SUPPORTING INFORMATION

Total Synthesis of the Antiviral Peptide Antibiotic Feglymycin

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1. Synthetic Procedures

1.1 General Information

All reactions were carried out under an atmosphere of argon in oven-dried glassware with magnetic stirring. Dry solvents were purchased from ACROS (Geel-Belgium). All other reagents were used as obtained (Sigma-Aldrich, Taufkirchen-Germany; ACROS, Geel-Belgium; Alfa Aesar, Karlsruhe-Germany; Merck, Darmstadt-Germany) unless otherwise noted. Flash chromatography was performed with Merck or ACROS silica gel (0,04-0,064 μm grade).

Analytical thin-layer chromatography was performed with commercial TLC aluminium sheets (E. Merck, TLC Silica gel 60 F$_{254}$). Compounds were visualized by UV-light at 254 nm and/or by dipping the plates in potassium permanganate-, ninhydrin- or ammonium molybdate-solution followed by heating.

Melting points were measured on a Leica Galen III melting point apparatus and were uncorrected.

Proton nuclear magnetic resonance (¹H-NMR) data were acquired on a Bruker AV 400 (400.14 MHz) in DMSO-d$_6$. Chemical shifts are reported in delta (δ) units, in parts per million (ppm) relative to the undeuterated solvent residual signal. Coupling constants (J) are reported in Hertz (Hz). Splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublet of doublet; br, broad. Proton decoupled Carbon-13 nuclear magnetic resonance (¹³C-NMR) data were acquired on a Bruker AV 400 (100.62 MHz) in DMSO-d$_6$. Chemical shifts are reported in delta (δ) units, in parts per million (ppm) relative to the undeuterated solvent residual signal. In some cases chemical shifts of ¹³C-signals were determined with the aid of 2D-NMR-experiments (HSQC, HMBC) measured on a Bruker DRX 500 NMR spectrometer.

Infrared (IR) data were recorded on a Perkin-Elmer 881 IR-Spectrometer. Absorbance frequencies are reported in reciprocal centimetres (cm$^{-1}$).

Chiral HPLC-analyses were performed on a Merck-Hitachi LaChrom system with a CHIRALPAK AD-H column using iso-PrOH and n-hexane as eluents (flow rate 0.8 ml/min).

Optical rotations were measured on a Perkin-Elmer Polarimeter 341 using a 10 cm cell with a Na 589 nm filter. The specific concentrations (in g/100 ml) are indicated.
**HPLC-ESI-MS coupled measurements** were performed on an *Applied Biosystems* Qtrap 2000 mass spectrometer in combination with an *Agilent* 1100 HPLC-system (column 1: Jupiter 4u Proteo90A, 30 x 1 mm; column 2: Jupiter 4u Proteo90A, 150 x 1 mm; solvent A: H₂O + 0.1 % HCOOH; solvent B: CH₃CN + 0.1 % HCOOH; flow rate 1.5 ml/min). ESI-MS spectra were acquired in the positive mode.

**Electron-impact ionisation (EI) mass spectra** (also HRMS) were recorded on *Finnigan* MAT 95 SQ or Variant 711 spectrometers. **HRMS** (electrospray ionisation) were recorded on a *Bruker* APEX-II ESI-FTICR spectrometer.

**Preparative HPLC** was performed on an *Agilent* 1100 system with 280 nm monitoring wavelength (column: C18 Grom-Sil 300 ODS-5 ST; solvent A: H₂O + 0.1 % HCOOH, solvent B: CH₃CN + 0.1 % HCOOH, flow rate 15 ml/min).

**UPLC-MS coupled measurements** were performed on a *Waters* Acquity UPLC System with sample manager, binary solvent manager and PDA (detector; 200 - 600 nm). As UPLC column a *Waters* Acquity UPLC BEH C18 (2.1 x 100 mm) was used (solvent A: H₂O, 6.5 mM ammoniumacetate; solvent B: CH₃CN, 6.5 mM ammoniumacetate; flow rate 0.6 ml/min). ESI-MS spectra were recorded with a *Bruker* µTOF LC MS and were acquired in the positive and negative mode.
1.2 Experimental Section

Synthesis of Feglymycin and its enantiomer:

Synthetic procedures in the following describe preparation of Feglymycin (1) and all intermediates. Composition of enantiomeric compounds differed very slightly concerning reaction yields wherefore reaction details in this case are omitted.

Purification of substances for biological testings by preparative HPLC under acidic conditions did not result in oxidation of compounds. No oxidized by-products or higher oligomers could be detected by analytical HPLC.

Feglymycin 1

Tridecapeptide 36 (35.00 mg, 0.02 mmol) was dissolved in 3.6 ml of methanol under an Ar-atmosphere. 20.00 mg Pd/C (10 %) were then added and the flask was flushed with H2. After stirring at room temperature for 1.5 h additionally 20.00 mg Pd/C (10 %) and two drops H2O were added. After a total reaction time of 5.5 h the reaction mixture was filtered through a syringe filter (Rotalibo®-syringe filter, PTFE, 0.45 μm). Volatiles of the so obtained clear solution were removed by rotary evaporation and after dissolving the remaining thin film in 5.0 ml of H2O lyophilisation afforded almost pure Feglymycin 1 as a fluffy solid (26.80 mg, 89 %) without further purification. Samples for biological testings were additionally purified by preparative HPLC (tR = 25.3 min [12 % to 26 % CH3CN in 30 min]): Rf = 0.6 (EtOAc/n-butanol/H2O/acetic acid = 2:1:1:1); mp > 230 °C; [α]D20 = -80.0 (c = 0.05, MeOH), 1′:[α]D20 = +80.0 (c = 0.05, MeOH), authentic Sample of 1: [α]D20 = -78.0 (c = 0.05, MeOH); IR (neat): 3285, 3080, 2971, 1639, 1635, 1600, 1530, 1513, 1456, 1370, 1266, 1151, 1004, 842 cm−1; 1H-NMR (400 MHz, DMSO): δ = 0.53 (t, J = 7.4 Hz, 6H), 0.59 (t, J = 6.6 Hz, 6H), 1.86 (br s, 1H), 2.81 (dd, J = 13.8, 9.3 Hz, 1H), 3.04 (dd, J = 13.3, 4.2 Hz, 2H), 4.34 – 4.38 (m, 3H), 4.48 – 4.54 (m, 1H), 4.95 (s, 1H), 5.29 (d, J = 7.5 Hz, 1H), 5.40 (d, J = 7.3 Hz, 1H), 5.45 (d, J
= 8.6 Hz, 1H), 5.48 (d, J = 7.5 Hz, 1H), 5.49 (d, J = 7.5 Hz, 1H), 5.52 (d, J = 7.5 Hz, 1H), 5.58 (d, J = 8.1 Hz, 1H), 5.60 (d, J = 8.0 Hz, 1H), 6.04 – 6.07 (m, 4H), 6.12 (br s, 1H), 6.16 (d, J = 1.3 Hz, 2H), 6.19 (d, J = 1.3 Hz, 2H), 6.24 (d, J = 1.3 Hz, 2H), 6.28 (d, J = 1.3 Hz, 2H), 6.35 (d, J = 1.6 Hz, 2H), 6.53 – 6.57 (m, 6H), 6.79 (d, J = 8.3 Hz, 2H), 6.92 (d, J = 8.6 Hz, 2H), 7.03 (d, J = 8.1 Hz, 2H), 7.05 (d, J = 8.3 Hz, 2H), 7.13 – 7.24 (m, 5H), 7.30 (d, J = 8.3 Hz, 2H), 7.96 (d, J = 9.4 Hz, 1H), 8.11 (d, J = 8.6 Hz, 1H), 8.36 (d, J = 7.5 Hz, 1H), 8.44 – 8.55 (m, 3H), 8.59 – 8.67 (m, 3H), 8.75 (d, J = 7.3 Hz, 1H), 8.90 (d, J = 7.8 Hz, 1H), 9.04 – 9.34 (m, 13H), 9.72 (br s, 1H); \(^{13}\)C-NMR (100 MHz, DMSO): \(\delta = 17.9, 18.5, 18.9, 19.4, 20.3, 31.8, 32.0, 37.9, 54.4, 55.4, 55.6, 56.3, 57.3, 57.4, 102.2, 106.3, 115.4, 116.1, 124.8, 127.0, 128.7, 129.9, 141.0, 158.2, 158.5, 169.4; MS (ESI) \(m/z = 950.6 \text{ (M+H)}^{+}\); HRMS (ESI): \(m/z\) calcd for C\(_{95}\)H\(_{97}\)N\(_{13}\)O\(_{30}\) (M+Na+H)\(^{++}\) 961.82146; found 961.82079.

Heptapeptide 2

Methylester 34 (100.00 mg, 0.08 mmol) and trimethyltin hydroxide (299.00 mg, 1.66 mmol) were suspended in 7.0 ml of dry 1,2-dichlorethane in an Ar-atmosphere. After heating up the mixture to 85 °C the obtained clear solution was stirred at this temperature for 4.0 h. The volatiles were removed by rotary evaporation and the crude residue was taken up in 10.0 ml of sat. NaHCO\(_3\). After washing three times with 5.0 ml of EtOAc the aqueous phase was acidified to pH 3 with citric acid and extracted three times with EtOAc (10.0 ml). The combined organic phases were washed once with 10 % citric acid and water. After washing twice with brine the organic phase was dried over Na\(_2\)SO\(_4\), filtered and concentrated by rotary evaporation. Pure heptapeptide 2 (83.50 mg, 84 %) was obtained as a slight yellowish solid and could be used without further purification in the next step. Deviating from the work up protocol resulted always in contamination of the product with trimethyltin hydroxide. Samples for biological testings were additionally purified by preparative HPLC \((t_R = 12.2 \text{ min})\).
[20 % to 70 % CH₃CN in 30 min]: Rᵣ = 0.3 (CH₂Cl₂/MeOH = 3:1); mp = 207-209 °C; [α]D²⁰ = -28.0 (c = 0.20, MeOH), 2⁺:[α]D²⁰ = +32.5 (c = 0.20, MeOH); IR (neat): 3273, 3088, 3067, 3033, 2977, 2929, 1751, 1698, 1678, 1637, 1605, 1514, 1455, 1391, 1367, 1334, 1216, 1173, 697 cm⁻¹; ¹H-NMR (400 MHz, DMSO): δ = 0.53 (d, J = 6.7 Hz, 3H), 0.56 (d, J = 6.7 Hz, 3H), 1.80 – 1.88 (m, 1 H), 4.38 (dd, J = 9.0, 6.0 Hz, 1H), 5.01 (s, 2H), 5.15 (d, J = 7.5 Hz, 1H), 5.30 (d, J = 8.9 Hz, 1H), 5.40 (d, J = 7.5 Hz, 1H), 5.53 (d, J = 8.3 Hz, 1H), 5.56 (d, J = 7.8 Hz, 1H), 5.62 (d, J = 8.3 Hz, 1H), 6.02 (t, J = 2.0 Hz, 1H), 6.06 (t, J = 1.9 Hz, 1H), 6.10 (t, J = 1.9 Hz, 1H), 6.17 (d, J = 2.2 Hz, 2H), 6.27 (d, J = 1.9 Hz, 2H), 6.33 (d, J = 1.9 Hz, 2H), 6.53 (d, J = 8.6 Hz, 2H), 6.67 (d, J = 7.8 Hz, 6H), 7.04 (d, J = 8.6 Hz, 2H), 7.10 (d, J = 8.6 Hz, 2H), 7.21 (d, J = 8.6 Hz, 2H), 7.26 – 7.37 (m, 5H), 7.80 (d, J = 8.9 Hz, 1H), 7.87 (d, J = 8.1 Hz, 1H), 8.47 (d, J = 7.5 Hz, 1H), 8.53 (d, J = 7.0 Hz, 1H), 8.69 (d, J = 8.3 Hz, 1H), 8.83 (d, J = 8.1 Hz, 1H), 8.90 (d, J = 7.8 Hz, 1H), 9.07 (s, 2H), 9.11 (br s, 1H), 9.18 (br s, 1H), 9.24 (s, 1H), 9.45 (br s, 1H); ¹³C-NMR (100 MHz, DMSO): δ = 17.4, 19.1, 31.5, 54.9, 55.5, 55.8, 56.4, 56.9, 57.5, 65.7, 101.7, 101.8, 105.5, 105.6, 105.7, 114.8, 115.1, 115.3, 126.9, 127.8, 127.9, 128.1, 128.4, 128.7, 128.8, 129.1, 137.0, 140.6, 140.9, 155.6, 156.6, 156.9, 157.3, 158.1, 158.2, 169.2, 169.4, 169.5, 169.8, 170.3, 172.3; MS (ESI) m/z = 1193.9, 1150.1, 1027.0, 862.0, 746.2, 713.3, 647.3, 548.4, 465.4, 449.2, 333.2, 138.1; HRMS (ESI): m/z calcd for C₆₁H₅₉N₇O₁₉ (M+Na)⁺ 1216.37590; found 1216.37551.

Hexapeptide 3

Tetrapeptide 23 (300.00 mg, 0.34 mmol) was treated with 2.3 ml of 4N HCl/dioxane in an Ar-atmosphere. After stirring for 1.0 h DC indicated complete conversion and the volatiles were removed under reduced pressure. Residual HCl was removed by adding 5.0 ml of diethylether to the hydrochloride with subsequent removal by rotary evaporation (repeat 3 times). Drying on high vacuum for 1.0 h afforded 24 in complete conversion (280.50 mg, theor. 278.18 mg). Obtained hydrochloride 24 (270.00 mg, 0.33 mmol) and dipeptide 6 (127.27 mg, 0.33 mmol)
were dissolved in 7.0 ml of dry DMF. After cooling the mixture to 0 °C NaHCO$_3$ (55.92 mg, 0.67 mmol) and DEPBT (197.16 mg, 0.66 mmol) were added subsequently. After stirring for 1.0 h at 0 °C the yellow solution was warmed to room temperature, stirred for additionally 20.5 h and poured in 20.0 ml of water. After extraction with four 20.0 ml portions of EtOAc the combined organic phases were washed subsequently with water (once), sat. NaHCO$_3$ (four times) and brine (once). Drying over Na$_2$SO$_4$, filtration and removal of volatiles by rotary evaporation afforded a yellowish solid which was further purified by flash chromatography (silica, CHCl$_3$/MeOH = 9:0.5) to obtain pure hexapeptide 3 (292.80 mg, 77 %): Samples for biological testings were additionally purified by preparative HPLC ($t_R$ = 24.1 min [20 % to 70 % CH$_3$CN in 30 min]): R$_f$ = 0.1 (CHCl$_3$/MeOH = 9:0.5); mp = 214 °C; [α]$_D^{20}$ = -26.3 (c = 0.36, MeOH), 3':[α]$_D^{20}$ = +22.3 (c = 0.36, MeOH); IR (neat): 3286, 3090, 3067, 2973, 2929, 2854, 1757, 1697, 1689, 1638, 1607, 1514, 1455, 1367, 1216, 1162, 1004, 851, 735, 696 cm$^{-1}$; $^1$H-NMR (400 MHz, DMSO): δ = 0.63 (d, $J$ = 5.4 Hz, 6H), 1.34 (s, 9H), 1.85 – 1.96 (m, 1H), 2.71 – 2.89 (m, 3H), 3.01 (dd, $J$ = 14.0, 4.6 Hz, 1H), 4.40 – 4.46 (m, 1H), 4.51 – 4.60 (m, 1H), 4.70 – 4.78 (m, 1H), 5.00 – 5.10 (m, 4H), 5.19 (d, $J$ = 8.2 Hz, 1H), 5.31 (d, $J$ = 7.9 Hz, 1H), 5.48 (d, $J$ = 7.6 Hz, 1H), 6.07 – 6.10 (m, 2H), 6.29 (d, $J$ = 2.4 Hz, 2H), 6.31 (d, $J$ = 2.4 Hz, 2H), 6.54 (d, $J$ = 8.8 Hz, 2H), 6.81 (d, $J$ = 8.1 Hz, 1H), 6.92 (d, $J$ = 8.8 Hz, 2H), 7.13 – 7.24 (m, 5H), 7.27 – 7.36 (m, 10H), 7.99 (d, $J$ = 9.2 Hz, 1H), 8.40 (d, $J$ = 8.5 Hz, 1H), 8.50 (d, $J$ = 8.1 Hz, 1H), 8.57 (d, $J$ = 7.9 Hz, 1H), 8.59 (d, $J$ = 7.9 Hz, 1H), 9.14 (s, 4H), 9.29 (s, 1H); $^{13}$C-NMR (100 MHz, DMSO): δ = 17.4, 19.0, 28.1, 31.5, 35.7, 48.5, 53.6, 55.6, 55.8, 56.7, 57.2, 65.9, 66.3, 78.2, 101.6, 101.7, 105.2, 105.6, 114.8, 126.3, 127.8, 128.0, 128.1, 128.2, 128.3, 128.4, 129.2, 135.7, 135.8, 137.5, 140.4, 141.4, 154.5, 156.6, 158.0, 169.2, 169.6, 169.8, 170.3, 170.4, 170.7; MS (ESI) m/z = 1139.3, 1039.1, 775.5, 726.4, 579.4, 551.3, 461.2, 430.2, 385.2, 371.3, 314.3, 287.2, 228.2; HRMS (ESI): m/z calcd for C$_{61}$H$_{66}$N$_{6}$O$_{16}$ (M+Na)$^+$ 1139.46081; found 1139.46132.

**Tripeptide 4**
Tripeptide 32 (227.20 mg, 0.43 mmol) was dissolved in 2.0 ml of 4N HCl/dioxane in an Ar-atmosphere and the clear solution was stirred for 55 min. After removal of volatiles by rotary evaporation the obtained hydrochloride was suspended in 5.0 ml of diethylether, followed by removal of solvent by rotary evaporation (repeat 3 times). After drying for 1.0 h under high vacuum the obtained hydrochloride was dissolved in 4.0 ml of a water/dioxane (1:1) mixture, followed by the addition of NaHCO$_3$ (86.00 mg, 1.03 mmol). To the vigorously stirred solution Cbz-Cl (73.00 mg, 0.06 ml, 0.43 mmol) in 1.0 ml of dioxane was added dropwise over a period of 45 min via syringe pump. After stirring for additionally 45 min at room temperature 20.0 ml of water were added, followed by washing the aqueous phase with three 10.0 ml portions of EtOAc. After acidification with 1N HCl to pH 3 the resulting suspension was extracted four times with EtOAc (15.0 ml). Combined organic phases were washed subsequently three times with 1N HCl (10.0 ml), three times with water (10.0 ml) and twice with brine (15.0 ml). After drying over Na$_2$SO$_4$, filtration and removal of the volatiles by rotary evaporation tripeptide 4 (209.70 mg, 87 %) was obtained in pure form over two steps as a white solid. Samples for biological testings were additionally purified by preparative HPLC ($t_R = 11.8$ min [20 % to 70 % CH$_3$CN in 30 min]): $R_f = 0.3$ (CHCl$_3$/MeOH = 9:2 [+0.1 % CH$_3$COOH]); mp = 130 °C; [$\alpha$]$_{D}^{20}$ = -78.7 (c = 0.37, MeOH), 4*:[$\alpha$]$_{D}^{20}$ = +76.9 (c = 0.36, MeOH); IR (neat): 3309, 3091, 3066, 3035, 2970, 2933, 2877, 1698, 1655, 1605, 1514, 1455, 1368, 1341, 1263, 1225, 1162, 1149 cm$^{-1}$; $^1$H-NMR (400 MHz, DMSO): $\delta = 0.68$ (d, $J = 6.5$ Hz, 3H), 0.71 (d, $J = 7.2$ Hz, 3H), 1.90 – 2.00 (m, 1H), 4.10 (dd, $J = 9.8$, 5.8 Hz, 1H), 5.02 (s, 2H), 5.31 (d, $J = 9.1$ Hz, 1H), 5.45 (d, $J = 7.8$ Hz, 1H), 6.11 (br s, 1H), 6.33 (br s, 2H), 6.66 (d, $J = 7.8$ Hz, 2H), 7.21 (d, $J = 9.1$ Hz, 1H), 7.31 – 7.34 (m, 5H), 7.82 (d, $J = 8.4$ Hz, 1H), 8.16 (d, $J = 9.1$ Hz, 1H), 8.52 (d, $J = 8.4$ Hz, 1H), 9.17 (s, 2H), 9.35 (s, 1H), 12.7 (br s, 1H); $^{13}$C-NMR (100 MHz, DMSO): $\delta = 17.6$, 18.9, 30.2, 55.9, 56.9, 57.4, 65.5, 101.7, 105.5, 114.9, 127.7, 127.8, 128.0, 128.3, 128.5, 128.9, 137.0, 140.8, 156.8, 158.0, 169.6, 169.8, 172.7; MS (ESI) $m/z$ = 566.4, 431.3, 383.1, 360.1, 332.2, 270.1, 268.2, 242.2, 211.2, 178.3, 150.2, 138.1, 91.0; HRMS (ESI): $m/z$ calcd for C$_{29}$H$_{31}$N$_3$O$_9$ (M+Na)$^+$ 566.21331; found 566.21343.
Dipeptide 5

Dipeptide 26 (900.00 mg, 1.44 mmol) was dissolved in an Ar-atmosphere in 64.0 ml THF and 1.40 g Pd/C (10 %) were added. After flushing the flask with H₂ the mixture was stirred for 4.0 h and filtered through a syringe filter (Rotalibo®-syringe filter, PTFE, 0.45 µm). Volatiles of the so obtained clear solution were removed by rotary evaporation and afforded pure dipeptide 5 as a fluffy white solid in quantitative yield (643.00 mg, theor. 641.14 mg) without further purification. Samples for biological testings were additionally purified by preparative HPLC ($t_R = 14.3$ min [15 % to 50 % CH₃CN in 30 min]): $R_f = 0.4$ (EtOAc/n-hexane = 1:1); mp = 98 °C; $[\alpha]_D^{20} = +26.1$ (c = 0.43, MeOH), 5*: $[\alpha]_D^{20} = -30.6$ (c = 0.43, MeOH); IR (neat): 3311, 2969, 2957, 2854, 1739, 1685, 1665, 1599, 1514, 1455, 1392, 1366, 1306, 1258, 1219, 1158 cm⁻¹; ¹H-NMR (400 MHz, DMSO): $\delta$ = 1.38 (s, 9H), 3.61 (s, 1H), 5.17 (d, $J$ = 8.8 Hz, 1H), 5.18 (d, $J$ = 6.7 Hz, 1H), 6.08 (t, $J$ = 2.0 Hz, 1H), 6.24 (d, $J$ = 2.0 Hz, 2H), 6.72 (d, $J$ = 9.4 Hz, 2H), 7.02 (d, $J$ = 8.6 Hz, 1H), 7.13 (d, $J$ = 8.6 Hz, 2H), 8.76 (d, $J$ = 8.0 Hz, 1H), 9.17 (s, 2H), 9.55 (s, 1H); ¹³C-NMR (100 MHz, DMSO): $\delta$ = 28.1, 52.1, 55.9, 57.0, 78.4, 101.7, 105.2, 115.3, 125.7, 129.0, 140.8, 157.5, 158.1, 170.0, 171.2; MS (ESI) $m/z$ = 447.2, 347.2, 330.2, 302.3, 270.1, 242.2, 215.1, 197.3, 183.3, 166.3, 138.1; HRMS (ESI): $m/z$ calcd for C₂₂H₂₆N₂O₈ (M+H)⁺ 447.17619; found 447.17606.

Dipeptide 6
Dipeptide **19** (1.24 g, 1.90 mmol) was dissolved in an Ar-atmosphere in 70.0 ml THF and 1.90 g Pd/C (10 %) were added. After flushing the flask with H₂ the mixture was stirred for 4.0 h and filtered through a syringe filter (Rotalibo®-syringe filter, PTFE, 0.45 µm). Volatiles of the so obtained clear solution were removed by rotary evaporation and afforded pure dipeptide **6** as a white solid in quantitative yield (730.00 mg, theor. 726.41 mg) without further purification. Samples for biological testings were additionally purified by preparative HPLC (tᵣ = 12.0 min [15 % to 50 % CH₃CN in 30 min]): Rᵣ = 0.4 (CHCl₃/MeOH = 9:2 [+0.1 % CH₃COOH]); mp = 97 °C; [α]D₂₀ = -60.6 (c = 0.49, MeOH), **6**: [α]D₂₀ = +60.8 (c = 0.49, MeOH); IR (neat): 3320, 2972, 2932, 2876, 2857, 1687, 1665, 1604, 1504, 1468, 1368, 1254, 1160, 1008 cm⁻¹; ¹H-NMR (400 MHz, DMSO): δ = 0.74 (d, J = 7.1 Hz, 3H), 0.78 (d, J = 7.4 Hz, 3H), 1.96 – 2.05 (m, 1H), 4.10 (dd, J = 8.4, 5.5 Hz, 1H), 5.16 (d, J = 6.7 Hz, 1H), 6.09 (t, J = 2.4 Hz, 1H), 6.28 (d, J = 1.8 Hz, 2H), 6.95 (d, J = 9.2 Hz, 1H), 8.17 (d, J = 9.2 Hz, 1H), 9.2 (br s, 2H); ¹³C-NMR (100 MHz, DMSO): δ = 17.2, 17.8, 28.2, 30.2, 57.2, 78.3, 101.6, 105.2, 114.2, 141.1, 158.1, 170.2, 172.9; MS (ESI) m/z = 383.1, 327.1, 283.8, 266.5, 248.5, 238.5, 220.5, 192.5, 178.4, 138.1; HRMS (ESI): m/z calcd for C₁₈H₂₆N₂O₇ (M+H)⁺ 383.18128; found 383.18137.

**Dipeptide 7**

Dipeptide **18** (1.25 g, 1.78 mmol) was dissolved in an Ar-atmosphere in 70.0 ml THF and 1.80 g Pd/C (10 %) were added. After flushing the flask with H₂ the mixture was stirred for 4.0 h and filtered through a syringe filter (Rotalibo®-syringe filter, PTFE, 0.45 µm). Volatiles of the so obtained clear solution were removed by rotary evaporation and afforded pure dipeptide **7** as a white solid in quantitative yield (780.00 mg, theor. 769.11 mg) without further purification. Samples for biological testings were additionally purified by preparative HPLC (tᵣ = 10.3 min [15 % to 50 % CH₃CN in 30 min]): Rᵣ = 0.1 (CHCl₃/MeOH = 9:2 [+0.1 % CH₃COOH]); mp = 103 °C; [α]D₂₀ = +28.0 (c = 0.40, MeOH), **7**: [α]D₂₀ = -30.0 (c =
Dipeptide 8

(S)-aspartic acid dibenzylester p-toluenesulfonate (20) (2.00 g, 4.12 mmol) was added to 17.0 ml dry DMF in an Ar-atmosphere and cooled to 0 °C. Subsequently (S)-N-Boc-phenylalanine (21) (1.09 g, 4.12 mmol), EDC-Cl (950.00 mg, 4.94 mmol), NaHCO$_3$ (350.00 mg, 4.12 mmol) and HOAt (670.00 mg, 4.94 mmol) were added and the mixture was stirred for 2.0 h at 0 °C. After a reaction time of 17.0 h at room temperature 30.0 ml of water were added and the aqueous phase was extracted four times with 40.0 ml of EtOAc. Combined organic phases were washed subsequently with 1N HCl (four times), sat. NaHCO$_3$ (four times), water (once) and brine (once). After drying over MgSO$_4$, filtration and removal of volatiles by rotary evaporation the crude mixture was purified by flash chromatography (silica, EtOAc/n-hexane = 1:2) to afford pure dipeptide 8 as a white solid (1.78 g, 77 %): $R_f = 0.3$ (EtOAc/n-hexane = 1:2); mp = 90 °C; [α]$_D^{20} = -14.1$ (c = 0.38, MeOH), 8':[α]$_D^{20} = +5.9$ (c = 0.38, MeOH); IR (neat): 3308, 3088, 3064, 3032, 2976, 2932, 2896, 1737, 1669, 1515, 1498, 1455, 1390, 1266, 1251, 1213, 1169, 1050 cm$^{-1}$; $^1$H-NMR (400 MHz, DMSO): $\delta = 1.28$ (s, 9H), 2.67 (dd, $J = 13.6, 10.5$ Hz, 1H), 2.78 – 2.85 (m, 1H), 2.88 – 2.97 (m, 2H), 4.16 – 4.23 (m, 1H), 4.75 – 4.81 (m, 1H), 5.09 (s, 4H), 6.92 (d, $J = 8.8$ Hz, 1H), 7.15 – 7.28 (m, 5H), 7.30 – 7.37 (m, 1H); $^1$H-NMR (400 MHz, DMSO): $\delta = 1.28$ (s, 9H), 2.67 (dd, $J = 13.6, 10.5$ Hz, 1H), 2.78 – 2.85 (m, 1H), 2.88 – 2.97 (m, 2H), 4.16 – 4.23 (m, 1H), 4.75 – 4.81 (m, 1H), 5.09 (s, 4H), 6.92 (d, $J = 8.8$ Hz, 1H), 7.15 – 7.28 (m, 5H), 7.30 – 7.37 (m, 1H); $^1$C-NMR (100 MHz, DMSO): $\delta = 28.1, 35.8, 37.4, 48.6, 55.4, 66.0, 66.4, 78.0, 126.1, 127.8, 128.0, 128.1, 128.4, 129.2, 135.7, 135.8,
138.1, 155.2, 169.9, 170.4, 171.9; MS (EI) m/z = 560.2, 504.1, 443.1, 409.1, 369.1, 278.1, 235.1, 178.1, 91.0; HRMS (ESI): m/z calcd for C$_{32}$H$_{36}$N$_2$O$_7$ (M+H)$^+$ 561.25953; found 561.25922.

**Styrol 10**

![Styrol 10](image)

To a suspension of methyltriphenylphosphoniumbromide (3.37 g, 94.20 mmol) in dry THF (192.0 ml) was added dropwise at -40 °C over a 30 min period 1.6 M n-BuLi in hexane (58.9 ml, 94.20 mmol). The resulting solution was warmed to -10 °C and stirred at this temperature for 50 min followed by cooling to -30 °C. A solution of aldehyde 9 (10.00 g, 31.41 mmol) in 15.0 ml dry THF was then added over a 10 min period. The orange solution was warmed to room temperature during 25 min and stirred vigorously at this temperature for further 2.0 h. Quenching the reaction by the addition of 100.0 ml of water resulted in the formation of two phases after addition of 100.0 ml of diethylether. The aqueous phase was extracted twice with 50.0 ml of EtOAc and the combined organic phases were washed twice with 82.0 ml of water and once with 100.0 ml of brine. Drying over MgSO$_4$, filtration and removal of the volatiles by rotary evaporation afforded a yellowish thick oil, which was further purified by flash chromatography (silica, EtOAc/n-hexane = 1:20) to obtain styrol 10 as a colorless thick oil (9.00 g, 91 %): R$_f$ = 0.3 (EtOAc/n-hexane = 1:8); IR (film): 3087, 3064, 3032, 3008, 2929, 2916, 2868, 1589, 1453, 1291, 1155, 1048 cm$^{-1}$; $^1$H-NMR (400 MHz, DMSO): $\delta$ = 5.06 (s, 4H), 5.27 (d, $J = 10.8$ Hz, 1H), 5.74 (d, $J = 17.6$ Hz, 1H), 6.57 (t, $J = 2.2$ Hz, 1H), 6.95 (dd, $J = 9.1, 9.1$ Hz, 1H), 7.00 (d, $J = 1.9$ Hz, 2H), 7.33 – 7.47 (m, 10H); $^{13}$C-NMR (100 MHz, DMSO): $\delta$ = 70.2, 101.8, 105.7, 114.6, 127.7, 128.1, 128.7, 136.9, 137.0, 160.2; MS (EI) m/z = 316.2, 225.1, 181.1, 91.0, 65.0; HRMS (EI): m/z calcd for C$_{22}$H$_{20}$O$_2$ (M)$^+$ 316.14633; found 316.14610.
Aminoalcohol 11

To a solution of tert-butyl carbamate 12 (9.88 g, 84.37 mmol) in 109.0 ml n-PrOH were added 195.6 ml of a NaOH solution (3.34 g, 83.39 mmol, in 205.0 ml H₂O; 9.4 ml of this solution is kept for later dissolving K₂[OsO₂(OH)₄]) and freshly prepared tert-butyl hypochlorite (9.66 g, 88.98 mmol). After 6 min the homogeneous (!) solution was cooled to -2 °C and a solution of ligand (DHQD)₂PHAL (1.36 g, 1.74 mmol) in 109.0 ml of n-PrOH was added. Thereafter a solution of styrol 10 (8.60 g, 27.18 mmol) in 191.0 ml of n-PrOH was poured into the mixture, immediately (!!) followed by a fresh (!! ) prepared purple solution of K₂[OsO₂(OH)₄] (401.00 mg, 1.09 mmol) in 9.4 ml of the above preserved NaOH solution. The white emulsion turns slight greenish and changes colour after a few minutes to yellow accompanied with precipitation of a white solid. After stirring at 0 °C for 1.0 h about 300.0 ml of n-PrOH were removed by rotary evaporation and the remainig green a queous solution was cooled to 0 °C. The formed white precipitate was filtered off, washed with 300.0 ml of cold water and dried under high vacuum over night. The crude mixture was purified by flash chromatography (silica, EtOAc/n-hexane = 1:3 to 1:2) to obtain aminoalkohol 11 as white solid (6.33 g, 52 %). Undesired regioisomer could not be isolated from the reaction mixture: Rₗ = 0.5 (EtOAc/n-hexane = 1:1); ee = 98 % (ee = 84 % for enantiomer 11’ respectively); mp = 109 °C; [α]D²⁰ = -37.9 (c = 0.53, MeOH), 11’: [α]D²⁰ = +38.1 (c = 0.53, MeOH); IR (neat): 3365, 3091, 3065, 3033, 2977, 2930, 2871, 1677, 1608, 1597, 1527, 1448, 1311, 1160, 1058 cm⁻¹; ¹H-NMR (400 MHz, DMSO): δ = 1.37 (s, 9H), 3.45 (t, J = 6.2 Hz, 2H), 4.52 – 4.50 (m, 1H), 4.78 (t, J = 6.0 Hz, 1H), 5.05 (s, 4H), 6.54 (t, J = 2.3 Hz, 1H), 6.58 (d, J = 1.8 Hz, 2H), 7.17 (d, J = 8.9 Hz, 1H), 7.30 – 7.46 (m, 10H); ¹³C-NMR (100 MHz, DMSO): δ = 28.3, 57.0, 64.9, 77.7, 99.9, 106.1, 127.8, 127.9, 128.4, 137.1, 144.3, 155.2, 159.3; MS (EI) m/z = 449.2, 418.2, 375.2, 362.1, 318.2, 181.1, 91.0, 57.0; HRMS (EI): m/z calcd for C₂₇H₃₁NO₅ (M)⁺ 449.22022; found 449.21999.
Aldehyde 14

Aminoalkohol 11 (4.90 g, 10.90 mmol) was dissolved in 110.0 ml of dry CH₂Cl₂, cooled to -5 °C and periodinane 13[1] (13.87 g, 32.70 mmol) was added. The mixture was slowly warmed to room temperature, stirred for 2.0 h, diluted with 200.0 ml of diethylether and poured into a solution of 500.0 ml of 10 % NaHCO₃ containing Na₂S₂O₃·5H₂O (18.67 g, 75.21 mmol). After 5 min of stirring the phases were separated and the aqueous phase was extracted three times with 200.0 ml of diethylether. Combined organic phases were washed twice with 300.0 ml portions of 10 % NaHCO₃ and once with 600.0 ml of brine. Drying over Na₂SO₄, filtration and removal of volatiles by rotary evaporation afforded crude aldehyde 14 (4.90 g; theor. 4.88 g) as a yellowish solid which was directly used in the next oxidation step due to its lability: Rᶠ = 0.6 (EtOAc/n-hexane = 1:1).

(R)-N-Boc-3,5-dibenzoxophenylglycine 15

A freshly prepared solution of NaClO₂ (11.58 g (80%), 102.46 mmol) and NaH₂PO₄·2H₂O (11.90 g, 76.30 mmol) in 418.0 ml of water was added over a 45 min period to a solution of crude aldehyde 14 (4.88 g, 10.90 mmol) in 1074.0 ml t-BuOH and 258.0 ml 2-methyl-2-butene at room temperature. The mixture was stirred for 40 min and organic solvents were removed by rotary evaporation. The aqueous residue was diluted with 500.0 ml of 5 % NaHCO₃ and was washed four times with 300.0 ml portions of diethylether. Due to the high hydrophobicity, the desired acid remained completely in the organic phases. The latter therefore were combined and washed twice with 300.0 ml of brine and dried over Na₂SO₄.

After filtration and removal of the volatiles by rotary evaporation \((R)-N\text{-Boc-3,5-dibenzoxyphenylglycine (15)}\) was obtained as white solid (4.90 g, 97 \%) without further purification: \(R_f = 0.2\) (CHCl\(_3\)/MeOH = 9:1); mp = ~ 95 °C; \([\alpha]_D^{20} = -64.2\) (c = 0.37, MeOH), 15':[\(\alpha\]D\(_{20}\) = +70.8 (c = 0.37, MeOH); IR (neat): 3396, 3327, 3091, 3064, 3033, 3005, 2976, 2931, 2872, 1694, 1596, 1498, 1453, 1378, 1367, 1159, 1055 cm\(^{-1}\); \(^1\)H-NMR (400 MHz, DMSO): \(\delta = 1.38\) (s, 9H), 4.92 (d, \(J = 8.2\) Hz, 1H), 5.05 (s, 4H), 6.57 (t, \(J = 2.0\) Hz, 1H), 6.58 (d, \(J = 1.7\) Hz, 2H), 7.28 (br s, 1H), 7.30 – 7.47 (m, 10H); \(^{13}\)C-NMR (100 MHz, DMSO): \(\delta = 28.2, 58.2, 69.3, 78.2, 100.6, 106.7, 127.7, 127.9, 128.4, 154.9, 159.3, 171.9\); MS (EI) \(m/z = 463.2, 449.2, 418.2, 362.1, 319.1, 318.2, 181.1, 91.0, 59.0\); HRMS (EI): \(m/z\) calcd for \(C_{27}H_{29}NO_6\) (M)\(^+\) 463.19949; found 463.02001.

\((S)\)-hydroxyphenylglycine \(N\)-benzylester hydrochloride 16

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\begin{align*}
\text{HCl}\cdot\text{H}_2\text{N}_\text{(S)}\cdot\text{O}\cdot\text{phenyl-3,5}}
\end{align*}
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A suspension of \((S)-4\)-hydroxyphenylglycine (700.00 mg, 4.19 mmol) in 26.0 ml of benzylalkohol was cooled to 0 °C. Thionylchloride (6.60 g, 4.0 ml, 55.10 mmol) was added dropwise under vigorous stirring, resulting at first in the formation of a clear solution and later of a colloid mixture. The latter was stirred for 40 min at 0 °C and then warmed slowly to room temperature. After stirring for 24.0 h the volatiles were removed by rotary evaporation (bath temperature 85 °C) and the oily residue was diluted with 100.0 ml of diethylether. The precipitated hydrochloride was filtered off and washed thoroughly with cold diethylether. After drying on high vacuum for 12.0 h \((S)\)-hydroxyphenylglycine benzylester hydrochloride (16) was obtained as a fluffy white solid (876.00 mg, 71 \%) without further purification: \(R_f = 0.5\) (CHCl\(_3\)/MeOH = 9:2); mp = 200 °C; \([\alpha]_D^{20} = +58.7\) (c = 0.53, MeOH), 16':[\(\alpha\]D\(_{20}\) = -53.7 (c = 0.53, MeOH); IR (neat): 3169, 3089, 3062, 3029, 2963, 2925, 2855, 2615, 1742, 1613, 1518, 1455, 1275, 1223, 1179, 1128 cm\(^{-1}\); \(^1\)H-NMR (400 MHz, DMSO): \(\delta = 4.46\) (s, 1H), 5.04 – 5.31 (m, 2H), 6.70 (d, \(J = 8.5\) Hz, 1H), 7.17 (d, \(J = 8.3\) Hz, 1H), 7.21 – 7.35 (m, 5H), 9.39 (br s, 1H); \(^{13}\)C-NMR (100 MHz, DMSO): \(\delta = 57.7, 65.6, 115.0, 127.6, 127.9, 128.0,\)
Dipeptide 18

(S)-hydroxyphenylglycine benzylester hydrochloride (16) (731.97 mg, 2.49 mmol) and (R)-N-Boc-3,5-dibenzoxypheynlglycine (15) (1.10 g, 2.37 mmol) were dissolved in 23.0 ml dry THF in an Ar-atmosphere. The mixture was cooled to 0 °C and successively NaHCO$_3$ (398.74 mg, 4.75 mmol) and DEPBT (1.44 g, 4.80 mmol) were added. After stirring for 1.0 h at 0 °C the suspension was warmed to room temperature and stirred vigorously for 20.0 h. After adding 64.0 ml of water the aqueous phase was extracted four times with 50.0 ml of EtOAc. The combined organic phases were washed thereafter once with 1 N HCl, twice with water, four times with sat. NaHCO$_3$ and once with brine. After drying over Na$_2$SO$_4$, filtration and removal of volatiles by rotary evaporation the crude yellowish solid was purified by flash chromatography (silica, diethylether/n-hexane = 2:1) to obtain dipeptide 18 (1.33 g, 80 %) as white solid. Infinitesimal amount of undesired diastereomer could not be isolated in pure form: R$_f$ = 0.5 (diethylether/n-hexane = 2:1); mp = 77 °C; [α]$_D^{20}$ = +1.6 (c = 0.38, MeOH), 18':[α]$_D^{20}$ = +/- 0 (c = 0.38, MeOH); IR (neat): 3313, 3091, 3064, 3033, 3006, 2973, 2925, 2870, 2855, 1739, 1695, 1662, 1595, 1514, 1367, 1160, 1052 cm$^{-1}$; $^1$H-NMR (400 MHz, DMSO): δ = 1.39 (s, 9H), 5.01 (s, 4H), 5.08 – 5.17 (m, 2H), 5.30 (d, $J = 6.9$ Hz, 1H), 5.34 (d, $J = 9.4$ Hz, 1H), 6.55 (t, $J = 2.2$ Hz, 1H), 6.71 (d, $J = 8.5$ Hz, 2H), 6.75 (d, $J = 2.2$ Hz, 2H), 7.14 (d, $J = 8.8$ Hz, 1H), 7.21 – 7.45 (m, 16H), 8.91 (d, $J = 7.1$ Hz, 1H), 9.55 (s, 1H); $^{13}$C-NMR (100 MHz, DMSO): δ = 28.2, 55.9, 57.2, 66.1, 69.3, 78.5, 100.7, 106.3, 115.3, 125.8, 127.5, 127.8, 128.0, 128.3, 128.4, 128.9, 135.8, 136.9, 141.9, 157.5, 159.3, 169.8, 170.6; MS (EI) m/z = 702.2, 602.2, 465.2, 362.1, 318.2, 228.1, 108.0, 91.0; HRMS (EI): m/z calcd for C$_{42}$H$_{42}$N$_2$O$_8$ (M)$^+$ 702.29411; found 702.29300.
Dipeptide 19

(S)-valine benzylester hydrochloride (17) (496.90 mg, 2.04 mmol) and (R)-N-Boc-3,5-dibenzoxyphenylglycine (15) (900.00 mg, 1.94 mmol) were dissolved in 19.5 ml dry THF in an Ar-atmosphere. The mixture was cooled to 0 °C and successively NaHCO₃ (326.24 mg, 3.88 mmol) and DEPBT (1.17 g, 3.93 mmol) were added. After stirring for 1.0 h at 0 °C the suspension was warmed to room temperature and stirred vigorously for 20.0 h. After adding 54.0 ml of water the aqueous phase was extracted four times with 42.0 ml of EtOAc. The combined organic phases were washed thereafter once with 1 N HCl, twice with water, four times with sat. NaHCO₃ and once with brine. After drying over Na₂SO₄, filtration and removal of volatiles by rotary evaporation the crude yellowish solid was purified by flash chromatography (silica, diethylether/n-hexane = 2:1) to obtain dipeptide 19 (1.24 g, 98 %) as white solid. Infinitesimal amount of undesired diastereomer could not be isolated in pure form: Rᵣ = 0.5 (diethylether/n-hexane = 2:1); mp = 59 °C; [α]D²⁰ = -55.9 (c = 0.39, MeOH), 19*: [α]D²⁰ = +56.1 (c = 0.39, MeOH); IR (neat): 3331, 3089, 3064, 3033, 3005, 2966, 2930, 2873, 1738, 1654, 1596, 1366, 1250, 1161, 1052, 696 cm⁻¹; H-NMR (400 MHz, DMSO): δ = 0.70 (d, J = 6.8 Hz, 3H), 0.75 (d, J = 6.8 Hz, 3H), 1.39 (s, 9H), 1.98 – 2.08 (m, 1H), 4.19 (dd, J = 8.2, 6.0 Hz, 1H), 5.05 (s, 4H), 5.10 – 5.20 (m, 2H), 5.34 (d, J = 9.0 Hz, 1H), 6.65 (t, J = 2.0 Hz, 1H), 6.83 (d, J = 2.0 Hz, 2H), 7.28 – 7.44 (m, 16H), 8.51 (d, J = 8.1 Hz, 1H); C-NMR (100 MHz, DMSO): δ = 17.8, 18.8, 28.2, 30.2, 57.3, 57.4, 66.0, 69.3, 78.4, 100.8, 106.3, 127.7, 127.8, 128.0, 128.1, 128.4, 135.8, 136.9, 141.4, 154.8 159.3, 170.3, 171.3; MS (EI) m/z = 652.3, 552.2, 418.3, 362.2, 318.2, 272.1, 228.1, 181.1, 91.0, 57.0; HRMS (EI): m/z calcd for C₃₉H₄₄N₂O₇ (M)⁺ 652.31485; found 652.31420.
Dipeptide 8 (622.50 mg, 1.11 mmol) was treated with 7.3 ml of 4N HCl/dioxane in an Ar-atmosphere. After stirring for 1.0 h DC indicated complete conversion and the volatiles were removed under reduced pressure. Residual HCl was removed by adding 15.0 ml of diethyl ether to the hydrochloride with subsequent removal by rotary evaporation (repeat 3 times). Drying on high vacuum for 1.0 h afforded 22 in complete conversion (558.00 mg, theor. 551.82 mg). The latter (551.82 mg, 1.11 mmol) and dipeptide 7 (400.00 mg, 0.93 mmol) were dissolved in 11.0 ml dry DMF in an Ar-atmosphere. The mixture was cooled to 0 °C and successively NaHCO$_3$ (186.51 mg, 2.22 mmol) and DEPBT (655.94 mg, 2.19 mmol) were added. After stirring for 1.0 h at 0 °C the suspension was warmed to room temperature and stirred vigorously for 20.0 h. After adding 40.0 ml of water the aqueous phase was extracted four times with 35.0 ml of EtOAc. The combined organic phases were washed thereafter once with water, four times with sat. NaHCO$_3$ and once with brine. After drying over Na$_2$SO$_4$, filtration and removal of volatiles by rotary evaporation the crude yellowish solid was purified by flash chromatography (silica, CHCl$_3$/MeOH = 9:0.5) to obtain tetrapeptide 23 (588.00 mg, 73 %) as white solid. Samples for biological testings were additionally purified by preparative HPLC ($t_R = 24.5$ min [20 % to 70 % CH$_3$CN in 30 min]): $R_f = 0.3$ (CHCl$_3$/MeOH = 9:0.5); mp = 120 °C; $[\alpha]_D^{20} = -9.6$ (c = 0.33, MeOH), 23':$[\alpha]_D^{20} = +11.7$ (c = 0.33, MeOH); IR (neat): 3273, 3088, 3067, 3033, 2977, 2929, 2854, 1751, 1698, 1637, 1514, 1216, 1202, 1173, 1165 cm$^{-1}$; $^1$H-NMR (400 MHz, DMSO): $\delta = 1.37$ (s, 9H), 2.71 – 2.91 (m, 2H), 3.04 (dd, $J = 14.3$, 4.3 Hz, 1H), 4.51 – 4.58 (m, 1H), 4.66 – 4.74 (m, 1H), 5.01 – 5.08 (m, 4H), 5.11 (d, $J = 9.2$ Hz, 1H), 5.24 (d, $J = 6.9$ Hz, 1H), 6.09 (t, $J = 2.0$ Hz, 1H), 6.26 (d, $J = 2.0$ Hz, 2H), 6.65 (d, $J = 8.5$ Hz, 1H), 6.91 (d, $J = 8.5$ Hz, 2H), 7.05 (d, $J = 7.9$ Hz, 1H), 7.14 – 7.24 (m, 5H), 7.27 – 7.36 (m, 10H), 8.29 (d, $J = 9.8$ Hz, 1H), 8.48 (d, $J = 7.4$ Hz, 1H), 8.50 (d, $J = 7.1$ Hz, 1H), 9.16 (s, 2H), 9.32 (s, 1H); $^{13}$C-NMR (100 MHz, DMSO): $\delta = 28.2, 35.7, 48.6, 53.6, 55.9, 57.4, 66.0, 66.4, 78.4, 101.7, 105.5, 114.8, 126.2,
Dipeptide 26

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\text{(S)-hydroxyphenylglycine methylester hydrochloride (25) (491.68 mg, 2.26 mmol) and (R)-N-Boc-3,5-dibenzyloxyphenylglycine (15) (1.00 g, 2.16 mmol) were dissolved in 20.0 ml dry DMF in an Ar-atmosphere. The mixture was cooled to 0 °C and successively NaHCO}_3 \text{(362.49 mg, 4.32 mmol) and DEPBT (1.30 g, 4.36 mmol) were added. After stirring for 1.0 h at 0 °C the suspension was warmed to room temperature and stirred vigorously for 22.0 h. After adding 58.0 ml of water the aqueous phase was extracted four times with 50.0 ml of EtOAc. The combined organic phases were washed thereafter twice with water, four times with sat. NaHCO}_3 \text{ and once with brine. After drying over Na}_2\text{SO}_4, \text{filtration and removal of volatiles by rotary evaporation the crude yellowish solid was purified by flash chromatography (silica, diethylether/n-hexane = 2:1) to obtain dipeptide 26 (1.05 g, 78 %) as white solid. Infinitesimal amount of undesired diastereomer could not be isolated in pure form: R}_f = 0.3 \text{ (diethylether/n-hexane = 2:1); mp = 154 °C; } [\alpha]_D^{20} = +17.6 \text{ (c = 0.47, MeOH), } 26':[\alpha]_D^{20} = -22.3 \text{ (c = 0.47, MeOH); IR (neat): 3321, 3091, 3064, 3033, 3006, 2975, 2955, 2932, 2874, 1744, 1695, 1661, 1606, 1596, 1515, 1498, 1453, 1367, 1216, 1160, 1052 cm}^{-1}; \text{ } {^1}\text{H-NMR (400 MHz, DMSO): } \delta = 1.39 \text{ (s, 9H), 3.62 (s, 3H), 5.01 (s, 4H), 5.24 (d, } J = 7.1 \text{ Hz, 1H), 5.32 (d, } J = 8.5 \text{ Hz, 1H), 6.55 (t, } J = 2.0 \text{ Hz, 1H), 6.71 (d, } J = 8.8 \text{ Hz, 2H), 6.74 (d, } J = 2.0 \text{ Hz, 2H), 7.12 (d, } J = 8.5 \text{ Hz, 1H), 7.20 – 7.47 (m, 16H), 8.88 (d, } J = 7.8 \text{ Hz, 1H), 9.55 (s, 1H); } {^{13}}\text{C-NMR (100 MHz, DMSO): } \delta = 28.2, 52.2, 55.7, 57.2, 69.3, 78.5, 100.7, 106.3, 115.3, 125.9, 127.4, 127.8, 127.9, 128.5, 128.9, 136.9, 141.1, 154.7, 157.5, 159.3, 169.7, 171.2; MS}
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(EI) $m/z = 626.2, 526.2, 362.1, 318.1, 228.1, 180.0, 91.0, 56.1$; HRMS (ESI): $m/z$ calcd for C$_{36}$H$_{38}$N$_2$O$_8$ (M+H)$^+$ 627.27009; found 627.27044.

Tetrapeptide 28

Dipeptide 5 (360.00 mg, 0.81 mmol) was treated with 5.4 ml of 4N HCl/dioxane in an Ar-atmosphere. After stirring for 1.5 h DC indicated complete conversion and the volatiles were removed under reduced pressure. Residual HCl was removed by adding 16.0 ml of diethylether to the hydrochloride with subsequent removal by rotary evaporation (repeat 3 times). Drying on high vacuum for 1.0 h afforded 27 in complete conversion (312.10 mg, theor. 308.67 mg). The latter (300.00 mg, 0.78 mmol) and dipeptide 7 (338.89 mg, 0.78 mmol) were dissolved in 19.0 ml dry DMF in an Ar-atmosphere. The mixture was cooled to 0 °C and successively NaHCO$_3$ (131.68 mg, 1.57 mmol) and DEPBT (464.28 mg, 1.55 mmol) were added. After stirring for 1.0 h at 0 °C the suspension was warmed to room temperature and stirred vigorously for 20.5 h. After adding 65.0 ml of water the aqueous phase was extracted four times with 40.0 ml of EtOAc. The combined organic phases were washed thereafter once with water, four times with sat. NaHCO$_3$ and once with brine. After drying over Na$_2$SO$_4$, filtration and removal of volatiles by rotary evaporation the crude yellowish solid was purified by flash chromatography (silica, CHCl$_3$/MeOH = 9:1) to obtain tetrapeptide 28 (322.60 mg, 54 %) as white solid. Samples for biological testings were additionally purified by preparative HPLC ($t_R = 13.2$ min [15 % to 60 % CH$_3$CN in 30 min]): $R_f = 0.2$ (CHCl$_3$/MeOH = 9:2); mp = 165 °C; $[\alpha]_D^{20} = +8.9$ (c = 0.23, MeOH), 28': $[\alpha]_D^{20} = -4.9$ (c = 0.23, MeOH); IR (neat): 3307, 2975, 2926, 2854, 1687, 1645, 1602, 1513, 1455, 1367, 1342, 1257, 1221, 1161, 1008 cm$^{-1}$; $^1$H-NMR (400 MHz, DMSO): $\delta = 1.38$ (s, 9H), 3.62 (s, 3H), 5.19 (d, $J = 9.2$ Hz, 1H), 5.22 (d, $J = 7.2$ Hz, 1H), 5.47 (d, $J = 8.4$ Hz, 1H), 5.58 (d, $J = 7.7$ Hz, 1H), 6.05 (t, $J = 2.4$ Hz, 1H), 6.09 (t, $J = 2.2$ Hz, 1H), 6.17 (d, $J = 1.9$ Hz, 2H), 6.27 (d, $J = 1.9$ Hz, 2H), 6.56 (d, $J = 8.4$ Hz, 2H), 6.70 (d, $J = 7.7$ Hz, 2H), 6.96 (d, $J = 
8.4 Hz, 1H), 7.05 (d, J = 8.4 Hz, 2H), 7.13 (d, J = 8.4 Hz, 2H), 8.52 (d, J = 7.6 Hz, 1H), 8.83 (d, J = 8.4 Hz, 1H), 8.97 (d, J = 7.7 Hz, 1H), 9.11 (s, 2H), 9.16 (s, 2H), 9.28 (s, 1H), 9.52 (s, 1H); $^{13}$C-NMR (100 MHz, DMSO): δ = 28.2, 52.1, 55.0, 55.5, 55.9, 57.2, 78.4, 101.6, 101.7, 105.3, 105.4, 114.7, 115.3, 125.8, 128.0, 128.6, 128.9, 140.2, 140.9, 156.6, 157.4, 158.0, 158.1, 169.4, 169.5, 169.7, 171.2; MS (ESI) m/z = 761.1, 705.1, 661.0, 629.3, 524.1, 479.2, 435.2, 347.3, 287.3, 183.2, 138.1; HRMS (ESI): m/z calcd for C$_{38}$H$_{40}$N$_4$O$_{13}$ (M+Na)$^+$ 783.24841; found 783.24811.

**Tripeptide 30**

Dipeptide 19 (1.00 g, 1.53 mmol) was treated with 10.0 ml of 4N HCl/dioxane in an Ar-atmosphere. After stirring for 55 min DC indicated complete conversion and the volatiles were removed under reduced pressure. Residual HCl was removed by adding 10.0 ml of diethylether to the hydrochloride with subsequent removal by rotary evaporation (repeat 3 times). Drying on high vacuum for 1.0 h afforded 29 in complete conversion (910.00 mg, theor. 902.48 mg). The latter (902.48 mg, 1.53 mmol) and (R)-N-Boc-4-hydroxyphenylglycine 31 (409.45 mg, 1.53 mmol) were dissolved in 38.0 ml dry DMF in an Ar-atmosphere. The mixture was cooled to 0 °C and successively NaHCO$_3$ (257.39 mg, 3.06 mmol) and DEPBT (907.53 mg, 3.03 mmol) were added. After stirring for 1.5 h at 0 °C the suspension was warmed to room temperature and stirred vigorously for 18.0 h. After adding 100.0 ml of water the aqueous phase was extracted four times with 60.0 ml of EtOAc. The combined organic phases were washed thereafter once with water, four times with sat. NaHCO$_3$ and once with brine. After drying over Na$_2$SO$_4$, filtration and removal of volatiles by rotary evaporation the crude yellowish solid was purified by flash chromatography (silica, EtOAc/n-hexane = 1:1) to obtain tripeptide 30 (971.00 mg, 79 %) as white solid: $R_f$ = 0.4 (EtOAc/n-hexane = 1:1); mp = 158 °C; [α]$_D^{20}$ = -71.5 (c = 0.38, MeOH); IR (neat): 3385, 3296, 3090, 3065, 3033, 3008, 3965, 2928, 2873, 2856, 1710, 1639, 1607, 1514, 1366, 1164,
1056 cm\(^{-1}\); \(^1\)H-NMR (400 MHz, DMSO): \(\delta = 0.67\) (d, \(J = 6.7\) Hz, 3H), 0.73 (d, \(J = 6.7\) Hz, 3H), 1.34 (s, 9H), 1.94 – 2.02 (m, 1H), 4.16 (dd, \(J = 8.5, 6.3\) Hz, 1H), 4.99 – 5.05 (m, 4H), 5.11 (s, 2H), 5.21 (d, \(J = 8.9\) Hz, 1H), 5.63 (d, \(J = 8.3\) Hz, 1H), 6.54 (br s, 1H), 6.65 (d, \(J = 8.6\) Hz, 2H), 6.81 (d, \(J = 1.9\) Hz, 2H), 7.20 (d, \(J = 8.6\) Hz, 2H), 7.28 – 7.46 (m, 15H), 8.56 (d, \(J = 8.3\) Hz, 1H), 8.64 (d, \(J = 8.3\) Hz, 1H), 9.38 (s, 1H); \(^{13}\)C-NMR (100 MHz, DMSO): \(\delta = 17.7, 18.8, 28.1, 30.2, 55.5, 57.3, 58.4, 66.0, 69.3, 78.4, 105.9, 114.9, 127.7, 127.9, 128.1, 128.2, 128.5, 128.6, 128.9, 135.8, 136.9, 141.5, 156.8, 159.3, 169.7, 170.1, 171.1; MS (ESI) \(m/z = 802.1, 702.2, 685.2, 591.2, 553.3, 540.2, 450.2, 391.2, 358.1, 318.2, 268.3, 208.3, 181.2, 91.0; \) HRMS (ESI): \(m/z\) calcd for C\(_{47}\)H\(_{51}\)N\(_3\)O\(_9\) (M+Na\(^+\)) 842.35230; found 824.35241.

**Tripeptide 32**

![Tripeptide 32](image)

Tripeptide 30 (881.00 mg, 1.10 mmol) was dissolved in an Ar-atmosphere in 40.0 ml THF and 1.10 g Pd/C (10 %) were added. After flushing the flask with H\(_2\) the mixture was stirred for 4.0 h and filtered through a syringe filter (Rotalibo®-syringe filter, PTFE, 0.45 \(\mu\)m). Volatiles of the so obtained clear solution were removed by rotary evaporation and afforded pure tripeptide 32 as a white solid in quantitative yield (586.00 mg, theor. 583.97 mg) without further purification: \(R_f = 0.2\) (CHCl\(_3\)/MeOH = 9:2 [+0.1 % CH\(_3\)COOH]); mp = 215 \(^\circ\)C; \([\alpha]_D^{20} = -8.1\) (c = 0.40, MeOH); IR (neat): 3309, 2961, 2924, 2871, 2854, 1685, 1653, 1603, 1456, 1392, 1367, 1254, 1222, 1159, 1049 cm\(^{-1}\); \(^1\)H-NMR (400 MHz, DMSO): \(\delta = 0.66\) (d, \(J = 6.7\) Hz, 3H), 0.70 (d, \(J = 6.7\) Hz, 3H), 1.35 (s, 9H), 1.90 – 1.98 (m, 1H), 4.07 (dd, \(J = 8.7, 5.5\) Hz, 1H), 5.18 (d, \(J = 8.9\) Hz, 1H), 5.43 (d, \(J = 8.1\) Hz, 1H), 6.09 (t, \(J = 2.0\) Hz, 1H), 6.31 (d, \(J = 1.9\) Hz, 2H), 6.65 (d, \(J = 8.6\) Hz, 2H), 7.16 (d, \(J = 8.6\) Hz, 2H), 7.26 (d, \(J = 8.9\) Hz, 1H), 8.19 (d, \(J = 8.9\) Hz, 1H), 8.42 (d, \(J = 8.1\) Hz, 1H), 9.16 (s, 2H), 9.36 (br s, 1H); \(^{13}\)C-NMR (100 MHz, DMSO): \(\delta = 17.6, 19.0, 28.2, 30.3, 55.8, 57.0, 57.1, 78.4, 101.7, 105.4, 114.9, 128.4, 129.2, 140.9, 154.7, 156.7, 158.0, 169.7, 169.8, 172.8; MS (ESI) \(m/z = 532.3, 432.4, \)
415.1, 387.1, 369.1, 293.1, 270.0, 245.3, 242.1, 178.4, 178.0, 150.2, 138.3, 122.1; HRMS (ESI): m/z calcd for C_{26}H_{33}N_{3}O_{9} (M+Na)^+ 554.21090; found 554.21084.

**Heptapetide 34**

![Chemical structure of Heptapetide 34](image)

Tetrapeptide 28 (170.00 mg, 0.22 mmol) was treated with 4.5 ml of 4N HCl/dioxane in an Ar-atmosphere. After stirring for 1.0 h DC indicated complete conversion and the volatiles were removed under reduced pressure. Residual HCl was removed by adding 4.0 ml of diethylether to the hydrochloride with subsequent removal by rotary evaporation (repeat 3 times). Drying on high vacuum for 1.0 h afforded tetrapeptide 33 in complete conversion (160.00 mg, theor. 155.78 mg). The latter (155.78 mg, 0.22 mmol) and tripeptide 4 (125.76 mg, 0.22 mmol) were dissolved in 4.0 ml dry DMF in an Ar-atmosphere. The mixture was cooled to 0 °C and successively NaHCO_3 (37.36 mg, 0.45 mmol) and DEPBT (131.73 mg, 0.44 mmol) were added. After stirring for 1.0 h at 0 °C the suspension was warmed to room temperature and stirred vigorously for 20.0 h. After adding 15.0 ml of water the aqueous phase was extracted four times with 20.0 ml of EtOAc. The combined organic phases were washed thereafter twice with water, four times with sat. NaHCO_3 and once with brine. After drying over Na_2SO_4, filtration and removal of volatiles by rotary evaporation the crude yellowish solid was purified by flash chromatography (silica, CH_2Cl_2/MeOH = 9:2) to obtain heptapeptide 34 (141.00 mg, 52 %) as white solid. Samples for biological testings were additionally purified by preparative HPLC (t_R = 13.9 min [20 % to 70 % CH_3CN in 30 min]): R_f = 0.1 (CH_2Cl_2/MeOH = 9:0.2); mp = 205-220 °C; [α]_D^{20} = -18.0 (c = 0.70, MeOH), 34':[α]_D^{20} = +19.1 (c = 0.70, MeOH); IR (neat): 3291, 3069, 2965, 2928, 2853, 2684, 1641, 1617, 1602, 1514, 1456, 1261, 1237, 1200, 1166, 1024 cm^{-1}; ^1H-NMR (400 MHz, DMSO): δ = 0.54 (d, J = 6.8 Hz, 3H), 0.57 (d, J = 6.8 Hz, 3H), 1.80 – 1.88 (m, 1 H), 3.62 (s, 3H), 4.38 (dd, J = 9.0, 6.0 Hz, 1H), 5.02 (s, 2H), 5.22 (d, J = 7.0 Hz, 1H), 5.30 (d, J = 8.7 Hz, 1H), 5.40 (d, J = 7.5 Hz, 1H), 5.52 (d, J = 8.2 Hz, 1H), 5.57 (d, J = 8.2 Hz, 1H), 5.64 (d, J = 8.0 Hz, 1H), 6.04
Hexapeptide 3 (100.00 mg, 0.09 mmol) was treated with 2.0 ml of 4N HCl/dioxane in an Ar-atmosphere. After stirring for 55 min DC indicated complete conversion and the volatiles were removed under reduced pressure. Residual HCl was removed by adding 8.0 ml of diethylether to the hydrochloride with subsequent removal by rotary evaporation (repeat 3 times). Drying on high vacuum for 1.0 h afforded 35 in complete conversion (98.00 mg, theor. 94.41 mg). The latter (70.00 mg, 0.07 mmol) and heptapeptide 2 (69.95 mg, 0.06 mmol) were dissolved in 0.9 ml dry DMF in an Ar-atmosphere. The mixture was cooled to 0 ℃ and successively NaHCO₃ (16.40 mg, 0.20 mmol) and DEPBT (58.42 mg, 0.20 mmol) were added. After stirring for 24.0 h at 0 ℃ the suspension was warmed to room temperature and stirred vigorously for 24.0 h. After adding 0.4 ml of water to the suspension the volatiles were removed under high vacuum and the orange residue was taken up in 1.0 ml of MeOH for purification via size exclusion chromatography (Sephadex® LH-20; column-hight: 80 cm;
column-diameter 5 cm; flow rate 0.4 ml MeOH/min) to obtain tridecapeptide 36 (61.10 mg, 42 %) as white solid. Samples for biological testings were additionally purified by preparative HPLC (t_R = 34.0 min [20 % to 40 % CH_3CN in 40 min]); R_f = 0.2 (CHCl_3/MeOH = 9:2); mp > 230 °C; [α]_D^{20} = -127.1 (c = 0.07, MeOH); IR (neat): 3290, 3089, 3067, 2963, 2924, 2852, 1636, 1513, 1377, 1260, 1092, 1022, 798 cm^{-1}; ^1H-NMR (400 MHz, DMSO): δ = 0.52 (d, J = 6.7 Hz, 3H), 0.55 (d, J = 6.7 Hz, 3H), 0.57 (d, J = 7.5 Hz, 3H), 0.59 (d, J = 8.0 Hz, 3H), 1.82 – 1.90 (m, 2H), 2.68 – 2.86 (m, 3H), 2.96 (dd, J = 13.6, 4.2 Hz, 1H), 4.33 – 4.39 (m, 2H), 4.51 – 4.56 (m, 1H), 4.66 – 4.71 (m, 1H), 4.95 – 5.09 (m, 6H), 5.29 (d, J = 8.9 Hz, 1H), 5.31 (d, J = 8.0 Hz, 1H), 5.39 (d, J = 7.3 Hz, 2H), 5.45 (d, J = 7.8 Hz, 1H), 5.51 (d, J = 7.8 Hz, 1H), 5.56 (d, J = 8.3 Hz, 1H), 5.58 (d, J = 8.0 Hz, 1H), 5.63 (d, J = 8.1 Hz, 1H), 6.01 – 6.09 (m, 4H), 6.10 (t, J = 1.9 Hz, 1H), 6.17 (d, J = 1.9 Hz, 2H), 6.20 (d, J = 1.6 Hz, 2H), 6.27 (d, J = 1.6 Hz, 2H), 6.29 (d, J = 1.9 Hz, 2H), 6.33 (d, J = 1.9 Hz, 2H), 6.50 – 6.58 (m, 6H), 6.66 (d, J = 8.3 Hz, 2H), 6.93 (d, J = 8.6 Hz, 2H), 6.03 (d, J = 9.4 Hz, 2H), 7.05 (d, J = 8.0 Hz, 2H), 7.10 – 7.23 (m, 7H), 7.24 – 7.35 (m, 15H), 7.80 (d, J = 8.3 Hz, 1H), 7.86 (d, J = 8.6 Hz, 1H), 8.08 (d, J = 8.6 Hz, 1H), 8.39 (d, J = 7.8 Hz, 1H), 8.42 – 8.57 (m, 5H), 8.61 (d, J = 7.0 Hz, 1H), 8.66 (d, J = 9.1 Hz, 1H), 8.69 (d, J = 8.0 Hz, 1H), 8.78 (d, J = 7.5 Hz, 1H), 9.07 (br s, 4H), 9.13 (s, 2H), 9.14 (s, 2H), 9.18 (s, 2H), 9.24 (s, 1H), 9.26 (s, 1H), 9.29 (s, 1H), 9.35 (s, 1H); ^13C-NMR (100 MHz, DMSO): δ = 17.5, 17.9, 19.4, 31.9, 35.9, 36.2, 48.9, 54.1, 55.3, 55.9, 56.1, 56.4, 56.8, 57.5, 57.9, 65.9, 66.3, 102.4, 105.7, 105.9, 115.1, 115.3, 128.3, 128.6, 129.3, 136.1, 156.9, 158.4, 169.9; MS (ESI) m/z calcd for C_117H_115N_13O_32 (M+H+K)^{++}; HRMS (ESI): m/z found 1126.87377; 1126.86969.
2. $^1$H-NMR Spectra

Solvent impurities: ethyl acetate: 1.16 (t, $J$ = 7.1 Hz, 0.6H), 1.98 (s, 0.6H), 4.02 (q, $J$ = 7.1 Hz, 0.4H); acetonitrile: 2.06 (s, 0.2H); methanol: 3.15 (d, $J$ = 5.1 Hz, 1.3H); 1,4-dioxane: 3.56 (s, 0.76H).
Dipeptide 8

Styrol 10
Aminoalcohol 11

(R)-N-Boc-3-5-dibenzoxyphenylglycine 15
(S)-hydroxyphenylglycine benzylester hydrochloride 16

Dipeptide 18
Heptapeptide 34

Tridecapeptide 36
3. HPLC-(MS)- and UPLC-MS-Data

3.1 HPLC-MS-Analytics of Hexapeptide 3 and Heptapeptide 34

Hexapeptide 3:

a) Total ion chromatogram (TIC scan; positive mode); b) Extracted ion chromatogram (XIC scan; m/z = 1139.0 – 1140.0); c) MS spectrum (9.27 – 9.48 min, positive mode); d) DAD chromatogram; e) UV/VIS spectrum (at 7.29 min).

Gradient: 0 min: 95% A → 10 min: 0% A.
Heptapeptide 34:

a) Total ion chromatogram (TIC scan; positive mode); b) Extracted ion chromatogram (XIC scan; m/z = 1208.0 - 1209.0); c) MS spectrum (7.53 - 7.71 min, positive mode); d) DAD chromatogram; e) UV/VIS spectrum (at 7.49 min).

Gradient: 0 min: 95% A → 10 min: 0% A.
3.2 HPLC-Analysis of Tridecapeptide 36

Tridecapeptide 36 after size exclusion chromatography (1\textsuperscript{st} chromatographic step):

Tridecapeptide 36 after preparative HPLC (2\textsuperscript{nd} chromatographic step):
3.3 UPLC-MS-Analytics of Synthetic Feglymycin (1) and an Authentic Sample of 1

Authentic sample of Feglymycin:

a) UV/VIS spectrum (at 5.46 min); b) MS spectrum (at 5.47 min, positive mode); c) MS spectrum (at 5.46 min, negative mode); d) Total ion chromatogram (TIC scan; positive mode); e) Total ion chromatogram (TIC scan, negative mode); f) UV/Vis scan (monitoring wavelength 210 nm); g) ADC 2D chromatogram.

Gradient: 0 min: 90% A → 15 min: 0% A

ESI-HRMS \([\text{M+H}]^+ = 1901.65668\)
Synthetic Feglymycin (1):

a) UV/VIS spectrum (at 5.48 min); b) MS spectrum (at 5.48 min, positive mode); c) MS spectrum (at 5.47 min, negative mode); d) Total ion chromatogram (TIC scan, positive mode); e) Total ion chromatogram (TIC scan, negative mode); f) UV/Vis scan (monitoring wavelength 210 nm); g) ADC 2D chromatogram.

Gradient: 0 min: 90% A → 15 min: 0% A

ESI-HRMS [M+H]^+ = 1901.65588
4. Antiviral Assay of Selected Compounds

The MT-4 cells were obtained from the American Type Culture Collection (Rockville, MD) and were cultured in RPMI-1640 medium (Invitrogen, Paisley, UK) supplemented with 10 % FBS (BioWhittaker Europe, Verviers, Belgium) and 2 mM glutamine (Invitrogen, Paisley, UK). The CXCR4-using (X4) HIV-1 molecular clone NL4.3 was obtained from the National Institutes of Health NIAID AIDS Reagent program (Bethesda, MD).

The antiviral activity of the drugs was determined using a tetrazolium-based colorimetric assay in MT-4 cells.[2] Briefly, fivefold dilutions of the test compounds in 100 µl of medium were added to duplicate wells of 96-well flat bottom plates (Iwaki, Japan). Then 6 × 10⁴ MT-4 cells were added in 50 µl of medium, and finally 50 µl of diluted HIV-1 stock was added to each well. Cytopathic effect induced by the virus was monitored by daily microscopic evaluation of the virus-infected cell cultures. At day 4-5 after infection, when strong cytopathic effect was observed in the positive control (i.e., untreated HIV-1-infected cells), the cell viability was assessed via the in situ reduction of the tetrazolium compound MTS, using the CellTiter 96® AQueous One Solution Cell Proliferation Assay (Promega, Madison, WI). The absorbance was then measured spectrophotometrically at 490 nm with a 96-well plate reader (Molecular Devices, Sunnyvale, CA) and compared with four cell control replicates (cells without virus and drugs) and four virus control wells (virus-infected cells without drugs). The 50 % inhibitory concentration (IC₅₀, i.e. the drug concentration that inhibits HIV-1-induced cell death by 50 %), was calculated for each compound from the dose-response curve. The CC₅₀ or 50 % cytotoxic concentration of each of the compounds was determined from the reduction of viability of uninfected MT-4 cells exposed to the compounds, as measured by the MTS method described above.

Table 1 shows anti-HIV-activity of the natural product Feglymycin (1) and of selected synthetic intermediates. Also the enantiomeric compounds are listed for comparison as well as IC₅₀ of the bicyclam fusion inhibitor AMD3100 as reference compound.

---

Table 1: Anti-HIV-1 activity of a selected group of compounds in MT-4 cells.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC$_{50}^{[a]}$ (µg/ml)</th>
<th>CC$_{50}^{[b]}$ (µg/ml)</th>
<th>enantiomeric compound</th>
<th>IC$_{50}$ (µg/ml)</th>
<th>CC$_{50}$ (µg/ml)</th>
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[a] 50 % Inhibitory concentration (IC$_{50}$), or drug concentration required to inhibit virus-induced cytopathic effect (CPE) by HIV-1 NL4.3 in human MT-4 cells by 50 %. [b] 50 % Cell cytotoxic concentration (CC$_{50}$), or drug concentration required to inhibit cell growth in MT-4 cells by 50 %. n.d. = not determined.
5. Antibacterial Assay of Selected Compounds

Compounds listed up in table 1 (1 - 36; except enantiomeric compounds) and an authentic sample of Feglymycin (1) were investigated concerning antibacterial activity against eight different bacteria strains (table 2). Heptapeptide 34 and Feglymycin (1, 1authentic) showed activity against methicillin-resistant Staphylococcus aureus (MRSA). Minimum inhibitory concentrations (MIC) and IC80-values of all other compounds were > 64 µg/ml and are therefore omitted. MIC-determinations provide one of the most simplest and yet most informative methods of assessing antimicrobial activity.

Table 2: Antibacterial activity of 1, 1authentic and 34 against different bacteria strains.

<table>
<thead>
<tr>
<th>bacteria strain</th>
<th>MIC (µg/ml)[a]</th>
<th>IC80 (µg/ml)[b]</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus ATCC29213</td>
<td>4</td>
<td>2.1</td>
</tr>
<tr>
<td>S. aureus ATCC33592</td>
<td>4</td>
<td>2.3</td>
</tr>
<tr>
<td>S. aureus ATCC13709</td>
<td>2</td>
<td>1.8</td>
</tr>
<tr>
<td>E. coli ATCC25922</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>E. faecalis ATCC2912</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>S. pyogenes ATCC12344</td>
<td>64</td>
<td>26.2</td>
</tr>
<tr>
<td>C. albicans FH 2173</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>M. smegmatis ATCC607</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
</tbody>
</table>

[a] The minimum inhibitory concentration (MIC) of an antibacterial is defined as the lowest concentration of an antimicrobial agent that prevents visible growth of a microorganism in an agar or broth dilution susceptibility test. [b] 80% Inhibitory concentration (IC80), or drug concentration required to inhibit bacterial growth by 80%.

5.1 Determination of IC80 by broth dilution method-microdilution

In this work the standard broth dilution method-microdilution was used to determine the in vitro susceptibility of bacteria growing aerobically according to the guidelines and standards that have been approved by the National Committee on Clinical Laboratory Standards (NCCLS M7-A7).[3] The IC80 value of the compound to be tested is calculated out of the inhibitory concentration of 80% at which 80% visible turbidity or the ATP level is inhibited.