Supporting Information:

Ring-Closing Alkyne Metathesis Approach toward the Synthesis of Alkyne Mimics of Thioether A, B, C and DE-ring Systems of the Lantibiotic Nisin Z

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Instruments and Methods. Unless stated otherwise, chemicals were obtained from commercial sources and used without any further purification. Peptide grade or pro analysi solvents were purchased from Biosolve (Valkenswaard, the Netherlands) and dried on 4Å MS (MeOH: 3Å MS) before use. *N*,*N*-Diisopropylethylamine (DIPEA) was distilled from ninhydrin and KOH-pellets. R_f values were determined by thin layer chromatography (TLC) on Merck precoated silicagel $60F_{254}$ plates. Spots were visualized with UV quenching, ninhydrin or with Cl_2-TDM.[1] Solid phase peptide synthesis was monitored with the Kaiser test[2] and the loading of the resin[3] was determined using a Heλios β UV/VIS-spectrophotometer at λ 300 nm. Solvents were removed by rotary evaporation under reduced pressure at 40 °C. Melting points were determined using a Büchi melting point apparatus accordig to dr. Tottoli and were uncorrected. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were measured on a Varian G-300 spectrometer. ^{1}H NMR 500 MHz spectra were recorded on a Varian INOVA-500 spectrometer. ^{1}H NMR chemical shifts are given in ppm (δ) relative to TMS and ^{13}C NMR chemical shifts are given in ppm (δ) relative to $CDCl_3$ (77.0 ppm). ^{13}C NMR spectra were recorded using the attached proton test (APT) pulse sequence. Analytical HPLC runs were performed on a Shimadzu automated HPLC (SPD-10A VP) system equipped with an evaporative light scattering detector (PL-ELS 1000, Polymer Laboratories) and a UV/VIS detector operating at 220/254 nm. Preparative HPLC runs were performed on a Gilson HPLC workstation. Chiral HPLC analysis was performed on an analytical reverse-phase column (Astec, Teicoplanin, Chirobiotic T, 250 × 4.6 mm) at a steady flow of

0.5 mL/min with 1% TEA in H_2O adjusted to pH 4 with AcOH/MeOH 1:1 v/v as eluens. Electrospray ionisation (ESI) mass spectrometry was carried out on a Shimadzu LCMS QP-8000 quadrupole benchtop spectrometer coupled to a QP-8000 data system. MS/MS-spectra were measured on a Micromass Quattro Ultima or a Micromass Q-TOF mass spectrometer.Optical rotations were measured on a Jasco P-1010 Polarimeter.

Solid phase peptide synthesis: Peptides **14** and **16** were synthesized manually on a 0.25 mmol scale on plain Argogel resin. Each synthetic cycle consisted of *N*-α-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 equivalents 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.[2] 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 KHSO4 (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 generally 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, 25 × 2.2 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 purities were evaluated by analytical HPLC on an Adsorbosphere XL C8 column (90Å pore size, 5 μm particle size, 25 × 0.46 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 and ^1H NMR (300 or 500 MHz). The mass of each analog 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 ^1H NMR COSY, TOCSY and/or ROESY spectra.

General procedure of ring closing alkyne metathesis (RCAM). All RCAM reactions were carried out under argon in flame-dried glassware using Schlenk techniques. A solution of the peptide and the catalyst ($(tBuO)_3W{\equiv}CCMe_3$) in toluene stirred at 80 ^oC till TLC analysis showed the completion of the reaction. Water (1 mL) was added to the reaction mixture and stirred for 10 min to quench the catalyst. After the evaporation of the solvent the product was purified by column chromatography.

Alkylation of the Gly/Ni/BPB-complex with 1-Bromo-2-butyne: The Gly/Ni/BPB-complex[4,5] (6 g, 12 mmol) and NaOH (1.2 g, 30 mmol) were dissolved in CH_3CN (60 mL) and 1-bromo-2-butyne (1.2 mL, 13.4 mmol) was added dropwise. The deeply red mixture was stirred for 1.5 h at room temperature. Then, the excess of NaOH was neutralized with HCl (0.1 M, 180 mL). The alkylation product was extracted into CH_2Cl_2. The combined organic layers were dried ($MgSO_4$) and evaporated to dryness. The product was purified by silicagel column chromatography (eluens $CHCl_3$/acetone 5:1 v/v) to obtain both diastereoisomers as red crystals with 61% yield (4 g)**.** R_f: 0.55 (CH_2Cl_2/acetone 2:1 v/v). $[\alpha]_D^{21}$ +268 (*c* 0.1 MeOH). ^1H NMR (300 MHz, $CDCl_3$) δ: 1.93-2.01 (s, 3H, _H-Bug); 2.02-2.18 (m, 2H, _Pro); 2.21-2.35 (m, 1H, βH-Bug); 2.44-2.68 (m, 2H, [1H, (βH-Bug), 1H, (γH-Pro)]; 2.72-2.94 (m, 1H, γH-Pro); 3.41-3.50 (m, 1H, δH-Pro); 3.51-3.61 (m, 1H, δH-Pro); 3.61-3.82 (m, 2H, [1H, d, (CH_2-Ph), 1H, m, (αH-Pro)]; 3.96-4.04 (q, 1H, αH-Bug); 4.42-4.52 (d, 1H, CH_2-Ph); 6.61-8.24 (m, 14H, Ar-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 4.1 (εC-Bug); 23.0 (γC-Pro); 23.9 (βC-Pro); 30.6 (βC-Bug); 56.4 (δC-Pro); 62.8 (Ph-CH_2-N); 68.1 (αC-Bug); 70.0 (αC-Pro); 73.8 (γC-Pro); 80.4 (δC-Pro); 120.6, 123.6, 126.5, 127.7, 128.8, 129.0, 129.7, 131.5, 132.3, 133.4 (Ar-CH); 126.3, 133.0, 133.9, 142.5 (Ar-C); 171.5 (C=N); 178.8 (C=O, Pro); 180.2 (C=O, Gly). ESI-MS: *m/z* 550.45 $[M+H]^+$, ($C_{31}H_{29}N_3NiO_3$: M: 549.16).

(*S*)-2-amino-5-hexynoic acid (H-Bug-OH) 1: The alkylated nickel-complex (10.1 g, 18.4 mmol) was dissolved in MeOH (300 mL) and heated till reflux. To this solution 2N HCl (200 mL) was added and the reaction mixture was refluxed. After 45 min the solvents were evaporated *in vacuo*. The green solid was dissolved in H_2O and neutralized with concentrated ammonia until pH 9 was reached and subsequently concentrated to dryness. The obtained solid was washed with acetone to recover the chiral auxillary (5.8 g, 82%). After that, the remaining solid was suspended in H_2O and centrifuged, the pellet was resuspended once and the insoluble material was collected by centrifugation. The obtained pellet was dried overnight in a vacuum dessicator to give 4.1 g of crude amino acid **1**. The combined aqueous layers were evaporated to dryness and the residue was treated with a cation exchange resin (Dowex 50X8 H^+ form) to obtain the pure amino acid (301 mg, 13%). The crude product was purified by reaction with $(Boc)_2O$ to give the Boc-protected amino acid (2.83 g, 12.4 mmol, 67%) after treatment with HCl in diethyl ether the hydrochloride of 1 was obtained (2.06 g, 12.4 mmol). R_f: 0.1 ($CHCl_3$/MeOH/HOAc 95:20:3 v/v/v). $[\alpha]_D^{21}$ -7.8 (*c* 1.22 MeOH). ^{1}H NMR (300 MHz, CD_3OD) δ: 3.77-3.80 (m, 1H, αH); 2.50-2.53 (m, 2H, βH); 1.45-1.46 (t, 3H, εH). ^{13}C NMR (75 MHz, CD_3OD) δ(: 170.4 (C=O); 82.2 (γC); 71.7(δC); 52.9 (αC); 21.6 (βC); 3.2 (εC).

(*S*)-*N*-(9-fluorenylmethyloxycarbonyl)-2-amino-5-hexynoic acid (Fmoc-Bug-OH) 4: HCl.H-Bug-OH **1** (0.83 mmol) was dissolved in H_2O (5 mL) and the pH was set to 9 with TEA. Fmoc-ONSu (269 mg, 0.8 mmol) was dissolved in CH_3CN (5 mL) and added to the basic solution. The reaction mixture was stirred at room temperature and the pH was kept at pH 8-8.5 with TEA. After 1.5 h the reaction was complete and CH_3CN was evaporated *in vacuo*. The mixture was acidified with 1N $KHSO_4$ to pH 1-2 and extracted with EtOAc. The organic layer was dried with Na_2SO_4 and evaporated. The crude product was purified by silicagel column chromatography (eluens: CH_2Cl_2/MeOH 98:2 → 90:10 v/v) to give **4** (221 mg, 75%) as a white solid. R_f: 0.51 ($CHCl_3$/MeOH/HOAc 95:20:3 v/v/v). $[\alpha]_D^{21}$ -17 (*c* 0.95 DMF). ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD$ 9:1 v/v) δ: 1.71-1.82 (s, 3H, εH-Bug); 2.63-2.88 (m, 2H, βH-Bug); 4.21-4.32 (t, 1H, CH-Fmoc); 4.40-4.48 (d, 2H, CH_2-Fmoc); 4.48-4.60 (m, 1H, αH-Bug); 7.22-7.82 (m, 8H, Ar-Fmoc). ^{13}C NMR (75 MHz, $CDCl_3/CD_3OD$ 9:1 v/v) δ: 172.5 (C=O, Bug); 156.0 (C=O, Fmoc), 143.5, 141.0, 127.5, 126.9, 124.9, 119.7 (Arom C, Fmoc); 78.8 (γC, Bug); 72.9 (δC, Bug); 66.9 (CH_2, Fmoc); 52.4 (αC, Bug); 46.8 (CH, Fmoc); 22.5 (βC, Bug); 3.1 (εC, Bug).

(*RS*)-*N*-(9-fluorenylmethyloxycarbonyl)-2-amino-5-hexynoic acid (Fmoc-Bug-OH) *rac*-4: To a suspension of K_2CO_3 (836 mg, 6.03 mmol) in CH_3CN (10 mL), tetrabutylammonium bromide (21 mg, 0.01 eq) followed by methyl *N*-(diphenylmethy1ene)-glycinate[6,7] (508 mg, 2 mmol) were added. After stirring for 20 min, 1-bromo-2-butyne (186 _L, 2 mmol, 1 eq) was added dropwise and the reaction

mixture was refluxed overnight. After filtration and extensively washing of the remaining solids with diethyl ether, the organic layer was concentrated *in vacuo*. The product was redissolved in diethyl ether, and the organic phase was washed with H_2O (2 × 25 mL). The organic layer was dried ($MgSO_4$) and evaporated to dryness. After purification by column chromatography using basic alumina (eluens: EtOAc/hexanes 9:1 v/v) the alkylation product was obtained in 61% (372 mg). The imine was dissolved in diethyl ether (10 mL) and treated with 1M HCl (2.5 mL) for 3 h. The organic phase was separated and subsequently washed with H_2O. The HCl solution and the combined H_2O washings were concentrated to dryness and the residue was crystallized from MeOH/EtOAc to give 170 mg (84%) of racemic HCl.H-Bug-OH. Fmoc protection of the amino group was carried out as described for **4**, yield: 71%.

Boc-Bug-Ile-Ala-Leu-Bug-OMe 6: Coupling and Boc-removal were carried out as described in the general procedure solution phase peptide synthesis.

*HCl.H-Bug-OMe **2**:* To a solution of HCl.H-Bug-OH (337 mg, 2 mmol) in MeOH (10 mL) was added dropwise $SOCl_2$ (362 _L, 5 mmol) at 0 ^oC. After 5 minutes of stirring the reaction mixture refluxed for 3 h. The methyl ester **2** was obtained after concentration *in vacuo* and coevaporation with CH_3CN and $CHCl_3$ in quantitative yield. R_f: 0.40 ($CHCl_3$/MeOH/HOAc 95:20:3 v/v/v). ^1H NMR (300 MHz, CD_3OD) δ: 4.11-4.14 (m, 1H, αH); 3.75 (s, OCH_3); 2.74-2.77 (m, 2H, βH); 1.67-1.70 (t, 3H, εH). ^{13}C NMR (75 MHz, CD_3OD) δ: 169.6 (C=O); 82.4 (γC); 71.5(δC); 53.9 (αC); 53.0 (O*C*H_3); 21.6 (βC); 3.2 (εC).

Boc-Leu-Bug-OMe: Yield: 91%. R_f(DCM/MeOH 9:1 v/v): 0.84; ^1H NMR ($CDCl_3$, 300 MHz) Leu: _ 5.39 (d, 1H, NH), 4.24 (m, 1H, C_H), 1.51-1.70 (m, 3H, C_H/CγH), 1.45 (s, 9H, Boc), 0.93-0.97 (m, 6H, C_H); Bug: _ 7.09 (d, 1H, NH), 4.64 (m, 1H, C_H), 3.77 (s, 3H, OCH_3), 2.67 (m, 2H, C_H), 1.76 (t, 3H, C_H); ES-MS: calcd for $C_{18}H_{31}N_2O_5$: 355.2, found: *m/z*: $[M + Na]^+$ 377.4, $[(M – C_5H_8O_2) + H]^+$ 255.3.

Boc-Ala-Leu-Bug-OMe: Yield: 93%. R_f(DCM/MeOH 9:1 v/v): 0.68; ^1H NMR ($CDCl_3$, 300 MHz) Ala: _ 5.54 (d, 1H, NH), 4.33 (m, 1H, C_H), 1.44 (s, 9H, Boc), 1.34 (d, 3H, C_H); Leu: _ 7.19 (d, 1H, NH), 4.58 (m, 1H, C_H), 1.49-1.70 (m, 3H, C_H/CγH), 0.91-0.95 (m, 6H, C_H); Bug: _ 7.19 (d, 1H, NH), 4.63 (m, 1H, C_H), 3.77 (s, 3H, OCH_3), 2.49-2.64 (m, 2H, C_H), 1.74 (s, 3H, C_H); ES-MS: calcd for $C_{21}H_{36}N_3O_6$: 426.3, found: *m/z*: $[M + H]^+$ 426.6, $[M + Na]^+$ 448.4, $[(M – C_4H_8) + H]^+$ 370.3, $[(M – C_5H_8O_2) + H]^+$ 326.3.

Boc-Ile-Ala-Leu-Bug-OMe: Coupling of Boc-Ile-OH with TFA.H-Ala-Leu-Bug-OMe was carried out in DMF. The tetrapeptide was isolated by trituration with EtOAc. Yield: 87%. R_f(DCM/MeOH 9:1 v/v): 0.62; ^1H NMR ($CDCl_3$, 300 MHz) Ile: _ 5.75 (d, 1H, NH), 4.20 (m, 1H, C_H), 1.80 (m, 1H,

C_H), 1.77 (m, 1H, CγH), 1.54 (m, 1H, CγH), 1.43 (s, 9H, Boc), 0.85-0.91 (m, 6H, Cγ'H/C_H); Ala: _ 7.79 (d, 1H, NH), 4.72 (m, 1H, C_H), 1.35 (d, 3H, C_H); Leu: _ 7.66 (d, 1H, NH), 4.72 (m, 1H, C_H), 1.60-1.70 (m, 2H, C_H), 1.04 (m, 1H, CγH), 0.85-0.91 (m, 6H, C_H); Bug: _ 7.57 (d, 1H, NH), 5.74 (m, 1H, CγH), 5.01 (m, 2H, C_H), 4.72 (m, 1H, C_H), 3.74 (s, 3H, OCH_3), 2.49-2.56 (m, 2H, C_H); 1.74 (s, 3H, C_H); ES-MS: calcd for $C_{27}H_{47}N_4O_7$: 539.3, found: *m/z*: $[M + H]^+$ 539.6, $[M + Na]^+$ 561.6, $[(M – C_4H_8) + H]^+$ 438.5, $[(M – C_5H_8O_2) + H]^+$ 439.5.

*Boc-Bug[1]-Ile[2]-Ala[3]-Leu[4]-Bug[5]-OMe (**6**):* Coupling of Boc-Bug-OH with TFA.H-Ile-Ala-Leu-Bug-OMe was carried out in DCM. The pentapeptide was isolated by trituration with EtOAc. Yield: 350 mg (73% over 7 steps); R_t: 17.6 min; R_f(DCM/MeOH 9:1 v/v): 0.60; ^{1}H NMR ($CDCl_3$/CD_3OH 14.5:1 v/v, 500 MHz) Bug[1]: _ 5.72 (br s, 1H, NH), 4.14 (m, 1H, C_H), 2.54 (m, 2H, C_H), 1.74 (s, 3H, C_H); 1.47 (s, 9H, Boc); Ile[2]: _ 7.18 (d, 1H, NH), 4.28 (m, 1H, C_H), 1.93 (m, 1H, CγH), 1.54 (m, 1H, CγH), 1.06 (m, 1H, C_H), 0.90 (m, 6H, Cγ'H/C_H); Ala[3]: _ 7.57 (br s, 1H, NH), 4.44 (m, 1H, C_H), 1.40 (d, 3H, C_H); Leu[4]: _ 7.44 (d, 1H, NH), 4.46 (m, 1H, C_H), 1.78 (m, 1H, CγH), 1.60 (m, 2H, C_H), 0.90 (m, 6H, C_H); Bug[5]: _ 7.36 (d, 1H, NH), 4.59 (m, 1H, C_H), 3.76 (s, 3H, OCH_3), 2.54 (m, 2H, C_H), 1.78 (s, 3H, C_H); ES-MS: calcd for $C_{33}H_{54}N_5O_8$: 648.4, found: *m/z*: $[M + H]^+$ 648.7, $[M + Na]^+$ 670.6, $[(M – C_4H_8) + H]^+$ 592.6, $[(M – C_5H_8O_2) + H]^+$ 548.9.

Boc-*cyclo*[Bug[1]-Ile[2]-Ala[3]-Leu[4]-Bug[5]]-OMe 7: Linear pentapeptide **6** (45.9 mg, 0.07 mmol) was dissolved in toluene (200 mL) and the catalyst (4.4 mg, 9.3 _mol) was added. The obtained mixture was heated to and stirred at 80 ^{o}C for two h. The product was purified by column chromatography (DCM/MeOH 97.5:2.5 v/v). Yield: 17.8 mg (42%); R_t: 16.4 min; R_f(DCM/MeOH 9:1 v/v): 0.56 ; ^{1}H NMR ($CDCl_3$/CD_3OH 14.5:1 v/v, 500 MHz) Bug[1]: _ 5.84 (d, 1H, NH), 4.27 (m, 1H, C_H), 2.67 (m, 2H, C_H), 1.45 (s, 9H, Boc); Ile[2]: _ 7.42 (m, 1H, NH), 4.18 (m, 1H, C_H), 1.91 (m, 1H, CγH), 1.58 (m, 1H, CγH), 1.06 (m, 1H, C_H), 0.92 (m, 6H, Cγ'H/C_H); Ala[3]: _ 7.72 (br s, 1H, NH), 4.09 (m, 1H, C_H), 1.51 (d, 3H, C_H); Leu[4]: _ 7.98 (d, 1H, NH), 4.18 (m, 1H, C_H), 1.81 (m, 1H, CγH), 1.62 (m, 2H, C_H), 0.92 (m, 6H, C_H); Bug[5]: _ 7.42 (m, 1H, NH), 4.60 (m, 1H, C_H), 3.79 (s, 3H, OCH_3), 2.67 (m, 2H, C_H). ES-MS: calcd for $C_{29}H_{48}N_5O_8$: 594.4, found: *m/z*: $[M + H]^+$ 594.7, $[M + Na]^+$ 617.5, $[(M – C_4H_8) + H]^+$ 538.6, $[(M – C_5H_8O_2) + H]^+$ 494.5. HRMS: calcd for $C_{29}H_{47}N_5O_8Na$: 616.33218. Found: 616.33223.

Boc-Bug-Pro-Gly-OMe 8: Boc-Pro-OH (495 mg, 2.3 mmol) and HCl.H-Gly-OMe (300 mg, 2.4 mmol) were coupled in the presence of BOP (1.02 g, 2.3 mmol) and DIPEA (962 _L) in DCM (25 mL) as described in the general procedure solution phase peptide synthesis.

Boc-Pro-Gly-OMe: Yield: 592 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, C_H), 3.49 (m, 2H, C_H), 2.14-2.20 (m, 2H, C_H), 1.86-1.98 (m, 2H, CγH), 1.46 (s, 9H, Boc); Gly: _ 7.01 and 7.39 (m, 1H, NH), 4.04 (m, 2H, C_H), 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, $[M + Na + ACN]^+$ 350.2,$[(M – C_4H_8) + H]^+$ 228.1, $[(M – C_5H_8O_2) + H]^+$ 187.1.

The protected dipeptide (300 mg, 1.1 mmol) was treated with TFA/DCM 1:1 v/v (4 mL) for 2 h to remove the Boc group. Then, the reaction mixture was concentrated *in vacuo* and coevaporated with toluene (2 × 10 mL), CH_3CN (2 × 10 mL) and DCM (2 × 10 mL) to remove any residual TFA. The TFA-salt was dissolved in DCM (15 mL) and to this solution were added: Boc-Bug-OH (228 mg, 1.0 mmol), BOP (442 mg, 1.0 mmol) and DiPEA (436 μL, 2.5 mmol) and the obtained reaction mixture was stirred overnight. After evaporation of DCM, the residue was dissolved in EtOAc (50 mL) and this solution was washed with 1N $KHSO_4$ (3 × 20 mL), 10% Na_2CO_3 (3 × 20 mL) and brine (1 × 20 mL), dried (Na_2SO_4), filtrated and evaporated *in vacuo*.

Boc-Bug-Pro-Gly-OMe ***8****:* was obtained in 75% yield (297 mg) after column chromatography (DCM/MeOH 97.5/2.5 v/v). R_f(DCM/MeOH 9:1 v/v): 0.60; ^{1}H NMR ($CDCl_3$, 300 MHz) Bug: δ 5.51 (d, 1H, NH), 4.60 (m, 1H, C_H), 2.51 (m, 2H, C_H), 1.74 (s, 3H, C_H), 1.44 (s, 9H, Boc); Pro: δ 4.69 (m, 1H, C_H), 3.66 (m, 2H, C_H), 2.35 (m, 2H, C_H), 2.04 (m, 2H, CγH); Gly: _ 7.33, 7.97 (m, 1H, NH), 3.95 (2xd, 2H, C_H), 3.73 (s, 3H, OCH_3); ES-MS: calcd for $C_{28}H_{32}N_3O_6$: 506.2, found: *m/z*: $[M + Na]^+$ 418.35,$[(M – C_4H_8) + H]^+$ 340.3, $[(M – C_5H_8O_2) + H]^+$ 296.2.

Boc-Bug^1-Pro^2-Gly^3-Bug^4-OMe 9: Boc-Bug-Pro-Gly-OMe **8** (240 mg, 0.6 mmol) was dissolved in THF (10 mL) and the methyl ester saponified with 0.2N LiOH (5 mL) during 2 h. Then, THF was 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*. Boc-Bug-Pro-Gly-OH was obtained in quantitative yield (214 mg) and was used in the next step without further purification. HCl.H-Bug-OMe (130 mg, 0.7 mmol) was dissolved in DCM (10 mL) and the tripeptide acid was added followed by BOP (265 mg, 0.6 mmol) and DIPEA (305 μL, 1.75 mmol) 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*. Tetrapeptide **9** was purified by column chromatography (DCM/MeOH 97.5:2.5) and obtained in 86% yield (260 mg). R_t:15.3 min; R_f(DCM/MeOH 9:1 v/v): 0.56. ^{1}H NMR ($CDCl_3$, 500 MHz); Bug^1: 5.42 and 5.47 (2 × d, 1H, NH), 4.63 (m, 1H, C_H), 2.54-2.67 (m, 2H, C_H), 1.42 (s, 9H, Boc); Pro^2: 4.63 (m, 1H, C_H), 3.88 (m, 2H, C_H), 2.29 (m, 2H, C_H),

2.06 (m, 2H, CγH); Gly^3: _ 7.31 and 8.80 (s, 1H, NH), 3.93-4.05 (m, 2H, C_H); Bug^4: _ 7.21 and 6.96 (d, 1H, NH), 4.63 (m, 1H, C_H), 3.78 (s, 3H, OCH_3), 2.46-2.63 (m, 2H, C_H), 1.79 (s, 3H, CεH). ES-MS: calcd for $C_{25}H_{37}N_4O_7$: 505.6 found: *m/z*: $[M + H]^+$ 505.4, $[M + Na]^+$ 527.5, $[(M – C_4H_8) + H]^+$ 449.5, $[(M – C_5H_8O_2) + H]^+$ 406.45.

Boc-*cyclo*[Bug^1-Pro^2-Gly^3-Bug^4]-OMe 10: Ring-closure was carried according the general procedure with 8% catalyst at the concentration of 2 mM in toluene. Cyclic pentapeptide **10** was purified by column chromatography (DCM/MeOH 97.5:2.5 v/v) and was obtained as a white powder in 82% yield (83.2 mg). R_t:14.0 min; R_f(DCM/MeOH 9:1 v/v): 0.43; ^{1}H NMR ($CDCl_3$, 500 MHz); Bug^1: 5.89 (d, 1H, NH), 4.66 (m, 1H, C_H), 2.47-2.60 (m, 2H, C_H), 1.50 (s, 9H, Boc); Pro^2: 4.40 (m, 1H, C_H), 3.59 (m, 2H, C_H), 1.96-2.37 (m, 4H, C_H + CγH), Gly^3: _ 7.21 (s, 1H, NH), 3.62-4.40 (dd, 2H, C_H); Bug^4: _ 8.03 (d, 1H, NH), 4.90 (m, 1H, C_H), 3.89 (s, 3H, OCH_3), 2.60-2.78 (m, 2H, C_H). ES-MS: calcd for $C_{21}H_{31}N_4O_7$: 551.5 found: *m/z*: $[M + H]^+$ 451.6, $[M + Na]^+$ 473.5, $[(M – C_4H_8) + H]^+$ 395.3, $[(M – C_5H_8O_2) + H]^+$ 351.3. HRMS: calcd for $C_{21}H_{30}N_4O_7Na$: 473.20096. Found: 473.20122.

Boc-Ala-D-Leu-Nle-Gly-Bug-OMe 11: To a solution of Boc-Ala-D-Leu-Nle-Gly-OH (236 mg, 0.5 mmol) in DCM (10 mL), HOBt.H_2O (100 mg, 0.6 mmol) and HCl.H-Bug-OMe (116 mg, 0.6 mmol) were added followed by DIPEA (223 μL). The mixture was cooled to –15^{o}C and EDCI (124.6 mg, 0.6 mmol) was added. The obtained reaction mixture was stirred overnight. Then, the reaction mixture was worked up using the standard procedures as described above. Yield after column purification (eluens: DCM/MeOH 95:5 v/v) was 246 mg (82%); R_f(DCM/MeOH 9:1 v/v): 0.36; ^{1}H NMR ($CDCl_3$, 300 MHz) Ala: _ 5.91 (d, 1H, NH), 4.34 (m, 1H, C_H), 1.41 (s, 9H, Boc), 1.35 (d, 3H, C_H); D-Leu: _ 7.76 (d, 1H, NH), 4.90 (m, 1H, C_H), 1.83 (m, 1H, CγH), 1.63 (m, 2H, C_H), 0.89-0.95 (m, 6H, C_H); Nle: _ 8.16 (br s, 1H, NH), 4.90 (m, 1H, C_H), 1.63 (m, 2H, C_H), 1.42-1.47 (m, 2H, CγH), 1.26-1.37 (m, 2H, C_H), 0.89-0.95 (m, 3H, C_H); Gly: _ 8.09 (m, 1H, NH), 4.12-4.39 (dd, 2H, C_H); Bug: _ 8.00 (d, 1H, NH), 4.71 (m, 1H, C_H), 3.77 (s, 3H, OCH_3), 2.67 (m, 2H, C_H), 1.74 (s, 3H, C_H). ES-MS: calcd for $C_{29}H_{50}N_5O_8$: 596.4, found: *m/z*: $[M + Na]^+$ 618.6.

Boc-Bug^1-Gly^2-Ala^3-D-Leu^4-Nle^5-Gly^6-Bug^7-OMe 12: Pentapeptide **11** (240 mg, 0.4 mmol) was dissolved in TFA/DCM 1:1 v/v (4 mL) to remove the Boc group and worked up as described. The obtained TFA-salt was dissolved in DCM (10 mL) and to this solution, Boc-Bug-Gly-OH (141 mg, 0.5 mmol) and HOBt.H_2O (76.7 mg, 0.5 mmol) were added. The obtained mixture was cooled to -15^{o}C and EDCI (97 mg, 0.5 mmol) followed by DiPEA (105 μL, 0.6 mmol) were added. After stirring for 16 h, DCM was removed under reduced pressure and **12** was purified by trituration with EtOAc. Yield: 191

mg (63 %); 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) Bug^1: _ 5.75 (d, 1H, NH), 4.17 (m, 1H, C_H), 2.61-2.67 (m, 2H, C_H), 1.78 (s, 3H, C_H), 1.45 (s, 9H, Boc); Gly$^{2/6}$: _ 7.90/7.63 (m, 1H, NH), 4.08/3.71 (2 × dd, 2H, C_H); Ala^3: _ 7.66 (m, 1H, NH), 4.34 (m, 1H, C_H), 1.36 (d, 3H, C_H); D-Leu^4: _ 7.66 (d, 1H, NH), 4.34 (m, 1H, C_H), 1.90 (m, 2H, C_H), 1.65 (m, 1H, CγH), 0.89 (m, 6H, C_H); Nle^5: _ 7.69 (d, 1H, NH), 4.34 (m, 1H, C_H), 1.90 (m, 2H, C_H), 1.56 (m, 2H, CγH), 1.32 (m, 2H, C_H), 0.89 (m, 3H, C_H); Bug^7: _ 7.49 (m, 1H, NH), 4.61 (m, 1H, C_H), 3.76 (s, 3H, OCH_3), 2.61-2.67 (m, 2H, C_H), 1.78 (s, 3H, C_H); ES-MS: calcd for $C_{37}H_{60}N_7O_{10}$: 762.9, found: *m/z*: $[M + H]^+$ 762.6, $[M + Na]^+$ 784.7, $[(M – C_4H_8) + H]^+$ 706.8, $[(M – C_5H_8O_2) + H]^+$ 662.6.

Boc-*cyclo*[Bug^1-Gly^2-Ala^3-D-Leu^4-Nle^5-Gly^6-Bug^7]-OMe 13: To a solution of the fully protected linear heptapeptide **12** (21.6 mg, 0.028 mmol) in toluene, 3.6 mg of catalyst was added and the mixture was stirred for 3 h at 80^{o}C. After concentrating *in vacuo*, the cyclic product was purified by column chromatography (DCM/MeOH 97.5:2.5 v/v). Yield: 3.6 mg (18%); R_f(DCM/MeOH 97.5:2.5 v/v) 0.42; R_t 15.5 min; ^{1}H NMR ($CDCl_3$/CD_3OH 14.5:1 v/v, 500 MHz) Bug^1: _ 6.03 (bs, 1H, NH), 4.34 (m, 1H, C_H), 2.62-2.85 (m, 2H, C_H), 1.46 (s, 9H, Boc); Gly^2: _ 8.16 (bs, 1H, NH), 3.95 (m, 2H, C_H); Ala^3; _ 7.49 (m, 1H, NH), 4.34 (m, 1H, C_H), 1.35 (d, 3H, C_H); D-Leu^4: _ 7.74 (br s, 1H, NH), 4.34 (m, 1H, C_H), 1.92 (m, 2H, C_H), 1.57 (m, 1H, CγH), 0.91 (m, 6H, C_H); Nle^5: _ 7.60 (br s, 1H, NH), 4.34 (m, 1H, C_H), 1.75 (m, 2H, C_H), 1.57 (m, 2H, CγH), 1.27 (m, 2H, C_H), 0.91 (m, 3H, C_H); Gly^6: _ 7.81 (bs, 1H, NH), 3.91-4.12 (dd, 2H, C_H); Bug^7: _ 7.85 (d, 1H, NH), 4.60 (m, 1H, C_H), 3.78 (s, 3H, OCH_3), 2.62-2.85 (m, 2H, C_H); ES-MS: calcd for $C_{33}H_{54}N_7O_{10}$: 708.4, found: *m/z*: $[M + H]^+$ 708.8, $[M + Na]^+$ 730.6, $[(M – C_4H_8) + H]^+$ 652.8, $[(M – C_5H_8O_2) + H]^+$ 608.8. HRMS: calcd for $C_{33}H_{53}N_7O_{10}Na$: 730.37478. Found: 730.37516.

Boc-Bug-Gly-OH: Coupling was carried out as described in the general procedure solution phase peptide synthesis. *Boc-Bug-Gly-OEt:* Yield 83% (155 mg); R_f(DCM/MeOH 95:5 v/v): 0.70; ^{1}H NMR ($CDCl_3$, 300 MHz) Bug: _ 5.40 (d, 1H, NH), 4.30 (m, 1H, C_H), 2.52-2.71 (m, 2H, C_H), _ 1.79 (t, 3H, C_H), _ 1.46 (s, 9H, CH_3 Boc); Gly: _ 7.01 (m, 1H, NH), 4.06 (m, 2H, C_H), 4.21 (q, 2H, O*C*$H_2$$CH_3$), 1.27 (t, 3H, OCH_2C*H*$_3$); ES-MS: calcd for $C_{15}H_{24}N_2O_5Na$: 335.2, found: *m/z*: $[M + Na]^+$ 335.2, $[M + Na + CH_3CN]^+$ 376.2, $[(M – C_5H_8O_2) + H]^+$ 213.2. *Boc-Bug-Gly-OH:* Boc-Bug-Gly-OEt (155 mg, 0.5 mmol) was dissolved in THF (5 mL) and the ethyl ester was saponified with 0.2N LiOH (4 mL) during 45 min at 0^{o}C. Then, THF was partially removed *in vacuo* and the aqueous solution was acidified with 1N $KHSO_4$ and subsequently extracted with EtOAc (3 × 10 mL). The combined EtOAc layers were washed with brine (1 × 15 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo*. Yield:

141 mg (quant); R_f(DCM/MeOH 95:5 v/v): 0; ^{1}H NMR ($CDCl_3$, 300 MHz) Bug: _ 5.60 (d, 1H, NH), 4.39 (m, 1H, C_H), 2.60-2.69 (m, 2H, C_H), 1.77 (t, 3H, C_H), 1.45 (s, 9H, CH_3 Boc); Gly: _ 8.62 (br s, 1H, OH), 7.28 (m, 1H, NH), 4.00-4,11 (m, 2H, C_H); ES-MS: calcd for $C_{13}H_{20}N_2O_5Na$: 306.1, found: *m/z*: $[M + Na]^+$ 307.2, $[M + Na + ACN]^+$ 348.2.

Boc-Bug^1-Ala^2-Bug^3-Bug^4-Asn(Trt)^5-Bug^6-OMe 14: The peptide was synthesized manually on a 0.20 mmol scale on plain Argogel resin as described in the general procedures. The peptide was purified by column chromatography (eluens: DCM/MeOH 97.5:2.5→95:5 v/v). Yield: 187 mg (99%); R_f 0.60 (DCM/MeOH 9:1 v/v); R_t 18.94 min; ^{1}H NMR (DMSO-d_6, 500 MHz) Bug^1: _ 6.98 (d, 1H, NH), 4.06 (m, 1H, C_H), 2.38-2.70 (m, 2H, C_H), 1.69 (s, 3H, C_H), 1.39 (s, 9H, Boc); Ala^2: _ 7.93 (d, 1H, NH), 4.32-4.42 (m, 1H, C_H), 1.21 (d, 3H, C_H); Bug^3: _ 7.98 (d, 1H, NH), 4.32-4.42 (m, 1H, C_H), 2.38-2.70 (m, 2H, C_H), 1.69 (s, 3H, C_H); Bug^4: _ 8.16 (d, 1H, NH), 4.32-4.42 (m, 1H, C_H), 2.38-2.70 (m, 2H, C_H), 1.69 (s, 3H, C_H); Asn(Trt)^5: _ 8.63 (m, 1H, _NH), _ 8.29 (d, 1H, _NH), 7.16-7.29 (m, 15H, arom Trt), 4.59 (m, 1H, C_H), 2.38-2.70 (m, 2H, C_H); Bug^6: _ 8.02 (d, 1H, NH), 4.32-4.42 (m, 1H, C_H), 3.63 (s, 3H, OCH_3), 2.38-2.70 (m, 2H, C_H), 2.16 (s, 3H, C_H). ES-MS: calcd for $C_{56}H_{67}N_7O_{10}$: 996.5, found: *m/z*: $[M + H]^+$ 996.8, $[M + Na]^+$ 1018.7.

Boc-Alg^1-Ala^2-Bug^3-Alg^4-Asn(Trt)^5-Bug^6-OMe 16: The peptide was synthesized manually on a 0.20 mmol scale on plain Argogel resin as described in the general procedures. The peptide was purified by column chromatography (eluens: DCM/MeOH 97.5:2.5→95:5 v/v). Yield = 180.6 mg (93%). R_f 0.60 (DCM/MeOH 9:1 v/v); R_t = 18.86 min; ^{1}H NMR (DMSO-d_6, 500 MHz) Alg^1: _ 6.86 (d, 1H, NH), 5.69 (m, 1H, CγH), 4.98-5.11 (m, 2H, C_H), 3.98 (m, 1H, C_H), 2.17-2.57 (m, 2H, C_H), 1.37 (s, 9H, Boc); Ala^2: _ 7.93 (d, 1H, NH), 4.33-4.40 (m, 1H, C_H), 1.21 (d, 3H, C_H); Bug^3: _ 8.16 (d, 1H, NH), 4.33-4.40 (m, 1H, C_H), 2.17-2.57 (m, 2H, C_H), 1.69 (s, 3H, C_H); Alg^4: _ 7.76 (d, 1H, NH), 5.69 (m, 1H, CγH), 4.98-5.11 (m, 2H, C_H), 4.33-4.40 (m, 1H, C_H), 2.17-2.57 (m, 2H, C_H); Asn(Trt)^5: _ 8.60 (m, 1H, _NH), _ 8.30 (d, 1H, _NH), 7.16-7.27 (m, 15H, arom Trt), 4.63 (m, 1H, C_H), 2.62 (m, 2H, C_H); Bug^6: _ 8.11 (d, 1H, NH), 4.33-4.40 (m, 1H, C_H), 3.63 (s, 3H, OCH_3), 2.17-2.57 (m, 2H, C_H), 2.16 (s, 3H, C_H). ES-MS: calcd for $C_{54}H_{66}N_7O_{10}$: 972.5, found: *m/z*: $[M + H]^+$ 972.7, $[M + Na]^+$ 994.6.

Boc-Alg^1-Ala^2-*cyclo*(Bug^3-Alg^4-Asn(Trt)^5-Bug^6)-OMe 17: Linear hexapeptide **16** (44.3 mg, 0.05 mmol) was dissolved in toluene (200 mL) and the catalyst (3.9 mg, 8.3 _mol) was added. The obtained mixture was heated to and stirred at 80^{o}C for 90 min. The product was purified by column chromatography (DCM/MeOH 97.5:2.5 v/v). Yield: 27.5 mg. This was a mixture of the desired product and a side product in a ratio 3:2. These two products were seperated by preparative HPLC chromatography to yield the desired product (4.6 mg) as jugded by MS/MS. R_f = 0.60 (DCM/MeOH

9:1 v/v); R_t 17.93 min; ^{1}H NMR ($CDCl_3$, 500 MHz) Alg[1]: _ 5.45 (bs, 1H, NH), 5.65-5.74 (m, 1H, CγH), 5.06-5.15 (m, 2H, C_H), 4.14 (m, 1H, C_H), 2.38-2.64 (m, 2H, C_H), 1.43 (s, 9H, Boc); Ala[2]: _ 7.41 (bs, 1H, NH), 4.42 (m, 1H, C_H), 1.37 (d, 3H, C_H); Bug[3]: _ 7.58 (bs, 1H, NH), 4.49 (m, 1H, C_H), 2.38-2.64 (m, 2H, C_H); Alg[4]: _ 7.95 (bs, 1H, NH), 5.65-5.74 (m, 1H, CγH), 4.06-5.15 (m, 2H, C_H), 4.22 (m, 1H, C_H), 2.65-2.74 (m, 2H, C_H); Asn(Trt)[5]: _ 7.58 (bs, 1H, _NH), _ 8.03 (d, 1H, _NH), 7.18-7.28 (m, 15H, arom Trt), 4.62 (m, 1H, C_H), 2.78 (m, 2H, C_H); Bug[6]: _ 7.34 (d, 1H, NH), 4.73 (m, 1H, C_H), 3.79 (s, 3H, OCH_3), 2.38-2.64 (m, 2H, C_H). ES-MS: calcd for $C_{50}H_{60}N_7O_{10}$: 918.4, found: *m/z*: $[M + H]^+$ 918.7, $[M + Na]^+$ 940.8.

References

1 E. von Arx, M. Faupel, M.J. Bruggen, *J. Chromatogr.* **1976**, *120*, 224.

2 E. Kaiser, R.L. Colescott, C.D. Bossinger, P.I. Cook, *Anal. Biochem.* **1970**, *34*, 595.

3 J. Meienhofer, M. Waki, E.P. Heimer, T.J. Lambros, R.C. Makofske, C.-D. Chang, *Int. J. Peptide Protein Res.* **1979**, *13*, 35.

4 Y.N. Belokon, *Janssen Chimica Acta* **1992**, *10*, 4.

5 S. Collet, P. Bauchat, R. Danion-Bougot, D. Danion, *Tetrahedron Asymm.* **1998**, *9*, 2121.

6 M.J. O'Donnell, J.M. Boniece, S.E. Earp, *Tetrahedron Lett.* **1978**, 2641; M.J. O'Donnell, T.M. Eckrich, *Tetrahedron Lett.* **1978**, 4625.

7 L.B. Wolf, T. Sonke, K.C.M.F. Tjen, B. Kaptein, Q.B. Broxterman, H.E. Schoemaker, F.P.J.T. Rutjes, *Adv. Synth. Catal.* **2001**, *343*, 662.