## Folded Conformation in Peptides Containing Furanoid Sugar Amino Acids

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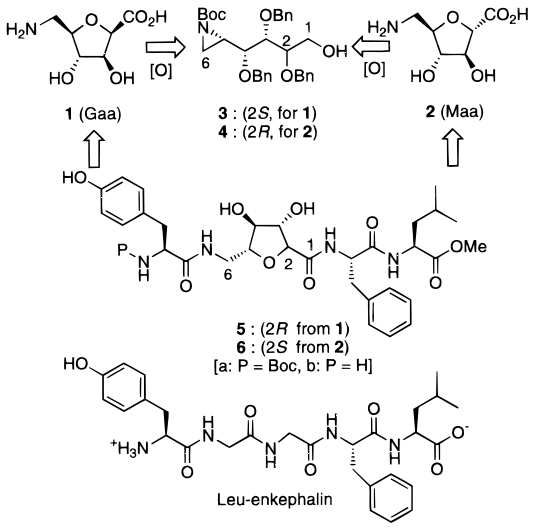
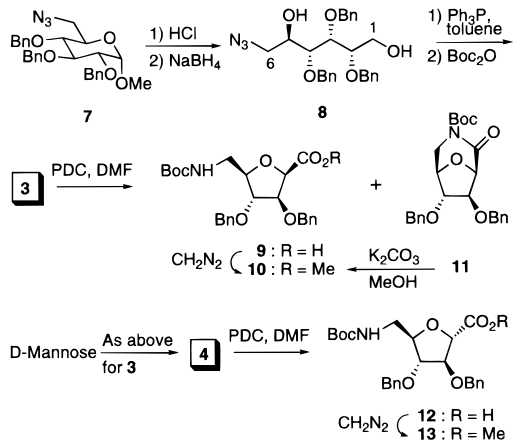
*Received May 14, 1998*

Introduction of conformationally restricted nonpeptide isosteres into the peptide backbone in order to achieve desirable secondary structures is of great interest in structure−activity relationship studies of peptides.[1] Furanoid sugar amino acids, 6-amino-2,5-anhydro-3,4-di-*O*-benzyl-6-deoxy-D-gluconic acid (**1**, Gaa) and its mannonic congener **2** (Maa), can serve as rigid scaffolds in the design and synthesis of carbopeptoids. The protected/unprotected hydroxyl groups of sugar rings can also influence the hydrophobic/hydrophilic nature of peptides.[2] Herein, we report an efficient synthesis of these furanoid sugar amino acids from their acyclic precursors **3** and **4**, followed by their incorporation into Leu-enkephalin in its Gly-Gly position as dipeptide isosteres leading to the formation of peptidomimetic analogues **5** and **6**. One of these analogues, Boc-Tyr-Gaa-Phe-Leu-OMe (**5a**), shows an unusual and interesting turn structure determined by CD and extensive NMR studies.




The synthesis followed a novel reaction path in which an intramolecular 5-exo opening of the hexose-derived aziridine ring by the $\gamma$-benzyloxy oxygen with concomitant debenzylation occurred during pyridinium dichromate (PDC) oxidation of the primary $\delta$-hydroxyl groups of **3** and **4**. Complete stereocontrol achieved in this electrophile-activated $S_N2$ opening of the aziridine ring under remarkably mild conditions outlines the essence of our synthesis. Scheme 1 unfolds the details of the route. The starting material, methyl 6-deoxy-6-azido-2,3,4-tri-*O*-benzyl-α-

**Scheme 1.** Synthesis of Protected Furanoid Sugar Amino Acids




D-glucopyranoside (**7**),[3] was transformed, in two steps, into intermediate diol **8** in 72% yield. Treatment of **8** with $Ph_3P$ in refluxing toluene led to the formation of an aziridine ring, which was protected in situ to give the target intermediate **3** in 85% yield. The crucial cycloetherification proceeded smoothly with simultaneous debenzylation and oxidation of the primary hydroxyl to the carboxylic acid moiety using an excess of PDC (5 molar equiv) in DMF as solvent. After workup, the crude acid **9** was treated with diazomethane. Chromatographic purification gave methyl 6-(*tert*-butyloxycarbonyl)amino-2,5-anhydro-3,4-di-*O*-benzyl-6-deoxy-D-gluconate (**10**) along with another compound, which was identified as bicyclic compound **11**, in about 75% yield (**10**:**11** ≈ 2:1).[4] Treatment of **11** with $K_2CO_3$/MeOH converted it to **10**, leading to an overall yield of 72% from **3**.

Similar oxidative rearrangement of **4**, prepared from D-mannose following the same route as outlined for **3**, furnished the expected mannonate **13** as the only product in 78% yield. In this case, the "2,5-trans" geometry in the furanoid framework prevented the formation of "**11**-type" bicyclic product.

Next, these furanoid gluconic and mannonic amino acids, **1** and **2**, respectively, were incorporated into Leu-enkephalin, replacing its Gly-Gly portion which is known to be flexible and amenable to different conformations depending on the binding environment.[5,6] The analogues **5** and **6** were synthesized by solution methods using EDCI/HOBt as coupling agents and DMF as solvent. Reaction of **9** with $H_2N$-Phe-Leu-OMe gave the protected tripeptide Boc-Gaa($Bzl_2$)-Phe- Leu-OMe, which was deprotected at the N-terminus with TFA/$CH_2Cl_2$ and subsequently coupled with Boc-Tyr(Br-Z) to give Boc-Tyr(Br-Z)-Gaa($Bzl_2$)-Phe-Leu-OMe. Finally, hydrogenation using $H_2$/Pd(OH)$_2$/C in methanol yielded the side-chain-deprotected compound Boc-Tyr-Gaa-Phe-Leu- OMe (**5a**). A similar reaction sequence starting with Boc-protected mannonic amino acid **12** led to the formation of Boc-Tyr-Maa-Phe-Leu-OMe (**6a**).[7]

Conformational analysis of these peptide analogues were carried out by studying their circular dichroism (CD) spectra in trifluoroethanol (TFE).[8] The CD spectrum of **5a** exhibits a strong

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(7) New compounds exhibited satisfactory spectral and exact mass data.

10.1021/ja9816685 CCC: $15.00 
Published on Web 11/25/1998

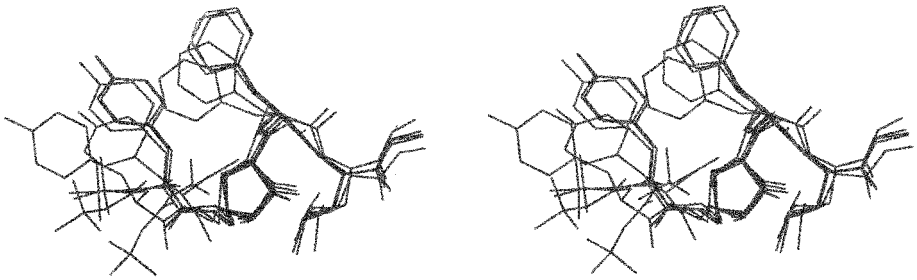

**Figure 1.** Stereoview of the five superimposed minimum energy conformations of **5a** selected from the 20 energy-minimized structures sampled during 20-ps MD simulations.

positive band at 216 nm characteristic of a type II $\beta$-turn.[9] The corresponding deprotected peptide $H_2N$-Tyr-Gaa-Phe-Leu-OMe (**5b**) also shows a positive band, although of reduced ellipticity at ~217 nm. The spectra of the Maa-containing analogues, both protected (**6a**) and deprotected $H_2N$-Tyr-Maa-Phe-Leu-OMe (**6b**), indicate a lower tendency to form turn structures.

Finally, solution conformation of **5a**, which shows the best turn structure in CD, was determined by studying, in detail, its $^1$H NMR spectra in DMSO-$d_6$ (10 mM). The resonance assignments were carried out with the help of TOCSY and ROESY experiments. The temperature coefficients of amide proton chemical shifts ($\Delta\delta/\Delta T$) were measured between 21 and 70 °C. The small $\Delta\delta/\Delta T$ for Leu amide (−1.3 ppb/K) shows its involvement in strong H-bonding, while other amide protons show medium to large values of temperature coefficients.

The peptide exhibits some unusual side-chain conformations, especially for Leu, whose side chain appears very rigid, as its $J_{\alpha,\beta(pro-R)} = 10.4$ Hz, $J_{\alpha,\beta(pro-S)} = 4.6$ Hz, $J_{\beta(pro-R),\gamma} = 4.6$ Hz, and $J_{\beta(pro-S),\gamma} = 9.6$ Hz indicate the presence of predominantly one single conformation about $\chi_1$ ($g^-$) and $\chi_2$ with an *anti* relationship between $\beta$H(*pro-S*) and $\gamma$H. The Phe and Tyr side chains also show the presence of $g^-$ conformation about $\chi_1$.

The small $J_{2-3}$ of 3.8 Hz and a ROESY cross-peak between sugar $C_2-H$ and $C_5-H$ indicates an envelope ($C_3$-*endo*) conformation of the sugar ring. Other cross-peaks between Leu$\alpha$H−Leu$\delta$CH$_3$(*pro-S*), Leu$\beta$H(*pro-R*)−sugar$C_3$-OH, Leu$\gamma$H−sugar$C_6$-H(*pro-R*), Leu$\delta$CH$_3$(*pro-R*)−sugar$C_3$-OH, and Leu$\delta$CH$_3$(*pro-R*)−sugar$C_6$-H(*pro-R*) show that these protons come close to each other and result in the Leu side chain getting locked in a single conformation. This conformation also shows H-bonding between LeuNH → sugar$C_3$-OH, leading to a nine-membered $\beta$-turn-like ring structure. The sugar-hydroxyl can also possibly act as a H-bond donor to Leu carbonyl or solvent molecules. There is a downfield shift of the sugar $C_3$-hydroxyl proton by 0.4 ppm compared to its chemical shift in the $^1$H NMR spectrum of Boc-Gaa-OMe in DMSO-$d_6$ (10 mM), whereas the $C_4$-OH signal did not change position. Amino acids with hydroxyl groups in their side chains (serine, threonine) serve as acceptors only ~30% of the time.[10] Moreover, a main-chain NH → side-chain OH H-bond leading to this type of turn structure is also very rare, mainly because of the free rotation about $\chi_1$ in these amino acids. In sugar amino acids, unlike in serine and threonine, the hydroxyls are conformationally restricted, forcing one of them to participate in the formation of an unusual secondary structure. This pseudo-$\beta$-turn is probably responsible for the strong positive band at 216 nm in the CD spectrum of the molecule. Another long-range ROESY cross-peak between Tyr$\beta$ protons and Phe aromatic protons suggests their close proximity.

Using ROESY cross-peak intensities as constraints, molecular dynamics (MD) simulations were carried out for a 20-ps period, sampling 20 frames at equal intervals which were energy-minimized. Five minimum energy conformations out of these 20 structures were selected and superimposed as shown in Figure 1.[11] A close look shows that the peptide takes a turn-like structure stabilized by aromatic−aromatic as well as hydrophobic interaction between the Leu side chain and the sugar ring. This structure very strongly resembles the conformation of Met-enkephalin in the presence of SDS micelles,[12] which is also stabilized by hydrophobic aromatic−aromatic interaction and corresponds to one of the two theoretical binding conformations[13] that enkephalin may adopt while interacting with cell membranes in which opioid receptors are located. We find that such a structure is preintroduced in **5a**, making it a potential ligand for the $\delta$-receptor which requires folded conformations with close proximity (<10 Å) of the two aromatic rings having nearly parallel orientation.[14]

The analgesic activities of these analogues were determined by mouse hot-plate test[15] following i.p. administration. Compounds **5a**,**b** showed activities ($ED_{50} = 1.14$ and 1.48 $\mu$mol/animal, respectively) similar to that of Leu-enkephalin methyl ester ($ED_{50} = 1.35$ $\mu$mol/animal). The same trend was observed when analgesic activities were assayed by the tail clip method.[16] Compounds **6a**,**b** showed no significant activity in either of these tests. These positive results are probably due to the above-mentioned preintroduced folded conformations present in these peptides which were absent in the biologically inactive pyranoid sugar amino acid-containing Leu-enkephalin analogues reported earlier.[2a]

In conclusion, furanoid sugar amino acids, now easily obtainable from hexose-derived aziridinyl precursors, can serve as useful templates to induce interesting secondary structures in peptides.

**Acknowledgment.** We thank Dr. J. A. R. P. Sharma for calculations and CSIR, New Delhi, for research fellowships (S.J. and S.R.B.J.) and a Young Scientist Award Research Grant (T.K.C.).

**Supporting Information Available:** Listing of selected physical data for **5a**, **6a**, **10**, **11**, and **13**; CD spectra of **5a**, **5b**, **6a**, and **6b**; $^1$H NMR spectra of **5a** and **6a**; TOCSY and ROESY spectra of **5a**; details of analgesic activity assay (11 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

JA9816685

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