Synthesis of bicyclic sugar azido acids and their incorporation in cyclic peptides

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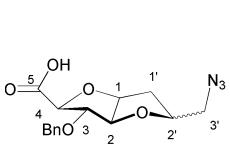
Bicyclic amino acids derived from the natural sugars D-arabinofuranose and D-fructofuranose have been synthesised; their ability to induce a turn conformation in peptides has been exploited in the preparation of a cyclic RGD loop mimetic.

The conformational rigidity of the pyran and furan rings makes sugar amino acids (SAAs) interesting building blocks in the induction of precise secondary structures in peptides and in the construction of peptidomimetics. In this context, β-D-glucopyranose was used as scaffold in the preparation of the first nonpeptide somatostatin mimetic;[1] in a different biological context the same sugar moiety was employed to present the guanidine group crucial for the biological activity of thrombin inhibitors.[2] By varying the mutual positions of the amino and carboxylic groups in the glucopyranose scaffold, a set of SAAs was designed and found to be capable of inducing linear or β-turn conformations when incorporated into Leu-enkephalin peptide analogues.[3] Oligomers of pyranose sugar amino acids[4] ('carbopeptoids') have been synthesised, and the furanose ring has also been exploited as a building block for carbopeptoid assembling.[5] Homo-oligomers of tetrahydrofuran amino acids derived from the arabinofuranose were shown to adopt a novel repeating β-turn type secondary structure in tetrameric units stabilized by intramolecular hydrogen bond.[6]

We present here the design and the synthesis of novel azido



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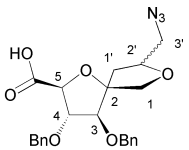

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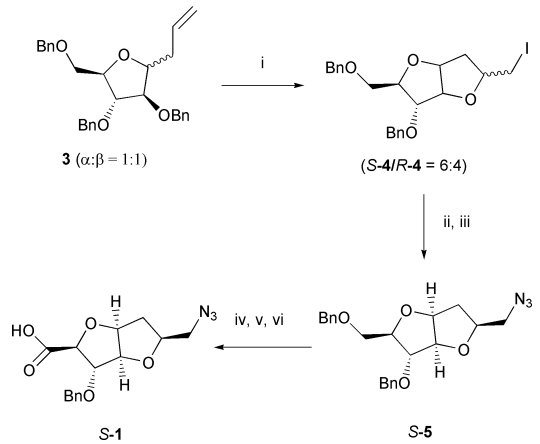
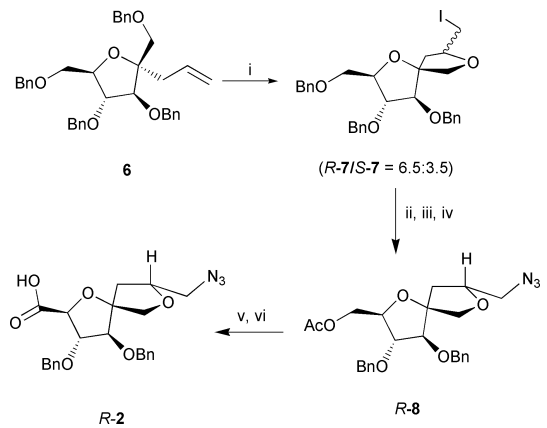
acids (**1** and **2**) with bicyclic structures, derived from D-arabinofuranose and D-fructofuranose respectively. Given that the azido group is chemically equivalent to a protected amine, these compounds can be used as building blocks for solid phase assembly of peptoids. Our main goal in the design of this type of SAAs was to lock the furanose ring in a rigid conformation through the formation of fused rings or spiro-bicycles, thus mimicking protein reverse turns (**1**) or constraining a linear peptide conformation (**2**). The transformation of the commercially available starting materials into the corresponding bicyclic azido acids are based on the same synthetic strategy, once the respective allyl-*C*-furanosides have been formed (Schemes 1 and 2).

The 2,3,5-tri-*O*-benzyl-D-arabinofuranose was converted into the anomeric acetate **3**, which was allylated by treatment with allyltrimethylsilane in the presence of catalytic amounts of BF_3–Et_2O. The reaction, effected at rt in dry acetonitrile, afforded **4** as a 1:1 mixture of α and β diastereomers (72% yield). Iodocyclization (Scheme 1) was then carried out with iodine in CH_2Cl_2 at 0 °C. This step is crucial to the formation of the bicyclic structures of the arabino- and fructo-derived compounds and consists of the opening of the intermediate iodonium ion by attack of the γ-benzyloxy groups in the 5-*exo*-



Scheme 1 *Reagents and conditions*: i, I_2, CH_2Cl_2, 0 °C, 12 h; ii, separation of diastereomers by flash chromatography; iii, Bu_4NN_3, toluene, 60 °C; iv, Ac_2O, CF_3COOH, 0 °C; v, MeONa, MeOH; vi, CrO_3–H_2SO_4, acetone–water.

mode with formation of a cyclic iodoether with debenzylation.[7] Obviously, only the β anomer of **4** reacted and this allowed the easy separation from the α-anomer. The bicyclic iodoether **5** was obtained as a mixture of diastereomers (95% yield based on the β anomer), the major isomer having the 2′-(*S*) configuration (20% d.e.). Compounds *R*-**5** and *S*-**5** were separated by flash chromatography and their relative configurations at the C-2′ assigned by NOESY analysis.[8] The reaction of the iododerivative **5** with tetrabutylammonium azide in toluene to give azidoderivative **5** (60 °C, 24 h, 87% yield), as well as the subsequent synthetic steps, were carried out separately on the two diastereoisomers with similar yields. In order to introduce the carboxylic function, **5** was regioselectively debenzylated at the primary hydroxy group by controlled acetolysis (Ac_2O, CF_3COOH, 0 °C), followed by saponification of the acetate and



Scheme 2 *Reagents and conditions*: i, I_2, CH_2Cl_2, 0 °C, 12 h; ii, separation of diastereomers by flash chromatography; iii, Bu_4NN_3, toluene, 60 °C; iv, Ac_2O, CF_3COOH, 0 °C; v, MeONa, MeOH; vi, CrO_3–H_2SO_4, acetone–water.

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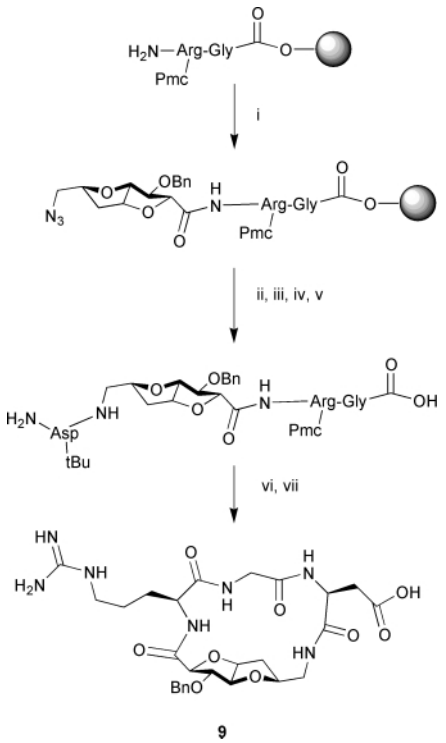
Jones oxidation. Compound **1** was obtained in 48% yield over three steps. The spiro-azido acid **2** was prepared in a similar way, as shown in Scheme 2, the iodocyclization of **6** affording stereoselectively *R*-**7** as the major product[9] (d.e. 33%). The iododerivative was reacted with tetrabutylammonium azide in toluene (60 °C, 24 h) then submitted to acetolysis affording *R*-**10**,[10] and finally converted into azido acid *R*-**2**. The overall yields for the transformation of **3** into **1** and of **6** into **2** were respectively 40% and 35%, *S*-**1**[11] and *R*-**2** being obtained as the major isomers.

NMR and molecular dynamics indicate that both arabino- and fructo-derived bicycles adopt rigid, spatially well-defined conformations. In particular the sugar-amino acids derived from precursors *S*-**1** and *R*-**1** are rigid turn mimetics, both molecules can be incorporated in peptide sequences and used to replace *i* + 1 and *i* + 2 residues of a protein β-turn.

Moreover, these conformationally constrained sugar-amino acids may be incorporated as rigid templates into cyclic peptides in order to lock a bioactive conformation. Several templates have been included in cyclic peptides containing the RGD loop with the aim of obtaining new inhibitors of the adhesive interaction between the $\alpha_v\beta_3$ and the $\alpha_{IIb}\beta_3$-type integrins and their ligands.[12] As example, cyclic peptide **9** was synthesised on solid phase (Scheme 3) by the well-established Fmoc protocol. The dipeptide Arg(Pmc)-Gly was assembled onto Sasrin resin with a loading of 0.47 mmol g^{-1}, then compound *S*-**1** was coupled in the presence of HBTU,[13] HO-Bt and DIPEA. The *N*-terminal azide reduction to the amine and the coupling with Fmoc-Asp(tBu) was effected one-pot in the presence of DIC, Bu_3P, HO-Bt in dry DMF–toluene (2 : 1) at rt for 24 h.[14] This reaction was found to be very efficient and high yielding (as assessed from the loading value calculated after the Fmoc deprotection of the aspartate), in sharp contrast with the inefficiency of other well-established methods we employed to reduce this azide. The tetrapeptide was then cleaved from the resin (1% TFA in CH_2Cl_2 with immediate neutralisation of the effluents with pyridine) maintaining all protecting groups, purified by flash chromatography on silica gel, cyclised in a dilute solution of DMF (peptide conc. 0.5 mM) in the presence of HBTU, HO-Bt and DIPEA and finally totally deprotected with TFA–TIS–water (95 : 2.5 : 2.5), affording **9**[15] in 63% yield over the last two steps.



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Scheme 3 *Reagents and conditions:* i, *S*-**1**, HBTU, HOBt, DIPEA, DMF, rt, 4 h; ii, Bu_3P, DIC, Fmoc-Asp(OtBu), rt, 24 h; iii, piperidine 20% in DMF; iv, 1% TFA in DCM, neutralisation of the effluent with pyridine; v, 0.5 mM peptide in DMF, HBTU, HOBt, DIPEA, 12 h, rt; vi, 95% TFA, 2.5% TIS, 2.5% H_2O, 10 h, rt.

The high efficiency of the synthesis of compounds **1** and **2** is likely to offer opportunities for the preparation of a wide range of carbohydrate-derived bicyclic amino acids with improved conformational rigidity to be used as templates, inducing precise secondary structure conformations in peptides. Detailed conformational analysis of these compounds was carried out by NMR and molecular dynamics and will be reported elsewhere.

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8 A consistent NOESY crosspeak (400 MHz, $CDCl_3$) was observed between H-2′ and H-3 protons for the diastereoisomer *R*-**5**, that was absent for *S*-**5**.

9 A sequential NOESY correlation allowed the unambiguous attribution of the configuration at C-2′: for diastereoisomer *R*-**5** H-3/H-1′b and H-2′/H-1′a NOESY crosspeaks were observed thus indicating that H-3 and H-2′ point towards different spatial regions. For diastereoisomer *S*-**8**, H-3/H-1′b and H-2′/H-1′b NOESY crosspeaks were observed.

10 *Selected data for R*-**9**: MALDI-TOF MS*: m/z* 467.9 (M), 491.3 (M + Na), 507.1 (M + K); δ_H (300 MHz, $CDCl_3$) 1.76 (1H, dd, *J* 12.9, 9.8, H-1′a), 2.04 (3H, s, CH_3CO), 2.18 (1H, dd, *J* 12.9, 6.0, H-1′b), 3.19 (1H, dd, *J* 12.9, 4.9, H-3′a) 3.47 (1H, dd, *J* 12.9, 4.0, H-3′b), 3.52–3.64 (1H, m, H-5), 3.82 (1H, d, *J* 9.8, H-1a), 3.87–3.92 (1H, m, H-4), 3.94 (1H, d, *J* 1.4, H-3), 4.08–4.20 (2H, m, H-6), 4.25 (1H, d, *J* 9.8, H-1b), 4.28–4.35 (1H, m, H-2′), 4.42–4.60 (4H, m, CH_2-Ph) 7.20–7.40 (10H, m, H_{arom}).

11 *Selected data for S*-**1**: MALDI-TOF MS: *m/z* 319.9 (M + H); 342.4 (M + Na), 358.4 (M + K); δ_H (300 MHz, $CDCl_3$) 2.09 (1H, m, H-1′b), 2.31 (1H, ddd, *J* 14.2, 6.1, 8.3, H-1′a), 3.30 (1H, dd, *J* 12.8, 3.8, H-3′b), 3.55 (1H, dd, *J* 12.8, 7.3, H-3′a), 4.13 (1H, m, H-2′), 4.35 (1H, bd, *J* 3.3, H-2), 4.42 (1H, bd, *J* 1.7, H-3), 4.61 (1H, bd, *J* 1.8, H-4), 4.66 (2H, AB system, CH_2-Ph), 4.90 (1H, m, H-1), 7.3–7.4 (5H, m, H_{arom}).

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13 *Abbreviations*: DIC, *N*,*N*′-diisopropylcarbodiimide; DIPEA, diisopropylethylamine; HBTU, 2-(1*H*-benzotriazole-1-yl-)-1,1,3,3-tetramethyluronium hexafluorophosphate; HOBt, *N*-hydroxybenzotriazole; Pmc, 2,2,5,7,8-pentamethylchroman-6-sulfonyl; TIS, triisopropylsilane.

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15 *Selected data for* **9**: MALDI-TOF MS*: m/z* 603.3 (M), 626.6 (M + Na), 642.1 (M + K).