Synthesis, NMR spectroscopy and conformational studies of the four anomeric methyl glycosides of the trisaccharide D-Glc*p*-(1→3)-[D-Glc*p*-(1→4)]-α-D-Glc*p*

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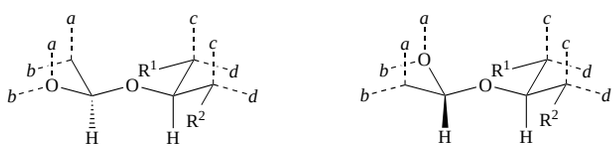
The four anomeric methyl glycosides of the vicinally disubstituted trisaccharide D-Glc*p*-(1→3)-[D-Glc*p*-(1→4)]-α-D-Glc*p* have been synthesized using silver trifluoromethanesulfonate mediated glycosylations. The ^{1}H and ^{13}C NMR resonances have been assigned and used for extraction of glycosylation shifts, *i.e.* the differences between chemical shifts for signals from the trisaccharides and those of the respective monomers, as well as those derived by addition of the glycosylation shifts for each disaccharide element. Glycosylation shifts are up to 0.5 ppm for proton and 10 ppm for carbon. Deviations from additivity are −0.2–0.1 ppm for proton and −4.5–2.3 ppm for carbon, usually confined to the atoms at the linkage positions. The conformational space spanned for the trisaccharides, and the constituent disaccharides, has been investigated by Metropolis Monte Carlo simulations using the HSEA force field. The α-linked glucosyl groups show larger conformational changes with multiple energy minima, whereas the β-linked glucosyl groups have a single energy minimum, close to that identified for the constituent disaccharide.

Introduction

Complex carbohydrates play important roles in many biochemical processes and the biological activity is a function of their surface properties which are governed by their structure and conformation, the latter arising mainly from rotation of the glycosidic linkages. Most monosaccharides have a defined ring shape, leaving flexibility only at the glycosidic linkage. This is true for most monosaccharides, with exceptions that are fairly easy to predict. The analysis of disaccharides, the simplest models for larger oligosaccharides, may be made, *inter alia*, with ^{1}H and ^{13}C NMR spectroscopy and computer modelling. NMR chemical shift assignments have been made for a large number of disaccharides and it has been concluded that a number of stereochemical factors influence the chemical shifts. Thus, collections of NMR data for disaccharides containing hexoses, deoxy-hexoses, 2-acetamido-sugars *etc*. can be found in the literature.[1,2] In our laboratory we have synthesized and, under similar conditions and temperature (mostly 70 °C), analysed several disaccharides. These include (1→2)-,[3–6] (1→3)-,[3–7] (1→4)-[8,9] and (1→6)-linkages.[3,10]

Conformational analyses of pyranosidic hexose disaccharides have shown that (1→2)-, (1→3)- and (1→4)-linked disaccharides have one main conformation. This conformation is essentially determined by the non-bonded interactions between atoms on linkage carbons and on neighboring atoms. In addition, changes contributed by the exoanomeric effect must also be considered. Expressed with dihedral angles φ (H1–C1–OA–CA, A = aglycon) and ψ (C1–OA–CA–HA), values are close to 60° (abs) and a low value near 0°, respectively. Apart from the absolute and anomeric configuration of the glycosyl group, the equatorial substituents on the neighboring carbons in the aglycon (R^1 and R^2, see below) are the most important factor. They may be hydrogen, hydroxy groups, hydroxymethyl groups or methyl groups. The latter three are similar in size and have roughly the same influence on the NMR spectra. In most cases these are either a proton and a larger group, or two larger groups. Hydrogens in both positions are seldom encountered. The substitution itself may also be equatorial or axial. The complexity of all these factors determining the conformation is best seen through some examples.

The disaccharides can be divided into two main groups. The difference between **I** and **II** is the position of the ring oxygen in



I **II**

the glycosyl group. If, in **I**, rings continue *via a* and *c*, a disaccharide with an α-D-sugar substituting an axial hydroxy group is obtained (*A*). For continuation *via a* and *d*, the same α-D-linkage is formed but substitution is now of an equatorial hydroxy group (*B*). If rings go through *b* and *c*, a β-L-sugar is obtained which substitutes an axial hydroxy group (*C*). Finally, if rings go through *b* and *d*, the β-L-sugar substitutes an equatorial hydroxy group (*D*). The similarity between α-D and β-L is then obvious and of course derives from the position of the ring oxygen. For the stereochemical arrangement in **II** the disaccharide formed *via a* and *c* gives an α-L-sugar substituting an axial hydroxy group (*E*), *via a* and *d* the same but substituting an equatorial hydroxy group (*F*) and for combinations *b* and *c*, and *b* and *d*, a disaccharide with a β-D-sugar substituting an axial (*G*) and an equatorial hydroxy group (*H*), respectively, is formed. The similarity here between α-L- and β-D-substitution also easily follows from **II**. For different combinations of sugars, different inter-residue interactions arise. The effect for three different groups of disaccharides will be given in order to show some typical glycosylation shifts, *i.e.* changes in chemical shift compared to those of monomers, for both ^{13}C and ^{1}H NMR spectroscopy.

Thus, if in **I**, R^1 is a hydrogen and R^2 is a hydroxy group or another large group an interaction between H1 and R^1 is present, a so-called γ-*gauche* interaction. This is manifested, *inter alia*, through an NOE between those protons. Disaccharides

with this stereochemistry are for instance α-D-Fuc-(1→3)-D-Gal where the α-D can be exchanged to β-L (type **I**) and α-L-Fuc-(1→3)-D-Man, where the α-L sugar can be exchanged to β-D (type **II**), and still the same interactions are present. For ^{13}C NMR spectra this stereochemistry brings about relatively small glycosylation shifts for the substituted carbons C1 and CA, and a relatively large negative glycosylation shift for the signal of the carbon to which R^1 is substituted. Significant glycosylation shifts for this and other disaccharides are obtained with only a few exceptions for signals from substituted (α-effect) or neighboring carbons (β-effect). For the α-D-Fuc-(1→3)-D-Gal disaccharide the values for Cl′, C3 and C4, are *ca.* 3.0, 5.0 and −3.5 ppm, respectively. The remaining carbon signal (for C2 in Gal) is shifted −1.5 ppm, a typical β-effect. The changes for ^1H NMR signals are for H1′, H2, H3 and H4, −0.14, 0.12, 0.04 and 0.20 ppm, respectively. The negative value is presumably a result of the proton–proton contact (H1′, H3/H4) giving the NOE, a shift that however is not observed for H4. As a comparison, for β-linked disaccharides the effects are normally less pronounced. Thus, β-L-Fuc-(1→3)-D-Gal has for C1′, C3 and C4 signals, the glycosylation shifts 4.4, 7.7 and −2.4 ppm.

If the aglycon changes from Gal to Man in the disaccharide from the first example to make α-D-Fuc-(1→3)-D-Man (type **I**) the proton–proton contact is removed and instead H1 will interact with a hydroxy group. Values for the same carbon signals as above are now 8.5, 8.0 and −0.8 ppm, *i.e.* relatively large and with no large negative value. The value for the C2 signal is now close to zero as compared to −1.5 ppm above. The changes for ^1H NMR signals are for H1′, H2, H3 and H4, −0.02, 0.08, 0.05 and 0.18 ppm, thus the large negative effect for H1′ is no longer present. Values for β-D-Glc-(1→3)-D-Gal (type **II**), an analogous 'mirror image' to β-L-Fuc-(1→3)-D-Man, are 8, 9.9 and −0.3 ppm for signals from C1′, C3 and C4, respectively, also relatively large.

The third and last group has no equatorial hydrogen and α-D-Glc-(1→3)-D-Glc is a typical representative for this group. Signals from C1′, C3 and C4, are shifted 7, 7.4 and 0.2 ppm respectively, and this is taken as an average starting point. The values for ^1H NMR glycosylation shifts are for H1′, H2, H3 and H4, 0.09, 0.10, 0.14 and 0.25 ppm. Disaccharides with groups other than hydroxy follow the general pattern but may vary slightly.

For the 6-linked disaccharides the situation is more complex as an additional degree of freedom is introduced, rotation around the C5–C6 linkage. The hydroxymethyl group occupies two out of three staggered conformations, different depending on the chirality at C4. The conformational space is likely to be shallow and a number of conformations may be occupied. Some preferences can however be seen, *e.g.* in general only one of the H6 protons has an NOE to H1 in the glycosyl group.[10] A distinction between the disaccharides which have an axial anomeric substitution and those that have an equatorial can be made and there is also a dependence on whether the two constituents are D or L. As stated above the effects are wholly dependent on the stereochemistry and therefore the effects are most similar if the sugars are α-DD or α-LL. The glycosylation shifts are around 5.9, −1.5 and 5.2 for C1′, C5 and C6 in α-D-Glc-(1→6)-D-Glc. Intermediate values are obtained for α- and β-LD combinations. Glycosylation shifts of around 6.7, −1.0 and 7.8 are found for the same carbon signals for β-D-Glc-(1→6)-D-Glc.

For larger oligosaccharides a first assumption can be made that the conformation adopted by the disaccharide is also kept in the oligosaccharide. It is quite clear that for most linear oligosaccharides this is true as data obtained for the disaccharide elements can be transferred to the oligosaccharide. It has been shown that additivity holds for the glycosylation shifts both for proton and carbon.[11] For vicinally branched trisaccharides data are available for 2,3-,[12,13] and 3,4-disubstituted[14–18] as well as formally linear but effectively branched trisaccharides with a 2-substituted residue in the middle, *i.e.* Sug-(1→2)-Sug-(1→X)-Sug.[19–21] In those oligosaccharides it could be expected that in several cases the glycosylation shifts should differ from those of the constituent disaccharides and from those calculated by additivity. One of the main reasons would of course be that the φ- and ψ-angles in the trisaccharide differ from those in the disaccharide because of interactions between the substituting sugars, or because the conformational freedom is limited. In general, few large glycosylation shifts are found apart from the expected α- and β-effects.

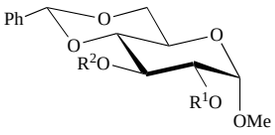
For NMR chemical shifts of trisaccharides a division into groups similar to that described for the disaccharides can be made. Thus, the substituting groups can take different anomeric and absolute configurations. The diols can be equatorial–equatorial or axial–equatorial. The highly unusual diaxial substitution is not taken into consideration. Further divisions can be made by allowing for variations at other places in the trisaccharide. For symmetry reasons some stereochemistries may give similar values. The trisaccharides used in previous studies generally had one of the substituting groups linked to an axial hydroxy group. To exemplify that some trisaccharide elements have substantial deviations of ^{13}C NMR data from those of the disaccharides, data for α-L-Fuc-(1→3)-[α-L-Fuc-(1→4)]D-Gal and α-D-Glc-(1→3)-[α-L-Fuc-(1→4)]D-Gal are compared. The former is an example of how large the changes in displacements may be. Signals from the anomeric carbon of the Sug-(1→3)- and Sug-(1→4)-groups, and C3 and C4 in the disubstituted residue, are 0, −2.8, −1.1 and −5.0 ppm, respectively, *i.e.* signals appear far more upfield than expected from comparisons with the disaccharides. If, on the other hand, the 3-*O*-glycosyl group is α-D-Glc instead the values are 0.9, −2.1, 0.5 and −2.6 ppm, thus significantly less upfield. There are examples, however, for which signals shift almost 2 ppm in the positive direction.

Results and discussion

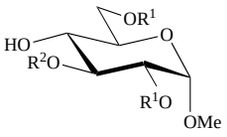
Synthesis of trisaccharides

In the synthesis of the four anomers of the trisaccharide D-Glc*p*-(1→3)-[D-Glc*p*-(1→4)]-α-D-Glc*p*-OMe **1**–**4**, three were made by glycosylation of a suitably protected derivative of the *O*-methyl glycoside, followed by selective deprotection and one more glycosylation. The last trisaccharide **4** was synthesized by di-glycosylation of a derivative of the *O*-methyl glycoside having two hydroxy groups free. All glycosylations were mediated by silver trifluoromethanesulfonate (AgOTf).[22,23] Diethyl ether was used as solvent in the formation of α-linked groups whereas for β-linked groups dichloromethane was used. The isolated yields in the glycosylation reactions were generally >70%. Data for the reaction conditions used in the conversions are given in Table 1.

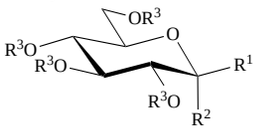
For the synthesis of the first two trisaccharides, **1** and **2**, both of which have an α-(1→3)-linkage, the key intermediate was a methyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside[24] (**9**). This compound was acetylated or allylated to give methyl 2-*O*-benzyl-3-*O*-acetyl-4,6-*O*-benzylidene-α-D-glucopyranoside (**10**) and methyl 2-*O*-benzyl-3-*O*-allyl-4,6-*O*-benzylidene-α-D-glucopyranoside (**11**), respectively, which were subsequently reductively opened by $NaCNBH_3$ in hydrochloric acid–THF[25] to give methyl 2,6-di-*O*-benzyl-3-*O*-acetyl-α-D-glucopyranoside (**12**) in 82% yield (over two steps) and methyl 2,6-di-*O*-benzyl-3-*O*-allyl-α-D-glucopyranoside (**13**) in 63% yield (over two steps), respectively. Glycosylation of **12** with 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl bromide[26] (**16**), generated *in situ* from ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside[26] (**15**), in diethyl ether at −30 °C gave the α-(1→4)-linked disaccharide **18** in 70% yield. Deprotection of **18** by Zemplen deacetylation (sodium methoxide in methanol) gave **19** to be used as an



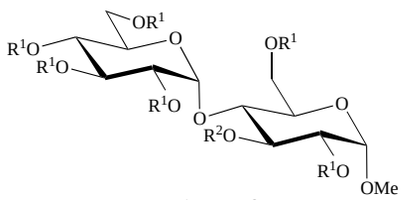
9 R^1 = Bn, R^2 = H
10 R^1 = Bn, R^2 = Ac
11 R^1 = Bn, R^2 = Allyl



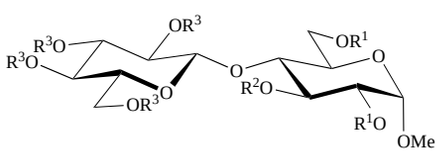
12 R^1 = Bn, R^2 = Ac
13 R^1 = Bn, R^2 = Allyl
14 R^1 = Bn, R^2 = H



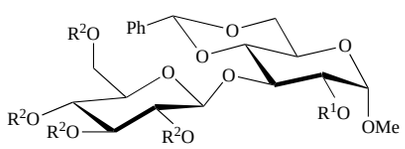
15 R^1 = SEt, R^2 = H, R^3 = Bn
16 R^1 = H, R^2 = Br, R^3 = Bn
17 R^1 = H, R^2 = Br, R^3 = Bz



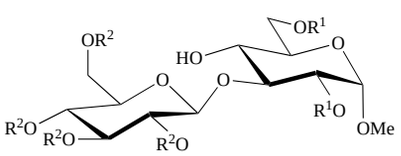
18 R^1 = Bn, R^2 = Ac
19 R^1 = Bn, R^2 = H



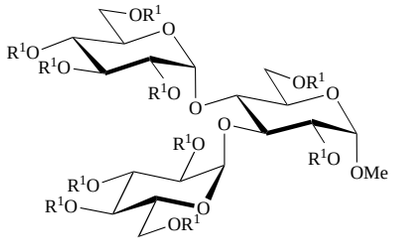
20 R^1 = Bn, R^2 = Allyl, R^3 = Bz
21 R^1 = Bn, R^2 = H, R^3 = Bz



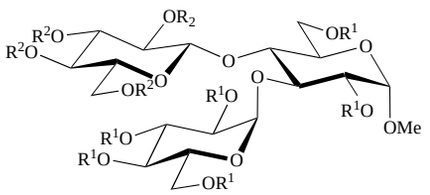
22 R^1 = Bn, R^2 = Bz



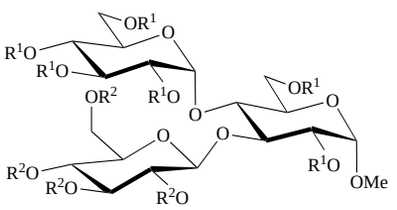
23 R^1 = Bn, R^2 = Bz



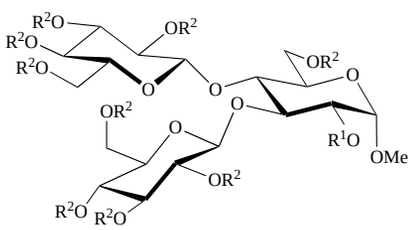
24 R^1 = Bn
1 R^1 = H



25 R^1 = Bn, R^2 = Bz
2 R^1 = R^2 = H



26 R^1 = Bn, R^2 = Bz
3 R^1 = R^2 = H



27 R^1 = Bn, R^2 = Bz
4 R^1 = R^2 = H

acceptor in the subsequent glycosylation. Compound **13** was condensed with 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide[27] (**17**) in dichloromethane at −30 °C to give the β-(1→4)-linked disaccharide **20** in 74% yield. The allyl group in compound **20** was rearranged[28] to give the prop-1-enyl isomer by reflux in ethanol using Wilkinson's catalyst [tris(triphenylphosphine)rhodium(I) chloride] and diisopropylethylamine (DIPEA), followed by treatment with toluene-*p*-sulfonic acid to give the deprotected **21** with a free hydroxy group. Glycosylations of the acceptors **19** and **21** with **16** as donor using the same conditions as above gave the trisaccharides **24** and **25** in 55 and 76% yield, respectively. Removal of the benzyl groups in **24** by hydrogenolysis using palladium on carbon gave, after gel filtration, compound **1** in 70% yield. Hydrogenolysis of **25**, followed by debenzoylation gave, after gel filtration, compound **2** in 85% yield.

For the synthesis of compound **3**, the β-(1→3)-linkage was formed first to give disaccharide **22**.[29] The 4,6-benzylidene group of **22** was subsequently opened by reductive cleavage to give the disaccharide **23** in 83% yield, with a free hydroxy group at O4. The α-(1→4)-linkage was formed by glycosylation of **23** with **16** in diethyl ether at −30 °C to give the trisaccharide **26**, in 79% yield. Hydrogenolysis of **26**, debenzoylation and gel filtration gave compound **3**, in 89% yield.

The last trisaccharide **4**, was formed by di-glycosylation of the selectively protected acceptor methyl 2,6-di-*O*-benzyl-α-D-glucopyranoside[30] (**14**) with 2.5 equiv. of 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide (**17**) in dichloromethane at −30 °C to give **27**, in 93% yield. Trisaccharide **27** was deprotected, as described for **26**, to give **4** in 85% yield.

The order of glycosylation played an important role, in particular for trisaccharide **2**, which has an α-(1→3)-linkage. For this compound the β-(1→4)-linkage was formed first since the reverse procedure led to a low yield of trisaccharide. Hence, the same order of glycosylation was used for compound **1**. The sequence was reversed for **3** in which the β-(1→3)-linkage was formed first, followed by the α-(1→4)-linkage. The last trisaccharide, **4**, could be formed in good yield by a di-glycosylation.

Monte Carlo calculations

The conformational flexibility of trisaccharides **1**–**4** and of the constituent disaccharides **5**–**8** was investigated by Metropolis Monte Carlo (MMC) calculations.[31,32] The global energy minimum for each molecule was also identified by energy minimization. Each MMC run employed 10^6 macro steps at 300 K. The results as averaged values of angles, angle differences between trisaccharides and the corresponding disaccharides, and rms deviations from average values of angles and energy minimized conformations are given in Table 2. The global energy conformers of **1**–**4** are shown in Fig. 1. The dihedral

Table 1 Data on reaction conditions, physical constants and selected NMR chemical shifts

Conversion[a]	Solvent (cm^3)	Reagents (mg, mmol)	t_{react}/h	T/°C[c]	Purif. (solv)[d,e]	Yield (%)	m/z	$[\alpha]_{578}$/° (c)[g]	$\delta_{^{13}C}$(anomeric)
9→10	CH_2Cl_2 (25)	**9** (1000, 2.69), pyridine (0.70 cm^3, 10.8), DMAP (33, 0.27)	2	rt	T–E 6:1	92			98.7
10→12	THF (50) 3 Å[b]	Acetyl chloride (0.29 cm^3, 4.04), **10** (900, 2.1), $NaCNBH_3$ (1680, 26.4), HCl–Ether	0.5	rt	T–E 4:1	89	439.2 $[M + Na]^+$	65 (0.83)	97.4
9→11	DMF (25)	**9** (2000, 5.39), NaH (272, 8.08), Allyl bromide (0.547 cm^3, 6.46)	1	rt	T–E 7:1	90			99.3
11→13	THF (50) 3 Å	**11** (1600, 3.9), $NaCNBH_3$ (3000, 48.3), HCl–Ether	0.5	rt	T–E 7:1–4:1	70	437.2 $[M + Na]^+$	16 (1.01)	98.2
15→16	CH_2Cl_2 (10)	**15** (906, 1.55), Br_2 (0.087 cm^3, 1.70)		0					
12 + 16→18	Ether (30)	**12** (500, 1.20), AgOTf (400, 1.55), collidine (0.079 cm^3, 0.59)	1	−30	T–E 7:1	70	961.7 $[M + Na]^+$	50 (1.14)	97.6 97.5
18→19	MeOH	**18** (720, 0.77), NaOMe (0.10 mol dm^{-1})	3	rt	T–E 7:1	90	919.5 $[M + Na]^+$	41 (0.80)	100.6 98.3
15→16	CH_2Cl_2 (10)	**15** (316, 0.54), Br_2 (0.030 cm^3, 0.59)		0					
19 + 16→24	Ether (30)	**19** (320, 0.357), AgOTf (138, 0.54), collidine (0.024 cm^3, 0.18)	0.75	−30	T–E 14:1	55	1441.5 $[M + Na]^+$	68 (0.77)	97.0 96.0 93.3
24→1	EtOAc–HOAc	**15** (240, 0.169), H_2/ Pd-C	20	rt	P2	70	517.2 $[M - H]^-$	117 (1.08)	
13 + 17→20	CH_2Cl_2	**17** (1030, 1.56), **13** (500, 1.2), AgOTf (402, 1.56), collidine (0.080 cm^3, 0.6)	2	−30	T–E 10:1	74	1015.5 $[M + Na]^+$	23 (0.95)	100.6 98.5
20→21	EtOH (40)	**20** (800, 0.806), DIPEA (0.15 cm^3, 0.887), $[(C_6H_5)_3P]_3$ RhCl (8.2, 0.0089), TPS[f]	3	90	T–E 7:1	78	975.6 $[M + Na]^+$	22 (1.01)	100.3 98.5
15→16	CH_2Cl_2 (10)	**15** (560, 1.18), Br_2 (0.066 cm^3, 1.29)		0					
21 + 16→25	Ether (30)	**21** (560, 0.59), AgOTf (303, 1.18), collidine (0.039 cm^3, 0.295)	0.5	−30	T–E 15:1	76	1497.9 $[M + Na]^+$	30 (1.11)	98.6 97.8 95.3
25→2		**25** (400, 0.27), H_2/ Pd-C, NaOMe (0.10 mol dm^{-1})		rt	P2		517.2 $[M - H]^-$	115 (0.83)	
22→23	THF (50) 3 Å	**22** (1500, 1.58), $NaCNBH_3$ (1830, 19.6), HCl–Ether	0.25	rt	T–E 4:1	83	975.7 $[M + Na]^+$		101.9 98.2
15→16	CH_2Cl_2 (10)	**15** (380, 0.63), Br_2 (0.036 cm^3, 0.69)		0					
23 + 16→26	Ether (30)	**23** (400, 0.42), AgOTf (163, 0.63), collidine (0.029 cm^3, 0.21)	0.5	−30	T–E 20:1 10:1	79	1474.6 $[M + Na]^+$	22 (1.53)	100.3 97.1 94.2
26→3	EtOAc MeOH	**26** (400, 0.27), H_2/ Pd-C, NaOMe (0.10 mol dm^{-1})	0.5	rt	P2	89	517.2 $[M - H]^-$	99 (1.04)	
14 + 17→27	CH_2Cl_2 (20) 4 Å	**14** (300, 0.802), **17** (1610, 2.44), AgOTf (628, 2.44), collidine (0.055 cm^3, 0.5)	0.75	−40/ −20	T–E 14:1	93	1553.6 $[M + Na]^+$	1 (1.01)	100.4 98.7 97.9
27→4	EtOAc MeOH	**27** (1.0, 0.65), H_2/ Pd-C, NaOMe (0.10 mol dm^{-1})	15 2	rt	P2	85	517.2 $[M - H]^-$	61 (1.46)	

[a] For coding see figures. [b] Molecular sieves, 3 Å. [c] rt = room temperature. [d] Solvents for chromatography separations; T = toluene, E = ethyl acetate. [e] Gel permeation chromatography on Bio-Gel P-2. [f] Toluene-*p*-sulfonic acid. [g] Concentration (g/100 cm^3).

Table 2 Conformational analysis of trisaccharides **1**–**4** and disaccharides **5**–**8** by Monte Carlo simulations[a] and energy minimizations

	Dihedral angles in glycosidic linkages/°							
	⟨MMC⟩				EM			
	(1→3)		(1→4)		(1→3)		(1→4)	
Molecule	φ	ψ	φ	ψ	φ	ψ	φ	ψ
α-D-Glc*p*(1→3)[α-D-Glc*p*(1→4)]α-D-Glc*p*-OMe (**1**)	−29	42	−43	−33	−34	31	−41	−31
	(10)[b]	(56)	(−16)	(−10)	(10)	(54)	(−9)	(−3)
	[8][c]	[12]	[11]	[11]				
α-D-Glc*p*(1→3)[β-D-Glc*p*(1→4)]α-D-Glc*p*-OMe (**2**)	−28	1	59	16	−32	14	61	10
	(11)	(15)	(7)	(15)	(12)	(37)	(7)	(8)
	[24]	[22]	[9]	[5]				
β-D-Glc*p*(1→3)[α-D-Glc*p*(1→4)]α-D-Glc*p*-OMe (**3**)	55	27	−24	−11	55	19	−17	8
	(5)	(21)	(3)	(12)	(2)	(14)	(15)	(36)
	[11]	[7]	[21]	[22]				
β-D-Glc*p*(1→3)[β-D-Glc*p*(1→4)]α-D-Glc*p*-OMe (**4**)	46	8	50	6	46	5	47	4
	(−4)	(2)	(−2)	(5)	(−7)	(0)	(−7)	(2)
	[9]	[6]	[9]	[5]				
α-D-Glc*p*(1→3)α-D-Glc*p*-OMe (**5**)	−39	−14			−44	−23		
	[12]	[17]						
α-D-Glc*p*(1→4)α-D-Glc*p*-OMe (**6**)			−27	−23			−32	−28
			[11]	[12]				
β-D-Glc*p*(1→3)α-D-Glc*p*-OMe (**7**)	50	6			53	5		
	[11]	[12]						
β-D-Glc*p*(1→4)α-D-Glc*p*-OMe (**8**)			52	1			54	2
			[11]	[10]				

[a] 10^6 Macro steps Metropolis Monte Carlo simulations at 300 K performed with a total acceptance ratio between 0.35 and 0.49. [b] Angle differences between trisaccharides and corresponding disaccharide in parentheses. [c] Rms deviation from average angles in square brackets.

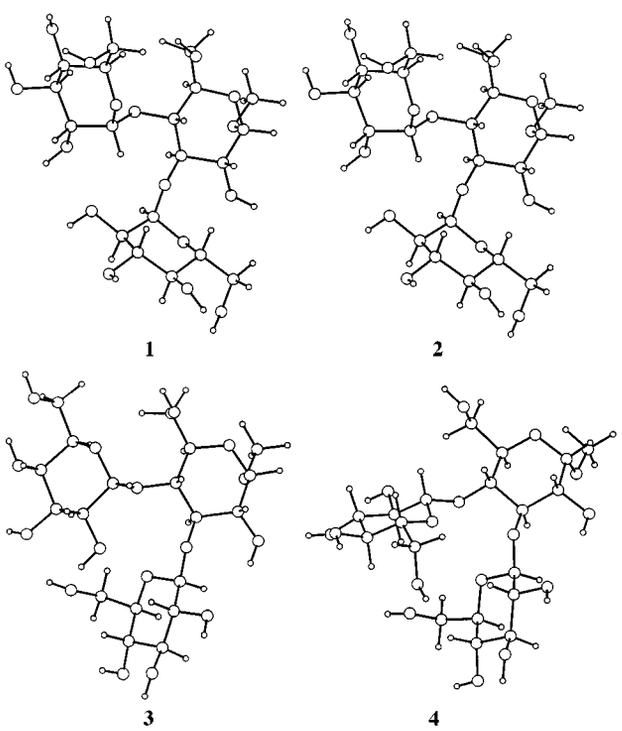


Fig. 1 Minimum energy conformers of trisaccharides **1**–**4**

angles for trisaccharides and their constituent disaccharides differ significantly for the α-linked glycosyl groups, in one case >50° for the ψ dihedral angle in the (1→3)-linkage of **1**. Trisaccharides **2** and **3**, which also contain α-linkages, show large and positive deviations. In contrast, the (1→4)-linkage in **1** shows small and negative deviations. All β-linked glycosyl groups have small differences between trisaccharides and disaccharides.

The averaged conformation from the MMC simulations is similar to that of the global energy minimum for **1**–**8**. Visualization of the conformational space sampled and the identification of conformational states can be obtained by scatter plots in which highly populated conformational states show clustering (Fig. 2). The β-linked residues show one conformational state at the global energy minimum region and low rms deviations of the dihedral angles over the MMC simulation. One conformational state is identified for the α-(1→3)-linkage in **1** and two states for the α-(1→4)-linkage, although only one is major. In **2** and **3** the α-linked sugar residues have an 'S-shaped' conformational space. For **2** three conformational states are readily identified whereas for **3** these are less pronounced. The rms fluctuations for the dihedral angles in the α-linkage in **2** and **3** are the largest for any of the trisaccharides, >20°, for both φ and ψ. The conformational flexibility of a molecule can be visualized by an overlay plot as shown for **2** in Fig. 3. The three conformational states of the α-(1→3)-linkage have been chosen and the overlay generated from the branch point residue. The larger flexibility for the α-linkage is contrasted to the lower flexibility for the β-linkage.

In the study of carbohydrate conformation two approaches have often been used, *i.e.* the application of sugar rings as rigid entities and non-charged atoms (hard sphere exo-anomeric, HSEA) or a full molecular mechanics force field with atoms carrying partial charges in which all degrees of freedom can be relaxed. In general, less conformational space is accessible in the former case. In the interpretation of experimental data, from NMR spectra for example, a choice of a force field has to be made, which may limit the extent to which conclusions can be drawn. These types of force fields do not account for geometrical effects of lone pairs or bond polarization, two factors which may influence the outcome of the NMR chemical shifts. By the use of *ab initio* methods chemical shifts can be calculated, although not yet with the accuracy to predict the chemical shifts of the trisaccharides in this study (*vide infra*).

^{1}H NMR glycosylation shifts

The ^{1}H NMR chemical shift data for compounds **1**–**4** are given in Table 3. The spectra were assigned by 1D TOCSY experiments with selective excitation at the anomeric protons. The glycosylation shifts for the trisaccharides (obs-mono) are obtained by subtraction of the monosaccharide chemical shift

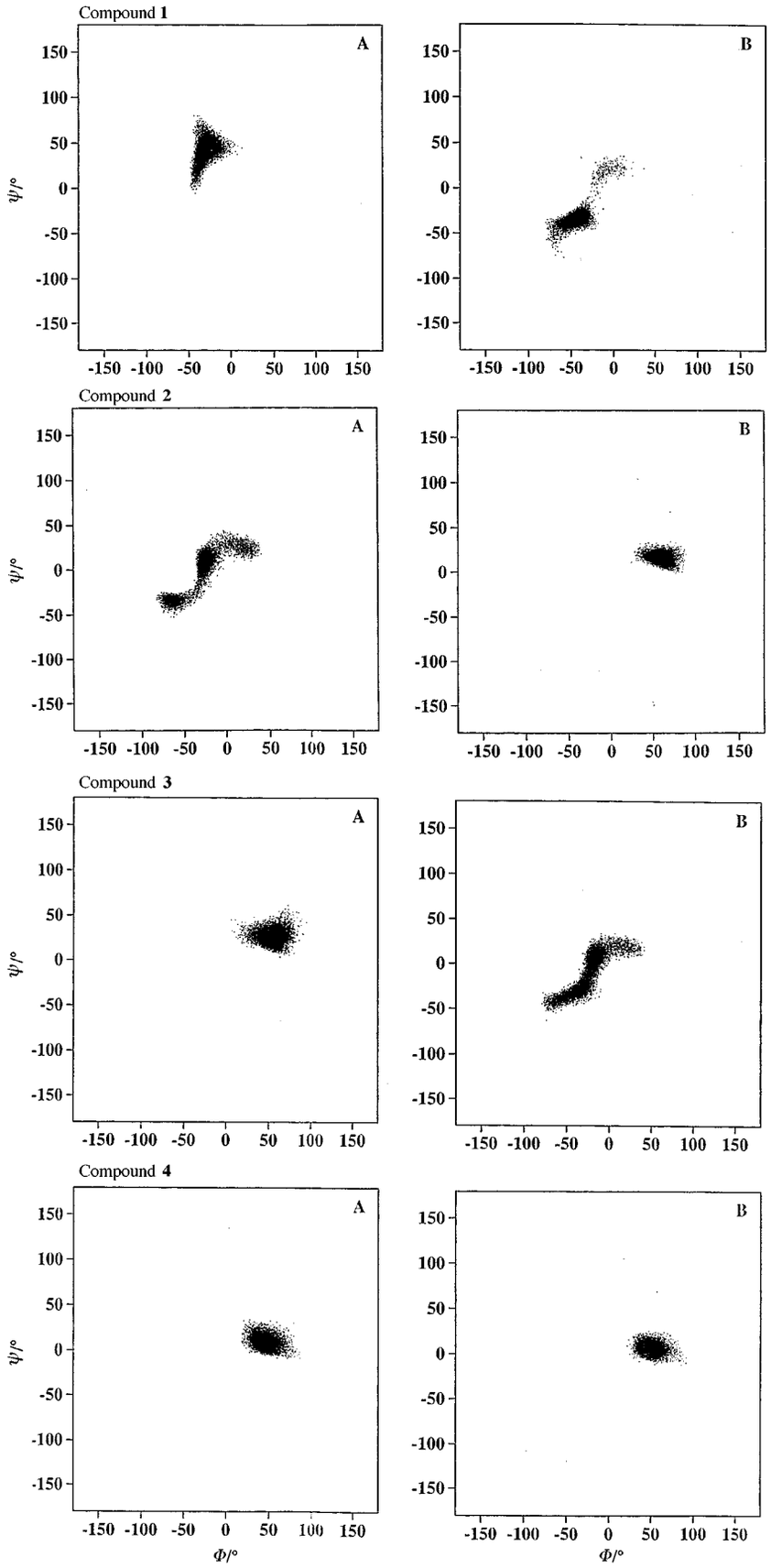


Fig. 2 Scatter plots from MMC simulations of trisaccharides **1**–**4**. (A) (1→3)-linkages and (B) (1→4)-linkages.

from those of the trisaccharides. A major aim of this study is the deviation from additivity of disaccharide glycosylation shifts (obs-calc). The deviation is the difference between actual spectra and what would have been obtained from monosaccharide chemical shifts to which are added glycosylation shifts specific for the glycosidic linkages in question. A negative value for the deviation indicates that the actual chemical shift is observed at a lower numerical value and *vice versa* for positive deviation. The deviations can be anticipated to derive from interactions between the substituting residues, although it is

Table 3 Chemical shifts of the signals in the ^{1}H NMR spectra at 70 °C of the trisaccharides **1**–**4** and the corresponding hexose and methyl hexosides, also listing calculated values of $\Delta\delta^a$ and of $\Delta\Delta\delta^b$

Sugar residue		H-1	H-2	H-3	H-4	H-5	H-6a	H-6b	OMe
α-D-Glc*p*(1→4)	(obs)	5.51	3.58	3.67	3.44	3.71	3.78	3.86	
	(obs-mono)	(0.28)	(0.04)	(−0.05)	(0.02)	(−0.13)	(0.02)	(0.02)	
	(obs-calc)	[0.15]	[−0.01]	[−0.02]	[0.00]	[−0.04]	[0.00]	[0.00]	
→3,4)α-D-Glc*p*-OMe	(obs)	4.82	3.76	4.02	3.86	3.78	3.87	3.93	3.43
	(obs-mono)	(0.01)	(0.20)	(0.34)	(0.45)	(0.14)	(0.11)	(0.06)	(0.00)
	(obs-calc)	[−0.01]	[0.05]	[−0.05]	[−0.01]	[0.01]	[0.07]	[0.05]	
α-D-Glc*p*(1→3)	(obs)	5.31	3.62	3.71	3.44	3.96	3.75	3.88	
	(obs-mono)	(0.08)	(0.08)	(−0.01)	(0.02)	(0.12)	(−0.01)	(0.04)	
(**1**)	(obs-calc)	[−0.01]	[0.04]	[−0.05]	[0.00]	[−0.01]	[−0.03]	[0.01]	
β-D-Glc*p*(1→4)	(obs)	4.56	3.30	3.53	3.41	3.49	3.79	3.96	
	(obs-mono)	(−0.08)	(0.05)	(0.03)	(−0.01)	(0.03)	(0.07)	(0.06)	
	(obs-calc)	[0.04]	[−0.03]	[0.00]	[−0.04]	[−0.01]	[0.05]	[0.03]	
→3,4)α-D-Glc*p*-OMe	(obs)	4.82	3.70	3.82	3.95	3.78	3.86	3.93	3.43
	(obs-mono)	(0.01)	(0.14)	(0.14)	(0.54)	(0.14)	(0.10)	(0.06)	(0.00)
	(obs-calc)	[0.00]	[−0.01]	[−0.12]	[0.07]	[0.00]	[0.01]	[0.02]	
α-D-Glc*p*(1→3)	(obs)	5.22	3.53	3.75	3.44	3.93	3.75	3.86	
	(obs-mono)	(−0.01)	(−0.01)	(0.03)	(0.02)	(0.09)	(−0.01)	(0.02)	
(**2**)	(obs-calc)	[−0.10]	[−0.05]	[−0.01]	[0.00]	[−0.04]	[−0.03]	[−0.01]	
α-D-Glc*p*(1→4)	(obs)	5.36	3.48	3.68	3.42	3.68	3.75	3.83	
	(obs-mono)	(0.13)	(−0.06)	(−0.04)	(0.00)	(−0.16)	(−0.01)	(−0.01)	
	(obs-calc)	[0.00]	[−0.11]	[−0.01]	[−0.02]	[−0.07]	[−0.03]	[−0.03]	
→3,4)α-D-Glc*p*-OMe	(obs)	4.78	3.80	4.19	3.72	3.75	3.86	3.92	3.42
	(obs-mono)	(−0.03)	(0.24)	(0.51)	(0.31)	(0.11)	(0.10)	(0.05)	(−0.01)
	(obs-calc)	[−0.06]	[0.00]	[0.07]	[−0.01]	[−0.03]	[0.05]	[0.01]	
β-D-Glc*p*(1→3)	(obs)	4.89	3.30	3.51	3.42	3.44	3.76	3.92	
	(obs-mono)	(0.25)	(0.05)	(0.01)	(0.00)	(−0.02)	(0.04)	(0.02)	
(**3**)	(obs-calc)	[0.21]	[−0.07]	[−0.03]	[−0.01]	[−0.06]	[0.02]	[0.03]	
β-D-Glc*p*(1→4)	(obs)	4.64	3.37	3.49	3.40	3.44	3.73	3.89	
	(obs-mono)	(0.00)	(0.12)	(−0.01)	(−0.02)	(−0.02)	(0.01)	(−0.01)	
	(obs-calc)	[0.12]	[0.04]	[−0.04]	[−0.05]	[−0.06]	[−0.01]	[−0.04]	
→3,4)α-D-Glc*p*-OMe	(obs)	4.78	3.79	4.13	3.86	3.80	3.90	3.90	3.41
	(obs-mono)	(−0.03)	(0.23)	(0.45)	(0.45)	(0.16)	(0.14)	(0.03)	(−0.02)
	(obs-calc)	[−0.05]	[−0.01]	[0.14]	[0.12]	[0.01]	[0.04]	[−0.04]	
β-D-Glc*p*(1→3)	(obs)	4.89	3.35	3.51	3.40	3.45	3.72	3.90	
	(obs-mono)	(0.25)	(0.10)	(0.01)	(−0.02)	(−0.01)	(0.00)	(0.00)	
(**4**)	(obs-calc)	[0.21]	[−0.02]	[−0.03]	[−0.03]	[−0.05]	[−0.02]	[−0.02]	
α-D-Glc*p*		5.23	3.54	3.72	3.42	3.84	3.76	3.84	
β-D-Glc*p*		4.64	3.25	3.50	3.42	3.46	3.72	3.90	
α-D-Glc*p*-OMe		4.81	3.56	3.68	3.41	3.64	3.76	3.87	3.43

[a] Glycosylation shifts are calculated by subtraction of the chemical shifts from those of the corresponding hexose and methyl hexoside, a positive difference indicates a downfield shift. [b] $\Delta\Delta\delta$ values 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 methylhexoside and then subtracting the resulting value from the measured chemical shift of the trisaccharide (for the methylhexoside residues $\Delta\delta$ values from both disaccharides are added).

difficult to quantify the magnitude of these. In general, for protons an upfield chemical shift displacement is observed upon a proton–proton interaction, whereas a downfield chemical shift displacement occurs for proton–oxygen interactions. A problem in the analysis is that different effects may cancel and the change may not be apparent. Previous work has included, *inter alia*, 3,4-disubstituted methyl galactosides and different 1,2-disubstituted sugar residues. The latter are linear oligosaccharides but formally vicinally disubstituted.

In compounds **1**–**4** glycosylation shifts are between −0.13 and 0.54 ppm. In examination of the trisaccharide chemical shifts and those obtained by the additivity approach a significance level of 0.1 ppm has been chosen for the present discussion. Only a few signals show differences of this magnitude, namely, H1″ in **1** (downfield), H3 and H1′ in **2** (upfield), H1′ in **3** (downfield), H2″ in **3** (upfield), and H3, H4, H1′ and H1″ in **4** (downfield), *i.e.* in all cases but one the deviations are confined to protons at glycosidic linkages.

In **1** the value of the anomeric proton resonance that shows a deviation from additivity is not at the glycosidic α-(1→3)-linkage that shows the largest difference in conformation compared to the constituent disaccharide, but at the α-(1→4)-linkage. One reason for this type of change has been discussed previously,[33] and may come from a changed localization of the lone pairs of the oxygen involved in a glycosidic linkage compared to its conformational preference in a hydroxy group. Thus, the downfield displacement of H1″ may be due to interactions with O3, through increased overlap of O3 lone pairs and H1″. In **2** the protons at the α-(1→3)-linkage show upfield displacements from additivity. Changes in positions in energy minima and their averages from the MMC calculations lead to a conformation with φ and ψ dihedral angles with smaller numerical values, *i.e.* closer to zero degrees. This in effect leads to a shorter H1′–H3 distance in **2**, 2.14 Å, than in **5**, 2.47 Å, as observed for the global energy minimum structures, as well as the average distance from the MMC simulation. The mutual upfield displacement can thus be explained by a shorter effective proton–proton distance. The β-(1→3)-linkage in **3** and **4** shows similarities as observed in the MMC and small deviations compared to its constituent disaccharide. As deduced from NMR data this linkage exhibits conformational similarities in **3** and **4** since H1′ and H3 show downfield shift displacements of *ca.* 0.2 and 0.1 ppm, respectively, together with a deviation for C2 of *ca.* 1.3 ppm (*vide infra*) which is not at a glycosylation position. Finally, for the β-(1→4)-linkage in **4**, significant

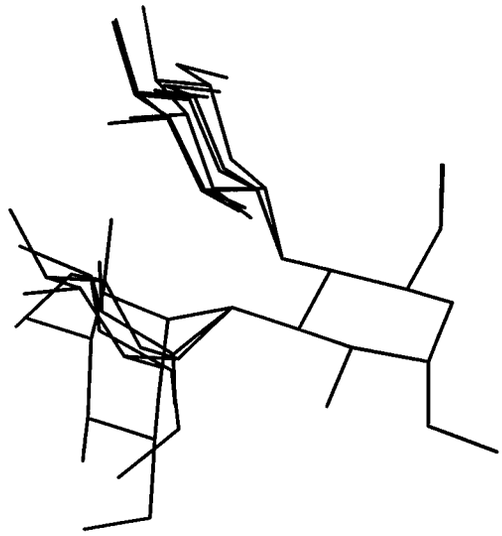


Fig. 3 Overlay plot for compound **2** of the conformers from the three low energy minima identified

deviations from additivity are also observed for the protons at the glycosidic linkage.

Since deviations from additivity of disaccharide glycosylations shifts are not larger than *ca*. 0.2 ppm, then it is possible to make reasonable predictions of ^{1}H NMR trisaccharide chemical shifts using only the disaccharide shifts.

^{13}C NMR glycosylation shifts

The ^{13}C NMR chemical shift data for compounds **1**–**4** are given in Table 4. The spectra were assigned by ^{1}H, ^{13}C HSQC and ^{1}H, ^{13}C HMBC experiments.† The significance level for the ^{13}C NMR chemical shifts was set to 1 ppm. Deviations are obtained for signals from glycosyloxylated carbons, namely, C3 (downfield) and C4 (upfield) in **1** and **2**, C3 (upfield) in **3** and C3 and C4 (upfield) in **4**. In **3** and **4**, which both have a β-(1→3)-linked glucosyl group the C2 resonance shows a downfield chemical shift displacement, *i.e.* the β-effect is quite large. Furthermore, the signals from anomeric carbons of the β-linked glucosyl groups in **4** have upfield chemical shift displacements.

In cases where significant changes from additivity take place for carbon signals and deviations also occur for protons, it can be observed that carbons with signals shifted upfield are associated with downfield displacements of the covalently linked protons and *vice versa*, compare for example H3/C3 in **2** and **4**. For the atoms at or next to glycosyloxylated carbons in compound **4** the direct α-effects and the adjacent β-effects add to an absolute deviation from additivity which is larger than 14 ppm. It would be detrimental not to include deviations of this magnitude in a simulation of a ^{13}C NMR spectrum of an oligo- or polysaccharide. For ^{13}C NMR chemical shifts the deviations are frequent and significant and in some cases large, ranging from −4.5 to 2.3 ppm.

Conclusions

The present study describes the synthesis of four trisaccharides which are used to investigate the vicinal disubstitution at the 3- and 4-positions in a glucopyranoside, the hydroxy groups of which have a *trans* relationship. It complements in particular the previous investigation of 3,4-disubstituted galactopyranosides with a *cis* relationship. Moderate deviations from additivity occur for ^{1}H NMR signals. Deviations for ^{13}C NMR spectra are large with both upfield and downfield displacements. For the analysis of complex NMR spectra of large oligo- and polysaccharides knowledge of these deviations will be indispensable. The conformational flexibility of the four anomeric combinations of the 3,4-disubstituted glucopyranoside was investigated by energy minimization and Metropolis Monte Carlo simulations which in general showed larger flexibility and larger deviations for the α-linked glucosyl groups than for the β-linked groups. Simulation using MMC leads to rapid identification of both oligosaccharide flexibility and possible changes in conformation between different oligosaccharides.

Experimental

General

Atoms in the (1→4)-linked glucosyl group are labeled by a double prime, in the (1→3)-linked glucosyl group by a prime and in the methyl glucoside the atoms are unprimed. Dichloromethane was distilled and dried over molecular sieves (4 Å) before use in coupling reactions. Diethyl ether was dried over sodium wire. Concentrations were performed under reduced pressure at temperatures <50 °C (bath). TLC was conducted on precoated plates (Merck Silica Gel 60 F_{254}) and detected by UV at 245 nm or developed by charring with 8% aqueous sulfuric acid. Column chromatography was carried out on Matrex silica gel 60 Å (35–70 μm, Amicon) and on Bio-Gel P-2. Optical rotations were determined at 578 nm with a Perkin-Elmer 241 polarimeter and measured in chloroform or water. $[\alpha]_{578}$ values are given in units of 10^3 deg cm^2 g^{-1}.

Synthesis

General procedure for α-glycosylation. To a cooled solution (0 °C) of ethyl 2,3,4,6-tetra-*O*-benzyl-thio-β-D-glucopyranoside (1.3 equiv.) in dichloromethane, bromine (10 cm^3, 1.4 equiv.) was added. After 10 min the reaction mixture was diluted with toluene (10 cm^3) and the mixture was concentrated *in vacuo* and coevaporated twice with toluene. The resulting 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl bromide as donor was dissolved in diethyl ether (20 cm^3). To the solution were added aglycon (1 equiv.), *s*-collidine ‡ (0.7 equiv.) and molecular sieves 4 Å (1 g). The mixture was stirred for 30 min, cooled to −30 °C, whereafter AgOTf (1.3 equiv.) was added and the temperature was kept at −30 °C. When TLC showed complete reaction, pyridine (0.5 cm^3) was added and the reaction mixture was filtered, concentrated and purified from small amounts of the β-anomer by silica gel column chromatography.

General procedure for β-glycosylation. A mixture of aglycon (1 equiv.), 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide (1.3 equiv., except for compound **14** for which 2.5 equiv. were used) was dissolved in dichloromethane (20 cm^3). To the solution were added *s*-collidine (0.7 equiv.) and molecular sieves 4 Å (1 g) and the mixture was stirred for 30 min, cooled to −30 °C, whereafter AgOTf (1.3 equiv.) was added and the temperature was kept at −30 °C. When TLC showed complete reaction, pyridine (0.5 cm^3) was added and the reaction mixture was filtered, concentrated and purified by silica gel column chromatography.

General procedure for reductive ring opening of a 4,6-benzylidene group. $NaCNBH_3$ (12.5 equiv.), the 4,6-benzylidene derivative (1 equiv.) and 3 Å molecular sieves were mixed in THF, and stirred at room temperature. After 30 min diethyl ether saturated with hydrogen chloride was added until the reaction mixture was acidic. After 15 (**12** and **23**) or 30 min (**13**), triethylamine was added. The quenched reaction mixture was filtered through Celite, concentrated and purified by silica gel column chromatography.

General procedure for removal of protecting groups. To remove benzoyl groups the compounds were dissolved in

† HSQC and HMBC are heteronuclear single quantum correlation and heteronuclear multiple bond correlation spectroscopy, respectively.

‡ *s*-Collidene = 2,4,6-trimethylpyridine.

Table 4 Chemical shifts of the signals in the ^{13}C NMR spectra at 70 °C of the trisaccharides **1**–**4** and the corresponding hexose and methyl hexosides, also listing calculated values of $\Delta\delta$[a] and of $\Delta\Delta\delta$[b]

Sugar residue		C-1	C-2	C-3	C-4	C-5	C-6	OMe
α-D-Glc*p*(1→4)	(obs)	99.91	72.34	73.58	70.40	73.58	61.49	
	(obs-mono)	(6.92)	(−0.13)	(−0.20)	(−0.31)	(1.21)	(−0.35)	
	(obs-calc)	[−0.74]	[−0.38]	[−0.31]	[−0.04]	[−0.02]	[−0.10]	
→3,4)α-D-Glc*p*-OMe	(obs)	99.62	71.31	84.10	76.28	71.02	61.76	55.88
	(obs-mono)	(−0.57)	(−0.92)	(10.00)	(5.60)	(−1.50)	(0.09)	(−0.05)
	(obs-calc)	[−0.48]	[0.69]	[2.34]	[−2.31]	[0.13]	[0.29]	
α-D-Glc*p*(1→3)	(obs)	99.80	72.44	73.74	70.61	73.44	61.49	
	(obs-mono)	(6.81)	(−0.03)	(−0.04)	(−0.10)	(1.07)	(−0.35)	
(**1**)	(obs-calc)	[−0.15]	[−0.22]	[−0.17]	[0.05]	[0.63]	[0.01]	
β-D-Glc*p*(1→4)	(obs)	102.56	74.36	76.55	70.61	77.30	61.73	
	(obs-mono)	(5.72)	(−0.84)	(−0.21)	(−0.10)	(0.54)	(−0.11)	
	(obs-calc)	[−0.73]	[0.23]	[−0.03]	[0.10]	[0.42]	[0.08]	
→3,4)α-D-Glc*p*-OMe	(obs)	99.94	71.47	81.75	75.71	71.47	60.55	55.91
	(obs-mono)	(−0.25)	(−0.76)	(7.65)	(5.03)	(−1.05)	(−1.12)	(−0.02)
	(obs-calc)	[−0.09]	[0.89]	[1.75]	[−4.32]	[0.54]	[−0.40]	
α-D-Glc*p*(1→3)	(obs)	100.88	72.96	74.36	70.53	73.23	61.46	
	(obs-mono)	(7.89)	(0.49)	(0.58)	(−0.18)	(0.86)	(−0.38)	
(**2**)	(obs-calc)	[0.93]	[0.30]	[0.45]	[−0.03]	[0.42]	[−0.02]	
α-D-Glc*p*(1→4)	(obs)	100.96	73.01	73.98[c]	70.45	73.69[c]	61.54	
	(obs-mono)	(7.97)	(0.54)	(0.20)	(−0.26)	(1.32)	(−0.30)	
	(obs-calc)	[0.31]	[0.29]	[0.09]	[0.01]	[0.88]	[0.06]	
→3,4)α-D-Glc*p*-OMe	(obs)	99.91	72.77	80.78	76.55	71.69	61.54	55.93
	(obs-mono)	(−0.28)	(0.54)	(6.68)	(5.87)	(−0.83)	(−0.13)	(0.00)
	(obs-calc)	[0.07]	[1.37]	[−3.21]	[−0.34]	[0.74]	[−0.09]	
β-D-Glc*p*(1→3)	(obs)	103.34	74.63	76.71	70.45	77.14	61.76	
	(obs-mono)	(6.50)	(−0.57)	(−0.05)	(−0.26)	(0.38)	(−0.08)	
(**3**)	(obs-calc)	[−0.33]	[0.21]	[0.11]	[−0.13]	[0.25]	[0.12]	
β-D-Glc*p*(1→4)	(obs)	101.56	73.93	76.52	70.42	76.79	61.71[c]	
	(obs-mono)	(4.72)	(−1.27)	(−0.24)	(−0.29)	(0.03)	(−0.13)	
	(obs-calc)	[−1.73]	[−0.20]	[−0.06]	[−0.09]	[−0.09]	[0.06]	
→3,4)α-D-Glc*p*-OMe	(obs)	99.86	72.66	77.87	73.85	71.80	61.17	55.91
	(obs-mono)	(−0.33)	(0.43)	(3.77)	(3.17)	(−0.72)	(−0.50)	(−0.02)
	(obs-calc)	[0.09]	[1.30]	[−4.36]	[−4.48]	[0.81]	[0.06]	
β-D-Glc*p*(1→3)	(obs)	102.37	74.23	76.65	70.56	77.03	61.76[c]	
	(obs-mono)	(5.53)	(−0.97)	(−0.11)	(−0.15)	(0.27)	(−0.08)	
(**4**)	(obs-calc)	[−1.30]	[−0.19]	[0.05]	[−0.02]	[0.14]	[0.12]	
α-D-Glc*p*		92.99	72.47	73.78	70.71	72.37	61.84	
β-D-Glc*p*		96.84	75.20	76.76	70.71	76.76	61.84	
α-D-Glc*p*-OMe		100.19	72.23	74.10	70.68	72.52	61.67	55.93

[a] Glycosylation shifts, in parentheses, are calculated by subtraction of the chemical shifts from those of the corresponding hexose and methyl hexoside, a positive difference indicates a downfield shift. [b] $\Delta\Delta\delta$ values 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 methylhexoside and then subtracting the resulting value from the measured chemical shift of the trisaccharide (for the methylhexoside residues $\Delta\delta$ values from both disaccharides are added). [c] Interchangeable pairs.

dichloromethane and 0.1 mol dm^{-3} sodium methoxide in methanol was added. The solution was stirred until TLC showed complete reaction, and passed through a DOWEX-50(H^+) ion-exchange column, after which the solvent was removed *in vacuo*. For the deprotection of the benzyl groups the compounds were dissolved in EtOAc–EtOH and hydrogenolysed over Pd–C (cat) at 90 psi for 5–12 h. The fully deprotected compounds were purified by gel permeation chromatography.

NMR spectroscopy

NMR spectra were recorded on JEOL GSX-270, Varian Unity 500 and Unity Plus 600 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, $\delta_H = 0.00$) and dioxane ($\delta_C = 67.40$). For assignments of signals of **1**–**4** different types of homo- and hetero-nuclear correlation spectroscopy (COSY) experiments were used as well as 1D TOCSY[34] experiments with different spin lock times of 30, 70, 90 and 100 ms. To establish the glycosidic connectivities long-range proton–carbon correlated experiments, HMBC,[35] were performed using a delay time of 50 ms.

Mass spectroscopy

Fast Atom Bombardment Mass Spectroscopy (FABMS) was performed at a resolution of 2500 on a JEOL SX-102 using a mixture of glycerol and thioglycerol, triethanolamine or *m*-nitrobenzyl alcohol as a matrix. Atmospheric Pressure Chemical Ionization (APCI) was recorded in the positive mode for compound **27** on a Quattro mass spectrometer (Fisons Instruments, UK).

Computational methods

The GEGOP program, version 2.6, was used for all calculations.[36] 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°. The *O*-methyl group was *gauche* to O5 and *anti* to C2. The Metropolis Monte Carlo (MMC) simulations of **1**–**8** were started from low energy conformations obtained from a 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 eight molecules. The parameters were adjusted

with test runs to obtain a total acceptance ratio between 35 and 49%. The maximum step length for the glycosidic angles φ and ψ was set to 20°. The bond angle τ and all dihedral angles φ, ψ, ω and those of the hydroxy and *O*-methyl groups, were optimized. The pyranose rings were treated as rigid units residing in the $^4C_1$ conformation.

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References

1 J. H. Bradbury and G. A. Jenkins, *Carbohydr. Res.*, 1984, **126**, 125.
2 K. Bock, *Adv. Carbohydr. Chem. Biochem.*, 1984, **42**, 193.
3 H. Baumann, P.-E. Jansson and L. Kenne, *J. Chem. Soc.*, *Perkin Trans. 1*, 1991, 2229.
4 P.-E. Jansson, L. Kenne and E. Schweda, *J. Chem. Soc.*, *Perkin Trans. 1*, 1988, 2729.
5 P.-E. Jansson, L. Kenne, K. Persson and G. Widmalm, *J. Chem. Soc.*, *Perkin Trans. 1*, 1990, 591.
6 P.-E. Jansson and G. Widmalm, *J. Chem. Soc.*, *Perkin Trans. 2*, 1992, 1085.
7 H. Baumann, P.-E. Jansson and L. Kenne, *J. Chem. Soc.*, *Perkin Trans. 1*, 1988, 209.
8 I. Backman, B. Erbing, P.-E. Jansson and L. Kenne, *J. Chem. Soc.*, *Perkin Trans. 1*, 1988, 889.
9 I. Backman, P. E. Jansson and L. Kenne, *J. Chem. Soc.*, *Perkin Trans. 1*, 1990, 1383.
10 P.-E. Jansson, L. Kenne and I. Kolare, *Carbohydr. Res.*, 1994, **257**, 163.
11 P.-E. Jansson, L. Kenne and G. Widmalm, *Acta Chem. Scand.*, 1991, **45**, 517.
12 G. M. Lipkind, N. E. Nifant'ev, A. S. Shashkov and N. K. Kochetkov, *Can. J. Chem.*, 1990, **68**, 1238.
13 P.-E. Jansson, A. Kjellberg, T. Rundlöf and G. Widmalm, *J. Chem. Soc.*, *Perkin Trans. 2*, 1996, 33.
14 H. Baumann, B. Erbing, P.-E. Jansson and L. Kenne, *J. Chem. Soc.*, *Perkin Trans. 1*, 1989, 2145.
15 H. Baumann, B. Erbing, P.-E. Jansson and L. Kenne, *J. Chem. Soc.*, *Perkin Trans. 1*, 1989, 2153.
16 H. Baumann, B. Erbing, P.-E. Jansson and L. Kenne, *J. Chem. Soc.*, *Perkin Trans. 1*, 1989, 2167.
17 K. Bock, J. F.-B. Guzman and R. Norrestam, *Carbohydr. Res.*, 1988, **179**, 97.
18 E. A. Khatuntseva, A. S. Shashkov and N. E. Nifant'ev, *Magn. Reson. Chem.*, 1997, **35**, 414.
19 A. Adeyeye, P.-E. Jansson, L. Kenne and G. Widmalm, *J. Chem. Soc.*, *Perkin Trans. 2*, 1991, 963.
20 K. Hermansson, P.-E. Jansson, L. Kenne, G. Widmalm and F. Lindh, *Carbohydr. Res.*, 1992, **235**, 69.
21 J. Ø. Duus, N. E. Nifant'ev, A. S. Shashkov, E. A. Khatuntseva and K. Bock, *Carbohydr. Res.*, 1996, **288**, 25.
22 F. J. Kronzer and C. Scheuerch, *Carbohydr. Res.*, 1973, **28**, 279.
23 S. Hanessian and J. Banoub, *Carbohydr. Res.*, 1977, **53**, C13.
24 P. J. Garegg, T. Iversen and S. Oscarson, *Carbohydr. Res.*, 1976, **50**, C12.
25 P. J. Garegg and H. Hultberg, *Carbohydr. Res.*, 1981, **93**, C10.
26 F. Weygand and H. Ziemann, *Liebigs Ann. Chem.*, 1962, **657**, 179.
27 R. K. Ness, H. G. Fletcher, Jr and C. S. Hudson, *J. Am. Chem. Soc.*, 1950, **72**, 2200.
28 E. J. Corey and W. J. Suggs, *J. Org. Chem.*, 1973, **38**, 3224.
29 K. Ekelöf and S. Oscarson, *Carbohydr. Res.*, 1995, **278**, 289.
30 H. Qin and T. B. Grindley, *J. Carbohydr. Chem.*, 1994, **13**, 475.
31 N. Metropolis, A. W. Rosenbluth, M. N. Rosenbluth, A. H. Teller and E. Teller, *J. Chem. Phys.*, 1953, **21**, 1087.
32 T. Peters, B. Meyer, R. Stuike-Prill, R. Somorjai and J.-R. Brisson, *Carbohydr. Res.*, 1993, **238**, 49.
33 H. Baumann, *Chem. Commun. Univ. Stockholm*, 1988, **9**, 1.
34 H. Kessler, H. Oschkinat and C. Griesinger, *J. Magn. Reson.*, 1986, **70**, 106.
35 A. Bax and M. F. Summers, *J. Am. Chem. Soc.*, 1986, **108**, 2093.
36 R. Stuike-Prill and B. Meyer, *Eur. J. Biochem.*, 1990, **194**, 903.

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