Synthesis and Structural Analysis of a β-Hairpin Peptide Containing a Sugar Amino Acid

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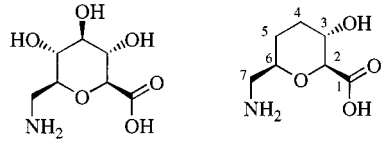
The solid-phase synthesis of the linear peptide **II** (Ac-KKYTVSI-SAA**2**-KKITVSI) containing the novel sugar amino acid SAA**2** [(2*S*,3*S*,6*R*)-6-aminomethyl-3-hydroxytetrahydropyran-2-carboxylic acid] is described. Structural analysis by NMR and CD in 50% aqueous methanol indicates that peptide **II** adopts a β-hairpin conformation, as reported earlier for the non-modified peptide **I** (Ac-KKYTVSI**NG**KKITVSI).

Introduction

It is well established that β-sheets are important secondary structure elements in proteins and play a crucial role in many biological processes such as electron transfer, protein dimerization, and DNA recognition.[1] In order to understand the mechanism of these processes, much attention[2] has been directed to the design and synthesis of model compounds displaying conformational properties resembling those of β-sheets. A β-hairpin, composed of two β-strands connected by a loop or turn region, is the simplest form of an antiparallel β-sheet secondary structure.[3] Recently,[4] several model peptides that form monomeric β-hairpin structures in aqueous media have been reported. It has been proposed that interstrand hydrogen bonding, hydrophobic interactions, and conformational preferences at the turn region are important factors in stabilizing β-hairpin structures.[5] For instance, replacement of one of the amino acids in the turn region of a β-hairpin may lead to non-native strand alignment and destabilization of the secondary structure.[6] On the other hand, the incorporation of a conformationally restricted β-turn mimic in a specific location of a peptide sequence promotes β-hairpin folding.[7] An interesting class of turn mimics are sugar amino acids (SAAs),[8] which may function as conformationally restricted dipeptide isosters. For instance, SAA**1** (Figure 1) was shown to mimic a β-turn in the cyclic peptide *cyclo*(-SAA**1**-Phe-D-Trp-Lys-Thr-).

Searle and co-workers showed[4d,9] that the tetrapeptide INGK adopts a β-turn motif in the linear peptide Ac-KKYTVS*INGK*KITVSI (i.e., peptide **I**, Figure 2). Moreover peptide **I**, designed to mimic the two-stranded antiparallel β-sheet DNA binding motif of the *met* repressor dimer,[1d] forms a stable autonomous β-hairpin structure in aqueous solution. It was shown[9] that the NG sequence has a natural propensity to form a two-residue turn, which appears to stabilize the β-hairpin conformation. We recently reported[10] that several modified peptides, in which partially deoxygenated sugar amino acids structurally related to SAA**2** (Figure 1) replace the amino acids Val-Phe in the tetrapeptide Cys-Val-Phe-Met (CVFM), showed inhibitory activity against the enzyme protein farnesyl transferase in the micromolar range. It has also been reported that the sequence CVFM of the heptapeptide KTKCVFM adopts a β-turn conformation upon binding to the same enzyme.[11] This knowledge was an incentive to investigate the effect of replacing the two amino acids NG in peptide **I** by the dipeptide isostere SAA**2**.



SAA**1** SAA**2**

Figure 1. Structures of the sugar amino acids SAA**1** and SAA**2**

Here we report the synthesis of peptide **II** (Ac-KKYTVSI-SAA**2**-KKITVSI, Figure 2), and provide evidence that **II**, with SAA**2** occupying the β-turn region, adopts a β-hairpin structure in solution.

Results And Discussion

Synthesis of the Sugar Amino Unit 8

We projected that the synthesis of target peptide **II** should readily be accomplished using standard Fmoc-based peptide chemistry, including acetylation of the N-terminus of the immobilized, fully assembled polypeptide prior to deprotection and cleavage. In order to prevent unwanted acetylation, the side chains of the amino acids lysine, serine,

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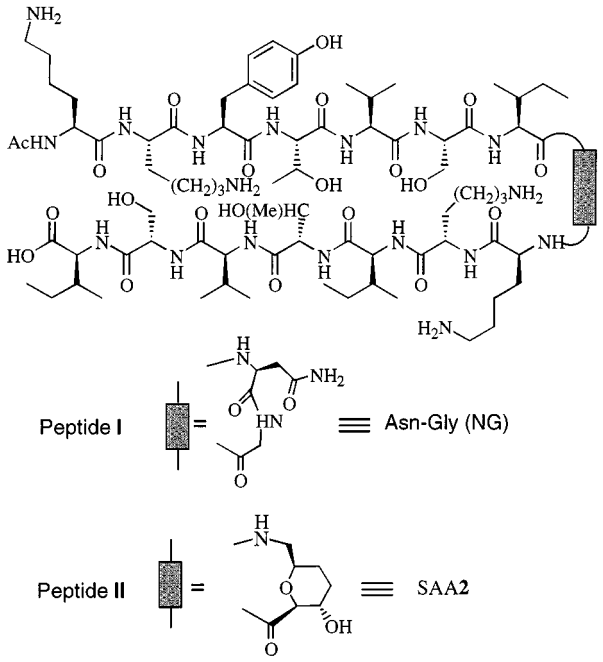
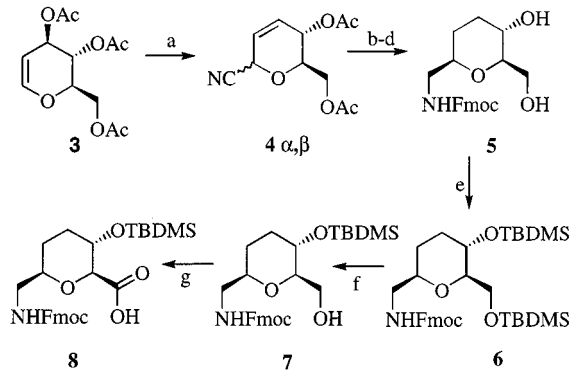


Figure 2. Structures of the β-hairpin peptide **I** and the modified peptide **II**



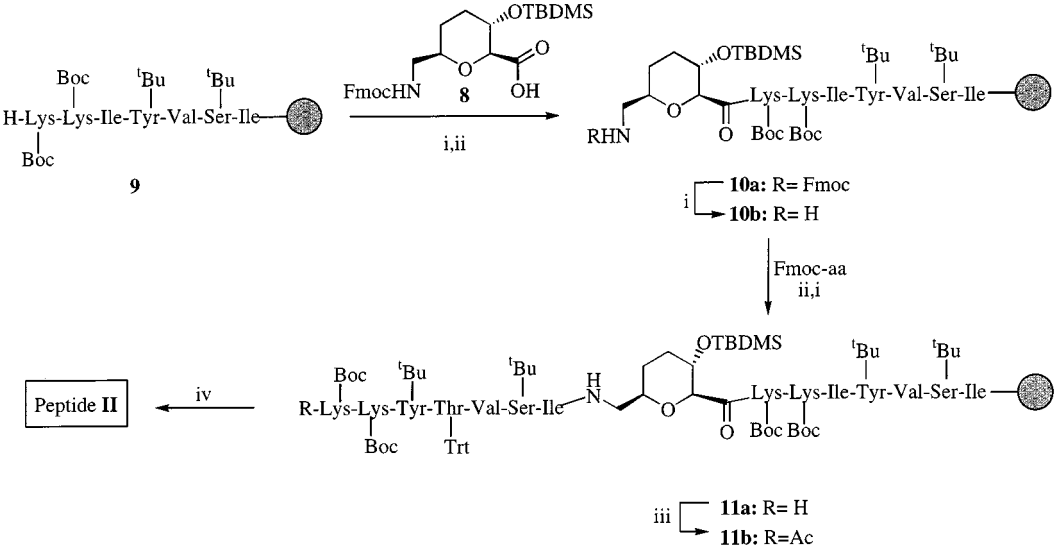
Scheme 1. Synthesis of sugar amino acid building block; *reagents and conditions:* (a) TMSCN, BF_3·Et_2O, DCM, room temp. 59% **4**α, 37% **4**β; (b) H_2, Pd/C, EtOAc, HCl; (c) *t*BuOK, MeOH; (d) Fmoc-OSu, $NaHCO_3$, dioxane/H_2O, room temp., 63% (three steps); (e) TBDMSCl, Im, DMF, room temp., 97%; (f) HOAc/THF/H_2O 13:7:1, 4 °C, 88%; (g) 1. oxalyl chloride, DMSO, Et_3N, DCM, −78 °C; 2. $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*BuOH/H_2O 1:1, room temp., 90% (2 steps)

tyrosine, and threonine were protected with acid-labile groups (Boc, *t*Bu, and Trt: see Experimental Section). For the same reason, the hydroxyl group of the sugar amino acid building block was protected as a *tert*-butyldimethylsilyl (TBDMS) ether. The synthesis of sugar amino acid **8**, starting from commercially available 3,4,6-tri-*O*-acetyl-D-glucal **3**, is outlined in Scheme 1.

Treatment of commercially available 2,3,6-tri-*O*-acetyl-D-glucal **3** with trimethylsilyl cyanide under acidic conditions gave a mixture of the anomeric cyanides **4**.[10] After separation, the β-anomer was transformed in three steps into diol **5**, in an overall yield of 63%. Thus, hydrogenolysis of **4β** in the presence of Pd/C under acidic conditions led to concomitant reduction of the double bond and cyanide function. Subsequent deacetylation with *t*BuOK in MeOH, followed by treatment with Fmoc succinimide, gave **5**. Silylation of both hydroxyl groups in **5** with TBDMS-Cl gave **6** in 97% yield. Regioselective removal of the silyl ether from the primary hydroxyl group was carried out under mildly acidic conditions to give **7** in good yield. Swern oxidation of **7** and treatment of the resulting aldehyde with sodium chlorite[12] in the presence of 2-methyl-2-butene furnished the sugar amino acid **8** in 90% yield over the two last steps. In comparison, direct oxidation of compound **7** with catalytic RuO_4[13] also provided **8**, albeit in lower yield (70%).

Synthesis of the Peptides I and II

The synthesis of polypeptide **II** was carried out on an automated peptide synthesizer, using standard Fmoc-based solid-phase peptide synthesis methodology (Scheme 2).



Scheme 2. Solid phase synthesis of peptide **II**; *reagents and conditions:* (i) 20% piperidine, NMP; (ii) BOP, HOBt, DIPEA, NMP; (iii) Ac_2O, DIPEA, HOBt, NMP; (iv) TFA/H_2O/TIS 95:2.5:2.5

In the first instance, the feasibility of condensing sugar amino acid building block **8** with immobilized peptide **9** was investigated. It turned out that immobilized derivative **10a** could be obtained by a repetitive coupling (2 times) of peptide **9** with excess **8** under the influence of Castro's reagent[14]/*N*-hydroxybenzotriazole and DIPEA. HPLC analysis of a sample obtained after cleavage of an aliquot of immobilized **10a** indicated complete consumption of the starting peptide **9**. Elongation of **10b** with the appropriately protected amino acids (**10b** to **11a**), followed by acetylation of the liberated N-terminus in **11a**, afforded the immobilized, fully protected pentadecamer **11b**. Treatment of this with TFA (95% in H_2O) in the presence of the cation scavenger triisopropylsilane resulted in deprotection and cleavage of the peptide from the solid support. Purification of the crude peptide by reverse phase HPLC furnished **II**, the mass spectrum of which was in full accord with the proposed structure. The reference peptide **I** was prepared using the same Fmoc-based SPPS methodology.

Structural Analysis by Circular Dichroism Spectroscopy

The CD spectra of the reference peptide **I** and the modified peptide **II** were recorded in a 1:1 mixture of MeOH:H_2O at pH = 3.9.

The spectrum of peptide **I**, as depicted in Figure 3, displays an ellipticity maximum around 214 nm. This CD curve is characteristic of a β-structure (both turn and sheet) and is in agreement with the results of Searle et al.[4d] The CD spectrum of peptide **II** shows a similar profile. Although the ellipticity maximum is slightly shifted to 210 nm and deeper than that of peptide **I** measured at the same peptide concentration (7.5 μM solution), these data strongly suggest that peptide **II** adopts a similar overall structure to that of peptide **I** in the solvent mixture studied.

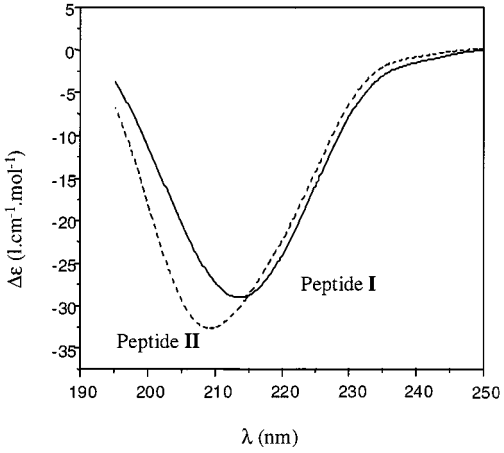


Figure 3. CD spectra of peptides **I** and **II** in MeOH/H_2O 1:1 (7.5 μM solution) at 4 °C

Structural Analysis by ^{1}H NMR

The resonance assignment of peptide **II** was accomplished in a standard manner, using 2D DQF-COSY, TOCSY, and NOESY experiments. ^{1}H chemical shift assignments of peptide **I** in a 1:1 mixture of CD_3OH and H_2O at 277 K are presented in Table 1 (see Experimental Section). Although still short of a complete tridimensional structure determination, NMR analysis can provide direct evidence for the existence of regular secondary structures through two parameters: the so-called secondary chemical shift and the appearance of characteristic cross-peaks in the NOESY spectrum.[15] The analysis of these parameters will be discussed in the following sections.

Analysis of Secondary Chemical Shift Perturbation of Peptide II

The secondary chemical shift (Δδ) is defined as the difference between the measured chemical shift for a given nucleus and the value expected in a random coil peptide. According to Wishart and co-workers,[16] the presence of three or more contiguous residues with $\Delta\delta H\alpha > 0.1$ ppm (i.e. the secondary chemical shift of the proton bound to Cα) is indicative of residues in an extended β-strand conformation.

Using the values of Wishart et al.,[16] the plot of ΔδHα for peptide **I** (see Figure 4), as observed earlier by Searle and co-workers, has the characteristic features of a β-hairpin, with two extended β-strands separated by a turn region. A similar analysis of the Hα chemical shifts of peptide **II** reveals a nearly identical pattern of secondary chemical shifts with large, positive deviations from the random coil values for nearly all amino acid residues (see Figure 4). Secondary chemical shift information for SAA**2** could not be included, due to the lack of reference values for the sugar amino acid residue. The calculated ΔδH for nearly all residues in peptide **II** is greater than 0.1 ppm, which is in agreement with an extended β-strand conformation.

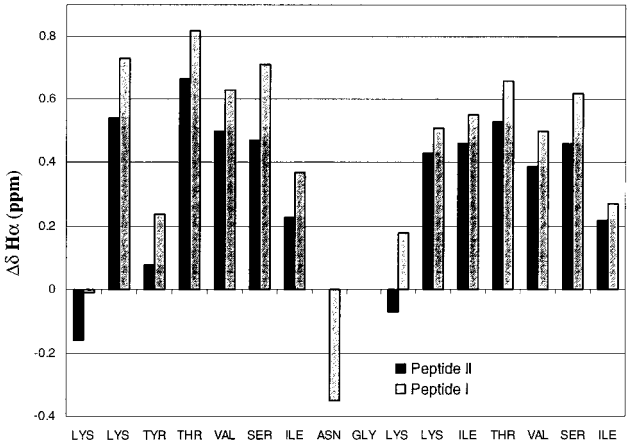


Figure 4. Plot of difference between Hα measured chemical shift for a given nucleus and the value expected in a random coil peptide for peptides **I** and **II**

Observation of Characteristic Nuclear Overhauser Effects (NOEs)

It is well established that the observation of long-range backbone NOE interactions, together with strong sequential $d_{\alpha N}$ NOEs combined with weak or absent d_{NN} peaks, is conclusive evidence for the presence of a β-sheet structure.[15]

Strong sequential $d_{\alpha N}$ NOEs were observed (Figure 5) in the NOESY spectrum of **II**. Moreover, as shown schematic-

ally in Figure 6, cross-peaks involving interstrand NH-NH (such as Tyr3-Val13), Hα-Hα (such as Ser6-Lys10) and Hα-NH interactions (such as Thr4-Val13) were prominent in NOESY spectra recorded with mixing times of 200 and 75 ms (data not shown).

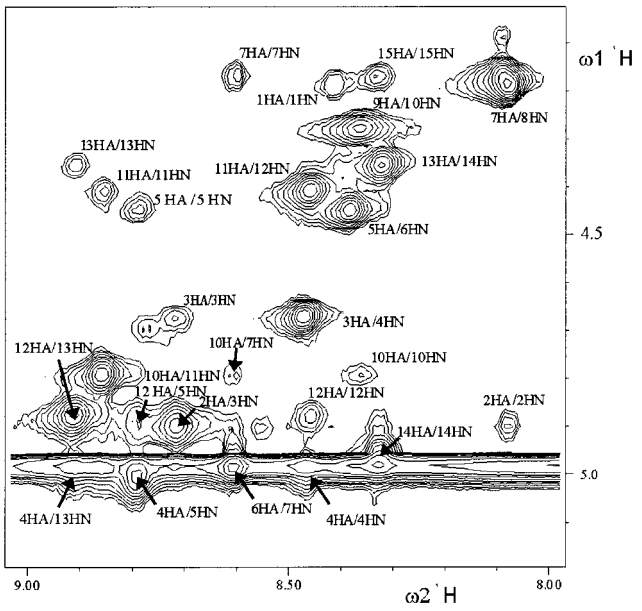


Figure 5. Portion of the fingerprint region of the 75 ms NOESY spectrum of peptide **II** in CD_3OH/H_2O (1:1) at 4 °C

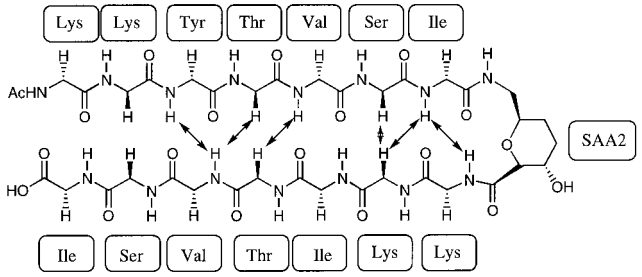


Figure 6. Observed backbone long range NOEs in peptide **II** (50% aqueous methanol, 277 °K)

The relevant NOEs in the turn region of peptide **I**, compared to the corresponding region in peptide **II**, are shown in Figure 7. The same NOEs were observed for both peptides, demonstrating that the backbone conformation of the peptide containing the sugar amino acid is similar to that of the Asn-Gly β-turn.

However, the relative intensities of some of the NOEs were lower than in the corresponding peptide **I**, indicating that the introduction of the sugar amino acid in the turn region causes subtle changes in the original structure. Thus, the NMR spectroscopic data taken as a whole strongly suggest that in 50% aqueous methanol the overall β-hairpin structure of peptide **I** − i.e., a β-turn flanked by two β-strands − is conserved in the sugar amino acid containing peptide **II**. While Searle and co-workers reported that β-hairpin **I** is about 30% populated in water at 278 K, direct comparison of the NMR spectra of **I** and **II** in water indicates that peptide **II** is unfolded under these conditions.[17]

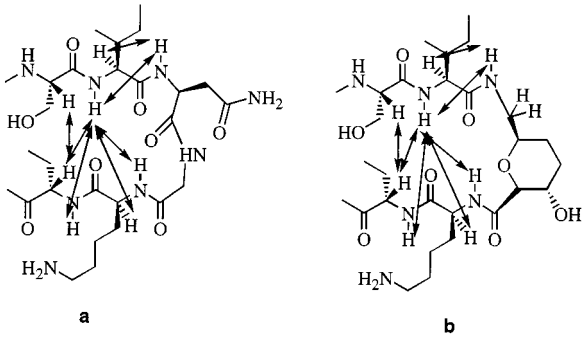


Figure 7. Relevant NOEs observed in the turn region a) peptide **I**; b) modified peptide **II**

Thus, the natural sequence is more stable than the analogue containing SAA-**2**.

Conclusions

A novel sugar amino acid building block **8** has been successfully synthesized and incorporated into a polypeptide chain by means of Fmoc chemistry, using an automated peptide synthesizer. The new peptide **II** has the same amino acid sequence as the β-hairpin peptide **I**, except for the residues involved in the turn (NG) which have been replaced by the sugar amino acid SAA**2**.

As ascertained by NMR experiments, peptide **II** is unfolded in water, but folds upon addition of methanol. The structure of peptide **II** was studied by circular dichroism and NMR spectroscopy in 50% aqueous methanol. In this solvent, the CD spectrum of **II** clearly differs from what would be expected for a random coil peptide and is similar to that observed for the β-hairpin peptide **I** under the same conditions. Analysis of the Hα chemical shift deviation from random coil values in modified peptide **II** has the characteristic pattern expected for residues in a β-strand conformation. Moreover, the presence of strong sequential $d_{\alpha N}$ NOEs (and weak d_{NN} NOEs) together with long range NOEs is conclusive evidence for a β-hairpin structure in the solvent mixture studied. The sugar amino acid SAA**2** can therefore be regarded as a β-turn mimic. To the best of our knowledge, peptide **II** (Ac-KKYTVSI-SAA**2**-KKITVSI) constitutes the first non-cyclic β-hairpin structure containing a sugar amino acid. The conformational behavior and biological relevance of peptides containing one or more sugar amino acids of various design and at predetermined positions is at present under investigation and will be reported in due course.

Experimental Section

Synthesis of the Sugar Amino Acid Building Block: TLC analysis was performed on Merck plastic silica gel 60 F_{254} plates, with detection by means of UV absorption (254 nm) and/or charring with ammonium molybdate (25 g/L) and ceric ammonium sulfate (10 g/L) in 10% aq. H_2SO_4. − Column chromatography was performed with Baker silica gel (0.063−0.200 mm). − Ethyl acetate (EtOAc)

and petroleum ether 60−80 (PE) were of technical grade and distilled before use. CH_2Cl_2 (p.a. Baker) and DMF (p.a. Baker) were stored over molecular sieves (4 Å). All reactions were performed under nitrogen atmosphere unless otherwise indicated. − For sugar derivatives, ^1H NMR spectra were recorded on a Bruker WM 300 (300 MHz) and ^{13}C NMR spectra on a Bruker AC 200 (50 MHz). Samples were measured in $CDCl_3$, using TMS as internal standard for ^1H NMR and $CDCl_3$ as internal standard for ^{13}C NMR. − Optical rotations were measured on a Propol automatic polarimeter (sodium D line, λ = 589 nm). − Mass spectra were performed using a JEOL JMS-SX/XS 102A coupled to a JEOL MS-MP7000.

5,7-Di-*O*-acetyl-2,6-anhydro-3,4-dideoxy-D-*arabino*-hept-3-enonitrile (4α) and 5,7-Di-*O*-acetyl-2,6-anhydro-3,4-dideoxy-D-*ribo*-hept-3-enonitrile (4β): Tri-*O*-acetyl-D-glucal (27.23 g, 100 mmol), coevaporated with 1,2-dichloroethane (2 × 25 mL), was dissolved in CH_2Cl_2 (380 mL), cooled to 0 °C, and treated with trimethylsilyl cyanide (14.75 mL, 110 mmol) and boron trifluoride diethyl etherate (3.55 mL, 28 mmol). The reaction mixture was stirred for 10 min, quenched with sat. aq. $NaHCO_3$ (300 mL), and diluted with diethyl ether (100 mL). The water layer was extracted with diethyl ether (2 × 100 mL). The combined organic phases were washed with sat. aq. $NaHCO_3$ (250 mL) and brine (250 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The crude product was triturated with a mixture of EtOAc/PE (1:1, 200 mL) to precipitate a proportion of the α-anomer. The mother liquid was purified by column chromatography on silica gel (PE/EtOAc 5:1) to give the α and β anomers in pure form, together with some mixed fractions. The mixed fractions were again triturated with EtOAc/PE (1:1) to precipitate the α anomer and the procedure was repeated until both anomers were completely separated. In this way, 14.1 g, (59%) of **4α** and 8.8 g (37%) of **4β** were obtained.

Compound 4α: ^1H NMR ($CDCl_3$): δ = 2.2−2.0 (br. s, 6 H, acetyl), 4.04 (dt, *J* = 9.1 Hz, *J* = 3.7 Hz, 1 H, 6-H), 4.27 (d, *J* = 3.7 Hz, 2 H, 7-H, 7′-H), 5.12−5.06 (m, 1 H, 2-H), 5.35 (ddd, *J* = 9.1 Hz, 1 H, 5-H), 5.89 (ddd, *J* = 10.2 Hz, *J* = 3.3 Hz, *J* = 1.8 Hz, 1 H, 4-H), 6.05 (dt, *J* = 10.2 Hz, *J* = 1.8 Hz, 1 H, 3-H). − ^{13}C NMR ($CDCl_3$): δ = 20.4, 20.6 (Me, acetyl), 62.0 (C-7), 62.4, 63.5 (C-5, C-6), 78.1 (C-2), 115.6 (CN), 123.6, 129.3 (C-3, C-4), 169.8, 170.4 (CO acetyl).

Compound 4β: ^1H NMR ($CDCl_3$): δ = 2.10, 2.12 (2 s, 6 H, acetyl), 3.82 (ddd, *J* = 8.1 Hz, *J* = 3.0 Hz, *J* = 5.9 Hz, 1 H, 6-H), 4.20 (dd, *J* = 12.4, *J* = 5.9 Hz, 1 H, 7′-H), 4.28 (dd, *J* = 12.4, *J* = 3.0 Hz, 1 H, 7-H), 5.14−5.12 (m, 1 H, 2-H), 5.30 (dq, *J* = 8.1 Hz, 1 H, 5-H), 5.93 (dt, *J* = 10.3 Hz, *J* = 1.8 Hz, 1 H, 4-H), 6.05 (dt, *J* = 10.3 Hz, *J* = 2.5 Hz, 1 H, 3-H). − ^{13}C NMR ($CDCl_3$): δ = 20.0, 20.2 (Me, acetyl), 62.0 (C-7), 62.4, 62.9 (C-5, C-6), 73.7 (C-2), 115.5 (CN), 123.9, 127.9 (C-3, C-4), 169.3, 169.9 (CO acetyl).

2,6-Anhydro-7-[*N*-(9-fluorenylmethoxycarbonyl)amino]-4,5,7-trideoxy-L-*ribo*-heptitol (5): Aqueous HCl (1 M, 7.2 mL) was added to a suspension of **4β** (1.55 g, 6.48 mmol) and 10% Pd/C (157 mg) in a mixture of EtOAc/2-propanol/ethanol (2:1:1, 39 mL), and the reaction mixture was shaken for 48 hours under a hydrogen atmosphere (40 Psi). The catalyst was removed by filtration over Hyflo, and the solvent was concentrated under reduced pressure. The residue was coevaporated with toluene (2 × 25 mL) and dissolved in MeOH (70 mL). *t*BuOK (390 mg, 3.47 mmol) was added and the reaction mixture was stirred at 20 °C for 1 h. The mixture was neutralized with Dowex-H^+, the resin was removed by filtration, and the solvent was concentrated under reduced pressure. $NaHCO_3$ (2.18 g, 25.95 mmol) and Na_2CO_3 (1.37 g, 12.93 mmol) were added to a solution of the residue in water (60 mL) at 0 °C. A solution of Fmoc-OSu (3.3 g, 9.76 mmol) in dioxane (60 mL) was added dropwise and the reaction mixture was stirred at 20 °C for 1 h. The mixture was neutralized with 1 M HCl, and the aqueous phase was extracted with EtOAc (3 × 100 mL). The combined organic layers were dried ($MgSO_4$) and the solvent was evaporated. The residue was purified by column chromatography (3% MeOH in CH_2Cl_2), to give **5** (1.60 g, 63%). $[\alpha]_D^{20}$ = −4.8 (*c* = 1, CH_2Cl_2). − HRMS (FAB+): calcd. for $C_{22}H_{26}NO_5$ (M + H) 384.1811; found 384.1808. − ^1H NMR ($CDCl_3$): δ = 1.30−1.56, 1.67−1.72, 2.05−2.22 (m, 4 H, 5-H, 5′-H, 4-H, 4′-H), 3.05−3.14, 3.19−3.24, (m, 2 H, 3-H, 2-H), 3.37−3.57 (m, 3 H, 7-H, 7′-H, 6-H), 3.83 (br. s, 2 H, 1-H, 1′-H), 4.22 (t, *J* = 6.6 Hz, 1 H, 9-H (fluorenyl)), 4.41−4.52 (m, 2 H, OCH_2-fluorenyl), 5.17 (t, *J* = 6.1 Hz, 1 H, NH), 7.25−7.43, 7.58−7.61, 7.76−7.78, (m, 8 H, Ar). − ^{13}C NMR ($CDCl_3$): δ = 27.7, 31.5 (C-5, C-4), 45.1 (O-CH_2-fluorenyl), 47.0 (CH-fluorenyl), 60.3 (C-1), 66.4 (C-7), 66.5, 76.1 (C-3, C-2), 81.5 (C-6), 119.8, 124.8, 126.9, 127.5 (aromatics), 141.1 (C-*ipso*), 143.7 (C-*ipso*), 156.7 (CO).

2,6-Anhydro-1,3-di-*O*-*tert*-butyldimethylsilyl-7-[*N*-(9-fluorenylmethoxycarbonyl)amino]-4,5,7-trideoxy-L-*ribo*-heptitol (6): Compound **5** (1.60 g, 4.17 mmol) was coevaporated with 1,2-dichloroethane (2 × 10 mL), and dissolved in dry DMF (10 mL) under Ar. Subsequently, imidazole (1.1 g, 16.9 mmol) and *tert*-butyldimethylsilyl chloride (2.65 g, 9.53 mmol) were added and the reaction mixture was stirred at 20 °C for 3 h. The reaction was quenched with MeOH, diluted with EtOAc (100 mL), and washed with saturated $NaHCO_3$ (2 × 100 mL). The organic phase was washed with ice/water (4 × 100 mL), dried ($MgSO_4$), and evaporated to dryness. The residue was purified by silica gel chromatography (PE/EtOAc 15:1→6:1) to give **6** (2.47 g, 97%). $[\alpha]_D^{20}$ = +14.4 (*c* = 1, $CHCl_3$). − HRMS (FAB+): calcd. for $C_{34}H_{54}NO_5Si_2$ (M + H) 612.3541; found 612.3540. − ^1H NMR ($CDCl_3$): δ = 0.06 (s, 6 H, CH_3-Si), 0.88 (s, 9 H, *t*Bu−Si), 0.91 (s, 9 H, *t*Bu−Si), 1.28−1.50, 1.61−1.73, 2.97−1.03 (m, 4 H, 5-H, 5′-H, 4-H, 4′-H), 3.03−3.17 (m, 2 H, 3-H, 2-H), 3.41−3.53 (m, 3 H, 7-H, 7′-H, 6-H), 3.72 (dd, *J* = 5.2, *J* = 11.2 Hz, 1 H, 1′-H), 3.85 (dd, *J* = 1.6, *J* = 11.2 Hz, 1 H, 1-H), 4.22 [t, *J* = 7.0 Hz, 1 H, 9-H (fluorenyl)], 4.33−4.41 (m, 2 H, OCH_2-fluorenyl), 5.20 (t, *J* = 6.6 Hz, 1 H, NH), 7.25−7.75, 7.75−7.77, 7.77−7.78 (m, 8 H, Ar). − ^{13}C NMR ($CDCl_3$): δ = −5.17 (CH_3-Si), −4.9 (CH_3-Si), −4.3 (CH_3-Si), 17.8 (C-Si), 18.3 (C-Si), 25.8 (CH_3-*t*Bu), 27.8, 32.6 (C-5, C-4), 45.2 (O-CH_2-fluorenyl), 47.1 (CH-fluorenyl), 60.1 (C-1), 66.4 (C-7), 66.3, 75.5 (C-3, C-2), 82.7 (C-6), 119.8 125.0, 126.9, 127.5 (aromatics), 141.2 (C-*ipso*), 143.9 (C-*ipso*), 156.4 (CO).

2,6-Anhydro-3-*O*-*tert*-butyldimethylsilyl-7-[*N*-(9-fluorenylmethoxycarbonyl)amino]-4,5,7-trideoxy-L-*ribo*-heptitol (7): Compound **6** (1.22 g, 1.99 mmol) was treated with a mixture of AcOH/H_2O/THF 13:7:3 (13 mL) over 36 hours at 4 °C. The mixture was neutralized with sat. aq. $NaHCO_3$. The organic layer was separated and the aqueous phase was extracted with EtOAc (3 times). The combined organics were washed with water, dried ($MgSO_4$), and evaporated to dryness. The residue was purified by column chromatography (PE/EtOAc 3:1→0:1) to give **7** (0.88 mg, 88%). $[\alpha]_D^{20}$ = +11.4 (*c* = 1, $CHCl_3$). − HRMS (FAB+): calcd. for $C_{28}H_{40}NO_5Si$ (M + H) 498.2676; found 498.2654. − ^1H NMR ($CDCl_3$): δ = 0.05 (s, 6 H, CH_3-Si). 0.87 (s, 9 H, *t*Bu−Si), 1.08−1.55, 1.68−1.72, 2.05−2.26 (m, 4 H, 5-H, 5′-H, 4-H, 4′-H), 3.10 (m, 1 H, 3-H), 3.47−3.49, 3.64−4.06 (m, 4 H, 7-H, 7′-H, 6-H, 2-H), 4.22 [t, *J* = 6.7 Hz, 1 H, 9-H (fluorenyl)], 4.37−4.54 (m, 2 H, OCH_2−fluorenyl), 5.36 (br. s, 1 H, NH), 7.17−7.41, 7.58−7.60, 7.74−7.76 (m, 8 H, Ar). − ^{13}C NMR ($CDCl_3$): δ = −5.0 (CH_3-

Si), −4.3 (CH_3-Si), 17.7 (C-Si), 25.7 (CH_3-*t*Bu), 27.7, 32.4 (C-5, C-4), 45.1 (O-CH_2-fluorenyl), 47.1 (CH-fluorenyl), 62.5 (C-1), 67.2 (C-7), 67.3, 76.1 (C-3, C-2), 82.0 (C-6), 119.8, 124.9, 126.9, 127.5 (aromatics), 141.1 (C-*ipso*), 143.8 (C-*ipso*), 156.6 (CO).

2,6-Anhydro-3-*O*-*tert*-butyldimethylsilyl-7-[*N*-(9-fluorenylmethoxycarbonyl)amino]-4,5,7-trideoxy-L-*ribo*-heptanoic Acid (8): A solution of DMSO (2 M in CH_2Cl_2, 1 mL) was added to a cooled (−78 °C) solution of oxalyl chloride (132 μL, 1.5 mmol) in CH_2Cl_2 (2 mL). After stirring for 5 min, a solution of alcohol **7** (362 mg, 0.72 mmol), was added dropwise. Stirring was continued for 30 min, after which triethylamine (501 μL, 3.60 mmol) was added. The reaction mixture was allowed to warm to −10 °C over 1 h. The mixture was diluted with EtOAc (100 mL), washed with water (50 mL), sat. aq. $NaHCO_3$ (50 mL), and water (50 mL), dried ($MgSO_4$), and concentrated to dryness. The crude product was dissolved in a *t*BuOH/water mixture (1:1, 26.50 mL), NaH_2PO_4 (662 mg, 4.80 mmol), $NaClO_2$ (578 mg, 6.39 mmol), and 2-methyl-2-butene (4.9 mL, 46.60 mmol) were added, and the reaction mixture was stirred at 20 °C for 2 h. The mixture was diluted with EtOAc (50 mL). The organic phase was separated and the water layer was extracted with EtOAc (3 × 50 mL). The combined organics were dried ($MgSO_4$) and concentrated. The residue was purified by silica gel column chromatography (PE/EtOAc 5:1→2:1) to give **8** (333 mg, 90%). $[\alpha]_D^{20} = +4.6$ (c = 1, CH_2Cl_2). − HRMS (FAB+): calcd. for $C_{28}H_{38}NO_6Si$ (M + H) 512.2468; found 512.2475. − ^{1}H NMR ($CDCl_3$): δ = 0.06 (s, 6 H, CH_3-Si), 0.88 (s, 9 H, *t*Bu-Si), 1.32−1.56, 1.63−1.70, 1.98−2.11 (m, 4 H, 5-H, 5′-H, 4-H, 4′-H), 3.04−3.13, 3.17−3.23, (m, 2 H, 3-H, 2-H), 3.37−3.52 (m, 3 H, 7-H, 7′-H, 6-H), 3.65 (m, 1 H, 1′-H), 3.82 (m, 1 H, 1-H), 4.21 [t, J = 6.7 Hz, 1 H, 9-H (fluorenyl)], 4.40−4.52 (m, 2 H, OCH_2-fluorenyl), 5.22 (t, J = 5.7 Hz, 1 H, NH), 7.26−7.42, 7.58−7.60, 7.75−7.76 (m, 8 H, Ar). − ^{13}C NMR ($CDCl_3$): δ = −5.1 (CH_3-Si), −4.5 (CH_3-Si), 17.7 (C-Si), 25.5 (CH_3-*t*Bu), 27.0, 32.4 (C-5, C-4), 44.8 (O-CH_2-fluorenyl), 47.0 (CH-fluorenyl), 66.7 (C-7), 68.7, 76.4, (C-3, C-2), 81.2 (C-6), 119.8, 124.6, 124.9, 126.9, 127.5 (aromatics), 141.1 (C-*ipso*), 143.7 (C-*ipso*), 173.9 (COOH), 156.6 (CONH).

Synthesis of Peptides: All solvents used in the automated peptide synthesis were of peptide synthesis grade and were purchased from Biosolve. Semipreparative RP HPLC was performed on an Alltima® C_{18} column (10 mm × 250 mm, 5 μm particle size, AllTech) at 23 mL/min (BioCAD "Vision" automated HPLC system). Analytical RP HPLC was performed using an Alltima® C_{18} column (4.6 × 150 mm, 5 μm particle size, AllTech) at 1 mL/min. Detection was performed at 214 nm (Jasco UV detector) or at 214 nm and 280 nm simultaneously (BioCAD "Vision"). Electrospray mass spectra were recorded using a Perkin−Elmer SCIEX API 165 Single Quadrupole LC/MS instrument. MALDI-TOF mass spectra were recorded on a Voyager DE-RP instrument (PerSpetive Biosystem) equipped with a 337 nm UV nitrogen laser producing 3 nm pulses in a linear mode.

Peptides **I** and **II** were synthesized on an ABI 433A (Applied Biosystems, division of Perkin−Elmer) peptide synthesizer employing the FastMoc® peptide synthesis technique (50 μm scale). Tentagel-S was used as solid support (0.26 mmol/g), to which Fmoc-Ile-OH was attached through the very acid-labile HMPB [4-(4-hydroxymethyl-3-methoxyphenoxy)butyric acid] linker.

The standard Fmoc-amino acids used in the synthesis were as follows: Fmoc-Ile-OH, Fmoc-Ser(O*t*Bu)-OH, Fmoc-Val-OH, Fmoc-Tyr(O*t*Bu)-OH, Fmoc-Lys(Boc)-OH, and Fmoc-Asn(Trt)-OH.

The couplings were performed in NMP, using BOP/HOBt/DIPEA. Fmoc-Val-OH and the sugar amino acid building block **8** were incorporated using double coupling cycles. The other residues were incorporated in single cycles (coupling time 1 h). Deprotection of the Fmoc group was performed with 20% piperidine in NMP. Fmoc deprotection was monitored online by UV detection. Final acetylation was carried out in a solution of 0.5 M acetic anhydride, 0.125 M DIPEA and 0.2% 1−hydroxybenzotriazole (HOBt) in NMP. The peptide was cleaved from the resin by treatment with a mixture of TFA:H_2O:triisopropylsilane (TIS) 95:2.5:2.5 at 20 °C for 2 h. After evaporation of the reaction mixture, the peptides were purified by RP HPLC (buffer A: 5% CH_3CN in 0.1% aq. TFA; buffer B: 75% CH_3CN in 0.1% aq. TFA). Purified samples were analyzed by analytical HPLC and mass spectrometry. Peptide **II**: MALDI-TOF m/z = 1807.24 (calculated 1806.9).

Table 1. Chemical shifts of peptide **I** in CD_3OD/H_2O 1:1 at 4 °C

Residue	NH	αH	βH	other
K1	8.42	4.20	1.63 1.57	γCH_2 1.23, 1.33 δCH_2 1.60, 1.60 εCH_2 2.84, 2.84 εNH_3 7.66
K2	8.08	4.90	1.42 1.51	γCH_2 1.29, 1.21 δCH_2 1.51, 1.51 εCH_2 2.80, 2.80 εNH_3 7.58
Y3	8.66	4.68	2.84 2.69	δH = 6.85 εH 6.53
T4	8.45	5.02	3.77	γCH_3 0.98
V5	8.78	4.45	1.93	γCH_3 0.78, 0.75
S6	8.38	4.97	3.49 3.49	
I7	8.61	4.18	1.79	γCH_2 1.01 γCH_3 0.75 δCH_3 0.72
SAA**2**	8.09	3.40		2-H 3.28 3-H 3.02 4-H 1.43, 1.32 5-H 1.64, 1.64 6-H 3.69
K9	7.82	4.29	1.78, 1.78	γCH_2 1.36, 1.28 δCH_2 1.59, 1.66 εεCH_2 2.85, 2.85 εNH_3 7.69
K10	8.34	4.79	1.62 1.62	γCH_2 1.35, 1.20 δCH_2 1.45, 1.45 εCH_2 2.82 εNH_3 7.58
I11	8.86	4.40	1.68	γCH_2 1.30, 0.99 γCH_3 0.74 δCH_3 0.82
T12	8.47	4.86	3.76	γCH_3 0.99
V13	8.90	4.34	1.73	γCH_3 0.74, 0.66
S14	8.32	4.96	3.66 3.55	
I15	8.33	4.17	1.77	γCH_2 1.33, 1.07 γCH_3 0.78 δCH_3 0.67

CD Measurements: Circular dichroism (CD) spectra were recorded at 4 °C on a Jasco spectropolarimeter. Stock solutions (1 mM) of peptides **I** and **II** in methanol/H_2O (1:1) were prepared, and samples for CD measurements were prepared by diluting the stock solutions with water/MeOH (1:1) to give 7.5 μM solutions. All samples were analyzed in 0.1 cm pathlength quartz cells, and a solvent blank was subtracted from each peptide spectrum. Each spectrum was obtained by averaging five scans in the 250−190 nm wavelength range, using a scan speed of 10 nm/min, a bandwidth of 1.0 nm, a resolution of 0.2 nm, and a response time of 2s.

NMR Measurements: For the peptides, all spectra were recorded at 4 °C on a Bruker DMX 600 spectrometer equipped with a pulsed field gradient accessory either in $H_2O/D_2O/CD_3OH$ (45:5:50) or in D_2O/CD_3OD (50:50), Table 1. Standard DQF-COSY (512c × 2048c) and TOCSY (400c × 2048c) spectra were recorded using presaturation for solvent suppression. NOESY spectra (400c × 2048c, τ_{mix} = 75, 200 ms) were recorded using the WATERGATE solvent suppression.[18] All spectra were recorded in phase-sensitive mode, using either the TPPI or States-TPPI for quadrature detection in the indirect dimension.

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[17] The lack of secondary structure for peptide **II** in water might suggest that SAA−**2**, unlike dipeptide Asn-Gly, is not predisposed to nucleate hairpin folding in this solvent. On the other hand, we observed that, under conditions that promote interstrand hydrogen bonding (50% aqueous methanol), the replacement of the Asn-Gly turn for SAA-**2** is compatible with a β-hairpin conformation. To investigate these aspects further, other modified peptides will be studied in the near future.

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