Synthesis, NMR, and Conformational Studies of some 1,2- and 1,3-Linked Disaccharides

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Eight 1,2- and 1,3-linked disaccharides have been synthesized in which the glycosidic linkages have different stereochemical environments. ^1H and ^{13}C NMR studies and conformational analysis, using the HSEA-approach, indicate a number of proton–oxygen and proton–proton interactions, resulting, *inter alia*, in downfield and upfield shifts of signals from protons involved in the glycosidic linkages and upfield shifts of signals from carbons which have their protons in γ-*gauche* contact. The differences of glycosylation shifts induced by a mannopyranosyl *versus* a glucopyranosyl group have been investigated.

Several recently published[1–6] NMR and conformational studies on di- and tri-saccharides have been aimed at understanding how glycosylation shifts are influenced by the type of sugar and the stereochemistry around the glycosidic bond. The data obtained were also used as the database for a computer program, CASPER,[7,8] which performs structural analysis of oligo- and poly-saccharides using NMR data and simple chemical analyses. The stereochemistry around the glycosidic linkage in the oligosaccharides was varied for the different compounds studied. We now report studies on eight 2- and 3-linked disaccharides, most of which have an axial 2-hydroxy group in the glycosyl part.

Experimental

General Methods.—Concentrations were performed under diminished pressure at temperatures <40 °C (bath). Optical rotations were measured with a Perkin-Elmer 241 polarimeter. TLC was performed on Silica Gel F_{254} (Merck) with detection by UV light when applicable, or by charring with sulphuric acid. Column chromatography was performed on Matrex Silica Si (0.035–0.07 mm, Amicon) and elution with toluene–ethyl acetate mixtures. Organic solvents were dried over $MgSO_4$ before use.

The substitution position of the glycosyl groups was determined by the synthetic route. The number and chemical shifts of signals in the ^1H and ^{13}C NMR spectra were also in agreement with the postulated structures. Anomeric configurations were deduced from the size of the coupling constant, $^3J_{H,H}$, of signals from anomeric protons.

The purity of intermediates was first analysed by TLC, by which they showed only one spot. Secondly, from their ^{13}C NMR spectra, the synthetic intermediates were estimated to be >95% pure. In the ^1H NMR spectra of the deprotected disaccharides, signals from contaminating components were <5% of the integral of the anomeric proton signals.

NMR spectra were recorded for solutions in $CDCl_3$ or D_2O using JEOL GSX-270 or GX-400 instruments. Chemical shifts are given in ppm and referenced to internal tetramethylsilane (δ_H, δ_C 0.00) for solutions in $CDCl_3$ at 25 °C. For solutions in deuterium oxide, the spectra were recorded at 70 °C using dioxane (δ_C 67.40) and sodium 3-(trimethylsilyl)-[$^2H_4$]-propionate (TSP, δ_H 0.00) as internal references.

For the assignment of signals in the spectra of final products, proton–proton and carbon–proton shift correlation spectroscopy (COSY) were used. ^1H NMR chemical shifts of overlapping signals were obtained from the centre of the cross-peaks in the proton-proton shift correlation spectra.

To estimate minimum energy conformations and rotational freedom, the HSEA program[9,10] was used which accounts for non-bonded interactions, as expressed by the Kitaigorodsky algorithm, together with a term for the exoanomeric effect. Atoms in the glycosyl group are labelled with a prime. The torsional angles φ and ψ, and their signs,[11] were defined in a 1,2-linked disaccharide by H(1′)–C(1′)–O(2)–C(2) and C(1′)–O(2)–C(2)–H(2), respectively. The bond angle τ [C(1′)–O(2)–C(2)] was set as 117°. In all sugars with a hydroxymethyl group the *gauche-trans* conformer [O(5)–C(5)–C(6)–O(6) = 60°] was chosen for the energy minimisations. Co-ordinate sets for methyl α-D-glucopyranoside,[12] methyl β-D-glucopyranoside,[13] α-L-rhamnopyranose,[14] and β-D-mannopyranose,[15] were obtained from crystal data, whereas the co-ordinate set for α-D-mannopyranose (*gauche-trans* conformer of hydroxymethyl group) was modified from methyl α-D-mannopyranoside[12] (*gauche-gauche* conformer of the hydroxymethyl group). Co-ordinate sets for β-L-fucopyranose and β-L-rhamnopyranose were obtained from the mirror images of modified β-D-galactopyranose[16] and modified methyl α-D-mannopyranoside,[12] respectively.

Methyl 2-O-(2,3,4,6-*Tetra*-O-*acetyl*-α-D-*mannopyranosyl*)-3,4,6-*tri*-O-*benzyl*-α-D-*glucopyranoside*.—2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl chloride[17] (407 mg, 1.25 mmol), methyl 3,4,6-tri-*O*-benzyl-α-D-glucopyranoside[18] (300 mg, 0.65 mmol) and molecular sieves were added to freshly distilled dichloromethane (3 ml) under nitrogen. The mixture was cooled to −20 °C and a solution of silver trifluoromethanesulphonate (330 mg, 1.28 mmol) in toluene (3 ml) was added with stirring. After 50 min, aqueous sodium thiosulphate was added. The mixture was filtered through Celite which was rinsed with dichloromethane. The filtrate was concentrated, and the residue separated on a silica gel column (toluene–ethyl acetate, 3:1) to yield, after concentration to dryness, the product as a syrup (398 mg, 81%), $[\alpha]_{578}^{22}$ + 82° (*c* 0.8, $CHCl_3$); δ_C 94.50, 96.21 (C-1, C-1′).

Methyl 2-O-α-D-*Mannopyranosyl*-α-D-*glucopyranoside* (**1**).—The above disaccharide was *O*-deacetylated with sodium methoxide in methanol (10 ml, 1M) at room temperature for 30 min. Dowex 50 (H^+) resin was used for neutralisation, and the residue was filtered and concentrated to dryness. The benzyl groups were then removed by hydrogenolysis in aqueous 80%

acetic acid (25 ml) at 400 kPa over 10% palladium-on-charcoal (Pd/C, 200 mg) for 18 h. After filtration and concentration, the product was purified by gel permeation chromatography on a column (2.5 × 80 cm) of Bio-Gel P-2 eluted with water. Compound (**1**) was obtained after freeze-drying as an amorphous powder (169 mg, 90%), $[\alpha]_{578}^{22}$ +136° (*c* 1.1, H_2O).

Methyl 2-O-(6-O-*Acetyl*-2,3,4-*tri*-O-*benzyl*-α-D-*mannopyranosyl*)-3-O-*benzyl*-4,6-O-*benzylidene*-β-D-*glucopyranoside* (**9**) and *Methyl* 2-O-(6-O-*Acetyl*-2,3,4-*tri*-O-*benzyl*-β-D-*mannopyranosyl*)-3-O-*benzyl*-4,6-O-*benzylidene*-β-D-*glucopyranoside* (**10**).—Silver zeolite (2.5 g) was added to a solution of methyl 3-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside[19] (0.72 g, 1.94 mmol) and 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl bromide[20] (1.40 g, 2.52 mmol) in dichloromethane (40 ml). After the mixture had been stirred for 100 min in the dark, the reaction was complete; the mixture was then filtered and concentrated. Column chromatography using toluene–ethyl acetate (10:1) as eluant yielded first (**9**) (0.36 g, 22%) and then (**10**) (1.22 g, 74%). Compound (**9**) δ_C 97.16 (C-1′), 101.10 (Ph*C*H), and 105.01 (C-1); compound (**10**) δ_C 101.02 (Ph*C*H), 102.26 (C-1′), and 103.20 (C-1).

Methyl 2-O-α-D-*Mannopyranosyl*-β-D-*glucopyranoside* (**2**).—A solution of compound (**9**) (0.36 g) in dichloromethane (7 ml) was treated with sodium methoxide in methanol (0.25M; 10 ml) for 1 h, neutralised with Dowex 50 (H^+), filtered, and concentrated to dryness. The residue was hydrogenolysed over 10% Pd/C in ethyl acetate–ethanol (1:1) at 400 kPa for 9 h. The mixture was filtered and the solution concentrated to dryness. Chromatography on a silica gel column (ethyl acetate–acetic acid–methanol–water, 12:3:3:2) followed by a column of Bio-Gel P-2 eluted with water yielded (**2**) (100 mg, 67%), $[\alpha]_{578}^{22}$ + 34° (*c* 1.9, H_2O).

Methyl 2-O-β-D-*Mannopyranosyl*-β-D-*glucopyranoside* (**3**).—Compound (**10**) (0.40g) was treated with sodium methoxide, the mixture worked up, hydrogenolysed over Pd/C in ethyl acetate–acetic acid (1:2) for 18 h, and purified as described for (**2**) to yield (**3**) (127 mg, 76%) $[\alpha]_{578}^{22}$ −45° (*c* 1.1, H_2O).

Methyl 2-O-(2,3,4-*Tri*-O-*acetyl*-β-L-*fucopyranosyl*)-3-O-*acetyl*-4,6-O-*benzylidene*-β-D-*glucopyranoside* (**11**).—2,6-Di-*t*-butyl-4-methylpyridine (132 mg, 0.64 mmol) was added to a mixture of methyl 3-*O*-acetyl-4,6-*O*-benzylidene-β-D-glucopyranoside[21] (209 mg, 0.64 mmol), ethyl 2,3,4-tri-*O*-acetyl-1-thio-β-L-fucopyranoside[22] (230 mg, 0.69 mmol) and molecular sieves (4 Å) in ether (15 ml). After the mixture had been stirred for 5 min methyl trifluoromethanesulphonate (0.36 ml, 3.2 mmol) was added. After 16 h at room temperature, the reaction was complete according to TLC, and triethylamine (0.9 ml) was added. The mixture was stirred for 25 min, filtered through Celite, and concentrated. Column chromatography using toluene–ethyl acetate (5:1) as eluant yielded derivative (**11**) (260 mg, 68%); δ_C 100.99 (C-1′), 101.47 (Ph*C*H), and 104.30 (C-1).

Methyl 2-O-β-L-*Fucopyranosyl*-β-D-*glucopyranoside* (**4**).—A solution of compound (**11**) (260 mg) in aqueous 70% acetic acid, was stirred at 80 °C for 50 min and then concentrated to dryness. The residue was *O*-deacetylated with sodium methoxide in methanol, worked up, filtered, and concentrated to dryness. Gel filtration on a column of Bio-Gel P-2 yielded compound (**4**) (101 mg, 65%), $[\alpha]_{578}^{22}$ −9° (*c* 1.7, H_2O).

Benzyl 3,4-*Di*-O-*benzoyl*-2-O-(2,3,4-*tri*-O-*acetyl*-β-L-*fucopyranosyl*)-α-L-*rhamnopyranoside* (**12**).—Methyl trifluoromethanesulphonate (0.42 ml, 3.6 mmol) was added dropwise to a solution of benzyl 3,4-di-*O*-benzoyl-α-L-rhamnopyranoside[23] (377 mg, 0.73 mmol), ethyl 2,3,4-tri-*O*-acetyl-1-thio-β-L-fucopyranoside (280 mg, 0.84 mmol) and ground molecular sieves (4 Å) in ether (15 ml) with stirring. The reaction was complete after 16 h and the reaction mixture was processed as described for (**11**). Column chromatography using toluene–ethyl acetate (20:1) as eluant yielded compound (**12**) (248 mg, 46%). δ_C 15.27 (C-6′), 17.67 (C-6), 96.92 (C-1), and 100.83 (C-1′).

2-O-β-L-*Fucopyranosyl*-α,β-L-*rhamnose* (**5**), (**6**).—A solution of compound (**12**) (248 mg) in dichloromethane (6 ml) was treated with sodium methoxide, and the mixture then worked up, filtered, and concentrated to dryness. The residue was hydrogenolysed with 10% Pd/C in aqueous 90% ethanol at 400 kPa for 2 h and purified as described for compound (**2**) to yield (**5**), (**6**) (51 mg, 49%), $[\alpha]_{578}^{22}$ +9° (*c* 0.7, H_2O). The ratio of (**5**) to (**6**) in aqueous solution was ~3:1 as seen from the NMR spectra.

Methyl 3-O-(6-O-*Acetyl*-2,3,4-*tri*-O-*benzyl*-α-D-*mannopyranosyl*)-2-O-*benzyl*-4,6-O-*benzylidene*-β-D-*glucopyranoside* (**13**) and *Methyl* 3-O-(6-O-*Acetyl*-2,3,4-*tri*-O-*benzyl*-β-D-*mannopyranosyl*)-2-O-*benzyl*-4,6-O-*benzylidene*-β-D-*glucopyranoside* (**14**).—Silver zeolite (1.7 g) was added to a solution of methyl 2-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside[19] (452 mg, 1.21 mmol) and 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl bromide[20] (985 mg, 1.77 mmol) in toluene (20 ml). After the mixture had been stirred for 2 h in the dark, the reaction was complete and the mixture was filtered and concentrated. The products were purified on silica gel using toluene–ethyl acetate (10:1) as eluant to yield first (**13**) (188 mg, 18%) and then (**14**) (334 mg, 33%) out of a total isolated yield of 71%. Compound (**13**) δ_C 97.43 (C-1′), 101.69 (Ph*C*H), 105.52 (C-1); Compound (**14**) δ_C 100.83, 102.47 (C-1′, Ph*C*H), 105.06 (C-1).

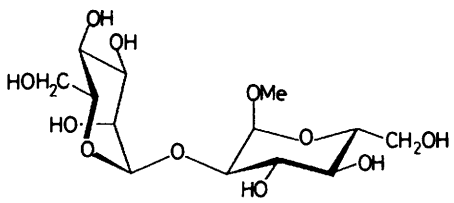
Methyl 3-O-α-D-*Mannopyranosyl*-β-D-*glucopyranoside* (**7**).—Compound (**13**) (188 mg) was *O*-deacetylated with sodium methoxide in methanol, hydrogenolysed by 10% Pd/C in ethyl acetate–ethanol (1:1), and purified as described for (**2**). The yield of (**7**) was 46 mg (58%), $[\alpha]_{578}^{22}$ +55° (*c* 0.8, H_2O).

Methyl 3-O-β-D-*Mannopyranosyl*-β-D-*glucopyranoside* (**8**).—Deprotection and purification of (**14**) (337 mg) was performed as described for (**2**). The yield was 91 mg (64%), $[\alpha]_{578}^{22}$ −38° (*c* 1.9, H_2O).

Results and Discussion

Synthesis.—The eight disaccharides, (**1**)–(**8**), were prepared by use of three different promoters for the glycosylation reactions, namely silver trifluoromethanesulphonate,[24,25] methyl trifluoromethanesulphonate,[26] and silver zeolite.[27] The disaccharide (**1**) was obtained by silver trifluoromethanesulphonate promotion in which 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl chloride was used as the glycosylating agent and methyl 3,4,6-tri-*O*-benzyl-α-D-glucopyranoside as the aglycone.

In the synthesis of β-D-mannopyranosides substantial amounts of α-D-mannopyranosides are usually formed. This was taken advantage of, since disaccharides with both anomeric forms of the central glycosidic linkage were required. For the synthesis of (**2**) and (**3**), 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl bromide was used as glycosyl donor and methyl 3-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside as the glycosyl acceptor in the presence of silver zeolite as promoter. Isolation of products after the glycosidation reaction yielded the α- and the β-linked disaccharide in a ratio of 1:3.4.



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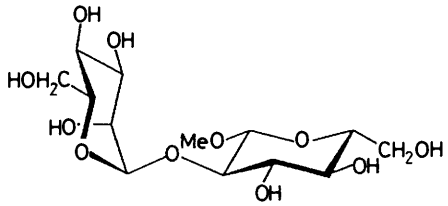

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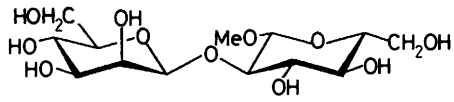

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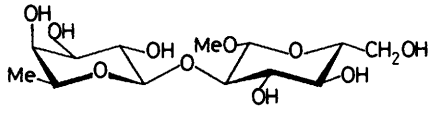

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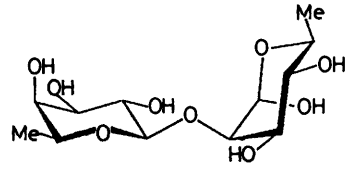

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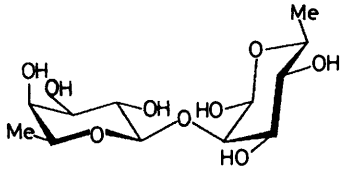

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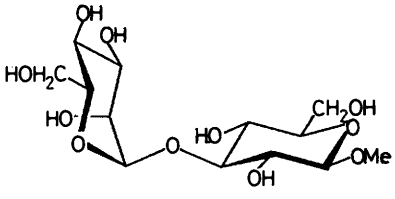

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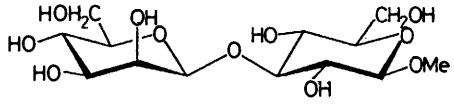

(8)

Table 1. Values for the φ and ψ angles, in degrees, together with inter-residue internuclear distances <3 Å in the minimum energy conformations of (1)–(8), as indicated by HSEA-calculations.

Substance	φ/ψ Angles	Inter-residue internuclear distances (minimum energy molecule) 1′-H	5′-H	O-5′
α-D-Man*p*-(1→2)α-D-Glc*p*OMe	−50/−35	2.27 (1-H)	2.61 (O-3)	2.47 (2-H)
(1)		2.59 (2-H)	2.87 (2-H)	
α-D-Man*p*-(1→2)β-D-Glc*p*OMe	−45/−25	2.45 (O-1)	2.46 (O-3)	2.62 (2-H)
(2)		2.47 (2-H)		
β-D-Man*p*-(1→2)β-D-Glc*p*OMe	60/−5	2.39 (2-H)		2.82 (O-1)
(3)				2.54 (2-H)
β-L-Fuc*p*-(1→2)β-D-Glc*p*OMe	−55/−10	2.80 (O-1)		2.49 (2-H)
(4)		2.44 (2-H)		
β-L-Fuc*p*-(1→2)α-L-Rha*p*OH	−55/−5	2.84 (1-H)		2.54 (2-H)
(5)		2.42 (2-H)		
β-L-Fuc*p*-(1→2)β-L-Rha*p*OH	−55/−5	2.88 (O-1)		2.48 (2-H)
(6)		2.35 (2-H)		
α-D-Man*p*-(1→3)β-D-Glc*p*OMe	−40/−20	2.54 (O-4)	2.44 (O-2)	2.76 (3-H)
(7)		2.40 (3-H)		
β-D-Man*p*-(1→3)β-D-Glc*p*OMe	60/0	2.93 (O-2)		2.89 (O-4)
(8)		2.46 (3-H)		2.54 (3-H)

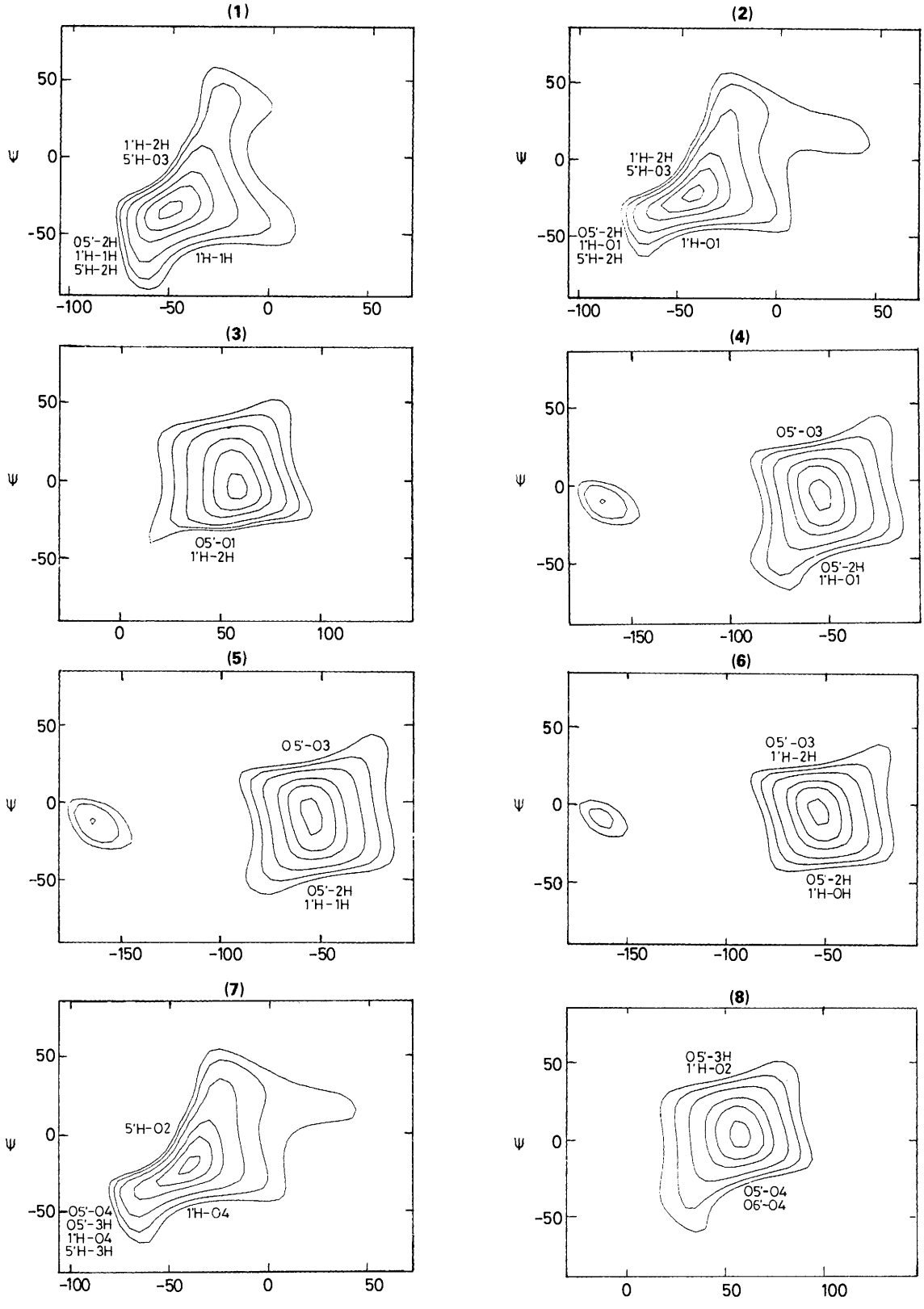

Figure. Conformational energy plots for disaccharides (**1**)–(**8**) with pronounced inter-residue contacts. Isocontour levels are indicated at 0.4, 2.1, 4.2, 8.4, 12.6, and 16.8 kJ (0.1, 0.5, 1, 2, 3, and 4 kcal) above the minimum energy conformation.

In the synthesis of disaccharides with β-L-fucopyranosyl groups, (**4**)–(**6**), ethyl 2,3,4-tri-*O*-acetyl-1-thio-β-L-fucopyranoside was the glycosyl donor in a methyl trifluoromethanesulphonate promoted coupling. The glycosyl acceptors were methyl 3-*O*-acetyl-4,6-*O*-benzylidene-β-D-glucopyranoside in the synthesis of (**4**) and benzyl 3,4-di-*O*-benzoyl-α-L-rhamnopyranoside of (**5**) and (**6**). For (**7**) and (**8**) the same procedure was used as that in the synthesis of (**2**) and (**3**) but with methyl 2-

O-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside as acceptor. Isolation of products after the glycosidation reaction yielded the α- and the β-linked disaccharide in a ratio of 1:1.8.

HSEA Calculations.—The φ/ψ-energy plots together with proximities between atoms over the glycosidic bond, derived from changes in φ and ψ, of the disaccharides (**1**)–(**8**) are shown in the Figure. All inter-residue internuclear distances <3 Å for the minimum energy conformations are given in Table 1. The disaccharides (**1**), (**2**), and (**7**) are termed α-glycosides with reference to the central bond and, consequently, the remaining compounds are termed β-glycosides.

The values for φ in the minimum energy conformations of (**1**)–(**8**) are −50° and 50° (±10°), for α-D/β-L and β-D-glycosyl groups, respectively, whereas the values for ψ vary between 0° and −35°. As observed from the energy maps (Figure), the restricted rotational freedom of disaccharide (**1**)–(**8**) is caused by different interatomic contacts. For the α-glycosides (**1**), (**2**), and (**7**), the anomeric proton 1′-H will, for conformations with ψ-values < −50°, interact strongly with the equatorial substituent on the opposing carbon, 1-H, O-1, and O-4, respectively, causing increased energy. On the other hand, for conformations with φ-values < −50° and ψ-values > −20°, higher energy is caused by the contact between 5′-H and the equatorial substituent on the carbon on the other side of the linkage, O-3 and O-2, respectively. From the energy plots it is evident that the β-glycosides have more rotational freedom than the α-glycosides but restrictions to rotation around the glycosidic bond are due to inter-residue contacts between O-5′ and 1′-H in the glycosyl group and the equatorial substituents on the carbons next to the linkage. No significant differences between the energy maps for disaccharides (**4**)–(**6**) with the β-L-fucosyl groups linked to equatorial or axial positions were observed, showing that no additional severe contacts occur for the axial substitution.

The influence of an axial hydroxy group at C-2′ on the conformation of the glycosidic linkage can be studied by comparison of data from the mannopyranosyl compounds (**1**)–(**3**), (**7**), and (**8**), with those from the corresponding *gluco*-derivatives.[4] This shows that compounds (**1**)–(**3**), (**7**), and (**8**) have virtually the same minimum energy conformations and inter-residual internuclear distances as their *gluco*-counterparts. For (**2**), however, which has φ and ψ slightly altered in the minimum energy conformation, differences in inter-residual atomic distances up to 0.15 Å are observed. For (**3**), 1′-H is 0.09 Å closer to 2-H and the distance to O-3 is larger than in the corresponding *gluco*-counterpart.[4]

For all the disaccharides (**1**)–(**8**) common interactions over the glycosidic linkage include the proton on the linkage carbon, 2-H or 3-H, which is close to 1′-H and O-5′. For all α-glycosides 5′-H is calculated to be close to the opposing equatorial substituent, O-3 or O-2, in the methyl glycopyranoside residue. These interactions have also been observed in previous studies.[2–6] Two energy minima are found for (**4**)–(**6**), the conformation with the lowest energy (φ/ψ *ca.* −55/−5) having the largest rotational freedom. The second minimum (φ/ψ *ca.* −160/−10) has >10 kJ higher energy and should, therefore, not be significantly populated.

^{1}H *NMR Glycosylation Shifts.*—The ^{1}H NMR chemical shifts and the glycosylation shifts (Δδ, induced chemical shift differences relative to the chemical shifts of the respective monomers) are give in Table 2. Chemical shifts of signals which are not of first order are approximate only. All coupling constants were of the expected size, showing that no conformational changes of the pyranosidic rings had occurred.

Signals from protons at the linkage carbons and at those next to these and signals for 5′-H in the α-glycosides have the largest shifts, a pattern which also has been observed for the oligosaccharides studied earlier.[2–6]

To show if glycosylation shifts induced by mannopyranosyl groups in disaccharides can be approximated from disaccharides with glucopyranosyl groups some disaccharides of the former type were included in this study. Comparison between spectra from disaccharides with mannopyranosyl groups and those from disaccharides with glucopyranosyl groups shows that the glycosylation shifts for signals from 1′-H, 2′-H, and 3′-H are different. Thus, disaccharides with a mannopyranosyl group have glycosylation shifts that are approximately −0.05, 0.05, and −0.05 ppm different from the values obtained for the glucopyranosyl compounds. This is true for (**2**), (**3**), (**7**), and (**8**) but (**1**) is an exception for which instead these glycosylation shift differences are 0.01, 0.00, and −0.04 ppm, respectively.

The glycosylation shifts for the methyl glucopyranoside residues of the disaccharides substituted with a mannopyranosyl group, (**1**)–(**3**), (**7**), and (**8**), show differences compared to disaccharides in which the glycosyl group has the *gluco*-configuration. The differences are observed for signals from protons at or vicinal to the glycosidic linkage, ranging from −0.10 to 0.07 ppm. These observations show that care must be taken in approximation of glycosylation shifts when there are differences in stereochemistry close to the glycosidic bonds.

The glycosylation shifts for signals from 5′-H in the α-glycosides, 0.05–0.14 ppm, can be correlated to the distance in the minimum energy conformation between 5′-H and an oxygen in the aglycone (Table 1). The closer 5′-H is to the oxygen the larger the downfield shift is. In the disaccharide (**1**) an upfield shift of the 1′-H signal is observed which is due to proton–proton interactions.[3] The glycosylation shift for the 1′-H signal in disaccharide (**5**) is −0.14 ppm whereas for the corresponding signal in disaccharide (**6**) it is −0.07 ppm. This may be caused by the substitution to an axial position in the rhamnopyranose residue. The extra upfield shift of the 1′-H signal in (**5**) could derive from the interaction between 1′-H and 1-H but in (**6**) 1′-H is devoid of a similar proton–proton interaction.

^{13}C *NMR Glycosylation Shifts.*—The ^{13}C NMR chemical shifts for compounds (**1**)–(**8**) and relevant monomers together with the glycosylation shifts, (Δδ), obtained upon comparison with the chemical shifts of the respective monomers, are given in Table 3.

Significant Δδ-values, >0.5 ppm, are observed for signals of linkage carbons and for most of the carbons next to these which is the same pattern as observed for other disaccharides.[1–5]

To study if the ^{13}C glycosylation shifts for mannopyranosyl groups in disaccharides can be approximated from disaccharides with glucopyranosyl groups, the two were compared as described for ^{1}H NMR glycosylation shifts. The presence of an axial OH-2′, *e.g.*, as in a mannopyranosyl group, causes only minor differences in glycosylation shifts in comparison with corresponding glucopyranosyl disaccharides except for the signals for C-2′ and the linkage carbons. The glycosylation shifts for the C-2′ signals of the α-mannosides (**1**), (**2**), and (**7**) are −0.33, −0.38, and −0.60 ppm respectively, different from the corresponding *gluco*-values. For the β-mannosides (**3**) and (**8**) only smaller glycosylation shift differences between *manno*- and *gluco*-disaccharides are observed for the C-2′ signals. The glycosylation shifts for the C-1′ signals for the D-mannosides show only minor differences compared to their *gluco*-counterparts except for disaccharide (**1**) in which the difference is −1.06 ppm. For the signals from the linkage carbons in the aglycone smaller glycosylation shifts are observed for disaccharides with mannosyl groups than with glucosyl groups. The difference in the glycosylation shifts ranges

Table 2. ^{1}H NMR chemical shifts of disaccharides and pertinent monosaccharides obtained at 70 °C relative to internal TSP (δ_H 0.00). Chemical shift differences[a] are given in parentheses.

Substance	1′-H[b]	2′-H	3′-H	4′-H	5′-H	6′-H	6′-H	1-H	2-H	3-H	4-H	5-H	6-H	6-H	OMe
α-D-Man*p*-(1→2)α-D-Glc*p*OMe	5.01	3.96	3.88	3.71	3.87	3.77[c]	3.88	5.03	3.68	3.73	3.46	3.64	3.77	3.88	3.45
(**1**)	(−0.17)	(0.02)	(0.02)	(0.03)	(0.05)	(0.03)	(0.02)	(0.22)	(0.12)	(0.05)	(0.05)	(0.00)	(0.01)	(0.01)	(0.02)
α-D-Man*p*-(1→2)β-D-Glc*p*OMe	5.19	3.98	3.86	3.72	3.94	3.78	3.86	4.46	3.42	3.56	3.43[c]	3.44[c]	3.73	3.92	3.58
(**2**)	(0.01)	(0.04)	(0.00)	(0.04)	(0.12)	(0.04)	(0.00)	(0.09)	(0.14)	(0.06)	(0.03)	(−0.02)	(−0.01)	(0.00)	(0.00)
β-D-Man*p*-(1→2)β-D-Glc*p*OMe	4.94	4.09	3.64	3.62	3.35	3.76	3.92	4.50	3.51	3.64	3.42	3.44	3.73	3.92	3.58
(**3**)	(0.05)	(0.14)	(−0.02)	(0.02)	(−0.03)	(0.01)	(0.01)	(0.13)	(0.23)	(0.14)	(0.02)	(−0.02)	(−0.01)	(0.00)	(0.00)
β-L-Fuc*p*-(1→2)β-D-Glc*p*OMe	4.59	3.52	3.65	3.75	3.77	1.27		4.52	3.48	3.61	3.44[c]	3.46[c]	3.74	3.93	3.58
(**4**)	(0.04)	(0.06)	(0.02)	(0.01)	(−0.02)	(0.01)		(0.15)	(0.20)	(0.11)	(0.04)	(0.00)	(0.00)	(0.01)	(0.00)
β-L-Fuc*p*-(1→2)α-L-Rha*p*OH	4.41	3.55	3.65	3.76	3.79	1.27		5.24	4.05	3.82	3.44	3.87	1.29		
(**5**)	(−0.14)	(0.09)	(0.02)	(0.02)	(0.00)	(0.01)		(0.12)	(0.13)	(0.01)	(−0.01)	(0.01)	(0.01)		
β-L-Fuc*p*-(1→2)β-L-Rha*p*OH	4.48	3.64[c]	3.67[c]	3.76	3.82	1.28		4.93	4.11	3.60	3.36	3.42	1.31		
(**6**)	(−0.07)	(0.18)	(0.04)	(0.02)	(0.03)	(0.02)		(0.08)	(0.18)	(0.01)	(−0.02)	(0.03)	(0.01)		
α-D-Man*p*-(1→3)β-D-Glc*p*OMe	5.24	4.05	3.86	3.73	3.96	3.78	3.86	4.39	3.35	3.66	3.55	3.47	3.74	3.92	3.57
(**7**)	(0.06)	(0.11)	(0.00)	(0.05)	(0.14)	(0.04)	(0.00)	(0.02)	(0.07)	(0.16)	(0.15)	(0.01)	(0.00)	(0.00)	(−0.01)
β-D-Man*p*-(1→3)β-D-Glc*p*OMe	4.89	4.13	3.65	3.61	3.40	3.74	3.92	4.40	3.41	3.72	3.52	3.47	3.74	3.92	3.58
(**8**)	(0.00)	(0.18)	(−0.01)	(0.01)	(0.02)	(−0.01)	(0.02)	(0.03)	(0.13)	(0.22)	(0.12)	(0.01)	(0.00)	(0.00)	(0.00)
α-D-Man*p*	5.18	3.94	3.86	3.68	3.82	3.74	3.86								
β-D-Man*p*	4.89	3.95	3.66	3.60	3.38	3.75	3.91								
β-L-Fuc*p*	4.55	3.46	3.63	3.74	3.79	1.26									
α-D-Glc*p*OMe								4.81	3.56	3.68	3.41	3.64	3.76	3.87	3.43
β-D-Glc*p*OMe								4.37	3.28	3.50	3.40	3.46	3.74	3.92	3.58
α-L-Rha*p*								5.12	3.92	3.81	3.45	3.86	1.28		
β-L-Rha*p*								4.85	3.93	3.59	3.38	3.39	1.30		

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

Table 3. ^{13}C NMR chemical shifts of disaccharides and pertinent monosaccharides obtained at 70 °C relative to internal dioxane (δ_C 67.40). Chemical shift differences[a] are given in parentheses.

Substance	C-1′[b]	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-Man*p*-(1→2)α-D-Glc*p*OMe	98.46	71.15	71.26	67.64	73.75	61.84[c]	97.27	75.94	72.55	70.59	72.37	61.61[c]	55.67
(1)	(3.52)	(−0.54)	(0.01)	(−0.30)	(0.41)	(−0.15)	(−2.92)	(3.71)	(−1.55)	(−0.09)	(−0.15)	(−0.06)	(−0.26)
α-D-Man*p*-(1→2)β-D-Glc*p*OMe	100.59	71.16	71.36	67.67	73.70	61.84	104.60	78.97[d]	75.45	70.82[d]	76.66	61.84	58.00
(2)	(5.65)	(−0.53)	(0.11)	(−0.27)	(0.36)	(−0.15)	(0.47)	(4.98)	(−1.33)	(0.13)	(−0.12)	(0.02)	(0.12)
β-D-Man*p*-(1→2)β-D-Glc*p*OMe	101.04	71.37	73.92[d]	67.68	77.26	61.97	103.00	80.65	76.68[d]	70.61	76.68	61.78	57.70
(3)	(6.49)	(−0.76)	(−0.11)	(−0.01)	(0.26)	(−0.02)	(−1.13)	(6.66)	(−0.10)	(−0.08)	(−0.10)	(−0.04)	(−0.18)
β-L-Fuc*p*-(1→2)β-D-Glc*p*OMe	103.04	71.76	73.78	72.18	71.59	16.25	103.87	80.90	75.26	70.54	76.58	61.74	58.01
(4)	(5.89)	(−0.97)	(−0.15)	(−0.17)	(−0.05)	(−0.08)	(−0.26)	(6.91)	(−1.52)	(−0.15)	(−0.20)	(−0.08)	(0.13)
β-L-Fuc*p*-(1→2)α-L-Rha*p*OH	103.27	71.26	73.70	72.14	71.78	16.19	93.16	79.86	70.23	73.80	69.26	17.61[e]	
(5)	(6.12)	(−1.47)	(−0.23)	(−0.21)	(0.14)	(−0.14)	(−1.68)	(8.05)	(−0.77)	(0.61)	(0.14)	(−0.06)	
β-L-Fuc*p*-(1→2)β-L-Rha*p*OH	105.06	71.89	73.75	72.14	72.00	16.19	94.28	81.90	72.94	73.41	73.05	17.56[e]	
(6)	(7.91)	(−0.84)	(−0.18)	(−0.21)	(0.36)	(−0.14)	(−0.09)	(9.67)	(−0.82)	(0.58)	(0.22)	(−0.05)	
α-D-Man*p*-(1→3)β-D-Glc*p*OMe	101.62	71.22	71.42	67.69	73.74	61.80[c]	104.14	72.77	83.39	70.95	76.56	61.66[c]	57.88
(7)	(6.68)	(−0.47)	(0.17)	(−0.25)	(0.40)	(−0.19)	(0.01)	(−1.22)	(6.61)	(0.26)	(−0.22)	(−0.16)	(0.00)
β-D-Man*p*-(1→3)β-D-Glc*p*OMe	101.30	71.40	73.88	67.72	77.21	61.94[c]	103.95	73.50	85.59	69.25	76.39	61.83[c]	57.88
(8)	(6.75)	(−0.73)	(−0.15)	(0.03)	(0.21)	(−0.05)	(−0.18)	(−0.49)	(8.81)	(−1.44)	(−0.39)	(0.01)	(0.00)
α-D-Man*p*	94.94	71.69	71.25	67.94	73.34	61.99							
β-D-Man*p*	94.55	72.13	74.03	67.69	77.00	61.99							
β-L-Fuc*p*	97.15	72.73	73.93	72.35	71.64	16.33							
α-D-Glc*p*OMe							100.19	72.23	74.10	70.68	72.52	61.67	55.93
β-D-Glc*p*OMe							104.13	73.99	76.78	70.69	76.78	61.82	57.88
α-L-Rha*p*							94.84	71.81	71.00	73.19	69.12	17.67	
β-L-Rha*p*							94.37	72.23	73.76	72.83	72.83	17.61	

[a] Chemical shift differences are calculated by subtraction of the chemical shifts of the corresponding hexose and methyl hexoside or reducing residue for the glycosyl part and the aglycone, respectively, and a positive difference indicates a downfield shift. [b] Primed labels refer to the glycopyranosyl group and unprimed to the methyl glycoside or reducing residue. [c] May be interchanged. [d] Reasonable assignment. [e] May be interchanged.

Table 4. ^{13}C Chemical shift differences in ppm on variation of temperature[a]

	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)	0.29	0.13	0.17	0.24	0.14	0.17[b]	0.27	0.44	0.15	0.26	0.10	0.22[b]	0.14
(2)	0.11	0.09	0.16	0.27	0.10	0.20	−0.03	0.39	0.18	0.19	0.04	0.20	−0.09
(3)	−0.08	−0.01	0.13	0.20	−0.01	0.12	0.13	0.05	0.04	0.23	0.04	0.21	−0.11
(4)	0.05	0.12	0.19	0.04	0.09	−0.04	−0.07	0.30	0.15	0.20	0.06	0.18	−0.12
(5)	0.06	0.08	0.18	0.06	0.07	−0.06	0.06	0.18	0.13	0.21	0.05	0.04	
(6)	−0.02	0.01	0.16	0.06	0.12	−0.06	−0.04	0.05	0.14	0.18	−0.02	0.04	
(7)	−0.07	0.08	0.15	0.29	0.14	0.24[b]	0.06	0.09	0.39	0.04	0.08	0.21[b]	−0.11
(8)	−0.02	0.04	0.17	0.15	0.01	0.09[b]	0.11	0.00	0.13	0.15	0.10	0.21[b]	−0.10

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

from −0.36 to −0.92 ppm for disaccharides (**1**), (**3**), (**7**), and (**8**), but for compound (**2**) a positive glycosylation shift difference (0.30 ppm) is observed. The differences in stereochemistry between *manno*- and *gluco*-pyranosyl groups lead to changes in glycosylation shifts which should be treated with caution when making approximations.

The effect of glycosidation with a D-sugar compared to that of an L-sugar can be seen from values of disaccharides (**3**) and (**4**). For disaccharide (**3**) with a D-glycosyl group the glycosylation shifts for the C-1 and C-3 signals are −1.1 and −0.1 ppm, respectively. For the disaccharide (**4**) with an L-glycosyl group, the glycosylation shifts for the C-1 and C-3 signals are −0.3 and −1.5 ppm, respectively, *i.e.* they are mirrored with similar glycosylation shift magnitudes. The substitution of the axial hydroxy group at C-2 of the L-rhamnose residue in compounds (**5**) and (**6**) leads to two disaccharides with different stereochemical surroundings, (Table 1) and different glycosylation shifts of signals from the linkage carbons are observed. The relatively large glycosylation shift for the signal for C-4, an atom which is further away from the glycosidic linkage, may be due to that O-2 and 4-H are 1,3-diaxially positioned. The upfield shift of the C-4 signal, caused by this interaction (*γ-gauche*),[28] seems to be decreased by the substitution of O-2. The C-1′ signals of compounds (**5**) and (**6**) both have medium-sized downfield glycosylation shifts. The difference, 1.79 ppm, in these glycosylation shifts could be a result of the interaction between 1′-H and 1-H in (**5**) causing upfield shifts of the corresponding C-1′ and C-1 signals (*γ-gauche* effect[28,29]).

Temperature Dependence of the ^{13}C *NMR Chemical Shifts.*— The differences in chemical shifts for the signals on changing the temperature from 30 to 70 °C are given in Table 4. The values are relative to the signal from internal dioxane, which has the same chemical shift, δ 67.40, at both temperatures.

On heating, most signals are shifted to lower field, and no differences are larger than 0.45 ppm. When there is a large shift for the C-1′ signal it is paired with a large shift of the signal from the substituted carbon in the aglycone. When the signals from the 1′-carbons have an upfield shift the signals from the substituted carbons also have a smaller shift, but for disaccharide (**7**) which do not follow this trend. An exception from the general large downfield shifts of the C-4 and C-6 signals for *gluco*-derivatives is observed for the C-4 signal in disaccharide (**7**). The temperature variation for this signal is only 0.04 ppm. The corresponding disaccharide with a glucosyl group[4] has an even smaller shift for the C-4 signal (0.00 ppm).

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