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 analysis 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. Rf values were determined by thin layer chromatography (TLC) on Merck precoated silicagel 60F254 plates. Spots were visualized with UV quenching, ninhydrin or with Cl2-TDM.1 Solid phase peptide synthesis was monitored with the Kaiser test2 and the loading of the resin3 was determined using a Helios 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. 1H NMR (300 MHz) and 13C NMR (75 MHz) spectra were measured on a Varian G-300 spectrometer. 1H NMR 500 MHz spectra were recorded on a Varian INOVA-500 spectrometer. 1H NMR chemical shifts are given in ppm (δ) relative to TMS and 13C NMR chemical shifts are given in ppm (δ) relative to CDCl3 (77.0 ppm). 13C 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 H₂O 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% Na₂CO₃ (3 × 25 mL) and brine (1 × 25 mL), dried (Na₂SO₄), filtrated and evaporated *in vacuo*. The obtained crude product was analyzed by TLC, ¹H 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), CH₃CN (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 CH₂CN/H₂O 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 H₂O,
buffer B: 0.1% TFA in CH$_3$CN/H$_2$O 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 x 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$_2$O; buffer B: 0.1% TFA in CH$_3$CN/H$_2$O 95:5 v/v).

**Peptide characterization:** The peptides were characterized by mass spectrometry and $^1$H 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 assignments were based on $^1$H 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)$_3$W=CCMe$_3$) in toluene stirred at 80 °C 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$_3$CN (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$_2$Cl$_2$. 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$_2$Cl$_2$/acetone 2:1 v/v). $[^{1}$H$]$NMR (300 MHz, CDCl$_3$) $\delta$: 1.93-2.01 (s, 3H, _H-Bug); 2.02-2.18 (m, 2H, _Pro); 2.21-2.35 (m, 1H, $\beta$H-Bug); 2.44-2.68 (m, 2H, [1H, ($\beta$H-Bug), 1H, ($\gamma$H-Pro)]; 2.72-2.94 (m, 1H, $\gamma$H-PRO); 3.41-3.50 (m, 1H, $\delta$H-PRO); 3.51-3.61 (m, 1H, $\delta$H-PRO); 3.61-3.82 (m, 2H, [1H, d, (CH$_2$-Ph), 1H, m, ($\alpha$H-Pro)]; 3.96-4.04 (q, 1H, $\alpha$H-Bug); 4.42-4.52 (d, 1H, CH$_2$-Ph); 6.61-8.24 (m, 14H, Ar-H). $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$: 4.1 (c-C-Bug); 23.0 ($\gamma$C-Pro); 23.9 ($\beta$C-Pro); 30.6 ($\beta$C-Bug); 56.4 ($\delta$C-Pro); 62.8 (Ph-CH$_2$-N); 68.1 ($\alpha$C-Bug); 70.0 ($\alpha$C-Pro); 73.8 ($\gamma$C-Pro); 80.4 ($\delta$C-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$_3$NiO$_3$: 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 H2O 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 auxiliary (5.8 g, 82%). After that, the remaining solid was suspended in H2O 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). Rf: 0.1 (CHCl3/MeOH/HOAc 95:20:3 v/v/v). [α]D 21 -7.8 (c 1.22 MeOH). 1H NMR (300 MHz, CD3OD) δ: 3.77-3.80 (m, 1H, αH); 2.50-2.53 (m, 2H, βH); 1.45-1.46 (t, 3H, εH). 13C NMR (75 MHz, CD3OD) 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 H2O (5 mL) and the pH was set to 9 with TEA. Fmoc-ONSu (269 mg, 0.8 mmol) was dissolved in CH3CN (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 CH3CN was evaporated in vacuo. The mixture was acidified with 1N KHSO4 to pH 1-2 and extracted with EtOAc. The organic layer was dried with Na2SO4 and evaporated. The crude product was purified by silicagel column chromatography (eluens: CH2Cl2/MeOH 98:2 → 90:10 v/v) to give 4 (221 mg, 75%) as a white solid. Rf: 0.51 (CHCl3/MeOH/HOAc 95:20:3 v/v/v). [α]D 21 -17 (c 0.95 DMF). 1H NMR (300 MHz, CDCl3/CD3OD 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, CH2-Fmoc); 4.48-4.60 (m, 1H, αH-Bug); 7.22-7.82 (m, 8H, Ar-Fmoc). 13C NMR (75 MHz, CDCl3/CD3OD 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 (CH2, 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 K2CO3 (836 mg, 6.03 mmol) in CH3CN (10 mL), tetrabutylammonium bromide (21 mg, 0.01 eq) followed by methyl N-(diphenylmethylene)-glycinate6,7 (508 mg, 2 mmol) were added. After stirring for 20 min, 1-bromo-2-butylene (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 H2O (2 × 25 mL). The organic layer was dried (MgSO4) 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 H2O. The HCl solution and the combined H2O 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 SOCl2 (362 _L, 5 mmol) at 0 °C. 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 CH3CN and CHCl3 in quantitative yield. Rf: 0.40 (CHCl3/MeOH/HOAc 95:20:3 v/v/v). 1H NMR (300 MHz, CD3OD) δ: 4.11-4.14 (m, 1H, αH); 3.75 (s, OCH3); 2.74-2.77 (m, 2H, βH); 1.67-1.70 (t, 3H, εH). 13C NMR (75 MHz, CD3OD) δ: 169.6 (C=O); 82.4 (γC); 71.5(δC); 53.9 (αC); 53.0 (OCH3); 21.6 (βC); 3.2 (εC).

**Boc-Leu-Bug-OMe:** Yield: 91%. Rf(DCM/MeOH 9:1 v/v): 0.84; 1H NMR (CDCl3, 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, OCH3), 2.67 (m, 2H, C_H), 1.76 (t, 3H, C_H); ES-MS: calcd for C18H31N2O5: 355.2, found: m/z: [M + Na]+ 377.4, [(M – C3H8O2) + H]+ 255.3.

**Boc-Ala-Leu-Bug-OMe:** Yield: 93%. Rf(DCM/MeOH 9:1 v/v): 0.68; 1H NMR (CDCl3, 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, OCH3), 2.49-2.64 (m, 2H, C_H), 1.74 (s, 3H, C_H); ES-MS: calcd for C21H36N3O6: 426.3, found: m/z: [M + H]+ 426.6, [M + Na]+ 448.4, [(M – C4H8) + H]+ 370.3, [(M – C3H6O2) + 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%. Rf(DCM/MeOH 9:1 v/v): 0.62; 1H NMR (CDCl3, 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, OCH3), 2.49-2.56 (m, 2H, C_H); 1.74 (s, 3H, C_H); ES-MS: caked for C2H47N4O7: 539.3, found: m/z: [M + H]+ 539.6, [M + Na]+ 561.6, [(M – C4H8) + H]⁺ 438.5, [(M – C5H8O2) + H]⁺ 439.5.

**Boc-Bug⁴-Ile²-Ala³-Leu⁴-Bug⁵-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 titration with EtOAc. Yield: 350 mg (73% over 7 steps); Rf: 17.6 min; RfDCF/DMeOH: 9:1 v/v): 0.60; 1H NMR (CDCl3/CD3OH) 14.5:1 v/v, 500 MHz) Bug¹: _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); Ile²: _7.18 (d, 1H, NH), 4.28 (m, 1H, C_H), 1.93 (m, 1H, CγH), 1.54 (1H, CγH), 1.06 (m, 1H, C_H), 0.90 (m, 6H, Cγ’H/C_H); Ala³: _7.57 (br s, 1H, NH), 4.44 (m, 1H, C_H), 1.40 (d, 3H, C_H); Leu⁴: _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); Bug⁵: _7.36 (d, 1H, NH), 4.59 (m, 1H, C_H), 3.76 (s, 3H, OCH3), 2.54 (m, 2H, C_H), 1.78 (s, 3H, C_H); ES-MS: caked for C33H54N5O8: 648.4, found: m/z: [M + H]+ 648.7, [M + Na]+ 670.6, [(M – C4H8) + H]⁺ 592.6, [(M – C5H8O2) + H]⁺ 548.9.

**Boc-cyclo[Bug¹-Ile²-Ala³-Leu⁴-Bug⁵]-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 °C for two h. The product was purified by column chromatography (DCM/MeOH 97.5:2.5 v/v). Yield: 17.8 mg (42%); Rf: 16.4 min; Rf(DCM/MeOH 9:1 v/v): 0.56; 1H NMR (CDCl3/CD3OH) 14.5:1 v/v, 500 MHz) Bug¹: _5.84 (d, 1H, NH), 4.27 (m, 1H, C_H), 2.67 (m, 2H, C_H), 1.45 (s, 9H, Boc); Ile²: _7.42 (m, 1H, NH), 4.18 (m, 1H, C_H), 1.91 (m, 1H, CγH), 1.58 (m, 1H, C_H), 1.06 (m, 1H, C_H), 0.92 (m, 6H, Cγ’H/C_H); Ala³: _7.72 (br s, 1H, NH), 4.09 (m, 1H, C_H), 1.51 (d, 3H, C_H); Leu⁴: _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); Bug⁵: _7.42 (m, 1H, NH), 4.60 (m, 1H, C_H), 3.79 (s, 3H, OCH3), 2.67 (m, 2H, C_H). ES-MS: caked for C29H48N5O8: 594.4, found: m/z: [M + H]+ 594.7, [M + Na]+ 617.5, [(M – C4H8) + H]⁺ 538.6, [(M – C5H8O2) + H]⁺ 494.5. HRMS: caked for C29H47N5O8Na: 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: \( \delta \) 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, C\_H), 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\(_2\)O\(_5\)Na: 309.1, found: \( m/z \): [M + Na]\(^+\) 309.2, [M + Na + ACN\]^+\) 350.2, [(M – C\(_4\)H\(_8\)) + H]\(^+\) 228.1, [(M – C\(_4\)H\(_8\)O\(_2\)) + 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 \textit{in vacuo} and coevaporated with toluene (2 × 10 mL), CH\(_3\)CN (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\(_2\)CO\(_3\) (3 × 20 mL) and brine (1 × 20 mL), dried (Na\(_2\)SO\(_4\)), filtrated and evaporated in \textit{vacuo}.

\textbf{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: \( \delta \) 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: \( \delta \) 4.69 (m, 1H, C\_H), 3.66 (m, 2H, C\_H), 2.35 (m, 2H, C\_H), 2.04 (m, 2H, C\_H); 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\(_3\)O\(_6\): 506.2, found: \( m/z \): [M + Na]\(^+\) 418.35, [(M – C\(_4\)H\(_8\)) + H]\(^+\) 340.3, [(M – C\(_4\)H\(_8\)O\(_2\)) + H]\(^+\) 296.2.

\textbf{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 \textit{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\(_2\)SO\(_4\)), filtered and concentrated \textit{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 \textit{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\(_2\)CO\(_3\) (3 × 5 mL) and brine (1 × 5 mL), dried (Na\(_2\)SO\(_4\)), filtrated and evaporated \textit{in vacuo}. Tetrapeptide 9 was purified by column chromatography (DCM/MeOH 97.5:2.5) and obtained in 86% yield (260 mg). \( R_f:15.3 \) 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),
Boc-cyclo[Bug\textsuperscript{1-Pro\textsuperscript{2}-Gly\textsuperscript{3}-Bug\textsuperscript{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; $R_t$(DCM/MeOH 9:1 v/v): 0.43; $^1$H NMR (CDCl$_3$, 500 MHz); Bug\textsuperscript{1}: 5.89 (d, 1H, NH), 4.66 (m, 1H, C$_\text{H}$), 2.47-2.60 (m, 2H, C$_\text{H}$), 1.50 (s, 9H, Boc); Pro\textsuperscript{2}: 4.40 (m, 1H, C$_\text{H}$), 3.59 (m, 2H, C$_\text{H}$), 1.96-2.37 (m, 4H, C$_\text{H}$ + C$_\gamma$H), Gly\textsuperscript{3}: 7.21 (s, 1H, NH), 3.62-4.40 (dd, 2H, C$_\text{H}$); Bug\textsuperscript{4}: 8.03 (d, 1H, NH), 4.90 (m, 1H, C$_\text{H}$), 3.89 (s, 3H, OCH$_3$), 2.60-2.78 (m, 2H, C$_\text{H}$). ES-MS: calcd for C$_{25}$H$_{37}$N$_4$O$_7$: 505.6 found: $m/z$: [M + H]$^+$ 505.4, [M + Na]$^+$ 527.5, [(M – C$_4$H$_8$) + H]$^+$ 449.5, [(M – C$_4$H$_8$O$_2$) + H]$^+$ 406.45.

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$_2$O (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$^\circ$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%); $R_t$(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$_\text{H}$), 1.41 (s, 9H, Boc), 1.35 (d, 3H, C$_\text{H}$); d-Leu: 7.76 (d, 1H, NH), 4.90 (m, 1H, C$_\text{H}$), 1.83 (m, 1H, C$_\gamma$H), 1.63 (m, 2H, C$_\text{H}$), 0.89-0.95 (m, 6H, C$_\text{H}$); Nle: 8.16 (br s, 1H, NH), 4.90 (m, 1H, C$_\text{H}$), 1.63 (m, 2H, C$_\text{H}$), 1.42-1.47 (m, 2H, C$_\gamma$H), 1.26-1.37 (m, 2H, C$_\text{H}$), 0.89-0.95 (m, 3H, C$_\text{H}$); Gly: 8.09 (m, 1H, NH), 4.12-4.39 (dd, 2H, C$_\text{H}$); Bug: 8.00 (d, 1H, NH), 4.71 (m, 1H, C$_\text{H}$), 3.77 (s, 3H, OCH$_3$), 2.67 (m, 2H, C$_\text{H}$), 1.74 (s, 3H, C$_\text{H}$). ES-MS: calcd for C$_{29}$H$_{50}$N$_8$O$_8$: 596.4 found: $m/z$: [M + Na]$^+$ 618.6.

Boc-Bug\textsuperscript{1-Gly\textsuperscript{2}-Ala\textsuperscript{3-d-Leu\textsuperscript{4}-Nle\textsuperscript{5}-Gly\textsuperscript{6}-Bug\textsuperscript{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$_2$O (76.7 mg, 0.5 mmol) were added. The obtained mixture was cooled to -15$^\circ$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 %); \( R_t(\text{DCM/MeOH 9:1 v/v}) : 0.46; R_i : 16.6 \) min. \(^1\text{H NMR (CDCl}_3/\text{CD}_2\text{OH 14.5:1 v/v, 500 MHz)} \) Bug: \_ 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\(^{26} \): \_ 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, C\_H), 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, C\_H), 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: calcld for C\(_{37}\)H\(_{60}\)N\(_7\)O\(_{10}\): 762.9, found: \( m/z \): [M + H]\(^+\) 762.6, [M + Na]\(^+\) 784.7, [(M – C\(_4\)H\(_8\)) + H]\(^+\) 706.8, [(M – C\(_3\)H\(_6\)O\(_2\)) + 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°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%); \( R_t(\text{DCM/MeOH 97.5:2.5 v/v}) 0.42; R_i 15.5 \) min; \(^1\text{H NMR (CDCl}_3/\text{CD}_2\text{OH 14.5:1 v/v, 500 MHz)} \) Bug: \_ 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: \_ 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, C\_H), 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, C\_H), 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: calcld for C\(_{33}\)H\(_{54}\)N\(_7\)O\(_{10}\): 708.4, found: \( m/z \): [M + H]\(^+\) 708.8, [M + Na]\(^+\) 730.6, [(M – C\(_4\)H\(_8\)) + H]\(^+\) 652.8, [(M – C\(_3\)H\(_6\)O\(_2\)) + H]\(^+\) 608.8. HRMS: calcld for C\(_{33}\)H\(_{54}\)N\(_7\)O\(_{10}\)Na: 730.3748. 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_t(\text{DCM/MeOH 95:5 v/v}) 0.70; \(^1\text{H NMR (CDCl}_3, 300 \) 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, OCH\(_2\)CH\(_3\)), 1.27 (t, 3H, OCH\(_2\)CH\(_3\)); ES-MS: calcld for C\(_{13}\)H\(_{24}\)N\(_2\)O\(_3\)Na: 335.2, found: \( m/z \): [M + Na]\(^+\) 335.2, [M + Na + CH\(_3\)CN]\(^+\) 376.2, [(M – C\(_3\)H\(_6\)O\(_2\)) + 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°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\(_2\)SO\(_4\)), filtered and concentrated in vacuo. Yield:
141 mg (quant); \( R_t (\text{DCM/MeOH} \, 95:5 \, \text{v/v}) = 0 \); \( ^1\text{H NMR (CDCl}_3, \, 300 \, \text{MHz}) \) 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); ESI: \( \_8.62 \) (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\(_2\)O\(_4\)Na: 306.1, found: \( m/z: [\text{M} + \text{Na}]^+ 307.2, [\text{M} + \text{Na} + \text{ACN}]^+ 348.2 \).

**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 \, \text{v/v}). Yield: 187 mg (99%); \( R_t 0.60 \) (DCM/MeOH 9:1 \, \text{v/v}); \( R_{t} 18.94 \, \text{min}; \, ^1\text{H NMR (DMSO-d}_6, \, 500 \, \text{MHz}) \) 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); Bug\(^5\): \( \_8.63 \) (m, 1H, NH), 7.93 (d, 1H, NH), 5.69 (m, 1H, C-H), 4.98-5.11 (m, 2H, C-H), 1.37 (s, 9H, Boc); Ala\(^5\): \( \_7.93 \) (d, 1H, NH), 4.33-4.40 (m, 1H, C-H), 1.21 (d, 3H, C-H); Bug\(^6\): \( \_7.76 \) (d, 1H, NH), 5.69 (m, 1H, C-H); 4.98-5.11 (m, 2H, C-H), 1.69 (s, 3H, C-H); Bug\(^5\): \( \_8.60 \) (m, 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\(_{56}\)H\(_{67}\)N\(_2\)O\(_{10}\): 996.5, found: \( m/z: [\text{M} + \text{H}]^+ 996.8, [\text{M} + \text{Na}]^+ 1018.7 \).

**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 \, \text{v/v}). Yield = 180.6 mg (93%). \( R_t 0.60 \) (DCM/MeOH 9:1 \, \text{v/v}); \( R_{t} 18.86 \, \text{min}; \, ^1\text{H NMR (DMSO-d}_6, \, 500 \, \text{MHz}) \) Alg\(^1\): \( \_6.86 \) (d, 1H, NH), 5.69 (m, 1H, C-H), 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); Alg\(^2\): \( \_7.93 \) (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, C-H); 4.98-5.11 (m, 2H, C-H), 1.69 (s, 3H, C-H); Alg\(^5\): \( \_8.60 \) (m, 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\(_{56}\)H\(_{66}\)N\(_2\)O\(_{10}\): 972.5, found: \( m/z: [\text{M} + \text{H}]^+ 972.7, [\text{M} + \text{Na}]^+ 994.6 \).

**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°C for 90 min. The product was purified by column chromatography (DCM/MeOH 97.5:2.5 \, \text{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 separated by preparative HPLC chromatography to yield the desired product (4.6 mg) as judged by MS/MS. \( R_t = 0.60 \) (DCM/MeOH
9:1 v/v); Rf 17.93 min; $^1$H NMR (CDCl$_3$, 500 MHz) Alg$^1$: _5.45 (bs, 1H, NH), 5.65-5.74 (m, 1H, C$_7$H), 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), 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$_7$O$_{10}$: 918.4, found: m/z: [M + H]$^+$ 918.7, [M + Na]$^+$ 940.8.

References