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

1. Structure of 11 oligosaccharides having the GlcNAcβ(1→3)Galβ(1→4)GlcNAcβ(1→6)GalNAc-ol structural element in common

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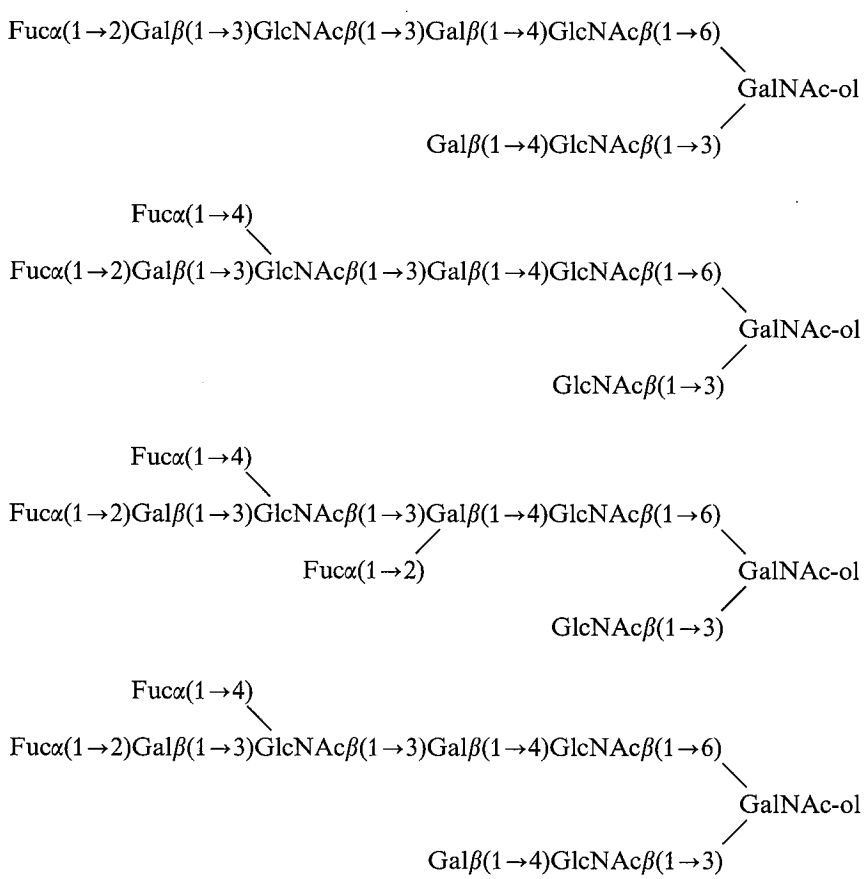
The carbohydrate chains of the respiratory-mucus glycoproteins of a patient (blood group O) suffering from bronchiectasis due to Kartagener's syndrome, were released by alkaline borohydride treatment of a pronase digest. Neutral oligosaccharides were obtained after ion-exchange chromatography and were subsequently separated utilizing gel filtration, HPLC on normal-phase alkylamine-bonded silica and reverse-phase HPLC, into 46 fractions. From these fractions oligosaccharide-alditols have been characterized by employing 500-MHz ^{1}H-NMR spectroscopy, in conjunction with fast-atom-bombardment mass spectroscopy, methylation analysis and sugar analysis.

Here 11 novel oligosaccharide structures are described. Five of them have the common element Fucα(1→2)Galβ(1→3)GlcNAcβ(1→3)Galβ(1→4)GlcNAcβ(1→6)GalNAc-ol, they are:



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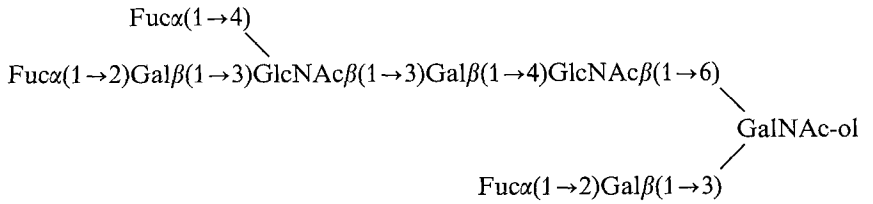
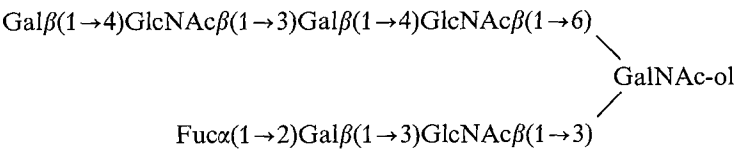
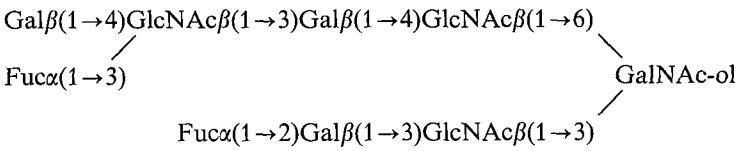
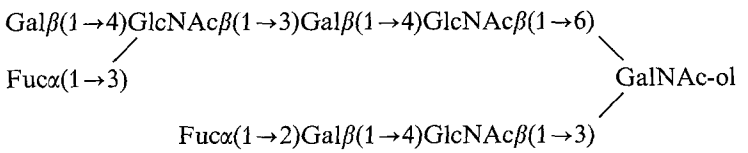
Abbreviations. Fuc, L-fucose; GalNAc, *N*-acetyl-D-galactosamine; GalNac-ol, *N*-acetyl-D-galactosaminitol; FAB-MS, fast-atom-bombardment mass spectrometry; 2D-HOHAHA, two-dimensional homonuclear Hartman-Hahn spectroscopy.

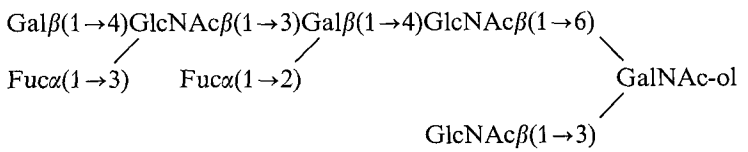


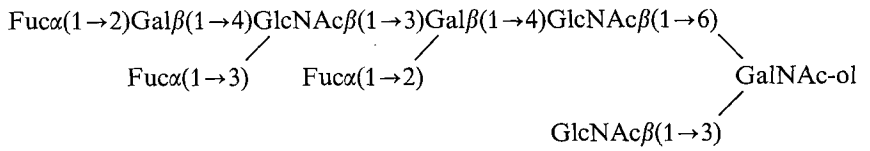
Another six possess Galβ(1→4)GlcNAcβ(1→3)Galβ(1→4)GlcNAcβ(1→6)GalNAc-ol as common element, they are:

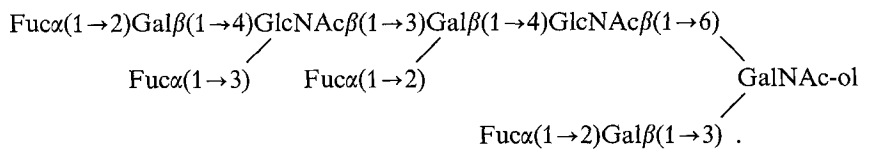












Human bronchial mucins are high-molecular-mass glycoproteins that appear as polydisperse flexible extended threads and are characterized by the large number of carbohydrate chains O-glycosidically linked to the peptide backbone [1]. Several studies indicate that the carbohydrate moiety of the mucins may be involved in the non-immune protection of the respiratory epithelium and in bacterial recognition mechanisms [2]. The diversity of the mucin oligosaccharides is proposed to represent a mosaic of binding sites, thus being important in the first step of the bacterial clearance of the respiratory airways [3, 4].

Oligosaccharides possessing more than eight sugar residues have been described only in human colonic [5], gastric [6] and ovarian cyst mucins [7]. In human bronchial mucins, the average length of the carbohydrate chains is eight sugar residues as derived from the chemical composition [8]. In previous studies, 59 low-molecular-mass neutral and monosialylated oligosaccharide-alditols were isolated and charac-

terized from the secretion of a single patient suffering from bronchiectasis due to Kartagener's syndrome [9–11]; these glycans possessed less than eight sugar residues. The fractions corresponding to the higher-molecular-mass oligosaccharide-alditols had not been studied.

Now we describe the isolation and the characterization of a new series of oligosaccharide-alditols with 7–10 sugar residues from the mucins of the same patient. Here we present the structure of 11 neutral oligosaccharide-alditols, possessing the GlcNAcβ(1→3)Galβ(1→4)GlcNAcβ(1→6)GalNAc-ol structural element in common.

MATERIALS AND METHODS

Materials

Pronase was from Calbiochem (Behring Diagnostics, La Jolla, CA, USA); Sepharose CL-2B was from Pharmacia (Uppsala, Sweden); guanidinium chloride was from Fluka (Buchs, Switzerland); AG50WX8 (100–200 mesh) and AG1X2 (100–200 mesh) ion-exchange resins, Bio-Gel P-4 (200–400 mesh) were from Bio-Rad Laboratories (Richmond, CA, USA); HPLC was performed with a Spectroflow 400 solvent-delivery system equipped with a Spectroflow 783 detector (Kratos, Ramsey, NY, USA); the Lichrosorb-NH_2 column was from Merck (Darmstadt, FRG); Ultrasphere ODS was from Beckman (Berkeley, CA, USA); HPLC solvents were from Carlo Erba (Milano, Italy).

Collection and dilution of mucus

Human sputum (2100 ml) was collected everyday from a patient with blood-group O suffering from bronchiectasis due to Kartagener's syndrome. It was kept frozen until use. These bronchial secretions were thawed at 4 °C, diluted 1:12 with deionized water and stirred overnight at 4 °C. The diluted mucus was then centrifuged at 3000 × *g* for 30 min.

Pronase digestion of lyophilized mucus supernatant and fractionation by chromatography on Sepharose CL-2B

Mucin glycopeptides (fraction P2) were obtained as described previously [9].

Purification of oligosaccharides

Alkaline borohydride reductive degradation of 3 g bronchial glycopeptides (fraction P2) was performed according to [9] and led to an heterogeneous population of glycopeptides and reduced oligosaccharides. This mixture was fractionated by ion-exchange chromatography and by gel filtration on Bio-Gel P4 as reported [12], yielding a pool of medium-size neutral oligosaccharide-alditols Ib.

Fractionation of neutral oligosaccharide-alditols of fraction Ib was carried out by HPLC on a Lichrosorb NH_2 column (25 × 0.46 cm internal diameter; particle size 5 μm) (Fig. 2). Elution was performed with a linear gradient of 65/35 to 53/47 (by vol.) acetonitrile/water, during 90 min at room temperature and at a flow rate of 1 ml/min. Further separation was realized by reverse-phase chromatograhy on an Ultrasphere ODS column (25 × 0.46 cm; particle size 5 μm); elution was performed with water during 30 min, followed by a linear gradient of 0/100 to 5/95 (by vol.) acetonitrile/water, during 60 min at a flow rate of 1 ml/min at room temperature. Oligosaccharides were detected by absorption at 206 nm.

Quantitative sugar analysis

Quantitative sugar analysis was performed according to [13].

Analysis of permethylated oligosaccharide-alditols

The oligosaccharide-alditols were permethylated according to [14] with methyl iodide, solid NaOH and methylsulfoxide. The permethylated oligosaccharide-alditols were analyzed by fast-atom-bombardment mass spectrometry (FAB-MS).

A Kratos concept EBEB high-resolution mass spectrometer (Kratos Analytical Instrument, Urmstom, Manchester, UK) equipped with a DS 90 (DGDG/30) data system was used. The mass spectrometer was operated at 8-keV accelerating potential. An Ion Tech model B 11 NF saddle field fast atom source energized with the B 50 current regulated power supply, was used with xenon as the bombarding atom (operating conditions: 7.3 kV, 1.2 mA). The mass range 2000–200 Da was scanned at 10 s/decade. The permethylated oligosaccharide-alditols were dissolved in methanol and loaded on the copper tip with thioglycerol as matrix.

The partially methylated and acetylated methyl glycosides were identified by GC/MS according to [15]. Combined GC/MS was carried out on a capillary column CP-SIL 5CB (50 m × 0.32 mm internal diameter) with a temperature program (150 °C to 280 °C, 5 °C/min) and a Riber Mag 10-10 mass spectrometer. The ionizing potential was 70 eV and the mass range 40–950 Da. The monosaccharides were identified by comparison of their relative retention times and their mass spectra with those of authentic standards.

^{1}H-NMR spectroscopy

Prior to ^{1}H-NMR spectroscopic analysis, the HPLC-fractionated neutral oligosaccharide-alditols were repeatedly treated with ^2H_2O at room temperature. After each exchange treatment, the materials were lyophilized. Finally each sample was redissolved in 0.4 ml ^2H_2O (99.96 atom % ^{2}H, Aldrich). 500-MHz ^{1}H-NMR spectroscopy was performed on a Bruker AM-500 spectrometer (Department of NMR Spectroscopy, University of Utrecht), and 600-MHz ^{1}H-NMR spectroscopy on a Bruker AM-600 spectrometer (Department of Biophysical Chemistry, Nijmegen University, The Netherlands) each operating under control of an Aspect 3000 computer. Resolution enhancement of the spectra was achieved by Lorentzian-to-Gaussian transformation. The probe temperature was kept at 27.0 (± 0.1) °C unless stated otherwise. Chemical shifts (δ) are expressed downfield from internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate, but were actually measured by reference to internal acetone (δ = 2.225 ppm in ^2H_2O at all temperatures), with an accuracy of 0.002 ppm.

Two-dimensional homonuclear Hartman-Hahn (2D-HOHAHA) spinlock experiments [16–18] were performed using the pulse sequence 90°-t_1-SL-acq, where SL stands for a multiple of the MLEV-17 sequence [16] preceded and followed by a 2-ms spinlock pulse with constant phase. The spinlock field-strength corresponded to a 90° pulse-width of 25 ms and the total spinlock mixing time was chosen between 80–120 ms.

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.
3.8	1.6	1.7	3.3	1	14.40	69
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
4.5	1.7	2.1	3.1	1	10.03	101
4.8	1.6	2.9	1.7	1	44.20	48
4.9	1.2	3.2	2.7	1	46.53	41
5.5	n.d.	n.d.	n.d.	n.d.	12.92	n.d.
5.7	n.d.	n.d.	n.d.	n.d.	24.00	n.d.
5.8	n.d.	n.d.	n.d.	n.d.	29.73	n.d.
6.3	n.d.	n.d.	n.d.	n.d.	5.03	n.d.
6.5	2.6	2.3	2.9	1	8.21	152
6.6	0.2	4.1	3.3	1	9.48	289
6.8	1.7	2.8	2.6	1	15.91	183
6.10	2.9	3.2	2.4	1	21.21	41
6.11	2.4	3.4	2.6	1	23.70	71
6.13	2.5	2.7	2.0	1	27.98	105
6.14	2.6	2.8	2.3	1	40.54	102
6.15	1.6	2.6	2.7	1	45.33	57
6.17	2.3	3.0	3.1	1	50.05	57
6.18	3.6	3.2	2.0	1	51.11	97

Table 2. *Methyl-glycosides present in the methanolysates of the permethylated oligosaccharide fractions*

Monosaccharide methylethers	Present in oligosaccharide fractions					
	4.9	4.5	6.5	6.8	6.13	3.8
2,3,4-Me_3Fuc	+	+	+	+	+	+
2,3,4,6-Me_4Gal	+		±	+		+
2,4,6-Me3Gal	+	+		+	+	
3,4,6-Me_3Gal	+	+	+	+	+	
4,6-Me_2Gal			+			+
3,4,6-Me_3GlcNAc(Me)		+	+			+
3,6-Me_2GlcNAc(Me)	+	+	+	+	+	+
4,6-Me_2GlcNAc(Me)	+					
6-MeGlcNAc(Me)		+	+	+	+	+
1,4,5-Me_3GalNAc(Me)-ol + 1,4,5-Me_3-3,6anhydroGalNAc(Me)-ol	+	+	+	+	+	+

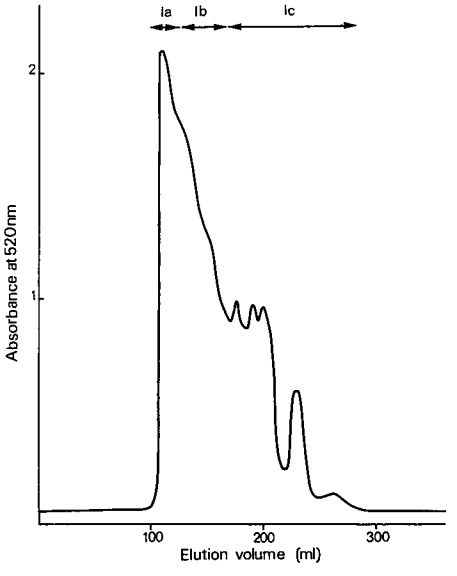


Fig. 1. *Bio-Gel P-4 (200–400 mesh) elution profile of fraction I obtained after reductive alkaline treatment of fraction P2.* The column (2 × 98 cm) was eluted with 0.1 M acetic acid. Aliquots were analyzed for neutral sugar

RESULTS

Isolation and purification of neutral oligosaccharide-alditols

Bronchial mucus glycopeptides (fraction P2, cf. [9]) were prepared from the sputum of a patient with Kartagener's syndrome. Fraction P2 (3 g) was submitted to alkaline-borohydride degradation and then separated into four fractions (I–IV) [9]. Fraction I, which was eluted from Dowex AG1X2 with 0.5 M acetic acid, was subfractionated into Ia, Ib and Ic by chromatography on Bio-Gel P4 (Fig. 1). Fraction Ib (191 mg) contained medium-size neutral oligosaccharide-alditols. HPLC on Lichrosorb-NH_2 was employed to fractionate Ib into 13 fractions, denoted 1–13 (Fig. 2). Fractions 3, 4, 5 and 6 were subfractionated on a reverse phase column and 46 fractions were obtained by this procedure, 3.1–3.11, 4.1–4.9, 5.1–5.8, 6.1–6.18 (Fig. 3A, 3B, 3C, 3D), respectively.

Structure determination

The molar carbohydrate compositions of the oligosaccharide-alditol fractions, obtained after HPLC separation of fraction Ib, were determined by sugar analysis. Table 1 lists the carbohydrate compositions of only those fractions of which the primary structure(s) has been deduced from mass spectrometry and ^{1}H-NMR spectroscopy. The amount of material of fractions 3.1, 5.5, 5.7, 5.8 and 6.3 were too low for quantitative sugar analysis.

500-MHz ^{1}H-NMR spectra of the 47 fractions obtained by HPLC have been recorded; 15 contained too low amounts of material to permit NMR analysis. Spectra of the fractions 3.2, 3.4, 3.5, 4.6, 4.7, 6.7, 6.9, 6.12 and 6.16 indicated the presence of carbohydrate material, but the chemical shifts of the structural reporters groups could not be interpreted in terms of primary structures, since these fractions were too heterogeneous. For the remaining fractions FAB-MS and methylation analysis were used to confirm the NMR data if the amount of material was sufficient. The order wherein the fractions are discussed is based on common structural elements, as determined by ^{1}H-NMR spectroscopy.

Structures that possess the Fucα(1→2)Galβ(1→3)GlcNAcβ(1→3) Galβ(1→4)GlcNAcβ(1→6)GalNAc-ol common element

Five of the HPLC-purified oligosaccharide-alditol fractions contain Fucα(1→2)Galβ(1→3)GlcNAcβ(1→3)-

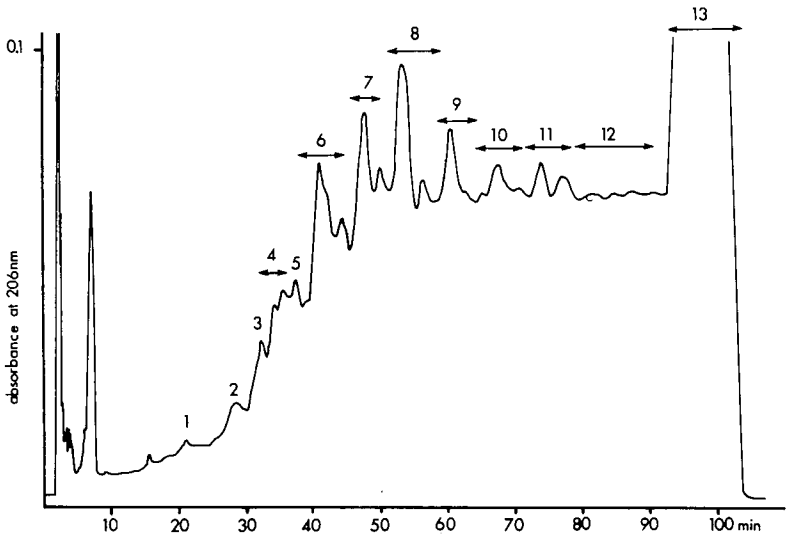


Fig. 2. *HPLC elution profile of bronc●al oligosaccharide-alditols of fraction Ib on a 5-μm Lichrosorb-NH_2 column.* The column was eluted with an acetonitrile/water gradient (65/35 to 53/47, by vol.)

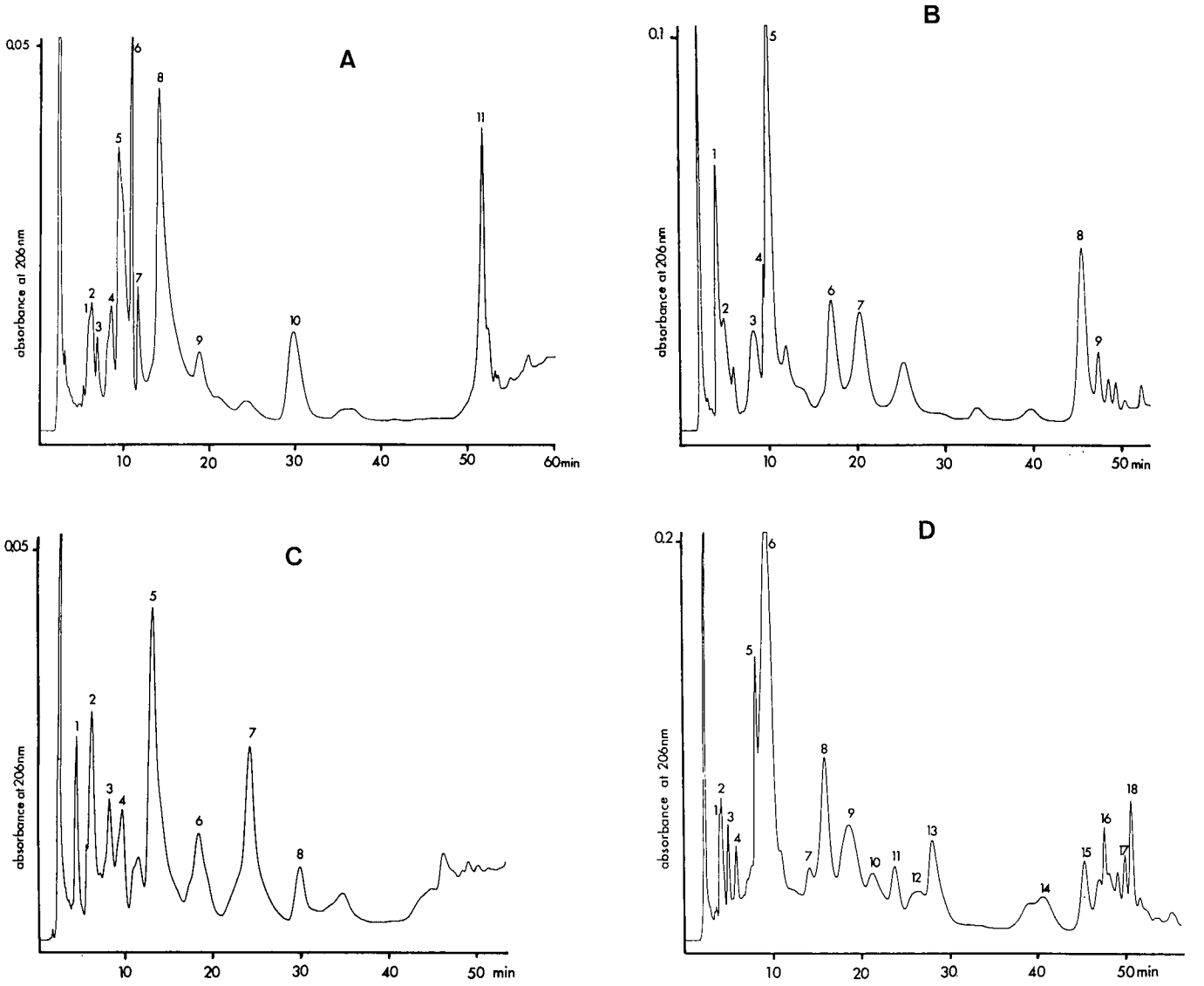


Fig. 3. *Subfractionation of fractions 3 (A), 4 (B), 5 (C) and 6 (D) by a second HPLC run on an Ultrasphere ODS column.* The column was eluted with water and, after 30 min, by a gradient of acetonitrile/water (0/100 to 5/95, by vol.)

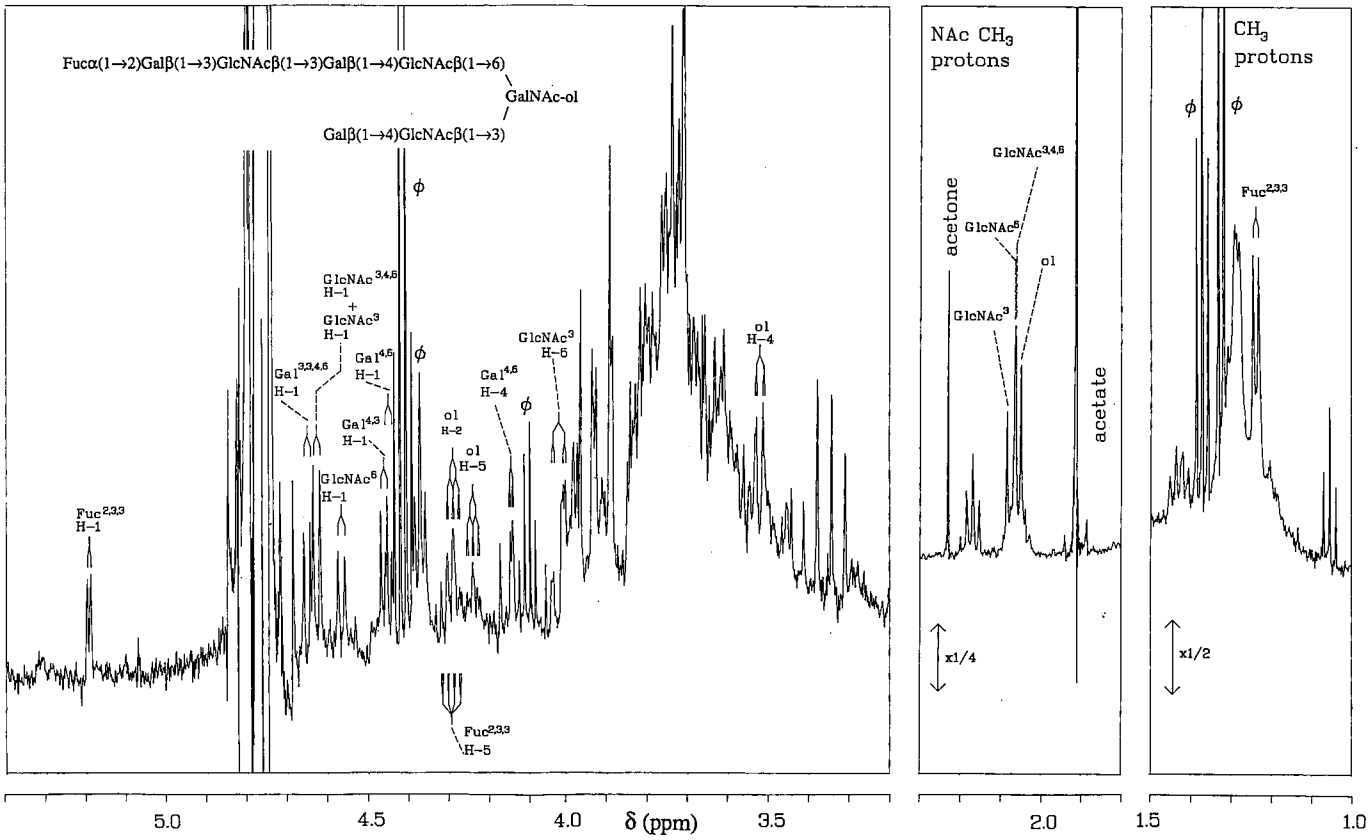


Fig. 4. *Resolution-enhanced 500-MHz spectrum (^2H_2O, 27°C) of fraction 4.9, obtained from the pool of neutral oligosaccharide-alditols Ib from the sputum of a patient with Kartagener's syndrome.* The relative intensity scale of the *N*-acetyl and Fuc methyl proton region of the spectrum differs from that of the other parts, as indicated. Signals marked by ϕ stem from a frequently occurring, non-protein non-carbohydrate contaminant

Galβ(1→4)GlcNAcβ(1→6)GalNAc-ol as common element. The structures of these compounds are listed in Scheme 1, the results of the methylation analyses are depicted in Table 2 and the ^{1}H-NMR structural-reporter groups are compiled in Table 3. The interpretation of the FAB-MS data is carried out guided by the sugar analysis data.

Fraction 4.9. FAB-MS analysis of the permethylated fraction 4.9 shows the presence of a high intensity ion $(M+Na)^+$ which is observed at *m/z* 1853. Altogether with the chemical composition (Table 1), this indicates an octasaccharide constituted of GalNAc-ol, Gal, GlcNAc, Fuc in a ratio of 1:3:3:1. Some low-intensity ions are also present in the FAB-MS spectrum and indicate the presence of contaminants. The data of the methylation analysis are compiled in Table 2. The substitution of *N*-acetylgalactosaminitol at C-3 and C-6 was confirmed by the presence of tri-*O*-methyl anhydrogalactosaminitol [19]. The ^{1}H-NMR spectrum of this fraction (Fig. 4) reveals the signals of a major carbohydrate chain together with other signals, belonging to low amounts of contamination (marked with ϕ in the spectrum). The major component of fraction 4.9 contains →4)GlcNAcβ(1→6)[Galβ(1→4)-GlcNAcβ(1→3)]GalNAc-ol, which can be deduced by comparison of the ^{1}H-NMR chemical shifts of this component with the corresponding chemical shifts of compound **15b** described in [10]. GlcNAc^6 is extended with Fucα(1→2)Galβ(1→3)GlcNAcβ(1→3)Galβ(1→4). This follows from the similarity of the ^{1}H-NMR chemical shifts of the Fucα(1→2)-Galβ(1→3)GlcNAcβ(1→3)Galβ(1→4)GlcNAcβ(1→6) element observed for the major component of fraction 4.9 and compound **A5** reported in [20]. This results point to a new structure, which is given in Scheme 1.

Fraction 4.5. FAB-MS analysis of the permethylated fraction 4.5 shows the presence of one ion $(M+Na)^+$ which is observed at *m/z* 1823. Altogether with the chemical composition (Table 1), this indicates an octasaccharide constituted of GalNAc-ol, Gal, GlcNAc and Fuc in the ratio of 1:2:3:2. The results of the methylation analysis are presented in Table 2. The ^{1}H-NMR spectrum of this fraction (Fig. 5) shows that it contains one oligosaccharide. To obtain more detailed insight into the ^{1}H-NMR features of this fraction, a 2D-HOHAHA experiment was carried out with a spinlock mixing time of 120 ms, yielding a data matrix of 400 × 2K with 80 scans for each experiment. Prior to Fourier transformation the matrix was zerofilled to 2K × 2K (Fig. 6). The relevant ^{1}H-NMR parameters are listed together with those of the 1D experiment in Table 3. Compound 4.5 contains the →4)GlcNAcβ(1→6)[GlcNAcβ(1→3)]GalNAc-ol core, as can be concluded by comparision of the spectra of compound 4.5 and reference compound **10b** in [20]. This core is extended with →3)Galβ(1→4) leading to →3)Galβ(1→4)GlcNAcβ-(1→6)[GlcNAcβ(1→3)]GalNAc-ol (compare the ^{1}H-NMR parameters of compound 4.5 and the major component of fraction 4.9 in Table 2). The presence of a Le^b determinant (Fucα(1→2)Galβ(1→3)[Fucα(1→4)]GlcNAcβ(1→) in compound 4.5 is inferred by comparing the corresponding chemical shifts with those of milk oligosaccharide compound **3** in [21]. These elements together establish a novel structure, which is presented in Scheme 1.

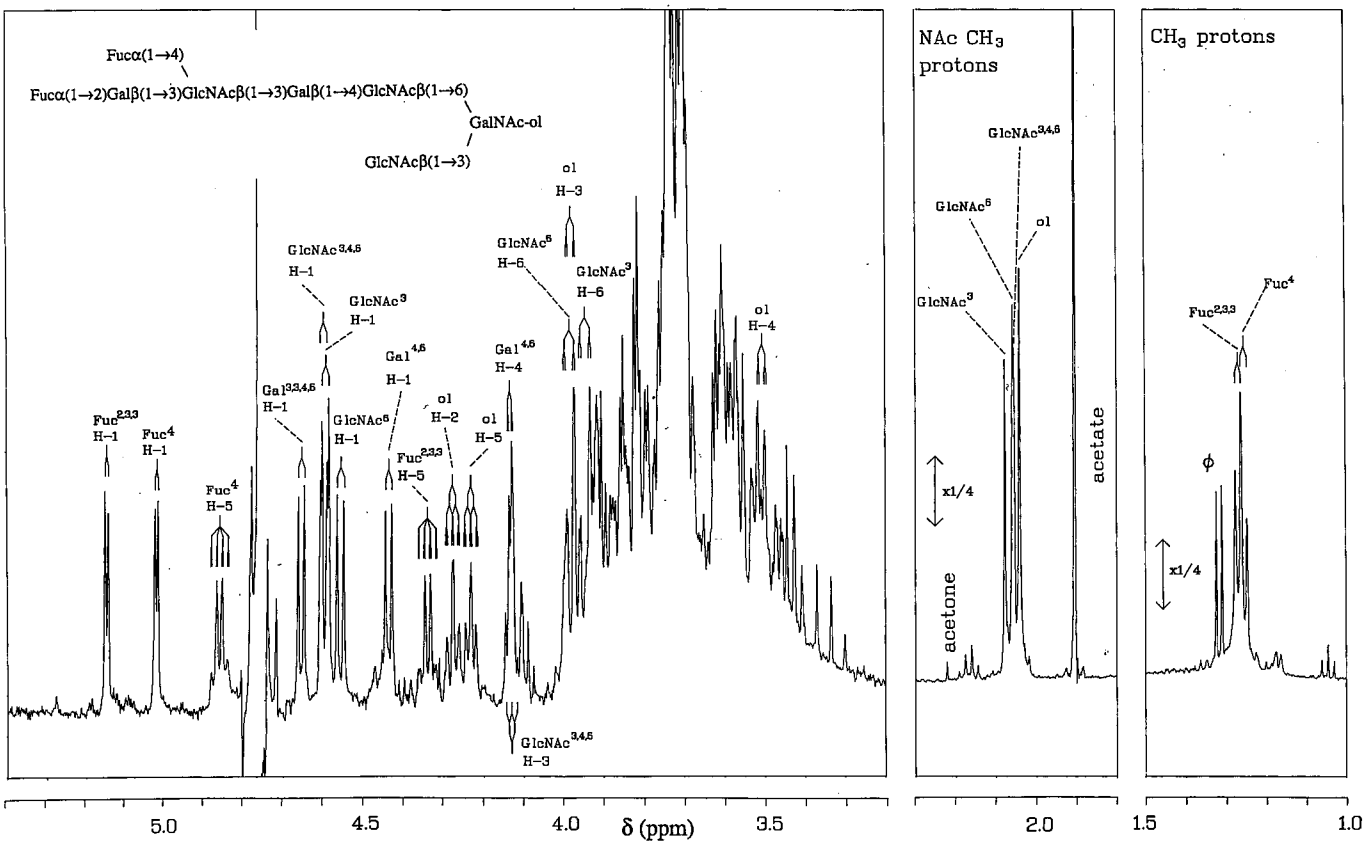


Fig. 5. *Resolution-enhanced 500-MHz spectrum (^2H_2O, 27°C) of fraction 4.5, obtained from the pool of neutral oligosaccharide-alditols Ib from the sputum of a patient with Kartagener's syndrome.* The relative intensity scale of the *N*-acetyl and Fuc methyl proton region of the spectrum differs from that of the other parts, as indicated. Signals marked by ϕ stem from a frequently occurring, non-protein non-carbohydrate contaminant

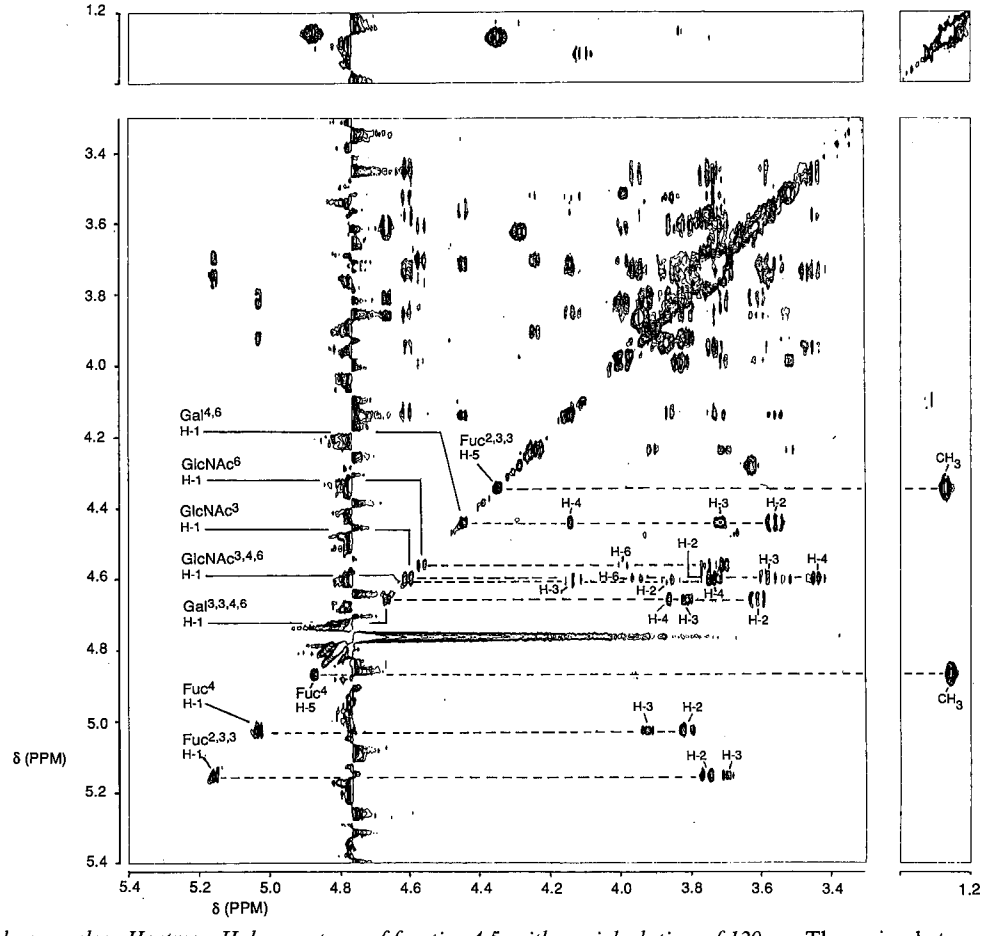


Fig. 6. *500-MHz homonuclear Hartman-Hahn spectrum of fraction 4.5, with a spinlock time of 120 ms.* The region between 3.3 and 1.4 ppm has been omitted

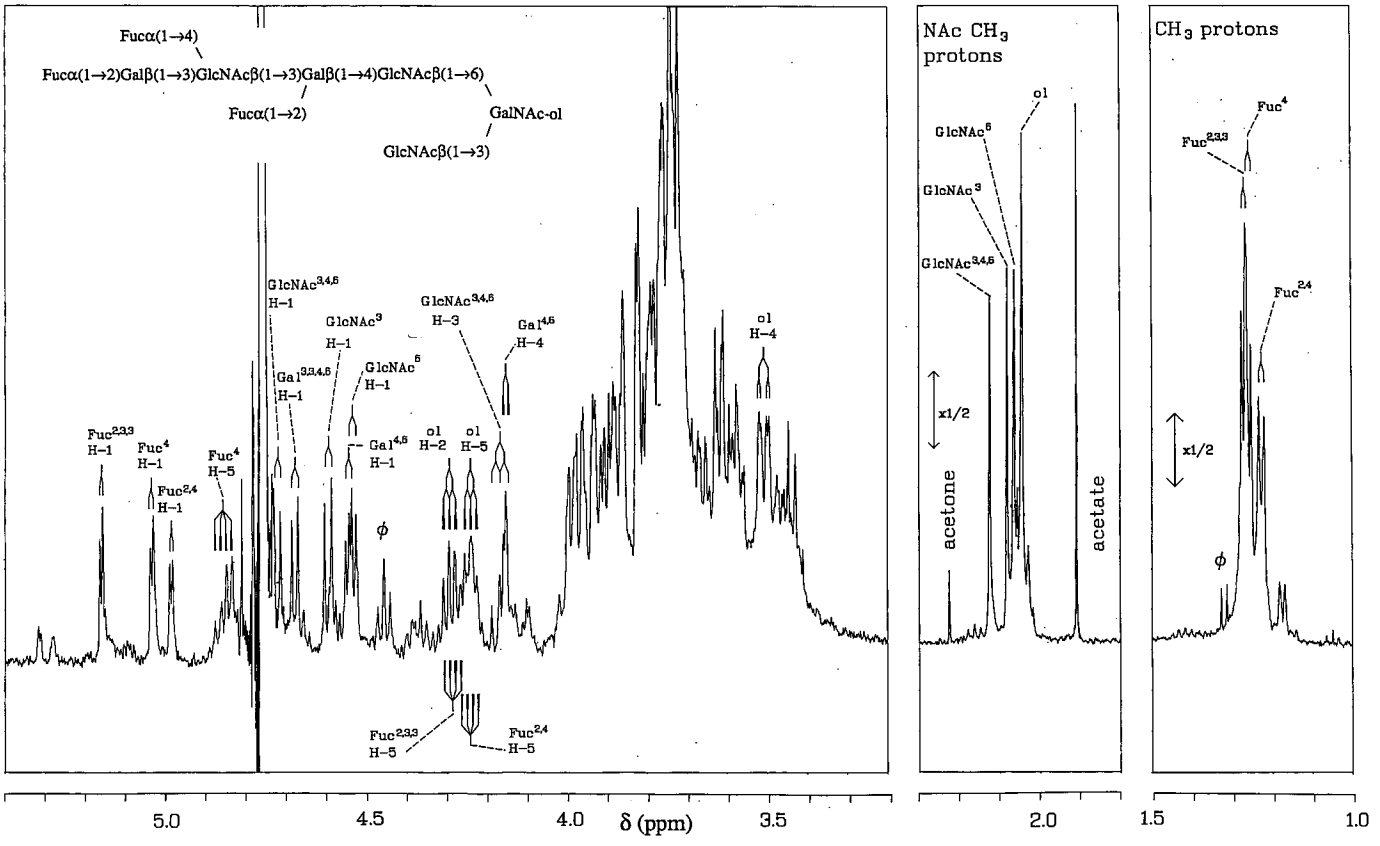


Fig. 7. *Resolution-enhanced 500-MHz spectrum (^2H_2O, 27°C) of fraction 6.5, obtained from the pool of neutral oligosaccharide-alditols Ib from the sputum of a patient with Kartagener's syndrome.* The relative intensity scale of the *N*-acetyl and Fuc methyl proton region of the spectrum differs from that of the other parts, as indicated. Signals marked by ϕ stem from a frequently occurring, non-protein non-carbohydrate contaminant

Fraction 6.5. FAB-MS analysis of the permethylated fraction 6.5 shows the presence of one ion $(M+Na)^+$ which is observed at *m/z* 1997. Altogether with the chemical composition (Table 1), this indicates a nonasaccharide constituted of GalNAc-ol, Gal, GlcNAc and Fuc in the ratio of 1:2:3:3. The methylation analysis is shown in Table 2. The ^{1}H-NMR spectrum (Fig. 7) reveals that this fraction contains mainly one component. The structures of the minor component(s) could not be determined due to the low amount of material. The chemical shifts of the structural-reporter groups of the main component are compiled in Table 3, together with the relevant chemical shifts derived from a 2D-HOHAHA experiment of this compound. The 2D-HOHAHA experiment was carried out at 600 MHz at 283 K with a spinlock mixing time of 80 ms, yielding a data matrix of 160 × 2K with 208 scans for each experiment. The matrix was zerofilled to 2K × 2K prior to Fourier transformation (spectrum not shown). The main component of fraction 6.5 can be conceived as an extension of compound 4.5 with a Fuc residue α(1→2) linked to $Gal^{4,6}$. This is in agreement with the methylation analysis which demonstrated that a Gal residue was substituted at C-2 and C-3. The chemical shifts of the structural-reporter groups of this Fuc residue (H-1, δ = 4.993 ppm; H-5, δ = 4.27 ppm; CH_3, δ = 1.226 ppm) have not been reported before. However, this set, and especially the combination of the H-5 and CH_3 chemical shifts, is typical for Fuc^2 [9, 10]. Owing to this substitution, the signals of H-1, H-2, H-3 and H-4 of $Gal^{4,6}$ and of H-1, H-3, and NAc of $GlcNAc^{3,4,6}$ are all shifted downfield, going from compound 4.5 to the major component of fraction 6.5. These results provide a novel structure listed in Scheme 1.

Fractions 6.8 and 6.13. Since the ^{1}H-NMR spectra of fractions 6.8 and 6.13 are very similar, they are discussed together. The spectrum of fraction 6.8 (Fig. 8) shows the presence of only one component. The spectrum of fraction 6.13 (Fig. 9) reveals that this fraction is a mixture, consisting of one major component (90%) and some minor components of which the structures could not be unraveled, due to the low amounts of material. These fractions both contain the Fucα(1 → 2)Galβ(1 → 3)[Fucα(1 → 4)]GlcNAcβ(1 → 3)Galβ-(1→4)GlcNAcβ(1→6) branch, linked to GalNAc-ol, for which the ^{1}H-NMR features correspond to those of compound 4.5. In addition the spectrum of compound 6.8 contains the structural-reporter group signals of the Galβ(1→4)-GlcNAcβ(1→3) element linked to GalNAc-ol. The characteristic resonances of this element have been described for the major component of fraction 4.9. Combination of the aforementioned structural elements affords a novel structure for component 6.8, which is presented in Scheme 1. The FAB-MS analysis of the permethylated fraction 6.8 [presence of the ion $(M+Na)^+$ at *m/z* 2027] and the methylation analysis data corroborate this new structure. The structure of the major component of fraction 6.13 contains the lower branch Fucα(1→2)Galβ(1→3) linked to GalNAc-ol, which is deduced from the comparison of the ^{1}H-NMR features of the major component of fraction 6.13 with those of reference compound **8d** in [9]. These conclusions, together with the FAB-MS data $[M+Na)^+$ at m/z 1956] and the methylation analysis

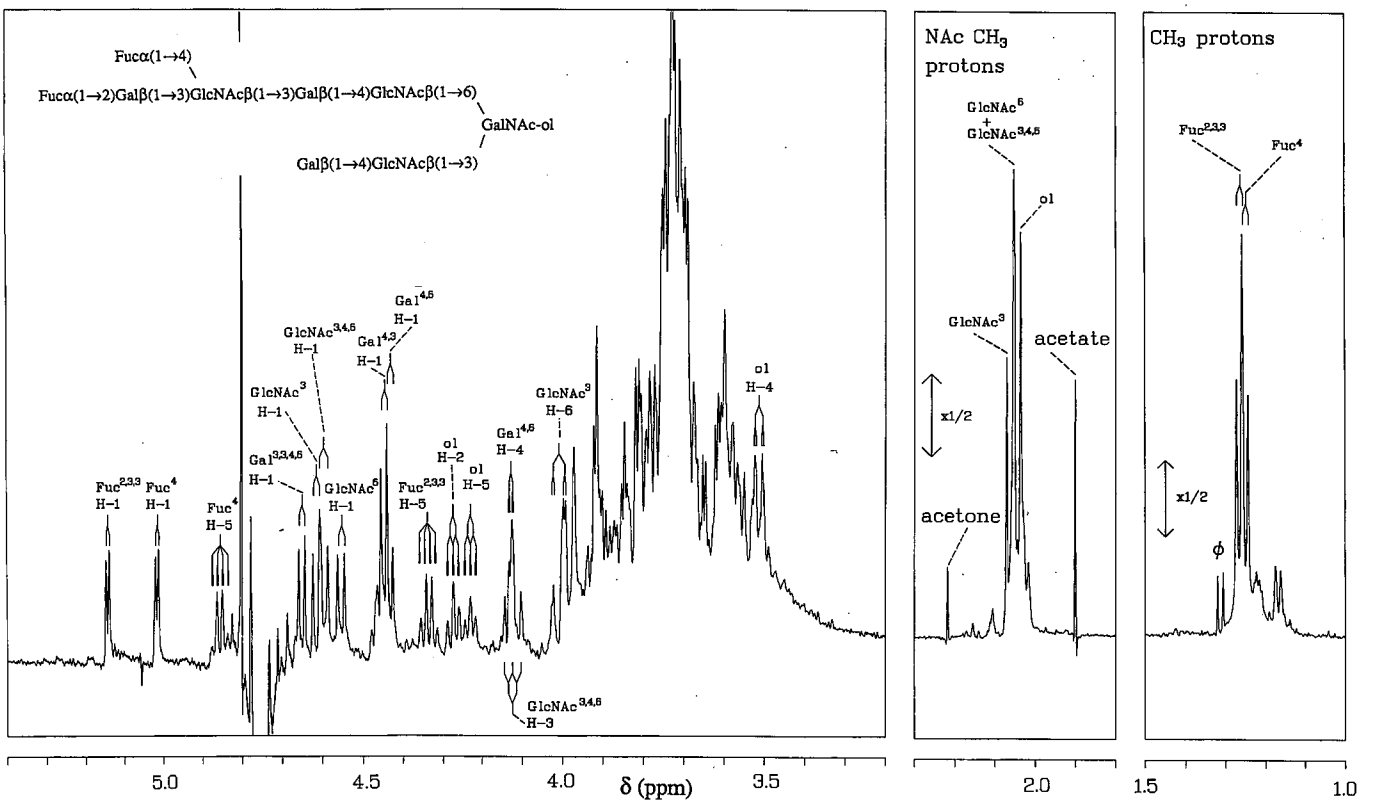


Fig. 8. *Resolution-enhanced 500-MHz spectrum (^2H_2O, 27°C) of fraction 6.8, obtained from the pool of neutral oligosaccharide-alditols Ib from the sputum of a patient with Kartagener's syndrome.* The relative intensity scale of the *N*-acetyl and Fuc methyl proton region of the spectrum differs from that of the other parts, as indicated. Signals marked by ϕ stem from a frequently occurring, non-protein non-carbohydrate contaminant

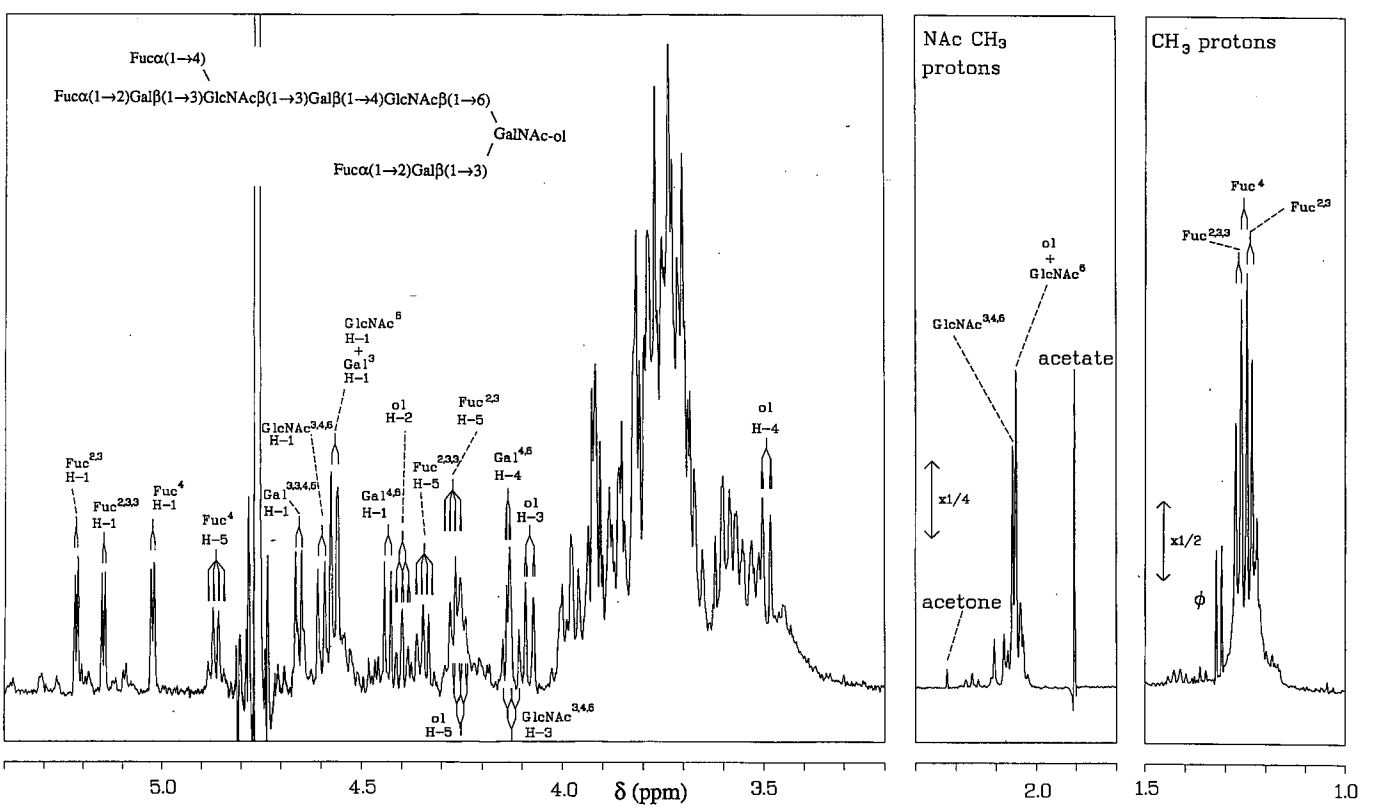


Fig. 9. *Resolution-enhanced 500-MHz spectrum (^2H_2O, 27°C) of fraction 6.13, obtained from the pool of neutral oligosaccharide-alditols Ib from the sputum of a patient with Kartagener's syndrome.* The relative intensity scale of the *N*-acetyl and Fuc methyl proton region of the spectrum differs from that of the other parts, as indicated. Signals marked by ϕ stem from a frequently occurring, non-protein non-carbohydrate contaminant

Table 3. ^{1}*H-chemical shifts of structural-reporter groups of constituent monosaccharides for the HPLC-fractionated oligosaccharide-alditols possessing the Galβ(1→3)GlcNAcβ(1→3)Galβ(1→4)GlcNAcβ(1→6)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. [13]); ◇ = 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 determined 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. [27]). 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				
		4.9 	4.5 	6.5 	6.8 	6.13 
		ppm				
GalNAc-ol	H-2	4.284	4.282	4.296	4.282	4.401
	H-3	n.d.	3.986	n.d.	n.d.	4.083
	H-4	3.518	3.515	3.511	3.522	3.496
	H-5	4.236	4.237	4.245	4.237	4.243
	NAc	2.045	2.045	2.044	2.044	2.054
GlcNAc^6	H-1	4.561	4.561	4.535	4.562	4.570
	H-6	n.d.	4.000	n.d.	n.d.	n.d.
	NAc	2.060	2.062[a]	2.063	2.061	2.054
Gal^{4,6}	H-1	4.444	4.440	4.545	4.441	4.438
	H-2	n.d.	3.55[b]	3.71[c]	n.d.	n.d.
	H-3	n.d.	3.71[b]	3.99[c]	n.d.	n.d.
	H-4	4.139	4.138	4.158	4.137	4.136
GlcNAc^{3,4,6}	H-1	4.623	4.600[d]	4.722	4.605	4.602
	H-2	n.d.	3.84[b]	3.85[c]	n.d.	n.d.
	H-3	n.d.	4.134	4.171	4.134	4.133
	H-4	n.d.	3.72b	3.50[c]	n.d.	n.d.
	NAc	2.058	2.058[a]	2.124	2.061	2.062
Gal^{3,3,4,6}	H-1	4.646	4.659	4.678	4.659	4.658
	H-2	n.d.	3.60[b]	3.65[c]	n.d.	n.d.
	H-3	n.d.	3.81[b]	3.82[c]	n.d.	n.d.
	H-4	n.d.	3.86[b]	3.85[c]	n.d.	n.d.
GlcNAc^3	H-1	4.623	4.598[d]	4.596	4.623	
	H-2	n.d.	3.74[b]	3.76[c]	n.d.	
	H-3	n.d	3.58[b]	3.58[c]	n.d.	
	H-4	n.d.	3.43[b]	3.47[c]	n.d.	
	H-6	4.019	3.948	n.d.	4.018	
	NAc	2.079	2.081	2.081	2.079	
Gal^{4,3}	H-1	4.455			4.455	
Gal^3	H-1					4.570
Fuc^{2,4}	H-1			4.993		
	H-5			4.27[c]		
	CH_3			1.226		
Fuc^{2,3,3}	H-1	5.189	5.152	5.161	5.152	5.151
	H-2	n.d.	3.75[b]	3.73[c]	n.d.	n.d.
	H-3	n.d.	3.69[b]	n.d.	n.d.	n.d.
	H-5	4.291	4.344	4.30[c]	4.343	4.343
	CH_3	1.233	1.273	1.269	1.272	1.272
Fuc^{2,3}	H-1					5.221
	H-5					4.275
	CH_3					1.244
Fuc^4	H-1		5.025	5.035	5.025	5.025
	H-2		3.80[b]	3.82[c]	n.d.	n.d.
	H-3		3.93[b]	n.d.	n.d.	n.d.
	H-5		4.866	4.855	4.866	4.865
	CH_3		1.258	1.259	1.258	1.258

[a] Assignments may have to be interchanged.
[b] From HOHAHA experiment.
[c] From HOHAHA experiment, recorded at 10 °C at 600 MHz.
[d] Assignments may have to be interchanged.

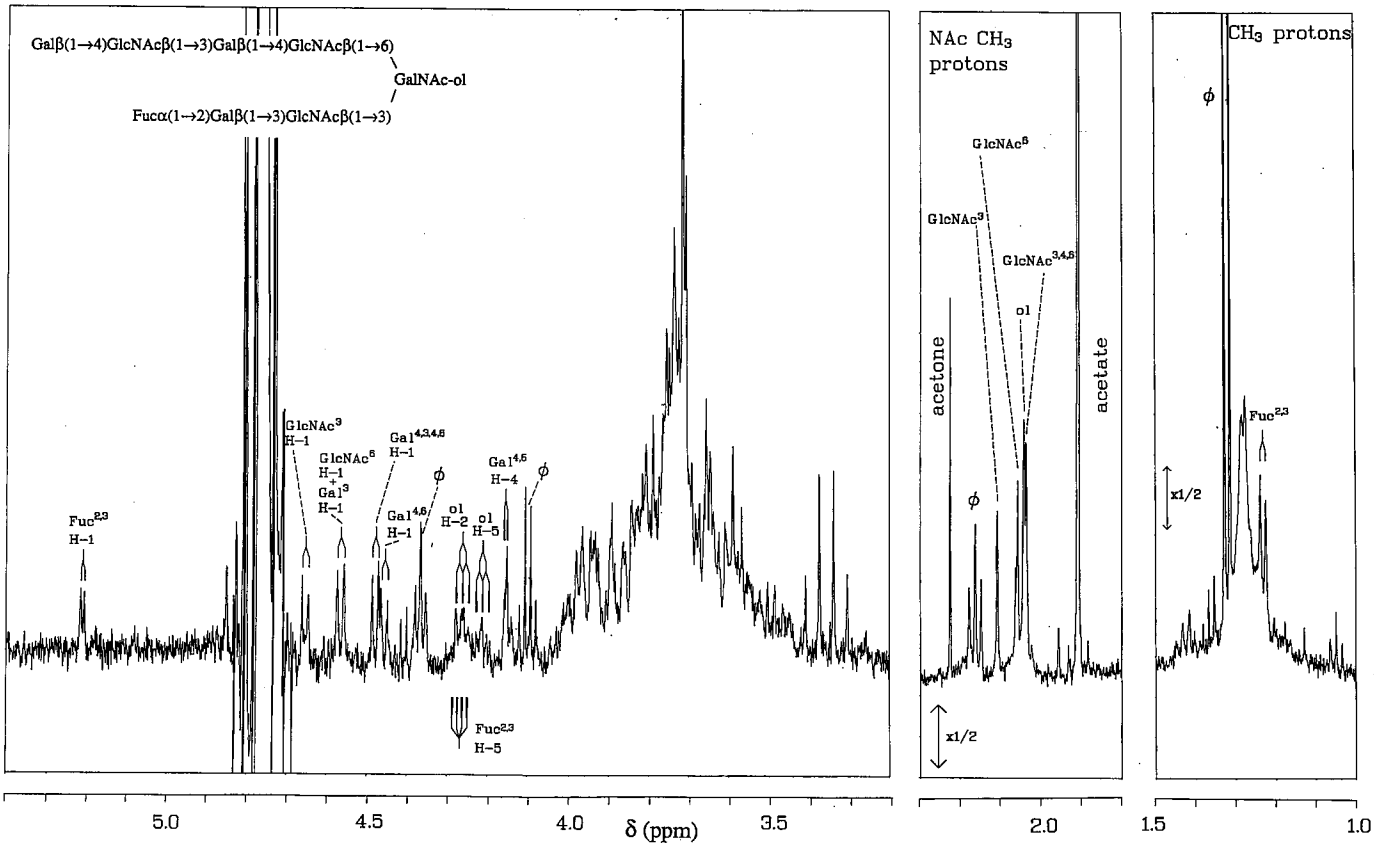


Fig. 10. *Resolution-enhanced 500-MHz spectrum (^2H_2O, 27°C) of fraction 5.8, obtained from the pool of neutral oligosaccharide-alditols Ib from the sputum of a patient with Kartagener's syndrome.* The relative intensity scale of the *N*-acetyl and Fuc methyl proton region of the spectrum differs from that of the other parts, as indicated. Signals marked by ϕ stem from a frequently occurring, non-protein non-carbohydrate contaminant

(Table 2), define for the major component of 6.13 a novel structure (Scheme 1).

Structures that possess the Galβ(1→4)GlcNAcβ(1→3)-Galβ(1→4)GlcNAcβ(1→6)GalNAc-ol common element

Six of the HPLC-purified fractions have the Galβ(1→4)-GlcNAcβ(1→3)Galβ(1→4)GlcNAcβ(1→6)GalNAc-ol chain as common element. The six structures are listed in Scheme 2 and the relevant 500-MHz ^{1}H-NMR data are compiled in Table 4. Methylation analysis data of fraction 3.8 are summarized in Table 2.

Fraction 5.8. According to the ^{1}H-NMR spectrum, fraction 5.8 contains a single component. Comparison of the spectra of compound 5.8 (Fig. 10) and reference compound **9** (Fucα(1→2)Galβ(1→3)GlcNAcβ(1→3)GalNAc-ol [10]) demonstrates the occurrence of this structural element in compound 5.8. This element is extended with the upper branch Galβ(1→4)GlcNAcβ(1→3)Galβ(1→4)GlcNAcβ(1→6), as indicated by the comparison of the ^{1}H-NMR features with those of compound **A-4** from human milk sIgA hinge region [20]. Compound 5.8 has a novel structure which is listed in Scheme 2.

Fraction 6.11. The ^{1}H-NMR spectrum of 6.11 (Fig. 11) proves that, in addition to the main component, at least one minor component is present. Owing to the low amount of material the structure of the latter could not be elucidated. The major component (Fig. 11) can be conceived as an extension of compound 5.8 with a Fuc residue α(1→3) linked to GlcNAc^{3,4,6}. The set of ^{1}H-NMR structural reporter groups of Fuc^3 (H-1, δ = 5.128 ppm; H-5, δ = 4.834 ppm; CH_3, δ = 1.175 ppm) are typical of this linkage (compare these signals with the corresponding signals of compound **14** [9]). The substitution of GlcNAc^{3,4,6} by Fuc is marked by the upfield shifts of the NAc signal ($\Delta\delta$ = −0.012 ppm) and of the H-1 signal of Gal^{4,3,4,6} ($\Delta\delta$ = −0.017 ppm), when compared to the afuco unit (compound 5.8). Similar chemical shift effects are found in the comparison of compound **10a** (Galβ(1→4) GlcNAcβ(1→3)Galβ(1→3)GalNAc-ol) with compound **14** (Galβ(1→4)[Fucα(1→3)]GlcNAcβ(1→3)-Galβ(1→3)GalNAc-ol) [9] ($\Delta\delta$ = −0.010 and −0.020 ppm, respectively). The primary structure of the main component of fraction 6.11 is new and is given in Scheme 2.

Fraction 6.17. The ^{1}H-NMR spectrum of fraction 6.17 (Fig. 12) points to the presence of a mixture containing one major component (80%). The Galβ(1→4) [Fucα(1→3)]-GlcNAcβ(1→3)Galβ(1→4)GlcNAcβ(1→6)GalNAc-ol structural element is present in the major component of fraction 6.17 as can be inferred from the comparison with the major compound of fraction 6.11. In addition, the ^{1}H-NMR parameters of another structural element are found corresponding to Fucα(1→2)Galβ(1→4)GlcNAcβ(1→3) linked to GalNAc-ol. This was evidenced by the similarity of the NMR

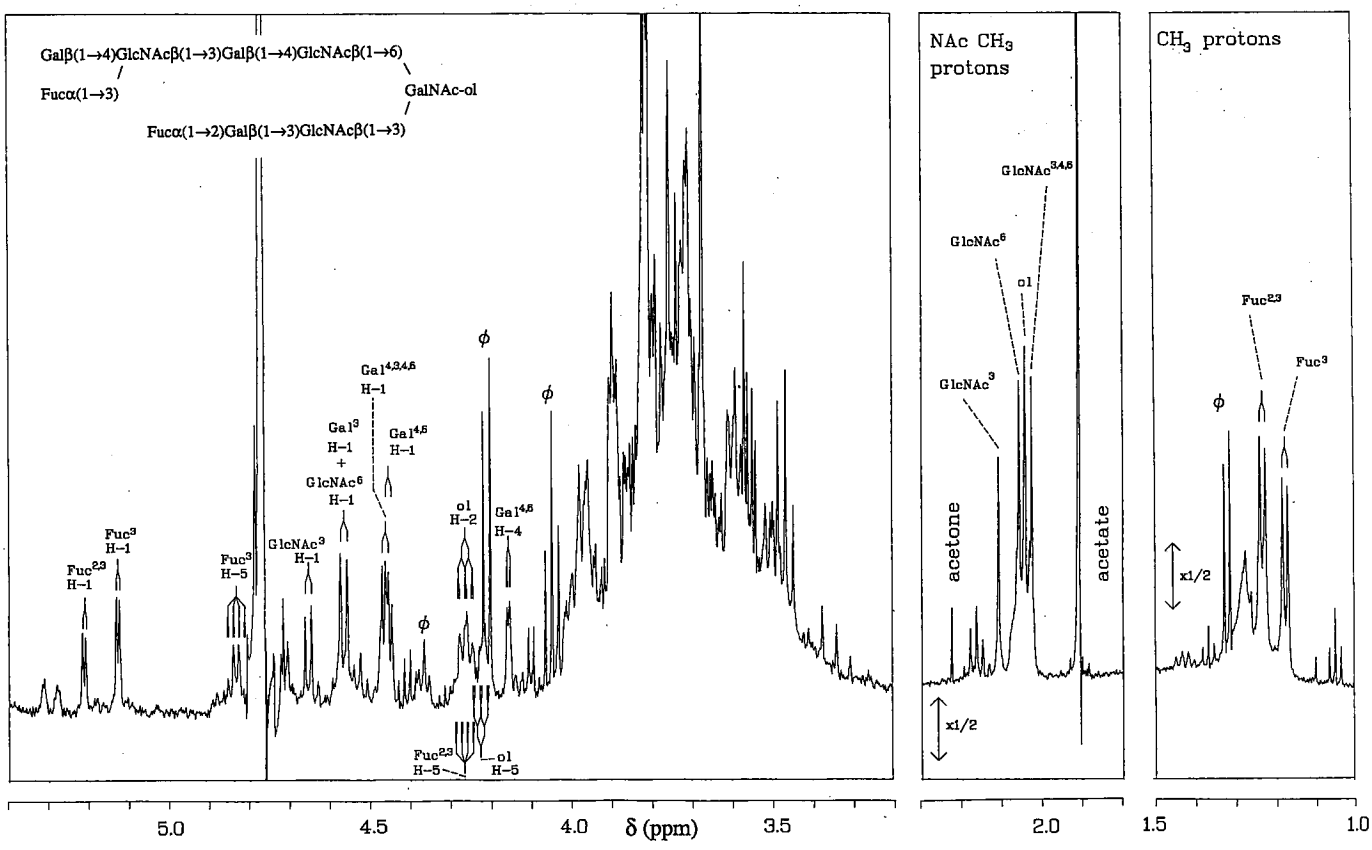


Fig. 11. *Resolution-enhanced 500-MHz spectrum (^2H_2O, 27°C) of fraction 6.11, obtained from the pool of neutral oligosaccharide-alditols Ib from the sputum of a patient with Kartagener's syndrome.* The relative intensity scale of the *N*-acetyl and Fuc methyl proton region of the spectrum differs from that of the other parts, as indicated. Signals marked by ϕ stem from a frequently occurring, non-protein non-carbohydrate contaminant

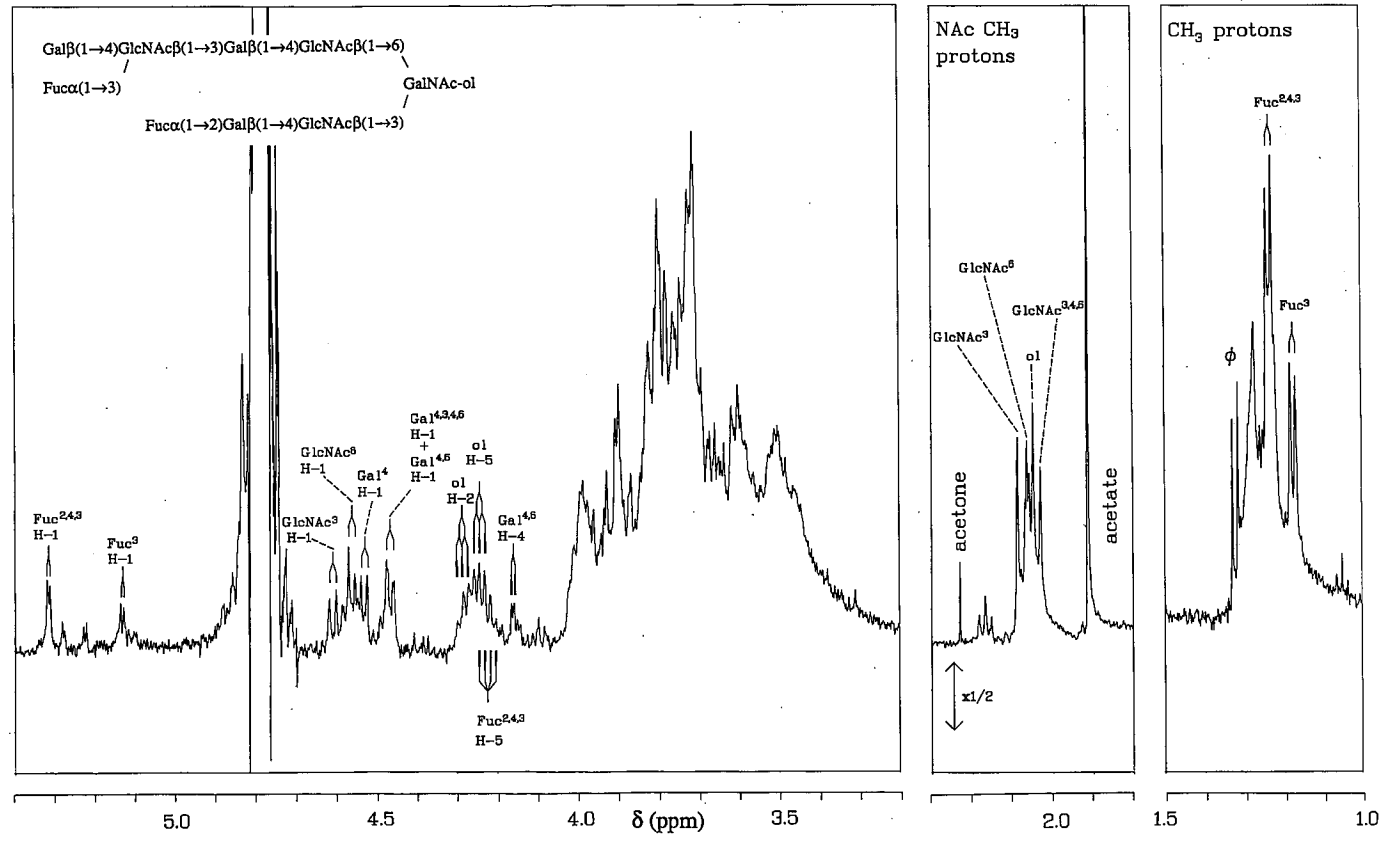


Fig. 12. *Resolution-enhanced 500-MHz spectrum (^2H_2O, 27°C) of fraction 6.17, obtained from the pool of neutral oligosaccharide-alditols Ib from the sputum of a patient with Kartagener's syndrome.* The relative intensity scale of the *N*-acetyl and Fuc methyl proton region of the spectrum differs from that of the other parts, as indicated. Signals marked by ϕ stem from a frequently occurring, non-protein non-carbohydrate contaminant

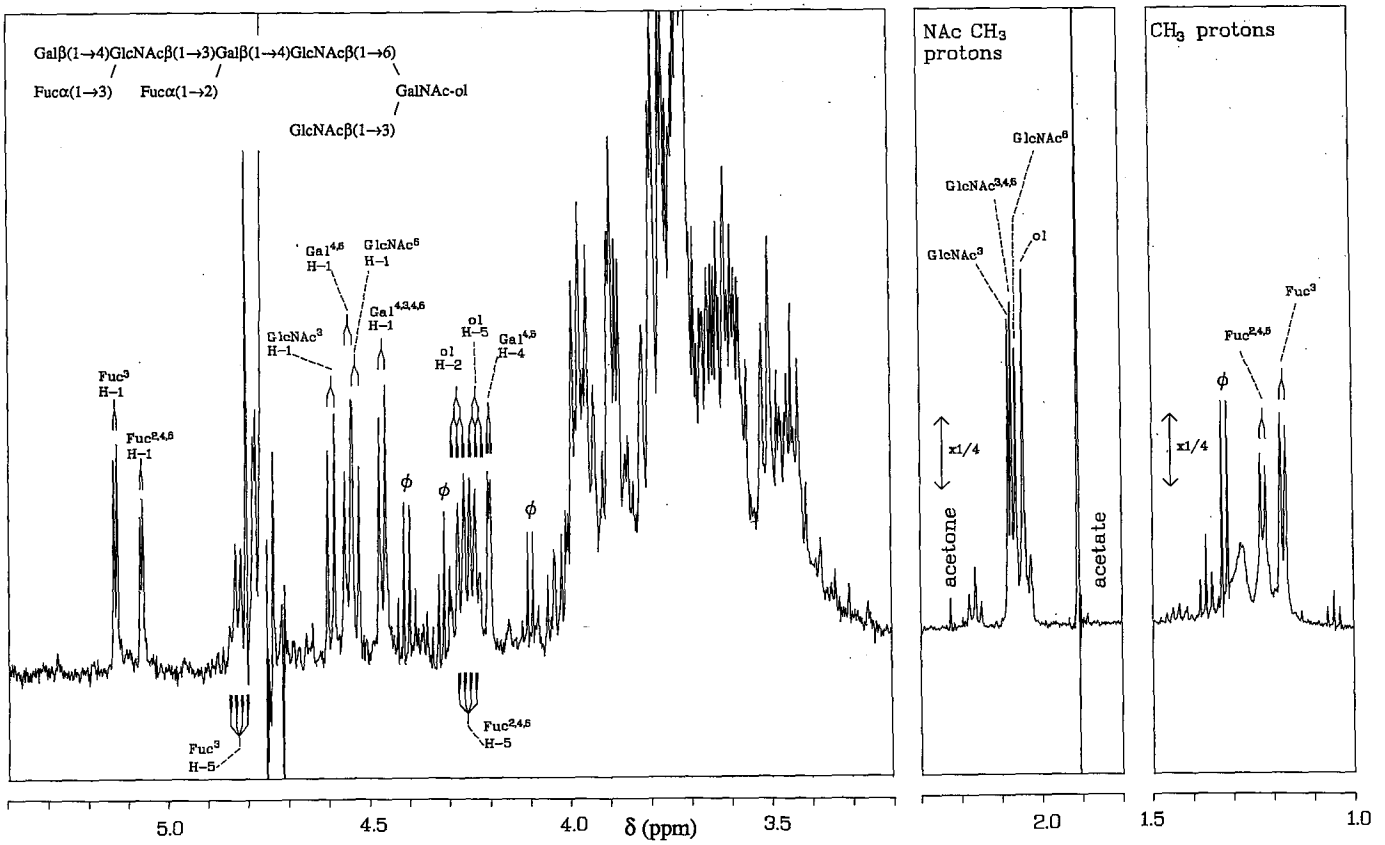


Fig. 13. *Resolution-enhanced 500-MHz spectrum (^2H_2O, 27°C) of fraction 3.8, obtained from the pool of neutral oligosaccharide-alditols Ib from the sputum of a patient with Kartagener's syndrome.* The relative intensity scale of the *N*-acetyl and Fuc methyl proton region of the spectrum differs from that of the other parts, as indicated. Signals marked by ϕ stem from a frequently occurring, non-protein non-carbohydrate contaminant

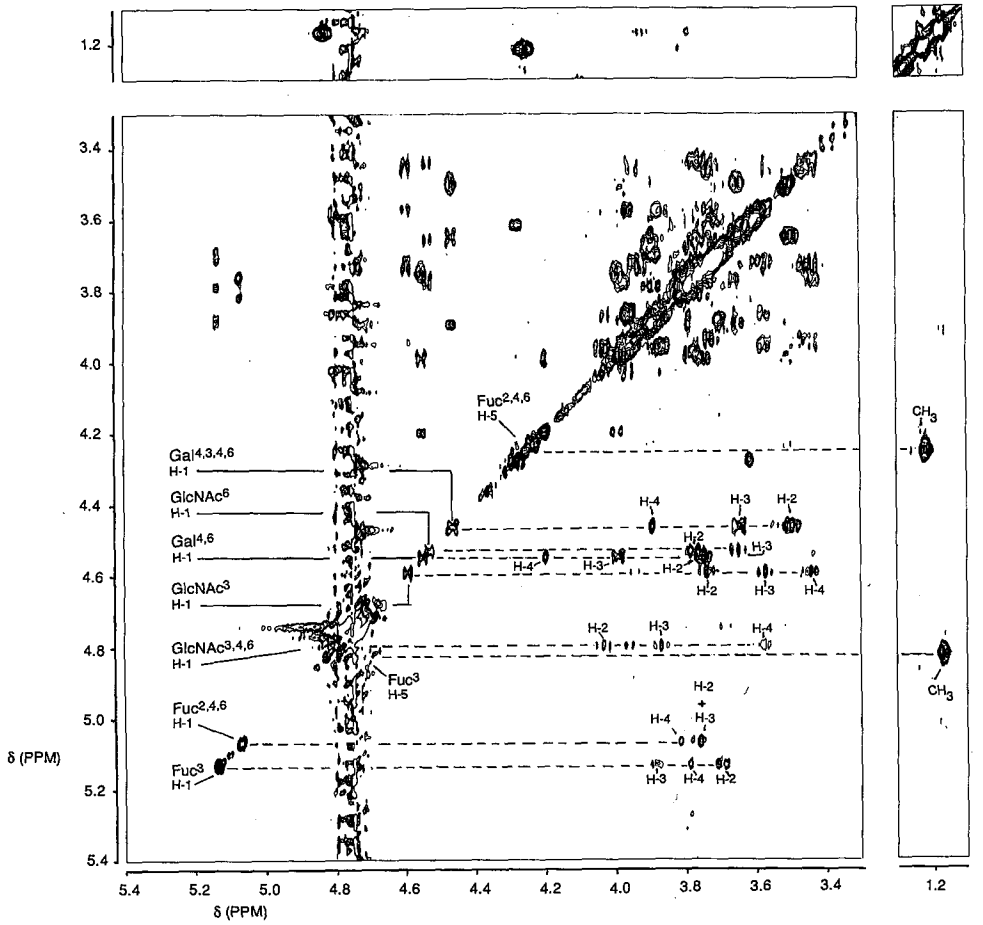


Fig. 14. *500-MHz homonuclear Hartman-Hahn spectrum of fraction 3.8, with a spinlock time of 100 ms.* The region between 3.3 – 1.4 ppm has been left out

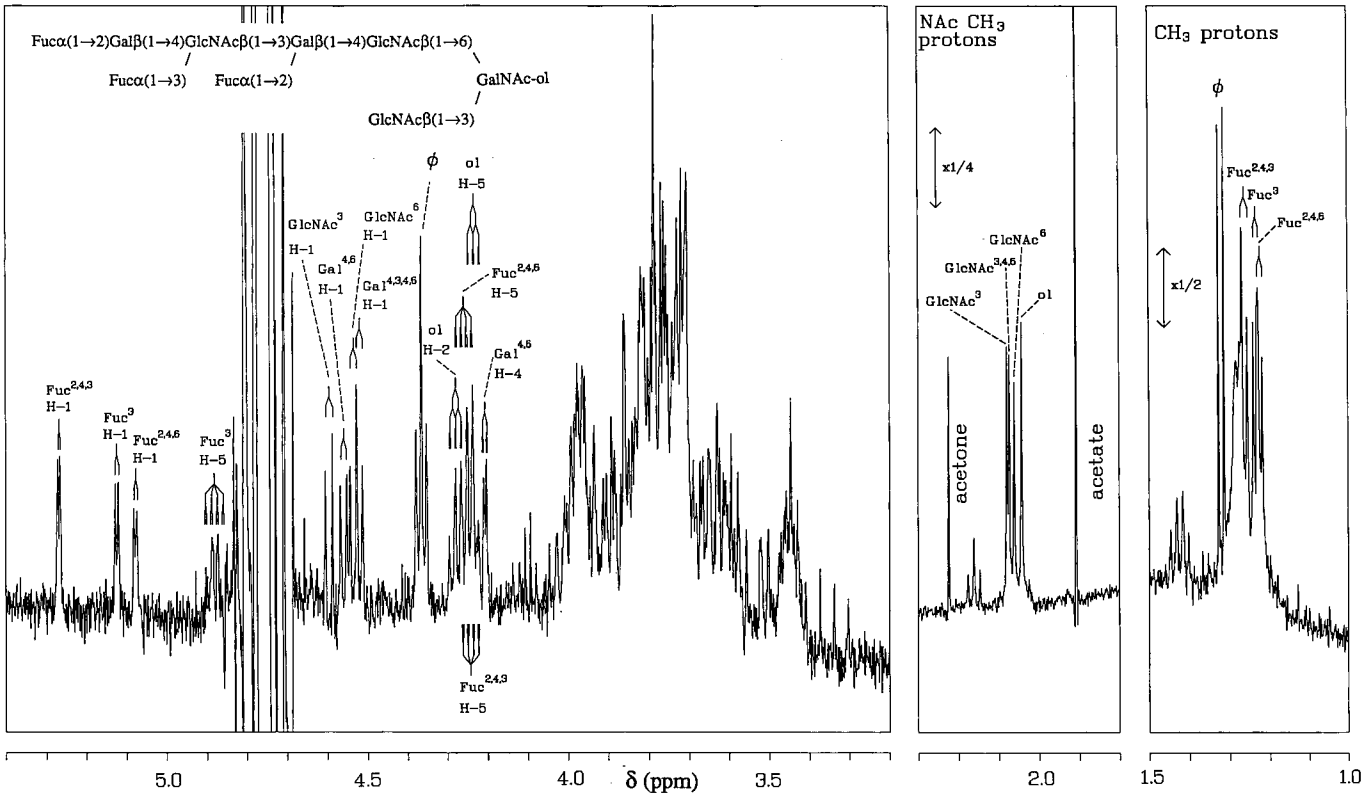


Fig. 15. *Resolution-enhanced 500-MHz spectrum (^2H_2O, 27°C) of fraction 5.7, obtained from the pool of neutral oligosaccharide-alditols Ib from the sputum of a patient with Kartagener's syndrome.* The relative intensity scale of the *N*-acetyl and Fuc methyl proton region of the spectrum differs from that of the other parts, as indicated. Signals marked by ϕ stem from a frequently occurring, non-protein non-carbohydrate contaminant

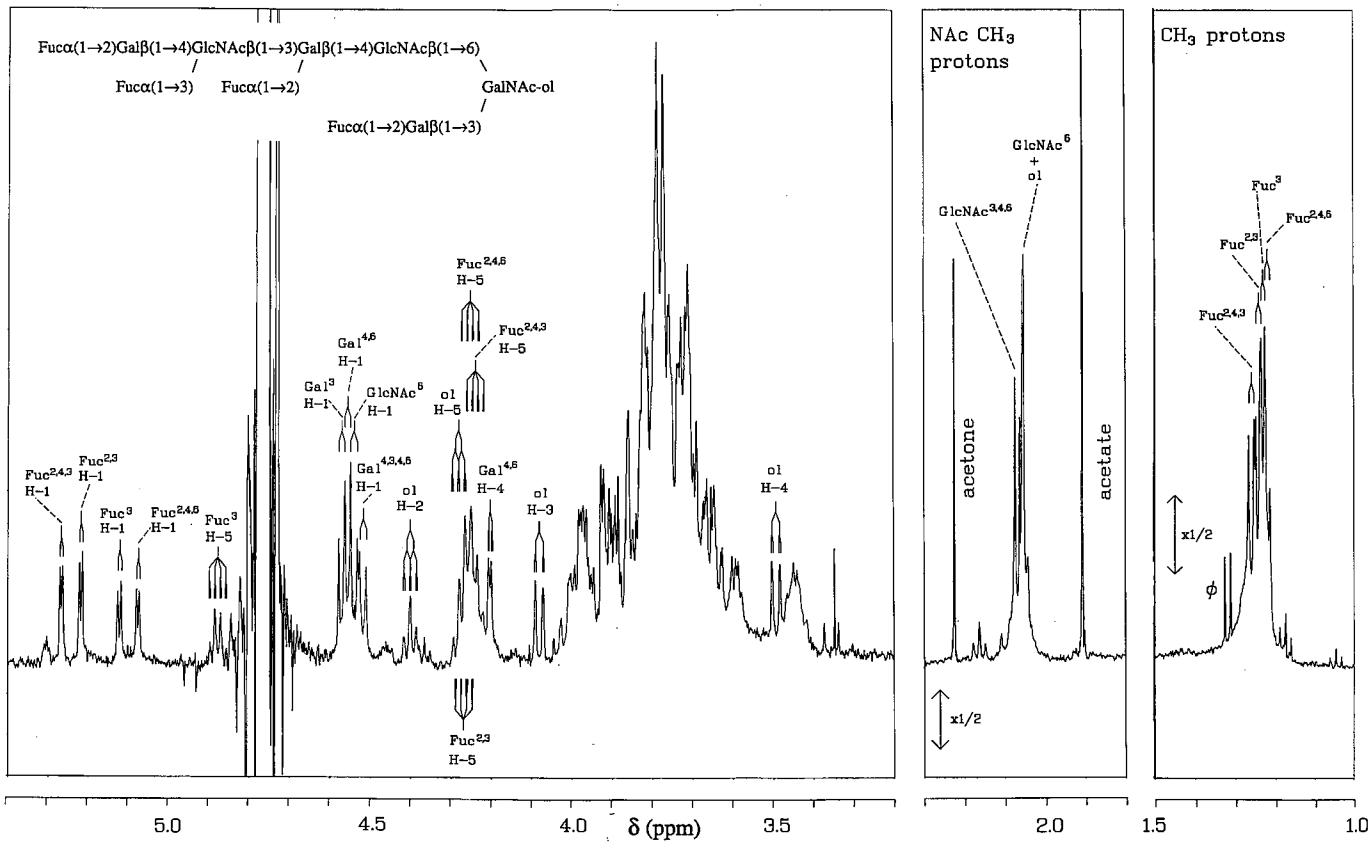


Fig. 16. *Resolution-enhanced 500-MHz spectrum (^2H_2O, 27°C) of fraction 6.18, obtained from the pool of neutral oligosaccharide-alditols Ib from the sputum of a patient with Kartagener's syndrome. The relative intensity scale of the N-*acetyl and Fuc methyl proton region of the spectrum differs from that of the other parts, as indicated. Signals marked by ϕ stem from a frequently occurring, non-protein non-carbohydrate contaminant

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

For explanation of the notation, see Table 3. n.d., value could not be determined 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. [27]). 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					
		5.8 	6.11 	6.17 	3.8 	5.7 	6.18 
		ppm					
GalNAc-ol	H-2	4.259	4.259	4.279	4.279	4.279	4.402
	H-3	n.d.	n.d.	n.d.	n.d.	n.d.	4.082
	H-4	n.d.	n.d.	n.d.	n.d.	n.d.	3.494
	H-5	4.213	4.23	4.23	4.235	4.236	4.26
	NAc	2.042	2.042	2.043	2.043	2.043	2.054
GlcNAc^6	H-1	4.566	4.565	4.556	4.535	4.534	4.541
	H-2	n.d.	n.d.	n.d.	3.77[a]	n.d.	n.d.
	H-3	n.d.	n.d.	n.d.	3.64[a]	n.d.	n.d.
	NAc	2.058	2.057	2.060	2.062	2.062	2.054
Gal^{4,6}	H-1	4.457	4.453	4.461	4.552	4.559	4.557
	H-2	n.d.	n.d.	n.d.	3.76[a]	n.d.	n.d.
	H-3	n.d.	n.d.	n.d.	3.99[a]	n.d.	n.d.
	H-4	4.148	4.151	4.157	4.202	4.206	4.206
GlcNAc^{3,4,6}	H-1	4.695[b]	4.699[b]	4.696[b]	4.802[c]	4.788[b]	4.787[b]
	H-2	n.d.	n.d.	n.d.	4.03[a]	n.d.	n.d.
	H-3	n.d.	n.d.	n.d.	3.87[a]	n.d.	n.d.
	H-4	n.d.	n.d.	n.d.	3.57[a]	n.d.	n.d.
	NAc	2.037	2.025	2.026	2.073	2.074	2.074
Gal^{4,3,4,6}	H-1	4.479	4.462	4.461	4.468	4.519	4.520
	H-2	n.d.	n.d.	n.d.	3.49[a]	n.d.	n.d.
	H-3	n.d.	n.d.	n.d.	3.64[a]	n.d.	n.d.
	H-4	n.d.	n.d.	n.d.	3.89[a]	n.d.	n.d.
GlcNAc^3	H-1	4.654	4.654	4.603	4.595	4.595	
	H-2	n.d.	n.d.	n.d.	3.77[a]	n.d.	
	H-3	n.d.	n.d.	n.d.	3.57[a]	n.d.	
	H-4	n.d.	n.d.	n.d.	3.44[a]	n.d.	
	NAc	2.108	2.108	2.083	2.080	2.080	
Gal^3	H-1	4.566	4.565				4.571
Gal^4	H-1			4.526			
Fuc^{2,4,6}	H-1				5.071	5.077	5.079
	H-2				3.76[a]	n.d.	n.d.
	H-3				3.76[a]	n.d.	n.d.
	H-4				3.81[a]	n.d.	n.d.
	H-5				4.257	4.26	4.26
	CH_3				1.224	1.222	1.221
Fuc^{2,4,3}	H-1			5.312		5.268	5.268
	H-5			4.23		4.25	4.25
	CH_3			1.234		1.261	1.261
Fuc^{2,3}	H-1	5.212	5.212				5.219
	H-5	4.269	4.269				4.27
	CH_3	1.231	1.231				1.243[d]
Fuc^3	H-1		5.128	5.128	5.135	5.123	5.124
	H-2		n.d.	n.d.	3.70[a]	n.d.	n.d.
	H-3		n.d.	n.d.	3.89[a]	n.d.	n.d.
	H-4		n.d.	n.d.	3.78[a]	n.d.	n.d.
	H-5		4.834	4.835[b]	4.831	4.880	4.880
	CH_3		1.175	1.175	1.173	1.234	1.231[d]

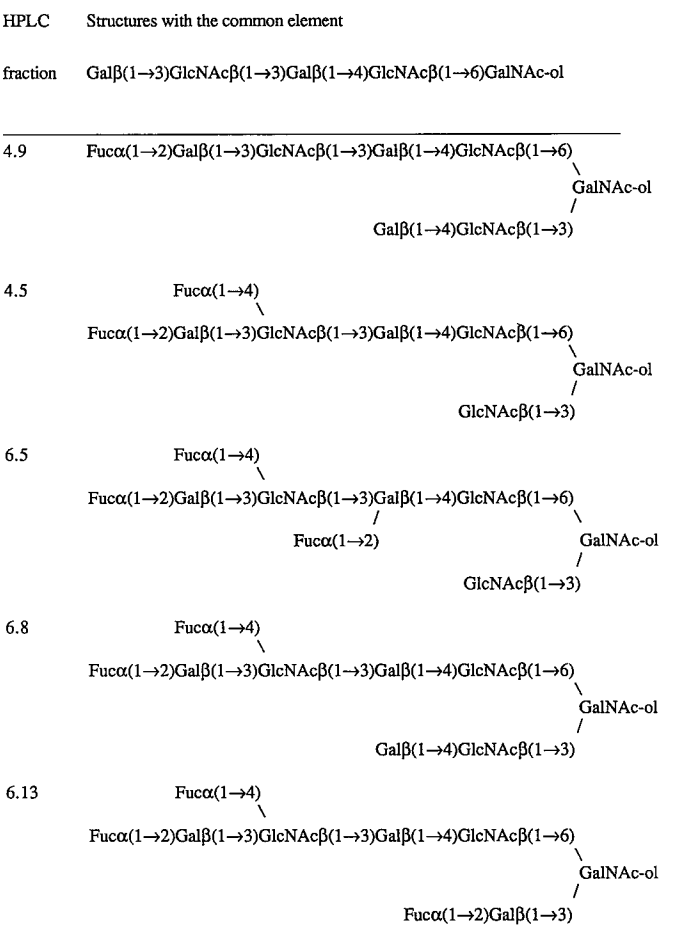
[a] From HOHAHA experiment.
[b] Spectrum recorded at 15°C.
[c] Spectrum recorded at 12°C.
[d] Assignments may have to be interchanged.

parameters of reference compound **18.2** in [10]. The combination of these two elements establish a new structure, which is depicted in Scheme 2.

Fraction 3.8. FAB-MS analysis of the permethylated fraction 3.8 shows the presence of one ion $(M+Na)^+$ which was observed at *m/z* 1823. Altogether with the chemical composition (Table 1), this indicates an octasaccharide constituted of GalNAc-ol, Gal, GlcNAc and Fuc in a ratio of 1:2:3:2. The methylation analysis data are presented in Table 2. The ^{1}H-NMR spectrum of the single component of fraction 3.8 is shown in Fig. 13. To verify and complete the assignments, a 2D-HOHAHA experiment was performed using a spinlock time of 100 ms. A data matrix of 400×2K was obtained with 96 scans for each experiment. The matrix was zero-filled to $2K \times 2K$ prior to Fourier transformation (Fig. 14). The core structure of the compound 3.8, →4)GlcNAcβ(1→6)-[GlcNAcβ(1→3)]GalNAc-ol, was deduced by comparing the ^{1}H-NMR parameters of compound 3.8 with those of compounds 4.5 and 6.5 (Table 2). The upper branch of compound 3.8 can be conceived as an extension of Galβ(1→4)-[Fucα(1→3)]GlcNAcβ(1→3)Galβ(1→4)GlcNAcβ(1→6) found in the major component of fraction 6.17 with a Fuc residue α(1→2) linked to Gal^{4,6}. The Fuc^2 structural-reporter group chemical shifts can be detected at δ = 5.071 ppm (H-1), δ = 4.257 ppm (H-5) and δ = 1.224 ppm (CH_3). The protons H-1, H-2 and H-3 of Gal^{4,6} resonate at essentially the same positions for compound 3.8 and the major component of fraction 6.5 (Table 3). In both compounds, Gal^{4,6} is substituted by Fucα(1→2) and GlcNAcβ(1→3). The linkage of Fuc^2 to Gal^{4,6} is corroborated by the presence of an interglycosidic NOE between Fuc^2 H-1 and H-2 of Gal^{4,6}. Finally, comparison of compound 3.8 with the major components of fractions 6.11 and 6.17, indicates the presence of the Galβ(1→4)[Fucα(1→3)]GlcNAcβ(1→3) element in this compound. The structural-reporter group signals of GlcNAc^{3,4,6} have been shifted downfield (H-1, $\Delta\delta$ = 0.103 ppm; NAc, $\Delta\delta$ = 0.048 ppm) going from the major component of fraction 6.11 to compound 3.8, due to the presence of a neighbouring Fuc residue. Similar shift effects were observed going from compound 4.5 to 6.5 (H-1, $\Delta\delta$ = 0.122 ppm; NAc, $\Delta\delta$ = 0.066 ppm). From these data, the novel structure of component 3.8 can be inferred as described in Scheme 2.

Fraction 5.7. The ^{1}H-NMR spectrum of fraction 5.7 (Fig. 15) demonstrates the occurrence of one component, corresponding to the structure of component 3.8 extended by a Fucα(1→2)-linked to Gal^{4,3,4,6}, completing the Y determinant, Fucα(1→2)Galβ(1→4)[Fucα(1→3)]GlcNAcβ(1→), of which the ^{1}H-NMR chemical shifts have been described [9]. The remaining structural-reporter groups of compound 5.7 resonate essentially at the same positions as those of compound 3.8. These observations establish the structure of component 5.7 as a new structure, which is described in Scheme 2.

Fraction 6.18. This fraction 6.18 contains one compound as can be inferred from the ^{1}H-NMR spectrum (Fig. 16). It shows essentially the same structural reporter group signals for the Fucα(1→2)Galβ(1→4)[Fucα(1→3)]GlcNAcβ(1→3)-[Fucα(1→2)]Galβ(1→4)GlcNAcβ(1→6) upper branch as described for compound 5.7. The NAc signal of GalNAc-ol, however, is shifted downfield to δ = 2.054 ppm in compound 6.18, due to another core type. This core type and the lower branch, can be deduced from the comparison of compounds 6.18 with the major component of fraction 6.13 (Table 3), and is established to be Fucα(1→2)Galβ(1→3)[→6]GalNAc-ol. The resulting novel structure is given in Scheme 2.



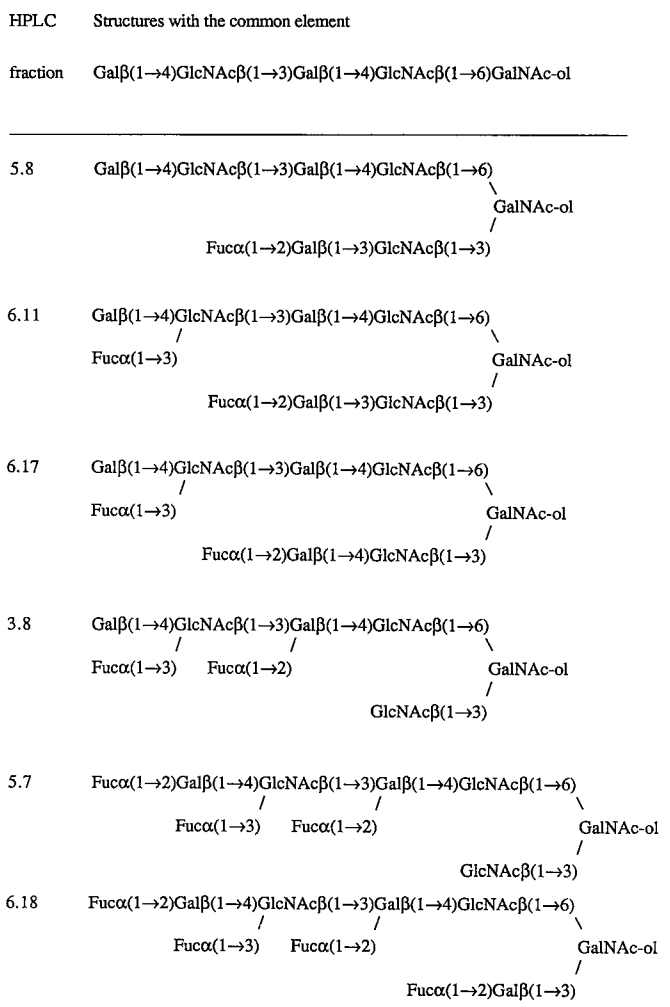
Scheme 1. *Structures of neutral oligosaccharide-alditols with the Galβ(1→3)GlcNAcβ(1→3)Galβ(1→4)GlcNAcβ(1→6)GalNAc-ol common element, obtained by HPLC fractionation of a pool of neutral oligosaccharide-alditols from Kartageners syndrome sputum*

DISCUSSION

In this study 11 oligosaccharide-alditols are characterized stemming from human bronchial mucin glycoproteins of a patient suffering from bronchiectasis. A pronase digest of the respiratory secretion of this patient was treated with alkaline borohydride, and the resulting oligosaccharide-alditols were fractionated by ion-exchange chromatography and gel filtration. The neutral chains were separated by HPLC on normal-phase alkylamine-bonded silica and reverse-phase HPLC into 46 subfractions. These fractions were investigated with high-field ^{1}H-NMR in conjunction with FAB-MS, methylation analysis and sugar analysis.

The ^{1}H-NMR spectral analysis was based on the structural-reporter group concept, wherein the positions and patterns of well defined signals are characteristic for the primary structure of oligosaccharides. Nowadays the results of this method can be corroborated and extended by the information obtained from 2D-HOHAHA experiments, as this technique provides subspectra of the monosaccharide residues in oligosaccharide chains.

The present article deals with structures that possess the Galβ(1 → 3/4)GlcNAcβ(1 → 3)Galβ(1 → 4)GlcNAcβ(1 → 6)-[Xβ(1→3)]GalNAc-ol structural element, where X stands for GlcNAc, Galβ(1→4)GlcNAc or Fucα(1→2)Galβ(1→3/4)-



Scheme 2. *Structures of neutral oligosaccharide-alditols with the Galβ(1→4)GlcNAcβ(1→3)Galβ(1→4)GlcNAcβ(1→6)GalNAc-ol common element, obtained by HPLC fractionation of a pool of neutral oligosaccharide-alditols from Kartageners syndrome sputum*

GlcNAc, which belong to core-type 4 according to [22], or X stands for Fucα(1→2)Gal, which represents core-type 2. The upper branch is characterized by a disaccharide unit of either type 1, Galβ(1→3)GlcNAc, or type 2, Galβ(1→4)GlcNAc. Further differences in this branch are defined by the number and positions of the Fuc residues, resulting in different determinants such as: H, Y, X, Le^b and internal H. The internal H determinant Galβ(1→4)GlcNAcβ(1→3)[Fucα(1→2)]Galβ-(1→4)GlcNAc is novel for oligosaccharides derived from glycoproteins. A variant of the internal H determinant, the internal blood group A glycolipid, has been described [23]. This increases the number of known H determinants [24]. Questions can be raised about the biosynthesis of this determinant. Is the Fuc residue added first to the Gal before elongation of the glycan by addition of a GlcNAc residue, or is the Fuc residue added later on to the backbone of the glycan. It should be noted that the biosynthesis of the H determinant as such is not well understood. So far, two types of α(1→2)fucosyl transferase activities have been described in the submaxillary glands of secretor and non secretor individuals. The secretor α(1→2)fucosyl transferase has a preference for acceptors of the lacto-type 1 chains, whereas the non-secretor transferase prefers lacto-type 2 chains [25]. Two different α(1→2)fucosyl transferases have also been found in human plasma and in milk, respectively [26]. The mode of introduction of the internal Fuc requires further investigation.

This report illustrates the extreme heterogeneity of human bronchial mucin oligosaccharides. For the 11 carbohydrate structures investigated here, the structural diversity is mainly due to the Fuc substitution patterns, which results in a variety of determinants.

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REFERENCES

1. Slayter, H. S., Lamblin, G., Le Treut, A., Galabert, C., Houdret, N., Degand, P. & Roussel, P. (1984) *Eur. J. Biochem. 142*, 209–218.
2. Tabak, L. A., Levine, M. J., Mandell, I. D. & Ellison, S. A. (1982) *J. Oral Pathol. 11*, 1–17.
3. Roussel, P., Lamblin, G., Lhermitte, M., Houdret, N., Lafitte, J. J., Perini, J. M., Klein, A. & Scharfman, A. (1988) *Biochimie 70*, 1471–1482.
4. Vishwanath, S. & Ramphal, R. (1985) *Infect. Immun. 48*, 331–335.
5. Podolsky, D. K. (1985) *J. Biol. Chem. 260*, 8262–8271.
6. Slomiany, B. L., Zdebska, E. & Slomiany, A. (1984) *J. Biol. Chem. 259*, 2863–2869.
7. Dua, V. K., Rao, N., Wu, S. S., Dube, V. E. & Bush, A. (1986) *J. Biol. Chem. 261*, 1599–1608.
8. Lamblin, G., Lhermitte, M., Degand, P. & Roussel, P. (1979) *Biochimie 61*, 23–43.
9. Klein, A., Lamblin, G., Lhermitte, M., Roussel, P., Breg, J., Van Halbeek, H. & Vliegenthart, J. F. G. (1988) *Eur. J. Biochem. 171*, 631–642.
10. Breg, J., Van Halbeek, H., Vliegenthart, J. F. G., Klein, A., Lamblin, G. & Roussel, P. (1988) *Eur. J. Biochem. 171*, 643–654.
11. Van Halbeek, H., Breg, J., Vliegenthart, J. F. G., Klein, A., Lamblin, G. & Roussel, P. (1988) *Eur. J. Biochem. 177*, 443–460.
12. Roussel, P., Lamblin, G., Degand, P., Walker-Nasir, E. & Jeanloz, R. W. (1975) *J. Biol. Chem. 250*, 2114–2122.
13. Lamblin, G., Boersma, A., Klein, A., Roussel, P., Van Halbeek, H. & Vliegenthart, J. F. G. (1984) *J. Biol. Chem. 259*, 9051–9058.
14. Ciucanu, I. & Kerek, F. (1984) *Carbohydr. Res. 131*, 209–217
15. Fournet, B., Strecker, G., Leroy, Y. & Montreuil, J. (1981) *Anal. Biochem. 116*, 489–502.
16. Braunschweiler, L. & Ernst, R. R. (1983) *J. Magn. Reson. 53*, 521–528.
17. Davis, D. G. & Bax, A. (1985) *J. Am. Chem. Soc. 107*, 2820–2821.
18. Bax, A. & Davis, D. G. (1985) *J. Magn. Reson. 65*, 355–360.
19. Wieruszeski, J. M., Michalski, J. C., Montreuil, J., Strecker G., Katalinic, J. P., Egge, H., Van Halbeek, H., Mutsaers, J. H. G. M. & Vliegenthart, J. F. G. (1987) *J. Biol. Chem. 262*, 6650–6657.
20. Pierce-Cretel, A., Decottignies, J.-P., Wieruszeski, J. M., Strecker, G., Montreuil, J. & Spik, G. (1989) *Eur. J. Biochem. 182*, 457–476.

21. Breg, J., Romijn, D., Vliegenthart, J. F. G., Strecker, G. & Montreuil, J. (1988) *Carbohydr. Res. 183*, 19–34
22. Brockhausen, I., Matta, K. L., Orr, J., & Schachter, H. (1985) *Biochemistry 24*, 1866–1874.
23. Clausen, H., Levery, S. B., Kannagi, R. & Hakomori, S. H.(1986) *J. Biol. Chem. 261*, 1380–1387.
24. Clausen, H., Holmes, E. & Hakomori, S. H. (1986) *J. Biol. Chem. 261*, 1388–1392.
25. Betteridge, A. & Watkins, W. M. (1985) *Glycoconjuguate J. 2*, 61–78.
26. Kumazaki, T. & Yoshida, A. (1984) *Proc. Natl Acad. Sci. USA 81*, 4193–4197.
27. Van Halbeek, H., Dorland, L., Vliegenthart, J. F. G., Hull, W. E., Lamblin, G., Lhermitte, M., Boersma, A. & Roussel P. (1982) *Eur. J. Biochem. 127*, 7–20.