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500-MHz ^{1}H-N.M.R. AND CONFORMATIONAL STUDIES OF FUCOSYL-OLIGOSACCHARIDES RECOGNISED BY MONOCLONAL ANTIBODIES WITH SPECIFICITIES RELATED TO Le^a, Le^b, AND SSEA-1*

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ABSTRACT

500-MHz ^{1}H-n.m.r. spectroscopy has been used to examine several fucosylated oligosaccharides in studies to characterise carbohydrate antigenic determinants recognised by monoclonal antibodies. Reduction of the oligosaccharides to give additional variants for analysis showed that oligosaccharides having an α-L-fucosyl group linked to the reducing end residue have markedly different chemical shifts, and in some instances different antigenic activity, compared to their alditols. This information was incorporated into space filling molecular models of the oligosaccharides in order to predict the topography of atoms recognised by the antibody combining sites. These studies are an intermediate stage in the full characterisation of oligosaccharide conformation and molecular recognition by methods which accurately determine torsional angles and through-space internuclear distances.

INTRODUCTION

Human milk is a relatively abundant source of oligosaccharides which have structural homologies with components of cell surface and secreted glycoproteins and glycolipids. As such, this material has been invaluable in providing oligosaccharides for studies on the molecular assignment of blood group activity[1–3], elucidation of the specificities of glycosyltransferases involved in blood group

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β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc
Lacto-*N*-tetraose (**1**)

β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc
Lacto-*N*-*neo*-tetraose (**2**)

α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc
Lacto-*N*-fucopentaose I (**3**)

β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc
4
↑
1
α-L-Fuc*p*
Lacto-*N*-fucopentaose II (**4**)

β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc
3
↑
1
α-L-Fuc*p*
Lacto-*N*-fucopentaose III (**5**)

α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc
4
↑
1
α-L-Fuc*p*
Lacto-*N*-difucohexaose I (**6**)

β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc
4 3
↑ ↑
1 1
α-L-Fuc*p* α-L-Fuc*p*
Lacto-*N*-difucohexaose II (**7**)

β-D-Gal*p*-(1→4)-D-GlcNAc
3
↑
1
α-L-Fuc*p*
3-Fucosyllactosamine (**8**)

α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-(1→
4
↑
1
α-L-Fuc*p*
9

α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→
3
↑
1
α-L-Fuc*p*
10

antigen biosynthesis[4,5], and the characterisation of carbohydrate antigenic determinants recognised by monoclonal antibodies to glycoconjugates[6–11]. These studies have illustrated the importance of structurally defined oligosaccharides in immunochemical and biochemical analysis, and 500-MHz ^{1}H-n.m.r. spectroscopy has emerged as an efficient non-destructive method to determine purity and structure. Further, from n.m.r. analysis, it is possible to make certain predictions as to oligosaccharide conformation which gives additional insight into carbohydrate recognition in biological systems.

We describe herein the ^{1}H-n.m.r. chemical shift data for the oligosaccharides lacto-*N*-fucopentaose I (**3**) and II (**4**), lacto-*N*-difucohexaose I (**6**) and II (**7**) from human milk and chemically synthesised 3-fucosyllactosamine (**8**). The oligosaccharides were also analysed after reduction in order to obtain additional chemical shift information. This series of oligosaccharides and their alditols were used in the characterisation of antibodies to mammary epithelial antigens[10,11], designated D11, H10, C2, G3, and E6. For antibodies D11 and H10, the best inhibitors of binding were the oligosaccharides **6** and its alditol derivative which have the blood group Le^b structure **9** at their nonreducing end.

For antibodies C2 and G3, the best inhibitor of binding was the oligosaccharide **7**, which has the blood group Le^a trisaccharide at the nonreducing end and, as reducing end, a 3-fucosyllactose sequence related to the SSEA-1 antigen which has been characterised as 3-fucosyllactosamine[6,7]. The importance of the reducing end of the molecule in the recognition by antibodies C2 and G3 was shown by the significantly decreased reactivity of the alditol derivative of **7** (by more than 15 fold)[10,11]. For another antibody, E6, the nonreducing end of the molecule appeared to be more important for recognition as the oligosaccharides **4** (lacking the α-L-fucosyl group linked to the D-glucose residue), **7**, and their alditol derivatives had similar inhibitory activity[11]

From the relative reactivities of the oligosaccharides in the inhibition assays, it was possible to make certain predictions as to the molecular features recognised by the antibody combining site. In order to visualise these features, space-filling molecular models have been constructed incorporating conformational aspects suggested by the n.m.r. data.

EXPERIMENTAL

N.m.r. spectroscopy. — Oligosaccharides were freeze-dried four times from ^2H_2O solution to replace all exchangeable hydrogen with deuterium. Samples were then made up in ^2H_2O (400 μL) with acetone as internal standard. Chemical shifts (δ) were measured in p.p.m. from the acetone signal and given with reference to sodium 4,4-dimethyl-4-silapentane-1-sulphonate taken as δ 2.225 from the acetone signal at 295 K, the temperature of the probe at which all experiments were carried out. The 500-MHz ^{1}H-n.m.r. spectra were obtained with a Bruker AM500 spectrometer operating in the F.t. mode and equipped with an Aspect 3000 computer as

described previously[12]. Chemical shifts were assigned by spin-decoupling experiments and by comparison of the data for the series of oligosaccharides and their alditols discussed in the present report, and also for lacto-*N*-fucopentaose III (**5**) and 3-fucosyllactose and their alditols[13], and lacto-*N*-tetraose (**1**) and lacto-*N*-*neo*-tetraose (**2**), and their alditols (largely as described[12,14]). The anomeric signals of the *N*-acetylglucosamine residues could be readily distinguished by their consistently larger coupling constants (~8.4 Hz) compared to those of D-galactose and D-glucose (~7.7 Hz). Signals for the anomeric protons of the D-galactose residues linked to the 2-acetamido-2-deoxy-D-glucose residue and those linked to the D-glucose residue could be distinguished by comparison of the shifts for each oligosaccharide and its alditol where the former signals differ by <0.01 p.p.m. and the latter by >0.04 p.p.m.

Oligosaccharides. — The oligosaccharides of human milk were purified as described by Anderson and Donald[15], and Donald and Feeney[13]. Chemically synthesised 3-fucosyllactosamine was a gift from Prof. P. Sinaÿ, University of Orléans, France. Reduction of the oligosaccharides was carried out with a 10–100-fold molar excess of $NaBH_4$ in 0.1M BO_4^{3-} buffer, pH 9.2 for 6 h at 20°. Samples were then desalted on mixed-bed, ion-exchange resins (Dowex 50W-X12, 200–400 mesh, H^+ and Dowex AG 1-X8, 200–400 mesh, OAc^-) and evaporated repeatedly with methanol under a stream of N_2 to remove residual borate ions.

Space-filling molecular models. — The space-filling molecular models were obtained from Ealing Beck Ltd., Watford, England. Models were constructed with the $^4C_1$(D) form for D-galactose, 2-acetamido-2-deoxy-D-glucose, and D-glucose, and the $^1C_4$(L) form for L-fucose[16]. From the work of Lemieux and others[17–20] on the consequences of the exoanomeric effect on the preferred solution conformation of oligosaccharides, the configuration around the glycoside bonds was set such that the anomeric and aglyconic protons of each linkage were adjacent to each other on the same side of the molecule giving a ϕ^H angle of $\sim+60°$ for β-D-glycosides and of $\sim-60°$ for α-D-glycosides. Large downfield shifts for a particular proton relative to a corresponding proton in a related oligosaccharide were interpreted as indicating the proximity of this proton to oxygen atoms in the molecule, thus causing deshielding of the nucleus[20–23]. Similar ^{1}H-chemical shifts for corresponding residues in differently substituted oligosaccharides have been interpreted as indicating that these residues have similar local conformations and are not showing any through-space anisotropic-shielding interactions.

RESULTS AND DISCUSSION

*^{1}H-N.m.r. analysis and conformations of lacto-*N*-difucohexaose I* (**6**) *and its alditol* (**6**-*ol*). — The ^{1}H-n.m.r. data shown in Table I for **3**, **4**, and **6** and their alditols were examined in order to predict the orientation of the α-L-Fuc*p*-(1→2) and -(1→4) groups with respect to other residues in **6** and its alditol because these two L-fucopyranosyl groups have been shown to be necessary for the binding of

TABLE I

^{1}H-CHEMICAL SHIFTS (δ) OF THE STRUCTURAL REPORTER GROUPS OF FUCOSYLOLIGOSACCHARIDES BASED ON TYPE 1 SEQUENCE AND THE DIFFERENCES IN CHEMICAL SHIFT OF THEIR NATIVE AND REDUCED FORMS

Reporter group	*Atom*	*Compound*											
		3	**3**-*ol*	*Diff.*	**4**	**4**-*ol*	*Diff.*	**6**	**6**-*ol*	*Diff.*	**7**	**7**-*ol*	*Diff.*
α-L-Fuc*p*-(1→2)	H-1	5.189	5.189	0				5.145	5.152	0.007			
	H-5	4.293	4.292	0.001				4.341	4.342	0.001			
	H_3-6	1.233	1.233	0				1.272	1.273	0.001			
α-L-Fuc*p*-(1→3) to D-Glc	H-1α,β										5.373	5.064	0.309
											5.431		0.367
	H-5										4.827	4.276	0.551
	H_3-6α,β										1.166	1.211	0.045
											1.160		0.051
α-L-Fuc*p*-(1→4) to D-GlcNAc	H-1				5.028	5.029	0.001	5.028	5.028	0	5.031	5.024	0.007
	H-5				4.882	4.870	0.012	4.865	4.873	0.008	4.884	4.883	0.001
	H_3-6				1.180	1.179	0.001	1.258	1.258	0	1.179	1.180	0.001
β-D-Gal*p*-(1→3) to D-GlcNAc	H-1	4.647	4.648	0.001	4.505	4.507	0.002	4.662	4.661	0.001	4.513	4.507	0.006
	H-2	3.5–4	3.480	[a]	3.485	3.486	0.001	3.58	3.5–4		3.484	3.486	0.002
β-D-Gal*p*-(1→4) to D-Glc	H-1	4.427	4.475	0.048	4.433	4.489	0.056	4.418	4.471	0.053	4.413	4.480	0.067
	H-4	4.139	4.133	0.006	4.156	4.152	0.004	4.137	4.135	0.002	4.097	4.146	0.049
β-D-GlcNAc	H-1α,β	4.628[b]	4.624	0.004	4.706	4.697	0.009	4.605	4.604	0.001	4.691	4.699	0.009
		4.624		0	4.695		0.002	4.602		0.002	4.689		0.010
	$NHCOCH_3$	2.057	2.056	0.001	2.032	2.032	0	2.061	2.061	0	2.029	2.035	0.006
α-D-Glc	H-1	5.220			5.220			5.220			5.182		
β-D-Glc	H-1	4.662			4.663			4.661			4.651		
	H-2	3.279			3.279			3.278			3.461		
D-Glcol	H-2											4.149	
	H-3											4.056	

[a]Not determined. [b]Shifts arising by an anomerisation affect of the reducing end were assigned by their intensity as compared to that for H-1 of α- and β-D-glucose.

antibodies D11 and H10 (**3**, **4**, and **1** are more than 75, 250, and 500 times less active, respectively, as inhibitors of binding compared to **6**, whereas **6**-ol has approximately the same inhibitory activity[10,11] as **6**).

Comparison of the chemical shifts for the α-L-Fuc*p*-(1→4) group of **4** and **6** showed that those of H-1 are the same (δ 5.028) and those of H-5 (δ 4.882 and 4.865, respectively) are significantly downfield of H-5 of the α-L-Fuc*p*-(1→2) group of **3** and **6** (δ 4.293 and 4.341, respectively). It has been proposed by others[20,25] that these shifts are characteristic of H-5 of an α-L-Fuc*p*-(1→4) group being deshielded by close proximity to the oxygen atom of the β-D-Gal*p*-(1→3)-D-GlcNAc glycoside bond and the ring oxygen atom of the nonreducing β-D-Gal*p* end group. H-1 is then adjacent to the $-CH_2OH$ group of GlcNAc. An explanation for the significant differences between the shift values shown in Table I for H_3-6 of the α-L-Fuc*p*-(1→4) group in **4** and **6** (0.08 p.p.m.) and between the values for H-1, H-5, and H_3-6 protons of the α-L-Fuc*p*-(1→2) group in **3** and **6** (>0.04 p.p.m.) is that the two α-L-fucopyranosyl groups are in close proximity to each other on one face of the molecule, as described for chemically synthesised Le^b-active oligosaccharides[24,25]. The same orientation of the α-L-Fuc*p*-(1→2) group with respect to the D-GlcNAc residues has been reported for blood group H-active oligosaccharides of ovarian cyst[26]. These features have been incorporated into the space-filling molecular model of **6** given in Fig. 1.

The similarity of the chemical shift data for **6** and its alditol suggests that the overall conformations of these oligosaccharides are the same, for example the α-L-fucopyranosyl groups of **6**-ol are expected to have the same orientation with respect to each other as that discussed above for **6** (Fig. 1). Previous studies[27] have predicted that the backbone Gal→GlcNAc→Gal sequence adopts a similar conformation in **6**-ol as that shown for **6** with D-glucitol in the same relative position as D-glucose with respect to the rest of the molecule (although with a different orientation of hydroxyl groups).

*^{1}H-N.m.r. analysis and conformations of lacto-*N*-difucohexaose II* (**7**) *and its alditol* (**7**-*ol*). — The similarity of the n.m.r. data for comparable residues in **7**-ol, and **3**, **4**, **6** and their alditols (Table I) suggested that the overall conformation of **7**-ol approximates to that for **6** and **6**-ol as discussed above. Further, from these data it can be predicted that the α-L-Fuc*p*-(1→3) group of **7**-ol is orientated away from the rest of the molecule, a shown in Fig. 1, as the presence of this α-L-fucopyranosyl group does not cause significant shift differences in groups at the nonreducing end of the molecule.

In contrast, comparison of the ^{1}H-n.m.r. data for **7** and **7**-ol (Table I) showed marked differences in the chemical shifts of the β-D-Gal*p*-(1→4)-[α-L-Fuc*p*-(1→3)]-D-Glc sequence. The largest difference of 0.551 p.p.m. for H-5 of the α-L-Fuc*p*-(1→3) group represents a similar downfield shift to that of H-5 of the α-L-Fuc*p*-(1→4) group in **4** compared to the α-L-Fuc*p*-(1→2) group in **3** (0.589 p.p.m.), and of these protons in **6** (0.524 p.p.m.), for example. Therefore, by analogy with the situation for **4** and **6** discussed above, the model for **7** given in Fig. 1 has H-5 of the

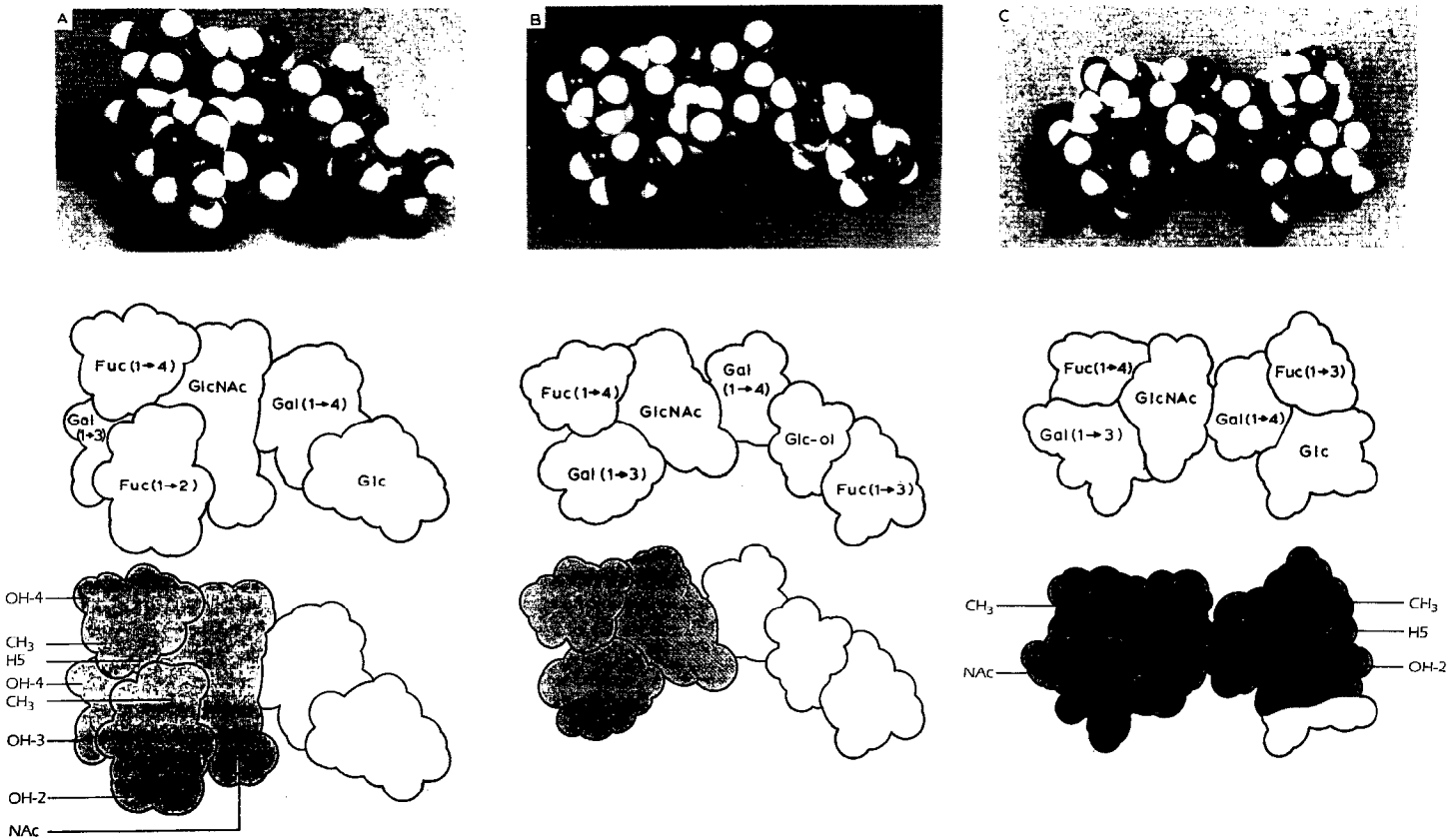

Fig. 1. The molecular models of lacto-*N*-difucohexaose I (panel A, structure **6**), and lacto-*N*-difucohexaose II (panel C, structure **7**) and its alditol (panel B, structure **7**). The outlines of the models indicate the relative positions of the constituent monosaccharides and the shaded areas indicate the proposed contact-residues which include molecular features predicted to be recognised by antibodies D11 and H10 (**6**), antibody E6 (**7**-ol), and antibodies C2 and G3 (**7**).

α-L-Fuc*p*-(1→3) group in close proximity to the oxygen atom of the β-D-Gal*p*-(1→4)-D-Glc glycoside bond and the ring oxygen atom of β-D-Gal*p*-(1→4). In this model, H-1 of the α-L-Fuc*p*-(1→3) group is adjacent to OH-2 of D-Glc which, being coupled to C-1, may explain the large anomerisation effect on this proton (0.058 p.p.m. difference in the α and β anomers of the molecule).

The similarity of the chemical shifts for the β-D-Gal*p*-(1→3)-[α-L-Fuc*p*-(1→4)]-D-GlcNAc sequence of **4, 7**, and their alditols suggests that this sequence has a similar conformation in each molecule. For **4**, it has been suggested[27] that this conformation approximated that shown in Fig. 1 for the same trisaccharide sequence in **6**, and therefore a comparable conformation is given for the nonreducing and nonreduced ends of **7** and **7**-ol in Fig. 1.

The difference in the chemical shift of H-4 of β-D-Gal-(1→4) of **7**, as compared to the same proton in **7**-ol (δ 4.097 and 4.146, respectively), is in contrast to the minor differences in shift observed for this proton between other oligosaccharides studied and their alditols; such oligosaccharides with an α-L-Fuc*p*-(1→2) group (**3** and **6**) have β-D-Gal*p*-(1→4) H-4 shifts of δ 4.139 and 4.137, respectively, for the unreduced compounds, and δ 4.133 and 4.135, respectively, for their alditols (Table II). For oligosaccharides lacking this α-L-fucopyranosyl group, *e.g.*, (Table II), **1, 2** (ref. 28), and **5** (ref. 13), the β-D-Gal*p*-(1→4) chemical shifts have values between δ 4.154 and 4.156, and between δ 4.144 and 4.152 for the corresponding proton in the alditols. The chemical shift for this proton in **7**-ol falls within the range of that for the other alditols lacking the α-L-Fuc*p*-(1→2) group, whereas that for **7** is significantly different. This may be a direct effect caused by the orientation of the α-L-Fuc*p*-(1→3) group suggested in Fig. 1, or it may reflect a different conformation in the backbone around the β-D-Glc*p*NAc-(1→3)-D-Gal glycoside bond in **7** and **7**-ol, which may also be slightly altered on reduction of the other oligosaccharides in the series giving small differences in chemical shifts of H-4 of β-D-Gal*p*-(1→4). A similar overall conformation for the backbones of **1, 3, 4, 5, 6**, and their alditols has been predicted by potential-energy calculations[27].

^{1}H-N.m.r. analysis and conformation of 3-fucosyllactosamine (**8**) *and its alditol* (**8**-*ol*). — The conformation shown in Fig. 1 for the reducing end trisaccharide β-D-Gal*p*-(1→4)-[α-L-Fuc*p*-(1→3)]-D-Glc of **7** is similar to that predicted for the nonreducing end group 3-fucosyllactosamine in chemically synthesised oligosaccharides[22,24,29]. We have therefore carried out the ^{1}H-n.m.r. analysis of **8** and **8**-ol to find if a similar change in chemical shifts occurs on reduction of **8** as that found for **7**, and hence provide further evidence for the proposed conformation.

Comparison of the chemical shift data for the α-L-Fuc*p*-(1→3) group of **7** (Table I) and **8** (Table II) showed that the values for H-5 and H_3-6 were similar (δ 4.827 and 4.848 for H-5, and on average δ 1.163 and 1.176 for H_3-6), whereas those for H-1 differ (δ 5.373 and 5.107 for the α anomers, and δ 5.431 and 5.099 for the β anomers of the oligosaccharides). This is consistent with both oligosaccharides

TABLE II

^{1}H-CHEMICAL SHIFTS (δ) OF THE STRUCTURAL REPORTER GROUPS OF 3-FUCOSYLLACTOSAMINE (**8**) AND ITS ALDITOL (**8**-OL) AND THE DIFFERENCES IN THEIR CHEMICAL SHIFTS AND THE CHEMICAL SHIFTS FOR THE NON-REDUCING END TRISACCHARIDE SEQUENCE OF LACTO-*N*-FUCOPENTAOSE III (**4**)

Reporter group	*Atom*	*Compound*			
		8	**8**-*ol*	*Difference*	**4**
L-Fuc*p*-(1→3)	H-1α	5.107	5.065	0.042	5.127
	H-1β	5.099		0.034	
	H-5	4.848	4.301	0.547	4.830
	H_3-6α	1.178	1.211	0.033	1.174
	H_3-6β	1.173		0.038	
D-Gal*p*-(1→4)	H-1α	4.467	4.512	0.045	4.462
	H-1β	4.457		0.055	
	H-2α	3.507	3.543	0.036	3.497
	H-2β	3.496		0.047	
	H-3	3.651	3.647	0.004	3.653
	H-4	3.914	3.919	0.005	3.897
D-GlcNAc or D-GlcNAcol	H-1α	5.099		4.715	
	H-1β	4.729			4.710
	H-2	3.987	4.425	0.428	
	NHCOCH_3	2.032	2.044	0.012	2.022

having a similarly deshielded H-5 caused by its close proximity to the oxygen atom of the β-D-Gal*p*-(1→4)-D-Glc or D-GlcNAc glycoside bond and the ring oxygen atom of the β-D-Gal residue. The resulting conformation has H-1 of the α-L-Fuc*p*-(1→3) group in close proximity to OH-2 of the D-Glc residue in **7** (Fig. 1) and NH-2 in **8**, thus giving rise to the difference in their H-1 chemical shifts.

The comparable differences in chemical shifts for H-5 of the α-L-Fuc*p*-(1→3) group in **7** and **8**, and their alditols (0.551 and 0.547 p.p.m., respectively, Tables I and II) suggest a similar change in conformation on reduction of **8** as deduced for **7** which, it is proposed, leads to the α-L-Fuc*p*-(1→3) group being orientated away from the β-D-Gal*p*-(1→4) residue as shown for **7**-ol (Fig. 1).

That **8** adopts the conformation proposed for the nonreducing 3-fucosyl-lactosamine sequence of chemically synthesised oligosaccharides is suggested by the similarity of the data given in Table II for the α-L-Fuc*p*-(1→3) group and β-D-Gal*p*-(1→4) residue of **8** and **5**. The data given for **5** are taken from studies on the milk oligosaccharide[13] and are comparable[12] to that reported by Dua and Bush[14], and from studies on the chemically synthesised oligosaccharides discussed[22].

*Recognition of lacto-*N*-difucohexaose I* (**6**) *and its alditol* (**6**-*ol*) *by antibodies D11 and H10.* — Because of the immunodominance of the two α-L-fucopyranosyl groups for antibodies D11 and H10, it is suggested that these antibodies recognise the face of the molecule **6** shown in Fig. 1 containing the fucose methyl groups. The 2-acetamido-2-deoxy-D-glucose residue is thought to be included in the recognition site because an oligosaccharide with the Type 2-based structure **10** was not

inhibitory[11]. For a synthetic molecule having this oligosaccharide sequence attached to a long chain aglycon, the orientation of the α-L-fucopyranosyl groups with respect to each other was found to be the same as in **6**, whereas their orientation with respect to the 2-acetamido-2-deoxy-D-glucose residue differs[24]. The approximately equal inhibitory activity of **6** and **6**-ol with antibodies D11 and H10 further suggests that they are recognising features in the nonreducing and nonreduced ends of tetra- or penta-saccharides. From other studies of oligosaccharide binding by lectins[22,24,29], antibodies[25,30–32], and carbohydrate-binding proteins[33,34], it is proposed that areas with a preponderance of protons interact with hydrophobic amino acids in the combining site and that surrounding hydroxyl groups form specific hydrogen bonds with polar amino acids. For wheat germ-agglutinin binding to a sialyloligosaccharide, the dominant forces stabilising the associated complex are thought to be hydrogen bonds and van der Waals' forces[35].

From the present studies, it is not possible to predict which of the hydroxyl groups are necessary for antibody binding, as such an investigation requires oligosaccharides with site-specific modifications as described by others[24,25,29–32]. From such an investigation for a lectin[24] and a monoclonal antibody[25] with Le^b specificity, it has been shown that the former requires OH-4 of the α-L-Fuc*p*-(1→4) group and OH-3 and OH-4 of the β-D-Gal*p*-(1→3) residue for binding, and the latter OH-2 of the α-L-Fuc*p*-(1→2) group and OH-3 of the β-D-Gal*p*-(1→3) residue. As shown in Fig. 1 for the model of **6**, these hydroxyl groups surround the nonreducing end of the molecule.

Recognition of **7** *by antibodies C2 and G3.* — From inhibition of binding assays[10,11] with several oligosaccharides, it was deduced that the monoclonal antibodies designated C2 and G3 recognise the combined Le^a and 3-fucosyllactose structure of **7**, as **4** which lacks the α-L-fucopyranosyl group (1→3)-linked to glucose was 3–7 fold less active than **7** as an inhibitor, and **7**-ol showed a far greater decrease in activity (15–24 fold). In the conformation shown for **7** but not for **7**-ol (Fig. 1), the face of the molecule having the methyl group of the α-L-Fuc*p*-(1→4) group and the acetamido group of the 2-acetamido-2-deoxy-D-glucopyranosyl residue also contains the methyl group of the second α-L-fucopyranosyl group (1→3)-linked to glucose. This face of the molecule is, therefore, predicted to contain the features recognised by the antibody-combining site of antibodies C2 and G3.

As the α-L-Fuc*p*-(1→3) group appears to be an immunodominant feature, it is suggested that the antibodies bind to residues internal to the nonreducing end, including features in the shaded area of **7** shown in Fig. 1. It is, then, unlikely that the differently orientated glucose or glucitol residue of **4**, **4**-ol, and **7**-ol could be accommodated in the combining site, hence their lower activities.

From the comparison of the ^{1}H-n.m.r. data for **8** and **7**, it is suggested that, in a glycoconjugate chain where the glucose residue of **7** would be replaced by a 2-acetamido-2-deoxy-D-glucose residue, the β-D-Gal*p*-(1→4)-[α-L-Fuc*p*-(1→3)]-D-GlcNAc sequence adopts a conformation similar to that proposed in Fig. 1 for the

reducing end of **7**, with OH-2 of the glucose residue (Fig. 1) replaced by an acetamido group, which may thus be part of an extended carbohydrate-binding site in glycoproteins and glycolipids recognised by the antibodies.

Recognition of lacto-N-fucopentaose II (**4**) *by antibody E6.* — For antibody E6, the best inhibitors of binding were **4** and **7** which had comparable activity; **7**-ol, **4**-ol, and **6** were 2-, 4-, and 40-fold less active than **4**, respectively[11]. This suggests that antibody E6 is primarily recognising features in the nonreducing end trisaccharide sequence which is not in close proximity to the α-L-Fuc*p*-(1→3) group of **7** (Fig. 1) but is present in **4** and **7**, and their alditols (*i.e.*, is an anti-Le^a antibody). The lower inhibitor activity of the alditols could then be due to the glucitol residue interfering with antibody binding.

In conclusion, we have used the chemical shift data of a series of related oligosaccharides, their relative activities as inhibitors of antibody binding, and empirical conformational rules of others[17–27] to deduce the topography of atoms recognised by the antibody-combining sites. Substantiation of the proposed conformations awaits additional experimentation, including analysis of the oligosaccharide nuclear Overhauser effects.

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