Synthesis, NMR spectroscopy and conformational studies of two vicinally disubstituted trisaccharides

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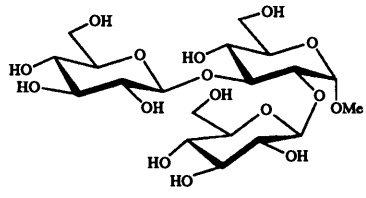
Two trisaccharides with 2,3-vicinal disubstitution have been synthesized and analysed by NMR spectroscopy and Monte Carlo simulations. When vicinal disubstitution occurs in trisaccharides, experimental NMR spectra often differ significantly from those calculated by simple additivity schemes. In the present study, small deviations from additivity occurred for the carbons at the glycosylation positions of the disubstituted sugar residues in β-D-Glc*p*(1→2)[β-D-Glc*p*(1→3)]α-D-Glc*p*-OMe (1) and β-D-Glc*p*(1→2)[β-D-Glc*p*(1→3)]α-D-Man*p*-OMe (2). Monte Carlo simulations, using the HSEA force field, showed a change in the average values and in the rms fluctuations of the ψ-dihedral angles of 2 compared to the corresponding disaccharides, whereas for 1 smaller changes were observed.

Computer assisted analysis of regular polysaccharides have been developed by different groups.[1–3] The methods are based on a similar scheme: (1) given a certain monosaccharide and linkage composition all possible structures are generated; (2) the NMR spectra of these structures are simulated according to an additive scheme and (3) all simulated spectra are in turn compared to the experimental spectrum and a fitting value calculated. In several cases it has been shown that the structure with the best fit or one of the best fits corresponded to the correct structure.

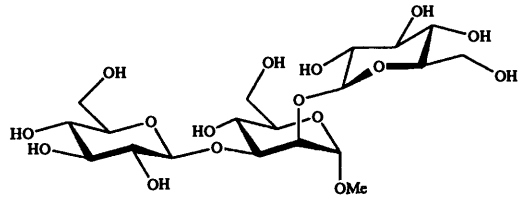
The NMR spectra are simulated from the monosaccharide chemical shifts to which the glycosylation shifts should be added. The glycosylation shifts are the changes in chemical shift, compared to the corresponding monomer, deriving from a certain glycosidic linkage. For many polysaccharides it is sufficient to assume that no long range interactions are present and that only the disaccharide glycosylation shifts should be added for each of the glycosidic linkages present.

For branch point residues which are vicinally substituted, however, it has been shown that deviations from additivity often occur. The probable reason for this is that interaction between the two branches causes deviations from the disaccharide conformation, which in turn generates new interactions and changed chemical shifts. Several investigations have shown that the deviations range from several ppm lower chemical shift values than those expected, to some ppm higher values.[4–6] Different 3,4-disubstituted galactose derivatives and 2,3-disubstituted rhamnose, galactose and glucose derivatives have been synthesized and studied by NMR spectroscopy.[4,5,7] We have now synthesized and studied two trisaccharides with a glucose or mannose branch point residue, 2,3-di-*O*-substituted with β-D-glucosyl groups, *i.e.* β-D-Glc*p*(1→2)[β-D-Glc*p*-(1→3)]α-D-Glc*p*-OMe (**1**) and β-D-Glc*p*(1→2)[β-D-Glc*p*-(1→3)]α-D-Man*p*-OMe (**2**). For comparison the disaccharide β-D-Glc*p*(1→3)α-D-Man*p*-OMe (**6**) was needed, and thus synthesized. In order to facilitate NMR interpretation of **2**, β-D-Glc*p*-[1,2,3,4,5,6,6′-$^2H_7$](1→2)[β-D-Glc*p*(1→3)]α-D-Man*p*-OMe (**2d**) was also synthesized. The ^1H and ^{13}C NMR spectra have been studied and the conformations calculated by Monte-Carlo simulations, using the HSEA force field.[8,9,10]

Similar compounds, with 2,3-disubstitution,[11] have been studied previously. Also, trisaccharides with the generic formula D-Glc*p*(1→2)-D-Glc*p*(1→3)-α-D-Glc*p*-OMe have been studied previously, since one would expect that any type of vicinal branching should potentially cause deviations, *i.e.*, also substitution in positions 1 and 2 of a sugar.[11] Indeed, the trisaccharide β-D-Glc*p*(1→2)-β-D-Glc*p*(1→3)-α-D-Glc*p*-OMe gave ^{13}C NMR spectra with deviations up to almost 2 ppm compared to what was expected by additivity alone.



1



2

In branched trisaccharides the absolute configuration of the substituting groups is an essential parameter. Furthermore, whether the substituted hydroxy groups are equatorial or axial, *i.e.*, the configuration at the anomeric and linkage carbons, are also essential parameters. The chirality of the non-anomeric carbons of the substituting glycosyl group is of less importance, since when the chirality of a non-anomeric carbon is changed, the pyranose ring still appears in approximately the same position, as indicated by computer calculations.

It cannot be excluded that in a polysaccharide with 2,3-disubstitution, the glycosyl group at O-1 of the branch point residue also effects the overall conformation. The parameter is not taken into consideration in this investigation at O-1 only carries a methyl group. We have, in the present study, chosen the Monte-Carlo approach, as used in the GEGOP program, in which all glycosidic linkages can be monitored in

Table 1 ^{1}H NMR chemical shifts (ppm) at 70 °C for trisaccharides **1** and **2**, disaccharide **6** and the corresponding hexose and methyl hexosides, also listing chemical shift differences $\Delta\delta^a$ and $\Delta\Delta\delta^b$

	Sugar residue		H-1	H-2	H-3	H-4	H-5	H-6a	H-6b	OMe
1	β-D-Glc*p*(1→3)	(obs)	4.77	3.33	3.50	3.43	3.48	3.73	3.92	
		(obs-mono)	(0.13)	(0.08)	(0.00)	(0.01)	(0.02)	(0.01)	(0.02)	
		(obs-calc)	[0.09]	[−0.04]	[−0.04]	[0.00]	[−0.02]	[−0.01]	[0.00]	
	→2,3)α-D-Glc*p*-OMe	(obs)	5.01	3.84	3.99	3.55	3.67	3.77	3.88	3.42
		(obs-mono)	(0.20)	(0.28)	(0.31)	(0.14)	(0.03)	(0.01)	(0.01)	(−0.01)
		(obs-calc)	[0.00]	[−0.02]	[−0.04]	[−0.01]	[−0.01]	[−0.01]	[−0.01]	[−0.01]
	β-D-Glc*p*(1→2)	(obs)	4.66	3.35	3.49	3.43	3.45	3.76	3.92	
		(obs-mono)	(0.02)	(0.10)	(−0.01)	(0.01)	(−0.01)	(0.04)	(0.02)	
		(obs-calc)	[0.04]	[−0.02]	[−0.03]	[0.00]	[0.02]	[0.00]	[0.01]	
2	β-D-Glc*p*(1→3)	(obs)	4.64	3.38	3.53	3.45	3.48	3.75	3.92	
		(obs-mono)	(0.00)	(0.13)	(0.03)	(0.03)	(0.02)	(0.03)	(0.02)	
		(obs-calc)	[0.07]	[0.01]	[0.00]	[−0.01]	[0.01]	[0.02]	[0.00]	
	→2,3)α-D-Man*p*-OMe	(obs)	4.92	4.28	4.07	3.83	3.65	3.83	3.91	3.44
		(obs-mono)	(0.15)	(0.34)	(0.30)	(0.16)	(0.04)	(0.05)	(0.01)	(0.02)
		(obs-calc)	[0.00]	[0.00]	[0.06]	[0.03]	[0.00]	[0.01]	[0.02]	[0.00]
	β-D-Glc*p*(1→2)	(obs)	4.54	3.34	3.51	3.45	3.43	3.75	3.92	
		(obs-mono)	(−0.10)	(0.09)	(0.01)	(0.03)	(−0.03)	(0.03)	(0.02)	
		(obs-calc)	[0.04]	[−0.01]	[0.01]	[0.02]	[−0.01]	[0.01]	[0.02]	
6	→3)α-D-Man*p*-OMe	(obs)	4.81	4.12	3.96	3.79	3.64	3.81	3.91	3.43
		(obs-mono)	(0.04)	(0.18)	(0.19)	(0.12)	(0.03)	(0.03)	(0.01)	(0.01)
	β-D-Glc*p*(1→3)	(obs)	4.57	3.37	3.53	3.46	3.47	3.73	3.92	
		(obs-mono)	(−0.07)	(0.12)	(0.03)	(0.04)	(0.01)	(0.01)	(0.02)	
	β-D-Glc*p* [1]		4.64	3.25	3.50	3.42	3.46	3.72	3.90	
	α-D-Glc*p*-OMe [2]		4.81	3.56	3.68	3.41	3.64	3.76	3.87	3.43
	α-D-Man*p*-OMe [2]		4.77	3.94	3.77	3.67	3.61	3.78	3.90	3.42

[a] Glycosylation shift differences (obs-mono), in parentheses, are calculated by substraction of chemical shifts of the corresponding hexose and methyl hexoside from **1**, *etc.*; a positive difference indicates a downfield shift. [b] $\Delta\Delta\delta$ Values (obs-calc) for the trisaccharides, in square brackets, are calculated by adding the $\Delta\delta$ values of the corresponding disaccharides to the chemical shift of the hexose or methyl hexoside and then substracting the resulting value from the measured chemical shift of the trisaccharide (for the methyl hexoside residues $\Delta\delta$ values from both disaccharides are added).

the same run. Both average and low energy conformations are estimated.

Results and discussion

The synthesis of trisaccharides **1**, **2** as well as disaccharide **6** were accomplished using silver triflate mediated glycosylations. To enable unambiguous assignment of the ^{1}H and ^{13}C NMR spectra of trisaccharide **2** an analogue, trisaccharide **2d**, in which the 2-*O*-β-D-glucosyl group is perdeuteriated, was synthesized using a DMTST mediated glycosylation. Assignments could also be accomplished using the ^{1}H detected HSQC-TOCSY experiment,[12,13] which was applied to **1** and **2**.

^{1}H NMR spectra of disaccharide 6 and trisaccharides 1–2

The chemical shifts, the glycosylation shifts (obs-mono) and the deviations from simulated spectra of the trisaccharides (obs-calc) are given in Table 1. Data for the disaccharides except β-D-Glc*p*(1→3)-α-D-Man*p*-OMe (**6**) was obtained from the literature.[14,15] The spectrum of disaccharide **6** was therefore analysed. Significant glycosylation shifts (0.1–0.25 ppm) for signals from H-2–H-4 in the methyl glycoside residue and a shift of *ca.* 0.1 ppm for the H-2 signal in the glycosyl group were observed. These values match well what is observed for the disaccharide glycoside β-L-Fuc(1→3)-α-D-Gal*p*-OMe,[16] which has similar stereochemistry at the glycosidic linkage. In trisaccharide **1** it can be observed that for positions not engaged in the glycosidic linkage, *i.e.*, other than H-1 in the substituting groups and H-2 and H-3 in the methyl glucoside residue, the deviation ($\Delta\Delta\delta$) is $\leqslant 0.04$ ppm. For signals from protons involved in the glycosidic linkage the shifts were also fairly small with the largest value of 0.09 ppm found for the anomeric proton signal of the β-D-Glc*p* group substituting position three of the branch point residue in **1**. This indicates that the disaccharide equilibrium φ,ψ-values are kept in the trisaccharide with no or small deviations.

Comparison with data for β-D-Gal*p*(1→2)[β-D-Gal*p*-(1→3)]α-D-Glc*p*-OMe [16] shows good agreement with respect to glycosylation shifts. For **2** no changes from expected δ-values were larger than 0.07 ppm, *i.e.*, here also φ,ψ-values can be expected to be similar in both the disaccharides and in the trisaccharide.

^{13}C NMR spectra of disaccharide 6 and trisaccharides 1–2

The chemical shifts, the glycosylation shifts (obs-mono) and the deviations from simulated spectra of trisaccharides (obs-calc) are given in Table 2. As for ^{1}H NMR values, the ^{13}C NMR glycosylation values for disaccharide **6** match well with what is observed for the disaccharide glycoside β-L-Fuc(1→3)-α-D-Gal*p*-OMe which has similar stereochemistry at the glycosidic linkage. For the trisaccharides, small but significant deviations from additivity are observed for signals from C-3 in **1**, C-2 and C-3 in **2**, of *ca.* −1 ppm, *i.e.*, the glycosylation shifts are smaller than expected. Upfield chemical shift displacements often occur as a result of steric interaction between sugar residues. In the present cases though, the effect is small. Comparison of **1** with data for β-D-Gal*p*(1→2)-[β-D-Gal*p*(1→3)]α-D-Glc*p*-OMe[16] shows good agreement with respect to glycosylation shifts.

Monte Carlo calculations

The result, as average angles and energy minimized conformations, from a run with 10^6 Monte Carlo macro steps

Table 2 ^{13}C NMR chemical shifts (ppm) at 70 °C for trisaccharides **1** and **2**, disaccharide **6** and the corresponding hexose and methyl hexosides, also listing chemical shift differences $\Delta\delta^a$ and $\Delta\Delta\delta^b$

Sugar residue		C-1	C-2	C-3	C-4	C-5	C-6	OMe
1 β-D-Glc*p*(1→3)	(obs)	103.26	74.47	76.83	70.62	76.87[c]	61.69[c]	
	(obs-mono)	(6.42)	(−0.73)	(0.07)	(−0.09)	(0.11)	(−0.15)	
	(obs-calc)	[−0.41]	[0.05]	[0.23]	[0.04]	[−0.02]	[0.05]	
→2,3)α-D-Glc*p*-OMe	(obs)	99.73	80.46	81.49	69.28	72.05	61.57	55.74
	(obs-mono)	(−0.46)	(8.23)	(7.39)	(−1.40)	(−0.47)	(−0.10)	(−0.19)
	(obs-calc)	[0.07]	[−0.02]	[−1.15]	[0.26]	[−0.01]	[−0.08]	[0.05]
β-D-Glc*p*(1→2)	(obs)	104.26	74.25	76.86[c]	70.53	76.78	61.78[c]	
	(obs-mono)	(7.42)	(−0.95)	(0.10)	(−0.18)	(0.02)	(−0.06)	
	(obs-calc)	[−0.33]	[0.00]	[0.21]	[−0.04]	[0.02]	[−0.05]	
2 β-D-Glc*p*(1→3)	(obs)	100.97	74.06	76.66	70.52	76.89	61.63	
	(obs-mono)	(4.13)	(−1.14)	(−0.10)	(−0.19)	(0.13)	(−0.21)	
	(obs-calc)	[−0.38]	[0.19]	[0.06]	[−0.03]	[−0.03]	[−0.05]	
→2,3)α-D-Man*p*-OMe	(obs)	99.62	74.96	77.55	66.00	73.39	61.52	55.77
	(obs-mono)	(−2.13)	(4.11)	(5.99)	(−1.79)	(−0.06)	(−0.40)	(0.22)
	(obs-calc)	[0.04]	[−1.09]	[−1.05]	[−0.33]	[−0.01]	[−0.02]	[0.00]
β-D-Glc*p*(1→2)	(obs)	102.53	73.66	76.47	70.42	76.85	61.57	
	(obs-mono)	(5.69)	(−1.54)	(−0.29)	(−0.29)	(0.09)	(−0.27)	
	(obs-calc)	[−0.13]	[0.01]	[0.03]	[−0.07]	[−0.03]	[−0.07]	
6 →3)α-D-Man*p*-OMe	(obs)	101.47	68.51	79.48	66.07	73.41	61.90	55.64
	(obs-mono)	(−0.28)	(−2.34)	(7.92)	(−1.72)	(−0.04)	(−0.02)	(0.09)
β-D-Glc*p*(1→3)	(obs)	101.35	73.87	76.60	70.55	76.92	61.68	
	(obs-mono)	(4.51)	(−1.33)	(−0.16)	(−0.16)	(0.16)	(−0.16)	
β-D-Glc*p*[1]		96.84	75.20	76.76	70.71	76.76	61.84	
α-D-Glc*p*-OMe[2]		100.19	72.23	74.10	70.68	72.52	61.67	55.93
α-D-Man*p*-OMe[2]		101.75	70.85	71.56	67.79	73.45	61.92	55.55

[a] Glycosylation shift differences (obs-mono), in parentheses, are calculated by substraction of chemical shifts of the corresponding hexose and methyl hexoside from **1**, *etc.*; a positive difference indicates a downfield shift. [b] $\Delta\Delta\delta$ Values (obs-calc) for the trisaccharides, in square brackets, are calculated by adding the $\Delta\delta$ values of the corresponding disaccharides to the chemical shift of the hexose or methyl hexoside and then subtracting the resulting value from the measured chemical shift of the trisaccharide (for the methyl hexoside residues $\Delta\delta$ values from both disaccharides are added). [c] Tentative assignment.

Table 3 Conformational analysis of trisaccharides **1–2** and disaccharides **3–6** by Monte Carlo simulations[a] and energy minimizations

	Dihedral angles in glycosidic linkages							
	⟨MMC⟩				EM			
	(1→2)		(1→3)		(1→2)		(1→3)	
Molecule	φ	ψ	φ	ψ	φ	ψ	φ	ψ
β-D-Glc*p*(1→2)[β-D-Glc*p*(1→3)]α-D-Glc*p*-OMe (**1**)	53	−7	53	8	53	2	52	13
	(4)[b]	(5)	(2)	(2)	(3)	(21)	(−4)	(8)
	[10][c]	[19]	[9]	[14]				
β-D-Glc*p*(1→2)[β-D-Glc*p*(1→3)]α-D-Man*p*-OMe (**2**)	50	25	56	30	54	21	57	48
	(−1)	(16)	(4)	(11)	(6)	(24)	(3)	(39)
	[13]	[11]	[12]	[22]				
β-D-Glc*p*(1→2)α-D-Glcp-OMe (**3**)	49	−12	—	—	50	−19	—	—
	[12]	[22]						
β-D-Glc*p*(1→3)α-D-Glc*p*-OMe (**4**)	—	—	51	6	—	—	56	5
			[11]	[13]				
β-D-Glc*p*(1→2)α-D-Man*p*-OMe (**5**)	51	9	—	—	48	−3	—	—
	[11]	[15]						
β-D-Glc*p*(1→3)α-D-Man*p*-OMe (**6**)	—	—	52	19	—	—	54	9
			[12]	[19]				

[a] 10^6 Macro steps Metropolis Monte Carlo simulations at 300 K performed with a total acceptance ratio between 0.3 and 0.6. [b] Angle differences between trisaccharide and corresponding disaccharide in parentheses. [c] Rms deviation from average angles in square brackets.

at 300 K for trisaccharides **1–2** and disaccharides **3–6** are given in Table 3. Only the conformational region close to the global energy minimum was investigated for the oligosaccharides. It can thus be observed that there is, in general, a good correspondence between the angles for the average and the low energy values for disaccharides **3–6**. Averaged values of φ and ψ are for trisaccharide **1** close to those of the disaccharides with differences $\leqslant 5°$. Changes, $> 10°$, have occurred for the ψ-dihedral angles in trisaccharide **2** for both glycosyl groups. The dihedral angles at the global energy minimum for **1–2** are changed in a similar way to the averaged values, *i.e.*, the differences occurring for the ψ-dihedral angle are smaller for **1** and larger for **2** of up to ~40°. The scatter plots (not shown) emphasize the above data and a reduction of the conformational

space available for the (1→2) linkage of **2** was readily observed. Root mean square deviations (rmsd) calculated from the MMC simulations showed the largest reduction, both in absolute values and in relative amounts, for the ψ-dihedral angle in the (1→2) linkage of **2** (Table 3). For the other dihedral angles the rmsd values were changed to a lesser extent.

Conclusions

The present study emphasises the fact that vicinal disubstitution of glycosyl residues can lead to non-additivity of NMR glycosylation shifts, whereas in other cases pure additivity is sufficient for predicting NMR chemical shifts upon glycosylation. The changes from additivity for the two trisaccharides in this study are small for ^{1}H NMR data, < 0.1 ppm. At the two glycosylation positions of each branch point sugar residue, only small differences in carbon-13 glycosylation shifts compared to those anticipated are observed at three of the four positions, which may be interpreted as a small reduction in conformational space for the terminal groups. MMC calculations using the HSEA force field showed small changes in φ for the energy minimized global conformations of both **1** and **2**, compared to their constituent disaccharides. For the dihedral angle ψ, the MMC average does not change for **1**, but for **2** differences > 10° are observed. In the energy minimized global conformations the differences in ψ are larger for **2** than for **1**. From the MMC calculations a reduction of the conformational space is observed especially for the ψ dihedral angle of the element β-D-Glc*p*(1→2)-α-D-Man*p* in trisaccharide **2**.

Furthermore, it should be possible to investigate rapidly, by computer calculation, which cases of vicinal substitution that should lead to changes in conformation and subsequently indicate differences in NMR chemical shifts. In such a case, preferably using averaged data as the NMR spectra are time averaged.

Experimental

General

Dichloromethane was distilled and dried over molecular sieves (4 Å) before being used in coupling reactions. The aglycone and the alcohol were dissolved in dry toluene, concentrated and dried under reduced pressure before being dissolved in dichloromethane. Evaporations were performed under reduced pressure at < 50 °C.

In addition to NMR spectroscopy the substitution pattern was also determined using methylation analysis.[17,18] GC-MS was performed on a Hewlett Packard 5970 MSD instrument using an HP-5MS column and a temperature program from 190 (3 min) to 250 °C at 3 °C min^{-1}.

NMR spectra were recorded on JEOL GSX-270 and Alpha-400 instruments using $CDCl_3$ or D_2O as solvents. For solutions in D_2O, spectra were recorded at 70 °C and chemical shifts referred to internal TSP {sodium 3-(trimethylsilyl)-[2,2,3,3-$^2H_4$]propanoate, δ_H = 0.00} and dioxane (δ_C = 67.40). To assign the NMR signals different types of homo- and heteronuclear correlation spectroscopy (COSY) experiments were used as well as an HSQC-TOCSY experiment with a spin lock time of 30 ms.

Coupling reactions

The aglycone, methyl 4,6-benzylidenehexoside (250–500 mg), the glycosyl donor, 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide and s-collidine were stirred in dichloromethane (10 cm^3) with molecular sieves (4 Å, 1 g) at room temperature. The molar excess of glycosyl donor was *ca.* 3 for compounds **1** and **2** and 1.2 for **6**. The mixture was cooled to −20 °C and stirred while a solution of silver trifluoromethanesulfonate (silver triflate) in dichloromethane-toluene 1:1 was added. After 30 min, pyridine (0.5 cm^3) was added and the mixture was immediately purified by silica gel chromatography. For synthesis of trisaccharide **2d**, the aglycone, 2,3,4,6-tetra-*O*-benzoyl-β-D-Glc*p*-(1→3)-4,6-benzylidene-α-D-Man*p*-*O*-Me (protected disaccharide **6**, 300 mg) and 1.1 equiv. [1,2,3,4,5,6,6′-$^2H_7$]ethyl 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside[19] was stirred in dichloromethane (10 cm^3) with ground molecular sieves (4 Å, 0.5 g) at room temperature, whereafter 3 equiv. dimethyl(methylthio)sulfonium triflate (DMTST) was added. The crude product was purified by silica gel chromatography.

Removal of protecting groups

The protected oligosaccharides were kept in 0.1 mol dm^{-3} sodium methoxide in methanol to remove benzoyl groups, worked-up and then debenzylidenated by treatment with aqueous 90% trifluoroacetic acid. Chromatography on a Bio-Gel P-2 column using a pyridinium acetate buffer as eluent gave **1**, **2**, **2d** and **6**. Final yields varied between 20 and 40%.

MS

Fast atom bombardment MS was performed on a JEOL SX-102 mass spectrometer in the positive mode. Pseudomolecular ions $[M + H]^+$ were observed for **1** and **2** at *m/z* 519, for **2d** at *m/z* 526 and for **6** at *m/z* 357, in complete agreement with postulated structures.

Metropolis Monte Carlo simulations

The GEGOP program, version 2.6, was used for all calculations.[8,9] The bond angle τ and dihedral angles φ,ψ and ω were defined as follows: τ = C1-O1-Cx, φ = H1-C1-O1-Cx, ψ = C1-O1-Cx-Hx and ω = O5-C5-C6-O6, where x is the linkage position. The bond angle τ was optimized starting from 117°. For ω the three staggered rotamers were denoted as gg (−60°), gt (60°) and tg (180°). The *O*-methyl group was gauche to O5 and anti to C2. The MMC simulations of **1**–**6** were started from low energy conformations obtained from grid-based conformational search of the compounds. Optimized angles of the hydroxy groups were also used in the starting conformations for the Monte Carlo simulations.

Simulations were performed at 300 K with 10^6 macro steps for each of the six molecules. The parameters were adjusted with test runs to obtain a total acceptance ratio between 30 and 60%. The maximum step length for the glycosidic angles φ, and ψ, was set to 20°. The bond angle τ, the dihedral angles φ,ψ,ω and those of the hydroxy and *O*-methyl groups, were optimized. The hydroxymethyl groups were mostly populating the gg conformer. The pyranose rings were treated as rigid units residing in the $^4C_1$ conformation using coordinates from neutron diffraction data.

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