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Primary structure of neutral oligosaccharides derived from respiratory-mucus glycoproteins of a patient suffering from bronchiectasis, determined by combination of 500-MHz ^{1}H-NMR spectroscopy and quantitative sugar analysis

2. Structure of 19 oligosaccharides having the GlcNAcβ(1→3)GalNAc-ol core (type 3) or the GlcNAcβ(1→3)[GlcNAcβ(1→6)]GalNAc-ol core (type 4)

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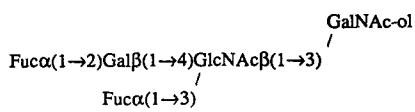
A pool of neutral carbohydrate chains was prepared from respiratory mucins of a patient suffering from bronchiectasis. Fractionation by HPLC led to 35 smaller-size oligosaccharide-alditols; the structure of 16 oligosaccharide-alditols with core type 1 or type 2 has been established (Klein, A., Lamblin, G., Lhermitte, M., Roussel, P., Breg, J., Van Halbeek, H. & Vliegenthart, J. F. G., preceding paper in this journal).

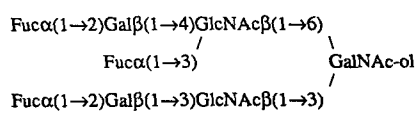
In this second part, we identified 19 oligosaccharide-alditols possessing core types 3 and 4. Nine of the structures (1, 5, 9, 6, 10b, 13, 19, 15b and 18.1) have been described previously to be present in cystic fibrosis mucins [Lamblin, G., Boersma, A., Lhermitte, M., Roussel, P., Mutsaers, J. G. H. M., Van Halbeek, H. & Vliegenthart, J. F. G. (1984) *Eur. J. Biochem. 143*, 227–234]. The remaining ten are new structures isolated from bronchial mucins; they are all extensions of the above-mentioned nine oligosaccharides. These compounds are octasaccharide-alditols containing the Y determinant together with the H determinant of either backbone-type 1 or 2, and partial structures thereof:

11b



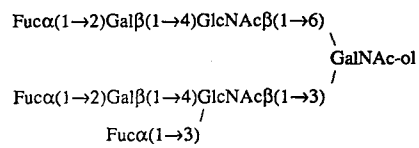
and **8c**, which is **11b** without Fucα(1→3),

24

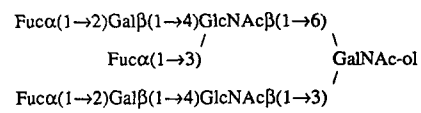


and **17b**, which is **24** without Fuc and Gal in the 3-branch, and **22c**, which is **24** without Fucα(1→3)

23b



23c



and 21b, which is 23c without Fucα(1→3), 18.2, which is 23c without any Fuc in the 6-branch, and 22b, which is 23c without Fuc in the 3-branch.

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Abbreviations. Fuc, L-fucose; Gal, D-galactose; GlcNAc, *N*-acetyl-D-glucosamine; GalNAc, *N*-acetyl-D-galactosamine; GalNAc-ol, *N*-acetyl-D-galactosaminitol.

Previously, 35 oligosaccharide-alditols have been isolated from the respiratory mucins secreted by a patient suffering from bronchiectasis due to a Kartagener's syndrome [1]. The characterization of 16 oligosaccharides possessing core type 1 or type 2 was described in the preceding paper [1]. The ^{1}H-NMR parameters that characterize the Y determinant structural element, i.e. Fucα(1→2)Galβ(1→4)[Fucα(1→3)]-GlcNAcβ(1→) have also been given in that paper.

In this second part we describe a series of 19 oligosaccharide-alditols possessing a GlcNAc residue linked β(1→3) to GalNAc-ol, the carbohydrate residue that was originally involved in the carbohydrate-peptide linkage (type 3 and type 4 core).

MATERIALS AND METHODS

Materials and methods are identical to those previously described [1]. Briefly, human bronchial sputum was obtained from a patient with blood group O and suffering from bronchiectasis due to a Kartagener's syndrome. From this crude material a fraction of mucus glycopeptides was obtained, which was submitted to alkaline borohydride reductive degradation. The resulting pool of glycopeptides and reduced oligosaccharides was fractionated in two successive steps according to acidity and molecular size. Of the fractions obtained, the one containing neutral oligosaccharide-alditols, denoted Ic, was used for further purification. As the final chromatography step the oligosaccharide-alditols in fraction Ic were fractionated by HPLC using successively a Lichrosorb-NH_2 and a µBondapack TM C_{18} column. The resulting HPLC samples were then submitted to quantitative sugar analysis and to 500-MHz ^{1}H-NMR spectroscopic analysis [1].

RESULTS

Structure determination

The results of the fractionation by HPLC have been presented in the accompanying paper [1]. A list of the sugar-analysis data of all HPLC subfractions, together with the retention times on the µBondapak TM C_{18} column, have been given there as well (Table 2 in [1]). After the discussion in the accompanying paper of the oligosaccharides having core type 1 or 2, i.e. GalNAc-ol with Gal in β(1→3) linkage, this paper deals with only those compounds having core type 3 or 4, i.e. GalNAc-ol with GlcNAc in β(1→3) linkage.

In Schemes 1, 2 and 3 the structures of the oligosaccharide-alditols with core type 3 or 4 have been listed that have been deduced from the 500-MHz ^{1}H-NMR spectra. The chemical shifts of the structural reporter-groups have been compiled in Tables 2, 3 and 4, while Table 1 lists the carbohydrate composition of the fractions concerned (see also Table 2 in [1]).

Structures with the GlcNAcβ(1→3)GalNAc-ol core

In the HPLC fractions, six compounds were identified with structures containing GalNAc-ol monosubstituted with GlcNAc at C-3 (core type 3). Their structures are listed in Scheme 1, and the ^{1}H-NMR parameters of these compounds have been compiled in Table 2. Oligosaccharide-alditols with core type 3 are characterized by the specific GalNAc-ol H-2 and H-5 signals at δ = 4.26–4.29 ppm and δ = 4.11–4.14 ppm respectively, and the NAc signal at δ = 2.030–2.038 ppm. The precise chemical shifts of these structural reporters as well as those of GlcNAc^3 are dependent on substitutions in GlcNAc^3. The ^{1}H-NMR spectra of fractions 1, 5, 6 and 9 point each to the presence of a single oligosaccharide. The structural-reporter groups are identical to those observed previously for four neutral oligosaccharide-alditols obtained from cystic fibrosis sputum [2, 3] (oligosaccharides 1, 4, 5 and 8 of [2]). A description of the deduction of the primary structures of the oligosaccharide-alditols has been published [3]. This identifies the compounds 1, 5, 6 and 9 as indicated in Scheme 1.

HPLC fraction	Structures with the common element GlcNAcβ(1→3)GalNAc-ol
1	GalNAc-ol / GlcNAcβ(1→3)
5	GalNAc-ol / Galβ(1→3)GlcNAcβ(1→3)
9	GalNAc-ol / Fuc(1→2)Galβ(1→3)GlcNAcβ(1→3)
6	GalNAc-ol / Galβ(1→4)GlcNAcβ(1→3)
8c	GalNAc-ol / Fuc(1→2)Galβ(1→4)GlcNAcβ(1→3)
N-1[a]	GalNAc-ol / Galβ(1→4)GlcNAcβ(1→3) / Fucα(1→3)
11b	GalNAc-ol / Fuc(1→2)Galβ(1→4)GlcNAcβ(1→3) / Fucα(1→3)

Scheme 1. *Structures of neutral oligosaccharide-alditols of the GlcNAcβ(1→3)GalNAc-ol core type (type 3), obtained by HPLC fractionation of a pool of neutral oligosaccharide-alditols from Kartagener's syndrome sputum.* [a] Compound N-1 has been added to the scheme as a reference compound [5]

The ^{1}H-NMR spectrum of fraction 8c (see Fig. 1) together with the results of the sugar analysis indicate the presence of a tetrasaccharide-alditol composed of Gal, GlcNAc, Fuc and GalNAc-ol in the molar ratio of 1:1:1:1. The core structure, as derived from the chemical shifts of H-2 and H-5 of GalNAc-ol at δ = 4.282 ppm and 4.143 ppm, respectively, is GlcNAcβ(1→3)GalNAc-ol [2, 3]. The presence of Fuc in α(1→2) linkage to Gal in an *N*-acetyllactosamine unit is revealed by the Fuc structural-reporters: H-1 at δ = 5.310 ppm, H-5 at δ = 4.219 ppm and CH_3 at δ = 1.234 ppm [2, 4]. A β(1→3) type of linkage between Gal and GlcNAc in the 3-branch is excluded since this would have been accompanied by a signal for H-3 of GlcNAc^3 at $\delta \approx 4.02$ ppm, together with NAc of GlcNAc^3 at $\delta \approx 2.11$ ppm and different positions of the Fuc reporter-groups (see compound 9) [3]. Combination of these observations leads to the following structure for compound 8c: Fucα(1→2)Galβ(1→4)GlcNAcβ(1→3)GalNAc-ol. Compound 8c can be conceived as an extension of 6 with Fucose in α(1→2) linkage to Gal. The shift effects on the ^{1}H-NMR characteristics of 6 are accordingly: $\Delta\delta$ for H-1 of Gal^4 and GlcNAc^3 are +0.069 ppm and +0.022 ppm,

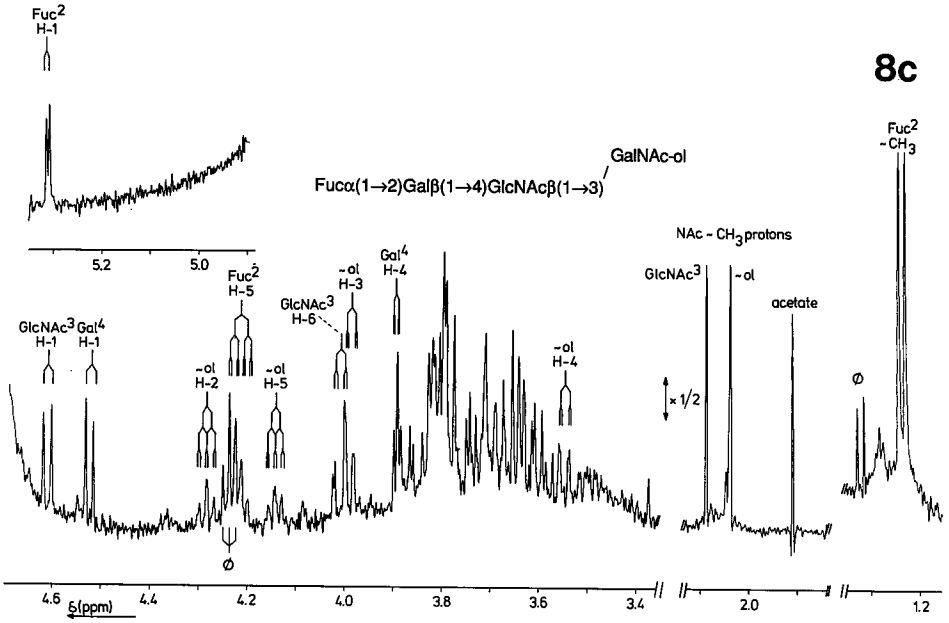


Fig. 1. *Resolution-enhanced 500-MHz ^{1}H-NMR spectrum (^2H_2O, 27°C) of HPLC fraction 8c, obtained from the pool of neutral oligosaccharide-alditols Ic from Kartagener syndrome sputum.* The relative-intensity scale of the *N*-acetyl methyl proton region of the spectrum differs from that of the other parts, as indicated. Signals marked by ϕ stem from a frequently occurring, non-protein non-carbohydrate contaminant

Table 1. *Molar carbohydrate composition of the neutral oligosaccharide-alditols that possess the GlcNAcβ(1→3)GalNAc-ol core type (type 3) or the GlcNAcβ(1→3)[GlcNAcβ(1→6)]GalNAc-ol core type (type 4)*
The molar composition of the oligosaccharide-alditols was calculated on the basis of one residue of GalNAc-ol per molecule

Oligosaccharide-alditol fraction	Molar ratios of monosaccharides			
	Fuc	Gal	GlcNAc	GalNAc-ol
1	–	–	0.9	1
5	n.d.	n.d.	n.d.	n.d.
6	–	0.9	0.9	1
8c	0.8	0.9	0.8	1
9	1.0	1.3	0.8	1
10b	–	1.0	1.8	1
11b	n.d.	n.d.	n.d.	n.d.
13	0.9	1.3	1.8	1
15b	–	2.1	1.8	1
17b	1.4	1.2	2.0	1
18	1.0	2.3	1.8	1
19	0.8	1.5	1.7	1
21b	n.d.	n.d.	n.d.	n.d.
22b	n.d.	n.d.	n.d.	n.d.
22c	1.7	2.3	1.4	1
23b	2.2	2.0	1.9	1
23c	n.d.	n.d.	n.d.	n.d.
24	3.6	2.5	1.8	1

respectively, and for the NAc signal of GlcNAc^3, $\Delta\delta$ is +0.003 ppm [2].

The ^{1}H-NMR spectrum of fraction 11b (see Fig. 2) together with the sugar analysis data (see Table 1) indicates the presence of a single oligosaccharide-alditol containing Gal, GlcNAc, Fuc and GalNAc-ol in the molar ratio of 1:1:2:1 respectively. The chemical shifts of H-2 and H-5 of GalNAc-ol are indicative of GalNAc-ol being monosubstituted with GlcNAc in β(1→3) linkage [2, 3]. The total set of structural-reporter groups of the two Fuc residues, i.e. H-1, H-5 and CH_3 for each residue (see Table 2), is indicative of the presence of the Y determinant: Fucα(1→2)Galβ(1→4)-[Fucα(1→3)]GlcNAcβ(1→), where the chemical shift of Fuc^3 H-1 at δ = 5.122 ppm points to the GlcNAc residue being involved in a β(1→3) linkage (compare 17a in [1]). This identifies 11b as Fucα(1→2)Galβ(1→4)[Fucα(1→3)]GlcNAcβ(1→3)GalNAc-ol. Structure 11b represents the extension of compound 8c with Fuc in α(1→3) linkage to GlcNAc^3. The expected shift effects accompanying this structure enlargement are observed (compare 16c and 20 in [1]). There are upfield shifts for Fuc^2 H-1, Gal^4 H-1 and GlcNAc^3 NAc of 0.032 ppm, 0.043 ppm and 0.011 ppm, respectively, and downfield shifts for Fuc^2 H-5 and Fuc^2 CH_3 of 0.033 ppm and 0.040 ppm respectively. Compound 11b can also be regarded as an extension of tetrasaccharide N-1 (Scheme 1) with Fuc in α(1→2) linkage to Gal^4. This tetrasaccharide was obtained by desialylation of a sialyl-oligosaccharide-alditol A-1 obtained from bronchial mucins from sputum of cystic fibrosis patients [5]. For comparison the ^{1}H-NMR parameters of compound N-1 have been added to Table 2. The shift effects that characterize the α(1→2) fucosylation of N-1 are indeed observed, namely, an upfield shift of Fuc^3 H-1 of 0.018 ppm and downfield shifts for Fuc^3 H-5 and CH_3, for Gal^4 H-1 and for the GlcNAc^3 NAc signal for 0.053, 0.061, 0.050 and 0.002 ppm respectively. This is comparable to the fucosylation of compounds 14 and 16c giving structures 17a and 20, respectively.

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

Three oligosaccharide-alditols have as common element GlcNAcβ(1→3)[Galβ(1→4)GlcNAcβ(1→6)]GalNAc-ol, in which GlcNAc^3 occupies a terminal position. The three structures are, together with four compounds with a different common element (see below), listed in Scheme 2; their 500-MHz

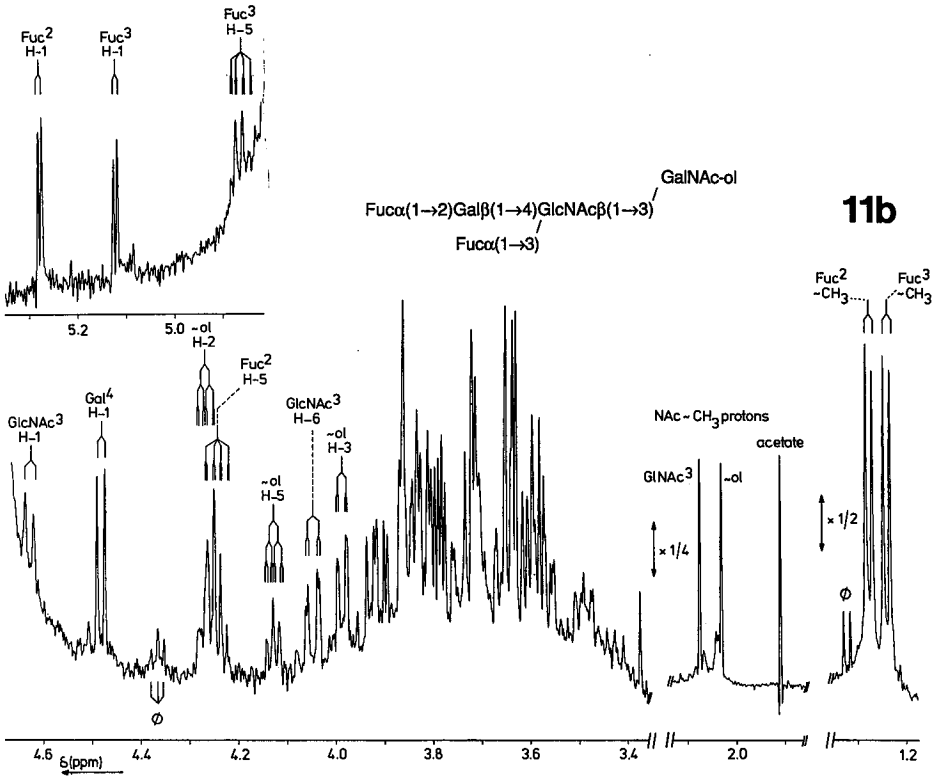


Fig. 2. *Resolution-enhanced 500-MHz ^{1}H-NMR spectrum (^2H_2O, 27°C) of HPLC fraction 11b, obtained from the pool of neutral oligosaccharide-alditols Ic from Kartagener syndrome sputum.* The relative-intensity scale of the *N*-acetyl and Fuc methyl proton region of the spectrum differs from that of the other parts, as indicated. Signals marked by ϕ stem from a frequently occurring, non-protein non-carbohydrate contaminant

HPLC fraction	Structures with the common element GlcNAcβ(1→3)[Galβ(1→4)GlcNAcβ(1→6)]GalNAc-ol
10b	Galβ(1→4)GlcNAcβ(1→6) GalNAc-ol GlcNAcβ(1→3)
13	Fucα(1→2)Galβ(1→4)GlcNAcβ(1→6) GalNAc-ol GlcNAcβ(1→3)
$3a_2$[a]	Galβ(1→4)GlcNAcβ(1→6) Fucα(1→3) GalNAc-ol GlcNAcβ(1→3)
17b	Fucα(1→2)Galβ(1→4)GlcNAcβ(1→6) Fucα(1→3) GalNAc-ol GlcNAcβ(1→3)
19	Galβ(1→4)GlcNAcβ(1→6) GalNAc-ol Fucα(1→2)Galβ(1→3)GlcNAcβ(1→3)
22c	Fucα(1→2)Galβ(1→4)GlcNAcβ(1→6) GalNAc-ol Fucα(1→2)Galβ(1→3)GlcNAcβ(1→3)
24	Fucα(1→2)Galβ(1→4)GlcNAcβ(1→6) Fucα(1→3) GalNAc-ol Fucα(1→2)Galβ(1→3)GlcNAcβ(1→3)

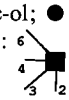
Scheme 2. *Structures of neutral oligosaccharide-alditols of the GlcNAcβ(1→3)[Galβ(1→6)]GalNAc-ol core type (type 4), obtained by HPLC fractionation of a pool of neutral oligosaccharide-alditols from Kartagener's syndrome sputum.* [a] Compound $3a_2$ has been added to the scheme as a reference compound [3]

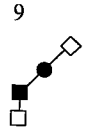
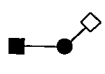
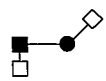
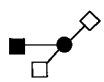
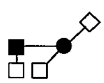
^{1}H-NMR parameters have been compiled in Table 3. Recurrent features in the structural-reporter group chemical shifts of this series are H-2, H-5 and NAc of GalNAc-ol at $\delta \approx 4.28$ ppm, 4.23 – 4.24 ppm and 2.043 – 2.046 ppm, respectively, together with H-1 and NAc of GlcNAc^3 at $\delta \approx 4.60$ ppm and 2.080 – 2.082 ppm respectively. The chemical shifts of the structural-reporter groups of GlcNAc^6 and Gal^4 experience major influences from extensions of the 6-branch with Fuc.

The ^{1}H-NMR spectra of fractions 10b and 13 have been observed previously, for oligosaccharide-alditols (oligosaccharides 10A and 13 of [2]) obtained from cystic fibrosis sputum. The primary structure of these compounds was identified as indicated for 10b and 13 in Scheme 2. The ^{1}H-NMR spectrum of fraction 13 points to the presence of also a minor compound, the ^{1}H-NMR parameters of which indicate the structure to be identical to that of compound 12.

The ^{1}H-NMR spectrum of fraction 17b (see Fig. 3) together with the sugar analysis (see Table 1) show the presence of a hexasaccharide-alditol containing Gal, GlcNAc, Fuc and GalNAc-ol in the molar ratio of 1:2:2:1 respectively. The core, GlcNAcβ(1→3)[GlcNAcβ(1→6)]GalNAc-ol, follows from the apparent position of H-2 and H-5 of GalNAc-ol at about 4.28 ppm and 4.24 ppm, respectively. The exact positions of these signals can not be determined at first glance, because of overlap with a Fuc H-5 signal and signals from a minor non-carbohydrate contaminant. The total set of structural-reporter chemical shifts of the two Fuc residues (see Table 3) is indicative of the Y determinant, i.e. Fucα(1→2)Galβ(1→4)[Fucα(1→3)]GlcNAcβ(1→) (compare compound 20 in [1]). The position of H-1 of Fuc^3 points to GlcNAc being β(1→6)-linked, as in compound 20. The combination of the foregoing findings identifies compound

Table 2. *^1H chemical shifts of structural reporter groups of constituent monosaccharides for the HPLC-fractionated, neutral bronchial oligosaccharide-alditols with GalNAc-ol bearing β(1→3) linked GlcNAc as a single substituent*

Chemical shifts expressed in ppm, are relative to internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate (using internal acetone at $\delta = 2.225$ ppm) in ^2H_2O at 27°C, acquired at 500 MHz. Data for compound N-1 have been added as reference [5]. For the complete structures of the compounds, see Scheme 1. In the table-heading, the structures are represented by short-hand symbolic notation (cf. [5]); ◇ = GalNAc-ol; ● = GlcNAc; ■ = Gal and □ = Fuc. The type of linkage in this notation is specified by the angle of the connecting bar as follows:  n.d., value could not be determined merely by inspection of the spectrum

Oligosaccharide-alditol fraction	Reporter group	Chemical shift in compound						
		1 	5 	9 	6 	8c 	N-1 	11b 
		ppm						
GalNAc-ol	H-2	4.286	4.287	4.263	4.288	4.282	4.272	4.266
	H-3	3.995	4.011	3.981	4.000	3.989	3.996	3.986
	H-4	3.545	3.560	3.589	3.550	3.542	3.564	n.d.
	H-5	4.141	4.136	4.113	4.142	4.143	4.126	4.128
	NAc	2.037	2.034	2.037	2.038	2.037	2.031	2.030
GlcNAc[3]	H-1	4.604	4.653	4.655	4.631	4.609	4.643	4.627
	H-6	3.948	3.953	3.952	4.020	4.010	4.034	4.046
	NAc	2.085	2.073	2.113	2.083	2.086	2.073	2.075
Gal[3]	H-1	–	4.459	4.578	–	–	–	–
	H-4	–	3.919	3.892	–	–	–	–
Gal[4]	H-1	–	–	–	4.454	4.523	4.430	4.480
	H-4	–	–	–	3.926	3.894	3.897	3.864
Fuc[2]	H-1	–	–	5.208	–	5.310	–	5.278
	H-5	–	–	4.269	–	4.219	–	4.252
	CH_3	–	–	1.231	–	1.234	–	1.274
Fuc[3]	H-1	–	–	–	–	–	5.140	5.122
	H-5	–	–	–	–	–	4.813	4.866
	CH_3	–	–	–	–	–	1.177	1.238

17b as GlcNAcβ(1→3)[Fucα(1→2)Galβ(1→4)[Fucα(1→3)]-GlcNAcβ(1→6)]GalNAc-ol. Compound 17b is an extension of 13 wherein GlcNAc[6] is substituted with Fuc in α(1→3) linkage. The observed chemical shift effects match exactly those accompanying analogous extensions with Fuc, i.e. compounds 16c and 20 in [1] and compounds 8c and 11b in the present report. Compound 13 can also be regarded as an extension of reference compound $3a_2$ (isolated from cystic fibrosis sputum [3]), wherein Fuc has been added in α(1→2) linkage to Gal[4]. The concomitant shift effects are in fact observed (compare the enlargement of 14 to 17a and of 16d to 20 in [1], and the extension of N-1 to 11b in the present report).

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

In the series of HPLC fractions four oligosaccharides have been identified with the GlcNAcβ(1→3)[GlcNAcβ(1→6)]-GalNAc-ol core (type 4), wherein the 3-branch is elongated by Fucα(1→2)Galβ(1→3). Their primary structures are added to Scheme 2, while the chemical shifts of the structural-reporter groups of these compounds are listed in Table 3. The chemical shifts of the 3-branch substitutions are recurrent features in the ^1H-NMR spectra, i.e. GlcNAc[3] H-1 and NAc at $\delta = 4.65$ ppm and 2.108 ppm, respectively, Gal[3] H-1 at $\delta = 4.56-4.57$ ppm and Fuc[2,3] H-1, H-5 and CH_3 at $\delta =$ 5.21 ppm, 4.27 ppm and 1.23 ppm respectively. The observable chemical shifts of the Gal[4] and GlcNAc[6] anomeric protons are strongly influenced by extension of the 6-branch i.e. by Fuc in α(1→2) linkage to Gal[4,6] and/or by Fuc in α(1→3) linkage to GlcNAc[6].

The ^1H-NMR spectral parameters of the major oligosaccharide present in fraction 19 are identical to those observed earlier for an oligosaccharide-alditol (oligosaccharide 19 of [2]) derived from cystic fibrosis sputum (see Table 3). The deduction from the ^1H-NMR spectrum of the primary structure of this compound has been described there as well. This determines compound 19 to be Fucα(1 → 2)Galβ(1 → 3)GlcNAcβ(1 → 3)[Galβ(1 → 4)GlcNAcβ(1→6)]GalNAc-ol. The ^1H-NMR spectrum of fraction 19 contains also signals of low intensity pointing to the presence of a minor component (≈15%). Owing to lack of reference data for the observed chemical shifts, the structure of this compound could not be deduced from its ^1H-NMR parameters.

The ^1H-NMR spectrum of fraction 22c (see Fig. 4) together with the results of the sugar analysis (see Table 1) demonstrate the presence of a heptasaccharide-alditol containing Gal, GlcNAc, Fuc and GalNAc-ol in a molar ratio of 2:2:2:1 respectively. The core of this oligosaccharide-alditol, GlcNAcβ(1→3)[GlcNAcβ(1→6)]GalNAc-ol, follows from the positions of H-2 and H-5 of GalNAc-ol at $\delta \approx 4.26$ ppm and 4.22 ppm respectively. The exact positions of the latter two resonances are obscured by two Fuc H-5 signals. One

Table 3. *^{1}H chemical shifts of structural-reporter groups of constituent monosaccharides for the HPLC-fractionated, neutral bronchial oligosaccharide-alditols possessing the GlcNAcβ(1→3)[Galβ(1→4)GlcNAcβ(1→6)]GalNAc-ol or the Fucα(1→2)Galβ(1→3)GlcNAcβ-(1→3)[Galβ(1→4)GlcNAcβ(1→6)]GalNAc-ol common element*

Data for compound $3a_2$ have been added as reference [3]. For explanation of the notation, see Table 2. n.d., values could not be determined merely by inspection of the spectrum

Residue	Reporter group	Chemical shift in compound						
		10b	13	$3a_2$	17b	19	22c	24
		ppm						
GalNAc-ol	H-2	4.280	4.282	4.280	4.282	4.264	4.26	4.290
	H-3	3.985	3.986	3.988	3.985	n.d.	n.d.	n.d.
	H-4	3.516	3.517	3.5–3.6	3.503	n.d.	n.d.	n.d.
	H-5	4.236	4.241	4.230	4.231	4.214	4.22	4.202
	NAc	2.046	2.044	2.046	2.044	2.042	2.042	2.042
GlcNAc^3	H-1	4.597	4.597	4.597	4.595	4.653	4.652	4.653
	H-6	3.950	3.949	3.966	3.947	n.d.	n.d.	n.d.
	NAc	2.082	2.081	2.081	2.080	2.108	2.108	2.106
GlcNAc^6	H-1	4.563	4.546	4.570	4.553	4.564	4.551	4.56[b]
	H-6	3.996	3.991	4.0[a]	4.026	n.d.	n.d.	4.022
	NAc	2.061	2.065	2.052	2.055	2.060	2.065	2.057
Gal^4	H-1	4.472	4.540	4.453	4.501	4.468	4.535	4.498
	H-4	3.926	3.891	3.921	n.d.	3.925	3.89[a]	n.d.
Gal^3	H-1	–	–	–	–	4.569	4.562	4.560
	H-4	–	–	–	–	3.89	3.89	3.89
Fuc^{2,3}	H-1	–	–	–	–	5.210	5.210	5.210
	H-5	–	–	–	–	4.270	4.270	4.271
	CH_3	–	–	–	–	1.232	1.232	1.233
Fuc^{2,4}	H-1	–	5.307	–	5.275	–	5.304	5.274
	H-5	–	4.225	–	4.259	–	4.224	4.256
	CH_3	–	1.233	–	1.273	–	1.232	1.271
Fuc^3	H-1	–	–	5.118	5.102	–	–	5.098
	H-5	–	–	4.83	4.872	–	–	4.874
	CH_3	–	–	1.177	1.236	–	–	1.233

[a] Value could not be determined more accurately owing to overlap with other carbohydrate originating signals.
[b] Value could not be determined more accurately, probably because of virtual coupling.

Fuc residue forms part of the element Fucα(1→2)Galβ(1→3)GlcNAcβ(1→3), as is demonstrated by Fuc^{2,3} H-1 and CH_3 at δ = 5.210 ppm and 1.232 ppm, respectively, and by the characteristic Gal^3 H-1 and GlcNAc^3 H-1 and NAc signal at δ = 4.562 ppm, 4.652 ppm and 2.108 ppm respectively (comparable to the 3-branch of compound 19). The second set of Fuc resonances (see Table 3) is characteristic of Fucα(1→2) linked to Gal in an *N*-acetyllactosamine unit [2, 4] and is comparable to the 6-branch of structure 13. Combination of the above-mentioned observations provides the structure of the oligosaccharide alditol in fraction 22c as Fucα(1→2)Galβ(1 → 3)GlcNAcβ(1 → 3)[Fucα(1 → 2)Galβ(1 → 4)GlcNAcβ(1→6)]GalNAc-ol. This is an extension of 19 with Fuc in α(1→2) linkage to Gal^4 and all concomitant shift effects are observed: $\Delta\delta$ for Gal^4 H-1 and for GlcNAc^6 H-1 and NAc are +0.067, −0.013 and +0.005 ppm respectively. The same structural elements and shift effects are observed for 10b and 13. The ^{1}H-NMR spectrum indicates the presence of a minor carbohydrate compound (≈15%), the structure of which could not be unravelled from the ^{1}H-NMR spectrum sofar, due to lack of reference data.

The ^{1}H-NMR spectrum of fraction 24 (see Fig. 5), together with the results of the sugar analysis (see Table 1) reveal the presence in this fraction of an octasaccharide-alditol made up of Gal, GlcNAc, Fuc and GalNAc-ol in a ratio of 2:2:3:1 respectively. A very characteristic GlcNAc^3 NAc signal at δ = 2.106 ppm, together with Fuc H-1 and CH_3 at δ = 5.210 ppm and 1.233 ppm, respectively, point to the structural element Fucα(1→2)Galβ(1→3)GlcNAcβ(1→3)-GalNAc-ol. The same element is present in compounds 19 and 22c. The total set of two other Fuc structural reporters, i.e. H-1 at δ = 5.274 ppm and 5.098 ppm, H-5 at δ = 4.259 ppm and 4.874 ppm and CH_3 at δ = 1.271 ppm and 1.233 ppm is indicative of the Y determinant i.e. Fucα(1→2)Galβ(1→4)[Fucα(1→3)]GlcNAcβ(1→). The position of Fuc^3 H-1 (δ = 5.098 ppm) indicates GlcNAc to be involved in a β(1→6) linkage, as observed for the 6-branch in compounds 17b and 20, in this paper and [1]. A combination of these two clearly recognizable elements renders structure 24 as Fucα(1→2)Galβ(1→3)GlcNAcβ(1→3)[Fucα(1→2)-Galβ(1→4)[Fucα(1→3)]GlcNAcβ(1→6)]GalNAc-ol. Compound 24 can be conceived as an extension of compound 22c, with Fuc α(1→3) linked to GlcNAc^6. An analogous extension of the structural element Fucα(1→2)Galβ(1→4)GlcNAc-β(1→) is observed when comparing compounds 13 and 17b or 8c and 11b. The shift effects accompanying this extension are again present for compounds 22c and 24. The Gal and GlcNAc anomeric signals for compound 24 are assigned

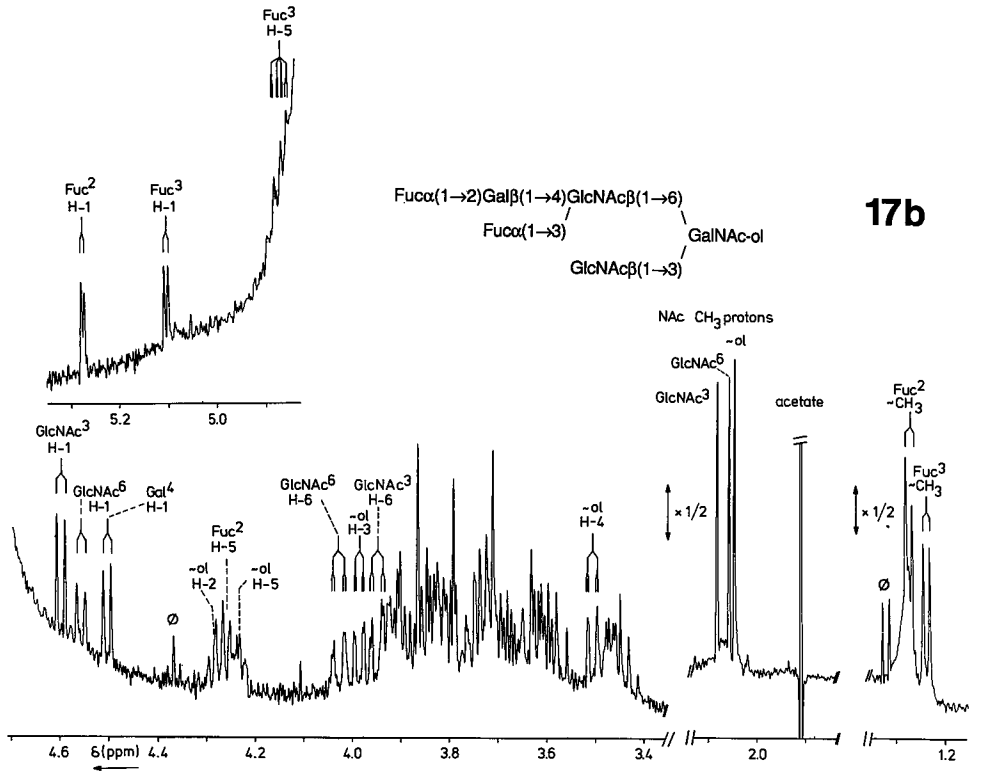


Fig. 3. *Resolution-enhanced 500-MHz ^{1}H-NMR spectrum (^2H_2O, 27°C) of HPLC fraction 17b, obtained from the pool of neutral oligosaccharide-alditols Ic from Kartagener syndrome sputum.* The relative-intensity scale of the *N*-acetyl and Fuc methyl proton region of the spectrum differs from that of the other parts, as indicated. Signals marked by ϕ stem from a frequently occurring, non-protein non-carbohydrate contaminant

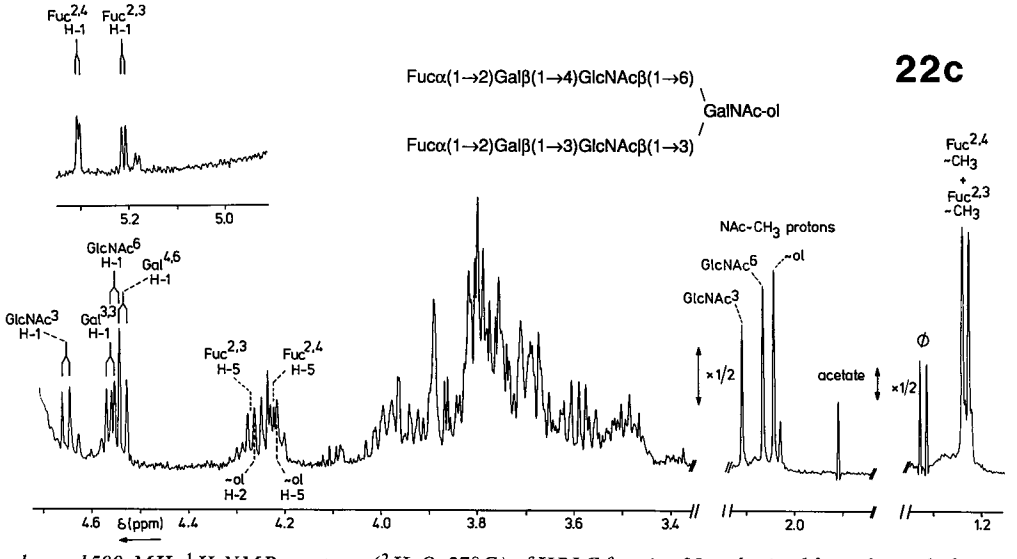


Fig. 4. *Resolution-enhanced 500-MHz ^{1}H-NMR spectrum (^2H_2O, 27°C) of HPLC fraction 22c, obtained from the pool of neutral oligosaccharide-alditols Ic from Kartagener syndrome sputum.* The relative-intensity scale of the *N*-acetyl and Fuc methyl proton region of the spectrum differs from that of the other parts, as indicated. Signals marked by ϕ stem from a frequently occurring, non-protein non-carbohydrate contaminant

through comparison with 17b and 22c. The disturbance in the intensity of the GlcNAc^6 H-1 doublet at δ = 4.56 ppm can be ascribed to a virtual coupling with its H-3 signal in the bulk region of the spectrum [6] (compare structure 20, in [1]).

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

From the ^{1}H-NMR spectra, together with the results of the sugar analysis, seven oligosaccharide-alditols have been identified with the common element Galβ(1→4)GlcNAc-β(1→3)[Galβ(1→4)GlcNAcβ(1→6)]GalNAc-ol. The structures of these compounds are compiled in Scheme 3, and their ^{1}H-NMR characteristics have been listed in Table 4. Typical ^{1}H-NMR signals for the common moieties in this group originate from GalNAc-ol, i.e. H-2, H-5 and NAc at δ = 4.27–4.28 ppm, δ = 4.22–4.24 ppm and δ = 2.040–2.045 ppm respectively. Other ^{1}H-NMR characteristics arise from the two GlcNAc residues that are linked to GalNAc-ol, i.e. H-1 and NAc of GlcNAc^3 at δ = 4.60–4.62 ppm and 2.071–2.082 ppm, respectively, and H-1 and NAc of GlcNAc^6 at

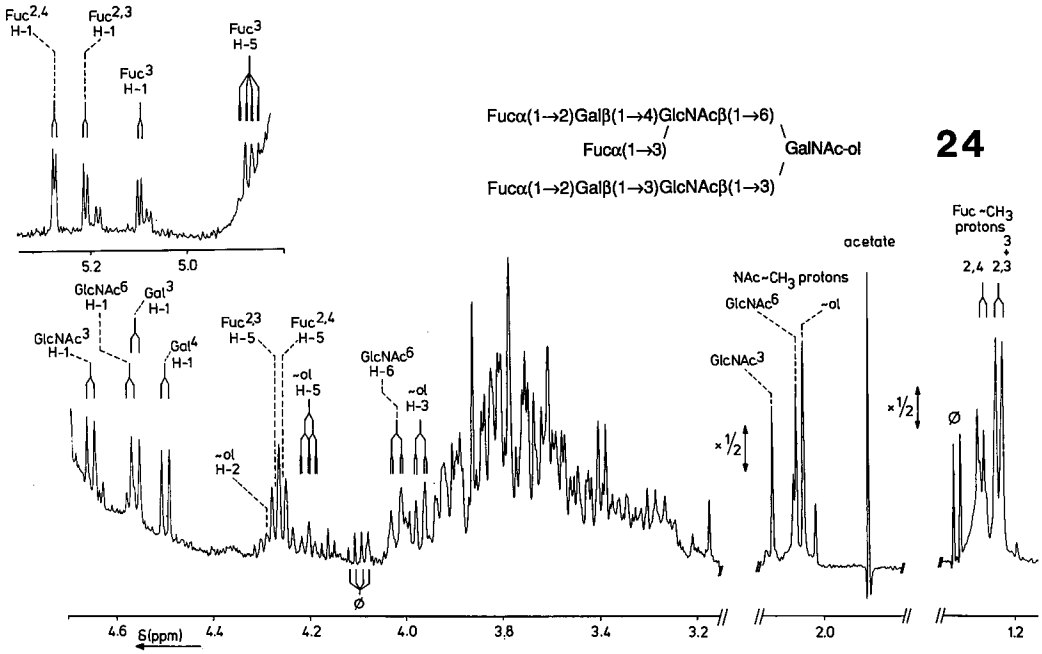


Fig. 5. *Resolution-enhanced 500-MHz ^{1}H-NMR spectrum (^2H_2O, 27°C) of HPLC fraction 24, obtained from the pool of neutral oligosaccharide-alditols Ic from Kartagener syndrome sputum.* The relative-intensity scale of the *N*-acetyl and Fuc methyl proton region of the spectrum differs from that of the other parts, as indicated. Signals marked by ϕ stem from a frequently occurring, non-protein non-carbohydrate contaminant

HPLC fraction	Structures with the common element Galβ(1→4)GlcNAcβ(1→3)[Galβ(1→4)GlcNAcβ(1→6)]GalNAc-ol
15b	Galβ(1→4)GlcNAcβ(1→6) \ GalNAc-ol / Galβ(1→4)GlcNAcβ(1→3)
18.1	Fucα(1→2)Galβ(1→4)GlcNAcβ(1→6) \ GalNAc-ol / Galβ(1→4)GlcNAcβ(1→3)
18.2	Galβ(1→4)GlcNAcβ(1→6) \ GalNAc-ol / Fucα(1→2)Galβ(1→4)GlcNAcβ(1→3)
21b	Fucα(1→2)Galβ(1→4)GlcNAcβ(1→6) \ GalNAc-ol / Fucα(1→2)Galβ(1→4)GlcNAcβ(1→3)
22b	Fucα(1→2)Galβ(1→4)GlcNAcβ(1→6) / \ GalNAc-ol Fucα(1→3) / Galβ(1→4)GlcNAcβ(1→3)
23b	Fucα(1→2)Galβ(1→4)GlcNAcβ(1→6) \ GalNAc-ol / Fucα(1→2)Galβ(1→4)GlcNAcβ(1→3) Fucα(1→3)
23c	Fucα(1→2)Galβ(1→4)GlcNAcβ(1→6) / \ GalNAc-ol Fucα(1→3) / Fucα(1→2)Galβ(1→4)GlcNAcβ(1→3)

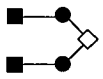
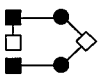
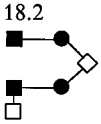
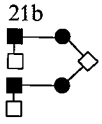
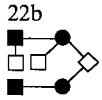
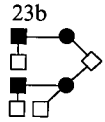
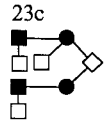
Scheme 3. *Structures of neutral oligosaccharide-alditols of the GlcNAcβ(1→3)[GlcNAcβ(1→6)]GalNAc-ol core type (type 4), obtained by HPLC fractionation of a pool of neutral oligosaccharide-alditols from Kartagener's syndrome sputum*

δ = 4.54–4.56 ppm and 2.056–2.070 ppm respectively. The relatively large variations in the chemical shifts mentioned are due to the many different Fuc substitutions, not being confined to a specific peripheral element. The ^{1}H-NMR chemical shifts of the Gal^{4,3} and Gal^{4,6} residues are largely influenced by extensions of the 3 or 6-branch respectively.

The ^{1}H-NMR spectrum of fraction 15b and that of the major component in fraction 18 (80%, compound 18.1), are identical to spectra observed in the analysis of oligosaccharide-alditols obtained from human bronchial cystic fibrosis mucins (oligosaccharides 15.2A and 17 of [2]). A description of the primary structures of these oligosaccharide-alditols from the ^{1}H-NMR spectra has been given [2]. Therefore compound 15b is identified as Galβ(1→4)GlcNAcβ(1→3)-[Galβ(1→4)GlcNAcβ(1→6)]GalNAc-ol, which is the above-mentioned common element without any extensions, and 18.1 is the common element extended with Fuc in α(1→2) linkage to Gal^{4,6} (see Scheme 3).

The presence of a minor component in fraction 18 (compound 18.2) is inferred from low-intensity signals in the anomeric, the *N*-acetyl and the Fuc CH_3 regions. The low-intensity signal in the latter region, at δ = 1.229 ppm, together with the H-1 signal at δ = 5.305 ppm (see Table 4), coinciding with an analogous signal of the main component, points to the presence of the element Fucα(1→2)Gal-β(1→4)GlcNAcβ(1→) [2, 4]. This structural element is further demonstrated by minor-intensity signals at δ = 4.603 ppm for GlcNAc H-1 and at δ = 4.523 ppm for Gal^4 H-1 (compare compound 8c). The shift effects of Fucα(1→2) attachment to Gal in the *N*-acetyllactosamine element are well-described [2, 4]. Taking these into account the aforementioned element is evidently β(1→3) linked to GalNAc-ol. The same 3-branch is encountered for compounds 21b and 23c (see below). Two anomeric signals of minor intensity at δ = 4.475 ppm and δ = 4.560 ppm are indicative of an unsubstituted

Table 4. ^1H *chemical shift structural-reporter groups of constituent monosaccharides for the HPLC-fractionated, neutral oligosaccharide-alditols possessing the common element Galβ(1→4)GlcNAcβ(1→3)[Galβ(1→4)GlcNAcβ(1→6)]GalNAc-ol*
For explanation of the notation, see Table 2. n.d., values could not be determined merely by inspection of the spectrum

Residue	Reporter group	Chemical shift in compound						
		15b 	18.1 	18.2 	21b 	22b 	23b 	23c 
		ppm						
GalNAc-ol	H-2	4.281	4.283	4.27	4.28	4.27	4.26	4.27
	H-5	4.237	4.234	4.23	4.23	4.22	4.23	4.22
	NAc	2.045	2.044	2.043	2.043	2.045	2.038	2.044
$GlcNAc^3$	H-1	4.623	4.621	4.603	4.602	4.621	4.617	4.601
	NAc	2.079	2.079	2.083	2.082	2.078	2.070	2.082
$GlcNAc^6$	H-1	4.564	4.546	4.560	4.541	4.55[a]	4.538	4.551
	NAc	2.062	2.066	2.062	2.066	2.057	2.070	2.056
$Gal^{4,6}$	H-1	4.473	4.540	4.475	4.539	4.501	4.538	4.501
	H-4	3.926	3.894	3.93	3.89	n.d.	3.89	n.d.
$Gal^{4,3}$	H-1	4.454	4.454	4.523	4.525	4.456	4.481	4.526
	H-4	3.926	3.927	3.89	3.89	n.d.	n.d.	n.d.
$Fuc^{2,4,3}$	H-1	–	–	5.305	5.311	–	5.279	5.309
	H-5	–	–	4.23	4.23	–	4.24	n.d.
	CH_3	–	–	1.229	1.235	–	1.276	1.230
$Fuc^{2,4,6}$	H-1	–	5.305	–	5.304	5.275	5.306	5.273
	H-5	–	4.227	–	4.23	n.d.	4.23	n.d.
	CH_3	–	1.235	–	1.235	1.273	1.237	1.273
Fuc^3	H-1	–	–	–	–	5.104	5.118	5.104
	H-5	–	–	–	–	n.d.	4.867	n.d.
	CH_3	–	–	–	–	1.236	1.237	1.234

[a] Value could not be determined more accurately, probably due to virtual coupling.

N-acetyllactosamine unit occurring in β(1→6) linkage to GalNAc-ol, which is identical to the 6-branch in compounds 10b, 15b and 19. The core of the minor component must thus be identical to that of the main component, namely, GlcNAcβ(1→3)[GlcNAcβ(1→6)]GalNAc-ol. The above observations suggest compound 18.2 to be Galβ(1→4)GlcNAcβ(1→3)[Fucα(1→2)Galβ(1→4)GlcNAcβ(1→6)]GalNAc-ol (see Scheme 3). This is a structural isomer of 18.1, with Fuc in α(1→2) linkage to $Gal^{4,3}$ instead of to $Gal^{4,6}$ for 18.1. The shift effects, as observed in the 6-branch when comparing 18.1 and its afuco analog, 15b, are observed in the 3-branch in the case of 18.2.

The ^{1}H-NMR spectrum of fraction 21b, together with the results of the sugar analysis (Table 1) indicate the presence of a heptasaccharide-alditol containing Gal, GlcNAc, Fuc and GalNAc-ol in a molar ratio of 2:2:2:1 respectively. The positions of H-2 and H-5 of GalNAc-ol at $\delta = 4.28$ ppm and $\delta = 4.23$ ppm, respectively, are typical for GalNAc-ol being substituted at C-3 and C-6 by βGlcNAc [2, 3]. The two sets of Fuc structural reporters at $\delta = 5.311$ ppm and 1.235 ppm and at $\delta = 5.304$ ppm and 1.235 ppm are each indicative of Fuc in α(1→2) linkage to the Gal residue of an *N*-acetyllactosamine unit [2, 4]. Two nearly coinciding doublets, at $\delta = 4.541$ ppm and 4.539 ppm, together with an *N*-acetyl signal at $\delta = 2.066$ ppm, support one Fucα(1→2)-Galβ(1→4)GlcNAcβ(1→) unit to be β(1→6) linked to GalNAc-ol (as in compounds 18.2, 13 and 22c, and in 15a in [1]). The other two anomeric doublets, at $\delta = 4.602$ ppm and 4.525 ppm, together with an *N*-acetyl singlet at $\delta = 2.082$ ppm, point to the second Fucα(1→2)Galβ(1→4)GlcNAcβ(1→) unit to be β(1→3) linked to GalNAc-ol (compare 18.2 and 8c). By combination of these partial structures, compound 21b is identified as Fucα(1→2)Galβ(1→4)GlcNAcβ(1→3)[Fucα(1→2)Galβ(1→4)GlcNAcβ(1→6)]GalNAc-ol. Compound 21b can be considered as an extension of 18.1 or 18.2 with a second Fuc linked α(1→2) to $Gal^{4,3}$ or $Gal^{4,6}$ respectively. The shift effects for such a fucosylation, i.e. an upfield shift for GlcNAc H-1 of 0.019 ppm and a downfield shift for Gal H-1 and GlcNAcNAc of 0.068 ppm and 0.004 ppm, respectively, are indeed observed.

The ^{1}H-NMR spectrum of fraction 22b indicates the presence of two Fuc residues, of which the total set of structural reporter groups, i.e. H-1 signals at $\delta = 5.275$ ppm and 5.104 ppm together with two Fuc CH_3 signals at $\delta = 1.273$ ppm and 1.236 ppm point to the Y determinant i.e. Fucα(1→2)Galβ(1→4)[Fucα(1→3)]GlcNAcβ(1→). The position of Fuc^3 H-1 proves the GlcNAc residue in this determinant to be β(1→6) linked (compare the same structural element in compounds 17b, 20 and 24). The NAc signal for $GlcNAc^6$ at $\delta = 2.057$ ppm and the H-1 signal of $Gal^{4,6}$ at $\delta = 4.501$ ppm also point to the presence of this element. The anomeric doublet of $GlcNAc^6$, at $\delta = 4.55$ ppm, is, like the spectra of compounds 20 and 24, severely distorted, probably due to virtual coupling. Two anomeric signals, at $\delta = 4.621$ and 4.456 ppm, together with a *N*-acetyl signal at $\delta = 2.078$ ppm, suggest the presence of a *N*-acetyllactosamine unit β(1→3) linked to GalNAc-ol, as also observed in compounds 15b and 18.1. Combination of these elements suggests compound 22b to be Galβ(1→4)GlcNAcβ(1→3)[Fucα(1→2)Galβ(1→4)[Fucα(1→3)]GlcNAcβ(1→6)]GalNAc-ol.

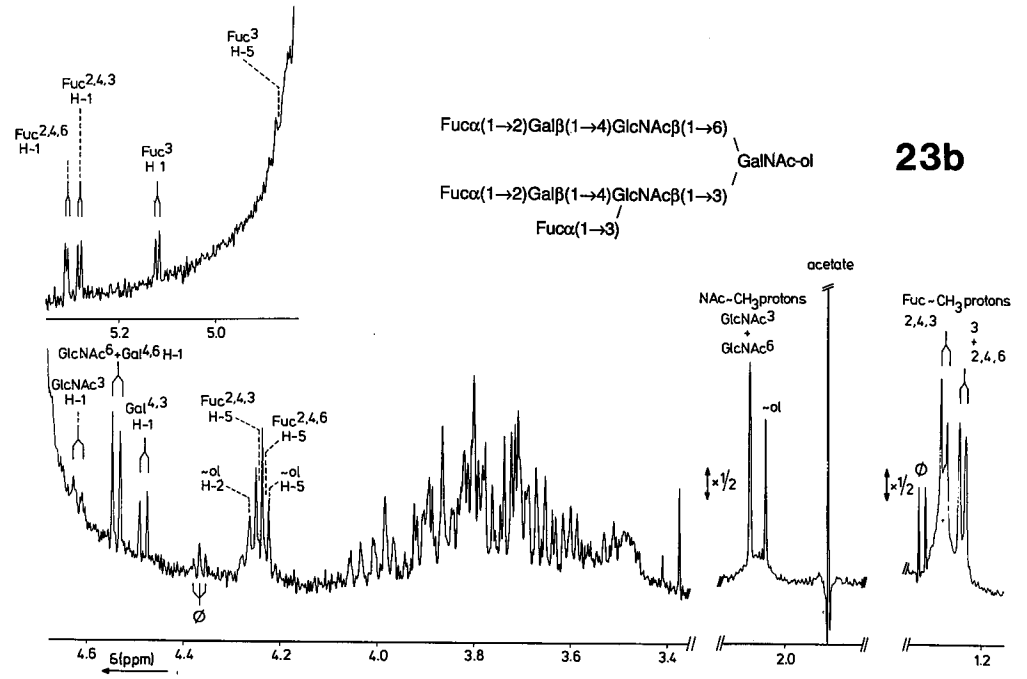


Fig. 6. *Resolution-enhanced 500-MHz ^{1}H-NMR spectrum (^2H_2O, 27°C) of HPLC fraction 23b, obtained from the pool of neutral oligosaccharide-alditols Ic from Kartagener syndrome sputum.* The relative-intensity scale of the *N*-acetyl and Fuc methyl proton region of the spectrum differs from that of the other parts, as indicated. Signals marked by ϕ stem from a frequently occurring, non-protein non-carbohydrate contaminant

The core of the structure can not be confirmed by H-2 and H-5 signals of GalNAc-ol owing to interference of non-carbohydrate contaminants. Compound 22b can be thought of as 18.1 extended with Fuc in α(1→3) linkage to GlcNAc^6 and all shift effects characterizing this extension are in fact observed (compare the extension of 13 to 17b and of 22c to 24).

The ^{1}H-NMR spectrum of fraction 23b (see Fig. 6), together with the sugar composition (see Table 1) point to the presence of an octasaccharide-alditol containing Gal, GlcNAc, Fuc and GalNAc-ol in a molar ratio of 2:2:3:1, respectively. From the total set of structural reporter groups of two Fuc residues, i.e. H-1 at δ = 5.279 ppm and 5.118 ppm, and CH_3 at δ = 1.276 ppm and 1.237 ppm, a structural element comprising the Y determinant can be inferred. The Fuc^3 H-1 shift value is indicative of the Y determinant to be involved in a (1→3) linkage, analogous to the same element in compound 11b and in 17a in [1]. This partial structure is supported by the GlcNAc^3 NAc signal at δ = 2.070 ppm and the anomeric doublet for Gal^{4,3} at δ = 4.481 ppm. The GlcNAc^3 H-1 doublet at δ = 4.617 ppm is heavily distorted, probably due to virtual coupling with its H-3, as in compound 24. A third Fuc is α(1→2) linked to Gal in a *N*-acetyllactosamine unit, as can be deduced from its H-1 and CH_3 signals at δ = 5.306 ppm and 1.237 ppm respectively. The anomeric signals of Gal and GlcNAc in this *N*-acetyllactosamine unit coincide at δ = 4.538 ppm and indicate the structural element to be 6-linked to GalNAc-ol. This is comparable to the 6-branch in compound 21b. H-2 and H-5 of GalNAc-ol are partly obscured by two Fuc H-5 signals. Combination of these data demonstrates compound 23b to be Fucα(1→2)Galβ(1→4)[Fucα(1→3)]GlcNAcβ(1→3)[Fucα(1→2)Galβ(1→4)GlcNAcβ(1→6)]GalNAc-ol. This structure is an extension of 21b with Fuc in α(1→3) linkage to GlcNAc^3. The shift effects on the ^{1}H-NMR parameters of 21b are completely analogous to those observed upon enlargement of 8c to 11b, i.e. upfield shifts for NAc of GalNAc-ol and GlcNAc^3 of 0.005 ppm and 0.012 ppm, respectively, upfield shifts for H-1 of Gal^{4,3} and Fuc^{2,4,3} of 0.044 ppm and 0.032 ppm, respectively, and downfield shifts for H-1 of GlcNAc^3 and CH_3 of Fuc^{2,4,3} of 0.015 ppm and 0.041 ppm respectively.

The ^{1}H-NMR spectrum of fraction 23c indicates the presence of three Fuc residues. The set of two Fuc reporter groups, i.e. H-1 at δ = 5.273 ppm and 5.104 ppm, and CH_3 signals at δ = 1.273 ppm and 1.234 ppm, are indicative of the Y determinant, i.e. Fucα(1→2)Galβ(1→4)[Fucα(1→3)]GlcNAcβ(1→6)]. The 1→6 linkage of GlcNAc is indicated by the exact position of Fuc^3 H-1 at δ = 5.104 ppm, as in the same element in compounds 17b, 22b and 24 and in compound 20 in [1]. The same analogy to the aforementioned oligosaccharide-alditols holds for the H-1 and NAc of GlcNAc^6 at δ = 4.551 ppm and 2.056 ppm, respectively, and for the H-1 of Gal^{4,6} at δ = 4.501 ppm. The third Fuc residue, as indicated by its H-1 and CH_3 at δ = 5.309 ppm and 1.230 ppm, respectively, is part of the element Fucα(1→2)Galβ(1→4)GlcNAcβ(1→). The anomeric signals of GlcNAc and Gal^4 in this element are observed at δ = 4.601 ppm and 4.526 ppm, respectively, which is comparable to the 3-branch in compounds 18.2 and 21b. Combination of these partial structures identifies the structure as Fucα(1→2)Galβ(1→4)GlcNAcβ(1→3)[Fucα(1→2)Galβ(1→4)[Fucα(1→3)]GlcNAcβ(1→6)]GalNAc-ol.

DISCUSSION

HPLC is a powerful tool for the separation of oligosaccharide-alditols [1, 2, 5]. The combination of two steps of HPLC, i.e. separation on a column of alkylamine-modified silica, followed by a second run on an octadecyl-bonded packing, has now been applied to fractionate a pool of neutral oligosaccharide-alditols. These alditols have been prepared by alkaline borohydride treatment of bronchial mucus glyco-

peptides and vary in size from two to eight sugars. The first column allows separation of, at least, 24 fractions. Nine of the latter have been separated into two to four subfractions by HPLC on a reverse-phase column. Finally the structure of 35 neutral oligosaccharide-alditols has been established by 500-MHz ^{1}H-NMR spectroscopy in combination with sugar analysis.

By application of empirical rules derived earlier for correlating ^{1}H-NMR features of mucin-type oligosaccharide-alditols with their primary structure [2, 4, 5, 7], the identity of nineteen neutral oligosaccharide-alditols that have core type 3 or 4, has been established. The structure determination of oligosaccharide-alditols that possess core type 1 or 2 has been described in the accompanying article [1]. Our studies show that the recognition of Fuc-containing elements like the H determinant (type 1 or type 2) as well as the X determinant can straightforwardly be carried out on the basis of previously obtained data. In addition, here the ^{1}H-NMR characteristics for the Y determinant have been described. It should be noted that the structural reporters for the Fuc residues in this structural element form a unique total set of parameters. The latter can not simply be conceived as a summation of two monofucosyl partial structures.

The comparison of the retention times of the oligosaccharide-alditols renders possible the identification of a few structural features responsible for the chromatographic behaviour of these compounds. As already shown by Boersma et al. [8] and Blanken et al. [9], the retention times on alkylamine-bonded columns depend on the number of sugar residues (see Fig. 3 of [1]). Moreover, within a series of oligosaccharide-alditols having the same number of sugar residues, the retention times of trimers and tetramers are lower when a fucose residue is present (compare the trimers 3, 5, 6 and 7 and the tetramers 8c, 8d, 9, 10a and 11a in Table 2 of [1]). Within the series of pentasaccharide or hexasaccharide-alditols, the compounds with two fucose residues usually have the lowest retention times, although, from a comparison of compounds 15b and 16a, this rule does not seem to be very strict. Within a series of reducing oligosaccharides a $\beta(1\rightarrow6)$ linkage usually induces a higher retention time as compared to other types of linkage [9]. This does not seem to be the case for oligosaccharide-alditols (compare compounds 8d with 9, and 16c or 16d with 17a).

The comparison of the retention times of oligosaccharide-alditols separated on an octadecyl-bonded column is also interesting. As already shown by Dua et al. [10], the elution of compounds with the Fuc$\alpha(1\rightarrow2)$Gal$\beta(1\rightarrow3)$GalNAc-ol element is strongly retarded (see retention times of 3, 8d, 12, 16c, 16d and 20 in Table 2 of [1]). A similar retarding effect arises from the element Fuc$\alpha(1\rightarrow2)$Gal$\beta(1\rightarrow4)$GlcNAc$\beta(1\rightarrow)$, as in fractions 8c, 13, 16d, 18, 21b, 22c and 23c. However, oligosaccharides possessing the element Fuc$\alpha(1\rightarrow2)$Gal$\beta(1\rightarrow3)$GlcNAc$\beta(1\rightarrow)$ (compounds 9, 19, 22c and 24) are less retarded on a reverse-phase column than analogous compounds with Fuc$\alpha(1\rightarrow2)$Gal$\beta(1\rightarrow4)$GlcNAc$\beta(1\rightarrow)$ (compounds 8c, 18, 21b and 23c). Retarding effects of different Fuc-containing elements may be added up, as observed for 16d, 21b and 22c. The element Gal$\beta(1\rightarrow4)$[Fuc$\alpha(1\rightarrow3)$]GlcNAc$\beta(1\rightarrow)$ is slightly less retarded than its afuco analog, as shown by compounds 14, 16a and 23a. Fuc $\alpha(1\rightarrow3)$ linkage to GlcNAc in the element Fuc$\alpha(1\rightarrow2)$Gal$\beta(1\rightarrow4)$GlcNAc$\beta(1\rightarrow)$, thereby completing the Y determinant partial structure, annuls the retarding influence of the initial Fuc$\alpha(1\rightarrow2)$ linkage (see compounds 11b, 17b, 22b, 23b, 23c and 24). This modulation of the retarding influence of $\alpha(1\rightarrow2)$-linked Fuc does not occur when the $\alpha(1\rightarrow2)$ and $\alpha(1\rightarrow3)$ linkages are in different branches. This suggests an influence at short distance within the molecule, i.e. by masking a binding site. The latter might be the result of a conformational change in the whole structural element, as is supported by the multiple NMR chemical-shift effects of the completion of the Y determinant structural element by addition of Fuc in $\alpha(1\rightarrow3)$ linkage (see Table 6 in [1]).

The data presented in this paper and in [1] illustrate the high degree of carbohydrate chain heterogeneity of human bronchial mucins. This is the first time that the four types of cores [11] are identified in the respiratory mucus glycopeptides of a single individual. It should be noted that, if such a diversity is already observable for the fraction of smallest neutral oligosaccharides (see Materials and Methods), the number of different carbohydrate chains in human bronchial mucins from a single individual might be in the order of several hundreds. Whether these different carbohydrate chains are attached to the same mucus peptide or to different mucin peptide isoforms is still unknown.

Most of the oligosaccharide chains (25 out of 35) of the mucus glycopeptides from this individual with blood-group O contain either H, or X [12] or Y [13] determinants. A few oligosaccharides have a X or Y determinant on one branch and an H determinant on the other. Oligosaccharides containing the Y determinant have not been found previously in mucins from patients suffering from cystic fibrosis [2]. These oligosaccharides, however, correspond in the present study to the most retarded fractions. These fractions have not yet been studied for cystic fibrosis mucus.

The carbohydrate chains of mucins from other sources exhibit also a large structural diversity. From a series of fourteen neutral oligosaccharides identified in swine trachea mucus glycoproteins [14, 15], five are identical to compounds described in the accompanying paper [1], i.e. 3, 7, 8d, 11a and 16d. Compounds 2, 3, 16c and 20, the latter lacking Fuc in the 3-branch [1], have been identified in low-molecular-mass human salivary mucin, oligosaccharides 2 and 3 being the most predominant [15]. Ovarian cyst mucin is a rich source of neutral oligosaccharides [10, 17]. In this material structures corresponding to compounds 1, 2, 3, 7, 8d, 11a, 12, 15a, 16d and 20 have been identified; with the exception of compound 9, these oligosaccharide-alditols have been described in [1]. The only structures that are common to gastrointestinal mucins [19, 20] and to human respiratory mucins are compounds 1 and 6. In gastrointestinal mucins [19, 20] and in swine trachea mucins [14, 15], often carbohydrate chains are found in which Gal is substituted both on the 3 and on the 6 position, thus serving as a new branching point. This structural element has not been identified among carbohydrate chains of human respiratory mucins. Such oligosaccharides may exist in the higher-molecular-mass oligosaccharides that have not been studied so far (fractions Ia and Ib of [1]). Finally one should notice that structures 1, 7 and 11a have also been identified previously in plasma membrane glycoproteins from ascites hepatoma [21] and structures 11a and 15a in surface glycoproteins from mammary adenocarcinoma [22].

This study will allow the future comparison of the smallest neutral carbohydrate chains of respiratory mucins from normal individuals and from patients with other bronchial diseases in order to find out whether or not the glycosylation of mucins may be altered in some pathological conditions.

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