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SYNTHETIC RECEPTOR ANALOGUES: THE CONFORMATION OF METHYL 4-*O*-α-D-GALACTOPYRANOSYL-β-D-GALACTOPYRANOSIDE (METHYL β-D-GALABIOSIDE) AND RELATED DERIVATIVES, DETERMINED BY N.M.R. AND COMPUTATIONAL METHODS*

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ABSTRACT

The conformations of galabiose and its methyl and ethyl β-glycosides as well as the 3-deoxy, 3-*O*-methyl, 3-deoxy-3-*C*-methyl, 3-deoxy-3-*C*-ethyl, and 6-deoxy analogues were investigated by n.m.r. (^{1}H, ^{13}C, n.O.e.) and computational (HSEA) methods. A good correlation was found between the computational data and the n.m.r. data for aqueous solutions. The conformations in aqueous solution were similar, whereas crystalline galabiose or methyl β-D-galabioside in solution in methyl sulfoxide adopted different conformations that showed intramolecular hydrogen bonds (O-5′ · · · O-3 and O-2′ · · · O-6, respectively).

INTRODUCTION

Galabiose (4-*O*-α-D-galactopyranosyl-D-galactopyranose)-containing glycolipids define the P blood-group system[1] and function as specific ligands towards receptors of uropathogenic *E. coli*[2] and the *Shigella dysenteriae* toxin[3]. In addition, globotriaosyl ceramide was indicated to be a tumour antigen in connection with Burkitt's lymphoma[4].

A detailed knowledge of the conformation of these saccharides is important for an understanding of molecular recognition phenomena. Furthermore, such insight would make it possible to construct analogues with altered over-all hydrophilic/hydrophobic and hydrogen bonding, and, hence, binding characteristics (*cf.* the evaluation of the binding of blood-group antigen analogues with antibodies and lectins[5]). It is probably important that, in such analogues, the over-all conformation of the parent sugar is preserved in order to maintain the binding specificity towards the receptor.

*Part 2, For Part 1, see ref. 7a.

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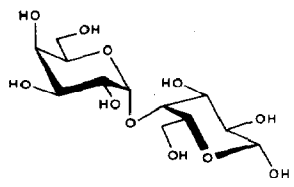
Determination of the crystal structure of β- (**1**) and α-galabiose[6] (**2**) revealed an intramolecular hydrogen bond between HO-3 of the reducing unit and the ring O-5′ of the non-reducing unit. In order to investigate the importance of this hydrogen bond for the conformation and biological activity of galabiose-containing oligosaccharides, derivatives of methyl β-D-galabioside were synthesised[7a] in which HO-3 was replaced by H (**5**), MeO (**6**), and CH_3 (**7**). Furthermore, derivatives with HO-3 replaced by CH_2CH_3 (**8**)[7b] and with HO-6 replaced by H (**9**)[7c,d] were also synthesised as well as 6*S*-deuterated methyl β-D-galabioside (**4**).



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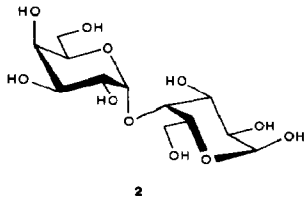

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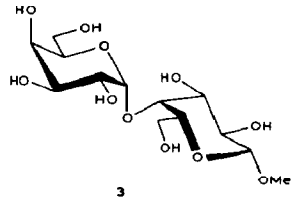

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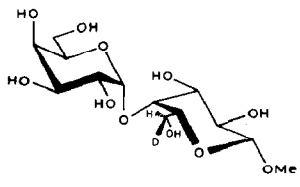

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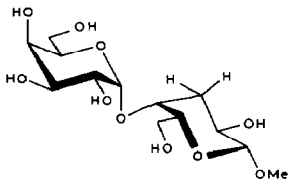

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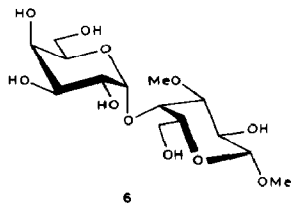

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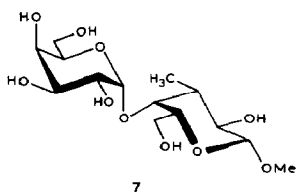

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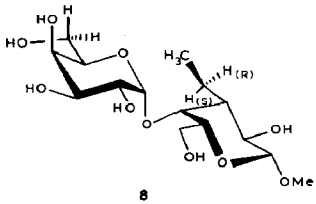

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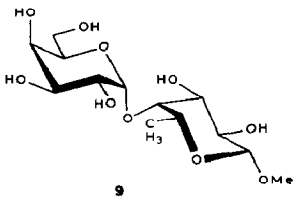

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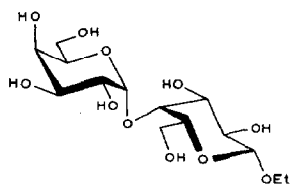

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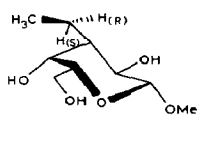

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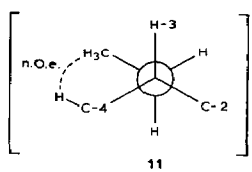

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Determination of the crystal structures of lactose[8], *N*-acetyl-lactosamine[9], and mannobiose[10] revealed intramolecular hydrogen bonds (HO-3 · · · O-5′), similar to that of galabiose[6]. Since the P^k-, P_1-, and P-antigen saccharides each contain both galabiose and lactose/*N*-acetyl-lactosamine units, it was anticipated[11a], and indicated by HSEA calculations[11b], that the conformational characteristics of the disaccharide portions should be transferred to the complete antigens.

Conformational analysis of oligosaccharides by n.m.r. spectroscopy and by computational methods is well established[12] and we now report on the conformational properties of galabiose (**1** and **2**), methyl β-D-galabioside (**3**), and its analogues (**5–10**) as revealed by ^{1}H- and ^{13}C-n.m.r. spectroscopy (including n.O.e. enhancements). The conformations were calculated, using the hard-sphere exo-anomeric (HSEA) approach, and compared with the experimental n.m.r. data as described previously[13]. The results were also compared with the data from the X-ray crystallographic investigation of galabiose[6].

The investigation of the biological activities of the galabiose analogues **5–9** will be reported elsewhere. The biological activities of some galabiose-containing di- and tri-saccharides have been reported[14].

RESULTS

The results of a conformational analysis using HSEA calculations[12a,13], with the GESA program[15], for **3** and **5–9** are shown in Table I (see Experimental for details).

Data from the X-ray analysis[6] of galabiose (**1** and **2**) are included in Table I. The conformation is substantially different from that resulting from the HSEA cal-

TABLE I

MINIMUM ENERGY CONFORMATIONS OF METHYL 4-*O*-α-D-GALACTOPYRANOSYL-β-D-GALACTOPYRANOSIDE AND ANALOGUES AS CALCULATED BY THE HSEA METHOD[12a,13]

Compound	$\phi_H{}^a/\psi_H{}^b$ (°)	$\omega_1{}^c$ (°)	$\omega_2{}^d$ (°)	*Calculated energy (kcal/mole)*
1,2[e]	−18/+35			0.0
3	−39/−15	63	68	−1.8
5	−46/−9	63	65	−2.6
6	I[f] −29/−27	−50	69	0.2
	II[f] −31/−25	170	69	−0.2
7	−32/−28	63	69	1.1
8	I[g] −16/−9	74	99	2.1
	II[g] −22/−18	176	88	2.3
9	−40/−14	62		−1.6

[a]ϕ_H = H-1′–C-1′–O-1′–C-4. [b]ψ_H = H-4–C-4–O-1′–C-1′. [c]ω_1 = O-5′–C-5′–C-6′–O-6′. [d]ω_2 = O-5–C-5–C-6–O-6. [e]Data from ref. 6. [f]The conformation of the OMe group is the same as determined for the CH_3CH_2 group of **8**. [g]Conformations I and II are approximately equally populated.

TABLE II

SELECTED INTER-ATOMIC DISTANCES IN THE CALCULATED MINIMUM-ENERGY CONFORMATION OF **3** AND **5–9**

Distances between atomic centers (Å)	*X-ray* (**1,2**)[a]	**3**	**5**	**6**[b]	**7**	**8**[c]	**9**	*Experimental support by n.m.r. data*[d]
H-1'---H-4	2.12	2.30	2.30	2.31	2.35	2.05	2.31	^{1}H-n.O.e. experiment
H-5'---O-3	3.41	2.39		2.75	2.74[e]	3.06[f]	2.38	^{1}H-n.m.r. deshielding observed
H-1'---O-3	2.77	4.25		4.33	4.43[e]	3.92[f]	4.26	^{1}H-n.m.r. deshielding not observed
O-2'---H-6(*R*)[g]	2.97	2.61	2.77	2.32	2.36	2.53		^{1}H-n.m.r. deshielding observed
H-1'---H-6(*R*)[g]	4.04	2.55[h]	2.62	2.33	2.22	2.30		^{1}H-n.O.e. experiment
O-5'---O-3	2.55	3.39		3.67	3.75[e]	3.38[f]	3.38	H–D exchange[i]
O-2'---O-6	3.52	4.66	4.87	4.34	4.38	4.03		H–D exchange[i]

[a]Data from ref. 6. [b]Data from conformer II; see Table I. [c]Data from conformer I; see Table I. [d]See underlined inter-atomic distances in each row. [e]Distance to the carbon atom in the CH_3 group. [f]Distance to the carbon atom in the CH_2 group. [g]With the CH_2OH group of the β-D-galactopyranosyl unit in the *gauche–trans* conformation. [h]^{1}H-n.O.e. observed for **4**. [i]Solution in $(CD_3)_2SO$ (*cf.* refs. 20–22).

culations (21° in ϕ and 50° in ψ). This difference warrants a more detailed analysis of the preferred solution conformation of **1** and **2** and its derivatives (**3, 5–9**) using n.m.r. parameters. Table II shows selected calculated H/H, H/O, and O/O interatomic distances, which were augmented by n.O.e. experiments, proton chemical shift deshielding, or hydrogen–deuterium exchange.

The ^{1}H- and ^{13}C-n.m.r. data for solutions of **1–11** in D_2O are recorded in Tables III and IV, respectively, and the n.O.e. data in Table V. The assignments of the ^{1}H resonances are based on ^{1}H-COSY and relayed COSY experiments which, together with the use of partially relaxed spectra, allowed a complete and unambiguous assignment of all chemical shifts and coupling constants (Table III). The assignments of the ^{13}C resonances are based on 1D correlated experiments (CHORTLE[16,17]), which gave a complete and unambiguous assignment of all the ^{13}C-n.m.r. chemical shifts (Table IV). The n.O.e.'s were measured in the difference mode using acetone-d_6 (5%) as the lock substances. This technique gives a good cancellation of unperturbed signals and allows measurements of small (~1%) n.O.e.'s with good accuracy (Table V).

Table VI shows a comparison between the observed (relative)[13a] n.O.e.'s (% of total) for **8** and the calculated (relative)[13a] enhancements for the two energy-minimised conformations (ratio 1:1; *cf.* Table I) found by HSEA calculations.

The chemical shifts of the ^{1}H and ^{13}C resonances for solutions of **3** and **9** in $(CD_3)_2SO$ are shown in Table VII.

DISCUSSION

Compounds **3** and **5–9** have similar conformations as calculated by the HSEA method[12a,13] (Table I), which differ significantly from that adopted by galabiose (**1** and **2**) in the crystal[6].

An analysis of the n.O.e.'s observed for **3–10** (Table V) shows a strong enhancement (6.6–11.6%) of the resonance of H-4 when H-1′ is saturated, together with a strong enhancement (10.1–15.0%) of the resonance of H-2′. These data require that H-1′ and H-4 be in close proximity (<2.4 Å separation distance). In both the structures in the crystal and in those calculated, conformations are adopted where this condition is fulfilled (Table II). The hydrogen atom H-5′ is strongly deshielded (~0.45 p.p.m.) in **1–4, 6, 9,** and **10** as compared tc H-5 in methyl α-D-galactopyranoside[18] or the 3-deoxy compound **5**. This change in chemical shift requires[13a] that H-5′ be in repulsive van der Waals interaction with O-3 (separation <2.7 Å). In the minimum-energy conformations obtained by the HSEA calculations, O-3 was found to be only 2.38–2.75 Å (Table II) away from H-5′, whereas, in the crystal of galabiose, the distance is 3.41 Å. Similarly, when HO-3 in methyl β-D-galabioside (**3**) is replaced by CH_3 (→**7**) or CH_2CH_3 (→**8**), the deshielding is decreased by ~0.25 p.p.m. If the crystal conformation for galabiose was preponderant, H-1′ would be expected to be deshielded by O-3. However, inspection of the data in Table III reveals that the chemical shift of the resonance of H-1′ varies only

TABLE III

^{1}H-N.M.R. DATA IN D_2O FOR DISACCHARIDES RELATED TO 4-*O*-α-D-GALACTOPYRANOSYL-D-GALACTOPYRANOSE

Compound[a]	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	*H-6'*	OMe
1								
N	4.95[b]	3.83	3.92	4.02	4.35	3.69	3.72	
	3.6[c]	10.0	3.2	0.8		6.4	6.4	
							12.3	
R	4.60	3.53	3.71	4.01	3.77	3.89	3.82	
	8.0	10.0	3.2	0.8		8.0	4.8	
							11.2	
2								
N	4.96	3.83	3.93	4.02	4.32	3.69	3.72	
	3.6	10.0	3.2	0.8		6.4	6.4	
							12.3	
R	5.28	3.84	3.90	4.08	4.15	3.89	3.82	
	3.6	10.0	3.2	0.8		8.0	4.8	
							11.2	
3								
N	4.95	3.83	3.90	4.03	4.34	3.69	3.71	
	3.6	10.0	3.5	0.8		7.0	6.0	
							12.5	
R	4.37	3.53	3.72	4.03	3.77	3.90	3.84	3.57
	8.0	10.0	3.6	0.8		7.5	5.0	
							11.5	
4								
N	4.95	3.83	3.90	4.03	4.34	3.69	3.71	
	3.6	10.0	3.5	0.8		7.0	6.0	
							12.5	
R	4.37	3.53	3.72	4.03	3.77	3.88		3.57
	8.0	10.0	3.6	0.8		7.5		
5								
N	5.00	3.79	3.88	3.99	3.97	3.73	3.72	
	3.6	10.0	3.6					
R	4.33	3.69	2.40(*e*)	3.98	3.75	3.83	3.79	
	8.0	5.1	3.2			7.0	5.0	
		12.0	14.0				11.8	
			1.70 (*a*)					
			2.8					
6								
N	4.94	3.79	3.89	4.05	4.24	3.75	3.68	
	3.8	10.0	3.6	0.8		7.1	5.6	
							11.5	
R	4.37	3.57	3.35	4.28	3.71	3.92	3.84	3.52
	7.9	10.0	3.2			7.9	5.4	3.61
							11.5	
7								
N	4.94	3.82	3.89	4.03	4.14	3.73	3.70	
	3.9	10.2	3.4	0.8		5.6	6.5	
							12.0	
R	4.36	3.35	1.89	3.89	3.78	3.92	3.86	3.57
	8.0	11.5	2.5	0.6		8.0	4.8	
			6.7				11.5	
Me-3	1.18							

Table III (continued)

Compound[a]	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	*H-6'*	OMe
8								
N	4.96	3.82	3.90	4.05	4.10	3.75	3.68	
	4.0	10.6	3.6	0.8		6.8	6.1	
							11.5	
R	4.36	3.40	1.58	4.06	3.75	3.97	3.87	3.57
	7.8	11.2	3.6	0.8		8.0	5.0	
							11.0	
Et-3	1.42 [$CH_2(S)$]		1.75[$CH_2(R)$]		1.00 (CH_3)			
	10.0		3.0		7.5			
			13.5					
9								
N	5.04	3.82	3.91	4.02	4.39	3.66	3.71	
	3.6	10.0	3.2	0.8		6.2	6.2	
							12.0	
R	4.31	3.48	3.70	3.83	3.85	1.34		3.58
	7.8	10.0			6.6			
10								
N	4.94	3.81	3.88	4.01	4.32	3.68	3.70	
	3.6	10.0	3.5	0.8		7.0	6.0	
							12.5	
R	4.44	3.51	3.69	4.01	3.72	3.87	3.80	
	8.0	10.0	3.2	0.8		7.5	5.0	
							11.5	
OEt	3.94	3.72	1.25					
	10.0		7.3					
11								
	4.32	3.33	1.54	3.94	3.72	3.81	3.77	3.60
	7.8	11.1	3.0	0.8		7.5	4.5	
							11.8	
Et-3	1.42 [$CH_2(S)$]		1.77 [$CH_2(R)$]		0.96 (CH_3)			
	11.1		3.0		7.5			
			13.5					

[a]N, non-reducing unit; R, reducing unit. [b]δ, p.p.m. [c]J, Hz.

TABLE IV

^{13}C-N.M.R. CHEMICAL SHIFTS (δ, p.p.m.) FOR SOLUTIONS IN D_2O FOR DISACCHARIDES RELATED TO 4-*O*-α-D-GALACTOPYRANOSYL-D-GALACTOPYRANOSE

Compound[a]	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*OR*	
1								
N	101.6	70.2	70.4	70.3	72.2	61.8		
R	98.0	73.2	73.8	78.8	76.5	61.6		
2								
N	101.8	70.0	70.4	70.3	72.2	61.8		
R	93.7	69.9	70.3	80.3	72.3	61.9		
3								
N	101.6[b]	70.0	70.4	70.3	72.2	61.8		
R	105.2	72.3	73.7	78.7[c]	76.3	61.5	58.5	
4								
N	101.6	70.0	70.5	70.3	72.2	61.9		
R	105.2	72.4	73.8	78.8	76.3	61.2	58.5	
5								
N	101.5[b]	69.7	70.3	70.3	72.6	61.6		
R	106.6	67.1	36.9	75.7	79.1	62.2	57.7	
6								
N	101.8[b]	70.4	70.6	70.3	72.0	61.6		MeO-3
R	105.5	71.6	83.8	76.9	73.7	61.7	58.8	59.2
7								
N	101.7[b]	70.3	70.8	70.8	72.6	62.0		Me-3
R	107.0	73.2	42.0	79.6	80.4	62.4	58.5	15.8
8								
N	101.8[b]	70.1	70.4	70.2	72.3	61.6		Et-3
R	106.8	71.9	49.1	76.1	80.5	61.9	58.3	20.7, 12.6
9								
N	101.9	70.2	70.6	70.2	72.2	61.6		
R	105.1	71.8	73.9	80.7	72.0	16.8	58.2	
10								
N	101.6[b]	70.1	70.5	70.4	72.2	61.9		
R	103.8	72.4	73.8	78.6[c]	76.3	61.5	67.4, 15.9	
11	106.9	71.6	48.3	66.9	80.0	62.9	58.3	Et-3
								20.4, 11.6

[a]N, non-reducing unit; R, reducing unit. [b]A long-range coupling constant of 5.2 Hz was observed between C-1′ and H-4 in these compounds. [c]A long-range coupling constant of 1.5 Hz was observed between C-4 and H-1′ in these compounds.

between 4.94 and 5.04 p.p.m. and is independent of the 3-substituent (hydroxyl, methoxyl, deoxy, methyl, or ethyl). This finding indicates that the conformation in the crystal does not occur to a significant degree in solution, which is in agreement with the calculated H-1′---O-3 distances (Table II). Furthermore, the calculated conformations are supported by the deshielding, for **3**, of H-6(*R*) by O-2′, which are found 2.61 Å apart in the HSEA-minimum-energy conformation. The assignments of H-6(*R*) and H-6(*S*) in the ^{1}H-n.m.r. spectrum of **3** are based on the

TABLE V

N.O.E EXPERIMENTS FOR **3–11**

Compound	*Proton saturated*	*N.O.e. observed (%)*	
		Intra-ring	*Inter-ring*
3	H-1′	H-2′ (15.0)	H-4 (11.6)
	H-5′ + H-1	H-3′ (2.1), H-5 (3.0), H-2 (3.5), H-4′ (2.0), H-6′, H-6′, H-3 (3.0), OMe (3.7)	
4	H-1′	H-2′ (11.8)	H-4 (10.4) H-6(*R*) (3.9)
	H-5′ + H-1	H-4′ (1.3), H-3′ (2.3), H-5 (3.2), H-2 (3.7), H-6′, H-6′, H-3 (4.2), OMe (3.2)	
	H-2[a]	H-1	H-5′
5	H-1′	H-2′ (14.1)	H-4 (9.1)
	H-3*a*	H-3*e* (27.0), H-1 (3.4), H-4 (3.7), H-5 (4.2)	
	H-3*e*	H-3*a* (21.8), H-2 (7.8), H-4 (4.2)	
6	H-1′	H-2′ (12.6)	H-4 (9.2), H-6 (1.5), H-6 (1.5)
	H-5′ + H-4[a]	H-3′, H-6′, H-2, H-3, H-5	
	OMe + H-2[a]	H-4, H-3, H-1	H-5′, H-1′
7	H-1′	H-2′ (12.5)	H-4 (10.0)
	H-4[a]	Me-3	
	H-5′	H-4′ (ors)[b], H-3′ (7.6), H-6′ (9.0)	H-2 (3.6), Me-3 (12.4)
	Me-3	H-4 (1.7), H-2 (3.5), H-3 (2.6)	H-5′ (3.5)
8	H-1′	H-2′ (10.4)	H-4 (6.6), H-6(*R*) (1.0), H-6(*S*) + H-3′ (3.3)
	H-1	H-5 (6.0), H-2 (2.0), H-3 (3.1), OMe (7.6)	
	C-3-CH_2CH_3	C-3-CH_2(*R*) (1.2), C-3-CH_2(*S*) (0.7), H-3 (1.0)	H-1′ (0.2), H-6′ (0.7), H-5′ + H-4 (1.9)
	H-2	H-1 (2.0), C-3-CH_2(*R*) (0.8), C-3-CH_2(*S*) (1.5), H-3 (1.1)	
	H-3	H-1 (3.9), H-2 (2.6), H-4 (4.3), H-5 (4.5) C-3-CH_2(*R*) (1.6), C-3-CH_2(*S*) (ors), C-3-CH_2CH_3 (1.8)	
	C-3-CH_2(*R*)	C-3-CH_2(*S*) (15.6), H-2 (3.7), C-3-CH_2CH_3 (3.4), H-3 (ors)	
	C-3-CH_2(*S*)	H-4 (1.0), H-2 (2.6), C-3-CH_2(*R*) (12.0), H-3 (ors)	H-5′ (7.8)

Table V (continued)

Compound	*Proton saturated*	*N.O.e. observed (%)*	
		Intra-ring	*Inter-ring*
9	H-1′	CH_3-6 (3.0)	H-4 + H-2′ (13.0)
	H-5′ + H-1	H-4′ (5.2), H-3′ (5.7), H-6′ + H-3 (3.5), H-5 (2.0), OMe (2.2)	
	CH_3-6	H-4 + H-5 (4.6)	H-1′ (6.1)
	H-2 + OMe	H-1 (8.0)	H-5′ (5.0)
10	H-1′	H-2′ (11.5)	H-4 (7.0)
	H-5′	H-4′ (6.0), H-3′ (6.3), H-6′ (5.0)	H-2 (3.0)
11	H-1	H-2 (2.1), H-3 (4.5), H-5 (8.7), H-4 (−2.3), OMe (5.7)	
	H-4	H-3 (7.7), C-3-CH_2(*S*) (1.4), C-3-CH_2CH_3 (5.9)	
	H-2	H-1 (1.9), H-3 (1.4), C-3-CH_2(*R*) (2.7), C-3-CH_2(*S*) (0.6), C-3-CH_2CH_3 (−)	
	C-3-CH_2CH_3	C-3-CH_2(*R*) (1.0), H-3 (1.0), C-3-CH_2(*S*) (1.4), H-4 (2.5), H-2 (−)	

[a]Data without numbers indicate qualitative observations which cannot be quantified due to off-resonance saturation of neighbouring lines. [b]Off-resonance saturation.

TABLE VI

OBSERVED AND CALCULATED[a] RELATIVE[13a] N.O.E (% OF TOTAL) FOR **8**

Proton saturated	*Observed relative n.O.e. (% of total)* / *Calculated[a] relative[13a] n.O.e. (% of total)*					
H-1′	H-4	H-6(*S*)	H-6(*R*)	H-2′		
	31	5	15	49		
	21	2	18	59		
H-1	H-2	H-3	H-5			
	18	28	54			
	21	22	57			
H-2	H-1	C-3-CH_2(*S*)	C-3-CH_2(*R*)	H-3		
	37	28	15	20		
	43	26	11	21		
H-3	H-1	H-4	H-5	H-2	C-3-CH_2(*R*)	C-3-CH_2CH_3
	21	23	24	14	9	9
	31	12	23	15	11	8
C-3-CH_2CH_3	H-3	C-3-CH_2(*S*)	C-3-CH_2(*R*)	H-4 + H-5′	H-6′(*R*)	H-1′
	20	11	16	42	7	4
	17	12	17	38	8	8
C-3-CH_2(*R*)	C-3-CH_2(*S*)	H-2	C-3-CH_2CH_3			
	69	16	15			
	66	17	17			
C-3-CH_2(*S*)	H-2	C-3-CH_2(*R*)	H-5′			
	12	53	35			
	26	42	32			

[a]Based on a 1:1 ratio of the conformers I and II described in Table I.

TABLE VII

^{1}H-[a] AND ^{13}C-N.M.R.[b] DATA FOR SOLUTIONS OF **3** AND **9** IN $(CD_3)_2SO$

Compound[c]	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	*H-6'*	*OMe*
3 N	4.92	3.73	3.65	3.84	4.15	3.60	3.55	
	3.9	10.0	3.2	0.8		6.3	6.3	
							12.0	
OH		4.83	4.68	4.42		4.46		
		5.7	5.5	4.3		5.2		
3 R	4.13	3.35	3.43	3.90	3.58	3.64[d]	3.80[e]	3.48
	7.8	10.0	3.0	0.8		5.0	7.5	
							11.0	
OH		4.97	4.50			4.78		
		4.9	6.6			6.1		
9 N	4.86	3.79	3.71	3.87	4.23	3.60	3.55	
	3.7	10.0	3.2	0.8		6.3	6.3	
							12.0	
OH		4.55	4.67	4.44		4.46		
		5.7	5.5	4.2		5.9		
9 R	4.13	3.33	3.45	3.67	3.68	1.38		3.48
	7.5	10.0	3.0		6.5			
OH		4.96	4.56					
		4.8	6.3					

	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*OMe*
3 N	103.0	71.2[f]	71.8	71.3[f]	73.3	62.8	
3 R	106.9	73.6	75.5	79.6	76.9	61.8	58.4
9 N	103.6	71.3	71.7	71.3	73.4	62.9	
9 R	106.8	73.0	75.6	82.6	72.6	19.0	58.3

[a]Measured at 500 MHz at 300 K, using the solvent signal as internal reference (2.60 p.p.m.). [b]Measured at 125.7 MHz at 300 K, using the solvent line as internal reference (42.0 p.p.m.). [c]N, non-reducing unit; R, reducing unit. [d]Pro-*R*-proton as determined from the ^{1}H-n.m.r. spectrum of **4**. [e]Pro-*S*-proton as determined from the ^{1}H-n.m.r. spectrum of **4**. [f]Assignments may be reversed.

spectrum of its deuterated [^{2}H-6(*S*)] analogue **4**. A similar deshielding of one H-6 is displayed by all derivatives (Table III), except the 6-deoxy derivative **9**, and correlates well with the O-2'---H-6(*R*) distances displayed in Table II.

Compound **4** also allows the observation of an n.O.e. of 3.9% for H-6(*R*) when H-1' is saturated, requiring these hydrogen atoms to be in close proximity (<2.4 Å separation). This is not so in the conformation of galabiose in the crystal (Table II). Such an n.O.e. is normally not observable in the corresponding protio compounds due to efficient relaxation between H-6,6. The deuterium substitution in **4** made it possible to show that the *gauche–trans** conformation of the 5-hydroxymethyl group is populated to an extent of ~70%[19].

*C-6–O-6 is *gauche* to C-5–O-5 and *trans* to C-4–C-5.

The calculated (HSEA) dotted areas shown in Fig. 1A–C define the torsional angles ϕ and ψ for **3** when each of the interatomic distances H-1′---H-4, H-5′---O-3, and H-1′---H-6(*R*) were restricted, as required by the experimental n.m.r. data discussed above. These three regions overlap in one small area (encircled in Fig. 1D) where the HSEA-minimum-energy conformation that was calculated without restrictions of interatomic distances was found. This augments the calculated conformations of **3** and its analogues (**5–9**) reported in Table I.

Furthermore, the strong n.O.e.'s observed for H-5′ on saturation of MeO-3 in **6** and for H-1′ on saturation of Me-3 (and *vice versa*) in **7** are only compatible with the conformations calculated by the HSEA method (Table I).

Finally, the observed ^{13}C–^{1}H three-bond coupling[20] for C-1′ to H-4 of 5.2 Hz for **3, 5–8**, and **10** indicate that the ψ angle is small[20], which was predicted by the HSEA calculations. Furthermore, the observation for **3** and **10** of a C-4 to H-1′ long-range coupling[20] of 1.4 Hz supports the numerically larger ϕ angle ($\sim -40°$) calculated by the HSEA method and not the small ϕ angle observed in the crystal.

In considering the conformation of the ethyl group in **8** and **11**, the H-3---CH_2(*S*)CH_3 and H-3---CH_2(*R*)CH_3 coupling constants of 10.0 and 3.0 Hz for **8**, and 11.1 and 3.0 Hz for **11**, indicate that one of the staggered conformations with CH_3 *gauche* to H-3 preponderates. For **11**, saturation of H-4 gives an n.O.e. of 5.9% for the resonance of the CH_3CH_2 group (saturation of CH_3CH_2 gave an n.O.e. of 2.5% on the resonance for H-4), indicating that the conformation shown (**11**) is populated to a significant degree in aqueous solution (Table V).

Similarly, a total n.O.e. for the resonances of H-5′ and H-4 of 1.9% on saturation of CH_3CH_2 in **8** as well as n.O.e.'s for the resonances of H-5′ (7.8%) and H-4 (1.0%) on saturation of CH_2(*S*)CH_3 indicated a conformation shown in **8**. The agreement between the observed and the calculated n.O.e. of **8** is good (Table VI). The small n.O.e. (0.7%) for H-6′ on saturation of CH_3CH_2 indicates that the *trans–gauche** conformer is populated to a larger extent in this compound, probably due to hydrophobic interactions of the CH_3CH_2-3 and CH_2OH-5′.

The n.m.r. data for solutions of **3** and **9** in $(CD_3)_2SO$ are given in Table VII. When a small amount of CD_3OD was added to exchange the OH protons of **3** to an extent of 50%[21–23], doubling of the signals for HO-2′ and HO-6 was observed (Fig. 1) due to isotope-induced chemical shifts caused by an intramolecular hydrogen bond between these hydroxyl groups.

The conformation of the CH_2OH-5 group of **3** was determined from the $J_{5,6}$ values of the specifically deuterated analogue **4**. In aqueous solution, the conformation is *gauche–trans*, as it is in the crystal (**1** and **2**)[6]. The O-2′---O-6 distances (4.66 Å and 3.52 Å, respectively) in **3** and **1** and **2** then rule out the possibility of an intramolecular hydrogen bond between HO-2′ and HO-6. In $(CD_3)_2SO$, the *trans–gauche* conformation is preferred, probably due mainly to the intramolecular hydrogen bond discussed above.

*C-6′–O-6′ is *trans* to C-5′–O-5′ and *gauche* to C-4′–C-5′.

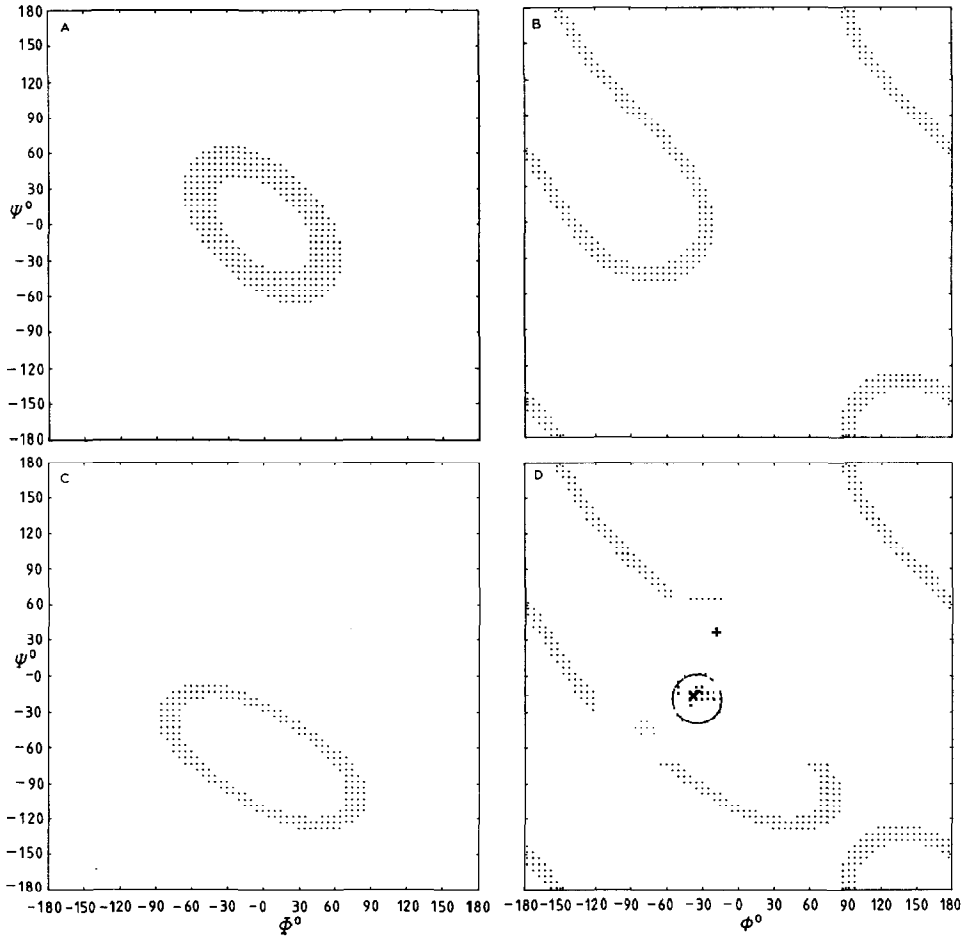

Fig. 1. Calculated (HSEA) conformations for **3** in which *A*, the H-1′ to H-4 distance is restricted to 2.40 ±0.15 Å; *B*, the H-5′ to O-3 distance is restricted to 2.70 ±0.25 Å; *C*, the H-1′ to H-6(*R*) distance (in the "gt" conformer) is restricted to 2.40 ±0.15 Å; *D*, all three distance restrictions are included. The ×-sign in *D* shows the minimum energy conformation calculated (HSEA) without restrictions (*cf.* Table I) and the + sign shows the crystal conformation.

The HSEA-calculated minimum-energy conformation of **3**, with the CH_2OH-5 group in the *trans–gauche* conformation, revealed an O-2′---O-6 distance of 4.1 Å, which is too long to accommodate a hydrogen bond. However, a *trans–gauche* conformation *with* an intramolecular hydrogen bond was clearly demonstrated by n.m.r. spectroscopy (see above) for a solution in $(CD_3)_2SO$. Therefore, the over-all conformation of **3** in solution in $(CD_3)_2SO$ was not reproduced well by the HSEA calculation and an exact description of the conformation cannot be given.

Based on the deuterium isotope-induced chemical shifts (see Fig. 2) and the arguments put forward in refs. 21 and 22, it is suggested that the intramolecular hydrogen bond of **3** in solution in Me_2SO has the form $Me_2SO \rightarrow$ HO-2′ $\rightarrow$ HO-6.

Comparison of the ^1H- and ^{13}C-data for **3** and **9** in Table VII shows that the

only major difference is an upfield shift of 0.28 p.p.m. for the resonance of HO-2′ of **9**, which now is not participating in an intramolecular hydrogen bond. A tentative conclusion then is that **3** and **9** adopt similar conformations in solution in $(CD_3)_2SO$ and that the HO-2′ · · · HO-6 intramolecular hydrogen bond is not of decisive importance for the conformation of **3**.

Thus, galabiose (**1** and **2**) displays an O-5′ · · · O-3 hydrogen bond in the crystal, which, for the methyl glycoside **3** in solution in $(CD_3)_2SO$ is replaced by an O-2′ · · · O-6 hydrogen bond. In solution in D_2O, the interatomic distances O-5′---O-3 and O-2′---O-6, for **1–3**, are too long to permit hydrogen bonds. For solutions in D_2O, there is good agreement between the calculated conformations for **3** and **5–9** and the experimental n.m.r. data. Each of the analogues **5–9** were shown to adopt a conformation similar to that of galabiose. Replacement of hydro-

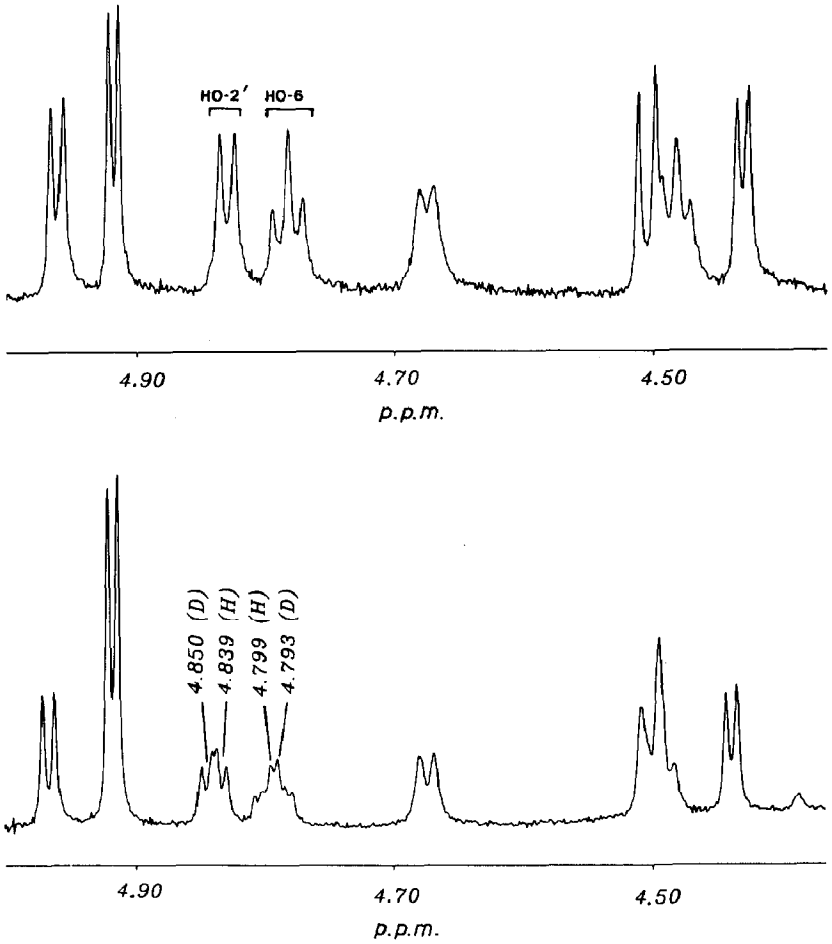

Fig. 2. Isotope-induced doubling[21–23] of the signals for HO-2′ and HO-6 of **3** in solution in $(CD_3)_2SO$ containing a small amount of CD_3OD; ~50% of the OH groups were replaced by OD groups. Note the small solvent-induced chemical shifts compared to the data of Table VII.

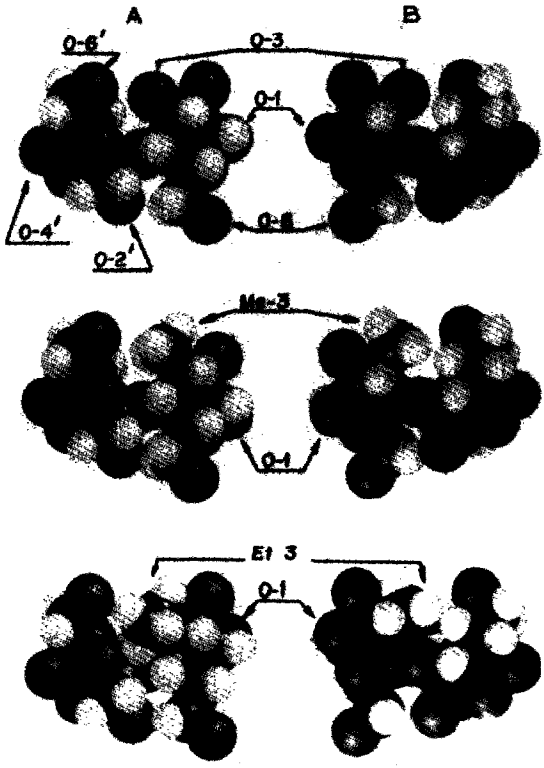

Fig. 3. HSEA-calculated minimum-energy CPK models of the disaccharides corresponding to **3, 7,** and **8**, showing the increase in hydrophobic surface area on substituting HO-3 by Me and Et. Hydroxyl protons are not shown. For clarity, less-than-normal van der Waals volumes were used. *A*, Front view showing the postulated[11b] binding surface. *B*, Rear-side view (180°).

xyl groups by hydrogen or alkyl groups therefore seems to be of little consequence with respect to the preferred conformations of galabiose analogues in aqueous solution. Consequently, it seems possible to change the size of the hydrophobic surface[5] of galabiose without seriously altering the over-all conformation in aqueous solution. This is of potential importance in creating oligosaccharide analogues with improved affinity towards lectins, antibodies, and microbes. Fig. 3 clearly displays the difference in hydrophobic area size between **3, 7,** and **8**.

EXPERIMENTAL

The syntheses of **1–10** were performed as follows: 4-*O*-α-D-galactopyranosyl-D-galactopyranose (**1** and **2**) by enzymic cleavage of "polygalacturonic acid" and reduction of the resulting "digalacturonic acid"[24]; methyl 4-*O*-α-D-galactopyranosyl-β-D-galactopyranoside (**3**) and methyl 4-*O*-α-D-galactopyranosyl-β-D-[6(*S*)-^{2}H]galactopyranoside (**4**) by glycoside synthesis[7d] using 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl chloride and methyl 2,3,6-tri-*O*-benzoyl-β-D-galacto-

pyranoside or the corresponding deuterated compound; methyl 3-deoxy-4-*O*-α-D-galactopyranosyl-β-D-*xylo*-hexopyranoside (**5**), methyl 4-*O*-α-D-galactopyranosyl-3-*O*-methyl-β-D-galactopyranoside (**6**), and methyl 3-deoxy-4-*O*-α-D-galactopyranosyl-3-*C*-methyl-β-D-galactopyranoside (**7**) by glycoside synthesis[7a] using 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl bromide and analogues of methyl β-D-galactopyranoside; methyl 3-deoxy-3-*C*-ethyl-4-*O*-α-D-galactopyranosyl-β-D-galactopyranoside (**8**) as for **5**–**7** but using the chloride as glycosyl donor[7b]; methyl 4-*O*-α-D-galactopyranosyl-β-D-fucopyranoside (**9**) by modification[7c] of protected **3** by a variation of the method described[7d]; and ethyl 4-*O*-α-D-galactopyranosyl-β-D-galactopyranoside (**10**) by hydrogenolysis of the corresponding 2-bromoethyl glycoside[25]. The synthesis of the specifically deuterated starting glycoside for the preparation of **4** has been reported[26].

^{1}H-N.m.r. spectra were obtained for 0.03M solutions in D_2O (internal acetone, 2.22 p.p.m.) at 300 K and 500 MHz with a Bruker AM 500 n.m.r. instrument. Generally, a sweep width of 4000 Hz, 32k datapoints, and 90° pulses (9 μs) were used in sampling the data. The 2D-experiments, COSY and relayed COSY, were performed[27,28] using a data matrix of 1k × 1k.

^{13}C-N.m.r. spectra were obtained on 0.03M solutions in D_2O (internal 1,4-dioxane, 67.4 p.p.m.) at 300 K with the same instrument operating at 125.77 MHz. Generally, a sweep width of 25 kHz, 64k data points, and 90° pulses (8.5 μs) were used in sampling the data. The correlated experiments (CHORTLE) were performed[16] using data transfer and data analysis with programs (ASCOM) and (CHASP) written for a personal computer[17]. The ^{13}C–^{1}H coupled spectra were obtained in the gated mode.

HSEA calculations[13] were made on an IBM 8083 computer using the program GESA[15]. Coordinates for α- and β-D-galactopyranose units were taken from the neutron diffraction data[29] for methyl β-D-galactopyranoside, using tetrahedral geometry and staggered conformations; C–H and C–C bond lengths of 1.10 and 1.53 Å, respectively, were used. During the energy minimisation, the Et-3 group of **8** was kept in the staggered conformation indicated in **11**, based on the observed vicinal coupling constants and n.O.e.'s (*cf.* Tables III and V, respectively). The conformation of the 5-hydroxymethyl group in the disaccharides was kept in the *gauche–trans* conformation based on the results from methyl β-D-[6(*S*)-^{2}H]galactopyranoside ($J_{5,6R}$ 8.2 Hz) which indicate[19] that the *gauche–trans* conformation is populated to an extent of ~70%. This assumption was supported by the $J_{5,6R}$ value of 7.5 Hz for the 6(*S*)-^{2}H-deuterated disaccharide **4**. Furthermore, each of the disaccharides, with the exception of the 3-deoxy analogue **5**, showed one $J_{5,6}$ value >7.5 Hz, suggesting a preponderance for the *gauche–trans* conformation. For **8**, experimentally observed and calculated relative n.O.e. values were correlated (*cf.* Table VI) as described previously[13]. Experimentally observed relative n.O.e.'s were calculated by summation of the n.O.e.'s observed on saturation of a certain hydrogen atom, followed by calculation of the percentages of the individual n.O.e.'s. Relative n.O.e. values for **8** were also calculated directly from the confor-

mational equilibrium obtained by the HSEA-calculations (conformations I and II in a 1:1 ratio, *cf.* Table I).

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