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Sugar furanoid *trans*-vicinal diacid as a γ-turn inducer: synthesis and conformational study†

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A simple method for the synthesis of a sugar furanoid *trans* vicinal diacid and its incorporation into the N-terminal tetrapeptide sequence (H-Phe-Trp-Lys-Thr-OH) to get glycopeptide 8 has been described. 2D NMR and MD simulation studies of 8 clearly show that the sugar diacid adopts a γ-turn conformation towards the N-terminus.

Biological receptors recognise peptides in a well defined, compact and predictable three-dimensional structure which is often composed of secondary structures such as β-turns[1] and γ-turns.[2] The β-turns consist of a tetrapeptide sequence inducing a reversal in chain direction in which the distance between the $C_\alpha(i)$ and the $C_\alpha(i + 3)$ is <7 Å, while the γ-turns consist of a tripeptide sequence. In general, β/γ-turns are stabilized by a hydrogen bond between C=O of residue *i* and NH of residue $(i + 3)/(i + 2)$, forming a pseudo ten-[3]/seven-membered ring.[4] These turn structures in peptides are important for understanding the molecular mechanism of peptide–protein or protein–protein interactions that enforce a specific backbone conformation.[5] In this direction, various peptidic and non-peptidic scaffolds were designed to assess their potential as β-turn[6] or γ-turn mimetics[7] although the latter are relatively rare. Among various peptide mimetics, furanoid sugars[8] have gained much acclaim in DNA/RNA due to the puckered conformation of the furanose ring. Moreover, sugar furanoid frameworks are found to be inducers of secondary structures in oligomers[9] as well as in short peptide sequences.[10] In this respect, a number of constrained amino acid analogues[11] as well as α-/β-sugar amino acids,[12] I/II (Fig. 1), are known to be stabilizers of β-turns[13] or helix inducers in homooligomers.[14] In addition, segments containing diacid functionalities, suitable for C-terminus linkages, have been explored and some of them showed parallel sheet folding between attached peptide strands. For example Gellmann *et al.* reported the synthesis of *cis*-1,2-cyclohexane dicarboxylic acid III as a linker that promotes parallel β-sheet secondary structure in water.[15] Chakraborty *et al.* have demonstrated the bi-directional elongation of sugar diacid[16] IV or its carbasugar/pyrrolidine analogue with identical peptide strands that led to the formation of a *C*2-symmetric reverse-turn mimetic or favor a pseudo β-turn.[17] Although a number of linkages containing diacids or equivalents have been explored in organic/aqueous solvents,[18] no report on the use of a sugar vicinal diacid as a promoter of secondary structures, to the best of our knowledge, is known so far. As a part of our continuous interest in sugar amino acids[19]/iminosugars[20] we report here a new sugar furanoid *trans* vicinal type diacid V (Fig. 1) and demonstrate its first applicability by incorporating it into the tetrapeptide (H-Phe-Trp-Lys-Thr-OH) N-terminus that induces γ-turns. Our efforts in this direction are depicted below.

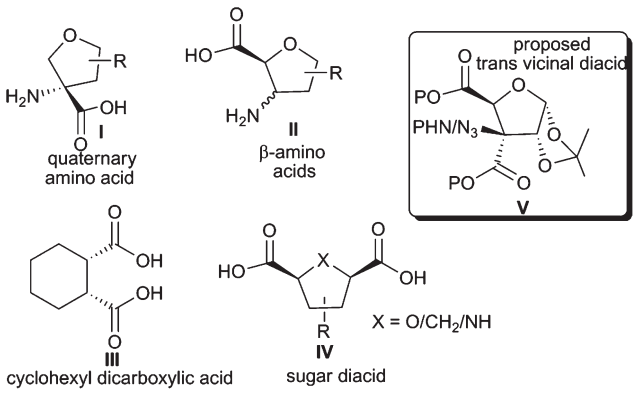

Fig. 1 Known furanoid α,β-sugar amino acids and diacids.

The required **V** was synthesized starting from the diacetonide D-glucose (Scheme 1). Thus, the PCC oxidation of

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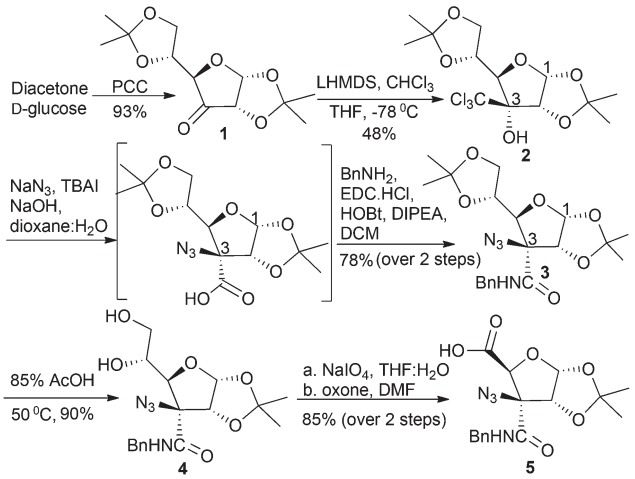
†Electronic supplementary information (ESI) available: Copies of ^1H and ^{13}C NMR, HRMS spectra of compounds **2–5**; IR of **7**; HPLC, HRMS of **8**; 2DNMR data of **8**; crystallographic data (CIF) of compounds **2** and **3**. CCDC 936034 and 936035. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c3ob41462k



Scheme 1 Synthesis of a *trans* vicinal sugar furanoid acid **5**.

diacetone D-glucose gave ketone **1**. Reaction of ketone **1** with trichloromethyl anion, generated using $CHCl_3$/LHMDS, afforded trichloromethyl alcohol **2** in 48% yield as the only diastereomer. The '*R*' configuration at the C-3 centre of **2** was confirmed by X-ray crystallography‡ (see data files in ESI†) and by comparing the analytical data with those reported.[21] Treatment of **2** with NaN_3 and NaOH (4 eq.) in dioxane–water afforded α-azido acid as is evident from the IR and ^{1}H NMR of crude product. The formation of azido acid proceeded by *in situ* formation of epoxychloride followed by the attack of NaN_3 to give azido acid chloride that was hydrolyzed to azido acid.[22,23] At this stage, crude α-azido acid was directly subjected to condensation with benzylamine using EDC–HOBt, to give amide **3**. The '*S*' absolute configuration at the newly generated stereocentre C-3 in **3** was confirmed by X-ray crystallography‡ (Fig. 2) (see data files in ESI†). The protection of C-3 acid group with benzylamine not only allowed us to determine the absolute configuration at C-3 but also gave the option of easy manipulation of the 5,6-isopropylidene group to get C-4 acid functionality that could be incorporated into the tetrapeptide so as to determine possible H-bonding and turn mimetics.

Thus, the 5,6-isopropylidene group in **3** was deprotected with 85% AcOH to give diol **4** in 90% yield. The diol **4** on oxidative cleavage using $NaIO_4$ in THF–H_2O gave an aldehyde which on oxidation with oxone[24] gave sugar acid **5**. The unique features of compound **5** are

(i) Vicinal diacid functionality offering selective protection of either of the carboxyl groups.
(ii) Presence of *cis* β-azido acid as a precursor for β-amino acid with a sugar framework.
(iii) Presence of α-azido acid functionality with a cyclic quaternary centre at C-3 as a synthon for sugar α-amino acid.

Having sugar acid **5** in hand, we planned the introduction of **5** into the N-terminus of the peptide sequence by keeping azide functionality intact to avoid the possibility of additional secondary structures. Kessler *et al.* replaced the Phe-Pro bridge of the Veberpeptide (cyclo[-Phe-Pro-Phe-D-Trp-Lys-Thr-]) with

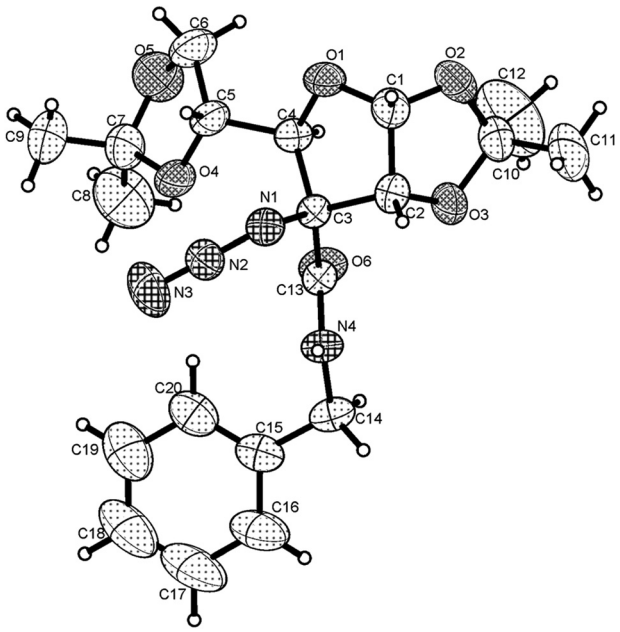
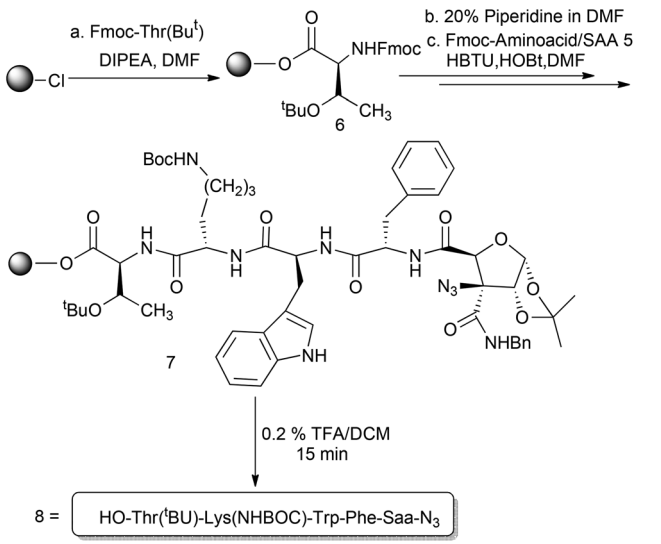


Fig. 2 ORTEP diagram of compound **3**.

trans β-sugar amino acid as a structure-inducing template to make cyclic somatostatin analogues.[25] Inspired by this observation, we incorporated the sugar acid **5** into a tetrapeptide sequence (H-Phe-Trp-Lys-Thr-OH) using 2-chlorotrityl chloride resin (0.8 mmol g^{-1}) by manual solid phase synthesis (Scheme 2). The attachment of the first amino acid, *i.e.*, Fmoc-Thr(Bu^t) (2 eq.), to a solid polymer was done using DIPEA. Fmoc deprotection was done using 20% piperidine in DMF and the coupling of succeeding amino acids, *i.e.*, Fmoc-Lys-(Boc), Fmoc-Trp and Fmoc-Phe, was done using HBTU, HOBt and DIPEA. Subsequently, sugar acid **5** was incorporated into the N-terminus of the tetrapeptide under the same coupling conditions to give resin bound glycopeptide **7**. In the next step,



Scheme 2 Solid phase synthesis of a glycopeptide.

7 was treated with 0.2% TFA in DCM to yield glycopeptide **8**, with all the protecting groups intact. The crude compound was then purified using RP-HPLC and analyzed by HRMS (S18–S20 of ESI†), yielding the glycopeptide **8** in more than 95% purity and in 30–41% yield.

In order to understand the influence of vicinal diacid linkage, conformational analysis of glycopeptide **8** was performed. We determined the solution structure of **8** by 2D NMR spectroscopy. Complete proton and carbon signal assignments of **8** were done on a 4 mM solution in CD_3CN using a combination of 2D COSY, HMBC, HSQC, NOESY and TOCSY (see data in ESI†).

The backbone has been well defined, suggesting a predominantly single conformation. Assignment of all the amide protons was done using ^{15}N HSQC spectroscopy and was found to be in the range of 5.2–9.5 ppm. The NOESY spectrum was characterized by numerous strong and medium cross peaks (Fig. 3). Sequential NOEs were observed between NHI–6CH, NHII–12CH, NHIII–23CH, NHIII–24CH_2 (weak NOE) and NHIV–32CH. In the sugar residue NOEs were observed between NHV–32CH, 35CH–38CH, 34CH–38CH, and 32CH–37CH. In addition, we observed some interresidual NOEs between NHV–19CH, 17CH–6CH, and equivalent ^{t}Bu 53/54/55CH_3–14/17/18CH, suggesting a possible secondary structure. The NOE cross peak between NHV–32CH indicated the possibility of 7-membered H-bonding formation leading to a γ-turn between NHV–31CO. In order to confirm this fact, we studied the effect of solvent on the changes in chemical shift of NH protons.

Thus, a solvent titration study of compound **8** in CD_3CN was performed using 5 μL of DMSO-d_6 for each addition. The chemical shift changes of all the amide protons are presented in a graph (S39 of ESI†). It is evident from the graph that the chemical shifts of NHI–IV, VI, and VII showed changes in shift $\Delta\delta$ in the range of 0.49–0.73 ppm suggesting their involvement in intermolecular hydrogen bonding. In contrast, the NHV showed very little changes in shift $\Delta\delta \sim 0.16$ ppm suggesting its involvement in intramolecular hydrogen bonding between NHV and 31CO, in accordance with the observed strong NOE correlation between NHV and 32CH (Fig. 4).

This fact is further substantiated by the molecular dynamics (MD) simulations for **8** that were carried out at 295 K using Schrodinger software on macromodel 9.9 implementing the OPLS_2005 force field in CD_3CN (dielectric constant 37.5). Monte Carlo conformational analyses were performed by generating 3000 starting structures, followed by minimization of these structures to a gradient of 0.05 kJ $Å^{-1}$ mol^{-1} with an RMS deviation of 0.09 kcal mol^{-1}. For minimization, ten distance restraints obtained from the NOESY experiment (S40 of ESI†) were used that generated 35 conformers out of which 15 conformations within 1 kcal mol^{-1} of the global minimum energy were considered. Superimposition of 15 lower energy structures showed the existence of 7-membered hydrogen bonding, forming an intramolecular C7 turn around NHV and 31CO at a distance of 1.9 Å (Fig. 5).

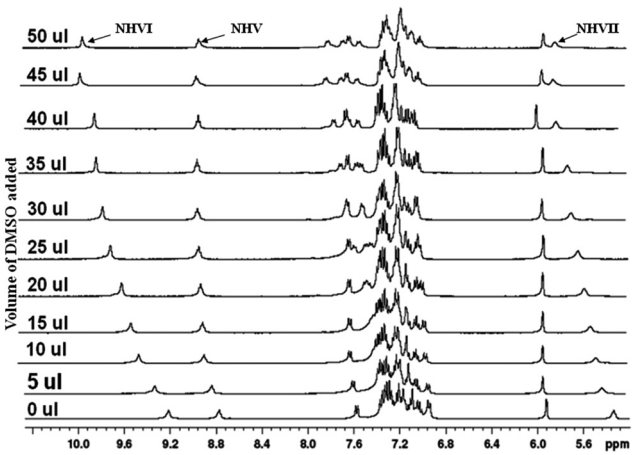


Fig. 4 DMSO titration studies.

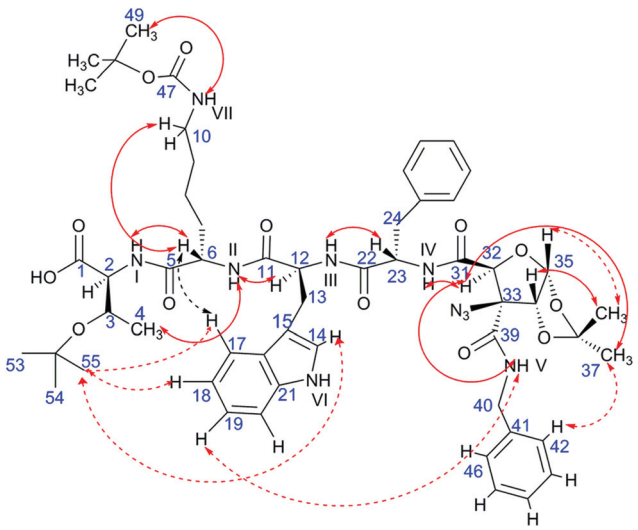


Fig. 3 NOE cross peaks in glycopeptide **8** (thick line – strong NOE; dotted line – medium NOE).

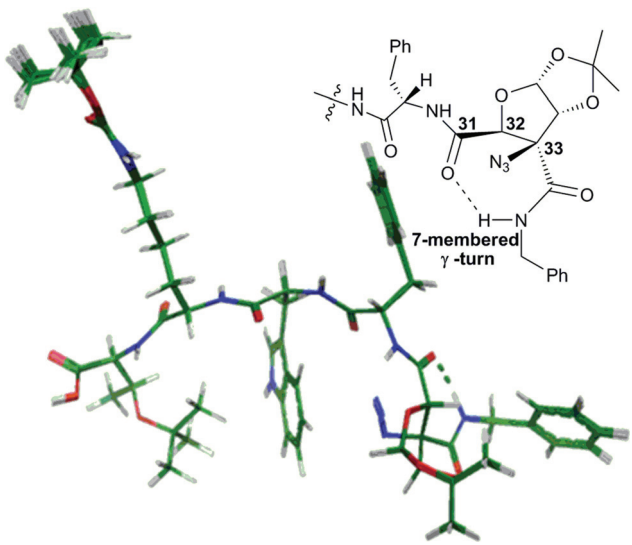


Fig. 5 Superimposed 15 lowest energy conformations forming a γ-turn.

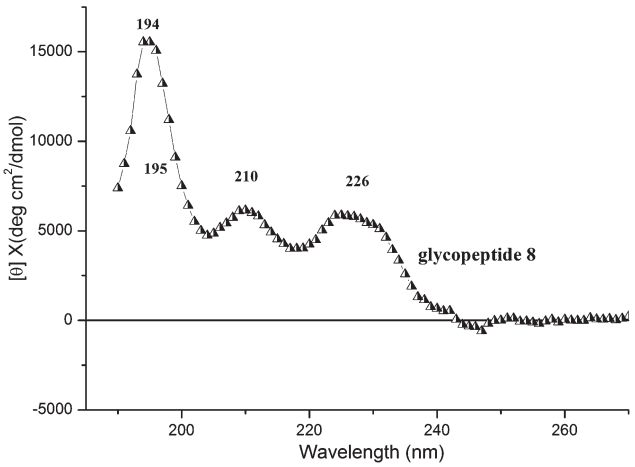


Fig. 6 CD signature of glycopeptide **8** in CH_3CN.

The conformational analysis of **8** was also performed by recording their circular dichroism (CD) spectra in CH_3CN at 150 μM concentration. Glycopeptide **8** displayed only positive bands at 195 nm, 210 nm and 228 nm. The positive band at 210/226 nm, although of reduced ellipticity, indicates the tendency to form a turn structure in the molecule (Fig. 6).[16,17a]

Conclusions

In conclusion, we have synthesized a sugar furanoid *trans* vicinal acid **5** from diacetone D-glucose in overall 28.6% yield. In the sugar acid **5**, the C-3 acid was converted to benzylamide as a continuation of the peptide sequence and C-4 acid was incorporated into a tetrapeptide sequence (H-Phe-Trp-Lys-Thr-OH) for conformational analysis. The 2D NMR and MD simulation studies of glycopeptide **8** clearly demonstrated that constrained sugar acid **5** can serve as an efficient inducer of γ-turns. Further application of this highly functionalized sugar acid **5** as a mixed α, β-sugar azido/amino acid will be exploited in the fine tuning of secondary structures in the area of peptidomimetics and glycopeptide synthesis. Work in this direction is in progress and will be reported in due course.

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Notes and references

‡Crystallographic data of compounds **2** and **3** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 936034 and 936035 respectively.

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