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Synthesis and structural studies of branched 2-linked trisaccharides related to blood group determinants

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Abstract

A series of trisaccharide glycosides, Fuc-(1 → 2)-β-Gal-(1 → 3)-β-X-OMe (X = GlcNAc, Glc, 2-deoxy-Glc) related to the blood group determinant Le^d have been synthesised both as their α- and β-Fuc anomers together with the component disaccharide starting compounds. The conformational properties of the six trisaccharides together with their parent disaccharides have been investigated by NMR spectroscopy (proton and carbon chemical shifts and proton NOEs) in combination with computer modeling using the Monte Carlo approach and the HSEA force field using the GEGOP programme. The interaction between the terminal fucose unit and the reducing unit was probed by substitution of the bulky NAc group with hydroxyl and deoxy substituents, respectively. Compounds with severe steric interactions were identified by the non-additivity of their carbon chemical shifts. This was subsequently confirmed by the detailed conformational assessment by NOE spectroscopy and computer modeling. The most severe contacts arose in the α-L-Fuc derivatives, whereas the β-linked L-Fuc derivatives only in one case exhibit severe steric interaction as probed by the NMR parameters. © 1996 Elsevier Science Ltd.

Keywords: Oligosaccharides; Synthesis; NMR spectroscopy; Conformations

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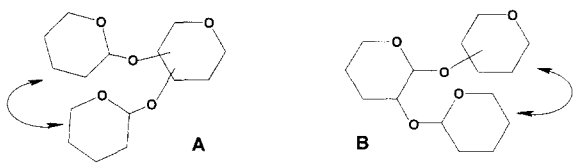
1. Introduction

Complex carbohydrates, as glycoproteins or polysaccharides, play an important role in different biochemical processes, and therefore the investigation of their spectral and conformational properties and the underlying principles has been a challenge in the area of carbohydrate research during the last two decades [1]. In this context a main interest has focused on oligosaccharides with intramolecular interactions of importance for the conformational preferences of the molecule, even though complex carbohydrates generally do not exhibit extensive backfolding and long range interactions as found in proteins. During the last years special attention has been paid to investigation of vicinally branched oligosaccharides (**A**), the conformational behaviour of which depends on the interactions between terminal monosaccharide residues [2–11].

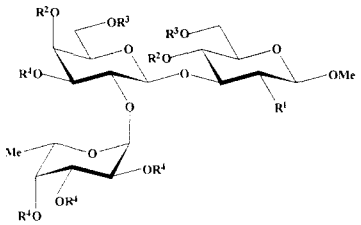


Furthermore, it has been shown that ^{13}C NMR data of the trisaccharide **1** [12] and several other oligosaccharides [13–15], i.e., structurally well defined linear trisaccharide sequences of type **B** with (1 → 2)-linkage at the non-reducing end were non-additive. In order to investigate this phenomenon in more detail, NMR and conformational studies have been performed of the trisaccharide **1**, its β-fucosylated analog **2**, and of a series of their derivatives **3–6**. This series of components allows an investigation of steric demands of the different substituents at C-2 of the monosaccharide unit at the reducing varied between NHAc, OH and H. Among other, such results are required for the development of computer-assisted methods used in the structural analysis of oligo- and polysaccharides [13,16]. The results of such investigations of the trisaccharides **1–6** and their respective disaccharide fragments **7–11** are discussed in the present paper

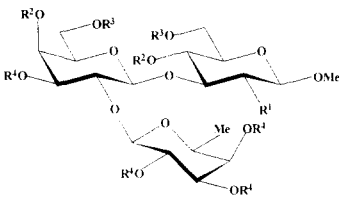
α-L-Fuc-(1 → 2)-β-D-Gal-(1 → 3)-β-D-GlcNAc-OMe	**1**
β-L-Fuc-(1 → 2)-β-D-Gal-(1 → 3)-β-D-GlcNAc-OMe	**2**
α-L-Fuc-(1 → 2)-β-D-Gal-(1 → 3)-β-D-Glc-OMe	**3**
β-L-Fuc-(1 → 2)-β-D-Gal-(1 → 3)-β-D-Glc-OMe	**4**
α-L-Fuc-(1 → 2)-β-D-Gal-(1 → 3)-2-deoxy-β-D-Glc-OMe	**5**
β-L-Fuc-(1 → 2)-β-D-Gal-(1 → 3)-2-deoxy-β-D-Glc-OMe	**6**
β-D-Gal-(1 → 3)-β-D-GlcNAc-OMe	**7**
β-D-Gal-(1 → 3)-β-D-Glc-OMe	**8**
β-D-Gal-(1 → 3)-2-deoxy-β-D-Glc-OMe	**9**
α-L-Fuc-(1 → 2)-β-D-Gal-OMe	**10**
β-L-Fuc-(1 → 2)-β-D-Gal-OMe	**11**.

2. Results and discussion

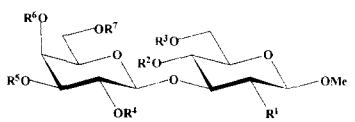
Synthesis of model oligosaccharides.—The main synthetic pathways in the prepara-



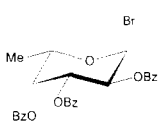
1 R^1=NHAc; R^2=R^3=R^4=H
3 R^1=OH; R^2=R^3=R^4=H
5 R^1=R^2=R^3=R^4=H
12 R^1=OBnCl-p; R^2,R^3=PhCH; R^4=Bz
13 R^1=H; R^2,R^3=PhCH; R^4=Bz



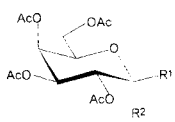
2 R^1=NHAc; R^2=R^3=R^4=H
4 R^1=OH; R^2=R^3=R^4=H
6 R^1=R^2=R^3=R^4=H
14 R^1=NPhth; R^2=R^3=H; R^4=Bz
15 R^1=OBnCl-p; R^2,R^3=PhCH; R^4=Bz
16 R^1=H; R^2,R^3=PhCH; R^4=Bz



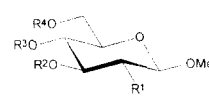
7 R^1=NHAc; R^2=R^3=R^4=R^5=R^6=R^7=H
8 R^1=OH; R^2=R^3=R^4=R^5=R^6=R^7=H
9 R^1=H; R^2=R^3=R^4=R^5=R^6=R^7=H
17 R^1=NPhth; R^2,R^3=R^6,R^7=PhCH; R^4=H; R^5=Bz
18 R^1=OBnCl-p; R^2,R^3=PhCH; R^4=R^5=R^6=R^7=Ac
19 R^1=OBnCl-p; R^2,R^3=R^6,R^7=PhCH; R^4=R^5=H
20 R^1=OBnCl-p; R^2,R^3=R^6,R^7=PhCH; R^4=H; R^5=Bz
21 R^1=H; R^2,R^3=PhCH; R^4=R^5=R^6=R^7=Ac
22 R^1=H; R^2,R^3=R^6,R^7=PhCH; R^4=R^5=H
23 R^1=H; R^2,R^3=R^6,R^7=PhCH; R^4=H; R^5=Bz



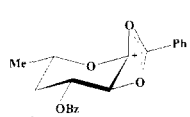
24



25 R^1=OAc; R^2=H
26 R^1=H; R^2=Br



27 R^1=OBnCl-p; R^2=H; R^3,R^4=PhCH
28 R^1=H; R^2=R^3=R^4=Ac
29 R^1= R^2=H; R^3,R^4=PhCH



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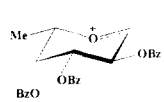

31

Table 1
^{1}H NMR data for substituted derivatives **12**, **13**, **15**, **16**, **20–23** and **29** ($CDCl_3$)

Compound	Residue	Chemical shifts (δ, ppm)									
		H-1	H-2	H-2′	H-3	H-4	H-5	H-6	H-6′	C(O)CH_3	O–CH_3
12	α-Fuc	5.69					5.04	1.28			
	β-Gal	5.07	4.46		5.12						
	β-Glc	4.42									3.54
13	α-Fuc	5.66	5.71		5.83	5.75	5.86	1.26			
	β-Gal	4.76	4.40		5.06	4.29	3.07	4.07	3.70		
	β-2-deoxy-Glc	4.64	2.41	1.88	4.21	3.84	3.51	4.37	3.89		3.58
15	β-Fuc	5.14	5.77		5.35	5.45		0.78			
	β-Gal				5.25						
	β-Glc										3.52
16	β-Fuc	5.05					3.83	1.17			
	β-Gal	4.55	4.27		5.07	4.39	3.73				
	β-2-deoxy-Glc										3.46
20	β-Gal	4.60	4.07		5.05	4.43					
	β-Glc										
21	β-Gal	4.64	5.20		4.97	5.33	3.72	4.08	3.95	1.9–2.2	
	β-2-deoxy-Glc	4.50	2.25	1.71	3.95	3.83	3.35				3.52
22	β-Gal	4.49				4.18					
	β-2-deoxy-Glc	4.55	2.46	1.81	4.17	3.68	3.43	4.36	3.84		3.53
23	β-Gal	4.65	4.26		5.05	4.47	3.48	4.26	3.99		
	β-2-deoxy-Glc	4.55	2.46	1.83	4.25	3.70	3.44	4.36	3.85		3.52
29	β-2-deoxy-Glc	4.56	2.32	1.70	3.93	3.82	3.40	4.36	3.46		3.53

tion of the trisaccharides **1** and **2** by fucosylation of selectively protected disaccharide **25** by benzobromofucose **24** under Helferich conditions and subsequent deblocking have been published previously [17], except for the transformation of the substituted derivative **17** to the trisaccharide **2**, which is given in the Experimental section.

Glycosylation of methyl 4,6-*O*-benzylidene-2-*O*-(4-chlorobenzyl)-β-D-glucopyranoside (**27**) [18] by 1,2,3,4,6-penta-*O*-acetyl-β-galactopyranose (**25**) in the presence of trimethylsilyl triflate [19,20] afforded 85% of the β-linked disaccharide derivative **18**. Analogous galactosylation of methyl 4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (**29**) was unsuccessful due to its instability under the reaction condition. In order to prepare the disaccharide **21**, the acceptor **29** was therefore galactosylated with acetobromogalactose **26** to give **21**, in 98% yield. The β-configured galactosyl unit in **18** and **21** was established from the ^{1}H NMR data presented in Table 1 ($J_{1,2}$ 7.6 and 7.5 Hz, respectively).

O-Deacetylation of **18** gave the corresponding tetraol which, on treatment with benzaldehyde dimethyl acetal, gave the 4,6:4′,6′-di-*O*-benzylidene derivatives **19** in high yield. Treatment of **19** with benzoyl cyanide in the presence of a catalytic amount of triethylamine [21] gave the 3′-benzoate **20** in 72% overall yield.

Similarly, *O*-deacetylation of **21** and subsequent benzylidenation gave 54% of the 4,6:4′,6′-di-*O*-benzylidene derivatives **22**. The rather low yield of **22** (as compared to **19**) is associated with a marked formation (22%) of a mixture of isomers of **22** which vary

by the orientation of Ph-groups in the respective benzylidene fragments. The formation of these compounds was established by the fact that the ^{1}H NMR spectrum of the mixture contains PhCH-proton signals as two pairs of singlets (in ratio of 3:1) with chemical shifts of 5.54 and 5.59 ppm for the major component, and 5.57 and 5.96 ppm for the minor, respectively. Treatment of **22** with benzoyl cyanide in the presence of a catalytic amount of triethylamine gave the 3′-benzoate **23**. The location of the benzoyl group in **20** and **23** at *O*-3′ was indicated by low-field chemical shift of the resonance for H-3′ (δ 5.05 ppm, Table 1).

Glycosylation of **20** and **23** was accomplished by benzobromofucose under Helferich conditions [22] in order to obtain both products of α- and β-fucosylation in one

Table 2
^{1}H NMR data for oligosaccharides **1–11** (D_2O)

Compound	Residue	Chemical shifts (δ, ppm)									
		H-1	H-2	H-2′	H-3	H-4	H-5	H-6	H-6′	$C(O)CH_3$	$O-CH_3$
1	α-Fuci	5.195	3.781		3.711	3.770	4.310	1.227			
	β-Gal	4.635	3.602		3.839	3.906	3.684	3.806	3.755		
	β-GlcNAc	4.357	3.795		3.996	3.487	3.505	3.950	3.773	2.058	3.497
2	β-Fuc	4.478	3.528		3.669	3.766	3.823	1.272			
	β-Gal	4.641	3.662		3.697	3.984	3.675	3.801	3.750		
	β-GlcNAc	4.563	3.737		3.981	3.553	3.500	3.943	3.767	2.063	3.510
3	α-Fuc	5.274	3.802		3.879	3.810	4.484	1.220			
	β-Gal	4.797	3.677		3.871	3.916	3.710	3.804	3.761		
	β-Glc	4.383	3.369		3.812	3.479	3.505	3.934	3.759		3.563
4	β-Fuc	4.696	3.536		3.674	3.758	3.819	1.273			
	β-Gal	4.799	3.778		3.737	3.995	3.753	3.807	3.762		
	β-Glc	4.429	3.481		3.747	3.534	3.496	3.930	3.748		3.575
5	α-Fuc	5.247	3.795		3.815	3.815	4.326	1.217			
	β-Gal	4.604	3.632		3.859	3.905	3.693	3.75	3.75		
	β-2-deoxy-Glc	4.607	2.444	1.420	3.978	3.387	3.416	3.934	3.746		3.523
6	β-Fuc	4.633	3.499		3.661	3.747	3.786	1.266			
	β-Gal	4.690	3.743		3.727	3.982	3.708	3.76	3.76		
	β-2-deoxy-Glc	4.613	2.468	1.569	3.959	3.442	3.403	3.938	3.756		3.524
7	β-Gal	4.430	3.536		3.646	3.927	3.715	3.77	3.77		
	β-GlcNAc	4.508	3.831		3.799	3.548	3.506	3.948	3.777	2.033	3.524
8	β-Gal	4.652	3.601		3.682	3.932	3.724	3.794	3.760		
	β-Glc	4.417	3.470		3.754	3.512	3.486	3.932	3.746		3.579
9	β-Gal	4.493	3.541		3.657	3.931	3.703	3.75	3.75		
	β-2-deoxy-Glc	4.619	2.409	1.521	3.947	3.432	3.412	3.941	3.795		3.532
10	α-Fuc	5.138	3.796		3.871	3.819	4.249	1.222			
	β-Gal	4.416	3.543		3.838	3.930	3.688	3.807	3.762		3.586
11	β-Fuc	4.596	3.494		3.656	3.746	3.776	1.261			
	β-Gal	4.468	3.685		3.723	3.980	3.689	3.800	3.759		3.577
β-Gal-OMe	β-Gal	4.369	3.566		3.695	3.985	3.745	3.83	3.83		3.631
β-2-deoxy	β-2-deoxy-Glc	4.630	2.261	1.487	3.719	3.273	3.386	3.936	3.741		3.531

glycosylation reaction. It should be noted that, to favour the α-fucosylation [22] acceptors **20** and **23** were used having a Bz-substituent in the neighbouring position to the glycosylated OH group.

As expected fucosylation of **20** and **23** was not stereospecific and gave both (α and β) trisaccharides, which were separated as their benzylidene derivatives **12** and **15**, and as their tetraols, **13** and **16**. It is interesting to note that the ratio of α- and β-fucosyl trisaccharides (2:3 and 1:2, respectively) which were obtained from **20** and **23**, varied significantly from the ratio 9:1 [17] which was obtained by fucosylation of the galactosyl glucosamine derivative **17**.

To rationalize the difference in the stereochemical outcome of these reactions the interaction between the disaccharide glycosyl acceptors **17**, **20**, and **23** with the dioxolenium cation **30** and the glycosyl cation **31** were modelled manually followed by energy minimization using standard procedures and default potentials and partial changes. Therefore, the results can only be interpreted qualitatively. Docking of the low-energy

Table 3
^{13}C NMR data for oligosaccharides **1–11** (D_2O)

Compound	Residue	Chemical shifts (δ, ppm)								
		C-1	C-2	C-3	C-4	C-5	C-6	$NHCOCH_3$	$NHCOCH_3$	$O-CH_3$
1	α-Fuc	100.4	69.0	70.3	72.7	67.3	16.0			
	β-Gal	101.0	77.5	74.4	70.0	76.0	62.0			
	β-GlcNAc	103.5	55.6	78.2	69.7	76.4	61.8	174.6	23.1	58.1
2	β-Fuc	104.1	71.6	73.4	71.9	71.8	16.1			
	β-Gal	101.6	81.0	72.6	69.0	76.0	61.9			
	β-GlcNAc	102.3	56.2	79.9	69.3	76.3	61.6	175.6	23.2	57.9
3	α-Fuc	100.2	69.2	70.4	72.6	67.6	16.2			
	β-Gal	101.9	77.7	74.3	69.8	75.9	61.7			
	β-Glc	104.1	74.1	83.6	69.2	76.3	61.6			58.1
4	β-Fuc	103.2	71.8	73.8	72.5	71.7	16.1			
	β-Gal	103.3	79.6	72.1	68.9	76.0	61.7			
	β-Glc	103.5	73.3	86.1	69.0	76.3	61.6			58.0
5	α-Fuc	100.3	69.1	70.4	72.6	67.6	16.1			
	β-Gal	99.6	77.5	74.6	69.8	76.1	61.8			
	β-2-deoxy-Glc	101.4	36.3	78.4	70.1	76.6	61.6			57.3
6	β-Fuc	103.0	71.7	73.6	72.2	71.6	16.2			
	β-Gal	100.9	78.4	72.5	69.0	75.9	61.8			
	β-2-deoxy-Glc	101.5	36.4	79.5	70.2	76.6	61.8			57.3
7	β-Gal	104.1	71.3	73.2	69.1	75.9	61.6			
	β-GlcNAc	102.3	55.0	83.3	69.4	76.0	61.4	175.4	22.8	57.6
8	β-Gal	104.1	72.0	73.4	69.4	76.1	61.8			
	β-Glc	103.7	73.4	85.6	69.1	76.3	61.6			57.9
9	β-Gal	101.4	71.4	73.5	69.4	76.0	61.8			
	β-2-deoxy-Glc	101.5	36.3	79.1	70.0	76.6	61.8			57.3
10	α-Fuc	100.7	69.1	70.3	72.5	67.5	15.8			
	β-Gal	103.4	78.9	73.9	69.4	75.5	61.6			57.7
11	β-Fuc	103.2	71.7	73.6	72.1	71.6	16.2			
	β-Gal	104.4	78.9	72.3	69.1	75.7	61.7			58.0
β-Gal-OMe	β-Gal	104.7	71.6	73.7	69.5	75.9	61.8			57.9
β-2-deoxy	β-2-deoxy-Glc	101.5	38.9	71.2	71.9	76.9	61.9			57.3

conformers of the molecules was carried out using the Insight II software (Biosym, San Diego) after energy minimization using Discover software (Biosym, San Diego) with CVFF or ESFF force fields. It was shown that the assembly of the trisaccharide from the acceptor **17** and dioxolenium cation is more sterically demanding than using the glycosyl cation **31** due to the presence of bulky phthalimido group, in agreement with previous experimental observations [23]. It is therefore most likely, that this restriction causes preferential progress of the reaction via glycosyl cation intermediate and thus favors the formation of larger portion of α-product, which cannot be formed via the dioxolenium intermediate.

Removal of the blocking groups in the substituted derivatives **12–16** and **21** gave the target oligosaccharides **2–6** and **9**, which were subsequently purified by gel-filtration. Preparation of oligosaccharides **1** [17], **7** [18], **8** [17], **10** [18], and **11** [18] has been described previously.

^{1}H and ^{13}C NMR spectroscopy.—^{1}H and ^{13}C NMR data for oligosaccharides **1–11** are presented in Tables 2 and 3, respectively. Assignment of the signals in the ^{1}H and ^{13}C NMR spectra was performed as previously reported by both homo- and heteronuclear 2D experiments [24].

One of the main aims in this work was an examination of the deviations from additivity values (ΔΔ) of chemical shifts in ^{13}C NMR spectra of oligosaccharides **1–6**. The ΔΔ values represent the difference between the experimental ^{13}C chemical shifts

Table 4
Deviations from additivities (ppm) [a] in ^{13}C NMR spectra (D_2O) of trisaccharides **1–6**

Compound	Residue	ΔΔC-1	ΔΔC-2	ΔΔC-3	ΔΔC-4	ΔΔC-5	ΔΔC-6
	α-Series						
1	α-Fuc	−0.3	−0.1	0	0.2	−0.2	0.2
3	α-Fuc	−0.5	0.1	0.1	0.1	0.1	0.4
5	α-Fuc	−0.4	0	0.1	0.1	0.1	0.3
1	β-Gal	**−1.8**	−1.1	1.0	1.0	0.5	0.6
3	β-Gal	−0.9	−1.6	0.7	0.5	0.2	0.1
5	β-Gal	−0.5	−1.2	0.9	0.5	0.5	0.2
1	β-GlcNAc	1.2	0.6	**−5.1**	0.3	0.4	0.3
3	β-Glc	0.4	0.7	**−2.0**	0.1	0	0
5	β-2-deoxy-Glc	−0.1	0	**−0.7**	0.1	0	−0.2
	β-Series						
2	β-Fuc	0.9	−0.1	−0.2	−0.2	0.2	−0.1
4	β-Fuc	0	0.1	0.2	0.4	0.1	−0.1
6	β-Fuc	−0.2	0	0	0.1	0	0
2	β-Gal	**−2.2**	**2.4**	0.8	0.3	0.3	0.4
4	β-Gal	−0.5	0.3	0.1	−0.1	0.1	0
6	β-Gal	−0.2	−0.3	0.4	0	0.1	0.1
2	β-GlcNAc	0	**1.2**	**−3.4**	−0.1	0.3	0.2
4	β-Glc	−0.2	−0.1	0.5	−0.1	0	0
6	β-2-deoxy-Glc	0	0	0.4	0.2	0	0

[a] See equation in text.

Table 5
Absolute (abs.) and relative (rel.) [a] NOEs from 1D NOESY measurements with a mixing time of 900 ms and corresponding calculated values from Monte Carlo simulation (MC)

Compound	Proton	Intraunit NOEs				Interunit NOEs			
	saturated	observed	abs.	rel.	MC	observed	abs.	rel.	MC
1	H-1a/H-2 [b] (6.8) [c]	H-3a	0.8	12	4.2	H-2b	7.8	115	92
		H-5a	0.3	4.4	5.5	H-3b	0.4	5.9	7.8
		H-6a	0.1	1.5	1.5	H-4b	(0.2)	2.9	0.5
	H-5a/H-4a (7.3)	H-3a	6.3	86	104	H-2c	4.6	63	54
		H-6a	2.5	34	43				
	H-6a/H-5a (4.0)	H-4a	2.0	50	68	H-2c	0.9	23	35
						H-4, 5c + OMe	2.0	50	48
	H-1b/H-3b (4.1)	H-2b	1.5	37	40	H-3c	6.9	168	120
		H-5b	8.3	202	175	H-4c	0.4	10	3
						NAc	0.5	12	13
						H-5b + H-3c	*15.2*	*370*	*295*
	NAc/H-1c (0.4)	H-2c	0.2	50	62	H-3a	0.8	200	435
3	H-1a/H-2a (6.8)	H-6a	0.13	1.9	1.5	H-2b	8.0	118	91
						H-3b	1.3	19	8
	H-5a/H-4a (9.0)	H-3a	7.3	81	107	H-2c	4.3	48	65
		H-6a	2.5	28	42				
	H-6a/H-5a (3.9)	H-4a	2.2	56	71	H-2c	1.2	31	38
						H-4c	0.8	21	40
	H-1b/H-3b (5.0)	H-2b	1.4	28	40	H-2c	0.8	16	11
		H-5b	7.2	144	175	H-3c	8.7	174	145
						H-6a	0.2	4	1
						H-5b + H-3c	*15.9*	*318*	*320*
5	H-1a/H-2a (8.5)	H-3a	0.6	7.0	4.3	H-2b	8.0	94	94
		H-5a	0.3	3.5	5.2				
		H-6a	0.07	0.8	1.5				
	H-5a/H-3 + 4a (13.9)	H-1a	0.5	3.5	3.5	H-2b	1.6	12	10
		H-6a	2.6	18	20	H_A-2c	2.1	15	15
						H_E-2c	0.9	7	2
						H-5c	0.8	6	0
	H-6a/H-5a (3.8)	H-4a	2.0	53	72	H_A-2c	0	0	13
						H-4c	0.7	18	44
	H-1b/H-3b	H-2b	[d]	[d]	40	H_A-2c	[d]	[d]	2
		H-5b	[d]	[d]	174	H_E-2c	[d]	[d]	34
						H-3c	[d]	[d]	114
						H-4c	[d]	[d]	3
						H-5b + H-3c			*288*
	H_A-2c/H-4c (7.3)	H_E-2c	28.5	390	385	H-5a	5.8	79	69
	H_E-2c/H-3c (8.0)	H_A-2c	34.6	433	348	H-5a	1.6	20	10
						H-6a	0.2	3	0
						H-1b,1c,HDO	14.0	175	78
						OMe	0.17	2.1	3.5
7	H-1b/H-3b (5.4)	H-5b	8.4	156	176	H-2 + 3c	11.3	209	171
						H-5b + H-2 + 3c	*19.7*	*365*	*347*

Table 5 (continued)

Compound	Proton	Intraunit NOEs				Interunit NOEs			
	saturated	observed	abs.	rel.	MC	observed	abs.	rel.	MC
8	H-1b/H-3b (5.4)	H-2b	1.9	35	46	H-5b + H-3c	18.1	335	338
						H-3c			160
						H-5c	0.2	4	−1
9	H-1b/H-3b (5.2)	H-5b	7.8	150	175	H_A-2c	0	0	2
						H_E-2c	3.5	67	55
						H-3c	7.6	146	120
						H-4c	1.5	29	5
						OMe + H-2b	1.8	36	45
						H-5b + H-3c	*15.4*	*296*	*295*
	H_E-2c/H-3c (5.9)	H_A-2c	36.1	612	430	H-1b	4.4	75	73
		H-1c + HDO	> 2.4	> 40	115				
10	H-1a/H-2a (7.1)					H-2b	8.6	121	117
	H-5a/H4-a (7.2)	H-3a	5.3	72	108	H-2b	0.9	13	14
		H-6a	2.6	35	50				
	H-1b/H-3b (4.1)	H-5b	7.6	185	180	H-5a	0.7	17	14
						OMe	2.0	49	57

[a] Relative (rel.) is % of the absolute NOEs of the reference NOE.

[b] For compounds **1**, **3**, **5**, and **10** residue a is α-Fuc; for compounds **1–9** residue b is β-Gal; for compounds **1** and **7** residue c is β-GlcNAc; for compounds **3** and **8** residue c is β-Glc; for compounds **5** and **9** residue c is β-2-deoxy-Glc.

[c] The value given in parenthesis is the absolute NOE to the proton used for calibration.

[d] The values could not be measured due to overlap.

and that calculated according to an additive scheme. Conventional ΔΔ values were calculated according to eqs (1)–(3) [6–8] in the spectra of trisaccharides of type **TS** containing the two disaccharide fragments **DS1** and **DS2**. In the models **TS**, **DS1**, and **DS2** the substituent **X** represents α- and β-Fuc, **Y** Gal and **Z** GlcNAc, Glc, and 2-deoxy residues, respectively. The index i in eqs (1)–(3) represents the number of the carbon atom.

$$\begin{array}{lll} \mathbf{X} \rightarrow \mathbf{Y} \rightarrow \mathbf{Z}\text{-}\beta\text{-OMe} & \mathbf{X} \rightarrow \mathbf{Y}\text{-}\beta\text{-OMe} & \mathbf{Y} \rightarrow \mathbf{Z}\text{-}\beta\text{-OMe} \\ \mathbf{TS} & \mathbf{DS1} & \mathbf{DS2} \end{array}$$

$$\begin{aligned} \Delta\Delta\mathbf{Y}_{(\mathbf{TS})}i &= \delta\mathbf{Y}_{(\mathbf{TS})}i_{\mathrm{exp}} - \delta\mathbf{Y}_{(\mathbf{TS})}i_{\mathrm{calc}} \\ &= \delta\mathbf{Y}_{(\mathbf{TS})}i_{\mathrm{exp}} - \left[\delta\mathbf{Y}_{(\mathbf{DS2})}i_{\mathrm{exp}} + \Delta\delta\mathbf{Y}_{(\mathbf{DS1})}i_{\mathrm{calc}}\right] \\ &= \delta\mathbf{Y}_{(\mathbf{TS})}i_{\mathrm{exp}} \\ &\quad - \left[\delta\mathbf{Y}_{(\mathbf{DS1})}i_{\mathrm{exp}} + \delta\mathbf{Y}_{(\mathbf{DS2})}i_{\mathrm{exp}} - \delta(\mathbf{Y} - \beta - \mathrm{OMe})\,i_{\mathrm{exp}}\right] \end{aligned} \quad (1)$$

$$\Delta\Delta\mathbf{X}_{(\mathbf{TS})}i = \delta\mathbf{X}_{(\mathbf{TS})}i_{\mathrm{exp}} - \delta\mathbf{X}_{(\mathbf{DS1})}i_{\mathrm{exp}} \quad (2)$$

$$\Delta\Delta\mathbf{Z}_{(\mathbf{TS})}i = \delta\mathbf{Z}_{(\mathbf{TS})}i_{\mathrm{exp}} - \delta\mathbf{Z}_{(\mathbf{DS2})}i_{\mathrm{exp}} \quad (3)$$

The largest ΔΔC values were observed for the C-3 in the GlcNAc residue of compounds **1** and **2** (−5.1 and −3.4 ppm, respectively, cf. Table 4). A significant value of ΔΔC3 (−2.0 ppm) was also detected for the Glc residue in compound **3**. In other

trisaccharides, ΔΔC values for the residue on the reducing end are less significant (< 1 ppm in magnitude).

Analysis of the ΔΔC2 values in the Gal residue of compounds **1**, **3** and **5** shows that for compounds which contain the α-L-Fuc residue this value does not depend much on the size of the substituent at C-2 of the residue on the reducing end. In contrast, only one significant value of ΔΔC2 in the Gal residue is observed in the case of trisaccharides that contain the β-L-Fuc residue (compound **2**).

Significant values of ΔΔC were observed for C-1 in the Gal residue in compounds **1** and **2**, and in the GlcNAc residue of compound **1**. These deviations from additivity proved to be in qualitative correlation with the theoretically calculated and available experimental NOE data discussed below (Table 5).

Conformational analysis.—The conformational analysis using both experimental data (1D NOESY) and molecular modelling was focused on the α-L-Fuc compounds. This series contains the trisaccharides **1**, **3** and **5** and the disaccharide fragments **7**, **8**, **9**, and **10**. The experimental data used are mainly the NOE values from 1D NOESY [25] with a mixing time of 900 ms acquired at 600 MHz (Table 5) and the ^{1}H- and ^{13}C-chemical shifts (Tables 2–4). The three bond ^{1}H–^{1}H coupling constants are not presented. For all the unprotected oligosaccharides these coupling constants only show very small variations between the corresponding values for the different oligosaccharides and therefore confirm that the normal chair conformation predominates in all compounds investigated.

The use of 1D NOESY spectra have been important for the detection of important long range NOEs in addition to the NOEs, normally observed across the glycosidic linkages. These are, e.g., from H-5a and H-6a of the fucose residue to the H-2c of the GlcNAc residue (see Fig. 1, also for labelling of units). These NOEs are very informative for defining the 3-dimensional structure. As the experimental NOE data alone cannot define the conformation of the oligosaccharides, a molecular modelling study was carried out. This was performed using the GEGOP program [26] based on the HSEA force field as described before using both determination of minimum energy conformation (Table 6) and sampling of the conformational space using Metropolis Monte Carlo (MC) simulations [27]. The conformational flexibility is shown in the population maps in Fig. 2 and also presented by the overlap of 25 low energy

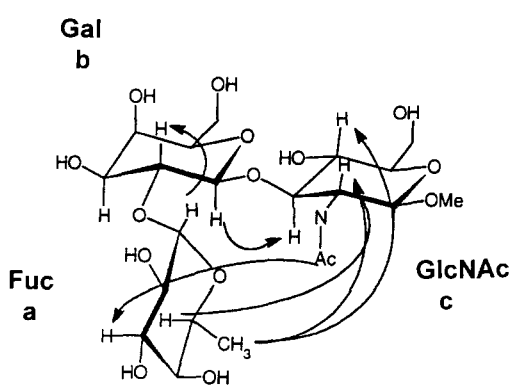

Fig. 1. A schematic representation of the interresidue NOE interactions observed for compound **1**.

Table 6
Dihedral angles (ϕ_H / ψ_H) from GEGOP calculation in the minimum energy conformation and from Monte Carlo simulations

Compound	α-Fuc-(1 → 2)-β-Gal		α-Gal-(1 → 3)-β-Glc-2X		X
	min	avr. MC	min	avr. MC	
1	49/11	49/16	52/5	55/9	NAc
3	49/11	51/18	52/5	52/7	OH
5	51/13	51/18	51/26	52/17	H
7			50/14	51/6	NAc
8			52/5	49/5	OH
9			52/13	51/18	H
10	47/6	44/8			

conformations for compound **1** randomly selected during the MC simulation in Fig. 3. Based on the MC simulations the NOEs were calculated using the full matrix approach and the appropriate mixing time of the experiments. The rotational correlation time used

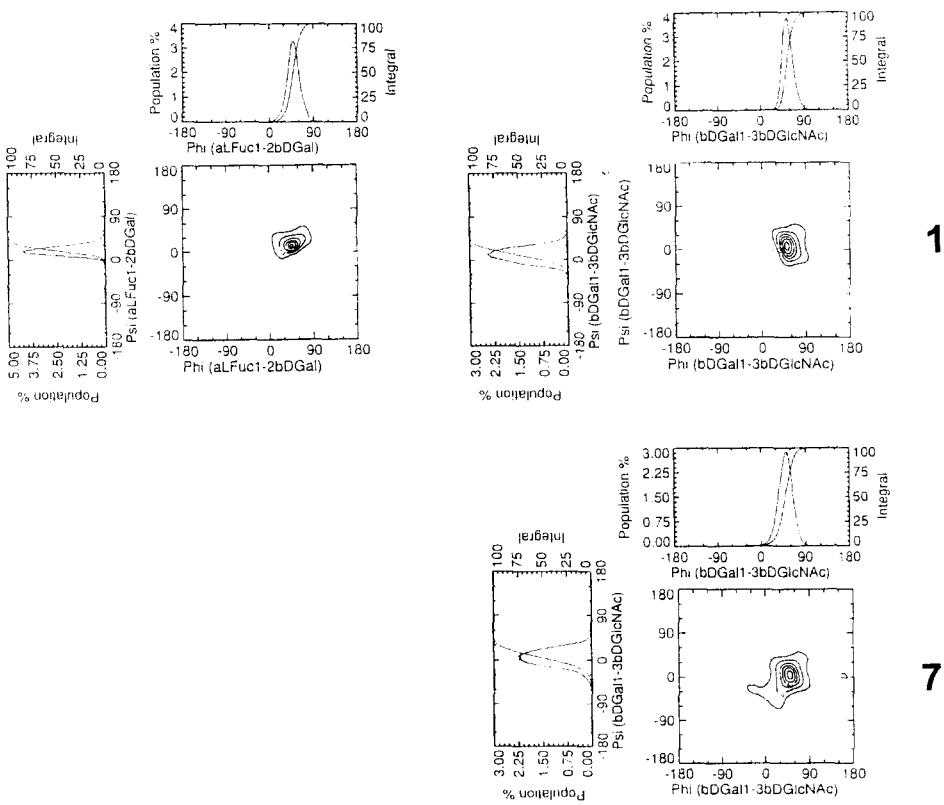

Fig. 2. Population ϕ_H / ψ_H maps for compounds, **1**, **3**, **5** and **7**–**10** for the α-Fuc-(1 → 2)-β-Gal linkage in the left column and for the β-Gal-(1 → 3)-β-Glc-2X linkage in the right column.

were 1.4×10^{-10} s for the di- and 1.7×10^{-10} s for the trisaccharides, after manual optimization of the correlation time to get the best agreement between calculated and measured intra residue NOEs.

In Table 5 NOEs are only included from Monte Carlo simulations, as the correlation between measured and calculated NOEs was generally better, when the full conformational space was included [27]. This improvement was most clearly seen for the 'long range' NOEs between the fucose residue and the reducing end residue in the trisaccharides, which are most sensitive to changes in conformation and flexibility. A clear example is the NOE from H-5a to H-2c in compound **1** (Table 5) which amounts to 63% of that from H-5a to H-4a. The corresponding relative NOE calculated in the minimum energy conformation was 110, while averaging over the full conformation space using MC methods gave a value of 54. Similar trends were seen for the long range NOEs in the compound **3** and **5**, with the NOEs from H-5a being about twice the value in the minimum compared to the MC simulation. The agreement between calculated and measured NOEs across the glycosidic linkages were also improved for some linkages by including the full conformational space, but not for others. The overall agreement is in general compatible with the experimental uncertainty ($\pm 5\%$) and within the scope of such an approach for the assessment of conformational preferences in oligosaccharides.

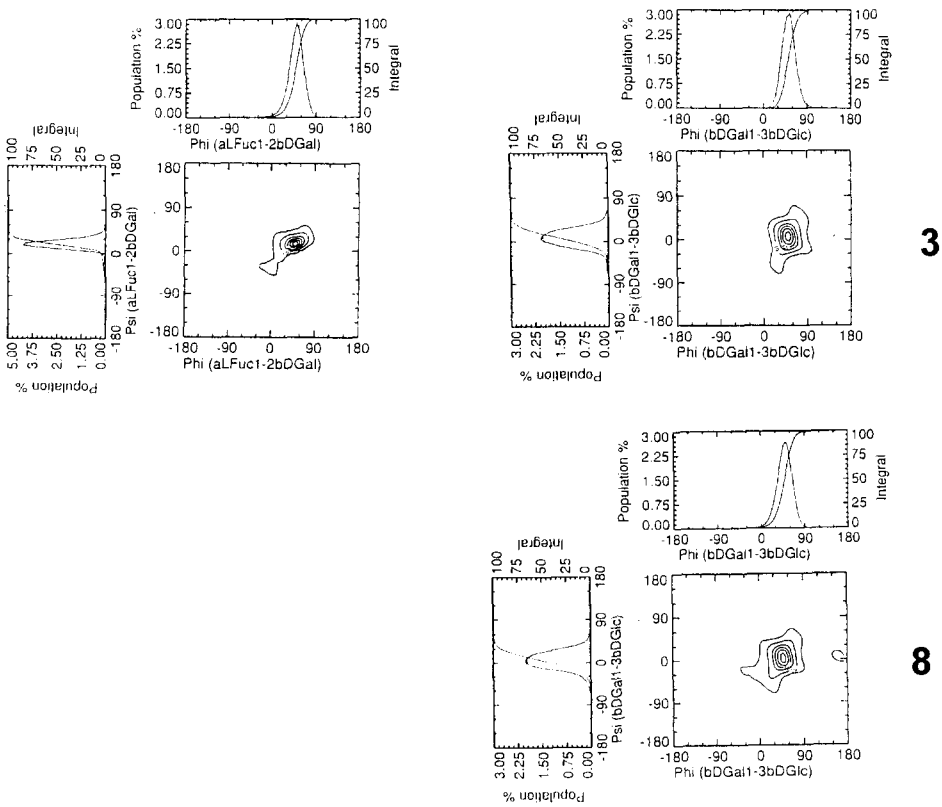

Fig. 2 (continued).

The main interresidue NOE obtained by saturating H-1a of the Fuc is the H-2b of Gal and the magnitude of this NOE relative to H-2a only shows minor variations between the three trisaccharides and the disaccharide **10**. The main deviation is seen for the deoxy trisaccharide **5**, having a slightly smaller NOE from H-1a to H-2b, as also predicted by the Monte Carlo simulation (Table 5).

The NOE from H-1b to the reducing end residue (GlcNAc, Glc or 2-deoxy Glc) is

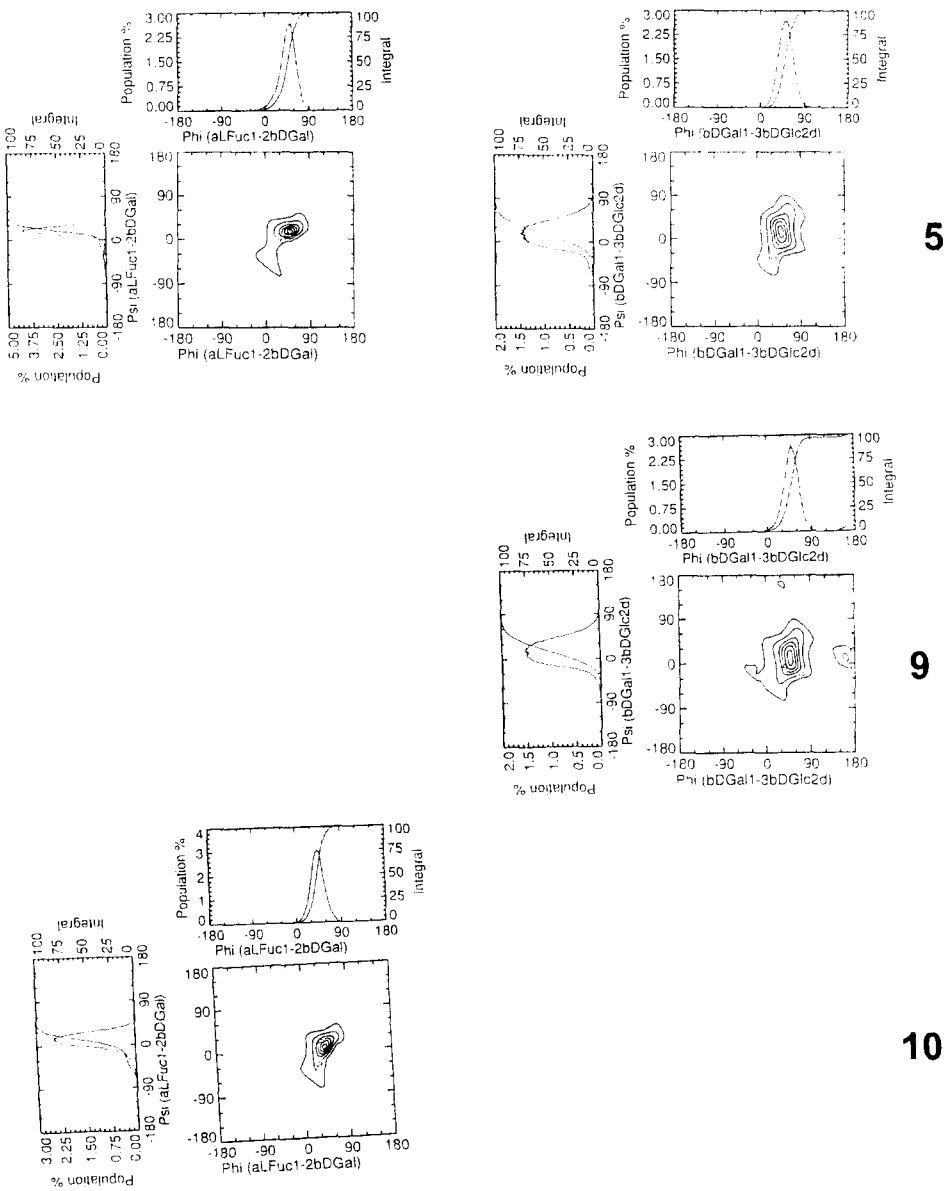

Fig. 2 (continued).

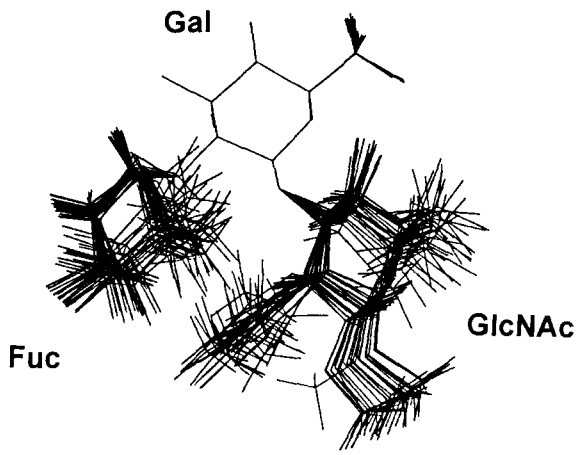

Fig. 3. The overlay of 25 structures samples during a 600 K Monte Carlo simulation of compound **1**. The central β-Gal residues are superimposed.

unfortunately not resolved for all the investigated compounds, thus making it more difficult to compare these data. However, the agreement between calculated and measured NOEs for the H-1b to H-3c and H-2c are not in as good, as for the other NOEs. This can be due to several factors, one of which is, that some anisotropy might exist in the tumbling and thus limits the accuracy of the calculated values. Furthermore, the NOEs observed are strong and correspond to very short distances making a small deviation in the calculated distances resulting in a rather large error in the NOEs. However, the agreement is still reasonable and the good agreement for the 'long range' NOEs indicate that the calculated structures are a good representation of the conformational behavior of the investigated compounds in solution.

The NOEs from H-5a and H-6a of the Fuc residue to the reducing end residue show a strong interaction between these and this changes slightly when the strong interaction between the Fuc residue and N-acetyl group is not present in compounds **3** (OH-group) and **5** (deoxy), respectively.

The 'long range' interactions between the α-Fuc residue and the 'reducing end ' residue are furthermore confirmed by several changes in the ^{1}H chemical shifts of the Fuc unit in the trisaccharides relative to the corresponding disaccharides. A significant shift is seen for H-3a of α-Fuc of compound **1**, which is 0.16 ppm upfield from the H-3 in compound **10**, while only small deviations are seen for the compounds **3** and **5**, respectively. This is most likely related to an interaction with the NAc of the reducing end. For compound **3** the major deviation is seen for H-5 of the α-Fuc residue (0.24 ppm downfield, relative to **10**), as expected for the interaction between the H-5 and O-2 of the β-Glc residue.

For several of the deviations from additivity of the carbon chemical shifts a clear correlation to the observed or calculated NOEs can be observed. For the trisaccharide **1** and the corresponding disaccharide **7** both the results of theoretical NOE calculations and the experimental data show the difference in conformational population about the glycosidic bond in the Gal(1-3)GlcNAc residue. Both the calculated and the experimen-

tal NOEs from H-1b of Gal to the H-3c of GlcNAc in compound **1** are significantly larger than that observed for the corresponding disaccharide **7**. This means a larger population of the conformers with closer contact between anomeric and trans-glycosidic protons in trisaccharide **1**, where the most considerable deviations are observed for carbon atoms about the glycosidic linkage (C-1 of Gal and C-3 of GlcNAc) in comparison with that in disaccharide **7**. The same trend can be seen for the trisaccharide **3** and disaccharide **8**. In case of trisaccharide **5** and disaccharide **9** the calculated NOE values for H-3c are almost the same, and the corresponding deviation from additivity for C-3 of 2-deoxy-Glc and for C-1 of Gal is very small.

The calculated ϕ_H and ψ_H (Table 6) angles or the distances corresponding to NOEs can not account for all deviations observed for compound **1** in itself, and it is therefore most likely that the large deviation for C-3 of GlcNAc is also related to a change in the relative orientation of the N-acetyl group. A small change here can have large effects, due to the magnetic anisotropy of the carbonyl group. This origin of the chemical shift difference is also confirmed by the other changes in chemical shifts, e.g., C-1 of GlcNAc ($\Delta\delta = 1.2$ ppm) and even the OMe carbon signal has a small change in chemical shift ($\Delta\delta = 0.5$ ppm).

All trisaccharides with α-Fuc (**1**, **3**, and **5**) display small negative deviations from additivity for C-2 of Gal. Calculated relative NOE for H-2 in trisaccharides and in the corresponding disaccharide **10** predict small negative deviations from additivity in this case like in the above-mentioned cases. However, the experimental NOE values are at least not in contradiction with these observed deviations.

The qualitative correlation between the values of the deviations from additivity and the calculated and/or experimental NOE values for oligosaccharides may be explained by the Grant and Cheney equation [28]:

$$\Delta\delta^{13}C = 1680 * \exp(-2.671r) * \cos\Theta, \quad (4)$$

which describes the influence of the proton–proton distance (r) on the chemical shifts of the carbons bearing these protons. The increase of the distance between two protons causes the decrease of chemical shifts of the corresponding carbon atoms of the same sign and order as a function of the $\cos\Theta$ term (describing the angle between the carbon–proton vector and the proton–proton vector) as indicated in Fig. 4. As a consequence, all the deviations proved to be in good qualitative agreement with the observed NOE-data.

The other variations in chemical shifts can be related to small changes in the

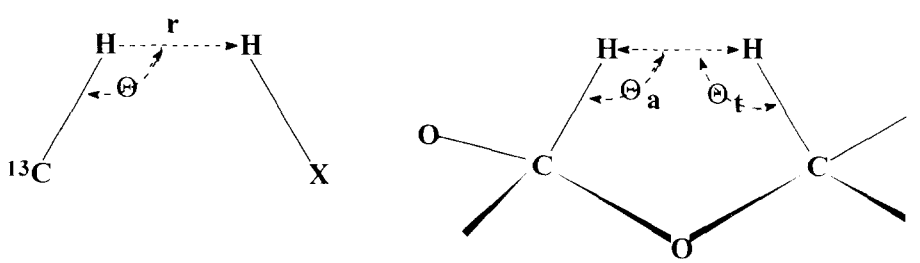

Fig. 4. Dependence of carbon chemical shifts with proton–proton distance as proposed by Grant and Cheney [28].

conformational population of the glycosidic linkage, as clearly seen in the population maps in Fig. 2. Even though the minimum energy conformations and average angles are quite similar for the trisaccharides and corresponding disaccharides, the conformational maps are slightly different. The flexibility is clearly increased going from the trisaccharides to the disaccharides. The flexibility is also clearly increased as the N-acetyl group is replaced by OH and further to H, going from compounds **1** to **3** and to **5**.

The β-Fuc series has not been investigated as closely as the α series, due to severe spectral overlap, but some interesting deviations from additivity can be noted (Table 4). The results for the trisaccharide **2**, which contains β-L-Fuc at position 2 of the Gal and disaccharide **7** proved to be very similar to that observed for the compound **1** and **7**. Significant difference in the relative NOE values of H-3c (72 for **7** and 52 for **2**, which were calculated using the NOE value for H-2, 3, 5 b as a reference) is in agreement with the large deviations from additivity for C-3c (−3.4 ppm) and C-1b (−2.2 ppm), respectively.

A rather the significant positive value of ΔΔC2 of the Gal residue was observed for the trisaccharide **2**. Comparison of experimental relative NOE values for H-2b of compounds **2** and **11** (194 and 113, respectively, taking the NOE value of H-2,3a as a reference) shows that the population of the conformer with a small distance between H-1a and H-2b is larger than in the corresponding disaccharide **7**. In this case a positive deviation from additivity is observed for carbon atoms around the glycosidic linkage (C-2 Gal to C-1 Fuc).

Conclusion.—The results of the detailed NMR investigations presented above clearly demonstrate that the conceptual linear 1–2 linked trisaccharides like compounds **1–6** with respect to conformational preferences and steric interactions have to be treated as branched trisaccharides. The most severe interactions are observed in the α-linked L-Fuc derivatives with an NAc group in the 2-position of the GlcNAc unit, but it can be relieved by substitution by a proton, i.e. the deoxy analogue compound **3**. On the contrary, only few of the β-linked L-Fuc derivatives exhibit severe steric interactions, and only in compound **4** some deviation from additivity of the carbon shift is observed and confirmed by comparison with both chemical shift and NOE data from the corresponding deoxy analogue.

3. Experimental

General methods.—TLC was performed on Silica Gel 60 F_{254} (Merck) with EtOAc-toluene (A, 1:4; B, 1:2), EtOAc (C), and EtOAc-heptane (D, 1:1), and with detection by charring with H_3PO_4. Medium pressure liquid chromatography was performed on Silica Gel L 40–100 μm (C.S.F.R.) by gradient elution with benzene-EtOAc. Optical rotations for substituted compounds **12–23** and **29** were determined on solutions in $CHCl_3$, and for oligosaccharides **2–6** and **9** in water with a Jasco DIP-360 digital polarimeter at 26–30 °C. All solvents used for syntheses were purified according to appropriate procedures. Glycosylation reactions were carried out under argon with freshly distilled solvents.

^{1}H NMR spectra for substituted compounds **12–23** and **29** were recorded in $CDCl_3$ on a Bruker AMX 300 spectrometer at 303 K. ^{1}H NMR spectra for oligosaccharides

1–11 were recorded in D_2O at 316 K on a Bruker AMX 600 NMR instrument and ^{13}C spectra on a Bruker AM500 operating at 125.7 MHz for ^{13}C. One- and two-dimensional spectra were acquired using standard Bruker software.

The molecular modelling was performed using the GEGOP program [26]. Metropolis Monte Carlo simulations were performed [27] at 500 K for the di- and 600 K for the trisaccharides with at least 10^6 Monte Carlo steps. The NOEs were calculated using the r^{-6} average full matrix approach, as described previously [27]. The coordinates for the 2-deoxy-β-Glc residue were constructed from β-Glc using standard bond length and angles with the InsightII program (Biosym, San Diego).

*Methyl 2-acetamido-2-deoxy-[*O*-β-*L*-fucopyranosyl-(1 → 2)*-O*-β-*D*-galactopyranosyl-(1 → 3)]-β-*D*-glucopyranoside* (**2**).—A solution of methyl *O*-(2,3,4-tri-*O*-benzoyl-β-L-fucopyranosyl)-(1 → 2)-*O*-(3-*O*-benzoyl-β-D-galactopyranosyl)-(1 → 3)-2-deoxy-2-phthalimido-β-D-glucopyranoside (**14**) [17] (12.6 mg, 11 μmol) in aq 96% EtOH (10 mL) and 99% hydrazine hydrate (2 mL) was boiled under reflux for 10 h. The mixture was concentrated and water (3 × 3 mL) was codistilled from the residue, which subsequently in MeOH (10 mL) and water (2 mL) was treated with Ac_2O (4 mL) for 17 h at 20°, and finally concentrated. A solution of the product was subjected to gel filtration on fracto-gel TSK HW-40(S) (25–40 mm, V_0 50 mL), in 0.01 M acetic acid, to give amorphous **2** (5.2 mg, 81%) $[\alpha]_D$ +15° (*c* 0.3, H_2O). ^1H and ^{13}C NMR data are presented in Tables 2 and 3.

*Methyl 4,6-*O*-benzylidene-2-(4-chlorobenzyl)-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranoside* (**18**).—A mixture of **27** [18] (206 mg, 0.52 mmol), 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose (**25**) (243 mg, 0.624 mmol), powdered molecular sieves 4A, and CH_2Cl_2 (4 mL) was stirred for 30 min at 20 °C under Ar. The mixture was cooled to −20 to −30 °C and trimethylsilyl triflate (1.4 mmol, 270 μL) was added portionwise during 3 h. The mixture was stirred for 5 h at −20 to −30 °C, filtered through Celite, and washed with 40 mL of $CHCl_3$. The filtrate was washed with water (20 mL), aq $NaHCO_3$ (2 × 15 mL) and water (20 mL), and concentrated. The product was subjected to column chromatography to give **18** (323 mg, 85%), $[\alpha]_D$ −32° (*c* 2, $CHCl_3$), R_F 0.32 (solvent A). The ^1H NMR data are presented in Table 1.

*Methyl 4,6-*O*-benzylidene-3-*O*-(3-*O*-benzoyl-4,6-*O*-benzylidene-β-*D*-galactopyranosyl)-2-(4-chlorobenzyl)-β-*D*-glucopyranoside* (**20**).—A solution of **18** (316 mg, 0.437 mmol) in methanolic 0.1 M MeONa (19 mL) was kept for 15 min at room temp. until *O*-deacetylation was completed (the tetraol has R_F 0.16 (solvent C)). The precipitate was added $CHCl_3$ (10 mL), and the mixture was neutralised with KU-2 (H^+) resin, filtered, and concentrated to dryness. To a solution of the crude residue in MeCN (5 mL) benzaldehyde diethyl acetal (0.15 mL, 1 mmol) and $TsOH \cdot H_2O$ (8 mg) were added. The mixture was stirred for 3 h at 20 °C, MeOH (0.2 mL) was added, stirring was continued for 5 min and pyridine (0.01 mL) was added. The mixture was diluted with $CHCl_3$ (20 mL), washed with water (20 mL), aq $NaHCO_3$ (2 × 15 mL) and water (20 mL). The organic layer was separated, filtered through cotton and concentrated. The residue was washed with heptane (5 × 2 mL) and dried in vacuo. To a stirred solution of the residue in MeCN (12 mL) benzoyl cyanide (60 mg, 0.45 mmol) and triethylamine (1 drop) were added. The mixture was stirred for 2 h, MeOH (1 mL) was added, the

mixture was stirred for 5 min, the solvent was concentrated, and MeOH (5 mL) was coevaporated from the residue. Column chromatography of the product gave **20** (234 mg, 72%), $[\alpha]_D$ +72° (*c* 1, $CHCl_3$), R_F 0.25 (solvent A). The ^1H NMR data are presented in Table 1.

Methyl O-*(2,3,4-tri*-O-*benzoyl*-α-L-*fucopyranosyl)-(1 → 2)*-O-*(3*-O-*benzoyl-4,6*-O-*benzylidene*-β-D-*galactopyranosyl)-(1 → 3)-4,6*-O-*benzylidene-2-(4-chlorobenzyl)*-β-D-*glucopyranoside* (**12**) and methyl O-(2,3,4-tri-O-benzoyl-β-L-*fucopyranosyl)-(1 → 2)*-O-*(3*-O-*benzoyl-4,6*-O-*benzylidene*-β-D-*galactopyranosyl)-(1 → 3)-4,6*-O-*benzylidene-2-(4-chlorobenzyl)*-β-D-*glucopyranoside* (**15**).—A solution of **20** (234 mg, 0.31 mmol), $Hg(CN)_2$ (157 mg, 0.62 mmol), $HgBr_2$ (40 mg), and molecular sieves 4A in MeCN (1 mL) was stirred for 45 min at 20° under Ar. Using a syringe, a solution of **15** (prepared [29] from tetra-*O*-benzoyl-L-fucopyranose (360 mg, 0.62 mmol)) in MeCN (1.5 mL) was added portionwise during 1 h. The mixture was stirred for 5 h, and $CHCl_3$ (10 mL) and satd aq KBr (10 mL were added). The mixture was stirred for 10 min, then filtered through Celite. The organic layer was separated, washed with satd aq KBr and $NaHCO_3$, then filtered through cotton, dried and concentrated. Column chromatography of the residue gave amorphous **12** (67 mg, 18%), $[\alpha]_D$ −64° (*c* 2, $CHCl_3$), R_F 0.40 (solvent A), and **15** (109 mg, 29%), $[\alpha]_D$ −71° (*c* 2, $CHCl_3$), R_F 0.54 (solvent A), and the starting material **20** (103 mg, 45%). The ^1H NMR data for **12** and **15** are presented in Table 1.

Methyl O-α-L-*fucopyranosyl-(1 → 2)*-O-β-D-*galactopyranosyl-(1 → 3)*-β-D-*glucopyranoside* (**3**).—Compound **12** (66 mg, 0.055 mmol) was subjected to catalytic hydrogenolysis in EtOH–EtOAc (1:2, 12 mL) with 10% Pd–C at 41 °C and atm. pressure for 20 h. The mixture was filtered, the solvent was evaporated in vacuo, and the residue was then *O*-deacetylated as described for the preparation of **20** and subjected to gel filtration as described for **2** to give amorphous **3** (25 mg, 91%), $[\alpha]_D$ −68° (*c* 1, H_2O). The ^1H and ^{13}C NMR data are presented in Tables 2 and 3.

Methyl O-β-L-*fucopyranosyl-(1 → 2)*-O-β-D-*galactopyranosyl-(1 → 3)*-β-D-*glucopyranoside* (**4**).—Compound **15** (109 mg, 0.09 mmol) was deblocked as described for the preparation of **3**, to give amorphous **4** (39 mg, 87%), $[\alpha]_D$ +6° (*c* 1, H_2O). The ^1H and ^{13}C NMR data are presented in Tables 2 and 3.

Methyl 4,6-O-*benzylidene-2-deoxy*-β-D-*glucopyranoside* (**29**).—2,3,4-Tri-*O*-acetyl-glucal (1.63 g, 6.0 mmol) was chloromercurated and reduced with $NaBH_4$, as described [30,31], but without crystallization of intermediates, to give a mixture of methyl 2,3,4-tri-*O*-acetyl-2-deoxy-D-glucosides. The product was subjected to column chromatography, followed by crystallization from EtOAc (5 mL)–heptane (5 mL) to give crystalline **28** (390 mg, 21%). Compound **28** (262 mg, 0.86 mmol) was deacetylated and benzylidenated as described above for the preparation of **20** to give **29** (126 mg, 55%). ^1H NMR data are presented in Table 1.

Methyl 4,6-O-*benzylidene-3*-O-*(2,3,4,6-tetra*-O-*acetyl*-β-D-*galactopyranosyl)-2-deoxy*-β-D-*glucopyranoside* (**21**).—A solution of **29** (58 mg, 0.22 mmol), $Hg(CN)_2$ (111 mg, 0.44 mmol), $HgBr_2$ (10 mg), and molecular sieves 4A in MeCN (1 mL) was stirred for 45 min at 20° under Ar. Using a syringe, a solution of **26** (prepared from penta-*O*-acetyl-D-galactopyranose (172 mg, 0.44 mmol)) in MeCN (1 mL) was added portionwise during 1 h. The mixture was stirred for 5 h, and $CHCl_3$ (10 mL) and satd aq

KBr (10 mL) were added. The mixture was stirred for 10 min and filtered through Celite. The organic layer was separated, washed with satd aq KBr and $NaHCO_3$, filtered through cotton, dried and concentrated. Column chromatography of the residue gave amorphous **21** (133 mg, 98%), $[\alpha]_D$ −24° (*c* 1, $CHCl_3$). R_F 0.20 (solvent D). The ^1H NMR data are presented in Table 1.

Methyl 4,6-O-benzylidene-3-O-(4,6-O-benzylidene-β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranoside (**22**).—A solution of **21** (133 mg, 0.22 mmol) was deacetylated and benzylidenated as described for the preparation of **20**. Column chromatography gave amorphous **22** (62 mg, 54%), $[\alpha]_D$ −44° (*c* 1, $CHCl_3$), R_F 0.15 (solvent B). The ^1H NMR data are presented in Table 1.

Methyl 3-O-(3-O-benzoyl-4,6-O-benzylidene-β-D-galactopyranosyl)-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (**23**).—Compound **22** (62 mg, 0.12 mmol) was benzoylated with benzoyl cyanide (16.2 mg, 0.13 mmol) in the presence of triethylamine as described for preparation of **20** to give amorphous **23** (36 mg, 54%), $[\alpha]_D$ +43° (*c* 1, $CHCl_3$), R_F 0.30 (solvent B). The ^1H NMR data are presented in Table 1.

Methyl O-*(2,3,4-tri-O-benzoyl-α-L-fucopyranosyl)-(1 → 2)-O-(3-O-benzoyl-4,6-O-benzylidene-β-D-galactopyranosyl)-(1 → 3)-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside* (**13**) and *methyl* O-*(2,3,4-tri-O-benzoyl-β-L-fucopyranosyl)-(1 → 2)-O-(3-O-benzoyl-4,6-O-benzylidene-β-D-galactopyranosyl)-(1 → 3)-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside* (**16**).—Glycosylation of **23** (23 mg, 0.037 mmol) with **13** {prepared [29] from tetra-*O*-benzoyl-L-fucopyranose (43 mg, 0.074 mmol)}, as for the synthesis of **12** and **15**, gave amorphous **13** (9 mg, 22%), $[\alpha]_D$ −35° (*c* 0.5, $CHCl_3$), R_F 0.34 (solvent D), and **16** (17 mg, 43%) $[\alpha]_D$ −13° (*c* 1, $CHCl_3$), R_F 0.39 (solvent D). The ^1H NMR data for **13** and **16** are presented in Table 1.

Methyl O-*α-L-fucopyranosyl-(1 → 2)-O-β-D-galactopyranosyl-(1 → 3)-2-deoxy-β-D-glucopyranoside* (**5**).—A solution of compound **13** (8.6 mg, 8 μmol) in $CHCl_3$ was treated with aq 90% trifluoroacetic acid (0.2 mL) during 30 min at room temperature and toluene (3 × 5 mL) was codistilled from the mixture, and it was concentrated to dryness. Deacetylation of the residue as described above for the preparation of **20** gave amorphous **5** (3.4 mg, 88%), $[\alpha]_D$ −65° (*c* 0.2, H_2O). The ^1H and ^{13}C NMR data are presented in Tables 2 and 3.

Methyl O-*β-L-fucopyranosyl-(1 → 2)-O-β-D-galactopyranosyl-(1 → 3)-2-deoxy-β-D-glucopyranoside* (**6**).—Compound **16** (17.0 mg, 0.015 mmol) was deblocked as described for the preparation of **5**, to give amorphous **6** (6.5 mg, 90%), $[\alpha]_D$ −6° (*c* 0.4, H_2O). The ^1H and ^{13}C NMR data are presented in Tables 2 and 3.

Methyl 3-O-(β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranoside (**9**).—Hydrolysis and saponification of the compound **20** (25 mg, 0.04 mmol) followed by gel-filtration as described above gave amorphous **9** (12 mg, 95%), $[\alpha]_D$ −17° (*c* 1, H_2O). The ^1H and ^{13}C NMR data are presented in Tables 2 and 3.

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