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Structure of the exceptionally large nonrepetitive carbohydrate backbone of the lipopolysaccharide of *Pectinatus frisingensis* strain VTT E-82164

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The structures of the oligosaccharides obtained after acetic acid hydrolysis and alkaline deacylation of the rough-type lipopolysaccharide (LPS) from *Pectinatus frisingensis* strain VTT E-82164 were analysed using NMR spectroscopy, MS and chemical methods. The LPS contains two major structural variants, differing by a decasaccharide fragment, and some minor variants lacking the terminal glucose residue. The largest structure of the carbohydrate backbone of the LPS that could be deduced from experimental results consists of 25 monosaccharides (including the previously found Ara4N*P* residue in lipid A) arranged in a well-defined nonrepetitive structure:

β-Ara4N-(1-*P*-6)-α-GlcN-(1-4)-α-Kdo-(2-4)┐
α-Glc-(1-2)-α-Glc-(1-3)-α-Gal-(1-2)-α-Man-(1-4)-α-Man-(1-5)-α-Kdo-(2-6)-β-GlcN-(1-6)-α-GlcN1*P*
R[1]-3)-α-Glc-(1-3)-α-Man-(1-6)┘└(3-2)-α-Kdo

where **R**[1] is H or

α-GlcNAc-(1-3)┐
β-GlcNAc-(1-4)-α-Fuc-(1-2)-β-GlcA-(1-4)-α-Fuc-(1-3)-α-GalNAc-(1-4)-β-ManNAc-(1-4)-α-Gal-(1-3)-β-GalNAc-(1-
2-O-Ac-3-O-Me-α-6dTal-(1-3)┘

We presume that the shorter variant with $R^1 = H$ represents the core-lipid A part of the LPS, and the additional fragment is present instead of the O-specific polysaccharide. Structures of this type have not been previously described. Analysis of the deacylation products obtained from the LPS of the smooth strain, VTT E-79100[T], showed that it contains a very similar core but with one different glycosidic linkage.

Keywords: core; lipid A; lipopolysaccharide; *Pectinatus frisingensis*.

Strictly anaerobic Gram-negative rod-shaped bacteria causing turbidity and off flavours in bottled beer were initially isolated in 1978 and described as *Pectinatus cerevisiiphilus* [1]. Another species, *Pectinatus frisingensis*, which differed from *P. cerevisiiphilus* in a number of biochemical characteristics was later described [2]. To date, the VTT culture collection (Espoo, Finland) has 32 *Pectinatus* isolates from spoiled beer originating from Belgium, Finland, Germany, the Nederlands and the USA; 24 have been identified as *P. frisingensis* and eight as *P. cerevisiiphilus* by conventional tests, ribotyping and partial 16S rDNA sequence analysis [3].

The lipopolysaccharides (LPS) of type strains of *P. cerevisiiphilus* and *P. frisingensis* possess a number of remarkable properties, including the predominance of odd-numbered fatty acids in lipid A [4] and the presence of furanosidic 6-deoxysugars in the O-specific chains [5]. The lipid A was shown to be quantitatively substituted at the 4′-phosphate and partially at the glycosidic phosphate by 4-amino-4-deoxy-β-L-arabinose [6]. There are no structural data on

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Abbreviations: LPS, lipopolysaccharide; Kdo, 3-deoxy-D-*manno*-oct-2-ulosonic acid; Ara4N, 4-amino-4-deoxy-L-arabinose; HPAEC, high-performance anion-exchange chromatography; ESI MS, electrospray ionization mass spectrometry.

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Pectinatus core structures, except a report that LPS of both *P. frisingesis* and *P. cerevisiiphilus* contain a disaccharide structure, a phosphorylated GlcN linked to O4 of a Kdo residue, tentatively assigned to the core region [7].

Screening of *Pectinatus* strains other than type strains has revealed that the LPS from certain strains exhibit only two distinct bands on PAGE, with no polymeric O chains (I. M. Helander, unpublished data). This indicates the presence of two structurally distinct LPS molecules. We describe here the chemical structure of the LPS carbohydrate backbone of one such isolate, *P. frisingensis* VTT-E-82164, which has 99.8% similarity of partial 16S rDNA to the *P. frisingensis* type strain.

Materials and methods

Bacterial strains and growth conditions

P. frisingensis VTT E-82164 and VTT E-79100^T and *P. cerevisiiphilus* E-79103^T were obtained from VTT Biotechnology (Espoo, Finland) [3]. Cells were grown anaerobically at 32 °C without shaking in Man Rogosa Sharpe broth (Difco), pH 6.5, in the presence of a reducing agent (Na_2S, 12.5 mM) and resazurin (1 mg·mL^{-1}), and collected at the stationary growth phase.

LPS isolation

Bacterial cells were washed with ethanol, acetone, and light petroleum, and LPS was extracted from the dried cells with phenol/chloroform/petroleum ether (60–95 °C) (5 : 5 : 8, v/v) with acetone precipitation [4,8].

NMR spectroscopy and general methods

NMR spectra were recorded at 25 °C in D_2O on a Varian Unity Inova 800 instrument at 799.96 MHz for proton and 201.12 MHz for carbon, using acetone as reference for proton (2.225 p.p.m.) and 1,4-dioxane for carbon (67.4 p.p.m.). Varian standard programs tndqcosy, tnnoesy (mixing time of 100 ms), tntocsy (spinlock time 80 ms), gHSQC, gHSQCTOCSY (spinlock time 80 ms), gHSQCNOESY (mixing time 200 ms) and gHMBC were used with digital resolution in F2 dimension < 2 Hz·pt^{-1}. Spectra were assigned using the computer program PRONTO [9].

Analytical methods

PAGE was performed with deoxycholate as the detergent. The separation gel contained 18% acrylamide, 0.5% (w/v) deoxycholate, and 375 mM Tris/HCl, pH 8.8, and stacking gel contained 4% acrylamide and 127 mM Tris/HCl, pH 6.8. LPS samples were prepared at a concentration of 0.1% (w/v) in sample buffer [127 mM Tris/HCl, pH 6.8, 10% (v/v) glycerol, 0.025% (w/v) bromphenol blue dye]. The electrode buffer was composed of deoxycholate (2.5 g·L^{-1}), glycine (21.7 g·L^{-1}), and Tris (4.5 g·L^{-1}). Electrophoresis was performed at a constant current of 15 mA per gel with cooling. Immediately after the electrophoresis run, the gel was soaked in the fixing solution containing ethanol (40%, w/w) and acetic acid (5%, w/w). The solution was changed after 30 min, and fixation continued overnight. LPS bands were visualized by silver staining as described by Tsai & Frasch [10].

Hydrolysis was performed with 4 M trifluoroacetic acid (110 °C, 3 h). Monosaccharides were conventionally converted into the alditol acetates and analysed by GC on a Agilent 6850 chromatograph equipped with a DB-17 fused-silica column (30 m × 0.25 mm) using a temperature gradient of 180 °C (2 min) → 240 °C at 2 °C·min^{-1}. For the determination of the absolute configuration of 3-*O*-methyl-6-deoxytalose, GC was performed in isothermal conditions at 150 °C. GC-MS was performed on a Varian Saturn 2000 system with ion-trap mass spectral detector using the same column. Electrospray ionization (ESI) MS was carried out as described previously [11].

Gel chromatography was carried out on columns (2.5 × 95 cm) of Sephadex G-50 in pyridinium/acetate buffer, pH 4.5 (4 mL pyridine and 10 mL acetic acid in 1 L water) and BioGel P4 (1 × 90 cm) in water. The eluate was monitored with a refractive index detector.

Methylation analysis was performed by the Ciucanu-Kerek procedure [12]. Methylated products were hydrolysed and monosaccharides converted into 1d-alditol acetates by conventional methods and analysed by GC-MS.

High-performance anion-exchange chromatography (HPAEC) was performed on a CarboPac PA1 column (9 × 250 mm) with pulsed amperiometric detection, equilibrated in 0.1 M NaOH, using a linear gradient of 1 M sodium acetate in 0.1 M NaOH from 5% to 80% of acetate in 60 min at 3 mL·min^{-1}. Fractions of volume 3 mL were collected and analysed using the Dionex system with an analytical CarboPac PA1 column (4.6 × 250 mm) at 1 mL·min^{-1}. Separated oligosaccharides were desalted on a Sephadex G-50 column.

De-O,N-acylation of LPS and preparation of backbone oligosaccharides [13]

LPS (120 mg) was dissolved in 4 M KOH (4 mL), and the solution was heated at 120 °C for 16 h, cooled, neutralized with 2 M HCl. The precipitate was removed by centrifugation, and the supernatant desalted by gel chromatography on Sephadex G-50. Two oligosaccharide fractions with K_{av} 0.60 and 0.47 were obtained and further separated by HPAEC on a semipreparative CarboPac PA1 column to give oligosaccharides **1a**, **1b** and a mixture of **2** and **3**.

Deamination of the de-O,N-acylated LPS and preparation of oligosaccharides 4 and 5

The mixture of oligosaccharides obtained after alkaline deacylation of the LPS (200 mg) was treated with 300 mg $NaNO_2$ in 10% acetic acid (10 mL, 25 °C, 24 h), desalted on a Sephadex G-50 column, reduced with $NaBH_4$, desalted, and oligosaccharides **4** and **5** isolated by HPAEC.

Acetic acid hydrolysis of LPS

LPS (100 mg) was treated with 2% acetic acid (5 mL, 100 °C, 3 h). The precipitate was removed by centrifugation, and the soluble products were separated on a Sephadex

G-50 column to give three oligosaccharide fractions. These were $NaBH_4$-reduced, desalted, and separated by HPAEC to give oligosaccharides **6**–**8**.

Isolation of 3-*O*-methyl-6-deoxy-D-talose (11)

LPS (300 mg) was hydrolysed with 3 M trifluoroacetic acid (100 °C, 1.5 h), and the cooled dark solution was treated with activated carbon, filtered, and evaporated to dryness. An aqueous solution of the residue was passed through a column (0.8 × 15 cm) of Dowex 50W8 (× 200; H^+), then through a column of Dowex 2 (AcO^-). The monosaccharides were separated by paper chromatography on Whatman 3MM paper in pyridine/butanol/water (6 : 4 : 3, v/v/v). Sugars were detected on a small strip with $AgNO_3$/NaOH reagent, and eluted with water. The portions of the fractions mainly containing Man, Glc, Gal, Fuc, and pure 3-*O*-methyl-6-deoxy-D-talose were treated with (*S*)-2-butanol/acetyl chloride (10 : 1, v/v; 2 h; 85 °C), dried under a stream of air, acetylated, and analysed by GC. 3-*O*-Methyl-6-deoxy-D-talose (3 mg) was obtained in pure form (moves close to front on paper); $[\alpha]_D$ + 2° (*c* 0.3, water), lit. for L-isomer (trivial name acovenose) −14.2° (*c*1.2, water) [14].

Amino sugars were eluted from Dowex 50 with 0.5 M HCl, N-acetylated (5 mL saturated $NaHCO_3$, 0.5 mL acetic anhydride; 20°, 1 h with stirring), converted into (*S*)-2-butyl glycoside acetates as described above, and analysed by GC.

Synthesis of methyl 3-*O*-methyl-6-deoxy-α-D-talopyranoside (9) and methyl 3-*O*-methyl-6-deoxy-β-D-talopyranoside (10)

3-*O*-Methyl-D-glucose (a gift from M. Perry, NRC Canada) (500 mg) was converted into an approximately 4 : 1 mixture of α-methyl and β-methyl glycosides by methanolysis (1 M HCl/MeOH; 85 °C; 24 h), brominated at C6 using CBr_4/imidazole/triphenyl phospine (1 : 1 : 2.5, v/v; 16 h; 25 °C; product isolated by column chromatography on SiO_2 in 5% MeOH in $CHCl_3$), and debrominated by hydrogenolysis over Pd/C in MeOH to yield methyl 3-*O*-methyl-6-deoxy-α,β-glucopyranosides. These were converted into methyl 3-*O*-methyl-2,4-di-*O*-trifluorosulfonyl-6-deoxy-α,β-D-glucopyranoside [$(CF_3SO_3)_2O$/Py; −20 °C to +25 °C) and treated with excess Et_4NOAc in dimethylformamide (100 °C; 3 h). The reaction mixture was diluted 10 times with water, passed through Dowex 50 (H^+) to remove Et_4N^+, evaporated to dryness, and compounds **9** and **10** were isolated by C_{18} RP-HPLC in water (45 and 8 mg, respectively).

Results

The LPS from *P. frisingensis* VTT E-82164 did not exhibit the typical ladder-like pattern of smooth LPS on deoxycholate-PAGE, but showed two main strongly stained rapidly migrating bands (Fig. 1).

Monosaccharide analysis of the whole LPS indicated the presence of fucose, 3-*O*-methyl-6-deoxyhexose, glucose, galactose, mannose, glucosamine, galactosamine, and mannosamine in the proportions 1 : 0.6 : 1.5 : 1.2 : 1.4 : 1.5 : 0.6 : 0.4.

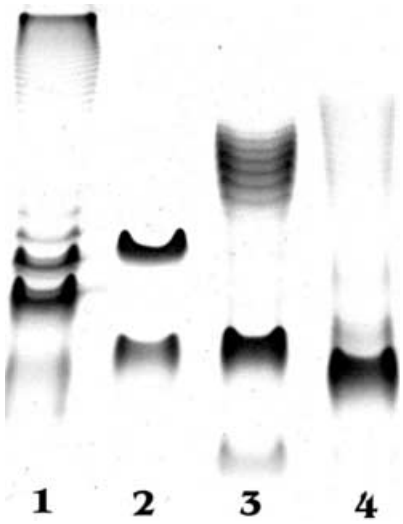


Fig. 1. Deoxycholate-PAGE profiles of the LPS. Lane 1, *Salmonella enteriditis*; lane 2, *P. frisingensis* E-82164; lane 3, *P. frisingensis* type strain E-79100T; lane 4, *P. cerevisiiphilus* type strain E-79103T.

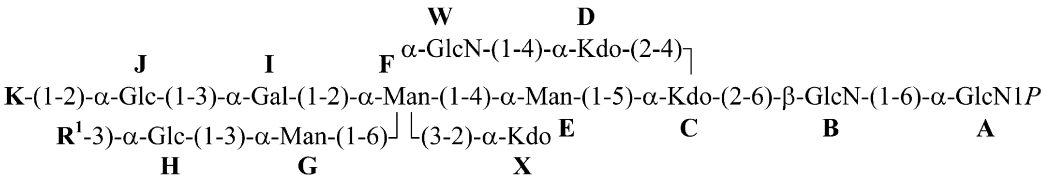
The LPS was O,N-deacylated by strong alkaline treatment. Gel chromatographic separation of the products on Sephadex G-50 gave two main peaks, which were further separated by HPAEC to give oligosaccharides **1a**, **1b**, and a mixture of **2** and **3** (Scheme 1).

In another experiment, the oligosaccharides obtained after deacylation and Sephadex G-50 separation were deaminated with nitrous acid and reduced with $NaBH_4$. This led to removal of all amino sugar residues except B and O, which were transformed into 2,5-anhydromannitol and 2,5-anhydrotalitol, respectively. The products were separated by HPAEC and the oligosaccharides **4** and **5** were isolated.

Mild hydrolysis of the LPS with acetic acid and subsequent separation of the products by gel chromatography gave three oligosaccharide fractions. These were reduced with $NaBH_4$ and purified by HPAEC to give oligosaccharides **6**, **7a**, and **8**. The longer oligosaccharide **7b** was also analysed by NMR without reduction and HPAEC, which allowed detection of O-acetylation. Separation by HPAEC led to O-deacetylation of the reduced oligosaccharide **7b** because of the alkaline chromatography conditions.

In 1D and 2D NMR spectra of compound **1a** (Fig. 2), spin systems of 13 monosaccharides were identified. These were three α-Glc residues (H, J, K), three α-Man residues (E, F, G), one α-Gal residue (I), two α-GlcN (A, W) residues, one β-GlcN (B) residue, and three Kdo residues (C, D, X). The spectra were completely assigned (Table 1), and the sequence of the hexoses was determined from NOE and HMBC data, in which all respective strong transglycosidic correlations were observed. Assignments were made using methodology outlined in [15]. Oligosaccharides **1a** and **1b** are the fragments of the larger structure **2**, thus the NMR data for **1a,b** are very close to those for the respective residues in **2** and are not presented.

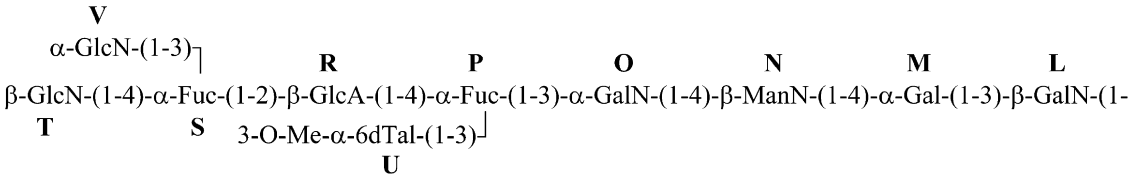
The position and anomeric configuration of Kdo residues was not as easy to assign. The ^1H and ^{13}C chemical shifts of Kdo residues C and X agreed well with



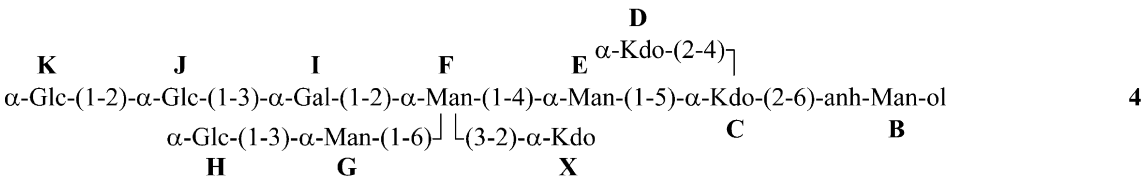
1a: **K** = α-Glc, **R**^{1} = H

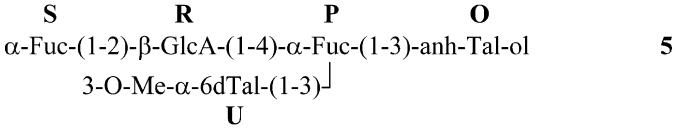
1b: **K** = H, **R**^{1} = H

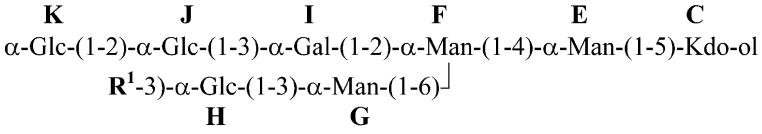
2: **K** = α-Glc, **R**^{1} =



3: - same as **2** with **R** = α-GlcA.

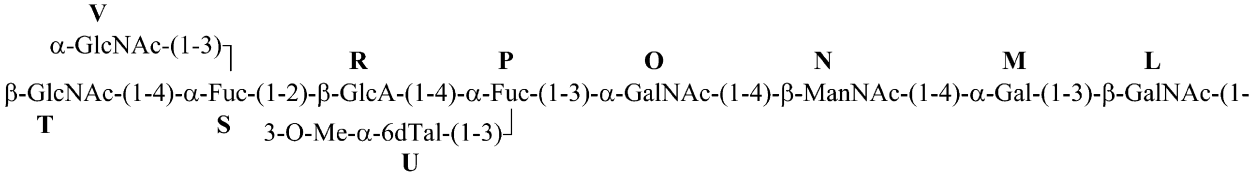






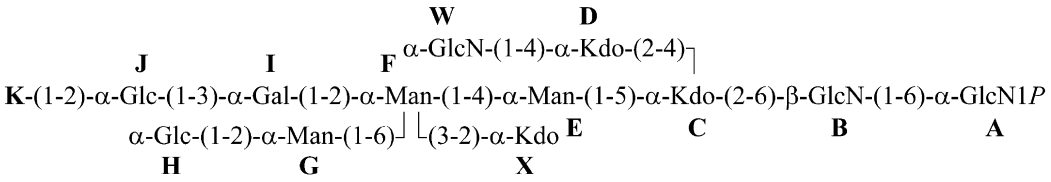
6 R^1 = H

7a R^1 =



7b: same as **7a** but **U** = 2-O-Ac-3-O-Me-α-6dTal, **C** = Kdo

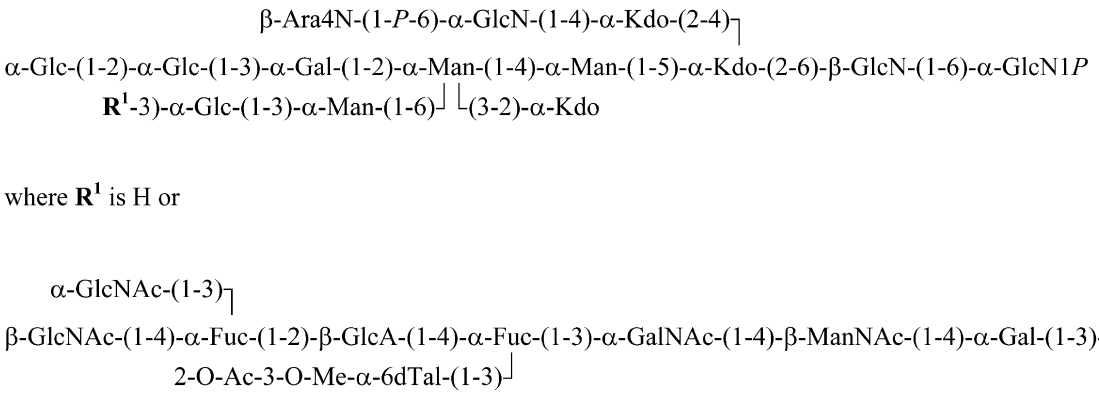
Scheme 1. Structures of the isolated compounds and proposed structure of the carbohydrate backbone of *P. frisingensis* VTT E-82164 LPS.



12a: **K** = α-Glc

12b: **K** = H

Proposed structure of the carbohydrate backbone of *P. frisigensis* LPS:



where $\mathbf{R^1}$ is H or

Scheme 1. (Continued).

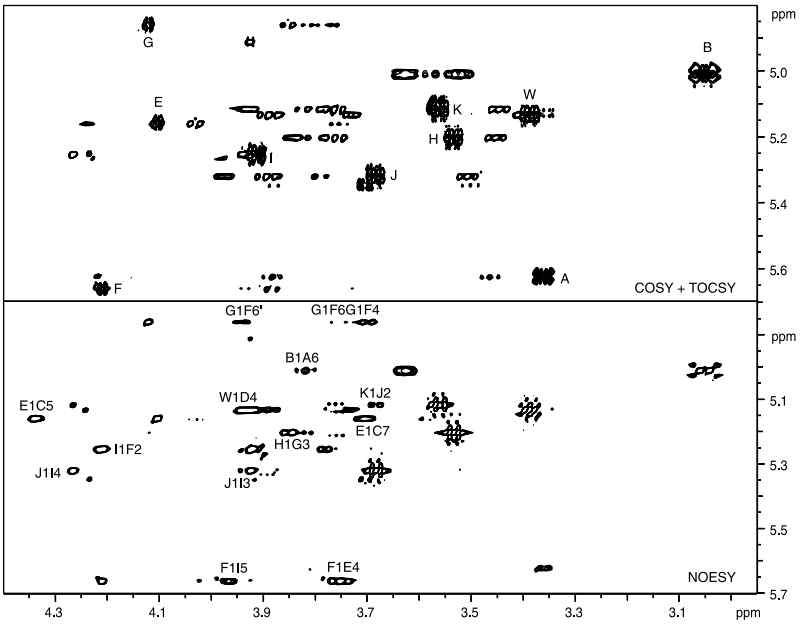


Fig. 2. Sections of COSY, TOCSY, and NOESY spectra of the oligosaccharide 1a, containing correlations from anomeric protons.

their α-configuration [16], while the H3 signals of Kdo D appeared at 1.97 (ax) and 2.53 (eq) p.p.m., which may correspond to a β-configuration [16]. However, a NOE correlation observed between H3 of Kdo C and H6 of Kdo D is possible only in the case of an α-configuration of residue D, linked to O4 of Kdo C, as follows from molecular modeling. The unusual position of the H3 signals of residue D in product **1a** (as well as in **1b**, **2**, and **3**)

Table 1. Assigned NMR spectral data for the isolated oligosaccharides obtained in ^2H_2O at 25 °C. Residue nomenclature and oligosaccharide structures are given in Scheme 1.

Unit, compound	Nucleus	**1**	**2** (**3**eq)	**3** (**3**ax)	**4**	**5**	**6** (**5b**)	**7** (**6b**)	**8a** (OMe)	**8b**
α-GlcNP A, **2, 3**	^1H	5.61	3.35	3.87	3.45	4.13	4.21	3.79		
	^{13}C	91.8	55.6	70.8	70.9	74.1	70.2			
β-GlcN B, **2, 3**	^1H	4.96	3.04	3.61	3.49	3.61	3.58	3.54		
	^{13}C	100.8	56.8	73.5	71.1	75.7	62.5			
α-Kdo C, **2, 3**	^1H		2.04	1.99	4.17	4.32	3.58	3.69	3.89	3.58
	^{13}C		101.5	35.4	70.5	72.7	73.8	70.5	64.8	
Kdo-ol C, **7a,b**	^1H		1.98	2.04		3.95	3.90	3.66	3.69	3.87
	^{13}C			38.8		81.6	71.3	72.1	64.2	
α-Kdo D, **2, 3**	^1H		2.63	1.91	3.95	4.23	3.74	3.97	3.95	3.81
	^{13}C		100.4	34.9	78.6	66.3	73.0	71.2	64.0	
α-Kdo-ol D, **8**	^1H		4.14	2.14/2.08	4.19	4.14	3.75	3.75	3.69	3.86
	^{13}C		71.4	38.0	80.5	71.4	72.1[a]	73.6[a]	65.1	
α-GlcN W, **2, 3**	^1H	5.12	3.38	3.88	3.34	3.73	3.86	3.78		
	^{13}C	98.5	55.1	70.8	71.1	74.9	62.3			
α-GlcN6P W, **8**	^1H	5.43	3.37	3.93	3.62	4.04	4.15	4.21		
	^{13}C	97.4	56.6	71.8	71.3	73.8	66.4			
α-Kdo X, **2, 3**	^1H		2.09	1.85	4.07	4.02	3.76	3.93	3.85	3.69
	^{13}C		103.8	35.5	66.6	67.4	73.5	70.3	63.3	
α-Man E, **2, 3**	^1H	5.13	4.09	4.01	3.68	4.26	3.74	4.02		
	^{13}C	100.2	71.7	72.3	76.4	71.5	63.6			
α-Man E, **7a,b**	^1H	5.07	4.04	4.06	3.83	3.97				
	^{13}C	103.3	72.0	72.0	76.3	73.1				
α-Man F, **2, 3**	^1H	5.58	4.20	3.86	3.84	3.75	3.86	3.75		
	^{13}C	101.0	80.3	76.0	66.6	74.0	67.6			
α-Man F, **7a,b**	^1H	5.63	4.12	3.96	3.81	3.91	3.79	4.02		
	^{13}C	101.1	82.0	71.7	68.0	72.9	67.1			
α-Man G, **2, 3**	^1H	4.84	4.15	3.89	3.81	3.82	3.85	3.76		
	^{13}C	100.6	70.7	81.9	66.9	73.7	62.3			
α-Man G, **7a,b**	^1H	4.91	4.16	3.89	3.88	3.78				
	^{13}C	100.8	70.8	81.7	66.9	73.9				
α-Glc H, **2, 3**	^1H	5.20	3.78	4.01	3.56	3.87	3.82	3.78		
	^{13}C	102.2	72.8	83.0	68.9	73.4	61.1			
α-Glc H, **7a,b**	^1H	5.22	3.62	3.97	3.57	3.90	3.83			
	^{13}C	102.7	72.8	83.2	69.3	72.9	61.4			
α-Gal I, **2, 3**	^1H	5.25	3.90	3.91	4.24	3.98	3.73	3.69		
	^{13}C	102.3	68.1	75.3	66.4	73.4	62.3			
α-Gal I, **7a,b**	^1H	5.19	4.01	3.95	4.32	4.07				
	^{13}C	102.7	68.3	75.2	66.2	72.6	62.3			
α-Glc J, **2, 3**	^1H	5.30	3.68	3.88	3.48	3.95	3.86	3.77		
	^{13}C	93.1	76.7	72.3	70.6	72.1	61.5			
α-Glc J, **7a,b**	^1H	5.36	3.69	3.93	3.52	3.98	3.80	3.89		
	^{13}C	92.8	76.6	72.4	70.6	72.7	61.9			
α-Glc K, **2, 3**	^1H	5.09	3.55	3.74	3.42	3.93	3.81	3.76		
	^{13}C	97.7	72.3	73.9	70.5	73.0	61.5			
α-Glc K, **7a,b**	^1H	5.14	3.60	3.80	3.50	3.96				
	^{13}C	97.6	72.4	74.0	70.5	73.0	61.5			
β-GalN L, **2, 3**	^1H	4.88	3.38	4.00	4.25	3.71	3.82	3.75		
	^{13}C	101.6	53.5	76.2	64.7	76.3	62.1			
β-GalN L, **7a,b**	^1H	4.78	4.11	3.86	4.18	3.71				
	^{13}C	103.0	52.3	77.0	65.3	75.9				
α-Gal M, **2, 3**	^1H	5.25	3.93	4.09	4.28	3.96	3.73	3.69		
	^{13}C	96.4	69.0	70.1	77.3	72.7	62.3			
α-Gal M, **7a,b**	^1H	5.10	3.82	3.90	4.23	3.90				
	^{13}C	97.0	69.1	70.5	77.3	71.8				
β-ManN N, **2, 3**	^1H	5.11	3.87	4.27	3.79	3.58	3.94	3.83		
	^{13}C	103.0	56.1	71.1	74.3	75.8	61.8			
β-ManN N, **7a,b**	^1H	4.98	4.61	4.16	3.68	3.53	3.92	3.99		
	^{13}C	100.9	54.8	73.7	76.7	76.1	61.6			

Table 1. (Continued).

Unit, compound	Nucleus	**1**	**2** (**3**eq)	**3** (**3**ax)	**4**	**5**	**6** (**5b**)	**7** (**6b**)	**8a** (OMe)	**8b**
α-GalN O, **2**	^{1}H	5.59	3.66	4.04	4.08	4.07	3.79	3.73		
	^{13}C	99.0	51.2	77.4	69.4	72.6	62.2			
α-GalN O, **3**	^{1}H	5.61	3.69	4.06	4.13	4.08	3.79	3.73		
	^{13}C	98.7	51.1	77.7	69.3	72.6	62.2			
α-GalN O, **7a,b**	^{1}H	5.22	4.44	3.98	4.04	4.09	3.79	3.83		
	^{13}C	100.9	50.2	75.2	69.9	73.1	62.3			
α-Fuc P, **2**	^{1}H	5.11	3.81	4.08	4.04	4.21	1.34			
	^{13}C	98.6	69.5	76.0	80.2	69.1	16.9			
α-Fuc P, **3**	^{1}H	5.09	3.89	4.07	4.08	4.26	1.30			
	^{13}C	103.0	73.2	76.0	80.0	69.4	17.1			
α-Fuc P, **7a,b**	^{1}H	5.10	3.71	4.08	4.02	4.17	1.39			
	^{13}C	101.9	69.4	76.1	80.6	68.4	16.9			
β-GlcA R, **2**	^{1}H	4.62	3.68	3.71	3.71	3.61				
	^{13}C	102.9	76.3	78.3	72.0	79.4				
β-GlcA R, **7a,b**	^{1}H	4.72	3.68	3.78	3.70	3.86				
	^{13}C	103.2	75.4	78.1	72.5	77.0	173.3			
α-GlcA R, **3**	^{1}H	5.02	3.75	3.86	3.82	4.39				
	^{13}C	100.5	74.2	70.8	71.6	72.2				
α-6dTal U, **2**	^{1}H	5.07	4.05	3.57	3.93	4.23	1.18		3.47	
	^{13}C	104.2	68.0	75.0	70.3	68.6	16.6		56.1	
α-6dTal U, **3**	^{1}H	5.10	4.06	3.51	3.90	4.33	1.221		3.45	
	^{13}C	104.0	68.2	75.3	70.3	68.8	16.7		56.2	
α-6dTal U, **7b**	^{1}H	5.08	5.24	3.67	3.82	4.21	1.22		3.44	
	^{13}C	102.8	68.5	74.8	69.2	68.2	16.7		56.8	
α-6dTal U, **7a**	^{1}H	5.13	4.14	3.55	3.82	4.20	1.24		3.51	
	^{13}C	104.0	67.6	75.3	70.9	68.4	16.6		56.1	
9	^{1}H	4.83	4.01	3.54	3.95	4.00	1.29		3.46	
9, $J_{n,n+1}$, Hz		1	3.5	3.5	~1	6.6				
9	^{13}C	102.5	68.1	75.7	70.4	68.2	16.6		56.1	
10	^{1}H	4.48	4.13	3.46	3.88	3.69	1.32		3.56	
	^{13}C	102.7	68.9	78.6	70.0	72.7	16.5		57.9	
α-11	^{1}H	5.24	4.00	3.61	3.95	4.18	1.27		3.45	
	^{13}C	95.6	68.8	75.2	70.4	68.1	16.7		56.3	
β-11	^{1}H	4.78	4.06	3.48	3.88	3.70	1.30		3.45	
	^{13}C	95.0	69.6	78.5	69.5	72.3	16.6		56.3	
α-Fuc S, **2**	^{1}H	5.56	4.05	4.08	4.20	4.56	1.27			
	^{13}C	99.4	69.2	73.6	78.8	68.0	17.4			
α-Fuc S, **3**	^{1}H	5.38	4.03	4.11	4.24	4.39	1.25			
	^{13}C	98.9	69.2	74.1	78.8	68.5	17.2			
α-Fuc S, **7a,b**	^{1}H	5.50	4.01	4.09	4.08	4.56	1.29			
	^{13}C	99.6	69.4	72.7	79.0	67.9	17.4			
β-GlcN T, **2,3**	^{1}H	4.77	3.02	3.58	3.43	3.43	3.93	3.76		
	^{13}C	100.7	57.8	74.3	70.8	77.8	61.5			
β-GlcN T, **7a,b**	^{1}H	4.71	3.85	3.62	3.44	3.47	3.76	4.04		
	^{13}C	102.2	57.2	74.9	71.6	77.3	62.1			
α-GlcN V, **2**	^{1}H	5.43	3.34	3.88	3.57	3.88	3.82	3.75		
	^{13}C	97.8	55.3	73.2	70.3	73.2	61.1			
α-GlcN V, **3**	^{1}H	5.44	3.33	3.91	3.51	3.88	3.82	3.75		
	^{13}C	97.8	55.3	73.4	70.6	73.2	61.1			
α-GlcN V, **7a,b**	^{1}H	5.24	3.96	3.84	3.66	3.90				
	^{13}C	99.3	54.7	72.5	70.4	72.8				
β-Ara4N Y, **8**	^{1}H	5.56	3.79	4.21	3.76	3.88	4.26			
	^{13}C	97.6	70.1	67.5	54.1	61.5				

[a] Assignments might be interchanged.

was probably due to its substitution by an α-GlcN residue. A similar effect was observed for the products obtained from *Acinetobacter* LPS [17,18]. Indeed, the configuration of Kdo D was unambiguously determined on the basis of NMR analysis of oligosaccharide **4**, in which Kdo D was not substituted and its H3 signals appeared at 1.70 and 1.94 p.p.m., corresponding to an α-configuration.

The position of the Kdo residue X was identified on the basis of the NOE correlation between its H6 and H2 of the Man residue F (which is analogous to the NOE between protons C3 and D6). This conclusion was confirmed by the results of the methylation analysis of compound **4**. The methylated oligosaccharide was hydrolyzed, and the monosaccharides converted into alditol acetates with deuterium label at C1 using $NaBD_4$ reduction, acetylated, and analyzed by GC-MS, which allowed identification of all partially methylated alditol acetates expected for structure **4**.

The ^{31}P-NMR spectrum of **1a** contained only one signal at 2 p.p.m., correlating with H1 of the α-GlcN residue A, with a coupling constant of 6.5 Hz. Thus oligosaccharide **1a** was phosphorylated at A1.

The negative ion mode ES mass spectrum of **1a** gave a molecular mass of 2378 Da, which corresponded to the expected composition $Hex_7HexN_3Kdo_3P_1$.

The minor product **1b** contained one hexose residue less than **1a** according to the mass spectrum (molecular mass of 2216 Da, $Hex_6HexN_3Kdo_3P_1$). This is confirmed in the NMR data by the absence of the glucose residue K, consistent with the structures shown in Scheme 1.

Oligosaccharides **2** and **3** were isolated in a mixture at a ratio of about 5 : 1. Analysis of the major series of signals in the NMR spectra of this mixture led to the identification of all components of oligosaccharide **1a** and also 10 monosaccharide spin systems (Fig. 3). The NMR spectra of this product were complex, but, at 800 MHz with the use of the standard 2D techniques DQFCOSY, TOCSY, NOESY, HSQC, HMBC, HSQC-TOCSY, HSQC-NOESY, the signal spread was sufficient for identification of all monosaccharides and linkages between them, as presented in Scheme 1. The most problematic assignment was related to the group of signals near 5.1 p.p.m., belonging to ManN N, Fuc P, 3-*O*-methyl-6-deoxytalose U (from **3**), GlcN W, and Glc K. Assignment of the signals of residue N and determination of its position in the structure was possible using ^{1}H-^{13}C correlation spectra (HSQC, HMBC, HSQC-TOCSY, HSQC-NOESY). The monosaccharide sequence was deduced from the observed transglycosidic correlations from proton-NOE to proton(s)/HMBC to carbon: B1-A6/A6; E1-C5,C7,D7/C5; W1-D4,D5/D4; I1-F2,X6/F2; F1-E4/E4; F2-X6; G1-F6/F6; H1-G2,G3/G3; J1-I3,I4/I3; K1-J2,I4/J2; L1-H3/H3; M1-L3,L4/L3; N1-M4/M4; P1-O3/O3; R1-P4/P4; U1-P3/–; S1-R2/R2; T1-S4/S4; V1-S3/S3. Determination of the substitution position of glucuronic acid R was difficult because of extensive overlapping of its ^{1}H and ^{13}C NMR signals. It was found to be substituted at O2 from the methylation analysis and from the data for other oligosaccharides. The problems with residues N, R, U were resolved in the analysis of the oligosaccharide **7**, which showed no signal overlap for the corresponding residues. In general, all assignments were confirmed by methylation analysis.

The residue 3-*O*-methyl-6-deoxyhexose (U) had all small intra-ring coupling constants (< 3 Hz) in the ^{1}H-NMR spectrum, which could correspond to an α-*talo*- or an α-*gulo*- configuration. For the reliable determination of its configuration, a model compound methyl 3-*O*-methyl-6-deoxy-α-D-talopyranoside (**9**), and its β-anomer (**10**), were synthesized. This was achieved by configuration inversion at C2 and C4 in the methyl 3-*O*-methyl-2,4-di-*O*-trifluoromethylsulfonyl-6-deoxy-α,β-D-glucopyranoside. NMR data (^{1}H and ^{13}C chemical shifts and vicinal coupling constants) for the synthetic compound **9** were close to those of the residue U in the oligosaccharides (Table 1). Monosaccharides were furthermore identified by GC as alditol acetates. Thus the residue of 3-*O*-methyl-6-deoxyhexose had a *talo*-configuration. 3-*O*-Methyl-6-deoxy-D-talopyranose, **11**, was isolated from the hydrolysate of the LPS. It contained α-pyranose and β-pyranose anomeric forms (NMR data in Table 1), and a smaller amount of furanoside forms (data for furanoses not presented).

In addition, a minor series of signals in the spectra of the **2** + **3** mixture could be attributed to structure **3**, with a single difference from **2** to an altered anomeric

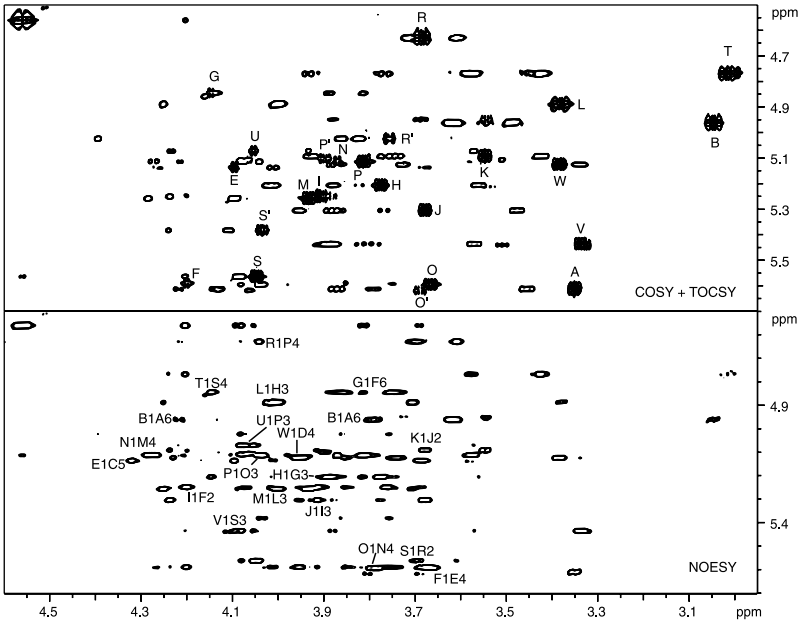


Fig. 3. Sections of COSY, TOCSY, and NOESY spectra of the mixture of the oligosaccharides 2 (letter labels) and 3 (letters with apostrophe labels), containing correlations from anomeric protons.

configuration of the residue of GlcA R, being α in **3**. The origin of α-GlcA is not clear; it was not present among the products of mild acid hydrolysis and thus may be an artefact of alkaline treatment.

Structures **2** and **3** were in agreement with ESI-MS data, which determined a molecular mass of 3973.5 Da ($Hex_8HexN_8HexA_1dHex_3Kdo_3P_1Me_1$).

Methylation analysis of the O-deacylated LPS was performed using the Ciucanu-Kerek method [12]. Methylated product was converted into a mixture of partially methylated alditol acetates by acid hydrolysis, reduction with $NaBD_4$, and acetylation. On another sample, the methylated product was depolymerized by acid methanolysis, treated with $NaBD_4$ to reduce carboxy groups, hydrolysed, reduced with $NaBD_4$, and acetylated. The second procedure led to the reduction of the GlcA residue with the introduction of two deuterium labels at C6. Comparison of the two chromatograms allowed unambigous confirmation that GlcA is substituted at position 2. The substitution positions of all the other monosaccharides were confirmed by GC-MS data of the methylated products to be as presented in Scheme 1.

Deamination of the products of complete deacylation of the LPS led to the oligosaccharides **4** and **5**, representing undecasaccharide and pentasaccharide fragments of oligosaccharides **1a** and/or **2**. These products were isolated by HPAEC (after borohydride reduction) and analysed by NMR spectroscopy, ESI MS, and methylation. The most important result obtained from NMR analysis of compound **4** was the determination of the anomeric configuration of Kdo D (see above).

Mild acid hydrolysis of the LPS with subsequent borohydride reduction and separation of the products by HPAEC in alkaline buffer led to the isolation of three main compounds **6**, **7a**, and **8**. The 18-residue oligosaccharide **7a** contained all the components of oligosaccharide **2**, except the Kdo residues D and X, GlcN residues A, B, and W. All amino sugars were N-acetylated. NMR spectra of this oligosaccharide were analysed (Table 1) and found to be consistent with the structure presented in Scheme 1. Especially useful for the assignment was the well-separated position of the H1 signal of ManN N, which allowed unambigous determination of its anomeric configuration as β, based on the intraresidual NOE between H1 and H3,5 (all axial) and the low-field position of its C5 at 76.1 p.p.m. No α-GlcA was found in the products, thus we conclude that α-GlcA in product **3** was a result of configuration inversion during strong alkaline treatment. ESI MS data confirmed the structure of **7a** (observed mass of 3181 Da) and showed that it contained minor amount of the structure with missing hexose. As in products **1**–**3**, Glc residue K was missing.

Analysis of the oligosaccharide **7b**, obtained after mild acid hydrolysis by gel chromatography without reduction, showed that it contains an O-acetyl group on O2 of 3-*O*-Me-α-6dTal residue U. Acetylation of O2 led to low-field shift of the residue U H2 signal to 5.24 p.p.m. (compare with 4.14 p.p.m. in **7a**). Its C1 signal was shifted 2 p.p.m. to high field in **7b** compared with **7a** (Table 1) because of the β-effect of the acetylation. Acetylation of **7b** was confirmed by ESI MS data, which gave the expected mass of 3221.4 Da.

The spectra of oligosaccharide **6** were completely assigned, and its structure was determined as presented in Scheme 1 (NMR data not shown). A variant of **6** without Glc K was also isolated as a minor compound.

The product **8** contained the residue of 4-amino-4-deoxyarabinose (Y), which was not found in the products of alkaline deacylation of the LPS. It was linked to position 6 of the GlcN residue by a phosphodiester bond (^{31}P signal at −0.3 p.p.m., correlating with H6 of GlcN and H1 of Ara4N). The residue of Ara4N1*P* was lost after KOH deacylation and therefore was not present in oligosaccharides **1**–**3**.

The NOE spectra of oligosaccharides **2**, **3**, and **7a,b** contained a number of correlations from H6 of 6-deoxysugars (Fig. 4). This fact was used as additional proof of the structural assignment. The terminal heptasaccharide fragment including monosaccharide residues from O to V was

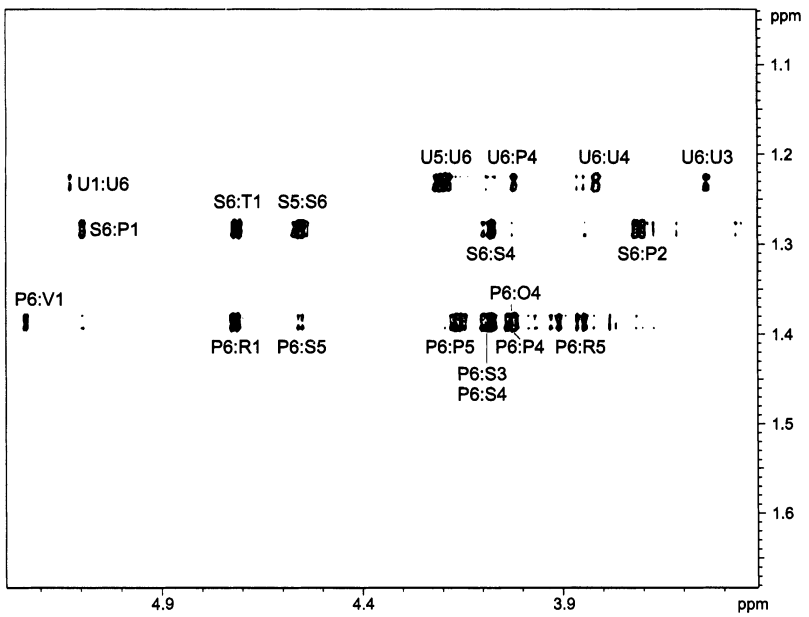


Fig. 4. Part of the NOESY spectrum of compound 7b, containing correlations of H6 of 6-deoxysugars.

modeled using the InsightII-Discover program, and minimum energy conformation was obtained using cvff force field. The minimum energy structure indeed explained most of the observed NOE contacts; calculated distances were within a range of 2.5–4 Å. Only the NOE between protons P6 and V1 remained unexplained. The distance between these protons was ≈9 Å and it was not clear how the molecule can be modified in order to shorten this distance. Modeling also confirmed a D-configuration for 3-*O*-methyl-6-deoxytalose, as setting the L-isomer instead of the D-isomer resulted in the disappearance of the contact between protons U6 and P4.

To determine the absolute configurations of the monosaccharides, LPS (300 mg) was hydrolysed with 3 M trifluoroacetic acid. The product was treated with activated carbon and sequentially passed through cationite in H^+ form and then anionite in AcO^- form. Neutral sugars were separated by paper chromatography, and fractions with a predominance of Gal, Glc, and Man, as well as pure Fuc and 3-*O*-methyl-6-deoxy-D-talose were isolated. They were converted into acetates of (*S*)-2-butyl glycosides and analysed by GC using the corresponding standard derivatives prepared with (*S*)-2-butanol and (*R*)-2-butanol. Thus Glc, Gal, Man, and 3-*O*-methyl-6-deoxytalose were found to have the D-configuration, and Fuc had the L-configuration. 3-*O*-Methyl-6-deoxytalose had a positive optical rotation, which confirms its D-configuration. However, the value of the optical rotation was much smaller than expected: + 2° *v* −14° published for the L-isomer [14].

Amino sugars were eluted from cationite with 0.5 M HCl, N-acetylated, converted into (*S*)-2-butyl glycoside acetates, and analysed by GC. Thus the D-configuration of GlcN and GalN was established. ManN was present in this mixture in small amounts, and its configuration could not be reliably determined; it was deduced to be D from NMR data.

To confirm the absolute configurations of the monosaccharides, ^{13}C-NMR spectra of linear trisaccharide substructures with different combinations of the absolute configurations of the components were calculated [19] and the results compared with observed spectra. Chemical shifts of C1 and carbon atoms at substitution and neighboring positions were taken into consideration. The results agreed with the presented structure and showed, in particular, the configuration of ManNAc to be D.

From the combined the data on the structures of the isolated oligosaccharides, the overall structure of the *P. frisingensis* LPS carbohydrate backbone shown in Scheme 1 is proposed.

Smooth LPS from *P. frisingensis* type strain E-79100^T was de-O,N-acylated by strong alkaline treatment, the products separated by gel chromatography on Sephadex G-50, and the major oligosaccharides **12a,b** isolated by HPAEC as described above. The structures of the oligosaccharides were analysed by NMR and MS. Negative mode ESI mass spectra of oligosaccharides **12a** and **12b** corresponded to molecular masses of 2378 and 2216 Da, identical with those of oligosaccharides **1a** and **1b**, respectively. NMR analysis revealed one difference from oligosaccharides **1a,b**: altered glycosylation position of the Man residue G, being O3 in **1a,b** and O2 in **12a,b**.

Discussion

The carbohydrate backbone of the *P. frisingensis* VTT E-82164 LPS was shown to comprise two major components, a 24-saccharide chain and a 14-saccharide chain (Scheme 1), and corresponding minor components lacking terminal glucose residue K. The presence of two oligosaccharides of different length is in agreement with the electrophoretic pattern of the LPS of this strain, exhibiting two well-resolving bands (Fig. 1). Smooth type LPS molecules from other *Pectinatus* strains show a low molecular mass band of the same mobility as in the strain E-82164, and a ladder-like pattern, characteristic of the presence of the O-chain. No bands analogous to the high-molecular-mass band of strain E-82164 LPS is present on PAGE of smooth LPS molecules. We thus conclude that the shorter structure with the backbone of oligosaccharide **1** corresponds to a core-lipid fragment of this LPS, and the additional components present in oligosaccharide **2** replace the O-specific polysaccharide part. This unusual construction would be better named lipo-oligosaccharide or LOS, although usually the term LOS is used to denote LPS from natural rough strains with core-lipid A parts only [18]. This conclusion is supported by the discovery of a similar core part in the polysaccharide O-chain-containing strain E-79100^T (O-chain structure described in [5]).

The inner-core region of the LPS analysed included the usual α-Kdo-(2–4)-α-Kdo- fragment, linked to lipid A disaccharide. Here the sugar chain extending from the Kdo region contained mannose residues and no heptose. Similar structures with Kdo replaced by mannose residues have been reported in several micro-organisms, including *Legionella pneumophila*, different *Rhizobium* species, and other bacteria [20]. In *P. frisingensis* VTT E-82164, the Kdo-proximal region consists of three mannose units in a branched structure, one carrying an additional α-Kdo residue. The outer part of the oligosaccharide is rich in amino sugars (five residues), including three different aminohexoses GalN, GlcN and ManN. Relatively small amounts of structural variants were found, mostly missing one glucose residue. The previously discovered α-D-GlcN6*P*-(1–4)-Kdo disaccharide [7] obviously corresponds to the fragment W-D. We found that the phosphate group at position 6 of GlcN carries the residue of β-Ara4N.

An interesting feature of the structure determined is that it contains a trisaccharide fragment, in common with the following part of the *Rhizobium etli* LPS structure [21]:

-β-D-GlcA-(1-4)-α-L-Fuc-
3-O-Me-α-6dTal-(1-3)-┘

The absolute configuration of the 3-*O*-methyl-6-deoxytalose residue in *R. etli* LPS has not been determined, however. This monosaccharide was found in other sources as the D (tentatively, in *Pseudomonas maltophila*) and (usually in plant sources) L isomers and has been given the trivial name acovenose [14,22].

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References

1. Lee, S.Y., Mabee, M.S. & Jangaard, N.O. (2002) Pectinatus, *a new genus of the family* Bacteroidaeceae. *Int. J. Syst. Bacteriol.* **28**, 582–594.
2. Haikara, A. & Helander, I. (1992) Pectinatus, Megasphaera and Zymophilus. In *The Prokaryotes. A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications* (Balows, A., Truper, H.G., Dworkin, M.S., Harder, W. & Schleifer, K.-H., eds), Springer Verlag.
3. Suihko, M.-L. & Haikara, A. (2001) Characterization of *Pectinatus* and *Megasphaera* strains by automated ribotyping. *J. Inst. Brew.* **107**, 175–184.
4. Helander, I.M., Hurme, R., Haikara, A. & Moran, A.P. (1992) Separation and characterization of two chemically distinct lipopolysaccharides in two *Pectinatus* species. *J. Bacteriol.* **174**, 3348–3354.
5. Senchenkova, S.N., Shashkov, A.S., Moran, A.P., Helander, I.M. & Knirel, Y.A. (1995) Structures of the O-specific polysaccharide chains of *Pectinatus cerevisiiphilus* and *Pectinatus frisingensis* lipopolysaccharides. *Eur. J. Biochem.* **232**, 552–557.
6. Helander, I.M., Kilpelâinen, I., Vaara, M., Moran, A.P., Lindner, B. & Seydel, U. (1994) Chemical structure of the lipid A component of lipopolysaccharides of the genus *Pectinatus*. *Eur. J. Biochem.* **224**, 63–70.
7. Helander, I.M., Moll, H. & Zähringer, U. (1993) 4-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-3-deoxy-D-manno-2-octulosonic acid, a constituent of lipopolysaccharides of the genus *Pectinatus*. *Eur. J. Biochem.* **213**, 377–381.
8. Brade, H. & Galanos, C. (1982) Isolation, purification, and chemical analysis of the lipopolysaccharide and lipid A of *Acinetobacter calcoaceticus* NCTC 10305. *Eur. J. Biochem.* **122**, 233.
9. Kjaer, M., Andersen, K.V. & Poulsen, F.M. (1994) Automated and semiautomated analysis of homo- and heteronuclear multidimensional nuclear magnetic resonance spectra of proteins: the program PRONTO. *Methods Enzymol.* **239**, 288–308.
10. Tsai, C.M. & Frasch, C.E. (1982) A sensitive silver stain for detecting lipopolysaccharides in polyacrylamide gels. *Anal. Biochem.* **119**, 115–119.
11. Vinogradov, E.V., Cedzynski, M., Ziolkowski, A. & Swierzko, A. (2001) The structure of the core region of the lipopolysaccharide from *Klebsiella pneumoniae* O3. 3-Deoxy-α-D-manno-octulosonic acid (α-Kdo) residue in the outer part of the core, a common structural element of *Klebsiella pneumoniae* O1, O2, O3, O4, O5, O8, and O12 lipopolysaccharides. *Eur. J. Biochem.* **268**, 1722–1729.
12. Ciucanu, I. & Kerek, F. (1984) A simple and rapid method for the permethylation of carbohydrates. *Carbohydr. Res.* **131**, 209–217.
13. Holst, O., Thomas-Oates, J.E. & Brade, H. (1994) Preparation and structural analysis of oligosaccharide monophosphates obtained from lipopolysaccharide of recombinant strains of *Salmonella minnesota* and *Escherichia coli*, expressing the genus-specific epitope of *Chlamydia* lipopolysaccharide. *Eur. J. Biochem.* **222**, 183–194.
14. Kapur, B.M. & Allgeier, H. (1968) 3-*O*-Methyl-6-deoxy-L-talose, Synthese und Identifizierung mit L-Acovenose. *Helv. Chim. Acta* **51**, 89–94.
15. Duus, J.Ø., Gotfredsen, C.H. & Bock, K. (2000) Carbohydrate structural determination by NMR spectroscopy: modern methods and limitations. *Chem. Rev.* **100**, 4589–4614.
16. Birnbaum, G.I., Roy, R., Brisson, J.R. & Jennings, H.J. (1987) Conformations of ammonium 3-deoxy-D-manno-2-octulosonate (KDO) and methyl α-and β-ketopyranosides of KDO: X-ray structure and ^{1}H NMR Analyses. *J. Carbohydr. Chem.* **6**, 17–39.
17. Vinogradov, E.V., Duus, J.Ø., Brade, H. & Holst, O. (2002) The structure of the carbohydrate backbone of the lipopolysaccharide from *Acinetobacter baumannii* strain ATCC 19606. *Eur. J. Biochem.* **269**, 422–430.
18. Vinogradov, E.V., Petersen, B., Thomas-Oates, J., Brade, H. & Holst, O. (1998) Characterization of a novel branched tetrasaccharide of 3-deoxy-D-manno-oct-2-ulopyranosonic acid. The structure of the carbohydrate backbone of the lipopolysaccharide from *Acinetobacter baumannii* strain NCTC 10303 (ATCC 17904). *J. Biol. Chem.* **273**, 28122–28131.
19. Lipkind, G.M., Shashkov, A.S., Knirel, Y.A., Vinogradov, E.V. & Kochetkov, N.K. (1988) A computer-assisted structural analysis of regular polysaccharides on the basis of ^{13}C-NMR data. *Carbohydr. Res.* **175**, 59–75.
20. Holst, O. (1999) Chemical structure of core region of lipopolysaccharides. In *Endotoxin in Health and Disease* (Brade, H., Morrison, D.C., Opal, S.M. & Vogel, S., eds), pp. 115–154. Marcel Dekker Inc., New York.
21. Forsberg, L.S., Bhat, U.R. & Carlson, R.W. (2000) Structural characterization of the O-antigenic polysaccharide of the lipopolysaccharide from *Rhizobium etli* Strain CE3. A unique *O*-acetylated glycan of discrete size, containing 3-*O*-methyl-6-deoxy-L-talose and 2,3,4-tri-*O*-methyl-L-fucose. *J. Biol. Chem.* **275**, 18851–18863.
22. Weckesser, J., Drews, G. & Mayer, H. (1979) Lipopolysaccharides of photosynthetic prokaryotes. *Annu. Rev. Microbiol.* **33**, 215–239.