Isolation and structural characterization of low-molecular-mass monosialyl oligosaccharides derived from respiratory-mucus glycoproteins of a patient suffering from bronchiectasis

Herman VAN HALBEEK [1,2], Jan BREG [2], Johannes F. G. VLIEGENTHART [2], André KLEIN [3], Geneviève LAMBLIN [3] and Philippe ROUSSEL [3]

[1] Complex Carbohydrate Research Center, University of Georgia
[2] Department of Bio-Organic Chemistry, University of Utrecht
[3] Unité des Protéines, Institut National de la Santé et de la Recherche Médicale no. 16, Lille

The carbohydrate chains of the respiratory-mucus glycoproteins of a patient suffering from bronchiectasis due to Kartagener's syndrome were released by alkaline borohydride treatment. Low-molecular-mass, monosialyl oligosaccharide-alditols were isolated by anion-exchange chromatography and fractionated by consecutive straight-phase high-performance liquid chromatography (HPLC) on a silica-based alkylamine column, and reverse-phase HPLC on a silica-based octadecyl column, respectively. The structures of the oligosaccharide-alditols were determined by 500-MHz ^{1}H-NMR spectroscopy in combination with sugar composition analysis. The 24 structures established range in size from disaccharides to heptasaccharides. Novel oligosaccharides obtained from the bronchiectasis mucus glycoproteins are:
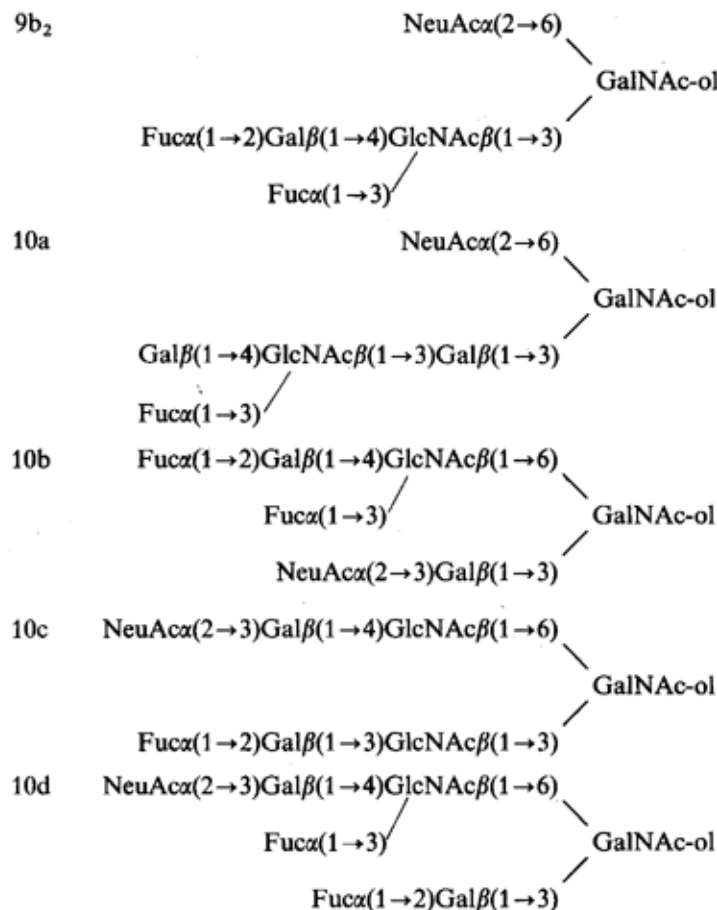
23 of the 24 monosialyl oligosaccharides characterized can be conceived of as extensions of neutral oligosaccharides purified from the bronchial mucus of this patient [Klein, A. et al. (1988) *Eur. J. Biochem. 171*, 631 – 642; Breg, J. et al. (1988) *Eur. J Biochem. 171*, 643 – 654]. The sialic acid residue was found to occur either in α(2→3)- or α(2→6)-linkage to a galactosyl residue or in α(2→6)-linkage to GalNAc-ol.

Correspondence to P. Roussel, Unité de Recherches sur la Biochimie des Protéines, Institut National de la Santé et de la Recherche Medicale no. 16, Place de Verdun, F-59045 Lille Cedex, France

Abbreviations. Fuc, L-fucose; GalNAc, *N*-acetyl-D-galactosamine; GalNAc-ol, *N*-acetyl-D-galactosaminitol; GlcNAc, *N*-acetyl-D-glucosamine; NeuAc, *N*-acetylneuraminic acid.

Human respiratory mucus forms a thin, gelatinous layer which covers the bronchial epithelium and protects it, e. g. against bacteria, viruses, and chemical agents. The major components of respiratory mucus are highly glycosylated proteins called mucins. The carbohydrate chains are believed to play an important part both in the physicochemical properties and the biological function of the mucus.

Previous studies have shown an extremely large heterogeneity in the structures of the oligosaccharide chains isolated from human respiratory mucins secreted by patients suffering from cystic fibrosis or chronic bronchitis [1 – 3]. Since carbohydrate chains are receptors for various micro-organisms [4 – 6], one may speculate that the diversity in oligosaccharide chains of respiratory mucins plays a role in the defense mechanism of respiratory mucosa by trapping various kinds of bacteria and viruses. To gain further insight into the role of respiratory mucin carbohydrates, it is of interest to determine whether mucins secreted by patients with different bronchial diseases differ in their carbohydrate structures.

A patient with blood group 0 and suffering from a Kartagener's syndrome was selected for our studies. Patients with Kartagener's syndrome have *situs inversus*, chronic sinusitis, and a chronic airway infection resulting in bronchiectasis, the latter abnormality being due to congenital immotility of the cilia [7].

Recently, we reported the isolation of the respiratory mucins from the sputum of the bronchiectasis patient, and the structural characterization of a series of their neutral oligosaccharides [8, 9]. In this article, we present the purification and structural characterization of their low-molecular-mass, monosialyl oligosaccharides.

MATERIALS AND METHODS

Isolation of bronchial oligosaccharides

The collection of sputum from the bronchiectasis patient and the subsequent preparation of bronchial mucus glycopeptides from it have been described recently [8]. Alkaline borohydride reductive degradation of bronchial glycopeptides (fraction P2) was performed as described [1]. The resulting mixture of glycopeptides and reduced oligosaccharides was applied to a Dowex AG50WX8 column. The eluted oligosaccharides were separated by ion-exchange chromatography on a Dowex AG1X2 column [1]. Four fractions were obtained: fraction I (neutral), fraction II (sialylated) and fractions III and IV (sulfated oligosaccharides). Fraction II was subfractionated by gel filtration on Bio-Gel P4 into high, medium, and low-molecular-mass compounds as described [1]. A pool of relatively small (average M_r < 1000), monosialyl oligosaccharide-alditols (IIc) was obtained by this procedure.

Further fractionation of pool IIc was carried out by HPLC on a Lichrosorb-NH$_2$ column (25 × 0.46 cm internal diameter, particle size 5 µm). Elution was performed with a linear gradient of 80/20 (solvent A) to 50/50 (by vol.) (solvent B) acetonitrile/water containing 2.5 mM ammonium bicarbonate, during 70 min at room temperature and at a flow rate of 1 ml/min [10]. Oligosaccharides were detected by absorption at 206 nm. All peaks in the elution profile were not well separated from their adjacent peaks. The corresponding oligosaccharide fractions were repurified by isocratic chromatography, either on 5-µm Lichrosorb-NH$_2$ with a mixture of solvent A (55%) and B (45%), or on Rsil-NH$_2$ (Alltech Europe, Eke, Belgium) (25 × 0.46 cm internal diameter; particle size 5 µm) with a mixture of solvent A (15%) and B

(85%), or by reverse-phase chromatography on a µBondapak TM C$_{18}$ column (30 × 0.39 cm internal diameter, particle size 10 µm) run isocratically with water as eluent at a flow rate of 0.5 ml/min at room temperature.

Analytical methods

Structural characterization of the purified oligosaccharide-alditols was performed by a combination of 500-MHz ^{1}H-NMR spectroscopy (SON NMR facility, University of Nijmegen, The Netherlands) and sugar composition analysis, as recently described [8].

RESULTS

Isolation and purification of monosialyl oligosaccharides

Bronchial mucus glycopeptides (fraction P2) were prepared from the sputum of the bronchiectasis patient; the chemical composition of fraction P2 is given in Table 1. Fraction P2 (900 mg) was submitted to alkaline-borohydride degradation and then fractionated into four fractions (I – IV) [1]. Fraction II, which was eluted from Dowex AG1X2 with 0.5 M formic acid, was subfractionated into IIa, IIb, and IIc by chromatography on Bio-Gel P4 (Fig. 1). Fraction IIc (28.2 mg) contained monosialyl oligosaccharide-alditols. HPLC on Lichrosorb-NH$_2$ was employed to fractionate IIc into 10 oligosaccharide-alditol fractions (Fig. 2). Some of them were not adequately separated from each other; these fractions were rechromatographed as follows. By isocratic rechromatography on Lichrosorb-NH$_2$, fraction 6 was subfractionated into 6a and 6b (Fig. 3A). Fractions 8 and 9 were chromatographed isocratically on Rsil-NH$_2$ and subfractionated into 8a, 8b, 8c, and 9a and 9b, respectively (Fig. 3B and C). A µBondapak reverse-phase column eluted with water was used to purify fraction 5 which was contaminated by fraction 4 (Fig. 4A). The same technique was employed for the subfractionation of fractions 8b, 9a, and 10 and gave fractions: 8b$_1$, 8b$_2$, 8b$_3$ (Fig. 4B), 9a$_1$, 9a$_2$, 9a$_3$ (Fig. 4C), and 10a, 10b, 10c, 10d (Fig. 4D).

Structure determination of the monosialyl oligosaccharide-alditols

The carbohydrate compositions of the HPLC-separated (sub)fractions of pool IIc of respiratory-mucus oligosaccharide-alditols from the bronchiectasis patient have been compiled in Table 2. All fractions contain GalNAc-ol and NeuAc; most also contain Gal and/or GlcNAc. About 65% contain additional Fuc. Based on the assumption that one residue of GalNAc-ol per oligosaccharide-alditol molecule occurs, the compounds obtained range from monosialyl disaccharides to heptasaccharides.

For structural characterization, the intact oligosaccharide-alditols were subjected to 500-MHz ^{1}H-NMR spectroscopic analysis. The structures of the oligosaccharide-alditols were grouped into five categories on the basis of the core unit. The core structure was identified from the set of chemical shifts of the GalNAc-ol H-2 and H-5 atoms and the NeuAc H-3ax and H-3eq atoms. The triplet-like resonances of the GalNAc-ol H-2 and H-5 atoms are readily recognizable in the 4.1 < δ < 4.4 region of each spectrum, and the actual positions of these signals are known [2, 3, 11 – 13] to be highly characteristic of the type of core unit of the oligosaccharide-alditols. The chemical shifts of H-3ax and H-3eq of NeuAc α(2→6)-

Table 1. *Chemical composition of mucus glycopeptide fraction P2 from bronchiectasis sputum*

Components	Amount	Total
	μmol/g	% (by mass)
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	
Histidine	24	
Arginine	29	
Total amino acids	1207	12.2
N-acetylneuraminic acid	256	
Fucose	567	
Galactose	1389	
N-acetylglucosamine	805	
N-acetylgalactosamine	421	
Total carbohydrates	3438	69.8
Sulfate	358	2.9

Fig. 1. *Bio-Gel P4 elution profile of fraction II obtained after alkaline borohydride treatment of bronchial-mucin glycopeptide fraction P2 from sputum of a bronchiectasis patient.* The column (200 – 400 mesh; 2 × 98 cm) was equilibrated in and eluted by acetic acid (0.1 M). 5-ml fractions were collected. Aliquots were analyzed for neutral sugars

Fig. 2. *HPLC elution profile of bronchial oligosaccharide-alditols (IIc) from sputum of a bronchiectasis patient on a 5-μm Lichrosorb-NH₂ column.* Elution was performed by a linear acetonitrile/water gradient (80/20 up to 50/50, by vol.), containing 2.5 mM ammonium bicarbonate

linked to GalNAc-ol are significantly different from those of NeuAc α(2→6)-linked to Gal of an *N*-acetyllactosamine unit, and of NeuAc α(2→3)-linked to Gal [13→23].

The structures in category (i) possess a C-3-monosubstituted GalNAc-ol residue; the substituent monosaccharide is β-linked Gal. The spectra of these oligosaccharide-alditols have in common the presence of the GalNAc-ol H-2/H-5 signals at δ 4.39/4.18, respectively [2, 3, 12, 13]. Category (ii) involves structures of the NeuAcα(2→6)[Galβ(1→3)]Gal-NAc-ol core type, which are characterized by the GalNAc-ol H-2/H-5 chemical shifts being δ 4.38/4.23, and the NeuAc H-3ax/H-3eq chemical shifts being δ 1.69/2.73, respectively; concomitantly, the GalNAc-ol H-6' atom gives rise to a doublet-of-doublets at δ ≈ 3.48, clearly upfield from the envelope of most of the skeleton proton signals [3, 13 – 23]. Group (iii) of structures is of the NeuAcα(2→6)[GlcNAcβ(1→3)]-GalNAc-ol core type; the GalNAc-ol H-2 and H-5 signals in the corresponding NMR spectra are found at δ 4.25 and δ 4.18, respectively; NeuAc H-3ax and H-3eq for this core resonate at δ 1.70 and δ 2.73, respectively; again, the GalNAc-ol H-6' atom resonates at δ ≈ 3.48. Set (iv) of oligosaccharide-alditols has the GlcNAcβ(1→6)[Galβ(1→3)]GalNAc-ol unit as core structural element. Typical chemical shifts for GalNAc-ol H-2 and H-5 atoms of compounds in this category are δ 4.39 and 4.26, respectively [2, 3, 11 – 13]. Class (v) of compounds contains the GlcNAcβ(1→6)[GlcNAcβ(1→3)]-GalNAc-ol core type; here, the typical set of GalNAc-ol H-2/H-5 chemical shifts is δ 4.27/4.22 [2, 3, 12, 13]. According to this classification, the chemical shifts of the structural-reporter groups for the various monosialyl oligosaccharide-alditols obtained from bronchiectasis sputum have been com-
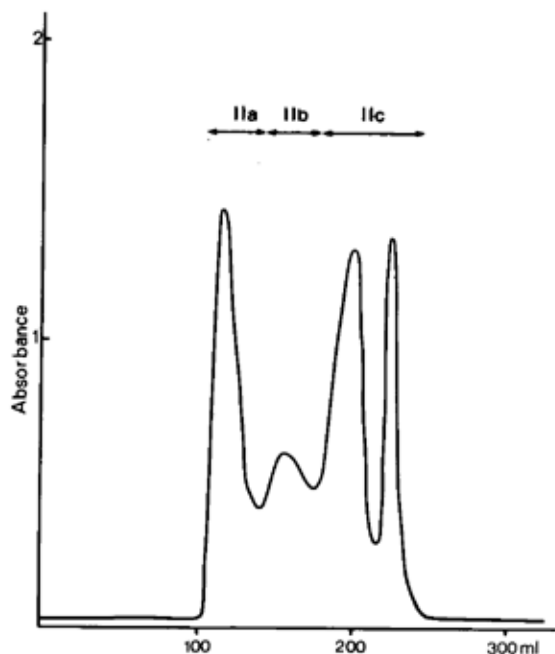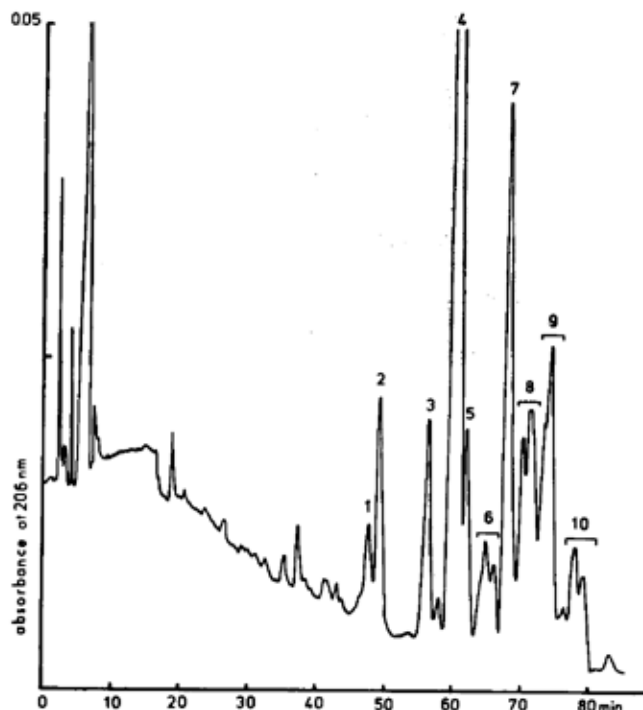
piled in Tables 3 – 7. The structures of the identifiable components of HPLC fractions 1 to 10d are summarized in Scheme 1, arranged in the order of their elution sequence.
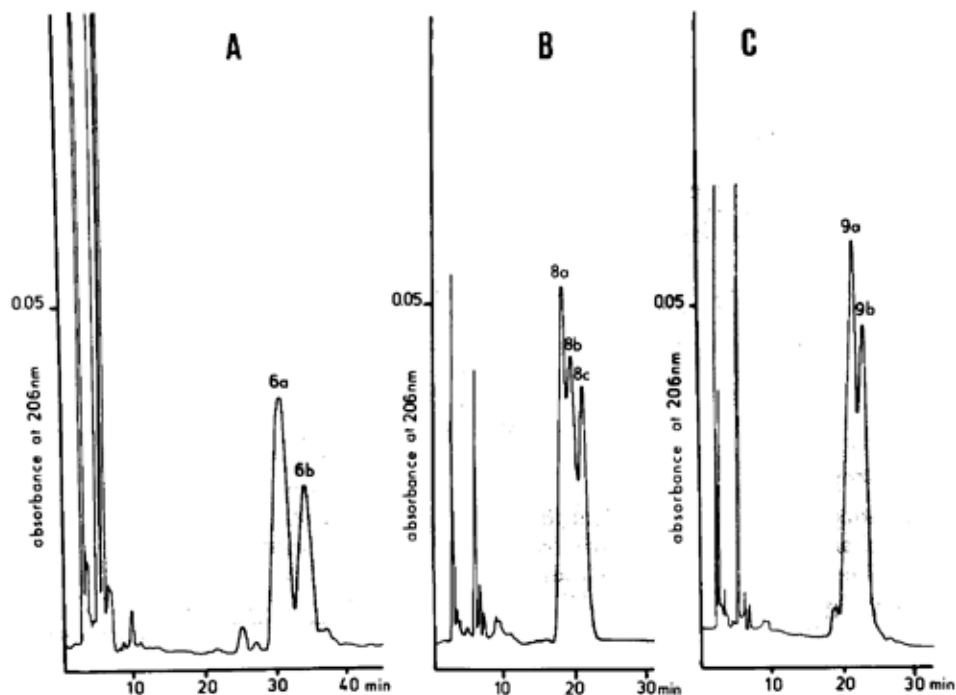
Fig. 3. *Repurification of fractions 6, 8 and 9.* (A) Fraction 6 was repurified by a second HPLC run on a 5-µm Lichrosorb-NH₂ column. Elution was performed by acetonitrile/water (66.5/33.5, v/v), containing 2.5 mM ammonium bicarbonate. (B) Fraction 8 and (C) fraction 9 were repurified by HPLC on a 5-µm Rsil-NH₂ column. Elution was performed by acetonitrile/water (54.5/45.5, by vol.) containing 2.5 mM ammonium bicarbonate
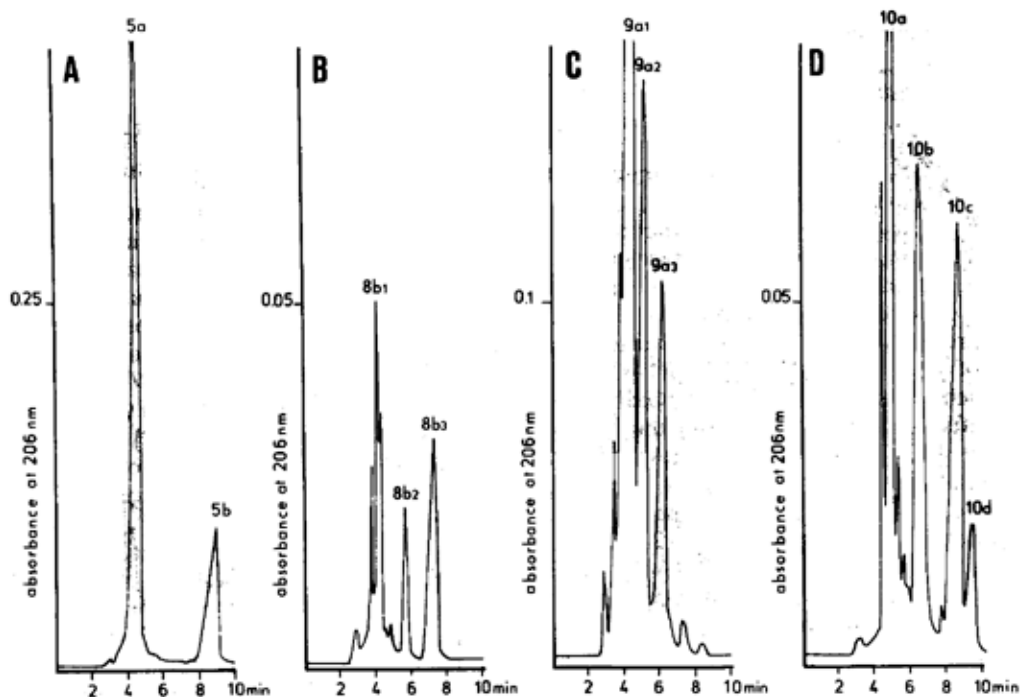

Fig. 4. *Repurification of fractions (A) 5, (B) 8b, (C) 9a, and (D) 10 by HPLC on a 10-µm µBondapak TM C₁₈ column.* Elution was performed isocratically by water

(i) Structures of the Galβ(1→3)GalNAc-ol core type which do not contain a substituent monosaccharide attached to the alditol at C-6

Structure 2. Fraction 2 contains a trisaccharide consisting of GalNAc-ol, Gal and NeuAc (see Table 2). The ¹H-NMR characteristics of this compound (Table 3) prove it to be the well-known linear trisaccharide-alditol NeuAcα(2→3)Galβ-(1→3)GalNAc-ol. The NMR features of this trisaccharide have been described previously, the compound being obtained from different sources [14, 16−21].

Structures 8a₁ and 8a₂. The NMR spectrum of fraction 8a is depicted in Fig. 5. This fraction appears to contain a mixture of two oligosaccharide-alditols (8a₁ and 8a₂). Evidence stems

Table 2. *Molar carbohydrate composition of the sialyl oligosaccharide-alditol fractions obtained by HPLC of pool IIc from bronchiectasis sputum*

The molar composition of the oligosaccharide-alditols was calculated on the basis of one residue of GalNAc-ol per molecule. n.d., not determined

Oligo-saccharide-alditol fraction	Molar ratio of monosaccharides					Amount
	NeuAc	Fuc	Gal	Glc-NAc	Gal-NAc-ol	
						μg
1	1.3	–	–	–	1	159
2	1.3	–	1.1	–	1	751
3	0.7	–	–	0.7	1	222
4	0.8	–	1.2	–	1	969
5a	0.8	–	1.1	–	1	79
5b	1.2	0.6	1.1	–	1	526
6a	1.1	0.1	1.4	1.0	1	330
6b	0.9	–	1.2	1.7	1	138
7	1.3	0.1	1.8	0.8	1	812
8a	1.0	0.2	1.6	0.8	1	201
8b$_1$	0.9	0.5	1.2	0.7	1	151
8b$_2$	0.9	0.5	1.7	1.0	1	101
8b$_3$	1.1	0.8	2.3	0.8	1	153
8c	1.0	0.7	1.2	0.8	1	303
9a$_1$	1.1	0.7	1.7	1.0	1	221
9a$_2$	0.9	1.3	1.6	0.7	1	138
9a$_3$	1.1	0.4	1.7	1.1	1	127
9b	0.9	0.4	1.2	0.7	1	258
10a	1.1	0.8	1.9	0.9	1	229
10b	1.1	1.3	1.8	1.1	1	216
10c	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
10d	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

from the presence in the spectrum of two NeuAc H-3ax signals observable at $\delta \approx 1.75$, and two NeuAc H-3eq signals at $\delta \approx 2.7$. Identification of the structures of 8a$_1$ and 8a$_2$ was facilitated because each of the compounds had been previously characterized by NMR among the monosialyl oligosaccharide-alditols obtained from the respiratory mucins of cystic fibrosis patients (see compounds B-6.2 [22] and A-3 [3], respectively). Both compounds contain the Galβ(1→3)-GalNAc-ol core unit (for chemical shifts of GalNAc-ol H-2 and H-5, see Table 3), extended by an *N*-acetyllactosamine unit in β(1→3)-linkage to the Gal^3 residue. The position of the Gal^3 H-4 signal (δ 4.129) is indicative of the β(1→3)-linkage [2, 3, 12, 13]. The two components of fraction 8a differ from each other *inter alia* in the type of linkage of the NeuAc residue. Compound 8a$_1$ has NeuAc in α(2→6)-linkage to Gal^4, as is readily concluded from the chemical shifts of H-3ax (δ 1.723) and H-3eq (δ 2.673; see Table 3, compare [22, 23]). The structure of compound 8a$_1$ is thus:

NeuAcα(2→6)Galβ(1→4)GlcNAcβ(1→3)-
Galβ(1→3)GalNAc-ol.
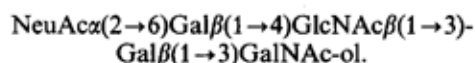
Compound 8a$_2$ has NeuAc α(2→3)-attached to Gal^4, as demonstrated by its set of H-3ax/H-3eq chemical shifts (δ 1.793/2.765), and in addition contains Fuc (δ H-1 5.131) in α(1→3)-linkage to the GlcNAc^3 residue of the *N*-acetyllactosamine moiety. The substitution of the *N*-acetyllactosamine unit simultaneously by NeuAc^3 and Fuc^3 is corroborated by the chemical shift of the Gal^4 H-1 signal at δ 4.528 (cf. [3]). Therefore, we conclude that 8a$_2$ contains the sialylated and fucosylated *N*-acetyllactosamine unit known as the sialyl X-

determinant (compare structures A-1, and A-3 to A-6 from the cystic fibrosis pool IIc [3]):

NeuAcα(2→3)Galβ(1→4)GlcNAcβ(1→3)Galβ(1→3) ⟶ GalNAc-ol

Fucα(1→3) ⟶
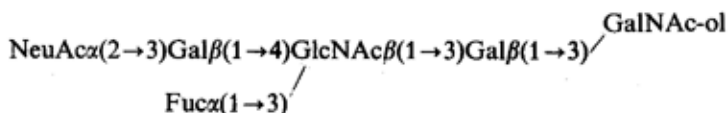
The two compounds 8a$_1$ and 8a$_2$ make up 55% and 45% of fraction 8a, respectively, as is estimated from the relative intensities of the NeuAc structural-reporter-group signals (at δ 1.723/1.793 and δ 2.673/2.765, see Fig. 5).

Structure 6a$_2$. Fraction 6a consists of a rather complex mixture of at least three components, all differing in their type of core (see below). The number of components has to be at least three, because of the occurrence of three sets of NeuAc H-3 signals in intensity ratio 7:2:1. The major (6a$_1$) and the minor component (6a$_3$) were recognized from their NMR characteristics to be identical to oligosaccharide-alditols that were previously identified by 500-MHz ^{1}H-NMR spectroscopy (see below). This highly facilitates the characterization of the component denoted 6a$_2$, making up 20% of the mixture. Component 6a$_2$, which has its H-3ax signal at δ 1.797, is discussed in this section because it has the Galβ(1→3)GalNAc-ol core type. The presence of this core element was deduced from the GalNAc-ol H-2 signal observable at δ 4.394. Again, as in the 8a compounds, the core disaccharide in 6a$_2$ is extended by an *N*-acetyllactosamine unit in β(1→3)-linkage to Gal^3, as evidenced by the Gal H-4 signal at δ 4.126. The Gal^4 residue bears NeuAc in α(2→3)-linkage, as in 8a$_2$, but now the Fuc residue is missing. The differences in chemical shift observed for the GlcNAc^3 NAc signal ($\Delta\delta$ 0.013 ppm) and also for the Gal^4 H-1 signal ($\Delta\delta$ 0.03 ppm) between compounds 6a$_2$ and 8a$_2$ are attributable to the absence of the Fuc^3 residue [3, 23]. Thus, the aforementioned reporter-group signals (see also Table 3) provide sufficient evidence to allow the deduction of the structure of compound 6a$_2$:

NeuAcα(2→3)Galβ(1→4)GlcNAcβ(1→3)-
Galβ(1→3)GalNAc-ol.
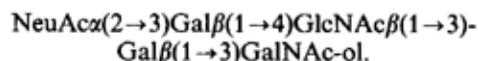
(ii) Structures of the NeuAcα(2→6)[Galβ(1→3)]$_{0-1}$GalNAc-ol core type

Structure 1. Carbohydrate composition analysis shows that fraction 1 contains a disaccharide consisting of GalNAc-ol and NeuAc (see Table 2). The NMR data of compound 1 (Table 4) match those of the disaccharide-alditol NeuAcα-(2→6)GalNAc-ol published previously (compound A-4-1 in [15]; see also [14, 17]). The set of chemical shifts of H-3ax (δ 1.70) and H-3eq (δ 2.73) of NeuAc is typical of its α(2→6)-linkage to GalNAc-ol, and is observed throughout the series of compounds to be discussed in this section.

The remaining oligosaccharide-alditols in this category have in common the occurrence of the GalNAc-ol H-2 and H-5 signals in their spectra at δ 4.38 and δ 4.23, respectively (see Table 4). That implies that these compounds have, in addition to NeuAc at C-6, a β-linked Gal residue attached to C-3 of GalNAc-ol.

Structure 4. The NMR features of fractions 4 and 5a are identical; moreover, they are the same as those reported previously for the branched trisaccharide-alditol NeuAcα-(2→6)[Galβ(1→3)]GalNAc-ol (compound A-4-2 in [15]; see also [14, 16, 17, 21]). The carbohydrate composition of the two fractions (Table 2) is in keeping with this structure. In the first HPLC run, fraction 5 (a minor fraction) was not
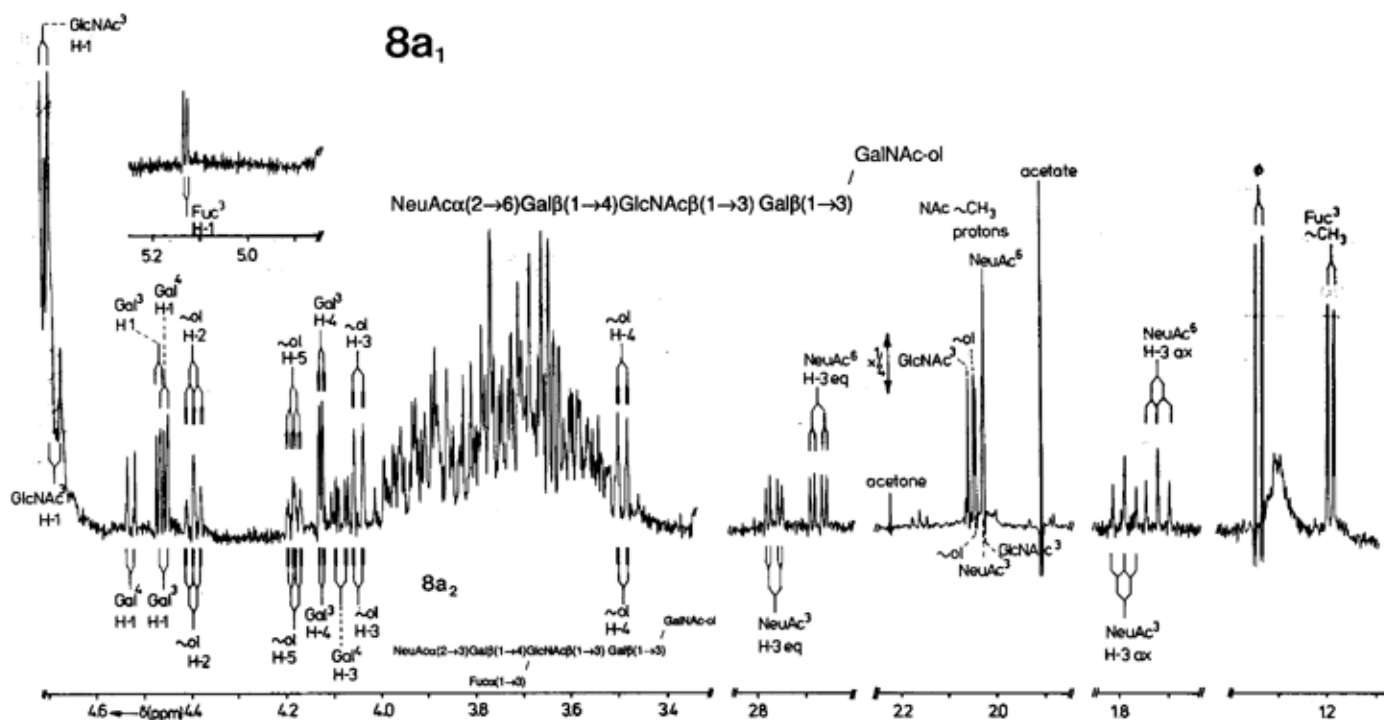
Fig. 5. *500-MHz ^{1}H-NMR spectrum ([2]H_2O, p[2]H 7, 27°C) of monosialyl oligosaccharide-alditol fraction 8a obtained from sputum of a bronchiectasis patient.* The two identifiable components, 8a_1 and 8a_2, occur in ratio 55:45. The signals attributed to 8a_1 are marked on top of the spectrum; those belonging to 8a_2 have been indicated on the bottom. The relative-intensity scale of the *N*-acetyl methyl proton region ($1.85 < \delta < 2.30$) deviates from that of the other parts of the spectrum. Resonances marked by Φ stem from non-carbohydrate contaminants

completely separated from fraction 4 (the major one) (see Fig. 2). It is therefore not surprising that one of the structures in fraction 5 is identical to the compound in fraction 4.

Structure 5b. From its sugar composition (Table 2) it is deduced that fraction 5b contains a tetrasaccharide-alditol that consists of GalNAc-ol, Gal, NeuAc and Fuc. The NMR spectrum of fraction 5b matches that of compound A-4-3 obtained from cervical mucus of a bonnet monkey [15], and therefore permits unambiguous identification of this oligosaccharide-alditol as NeuAcα(2→6)[Fucα(1→2)Galβ(1→3)]-GalNAc-ol (see Scheme 1).

Structure 9b_1. Fraction 9b was found by NMR analysis to be a mixture of three components. This is immediately clear from the NeuAc H-3 regions ($\delta \approx 1.75$ and $\delta \approx 2.75$) in the NMR spectrum (Fig. 6). The region around δ 2.75 shows three different H-3eq signals, namely at δ 2.725, 2.735 and 2.774, in intensity ratio 5:4:1. The region around δ 1.75 shows three H-3ax signals, namely at δ 1.689, 1.699 and 1.800, also in the intensity ratio of 5:4:1, respectively. All three components could be identified from the NMR spectrum. The main oligosaccharide-alditol present in fraction 9b (denoted 9b_1, accounting for 50% of the mixture) was grouped into the core class NeuAcα(2→6)[Galβ(1→3)]GalNAc-ol, based on the occurrence of the NeuAc H-3ax and H-3eq signals at δ 1.689 and 2.725, in conjunction with the GalNAc-ol H-2 and H-5 signals at δ 4.380 and 4.231; these four signals have equal intensities. The core Gal[3] residue of structure 9b_1 is extended by an *N*-acetyllactosamine unit in $\beta(1→3)$-linkage. Evidence for this structural element was obtained by careful comparison of the data of compound 9b_1 with those of its neutral analogue, namely, Galβ(1→4)GlcNAcβ(1→3)Galβ(1→3)Gal-NAc-ol (oligosaccharide-alditol 10B, found among pool Ic of small-size neutral oligosaccharide-alditols from cystic fibrosis
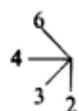
respiratory mucins [2]). The H-4 signal of Gal[3] (at δ 4.119) was indicative of the $\beta(1→3)$-substitution of the core Gal[3] residue in compound 9b_1. Furthermore, the position of the NAc signal of GlcNAc[3] (δ 2.042) is typical for this structural element [2] (compare with so-called 'additional' *N*-acetyllactosamine units in *N*-linked oligosaccharides [23, 24]). The terminal Gal residue is present in $\beta(1→4)$ [rather than $\beta(1→3)$] linkage to GlcNAc[3] based on the chemical shift of its anomeric proton (δ 4.480) (compare [13, 25−27]), and the position of the GlcNAc[3] NAc signal. Combination of the data affords the following structure for pentasaccharide 9b_1:

NeuAcα(2→6)[Galβ(1→4)GlcNAcβ(1→3)-
Galβ(1→3)]GalNAc-ol.
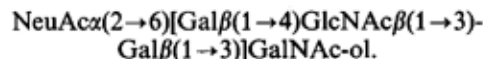
Structure 10a. The carbohydrate composition (Table 2) of fraction 10a, repurified by reverse-phase HPLC (Fig. 4D), points to the presence of a hexasaccharide consisting of GalNAc-ol, Gal, GlcNAc, NeuAc and Fuc in the ratio of 1:2:1:1:1. The ^{1}H chemical shifts of the structural-reporter groups of 10a have been included in Table 4, because the positions of the NeuAc H-3ax (δ 1.688) and H-3eq (δ 2.724) signals in the spectrum of fraction 10a are characteristic [13, 14] for a NeuAc residue that is $\alpha(2→6)$-linked to GalNAc-ol, and the positions of H-2 (δ 4.379) and H-5 (δ 4.225) of GalNAc-ol point to a Gal residue in $\beta(1→3)$-linkage to GalNAc-ol. As in compounds 6a_2, 8a_1, 8a_2, and 9b_1, the core trisaccharide in 10a was found to be extended by an *N*-acetyllactosamine unit $\beta(1→3)$-linked to Gal[3]. In turn, the *N*-acetyllactosamine unit bears a Fuc residue in $\alpha(1→3)$-linkage to GlcNAc. The latter is inferred from the chemical shifts of the Fuc H-1 (δ 5.136), H-5 (δ 4.833), and CH_3 signals (δ 1.176), in conjunction with the chemical shift of the GlcNAc[3] NAc signal (δ 2.032; $\Delta\delta$ − 0.01 ppm compared to the corre-

Table 3. ^1H-*NMR chemical shifts of structural-reporter groups of constituent monosaccharides for the bronchiectasis respiratory-mucus oligosaccharide-alditols which possess the Galβ(1→3)GalNAc-ol core unit*

Chemical shifts are relative to internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate (using internal acetone at δ 2.225 ppm) in ^2H_2O at 27°C (δ HO^{2}H 4.76 ppm), acquired at 500 MHz. For the complete structures of the compounds, see Scheme 1. In the tableheading, the structures are represented by short-hand symbolic notation (cf. [8, 9]); ◇ = GalNAc-ol; ■— = Gal; ●— = GlcNAc; □— = Fuc; △— = NeuAc. The linkage position is specified by the direction of the connecting bars as follows:

Thus, at variance with our previous system of notation [2, 3, 12], the branch which is linked to C-6 of GalNAc-ol is pointing upward, and the branch linked to C-3 of GalNAc-ol downward

Residue[a]	Reporter group	Chemical shift in compound			
			6a₂	8a₁	8a₂
		ppm			
GalNAc-ol	H-2	4.388	4.394	4.396	4.396
	H-3	4.073	4.052	4.050	4.049
	H-4	3.496	3.491	3.494	3.492
	H-5	4.186	4.185	4.185	4.185
	NAc	2.045	2.047	2.050	2.046
Gal³(a)	H-1	4.545	4.464	4.466[b]	4.460
	H-3	4.121	n.d.[c]	n.d.[c]	n.d.[c]
	H-4	3.930	4.126	4.129	4.129
GlcNAc³	H-1	—	4.698	4.708	4.688
	NAc	—	2.039	2.062	2.029
Gal⁴	H-1	—	4.557	4.458[b]	4.528
	H-3	—	4.114	n.d.[c]	4.084
Fuc³	H-1	—	—	—	5.131
	H-5	—	—	—	4.82
	CH₃	—	—	—	1.170
NeuAc³	H-3ax	1.800	1.797	—	1.793
	H-3eq	2.774	2.759	—	2.765
	NAc	2.034	2.031	—	2.032
NeuAc⁶	H-3ax	—	—	1.723	—
	H-3eq	—	—	2.673	—
	NAc	—	—	2.029	—

[a] A superscript at the name of a monosaccharide residue indicates to which position of the adjacent monosaccharide it is glycosidically linked.
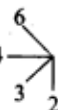
[b] Assignments may have to be interchanged.

[c] Value could not be determined merely by inspection of the spectrum.

Table 4. ^1H-*NMR chemical shifts of structural-reporter groups of constituent monosaccharides for the bronchiectasis respiratory-mucus oligosaccharide-alditols which possess the NeuAcα(2→6)[Galβ-(1→3)]₀₋₁GalNAc-ol core unit*

Chemical shifts are relative to internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate (using internal acetone at δ 2.225 ppm) in ^2H_2O at 27°C (δ HO^{2}H 4.76 ppm), acquired at 500 MHz. For the complete structure of the compounds, see Scheme 1. In the tableheading, the structures are represented by short-hand symbolic notation (cf. [8, 9]); ◇ = GalNAc-ol; ■— = Gal; ●— = GlcNAc; □— = Fuc; △— = NeuAc. The linkage position is specified by the direction of the connecting bars as follows:


Thus, at variance with our previous system of notation [2, 3, 12], the branch which is linked to C-6 of GalNAc-ol is pointing upward, and the branch linked to C-3 of GalNAc-ol downward

Residue[a]	Reporter group	Chemical shift in compound				
			4 (= 5a)	5b	9b₁	10a
		ppm				
Gal-NAc-ol	H-2	4.246	4.377	4.383	4.380	4.379
	H-3	n.d.[b]	4.057	4.082	4.041	4.041
	H-4	3.413	3.534	3.545	3.5	n.d.[b]
	H-5	4.020	4.244	4.220	4.231	4.225
	H-6'	3.533	3.489	3.482	3.479	3.47
	NAc	2.055	2.047	2.042	2.045	2.045
Gal³(a)	H-1	—	4.473	4.585	4.461	4.464
	H-4	—	3.895	3.918	4.119	4.119
Glc-NAc³	H-1	—	—	—	4.69	4.69
	NAc	—	—	—	2.042	2.032
Gal⁴	H-1	—	—	—	4.480	4.458
	H-4	—	—	—	n.d.[b]	n.d.[b]
Fuc²	H-1	—	—	5.270	—	—
	H-5	—	—	4.270	—	—
	CH₃	—	—	1.237	—	—
Fuc³	H-1	—	—	—	—	5.136
	H-5	—	—	—	—	4.833
	CH₃	—	—	—	—	1.176
Neu-Ac⁶	H-3ax	1.701	1.693	1.696	1.689	1.688
	H-3eq	2.729	2.728	2.730	2.725	2.724
	NAc	2.033	2.034	2.034	2.033	2.032

[a] A superscript at the name of a monosaccharide residue indicates to which position of the adjacent monosaccharide it is glycosidically linked.

[b] Value could not be determined merely by inspection of the spectrum.

sponding afuco unit in 9b₁) [2, 3, 23]. It should be emphasized that the chemical shift of H-1 of Fuc not only indicates the occurrence of the X-determinant, but, in addition, that the GlcNAc residue forming part of the X-determinant is involved in a β(1→3)-linkage to another residue [13] [compare, for example, compounds 8b₂, 10b, and 10d (Table 6 below) con-

taining the X-determinant β(1→6)-linked to GalNAc-ol]. Combination of NMR and sugar analyses leads to the following structure for oligosaccharide-alditol 10a:

NeuAcα(2→6){Galβ(1→4)[Fucα(1→3)]-
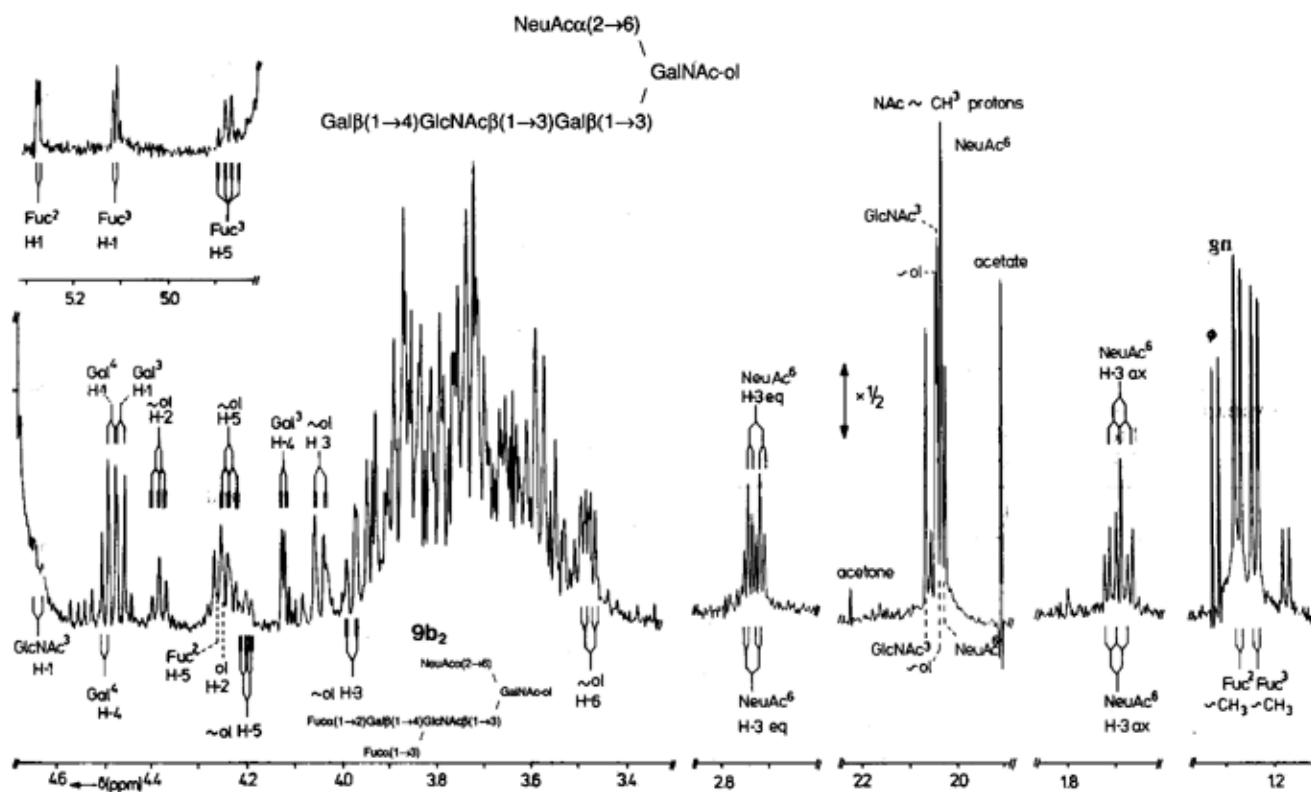GlcNAcβ(1→3)Galβ(1→3)}GalNAc-ol.
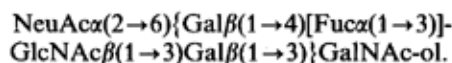
Fig. 6. *500-MHz ^{1}H-NMR spectrum (^2H_2O, p^2H 7, 27°C) of monosialyl oligosaccharide-alditol fraction 9b obtained from sputum of a bronchiectasis patient.* The three identifiable components, $9b_1$, $9b_2$, and $9b_3$, occur in ratio 5:4:1. The signals attributed to $9b_1$ are marked on top of the spectrum; those belonging to $9b_2$ have been indicated on the bottom. The signals belonging to $9b_3$ have not been indicated in this spectrum; the subspectrum of $9b_3$, however, is identical to the spectrum of $9a_1$ (see Fig. 10). The relative-intensity scale of the *N*-acetyl methyl proton region ($1.85 < \delta < 2.30$) deviates from that of the other parts of the spectrum. Resonances marked by Φ stem from non-carbohydrate contaminants

(iii) Structures of the
NeuAcα(2→6)$_{0-1}$[GlcNAcβ(1→3)]GalNAc-ol core type

Structure 3. The carbohydrate composition of fraction 3 (see Table 2) indicates the presence of a trisaccharide consisting of GalNAc-ol, GlcNAc, and NeuAc. The 500-MHz ^{1}H-NMR spectrum of oligosaccharide-alditol 3 is shown in Fig. 7. The chemical shift of the GalNAc-ol H-2 atom (δ 4.260) is indicative of the $\beta(1→3)$-linkage of GlcNAc to GalNAc-ol [2, 12, 13]. The chemical shifts of the GlcNAc³ H-1 and NAc signals point to the terminal position of the GlcNAc residue in the oligosaccharide. The chemical shift of the NeuAc H-3ax atom (δ 1.697; see Table 5) is in the range usually observed for NeuAc residues involved in an $\alpha(2→6)$-linkage (compare Table 4) [14, 23]. The occurrence of the GalNAc-ol H-6' signal at δ 3.487, clearly outside the bulk signal of the majority of sugar-skeleton-proton resonances, in combination with the GalNAc-ol H-5 signal at δ 4.185 (shifted from δ 4.145 observed for the disaccharide GlcNAcβ(1→3)-GalNAc-ol [2, 12]), prove NeuAc to be $\alpha(2→6)$-linked to GalNAc-ol. The set of chemical shifts of the NeuAc H-3 atoms (δ 1.697 and δ 2.733) is unique thus far (cf. [14]), and can therefore be considered as typical of the NeuAcα(2→6)-[GlcNAcβ(1→3)]GalNAc-ol type of core. The average values of the chemical shifts of H-3ax (δ 1.698) and H-3eq (δ 2.733) for compounds listed in Table 5 differ subtly but significantly from the corresponding values for compounds listed in Table 4 that share the NeuAcα(2→6)[Galβ(1→3)]GalNAc-ol

core element: δ 1.690 and δ 2.726, respectively. The fact that the set of NeuAc H-3ax and H-3eq chemical shifts appears not to be affected by extension of the trisaccharide NeuAcα(2→6)[GlcNAcβ(1→3)]GalNAc-ol at one or more positions of GlcNAc³ has already been successfully applied in the structural determination of some of the monosialyl oligosaccharide-alditols from the cystic fibrosis bronchial mucin pool IIc [22].

Structure $6a_1$. As pointed out before, fraction 6a contains a mixture of at least three components, differing in the set of chemical shifts of H-3ax and H-3eq of their sialic acid residue. The identifiable three components occur in ratio 7:2:1. The structure of $6a_2$ has been described (see above). The structural-reporter-group signals of the major (70%) component of 6a (denoted $6a_1$) are clearly recognizable from the ^{1}H-NMR spectrum. Their chemical shifts are identical to those observed for the monosialyl oligosaccharide-alditol (compound B-2/C-4 in [22]) obtained from cystic fibrosis mucins:

NeuAcα(2→6)[Galβ(1→4)GlcNAcβ(1→3)]GalNAc-ol.

Structure $6a_3$. Sugar analysis of fraction 6a (Table 2) indicates that a minor component ($\approx$ 10%) of the fraction contains Fuc. Since compounds $6a_1$ and $6a_2$, as previously discussed, account for 70% and 20% of the mixture 6a, respectively, and both of them are devoid of Fuc (Scheme 1), the third component (10%) in the mixture, denoted $6a_3$, contains Fuc. In the NMR spectrum of fraction 6a, the structural-
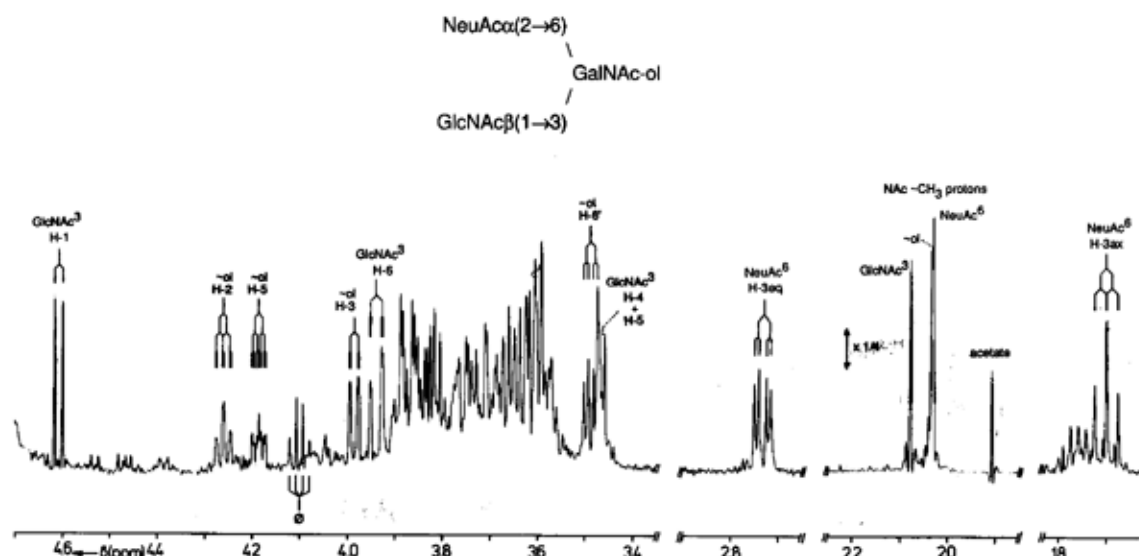
3

Fig. 7. *500-MHz* ¹*H-NMR spectrum* (²H₂O, p²H 7, 27°C) *of monosialyl oligosaccharide-alditol fraction 3 obtained from sputum of a bronchiectasis patient.* The relative-intensity scale of the *N*-acetyl methyl proton region (1.85 < δ < 2.30) deviates from that of the other parts of the spectrum. Resonances marked by Φ stem from non-carbohydrate contaminants

reporter groups of the Fuc residue show up at δ 5.131 (H-1) and δ 1.170 (CH₃). In combination with the low-intensity set of NeuAc H-3ax (δ 1.792) and H-3eq (δ 2.760) signals, these parameters indicate the occurrence of the sialyl X-determinant in compound 6a₃. This led to the conclusion that compound 6a₃ is identical to the pentasaccharide-alditol A-1 obtained from cystic fibrosis respiratory mucins:

NeuAcα(2→3)Galβ(1→4)[Fucα(1→3)]-
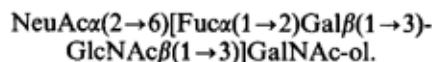GlcNAcβ(1→3)GalNAc-ol.
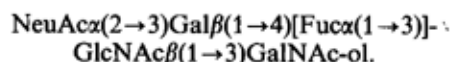
Structure 8b₁. Fraction 8b₁ contains, as major component, a pentasaccharide-alditol consisting of GalNAc-ol, Gal, GlcNAc, NeuAc and Fuc (see Table 2). Two minor components present in this fraction appeared to be compounds 8a₁ and 8a₂. From the chemical shifts of the NeuAc H-3ax and H-3eq atoms in combination with those of the GalNAc-ol H-2 and H-5 atoms of the major component (see Table 5), compound 8b₁ is concluded to belong to the core type NeuAcα(2→6)[GlcNAcβ(1→3)]GalNAc-ol. However, the Fuc H-1 and CH₃ signals are now observed at δ 5.132 and δ 1.177, respectively. This indicates that the Fuc residue is α(1→3)-linked to the GlcNAc³ residue, which is simultaneously substituted by Gal in β(1→4)-linkage (X-determinant) [2, 3, 12, 13, 23]. The structure of compound 8b₁ is therefore identical to that of compound B-6.1, obtained from cystic fibrosis mucins [9]:

NeuAcα(2→6){Galβ(1→4)[Fucα(1→3)]-
GlcNAcβ(1→3)}GalNAc-ol.
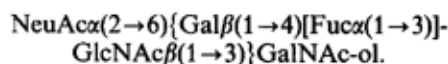
Structure 8c. The carbohydrate composition of fraction 8c points to the presence of yet another pentasaccharide-alditol containing GalNAc-ol, Gal, GlcNAc, NeuAc and Fuc as constituents. The 500-MHz ¹H-NMR spectrum of fraction 8c (Fig. 8) revealed the pentasaccharide-alditol to belong to the NeuAcα(2→6)[GlcNAcβ(1→3)]GalNAc-ol core type (see

Table 5, in particular δ H-2 and δ H-5 of GalNAc-ol, and δ H-3ax and δ H-3eq of NeuAc). Moreover, it shows a set of Fuc structural-reporter-group signals (δ H-1 5.206, δ H-5 4.273, δ CH₃ 1.235) that are characteristic of the presence of the blood group H type-1 structural element Fucα(1→2)Galβ-(1→3)GlcNAcβ(1→●). That the GlcNAc residue involved in this determinant is the GlcNAcβ(1→3) of the core is proven unequivocally by the position of the NAc signal of the latter residue, namely, δ 2.107 [2, 12]. Therefore, the structure of compound 8c is identical to that of compound D-3 obtained from cystic fibrosis bronchial mucins [9]:

NeuAcα(2→6)[Fucα(1→2)Galβ(1→3)-
GlcNAcβ(1→3)]GalNAc-ol.

Structure 9b₂. As mentioned before, fraction 9b contains a mixture of at least three oligosaccharide-alditols. Component 9b₂ (40% of the mixture) belongs in the NeuAcα(2→6)-[GlcNAcβ(1→3)]GalNAc-ol core category, on the basis of the chemical shifts of the medium-intensity NeuAc H-3ax and H-3eq signals: δ 1.699 and δ 2.735. Furthermore, in the NMR spectrum of 9b (Fig. 6), two Fuc H-1 signals of equal intensity are observed at δ 5.276 and δ 5.113; these signals, in conjunction with the CH₃ doublets at δ 1.274 and δ 1.238, are indicative of the occurrence of two Fuc residues, one being α(1→2)-linked to Gal⁴, the other α(1→3) to GlcNAc of the same *N*-acetyllactosamine unit. Together, the difucosyl *N*-acetyllactosamine unit is the so-called Y-determinant sequence [27, 28]. Combination of the structural elements mentioned leads to the following structure for compound 9b₂ (see Scheme 1):

NeuAcα(2→6){Fucα(1→2)Galβ(1→4)[Fucα(1→3)]-
GlcNAcβ(1→3)}GalNAc-ol.
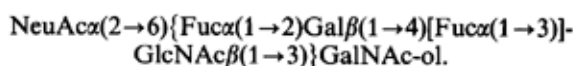
The asialo analogue of compound 9b₂ was characterized previously among the neutral oligosaccharide-alditols obtained

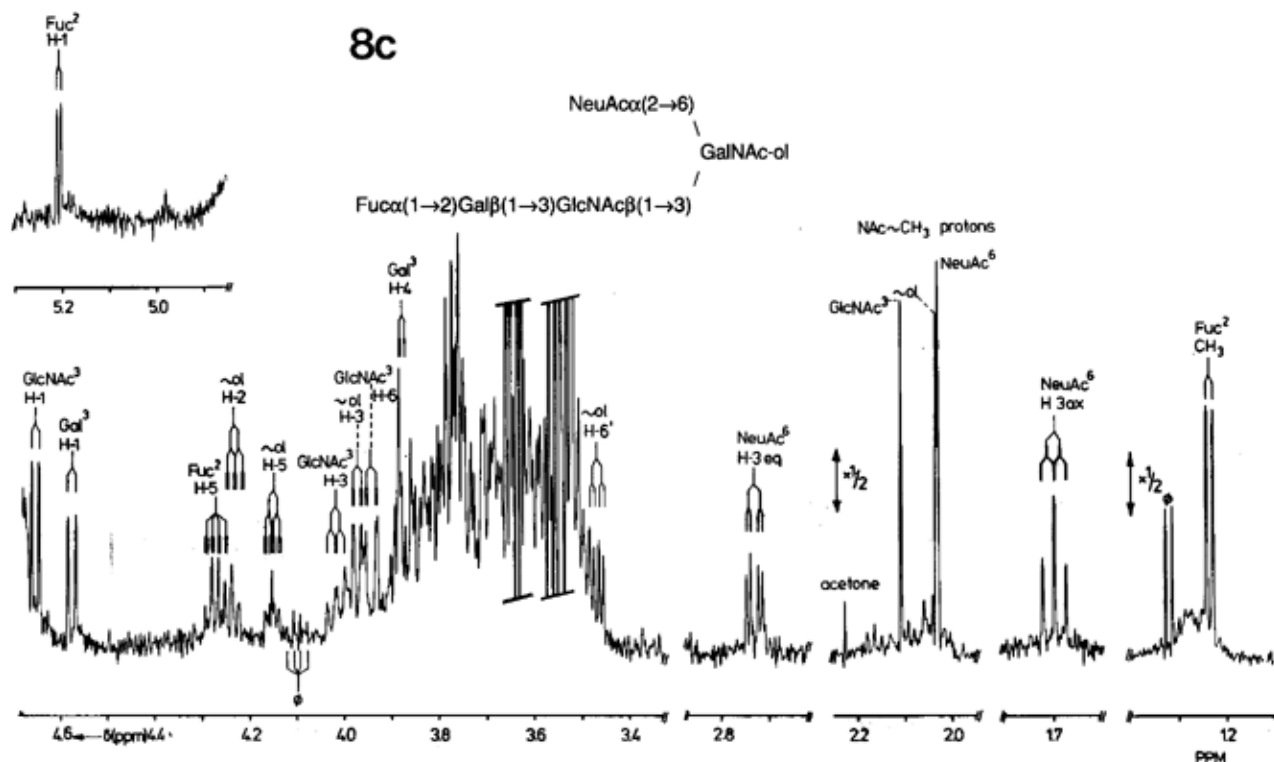Fig. 8. *500-MHz* ^{1}*H-NMR spectrum* (^{2}*H*$_2$*O, p*^{2}*H 7, 27°C) of monosialyl oligosaccharide-alditol fraction 8c obtained from sputum of a bronchiectasis patient.* The relative-intensity scale of the N-acetyl methyl proton region (1.85 < δ < 2.30) deviates from that of the other parts of the spectrum. Resonances marked by Φ stem from non-carbohydrate contaminants

from the respiratory mucins of the bronchiectasis patient (compound 11b in [9]). The NMR parameters of the Y-determinant in compound 9b$_2$ and in its asialo analogue are virtually identical.

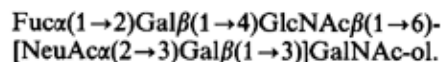*(iv) Structures of the GlcNAcβ(1→6)[Galβ(1→3)]GalNAc-ol core type*

Structure 7. The sugar composition of fraction 7 (Table 2) indicates that it contains (at least as its main component) a pentasaccharide-alditol consisting of GalNAc-ol, Gal, GlcNAc, and NeuAc in the ratio 1:2:1:1. Compound 7 was readily identified as

$$Galβ(1→4)GlcNAcβ(1→6)[NeuAcα(2→3)-$$
$$Galβ(1→3)]GalNAc-ol$$
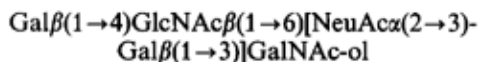
because its NMR characteristics (Table 6) match those reported before for this structure (compound B-4/C-5 in the cystic fibrosis pool IIc of oligosaccharide-alditols [22]). The presence of Gal in β(1→3)-linkage to GalNAc-ol is reflected by the chemical shift of GalNAc-ol H-2 (δ 4.388; Table 6; compare the data in Tables 3 and 4). The chemical shift of GalNAc-ol H-5 is found to be δ 4.269; this is decisive for the presence of GlcNAc in β(1→6)-linkage to GalNAc-ol [2, 12, 13]. It should be noted that the NeuAc residue in compound 7 (NeuAc^{3,3}) is characterized by the set of H-3ax and H-3eq chemical shifts: δ 1.801 and δ 2.775. These values specify not only its type of linkage to Gal to be α(2→3), but also its location in the Gal^3 rather than Gal^4 branch [20, 22, 29].

Structure 8b$_2$. Fraction 8b$_2$ was separated from 8b$_1$ and 8b$_3$ by an additional HPLC run on the µBondapak reverse-phase column (see Fig. 4B). It appears to contain a hexasaccharide consisting of GalNAc-ol, Gal, GlcNAc, NeuAc, and
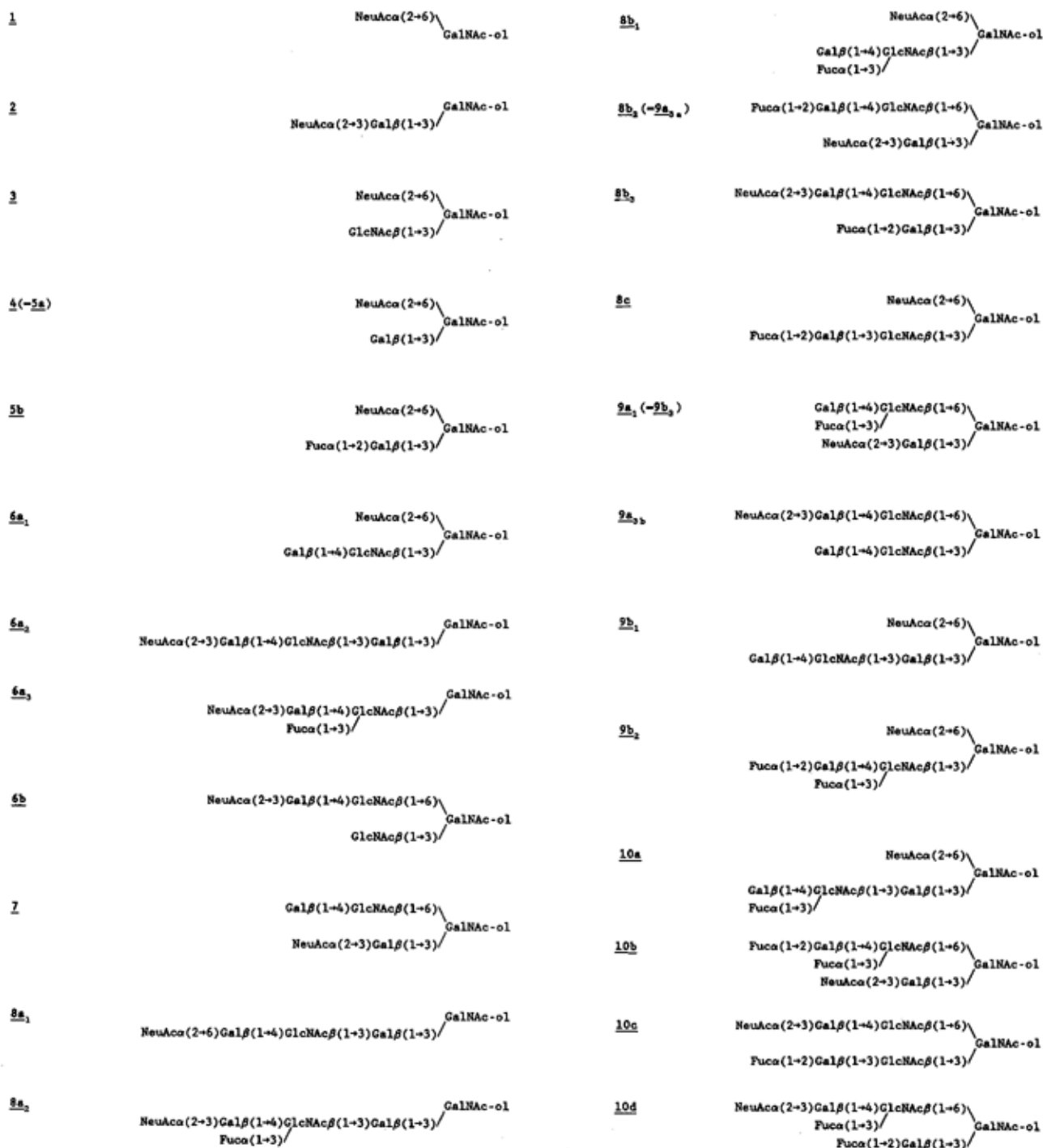
Fuc in the ratio 1:2:1:1:1 (see Table 2). From the chemical shifts of GalNAc-ol H-2 and H-5 in the NMR spectrum of 8b$_2$ (Table 6) it is evident that the compound belongs to the GlcNAcβ(1→6)[Galβ(1→3)]GalNAc-ol type of core. The sialic acid in 8b$_2$ is, as in structure 7, α(2→3)-linked to the core Gal^3 residue. Actually, compound 8b$_2$ was identified as the extension of compound 7 with Fuc α(1→2)-linked to Gal^4 of the N-acetyllactosamine moiety in the β(1→6) branch:

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

This is proven by the chemical shifts of the Fuc structural-reporter groups (δ H-1 5.307, δ H-5 4.230, δ CH$_3$ 1.231), which are indicative of the type-2 blood group H determinant (cf. [2, 12, 30]). The NMR features of compound 8b$_2$ have been described previously for compound D-4 in [22], obtained from cystic fibrosis sputum.

Structure 9a$_{3a}$. Fig. 9 shows the 500-MHz ^{1}H-NMR spectrum of fraction 9a$_3$, which was obtained from fraction 9 by repurification on Rsil-NH$_2$ (Fig. 3C), and then by reverse-phase HPLC from 9a (Fig. 4C). In the NMR spectrum two GalNAc-ol H-2 signals are observed, namely, at δ 4.388 and δ 4.28. Furthermore, two NeuAc H-3eq signals are seen at δ 2.774 and δ 2.758, in the intensity ratio 2:1. This indicates that fraction 9a$_3$ contains a mixture of at least two oligosaccharide-alditols, occurring in the approximate ratio 2:1. The high-intensity signals in the spectrum are attributed to the main component 9a$_{3a}$, which will be discussed here. The low-intensity signals are ascribed to compound 9a$_{3b}$, which belongs to the class of compounds sharing the GlcNAcβ(1→6)[GlcNAcβ(1→3)]GalNAc-ol core element (see below).

Compound 9a$_{3a}$ could be readily identified because its NMR subspectrum (Fig. 9) matches exactly that of compound

1   NeuAcα(2→6)\GalNAc-ol

2   NeuAcα(2→3)Galβ(1→3)/GalNAc-ol

3   NeuAcα(2→6)\
        GlcNAcβ(1→3)/ GalNAc-ol

4(=5a)   NeuAcα(2→6)\
             Galβ(1→3)/ GalNAc-ol

5b   NeuAcα(2→6)\
         Fucα(1→2)Galβ(1→3)/ GalNAc-ol

6a₁   NeuAcα(2→6)\
          Galβ(1→4)GlcNAcβ(1→3)/ GalNAc-ol

6a₂   NeuAcα(2→3)Galβ(1→4)GlcNAcβ(1→3)Galβ(1→3)/ GalNAc-ol

6a₃   NeuAcα(2→3)Galβ(1→4)GlcNAcβ(1→3)/
          Fucα(1→3)/ GalNAc-ol

6b   NeuAcα(2→3)Galβ(1→4)GlcNAcβ(1→6)\
         GlcNAcβ(1→3)/ GalNAc-ol

7   Galβ(1→4)GlcNAcβ(1→6)\
        NeuAcα(2→3)Galβ(1→3)/ GalNAc-ol

8a₁   NeuAcα(2→6)Galβ(1→4)GlcNAcβ(1→3)Galβ(1→3)/ GalNAc-ol

8a₂   NeuAcα(2→3)Galβ(1→4)GlcNAcβ(1→3)Galβ(1→3)/
          Fucα(1→3)/ GalNAc-ol

8b₁   NeuAcα(2→6)\GalNAc-ol
          Galβ(1→4)GlcNAcβ(1→3)/
          Fucα(1→3)/

8b₂(=9a₃ₐ)   Fucα(1→2)Galβ(1→4)GlcNAcβ(1→6)\
                 GalNAc-ol
                 NeuAcα(2→3)Galβ(1→3)/

8b₃   NeuAcα(2→3)Galβ(1→4)GlcNAcβ(1→6)\
          GalNAc-ol
          Fucα(1→2)Galβ(1→3)/

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

9a₁(=9b₃)   Galβ(1→4)GlcNAcβ(1→6)\
                Fucα(1→3)/ GalNAc-ol
                NeuAcα(2→3)Galβ(1→3)/

9a₃b   NeuAcα(2→3)Galβ(1→4)GlcNAcβ(1→6)\
           GalNAc-ol
           Galβ(1→4)GlcNAcβ(1→3)/

9b₁   NeuAcα(2→6)\GalNAc-ol
          Galβ(1→4)GlcNAcβ(1→3)Galβ(1→3)/

9b₂   NeuAcα(2→6)\GalNAc-ol
          Fucα(1→2)Galβ(1→4)GlcNAcβ(1→3)/
          Fucα(1→3)/

10a   NeuAcα(2→6)\GalNAc-ol
          Galβ(1→4)GlcNAcβ(1→3)Galβ(1→3)/
          Fucα(1→3)/

10b   Fucα(1→2)Galβ(1→4)GlcNAcβ(1→6)\
          Fucα(1→3)/ GalNAc-ol
          NeuAcα(2→3)Galβ(1→3)/

10c   NeuAcα(2→3)Galβ(1→4)GlcNAcβ(1→6)\
          GalNAc-ol
          Fucα(1→2)Galβ(1→3)GlcNAcβ(1→3)/

10d   NeuAcα(2→3)Galβ(1→4)GlcNAcβ(1→6)\
          Fucα(1→3)/ GalNAc-ol
          Fucα(1→2)Galβ(1→3)/

Scheme 1. *Structures of low-molecular-mass monosialyl oligosaccharide-alditols (fraction IIc) obtained from respiratory mucins of a patient with bronchiectasis*
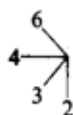
8b₂ which was discussed above. Therefore, it is concluded that the structures of 8b₂ and 9a₃ₐ are the same (Scheme 1).

Structure 9a₁. The 500-MHz ¹H-NMR spectrum of fraction 9a₁ is depicted in Fig. 10. According to its carbohydrate composition (Table 2), fraction 9a₁ contains another hexasaccharide-alditol consisting of GalNAc-ol, Gal, GlcNAc, NeuAc, and Fuc in the ratio 1:2:1:1:1. The ¹H-NMR spectrum of 9a₁ illustrates that a virtually pure compound is involved which belongs to the GlcNAcβ(1→6)[Galβ(1→3)]-GalNAc-ol core class (for δ H-2 and δ H-5 of GalNAc-ol, see Table 6). As with structures 7, 8b₂, and 9a₃ₐ, the sialic acid

residue in 9a₁ is found to be α(2→3)-linked to Gal³ in the core. This conclusion is based upon the chemical shift of NeuAc H-3eq (δ 2.774). The chemical shifts of the Fuc structural-reporter groups (δ H-1 5.107; δ CH₃ 1.174) reveal compound 9a₁ to be another extension of compound 7, which possesses a Fuc residue linked to the *N*-acetyllactosamine unit in the β(1→6)-arm; in 9a₁, Fuc is present in α(1→3)-linkage to GlcNAc⁶ rather than in α(1→2)-linkage to Gal. The effect on the chemical shift of the GlcNAc⁶ NAc signal brought about by the apparent attachment of Fuc³ to structure 7 (Δδ − 0.010 ppm, compare [2, 12, 23]) corroborates the pres-

Table 5. *^{1}H-NMR chemical shifts of structural-reporter groups of constituent monosaccharides for the bronchiectasis respiratory-mucus oligosaccharide-alditols which possess the NeuAc$\alpha(2\to6)_{0-1}$[GlcNAc$\beta(1\to3)$]GalNAc-ol core unit*

Chemical shifts are relative to internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate (using internal acetone at δ 2.225 ppm) in ^{2}H$_2$O at 27 °C (δ HO^{2}H 4.76 ppm), 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. [8, 9]); $\diamond$ = GalNAc-ol; $\blacksquare$— = Gal; $\bullet$— = GlcNAc; $\square$— = Fuc; $\triangle$— = NeuAc. The linkage position is specified by the direction of the connecting bars as follows:

Thus, at variance with our previous system of notation [2, 3, 12], the branch which is linked to C-6 of GalNAc-ol is pointing upward, and the branch linked to C-3 of GalNAc-ol downward

Residue[a]	Reporter group	Chemical shift in compound					
		3[b]	6a$_1$	6a$_3$	8b$_1$	8c	9b$_2$
		ppm					
GalNAc-ol	H-2	4.260	4.260	4.277	4.247	4.237	4.250
	H-3	3.985	3.990	n.d.	3.984	3.974	3.976
	H-5	4.185	4.185	4.126	4.179	4.153	4.198
	H-6′	3.487	3.491	n.d.[f]	3.489	3.468	3.48
	NAc	2.035	2.035	2.031	2.034[c]	2.034	2.033[d]
GlcNAc$^{3(a)}$	H-1	4.608	4.635	4.634	4.66	4.653	4.635[e]
	H-3	n.d.[f]	n.d.[f]	n.d.[f]	n.d.[f]	4.018	n.d.[f]
	H-6	3.939	4.009	4.052	4.021	3.944	n.d.[f]
	NAc	2.079	2.076	2.070	2.066	2.107	2.069
Gal^3	H-1	—	—	—	—	4.574	—
	H-4	—	—	—	—	3.885	—
Gal^4	H-1	—	4.464	4.496	4.444	—	4.493
	H-4	—	3.927	3.926	n.d.[f]	—	n.d.[f]
Fuc^2	H-1	—	—	—	—	5.206	5.276
	H-5	—	—	—	—	4.273	4.258
	CH$_3$	—	—	—	—	1.235	1.274
Fuc^3	H-1	—	—	5.131	5.132	—	5.113
	H-5	—	—	n.d.[f]	n.d.[f]	—	4.874
	CH$_3$	—	—	1.170	1.177	—	1.238
NeuAc^3	H-3ax	—	—	1.792	—	—	—
	H-3eq	—	—	2.760	—	—	—
	NAc	—	—	2.031	—	—	—
NeuAc^6	H-3ax	1.697	1.697	—	1.698	1.696	1.699
	H-3eq	2.733	2.733	—	2.732	2.730	2.735
	NAc	2.031	2.031	—	2.025[c]	2.030	2.026[d]

[a] A superscript at the name of a monosaccharide residue indicates to which position of the adjacent monosaccharide it is glycosidically linked.

[b] The data for compound 3 were reported in [22] (reference compound R-1).

[c] Assignments may have to be interchanged.

[d] Assignments may have to be interchanged.

[e] The shape of this GlcNAc H-1 doublet is distorted due to virtual coupling (δ H-2 $\approx$ δ H-3).

[f] Value could not be determined merely by inspection of the spectrum.

ence of this structural element. Thus, the structure of compound 9a$_1$ has been established to be:
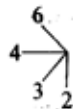
Gal$\beta(1\to4)$[Fuc$\alpha(1\to3)$]GlcNAc$\beta(1\to6)$-
[NeuAc$\alpha(2\to3)$Gal$\beta(1\to3)$]GalNAc-ol.
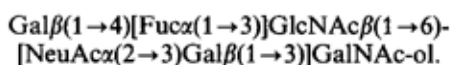
The NMR characteristics of the hexasaccharide-alditol 9a$_1$ have been observed before (compound B-8.1 in [22]).

Structure 9b$_3$. Compound 9b$_3$, being the minor (10%), third constituent of fraction 9b (see above), was concluded to be identical to 9a$_1$, because all resonances just described as being typical of 9a$_1$ are found in the spectrum of 9b (Fig. 6) with the low intensity typical of a minor component's reporter-group resonances.

Structure 10b. Fraction 10b, as suggested by its carbohydrate composition (Table 2), contains a heptasaccharide

Table 6. *^{1}H-NMR chemical shifts of structural-reporter groups of constituent monosaccharides for the bronchiectasis respiratory-mucus oligosaccharide-alditols which possess the GlcNAcβ(1→6)[Galβ(1→3)]GalNAc-ol core unit*

Chemical shifts are relative to internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate (using internal acetone at δ 2.225 ppm) in ^2H_2O at 27°C (δ HO^{2}H 4.76 ppm), 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. [8, 9]); ◇ = GalNAc-ol; ■— = Gal; ●— = GlcNAc; □— = Fuc; △— = NeuAc. The linkage position is specified by the direction of the connecting bars as follows:

Thus, at variance with our previous system of notation [2, 3, 12], the branch which is linked to C-6 of GalNAc-ol is pointing upward, and the branch linked to C-3 of GalNAc-ol downward

Residue[a]	Reporter group	Chemical shift in compound					
		8a	8b₂ (= 9a₃ₐ)	9a₁ (= 9b₃)	10b	8b₃	10d
		ppm					
GalNAc-ol	H-2	4.388	4.388	4.387	4.387	4.400	4.399
	H-3	4.067	4.067	4.067	4.067	4.082	4.083
	H-4	3.437	3.442	3.426	n.d.	3.49	n.d.
	H-5	4.269	4.271	4.258	4.260	4.253	4.249
	NAc	2.066	2.067[b]	2.066	2.064	2.054	2.055[d]
Gal[3(a)]	H-1	4.530	4.533	4.530	4.529	4.572	4.568
	H-3	4.114	4.115	4.114	4.112	n.d.[c]	n.d.[c]
	H-4	3.930	3.928	3.933	3.929	3.922	n.d.[c]
GlcNAc[6]	H-1	4.557	4.537	4.559	4.544	4.567	4.570
	H-6	3.994	3.988	4.006	4.025	4.018	4.023
	NAc	2.066	2.064[b]	2.056	2.056	2.054	2.044[d]
Gal[4]	H-1	4.468	4.537	4.447	4.498	4.550	4.515
	H-3	n.d.[c]	n.d.[c]	n.d.[c]	n.d.[c]	4.114	4.083
	H-4	3.924	3.891	3.895	n.d.[c]	3.957	n.d.[c]
Fuc[2]	H-1	—	5.307	—	5.280	5.224	5.222
	H-5	—	4.230	—	4.251	4.273	4.274
	CH₃	—	1.231	—	1.271	1.245	1.244
Fuc[3]	H-1	—	—	5.107	5.094	—	5.105
	H-5	—	—	4.829	4.870	—	4.82
	CH₃	—	—	1.174	1.235	—	1.168
NeuAc[3]	H-3ax	1.801	1.800	1.800	1.800	1.798	1.795
	H-3eq	2.775	2.774	2.774	2.773	2.756	2.763
	NAc	2.034	2.034	2.033	2.033	2.031	2.032

[a] A superscript at the name of a monosaccharide residue indicates to which position of the adjacent monosaccharide it is glycosidically linked.

[b] Assignments may have to be interchanged.

[c] Value could not be determined merely by inspection of the spectrum.

[d] Assignments may have to be interchanged.

that possesses a second Fuc residue in addition to the monosaccharides that are present in 8b₂/9a₃ₐ and 9a₁/9b₃. From the ^{1}H-NMR spectrum of fraction 10b (see Fig. 11) it is obvious that an essentially pure compound that belongs to the GlcNAcβ(1→6)[Galβ(1→3)]GalNAc-ol core class is involved (see chemical shifts of GalNAc-ol H-2 and H-5 in Table 6). The core Gal³ residue bears NeuAc in α(2→3)-linkage (δ H-3eq 2.773). Two Fuc H-1 signals are observed, at δ 5.280 and δ 5.094, respectively. Also, two Fuc CH₃ doublets are present, at δ 1.271 and δ 1.235. From the chemical shifts of this dual set of Fuc structural-reporter groups, it is deduced that one of the Fuc residues of compound 10b is α(1→2)-

attached to Gal⁴, the other one α(1→3)-linked to GlcNAc⁶. Together, this difucosyl *N*-acetyllactosamine unit constitutes the Y-determinant. Thus, the structure of compound 10b is as follows:

Fucα(1→2)Galβ(1→4)GlcNAcβ(1→6)
Fucα(1→3)   GalNAc-ol.
NeuAcα(2→3)Galβ(1→3)
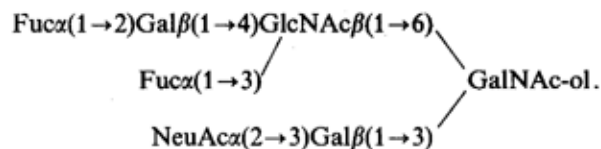
The Y determinant has been previously characterized by NMR in milk oligosaccharides [31], in *N*-type glycopeptides

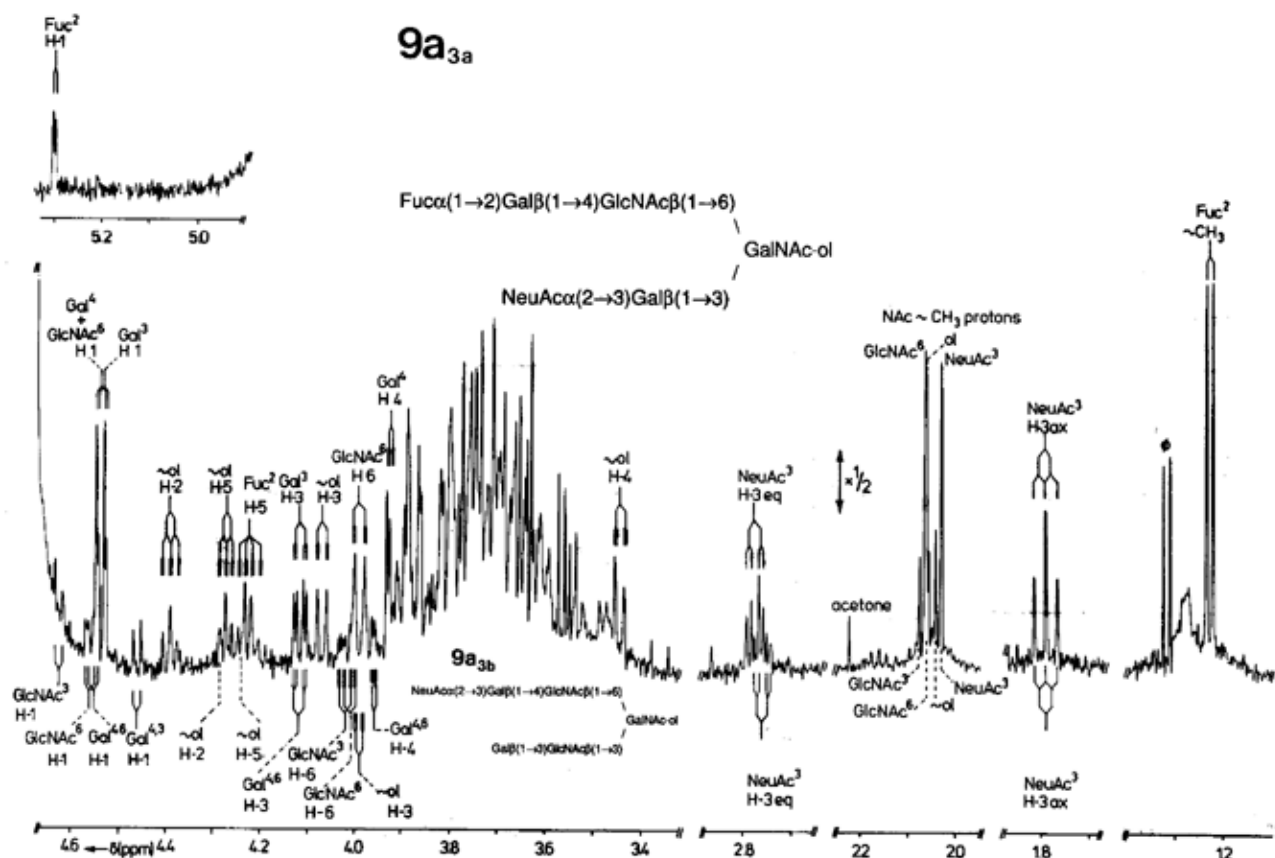Fig. 9. *500-MHz* [^1]*H-NMR spectrum (*[^2]*H₂O, p*[^2]*H 7, 27°C) of monosialyl oligosaccharide-alditol fraction 9a₃ obtained from sputum of a bronchiectasis patient*. The two identifiable components, 9a₃ₐ and 9a₃b, occur in ratio 2:1. The signals attributed to 9a₃ₐ are marked on top of the spectrum; those belonging to 9a₃b have been indicated on the bottom. The relative-intensity scale of the *N*-acetyl methyl proton region (1.85 < δ < 2.30) deviates from that of the other parts of the spectrum. Resonances marked by Φ stem from non-carbohydrate contaminants
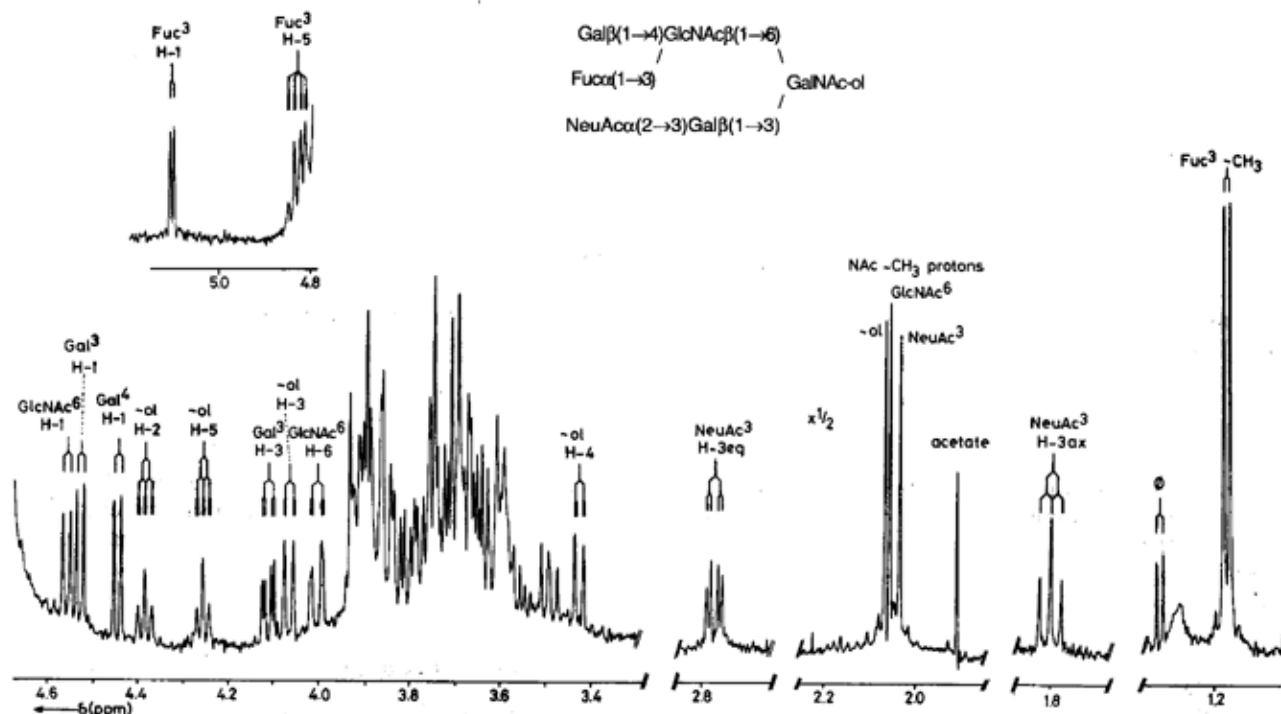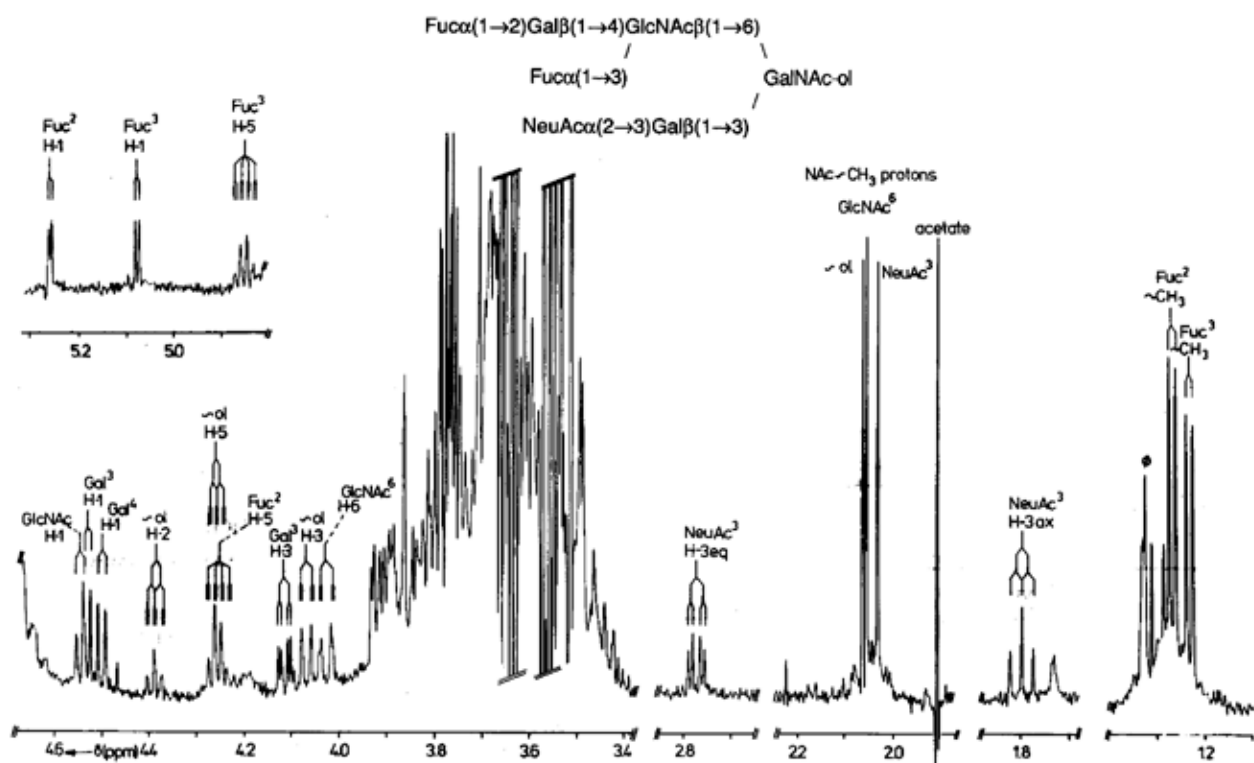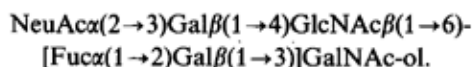

Fig. 10. *500-MHz* [^1]*H-NMR spectrum (*[^2]*H₂O, p*[^2]*H 7, 27°C) of monosialyl oligosaccharide-alditol fraction 9a₁ obtained from sputum of a bronchiectasis patient*. The relative-intensity scale of the *N*-acetyl methyl proton region (1.85 < δ < 2.30) deviates from that of the other parts of the spectrum. Resonances marked by Φ stem from non-carbohydrate contaminants

10b
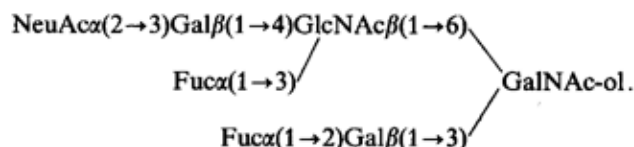

Fig. 11. *500-MHz ^{1}H-NMR spectrum (^2H_2O, p^2H 7, 27°C) of monosialyl oligosaccharide-alditol fraction 10b obtained from sputum of a bronchiectasis patient.* The relative-intensity scale of the N-acetyl methyl proton region ($1.85 < \delta < 2.30$) deviates from that of the other part of the spectrum. Resonances marked by Φ stem from non-carbohydrate contaminants

obtained from γ-seminal protein [28], and in oligosaccharide-alditols from ovarian-cyst mucins [27]. The ^{1}H-NMR spectroscopic characteristics of the Y determinant, in particular the chemical shift of the Fuc^3 H-1 signal, differ slightly when comparing 10b (Table 6) to 9b$_2$ (Table 5), in which the Y-determinant is $\beta(1\rightarrow3)$- rather than $\beta(1\rightarrow6)$-linked to GalNAc-ol. This is in accordance with the ^{1}H-NMR data reported for the Y-determinant in neutral oligosaccharide-alditols [8, 9].

Structure 8b$_3$. The 500-MHz ^{1}H-NMR spectrum of fraction 8b$_3$, presumably (see Table 2) containing a hexasaccharide, shows the GalNAc-ol H-2 and H-5 signals at δ 4.400 and 4.253, respectively. This identifies the core type of compound 8b$_3$ as GlcNAc$\beta(1\rightarrow6)$[Gal$\beta(1\rightarrow3)$]GalNAc-ol. The H-3ax and H-3eq signals of the NeuAc residue in 8b$_3$ are observed at δ 1.798 and 2.756, respectively. In particular the latter of the two values indicates that NeuAc is $\alpha(2\rightarrow3)$-linked to a Gal residue involved in a $\beta(1\rightarrow4)$-linkage to GlcNAc [14, 20, 23, 29]; the NeuAc residue is therefore located in the $\beta(1\rightarrow6)$-arm of structure 8b$_3$. The structural-reporter groups of Fuc are observed at the same positions as for compound 5b (see Table 4), implying that Fuc is $\alpha(1\rightarrow2)$-linked to the core Gal^3. In combination with the sugar composition of fraction 8b$_3$, this completes the structure of the compound to be:

NeuAc$\alpha(2\rightarrow3)$Gal$\beta(1\rightarrow4)$GlcNAc$\beta(1\rightarrow6)$-
[Fuc$\alpha(1\rightarrow2)$Gal$\beta(1\rightarrow3)$]GalNAc-ol.

Structure 10d. The carbohydrate composition of compound 10d suggests the presence of a heptasaccharide

consisting of GalNAc-ol, Gal, GlcNAc, NeuAc and Fuc in the ratio 1:2:1:1:2 (see Table 2). The chemical shifts of GalNAc-ol H-2 and H-5 in the NMR spectrum of fraction 10d (δ 4.399 and 4.249; see Table 6) classify the compound as belonging to the GlcNAc$\beta(1\rightarrow6)$[Gal$\beta(1\rightarrow3)$]GalNAc-ol core type. We conclude that the core Gal^3 residue is substituted by Fuc in $\alpha(1\rightarrow2)$-linkage (δ H-1 5.222; δ H-5 4.274; δ CH$_3$ 1.244) for reasons outlined above for compounds 5b and 8b$_3$. The second Fuc residue (δ H-1 5.105; δ CH$_3$ 1.168) is $\alpha(1\rightarrow3)$-linked to the GlcNAc^6 residue in the core, forming part of an N-acetyllactosamine unit (compare compound 9a$_1$, Table 6). The NeuAc residue in 10d is $\alpha(2\rightarrow3)$-linked to the Gal^4 residue of the N-acetyllactosamine moiety (δ H-3eq 2.763). Thus, the $(1\rightarrow6)$-linked branch of compound 10d contains the sialyl X-determinant (compare compounds A-4 and A-6 [3]):

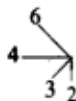NeuAc$\alpha(2\rightarrow3)$Gal$\beta(1\rightarrow4)$GlcNAc$\beta(1\rightarrow6)$

Fuc$\alpha(1\rightarrow3)$ ╲ GalNAc-ol.

Fuc$\alpha(1\rightarrow2)$Gal$\beta(1\rightarrow3)$ ╱

(v) Structures of the GlcNAcβ(1→6)[GlcNAcβ(1→3)]GalNAc-ol core type

Structure 6b. The carbohydrate composition of fraction 6b (Table 2) suggests that a pentasaccharide is involved consisting of GalNAc-ol, Gal, GlcNAc, and NeuAc in the ratio of 1:1:2:1. Its ^{1}H-NMR data (Table 7) match those of

Table 7. *^{1}H-NMR chemical shifts of structural-reporter groups of constituent monosaccharides for the bronchiectasis respiratory-mucus oligosaccharide-alditols which possess the GlcNAcβ(1→6)[GlcNAcβ-(1→3)]GalNAc-ol core unit*

Chemical shifts are relative to internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate (using internal acetone at δ 2.225 ppm) in ^2H_2O at 27°C (δ HO^{2}H 4.76 ppm), 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. [8, 9]); ◇ = GalNAc-ol; ■— = Gal; ●— = GlcNAc; □— = Fuc; △— = NeuAc. The linkage position is specified by the direction of the connecting bars as follows:

```
        6
        ↑
   4 ——→
        ↓
       3 2
```

Thus, at variance with our previous system of notation [2, 3, 12], the branch which is linked to C-6 of GalNAc-ol is pointing upward, and the branch linked to C-3 of GalNAc-ol downward

Residue[a,b]	Reporter group	Chemical shift in compound 6b	9a₃ᵦ	10c
		ppm		
GalNAc-ol	H-2	4.279	4.28	4.257
	H-3	3.985	n.d.[c]	3.983
	H-4	3.514	n.d.[c]	n.d.[c]
	H-5	4.234	4.23–4.24	4.204
	NAc	2.044	2.043	2.042
GlcNAc³⁽ᵃ⁾	H-1	4.597	4.623	4.650
	H-6	3.954	4.021	3.948
	NAc	2.081	2.078	2.108
GlcNAc⁶	H-1	4.556	4.558	4.564
	H-6	4.011	4.009	4.003
	NAc	2.058	2.059	2.057
Gal³	H-1	—	Gal⁴·³ 4.456	4.564
	H-4	—	n.d.[c]	3.961
Gal⁴	H-1	4.551	Gal⁴·⁶ 4.551	4.547
	H-3	4.113	4.115	4.115
	H-4	3.960	3.960	n.d.[c]
Fuc²	H-1	—	—	5.211
	H-5	—	—	4.273
	CH₃	—	—	1.231
NeuAc³	H-3ax	1.798	1.800	1.799
	H-3eq	2.757	2.758	2.758
	NAc	2.032	2.033	2.032

[a] A superscript at the name of a monosaccharide residue indicates to which position of the adjacent monosaccharide it is glycosidically linked.

[b] A second superscript is used to discriminate between identically bound Gal residues. It indicates the type of linkage of the monosaccharide adjacent to this Gal.

[c] Value could not be determined merely by inspection of the spectrum.

oligosaccharide A-2 from cystic fibrosis respiratory mucins [3]. Therefore, the structure of pentasaccharide-alditol 6b is:
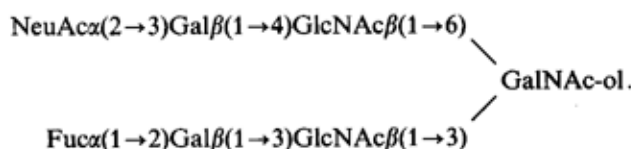
NeuAcα(2→3)Galβ(1→4)GlcNAcβ(1→6)-
[GlcNAcβ(1→3)]GalNAc-ol.
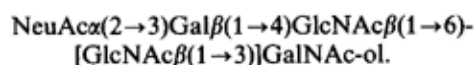
Structure 9a₃ᵦ. As mentioned above, fraction 9a₃ contains a mixture of (at least) two oligosaccharide-alditols, in ratio 2:1. The minor (33%) constituent, designated 9a₃ᵦ, is of the GlcNAcβ(1→6)[GlcNAcβ(1→3)]GalNAc-ol core type, as is inferred from the set of GalNAc-ol H-2 and H-5 signals at δ 4.28 and δ 4.24, respectively. Corroborating evidence is the occurrence of three lower-intensity NAc singlets in the spectrum (see Fig. 9), attributable to GlcNAc³, GlcNAc⁶, and GalNAc-ol. The β(1→6)-linked arm of structure 9a₃ᵦ is identical to that of 6b, that is, an α(2→3)-sialylated N-acetyllactosamine unit (compare NMR data of 9a₃ᵦ and 6b in Table 7). The difference between 9a₃ᵦ and 6b is in the substitution of the core GlcNAc³ residue by a Gal residue in β-linkage in 9a₃ᵦ. The presence of the Gal residue is evident from the chemical shift of an additional H-1 signal at δ 4.456 (Fig. 11). In combination with the shift effects observed on the H-1 and NAc signals of GlcNAc³ compared to 6b (see Table 7) the δ H-1 value points to the terminal position of this Gal residue in the chain, and also to its β(1→4)-linkage to GlcNAc³ [13, 25, 32]. Combination of the data leads to the following structure for 9a₃ᵦ:

NeuAcα(2→3)Galβ(1→4)GlcNAcβ(1→6)-
[Galβ(1→4)GlcNAcβ(1→3)]GalNAc-ol.
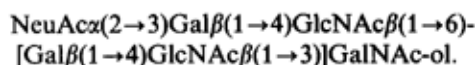
It should be mentioned that the NMR features of compound 9a₃ᵦ were described before (compound B-8.2 in [22]). The possible alternative structure in which the NeuAc residue would be located in the β(1→3) rather than the β(1→6)-branch of the hexasaccharide could be ruled out for 9a₃ᵦ on the basis of the chemical shift of the H-1 of the terminal Gal⁴: δ 4.456 is known [2] to be characteristic for terminal Gal⁴·³, while δ ≈ 4.473 would be observed for terminal Gal⁴·⁶ (see compound 15.2A in [2]; compare also compound 7, Table 6). This conclusion is supported by the chemical shifts of the NAc signals of GlcNAc³ (δ 2.078) and GlcNAc⁶ (δ 2.059) for 9a₃ᵦ (see Table 7). The latter chemical shift shows the small but significant effect (Δδ − 0.003 ppm, compared to 15.2 A [2]) known [14, 23] to be characteristic for α(2→3)-sialylation of the branch, while the former one does not show that effect.

Structure 10c. The ^{1}H-NMR spectrum of fraction 10c indicates the presence of a virtually pure compound that contains the GlcNAcβ(1→6)[GlcNAcβ(1→3)]GalNAc-ol core trisaccharide (see the chemical shifts of GalNAc-ol H-2 and H-5, Table 7). The β(1→6)-linked branch is identical to that described before for compounds 6b and 9a₃ᵦ, namely, NeuAcα(2→3)Galβ(1→4)GlcNAcβ(1→6). This is illustrated by the perfect match of the NMR features of this part of structure 10c with those of the corresponding structural-reporter groups of 6b and 9a₃ᵦ (Table 7). The core GlcNAcβ(1→3) residue bears a Fucα(1→2)Galβ(1→3) moiety. The location of this moiety is inferred from the chemical shifts of the Fuc H-1 and CH₃ signals, but, most of all, from the position of the NAc signal of GlcNAc³ (δ 2.108; compare compound 8c, Table 6), which is known to be [2, 12] highly characteristic for the entire structural element Fucα(1→2)-Galβ(1→3)GlcNAcβ(1→3)GalNAc-ol. The structure of compound 10c was thus established to be:

NeuAcα(2→3)Galβ(1→4)GlcNAcβ(1→6)
GalNAc-ol.
Fucα(1→2)Galβ(1→3)GlcNAcβ(1→3)

(vi) Structure 9a₂. The sugar composition of fraction 9a$_2$ (Table 2) suggests that, under the assumption that just one compound is involved, the fraction contains a heptasaccharide consisting of GalNAc-ol, Gal, GlcNAc, Fuc and NeuAc in the ratio of 1:2:1:2:1. The NeuAc residue is present in $\alpha(2\rightarrow3)$-linkage to a Gal^4 residue, as is evident from the chemical shifts of its H-3ax and H-3eq atoms. The GlcNAc residue is present in $\beta(1\rightarrow3)$-linkage (δ H-1 4.704). The structural-reporter groups of one of the two Fuc residues in 9a$_2$ are observed at δ 5.119 (H-1) and δ 1.167 (CH$_3$); we conclude that this Fuc residue is $\alpha(1\rightarrow3)$-linked to GlcNAc^3. The second Fuc residue, however, is characterized by its H-1/H-5/CH$_3$ signals at δ 5.158/4.28/1.202; such a combination of chemical shifts has not been observed before in mucin-type oligosaccharide-alditols. Therefore, the location of the second Fuc residue in the structure was not possible by NMR alone. Unfortunately, the amount of material of 9a$_2$ available was too small to permit methylation analysis before and after defucosylation. Therefore, the structure of compound 9a$_2$ could not be elucidated.

DISCUSSION

Alkaline borohydride reductive treatment of bronchial-mucus glycopeptides isolated from the sputum of a bronchiectasis patient yielded a mixture of glycopeptides and oligosaccharide-alditols. Fractionation by ion-exchange chromatography and gel filtration afforded, *inter alia*, a pool of relatively small, monosialyl oligosaccharide-alditols designated IIc [8]. Fraction IIc was further partitioned by HPLC. Initial straight-phase HPLC of IIc on alkylamine-bonded silica led to 10 subfractions. Repeated straight-phase HPLC and/or subsequent reverse-phase HPLC of most of the 10 fractions yielded a total of 22 subfractions. The merits of both straight-phase and reverse-phase HPLC for the separation of mucin-type oligosaccharide-alditols, and the structural features that govern the sequence of their elution, have been discussed extensively [2, 3, 8 – 11, 15, 22, 26, 27]. In particular, those structures that contain the Fuc$\alpha(1\rightarrow2)$Gal$\beta(1\rightarrow3)$-GalNAc-ol element (e.g. 5b, 8b$_3$, and 10d) are slowly eluted from the reverse-phase C$_{18}$ column (compare [11]). Mixtures of positional isomers, like 8b$_2$ and 8b$_3$, 9a$_1$ and 9a$_3$, 10b and 10d, were adequately separated by reverse-phase HPLC. It should be noted, however, that significant variations in retention times of oligosaccharide-alditols occur when the HPLC columns are aging.

500-MHz ^{1}H-NMR spectroscopy, in combination with quantitative sugar composition analysis of the 22 subfractions, afforded the structures of 24 monosialyl oligosaccharide-alditols (see Scheme 1). The structure of the constituent(s) of one fraction, 9a$_2$, could not be determined. Five different substitution patterns of GalNAc-ol were established among the series of 24 sialyl oligosaccharide-alditols, namely, (i) Gal$\beta(1\rightarrow3)$GalNAc-ol, (ii) NeuAc$\alpha(2\rightarrow6)$[Gal$\beta(1\rightarrow3)$]-GalNAc-ol, (iii) NeuAc$\alpha(2\rightarrow6)$[GlcNAc$\beta(1\rightarrow3)$]GalNAc-ol, (iv) GlcNAc$\beta(1\rightarrow6)$[Gal$\beta(1\rightarrow3)$]GalNAc-ol, and (v) GlcNAc$\beta(1\rightarrow6)$[GlcNAc$\beta(1\rightarrow3)$]GalNAc-ol. The ^{1}H-NMR characteristics for compounds with the NeuAc$\alpha(2\rightarrow6)$[GlcNAc$\beta(1\rightarrow3)$]GalNAc-ol type of core have been firmly established.

The peripheral regions of the oligosaccharide-alditols are comprised of blood group antigenic determinants, such as H type-1, H type-2, X, or Y determinants, or contain sialic acid. 13 of the monosialyl oligosaccharide-alditols contain NeuAc in $\alpha(2\rightarrow3)$-linkage to Gal, 1 possesses NeuAc in $\alpha(2\rightarrow6)$-linkage to Gal, and the remaining 10 have NeuAc $\alpha(2\rightarrow6)$-linked to GalNAc-ol. While NeuAc$\alpha(2\rightarrow3)$Gal$\beta(1\rightarrow\bullet)$ and NeuAc$\alpha(2\rightarrow6)$GalNAc-ol are fairly common elements in mucin-type oligosaccharides, NeuAc$\alpha(2\rightarrow6)$Gal$\beta(1\rightarrow4)$ is not observed in such structures as frequently. Previously, the latter structural element has only been characterized in hog and rat salivary mucus [33, 34] and in a minor oligosaccharide derived from cystic-fibrosis-bronchial mucus [22]. The corresponding neutral analogues of most of the 24 structures listed in Scheme 1 were found among pool Ic of neutral oligosaccharide-alditols obtained from the bronchial mucus of the bronchiectasis patient [8, 9]. Only two of the 24 structures, namely, 6a$_3$ and 10b, were not characterized in their desialylated form. Three of the 24 structures end in the sialyl X-determinant. In contrast to the monosialyl oligosaccharide-alditols from cystic-fibrosis-bronchial-mucus fraction IIc [3, 22], the sialyl X-determinant seems to occur preferentially in the branch linked to C-3 of GalNAc-ol in bronchiectasis-mucus glycoproteins.

The NMR parameters of both the NeuAc and Fuc residue permit, independent from each other, the location of each of these residues in a particular branch of the oligosaccharide-alditol in question. Comparison of the NMR features of 8b$_2$ and 8b$_3$ (see Table 6) illustrates the power of ^{1}H-NMR spectroscopy in this respect. In particular, δ H-1 of Fuc and δ H-3eq of NeuAc are useful parameters for this purpose. Another example of the chemical shift of one particular reporter-group signal being indicative of a structural entity of four monosaccharides is the δ NAc value of core GlcNAc$\beta(1\rightarrow3)$: when observed at $\delta \approx 2.11$, this signal is typical of Fuc$\alpha(1\rightarrow2)$Gal$\beta(1\rightarrow3)$GlcNAc$\beta(1\rightarrow3)$GalNAc-ol.

Since the present study was performed on the bronchial mucins stemming from a single bronchiectasis patient, the heterogeneity in the carbohydrate structures cannot be ascribed to pooling of material from different individuals. The heterogeneity in the carbohydrates appears to be an intrinsic phenomenon of bronchial mucus glycoproteins, which may be due in part to the different origins of the mucus glycoproteins [35], but which may also reflect the competition of various glycosyltransferases for the same substrate during the biosynthesis of these glycoproteins.

There are some differences in the structures and relative abundances of the monosialyl oligosaccharide-alditols when comparing the bronchial-mucus glycoproteins of the bronchiectasis patient to the cystic fibrosis patients investigated previously [3, 22]. However, it would be premature to claim that these differences, e.g. the preponderance of the sialyl X-determinant in the C-6 branch of cystic fibrosis oligosaccharide-alditols, correspond to biosynthetic features specific for this disease.

REFERENCES

1. Roussel, P., Lamblin, G., Degand, P., Walker-Nasir, E. & Jeanloz, R. W. (1975) *J. Biol. Chem. 250*, 2114 – 2122.

2. Lamblin, G., Boersma, A., Lhermitte, M., Roussel, P., Mutsaers, J. H. G. M., Van Halbeek, H. & Vliegenthart, J. F. G. (1984) *Eur. J. Biochem. 143*, 227–236.

3. Lamblin, G., Boersma, A., Klein, A., Roussel, P., Van Halbeek, H. & Vliegenthart, J. F. G. (1984) *J. Biol. Chem. 259*, 9051–9058.

4. Carroll, S. M., Higa, H. H. & Paulson, J. C. (1981) *J. Biol. Chem. 256*, 8357–8363.

5. Ramphal, R. J. & Pyle, M. (1983) *Infect. Immun. 41*, 339–344.

6. Loones, L. M., Uemura, K., Childs, R. A., Paulson, J. C., Rogers, G. N., Scudder, P. R., Michalski, J. C., Hounsell, E. F., Taylor-Robinson, D. & Feizi, T. (1984) *Nature (Lond.) 307*, 560–563.

7. Eliasson, R., Mossberg, B., Camner, P. & Afzelius, B. A. (1977) *N. Engl. J. Med. 297*, 1–6.

8. Klein, A., Lamblin, G., Lhermitte, M., Roussel, P., Breg, J., Van Halbeek, H. & Vliegenthart, J. F. G. (1988) *Eur. J. Biochem. 171*, 631–642.

9. Breg, J., Van Halbeek, H., Vliegenthart, J. F. G., Klein, A., Lamblin, G. & Roussel, P. (1988) *Eur. J. Biochem. 171*, 643–654.

10. Lamblin, G., Klein, A., Boersma, A., Nasir-ud-Din & Roussel, P. (1983) *Carbohydr. Res. 118*, c1–c4.

11. Dua, V. K., Dube, V. E. & Bush, C. A. (1984) *Biochim. Biophys. Acta 802*, 29–40.

12. Van Halbeek, H., Dorland, L., Vliegenthart, J. F. G., Hull, W. E., Lamblin, G., Lhermitte, M., Boersma, A. & Roussel, P. (1982) *Eur. J. Biochem. 127*, 7–20.

13. Van Halbeek, H. (1984) *Biochem. Soc. Trans. 12*, 601–605.

14. Vliegenthart, J. F. G., Dorland, L., Van Halbeek, H. & Haverkamp, J. (1982) *Cell Biol. Monogr. 10*, 127–172.

15. Nasir-ud-Din, Jeanloz, R. W., Lamblin, G., Roussel, P., Van Halbeek, H., Mutsaers, J. H. G. M. & Vliegenthart, J. F. G. (1986) *J. Biol. Chem. 261*, 1992–1997.

16. Van Halbeek, H., Dorland, L., Vliegenthart, J. F. G., Fiat, A.-M. & Jollès, P. (1980) *Biochim. Biophys. Acta 623*, 295–300.

17. Vliegenthart, J. F. G., Van Halbeek, H. & Dorland, L. (1981) *Pure Appl. Chem. 53*, 45–77.

18. Van Halbeek, H., Dorland, L., Vliegenthart, J. F. G., Fiat, A.-M. & Jollès, P. (1981) *FEBS Lett. 133*, 45–50.

19. Savage, A. V., Koppen, P. L., Schiphorst, W. E. C. M., Trippelvitz, L. A. W., Van Halbeek, H., Vliegenthart, J. F. G. & Van den Eijnden, D. H. (1986) *Eur. J. Biochem. 160*, 123–129.

20. Korrel, S. A. M., Clemetson, K. J., Van Halbeek, H., Kamerling, J. P., Sixma, J. J. & Vliegenthart, J. F. G. (1984) *Eur. J. Biochem. 140*, 571–576.

21. Nato, F., Goulut, C., Bourrillon, R., Van Halbeek, H. & Vliegenthart, J. F. G. (1986) *Eur. J. Biochem. 159*, 303–308.

22. Breg, J., Van Halbeek, H., Vliegenthart, J. F. G., Lamblin, G., Houvenaghel, M. C. & Roussel, P. (1987) *Eur. J. Biochem. 168*, 57–68.

23. Vliegenthart, J. F. G., Dorland, L. & Van Halbeek, H. (1983) *Adv. Carbohydr. Chem. Biochem. 41*, 209–374.

24. Pierce-Crétel, A., Debray, H., Montreuil, J., Spik, G., Van Halbeek, H., Mutsaers, J. H. G. M. & Vliegenthart, J. F. G. (1984) *Eur. J. Biochem. 139*, 337–349.

25. Mutsaers, J. H. G. M., Van Halbeek, H., Vliegenthart, J. F. G., Wu, A. M. & Kabat, E. A. (1986) *Eur. J. Biochem. 157*, 139–146.

26. Dua, V. K., Rao, B. N. N., Wu, S. S., Dube, V. E. & Bush, C. A. (1986) *J. Biol. Chem. 261*, 1599–1608.

27. Dua, V. K., Dube, V. E., Li, Y. T. & Bush, C. A. (1985) *Glycoconjugate J. 2*, 17–30.

28. Van Halbeek, H., Gerwig, G. J., Vliegenthart, J. F. G., Tsuda, R., Hara, M., Akiyama, K. & Schmid, K. (1985) *Biochem. Biophys. Res. Commun. 131*, 507–514.

29. Akiyama, K., Simons, E. R., Bernasconi, P., Schmid, K., Van Halbeek, H., Vliegenthart, J. F. G., Haupt, H. & Schwick, H. G. (1984) *J. Biol. Chem. 259*, 7151–7154.

30. Van Halbeek, H., Dorland, L., Vliegenthart, J. F. G., Kochetkov, N. K., Arbatsky, N. P. & Derevitskaya, V. A. (1982) *Eur. J. Biochem. 127*, 21–29.

31. Hindsgaul, O., Norberg, T., Le Pendu, J. & Lemieux, R. U. (1982) *Carbohydr. Res. 109*, 109–142.

32. Bernard, N., Engler, R., Strecker, G., Montreuil, J., Van Halbeek, H. & Vliegenthart, J. F. G. (1984) *Glycoconjugate J. 1*, 123–140.

33. Baig, M. M. & Aminoff, D. (1972) *J. Biol. Chem. 247*, 6111–6118.

34. Slomiany, A. & Slomiany, B. L. (1978) *J. Biol. Chem. 253*, 7301–7306.

35. Mazzuca, M., Lhermitte, M., Lafitte, J. J. & Roussel, P. (1982) *J. Histochem. Cytochem. 30*, 956–966.