An Unusual Reverse Turn Structure Adopted by a Furanoid Sugar Amino Acid Incorporated in Gramicidin S

Gijsbert M. Grotenbreg, Mattie S. M. Timmer, Antonio L. Llamas-Saiz, Martijn Verdoes, Gijsbert A. van der Marel, Mark J. van Raaij, Herman S. Overkleeft and Mark Overhand

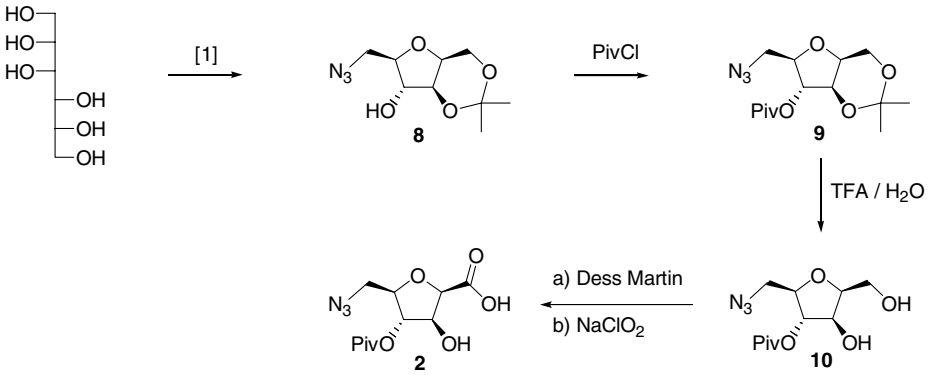
Supporting Information :

Contents :

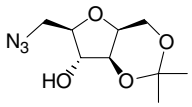
A. General :

All reactions were performed under an inert atmosphere and at ambient temperature unless stated otherwise. Reactions were monitored by TLC-analysis using DC-fertigfolien (Schleicher & Schuell, F1500, LS254) with detection by spraying with 20% H_2SO_4 in ethanol followed by charring at ~150°C or by spraying with a solution of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (25 g/L) and $(NH_4)_4Ce(SO_4)_4\cdot 2H_2O$ (10 g/L) in 10% sulfuric acid followed by charring at ~150°C. Column chromatography was performed on Merck silicagel (0.040 – 0.063 nm) and size exclusion chromatography on Sephadex™ LH-20.
Mass spectra were recorded on a PE/Sciex API 165 instrument with a custom-build Electrospray Ionisation (ESI) interface and HRMS (SIM mode) were recorded on a TSQ Quantum (Thermo Finnigan) fitted with an accurate mass option, interpolating between PEG-calibration peaks. For LC/MS analysis, a Jasco HPLC-system (detection simultaneously at 214 and 254 nm) equipped with an analytical Alltima C_{18} colomn (Alltech, 4.6 mmD × 250 mmL, 5µ particle size) in combination with buffers A: H_2O, B: MeCN and C: 0.5% aq. TFA and coupled to a Perkin Elmer Sciex API 165 mass instrument with a custom-made Electronspray Interface (ESI) was used. For RP-HPLC purification of the peptide, a BioCAD "Vision" automated HPLC system (PerSeptiveBiosystems, inc.) supplied with a semi-preperative Alltima C_{18} column (Alltech, 10.0 mmD × 250 mmL, 5µ particle size) was used. The applied buffers were A: H_2O, B: MeCN and C: 1.0% aq. TFA.
^{1}H- and ^{13}C-APT-NMR spectra were recorded on a Brüker AV-400 (400/100 MHz) and the peptide **7** was analyzed using a Brüker DMX 600 spectrometer equipped with a pulsed field gradient accessory. Chemical shifts are given in ppm (δ) relative to tetramethylsilane as internal standard (^{1}H-NMR) or $CDCl_3$ (^{13}C-NMR). Coupling constants are given in Hz. All presented ^{13}C-APT spectra are proton decoupled. Optical rotations were measured on a Propol automatic polarimeter (Sodium D line, λ = 589 nm) and ATR-IR spectra were recorded on a Shimadzu FTIR-8300 fitted with a single bounce DurasamplIR diamond crystal ATR-element.

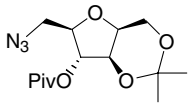
B. Experimental Procedures of the Sugar Amino Acid Synthesis



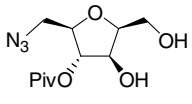
Scheme 1: Synthesis of the Sugar Amino Acid



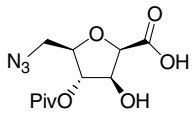
2,5-Anhydro-6-azido-6-deoxy-1,3-*O*-isopropylidene-D-glucitol (8)
Prepared as described by Timmer et al.[1]



2,5-Anhydro-6-azido-6-deoxy-1,3-*O*-isopropylidene-4-*O*-pivaloyl-D-glucitol (9)
Azide **8** (100 mmol, 22.9 g) was coevaporated twice with pyridine and dissolved in pyridine (500 mL). After pivaloylchloride (PivCl) (1.2 equiv., 120 mmol, 14.7 mL) was added, the reaction mixture was stirred overnight before being concentrated. The residue was dissolved in EtOAc and washed with 1M aq. HCl, water and brine. The EtOAc layer was dried over $MgSO_4$ and concentrated. Silica colomn chromatography (20% EtOAc in light PE) yielded fully protected glucitol **9** quantitatively (100 mmol, 31.4 g) as a transparant oil. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 4.85 (d, 1H, H_4, $J_{4,5}$ = 1.7 Hz), 4.25 (d, 1H, H_3, $J_{3,2}$ = 2.6 Hz), 4.10 (dd, 1H, H_{1a}, $J_{1a,2}$ = 2.5 Hz, $J_{1a,1b}$ = 13.4 Hz), 4.03 (dd, 1H, H_{1b}, $J_{1b,2}$ = 1.6 Hz, $J_{1b,1a}$= 13.4 Hz), 3.98 (ddd, 1H, H_5, $J_{5,4}$ = 1.7 Hz, $J_{5,6b}$ = 4.7 Hz, $J_{5,6a}$ = 7.8 Hz), 3.89 (ddd, 1H, H_2, $J_{2,1b}$ = 1.6 Hz, $J_{2,1a}$ = $J_{2,3}$ = 2.6 Hz), 3.66 (dd, 1H, H_{6a}, $J_{6a,5}$ = 7.8 Hz, $J_{6a,6b}$ = 12.6 Hz), 3.50 (dd, 1H, H_{6b}, $J_{6b,5}$ = 4.7 Hz, $J_{6b,6a}$ = 12.6 Hz), 1.44 (s, 3H, CH_3 *i*Pr), 1.41 (s, 3H, CH_3 *i*Pr), 1.20 (s, 9H, 3 × CH_3 Piv) ^{13}C-NMR (100 MHz, $CDCl_3$): δ 177.2 (C=O Piv), 97.6 (C_q *i*Pr), 83.6 (C_5), 80.1 (C_4), 73.6 (C_3), 73.3 (C_2), 60.1 (C_1), 52.7 (C_6), 38.5 (C_q Piv), 28.7 (CH_3 *i*Pr), 26.9 (CH_3 Piv), 18.8 (CH_3 *i*Pr). ATR-IR (thin film) 2977.9, 2096.5, 1732.0, 1481.2, 1375.2, 1280.6, 1143.7, 1091.6, 929.6, 846.7 cm^{-1}. $[\alpha]_D^{23}$ +22.4 (c = 1.00, $CHCl_3$) MS (ESI): *m/z* 314.3 $[M+H]^+$, 336.1 $[M+Na]^+$. HRMS: calcd for $C_{14}H_{23}N_3O_5NH_4$ 331.1981, found 331.1968.



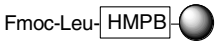
2,5-Anhydro-6-azido-6-deoxy-4-*O*-pivaloyl-D-glucitol (10)
Glucitol **9** (100 mmol, 31.4 g) was dissolved in a mixture of water/TFA (400 mL, 2/1, v/v). The resulting white suspension was stirred overnight to give a homogeneous yellow solution. The reaction mixture was concentrated and coevaporated with toluene before being purified by column chromatography (toluene → 30% EtOAc in toluene) furnishing the title compound **10** (17.2 g, 63 mmol, 63%) as a transparant oil. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 4.81 (dd, 1H, H_4, $J_{4,3}$ = 1.8 Hz, $J_{4,5}$ = 3.4 Hz), 4.28 (dd, 1H, H_3, $J_{3,4}$ = 1.8 Hz, $J_{3,2}$ = 4.3 Hz), 4.05 (dd, 1H, H_2, $J_{2,3}$ = 2.5 Hz, $J_{2,1}$ = 8.6 Hz), 3.97 (m, 3H, H_5 and 2 × H_1) 3.63 (d, 2H, 2 × H_6, $J_{6,5}$ = 4.8 Hz), 1.20 (s, 9H, 3 × CH_3 Piv) ^{13}C-NMR (100 MHz, $CDCl_3$): δ 178.3 (C=O Piv), 81.8 (C_5), 81.5 (C_4), 80.9 (C_2), 76.5 (C_3), 61.0 (C_1), 52.4 (C_6), 38.5 (C_q Piv), 26.8 (CH_3 Piv). ATR-IR (thin film) 3328.9, 2974.0, 2098.4, 1726.2, 1481.2, 1280.6, 1149.5, 1078.1, 1035.7 cm^{-1}. $[\alpha]_D^{23}$ +41.6 (c = 1.00, $CHCl_3$) MS (ESI): *m/z* 273.9 $[M+H]^+$, 296.2 $[M+Na]^+$. HRMS: calcd for $C_{11}H_{19}N_3O_5H$ 274.1403, found 274.1409.



2,5-Anhydro-6-azido-6-deoxy-4-*O*-pivaloyl-D-gluconic acid (2).

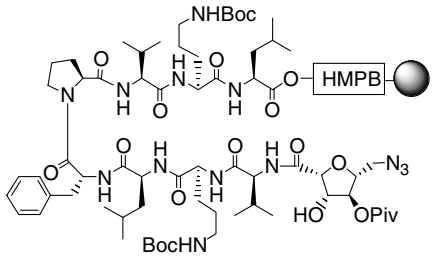
Diol **10** (5.8 g, 20 mmol) was coevaporated twice with toluene, dissolved in DCM (100 mL), placed under an argon atmosphere and cooled to 0°C, before Dess-Martin periodinane (1.1 equiv., 9.35 g, 22 mmol) was added under vigorous stirring. The reaction mixture was stirred for 30 min. before a sat. aq. NaS_2O_3 / sat. aq. $NaHCO_3$ solution (100 mL, 7 / 3 (v/v)) was added and stirred for an additional 15 min. Then, the DCM layer was separated, washed with H_2O and brine, dried over $MgSO_4$ and concentrated. The residue was coevaporated with toluene and purified by column chromatography (toluene → 5 % (v/v) EtOAc in toluene) yielding 6-azido-6-deoxy-4-pivaloyl-2,5-anhydro-D-glucose (4.97 g, 18.3 mmol, 91.5 %). The aldehyde (2.56 g, 9.4 mmol) was dissolved in a solution of *tert*-butanol (80 mL), 2-methyl-2-butene (20 mL) and water (80 m), before NaH_2PO_4 (8.0 g) and $NaClO_2$ (8.0 g) were added. The reaction was stirred overnight, before the solution was acidified and extracted with EtOAc (2 ×). The EtOAc layers were dried over $MgSO_4$, concentrated and purified by column chromatography (toluene → 1% AcOH in EtOAc) yielding the title compound **2** (1.55 g, 5.38 mmol, 57% (52 %, 2 steps)). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 4.86 (dd, 1H, H_4, $J_{4,3}$ = 1.0 Hz, $J_{4,5}$ = 2.0 Hz), 4.58 (d, 1H, H_2, $J_{2,3}$ = 4.0 Hz), 4.41 (dd, 1H, H_3, $J_{3,4}$ = 1.0 Hz, $J_{3,2}$ = 4.0 Hz), 4.00 (ddd, 1H, H_5, $J_{5,4}$ = 2.0 Hz, $J_{5,6b}$= 4.3 Hz, $J_{5,6a}$= 5.5 Hz), 3.73 (dd, 1H, H_{6a}, $J_{6a,5}$ = 5.5 Hz, $J_{6a,6b}$ = 12.8 Hz), 3.63 (dd, 1H, H_{6b}, $J_{6b,5}$ = 4.3 Hz, $J_{6b,6a}$ = 12.8 Hz), 1.15 (s, 9H, 3 × CH_3 Piv). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 177.9 (C=O Piv), 171.5 (COOH) 83.3 (C_5), 81.4 (C_2), 80.1 (C_4), 76.0 (C_3), 52.0 (C_6), 38.6 (C_q Piv), 26.8 (CH_3 Piv). ATR-IR (thin film) 3421.6, 2976.0, 2102.3, 1728.1, 1481.2, 1282.6, 1143.7, 1097.4, 1037.6 cm^{-1}. $[\alpha]_D^{23}$ +30.0 (c 1.00, $CHCl_3$). MS (ESI): *m/z* 288.2 $[M+H]^+$, 310.1 $[M+Na]^+$. HRMS: calcd for $C_{11}H_{17}N_3O_6$ H 288.1196, found 288.1240.

C. Experimental Procedures of the Peptide Synthesis



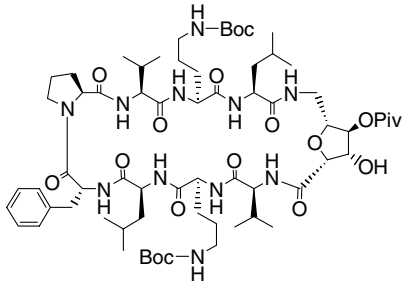
Fmoc-Leu-HMPB-MBHA resin (3)

Commercially available 4-methylbenzhydrylamine (MBHA) functionalized polystyrene resin (2.22 g, 0.9 mmol/g, 2.0 mmol) was shaken with NMP (30 mL, 3×, 3 min.) followed by addition of a pre-activated mixture of 4-(4-hydroxymethyl-3-methoxyphenoxy)-butyric acid (HMPB) (3 equiv., 1.44 g, 6.0 mmol), benzotriazole-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP) (3 equiv., 2.652 g, 6.0 mmol) and *N,N*-diisopropylethylamine (DiPEA) (6 equiv., 2.09 mL, 12.0 mmol) in NMP (25 mL). Shaking was continued overnight after wich the resin was washed with NMP (30 mL, 3×, 3 min.) and DCM (30 mL, 3×, 3 min.). Next, the resin was transferred to a flask, coevaporated with DCE (30 mL, 3×) and condensed with Fmoc-Leu-OH (3 equiv., 2.12 g, 6.0 mmol) under de influence of *N,N'*-diisopropylcarbodiimide (DIC) (3.3 equiv., 1.03 mL, 6.6 mmol) and a catalytic amount of 4-dimethylaminopyridine (DMAP) (40 mg, 0.33 mmol) for two hours. The resin was then filtered and washed with DCM (30 mL, 3×, 3 min.) and subjected to a second condensation sequence, gaining fully loaded resin **3**. The loading of the resin was determined to be 0.50 mmol/g by spectrophotometric analysis.



SAA(Piv)-Val-Orn(Boc)-Leu-^{D}Phe-Pro-Val-Orn(Boc)-Leu-HMPB-MBHA resin (4)

Resin **3** (0.2 g, 0.5 mmol/g, 0.1 mmol) was submitted to seven cycles of Fmoc solid-phase synthesis with Fmoc-Orn$_8$(Boc)-OH, Fmoc-Val$_7$-OH, Fmoc-Pro$_6$-OH, Fmoc-^{D}Phe$_5$-OH, Fmoc-Leu$_4$-OH, Fmoc-Orn$_3$(Boc)-OH and Fmoc-Val$_2$-OH, respectively, as follows: a) deprotection with piperidine / NMP (1/4, v/v, 5 mL, 15 min.); b) wash with NMP (5 mL, 3×, 3 min.); c) coupling of the appropriate Fmoc amino acid (2.5 equiv., 0.25 mmol) in the presence of BOP (2.5 equiv., 0.25 mmol, 0.11 g), *N*-hydroxybenzotriazole (HOBt, 2.5 equiv., 0.25 mmol, 34 mg) and DiPEA (3 equiv., 0.3 mmol, 0.051 mL) which was preactivated for 2 min. in NMP (5 mL) and shaken for 90 min.; d) wash with NMP (5 mL, 3×, 3 min.). Couplings were monitored for completion by the Kaiser test.[2] Finally, the *N*-terminal amine was liberated by Fmoc-deprotection with piperidine / NMP (1/4, v/v, 5 mL, 15 min.) followed by washing with NMP (5 mL, 3×, 3 min.). To the resin bound octapeptide, a preactivated solution of SAA **2** (3.6 equiv., 105 mg, 0.366 mmol), BOP (6 equiv., 266 mg, 0.6 mmol), HOBt (6 equiv., 81 mg, 0.6 mmol) and DiPEA (6.5 equiv., 110 µL, 0.65 mmol) in NMP (3 mL) was added and the resulting suspention was shaken for 16h. The resin was finally washed with NMP (5 mL, 3×, 3 min.) to give title compound **3**.

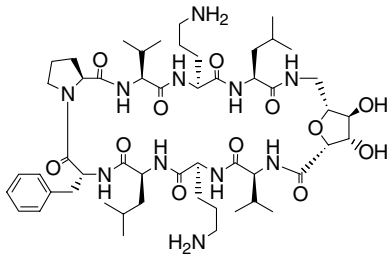


***cyclo*-[SAA(Piv)-Val-Orn(Boc)-Leu-^{D}Phe-Pro-Val-Orn(Boc)-Leu] (5)**

Resin bound nonapeptide **4** was washed with 1,4-dioxane (5 mL, 3×, 3 min.) and taken up in 1,4-dioxane (10 mL) to which trimethylphosphine (16 equiv., 1.6 mL, 1.6 mmol, 1 M in toluene) was added. Subsequently, the resin was shaken for 2h., water (1 mL) was added and shaken another 4h. The resin was then washed with 1,4-dioxane (5 mL, 3×, 3 min.) and DCM (5 mL, 3×, 3 min.) after which the peptide was released from the resin by mild acetic cleavage (TFA/DCM, 1/99, v/v, 10 mL, 3×, 10 min.). The fractions were collected and coevaporated with toluene (50 mL) for three times to give the crude linear peptidic construct which was cyclized directly without further purification.

For the cyclization of the crude linear peptide, it was taken up in DMF (5 mL) and added dropwise over the course of an hour to a solution of benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP) (5 equiv., 270 mg, 0.5 mmol), HOBt (5 equiv., 67 mg, 0.5 mmol) and DiPEA (15 equiv., 254 µL, 1.5 mmol) in DMF (70 mL) and allowed to stir for 16h. The solvent was removed *in vacuo* and the resulting

mixture was applied to a Sephadex® size exclusion colomn (50.0 mmD × 1500 mmL) and eluted with MeOH yielding pure peptide **5** as white amorphous solid (128 mg, 96 µmol, 96%).
^{1}H-NMR (400 MHz, DMSO-D6, 300 K): δ 9.11 (bs, 1H, NH ^{D}Phe), 8.65 (d, 1H, NH_α Orn, J_{NH,H_α} = 8.8 Hz), 8.57 (d, 1H, NH_α Orn, J_{NH,H_α} = 8.6 Hz), 8.29 (d, 1H, NH Leu, J_{NH,H_α} = 8.7 Hz), 7.97 (d, 1H, NH Leu, J_{NH,H_α}= 7.6 Hz), 7.42 (m, 2H, NH SAA, NH Val), 7.28 (m, 1H, NH Val), 7.27–7.13 (m, 5H, H_{ar}), 6.86 (bs, 1H, NH_δ Orn), 6.55 (bs, 1H, NH_δ Orn), 5.90 (d, 1H, C_3-OH SAA, $J_{OH,\ H3}$ = 4.8 Hz), 4.87 (m, 1H, H_α Orn), 4.73 (bs, 1H, H_2 SAA), 4.63 (m, 1H, H_α Leu), 4.53 (m, 1H, H_α Pro), 4.46 (m, 1H, H_α Val), 4.37 (d, 1H, H_4 SAA J = 3.3 Hz), 4.33 (m, 1H, H_α ^{D}Phe), 4.22 (m, 2H, H_α Val, H_α Orn), 4.16 (m, 3H, H_α Leu, H_3 SAA, H_5 SAA), 3.51 (m, 1H, $H_{\delta d}$ Pro), 3.39 (m, 1H, H_{6d} SAA), 3.28 (m, 1H, H_{6u} SAA), 2.98–2.78 (m, 6H, H_β ^{D}Phe, H_δ Orn), 2.42 (m, 1H, $H_{\delta u}$ Pro), 2.00 (m, 4H, H_β Val, H_β Pro), 1.75–1.25 (m, 16H, H_β Orn, H_γ Orn, H_β Leu, H_γ Leu, H_γ Pro), 1.33 (s, 18H, CH_3 Boc) 1.14 (s, 9H, CH_3 Piv) 0.91-0.66 (m, 24H, H_γ Val, H_δ Leu). ATR-IR (thin film) 3274.9, 2960.5, 2931.6, 2873.7, 1633.6, 1525.6, 1450.4, 1390.6, 1365.5, 1276.8, 1251.7, 1164.9, 1093.6, 1037.6, 910.3, 729.0, 702.0, 646.1 cm^{-1}. MS (ESI): *m/z* 1341.0 $[M+H]^+$, 1363.0 $[M+Na]^+$ HRMS: calcd for $C_{67}H_{109}N_{11}O_{17}NH_4$ 1357.8347, found 1357.8325.



***cyclo*-[SAA-Val-Orn-Leu-^{D}Phe-Pro-Val-Orn-Leu] (7)**
Cyclic peptide **5** (64 mg, 48 µmol) was taken up in anhydrous MeOH (2.5 mL), sodium methoxide (7.7 equiv., 20 mg, 370 µmol) was added and the mixture was allowed to stir for 16h. Subsequently, the solution was neutralized using Amberlite® exchange resin (H^+-form) and concentrated *in vacuo*. A portion of the deprotected cyclic peptide **6** (36 mg, 29 µmol) was directly dissolved in DCM (5 mL) and cooled to 0 ºC. Then, trifluoroacetic acid (5 mL) was added and the mixture was allowed to warm to ambient temperature over a period of 30 min. To the solution was added toluene (15 mL) and concentrated. The resulting peptide was analyzed by LC/MS (R_t 17.56 min, linear gradient 20→ 60% B in 20 min; *m/z* = 1057.1 $[M+H]^+$, 529.1 $[M+H]^{2+}$) and purified by semi-preperative RP-HPLC (linear gradient of 4.0 CV; 30→50% B; R_t 4.0 CV). Lyophilization of the combined fractions furnished title compound **7** (22.0 mg, 17.1 µmol, 59%) as white amorphous powder. ^{1}H-NMR (600 MHz, CD_3OH): δ 8.95 (d, 1H, NH ^{D}Phe$_5$, J_{NH,H_α} = 3.3 Hz), 8.64 (d, 1H, NH Leu$_4$, J_{NH,H_α} = 9.1 Hz), 8.62 (d, 2H, NH_α Orn$_{3,8}$, J_{NH,H_α} = 8.7 Hz), 8.33 (d, 1H, NH Leu$_9$, J_{NH,H_α} = 8.5 Hz), 8.00 (t, 1H, NH SAA$_1$, $J_{NH,6}$ = 5.3 Hz), 7.83 (bs, 2H, NH_δ Orn$_3$), 7.80 (bs, 2H, NH_δ Orn$_8$), 7.77 (d, 1H, NH Val$_7$, J_{NH,H_α} = 8.7 Hz), 7.46 (d, 1H, NH Val$_2$, J_{NH,H_α} = 8.8 Hz), 7.40 – 7.21 (m, 5H, H_{ar}), 4.99 (m, 1H, H_α Orn$_3$), 4.67 (m, 1H, H_α Orn$_8$), 4.63 (m, 1H, H_α Leu$_4$), 4.53 (d, 1H, H_2 SAA$_1$, $J_{2,3}$ = 3.9 Hz), 4.48 (m, 1H, H_α ^{D}Phe$_5$), 4.46 (m, 1H, H_α Leu$_9$), 4.33 (m, 1H, H_α Pro$_6$), 4.30 (m, 1H, H_α Val$_2$), 4.20 (dd, 1H, H_3 SAA$_1$, $J_{3,4}$ = 1.6 Hz, $J_{3,2}$ = 3.9 Hz), 4.10 (m, 1H, H_5 SAA$_1$), 4.03 (m, 1H, H_α Val$_7$), 3.93 (dd, 1H, H_4 SAA$_1$, $J_{4,3}$ = 1.6 Hz, $J_{4,5}$ = 1.6 Hz), 3.72 (m, 1H, $H_{\delta d}$ Pro$_6$), 3.59 (ddd, 1H, H_{6d} SAA$_1$, $J_{6d,5}$ = 3.8 Hz, $J_{6d,NH}$ = 5.3 Hz, $J_{6d,6u}$ = 14.3 Hz), 3.44 (ddd, 1H, H_{6u} SAA$_1$, $J_{6u,NH}$ = 5.3 Hz, $J_{6u,6d}$ = 14.3 Hz), 3.08 (dd, 1H, $H_{\beta d}$ ^{D}Phe$_5$, $J_{\beta d,\beta u}$ = 12.6 Hz, $J_{\beta d,\alpha}$ = 5.0 Hz), 2.99 (m, 1H, $H_{\delta d}$ Orn$_3$), 2.93 (m, 4H, H_δ Orn$_8$, $H_{\delta u}$ Orn$_3$, $H_{\beta u}$ ^{D}Phe$_5$), 2.47 (m, 1H, $H_{\delta u}$ Pro$_6$), 2.28 (m, 1H, H_β Val$_7$), 2.07 (m, 1H, H_β Val$_2$), 1.98 (m, 2H, $H_{\beta d}$ Pro$_6$, $H_{\beta d}$ Orn$_3$), 1.84 (m, 1H, $H_{\beta d}$ Orn$_8$), 1.76 (m, 3H, $H_{\beta u,\ \gamma}$ Orn$_3$), 1.68 (m, 2H, $H_{\beta u,\ \gamma d}$ Pro$_6$), 1.67 (m, 2H, H_γ Orn$_8$), 1.65 (m, 3H, $H_{\beta,\ \gamma}$ Leu$_9$), 1.63 (m, 1H, $H_{\beta u}$ Orn$_8$), 1.57 (m, 1H, $H_{\gamma u}$ Pro$_6$), 1.54 (m, 2H, $H_{\beta d,\ \gamma}$ Leu$_4$), 1.40 (m, 1H, $H_{\beta u}$ Leu$_4$), 0.95 (m, 3H, $H_{\gamma d}$ Val$_7$), 0.93 (m, 6H, H_γ Val$_2$), 0.89 (m, 6H, H_δ Leu$_4$), 0.87 (m, 3H, $H_{\gamma u}$ Val$_7$), 0.85 (m, 6H, H_δ Leu$_9$). The amide region of the ROESY-experiment is depicted in Figure 1. ATR-IR (thin film) 3270.0, 3066.7, 2958.7, 2935.1, 2874.8, 1733.9, 1670.4, 1639.2, 1533.3, 1456.3, 1202.2, 1181.2, 1133.5, 1033.3, 837.4, 799.5, 748.6, 722.4, 702.4 cm^{-1}. HRMS: calcd for $C_{52}H_{85}N_{11}O_{12}H$ 1056.6457, found 1056.6382.

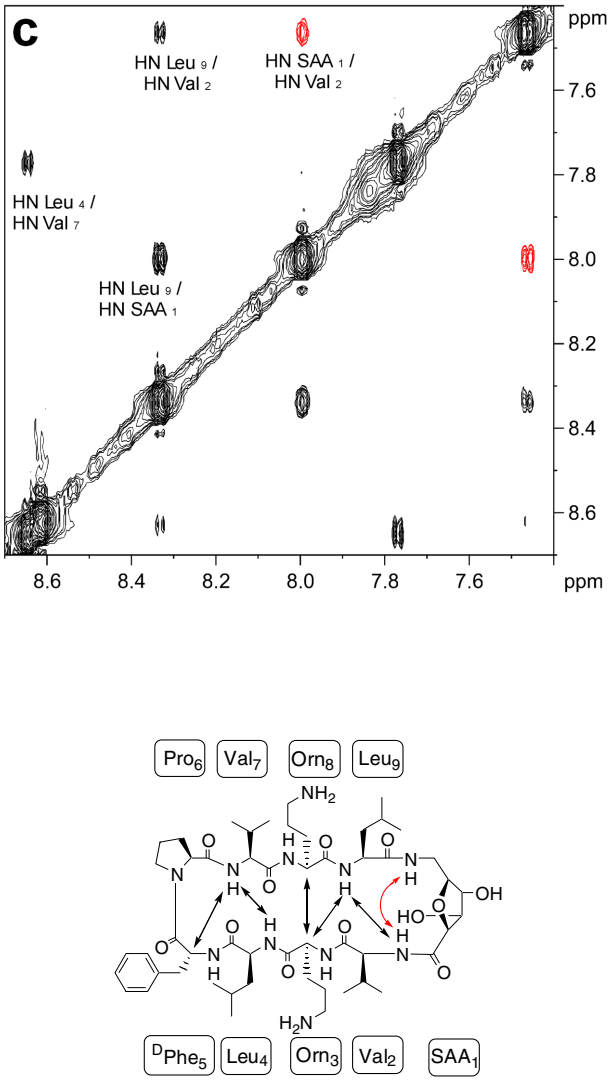


Figure 1 *:Amide region of the ROESY experiment of* ***7*** *(CD_3OH, 600 MHz).*

D. X-ray crystallographic data

Lyophilized peptide **7** (1,40 mg, 1.09 µmol) was dissolved in 200 µL MeOH / H_2O (1/1, v/v), after which 5 µL of the solution was injected onto a 96-well microtiter plate that was previously filled with n-decane. To the sample, 1 µl spermidine tri-HCl (0.1 M) was added (addition of 1 µl 1,5-diaminopentane di-HCl (30 % w/v) gave similar results), after which the microtiter plate was covered with a mixture of parafine and silicone oil (10/9, v/v) and allowed to stand for a period of 2 weeks. The crystals that formed (Figure 2) were then analyzed and the structure refined (Table 1).

A complete dataset was collected from one crystal (0.8 x 0.08 x 0.04 mm) at 100 K using a Bruker-Nonius FR591 rotating anode generator equipped with kappa-CCD2000 detector and MONTEL multilayer graded x-ray optics, CuKα radiation (λ=1.54184 Å). Data were processed using HKL Denzo and Scalepack.[3] The structure was solved by direct methods (SIR-97)[4] and refined with full-matrix least-squares analysis on F^2 using SHELXL-97.[5] Due to the limited resolution of 1.2Å, local disorder and the presence of solvent channels in the crystal, hydrogens were not always added and some atoms were refined at multiple positions. Atoms with occupancies lower than unity, disordered side chains and solvent atoms were refined isotropically. Semi-empirical absorption correction from equivalents using SORTAV.[6] CCDC-216610 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

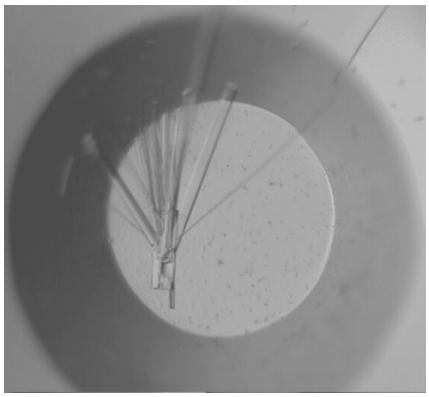


Figure 2: *Crystal of GS analogue **7**.*

Table 1: Crystal data and structure refinement for GS analogue **7**.

Identification code	gsd17e21	
Empirical formula	$C_{52}H_{85}N_{11}O_{12}$ 5.75(H_2O)	
Formula weight	1149.64	
Temperature	100 K	
Wavelength	1.5418 Å	
Crystal system,	Hexagonal	
Space group	P6	
Unit cell dimensions	a = 31.3930(4) Å	α = 90º
	b = 31.3930(4) Å	β = 90º
	c = 12.7243(2) Å	γ = 120º
Volume	10860.0(3) $Å^3$	
Z	6	
Calculated density	1.055 Mg/m^3	
Absorption coefficient	0.666 mm^{-1}	
F(000)	3700	
Crystal size	0.8 x 0.08 x 0.04 mm	
Theta range for data collection	3.47º to 50.32º	
Limiting indices	0<=h<=27	
	0<=k<=26	
	-12<=l<=11	
Reflections collected / unique	49791 / 7378 [R(int) = 0.075]	
Completeness to theta = 50.32º	99 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.980 and 0.911	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	7378 / 38 / 709	
Goodness-of-fit on F^2	1.072	
Final R indices (F_o>4σ(F_o)	R1 = 0.0982, wR2 = 0.2423	
R indices (all data)	R1 = 0.1093, wR2 = 0.2590	
Absolute structure parameter	0.0(4)	
Extinction coefficient	0.0021(2)	
Largest diff. peak and hole	0.624 and -0.549 $eÅ^{-3}$	

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