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

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Peptides and proteins display an extraordinary structural diversity and are instrumental in numerous biological events. Correct folding of these biomolecules is imperative for their functioning. Key components contributing to the overall folding are secondary structure elements such as helices, sheets, and turns.[1] Adoption of nonproteinogenic residues or sequences has appreciably contributed to further our understanding of the factors that are at the basis of secondary structure. Besides mimicking spatial arrangements found in polypeptides, peptide-like molecules have been designed with the aim to enhance resistance to proteolytic activity, to attain structural stabilization, and to introduce additional functionalization sites.[2] Synthetic peptide analogues are now widely recognized as important lead compounds, both in the development of new materials[3] and in the generation of therapeutic agents.[4]

Increasing research efforts have been devoted to the study of reverse turns. In this common motif, the polypeptide chain reverses its overall direction. The γ- and β-turns describe three and four consecutive residues, respectively, in which the C═O of the first residue i is H-bonded to the NH of residue $i + 2$ or $i + 3$. Further classification of the turn motifs can be made on the basis of their peptide backbone geometry with specific angular and torsional parameters.[5] Factors influencing turn motifs include hydrophobic interactions, conformational bias, side chain participation, and intra- and interresidue interactions.

Recently, sugar amino acids (SAAs), carbohydrate derivatives featuring an amine and a carboxylic acid, have emerged as a promising new class of peptidomimetics.[6] Oligopeptides containing SAA building blocks have been assembled with the aim to improve their biostability. Furthermore, examples of these structurally and functionally diverse molecular scaffolds have been found to induce well-defined secondary structures in oligomeric constructs, including reverse turns.[7,8] An attractive feature of the use of carbohydrate-based peptidomimetics as turn motifs is the presence of additional functionalities on the furanose or pyranose core stemming from the parent sugar, enabling further functionalization. For instance, Smith et al. demonstrated the incorporation of a pyranoid SAA as β-turn inducer in a heptapeptide corresponding to the C-terminus of the R2 subunit of mammalian ribonucleotide reductase. The remaining hydroxyl functions were equipped with methylene carboxylate (mimicking aspartic acid) and isobutyl (mimicking leucine) functionalities, resulting in an artificial ensemble that closely resembles the native peptide sequence.[9] Notably, the residual functionalities present at the parent core of SAAs may also prohibit the formation of the targeted secondary structural motif. The latter is exemplified by the finding of Chakraborty and co-workers that the incorporation of furanoid SAA **1** (Scheme 1) in short linear peptide sequences does not lead to regular β-turn structures.[10] Instead, one of the hydroxyl functionalities (C_3−OH) on the furanoid SAA proved to be actively involved in stabilizing the observed secondary structure by acting as hydrogen acceptor.

Our focus in the area of peptidomimetics is directed at the determination of the structural consequences of incorporating SAA building blocks in selected oligopeptides. Ultimately, we aim to attain tailor-made peptidomimetic building blocks able to induce the desired secondary structure combined with the opportunity to introduce extra functionalities on the turn region. To this end, we have selected gramicidin S (GS), a cyclic decapeptide antibiotic with the primary sequence *cyclo*-(Pro−Val−Orn−Leu−^{D}Phe)$_2$, as a suitable model peptide. GS adopts a C_2-symmetric amphiphilic antiparallel β-sheet structure[11] with two type II′ β-turns with ^{D}Phe and Pro at positions $i + 1$ and $i + 2$, respectively, and is widely recognized as a good system to study the effect of potential artificial reverse turn inducers.[12] We here report the in-depth study, through NMR and X-ray analysis, of synthetic GS analogue **7**, with SAA **1** as a replacement of one of the ^{D}Phe−Pro dipeptides in GS. NMR and crystallographic analysis of **7** revealed the involvement of C_3−OH in the final overall secondary structure by inducing an unprecedented turn motif. The implications of this secondary structure element on the overall structure, as well as oligomeric assemblies of **7**, are disclosed.

The synthesis of cyclic peptide **7** was accomplished as follows (Scheme 1). Fmoc−Leu−OH was condensed with the 4-(4-hydroxymethyl-3-methoxyphenoxy)-butyric acid (HMPB)-functionalized 4-methylbenzhydrylamine (MBHA)-resin under the agency of *N*,*N*′-diisopropylcarbodiimide (DIC) and a catalytic amount of 4-(dimethylamino)pyridine (DMAP) to furnish **3**. Standard Fmoc-based solid-phase peptide synthesis (condensating agents; Castro's reagent,[13] *N*-hydroxybenzotriazole (HOBt) and *N*,*N*′-diisopropylethylamine (DiPEA), Fmoc cleavage; 20% piperidine in NMP) using the appropriate amino acid building blocks (Fmoc−Orn(Boc)−OH, Fmoc−Val−OH, Fmoc−^{D}Phe−OH, Fmoc−Pro−OH, and Fmoc−Leu−OH) followed by analogous condensation of azido acid **2** furnished immobilized nonapeptide **4**. At this stage, the azide functionality was converted to the corresponding amine employing Staudinger reduction conditions (PMe_3, 1,4-dioxane, and H_2O). Mild acidic cleavage from the resin (1% TFA in CH_2Cl_2) afforded the partially protected linear peptide which was directly cyclized using Castro's reagent, HOBt, and DiPEA under highly dilute conditions. The thus obtained cyclic peptide was purified by size-exclusion chromatography (Sephadex LH-20) to furnish the homogeneous target compound **5** in 96% overall yield, based on **3**. Removal of the pivaloyl- and Boc-protecting groups (1% NaOMe in MeOH and 50% TFA in CH_2Cl_2, respectively) and

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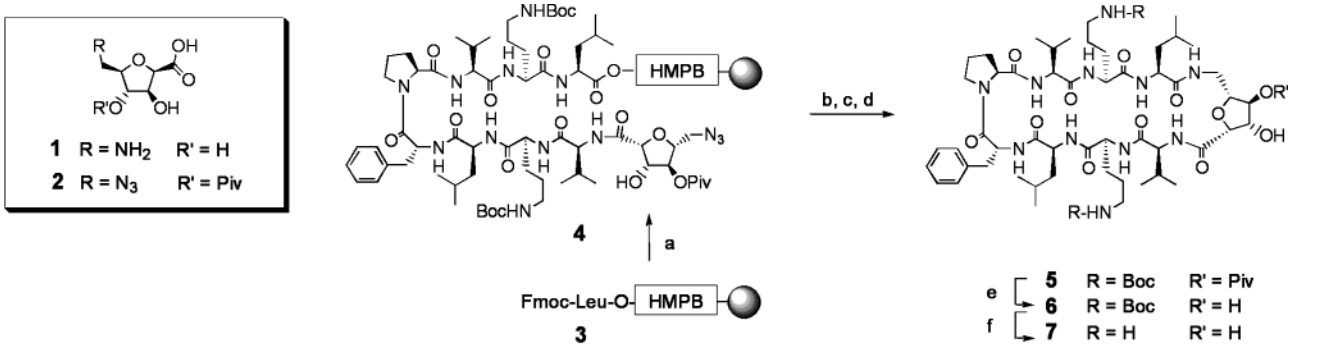
Scheme 1. Synthesis of GS Analogue **7**[a]



[a] (a) Sequential coupling (Xaa or **2**, BOP, HOBt, DiPEA) and deprotection (piperidine/NMP 1/4 v/v) steps; (b) PMe_3, 1,4-dioxane, H_2O; (c) TFA/DCM (1/99 v/v); (d) BOP, HOBt, DiPEA; (e) NaOMe, MeOH; (f) TFA/DCM (1/1 v/v).

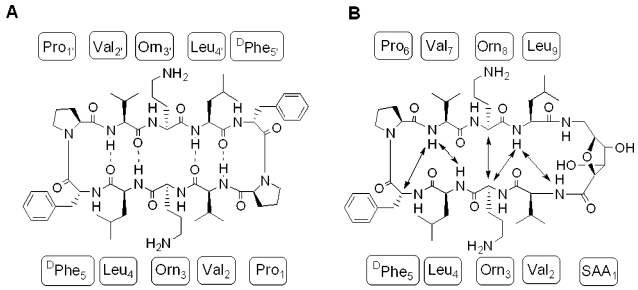


Figure 1. (A) GS; (B) observed long-range NOEs for **7**.

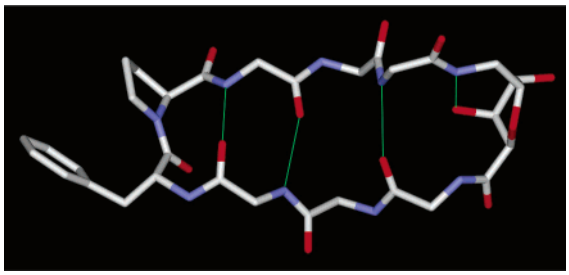


Figure 2. Pleated sheet structure of **7** with the intramolecular H-bonds depicted in green. Water molecules, Leu-, Val-, and Orn-side chains as well as hydrogens were omitted for clarity.

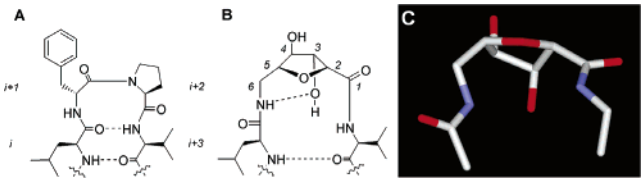


Figure 3. (A) Turn region of GS; (B) turn region of **7**; (C) crystal structure of turn region of **7**. Side chains and hydrogens were omitted for clarity.

subsequent reversed-phase HPLC purification finally gave GS analogue **7** in 59% yield.

The ^{1}H NMR resonance assignment of peptide **7** was accomplished using a combination of COSY, TOCSY, and ROESY data sets. Subsequently, we compared the thus obtained structural information with the antiparallel β-sheet structure adopted by GS.[11] The structure of GS is characterized, apart from the two ^{D}Phe−Pro turn regions, by four H-bonds, two shared between the Leu_4 and $Val_{2'}$ residues and two between the $Leu_{4'}$ and Val_2 residues (Figure 1A). Besides numerous short-range NOEs, the observation of interstrand NH−NH (Val_2−Leu_9 and Val_7−Leu_4), NH−Hα (Val_7−^{D}Phe$_5$ and Leu_9−Orn_3), and Hα−Hα (Orn_3−Orn_8) NOEs in **7** confirms the preservation of the overall β-sheet structure and indicates the presence of three H-bonds (Figure 1B).[14] However, a strong NH−NH NOE between SAA_1−NH and Val_2−NH was observed, indicating their close proximity. The latter observation strongly suggests that residue SAA_1 does not induce a regular β-turn conformation.

With the aim to create a better understanding of the overall structure and the implications of the introduction of SAA **1** in one of the turn regions of GS, we obtained and analyzed crystallographic data of **7**. To this end, a solution of **7** in a 1:1 mixture of MeOH and H_2O in the presence of spermidine tri-HCl (or 1,5-diaminopentane di-HCl) was allowed to evaporate slowly under oil. The resulting needle-shaped crystals were subjected to X-ray diffraction analysis. The structure was solved and refined to 1.2 Å resolution (the diffraction limit of the crystals).

As can be seen in Figure 2, peptide **7** adopts a pleated sheet structure with two H-bonds shared between the Leu_4 and Val_7 residues and one between Leu_9−NH and Val_2−carbonyl similar to that reported for GS, but with a larger right-handed twist[15] in the overall β-sheet structure. Interestingly, the SAA residue induces an unusual turn structure with the C_3−OH in close proximity to the SAA_1−NH (Figure 3). The protrusion of this hydroxyl function into the turn region, enabled by the C_3-*endo* conformation[16] adopted by the furanose moiety, allows it to function as a H-bond acceptor. The structure that results from formation of a H-bond with SAA_1−NH is in full agreement with the data obtained from the NMR studies. As a consequence, the amide bond linking residues Leu_9 and SAA_1 is flipped, causing the SAA_1−NH to extend into the turn region leading to a novel secondary structure with a H-bond between a side chain functionality and the amide NH of the synthetic dipeptide isostere incorporated (Figure 3). The structure adopted by SAA **1** in compound **7** constitutes, to our knowledge, an unprecedented turn structure.

Perusal of the molecular packing of **7** reveals the presence of cyclic assemblies of six crystallographically equivalent molecules, with the hydrophilic Orn side chain residues extending into the core and the Val, Leu, and ^{D}Phe residues forming a hydrophobic periphery (Figure 4A). The structure is stabilized by intermolecular H-bonds between SAA_1−C═O and Orn_3−NH of one β-sheet with Orn_8−NH and Pro_6−C═O of the next, respectively (Figure 4B). This results in a novel hexameric β-barrel-like structure corresponding to a 12-stranded β-barrel of approximately 13 Å height (the length of the unit cell *c*-axis).

It has been reported that the parent cyclodecapeptide GS itself adopts oligomeric structures of a different nature. X-ray analysis of a crystal structure of a GS−urea−water complex revealed channel-like structures composed of six crystallographically equivalent GS molecules assembled in a double spiral of two left-handed

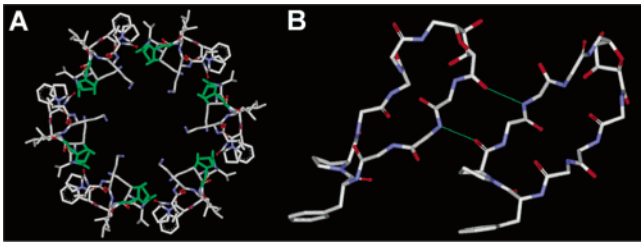


Figure 4. (A) Top view of the hexameric assembly of **7** with the SAA residues highlighted in green; (B) side view of the assembly, showing two peptides **7** with intermolecular H-bonds depicted in green. Water molecules, Leu-, Val-, and Orn-side chains and hydrogens were omitted for clarity.

helices.[17] Changes in the turn region, while of relatively small consequence on the secondary structure of the cyclic peptide itself (both GS and **7** adopt a pleated β-sheet), may therefore have a profound effect on oligomeric assemblies thereof, at least in their crystal structures. Interestingly, β-barrels are found to be at the basis of the mode of action of many pore-forming proteins, including cytolytic bacterial toxins such as perfringolysin O and α-hemolysin.[18] The results presented here may therefore be of use for the future development of novel transmembrane channels and may contribute in the design of artificial β-barrel-like molecules based on cyclic peptides with applications such as bactericidal agents.[19,20]

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Supporting Information Available: Experimental procedures and spectral data for all new compounds (PDF). This material is available free of charge via the Internet at http://pubs.acs.org. CCDC-216610 contains the 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).

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