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Synthesis and NMR Studies of Cyclopeptides Containing a Sugar Amino Acid

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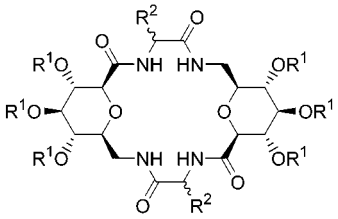
ABSTRACT



R^1 = H or Bn

R^2 = H, Me, Bn or aminobutyl

Cyclopeptides containing Glucuronic acid methylamine (Gum) alternating with Gly, L-Ala, D-Ala, L-Phe, D-Phe, L-Lys, or D-Lys were synthesized by a combination of solid-phase synthesis and solution chemistry. A more effective pathway to synthesize the sugar amino acid Gum in higher yields and in a shorter period of time was developed. Gum is employed in the benzylated and deprotected form. The cyclopeptides were characterized by NMR and the structure of one deprotected cyclic peptide solved.

The aim of this work is the synthesis of new cyclic peptides containing sugar amino acids (SAAs) and amino acids (AAs) to provide new structural scaffolds. SAAs are pyranoid and furanoid sugars, which contain at least one amino and one carboxyl group.[1] They are used as turn mimetica,[1] peptidomimetic, and glycomimetic scaffolds[1,2] and in combinatorial chemistry.[1,3] Modification of backbone or side chains of cyclopeptide agents by SAAs could improve their pharmacological properties. The SAA Gum was designed in our laboratory as a dipeptide isostere of the Gly-Ser sequence held in a "flexible" β-turn conformation.[1a,b]

Cyclic compounds containing SAAs could be used to modify cyclic peptides to provide a scaffold for template-assembled synthetic proteins (TASP).[4] The use of sugar rings has distinct advantages over that of conventional AAs, including hydrophilicity and introduction of lipophilicity by use of protected SAAs.[1d,e] The easiest way to present functional groups for the attachment of protein-mimicking elements is to use conventional amino acids. To explore the synthesis and conformation of such ring systems we present here cyclic oligomers (**1**−**16**) of alternating Gum as SAA and Xaa, where Xaa is an amino acid (Gly, Ala, Phe, or Lys) in the D- or L-conformation (Table 1).

The tetrameric ring size was chosen to mimic cyclic hexapeptides. This comparison is based on the chain length between the Gum amino group and the carboxylate carbon

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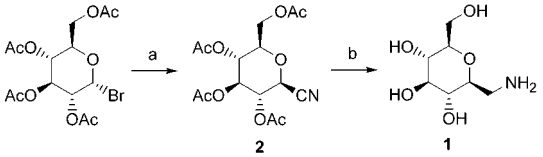
Table 1. Synthesized Cyclic Oligomers Cyclo[Gum(R)$_3$-Xaa1-Gum(R)$_3$-Xaa2] (R = H or Bn; XaaX = Gly, L-Ala, D-Ala, L-Phe, D-Phe, L-Lys, or D-Lys)

cyclo[Gum(R)$_3$-Xaa1-Gum(R)$_3$-Xaa2]			
R = H	R = Bn	Xaa1	Xaa2
1	**5**	Gly	Gly
2	**6**	L-Ala	L-Ala
3	**7**	L-Ala	D-Ala
4	**8**	D-Ala	D-Ala
9		L-Lys	L-Phe
10		L-Lys	D-Phe
11		D-Lys	L-Phe
12		D-Lys	D-Phe
	13	L-Lys(Boc)	L-Phe
	14	L-Lys(Boc)	D-Phe
	15	D-Lys(Boc)	L-Phe
	16	D-Lys(Boc)	D-Phe

(six atoms, similar to a dipeptide unit; see above). Lys was chosen to allow further side chain functionalization, whereas Phe was used to simplify the procedure of HPLC separation, since Phe can easily be detected by UV spectroscopy. Furthermore, D-Phe shows structurally induced tendencies and exhibits better separation of NMR signals. Similar compounds with furanoid SAAs were already described previously.[5]

For the synthesis of Gum, β-D-glucopyranosylmethylamine **17** is an important intermediate product for which we present here a more efficient synthesis (Scheme 1). 2,3,4,6-Tetra-

Scheme 1. Improved Synthesis of β-D-Glucopyranosylmethylamine **17**[a]

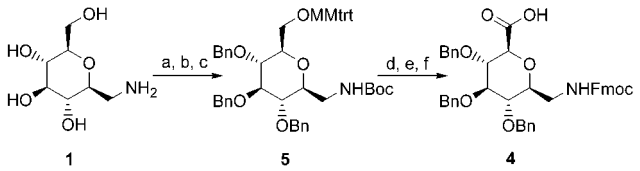


[a] Reaction conditions: (a) $Hg(CN)_2$, melt, 85 °C, argon atmosphere, 1 h, 81%; (b) $LiAlH_4$, THF, 0 °C.

O-acetyl-α-D-glucopyranosyl bromide reacts with $Hg(CN)_2$ in melt[6] in only 1 h and excellent yields (81%) to form 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylcyanide **18**, followed by reduction to compound **17**. This pathway is much more efficient than all alternative routes such as the synthesis of **18** by reaction of pentaacetylglucose with trimethylsilylcyanide (TMSCN)[7] or the synthesis of compound **17** by reduction of β-D-glucopyranosylnitromethane, which is prepared by nitroaldol reaction of glucose in low yield (27%) with high experimental effort.[1a]

Fmoc protection followed by TEMPO oxidation (TEMPO = 2,2,6,6-tetramethyl-piperidine-1-oxyl) leads to unprotected Fmoc-Gum-OH.[1a] To date, Fmoc-Gum(Bn)$_3$-OH **19** was synthesized in a 12-step synthesis starting from D-glucopyranose (overall yield 3%).[1d,e] Herein we present an alternative pathway starting from compound **17** (Scheme 2).

Scheme 2. Synthesis of Fmoc-Gum(Bn)$_3$-OH **19**[a]



[a] Reaction conditions: (a) Boc_2O, THF, H_2O, 81% starting from compound **18**. (b) MMTr-Cl, TEA, DMAP, DMF, 78%. (c) BnBr, 18-crown-6, KOH, THF, 63%. (d) (1) 20 vol % TFA in DCM, H_2O; (2) Fmoc-Cl, $NaHCO_3$, THF, H_2O, 71%. (e) TEMPO, KBr, TBABr, NaOCl, DCM, NaCl, $NaHCO_3$, H_2O, 96%.

Boc protection of **17** was realized in situ under standard conditions[8] with Boc_2O. Reaction of monomethoxytrityl chloride (1.1 equiv) with Boc-*N*-(β-D-glucopyranosyl)-methylamine in DMF solution overnight at room temperature in the presence of catalytic amounts of 4-(dimethylamino)-pyridine (DMAP) and triethylamine (TEA, 1.5 equiv) produced Boc-*N*-(6-*O*-MMTr-β-D-glucopyranosyl)methylamine in good yield (78%). Compound **20** was obtained in 63% yield by treating the last compound in THF solution overnight at room temperature with benzylbromide (3.3 equiv), KOH powder (5.4 equiv), and catalytic amounts of 18-crown-6 under argon. After simultaneous cleavage of the monomethoxy triphenylmethyl ether and Boc deprotection, the intermediate was Fmoc protected with Fmoc chloride to yield Fmoc-*N*-(2,3,4-tri-*O*-benzyl-β-D-glucopyranosyl)methylamine in 71% over two steps. Finally, TEMPO oxidation in a two-phase system (water/dichloromethane) with tetrabutylammonium bromide (TBABr) as a phase transfer catalyst led to the final product Fmoc-Gum(Bn)$_3$-OH **19** in excellent yields (96%). The overall yield starting from 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide was 22%.

Oligomerization of Gum(Bn)$_3$-OH alternating with L- and D-forms of Ala, Lys, Phe, or Gly, respectively, was realized by solid-phase synthesis with the Fmoc strategy using TentaGel S Trt resin (Rapp Polymere), *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyl-uronium hexafluorophosphate (HATU),[9] and 1-hydroxy-7-azabenzotriazole (HOAt)[10] as coupling reagents and 2,4,6-collidine as a base in DMF (Scheme 3).

The azabenzotriazol-based coupling reagents were the most suitable, since they permit high coupling yields and low

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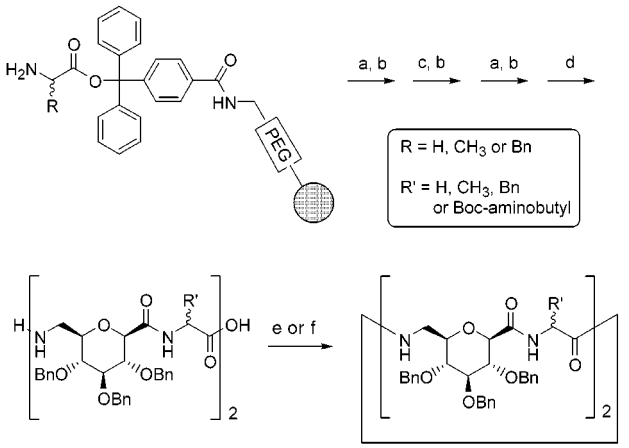
Scheme 3. Oligomerization and Cyclization of Gum(Bn_3)-OH Alternating with L-Ala, D-Ala, L-Phe, D-Phe, L-Lys, D-Lys, or Gly on TentaGel S Trt resin[a]



[a] Reaction conditions: (a) Fmoc-Gum(Bn_3)-OH (2 equiv), HATU (2 equiv), HOAt (2 equiv), 2,4,6-collidine (20 equiv), DMF, 15 h, rt; (b) 1:4 piperidine/DMF, 2 × 30 min, rt; (c) Fmoc-AA-OH (3 equiv), HATU (3 equiv), HOAt (3 equiv), 2,4,6-collidine (30 equiv), DMF, 15 h, rt; (d) 1:1:3 TFE/AcOH/DCM or 20 vol % HFIP in DCM. (e) DIC (10 equiv), HOAt (1 equiv), NMM (3 equiv), 9:1 DCM/DMF, 4 days, 0−4 °C; (f) HATU (1 equiv), HOAt (1 equiv), 2,4,6-collidine (10 equiv), DMF, overnight, 0−4 °C.

racemization. As solution studies have shown,[11] racemization is lower when using 2,4,6-collidine rather than diisopropyl ethylamine (DIPEA). Our studies on solid-phase synthesis have confirmed this.[1e]

Oligomers were cleaved from resin with 1:1:3 2,2,2-trifluoroethanol (TFE)/AcOH/DCM or with 20 vol % hexafluoro 2-propanol in DCM. Cyclic tetramers were obtained by cyclization of linear oligomers with diisopropyl carbodiimide (DIC), HOAt, and *N*-methylmorpholine (NMM) in a very diluted (0.1−0.2 mM) solution of 9:1 DCM/DMF at 4 °C in 4 days or overnight by treatment of the linear oligomers with HATU (1 equiv), HOAt (1 equiv), and 2,4,6-collidine in a highly diluted (0.2 mM) solution in DMF. Yields between 60 and 75% were obtained. Deprotected cycles were received by hydrogenolytic cleavage of benzylic ethers with Pd/C (5%, dry) and H_2 (50 bar) overnight in 7:2:1 *N*,*N*-dimethylacetamid/MeOH/AcOH in excellent yields (95%). We obtained unprotected cyclic oligomers also by the described coupling protocol in Scheme 3 with the unprotected Fmoc-Gum-OH. But the yields were only between 30 and 50% for oligomerization and 30−60% for cyclization. Boc deprotection of the lysine side chain was obtained by treatment with 50 vol % TFA in DCM almost quantitatively.

The cyclic oligomers were characterized by NMR spectroscopy in DMSO (protected cycles **5**−**8** and **13**−**16**) and water or D_2O, respectively (unprotected cycles **1**−**4** and **9**−**12**). NMR spectra of the cyclopeptides containing only Gly, L-Ala, or D-Ala alternated with Gum indicate a symmetrical conformation on the NMR time scale at 300 K. Even the unprotected, mixed cycle of L-Ala and D-Ala **3** shows only a very small asymmetric tendency. As expected, the incorporation of Phe induced a better separation of NMR signals. The tetrameric ring size of the cyclic oligomers described here was chosen to mimic cyclic hexapeptides, with Gum as a β-turn-inducing agent (see above). To test, whether the Gum residue displays its β-turn-inducing properties also in these compounds, we further investigated the conformation of the unprotected oligomer cyclo(Gum-L-Lys-Gum-D-Phe) **10** using distance geometry and molecular modeling.

A total of 18 distance restraints derived from the ROESY spectrum were used in combination with selected coupling constants for metric matrix distance geometry calculations of 1000 starting structures using a modified[12] version of DISGEO.[13] After a cluster algorithm[14] was applied to the backbone atoms (including sugar atoms within the backbone ring), this first pool of conformations was used as a starting point for further structural refinement. A 150 ps restrained molecular dynamics simulation of cyclo(Gum-L-Lys-Gum-D-Phe) **10** was then carried out in the CVFF force field as incorporated in the Discover program package.[15] The simulation was performed in an explicit water box (3199 H_2O molecules, box size 46 × 46 × 46 Å^3, cubical) with an explicit image of periodic boundary conditions. All ROE violations, calculated over the trajectory with $\langle r^{-3}\rangle^{1/3}$ averaging, remained below 0.13 Å. The averaged and energy-minimized structure from the restrained MD trajectory (see Figure 1) is in agreement with the temperature gradients of the amide protons, which indicate good solvent accessibility for all H^N.

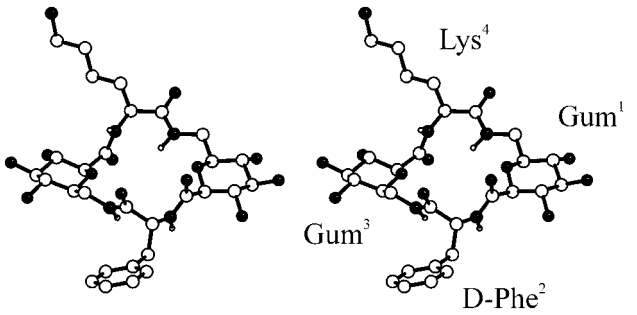


Figure 1. Stereopicture of the three-dimensional structure of **10**.

To obtain more insight into the conformational flexibility of **10**, a subsequent 150 ps MD simulation in water without applying any experimental restraints (free MD) was then carried out. The structure remained stable and very similar

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to the structure calculated from the restrained simulation. The distance RMSD between the averaged and minimized structures from both the free and the restrained simulations is 0.46 Å for the backbone atoms. This good agreement proves that the solution structure calculated from NMR data represents a stable low-energy conformation in the CVFF force field.

Against our expectations, Gum does not act as a β-turn mimetic in **10**. This can be explained by the comparison between an ideal βI′-turn in a regular tetrapeptide and the analogous turn in Xaa-Gum-Xaa. In the tetrapeptide, the amide bond between the amino acids in positions $i + 1$ and $i + 2$ is planar, whereas the analogous bond in Xaa-Gum-Xaa resembles an aliphatic chain. Therefore, the distance of the CO(i)−HN($i + 2$) hydrogen bond in the tetrapeptide measures 1.85 Å, as opposed to the corresponding distance of 2.43 Å in the Xaa-Gum-Xaa turn (see Supporting Information). It therefore seems that the Gum replacement of a single Xaa-Xaa sequence in a regular cyclic hexapeptide results in an additional cyclic constraint, which can be compensated for by the rest of the molecule, while still maintaining β-turn geometry. On the contrary, the replacement of two adjacent Xaa-Xaa sequences leads to a very high ring tension in the usual conformation of two adjacent β-turns, so this conformation becomes energetically unfavored.

For a better rationalization of the conformation, we have reduced the structure of **10** according to the principle of Dunitz and Waser.[16] Cyclic hexapeptides in such a model resemble cyclohexane-like conformations.[17] The Gum sugar ring possesses five large substituents that can be in either an all-axial or an all-equatorial position, with the latter being highly energetically favored. Therefore, the Gum methylene carbon C, the three ring atoms C1, O, and C5, and the Gum carbonyl carbon C6 can be regarded as sterically fixed in space relative to each other. Thus, it is possible to reduce C1, O, and C5 to a single pseudoatom X (for further details see Supporting Information). In combination with the standard reduction according to Dunitz and Waser, this procedure results in a hexacycle, consisting of the Xaa-C$^{\alpha}$ and the Gum C and X atoms. For this hexacycle, we have found a nearly ideal twisted boat conformation (dihedral angles (calcd/ideal), starting from Lys^{4}C$^{\alpha}$−Gum^{1}C−Gum^{1}X−D-Phe^{2}C$^{\alpha}$: −53.1/−54.8; 17.7/27.0; 43.9/27.0; −64.6/−54.8; 41.9/27.0; 13.1/27.0).

A close inspection of the ROESY spectrum reveals a second conformation, which interchanges slowly on the NMR time scale with the main conformation. The structure of the second conformation could not be elucidated, since the signals of the two conformations are not separated well enough. The population of the second conformer increases with the temperature (approximately 3% at 293 K until 5% at 320 K). It should be noted that a conformation with D-Phe in the standard position $i + 1$ of a βII′-turn cannot be formed in **10**. In such a conformation, there would exist two very unfavorable, close contacts between the CO(i) and O($i + 4$) oxygen atoms, as opposed to the stabilizing hydrogen bonds in a standard cyclic hexapeptide. However, a conformation that corresponds to a chair conformation according to Dunitz and Waser would explain the slow interchange of the two conformers, since there is a high energy barrier between the boat and the chair conformations in such a hexacycle.

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Supporting Information Available: Characterization and detailed descriptions of the synthesis of key compounds; tables of coupling constants, ROE data, and amide proton temperature gradients of cyclo(Gum-D-Phe-Gum-L-Lys) **10** in the conformation described; all molecular dynamics protocols; and a detailed description of the Dunitz−Waser reduction of our calculated structure. This material is available free of charge via the Internet at http://pubs.acs.org.

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