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Note

Structure of an acidic O-specific polysaccharide of *Pseudoalteromonas haloplanktis* type strain ATCC 14393 containing 2-acetamido-2-deoxy-D- and -L-galacturonic acids and 3-(*N*-acetyl-D-alanyl)amino-3,6-dideoxy-D-glucose☆

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Abstract

An acidic O-specific polysaccharide was obtained from the lipopolysaccharide of *Pseudoalteromonas haloplanktis* ATCC 14393 and found to contain D-galactose, 3-(*N*-acetyl-D-alanyl)amino-3,6-dideoxy-D-glucose (D-Qui3NDAlaAc), 2,4-diacetamido-2,4,6-trideoxy-D-glucose (D-QuiNAc4NAc), 2-acetamido-2-deoxy-D- and -L-galacturonic acids (D- and L-GalNAcA), and *O*-acetyl groups. On the basis of Smith degradation and ^{1}H and ^{13}C NMR spectroscopic studies, including 2D COSY, TOCSY, NOESY, ^{1}H, ^{13}C HMQC, and HMBC experiments, the following structure of the pentasaccharide repeating unit of the polysaccharide was established:

→4)-α-L-Gal*p*NAcA-(1→3)-β-D-Qui*p*NAc4NAc-(1→2)-β-D-Qui*p*3NDAlaAc-(1→4)-α-D-Gal*p*NAcA-(1→4)

-α-D-Gal*p*2,6Ac_2-(1→

where O-acetylation of the galactose residue at each position is partial (50–70%).

Keywords: *Pseudoalteromonas haloplanktis*; O-specific polysaccharide; 2-Acetamido-2-deoxy-D-galacturonic acid; 2-Acetamido-2-deoxy-L-galacturonic acid; 2,4-Diacetamido-2,4,6-trideoxy-D-glucose; 3-(*N*-Acetyl-D-alanyl)amino-3,6-dideoxy-D-glucose

Gram-negative bacteria of the genus *Pseudoalteromonas* are aerobic, motile, heterotrophic, non-fermentative prokaryotes. They are widespread obligatory marine microorgan-

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isms and require a seawater base for their growth. The species *Pseudoalteromonas haloplanktis* was initially classified as *Alteromonas haloplanktis* but later transferred to the new genus *Pseudoalteromonas* [1,2], while the species strain is *P. haloplanktis* ATCC 14393. Data on the cell-surface lipopolysaccharide structure of *Pseudoalteromonas* are limited (Ref. [3] and references cited therein). Recently, we have determined the structure of a highly acidic O-specific polysaccharide chain (O-antigen) of the lipopolysaccharide of *P. haloplanktis* KMM 223 (44-1) and found it to contain D-glucuronic and L-iduronic acids [3]. Now we report the structure of another acidic O-specific polysaccharide isolated from the lipopolysaccharide of the type strain of *P. haloplanktis*, ATCC 14393. Bacterial cells were extracted with aq 45% phenol, and the lipopolysaccharide was recovered from the aqueous phase and degraded with dilute acetic acid to give the O-specific polysaccharide (PS-1). Sugar analysis of PS-1 revealed galactose as the only neutral monosaccharide. Analysis of the polysaccharide hydrolysate using an amino acid analyser resulted in identification of alanine and two amino sugars, one having the same retention time as 2-amino-2-deoxygalacturonic acid (GalNA) and the other as 3-amino-3,6-dideoxyglucose (Qui3N). After carboxyl reduction, 2-amino-2-deoxygalactose was identified. GLC of the acetylated glycosides with chiral alcohols demonstrated that Gal and Qui3N have the D configuration, whereas both D-GalN and L-GalN are present. Alanine has the D configuration, as shown by GLC of its N-acetylated (*S*)-2-butyl ester. One more monosaccharide component of PS-1, 2,4-diamino-2,4,6-trideoxy-D-glucose (QuiN4N, bacillosamine), was not detected; it was, however, identified in the course of NMR spectroscopic studies of the polysaccharide.

The ^{13}C NMR spectrum of PS-1 contained signals of different intensities, thus indicating structural irregularity. It could be suggested that this was caused by non-stoichiometric O-acetylation because there were three peaks for *O*-acetyl groups at δ 21.2–21.6 (CH_3). After O-deacetylation with aqueous ammonia, the ^{13}C NMR spectrum of the resultant modified polysaccharide (PS-2) became typical of a regular polysaccharide (Fig. 1). It contained signals for five anomeric carbons at δ 99.1–104.3, six carbons bearing nitrogen at δ 50.7–57.8, one $HOCH_2$–C group at δ 60.7 (C-6 of Gal, data of the attached-proton test [4]), three CH_3–C groups of two 6-deoxy sugars and alanine at δ 17.9–18.0, five CH_3–CON groups at δ 23.0–23.6, eight CO groups, including those of six *N*-acyl groups (five *N*-acetyl and one *N*-alanyl) and two COOH groups (C-6 of GalNA) at δ 173.9–174.4. Signals for other sugar ring carbons were in the region δ 68.7–80.7. The absence from the ^{13}C NMR spectrum of any signals for non-anomeric sugar ring carbons in a

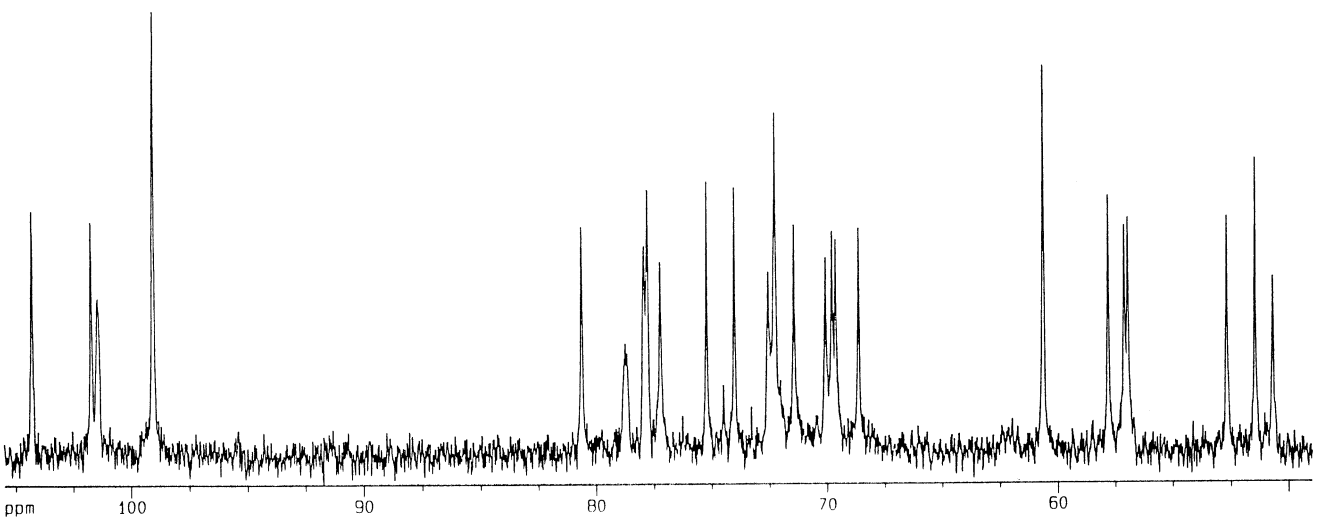

Fig. 1. ^{13}C NMR spectrum of the O-specific polysaccharide (PS-1).

Table 1
^{1}H NMR chemical shifts (δ in ppm) [a]

Sugar residue	H-1a H-1b	H-2	H-3	H-4a H-4b	H-5	H-6a H-6b
O-Deacetylated polysaccharide (PS-2)						
→4)-α-L-Gal*p*NAcA^{I}-(1→	5.20	4.31	3.89	4.45	4.27	
→3)-β-D-Qui*p*NAc4NAc-(1→	4.52	3.83	3.83	3.59	3.56	1.09
→2)-β-D-Qui*p*3NDAlaAc-(1→	4.45	3.57	3.87	3.02	3.36	1.22
→4)-α-D-Gal*p*NAcA^{II}-(1→	5.08	4.05	4.03	4.52	4.89	
→4)-α-D-Gal*p*-(1→	5.30	3.85	3.95	4.05	3.99	3.64 3.51
Oligosaccharide (OS)						
α-L-Gal*p*NAcA^{I}-(1→	5.21	4.19	3.78	4.21	4.17	
→3)-β-D-Qui*p*NAc4NAc-(1→	4.55	3.83	3.83	3.60	3.55	1.10
→2)-β-D-Qui*p*3NDAlaAc-(1→	4.48	3.57	3.88	3.02	3.37	1.22
→4)-α-D-Gal*p*NAcA^{II}-(1→	5.20	4.02	4.03	4.53	4.56	
→2)-Threitol	3.71 3.66	3.73	3.83	3.62 3.59		

[a] Additional chemical shifts: CH_3CON at δ 1.84–2.09; Ala H-2 and H-3 at δ 4.20–4.21 and 1.38–1.39, respectively.

lower field than δ 81 demonstrated the pyranoid form of all sugar residues [5].

The $^1J_{C\text{-}1,H\text{-}1}$ coupling constant values determined from the gated-decoupling spectrum of the polysaccharide were less than 173 Hz and thereby confirmed the pyranoid form of the monosaccharides [6]. These data showed that three of the monosaccharides are α-linked ($^1J_{C\text{-}1,H\text{-}1}$ 171–172 Hz for the signals at δ 99.1 (2C) and 101.5), and two others β-linked ($^1J_{C\text{-}1,H\text{-}1}$ 162–163 Hz for the signals at δ 101.8 and 104.3) [7].

Accordingly, the ^{1}H NMR spectrum of PS-2 contained, inter alia, signals for five *N*-acetyl groups at δ 1.84–2.09 (all singlets), three CH_3–C groups of Qui3N, QuiN4N and Ala at δ 1.09, 1.22 and 1.38 (all doublets), and anomeric protons of three α-pyranosides at δ 5.08, 5.20 and 5.30 (all broadened singlets). Signals for anomeric protons of two β-pyranosides coincided with other signals, most likely, at δ 4.45 and 4.52.

The ^{1}H NMR spectrum of PS-2 was assigned using 2D COSY and TOCSY experiments (Table 1); the ^{13}C NMR spectrum of PS-2 was then assigned using 2D H-detected ^{1}H, ^{13}C HMQC and HMBC experiments (Table 2). Based on the $J_{2,3}$, $J_{3,4}$ and $J_{4,5}$ coupling constant values and correlations of the protons at carbons bearing nitrogen (H-3 of Qui3N, H-2 and H-4 of QuiN4N) to the corresponding carbons at δ 57.0–57.8, two of the sugar spin systems were assigned to the β-linked amino sugars with the *gluco* configuration. Three other sugar spin systems belonged to the α-linked monosaccharides with the *galacto* configuration, namely, to one Gal and two GalNA residues (GalNA^{I} and GalNA^{II}). The GalNA residues were distinguished by typical singlet signals for H-5 and low-field position of the signals for H-4 and H-5 (δ 4.27–4.89, cf. those of Gal: δ 4.05 and 3.99, respectively), as well as by the position of the signals for C-2 in the region of carbons bearing nitrogen (δ 50.7 and 52.7). ^{1}H and ^{13}C NMR signals for alanine were also found (Tables 1 and 2). The signals for COOH groups (C-6 of GalNA^{I} and GalNA^{II}) were assigned by the C-6/H-5 correlations at δ 173.9/4.27 and 174.4/4.89, respectively, which were revealed by the HMBC spectrum. Similarly, the signal for the CO group of alanine (C-1) was assigned by the C-1/H-2,3 correlations at δ 176.7/4.20 and 176.7/1.38.

Therefore, the polysaccharide has a pentasaccharide repeating unit containing one residue each of α-D-Gal*p*, β-D-Qui*p*3N, β-D-Qui*p*N4N, α-D-Gal*p*NA and α-L-Gal*p*NA. Most amino groups of the amino sugars are acetylated, and one is acylated by a D-alanyl residue; the amino group of alanine is evidently acetylated as well.

Sequence and linkage analyses of PS-2 were performed using 2D NOESY and HMBC experiments. The NOESY spectrum showed the following strong inter-residue cross-peaks:

GalNA^{I} H-1/QuiN4N H-3 at δ 5.20/3.83; QuiN4N H-1/Qui3N H-2 at δ 4.52/3.57; Qui3N H-1/GalNA^{II} H-4 at δ 4.45/4.52; GalNA^{II} H-1/Gal H-4,6a,6b at δ 5.08/4.05, 5.08/3.64, and 5.08/3.51, respectively, and Gal H-1/GalNA^{I} H-4 at δ 5.30/4.45. All the cross-peaks were those between transglycosidic protons, except for the GalNA^{II} H-1/Gal H-6a,6b cross-peaks, which are typical of α-(1→4)-linked disaccharides with the same absolute configuration of the constituent monosaccharides [8]. This finding suggested that GalNA^{II} has the same D configuration as Gal. In addition, two weak cross-peaks, with Qui3N H-2 and H-4, were observed for Ala H-2. Hence, the *N*-alanyl group is attached to Qui3N.

The HMBC spectrum displayed all expected correlations confirming the substitution pattern and full sequence of the monosaccharides. The following were cross-peaks for C-1: GalNA^{I} C-1/QuiN4N H-3 at δ 99.1/3.83; QuiN4N C-1/Qui3N H-2 at δ 101.8/3.57; Qui3N C-1/GalNA^{II} H-4 at δ 104.3/4.52; GalNA^{II} C-1/Gal H-4 at δ 99.1/4.05; Gal C-1/GalNA^{I} H-4 at δ 101.5/4.45; and for H-1: Qui3N H-1/GalNA^{II} C-4 at δ 4.45/80.7; and GalNA^{II} H-1/Gal C-4 at δ 5.08/78.0. A weak cross-peak between Ala C-1 and Qui3N H-3 at δ 176.7/3.87 confirmed the location of the *N*-acetylalanyl group at N-3 of Qui3N. Therefore, the amino groups of the other amino sugars are acetylated.

The ^{13}C NMR chemical shift data allowed determination of the absolute configurations of the GalNA residues and QuiN4N. An upfield shift of the signal for D-Gal C-6 (-1.5 ppm) caused by its glycosylation with α-GalNA^{II} at position 4 indicated the same absolute configuration of the constituent monosaccharides [9,10], thus confirming the D configuration of GalNA^{II} and, consequently, the L configuration of GalNA^{I}. This shift, as the NOE correlation pattern between these two sugars (see above), resulted from the spatial proximity of GalNA^{II} H-1 and Gal H-6a,6b [8]. A relatively large, by the absolute value, negative β-effect of glycosylation for QuiN4N C-4 (-1.0 ppm), caused by its glycosylation by α-L-GalNA^{I} at position 3, proved that the two monosaccharides have different absolute configurations [9–11]. Therefore, QuiN4N has the D configuration.

The data obtained showed that PS-2 has the following structure:

→4)-α-L-Gal*p*NAcA^{I}-(1→3)-β-D-Qui*p*NAc4-NAc-(1→2)-β-D-Qui*p*3NDAlaAc-(1→4)-α-D-Gal*p*NAcA^{II}-(1→4)-α-D-Gal*p*-(1→

In order to confirm the structure, Smith degradation of PS-2 was performed, which resulted in selective destruction of Gal to yield an oligosaccharide, threitol (OS). Studies using the same NMR spectroscopic techniques as described above for PS-2, including full assignment of the ^{1}H and ^{13}C NMR spectra (Tables 1 and 2), allowed determination of the following structure of OS, which is in full

Table 2
^{13}C NMR chemical shifts (δ in ppm) [a]

Sugar residue	C-1	C-2	C-3	C-4	C-5	C-6
O-Deacetylated polysaccharide (PS-2)						
→4)-α-L-Gal*p*NAcA^{I}-(1→	99.1	50.7	69.6	78.8	72.3	173.9
→3)-β-D-Qui*p*NAc4NAc-(1→	101.8	57.1	77.3	57.0	72.3	17.9
→2)-β-D-Qui*p*3NDAlaAc-(1→	104.3	77.8	57.8	75.2	74.0	18.0
→4)-α-D-Gal*p*NAcA^{II}-(1→	99.1	52.7	68.7	80.7	71.5	174.4
→4)-α-D-Gal*p*-(1→	101.5	70.1	69.8	78.0	72.6	60.7
Oligosaccharide (OS)						
α-L-Gal*p*NAcA^{I}-(1→	99.1	50.1	68.9	71.0	73.2	175.4
→3)-β-D-Qui*p*NAc4NAc-(1→	101.9	57.2	78.0	56.9	72.4	17.9
→2)-β-D-Qui*p*3NDAlaAc-(1→	104.2	77.9	57.8	75.3	74.1	18.0
→4)-α-D-Gal*p*NAcA^{II}-(1→	99.0	52.8	68.8	80.7	71.2	174.0
→2)-Threitol	62.4	81.3	72.6	63.6		

[a] Additional chemical shifts: CH_3CON at δ 23.0–23.6; CH_3CON at δ 174.9–175.8; Ala C-1, C-2, and C-3 at δ 176.7–176.8, 51.5, and 18.1, respectively.

agreement with the structure of the polysaccharide:

α-L-Gal*p*NAcA^{I}-(1→3)-β-D-Qui*p*NAc4NAc-(1→2)-β-D-Qui*p*3NDAlaAc-(1→4)-α-D-Gal*p*-NAcA^{II}-(1→2)-threitol

The location of the OAc groups was determined by comparison of the ^{13}C NMR spectra of the initial PS-1 and O-deacetylated PS-2 using published data on effects of O-acetylation [12]. In addition to a single signal at δ 60.7 for the HO*C*H_2–C group in the spectrum of PS-2, the spectrum of PS-1 contained signals at δ 60.6 and 62.1–62.2, thus indicating partial acetylation of Gal at position 6. Similarly, the intensity of the signal at δ 101.5 for Gal C-1 decreased and new signals appeared at δ 97.8–98.0; hence, Gal is partially O-acetylated at position 2 as well. As judged by the ratios of the integral intensities of the signals for the O-acetylated and non-O-acetylated residues, the degree of O-acetylation could be estimated as 50–70% at each position. Splitting of the signals for C-1 and C-6 of the O-acetylated Gal residue, as well as of those for the *O*-acetyl groups themselves (Me at δ 21.3, 21.5, and 21.6), could be accounted for by the occurrence of both mono-O-acetylated at each position and di-O-acetylated residues of Gal. However, additional minor O-acetylation of Gal at position 3 could not be strictly excluded.

Therefore, the O-specific polysaccharide of *P. haloplanktis* type strain ATCC 14393 has the following structure:

→4)-α-L-Gal*p*NAcA-(1→3)-β-D-Qui*p*NAc4NAc-(1→2)-β-D-Qui*p*3NDAlaAc-(1→4)-α-D-Gal*p*NAcA-(1→4)-α-D-Gal*p*2,6Ac_2-(1→

To the best of our knowledge, this O-antigen is the first bacterial polysaccharide reported to contain both D and L isomers of 2-amino-2-deoxygalacturonic acid. A derivative of another rarely occurring amino sugar, 3-(*N*-acetyl-D-alanyl)amino-3,6-dideoxy-D-glucose, has been hitherto found only once, as a component of the O-specific polysaccharide of *Proteus penneri* 14 [13]. The fourth unusual amino sugar present, 2,4-diacetamido-2,4,6-trideoxy-D-glucose, has been reported as a constituent of a number of O-antigens [14].

1. Experimental

Bacterial strain, growth, isolation of the lipopolysaccharide and O-specific polysaccharide.—*P. haloplanktis* ATCC 14393 was obtained from the Institute of Applied Microbiology (Tokyo, Japan). The strain is maintained in the collection of the Pacific Institute of Bioorganic Chemistry (KMM). The bacterium was grown in the modified medium of Youshimizu–Kimura [15].

The lipopolysaccharide (LPS) was isolated in a yield of 7.8% from dry bacterial cells, 16 g of which gave 1.2 g of LPS by phenol–water extraction [16] followed by removal of nucleic acid by precipitation with cold aq 50% CCl_3CO_2H. The O-specific polysaccharide (PS-1) was obtained by mild acid degradation of the LPS (aq 1% HOAc, 100 °C, 2 h) followed by GPC on TSK HW-50 in aq 0.3% HOAc. A total of 450 mg of LPS gave 310 mg of polysaccharide.

O-Deacetylation and Smith degradation.—O-Deacetylation of PS-1 (150 mg) was carried out with aq 12% ammonia at 60 °C for 2 h; the resultant modified polysaccharide (PS-2, 30 mg) was isolated by GPC on TSK HW-50.

Smith degradation of PS-2 (160 mg) was performed with 0.15 M $NaIO_4$ for 74 h at 20 °C in the dark. An excess of $NaBH_4$ was added and after 17 h destroyed with concentrated HOAc. After desalting by GPC on TSK HW-40, the modified polysaccharide was hydrolysed with aq 0.5 M HCl for 72 h at ambient temperature, and an oligosaccharide product (OS, 38 mg) was isolated by GPC on the same gel.

Sugar analyses.—Hydrolysis of PS-2 was performed with 2 M CF_3CO_2H at 120 °C for 2 h. Gal was identified by GLC as galactitol acetate and also on a Biotronik LC-2000 sugar analyser, using a Dionex A × 8-11 anion-exchange resin and 0.5 M sodium borate buffer (pH 8) at 70 °C. Amino sugars and alanine were analysed on a Biotronik LC-2000 amino acid analyser, using an Ostion LG AN B cation-exchange resin and conventional sodium citrate buffers at 80 °C. GalNA was identified also by GLC after methanolysis with a mixture 1:10 AcCl–MeOH at 100 °C for 3 h followed by acetylation. Reference

compounds L-GalNA and D-Qui3N were obtained from the O-specific polysaccharides of *Pseudomonas aeruginosa* NCTC 8505 [17] and *Providencia alcalifaciens* O5 [18], respectively.

For determination of the absolute configuration of GalNA, PS-2 was methanolysed with 1 M HCl in MeOH at 80 °C for 16 h; products were carboxyl reduced with $LiBH_4$ in aq 70% 2-propanol at 20 °C for 16 h, and hydrolysed as above. The absolute configurations of the monosaccharides and alanine were determined by GLC of the acetylated (+)-2-butyl (for GalNA) and (+)-2-octyl (for Gal and Qui3N) glycosides and (+)-2-butyl ester (for Ala) as described [19–21], using a Hewlett–Packard 5890 instrument equipped with a glass capillary column (13 m × 0.25 mm) coated with DB-5 stationary phase and a temperature program of 160 °C (3 min) to 250 °C at 10 °C/min.

NMR spectroscopy.—Samples were deuterium exchanged by freeze-drying three times from ^2H_2O and then examined in solutions of 99.97% ^2H_2O. Spectra were recorded using internal acetone (δ_H 2.225, δ_C 31.45) as reference, on a Bruker DRX-500 spectrometer at 60 °C where data were acquired and performed using XWINNMR version 1.1. Spectra were also recorded on a Jeol 400 MHz spectrometer equipped with a DEC AXP 300 computer. The parameters used for 2D experiments were as follows: COSY, 512 × 1024 data matrix, zero-filled to 1024 data points in t_1, 8 scans per t_1 value, spectral width 3501 Hz, recycle delay 1 s, unshifted sine-bell filtering in t_1 and t_2; NOESY, 512 × 1024 data matrix, zero-filled to 1024 data points in t_1, 16 scans per t_1 value, spectral width 3501 Hz, mixing time 300 ms, shifted sine-squared filtering in t_1 and t_2; TOCSY, 512 × 1024 data matrix, zero-filled to 1024 data points in t_1, 16 scans per t_1 value, the duration of the MLEV17 spin-lock 60 ms; H-detected ^1H, ^{13}C HMQC, 256 × 1024 data matrix, zero-filled to 512 data points in t_1, 40 scans per t_1 value, spectral width in t_1 3501 Hz and in t_2 11875 Hz, recycle delay 1.0 s, shifted sine-squared filtering in t_1 and t_2. A HMBC spectrum was recorded at 60 °C using a Jeol 400 MHz spectrometer equipped with a DEC AXP 300 computer; the parameters used were: 512 × 1024 data matrix, zero-filled to 1024 data points in t_1, 80 scans per t_1 value, spectral width in t_1 1873.0 Hz and in t_2 17530 Hz, recycle delay 1.5 s, shifted sine-squared filtering in t_1 and t_2.

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References

[1] G. Gauthier, M. Gauthier, R. Christen, *Int. J. Syst. Bacteriol.*, 45 (1995) 755–761.
[2] P. Baumann, M.J. Gauthier, L. Baumann, in J.C. Holt (Ed.), *Bergey's Manual of Systematic Bacteriology*, Vol. 1, Williams and Wilkins, Baltimore, 1984, pp. 343–352.
[3] O.M. Hanniffy, A.S. Shashkov, S.N. Senchenkova, S.V. Tomshich, N.A. Komandrova, L.A. Romanenko, Y.A. Knirel, A.V. Savage, *Carbohydr. Res.*, 307 (1998) 291–298.
[4] S.L. Patt, J.N. Shoolery, *J. Magn. Reson.*, 46 (1982) 535–539.
[5] K. Bock, C. Pedersen, *Adv. Carbohydr. Chem. Biochem.*, 41 (1983) 27–65.
[6] N. Cyr, A.S. Perlin, *Can. J. Chem.*, 57 (1979) 2504–2511.
[7] K. Bock, C. Pedersen, *J. Chem. Soc., Perkin Trans. 2*, (1974) 293–297.
[8] G.M. Lipkind, A.S. Shashkov, S.S. Mamyan, N.K. Kochetkov, *Carbohydr. Res.*, 181 (1988) 1–12.
[9] G.M. Lipkind, A.S. Shashkov, Y.A. Knirel, E.V. Vinogradov, N.K. Kochetkov, *Carbohydr. Res.*, 175 (1988) 59–75.
[10] A.S. Shashkov, G.M. Lipkind, Y.A. Knirel, N.K. Kochetkov, *Magn. Reson. Chem.*, 26 (1988) 735–747.
[11] Y.A. Knirel, N.A. Kocharova, A.S. Shashkov, N.K. Kochetkov, E.V. Kholodkova, E.S. Stanislavsky, *Eur. J. Biochem.*, 166 (1987) 189–197.
[12] P.-E. Jansson, L. Kenne, E. Schweda, *J. Chem. Soc. Perkin Trans. 1*, (1987) 377–383.
[13] E.V. Vinogradov, A.S. Shashkov, Y.A. Knirel, N.K. Kochetkov, Z. Sidorczyk, A. Swierzko, *Carbohydr. Res.*, 219 (1991) C1–C3.
[14] Y.A. Knirel, N.K. Kochetkov, *Biochemistry* (*Moscow*), 59 (1994) 1325–1383.
[15] M. Youschimizu, T. Kimura, *Fish Pathol.*, 10 (1976) 243–259.
[16] O. Westphal, O. Lüderitz, F. Bister, *Z. Naturforsch. Teil B*, 7B (1952) 148–155.
[17] Y.A. Knirel, E.V. Vinogradov, A.S. Shashkov, S.G. Wilkinson, Y. Tahara, B.A. Dmitriev, N.K. Kochetkov, E.S. Stanislavsky, G.M. Mashilova, *Eur. J. Biochem.*, 155 (1986) 659–669.

[18] G.V. Zatonsky, O.V. Bystrova, N.A. Kocharova, A.S. Shashkov, Y.A. Knirel, E.V. Kholodkova, E.S. Stanislavsky, *Biochemistry (Moscow)*, 64 (1999) 523–527 (English translation).
[19] G.J. Gerwig, J.P. Kamerling, J.F.G. Vliegenthart, *Carbohydr. Res.*, 77 (1979) 1–7.
[20] K. Leontein, B. Lindberg, J. Lönngren, *Carbohydr. Res.*, 62 (1978) 359–362.
[21] A.S. Shashkov, S.N. Senchenkova, E.L. Nazarenko, V.A. Zubkov, N.M. Gorshkova, Y.A. Knirel, R.P. Gorshkova, *Carbohydr. Res.*, 303 (1997) 333–338.