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

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Both ^{1}H and ^{13}C n.m.r. studies and conformational analyses have been performed on methyl α-D-galactopyranosides substituted in the 3-, 4-, and 3,4-positions with different anomeric forms of either L-fucopyranosyl or D-glucopyranosyl groups. Conformational analysis using the HSEA and GESA approaches indicated restricted rotational freedom around the glycosidic bonds of the trisaccharides compared to those of the disaccharides. It also indicated a number of proton-oxygen and proton-proton interactions which could be correlated to downfield and upfield glycosylation shifts, respectively, of the proton signals in both the di- and the tri-saccharides. Similar inter-residue atomic interactions could also be correlated to the ^{13}C n.m.r. glycosylation shifts observed for the disaccharides and to some extent for the trisaccharides. 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 for the signals for many linkage carbons.

The increasing use and importance of n.m.r. spectroscopy in structural and conformational studies of oligo- and poly-saccharides requires a better understanding of the origin of glycosylation shifts, *i.e.* the change in chemical shift upon glycosylation, for different types of linkages. Recently, n.m.r. studies have been reported on 1,2-,[1] 1,3-,[1,2] 1,4-,[3] and 1,6-linked[4] disaccharide glycosides in which some of the n.m.r. data was also correlated with theoretically-derived conformations. The purpose of the studies was to provide data for a computer-based analysis of n.m.r. spectra from oligo- and polysaccharides[5] and to understand how glycosylation affects the chemical shifts for different stereochemistries around the glycosidic bond. The glycosylation shifts obtained from the disaccharides were used for the calculation of ^{13}C n.m.r. spectra for some polysaccharides.[1-5] It was found that for linear polysaccharides glycosylation shifts similar to those observed in the disaccharides were obtained. This shows that the glycosylation shifts are dominated by the type and stereochemistry of the two closest neighbours.

Several conformational studies of glycosides have been performed (*e.g.* refs. 6—9). In these studies, results from theoretical calculations, n.m.r. chemical shifts, and nuclear Overhauser effects (n.O.e.s) have been used to estimate preferred conformations. Conformational states and n.O.e.s of some 3-*O*- and 4-*O*-substituted methyl β-D-galactopyranosides have recently been described.[10] Proton-proton contacts (γ-*gauche*) were indicated in the lower energy conformations when only small glycosylation shifts for the signals of the anomeric carbons were observed.[8] A correlation between glycosylation shifts for the signals of the linkage carbons and the value of the ψ-angle of the minimum energy conformation obtained by HSEA calculations has been described.[11]

A ^{13}C n.m.r. study[12] of some *Shigella flexneri O*-polysaccharides, in which the structures of branched polysaccharides were also investigated, showed that for sugar residues disubstituted in the 3- and 6-positions the glycosylation shifts obtained from model disaccharides were additive. However, no additivity was observed for the signals from linkage carbons when sugar residues were disubstituted in vicinal positions.

As branched oligo- and poly-saccharides constitute a large proportion of naturally-occurring carbohydrates, better knowledge about the magnitude and origin of the glycosylation shifts for sugars involved in branching is needed. We now report n.m.r. and conformational studies of some 3-*O*-, 4-*O*-, and 3,4-di-*O*-glycosyl-substituted methyl α-D-galactopyranosides in which a comparison is made between data obtained from the 'branched' trisaccharides and from each disaccharide element. The correlation between n.m.r. glycosylation shifts and theoretically-derived conformations is also described. As the di- and tri-saccharides are built from α- and β-L-fucopyranosyl and α- and β-D-glucopyranosyl groups, stereochemically different surroundings of the glycosidic bond are obtained.

Experimental

General.—The syntheses of the di- and tri-saccharides (**1**)—(**16**) have been described.[13] ^{1}H N.m.r. spectra (400 MHz) and ^{13}C n.m.r. spectra (100 MHz) were recorded for 0.04M and 0.2M deuterium oxide solutions at 30 and 70 °C, respectively, with a JEOL GX 400 spectrometer. Chemical shifts are given in p.p.m. using sodium [2,2,3,3-$^2H_4$]-3-(trimethylsilyl)propanoate (TSP, δ_H 0.00) and dioxane (δ_C 67.40) as internal references. For the assignment of proton signals, proton-proton shift correlated techniques were used (COSY, relayed COSY,[14] double relayed COSY,[14] NOESY). For the assignment of carbon signals, proton-carbon shift correlated spectroscopy (H,C-COSY) and induced temperature shifts were used (see below). Chemical shifts of overlapping signals in the ^{1}H n.m.r. spectra were obtained from the centre of the cross-peaks in the proton-proton correlation spectra.

The GESA program[15] was used to estimate minimum energy conformations of all di- and tri-saccharides. Different starting torsion angles (φ, ψ, ω) were used to obtain the global energy minimum and the hydroxymethyl groups were allowed to rotate. 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 were obtained from the crystal structures of α-

(1) α-L-Fuc*p*-(1→3)-α-D-Gal*p*
(2) β-L-Fuc*p*-(1→3)-α-D-Gal*p*
(3) α-L-Fuc*p*-(1→4)-α-D-Gal*p*
(4) β-L-Fuc*p*-(1→4)-α-D-Gal*p*
(5) α-D-Glc*p*-(1→3)-α-D-Gal*p*
(6) β-D-Glc*p*-(1→3)-α-D-Gal*p*
(7) α-D-Glc*p*-(1→4)-α-D-Gal*p*
(8) β-D-Glc*p*-(1→4)-α-D-Gal*p*

α-L-Fuc*p*-(1→4)
(9) α-D-Gal*p*
α-L-Fuc*p*-(1→3)
β-L-Fuc*p*-(1→4)
(10) α-D-Gal*p*
α-L-Fuc*p*-(1→3)
α-L-Fuc*p*-(1→4)
(11) α-D-Gal*p*
β-L-Fuc*p*-(1→3)
β-L-Fuc*p*-(1→4)
(12) α-D-Gal*p*
β-L-Fuc*p*-(1→3)

α-D-Glc*p*-(1→4)
(13) α-D-Gal*p*
α-D-Glc*p*-(1→3)
β-D-Glc*p*-(1→4)
(14) α-D-Gal*p*
α-D-Glc*p*-(1→3)
α-D-Glc*p*-(1→4)
(15) α-D-Gal*p*
β-D-Glc*p*-(1→3)
β-D-Glc*p*-(1→4)
(16) α-D-Gal*p*
β-D-Glc*p*-(1→3)

Scheme. Compounds (1)—(16) are the methyl glycosides of the above di- and tri-saccharides

D-glucopyranose,[18] β-D-glucopyranose,[19] α-L-fucopyranose,[20] and α-D-galactopyranose.[21] If no values for hydrogen atoms were present these were generated by the HSEA-program, using a C–H distance of 1.1 Å. β-D-Galactopyranose was converted to methyl α-D-galactopyranoside by the addition of a methyl group at φ = 50°. Co-ordinates for β-L-fucopyranose were obtained from the mirror image of modified β-D-galactopyranose.[22] The co-ordinates of α- and β-D-glucopyranoses were changed by the HSEA-programe from the *gauche-trans* conformation ω = 60°, to the *gauche-gauche* conformation ω = −60°, as this conformation has been found to predominate in solution.[23] The new co-ordinates were than used in the GESA calculations.

Energy maps, showing the rotational freedom around the glycosidic bonds, were obtained by the HSEA program.[16,17] During these energy calculations, the φ,ψ-values of one of the glycosidic bonds were varied whereas the other glycosidic bond remained at the φ,ψ-values obtained by the GESA calculations for the trisaccharides. The same procedure was then repeated for the other glycosidic bond.

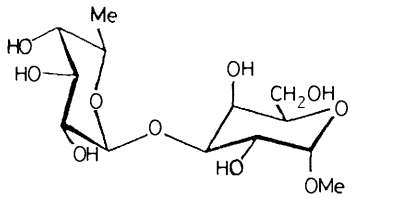
Results and Discussion

The methylglycosides of the oligosaccharides shown in the Scheme were used in the studies.

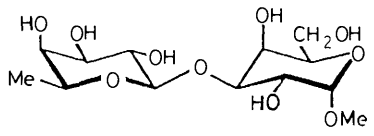
GESA and HSEA Calculations for Disaccharides (1)—(8).— The φ-, ψ-, and ω-angles and inter-residue atomic distances (< 3.5 Å) of the minimum energy conformations derived from GESA calculations of disaccharides (1)—(8) are given in Table 1. The conformational energy plots, obtained by HSEA calculations, and internuclear contacts derived from changes in φ- and ψ-angles are given in Figure 1. These contacts are not always present in the minimum energy conformer but are present for higher energy conformers, and consequently cause restriction to the rotational freedom.

In the following the phrases 'vicinal' and 'interactions' are used for calculated interatomic distances that are shorter than 3.5 Å. This may not always be the required distance for overlap of van der Waals spheres but due to the dynamics of the molecule, generating contacts in other highly populated conformations, effects may anyhow be observed in the n.m.r. spectra.

For all disaccharides, energy maps (Figure 1) are obtained in



(1)



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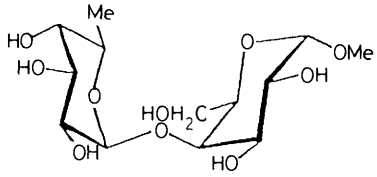

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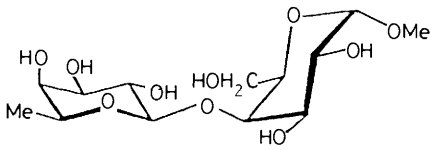

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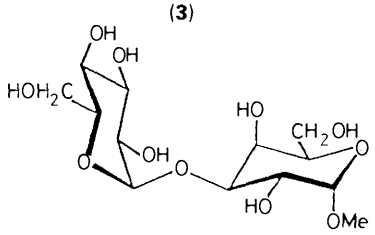

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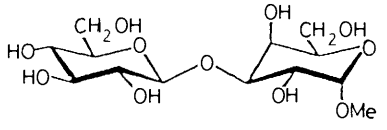

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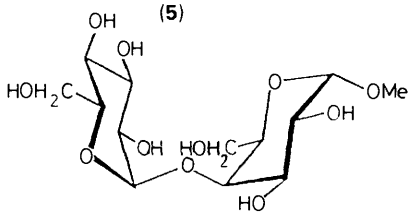

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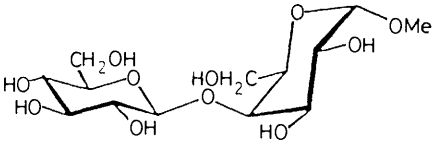

(8)

Table 1. Values for φ, ψ, and ω angles of minimum energy conformations and inter-residue atomic distances < 3.5 Å in disaccharides (**1**)—(**8**) obtained by GESA calculations

	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)
(**1**)					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)
(**2**)					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)
(**3**)					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)
(**4**)					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)
(**5**)					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)
(**6**)					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)
(**7**)					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)
(**8**)					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).

which the minimum energy conformation has a φ-angle of ~50° or ~ −50° whereas the values for ψ-angles vary between −35° and 25°. In addition to this minimum, a minimum at φ-angles of ~170° or ~ −170° was observed for the β-glycosides. However, this minimum has an energy 5—13 kJ higher than that above. From the energy maps it is concluded that the 3-linked disaccharides have a greater degree of freedom for rotation around the O(3)–C(3) bond (ψ), whereas the 4-linked disaccharides have a greater degree of freedom around the C(1′)–O(4) bond (φ).

All disaccharides have in common an interaction between 1′-H and O-5′ in the glycosyl group on one hand, and the proton on the linkage carbon (3-H or 4-H) on the other hand. In addition, the anomeric proton, 1′-H, is vicinal to one of the equatorial substituents on the carbon adjoining the linkage carbon in the methyl galactoside residue. In the 3-linked disaccharides this can be either O-2 or 4-H, and in the 4-linked disaccharides O-3 or protons in the hydroxymethyl group. Similar types of short interatomic distances are observed for α-D-,β-L-glycosides and for α-L-,β-D-glycosides. For the α-glycosides 5′-H is vicinal either to a proton [as for (**1**) and (**3**)] or to an oxygen atom [as for (**5**) and (**7**)].

^{1}H *N.m.r. Glycosylation Shifts of Disaccharides* (**1**)—(**8**).—The ^{1}H n.m.r. chemical shifts and the induced chemical shift differences (glycosylation shifts) relative to the chemical shifts of the corresponding monomers are given in Table 2.

In the glycosyl group, the chemical shift of the signal from the anomeric proton is indicative of its environment.[3,16] Thus anomeric protons which are calculated to be vicinal to oxygen atoms, as in hydroxy groups, in the minimum energy conformation, have their signals shifted downfield. If the vicinal atoms (Table 1) are protons the signals are shifted upfield. Thus, for the 4-linked disaccharides (**4**) and (7) substantial upfield shifts for the anomeric proton signals are observed corresponding to interactions between the protons on C-4 and C-6 and the anomeric proton. Somewhat smaller shifts (−0.08 and −0.12 p.p.m.) are observed for (**3**) and (**5**) in which contacts are present between the anomeric proton and 4-H in (**3**) and 3-H and 4-H in (**5**). For compound (**3**), in addition to this interaction, an interaction between 1′-H and an oxygen (O-3) at a short distance (2.46 Å) is indicated. As an upfield shift is observed it is indicated that the minimum energy conformation is not representative. For other favourable conformers with somewhat higher energy, φ- and ψ-angles have values near 0°, the proton-oxygen distance is longer and the proton-proton distance is shorter, and shielding of the anomeric proton is observed.

For α-glycosides, the signal for 5′-H is shifted upfield or downfield depending upon whether 5′-H is vicinal to a hydrogen or an oxygen atom, analogous to changes for the 1′-H signal. Downfield shifts (0.09 and 0.22 p.p.m.) are observed for the signal from 5′-H in (**5**) and (**7**). In these compounds interactions are present between 5′-H and O-2 in (**5**) and O-3 in (**7**). An analogous upfield shift (−0.08 p.p.m.) for the signal from 5′-H in compound (**3**) corresponds to proximity to 6-H.

For most of the compounds, only smaller shifts (<0.05 p.p.m.) are observed for signals from 2-, 3-, 4-, and 6-H in the glycosyl group. Exceptions are the 2′-H signals of the β-glycosides, which are shifted downfield ~0.1 p.p.m., and the 3-H signal of compounds (**1**) and (**5**).

Glycosylation shifts for the signals of the methyl galactoside residue followed the same general pattern as previously observed for 2-, 3-, and 4-linked disaccharides.[1–3] Substantial shifts are mainly observed for signals of protons on the linkage and neighbouring carbons. The shift values for the signals for the protons on the linkage carbon (3-H, 4-H) vary between 0.04 and 0.22 p.p.m., with larger shifts for the β-linked compared to the α-linked disaccharides. This corresponds to a shorter distance between the protons on the linkage carbon and O-5′ in the minimum energy conformation of the β-linked disaccharides. For compounds (**5**) and (**6**) the distances are similar and a smaller shift difference is observed. The shift values for the signals of the neighbouring protons (2-H and 4-H in the 3-linked and 3-H and 5-H in the 4-linked disaccharides) vary between −0.02 and 0.23 p.p.m. Larger shifts are observed for the signals from the 3-linked than from the 4-linked disaccharides. For the 3-linked disaccharides the signal for the proton which is '*trans*' to O-5′ in the glycosyl group (Figure 2), always has a large (>0.18 p.p.m.) downfield shift. Such effects have also been observed for other disaccharides.[1–3] These shifts probably originate from an interaction with the lone pairs of the glycosidic oxygen, different from that present in the unsubstituted monosaccharide. The shift is generally larger than that observed for the signal of the other neighbouring '*cis*'

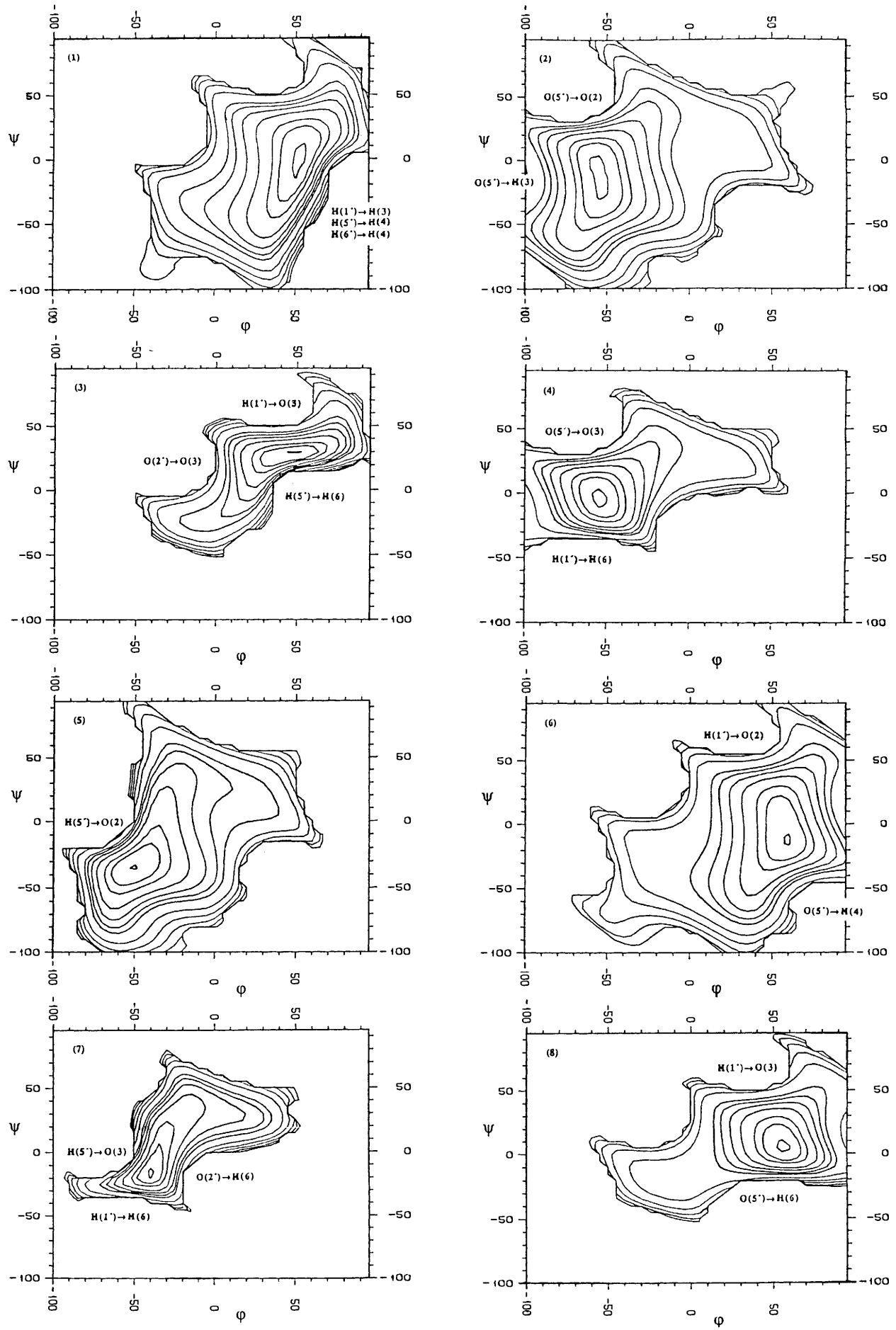

Figure 1. Conformational energy plots for disaccharides (**1**)—(**8**) with pronounced inter-residue contacts. Isocontour levels are indicated at 0.4, 2.1, 4.2, 8.4, 12.6, 16.8, 21.0, 33.6, 46.2, 58.7, 71.3, and 83.9 kJ (0.1, 0.5, 1, 2, 3, 4, 5, 8, 11, 14, 17, and 20 kcal) above the minimum energy conformation

Table 2. ^{1}H N.m.r. chemical shifts and chemical shift differences of the disaccharides (**1**)—(**8**) at 70 °C relative to internal TSP (δ_H 0.00)

	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-L-Fucp(1→3)α-D-Galp-OMe	5.16	3.80	3.94	3.82	4.18	1.22		4.86	4.03	3.85	4.07	3.92	3.73[b]	3.73[b]	3.44
(**1**)	−0.04[c]	0.03	0.08	0.01	−0.02	0.01		0.01	0.19	0.04	0.08	0.03	−0.03	−0.03	0.01
β-L-Fucp(1→3)α-D-Galp-OMe	4.50	3.57	3.67	3.76	3.79	1.27		4.90	3.96	4.00	4.19	3.89	3.78[b]	3.78[b]	3.44
(**2**)	−0.05	0.11	0.04	0.02	0.00	0.01		0.05	0.12	0.19	0.20	0.00	0.02	0.02	0.01
α-L-Fucp(1→4)α-D-Galp-OMe	5.12	3.85	3.86	3.82	4.12	1.23		4.85	3.90	3.89	4.04	3.94	3.74[b]	3.74[b]	3.42
(**3**)	−0.08	0.08	0.00	0.01	−0.08	0.02		0.00	0.06	0.08	0.05	0.05	−0.02	−0.02	−0.01
β-L-Fucp(1→4)α-D-Galp-OMe	4.34	3.56	3.65	3.76	3.81	1.27		4.86	3.82	3.79	4.16	3.98	3.83[b]	3.83[b]	3.43
(**4**)	−0.21	0.10	0.02	0.02	0.02	0.01		0.01	−0.02	−0.02	0.17	0.09	0.07	0.07	0.00
α-D-Glcp(1→3)α-D-Galp-OMe	5.11	3.59	3.80	3.44	3.93	3.85	3.76	4.87	3.98	3.91	4.21	3.88	3.76[b]	3.76[b]	3.44
(**5**)	−0.12	0.05	0.08	0.02	0.09	0.01	0.00	0.02	0.14	0.10	0.22	−0.01	0.00	0.00	0.01
β-D-Glcp(1→3)α-D-Galp-OMe	4.67	3.39	3.50	3.44	3.46	3.89	3.74	4.86	4.02	3.96	4.22	3.92	3.77[b]	3.77[b]	3.44
(**6**)	0.03	0.14	0.00	0.02	0.00	−0.01	0.02	0.01	0.18	0.15	0.23	0.03	0.01	0.01	0.01
α-D-Glcp(1→4)α-D-Galp-OMe	4.96	3.57	3.74	3.45	4.06	3.79[b]	3.79[b]	4.88	3.88	3.89	4.08	3.96	3.87[b]	3.87[b]	3.44
(**7**)	−0.27	0.03	0.02	0.03	0.22	−0.05	0.03	0.03	0.04	0.08	0.09	0.07	0.11	0.11	0.01
β-D-Glcp(1→4)α-D-Galp-OMe	4.64	3.36	3.52	3.41	3.44	3.91	3.74	4.86	3.92[b]	3.92[b]	4.21	3.93	3.83	3.76	3.43
(**8**)	0.00	0.11	0.02	−0.01	−0.02	0.01	0.02	0.01	0.08	0.11	0.22	0.04	0.07	0.00	0.00

[a] Primed labels refer to the 3-*O*- or 4-*O*-glycopyranosyl group and unprimed to the methyl galactoside residue. [b] Approximate chemical shifts due to higher order spectrum. [c] Chemical shift differences are calculated by subtraction of chemical shifts of the corresponding hexose and methyl hexoside for the glycosyl part and the aglycone, respectively, and a positive difference indicates a downfield shift.

(*a*) (*b*)

Figure 2. (*a*) Cis-, and (*b*) trans-relation of O-5′ and the hydrogen on the carbon adjacent to the linkage of the methyl glycopyranoside residue

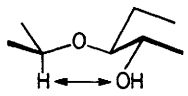

Figure 3. Interaction between the anomeric proton and the oxygen of the neighbouring equatorial hydroxy group of the methyl galactopyranoside residue

proton. The shift of this '*cis*' proton varies substantially, depending upon whether it is equatorial or axial. The larger downfield shift for the signals of 4-H in (**1**) and (**6**) could be due to the short distance between this proton and O-5′ in the minimum energy conformations. This distance is shorter in (**6**) than in (**1**), corresponding to the somewhat larger shift.

Signals for 6-H_a and 6-H_b in disaccharides (**4**) and (**7**) are shifted 0.07 and 0.11 p.p.m. downfield. This may derive from interactions of these protons with 1′-H and O-2′.

^{13}C *N.m.r. Glycosylation Shifts of Disaccharides* (**1**)—(**8**).—The ^{13}C n.m.r. chemical shifts and the chemical shift differences relative to those of the corresponding monomers are given in Table 3. In the following the shift values are given in the text with one decimal only for simplicity.

In the glycosyl group the signals for C-1′, C-2′, and C-5′ may be significantly shifted (>0.5 p.p.m.). Low values (4.4 p.p.m. and 3.0 p.p.m.) are observed for the shifts of the C-1′ signals in (**2**) and (**5**). Compared to other C-1′ signals, for which the shift is about 8 ± 2 p.p.m., they appear shifted upfield. This may be attributed to a proton-proton interaction (γ-*gauche* effect) between 1′-H and 4-H which has been described earlier.[8] The smallest shift is observed for the disaccharide that has the shortest calculated distance between 1′-H and 4-H.

The signals for C-2′ in β-linked disaccharides are shifted upfield by ~ −1 p.p.m. This is accompanied by a downfield shift of the corresponding proton signal (Table 2). Signals from C-5′ are little shifted (<0.3 p.p.m.) for the β-glycosides but are shifted downfield (0.4—1.2 p.p.m.) for the α-glycosides. This may derive from interactions of 5′-H with the lone pairs on the glycosidic oxygen and/or atoms in the opposing ring which are close to 5′-H. The shift of the C-5′ signal is small when the signal for 5′-H shifts downfield and larger when the signal for 5′-H is shifted upfield. Such effects have been attributed to bond polarisation.[24]

Significant shifts (>0.5 p.p.m.) for signals from the methyl galactoside residue are mainly observed for signals from linkage carbons or for carbons vicinal to these. In addition signals from C-2 in the 4-linked disaccharides (**3**), (**4**), (**7**), and (**8**) are shifted upfield by up to −0.9 p.p.m. This may derive from an interaction between the lone pair on the glycosidic oxygen and 2-H, different from that in the unsubstituted monosaccharide γ-*gauche* effect. The signals for C-6 in the 4-linked disaccharides (**4**) and (**7**) are shifted upfield significantly, whereas the corresponding proton signals are shifted downfield, something which has been observed for other 4-linked disaccharides.[3] This may be due to interactions between 6-H and 1′-H or 6-H and O-2′ or to a change in the rotamer distribution around the C-5)–C(6) bond. Such long-range interactions can give both positive and negative shifts,[25] *c.f.* downfield shifts for the C-5′ signals and upfield shifts caused by the γ-*gauche* effect.

Compounds (**2**) and (**5**) for which the shifts of the signal from the linkage carbon, C-3, are 7.7 and 5.1 p.p.m., respectively, have significant upfield shifts for the signal from C-4 (−2.4 and −3.6 p.p.m.) deriving from interactions between 1′-H and 4-H (the γ-*gauche* effect). For (**2**), larger downfield shifts of the signals from the linkage carbons, C-1′ and C-3, and larger upfield shifts of the signal from C-4 are observed than for compound (**5**). This corresponds to a shorter calculated distance between 1′-H and 4-H in compound (**5**), and different ψ-values[11] for the minimum energy conformation.

For disaccharides in which the methyl glycoside is glycosylated on an equatorial hydroxy group, it was observed[3] that the signal for a carbon next to the linkage was shifted upfield by ~ −1.5 p.p.m. if the oxygen substituent on this carbon had no interaction with 1′-H (calculated distance >3.5 Å, Figure 3). Shifts of *ca.* −1.5 p.p.m. are observed for the C-2 signals in (**2**) and (**5**), in good agreement with the earlier observations. However, if there is an interaction between the oxygen substituent and 1′-H the shifts are −1.5—0.5 p.p.m. with correlation between the shift and the distance. For the C-2 signals in compounds (**1**) and (**6**), shifts of −0.7 and −1.0 p.p.m.

Table 3. ^{13}C N.m.r. chemical shifts and chemical shift differences of the disaccharides (**1**)—(**8**) at 70 °C relative to internal dioxane (δ_C 67.40)

	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-L-Fuc*p*(1→3)α-D-Gal*p*-O-Me	101.52	69.40	70.51	72.71	67.90	16.15	100.33	68.44	78.62	70.26	71.54	61.94	55.96
(**1**)	8.40[b]	0.31	0.22	−0.08	0.80	−0.18	−0.02	−0.73	8.16	0.07	0.00	−0.12	0.00
β-L-Fuc*p*(1→3)α-D-Gal*p*-O-Me	101.51	71.45	73.84	72.19	71.90	16.16	100.07	67.67	78.12	67.79	71.31	61.96	55.90
(**2**)	4.36	−1.28	−0.09	−0.16	0.26	−0.17	−0.28	−1.50	7.66	−2.40	−0.23	−0.10	−0.06
α-L-Fuc*p*(1→4)α-D-Gal*p*-O-Me	102.26	69.69	70.66	72.66	68.27	16.13	100.36	69.85	71.24	80.22	71.82	62.26	56.04
(**3**)	9.14	0.60	0.37	−0.13	1.17	−0.20	0.01	0.68	0.78	10.03	0.28	0.20	0.08
β-L-Fuc*p*(1→4)α-D-Gal*p*-O-Me	104.16	71.70	73.79	72.10	71.86	16.16	100.46	70.04	69.79	79.91	71.67	61.37	56.07
(**4**)	7.01	−1.03	−0.14	−0.25	0.22	−0.17	0.11	0.87	−0.67	9.72	0.13	−0.69	0.11
α-D-Glc*p*(1→3)α-D-Gal*p*-O-Me	96.04	72.20	73.84	70.58	72.74	61.55	100.36	67.54	75.60	66.60	71.31	62.03	55.97
(**5**)	3.05	−0.27	0.06	−0.13	0.37	−0.29	0.01	−1.63	5.14	−3.59	−0.23	−0.03	0.01
β-D-Glc*p*(1→3)α-D-Gal*p*-O-Me	104.43	74.34	76.62	70.51	76.72	61.64	100.25	68.21	80.32	69.86	71.24	61.98	55.91
(**6**)	7.59	−0.86	−0.14	−0.20	−0.04	−0.20	−0.10	−0.96	9.86	−0.33	−0.30	−0.08	−0.05
α-D-Glc*p*(1→4)α-D-Gal*p*-O-Me	101.06	72.69	73.67	70.51	73.05	61.42	100.40	69.42	70.13	79.70	71.92	61.37	56.09
(**7**)	8.07	0.22	−0.11	−0.20	0.68	−0.42	0.05	0.25	−0.33	9.51	0.38	−0.69	0.13
β-D-Glc*p*(1→4)α-D-Gal*p*-O-Me	104.62	74.69	76.80	70.66	76.80	61.80	100.37	69.64	70.86	79.18	70.99	61.80	56.05
(**8**)	7.78	−0.51	0.04	−0.05	0.04	−0.04	0.02	0.47	0.40	8.99	−0.55	−0.26	0.09

[a] Primed labels refer to the 3-*O*- or 4-*O*-glycopyranosyl group and unprimed to the methyl galactoside residue. [b] Chemical shift differences are calculated by substraction of chemical shifts of the corresponding hexose and methyl hexoside for the glycosyl part and the aglycone, respectively, and a positive difference indicates a downfield shift.

are observed and the calculated distances are 3.1 and 3.4 Å.

For the axially 4-*O*-substituted derivatives (**3**), (**4**), (**7**), and (**8**), several downfield shifts for signals from neighbouring carbons are observed, contrary to the more commonly observed upfield shifts. In these compounds several inter-residue interactions at a relatively short distance are present. In compounds with no or minor interactions for substituents on neighbouring carbons, upfield shifts of ~ −0.6 p.p.m. are observed.

GESA and HSEA Calculations For Trisaccharides (**9**)—(**16**).—To make a complete search of the conformational space for trisaccharides, which have four degrees of freedom of the glycosidic bonds, φ and ψ, together with rotational freedom of the hydroxymethyl groups, ω, a large number of calculations would be required which is impractical with limited computer capacity. An iterative procedure like the one used in the GESA program is more suitable and was adopted in this study. There are two obvious disadvantages with this approach, however. If the starting torsion angles are substantially different from those in the global energy minimum, the calculations may yield a conformation of a local energy minimum. To avoid this, the iteration must be started from several different conformations. Furthermore, the rotational freedom around the glycosidic bond can not be assessed from a single final conformation obtained by the GESA calculations. However, a fair estimation of the rotational freedom of the trisaccharide can be obtained if rotation around one of the glycosidic bonds is allowed while the other bond remains at the φ,ψ-values obtained by the GESA calculations of the trisaccharides. This was performed with the HSEA program and energy maps were obtained for rotation of each glycosidic bond in the trisaccharides (Figure 4).

The φ,ψ-angles for compounds (**9**)—(**16**) and inter-residue atomic distances between the glycosyl groups and the methyl galactoside residue are given in Table 4. Short inter-residue atomic distances between the two glycosyl groups are given in Table 5. To differentiate between the glycosyl groups, that linked to O-3 is labelled with a prime and that to O-4 with a double prime. In addition to the minimum given in the Table a minimum at φ-angles of ~170° or ~ −170° was observed for the β-glycosides. However, this minimum had an energy of more than 6 kJ over that given in the Table.

Most of the trisaccharides have one or two of the glycosidic torsion angles different from the values in the corresponding disaccharide, with larger deviations generally observed for the ψ- than for the φ-angle. In all trisaccharides, including those which have their glycosidic torsion angles at or near the values of the corresponding disaccharide, atomic interactions (< 3.5 Å) between the two glycosyl groups are observed. It can be observed in the energy maps that the rotational freedom around the glycosidic bonds is restricted for the trisaccharides compared to that for the disaccharides. For all glycosyl groups linked to the 3-position the rotational freedom is restricted, as certain ψ,φ-angles give high energy conformers due to collision between the glycosyl groups. This results in a more pronounced orientation of the lone pair of the glycosidic oxygen towards the 4-*O*-glycosyl group.

For the 4-*O*-α-L- or β-D-glycosyl group in (**9**), (**11**), (**14**), and (**16**), the rotational freedom is similar to that of the corresponding disaccharide and approximately the same φ- and ψ-values are obtained in the minimum energy conformations. In compound (**16**) a contact from 6′-H to 3″-H and 5″-H for high φ- and ψ-values restricts the rotaton to some extent.

For the 4-*O*-β-L-fucosyl group in (**10**) and (**12**), several inter-residue atomic contacts restrict the rotational freedom (Figure 4). For compound (**13**) with two α-D-glucosyl groups, the φ- and ψ-values for the 4-*O*-glucosyl groups in the minimum energy conformation are changed drastically, by −35° for the φ-angle and 55° for the ψ-angle. In compound (**15**), which also has a 4-*O*-α-D-glucosyl group, the situation is similar to that in trisaccharide (**13**) but the 4-*O*-glucosyl group still has some rotational freedom around the energy minimum with φ- and ψ-angles close to those of the corresponding disaccharide (Figure 5).

In the 4-*O*-glycosyl group, mostly 1″-H or atoms on C-5″ or C-6″ interact with the 3-*O*-glycosyl group in which several hydrogen and oxygen atoms can be involved in the interactions.

^1H *N.m.r. Glycosylation Shifts of Trisaccharides* (**9**)—(**16**).—The ^1H n.m.r. chemical shifts and the chemical shift differences for compounds (**9**)—(**16**) are given in Table 6. The chemical shift differences (obs. − mono.) are obtained by comparison between experimental data for the trisaccharides and those for the appropriate monomers. The chemical shift differences (obs. − calc.) are obtained for the glycosyl groups by comparison between experimental data and those of the corresponding disaccharide. For the methyl galactoside residue, the

Table 4. Values for φ, ψ, and ω angles of the minimum energy conformations and inter-residue atomic distances <3.5 Å between the terminal groups and the methyl galactoside residue in the trisaccharides (**9**)—(**16**) obtained by GESA calculations

		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″	O-6″
α-L-Fuc*p*-(1→4)		46	26		O-2 (2.76)	3-H (3.30)	4-H (3.20)	3-H (2.53)	O-3 (2.39)	6-H (3.40)	4-H (3.33)		2-H (3.22)	4-H (2.56)	
α-D-Gal*p*-OMe	(**9**)			70	3-H (2.46)	4-H (2.42)		4-H (3.44)	4-H (2.44)		6-H_a (2.43)	6-H (2.66)			
α-L-Fuc*p*-(1→3)		55	12								6-H_b (2.28)				
β-L-Fuc*p*-(1→4)		−54	−13		O-2 (2.73)	3-H (3.30)	4-H (3.25)	3-H (2.53)	4-H (2.42)				6-H (3.15)	0.3 (3.30)	
α-D-Gal*p*-OMe	(**10**)			64	3-H (2.47)	4-H (2.45)			6-H_a (2.39)					4-H (2.52)	
α-L-Fuc*p*-(1→3)		54	13						6-H_b (2.54)						
α-L-Fuc*p*-(1→4)		39	21		3-H (2.62)				O-3 (2.55)		6-H_a (2.25)	6-H (2.52)	O-3 (3.50)	4-H (2.67)	
α-D-Gal*p*-OMe	(**11**)			74	4-H (2.51)			3-H (2.40)	4-H (2.30)		6-H_b (2.57)	2-H (3.18)	6-H (3.15)		
β-L-Fuc*p*-(1→3)		−57	−25												
β-L-Fuc*p*-(1→4)		−56	−13		O-4 (3.31)			3-H (2.3)	4-H (2.43)				6-H (3.18)	O-3 (3.30)	
α-D-Gal*p*-OMe	(**12**)			64	3-H (2.82)				6-H_a (2.54)					4-H (2.41)	
β-L-Fuc*p*-(1→3)		−58	−42		4-H (2.20)				6-H_b (2.40)						
α-D-Glc*p*-(1→4)		−16	39	−59	3-H (2.14)	O-2 (2.50)	O-2 (2.84)	O-2 (3.00)	O-3 (2.72)	6-H (3.48)			4-H (3.49)	O-3 (2.60)	
α-D-Gal*p*-OMe	(**13**)			66	4-H (3.28)	2-H (3.18)		3-H (2.95)	4-H (2.10)		2-H (2.34)	2-H (3.36)	6-H_a (3.39)	2-H (3.30)	
α-D-Glc*p*-(1→3)		−35	4	−56									6-H_b (3.43)		
β-D-Glc*p*-(1→4)		54	7	−54	3-H (2.74)	O-2 (2.67)		3-H (2.48)	O-3 (2.92)			6-H (3.20)		4-H (2.49)	6-H (3.31)
α-D-Gal*p*-OMe	(**14**)			63	4-H (2.26)	3-H (2.80)			4-H (2.37)					6-H_a (2.70)	
α-D-Glc*p*-(1→3)		−55	−40	−56	O-4 (3.44)									6-H_b (2.92)	
α-D-Glc*p*-(1→4)		−42	−22	−61	O-2 (3.38)			3-H (2.74)	4-H (2.36)		O-3 (2.44)		6-H (2.73)	4-H (2.60)	
α-D-Gal*p*-OMe	(**15**)			63	3-H (2.30)			4-H (2.74)	6-H_a (2.22)		4-H (3.27)				
β-D-Glc*p*-(1→3)		51	−10	−53					6-H_b (2.37)						
β-D-Glc*p*-(1→4)		54	18	−54	3-H (2.38)		4-H (3.29)	3-H (2.70)	O-3 (2.61)					4-H (2.41)	4-H (3.43)
α-D-Gal*p*-OMe	(**16**)			66				4-H (2.43)	4-H (2.48)					6-H_a (2.99)	
β-D-Glc*p*-(1→3)		61	−21	−55										6-H_b (3.34)	

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

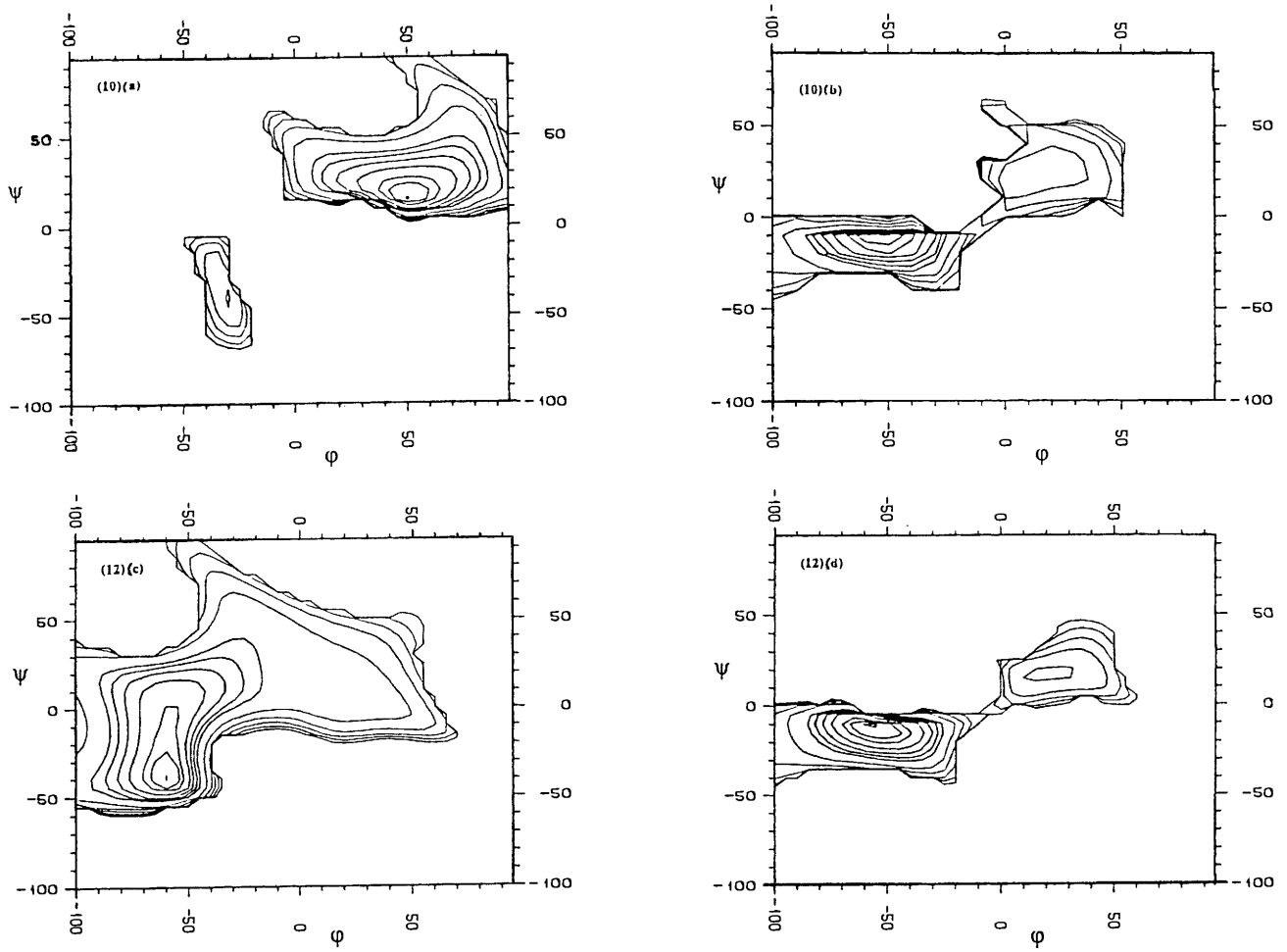

Figure 4. Conformational energy plots for trisaccharides (**10**) (*a*,*b*) and (**12**) (*c*,*d*). Rotation of the 3-*O*-fucosyl group is shown in (*a*) and (*c*) and of the 4-*O*-fucosyl group in (*b*) and (*d*). Comparison should be made with plots in Figure 1 as the following: (*a*) with (**1**), (*b*,*d*) with (**4**) and (*c*) with (**2**). Isocontour levels are as depicted in Figure 1

Table 5. Interatomic distances <3.5 Å between the 3-*O*-glycosyl group and the 4-*O*-glycosyl group in the minimum energy conformation of trisaccharides (**9**)—(**16**) 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′
(**9**)			1″-H (2.29)[a]		O-5″ (3.27) 1″-H (2.29)		1″-H (2.39)			
(**10**)			O-5″ (2.96) 6″-H_a (3.21) 6″-H_b (2.39)	6″-H_a (2.49) 6″-H_b (2.66)	O-5″ (2.56) 6″-H_a (2.39) 6″-H_b (3.07)	6″-H (2.69)	O-5″ (3.30)			
(**11**)	1″-H (2.64)						O-2″ (3.50) 1″-H (2.55)	1″-H (2.55)		
(**12**)	O-5″ (2.52) 6″-H_a (2.85) 6″-H_b (3.35)		6″-H_a (2.72) 6″-H_b (2.73)				O-5″ (3.30)	O-4″ (3.48) O-5″ (3.02) 6″-H (2.55)		
(**13**)			O-5″ (2.56) O-6″ (2.65) 6″-H (2.44)		6″-H (2.57)		O-5″ (2.60) 1″-H (2.72)	O-5″ (3.28) 1″-H (2.64)		
(**14**)	1″-H (2.56)						1″-H (2.92)	O-2″ (3.40) 1″-H (2.49)		
(**15**)		5″-H (2.52) 6″-H_a (2.35) 6″-H_b (2.59)		6″-H_a (2.32) 6″-H_b (3.48)			5″-H (2.44)		5″-H (3.35) 6″-H (2.82)	6″-H (2.76)
(**16**)		1″-H (2.68)					1″-H (2.61)		1″-H (2.81)	5″-H (2.71) 6″-H (2.92)

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

Table 6. ^{1}H N.m.r. data of the trisaccharides (**9**)—(**16**) at 70 °C relative to internal TSP (δ_H 0.00)

		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-L-Fuc*p*-(1→4)	(obs.)	5.19	3.82	3.80	3.83	4.20	1.22		5.40	3.80	3.87	3.83	4.00	1.24		4.89	4.22	4.03	4.15	4.03	3.75[c]	3.75[c]	3.45
α-D-Gal*p*-O-Me (**9**)	(obs. − mono.)[b]	−0.01	0.05	−0.06	0.02	0.00	0.01		0.20	0.03	0.01	0.02	−0.20	0.03		0.04	0.38	0.22	0.16	0.14	−0.01	−0.01	0.02
α-L-Fuc*p*-(1→3)	(obs. − calc.)[b]	0.03	0.02	−0.14	0.01	0.02	0.00		0.28	−0.05	0.01	0.01	−0.12	0.01		0.03	0.13	0.10	0.03	0.06	0.04	0.04	0.02
β-L-Fuc*p*-(1→4)	(obs.)	5.23	3.80	3.94	3.81	4.27	1.22		4.36	3.54	3.63	3.73	3.72	1.24		4.88	4.11	3.95	4.19	3.99	3.86[c]	3.86[c]	3.44
α-D-Gal*p*-O-Me (**10**)	(obs. − mono.)	0.03	0.03	0.08	0.00	0.07	0.01		−0.19	0.08	0.00	−0.01	−0.07	−0.02		0.03	0.27	0.14	0.20	0.10	0.10	0.10	0.01
α-L-Fuc*p*-(1→3)	(obs. − calc.)	0.07	0.00	0.00	−0.01	0.09	0.00		0.02	−0.02	−0.02	−0.03	−0.09	−0.03		0.01	0.10	0.12	−0.05	−0.02	0.06	0.06	0.00
α-L-Fuc*p*-(1→4)	(obs.)	4.51	3.56	3.66	3.76	3.79	1.27		5.38	3.80	3.87	3.83	4.06	1.23		4.93	4.10	4.11	4.25	3.96	3.76[c]	3.76[c]	3.44
α-D-Gal*p*-O-Me (**11**)	(obs. − mono.)	−0.04	0.10	0.03	0.02	0.00	0.01		0.18	0.03	0.01	0.02	−0.14	0.02		0.08	0.26	0.30	0.26	0.07	0.00	0.00	0.01
β-L-Fuc*p*-(1→3)	(obs. − calc.)	0.01	−0.01	−0.01	0.00	0.00	0.00		0.26	−0.05	0.01	0.01	−0.06	0.00		0.03	0.08	0.03	0.01	0.02	0.00	0.00	0.01
β-L-Fuc*p*-(1→4)	(obs.)	4.54	3.57	3.66	3.76	3.80	1.27		4.46	3.55	3.63	3.73	3.76	1.29		4.93	4.00	4.04	4.41	3.94	3.85[c]	3.85[c]	3.44
α-D-Gal*p*-O-Me (**12**)	(obs. − mono.)	−0.01	0.11	0.03	0.02	0.01	0.01		−0.09	0.11	0.00	−0.01	−0.03	0.03		0.08	0.16	0.23	0.42	0.05	0.09	0.09	0.01
β-L-Fuc*p*-(1→3)	(obs. − calc.)	0.04	0.00	−0.01	0.00	0.01	0.00		0.12	−0.01	−0.02	−0.03	−0.05	0.02		0.02	0.06	0.06	0.05	−0.04	0.00	0.00	0.00
α-D-Glc*p*-(1→4)	(obs.)	5.16	3.55	3.76	3.44	3.97	3.85	3.74	5.07	3.56	3.76	3.46	3.86	3.83[c]	3.83[c]	4.91	4.05	3.98	4.35	3.93	3.89[c]	3.89[c]	3.44
α-D-Gal*p*-O-Me (**13**)	(obs. − mono.)	−0.07	0.01	0.04	0.02	0.13	0.01	−0.02	−0.16	0.02	0.04	0.04	0.02	−0.01	0.07	0.06	0.21	0.17	0.36	0.04	0.13	0.13	0.01
α-D-Glc*p*-(1→3)	(obs. − calc.)	0.05	−0.04	−0.04	0.00	0.04	0.00	−0.02	0.11	−0.01	0.02	0.01	−0.20	0.04	0.04	0.01	0.03	−0.01	0.05	−0.02	0.02	0.02	−0.01
β-D-Glc*p*-(1→4)	(obs.)	5.15	3.60	3.78	3.46	4.01	3.86	3.76	4.77	3.33	3.44	3.40	3.44	3.92	3.74	4.88	4.12	4.00	4.40	3.91	3.83	3.75	3.44
α-D-Gal*p*-O-Me (**14**)	(obs. − mono.)	−0.08	0.06	0.06	0.04	0.17	0.02	0.00	0.13	0.08	−0.06	−0.02	−0.02	0.02	0.02	0.03	0.28	0.19	0.41	0.02	0.07	−0.01	0.01
α-D-Glc*p*-(1→3)	(obs. − calc.)	0.04	0.01	−0.02	0.02	0.08	0.01	0.00	0.13	−0.03	−0.08	−0.01	0.00	0.01	0.00	0.00	0.06	−0.02	−0.03	−0.01	0.00	−0.01	0.00
α-D-Glc*p*-(1→4)	(obs.)	4.65	3.29	3.51	3.40	3.44	3.92	3.74	5.00	3.53	3.77	3.52	4.16	3.84[c]	3.84[c]	4.90	4.10	4.02	4.29	4.00	3.85[c]	3.85[c]	3.44
α-D-Gal*p*-O-Me (**15**)	(obs. − mono.)	0.01	0.04	0.01	−0.02	−0.02	0.02	0.02	−0.23	−0.01	0.05	0.10	0.32	0.00	0.08	0.05	0.26	0.21	0.30	0.11	0.09	0.09	0.01
β-D-Glc*p*-(1→3)	(obs. − calc.)	−0.02	−0.10	0.01	−0.04	−0.02	0.03	0.00	0.04	−0.04	0.03	0.07	0.10	0.05	0.05	0.01	0.04	−0.02	−0.02	0.01	−0.03	−0.03	−0.01
β-D-Glc*p*-(1→4)	(obs.)	4.67	3.38	3.52	3.42	3.47	3.92	3.73	4.84	3.34	3.49	3.42	3.40	3.92	3.73	4.88	4.12	4.05	4.43	3.95	3.80	3.72	3.44
α-D-Gal*p*-O-Me (**16**)	(obs. − mono.)	0.03	0.13	0.02	0.00	0.01	0.02	0.01	0.20	0.09	−0.01	0.00	−0.06	0.02	0.01	0.03	0.28	0.24	0.44	0.06	0.04	−0.04	0.01
β-D-Glc*p*-(1→3)	(obs. − calc.)	0.00	−0.01	0.02	−0.02	0.01	0.03	−0.01	0.20	−0.02	−0.03	0.01	−0.04	0.01	−0.01	0.01	0.02	−0.02	−0.01	−0.01	−0.04	−0.05	0.00

[a] Primed labels refer to the 3-*O*-glycosyl group 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 spectrum.

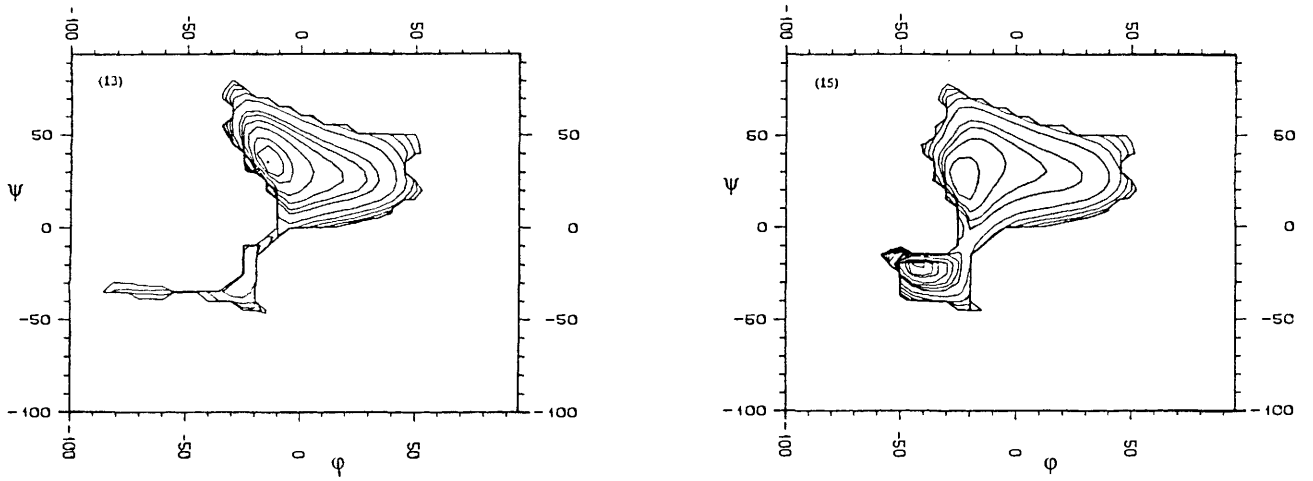

Figure 5. Conformational energy plots for trisaccharides (**13**) and (**15**) with rotation around the glycosidic linkage of the 4-*O*-α-D-glucopyranosyl group. Isocontour levels are as depicted in Figure 1

comparison is made with chemical shifts obtained for the monomer to which two sets of chemical shift differences (glycosylation shifts) are added, namely those deriving from the two appropriate disaccharides (Table 2). Thus a comparison of observed chemical shifts with those derived from pure additivity of glycosylation shifts is made. As a guidance to the interpretation of Table 6, the following should be observed: a small value of (obs. − calc.) implies that no changes in the interactions have taken place: a large value for (obs. − calc.) indicates that new or stronger interactions are present in the trisaccharide.

For signals from the 3-*O*-glycosyl group, good agreement (obs. − calc. <0.06 p.p.m.) is obtained with calculated values except for siganls for protons which have interactions with atoms in the 4-*O*-glycosyl group or with changed interactions to atoms in the methyl galactoside residue. The latter may be caused by different conformations around the glycosidic bond in the trisaccharides compared to those of the corresponding disaccharides. Interactions of 3′-H in (**9**) and 2′-H in (**15**) with protons of the other glycosyl group are observed and the corresponding signals are shifted −0.14 and −0.10 p.p.m., respectively. The signal for anomeric protons in the 3-*O*-fucosyl group is only substantially changed (>0.05 p.p.m.) in (**10**). In the calculated minimum energy conformation, 1′-H has an increased interaction with O-2 in the galactosyl residue which could account for the shift. For compound (**9**) the same effect, but smaller, is observed.

The signal for 5′-H in (**10**) is shifted 0.09 p.p.m. downfield, corresponding to a contact between 5′-H and O-5″ (2.56 Å). In compound (**14**), there is an extra downfield shift of 0.08 p.p.m. for the signal from 5′-H which could not be correlated to changed interactions in the minimum energy conformations. In the energy map, however, (Figure 6), restricted rotation of the glycosidic bond is indicated. This restriction increases the population of conformers with $\varphi < -50°$, in which there are contacts between 5′-H and O-2.

The 4-*O*-glycosyl groups give several signals that experience extra substituent shifts. The largest deviations from calculated values are found for signals from 1″-H and 5″-H. The signals for 1″-H in compounds (**9**), (**11**), (**14**), and (**16**) (α-L-/β-D-) shift downfield by 0.13—0.28 p.p.m. The observed downfield shift may be due to the enhanced contact between 1″-H and the lone pair on the adjacent O-3 (see GESA calculations) and/or to the contacts with oxygen atoms in the 3-*O*-glycosyl group (Table 5). Some additional contacts with protons do not seem to have any effect or are weaker than the effects obtained for contacts with vicinal oxygen atoms.

In compounds (**10**) and (**15**), 1″-H had no new interactions and the chemical shifts of the corresponding signals are close to the calculated values. The signal for 1″-H in (**12**) and (**13**) shifts ~0.1 p.p.m. downfield relative to the calculated values. There are new interactions to both O-2′ and O-3 and (O-1′ in Table 5) in (**13**) but no pronounced short distances can explain the shift in (**12**). However, high energies for conformers with ψ-values >0°, and consequently restricted rotation, are indicated in the energy map (Figure 4). The signal from 5″-H in all fucose containing trisaccharides, (**9**)—(**12**), has an additional upfield shift from −0.05 to −0.12 p.p.m. For the 4-*O*-α-L-fucosyl derivatives (**9**) and (**11**), this can be explained by proximity to protons in the hydroxymethyl group. For the 4-*O*-β-L-fucosyl derivatives (**10**) and (**12**), no short inter-residue atomic distances correspond to the upfield shifts. However, the shifts could be due to the restricted rotation around the glycosidic bond as high energy conformers with ψ-values >0° are indicated in the energy maps. The same conformers had lower energies for the corresponding disaccharide (**4**) and consequently were more populated.

The upfield shift of the signal for 5″-H in (**13**), −0.20 p.p.m., correlates with the GESA-minimised conformation in which the short distance to O-3, 2.40 Å, which gave a downfield shift of 0.22 p.p.m. in the disaccharide, is increased to >3.5 Å in the trisaccharide which decreases the deshielding effect from O-3. In compound (**15**) a downfield shift of 0.1 p.p.m. for the 5″-H signal correlates well with increased contact with the lone pairs of O-3 due to restricted rotation of the glycosidic bond at O-3 (Figure 7).

In the methyl galactoside residue, the calculated chemical shifts are generally in good agreement with the observed shifts, especially for the glucose containing trisaccharides (**13**)—(**16**). Values for the fucose containing compounds (**9**)—(**12**) deviates somewhat more from the calculated shifts. In most cases there is a larger downfield shift than that calculated, *e.g.* ~0.1 p.p.m. for the 2-H signal. This could be due to a different contact to O-3 and O-4 with their oriented lone pairs in comparison to the contact in the corresponding disaccharides.

The signals for 3-H in the 3-*O*-α-L-fucosyl trisaccharides (**9**) and (**10**) shift downfield ~0.1 p.p.m. corresponding to a shorter distance between 3-H and O-5′ in the minimum energy conformation. For compound (**13**), which has a significantly changed conformation, only minor deviations from calculated values are observed.

^{13}C *N.m.r. Glycosylation Shifts of Trisaccharides* (**9**)—(**16**).—The ^{13}C n.m.r. chemical shifts and the chemical shift differences,

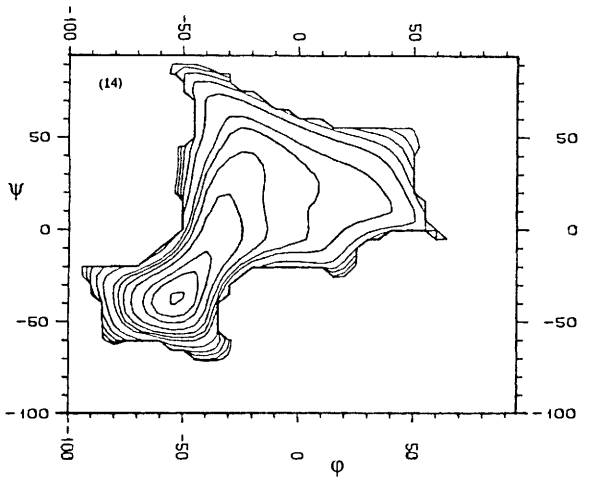

Figure 6. Conformational energy plots for trisaccharide (**14**) with rotation around the glycosidic linkage of the 3-*O*-β-D-glucopyranosyl group. Isocontour levels are as depicted in Figure 1

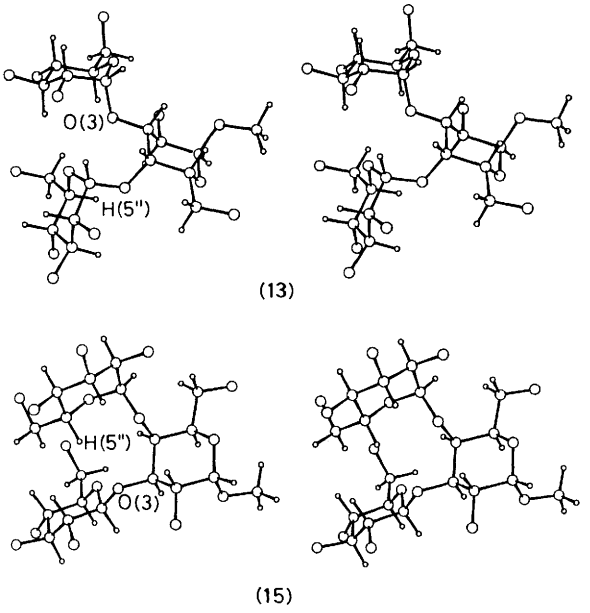

Figure 7. Stereoplots of the minimum energy conformation of trisaccharides (**13**) and (**15**) as derived by GESA calculations

obtained as described earlier for ^{1}H n.m.r. glycosylation shifts, are given in Table 7.

The glycosylation shifts for signals from carbons involved in the glycosidic linkages have the largest deviations from the calculated values. The shifts observed for signals from the 1,4-linkage, *i.e.* C-1″ and C-4, generally deviate more from the calculated values than do the shifts for the corresponding 1,3-linkage. Large additional upfield shifts from −1.0 to −2.8 p.p.m. are observed for the signal from C-1″ in (**9**), (**11**), (**14**), and (**16**) (α-L/β-D). All these compounds have the signal for 1″-H shifted downfield (Table 6). An upfield shift of −1.0 p.p.m., but without the corresponding shift of the 1″-H signal, is observed for (**15**).

For the C-4 signal in the methyl galactoside residue there is an upfield shift of −0.9 to −5.0 p.p.m., except for the signal for (**13**) for which a downfield shift of 1.7 p.p.m. is observed. These shifts can not be rationalised using these models. The downfield shift for the C-4 signal of (**13**) can be explained by a calculated longer distance between 1′-H and 4-H (Table 1), as discussed below.

Additional downfield shifts of 0.4—1.1 p.p.m. for the C-1′ signals of the 3-*O*-glycosyl groups are observed for compound (**11**)—(**14**) (β-L/α-D). In the corresponding disaccharides (**2**) and (**5**), a γ-*gauche* interaction was indicated between 1′-H and 4-H (Table 1) and only small glycosylation shifts were observed (Table 3). Larger glycosylation shifts were observed for the trisaccharides for which weaker interactions were indicated, *i.e.* longer distances between these protons. For compound (**13**) the calculated distance is longer and thus corresponds to the increased glycosylation shift for the C-1′ signal. However, shorter distances are present in the calculated minimum energy conformations of compounds (**11**), (**12**), and (**14**). The energy minima are, however, close to an 'energy wall' and it can be assumed that the averaged molecule is shifted away. The allowed regions have conformers with increased 1′-H–4-H distances supporting the observation of larger glycosylation shifts than those observed for the corresponding disaccharides.

In the methyl galactoside residue, large upfield shifts of −0.9—−2.8 p.p.m. for the C-3 signals relative to calculated values are observed for the α-L-/β-D-compounds (**9**), (**10**), (**15**), and (**16**). These compounds have φ- and ψ-angles for the 1,3-linkage similar to those in the disaccharides (**1**) and (**6**). However, restricted rotational freedom for the 1,3-glycosidic bond is indicated by the energy maps causing the averaged molecule to shift away from the position of the minimum energy conformation, possibly causing the observed shifts.

Signals for C-5 only vary significantly in the 4-*O*-glycosyl group. Upfield shifts are observed for all compounds except for the α-D/β-L-substituted (**12**) and (**13**). The largest changes are 0.6 and −0.6 p.p.m. for (**13**) and (**15**), respectively.

In compound (**10**), 6″-H has a large number of contacts with protons in the 3-*O*-fucosyl group, probably causing the 0.6 p.p.m. downfield shift of the C-6″ signal.

Effects of Temperature Variation on Chemical Shifts.—The temperature shifts for disaccharides have been reported [1,3,4] and were also used to aid the assignment of the ^{13}C n.m.r. spectrum of stachyose.[4]

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 8. Most signals are shifted downfield with reference to the internal standard, dioxane, to which a constant chemical shift of δ 67.40 is assigned. In the disaccharides the largest shifts are observed for signals from C-4 and C-6 of the D-glucopyranosyl groups and for C-3 of the L-fucopyranosyl groups. As only small shift differences are observed for the C-6 signal of the methyl galactoside residue, differentiation between the C-6 signals from gluco- and galacto-pyranosyl residues can be performed. This shift difference was used for the assignment of signals from C-6 and C-6′ in disaccharide (**7**).

The signals from the anomeric carbons in the glycosyl groups of the disaccharides can be shifted either downfield or upfield. Substantial upfield shifts (~ −0.2 p.p.m.) are observed for signals from the carbons involved in the 3-*O*-α-L- and β-D-linkages, whereas almost no shifts are observed for the corresponding signals from the 4-*O*-α-L- and β-D-linkages. For the signals from the linkage carbons in the 3-*O*- and 4-*O*-β-L- and α-D-linkages downfield shifts are observed. For these compounds smaller shifts are generally observed for the 1,4-linkages.

The pattern of temperature shifts observed for the trisaccharides is different from that of the corresponding disaccharides, especially with reference to signals from linkage carbons. These shifts are dependent upon the type of 3-*O*- and 4-*O*-glycosyl group. Large up- or down-field shifts for signals from anomeric carbons in the glycosyl groups are, in most cases, accompanied by similar shifts of the corresponding linkage carbons. For example for signals from C-1′ — C-3 and C-1″ —

Table 7. ^{13}C N.m.r. data of the trisaccharides (**9**)—(**16**) at 70 °C relative to internal dioxane (δ_C (67.40)

			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-L-Fuc*p*-(1→4)		(obs.)	101.35	69.20	70.65	72.64	68.11	16.13	99.42	69.48	70.54	72.67	67.84	16.13	100.46	69.38	78.27	75.30	72.49	62.08	56.06
α-D-Gal*p*-O-Me	(**9**)	(obs. − mono.)[b]	8.23	0.11	0.36	−0.15	1.01	−0.20	6.30	0.39	0.25	−0.12	0.74	−0.20	0.11	0.21	7.81	5.11	0.95	0.02	0.10
α-L-Fuc*p*-(1→3)		(obs. − calc.)[b]	−0.17	−0.20	0.14	−0.07	0.21	−0.02	−2.84	−0.21	−0.12	0.01	−0.43	0.00	0.12	0.26	−1.13	−4.99	0.67	−0.06	0.02
β-L-Fuc*p*-(1→4)		(obs.)	100.95	69.37	70.34	72.81	67.62	16.06	104.08	72.17	73.79	72.27	71.54	16.74	100.40	69.43	75.10	77.49	72.39	60.95	56.08
α-D-Gal*p*-O-Me	(**10**)	(obs. − mono.)	7.83	0.28	0.05	0.02	0.52	−0.27	6.93	−0.56	−0.14	−0.08	−0.10	0.41	0.05	0.26	4.64	7.30	0.85	−1.11	0.12
α-L-Fuc*p*-(1→3)		(obs. − calc.)	−0.57	−0.03	−0.17	0.10	−0.28	−0.09	−0.08	0.47	0.00	0.17	−0.32	0.58	−0.04	0.12	−2.85	−2.49	0.72	−0.30	0.01
α-L-Fuc*p*-(1→4)		(obs.)	102.26	71.48	73.91	72.19	71.90	16.17	100.46	69.54	70.47	72.63	68.07	16.10	100.10	68.01	79.10	75.40	72.15	62.24	55.97
α-D-Gal*p*-O-Me	(**11**)	(obs. − mono.)	5.11	−1.25	−0.02	−0.16	0.26	−0.16	7.34	0.45	0.18	−0.16	0.97	−0.23	−0.25	−1.16	8.64	5.21	0.61	0.18	0.01
β-L-Fuc*p*-(1→3)		(obs. − calc.)	0.75	0.03	0.07	0.00	0.00	0.01	−1.80	−0.15	−0.19	−0.03	−0.20	−0.03	0.02	−0.34	0.20	−2.42	0.56	0.08	−0.01
β-L-Fuc*p*-(1→4)		(obs.)	102.56	71.56	73.91	72.22	72.01	16.39	103.75	72.17	73.80	72.26	71.94	16.16	99.96	67.92	77.84	74.76	71.87	61.15	55.98
α-D-Gal*p*-O-Me	(**12**)	(obs. − mono.)	5.41	−1.17	−0.02	−0.13	0.37	0.06	6.60	−0.56	−0.13	−0.09	0.30	−0.17	−0.39	−1.25	7.38	4.57	0.33	−0.91	0.02
β-L-Fuc*p*-(1→3)		(obs. − calc.)	1.05	0.11	0.07	0.03	0.11	0.23	−0.41	0.47	0.01	0.16	0.08	0.00	−0.22	−0.62	0.39	−2.75	0.43	−0.12	−0.03
α-D-Glc*p*-(1→4)		(obs.)	96.88	72.62	74.21	70.54	72.76	61.53	101.40	72.84	73.52	70.54	73.62	61.64	100.42	67.99	75.71	77.80	71.95	61.80	56.04
α-D-Gal*p*-O-Me	(**13**)	(obs. − mono.)	3.89	0.15	0.43	−0.17	0.39	−0.31	8.41	0.37	−0.26	−0.17	1.25	−0.20	0.07	−1.18	5.25	7.61	0.41	−0.26	0.08
α-D-Glc*p*-(1→3)		(obs. − calc.)	0.84	0.42	0.37	−0.04	0.02	0.02	0.34	0.15	−0.15	0.03	0.57	0.22	0.01	0.20	0.44	1.69	0.25	0.46	−0.06
β-D-Glc*p*-(1→4)		(obs.)	96.43	72.57	74.01	70.66	72.60	61.60	103.58	74.39	76.85	70.92	76.62	61.99	100.49	67.84	75.66	74.69	71.05	61.71	56.03
α-D-Gal*p*-O-Me	(**14**)	(obs. − mono.)	3.44	0.10	0.23	−0.05	0.23	−0.24	6.74	−0.81	0.09	0.21	−0.14	0.15	0.14	−1.33	5.20	4.50	−0.49	−0.35	0.07
α-D-Glc*p*-(1→3)		(obs. − calc.)	0.39	0.38	0.17	0.08	−0.14	0.05	−1.04	−0.30	0.05	0.26	−0.18	0.19	0.11	−0.17	−0.34	−0.90	0.29	−0.06	−0.03
β-D-Glc*p*-(1→4)		(obs.)	105.12	74.48	76.77	70.76	76.74	61.86	100.04	72.83	73.64	70.31	72.41	61.30	100.28	68.82	79.06	77.59	72.30	61.06	56.02
α-D-Gal*p*-O-Me	(**15**)	(obs. − mono.)	8.28	−0.72	0.01	0.05	−0.02	0.02	7.05	0.36	−0.14	−0.40	0.04	−0.54	−0.07	−0.35	8.60	7.40	0.76	−1.00	0.06
α-D-Glc*p*-(1→3)		(obs. − calc.)	0.69	0.14	0.15	0.25	0.02	0.22	−1.02	0.14	−0.03	−0.20	−0.64	−0.12	−0.02	0.36	−0.93	−1.78	0.68	−0.28	−0.02
β-D-Glc*p*-(1→4)		(obs.)	104.77	74.52	76.61	70.79[c]	76.83	61.95	103.29	74.52	76.87	70.84[c]	76.65	61.95	100.31	68.54	79.76	77.26	70.96	61.71	56.02
α-D-Gal*p*-O-Me	(**16**)	(obs. − mono.)	7.93	−0.68	−0.15	0.08	0.07	0.11	6.45	−0.68	0.11	0.13	−0.11	0.11	−0.04	−0.63	9.30	7.07	−0.58	−0.35	0.06
β-D-Glc*p*-(1→3)		(obs. − calc.)	0.34	0.18	−0.01	0.33	0.11	0.31	−1.33	−0.17	0.07	0.13	−0.15	0.15	0.04	−0.14	−0.96	−1.59	0.27	−0.01	0.02

[a] Primed labels refers 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] Assignment may be reversed.

Table 8. Chemical shift differences in p.p.m. for oligosaccharides (**1**)—(**16**) 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**)	−0.17	0.09	0.21	0.07	−0.04	−0.04							0.12	0.19	−0.10	0.06	−0.05	−0.01	0.08
(**2**)	0.11	0.10	0.17	0.06	0.04	−0.05							0.14	0.07	0.18	0.17	−0.02	0.01	0.09
(**3**)							−0.01	0.13	0.19	0.07	−0.08	−0.04	0.10	0.14	0.07	−0.04	0.08	0.02	0.06
(**4**)							0.11	0.08	0.20	0.06	0.07	−0.06	0.10	0.17	0.08	0.08	−0.05	0.01	0.06
(**5**)	0.23	0.04	0.15	0.25	0.16	0.29							0.06	0.14	0.36	0.23	−0.04	0.02	0.11
(**6**)	−0.16	0.16	0.21	0.25	0.13	0.28							0.15	0.15	−0.16	0.09	0.00	0.00	0.08
(**7**)							0.03	0.07	0.12	0.32	0.23	0.33	0.07	0.25	0.22	0.23	−0.03	0.12	0.06
(**8**)							0.02	0.12	0.09	0.22	0.14	0.20	0.09	0.14	0.09	−0.03	0.05	−0.01	0.07
(**9**)	0.04	0.12	0.16	0.07	−0.01	−0.04	0.27	0.08	0.23	0.06	−0.02	−0.07	0.03	0.12	0.20	0.51	0.01	0.03	0.05
(**10**)	0.20	0.10	0.23	0.07	−0.02	−0.06	0.03	0.17	0.22	0.08	0.05	−0.12	0.06	0.08	0.74	0.01	−0.05	0.02	0.03
(**11**)	0.37	0.03	0.17	0.05	0.06	−0.04	0.21	0.15	0.25	0.06	−0.01	−0.05	0.09	0.15	0.33	0.58	0.01	−0.02	0.06
(**12**)	0.01	0.05	0.14	0.06	0.02	−0.08	−0.03	0.10	0.19	0.07	0.00	−0.07	0.12	0.09	−0.02	0.06	0.01	0.14	0.08
(**13**)	0.53	0.04	0.11	0.21	0.16	0.30	−0.26	0.18	0.22	0.33	0.08	0.22	0.04	0.28	0.78	−0.39	0.10	−0.13	0.12
(**14**)	0.48	0.03	0.14	0.32	0.18	0.30	−0.07	0.17	0.15	0.24	0.08	0.25	0.08	0.22	0.62	0.02	0.06	−0.04	0.07
(**15**)	−0.08	0.10	0.18	0.21	0.10	0.26	−0.02	0.17	0.19	0.41	0.15	0.43	0.10	0.17	0.08	0.06	−0.07	0.07	0.06
(**16**)	−0.07	0.18	0.19	0.21	0.09	0.23	0.09	0.18	0.17	0.21	0.11	0.23	0.11	0.15	−0.01	0.10	0.03	−0.01	0.07

[a] $\Delta\delta = \delta(70\ °C) - \delta(30\ °C)$. Dioxane was taken as δ 67.40 p.p.m. for all temperatures.

C-4 in (**13**) and (**16**) the observed values are 0.53—0.78 p.p.m., −0.26——0.39 p.p.m. for (**13**), and −0.07——0.01 p.p.m. and 0.09—0.10 p.p.m. for (**16**).

Conclusions

From the investigation of the di- and tri-saccharides (**1**)—(**16**) it is concluded that a typical set of substituent shifts in ^1H and ^{13}C n.m.r. spectroscopy is obtained on glycosylation at the 3- and/or 4-position of a galactopyransyl residue. The shifts depend mainly upon the stereochemistry around the glycosidic linkages. The ^1H n.m.r. shifts could be explained for all compounds by different interactions over the glycosidic bonds but for the ^{13}C n.m.r. shifts only few satisfactory correlations between glycosylation shifts and the atomic interactions could be found for the trisaccharides. In the simulation of n.m.r. spectra of oligo- and poly-saccharides containing vicinally disubstituted branching sugar residues using the computer program CASPER,[5,26] glycosylation shifts obtained from authentic trisaccharides must be used instead of using only values from each disaccharide element. The effects of temperature variation show that the same temperature must be used for all measurements if comparison of chemical shifts should be performed as large temperature shifts, often with different signs, of −0.39—0.78 p.p.m. are obtained.

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References

1 P.-E. Jansson, L. Kenne, and E. Schweda, *J. Chem. Soc., Perkin Trans. 1*, 1988, 2729.
2 H. Baumann, P.-E. Jansson, and L. Kenne, *J. Chem. Soc., Perkin Trans. 1*, 1988, 209.
3 I. Backman, B. Erbing, P.-E. Jansson, and L. Kenne, *J. Chem. Soc., Perkin Trans. 1*, 1988, 889.
4 M. Forsgren, P.-E. Jansson, and L. Kenne, *J. Chem. Soc., Perkin Trans. 1*, 1985, 2383.
5 P.-E. Jansson, L. Kenne, and G. Widmalm, *Carbohydr. Res.*, 1987, **168**, 67.
6 R. U. Lemieux and K. Bock, *Arch. Biochem. Biophys.*, 1983, **221**, 125.
7 K. Bock, *Pure Appl. Chem.*, 1983, **55**, 605.
8 G. M. Lipkind and N. K. Kochetkov, *Bioorg. Khim.*, 1984, **10**, 1129.
9 I. Tvaroska and S. Pérez, *Carbohydr. Res.*, 1986, **149**, 389.
10 G. M. Lipkind, S. S. Mamyan, A. S. Shashkov, O. A. Nechaev, V. I. Torgov, V. N. Shibaev, and N. K. Kochetkov, *Bioorg. Khim.*, 1988, **14**, 340.
11 K. Bock, A. Brignole, and B. W. Sigurskjold, *J. Chem. Soc., Perkin Trans. 2*, 1986, 1711.
12 P.-E. Jansson, L. Kenne, and T. Wehler, *Carbohydr. Res.*, 1988, **179**, 359.
13 H. Baumann, B. Erbing, P.-E. Jansson, and L. Kenne, *J. Chem. Soc., Perkin Trans. 1*, preceding paper.
14 A. Bax and G. Drobny, *J. Magn. Reson.*, 1985, **61**, 306.
15 H. Paulsen, T. Peters, V. Sinnwell, R. Lebuhn, and B. Meyer, *Justus Liebigs Ann. Chem.*, 1985, 489.
16 R. U. Lemieux, K. Bock, L. T. J. Delbaere, S. Koto, and V. S. Rao, *Can. J. Chem.*, 1980, **58**, 631.
17 H. Thøgersen, R. U. Lemieux, K. Bock, and B. Meyer, *Can. J. Chem.*, 1982, **60**, 44.
18 G. M. Brown and H. A. Levy, *Acta. Crystallogr., Sect. B*, 1979, **35**, 656.
19 F. Arene, A. Neuman, and F. Longchambon, *C.R. Seances Acad. Sci.*, 1979, **288**, 331.
20 W. J. Cook and C. E. Bugg, *Biochim. Biophys. Acta*, 1975, **389**, 428.
21 B. Sheldrick, *Acta Crystallogr., Sect. B*, 1976, **32**, 1016.
22 S. Takagi and G. A. Jeffrey, *Acta Crystallogr., Sect. B*, 1979, **35**, 902.
23 Y. Nishida, H. Hori, H. Ohrui, and H. Meguro, *J. Carbohydr. Chem.*, 1988, **7**, 239.
24 A. S. Perlin, 'International Review of Science: Carbohydrates,' ed. G. O. Aspinall, Butterworths, 1976, vol. 7, p. 1.
25 F. W. Wehrli and T. Wirthlin, 'Interpretation of Carbon-13 NMR Spectra,' Heyden, 1976, p. 22.
26 P.-E. Jansson, L. Kenne, and G. Widmalm, *Carbohydr. Res.*, 1989, **188**, 169.

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