N.m.r. and Conformational Studies of some 1,4-Linked Disaccharides

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^{1}H and ^{13}C N.m.r. studies and conformational analysis have been performed on eight 1,4-linked disaccharides in which the glycosidic linkages are in different stereochemical environments. The disaccharide glycosides have been divided into two groups, one containing α-DD-, β-LD-, and β-DL-glycosides, and one containing β-DD-, α-LD-, and α-DL-glycosides with typical chemical shift differences for each group. The conformational analysis, using the HSEA-approach, indicates a number of proton–oxygen and proton–proton interactions resulting in downfield and upfield shifts of the proton signals, respectively. The ^{13}C n.m.r. glycosylation shifts obtained have been used to simulate spectra of polysaccharides containing 1,4-linkages.

In order to gain more information from n.m.r. spectra of oligo- and poly-saccharides, it is necessary to understand the origin of glycosylation shifts, *i.e.* the change in chemical shift upon glycosylation, for different types of linkages. We have previously reported n.m.r. studies on 1,6- and 1,3-linked disaccharide glycosides.[1,2] In the latter study, n.m.r. data were correlated with theoretically derived conformations. The purpose of the studies was to provide data for a computer-based analysis of n.m.r. spectra from polysaccharides and larger oligosaccharides[3] and to understand how glycosylation affected the chemical shifts. We now report synthesis, n.m.r., and conformational studies of eight 1,4-linked disaccharide glycosides in which the anomeric and absolute configuration of the two sugar residues have been varied, thus giving stereochemically different surroundings around the glycosidic bond between the two sugars.

^{13}C N.m.r. data have been reported for maltosides and cellobiosides[4,5] but assignments and recording conditions have varied. Some ^{1}H n.m.r. data are available[6] and theoretical conformations of maltose, cellobiose, and derivatives thereof have been calculated.[7–10] Various methods of calculation have been used and in some examples conformations representing energy minima have been correlated with experimental data such as n.O.e. and optical rotation.[8] The conformational dependence of ^{13}C n.m.r. chemical shifts in oligosaccharides has been investigated and a correlation between glycosylation shifts and one of the dihedral angles in the glycosidic linkage in the minimum energy conformation has been observed.[11]

Experimental

General.—Evaporations were carried out under reduced pressure at <40 °C. Column chromatography was performed on silica gel 0.035—0.070 mm. ^{1}H N.m.r. spectra (400 MHz) and ^{13}C n.m.r. spectra (100 MHz) were recorded for 0.05M- and 0.1M-deuterium oxide solutions at 85 and 70 °C, respectively, with a JEOL GX400 spectrometer. Chemical shifts are given in p.p.m. using sodium [2,2,3,3-$^2H_4$]-3-(trimethylsilyl)propanoate (TSP, δ_H 0.00) and dioxane (δ_C 67.40) as internal references. For the assignment of signals proton–proton and proton–carbon shift correlated spectroscopy (COSY) were used. Chemical shifts of overlapping signals were obtained from the centre of the crosspeaks in the proton–proton shift correlation spectra. The HSEA-program[12,13] was used to estimate minimum energy conformations and rotational freedom. This program takes into account non-bonded interactions as expressed by the Kitaigorodsky algorithm, together with a term for the exoanomeric effect. The torsional angles φ and ψ were defined by H(1′)–C(1′)–O(4)–C(4) and C(1′)–O(4)–C(4)–H(4) respectively. The bond angle τ [C(1′)–O(4)–C(4)] was set at 117°. Co-ordinate sets for α-D-glucopyranose,[14] methyl α-D-glucopyranoside,[15] β-D-glucopyranose,[16] and α-L-fucopyranose[17] were obtained from the crystal structures, whereas the co-ordinates for β-L-fucopyranose were obtained from the mirror image of modified β-D-galactopyranose.[18] Co-ordinates for methyl α-L-rhamnopyranoside were obtained from the crystal data of α-L-rhamnopyranose[19] to which a methyl group at φ = 50° was added.

Synthesis.—Compounds (**1**)—(**8**) are listed in Table 2. The disaccharides (**1**), (**5**), (**6**), (**7**), and (**8**) were obtained under conditions of methyl trifluoromethanesulphonate promotion[20] in diethyl ether at room temperature, using ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside[21] for (**1**), (**7**), and (**8**), ethyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside[20] for (**5**) and ethyl 2,3,4-tri-*O*-benzoyl-1-thio-β-L-fucopyranoside for (**6**), as glycosyl donors. The latter fucose derivative was prepared by analogy with published methods.[20,21] Syntheses of (**2**), (**3**), and (**4**) were promoted by silver trifluoromethanesulphonate[22,23] in dichloromethane at −30 °C using the acetylated disaccharide glycosyl bromides for (**2**) and (**4**) and 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide for (**3**). Methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside[24] was used as the aglycone for the synthesis of disaccharides (**1**), (**3**), (**5**), and (**6**), methanol for (**2**) and (**4**), and methyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside for (**7**) and (**8**). The synthesis of (**1**) gave an anomeric mixture (α:β 1:1) that was separated on silica gel using chloroform–light petroleum–acetone (20:8:1) as the eluant. An anomeric mixture of (**7**) and (**8**) (α:β 3:2) was separated on silica gel using iso-octane–ethyl acetate (7:2) as the eluant. Syntheses of (**1**),[25,26] (**2**),[27] (**3**),[28] (**4**),[27] (**7**),[29] and (**8**)[30] have been reported, therefore no experimental details are given here.

Methyl 4-O-α-L-*Fucopyranosyl*-α-D-*glucopyranoside* (**5**).—Methyl trifluoromethanesulphonate (0.65 ml) was added to a stirred solution of ethyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside (540 mg), methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (350 mg), and ground molecular sieves (4 Å; 7 g) in diethyl ether (15 ml) at room temperature.[20] Triethylamine (0.8 ml) was added after 1.5 h and the mixture was stirred for another 30 min, then filtered through a layer of Celite. The filtrate was concentrated and purified on silica gel [iso-octane–ethyl acetate (3:1)] to yield the protected disaccharide as a syrup (610 mg, 92%), δ_C 97.7 and 97.6 (anomeric carbons).

The product (610 mg) in aqueous 90% acetic acid was hydrogenolysed at 400 kPa over Pd–C for 16 h, filtered, and

Table 1. Values for the φ and ψ angles of the minimum energy conformations and inter-residue atomic distances < 3 Å in (**1**), (**3**), and (**5**)—(**8**) obtained by HSEA-calculations. Obtained atomic distances at the 8 kJ level above the minimum energy conformations are also given

Substance	φ/ψ	1′-H	5′-H	O-5′	6′-H	O-2′
(**1**)	−65/−45	2.26 (O-3)	2.39 (4-H)	2.40 (4-H)		
		2.98 (4-H)	2.60 (6-H)			
			2.09 (6-H)			
	−15/−30	2.57 (O-3)	2.15 (6-H)			
		2.24 (4-H)				
(**3**)	55/−5	2.40 (4-H)		2.65 (4-H)		2.99 (6-H)
		2.47 (6-H)				
(**5**)	45/0	2.28 (4-H)	2.60 (0-3)	2.79 (4-H)	2.64 (0-3)	2.70 (6-H)
		2.37 (6-H)				
(**6**)	−55/−15	2.73 (0-3)		2.50 (4-H)		
		2.52 (4-H)		2.59 (6-H)		
(**7**)	−40/−15	2.33 (4-H)	2.44 (O-3)	2.73 (4-H)		
		2.86 (6-H)				
(**8**)	55/10	2.85 (O-3)		2.51 (4-H)		
		2.48 (4-H)				
	φ/ψ-Range					
(**1**)	−75→−3	→2.1[a] (O-3)	→2.0 (4-H)	→2.3 (4-H)		
	−70→−5	→2.6 (3-H)	→2.3 (6-H)	→2.4 (6-H)		
		→2.1 (4-H)	→1.8 (6-H)			
(**3**)	34→73	2.7—2.2 (4-H)		→2.3 (4-H)		→2.5 (6-H)
	12→30	→2.8 (6-H)				
		→2.0 (6-H)				
(**5**)	46→27	→2.7 (O-3)	→2.7 (3-H)	→2.7 (4-H)	→2.3 (O-3)	2.9—2.3 (6-H)
	12→−42	2.4—2.1 (4-H)	→2.6 (O-3)			
		→2.8 (6-H)				
		→2.0 (6-H)				
(**6**)	−74→−33	→2.2 (O-3)		2.8—2.2 (4-H)		
	−5→−41	2.9—2.3 (4-H)		→2.2 (6-H)		
(**7**)	−61→−18	→2.9 (O-3)	→2.7 (4-H)	→2.4 (4-H)		
	−29→37	2.7—2.0 (4-H)	→2.9 (3-H)			
		→2.4 (6-H)	→2.3 (O-3)			
(**8**)	74→33	→2.2 (O-3)		2.9—2.2 (4-H)		
	37→12	2.8—2.2 (4-H)		→2.7 (6-H)		

[a] →2.1 Indicates distances between 3 and 2.1 Å. In addition to the contacts listed in Table 1 a few contacts appear in (**1**) between 3′- and 6-H (2.7—2.5 Å), O-6′ and 4-H (→2.8 Å) and between O-6′ and 6-H (→2.5 Å). In (**6**) an additional contact between 2′-H and 6-H (→2.8 Å) is observed.

purified on a column of silica gel [eluant: ethyl acetate–acetic acid–methanol–water (12:3:3:2)] followed by a column of Biogel P-2 (eluant: water). After having been freeze-dried (**5**) was obtained as an amorphous solid (110 mg, 47%), $[\alpha]_{578}^{20}$ −17° (*c* 1.0, water).

Methyl 4-O-β-L-*Fucopyranosyl*-α-D-*glucopyranoside* (**6**).—Methyl trifluoromethanesulphonate (0.85 ml) was added to a stirred solution of ethyl 2,3,4-tri-*O*-benzoyl-1-thio-β-L-fucopyranoside (865 mg), methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (390 mg), and ground 4 Å molecular sieves in diethyl ether. Triethylamine (0.9 ml) was added after 2 h and the mixture was stirred for another 30 min. Purification on silica gel [iso-octane–ethyl acetate (2:1)] gave the protected β-glycoside as a syrup (610 mg, 82%), δ_C 100.5 and 97.6 (anomeric carbons).

The product (610 mg) in dichloromethane (10 ml) was treated with sodium methoxide in methanol (0.25M; 10 ml) at 25 °C for 16 h, neutralised with ion exchange resin, Dowex 50 (H^+), and concentrated to dryness. The residue was subjected to catalytic hydrogenolysis as described above. Purification of the product on a column of Biogel P-2 yielded (**6**) as an amorphous solid (160 mg, 68%), $[\alpha]_{578}^{20}$ 102° (*c* 1.0, water).

Results and Discussion

HSEA Calculations.—In the conformational analysis of glucose-containing oligosaccharides the hydroxymethyl group may be assumed to be *gauche–trans*, or *gauche–gauche* as observed in many crystal structures, or it may be treated as a methyl group. All alternatives are found in the literature and we have chosen the *gauche–trans* conformer for the calculation. In common with all conformational investigations of maltose and cellobiose is the shape of the energy well and a region where the minimum energy conformer is found. Depending on the co-ordinate sets and calculation algorithm, the position of the energy well and the number of minima for maltose will vary slightly. It is therefore difficult to determine the population distribution. The choice of co-ordinates is, however, normally not critical. The HSEA method was used in the present study as this method is comparatively simple and gives the essential information namely the shape of the energy well and the type of inter-residue contacts present.

The minimum energy conformations of (**1**), (**3**), and (**5**)—(**8**) are shown in Figure 1 and the conformational energy plots of the same compounds together with pronounced inter-residue contacts are given in Figure 2. φ/ψ-Values and interatomic distances of the minimum energy conformation are given in Table 1. For the φ/ψ-extremes on the 8 kJ level the maximum and minimum atomic distances below 3Å are also given in Table 1. These distances were compiled and used to estimate atomic interactions of the molecule. Values for (**2**) and (**4**) are omitted as they were virtually the same as those for (**1**) and (**3**), respectively.

Apart from (**1**) all compounds have energy maps in which the

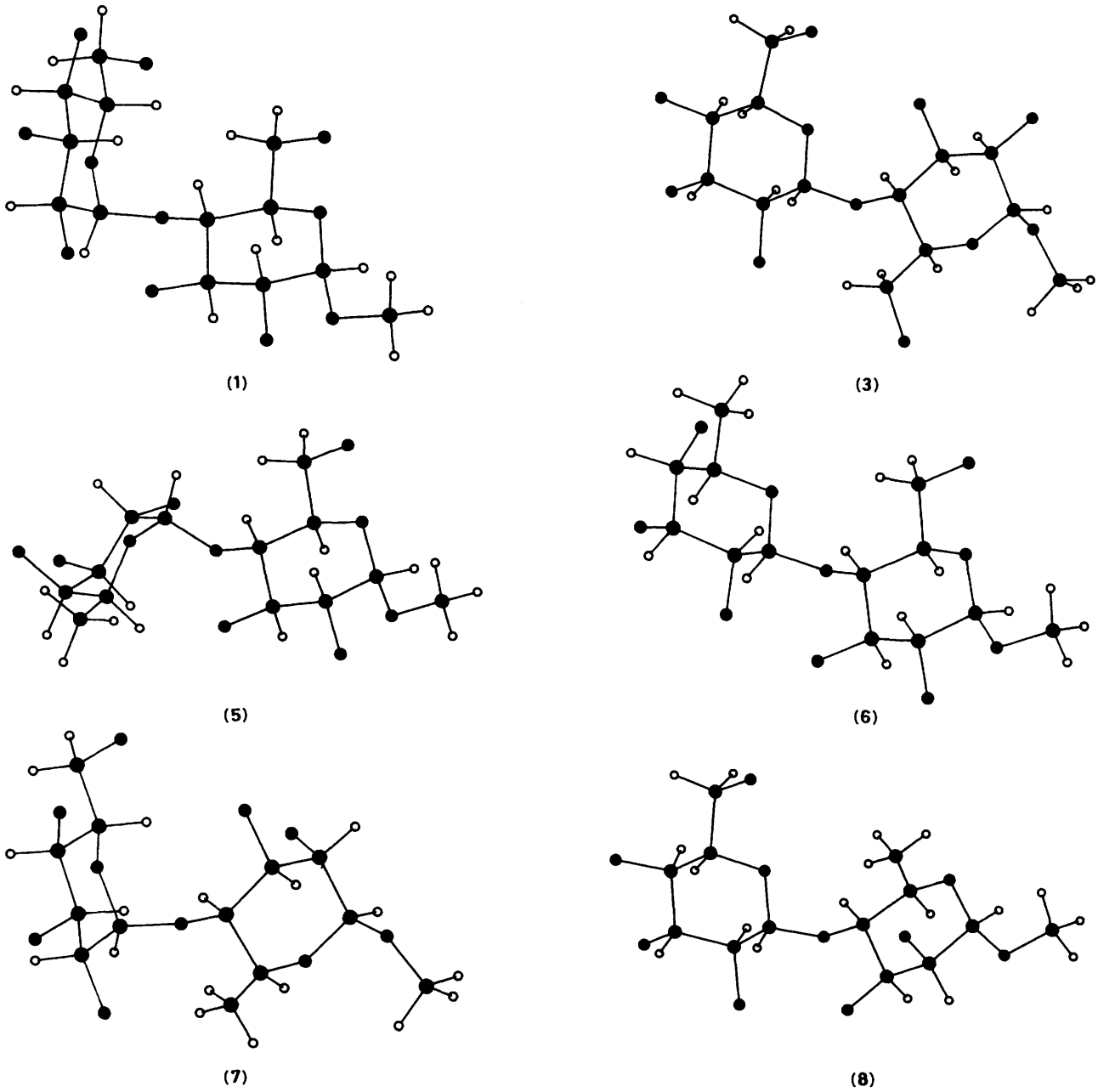


Figure 1. The minimum energy conformation of (**1**), (**3**), and (**5**)—(**8**) as derived by HSEA-calculations

minimum energy molecule is located at φ/ψ ~50/0° or φ/ψ ~ −50/0°.

In principle, it should be possible to compare (**1**) with (**6**), as the same atoms oppose each other across the glycosidic bond in both residues. Severe interactions between 5′-H and the opposing hydroxymethyl group, however, exclude part of the conformational space in (**1**), and two energy minima are obtained for (**1**) and only one for (**6**). The steep rise in energy towards low φ high ψ in (**1**) (Figure 2) is a consequence of these interactions. For (**6**) less-pronounced interactions between O-5′ and the hydroxymethyl group are observed on going to higher ψ values from the minimum energy molecule. In all compounds the anomeric proton 1′-H is close to 4-H. In (**1**), (**6**), and (**8**) an interaction with O-3 is indicated for 1′-H, and in (**3**), (**5**), and (**7**) for 1′-H with the 6-protons. The interactions of 5′-H could be to the hydroxymethyl group in (**1**) or to O-3 in (**5**) and (**7**) depending upon the absolute configurations of the sugars. The ring oxygen, O-5′, is also close to some opposing atoms, mainly to 4-H.

The general features outlined above for 1,4-linked disaccharides are most probably valid when these residues are present in large saccharides. The presence of other residues could, however, change the average conformation. Thus less-populated separate minima will probably lose their importance in polymers as a large part of the chain must rotate to attain such conformations.

The orientation of the hydroxymethyl group could also be of importance for the conformational preferences as well as for the ^{13}C n.m.r. chemical shifts. It is reasonable to assume that both hydroxymethyl rotamers are present in (**1**)—(**8**) as the coupling

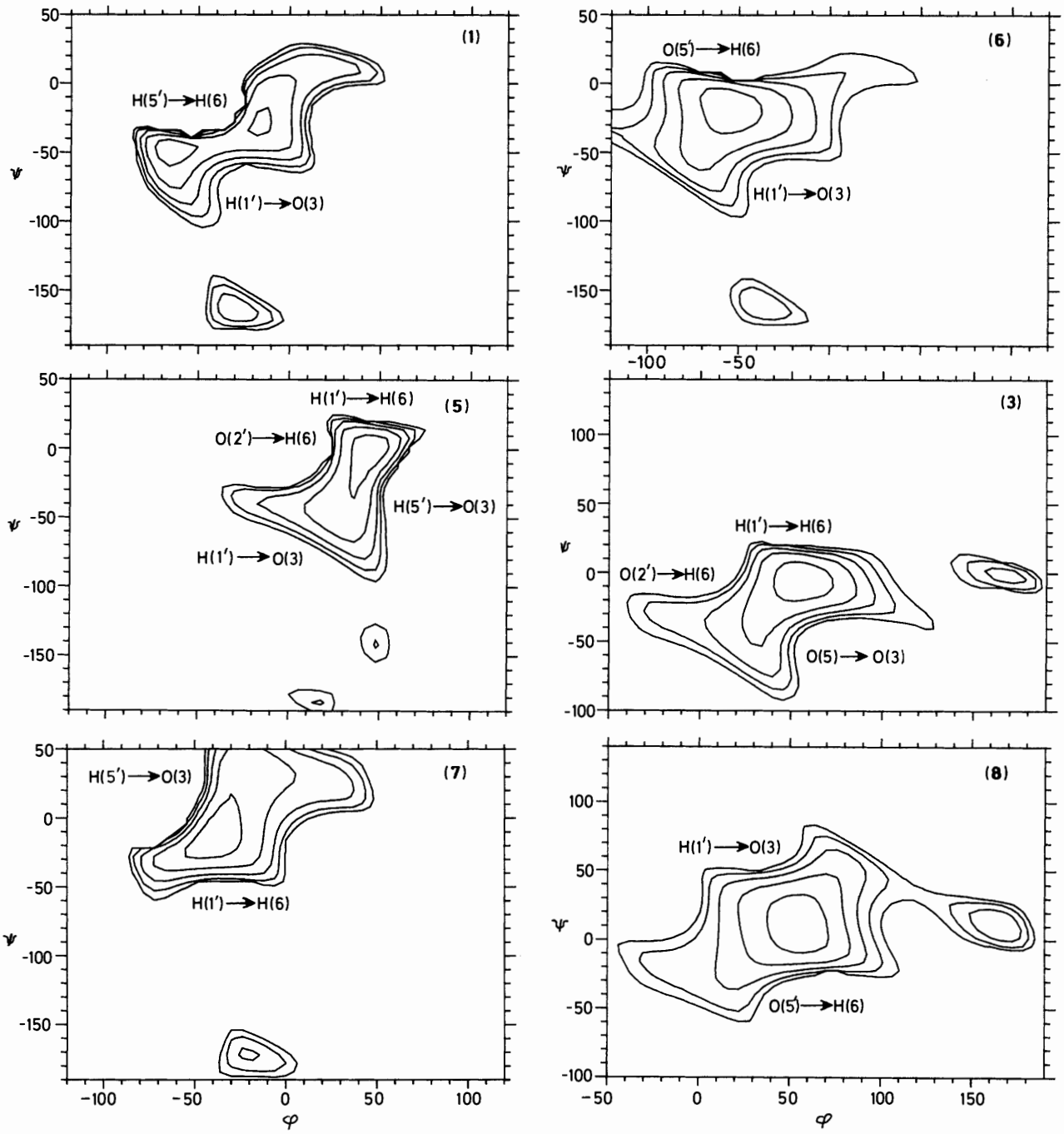


Figure 2. Conformational energy plots for (**1**), (**3**), and (**5**)—(**8**) with pronounced inter-residue contacts. Isocontour levels are indicated at 4.2, 12.6, 21.0, 29.4, and 37.8 kJ above the minimum energy molecule

constants for 5-H (not listed) are similar to those in glucose, in which there are approximately equal proportions of the two rotamers.[31]

^{1}H *N.m.r. Chemical Shifts.*—The ^{1}H n.m.r. chemical shifts of (**1**)—(**8**) and relevant monomers are given in Table 2 together with the chemical shift differences obtained relative to the respective monomers. No attempts were made to assign the two protons of the hydroxymethyl groups but signals were assumed to appear in the same relative order as in the monosaccharides.

The induced chemical shift differences range from −0.22 p.p.m. to 0.28 p.p.m. The largest values are observed for the protons on the substituted carbons, 1′- and 4-H, and for the neighbouring protons, 3- and 5-H respectively. There are, however, exceptions which may be due to inter-residue through-space interactions.

It is evident from a comparison of the pairs (**1**) and (**2**), and (**3**) and (**4**), that the anomeric configuration in the methyl glycoside residue exerts only minor effects upon the induced shifts. The chemical shifts of the protons in the methyl glycoside residue, however, are affected by the glycosyl group. Thus substitution of methyl α-D-glucopyranoside with either α-D- or β-D-glucose [(**1**)

Table 2. ^{1}H N.m.r. chemical shifts of the disaccharides (**1**)—(**8**) and appropriate monosaccharides obtained at 85 °C relative to internal TSP (δ_H 0.00). Chemical shift differences[b] are given in parentheses

Substance	1′-H[a]	2′-H	3′-H	4′-H	5′-H	6′-H	6′-H	1-H	2-H	3-H	4-H	5-H	6-H	6-H	OMe
α-D-Glc*p*(1→4)αD-Glc*p*OMe	5.35	3.59	3.70	3.43	3.73	3.86[c]	3.77[c]	4.82	3.61	3.93	3.62	3.75	3.90[c]	3.80[c]	3.44
(**1**)	(0.13)	(0.05)	(−0.03)	(0.02)	(−0.09)	(0.02)	(0.02)	(0.01)	(0.05)	(0.25)	(0.21)	(0.11)	(0.03)	(0.04)	(0.02)
α-D-Glc*p*(1→4)βD-Glc*p*OMe	5.35	3.58	3.69	3.42	3.73	3.86	3.78	4.38	3.32	3.78	3.61[d]	3.58[d]	3.95	3.78	3.57
(**2**)	(0.13)	(0.04)	(−0.04)	(0.01)	(−0.09)	(0.02)	(0.03)	(0.01)	(0.04)	(0.28)	(0.21)	(0.12)	(0.03)	(0.04)	(0.00)
β-D-Glc*p*(1→4)αD-Glc*p*OMe	4.51	3.33	3.52	3.44	3.50	3.92	3.74	4.81	3.61	3.80	3.63	3.76	3.93	3.85	3.44
(**3**)	(−0.12)	(0.08)	(0.03)	(0.03)	(0.04)	(0.03)	(0.02)	(0.00)	(0.05)	(0.12)	(0.22)	(0.12)	(0.06)	(0.09)	(0.02)
β-D-Glc*p*(1→4)βD-Glc*p*OMe	4.52	3.34	3.53	3.43	3.51	3.92	3.74	4.39	3.33	3.63	3.61	3.60	3.99	3.82	3.58
(**4**)	(−0.11)	(0.09)	(0.04)	(0.02)	(0.05)	(0.03)	(0.02)	(0.02)	(0.05)	(0.13)	(0.21)	(0.14)	(0.07)	(0.08)	(0.01)
α-L-Fuc*p*(1→4)αD-Glc*p*OMe	4.98	3.82	3.84[d]	3.83[d]	4.33	1.21		4.81	3.59	3.77	3.52	3.76	3.92	3.86	3.43
(**5**)	(−0.22)	(0.05)	(−0.02)	(0.02)	(0.14)	(0.00)		(0.00)	(0.03)	(0.09)	(0.11)	(0.12)	(0.05)	(0.10)	(0.01)
β-L-Fuc*p*(1→4)αD-Glc*p*OMe	4.61	3.50	3.63	3.73	3.76	1.26		4.79	3.57	3.86	3.63	3.68	~3.87	~3.87	3.42
(**6**)	(0.06)	(0.04)	(0.00)	(−0.02)	(−0.04)	(0.00)		(−0.02)	(0.01)	(0.18)	(0.22)	(0.04)	(0.00)	(0.11)	(0.00)
α-D-Glc*p*(1→4)αL-Rha*p*OMe	5.07	3.58	3.70	3.45	3.97	3.84	3.77	4.70	3.95	3.84	3.52	3.82	1.39		3.41
(**7**)	(−0.15)	(0.04)	(−0.03)	(0.04)	(0.15)	(0.00)	(0.02)	(0.01)	(0.02)	(0.12)	(0.07)	(0.16)	(0.09)		(0.01)
β-D-Glc*p*(1→4)αL-Rha*p*OMe	4.70	3.32	3.52	3.42	3.45	3.91	3.75	4.69	3.95	3.94	3.69	3.75	1.36		3.41
(**8**)	(0.07)	(0.07)	(0.03)	(0.01)	(−0.01)	(0.02)	(0.03)	(0.00)	(0.02)	(0.22)	(0.24)	(0.09)	(0.06)		(0.01)
α-D-Glucopyranose	5.22	3.54	3.73	3.41	3.82	3.84	3.75								
β-D-Glucopyranose	4.63	3.25	3.49	3.41	3.46	3.89	3.72								
α-L-Fucopyranose	5.20	3.77	3.86	3.81	4.19	1.21									
β-L-Fucopyranose	4.55	3.46	3.63	3.75	3.80	1.26									
Methyl α-D-glucopyranoside								4.81	3.56	3.68	3.41	3.64	3.87	3.76	3.42
Methyl β-D-glucopyranoside								4.37	3.28	3.50	3.40	3.46	3.92	3.74	3.57
Methyl α-L-rhamnopyranoside								4.69	3.93	3.72	3.45	3.66	1.30		3.40

[a] Primed labels refer to the glycopyranosyl group and unprimed to the methyl glycoside residue. [b] Chemical shift differences 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. [c] Chemical shift was obtained from the *J*-resolved spectrum. [d] Chemical shift was obtained from the C,H-correlated spectrum.

Table 3. ^{13}C N.m.r. chemical shifts of the disaccharides (**1**)—(**8**) and appropriate monosaccharides relative to internal dioxane (δ_C 67.40). Chemical shift differences are given in parentheses

Substance	C-1′[a]	C-2′	C-3′	C-4′	C-5′	C-6′	C-1	C-2	C-3	C-4	C-5	C-6	OMe
α-D-Glc*p*(1→4)-α-DGlc*p*OMe	100.65	72.72	73.89	70.44	73.60	61.59	99.99	72.02	74.35	78.44	71.12	61.59	55.97
(**1**)	(7.66[b])	(0.25)	(0.11)	(−0.27)	(1.23)	(−0.25)	(−0.20)	(−0.21)	(0.25)	(7.76)	(−1.40)	(−0.08)	(0.04)
α-D-Glc*p*(1→4)-β-D-Glc*p*OMe	100.58	72.64	73.84	70.41	73.61	61.60	103.99	73.84	77.05	78.30	75.56	61.77	57.87
(**2**)	(7.59)	(0.17)	(0.06)	(−0.30)	(1.24)	(−0.24)	(−0.14)	(−0.15)	(0.27)	(7.61)	(−1.22)	(−0.05)	(−0.01)
β-D-Glc*p*(1→4)-α-D-Glc*p*OMe	103.29	74.13	76.58	70.51	76.88	61.65	99.92	71.98	72.59	79.88	71.16	61.07	56.01
(**3**)	(6.45)	(−1.07)	(−0.18)	(−0.20)	(0.12)	(−0.19)	(−0.27)	(−0.25)	(−1.51)	(9.20)	(−1.36)	(−0.60)	(0.08)
β-D-Glc*p*(1→4)-β-D-Glc*p*OMe	103.36	74.14	76.60	70.53	76.90	61.67	103.96	73.77	75.32	79.88	75.64	61.26	57.91
(**4**)	(6.52)	(−1.06)	(−0.16)	(−0.18)	(0.14)	(−0.17)	(−0.17)	(−0.22)	(−1.46)	(9.19)	(−1.14)	(−0.56)	(0.03)
α-L-Fuc*p*(1→4)-α-D-Glc*p*OMe	100.23	69.03	70.38	72.76	67.90	16.08	99.95	72.38	72.74	78.54	71.60	61.13	55.95
(**5**)	(7.11)	(−0.06)	(0.09)	(−0.03)	(0.80)	(−0.25)	(−0.24)	(0.15)	(−1.36)	(7.86)	(−0.92)	(−0.54)	(0.02)
β-L-Fuc*p*(1→4)-α-D-Glc*p*OMe	104.40	72.16	73.84	72.13	71.78	16.15	100.06	72.08	73.87	78.46	71.10	61.62	55.98
(**6**)	(7.25)	(−0.57)	(−0.09)	(−0.22)	(0.14)	(−0.18)	(−0.13)	(−0.15)	(−0.23)	(7.78)	(−1.42)	(−0.05)	(0.05)
α-D-Glc*p*(1→4)-α-L-Rha*p*OMe	100.29	72.48	73.76	70.52	72.86	61.41	101.39	71.14	69.96	82.05	68.36	17.80	55.60
(**7**)	(7.30)	(0.01)	(−0.02)	(−0.19)	(0.49)	(−0.43)	(−0.35)	(0.20)	(−1.34)	(9.04)	(−0.87)	(0.34)	(0.06)
β-D-Glc*p*(1→4)-α-L-Rha*p*OMe	104.02	74.85	76.77	70.60	76.83	61.76	101.57	70.94	71.21	82.03	67.83	17.69	55.64
(**8**)	(7.18)	(−0.35)	(0.01)	(−0.11)	(0.07)	(−0.08)	(−0.17)	(0.00)	(−0.09)	(9.02)	(−1.40)	(0.23)	(0.10)
α-D-Glucopyranose	92.99	72.47	73.78	70.71	72.37	61.84							
β-D-Glucopyranose	96.84	75.20	76.76	70.71	76.76	61.84							
α-L-Fucopyranose	93.12	69.09	70.29	72.79	67.10	16.33							
β-L-Fucopyranose	97.15	72.73	73.93	72.35	71.64	16.33							
Methyl α-D-glucopyranoside							100.19	72.23	74.10	70.68	72.52	61.67	55.93
Methyl β-D-glucopyranoside							104.13	73.99	76.78	70.69	76.78	61.82	57.88
Methyl α-L-rhamnopyranoside							101.74	70.94	71.30	73.01	69.23	17.46	55.54

[a] Primed labels refer to the D-glucopyranosyl group and unprimed to the methyl glycoside residue. [b] Primed shift differences 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.

and (3)] results in a downfield shift of the 3-H signal of 0.25 and 0.12 p.p.m., respectively, whereas the signals for 4- and 5-H show similar shifts in both compounds. A small difference is also observed for the 6-protons. For the methyl glycoside residue in (5) and (6), in which the glycosyl group is α-L- or β-L-fucose, respectively, the glycosylation shifts for 3-, 4-, and 5-H differ significantly. The Δδ values are 0.09, 0.11, and 0.12, in (5), and 0.18, 0.22, and 0.04 in (6). In (7) and (8), in which α-D- or β-D-glucose is linked to methyl α-L-rhamnoside, the Δδ values for 3-, 4-, and 5-H are 0.12, 0.07, and 0.16, respectively, in (7), compared with 0.22, 0.24, and 0.09 p.p.m., respectively, in (8). Thus, one pattern for the induced chemical shift differences of 3- and 5-H is obtained for α-DD-, β-LD-, and β-DL-pairs [(1), (6), and (8)] and another for β-DD-, α-LD-, and α-DL-pairs [(3), (5), and (7)]. The HSEA-calculations show that in the former group, the ring oxygen opposes the C-5 substituent and O-3 in the latter. The shift differences probably originate from different interactions and the orientation of the lone pairs of the glycosidic oxygen.

As is evident from the atomic distances (Table 1) and contacts (Figure 2), 5′-H in (5) and (7) is close to O-3, resulting in a downfield shift, but 5′-H in (1) and (2) is close to other protons, resulting in an upfield shift. This illustrates how chemical shifts are affected by the stereochemistry around the glycosidic linkage.

For all pairs which differ only in the anomeric configuration of the glycosyl group, *e.g.* (1) and (3) the induced shifts for 1′-H are positive for one and negative for the other anomer. The reason for this is a proton–oxygen interaction only present in one of the anomers. The φ-angle in these compounds is *ca.* 60 or *ca.* −60° resulting in different inter-residue contacts for 1′-H. When 1′-H is close to O-3, as in (1), a downfield shift is observed but when it is close to 4-H and to a proton in the hydroxymethyl or methyl group, as in (3), an upfield shift is obtained. A similar situation exists in α- and β-kojibiose, for which the 1′-H signals differ by 0.31 p.p.m.[9] The highest downfield shift is observed for (1) and (2) in agreement with the short H(1′)–O(3) distance (Table 1).

For glycopyranosyl groups or residues linked to O-4 of a hexose or a 6-deoxyhexose, the 1-H signal may differ by ±0.2 p.p.m. from that of the corresponding pyranose. When O-2 in this residue is equatorial, the signals due to 1-H of α- and β-glycopyranosyl groups are well-separated and the coupling constants significantly different. When O-2 is axial, however, the chemical shift regions for the anomeric protons may overlap, and the coupling constants are small and similar. Assignments of anomeric configurations of such residues from ^{1}H n.m.r. spectra are therefore less obvious.

^{13}C *N.m.r. Chemical Shifts.*—The ^{13}C n.m.r. chemical shifts for (1)—(8) and relevant monomers and the chemical shift differences (Δδ) obtained upon comparison with the respective monomers are given in Table 3.

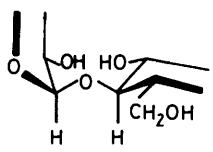
The common pattern with significant shifts for signals from the glycosyloxylated carbons and for the carbons next to these, is observed. Shifts for other signals *e.g.* those given by C-5′ and C-6 may also be large. In the glycosyl groups, shifts for signals from C-1′ vary between 6.4 and 7.7 p.p.m., those for C-2′ between −1.1 and 0.2 p.p.m., and those for C-5′ between 0.1 and 1.2 p.p.m. For the signals due to C-2′ and C-5′, differences in shift are observed for α- and β-glycopyranosyl groups. Small up- or down-field shifts for C-2′ signals and larger downfield shifts for C-5′ signals are observed for the α-glycopyranosyl groups, whereas only upfield and small downfield shifts are observed for C-2′ and C-5′, respectively, of the β-linked disaccharides. Inter-residue contacts (Table 1) between 5′-H in and α-linked glycosyl groups and 4-H, 6-H and O-3 in the next sugar residue may be the reason for the substantial downfield shift of the C-5 signals.

The shifts of the C-3, C-4, and C-6 signals differ considerably when the glycopyranosyl group linked to O-4 has an α-configuration, as in (1) or a β-configuration, as in (3). In (1) and (5) the glycosyl groups, α-D-glucopyranose and α-L-fucopyranose have different absolute configurations. Other differences are an equatorial or axial hydroxy group at C-4′ and a hydroxymethyl or a methyl group at C-5′. According to molecular models, these groups are not proximal to any part of the methyl glycoside residue. The different shifts observed for the signals in the methyl glycoside residues in (1) and (5), in particular those of C-3, should therefore reflect the influence of the absolute configuration of the glycosyl group. Analogous results are obtained for the β-linked disaccharides, *e.g.* (3) and (6).

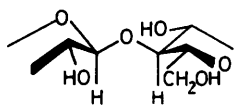
The configurations of the DL- and LD-disaccharides (5)—(8), and consequently the inter-residue interactions, differ significantly from those of the DD-disaccharides (1)—(4), as shown for the substructures of (1), (3), (5), and (6), given below.

It is evident from the substructures and shifts of the C-3 and C-5 signals that 1,4-linked disaccharides could be divided into two groups, one containing α-DD, β-LD, and β-DL, and the other β-DD, α-LD, and α-DL, with similar properties within each group.

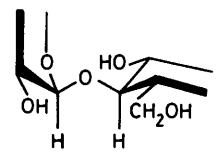
When an axial hydroxy group is present on C-2 in the methyl



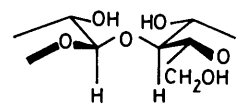
(1)



(3)



(5)



(6)

Table 4. Chemical shift differences in p.p.m. from variation in temperature[a]

	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′	C-1	C-2	C-3	C-4	C-5	C-6	OMe
(1)	0.13	0.13	0.16	0.23	0.07	0.18[b]	0.06	0.13	−0.01	0.57	0.17	0.23[b]	0.07
(2)	0.13	0.13	0.12[c]	0.20	0.06	0.22	0.07	0.04[c]	0.00	0.50	0.14	0.15	−0.11
(3)	−0.04	0.11	0.19	0.18	0.05	0.19	0.08	0.15	0.09	0.30	0.07	0.30	0.06
(4)	−0.03	0.11	0.19	0.18	0.07	0.19	0.06	0.07	0.12	0.22	0.05	0.27	−0.11
(5)	−0.07	0.08	0.13	0.03	0.10	−0.01	0.04	0.07	0.10	0.54	−0.02	0.40	0.06
(6)	0.02	0.11	0.14	0.10	0.01	−0.03	0.05	0.12	0.12	0.45	0.11	0.22	0.06
(7)	−0.21	0.08	0.13	0.32	0.20	0.38	0.01	0.02	0.12	0.09	−0.04	0.11	0.03
(8)	0.00	0.12	0.16	0.21	0.06	0.28	0.03	0.09	0.09	0.15	0.07	0.01	0.01

[a] Δδ = δ(70 °C) − δ(30 °C). Dioxane was taken as δ 67.40 p.p.m. for all temperatures. [b,c] These values are interchangeable.

Table 5. Observed and calculated chemical shifts of amylose, cellopentaose, pullulan, and elsinan. For information on structures and method of calculation see text

	C/H																	
δ_C	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Amylose																		
Obs.	100.5	72.5	74.2	78.3	72.2	61.5												
Calc.	100.4	72.5	74.1	78.2	72.2	61.5												
Obs − calc.	0.1		0.1	0.1														
Cellopentaose																		
Obs.	103.1	73.9	75.0	79.5	75.6	61.0												
Calc.	103.2	73.9	75.1	79.7	75.8	61.1												
Obs. − calc.	−0.1		−0.1	−0.2	−0.2	−0.1												
	→4)-α-D-Glc(1→6						→6)-α-D-Glc-(1→						→4)-α-D-Glc-(1→4					
Pullulan																		
Obs.	98.9	72.2[a]	74.3	78.9	71.4[b]	61.6[c]	101.1	72.7[a]	74.0	70.7	72.5	67.7	100.7	72.6	74.2	79.2	72.4[b]	61.8[c]
Calc.	98.6	72.2	74.4	78.4	71.4	61.6	100.7	72.7	74.1	70.4	72.1	66.8	100.4	72.5	74.1	78.2	72.2	61.5
Obs − calc.	0.3		−0.1	0.5			0.4		0.1	0.3	0.4	0.9	0.3	0.1	0.1	1.0	0.2	0.3
Elsinan[d]																		
Obs.	101.1	100.5	99.8	81.0	78.6	78.6	74.1	74.1	73.5	72.5	72.5	72.2	71.4	71.4	70.4	61.5	61.5	61.5
Calc.	100.8	100.5	99.8	81.6	78.6	78.2	74.3	74.1	74.0	72.5	72.3	72.2	71.4	70.9	70.5	61.6	61.5	61.5
Obs. − calc.	0.3			−0.6		0.4	−0.2		−0.5		0.2			0.5	−0.1	−0.1		
δ_H																		
Amylose	5.37	3.65	3.98	3.65	3.84	~3.9 ~3.8												
Cellopentaose	4.54	3.35	~3.65	~3.65	~3.65	3.99 3.83												
Pullulan	4.96	3.62	4.01	3.66	3.86	~3.8 ~3.9	5.34	3.62	3.72	3.46	3.93	3.81 3.93	5.38	3.65	3.96	3.61	3.86	~3.8 ~3.9

[a,b,c] These values are interchangeable. [d] Descending unassigned chemical shifts.

glycoside residue, as in (**7**) and (**8**), a larger glycosylation shift for C-4 is obtained than when there is an equatorial substituent in the same position, as in (**4**) and (**5**). An analogous effect is observed for C-3 of α- and β-glycosides of 1,3-linked disaccharides.[2]

Comparison of the shifts of the C-3 and C-5 signals with the shifts for 3- and 5-H in (**1**)—(**8**) shows that increased upfield shifts for carbon signals are accompanied by increased downfield shifts for proton signals. Such 'bond polarisation' has been observed in studies of carbohydrates and cyclohexane derivatives.[3,2]

Effects of Temperature Variation on Chemical Shifts.—The temperature shifts for 1,6-linked disaccharides have been reported and were used to aid the assignment of the ^{13}C n.m.r. spectrum of stachyose.[1]

Values for chemical shift differences (in p.p.m.) obtained from ^{13}C n.m.r. spectra recorded at 30, 50, 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 of δ 67.40 is assigned. Exceptions are observed mainly for anomeric carbons in the glycosyl group, the largest negative value being −0.21 p.p.m. Downfield shifts ≥0.2 p.p.m. are observed for the signals due to C-4 and C-6 in glucose residues, the methyl glucoside residue being most affected. As only small shift differences are observed for the 6-deoxy hexose residues, these temperature shifts are most probably due to changes in the rotamer distribution.

The values for the anomeric carbon signals follow the same pattern as the ^{13}C and ^{1}H glycosylation-induced shifts. Downfield shifts occur for signals from C-1′ of the α-DD-, β-LD-, and β-DL-disaccharides and upfield shifts for the β-DD-, α-LD-, and α-DL-disaccharides. For 1,6-linked disaccharides[1] a similar pattern was observed with larger values for the α-DD-disaccharides (0.11—0.13 p.p.m.) and only small values for the β-DD-disaccharides (0.03—0.05 p.p.m.). These temperature shifts could be useful for signal assignments in spectra of complex oligo- and poly-saccharides.

The use of Substituent Shifts for Predicting Chemical Shifts in Oligo- and Poly-saccharides.—Substituent shifts obtained from disaccharide models have been used to predict ^{13}C n.m.r. spectra of 1,6-linked polysaccharides.[1] Similar calculations have now been performed for amylose, cellopentaose (as a soluble model for cellulose), pullulan, and elsinan, all of which contain 1,4-linkages. Pullulan and elsinan are composed of maltotriose units linked through α-1,6- and α-1,3-linkages, respectively. Both further contain maltotetraose units which, however, do not complicate the interpretation of the n.m.r. spectra. The calculated and experimental ^{1}H and ^{13}C n.m.r. spectra are given in Table 5.

The signals in the ^{13}C n.m.r. spectra of amylose and cellodextrins have been assigned[33] and assignments for the spectrum of pullulan were obtained *via* homo- and hetero-nuclear correlation spectra. For elsinan no assignments were made and comparison was performed by superposition only. Owing to long-range effects caused by maltotriose and maltotetraose units, the elsinan signals for linkage carbons appear as 'multiplets' and the chemical shifts were taken from the centre of the signals.

In amylose, starting from the chemical shifts of α-D-glucose, the glycosylation shifts for C-1′ to C-6′ of (**1**) (Table 3) are added to account for the presence of a 1,4-linked α-D-glucopyranosyl aglycone residue. Similarly, the effect of an α-D-glucopyranosyl group linked to O-4 of the α-D-glucopyranosyl residue is accounted for by the addition of the glycosylation shifts for C-1 to C-6 in (**1**). The addition of these values, gives the predicted spectrum of amylose. The fit with the experimental values is good, indicating that glycosylation shifts are similar and additive in the spectrum of the polysaccharide. The resonances of the three inner glycosyl residues of cellopentaose coincide and were used for the comparison. The calculated and the experimental spectrum show good agreement. For pullulan with one α-1,6-linkage and two α-1,4-linkages the chemical shifts of three α-D-glucose residues were used as a starting point. For each of these residues the glycosylation shifts obtained from the corresponding 1,4- or 1,6-linked disaccharide elements were added. The observed chemical shifts are at lower fields than the calculated values with the largest deviations for the linkage carbons. A different rotamer distribution around the 1,6-linkage in isomaltose and in pullulan is a possible explanation to the discrepancy.

The simulated spectrum of elsinan was constructed from the shifts of (**1**) and methyl α-nigeroside[34] and the chemical shifts of α-D-glucose. Comparison with experimental data, as shown in Table 5, indicates a good fit, with deviations not larger than ±0.6 p.p.m. As the signals in the ^{13}C n.m.r. spectrum were not assigned, the real deviations may be larger.

From the ^{1}H n.m.r. chemical shifts (Table 5) good agreement for the 4-linked residues is found with calculated ^{1}H n.m.r. spectra, using data from Table 2 and the procedure described for simulation of ^{13}C n.m.r. spectra.

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