Supporting Information

Synthesis of 1,5-triazole bridged vancomycin CDE-ring bicyclic mimics using RuAAC macrocyclization

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1. Synthetic Procedures

1.1 General Experimental Procedures

Chemicals were used as obtained from commercial sources without further purification unless stated otherwise. Azidoamino acids 16 and 17 were synthesized using literature procedures starting from the corresponding diamino acid residues. The solvents were obtained as peptide synthesis grade and stored over molecular sieves (4 Å) prior to use.

Column chromatography was performed on Silicycle SiliFlash P60 silica gel (particle size 40-63 μm).

Thin Layer Chromatography was performed on Merck precoated silica gel 60F254 glass plates. Compound spots were visualized by UV-quenching, ninhydrin, or Cl₂/TDM.

Optical rotations were measured on a JASCO P-1010 Polarimeter using a 10 cm cell with a Na 589 nm filter. The specific concentrations (in g/100 mL) are indicated.

H-NMR data were acquired on a Varian Mercury 300 MHz or a Varian Innova 500 MHz spectrometer in CDCl₃ or CD₂OD as solvent. Chemical shifts are reported in delta (δ) units, in parts per million (ppm) relative to TMS (0.00 ppm). Coupling constants (J) are reported in Hertz (Hz). Splitting patterns are designated as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), doublet of doublet (dd), and broad (br). C-NMR data were acquired on a Varian Mercury 75 MHz or a Varian Innova 125 MHz spectrometer in CDCl₃ or CD₂OD as solvent. Chemical shifts are reported in delta (δ) units, in parts per million (ppm) relative to the solvent residual signal, CDCl₃ (77.0 ppm) or CD₂OD (49.0 ppm).

Analytical HPLC was performed on an automated HPLC system (Shimadzu) equipped with a UV/Vis detector operating at 220/254 nm and an evaporative light scattering detector using a Dr. Maisch Reprosil-Pur C18-AQ column (pore size: 100 Å, particle size: 5 μm; 250×4.6 mm) at a flow rate of 1.0 mL/min (100% buffer A (0.1% TFA in CH₃CN/H₂O 5:95 v/v) to 100% buffer B (0.1% TFA in CH₃CN/H₂O 95:5 v/v) in 60 min).

Preparative HPLC was performed on an automated preparative HPLC system (Applied Biosystems) equipped with a UV/Vis detector operating at 214 nm using a Dr. Maisch Reprosil-Pur C18-AQ column (pore size: 100 Å, particle size: 10 μm; 250×22 mm) at a flow rate of 2.0 mL/min (100% buffer A (0.1% TFA in CH₃CN/H₂O 95:5 v/v) to 100% buffer B (0.1% TFA in CH₃CN/H₂O 95:5 v/v) in 90 min).

ESI-MS was performed on a Shimadzu LCMS-QP8000 electrospray ionization mass spectrometer.

MALDI-TOF MS was performed on a Shimadzu Kratos AXIMA-CFR mass spectrometer using α-cyano-4-hydroxycinnamic acid (CHCA) as a matrix and human ACTH (18-39) as the reference.
1.2 Syntheses Schemes

Scheme S11. Synthesis of, (I) (i) the C-terminal dipeptide: a) MeNH₂, BOP, DCM, 20 h; b) TFA/DCM, 1 h; c) Boc-D-Leu-OH, BOP, DIPEA, DCM, 3 h, 82% over three steps; (II) the N-terminal dipeptide: d) H-Ala-OtBu, BOP, DIPEA, DCM, 3 h, 80%; e) TFA/DCM, 1 h, quant.; and (III) the central amino acid: f) Ac₂O, aq. 1 N NaOH/DCM, 3 h, 88%; g) TIPS-acetylene, [Pd(PPh₃)₄], CuI, DIPEA, THF, 20 h, 91%.

Scheme S12. The carbon atoms αC', αC², αC³, αC⁴, αC⁵, arom-C⁶, arom-C⁷ and triazole-C⁸ and C⁹ have been used as fixed coordinates for superimposition (this Scheme belongs to Figure 1 of the main manuscript).
1.3 Syntheses and Compound Analyses

**Boc-d-Leu-Lys(N3)-NHMe 4:** Boc-Lys(N3)-OH 16 (2.18 g, 8.0 mmol) was dissolved in DCM (80 mL). To this solution, BOP (3.90 g, 8.8 mmol) was added, and followed by the addition of MeNH2 (2M in THF, 12 mL, 24 mmol) dropwise during 20 min at 0 °C. The mixture was allowed to warm to room temperature and stirred for 20 h. Then, the solvents were removed by evaporation and the residue was redissolved in EtOAc (150 mL). The resulting solution was washed with 1 N KHSO4 (100 mL, twice), saturated NaHCO3 (100 mL, twice) and brine (100 mL, once), and dried over anhydrous Na2SO4. After filtration and removal of the solvent, the residue was purified by column chromatography (EtOAc/hexane, 1:3 to 1:1, v/v). Boc-Lys(N3)-NHMe was obtained as white solid (2.10 g, 92%). Subsequently, the obtained amide (1.43 g, 5.0 mmol) was dissolved in DCM (50 mL), and to this solution TFA (30 mL) was added. The reaction mixture was stirred for 1 h, after which the volatiles were removed by evaporation and the residual TFA was removed by coevaporation with DCM (30 mL, twice). After drying for 1 h at high vacuum, the free amine was dissolved in DCM (80 mL). To this solution Boc-d-Leu-OH (1.27 g, 5.5 mmol) and BOP (2.43 g, 5.5 mmol) were added, followed by the addition of DiPEA (2.59 mL, 15.0 mmol). The reaction mixture was stirred for 3 h. Then, the solvent was removed and the residue was redissolved in EtOAc (100 mL). The resulting solution was successively washed with 1 N KHSO4 (100 mL, twice), saturated NaHCO3 (100 mL, twice) and brine (100 mL, once), and dried over anhydrous Na2SO4. After filtration and removal of the solvent, the residue was purified by column chromatography (hexane/EtOAc, 3:1 to 1:1, v/v). Boc-d-Leu-Lys(N3)-NHMe 4 was obtained as viscous oil (1.64 g, 82%); Rf = 0.64 (DCM/MeOH, 9:1, v/v); [α]D20 = -9.0 (c = 1.0 MeOH); 1H NMR (300 MHz, CDCl3) δ = 7.01 (s, 1H), 6.93 (d, J = 8.3 Hz, 1H), 5.25 (d, J = 5.7 Hz, 1H), 4.41 (q, J = 8.3 Hz, 1H), 4.12 – 3.92 (m, 1H), 3.24 (t, J = 6.6 Hz, 2H), 2.74 (d, J = 4.5 Hz, 3H), 1.91 (s, 1H), 1.75 – 1.52 (m, 5H), 1.52 – 1.30 (m, 12H), 0.91 (t, J = 5.2 Hz, 6H); 13C NMR (75 MHz, CDCl3) δ = 173.2, 172.0, 156.1, 80.3, 53.8, 53.0, 51.1, 40.8, 31.4, 28.4, 28.3, 26.2, 24.7, 22.9, 22.7, 22.1; MS (ESI) m/z calcd for C18H32N6O4 [M+H]+ 399.27, found 399.45; calcd for C18H32N6NaO4 [M+Na]+ 421.25, found 421.20.

**The synthesis of Fmoc-d-Lys(N3)-Ala-O’Bu (6) and Fmoc-d-Lys(N3)-Ala-OH (10):** Fmoc-d-Lys(N3)-OH 17 (680 mg, 1.72 mmol) was dissolved in DCM (30 mL). To this solution H-Ala-O’Bu·HCl (343 mg, 1.89 mmol) and BOP (836 mg, 1.89 mmol) were added, followed by the addition of DiPEA (743 µL, 4.30 mmol). The reaction mixture was stirred for 3 h. Then, the solvent was removed and the residue was redissolved in EtOAc (100 mL). The resulting solution was successively washed with 1 N KHSO4 (100 mL, twice), saturated NaHCO3 (100 mL, twice) and brine (100 mL, once), and dried over anhydrous Na2SO4. After filtration and removal of the solvent, the residue was purified by column chromatography (hexane/EtOAc, 4:1, v/v). Fmoc-d-Lys(N3)-Ala-O’Bu 6 was obtained as a white solid (720 mg, 80%); Rf = 0.76 (DCM/MeOH, 9:1, v/v); [α]D20 = +1.3 (c = 1.0 CHCl3); 1H NMR (300 MHz, CDCl3) δ = 7.76 (d, J = 7.4 Hz, 2H), 7.59 (d, J = 7.4 Hz, 2H), 7.40 (dd, J = 7.4, 6.9 Hz, 2H), 7.31 (td, J = 7.4, 1.2 Hz, 2H), 6.58 (d, J = 6.0 Hz, 1H), 5.39 (d, J = 8.0 Hz, 1H), 4.44 (dt, J = 9.5, 4.8 Hz, 3H), 4.22 (t, J = 6.8 Hz, 2H), 3.26 (t, J = 6.7 Hz, 2H), 1.98 – 1.78 (m, 1H), 1.76 – 1.51 (m, 3H), 1.48 – 1.40 (m, 12H), 1.37 (d, J = 7.0 Hz, 3H); 13C NMR (75 MHz,
CDCl$_3$ $\delta$ = 172.1, 170.9, 156.3, 144.0, 144.0, 141.5, 128.0, 127.3, 125.3, 120.2, 82.5, 67.3, 54.8, 51.3, 49.0, 47.4, 32.6, 28.7, 28.2, 22.8, 18.8; MS (ESI) $m/z$ calcd for C$_{28}$H$_{36}$N$_2$O$_5$ [M+H]$^+$ 522.27, found 522.00; calcd for C$_{28}$H$_{35}$NaO$_5$ [M+Na]$^+$ 544.25, found 544.55.

Fmoc-D-Lys(N$_2$)-Ala-OH 10 was obtained in quantitative yield by treatment of dipeptide tert-butyler ester 6 with TFA and used without further purification.

(R)-N-$\alpha$-Boc-(4-hydroxy-3,5-diiodophenylglycine 19 was synthesized according to a literature procedure.$^{[2]}$ D-4-Hydroxyphenylglycine 18 (10.5 g, 63.0 mmol) was dissolved in AcOH (90 mL). To this solution ICl (22.5 g, 138.6 mmol) in AcOH (5.0 mL) was added dropwise during 10 min under argon. After stirring for 72 h at room temperature, the reaction mixture was poured into ice water (1000 mL). The precipitated crystals were filtered off, washed with EtOH (100 mL twice) to provide 3,5-diiodo-D-4-hydroxyphenylglycine (22.1 g, 85%) as light brown crystals and was used without further purification in the next step. 3,5-Diiodo-D-4-hydroxy-phenylglycine (4.19 g, 10 mmol) was dissolved in H$_2$O/dioxane (60 mL, 1:1, v/v). To this solution Boc$_2$O (2.62 g, 12 mmol) and Et$_3$N (2.1 mL, 15 mmol) were added. The reaction mixture was stirred for 4 h. The reaction mixture was diluted with EtOAc (50 mL) and the resulting solution was extracted with H$_2$O (50 mL, twice). The aqueous phase was combined and acidified with KHSO$_4$ to pH 2-3, extracted with EtOAc (100 mL, twice). The organic phase was washed with brine (150 mL, once), and dried over anhydrous Na$_2$SO$_4$. After filtration and removal of the solvent, the residue was purified by column chromatography (hexane/EtOAc, 3:1, v/v, with 0.1% AcOH). Diiodo compound 19 was obtained as a light yellowish solid (3.54 mg, 68%); $R_f$ = 0.57 (hexane/EtOAc, 1:1, v/v, with 0.1% AcOH); $[\alpha]_D^{20} = -94.3$ (c = 1.0 CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ = 8.02 (s, 1H), 7.75 (s, 2H), 4.97 (d, $J$=4.8 Hz, 1H), 1.29 (s, 9H).

(R)-N-$\alpha$-Boc-(4-acetoxy-3,5-diiodophenylglycine 20 was synthesized according to a literature procedure.$^{[3]}$ Hydroxy compound 19 (2.60 g, 5.00 mmol) was dissolved in ice-cold aq. 1N NaOH/DCM (60 mL, 1:1, v/v) and to this mixture, Ac$_2$O (2.36 mL, 25.0 mmol) was added dropwise at 0 °C. Then, the reaction mixture was stirred at room temperature for 3 h. Subsequently, the aqueous solution was acidified with KHSO$_4$ to pH 2-3 and extracted with DCM (30 mL, twice). The organic phases were combined and dried over anhydrous Na$_2$SO$_4$. After filtration and removal of the solvent, the residue was purified by column chromatography (EtOAc/hexane, 1:3 to 1:2, v/v, with 0.1% HOAc). Acetyl ester 20 was obtained as a white solid (2.47 g, 88%); $R_f$ = 0.61 (hexane/EtOAc, 1:1, v/v, with 0.1% HOAc); $[\alpha]_D^{20} = -72.6$ (c = 1.0, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ = 8.08 (s, 1H), 7.86 (s, 2H), 5.02 (d, $J$ = 3.7 Hz, 1H), 2.39 (s, 3H), 1.26 (s, 9H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ = 172.5, 167.4, 157.0, 151.5, 139.5, 138.3, 90.5, 83.1, 57.3, 28.3, 21.6.

Bis-alkyne 8: Diiodo compound 20 (2.24 g, 4.0 mmol), [Pd(PPh$_3$)$_4$] (462 mg, 0.4 mmol), and CuI (228 mg, 1.2 mmol) were placed in a flask sealed with a rubber septum. The flask was evacuated and refilled with dry N$_2$ (repeated for three times). THF (40 mL) (purged with dry N$_2$ for 1 h prior to use) was added to the flask via a syringe. The resulting solution was degassed again using a freeze-pump-thaw procedure (repeated three times). Then, DiPEA (1.39 mL, 8.0 mmol) and TIPS-acetylene (3.60 mL, 16 mmol) were added to the mixture via a
solvent, brine (successively evaporation). The resulting solution was successively washed with 1 N KHSO₄ (100 mL, twice), saturated NaHCO₃ (100 mL, twice) and brine (100 mL, once), and dried over anhydrous Na₂SO₄. After filtration and removal of the solvent, the residue was purified by column chromatography (EtOAc/hexane, 1:4 to 1:2, v/v, with 0.1% AcOH). Bis-alkyne 8 was obtained as a yellowish solid (2.45 g, 91%). Rf = 0.26 (DCM/MeOH, 9:1, v/v); [α]D²⁰ = -51.4 (c = 1.0, CHCl₃); ¹H NMR (300 MHz, CDCl₃) δ = 7.99 (s, 1H), 7.51 (s, 2H), 5.04 (s, 1H), 2.28 (s, 3H), 1.26 (s, 9H), 1.11 (s, 42H); ¹³C NMR (75 MHz, CDCl₃) δ = 172.6, 167.4, 156.9, 152.6, 135.9, 132.4, 118.6, 103.4, 96.6, 82.7, 58.2, 27.9, 20.6, 18.6, 11.2; MS (ESI) m/z calcd for C₇₇H₅₀N₉O₆Si₂ [M+Na]⁺ 962.38, found 962.25. This compound is abbreviated as: D-Phg(4-OAc-3,5-bis-TIPS-alkyne).

Boc-d-Phg(4-OAc-3,5-bis-TIPS-alkyne)-d-Leu-Lys(N₃)-NHMe 9:
Boc-d-Leu-Lys(N₃)-NHMe 4 (877 mg, 2.2 mmol) was dissolved in DCM (15 mL), and to this solution, TFA (15 mL) was added. The reaction mixture was stirred for 1 h, after which the volatiles were removed by evaporation and the residual TFA was removed by coevaporation with DCM (20 mL, twice). After drying for 1 h at high vacuum, the free amine was dissolved in DCM (60 mL). To this solution Boc-d-Phg(4-OAc-3,5-bis-TIPS-alkyne) compound 8 (1.34 g, 2.0 mmol) and BOP (973 mg, 2.2 mmol) were added, followed by the addition of DIPEA (1.04 mL, 6.0 mmol). This reaction mixture was stirred for 3 h. Then, the solvent was removed by evaporation and the residue was redissolved in EtOAc (100 mL). The resulting solution was successively washed with 1 N KHSO₄ (50 mL, twice), saturated NaHCO₃ (50 mL, twice) and brine (50 mL, once), and dried over anhydrous Na₂SO₄. After filtration and removal of the solvent, the residue was purified by column chromatography (hexane/EtOAc, 3:1 to 1:1, v/v). Boc-d-Phg(4-OAc-3,5-bis-TIPS-alkyne)-d-Leu-Lys(N₃)-NHMe 9 was obtained as a diastereoisomeric mixture: RRR:SSS = 5:4:1 based on analytical HPLC (1.60 g, 84%); MS (ESI) m/z calcd for C₅₀H₄₄N₉O₆Si₂ [M+H]⁺ 950.60, found 951.00.

Fmoc-d-Lys(N₃)-Ala-d-Phg(4-OAc-3,5-bis-TIPS-alkyne)-d-Leu-Lys(N₃)-NHMe 11:
Fmoc-d-Phg(4-OAc-3,5-bis-TIPS-alkyne)-d-Leu-Lys(N₃)-NHMe 9 (300 mg, 0.316 mmol) was dissolved in 8 mL of DCM, and to this solution TFA (2 mL) was added. The obtained reaction mixture was stirred for 1 h. Then, the volatiles were removed by evaporation and the residual TFA was removed by coevaporation with DCM (10 mL, twice). After drying for 1 h at high vacuum, the free amine was dissolved in DCM (10 mL). To this solution, Fmoc-d-Lys(N₃)-Ala-OH 10 (161 mg, 0.347 mmol), EDCI (66.3 mg, 0.347 mmol), and HOBr (46.9 mg, 0.347 mmol) were added, followed by the addition of DIPEA (192 µL, 1.11 mmol), and the reaction mixture was stirred for 4 h. Subsequently the solvent was removed by evaporation and the residue was redissolved in EtOAc (100 mL). The resulting solution was successively washed with 1 N KHSO₄ (50 mL, twice), saturated NaHCO₃ (50 mL, twice) and brine (50 mL, once), and dried over anhydrous Na₂SO₄. After filtration and removal of the solvent, the residue was purified by column chromatography (MeOH/DCM, 1:99 to 2:98, v/v). Fmoc-d-Lys(N₃)-Ala-d-Phg(4-OAc-3,5-bis-TIPS-alkyne)-d-Leu-Lys(N₃)-NHMe 11 was obtained as a white solid (230 mg, 56%); Rf = 0.68 (DCM/MeOH, 9:1, v/v); [α]D²⁰ = +19.7 (c
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595.0x842.0

Once) and brine (solution was stirred for 24 h. 0.738 mmol) and K

1

Boc

12H), 1.47 (s, 12H), 1.37 (d,

4.48 (m, 1H), 4.40 – 3.92 (m, 1H), 3.27 – 3.18 (m, 4H), 2.75 (d, J = 3.7 Hz, 3H), 2.31 (s, 3H), 1.95 – 1.65 (m, 6H), 1.60 – 1.52 (m, 4H), 1.47 – 1.34 (m, 8H), 1.11 (s, 42H), 0.92 (s, 6H).\[^{13}\]C NMR (75 MHz, CDCl\(_3\)) \(\delta = 174.0, 173.5, 173.0, 172.7, 169.0, 167.6, 157.8, 153.5, 143.9, 143.6, 141.3, 133.3, 127.7, 127.1, 127.0, 125.2, 125.1, 119.9, 119.6, 100.0, 98.0, 67.1, 59.1, 54.0, 53.6, 52.0, 51.1, 50.6, 47.2, 39.2, 30.4, 29.7, 28.5, 28.4, 26.2, 25.2, 23.3, 23.1, 23.0, 20.7, 20.6, 18.6, 16.3, 11.2, -0.0; MS (ESI) m/z calcd for C\(_{60}\)H\(_{101}\)N\(_{13}\)O\(_3\)S\(_2\) [M+H\(^+\)]: 1297.74, found 1297.75.

Boc-N-Me-d-Leu-d-Lys(N\(_3\))-Ala-d-Phg(4-OH-3,5-bis-TIPS-alkyne)-d-Leu-Lys(N\(_3\))-NH Me 12: Fmoc-d-Lys(N\(_3\))-Ala-d-Phg(4-OAc-3,5-bis-TIPS-alkyne)-d-Leu-Lys(N\(_3\))-NHMe 11 (430 mg, 0.331 mmol) was dissolved in THF (10 mL) and to this solution (CH\(_3\)\(_2\))\(_2\)NH (8.28 mL, 2M in THF, 16.6 mmol) was added, followed by the addition of 1-propanethiol (292 µL, 3.31 mmol).\(^{[9]}\) The resulting reaction mixture was stirred for 1 h. After removal of the solvent under reduced pressure, the free amine was obtained and after drying for 1 h at high vacuum, the free amine was dissolved in DCM (20 mL), and to this solution Boc-N-Me-d-Leu-OH (81 mg, 0.331 mmol), EDCI (69.5 mg, 0.364 mmol), and HOBT (49.2 mg, 0.364 mmol) were added, followed by the addition of DiPEA (126 µL, 0.728 mmol). The reaction mixture was stirred for 3 h. Then, the solvent was removed by evaporation and the residue was redissolved in EtOAc (100 mL). The resulting solution was successively washed with 1 N KHSO\(_4\) (50 mL, twice), saturated NaHCO\(_3\) (50 mL, twice) and brine (50 mL, once), and dried over anhydrous Na\(_2\)SO\(_4\). After filtration and removal of the solvent, the residue was purified by column chromatography (MeOH/DCM, 1:99 to 2:98, v/v). Hexapeptide, Boc-N-Me-d-Leu-d-Lys(N\(_3\))-Ala-d-Phg(4-OH-3,5-bis-TIPS-alkyne)-d-Leu-Lys(N\(_3\))-NHMe 12 was obtained as a white solid (310 mg, 74%); R\(_f\) = 0.55 (DCM/MeOH, 9:1, v/v); [\(\alpha\)]\(_D\)\(^{20}\) = +25.5 (c = 1.0 CHCl\(_3\)); \(^1\)H NMR (300 MHz, CD\(_3\)OD) \(\delta = 7.43\) (s, 2H), 5.14 (s, 1H), 4.65 – 4.48 (m, 1H), 4.40 – 4.11 (m, 3H), 3.29 (d, J = 6.4 Hz, 4H), 2.73 (s, 6H), 1.58 – 1.85 (m, 12H), 1.47 (s, 12H), 1.37 (d, J = 6.8 Hz, 4H), 1.15 (s, 44H), 1.01 – 0.82 (m, 12H);\(^{13}\)C NMR (75 MHz, CDCl\(_3\)/CD\(_3\)OD) \(\delta = 177.8, 177.1, 177.0, 176.4, 175.4, 162.8, 136.6, 131.3, 115.4, 104.9, 101.0, 84.2, 62.2, 59.6, 58.0, 57.2, 56.5, 54.9, 54.8, 53.0, 43.1, 40.3, 34.8, 33.8, 32.2, 31.8, 31.5, 29.4, 28.6, 28.5, 26.9, 26.7, 26.6, 26.4, 25.0, 24.4, 21.9, 20.3, 15.0; MS (ESI) m/z calcd for C\(_{68}\)H\(_{110}\)N\(_{13}\)O\(_3\)S\(_2\) [M+H\(^+\)]\(^+\): 1260.81, found 1261.20.

Boc-N-Me-d-Leu-d-Lys(N\(_3\))-Ala-d-Phg(4-OMe-3,5-bis-TIPS-alkyne)-d-Leu-Lys(N\(_3\))-NH Me 13:
Boc-N-Me-d-Leu-d-Lys(N\(_3\))-Ala-d-Phg(4-OMe-3,5-bis-TIPS-alkyne)-d-Leu-Lys(N\(_3\))-NHMe 12 (310 mg, 0.246 mmol) was dissolved in acetone (20 mL). To this solution, Mel (46 µL, 0.738 mmol) and K\(_2\)CO\(_3\) (102 mg, 0.738 mmol) were added. The obtained reaction mixture was stirred for 24 h. After the reaction was complete, as judged by ESI-MS, the solvent was removed by evaporation and the residue was redissolved in EtOAc (100 mL). The resulting solution was successively washed with 1 N KHSO\(_4\) (50 mL, once), saturated NaHCO\(_3\) (50 mL, once) and brine (50 mL, once), and dried over anhydrous Na\(_2\)SO\(_4\). After filtration and
removal of the solvent, the residue was purified by column chromatography (MeOH/DCM, 2:98, v/v). The protected hexapeptide Boc-N-Me-d-Leu-d-Lys(N3)-Ala-d-Phg(4-OMe-3,5-bis-TIPS-alkyne)-d-Leu-Lys(N3)-NHMe 13 was obtained as a white solid (295 mg, 94%); Rf = 0.55 (DCM/MeOH, 9:1, v/v); [α]D20 = +26.9 (c = 1.0 CHCl3); 1H NMR (300 MHz, CDCl3) δ = 7.63 (s, 1H), 7.50 (s, 1H), 7.34 (s, 2H), 6.89 (s, 1H), 6.68 (d, J = 17.3 Hz, 1H), 5.19 (s, 1H), 4.53 (s, 1H), 4.42 (s, 1H), 4.26 (s, 1H), 4.01 (s, 1H), 3.99 (s, 3H), 3.26 (d, J = 3.1 Hz, 4H), 2.86 – 2.71 (m, 6H), 1.77 (d, J = 11.7 Hz, 8H), 1.57 – 1.64 (m, 4H), 1.43 (s, 12H), 1.39 (d, J = 6.4 Hz, 4H), 1.12 (s, 44H), 0.93 (s, 12H); 13C NMR (75 MHz, CDCl3) δ = 173.5, 173.0, 172.4, 169.3, 163.2, 133.4, 131.0, 119.0, 101.5, 96.8, 80.3, 77.2, 61.1, 58.8, 57.5, 53.5, 51.9, 51.1, 51.0, 50.3, 39.8, 37.0, 30.7, 28.4, 28.3, 26.1, 25.2, 25.0, 23.4, 23.2, 23.0, 21.8, 20.8, 18.6, 16.0, 11.6, 11.3, 11.2, 10.9, -0.0; MS (ESI) m/z calcd for C65H112N13O38Si2 [M+H]+ 1274.82, found 1275.05.

Boc-N-Me-d-Leu-d-Lys(N3)-Ala-d-Phg(4-OMe-3,5-bis-alkyne)-d-Leu-Lys(N3)-NHMe 14:  Boc-N-Me-d-Leu-d-Lys(N3)-Ala-d-Phg(4-OMe-3,5-bis-TIPS-alkyne)-d-Leu-Lys(N3)-NHMe 13 (275 mg, 0.216 mmol) was dissolved in THF/MeOH (20 mL, 19:1, v/v), and to this solution TBAF (0.65 mL, 1M in THF, 0.647 mmol) was added. After stirring for 1 h, another portion of TBAF (0.65 mL, 1M in THF) was added. The obtained reaction mixture was stirred for another 2h. Based on TLC analysis, the reaction was complete. Subsequently the solution was diluted with EtOAc (100 mL) and the resulting solution was successively washed with 1 N KH2PO4 (50 mL, once), saturated NaHCO3 (50 mL, once) and brine (50 mL, once), and dried over anhydrous Na2SO4. After filtration and removal of the solvent, the residue was purified by column chromatography (MeOH/DCM, 2:98 to 4:96, v/v), and Boc-N-Me-d-Leu-d-Lys(N3)-Ala-d-Phg(4-OMe-3,5-bis-alkyne)-d-Leu-Lys(N3)-NHMe 14 was obtained as a white solid (190 mg, 91%); Rf = 0.49 (DCM/MeOH, 9:1, v/v); [α]D20 = -15.0 (c = 1.0 MeOH); 1H NMR (300 MHz, CD3OD) δ = 7.52 (s, 2H), 5.28 (s, 1H), 4.73 – 4.48 (m, 1H), 4.27 (t, J = 9.2 Hz, 4H), 3.99 (s, 3H), 3.79 (s, 2H), 3.42 – 3.17 (m, 4H), 2.72 (d, J = 4.0 Hz, 6H), 1.96 – 1.51 (m, 10H), 1.51 – 1.22 (m, 20H), 0.93 (dd, J = 15.2, 6.1 Hz, 12H); 13C NMR (75 MHz, CDCl3) δ = 177.6, 177.1, 177.0, 176.5, 174.9, 166.8, 137.7, 135.7, 120.8, 86.9, 84.1, 82.2, 81.8, 64.3, 61.2, 59.9, 57.6, 57.3, 57.2, 56.7, 54.8, 54.8, 53.0, 43.2, 40.6, 34.7, 34.6, 33.6, 32.1, 31.9, 31.2, 29.1, 28.5, 26.9, 26.7, 26.2, 25.9, 24.4, 21.7, 20.2; MS (ESI) m/z calcd for C47H73N13O38 [M+H]+ 962.56, found 963.05; calcd for C47H73N13NaO3 [M+Na]+ 984.54, found 985.32; MALDI-TOF MS m/z calcd for C47H73N13NaO3 [M+Na]+ 984.540, found 984.813; calcd for C47H73K13NO3 [M+K]+ 1000.513, found 1000.788.

N-Boc protected bicyclic hexapeptide 15: Boc-N-Me-d-Leu-d-Lys(N3)-Ala-d-Phg(4-OMe-3,5-bis-alkyne)-d-Leu-Lys(N3)-NHMe 14 (75 mg, 0.078 mmol) and [Cp*RuCl2]2 (25.2 mg, 0.0234 mmol) were placed in a capped flask. The flask was evacuated and refilled with dry N2 (repeated three times). Then, THF/MeOH (15.6 mL, 19:1, v/v) was added to the flask via a syringe. The solvents were purged with dry N2 for 1 h prior to use. The resulting solution was degassed using a free-pump-thaw procedure (repeated three times). Then, the reaction mixture was stirred for 24 h at 50 °C under N2, after which the solvents were removed under reduced pressure and the residue was absorbed on silica gel and purified by column chromatography (MeOH/DCM, 5:95 to 10:90, v/v). A product fraction was obtained which was further
purified with preparative RP-HPLC. N-Boc-protected bicyclic hexapeptide 15 was obtained as a white solid after lyophilization (30 mg, 40%); R_f = 0.25 (DCM/MeOH, 9:1, v/v); ^1H NMR (500 MHz, CD_3OD) δ = 8.02 (s, 1H), 7.86 (s, 1H), 7.82 (s, 1H), 7.55 (s, 1H), 5.73 (s, 1H), 4.59 (s, 1H), 4.45 – 4.28 (m, 3H), 4.28 – 4.05 (m, 5H), 3.96 – 3.86 (m, 1H), 3.19 (s, 3H), 2.89 (s, 3H), 2.69 (s, 3H), 2.06 – 1.77 (m, 5H), 1.75 – 1.63 (m, 6H), 1.57 – 1.49 (m, 3H), 1.47 (s, 9H), 1.42 (d, J = 7.3 Hz, 3H), 1.29 (s, 3H), 1.23 – 1.04 (m, 2H), 0.96 (d, J = 6.5 Hz, 11H), 0.90 (d, J = 6.4 Hz, 6H); MS (ESI) m/z calcd for C_{47}H_{72}N_{13}O_{9} [M+H]^+ 962.56, found 963.05; calcd for C_{47}H_{71}N_{13}NaO_{9} [M+Na]^+ 984.54, found 984.60; MALDI-TOF MS m/z calcd for C_{47}H_{71}N_{13}NaO_{9} [M+Na]^+ 984.540, found 984.740; calcd for C_{47}H_{71}KN_{13}NO_{9} [M+K]^+ 1000.513, found 1000.701.

**Bicyclic hexapeptide 2:** N-Boc-protected bicyclic hexapeptide 15 (20 mg, 0.021 mmol) was treated with TFA (1 mL) in DCM (1 mL) for 1 h, after which the volatiles were removed under reduced pressure. The residue was purified with preparative RP-HPLC. Bicyclic hexapeptide 2 was obtained as a white solid after lyophilization (10.2 mg, 55%); HPLC analysis, R_t = 18.9 min; MS (ESI) m/z calcd for C_{42}H_{64}N_{13}O_{7} [M+H]^+ 862.51, found 862.85; MALDI-TOF MS m/z calcd for C_{42}H_{64}N_{13}O_{7} [M+H]^+ 862.505, found 862.685; calcd for C_{42}H_{63}N_{13}NaO_{7} [M+Na]^+ 884.487, found 862.685.

### 1.4 References

2. ITC Experiments

Binding affinity measurement was determined by using microcalorimetry, which was performed on automated MicroCal Auto-iTC200 equipment.

ITC (isothermal titration calorimetry) experiment was carried out by injection the ligand solution (10-15 mM) into the cell containing the solution of the synthesized mimics or vancomycin (0.1-0.3 mM) dissolved in a 0.02 M Na-citrate/citric acid buffer (pH 5.1). The typical experiment contains 16 injections in 40 min and the resulting data was analyzed by non-linear fitting in Origin software.
Figure S1. ITC experiment of vancomycin (1) + Ac-D-Ala.
Figure S2. ITC experiment of vancomycin (1) + Ac-D-Ala-D-Ala.
Figure S3. ITC experiment of vancomycin (1) + Ac-Lys-D-Ala-D-Ala.
Figure S4. ITC experiment of vancomycin (1) + Ac-Lys-D-Ala-D-Lac.
Figure S5. ITC experiment of vancomycin mimic 2 + Ac-D-Ala.
Figure S6. ITC experiment of vancomycin mimic 2 + Ac-D-Ala-D-Ala.
**Figure S7.** ITC experiment of vancomycin mimic 2 + Ac-Lys-D-Ala-D-Ala.
Figure S8. ITC experiment of vancomycin mimic 2 + Ac-Lys-D-Ala-D-Lac.
**Figure S9.** ITC experiment of vancomycin (I) + Ac-L-Ala-OH.
Figure S10. ITC experiment of vancomycin (1) + Ac-L-Ala-L-Ala-OH.
3. Copies of the $^1$H-NMR and $^{13}$C-NMR Spectra and the 2D HSQC NMR Spectrum

![NMR Spectra](image)

**Figure S11 and S12.** $^1$H and $^{13}$C NMR spectrum of Boc-D-Leu-Lys(N$_3$)-NHMe 4.
Figure S13 and S14. $^1$H and $^{13}$C NMR spectrum of Fmoc-D-Lys(N$_3$)-Ala-O'Bu 6.
Figure S15 and S16. $^1$H and $^{13}$C NMR spectrum of (R)-$\alpha$-Boc-(4-acetoxy-3,5-diiodo)phenylglycine 20 (indicated as 13).
**Figure S17 and S18.** $^1$H and $^{13}$C NMR spectrum of Boc-D-Phg(4-OAc-3,5-bis-TIPS-alkyne) 8 (indicated as 14).
Figure S19 and S20. $^1$H and $^{13}$C NMR spectrum of Fmoc-D-Lys(N$_3$)-Ala-D-Phg(4-OAc-3,5-bis-TIPS-alkyne)-D-Leu-Lys(N$_3$)-NHMe 11 (indicated as 16).
Figure S21 and S22. $^1$H and $^{13}$C NMR spectrum of Boc-$N$-Me-$d$-Leu-$d$-Lys($N_3$)-Ala-$d$-Phg(4-OH-3,5-bis-TIPS-alkyne)-$d$-Leu-Lys($N_3$)-NHMe 12 (indicated as 17).
Figure S23 and S24. $^1$H and $^{13}$C NMR spectrum of Boc-$\alpha$-Me-$\delta$-$\alpha$-Leu-$\delta$-$\alpha$-Leu-$\delta$-$\alpha$-Lys(3)-NHMe 13 (indicated as 18).
Figure S25 and S26. $^1$H and $^{13}$C NMR spectrum of Boc-N-Me-d-Leu-d-Leu-Lys(N$_3$)-Ala-d-Phg(4-OMe-3,5-bis-alkyne)-d-Leu-Lys(N$_3$)-NHMe 14 (indicated as 19).
**Figure S27.** $^1$H NMR spectrum of N-Boc-protected bicyclic hexapeptide 15 (indicated as 20).
Figure S28. 2D-HSQC spectrum of N-Boc-protected bicyclic hexapeptide 15.
4. Copies of the Analytical HPLC Chromatograms and MALDI-TOF MS Spectra

**Figure S29.** HPLC analysis of (a) the linear hexapeptide 14 (Boc-N-Me-D-Leu-D-Leu-D-Lys(N3)-Ala-D-Phg(4-OMe-3,5-bis-alkyne)-D-Leu-Lys(N3)-NH Me) and (b) the reaction mixture after RuAAC macrocyclization of linear hexapeptide 14.
**Figure S30.** HPLC analysis of N-Boc-protected bicyclic hexapeptide 15.

**Figure S31.** HPLC analysis of bicyclic hexapeptide 2.
Figure S32. MALDI-TOF MS spectrum of compound 14 (Boc-N-Me-D-Leu-D-Lys(N$_3$)-Ala-D-Phg(4-OMe-3,5-bis-alkyne)-D-Leu-Lys(N$_3$)-NHMe).

Figure S33. MALDI-TOF MS spectrum of compound 14 after treatment with TCEP.

Figure S34. MALDI-TOF MS spectrum of N-Boc-protected bicyclic hexapeptide 15.
**Figure S35.** MALDI-TOF MS spectrum of N-Boc-protected bicyclic hexapeptide 15 after treatment with TCEP.

**Figure S36.** MALDI-TOF MS spectrum of bicyclic hexapeptide 2.
Figure S37. HPLC analysis of the TCEP reduction experiment: (a) linear hexapeptide 14 (indicated as hexapeptide 41), (b) linear hexapeptide 14 after treatment with TCEP, (c) bicyclic hexapeptide 2 (indicated as bicyclic compound 43) and (d) bicyclic hexapeptide 2 after treatment with TCEP.

Linear hexapeptide 14: Boc-\textit{N}-Me-D-Leu-D-Lys\textsubscript{(N\textsubscript{3})}-Ala-D-Phg(4-OMe-3,5-bis-alkyne)-D-Leu-Lys\textsubscript{(N\textsubscript{3})}-NHMe.