N.m.r. and Conformational Studies on some 1,3-linked Disaccharides

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^{13}C and ^{1}H N.m.r. data have been obtained from 10 methyl 6-deoxyhexosylhexosides in which the 6-deoxysugar is the L- or D-enantiomer of fucose or rhamnose. A large variation in the magnitude of displacement shifts of signals for α- and β-carbons is observed. For all compounds Hard Sphere Exo Anomeric effect (HSEA) calculations have been performed to estimate favoured conformations. From the derived minimum energy conformations, a number of ^{1}H n.m.r. chemical shifts can be rationalised.

N.m.r. spectroscopy, including one- and two-dimensional ^{1}H and ^{13}C n.m.r. spectroscopy has become increasingly important in structural studies of oligo- and poly-saccharides. However, even high-field ^{1}H n.m.r. spectra generally have several overlapping signals, whereas totally resolved ^{13}C n.m.r. spectra can often be obtained. For ^{1}H n.m.r. spectroscopy, Vliegenthart *et al*[1] have created the concept of 'structural-reporter-groups', that is, groups which give signals outside the bulk region in ^{1}H n.m.r. spectra. Chemical shifts of these give valuable structural information.

For the complete assignment of ^{13}C n.m.r. spectra of polysaccharides, the spectra of suitable model compounds are usually required. Thus glycosylation shifts have been estimated using alkyl ethers as models. This is not very satisfactory, however, because steric effects of the more bulky glycosyl groups are neglected. In order to obtain better glycosidation shifts, oligosaccharides with similar stereochemistry around the glycosidic bond should be investigated. ^{13}C N.m.r. data for a large number of oligosaccharides have been collected by Bock *et al.*[2] Some useful rules have also been established[3–6] and a set of empirical rules for the structural analysis of trisaccharides by ^{13}C n.m.r. has been described.[7]

Lemieux and co-workers have found that conformations derived by ^{1}H n.m.r. are well anticipated by theoretical calculations using the HSEA-program.[8] This takes into account non-bonded interactions (van der Waals interactions), corrected for the influence of the exoanomeric effect. In some instances, HSEA-calculations have provided conformations in which strongly deshielded protons are found close to oxygen atoms in other sugar units[9] which explains chemical shifts of signals at otherwise unexpected fields.

We have previously described[10] an n.m.r. study of some 1,6-linked disaccharides which was performed in order to develop computer-assisted structural analysis of oligo- and poly-saccharides. From this data, the ^{13}C n.m.r. spectra of 1,6-linked polysaccharides could be simulated and these showed good agreement with experimental ones.

We now report on syntheses, n.m.r. studies and HSEA-calculations, of ten 1,3-linked disaccharide methyl glycosides (**1**)—(**10**) (see Table 1).

The discussion will mainly be concerned with the various chemical shift displacements deriving from differences in stereochemistry around the glycosidic bond. For the calculation of the chemical shifts of unknown polysaccharides the glycosidation shifts do not necessarily have to be determined for all types of disaccharide elements involved but can often be approximated from compounds with similar stereochemistry in the vicinity of the glycosidic linkage.

† Sodium 3-(trimethylsilyl)tetradeuteriopropionate.

Experimental

General Methods.—M.p.s are corrected. Concentrations were performed at reduced pressure at <40 °C. Column chromatography was performed on silica gel 60 (0.040—0.063 mm, Merck). ^{1}H N.m.r. spectra (400 MHz) and ^{13}C n.m.r. spectra (100 MHz) were recorded for deuterium oxide solutions at 70 °C with a JEOL GX-400 spectrometer. Chemical shifts are given in p.p.m. using internal TSP,† δ 0.00 p.p.m. (^{1}H n.m.r.) and internal dioxane, δ 67.40 (^{13}C n.m.r.) as references.

Methyl 2-O-*Benzyl*-4,6-O-*benzylidene*-3-O-(2,3,4-*tri*-O-*benzyl*-α-L-*fucopyranosyl*)-α-D-*glucopyranoside.*—Methyl trifluoromethylsulphonate (60 μl) was added to a stirred mixture of methyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside[11] (30 mg), ethyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside[12] (50 mg) and ground molecular sieves (4Å, 0.5 g) in diethyl ether (5 ml) at room temperature. Triethylamine (200 μl) was added after 2 h and the mixture was stirred for another 30 min, filtered through a layer of Celite, concentrated, and purified on silica gel (iso-octane–ethyl acetate, 3:1), to yield the product as a syrup (53 mg, 84%), $[\alpha]_{578}^{22}$ −62° (*c* 1.0, $CHCl_3$).

Methyl 3-O-α-L-*Fucopyranosyl*-α-D-*glucopyranoside* (**1**).—The above product from a large-scale preparation of the previous step (408 mg) was dissolved in aqueous 90% acetic acid (20 ml) and hydrogenolysed at 400 kPa over 10% palladium-on-charcoal (200 mg) for 18 h. After filtration and concentration the product was purified on silica gel (ethyl acetate–acetic acid–methanol–water, 12:3:3:2) followed by purification on a column (2.5 × 80 cm) of Bio-Gel P-2 irrigated with water. After freeze-drying, compound (**1**) was obtained as an amorphous powder (125 mg, 71%), $[\alpha]_{578}^{22}$ −22° (*c* 1.0, water) (Lit., $[\alpha]_D$ −20°).[13]

Methyl 2-O-*Benzyl*-4,6-O-*benzylidene*-3-O-(2,3,4-*tri*-O-*benzyl*-α-D-*fucopyranosyl*)-α-D-*glucopyranoside.*—Glycosidation and purification as described above but using ethyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-fucopyranoside gave the title compound (58 mg, 91%), $[\alpha]_{578}^{22}$ + 54° (*c* 0.6, $CHCl_3$).

Methyl 3-O-α-D-*Fucopyranosyl*-α-D-*glucopyranoside* (**2**).—Hydrogenolysis of a large-scale preparation of the protected disaccharide (850 mg) and purification as above gave compound (**2**) as an amorphous powder (238 mg, 65%). Crystallisation from ethanol gave (**2**), m.p. 192—195 °C; $[\alpha]_{578}^{22}$ 243° (*c* 1.0, water).

Methyl 3-O-α-L- *and* D-*Fucopyranosyl*-β-D-*glucopyranoside* (**3**) *and* (**4**).—Glycosidation and purification as described above but with the corresponding β-isomer as aglycone[11] and the thioglycosides with the L- and D-configuration, respectively,

yielded the protected disaccharide glycosides (69% and 80% respectively), (LD) m.p. 131—132 °C (ethanol); $[\alpha]_{578}^{22}$ −66° (*c* 1.0, $CHCl_3$) and (DD) +32° (*c* 1.0, $CHCl_3$). Deprotection and purification as described above yielded (**3**), (81%) $[\alpha]_{578}^{22}$ −138° (*c* 1.0, water) and **4** (87%) m.p. 91—92 °C (isopropyl alcohol), $[\alpha]_{578}^{22}$ +105° (*c* 1.1, water).

Methyl 2-O-*Acetyl*-4,6-O-*benzylidene*-3-O-(2,3,4-*tri*-O-*benzoyl*-α-L-*rhamnopyranosyl*)-α-D-*glucopyranoside*.—A solution of silver trifluoromethylsulphonate[14,15] (0.9 g) in nitromethane–toluene-dichloromethane (10 ml, 1:1:1) was added to a stirred mixture of methyl 2-*O*-acetyl-4,6-*O*-benzylidene-α-D-glucopyranoside[16] (648 mg), 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl bromide[17] (1.4 g) and ground molecular sieves (4Å, 6 g) in the same solvent (20 ml) and the reaction mixture was kept at −20 °C. The mixture was stirred for 15 min after which aqueous 10% sodium thiosulphate (30 ml) and dichloromethane (50 ml) were added. The mixture was filtered through a layer of Celite, the organic layer washed with water, sulphuric acid (2M), water, sodium hydrogen-carbonate and water, dried (Na_2SO_4), and concentrated to dryness. The material was purified on silica gel (light petroleum–ethyl acetate, 3:1) to yield the product as a glass (1.33 g, 85%), $[\alpha]_{578}^{22}$ +104° (*c* 1.0, $CHCl_3$).

Methyl 3-O-α-L-*Rhamnopyranosyl*-α-D-*glucopyranoside* (**5**).—A catalytic amount of sodium was added to a solution of the protected disaccharide (800 mg) in dry methanol (25 ml). The mixture was left at room temperature for 18 h, neutralised with Dowex 50 (H^+) resin, and concentrated to dryness. The product was then hydrogenolysed and purified as described above. Compound (**5**) was obtained as an amorphous powder (241 mg, 69%), $[\alpha]_{578}^{22}$ +38° (*c* 1.0, water).

Methyl 2-O-*Benzyl*-4,6-O-*benzylidene*-3-O-(2,3,4-*tri*-O-*acetyl*-α-D-*rhamnopyranosyl*)-α-D-*glucopyranoside*.—The reaction of methyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (600 mg) and 2,3,4-tri-*O*-acetyl-α-D-rhamnopyranosyl bromide[18] (800 mg) using silver trifluoromethylsulphonate as promoter, was carried out as described above. Purification on silica gel (toluene–ethyl acetate, 4:1), gave the product (870 mg, 84%), m.p. 188—189 °C (iso-octane–ethyl acetate), $[\alpha]_{578}^{22}$ +49° (*c* 1.0, $CHCl_3$).

Methyl 3-O-α-D-*Rhamnopyranosyl*-α-D-*glucopyranoside* (**6**).—The protected disaccharide (820 mg) was treated with sodium methoxide in methanol and was then hydrogenolysed and purified as described above. Compound (**6**) was obtained as an amorphous powder (285 mg, 66%), $[\alpha]_{578}^{22}$ +147° (*c* 1.0, water).

Methyl 2,6-*Di*-O-*benzyl*-3-O-(2,3,4-*tri*-O-*benzyl*-α-L-*fucopyranosyl*)-α-D-*galactopyranoside*.—Methyl 2,6-di-*O*-benzyl-α-D-galactopyranoside[19] (570 mg) and ethyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside (725 mg) were reacted by using methyl trifluoromethylsulphonate (840 μl) as promoter, as described above. Purification on silica gel (toluene–ethyl acetate, 3:1) gave the product as a syrup (625 mg, 52%) $[\alpha]_{578}^{22}$ −35° (*c* 0.9, CH_2Cl_2). A minor faster moving component was identified as the 3,4-disubstituted trisaccharide.

Methyl 3-O-α-L-*Fucopyranosyl*-α-D-*galactopyranoside* (**7**).—Hydrogenolysis of the previous product (625 mg) in aqueous 90% acetic acid (20 ml) as described above, gave, after purification, compound (**7**) as an amorphous powder (165 mg, 62%), $[\alpha]_{578}^{22}$ +22° (*c* 1.0, water).

Methyl 2,6-*Di*-O-*benzyl*-3-O-(2,3,4-*tri*-O-*benzyl*-α-D-*fucopyranosyl*)-α-D-*galactopyranoside*.—Methyl 2,6-di-*O*-benzyl-α-D-galactopyranoside (510 mg) and ethyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-fucopyranoside (650 mg) were allowed to react as described above by using methyl trifluoromethylsulphonate (750 μl) as promoter. Purification on silica gel (toluene–ethyl acetate, 4:1) gave the disaccharide as a syrup (625 mg, 58%), $[\alpha]_{578}^{22}$ +63° (*c* 1.1, $CHCl_3$).

Methyl 3-O-α-D-*Fucopyranosyl*-α-D-*galactopyranoside* (**8**).—Hydrogenolysis of the protected disaccharide (624 mg) and purification as described above for the preparation of compound (**2**) gave compound (**8**) as an amorphous powder (171 mg, 64%), $[\alpha]_{578}^{22}$ +241° (*c* 1.0, water).

Methyl 4,6-*Di*-O-*benzyl*-3-O-(2,3,4-*tri*-O-*benzyl*-α-L-*fucopyranosyl*)-α-D-*mannopyranoside*.—Methyl trifluoromethylsulphonate (1 ml) was added to a stirred mixture of methyl 4,6-di-*O*-benzyl-α-D-mannopyranoside[20] (600 mg), ethyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside (800 mg) and ground molecular sieves (4 Å, 4 g) in benzene (20 ml) at room temperature. Work-up as described for the precursor of compound (**1**) and purification on silica gel (toluene–ethyl acetate, 4:1) yielded the product as a syrup (460 mg, 36%), $[\alpha]_{578}^{22}$ −44° (*c* 1.5, $CHCl_3$)

Methyl 3-O-α-L-*Fucopyranosyl*-α-D-*mannopyranoside* (**9**).—Hydrogenolysis of the above protected disaccharide (460 mg) and purification as described above gave compound (**9**) as an amorphous powder (136 mg, 69%), $[\alpha]_{578}^{22}$ −65° (*c* 1.0, water).

Methyl 2-O-*Benzyl*-4,6-O-*benzylidene*-3-O-(2,3,4-*tri*-O-*benzyl*-α-D-*fucopyranosyl*)-α-D-*mannopyranoside*.—The reaction of methyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside[21] (400 mg) and ethyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-fucopyranoside (800 mg) was carried out as described above using methyl trifluoromethylsulphonate (0.9 ml) as promoter. Purification on silica gel (iso-octane–ethyl acetate, 2:1) gave the disaccharide as a syrup (530 mg, 63%), $[\alpha]_{578}^{22}$ +63° (*c* 1.0, $CHCl_3$).

Methyl 3-O-α-D-*Fucopyranosyl*-α-D-*mannopyranoside* (**10**).—Hydrogenolysis of the protected disaccharide (529 mg) and purification as described above, gave compound (**10**) as an amorphous powder (109 mg, 53%), $[\alpha]_{578}^{22}$ +148° (*c* 1.0, water).

HSEA-Calculations.—These were performed as described earlier[8] using crystal co-ordinates for the respective sugars. The co-ordinate sets for α-D-fucose and α-D-rhamnose were obtained from the corresponding L-isomer by modification of co-ordinates. A value of 117° was used for the glycosidic bond angle, τ, and φ/ψ-values were incremented by 5° in the calculations. The plots were prepared using CHEM-X.* Isocontour levels were set at 0.1, 0.5, 1, 2, 3, and 4 kcal above the energy of the minimum energy molecule.

Results and Discussion

Synthesis.—For the synthesis of the α-fucopyranosides (**1**)—(**4**) and (**7**)—(**10**), ethyl 2,3,4-tri-*O*-benzyl-1-thio-β-L- or D-fucopyranoside was used as glycosylating agent and methyl trifluoromethylsulphonate[12] as promoter. When methyl 2,6-di-*O*-benzyl-α-D-galactopyranoside was used as aglycon, glycosylation occurred predominantly at the 3-position, as evident from methylation analysis of the deprotected product, (**7**) and (**8**). Substance (**9**) was prepared using the same glycosylating agent in the L-form and methyl 4,6-di-*O*-benzyl-D-mannopyranoside as aglycon. Reaction occurred predominantly at the 3-position, as evident from methylation analysis of the deprotected

* Developed and distributed by Chemical Design, Oxford.

product. When, however, the D-form of the glycosylating agent was used, substitution occurred predominantly at the 2-position, and a fully protected aglycon, methyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside had to be used for the synthesis of (**10**).

For the synthesis of compounds (**5**) and (**6**), 2,3,4-tri-*O*-benzoyl-α-L-or 2,3,4-tri-*O*-acetyl-α-D-rhamnopyranosyl bromide was used as glycosylating agent and silver trifluoromethanesulphonate[14,15] as promoter. Further details are given in the experimental section.

N.m.r. assignments.—The signals in the ^{1}H n.m.r. spectra were assigned from the COSY-spectra and the ^{13}C n.m.r. signals from the C,H-correlation spectra. The signal for the methyl group in the 6-deoxyhexose residue was taken as the unambiguous starting point in the assignment of the COSY-spectra. The chemical shifts of overlapping signals were taken from the COSY-spectra, using the chemical shift of the methoxy signal as reference. In the ^{1}H n.m.r. spectra of compounds (**1**) and (**5**), the signals for 2-H and 5-H overlap, making the assignment of C-2 and C-5 difficult. This problem also occurred for 5-H and 2′-H and the corresponding carbons in (**9**). No further attempt was made to assign these signals as their chemical shifts were almost the same.

^{13}C *N.m.r. spectra.*—The ^{13}C n.m.r. chemical shifts and chemical shift differences for pyranosides (**1**)—(**10**) are given in Table 1. The chemical shift differences were obtained for the glycosyl group by comparison with the corresponding reducing sugar and for the methyl glycoside residue by comparison with the corresponding methyl glycoside. Chemical shifts of the relevant monomers are also given in Table 1.

In the following, the convention of designating substituted carbons α-carbons and their vicinal carbons β-carbons is used. In general, significant chemical shift changes (>1 p.p.m.) are obtained only for the anomeric carbon of the glycosyl group (C-1′), the substituted carbon (C-3), and the carbons next to these (C-2 and C-4). The displacements for C-1′ and C-3 (α-carbons) vary between 3.2 and 8.5 p.p.m. The induced shifts of the β-carbons also show a large variation, between 0.4 and −3.5 p.p.m.

On L-fucosylation of O-3 in methyl α-D-glucopyranoside, (**1**), changes in the chemical shift (>0.5 p.p.m.) are observed for C-1′ (7.3 p.p.m.) and C-5′ (0.6 p.p.m.) in the glycosyl group and for C-3 (7.6 p.p.m.) and C-4 (−1.5 p.p.m.) in the methyl glucoside residue. A smaller downfield shift of 0.4 p.p.m. is observed for C-2.

On fucosylation with the D-isomer the changes in chemical shift for C-1′, C-5′, and C-3 are similar to those observed on L-fucosylation, but the signal for C-2 is displaced upfield (−1.3 p.p.m.) and that for C-4 downfield (0.2 p.p.m.).

On comparison of the spectra of compounds (**1**) and (**3**) with compounds (**2**) and (**4**), respectively, an estimate of the influence of the anomeric configuration in the methyl glycoside can be obtained. Signals for the fucosyl carbons and the β-carbons in the glycopyranosyl residue are essentially the same. The displacement of the α-carbon in the glucosyl residue is somewhat smaller in compounds (**3**) and (**4**) than in compounds (**1**) and (**2**) (~0.4 p.p.m.).

The difference in the ^{13}C n.m.r. spectra of 3-*O*-α-L- and D-rhamnosylated methyl α-D-glucopyranosides, (**5**) and (**6**), gives an indication of substituent effects for glycosyl groups with an axial hydroxyl group on C-2. In the spectra for compounds (**5**) and (**6**), similar displacements of the chemical shifts for C-1′ and C-3 are observed. The displacement on α-L-rhamnosylation are 0.3 and −1.5 p.p.m. for C-2 and C-4, respectively, and these values are reversed on α-D-rhamnosylation (−1.3 and 0.3 p.p.m.). In the D-isomer, upfield shifts of C-5 and C-6 are observed and this effect is also observed in spectra of (**2**) and (**4**). The main influence of the configurational change for C-2′ is observed for C-1′ and C-3, for which the chemical shifts are displaced somewhat less in compounds (**5**) and (**6**) than in compounds (**1**) and (**2**).

Upon fucosylation of methyl α-D-galactopyranoside and methyl α-D-mannopyranoside which have axial hydroxyl groups at C-4 and C-2, respectively, the substituent shifts for all α- and β-carbons are different to those observed for the corresponding D-*gluco*-derivatives. As the only difference is the configuration at C-2 or C-4, the different chemical shift displacements must derive from steric interactions between the two sugar residues over the glycosidic bond. As no major changes in the conformation (Table 3) were obtained between compounds (**1**) and (**7**) and (**2**) and (**8**), respectively, the main part of the different displacements must derive from interactions between different atoms which are in van der Waals contact.

The chemical shift displacements for compounds (**7**) and (**10**) are similar and differ from those of (**8**) and (**9**), which are also similar. This is not unexpected as the stereochemistry around the glycosidic linkage in each pair is similar (Figure 1).

Thus, in compounds (**7**) and (**10**), the signals for the α-carbons, C-1′ and C-3, are shifted ~8.5 and ~8.1 p.p.m., respectively, whereas in (**8**) and (**9**) the corresponding values are only ~3.5 and ~5.5 p.p.m., respectively. Signals for the β-carbons, C-2 and C-4 are shifted only −0.8 and 0.1 p.p.m. in (**7**)

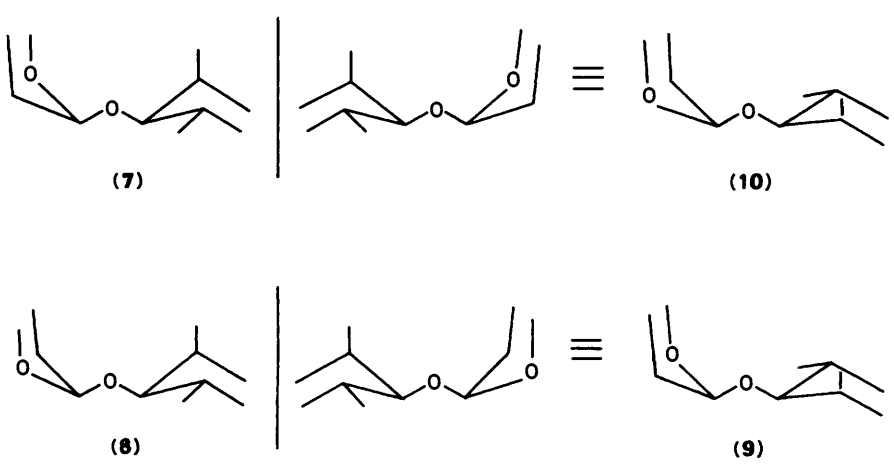

Figure 1. The similarity of the stereochemistry around the glycosidic linkage of compounds (**7**) and (**10**) and compounds (**8**) and (**9**), respectively, shown by the mirror-image isomers

Table 1. ^{13}C N.m.r. chemical shifts of disaccharides (**1**)—(**10**) and appropriate monosaccharides all obtained at 70 °C. Chemical shift differences are given in parentheses

Compound	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-L-Fuc*p*(1 ⟶ 3)α-D-Glc*p*OMe	100.42	69.33	70.59	72.78	67.70	16.08	100.13	72.58[c]	81.65	69.20	72.50[c]	61.66	55.91
(**1**)	(7.30)[b]	(0.24)	(0.30)	(−0.01)	(0.60)	(−0.25)	(−0.06)	(0.35)	(7.55)	(−1.48)	(−0.02)	(−0.01)	(−0.02)
α-D-Fuc*p*(1 ⟶ 3)α-D-Glc*p*OMe	100.32	69.36	70.56	72.79	67.70	16.10	100.32	70.92	81.48	70.92	72.28	61.48	55.90
(**2**)	(7.20)	(0.27)	(0.27)	(0.00)	(0.60)	(−0.23)	(0.13)	(−1.31)	(7.38)	(0.24)	(−0.24)	(−0.19)	(−0.03)
α-L-Fuc*p*(1 ⟶ 3)β-D-Glc*p*OMe	100.35	69.31	70.56	72.77	67.78	16.11	103.92	74.30	83.97	69.31	76.76	61.83	57.93
(**3**)	(7.23)	(0.22)	(0.27)	(−0.02)	(0.68)	(−0.22)	(−0.21)	(0.31)	(7.19)	(−1.38)	(−0.02)	(0.01)	(0.05)
α-D-Fuc*p*(1 ⟶ 3)β-D-Glc*p*OMe	100.36	69.29	70.54	72.79	67.76	16.10	104.19	72.79	83.92	70.93	76.48	61.64	57.92
(**4**)	(7.24)	(0.20)	(0.26)	(0.00)	(0.66)	(−0.23)	(0.06)	(−1.20)	(7.14)	(0.24)	(−0.30)	(−0.18)	(0.04)
α-L-Rha*p*(1 ⟶ 3)α-D-Glc*p*OMe	101.89	71.25	71.21	72.97	69.63	17.40	100.27	72.52[c]	81.14	69.14	72.58[c]	61.61	55.95
(**5**)	(7.05)	(−0.56)	(0.21)	(−0.22)	(0.51)	(−0.27)	(0.08)	(0.29)	(7.04)	(−1.54)	(0.06)	(−0.06)	(0.02)
α-D-Rha*p*(1 ⟶ 3)α-D-Glc*p*OMe	101.80	71.30	71.19	73.02	69.65	17.46	100.32	70.92	80.94	70.97	72.38	61.46	55.91
(**6**)	(6.96)	(−0.51)	(0.19)	(−0.17)	(0.53)	(−0.21)	(0.13)	(−1.31)	(6.84)	(0.29)	(−0.14)	(−0.21)	(−0.02)
α-L-Fuc*p*(1 ⟶ 3)α-D-Gal*p*OMe	101.52	69.40	70.51	72.71	67.90	16.15	100.33	68.44	78.62	70.26	71.54	61.94	55.96
(**7**)	(8.40)	(0.31)	(0.22)	(−0.08)	(0.80)	(−0.18)	(−0.02)	(−0.73)	(8.16)	(0.07)	(0.0)	(−0.12)	(0.0)
α-D-Fuc*p*(1 ⟶ 3)α-D-Gal*p*OMe	96.27	68.89	70.50	72.78	67.73	16.08	100.36	67.59	75.62	66.66	71.31	62.05	55.98
(**8**)	(3.15)	(−0.20)	(0.21)	(−0.01)	(0.63)	(−0.25)	(0.01)	(−1.58)	(5.16)	(−3.53)	(−0.23)	(−0.01)	(0.02)
α-L-Fuc*p*(1 ⟶ 3)α-D-Man*p*OMe	96.88	68.93	70.53	72.78	67.68[c]	16.04	101.44	67.64[c]	77.22	66.11	73.51	61.94	55.65
(**9**)	(3.76)	(−0.16)	(0.24)	(−0.01)	(0.58)	(−0.29)	(−0.31)	(−3.21)	(5.66)	(−1.68)	(0.06)	(0.02)	(0.09)
α-D-Fuc*p*(1 ⟶ 3)α-D-Man*p*OMe	101.65	69.44	70.53	72.72	67.80	16.18	101.59	70.87	79.59	66.99	73.59	61.84	55.61
(**10**)	(8.53)	(0.35)	(0.24)	(−0.07)	(0.70)	(−0.15)	(−0.16)	(0.02)	(8.03)	(−0.80)	(0.14)	(−0.08)	(0.05)
α-L-Fucopyranose	93.12	69.09	70.29	72.79	67.10	16.33							
α-L-Rhamnopyranose	94.84	71.81	71.00	73.19	69.12	17.67							
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-D-galactopyranoside							100.35	69.17	70.46	70.19	71.54	62.06	55.96
Methyl α-D-mannopyranoside							101.75	70.85	71.56	67.79	73.45	61.92	55.56

Chemical shifts are given in p.p.m. relative to internal dioxane (67.40 p.p.m.) [a] Primed labels refer to the D-glucopyranosyl group and unprimed to the methyl glycoside residue. [b] Chemical shift differences are calculated by subtraction of chemical shifts of α-L-fucose and methyl α-D-glucopyranoside from (**1**) etc. and a positive difference indicates a downfield shift. [c] Pairs of chemical shifts that may be interchanged.

Table 2. ^{1}H N.m.r. chemical shifts of disaccharides (**1**)—(**10**) and appropriate monosaccharides, all obtained at 70 °C. Chemical shift differences are given in parentheses.

Compound	1′-H	2′-H	3′-H	4′-H	5′-H	6′-H	1-H	2-H	3-H	4-H	5-H	6-H	6-H	OMe
α-L-Fuc*p*(1 ⟶ 3)α-D-Glc*p*OMe	5.20	3.81	3.88	3.81	4.32	1.20	4.78	3.73	3.73	3.49	3.66	3.76	3.87	3.43
(**1**)	(0.0)	(0.04)	(0.02)	(0.0)	(0.12)	(−0.01)	(−0.03)	(0.17)	(0.05)	(0.08)	(0.02)	(0.0)	(0.0)	(0.0)
α-D-Fuc*p*(1 ⟶ 3)α-D-Glc*p*OMe	5.25	3.80	3.88	3.80	4.32	1.21	4.81	3.62	3.76	3.60	3.64	3.75	3.85	3.43
(**2**)	(0.05)	(0.03)	(0.02)	(−0.01)	(0.12)	(0.0)	(0.0)	(0.06)	(0.08)	(0.19)	(0.0)	(−0.01)	(−0.02)	(0.0)
α-L-Fuc*p*(1 ⟶ 3)β-D-Glc*p*OMe	5.24	3.81	3.88	3.80	4.32	1.21	4.38	3.45	3.60	3.48)	3.47	3.73	3.92	3.57
(**3**)	(0.04)	(0.04)	(0.02)	(−0.01)	(0.12)	(0.0)	(0.01)	(0.17)	(0.10)	(0.08)	(0.01)	(−0.01)	(0.0)	(−0.01)
α-D-Fuc*p*(1 ⟶ 3)β-D-Glc*p*OMe	5.25	3.82	3.89	3.81	4.33	1.21	4.39	3.35	3.59	3.59	3.47	3.70	3.91	3.57
(**4**)	(0.05)	(0.05)	(0.03)	(0.0)	(0.13)	(0.0)	(0.02)	(0.07)	(0.09)	(0.19)	(0.01)	(−0.04)	(−0.01)	(−0.01)
α-L-Rha*p*(1 ⟶ 3)α-D-Glc*p*OMe	5.08	4.04	3.78	3.45	3.98	1.27	4.77	3.64	3.73	3.45	3.64	3.75	3.86	3.42
(**5**)	(−0.04)	(0.12)	(−0.03)	(0.0)	(0.12)	(−0.01)	(−0.04)	(0.08)	(0.05)	(0.04)	(0.0)	(−0.01)	(−0.01)	(−0.01)
α-D-Rha*p*(1 ⟶ 3)α-D-Glc*p*OMe	5.15	4.05	3.80	3.46	3.99	1.28	4.81	3.61	3.77	3.52	3.65	3.75	3.86	3.43
(**6**)	(0.03)	(0.13)	(−0.01)	(0.01)	(0.13)	(0.0)	(0.0)	(0.05)	(0.09)	(0.11)	(0.01)	(−0.01)	(−0.01)	(0.0)
α-L-Fuc*p*(1 ⟶ 3)α-D-Gal*p*OMe	5.16	3.80	3.94	3.82	4.18	1.22	4.86	4.03	3.85	4.07	3.92	3.73[b]	3.73[b]	3.44
(**7**)	(−0.04)	(0.03)	(0.08)	(0.01)	(−0.02)	(0.01)	(0.01)	(0.19)	(0.04)	(0.08)	(0.03)	(−0.03)	(−0.03)	(0.01)
α-D-Fuc*p*(1 ⟶ 3)α-D-Gal*p*OMe	5.06	3.82	3.93	3.82	4.27	1.21	4.87	3.96	3.85	4.19	3.88	3.77[b]	3.77[b]	3.43
(**8**)	(−0.14)	(0.05)	(0.07)	(0.01)	(0.07)	(0.0)	(0.02)	(0.12)	(0.04)	(0.20)	(−0.01)	(0.01)	(0.01)	(0.00)
α-L-Fuc*p*(1 ⟶ 3)α-D-Man*p*OMe	5.03	3.83	3.94	3.83	4.30	1.21	4.81	4.11	3.80	3.80	3.65	3.80	3.91	3.43
(**9**)	(−0.17)	(0.06)	(0.08)	(0.02)	(0.10)	(0.0)	(0.04)	(0.17)	(0.03)	(0.13)	(0.05)	(0.02)	(0.01)	(0.01)
α-D-Fuc*p*(1 ⟶ 3)α-D-Man*p*OMe	5.18	3.83	3.93	3.81	4.18	1.23	4.75	4.02	3.82	3.85	3.65	3.79	3.90	3.43
(**10**)	(−0.02)	(0.06)	(0.07)	(0.0)	(−0.02)	(0.02)	(−0.02)	(0.08)	(0.05)	(0.18)	(0.05)	(0.01)	(0.0)	(0.01)
α-L-Fucopyranose	5.20	3.77	3.86	3.81	4.20	1.21								
α-L-Rhamnopyranose	5.12	3.92	3.81	3.45	3.86	1.28								
α-D-Glc*p*OMe							4.81	3.56	3.68	3.41	3.64	3.76	3.87	3.43
β-D-Glc*p*OMe							4.37	3.28	3.50	3.40	3.46	3.74	3.92	3.58
α-D-Gal*p*OMe							4.85	3.84	3.81	3.99	3.89	3.76[b]	3.76[b]	3.43
α-D-Man*p*OMe							4.77	3.94	3.77	3.67	3.60	3.78	3.90	3.42

[a] Chemical shifts are given in p.p.m. relative to internal TSP (δ 0.00). [b] Approximate values.

Table 3. Values for the φ and ψ angles of the minimum energy molecules and inter-residual atomic distances < 3.5 Å in compounds (**1**)—(**10**). The data was obtained by HSEA calculations

Compound	φ/ψ	1′-H	5′-H	O-5′
(**1**)	50/15	2.44 (H-3) 2.71 (O-2)	2.55 (O-4)	2.60 (H-3)
(**2**)	−50/−20	2.48 (H-3) 2.62 (O-4)	2.51 (O-2)	2.58 (H-3)
(**3**)	45/10	2.36 (H-3) 2.71 (O-2)	2.64 (O-4)	2.73 (H-3)
(**4**)	−45/−15	2.40 (H-3) 2.64 (O-4)	2.52 (O-2)	2.71 (H-3)
(**5**)	50/15	2.43 (H-3) 2.69 (O-2)	2.49 (O-4)	2.60 (H-3)
(**6**)	−50/−20	2.47 (H-3) 2.60 (O-4)	2.48 (O-2)	2.58 (H-3)
(**7**)	50/−5	2.28 (H-3) 3.22 (O-2)	2.38 (H-4)	2.72 (H-3)
(**8**)	−55/−30	2.65 (H-3) 2.40 (H-4)	2.67 (O-2)	2.48 (H-3)
(**9**)	50/30	2.60 (H-3) 2.39 (H-2)	2.72 (O-4)	2.58 (H-3)
(**10**)	−50/5	2.30 (H-3) 3.13 (O-4)	2.43 (H-2)	2.74 (H-3)

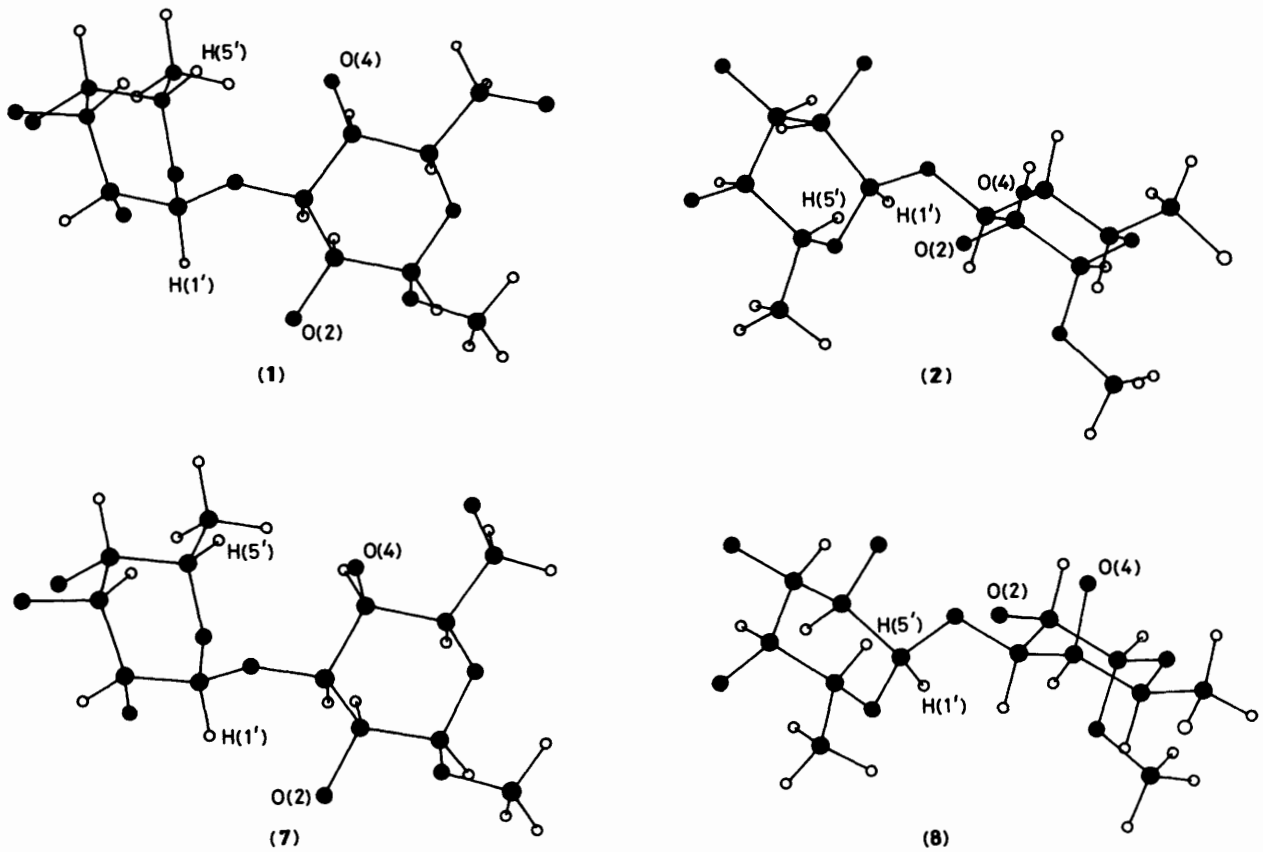

Figure 2. The minimum energy conformation of compounds (**1**), (**2**), (**7**), and (**8**) as derived by HSEA-calculations

and are reversed in (**10**). In (**8**) the corresponding shifts are −1.6 and −3.5 p.p.m., respectively, and are reversed in (**9**).

^{1}H *N.m.r. spectra.*—Chemical shifts and chemical shift differences for all protons relative to those of the parent monomers are given in Table 2. In addition to the signals for the anomeric and the deoxy protons, the equatorial 2-H in rhamnose and mannose, 4-H in galactose, and 5-H in the deoxy sugars are separated from the 'bulk' region and thus easily observable also in one dimensional n.m.r. spectra. The chemical shift displacements vary between 0.20 p.p.m. for 4-H in galactose in compound (**8**) to −0.17 p.p.m. for 1-H in fucose in compound (**9**).

Fucosyl 5′-H appears at δ ~4.32 for (**1**)—(**4**) which is a shift

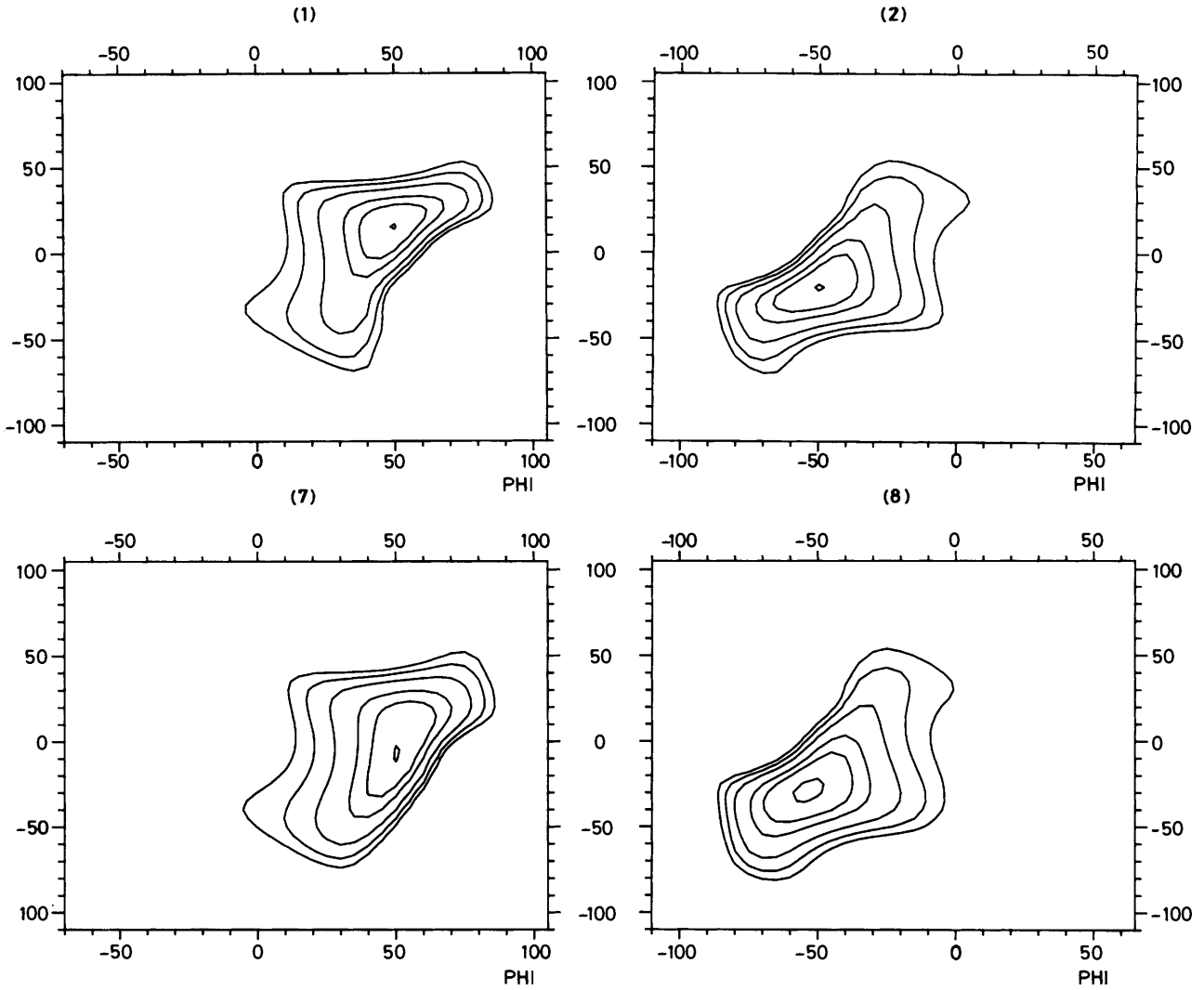

Figure 3. Conformational energy plots for compounds (**1**), (**2**), (**7**), and (**8**). Isocontour levels are indicated at 0.4, 2.1, 4.2, 8.4, 12.6, and 16.8 kJ (0.1, 0.5, 1, 2, 3, and 4 kcal) above the minimum energy molecule

of 0.12 p.p.m. downfield of the chemical shift of 5-H in α-fucopyranose. A possible explanation for this displacement and of protons in similar arrangements has been given by Lemieux *et al.*[8,22] Downfield shifts may be obtained when the proton in question is close to one or two oxygen atoms in the neighbouring sugar residues. This effect is further discussed below.

The displacements for the signals of the α-proton and the two β-protons in the methyl glucoside residue of (**1**)—(**4**) are appreciable. All shifts are positive, *i.e.* downfield, and the largest, 0.19 p.p.m., is that for 4-H in the α-D-fucosides (**2**) and (**4**). In the corresponding α-L-fucosides, the shift for 2-H and 4-H is reversed. In the α-fucosyl groups of (**1**)—(**4**), small downfield shifts are obtained for most protons. The largest shift occurs for 5′-H (0.12 p.p.m.). The smaller shift of the anomeric proton, 1^1-H, (0—0.05 p.p.m.) is of structural significance as discussed later. Similar effects on 5′-H are obtained for the rhamnosyl group in compounds (**5**) and (**6**) and a downfield shift for the equatorial 2′-H (~0.12 p.p.m.) is also observed. The anomeric proton signal is shifted somewhat upfield in (**5**) and downfield in (**6**). In the methyl glucoside residue, smaller displacements are observed for 2-H in (**5**) and 4-H in (**6**) than for corresponding protons in the α-fucopyranosides (**1**)—(**4**).

The anomeric proton in the glycosyl residue of compound (**7**), in which the aglycon is galactose, appears at a chemical shift upfield to that observed for the corresponding proton in (**1**). A possible explanation for this is given by the HSEA-calculations described in the next section. The signal for 5′-H in (**7**) is unaffected, but in compound (**8**) it is shifted almost as much as in compounds (**1**)—(**4**). For the signals of the α- and β-protons in the methyl galactoside residue of compound (**7**) downfield shifts are observed for all three protons, small for 3-H and 4-H and larger, 0.19 p.p.m. for 2-H. The reverse, a small shift for 2-H and 3-H and a larger shift for 4-H, is observed for compound (**8**).

For symmetry reasons discussed above and indicated in Figure 1, one would expect similar substituent shifts in the pair (**7**) and (**10**) as compared to the pair (**8**) and (**9**), and this was also observed.

Correlations with Theoretical Calculations.—The minimum energy conformatons of (**1**), (**2**), (**7**), and (**8**) are shown in Figure 2, the energy plots of the same compounds in Figure 3 and φ/ψ-values together with short inter-atomic distances for all compounds are given in Table 3.

The energy plots and the minimum energy conformation were obtained as described in the Experimental section using

the HSEA-program. In this approach only van der Waals-interactions together with the exo-anomeric effect are evaluated and the rigid-body assumption is made, *i.e.* an atom in a monomer is fixed relative to the other atoms in the monomer.

The general shape of the energy wells is ellipsoid rather than circular and shows a somewhat larger degree of freedom for ψ than for φ. The maps for compounds (**1**) and (**2**) are almost mirror images. The flexibility is rather large at a level of 8.2 kJ (2.0 kcal) above that of the minimum energy molecule and is approximately 60° for φ and 80° for ψ. A prerequisite for taking the minimum energy conformation as a weighted average is that the energy well is symmetrical. This is only fulfilled to some extent as the increase in energy is larger on going to high φ, low ψ in (**1**) and (**7**) and to the opposite direction in (**2**) and (**8**). The degrees of freedom are approximately the same for (**7**) and (**8**) as for (**1**) and (**2**). Rotational freedom is, however, also a function of τ, the glycosidic bond angle which has been set to 117°, a relatively large value compared to those found in crystal structures. On decrease of τ, the calculated energy increases but the values of φ and ψ for the minimum energy molecule remain constant.[8] On comparison of the maps for (**1**) and (**7**), the effect of an axial hydroxyl group on a β-carbon in the methyl glycoside residue can be observed as a shift of the position of high energy conformers. Thus for compound (**1**) such conformers are obtained on going to high φ, low ψ. In compound (**7**), more of this space is accessible. An explanation for this is that the equatorial hydroxyl group 2-OH in compound (**1**) has been exchanged for a hydrogen atom [2-H in (**7**)], thereby causing less interaction on 3-substitution with an α-L-fucosyl group. This effect is not observed when an α-D-fucosyl group is substituted to the D-glycoside residue.

Conformational dependence of ^{13}C n.m.r. chemical shifts has been observed.[3,23] Bock *et al.*[24] have shown that there is some correlation between the induced chemical shift differences of C-1 for α-D-gluco- and α-D-galactopyranosyl residues in oligosaccharides and the ψ-angle. For low values of ψ, a low value of the chemical shift is observed going from 94.0 p.p.m. at ψ = −50° to 102.3 p.p.m. at ψ = −8°. Such a correlation is also observed for the aglycon carbon. It was suggested that a similar dependence should be valid for other glycosides. In agreement with the assumption, the ψ-values for compounds (**8**), (**2**), and (**10**) are −30°, −20°, and 5°, and the corresponding chemical shifts 96.3, 100.3, and 101.6 p.p.m., respectively. The corresponding disaccharides in the L-series also have such a dependence, and compounds (**9**), (**1**), and (**7**) have ψ-values of 30°, 15°, and −5° and C-1′ chemical shifts at δ 96.9, 100.4, and 101.5 p.p.m., respectively.

Recently Lipkind and Kochetkov[25] presented conformational analyses of some 1,3-linked disaccharides using theoretical calculations and measurements of the nuclear Overhauser effect obtained by saturation of the anomeric proton. A large population of conformers of low energy and short distances between 1′-H and 4-H or 1′-H and 2-H was observed for disaccharides with the same stereochemistry around the glycosidic bond as that in compounds (**8**) or (**9**), respectively. This observation suggested that the steric γ-gauche effect was the reason for the strong upfield shift of signals for C-4 and C-2, respectively, and for the corresponding small glycosidation shift of C-1′ in these disaccharides. Similar short distances could also be observed in the minimum energy conformation of compounds (**8**) and (**9**) (Table 3) and in conformers with low energy (Figure 3).

From the molecular models of compounds (**1**) and (**2**) in their minimum energy conformation, a short distance (~2.5 Å) between 5′-H and either O-4 as in (**1**) or O-2, as in (**2**), is observed. This is also observed for (**5**) and (**6**). The short distance may be responsible for the downfield shift observed in all compounds except compounds (**7**) and (**10**). From the model of (**7**) one can observe that in these compounds, 5′-H opposes a hydrogen and not an oxygen (Table 3).

Recent studies[22,26] have shown that the signal of an anomeric proton which is close to an oxygen is shifted downfield. We have also observed that proximity with protons causes an upfield shift.[26] Compounds (**1**)—(**7**), and (**10**) belong to the former group and (**8**) and (**9**) to the latter. In the former group, the shifts vary between −0.04 and 0.05 p.p.m. The upfield shifts of 1′-H may be caused by the short distance to 3-H. For compounds (**8**) and (**9**), the corresponding values are −0.14 and −0.17 p.p.m.

Conclusion

From the investigation of the disaccharide glycosides (**1**)—(**10**) it is concluded that a typical set of substituent shifts in ^{1}H and ^{13}C n.m.r. spectroscopy is obtained on glycosylation at the 3-position in a sugar residue. The shifts depend upon the stereochemistry around the glycosidic linkage and, to only a small extent, upon the anomeric configuration of the methyl glycoside residue. It should therefore be possible to calculate chemical shift differences for disaccharide residues which are similar in these respects. The results make up part of the material that will be used for the construction of a computer program, CASPER,[27] by which spectra of oligosaccharides and polysaccharides containing oligosaccharide repeating units may be simulated.

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