47% yield (131 mg). R_f (DCM/MeOH 9:1 *v*/*v*): 0.39 and 0.42; R_t: 17.5 min; (500 MHz,$CDCl_3$/CD_3OH 14.5:1 *v*/*v*): Ile1: *d* = 5.06 (d, 1H, NH), 3.91 (m, 1H, CαH), 1.74 (m, 1H, CβH), 1.50 (m, 1H, CγH), 1.26 (m, 1H, CγH), 1.43 (s, 9H, Boc), 0.99-0.86 (m, 6H, Cγ'H/CδH); Ala2: 8.25 (br s, 1H, NH), 4.55-4.52 (m, 1H, CαH), 1.39 (m, 3H, CβH); D-Alg3: 7.59 (d, 1H, NH), 5.66-5.51 (m, 1H, CγH), 4.55-4.52 (m, 1H, CαH), 2.98-2.96 (m, 1H, CβH), 2.13-2.09 (m, 1H, CβH); Ile4: 7.38 (d, 1H, NH), 4.24-4.20 (m, 1H, CαH), 1.74 (m, 1H, CβH), 1.50 (m, 1H, CγH), 1.26 (m, 1H, CγH), 0.99-0.86 (m, 6H, Cγ'H/CδH); Ala5: 7.68 (d, 1H, NH), 4.55-4.52 (m, 1H, CαH), 1.39 (m, 3H, CβH); Leu6: 7.17 (d, 1H, NH), 4.55-4.52 (m, 1H, CαH), 1.68 (m, 2H, CβH), 1.26 (m, 1H, CγH), 0.99-0.86 (m, 6H, CδH/Cδ'H); Alg7: 7.94 (d, 1H, NH), 5.66-5.51 (m, 1H, CγH), 4.55-4.52 (m, 1H, CαH), 3.80 (s, 3H, OCH_3), 2.69-2.63 (m, 1H, CβH), 2.20-2.17 (m, 1H, CβH); ES-MS: calcd for $C_{38}H_{66}N_7O_{10}$: 780.5, found: *m*/*z*: $[M + H]^+$ 780.7, $[M + Na]^+$ 803.2, $[(M - C_4H_8) + H]^+$ 725.2, $[(M - C_5H_8O_2) + H]^+$ 680.5; MALDI-TOF: $[M + Na]^+$ 802.5, $[M + K]^+$ 818.5.

Fmoc-Pro-Gly-OH (30): Fmoc-Pro-OH (710 mg, 2.1 mmol), HCl.H-Gly-O^{t}Bu (412 mg, 2.5 mmol) and DIPEA (1 mL, 5.7 mmol) were dissolved in DCM (15 mL). To this solution, BOP (930 mg, 2.1 mmol) was added and the obtained reaction mixture was stirred for 16 h. The reaction work-up was carried out as described in the general procedures. Dipeptide Fmoc-Pro-Gly-O^{t}Bu was isolated in 89% yield (0.87 g). Then, the *tert*-butyl ester was dissolved in TFA/DCM (4 mL, 1:1 *v*/*v*) and stirred for 1 h and subsequently evaporated to dryness and coevaporated to remove any TFA. The crude protected dipeptide acid was used in the next synthesis steps.

Fmoc-Pro-Gly-Alg-Lys(Boc)-OMe (31): Fmoc-Alg-Lys(Boc)-OMe (**17**, 931 mg, 1.6 mmol) was dissolved in THF (2 mL) and Et_2NH (2 mL) was added to remove the Fmoc functionality. After stirring for 1 h, the volatiles were removed and the residue was coevaporated with toluene (2 × 5 mL) and $CHCl_3$ (2 × 5 mL) to remove any residual diethylamine. Then, H-Alg-Lys(Boc)-OMe (**18**) was dissolved in DMF (15 mL) and Fmoc-Pro-Gly-OH (**30**, 781 mg, 1.7 mmol) followed by BOP (751 mg, 1.7 mmol) and DIPEA (600 μL, 3.4 mmol) were added and the reaction mixture was stirred overnight. The solvent was removed in vacuo and the workup of the residue was as described in the general procedures. After purification by column chromatography on silica gel (eluens: DCM/MeOH 98/2 → DCM/MeOH 95:5 *v*/*v*), tetrapeptide **31** was obtained as a white solid in 80% yield (940 mg). R_f(DCM/MeOH 9:1 *v*/*v*): 0.47; ^{1}H NMR (500 MHz, $CDCl_3$) Pro: *d* = 7.77-7.29 (m, 8H, arom H Fmoc), 4.44 (m, 1H, CαH), 4.41-4.22 (m, 3H, CH_2/CH Fmoc), 3.74-3.53 (m, 2H, CδH), 2.13 (m, 2H, CβH), 2.04-1.91 (m, 2H, CγH); Gly: 8.35 (br s, <1H, NH), 7.58-7.50 (m, <1H, NH), 4.44 (m, 1H, CαH), 3.74-3.53 (m, 1H, CαH); Alg: 7.58-7.50 (m, 1H, NH), 5.74-5.70 (m, 1H, CγH), 5.07-5.00 (m, 2H, CδH), 4.57-4.47 (m, 1H, CαH), 2.65-2.48 (m, 2H, CβH); Lys: 7.03/6.91 (double br s, 1H, αNH), 5.45/4.87 (double br s, 1H, εNH), 4.57-4.47 (m, 1H, CαH), 3.68 (s, 3H, OCH_3), 3.03/2.93 (double m, 2H, CεH), 2.02-1.54 (br m, 4H, CβH/CγH), 1.42 (s, 9H, Boc), 1.37-1.31 (m, 2H, CδH); ES-MS: calcd for $C_{39}H_{51}N_5O_9$: 734.4 found: *m*/*z*: $[M + H]^+$ 734.6, $[M + Na]^+$ 756.6, $[(M - C_4H_8) + H]^+$ 678.7, $[(M - C_5H_8O_2) + H]^+$ 634.6.

Fmoc-D-Alg-Pro-Gly-Alg-Lys(Boc)-OMe (32): Tetrapeptide **31** (421 mg, 0.57 mmol) was dissolved in THF (3 mL) and Et_2NH (3 mL) was added and this solution was stirred for 1 h. Then, the solvents were removed in vacuo and the residue was coevaporated with toluene (2 × 5 mL) and $CHCl_3$ (2 × 5 mL) to remove any residual diethylamine. The obtained free amine was dissolved in DMF (10 mL) and Fmoc-D-Alg-OH (192 mg, 0.57 mmol), BOP (266 mg, 0.60 mmol) followd by DIPEA (160 mL, 0.90 mmol) were added and the reaction mixture was stirred for 16 h. The reaction mixture was concentrated in vacuo and the residue was redissolved in EtOAc (20 mL). This solution was subsequently washed with 1 N $KHSO_4$ (3 × 5 mL), 10% Na_2CO_3 (3 × 5 mL) and brine (3 × 5 mL), dried (Na_2SO_4) and evaporated in vacuo. After purification by column chromatography on silica gel (eluens: DCM/MeOH 98/2 → DCM/MeOH 95:5 *v*/*v*) pentapeptide **32** was obtained in 74% yield (370 mg). R_f (DCM/MeOH 9:1

v/*v*): 0.56; R_t: 18.6 min; ^{1}H NMR (500 MHz, $CDCl_3$) D-Alg: *d* = 7.76-7.30 (m, 8H, arom H Fmoc), 5.74-5.72 (m, 1H, CγH), 5.62 (br s, 1H, NH), 5.19-5.01 (m, 2H, CδH), 4.61 (m, 1H, CαH), 4.30-4.18 (m, 3H, CH_2/CH Fmoc), 2.64-2.44 (m, 2H, CβH); Pro: 4.30-4.18 (m, 1H, CαH), 3.98/3.62 (double m, 2H, CδH), 2.21-2.01 (m, 4H, CβH/CγH); Gly: 9.07/7.68 (double br s, 1H, NH), 3.98/3.62 (double m, 2H, CαH); Alg: 7.56/7.40 (double br s, 1H, NH), 5.74-5.72 (m, 1H, CγH), 5.19-5.01 (m, 2H, CδH), 4.30-4.18 (m, 1H, CαH), 2.64-2.44 (m, 2H, CβH); Lys: 6.98/6.21 (double br s, 1H, αNH), 5.06 (m, 1H, εNH), 4.30-4.18 (m, 1H, CαH), 3.73/3.71 (double s, 3H, OCH_3), 3.07/2.91 (double m, 2H, CεH), 1.64-1.85 (m, 2H, CβH), 1.54 (m, 2H, CγH), 1.46/1.42 (double s, 9H, Boc), 1.37 (m, 2H, CδH); ES-MS: calcd for $C_{44}H_{59}N_6O_{10}$: 831.4 found: *m*/*z*: $[M + H]^+$ 831.6, $[M + Na]^+$ 853.6, $[(M - C_4H_8) + H]^+$ 775.7, $[(M - C_5H_8O_2) + H]^+$ 731.7.

Fmoc-D-Alg(βMe)-Pro-Gly-Alg-Lys(Boc)-OMe (33): The synthesis of this compound was carried out as described for **32**. Quantities used: Fmoc-Pro-Gly-Alg-Lys-(Boc)-OMe (**31**, 421 mg, 0.57 mmol), Fmoc-D-Alg(βMe)-OH[5] (212 mg, 0.60 mmol). Pentapeptide **33** was obtained in 75% yield (360 mg). R_f (DCM/MeOH 9:1 *v*/*v*): 0.55; R_t: 19.2 min; ^{1}H NMR (500 MHz, $CDCl_3$) D-Alg(βMe): *d* = 7.75-7.07 (m, 8H, arom H Fmoc), 5.42/5.26 (double br s, 1H, NH), 5.73-5.70 (m, 1H, CγH), 5.22-5.05 (m, 2H, CδH), 4.49-4.39 (m, 1H, CαH), 4.44-4.28 (m, 3H, CH_2/CH Fmoc), 2.49 (m, 1H, CβH), 1.29-1.04 (m, 3H, Cβ'H); Pro: 4.49-4.39 (m, 1H, CαH), 3.75-3.65 (m, 2H, CδH), 2.28-2.05 (m, 4H, CβH/CγH); Gly: 9.04/7.45 (double br s, 1H, NH), 4.02/3.65 (double m, 2H, CαH); Alg: 7.54/7.31 (double br s, 1H, NH), 5.73-5.69 (m, 1H, CγH), 5.22-5.05 (m, 2H, CδH), 4.49-4.39 (m, 1H, CαH), 2.80-2.39 (m, 2H, CβH); Lys: 7.00/6.60 (double br s, 1H, αNH), 5.00 (m, 1H, εNH), 4.49-4.39 (m, 1H, CαH), 3.68 (s, 3H, OCH_3), 3.28-3.08 (m, 2H, CεH), 1.86-1.63 (m, 4H, CβH/CγH), 1.44 (s, 9H, Boc), 1.46-1.42 (m, 2H, CδH); ES-MS: calcd for $C_{45}H_{61}N_6O_{10}$: 845.4 found: *m*/*z*: $[M + H]^+$ 845.6, $[M + Na]^+$ 867.5, $[(M - C_4H_8) + H]^+$ 789.4, $[(M - C_5H_8O_2) + H]^+$ 745.7.

Fmoc-*cyclo*[D-Alg-Pro-Gly-Alg]-Lys(Boc)-OMe (34): Linear peptide **32** (270 mg, 0.33 mmol) was dissolved in DCE (200 mL) and the solution was flushed with N_2 while it was heated to 60°C. After 30 min, second generation Grubbs' catalyst (31 mg, 0.036 mmol) was added and the reaction mixture was stirred for 5 h at 60°C. Then, the solvent was removed by evaporation and the residue was purified by column chromatography on silica gel (eluens: DCM/MeOH 98/2 → DCM/MeOH 95:5

v/*v*). Cyclic peptide **34** was obtained as a brownish foam in 76% yield (202 mg). R_f (DCM/MeOH 9:1 *v*/*v*): 0.54/0.53; R_t: 18.1 min; ES-MS: calcd for $C_{42}H_{54}N_6O_{10}$: 803.4, found: *m*/*z*: $[M + H]^+$ 803.7, $[M + Na]^+$ 825.6, $[(M – C_4H_8) + H]^+$ 747.6, $[(M – C_5H_8O_2) + H]^+$ 703.6.

Fmoc-*cyclo*[D-Alg(β-Me)-Pro-Gly-Alg]-Lys(Boc)-OMe (35): Linear peptide **33** (270 mg, 0.32 mmol) was dissolved in DCE (200 mL) and the solution was flushed with N_2 while it was refluxed. After 30 min, second generation Grubbs' catalyst (27 mg, 0.032 mmol) was added and the reaction mixture was refluxed for 16 h. After 3 h and 6 h, respectively, an additional amount of catalyst was added: 17 mg (0.020 mmol) and 13 mg (0.015 mg), respectively. Although the reaction was not complete according to TLC analysis, the solvent was removed in vacuo and the residue was purified by column chromatography on silica gel (eluens: DCM/MeOH 98/2 → DCM/MeOH 95:5 *v*/*v*). Cyclic peptide **35** was obtained in 29% yield (75 mg). R_f(DCM/MeOH 9:1 *v*/*v*): 0.53; R_t: 18.5 min; ES-MS: calcd for $C_{44}H_{57}N_6O_{10}$: 817.4 found: *m*/*z*: $[M + H]^+$ 817.8, $[M + Na]^+$ 839.6, $[(M – C_5H_8O_2) + H]^+$ 717.7; MALDI-TOF: $[M + Na]^+$ 839.574; $[M + K]^+$ 855.528.

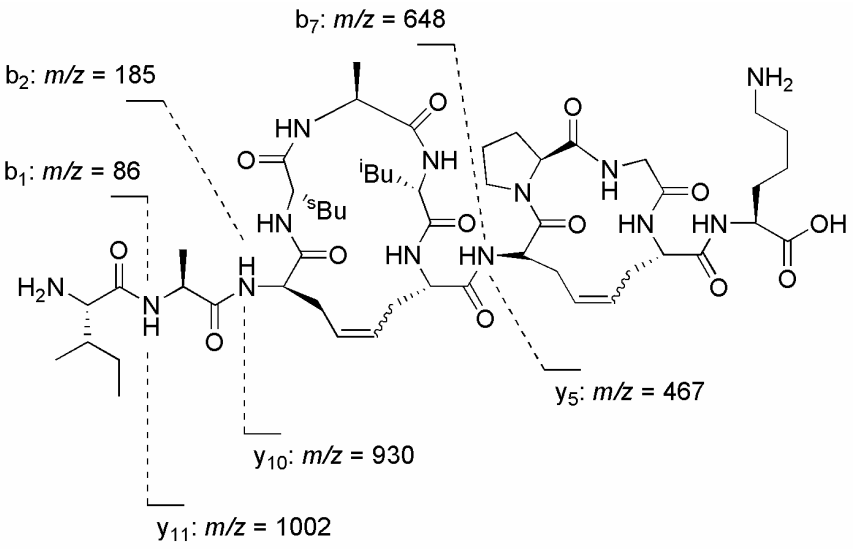
Boc-Ile-Ala-*cyclo*[D-Alg-Ile-Ala-Leu-Alg]-OH (36): This compound was synthesized in situ as described in the synthesis of compound **4**.

H-*cyclo*[D-Alg-Pro-Gly-Alg]-Lys(Boc)-OMe (37): This compound was synthesized in situ as described in the synthesis of compound **4**.

H-Ile-Ala-*cyclo*[D-Alg-Ile-Ala-Leu-Alg]-*cyclo*[D-Alg-Pro-Gly-Alg]-Lys-OH (4): Peptide methyl ester **29** (136 mg, 0.17 mmol) was saponified in THF/20% aqueous LiOH (4.5 mL, 2:1 *v*/*v*) during 3 h at 0°C. Subsequently, the reaction mixture was acidified with 1 M HCl to pH 2 and the solvents were removed in vacuo. The residue was redissolved in H_2O and lyophilized. The obtained peptide acid **36** was used in the next reaction steps without further purification.

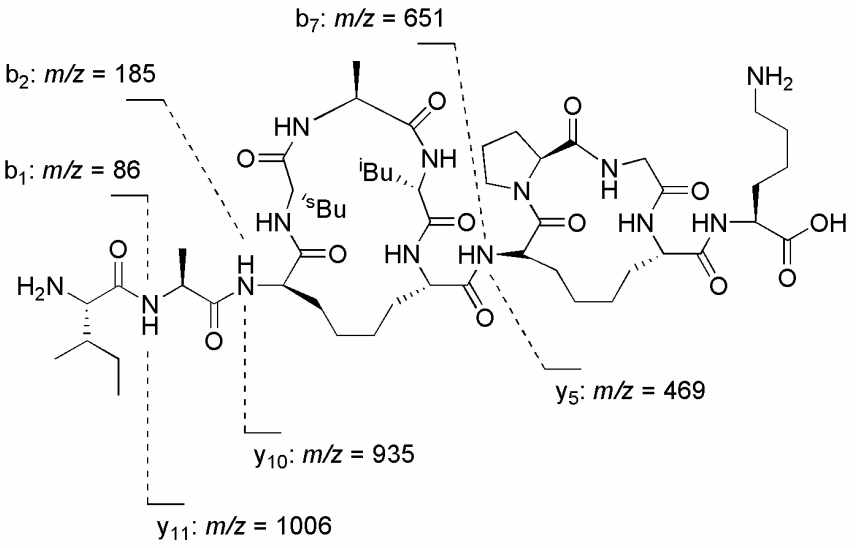
Fmoc-protected peptide **34** (50 mg, 62 μmol) was dissolved in THF (2 mL) and Et_2NH (2 mL) was added. The obtained reaction mixture was stirred for 1 h. Then, the solvents were removed in vacuo and the residue was coevaporated with toluene (2 × 5 mL), $CHCl_3$ (2 × 5 mL) and DCM (2 × 5 mL) to remove any residual diethylamine. The obtained free amine **37** was used as such in the next reaction step.

Amine **37** (62 µmol) was dissolved in DMF and acid **36** (72 mg, 80 µmol) and HOBt•H_2O (13 mg, 85 µmol) were added and the solution was cooled to -15°C. Finally, EDCI (15 mg, 80 µmol) was added and the reaction mixture was stirred for 1 h at -15°C followed by 16 h at room temperature. Then, DMF was removed in vacuo and the residue was redissolved in EtOAc (20 mL). This solution was subsequently washed with 1 N $KHSO_4$ (3 × 5 mL), 10% Na_2CO_3 (3 × 5 mL) and brine (3 × 5 mL), dried (Na_2SO_4) and evaporated at reduced pressure to yield intermediate **39**. As described above, the methyl ester was saponified by treatment with THF/20% aqueous LiOH (4.5 mL, 2:1 *v*/*v*) for 3 h at 0°C. After acidic workup the peptide acid was lyophilized and subsequently dissolved in TFA/TIS/H_2O (2 mL, 95:2.5:2.5 *v*/*v*/*v*) and stirred for 2 h at room temperature. The peptide was precipitated with MTBE/hexane 1:1 *v*/*v* at -20°C and finally lyophilized from CH_3CN/H_2O 1:1 *v*/*v*. After purification by HPLC bicyclic peptide **4** was obtained in an overall yield of 26% (11 mg) over five reaction steps. R_t: 14.2 min; ES-MS: calcd for $C_{53}H_{88}N_{13}O_{13}$: 1114.70, found: *m*/*z*: $[M + H]^+$ 1115.25, $[M + 2H]^{2+}$ 558.33; MALDI-TOF: $[M + H]^+$ 1114.65, $[M + Na]^+$ 1136.58; MS/MS:



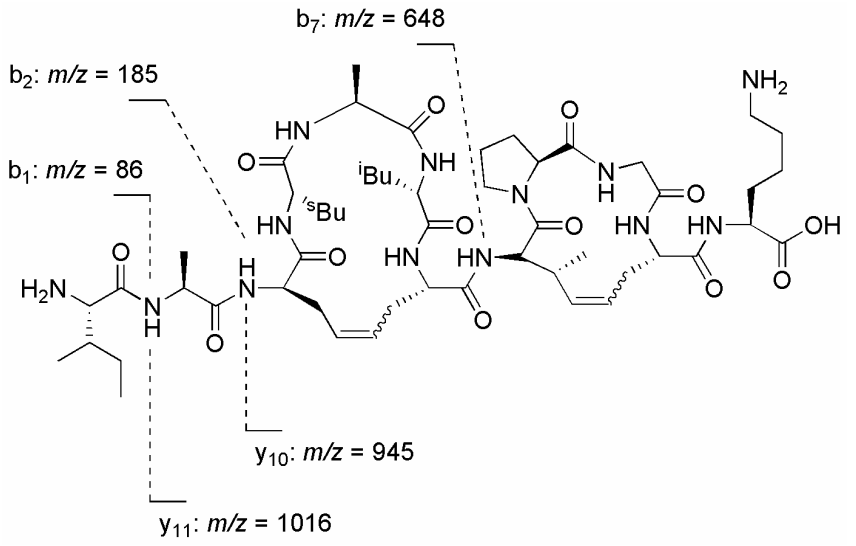
Bicyclic alkane-bridged compound 5: Bicyclic alkene-bridged compound **4** was dissolved in *tert*-butanol/H_2O 1:1 *v*/*v* (2 mL) and a catalytic amount of Pd (10%)/carbon was added. The reaction mixture was shaken in a Parr apparatus for 16 h in a H_2 atmosphere. The catalyst was removed by filtration over Hyflo and compound **5** was obtained in 33% yield. R_t: 16.2 min; ES-MS: calcd for $C_{53}H_{92}N_{13}O_{13}$: 1118.70, found:

m/z: $[M + H]^+$ 1118.90, $[M + 2H]^{2+}$ 560.40; MALDI-TOF: $[M + H]^+$ 1118.6, $[M + Na]^+$ 1140.5; MS/MS:

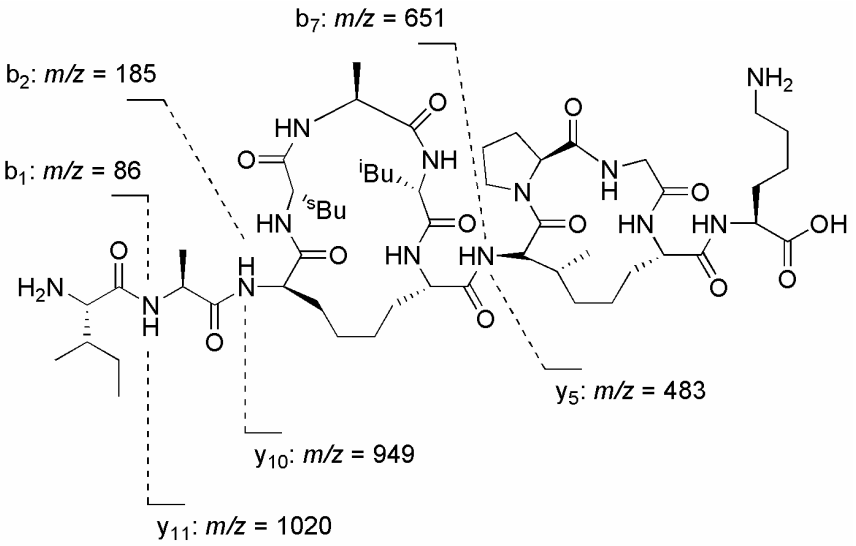


H-*cyclo*[D-Alg(β Me)-Pro-Gly-Alg]-Lys(Boc)-OMe (38): This compound was synthesized in situ as described in the synthesis of compound **6**.

H-Ile-Ala-*cyclo*[D-Alg-Ile-Ala-Leu-Alg]-*cyclo*[D-Alg(β Me)-Pro-Gly-Alg]-Lys-OH (6): The synthesis of this compound was carried out as described for bicyclic peptide **4**. Quantities used: peptide acid **36** (72 mg, 80 μmol) and Fmoc-protected peptide **35** (51 mg, 62 μmol). After purification by HPLC bicyclic peptide **6** was obtained in an overall yield of 35% (12 mg). R_t: 17.8 min; ES-MS: calcd for $C_{54}H_{90}N_{13}O_{13}$: 1128.67, found: *m/z*: $[M + H]^+$ 1128.95, $[M + 2H]^{2+}$ 565.35; MALDI-TOF: $[M + H]^+$ 1128.89, $[M + Na]^+$ 1150.85; $[M + K]^+$ 1166.81; MS/MS:



Bicyclic alkane-bridged compound 7: This compound was synthesized as described for **5**. Yield: 33%; R_t: 12.8 min; ES-MS: calcd for $C_{54}H_{94}N_{13}O_{13}$: 1132.67, found: *m/z*: $[M + H]^+$ 1132.70, $[M + 2H]^{2+}$ 567.5; MALDI-TOF: $[M + H]^+$ 1132.80, $[M + Na]^+$ 1154.70; $[M + K]^+$ 1170.70; MS/MS:



Computational modeling: The modeling experiments were performed with MacroModel 7.0[6] on a SiliconGraphics O_2 workstation using the organic builder and the peptide builder in the grow mode. MMFF was used as forcefield.[7] Structure minimization was performed on a Silicon Graphics Origin 200 Server and molecular mechanics calculations were performed with the next settings: MMFF (planar N's), PRCG, CCrit 0.01 kJ/molÅ, Iterations > max. Finally, a conformational search was carried out starting with the minimized structure using a Monte Carlo run which generated 1000 structures (all appropriate single bonds will become variable, all double bonds, amide bonds and ester bonds will become constrained, potential chiral centers will be set and flexible rings will be opened). The goal of conformational searching was to locate the low-energy conformations of the structure of interest. The settings of a standard conformational search: Monte Carlo Multiple Minimum (MCMM); Number of Steps: 1000; Solvent: water. After each MCMM step, the structure was again minimized (with the same settings as above).

References and Notes

[1] E. von Arx, M. Faupel, M.J. Brugger, *J. Chromatogr.* **1976**, *120*, 224-228.

[2] R. Knorr, A. Trzeciak, W. Bannwarth, D. Gillesen, *Tetrahedron Lett.* **1989**, *30*, 1927-1930.

[3] B. Castro, J.R. Dormoy, G. Evin, C. Selve, *Tetrahedron* **1975**, *31*, 1219-1222.

[4] E. Kaiser, R.L. Colescott, C.D. Bossinger, P.I. Cook, *Anal. Biochem.* **1970**, *34*, 595-598.

[5] This amino acid was synthesized according to: a) K. Sakaguchi, H. Suzuki, Y. Ohfune, *Chirality* **2001**, *13*, 357-365; b) I. Izzo, E. Avallone, L. Della Corte, N. Maulucci, F. De Riccardis, *Tetrahedron Asymm.* **2004**, *15*, 1181-1186; c) Y. Morimoto, M. Takaishi, T. Kinoshita, K. Sakaguchi, K. Shibata, *Chem Commun.* **2002**, 42-43.

[6] F. Mohamadi, N.C.J. Richards, W.C. Guida, R. Liskamp, M. Lipton, C. Caufield, G. Chang, T. Hendrickson, W.C. Still, *J. Comput. Chem.* **1990**, *11*, 440-467.

[7] a) J. Halgren, *J. Comput Chem.* **1996**, *17*, issues 5 and 6. (will contain five articles introducing MMFF94 as a good force field for biopolymers (peptides and proteins) and many organic molecules); b) RCG: Conjugate gradient minimization using the Polak-Ribiere first derivative method with restarts every 3*N* iterations. Should not find saddle points. Best general minimization method for energy minimization. BatchMin code for carrying out this method is highly vectorized for efficient operation on vector hardware: E. Polak and G. Ribiere, *Revue Française Informat. Recherche Operationelle* **1969**, *16*, 35; c) Convergence criterion - energy, movement or gradient. Default is gradient (first derivative RMS) convergence (criterion = 0.01 kJ/molÅ); d) Iterations/stop, sets the maximum number of iterations BatchMin will use to energy minimize a structure.