r. J. Biochem. *171*, 631 – 642 (1988)
FEBS 1988

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

Structure of 16 oligosaccharides having the Galβ(1→3)GalNAc-ol core (type 1) the Galβ(1→3)[GlcNAcβ(1→6)]GalNAc-ol core (type 2)

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Received July 27/September 29, 1987) – EJB 87 0866

Carbohydrate chains of respiratory-mucus glycopeptides from a patient (blood group O) suffering from bronchiectasis with a Kartagener's syndrome have been released by alkaline borohydride treatment. Application of high-performance liquid chromatography using subsequently two silica columns, one bonded with aminopropyl groups and the other with octadecyl groups, afforded 39 neutral fractions; 35 oligosaccharide-alditol structures have been characterized by employing 500-MHz ^{1}H-NMR spectroscopy in conjunction with sugar analysis.

Here, 16 oligosaccharide structures, possessing a core consisting of Galβ(1→3)GalNAc-ol with or without branching through a GlcNAc residue linked β(1→6) to the GalNAc residue (core type 2 or core type 1, respectively), are described. Ten oligosaccharide-alditols with these types of cores (2, 3, 10a, 14, 7, 11a, 15a, 16a, 12 and 16c) have been identified previously in human bronchial mucins of patients suffering from cystic fibrosis [Lamblin, G., Boersma, A., Lhermitte, M., Roussel, P., Mutsaers, J. H. G. M., Van Halbeek, H. and Vliegenthart, J. F. G. (1984) *Eur. J. Biochem. 143*, 227 – 236].

Of the remaining six compounds, one is a partial structure of oligosaccharides previously described:

8d
```
GlcNAcβ(1→6)
            \
             GalNAc-ol
            /
Fucα(1→2)Galβ(1→3)
```

and five compounds are elongations of oligosaccharides previously described:

21a
```
                  Galβ(1→4)GlcNAcβ(1→6)
                                      \
                                       GalNAc-ol
                                      /
Galβ(1→4)GlcNAcβ(1→3)Galβ(1→3)
```

23a
```
                  Galβ(1→4)GlcNAcβ(1→6)
                                      \
                                       GalNAc-ol
                                      /
Galβ(1→4)GlcNAcβ(1→3)Galβ(1→3)
        /
Fucα(1→3)
```

16c
```
Galβ(1→4)GlcNAcβ(1→6)
        /           \
Fucα(1→3)            GalNAc-ol
                    /
   Fucα(1→2)Galβ(1→3)
```

17a
```
                                        GalNAc-ol
                                       /
Fucα(1→2)Galβ(1→4)GlcNAcβ(1→3)Galβ(1→3)
                 /
        Fucα(1→3)
```

20
```
Fucα(1→2)Galβ(1→4)GlcNAcβ(1→6)
                 /           \
        Fucα(1→3)             GalNAc-ol
                             /
            Fucα(1→2)Galβ(1→3)
```

The structures 17a and 20 contain the Y determinant, i.e., Fucα(1→2)Galβ(1→4)[Fucα(1→3)]GlcNAcβ(1→). High-resolution ^{1}H-NMR spectroscopy is able to distinguish whether the Y determinant is β(1→3) or β(1→6) linked in such oligosaccharide-alditols.

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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.

Tracheobronchial mucus is the principal component of the mucociliary system and protects the bronchial epithelium. It contains a major class of high-molecular-mass glycoproteins (so-called mucins) which are characterized by their high content in O-linked oligosaccharides. These oligosaccharides can be fractionated as oligosaccharide-alditols, after alkaline borohydride degradation of the mucins, by ion-exchange chromatography and HPLC on a alkylamine-bonded silica column [1 – 3].

In previous studies we have characterized twenty neutral oligosaccharide structures from acidic mucins secreted by six patients suffering from cystic fibrosis and having blood-group O, by a combination of quantitative sugar analysis and 500-MHz ^{1}H-NMR spectroscopy [1].

In the present work we describe the isolation and characterization of a series of oligosaccharide-alditols obtained from the bronchial mucins of a single patient with blood-group O, suffering from bronchiectasis due to Kartageners' syndrome. These oligosaccharides were separated by HPLC. In this paper we present the structures of 16 oligosaccharides possessing a galactose residue linked $\beta(1\rightarrow3)$ to an *N*-acetylgalactosaminitol residue, derived from the *N*-acetylgalactosamine residue originally involved in the carbohydrate-peptide linkage (type 1 and type 2 cores according to [4]).

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 Varian (Walton-on-Thames, UK), model 5000; the Lichrosorb-NH_2 column was from Merck (Darmstadt, FRG); the µBondapak TM C_{18} column was from Waters Associates (Milford, MA 01757, USA); HPLC solvents were from Carlo Erba (Milano, Italy).

Collection and dilution of mucus

Human sputum (618 ml) was collected everyday from a patient with blood-group O suffering from bronchiectasis due to a Kartagener's syndrome. It was kept frozen until used. These bronchial secretions were thawed at 4°C, diluted 1 to 12 with deionized water and stirred overnight at 4°C. The diluted mucus was then centrifuged at 3000 × *g* for 30 min. The dialyzed and lyophilized pellet and supernatant corresponded to 4.82 g and 7.46 g respectively.

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

The lyophilized supernatant was digested with pronase in 0.01 M calcium acetate for 48 h at 37°C using an enzyme/substrate ratio of 1/40 and a fresh addition of enzyme at 24 h. After 48 h, the mixture was centrifuged to give a pellet (1.521 g) and a supernatant (7.2 g lyophilized material corresponding to 2.16 g dialyzed and lyophilized material).

Aliquots (150 mg) of freeze-dried supernatant (7.2 g) were submitted to gel filtration on a column of Sepharose CL-2B (2.5 × 48 cm) equilibrated and eluted with 6 M guanidinium chloride. Fractions (5 ml) were collected and analyzed for absorbance at 278 nm and for hexose by an automated orcinol assay [2]. Three fractions were obtained; after dialysis and freeze-drying, the yields amounted to: P1 (66 mg), P2 (900 mg) and P3 (1119 mg) (Fig. 1).

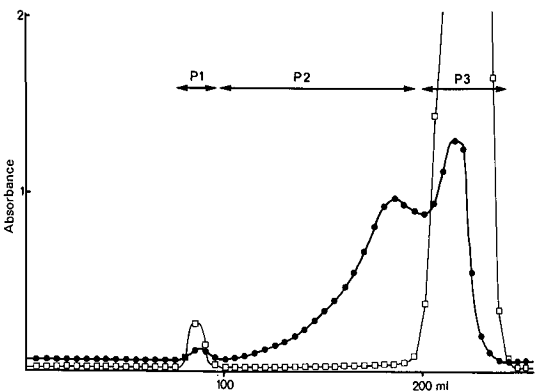


Fig. 1. *Sepharose CL-2B (2.5 × 48 cm) chromatography of pronase-treated mucus supernatant (150 mg).* The eluting solution was 6 M guanidinium hydrochloride. Representative aliquots were analyzed for neutral sugars (●) and for absorbance at 278 nm (□) [2]

Table 1. *Composition of mucus glycopeptide fraction P2*

Components	Content
	µmol/g
Amino acids	1207
Aspartic acid	53
Threonine	304
Serine	178
Glutamic acid	79
Proline	132
Glycine	108
Alanine	109
Valine	51
Methionine	3
Isoleucine	29
Leucine	58
Tyrosine	9
Phenylalanine	20
Lysine	21
Hystidine	24
Arginine	29
N-Acetylneuraminic acid	256
Fucose	567
Galactose	1389
N-Acetylglucosamine	805
N-Acetylgalactosamine	421
Sulfate	358

The chemical composition of fraction P2 is typical of mucus glycopeptides (Table 1): it contains 70.9% carbohydrate, 2.9% sulfate and 12.1% amino acid, serine and threonine representing 39.4 residues out of 100 amino acid residues. Polyacrylamide gel electrophoresis of fraction P2 showed a typical pattern of mucus glycopeptides when stained by Schiff/periodate (data not shown).

Purification of oligosaccharides

Alkaline borohydride reductive degradation of bronchial glycopeptides (fraction P2) led to a heterogeneous population

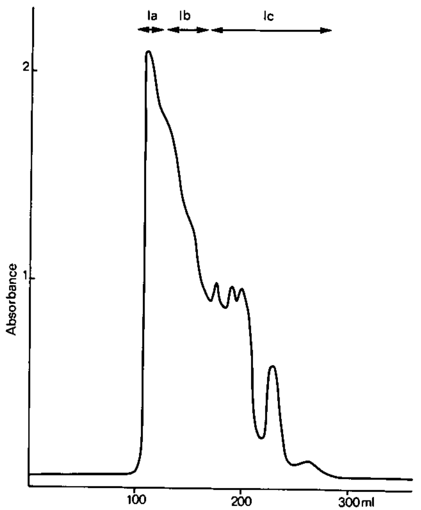


Fig. 2. *Bio-Gel P-4 (200–400 mesh) elution profile of fraction I obtained after alkaline treatment of fraction P2.* The column (2 × 98 cm) was eluted with 0.1 M acetic acid. Aliquots were analyzed for neutral sugars [2]

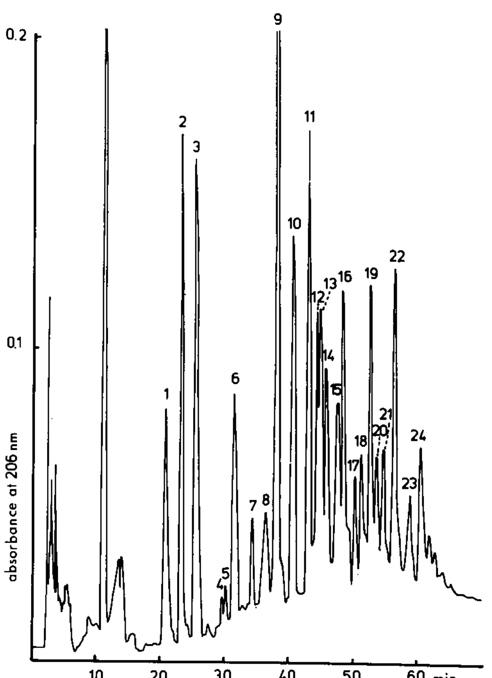


Fig. 3. *HPLC elution profile of bronchial oligosaccharide-alditols of fraction Ic on a 5 μm Lichrosorb-NH_2 column, eluted with a linear acetonitrile/water gradient (85/15 up to 60/40)*

of glycopeptides and reduced oligosaccharides. This mixture was applied to a column of AG50WX8 and the fraction eluted by water was further separated by ion-exchange chromatography on a Dowex AG1X2 column according to acidity as previously described [2]. Four fractions were obtained: I (neutral), II (sialylated) and III and IV (sulfated). Fraction I was subfractionated by chromatography on Bio-Gel P4 (Fig. 2). A pool of smaller-size neutral oligosaccharide-alditols Ic (65.1 mg) was obtained by this procedure.

Fractionation of neutral oligosaccharide-alditols of fraction Ic was carried out by HPLC on a Lichrosorb-NH_2 column (25 × 0.46 cm internal diameter, particle size 5 μm) (Fig. 3). Elution was performed with a linear gradient of 85/15 to 60/40 (v/v) acetonitrile/water, during 60 min at room temperature and at a flow rate of 1 ml/min [3]. Oligosaccharide peaks were detected by absorption at 206 nm after which 24 fractions were obtained. A reverse-phase column was used for further separation. Fractions 8, 10, 11, 15, 16, 17, 21, 22 and 23 were subfractionated on a μBondapak TM C_{18} column (30 × 0.39 cm internal diameter, particle size 10 μm), eluted with water at a flow rate of 1 ml/min at room temperature, and gave fractions 8a–d; 10a, b; 11a, b; 15a, b; 16a–d; 17a, b; 21a, b; 22a–c; 23a–c respectively (Fig. 4).

Analytical methods

Amino acid analysis was performed according to Houdret et al. [5]. Sulfate and sialic acid were measured as described [2]. Quantitative sugar analysis was carried out as described before [6].

^{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 WM-500 or AM-500 spectrometer (SON hf-NMR facility, Department of Biophysical Chemistry, University of Nijmegen, The Netherlands), operating under control of an Aspect-2000 or Aspect-3000 computer. Experimental details have been described [6–9]. Resolution enhancement of the spectra was achieved by Lorentzian-to-Gaussian transformation. The probe temperature was kept at 27.0 (± 0.1) °C. Chemical shifts (δ) are expressed in ppm 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 27 °C), with an accuracy of 0.002 ppm.

RESULTS

Structure determination

The molar carbohydrate compositions of all oligosaccharide-alditol fractions, obtained after HPLC (sub)fractionation of the pool of neutral oligosaccharide-alditols, were determined by sugar analysis. Table 2 lists the carbohydrate compositions of only those fractions of which the primary structure of the constituting oligosaccharide-alditol(s) has been deduced from the combination of sugar-analysis and 500-MHz ^{1}H-NMR spectroscopy. The amounts of material of fractions 4, 5, 8a, 11b, 21b and 22a were too low for quantitative sugar analysis.

500-MHz ^{1}H-NMR spectra have been recorded of all HPLC fractions or subfractions, except for fractions 8a, which contained too low an amount of material to permit NMR analysis. The spectra of fractions 4, 8b, 16b and 22a indicate the presence of carbohydrate material, but the chemical shifts of the structural-reporter groups can not be interpreted in terms of a primary structure of one or more oligosaccharide-alditols, owing to lack of reference data or to the presence of more than compound in nearly equal amounts. The structures of the oligosaccharide-alditols of the Galβ(1→3)GalNAc-ol type (core types 1 and 2), that have been deduced from the ^{1}H-NMR spectra, are compiled in Schemes 1–3. The struc-

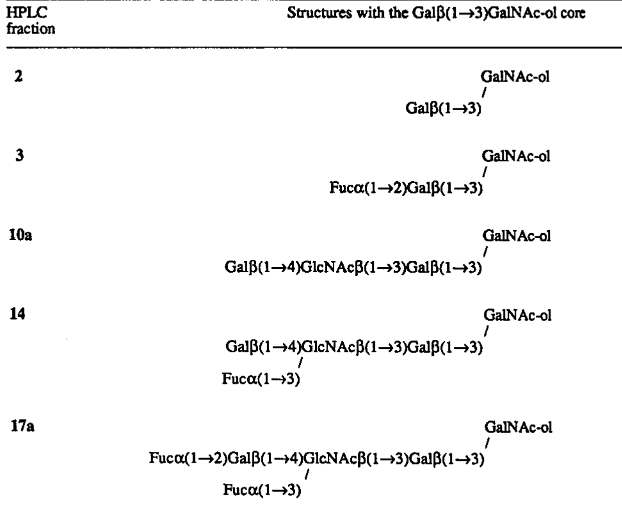
Fig. 4. *Subfractionation of fractions 8, 10, 11, 15, 16, 17, 21, 22 and 23 by a second HPLC run.* Elution was performed isocratically with water on a 10-µm µBondapack TM C_{18} column. Oligosaccharide peaks were detected by absorbance at 206 nm

tures of the oligosaccharide-alditols that are of the GlcNAcβ(1→3) core type (GlcNAcβ(1→3)GalNAc-ol i. e. core types 3 and GlcNAcβ(1→3)[GlcNAcβ(1→6)]GalNAc-ol i. e. core type 4) are described in the accompanying paper [10].

The structures are arranged according to a common structural element for reasons of surveyability and to facilitate the discussion of the deduction of the primary structures of the oligosaccharide-alditols from the ^{1}H-NMR spectra. The chemical shifts of the structural-reporter groups are compiled in Tables 3–5.

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

In the series of oligosaccharide-alditols identified in the HPLC-purified fractions, five contain GalNAc-ol being monosubstituted with Gal in β(1→3) linkage. The structures of these compounds are listed in Scheme 1, while the ^{1}H-NMR parameters are compiled in Table 3. Oligosaccharide-alditols with this core are characterized by the H-2 and H-5 signal of GalNAc-ol at $\delta \approx 4.39-4.40$ ppm and $\delta \approx 4.16-4.19$ ppm, respectively [7]. For compounds wherein Gal^3 is substituted, the position of the NAc signal of GalNAc-ol at about 2.047 ppm is also characteristic. Extensions of this core by substitutions on Gal^3 determine the exact position of H-2 and H-5 of GalNAc-ol. The ^{1}H-NMR spectra of fractions 2, 3, 10a and 14 match those for oligosaccharide-alditols obtained from cystic fibrosis sputum (oligosaccharides 2, 3, 10b and 14 of [1]); the primary structures of these compounds were identified as listed in Scheme 1.



HPLC fraction	Structures with the Galβ(1→3)GalNAc-ol core
2	Galβ(1→3)GalNAc-ol
3	Fucα(1→2)Galβ(1→3)GalNAc-ol
10a	Galβ(1→4)GlcNAcβ(1→3)Galβ(1→3)GalNAc-ol
14	Galβ(1→4)[Fucα(1→3)]GlcNAcβ(1→3)Galβ(1→3)GalNAc-ol
17a	Fucα(1→2)Galβ(1→4)[Fucα(1→3)]GlcNAcβ(1→3)Galβ(1→3)GalNAc-ol

Scheme 1. *Structures of neutral oligosaccharide-alditols of the Galβ(1→3)GalNAc-ol core type (type 1), obtained by HPLC fractionation of a pool of neutral oligosaccharide-alditols from Kartageners' syndrome sputum*

From the ^{1}H-NMR spectrum of fraction 17a (Fig. 5) together with the results of sugar analysis (see Table 2) it is concluded that the fraction contains a hexasaccharide-alditol composed of Gal, GlcNAc, Fuc and GalNAc-ol in the molar

HPLC fraction	Structures with the Galβ(1→3)[GlcNAcβ(1→6)]GalNAc-ol core
7	GlcNAcβ(1→6) \ GalNAc-ol / Galβ(1→3)
11a	Galβ(1→4)GlcNAcβ(1→6) \ GalNAc-ol / Galβ(1→3)
15a	Fucα(1→2)Galβ(1→4)GlcNAcβ(1→6) \ GalNAc-ol / Galβ(1→3)
16a	Galβ(1→4)GlcNAcβ(1→6) / Fucα(1→3) \ GalNAc-ol / Galβ(1→3)
21a	Galβ(1→4)GlcNAcβ(1→6) \ GalNAc-ol / Galβ(1→4)GlcNAcβ(1→3)Galβ(1→3)
23a	Galβ(1→4)GlcNAcβ(1→6) \ GalNAc-ol / Galβ(1→4)GlcNAcβ(1→3)Galβ(1→3) / Fucα(1→3)

Scheme 2. *Structures of neutral oligosaccharide-alditols of the Galβ(1→3)[GlcNAcβ(1→6)]GalNAc-ol core type (type 2), obtained by HPLC fractionation of a pool of neutral oligosaccharide-alditols from Kartageners' syndrome sputum*

HPLC fraction	Structures with the common element Fucα(1→2)Galβ(1→3)[GlcNAcβ(1→6)]GalNAc-ol
8d	GlcNAcβ(1→6) \ GalNAc-ol / Fucα(1→2)Galβ(1→3)
12	Galβ(1→4)GlcNAcβ(1→6) \ GalNAc-ol / Fucα(1→2)Galβ(1→3)
16c	Galβ(1→4)GlcNAcβ(1→6) / Fucα(1→3) \ GalNAc-ol / Fucα(1→2)Galβ(1→3)
16d	Fucα(1→2)Galβ(1→4)GlcNAcβ(1→6) \ GalNAc-ol / Fucα(1→2)Galβ(1→3)
20	Fucα(1→2)Galβ(1→4)GlcNAcβ(1→6) / Fucα(1→3) \ GalNAc-ol / Fucα(1→2) Galβ(1→3)

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

ratio 2:1:2:1. GalNAc-ol is substituted at C-3 by βGal, as can be inferred from the H-2 and H-5 signal of GalNAc-ol at $\delta = 4.396$ ppm and $\delta = 4.177$ ppm respectively [1]. This Gal^3 is substituted at C-3 as is evident from the position of H-1

Table 2. *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 of the compounds on μBondapak TM C_{18} have been included. n.d. is not determined

Oligo-saccharide-alditol fraction	Molar ratio of monosaccharides				Retention times	Amount
	Fuc	Gal	GlcNAc	GalNAc-ol	min	μg
1	–	–	0.9	1	4.3	144
2	–	1.1	–	1	3.3	570
3	0.7	1.5	–	1	12.5	531
4	n.d.	n.d.	n.d.	n.d.	5.9	n.d.
5	n.d.	n.d.	n.d.	n.d.	5.2	n.d.
6	–	0.9	0.9	1	5.0	78
7	–	1.2	0.9	1	3.5	117
8a	n.d.	n.d.	n.d.	n.d.	4.3	n.d.
8b	0.8	0.8	1.1	1	5.3	45
8c	0.8	0.9	0.8	1	9.4	123
8d	0.6	0.7	0.7	1	13.5	72
9	1	1.3	0.8	1	5.4	546
10a	–	2.1	0.9	1	4.0	360
10b	–	1	1.8	1	5.7	255
11a	–	2	0.8	1	3.8	405
11b	n.d.	n.d.	n.d.	n.d.	4.8	n.d.
12	0.8	1.9	0.8	1	14.3	810
13	0.9	1.3	1.8	1	10.3	63
14	0.7	1.7	1.1	1	3.6	186
15a	0.9	2.3	0.8	1	5.5	120
15b	–	2.1	1.8	1	7.0	240
16a	0.8	1.8	0.8	1	3.6	102
16b	0.9	1.6	0.9	1	5.2	54
16c	2.0	2.2	1.3	1	16.8	9
16d	1.8	2.0	0.9	1	25.5	477
17a	1.4	1.9	0.9	1	4.5	90
17b	1.4	1.2	2.0	1	7.8	78
18	1.0	2.3	1.8	1	16.6	171
19	0.8	1.5	1.7	1	8.5	246
20	2.1	1.8	1.0	1	22.5	70
21a	–	2.8	1.7	1	5.0	97
21b	n.d	n.d.	n.d.	n.d.	45.0	n.d.
22a	n.d	n.d.	n.d.	n.d.	8.6	n.d.
22b	n.d.	n.d.	n.d.	n.d.	10.1	n.d.
22c	1.7	2.3	1.4	1	17.1	300
23a	1.0	3.1	1.8	1	4.5	78
23b	2.2	2.0	1.9	1	17.8	96
23c	n.d.	n.d	n.d.	n.d.	24.3	n.d.
24	3.6	2.5	1.8	1	11.0	307

($\delta = 4.463$ ppm) and H-4 ($\delta = 4.111$ ppm) of this sugar (cf. compound 14). The ^{1}H-NMR spectrum indicates the presence of two different Fuc residues. One set of Fuc structural reporters, i.e., H-1, H-5 and CH_3 at $\delta = 5.273$ ppm, 4.248 ppm and 1.266 ppm, respectively, indicates for Fuc in an α(1→2) linkage to Gal [7, 11], while the other set, i.e. H-1, H-5 and CH_3 at $\delta = 5.127$ ppm, 4.873 ppm and 1.236 ppm, respectively, points to Fuc in an α(1→3) type of linkage [7]. The assignment of the CH_3 signals of Fuc^2 and Fuc^3 was verified by irradiation of Fuc^2 H-5 at $\delta = 4.248$ ppm. The exact positions of these Fuc structural reporters, together with the Gal H-1 signal at $\delta = 4.510$ ppm, demonstrate the presence in structure 17a of the Y determinant i.e. Fucα(1→2)Galβ(1→4)-[Fucα(1→3)]GlcNAcβ(1→) [12, 13]. These observations together determine compound 17a to be Fucα(1→2)Galβ-

Table 3. *^{1}H chemical shifts of structural-reporter groups of constituent monosaccharides for the HPLC-fractionated, neutral bronchial oligosaccharide-alditols with GalNAc-ol bearing Gal in β(1→3) linkage as single substituent*
Chemical shifts, expressed in ppm, are relative to internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate (using internal acetone a 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. [6]); ◇ = GalNAc-ol; ● = GlcNAc; ■ = Gal and □ = Fuc. The position of linkage in this notation is specified by the angle of the connecting bar as follows: [6, 4, 3, 2 diagram]. A superscript at the name of a sugar indicates to which position of the adjacent monosaccharide it is glycosidically linked (cf. [7])

Residue	Reporter group	Chemical shift in compound				
		2	3	10a	14	17a
		ppm				
GalNAc-ol	H-2	4.392	4.397	4.396	4.397	4.396
	H-3	4.064	4.089	4.044	4.050	4.046
	H-4	3.507	3.521	3.497	3.498	3.496
	H-5	4.194	4.161	4.184	4.181	4.177
	NAc	2.051	2.046	2.048	2.047	2.046
Gal^3	H-1	4.477	4.582	4.463	4.465	4.463
	H-4	3.901	3.926	4.126	4.126	4.111
GlcNAc^3	H-1	–	–	4.688	4.697	4.75
	H-6	–	–	3.953	3.967	3.992
	NAc	–	–	2.042	2.032	2.035
Gal^4	H-1	–	–	4.480	4.460	4.510
	H-4	–	–	3.928	3.900	3.87
Fuc^2	H-1	–	5.254	–	–	5.273
	H-5	–	4.276	–	–	4.248
	CH_3	–	1.244	–	–	1.266
Fuc^3	H-1	–	–	–	5.139	5.127
	H-5	–	–	–	4.833	4.873
	CH_3	–	–	–	1.177	1.236

(1 → 4)[Fucα(1 → 3)]GlcNAcβ(1 → 3)Galβ(1 → 3)GalNAc-ol. Compound 17a can be considered an extension of compound 14 with Fuc in α(1→2) linkage to Gal^4. The shift effects of this fucosylation on the ^{1}H-NMR parameters of compound 14 are an upfield shift for H-1 of Fuc^3 of 0.012 ppm and downfield shifts for CH_3 of Fuc^3, for H-1 of Gal^4 and for NAc of GlcNAc^3 of 0.059 ppm, 0.050 ppm and 0.003 ppm respectively. These chemical-shift differences are comparable to what was observed for synthetic blood-group antigenic X and Y determinants [13, 14]. It should be noted that, in the 300-MHz ^{1}H-NMR spectrum of the Y-determinant-containing oligosaccharide-alditol, obtained from ovarian cyst mucins [15], the assignment of the CH_3 signals of Fuc^2 and Fuc^3 has to be interchanged (see also compound 20).

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

The structures of the oligosaccharide-alditols that have been found to contain the Galβ(1→3)[GlcNAcβ(1→6)]-GalNAc-ol core are listed in Scheme 2, and their ^{1}H-NMR parameters are compiled in Table 4. *O*-Glycosidic structures with this core are characterized by the H-2 and H-5 signals of GalNAc-ol at $\delta = 4.39-4.40$ ppm and $\delta = 4.27-4.28$ ppm, respectively, and by the NAc signal of GalNAc-ol at about 2.067 ppm [7]. The positions of the structural-reporter groups of Gal^3 and GlcNAc^6 are highly dependent on substitutions on these sugars. The ^{1}H-NMR spectra of fractions 7, 11a, 15a and 16a are identical to those observed earlier for oligosaccharide-alditols (6, 11, 15.1A and 15.2B) obtained from cystic fibrosis sputum [1]. The corresponding primary structures are summarized in Scheme 3. The deduction of these structures from the ^{1}H-NMR spectra has been given with these spectra (7, 11a and 15a in [7] and 16a in [1]).

The ^{1}H-NMR spectrum, together with the sugar analysis, of fraction 21a (Fig. 6) indicates the presence of a hexasaccharide-alditol containing Gal, GlcNAc and GalNAc-ol in the molar ratio of 3:2:1. The core element of the oligosaccharide-alditol is Galβ(1→3)[GlcNAcβ(1→6)]-GalNAc-ol as is shown by the chemical shifts of GalNAc-ol H-2 at $\delta = 4.397$ ppm and of H-5 at $\delta = 4.266$ ppm. The GlcNAc^6 residue is substituted by Gal in β(1→4) linkage, as is evident from the chemical shift of H-1 of GlcNAc^6 (δ = 4.554 ppm), while the terminal position of Gal^4 can be inferred from its H-1 signal at $\delta = 4.468$ ppm (compare Gal^4 in compound 11a). The chemical shift of Gal^3 H-4 at δ = 4.125 ppm is characteristic for the →)GlcNAcβ(1→3)Galβ(→ sequence [8]. GlcNAc^3 in this sequence is substituted by Gal in a β(1→4) type of linkage, as can be deduced from the position of H-1 of this terminal Gal (δ = 4.481 ppm), together

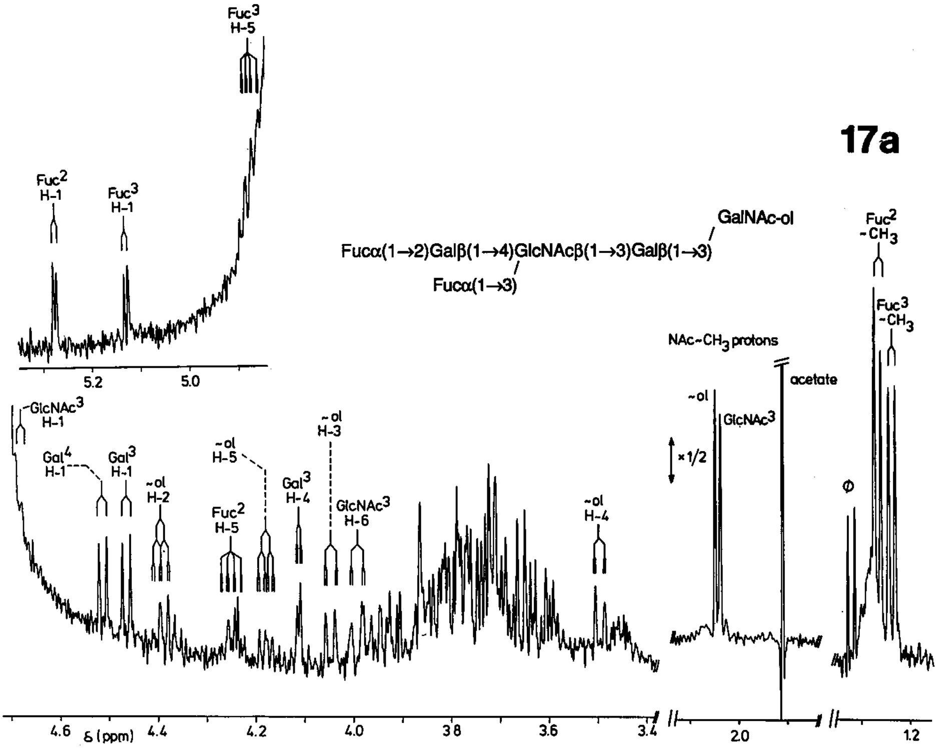


Fig. 5. *Resolution-enhanced 500-MHz ^{1}H-NMR spectrum (^2H_2O, 27°C) of HPLC fraction 17a, obtained from the pool of neutral oligosaccharide-alditols Ic from bronchiectasis sputum.* The relative-intensity scale of the *N*-acetyl methyl proton region of the spectrum differs from that of the other parts, as indicated. Signals marked by ϕ stem from a frequently occurring, non-protein non-carbohydrate contaminant

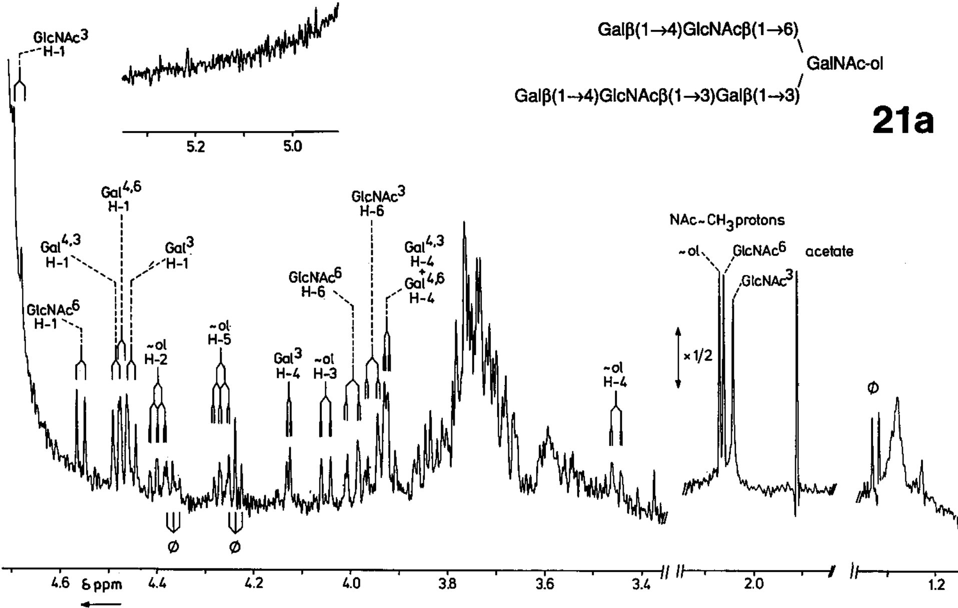


Fig. 6. *Resolution-enhanced 500-MHz ^{1}H-NMR spectrum (^2H_2O, 27°C) of HPLC fraction 21a, obtained from the pool of neutral oligosaccharide-alditols Ic from bronchiectasis sputum.* The relative-intensity scale of the *N*-acetyl methyl proton region of the spectrum differs from that of the other parts, as indicated. Signals marked by ϕ stem from a frequently occurring, non-protein non-carbohydrate contaminant

with the position of the NAc signal of GlcNAc[3] at $\delta =$ 2.039 ppm [16]. The 3-branch is thus identical to 10a. Combination of the aforementioned structural elements affords structure 21a to be Galβ(1→4)GlcNAcβ(1→3)Galβ(1→3)-[Galβ(1→4)GlcNAcβ(1→6)]GalNAc-ol.

The ^{1}H-NMR spectrum of fraction 23a (see Fig. 7) indicates the presence of a small amount of carbohydrate material consisting of a major and a minor compound ($\approx 25\%$). Owing to the low amount of material, the structure of the minor component could not be derived from the spectrum. The core structure of the major component of 23a is Galβ-(1→3)[GlcNAcβ(1→6)]GalNAc-ol, as revealed by the positions of H-2 and H-5 of GalNAc-ol at $\delta = 4.397$ ppm and $\delta = 4.266$ ppm, respectively. The GlcNAc[6] residue is part of

Table 4. *^{1}H chemical shifts of structural-reporter groups of constituent monosaccharides for the HPLC-fractionated, neutral bronchial oligosaccharide-alditols possessing the Galβ(1→3)[GlcNAcβ(1→6)]GalNAc-ol core 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. [7])

Residue	Reporter group	Chemical shift in compound					
		7	11a	15a	16a	21a	23a
		ppm					
GalNAc-ol	H-2	4.391	4.391	4.392	4.391	4.397	4.397
	H-3	4.069	4.060	4.060	4.059	4.048	4.048
	H-4	3.468	3.466	3.467	3.454	3.50	n.d.
	H-5	4.277	4.281	4.281	4.269	4.266	4.266
	NAc	2.066	2.067	2.067	2.068	2.066	2.066
Gal^3	H-1	4.464	4.465	4.464	4.462	4.449	4.447
	H-4	3.900	3.900	3.89	3.90	4.125	4.126
GlcNAc^6	H-1	4.537	4.559	4.538	4.560	4.554	4.554
	H-6	3.931	3.997	3.990	4.010	3.993	3.995
	NAc	2.066	2.065	2.067	2.056	2.058	2.059
GlcNAc^3	H-1	–	–	–	–	4.684	4.69
	H-6	–	–	–	–	3.952	3.966
	NAc	–	–	–	–	2.039	2.029
Gal^{4,6}	H-1	–	4.470	4.537	4.446	4.468	4.466
	H-4	–	3.925	3.90	3.92	3.925	3.925
Gal^{4,3}	H-1	–	–	–	–	4.481	4.464
	H-4	–	–	–	–	3.925	3.900
Fuc^2	H-1	–	–	5.308	–	–	–
	H-5	–	–	4.225	–	–	–
	CH_3	–	–	1.233	–	–	–
Fuc^3	H-1	–	–	–	5.108	–	5.138
	H-5	–	–	–	4.835	–	n.d.
	CH_3	–	–	–	1.174	–	1.176

a terminal *N*-acetyllactosamine unit, as is evident from the chemical shifts of H-1 of GlcNAc^6 at $\delta = 4.554$ ppm and of Gal^{4,6} at $\delta = 4.466$ ppm (see also the 6-branch in 11a). The chemical shift of Gal^3 H-4 at $\delta = 4.126$ ppm indicates the 3-branch of GalNac-ol to be elongated by β(1→3) linked GlcNAc. The H-1 signal of GlcNAc^3 at $\delta = 4.69$ ppm (partly obscured by the HDO line), together with the structural-reporter groups of a single Fuc residue, i.e. H-1 ($\delta = 5.138$ ppm) and CH_3 ($\delta = 1.176$ ppm), are indicative of the X determinant Galβ(1→4)[Fucα(1→3)]GlcNAcβ(1→) [7]. This element is further demonstrated by the H-1 signal of Gal^4 at $\delta =$ 4.464 ppm. This determines the structure of the major oligosaccharide-alditol in fraction 23a to be Galβ(1→4)[Fucα-(1 → 3)]GlcNAcβ(1 → 3)Galβ(1 → 3)[Galβ(1 → 4)GlcNAcβ-(1→6)]GalNAc-ol. Structure 23a is an extension of 21a with Fuc in α(1→3) linkage to GlcNAc^3 and the effects of this extension are similar to those observed in the ^{1}H-NMR spectrum when going from 10a to 14, i.e. an upfield shift for NAc of GlcNAc^3 ($\Delta\delta = -0.010$ ppm) and for H-1 of Gal^{4,3} ($\Delta\delta = -0.017$ ppm), and an unaltered chemical shift of Gal^3 H-1.

Structures with the common element Fucα(1→2)Galβ1→3)[GlcNAcβ(1→6)]GalNAc-ol

Scheme 3 lists the compounds identified in the HPLC fractions, the structures of which are extensions of Fucα(1→2)-Galβ(1→3)[GlcNAcβ(1→6)]GalNAc-ol. The ^{1}H-NMR chemical shifts of the structural-reporter groups of these oligosaccharide-alditols are presented in Table 5. The ^{1}H-NMR spectra of compounds in this group are characterized by a series of constant spectral features stemming from the 3-branch, whereas variations are due to different extensions of the 6-branch. Typical chemical shifts are the H-2, H-5 and the NAc signals of GalNAc-ol at $\delta \approx 4.40$ ppm, 4.24–4.26 ppm and 2.054–2.055 ppm respectively. Furthermore H-1 of Gal^3 at $\delta \approx 4.57$ ppm and H-1, H-5 and CH_3 of Fuc^{2,3} at $\delta \approx 5.22$ ppm, 4.27 ppm and 1.23–1.24 ppm, respectively, are recurrent signals. The positions of the structural-receptor groups of GlcNAc^6 are strongly influenced by substitutions on this sugar.

The ^{1}H-NMR spectrum of fraction 8d (see Fig. 8), together with the sugar analysis (see Table 2), indicate the presence of a tetrasaccharide-alditol containing Gal, GlcNAc, Fuc and GalNAc-ol in a molar ratio of 1:1:1:1. The core, as revealed by the position of H-2 and H-5 of GalNAc-ol at $\delta = 4.401$ ppm and 4.236 ppm, respectively, is Galβ(1→3)[GlcNAcβ(1→6)]GalNAc-ol [7]. The Fuc residue in compound 8d is α(1→2)-linked to Gal^3, as can be deduced from the typical set of chemical shifts of Fuc, i.e. CH_3, H-1 and H-5 at $\delta = 1.244$ ppm, 5.221 ppm and 4.274 ppm respectively. Therefore, the structure of 8d is Fucα(1→2)Galβ(1→3)-

Table 5. *^{1}H chemical shifts of structural-reporter groups of constituent monosaccharides for the HPLC-fractionated, neutral bronchial oligosaccharide-alditols possessing the Fucα(1→2)Galβ(1→3)[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. [7]). For reference purposes compound 7 is repeated from Table 4

Residue	Reporter group	Chemical shift in compound					
		7	8d	12	16c	16d	20
		ppm					
GalNAc-ol	H-2	4.391	4.401	4.402	4.401	4.401	4.401
	H-3	4.069	4.085	4.083	4.085	4.083	4.081
	H-4	3.468	3.501	3.499	n.d.	3.498	3.486
	H-5	4.277	4.236	4.256	4.24	4.255	4.249
	NAc	2.066	2.054	2.055	2.055	2.054	2.054
Gal^{3}	H-1	4.464	4.572	4.572	4.575	4.573	4.570
	H-4	3.900	3.924	3.924	3.923	3.924	3.921
GlcNAc^{6}	H-1	4.537	4.551	4.572	4.569	4.554	4.563
	H-6	3.931	3.937	3.998	4.010	3.993	4.030
	NAc	2.066	2.057	2.055	2.047	2.059	2.049
Gal^{4}	H-1	–	–	4.470	4.448	4.537	4.497
	H-4	–	–	3.924	3.92	3.924	n.d.
Fuc^{2,3}	H-1	–	5.221	5.221	5.218	5.220	5.217
	H-5	–	4.274	4.273	4.273	4.274	4.273
	CH_3	–	1.244	1.245	1.233	1.244	1.244
Fuc^{2,4}	H-1	–	–	–	–	5.309	5.279
	H-5	–	–	–	–	4.226	4.242
	CH_3	–	–	–	–	1.232	1.271
Fuc^{3}	H-1	–	–	–	5.112	–	5.100
	H-5	–	–	–	n.d.	–	4.871
	CH_3	–	–	–	1.175	–	1.235

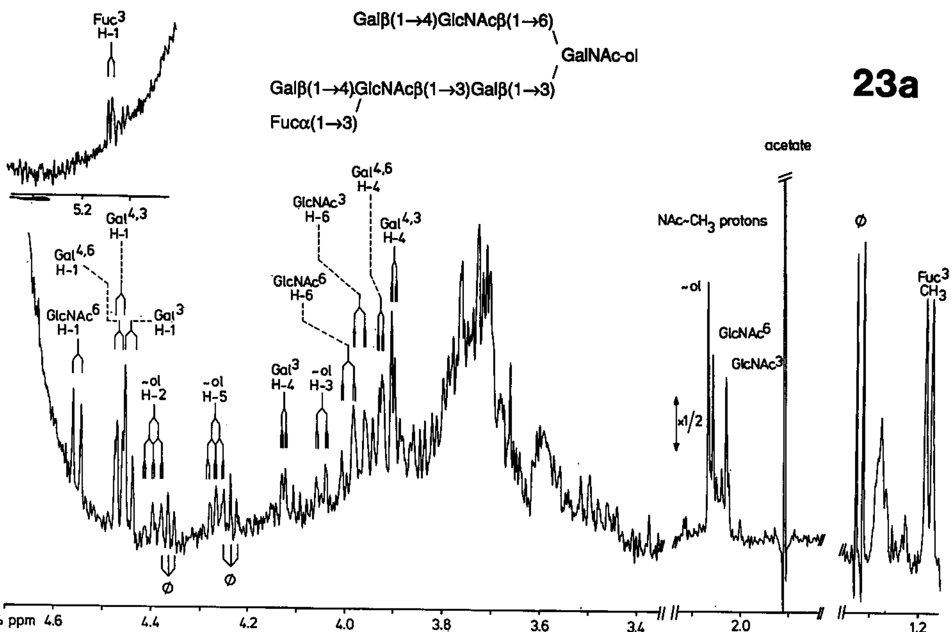


Fig. 7. *Resolution-enhanced 500-MHz ^{1}H-NMR spectrum ($^{2}H_2O$, 27°C) of HPLC fraction 23a, obtained from the pool of neutral oligosaccharide-alditols Ic from bronchiectasis sputum.* The relative-intensity scale of the *N*-acetyl methyl proton region of the spectrum differs from that of the other parts, as indicated. Signals marked by ϕ stem from a frequently occurring, non-protein non-carbohydrate contaminant

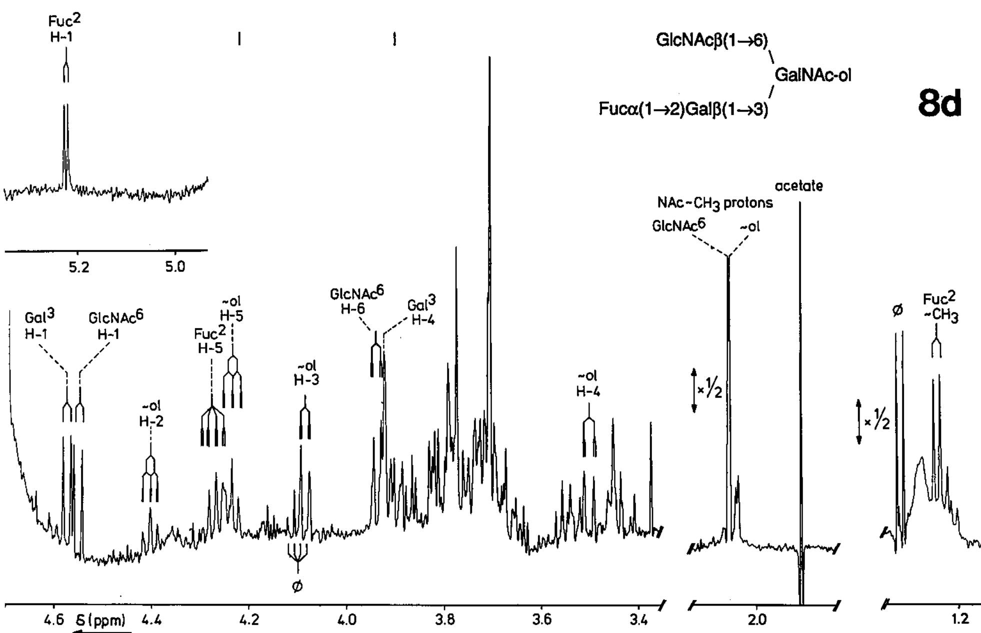


Fig. 8. *Resolution-enhanced 500-MHz ^{1}H-NMR spectrum (^2H_2O, 27°C) of HPLC fraction 8d, obtained from the pool of neutral oligosaccharide-alditols Ic from bronchiectasis sputum.* The relative-intensity scale of the *N*-acetyl and Fuc methyl proton regions of the spectrum differs from that of the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

[GlcNAcβ(1→6)]GalNAc-ol; it is the common element for the structures in this group (compounds 12, 16c, 16d and 20). Structure 8d is the analogue of compound 7, bearing fucose in α(1→2) linkage (see Table 4). The effects of this Fuc attachment on the chemical shift of the anomeric protons of Gal^3 and GlcNAc^6, being + 0.108 ppm and + 0.014 ppm respectively, are analogous to the shift effects observed for the same protons when comparing 2 and 3; 11 and 12; and 15.1A and 15.3 in [1]. Compared to the spectral interpretation by Dua et al. [15] for the structure 8d, the assignment for H-1 of Gal^3 and GlcNAc^6 has been reversed. The chemical shift for H-1 of Gal^3 is now in line with that for the other structures in Table 5 containing the same 3-branch. Furthermore, with the present interpretation the shift-effects for Fuc attachment are in full agreement with what has been observed before [1]. The assignment of the NAc signals was achieved by comparison of the chemical shifts of the NAc signals of 8d and 12; analogously to the observation made for 7 and 11a, extension of the 6-branch by Gal in β(1→4) linkage induces a small upfield shift for NAc of GlcNAc^6 (cf. [8]) and a minor downfield shift for NAc of GalNAc-ol.

The ^{1}H-NMR spectral features of fractions 12 and 16d are identical to those of two oligosaccharide-alditols identified in HPLC fractions (12 and 15.3) of reduced bronchial mucus of cystic fibrosis patients [1]. The structures of compounds 12 and 16d are therefore as indicated in Scheme 3. Oligosaccharide-alditol 12 is an extension of 8d with Gal in β(1→4) linkage to GlcNAc^6. The observed shift effects for the structural reporter groups of GlcNAc^6 agree with this, i.e. a downfield shift for H-1 of 0.021 ppm and a slight upfield shift for the NAc signal of 0.002 ppm [1]. The shift effect on the NAc signal of GalNAc-ol is negligible. Compound 16d can be regarded as an analog of 12 with Fuc in α(1→2) linkage to Gal^4.

The ^{1}H-NMR spectrum of fraction 16c indicates the presence of two Fuc residues, one of which is α(1→2) linked to Gal^3 as is evident from the chemical shifts of Fuc^2 H-1 at δ = 5.218 ppm and of Fuc^2 CH$_3$ at δ = 1.233 ppm [1]. Together with signals for H-2 and NAc of GalNAc-ol at δ = 4.401 ppm and δ = 2.055, a signal for H-1 of Gal^3 at δ = 4.575 ppm and a signal for Fuc^2 H-5 at δ = 4.273 ppm this provides sufficient evidence to propose the occurrence of the element Fucα(1→2)Galβ(1→3)GalNAc-ol (compare the 3-branch of the three foregoing structures). The position of H-5 of GalNAc-ol could not be determined owing to the presence of signals from non-carbohydrate contaminants in the region of the spectrum where this signal is expected. The ^{1}H-NMR parameters of the second Fuc residue, i.e. H-1 at δ = 5.112 ppm and CH$_3$ at δ = 1.175 ppm are indicative of Fuc to be involved in the element Galβ(1→4)[Fucα(1→3)]-GlcNAcβ(1→6). This is supported by the Gal^4 and GlcNAc^6 H-1 signals at δ = 4.448 ppm and 4.569 ppm respectively (compare the 6-branch of 16a). Combination of the above-mentioned elements suggests structure 16c to be Fucα(1→2)-Galβ (1 → 3) [Galβ (1 → 4) [Fucα (1 → 3)] GlcNAcβ - (1→6)]GalNAc-ol. This structure can be regarded as an extension of compound 12 with Fuc in α(1→3) linkage to GlcNAc^6 and in fact the concomitant shift effects confirm this [6], i.e. an upfield shift for NAc of GlcNAc^6 and for H-1 of Gal^4 of 0.008 ppm and 0.022 ppm, respectively, and negligible shift effects on NAc of GalNAc-ol and H-1 of GlcNAc^6.

The ^{1}H-NMR spectrum of fraction 20 (see Fig. 9), together with the results of the sugar analysis (see Table 2) indicates the presence of a heptasaccharide-alditol with Gal, GlcNAc, Fuc and GalNAc-ol in a molar ratio of 2:1:3:1. The ^{1}H-NMR spectrum suggests also the presence of a minor compound, the primary structure of which could not be determined due to the small amount of material. The presence in the main component of the structural element Fucα(1→2)Galβ(1→3)GalNAc-ol is evident from the discernible resonances of H-1 and CH$_3$ of Fuc^{2,3} at δ = 5.217 ppm and δ = 1.244 ppm, respectively, from the position of H-1 of Gal^3 at δ = 4.570 ppm, and from the position of H-2 and of NAc of GalNAc-ol at δ = 4.401 ppm and δ = 2.054 ppm respectively. Two additional Fuc residues are present, one being attached in α(1→3) linkage to GlcNAc^6, as is indicated

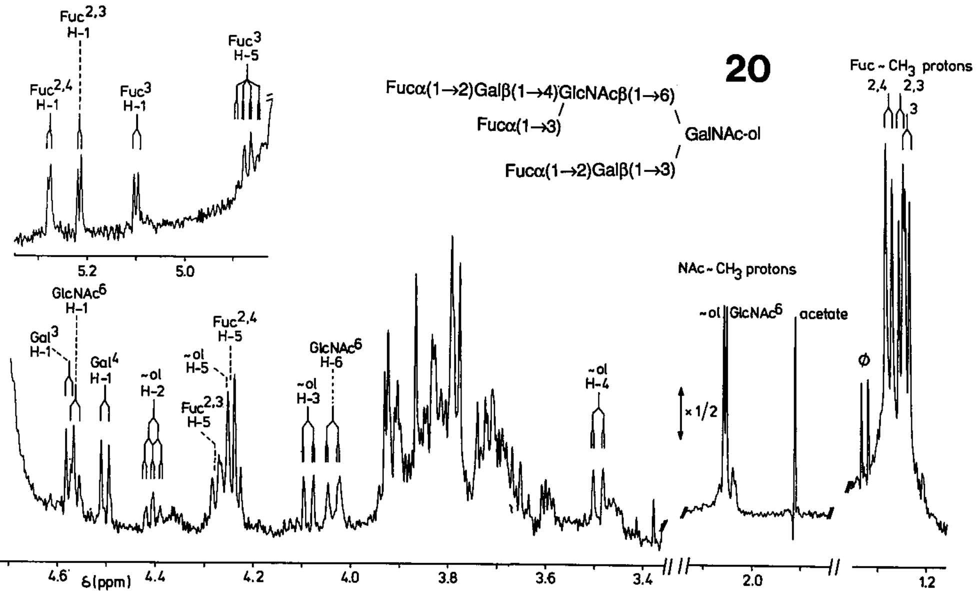


Fig. 9. *Resolution-enhanced 500-MHz ^1H-NMR spectrum (^2H_2O, 27°C) of HPLC fraction 20, obtained from the pool of neutral oligosaccharide-alditols Ic from bronchiectasis sputum.* The relative-intensity scale of the *N*-acetyl methyl proton region of the spectrum differs from that of the other parts, as indicated. Signals marked by ϕ stem from a frequently occurring, non-protein non-carbohydrate contaminant

by the position of H-1 and H-5 of this Fuc residue at $\delta = 5.100$ ppm and $\delta = 4.871$ ppm [6], respectively, the other being α(1→2) linked to Gal^4, as is suggested by the position of H-1 of this Fuc residue at $\delta = 5.279$ ppm [1]. The latter two Fuc residues form part of the Y determinant structural element, Fucα(1→2)Galβ(1→4)[Fucα(1→3)]GlcNAcβ(1→6), comparable to compound 17a. Combination of the above-indicated partial structures affords oligosaccharide 20 to be Fucα(1→2)Galβ(1→3)[Fucα(1→2)Galβ1→4)[Fucα(1→3)] GlcNAcβ(1→6)]GalNAc-ol. With respect to the Y determinant partial structure, the extension of compound 16c with Fuc in α(1→2) linkage to Gal^4 rendering compound 20, is comparable to the enlargement of 14 to form compound 17a. The shift effects on the ^1H-NMR parameters of 16c are similar, i.e. an downfield shift for CH_3 of Fuc^3 ($\Delta\delta = +0.060$ ppm), for H-1 of Gal^4 ($\Delta\delta = +0.049$ ppm) and for the NAc signal of $GlcNAc^6$ ($\Delta\delta = +0.002$ ppm). Structure 20 can also be conceived of as an extension of 16d by Fuc in α(1→3) linkage to $GlcNAc^6$. The concomitant shift effects are analogous to those observed before for synthetic blood-group antigenic determinants [13]: an upfield shift for H-1 of Gal^4 ($\Delta\delta = -0.040$ ppm), for H-1 of $Fuc^{2,4}$ ($\Delta\delta = -0.030$ ppm) and for NAc of $GlcNAc^6$ ($\Delta\delta = -0.010$ ppm) and a downfield shift for H-5 and CH_3 of $Fuc^{2,4}$ ($\Delta\delta = +0.016$ ppm and $+0.039$ ppm respectively). It should be noted that the H-1 signal of $GlcNAc^6$ has a distorted line shape, which is probably due to virtual coupling with its H-3 [17]. The assignment of GlcNAc H-2 and H-3 at 0.01 ppm distance from one another in the Y determinant [13] supports this suggestion.

DISCUSSION

Alkaline borohydride treatment of bronchial mucus glycopeptides isolated from the respiratory secretion of a patient suffering from bronchiectasis due to a Kartagener's syndrome afforded a mixture of glycopeptides and reduced oligosaccharide-alditols which was fractionated by ion-exchange chromatography and gel filtration. This procedure yielded a pool of neutral oligosaccharide-alditols (Ic), as previously described [2]. Fraction Ic was fractionated by HPLC using subsequently two columns, one containing an alkylamine-bonded phase, which led to 24 fractions, and another containing an octadecyl-bonded phase which allowed the subfractionation of the 24 fractions into 39 subfractions, of which 35 were obtained in sufficient amount for structural analysis. 500-MHz ^1H-NMR spectroscopy in combination with sugar analysis of oligosaccharides, afforded the primary structure of 35 neutral mucin-type oligosaccharide-alditols. 19 neutral oligosaccharides that have already been described for bronchial mucins from cycstic fibrosis patients [1] are found again in this patient with bronchiectasis, suggesting that the structures as such and their variability are intrinsic phenomena of bronchial mucins.

The 35 oligosaccharide structures can be classified into four groups according to their core region (cf. [4]). The present article deals with structures that belong to core types 1 and 2, whereas the accompanying article [10], describes oligosaccharide-alditols that are of core type 3 and 4, i.e. GlcNAcβ(1→3)GalNAc-ol or GlcNAcβ(1→3)[GlcNAcβ-(1→6)]GalNAc-ol, respectively.

The empirical rules developed earlier [1, 6, 7, 16] for correlating ^1H-NMR parameters of mucin-type oligosaccharide-alditols with their structure, have been applied and extended further. In this report and in the accompanying one eight new oligosaccharides (four in this paper and four in the following) are characterized by a combination of sugar analysis and comparison with available data on ^1H-NMR reporter groups of neutral oligosaccharide-alditols. These compounds are all extensions or partial structures of well-known structures. Eight other oligosaccharides (two in this paper and six in the following paper) contain a set of two Fuc residues, the parameters of which do not fit into the aforementioned set of empirical rules. However, they can be ascribed to the presence in these oligosaccharides of the Y determinant, i.e. Fucα(1→2)Galβ(1→4)[Fucα(1→3)]GlcNAcβ(1→). The shift effects in the ^1H-NMR spectrum of an oligosaccharide-alditol with the type-2 H determinant or with the X determinant when completing the Y determinant are listed in Table 6. The resonance position of GlcNAc NAc, of Gal H-1 and of the two sets of Fuc H-1 and CH_3 are easy to recognize, since they

Table 6. *^1H chemical-shift differences for pertinent reporter groups, comparing blood-group H and X to the Y determinant sequence*
Chemical-shift differences are expressed in ppm. A positive value indicates a downfield shift. In the table heading the shorthand symbolic notation is used as described with Table 3. This table is obtained from ^{1}H-NMR data of two Y-determinant-containing oligosaccharide-alditols in this report and six Y-determinant-containing oligosaccharide-alditols in the accompanying article [10]. 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. [7])

Residue	Reporter group	Chemical shift differences for	
		H ⟶ Y	X ⟶ Y
		ppm	
Gal	H-1	−0.040	+0.050
	H-4	n.d.	−0.030
GlcNAc	H-1	+0.015	−0.010
	H-6	+0.035	+0.020
	NAc	−0.010	+0.003
Fuc^2	H-1	−0.030	–
	H-5	+0.025	–
	CH_3	+0.040	–
Fuc^3	H-1	–	−0.015
	H-5	–	+0.045
	CH_3	–	+0.060

usually do not show overlap with other signals that originate from carbohydrates. These resonances provide a reliable set of reporters for identification of the Y determinant in an oligosaccharide alditol (cf. [12, 13]). In addition to these shift effects, listed in Table 6, the position of Fuc^3 H-1 appears to be sensitive to the linkage type of the GlcNAc residue in the Y determinant, i.e. Fuc^3 H-1 at $\delta \approx 5.12$ ppm for a $\beta(1\rightarrow3)$ linkage and $\delta \approx 5.10$ ppm for a $\beta(1\rightarrow6)$ linkage. These empirical rules concerning the presence of the Y determinant in neutral oligosaccharide-alditols, as described above, will in turn be of help when analyzing other larger carbohydrate structures. This holds for neutral and sialylated compounds of both *N* and *O*-type of carbohydrate chain.

For smaller oligosaccharide-alditols, the core structure, i.e. the substitution of GalNAc-ol, is usually inferred from the resonance position of H-2 and H-5 of GalNAc-ol [6]. Starting from there, the total structure is then deduced from additional structural-reporter groups [1, 6, 7]. This report indicates that, when analyzing larger structures, the H-2 and H-5 resonances of GalNAc-ol may be obscured by other carbohydrate signals. The complete structure is then in fact assembled from separately recognizable peripheral structural elements. The combination of shift-effects arising from partial structures should account for the observed spectrum of the compound. Obviously the total structure must be in accordance with restrictions imposed by sugar analysis and apparent size from chromatographic behaviour. Additional information on the presence of structural groups might be obtained from retention times on specific HPLC columns [15].

This investigation was supported by the *Fondation pour la Recherche Médicale*, the Netherlands Foundation for Chemical Research (SON/ZWO), and grant UUKC 83-13 from the Netherlands Foundation for Cancer Research. Thanks are due to M. C. Houvenaghel (Lille) for skillful technical assistence.

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