Carbohydrate Research, 226 (1992) 1–14
Elsevier Science Publishers B.V., Amsterdam

Primary structure of four human milk octa-, nona-, and undeca-saccharides established by ^{1}H- and ^{13}C-nuclear magnetic resonance spectroscopy

Gérard Strecker*, Sophie Fièvre, Jean-Michel Wieruszeski, Jean-Claude Michalski, and Jean Montreuil

Laboratoire de Chimie Biologique (Unité Mixte de Recherche du C.N.R.S. No 111), Université des Sciences et Techniques de Lille Flandres–Artois, F-59655 Villeneuve d'Ascq (France)

(Received August 19th, 1990; accepted in revised form July 21st, 1991)

ABSTRACT

The structures of two octasaccharides, one nonasaccharide, and one undecasaccharide, isolated from human milk, have been investigated by ^{1}H- and ^{13}C-nuclear magnetic resonance spectroscopy. The structures of these oligosaccharides are: β-D-Gal*p*-(1→4)-[α-L-Fuc*p*-(1→3)]-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-[α-L-Fuc*p*-(1→3)]-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc; β-D-GAL*p*-(1→3)-[α-L-Fuc*p*-(1→4)]-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-[α-L-Fuc*p*-(1→3)]-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc; β-D-Gal*p*-(1→4)-[α-L-Fuc*p*-(1→3)]-β-D-Glc*p*NAc-(1→6)-{α-L-Fuc*p*-(1→2)-β-D-Gal-(1→3)-[α-L-Fuc*p*-(1→4)]-β-D-Glc*p*NAc-(1→3)}-β-D-Gal*p*-(1→4)-D-Glc; and 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)-[α-L-Fuc*p*-(1→3)]-β-D-Glc*p*NAc-(1→6)-[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. The two octasaccharides have been previously isolated from human milk as a mixture, and in a pure form from new-born feces, but the n.m.r. data were not provided. These two octasaccharides display the di-Lewis X and the composite Lewis A–Lewis X antigenic determinant, previously described as neo-antigens of adenocarcinoma cell lines.

INTRODUCTION

The combination of gel filtration, preparative paper chromatography, and high performance liquid chromatography on reverse-phase octadecyl column recently led us to characterize about 70 neutral human milk oligosaccharidic fractions[1]. Despite the ability of h.p.l.c. to separate compounds having similar composition, numerous fractions remained heterogenous. Nevertheless, by recycling the material, we have been able to isolate new isomers. The present paper describes the isolation of four oligosaccharides and the assignment of most of their ^{1}H- and ^{13}C-n.m.r. parameters.

MATERIALS AND METHODS

Fractionation of milk oligosaccharides that led to the isolation of the so-called "Fractions V, VI, and VIII" has been previously described[1]. The data of the n.m.r.

* Author for correspondence.

0008-6215/92/$ 05.00

spectroscopy, f.a.b.–mass spectrometry, and methylation analysis have been published previously[1].

RESULTS

Isolation of the four oligosaccharides. — Fractions V, VI, and VIII, obtained by preparative paper chromatography[1], were respectively fractionated into 8, 3, and 17 peaks (Fig. 1) on a 5-μm ODS Zorbax column (25 × 0.95 cm), with water as eluent. Compounds V-1,2 and V-3,5 were recycled two times on the same column, until total purification. Finally, 17 mg of V-1,2, 6 mg of V-3,5, 112 mg of VI-2, and 5 mg of VIII-16,17 were obtained from 20 L of combined samples of milk. Two h.p.l.c. peaks were obtained for each oligosaccharide, which correspond to the β (first peak) and the α anomer (second peak) of the compound. The other fractions remained too heterogenous for structural analysis.

Structure of compound V-1,2. — From the ^{1}H-n.m.r. spectrum of oligosaccharide V-1,2 (Fig. 2 and Table I), it could be concluded that it is an octasaccharide (**1**) containing two fucose units (δ 5.132 and 5.111), H-1, three galactose units (δ 4.464, H-1, 3.494, H-2; 4.442, H-1, 3.502, H-2; 4.432, H-1, 3.578, H-2), two 2-acetamido-2-deox-

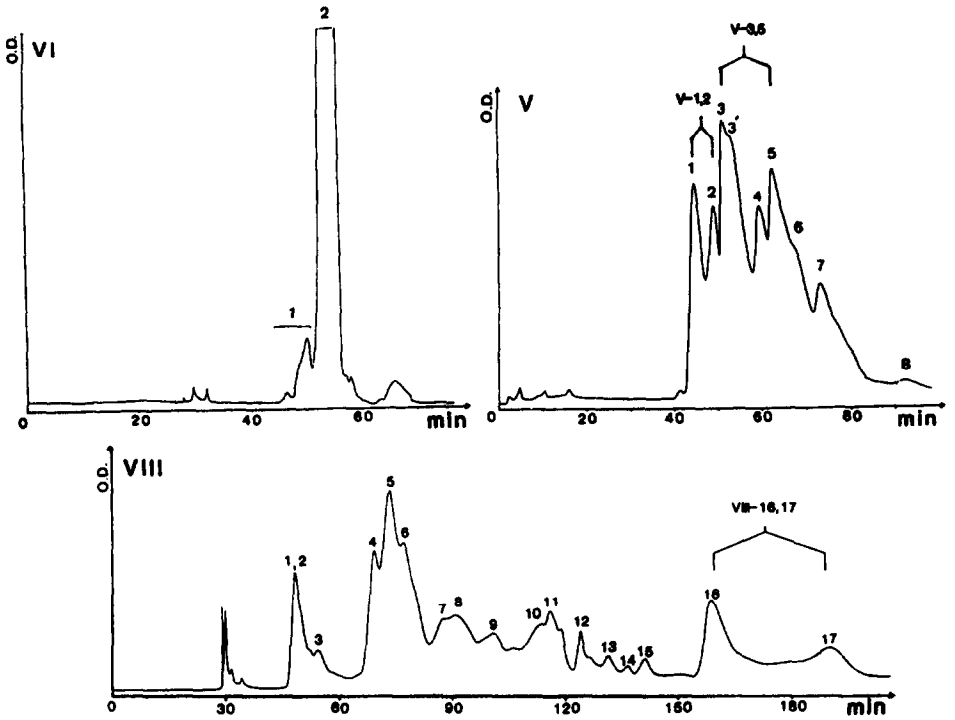


Fig. 1. H.p.l.c. chromatogram of Fractions V, VI, and VIII using a 0.5 μm ODS Zorbax column (25 × 0.95 cm) (Du Pont Instruments, Paris) with water as eluent; flow rate, 0.5 mL/min.

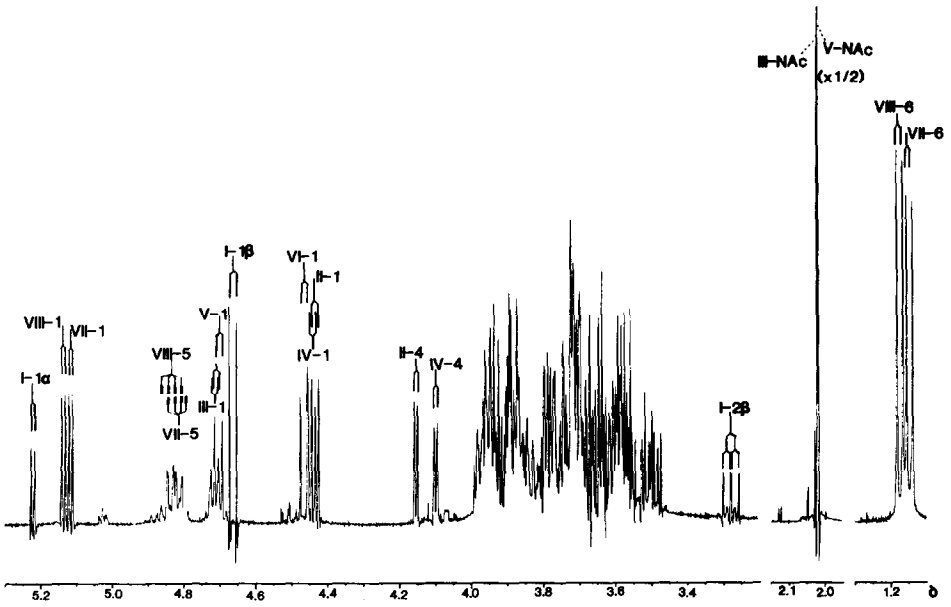


Fig. 2. 400-MHz ^{1}H-n.m.r. spectrum of compound V-1,2 (**1**).

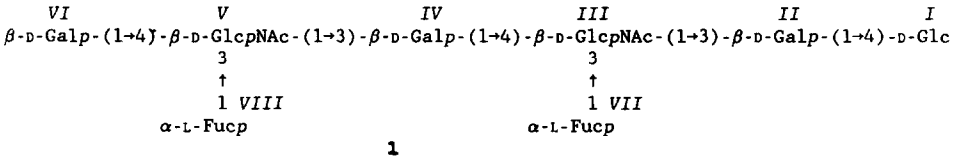
TABLE I

^{1}H- and ^{13}C-n.m.r. data for compound V-1,2 (**1**)

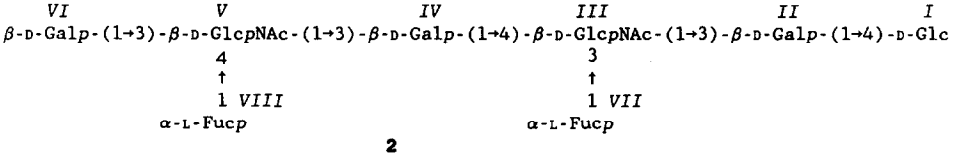
Residue or group	*Linkage*	*Chemical shift (δ)*						
		H-1	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	*NHCOCH$_3$*
VI β-Gal	1→4	4.464	3.494	3.648	3.897	3.600	3.718	
VIII α-Fuc	1→3	5.132	3.687	3.906	3.788	4.835	1.175	
V β-GlcNAc	1→3	4.701	3.956	3.868	3.956	3.569	3.823 3.982	2.016
IV β-Gal	1→3	4.442	3.502	3.700	4.097	3.587	3.718	
VII α-Fuc	1→3	5.111	3.687	3.882	3.770	4.812	1.147	
III β-GlcNAc	1→3	4.714(α) 4.711(β)	3.956	3.868	3.938	3.569	3.823 3.982	2.020
II β-Gal	1→4	4.432	3.578	3.709	4.154	3.701	3.762	
I α-Glc		5.218	3.573	3.825	3.636	3.946	3.859	
I β-Glc		4.660	3.276	3.630	3.636	3.596	3.815	
		C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*CO*	*CH$_3$*
VI β-Gal	1→4	103.04	72.35	73.77	69.65	76.21	62.81		
VIII α-Fuc	1→3	99.99	69.00	70.48	73.21	67.99	16.59		
V β-GlcNAc	1→3	103.79	57.26	76.05	74.32	76.38	60.94	175.97	23.57
IV β-Gal	1→4	103.04	71.82	82.92	69.55	75.74	62.76		
VII α-Fuc	1→3	99.87	68.94	70.48	73.16	67.99	16.63		
III β-GlcNAc	1→3	103.79	57.26	76.05	74.08	76.40	60.94	175.91	23.57
II β-Gal	1→4	104.22(α) 71.27(β)	71.03	83.35	69.65	76.16	62.27		
I α-Glc		93.11	72.43	72.70	79.67	71.42	61.24		
I β-Glc		97.02	75.08	75.65	79.55	76.10	61.38		

yglucose units (δ 4.701, H-1, 3.956, H-2; 4.711, H-1, 3.956, H-2), and one glucose unit (δ 5.218, H-1α, 4.460, H-1β).

The H-1, H-5, and H-6 resonances of the two L-fucosyl groups are characteristic for α-L-(1→3)-linked residues and consequently, the octasaccharide displays two Lewis X determinants. In addition, the presence of two galactose H-4 signals, deshielded at δ 4.097 and 4.154, respectively, showed that the compound contains two galactose units substituted at O-3, allowing the definition of oligosaccharide **1** as a linear structure. The value of the Gal^{II} H-4 resonance is remarkably constant in the series of lactose-containing milk oligosaccharides previously analyzed by ^{1}H-n.m.r. spectroscopy (δ 4.12–4.15)[2,3], and this well-known parameter allowed us to establish the H-1 and H-4 resonances of Gal^{II} and Gal^{IV} through a COSYDR spectrum, and the Gal^{VI} H-4 signal, which occurred at δ 3.897, was correlated with the corresponding H-1β resonance at δ 4.464. The anomeric protons of the two 2-acetamido-2-deoxyglucose units were distinguished on the previous observations[2-5] that the anomerization effect affects the H-1 signal of GlcNAc^{III} owing to the spatial proximity of this H-1 to the reducing end of the oligosaccharide. The GlcNAc^{III} H-1 resonance was also deduced from a comparison of the spectrum with that of compound V-3,5 (Table I). Concerning the fucosyl groups, one of the H-1 resonance (δ 5.111) was absolutely identical to that observed for the α-L-fucosyl group (1→3)-linked to GlcNAc^{III} in oligosaccharides bearing the X determinant[1,4,5] (see also below compound V-3,5). Consequently, the n.m.r. parameters of the two L-fucosyl groups *VII* and *VIII* (see Fig. 2) could be ascribed without ambiguity. According to this ^{1}H-n.m.r. spectroscopy analysis, the structure of V-1,2 was established as **1**.



1



2

The ^{1}H–^{13}C COSY spectrum of compound V-1,2 (Fig. 3) showed all the ^{1}H- and ^{13}C-n.m.r. resonances of the compound, which are reported in Table I. This structure was confirmed by f.a.b.–m.s. (Fig. 4), which showed a pseudomolecular ion (M + 1) at *m/z* 1701. The primary-sequence ion (*m/z* 1261) and the secondary ion (*m/z* 1055) arising by preferential elimination of the substituent at O-3 of the 2-acetamido-2-deoxyglucose unit indicated that GlcNAc^{III} was substituted at O-3 by a fucosyl group. The other ions

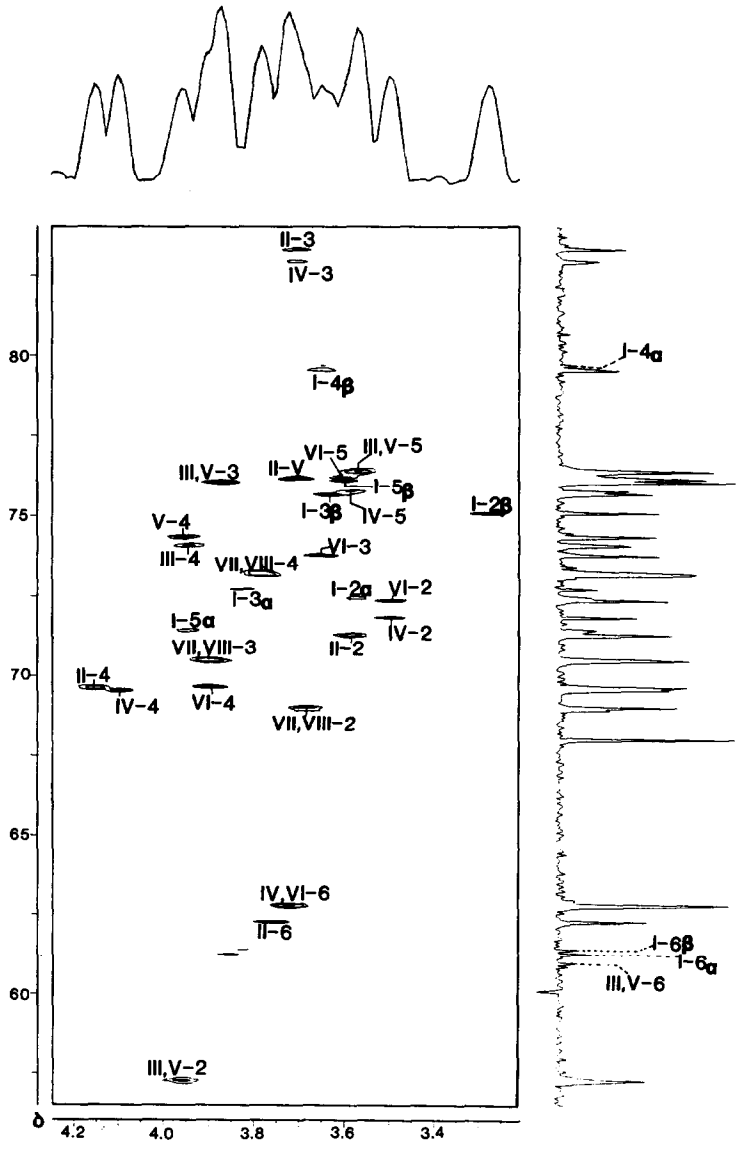


Fig. 3. Heteronuclear-correlated n.m.r. spectrum of compound V-1,2 (**1**).

having *m/z* 638 and 432 indicated the sequence, β-D-Gal*p*-(1→4)-[α-L-Fuc*p*-(1→3)]-α-D-Glc*p*NAc. The partially methylated and acetylated methyl glycosides obtained by methanolysis of the permethylated compound are listed in Table II. Therefore, the structure deduced by ^{1}H- and ^{13}C-n.m.r. analysis was fully confirmed by f.a.b.–m.s. and methylation analysis.

TABLE II

Partially methylated methyl glycoside acetates obtained from the methanolyzates of permethylated milk oligosaccharides

Sugar derivative	*Compound*			
	V-1,2 (**1**)	*V-3,5* (**2**)	*VI-2* (**3**)	*VIII-16,17* (**4**)
2,3,4-Me_3-Fuc	1.8	1.7	2.6	2.6
2,3,4,6-Me_4-Gal	0.9	0.9	0.9	0
2,4,6-Me_3-Gal	2.0[a]	2.0[a]	0	1[a]
3,4,6-Me_3-Gal	0	0	1[a]	2.1
2,4-Me_2-Gal	0	0	1.1	0.9
2,3,6-Me_3-Glc	0.9	0.9	0.9	0.9
4,6-Me_2-GlcN(Me)Ac	0	0	0	1.8
6-Me-GlcN(Me)Ac	0.7	0.8	0.8	0.8

[a] Values taken as basis of calculation. The response factors were obtained by methylation analysis of known milk oligosaccharides.

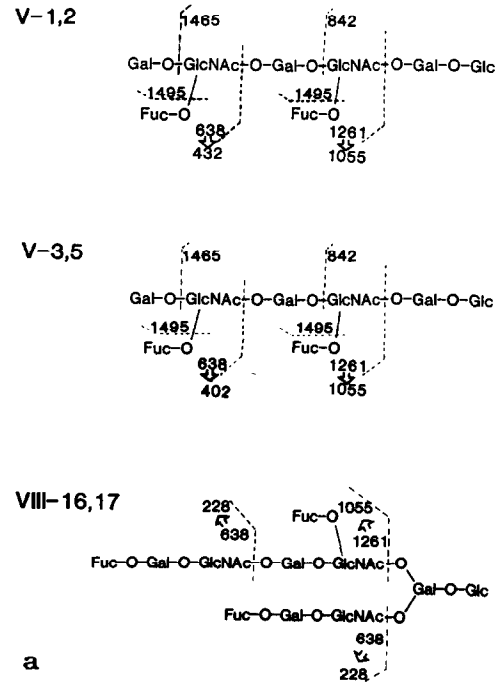
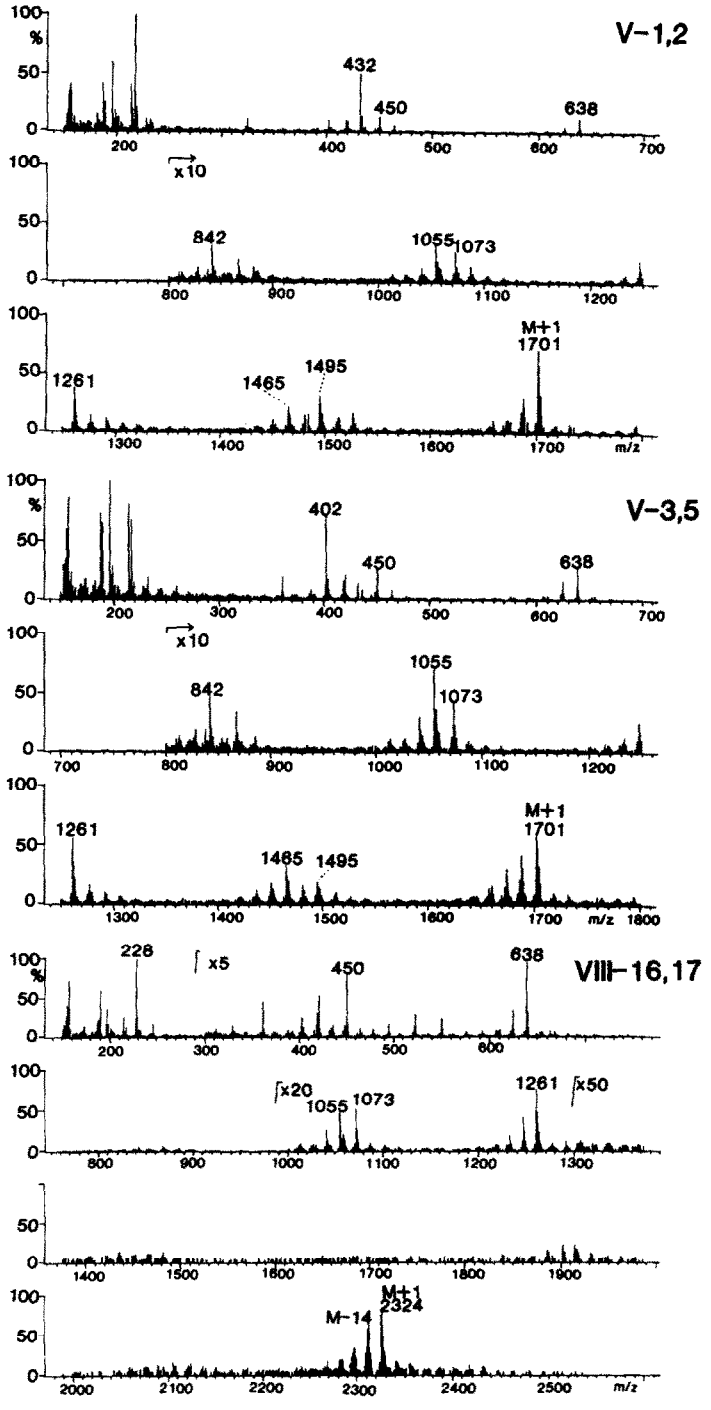


Fig. 4. (a and b) F.a.b.–m.s. of compounds V-1,2 (**1**), V-3,5 (**2**), and VIII-16,17 (**4**).


V-1,2
432
450
638
x10
842
1055
1073
M+1
1701
1261
1465
1495
m/z
V-3,5
402
450
638
x10
842
1055
1073
1261
M+1
1701
1465
1495
m/z
VIII-16,17
228
x5
450
638
x20
1055
1073
1261
x50
M+1
2324
M-14
m/z

b

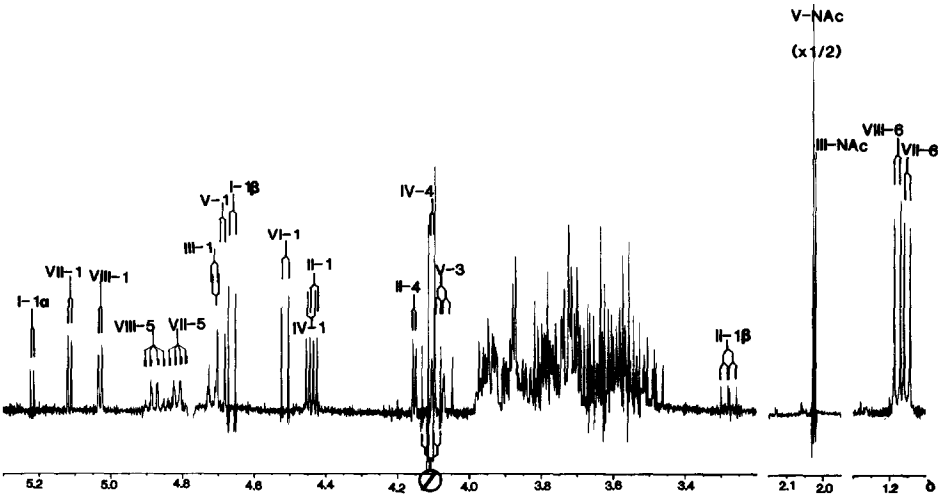


Fig. 5. 400-MHz ^{1}H-n.m.r. spectrum of compound V-3,5 (**2**).

TABLE III

^{1}H- and ^{13}C-n.m.r. data for compound V-3,5 (**2**)

Residue or group	*Linkage*	*Chemical shifts (δ)*						
		H-1	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	*NHCOCH$_3$*
VI β-Gal	1→3	4.512	3.482	3.616	3.874	[a]	[a]	
VIII α-Fuc	1→4	5.026	3.796	3.884	3.788	4.875	1.178	
V β-GlcNAc	1→3	4.691	3.946	4.070	3.946	[a]	[a]	2.027
IV β-Gal	1→4	4.443	3.511	3.704	4.098	[a]	[a]	
VII α-Fuc	1→3	5.112	3.686	3.886	3.783	4.811	1.152	
II β-GlcNAc	1→3	4.718(α) 4.713(β)	3.941	3.866	3.941	[a]	[a]	2.020
II β-Gal	1→4	4.433	3.574	3.700	4.150	[a]	[a]	
I α-Glc		5.218	3.572	3.832	3.636	[a]	[a]	
I β-Glc		4.660	3.277	3.654	3.654	[a]	[a]	

		C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*CO*	*CH$_3$*
VI β-Gal	1→3	104.29	71.92	73.74	69.77	75.88	63.07		
VIII α-Fuc	1→4	99.41	69.22	70.56	73.37	68.26	16.80		
V β-GlcNAc	1→3	103.96	57.41	77.35	73.53	76.61	61.04	176.09	23.72
IV β-Gal	1→4	103.14	71.92	83.46	69.77	75.76	62.87		
VII α-Fuc	1→3	100.88	69.08	70.61	73.28	68.10	16.72		
III β-GlcNAc	1→3	104.34	57.29	76.16	74.25	76.27	61.08	176.09	23.09
II β-Gal	1→4	104.23	71.39	83.09	69.64	75.76	62.38		
I α-Glc		93.22	72.37	72.55	79.80	71.53	61.38		
I β-Glc		97.14	75.06	75.21	79.69	75.16	61.51		

[a] Not determined.

Structure of compound V-3,5. — The ^{1}H-n.m.r. spectrum of compound V-3,5 (Fig. 5) indicated the presence of two fucose, three galactose, two 2-acetamido-2-deoxyglucose, and one glucose units. The H-1, H-5, and H-6 resonances of the α-L-fucosyl groups showed that they are (1→3) and (1→4) linked. The H-1 and H-4 resonances of Gal^{II} could be easily identified owing to the characteristic chemical shift values (see above for compound V-1,2). The second Gal H-4 signal at δ 4.098 is significant for another

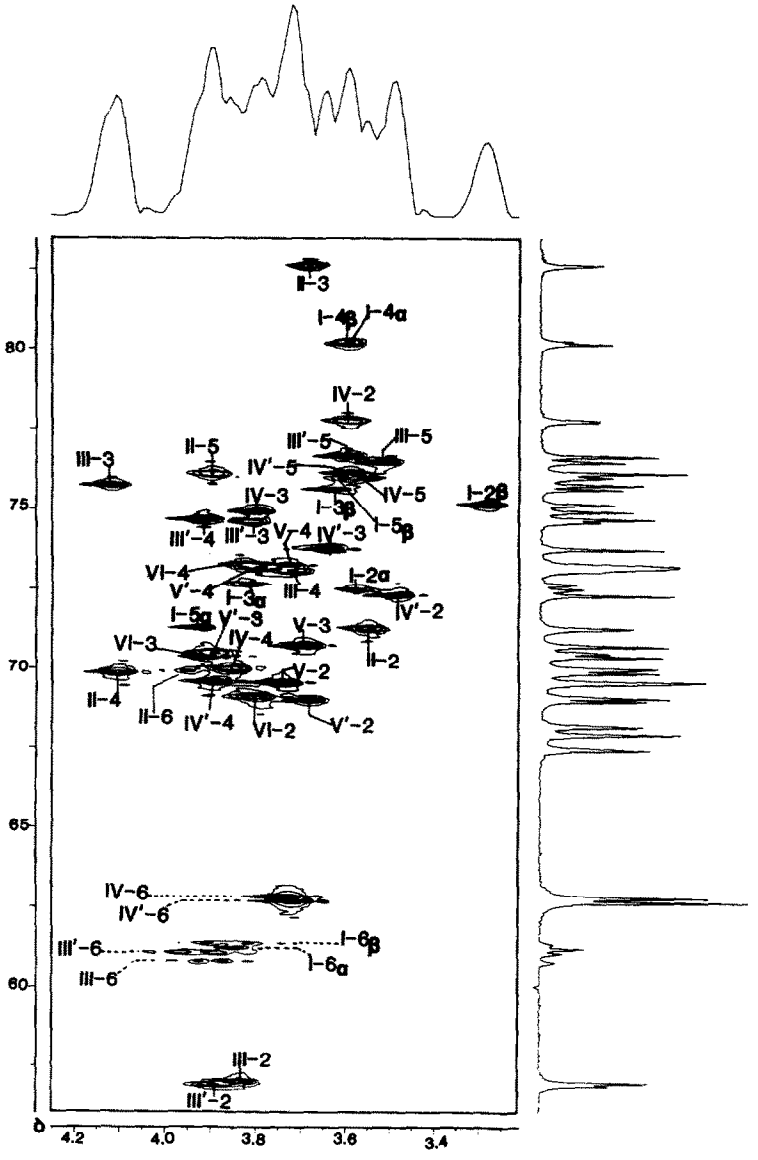


Fig. 6. Heteronuclear-correlated n.m.r. spectrum of compound VI-2 (2).

O-3-substituted galactose unit (Gal^{IV}). Consequently, compound V-3,5 also possesses a linear structure. The H-1 signal of $GlcNAc^{III}$ is affected by the anomerization effect, which allowed us to distinguish this unit from $GlcNAc^{V}$. The H-3 signal of $GlcNAc^{V}$ is deshielded at δ 4.070, thus confirming the substitution at C-4 by a fucosyl group (Le^a determinant)[5]. On the basis of these results, the structure of compound V-3,5 was established as **2**, which harbors the Le^a–Le^x hybride determinant.

The ^{13}C-n.m.r. parameters of compound V-3,5 are listed in Table III. As shown in Fig. 4b, the f.a.b.–m.s. spectrum confirmed this structure; it indicated, *inter alia*, the position of the two fucosyl groups, respectively linked to O-3 of $GlcNAc^{III}$ (*m/z* 1261→1055) and to O-4 of $GlcNAc^{V}$ (*m/z* 638→402).

Structure of compound VI-2. — Compound VI-2 was previously isolated from the paper chromatogram of Fraction VII, in an amount too low to obtain meaningful

TABLE IV

^{1}H- and ^{13}C-n.m.r. data for compound VI-2 (**3**)

Residue or group	*Linkage*	*Chemical shift (δ)*						
		H-1	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	*NHCOCH$_3$*
*IV'*β-Gal	1→4	4.446	3.489	3.642	3.888	3.590	3.721	
*V'*α-Fuc	1→3	5.097	3.678	3.897	3.783	4.828	1.170	
*III'*β-GlcNAc	1→6	4.630	3.897	3.812	3.923	3.598	3.844(6a) 4.003(6b)	2.044(α) 2.049(β)
V α-Fuc	1→2	5.145	3.748	3.686	3.739	4.335	1.270	
IV β-Gal	1→3	4.651	3.598	3.801	3.853	3.555	3.730	
VI α-Fuc	1→4	5.024	3.801	3.915	3.818	4.863	1.254	
III β-GlcNAc	1→3	4.598(α) 4.594(β)	3.836	4.123	3.725	3.511	3.844(6a) 3.959(6b)	2.055
II β-Gal	1→4	4.403	3.546	3.686	4.114	3.897	3.94(6a) (6b)[a]	
I α-Glc		5.215	3.851	3.818	3.598	3.932	3.844	
I β-Glc		4.661	3.284	3.634	3.598	3.598	3.774(6a) 3.941(6b)	

		C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*CO*	*CH$_3$*
*IV'*β-Gal	1→4	103.10	72.29	73.71	69.60	76.16	62.76		
*V'*α-Fuc	1→3	99.89	68.97	70.48	73.17	67.99	16.61		
*III'*β-GlcNAc	1→6	102.07	56.92	74.61	74.61	76.63	61.04	175.51	23.78(α) 23.80(β)
V α-Fuc	1→2	100.81	69.53	70.70	73.24	67.51	16.61		
IV β-Gal	1→3	101.88	77.74	74.88	70.00	76.01	62.87		
V α-Fuc	1→4	99.03	69.06	70.36	73.24	68.29	16.66		
III β-GlcNAc	1→3	104.45	57.00	75.73	73.02	76.43	60.73	175.38	23.46
II β-Gal	1→4	104.32	71.28	82.56	69.93	76.10(α) 76.13(β)	69.95		
I α-Glc		93.05	72.46	72.65	80.18	71.28	61.18		
I β-Glc		96.95	75.12	75.61	80.09	76.01	61.31		

[a] Not determined.

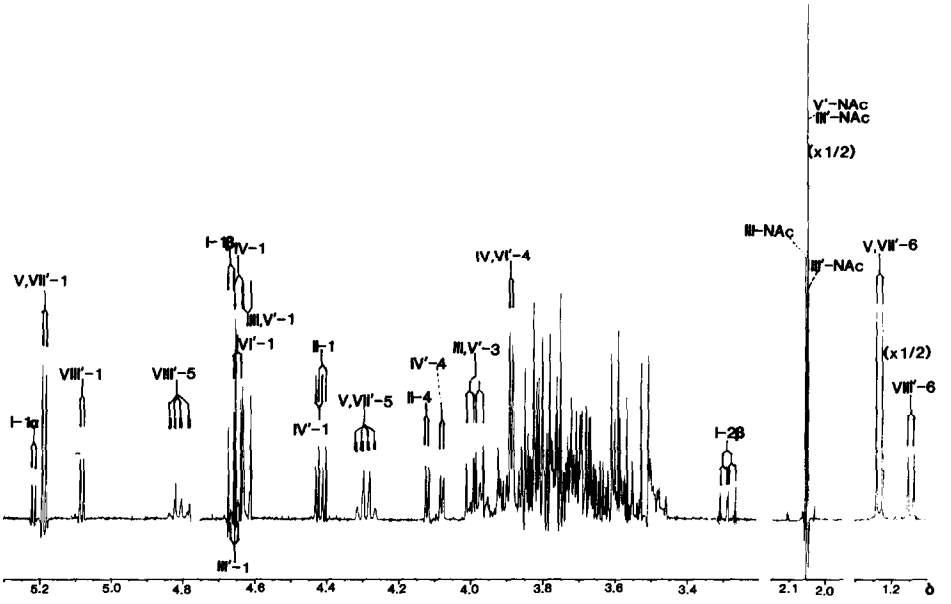
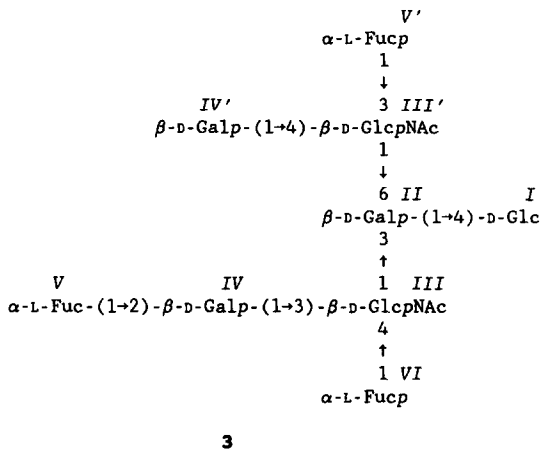


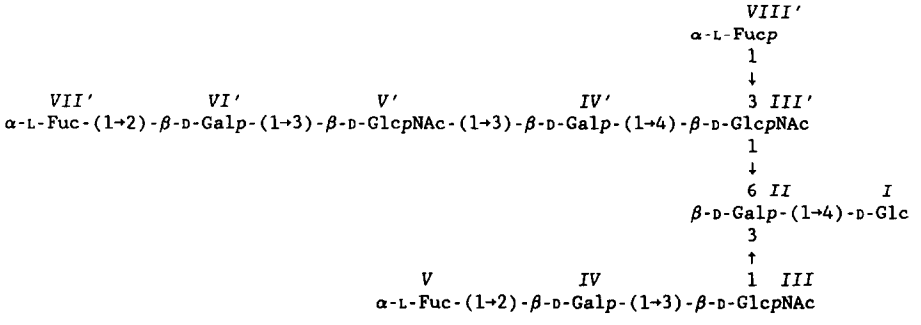
Fig. 7. 400-MHz ^{1}H-n.m.r. spectrum of compound VIII-16,17 (**4**).

^{13}C-n.m.r. data[4]. Its isolation in an higher amount from Fraction VI allowed us to fully assign the ^{1}H- and ^{13}C-n.m.r. parameters (Fig. 6 and Table IV), which correspond to structure **3**.

Structure of compound VIII-16,17. — Compound VIII – 16,17 contains three fucose, four galactose, three 2-acetamido-2-deoxyglucose, and one glucose units according to the 1D ^{1}H-n.m.r. spectrum (see Fig. 7). Two fucosyl groups are linked to O-2 of a galactose unit (H determinant), and one to O-3 of a 2-acetamido-2-deoxyglucose units (Le^x determinant), as shown by the chemical shift values of H-1, H-5, and H-6 (Table V), and the relative intensity of these signals. The ^{1}H-n.m.r. COSY spectrum showed that H-3 atoms of both GlcNAc^{III} and GlcNAc^{V} are deshielded and possess the same chemical shift value (δ 3.987), which is characteristic of a 2-acetamido-2-deoxyglucose unit substituted at O-3 by a galactose unit. The third GlcNAc$^{III'}$ residue is substituted with an (1→3)-linked α-L-fucosyl group and displays the ^{1}H-n.m.r. parameters of a (1→6)-linked β-D-GlcNAc unit, observed in similar oligosaccharides[4,7]. The two H-4 resonances for Gal^{II} (δ 4.118) and Gal^{IV} (δ 4.078) are indicative of a galactose unit substituted at O-3, one of them being characteristic of the Gal^{II} unit of lactose[4,7]. The ^{1}H-n.m.r. parameters of Gal^{IV} and GlcNAc^{III} are identical to those observed for IV^{2}FucLnOse$_4$ (ref. 5) and, consequently, were attributed to the sugar unit of the (1→3) branch. Finally, the oligosaccharide contains two α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-(1→3) units as chain terminals, associated with the inner Le^x determinant, R-(1→3)-β-D-Gal*p*-(1→4)-[α-L-Fuc*p*-(1→3)]-β-D-Glc*p*NAc-(1→6). Therefore, the structure of compound VIII-16,17 could be established as **4**.



3



4

The ^{1}H- and ^{13}C-n.m.r. signals of compound VIII-16,17 were assigned by use of DEPT, multistep ^{1}H–^{1}H COSY, and ^{1}H–^{13}C COSY experiments (Table IV). The structure was confirmed by f.a.b.–mass spectrometry, which gave primary fragments having *m/z* 1261 and 638 (Fig. 4), together with the pseudomolecular ion (M + 1) at *m/z* 2324.

DISCUSSION

Milk oligosaccharides V-1,2 ($V^{3}FucIII^{3}Fucp Lc_{6}$) and V-3,5 ($V^{4}FucIII^{3}Fucpn Lc_{6}$) have been previously characterized as a mixture[6], and more recently compound V-3,5 was isolated from feces of preterm infants fed on breast milk[7]. Compound VI-2 ($III'^{3}FucIV^{2}FucIII^{4}FucLc_{6}$) has also been described[4,8], but its ^{1}H- and ^{13}C-n.m.r. signals are fully assigned in the present work. Compound VIII-16,17 ($VI'^{2}FucIII'^{3}FucIV^{2}Fuc$-$iLc_{8}$) is a novel milk oligosaccharide.

TABLE V

^{1}H- and ^{13}C-n.m.r. data for compound VIII-16,17 (4)

Residue or group	*Linkage*	*Chemical shift (δ)*							
		H-1	*H-2*	*H-3*	*H-4*	*H-5*	*H-6a*	*H-6b*	*NHCOCH$_3$*
α-Fuc[a]	1→2	5.186	3.772	3.656	3.729	4.288	1.232		
*VI'*β-Gal	1→3	4.647	3.587	3.83	3.886	[b]	[b]	[b]	
*V'*β-GlcNAc	1→3	4.620	3.80	3.987	3.527	3.52	3.78	3.90	2.046
*IV'*β-Gal	1→4	4.419	3.480	3.707	4.078	[b]	[b]	[b]	
*VIII'*α-Fuc	1→3	5.080	3.690	3.875	3.768	4.811	1.144		
*III'*β-GlcNAc	1→6	4.655	[b]	[b]	[b]	[b]	[b]	[b]	2.046(α) 2.049(β)
α-Fuc[a]	1→2	5.186	3.772	3.656	3.729	4.288	1.232		
*IV*β-Gal	1→3	4.642	3.587	3.83	3.886	[b]	[b]		
*III*β-GlcNAc	1→3	4.623(α) 4.620(β)	3.80	3.987	3.527	3.52	3.78	3.90	2.052
*II*β-Gal	1→4	4.411	3.553	3.707	4.118	3.84	[b]	[b]	
*I*α-Glc		5.215	3.583	3.832	3.636	[b]	[b]	[b]	
*I*β-Glc		4.662	3.286	3.630	3.60	[b]	[b]	[b]	

		C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*CO*	*CH$_3$*
α-Fuc[a]	1→2	100.76	69.24	70.71	73.13	67.73	16.51[c]		
*VI'*β-Gal	1→3	101.51	77.90	74.77	70.39	76.34	62.38		
*V'*β-GlcNAc	1→3	104.43	56.23	78.50	69.74	76.48	61.72	175.42	23.43
*IV'*β-Gal	1→4	103.04	72.04	82.50	69.74	75.63	62.67		
*VIII'*α-Fuc	1→3	100.02	68.91	70.48	73.31	67.95	16.57		
*III'*β-GlcNAc	1→6	102.02	56.96	74.67(α) 74.61(β)	74.23	75.63	61.07	175.55	23.73(α) 23.76(β)
α-Fuc[a]	1→2	100.76	69.33	70.71	73.13	67.73	16.54[c]		
*IV*β-Gal	1→3	101.51	77.90	74.77	70.39	76.34	62.38		
*III*β-GlcNAc	1→3	104.43	56.28	78.50	69.88	76.55	61.72	175.42	23.43
*II*β-Gal	1→4	104.32	71.31	82.58	69.74	76.19	69.92		
*I*α-Glc		93.07	72.47	72.66	80.25	71.28	61.22		
*I*β-Glc		96.77	75.13	75.63	80.18	75.98	61.36		

[a] *VII'*Fuc or *V* Fuc. [b] Not determined. [c] These values may be interchanged.

Oligosaccharide V-1,2 displays the dimeric Lewis X determinant, previously found in glycolipids isolated from human colonic and liver adenocarcinoma[9]. A rat monoclonal antibody against this oligosaccharide (624 H 12) was recently found to detect the presence of lung cancer two years prior to its detection by conventional diagnostic techniques[10,11]. The chemical synthesis of the dimeric Lewis X hexasaccharide has been also achieved[12].

Oligosaccharide V-3,5 (Lewis A–Lewis X hybrid determinant) corresponds to the carbohydrate structure recognized by the mouse monoclonal antibody ST-421, which is highly specific for human stomach adenocarcinoma[13]. These tumor-associated carbohydrate structures can only be obtained in small amounts from tumor cells. Although they are generally absent, or present in undetectable levels in normal cells, they are easily obtainable as free oligosaccharides from human milk and thus, can be used for further immunological experiments.

ACKNOWLEDGMENTS

This research was supported, in part, by the Centre National de la Recherche Scientifique (Unité Mixte No 111: "Relations structure–fonction des constituants membranaires"; Director: Professor André Verbert), by the Université des Sciences et Techniques de Lille Flandres–Artois, and by the Ministère de l'Éducation Nationale.

The authors are grateful to the Conseil Régional du Nord Pas-de-Calais, the Centre National de la Recherche Scientifique, the Ministère de la Recherche et de l'Enseignement Supérieur, the Ministère de l'Éducation Nationale, and the Association pour la Recherche sur le Cancer for their contribution in the acquisition of the 400-MHz n.m.r. instrument. They are indebted to Miss Catherine Alonso (C.N.R.S. technician) for her skilful technical assistance and to Mr. Jérôme Lemoine for the mass spectrometric analyses. They thank Prof. P. Maubois (I.N.R.A. Rennes) and Dr. V. Barrois (Lactarium, Paris) for providing human milk samples.

REFERENCES

1 G. Strecker, J. M. Wieruszeski, J. C. Michalski, and J. Montreuil, *Glycoconjugate J.*, 5 (1988) 385–396.
2 S. Sabesan and J. C. Paulson, *J. Am. Chem. Soc.*, 108 (1986) 2068–2080.
3 G. Strecker, J. M. Wieruszeski, J. C. Michalski, and J. Montreuil, *Glycoconjugate J.*, 6 (1989) 67–83.
4 G. Strecker, J. M. Wieruszeski, J. C. Michalski, and J. Montreuil, *Glycoconjugate J.*, 6 (1989) 169–182.
5 J. Breg, D. Romijn, J. F. G. Vliegenthart, G. Strecker, and J. Montreuil, *Carbohydr. Res.*, 183 (1988) 19–34.
6 K. Yamashita, Y. Tachibana, and A. Kobata, *J. Biol. Chem.*, 252 (1977) 5408–5411.
7 H. Sabharwal, B. Nilsson, G. Grönberg, M. A. Chester, J. Dakour, S. Sjöblad, and A. Lundblad, *Arch. Biochem. Biophys.*, 265 (1988) 390–406.
8 H. Sabharwal, B, Nilsson, M. A. Chester, F. Lindh, G. Grönberg, S. Sjöblad, and A. Lundblad, *Carbohydr. Res.*, 178 (1988) 145–154.
9 S. Hakomori, E. Nudelman, S. B. Levery, and R. Kannagi, *J. Biol. Chem.*, 259 (1984) 4672–4680.
10 M. Kyogashima, J. Mulshine, R. I. Linnoila, S. Jensen, J. L. Magnani, E. Nudelman, S. Hakomori, and V. Ginsburg, *Arch. Biochem. Biophys.*, 275 (1989) 309–314.
11 M. S. Tockman, P. K. Gupta, J. D. Myers, J. K. Frost, S. B. Baylin, E. B. Gold, A. M. Chase, P. H. Wilkinson, J. L. Mulshcine, *J. Clin. Oncol.*, 6 (1988) 1685–1693.
12 M. Nilsson and T. Norberg, *Carbohydr. Res.*, 183 (1988) 71–82.
13 M. R. Stroud, E. Nudelman, S. B. Levery, M. E. K. Salyan, M. Watanabe, S. Hirohashi, and S. Hakomori, *X^th Int. Symp. Glycoconjugates, Abstr.*, (1989) 263–264.