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

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^{1}H and ^{13}C NMR spectroscopic studies have been performed on eight 1,2- and 1,3-linked disaccharides in which the glycosidic linkages have different stereochemical surroundings. The changes in glycosylation shift induced by an equatorial *N*-acetyl group at C-2′ or C-2 or an axial hydroxy group at C-2′ *vs.* those from compounds with an equatorial hydroxy group were investigated. It was found that the difference in ^{1}H NMR glycosylation shift between compounds with an equatorial hydroxy group and an axial was only minor. Compounds with an *N*-acetyl group had in some cases significantly changed ^{1}H NMR glycosylation shifts. An upfield glycosylation shift of the C-2′ or C-2 signal was observed for the oligosaccharides containing 2-acetamido-2-deoxy-glucose and 2-acetamido-2-deoxy-galactose residues compared to oligosaccharides containing a glucose and a galactose residue, respectively. The minimum energy conformation was also calculated, using the HSEA-approach. It could, *inter alia*, be shown that signals from protons calculated to be near an NH-grouping were shifted upfield compared to analogous oligosaccharides containing only hydroxy groups.

We have reported NMR spectroscopic and conformational studies on several di- and tri-saccharides.[1] The aim of the studies has been to understand how glycosylation shifts are influenced by type of sugar and stereochemistry around the glycosidic bond. The glycosylation shifts have also been used in a database in the computer program CASPER,[2] by which structural analysis of oligo- and poly-saccharides can be performed on the basis of NMR data and simple chemical analyses. We now report on additional studies on disaccharides that have glycosidic linkages for which the glycosylation shifts are not known. Most of the oligosaccharides contain a 2-acetamido-2-deoxy-sugar. In addition the low energy conformations as shown by the HSEA program have been calculated.

Experimental

General Methods.—NMR spectra were recorded for solutions in D_2O using JEOL GSX-270 or GX-400 instruments. Chemical shifts are given in ppm and the spectra were recorded at 70 °C using dioxane (δ_C 67.40) or sodium 3-(trimethylsilyl)-[$^2H_4$]propanoate (TSP, δ_H 0.00) as internal references. For the assignment of signals, H,H- and C,H-COSY experiments were used. ^{1}H NMR chemical shifts of overlapping signals were obtained from the centre of the cross-peaks in the H,H-COSY spectra. The β-anomer of 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1 ⟶ 2)-D-mannopyranose was only present in minute amounts (*ca.* 10%) and therefore not further characterized.

To estimate minimum energy conformations and rotational freedom, the HSEA program[3–5] was used which accounts for non-bonded interactions, as expressed by the standard Kitaigorodsky algorithm, together with a term for the exo-anomeric effect. Explicit hydrogens were used for all residues. The torsional angles φ and ψ were defined in a 1,2-linked disaccharide by H(1′)–C(1′)–O(2)–C(2) and C(1′)–O(2)–C(2)–H(2), respectively. The bond angle τ [C(1′)–O(2)–C(2)] was set at 117°. Coordinate sets for methyl α-D-mannopyranoside,[6] methyl α-D-glucopyranoside,[6] and α-L-rhamnopyranose,[7] were obtained from crystal data, whereas the coordinate sets for β-D-galactopyranose, α-D-mannopyranose and 2-acetamido-2-deoxy-β-D-glucopyranose were obtained from those of methyl β-D-galactopyranoside,[8] methyl α-D-mannopyranoside[5] and the disaccharide β-*N*,*N*′-diacetylchitobiose,[9] respectively. Conversion of the coordinate set of α-D-galactopyranose,[10] β-D-galactopyranose, 2-acetamido-2-deoxy-α-D-galactopyranose,[11] and 2-acetamido-2-deoxy-β-D-glucopyranose to that of the corresponding methyl glycoside was achieved by adding the coordinates for a methyl group at $\varphi = -60°$ or 60°, for α- and β-linked disaccharides, respectively.

Disaccharides.—Disaccharides **2**, **4**, **6** and **8** were obtained from the Swedish Sugar Company. Compound **7** was provided by Dr. T. Norberg, BioCarb AB, Lund. The other disaccharides were gifts from Dr. H. Ottosson (**1**), Mr. C. Hällgren (**3**) and Dr. F. Dasgupta (**5**), Department of Organic Chemistry, Stockholm University.

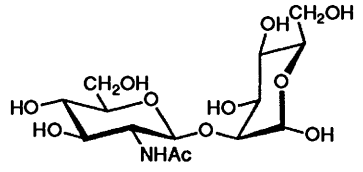
Results and Discussion

HSEA Calculations.—All inter-residue internuclear distances < 3 Å for the minimum energy conformations of **1**–**8** are given in Table 1. Of the disaccharides, **2**–**4** are termed α-glucosides with reference to the central bond and the remaining compounds β-glycosides. The C(2)–N bond in 2-acetamido-2-deoxy-D-hexose residues was always fixed.

Comparisons below and in the sections on NMR spectroscopy, are made to disaccharides that have a hydroxy group instead of an *N*-acetyl group, or for **2** and **4** to compounds that have a glycosyl group with an equatorial hydroxy group at C-2′. For compound **3** no comparisons were made. Thus, **1** is compared with β-D-Glc(1 ⟶ 2)α-D-Man-OMe, **2** with α-D-Glc(1 ⟶ 2)α-D-Man-OMe, **4** with α-D-Fuc(1 ⟶ 3)α-D-Man-OMe, **5** with β-D-Glc(1 ⟶ 3)α-D-Glc-OMe, **6** and **8** with β-D-Glc(1 ⟶ 3)α-D-Gal-OMe, and **7** with β-D-Glc-(1 ⟶ 3)β-D-Glc-OMe. These oligosaccharides are referred to as the reference compounds.

The absolute value of φ in the minimum-energy conformations of **1**, **3** and **5**–**8** is 55° (±5°), and of **2** and **4** −50° whereas the value for ψ varies between 15° and −20°. From the conformational energy plots (data not shown) it can be concluded that the α-glycosides show a more restricted rotation around the glycosidic linkage than the β-glycosides. This pattern has also been observed in previous studies.

For **1**, ψ is 5° larger than the value from the reference compound.[1] This results in 0.16 Å shorter distance between 1′-H and 1-H. The influence of an axial OH-group at C-2′ can be



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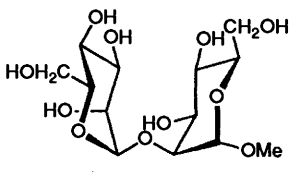

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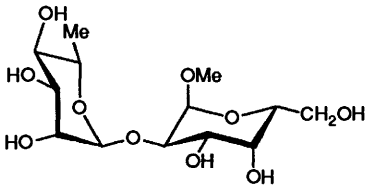

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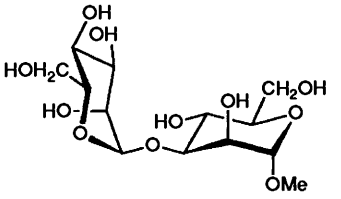

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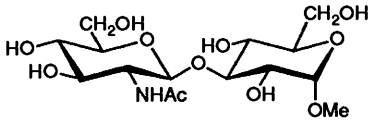

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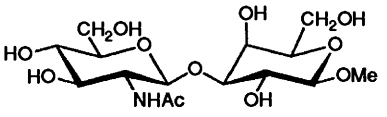

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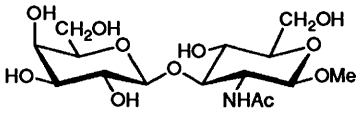

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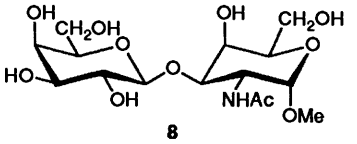

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Table 1 Values for the φ and ψ angles (in degrees) together with inter-residual internuclear distances < 3 Å in the minimum energy conformations of **1**–**8**, as indicated by HSEA-calculations

Compound[a]	φ/°	ψ/°	1′-H	5′-H	O-5′
β-D-Glc*p*NAc-(1 ⟶ 2)-α-D-Man*p* **1**	55	10	2.71 (1-H) 2.47 (2-H)		2.54 (2-H)
α-D-Man*p*-(1 ⟶ 2)-α-D-Man*p*OMe **2**	−50	−20	2.59 (O-3) 2.51(2-H)	2.23 (1-H)	2.58 (2-H)
α-L-Rha*p*-(1 ⟶ 2)-α-D-Gal*p*OMe **3**	50	10	2.79 (O-3) 2.37(2-H)	2.35 (1-H)	2.56 (2-H)
α-D-Man*p*(1 ⟶ 3)-α-D-Man*p*OMe **4**	−50	−10	2.75 (O-4) 2.41 (3-H)	2.27 (2-H)	2.62 (3-H)
β-D-Glc*p*NAc-(1 ⟶ 3)-α-D-Glc*p*OMe **5**	55	0	2.39 (3-H)		2.96 (O-4) 2.61 (3-H)
β-D-Glc*p*NAc-(1 ⟶ 3)-β-D-Gal*p*OMe **6**	60	−10	2.34 (3-H)		2.56 (3-H) 2.61 (4-H)
β-D-Gal*p*-(1 ⟶ 3)-β-D-Glc*p*NAcOMe **7**	55	10	2.75 (N-2) 2.46 (3-H)		2.51 (3-H)
β-D-Gal*p*-(1 ⟶ 3)-α-D-Gal*p*NAcOMe **8**	55	15	2.74 (N-2) 2.47 (3-H)		2.45 (3-H)

[a] Primed labels refer to the glycosyl group and unprimed to the methyl hexoside or reducing residue.

studied from data on **2** and **4**. The differences in φ/ψ compared to the reference compounds α-D-Glc-(1 ⟶ 2)-α-D-Man-OMe,[1] and α-D-Fuc-(1 ⟶ 3)-α-D-Man-OMe,[12] are small for **2** but up to 15° for **4**. This leads to small changes in internuclear distances for **2**, but changes up to *ca.* 0.4 Å for **4**. For **8**, containing an *N*-acetyl group at C-2′, a difference in ψ towards a positive value (−12° ⟶ 15°) is calculated, in comparison with the reference compound, β-D-Glc-(1 ⟶ 3)-α-D-Gal-OMe.[13] The change in ψ also leads to an increase of the distance 1′-H–3-H of *ca.* 0.1 Å. For **3**, for which no comparison is made, significant short proton-proton distances are calculated for 1′-H and 2-H (2.37 Å), and 5′-H and 1-H (2.35 Å).

^{1}H *NMR Glycosylation Shifts.*—The ^{1}H NMR chemical shifts and the glycosylation shifts, *i.e.* the induced chemical shift differences relative to the chemical shifts of the respective monomers, are given in Table 2. Differences in glycosylation shifts to the reference compounds (see above) are also given in Table 2. Comparison values, in parentheses in Table 2, are obtained by subtraction of glycosylation shifts for reference compounds from the new values, *i.e.* the glycosylation shifts in this study. Chemical shifts of signals which are not of first order are approximate only. Coupling constants were all of the expected order for pyranose rings in the same conformation as the respective monomers.

Table 2 ^{1}H NMR chemical shifts and glycosylation shifts[a] of disaccharides **1–8** and pertinent monosaccharides obtained at 70 °C relative to internal TSP (δ_H 0.00)

Compound	1′-H[b]	2′-H	3′-H	4′-H	5′-H	6′-Ha	6′-Hb	Me′	1-H	2-H	3-H	4-H	5-H	6-Ha	6-Hb	Me	OMe
β-D-Glc*p*NAc-(1 ⟶ 2)-α-D-Man*p*	4.59	3.70	3.59	3.46[c]	3.45[c]	3.76	3.92	2.05	5.19	4.03	3.86	3.55	3.77	3.68	3.87		
1	−0.13	0.05	0.03	0.00	−0.01	0.01	0.01	−0.01	0.01	0.09	0.00	−0.13	−0.05	−0.06	0.01		
	(0.01)[d]	(−0.05)	(0.03)	(—0.01)	(0.01)	(—0.01)	(0.01)		(—0.10)	(—0.07)	(—0.05)	(—0.14)	(—0.06)	(—0.07)	(0.03)		
α-D-Man*p*-(1 ⟶ 2)-α-D-Man*p*OMe	5.05	4.07	3.85	3.66	3.76[c]	3.77[c]	3.89[c]		4.97	3.96	3.87	3.69	3.60	3.78	3.90		3.42
2	−0.13	−0.13	−0.01	−0.02	−0.06	0.03	0.03		0.20	0.20	0.10	0.02	−0.01	0.00	0.00		0.00
	(−0.02)	(0.11)	(−0.04)	(−0.01)	(−0.03)	(0.05)	(0.04)		(−0.02)	(0.01)	(0.00)	(−0.06)	(0.00)	(0.02)	(0.00)		(0.00)
α-L-Rha*p*-(1 ⟶ 2)-α-D-Gal*p*OMe	4.94	4.05	3.78	3.46	3.73	1.31			4.91	3.82	3.89	4.02	3.88[c]	3.76[c]	3.76[c]		3.43
3	−0.18	0.13	−0.03	0.01	−0.13	0.03			0.06	−0.02	0.08	0.03	−0.01	0.00	0.00		0.00
α-D-Man*p*-(1 ⟶ 3)-α-D-Man*p*OMe	5.11	4.06	3.87	3.67	3.75[c]	3.77[c]	3.87[c]		4.73	4.06	3.85	3.77	3.64	3.78	3.88		3.41
4	−0.07	0.12	0.01	−0.01	−0.07	0.03	0.01		−0.04	0.12	0.08	0.10	0.03	0.00	−0.02		−0.01
	(−0.05)	(0.06)	(−0.06)	(−0.01)	(−0.05)	(0.01)	(0.01)		(−0.02)	(0.04)	(0.03)	(−0.08)	(−0.01)	(−0.01)	(−0.02)		(−0.02)
β-D-Glc*p*NAc-(1 ⟶ 3)-α-D-Glc*p*OMe	4.75	3.74	3.60	3.48[c]	3.48[c]	3.76	3.93	2.04	4.78	3.63	3.78	3.48	3.65	3.76	3.87		3.43
5	0.03	0.09	0.04	0.02	0.02	0.01	0.02	−0.02	−0.03	0.07	0.10	0.07	0.01	0.00	0.00		0.00
	(−0.01)	(−0.03)	(0.00)	(0.01)	(−0.02)	(−0.01)	(0.00)		(−0.05)	(−0.12)	(−0.09)	(−0.04)	(−0.02)	(−0.01)	(−0.01)		(−0.01)
β-D-Glc*p*NAc-(1 ⟶ 3)-β-D-Gal*p*OMe	4.74	3.73	3.59	3.47[c]	3.47[c]	3.76	3.91	2.04	4.31	3.58	3.70	4.12	3.67	3.78[c]	3.78[c]		3.57
6	0.02	0.08	0.03	0.01	0.01	0.01	0.00	−0.02	0.00	0.06	0.06	0.19	−0.01	0.00	0.00		−0.01
	(−0.01)	(−0.06)	(0.03)	(−0.01)	(0.01)	(−0.01)	(0.01)		(−0.01)	(−0.12)	(−0.09)	(−0.04)	(−0.04)	(−0.01)	(−0.01)		(−0.02)
β-D-Gal*p*-(1 ⟶ 3)-β-D-Glc*p*NAcOMe	4.42	3.55	3.64	3.93	3.70	3.78[c]	3.78[c]		4.52	3.80	3.81	3.54	3.49	3.77	3.94	2.03	3.52
7	−0.11	0.10	0.05	0.04	0.05	0.14	0.06		0.06	0.13	0.25	0.09	0.04	0.02	0.01	−0.01	0.01
	(−0.18)	(−0.03)	(0.01)	(0.03)	(0.02)	(0.12)	(0.03)		(0.03)	(−0.07)	(0.01)	(−0.01)	(0.01)	(0.02)	(0.00)		(0.01)
β-D-Gal*p*-(1 ⟶ 3)-α-D-Gal*p*NAcOMe	4.48	3.55	3.63	3.93	3.66	3.77[c]	3.77[c]		4.80	4.35	4.00	4.23	3.94	3.78[c]	3.78[c]	2.03	3.41
8	−0.05	0.10	0.04	0.04	0.01	−0.13	0.05		−0.01	0.17	0.16	0.23	0.04	0.00	0.00	−0.02	0.01
	(−0.08)	(−0.04)	(0.04)	(0.02)	(0.01)	(0.11)	(0.06)		(−0.02)	(−0.01)	(0.01)	(0.00)	(0.01)	(−0.01)	(−0.01)		(0.00)
α-D-Man*p*	5.18	3.94	3.86	3.68	3.82	3.74	3.86										
α-L-Rha*p*	5.12	3.92	3.81	3.45	3.86	1.28											
β-D-Gal*p*	4.53	3.45	3.59	3.89	3.65	3.64	3.72										
β-D-Glc*p*NAc	4.72	3.65	3.56	3.46[c]	3.46[c]	3.75	3.91	2.06									
α-D-Man*p*OMe									4.77	3.94	3.77	3.67	3.61	3.78	3.90		3.42
α-L-Rha*p*OMe									4.69	3.93	3.72	3.45	3.66	1.30			3.40
α-D-Gal*p*OMe									4.85	3.84	3.81	3.99	3.89	3.76[c]	3.76[c]		3.43
α-D-Glc*p*OMe									4.81	3.56	3.68	3.41	3.64	3.76	3.87		3.43
β-D-Gal*p*OMe									4.31	3.52	3.64	3.93	3.68	3.78[c]	3.78[c]		3.58
β-D-Glc*p*NAcOMe									4.46	3.67	3.56	3.45[c]	3.45[c]	3.75	3.93	2.04	3.51
α-D-Gal*p*NAcOMe									4.81	4.18	3.84	4.00	3.90	3.78	3.78	2.05	3.40

[a] Glycosylation shifts are calculated by subtraction of the chemical shifts of the corresponding hexose and methyl hexoside or reducing residue for the glycosyl part and the aglycone, respectively, and a positive difference indicates a downfield shift. [b] Primed labels refer to the glycopyranosyl group and unprimed to the methyl glycoside residue or reducing residue. [c] Approximate values. [d] Obtained from comparison with reference compounds, for explanation see details in the results and discussion section.

Table 3 ^{13}C NMR chemical shifts and glycosylation shifts of the disaccharides **1**–**8** and appropriate monosaccharides obtained at 70 °C relative to internal dioxane (δ_C 67.40)

Compound	C-1′[b]	C-2′	C-3′	C-4′	C-5′	C-6′	Me′	C=O′	C-1	C-2	C-3	C-4	C-5	C-6	Me	C=O	OMe
β-D-Glc*p*NAc-(1 ⟶ 2)-α-D-Man*p*OH	100.74	56.41	74.36	70.97[c]	76.79[c]	61.67[d]	23.19	175.45	92.28	78.60	70.29	68.45	73.55	62.45[d]			
1	4.89	−1.45	−0.45	−0.09	−0.03	−0.18	0.09	−0.04	−2.66	6.91	−0.96	0.51	0.21	0.46			
	(−0.93)[e]	(0.10)	(−0.13)	(0.13)	(−0.15)	(0.02)			(−0.77)	(−0.63)	(−0.08)	(0.25)	(0.22)	(0.82)			
α-D-Man*p*-(1 ⟶ 2)-α-D-Man*p*OMe	102.99	70.87	71.33	67.87	74.13	62.03[d]			100.34	79.09	71.21	68.01	73.52	61.95[d]			55.71
2	8.05	−0.82	0.88	−0.07	0.79	0.04			−1.41	8.24	−0.35	0.22	0.07	0.03			0.16
	(−0.65)	(−1.08)	(0.04)	(−0.05)	(−0.09)	(0.16)			(−0.04)	(−1.09)	(−0.32)	(−0.16)	(0.06)	(0.01)			(−0.01)
α-L-Rha*p*-(1 ⟶ 2)-α-D-Gal*p*OMe	103.46	70.93	71.09	72.83	69.87	17.56			99.73	78.03	69.33	70.23	71.30	61.92			55.70
3	8.48	−0.88	0.09	−0.36	0.75	−0.11			−0.62	8.86	−1.13	0.04	−0.24	−0.14			−0.26
α-D-Man*p*-(1 ⟶ 3)-α-D-Man*p*OMe	102.97	70.97	71.37	67.80	74.14	61.81[d]			101.69	70.44	79.16	67.05	73.63	61.93[d]			55.62
4	8.03	−0.72	0.12	−0.14	0.80	−0.18			−0.06	−0.41	7.60	−0.74	0.18	0.01			0.07
	(−0.50)	(−1.07)	(−0.12)	(−0.07)	(0.10)	(−0.03)			(0.10)	(−0.43)	(−0.43)	(0.06)	(0.04)	(0.09)			(0.02)
β-D-Glc*p*NAc-(1 ⟶ 3)-α-D-Glc*p*OMe	102.56	56.78	74.71	70.96[c]	76.74[c]	61.72[d]	23.14	175.54	100.25	71.72	83.56	69.12	72.20	61.66[d]			55.97
5	6.71	−1.08	−0.10	−0.10	−0.08	−0.13	0.04	0.05	0.06	−0.51	9.46	−1.56	−0.32	−0.01			0.04
	(−0.12)	(−0.30)	(0.06)	(0.03)	(−0.21)	(0.07)			(0.21)	(0.11)	(−0.18)	(−0.01)	(−0.15)	(−0.05)			(0.05)
β-D-Glc*p*NAc-(1 ⟶ 3)-α-D-Gal*p*OMe	103.24	56.75	74.62	70.85	76.65	61.63	23.11	175.66	104.72	70.67	83.02	69.23	75.52	61.78			57.90
6	7.39	−1.11	−0.19	−0.21	−0.17	−0.22	0.01	0.17	0.00	−0.97	9.23	−0.39	−0.40	−0.06			0.02
	(−0.20)	(−0.25)	(−0.05)	(−0.01)	(−0.13)	(−0.02)			(0.10)	(−0.01)	(−0.63)	(−0.06)	(−0.10)	(0.02)			(0.07)
β-D-Gal*p*-(1 ⟶ 3)-β-D-Glc*p*NAcOMe	104.30	71.65	73.52	69.45	76.13	61.81			102.51	55.32	83.66	69.78	76.34	61.81	23.15	175.40	57.80
7	6.93	−1.31	−0.26	−0.24	0.20	−0.03			−0.25	−1.07	8.76	−1.28	−0.45	−0.01	0.09	0.05	0.06
	(0.13)	(−0.51)	(−0.07)	(−0.10)	(0.04)	(0.09)			(0.02)	(−0.59)	(−0.41)	(0.18)	(−0.11)	(0.01)			(0.01)
β-D-Gal*p*-(1 ⟶ 3)-α-D-Gal*p*NAcOMe	105.28	71.64	73.55	69.53	75.83	61.85[d]			99.24	49.49	78.15	69.53	71.27	62.06[d]	22.94	175.32	56.04
8	7.91	−1.32	−0.23	−0.16	−0.10	0.01			0.13	−1.33	9.35	0.05	−0.30	−0.04	0.12	0.02	−0.02
	(0.32)	(−0.46)	(−0.09)	(0.04)	(−0.06)	(0.21)			(0.23)	(−0.37)	(−0.51)	(0.38)	(0.00)	(0.04)			(0.03)
α-D-Man*p*	94.94	71.69	71.25	67.94	73.34	61.99											
α-L-Rha*p*	94.98	71.81	71.00	73.19	69.12	17.67											
β-D-Gal*p*	97.37	72.96	73.78	69.69	75.93	61.84											
β-D-Glc*p*NAc	95.85	57.86	74.81	71.06	76.82	61.85	23.10	175.49									
α-D-Man*p*OMe									101.75	70.85	71.56	67.79	73.45	61.92			55.55
α-L-Rha*p*OMe									101.74	70.94	71.30	73.01	69.23	17.46			55.54
α-D-Gal*p*OMe									100.35	69.17	70.46	70.19	71.54	62.06			55.96
α-D-Glc*p*OMe									100.19	72.23	74.10	70.68	72.52	61.67			55.93
β-D-Gal*p*OMe									104.72	71.64	73.79	69.62	75.92	61.84			57.88
β-D-Glc*p*NAcOMe									102.76	56.39	74.90	71.06	76.79	61.82	23.06	175.35	57.74
α-D-Gal*p*NAcOMe									99.11	50.82	68.80	69.48	71.57	62.10	22.82	175.30	56.06

[a] Glycosylation shifts are calculated by subtraction of the chemical shifts of the corresponding hexose and methyl hexoside or reducing residue for the glycosyl part and the aglycone, respectively, and a positive difference indicates a downfield shift. [b] Primed labels refer to the glycopyranosyl group and unprimed to the methyl glycoside residue or reducing residue. [c] Tentative assignment. [d] May be interchanged. [e] Obtained from comparison with reference compounds, for explanation see details in the results and discussion section.

Table 4 ^{13}C NMR chemical shift differences (ppm) on variation of temperature for compounds **1–8**[a]

Compound	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′	Me′	C=O	C-1	C-2	C-3	C-4	C-5	C-6	Me	C=O	OMe
β-D-Glc*p*NAc-(1 ⟶ 2)-α-D-Man*p* **1**	0.19	0.17	0.16	0.21	0.13	0.20	0.03	−0.18	0.26	0.44	0.15	0.21	0.14	0.06			
α-D-Man*p*-(1 ⟶ 2)-α-D-Man*p*OMe **2**	−0.11	0.08	0.17	0.13	0.03	0.16			0.16	−0.21	0.14	0.21	0.12	0.06			0.05
α-L-Rha*p*-(1 ⟶ 2)-α-D-Gal*p*OMe **3**	−0.11	0.16	0.24	0.21	−0.05	0.04			0.16	−0.05	0.11	0.19	−0.04	0.00			0.12
α-D-Man*p*-(1 ⟶ 3)-α-D-Man*p*OMe **4**	−0.18	0.05	0.16	0.22	0.02	0.14			0.08	0.10	0.11	0.11	0.10	0.13			0.04
β-D-Glc*p*NAc-(1 ⟶ 3)-α-D-Glc*p*OMe **5**	−0.13	0.18	0.16	0.25	0.09	0.20	0.07	−0.09	0.00	0.02	0.32	0.14	0.14	0.23			0.05
β-D-Glc*p*NAc-(1 ⟶ 3)-α-D-Gal*p*OMe **6**	−0.24	0.17	0.15	0.27	0.11	0.25	0.07	−0.12	0.00	0.09	0.00	0.03	0.00	0.04			−0.11
β-D-Gal*p*-(1 ⟶ 3)-β-D-Glc*p*NAcOMe **7**	−0.09	0.13	0.16	0.08	0.02	0.21[b]			−0.02	0.10	0.17	0.16	0.10	−0.02[b]	0.06	−0.16	−0.12
β-D-Gal*p*-(1 ⟶ 3)-α-D-Gal*p*NAcOMe **8**	−0.26	0.18	0.16	0.11[b]	0.03	0.03			0.08	0.06	0.01	−0.05[b]	−0.02	−0.01	0.07	−0.11	0.09

[a] Dd = δ(70 °C) − δ(30 °C). Dioxane was taken as δ 67.40 ppm for all temperatures. [b] These values could be interchanged.

Together with signals from protons at the linkage carbons and those next to these, the signals for 5′-H in the α-glycosides are significantly shifted compared to the monomers, a pattern which has been observed for all disaccharides studied earlier.[1] The glycosylation shift of the 1-H signal for **1** is only 0.01 ppm compared to 0.11 ppm for the reference compound, which correlates to the shorter distance between 1′-H and 1-H in **1** causing an upfield shift. The observed difference may also partly be due to the fact that the comparison is made between a reducing sugar residue and a methyl glycoside. The signal from 4-H in **1** also shows a significantly changed glycosylation shift, −0.14 ppm. For **2** a significant change for the signal from 2′-H, >0.1 ppm, is observed, as well as for the 4-H signal (−0.06 ppm) compared to the reference compound. In **3** an upfield shift, compared to the monomers, of the 1′-H signal is observed due to the proton-proton interaction of 1′-H to 2-H with a distance of 2.37 Å as seen from the HSEA-calculations. The signals for 5′-H for **2** and **4** show somewhat larger upfield shifts due to shorter proton-proton distances over the glycosidic linkage. In **4** shifts for signals from 1′-H, 2′-H and 4-H are −0.05, 0.06 and −0.08 ppm, respectively, compared to the reference compound. The changes are small but probably related to the difference in the minimum energy conformation, as discussed in the previous section. In **5** and **6** changed glycosylation shifts, compared to the reference compound, of *ca.* 0.1 ppm for the signals from 2-H are introduced from the *N*-acetyl group. A similar comparison shows that the 1′-H signals for **7** and **8** are shifted towards negative glycosylation shifts due to the proximity between 1′-H and N–H.

^{13}C NMR Glycosylation Shifts.—The ^{13}C NMR chemical shifts for compounds **1**–**8** and relevant monomers and the glycosylation shifts ($\Delta\delta$) are given in Table 3. Differences in glycosylation shifts to similar oligosaccharides (see above) are also given in Table 3.

Significant glycosylation shifts, >0.5 ppm, compared to the monomers, are observed for signals of linkage carbons and for most of the carbons next to these which is the general pattern as observed for other disaccharides.[1]

In **1**, a γ-gauche effect is the origin of the small downfield glycosylation shifts of the C-1 signal. For **2**, signals for C-2′ as well as for C-2 have glycosylation shifts that are smaller (or more negative, *ca.* 1 ppm) compared to the reference disaccharide with a glucosyl group. The influence of an *N*-acetyl group on the ^{13}C NMR glycosylation shifts is small and usually an upfield change of *ca.* 0.4 ppm is observed for C-2′ or C-2 compared to values from the reference compounds. In the ^{13}C NMR signals from the *N*-acetyl group, small downfield changes compared to the monosaccharides are obtained.

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

On heating, most signals are shifted to lower field. The range of temperature shifts is −0.26 to 0.44 ppm. The signals for C-1 are shifted upfield except for **1**. In the 2-linked residues the signal for C-2 shows a downfield shift when the residue is glycosylated by a β-linked sugar whereas the α-linkages show upfield shifts. The ^{13}C NMR signals from the *N*-acetyl group show downfield shifts for the signals from the methyl carbon and upfield shifts for that from the carbonyl carbon.

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