

## N.m.r. and Conformational Studies of the Methyl Glycosides of some 1,2- and 1,3-Linked Disaccharides

Per-Erik Jansson, Lennart Kenne, and Elke Schweda

Department of Organic Chemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden

$^1\text{H}$  and  $^{13}\text{C}$  N.m.r. studies and conformational analysis have been performed on the methyl glycosides of ten 1,2- and 1,3-linked disaccharides in which the glycosidic linkages have different stereochemical surroundings. Conformational analysis, using the HSEA-approach, indicates a number of proton-oxygen and proton-proton interactions, resulting, *inter alia*, in downfield and upfield shifts of anomeric proton signals, respectively. The  $^{13}\text{C}$  n.m.r. glycosylation shifts obtained have been used to simulate spectra of 1,3-linked  $\alpha$ - and  $\beta$ -D-glucan.

In previous reports, a number of methyl glycosides of 1,3-,<sup>1</sup> 1,4-,<sup>2</sup> and 1,6-linked<sup>3</sup> disaccharides were characterised by  $^1\text{H}$  and  $^{13}\text{C}$  n.m.r. spectroscopy in order to establish a database for a computer program predicting n.m.r. spectra from oligo- and poly-saccharides<sup>4</sup> and to increase the knowledge about the spectral characteristics of oligosaccharides with different types of glycosidic linkages. Attempts have also been made to correlate data from computer-calculated conformations with  $^1\text{H}$  and  $^{13}\text{C}$  n.m.r. chemical shifts.<sup>1,2</sup> In this work we present data obtained from some 1,2- and 1,3-linked disaccharide glycosides, namely  $^1\text{H}$  and  $^{13}\text{C}$  n.m.r. chemical shifts and the temperature dependence of the  $^{13}\text{C}$  n.m.r. chemical shifts. The n.m.r. data have also been correlated with the internuclear distances in highly populated conformations, as estimated by HSEA-calculations.<sup>5,6</sup> For some of the disaccharide glycosides or the corresponding reducing oligosaccharides, tentative  $^{13}\text{C}$  n.m.r. assignments had been made<sup>7</sup> and some  $^1\text{H}$  n.m.r. data were also available.<sup>8</sup>

### Experimental

**General Methods.**—Concentrations were performed under reduced pressure at  $<40^\circ\text{C}$ . Column chromatography was performed on silica gel 60 (0.040–0.063 mm; Merck).  $^1\text{H}$  N.m.r. spectra (400 MHz) and  $^{13}\text{C}$  n.m.r. spectra (100 MHz) were recorded for solutions in deuterium oxide at  $70^\circ\text{C}$  with a JEOL GX-400 spectrometer. Chemical shifts are given in p.p.m. using sodium [ $^2\text{H}_4$ ]-3-trimethylsilylpropanoate ( $\delta$  0.00,  $^1\text{H}$  n.m.r.) and dioxane ( $\delta_{\text{C}}$  67.40) as internal references. For the assignment of signals proton-proton and proton-carbon shift-correlation spectroscopy (COSY) were used. Chemical shifts of overlapping signals were obtained from the centre of the cross-peaks in the proton-proton shift correlation spectra. The HSEA program<sup>5,6</sup> was used to estimate minimum energy conformations and rotational freedom. This program accounts for non-bonded interactions as expressed by the Kitaigorodsky algorithm, together with a term for the exoanomeric effect. The torsional angles  $\varphi$  and  $\psi$  were defined in a 1,2-linked disaccharide by  $\text{H}(1')\text{-C}(1')\text{-O}(2)\text{-C}(2)$  and  $\text{C}(1')\text{-O}(2)\text{-C}(2)\text{-H}(2)$ , respectively. The bond angle  $\tau$  [ $\text{C}(1')\text{-O}(2)\text{-C}(2)$ ] was set as  $117^\circ$ . Co-ordinate sets for  $\alpha$ -D-glucopyranose,<sup>9</sup> methyl  $\alpha$ -D-glucopyranoside,<sup>10</sup>  $\beta$ -D-glucopyranose,<sup>11</sup> methyl  $\beta$ -D-glucopyranoside,<sup>12</sup> and  $\alpha$ -L-fucopyranose<sup>13</sup> were obtained from crystal structure data.

**Synthesis.**—Compounds (1)–(10) are listed in Table 2. The  $\alpha$ -linked disaccharides (1), (2), (5), and (6) were obtained by reaction of 2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl chloride

with the appropriate aglycone and silver trifluoromethanesulphonate.<sup>14</sup>

The  $\beta$ -linked disaccharides (3), (4), (7), and (8) were obtained by the reaction of the appropriate aglycones with 2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl bromide and silver trifluoromethanesulphonate.<sup>15,16</sup> The disaccharides (1)–(8) have been synthesized before<sup>17–22</sup> and therefore only a general experimental description is given. For (9) and (10) full experimental details are given below.

**Synthesis of the  $\alpha$ -Linked Disaccharides (1), (2), (5), and (6).**—For the synthesis of (1) a solution of freshly prepared 2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl chloride<sup>23</sup> (345 mg, 0.62 mmol) in dichloromethane was added, with stirring, to a mixture of methyl 3-*O*-acetyl-4,6-*O*-benzylidene- $\alpha$ -D-glucopyranoside<sup>24</sup> (100 mg, 0.31 mmol), silver trifluoromethanesulphonate (158 mg, 0.62 mmol), ground 4 Å molecular sieves and 2,4,6-trimethylpyridine (0.04 ml) in dichloromethane kept at room temperature. The reaction was monitored by t.l.c. and after completion the mixture was filtered, and the solution was washed consecutively with 10% aqueous sodium thiosulphate and water, dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated to dryness. Chromatography of the residue on silica gel using toluene-ethyl acetate (4:1) as the eluant gave the pure disaccharide derivative. Deprotection was effected by hydrogenolysis with 10% Pd-C in aqueous 90% acetic acid at 400 kPa for 16 h, followed by de-*O*-acetylation with 0.1M sodium methoxide in methanol. The solution was deionized by passage through Dowex 50 ( $\text{H}^+$ ) and then concentrated to dryness. The product was purified on a column of Biogel P-2 eluting with water. The disaccharides (2), (5), and (6) were synthesized analogously. Yields of (1), (2), (5), and (6) were 31, 87, 60, and 86%, respectively, as calculated from the amount of aglycone in the coupling reaction. Physical data were in accordance with literature values.

**Synthesis of the  $\beta$ -Linked Disaccharides (3), (4), (7), and (8).**—For the synthesis of (3) silver trifluoromethanesulphonate (210 mg, 0.96 mmol) was added to a stirred solution kept at  $-25^\circ\text{C}$ , of 2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl bromide (461 mg, 0.70 mmol) and methyl 3-*O*-acetyl-4,6-*O*-benzylidene- $\alpha$ -D-glucopyranoside (206 mg, 0.64 mmol) in nitromethane-toluene (1:1; 25 ml) containing ground 4 Å molecular sieves. The reaction was monitored by t.l.c. and when complete (15 min) pyridine (1 ml) was added and the mixture was allowed to reach room temperature. It was then diluted with nitromethane-toluene and filtered. The filtrate was washed successively with 10% aqueous sodium thiosulphate, water, 2M sulphuric acid, saturated aqueous sodium hydrogen carbonate, and water. The

dried ( $\text{MgSO}_4$ ) extract was filtered and concentrated and the residue was subjected to chromatography on silica gel [toluene-ethyl acetate (4:1)]. The pure disaccharide derivative was de-*O*-acetylated by treatment with 0.1M-sodium methoxide in methanol and the benzylidene group was removed by treatment with aqueous 90% trifluoroacetic acid. The product was purified on a column of Biogel P-2. The yields of (3), (4), (7), and (8) were 63, 87, 73, and 80%, respectively, as calculated from the amounts of aglycone in the coupling reaction.

*Synthesis of (9).*—Methyl trifluoromethanesulphonate (0.8 ml) was added to a solution of ethyl 2,3,4-tri-*O*-benzyl-1-thio- $\beta$ -L-fucopyranoside<sup>25</sup> (335 mg, 0.70 mmol) and methyl 3-*O*-acetyl-4,6-*O*-benzylidene- $\alpha$ -D-glucopyranoside (150 mg, 0.50 mmol) in

dichloromethane (25 ml) containing ground 4 Å molecular sieves.<sup>25</sup> When, according to t.l.c., the reaction was complete, triethylamine (1 ml) was added. The reaction mixture was then filtered through Celite, concentrated to dryness and the product was purified by chromatography on silica gel with toluene-ethyl acetate (4:1) as the eluant. The protected disaccharide (172 mg, 50%) showed m.p. 164 °C (crystallised from iso-octane-toluene) and  $[\alpha]_{578}^{20} -17^\circ$  (*c* 1.0,  $\text{CH}_2\text{Cl}_2$ ). De-*O*-acetylation and hydrogenolysis were performed as above and the product purified on a column of Biogel P-2 yielding (9),  $[\alpha]_{578}^{20} -16^\circ$  (*c* 1.0, water).

*Synthesis of (10).*—Methyl trifluoromethanesulphonate (0.8 ml) was added at 0 °C to a stirred solution of ethyl 2,3,4-tri-*O*-

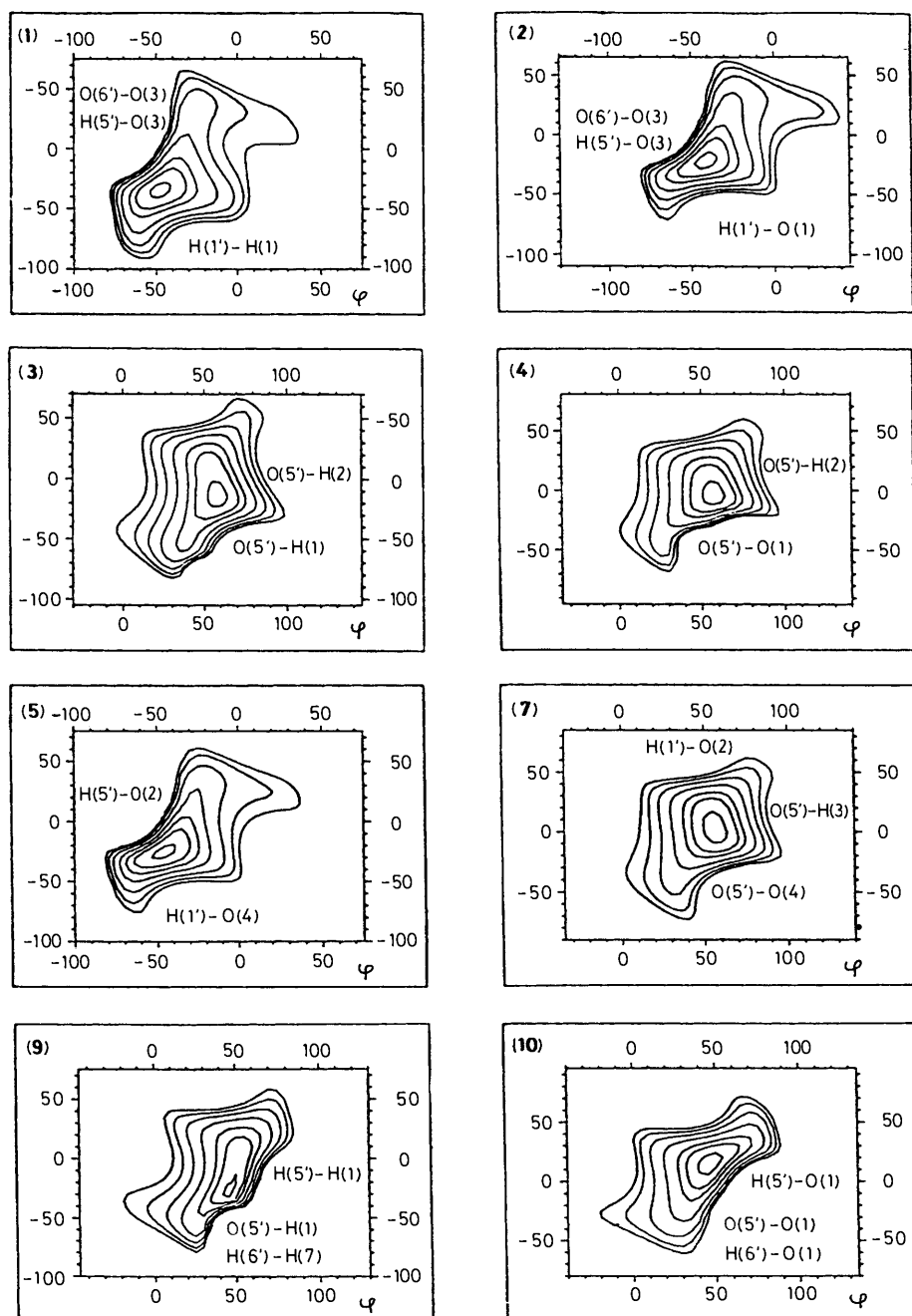


Figure 1.

benzyl-1-thio- $\beta$ -L-fucopyranoside (325 mg, 0.68 mmol) and methyl 3-*O*-benzyl-4,6-*O*-benzylidene- $\beta$ -D-glucopyranoside<sup>27</sup> (179 mg, 0.50 mmol) in dichloromethane (25 ml) containing 4 Å molecular sieves. After 45 min triethylamine (1 ml) was added. The reaction mixture was filtered through Celite, concentrated to dryness and purified by chromatography on silica gel using toluene-ethyl acetate (6:1) as the eluant. The amorphous product (143 mg, 37%),  $[\alpha]_{578}^{20} -71^\circ$  (*c* 2.4, CH<sub>2</sub>Cl<sub>2</sub>) was subjected to hydrogenolysis and purified on a column of Biogel P-2 to yield (10),  $[\alpha]_{578}^{20} -99^\circ$  (*c* 1.0, water).

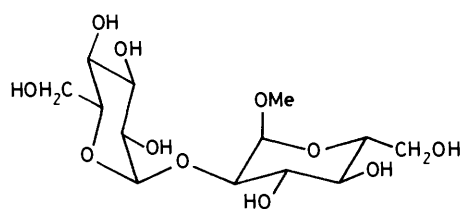
**Preparation of  $\alpha$ -1,3- and  $\beta$ -1,3-Linked D-Glucans.**—A solution of  $\alpha$ -glucan (300 mg) from *Streptococcus salivarius* LP-1<sup>27</sup> in 0.14M acetate buffer (pH 5.7; 39 ml) was treated with sodium metaperiodate (1.26 g) at 5 °C for 5 days in the dark. The excess of periodate was then reduced with ethylene glycol (1.5 ml) and the solution was dialysed overnight and freeze-dried. The product was dissolved in water (20 ml) and reduced with sodium borohydride (100 mg) for 4 h, whereafter the solution was neutralised, dialysed and freeze-dried. The product was hydrolysed with trifluoroacetic acid (0.5M; 50 ml) at room temperature for 16 h and the hydrolysate was diluted with water and freeze-dried. By this procedure side-chains in the  $\alpha$ -glucan and eventual  $\alpha$ -1,6-glucan were removed.

A  $\beta$ -1,3-glucan suitable for n.m.r. studies was obtained by solvolysis of a curdlan<sup>28</sup> (200 mg) with 90% formic acid (10 ml) for 5 h at 100 °C followed by reduction with sodium borohydride and hydrolysis of formate esters with aqueous 0.1M sodium hydroxide. The product was fractionated on a column of Sephadex G-25 eluted with water, and the high molecular fraction was collected and freeze-dried.

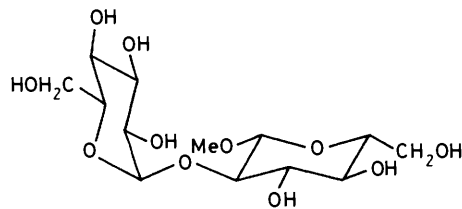
## Results and Discussion

**HSEA Calculations.**—The  $\phi/\psi$ -energy plots together with proximities derived from changes in  $\phi$  and  $\psi$  of (1)—(5), (7), (9), and (10) are shown in the Figure. All inter-residue internuclear distances below 3.0 Å for the minimum energy conformations and the minimum internuclear distances for conformations at the 8.3 kJ level above the minimum for (1)—(10) are given in Table 1. Of these (1), (2), (5), (6), (9), and (10) are termed  $\alpha$ -glycosides with reference to the central bond and consequently the remaining compounds are termed  $\beta$ -glycosides.

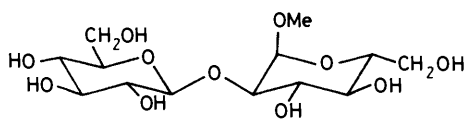
The absolute value of  $\phi$  in the minimum energy conformations of (1)—(10) is 50° ( $\pm 10^\circ$ ) whereas the values for  $\psi$  vary within a range of 50°. As observed from the energy maps (Figure) restricted rotational freedom for smaller  $\phi/4$  values for the  $\alpha$ -D-glycosides are present due to contacts from 5'(H). The



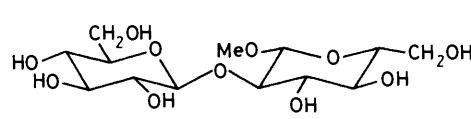
(1)



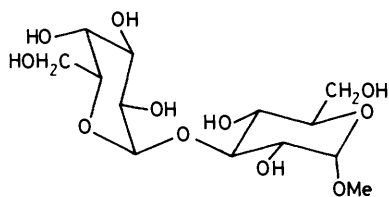
(2)



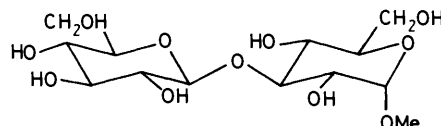
(3)



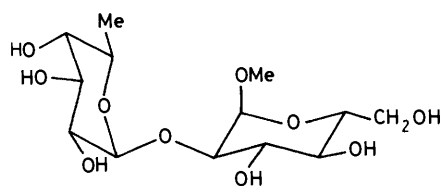
(4)



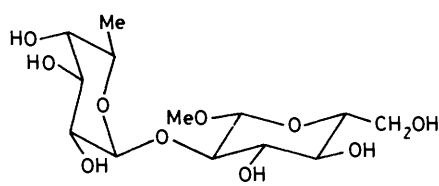
(5)



(7)



(9)



(10)

**Table 1.** Values for the  $\phi$  and  $\psi$  angles, in degrees, together with inter-residual internuclear distances  $< 3 \text{ \AA}$  in the minimum energy conformations of (1)–(10), as indicated by HSEA calculations. The minimum internuclear distances at the 8.3 kJ level are also given

Substance	$\phi/\psi$ Angles	Inter-residual internuclear distances (minimum energy molecule)				Range of $\phi/\psi$ angles	Inter-residual internuclear distances (at the 8.3 kJ level)				
		1'-H	5'-H	O-5'	6'-H		1'-H	5'-H	O-5'	6'-H	O-6'
$\alpha$ -D-Glcp(1 $\rightarrow$ 2) $\beta$ -D-GlcpOMe (1)	-50/-35	2.29 (1-H)	2.58 (O-3)	2.45 (2-H)	6'-H	-72/-14 <sup>a</sup>	-1.89 <sup>c</sup> (1-H)	-2.08 (O-3)	-2.19 (2-H)	0-6'	-2.89 (2-H)
$\alpha$ -D-Glcp(1 $\rightarrow$ 2) $\beta$ -D-GlcpOMe (2)	-40/-20	2.60 (2-H)	2.88 (2-H)	2.70 (2-H)	6'-H	-71/12 <sup>b</sup>	-2.03 (2-H)	-2.01 (2-H)	-2.64 (1-H)	0-6'	-2.86 (2-H)
$\beta$ -D-Glcp(1 $\rightarrow$ 2) $\alpha$ -D-GlcpOMe (3)	60/-15	2.37 (2-H)	2.48 (O-3)	2.60 (O-1)	6'-H	-73/-14	-2.02 (2-H)	-2.12 (O-3)	-2.24 (2-H)	0-6'	-2.86 (2-H)
$\beta$ -D-Glcp(1 $\rightarrow$ 2) $\beta$ -D-GlcpOMe (4)	55/5	2.48 (2-H)	2.54 (2-H)	2.58 (2-H)	6'-H	-48/-37	-2.09 (O-1)	-2.86 (3-H)	-2.14 (2-H)	0-6'	-2.99 (1-H)
$\alpha$ -D-Glcp(1 $\rightarrow$ 3) $\alpha$ -D-GlcpOMe (5)	-45/-25	2.91 (O-3)	2.49 (3-H)	2.62 (3-H)	6'-H	26/82	-2.04 (2-H)	-2.09 (3-H)	-2.11 (O-2)	0-6'	-2.97 (7-H)
$\alpha$ -D-Glcp(1 $\rightarrow$ 3) $\beta$ -D-GlcpOMe (6)	-40/-20	2.53 (O-4)	2.41 (3-H)	2.75 (3-H)	6'-H	-70/37	-2.14 (O-3)	-2.93 (2-H)	-2.07 (1-H)	0-6'	-2.45 (7-H)
$\beta$ -D-Glcp(1 $\rightarrow$ 3) $\alpha$ -D-GlcpOMe (7)	55/0	2.41 (3-H)	2.44 (O-2)	2.59 (3-H)	6'-H	30/80	-2.12 (1-H)	-2.11 (O-2)	-2.16 (2-H)	0-6'	-2.45 (7-H)
$\beta$ -D-Glcp(1 $\rightarrow$ 3) $\beta$ -D-GlcpOMe (8)	55/5	2.47 (3-H)	2.58 (3-H)	2.58 (3-H)	6'-H	-29/34	-2.13 (2-H)	-2.09 (O-2)	-2.22 (3-H)	0-6'	-2.83 (3-H)
$\alpha$ -L-Fucp(1 $\rightarrow$ 2) $\alpha$ -D-GlcpOMe (9)	45/-30	2.85 (O-2)	2.13 (2-H)	2.67 (1-H)	6'-H	-75/-16	-2.15 (3-H)	-2.10 (3-H)	-2.23 (3-H)	0-6'	-2.83 (3-H)
$\alpha$ -L-Fucp(1 $\rightarrow$ 2) $\beta$ -D-GlcpOMe (10)	45/-30	2.13 (2-H)	2.94 (7-H)	2.48 (1-H)	6'-H	-52/-36	-2.20 (O-4)	-2.84 (2-H)	-2.27 (3-H)	0-6'	-2.83 (3-H)
$\alpha$ -L-Fucp(1 $\rightarrow$ 2) $\beta$ -D-GlcpOMe (10)	45/15	2.41 (2-H)	2.58 (O-1)	2.27 (2-H)	6'-H	-74/-14	-2.93 (4-H)	-2.11 (O-2)	-2.27 (3-H)	0-6'	-2.83 (3-H)
$\alpha$ -L-Fucp(1 $\rightarrow$ 2) $\beta$ -D-GlcpOMe (10)	45/15	2.65 (O-3)	2.65 (O-3)	2.27 (2-H)	6'-H	-55/37	-2.05 (3-H)	-2.10 (3-H)	-2.27 (3-H)	0-6'	-2.83 (3-H)

<sup>a</sup>  $\phi_{\text{min.}/\text{max.}}$ , <sup>b</sup>  $\psi_{\text{min.}/\text{max.}}$ , <sup>c</sup>  $\rightarrow 1.89$  indicates a distance  $> 3 \text{ \AA}$  down to 1.89  $\text{\AA}$ .

**Table 2.**  $^1\text{H}$  N.m.r. chemical shifts<sup>a</sup> of the disaccharides (1)–(10) at 70 °C. Chemical shift differences are given in parentheses

Substance	1'-H	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
(1)	5.07 (-0.16)	3.56 (0.02)	3.78 (0.06)	3.45 (0.03)	3.92 (0.08)	3.85 (0.01)	3.77 (0.01)	5.01 (0.20)	3.66 (0.10)	3.78 (0.10)	3.47 (0.06)	3.66 (0.02)	3.88 (0.01)	3.77 (0.01)	3.45 (0.01)
(2)	5.28 (0.05)	3.54 (0.0)	3.75 (0.03)	3.44 (0.02)	4.00 (0.16)	3.83 (-0.01)	3.77 (0.01)	4.51 (0.14)	3.40 (0.12)	3.60 (0.10)	3.42 (0.02)	3.45 (-0.01)	3.93 (0.01)	3.74 (0.0)	3.57 (0.0)
(3)	4.62 (-0.02)	3.37 (0.12)	3.52 (0.02)	3.43 (0.01)	3.43 (-0.03)	3.91 (0.01)	3.76 (0.04)	4.99 (0.18)	3.67 (0.11)	3.84 (0.16)	3.45 (0.04)	3.65 (0.01)	3.88 (0.01)	3.77 (0.01)	3.42 (0.01)
(4)	4.76 (0.12)	3.34 (0.09)	3.52 (0.02)	3.43 (0.01)	3.41 (-0.05)	3.92 (0.02)	3.74 (0.02)	4.48 (0.11)	3.56 (0.28)	3.70 (0.20)	3.43 (0.03)	3.44 (-0.02)	3.91 (-0.01)	3.75 (0.01)	3.57 (0.01)
(5)	5.32 (0.09)	3.58 (0.04)	3.76 (0.04)	3.44 (0.02)	3.97 (0.13)	3.87 (0.03)	3.78 (0.02)	4.82 (0.01)	3.66 (0.10)	3.82 (0.14)	3.66 (0.25)	3.66 (0.02)	3.85 (-0.02)	3.76 (0.0)	3.44 (0.0)
(6)	5.32 (0.09)	3.58 (0.04)	3.76 (0.04)	3.45 (0.03)	3.98 (0.14)	3.84 (0.0)	3.77 (0.01)	4.40 (0.03)	3.40 (0.12)	3.65 (0.15)	3.65 (0.25)	3.49 (0.03)	3.92 (0.0)	3.74 (0.0)	3.58 (0.0)
(7)	4.68 (0.04)	3.37 (0.12)	3.54 (0.04)	3.43 (0.01)	3.50 (0.04)	3.92 (0.02)	3.74 (0.02)	4.83 (0.02)	3.75 (0.19)	3.87 (0.19)	3.52 (0.11)	3.67 (0.03)	3.88 (0.01)	3.77 (0.01)	3.44 (0.01)
(8)	4.71 (0.07)	3.38 (0.13)	3.54 (0.04)	3.43 (0.01)	3.49 (0.03)	3.93 (0.03)	3.74 (0.02)	4.40 (0.03)	3.48 (0.20)	3.74 (0.24)	3.50 (0.10)	3.49 (0.03)	3.93 (0.01)	3.74 (0.0)	3.58 (0.0)
(9)	5.07 (-0.13)	3.79 (0.02)	3.89 (0.03)	3.83 (0.02)	4.10 (-0.10)	1.25 (0.04)	4.90 (0.09)	3.54 (-0.02)	3.54 (0.13)	3.81 (0.07)	3.48 (0.07)	3.63 (-0.01)	3.88 (0.01)	3.77 (0.01)	3.45 (0.01)
(10)	5.17 (-0.03)	3.81 (0.04)	3.86 (0.0)	3.81 (0.0)	4.23 (0.03)	1.22 (0.01)	4.45 (0.08)	3.36 (0.08)	3.69 (0.19)	3.41 (0.01)	3.44 (-0.02)	3.92 (0.0)	3.74 (0.0)	3.57 (0.0)	
$\alpha$ -D-Gluco- pyranose	5.23	3.54	3.72	3.42	3.84	3.84	3.76								
$\beta$ -D-Gluco- pyranose	4.64	3.25	3.50	3.42	3.46	3.90	3.72								
$\alpha$ -L-Fuco- pyranose	5.20	3.77	3.86	3.81	4.20	1.21									
Methyl $\alpha$ -D-gluco- pyranoside								4.81	3.56	3.68	3.41	3.64	3.87	3.76	3.39
Methyl $\beta$ -D-gluco- pyranoside								4.37	3.28	3.50	3.40	3.46	3.92	3.74	3.57

<sup>a</sup> Chemical shifts are given in p.p.m. relative to internal sodium [ $^2\text{H}_4$ ]-3-trimethylsilylpropanoate (0.00 p.p.m.).

shape of the map for (9) and (10) are similar to (3) and (4), respectively, and not to (1) and (2), showing that  $\alpha$ -L/D- and  $\beta$ -D/D-disaccharides are similar. This pattern was also observed for 1,4-linked disaccharides.<sup>2</sup> In compounds (1)–(10) there are several stereochemical arrangements around the glycosidic linkage and the interacting atoms differ. The 1,3-linked pairs (5), (6), and (7), (8) only differ at the anomeric centre of the methyl glycoside residue and give virtually the same energy maps and have similar internuclear interactions. The pairs (2), (6) and (4), (8) have the same anomeric configuration and the glycosyl group substitutes an equatorial position which has two neighbouring equatorial substituents. One important difference between the pairs is, however, that in (2) and (4) the methyl group, as aglycone, is affected by the *exo*-anomeric effect. As a consequence, not only the methyl group but also the lone-pairs of O(1) should have preferred orientations. The fact that O(1) bears a methyl group has no significant effect on the result of the calculations. The HSEA calculations, which do not include oxygen lone-pairs or hydrogens linked to oxygens, do not show any significant differences between (2), (6) or (4), (8). The 1,2-linked (2) and (10) are also similar but with proximity of 1'(H) to O(1) in (2) and to O(3) in (10). The energy maps of these compounds are also similar after mirror imaging of both  $\phi$  and  $\psi$  to compensate for the different absolute configurations of the glycosyl group.

The  $\alpha$ -linked disaccharides have, in contradistinction to the  $\beta$ -linked disaccharides, a long-range interaction from 5'(H) to the opposing equatorial substituent next to the linkage in the other ring. The anomeric proton, 1'(H) and the ring oxygen, O(5'), are always close to the proton on the linkage carbon and the anomeric proton is in most examples also in proximity with one of the neighbouring substituents.

<sup>1</sup>H N.m.r. Chemical Shifts.—The  $^1\text{H}$  n.m.r. chemical shifts and the induced chemical shift differences relative to the chemical shifts of the respective monomers are given in Table 2. Chemical shifts of signals which are not of first order are approximate only.

In the glycosyl groups upfield shifts for signals of anomeric protons are observed for (1), (3), (9), and (10). Similar shifts have been observed for those 1,4-linked disaccharides for which HSEA-calculated conformations indicate additional proton interactions to 1'(H).<sup>2</sup> Such interaction is clearly indicated in (1) and to lesser extent in (9). For (3) and (10) proximity to both hydrogen and oxygen for 1'(H) are indicated and the anomeric signals have only a minor shift. For the compounds in which the anomeric proton is shifted downfield an interaction with oxygen is indicated in the HSEA-calculations (Table 1). In addition to the effects observed for the anomeric protons downfield shifts for 2'(H) of the  $\beta$ -linked disaccharides are observed. Signals for 5'(H) are shifted downfield in the  $\alpha$ -linked D-glycosides but are almost unaffected or shifted upfield in the  $\beta$ -linked disaccharides. Calculated proximities for 5'(H) to oxygen or hydrogen are correlated to downfield or upfield shifts, respectively.

In the methyl glycoside residues substituent shifts between 0.1 and 0.3 p.p.m. are observed for protons on the glycosyloxylated and/or on the adjacent carbons except for (9) and (10). The stereochemical similarity between (2) and (6) was indicated by the HSEA calculations but 1(H) and 3(H) in (2) correspond to 4(H) and 2(H) in (6), respectively. The signal for 1(H) in (2) is, however, less shifted than the signal for 4(H) in (6). In (4) and (8), which also have similar stereochemistry, there is better agreement between the corresponding shifts.

For all compounds in which 1'(H) has a calculated proximity to an oxygen in the methyl glycoside residue, e.g. to O(3), the

**Table 3.**  $^{13}\text{C}$  N.m.r. chemical shifts of disaccharides (1)–(10) and pertinent monosaccharides, all obtained at 70 °C. Chemical shift differences are given in parentheses

Substance	C-1' <sup>a</sup>	C-2'	C-3'	C-4'	C-5'	C-6'	C-1	C-2	C-3	C-4	C-5	C-6	OMe
(1)	97.57 (4.58)	72.26 (-0.21)	73.76 (-0.02)	70.54 (-0.17)	72.85 (0.48)	61.51 (-0.33)	97.43 (-2.76)	76.86 (4.63)	72.47 (-1.63)	70.66 (-0.02)	72.38 (-0.14)	61.63 (-0.04)	55.75 (-0.18)
(2)	98.64 (5.65) <sup>b</sup>	72.32 (-0.15)	73.77 (-0.01)	70.50 (-0.21)	72.62 (0.25)	61.46 (-0.38)	104.64 (0.51)	78.67 (4.68)	75.41 (-1.37)	70.86 (0.17)	76.57 (-0.21)	61.84 (0.02)	57.97 (0.09)
(3)	104.59 (7.75)	74.25 (-0.95)	76.65 (-0.11)	70.57 (-0.14)	76.76 (0.00)	61.83 (-0.01)	99.81 (-0.38)	81.10 (8.87)	73.00 (-1.10)	70.57 (-0.11)	72.23 (-0.29)	61.61 (-0.06)	55.70 (-0.23)
(4)	103.38 (6.54)	74.58 (-0.62)	76.63 (-0.13)	70.54 (-0.17)	76.93 (0.17)	61.74 (-0.10)	102.73 (-1.40)	81.28 (7.29)	76.79 (0.01)	70.54 (-0.15)	76.63 (-0.15)	61.74 (-0.08)	57.71 (-0.17)
(5)	99.95 (6.96)	72.66 (0.19)	73.91 (0.13)	70.56 (-0.15)	72.81 (0.44)	61.48 (-0.36)	100.30 (0.11)	70.83 (-1.40)	81.51 (7.41)	70.83 (0.15)	72.29 (-0.23)	61.55 (-0.12)	55.92 (-0.01)
(6)	99.94 (6.95)	72.60 (0.13)	73.91 (0.13)	70.50 (-0.21)	72.83 (0.46)	61.47 (-0.37)	104.15 (0.02)	72.71 (-1.28)	83.93 (7.15)	70.87 (0.18)	76.46 (-0.32)	61.65 (-0.17)	57.91 (0.03)
(7)	103.67 (6.83)	74.42 (-0.78)	76.60 (-0.16)	70.58 (-0.13)	76.89 (0.13)	61.64 (-0.20)	100.04 (-0.15)	71.61 (-0.62)	83.74 (9.64)	69.13 (-1.55)	72.35 (-0.17)	61.71 (0.04)	55.92 (-0.01)
(8)	103.64 (6.80)	74.40 (-0.80)	76.57 (-0.19) <sup>c</sup>	70.57 (-0.14)	76.92 <sup>c</sup> (0.16)	61.72 <sup>c</sup> (-0.12)	103.86 (-0.27)	73.51 (-0.48)	85.95 (9.17)	69.23 (-1.46)	76.44 <sup>c</sup> (-0.34)	61.80 <sup>c</sup> (-0.02)	57.93 (0.05)
(9)	102.15 (9.03)	69.27 (0.18)	70.45 (0.16)	72.65 (-0.14)	68.01 (0.91)	16.31 (-0.02)	99.66 (-0.53)	81.23 (9.00)	72.99 (-1.11)	70.52 (-0.16)	72.28 (-0.24)	61.61 (-0.06)	55.67 (-0.26)
(10)	100.78 (7.66)	69.39 (0.30)	70.62 (0.33)	72.80 (0.01)	67.69 (0.59)	15.99 (-0.34)	103.21 (-0.92)	80.89 (6.90)	77.19 (0.41)	70.58 (-0.11)	76.57 (-0.21)	61.81 (-0.01)	57.87 (-0.01)
$\alpha$ -D-Glucopyranose	92.99	72.47	73.78	70.71	72.37	61.84							
$\beta$ -D-Glucopyranose	96.84	75.20	76.76	70.71	76.76	61.84							
$\alpha$ -L-Fucopyranose	93.12	69.09	70.29	72.79	67.10	16.33							
Methyl $\alpha$ -D-glucopyranoside							100.19	72.23	74.10	70.68	72.52	61.67	55.93
Methyl $\beta$ -D-glucopyranoside							104.13	73.99	76.78	70.69	76.78	61.82	57.88

Chemical shifts are given in p.p.m. relative to internal dioxane (67.40 p.p.m.) for solutions in  $\text{D}_2\text{O}$ . <sup>a</sup> Primed labels refer to the D-glycopyranosyl group and unprimed to the methyl glycoside residue. <sup>b</sup> Chemical shift differences are calculated by subtraction of chemical shifts of  $\alpha$ -D-glucose and methyl  $\alpha$ -D-glucopyranoside from (1) *etc.* and a positive difference indicates a downfield shift. <sup>c</sup> Interchangeable pairs.

corresponding proton, 3(H) has the largest downfield shift of the two protons adjacent to the linkage.

It can be concluded from comparison between substituent shifts for signals of protons on the linkage carbon and the adjacent carbon atoms that it should be possible to determine linkage positions from the magnitudes of these shifts. With an extensive database sequence information for oligo- and polysaccharides could thus be obtained.

**$^{13}\text{C}$  N.m.r. Chemical Shifts.**—The  $^{13}\text{C}$  n.m.r. chemical shifts for (1)–(10) and relevant monomers and the chemical shift differences ( $\Delta\delta$ ) obtained upon comparison with the chemical shifts of the respective monomers are given in Table 3. Significant  $\Delta\delta$ -values are observed for signals of linkage carbons and for most of the carbons next to these. In addition signals for C(5') in the  $\alpha$ -linked disaccharides are shifted considerably downfield.

The downfield shift of the C(1') signal in (1) is smaller than for the other C(1') signals, which may be due to the 1'(H)—1(H)  $\gamma$ -*gauche* interaction, as indicated by the calculations. In (2) the anomeric oxygen has almost the same position as 1(H) in (1) and only a small shift for the C(1') signal is observed. It is evident from the substituent shifts of C(1') and C(2) that for 2-substituted disaccharides, the anomeric centre plays an important role by possibly affecting the interactions across the glycosidic linkage.

The largest shift for a C(1') signal is observed in (9) and the chemical shift, 102.15 p.p.m. appears in a region where the

signals for anomeric carbons of  $\beta$ -linked disaccharides could be observed and of mannosides and rhamnosides generally are observed.

For the  $\beta$ -linked disaccharides (3), (4), (7), and (8), an upfield shift for the C(2') signal between 0.6 and 1 p.p.m. is observed, whereas this shift for the corresponding  $\alpha$ -linked disaccharides is very small.

In the methyl glycoside residue of (1) a relatively small downfield shift for the signal of the linkage carbon and a relatively large upfield shift for the signal from the neighbouring carbon, C(1), bearing the axial substituent is observed. This is in agreement with what is observed for other glycosides<sup>1,29</sup> in which a similar stereochemical arrangement of substituents around the glycosidic linkage is present and for which HSEA calculations indicate proton–proton interactions. Similar substituent shifts for (2), (5), and (6) which have two equatorial neighbouring substituents, are observed except for the signal of C(2) in (2), which has a relatively small downfield shift.

One of the substituents vicinal to the linkage position is relatively unaffected by the substituting glycosyl group and for this substituent no short inter-residue internuclear distances to 1'(H) are found by the HSEA calculations (Table 1, Figure). The signal for the corresponding carbon on this side is always shifted upfield, between -1 and -1.5 p.p.m. The signal given by the other neighbouring carbon, which has a proton with calculated short inter-residue atom–atom distances, is shifted between -1.10 and 0.51 p.p.m. except for (1).

**Table 4.**  $^{13}\text{C}$  Chemical shift differences in p.p.m. with variation in temperature<sup>a</sup>

	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	0.11	0.21	0.27	0.16	0.30	0.18	0.37	0.17	0.24	0.08	0.22	0.05
(2)	-0.02	0.12	0.20	0.31	0.16	0.35	-0.11	0.28	0.15	0.18	0.04	0.18	-0.14
(3)	-0.08	0.15	0.19	0.23	0.08	0.25	0.10	-0.17	0.17	0.27	0.11	0.24	0.08
(4)	0.03	0.18	0.18	0.18	0.02	0.19	0.17	0.33	0.00	0.18	0.07	0.19	-0.05
(5)	0.12	0.15	0.21	0.30	0.23	0.26	0.06	0.16 <sup>b</sup>	0.86	0.03 <sup>b</sup>	0.10	0.33	0.08
(6)	-0.01	0.13	0.18	0.33	0.21	0.22	0.06	0.09	0.76	0.00	0.03	0.35	-0.10
(7)	-0.03	0.11	0.17	0.16	0.07	0.24	0.05	0.05	0.26	0.21	0.12	0.17	0.06
(8)	0.21	0.12	0.20	0.15	0.07	0.17	0.08	-0.05	0.34	0.17	0.07	0.25	-0.10
(9)	-0.12	0.11	0.24	-0.18	0.11	-0.05	0.11	-0.15	0.19	0.31	0.10	0.23	0.10
(10)	-0.05	0.11	0.16	0.03	-0.03	-0.06	0.08	0.12	0.09	0.16	0.07	0.18	-0.07

<sup>a</sup>  $\delta = \delta(70^\circ\text{C}) - \delta(30^\circ\text{C})$ . Dioxane was taken as  $\delta$  67.40 for all temperatures. <sup>b</sup> These values could be interchanged.

**Table 5.** Calculated and observed  $^{13}\text{C}$  n.m.r. chemical shifts of an  $\alpha$ -1,3-glucan and a  $\beta$ -1,3-glucan. Dioxane ( $\delta$  67.4 p.p.m.) was used as an internal standard

	C-1	C-2	C-3	C-4	C-5	C-6
$\alpha$ -1,3-Glucan (obs.)	100.3	71.4	81.7	70.8	72.7	61.5
(calc.)	100.1	71.3	81.3	70.7	72.6	61.4
$\beta$ -1,3-Glucan (obs.)	103.4	74.0	85.7	69.2	76.6	61.7
(calc.)	103.4	73.9	85.7	69.1	76.6	61.7

#### Temperature Dependence of $^{13}\text{C}$ N.m.r. Chemical Shifts.—

The temperature shifts obtained on changing the temperature from 30 to 70 °C are given in Table 4. The values are relative to internal dioxane, which has chemical shift  $\delta$  67.40 at both temperatures.

Most signals were shifted to lower field on heating, the lowest value (0.86 p.p.m.) being observed for the HC(3) signal in (5). Several sets of values are different enough to be of diagnostic value. Thus the temperature shifts are quite different for the  $\alpha$ -linked and the  $\beta$ -linked disaccharides. Large shifts are observed for all C(4) and C(6) signals in the glucopyranosyl residues. The linkage carbon, C(2) or C(3) is also affected and a similar pattern is observed for  $\alpha$ -L/D and  $\beta$ -D/D-oligosaccharides.

As earlier mentioned the pairs (2), (6) and (4), (8) are similar with respect to the substituents close to the glycosidic linkage and also with respect to their minimum energy conformation. The shift values for protons in the same relative positions differ in the first pair but not in the second pair. The same is observed for the temperature shifts. Values for (2) and (6) differ but those for (4) and (8) are similar.

**Calculation of  $^{13}\text{C}$  N.m.r. Spectra of Polysaccharides using Substituent Shifts.**—In order to decide whether substituent shifts for disaccharides are similar to those in polysaccharides the spectra of an  $\alpha$ -1,3-D-glucan and a  $\beta$ -1,3-D-glucan were recorded and compared with the simulated spectra. These results are given in Table 5. Such predictions have earlier been performed for linear  $\alpha$ -1,6-,  $\beta$ -1,6-,<sup>3</sup>  $\alpha$ -1,4-, and  $\beta$ -1,4-linked<sup>2</sup> glucans and for some bacterial heteropolysaccharides from *Klebsiella*.<sup>4</sup>

In simulating the n.m.r. spectra it is assumed that short-range interactions are the most important and that substituent shifts are additive. Thus for a linear glycan the chemical shifts given by a glycosyl residue are affected by its two nearest neighbours. The substituent shifts all taken from disaccharides with similar stereochemistry are added to the chemical shifts of the corresponding glucose.

Starting from the chemical shifts of  $\alpha$ -D-glucose and adding the appropriate shifts from (5), first to account for the  $\alpha$ -D-glucosyl residue in the 3-position ( $\Delta\delta_{1,6}$ ) and then to account for the  $\alpha$ -D-glucosyl residue to which it is linked ( $\Delta\delta_{1,6}$ ) yields

the simulated chemical shifts for the  $\alpha$ -1,3-D-glucan (Table 5). A similar calculation was performed for a  $\beta$ -glucan. The differences between observed and calculated spectra are small indicating that data from 1,3-linked disaccharides could be used for the analysis of n.m.r. spectra of polysaccharides containing these linkages. The agreement is better than for polysaccharides containing both 1,4- and 1,6-linkages,<sup>2</sup> probably because such linkages affect the distribution of conformers around C(5)–C(6).

We have now investigated 1,2-, 1,3-, 1,4-, and 1,6-linked pyranosidic oligosaccharides and are able to simulate n.m.r. spectra of linear polysaccharides.<sup>4</sup> Branched polysaccharides may cause special problems and a number of trisaccharides representative of branching points are currently being investigated in our laboratory.

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