Synthesis and Structural Studies of "Branched" 2-Linked Trisaccharides Related to H-Type 2 Blood Group Determinants

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Abstract. A series of fucosylated trisaccharides L-Fuc-(1→2)-β-D-Gal-(1→4)-β-X-OMe (**1**–**6**, X = D-GlcNAc, D-Qui (6-deoxy-Glc), D-Xyl) related to H type 2 blood group determinant have been synthesized both as their α- and β- L-Fuc anomers together with the component disaccharide starting compounds (**7–11**). The conformational properties of the 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 program with the main focus on the α-linked fucose series.

The series of compounds allow for the investigation of interaction between the sugar units in the—in principle—linear structures, which in practice behave as branched trisaccharides.

The interaction between the terminal fucose unit and the unit at the reducing end has been probed by substitution of the bulky CH_2OH group with CH_3 and H substituents, respectively. Compounds with severe steric interactions can be identified by the non-additivity of their carbon chemical shifts and subsequently confirmed by the detailed conformational assessment by NOEs and computer modeling. The most severe contacts arise in the GlcNAc and Qui trisaccharide series, whereas the Xyl-containing trisaccharide derivatives only exhibit weak steric interaction as probed by the NMR parameters.

INTRODUCTION

Complex carbohydrates, such as glycoproteins or polysaccharides, play an important role in different natural biochemical processes.[1] Common for most of these is the carbohydrate–protein interaction, as pioneered by Lemieux.[2] The investigation of oligosaccharide spectral and conformational properties, as well as of the principles determining their spatial organization, has been a constantly challenging field in the area of carbohydrate research.[3,4] In this context, of major interest are the oligosaccharides which are characterized by intramolecular interactions regulating the conformational shape of the molecule even though complex carbohydrates generally do not exhibit the extensive backfolding and long-range interactions found in proteins. We investigated the conformations of the methyl glycoside of H type 1 (Le^d) trisaccharide α-L-Fuc-(1→2)-β-D-Gal-(1→3)-β-D-GlcNAc-OMe, and its Glc, 2-deoxy-Glc, and β-fucosyl analogues, and showed that the spatial interaction of the Fuc unit and the substituent at C-2 of the monosaccharide residue at the reducing end influence the conformational shape of the whole molecule and distinguish it from the shape of constituent disaccharides.[5] In a continuation of the research of NMR and conformational properties of linear trisaccharide molecules with (1→2)-linkage at non-reducing end, we studied the methyl glycoside of H-type 2 trisaccharide (**1**) and its derivatives **2**–**6**. Here the anomeric configuration of the Fuc unit and the nature of the substituent at C-5 of the residue at the reducing end were varied. The

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H-type 2 structure is of great general interest and especially as Lemieux and coworkers[6] synthesized **1** and **5**, among other analogues, and studied the interaction with three lectins to identify the key polar groups.

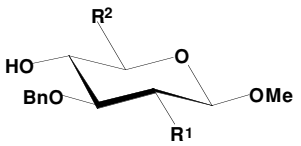
The present studies were performed to assess the spatial interactions between the units at reducing and non-reducing ends in the linear trisaccharides with the (1→2)-(1→4) sequence of glycosidic linkages and their influence on the conformational behavior of the molecules. The investigations were concluded with NMR studies of the oligosaccharides and aimed to rationalize some special effects, in particular the deviations from additivity of ^{13}C chemical shifts[5,7–10] that could be observed in ^{13}C NMR spectra of oligosaccharides with (1→2)-linkage at the non-reducing end. Among others, such results are required for the development of computer-assisted methods used in the structural analysis of oligo- and polysaccharides.[8,11] The results of such investigations of the trisaccharides **1**–**6** and their constituent disaccharide fragments **7**–**11** are presented here.

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

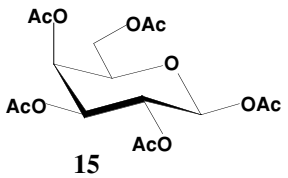
RESULTS AND DISCUSSION

Synthesis of Model Oligosaccharides

Glycosylation of methyl 2-deoxy-3,6-di-*O*-benzyl-2-phthalimido-β-D-glucopyranoside (**12**),[12] methyl 2,3-di-*O*-benzyl-β-D-quinovopyranoside (**13**),[13] and methyl 2,3-di-*O*-benzyl-β-D-xylopyranoside (**14**) by 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose (**15**) in the pres-



12 R^1=NPhth, R^2 =CH_2OBn;
13 R^1=OBn, R^2 =CH_3;
14 R^1=OBn, R^2 =H;



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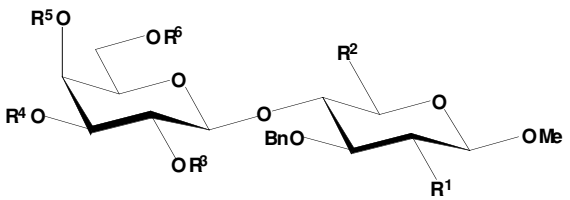
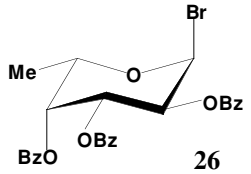


16 R^1=NPhth, R^2=CH_2OBn, R^3=R^4=R^5=R^6=Ac
8 ← **17** R^1=OBn, R^2=CH_3, R^3=R^4=R^5=R^6=Ac
9 ← **18** R^1=OBn, R^2=H, R^3=R^4=R^5=R^6=Ac
19 R^1=NPhth, R^2=CH_2OBn, R^3=R^4=H, R^5+R^6=PhCH
20 R^1=OBn, R^2=CH_3, R^3=R^4=H, R^5+R^6=PhCH
21 R^1=OBn, R^2=H, R^3=R^4=H, R^5+R^6=PhCH
22 R^1=NPhth, R^2=CH_2OBn, R^3=Bz, R^4=H, R^5+R^6=PhCH
23 R^1=OBn, R^2=CH_3, R^3=H, R^4=Bz, R^5+R^6=PhCH
24 R^1=OBn, R^2=H, R^3=H, R^4=Bz, R^5+R^6=PhCH
25 R^1=NPhth, R^2=CH_2OBn, R^3=H, R^4=Bz, R^5+R^6=PhCH



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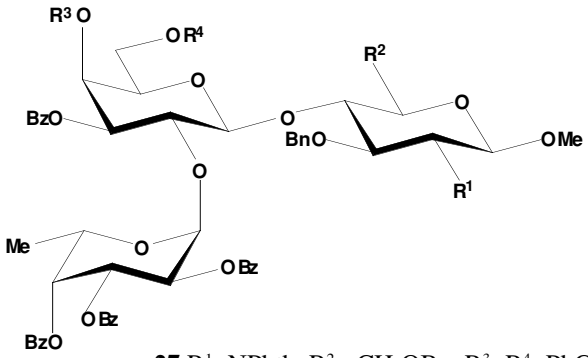
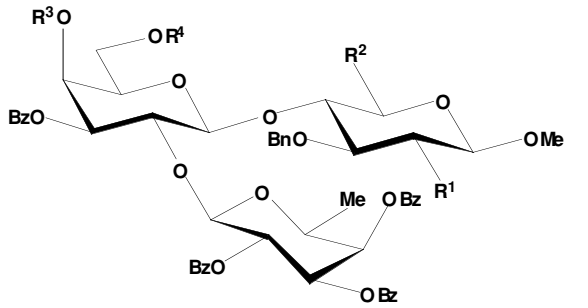
Scheme 1



27 R^1=NPhth, R^2 =CH_2OBn, R^3+R^4=PhCH
1 ← **28** R^1=NPhth, R^2=CH_2OBn, R^3=R^4=Ac
3 ← **29** R^1=OBn, R^2=CH_3, R^3=R^4=H
5 ← **30** R^1=OBn, R^2=R^3=R^4=H



31 R^1=NPhth, R^2 =CH_2OBn, R^3+R^4=PhCH
2 ← **32** R^1=NPhth, R^2=CH_2OBn, R^3=R^4=Ac
4 ← **33** R^1=OBn, R^2=CH_3, R^3=R^4=H
6 ← **34** R^1=OBn, R^2=R^3=R^4=H

Scheme 2

ence of trimethylsilyl triflate afforded disaccharide derivatives **16**–**18** in 85, 86, and 42% yields, respectively.[14] The β-configuration of the Gal unit in **16**–**18** was established from the ^{1}H NMR data presented in Table 1 ($J_{1,2}$ 7.5, 7.5, and 8.0 Hz, respectively).

O-Deacetylation of **16**–**18** afforded the corresponding tetraols which, on treatment with benzaldehyde dimethyl acetal, gave the 4′,6′-*O*-benzylidene derivatives **19**–**21**. By selective benzoylation upon treatment with benzoyl cyanide in the presence of a catalytic amount of triethylamine,[15] diols **19**–**21** were transformed into the respective 3′-benzoates **22**–**24** in 75, 66, and 77% yields, respectively. Benzoylation of **19** was also accompanied by formation of 20% of 2′-benzoylated product **25**. The location of benzoyl groups was indicated by downfield ^{1}H NMR chemical shifts of the H-3′ resonances in spectra **22**–**24** and of H-2′ resonance in the spectrum of **25** (Table 1).

Glycosylation of **22**–**24** was accomplished by benzobromofucose **26** under Helferich conditions[16] in order to obtain products of both α- and β-fucosylation in the same glycosylation reaction. It should be noted that, to favor the α-fucosylation,[16] acceptors **22**–**24** with Bz-substituent in the neighboring position to the glycosylated OH group were used.

As expected, fucosylation of **22**–**24** was not stereospecific and gave pairs of isomeric trisaccharides in 70–80% yields with α:β ratios of 1:2 in the case of acceptors **22** and **23** and 1:4 in the case of **24**. Lower selectivity of β-fucosylation **22** and **23** may be connected[16] with the shielding effect of benzyloxymethyl and methyl groups at C-5 of GlcNAc and Qui residues. Isomers **27** and **31** were separated in the form of their respective 4′,6′-di-O-acetates **28** and **32**, which were obtained after removal of the benzylidene group and subsequent acetylation. The products of fucosylation of **23** and **24** were separated

Table 1. ^{1}H NMR data for substituted derivatives **13, 14, 17, 18, 22–25, 28–30,** and **32–34**($CDCl_3$)

		chemical shifts (δ, ppm)									
compound	residue	H-1	H-2	H-3	H-4	H-5a	H-5e	H-6	H-6'	$C(O)CH_3$	$O\text{-}CH_3$
13	β-Qui	4.35	3.43–3.50		3.33–3.41			1.37			3.62
14	β-Xyl	4.39	3.41–3.52		3.70	3.30	4.02				3.57
17	β-Gal	4.83	5.20	4.96	5.30	3.63		4.00	3.80	1.8–2.1	
	β-Qui	4.29	3.40			3.52		1.33			3.56
18	β-Gal	4.63	5.19	5.02	5.37	3.85		4.15	4.00	2.0–2.2	
	β-Xyl	4.26	3.35	3.56	3.88	3.20	3.95				3.54
22	β-Gal	4.72	4.08	4.99	4.36			4.20	3.88		
	β-GlcNAc	5.04	4.45	4.15–4.26		3.68		4.08	3.82		3.39
23	β-Gal	4.72	4.22	5.13	4.42	3.57		4.22	3.90		
	β-Qui	4.34	3.49	3.69		3.52		1.50			3.58
24	β-Gal	4.60	4.24	5.14	4.48	3.59		4.31	4.04		
	β-Xyl	4.33	3.45	3.66	4.04	3.34	4.15				3.56
25	β-Gal	4.79	5.40	3.73	4.18	3.99		4.36	4.17		
	β-GlcNAc	4.98	4.17	4.33	4.15	3.43		3.86	3.66		3.34
28	α-Fuc	5.73	5.68	5.80	5.83	4.79		1.40			
	β-Gal	4.74	4.31	5.28	5.47			4.50		2.0–2.18	
	β-GlcNAc	5.25	4.20								3.53
29	α-Fuc	5.67	5.72	5.83	5.77	4.89		1.28			
	β-Gal	4.75	4.40	5.09	4.19						
	β-Qui	4.35	3.44	3.66	3.50	3.46					3.65
30	α-Fuc	5.66	5.74	5.85	5.75	4.88		1.16			
	β-Gal	4.70	4.36	5.09	4.25						
	β-Xyl	4.40	3.45	3.62							3.61
32	β-Fuc	5.03	5.67	5.43	5.53			0.95			
	β-Gal	4.67			5.55					1.9–2.1	
	β-GlcNAc	4.87									3.40
33	β-Fuc	5.07	5.70	5.41	5.52	3.55		0.9			
	β-Gal	4.63	4.26	5.24	4.23	3.42		3.65	3.65		
	β-Qui	4.06	3.20			3.20		1.25			3.51
34	β-Fuc	5.04	5.65	5.47	5.56	3.72		1.09			
	β-Gal	4.51	4.24	5.14	4.29	3.46		3.75	3.75		
	β-Xyl	4.12	3.15–3.26		3.74	3.08	3.81				3.50

Table 1. *continued*

compound	residue	coupling constants (Hz)								
		$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5a}$	$J_{4,5e}$	$J_{5,5'}$	$J_{5,6}$	$J_{5,6'}$	$J_{6,6'}$
13	β-Qui									6.0
14	β-Xyl	6.0			8.5	4.5	11.0			
17	β-Gal	7.5	10.0	3.5	<1			8.0	5.5	11.0
	β-Qui	7.5	9.0					6.0		
18	β-Gal	8.0	10.0	3.5	<1			7.5	6.0	11.0
	β-Xyl	7.5	9.0							
22	β-Gal	7.5	10.5	3.0	<1				2.5	
	β-GlcNAc	8.0	11.0						2.6	
23	β-Gal	7.5	10.0	3.5	<1			7.5	1.5	10.0
	β-Qui	7.5	9.0					6.0		
24	β-Gal	7.5	10.0	3.5	<1			<1	1.5	13.0
	β-Xyl	7.0	8.5	8.5	7.5	5.0	11.5			
25	β-Gal	7.5	10.0					<1		
	β-GlcNAc	8.0						<1	3.5	11.5
28	α-Fuc	3.5								
	β-Gal	7.5	10.5	3.5	11.0					
	β-GlcNAc	7.5								
29	α-Fuc	3.5	10.0	3.0	<1			6.5		
	β-Gal	8.0	10.0	3.0	<1					
	β-Qui	7.5						6.0		
30	α-Fuc	3.5	10.5	3.0	<1			6.5		
	β-Gal	7.5	10.0							
	β-Xyl	7.0	9.5							
32	β-Fuc	7.5	10.0							
	β-Gal	7.5								
	β-GlcNAc	7.5								
33	β-Fuc	8.0	10.5	3.5	<1			6.5		
	β-Gal	7.5	10.0	3.5	<1					
	β-Qui	7.5						6.0		
34	β-Fuc	7.5	10.0	3.0	<1			6.5		
	β-Gal	8.0	10.0	3.5	<1			4.5	4.5	
	β-Xyl	7.0							9.5	11.5

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

		chemical shifts (δ, ppm)									
compound	residue	H-1	H-2	H-3	H-4	H-5	H-5'	H-6	H-6'	$C(O)CH_3$	$O\text{-}CH_3$
1	α-Fuc	5.310	3.795	3.820	3.820	4.228		1.233			
	β-Gal	4.537	3.678	3.876	3.906	3.704		3.794	3.736		
	β-GlcNAc	4.456	3.743	3.670	3.768	3.474		3.991	3.809	2.040	3.508
2	β-Fuc	4.569	3.521	3.651	3.756	3.799		1.274			
	β-Gal	4.668	3.773	3.740	3.990	3.715		3.784	3.737		
	β-GlcNAc	4.469	3.738	3.724	3.781	3.606		4.027	3.908	2.040	3.511
3	α-Fuc	5.297	3.815	3.816	3.819	4.266		1.224			
	β-Gal	4.611	3.675	3.875	3.905	3.693		3.741	3.802		
	β-Qui	4.346	3.319	3.523	3.479	3.519		1.389			3.548
4	β-Fuc	4.587	3.521	3.655	3.758	3.798		1.276			
	β-Gal	4.716	3.786	3.732	3.991	3.710		3.760	3.812		
	β-Qui	4.370	3.315	3.591	3.479	3.652		1.419			3.558
5	α-Fuc	5.246	3.794	3.829	3.819	4.259		1.232			
	β-Gal	4.592	3.627	3.850	3.906	3.696		3.748	3.803		
	β-Xyl	4.319	3.306	3.562	3.876	3.329	4.148				3.542
6	β-Fuc	4.593	3.502	3.659	3.747	3.782		1.264			
	β-Gal	4.660	3.724	3.722	3.981	3.708		3.758	3.808		
	β-Xyl	4.342	3.299	3.616	3.856	3.452	4.162				3.546
7	β-Gal	4.477	3.550	3.671	3.937	3.753		3.783	3.750		
	β-GlcNAc	4.477	3.732	3.730	3.710	3.600		4.004	3.839	2.038	3.512
8	β-Gal	4.500	3.551	3.667	3.938	3.723		3.759	3.798		
	β-Qui	4.378	3.332	3.583	3.397	3.638		1.379			3.559
9	β-Gal	4.470	3.510	3.645	3.925	3.706		3.752	3.815		
	β-Xyl	4.347	3.303	3.601	3.837	3.411	4.109				3.548
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		3.830	3.830		3.631

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

		chemical shifts (δ, ppm)							
compound	residue	C-1	C-2	C-3	C-4	C-5	C-6	$NHCO\underline{C}H_3$	$O\text{-}CH_3$
1	α-Fuc	100.2	69.1	70.4	72.5	67.7	16.1		
	β-Gal	101.1	77.2	74.4	69.9	76.1	61.8		
	β-GlcNAc	102.7	56.0	73.2	77.2	76.1	61.0	22.7	57.9
2	β-Fuc	103.0	71.7	73.8	72.1	71.7	16.1		
	β-Gal	101.8	79.1	72.4	69.0	75.9	61.8		
	β-GlcNAc	102.6	56.0	73.1	77.8	75.7	61.1	23.0	57.8
3	α-Fuc	100.0	68.9	70.3	72.3	67.5	15.8		
	β-Gal	101.1	77.1	74.2	69.8	75.8	61.7		
	β-Qui	103.7	73.7	74.8	81.7	71.8	17.3		57.7
4	β-Fuc	102.8	71.5	73.5	71.9	71.4	15.9		
	β-Gal	101.7	78.6	72.3	68.9	75.7	61.6		
	β-Qui	103.6	73.8	74.7	82.5	71.8	17.7		57.8
5	α-Fuc	100.1	69.0	70.2	72.4	67.5	15.8		
	β-Gal	100.4	77.4	74.2	69.5	75.7	61.6		
	β-Xyl	104.6	73.4	74.8	76.7	63.5			57.6
6	β-Fuc	102.8	71.4	73.4	71.9	71.3	16.0		
	β-Gal	101.5	78.3	72.2	68.8	75.7	61.6		
	β-Xyl	104.4	73.4	74.7	77.4	63.6			57.7
7	β-Gal	103.7	71.8	73.3	69.4	76.1	61.8		
	β-GlcNAc	102.6	55.8	73.3	79.6	75.6	61.0	23.0	57.8
8	β-Gal	103.8	71.7	73.2	69.2	75.9	61.6		
	β-Qui	103.6	73.6	74.9	84.5	71.5	17.2		57.8
9	β-Gal	102.4	71.3	73.3	69.2	75.9	61.7		
	β-Xyl	104.4	73.4	74.6	77.2	63.6			57.7
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

after O-debenzylidenation in the form of diols **29** and **33**, and **30** and **34**, respectively. The anomeric configuration of Fuc residues in **27–34** was established on the basis of the values of the coupling constants $J_{1,2}$ in their ^{1}H NMR spectra (Table 1).

Removal of the blocking groups in the substituted derivatives **17**, **18**, **28–30** and **32–34** gave the target oligosaccharides **8**, **9,** and **1–6**, which were purified by gel filtration. Preparation of oligosaccharides **7**,[17,18] **10**,[19] and **11**[19] was 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 ^{1}H and ^{13}C NMR spectra was performed as previously reported by both homo- and heteronuclear 2-D experiments.[20] The assignment of ^{1}H for **1** and **5** is in good agreement with the previous partial assignment[6] and likewise the tentative ^{13}C chemical shift assignment by Lemieux and coworkers[6] was confirmed here using 2-D heteronuclear experiments.

One of the main aims in this work was examination of the deviations from additivity values (ΔΔ) of chemical shifts in ^{13}C NMR spectra of oligosaccharides **1–6**. ΔΔ values represent the difference between the experimental ^{13}C chemical shifts and that calculated according to an additive scheme. Conventional ΔΔ values were calculated according to eqs 1–3 [*on opposite page*] which are for calculation of such parameters[5] in the spectra of trisaccharides of type **TS** and containing two disaccharide fragments **DS1** and **DS2**. In the models **TS**, **DS1**, and **DS2** the substituent ***X*** represents α- and β-Fuc units, ***Y*** is Gal, and ***Z*** represents GlcNAc, Qui, and Xyl moieties. Index *i* in eqs 1–3 is the number of the carbon atom.

Significant ΔΔ values of –1.3 to –2.8 ppm (Table 4) were observed for the GlcNAc and Qui trisaccharide series: for C-1 Gal, C-2 Gal, and C-4 GlcNAc in the case of α-fucosylated derivatives **1** and **3**, and for C-1 Gal and C-4 GlcNAc for compounds **2** and **4** with β-fucosyl units. In the spectra of Xyl-containing trisaccharide **5** and **6** the deviations from additivity were less significant, and the largest one, of –1.2 ppm, was observed for C-2 Gal in **5**.

Deviations from additivity of chemical shifts of respective carbons in the spectra of trisaccharides from the GlcNAc and Qui series were of similar values. This indicates that the hydroxymethyl and methyl groups at C-5 of the unit at the reducing end influence to an equal

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

compound	residue	C-1	C-2	C-3	C-4	C-5	C-6
	α-Series						
1	α-Fuc	–0.5	0	0.1	0	0.2	0.3
3	α-Fuc	–0.7	–0.2	0	–0.2	0	0
5	α-Fuc	–0.6	–0.1	–0.1	–0.1	0	0
1	β-Gal	**–1.3**	**–1.9**	0.9	0.6	0.4	0.2
3	β-Gal	**–1.4**	**–1.9**	0.8	0.7	0.3	0.3
5	β-Gal	–0.7	**–1.2**	0.7	0.4	0.2	0.1
1	β-GlcNAc	0.1	0.2	–0.1	**–2.4**	0.5	0
3	β-Qui	0.1	0.1	–0.1	**–2.8**	0.3	0.1
5	β-Xyl	0.2	0	0.2	–0.5	–0.1	
	β-Series						
2	β-Fuc	–0.2	0	0.2	0	0.1	–0.1
4	β-Fuc	–0.4	–0.2	–0.1	–0.2	–0.2	–0.3
6	β-Fuc	–0.4	–0.3	–0.2	–0.2	–0.3	–0.2
2	β-Gal	**–1.6**	0	0.5	0	0	0.1
4	β-Gal	**–1.8**	–0.4	–0.4	0.1	0	0.1
6	β-Gal	–0.7	–0.3	0.4	0	0	0
2	β-GlcNAc	0	–0.2	–0.2	**–1.8**	0.1	0.1
4	β-Qui	0	0.2	–0.2	**–2.0**	0.3	0.5
6	β-Xyl	0	0	0.1	0.2	0	

[a] See formula in text.

	X*→*Y*→*Z-β-OMe	***X*→*Y***-β-OMe	***Y*→*Z***-β-OMe
	TS	DS1	DS2

$$\Delta\Delta\delta\boldsymbol{Y}(\mathbf{TS})i = \delta\boldsymbol{Y}(\mathbf{TS})i_{exp} - \delta\boldsymbol{Y}(\mathbf{TS})i_{calc} = \delta\boldsymbol{Y}(\mathbf{TS})i_{exp} - [\delta\boldsymbol{Y}(\mathbf{DS2})i_{exp} + \Delta\delta\boldsymbol{Y}(\mathbf{DS1})i_{calc}] =$$
$$= \delta\boldsymbol{Y}(\mathbf{TS})i_{exp} - [\delta\boldsymbol{Y}(\mathbf{DS2})i_{exp} + \delta\boldsymbol{Y}(\mathbf{DS2})i_{exp} - \delta(\mathbf{Y}\text{-}\beta\text{-OMe})i_{exp}] \quad (1)$$

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

$$\Delta\Delta\delta\boldsymbol{Z}(\mathbf{TS})i = \delta\boldsymbol{Z}(\mathbf{TS})i_{exp} - \delta\boldsymbol{Z}(\mathbf{DS1})i_{exp} \quad (3)$$

extent the NMR and conformational properties of trisaccharides. The substitution by H (transfer to Xyl series with additive ^{13}C NMR spectra) partly removes these conformational restrictions.

Conformational Analysis

The conformational analysis based on 1D NOESY[21] data and GEGOP Monte Carlo (MC) simulation[22,23] has been focused on the α-linked fucose compounds, trisaccharides **1**, **3**, **5**, and the corresponding reference disaccharides **7**, **8**, **9**, and **10**. The use of 1D NOESY measurements (900 ms mixing time, 600 MHz; see Table 5) generally provides many high quality data relating to the three-dimensional structure. Unfortunately, severe overlap is seen for several compounds, especially for the disaccharide **7**, where the two anomeric protons have exactly the same chemical shifts (Table 2). For all compounds the chemical shifts are assigned (Tables 2 and 3). The three-bond ^{1}H–^{1}H coupling constants are measured, but are not reported as these generally have standard values for the types of monosaccharide residues and thereby indicate that normal ring conformations are present.

The measured NOESY data are compared to calculated values from GEGOP MC simulations,[22,23] based on the HSEA force field.[24] Only calculated values from full MC simulations are presented (Table 5), as both this study and previous results[5] show that these give better agreement with experimental data than NOESY values calculated only from the global energy minimum (Table 6). The NOESY values are calculated based on a full matrix relaxation model assuming isotropic tumbling (mixing time 900 ms, rotational correlation time 1.4×10^{-10} s for the disaccharides and 1.7×10^{-10} s for the trisaccharides). No attempts to introduce internal motion or non-isotropic tumbling models were carried out, as the correlation between measured and calculated data doesn't validate a more complex model, keeping in mind also a limited accuracy in any experimental measurement. The population maps for the compounds investigated are presented in Fig. 1.

For the compounds investigated, the NOESY data provide valuable information about the conformational properties when used in combination with the MC simulations. When well separated signals are observed the correlation between measured and calculated data is good. The expected NOEs between anomeric protons and the protons at the point of attachment are always seen and also smaller NOEs to protons at adjacent positions, e.g., in compound **1** NOEs from H-1a to H-2b and H-3b (see Fig. 2). The relative size of these NOEs are in good agreement with the calculated data from the MC simulation. The many so-called long-range NOEs between residues not directly linked, i.e., between Fuc and the "reducing" end residue (GlcNac, Qui or Xyl) are very informative about the conformation of the trisaccharides. Here, both the Fuc residue and the Qui residue are well suited for NOESY measurement, with well dispersed chemical shifts values and especially the methyl groups. A good example of such a NOE is the correlation between H-6a and H-5c in compound **1** (see Fig. 2). The calculated relative values for these "long-range" NOEs also show good agreement with the observed ones. All these data indicate that the sampling of the conformational space using the HSEA force field and MC simulation provides a good model for the conformational behavior of the oligosaccharides investigated. The conformational behavior of the di- and trisaccharides, as shown in the population maps in Fig. 1, can then be discussed with respect to the observed differences in glycosylations shifts. This analysis is necessary when no direct comparison in measured NOEs is possible, e.g., for the trissaccharide **1** and the corresponding disaccharide **7**.

Clearly, both the observed NOEs and population maps show that the overall conformational properties of the trisaccharides **1** and **3**, having either a hydroxyl methyl group or a methyl group attached at the C-5, are the same. An inspection of the three-dimensional models shows that the substituents at C-2 (–OH or –NAc) make no contact with the other residues. These results are in good agreement with the observed deviations from additivity (Table 4) being essentially the same for **1** and **3**, with the major deviations being –1.3 ppm for C-1b, –1.9 ppm for C-2b, and –2.4 to –2.9 ppm for C-4c. These positions are expected to be sensitive to changes in the

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 saturated	intraunit NOEs observed	abs.	rel.	MC	interunit NOEs observed	abs.	rel.	MC
1	H-1a/H-2a[b]					H-2b	7.1	123	88
	(5.8)[c]					H-3b	0.56	9.7	8.5
	H-5a/H-3,4a	H-1a	0.54	4.0	3.5	H-2b,3c	1.7	13	14
	(13.5)	H-6a	7.3	54	62	H-5c	3.0	22	22
						H-6Ac	1.3	9.5	6.6
	H-6a/H-5a	H-3,4a	7.2	73	76	H-2b	1.7	18	15
	(9.9)					H-5c	2.9	29	32
	H-1b/H-3b	H-5b+3c	11.4	225	178	H-1a	0.71	14	3.4
	(5.1)					H-2a	1.0	21	0.7
						H-4c	6.5	129	150
						H-6c	1.9	38	48
	H-1c/H-3c	H-2c	1.8	48	42	H-6a	0.39	11	6
	(3.7)	H-5c	4.3	118	119	OMe	4.8	130	167
		NAc	0.57	16	6				
3	H-1a/H-2,3,4a					H-2b	8.0	111	84
	(7.2)					H-3b	0.56	8	8
	H-5a/H-6a	H-2,3,4a	13.6	555	495	H-2b	1.7	69	55
	(2.5)					H-6c	2.3	31	24
	H-6a/H-5a	H-3,4a	2.28	66	75	H-3,4,5c	1.8	50	75
	(3.5)								
	H-1b/H-3b	H-2,5b	2.62	147		H-3,4c	2.3	129	138
	(1.8)					H-6c	1.18	66	42
	H-6c/H-3,4,5b[d]	H-1c	4.7[d]	19	1.8	H-1a	4.4[d]	18	0.6
	(34.8)					H-2a,3a,4a	12.6[d]	51	22
						H-5a	4.4	18	14
						H-4b4.9[d]		20	0
5	H-1a/H-2,3,4a					H-2b	8.6	99	90
	(8.6)								
	H-5a/H-6a	H-2,3, 4a +3b	13.7	570	501	H-2b	0.91	38	51
	(2.4)					H-5Ac	1.7	71	56
	H-6a/H-5a	H-2,3,4a	5.6	49	74	H-3c	0.84[d]	7	44
	(11.4)								
	H-1b/H-5b	H-3b+ 2,3, 4a+4c	14.8	187	138	H-6a	0.88	11	1
	(7.9)					H-5Ec	3.0	37	37
	H-2b/H-3b								
	(1.8[d])	H-1b0.26[d]		14	82	H-1a	7.1	394	423
	H-3c/H-1c	H-4c	1.3	35	42	H-6a	0.78	21	24
	(3.6)								
7[e]	δ1b=δ1c								
8	H-1b/H-3b								
	(4.8)	H-5b	7.9	165	176	H-3c	<1[d]	<21	3.1
						H-4c	10.2	213	156
						H-6c	3.1	65	38
	H-6c/H-5c								
	(12.3)	H-4c	6.3	51	63	H-1b	5.7	40	47
9	H-1b/H-3b	H-5b	7.1	200	174	H-4c	7.1	200	144
	(3.6)					H-5Ec	2.9	83	60

Table 5. *continued*

compound	proton saturated	intraunit NOEs observed	abs.	rel.	MC	interunit NOEs observed	abs.	rel.	MC
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 (abs.) NOEs of the reference NOE.
[b] For compounds **1**,**3**,**5**, and **10** residue a is α-Fuc; for compounds **1**–**10** residue b is β-Gal; for compounds **1** and **7** residue c is β-GlcNAc; for compounds **3** and **8** residue c is β-Qui; for compounds **5** and **9** residue c is β-Xyl.
[c] The value given in parenthesis is the absolute NOE to the proton used for calibration.
[d] The values measured have lower accuracy due to overlap or dispersive lineshape.
[e] No useful data could be obtained due to overlapping resonances, e.g., δ1b = δ1c.

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

compound	α-Fuc-(1→2)-β-Gal min	av. MC	β-Gal-(1→4)-Xyl-5X min	av. MC	X
1	51/12	50/20	53/4	57/0	CH_2OH
3	49/12	50/20	54/2	55/1	CH_3
5	48/10	48/18	54/11	52/15	H
7			53/2	51/–5	CH_2OH
8			52/1	51/–3	CH_3
9			51/17	52/13	H
10	47/6	44/8			

conformational behavior of the two glycosidic linkages. When comparing the population maps for **1** and **3** with the corresponding maps the disaccharides **7**, **8**, and **10**, it is clearly seen that these correlate well with much more restricted conformational space for the trisaccharides for both linkages. When inspecting the conformations of the global energy minima, as presented by the ϕ_H/ψ_H values, it is seen that the deviations from additivity cannot be explained by the minima alone, as only insignificant differences are seen between the di- and trisaccharides. The differences in sampling the conformational space seen in the populations maps can also be detected by the average ϕ_H/ψ_H values (Table 6), but more complete information can be obtained from the full maps.

When replacing the CH_2OH or CH_3 groups at C-5c in **1** and **3**, respectively, with an H in **5**, the first observation is that only one small deviation from additivity is observed for **5,** –1.2 ppm for C-2b. This can be explained by the conformational analysis, where comparison of the population maps of **5** to the maps of the disaccharides **9** and **10** shows that only a weak restriction is imposed on the conformation in the trisaccharide relative to the disaccharides. The only difference is seen for the 1-2 linkage, where the average ψ_H value seen in the maps and in Table 6 is somewhat larger for the trisaccharide **5** than for the disaccharide **10**. The change is the same as seen for the other two trisaccharides **1** and **3**, but of a somewhat smaller size in correspondence with a smaller deviation of –1.2 ppm compared to –1.9 ppm.

For the 1-4 linkage no restriction is observed for **5** relative to **9**, in agreement with the fact that no significant deviation from additivity was observed. Unfortunately, a direct comparison between NOEs obtained for **5** and **9** or **10** cannot be made due to overlapping signals, where only the sum of several NOEs can be obtained. These combined NOEs, however, are in good agreement with the MC simulations, indicating this to be a good model for the conformational behavior.

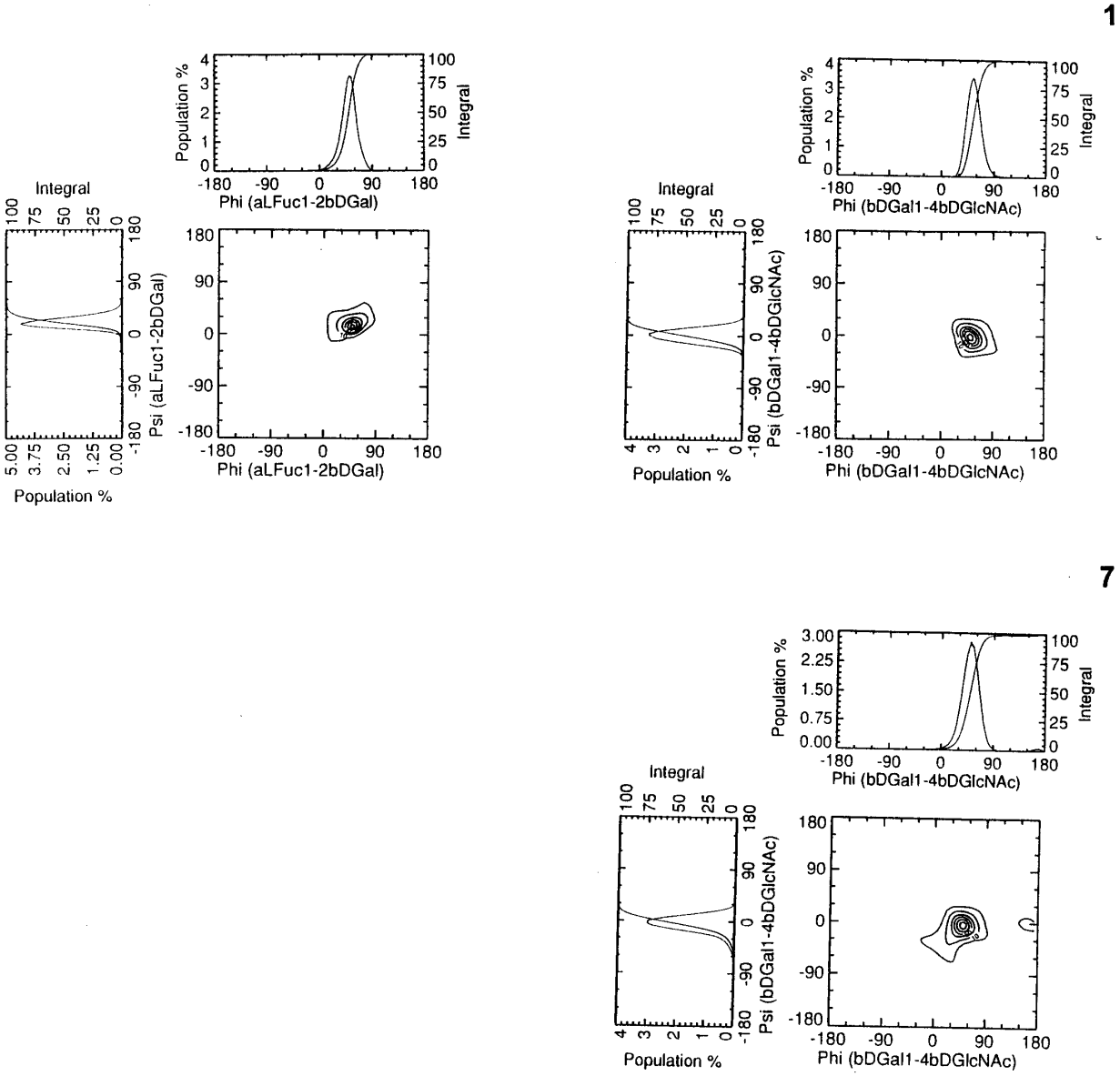


Fig. 1. Population maps (ϕ_H /ψ_H) for compound **1**, **3**, **5,** and **7**–**10**. In the left column are shown the α-L-(1→2) linkage and in the right column the β-(1→4) linkage. *(Figure continues on opposite page.)*

The trend in ΔΔ for the β series is similar to what is observed for the α series for the carbons related to the 1-4 linkage, i.e., C-1b and C-4c. Here also, the picture is that the compounds **2** and **4** with CH_2OH and CH_3 at C-5c show the same deviations, while **6** with only H at C-5c shows no significant ΔΔ. For position C-1a and C-2b related to the 1-2 linkage, no significant ΔΔ is observed.

CONCLUSION

The results show that the linear 1-2, 1-4 linked trisaccharides behave conformationally as branched oligosaccharides with clear restrictions in the conformational space compared to the corresponding disaccharides. The substituent at the carbon adjacent to the 1-4 linkage, C-5c, is important for the restrictions imposed, with large restrictions seen for the trisaccharides **1** and **3** having either CH_2OH or CH_3 at C-5c and only weak restrictions for **5** with only an H at C-5c.

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 with detec-

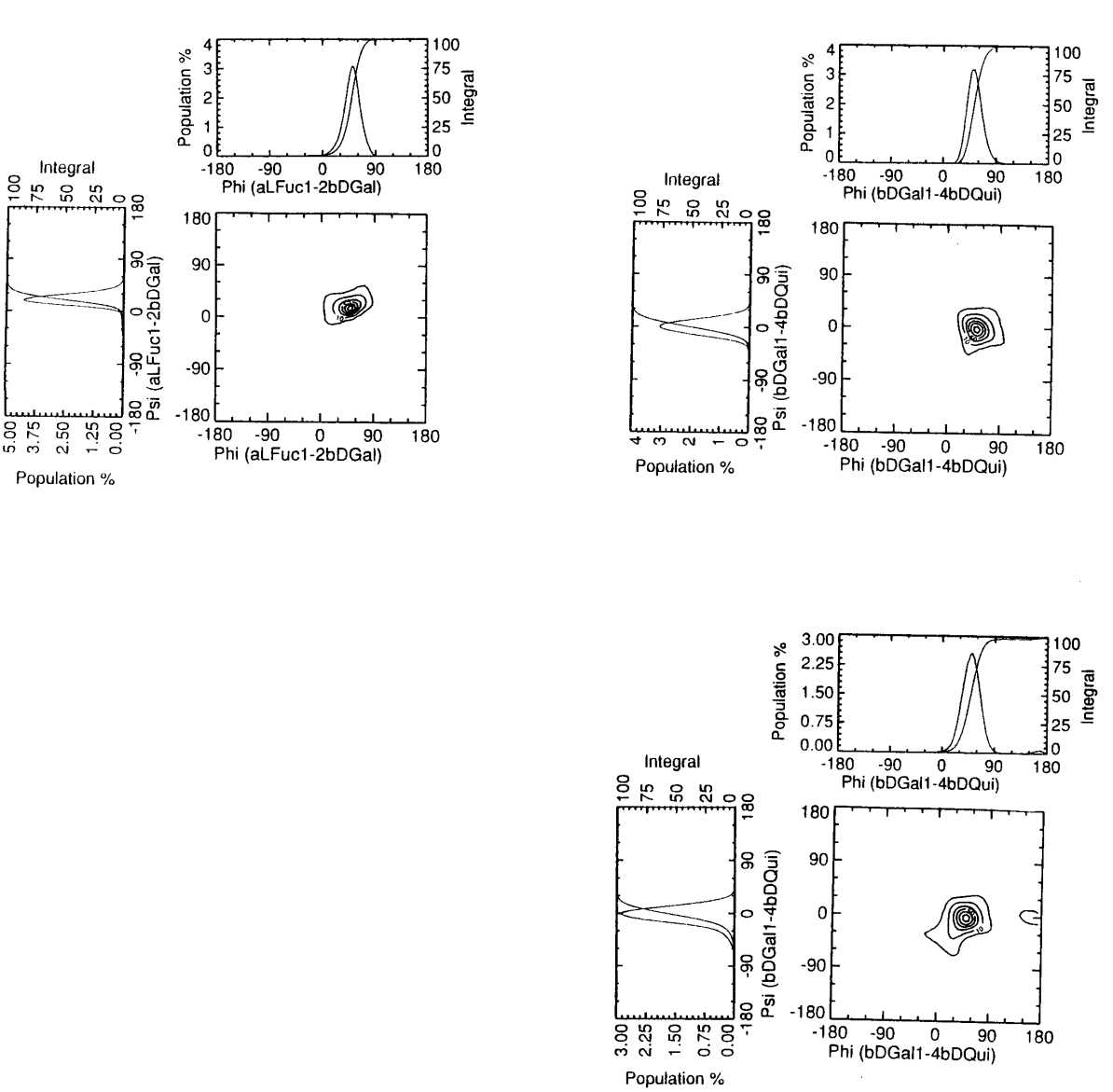


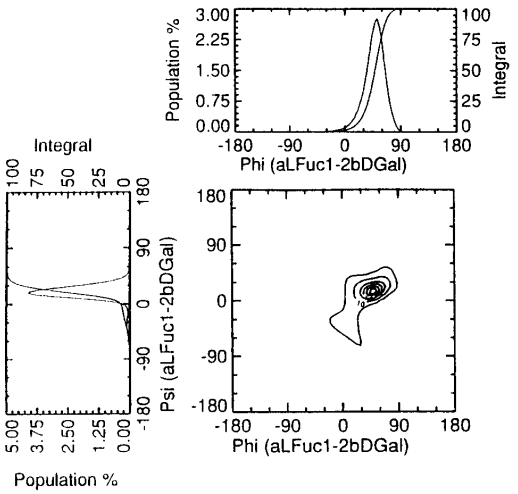
Fig. 1. *continued*

tion 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 were determined 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

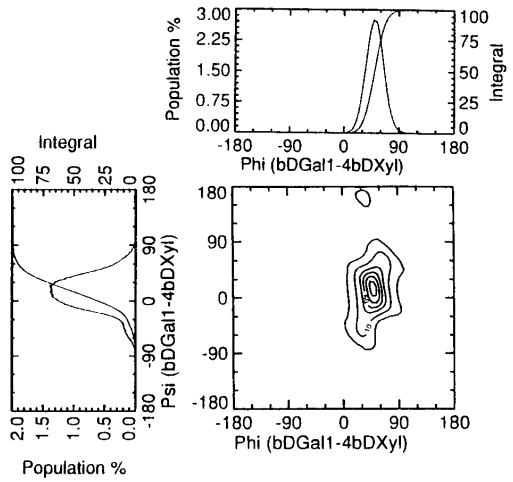
^{1}H NMR spectra for substituted compounds **12**–**23** and **29** were recorded in $CDCl_3$ on a Bruker AMX 300 spectrometer at 303 K. ^{1}H NMR spectra for oligosaccharides **1**–**11** were recorded in D_2O at 316 K on a Bruker AMX 600 NMR instrument and ^{13}C spectra on a Bruker AM500 operating at 125.7 MHz for ^{13}C. One- and two-dimensional spectra were acquired using standard Bruker software.

The molecular modeling was performed using the GEGOP program.[22] Metropolis Monte Carlo simulations were performed[23] at 500 K for the disaccharides and 600 K for the trisaccharides with at least 2×10^6 Monte Carlo steps. The NOEs were calculated using the r^{-6} average full matrix approach, as described previously.[23] The coordinates for the β-Qui residue were constructed from β-Glc using standard bond lengths and angles with the InsightII program (Biosym, San Diego, CA).

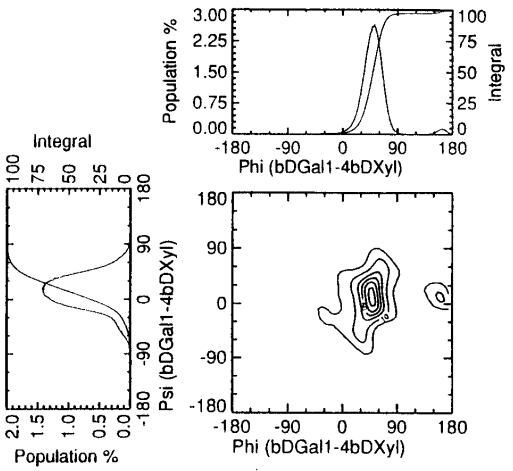
*Methyl 2,3-di-*O*-benzyl-β-*D*-xylopyranoside* (**14**). A solution of methyl 2,3-di-*O*-benzyl-4-O-trityl-β-D-xylopyrano-



5



9



10

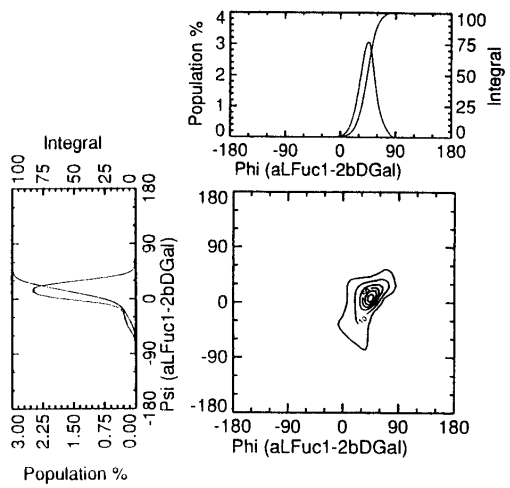


Fig. 1. *continued*

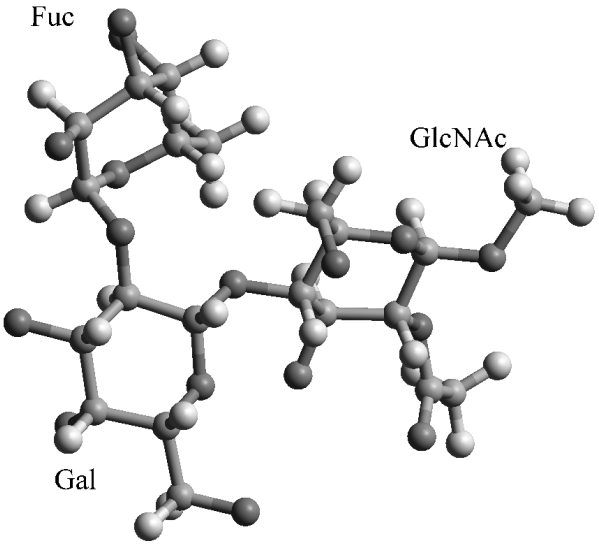


Fig. 2. Minimum energy conformation of compound **1** (O-methyl group to the right), showing the close proximity of the Fuc and GlcNAc residues.

side[25] (500 mg, 0.85 mmol) in chloroform (4 mL) was treated with 90% aq trifluoacetic acid (0.5 mL); the mixture was stirred for 30 min at r.t., diluted with chloroform (5 mL), and washed with water (25 mL), aq $NaHCO_3$ (30 mL), and water (30 mL), filtered through cotton, and concentrated. The product was subjected to column chromatography to give **14** (283 mg, 97%), $[\alpha]_D$ –32° (*c* 2, $CHCl_3$), R_f 0.31 (solvent C). The ^{1}H NMR data are presented in Table 1.

Methyl 2,3-di-O-*benzyl-2-deoxy-4*-O-*(2,3,4,6-tetra*-O-*acetyl*-O-*β*-D-*galactopyranosyl)-2-phthalimido-b*-D-*glucopyranoside* (**16**). A mixture of **12**[12] (612.5 mg, 1.21 mmol), 1,2,3,4,6-penta-*O*-acetyl-*β*-D-galactopyranose (**25**) (566 mg, 1.452 mmol), powdered molecular sieves 4A, and CH_2Cl_2 (10 mL) was stirred for 30 min at r.t. under Ar. The mixture was cooled to –20 to –30 °C and trimethylsilyl triflate (516 μL, 2.68 mmol) 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 (40 mL), aq $NaHCO_3$ (2 × 30 mL) and water (40 mL), and concentrated. The product was subjected to column chromatography to give **16** (838.5 mg, 84%), $[\alpha]_D$ +20° (*c* 1, $CHCl_3$), R_f 0.15 (solvent D). Lit.[12]: $[\alpha]_D$ +23° (*c* 1, $CHCl_3$). The ^{1}H NMR data for **16** are presented in Table 1.

Methyl 2,3-di-O-benzyl-4-O-*(2,3,4,6-tetra*-O-*acetyl*-O-*β*-D-*galactopyranosyl)-β*-D-*quinovopyranoside* (**17**). Glycosylation of **13**[13] (36 mg, 0.1 mmol) with **15** (47 mg, 0.12 mmol) in the presence of trimethylsilyl triflate (43 μL, 0.22 mmol) as described for **16** gave amorphous **17** (59 mg, 86%), $[\alpha]_D$ 9° (*c* 2, $CHCl_3$), R_f 027. (solvent C). The ^{1}H NMR data are presented in Table 1.

Methyl 2,3-di-O-*benzyl-4*-O-*(2,3,4,6-tetra*-O-*acetyl*-O-*β*-D-*galactopyranosyl)-β*-D-*xylopyranoside* (**18**). Glycosylation of **14** (59 mg, 0.17 mmol) with **15** (74 mg, 0.19 mmol) in the presence of trimethylsilyl triflate (73 mL, 0.162 mmol) as described for **16** gave amorphous **18** (49 mg, 42%), $[\alpha]_D$ –1° (*c* 2, $CHCl_3$), R_f 0.21 (solvent C). The ^{1}H NMR data for **18** are presented in Table 1.

Methyl 2,3-di-O-*benzyl-4*-O-*(3*-O-*benzoyl-4,6-benzylidene-β*-D-*galactopyranosyl)-2-deoxy-2-phthalimido-β*-D-*glucopyranoside* (**22**). A solution of **16** (838 mg, 1.023 mmol) in methanolic 0.1 M MeONa (15 mL) was kept for 15 min at r.t. and then was neutralized with KU-2 (H^+) resin, filtered, and concentrated to dryness. To a solution of the crude residue in MeCN (1.5 mL) benzaldehyde diethyl acetal (0.47 mL, 3.07 mmol) and $TsOH{\cdot}H_2O$ (5 mg) were added. The mixture was stirred for 3 h at r.t., MeOH (0.8 mL) was added, stirring was continued for 5 min, and pyridine (0.01 mL) was added. The mixture was diluted with $CHCl_3$ (40 mL), washed with water (40 mL), aq $NaHCO_3$ (2 × 30 mL), and water (40 mL). The organic layer was separated, filtered through cotton, and concentrated. The residue was washed with heptane (5 × 4 mL) and dried in vacuo to give crude **22**. To its solution in MeCN (10 mL) benzoyl cyanide (95 mg, 0.7 mmol) and triethylamine (1 drop) were added under stirring. The mixture was stirred for 3 h, MeOH (4 mL) was added, the mixture was kept for 5 min, the solvent was concentrated, and MeOH (4 mL) was evaporated from the residue. Column chromatography of the product gave **22** (426 mg, 75%), [a]$_D$ +70.5° (*c* 2, $CHCl_3$), R_f 0.5 (solvent B) and **25** (112 mg, 20%), [a]$_D$ +1° (*c* 2, $CHCl_3$), R_f 0.38 (solvent B). The ^{1}H NMR data for isomers **22** and **25** are presented in Table 1.

Methyl 2,3-di-O-*benzyl-4*-O-*(3*-O-*benzoyl-4,6-benzylidene-β*-D-*galactopyranosyl)-β*-D-*quinovopyranoside* (**23**). Disaccharide **17** (267 mg, 0.38 mmol) was deacetylated, benzylidenated, and 3′-*O*-benzoylated, as described for preparation of **22**, to give amorphous **23** (180 mg, 67%), $[\alpha]_D$ +54° (*c* 2, $CHCl_3$), R_f 0.23 (solvent A). The ^{1}H NMR data are presented in Table 1.

Methyl 4-O-*(3*-O-*benzoyl-4,6-benzylidene-β*-D-*galactopyranosyl)- 2,3-di*-O-*benzyl-β*-D-*xylopyranoside* (**24**). Disaccharide **18** (118 mg, 0.175 mmol) was deacetylated and benzylidenated, and compound **21** was benzoylated with benzoyl cyanide (24 mg, 0.18 mmol) in the presence of triethylamine, as described for preparation of **23**, to give amorphous **24** (80.5 mg, 77%), $[\alpha]_D$ +46° (*c* 2, $CHCl_3$), R_f 0.22 (solvent A). The ^{1}H 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-di*-O-*acetyl-β*-D-*galactopyranosyl)-(1→4)-4,6-di*-O-*benzyl-2-deoxy-2-phthalimido-β*-D-*glucopyranoside* (**28**) and *methyl* O-*(2,3,4-tri*-O-*benzoyl-β*-L-*fucopyranosyl)-(1→2)*-O-*(3*-O-*benzoyl-4,6-di*-O-*acetyl-β*-D-*galactopyranosyl)-(1→4)-2-phthalimido-4,6-di*-O-*benzyl-2-deoxy-β*-D-*glucopyranoside* (**32**). A solution of **22** (195 mg, 0.228 mmol), $Hg(CN)_2$ (123 mg, 0.48 mmol), $HgBr_2$ (70 mg), and molecular sieves 4A in MeCN (1.7 mL) was stirred for 45 min at 20° under Ar. Using a syringe, a solution of fucozyl bromide **26** {prepared [26] from tetra-*O*-benzoyl-L-fucopyranose (278 mg, 0.48 mmol)} in MeCN (1.7 mL) was added

portionwise during 1 h. The mixture was stirred for 5 h, and $CHCl_3$ (10 mL) and satd aq KBr (5 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, and concentrated. The residue was dissolved in chloroform (2 mL), treated with 90% aq trifluoacetic acid (0.5 mL), and, after being kept for 30 min at r.t., the solution was concentrated, and toluene (3 × 5 mL) was evaporated from the residue. A solution of the product in chloroform (1 mL), pyridine 3 mL, and acetic anhydride (1 mL) was kept for 3 h at r.t., and toluene (3 × 10 mL) was evaporated from the residue. The mixture 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, and the solvent was evaporated in vacuo. Column chromatography of the product gave amorphous **28** (80 mg, 26%), $[\alpha]_D$ –81° (*c* 0.8, $CHCl_3$), R_f 0.24 (solvent B), and **32** (159 mg, 53%), $[\alpha]_D$ -122° (c 1, $CHCl_3$), R_F 0.15 (solvent B). The ^1H NMR data for **28** and **32** are presented in Table 1.

Methyl O-*(2,3,4-tri*-O-*benzoyl-a-L-fucopyranosyl)-(1→2)*-O-*(3*-O-*benzoyl-b-D-galactopyranosyl)-(1→4)-2,3-di*-O-*benzyl-b-D-quinovopyranoside* (**29**) and *methyl* O-*(2,3,4-tri*-O-*benzoyl-α-L-fucopyranosyl)-(1→2)*-O-*(3*-O-*benzoyl-β-D-galactopyranosyl)-(1→4)-2,3-di*-O-*benzyl-6-deoxy-b-D-glucopyranoside* (**33**). Glycosylation of **23** (62 mg, 0.087 mmol) with **26** {prepared[26] from tetra-*O*-benzoyl-L-fucopyranose (101 mg, 0.174 mmol)} and treatment with trifluoroacetic acid, as for the synthesis of **28** and **32**, gave amorphous **29** (24 mg, 26%), $[\alpha]_D$ –81° (*c* 0.5, $CHCl_3$), R_f 0.21 (solvent C), and **33** (48 mg, 52%) $[\alpha]_D$ –100° (*c* 0.5, $CHCl_3$), R_F 0.17 (solvent C). The ^1H NMR data for **28** and **33** are presented in Table 1.

Methyl O-*(2,3,4-tri*-O-*benzoyl-α-L-fucopyranosyl)-(1→2)*-O-*(3*-O-*benzoyl-β-D-galactopyranosyl)-(1→4)-2,3-di*-O-*benzyl-β-D-xylopyranoside* (**30**) and *methyl* O-*(2,3,4-tri*-O-*benzoyl-α-L-fucopyranosyl)-(1→2)*-O-*(3*-O-*benzoyl-β-D-galactopyranosyl)-(1→4)-2,3-di*-O-*benzyl-b-D-xylopyranoside* (**34**). Glycosylation of **24** (29 mg, 0.041 mmol) with **26** {prepared[26] from tetra-*O*-benzoyl-L-fucopyranose (48 mg, 0.082 mmol)} and treatment with trifluoroacetic acid, as for the synthesis of **28** and **32**, gave amorphous **30** (7 mg, 16%), $[\alpha]_D$ –102° (*c* 1, $CHCl_3$), R_F 0.25 (solvent C), and **34** (23.5 mg, 54%) $[\alpha]_D$ –78° (*c* 2, $CHCl_3$), R_f 0.2 (solvent C). The ^1H NMR data for **30** and **34** are presented in Table 1.

*Preparation of Non-substituted Oligosaccharide Methyl Glycosides **1–6**, **8**, and **9***

Methyl-O-*(α-L-fucopyranosyl)-(1→2)*-O-*(β-D-galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside* (**1**). Compound **28** (20 mg, 0.015 mmol) was subjected to catalytic hydrogenolysis in EtOH–AcOH (1:2, 12 mL) with Pd-C as catalyst at 40 °C and atm. pressure for 20 h. The mixture was filtered and filtrate was concentrated. Solution of the residue 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 distilled from the residue, which then was dissolved in MeOH (10 mL) and water (2 mL), 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_o 50 ml), in 0.01 M acetic acid, to give amorphous **1** (7.5 mg, 80%), $[\alpha]_D$ -88° (*c* 0.5, H_2O). Lit. [22]: $[\alpha]_D$ –90.2° (*c* 0.5, H_2O). ^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→4)-2-acetamido-2-deoxy-β-D-glucopyranoside* (**2**). Hydrolysis and saponification of the compound **32** (35 mg, 0.027 mmol), followed by gel filtration as described above, gave amorphous **2** (14 mg, 82%), $[\alpha]_D$ 131° (*c* 0.5, H_2O). The ^1H and ^{13}C NMR data are presented in Tables 2 and 3.

Methyl O-*(α-L-fucopyranosyl)-(1→2)*-O-*(β-D-galactopyranosyl)-(1→4)-β-D-quinovopyranoside* (**3**). Compound **29** (8.1 mg, 0.008 mmol) was debenzylated as described for the preparation of **1**, O-deacylated by treatment with methanolic 0.1M MeONa as described for preparation of **19**, and subjected to gel filtration on fracto-gel TSK HW-40(S) (25-40 mm, V_o 50 mL), in water, to give amorphous **3** (3.5 mg, 93%), $[\alpha]_D$ –59° (*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→4)-β-D-quinovopyranoside* (**4**). Compound **33** (9 mg, 0.009 mmol) was deblocked, as described for the preparation of **3**, to give amorphous **4** (4 mg, 95%), $[\alpha]_D$ –88° (*c* 0.3, H_2O). The ^1H and ^{13}C NMR data are presented in Tables 2 and 3.

Methyl O-*(α-L-fucopyranosyl)-(1→2)*-O-*(β-D-galactopyranosyl)-(1→4)-β-D-xylopyranoside* (**5**). Compound **30** (29 mg, 0.027 mmol) was deblocked, as described for the preparation of **3**, to give amorphous **5** (12 mg, 94%), $[\alpha]_D$ –3° (*c* 1, H_2O). The obtained value of optical rotation for **5** varied from that reported previously in ref 6 [–90.2° (*c* 0.5, H_2O)]; nevertheless the NMR data for **5** (Tables 2 and 3) coincide well with that reported in ref 6.

Methyl O-*(β-L-fucopyranosyl)-(1→2)*-O-*(β-D-galactopyranosyl)-(1→4)-β-D-xylopyranoside* (**6**). Compound **34** (36 mg, 0.034 mmol) was deblocked, as described for the preparation of **3**, to give amorphous **6** (15 mg, 96%), $[\alpha]_D$ –52° (*c* 1, H_2O). The ^1H and ^{13}C NMR data are presented in Tables 2 and 3.

Methyl 4-O-*(β-D-galactopyranosyl)-β-D-glucopyranoside* (**8**). Compound **17** (21.5 mg, 0.031 mmol) was deblocked, as described for the preparation of **3**, to give amorphous **8** (10 mg, 94%), $[\alpha]_D$ –5° (*c* 0.5, H_2O). The ^1H and ^{13}C NMR data are presented in Tables 2 and 3.

Methyl 4-O-*(β-D-galactopyranosyl)-β-D-xylopyranoside* (**9**). Compound **18** (22 mg, 0.032 mmol) was deblocked, as described for the preparation of **3**, to give amorphous **9** (10 mg, 95%), $[\alpha]_D$ –30° (*c* 0.5, H_2O). The ^1H and ^{13}C NMR data are presented in Tables 2 and 3.

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