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Synthesis and conformational analysis of carbasugar bioisosteres of α-L-iduronic acid and its methyl glycoside

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ABSTRACT

The synthesis of two novel carbasugar analogues of α-L-iduronic acid is described in which the ring-oxygen is replaced by a methylene group. In analogy with the conformational equilibrium described for α-L-IdopA, the conformation of the carbasugars was investigated by ^{1}H and ^{13}C NMR spectroscopy. Hadamard transform NMR experiments were utilised for rapid acquisition of ^{1}H,^{13}C-HSQC spectra and efficient measurements of heteronuclear long-range coupling constants. Analysis of ^{1}H NMR chemical shifts and $J_{H,H}$ coupling constants extracted by a total-lineshape fitting procedure in conjunction with $J_{H,C}$ coupling constants obtained by three different 2D NMR experiments, viz., ^{1}H,^{13}C-HSQC-HECADE, J-HMBC and IPAP-HSQC-TOCSY-HT, as well as effective proton–proton distances from 1D ^{1}H,^{1}H T-ROE and NOE experiments showed that the conformational equilibrium $^{4}C_1 \rightleftharpoons {}^{2}S_{5a} \rightleftharpoons {}^{1}C_4$ is shifted towards $^{4}C_1$ as the predominant or exclusive conformation. These carbasugar bioisosteres of α-L-iduronic acid do not as monomers show the inherent flexibility that is anticipated to be necessary for biological activity.

1. Introduction

Monosaccharides are usually assumed to have a rigid ring-conformation. This is a good approximation for hexapyranoses with up to one non-anomeric axial hydroxy group, which includes the majority of monosaccharide residues found in oligosaccharides of biological or biochemical importance. Iduronic acid, which is found α-(1→4)-linked in nature in the glycosaminoglycans heparin, heparan sulfate and dermatan sulfate, is unusual among biologically relevant hexoses in that in its pyranose form it does not exclusively occupy the $^{4}C_1$ conformation, but rather is a flexible entity with more than one low-energy conformation accessible.[1] The relative population of the three low-energy conformations $^{4}C_1$, $^{2}S_O$ and $^{1}C_4$ (Fig. 1) is dependent on the surrounding residues and sulfation.[2,3] It has been proposed that the flexibility of iduronic acid is key to the strong binding of certain of these glycans to their receptors.[1] One important interaction is the binding of a heparin pentasaccharide to antithrombin.[4] Different conformationally constrained mimics of iduronic acid have been synthesised and incorporated into heparin sequences whose affinities for antithrombin indicate that the $^{2}S_O$ skew conformation is important for this binding event.[5–7] The conformational preferences of iduronic acid and heparin-derived oligosaccharides have been studied by NMR spectroscopy, molecular modelling, molecular dynamics simulations, ab initio quantum mechanical computations and density functional theory methodology.[8–16]

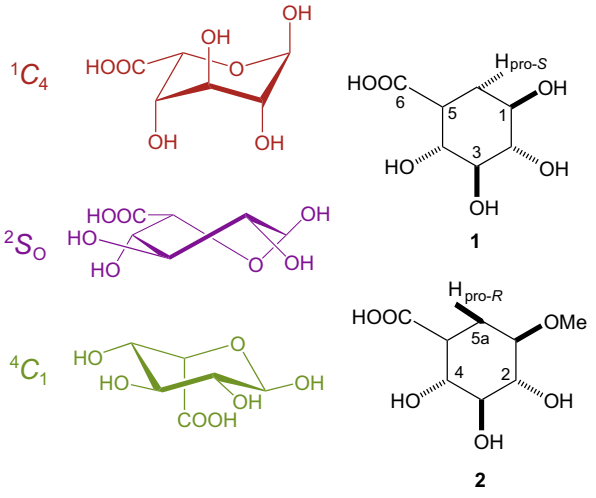

Figure 1. Schematic of possible conformations for α-L-iduronic acid (left column) and the carbasugar analogues thereof synthesised herein (right column).

Carbasugars (formerly pseudosugars) are carbohydrate-like molecules in which the ring-oxygen has been formally replaced by carbon.[17–19] They thus differ from natural glycosides in that pseudodisaccharides based on carbasugars will be hydrolytically

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stable, an important factor when considering enzyme inhibitor design. Some *ido*-configured carbasugars have been synthesised before: both anomers of carbaidose have been described, as have some C1 ether pseudodisaccharides.[20–25] Only two carbaiduronic acid derivatives have been synthesised, both as protected derivatives only, and both with the non-natural β-D-configuration.[26,27] Recently, two novel carbasugars were isolated from *Streptomyces lincolnesis*.[28]

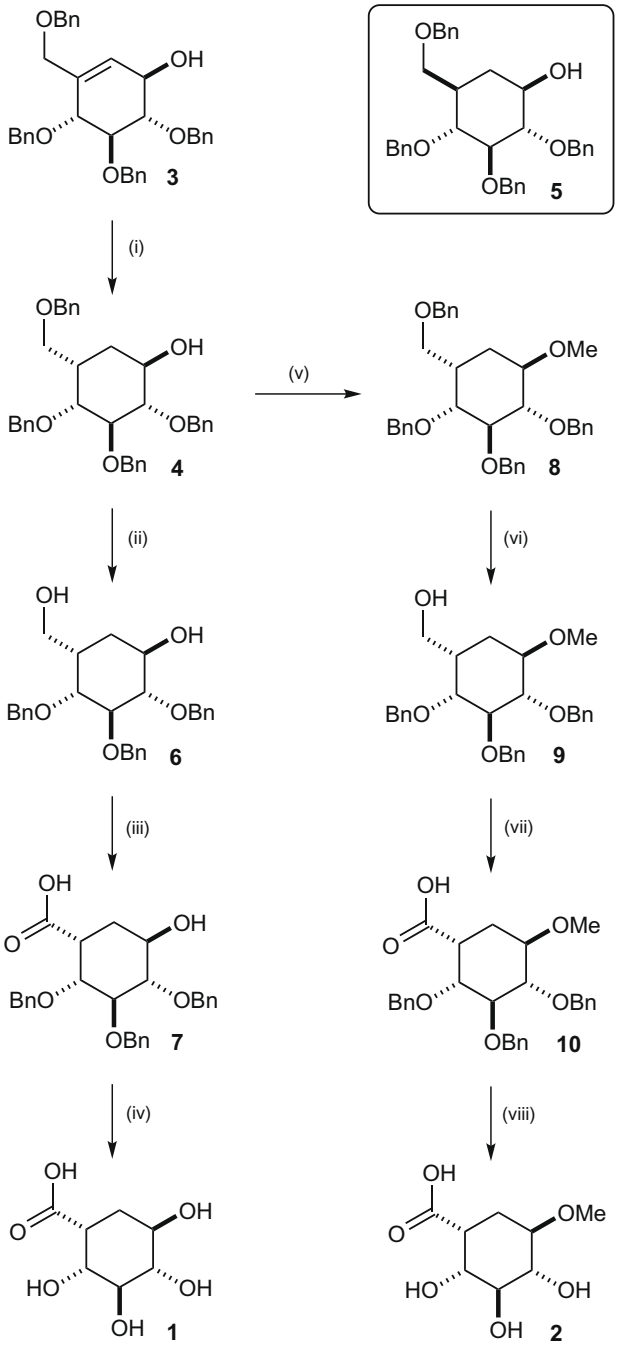
We were interested as to whether carbaiduronic acid derivatives would have a conformational profile that would give them the potential to be useful mimics of iduronic acid, and we therefore undertook the synthesis of carbasugar **1** and its 1-*O*-methyl ether derivative **2** (Fig. 1) and investigated their ring-conformation(s), the results of which we report in this paper.

2. Results and discussion

2.1. Synthesis

Our synthesis started from the known cyclohexene derivative **3** that is prepared from L-sorbose in nine steps (Scheme 1).[29] Hydrogenation of the C=C double bond proceeded stereoselectively with addition of hydrogen from the same face as the free OH group to give the protected carbaidose **4** as the major product, separable by chromatography from the minor *gluco* compound **5** (*ido*:*gluco*; **4**:**5**; 4:1).[30] Selective cleavage of the primary benzyl ether from O6 in **4** was achieved by acetolysis with TFA and Ac_2O,[31] followed by deacetylation with NaOMe in MeOH to give the known diol **6**.[30] Initial attempts at selective oxidation to iduronic acid **7** failed however: treatment of diol **6** with TEMPO and diacetoxyiodobenzene under two-phase conditions with CH_2Cl_2 and water gave clean formation of the C6 aldehyde,[32] which was not transformed into the acid **7** even after several hours. Using a mixture of acetone and water as reaction solvent in the oxidation did not change the outcome. Nevertheless, the crude aldehyde could be oxidised to the carboxylic acid **7** with sodium chlorite.[33] The secondary alcohol at C1 remained untouched during these two steps. Hydrogenolysis of the benzyl ethers gave the deprotected carbasugar **1**.

In order to access the carbasugar analogue **2** of the methyl glycoside, OH1 of carbaidose **4** was methylated with methyl iodide to give the methyl ether **8**. The OH6 protection was selectively removed as described above to give the primary alcohol **9**. In this case, treatment with TEMPO and diacetoxybenzene in acetone/water gave the carboxylic acid **10** directly, although a rather long reaction time (48 h) was needed for complete conversion. Debenzylation as described above gave the methyl glycoside analogue **2**.



Scheme 1. Reagents and conditions: (i) H_2, Pd (C), Et_3N, EtOAc; **4**, 78%; **5**, 19%; (ii) TFA, Ac_2O; then NaOMe, MeOH, 84%; (iii) TEMPO, $PhI(OAc)_2$, CH_2Cl_2, water; then DMSO, *t*BuOH, dimethoxybenzene, $NaClO_2$, NaH_2PO_4, 67%; (iv) $Pd(OAc)_2$, H_2, EtOH, AcOH, 96%; (v) NaH, MeI, THF, 92%; (vi) TFA, Ac_2O; then NaOMe, MeOH, 65%; (vii) TEMPO, $PhI(OAc)_2$, acetone, water, 64%; (viii) $Pd(OAc)_2$, H_2, MeOH, AcOH, 89%.

2.2. Conformational analysis

The conformational analysis of carbasugar **1** and its 1-*O*-methyl ether derivative **2** (Fig. 1) is based on NMR spectroscopy and molecular modelling. ^1H and ^{13}C NMR chemical shift assignments of the novel compounds used, besides standard 1D ^1H and ^{13}C experiments, 2D $^1H,^1H$-TOCSY, $^1H,^{13}C$-H2BC,[34] $^1H,^{13}C$-HMBC, and Hadamard transform (HT) $^1H,^{13}C$-HSQC experiments.[35] The $^1H,^{13}C$-HSQC-HT experiment was used to rapidly and efficiently obtain the one-bond $^1H,^{13}C$-correlations. Since the ^{13}C resonances from both **1** and **2** were resolved in the 1D ^{13}C NMR spectra and the compounds have, respectively, six and seven carbons that carry protons, a Hadamard-8 matrix was optimal for irradiation. In particular, as the 'all-plus' column of the Hadamard matrix is normally not used since it is not effective in reducing instrumental artefacts.[36] The $^1H,^{13}C$-HSQC-HT spectra of **1** are presented in Figure 2 and the ^1H and ^{13}C NMR chemical shift assignments of the two compounds are compiled in Table 1. Analysis of the homo- and heteronuclear coupling constants (vide infra) showed that the conformational equilibria in the two compounds are very similar, and therefore the detailed conformational analysis is focussed on compound **1**.

The conformational analysis is based on the following: $^3J_{H,H}$ values, $^3J_{H,C}$ values, effective $^1H,^1H$-distances derived from cross-relaxation rates in 1D T-ROESY and NOESY experiments and ^1H chemical shifts. The $J_{H,H}$ coupling constants were extracted by a total-lineshape analysis[37] carried out with the PERCH NMR spin simulation software, which resulted in an excellent agreement between the experimental and the simulated ^1H NMR spectra