Synthesis of and Nuclear Magnetic Resonance and Conformational Studies on some 1,2-Linked Disaccharide Methyl Glycosides containing D-Mannose and L-Rhamnose

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Syntheses have been performed of eight 1,2-linked disaccharide methyl glycosides in which the glycosidic linkages have different stereochemical surroundings. A factor in common has been that the substituted hydroxy group is axial. ¹H and ¹³C 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 at or near the glycosidic linkage, and upfield shifts of signals from carbons with their attached protons in γ-*gauche* contact.

To obtain correlations between the stereochemistry at a glycosidic linkage and the ¹H and ¹³C NMR chemical shifts for signals from the corresponding sugars a large number of disaccharides and trisaccharides have been characterized by NMR spectroscopy.[1-8] Theoretically derived inter-residue atomic distances were used to explain the glycosylation shifts. The glycosylation shifts obtained from the di- and trisaccharides have also been used successfully in a computerized approach to structure determination of oligo- and poly-saccharides.[9,10]

Among the previously studied disaccharides few have been with a substituted axial hydroxy group. Therefore in this study eight 1,2-linked disaccharides containing D-mannose or L-rhamnose have been synthesized and analysed by NMR spectroscopy, and their minimum-energy conformations calculated.

Experimental

General Methods.—Concentrations were performed under diminished pressure at temperatures <40 °C (bath). TLC and column chromatography was performed on silica gel. Organic solvents used in the syntheses were dried over molecular sieves before use.

The substitution position of the glycosyl groups was determined by the synthetic route. The number of signals and their chemical shifts in the ¹H and ¹³C NMR spectra were also in agreement with the postulated structures. Anomeric configurations of the products 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, and then by ¹³C NMR spectroscopy, from which the intermediates were estimated to be >95% pure. In the ¹H NMR spectra of the deprotected disaccharides, signals from contaminating components were <5% of those of the anomeric proton signals.

NMR spectra were recorded for solutions in CDCl₃ or D₂O on JEOL GSX-270 or GX-400 instruments. Chemical shifts are referenced to internal SiMe₄ (δ_H, δ_C 0.00) for solutions in CDCl₃ at 25 °C. For solutions in D₂O, the spectra were recorded at 70 °C using dioxane (δ_C 67.40) and sodium 3-(trimethylsilyl)-[2,2,3,3-²H₄]propanoate (TSP, δ_H 0.00) as internal references.

For the assignment of signals in the spectra of final products, different types of proton–proton and carbon–proton shift correlation spectroscopy (COSY) were used. ¹H NMR chemical shifts of overlapping signals were obtained from the centre of the cross-peaks in the proton–proton shift correlation spectra.
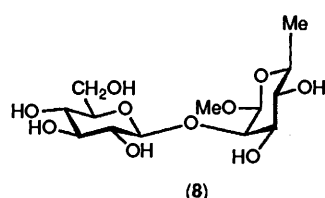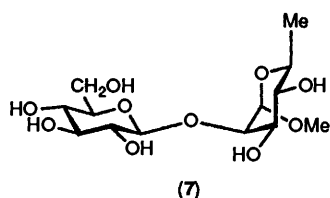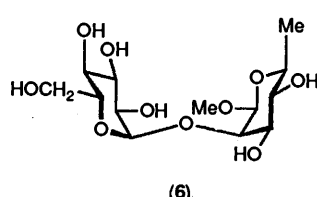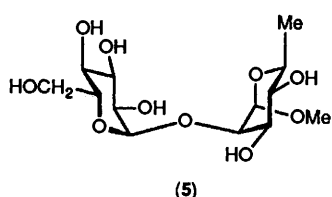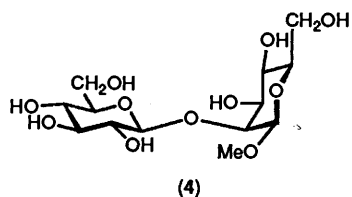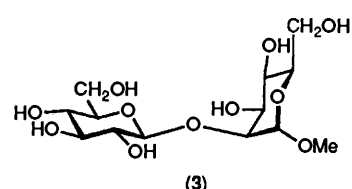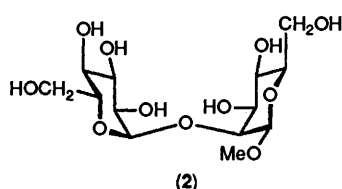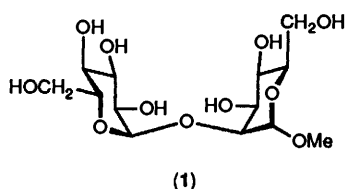
To estimate minimum-energy conformations and rotational freedom of the glycosidic bond, the HSEA program[11,12] was used. Atoms in the glycosyl group are labelled with a prime. The torsional angles φ and ψ were defined by H(1')–C(1')–O(2)–C(2) and C(1')–O(2)–C(2)–H(2), respectively. The bond angle τ [C(1')–O(2)–C(2)] was set at 117°. Co-ordinate sets for α-D-glucopyranose,[13] β-D-glucopyranose,[14] methyl α-D-mannopyranoside,[15] and methyl α-L-rhamnopyranoside[16] were obtained from crystal data. The co-ordinate set for methyl β-D-mannopyranoside was obtained from a β-D-mannopyranose residue in a trisaccharide[17] and the addition of a methyl group at φ 50°, and that for methyl β-L-rhamnopyranoside by modification of the co-ordinates from the β-D-mannopyranose residue.

Synthesis.—The disaccharides were obtained *via* coupling reactions using materials and conditions as given in Table 1. Deprotection conditions are also given in Table 1.

Coupling reaction A. A mixture of ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-α-D-glucopyranoside, the alcohol, and molecular sieves (4 Å, ~0.5 g) in dichloromethane (~5 ml) under dry nitrogen was stirred at room temperature for 10 min. Excess of dimethyl (methylthio)sulphonium trifluoromethanesulphonate (DMTST) (~3 mol equiv.)[18,19] was added in three portions during 2 h to the stirred mixture and then triethylamine (~0.5 ml) was added. The mixture was filtered through Celite, diluted with dichloromethane, and washed with aq. 8% NaHCO₃, the organic phase was dried with Na₂SO₄, and the solvents were evaporated. The products were purified by silica gel chromatography.

Coupling reaction B. A mixture of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl bromide, the alcohol, and molecular sieves (4 Å, ~0.5 g) was stirred in dichloromethane (~5 ml) under dry nitrogen at room temperature for 10 min. The mixture was cooled to −20 °C and stirred while a solution of silver trifluoromethanesulphonate (~1.5 mol equiv.) and *s*-collidine (2,4,6-trimethylpyridine) (1 mol equiv.) in toluene–dichloromethane (1:1; ~5 ml) was added.[20-22] After 30 min pyridine (~0.5 ml) was added and the mixture was filtered through Celite, diluted with dichloromethane, and washed successively with aq. 5% Na₂S₂O₃ and 8% NaHCO₃. The organic phase was dried with Na₂SO₄, then evaporated, and the product was purified by silica gel chromatography.

Coupling reaction C. A solution of the thioglycoside in dichloromethane and 1M-methanolic sodium methoxide (2:1; ~5 ml) were stirred together at room temperature for 10 min.

(1)

(2)

(3)

(4)

(5)

(6)

(7)

(8)

(1) α-D-Glcp-(1 ⟶ 2)-α-D-Manp-OMe
(3) β-D-Glcp-(1 ⟶ 2)-α-D-Manp-OMe
(5) α-D-Glcp-(1 ⟶ 2)-α-L-Rhap-OMe
(7) β-D-Glcp-(1 ⟶ 2)-α-L-Rhap-OMe

(2) α-D-Glcp-(1 ⟶ 2)-β-D-Manp-OMe
(4) β-D-Glcp-(1 ⟶ 2)-β-D-Manp-OMe
(6) α-D-Glcp-(1 ⟶ 2)-β-L-Rhap-OMe
(8) β-D-Glcp-(1 ⟶ 2)-β-L-Rhap-OMe

Bromine was then added until a permanently coloured mixture was obtained. After 5 min aq. 5% Na$_2$S$_2$O$_3$ was added and the reaction mixture was diluted with dichloromethane. The organic phase was washed with aq. 8% NaHCO$_3$, dried, and evaporated, and the product was purified by silica gel chromatography.

De-O-acylation (*D*). The derivative was treated with methanolic sodium methoxide (~0.2M) at room temperature overnight, and the solution was neutralized with cation exchange resin (H$^+$), then filtered, and the solvent was evaporated off.

Hydrogenolysis (*E*). The derivative was dissolved in aq. 80% acetic acid and hydrogenolysed over 10% Pd/C at 400 kPa overnight, the solution was filtered through Celite, and the solvent was evaporated off. The product was then purified on a Bio-Gel P-2 column irrigated with water.

Results and Discussion

Synthesis.—Data on coupling reactions, deprotection procedures, yields, physical constants, and selected NMR chemical shifts are given in Table 1.

All anomeric combinations of the methyl D-glucopyranosyl-(1 ⟶ 2)-D-mannopyranoside and the methyl D-glucopyranosyl(1 ⟶ 2)-L-rhamnopyranoside disaccharides were desired. The D-mannose-containing disaccharides were synthesized by coupling to the 3,4,6-protected methyl α- or β-D-mannoside, followed by separation of the anomeric mixtures. Thus methyl 3-O-benzoyl-4,6-O-benzylidene-α- or -β-D-mannoside was con-

densed with perbenzylated thioethyl glucopyranoside under DMTST promotion (Table 1). After deprotection and purification the disaccharides (1), (2), (3), and (4) were obtained. The methyl glycoside of the L-rhamnose-containing disaccharides was made at the disaccharide level. Thus perbenzylated glucopyranosyl bromide was condensed with ethyl 3,4-di-O-benzyl-1-thio-α-L-rhamnoside under silver trifluoromethanesulphonate promotion to yield an α,β-mixture of the thioethyl disaccharides. After separation these were treated with sodium methoxide and bromine to give the disaccharide methyl glycosides with a preponderance of the β-glycoside. After deprotection and purification the disaccharides (5), (6), (7), and (8) were obtained.

HSEA Calculations.—The φ/ψ-energy plots for disaccharides (1)–(8) are shown in Figure 1. All inter-residue atomic distances < 3 Å for the minimum-energy conformations are given in Table 2. Disaccharides (1), (2), (5), and (6) are termed α-glycosides with reference to the central bond and consequently the remaining compounds are termed β-glycosides. The energy maps in Figure 1 show for the α-glycosides (1), (2), (5), and (6) the typical behaviour of a glycoside with a linkage consisting of two axial CO bonds; *i.e.*, the most populated φ/ψ-region is relatively small.[5] The presence of one equatorial CO bond in the glycosidic linkage, as for the β-glycosides (3), (4), (7), and (8), gives more freedom for rotation and also an additional minimum of somewhat higher energy at φ ~ 160° and ψ ~0°. In common for glycosides (1)–(8) regarding inter-residue atomic distances is the short distance between 1′-H and 2-H and

(9) 2,3,4,6-Tetra-O-benzyl-α-D-Glcp-(1 ⟶ 2)-3-O-benzoyl-4,6-O-benzylidene-α-D-Manp-OMe
(10) 2,3,4,6-Tetra-O-benzyl-α-D-Glcp-(1 ⟶ 2)-3-O-benzoyl-4,6-O-benzylidene-β-D-Manp-OMe
(11) 2,3,4,6-Tetra-O-benzyl-β-D-Glcp-(1 ⟶ 2)-3-O-benzoyl-4,6-O-benzylidene-α-D-Manp-OMe
(12) 2,3,4,6-Tetra-O-benzyl-β-D-Glcp-(1 ⟶ 2)-3-O-benzoyl-4,6-O-benzylidene-β-D-Manp-OMe
(13) 2,3,4,6-Tetra-O-benzyl-α-D-Glcp-(1 ⟶ 2)-3,4-di-O-benzyl-α-L-Rhap-SEt
(14) 2,3,4,6-Tetra-O-benzyl-β-D-Glcp-(1 ⟶ 2)-3,4-di-O-benzyl-α-L-Rhap-SEt
(15) 2,3,4,6-Tetra-O-benzyl-α-D-Glcp-(1 ⟶ 2)-3,4-di-O-benzyl-α-L-Rhap-OMe
(16) 2,3,4,6-Tetra-O-benzyl-α-D-Glcp-(1 ⟶ 2)-3,4-di-O-benzyl-β-L-Rhap-OMe
(17) 2,3,4,6-Tetra-O-benzyl-β-D-Glcp-(1 ⟶ 2)-3,4-di-O-benzyl-α-L-Rhap-OMe
(18) 2,3,4,6-Tetra-O-benzyl-β-D-Glcp-(1 ⟶ 2)-3,4-di-O-benzyl-β-L-Rhap-OMe
(19) 3-O-Benzoyl-4,6-O-benzylidene-α-D-Manp-OMe
(20) 3-O-Benzoyl-4,6-O-benzylidene-β-D-Manp-OMe
(21) 2,3,4,6-Tetra-O-benzyl-α-D-Glcp-SEt
(22) 2,3,4,6-Tetra-O-benzyl-α-D-glucopyranosyl bromide
(23) 3,4-Di-O-benzyl-α-L-Rhap-SEt

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

Compound	Glycosyl donor (mg)	Aglycone precursor (mg)	Glycosidation method[a]	TLC[b]	R_F	Yield mg (%)	δ_C[d] C-1'	C-1
(9)/(11)	(21) (410)	(19) (180)	A	T:E (6:1)	0.57	162 (38)	100.40	99.07
					0.45	96 (23)	99.83	103.18
(10)/(12)	(21) (670)	(20) (260)	A	T:E (4:1)	0.56	235 (38)	101.42	97.21
					0.51	130 (21)	103.34	102.42
(13)/(14)	(22) (450)	(23) (200)	B	P:E (4:1)	0.37	415 (88)[c]	95.73	81.68[e]
					0.42		98.80	83.07
(15)/(16)	(13) (140)	MeOH	C	P:E (4:1)	0.17	670 (50)[c]	96.58	98.33
					0.18		96.27	102.49
(17)/(18)	(14) (410)	MeOH	C	T:E (4:1)	0.68	200 (50)[c]	104.66	100.64
					0.32		102.53	100.91

Compound	Protected precursor (mg, deprot. method)[a]		Yield mg (%)
(1)	(9)	(235; D + E)	58 (63)
(2)	(10)	(162; D + E)	48 (75)
(3)	(11)	(120; D + E)	24 (51)
(4)	(12)	(104; D + E)	38 (92)
(5)/(6)	(15)/(16)	(97; E)	25 (67)
(7)	(17)	(42; E)	16 (98)
(8)	(18)	(67; E)	15 (58)

[a] See Experimental section for details. [b] T = toluene, E = ethyl acetate, P = light petroleum (boiling range 60–71 °C). [c] Yields of α + β. Separation could not be accomplished quantitatively. [d] Tentative assignments. [e] Alternative signal at δ 81.44. [f] As in c and separation on silica gel before Bio-Gel P-2.

Table 2. Values for φ- and ψ-angles, in degrees, in the minimum-energy conformation, and inter-residue distances <3 Å in compounds (1)–(8) obtained by HSEA calculations.

Compound	φ/ψ	1'-H	5'-H	O-5'
(1) α-D-Glcp(1 ⟶ 2)-α-D-Manp-OMe	−45/−20	2.62 (O-3) 2.45 (2-H)	2.30 (1-H)	2.65 (2-H)
(2) α-D-Glcp(1 ⟶ 2)-β-D-Manp-OMe	−40/−18	2.60 (O-3) 2.30 (2-H)	2.40 (O-1)	2.66 (2-H)
(3) β-D-Glcp(1 ⟶ 2)-α-D-Manp-OMe	55/5	2.87 (1-H) 2.46 (2-H)		2.56 (2-H)
(4) β-D-Glcp(1 ⟶ 2)-β-D-Manp-OMe	54/8	2.84 (O-1) 2.39 (2-H)		2.48 (2-H)
(5) α-D-Glcp(1 ⟶ 2)-α-L-Rhap-OMe	−44/−26	2.36 (1-H) 2.49 (2-H)	2.48 (O-3)	2.64 (2-H)
(6) α-D-Glcp(1 ⟶ 2)-β-L-Rhap-OMe	−40/−22	2.50 (O-1) 2.34 (2-H)	2.46 (O-3)	2.64 (2-H)
(7) β-D-Glcp(1 ⟶ 2)-α-L-Rhap-OMe	58/−4	2.42 (2-H)		2.71 (1-H) 2.58 (2-H)
(8) β-D-Glcp(1 ⟶ 2)-β-L-Rhap-OMe	56/−2	2.34 (2-H)		2.78 (O-1) 2.53 (2-H)

between 1'-H and the equatorial substituent on one of the neighbouring carbons, i.e. O-3, H-1, or O-1. For all compounds O-5' is found close to the proton on the linkage carbon, 2-H, and for the α-glycosides (1), (2), (5), and (6) a short distance

between 5'-H and the equatorial 1-H, O-1, or O-3 is indicated.

^{1}H *NMR Glycosylation Shifts.*—The ^{1}H NMR chemical shifts and the glycosylation shifts (chemical-shift differences relative
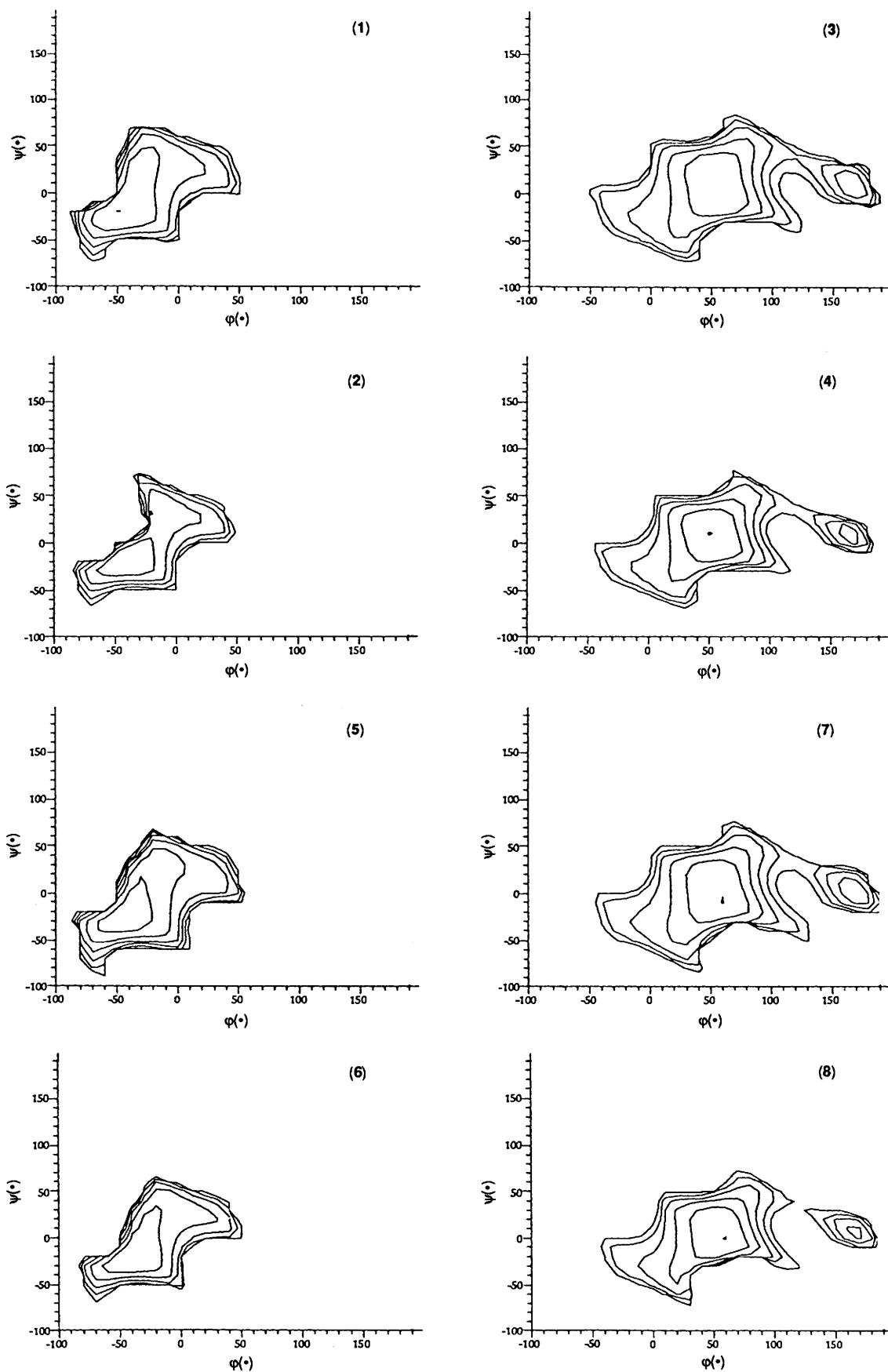
Figure 1. Conformational energy plots for disaccharides (**1**)—(**8**). Isocontour levels are indicated at 0.08, 8.4, 16.8, 25.1, 33.4, and 41.8 kJ above the minimum-energy conformation.

Table 3. ¹H NMR chemical shifts of disaccharides (1)–(8)[a] and appropriate monosaccharides obtained at 70 °C relative to internal TSP (δ 0.00). Glycosylation shifts[b] are given in parentheses.

	1'-H[a]	2'-H	3'-H	4'-H	5'-H	6'-H	1-H	2-H	3-H	4-H	5-H	6-H	OMe
(1) α-D-Glcp(1⟶2)-α-D-Manp-OMe	5.12 (-0.11)	3.56 (0.02)	3.75 (0.03)	3.41 (-0.01)	3.81 (-0.03)	3.74 (-0.02)	4.99 (0.22)	3.95 (0.01)	3.87 (0.10)	3.75 (0.08)	3.60 (-0.01)	3.76 (-0.02)	3.42 (0.00)
(2) α-D-Glcp(1⟶2)-β-D-Manp-OMe	5.16 (-0.07)	3.57 (0.03)	3.78 (0.06)	3.44 (0.02)	4.00 (0.16)	3.79 (-0.05)	4.59 (0.05)	4.08 (0.10)	3.73 (0.13)	3.67 (0.07)	3.40 (0.05)	3.76 (0.02)	3.53 (0.00)
(3) β-D-Glcp(1⟶2)-α-D-Manp-OMe	4.50 (-0.14)	3.35 (0.10)	3.50 (0.00)	3.43 (0.01)	3.44 (-0.02)	3.74 (0.02)	4.88 (0.11)	4.10 (0.16)	3.82 (0.05)	3.68 (0.01)	3.62 (0.01)	3.79 (0.01)	3.43 (0.01)
(4) β-D-Glcp(1⟶2)-β-D-Manp-OMe	4.57 (-0.07)	3.37 (0.12)	3.50 (0.00)	3.40 (-0.02)	3.45 (-0.01)	3.73 (0.01)	4.64 (0.10)	4.22 (0.24)	3.65 (0.05)	3.57 (-0.03)	3.40 (0.05)	3.76 (0.02)	3.55 (0.02)
(5) α-D-Glcp(1⟶2)-α-L-Rhap-OMe	5.02 (-0.21)	3.55 (0.01)	3.78 (0.06)	3.45 (0.03)	4.00 (0.16)	3.76 (0.00)	4.82 (0.13)	3.97 (0.04)	3.83 (0.11)	3.50 (0.05)	3.69 (0.03)	1.33 (0.03)	3.42 (0.02)
(6) α-D-Glcp(1⟶2)-β-L-Rhap-OMe	5.14 (-0.09)	3.50 (-0.04)	3.77 (0.05)	3.44 (0.02)	4.04 (0.20)	3.80 (0.04)	4.61 (0.08)	4.10 (0.11)	3.65 (0.08)	3.42 (0.04)	3.43 (0.05)	1.35 (0.04)	3.53 (0.01)
(7) β-D-Glcp(1⟶2)-α-L-Rhap-OMe	4.61 (-0.03)	3.36 (0.11)	3.51 (0.01)	3.44 (0.02)	3.44 (-0.02)	3.73 (0.01)	4.95 (0.26)	4.04 (0.11)	3.81 (0.09)	3.48 (0.03)	3.67 (0.01)	1.31 (0.01)	3.41 (0.01)
(8) β-D-Glcp(1⟶2)-β-L-Rhap-OMe	4.74 (0.10)	3.36 (0.11)	3.51 (0.01)	3.42 (0.00)	3.43 (-0.03)	3.73 (0.01)	4.57 (0.04)	4.24 (0.25)	3.67 (0.10)	3.46 (0.08)	3.39 (0.01)	1.33 (0.02)	3.52 (0.00)
α-D-Glucopyranose	5.23	3.54	3.72	3.42	3.84	3.76						3.84	
β-D-Glucopyranose	4.64	3.25	3.50	3.42	3.46	3.72						3.90	
Methyl α-D-mannopyranoside							4.77	3.94	3.77	3.67	3.61	3.90	3.42
Methyl β-D-mannopyranoside							4.54	3.98	3.60	3.60	3.35	3.92	3.53
Methyl α-L-rhamnopyranoside							4.69	3.93	3.72	3.45	3.66	1.30	3.40
Methyl β-L-rhamnopyranoside							4.53	3.99	3.57	3.38	3.38	1.31	3.52

[a] Primed labels refer to the D-glycopyranosyl group, and unprimed to the methyl glycoside residue. [b] Glycosylation shifts are calculated by subtraction of the chemical shifts from those of the corresponding hexose and methyl hexoside for the glycosyl part and the aglycone, respectively, a positive difference indicates a downfield shift.

Table 4. ^{13}C NMR chemical shifts of disaccharides (1)–(8)^a and appropriate monosaccharides obtained at 70 °C relative to internal dioxane (δ 67.40). Glycosylation shifts are given in parentheses.^{b}

	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
(1) α-D-Glcp(1⟶2)-α-D-Manp-OMe	101.69 (8.70)	72.73 (0.26)	73.82 (0.04)	70.69 (−0.02)	73.25 (0.88)	61.72 (−0.12)	100.38 (−1.37)	80.18 (9.33)	71.53 (−0.03)	68.17 (0.38)	73.46 (0.01)	61.94 (0.02)	55.72 (0.17)
(2) α-D-Glcp(1⟶2)-β-D-Manp-OMe	101.72 (8.73)	73.02 (0.55)	74.02 (0.24)	70.40 (−0.31)	72.67 (0.30)	61.40 (−0.44)	101.13 (−0.76)	78.80 (7.70)	74.62 (0.69)	68.54 (0.65)	77.35 (0.25)	62.05 (0.03)	57.62 (0.01)
(3) β-D-Glcp(1⟶2)-α-D-Manp-OMe	102.66 (5.82)	73.65 (−1.55)	76.44 (−0.32)	70.49 (−0.22)	76.88 (0.12)	61.64 (−0.20)	99.86 (−1.89)	78.39 (7.54)	70.68 (−0.88)	68.05 (0.26)	73.44 (−0.01)	61.56 (−0.36)	55.68 (0.13)
(4) β-D-Glcp(1⟶2)-β-D-Manp-OMe	104.16 (7.32)	74.36 (−0.84)	76.40 (−0.36)	70.46 (−0.25)	76.82 (0.06)	61.63 (−0.21)	102.07 (−0.18)	79.23 (8.13)	73.04 (−0.89)	68.37 (0.48)	77.33 (0.23)	61.87 (−0.15)	57.78 (0.17)
(5) α-D-Glcp(1⟶2)-α-L-Rhap-OMe	98.55 (5.56)	72.28 (−0.19)	73.71 (−0.07)	70.56 (−0.15)	72.90 (0.53)	61.48 (−0.36)	99.24 (−2.50)	77.09 (6.15)	70.72 (−0.58)	73.01 (0.00)	69.56 (0.33)	17.46 (0.00)	55.69 (0.15)
(6) α-D-Glcp(1⟶2)-β-L-Rhap-OMe	100.75 (7.76)	73.02 (0.55)	74.02 (0.24)	70.46 (−0.25)	72.89 (0.52)	61.46 (−0.38)	102.35 (0.47)	78.70 (7.48)	73.13 (−0.54)	73.13 (0.14)	73.32 (0.33)	17.46 (−0.03)	57.73 (0.14)
(7) β-D-Glcp(1⟶2)-α-L-Rhap-OMe	105.02 (8.18)	74.40 (−0.80)	76.61 (−0.15)	70.48 (−0.23)	76.78 (0.02)	61.67 (−0.17)	100.78 (−0.96)	80.53 (9.59)	71.16 (−0.14)	73.32 (0.31)	69.21 (−0.02)	17.46 (0.00)	55.70 (0.16)
(8) β-D-Glcp(1⟶2)-β-L-Rhap-OMe	103.83 (6.99)	74.48 (−0.72)	76.70 (−0.06)	70.48 (−0.23)	76.97 (0.21)	61.70 (−0.14)	101.15 (−0.73)	78.56 (7.34)	74.29 (0.62)	73.45 (0.46)	73.32 (0.33)	17.51 (0.02)	57.48 (−0.11)
α-D-Glucopyranose	92.99	72.47	73.78	70.71	72.37	61.84							
β-D-Glucopyranose	96.84	75.20	76.76	70.71	76.76	61.84							
Methyl α-D-mannopyranoside							101.75	70.85	71.56	67.79	73.45	61.92	55.55
Methyl β-D-mannopyranoside							101.89	71.10	73.93	67.89	77.10	62.02	57.61
Methyl α-L-rhamnopyranoside							101.74	70.94	71.30	73.01	69.23	17.46	55.54
Methyl β-L-rhamnopyranoside							101.88	71.22	73.67	72.99	72.99	17.49	57.59

^{a} Primed labels refer to the D-glycopyranosyl group, and unprimed to the methyl glycoside residue. ^{b} Glycosylation shifts are calculated by subtraction of the corresponding hexose and methyl hexoside chemical shifts for the glycosyl part and the aglycone, respectively, and a positive difference indicates a downfield shift.

Table 5. ^{13}C Chemical shift differences (ppm) with variation in 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) α-D-Glcp-(1 ⟶ 2)-α-D-Manp-OMe	−0.06	0.07	0.23	0.13	0.12	0.15	0.21	−0.25	0.18	0.27	0.10	0.15	0.06
(2) α-D-Glcp-(1 ⟶ 2)-β-D-Manp-OMe	0.08	0.13	0.21	0.30	0.08	0.30	0.03	0.08	0.08	0.25	0.00	0.16	−0.08
(3) β-D-Glcp-(1 ⟶ 2)-α-D-Manp-OMe	0.10	0.12	0.19	0.14	0.10	0.16	0.11	0.22	0.14	0.37	0.17	0.31	0.02
(4) β-D-Glcp-(1 ⟶ 2)-β-D-Manp-OMe	0.04	0.13	0.16	0.15	0.13	0.15	−0.04	0.31	0.15	0.26	0.04	0.18	−0.01
(5) α-D-Glcp-(1 ⟶ 2)-α-L-Rhap-OMe	0.12	0.09	0.18	0.27	0.20	0.34	0.13	0.25	0.26	0.31	0.02	0.07	0.03
(6) α-D-Glcp-(1 ⟶ 2)-β-L-Rhap-OMe	−0.03	0.09	0.23	0.28	0.24	0.37	0.06	0.13	0.26	0.24	0.01	0.06	0.04
(7) β-D-Glcp-(1 ⟶ 2)-α-L-Rhap-OMe	−0.07	0.19	0.25	0.22	0.11	0.25	0.22	−0.35	0.24	0.23	0.02	0.05	0.01
(8) β-D-Glcp-(1 ⟶ 2)-β-L-Rhap-OMe	0.09	0.12	0.15	0.19	0.05	0.21	0.19	0.02	0.06	0.17	0.04	0.02	−0.02

[a] $\delta = \delta(70°) - \delta(30°)$. Dioxane was taken as having δ_C 67.40 for all temperatures.

to the chemical shifts of the respective monomers) are given in Table 3. 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.

The glycosylation shifts range from −0.21 to 0.26 ppm. Significant ^1H NMR glycosylation shifts (>0.05 ppm) are confined not only to signals from protons on linkage carbons and carbons next to these but are found *inter alia* also for the signal from 5'-H in the α-glycosides (2), (5), and (6) and from 4-H in the α-glycosides (1) and (2), and the β-glycoside (8). For the glycosyl group in compounds (1)–(6), the glycosylation shifts for the 1'-H signals are between −0.07 and −0.21 ppm, whereas in β-glycosides in (7) and (8) the shift is small or downfield. According to Table 2 a short distance between 1'-H and 1-H is calculated for the minimum-energy conformation of compounds (3) and (5) and this could be correlated to a large upfield shift, −0.14 and −0.21 ppm, respectively, for the corresponding anomeric signals. In compounds (1), (2), (4), and (6) 1'-H is near both 2-H and an oxygen, O-1 or O-3, but there is still a net upfield shift. Similar shifts have been observed also for 4-substituted D-galactosides in which a short distance between 1'-H and the adjacent oxygen is also found.[5] A downfield shift of the 1'-H signal in β-glycoside (8) does not correlate to a short distance to oxygen in the minimum-energy conformation. For the α-glycosides a downfield shift of the 5'-H signal could be correlated to the short distance between 5'-H and O-1 in compound (2), and O-3 in compound (5) and (6). In α-glycoside (1) the small upfield shift of the 5'-H signal corresponds to the short distance between 5'-H and 1-H instead. For the β-glycosides the commonly observed downfield shift of ∼0.10 ppm for the 2'-H signal is again found.

For the methyl glycoside residue in glycosides (1)–(8) signals are mainly shifted for protons at or next to the linkage carbon. There is a correlation between the glycosylation shift for the 2-H signal and the calculated distance between O-5' and 2-H. A shorter distance corresponds to a larger downfield shift. Compounds (1)–(8) constitute a set of glycosidic linkages with different stereochemistry not previously investigated, and few comparisons can be made with other disaccharides. It may be noted, however, that the glycosylation shifts for the 1-H–3-H signals in the α-glycosides (1) and (2) are different, but those from the β-glycosides (3) and (4) are similar. The same pattern is observed for compounds (5)–(8) but with similarities for the α-glycosides and differences for the β-glycosides instead. The large glycosylation shift of the 1-H signal in glycosides (1) and (7) could be the result of O-5' being opposite to the equatorial 1-H. When 1-H is axial as in compounds (2) and (8) a smaller shift is observed. The glycosylation shift for 4-H varies between −0.03 and 0.08 ppm. The shift for the signal from 4-H, which is 1,3-diaxially disposed to O-2, has been postulated[5,6] to derive from the change of 2-OH to 2-O-glycosyl with the concomitant change

of the direction of the lone pairs on the glycosidic oxygen (= O-2) and this may be the reason here also.

^{13}C *NMR Glycosylation Shifts.*—The ^{13}C NMR chemical shifts for compounds (1)–(8) and relevant monomers together with the glycosylation shifts, obtained upon comparison with the chemical shifts of the respective monomers, are given in Table 4.

For most compounds large shifts are found for the signals from anomeric carbons, relative to, *e.g.*, that in cellobiose, 6.5 ppm, and maltose, 7.6 ppm, indicating that an axial oxygen is substituted and involved in the glycosidic linkage. For compounds (3) and (5), however, a short distance between 1'-H and the equatorial 1-H is calculated (γ-*gauche*) and, as observed earlier,[3,23] a relative smaller shift, 5.82 and 5.56 ppm, respectively, is found. For β-glycosides an upfield shift of the C-2' signal is commonly found; for compounds (3), (4), (7), and (8) it ranges from −0.72 to −1.55 ppm.

For signals from carbons adjacent to the linkage large upfield shifts have been observed for signals from carbons with a proton engaged in a γ-*gauche* interaction[3,23] and for compounds (3) and (5) shifts of −1.89 and −2.50 ppm are observed for the C-1 signal. The range of shifts for signals from other neighbouring carbons is between −1.4 and 0.7 ppm. Such large positive values have only been observed previously for axially 4-substituted galactosides.[5] The same pattern of the glycosylation shifts for signals from C-1–C-3 are observed for compounds (1), (2), (7), and (8) which are α-D/D- and β-D/L-glycosides, and compounds (3), (4), (5), and (6) which are β-D/D- and α-D/L-glycosides, respectively. Differences are observed for the glycosylation shifts for the methyl α- and β-glycosides, affecting all three C-1–C-3 signals. The short distances, <3 Å, in the minimum-energy conformation are the same but the interaction between O-5' and the opposing substituent on the methyl glycoside residue differs. Significant downfield shifts of signals from almost all C-4 atoms are observed, probably because of differences in the direction of the lone pairs on O-2, something which was also discussed for the ^1H NMR glycosylation shifts.

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 5. 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 difference is larger than −0.35 or 0.37 ppm. For compounds (1) and (7) there is a negative shift for the C-1' signal, which is paired with a negative shift of the signal from the substituted carbon, C-2, in the aglycone. Significant shifts for signals from C-4 are noteworthy.

Acknowledgements

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