# Synthesis, NMR and Conformational Studies of some 1,4- Linked Disaccharides

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Synthesis of, and NMR and conformational studies on the methyl glycosides of some 1,4-linked disaccharides, containing rhamnose and fucose in the methyl glycoside residue, have been performed. The glycosylation shifts were correlated with inter-residue atomic distances found in the calculated minimum energy conformations. It is concluded that a typical set of glycosylation shifts are obtained for the disaccharides thus making it possible to use these in the analysis of complex carbohydrates.

In order to obtain correlations between the stereochemical surrounding of a glycosidic linkage and the <sup>1</sup>H and <sup>13</sup>C NMR chemical shifts of the signals from protons and carbons in the constituent sugars, a large number of differently linked di- and tri-saccharides have been characterized by NMR spectros-copy.<sup>1-8</sup> Theoretically derived inter-residue atomic distances in the minimum energy conformation were used in attempts to explain the glycosylation shifts. These shifts have also been used successfully in a computerized approach to structure determination of oligo- and poly-saccharides.<sup>9,10</sup>

In order to extend the knowledge of certain glycosidic linkages the methyl glycosides of six 4-linked disaccharides containing 6-deoxysugars have been synthesized, studied by NMR spectroscopy and their preferred conformation analysed by HSEA-calculations. Four of these are linked through an axial oxygen (fucosides) and the other two have an axial hydroxy group in a 1,3-position to the linkage (rhamnosides). This is a group of glycosides currently under investigation. We present here the NMR data obtained and the correlations between these and short inter-residue atomic distances present in the minimum energy conformations of the disaccharides.

## **Experimental**

General Methods.—<sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded for 0.05M and 0.1M deuterium oxide solutions, respectively, at 70 °C with JEOL GX-400 and GSX-270 spectrometers. Chemical shifts are given in ppm using sodium [2,2,3,3-<sup>2</sup>H<sub>4</sub>]-3-(trimethylsilyl)propanoate (TSP,  $\delta_{\rm H}$  0.00 ppm) and dioxane ( $\delta_{\rm C}$ 67.40 ppm) as internal references. For the assignment of signals different types of proton-proton and proton-carbon shift correlated spectroscopy (COSY) were used.

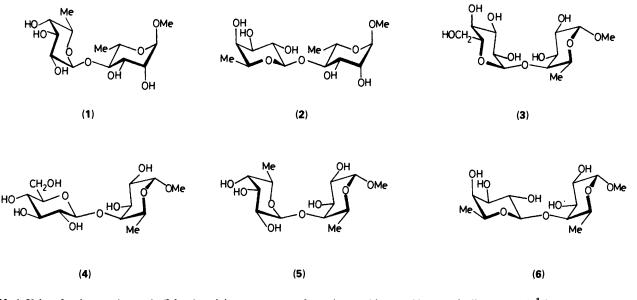
The HSEA-program<sup>11,12</sup> was used to estimate minimum energy conformations and the rotational freedom around the glycosidic bond. The torsional angles  $\varphi$  and  $\psi$  were defined by H(1')-C(1')-O(4)-C(4) and C(1')-O(4)-C(4)-H(4), respectively and a positive angle is obtained when the substituent in the rear, as observed in a Newton projection, is rotated clockwise. The bond angle  $\tau$  [C(1')-O(4)-C(4)] was set at 117°. Co-ordinate sets for  $\alpha$ -D-glucopyranose,<sup>13</sup>  $\beta$ -D-glucopyranose,<sup>14</sup> and  $\alpha$ -L-fucopyranose<sup>15</sup> were obtained from the crystal structures whereas the co-ordinates for  $\beta$ -L-fucopyranose were obtained from the mirror image of modified methyl  $\beta$ -Dgalactopyranoside.<sup>16</sup> Co-ordinates for methyl  $\alpha$ -L-fucopyranoside and methyl  $\alpha$ -L-rhamnopyranoside were obtained from the crystal data of  $\alpha$ -L-fucopyranose and  $\alpha$ -L-rhamnopyranose<sup>17</sup> to which methyl groups at  $\varphi = 50^{\circ}$  were added.

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

The purity of the intermediates was first analysed by TLC, by which only one spot was evident; from the <sup>13</sup>C NMR spectra they were estimated to be > 95% pure. In the <sup>1</sup>H NMR spectra of the deprotected disaccharides, signals from contaminating components were <5% of the integral of the anomeric proton signals.

Methyl 4-O- $\alpha$ -L-Fucopyranosyl- $\alpha$ -L-rhamnopyranoside (1) and Methyl 4-O- $\beta$ -L-Fucopyranosyl- $\alpha$ -L-rhamnopyranoside (2). Methyl trifluoromethanesulphonate (0.8 ml) was added to a stirred solution of ethyl 2,3,4-tri-O-benzyl-1-thio-B-L-fucopyranoside<sup>18</sup> (1.0 g), methyl 2,3-O-isopropylidene-α-L-rhamnopyranoside (320 mg), and ground molecular sieves (4 Å, 6 g) in diethyl ether (10 ml) at room temperature. Triethylamine (1 ml) was added after 2 h and the mixture was stirred for a further 30 min, before being diluted with dichloromethane and filtered through a layer of Celite. After filtration and concentration the products were purified on a column of silica gel (toluene-ethyl acetate, 4:1) to yield the protected  $\alpha$ -linked disaccharide (720 mg, 77%),  $\delta_c$  97.6 and 95.6 ppm (C-1, C-1'), and the  $\beta$ -linked disaccharide (115 mg, 12%),  $\delta_{\rm C}$  103.2 and 98.4 ppm (C-1', C-1). The products, (720 mg) and (115 mg) were separately subjected first to treatment with aqueous 90% acetic acid (10 ml) at 90 °C for 3 h and then to hydrogenolysis in the same solvent at 400 kPa over Pd-C for 13 h. The mixtures were then filtered and the products purified on columns of silica gel (ethyl acetate-acetic acid-methanol-water, 12:3:3:2) followed by chromatography on Bio-Gel P-2 using water as eluant. After freeze-drying the disaccharides (1) (340 mg, 72%)  $[\alpha]_D - 140^\circ$  (c 1.0 in water) and (2) (54 mg, 11%)  $[\alpha]_D - 22^\circ$  (c 0.9, water) were obtained.

Methyl 4-O- $\alpha$ -D-Glucopyranosyl- $\alpha$ -L-fucopyranoside (3).— The glycosidation reaction between ethyl 2,3,4,6-tetra-O-benzyl-1-thio- $\beta$ -D-glucopyranoside<sup>19</sup> (530 mg) and methyl 2,3-di-Obenzoyl- $\alpha$ -L-fucopyranoside<sup>20</sup> (260 mg) was performed using methyl trifluoromethanesulphonate (0.3 ml) and worked up as described above. The product was purified on silica gel (tolueneethyl acetate, 5:1) to yield the protected disaccharide (490 mg, 80%),  $\delta_c$  97.6 and 97.5 ppm (C-1', C-1). This material (490 mg) in dichloromethane (5 ml) was treated with sodium methoxide in methanol (0.25M; 5 ml) at 25 °C for 8 h and the solution was neutralized with ion exchange resin, Dowex 50 (H<sup>+</sup>), and deprotected as described above to yield after freeze-drying, the disaccharide (3) (160 mg, 71%),  $[\alpha]_D - 52^\circ$  (c 0.9 in water).



**Table 1.** Values for the  $\varphi$  and  $\psi$  angles/° for the minimum energy conformations and inter-residue atomic distances < 3 Å in (1)–(6) obtained by HSEA-calculations.

:	Substance		φ/ψ	1′-H	5′-H	O-5′
(	α-L-Fucp(1→4)α-L-RhapOMe	<b>(1)</b> <sup><i>a</i></sup>	42/20	2.58 (O-3) 2.38 (4-H)	2.82 (6-HB) 1.92 (6-HC)	2.70 (4-H)
ĺ	β-L-Fucp(1→4)α-L-RhapOMe	(2)	- 58/2	2.37 (4-H) 2.69 (6-HB) 2.86 (6-HC)		2.59 (4-H)
(	α-D-Glcp(1→4)α-L-FucpOMe	(3)	-30/-22	2.55 (O-3) 2.28 (4-H)	2.05 (6-H)	2.86 (4-H)
ĺ	β-D-Glcp(1→4)α-L-FucpOMe	(4)	56/8	2.47 (4-H) 2.52 (6-H) 2.91 (6-H)		2.50 (4-H)
(	x-L-Fucp(1→4)α-L-FucpOMe	<b>(5)</b> <sup><i>a</i></sup>	42/14	2.32 (4-H) 2.40 (6-H) 2.72 (6-H)	2.40 (O-3)	2.72 (4-H)
ſ	β-L-Fucp(1→4)α-L-FucpOMe	(6)	- 58/-4	2.87 (O-3) 2.44 (4-H)		2.49 (4-H) 2.71 (6-H) 2.95 (6-H)

<sup>a</sup> The distance between 6'-H<sub>A</sub> and 6-H<sub>c</sub> in (1) is 2.73 Å and between O-2' and 6-H in (5) 2.99 Å.

Methyl 4-O- $\beta$ -D-Glucopyranosyl- $\alpha$ -L-fucopyranoside (4).--Silver trifluoromethanesulphonate (700 mg) was added to a stirred solution of ethyl 2,3,4,6-tetra-O-benzoyl-1-thio-β-Dglucopyranoside<sup>19</sup> (650 mg), methyl 2,3-di-O-benzoyl-a-Lfucopyranoside (200 mg), collidine (65 µl) and ground molecular sieves (4 Å, 6 g) in dichloromethane (10 ml) at - 30 °C.<sup>21,22</sup> The mixture was stirred for 2 h and then filtered through a layer of Celite. The solution was concentrated and the product purified on silica gel (toluene-ethyl acetate, 10:1) to yield the protected disaccharide (325 mg, 65%),  $\delta_{\rm C}$  102.0 and 97.3 ppm (C-1', C-1). This product (325 mg) in dichloromethane (5 ml) was treated with sodium methoxide in methanol (0.25m; 5 ml) at 25 °C for 8 h, neutralized with ion exchange resin, Dowex 50  $(H^+)$ , and then concentrated to dryness. The resulting disaccharide was purified on a column of Bio-Gel P-2 irrigated with water to yield, after freeze-drying, compound (4) (93 mg, 53%),  $[\alpha]_D - 128^\circ$  (c 1.3 in water).

As the syntheses of compounds (5) and (6) have been reported earlier<sup>23</sup> no experimental details are given here.

### **Results and Discussion**

Synthesis.—Compounds (1)-(6) are listed in Table 1. The

disaccharides (1)-(3) were obtained under conditions of methyl trifluoromethanesulphonate promotion<sup>18</sup> using the glycosyl donors ethyl 2,3,4-tri-O-benzyl-1-thio- $\beta$ -L-fucopyranoside for the synthesis of (1) and (2) and ethyl 2,3,4,6-tetra-O-benzyl-1-thio- $\beta$ -D-glucopyranoside for the synthesis of (3). Synthesis of (4) was promoted by silver trifluoromethanesulphonate<sup>21,22</sup> using 2,3,4,6-tetra-O-benzoyl- $\alpha$ -D-glucopyranosyl bromide as glycosyl donor. Methyl 2,3-O-isopropylidene- $\alpha$ -L-rhamnopy-ranoside was used as the aglycone for the synthesis of disaccharides (1) and (2), and methyl 2,3-di-O-benzoyl- $\alpha$ -L-fucopyranoside for (3) and (4).

HSEA Calculations.—The  $\phi/\psi$ -energy plots of disaccharides (1)–(6) together with pronounced inter-residue contacts are shown in the Figure. All inter-residue atomic distances < 3 Å for the minimum energy conformations are given in Table 1. Disaccharides (1), (3), and (5) are termed  $\alpha$ -glycosides with reference to the central bond and consequently the remaining compounds are termed  $\beta$ -glycosides.

The results of the calculations on the disaccharides (1)-(6) show short distances between the atoms commonly involved in interactions in disaccharides, namely between the anomeric proton, 1'-H, in the glycosyl group and the proton on the

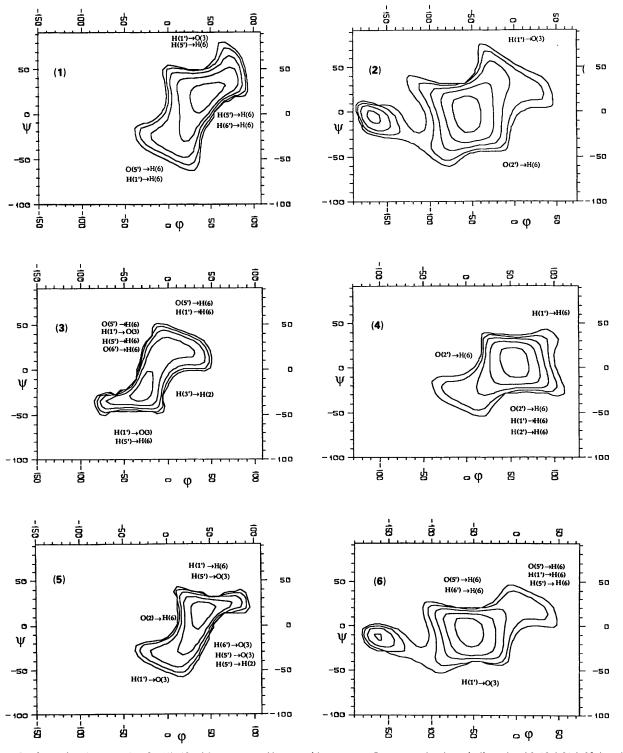


Figure. Conformational energy plots for (1)-(6) with pronounced inter- residue contacts. Isocontour levels are indicated at 4.2, 12.6, 21.0, 29.4, and 37.8 kJ (1, 3, 5, 7, and 9 kcal) above the minimum energy conformation.

linkage carbon, 4-H, and one of the equatorial substituents adjacent to C-4, *i.e.* O-3 or 6-Me. It can be concluded from the present data and those obtained earlier<sup>3,5</sup> that disaccharides with a 1,4-linkage and with the configuration  $\alpha$ -LL,  $\alpha$ -DD,  $\beta$ -LD, and  $\beta$ -DL (L-Fuc $\rightarrow$ L-Rha, D-Glc $\rightarrow$ D-Glc, L-Fuc $\rightarrow$ D-Glc, D-Glc $\rightarrow$ L-Rha) with equatorial O-4, and  $\alpha$ -DL and  $\beta$ -LL (D-Glc $\rightarrow$ L-Fuc, L-Fuc $\rightarrow$ L-Fuc) with axial O-4 have a short distance between 1'-H and O-3. The disaccharides with the other combinations *i.e.*  $\beta$ -LL *etc.* have a short distance between 1'-H and 6-H. For disaccharides (1)–(6) O-5' is near 4-H for all disaccharides and in (6) also near 6-H. In the  $\alpha$ -glycosides 5'-H is near 6-Me in (1) and (3), and O-3 in (5).

The  $\beta$ -glycosides have more rotational freedom around the glycosidic bond than the  $\alpha$ -glycosides according to the energy plots (Figure). This is also found for the glycosides linked through an equatorial O-4, (1) and (2), compared to those

$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	5.23 (0.03) 4.43 (-0.12) 5.20 (-0.03) 4.48 (-0.03) 4.48 (-0.04) 5.20 4.55 5.23 4.51 (-0.04) 5.20 4.55 5.23 4.64 4.64 4.64 5.20 6.52 6.53 6.53 6.53 6.53 6.53 6.53 6.53 6.53	3.83° (0.06) (0.06) (0.06) (0.06) (0.06) (0.06) (0.06) (0.09) (0.09) (0.09) (0.09) (0.09) (0.09) (0.09) (0.09) (0.09) (0.09) (0.09) (0.09) (0.06) (0.	3.83° (-0.03) 3.66 (0.03) 3.76 (0.04) 3.52 (0.00) 3.63 3.63 3.63 3.63 3.63 3.63 3.63 3.6	3.81 <sup>4</sup> (0.01) 3.77 (0.03) 3.43 (0.01) 3.43 (0.01) 3.43 (0.01) 3.83 (0.01) 3.83 (0.01) 3.83 (0.02) 3.83 (0.02) 3.83 (0.02) 3.83 (0.01) 3.81 (0.01) 3.42 (0.01) 3.42 (0.01) 3.42 (0.01) 3.43 (0.02) 3.43 (0.02) 3.43 (0.02) 3.42 (0.02) (0.02) 3.42 (0.02) (0.0	(-0.06) 3.83 (0.04) 3.83 (0.00) 3.84 (0.00) 4.46 (0.00) 4.46 (0.00) 4.46 (0.00) 4.46 (0.00) 4.46 (0.00) 4.46 (0.00) 3.79 3.79 3.79 3.79 3.79 3.79 3.79 3.79	$331^4$ $4.14$ $1.22$ $4.66$ $377$ $3.31$ $1.128$ $4.71$ $56$ $3.77$ $3.83$ $1.28$ $4.71$ $317$ $3.83$ $1.28$ $4.71$ $56$ $3.77$ $3.83$ $1.28$ $4.71$ $66$ $3.43$ $3.84$ $3.84$ $3.77$ $4.75$ $471$ $0.001$ $0.001$ $0.001$ $0.001$ $0.001$ $22$ $3.43$ $3.46$ $3.91$ $3.75$ $4.81$ $22$ $3.43$ $3.46$ $3.91$ $3.75$ $4.81$ $22$ $3.83$ $4.46$ $1.19$ $0.03$ $0.02$ $20$ $0.01$ $0.001$ $0.001$ $0.003$ $0.02$ $233$ $3.44$ $3.76$ $4.71$ $4.71$ $233$ $3.72^{\circ}$ $1.24$ $3.76$ $4.71$ $233$ $3.72^{\circ}$ $1.24$ $3.76$ $4.71$ $233$ $3.72^{\circ}$ $1.24$ $3.76$ $4.75$ $3.72$	) 3.77 (0.01) 3.75 (0.03) 3.75 3.72 3.72 hexoside fe	4.68 4.71 4.71 (0.02) (0.02) 4.79 (0.02) (0.02) (0.03) 4.79 4.81 (0.04) (0.04) (0.04) (0.04) (0.00) (0.04) (0.00) (0.00) (0.00) (0.01) (0.02) (0.02) (0.03) (0.03) (0.03) (0.03) (0.04) (0.03) (0.04) (0.03) (0.04) (0.03) (0.04) (0.03) (0.04) (0.03)(	8 3.93 <sup>c</sup> 3.9 1 (0.00) (0.2 2 (0.07) (0.0 9 3.90 <sup>c</sup> 3.8 2 (0.10) (0.0 3 78 <sup>c</sup> 3.8 1 3.83 3.8 1 3.83 3.8 1 (-0.02) (0.0 3 78 <sup>c</sup> 3.8 3 .8 3 .8 3 .8 3 .8 3 .7 3 .8 3 .8 3 .8 3 .8 3 .8 3 .7 3 .8 3 .8 1 .	3.93 <sup>6</sup> (0.21) 3.80 (0.08) 3.89 <sup>6</sup> 3.81 <sup>6</sup> (0.09) 3.86 <sup>6</sup> (0.09) 3.86 <sup>6</sup> (0.06) (0.06) 3.86 <sup>6</sup> (0.06) 3.86 <sup>6</sup> (0.06) 3.872 3.80 <sup>6</sup> (0.06) ctra. <sup>4</sup> Cheta	$3$ $3.53$ $3.75$ $1.33$ $0$ $3.56$ $3.79$ $1.37$ $8$ $(0.11)$ $(0.13)$ $(0.07)$ $9^{\circ}$ $3.93$ $4.07$ $1.28$ $9^{\circ}$ $0.13$ $(0.05)$ $(0.07)$ $9^{\circ}$ $3.93$ $4.07$ $1.28$ $9^{\circ}$ $0.13$ $(0.05)$ $(0.05)$ $1^{\circ}$ $4.01$ $4.10$ $1.32$ $1^{\circ}$ $4.01$ $4.10$ $1.32$ $9^{\circ}$ $3.85$ $4.07$ $1.32$ $9^{\circ}$ $3.85$ $4.07$ $1.32$ $9^{\circ}$ $0.05$ $(0.09)$ $(0.09)$ $6^{\circ}$ $0.17$ $(0.01)$ $(0.03)$ $(0.02)$ $6^{\circ}$ $0.17$ $(0.01)$ $(0.03)$ $(1.26)$ $9^{\circ}$ $3.45$ $4.02$ $1.23$ $9^{\circ}$ $3.86$ $4.02$ $1.23$ $9^{\circ}$ $3.66$ $1.23$ $0.023$ $0^{\circ}$ $3.86$ $4.02$ $1.23$ $9^{\circ}$	3.75 (0.09) 3.79 (0.13) 4.07 (0.05) 4.10 (0.08) 4.10 (0.01) (0.01) (0.01) (0.01) 3.66 4.03 (0.01) (0.01) (0.01)	1.33 (0.07) 1.37 (0.07) 1.28 (0.09) 1.28 (0.09) 1.28 (0.09) 1.26 (0.03) 1.26 (0.03) 1.26 (0.03) 1.26 (1.23 1.26 (1.23) 1.23 (1.23) 1.23 (1.23)	3.40 (0.00) 3.41 (0.01) 3.41 (0.00) 3.41 (0.00) 3.42 (0.01) 3.39 (0.01) 3.39 (0.01) 3.39 (0.01) 3.40 3.41 (0.02) 3.41 (0.01) 3.41 (0.01) 3.41 (0.01) 3.41 (0.01) 3.41 (0.01) 3.41 (0.01) 3.41 (0.01) 3.41 (0.00) (0.00) 3.41 (0.00) (0
<ul> <li>β-L-Fucp(14)α-L-RhapOMe (2)</li> <li>α-D-Glcp(14)α-L-FucpOMe (3)</li> <li>β-D-Glcp(14)α-L-FucpOMe (4)</li> <li>α-L-Fucp(14)α-L-FucpOMe (5)</li> <li>β-L-Fucopyranose</li> <li>β-L-fuco</li></ul>	(0.03) 4.43 (-0.12) 8.43 (-0.03) 4.48 (-0.12) 4.48 (-0.16) 4.48 (-0.16) 4.45 (-0.23) 4.51 (-0.04) 5.20 4.55 5.23 4.64 4.55 5.23 4.64 4.55 5.23 4.64 5.20 4.64 5.20 6.520 6.520 6.520 6.64 5.20 6.64 5.20 6.64 5.20 6.64 5.20 6.64 5.20 6.64 5.20 6.64 6.65 7.65 7.65 7.65 7.65 7.65 7.65 7.65	(0.06) 3.52 (0.06) 3.60 (0.06) 3.41 (0.16) 3.83 3.41 (0.06) 3.55 (0.09) 3.55 (0.09) 3.55 3.54 3.54 3.54 3.54 3.54 3.54 3.54	(0.03) 3.66 (0.03) 3.56 (0.04) 3.52 (0.04) 3.52 (0.05) 3.53 3.53 3.63 3.63 3.63 3.63 3.63 3.63	(0.01) 3.77 (0.03) 3.43 (0.01) 3.43 (0.01) 3.43 (0.01) 3.43 (0.02) 3.43 3.42 3.42 3.42 3.42 3.42 3.42 3.42	(-006) 3.83 (0.04) 3.84 (0.00) 3.46 (0.00) 3.46 (0.26) 3.72 <sup>c</sup> (-0.07) 3.79 3.79 3.79 3.79 3.79 3.79 3.76 (-0.07) 3.76 (-0	(0.01) 1.28 (0.02) 3.84 (0.00) 3.91 (0.01) 1.19 (.001) 1.19 (.1.02) 1.24 1.24 1.24 1.24 1.24 1.24 1.24 3.30 3.30 3.30 3.30 1.21 1.26 3.34 3.90 3.30 1.26 1.26 1.21 1.26 3.34 3.90 3.90 1.21 1.26 1.21 1.26 1.21 1.21 1.21 1.21	) 3.77 (0.01) 3.75 (0.03) (0.03) 3.75 3.72 3.72 3.72	(-0.01) (0.02) (0.02) (0.03) (0.04) (0.04) (0.04) (0.04) (0.04) (0.00) (0.00) (0.00) (0.00) (0.00) (0.00) (0.00) (0.00) (0.00) (0.00) (0.00) (0.00) (0.00) (0.02) (0.03) (0.04) (0.03) (0.03) (0.03) (0.03) (0.03) (0.03) (0.03) (0.03) (0.03) (0.04) (0.03) (0.03) (0.04) (0.03) (0.04) (0.03) (0.03) (0.04) (0.03) (0.04) (0.03) (0.03) (0.03) (0.04) (0.03) (0.04) (0.03) (0.03) (0.04) (0.03) (0.03) (0.03) (0.04) (0.03) (0.04) (0.04) (0.03) (0.03) (0.03) (0.04) (0.03) (0.04) (	(0.00) (0.07) (0.07) (0.10) (0.10) (0.10) (0.10) (0.10) (0.05) (0.06) (0.06) (0.06) (0.06) (0.06) (0.06) (0.06) (0.06) (0.06) (0.06) (0.07) (0.07) (0.07) (0.07) (0.07) (0.10) (0	(0.21) 3.80 (0.08) 3.89° (0.09) 3.81° (0.09) 3.80° (0.06) (0.06) 3.80° (0.06) (0.06) 3.72 3.80° (0.06) (0.06) 3.72 3.80° (0.06) (0.06) 3.72 3.80° (0.09) 3.80° (0.00) 3.80° (0	(0.08) 3.56 (0.11) 3.93 (0.13) 4.01 (0.21) 3.85 (0.21) 3.85 (0.17) (0.17) (0.17) (0.17) (0.17) (0.17) (0.17) (0.17) (0.17) (0.17) (0.17) (0.18) (0.11) 3.86 (0.11) 3.86 (0.11) 3.86 (0.11) 3.86 (0.11) 3.86 (0.11) 3.85 (0.11) (0.11) 3.85 (0.11) (	(0.09) 3.79 (0.13) 4.07 (0.05) 4.07 (0.08) 4.07 (0.08) 4.07 (0.08) 4.07 (0.01) (0.01) (0.01) (0.01) (0.01) (0.01) (0.01)	(0.03) 1.37 (0.07) 1.28 (0.05) 1.32 (0.09) 1.32 1.32 (0.09) 1.32 1.23 1.	(0.00) 3.41 (0.01) 3.41 (0.00) 3.41 (0.00) 3.42 (0.01) 3.42 (0.01) 3.42 (0.01) 3.40 3.40 3.40 (0.01) 3.41 (0.02) (0.02) (0.02) (0.01) 3.41 (0.01) 3.42 (0.01) 3.41 (0.01) 3.42 (0.01) 3.41 (0.01) 3.40
p-L-Fucp(1 $\rightarrow$ 4)x-L-KnapOMe (2) $\alpha$ -D-Glcp(1 $\rightarrow$ 4)x-L-FucpOMe (3) $\beta$ -D-Glcp(1 $\rightarrow$ 4)x-L-FucpOMe (4) $\alpha$ -L-Fucp(1 $\rightarrow$ 4)x-L-FucpOMe (5) $\beta$ -L-Fucopyranose $\beta$ -L-Fucop	(-0.12) (-0.12) (-0.03) (-0.03) (-0.16) 4.97 (-0.16) 4.97 (-0.23) (-0.04) 5.20 4.55 5.23 4.64 4.55 5.20 4.55 5.23 4.64 t.64 5.20 (-0.04) 5.20 5.20 5.20 5.20 5.20 5.20 5.20 5.20	2.22 (0.06) 3.60 (0.06) 3.41 (0.16) 3.83 3.83 (0.06) 3.55 (0.09) 3.77 3.46 3.54 3.54 3.54 3.54 3.54 3.54 3.54 3.54	2.00 (0.04) (0.04) (0.04) (0.02) (0.02) (0.05) (0.06) (0.06) (0.06) (0.06) (0.06) (0.06) (0.06) (0.06) (0.06) (0.06) (0.02) (0.0	5.77 (0.03) 3.43 (0.01) 3.43 (0.01) 3.43 (0.01) 3.43 3.72 <sup>c</sup> (-0.02) 3.72 <sup>c</sup> (-0.02) 3.72 <sup>c</sup> 3.72 <sup>c</sup> 1.72 <sup>c</sup> 3.72 <sup>c</sup> 3	(0.00) 3.84 (0.00) 3.46 (0.00) 3.46 (0.00) (0.00) (0.00) 3.75 (-0.07) 3.79 3.79 3.79 3.79 3.79 3.79 3.79 3.79	$\begin{array}{c} 1.26 \\ (0.02) \\ 3.84 \\ (0.00) \\ 3.91 \\ (0.01) \\ 1.19 \\ 1.19 \\ 1.19 \\ 1.24 \\ 1.24 \\ 1.24 \\ 1.24 \\ 1.26 \\ 3.84 \\ 3.90 \\ 3.84 \\ 3.90 \\ 1.26 \\ 1.26 \\ 3.84 \\ 3.90 \\ 1.26 \\ 1.2$	3.77 (0.01) 3.75 (0.03) 3.76 3.76 3.72 3.72	4.71 (0.02) 4.79 (0.02) 4.80 (0.03) ( 4.81 (0.04) 4.77 (0.00) (0.00) (0.00) 4.77 4.69 4.77 (0.00) (0.00) 5 of the glycoi	4.00 3.90 3.78 (0.10) 3.78 3.78 3.83 3.86 (0.05) (0.06) (0.06) 3.86 (0.06) 3.86 (0.06) 3.86 (0.06) 3.89 3.93 3.93 3.93 3.93 3.93 3.93 3.93	, 5.80 (0.08) 3.89% (0.09) 3.81 (0.09) 3.88% (0.09) (0.06) (0.06) 3.72 3.72 3.87% (0.06) (0.06) (0.06) (1.09) 3.72 3.87% (1.09) (1.09) 3.87% (1.09) 3.87% (1.09) 3.88% (1.00) 3.88% (1.00)		3.79 (0.13) 4.10 (0.08) 4.10 (0.08) 4.07 (0.08) 4.07 (0.08) 4.07 (0.01) (0.01) (0.01) (0.01) (0.01) (0.01) (0.01) (0.01) (0.05) 4.07 (0.05) 4.07 (0.05) 4.10 (0.05) 4.02 (0.05) 4.02 (0.05) 4.02 (0.01) (0.05) (0.01) (0.01) (0.02) (0.01) (0.0	(0.03) (0.05) (1.28 (1.32) (1.32) (1.32) (1.32) (1.33) (1.30) (1.33) (1.33) (1.33) (1.33) (1.23) (1.23) (1.23) (1.23) (1.23) (1.23) (1.23) (1.23) (1.23) (1.23) (1.23) (1.23) (1.23) (1.28) (1.	3.41 (0.01) 3.41 (0.00) 3.41 (0.00) 3.42 (0.01) 3.42 (0.01) 3.42 (0.01) 3.42 (0.01) 3.42 (0.01) 3.40 (0.01) 3.40 (0.01) 3.41 (0.01) 3.41 (0.00) 3.41 (0.00) 3.42 (0.01) 3.42 (0.00) 3.41 (0.00) 3.41 (0.00) 3.41 (0.00) 3.41 (0.00) 3.41 (0.00) 3.41 (0.00) 3.41 (0.00) 3.41 (0.00) 3.41 (0.00) 3.42 (0.00) 3.40 (0.00) (
<ul> <li>α-D-Glcp(1→4)α-L-FucpOMe (3)</li> <li>β-D-Glcp(1→4)α-L-FucpOMe (4)</li> <li>α-L-Fucp(1→4)α-L-FucpOMe (5)</li> <li>β-L-Fucopyranose</li> <li>β-L-Fucopyranose</li> <li>β-L-Glucopyranose</li> <li>β-L-Glucopyranos</li></ul>	$\begin{array}{c} 5.20\\ (-0.03)\\ 4.48\\ (-0.16)\\ 4.97\\ (-0.23)\\ 4.51\\ (-0.04)\\ 5.20\\ 4.55\\ 5.23\\ 4.64\\ 4.55\\ 5.23\\ 4.64\\ 4.64\\ t.64\\ t.64\\$	3.60 (0.06) 3.41 (0.16) 3.83 (0.06) 3.55 (0.09) 3.77 3.46 3.54 3.55 (0.09) 3.77 3.55 (0.09) 3.55 3.55 (1009) 3.55 3.55 (1006)	3.76 (0.04) 3.52 (0.02) 3.52 (0.02) 3.63 3.63 3.63 3.63 3.63 3.63 3.63 3.6	3.43 (0.01) 3.43 (0.01) 3.83 3.83 (0.02) 3.72 <sup>6</sup> (-0.02) 3.72 <sup>6</sup> 3.72 <sup>6</sup> 3.72 3.42 3.42 3.42 3.42 3.42 3.42 3.42 3.4	3.84 (0.00) 3.46 (0.00) 4.46 (0.26) 3.72 (-0.07) 3.79 3.46 3.79 3.46 3.79 3.46 3.79 3.46 3.79 3.46 3.46 3.46 3.46 1.420 3.46 1.420 3.46 5.46 5.46 5.46 5.46 5.46 5.46 5.46 5	3.84 (0.00) 3.91 (0.01) 1.19 (-0.02) 1.24 (-0.02) 1.24 1.24 1.24 1.26 3.84 3.90 3.84 3.90 3.84 3.90 and methyl g	3.77 (0.01) 3.75 (0.03) 3.76 3.76 3.72 1.72	4.79 (0.02) 4.80 (0.03) ( 4.81 (0.04) 4.77 (0.00) (0.00) 4.77 (0.00) (0.00) (0.00) (0.00) (0.00) (0.00) (0.00) (0.00) (0.00) (0.00) (0.01) (0.02) (0.03) (0.03) (0.03) (0.03) (0.03) (0.03) (0.03) (0.03) (0.03) (0.03) (0.03) (0.03) (0.03) (0.03) (0.03) (0.03) (0.03) (0.03) (0.04) (0.04) (0.04) (0.04) (0.04) (0.04) (0.05) (0.05) (0.06) (0.05) (0.06) (0.06) (0.06) (0.06) (0.06) (0.07) (0.06) (0.	3.90 3.78 (0.10) 3.78 0.02) 3.83 (0.05) (0.06) (0.06) 3.86 (0.06) 3.86 (0.06) 3.86 (0.06) 3.86 (0.06) 3.86 (0.10) 3.78 3.78 (0.10) 3.78 (0.10) 3.78 (0.10) 3.78 (0.10) 3.78 (0.10) 3.78 (0.10) 3.78 (0.10) 3.78 (0.10) 3.78 (0.10) 3.78 (0.10) 3.78 (0.10) 3.78 (0.10) 3.78 (0.10) 3.78 (0.00) 3.78 (0.00) 3.83 (0.00) 3.86 (0.00) 3.80 (0.00) 3.80 (0.00) 3.80 (0.00) (0.00) 3.80 (0.00) (0.0)	3.89° (0.09) 3.81° (0.01) 3.89° (0.09) (0.09) (0.00) 3.72 3.72 (0.06) (0.06) (0.06) (0.06) (0.06) (0.06) (0.06) (0.06) (0.09) (0.09) (0.09) 3.87° (0.09) 3.88° (0.06) 3.88° (0.06) 3.80° (0.06) 3.80° (0.06) 3.80° (0	3.93 (0.13) 4.01 (0.21) 3.85 3.85 3.97 (0.17) (0.17) 3.45 3.45 3.45 3.45 3.45 3.45 3.45 3.45	4.07 (0.05) 4.10 (0.08) 4.07 4.07 (0.08) 4.07 (0.01) 3.66 4.03 (0.01) (0.01) (0.01) (0.01) (0.01) (0.01)	(0.05) (0.09) (1.32) (1.32) (1.32) (1.30) (1.30) (1.30) (1.33) (1.33) (1.33) (1.33) (1.33) (1.33) (1.33) (1.33) (1.33) (1.33) (1.33) (1.33) (1.32)	3.41 (0.00) (0.0
<ul> <li>β-D-Glcp(14)α-L-FucpOMe (4)</li> <li>α-L-Fucp(14)α-L-FucpOMe (5)</li> <li>β-L-Fucp(14)α-L-FucpOMe (6)</li> <li>α-L-Fucopyranose</li> <li>β-L-Fucopyranose</li> <li>α-D-Glucopyranose</li> <li>β-L-Glucopyranose</li> <li>α-L-fucopyranose</li> <li>α-D-Glucopyranose</li> <li>α-L-fucopyranose</li> <li>α-D-Glucopyranose</li> <li>α-D-Glucopyranose</li> <li>α-L-fucopyranose</li> <li>α-D-Glucopyranose</li> <li>α-D-Glucopyranose</li></ul>	(-0.03) 4.48 (-0.16) 4.97 (-0.23) 4.51 (-0.04) 5.20 4.55 5.23 4.64 4.64 4.64 4.64 4.64 4.64 4.64 4.6	(0.06) 3.41 (0.16) 3.83 (0.06) 3.83 (0.09) 3.77 3.46 3.54 3.54 3.54 3.54 3.54 3.54 3.54 3.54	(0.04) 3.52 (0.02) 3.92 (0.06) 3.63 3.63 3.63 3.63 3.63 3.63 3.63 3.	(0.01) (0.01) (0.01) (0.02) (0.02) (0.02) (0.02) (0.02) (0.02) (0.02) (0.02) (0.02) (0.02) (0.02) (0.01) (0.01) (0.01) (0.01) (0.01) (0.01) (0.01) (0.01) (0.01) (0.01) (0.01) (0.01) (0.02) (0	(0.00) 3.46 (0.00) 4.46 (0.26) 3.72 (-0.07) 3.79 3.79 3.46 3.79 3.46 3.79 3.46 3.79 3.46 (-0.07) 3.79 3.46 (-0.07) 3.79 (-0.07) 3.79 (-0.00) 3.79 (-0.00) 3.76 (-0.00) 4.26 (-0.00) 3.76 (-0.00) 3.76 (-0.00) 4.46 (-0.00) 4.46 (-0.00) 4.46 (-0.00) 4.46 (-0.00) 4.46 (-0.00) 4.46 (-0.00) 4.46 (-0.00) 4.46 (-0.00) 4.46 (-0.00) 4.46 (-0.00) 3.77 (-0.00) 3.77 (-0.00) 3.76 (-0.00) 3.76 (-0.00) 3.76 (-0.00) 3.76 (-0.00) 3.76 (-0.00) 3.77 (-0.00) 3.76 (-0.00) 3.66 (-0.00) 3.66 (-0.00) 3.66 (-0.00) 3.66 (-0.00) 3.66 (-0.00) 3.66 (-0.00) 3.66 (-0.00) 3.66 (-0.00) 3.66 (-0.00) 3.66 (-0.00) 3.66 (-0.00) 3.66 (-0.00) 3.66 (-0.00) 3.76 (-0.00) 3.66 (-0.00) 3.76 (-	(0.00) 3.91 (0.01) 1.19 (-0.02) 1.24 (-0.02) 1.24 1.21 1.26 3.84 3.84 3.90 3.90 and methyl the methyl g	(0.01) 3.75 (0.03) 3.76 3.72 3.72 hexoside fe	(0.02) 4.80 (0.03) ( 4.81 (0.04) 4.77 (0.00) (0.00) (0.00) 4.69 4.77 4.69 4.77 ar the glyco: sidue. <sup>c</sup> High	(0.10) 3.78 3.78 3.83 3.83 (0.03) 3.86 (0.06) (0.06) (0.06) 3.86 (0.06) 3.93 3.93 3.93 3.93 3.93 3.93 3.93 3.9	(0.09) 3.81 <sup>6</sup> (0.01) 3.89 (0.09) 3.86 <sup>6</sup> (0.06) (0.06) 3.36 <sup>6</sup> (0.06) (3.30 <sup>6</sup> (3.32 3.30 <sup>6</sup> (3.42) (3.42) (3.42) (3.42) (4.42)	(0.13) 4.01 (0.21) 3.85 (0.05) 3.97 (0.17) (0.17) (0.17) 3.45 3.45 3.80 <sup>c</sup> 3.80 <sup>c</sup> 	(0.05) 4.10 (0.08) 4.07 4.07 (0.01) 3.66 4.02 1.001 1.001 1.001	(0.05) (0.09) (1.32 (1.32 (1.32) (1.26) (1.26) (1.26) (1.23) (1.23) (1.23) (1.23) (1.23) (1.23) (1.23) (1.23) (1.23) (1.23) (1.23) (1.23) (1.23) (1.23) (1.23) (1.26) (1.2	(0.00) 3.41 (0.00) 3.42 (0.01) 3.39 (-0.02) (-0.02) 3.40 3.40 3.41 3.41
<ul> <li>β-D-Glcp(1→4)z-L-FucpOMe (4)</li> <li>α-L-Fucp(1→4)z-L-FucpOMe (5)</li> <li>β-L-Fucopyranose</li> <li>β-L-Fucopyranose</li> <li>β-L-Fucopyranose</li> <li>α-L-Glucopyranose</li> <li>β-D-Glucopyranose</li> <li>Methyl α-L-thannopyranoside</li> <li>Methyl α-L-fucopyranoside</li> <li>Methyl α-L-fucopyranoside</li> <li>and Glycosylation shifts are calculated by indicates a downfield shift.<sup>b</sup> Primed labe</li> </ul>	(-0.16) (-0.16) (-0.23) (-0.23) (-0.04) 5.20 4.55 5.23 4.64 4.64 4.64 4.64 4.64 4.64 4.64 4.6	3.41 (0.16) (0.16) (0.06) 3.85 (0.09) 3.77 3.46 3.54 3.54 3.54 3.54 3.54 3.54 3.57 3.54 3.57 3.57 3.57 3.57 3.57 3.57 3.57 3.57	3.52 (0.02) 3.92 (0.06) 3.63 3.63 3.63 3.72 3.50 3.50 3.50 3.50 3.50 3.50 3.50 3.50	3.43 (0.01) 3.83 (0.02) 3.72 <sup>e</sup> 3.72 <sup>e</sup> 3.74 3.81 3.42 3.42 3.42 3.42 3.42 3.42 3.42 3.42	3.46 (0.00) 4.46 (0.26) 3.72 (-0.07) 3.79 3.79 3.46 3.79 3.46 3.79 3.46 3.79 3.46 3.79 3.46 d abels to	3.91 (0.01) 1.19 (-0.02) 1.24 (-0.02) 1.24 1.21 1.26 3.84 3.84 3.90 3.90 and methyl the methyl g	3.75 (0.03) 3.76 3.72 hexoside fr	4.80 (0.03) ( 4.81 (0.04) 4.77 (0.00) (0.00) 4.69 4.77 5 of the glycol sidue. <sup>c</sup> High	3.78° - 0.02) 3.83 (0.03) 3.86° (0.06) (0.06) (0.06) 3.80° (0.06) 3.93 3.93 3.93 3.93 3.93 3.93 3.93 3.9	3.81 <sup>c</sup> (0.01) 3.89 (0.09) 3.86 <sup>c</sup> (0.06) (0.06) 3.72 3.80 <sup>c</sup> 3.72 3.80 <sup>c</sup> the aglycc the aglycc	4.01 (0.21) 3.85 (0.05) 3.97 (0.17) 3.45 3.45 3.80 <sup>c</sup> 3.80 <sup>c</sup> 	4.10 (0.08) 4.07 (0.05) 4.03 (0.01) (0.01) 3.66 4.02 tively, and from the C	1.32 (0.09) (0.09) (0.09) (0.03) (0.03) (0.03) (1.26 (0.03) (1.23 (1.23) (1.23) (1.23) (1.23)	3.41 (0.00) 3.42 (0.01) 3.39 (-0.02) (-0.02) (-0.02) (-0.02) (-0.02) (-0.02) (-0.02)
a-L-Fucp(14)z-L-FucpOMe (5) β-L-Fucp(14)z-L-FucpOMe (6) a-L-Fucopyranose β-L-Fucopyranose β-D-Glucopyranose a-D-Glucopyranose Methyl a-L-thamnopyranoside Methyl a-L-thucopyranoside Methyl a-L-fucopyranoside Methyl a-L-fucopyranoside Methyl a-L-fucopyranoside	(-0.10) 4.97 (-0.23) 4.51 (-0.04) 5.20 4.55 5.23 4.64 4.64 4.64 a.64 a.64 a.64 a.64 a.64	(0.10) 3.83 (0.06) 3.55 (0.09) 3.77 3.46 3.54 3.54 3.25 3.25 3.25 3.25 3.25 3.25 3.25 3.25	(0.02) 3.92 (0.06) 3.63 3.63 3.63 3.63 3.63 3.50 3.50 3.50 3.50 3.50 3.50 3.50	(0.01) (0.02) (0.02) (0.02) (-	(0.00) (4.06) (0.26) (0.26) (-0.07) (-	(0.01) 1.19 (-0.02) 1.24 1.21 1.21 1.26 3.84 3.90 3.90 and methyl the methyl g	3.76 3.72 hexoside fr	(0.00) (0.04) 4.77 (0.00) (0.0		(0.01) 3.89 (0.09) 3.86 (0.06) (0.06) 3.30 3.30 3.30 the aglycc	(0.11) 3.85 (0.05) 3.97 (0.17) (0.17) 3.45 3.45 3.80 <sup>c</sup> 3.80 <sup>c</sup> 	(0.08) 4.07 (0.05) 4.03 (0.01) (0.01) 3.66 4.02 tively, and from the C	(0.09) 1.32 (0.09) 1.26 (0.03) 1.26 1.23 1.23 1.23 1.23 1.23 1.23 1.23 1.23 1.23 1.23 1.23 1.23 1.25 1.26 1.20 1.26 1.20	(0.00) 3.42 (0.01) 3.39 (-0.02) (-0.02) (-0.02) (-0.02) (-0.02) (-0.02) (-0.02) (-0.02) (-0.02) (-0.02) (-0.02) (-0.01) 3.40 (-0.01) (-0.01) 3.40 (-0.01) 3.40 (-0.01) 3.42 (-0.01) 3.42 (-0.01) 3.42 (-0.01) 3.42 (-0.01) 3.42 (-0.01) 3.42 (-0.02) (
a-L-Fucp(14)a-L-FucpOMe (6) β-L-Fucopyranose β-L-Fucopyranose β-L-Fucopyranose a-D-Glucopyranose β-D-Glucopyranose Methyl a-L-thamopyranoside Methyl a-L-thucopyranoside a Glycosylation shifts are calculated by indicates a downfield shift. <sup>b</sup> Primed labb	(-0.23) 4.51 (-0.04) 5.20 4.55 5.23 4.64 4.64 4.64 abtraction o els refer to the els refer to the	(0.09) 3.55 3.55 3.77 3.77 3.46 3.54 3.25 3.25 3.25 3.25 3.25 3.25 3.25 3.25	(0.05 (0.06) (0.00) (0.00) (0.00) (0.00) (0.00) (0.00) (0.00) (0.00) (0.00) (0.00) (0.00) (0.00) (0.00) (0.00) (0.00) (0.06) (0.06) (0.06) (0.06) (0.06) (0.06) (0.00) (0.	(0.02) (0	(0.26) (0.26) (0.07) (-0.07) (	(-0.02) (-0.02) (-0.02) 1.21 1.26 3.84 3.90 3.90 and methyl the methyl g the methyl g	3.76 3.72 hexoside fr	(0.04) 4.77 (0.00) 4.69 4.77 4.77 5 cf e glyco: sidue. <sup>6</sup> High	(0.06) 3.86 (0.06) 3.93 3.93 3.93 3.93 3.80 <sup>c</sup> 1.93 3.80 <sup>c</sup> 1.93 3.80 <sup>c</sup> 1.93 3.80 <sup>c</sup>	(0.09) 3.86 (0.06) 3.72 3.72 3.87 1.42 3.80 ctra. <sup>4</sup> Che		(0.01) (0.01) (0.01) (0.01) (0.01) (0.01) (0.01) (0.01) (0.01) (0.01) (0.01) (0.01) (0.01) (0.01) (0.05) (0.05) (0.05) (0.05) (0.05) (0.05) (0.05) (0.05) (0.05) (0.05) (0.05) (0.05) (0.05) (0.05) (0.01) (0	(0.03) (0.03) (0.03) (0.03) (0.03) (0.03) (0.03) (0.03) (1.26) (1.23) (1.23) (1.23) (1.23) (1.23) (1.23) (1.23) (1.26) (1.22) (1.26) (1.22) (1	
<ul> <li>β-L-Fucp(14)z-L-FucpOMe (6)</li> <li>α-L-Fucopyranose</li> <li>β-L-Fucopyranose</li> <li>α-D-Glucopyranose</li> <li>α-D-Glucopyranose</li> <li>Methyl α-L-thannopyranoside</li> <li>Methyl α-L-fucopyranoside</li> <li>a Glycosylation shifts are calculated by indicates a downfield shift. <sup>b</sup> Primed labe</li> </ul>	(-0.04) 5.20 4.55 5.23 4.64 4.64 4.64 4.64 els refer to the els refer to the	3.25 3.77 3.77 3.46 3.54 3.25 3.25 3.25 3.25 3.25 3.25 3.25 3.25	3.60 (0.00) 3.86 3.63 3.72 3.50 3.50 3.50 3.50 3.50 3.50	(-0.02) (-0.02) 3.81 3.74 3.42 3.42 3.42 3.42 correspont and unprim	(	(-0.02) (-0.02) 1.21 1.26 3.84 3.90 3.90 and methyl the methyl g the to inten	3.76 3.72 hexoside fr	(0.00) (0.00) 4.77 4.77 5 the glyco: sidue. <sup>c</sup> High			3.97 (0.17) 3.45 3.80 <sup>c</sup> mc, respect mical shift	(0.01) (0.01) 3.66 4.02 tively, and from the C	(0.03) (0.03) (0.03) (1.26 (1.23) (1.23) a positive d	(100) (-0.02) (-0.02) 3.40 3.41 3.41 3.41
«-L-Fucopyranose β-L-Fucopyranose «-D-Glucopyranose Methyl «-L-rhamnopyranoside Methyl «-L-fucopyranoside «Glycosylation shifts are calculated by indicates a downfield shift. <sup>b</sup> Primed lab	(-0.04) 5.20 4.55 5.23 4.64 4.64 aubtraction o els refer to the els refer to the	(0.09) 3.77 3.46 3.54 3.25 3.25 3.25 f chemical glycopyrar	(0.00) 3.86 3.63 3.72 3.50 3.50 3.50 shifts of the nosyl group i	(-0.02) 3.81 3.74 3.42 3.42 3.42 correspon and unprim	(-0.07) 4.20 3.79 3.84 3.46 3.46 1ing hexose ied labels to	(-0.02) 1.21 1.26 3.84 3.90 and methyl the methyl g the methyl g	3.76 3.72 hexoside fr	(0.00) 4.69 4.77 5r the glycor sidue. <sup>6</sup> High	(0.06) 3.93 3.80 <sup>6</sup> 3.80 <sup>6</sup> syl part and ter order spe	(0.06) 3.72 3.80 <sup>c</sup> the aglycc tctra. <sup>d</sup> Che	(0.17) 3.45 3.80 <sup>c</sup> me, respect mical shift	(0.01) 3.66 4.02 tively, and from the C	(0.03) 1.30 1.23 a positive d ',H-COSY s <sub>i</sub>	(-0.02) 3.40 3.41 3.41 ifference
a-L-Fucopyranose β-L-Fucopyranose β-D-Glucopyranose β-D-Glucopyranose Methyl a-L-rhamnopyranoside Methyl a-L-fucopyranoside aflycosylation shifts are calculated by indicates a downfield shift. <sup>b</sup> Primed labe	5.20 4.55 5.23 4.64 4.64 abtraction o els refer to the els refer to the	3.77 3.46 3.54 3.25 3.25 f chemical glycopyrar	3.86 3.63 3.72 3.50 3.50 3.50 shifts of the nosyl group i	3.81 3.74 3.42 3.42 3.42 correspont and unprim	4.20 3.79 3.46 3.46 3.46 1ing hexose ied labels to	1.21 1.26 3.84 3.90 and methyl the methyl g the methyl g	3.76 3.72 hexoside fr ilycoside re:	4.69 4.77 or the glyco: sidue. <sup>6</sup> High	3.93 3.80 <sup>c</sup> syl part and the order spe	3.72 3.80 <sup>c</sup> the aglycc cctra. <sup>4</sup> Che	3.45 3.80 <sup>6</sup> nne, respect mical shift	3.66 4.02 from the C	1.30 1.23 a positive d	3.40 3.41 ifference ectrum.
<ul> <li>β-L-Fucopyranose</li> <li>α-D-Glucopyranose</li> <li>β-D-Glucopyranose</li> <li>β-D-Glucopyranose</li> <li>Methyl α-L-rhannopyranoside</li> <li>Methyl α-L-fucopyranoside</li> <li><sup>a</sup> Glycosylation shifts are calculated by indicates a downfield shift. <sup>b</sup> Primed labe</li> </ul>	4.55 5.23 4.64 4.64 subtraction o els refer to the els refer to the	3.46 3.54 3.25 f chemical glycopyran	3.63 3.72 3.50 3.50 shifts of the nosyl group (	3.74 3.42 3.42 3.42 correspont and unprim	3.79 3.84 3.46 3.46 ding hexose ed labels to	1.26 3.84 3.90 and methyl the methyl g the to intern	3.76 3.72 hexoside for the coside read	4.69 4.77 or the glyco: sidue. <sup>c</sup> High	3.93 3.80 <sup>c</sup> 3.81 part and syl part and	3.72 3.80 <sup>c</sup> the aglycc	3.45 3.80° ne, respect mical shift	3.66 4.02 tively, and from the C	1.30 1.23 a positive d	3.40 3.41 ifference ectrum.
<ul> <li>a-D-Glucopyranose</li> <li>B-D-Glucopyranose</li> <li>Methyl a-L-rhamnopyranoside</li> <li>Methyl a-L-fucopyranoside</li> <li>Glycosylation shifts are calculated by indicates a downfield shift. <sup>b</sup> Primed labe</li> </ul>	5.23 4.64 subtraction o els refer to the disaccharides	3.54 3.25 f chemical glycopyran	3.72 3.50 shifts of the nosyl group :	3.42 3.42 3.42 correspont and unprim	3.84 3.46 ing hexose ed labels to	3.84 3.90 and methyl the methyl g the to inten	3.76 3.72 a.72 hexoside for the coside re:	4.69 4.77 or the glyco sidue. <sup>6</sup> High	3.93 3.80° syl part and ter order spe	3.72 3.80 <sup>c</sup> the aglycc cctra. <sup>4</sup> Che	3.45 3.80° ne, respect mical shift	3.66 4.02 tively, and from the C	1.30 1.23 a positive d	3.40 3.41 ifference bectrum.
B-D-Glucopyranose Methyl <i>a</i> -L-rhamnopyranoside Methyl <i>a</i> -L-fucopyranoside "Glycosylation shifts are calculated by indicates a downfield shift. <sup>b</sup> Primed labe	4.64 subtraction of els refer to the disaccharides	3.25 3.25 f chemical glycopyrar	3.50 shifts of the nosyl group :	3.42 3.42 correspont and unprim	3.46 ing hexose ed labels to	3.90 and methyl the methyl g ative to inter	3.72 hexoside fo tycoside rei	4.69 4.77 or the glyco sidue. * Higt	3.93 3.80 <sup>c</sup> 3.81 part and her order spe	3.72 3.80 <sup>c</sup> 1.41c aglycc the aglycc	3.45 3.80 <sup>c</sup> me, respect mical shift	3.66 4.02 tively, and from the C	1.30 1.23 a positive d ,H-COSY si	3.40 3.41 ifference bectrum.
Methyl <i>a</i> -1-rhamnopyranoside Methyl <i>a</i> -1-fucopyranoside "Glycosylation shifts are calculated by indicates a downfield shift. <sup>b</sup> Primed labe	subtraction or els refer to the disaccharides	f chemical glycopyrar	shifts of the tosyl group (	correspont and unprim te monosac	ing hexose ed labels to	and methyl the methyl g tive to inten	hexoside fo	4.69 4.77 or the glyco: sidue. <sup>e</sup> High	3.93 3.80 <sup>c</sup> syl part and her order spe	3.72 3.80 <sup>c</sup> the aglycc cetra. <sup>d</sup> Che	3.45 3.80° me, respect mical shift	3.66 4.02 tively, and from the C	1.30 1.23 a positive d 'H-COSY si	3.40 3.41 ifference sectrum.
Methyl <i>a</i> -1-fucopyranoside <sup><i>a</i></sup> Glycosylation shifts are calculated by indicates a downfield shift. <sup>b</sup> Primed labe Toblo 2 13C NMD chemical shifts of the	subtraction of sls refer to the disaccharides	f chemical glycopyrar	shifts of the tosyl group (	correspond and unprim te monosac	ling hexose ed labels to	and methyl the methyl g the to inter	hexoside fo	4.77 ar the glyco: sidue. <sup>c</sup> High	3.80° syl part and ier order spe	3.80 <sup>c</sup> the aglycc cctra. <sup>d</sup> Che	3.80° ne, respect mical shift	4.02 tively, and from the C	1.23 a positive d ',H-COSY si	3.41 ifference sectrum.
<sup>a</sup> Glycosylation shifts are calculated by indicates a downfield shift. <sup>b</sup> Primed labe	subtraction of sls refer to the disaccharides	f chemical glycopyran	shifts of the tosyl group :	correspond and unprim te monosac	ling hexose ed labels to	and methyl the methyl g tive to inter	hexoside fo	or the glyco: sidue. ' High to 67.40).	syl part and ler order spe	the aglyco ctra. <sup>4</sup> Che	mical shift	tively, and from the C	a positive d ,H-COSY s	ifference bectrum.
Substances	C-1' <sup>b</sup>	C-2′	C-3′	C 4⁄	C-5′	C-6′	C-1	C-2	C-3	C-4	4 C-5	-5	C.6	OMe
					0000									
$\alpha$ -L-Fucp $(1 \rightarrow 4)\alpha$ -L-RhapOMe (1)	101.33	69.49 (0.40)	9C.07	(11)	68.09 (00.00)	16.03	101.62		9 /1.87		82.14 (9.13) (	67.87 - 1 36)	17.88	55.63 (0.00)
$R_{-1} - F_{11} cn(1 \rightarrow 4)n-1 - R hanOMe$ (2)	104.00	71.68	73.80	72.11	71.82			4 70.28				67.88	17.39	(0.0) 55.66
	(6.85)	(-1.05)	(-0.13)	(-0.24)	(0.18)	-	Ū	Ļ	÷	_	<u> </u>	-1.35)	-0.07)	(0.12)
$\alpha$ -D-Glcp(1	101.76	73.11	74.00	70.44	73.47				•		•	67.11	16.84	56.03
	(8.77)	(0.64) 7.25	(0.22)	(-0.27)	(1.10)	-	(0.04)	4) (0.54)	(0.79)		(10.13) (-	-0.06)	(0.72)	(0.07)
β-D-Glcp(1-+4)α-L-FucpOMe (4)	104.10	74.37	76.56	/0.48	76.83							67.40	16.11	56.08
(5) (5) (5) (5) (5) (5) (5) (5)	(07.1)	(co.n ) 69 14	70.40	(2.0-)	(10.07) 67.80	-	-	-	_		(C7.6) 81 03	(0.2.0) 67.83	- 0.01)	(0.12) \$6.08
	(8.19)	(0.05)	(0.10)	(0.02)	(0.70)	-			0		_	(0.66)	-0.02)	(0.12)
$\beta$ -L-Fucp $(1 \rightarrow 4)_{\alpha}$ -L-FucpOMe (6)	105.32	72.57	74.16	72.29	71.72			-	, ,			66.95	16.53	56.03
•	(8.17)	(-0.16)	(0.23)	(-0.06)	(0.08)	Ī	-		(0.61) (0.61)		÷	-0.22)	(0.41)	(0.07)
α-L-Fucopyranose	93.12	60.69	70.30	72.80	67.10									
β-L-Fucopyranose	97.15	72.73	73.93	72.35	49.17 29.25									
a-D-Glucopyranose	92.99 06.84	14.71	13.18 76.76	70.71	16.21	01.84 61 84								
Mothul Thomsonization	10.00	07.01	0.01	1.01			101 74					60 73	17 46	25 54
Methyl a-L-Inaunopyranoside Methyl a-L-fucopyranoside							100.44	4 68.97	70.64		72.72	67.17	16.12	55.96

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

Table 4. Chemical shift differences (ppm) from variation in temperature."

		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
α-L-Fucp(1 $\rightarrow$ 4)α-L-RhapOMe β-L-Fucp(1 $\rightarrow$ 4)α-L-RhapOMe α-D-Glcp(1 $\rightarrow$ 4)α-L-FucpOMe β-D-Glcp(1 $\rightarrow$ 4)α-L-FucpOMe α-L-Fucp(1 $\rightarrow$ 4)α-L-FucpOMe β-L-Fucp(1 $\rightarrow$ 4)α-L-FucpOMe	(1) (2) (3) (4) (5) (6)	$\begin{array}{r} 0.02 \\ -0.06 \\ -0.03 \\ 0.05 \\ 0.03 \\ -0.01 \end{array}$	0.15 0.12 0.13 0.08 0.29 0.10	0.20 0.20 0.14 0.19 0.15 0.14	0.06 0.08 0.23 0.22 0.03 0.09	-0.09 0.00 0.01 0.08 0.04 -0.06	-0.04 -0.04 0.21 0.19 0.08 -0.04	0.03 0.03 0.07 0.08 0.05 0.06	0.08 0.18 0.13 0.22 0.07 0.14	0.07 0.08 0.08 0.11 0.23 0.06	$\begin{array}{r} 0.17 \\ -0.07 \\ -0.05 \\ 0.22 \\ 0.22 \\ -0.10 \end{array}$	0.10 0.06 -0.03 0.00 -0.01 -0.03	-0.02 0.07 0.00 0.06 -0.02 0.01	0.03 0.00 0.02 0.03 0.02 0.03

<sup>a</sup>  $\Delta \delta = \delta$  (70 °C)  $- \delta$  (30 °C). Dioxane was taken as  $\delta$  67.40 ppm for all temperatures.

linked through an axial O-4, (3)-(6). The calculations give for two of the  $\beta$ -glycosides also a local energy minimum at  $\varphi$  ca. -160° and  $\psi$  ca. -10° but this is of higher energy (1 kcal) than that of the global minimum.

<sup>1</sup>H NMR Glycosylation Shifts.—The <sup>1</sup>H NMR chemical shifts and the glycosylation shifts ( $\Delta\delta$ , induced chemical shift differences relative to the chemical shifts of the respective monomers) of compounds (1)–(6) and relevant monomers are given in Table 2.

The glycosylation shifts for proton signals from the disaccharides range from -0.23-0.26 ppm. The significant glycosylation shifts (>0.05 ppm) are mainly found for signals from protons on linkage carbons and carbons next to these. In addition large shifts are found for signals from 5'-H in the glycosyl group, and 2-H and 6-H in the methyl glycoside residue, which are in a '1,3-diaxial' relation to the substituted O-4.

The signals from anomeric protons in the glycosyl group, which according to the HSEA calculations are close to protons and no oxygens in the methyl glycoside residue (Table 1), are shifted upfield -0.12, -0.16, and -0.23 ppm for compounds (2), (4), and (5), respectively. Remaining values for anomeric proton signals are found between -0.04 and 0.03 ppm. For signals from 2'-H a significantly larger shift is often observed for  $\beta$ -glycosides compared to that for  $\alpha$ -glycosides<sup>3-8</sup> but this is, however, not particularly evident for the disaccharides containing a L-fucopyranosyl group. The calculated short distance between 5'-H and O-3 in (5) corresponds to the large glycosylation shift, 0.26 ppm, observed for the 5'-H signal.

The stereochemistry around the glycosidic linkage in (1) and (2) is similar to that of  $\alpha$ - and  $\beta$ -D-Glcp-(1 $\rightarrow$ 4)-D-Glcp,<sup>3</sup> respectively, with the exception that the methyl groups in (1) and (2) correspond to hydroxymethyl groups in the latter disaccharides and that 4-H in (1) and (2) has a 1,3-diaxial relation to O-2. The glycosylation shifts of these disaccharides are similar for the signals from the protons adjacent to the linkage but *ca.* 0.5 that for the signal from 4-H. However, a comparison between (3)-(6) and compounds with similar stereochemistry around the glycosidic linkage *i.e.* L-Fucp-(1 $\rightarrow$ 4)-D-Galp and D-Glcp-(1 $\rightarrow$ 4)-D-Galp<sup>5</sup> shows, for signals from 3-H, 4-H, and 5-H, a high degree of similarity with differences in glycosylation shifts < 0.07 ppm.

For other disaccharides with the linkage through an equatorial oxygen it has been shown that glycosyl groups with an  $\alpha$ -D and  $\beta$ -L configuration or the  $\beta$ -D and  $\alpha$ -L configuration give similar glycosylation shifts for the signals from protons at or adjacent to the linkage.<sup>3,5</sup> This is not observed for (3) and (6) or for (4) and (5) and is most likely related to the fact that the linkage is through two axial positions in (3) and (5). However, similar shifts are observed in pairs for signals from 2-H and 6-H in (3) and (6) and in (4) and (5). This may be due to the fact that 2-H and 6-H have a '1,3-diaxial' relation to O-4 and that the 4-O-glycosyl group interacts similarly with these protons in respective disaccharide pairs. The shifts of signals from 2-H and 6-H can also be linearly correlated to changes in  $\psi$ -angles indicating that these shifts are dependent on the

distance between the lone-pairs on O-4 and the corresponding protons.

<sup>13</sup>C NMR Glycosylation Shifts.—The <sup>13</sup>C NMR chemical shifts for compounds (1)–(6) and relevant monomers together with the glycosylation shifts, obtained as described for the <sup>1</sup>H NMR glycosylation shifts, are given in Table 3. Significant  $\Delta\delta$ -values, >0.5 ppm, are mainly observed for signals of linkage carbons and for most of the adjacent carbons, which is the same pattern as that observed for other disaccharides.<sup>1-8</sup>

For signals from anomeric carbons the range of glycosylation shifts is rather small with most values >8 ppm. The  $\beta$ -glycosides have their C-2' signals significantly shifted upfield, whereas the  $\alpha$ -glycosides have their C-5' signals shifted downfield, common observations for  $\beta$ -glycosides and  $\alpha$ -glycosides, respectively.

In the methyl glycoside residue the glycosylation shifts for signals from linkage carbons are >8 ppm, as is also observed for the C-1' signals, which range from 8.3 to 10.2 ppm. A comparison of glycosylation shifts for signals from the linkage carbons, C-4, and the adjacent carbons, C-3 and C-5, shows the same pattern as previously observed,<sup>3.5</sup> with similar shifts for  $\alpha$ -D/ $\beta$ -L-, (3) and (6), and  $\beta$ -D/ $\alpha$ -L-substitution, (4) and (5).

As regards signals from more distant carbons, some of those from C-2 and C-6 have shifts > 0.5 ppm. The reason for this may be differences in the magnitude of the  $\gamma$ -gauche effect caused by the '1,3-diaxial' interaction between the lone-pairs on O-4 and 2-H or 6-H, when O-4 is unsubstituted or substituted with a glycosyl group. Similar shifts for the C-6 signal are observed for  $\alpha$ -D/ $\beta$ -L- (3) and (6), respectively, and  $\beta$ -D/ $\alpha$ -L- (4) and (5), substituted L-fucosides.

Effects of Temperature Variation on <sup>13</sup>C NMR Chemical Shifts.—Values for chemical shifts differences (in ppm) obtained from <sup>13</sup>C NMR spectra recorded at 30 and 70 °C are shown in Table 4. Most signals are shifted downfield with reference to the internal standard, dioxane, to which a constant chemical shift is assigned.

For signals from linkage carbons, which normally show the largest shift, only small shifts from -0.10 to 0.22 ppm, are observed. Similar shifts between (3) and (6) and between (4) and (5) are found, being negative for the C-1' and the C-4 signals for the first pair and positive for the second pair.

#### Conclusion

From the investigation of the disaccharide glycosides (1)-(6)and other 1,4-linked disaccharides<sup>3,5</sup> we conclude that a typical set of glycosylation shifts in the <sup>1</sup>H and <sup>13</sup>C NMR spectra is obtained on glycosidation of the 4-position in a sugar residue. It should therefore be possible to calculate the chemical shifts of disaccharide elements in oligo- and poly-saccharides, which are similar in stereochemistry around the glycosidic bond. The results make up part of the material that is used as the database of the computer program CASPER,<sup>9,10</sup> by which spectra of oligo- and poly-saccharides may be simulated.

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