Synthesis, N.m.r., and Conformational Studies of some 3,4-Di-*O*-glycopyranosyl- substituted Methyl α-D-Galactopyranosides

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Methyl α-D-galactopyranosides substituted in the 3- and 4-positions with one L-fucopyranosyl and one D-glucopyranosyl group have been synthesized, with all anomeric combinations. The trisaccharides were used for ^1H and ^{13}C n.m.r. studies. All ^1H and ^{13}C n.m.r. resonances were assigned and comparison was made between the observed glycosylation shifts of the trisaccharides and those calculated by using the glycosylation shifts for each disaccharide element. Large deviations, in most cases upfield shifts, were found in the ^{13}C n.m.r. spectra for signals from some of the linkage carbons. Conformational analysis has been performed using the HSEA and GESA approaches. This analysis indicated restricted rotational freedom around the glycosidic bonds of the trisaccharides relative to those of the disaccharides. A number of proton-oxygen and proton-proton interactions in the trisaccharides was indicated which could be correlated to downfield and upfield shifts, respectively, of the proton signals.

Recently a number of '3,4-branched' trisaccharides have been synthesized[1] and used for n.m.r. and conformational studies.[2] They were designed to mimic the branch points in oligo- and poly-saccharides. Methyl α-D-galactopyranoside was substituted with all anomeric combinations of two L-fucosyl or two D-glucosyl groups. We now report similar studies on '3,4-branched' trisaccharides consisting of methyl α-D-galactopyranoside substituted with one L-fucosyl and one D-glucosyl group in all combinations. This is part of a continuous project aimed at a better understanding of the origin of the glycosylation shifts for different stereochemistries around the glycosidic bonds[2–6] and the creation of a database for a computer assigned structural analysis of oligo- and polysaccharides.[7,8]

Experimental

General Methods.—The coupling reactions in the synthesis of di- and tri-saccharides were not optimized. Data on coupling reactions and deprotection procedures, yields, physical constants, and selected n.m.r. chemical shifts are given in Table 1. Concentrations were performed under reduced pressure at bath temperatures <40 °C. Optical rotations were recorded using a Perkin-Elmer 241 polarimeter. ^1H N.m.r. spectra (400 MHz) and ^{13}C n.m.r. spectra (25 and 100 MHz) were recorded for 0.04M and 0.2M deuterium oxide solutions, respectively, at 30 °C (^{13}C) and 70 °C (^1H and ^{13}C) or for $CDCl_3$ solutions, with JEOL FX 100 or GX 400 spectrometers. Chemical shifts are given in p.p.m. with sodium [2,2,3,3-$^2H_4$]-3-(trimethylsilyl)-propanoate (TSP, δ_H 0.00), dioxane (δ_C 67.40), and tetramethylsilane (TMS, δ_H, δ_C 0.00) as internal references. For the assignment of signals, techniques described earlier were used.[2] Column chromatography was performed on silica gel (Matre Silica Si 60 Å, 35—70 μ, Amicon).

The purity of intermediates was first analysed by t.l.c. where they showed only one spot, and then by ^{13}C n.m.r. spectroscopy from which the intermediates were estimated to be more than 95% pure. The number and chemical shifts of signals in the ^{13}C n.m.r. spectrum were consistent with the postulated structures and the chemical shifts of the C-1 signals showed the anomeric configurations.

In the ^1H n.m.r. spectra of the deprotected oligosaccharides, signal integrals from contaminating components were less than 5% of those of the anomeric proton signals. The position of substitution of the glycosyl groups was determined in most cases by the synthetic route. When diols were monoglycosylated the determination of an n.O.e. between the anomeric proton and the proton on the linkage carbon, or a methylation analysis, established the linkage position. The number and chemical shifts of signals in the ^1H and ^{13}C n.m.r. spectra were in agreement with the postulated structures. Anomeric configurations were deduced from the coupling patterns [$^3J_{H,H}$, $^1J_{C,H}$] of signals from anomeric protons and carbons.

Conformational Analysis.—The GESA program[9] was used to estimate minimum energy conformations for all of the di- and tri-saccharides. Different starting torsion angles (φ, ψ, ω) were used to calculate the global energy minimum and the hydroxymethyl groups were allowed to rotate. Energy maps, showing the rotational freedom around the glycosidic bonds, were obtained using the HSEA program.[10,11] During these energy calculations, the φ,ψ-values of one of the glycosidic bonds were varied whereas the other glycosidic bond was kept at the φ,ψ-values of the minimum energy conformation of the trisaccharide which was obtained from the GESA calculations. The same procedure was then repeated for the other glycosidic bond. 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, for which X could be either 3 or 4. The bond angle τ, defined by C(1′)–O(X)–C(X), was set as 117°. Co-ordinate sets of the sugars were the same as those used earlier.[2]

Glycosylation Methods.—Method A. Methyl trifluoromethanesulphonate (5 equiv.) was added to a stirred solution at 0 °C of thioglycoside (1.3 equiv.) and suitably protected methyl galactoside (1.0 equiv.) in diethyl ether containing ground molecular sieves (4 Å, 1—2 g),[12] and the mixture was then allowed to warm to 20 °C. When the reaction was complete by t.l.c. analysis (2—20 h), triethylamine (10 equiv.) was added and stirring continued for 30 min. The mixture was then diluted with dichloromethane, filtered through a layer of Celite, and washed

Table 1. Data on coupling reactions and deprotection procedures, yields, physical constants, and selected n.m.r. chemical shifts

(a) Compd.	Aglycone precursor (mg)	Glycosyl donor (mg)	Glycosidation method[a] (time h)	T.l.c. R_f (solvent)[b]	Column chromatography solvent	Yield mg (%)	δ_C C-1	C-1′	C-1″
(21)	(17) (400)	(15) (440)	A (4)	0.47 (P–E, 3:1)	T–E, 8:1	280 (42)	98.4	99.1	100.0
(22)	(17) (400)	(16) (500)	B (2)	0.30 (T–E, 8:1)	T–E, 8:1	520 (75)	98.5	98.1	100.2
(23)[c]	(18) (500)	(15) (550)	A (2)	0.30 (T–E, 1:4)	T–E, 1:4	195 (26)[d]	98.5	102.2	100.1
(24)[c]	(18) (500)	(15) (550)	A (2)	0.43 (T–E, 1:4)	T–E, 1:4	145 (19)[d]	98.5	100.4	103.9
(25)	(19) (450)	(11) (350)	A (5)	0.69 (T–E, 4:1)	T–E, 8:1	630 (96)	98.4	93.7	95.6
(26)	(19) (380)	(12b) (200)	A (16)	0.56 (T–E, 4:1)	T–E, 6:1	290 (59)	98.9	94.4	99.9
(27)	(20) (400)	(11) (300)	A (2)	0.62 (T–E, 4:1)	T–E, 6:1	550 (96)	98.5	102.5	95.5
(28)	(20) (510)	(12a) (400)	A (22)	0.59 (I–E, 4:1)	I–E, 8:1	490 (65)	99.0	102.9	100.9

(b) Compd.	Protected precursor (mg; deprotection method)[e]	Yield mg (%)	Optical rotation $[\alpha]_{578}^{22}$ (c in H_2O)	δ_{1-H} ($J_{1,2}$/Hz) 1-H	1′-H	1″-H	δ_C C-1	C-1′	C-1″
(1)	(21) (250; C)	78 (80)	55 (1.3)	4.88 (3.8)	5.26 (3.2)	5.03 (3.5)	100.4	101.0	100.8
(2)	(22) (500; D)	131 (70)	−2 (1.1)	4.86 (3.9)	5.23 (3.1)	4.68 (7.8)	100.4	101.1	103.4
(3)	(23) (240; C)	90 (99)	188 (1.1)	4.94 (2.7)	4.47 (7.7)	5.03 (3.6)	100.2	103.2	101.2
(4)	(24) (200; C)	70 (92)	111 (0.9)	4.90 (3.7)	4.52 (7.6)	4.77 (7.9)	100.2	101.4	103.7
(5)	(25) (600; C)	180 (77)	102 (1.1)	4.91 (3.9)	5.14 (3.8)	5.36 (4.1)	100.4	96.9	100.2
(6)	(26) (245; D)	93 (86)	181 (1.0)	4.91 (3.9)	5.29 (4.0)	4.45 (7.6)	100.3	96.0	103.7
(7)	(27) (530; D)	150 (75)	7 (0.9)	4.89 (3.8)	4.66 (7.8)	5.44 (4.0)	100.2	104.9	100.2
(8)	(28) (430; D)	130 (83)	88 (1.1)	4.89 (3.8)	4.66 (7.7)	4.52 (7.4)	100.3	104.8	104.2

[a] Two coupling methods were used, A and B. See Experimental for details. [b] T.l.c R_f values are given for the solvent system used; T = toluene, E = ethyl acetate, P = light petroleum, I = iso-octane. [c] Compounds (**23**) and (**24**) were isolated from the same reaction mixture after debenzoylation of the glycosidation product. [d] Calculated from amount of aglycone. [e] Two deprotection methods were used, C and D. See Experimental for details.

successively with aqueous 10% H_2SO_4, aqueous $NaHCO_3$, and water. After drying (Na_2SO_4) and evaporation to dryness, the product was purified on a silica gel column using the solvents given in Table 1.

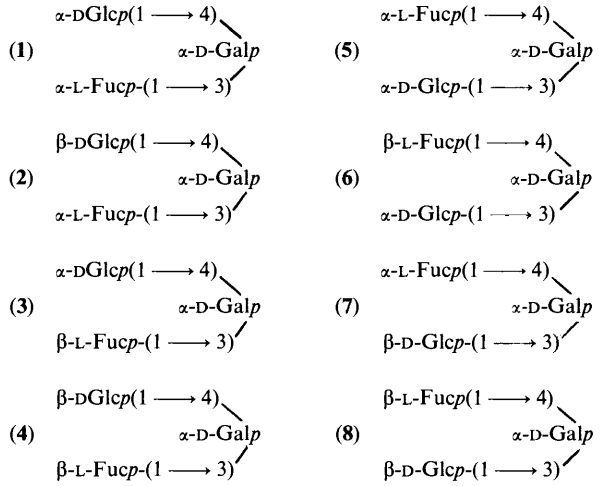
Method B. A solution of silver trifluoromethanesulphonate (2.25 equiv.) and 2,4,6-trimethylpyridine (2.25 equiv.) in dichloromethane was added to a stirred solution of per-*O*-benzoylated glucosyl bromide (1.5 equiv.), suitably protected methyl galactoside (1.0 equiv.), and ground molecular sieves (4 Å, 1 g) in dichloromethane at −20 °C under nitrogen.[13,14] When the reaction was complete (t.l.c.), the mixture was allowed to warm to 20 °C, filtered through a layer of Celite, and washed successively with aqueous 10% $Na_2S_2O_3$, water, 2M H_2SO_4, water, and aq. $NaHCO_3$, dried (Na_2SO_4), and the solvents evaporated. The crude product was purified on a silica gel column using solvents given in Table 1.

Deblocking and Purification Procedures.—Method C. The oligosaccharide derivative (200—600 mg) in aqueous acetic acid (90%, 20—40 ml) was hydrogenolysed at ~350 kPa over Pd/C (10%, 200—400 mg) for 6—18 h. After filtration and evaporation to dryness the product was chromatographed on a column silica gel (10 × 2 cm; ethyl acetate–acetic acid–methanol–water, 12:3:3:2) and then on a column of Bio-Gel P-2 (80 × 2.5 cm) using water as eluant. After freeze-drying, the substance was obtained as an amorphous powder.

Method D. The oligosaccharide derivative was de-*O*-acylated with sodium methoxide in 1:1 dichloromethane–methanol (0.025M; 10—30 ml). After neutralization with acetic acid or Dowex 50 (H^+) the solvent was evaporated and the material hydrogenolysed as described in Method C.

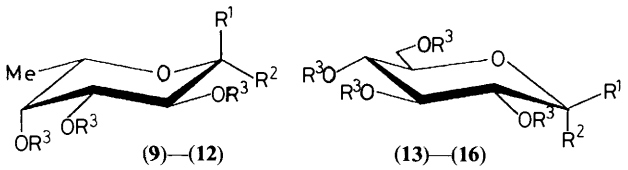
Results and Discussion

Synthesis of Trisaccharides (**1**)—(**8**).—All substances were numbered as shown in Scheme 1.



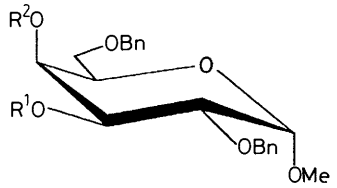
Scheme 1. Compounds (**1**)—(**8**) are the methyl glycosides of the above trisaccharides

The syntheses of trisaccharides (**1**)—(**8**) were accomplished *via* the disaccharides (**17**)—(**20**), the synthesis of which has been described earlier.[1] Data on coupling reactions and deprotection procedures, yields, physical constants, and selected n.m.r. chemical shifts are given in Table 1. Scheme 2 shows the syntheses starting from disaccharides (**17**) and (**18**), *i.e.* the suitably protected methyl 3-*O*-α-L-fucosyl- and 3-*O*-β-L-fucosyl-α-D-galactosides. Starting from (**17**), reaction with the benzylated thioglucuside (**15**) under methyl trifluoromethanesulphonate promotion (method A) gave, in moderate yield, the 4-*O*-α-D-glycosyl trisaccharide (**21**). Deprotection by hydrogenolysis yielded (**1**) for which the ^1H n.m.r. spectrum showed signals for three anomeric protons all with a coupling constant of 3—4 Hz, demonstrating the presence of two α-glycosyl groups in addition to the methyl α-D-galactoside residue.



(9)—(12) (13)—(16)

(9) R^1 = –, R^2 = H, R^3 = Bn
(10a) R^1 = H, R^2 = –, R^3 = Ac
(10b) R^1 = H, R^2 = –, R^3 = Bz
(10c) R^1 = H, R^2 = –, R^3 = H
(11) R^1 = H, R^2 = SEt, R^3 = Bn
(12a) R^1 = H, R^2 = SEt, R^3 = Bz
(12b) R^1 = H, R^2 = SEt, R^3 = Ac
(13) R^1 = H, R^2 = –, R^3 = Bn
(14a) R^1 = –, R^2 = H, R^3 = Bz
(14b) R^1 = –, R^2 = H, R^3 = Bn
(15) R^1 = SEt, R^2 = H, R^3 = Bn
(16) R^1 = H, R^2 = Br, R^3 = Bz



(17) R^1 = (9), R^2 = H
(18) R^1 = (10b), R^2 = H
(19) R^1 = (13), R^2 = H
(20) R^1 = (14a), R^2 = H
(21) R^1 = (9), R^2 = (13)
(22) R^1 = (9), R^2 = (14a)
(23) R^1 = (10c), R^2 = (13)
(24) R^1 = (10c), R^2 = (14b)
(25) R^1 = (13), R^2 = (9)
(26) R^1 = (13), R^2 = (10a)
(27) R^1 = (14a), R^2 = (9)
(28) R^1 = (14a), R^2 = (10b)

Reaction of (17) with perbenzoylated α-D-glucosyl bromide (16) under silver trifluoromethanesulphonate promotion gave the 4-*O*-β-D-glucosyl trisaccharide (22). Deprotection by treatment with sodium methoxide followed by a hydrogenolysis gave the trisaccharide (2), which showed a signal at δ 4.68 ($J_{1,2}$ 7.8 Hz) in its ^{1}H n.m.r. spectrum, confirming the presence of a β-linkage.

Reaction of (18), which contains a 3-*O*-β-L-fucosyl group, with the thioglycoside (15) using glycosylation method A gave an anomeric mixture which after de-*O*-benzoylation could be separated by chromatography on silica gel to give (23) and (24). The identity of these compounds was shown by ^{1}H n.m.r. spectroscopy of the deprotected products (3) and (4). The signals for anomeric protons showed that there was one α- and one β-linked glycosyl group in (3) and two β-linked glycosyl groups in (4) in addition to the methyl α-D-galactoside residue.

Compound (19), with a 3-*O*-α-D-glucosyl group, was condensed with (11) to give the trisaccharide (25) as shown in Scheme 3. After deprotection, the trisaccharide (5) was obtained, the ^{1}H n.m.r. spectrum of which showed only signals from α-linked residues. Reaction of (19) with the peracetylated thioethyl β-L-fucoside (12b) gave the 4-*O*-β-L-fucosyl derivative in higher yields than did the corresponding perbenzoylated derivative.

The 3-*O*-β-D-glucosyl disaccharide (20) was condensed with the glycosyl donor (11) to give the trisaccharide (27) and with (12a) to give the trisaccharide (28) using glycosylation method A. The identity of the deprotected products (7) and (8) was evident from the coupling constants of signals from the anomeric protons in the respective ^{1}H n.m.r. spectra.

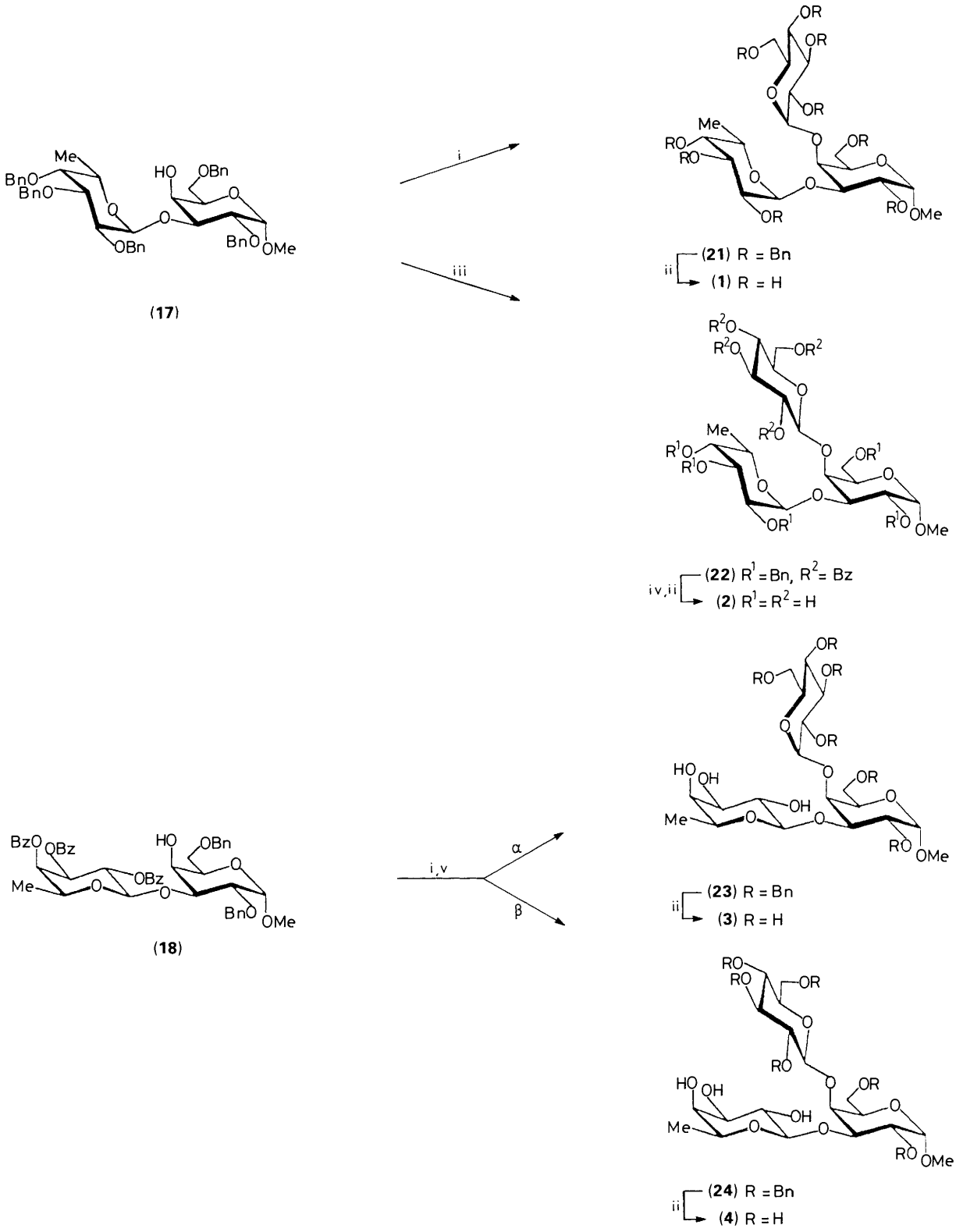
GESA and HSEA Calculations of Trisaccharides (1)—(8).—The φ-, ψ-, and ω-angles in the constituent disaccharides and in compounds (1)—(8), together with short inter-residue atomic distances between the glycosyl groups and the methyl galactoside residue in the minimum energy conformation, are given in Tables 2 and 3. The disaccharides were synthesized[1] and analysed[2] previously. Short inter-residue atomic distances between the two glycosyl groups in the trisaccharides are given in Table 4. To differentiate between the glycosyl groups, atoms in the 3-*O*-glycosyl groups are labelled with a prime and atoms in the 4-*O*-glycosyl groups with a double prime. In addition to the given energy minima, some other energy minima were obtained in the calculations. These were all of higher energy and are not included in the Tables.

An estimate of the rotational freedom of the glycosidic bonds in the trisaccharides was obtained by examination of the energy surface upon rotation around one of the glycosidic bonds. The other bond was kept at the φ,ψ-values of the minimum energy conformation obtained from the GESA calculations. The calculations were performed with the HSEA program and

Table 2. Values for φ, ψ, and ω angles of minimum energy conformations and inter-residue atomic distances <3.5 Å in disaccharides obtained by GESA calculations

Compd.	φ	ψ	ω[a]	ω[a]	1′-H	5′-H	6′-H	O-2′	O-5′
α-L-Fuc*p*-(1 ⟶ 3)α-D-Gal*p*-OMe	52	−3		66	O-2 (3.15)	O-4 (3.22)	4-H (2.72)		3-H (2.68)
					3-H (2.31)	4-H (2.34)			4-H (3.08)
β-L-Fuc*p*-(1 ⟶ 3)α-D-Gal*p*-OMe	−55	−7		66	3-H (2.42)				O-2 (3.14)
					4-H (2.92)				3-H (2.51)
α-L-Fuc*p*-(1 ⟶ 4)α-D-Gal*p*-OMe	43	24		72	O-3 (2.46)	6-H_a (2.26)	6-H (2.58)	2-H (3.20)	4-H (2.60)
					4-H (2.38)	6-H_b (2.47)			
β-L-Fuc*p*-(1 ⟶ 4)α-D-Gal*p*-OMe	−54	−4		65	4-H (2.33)			6-H (3.21)	4-H (2.49)
					6-H_a (2.58)				
					6-H_b (2.84)				
α-D-Glc*p*-(1 ⟶ 3)α-D-Gal*p*-OMe	−50	−34	−60	66	3-H (2.65)	O-2 (2.59)			3-H (2.52)
					4-H(2.34)	3-H (2.94)			
β-D-Glc*p*-(1 ⟶ 3)α-D-Gal*p*-OMe	59	−12	−60	66	O-2 (3.40)				3-H (2.63)
					3-H (2.38)				4-H (2.65)
α-D-Glc*p*-(1 ⟶ 4)α-D-Gal*p*-OMe	−49	−16	−56	65	4-H (2.27)	O-3 (2.40)	O-3 (3.39)	6-H (2.66)	O-3 (3.48)
					6-H_a (2.34)	2-H (3.48)			4-H (2.67)
					6-H_b (2.63)	4-H (3.41)			
β-D-Glc*p*-(1 ⟶ 4)α-D-Gal*p*-OMe[b]	55	5	−54	63	O-3 (2.95)		6-H (3.15)		4-H (2.47)
					4-H (2.38)				6-H_a (2.67)
									6-H_b (2.88)

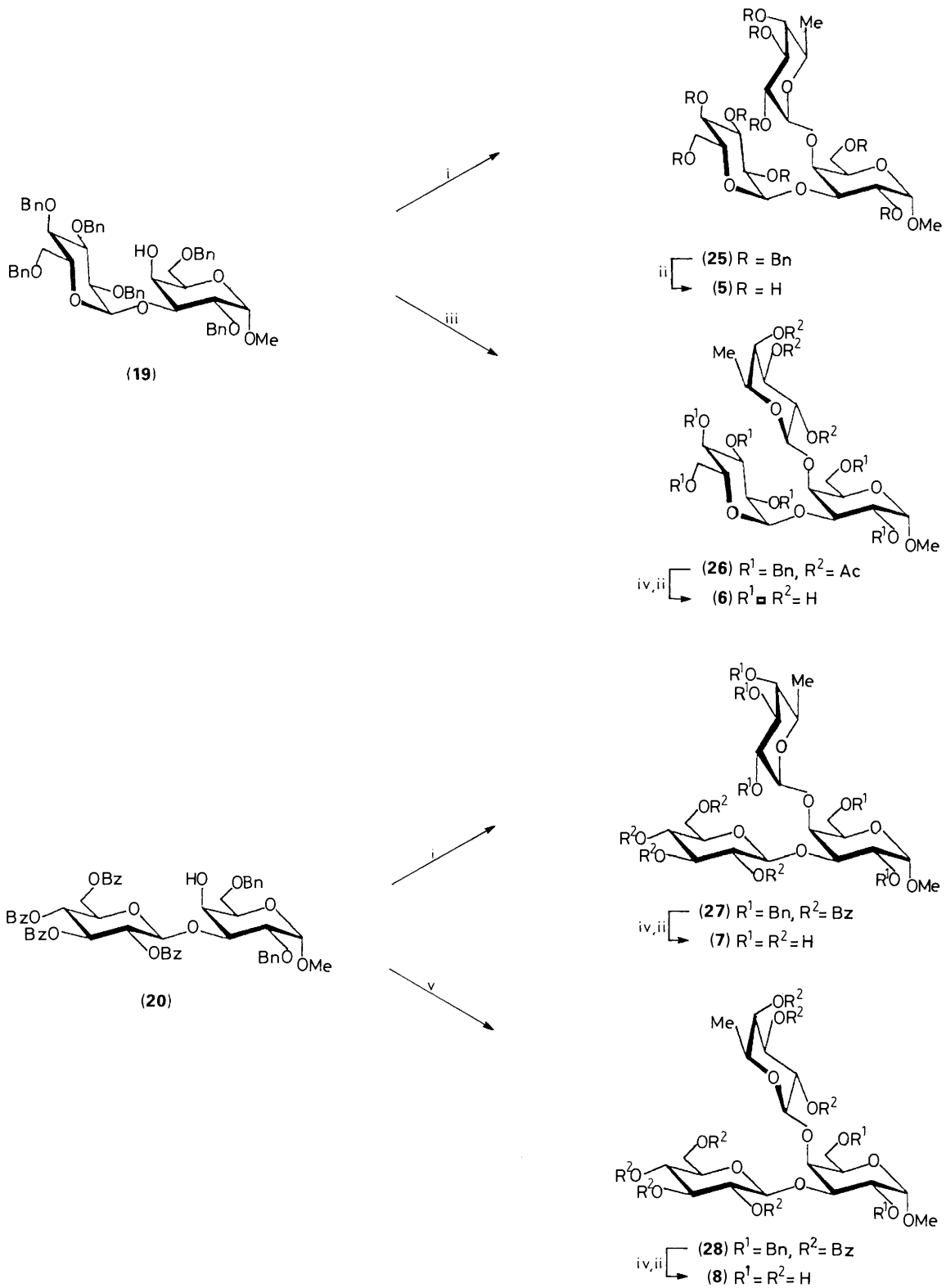
[a] The first value refers to the glycosyl group and the second value refers to the methyl galactoside residue. [b] In this compound also O-6′ and 6-H are vicinal (3.24 Å).



Scheme 2. Syntheses starting from disaccharides (**17**) and (**18**); *Reagents and conditions:* i, glycosyl donor (**15**), $F_3CSO_3CH_3$, Et_2O, room temp.; ii, H_2, Pd; iii, glycosyl donor (**16**), F_3CSO_3Ag, CH_2Cl_2, collidine, − 20 °C; iv, NaOMe, MeOH–CH_2Cl_2; v, NaOMe, MeOH–CH_2Cl_2, SiO_2 chromatography

energy maps were obtained for rotation of each glycosidic bond in the trisaccharides. The φ,ψ-energy maps for trisaccharides (**1**)—(**8**) are shown in the Figure.

Most of the glycosidic linkages have approximately the same φ- and ψ-angles in the trisaccharides (Table 3) as in the corresponding disaccharides (Table 2). The largest deviations observed (> 20°) were predominantly for the ψ-angles. For the 4-*O*-α-D-glucosyl group in compounds (**1**) and (**3**) the φ-angle also deviates. As two glycosyl groups are linked to vicinal positions, a change of the conformation relative to that of the



Scheme 3. Synthesis starting from disaccharides (**19**) and (**20**); *Reagents and conditions:* i, glycosyl donor (**11**), $F_3CSO_3CH_3$, Et_2O, room temp.; ii, H_2, Pd; iii, glycosyl donor (**12b**), $F_3CSO_3CH_3$, Et_2O, room temp.; iv, NaOMe, MeOH–CH_2Cl_2; v, glycosyl donor (**12a**), $F_3CSO_3CH_3$, Et_2O, room temp.

corresponding disaccharide is necessary to minimize severe contacts. Rotation around the O(X)–C(X) bond (ψ) makes the substituents in one glycosyl group attain positions further away from the substituents in the other glycosyl group, more than does rotation around the C(1)–O(X) bond (φ).

In all of the trisaccharides, including those with φ- and ψ-values similar to the values in the corresponding disaccharides, the energy maps indicate that conformational freedom is restricted due to severe atomic interactions between the two glycosyl groups. For all of the 3-*O*-glycosyl groups, the rotational freedom is restricted for rotamers with negative ψ-values. The same restriction was also observed for other 3,4-di-*O*-

Table 3. Values for the φ, ψ, and φ angles of the minimum energy conformations and the inter-residue atomic distances <3.5 Å between the terminal groups and the methyl galactoside residue in the trisaccharides (**1**)—(**8**), as obtained by GESA calculations

Compd.		φ	ψ	ω	1′-H[a]	5′-H	6′-H	O-5′	1″-H[a]	3″-H	5″-H	6″-H	O-2″	O-5″
α-D-Glc*p*-(1 ⟶ 4)		−15	39	−52	O-2 (2.70)	3-H (3.22)	3-H (3.41)	3-H (2.48)	O-3 (2.70)	6-H_b (3.46)		2-H (3.34)	4-H (3.47)	O-3 (2.62)
α-D-Gal*p*-OMe	(**1**)			66	3-H (2.51)	4-H (2.41)	4-H (3.28)	4-H (3.50)	4-H (2.10)				6-H_a (3.41)	2-H (3.30)
α-L-Fuc*p*-(1 ⟶ 3)		57	14										$6(\text{H}_b$ (3.39)	
β-D-Glc*p*-(1 ⟶ 4)		60	29	−54	O-2 (2.94)	O-4 (3.43)	4-H (2.96)	3-H (2.58)	O-3 (2.36)					4-H (2.28)
α-D-Gal*p*-OMe	(**2**)			66	3-H (2.39)	3-H (3.42)		4-H (3.27)	4-H (2.67)					6-H_a (3.28)
α-L-Fuc*p*-(1 ⟶ 3)		54	5			4-H (2.34)								
α-D-Glc*p*-(1 ⟶ 4)		−24	28	−52	3-H (2.41)			O-2 (3.10)	O-3 (3.14)		O-3 (3.03)		6-H_a (3.44)	O-3 (2.59)
α-D-Gal*p*-OMe	(**3**)			66	4-H (2.95)			3-H (2.52)	4-H (2.03)		4-H (2.31)		6-H_b (3.12)	4-H (3.31)
β-L-Fuc*p*-(1 ⟶ 3)		−55	−6											
β-D-Glc*p*-(1 ⟶ 4)		54	7	−54	O-4 (3.45)			3-H (2.42)	O-3 (2.91)			6-H (3.22)		4-H (2.48)
α-D-Gal*p*-OMe	(**4**)			63	3-H (2.73)				4-H (2.37)					6-H_a (2.71)
β-L-Fuc*p*-(1 ⟶ 3)		−55	−37		4-H (2.27)									6-H_b (2.94)
α-L-Fuc*p*-(1 ⟶ 4)		37	18		3-H (2.71)	O-2 (2.54)	3-H (3.42)	3-H (2.45)	O-3 (2.64)		6-H_a (2.63)	6-H_a (2.48)	O-3 (3.41)	4-H (2.72)
α-D-Gal*p*-OMe	(**5**)			76	4-H (2.34)	3-H (2.80)			4-H (2.72)		6-H_b (2.34)	6-H_b (3.46)	2-H (3.20)	6-H_a (3.07)
α-D-Glc*p*-(1 ⟶ 3)		−55	−34	−56										
β-L-Fuc*p*-(1 ⟶ 4)		−56	−12		O-4 (3.39)	O-2 (2.70)		3-H (2.45)	4-H (2.41)				6-H_b (3.19)	O-3 (3.26)
α-D-Gal*p*-OMe	(**6**)			64	3-H (2.79)	3-H (2.70)			6-H_a (2.43)					4-H (2.42)
α-D-Glc*p*-(1 ⟶ 3)		−56	−40	−56	4-H (2.25)				6-H_b (2.59)					
α-L-Fuc*p*-(1 ⟶ 4)		48	27		3-H (2.36)		4-H (3.29)	3-H (2.75)	O-3 (2.37)	6-H_b (3.34)	4-H (3.27)	6-H_a (2.68)	2-H (3.26)	4-H (2.52)
α-D-Gal*p*-OMe	(**7**)			69				4-H (2.42)	4-H (2.47)		6-H_a (2.38)			6-H_a (3.36)
β-D-Glc*p*-(1 ⟶ 3)		58	−22	−52							6-H_b (2.28)			
β-L-Fuc*p*-(1 ⟶ 4)		−52	−2		O-2 (3.25)			3-H (2.53)	4-H (2.29)				6-H_b (3.18)	O-3 (2.99)
α-D-Gal*p*-OMe	(**8**)			65	3-H (2.43)			4-H (2.80)	6-H_a (2.64)					4-H (2.54)
β-D-Glc*p*-(1 ⟶ 3)		61	−6	−52					6-H_b (2.93)					

[a] Primed labels refer to the 3-*O*-glycosyl group and double-primed to the 4-*O*-glycosyl group. Compound (**4**) also contains O-6″ ⟶ 6-H (3.32) and (**7**) also contains O′-6 ⟶ 4-H (3.42).

Table 4. Inter-atomic distances <3.5 Å between the 3-*O*-glycosyl and 4-*O*-glycosyl groups in the minimum energy conformation of trisaccharides (**1**)—(**8**), as obtained by GESA calculations

Compd.	1′-H[a]	2′-H	3′-H	4′-H	5′-H	6′-H	O-1′	O-2′	O-5′	O-6′
(1)			O-5″ (2.77) 1″-H (2.58)		1″-H (2.24)		O-5″ (2.62) 1″-H (2.70)	O-5″ (3.13) O-6″ (3.17) 6″-H (2.65)		
(2)			1″-H (2.18) 3″-H (3.10) 5″-H (2.87)	5″-H (2.85)	O-5″ (2.74) 1″-H (2.26) 5″-H (2.50) 6″-H (3.26)	5″-H (3.43) 6″-H (2.78)	1″-H (2.36)			
(3)		O-5″ (3.46) 6″-H_a (2.37) 6″-H_b (3.29)					O-5″ (2.59) 1″-H (3.14) 5″-H (3.03)	O-5″ (2.83) 1″-H (3.38) 6″-H_a (3.45) 6″-H_b (3.30)		
(4)	1″-H (2.57)						1″-H (2.91)	1″-H (2.64)		
(5)	1″-H (2.41)						O-2″ (3.41) 1″-H (2.64)	O-2″ (3.47) 1″-H (2.55) 2″-H (3.36)		
(6)	O-5″ (2.60) 6″H_a (2.85) 6″-H_b (3.39)	6″-H_a (3.11) 6″-H_b (3.40)					O-5″ (3.26)	O-4″ (3.47) O-5″ (3.00) 6″-H (2.60)		
(7)		1″-H (2.56)					1″-H (2.37)		1″-H (2.64)	O-5″ (3.34)
(8)		O-5″ (3.06)					O-5″ (2.99)		O-5″ (3.45)	6″-H (2.59)

[a] Primed labels refer to the 3-*O*-glycosyl group and double-primed to the 4-*O*-glycosyl group.

glycosyl substituted methyl α-D-galactopyranosides.[2] This restriction depends upon increased contacts between various atoms in the 3-*O*-glycosyl group and the anomeric proton (1″-H) and/or atoms on C-5″ and C-6″ in the 4-*O*-glycosyl group. This restriction in rotational freedom results in the lone-pair on the glycosidic oxygen (O-3) being oriented more towards the 4-*O*-glycosyl group than it was oriented towards 4-(O)H in the corresponding disaccharide.

On rotation around the 4-linkage in trisaccharides with a 4-*O*-α-L-fucosyl or 4-*O*-β-D-glucosyl group, energy maps similar to those of the corresponding disaccharides are obtained. For the 4-*O*-α-D-glucosyl group in compounds (**1**) and (**3**), the φ- and ψ-angles are changed 35° and 55°, respectively, in the trisaccharide relative to those in the parent disaccharides. This results, *inter alia*, in a change of the surroundings of 5″-H. A calculated short distance between 5″-H and O-3 in the disaccharide is substantially longer in the trisaccharide.

^{1}H *N.m.r. Glycosylation Shifts of Trisaccharides* (**1**)—(**8**).—The ^{1}H n.m.r. chemical shifts and the glycosylation shifts for compounds (**1**)—(**8**) are given in Table 5. The glycosylation shifts (obs. – mono.) are the difference between observed chemical shifts for signals from the trisaccharides and those from the appropriate monomers. The glycosylation shifts (obs. – calc.), are, for signals from the glycosyl groups, the difference between the observed chemical shifts (obs.) and those from the corresponding disaccharide. For the methyl galactoside residue, comparison is made with chemical shifts for signals from methyl α-D-galactoside to which two sets of glycosylation shifts are added, namely those deriving from the two appropriate disaccharides. Thus the difference between observed chemical shifts and those derived from pure additivity of glycosylation shifts is obtained.

For compounds (**1**)—(**8**) and the previously investigated trisaccharides[2] the most significant deviations from the calculated shifts are observed for the anomeric proton signals. For compounds (**1**)—(**8**), extra downfield shifts are present for signals from the anomeric protons except for those from the 3-*O*-β-L-fucosyl and 3-*O*-β-D-glucosyl groups. The downfield shift of signals from 1′-H in the trisaccharides (**1**), (**2**), and (**6**) corresponds to increased contact between the anomeric protons and O-2 in (**1**) and (**2**) (Table 3) and O-5″ in compound (**6**) (Table 4). The largest deviations are observed for signals from the 4-*O*-glycosyl groups, in particular the 4-*O*-α-L-fucosyl groups (0.24 and 0.32 p.p.m.). A reason for these large additional shifts could be the proximity of the anomeric proton (1″-H) to O-3 and the more directed lone-pairs of this linkage oxygen, as discussed above. Large additional shifts of 0.11 and 0.18 p.p.m. are also observed from the signal for 1″-H in (**6**) and (**8**), *i.e.* those compounds with a 4-*O*-β-L-fucosyl group.

For signals from other than anomeric protons in the 3-*O*-glycosyl groups, significant glycosylation shifts are only observed for the 3′-H and 5′-H signals from trisaccharides containing a 3-*O*-α-L-fucosyl group, (**1**) and (**2**). These protons are close to 1″-H in the minimum energy conformation (Table 4).

For signals from other than anomeric protons in the 4-*O*-glycosyl groups, significant deviations from the calculated shifts are only observed for the 5″-H signals, from −0.01 to −0.18 p.p.m. The largest of these upfield shifts could be attributed to a weaker contact between 5″-H and O-3 in trisaccharides (**1**) and (**3**), and to stronger contacts between 5″-H and several protons in the 3-*O*-fucosyl group in trisaccharide (**2**) compared with those in the corresponding disaccharides.

The calculated shifts of the signals for protons in the methyl galactoside residue are similar to the observed shifts. Only the signal for 2-H shows an extra downfield shift of 0.02—0.12 p.p.m. This may depend on changed interactions between 2-H and the lone-pairs on O-3 and O-4, as suggested for related trisaccharides.[2] The glycosylation shifts for the signals from 2-H, 3-H, and 4-H in the trisaccharides relative to those of the monomer (obs. – mono., Table 5) vary between 0.15 and 0.46 p.p.m. For almost every trisaccharide a typical set of values is obtained making them useful for the identification of the anomeric and absolute configuration of the substituting groups.

^{13}C *N.m.r. Glycosylation Shifts of Trisaccharides* (**1**)—(**8**).—The chemical shifts and the chemical shift differences, obtained as described above for ^{1}H n.m.r. glycosylation shifts, are given in Table 6.

In the previous study on trisaccharides[2] with only L-fucosyl

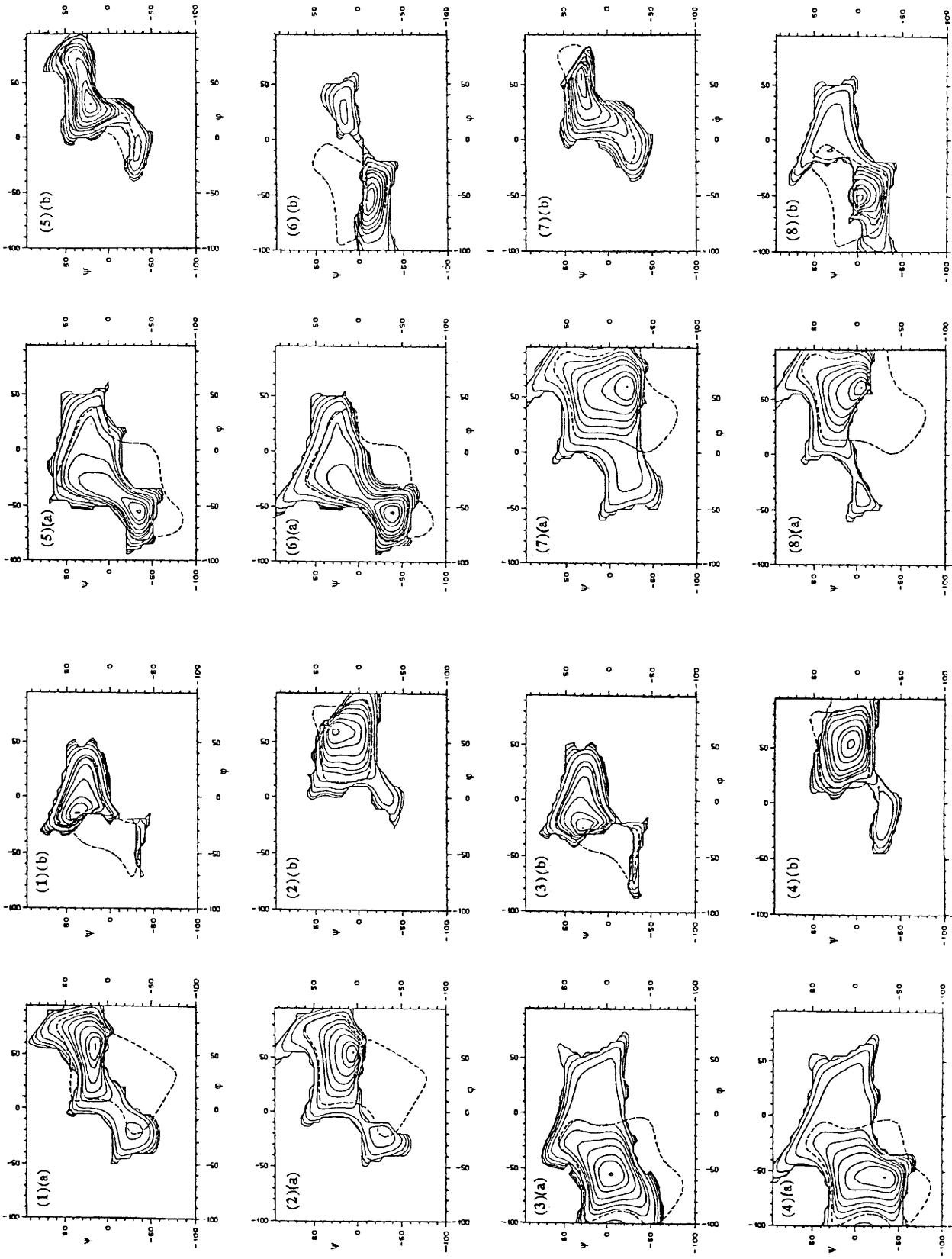


Figure. Conformational energy plots for trisaccharides (**1**)—(**8**). Energy maps obtained upon rotation of the 3-*O*-glycosyl group are shown in (*a*) and of the 4-*O*-glycosyl group in (*b*). Isocontour levels are indicated at 0.4, 2.1, 4.2, 8.4, 16.8, 21.0, 33.6, 46.2, 58.7, 71.3, and 83.9 kJ (0.1, 0.5, 1, 2, 3, 5, 8, 11, 14, 17, and 20 kcal) above the minimum energy conformation. Dashed lines indicate the 5 kcal energy level for the corresponding disaccharide

Table 5. ^{1}H N.m.r. data for the trisaccharides (**1**)—(**8**) at 70 °C relative to internal TSP (δ_H 0.00)

Compd.			1′-H[a]	2′-H	3′-H	4′-H	5′-H	6′-H$_a$	6′-H$_b$	1″-H[a]	2″-H	3″-H	4″-H	5″-H	6″-H$_a$	6″-H$_b$	1-H	2-H	3-H	4-H	5-H	6-H$_a$	6-H$_b$	OMe
α-D-Glc*p*-(1 ⟶ 4)		(obs.)	5.26	3.81	3.84	3.82	4.14	1.24		5.03	3.54	3.79	3.48	3.88	3.80[c]	3.80[c]	4.88	4.15	3.97	4.15	4.01	3.87[c]	3.87[c]	3.45
α-D-Gal*p*-O-Me	(**1**)	(obs. − mono.)[b]	0.06	0.04	−0.02	0.01	−0.06	0.03		−0.20	0.00	0.07	0.06	0.04	−0.04	0.04	0.03	0.31	0.16	0.16	0.12	0.11	0.11	0.02
α-L-Fuc*p*-(1 ⟶ 3)		(obs. − calc.)[b]	0.10	0.01	−0.10	0.00	−0.04	0.02		0.07	−0.03	0.05	0.03	−0.18	0.01	0.01	−0.01	0.08	0.04	−0.01	0.02	0.03	0.03	0.00
β-D-Glc*p*-(1 ⟶ 4)		(obs.)	5.23	3.83	3.85	3.85	4.11	1.25		4.68	3.35	3.47	3.41	3.32	3.90	3.73	4.86	4.20	4.01	4.30	3.97	3.80	3.73	3.44
α-D-Gal*p*-O-Me	(**2**)	(obs. − mono.)	0.03	0.06	−0.01	0.04	−0.09	0.04		0.04	0.10	−0.03	−0.01	−0.14	0.00	0.01	0.01	0.36	0.20	0.31	0.08	0.04	−0.03	0.01
α-L-Fuc*p*-(1 ⟶ 3)		(obs. − calc.)	0.07	0.03	−0.09	0.03	−0.07	0.03		0.04	−0.01	−0.05	0.00	−0.12	−0.01	−0.01	−0.01	0.09	0.05	0.01	0.01	0.00	0.00	0.00
α-D-Glc*p*-(1 ⟶ 4)		(obs.)	4.47	3.60	3.67	3.76	3.79	1.27		5.03	3.55	3.78	3.48	3.96	3.83[c]	3.83[c]	4.94	4.03	4.03	4.26	3.95	3.88[c]	3.88[c]	3.44
α-D-Gal*p*-O-Me	(**3**)	(obs. − mono.)	−0.08	0.14	0.04	0.02	0.00	0.01		−0.20	0.01	0.06	0.06	0.12	−0.01	0.07	0.09	0.19	0.22	0.27	0.06	0.12	0.12	0.01
β-L-Fuc*p*-(1 ⟶ 3)		(obs. − calc.)	−0.03	0.03	0.00	0.00	0.00	0.00		0.07	−0.02	0.04	0.03	−0.10	0.04	0.04	0.01	0.03	−0.05	−0.02	−0.01	−0.01	−0.01	−0.01
β-D-Glc*p*-(1 ⟶ 4)		(obs.)	4.52	3.59	3.67	3.76	3.78	1.27		4.77	3.34	3.47	3.39	3.43	3.86	3.72	4.90	4.06	4.12	4.38	3.85	3.82	3.74	3.43
α-D-Gal*p*-O-Me	(**4**)	(obs. − mono.)	−0.03	0.13	0.04	0.02	−0.01	0.01		0.13	0.09	−0.03	−0.03	−0.03	−0.04	0.00	0.05	0.22	0.31	0.39	−0.04	0.06	−0.02	0.00
β-L-Fuc*p*-(1 ⟶ 3)		(obs. − calc.)	0.02	0.02	0.00	0.00	−0.01	0.00		0.13	−0.02	−0.05	−0.02	−0.01	−0.05	−0.02	−0.01	0.02	0.01	−0.03	−0.08	−0.03	−0.04	−0.01
α-L-Fuc*p*-(1 ⟶ 4)		(obs.)	5.14	3.61	3.75	3.46	3.97	3.86	3.76	5.36	3.81	3.88	3.83	4.05	1.24		4.91	4.16	4.02	4.26	3.97	3.76[c]	3.76[c]	3.45
α-D-Gal*p*-O-Me	(**5**)	(obs. − mono.)	−0.09	0.07	0.03	0.04	0.13	0.02	0.00	0.16	0.04	0.02	0.02	−0.15	0.03		0.06	0.32	0.21	0.27	0.08	0.00	0.00	0.02
α-D-Glc*p*-(1 ⟶ 3)		(obs. − calc.)	0.03	0.02	−0.05	0.02	0.04	0.01	0.00	0.24	−0.04	0.02	0.01	−0.07	0.01		0.04	0.12	0.03	0.00	0.04	0.02	0.02	0.02
β-L-Fuc*p*-(1 ⟶ 4)		(obs.)	5.29	3.60	3.78	3.45	3.90	3.86	3.76	4.45	3.55	3.64	3.74	3.77	1.29		4.91	4.04	3.96	4.45	3.95	3.87[c]	3.87[c]	3.44
α-D-Gal*p*-O-Me	(**6**)	(obs. − mono.)	0.06	0.06	0.06	0.03	0.06	0.02	0.00	−0.10	0.09	0.01	0.00	−0.02	0.03		0.06	0.20	0.15	0.46	0.06	0.11	0.11	0.01
α-D-Glc*p*-(1 ⟶ 3)		(obs. − calc.)	0.18	0.01	−0.02	0.01	−0.03	0.01	0.00	0.11	−0.01	−0.01	−0.02	−0.04	0.02		0.03	0.08	0.07	0.07	−0.02	0.04	0.04	0.00
α-L-Fuc*p*-(1 ⟶ 4)		(obs.)	4.66	3.36	3.52	3.45	3.46	3.91	3.75	5.44	3.80	3.86	3.83	4.07	1.23		4.89	4.14	4.07	4.32	4.00	3.74[c]	3.74[c]	3.45
α-D-Gal*p*-O-Me	(**7**)	(obs. − mono.)	0.02	0.11	0.02	0.03	0.00	0.01	0.03	0.24	0.03	0.00	0.02	−0.13	0.02		0.04	0.30	0.26	0.33	0.11	−0.02	−0.02	0.02
β-D-Glc*p*-(1 ⟶ 3)		(obs. − calc.)	−0.01	−0.03	0.02	0.01	0.00	0.02	0.01	0.32	−0.05	0.00	0.01	−0.05	0.00		0.03	0.06	0.03	0.05	0.03	−0.01	−0.01	0.02
β-L-Fuc*p*-(1 ⟶ 4)		(obs.)	4.66	3.36	3.52	3.39	3.44	3.92	3.74	4.52	3.58	3.61	3.73	3.73	1.28		4.89	4.09	4.00	4.39	3.98	3.83[c]	3.83[c]	3.44
α-D-Gal*p*-O-Me	(**8**)	(obs. − mono.)	0.02	0.11	0.02	−0.03	−0.02	0.02	0.02	−0.03	0.12	−0.02	−0.01	−0.06	0.02		0.04	0.25	0.19	0.40	0.09	0.07	0.07	0.01
β-D-Glc*p*-(1 ⟶ 3)		(obs. − calc.)	−0.01	−0.03	0.02	−0.05	−0.02	0.03	0.00	0.18	0.02	−0.04	−0.03	−0.08	0.01		0.02	0.09	0.06	0.00	−0.03	−0.01	−0.01	0.00

[a] Primed labels refer to the 3-*O*-glycosyl group and double-primed labels to the 4-*O*-glycosyl group. [b] The method of calculating chemical shifts is described in Results and Discussion. [c] Approximate chemical shifts due to higher order spectrum.

Table 6. ^{13}C N.m.r. data for the trisaccharides (**1**)—(**8**) at 70 °C relative to internal dioxane (δ_C 67.40)

Compd.			C-1′[a]	C-2′	C-3′	C-4′	C-5′	C-6′	C-1″[a]	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 ⟶ 4)		(obs.)	101.01	69.34	70.74	72.68	67.93	16.15	100.77	72.95	73.54	70.54	73.49	61.62	100.43	69.63	76.69	78.90	72.33	61.42	56.06
α-D-Gal*p*-O-Me	(**1**)	(obs. − mono.)[b]	7.89	0.25	0.45	−0.11	0.83	−0.18	7.78	0.48	−0.24	−0.17	1.12	−0.22	0.08	0.46	6.23	8.71	0.79	−0.64	0.10
α-L-Fuc*p*-(1 ⟶ 3)		(obs. − calc.)[b]	−0.51	−0.06	0.23	−0.03	0.03	0.00	−0.29	0.26	−0.13	0.03	0.44	0.20	0.05	0.94	−1.60	−0.87	0.41	0.17	−0.03
β-D-Glc*p*-(1 ⟶ 4)		(obs.)	101.13	69.35	70.78	72.63	68.21	16.27	103.44	74.34	76.87	70.78	77.12	61.86	100.45	69.45	77.60	76.31	71.46	61.68	56.05
α-D-Gal*p*-O-Me	(**2**)	(obs. − mono.)	8.01	0.26	0.49	−0.16	1.11	−0.06	6.60	−0.86	0.11	0.07	0.36	0.02	0.10	0.28	7.14	6.12	−0.08	−0.38	0.09
α-L-Fuc*p*-(1 ⟶ 3)		(obs. − calc.)	−0.39	−0.05	0.27	−0.08	0.31	0.12	−1.18	−0.35	0.07	0.12	0.32	0.06	0.10	0.54	−1.42	−2.94	0.47	0.00	0.00
α-D-Glc*p*-(1 ⟶ 4)		(obs.)	103.22	71.21	73.69	72.12	71.97	16.23	101.24	72.85	73.54	70.55	73.16	61.47	100.17	68.20	78.26	78.38	72.12	61.35	55.99
α-D-Gal*p*-O-Me	(**3**)	(obs. − mono.)	6.07	−1.52	−0.24	−0.23	0.33	−0.10	8.25	0.38	−0.24	−0.16	0.79	−0.37	−0.18	−0.97	7.80	8.19	0.58	−0.71	0.03
β-L-Fuc*p*-(1 ⟶ 3)		(obs. − calc.)	1.71	−0.24	−0.15	−0.07	0.07	0.07	0.18	0.16	−0.13	0.04	0.11	0.05	0.05	0.28	0.47	1.08	0.43	0.08	−0.04
β-D-Glc*p*-(1 ⟶ 4)		(obs.)	101.43	71.60	73.92	72.25	71.76	16.21	103.70	74.45	76.75	70.86	76.61	62.00	100.16	67.94	77.39	75.42	71.07	61.69	55.99
α-D-Gal*p*-O-Me	(**4**)	(obs. − mono.)	4.28	−1.13	−0.01	−0.10	0.12	−0.12	6.86	−0.75	−0.01	0.15	−0.15	0.16	−0.19	−1.23	6.93	5.23	−0.47	−0.37	0.03
β-L-Fuc*p*-(1 ⟶ 3)		(obs. − calc.)	−0.08	0.15	0.08	0.06	−0.14	0.05	−0.92	−0.24	−0.05	0.20	−0.19	0.20	0.07	−0.20	−1.13	−1.36	0.31	−0.01	0.00
α-L-Fuc*p*-(1 ⟶ 4)		(obs.)	96.94	72.35	73.88	70.54	72.66	61.55	100.18	69.37	70.31	72.62	67.96	16.15	100.45	67.76	76.84	74.04	72.29	62.30	56.02
α-D-Gal*p*-O-Me	(**5**)	(obs. − mono.)	3.95	−0.12	0.10	−0.17	0.29	−0.29	7.06	0.28	0.02	−0.17	0.86	−0.18	0.10	−1.41	6.38	3.85	0.75	0.24	0.06
α-D-Glc*p*-(1 ⟶ 3)		(obs. − calc.)	0.90	0.15	0.04	−0.04	−0.08	0.00	−2.08	−0.32	−0.35	−0.04	−0.31	0.02	0.08	−0.46	0.46	−2.59	0.70	0.07	−0.03
β-L-Fuc*p*-(1 ⟶ 4)		(obs.)	96.01	72.24	74.18	70.45	72.67	61.54	103.70	72.24	73.80	72.12	71.94	16.20	100.26	67.76	74.59	72.93	71.82	61.11	56.06
α-D-Gal*p*-O-Me	(**6**)	(obs. − mono.)	3.02	−0.23	0.40	−0.26	0.30	−0.30	6.55	−0.49	−0.13	−0.23	0.30	−0.13	−0.09	−1.41	4.13	2.74	0.28	−0.95	0.10
α-D-Glc*p*-(1 ⟶ 3)		(obs. − calc.)	−0.03	0.04	0.34	−0.13	−0.07	−0.01	−0.46	0.54	0.01	0.02	0.08	0.04	−0.21	−0.65	−0.34	−3.39	0.38	−0.23	−0.02
α-L-Fuc*p*-(1 ⟶ 4)		(obs.)	104.92	74.41	76.58	70.48	76.72	61.75	100.20	69.57	70.70	72.65	68.03	16.14	100.24	68.49	80.69	77.32	71.87	62.23	55.98
α-D-Gal*p*-O-Me	(**7**)	(obs. − mono.)	8.08	−0.79	−0.18	−0.23	−0.04	−0.09	7.08	0.48	0.41	−0.14	0.93	−0.19	−0.11	−0.68	10.23	7.13	0.33	0.17	0.02
β-D-Glc*p*-(1 ⟶ 3)		(obs. − calc.)	0.49	0.07	−0.04	−0.03	0.00	0.11	−2.06	−0.12	0.04	−0.01	−0.24	0.01	−0.02	−0.40	−0.41	−2.57	0.35	0.05	−0.01
β-L-Fuc*p*-(1 ⟶ 4)		(obs.)	104.79	74.58	76.63	70.84	76.74	61.93	104.19	72.10	74.03	72.28	71.76	16.48	100.29	68.45	78.77	78.47	71.61	61.42	55.98
α-D-Gal*p*-O-Me	(**8**)	(obs. − mono.)	7.95	−0.62	−0.13	0.13	−0.02	0.09	7.04	−0.63	0.10	−0.07	0.12	0.15	−0.06	−0.72	8.31	8.28	0.07	−0.64	0.02
β-D-Glc*p*-(1 ⟶ 3)		(obs. − calc.)	0.36	0.24	0.01	0.33	0.02	0.29	0.03	0.40	0.24	0.18	−0.10	0.32	−0.07	−0.63	−0.88	−1.11	0.24	0.13	−0.04

[a] Primed labels refer to the 3-*O*-glycosyl group and double-primed labels to the 4-*O*-glycosyl group. [b] The method of calculating chemical shifts is described in Results and Discussion.

Table 7. Chemical shift differences (in p.p.m.) for trisaccharides (1)—(**8**) from variation in temperature[a]

Compd.	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
(**1**)	0.05	0.11	0.17	0.06	0.03	−0.05	−0.07	0.12	0.17	0.24	0.12	0.24	0.04	0.09	0.35	−0.05	0.04	0.04	0.06
(**2**)	0.11	0.13	0.17	0.08	−0.03	−0.03	0.11	0.15	0.17	0.17	−0.04	0.16	0.07	0.09	0.33	0.19	0.02	0.03	0.06
(**3**)	0.09	0.08	0.20	0.08	0.02	−0.06	−0.20	0.16	0.21	0.27	0.08	0.34	0.10	0.10	−0.15	−0.37	0.03	−0.03	0.05
(**4**)	0.43	0.01	0.21	0.05	0.09	−0.05	−0.09	0.21	0.19	0.21	0.09	0.29	0.10	0.14	0.51	0.06	0.06	−0.02	0.05
(**5**)	0.64	0.10	0.17	0.29	0.20	0.29	0.12	0.12	0.29	0.07	−0.01	−0.05	0.07	0.27	0.76	0.57	0.04	−0.03	0.07
(**6**)	0.51	0.13	0.15	0.27	0.15	0.26	−0.02	0.13	0.18	0.13	−0.05	0.01	0.08	0.19	0.56	0.28	0.01	−0.04	0.05
(**7**)	−0.12	0.13	0.21	0.24	0.15	0.23	0.34	0.11	0.25	0.07	−0.03	−0.05	0.09	0.21	−0.20	0.55	0.03	0.00	0.06
(**8**)	−0.05	0.09	0.16	0.16	0.09	0.22	−0.15	0.03	0.13	0.06	0.00	−0.09	0.12	0.16	−0.04	−0.56	0.00	−0.08	0.07

[a] $\Delta\delta = \delta(70\ °C) - \delta(30\ °C)$. The value for dioxane was taken to be δ 67.40 p.p.m. for all temperatures.

or only D-glucosyl groups, deviations of glycosylation shifts from calculated values were observed mainly for signals for those carbons involved in the glycosidic linkages. Similar results were obtained for the trisaccharides (**1**)—(**8**), which showed larger deviations for signals from the carbons in the 1,4-linkage, *i.e.* C-1″ and C-4, than for signals from the corresponding carbons in the 1,3-linkage. Thus large additional upfield shifts, from −0.9 to −2.1 p.p.m., were observed for the signal for C-1″ in trisaccharides (**2**), (**4**), (**5**), and (**7**) (α-L/β-D), and with the highest values for signals from C-1″ in the α-L-fucosyl group. For the other trisaccharides (α-D/β-L) smaller additional shifts, from −0.5 to 0.2 p.p.m., were found. Such similarities of glycosylation shifts between α-D/β-L-substituents have been found earlier.[2,5] In all compounds the deviations from calculated values were even more pronounced for the C-4 signal. Additional upfield shifts of between −0.9 and −3.4 p.p.m. were found, with the exception of compound (**3**) for which instead an additional downfield shift of 1.1 p.p.m. was observed. This compound has β-L-fucosyl and α-D-glucosyl groups in the 3- and 4-positions, respectively, of the methyl α-D-galactopyranoside residue. In the previous study on related compounds[2] we observed a downfield shift of 1.7 p.p.m. for the C-4 signal from the trisaccharide with an α-D-glucosyl group linked to both the 3- and 4-position, in contrast to the upfield shifts for the other trisaccharides. In the methyl glucosides of the two disaccharides α-D-Glc*p*(1 ⟶ 3)-α-D-Gal*p* and β-L-Fuc*p*(1 ⟶ 3)-α-D-Gal*p*, large upfield shifts for the signal of C-4 were observed.[2] These were attributed to the γ-*gauche* effect caused by the proximity between 1′-H and 4-H. In the two trisaccharides containing these disaccharide elements and for which the C-4 signals showed a downfield shift, the smaller upfield shift for the signal from C-1′ and C-4, observed as extra downfield shifts when compared to the calculated values, indicates weaker interaction between 1′-H and 4-H. The smaller contribution from the γ-*gauche* effect could also result in downfield shifts of the C-4 signal for other trisaccharides containing a 3-*O*-α-D- or 3-*O*-β-L-glycosyl group. The shift is, however, less obvious, as large upfield shifts of the C-4 signal caused by other interactions dominate. An increased distance between 1″-H and 4-H is not supported by calculated distances in the minimum energy conformation, however, but the average conformation could be different from the minimum energy conformation, and restricted rotation compared to the corresponding disaccharides is indicated (Figure).

Some significant deviations from the calculated values for signals for the carbons in the 1,3-linkage, C-1′ and C-3, were also observed. For the 3-*O*-α-L-fucosyl containing trisaccharides (**1**) and (**2**), and for similarly substituted trisaccharides in the previous investigation,[2] these signals have glycosylation shifts from −0.2 to −0.6 p.p.m. for C-1′, and from −1.1 to −2.8 p.p.m. for C-3. For 3-*O*-β-D-glucosyl containing trisaccharides in the two studies, the signals from the anomeric carbon have glycosylation shifts, from 0.4 to 0.7 p.p.m., and those from C-3 an additional upfield shift, from −0.4 to −1.0 p.p.m. For the 3-*O*-α-D/β-L-glycosyl containing trisaccharides an extra downfield shift for the C-1′ signal, from 0.4 to 1.0 p.p.m., was observed in the previous study.[2] For (**3**) and (**5**), which contain 3-*O*-α-D/β-L-glycosyl groups, a large extra downfield shift for the C-1′ signal was observed, whereas for (**4**) and (**6**) shifts similar to the calculated values were obtained. For trisaccharides (**3**) and (**5**), this could be interpreted as a weaker γ-*gauche* effect with a longer distance between 1′-H and 4-H, as discussed above.

Deviations from calculated values for signals from carbons other than linkage carbons could also be observed, namely for the C-2″ signals. For trisaccharides containing α-D-glycosyl and β-L-fucosyl groups linked to the 4-position an additional downfield shift, from 0.2 to 0.5 p.p.m., is observed, whereas for trisaccharides containing β-D-glucosyl and α-L-fucosyl groups of upfield shifts from −0.1 to −0.3 p.p.m. are observed. In the methyl galactoside residue some downfield and upfield shift deviations are also observed for the C-2 signals whereas only downfield deviations are found for signals from C-5. These shifts range from −0.7 to 0.9 p.p.m.

Effects of Temperature Variation on Chemical Shifts.—Values for chemical shift differences (in p.p.m.) obtained from ^{13}C n.m.r. spectra recorded at 30 and 70 °C are shown in Table 7. Most signals are shifted downfield with reference to the internal standard, dioxane, to which a constant chemical shift of δ_C 67.40 was assigned. The largest positive and the largest negative shifts are observed for signals from linkage carbons, and the remaining values are normally shifted <0.3 p.p.m. The largest shifts are found for signals from C-1′ and C-3, *i.e.* for two carbons engaged in the same glycosidic linkage. A number of carbon signals have minor or negative shifts, *e.g.* the C-6 signals from the fucosyl groups and the methyl galactoside residue, the C-1′ signal of the 3-*O*-β-D-glucosyl groups and several of the C-1″ signals. The upfield shifts observed for the signals for the linkage carbons, C-3 and C-4, are accompanied by upfield shifts of the corresponding anomeric carbon signals.

Conclusions

The present study confirms the results from the previous study on related '3,4-branched' trisaccharides. Thus, upon vicinal disubstitution of a glycose residue, changes in conformation and/or dynamics are present such that n.m.r. glycosylation shifts from the constituent disaccharides can rarely be added to give the chemical shifts of the trisaccharide. For most trisaccharides a typical set of chemical shift displacements are found, making it possible to use chemical shifts in structural analysis. It is also emphasized that identical conditions should be used in the n.m.r. experiments as the chemical shifts are temperature dependent. By using semi-empirical calculations to find minimum energy conformations a number of atom interactions could be correlated primarily to ^1H n.m.r. glycosylation shifts. It seems, however, as if the restricted rotation around the glycosidic linkages in the trisaccharides sometimes complicates the estimation of favourable conformations and that information on, *e.g.*, molecular dynamics would be needed.

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References

1 H. Baumann, B. Erbing, P.-E. Jansson, and L. Kenne, *J. Chem. Soc., Perkin Trans. 1*, 1989, 2145.
2 H. Baumann, B. Erbing, P.-E. Jansson, and L. Kenne, *J. Chem. Soc., Perkin Trans. 1*, preceding paper.
3 M. Forsgren, P.-E. Jansson, and L. Kenne, *J. Chem. Soc., Perkin Trans. 1*, 1985, 2383.
4 H. Baumann, P.-E. Jansson, and L. Kenne, *J. Chem. Soc., Perkin Trans. 1*, 1988, 209.
5 I. Backman, B. Erbing, P.-E. Jansson, and L. Kenne, *J. Chem. Soc., Perkin Trans. 1*, 1988, 889.
6 P.-E. Jansson, L. Kenne, and E. Schweda, *J. Chem. Soc., Perkin Trans. 1*, 1988, 2729.
7 P.-E. Jansson, L. Kenne, and G. Widmalm, *Carbohydr. Res.*, 1987, **168**, 67.
8 P.-E. Jansson, L. Kenne, and G. Widmalm, *Carbohydr. Res.*, 1989, **188**, 169.
9 H. Paulsen, T. Peters, V. Sinnwell, R. Lebuhn, and B. Meyer, *Justus Liebigs Ann. Chem.*, 1985, 489.

10 R. U. Lemieux, K. Bock, L. T. J. Delbaere, S. Koto, and V. S. Rao, *Can. J. Chem.*, 1980, **58**, 631.
11 H. Thøgersen, R. U. Lemieux, K. Bock, and B. Meyer, *Can. J. Chem.*, 1982, **60**, 44.
12 P. Fügedi, P. J. Garegg, H. Lönn, and T. Norberg, *Glycoconjugate J.*, 1987, **4**, 97.
13 R. U. Lemieux, T. Takeda, and B. Y. Chung, in 'Synthetic Methods for Carbohydrates,' *A.C.S. Symp. Ser.*, 1979, 39.
14 P. J. Garegg and T. Norberg, *Acta Chem. Scand., Ser. B*, 1979, **33**, 116.

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