Carbohydrate Research 345 (2010) 984–993

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

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

Elin Säwén, Mattias U. Roslund, Ian Cumpstey, Göran Widmalm *

Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, S-106 91 Stockholm, Sweden

ARTICLE INFO

Article history:
Received 18 December 2009
Received in revised form 15 February 2010
Accepted 1 March 2010
Available online 6 March 2010

Presented at the SMASH 2008 NMR Conference, Santa Fe, NM, USA, September 7–10, 2008

Keywords:
Heparin
Conformational equilibrium
NMR
Molecular modelling

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]

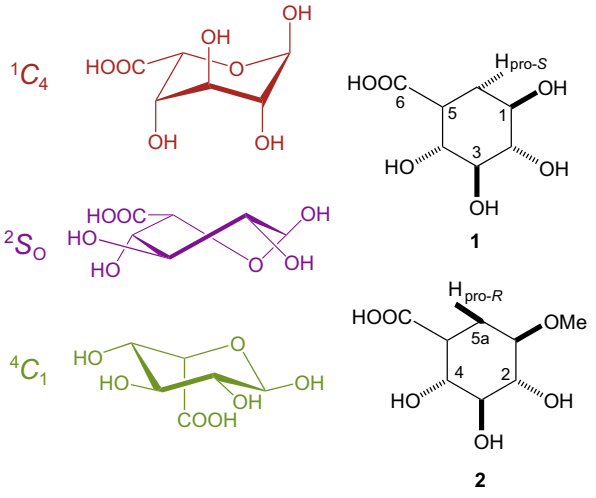

Figure 1. Schematic of possible conformations for α-L-iduronic acid (left column) and the carbasugar analogues thereof synthesised herein (right column).

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

* Corresponding author.
E-mail address: gw@organ.su.se (G. Widmalm).

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doi:10.1016/j.carres.2010.03.002

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]

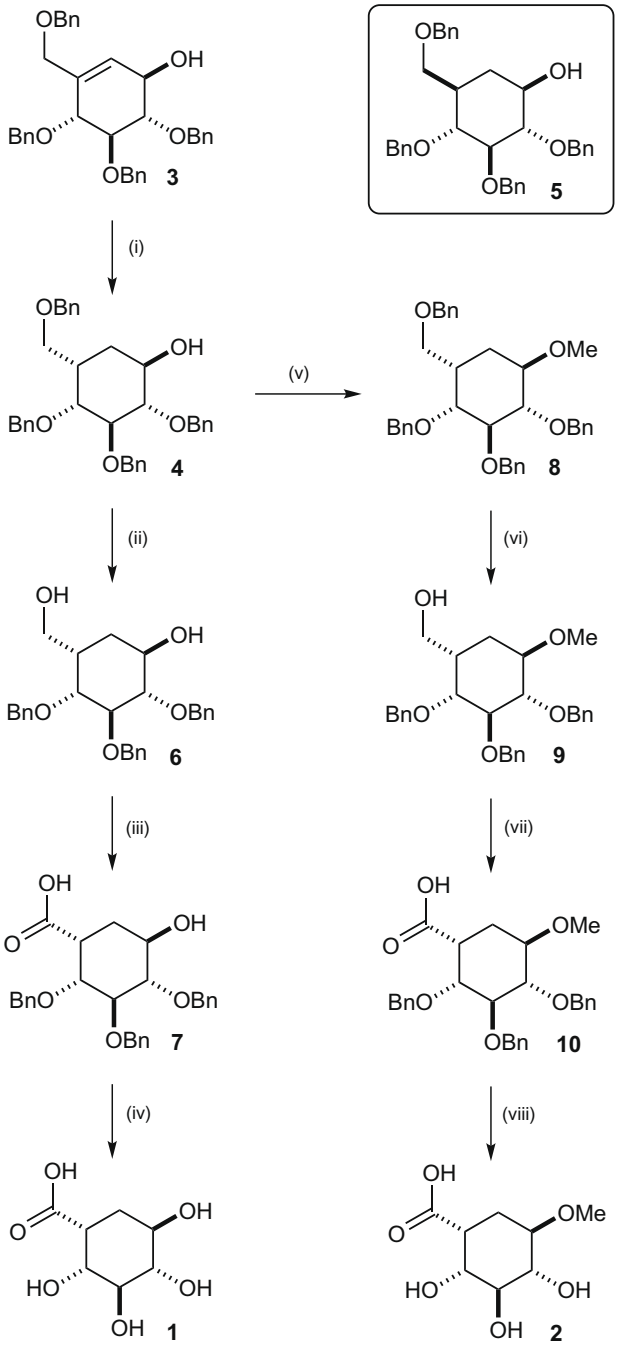
We were interested as to whether carbaiduronic acid derivatives would have a conformational profile that would give them the potential to be useful mimics of iduronic acid, and we therefore undertook the synthesis of carbasugar **1** and its 1-*O*-methyl ether derivative **2** (Fig. 1) and investigated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

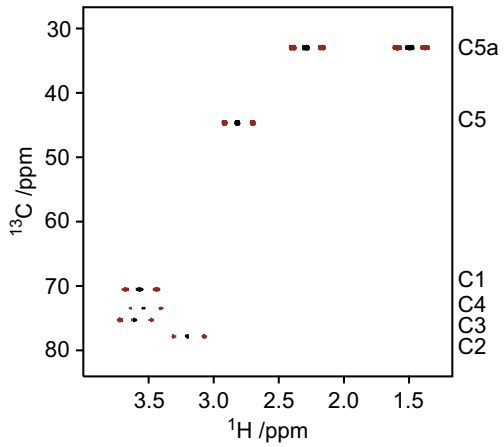


Figure 2. ^{1}H,^{13}C-HSQC-Hadamard transform spectra of **1** (^{1}H-decoupled: black cross-peaks; ^{13}C-coupled: red cross-peaks).

(Fig. 3a and b). The $^{3}J_{H,H}$ values thus obtained (Table 1) could be used in the conformational analysis. Based on previous conformational analyses of α-L-Ido*p*A and its derivatives, it is reasonable to consider the following three conformations in **1**: $^{4}C_{1}$, $^{2}S_{5a}$ and $^{1}C_{4}$. For a conformational equilibrium between the three ring-conformations, the NMR observables will be averaged by rapid exchange. An indication of the predominant conformation may be obtained by predicting the ^{1}H NMR chemical shifts and proton–proton coupling constants for each conformation. This was carried out using the PERCH NMR simulation software for which the $^{4}C_{1}$, $^{2}S_{5a}$ and $^{1}C_{4}$ energy-minimised conformations of **1** were given as input. Comparison of the predicted ^{1}H NMR spectra with the experimental ^{1}H NMR spectrum (Fig. 3c–e vs a) shows that the $^{4}C_{1}$ conformation agrees the best. This result indicates that this conformation should be present for **1**. A comparison between experimental and predicted $^{3}J_{H,H}$ values, using the Karplus-type relationships proposed by Haasnot et al.[38] and implemented in the Janocchio software,[39] confirms this conclusion (Fig. 4a). All the $J_{H,H}$ values of compound **2** (Table 1) are very similar to those of **1**. Consequently, whatever conformation or conformational equilibrium is present in **1** is also present in **2**.

^{1}H,^{13}C-Heteronuclear coupling constants give, like $^{n}J_{H,H}$, information on conformational preferences, in particular, of torsion angles via their Karplus-type relationships. Several approaches[40] to extract these *J*-couplings have been reported, e.g., from 1D NMR spectra.[41,42] In the present study we employ three different 2D NMR experiments to measure $^{n}J_{H,C}$, viz., (i) ^{1}H,^{13}C-HSQC-HECADE,[43] (ii) J-HMBC[44] and (iii) IPAP-HSQC-TOCSY-HT.[35,45,46] NMR spectra resulting from the three experiments are presented in Fig. 5a–c, respectively. For all spectra, $^{n}J_{H,C}$ values are obtained from a peak-to-peak separation. The signs of the coupling constants may be obtained from the relative peak separation in the HSQC-HECADE and IPAP-HSQC-TOCSY-HT experiments. If spectral overlap occurs, one may in the HSQC-HECADE or the J-HMBC experiments alter a scaling factor for the F_1-peak separation to resolve the overlap. The IPAP-HSQC-TOCSY-HT experiment requires significantly less experimental time than the other two experiments, which have conventional t_1-evolution times. The $^{n}J_{H,C}$ coupling constants, which were quantified and were consistent between the experimental spectra within ±0.5 Hz of the average, are compiled in Table 2. Two-bond coupling constants are negative whereas three-bond coupling constants are positive. In the same way as for the $^{3}J_{H,H}$ coupling constants, the $^{3}J_{H,C}$ coupling constants were analysed for the three ring-conformations using the Karplus-type relationship $^{3}J_{H,C} = 4.26 - 1.00\cos\theta + 3.56\cos 2\theta$ as proposed by Wasylishen and Schaefer.[47] The results for the three conformations are plotted in Figure 4b, and again the $^{4}C_{1}$ conformation shows the best agreement between experimental and calculated values from the energy-minimised structures.

Effective proton–proton distances may be obtained from 1D ^{1}H,^{1}H T-ROESY and ^{1}H,^{1}H NOESY experiments[48,49] in which proton resonances are selectively targeted and build-up curves are created as a function of the mixing times used (Fig. 6). The cross-relaxation rates (σ) are subsequently obtained from the initial slopes and the effective distances (r_{ij}) are derived from $r_{ij} = r_{\mathrm{ref}}(\sigma_{\mathrm{ref}}/\sigma_{ij})^{1/6}$, compiled in Table 3. The agreement between the effective proton–proton distances from T-ROE and NOE is excellent, and a comparison between experimentally derived distances and those obtained from the molecular models confirms that the $^{4}C_{1}$ conformation is the predominant one (Table 3 and Fig. 4c). In this analysis, the effective distances between H5 and H5a$_{\mathrm{pro}\text{-}S}$ and between H5a$_{\mathrm{pro}\text{-}R}$ and H2 differ substantially between the three conformations, whereas the distance between H5a$_{\mathrm{pro}\text{-}S}$ and H2 was approximately 3.83 Å in all three conformations and was not used to investigate conformational preferences.

Based on ^{1}H chemical shifts and homo- and heteronuclear coupling constants together with effective proton–proton distances, the equilibrium in **1** (and **2**) is shifted far towards the $^{4}C_{1}$ conformation (Fig. 7), which is the predominant or exclusive conformation with only small if any populations of $^{2}S_{5a}$ and $^{1}C_{4}$, since increased populations of these conformations would not lead to significantly better agreement with experimental data (cf. Fig. 4). The exchange of the ring-oxygen in α-L-Ido*p*A for a methylene group results in the absence of an anomeric effect that would stabilise the conformation with an axial C1–O1 bond, i.e., the $^{1}C_{4}$ conformer. Thus, the driving force to populate the $^{1}C_{4}$ conformation in a $^{4}C_{1} \rightleftharpoons {}^{2}S_{5a} \rightleftharpoons {}^{1}C_{4}$ equilibrium is not present to any significant extent in these carbasugars. The importance of the anomeric effect in general and the *exo*-anomeric effect in particular has also been noted for carba- and C-fucopyranosides[50,51] where, in its absence, conformations that would not be populated to any significant extent then are present. The fact that internal α-L-Ido*p*A and 2-*O*-sulfo-α-L-Ido*p*A in oligosaccharide sequences related to heparin only populate the $^{1}C_{4}$ and $^{2}S_{O}$ conformations whereas non-sulfated α-L-Ido*p*A as a terminal group populates also the $^{4}C_{1}$ conformation[3] indicates that the carbaiduronic acid bioisosteres may still be suitable as hydrolytically stable residues in synthetic oligosaccharides.

Table 1
^{1}H and ^{13}C NMR chemical shifts and ^{1}H,^{1}H coupling constants of compounds **1** and **2** at 27 °C in D_2O

			1	2	3	4	5	5a		6	Me
			$^{3}J_{H1,H5a(pro\text{-}R)/H5a(pro\text{-}S)}$	$^{3}J_{H2,H1/H3}$	$^{3}J_{H3,H4}$	$^{3}J_{H4,H5}$	$^{3}J_{H5,H5a(pro\text{-}R)/H5a(pro\text{-}S)}$	$^{2}J_{H5a(pro\text{-}R)/H5a(pro\text{-}S)}$			
1	^{1}H	δ ppm	3.56	3.20	3.60	3.53	2.81	1.49 (pro-*R*)	2.29 (pro-*S*)		
		$^{n}J_{H,H}$/Hz	11.94, 4.67	9.34, 9.30	9.78	5.57	4.95, 2.97	−13.54			
	^{13}C	δ ppm	70.5	78.0	75.2	73.6	44.1	32.0		181.2	
2	^{1}H	δ/ppm	3.267	3.274	3.61	3.53	2.84	1.34 (pro-*R*)	2.50 (pro-*S*)		3.44
		$^{n}J_{H,H}$/Hz	11.43, 4.36	9.57, 9.33	9.71	5.54	4.72, 3.10	−13.44			
	^{13}C	δ ppm	80.3	76.7	75.4	73.5	43.8	28.4		181.0	57.5

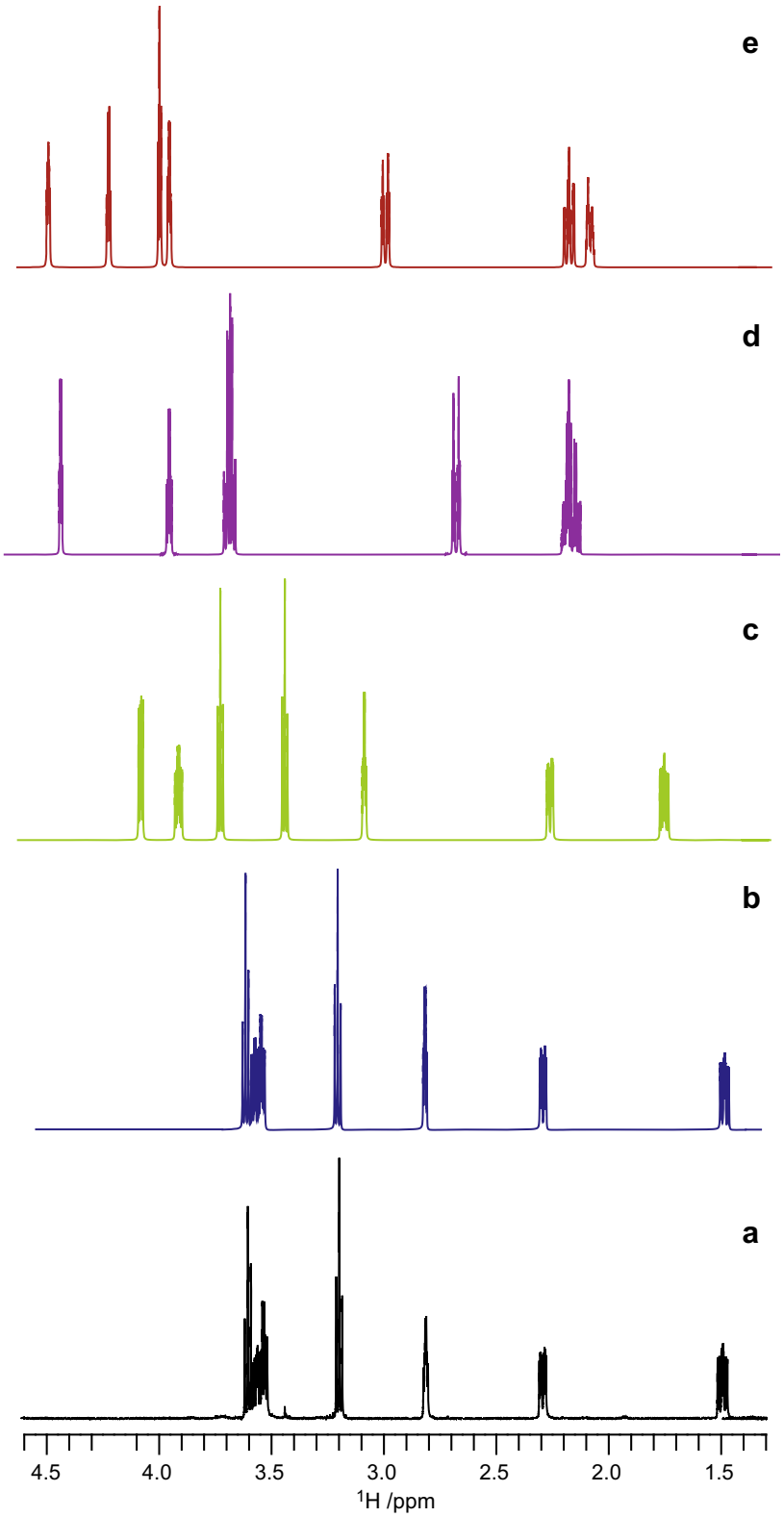


Figure 3. ^{1}H NMR analysis at 700 MHz of **1**: (a) experimental spectrum (black), (b) simulated spectrum by total-lineshape analysis using the PERCH NMR software to obtain ^{1}H,^{1}H coupling constants (blue), (c) predicted by PERCH NMR software for the $^4C_1$ conformation (green), (d) for the $^2S_{5a}$ conformation (purple) and (e) for the $^1C_4$ conformation (red).

3. Conclusions

The conformational preferences of the two novel carbaiduronic acid derivatives **1** and **2** of α-L-Ido*p*A were studied by ^{1}H NMR chemical shifts, $^3J_{H,H}$, and $^3J_{H,C}$ obtained by three different 2D NMR experiments as well as proton–proton distances from two different types of nuclear Overhauser experiments. The carbasugar bioisosteres do not exhibit the conformational flexibility that has

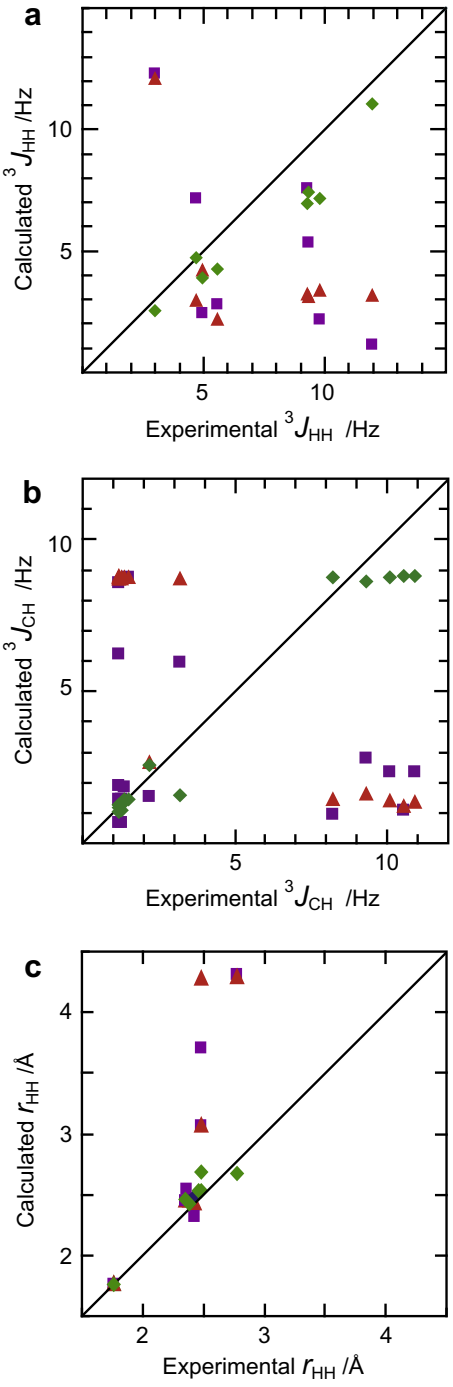


Figure 4. Experimental versus calculated data of **1** for the three conformations $^4C_1$ (green diamonds), $^2S_{5a}$ (purple squares) and $^1C_4$ (red triangles): (a) $J_{H,H}$; (b) $J_{C,H}$; (c) $^1H,^1H$ distance.

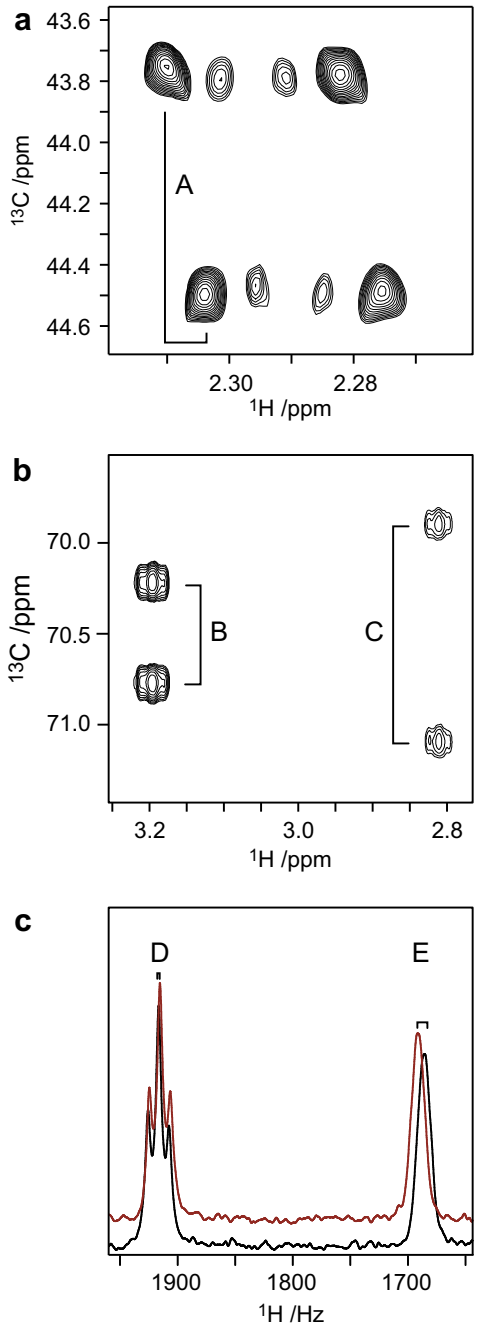


Figure 5. Two- and three-bond heteronuclear correlations in **1**: (a) $^1H,^{13}C$-HSQC-HECADE spectrum (mixing time 40 ms) showing the $H5a_{pro\text{-}S}$ to C5 correlation (A); (b) J-HMBC spectrum with $\kappa = 20.6$ showing the H2 to C1 (B) and the H5 to C1 (C) correlations (c) IPAP-HSQC-TOCSY-HT (mixing time 50 ms) showing the H2 to C5a (D) and the H5 to C5a (E) correlations.

been reported for α-L-iduronic acid in oligo- or polysaccharides and which is of great importance for its interaction with some protein receptors, such as antithrombin. The present study shows that the conformational equilibrium in the six-membered ring may be investigated in detail using different NMR techniques. To be able to use these carbasugars in (pseudo)oligosaccharides that mimic the biological activity of heparin, the influence by a 2-*O*-sulfo group or by sugar residues at O1 and O4 of the carbasugar on its conformation should be investigated. Substitution with a 2-*O*-sulfo group and incorporation of the carbasugar into a heparin-like oligosaccharide that may affect the conformational properties of the carbasugar, as has been seen for iduronic acid itself, would be the most obvious extension of this work. It is hoped that oligosaccharides having these chemically modified and hydrolytically stable residues may be of importance as therapeutic agents of medical significance related to cancer processes.[52]

4. Experimental section

4.1. General methods

Proton nuclear magnetic resonance (^1H) spectra (**4**–**10**) were recorded on Bruker Avance II 400 (400 MHz) and Varian Mercury

Table 2
Two- and three-bond heteronuclear coupling constants extracted from ^{1}H,^{13}C-HSQC-HECADE, J-HMBC and IPAP-HSQC-TOCSY-HT experiments

Carbon	Proton	Compound **1**			Compound **2**		
		HECADE	J-HMBC	IPAP-TOCSY	HECADE	J-HMBC	IPAP-TOCSY
		J/Hz	$\|J\|$/Hz	J/Hz	J/Hz	$\|J\|$/Hz	J/Hz
C1	H2	−5.1	4.7		−5.1	5.4	
	H3	1.0	1.1	1.5	1.3	1.8	2.3
	H5	10.0	10.2		10.6	10.1	
	H5a$_{pro\text{-}R}$	−6.0	6.2	−6.2	−6.0	6.3	
	H5a$_{pro\text{-}S}$	−5.7	5.7	−5.4	−5.0	5.5	
	OMe				>0	4.6	
C2	H1		4.1			5.2	−5.1
	H3	−4.5	4.6		−4.1	4.2	
	H4		1.2		1.1		
	H5a$_{pro\text{-}R}$	3.4	3.0		2.8	2.9	
	H5a$_{pro\text{-}S}$	10.4	10.7		10.6	10.7	
C3	H1		1.3				
	H2	−5.0	4.8				
	H4		6.2				
	H5	7.8	8.6		9.2	8.4	
C4	H2	1.2	1.3	1.4			
	H3	−5.8	5.8	−5.4		7.2	
	H5	−6.7	6.4		−6.6	6.5	−6.1
	H5a$_{pro\text{-}R}$	1.5	1.5		2.6	1.8	2.1
	H5a$_{pro\text{-}S}$	10.8	11.0		10.5	10.7	
C5	H1		1.2				
	H3		1.1	1.6	0.7	1.1	2.1
	H4	−2.4	2.7	−2.8	−2.6		−3.6
	H5a$_{pro\text{-}R}$	−3.6	3.4	−3.4	−3.5	3.6	−3.7
	H5a$_{pro\text{-}S}$	−4.4	4.3	−4.2	−4.6	4.2	−5.1
C5a	H1	−2.0	1.8	−2.2			
	H2	1.2	1.1	1.2			
	H4		1.2		>0		
	H5	−5.1	5.0	−5.5	−4.5	4.9	−4.6
C6	H4					9.3	
	H5		6.6			6.4	
	H5a$_{pro\text{-}R}$		9.3				
	H5a$_{pro\text{-}S}$		2.2				

400 (400 MHz) spectrometers; multiplicities are quoted as singlet (s), broad singlet (br s), doublet (d), doublet of doublets (dd), etc. or multiplet (m). Carbon nuclear magnetic resonance (^{13}C) spectra (**4**–**10**) were recorded on Bruker Avance II 400 (100 MHz) and Varian Mercury 400 (100 MHz) spectrometers. Spectra were assigned using COSY, HSQC and DEPT experiments. Chemical shifts and coupling constants were recorded in units of ppm and Hz. Residual solvent signals were used as an internal reference for $CDCl_3$: δ_H = 7.26 ppm and δ_C = 77.16 ppm for compounds **4**–**10**. In order to extract accurate coupling constant values, computational spectral analyses of all the ^{1}H NMR spectra were performed with the PERCH NMR software. For molecules with several benzyl groups, the substitution positions have not been determined but they are distinguished by different number of primes. Low- and high-resolution (HRMS) electrospray (ES) mass spectra were recorded using a Bruker Microtof instrument. Infra-red spectra were recorded on a Perkin–Elmer Spectrum One FT-IR spectrometer using the thin film method on NaCl plates. Optical rotations were measured on a Perkin–Elmer 241 polarimeter with a path length of 1 dm; concentrations are given in g/100 mL. Thin layer chromatography (TLC) was carried out on Merck Kieselgel sheets, pre-coated with $60F_{254}$ silica. The plates were visualised with UV light or developed using 10% sulfuric acid or an ammonium molybdate (10% w/v) and cerium (IV) sulfate (2% w/v) solution in 10% sulfuric acid. Flash column chromatography was carried out on silica gel (35–70 μm, Grace). Dichloromethane was distilled from calcium hydride. THF was distilled from sodium benzophenone ketyl radical. Reactions performed under an atmosphere of hydrogen or nitrogen were maintained by an inflated balloon.

4.1.1. 5a-Carba-2,3,4,6-tetra-*O*-benzyl-α-L-idopyranose (4)

Unsaturated alcohol **3** (157 mg, 0.29 mmol) was dissolved in EtOAc (4 mL), and palladium (10% on carbon, 15 mg) and triethylamine (41 μL, 0.29 mmol) were added. The mixture was degassed and stirred under an atmosphere of hydrogen. After 2 h, the mixture was filtered through Celite and concentrated in vacuo. The residue was purified by flash column chromatography (CH_2Cl_2/ether, 30:1) to give protected carbaidose **4** (123 mg, 78%) as a colourless oil. $[\alpha]_D^{22}$ −34.6 (*c* 1.0 in $CHCl_3$); ν_{max}(film)/cm^{-1} 3376 (br, OH st); δ_H (400 MHz, $CDCl_3$) 1.42 (1H, ddd, $J_{5a(pro\text{-}S),5a(pro\text{-}R)}$ −13.6 Hz, H5a$_{pro\text{-}S}$), 2.24 (1H, ddd, H5a$_{pro\text{-}R}$), 2.46 (1H, br s, OH1), 2.52 (1H, ddddd, $J_{5,5a(pro\text{-}S)}$ 4.8 Hz, $J_{5,5a(pro\text{-}R)}$ 4.3 Hz, $J_{5,6}$ 4.7 Hz, $J_{5,6'}$ 8.3 Hz, H5), 3.35 (1H, dd, $J_{2,3}$ 8.2 Hz, H2), 3.53 (1H, dd, H6′), 3.720 (1H, dd, $J_{4,5}$ 5.0 Hz, H4), 3.721 (1H, dd, $J_{6,6'}$ −9.4 Hz, H6), 3.73 (1H, dd, $J_{3,4}$ 8.6 Hz, H3), 3.89 (1H, ddd, $J_{1,2}$ 8.4 Hz, $J_{1,5a(pro\text{-}S)}$ 10.7 Hz, $J_{1,5a(pro\text{-}R)}$ 4.6 Hz, H1), 4.54, 4.57 (2H, 2 × d, J −12.2 Hz, PhCH_2), 4.62, 4.66 (2H, 2 × d, J −11.5 Hz, PhCH_2'), 4.69, 4.95 (2H, 2 × d, J −11.4 Hz, PhCH_2''), 4.73, 4.89 (2H, 2 × d, J −10.9 Hz, PhCH_2'''), 7.27–7.40 (20H, m, Ph); δ_C (100 MHz, $CDCl_3$) 29.7 (C5a), 34.9 (C5), 68.5 (C6), 68.7 (C1), 72.4, 73.3, 75.0, 75.2 (PhCH_2), 81.2, 81.6 (C3, C4), 85.3 (C2), 127.6, 127.7, 127.7, 127.7, 127.9, 127.9, 127.9, 128.0, 128.1, 128.4, 128.5, 128.5, 128.7 (Ph–CH), 138.5, 138.5, 138.6, 138.7 (Ph–C); HRMS (ES^+) Calcd for $C_{35}H_{38}O_5Na$ (MNa^+) 561.2611; Found 561.2617. Along with a mixture of *gluco* **5** and *ido* **4** isomers (6:1, 30 mg, 19%).

4.1.2. 5a-Carba-2,3,4-tri-*O*-benzyl-α-L-idopyranose (6)

Alcohol **4** (37 mg, 0.069 mmol) was dissolved in a mixture of Ac_2O (1 mL) and TFA (0.25 mL) and stirred at rt. After 55 min,

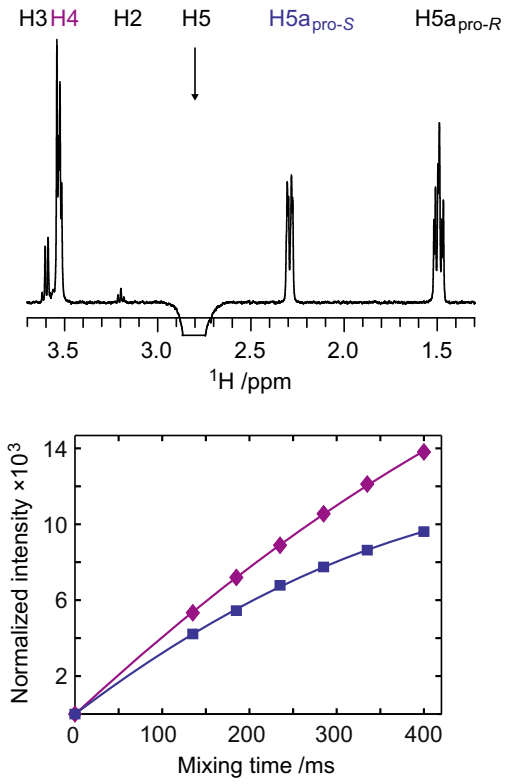


Figure 6. (top) 1D ^{1}H,^{1}H-NOESY spectrum of **1** with selective excitation of the resonance from H5 and a mixing time of 400 ms; (bottom) ^{1}H,^{1}H cross-relaxation build-up curves obtained from the 1D ^{1}H,^{1}H NOESY spectra of **1**; H5 to H5a$_{pro\text{-}S}$ (blue filled squares) and to H4 (cerise filled diamond).

TLC (pentane/EtOAc, 3:1) showed conversion to a major component (R_f 0.2) and little remaining starting material (R_f 0.1). The reaction mixture was diluted with CH_2Cl_2 (25 mL) and washed with $NaHCO_3$ (saturated aqueous, 30 mL). The aqueous phase was re-extracted with CH_2Cl_2 (15 mL). The combined organic extracts were dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was dried under high vacuum then dissolved in MeOH (1.6 mL). Na (8 mg, 0.35 mmol) was added to MeOH (1.6 mL) and the resulting solution was added to the sugar solution and the mixture was stirred at rt. After 2 h 40 min, TLC (pentane/EtOAc, 1:1) showed the formation of a major product (R_f 0.1). The reaction was quenched with ammonium chloride (saturated aqueous, 30 mL) and extracted with ether (30 mL + 15 mL). The combined organic extracts were dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (EtOAc/pentane, 3:2) to give the diol **6** (26 mg, 84%) as a colourless oil. $[\alpha]_D^{21}$ −22.1 (*c* 1.0 in $CHCl_3$) (lit.[30] −19.0 (*c* 1.07 in $CHCl_3$)); ν_{max}(film)/cm^{-1} 3384 (br, OH st); δ_H (400 MHz, $CDCl_3$) 1.45 (1H, ddd, $J_{5a(pro\text{-}S),5a(pro\text{-}R)}$ −13.7 Hz, H5a$_{pro\text{-}S}$), 1.99 (1H, ddd, H5a$_{pro\text{-}R}$), 2.42 (1H, ddddd, $J_{5,5a(pro\text{-}S)}$ 5.0 Hz, $J_{5,5a(pro\text{-}R)}$ 4.3 Hz, $J_{5,6}$ 4.9 Hz, $J_{5,6'}$ 8.2 Hz, H5), 2.63 (1H, br s, OH1), 2.63 (1H, br s, OH6), 3.31 (1H, dd, $J_{2,3}$ 8.3 Hz, H2), 3.59 (1H, dd, $J_{6,6'}$ −11.3 Hz, H6), 3.72 (1H, ddd, $J_{1,2}$ 8.4 Hz, $J_{1,5a(pro\text{-}S)}$ 11.0, $J_{1,5a(pro\text{-}R)}$ 4.6 Hz, H1), 3.76 (1H, dd, $J_{4,5}$ 5.1 Hz, H4), 3.81 (1H, dd, $J_{3,4}$ 8.7 Hz, H3), 3.95 (1H, dd, H6′), 4.67, 4.72 (2H, 2 × d, *J* −11.3 Hz, PhCH_2), 4.68, 4.94 (2H, 2 × d, *J* −11.4 Hz, PhCH_2'), 4.80, 4.88 (2H, 2 × d, *J* −10.9 Hz, PhCH_2''), 7.13–7.36 (15H, m, Ph); δ_C (100 MHz, $CDCl_3$) 30.3 (C5a), 36.9 (C5), 63.9 (C6), 68.8 (C1), 73.5, 75.2, 75.4 (PhCH_2), 81.4 (C3), 82.8 (C4), 85.4 (C2), 127.9, 128.0, 128.1, 128.1, 128.1, 128.6, 128.7, 128.8 (Ph–CH), 137.9, 138.4, 138.6 (Ph–C); HRMS (ES$^+$) Calcd for $C_{28}H_{32}O_5Na$ (MNa$^+$) 471.2142; Found 471.2130.

4.1.3. 5a-Carba-2,3,4-tri-*O*-benzyl-α-L-idopyranuronic acid (7)

Diol **6** (26 mg, 0.058 mmol) was dissolved in CH_2Cl_2 (1.5 mL), and water (0.6 mL) was added. TEMPO (4 mg, 0.026 mmol) and (diacetoxyiodo)benzene (46 mg, 0.15 mmol) were added, and the mixture was stirred at rt. After 1 h 30 min, TLC (EtOAc) showed the formation of a major product (R_f 0.7) and the complete consumption of starting material (R_f 0.5). After a further 5 h, no change

Table 3
^{1}H,^{1}H Distances and cross-relaxation rates for **1** derived from NMR experiments and molecular mechanics models

Atom pair		NMR experiment				Molecular mechanics models[a]		
		T-ROE[b]		NOE[b]		$^4C_1$	$^2S_{5a}$	$^1C_4$
		r_{eff}[c]/Å	$\sigma \times 10^3$/s^{-1}	r_{eff}[c]/Å	$\sigma \times 10^3$/s^{-1}	Distance/Å		
H5	H4	2.38	5.16	2.38	4.27	2.43	2.45	2.45
H5	H5a$_{pro\text{-}R}$	2.35	5.41[d]	2.35	4.66	2.46	2.54	2.47
H5	H5a$_{pro\text{-}S}$	2.47	4.07[d]	2.46	3.49	2.53	3.06	3.07
H5a$_{pro\text{-}R}$	H2	2.50	2.82	2.47	3.44	2.68	3.70	4.28
H5a$_{pro\text{-}R}$	H5a$_{pro\text{-}S}$	1.76[d,e]	30.8	1.76[d,e]	26.1	1.76	1.76	1.76
H5a$_{pro\text{-}S}$	H1	2.42	4.57	2.43	3.81	2.48	2.32	2.43

[a] Energy-minimised in the VEGA ZZ software and geometry optimised using the PERCH NMR software.
[b] Selective excitation of the first resonance in the atom pair.
[c] $r_{ij} = r_{ref}(\sigma_{ref}/\sigma_{ij})^{1/6}$.
[d] Mean values from excitations of both resonances in the atom pair.
[e] Reference distance.

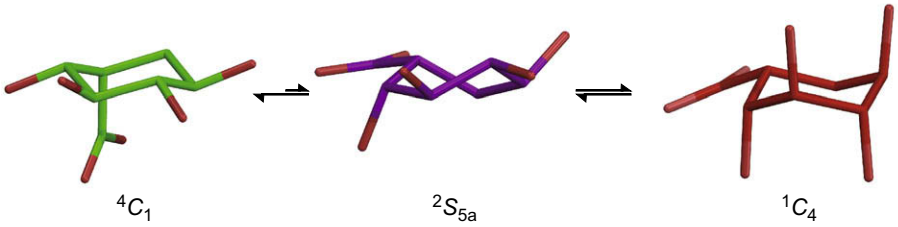


Figure 7. The conformational equilibrium is shifted far to the left with the $^4C_1$ conformation in **1** predominating over the $^2S_{5a}$ and the $^1C_4$ conformations.

was visible by TLC and the reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed with $Na_2S_2O_3$ (10% aqueous, 20 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated in vacuo.

The crude aldehyde was dissolved in a mixture of *n*-butanol (2.5 mL) and DMSO (2.5 mL). $NaClO_2$ (250 mg) was dissolved in water (5 mL) and the pH was adjusted to 4.5 by addition of sodium phosphate (monobasic). 1,3-Dimethoxybenzene (10 μL) was added to the aldehyde solution, then aqueous chlorite solution (0.44 mL) was added, and the mixture was stirred at rt. After 20 min, further 1,3-dimethoxybenzene (70 μL) and chlorite solution (0.44 mL) were added and the mixture was stirred further. After 1 h, ether (25 mL) was added, and the mixture was washed with water (25 mL) and brine (25 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated in vacuo. TLC (EtOAc) showed the presence of a major component (R_f 0.1) and a faster component (R_f 0.9). The residue was purified by flash column chromatography (EtOAc) to give the acid **7** (18 mg, 67%) as a colourless oil. $[\alpha]_D^{21}$ −31.5 (*c* 1.0 in $CHCl_3$); ν_{max}(film)/cm^{-1} 1728 (s, C=O st); δ_H (400 MHz, $CDCl_3$) 1.48 (1H, ddd, $J_{5a(pro\text{-}S),5a(pro\text{-}R)}$ −13.9 Hz, H5a$_{pro\text{-}S}$), 2.34 (1H, ddd, H5a$_{pro\text{-}R}$), 3.06 (1H, ddd, $J_{5,5a(pro\text{-}S)}$ 5.1 Hz, $J_{5,5a(pro\text{-}R)}$ 3.9 Hz, H5), 3.29 (1H, dd, $J_{2,3}$ 8.4 Hz, H2), 3.75 (1H, dd, $J_{4,5}$ 5.6 Hz, H4), 3.92 (1H, dd, $J_{3,4}$ 8.9 Hz, H3), 3.97 (1H, ddd, $J_{1,2}$ 8.6 Hz, $J_{1,5a(pro\text{-}S)}$ 10.8 Hz, $J_{1,5a(pro\text{-}R)}$ 4.6 Hz, H1), 4.68, 4.94 (2H, 2 × d, *J* −11.4 Hz, PhCH_2), 4.75 (2H, s, PhCH_2'), 4.79, 4.87 (2H, 2 × d, *J* −10.9 Hz, PhCH_2''), 7.28−7.35 (15H, m, Ph); δ_C (100 MHz, $CDCl_3$) 29.0 (C5a), 40.9 (C5), 68.7 (C1), 73.9, 75.2, 75.6 (PhCH_2), 80.1 (C4), 81.4 (C3), 84.9 (C2), 127.9, 128.0, 128.0, 128.2, 128.3, 128.4, 128.6, 128.7, 128.8 (Ph–CH), 137.2, 138.3, 138.6 (Ph–C), 175.4 (C=O); HRMS (ES$^+$) Calcd for $C_{28}H_{30}O_6Na$ (MNa$^+$) 485.1935; Found 485.1923.

4.1.4. 5a-Carba-α-L-idopyranuronic acid (1)

Protected acid **7** (15 mg, 0.032 mmol) was dissolved in a mixture of ethanol (2.25 mL) and acetic acid (0.25 mL). Palladium acetate (8 mg) was added and it dissolved to give a yellow solution. The mixture was degassed and stirred under an atmosphere of hydrogen. After 14 h, a black suspension had appeared and the solution was colourless. TLC ($CHCl_3$/MeOH/AcOH/H_2O, 60:30:3:5) showed the formation of a major polar product (R_f 0.2). The mixture was filtered through Celite and concentrated in vacuo. The residue was purified on a Waters Sep-Pak C-18 cartridge eluting with water, to give the deprotected carbasugar **1** (6 mg, 96%) as a colourless oil. $[\alpha]_D^{22}$ −38 (*c*, 0.3 in MeOH); ^{1}H NMR and ^{13}C NMR: See Table 1. HRMS (ES$^+$) Calcd for $C_7H_{12}O_6Na$ (MNa$^+$) 215.0526; Found 215.0530.

4.1.5. Methyl 5a-carba-2,3,4,6-tetra-*O*-benzyl-α-L-idopyranoside (8)

Alcohol **4** (70 mg, 0.13 mmol) was dissolved in THF (2 mL), and methyl iodide (16 μL, 0.26 mmol) and sodium hydride (60% in oil, 16 mg, 0.39 mmol) were added. The mixture was stirred under N_2 and further methyl iodide (16 μL, 0.26 mmol) was added after 2 h. After a further 3 h 30 min, TLC (pentane/EtOAc, 3:1) showed the formation of a major product (R_f 0.8), and almost no remaining starting material (R_f 0.3). The reaction was quenched by the addition of methanol, then silica was added and the solvent was removed in vacuo. The residue was purified directly by flash column chromatography (pentane/EtOAc, 6:1–4:1) to give the methyl ether **8** (66 mg, 92%) as a colourless oil. $[\alpha]_D^{21}$ −27.2 (*c* 1.0 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.24 (1H, ddd, $J_{5a(pro\text{-}S),5a(pro\text{-}R)}$ −13.6 Hz, H5a$_{pro\text{-}S}$), 2.34 (1H, ddd, H5a$_{pro\text{-}R}$), 2.49 (1H, ddddd, $J_{5,5a(pro\text{-}S)}$ 4.5 Hz, $J_{5,5a(pro\text{-}R)}$ 3.2 Hz, $J_{5,6}$ 4.2 Hz, $J_{5,6'}$ 9.4 Hz, H5), 3.38 (1H, dd, $J_{2,3}$ 9.1 Hz, H2), 3.448 (1H, ddd, $J_{1,2}$ 9.4 Hz, $J_{1,5a(pro\text{-}S)}$ 11.3 Hz, $J_{1,5a(pro\text{-}R)}$ 5.0 Hz, H1), 3.449 (3H, s, OMe), 3.49 (1H, dd, H6′), 3.61 (1H, dd, $J_{3,4}$ 9.5 Hz, H3), 3.63 (1H, dd, $J_{4,5}$ 5.2 Hz, H4), 3.70 (1H, dd, $J_{6,6'}$ −9.4 Hz, H6), 4.50, 4.59 (2H, 2 × d, *J* −12.2 Hz, PhCH_2), 4.61, 4.65 (2H, 2 × d, *J* −11.5 Hz, PhCH_2'), 4.76, 4.84 (2H, 2 × d, *J* −10.8 Hz, PhCH_2''), 4.80, 4.82 (2H, 2 × d, *J* −11.0 Hz, PhCH_2'''), 7.26–7.37 (20H, m, Ph); δ_C (100 MHz, $CDCl_3$) 27.9 (C5a), 35.4 (C5), 58.2 (OMe), 67.8 (C6), 72.5, 73.3, 75.7, 75.8 (PhCH_2), 79.5 (C1), 81.7, 82.5 (C3, C4), 85.9 (C2), 127.6, 127.6, 127.7, 127.7, 127.8, 127.8, 128.1, 128.1, 128.4, 128.4, 128.5, 128.6 (Ph–CH), 138.6, 138.7, 139.1, 139.1 (Ph–C); HRMS (ES$^+$) Calcd for $C_{36}H_{40}O_5Na$ (MNa$^+$) 575.2768; Found 575.2761.

4.1.6. Methyl 5a-carba-2,3,4-tri-*O*-benzyl-α-L-idopyranoside (9)

Fully protected **8** (66 mg, 0.12 mmol) was dissolved in a mixture of Ac_2O (2 mL) and TFA (0.5 mL) and stirred at rt. After 55 min, TLC (pentane/EtOAc, 3:1) showed conversion to a major component (R_f 0.5) and no remaining starting material (R_f 0.55). The reaction mixture was diluted with CH_2Cl_2 (25 mL) and washed with $NaHCO_3$ (saturated aqueous, 30 mL). The aqueous phase was re-extracted with CH_2Cl_2 (15 mL). The combined organic extracts were dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was dried under high vacuum then dissolved in MeOH (1.6 mL). Na (8 mg, 0.35 mmol) was added to MeOH (1.6 mL) and the resulting solution was added to the sugar solution and the mixture was stirred at rt. After 1 h, TLC (pentane/EtOAc, 1:1) showed the formation of a major product (R_f 0.5). The reaction was quenched by the addition of Dowex IR-120 (H$^+$) resin, filtered and concentrated in vacuo. The residue was purified by flash column chromatography (EtOAc/pentane, 4:5) to give the alcohol **9** (36 mg, 65%) as a colourless oil. $[\alpha]_D^{21}$ +4.8 (*c* 1.0 in $CHCl_3$); ν_{max} (film)/cm^{-1} 3440 (br, OH st); δ_H (400 MHz, $CDCl_3$) 1.34 (1H, ddd, $J_{5a(pro\text{-}S),5a(pro\text{-}R)}$ −13.8 Hz, H5a$_{pro\text{-}S}$), 2.07 (1H, ddd, H5a$_{pro\text{-}R}$), 2.43 (1H, ddddd, $J_{5,5a(pro\text{-}S)}$ 5.0 Hz, $J_{5,5a(pro\text{-}R)}$ 3.4 Hz, $J_{5,6}$ 5.1 Hz, $J_{5,6'}$ 8.3 Hz, H5), 2.72 (1H, br s, OH6), 3.35 (1H, ddd, $J_{1,2}$ 8.8 Hz, $J_{1,5a(pro\text{-}S)}$ 11.6 Hz, $J_{1,5a(pro\text{-}R)}$ 4.8 Hz, H1), 3.40 (1H, dd, $J_{2,3}$ 8.8 Hz, H2), 3.45 (3H, s, OMe), 3.58 (1H, dd, $J_{6,6'}$ −11.2 Hz, H6), 3.73 (1H, dd, $J_{4,5}$ 5.2 Hz, H4), 3.77 (1H, dd, $J_{3,4}$ 9.3 Hz, H3), 3.98 (1H, dd, H6′), 4.69, 4.74 (2H, 2 × d, *J* −11.3 Hz, PhCH_2), 4.81, 4.86 (2H, 2 × d, *J* −10.8 Hz, PhCH_2'), 4.85, 4.86 (2H, 2 × d, *J* −10.9 Hz, PhCH_2''), 7.28−7.36 (15H, m, Ph); δ_C (100 MHz, $CDCl_3$) 28.9 (C5a), 37.3 (C5), 58.2 (OMe), 63.8 (C6), 73.8, 75.7, 75.8 (PhCH_2), 79.7 (C1), 82.1 (C3), 83.2 (C4), 85.9 (C2), 127.7, 127.7, 128.0, 128.1, 128.1, 128.1, 128.5, 128.5, 128.7 (Ph–CH), 138.1, 138.9, 139.0 (Ph–C); HRMS (ES$^+$) Calcd for $C_{29}H_{34}O_5Na$ (MNa$^+$) 485.2298; Found 485.2303.

4.1.7. Methyl 5a-carba-2,3,4-tri-*O*-benzyl-α-L-idopyranosiduronic acid (10)

Alcohol **9** (35 mg, 0.076 mmol) was dissolved in acetone (2 mL), and water (1 mL) was added. TEMPO (5 mg, 0.030 mmol) and diacetoxyiodobenzene (65 mg, 0.19 mmol) were added, and the mixture was stirred at rt. After 3 h, TLC (EtOAc/pentane, 2:1) showed the formation of a major product (R_f 0.9) and the complete consumption of starting material (R_f 0.6). After a further 5 h, significant amounts of a new product (R_f 0.2) had appeared, but much of the initially formed product remained. After a further 18 h, further diacetoxyiodobenzene (37 mg, 0.11 mmol) was added. After a further 24 h, TLC (EtOAc/pentane, 2:1) showed the polar compound (R_f 0.2) to be the major reaction component, and very little of the initially formed product (R_f 0.9) remaining. The mixture was diluted with CH_2Cl_2 (30 mL) and washed with $Na_2S_2O_3$. HCl (1 M, 20 mL) was added to the aqueous phase, which was then extracted with CH_2Cl_2 (20 mL). The combined organic extracts were dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (EtOAc/pentane, 2:1) to give the acid **10** (23 mg, 64%) as a colourless oil. $[\alpha]_D^{21}$ −9.1 (*c* 1.0 in $CHCl_3$); ν_{max}(film)/cm^{-1} 1731 (s, C=O st); δ_H (400 MHz, $CDCl_3$) 1.31 (1H, dd, $J_{5a(pro\text{-}S),5a(pro\text{-}R)}$ −13.8 Hz, H5a$_{pro\text{-}S}$), 2.48 (1H, dd,

H5a$_{pro\text{-}R}$), 3.00 (1H, ddd, $J_{5,5a(pro\text{-}S)}$ 4.8 Hz, $J_{5,5a(pro\text{-}R)}$ 3.3 Hz, H5), 3.37 (1H, dd, $J_{2,3}$ 8.9 Hz, H2), 3.48 (3H, s, OMe), 3.59 (1H, ddd, $J_{1,2}$ 8.9 Hz, $J_{1,5a(pro\text{-}S)}$ 11.3 Hz, $J_{1,5a(pro\text{-}R)}$ 4.8 Hz, H1), 3.71 (1H, dd, $J_{4,5}$ 5.8 Hz, H4), 3.78 (1H, dd, $J_{3,4}$ 9.6 Hz, H3), 4.75, 4.80 (2H, 2 × d, J −11.5 Hz, PhCH_2), 4.79, 4.87 (2H, 2 × d, J −10.9 Hz, PhCH'_2), 4.81, 4.84 (2H, 2 × d, J −10.7 Hz, PhCH''_2), 7.26–7.35 (15H, m, Ph); δ_C (100 MHz, $CDCl_3$) 27.4 (C5a), 41.1 (C5), 58.4 (OMe), 74.1, 75.5, 75.9 (PhCH_2), 79.4 (C1), 80.1 (C4), 81.8 (C3), 85.1 (C2), 127.6, 127.8, 128.0, 128.1, 128.2, 128.3, 128.4, 128.5, 128.5, 128.8 (Ph–CH), 137.1, 138.6, 139.0 (Ph–C), 175.0 (C=O); HRMS (ES^+) Calcd for $C_{29}H_{32}O_6Na$ (MNa^+) 499.2091; Found 499.2116.

4.1.8. Methyl 5a-carba-α-L-idopyranosiduronic acid (2)

Protected acid **10** (13 mg, 0.027 mmol) was dissolved in a mixture of methanol (2.25 mL) and acetic acid (0.25 mL). Palladium acetate (7 mg) was added and it dissolved to give a yellow solution. The mixture was degassed and stirred under an atmosphere of hydrogen. After 22 h, a black suspension had appeared and the solution was colourless. TLC ($CHCl_3$/MeOH/AcOH/H_2O, 60:30:3:5) showed the formation of a major polar product (R_f 0.3). The mixture was filtered through Celite and concentrated in vacuo. The residue was purified on a Waters Sep-Pak C-18 cartridge, eluting with water to give the deprotected carbasugar **2** (5 mg, 89%) as a colourless oil. $[\alpha]_D^{22}$ −52 (*c* 0.2 in MeOH); *m/z* (ES^+) 435 (2 M+Na^+, 40), 229 (M+Na^+, 100%); ^{1}H NMR and ^{13}C NMR: See Table 1. HRMS (ES^+) Calcd for $C_{16}H_{28}O_{12}Na$ (2MNa^+) 435.1473; Found 435.1467.

4.2. NMR spectroscopy

NMR experiments on **1** (44 mM) and **2** (24 mM) in D_2O (pD ≈ 6) were performed at 27 °C on three different spectrometers, a Varian INOVA 600 MHz spectrometer equipped with a 5 mm PFG triple resonance probe, a Bruker AVANCE 500 MHz spectrometer and a Bruker AVANCE III 700 MHz spectrometer; the latter two equipped with 5 mm TCI Z-Gradient CryoProbes. ^{1}H chemical shifts were referenced to internal TSP (δ_H 0.00) and ^{13}C chemical shifts were referenced to external dioxane in D_2O (δ_C 67.40). ^{1}H chemical shift assignments were performed using ^{1}H,^{1}H-TOCSY experiments recorded over 5 ppm with 1466 × 128 points in $F_2 \times F_1$, respectively, and 8 scans per t_1 increment using the States-TPPI method. An MLEV-17 spin-lock of 12.7 kHz and two different mixing times, 10 ms and 50 ms, were used. Zero-filling was performed to 4096 × 512 points. Prior to Fourier transformation a 90° shifted sine-bell function was applied in the direct dimension and a 90° shifted squared sine-bell function was used in the indirect dimension. For **2** a ^{1}H,^{13}C-H2BC experiment[34] was recorded over 10 ppm in the direct dimension and 165 ppm in the indirect dimension with 2048 × 256 points and 64 scans per t_1 increment with the echo/anti-echo method. A constant time delay of 22 ms was used for $J_{H,H}$ evolution. Zero-filling to 2048 × 1024 points and a 90° shifted squared sine-bell function was applied prior to Fourier transformation. The H2BC spectrum was processed in magnitude mode. For **2** a ^{1}H,^{13}C-HMBC experiment was also recorded over 5 ppm in the direct dimension and 96 ppm in the indirect dimension with 2048 × 256 points and 64 scans per t_1 increment with the States-TPPI method. A 50 ms long evolution time for long-range coupling constants was used. Zero-filling to 2048 × 1024 points and a Gaussian line-broadening factor with the maximum at a fraction of 0.28 of the fid were applied prior to Fourier transformation. ^{1}H chemical shifts and $J_{H,H}$ were refined from 1D ^{1}H spectra with the PERCH NMR software[37]

^{13}C chemical shifts were assigned using coupled and decoupled ^{1}H,^{13}C-HSQC-HT experiments.[35] Prior to recording the Hadamard transform (HT) experiment, a ^{13}C spectrum with a spectral width of 96 ppm was performed and from peak-picking, six or seven ^{13}C frequencies were selected for **1** and **2**, respectively. The selected ^{13}C frequencies were irradiated by 45 ms 180° Gaussian shaped pulses (20 Hz bandwidth) and encoded as on or off with a Hadamard-8 matrix using 32 scans. Adiabatic 180° pulses were used for inversion and refocusing on ^{13}C. For ^{1}H decoupling, the same pulse length (45 ms) as for the irradiation was used with adiabatic 180° pulses of a 6.5 kHz sweep width.

The ^{1}H,^{13}C-HSQC-HECADE experiment[43] was performed over a spectral width of 14 ppm for ^{1}H and 65 ppm for ^{13}C. A 40 ms DIPSI-2 spin-lock with ($\gamma B_1/2\pi$ = 9.6 kHz) was applied and a t_1^*/t_1 scaling factor of unity. A 2D matrix of 4096 × 256 points, 16 scans per t_1 increment and the echo/anti-echo method were used. Prior to Fourier transformation zero-filling to 8192 × 1024 points was performed and 90° shifted squared sine-bell functions were applied in both dimensions.

In the J-HMBC experiment[44] a three-fold low-pass *J*-filter (J = 120 Hz, 150 Hz and 190 Hz) was used to suppress the $^1J_{C,H}$. Scale factors between 15 and 24, calculated from $\kappa = (0.6/{}^nJ_{CH}^{min})/t_1^{max}$, were used to scale the coupling constants in the indirect dimension. Spectral widths of 10 ppm for ^{1}H and 100 ppm for ^{13}C were used. The experiments were performed with 3072 × 512 points and 64 scans per t_1 increment with the echo/anti-echo method. Forward linear prediction to 1024 points in F_1 and subsequent zero-filling to 2048 × 8192 points was applied prior to Fourier transformation. Coupling constants were extracted from 1D-projections of the resonances of interest.

The IPAP-HSQC-TOCSY-HT experiment[35,45] was carried out with zero-quantum suppression as devised by Thrippleton and Keeler,[46] with 512 scans and a 50 ms long spin-lock ($\gamma B_1/2\pi$ = 5 kHz); otherwise the same parameters were used as in the HSQC-HT experiment. Zero-filling was carried out to 2048 × 256 points. In the ^{1}H dimension an exponential line-broadening factor of 1 Hz was applied. Following baseline correction in F_2, coupling constants were extracted from the 1D IPAP selections of the corresponding ^{13}C frequencies.

^{1}H,^{1}H cross-relaxation rates were measured using 1D ^{1}H,^{1}H-DPFGSE NOESY[48] with zero-quantum suppression and 1D ^{1}H,^{1}H-DPFGSE T-ROESY[49] experiments at 600 MHz. Selective excitations of H5, H5a$_{pro\text{-}R}$ and H5a$_{pro\text{-}S}$ were achieved using 60 Hz broad i-SNOB-2 shaped pulses of 28 ms duration. Six different mixing times between 50 ms and 300 ms were used. The spectral width was 5 ppm sampled with 24,314 points. A relaxation delay of 8.5 s was used. The T-ROESY spin-lock strength was 3.0 kHz. Pulsed field gradients of 10 ms were used. Zero-filling to 65,536 points and an exponential line-broadening of 1 Hz were used prior to Fourier transformation. All spectra were baseline corrected and integrated with the same limits in all mixing times.

Acknowledgements

This work was supported by grants from the Swedish Research Council (VR), The Knut and Alice Wallenberg Foundation, Magn. Bergvalls Stiftelse and Magnus Ehrnrooths Stiftelse (to M.U.R.). Drs. Ē. Kupče and T. Nishida are thanked for stimulating discussions and the pulse sequence for the IPAP-HSQC-TOCSY-HT NMR experiment.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2010.03.002.

References

1. Ferro, D. R.; Provasoli, A.; Ragazzi, M.; Torri, G.; Casu, B.; Gatti, G.; Jacquinet, J.-C.; Sinaÿ, P.; Petitou, M.; Choay, J. *J. Am. Chem. Soc.* **1986**, *108*, 6773.
2. Sanderson, P. N.; Huckerby, T. N.; Nieduszynski, I. A. *Biochem. J.* **1987**, *243*, 175.

3. Ferro, D. R.; Provasoli, A.; Ragazzi, M.; Casu, B.; Torri, G.; Bossennec, V.; Perly, B.; Sinaÿ, P.; Petitou, M.; Choay, J. *Carbohydr. Res.* **1990**, *195*, 157.
4. Jin, L.; Abrahams, J. P.; Skinner, R.; Petitou, M.; Pike, R. N.; Carrell, R. W. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 14683.
5. Das, S. K.; Mallet, J.-M.; Esnault, J.; Driguez, P.-A.; Duchaussoy, P.; Sizun, P.; Herault, J.-P.; Herbert, J.-M.; Petitou, M.; Sinaÿ, P. *Chem. Eur. J.* **2001**, *7*, 4821.
6. Kovensky, J.; Mallet, J.-M.; Esnault, J.; Driguez, P.-A.; Sizun, P.; Hérault, J.-P.; Herbert, J.-M.; Petitou, M.; Sinaÿ, P. *Eur. J. Org. Chem.* **2002**, 3595.
7. Herrera, A. J.; Beneitez, M. T.; Amorim, L.; Cañada, F. J.; Jiménez-Barbero, J.; Sinaÿ, P.; Blériot, Y. *Carbohydr. Res.* **2007**, *342*, 1876.
8. Cros, C.; Petitou, M.; Sizun, P.; Pérez, S.; Imberty, A. *Bioorg. Med. Chem.* **1997**, *5*, 1301.
9. Mikhailov, D.; Linhardt, R. J.; Mayo, K. H. *Biochem. J.* **1997**, *328*, 51.
10. Verli, H.; Guimarães, J. A. *Carbohydr. Res.* **2004**, *339*, 281.
11. Becker, C. F.; Guimarães, J. A.; Verli, H. *Carbohydr. Res.* **2005**, *340*, 1499.
12. Canales, A.; Angulo, J.; Ojeda, R.; Bruix, M.; Fayos, R.; Lozano, R.; Giménez-Gallego, G.; Martín-Lomas, M.; Nieto, P. M.; Jiménez-Barbero, J. *J. Am. Chem. Soc.* **2005**, *127*, 5778.
13. Remko, M.; von der Lieth, C.-W. *J. Chem. Inf. Model.* **2006**, *46*, 1194.
14. Hricoví, M. *Carbohydr. Res.* **2006**, *341*, 2575.
15. Hricoví, M.; Bízik, F. *Carbohydr. Res.* **2007**, *342*, 779.
16. Hricoví, M.; Scholtzova, E.; Bízik, F. *Carbohydr. Res.* **2007**, *342*, 1350.
17. McCasland, G. E.; Furuta, S.; Durham, L. J. *J. Org. Chem.* **1966**, *31*, 1516.
18. Ogawa, S. *Trends Glycosci. Glycotechnol.* **2004**, *16*, 33.
19. Arjona, O.; Gómez, A. M.; López, J. C.; Plumet, J. *Chem. Rev.* **2007**, *107*, 1919.
20. Ogawa, S.; Ara, M.; Kondoh, T.; Saitoh, M.; Masuda, R.; Toyokuni, T.; Suami, T. *Bull. Chem. Soc. Jpn.* **1980**, *53*, 1121.
21. Barton, D. H. R.; Géro, S. D.; Cléophax, J.; Salustiano Machado, A.; Quiclet-Sire, B. *J. Chem. Soc., Chem. Commun.* **1988**, 1184.
22. Yoshikawa, M.; Cha, B. C.; Nakae, T.; Kitagawa, I. *Chem. Pharm. Bull.* **1988**, *36*, 3714.
23. Larsen, D. S.; Lins, R. J.; Stoodley, R. J.; Trotter, N. S. *Org. Biomol. Chem.* **2004**, *2*, 1934.
24. Yu, S.-H.; Chung, S.-K. *Tetrahedron: Asymmetry* **2005**, *2729*, 16.
25. Ramana, C. V.; Chaudhuri, S. R.; Gurjar, M. K. *Synthesis* **2007**, 523.
26. Sollogoub, M.; Pearce, A. J.; Hérault, A.; Sinaÿ, P. *Tetrahedron: Asymmetry* **2000**, *11*, 283.
27. Boyd, D. R.; Sharma, N. D.; Llamas, N. M.; Malone, J. F.; O'Dowd, C. R.; Allen, C. C. R. *Org. Biomol. Chem.* **2005**, *3*, 1953.
28. Sedemera, P.; Halada, P.; Pospíšil, S. *Magn. Reson. Chem.* **2009**, *47*, 519.
29. Cumpstey, I.; Gehrke, S.; Erfan, S.; Cribiu, R. *Carbohydr. Res.* **2008**, *343*, 1676.
30. Paulsen, H.; Von Deyn, W. *Liebigs Ann. Chem.* **1987**, 125.
31. Prosperi, D.; Ronchi, S.; Lay, L.; Rencurosi, A.; Russo, G. *Eur. J. Org. Chem.* **2004**, 395.
32. van den Bos, L. J.; Codée, J. D. C.; van der Toorn, J. C.; Boltje, T. J.; van Boom, J. H.; Overkleeft, H. S.; van der Marel, G. A. *Org. Lett.* **2004**, *6*, 2165.
33. Benowitz, A. B.; Fidanze, S.; Small, P. L. C.; Kishi, Y. *J. Am. Chem. Soc.* **2001**, *123*, 5128.
34. Nyberg, N. T.; Duus, J. Ø.; Sørensen, O. W. *J. Am. Chem. Soc.* **2005**, *127*, 6154.
35. Kupče, E.; Nishida, T.; Freeman, R. *Prog. Nucl. Magn. Reson. Spectrosc.* **2003**, *42*, 95.
36. Kupče, Ē.; Freeman, R. *J. Magn. Reson.* **2003**, *163*, 56.
37. Laatikainen, R.; Niemitz, M.; Weber, U.; Sundelin, J.; Hassinen, T.; Väpsäläinen, J. *J. Magn. Reson., Ser. A* **1996**, *120*, 1.
38. Haasnoot, C. A. G.; de Leeuw, F. A. A. M.; Altona, C. *Tetrahedron* **1980**, *36*, 2783.
39. Evans, D. A.; Bodkin, M. J.; Baker, S. R.; Sharman, G. J. *Magn. Reson. Chem.* **2007**, *45*, 595.
40. Marquez, B. L.; Gerwick, W. H.; Williamsson, R. T. *Magn. Reson. Chem.* **2001**, *39*, 499.
41. Nishida, T.; Widmalm, G.; Sandor, P. *Magn. Reson. Chem.* **1995**, *33*, 596.
42. Vidal, P.; Esturau, N.; Parella, T.; Espinosa, J. F. *J. Org. Chem.* **2007**, *72*, 3166.
43. Koźmiński, W.; Nanz, D. *J. Magn. Reson.* **2000**, *142*, 294.
44. Meissner, A.; Sørensen, O. W. *Magn. Reson. Chem.* **2001**, *39*, 49.
45. Ottiger, M.; Delaglio, F.; Bax, A. *J. Magn. Reson.* **1998**, *131*, 373.
46. Thrippleton, M. J.; Keeler, J. *Angew. Chem., Int. Ed.* **2003**, *42*, 3938.
47. Wasylishen, R.; Schaefer, T. *Can. J. Chem.* **1973**, *51*, 961.
48. Stott, K.; Keeler, J.; Van, Q. N.; Shaka, A. J. *J. Magn. Reson.* **1997**, *125*, 302.
49. Kjellberg, A.; Widmalm, G. *Biopolymers* **1999**, *50*, 391.
50. Carpintero, M.; Fernández-Mayoralas, A.; Jiménez-Barbero, J. *Eur. J. Org. Chem.* **2001**, 681.
51. Carpintero, M.; Bastida, A.; García-Junceda, E.; Jiménez-Barbero, J.; Fernández-Mayoralas, A. *Eur. J. Org. Chem.* **2001**, 4127.
52. Jiménez-Barbero, J.; Díaz, M. D.; Nieto, P. M. *Anti-Cancer Agents Med. Chem.* **2008**, *8*, 52.