Synthesis and NMR and Conformational Studies of the Four Anomeric Methyl Glycosides of the Trisaccharide D-Glc*p*-(1→2)-D-Glc*p*-(1→3)-α-D-Glc*p*

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The four anomeric methyl glycosides of the trisaccharide D-Glc*p*-(1→2)-D-Glc*p*-(1→3)-α-D-Glc*p* have been synthesized and used for ^1H and ^{13}C NMR studies. All ^1H and ^{13}C NMR resonances were assigned and comparison was made between the observed glycosylation shifts, *i.e.* the differences between chemical shifts for signals from the trisaccharides and those of the respective monomers, and those derived by addition of the glycosylation shifts for each disaccharide element. With a few exceptions, only minor deviations were found and the differences were mostly confined to signals from linkage carbons and the attached protons. Conformational analysis was performed using the HSEA and GESA approaches and measurements of the nuclear Overhauser enhancements. The results indicate that minimum energy conformations are similar to those of the disaccharides but that rotational freedom around some of the glycosidic bonds is restricted.

NMR and conformational studies have previously been performed on a number of 2,3-[1] and 3,4-branched[2,3] trisaccharides with the purpose of studying models for vicinally disubstituted branchpoint regions in oligo- and poly-saccharides. The glycosylation shifts in the trisaccharides were shown to deviate from the sum of the glycosylation shifts of the constituent disaccharides, and for many of the signals from atoms at or near a linkage large deviations were observed. This was assumed to be due to atomic interactions between sugar residues, causing displaced energy minima with changed values for torsional angles, and restricted rotational freedom around the glycosidic bonds. As deviations from additivity of glycosylation shifts were observed for one type of vicinally disubstituted residues, and an understanding of the phenomenon was desired, studies on other related trisaccharides were implied. We now report synthesis of, and NMR and conformational studies on, the four anomers of the trisaccharide D-Glc*p*-(1→2)-D-Glc*p*-(1→3)-α-D-Glc*p*-OMe. The aim of the study is to investigate whether additivity of NMR glycosylation shifts holds at a 1,2-linked residue, which is not a branched residue but nevertheless vicinally disubstituted. This would help in the prediction of NMR spectra of 1,2-linked residues, and of neighbouring residues, as performed by the computer program CASPER[4,5] used for structural studies of oligo- and polysaccharides. The glycosylation shifts are needed in the computer analysis.

Experimental

Materials.—The structure of trisaccharides **1–4** are given in Table 1. The syntheses of the intermediates **5**,[6] **7**,[7] **11**,[8] **13** and **14**,[9] **18**,[10] **22**[11] and **24**[12] have been reported.

General Methods.—Concentrations were performed under reduced pressure at temperatures <40 °C (bath). Optical rotations were measured at 22 °C for solutions in chloroform or water (c = 1.0–1.7 mg cm^3) with a Perkin-Elmer 241 polarimeter. Silver trifluoromethanesulphonate-mediated couplings were performed essentially as described.[13–16] Data on coupling reactions and deprotection procedures, together with physical constants are given in Table 2. In the following text, and in the Tables, the atoms of the methyl glucoside residue are unprimed, those in the central residue primed, and those in the group at the non-reducing end double primed.

The purity of intermediates was first analysed by TLC, which showed only one spot. Secondly, from the ^{13}C NMR spectra the intermediates were estimated to be more than 95% pure (except for **23**). The number of signals in the ^{13}C NMR spectra and their chemical shifts were consistent with the postulated structures and the chemical shift of the C-1 signals showed the anomeric configurations. The substitution position of the glucosyl groups was usually determined by the synthetic route. When diols were monoglycosylated a methylation analysis[17] established the linkage position.

In the ^1H NMR spectra of the deprotected oligosaccharides, signals from contaminating components were <5% of those of the anomeric proton signals (except for **3**, see below). The number of signals and their chemical shifts in the ^1H and ^{13}C NMR spectra were also in agreement with the postulated structures. Anomeric configurations of the final products were deduced from the size of the coupling constant, $^3J_{H,H}$, of signals from anomeric protons.

NMR spectra were recorded for solutions in $CDCl_3$, CD_3OD or D_2O using JEOL FX 100, GSX 270 or GX 400 instruments. Chemical shifts are reported in ppm and referenced to internal $SiMe_4$ (δ_C 0.00) for solutions in $CDCl_3$ or CD_3OD at 25 °C. For solutions in D_2O the spectra were recorded at 70 °C using dioxane (δ_C 67.40 ppm) and sodium 3-(trimethylsilyl)-[2,2,3,3-$^2H_4$]-propanoate (TSP, δ_H 0.00 ppm) as internal references. For the assignment of signals in the spectra of the final products, different types of proton–proton and carbon–proton shift correlation spectroscopy, COSY, were used. ^1H NMR chemical shifts of overlapping signals were obtained from the centre of the cross-peaks in the proton–proton shift correlation spectra. Normally, proton connectivities from the anomeric protons were established from the COSY spectrum but when overlap was present, relayed and double relayed COSY spectra aided the analysis. These assignments were then used to assign the signals in the carbon–proton correlation spectrum. From this analysis it was also evident which set of signals belonged to which residue as large glycosylation shifts were obtained for signals for C-2 and C-3, *i.e.* the linkage carbons.

NOE was determined by NOE difference experiments, using 4 s pre-irradiation at the frequency of the respective anomeric proton, and confirmed by NOESY experiments, using 0.6 s mixing time.

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Table 1 Values for the φ and ψ angles, in degrees, together with inter-residue internuclear distances <3 Å in the minimum energy conformations as indicated by GESA calculations. The glycosidic linkage between the glucosyl group and the middle glucosyl residue is defined by φ_1/ψ_1 and between the middle residue and the methyl glycoside residue by φ_2/ψ_2. The differences compared to the corresponding disaccharides are given in parentheses, and a negative value indicates a shorter distance in the trisaccharide.

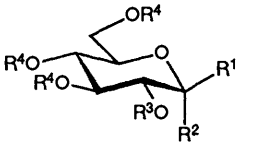
Compound	φ_1/ψ_1	φ_2/ψ_2	1″-H	5″-H	O-5″	1′-H	5′-H	O-5′
α-D-Glc*p*-(1→2)-α-D-Glc*p*-(1→3)-α-D-Glc*p*-OMe (**1**)	−48/−36 (0/−2)	−41/−23 (5/2)	2.24 (1′-H) (−0.06) 2.62 (2′-H) (0.06) 2.90 (O-4)	2.66 (0-3′) (0.06) 2.94 (2′-H) (−0.01)	2.51 (2′-H) (0.03)	2.58 (O-4) (0.06) 2.42 (3-H) (−0.06)	2.54 (O-2) (0.07)	2.70 (3-H) (0.09)
α-D-Glc*p*-(1→2)-β-D-Glc*p*-(1→3)-α-D-Glc*p*-OMe (**2**)	−53/−53 (−11/−31)	57/3 (−1/2)	1.97 (O-1′) (−0.58) 2.99 (1′-H) (−0.74) 2.92 (2′-H) (0.51) 2.91 (O-2) 2.61 (2-H)	2.66 (2′-H) (−0.67)	2.54 (2′-H) (−0.13)	2.43 (3-H) (0.00)		2.56 (3-H) (0.00)
β-D-Glc*p*-(1→2)-α-D-Glc*p*-(1→3)-α-D-Glc*p*-OMe (**3**)	58/−21 (−2/−7)	−41/−24 (5/1)	2.28 (2′-H) (0.00)		2.41 (1′-H) (−0.17) 2.74 (2′-H) (0.13)	2.55 (O-4) (0.03) 2.44 (3-H) (−0.06)	2.55 (O-2) (0.08)	2.68 (3-H) (0.07)
β-D-Glc*p*-(1→2)-β-D-Glc*p*-(1→3)-α-D-Glc*p*-OMe (**4**)	66/−17 (8/−20)	53/5 (−5/4)	2.44 (2′-H) (0.02)		2.57 (2′-H) (0.03)	2.39 (3-H) (−0.04)		2.62 (3-H) (0.04)

The GESA programme[18] was used to estimate minimum energy conformations of the trisaccharides and their constituent disaccharides. The torsion angles φ, ψ and ω were defined by H(1′)–C(1′)–O(X)–C(X), C(1′)–O(X)–C(X)–H(X) and O(5)–C(5)–C(6)–O(6), respectively, where X is the linkage position. The bond angle τ, defined by C(1′)–O(X)–C(X), was set at 117°. For **4**, ω″ was also allowed to change due to the proximity of the hydroxymethyl group to the methyl glucoside residue. Energy maps, showing the rotational freedom around the glycosidic bonds, were obtained using the HSEA programme.[19,20] During these energy calculations, the dihedral angles of one of the glycosidic bonds were fixed at the φ,ψ values obtained from the GESA calculations on the trisaccharides, while the other glycosidic bond was rotated. The same procedure was then repeated for the other glycosidic bond. Co-ordinate sets were obtained from crystal structures of α-D-glucopyranose,[21] β-D-glucopyranose[22] and methyl α-D-glucopyranoside.[23]

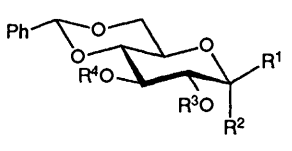
Results and Discussion

Synthesis.—For the preparation of the four anomers of the trisaccharide D-Glc*p*-(1→2)-D-Glc*p*-(1→3)-α-D-Glc*p*-OMe **1**–**4**, one route working towards the methyl glucoside residue and one towards the glucosyl group was employed. Silver trifluoromethanesulphonate-mediated glycosylations[13–16] were used in all steps. The first route was employed for the synthesis of α-D-Glc*p*-(1→2)-α-D-Glc*p*-(1→3)-α-D-Glc*p*-OMe (**1**). Ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside (**5**) was treated with bromine to yield 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl bromide (**6**), which was condensed with ethyl 3-*O*-acetyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (**8**) to yield disaccharide **9** in 63% yield. Treatment of **9** with bromine gave a disaccharide bromide (**10**), which was reacted with methyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (**11**) to yield trisaccharide **12** in 34% yield. The protecting groups were removed from **12** by treatment with methanolic sodium methoxide followed by catalytic hydrogenolysis with palladium on carbon to yield, after gel filtration, **1** in 95% yield.

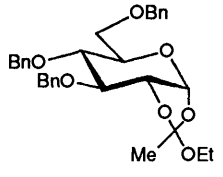
For the synthesis of α- and β-D-Glc*p*-(1→2)-β-D-Glc*p*-(1→3)-α-D-Glc*p*-OMe, **2** and **4**, the second route with the common disaccharide intermediate **16**, was used. 3,4,6-Tri-*O*-benzyl-1,2-*O*-(1-ethoxyethylidene)-α-D-glucopyranoside (**13**) was treated with hydrogen bromide in glacial acetic acid to yield 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl bromide (**14**) which was used in the glycosylation of **11** to yield 73% of disaccharide **15**.



5 R^1 = SEt, R^2 = H, R^3 = R^4 = Bn
6 R^1 = H, R^2 = Br, R^3 = R^4 = Bn
14 R^1 = H, R^2 = Br, R^3 = Ac, R^4 = Bn
18 R^1 = Cl, R^2 = H, R^3 = $COCCl_3$, R^4 = Ac
22 R^1 = H, R^2 = Br, R^3 = R^4 = Ac
24 R^1 = H, R^2 = Br, R^3 = R^4 = Bz



7 R^1 = SEt, R^2 = R^3 = R^4 = H
8 R^1 = SEt, R^2 = R^3 = H, R^4 = Ac
11 R^1 = H, R^2 = OMe, R^3 = Bn, R^4 = H



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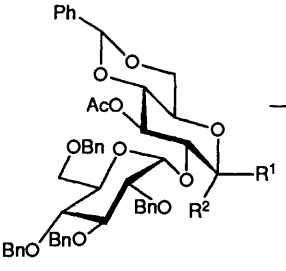

9 R^1 = SEt, R^2 = H
10 R^1 = H, R^2 = Br

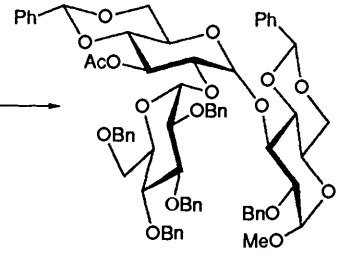

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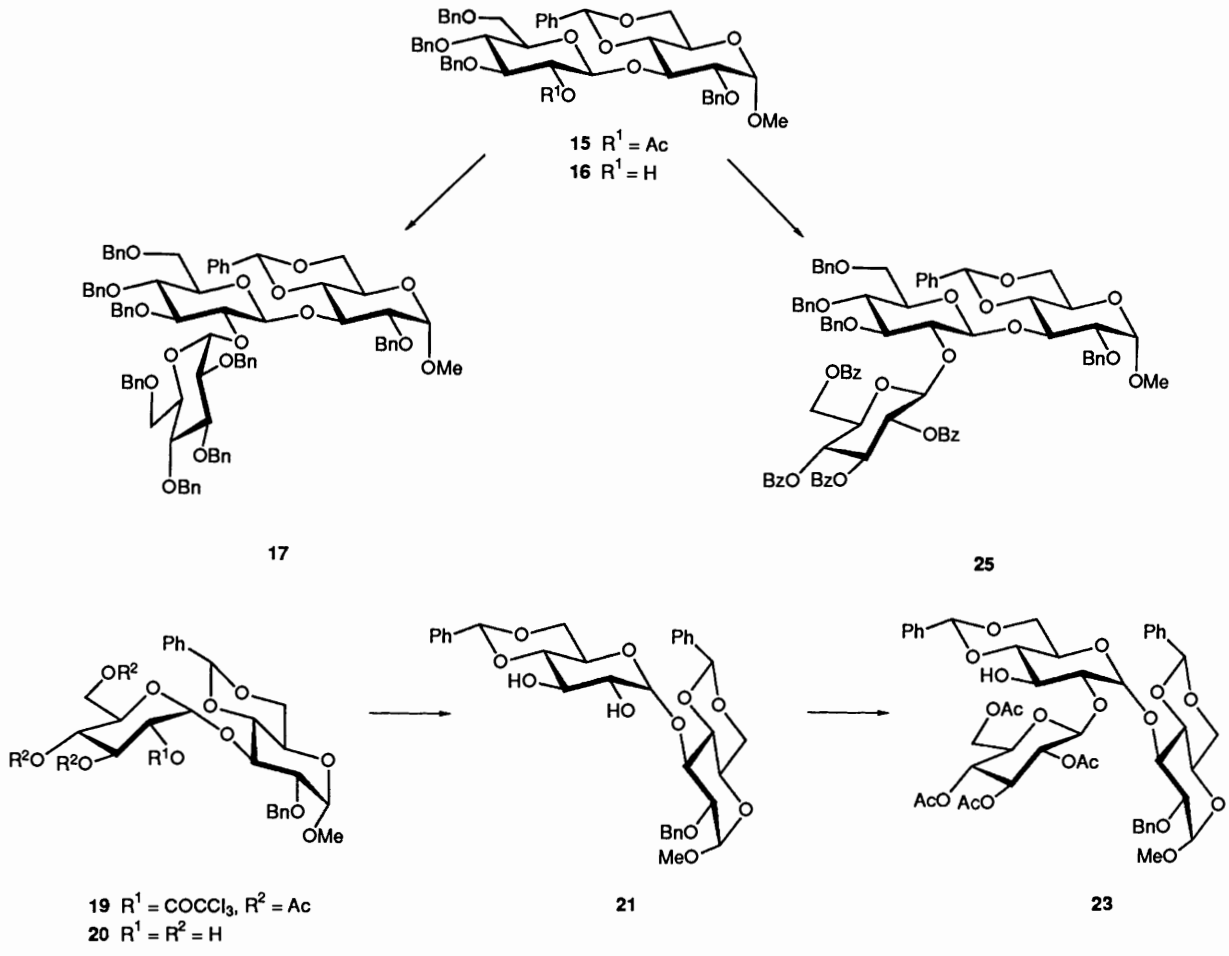
Table 2 Data on reaction conditions, physical constants and selected NMR chemical shifts

Conversion[a]	Solvent (cm^3)	Reagents (mg, mmol)	t_{react}/h	T/°C	Yield (%)	Purif. (solvent)	$[\alpha]_{578}/10^{-1}$ deg cm^2 g^{-1}	δ_C (anomeric)
7→8[25]								86.8 (C-1)
5→6	CH_2Cl_2/4 Å[b] (10)	(5) (850, 1.4) Br_2 (70 mm^3)	1	0	*c*			
6 + 8→9	CH_2Cl_2/4 Å (15)	(6) (–, 1.4) (8) (355, 1.0) AgOTf (590, 2.3) Collidine (0.3 cm^3, 2.3)	1	−40→22	63	T–E[d]	17	85.2 (C-1), 95.9 (C-1′)
9→10	CH_2Cl_2/4 Å (10)	(9) (1230, 1.4) Br_2 (300 mm^3)	1	0	*c*			
10 + 11→12	CH_2Cl_2/4 Å (10)	(10) (–, 1.4) (11) (390, 1.0) AgOTf (770, 3.0) Collidine (0.3 cm^3, 2.3)	0.5	−25	34	T–E	73	94.5 (C-1′), 95.6 (C-1″), 98.5 (C-1)
12→1	(a) MeOH	(12) (350, 0.3) NaOMe (0.1 mol dm^{-3})						
	(b) HOAc (80%)	H_2/Pd	16		95	E–A–M–H, 12:3:3:2	191	
14 + 11→15	CH_2Cl_2/4 Å (10)	(11) (470, 1.0) (14) (1000, 2.0) AgOTf (590, 2.3) Collidine (0.16 cm^3, 1.2)	0.25	−25	73	T–E	4	98.9 (C-1), 100.8 (C-1′)
15→16	CH_2Cl_2/MeOH (30, 1:1)	(15) (860, 1.0) NaOMe (1 mol dm^{-1} 1 cm^3)	16		87			98.3 (C-1), 104.5 (C-1′)
6 + 16→17	CH_2Cl_2/4 Å (10)	(6) (–, 2.0) (16) (980, 1.2) AgOTf (770, 3.0) Collidine (0.27 cm^3, 2.0)	0.25	−25	36	T–E	52	94.8 (C-1″), 98.2 (C-1), 102.4 (C-1′)
17→2	HOAc (80%)	(17) (200, 0.14) H_2/Pd	16		95	E–A–M–H, 12:3:3:2	119	
11 + 18→19	CH_2Cl_2/4 Å (15)	(11) (390, 1.0) (18) (640, 1.4) AgOTf (1540, 6.0) Collidine (0.5 cm^3, 3.8)		−40	38	P–E	85	94.6 (C-1′), 98.2 (C-1)
19→20	MeOH	(19) (810, 1.0) NaOMe (0.05 mol dm^{-1})	2					97.6, 100.3 (C-1, C-1′)
20→21	Benzaldehyde (1)	(20) (500, 1.1) PTS[e] (50)	16		60			98.4, 99.3 (C-1, C-1′)
21 + 22→23	CH_2Cl_2/4 Å (10)	(21) (590, 1.0) (22) (820, 2.0) AgOTf (590, 2.3)	0.5	−25	—			98.2, 98.4, 100.1 (C-1, C-1′, C-1″)
23→3	(a) MeOH	(23) (55, 0.06) NaOMe (0.05 mol dm^{-1})	1					
	(b) HOAc (80%)	H_2/Pd			84	E–A–M–H, 12:3:3:2		
16 + 24→25	CH_2Cl_2/4 Å (10)	(16) (980, 1.2) (24) (1200, 2.0) AgOTf (770, 3.0) Collidine (0.27 cm^3, 2.0)	0.25	−25	75	T–E	15	98.1 (C-1), 100.6, 100.7 (C-1′, C-1″)
25→4	(a) MeOH	(25) (350, 0.25) NaOMe (0.1 mol dm^{-1})						
	(b) HOAc (80%)	H_2/Pd	16		76	E–A–M–H, 12:3:3:2	54	

[a] For coding see figures. [b] Molecular sieves, 4 Å. [c] Used directly in subsequent step. [d] Solvents for chromatography separations; T = toluene, E = ethyl acetate, A = acetic acid, M = methanol, H = water, P = light petroleum (boiling range 60–71 °C). [e] *p*-Toluenesulphonic acid.

O-Deacetylation of **15** in methanolic sodium methoxide yielded **16** (87%). This disaccharide was condensed with **6**, to give **17** (36%) which was protected as described for **12** to yield **2** in 95% yield. For the synthesis of β-D-Glc*p*-(1→2)-β-D-Glc*p*-(1→3)-α-D-Glc*p*-OMe (**4**), disaccharide derivative **16** was condensed with tetra-*O*-benzoyl-α-D-glucopyranosyl bromide (**24**) to yield **25** (75%) which was deprotected as described above to yield **4** in 76% yield.

Preparation of β-D-Glc*p*-(1→2)-α-D-Glc*p*-(1→3)-α-D-Glc*p*-OMe (**3**) also employed the second route in which 2-*O*-trichloroacetyl-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl chloride (**18**) was condensed with **11** to yield **19** in 38% yield. Compound **19** was selectively *O*-detrichloroacetylated using ammonia in diethyl ether to yield a disaccharide which, however, could not be glycosylated with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**22**). A modified approach was therefore chosen. *O*-Deacetylation of **19** with methanolic sodium methoxide yielded **20** which was treated with benzaldehyde and zinc chloride to give the benzylidene acetal **21** (60%). This disaccharide was selectively glycosylated with **22** at O-2′, to yield **23** which was deprotected as described above to give **3** which was *ca.* 85% pure as determined by NMR spectroscopy. The major impurity was a



trisaccharide with the α-D-glucopyranosyl group linked to O-3′. The reaction mixture containing trisaccharides **23** or **3** resisted purification, *i.e.* both as protected and deprotected, and the mixture was therefore used for the NMR studies.

Conformational Calculations.—The angles φ and ψ for the minimum energy conformations of trisaccharides **1**–**4**, and inter-residue distances <3 Å, obtained by GESA calculations are given in Table 1 and the molecules are depicted in stereo in Fig. 1. Only one low energy conformation was found for each trisaccharide. For trisaccharides **1**–**3** the hydroxymethyl groups were kept in the *gauche–trans* conformation as these groups were devoid of inter-residue atomic interactions, but in **4** the hydroxymethyl group in the terminal non-reducing sugar was allowed to rotate during energy minimisations due to its proximity to the methyl glucoside residue (see below). All comparisons of torsion angles and atomic distances are made relative to the constituent disaccharide.

An estimation of the rotational freedom around the glycosidic bonds in the trisaccharides was obtained by examination of the φ/ψ-map upon rotation around one of the glycosidic bonds. The calculations were performed using the HSEA programme and energy maps were obtained for all glycosidic bonds.

For **1** the φ/ψ-angles of the minimum energy conformation were within 5°, and the inter-residue distances within 0.1 Å, of those of the constituent disaccharides. The energy maps for **1** (Fig. 2) show that the 1,3-glycosidic linkage exhibits a somewhat restricted rotational freedom.

The minimum energy conformation of **2** showed notable deviations for both φ and ψ in the α-1,2-linkage but not for the β-1,3-linkage. The distances most altered were those between 1″-H and various other atoms, including atoms in the methyl glucoside residue, and between 5″-H and 2′-H. The energy maps for **2** show some restriction of rotational freedom for the β-1,3-linkage relative to the respective disaccharide elements (Fig. 2).

The minimum energy conformation of **3** had φ/ψ-angles that were within 7° of those of the constituent disaccharides. The major differences for inter-residue distances were found for O-5″ which is closer to 1′-H and further from 2′-H than in the corresponding disaccharide. The energy maps for **3** show that the β-1,2-linkage exhibits restricted rotation around the glycosidic bond whereas the α-1,3-linkage is virtually unaffected.

Trisaccharide **4** has, in the minimum energy conformation, notably different values for the φ/ψ-angles in the β-1,2-linkage, and the terminal glucosyl group is brought closer to the methyl glucoside residue. The most pronounced changes are found for the hydroxymethyl group of the non-reducing sugar which is close to 2-H and 4-H in the methyl glucoside residue. Starting with ω″ at 60° (*gauche–trans*) for the hydroxymethyl group in the non-reducing terminal glucosyl group, a change from 60 to 70° is observed, with short distances (Fig. 1, data not included in Table 1) between the following atom pairs: O-6″ and 4-H; 6″-H_R and 2-H; 6″-H_R and 4-H (2.56 Å, 2.40 Å and 2.56 Å, respectively). When ω″ was set at −60 °C (*gauche–gauche*) it changed to −61° on minimisation, with interactions between the following atom pairs: O-6″ and 2-H; O-6″ and 4-H; 6″-H_S and 4-H (2.59 Å, 2.73 Å and 2.43 Å, respectively). The energy maps for **4** show that the rotational freedom of the β-1,2-glycosidic linkage is restricted, similar to that of the equivalent linkage in **3**. The β-1,3-glycosidic linkage has its rotational freedom severely restricted.

NOE Measurements.—To check the result of the conformational calculations NOE measurements were performed. These

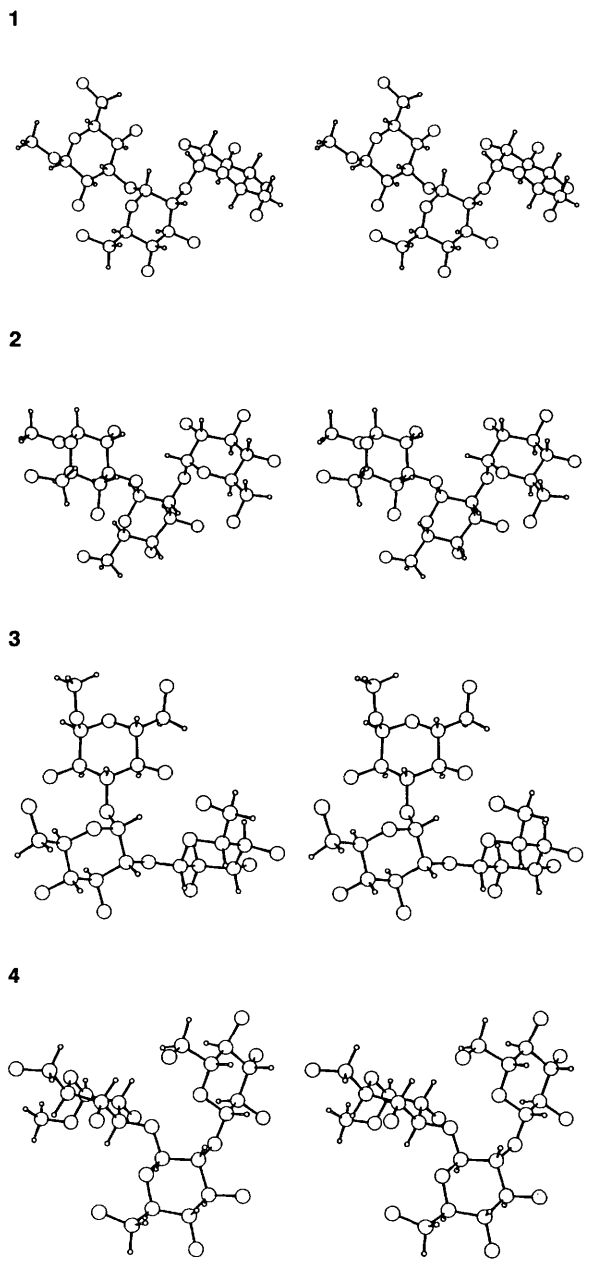


Fig. 1 Stereoplots of the minimum energy conformations of trisaccharides **1–4**

were done either as NOE difference experiments or, when overlap of signals occurred, as NOESY experiments and the results are given in Table 3.

On irradiation of 1″-H or 1′-H in **1** interglycosidic NOE to the proton on the linkage carbon and intra-residue NOE to 2″-H and 2′-H, respectively, were observed. Furthermore, NOE between 1″-H and 1′-H was observed. These NOEs are in agreement with the calculated conformation for **1**. In the NOESY spectrum no NOE crosspeak was observed between 5″-H and 2′-H.

From the NOE difference spectra of **2** the anticipated interglycosidic NOEs were identified, as well as intra-residue NOEs to 2″-H and 2′-H (α-configuration) and to 3′-H and 5′-H (β-configuration). No NOE was observed, however, between 1″-H and 1′-H, or between 1″-H and 2-H, which have calculated short distances of 2.99 Å and 2.61 Å, respectively. Conformations which have larger, *i.e.* less negative, values of φ and ψ for the α-1,2-glycosidic linkage are also in the low energy region (Fig. 2). These have increased distances between the above mentioned inter-residue protons and 1″-H and O-1′, indicating the possibility of a larger population of such conformers. These results thus indicate that for the 1,2-glycosidic linkage the conformation is closer to that of the constituent disaccharide than that calculated for the trisaccharide. In the NOESY spectrum no NOE crosspeak was observed between 5″-H and 2′-H. All interglycosidic NOEs for **3** were observed, thereby supporting the calculated conformation, which is close to that of the constituent disaccharides. Intra-residue NOEs to 3″-H and 5″-H for the sugar residue with β-configuration and 2′-H and 2-H for the sugar residues with α-configuration were also observed. In addition to the expected intra-residue NOEs, the inter-residue NOEs between neighbouring residues found for **4** (Fig. 3) were those corresponding to calculated short distances. However, no crosspeaks in the NOESY spectrum corresponding to the calculated long-range interaction between the hydroxymethyl group in the terminal non-reducing end group and protons in the methyl glucoside residue were observed. Thus the predicted contact is not supported by the NOESY experiment.

^{1}H *NMR Glycosylations Shifts*.—The ^{1}H NMR chemical shift data for compounds **1–4** are given in Table 4. The calculated chemical shifts (calc.) are obtained by addition of the glycosylation shifts of the appropriate disaccharides[24] to the chemical shifts of the monomers. The deviations from additivity of glycosylation shifts (obs. − calc.) are obtained by substraction of the calculated data from the experimental data. As a guidance to the interpretation of Table 4, the following should be observed. A small value for (obs. − calc.) indicates that no changes in the interactions have taken place, or that changes in the glycosylation shifts cancel. A larger positive or negative value of (obs. − calc.) indicates that new interactions, stronger or weaker, respectively, are present in the trisaccharide, relative to those for the respective disaccharide, causing changes in the glycosylation shifts. These extra glycosylation shifts, (obs. − calc.), are mostly small and only a few differ by ≥0.05 ppm. The signals from 5″-H in the glycosyl group are shifted an additional 0.04–0.10 ppm and the largest shift, 0.10 ppm, is observed for **4**. This may be a result of the indicated hydroxymethyl group rotation. In some cases atomic distances are changed but no significant shifts are observed. This may derive from a variety of sources such as angular dependence, cancellation due to both positive and negative effects *etc.*

For **2** a large extra downfield shift of 0.16 ppm is observed for the signal from 1″-H which correlates with the decrease in distance to O-1′. NOE data, presented above, indicated that the conformation at the α-1,2-linkage was closer to that of the disaccharide, than that calculated for the trisaccharide. It is still possible, however, that there is an increased proximity between 1″-H and O-1′ thereby explaining the downfield shift.

Extra shifts for **4** are observed for signals from 1′-H, 2-H and 3-H. The signals from 1′-H and 3-H at the β-1,3-glycosidic linkage are both shifted upfield by −0.05 ppm. This may be a result of the drastically restricted rotation around the β-1,3-linkage in the trisaccharide as indicated in Fig. 2. An attempt to calculate hydroxymethyl rotamer distributions, especially that of the terminal sugar, from $J_{5,6}$ values, was hampered by the fact that an almost complete overlap between the signals from that hydroxymethyl group and that of the middle residue was present. Extraction of approximate values indicated that J values, and therefore populations, were roughly equal to those in methyl β-D-glucopyranoside. Neither were any significant glycosylation shifts for the 6″-protons, which could be attributed to the calculated interaction with the methyl glucoside residue observed.

^{13}C *NMR Glycosylation Shifts*.—The calculated chemical shift

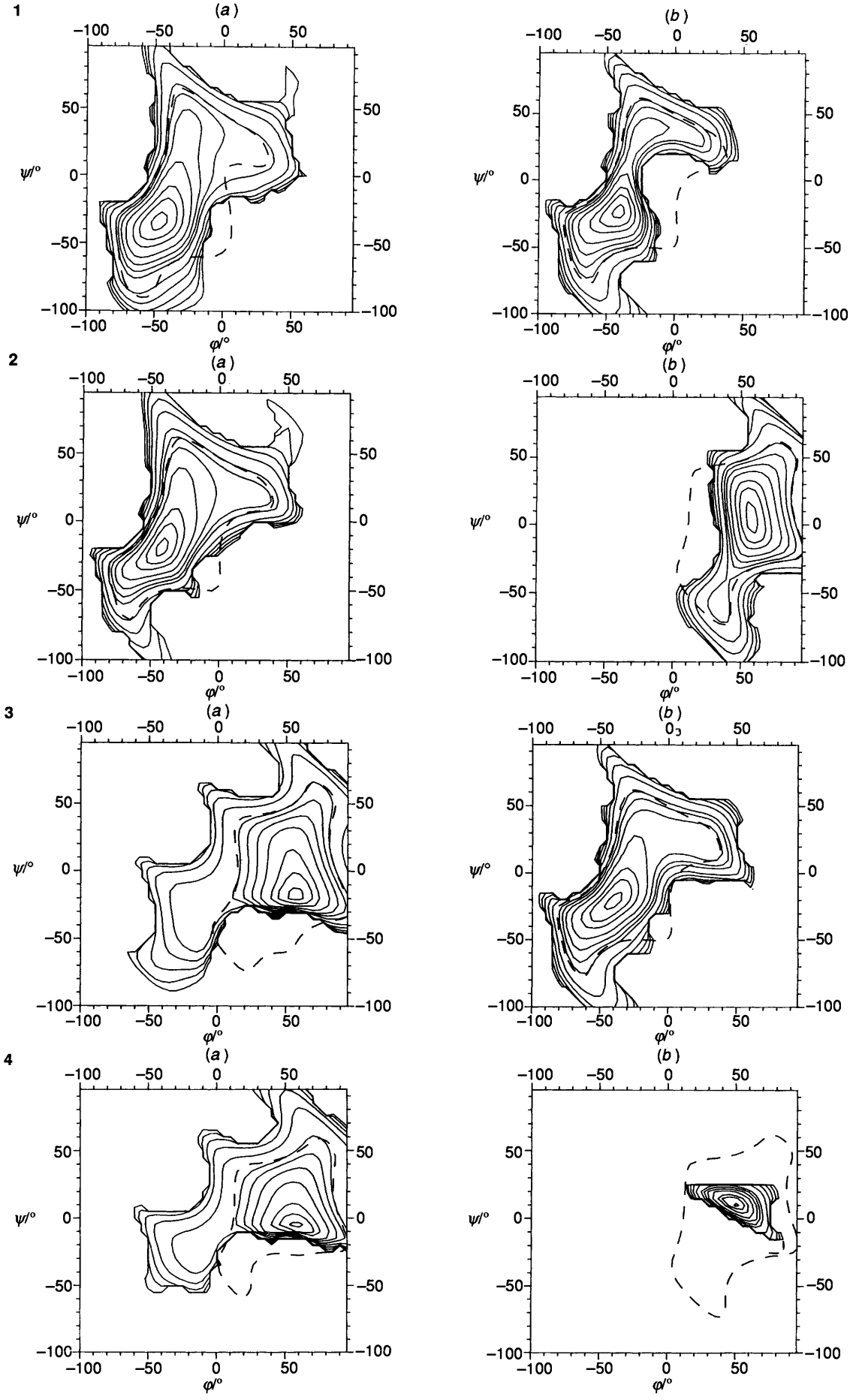


Fig. 2 Conformational energy plots for trisaccharides **1**–**4**. Energy map obtained upon rotation of the φ_1/ψ_1-linkage is shown in (*a*) and of the φ_2/ψ_2-linkage in (*b*). Isocontour levels are indicated at 0.4, 2.1, 4.2, 8.4, 12.6, 20.9, 33.5, 46.0, 58.6, 71.1 and 83.7 kJ above the minimum energy conformation. Dashed lines indicate the 20.9 kJ energy level for the corresponding disaccharide.

Table 3 NOEs observed from NOE difference spectra of trisaccharides **1**–**4**. The calculated short distances are given in brackets (in Å). Signal intensities (in %) are given in parentheses and are relative to the signal of the irradiated proton.

Compound	Atom irr.	NOE observed					
α-D-Glc*p*-(1→2)-α-D-Glc*p*-(1→3)-α-D-Glc*p*-OMe **1**	1″-H	1′-H	2′-H	2″-H			
	5.16 (−100%)	5.51 (9%) [2.24]	3.69 (8%) [2.62]	3.60 (16%) [2.38]			
	1′-H	1″-H	3-H	2′-H			
	5.51 (−100%)	5.16 (10%) [2.24]	3.83 (14%) [2.42]	3.69 (10%) [2.38]			
α-D-Glc*p*-(1→2)-β-D-Glc*p*-(1→3)-α-D-Glc*p*-OMe **2**	1″-H	2′-H	1′-H	2″-H			
	5.42 (−100%)	3.50 (12%) [2.92]	4.83 (0%) [2.99]	3.60 (15%) [2.38]			
	1′-H/1-H	1″-H	3-H	OCH_3	2-H	3′-H	5′-H/2′-H
	4.83 (−200%)	5.42 (0%) [2.99]	3.86 (17%) [2.43]	3.44 (10%) [2.34–3.51]	3.77 (14%) [2.48]	3.61 (10%) [2.51]	3.49/3.50 (12%) [2.33/2.38]
β-D-Glc*p*-(1→2)-α-D-Glc*p*-(1→3)-α-D-Glc*p*-OMe **3**	1″-H	2′-H	3″-H/5″-H				
	4.66 (−100%)	3.71 (18%) [2.28]	3.48/3.52 (16%) [2.51/2.33]				
	1′-H	3-H	2′-H				
	5.54 (−100%)	3.85 (13%) [2.44]	3.71 (11%) [2.38]				
β-D-Glc*p*-(1→2)-β-D-Glc*p*-(1→3)-α-D-Glc*p*-OMe **4**	1″-H	2′-H	3″-H/5″-H				
	4.78 (−100%)	3.63 (18%) [2.44]	3.54/3.51 (18%) [2.51/2.33]				
	1′-H	3-H	3′-H	5′-H			
	4.74 (−100%)	3.82 (20%) [2.39]	3.74 (8%) [2.51]	3.51 (~5%) [2.33]			

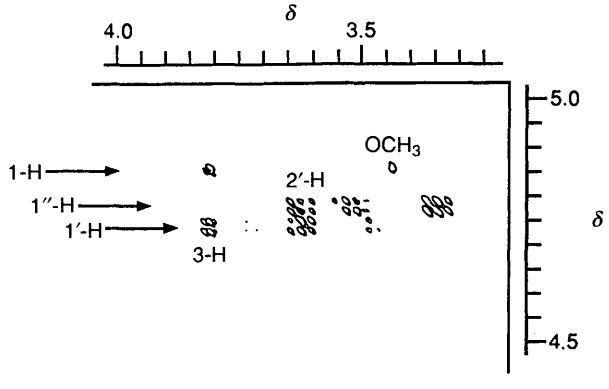


Fig. 3 Part of the NOESY spectrum of trisaccharide **4** showing the cross peaks for the anomeric protons. The inter-residue contacts are labelled.

data obtained as described for ^{1}H NMR spectroscopy, are given in Table 5.

In the 3,4-branched trisaccharides previously studied [2,3] extra glycosylation shifts of up to 5 ppm compared to the corresponding disaccharides were observed. In this study extra shifts are not larger than 1.7 ppm and the significant differences, ≥0.5 ppm, found in trisaccharides **1**, **2** and **4**, are for signals from linkage carbons. For **1** significant differences are only observed for signals from C-2′ and C-3 and almost all shifts are upfield. The largest shift for signals from **2** is found for the C-1′ signal which is shifted −0.64 ppm. Trisaccharide **3** had a conformation which was calculated to be near to that of the constituent disaccharides and no glycosylation shift larger than 0.41 ppm was observed. Trisaccharide **4** had notable changes in its conformation, and also had much restricted rotation, especially around the β-1,3-linkage. This resulted in only small changes for ^{1}H NMR data but for ^{13}C NMR signals larger changes are observed. Thus, signals from C-1″, C-2′ and C-3 are shifted by 0.84, 0.88 and 1.67 ppm, respectively. These changes are downfield, in contrast to almost all those described above, which are upfield.

Temperature Dependence of ^{13}C NMR Chemical Shifts.— Values for chemical shift differences (in ppm) obtained from ^{13}C NMR spectra recorded at 30 and 70 °C are shown in Table 6. Most signals are shifted downfield relative to the internal standard, dioxane, to which a constant chemical shift is assigned.

Shifts between −0.16 and 0.91 ppm of signals for carbons involved in linkages are observed. For other signals shifts vary between 0 and 0.39 ppm. The carbons involved in α-linkages generally give signals with the largest shifts. For α-linkages, signals from non-anomeric carbons also tend to have larger shifts.

Conclusions

In this study it has been shown that when 1,2-vicinal disubstitution occurs and the conformational changes are small, the variations in chemical shifts are also small. This implies that,

Table 4 ^{1}H NMR data of the trisaccharides **1**–**4** obtained at 70 °C. Chemical shifts are given relative to internal TSP (δ_H 0.00)

Compound		1″-H	2″-H	3″-H	4″-H	5″-H	6″-Ha	6″-Hb	1′-H	2′-H	3′-H	4′-H	5′-H	6′-Ha	6′-Hb
α-D-Glc*p*-(1→2)-α-D-Glc*p*-(1→3)-α-D-Glc*p*-OMe **1**	(obs.)	5.16	3.60	3.79	3.46	3.97	3.77[b]	3.85[b]	5.51	3.69	3.86	3.50	4.05	3.77[b]	3.85[b]
	(calc.)[a]	5.07	3.56	3.78	3.45	3.92	3.77	3.85	5.52	3.68	3.86	3.50	3.99	3.79	3.88
	(obs. − calc.)[a]	0.09	0.04	0.01	0.01	0.05	0.00	0.00	−0.01	0.01	0.00	0.00	0.06	−0.02	−0.03
α-D-Glc*p*-(1→2)-β-D-Glc*p*-(1→3)-α-D-Glc*p*-OMe **2**	(obs.)	5.42	3.60	3.75	3.47	4.04	3.78	3.84	4.83	3.50	3.61	3.45	3.49	3.73	3.93
	(calc.)[a]	5.28	3.54	3.75	3.44	4.00	3.77	3.83	4.82	3.49	3.64	3.45	3.49	3.74	3.93
	(obs. − calc.)[a]	0.16	0.04	0.00	0.03	0.04	0.01	0.01	0.01	0.01	−0.03	0.00	0.00	−0.01	0.00
β-D-Glc*p*-(1→2)-α-D-Glc*p*-(1→3)-α-D-Glc*p*-OMe **3**	(obs.)	4.66	3.37	3.52	3.43	3.48	3.75	3.91	5.54	3.71	3.91	3.50	3.98	3.76	3.86
	(calc.)[a]	4.62	3.37	3.52	3.43	3.43	3.76	3.91	5.50	3.69	3.92	3.48	3.98	3.79	3.88
	(obs. − calc.)[a]	0.04	0.00	0.00	0.00	0.05	−0.01	0.00	0.04	0.02	−0.01	0.02	0.00	−0.03	−0.02
β-D-Glc*p*-(1→2)-β-D-Glc*p*-(1→3)-α-D-Glc*p*-OMe **4**	(obs.)	4.78	3.35	3.54	3.40	3.51	3.73	3.91[d]	4.74	3.63	3.74	3.47	3.51[b]	3.73	3.93[d]
	(calc.)[a]	4.76	3.34	3.52	3.43	3.41	3.74	3.92	4.79	3.65	3.74	3.46	3.48	3.75	3.91
	(obs. − calc.)[a]	0.02	0.01	0.02	−0.03	0.10	−0.01	−0.01	−0.05	−0.02	0.00	0.01	0.03	−0.02	0.02

Compound		1-H	2-H	3-H	4-H	5-H	6-Ha	6-Hb	OMe
α-D-Glc*p*(1→2)-α-D-Glc*p*-(1→3)-α-D-Glc*p*-OMe **1**	(obs.)	4.82	3.66	3.83	3.65[b]	3.65[b]	3.77[b]	3.85[b]	3.44
	(calc.)[a]	4.82	3.66	3.82	3.66	3.66	3.76	3.85	3.44
	(obs. − calc.)[a]	0.00	0.00	0.01	−0.01	−0.01	0.01	0.00	0.00
α-D-Glc*p*-(1→2)-β-D-Glc*p*-(1→3)-α-D-Glc*p*-OMe **2**	(obs.)	4.82	3.77	3.86	3.51	3.67	3.77	3.87	3.44
	(calc.)[a]	4.83	3.75	3.87	3.52	3.67	3.77	3.88	3.44
	(obs. − calc.)[a]	−0.01	0.02	−0.01	−0.01	0.00	0.00	−0.01	0.00
β-D-Glc*p*-(1→2)-α-D-Glc*p*-(1→3)-α-D-Glc*p*-OMe **3**	(obs.)	4.82	3.69	3.85[c]	3.69[c]	3.66	3.76	3.86	3.44
	(calc.)[a]	4.82	3.66	3.82	3.66	3.66	3.76	3.85	3.44
	(obs. − calc.)[a]	0.00	0.03	0.03	0.03	0.00	0.00	0.01	0.00
β-D-Glc*p*-(1→2)-β-D-Glc*p*-(1→3)-α-D-Glc*p*-OMe **4**	(obs.)	4.86[e]	3.81	3.82	3.53	3.67	3.77	3.87	3.44
	(calc.)[a]	4.83	3.75	3.87	3.52	3.67	3.77	3.88	3.44
	(obs. − calc.)[a]	0.03	0.06	−0.05	0.01	0.00	0.00	0.00	0.00

[a] The method of calculating chemical shifts is described in Results and Discussion. [b] Approximate values. [c] Assignments from CH-correlation spectrum. [d] Tentative assignments. [e] The signal is distorted due to virtual coupling.

Table 5 ^{13}C NMR data of the trisaccharides **1**–**4** obtained at 70 °C. Chemical shifts are given relative to internal dioxane (δ_C 67.40).

Compound		C-1″	C-2″	C-3″	C-4″	C-5″	C-6″	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′
α-D-Glc*p*-(1→2)-α-D-Glc*p*-(1→3)-α-D-Glc*p*-OMe **1**	(obs)	97.18	72.22	73.81	70.44	72.74	61.45	97.25	76.78	72.18	70.53	72.40	61.45
	(calc.)[a]	97.57	72.26	73.76	70.54	72.85	61.51	97.19	77.29	72.28	70.54	72.67	61.44
	(obs. − calc.)[a]	−0.39	−0.04	0.05	−0.10	−0.11	−0.06	0.06	−0.51	−0.10	−0.01	−0.27	0.01
α-D-Glc*p*-(1→2)-β-D-Glc*p*-(1→3)-α-D-Glc*p*-OMe **2**	(obs.)	99.00	72.37	73.81	70.39[b]	72.73	61.41	103.54	79.36	75.62	70.73[b]	76.63	61.63[b]
	(calc.)[a]	98.64	72.32	73.77	70.50	72.62	61.46	104.18	79.10	75.23	70.75	76.68	61.66
	(obs. − calc.)[a]	0.36	0.05	0.04	−0.11	0.11	−0.05	−0.64	0.26	0.39	−0.02	−0.05	−0.03
β-D-Glc*p*-(1→2)-α-D-Glc*p*-(1→3)-α-D-Glc*p*-OMe **3**	(obs.)	104.84	74.37	76.52	70.35	76.90	61.60[b]	99.16	81.70	72.71	70.45	72.54	61.47[b]
	(calc.)[a]	104.59	74.25	76.65	70.57	76.76	61.83	99.57	81.53	72.81	70.45	72.52	61.42
	(obs. − calc.)[a]	0.25	0.12	−0.13	−0.22	0.14	−0.23	−0.41	0.17	−0.10	0.00	0.02	0.05
β-D-Glc*p*-(1→2)-β-D-Glc*p*-(1→3)-α-D-Glc*p*-OMe **4**	(obs.)	104.22	74.79	76.40	70.50	77.28[b]	61.79[b]	102.66	82.59	76.60	70.33	76.70[b]	61.54[b]
	(calc.)[a]	103.38	74.58	76.63	70.54	76.93	61.74	102.27	81.71	76.61	70.43	76.74	61.56
	(obs. − calc.)[a]	0.84	0.21	−0.23	−0.04	0.35	0.05	0.39	0.88	−0.01	−0.10	−0.04	−0.02

Compound		C-1	C-2	C-3	C-4	C-5	C-6	OMe
α-D-Glc*p*-(1→2)-α-D-Glc*p*-(1→3)-α-D-Glc*p*-OMe **1**	(obs.)	100.32	70.76	80.94	70.76[b]	72.22[b]	61.45	55.92
	(calc.)[a]	100.30	70.83	81.51	70.83	72.29	61.55	55.92
	(obs. − calc.)[a]	0.02	−0.07	−0.57	−0.07	−0.07	−0.10	0.00
α-D-Glc*p*-(1→2)-β-D-Glc*p*-(1→3)-α-D-Glc*p*-OMe **2**	(obs.)	100.05	71.52	83.40	68.98	72.37	61.69[b]	55.90
	(calc.)[a]	100.04	71.61	83.74	69.13	72.35	61.71	55.92
	(obs. − calc.)[a]	0.01	−0.09	−0.34	−0.15	0.02	−0.02	−0.02
β-D-Glc*p*-(1→2)-α-D-Glc*p*-(1→3)-α-D-Glc*p*-OMe **3**	(obs.)	100.25	70.71[b]	81.50	70.68[b]	72.16	61.47[b]	55.90
	(calc.)[a]	100.30	70.83	81.51	70.83	72.29	61.55	55.92
	(obs. − calc.)[a]	−0.05	−0.12	−0.01	−0.15	−0.13	−0.08	−0.02
β-D-Glc*p*-(1→2)-β-D-Glc*p*-(1→3)-α-D-Clc*p*-OMe **4**	(obs.)	99.63	71.23	85.41	69.07	72.43	61.57[b]	55.84
	(calc.)[a]	100.04	71.61	83.74	69.13	72.35	61.71	55.92
	(obs. − calc.)[a]	−0.41	−0.38	1.67	−0.06	0.08	−0.14	−0.08

[a] The method of calculating chemical shifts is described in Results and Discussion. [b] Tentative assignments.

Table 6 ^{13}C NMR chemical shift differences (ppm) on variation of temperature for compounds **1–4**[a]

Compound	Carbon atom																		
	C-1″	C-2″	C-3″	C-4″	C-5″	C-6″	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′	C-1	C-2	C-3	C-4	C-5	C-6	OMe
α-D-Glc*p*-(1→2)-α-D-Glc*p*-(1→3)-α-D-Glc*p*-OMe **1**	0.38	0.12	0.16	0.30	0.14	0.28[b]	0.07	0.59	0.17	0.39	0.16	0.36[b]	0.01	0.19	0.42	0.08	0.12	0.28[b]	0.06
α-D-Glc*p*-(1→2)-β-D-Glc*p*-(1→3)-α-D-Glc*p*-OMe **2**	0.17	0.16[b]	0.11	0.34	0.16	0.35	−0.14	0.52	0.19	0.16	0.04	0.23	0.07	0.00	0.37	0.22	0.05[b]	0.14	0.08
β-D-Glc*p*-(1→2)-α-D-Glc*p*-(1→3)-α-D-Glc*p*-OMe **3**	−0.10	0.15	0.18	0.23	0.12	0.23[b]	0.22	−0.11	0.20	0.33	0.22	0.24[b]	0.10	0.18[c]	0.91	0.01[c]	0.11	0.27[b]	0.07
β-D-Glc*p*-(1→2)-β-D-Glc*p*-(1→3)-α-D-Glc*p*-OMe **4**	0.03	0.12	0.19	0.13	0.05	0.18	−0.21	0.17	0.09	0.18	0.06	0.24[b]	0.14	0.01	−0.16	0.16	0.08	0.17[b]	0.06

[a] $\Delta\delta = \delta$ (70 °C) − δ (30 °C). Dioxane was taken as δ 67.40 for all temperatures. [b,c] These values could be interchanged.

to a first approximation, NMR spectra of trisaccharides with vicinal disubstitution with small interactions can be calculated as the sum of glycosylation shifts from their constituent disaccharides. However, other substitution patterns of sugar1-(1→2)-sugar2-(1→X)-sugar3 have been shown to give larger deviations[26] and the reason for this is present under investigation.

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