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A nuclear magnetic resonance spectroscopic and conformational study of eight pseudo-trehaloses (D-glucopyranosyl 5a-carba-D- and -L-glucopyranosides)

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ABSTRACT

N.m.r. data (^1H and ^{13}C) are presented for eight pseudo-trehalose derivatives in which the D-glucopyranosyl moiety is α or β and the 5a-carbaglucopyranoside moiety is α-D, β-D, α-L, or β-L. The differences in the chemical shifts and the n.O.e. effects have been correlated with the preferred conformations estimated from empirical force-field calculations (HSEA), which have been used to calculate the average parameters over the whole energy surface. Of the four α-D-glucopyranosyl derivatives, only that with a 5a-carba-β-D-glucopyranoside moiety (**3**) was a substrate for glucoamylase.

INTRODUCTION

Trehalose is widely distributed in Nature and plays an important role as an energy source as well as a carbohydrate reserve in insects[1]. Trehalose and its derivatives have been the target for systematic studies of their properties towards trehalase in order to determine the mechanism of the hydrolysis by this enzyme[2,3]. Furthermore, the conformational preferences in solution have been studied both by empirical calculations and n.m.r. spectroscopy[4–6] for both symmetrical and unsymmetrical derivatives of trehalose.

Recently, all eight isomers (**1–8**) of D-glucopyranosyl 5a-carba-D- or -L-glucopyranoside with each moiety α or β (pseudo-trehaloses) have been synthesised and characterised[7]. These compounds represent a unique series of structures which allow a systematic study of the interactions of the monosaccharide units that are important in the assessment of the conformation of oligosaccharides in solution as studied by n.m.r. spectroscopy and computer modelling. There have been several other attempts to address this problem[8–10] and the present data supplement the findings of these investigations. A detailed n.m.r. analysis (both ^1H and ^{13}C) of **1–8** is now reported together

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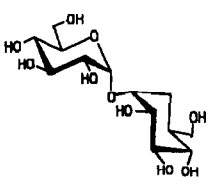
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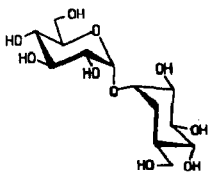
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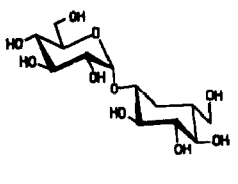
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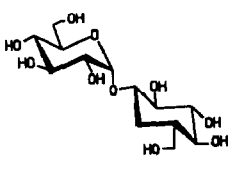
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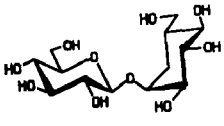
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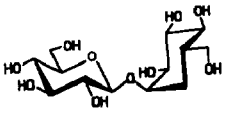
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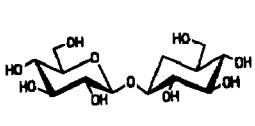
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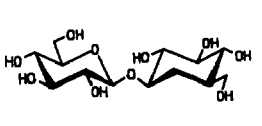
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with a correlation of the results with simple molecular modelling calculations. Furthermore, the α-glucosides **1–4** have been tested as substrates for glucoamylase[11].

RESULTS AND DISCUSSION

The octa-acetates of **1–8** were available from earlier synthesis work[7] and the ^{13}C-n.m.r. data are shown in Table I. The ^{1}H-n.m.r. data[7] have been confirmed by measurements at 500 MHz (not shown). *O*-Deacetylation of the octa-acetates was

TABLE I

^{13}C-N.m.r. chemical shift data (δ, p.p.m.) for the octa-acetates of **1–8**

Octa-acetate of	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	
1 αD,αL	96.12	70.38	69.56	68.71	67.62	61.86	
2 αD,αD	94.05	70.74	69.97	68.24	67.57	61.53	
3 αD,βD	95.77	70.55	69.48	70.35	67.69	61.95	
4 αD,βL	93.90	70.60	69.70	70.60	67.97	61.34	
5 βD,αD	101.59	70.97	72.22	68.37	71.77	61.72	
6 βD,βL	99.48	71.38	72.67	68.07	71.83	61.85	
7 βD,βL	100.74	70.85	72.59	68.23	71.77	61.59	
8 βD,βD	99.01	71.44	72.62	67.98	71.67	61.70	
	C-1'	*C-2'*	*C-3'*	*C-4'*	*C-5'*	*C-6'*	*C-7'*
1 αD,αL	72.28	73.52	71.52	70.99	34.47	62.76	30.18
2 αD,αD	72.78	72.33	71.40	71.29	34.35	62.88	27.90
3 αD,βD	74.17	74.66	73.39	68.48	36.41	62.63	31.01
4 αD,βL	74.46	73.53	73.27	68.08	36.10	62.82	28.90
5 βD,αD	74.43	73.70	71.33	70.86	34.30	62.73	30.44
6 βD,αL	73.14	72.67	71.05	71.35	34.28	63.18	28.67
7 βD,βL	75.86	74.48	73.18	70.48	36.34	62.66	31.06
8 βD,βD	76.56	72.92	72.88	70.76	36.13	62.90	29.51

accomplished in the usual fashion to give **1–8**, which were characterised by their ^{1}H- (500 MHz) and ^{13}C-n.m.r. (125.77 MHz) data (Tables II and III, respectively) obtained for solutions in D_2O at 27°. The assignments were based on homonuclear COSY, relayed COSY, and carbon–proton shift correlation experiments.

TABLE II

^{1}H-N.m.r. data for **1–8** [δ (p.p.m.)]

Compound	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6a*	*H-6b*
1 αD,αL	5.10	3.55	3.74	3.42	3.74	3.83	3.76
2 αD,αD	5.03	3.58	3.83	3.43	3.93	3.85	3.77
3 αD,βD	5.18	3.54	3.72	3.40	3.77	3.86	3.75
4 αD,βL	5.09	3.57	3.76	3.45	3.94	3.85	3.78
5 βD,αD	4.60	3.35	3.51	3.38	3.47	3.89	3.71
6 βD,αL	4.50	3.33	3.51	3.41	3.46	3.92	3.74
7 βD,βL	4.68	3.32	3.52	3.39	3.48	3.92	3.72
8 βD,βD	4.57	3.30	3.51	3.41	3.46	3.92	3.73
	J (*Hz*)						
	1,2	*2,3*	*3,4*	*4,5*	*5,6b*	*5,6a*	*6a,6b*
1 αD,αL	3.8	10.0	9.2	9.7	5.1	2.1	12.3
2 αD,αD	3.8	10.0	9.1	10.1	5.2	2.3	12.3
3 αD,βD	3.9	10.0	9.3	9.6	5.5	1.9	12.1
4 αD,βL	3.7	9.8	9.2	10.1	5.2	2.1	12.2
5 βD,αD	8.0	9.5	9.0	9.9	6.0	2.3	12.4
6 βD,αL	7.9	9.4	8.8	9.6	5.8	2.2	12.3
7 βD,βL	8.0	9.5	9.2	9.7	6.0	2.3	12.4
8 βD,βD	8.0	9.4	9.1	9.8	5.9	2.2	12.3

Compound	*H-1'*	*H-2'*	*H-3'*	*H-4'*	*H-5'*	*H-6'a*	*H-6'b*	*H-7'*eq	*H-7'*ax
1 αD,αL	4.08	3.49	3.67	3.31	1.90	3.74	3.66	2.09	1.44
1 αD,αD	4.11	3.54	3.72	3.32	1.91	3.75	3.67	2.11	1.32
3 αD,βD	3.62	3.45	3.32	3.31	1.63	3.75	3.65	2.17	1.41
4 αD,βL	3.64	3.41	3.34	3.34	1.59	3.78	3.67	2.19	1.21
5 βD,αL	4.18	3.49	3.65	3.30	1.96	3.74	3.65	2.18	1.41
6 βD,αL	4.26	3.50	3.63	3.33	1.95	3.74	3.69	2.08	1.37
7 βD,βL	3.75	3.44	3.34	3.31	1.64	3.77	3.65	2.23	1.35
8 βD,βD	3.79	3.40	3.33	3.34	1.60	3.78	3.67	2.16	1.30

	J (*Hz*)										
	1',2'	*1',7'*eq	*1',7'*ax	*2',3'*	*3',4'*	*4',5'*	*5',6'*b	*5',6'*a	*6',6'*	*5',7'*eq	*5',7'*ax	*7',7'*
1 αD,αL	3.2	3.7	2.1	10.0	9.1	10.8	5.8	3.3	11.3	3.7	13.5	14.7
2 αD,αD	3.2	3.7	1.9	10.0	9.0	10.8	6.0	3.6	11.2	3.7	13.2	14.9
3 αD,βD	9.3	4.6	11.7	9.3			6.0	3.5	11.2	3.8	13.0	13.0
4 αD,βL	9.3	4.5	11.7	9.1			6.0	3.5	11.2	3.8	12.6	13.0
5 βD,αD	3.2	3.7	2.0	10.0	9.1	10.9	6.1	3.6	11.2	3.7	13.3	14.8
6 βD,αL	3.4	3.8	1.9	10.0	9.2	10.6	5.8	3.6	11.2	3.8	13.2	14.9
7 βD,βL	9.3	4.7	11.9	9.3			6.0	3.6	11.2	3.7	13.0	13.1
8 βD,βD	9.1	4.7	11.6	9.3			6.0	3.8	11.2	3.6	13.0	13.1

TABLE III

^{13}C-N.m.r. data for **1–8** (δ, p.p.m.)

Compound	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
1 αD,αL	101.86	72.75	73.76	70.39	73.53	61.29
2 αD,αD	95.53	72.23	73.56	70.61	72.50	61.35
3 αD,βD	100.75	72.52	73.74	70.50	72.98	61.47
4 αD,βL	95.80	71.97	73.70	70.34	72.46	61.26
5 βD,αD	104.69	74.29	76.46	70.51	76.74	61.61
6 βD,αL	100.66	73.65	76.50	70.57	76.62	61.56
7 βD,βL	104.11	74.31	76.45	70.46	76.74	61.54
8 βD,βD	101.02	73.75	76.54	70.50	76.72	61.57

	C-1′	*C-2′*	*C-3′*	*C-4′*	*C-5′*	*C-6′*	*C-7′*
1 αD,αL	80.49	74.93	75.76	73.65	39.81	62.93	30.36
2 αD,αD	74.01	74.19	75.36	74.08	38.76	63.04	26.55
3 αD,βD	81.00	76.90	77.36	73.15	40.79	62.91	31.51
4 αD,βL	76.40	75.88	77.58	73.30	40.49	62.91	28.61
5 βD,αD	80.67	74.76	75.62	73.84	39.09	63.10	30.35
6 βD,αL	76.68	73.84	75.71	73.75	38.77	63.02	27.93
7 βD,βL	82.09	76.74	77.36	73.12	40.73	62.89	31.76
8 βD,βD	79.40	75.79	77.59	73.13	40.55	62.87	29.73

The n.m.r. data confirmed the structures of all of the compounds **1–8** discussed previously[7], except for **3** and **4**. The chemical shifts of the resonances of anomeric and aglyconic carbon atoms in these compounds showed that the structures assigned on the basis of differences in optical rotation of pairs of isomers have to be interchanged. This conclusion was confirmed by acetolysis of the octa-acetate of **3** (17B in ref. 7), which gave 5a-carba-β-D-glucopyranose penta-acetate, $[\alpha]_D$ +13° (lit.[12] $[\alpha]_D$ +13.8°). In the

TABLE IV

^{13}C Glycosylation shifts[a] (p.p.m.) and minimum-energy conformations for **1–8**

Compound	*C′-1*[b]	*C-1*	*C-1′*	*C-2*	*C-2′*	*C-7′*	*Φ*(°)	*Ψ*(°)	*C-1′–C-1*
1 αD,αL	5.9	9.0	10.9	0.3	0.3	0.8	−48	−9	1.9
2 αD,αD	3.9	2.6	4.4	−0.3	−0.4	−3.0	−51	−34	1.8
3 αD,βD	5.6	7.9	9.2	0.0	−0.8	−0.9	−50	−13	1.3
4 αD,βL	3.7	2.9	4.6	−0.5	−1.8	−3.8	−53	−35	1.7
5 βD,αD	6.0	8.0	11.1	−0.8	0.2	0.8	57	−13	3.1
6 βD,αL	3.9	4.0	7.1	−1.5	−0.8	−1.7	52	27	3.1
7 βD,βL	5.1	7.4	10.3	−0.8	−0.1	−0.6	59	−12	2.9
8 βD,αD	3.4	4.3	7.6	−1.3	−1.9	−2.7	53	20	3.3

[a] Chemical shifts for **1–8** less that for D-glucose taken from ref. 28 or pseudo-glucose taken from ref. 24.
[b] Chemical shifts for the octa-acetates of **1–8** less that for 2,3,4,6-tetra-*O*-acetyl-α- and -β-D-glucopyranose taken from ref. 29.

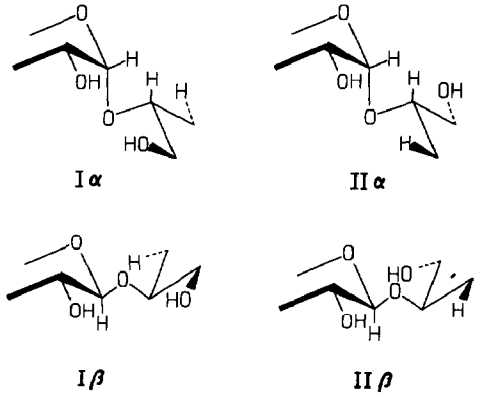
pairs of isomers **3,4** and **7,8**, the DL isomers are more dextrorotatory than the DD isomers[7], although 5a-carba-β-D-glucose is dextrorotary[12]. The relative optical rotations are inverted[7] for the octa-acetates of **7** and **8**, but not for those of **3** and **4**.

The conformational preferences for **1–8** were calculated using the HSEA[13,14] approach, and the results are shown in Table IV. The angles Φ and Ψ are defined as H-1–C-1–O-1–C-1′ and C-1–O-1–C-1′–H-1′, respectively. The isoenergy contour maps for **1–8** are shown in Fig. 1 and there is no significant difference when the absolute configuration of the aglycon is changed, except that the Ψ for **2, 4, 6**, and **8** is larger than for the corresponding isomers **1, 3, 5**, and **7**.

Furthermore, the flexibility in terms of Ψ is greater for all compounds (Fig. 1) than that of Φ, in accordance with the exo-anomeric effect[13]. The above results are in good agreement with the conformation recently determined by X-ray diffraction of leucrose[15], a disaccharide which has the same interactions as in **1**. The reported Φ/Ψ values[15] 68.8° and −93.9°, which correspond to −51.2° and 26.1° for **1**, compare well with those (−48° and −9°) calculated (Table IV). The isoenergy contour diagram (Fig. 1) shows that the change in Ψ from −9° to 26° requires <1 kcal/mol.

^{13}C-N.m.r. data. — Selected glycosylation shifts for the ^{13}C resonances of carbons close to the site of substitution are also shown in Table IV. the αD,αL compound (**1**), and those (**3, 5**, and **7**) that differ by an even number of structural factors (change of α to β or D to L) have large anomeric and aglyconic glycosylation shifts (7.4–9.0 and 9.2–11.1 p.p.m, respectively). The relative arrangement of atoms around the glycosidic linkage in this group of compounds is Iα for **1** and **3**, and Iβ for **5** and **7** (Scheme 1).

On the other hand, the αD,αD compound (**2**) and those (**4, 6** and **8**) that differ by an even number of structural factors have small anomeric and aglyconic glycosylation shifts (2.6–4.3 and 4.4–7.6 p.p.m., respectively). The distribution of atoms around the glycosidic linkage in this group of compounds is IIα for **2** and **4**, and IIβ for **6** and **8** (Scheme 1).



Scheme 1. Definition of structures type I and II, indicating the atoms responsible for the strongest interactions involved in the conformational preferences for compounds **1–8**.

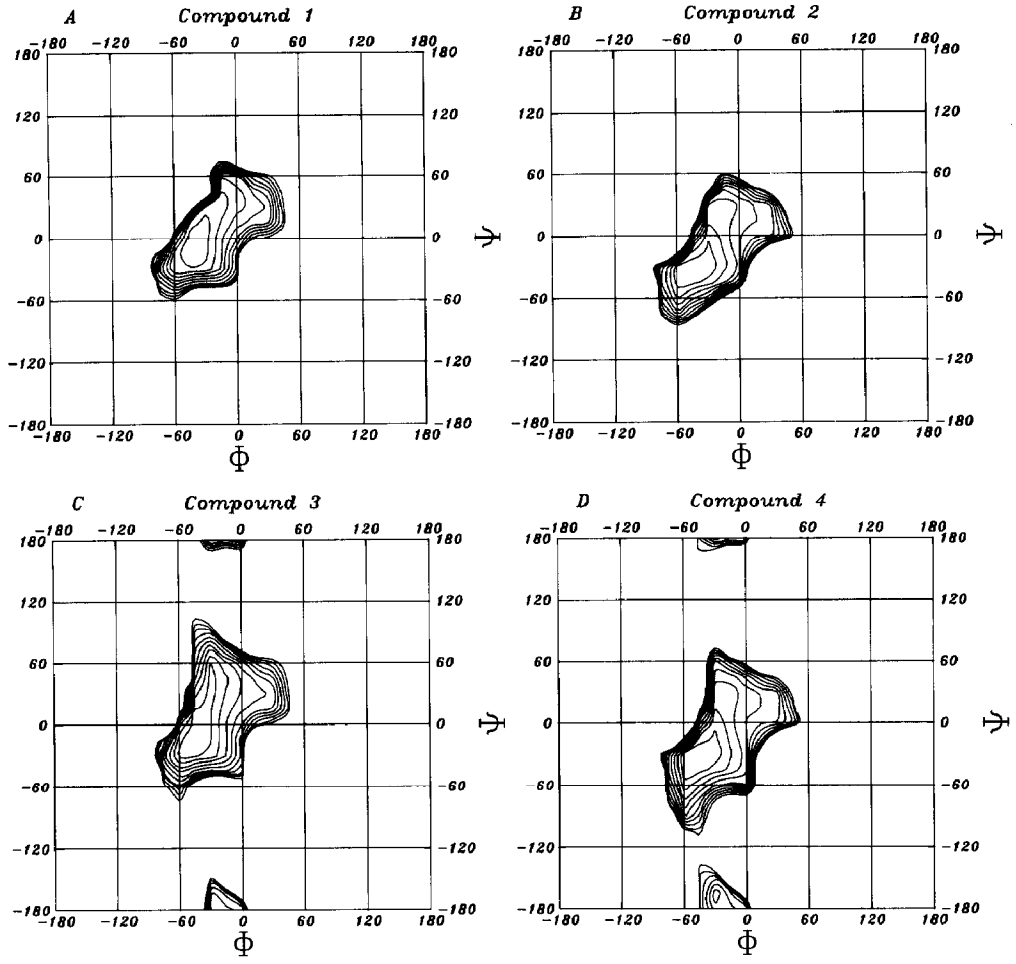
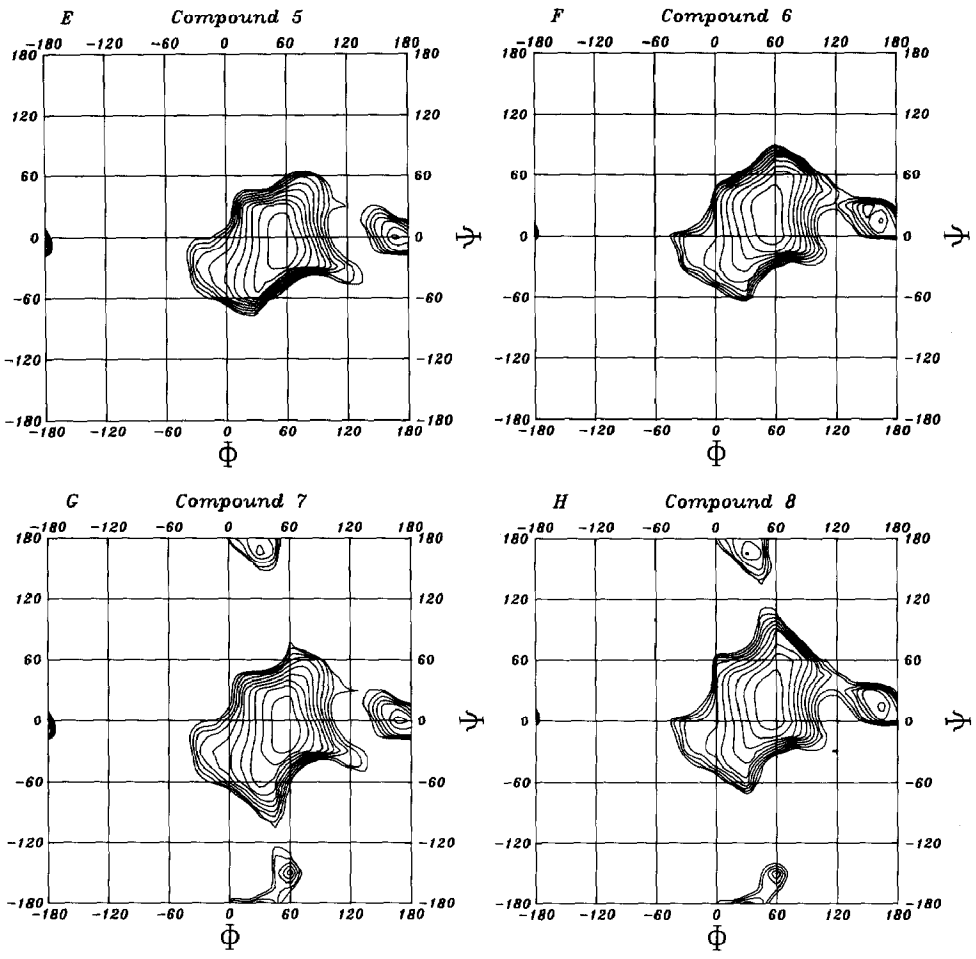


Fig. 1. Isoenergy contour diagrams (10 kcal limit, in 1 kcal/mol steps from the minimum) of the conformational flexibility for **1–8** in A–H, respectively.

The arrangement of structures Iα and Iβ can be considered as mirror images through a plane defined by O-5, C-1, and C-2, which also incudes O-2 and one of the lone-pairs of O-5. Therefore, it is expected that the conformational preferences will result in similar values of Φ and Ψ, H-1,1′ and H-1,HO-2′ distances, and glycosylation shifts. In fact, this expectation proved to be true for the glycosylation shifts (compare **1** with **5**, and **3** with **7**).

On the other hand, Φ and Ψ for each pair of compounds within each subtype Iα (**1** and **3**), Iβ (**5** and **7**), IIα (**2** and **4**), and IIβ (**6** and **8**) are nearly identical; hence, the conformational behaviour of an oligosaccharide is governed mainly by the equatorial substituents β to the glycosylated OH group of the aglycon. For example, the Φ/Ψ values reported for 2-*O*-α-D-glucopyranosyl-α-D-glucopyranoside[8] (−50°, −35°) and methyl 3-*O*-α-D-glucopyranosyl-α-D-galactopyranoside[10] (−50°, −34°) are similar to



those of **2** and **4**, and show that the axial OH or OMe β to the glycosylated OH does not exert a significant influence on the conformational preferences. Similarly, the Φ/Ψ values reported for methyl 2-*O*-β-D-glucopyranosyl-α-D-glucopyranoside[8] ($+60°$, $-15°$) and methyl 3-*O*-α-D-glucopyranosyl-α-D-galactopyranoside[10] ($+59°$, $-12°$) are nearly identical to those of **5** and **7** since they can be regarded as compounds of type Iβ.

Small up- or down-field glycosylation shifts are observed for C-2, C-2', and C-7' (Table IV). However, for each diastereoisomeric pair ($\alpha\alpha$, $\alpha\beta$, $\beta\alpha$, and $\beta\beta$), C-1, C-1', C-2, C-2', and C-7' are more deshielded for compounds of type I than for compounds of type II (Table V). The differences in chemical shifts (p.p.m.) are 2.7–6.5 for C-1,1', 0.5–0.6 for C-2, 0.7–1.0 for C-2', and 2.0–3.8 for C-7'. The differences for pairs of diastereoisomers are due most likely to the difference in interatomic effects and in the orientation of the lone pairs of the glycosylated oxygen[8], and, in contrast to direct glycosylation shifts, the indirect effects through bonds appear to be compensated.

TABLE V

^{13}C-Chemical shift differences (p.p.m.) for pairs of diastereoisomers $\alpha\alpha$, $\alpha\beta$, $\beta\alpha$, and $\beta\beta$ for **1–8**

Compound	**1–8**					*Octa-acetates of* **1–8**				
	C-1	*C-1′*	*C-2*	*C-2′*	*C-7′*	*C-1*	*C-1′*	*C-2*	*C-2′*	*C-7′*
1,2 $\alpha\alpha$	6.3	6.5	0.5	0.7	3.8	2.1	−0.5	−0.4	1.2	2.3
3,4 $\alpha\beta$	5.0	4.6	0.6	1.0	2.9	1.9	−0.3	−0.1	1.1	2.1
5,6 $\beta\alpha$	4.0	4.0	0.6	0.9	2.4	2.1	1.3	−0.4	1.0	1.8
7,8 $\beta\beta$	3.1	2.7	0.6	1.0	2.0	1.7	−0.7	−0.6	1.6	1.6

The compounds (type I: **1**, **3**, **5**, and **7**) with large anomeric and aglycon glycosylation shifts have small absolute values of $|\Psi|$ (9–13°), and those (type II: **2**, **4**, **6**, or **8**) with small glycosylation shifts have larger values of $|\Psi|$ (20–35°). These correlations are in agreement with the published dependence[16] for disaccharides containing an α-D-gluco- or α-D-galacto-pyranosyl non-reducing unit. However, the change in chemical shift of the resonances for C-1 (or C-1′) for pairs of diastereoisomers with change of Ψ is ~2.4 p.p.m./10° for α-glucosides (**1–4**) and ~1 p.p.m./10° for β-glucosides (**5–8**).

Glycosylation shifts for the resonances of anomeric and aglyconic carbon atoms can also be correlated with the distance between the protons attached to the glycosidic and aglyconic carbon atoms[16,17]. For compounds of type I, whith high glycosylation shifts, the H-1,1′ weighted[18,19] distances are ~2.32 Å, whereas, for compounds of type II, with small glycosylation shifts, the distances are ~2.55 Å. The change in chemical shift for the resonance of C-1 (or C-1′) for pairs of diastereoisomers with the change of the H-1,1′ distances is ~2 p.p.m./0.1 Å for α- and β-glucosides.

For compounds of type II, the H-1,7′*eq* weighted distance is small (~2.35 Å for α-glycosides, and ~2.68 Å for β-glycosides). This situation can be related with a substantial upfield glycosylation shift (−3.0 to −3.8 p.p.m. for α-glucosides and −1.7 to −2.7 p.p.m. for β-glucosides) of the C-7′ resonance. This shift may be explained by the γ-gauche-effect that arises when two protons separated by 5 bonds interact with each other, resulting in an upfield shift of the resonances of the respective carbon atoms. In fact, for compounds of type II, the downfield shift of the resonance of C-1 decreases as the H-1,7′*eq* distance decreases[10] (Tables IV and VI). However, this upfield contribution to the resonance for C-1 for compounds of type II would have to be added[17] to that coming from the larger H-1,1′ distances and this would make the difference in the glycosylation shift between the resonances of C-1 and C-1′ higher when C-7′ is shielded. However, this is not true, as these differences (Table IV) seem to depend only on the anomeric configuration (1.3–1.9 p.p.m. for α-glucosides and 2.9–3.3 p.p.m. for β-glucosides). Therefore, the dependence of the glycosylation shifts with torsion angles and the hybridisation of carbon atoms discussed above is more likely.

The glycosylation shifts discussed above for **1** (Table IV) compare well with those for leucrose[20] (C-1, 8.6; C-5′, 10.2; C-2, 0.7; C-4′, 0.7; and C-6′, −0.7 p.p.m.; *cf.* 9.0, 10.9, 0.3, 0.3, and 0.8 p.p.m. for **1**). Even though the conformational preferences in solution

TABLE VI

Selected interatomic distances from the minimum energy conformations of **1–8** and ensemble average distances calculated from the complete conformational energy surface

Compound	*H-1*	*M*[a]	*A*[b]	*H-5*	*M*	*A*	*O-5*	*M*	*A*
1 αD,αL	H-1′	2.30	2.26	H-7′*eq*	2.33	2.39	H-1′	2.68	2.81
	O-2	2.77	2.99				H-7′*eq*	3.30	3.18
2 αD,αD	H-1′	2.60	2.53	O-2′	2.50	2.61	H-1′	2.55	2.65
	H-7′*eq*	2.23	2.35						
3 αD,βD	H-1′	2.37	2.28	H-7′*eq*	2.25	2.50	H-1′	2.61	2.87
	O-2	2.67	3.15				H-7′*eq*	3.40	3.08
4 αD,βL	H-1′	2.64	2.57	O-2′	2.51	2.63	H-1′	2.52	2.62
	H-7′*eq*	2.25	2.36						
5 βD,αD	H-1′	2.33	2.37				H-1′	2.63	2.65
	O-2	3.36	3.21				H-7′*eq*	2.61	2.82
6 βD,αL	H-1′	2.56	2.54				H-1′	2.45	2.50
	H-7′*eq*	2.47	2.65						
7 βD,βL	H-1′	2.34	2.38				H-1′	2.63	2.64
	O-2′	3.36	3.22				H-7′*eq*	2.63	2.84
8 βD,βD	H-1′	2.51	2.54				H-1′	2.49	2.50
	H-7′*eq*	2.63	2.70						

[a] Value for the minimum energy conformation. [b] Values calculated from ensemble average conformational map.

are not necessarily the same as in the solid state, the agreement between these results supports the conclusions presented here about the conformational preferences for pseudo-trehaloses.

The glycosylation shifts for the resonances of the anomeric carbon atoms in the octa-acetate (Table IV) are larger for compounds of type I (6.0–5.1 p.p.m. for the octa-acetates of **1**, **3**, **5**, and **7**) than for compounds of type II (3.9–3.4 p.p.m. for the octa-acetates of **2**, **4**, **6**, and **8**). These results indicate that it is possible to assign the absolute configuration of the pseudo-glucose moiety on the basis of data for pseudo-trehalose octa-acetates. However, the differences in chemical shift for pairs of octa-acetate diastereoisomers are less consistent than those for the trehaloses (Table V), probably due to the contribution of AcO-2,2′ to the overall conformational properties[21].

^{1}H-N.m.r. data. — Differences in chemical shifts for the resonances of protons close to the glycosidic linkage relative to the corresponding values for glucose and pseudo-glucose are given in Table VII. The resonances of H-2,1′,2′,7′*eq* show downfield shifts, whereas those of H-1,5,7′*ax* show up- and down-field shifts. However, the differences in chemical shifts for pairs of diastereoisomers are clearly regular due to the analogy of structures within each relative arrangement for types Iα, IIα, Iβ, and IIβ.

A short distance between H-5 and O-2′ in **2** and **4** was observed (~2.62 Å) and is most likely responsible[13] for the downfield shifts observed for the H-5 resonance. On the other hand, the upfield shifts observed for the H-5 resonance in **1** and **3** might be explained by the short distance between H-5 and H-7′*eq* (~2.45 Å), as documented in other examples where proton resonances have been shifted upfield[10,22].

TABLE VII

^{1}H Glycosylation shifts[a] (p.p.m.) for **1–8** and differences in ^{1}H chemical shifts (p.p.m.) for pairs of diastereoisomers $\alpha\alpha$, $\alpha\beta$, $\beta\alpha$, and $\beta\beta$ for **1–8**

Compound	*H-1*	*H-2*	*H-5*	*H-1'*	*H-2'*	*H-7'*eq	*H-7'*ax
Glycosylation shifts							
1 αD,αL	−0.09	0.04	−0.08	−0.02	0.06	0.17	−0.03
2 αD,αD	−0.16	0.07	0.11	0.01	0.11	0.19	−0.15
3 αD,βD	−0.01	0.03	−0.05	0.04	0.21	0.16	0.13
4 αD,βL	−0.10	0.06	0.12	0.06	0.17	0.18	−0.07
5 βD,αD	−0.01	0.12	0.02	0.08	0.06	0.26	−0.06
6 βD,αL	−0.11	0.10	0.01	0.16	0.07	0.16	−0.10
7 βD,βL	0.07	0.09	0.03	0.17	0.20	0.22	0.07
8 βD,βD	−0.04	0.07	0.01	0.21	0.16	0.15	0.02
Differences in chemical shifts							
1,2 αα	0.07	−0.03	−0.19	−0.03	−0.05	−0.02	0.12
3,4 αβ	0.09	−0.03	−0.17	−0.02	0.04	−0.02	0.20
5,6 βα	0.10	0.02	0.01	−0.08	−0.01	0.07	0.04
7,8 ββ	0.11	0.02	0.02	−0.04	0.04	0.05	0.05

[a] ^{1}H-Chemical shifts for **1–8** less that for D-glucose from ref. 28 or for pseudo-glucose from ref. 24.

The anomeric protons H-1 are more shielded in compounds of type II (~0.10 p.p.m.) because they are close to H-7'*eq*, whereas, in compounds of type I, H-1 is close to O-2'. On the other hand, H-1' is more deshielded in compounds of type II because H-1' is closer to O-5 (~2.55 Å) than in structures of type I (~2.74 Å).

A short distance between H-7'*eq* and O-5 in **5** and **7** may explain the downfield shifts observed for the H-7'*eq* resonance compared to those for **6** and **8**. The H-7'*ax* resonance is shifted upfield in compounds of type II compared with those of type I, paralleling the upfield shift of the C-7' resonance in compounds of type II.

The observed n.O.e.'s for **1–8** are shown in Table VIII together with those calculated for the minimum energy conformation and for the weighted average conformation, which take into account all possible conformations with population derived from the relative energies[18]. A correlation time of 0.21×10^{-9} s has been used in order to obtain a good fit between observed and calculated intramolecular n.O.e.'s[19].

The changes in the calculated inter-ring n.O.e.'s from the minimum energy conformation to the weighted average, using a full matrix relaxation method[18,19,23] are in the direction expected from the corresponding interprotonic distances. The observed n.O.e.'s fit better with the calculated n.O.e.'s for the weighted conformation than with those for the minimum in almost every instance.

For **1–4** and **6**, the H-1,1' distances are smaller in the calculated weighted conformations compared to the minimum energy conformation corresponding to higher calculated n.O.e.'s. For **5**, **7**, and **8**, the H-1,1' distances are slightly larger in the calculated weighted conformation than in the minimum energy conformation with similar calculated n.O.e.'s.

TABLE VIII

Observed and calculated n.O.e. effects for **1–8** in the minimum energy conformation (M) and in the weighted ensemble average conformation (A)

Compound	*Proton*		*Intra-ring n.O.e.*				*Inter-ring n.O.e.*		
	Satd.	*Obs.*		*M*	*A*	*Obs.*		*M*	*A*
1-αD,αL	H-1	H-2	9.6	10.0	10.0	H-1′	9.9	6.7	7.8
	H-1′	H-2′	7.8	7.3	7.4	H-1	15.3	10.8	11.8
		H-7′*eq*	1.2	1.4	1.4				
		H-7′*ax*	1.8	1.6	1.6				
	H-7′*eq*	H-1′	3.3	3.4	3.2	H-1	1.1	−0.1	0.2
		H-7′*ax*	16.8	15.1	15.1	H-5	3.9	8.4	6.8
2-αD,αD	H-1	H-2	10.8	9.9	9.9	H-1′	4.6	3.5	4.8
		H-3	0.6	0.6	0.6	H-5′	0.9	0.5	0.5
		H-4	−0.4	−0.2	−0.2	H-7′*eq*	3.4	3.3	3.1
		H-5	0.5	0.5	0.5				
	H-1′	H-2′	6.8	7.4	7.4	H-1	5.1	3.7	5.2
		H-7′*eq*	0.8	1.3	1.3				
		H-7′*ax*	2.1	1.6	1.6				
	H-5′	H-1′	0.7	0.2	0.2	H-1	1.9	0.5	0.5
		H-3′, H-6′a	10.1	8.1	8.0				
		H-4′	1.9	1.5	1.6				
		H-6′b	1.5	3.0	3.0				
	H-7′*eq*	H-1′	4.0	3.5	3.2	H-1	8.2	9.4	8.3
		H-6′a	1.4	0.2	0.2				
		H-7′*ax*	16.4	15.1	15.1				
3-αD,βD	H-1	H-2	8.1	10.0	10.0	H-1′	7.4	6.1	7.7
		H-3	0.4	0.6	0.6	H-7′*ax*	0.9	0	0.1
	H-7′*eq*	H-1′, H-6′b	3.7	4.4	4.0	H-1	1.1	−0.2	0.5
		H-5′	4.3	4.5	4.5	H-5,H-6′a,	3.5	8.0	6.1
		H-7′*ax*	16.5	15.9	15.5				
	H-7′*ax*	H-1′, H-6′b	1.5	2.2	2.2	H-1	1.0	0.2	0
		H-2′	5.8	5.9	5.9	H-5,H-6′a,	1.3	−0.5	1.0
		H-4′	5.3	3.7	3.7				
		H-7′*eq*	13.0	14.2	14.5				
4-αD,βL	H-1	H-2	8.4	10.0	10.0	H-1′	4.6	3.3	4.5
						H-7′*eq*	3.0	3.2	3.0

Table continued overleaf.

TABLE VIII (continued)

Compound	*Proton*		*Intra-ring n.O.e.*				*Inter-ring n.O.e.*		
	Satd.	*Obs.*		*M*	*A*	*Obs.*		*M*	*A*
5-βD,αD	H-1	H-2	3.1	3.3	3.3	H-1′	7.3	6.4	6.3
		H-3	6.0	6.7	6.7	H-5′		0.1	0.1
		H-5	8.0	7.0	7.1	H-7′*eq*			0.1
						H-7′*ax*		−0.1	−0.1
	H-1′	H-2′	7.4	7.5	7.4	H-1	8.3	7.6	7.5
		H-5′		0.2	0.2				
		H-7′*eq*	1.1	1.6	1.6				
		H-7′*ax*	1.8	1.7	1.7				
	H-7′*eq*	H-1′	2.0	4.4	4.0	H-1	1.2	−0.2	0.5
		H-6′a	0.6	0.2	0.2				
		H-7′*ax*	10.4	15.9	15.5				
6-βD,αL	H-1	H-2	3.0	3.5	3.4	H-1′	4.0	4.1	4.8
		H-3,	12.3	13.7	13.7	H-5′		0.3	0.4
		H-5				H-7′*eq*	2.5	1.9	1.9
						H-7′*ax*		−0.2	−0.3
	H-1′	H-2′	6.4	7.5	7.5	H-1	4.8	3.9	4.5
		H-5′		0.2	0.2				
		H-7′*eq*	1.3	1.5	1.5				
		H-7′*ax*	1.6	1.6	1.6				
	H-7′*eq*	H-1′	3.1	3.9	3.7	H-1	4.0	4.7	4.5
	H-6′b+H-6′a[a]		3.1	0.2	0.2				
		H-7′*ax*	14.8	15.1	15.1				
7-βD,βL	H-1	H-2	1.5	3.5	3.4	H-1′	6.2	6.6	6.5
		H-3,	10.0	13.7	13.7	H-5′	−0.2	−0.1	−0.1
		H-5							
	H-7′*eq*	H-1′	3.0	4.5	4.5	H-1	0.6	0	0.1
		H-5′	2.1	4.5	4.5				
		H-7′*ax*	12.6	16.2	16.2				
8-βD,βD	H-1	H-2	3.6	3.5	3.5	H-1′	5.6	4.8	5.0
		H-3,	13.3	13.8	13.8	H-5′		0	−0.1
		H-5				H-7′*eq*	1.5	1.3	1.7
	H-7′*eq*	H-1′	4.8	4.8	4.7	H-1	4.0	3.3	4.1
		H-5′	3.7	4.5	4.5				
		H-7′*ax*	20.2	16.1	16.0				

[a] Off-resonance decoupling due to closely resonating signals.

The observed n.O.e.'s for H-1′ when H-1 was saturated, or for H-1 when H-1′ was saturated, fit better for the calculated ensemble average n.O.e.'s, although in general they are even bigger than expected for the weighted data.

For compounds of type I (**1**, **3**, **5**, and **7**), the observed enhancement of the H-1′ resonance when H-1 was saturated (9.9–6.2%) is higher than that observed for compounds of type II (5.6–4.0%). In the same way, the observed enhancements for the H-1 resonance when H-1′ was saturated are higher for compounds of type I (15.3–8.3%) than of type II (5.1–4.8%).

For **1** and **3**, the observed enhancement of the H-5 resonance when H-7′*eq* was saturated is smaller than calculated for the weighted form, but only half of that calculated for the minimum energy conformer in agreement with larger observed values than calculated n.O.e.'s between H-1 and H-1′.

For **2** and **4**, the enhancements of the H-7′*eq* resonance when H-1 was saturated and *vice versa* fit nicely the calculation ensemble average n.O.e. values.

Enzyme experiments. — Treatment[11] of **1–4** in acetate buffer at pH 4.75 with glucoamylase and following the reactions by ^{1}H-n.m.r. spectroscopy showed, in agreement with earlier observations[11], that only **3**, which has a structure that most closely resembles maltose, was hydrolysed. The finding confirms the importance of a proper three-dimensional arrangement of key polar hydroxyl groups in order for enzymic hydrolysis to occur.

EXPERIMENTAL

The numbering of atoms in the pseudo-glucose ring is as given in ref. 24.

Acetolysis of α-D-glycopyranosyl 5a-carba-β-D-glucopyranoside octa-acetate. — The title compound[7] (65 mg) was treated with 5:5:1 acetic acid-acetic anhydride–conc. sulfuric acid (2 mL) for 2 days at 60°. The mixture was neutralised with saturated aqueous sodium hydrogencarbonate and extracted with chloroform, and the extract was washed with water, dried (Na_2SO_4), and concentrated. The residue was eluted from a column of silica gel with ethyl acetate–hexane (1:4) to give a syrupy 5a-carba-β-D-glucopyranose penta-acetate (33 mg, 89%), m.p. 114° (from ether–light petroleum), $[\alpha]_D^{23}$ +13° (*c* 1.7, chloroform). The i.r. spectrum (chloroform) was superimposable on that of the authentic crystalline racemate [lit.[12] m.p. 115–116°, $[\alpha]_D^{23}$ +13.8° (*c* 1, chloroform)].

N.m.r. spectra. — A Bruker AM-500 spectrometer operating at 500 MHz was used for the ^{1}H-n.m.r. spectra on 0.1M solutions of **1–8** in D_2O at neutral pH and at 27° (internal acetone 2.22 p.p.m., DOH at 4.75 p.p.m.). A spectral width of 5 kHz, using 32k of computer memory giving a digital resolution of 0.3 Hz/pt., was used together with pulse angles of 10 μs (60°). COSY experiments were made using Bruker standard software, and the n.O.e. experiments were performed in the difference mode. The ^{13}C-n.m.r. spectra were recorded on the same spectrometer operating at 125.77 MHz (internal 1,4-dioxane 67.4 p.p.m.). A spectral width of 25 kHz, using a computer memory of 64k giving a digital resolution of 0.8 Hz/pt., was used together with a pulse angle of 5 μs (60°).

HSEA calculations. — These were performed[13] on an IBM PS/2 system model 80 with a 387 math-coprocessor. The calculation of the ensemble average n.O.e.'s was performed[23] on a TITAN (Ardent Computer Systems) computer as described[18,19]. The angles Φ and Ψ are defined as H-1–C-1–O-1–C-1′ and C-1–O-1–C-1′–H-1′, respectively. The co-ordinates for the α- and β-D-glucopyranosyl units were taken from the average X-ray structures calculated by Arnott and Scott[25], and the protons attached as described[26]. The pseudo-hexose units were constructed by bond modification of the above-mentioned glucose units, using the molecular modelling program Alchemy[27].

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