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Isolation and structural characterization of novel neutral oligosaccharide-alditols from respiratory-mucus glycoproteins of a patient suffering from bronchiectasis

2. Structure of twelve hepta-to-nonasaccharides, six of which possess the GlcNAcβ(1→3)[Galβ(1→4)GlcNAcβ(1→6)]Galβ(1→3)GalNAc-ol common structural element

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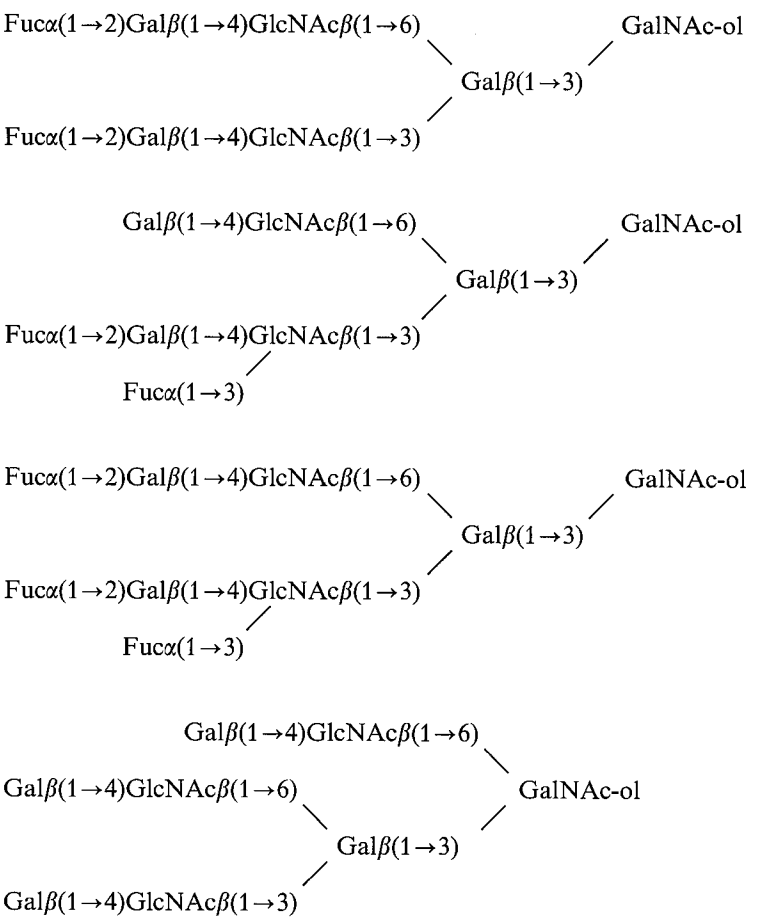
Oligosaccharide-alditols derived from the respiratory mucins of a patient suffering from bronchiectasis were separated by HPLC into 46 fractions. The structures of 11 oligosaccharides with GlcNAcβ(1→3)Galβ(1→4)-GlcNAcβ(1→6)GalNAc-ol in common have been established (preceding paper in this journal). In this second part the structures of 12 oligosaccharides were established 8 of which have not been described before. Of the 12 compounds, 6 possess the GlcNAcβ(1→3)[Galβ(1→4)GlcNAcβ(1→6)]Galβ(1→3)GalNAc-ol element :



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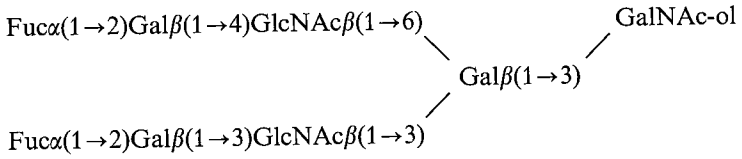
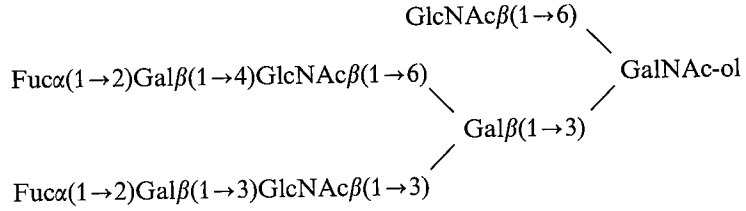
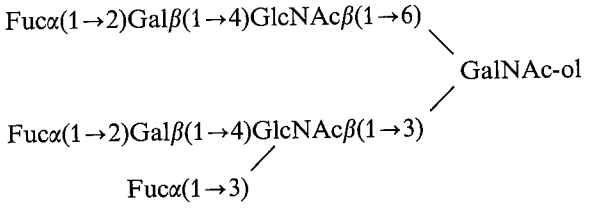
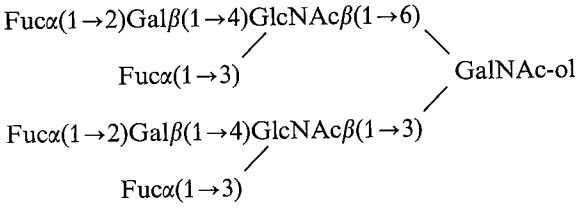
Abbreviations. Fuc, L-fucose; GalNAc, *N*-acetyl-D-galactosamine; GalNAc-ol, *N*-acetyl-D-galactosaminitol; FAB-MS, fast-atom-bombardment mass spectrometry; 2D-HOHAHA, two-dimensional homonuclear Hartman-Hahn spectroscopy.

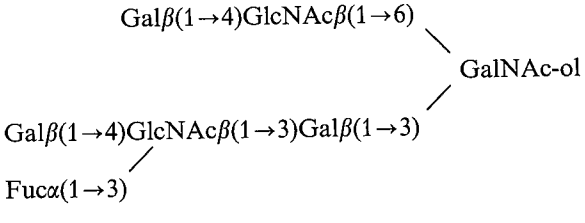


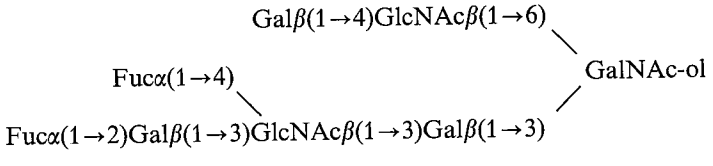


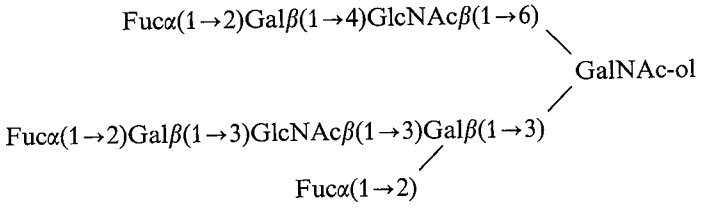
The other compounds, that could not be classified on basis of a common structural element, are :

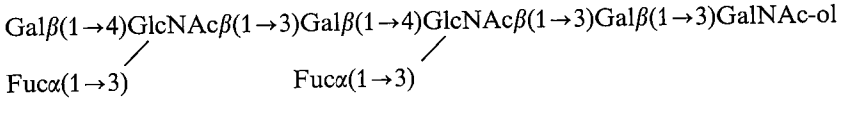












Previously, a large number of oligosaccharide-alditols have been isolated from the respiratory mucins secreted by a patient suffering from bronchiectasis due to a Kartagener's syndrome [1–3]. The diversity of the oligosaccharide structures provides a variety of determinants which may play a role in the bacterial clearance of the respiratory airways [4, 5]. The characterization of 11 oligosaccharide-alditols possessing GlcNAcβ-(1→3)Galβ(1→4)GlcNAcβ(1→6)GalNAc-ol in common was described in the preceding paper [6]. In the framework of the isolation and characterization of the enormous amount of compounds available from this source, we describe here a series of six oligosaccharide-alditols having the GlcNAcβ(1→3)[Galβ(1→4)GlcNAcβ(1→6)]Galβ(1→3)GalNAc-ol element in common, together with six other structures that could not be classified on the basis of a common structural element.

MATERIALS AND METHODS

Materials and methods are identical to those described in the preceding paper [6]. Human bronchial mucus was obtained from a patient with blood group O, suffering from bronchiectasis due to a Kartagener's syndrome. Mucus glycopeptides were prepared from the sputa as previously described [1] and submitted to alkaline-borohydride degradation. The resulting oligosaccharide-alditols and the glycopeptides resistant to alkaline-borohydride degradation were fractionated according to their charge and size. Medium size oligosaccharide-alditols were further fractionated by HPLC using successively a Lichrosorb-NH_2 and an Ultrasphere ODS column. The resulting samples were then submitted to quantitative sugar analysis and investigated by ^{1}H-NMR spectroscopy. FAB-MS and methylation analysis were applied when the quantities of oligosaccharides were sufficient.

RESULTS

The results of the HPLC fractionation have been described in the accompanying paper [6]. Relevant sugar analysis data together with the retention times on Ultrasphere ODS column are summarized in Table 1. The various compounds were identified as follows :

Structures with GlcNAcβ(1→3)[Galβ(1→4)GlcNAcβ-(1→6)]Galβ(1→3)GalNAc-ol as common element

Six oligosaccharide-alditols have been characterized with the common element GlcNAcβ(1→3)[Galβ(1→4)-GlcNAcβ(1→6)]Galβ(1→3)GalNAc-ol. The primary structures of these compounds are compiled in Scheme 1, the available methylation analysis results are presented in Table 2 and the ^{1}H-NMR chemical-shifts of the structural-reporter groups are listed in Table 3. The interpretation of the FAB-MS data is carried out guided by the sugar analysis data.

The presence of the core type Galβ(1→3)GalNAc-ol is indicated in the ^{1}H-NMR spectra by typical structural-reporter group signals for most structures. Although the exact positions may vary for each structure, the presence of a disubstituted Galβ(1→3) linked to GalNAc-ol can be deduced from the H-2 and NAc chemical shifts of GalNAc-ol, resonating at $\delta \approx 4.39$ and ≈ 2.04 ppm respectively, in combination with the H-1 and H-4 chemical shifts of Gal[3] which are found at $\delta \approx 4.46$ and ≈ 4.10 ppm, respectively. For compounds that were available in sufficient quantities, this was confirmed by the methylation analysis, showing the presence of a 2,4-Me_2Gal derivative (Table 2). The chemical shifts of the other structural-reporter groups of GlcNAc and Gal residues in the structural element are influenced by further extensions.

Fraction 3.11. The ^{1}H-NMR spectrum of fraction 3.11 reveals the presence of one compound, with features essentially the same as those reported for compound **14.5** from porcine blood-group H substance [7]. Therefore, it can be concluded that compounds 3.11 and **14.5** are identical (Scheme 1).

Fraction 4.3. FAB-MS analysis of the permethylated fraction 4.3 shows the presence of a high-intensity ion $(M+Na)^+$ which is observed at *m/z* 1782. Altogether with the chemical composition (Table 1), this indicates an octasaccharide constituted of GalNAc-ol, Gal, GlcNAc and Fuc in a ratio of 1:3:2:2. The ^{1}H-NMR spectrum of this fraction shows the occurrence of a single component (Fig. 1). The comparison of the spectra of compound 4.3 and of reference compound **17a** [1], indicates the presence of a common structural element Fucα (1→2)Galβ (1→4) [Fucα (1→3)] GlcNAcβ (1→3) Galβ (1→3)GalNAc-ol, as the corresponding structural-reporter

Table 1. *Molar carbohydrate composition and yields of neutral oligosaccharide-alditols obtained after HPLC*
The molar composition of the oligosaccharide-alditols was calculated on the basis of one residue of GalNAc-ol per molecule. The retention times t_R of the compounds on ODS reverse phase column have been included. n.d. is not determined.

Oligosaccharide-alditol fraction	Molar ratio of monosaccharides				t_R	Amount
	Fuc	Gal	GlcNAc	GalNAc-ol		
					min	μg
3.1	n.d.	n.d.	n.d.	n.d.	6.24	n.d.
3.10	2.9	2.1	2.0	1	29.97	58
3.11	1.9	3.1	1.9	1	51.92	41
4.1	1.7	2.7	2.2	1	4.24	54
4.3	1.7	2.7	2.0	1	8.16	36
4.8	1.6	2.9	1.7	1	44.20	48
5.5	n.d.	n.d.	n.d.	n.d.	12.92	n.d.
6.3	n.d.	n.d.	n.d.	n.d.	5.03	n.d.
6.6	0.2	4.1	3.3	1	9.48	289
6.10	2.9	3.2	2.4	1	21.21	41
6.14	2.6	2.8	2.3	1	40.54	102
6.15	1.6	2.6	2.7	1	45.33	57

Table 2. *Methyl glycosides present in the methanolysates of the permethylated oligosaccharide fractions*
(± = traces corresponding to minor compounds present in the fraction; nf = not found, due to the low amount of sugar)

Monosaccharide methylethers	Present in oligosaccharide fractions					
	4.8	6.15	5.5	6.3	6.14	4.1
2,3,4-Me_3 Fuc	+	+	+	+	+	+
2,3,4,6-Me_4 Gal	±		±	+	±	+
2,4,6-Me_3 Gal				+		+
3,4,6-Me_3 Gal	+	+	+	nf	+	
2,4-Me_2 Gal	+	+				
4,6-Me_2 Gal					+	
3,4,6-Me_3 GlcNAc(Me)		nf				
3,6-Me_2 GlcNAc(Me)	+	+		+	+	
4,6-Me_2 GlcNAc(Me)	+	+			+	
6-Me GlcNAc(Me)			+	+		+
1,4,5,6-Me_4 GalNAc(Me)-ol	+					+
1,4,5-Me_3 GalNAc(Me)-ol						
+ 1,4,5-Me_3-3,6 anhydroGalNAc(Me)-ol		+	+	+	+	

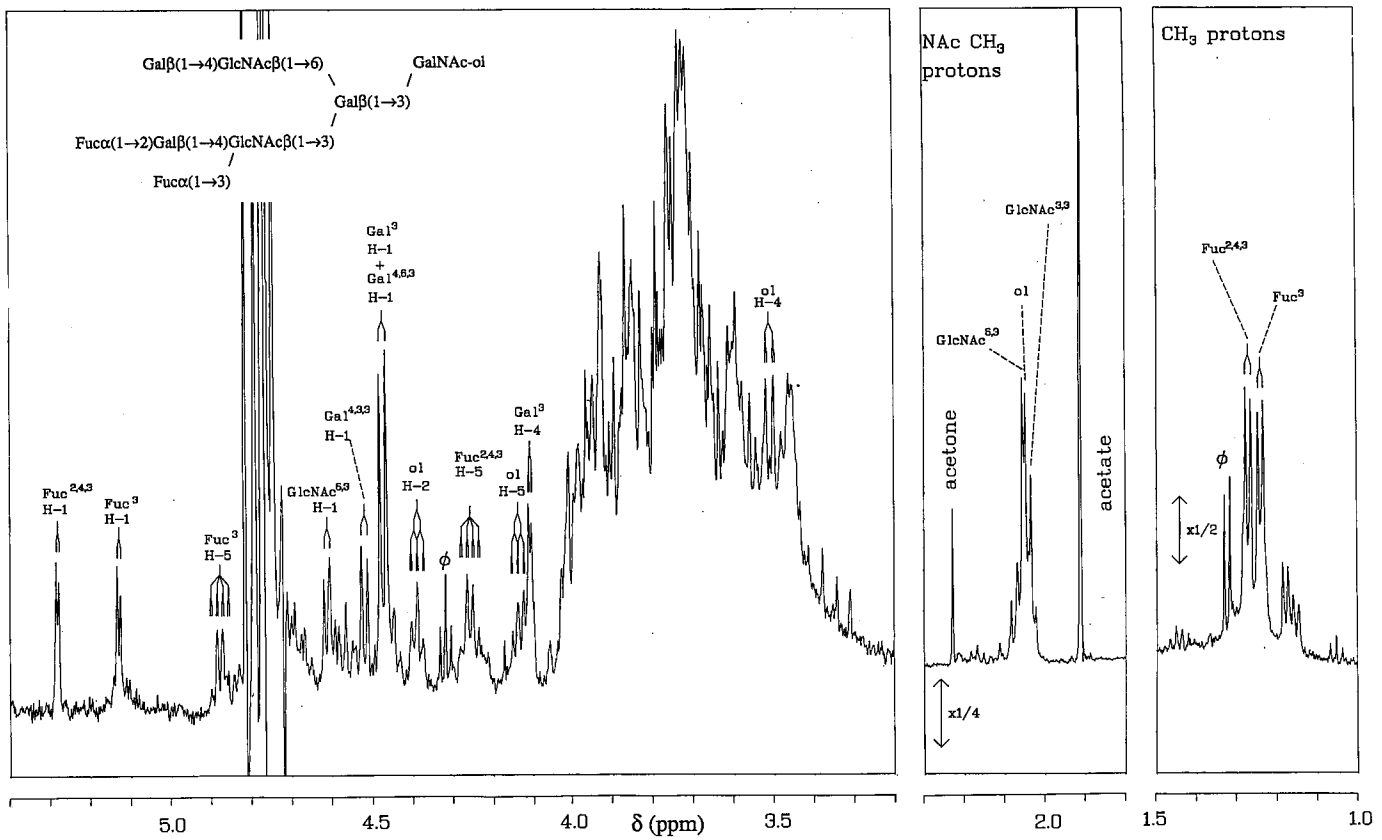


Fig. 1. *Resolution-enhanced 500-MHz spectrum (^2H_2O, 27°C) of fraction 4.3, obtained from the pool of neutral oligosaccharide-alditols Ib from the sputum of a patient with Kartagener's syndrome.* The relative intensity scale of the *N*-acetyl and Fuc methyl proton region of the spectrum differs from that of the other parts, as indicated. Signals marked by ϕ stem from a frequently occurring, non-protein non-carbohydrate contaminant

groups are resonating at virtually the same position. An exception is the H-5 signal of GalNAc-ol which is found at δ = 4.177 ppm for compound **17a** and at δ = 4.134 ppm for compound 4.3. The H-5 signal of GalNAc-ol points out an extension of compound 4.3 with the structural element Galβ(1→4)GlcNAcβ(1→6) linked to Gal^3 (compare with compound 3.11). The chemical shifts of the structural-reporter groups of Galβ(1→4)GlcNAcβ(1→6) almost coincide with the corresponding signals of reference compound **2B** from human ovarian-cyst glycoproteins [8]. Combination of the structural elements mentioned leads for compound 4.3 to a novel structure, which is depicted in Scheme 1.

Fraction 6.10. The ^{1}H-NMR spectrum of fraction 6.10 (Fig. 2) shows the presence of one component. This component can be conceived as an extension of compound 4.3 with Fuc α(1→2) linked to $Gal^{4,6,3}$ (compare with compound

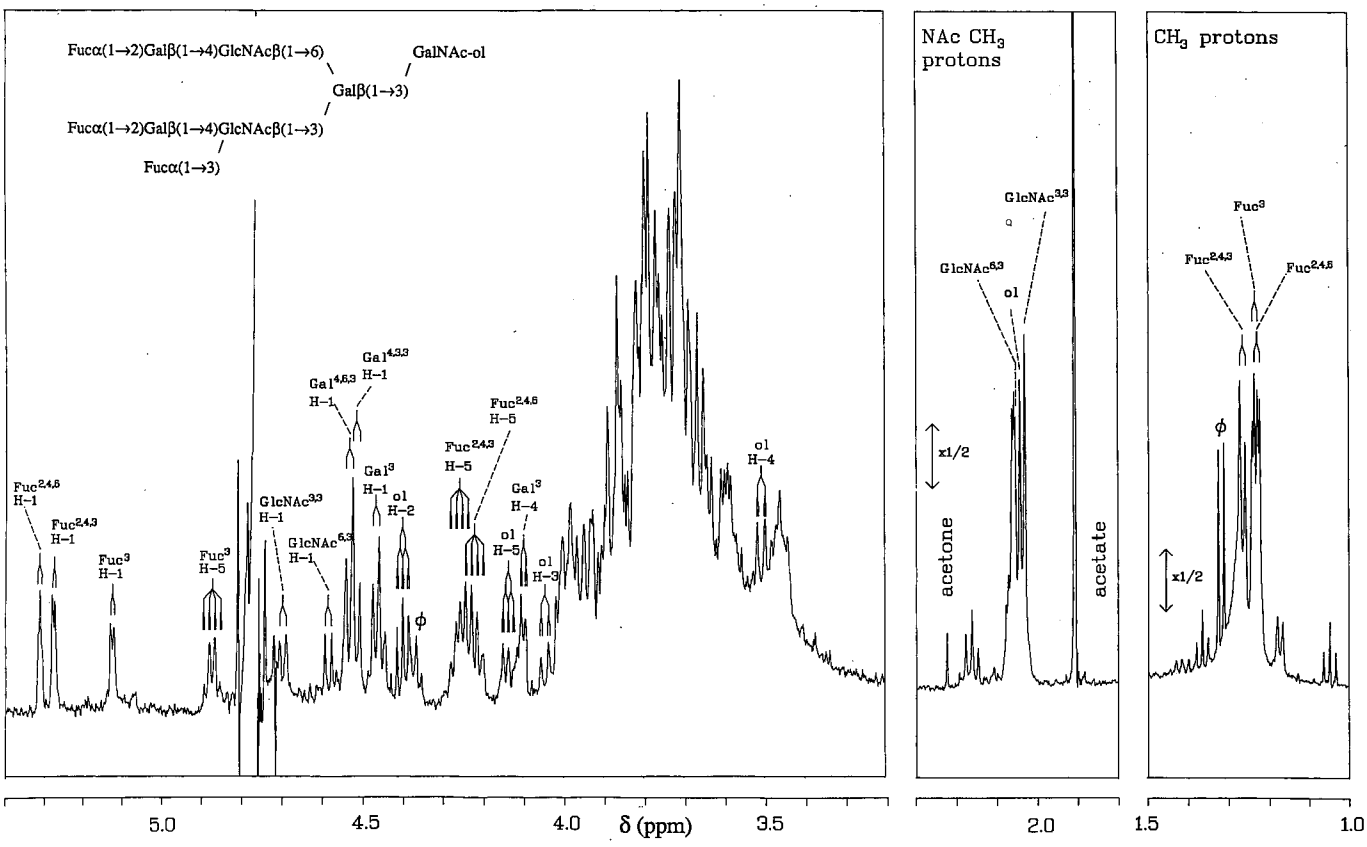


Fig. 2. *Resolution-enhanced 500-MHz spectrum (^2H_2O, 27°C) of fraction 6.10, obtained from the pool of neutral oligosaccharide-alditols Ib from the sputum of a patient with Kartagener's syndrome.* The relative intensity scale of the *N*-acetyl and Fuc methyl proton region of the spectrum differs from that of the other parts, as indicated. Signals marked by ϕ stem from a frequently occurring, non-protein non-carbohydrate contaminant

3.11). The resulting compound is the trifucosylated Galβ (1→3) GlcNAcβ (1→3) [Galβ (1→4) GlcNAcβ (1→6)] Galβ (1→3)GalNAc-ol structure possessing an H determinant in the upper branch and a Y determinant in the lower branch. This new structure is depicted in Scheme 1.

Fraction 6.6. The ^{1}H-NMR spectrum of fraction 6.6 demonstrates the presence of one compound. Since the chemical shifts of the structural-reporter group signals of compound 6.6 match completely those of compound **N-6B** from human milk sIgA hinge region measured at 400 MHz [9], these compounds are identical. The structure of compound 6.6 is described in Scheme 1.

Fraction 4.8. FAB-MS analysis of the permethylated fraction 4.8 shows the presence of a high intensity ion $(M+Na)^+$ which is observed at *m/z* 1782. Altogether with the chemical composition (Table 1), this indicates an octasaccharide constituted of GalNAc-ol, Gal, GlcNAc and Fuc in a ratio of 1:3:2:2. The ^{1}H-NMR spectrum (Fig. 3) demonstrates the presence of one compound which has almost the same structure as compound 3.11 . The results of the methylation analysis (Table 2) indicate a GlcNAc residue, substituted at C-3, which is not present in compound 3.11 . The ^{1}H-NMR data demonstrate that the difference in the structures is located in the lower branch wherein the Fucα(1→2)Gal element is β(1→4)-linked in compound 3.11 and β(1→3)-linked in compound 4.8. This follows from the set of Fuc^2 structural-reporter groups in 4.8 (H-1, δ = 5.192 ppm; H-5, δ = 4.291 ppm; CH3, δ = 1.235 ppm) which is typical for Fucα(1→2) linked to Gal^3. The Fucα(1→2)Galβ(1→3)GlcNAcβ(1→3) sequence has been described for compound **N-8** from human milk sIgA hinge region [9]. To obtain correct assignments of the GlcNAc^{3,3} and Gal^{3,3,3} H-1 signals, a 2D-HOHAHA experiment was carried out at 600 MHz, using an 80-ms spinlock mixing time. A data matrix of 160 × 2K with 176 scans for each experiment was obtained, which was zero filled to 1K × 2K prior to Fourier transformation (Fig. 4). As a β-D-Gal*p* residue can easily be recognized from its H-4 signal ($J_{4,5}$ < 1 Hz), the 2D-HOHAHA experiment reveals that the signal at δ = 4.650 ppm belongs to the H-1 of Gal^{3,3,3} and the proton resonating at δ = 4.599 ppm to the GlcNAc^{3,3} H-1 (for compound **N-8** [9] these signals have to be interchanged). The structure of compound 4.8, that has not been described previously, is given in Scheme 1.

Fraction 6.15. FAB-MS analysis of the permethylated fraction 6.15 reveals the presence of a high intensity ion $(M+Na)^+$ which is observed at *m/z* 2027. Altogether with the chemical composition (Table 1), this indicates a nonasaccharide constituted of GalNAc-ol, Gal, GlcNAc and Fuc in a ratio of 1:3:3:2. The results of the methylation analysis are summarized in Table 2. The ^{1}H-NMR spectrum of this fraction, which consists of one compound, is shown in Fig. 5 and the chemical shifts are listed in Table 3. Compound 6.15 can be considered as an extension of Fucα (1→2) Galβ (1→4) GlcNAcβ(1→6)[Fucα(1→2)Galβ(1→3)GlcNAcβ(1→3)]Galβ (1→3) GalNAc - ol (compound 4.8), with a GlcNAcβ(1→6) linked to GalNAc-ol. The chemical-shift effects on the H-4 and H-5 of GalNAc-ol, due to the attachment of GlcNAcβ(1→6) to GalNAc-ol ($\Delta\delta$ = −0.036 and 0.09 ppm,

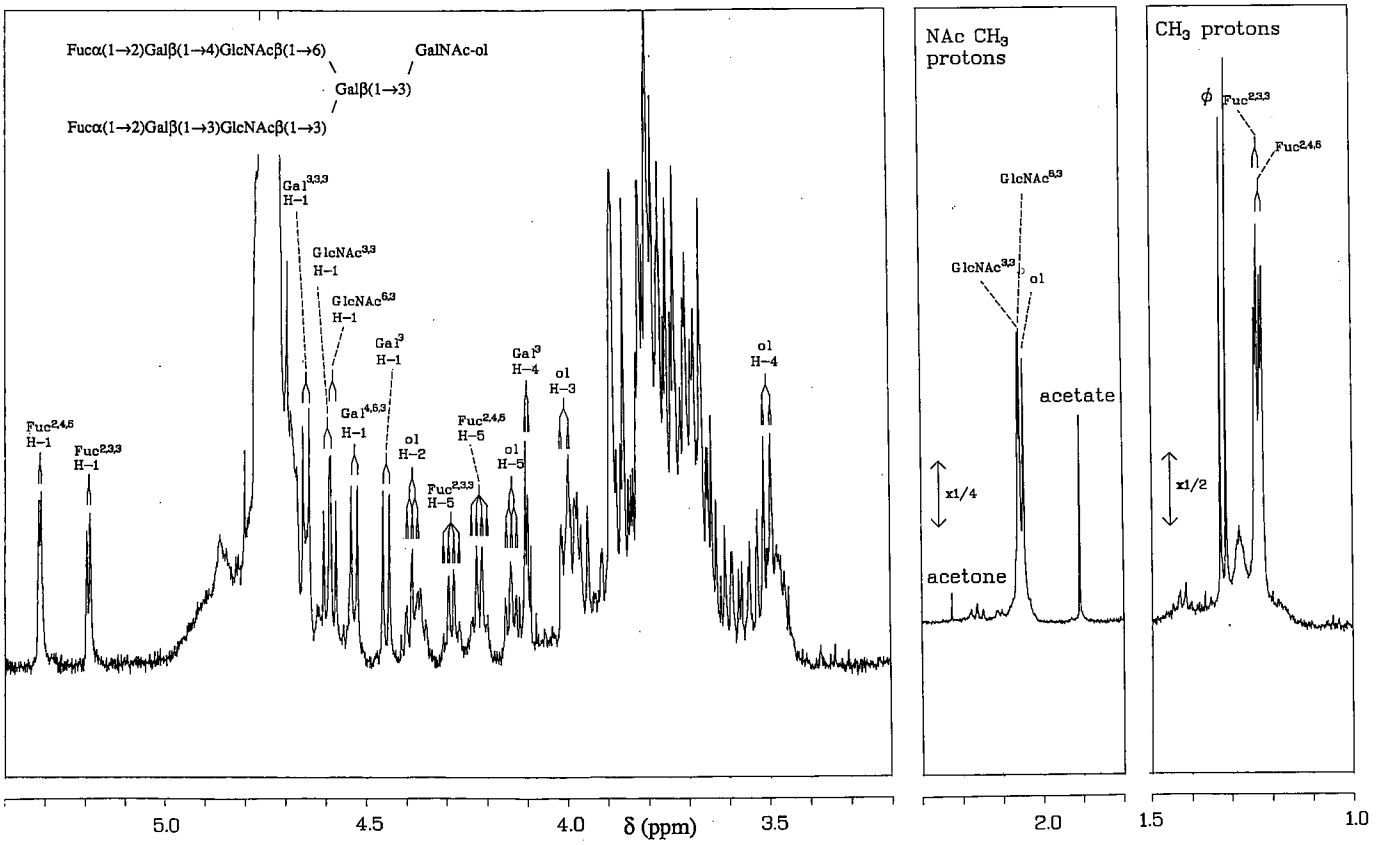


Fig. 3. *Resolution-enhanced 500-MHz spectrum (^2H_2O, 27°C) of fraction 4.8, obtained from the pool of neutral oligosaccharide-alditols Ib from the sputum of a patient with Kartagener's syndrome.* The relative intensity scale of the *N*-acetyl and Fuc methyl proton region of the spectrum differs from that of the other parts, as indicated. Signals marked by ϕ stem from a frequently occurring, non-protein non-carbohydrate contaminant

respectively), are similar as those found in the spectra of compounds **14.5** and **16.4** from porcine blood-group H substance ($\Delta\delta$ = −0.037 and 0.077 ppm, respectively) [7]. The chemical shifts of the structural-reporter groups of GlcNAc^6 in compound 6.15, H-1 and NAc resonating at δ = 4.534 and 2.067 ppm, respectively, are similar to those found for GlcNAc^6 in compound **16.4** (δ = 4.526 and 2.066 ppm, respectively). The new structure for compound 6.15 is presented in Scheme 1.

Remaining structures.

From the ^{1}H-NMR spectra of the HPLC fractionated oligosaccharide-alditols, six other compounds could be identified. These structures do not have a structural element in common with the preceding structures, nor could they be classified on the basis of a common structural element. Methylation analysis data are given in Table 2, the ^{1}H-NMR chemical shifts of the structural-reporter group signals are listed in Table 4, and the structures are given in Scheme 2. The interpretation of the FAB-MS data is carried out guided by the sugar analysis data.

Fraction 3.10. The ^{1}H-NMR spectrum of fraction 3.10 demonstrates the presence of one component. The chemical shifts of the structural-reporter group signals match completely those of compound **23b** [2]. Consequently, the structure of 3.10 is identical to that of compound **23b** and is described in Scheme 2.

Fraction 5.5. The ^{1}H-NMR spectrum of fraction 5.5 (Fig. 6), points to the presence of one major component. Due to the low amount of material, the structure of the minor component(s) could not be elucidated. The major component can be conceived as an extension of the structure of component 3.10 with Fucα (1→3) linked to GlcNAc^6. The shift effects due to this substitution, when comparing compound 3.10 with the major component of 5.5, are a downfield shift of the GlcNAc^6 H-1 signal ($\Delta\delta$ = 0.007 ppm), an upfield shift of the NAc signal ($\Delta\delta$ = −0.012 ppm) and an upfield shift of Gal^{4,6} H-1 ($\Delta\delta$ = −0.038 ppm). These effects are analogous to those observed for the structure of reference compounds **23b** versus **23c** in [2]. The structure of the major component of 5.5 is described in Scheme 2.

Fraction 3.1. The structural-reporter groups of the only compound of fraction 3.1 resonate at essentially the same position as those described for compound **23a** [1] and, in consequence, compounds 3.1 and **23a** are identical. The structure is given in Scheme 2.

Fraction 6.3. FAB-MS analysis of the permethylated fraction 6.3 shows the presence of a high intensity ion $(M+Na)^+$ which is observed at *m/z* 1783 indicating the presence of an octasaccharide constituted of *N*-acetylhexosaminitol, hexose, *N*-acetylhexosamine and Fuc in a ratio of 1:3:2:2. The ^{1}H-NMR spectrum of fraction 6.3, which is presented in Fig. 7, demonstrates the occurrence of one compound. The presence of an Le^b determinant, Fucα(1→2)Galβ(1→3)[Fucα(1→4)] GlcNAcβ(1→3), can be inferred by comparing the spectra

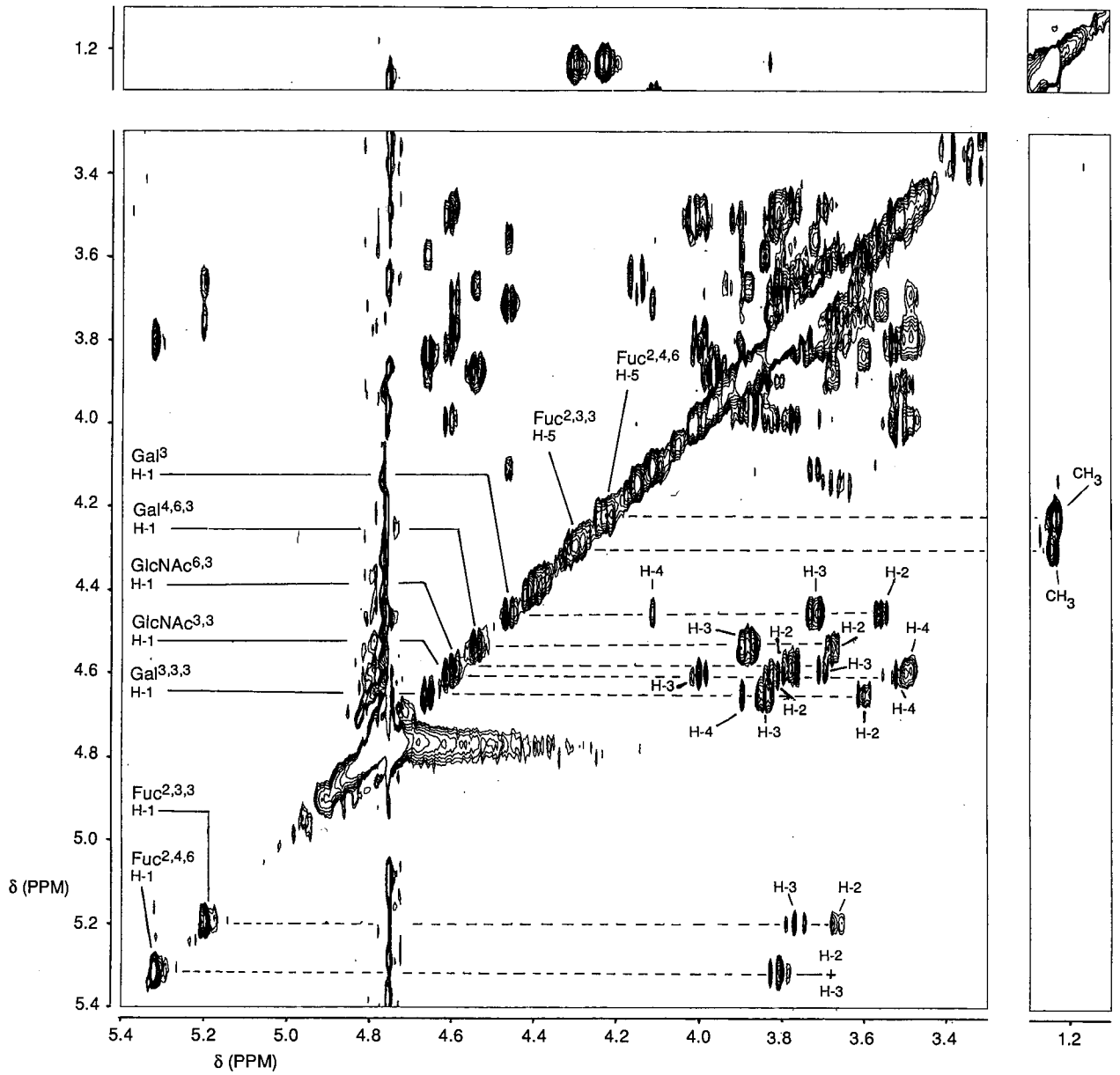
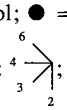


Fig. 4. *600-MHz homonuclear Hartman-Hahn spectrum of fraction 4.8, with a spinlock time of 80 ms.* The region between 3.3 and 1.4 ppm has been left out

of compounds 6.3 and 6.8 (accompanying paper [6]). The corresponding structural-reporter group signals resonate at almost the same positions for both compounds. Therefore, the core structure of compound 6.3 is →3)Galβ(1→3)[Galβ(1→4)GlcNAcβ(1→6)]GalNAc-ol, as can be concluded from the comparison of the corresponding chemical shifts of compound 6.3 with those of compound 3.1. The combination of these two elements leads to a new structure, which is given in Scheme 2.

Fraction 6.14. FAB-MS analysis of the permethylated fraction 6.14 shows the presence of a high intensity ion $(M+Na)^+$ observed at *m/z* 1956. Altogether with the chemical composition (Table 1), this indicates a nonasaccharide constituted of GalNAc-ol, Gal, GlcNAc and Fuc in a ratio of 1:3:2:3. The ^{1}H-NMR spectrum of this fraction (Fig. 8) shows that it consists of a mixture having one major component (>90%). Due to the low amount of material, the structure of the minor component(s) could not be elucidated. The presence of the Fucα(1→2)Galβ(1→4)GlcNAcβ(1→6) structural element can be inferred from the very typical set of Fuc structural-reporter group signals: H-1, δ = 5.311 ppm; H-5, δ = 4.224 ppm; CH_3, δ = 1.231 ppm, together with the Gal H-1 signal resonating at δ = 4.543 ppm and the GlcNAc H-1 signal resonating at δ = 4.586 ppm (compare with the data of the compounds 3.10, 4.8, 6.10 and 6.15). In the same way, the occurrence of also a Fucα(1→2)Galβ(1→3)GlcNAcβ(1→3) structural element can be deduced from the Fuc structural-reporter group signals: H-1, δ = 5.198 ppm; H-5, δ = 4.274 ppm; CH_3, δ = 1.231 ppm, in combination with the Gal H-1 signal resonating at δ = 4.668 ppm (compare with the data of the compounds 4.8 and 6.15). A third Fuc residue in the major compound of fraction 6.14 gives rise to a new set of Fuc structural-reporter group signals: H-1, δ = 5.081 ppm; H-5, δ = 4.274 ppm; CH_3, δ = 1.190 ppm, which is indicative

Table 3. *^{1}H-chemical shifts of structural-reporter groups of constituent monosaccharides for the HPLC-fractionated oligosaccharide-alditols possessing the Galβ(1→4)GlcNAcβ(1→6)[GlcNAcβ(1→3)]Galβ(1→3)GalNAc-ol common element*

Chemical shifts are relative to internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate (using internal acetone at δ = 2.225 ppm) in ^2H_2O at 27 °C, acquired at 500 MHz. For the complete structures of the compounds, see Scheme 1. In the table heading, the structures are represented by short-hand symbolic notation (cf.[15]) ◇; = GalNAc-ol; ● = GlcNAc; ■ = Gal and □ = Fuc. The position of linkage in this notation is specified by the angle of the connecting bars as follows:  ; n.d., value could not be determinded merely by inspection of the spectrum.

A superscript at the name of a sugar indicates to which position of the adjacent monosaccharide it is glycosidically linked (cf.[16]). Frequently, more than one superscript is used to discriminate between identically linked residues, by indicating the types of the next linkages in the sequence

Residue	Reporter group	Chemical shift in compound					
		3.11 	4.3 	6.10 	6.6 	4.8 	6.15 
		ppm					
GalNAc-ol	H-2	4.383	4.384	4.399	4.388	4.387	4.395
	H-3	3.991	n.d.	4.046	4.002	4.003	4.00
	H-4	3.511	3.505	3.508	3.467	3.504	3.468
	H-5	4.140	4.134	4.139	4.220	4.143	4.23
	NAc	2.043	2.044	2.044	2.045	2.046	2.046
Gal^3	H-1	4.463	4.469	4.466	4.455	4.452	4.442
	H-2	n.d.	3.53[a]	n.d.	n.d.	3.55[b]	n.d.
	H-3	n.d.	n.d.	n.d.	n.d.	3.71[b]	n.d.
	H-4	4.103	4.102	4.100	4.113	4.104	4.102
GlcNAc^{6,3}	H-1	4.584	4.608	4.584	4.595	4.582	4.576
	H-2	n.d.	3.78[a]	n.d.	n.d.	3.78[b]	n.d.
	H-3	n.d.	3.72[a]	n.d.	n.d.	3.71[b]	n.d.
	H-4	n.d.	n.d.	n.d.	n.d.	3.49[b]	n.d.
	NAc	2.055	2.052	2.055	2.049	2.055	2.051
Gal^{4,6,3}	H-1	4.532	4.469	4.531	4.468	4.530	4.534
	H-2	n.d.	3.67[a]	n.d.	n.d.	3.68[b]	n.d.
	H-3	n.d.	n.d.	n.d.	n.d.	3.88[b]	n.d.
	H-4	3.891	3.92[a]	n.d.	n.d.	n.d.	n.d.
GlcNAc^{3,3}	H-1	4.686	4.684[c]	4.697	4.683	4.599	4.592
	H-2	n.d.	3.83[a]	n.d.	n.d.	3.81[b]	n.d.
	H-3	n.d.	3.95[a]	n.d.	n.d.	3.99[b]	n.d.
	H-4	n.d.	n.d.	n.d.	n.d.	3.52[b]	n.d.
	NAc	2.043	2.031	2.032	2.036	2.060	2.057
Gal^{4,3,3}	H-1	4.549	4.515	4.514	4.483		
	H-4	3.891	n.d.	n.d.	n.d.		
Gal^{3,3,3}	H-1					4.650	4.651
	H-2					3.60[b]	n.d.
	H-3					3.84[b]	n.d.
	H-4					3.89[b]	n.d.
GlcNAc^6	H-1				4.556		4.534
	H-6				3.996		n.d.
	NAc				2.065		2.067
Gal^{4,6}	H-1				4.468		
Fuc^{2,4,6}	H-1	5.311		5.315		5.313	5.312
	H-2	n.d.		n.d.		3.81[b]	n.d.
	H-3	n.d.		n.d.		3.81[b]	n.d.
	H-5	4.221		4.223		4.221	4.225
	CH_3	1.230		1.231		1.229	1.232
Fuc^{2,4,3}	H-1	5.311	5.278	5.276			
	H-5	4.221	4.253	4.251			
	CH_3	1.230	1.268	1.266			
Fuc^{2,3,3}	H-1					5.192	5.199
	H-2					3.67[b]	n.d.
	H-3					3.77[b]	n.d.
	H-5					4.291	4.293
	CH_3					1.235	1.237
Fuc^3	H-1		5.125	5.126			
	H-5		4.874	4.874			
	CH_3		1.237	1.236			

[a] From HOHAHA experiment.
[b] From HOHAHA experiment recorded at 600 MHz.
[c] Spectrum recorded at 10 °C.

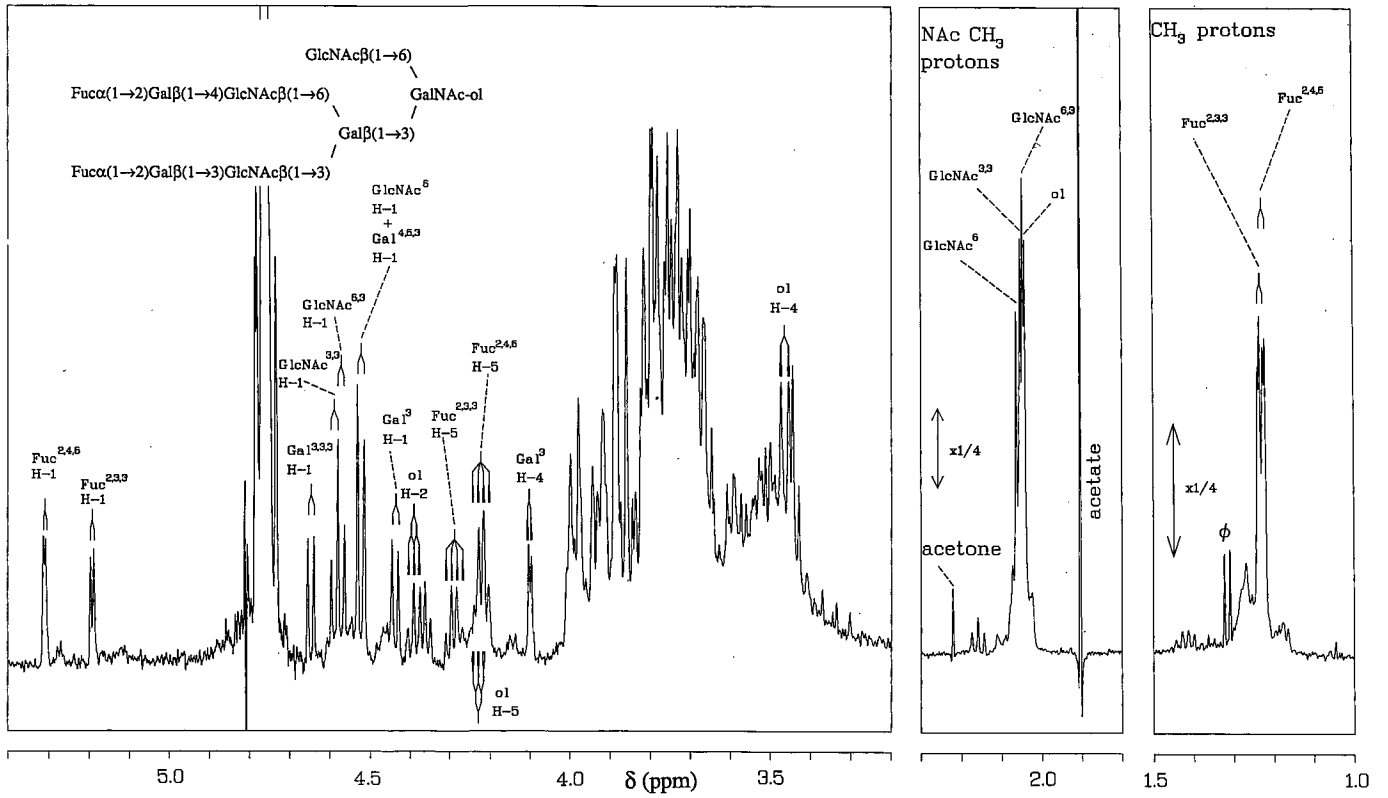


Fig. 5. *Resolution-enhanced 500-MHz spectrum (^2H_2O, 27°C) of fraction 6.15, obtained from the pool of neutral oligosaccharide-alditols Ib from the sputum of a patient with Kartagener's syndrome.* The relative intensity scale of the *N*-acetyl and Fuc methyl proton region of the spectrum differs from that of the other parts, as indicated. Signals marked by ϕ stem from a frequently occurring, non-protein non-carbohydrate contaminant

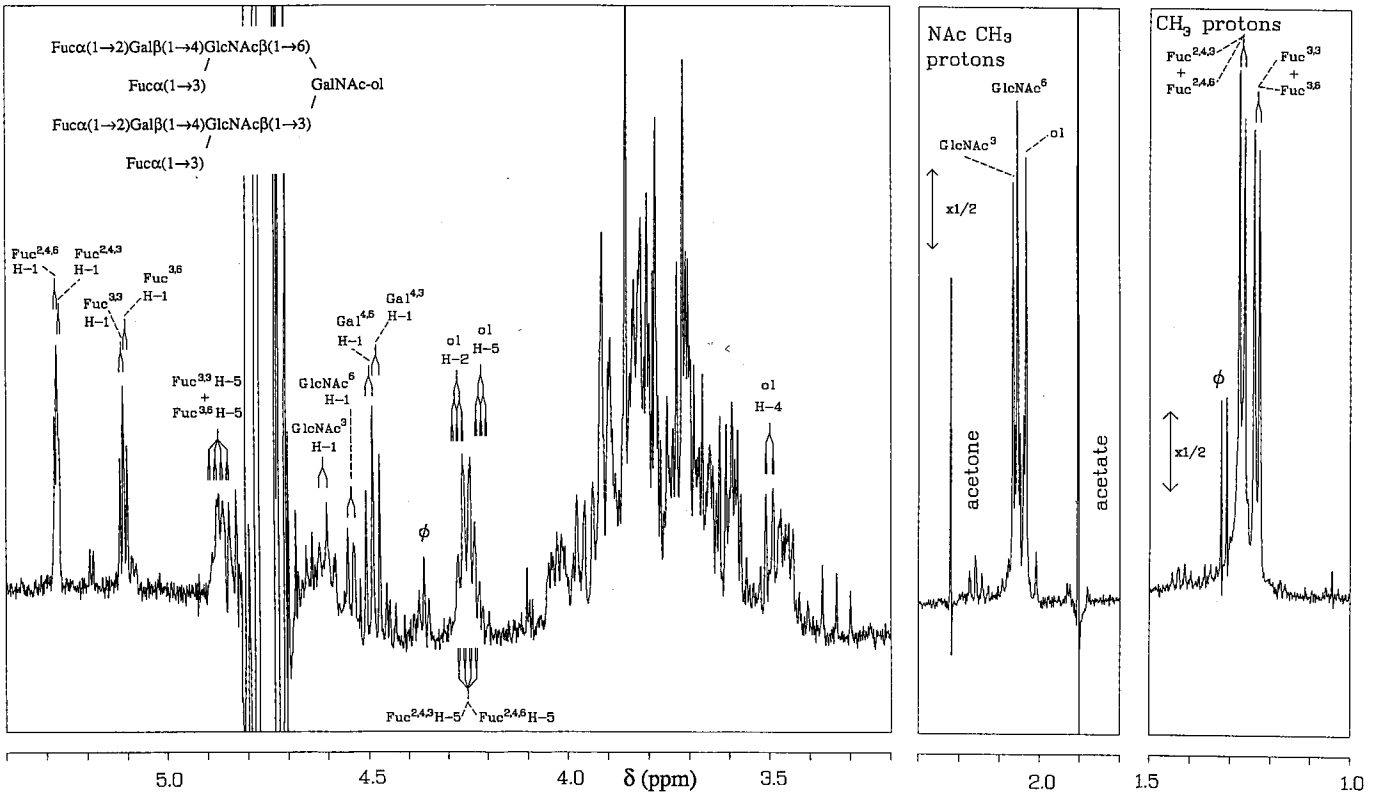


Fig. 6. *Resolution-enhanced 500-MHz spectrum (^2H_2O, 27°C) of fraction 5.5, obtained from the pool of neutral oligosaccharide-alditols Ib from the sputum of a patient with Kartagener's syndrome.* The relative intensity scale of the *N*-acetyl and Fuc methyl proton region of the spectrum differs from that of the other parts, as indicated. Signals marked by ϕ stem from a frequently occurring, non-protein non-carbohydrate contaminant

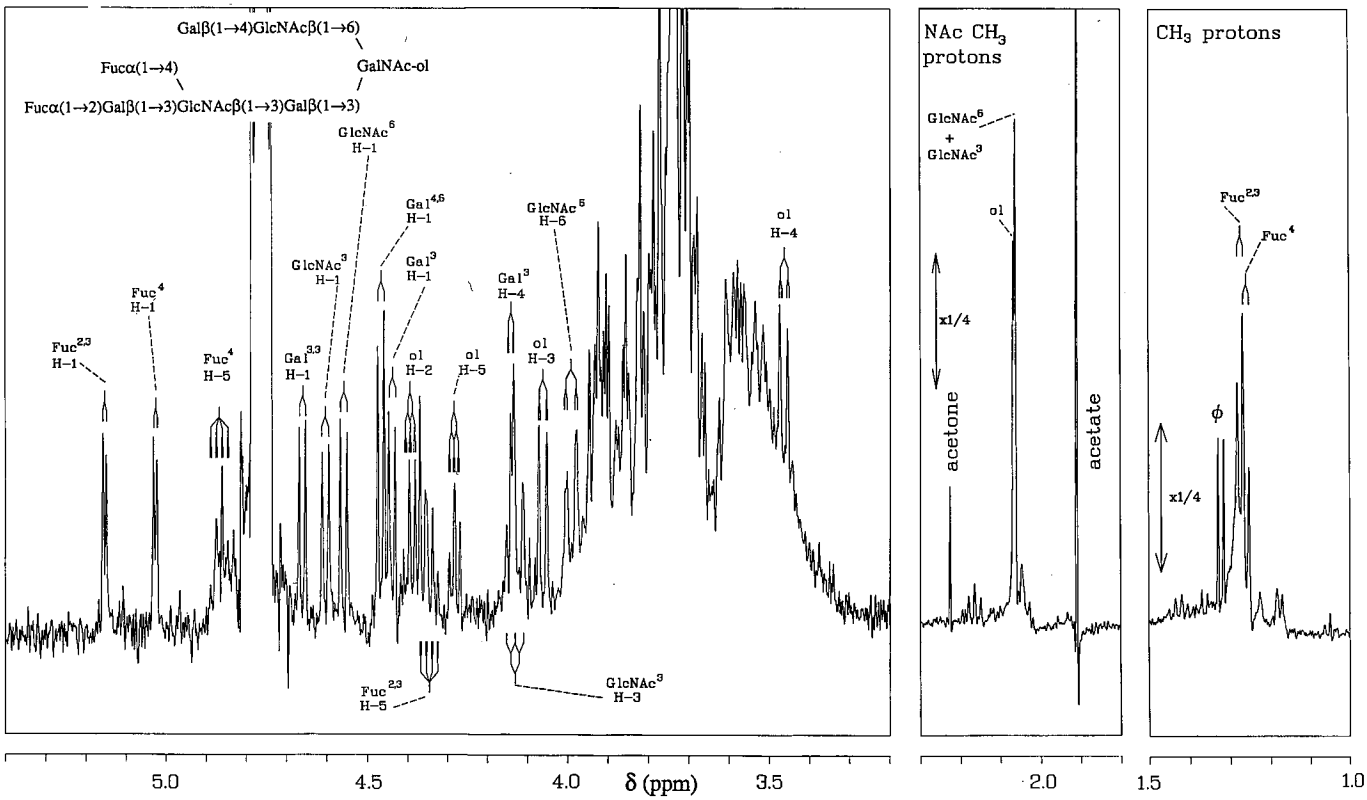


Fig. 7. *Resolution-enhanced 500-MHz spectrum (^2H_2O, 27°C) of fraction 6.3, obtained from the pool of neutral oligosaccharide-alditols Ib from the sputum of a patient with Kartagener's syndrome.* The relative intensity scale of the *N*-acetyl and Fuc methyl proton region of the spectrum differs from that of the other parts, as indicated. Signals marked by ϕ stem from a frequently occurring, non-protein non-carbohydrate contaminant

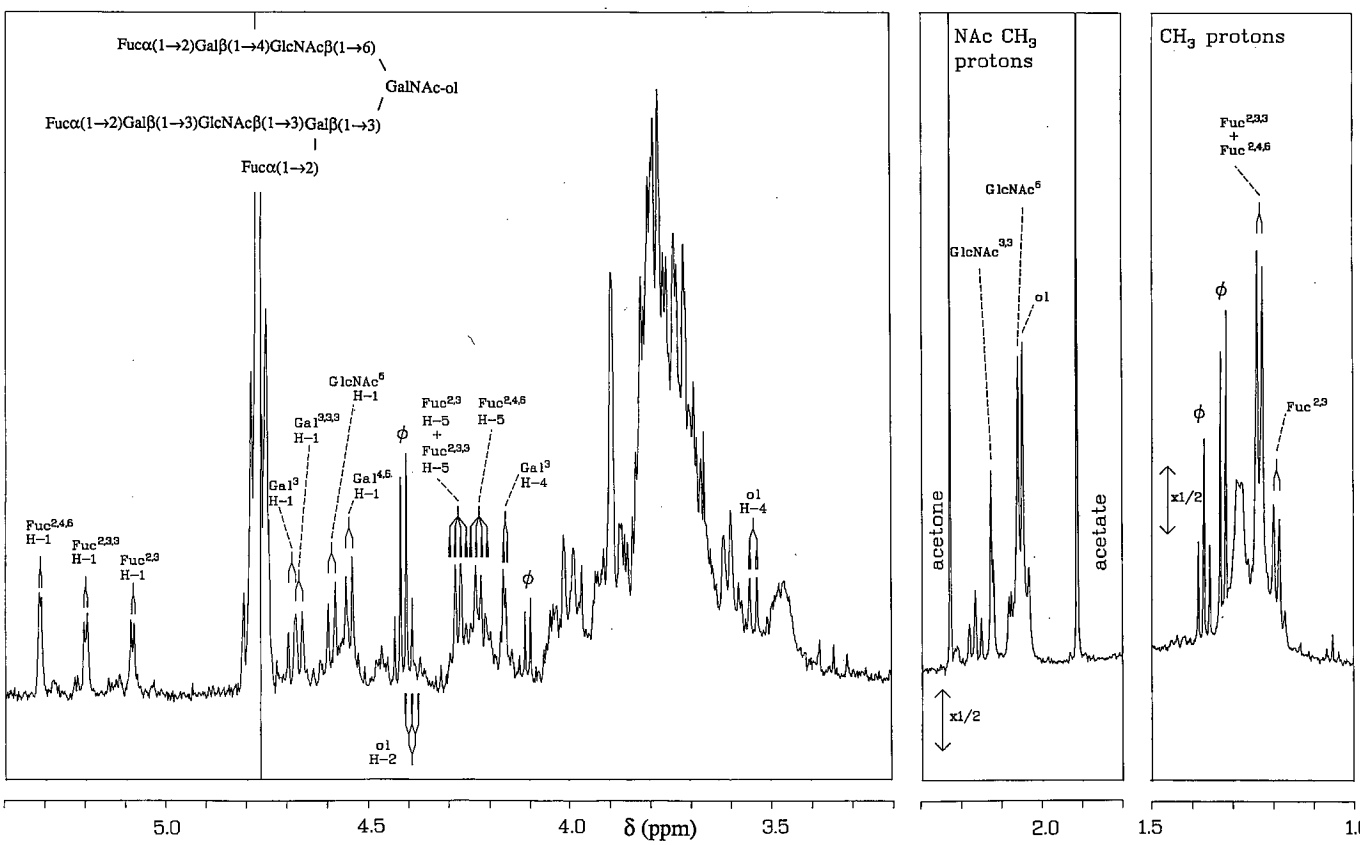


Fig. 8. *Resolution-enhanced 500-MHz spectrum (^2H_2O, 27°C) of fraction 6.14, obtained from the pool of neutral oligosaccharide-alditols Ib from the sputum of a patient with Kartagener's syndrome.* The relative intensity scale of the *N*-acetyl and Fuc methyl proton region of the spectrum differs from that of the other parts, as indicated. Signals marked by ϕ stem from a frequently occurring, non-protein non-carbohydrate contaminant

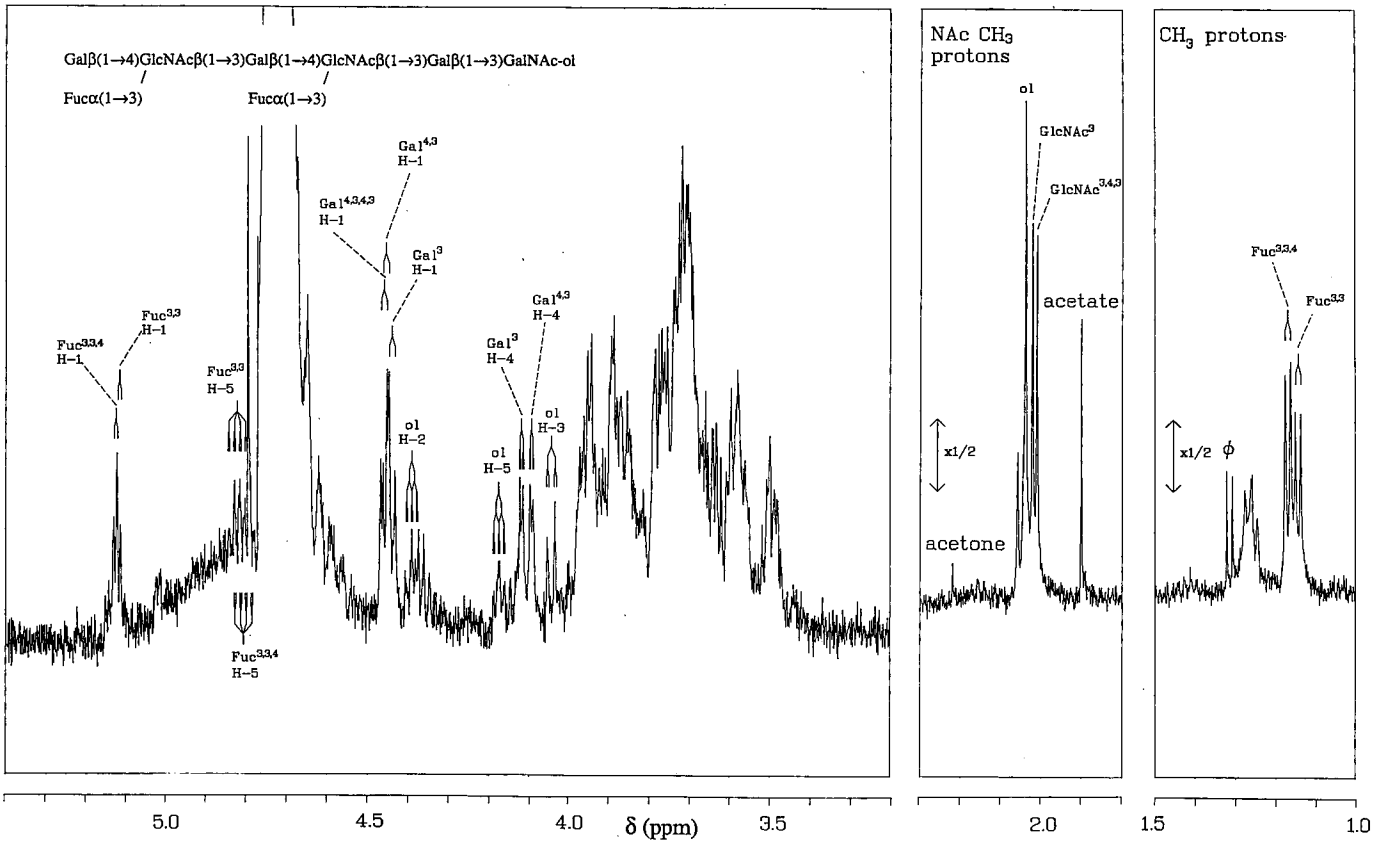


Fig. 9. *Resolution-enhanced 500-MHz spectrum (^2H_2O, 27°C) of fraction 4.1, obtained from the pool of neutral oligosaccharide-alditols Ib from the sputum of a patient with Kartagener's syndrome.* The relative intensity scale of the *N*-acetyl and Fuc methyl proton region of the spectrum differs from that of the other parts, as indicated. Signals marked by ϕ stem from a frequently occurring, non-protein non-carbohydrate contaminant

for a Fucα(1→2)[GlcNAcβ(1→3)]Galβ(1→ structural element (see the compounds 3.8, 5.7, and 6.18, and the major component of fraction 6.5 in the accompanying paper [6]). The chemical shift of Fuc H-5 was derived from the 2D-HOHAHA spectrum of fraction 6.14, recorded at 600 MHz at 288 K, using an 80-ms spinlock mixing time. A data matrix of 1K × 2K was obtained which was zero-filled to 2K × 2K prior to Fourier transformation. For each experiment 160 scans were recorded (data not shown). The H-4 signal of Gal (δ = 4.159 ppm) shows that this residue is substituted at C-2 and C-3. The GlcNAc H-1 and NAc signals at δ = 4.77 and 2.124 ppm, respectively, are similar to those of GlcNAc^{3,4,6} in compound 6.5 at δ = 4.722 ppm and 2.124 ppm, respectively. Similarly, in the major component of fraction 6.5 [6] GlcNAc is β(1→3)-linked to a Gal residue which is substituted with Fucα(1→2). Combination of these structural elements, together with the methylation analysis of this fraction (Table 2), leads to a novel structure for the major component of 6.14 as is given in Scheme 2.

Fraction 4.1. FAB-MS analysis of the permethylated fraction shows the presence of a high-intensity ion $(M+Na)^+$ observed at *m/z* 1783. Altogether with the chemical composition (Table 1), this indicates an octasaccharide constituted of GalNAc-ol, Gal, GlcNAc and Fuc in a ratio of 1:3:2:2. The results of the methylation analysis are given in Table 2. The ^{1}H-NMR spectrum of this fraction (Fig. 9) shows the presence of one major component. The structure of the minor component(s) could not be elucidated due to the low amount of material. The major component of fraction 4.1 has a →3)Galβ(1→3)GalNAc-ol core structure, which can be deduced from the GalNAc-ol structural-reporter groups H-2 (δ = 4.396 ppm), H-5 (δ = 4.178 ppm) and NAc (δ = 2.045 ppm) (compare the data of the major component of 4.1 with those of reference compound **14** [1]). Two sets of structural-reporter group signals for Fucα(1→3) linked to a GlcNAc residue are detected in the spectrum of fraction 4.1. The occurrence of two Gal residues, both substituted at C-3, is deduced from the presence of two Gal H-4 signals resonating at δ = 4.124 and 4.099 ppm, respectively, and of the results of the methylation analysis indicating a Gal residue substituted at C-3. Combination of these findings leads to a novel structure for the major component of fraction 4.1, with one repeating Galβ(1→4)[Fucα(1→3)]GlcNAcβ(1→3) element, which is presented in Scheme 2.

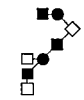
DISCUSSION

The use of a two-step HPLC fractionation allowed the purification of 46 fractions, 23 out of them contained sufficient amounts of material for structural analysis. This has enabled the characterization of the primary structure of 23 oligosaccharide-alditols.

The first fractionation step, performed on an alkylamine-bonded phase silica, yielded 13 fractions. On that type of column, the oligosaccharide retention times are based on the

Table 4. [1]*H-chemical shifts of structural-reporter groups of constituent monosaccharides for the remaining HPLC-fractionated oligosaccharide-alditols*

For explanation of the notation, see Table 3; n.d., value could not be determinded merely by inspection of the spectrum. A superscript at the name of a sugar indicates to which position of the adjacent monosaccharide it is glycosidically linked (cf.[16]). Frequently, more than one superscript is used to discriminate between identically linked residues, by indicating the types of the next linkages in the sequence

Residue	Reporter group	Chemical shift in compound					
		3.10 	5.5 	3.1 	6.3 	6.14 	4.1 
		ppm					
GalNAc-ol	H-2	4.265	4.27	4.402	4.393	4.401	4.396
	H-3	n.d.	n.d.	4.049	4.059	n.d.	4.049
	H-4	3.519	3.505	3.452	3.463	3.539	n.d.
	H-5	4.223	4.22	4.266	4.279	n.d.	4.178
	NAc	2.037	2.037	2.066	2.066[a]	2.044	2.045
GlcNAc^{6}	H-1	4.539	4.546	4.554	4.556	4.586	
	H-2	n.d.	n.d.	n.d.	n.d.	3.78[b]	
	H-3	n.d	n.d.	n.d.	n.d.	3.66[b]	
	H-4	n.d.	n.d.	n.d.	n.d.	3.46[b]	
	H-6	n.d.	n.d.	3.992	3.987	n.d.	
	NAc	2.071	2.059	2.057	2.061	2.056	
Gal^{4,6}	H-1	4.539	4.501	4.464	4.464	4.543	
	H-2	n.d.	n.d.	n.d.	n.d.	3.66[b]	
	H-3	n.d.	n.d.	n.d.	n.d.	3.88[b]	
	H-4	n.d.	n.d.	3.924	n.d.	n.d.	
Gal^{3}	H-1			4.448	4.435	4.685	4.445
	H-2			n.d.	n.d.	3.72[b]	n.d.
	H-3			n.d.	n.d.	4.04[b]	n.d.
	H-4			4.128	4.136	4.159	4.124
GlcNAc^{3}	H-1	4.620	4.616	4.69	4.601	4.77[b]	4.673[c,d]
	H-2	n.d.	n.d.	n.d.	n.d.	3.84[b]	n.d.
	H-3	n.d.	n.d.	n.d.	4.132	4.03[b]	n.d.
	H-4	n.d.	n.d.	n.d.	n.d.	3.47[b]	n.d.
	H-6	4.018	n.d.	3.969	n.d.	n.d.	n.d.
	NAc	2.071	2.070	2.029	2.061[a]	2.124	2.029[e]
Gal^{4,3}	H-1	4.482	4.484	4.464			4.459[f]
	H-4	n.d.	n.d.	3.901			4.099
Gal^{3,3}	H-1				4.653	4.668	
	H-2				n.d.	3.59[b]	
	H-3				n.d.	3.84[b]	
	H-4				n.d.	3.88[b]	
GlcNAc^{3,4,3}	H-1						4.661[c,d]
	NAc						2.017[e]
Gal^{4,3,4,3}	H-1						4.464[f]
Fuc^{2,3}	H-1					5.081	
	H-5					4.274	
	CH_3					1.190	
Fuc^{2,3,3}	H-1				5.151	5.198	
	H-5				4.344	4.274	
	CH_3				1.272	1.231	
Fuc^{2,4,3}	H-1	5.280	5.27				
	H-5	4.24	4.258				
	CH_3	1.275	1.275				
Fuc^{2,4,6}	H-1	5.307	5.27			5.311	
	H-2	n.d.	n.d.			3.81[b]	
	H-3	n.d.	n.d.			3.81[b]	
	H-5	4.23	4.258			4.224	
	CH_3	1.236	1.275			1.231	
Fuc^{3,3}	H-1	5.119	5.117[g]	5.139			5.125
	H-5	4.868	4.873	n.d.			4.865[c,h]
	CH_3	1.236	1.238	1.177			1.150
Fuc^{3,3,4}	H-1						5.133
	H-5						4.851[c,h]
	CH_3						1.174
Fuc^{3,6}	H-1		5.108[g]				
	H-5		4.873				
	CH_3		1.238				
Fuc^{4}	H-1				5.024		
	H-5				4.865		
	CH_3				1.257		

[a] Assignments may have to be interchanged.
[b] From HOHAHA experiment recorded at 15°C at 600 MHz.
[c] Spectrum recorded at 7°C.
[d–h] Assignments may have to be interchanged.

3.11 Fucα(1→2)Galβ(1→4)GlcNAcβ(1→6)[Fucα(1→2)Galβ(1→4)GlcNAcβ(1→3)]Galβ(1→3)GalNAc-ol

4.3 Galβ(1→4)GlcNAcβ(1→6)[Fucα(1→2)Galβ(1→4)GlcNAcβ(1→3)Fucα(1→3)]Galβ(1→3)GalNAc-ol

6.10 Fucα(1→2)Galβ(1→4)GlcNAcβ(1→6)[Fucα(1→2)Galβ(1→4)GlcNAcβ(1→3)Fucα(1→3)]Galβ(1→3)GalNAc-ol

6.6 Galβ(1→4)GlcNAcβ(1→6)[Galβ(1→4)GlcNAcβ(1→6)[Galβ(1→4)GlcNAcβ(1→3)]Galβ(1→3)]GalNAc-ol

4.8 Fucα(1→2)Galβ(1→4)GlcNAcβ(1→6)[Fucα(1→2)Galβ(1→3)GlcNAcβ(1→3)]Galβ(1→3)GalNAc-ol

6.15 GlcNAcβ(1→6)[Fucα(1→2)Galβ(1→4)GlcNAcβ(1→6)[Fucα(1→2)Galβ(1→3)GlcNAcβ(1→3)]Galβ(1→3)]GalNAc-ol

Scheme 1. *Structures of neutral oligosaccharide-alditols with the Galβ(1→4)GlcNAcβ(1→6)[GlcNAcβ(1→3)]Galβ(1→3)GalNAc-ol common element, obtained by HPLC fractionation of a pool of neutral oligosaccharide-alditols from Kartageners syndrome sputum*

3.10 Fucα(1→2)Galβ(1→4)GlcNAcβ(1→6)[Fucα(1→2)Galβ(1→4)GlcNAcβ(1→3)Fucα(1→3)]GalNAc-ol

5.5 Fucα(1→2)Galβ(1→4)GlcNAcβ(1→6)[Fucα(1→3)][Fucα(1→2)Galβ(1→4)GlcNAcβ(1→3)Fucα(1→3)]GalNAc-ol

3.1 Galβ(1→4)GlcNAcβ(1→6)[Galβ(1→4)GlcNAcβ(1→3)Galβ(1→3)Fucα(1→3)]GalNAc-ol

6.3 Galβ(1→4)GlcNAcβ(1→6)[Fucα(1→4)][Fucα(1→2)Galβ(1→3)GlcNAcβ(1→3)Galβ(1→3)]GalNAc-ol

6.14 Fucα(1→2)Galβ(1→4)GlcNAcβ(1→6)[Fucα(1→2)Galβ(1→3)GlcNAcβ(1→3)[Fucα(1→2)]Galβ(1→3)]GalNAc-ol

4.1 Galβ(1→4)GlcNAcβ(1→3)[Fucα(1→3)]Galβ(1→4)GlcNAcβ(1→3)[Fucα(1→3)]Galβ(1→3)GalNAc-ol

Scheme 2. *Structures of the remaining neutral oligosaccharide-alditols, obtained by HPLC fractionation of a pool of neutral oligosaccharide-alditols from Kartageners syndrome sputum*

number and type of sugar residues as described previously [2]. In the present study, the numerous structures present in fraction Ib, with sometimes very subtle structural differences, explain the pattern of the elution profile. It should be noted that in the second chromatographic step using a reverse-phase column, there were no overlapping fraction among fractions 3, 4, 5 and 6. The fractionation that was obtained follows other empirical rules that were partially defined in a previous publication [2]. The Fuc residues present in different structural elements (H, X, Y) modify the retention time of the oligosaccharides.

(a) Structures possessing the Fucα(1→2)Galβ(1→3)-GalNAc-ol structural element are strongly retained (see retention times of compounds 6.13 and 6.18).

(b) Structures possessing the Fucα(1→2)Galβ(1→4)-GlcNAcβ(1→) structural element induce a similar retarding effect (i.e compounds 3.11, 6.15 and 6.17).

(c) Structures possessing the Fucα(1→2)Galβ(1→3)-GlcNAcβ(1→) structural element are less retained than their analogs with the Fucα(1→2)Galβ(1→4)GlcNAcβ(1→) structural element (compare compound 6.11 with 6.17 and compound 3.11 with 4.8).

(d) Fucα(1→3) linked to GlcNAc shortens the retention time (compare compound 6.11 with its afuco analog 5.8) and, when linked to Fucα(1→2)Galβ(1→4)GlcNAcβ(1→), suppresses the retarding effect of Fucα(1→2) [2] in this element.

(e) Fucα(1→4) lowers the retarding effect of Fucα(1→2) in a Le^b determinant (Fucα(1→2)Galβ(1→3)[Fucα(1→4)]-GlcNAcβ(1→)) (i.e. compound 4.5, 6.3, 6.8).

(f) Internal Fucα(1→2) has little or no effect on the retention time of the reverse-phase column.

These different chromatographic patterns indicate that substitutions of oligosaccharides by Fuc deeply modify their polarity and thereby may play a role in the formation of a mosaic of bacterial binding sites at the mucin surface.

Among the 12 structures described in this paper, 6 presented a Gal^3 residue substituted by an *N*-acetyllactosamine at C-6, thereby giving rise to an I determinant [10]. This has

never been found before in human bronchial mucins. In O-linked oligosaccharides, such a branched Gal residue has been described in oligosaccharides isolated from different sources [7–9,11–13]. This type of Gal residue is so far invariably located on the branch substituting the GalNAc residue at C-3 in neutral oligosaccharide-alditols, whereas in sialylated compounds, possessing the structural element NeuAcα(2→3)-Galβ(1→3)GalNAc-ol, isolated from IgA [9], the I determinant is present on the branch substituting the GalNAc residue at C-6.

The different substitutions of carbohydrate chains by Fuc residues described here, correspond to H, X, Y, and Lewis b determinants. Fuc found α(1→2) linked to a non-terminal Gal of the core type 1 · (1→3)Galβ(1→3)GalNAc-ol in compound 6.14 defines a new type of H determinant, corresponding to the type 3 chain internal H determinant [14]. In compound 4.1 the dimeric X determinant Galβ(1→4)[Fucα(1→3)] GlcNAcβ(1 → 3)Galβ(1 → 4)[Fucα (1 → 3)]GlcNAcβ(1→), is observed for the first time in human bronchial glycans.

These 12 oligosaccharide-alditols bring the number of different structures isolated from the bronchial mucins of a single patient with blood group O suffering from bronchiectasis to 82.

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