

Structure of the Major O-Specific Polysaccharide from the Lipopolysaccharide of *Pseudomonas fluorescens* BIM B-582: Identification of 4-Deoxy-D-xylo-hexose As a Component of Bacterial Polysaccharides

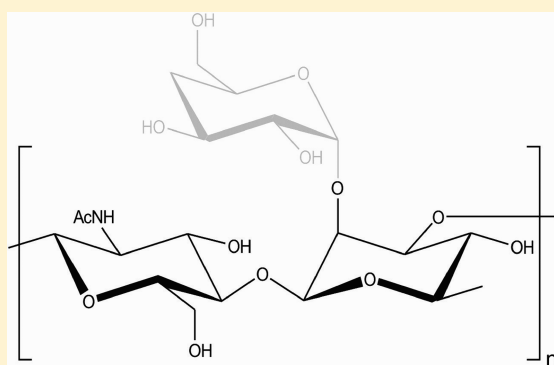
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ABSTRACT: A novel constituent of bacterial polysaccharides, 4-deoxy-D-xylo-hexose (D-4dxylHex), was found in the major O-specific polysaccharide from the lipopolysaccharide of *Pseudomonas fluorescens* BIM B-582. D-4dxylHex was isolated in the free state by paper chromatography after full acid hydrolysis of the polysaccharide and identified by GLC–mass spectrometry, ¹H and ¹³C NMR spectroscopy, and specific rotation. It occurs as a lateral substituent in ~40% of the oligosaccharide repeating units, making the polysaccharide devoid of strict regularity. The structure of the polysaccharide was established by sugar analysis, Smith degradation, and two-dimensional ¹H and ¹³C NMR spectroscopy. In addition, a minor polysaccharide was isolated from the same lipopolysaccharide and found to contain 4-O-methylrhamnose.



Pseudomonas fluorescens is a typical representative of the widely spread genus *Pseudomonas* from the family Pseudomonadaceae, which can be found in soil and water. On the basis of phenotypic characteristics, such as metabolic tests, fatty acid composition, and protein profiles, strains of *P. fluorescens* were subdivided into five biovars whose taxonomical status remains undefined.^{1,2} *P. fluorescens* genomes are highly diverse, the genomic heterogeneity between strains being reminiscent of a species complex rather than a single species.³

P. fluorescens possesses hemolytic activity⁴ and, as a result, may affect blood transfusions but is an unusual cause of disease in humans, affecting mainly immunocompromised patients. *Pseudomonas syringae* and *P. fluorescens* are important phytopathogens causing severe bacteriosis in a wide range of cultured plants, including orchard plants, cereals, melons, gourds, and others. Due to production of heat-stable extracellular enzymes that digest lipids, proteins, and other substances, *P. fluorescens* causes spoilage of food, including milk and meat.^{5,6} When attached to a surface, bacteria such as *P. fluorescens* form biofilms,⁷ which are inherently resistant to a variety of antibiotics. Therefore alternative methods to eradicate bacteria in the biofilm form, such as phage treatment, have been suggested.^{8,9}

Phages employ the lipopolysaccharide (LPS) receptor for adsorption on the cell surface of Gram-negative bacteria.¹⁰ The LPS is also involved in interactions of bacteria with plants and protection of the microorganisms from host defenses. It consists of an outermost repetitive glycan region called O-specific

polysaccharide (OPS) or O-antigen, which is linked to a lipid anchor (lipid A) through a central oligosaccharide designated as the core.¹¹ The O-antigen is the most variable LPS domain, and its fine structure defines the immunospecificity of strains, which is used for bacterial serotyping. Phage reception by the LPS often is associated with the enzymatic cleavage of the O-antigen.¹² Structures of the OPS of a number of *P. fluorescens* strains, both classified to biovars A–C^{13,14} and nonclassified,^{15–17} have been elucidated and found to be diverse even between representatives of the same biovar.

The nonclassified strain *P. fluorescens* BIM B-582 hosts 24 bacteriophages of phytopathogenic bacteria (*P. syringae*, *P. fluorescens*, *P. putida*). These phages are deposited in the Belarusian Collection of Nonpathogenic Microorganisms. A combination of five phages (Pentafag) is used in agriculture for prophylaxis against various diseases in orchard plants and vegetables. Strain BIM B-582 is utilized in the preparation of Pentafag. So far, the LPS of this strain has not been studied.

Aimed at elucidation of the nature of the phage receptor and the mechanism of the interaction between the phages and the bacterial cell, we established the structure of the OPS of *P. fluorescens* BIM B-582. A novel component of bacterial polysaccharides, 4-deoxy-D-xylo-hexose, was found in the OPS and identified. The results of this work may improve the

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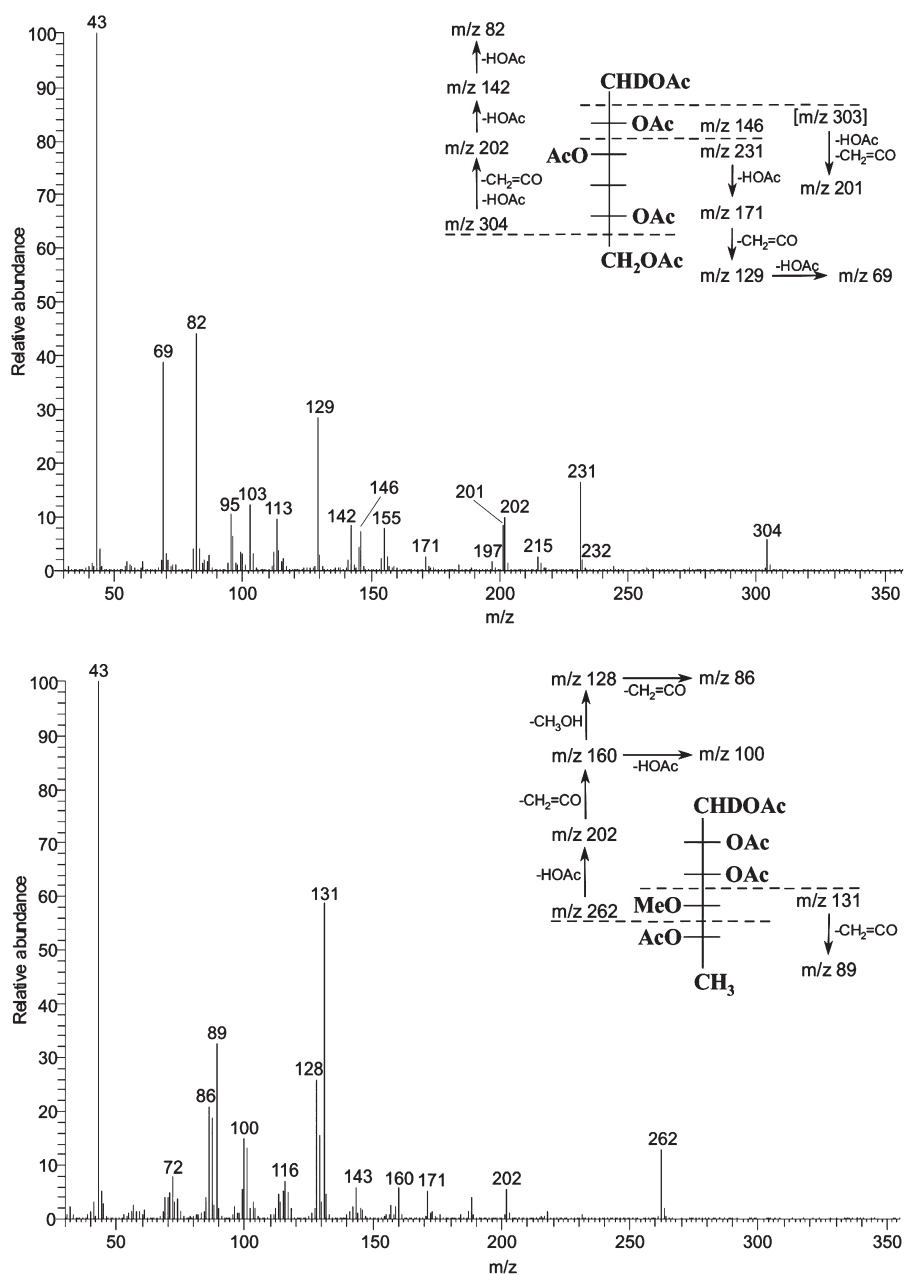


Figure 1. Electron impact mass spectra of 1,2,3,5,6-penta-*O*-acetyl-4-deoxyhexitol-1-*d* (top) and 1,2,3,5-tetra-*O*-acetyl-6-deoxy-4-*O*-methylhexitol-1-*d* (bottom). Structures and fragmentation pathways of the compounds are indicated in the insets. The putative parent ion with *m/z* 303 (indicated in square brackets) for the daughter ion with *m/z* 201 is evidently unstable and not seen in the spectrum of 1,2,3,5,6-penta-*O*-acetyl-4-deoxyhexitol-1-*d*.

process of production of useful phage preparations and will help the development of an O-antigen-based classification scheme of *P. fluorescens* necessary for environmental monitoring of strains.

RESULTS AND DISCUSSION

The LPS was isolated from dried cells of *P. fluorescens* BIM B-582 by the phenol-H₂O procedure and degraded under mild acidic conditions. This procedure cleaved an acid-labile linkage of the 3-deoxy-*D*-manno-oct-2-ulosonic acid residue, which connects the core oligosaccharide and lipid A. The carbohydrate portion thus released was fractionated by gel-permeation chromatography on Sephadex G-50 to yield a long-chain OPS.

For determination of the sugar composition, the OPS was hydrolyzed with 2 M CF₃CO₂H, and the monosaccharides were reduced with sodium borodeuteride, N,O-acetylated, and analyzed by GLC-MS. This revealed rhamnose (Rha), a 4-deoxyhexose, and *N*-acetylglucosamine (GlcNAc) as the major components. Rha and GlcNAc were identified by a comparison of the GLC retention times and mass spectra of the corresponding alditol acetates with authentic samples. The *L* configuration of rhamnose was established by GLC analysis of the acetylated (*S*)-2-octyl glycosides. The absolute configuration of GlcNAc was inferred as *D* using known regularities in glycosylation effects on ¹³C NMR chemical shifts (see below).

The 4-deoxyhexose was recognized by characteristic ion peaks at *m/z* 304 and 231 for the C-1–C-5 and C-3–C-6 fragments,

Table 1. ^1H and ^{13}C NMR Data for 4-Deoxy-D-xyllo-hexose Isolated from the OPS of *Pseudomonas fluorescens* BIM B-582

position	α -D-4dxylHexp		β -D-4dxylHexp	
	δ_{C} , type	δ_{H} (J in Hz)	δ_{C} , type	δ_{H} (J in Hz)
1	93.9, CH	5.25, d (3.7)	97.4, CH	4.56, d (7.9)
2	74.4, CH	3.45, dd (9.6, 3.7)	77.2, CH	3.14, d (9.2, 7.9)
3	68.1, CH	3.94, m	71.6, CH	3.73, m
4	35.4, CH ₂	1.43, m, 1.98, m	35.4, CH ₂	1.42, m, 1.96, m
5	69.7, CH	4.09, m	73.7, CH	3.70, m
6	64.9, CH ₂	3.58, dd (12.1, 6.2), 3.65, dd (12.1, 3.2)	64.8, CH ₂	3.59, dd (12.1, 6.2), 3.66, dd (12.1, 3.2)

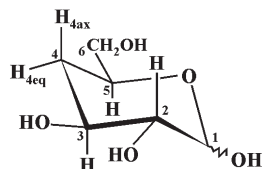


Figure 2. Structure of 4-deoxy-D-xyllo-hexose.

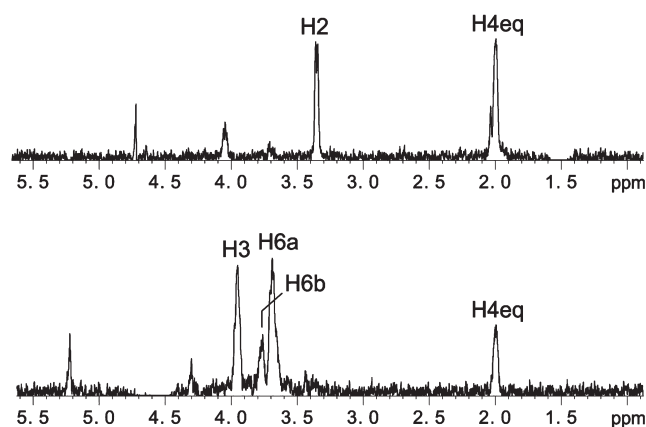
respectively, in the electron impact mass spectrum of the derived 1,2,3,5,6-penta-O-acetyl-4-deoxyhexitol-1-d (Figure 1, top). For the stereochemical assignment, the 4-deoxyhexose was isolated in the free state by preparative paper chromatography of a monosaccharide mixture obtained by full acid hydrolysis of the OPS.

The ^1H and ^{13}C NMR spectra of the 4-deoxyhexose were assigned (Table 1) using two-dimensional COSY and HSQC spectroscopy. As judged by the ratio of integral intensities of the signals in the NMR spectra, the monosaccharide exists as a $\sim 1:2$ mixture of α - and β -pyranoses. The position of the deoxy unit (ring methylene group) was confirmed by the chemical shifts for the H-4 protons (δ_{H} 1.42 and 1.96 in the major β -anomer) and C-4 carbon (δ_{C} 35.4). Relatively large $J_{2,3}$, $J_{3,4ax}$ and $J_{4ax,5}$ coupling constants of 9.2–12 Hz (Table 1) indicated a pyranose ring with the axial orientation of all these protons and thus the *xyllo* configuration of the 4-deoxyhexose (Figure 2).

Therefore, the isolated monosaccharide is 4-deoxy-xyllo-hexose (4dxylHex). This conclusion was confirmed by one-dimensional ROESY experiments with selective saturation of the H-4ax and H-5 protons (Figure 3). They revealed spatial proximity of H-4ax and H-2 as well as H-5 and H-3, which is possible only in the *xyllo* configuration. Finally, the absolute D configuration of 4dxylHex was established by the specific rotation $[\alpha]_{\text{D}}^{21} +43.0$ (c 0.14, H₂O) (compare with the published value $[\alpha]_{\text{D}}^{20} +40.5 \rightarrow 52.4$ (H₂O) for 4-deoxy-D-xyllo-hexose).¹⁸

The ^{13}C NMR spectrum of the OPS (Figure 4) showed, *inter alia*, major signals for five anomeric carbons at δ_{C} 101–104, CH₃–C groups (C-6 of Rha) at δ_{C} 17.6 and 17.9, a C–CH₂–C group (C-4 of 4dxylHex) at δ_{C} 35.3, HOCH₂–C groups (C-6 of GlcNAc and 4dxylHex) at δ_{C} 61.9, 62.0, and 64.8, nitrogen-bearing carbons (C-2 of GlcNAc) at δ_{C} 56.9 and 57.0, and N-acetyl groups at δ_{C} 23.3, 23.4 (both CH₃), 175.7, and 176.2 (both CO). The ^1H NMR spectrum of the OPS (Figure 5) contained major signals for anomeric protons at δ_{H} 4.6–5.3, CH₃–C groups (H-6 of Rha) at δ_{H} 1.29 and 1.32, a C–CH₂–C group (H-4 of 4dxylHex) at δ_{H} 1.51 (axial) and 1.99 (equatorial), other sugar ring protons in the region of δ_{H} 3.2–4.6, and N-acetyl groups at δ_{H} 2.02.

The ^1H NMR spectrum of the OPS was assigned by tracing connectivities in the two-dimensional COSY and TOCSY spectra, and five major sugar spin systems were revealed, including

Figure 3. Parts of the one-dimensional ROESY spectra of the OPS from *Pseudomonas fluorescens* BIM B-582 with selective saturation of H-4ax at δ_{H} 1.51 (top) and H-5 at δ_{H} 4.53 (bottom) of 4dxylHex.

two spin systems each for Rha (units A and C) and GlcNAc (B and D) and one spin system for 4dxylHex (E) (Table 2). Integral intensities of the nonoverlapping signals for units C, D, and E were ~ 1.5 times lower than those of units A and B. Typical $^3J_{\text{H,H}}$ coupling constants estimated from the two-dimensional spectra indicated that all sugar residues occur in the pyranose form. With the ^1H NMR spectrum assigned, the ^{13}C NMR spectrum of the OPS was assigned using an HSQC experiment (Table 2).

A relatively large $J_{1,2}$ coupling constant of ~ 7 Hz for the H-1 signal at δ_{H} 4.67 (both units B and D) showed that GlcNAc is β -linked. The H-1 signal of 4dxylHex at δ_{H} 5.22 was not resolved, and the α -linkage of this monosaccharide was inferred from the C-5 chemical shift of δ_{C} 69.5 (compare δ_{C} 69.7 and 73.7 for α -4dxylHexp and β -4dxylHexp, respectively, Table 1). Similarly, the β -configuration of Rha was inferred by a comparison of the C-5 chemical shifts of δ_{C} 73.5 and 73.9 (units A and C) with published data of δ_{C} 69.5 and 73.2 for α - and β -rhamnopyranosides, respectively.¹⁹ The presence in the one-dimensional ROESY spectrum of a H-1,H-2 correlation confirmed α -4dxylHex, whereas β -Rha and β -GlcNAc were corroborated by H-1,H-3 and H-1,H-5 correlations.

Downfield displacements of the ^{13}C NMR signals for C-3 of Rha A to δ_{C} 83.2 and C-4 of GlcNAc to δ_{C} 78.1 (B) and 79.1 (D), as compared with their positions in the spectra of the corresponding nonsubstituted monosaccharides at δ_{C} 74.0 and 71.2,¹¹ were due to glycosylation of these monosaccharides at O-3 and O-4, respectively. Both C-2 and C-3 signals of Rha C were shifted downfield to δ_{C} 77.4 and 80.6, respectively, thus showing that this sugar residue is at the branching point. The C-2–C-6 chemical shifts of 4dxylHex in the

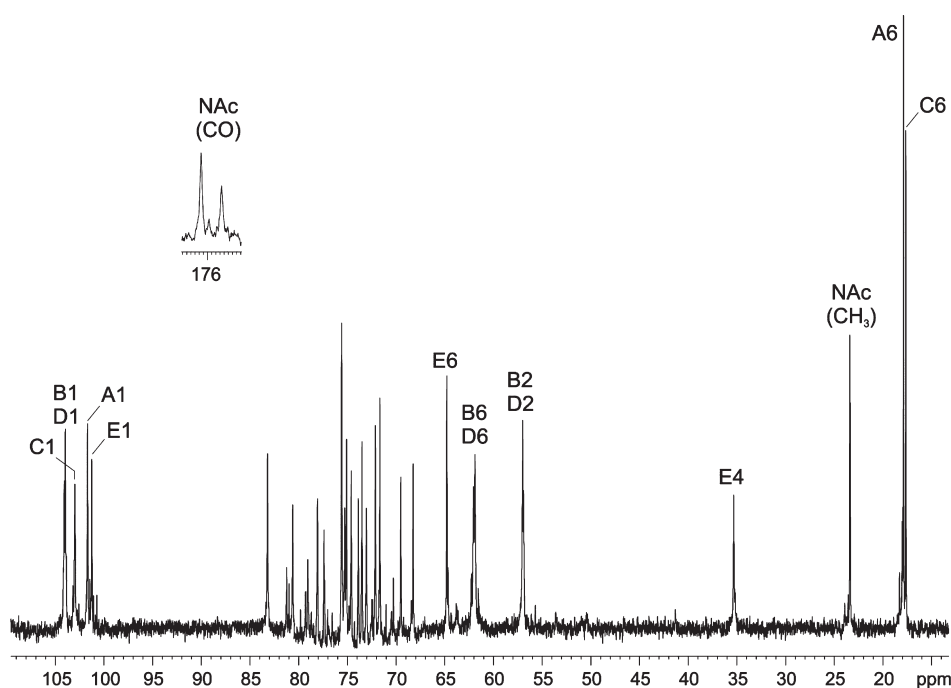


Figure 4. ^{13}C NMR spectrum of the OPS from *Pseudomonas fluorescens* BIM B-582. A–E refer to sugar units as shown in Table 2; NAc stands for the *N*-acetyl group.

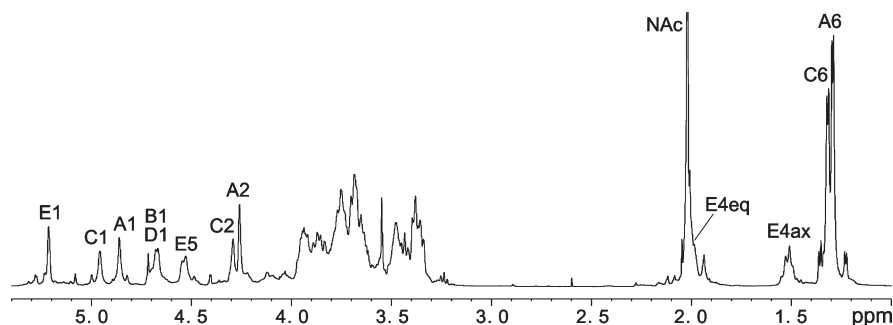


Figure 5. ^1H NMR spectrum of the OPS from *Pseudomonas fluorescens* BIM B-582. A–E refer to sugar units as shown in Table 2; NAc stands for the *N*-acetyl group.

OPS (Table 2) were close to those of free α -4dxyIHexp (Table 1); hence, 4dxyIHex is attached as a side chain.

The two-dimensional ROESY and HMBC spectra showed correlations between anomeric protons and anomeric carbons (HMBC) with the linkage atoms in the neighboring sugar residues (Table 3). These correlations were in agreement with the ^{13}C NMR chemical shift data (see above) and showed that the OPS has the linear $\rightarrow 3$ -Rha-(1 \rightarrow 4)-GlcNAc-(1 \rightarrow backbone, in which $\sim 40\%$ of the Rha residues (units C) bear 4dxyIHex E at position 2. The nonstoichiometric substitution with 4dxyIHex is the reason for splitting of the NMR signals for Rha and GlcNAc into two series (A/C and B/D, respectively). The ^1H NMR spectrum of the LPS showed the same abundance of 4dxyIHex as in the OPS, and hence, no 4dxyIHex was lost upon mild acid degradation of the LPS.

For confirmation of the structure, the OPS was subjected to Smith degradation,¹⁹ which resulted in oxidation followed by cleavage of the lateral 4dxyIHex residue, whereas the main polysaccharide chain was unaffected. As expected, the linear

$\rightarrow 4$)- β -GlcNAc-(1 \rightarrow 3)- β -Rhap-(1 \rightarrow polysaccharide was obtained, whose structure was established by two-dimensional NMR spectroscopy as described above for the OPS. The ^1H and ^{13}C NMR chemical shifts of the repeating unit of the Smith-degraded polysaccharide (Table 2) were close to those of the $\rightarrow \text{A} \rightarrow \text{B} \rightarrow$ disaccharide unit in the initial polysaccharide.

Substitution of L-Rha A at position 3 with β -GlcNAc B caused a relatively small upfield shift (β -effect of glycosylation) of the C-2 signal of Rha A (1 ppm as compared with the position of this signal in nonsubstituted β -Rhap²⁰). The shift of the C-1 signal of the linked GlcNAc B was relatively large (7.7 ppm as compared with that of free β -GlcNAc¹¹). These displacements are characteristic for different absolute configurations of the constituent monosaccharides in the β -GlcNAc-(1 \rightarrow 3)- β -L-Rhap disaccharide fragment, i.e., the D configuration of GlcNAc.²¹ Indeed, in the case of the same absolute configuration of the monosaccharides, the β -effect of glycosylation on C-2 of Rha A would be significantly higher (>2.5 ppm) and the effect on C-1 of GlcNAc B significantly lower (3.5 ± 1.1 ppm).²¹

Table 2. ^1H and ^{13}C NMR Chemical Shifts (δ , ppm) of the Polysaccharides from *Pseudomonas fluorescens* BIM B-582^a

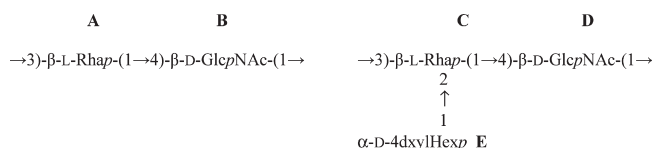
sugar residue		H-1	H-2	H-3	H-4 (4ax, 4eq)	H-5	H-6 (6a, 6b)
		C-1	C-2	C-3	C-4	C-5	C-6
Major Polysaccharide							
$\rightarrow 3$)- β -L-Rhap-(1 \rightarrow	A	4.86	4.26	3.64	3.38	3.38	1.29
		101.6	71.6	83.2	72.1	73.5	17.9
$\rightarrow 4$)- β -D-GlcpNAc-(1 \rightarrow	B	4.67	3.74	3.48	3.68	3.68	3.86, 3.90
		103.9	57.0	75.6	78.1	74.6	61.9
$\rightarrow 2,3$)- β -L-Rhap-(1 \rightarrow	C	4.96	4.29	3.73	3.44	3.40	1.32
		102.9	77.4	80.6	73.0	73.9	17.6
$\rightarrow 4$)- β -D-GlcpNAc-(1 \rightarrow	D	4.67	3.69	3.48	3.56	3.67	3.79, 3.92
		104.0	56.9	75.6	79.1	75.3	62.0
α -D-4dxylHexp-(1 \rightarrow	E	5.22	3.35	3.95	1.51, 1.99	4.53	3.70, 3.76
		101.2	75.1	68.2	35.3	69.5	64.8
Smith-Degraded Polysaccharide							
$\rightarrow 3$)- β -L-Rhap-(1 \rightarrow	A	4.88	4.28	3.66	3.39	3.40	1.31
		101.7	71.7	83.2	72.1	73.5	17.8
$\rightarrow 4$)- β -D-GlcpNAc-(1 \rightarrow	B	4.69	3.76	3.50	3.69	3.70	3.86, 3.90
		103.8	56.9	75.5	78.0	74.6	61.8
Minor Polysaccharide							
$\rightarrow 3$)- β -L-Rhap4Me-(1 \rightarrow		5.08	4.42	4.27	3.25	3.43	1.36
		101.4	70.3	81.2	81.0	72.4	17.9

^a Chemical shifts for the NAc groups on GlcNAc are δ_{H} 2.02–2.03 and δ_{C} 23.3–23.4 (CH₃), 175.7 and 176.2 (both CO). Chemical shifts for the O-methyl group on Rha4Me are δ_{H} 3.55 and δ_{C} 61.5.

Table 3. Interresidual Homonuclear (ROESY) and Heteronuclear (HMBC) Correlations for the Anomeric Atoms H-1 and C-1 in the Major Polysaccharide from *Pseudomonas fluorescens* BIM B-582

unit	^1H ^{13}C (anomeric)	correlations to		
		unit	ROESY	HMBC
$\rightarrow 3$)- β -L-Rhap-(1 \rightarrow	4.86	$\rightarrow 4$)- β -D-GlcpNAc-(1 \rightarrow	H-4 3.68	C-4 78.1
	101.6			H-4 3.68
$\rightarrow 4$)- β -D-GlcpNAc-(1 \rightarrow	4.67	$\rightarrow 3$)- β -L-Rhap-(1 \rightarrow	H-3 3.64	C-3 83.2
	103.9			not observed
$\rightarrow 2,3$)- β -L-Rhap-(1 \rightarrow	4.96	$\rightarrow 4$)- β -D-GlcpNAc-(1 \rightarrow	H-4 3.56	C-4 79.1
	102.9			H-4 3.56
$\rightarrow 4$)- β -D-GlcpNAc-(1 \rightarrow	4.67	$\rightarrow 2,3$)- β -L-Rhap-(1 \rightarrow	H-3 3.73	C-3 80.6
	104.0			not observed
α -D-4dxylHexp-(1 \rightarrow	5.22	$\rightarrow 2,3$)- β -L-Rhap-(1 \rightarrow	H-2 4.29	C-2 77.4
	101.2			H-2 4.29

Therefore, the OPS from the LPS of *P. fluorescens* BIM B-582 is built up of the linear disaccharide (major) and branched trisaccharide (minor) repeating units having the following structures:



The NMR spectra of the OPS also showed a number of minor signals with integral intensities less than 10% of the major signals, including a signal for an O-methyl group (δ_{H} 3.55,

singlet). It correlated with a proton signal at δ_{H} 3.25 (triplet) in the two-dimensional ROESY spectrum and a carbon signal at δ_{C} 81.0 in the HMBC spectrum. Tracing connectivities in the COSY, TOCSY, ROESY, and HSQC spectra showed that these are the H-4 and C-4 signals of a 4-O-methylated rhamnose residue designated as Rha4Me. This finding was confirmed by identification of a minor 6-deoxy-4-O-methylhexose on GLC-MS analysis of the OPS hydrolysate. The mass spectrum of the derived 1,2,3,5-tetra-O-acetyl-6-deoxy-4-O-methylhexitol-1-*d* showed characteristic ion peaks at m/z 262 and 131 for the C-1–C-4 and C-4–C-6 fragments, respectively (Figure 1, bottom).

The ^1H and ^{13}C NMR chemical shifts of Rha4Me were assigned (Table 2), and a low-field position of the C-4 signal at δ_{C} 81.0 confirmed the 4-O-methylation. As judged by the C-5 and C-3 chemical shifts of δ_{C} 72.4 and 81.2, respectively, Rha4Me is β -linked and 3-substituted (compare published data of β -Rhap²⁰). Some other minor NMR signals belonged to nonmethylated rhamnose residues, which probably enter into the same polysaccharide as Rha4Me. However, multiple coincidences of the minor and major signals precluded full structure elucidation of the minor polysaccharide.

A peculiar feature of the OPS studied is the presence of 4-deoxy-D-xylo-hexose. Deoxyhexoses are an important class of carbohydrates. 6-Deoxy-L-mannose (L-rhamnose) and 6-deoxy-L-galactose (L-fucose) are widespread in natural products, including bacterial polysaccharide antigens.²² Other 6-deoxyhexoses, their isomers with a deoxy unit in the sugar ring, dideoxy- and trideoxy-hexoses are less common. For 4-deoxyhexoses, only 4-deoxy-D-arabino-hexose has been hitherto reported as a component of the O-polysaccharides of the genus *Citrobacter* from the family Enterobacteriaceae.^{23–25} In this work, another isomer, 4-deoxy-D-xylo-hexose, was found and identified for the first time in nature, thus extending the list of sugar components of bacterial carbohydrates.

Most bacterial glycopolymers are regular polysaccharides built up of repeating oligosaccharide units. The major O-polysaccharide of *P. fluorescens* BIM B-582 lacks strict regularity, as it consists of two types of repeating units differing in the presence (minor) or absence (major) of 4-deoxy-D-xylo-hexose. When present, this monosaccharide occupies the lateral position in the polysaccharide chain, which suggests that adding 4-deoxy-D-xylo-hexose is a postpolymerization step in O-polysaccharide biosynthesis.

The terminal monosaccharides are known to be most accessible to antibodies directed to carbohydrates as well as to cellular and viral receptors. For instance, in accordance with this, lateral residues of 4-deoxy-D-arabino-hexose play the immunodominant role in the O-antigens of *Citrobacter*^{23,25} and various 3,6-dideoxyhexoses in those of *Salmonella* and *Yersinia pseudotuberculosis*.²⁶ The O-polysaccharide backbone of *P. fluorescens* BIM B-582 consists of commonly occurring monosaccharides, D-GlcNAc and L-Rha, and its decoration with such a unique sugar as 4-deoxy-D-xylo-hexose evidently provides specificity to the bacterial cell surface. As the O-antigen is the major target of the environmental factors, such as the immune system and bacteriophages, this trait may be important for bacterial survival and niche adaptation.

EXPERIMENTAL SECTION

General Experimental Procedures. NMR spectra were recorded at 30 °C on a Bruker Avance II 600 spectrometer using a 5 mm broadband inverse probehead for solutions in 99.95% D₂O after deuterium exchange by freeze-drying sample solutions in 99.9% D₂O. Sodium 3-(trimethylsilyl)propanoate-2,2,3,3-*d*₄ (δ_{H} 0.0) and acetone (δ_{C} 31.45) were used as internal calibration standards for ^1H and ^{13}C NMR chemical shifts, respectively. Two-dimensional NMR spectra were obtained using standard Bruker software, and the Bruker Topspin 2.1 program was used to acquire and process the NMR data. The two-dimensional TOCSY and one-dimensional ROESY spectra were recorded with 150 ms duration of MLEV-17 spin-lock and 100 ms mixing time, respectively. The ^1H , ^{13}C HMBC spectrum was recorded with 60 ms delay for evolution of long-range spin couplings. GLC experiments were carried out on an Agilent 7820A GC system using a temperature

program from 160 °C (1 min) to 290 °C at 7 °C min⁻¹. Gel-permeation chromatography was carried on a column (56 × 2.5 cm) of Sephadex G-50 Superfine (Amersham Biosciences) using 0.05 M pyridinium acetate pH 4.5 as eluent or a column (80 × 1.6 cm) of TSK HW-40 (S) (Merck) in aqueous 1% AcOH and monitored with a differential refractometer (Knauer).

Growth of Bacteria and Isolation of the Lipopolysaccharide and O-Polysaccharide. Cells were cultivated in a liquid medium (Mikrogen, Russia) containing pancreatic sprat hydrolysate (10.05 g L⁻¹) and NaCl (4.95 g L⁻¹) at 27 °C for 24 h under aerobic conditions and stirring. Cells were extracted using the phenol–H₂O procedure,²⁷ and the isolated crude material was purified by precipitation of nucleic acid and proteins by treatment with CCl₃CO₂H to yield an LPS preparation as 15% of dried cell mass. The OPS was obtained by degradation of the LPS with 2% HOAc–H₂O for 1.5 h at 100 °C. After centrifugation at 13000g the supernatant was fractionated by gel-permeation chromatography on a Sephadex G-50 column yielding 17.9%.

Sugar Analysis. Hydrolysis of the OPS was performed with 2 M CF₃CO₂H (120 °C, 2 h). The monosaccharides were conventionally reduced with NaBD₄, acetylated with a 1:1 acetic anhydride–pyridine mixture, and analyzed by GLC. The absolute configuration of rhamnose was determined by GLC of the acetylated (S)-2-octyl glycosides as described.²⁸

Isolation and Characterization of 4-Deoxy-D-xylo-hexose. An OPS sample (21 mg) was hydrolyzed with 2 M CF₃CO₂H (2 mL) at 120 °C for 2 h and lyophilized. Analytical descending paper chromatography of the hydrolysate (1 mg) on FN-11 paper (Filtrak) in a butanol–pyridine–H₂O (6:4:3, v/v/v) system for 20 h and conventional silver staining revealed Rha; 4dxyHex, R_{Rha} 0.86; and GlcN, R_{Rha} 0.54. 4dxyHex (2.4 mg) was isolated by preparative descending paper chromatography of the remaining hydrolysate on FN-18 paper (Filtrak) in the same system. The optical rotation of 4dxyHex was measured on a JASCO 360 polarimeter at 20 °C in H₂O.

Smith Degradation. An OPS sample (20 mg) was oxidized with 0.1 M NaIO₄ (1.0 mL) in the dark at 20 °C for 72 h. After reduction with an excess of NaBH₄ and desalting on a TSK HW-40 column, the product was hydrolyzed with 1% HOAc–H₂O at 100 °C for 2 h and fractionated by gel-permeation chromatography on TSK HW-40 to give the modified polysaccharide.

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