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Assignment of the ^{1}H- and ^{13}C-NMR Spectra of Eight Oligosaccharides of the Lacto-*N*-tetraose and Neotetraose Series

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The assignment of the ^{13}C- and ^{1}H-NMR spectra of eight oligosaccharides of the lacto-*N*-tetraose and neotetraose series was obtained from homonuclear and heteronuclear correlation spectroscopy. These analyses were performed on the following compounds:

1, Galβ1-3GlcNAcβ1-3Galβ1-4Glc;
2, NeuAcα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc;
3, Galβ1-3[NeuAcα2-6]GlcNAcβ1-3Galβ1-4Glc;
4, NeuAcα2-3Galβ1-3[NeuAcα2-6]GlcNAcβ1-3Galβ1-4Glc;
5, NeuAcα2-3Galβ1-3[Fucα1-4]GlcNAcβ1-3Galβ1-4Glc;
6, Fucα1-2Galβ1-3[NeuAcα2-6]GlcNAcβ1-3Galβ1-4Glc;
7, Galβ1-4GlcNAcβ1-3Galβ1-4Glc;
8, NeuAcα2-6Galβ1-4GlcNAcβ1-3Galβ1-4Glc.

Human milk oligosaccharides are currently used for studying the biosynthesis of I,i and Lewis blood group-related antigens [1-3]. An increase of interest has been devoted to their study since many of them are found covalently linked to ceramide and characterized as tumour associated antigens or as differentiation antigens [4, 5]. The combined use of mass spectrometry and high field NMR spectroscopy can result in a structural analysis of these compounds. In addition, a complete assignment of ^{1}H and ^{13}C-NMR spectra should be a pre-requisite for the detailed analysis of the conformation of these molecules in aqueous solution, which could lead to an understanding of the three dimensional shape recognized by antibodies or lectins. The present paper describes an assignment of the ^{1}H- and ^{13}C-NMR spectra of eight oligosaccharides of the lacto-*N*-tetraose and neotetraose series, including the sialylated Le^{a} and H determinants.

1	Galβ1-3GlcNAcβ1-3Galβ1-4Glc	LcOse$_4$	[8]
2	NeuAcα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc	IV^{3}NeuAc-LcOse$_4$	[9]
3	NeuAcα2 6 Galβ1-3GlcNAcβ1-3Galβ1-4Glc	III^{6}NeuAc-LcOse$_4$	[9]
4	NeuAcα2 6 NeuAcα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc	IV^{3}NeuAc,III^{4}NeuAc-LcOse$_4$	[10]
5	Fucα1 4 NeuAcα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc	IV^{3}NeuAc,III^{4}Fuc-LcOse$_4$	[11]
6	NeuAcα2 6 Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1-4Glc	IV^{2}Fuc,III^{6}NeuAc-LcOse$_4$	[11]
7	Galβ1-4GlcNAcβ1-3Galβ1-4Glc	nLcOse$_4$	[12]
8	NeuAcα2-6Galβ1-4GlcNAcβ1-3Galβ1-4Glc	IV^{6}NeuAc-nLcOse$_4$	[13]

Figure 1. Structures of oligosaccharides described in this study.

The ^{1}H-NMR parameters of six of the eight oligosaccharides studied in this paper have been partially established, and their ^{13}C-NMR parameters fully described [6, 7]. In order to give an accurate and more detailed assignment of the NMR spectra, we have repeated these analysis, using in particular multiple-relayed ^{1}H COSY and heteronuclear COSY experiments.

Experimental

Oligosaccharides presented in Fig. 1 were isolated from human milk according to previous reports [8-13]. The amounts of material were: **1:** 80 mg; **2:** 25 mg; **3:** 20 mg; **4:** 50 mg; **5:** 8 mg; **6:** 3 mg; **7:** 40 mg; **8:** 45 mg.

The 400 MHz ^{1}H-NMR experiments were performed on a Bruker AM-400 WB spectrometer equipped with a 5 mm ^{1}H/^{13}C mixed probe-head, operating in the pulse Fourier transform mode and controlled by an Aspect 3000 computer. Each oligosaccharide was dissolved in 0.4 ml ^2H_2O after three exchanges with ^2H_2O (99.96% atom ^{2}H, Aldrich, Milwaukee, WI, USA) and intermediate lyophilisations. The products were analysed at 300 K with a spectral width of 3000 Hz for 16 K frequency domain points and time domain data points giving a final digital resolution of 0.365 Hz/point.

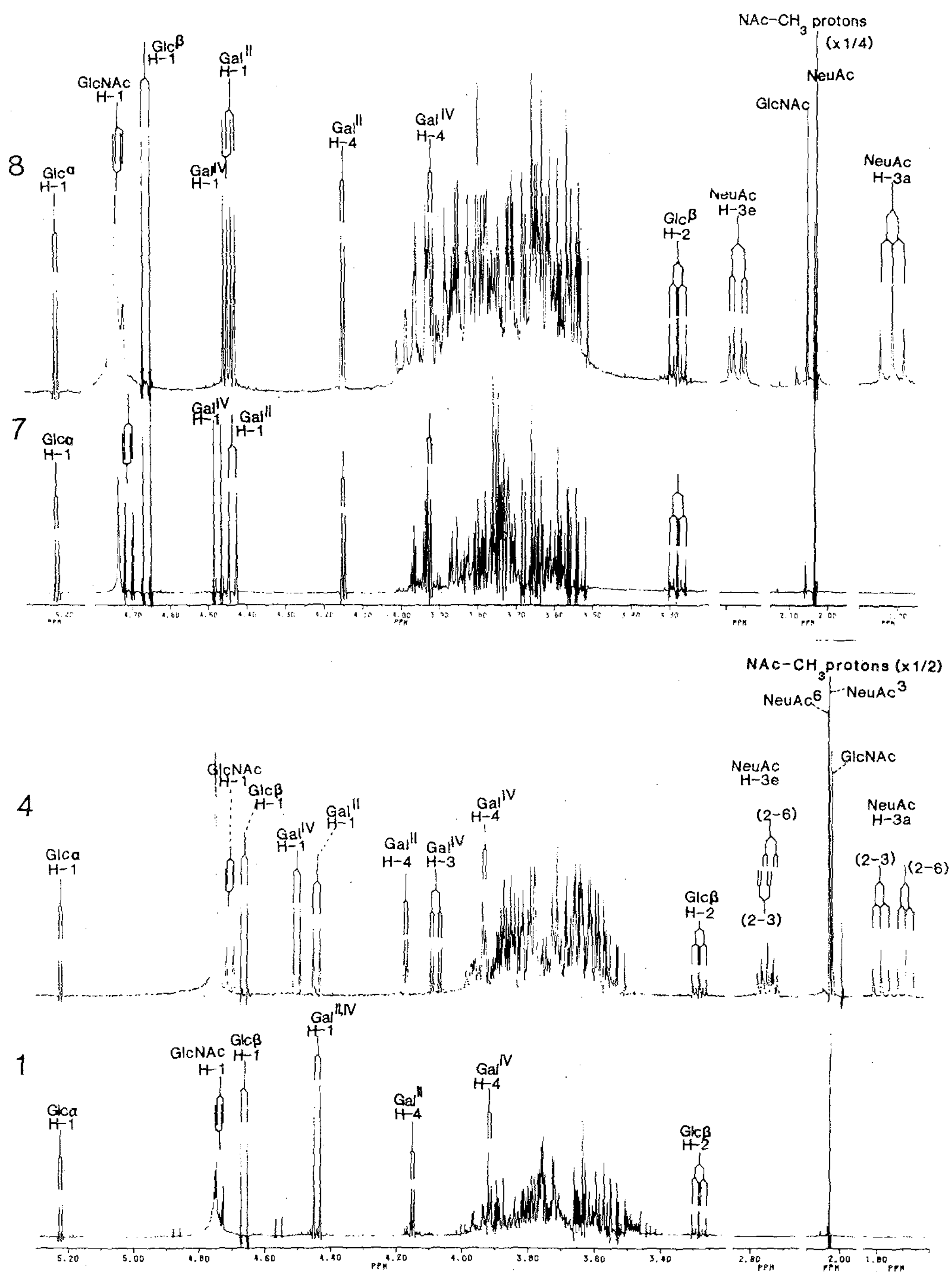

Figure 2. Comparison of the 400 MHz ¹H-NMR spectra of compounds **1, 4, 7** and **8.**

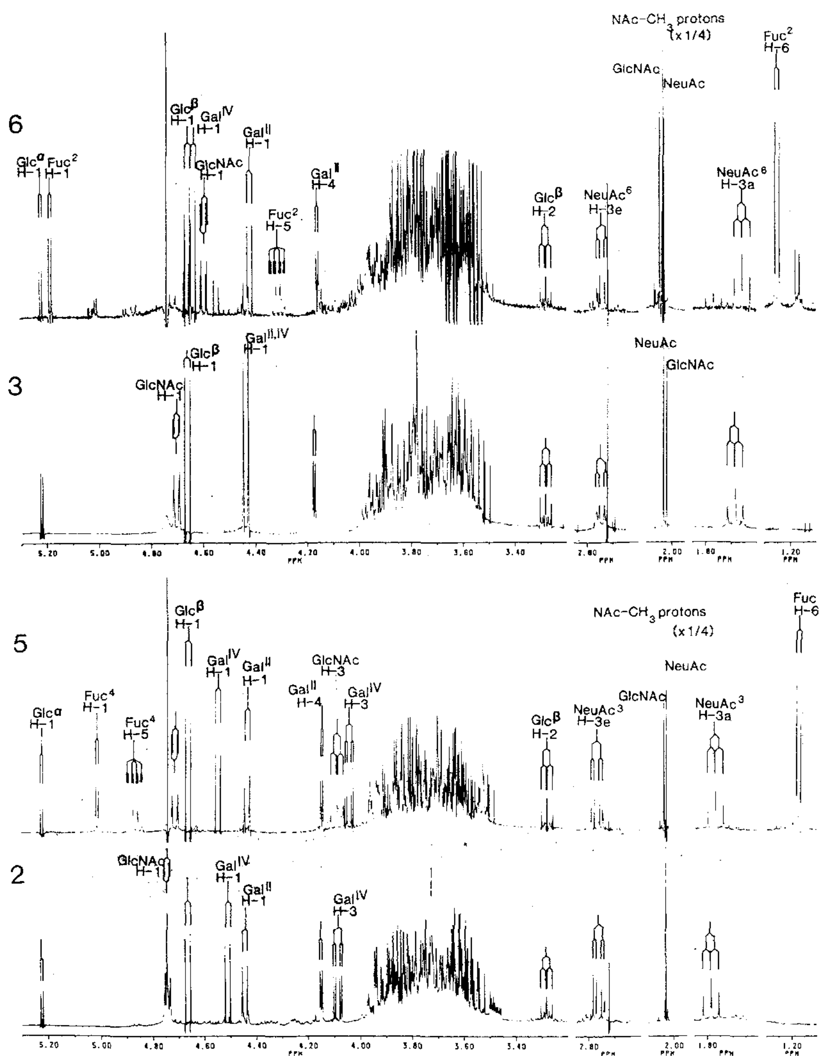

Figure 3. Comparison of the 400 MHz ^{1}H-NMR spectra of compounds **2, 5, 3** and **6.**

The 100 MHz ^{13}C-NMR experiments were obtained using the standard Bruker pulse program POWGATE with ^{1}H broad band composite-pulse decoupling. The spectral width was 25.000 Hz for 32 K frequency domain points and time domain giving a final digital resolution of 1.526 Hz/point. A 90° pulse (6 μs) and 0.5-1.0 sec recycle delay were used. The chemical shifts are given relative to sodium-4,4-dimethyl-4-silapentane-1-sulphonate, but were actually measured to methyl of acetone (δ = 2.225 ppm for ^{1}H and δ = 31.55 ppm for ^{13}C).

The 2D homonuclear COSY 45 experiments were performed using the standard Bruker pulse programme COSY. In these experiments the spectral width was 1800 Hz. The ^{1}H ninety

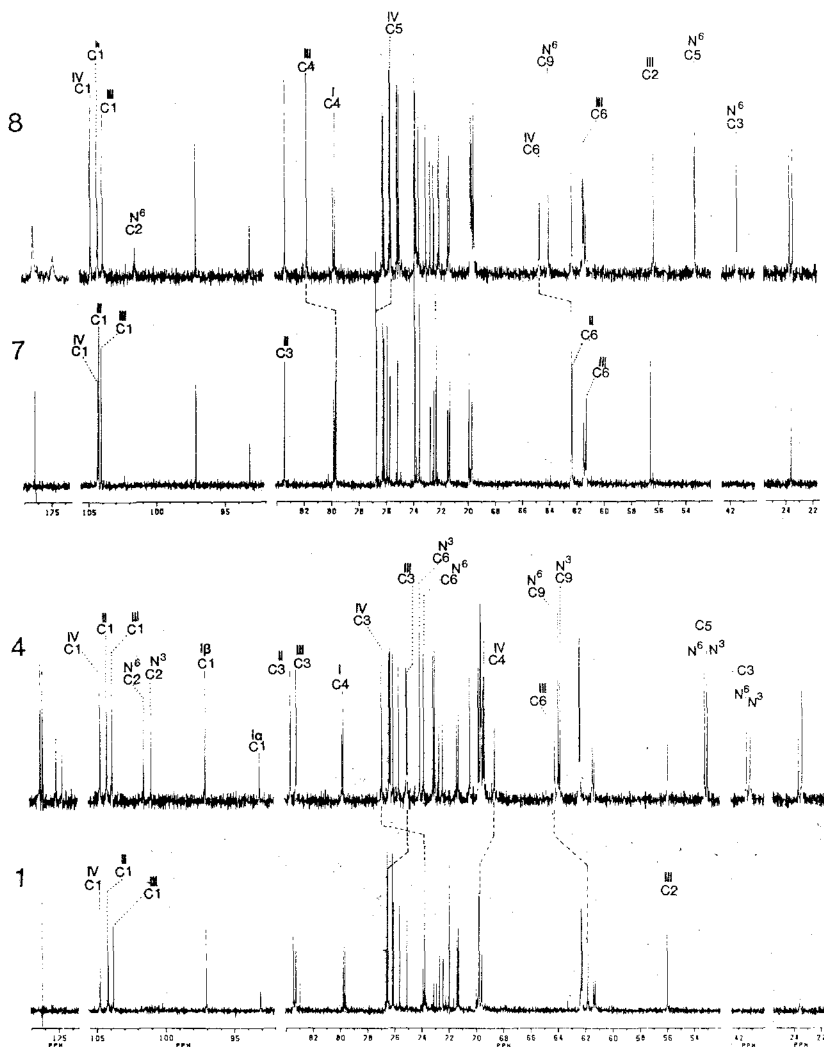

Figure 4. Comparison of the 100 MHz ^{13}C-NMR spectra of compounds **1, 4, 7** and **8.**

degrees pulse was 10.6 sec. 256 W x 2 K data matrix were acquired which were zerofilled prior to Fourier transformation to obtain a 1 K x 2 K spectral data matrix, a sine-bell squared function was used in both dimensions.

The 2D- homonuclear COSY with simple and double relay transfers were performed using the standard Bruker pulse programme COSYRCT and the pulse programme COSYDR (Bruno Perly, Cea Saclay, personal communication). For example, the COSYDR experiments were performed using the sequence: D_1-90-D_ϕ-90-D_2-180-D_2-90-D_3-180-D_3-90-FID, where D_1 = 2 sec; 90, 180 = 90°, 180° ^{1}H pulse (90° = 10.6 µsec); D_ϕ = incremental delay (initial = 3 µsec);

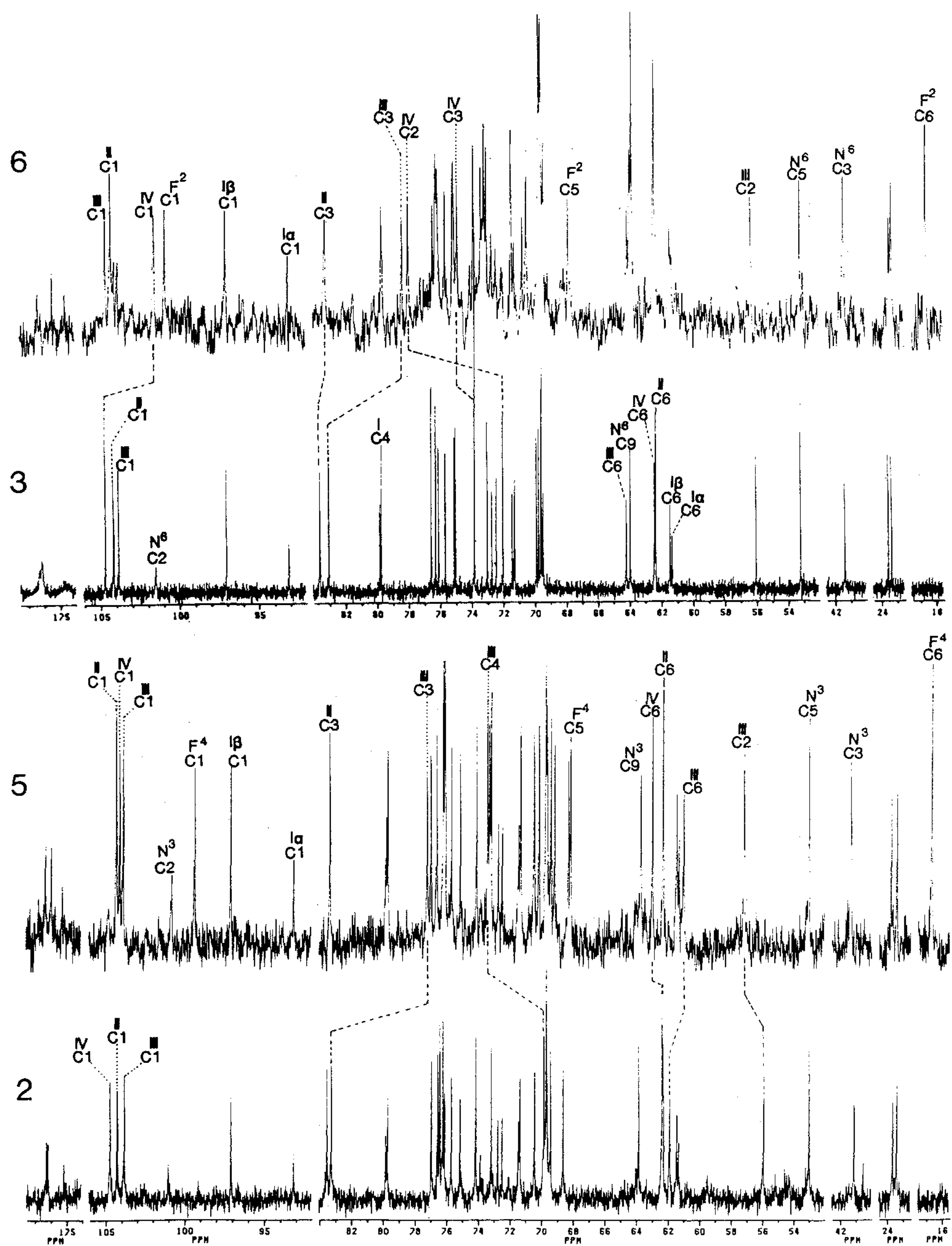

Figure 5. Comparison of the 100 MHz ^{13}C-NMR spectra of compounds **2, 5, 3,** and **6.**

$D_2 = D_3$ = 30 or 40 ms. In all experiments a spectral width of 1800 Hz, 256 W x 2 K data matrix was obtained, which were zerofilled to 1 K x 2 K prior to Fourier-transformation, a sine-bell squared function was used in both dimensions.

The 2D heteronuclear correlated experiments were performed with simultaneous ^1H decoupling [14, 15] using the standard Bruker pulse programme XHCORRD. In this experiment, the phase cycling of the refocusing pulse described by Wilde and Bolton [16] is used in addition. Refocusing delays were adjusted to an average J_{C-H} coupling constant of 150 Hz [17]. Spectral windows of 10000 Hz, with 4096 data points, for ^{13}C and 900 Hz, with 128 data points, for ^1H were employed. ^1H and ^{13}C-90° pulse width were 10.6 and 6 µsec respectively. A 128 W x 4 K data matrix was acquired which was zerofilled prior to Fourier-transformation to obtain a 512 W x 4 K spectral data matrix. The F_1 domain was multiplied by a sine-bell function and F_2 domain by a line-broadening function (1-2 Hz depending of the concentration of the product) prior to processing.

Results and Discussion

*^1H and ^{13}C-NMR Assignment of Compounds **1** to **8***

The ^1H and ^{13}C-NMR spectra of compounds **1** and **8** are shown in Figs. 2-5 and the chemical shifts are given in Tables 1 and 2. The H-1 signal of Glcα was easily recognized on the basis of its small coupling constant to H-2 ($J_{1,2}$ = 3.75 Hz), and, consequently, the H-2 to H-4 signals were successively assigned on the one and two-step relayed COSY spectra. The H-2 signal of Glcβ resonates at a frequency away from the main bulk of the protons signals (δ = 3.278-3.290 ppm), that allows identification of the H-1 resonance without ambiguity *via* the COSY spectrum. Gal^{II}, which is substituted at the C-3 position with an *N*-acetylglucosamine residue, presents a characteristic H-4 signal, deshielded at δ = 4.146-4.176 ppm.

As shown in Fig. 6, the H-1 to H-4 resonances of Gal^{II} and Gal^{IV} can be deduced from the relayed COSY spectra, starting from the two H-4 signals. No cross-peak between Gal H-4 and H-5 could be detected, due to the very small coupling constant $J_{4,5}$ (0.9 Hz), therefore assignments for the H-5 and H-6 protons were obtained through the heteronuclear-correlated spectra (see below).

For *N*-acetylglucosamine, the multi-step relayed COSY experiments furnished most of chemical shifts of the H-1 to H-4 signals. The H-5 and H-6 signals were also extracted from the COSY spectra, except for **3** and **4**, which required a ^{13}C-^1H heteronuclear-correlated 2D experiment.

The H-3 to H-6 signals of the *N*-acetylneuraminic acid residues were assigned by the same way, starting from the H-3ax and H-3eq signals. For the H-7 to H-9 resonances, which fall in the range 3.60-3.86 ppm, the ^{13}C-^1H heteronuclear-correlated experiments were sufficient.

For the fucose residues, the H-1 to H-4 and H-6, H-6 correlations observed on the COSY spectra allowed the full ^1H-NMR assignment of both fucosylated compounds **5** and **6**.

Table 1. ^{1}H Chemical Shifts for Compounds 1 to 8.[a]

		1	2	3	4	5	6	7	8
Gal_{ext}	H-1	4.436	4.507(+0.071)	4.436	4.499(+0.063)	4.548(+0.112)	4.637(+0.201)	4.476	4.450
	H-2	3.526	3.540(+0.014)	3.519	3.527	3.506(-0.020)	3.585(+0.059)	3.540	3.533
	H-3	3.640	4.084(+0.444)	3.635	4.076(+0.436)	4.043(+0.403)	3.821(+0.181)	3.668	3.662
	H-4	3.912	3.938(+0.026)	3.906	3.930(+0.018)	3.910	3.875	3.927	3.924
	H-5	3.70	3.67	3.69	3.66	3.61	n.d.[b]	3.94	3.82(-0.012)
	H-6	3.76	3.72	3.78	3.78	3.70	n.d.	3.77	3.57(-0.020)
									3.97(+0.020)
GlcNAc	H-1α	4.736	4.743	4.703	4.705	4.716	4.598	4.710	4.732
	β	4.732	4.740	4.703	4.702	4.714	4.594	4.706	4.730
	H-2	3.898	3.892	3.895	3.885	3.940(+0.042)	3.825	3.802	3.793
	H-3	3.816	3.810	3.805	3.793	4.088(+0.272)	3.967	3.724	3.78
	H-4	3.560	3.570	3.625	3.613	3.746(+0.186)	3.564	3.73	3.66
	H-5	3.477	3.480	3.56	3.592	3.533	3.55	3.590	3.60
	H-6	3.79	3.790	3.79	3.80	3.85	3.77	3.846	3.85
		3.88	3.900	3.90	3.96	3.96	3.96	3.950	3.92
	CH_3	2.025	2.030	2.020	2.020	2.038	2.047	2.033	2.051
Gal_{int}	H-1	4.436	4.447	4.436	4.436	4.436	4.419	4.436	4.442
									4.440
	H-2	3.596	3.600	3.585	3.588	3.588	3.56	3.588	3.604
	H-3	3.732	3.735	3.720	3.716	3.717	3.708	3.720	3.728
	H-4	4.146	4.146	4.171	4.168	4.148	4.160	4.149	4.152
	H-5	3.71	3.73	3.70	3.72	3.71	n.d.	3.71	3.73
	H-6	3.76	3.77	3.76	3.72	3.76	n.d.	3.77	3.77
Glc	H-1α	5.218	5.219	5.218	5.218	5.220	5.217	5.218	5.218
	β	4.655	4.662	4.658	4.660	4.663	4.658	4.660	4.661
	H-2α	3.573	3.575	3.578	3.577	3.574	3.576	3.574	3.574
	β	3.278	3.280	3.290	3.280	3.280	3.280	3.278	3.280
	H-3α	3.829	3.826	3.828	3.841	3.827	3.82	3.830	3.826
	β	3.640	3.636	3.636	3.639	3.64	3.635	3.639	3.638
	H-4α	3.636	3.638	3.64	3.621	3.636	3.634	3.636	3.637
	β	3.640	3.640	3.64	3.632	3.65	3.63	3.64	3.634

	H-5α	3.94	3.96	3.94	3.947	3.948	n.d.	3.94	3.94
	β	3.595	3.60	3.59	3.62	3.53	n.d.	3.60	3.60
	H-6α	3.87	3.87	3.87	3.87	n.d.	n.d.	3.92	3.92
	β	3.92	3.93	3.85	3.95	3.93	n.d.	3.92	3.92
NeuAc (α2-3)	H-3ax		1.783		1.779	1.768			
	H-3eq		2.760		2.754	2.768			
	H-4		3.702		3.626	3.656			
	H-5		3.834		3.840	3.840			
	H-6		3.665		3.64	3.63			
	H-7		3.59		3.58	3.61			
	H-8		3.875		3.845	3.85			
	H-9		3.66		3.66	3.68			
			3.82		3.86	3.81			
	CH_3		2.030		2.027	2.029			
NeuAc (α2-6)	H-3ax			1.685	1.685		1.658		1.713
	H-3eq			2.741	2.736		2.742		2.668
	H-4			3.68	3.668		3.67		3.666
	H-5			3.816	3.814		3.814		3.810
	H-6			3.68	3.67		3.67		3.67
	H-7			3.59	3.67		n.d.		3.56
	H-8			3.88	3.845		n.d.		3.87
	H-9			3.75	3.78		n.d.		3.67
				3.82	3.86				3.86
	CH_3			2.032	2.030		2.033		2.028
Fuc	H-1					5.010	5.180		
	H-2					3.790	3.768		
	H-3					3.878	3.680		
	H-4					3.792	3.752		
	H-5					4.864	4.310		
	H-6					1.174	1.251		

[a] In brackets are the chemical shift differences with the references compounds (**1** for **2** to **6** and **7** for **8**).
[b] n.d. = not determined.

Table 2. ^{13}C Chemical Shifts for Compounds 1 to 8[a, b]

		1	2	3	4	5	6	7	8
Gal_{ext}	C-1	104.70	104.64	104.66	104.64	104.01	101.51(-3.19)	104.19	104.66
	C-2	71.94	70.38(-1.56)	71.98	70.38(-1.56)	70.11(-1.83)	78.00(+6.06)	72.24	72.01
	C-3	73.73	76.90(+3.17)	73.77	76.88(+3.15)	76.94(+3.21)	74.88(+1.15)	73.80	73.80
	C-4	69.78	68.57(-1.21)	69.85	68.57(-1.21)	68.25(-1.53)	70.44	69.82	69.68
	C-5	76.51	76.37	76.53	76.33	76.08	76.43	76.61	74.94(-1.57)
	C-6	62.28	62.31	62.38	62.37	62.93	62.41	62.29	64.61(+2.32)
GlcNAc	C-1	103.73	103.73	103.83	103.76	103.78	104.58(+0.85)	103.95	103.79
	C-2	55.95	55.88	55.98	55.88	57.13(+1.18)	56.32	56.49	56.24
	C-3	83.38	83.17[b]	83.02[b]	83.13[b]	77.19(-6.29)	78.38(-5.00)	73.45	73.48
	C-4	69.72	69.78	69.71	69.74	73.35(+3.64)	69.70	79.54	81.67(+2.12)
	C-5	76.44	76.50	74.98(-1.46)	75.00(-1.44)	76.58	75.16(-1.28)	75.83	75.54
	C-6	61.80	61.84	64.15(+2.35)	64.16(+2.34)	60.96	63.81(+2.01)	61.20	61.44
	CO	176.13	176.13	176.08	176.03	175.85	175.44	176.13	176.14
	CH_3	23.54	23.62	23.55	23.62	23.73	23.50	23.54	23.59
Gal_{int}	C-1	104.12(α) 104.15(β)	104.19	104.14(α) 104.16(β)	104.15	104.22	104.25	104.14	104.17
	C-2	71.25(α) 71.27(β)	71.30	71.24(α) 71.26(β)	71.23	71.27	71.48	71.27(α) 71.24(β)	71.24
	C-3	83.21	83.47[b]	83.60[b]	83.55(α)[b] 83.56(β)	83.32	83.27	83.29	83.25
	C-4	69.55(α) 69.58(β)	69.63	69.43	69.41	69.60	69.58	69.61	69.58
	C-5	76.13	76.18	76.25	76.24	76.17	76.25	76.14	76.14
	C-6	62.21	62.25	62.29	62.29	62.26	62.41	62.23	62.23
Glc	C-1α	93.04	93.08	93.07	93.06	93.10	93.11	93.04	93.06
	β	96.98	97.01	97.00	96.99	97.02	97.03	96.98	96.99
	C-2α	72.40	72.43	72.40	72.40	72.43	72.44	72.42	72.40
	β	75.05	75.08	75.06	75.05	75.09	75.11	75.07	75.06
	C-3α	72.64	72.69	72.68	72.67	72.69	72.70	72.68	72.67
	β	75.60	75.64	75.64	75.69	75.65	75.66	75.63	75.61
	C-4α	79.73	79.78	79.79	79.80	79.78	79.79	79.73	79.75
	β	79.63	79.67	79.69	79.69	79.67	79.61	79.63	79.63

	C-5α	71.36	71.40	71.39	71.38	71.41	71.45	71.40	71.38
	β	76.02	76.07	76.06	76.05	76.03	76.13	76.06	76.04
	C-6α	61.26	61.27	61.27	61.26	61.26	61.21	61.26	61.24
	β	61.38	61.39	61.41	61.39	61.39	61.43	61.39	61.37
NeuAc (α2-3)	C-1		175.10		175.05	175.14			
	C-2		100.94		100.92	100.71			
	C-3		41.08		41.06	41.31			
	C-4		69.63		69.55	69.68			
	C-5		52.98		52.96	52.99			
	C-6		74.09		74.06	74.06			
	C-7		69.36		69.33	69.35			
	C-8		73.11		73.07	73.13			
	C-9		63.78		63.75	63.65			
	CH_3		23.36		23.34	23.36			
NeuAc (α2-6)	C-1			174.6	174.61		174.63		174.73
	C-2			101.48	101.46		101.51		101.41
	C-3			41.36	41.34		41.43		41.35
	C-4			69.55	69.55		69.70		69.45
	C-5			53.17	53.15		53.22		53.18
	C-6			73.77	73.77		73.84		73.70
	C-7			69.55	69.55		69.58		69.68
	C-8			72.97	72.96		73.02		72.98
	C-9			63.89	63.88		63.81		63.95
	CO			176.26	176.19		176.34		176.15
	CH_3			23.35	23.34		23.35		23.35
Fuc	C-1					99.27	100.81		
	C-2					69.11	69.36		
	C-3					70.41	70.75		
	C-4					73.24	73.16		
	C-5					68.11	67.84		
	C-6					16.64	16.65		

[a] In brackets are the chemical shift differences with the reference compounds (**1** for **2** to **6** and **7** for **8**).
[b] Assignments that are different from the published report [7].

NeuAc(α2-3)Gal^{IV}(β1-3)GlcNAc(β1-3)Gal^{II}(β1-4Glc

Fuc(α1-4)

Figure 6. Homonuclear two-step relayed COSY spectrum of compound **5.** Abbreviations: F, fucose; *N*, *N*-acetylneuraminic acid

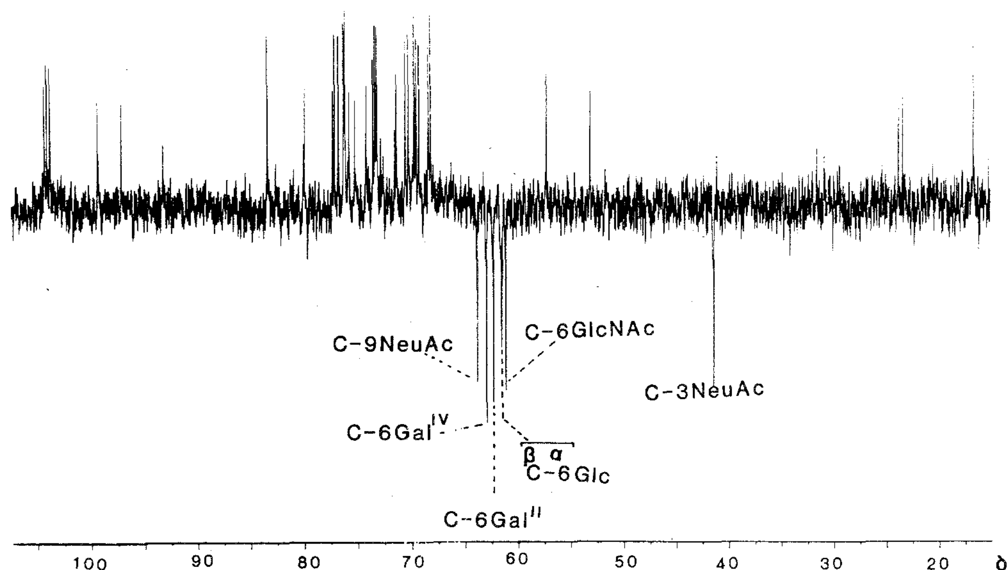

Figure 7. ^{13}C-NMR spectrum (DEPT) of compound **5**.

The ^{13}C assignments were deduced from the correlation of firmly assigned protons resonances in the ^{1}H-NMR spectra. The ^{13}C signals of the *N*-acetylneuraminic acid residues were found to resonate at δ values strictly depending on the linkage position (α2-3 or α2-6), and consequently, can be directly assigned by comparing the 1D ^{13}C-NMR spectra. Finally, the only signals which remained to be analysed are related to H-5, H-6 and C-5/C-6 of galactose and glucose residues. The C-6 atoms were assigned owing the examination of the DEPT spectrum (Fig. 7). The C-6 signals of Glcα and β were easily recognized owing the anomerization effect which furnishes the doubling of the resonance of 61.3-61.4 ppm, in a ratio 3:1. These resonances, which are not affected by the substitution of the rest of the molecule, are characterized by their remarkably constant δ values.

The two signals related to C-6 of Gal^{II} and Gal^{IV} were distinguished by the fact that the former is not influenced by the attachment of fucose and *N*-acetylneuraminic acid to the Galβ1-3/4GlcNAc sequence, and consequently was assigned in the range 62.21-62.29 ppm. The signals related to C-5 of Gal^{II} and Gal^{IV} possess similar chemical shifts, close to 76.10-76.60 ppm. One of them is correlated with a ^{1}H signal which has an identical δ value for the eight compounds investigated (δ = 3.70-3.73 ppm), while the second one has ^{1}H resonances varying from 3.61 to 3.94 ppm. Consequently, this second parameter was assigned for H-5 Gal^{IV}, which is largely influenced by the nature of the substitution by fucose or *N*-acetylneuraminic acid residues.

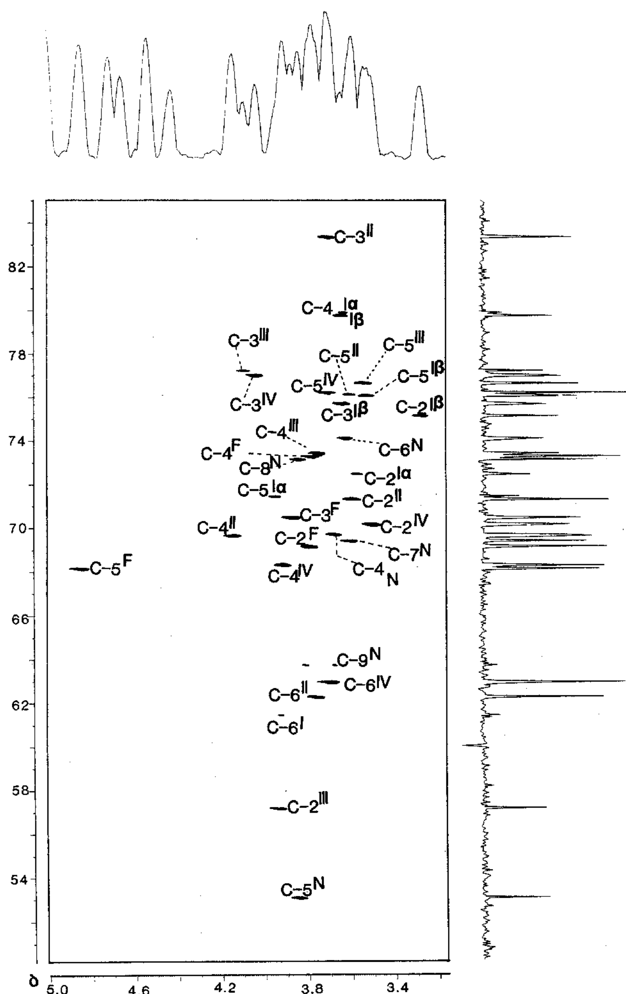

Figure 8. 2D $^{13}C/^{1}H$ correlated spectrum of compound **5**.

Comparison of ^{1}H and ^{13}C-NMR Chemical Shifts

Sequence effects on the chemical shifts of structural reporter group protons of constituent monosaccharides should be analysed in terms of spatially related neighbouring carbohydrates. The H-1 signal of Gal^{IV} is significantly deshielded by the attachment of fucose to C-2 ($\Delta\delta$ = +0.209 [18]) and the α(2-3)-linkage of *N*-acetylneuraminic acid ($\Delta\delta$ = + 0.071 ppm), while the attachment of *N*-acetylneuraminic acid to C-6 of *N*-acetylglucosamine (compound **2**) has no effect on this galactose H-1 resonance. For oligosaccharides **2, 4** and **5**, the attachment of *N*-acetylneuraminic acid to C-3 of Gal^{IV} results in an intense deshielding effect on the Gal^{IV} H-3 signal ($\Delta\delta$ = +4.404/+ 0.444 ppm), that resonates now at a frequency away

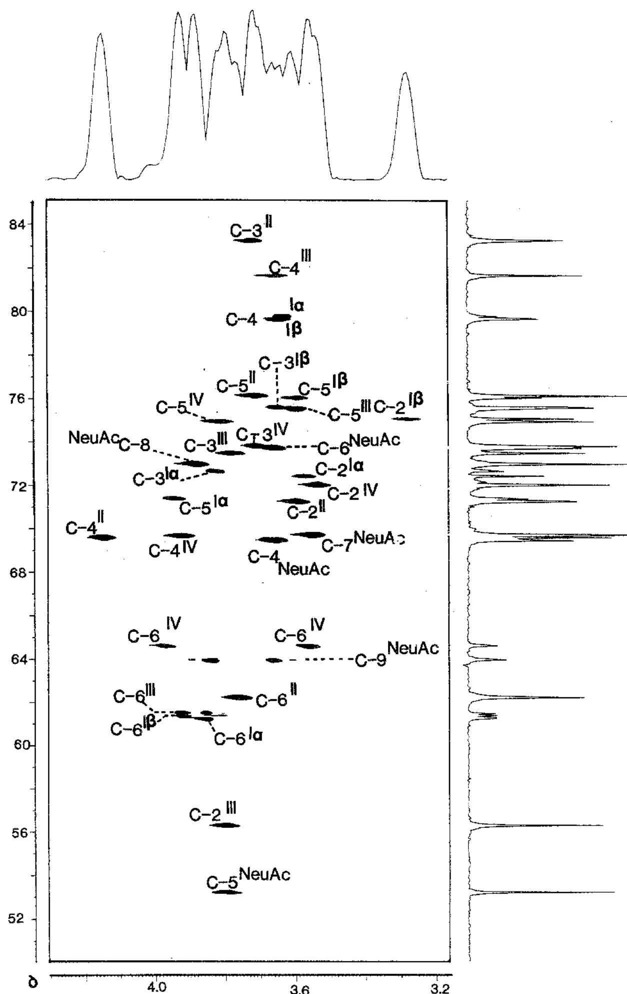

Figure 9. 2D $^{13}C/^{1}H$ correlated spectrum of compound **8**.

from the main bulk of the protons resonances. The shift increment observed (Fig. 8) for the *N*-acetylglucosamine H-4 resonance of compound **5** ($\Delta\delta = +0.272$ ppm) is also comparable with those observed for III^4-α-Fuc-LcOse$_4$ [Breg J, Romijn D, Vliegenthart JFG, Strecker G, Montreuil J, unpublished results]. Another interesting shift increment is observed when *N*-acetylneuraminic acid is attached to C-6 of Gal^{IV} (compound **8**), that results in the decoupling of galactose H-6 resonance at $\delta = 3.57$ and 3.97 ppm, instead of 3.77 ppm for asialo compound **7** (Fig. 9).

The analysis of the ^{13}C-NMR spectra also points to characteristic shift increments reliable to the type of substitution. Particularly, the effects of the attachment of fucose to galactose or

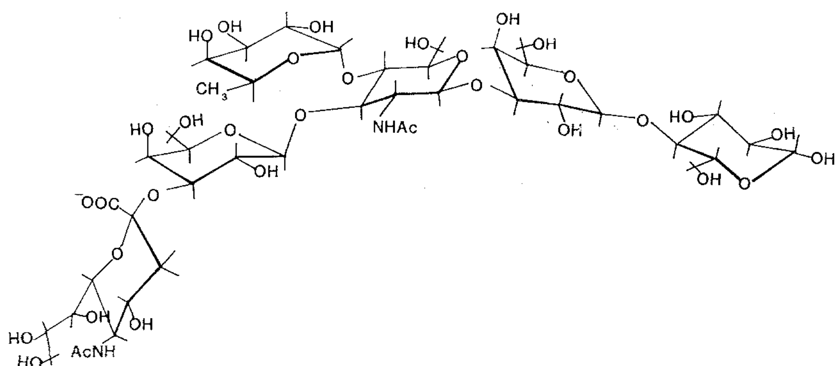

Figure 10. Proposed conformation of oligosaccharide **5** based on Galβ1-3[Fucα1-4]GlcNAc [19] and NeuAcα2-3Galβ1-4Glc [20] models.

N-acetylglucosamine residues results in an intense shift increment of the substituted carbon: $\Delta\delta$ = +6 ppm for C-2 Gal^{IV} in compound **6**; $\Delta\delta$ = + 3.7 ppm for C-4 of *N*-acetylglucosamine in compound **5**. In addition, the attachment of this fucose residue produces an important shielding effect on the adjacent carbon ($\Delta\delta$ = -3.20 ppm for C-1 Gal^{IV}; -5 ppm for C-3 *N*-acetylglucosamine). Similarly, the attachment of *N*-acetylneuraminic acid to C-3 or C-6 of Gal^{IV} and C-6 of *N*-acetylglucosamine also led to a deshielding effect on the substituted carbon and a shielding effect on the adjacent carbons.

The similarity in the chemical shifts observed for **5** and **6**, as compared to the reference asialo compounds lacto-*N*-fucopentaoses I and II [Breg J, Romijn D, Vliegenthart JFG, Strecker G, Montreuil J, unpublished results] suggests the presence of *N*-acetylneuraminic acid residue to have no influence on the conformation of the lacto-*N*-tetraose core. Indeed, it has been shown that the intense deshielding of the H-5 α(1-4)-linked fucose residue of lacto-*N*-fucopentaose II is due to the close proximity of this proton to the two oxygen atoms, those of the ring and the glycosidic bond of Gal^{IV} [19]. This conformational feature also occurs in compound **5**, which exhibits similar NMR parameters, and, consequently, the *N*-acetylneuraminic acid residues is probably extended away the lacto-*N*-tetraose core (Fig. 10).

For compounds **2, 3** and **4**, the absence of interaction of *N*-acetylneuraminic acid with the rest of the molecule may also be deduced from the remarkable stability of the NMR parameters of the atoms not directly involved in the glycosidic bounds. On the contrary, the comparison of the spectra of **7** and **8** indicates steric interactions between *N*-acetylneuraminic acid and *N*-acetylglucosamine, as shown by the significant downfield shift-effect on GlcNAc C-4 (δ = +2.12 ppm) and the chemical shift increments observed for the H-1, H-3, H-4 and NAc protons. These shift-effects are in accordance with those observed for larger oligosaccharides containing the same structural element [18].

Our ^{1}H-NMR parameters described for this series of oligosaccharides are fully in accordance with those previously reported by Sabesan and Paulson [7]. The ^{13}C-NMR parameters are also in good agreement, with only one exception concerning the assignment of the C-3 atoms of the *N*-acetylglucosamine and Gal^{II} residues, which must be interchanged for compounds **2, 3** and **4**. The new values were easily determined on the heteronuclear COSY spectra, while the previous ones were deduced from the comparison of the spectra with those of the asialo compound [7].

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