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NMR studies of some (1 → 6)-linked disaccharide methyl glycosides

Per-Erik Jansson [a,*], Lennart Kenne [b], Irene Kolare [a]

[a] *Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, S-106 91 Stockholm, Sweden*

[b] *Department of Chemistry, Swedish University of Agricultural Sciences, Box 7015, S-750 07 Uppsala, Sweden*

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Abstract

NMR studies have been performed on the methyl glycosides of some (1 → 6)-linked disaccharides. Observed $J_{5,6pro\text{-}R}$ and $J_{5,6pro\text{-}S}$ values indicate that, for the 6-substituted D-gluco- and D-galacto-pyranosides, the rotamer distribution around the C-5–C-6 bond deviates somewhat from that observed for the respective unsubstituted monosaccharide glycosides. There is also a difference between 6-*O*-α-D- or 6-*O*-β-L- on the one hand and 6-*O*-β-D- or 6-*O*-α-L-substituted glycosides on the other, with somewhat larger values for $J_{5,6pro\text{-}R}$ for the latter two indicating a higher proportion of the *gauche–trans* conformer. The glycosylation shifts observed for the signals from the 6-protons in the glycosidic linkage were dependent on the type of anomeric and absolute configuration of the glycosyl group. NOE measurements by irradiation of the anomeric proton indicated that sugars 6-substituted with α-D- or β-L-glycosyl groups have highly populated conformations in which H-1 and H-6*pro-S* are proximal, and for β-D- and α-L-glycosyl groups conformations in which H-1 and H-6*pro-R* are proximal.

1. Introduction

The (1 → 6)-glycosidic linkage has received considerable attention as the additional rotational freedom, present in the C-5–C-6 bond, makes it more flexible than the (1 → 2), (1 → 3), and (1 → 4) linkages. In the free glycopyranoses, the preferred conformers of the hydroxymethyl group are *gauche–gauche* (*gg*), *gauche–trans* (*gt*), and *trans–gauche* (*tg*) (Fig. 1).

* Corresponding author.

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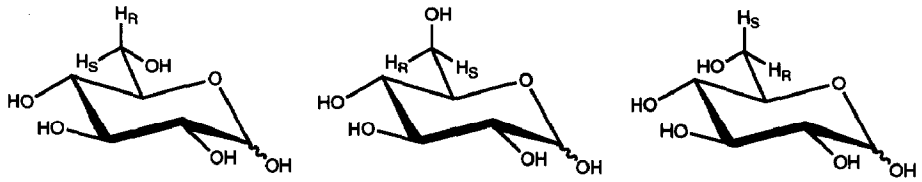

Fig. 1. Preferred rotamers of the hydroxymethyl group in D-glucopyranose: *gauche–trans* (*gt*), *gauche–gauche* (*gg*), and *trans–gauche* (*tg*). The prochiral methylene protons are indicated with H_R and H_S.

Studies [1,2] have been devoted to the determination of the rotamer population distribution of the hydroxymethyl group in hexopyranoses where the use of specifically C-6-deuterated sugars facilitated the analysis. In these studies, the proportions of the respective rotamers were determined from the $J_{5,6}$ values, using different generalised Karplus equations [3]. Thus, roughly equimolar proportions of the *gg* and *gt* conformers and only minor amounts of the *tg* conformer were found for the glucopyranoses. The latter conformer suffers from a steric 1,3-interaction between O-4 and O-6. The studies also showed that, for the galactopyranoses, all three conformers are present with a preponderence of the *gt* conformer over the *tg* conformer which is the second most populated. A ^{1}H NMR study of some (1 → 6)-linked disaccharides consisting of D-mannopyranosyl residues showed, from the $J_{5,6}$ values [4], that the rotamer distribution of the hydroxymethyl group involved in the linkage was similar to that of the monosaccharide and with the C-1–O-6–C-6–C-5 torsion angle near 180°. Cumming and Carver [5,6] investigated the preferred conformations of the C-5–C-6 bond of disaccharides containing D-mannopyranosyl residues, using $J_{5,6}$ values, NOE data, and empirical energy calculations for ensemble averaging, and found an equal proportion of *gg* and *gt* rotamers. Rotamer distributions similar to those in the respective monomers were also found for maltose and isomaltose [2] and for some mono- and di-galactopyranoside derivatives [7,8]. For the latter compounds, the solvent dependence of the rotamer population was also studied using $J_{5,6}$ values and a new set of equations for calculation of the proportion of the respective rotamer. Conformational analysis of some 4,6-branched trisaccharides demonstrated that a β-D-glucopyranosyl group at O-4 increases the proportion of the *gg* rotamer at the (1 → 6) linkage [9]. In another study, using $J_{5,6}$ values, of 3,6-disubstituted D-mannopyranosyl residues that form parts of oligosaccharides in glycopeptides, the presence of only the *gg* rotamer was indicated [10].

We have previously investigated a number of di- and tri-saccharides with respect to ^{1}H and ^{13}C NMR chemical shifts [11,12]. A correlation of the glycosylation shifts, i.e., the difference in chemical shift for the signals of the disaccharide compared to those of the monomers, to interresidue atomic distances in the calculated minimum energy conformations was found. The purpose of these

studies has been to increase our knowledge of the properties of the glycosidic bond, to aid the interpretation of NMR spectra of oligo- and poly-saccharides, and to supply data for the database of the computer program CASPER [13,14]. This program can, from information on components, linkages, and NMR chemical shifts, suggest the most likely structure which fits the available data.

In an earlier study of some (1 → 6)-linked disaccharide methyl glycosides, only ^{13}C NMR chemical shifts were analysed [15]. We now report, for these disaccharides and some additional (1 → 6)-linked disaccharides, on ^{1}H NMR data and preferred conformations, using $J_{5,6}$ values and NOE data.

2. Results and discussion

Compounds **1–14** (Table 3) were available in our laboratory [12,15]. Of these, **1**, **2**, **5**, **6**, **9**, **11**, and **13** are termed α-glycosides with reference to the central bond, and the remaining compounds are termed β-glycosides.

^{1}H NMR glycosylation shifts.—The ^{1}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 **1–10** and relevant monomers are given in Table 1. The hydroxymethyl protons, H-6*pro-R* and H-6*pro-S* are referred to as $H\text{-}6_R$ and $H\text{-}6_S$. The assignment of the corresponding NMR signals is based on literature data [1,2] which indicates that the signal with the smaller coupling can be assigned to $H\text{-}6_S$ and that with the larger coupling constant to $H\text{-}6_R$. All coupling constants of ring proton signals were of the expected size, showing that no significant conformational changes of the pyranosidic rings had occurred.

In the glycosyl group, significant glycosylation shifts (> 0.05 ppm) were observed only for signals from H-1′, H-2′, and H-5′. For the anomeric proton signal, the shift is ca. −0.27 ppm for all α-D-glycopyranosyl groups and ca. −0.12 ppm for β-D-glycopyranosyl groups with the exception of compound **10** which contains a β-D-mannopyranosyl group with a glycosylation shift of −0.20 ppm. The upfield shifts of the signals from all H-1′ can be correlated with a short distance to a 6-proton (see below). The H-2′ signals of the β-D-glycopyranosyl groups are shifted between 0.06 and 0.09 ppm and the H-5′ signals for the α-glycopyranosyl groups by ca. −0.10 ppm. The magnitude of the glycosylation shifts of the glycosyl groups is not influenced by the configuration at C-4 in the methyl glycoside residue, as observed by comparison of the glycosylation shifts for the disaccharides containing a methyl D-galactoside and a methyl D-glucoside residue, respectively.

For the methyl glycoside residue, significant glycosylation shifts are observed for signals from H-4, H-5, $H\text{-}6_R$, and $H\text{-}6_S$. For almost all signals, the shifts are positive, i.e., downfield, up to 0.29 ppm, except for the signal from $H\text{-}6_S$ of the glucopyranosides **1**, **2**, and **9** and galactopyranosides **5** and **6**, substituted with α-D-glycopyranosyl groups, which is shifted upfield by ca. −0.10 and −0.01 ppm, respectively. The magnitude of the glycosylation shifts of the $H\text{-}6_R$ and $H\text{-}6_S$ signals is correlated to the anomeric configuration of the glycopyranosyl group. A

Table 1
^{1}H NMR chemical shifts of the disaccharides **1–10** and appropriate monosaccharides obtained at 70°C and relative to internal TSP (δ 0.00). Glycosylation shifts are given in parenthesis

Substance	H-1′[b]	H-2′	H-3′	H-4′	H-5′	H-6′$_R$	H-6′$_S$	H-1	H-2	H-3	H-4	H-5	H-6$_R$	H-6$_S$	OMe
α-D-Glc*p*-(1 → 6)-α-D-Glc*p*OMe	4.96	3.57	3.74	3.44	3.73	3.77	3.86	4.82	3.58	3.68	3.51	3.81[c]	3.97	3.78[c]	3.44
1	(−0.27)	(0.03)	(0.02)	(0.02)	(−0.11)	(0.01)	(0.02)	(0.01)	(0.02)	(0.00)	(0.10)	(0.17)	(0.21)	(−0.09)	(0.01)
α-D-Glc*p*-(1 → 6)-β-D-Glc*p*OMe	4.96	3.57	3.73	3.44	3.73[c]	3.76[c]	3.86	4.39	3.29	3.51[c]	3.51[c]	3.63	3.96	3.82	3.57
2	(−0.27)	(0.03)	(0.01)	(0.02)	(−0.11)	(0.00)	(0.02)	(0.02)	(0.01)	(0.01)	(0.11)	(0.17)	(0.22)	(−0.10)	(−0.01)
β-D-Glc*p*-(1 → 6)-α-D-Glc*p*OMe	4.50	3.33	3.51	3.41	3.45	3.74	3.92	4.81	3.57	3.68	3.49	3.80	3.90	4.16	3.44
3	(−0.14)	(0.08)	(0.01)	(−0.01)	(−0.01)	(0.02)	(0.02)	(0.00)	(0.01)	(0.00)	(0.08)	(0.16)	(0.14)	(0.29)	(0.01)
β-D-Glc*p*-(1 → 6)-β-D-Glc*p*OMe	4.52	3.34	3.51	3.41	3.46	3.74	3.92	4.38	3.28	3.50	3.46	3.62	3.88	4.20	3.58
4	(−0.12)	(0.09)	(0.01)	(−0.01)	(0.00)	(0.02)	(0.02)	(0.01)	(0.00)	(0.00)	(0.06)	(0.16)	(0.14)	(0.28)	(0.00)
α-D-Glc*p*-(1 → 6)-α-D-Gal*p*OMe	4.95	3.57	3.71	3.43	3.75[c]	3.76[c]	3.86	4.84	3.83[c]	3.83[c]	4.02	4.06	3.87	3.75	3.43
5	(−0.28)	(0.03)	(−0.01)	(0.01)	(−0.09)	(0.00)	(0.02)	(−0.01)	(−0.01)	(0.02)	(0.03)	(0.17)	(0.11)	(−0.01)	(0.00)
α-D-Glc*p*-(1 → 6)-β-D-Gal*p*OMe	4.96	3.57	3.72	3.43	3.75[c]	3.76[c]	3.87	4.33	3.53	3.67	3.98	3.87	3.93	3.76	3.57
6	(−0.27)	(0.03)	(0.00)	(0.01)	(−0.09)	(0.00)	(0.03)	(0.02)	(0.01)	(0.03)	(0.05)	(0.19)	(0.15)	(−0.02)	(−0.01)
β-D-Glc*p*-(1 → 6)-α-D-Gal*p*OMe	4.51	3.31	3.51	3.40	3.47	3.73	3.92	4.85	3.83[c]	3.82[c]	4.02	4.08[c]	3.89	4.05[c]	3.44
7	(−0.13)	(0.06)	(0.01)	(−0.02)	(0.01)	(0.01)	(0.02)	(0.00)	(−0.01)	(0.01)	(0.03)	(0.19)	(0.13)	(0.29)	(0.01)
β-D-Glc*p*-(1 → 6)-β-D-Gal*p*OMe	4.53	3.31	3.51	3.40	3.46	3.74	3.92	4.33	3.53	3.65	3.97	3.87	3.93	4.06	3.58
8	(−0.11)	(0.06)	(0.01)	(−0.02)	(0.00)	(0.02)	(0.02)	(0.02)	(0.01)	(0.01)	(0.04)	(0.19)	(0.15)	(0.28)	(0.00)
α-D-Man*p*-(1 → 6)-α-D-Glc*p*OMe	4.91	3.98	3.83	3.70[c]	3.70[c]	3.78	3.89	4.80	3.56	3.68	3.48	3.78[c]	3.96	3.78[c]	3.43
9	(−0.27)	(0.04)	(−0.03)	(0.02)	(−0.12)	(0.04)	(0.03)	(−0.01)	(0.00)	(0.00)	(0.07)	(0.14)	(0.20)	(−0.09)	(0.00)
β-D-Man*p*-(1 → 6)-α-D-Glc*p*OMe	4.69	4.04	3.63[c]	3.63[c]	3.37	3.76	3.93	4.80	3.56	3.68	3.44	3.79	3.85	4.16	3.44
10	(−0.20)	(0.09)	(−0.03)	(0.03)	(−0.01)	(0.01)	(0.02)	(−0.01)	(0.00)	(0.00)	(0.03)	(0.15)	(0.09)	(0.29)	(0.01)
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								
α-D-Mannopyranose	5.18	3.94	3.86	3.68	3.82	3.74	3.86								
β-D-Mannopyranose	4.89	3.95	3.66	3.60	3.38	3.75	3.91								
Methyl α-D-glucopyranoside								4.81	3.56	3.68	3.41	3.64	3.76	3.87	3.43
Methyl β-D-glucopyranoside								4.37	3.28	3.50	3.40	3.46	3.74	3.92	3.58
Methyl α-D-galactopyranoside								4.85	3.84	3.81	3.99	3.89	3.76	3.76	3.43
Methyl β-D-galactopyranoside								4.31	3.52	3.64	3.93	3.68	3.78	3.78	3.58

[a] Glycosylation shifts are calculated by subtraction of the chemical shifts of the corresponding hexose and methyl hexoside from those of the glycosyl part and the "aglycon" part, respectively, and a positive difference indicates a downfield shift. [b] Primed labels refer to the glycopyranosyl group and unprimed to the methyl glycoside residue, respectively. [c] Signals of higher order.

larger glycosylation shift is observed for the H-6_R signal in the α-linked disaccharides and for the H-6_S signal in the β-linked disaccharides. For signals from the methyl galactopyranoside residues substituted with α-glycosyl groups, smaller glycosylation shifts of the H-6_R signal are observed than for the corresponding methyl glucopyranosides and practically no shifts of the H-6_S signals.

For the monomeric sugars, the H-6_S signal is downfield of the H-6_R signal for the glucosides and on similar chemical shifts for the galactosides. However, on 6-substitution, a large difference in glycosylation shifts occurs for the H-6_S signal, an upfield shift for the α-glycosides and a large downfield shift for the β-glycosides. On the other hand, for the H-6_R signal, a downfield shift occurs for both α- and β-glycosides. These changes cause the relative positions of the H-6_R and H-6_S signals to shift for the α-glycosides. In these, the signal for H-6_R is more downfield than that for H-6_S. For the H-5 signals, the glycosylation shifts are between 0.14 and 0.19 ppm, and for the H-4 signals between 0.03 and 0.11 ppm, with somewhat larger shifts of the latter for the α-glycosides than for the β-glycosides.

The glycosylation shifts for the fucosyl-containing disaccharides **11–14** follow the same pattern and these have been reported [12].

^{13}C NMR glycosylation shifts.—The ^{13}C NMR chemical shifts for compounds **9** and **10** and relevant monomers, the glycosylation shifts, ($\Delta\delta$), and the differences in chemical shifts on changing the temperature from 30 to 70°C are given in Table 2.

Comparison of the glycosylation shifts for the two mannosyl-containing disaccharides with data from glucosyl disaccharides [15] showed that the presence of the axial HO-2′ in the mannosyl group causes only minor differences (< 0.2 ppm) in the glycosylation shifts except for the signals from C-2′ and the linkage carbons. Thus, the signal from C-2′ in **9** is shifted −0.74 ppm compared to −0.04 ppm in the *gluco* derivative. Similar data [11,12] have been obtained for other disaccharides containing mannosyl or glucosyl groups.

The shifts of the signals upon changing the temperature are similar to those observed for the corresponding gluco-disaccharides [15].

Interresidue atomic contacts.—To find the degree of contact between H-1′ and the two 6-protons, NOE difference experiments were performed, as the magnitude of the dipolar relaxation is correlated to the distance between the protons (Table 3). On irradiation at the resonance frequency of H-1′ in the α-glycosides **1**, **2**, and **9**, a 5–7% enhancement of the H-6_S signal was obtained, whereas no enhancement of the H-6_R signal occurred. These data indicate that conformations with a short distance between H-1′ and H-6_S are preferred. This is also indicated from the upfield shift of the H-6_S signal. Furthermore, an interaction between O-5′ and H-6_R is indicated by the large downfield shift of the H-6_R signal (Table 1). These results support highly populated conformations as depicted in Fig. 2, which shows two gluco-disaccharides. Similar results are obtained for the 6-*O*-α-D-glucosylated D-galactosides, **5** and **6**, but for these the H-6_R signal was also somewhat enhanced (1%) in the NOE difference experiments. The glycosylation shift of the H-6_S signal is negligible and that of the H-6_R signal, 0.14 ppm, is somewhat smaller than that of the D-glucosides. These data indicate, however, that most of the highly popu-

Table 2
^{13}C NMR chemical shifts of the disaccharides **9** and **10** and appropriate monosaccharides relative to internal dioxane (δ_C 67.40). Glycosylation shifts [a] are given in parenthesis and chemical shift differences (in ppm) from variation of the temperature [b] are given in brackets

Substance	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′	C-1	C-2	C-3	C-4	C-5	C-6	Me
α-D-Man*p*-(1 → 6)-α-D-Glc*p*OMe	100.49	70.95	71.64	67.76	73.65	61.92	100.26	72.21	74.31	70.56	70.89	66.55	55.96
9	(5.55)	(−0.74)	(0.39)	(−0.18)	(0.31)	(−0.07)	(0.07)	(−0.02)	(0.21)	(−0.12)	(−1.63)	(4.88)	(0.03)
	[0.12]	[0.13]	[0.15]	[0.21]	[0.06]	[0.16]	[0.03]	[0.17]	[0.10]	[0.30]	[0.20]	[0.20]	[0.03]
β-D-Man*p*-(1 → 6)-α-D-Glc*p*OMe	101.48	71.28	73.97	67.88	77.18	62.04	100.25	72.19	74.08	70.72	71.58	69.35	56.07
10	(6.93)	(−0.85)	(−0.06)	(0.19)	(0.18)	(0.05)	(0.06)	(−0.04)	(−0.02)	(0.04)	(−0.94)	(7.68)	(0.14)
	[0.05]	[0.04]	[0.16]	[0.17]	[0.03]	[0.11]	[0.07]	[0.14]	[0.15]	[0.30]	[0.12]	[0.20]	[0.04]
α-D-Mannopyranose	94.94	71.69	71.25	67.94	73.34	61.99							
β-D-Mannopyranose	94.55	72.13	74.03	67.69	77.00	61.99							
Methyl α-D-glucopyranoside							100.19	72.23	74.10	70.68	72.52	61.57	55.93

[a] Glycosylation shifts are calculated by subtraction of the chemical shifts of the corresponding hexose and methyl hexoside for the glycosyl part and the "aglycon" part, respectively, and a positive difference indicates a downfield shift. [b] $\Delta\delta = \delta(70-30°C)$. Dioxane was taken as δ 67.40 for all temperatures. [c] Primed labels refer to the glycopyranosyl group and unprimed labels to the methyl glycoside residue.

Table 3
Values for the $^2J_{H\text{-}6_R,H\text{-}6_S}$, $^3J_{H\text{-}5,H\text{-}6_R}$, and $^3J_{H\text{-}5,H\text{-}6_S}$ coupling constants of disaccharides **1–14**, dextran, and pustulan, and enhancements of the signals from H-6_R and H-6_S on irradiation of H-1′, at 400 MHz. Coupling constants (in Hz) are obtained from the one-dimensional ^{1}H NMR spectrum or from spin simulation of spin-systems of higher order

Substance	$J_{5',6'_R}$	$J_{5',6'_S}$	$J_{6'_R,6'_S}$	$J_{5,6_R}$	NOE % (H-1′/H-6_R)	$J_{5,6_S}$	NOE % (H-1′/H-6_S)	$J_{6_R,6_S}$
α-D-Glc*p*-(1 → 6)-α-D-Glc*p*OMe **1**	5.0	2.5	−12.0	5.0 [b]	0	2.2 [b]	7	−11.5 [b]
α-D-Glc*p*-(1 → 6)-β-D-Glc*p*OMe **2**	5.0 [b]	2.0 [b]	−11.5 [b]	5.0	0	2.2	5	−11.2
β-D-Glc*p*-(1 → 6)-α-D-Glc*p*OMe **3**	5.5	2.2	−12.2	5.3	4	2.1	0	−11.5
β-D-Glc*p*-(1 → 6)-β-D-Glc*p*OMe **4**	5.5	2.2	−12.2	5.9	5	2.2	0	−11.7
α-D-Glc*p*-(1 → 6)-α-D-Gal*p*OMe **5**	5 [b]	2 [b]	−12 [b]	7.3	1	5.0	5	−10.8
α-D-Glc*p*-(1 → 6)-β-D-Gal*p*OMe **6**	5.0 [b]	2.5 [b]	−11.5 [b]	7.1	1	4.2	5	−10.0
β-D-Glc*p*-(1 → 6)-α-D-Gal*p*OMe **7**	5.8	2.3	−12.3	7.2 [b]	4	4.4 [b]	3	−10.8 [b]
β-D-Glc*p*-(1 → 6)-β-D-Gal*p*OMe **8**	5.7	2.3	−12.2	7.5	6	4.2	2	−11.0
α-D-Man*p*-(1 → 6)-α-D-Glc*p*OMe **9**	5.7 [a]	2.0	−12.7	5.4 [b]	0	2.8 [b]	7	−11.9 [b]
β-D-Man*p*-(1 → 6)-α-D-Glc*p*OMe **10**	5.0	2.0	−10.0	5.4	5	2.1	1	−12.3
α-L-Fuc*p*-(1 → 6)-α-D-Glc*p*OMe **11**				5.6 [b]	5	1.9 [b]	*c*	−11.5 [b]
β-L-Fuc*p*-(1 → 6)-α-D-Glc*p*OMe **12**				4.8	1	2.0	5	−11.6
α-L-Fuc*p*-(1 → 6)-α-D-Gal*p*OMe **13**				7.6	3	4	*c*	−10.6
β-L-Fuc*p*-(1 → b)-α-D-Gal*p*OMe **14**				6.7	1	5.1	4	−10.5
Dextran				4.9		2.0		−11.7
Pustulan				6.1		2.0		−11.8

[a] This coupling constant was taken from the cross-peaks in the COSY-spectrum. [b] Coupling constant from spin-simulation. [c] The NOE for this signal could not be separated from the internal NOE intensities for the overlapping H-2′.

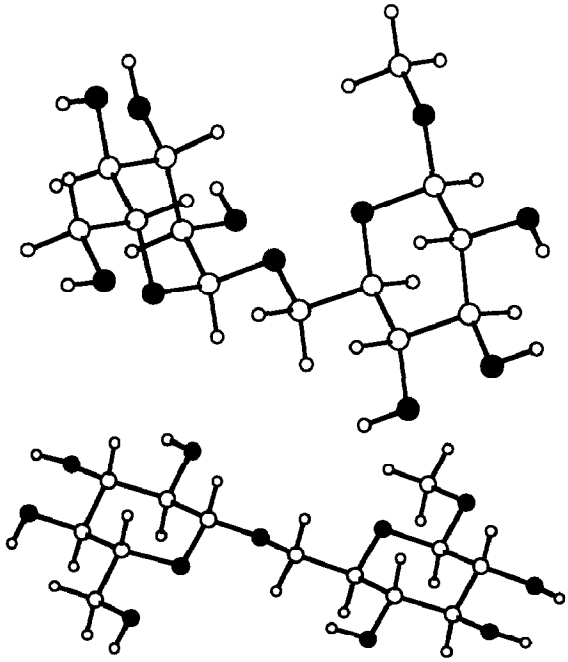

Fig. 2. Approximate conformations of 6-*O*-α-D- and 6-*O*-β-D-glucosylated methyl α-D-glucosides, demonstrating the proximity between interresidue atoms near the glycosidic linkage.

lated conformations are similar for both 6-*O*-α-D-glucosylated D-glucosides and D-galactosides.

The β-L-fucosyl-containing disaccharides **12** and **14**, with a stereochemistry at the glycosidic linkage similar to that of the α-D disaccharides [11], showed a ~5:1 ratio for the enhancements of the H-6_S and the H-6_R signals, respectively. This indicates that similar conformations as in the α-D-glycosides are most populated.

For the β-D-glycosides **3**, **4**, and **10** NOE data indicate a short distance between H-1′ and H-6_R for conformations that are highly populated, as enhancements of the signal for H-6_R are 4–5% and those of H-6_S 0–1%. Furthermore, the glycosylation shifts for the H-6_S signal indicate a short distance between O-5′ and H-6_S (Fig. 2). Similar results were obtained for the 6-*O*-β-D-glucosylated D-galactosides, **7** and **8**, but in addition the 6-H_S signal was also somewhat enhanced (2–3%) in the NOE difference experiment. These data indicate that most of the highly populated conformations are similar to those of the D-glucosides, but that a fair amount of conformations with a short distance between H-1′ and H-6_S also exists. The data from the stereochemically related α-L-fucosyl derivatives **11** and **13** cannot be fully interpreted because of overlapping signals, but the available data indicate a situation similar to that for the 6-*O*-β-D-substituted D-glycosides.

Conformation of the hydroxymethyl group.—An approximation of the rotamer distribution around the C-5–C-6 bond, using the $J_{5,6}$ values, was made with the graphical method described by Bock and Pedersen [2] (Fig. 3). The $J_{5,6_R}$ and $J_{5,6_S}$

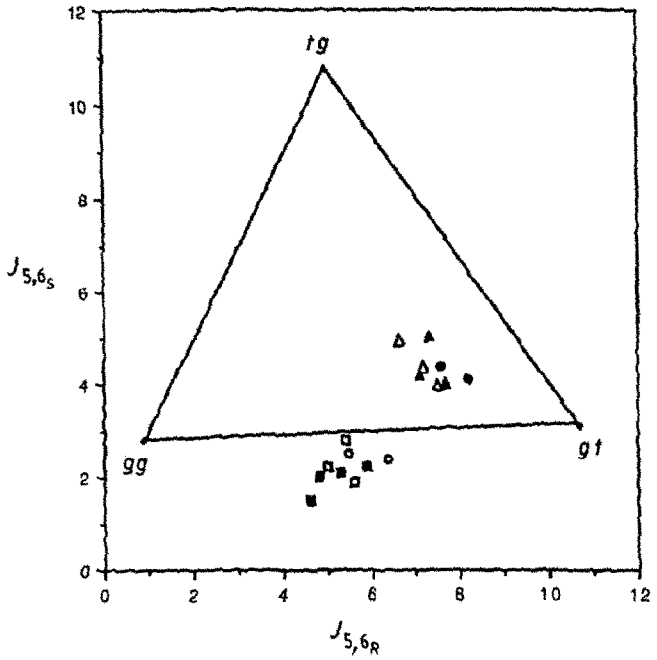

Fig. 3. Plot of the $J_{5,6_R}$ and $J_{5,6_S}$ values, using the limiting values of Haasnot et al. [3] to define the population of the conformers *tg*, *gg*, and *gt*, as suggested by Bock and Pedersen [2]. Compounds **1**, **2**, **9**, and **11** are indicated with a □; **3**, **4**, **10**, and **12** with a ■; **5**, **6**, and **13** with a ▲; and **7**, **8**, and **14** with a △. Values for methyl α- and β-D-glucopyranoside and methyl α- and β-D-galactopyranoside are also included, and marked ○ and ●, respectively.

values obtained for compounds **1**–**14** are given in Table 3 and Fig. 3, and show the rotamer distribution. Fig. 3 also contains values for D-glucose and D-galactose [16].

For the α-D-glycosides **1**, **2**, and **9**, containing a methyl glucoside residue, the plot demonstrates that the proportion of the *gg* rotamer is in the range 50 to 60% and that of the *gt* rotamer 40 to 50%, whereas no significant amount of *tg* is present. Similar proportions of rotamers are calculated for the stereochemically analogous β-L-fucopyranosyl glycoside **12**. For the β-D-glycosides **3**, **4**, and **10** and the analogous α-L-glycoside **11**, the proportion of *gg* rotamer ranges between 50 and 65%, that of *gt* rotamers between 35 and 50%, and the amount of *tg* rotamer is negligible. As can be seen from the graph, the proportion of the *gg* and *gt* conformers is determined from the size of the coupling between H-5 and H-6_R. For all the 6-substituted D-glucosides, a somewhat higher $J_{5,6_R}$ value is observed for the β-D- and α-L-substituted isomers than for the α-D- and β-L-substituted isomers (except **9** and **10**, which have the same value). This difference is even more pronounced in dextran [(1 → 6)-α-D-glucan] and pustulan [(1 → 6)-β-D-glucan] for which the $J_{5,6_R}$ values were 4.9 and 6.1 Hz, respectively. These results show that the population distribution of conformers of the C-5–C-6 bond is dependent on

the anomeric and absolute configuration of the substituent and that the *gg* : *gt* ratio is higher in the α-(1 → 6) linkage than in the β-(1 → 6) linkage. Also, the proportion of *gg* is higher in the disaccharides than in the monosaccharides.

The disaccharides **5–8** containing methyl D-galactoside residues all have, according to the $J_{5,6}$ values, a preponderance of the *gt* rotamer ranging from 55 to 60%. The *gg* rotamer is present in between 20 and 25% and the *tg* rotamer in between 15 and 25%. The related disaccharides **13** and **14** containing L-fucosyl groups also have the *gt* rotamer as the preponderant conformation. The $J_{5,6}$ values deviate somewhat from those of the respective monomers, showing that the population distribution is different in the disaccharide and the monosaccharide, respectively, with a higher proportion of the *gg* and *tg* conformers.

3. Conclusion

^{1}H and ^{13}C NMR data for a number of (1 → 6)-linked disaccharides have given glycosylation shifts which can facilitate the interpretation of more complex NMR spectra of oligo- and poly-saccharides. Glycosylation shifts typical for the (1 → 6) linkage were found for the H-1′ signal with upfield shifts between −0.11 and −0.27 ppm. The ^{1}H and ^{13}C chemical shifts of the signals for the 6-protons and

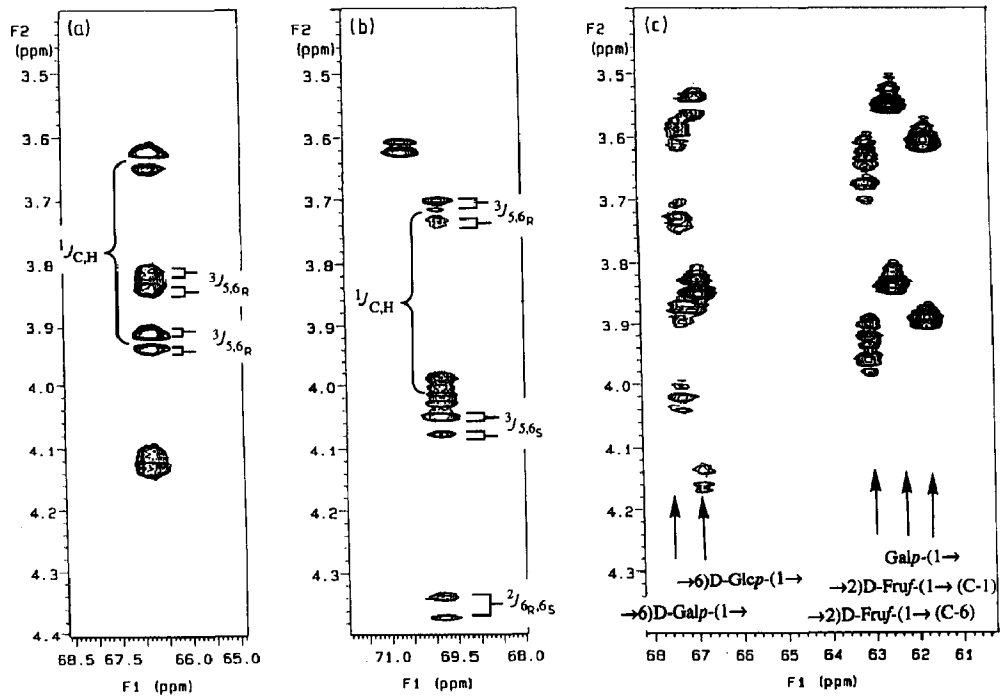

Fig. 4. Parts of the HMQC spectra of dextran (a), pustulan (b), and stachyose (c), showing the region for signals from C-6 and the 6-protons. The different coupling constants in (a) and (b) and the carbon signals in (c) are marked.

C-6 in combination with the $J_{5,6_R}$ and $J_{5,6_S}$ values can be used for assignment of signals and to obtain structural information. In the HMQC spectra of dextran, pustulan and stachyose (Fig. 4), it is possible to determine an α-(1 → 6) or a β-(1 → 6) linkage from the coupling pattern of the peaks derived from H-6_R and H-6_S. If the peaks with the largest coupling constants are more downfield than those with smaller coupling constants, it is an α-(1 → 6) linkage. If the opposite situation occurs, with the peaks with the larger coupling constants upfield of the peaks with smaller coupling constants, it is a β-(1 → 6) linkage. It is also possible to differentiate between the C-6 signals of the 1,6-substituted glucose and galactose residues as can be seen in the HMQC spectrum of stachyose (Fig. 4). The signal at δ 67.4 is assigned to C-6 of the 6-substituted D-galactopyranosyl residue as H-6_R and H-6_S are less separated than those corresponding to the C-6 signal of the 6-substituted D-glucopyranosyl residue at δ 66.9 (cf. Table 3). In addition, the $^3J_{5,6_R}$ and $^3J_{5,6_S}$ values are both larger than the corresponding values for the latter. The unsubstituted hydroxymethyl groups give signals at δ 61.9, 62.5, and 63.2 (Fru C-1 and C-6).

The $J_{5,6}$ values of signals from the methyl glycoside residue have shown that the rotamer distribution in some of the disaccharides differs somewhat between the α-D- and β-L-substituted, on the one hand, and β-D- and α-L-substituted, on the other hand, from those of the unsubstitued monomers. The NOE measurements indicate that the α-D-glycosides have H-1′ and H-6_S close to each other. For the β-D-glycosides, highly populated conformations instead have H-1′ and H-6_R close to each other.

4. Experimental

General methods.—^{1}H (400 MHz) and ^{13}C (100 and 67.8 MHz) NMR spectra were recorded for 0.05 M D_2O solutions at 70°C with Jeol GX-400 and GSX-270 spectrometers. The HMQC experiments were performed using a Varian Unity 500 instrument. Chemical shifts were given in ppm using sodium 3-(trimethylsilyl)propanoate-d_4 (TSP, δ_H 0.00) and dioxane (δ_C 67.40) as internal references. For the assignment of proton signals, different H,H-COSY techniques (COSY, relayed COSY, and double relayed COSY) were used. Chemical shifts from overlapping signals were obtained from the centre of the COSY cross-peaks. Coupling constants were measured from expanded one-dimensional spectra or, for signals of higher order, from spin simulation (Jeol program COMIC). The NOE-difference experiments were performed at 40°C with solutions in D_2O containing 10% of acetone-d_6. The acetone was used for stabilisation of the lock signal. Each experiment consisted of cycles of 16 accumulations for on- and off-resonance, respectively, using a pre-irradiation time of 6 s.

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