# Structures of the O-antigens of *Proteus* bacilli belonging to OX group (serogroups O1–O3) used in Weil-Felix test

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Received 14 May 1997

**Abstract Structures of the O-specific polysaccharide chains of lipopolysaccharides from *Proteus* group OX strains (serogroups O1–O3) used as antigens in Weil-Felix test for diagnosis of rickettsiosis, were established. From them, the acid-labile polysaccharide of *Proteus vulgaris* OX19 (O1) is built up of the following branched pentasaccharide repeating units connected via a phosphate group:**

α-L-Qui*p*NAc
1
↓
3
→4)-α-L-Qui*p*NAc-(1→3)-α-D-Glc*p*NAc-(1→4)-α-D-Gal*p*NAc-(1→4)-α-D-Gal*p*-1-*P*-(O→

**where QuiNAc stands for 2-acetamido-2,6-dideoxyglucose (*N*-acetylquinovosamine). The basis of serospecificity of the *Proteus* group OX antigens and their cross-reactivity with human antirickettsial antibodies is discussed.**



*Key words:* O-antigen; Lipopolysaccharide; Bacterial polysaccharide structure; Serological cross-reactivity; Rickettsiosis; Weil-Felix test; *Proteus*; *Rickettsia*

## 1. Introduction

A group of diseases caused by bacteria of the world-wide occurring genus *Rickettsia* poses serious medical problems in both developing and developed countries. An unspecific Weil-Felix test for serodiagnosis of rickettsiosis used since 1916 employs *Proteus* group OX strains as antigens which give characteristic agglutination patterns with sera from patients infected with various rickettsial agents [1–3]. Sera from patients with typhus and Rocky Mountain spotted fever react with *P. vulgaris* OX19, whereas *P. vulgaris* OX2 cells are agglutinated by sera from patients with spotted fever (except for Rocky Mountain spotted fever). *P. mirabilis* OXK cells bind antibodies from sera from patients with scrub typhus, which is caused by *Orientia tsutsugamushi* formerly known as *Rickettsia tsutsugamushi* reclassified on the basis of genetic data [4].

The serological specificity of Gram-negative bacteria is defined by the structure of the O-specific polysaccharide chain (OPS, O-antigen) of the outer-membrane lipopolysaccharide (LPS). Based on O-antigens, a serological classification of *Proteus* has been developed [5,6]. LPS is also responsible for the cross-reactivity between *Proteus* cells and antisera from patients with rickettsiosis [7–12]. Studies of chemical compositions of the *Proteus* group OX LPSs were performed to substantiate the Weil-Felix test at the molecular level [8,11], and we now report the structures of OPSs of these strains.

## 2. Materials and methods

### 2.1. Bacteria, growth, isolation of lipopolysaccharides and O-specific polysaccharides

Bacterial strains *P. vulgaris* OX19 (O1, PZH-24), *P. vulgaris* OX2 (O2) and *P. mirabilis* OXK (O3, PrO 10/52) came from the National Institute of Hygiene (Warsaw), The National Institute of Health (Tokyo) and the Czech National Collection of Type Cultures (Institute of Epidemiology and Microbiology, Prague), respectively. The bacteria were grown on a nutrient broth supplemented with 1% glucose as described [13]. LPSs were isolated by extraction of cells with hot aqueous phenol [14], purified and degraded with 1% HOAc at 100°C as described [15] to give OPSs isolated by gel-permeation chromatography on Sephadex G-50.

### 2.2. Chemical methods

Hydrolysis of OPSs was performed with 2 M $CF_3CO_2H$ at 121°C for 2 h. Amino components were detected using a Biotronik LC-2000 amino acid analyzer (Germany) and the standard 0.35 M sodium citrate buffer (pH 5.28) at 80°C. Neutral sugars and uronic acids were identified with a Biotronik LC-2000 sugar analyzer using a column of a Dionex A×8-11 anion-exchange resin and 0.5 M sodium borate buffer (pH 8.0) at 65°C or 0.02 M potassium phosphate buffer (pH 2.4) at 70°C, respectively. Amino sugars and neutral sugars were analyzed also as alditol acetates using GLC on a Hewlett-Packard 5890 chromatograph (USA) equipped with an Ultra 2 capillary column. The absolute configurations of monosaccharides were determined by GLC of acetylated (*S*)-2-octyl or (*S*)-2-butyl glycosides [16,17].

O-Deacetylation was performed with aqueous 12% ammonia at 50°C for 2 h.

### 2.3. NMR spectroscopy and mass spectrometry

NMR spectra were run with WM-250 ($^1$H) and AM-300($^{13}$C,$^{31}$P) spectrometers (Bruker, Germany) for solutions in $D_2O$ at 45 and 70°C, respectively, using acetone ($\delta_H$ 2.225, $\delta_C$ 31.45) as internal standard and aqueous 85% $H_3PO_4$ ($\delta_P$ 0) as external standard. Two-dimensional rotating-frame NOE (ROESY) experiments were performed on a modified Bruker WM-250 spectrometer using the proposed pulse sequence [18] and a mixing time of 0.23 s; HDO signal was suppressed by irradiation during 1 s. A mixing time of 1 s was used in one-dimensional NOE experiments. $^1$H-NMR spectra were assigned using selective spin-decoupling, two-dimensional COSY and H,H-relayed COSY. $^{13}$C-NMR spectra were assigned using two-dimensional heteronuclear $^{13}$C,$^1$H correlation (HETCOR). Sugar spin systems were identified based on the $^3J_{H,H}$ coupling constant values; amino sugars were distinguished by correlation of the protons at the carbons bearing nitrogen to the corresponding carbons.

A negative mode electrospray ionization mass spectrum was obtained on a VG Quattro mass spectrometer (Micromass, UK) using aqueous 50% acetonitrile as mobile phase.

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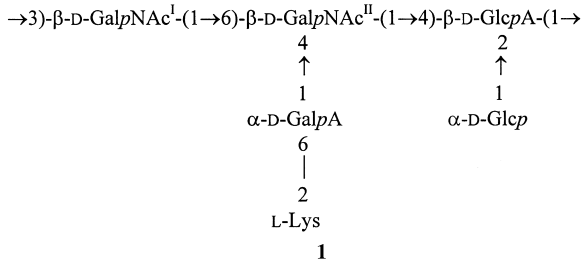
*PII* S0014-5793(97)00694-7

## 3. Results

### 3.1. Structural studies of the O-specific polysaccharide of P. mirabilis OXK

Sugar and amino acid analyses of OPS revealed glucose and equal amounts of glucuronic acid (GlcA) and galacturonic acid (GalA), as well as 2-amino-2-deoxygalactose (GalN) and lysine in the ratio ~2:1. The $^{13}$C-NMR spectrum of the *P. mirabilis* OXK OPS resembled much that of an unclassified *P. mirabilis* strain, which was studied by us earlier and found to have the same composition and structure **1** [19,20].



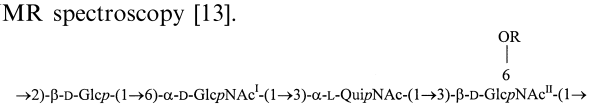

**1**

Previous structural analysis of the *P. mirabilis* S1959 OPS was performed mainly by chemical methods and analysis of one-dimensional $^{13}$C-NMR spectra which were assigned tentatively [19]. An independent structural study of the *P. mirabilis* OXK OPS was carried out using reliable two-dimensional $^{1}$H- and $^{13}$C-NMR spectroscopy, including ROESY.

The α-configuration of GalA and Glc and the β-configuration of GlcA and both GalNAc residues were determined by the coupling constant values $J_{1,2}$ 3.5 and 7.5–8 Hz, respectively. The monosaccharide sequence and the substitution pattern followed from the correlations between the transglycosidic protons (GalNAc$^{I}$ H1/GalNAc$^{II}$ H6; GalNAc$^{II}$ H1/GlcA H4; GlcA H1/GalNAc$^{I}$ H3; GalA H1/GalNAc$^{II}$ H4; Glc H1/GlcA H2) revealed by a ROESY experiment and were in agreement with the characteristic displacements of the carbon signals in the $^{13}$C-NMR spectrum of OPS [19,20]. These data proved finally that OPS of *P. mirabilis* OXK has structure **1**.

### 3.2. Structural studies of the O-specific polysaccharide of P. vulgaris OX2

Sugar analysis of OPS revealed 2-amino-2-deoxyglucose (GlcN) and 2-amino-2,6-dideoxyglucose (quinovosamine, QuiN) in the ratio ~2:1, as well as glucose. The $^{13}$C-NMR spectrum showed that OPS lacked strict regularity, most probably, owing to non-stoichiometric O-acetylation (there was a signal for an O-acetyl group at δ 21.8). After O-deacetylation, the spectrum became typical of a regular polymer and was found to be identical with the spectrum reported by us earlier for OPS of *P. vulgaris* OX19 [13]. Structure **2** was established for that OPS by partial acid hydrolysis, solvolysis with anhydrous hydrogen fluoride, and one-dimensional $^{1}$H- and $^{13}$C-NMR spectroscopy [13].




**2** R = H
**3** R = Ac (~70%) or H (~30%)

That O-deacylated OPS of *P. vulgaris* OX2 has the same structure followed from a two-dimensional COSY experiment which demonstrated the α-configuration of GlcNAc$^{I}$ and QuiNAc and the β-configuration of Glc and GlcNAc$^{II}$, and one-dimensional NOE experiments which revealed correlations between the transglycosidic protons (Glc H1/GlcNAc$^{I}$ H6; GlcNAc$^{I}$ H1/QuiNAc H3; QuiNAc H1/GlcNAc$^{II}$ H3; GlcNAc$^{II}$ H1/Glc H2) [21].

Comparison of the $^{13}$C-NMR spectra of the initial and O-deacetylated OPSs revealed partial displacements of the signals for C6 and C5 of GlcNAc$^{II}$ from δ 62.1 and 77.3 to δ 65.1 and 74.8, respectively, that was evidently due to O-acetylation at O6. Accordingly, partial downfield displacements (by ~0.5 ppm) caused by the deshielding effect of the O-acetyl group, was observed for the signals for H6a and H6b of GlcNAc$^{II}$ in the $^{1}$H-NMR spectrum. As judged by the relative intensities of the signals for C6 and H6 of the O-acetylated and non-O-acetylated GlcNAc$^{II}$ residues, the degree of O-acetylation is ~70%. Therefore, the O-acetyl group

Table 1
Data of $^{1}$H- and $^{13}$C-NMR spectra of *P. vulgaris* OX19 OPS

| Residue | Chemical shift (δ, ppm) | | | | | |
|---|---|---|---|---|---|---|
| | H1 | H2 | H3 | H4 | H5 | H6a; H6b |
| →4)-α-L-QuipNAc$^{I}$-(1→ | 4.97 | 3.99 | 3.84 | 3.87 | 4.21 | 1.29 |
| →3)-β-D-GlcpNAc-(1→ | 5.17 | 3.56 | 3.98 | 3.53 | 3.46 | 3.91; 3.76 |
| →4)-α-D-GalpNAc-(1→ | 4.87 | 4.45 | 4.09 | 4.35 | 4.46 | 3.74; 3.66 |
| 3 ↑ | | | | | | |
| →4)-α-D-Galp-1-*P*-(O→ | 5.63 | 3.95 | 3.95 | 4.11 | 4.17 | 3.71; 3.65 |
| α-L-QuipNAc$^{II}$-(1→ | 4.96 | 3.97 | 3.63 | 3.30 | 3.68 | 1.31 |
| | C1 | C2 | C3 | C4 | C5 | C6 |
| →4)-α-L-QuipNAc$^{I}$-(1→ | 98.4 [a] | 54.8 [b] | 71.1 | 81.8 | 68.2 | 17.8[c] |
| →3)-β-D-GlcpNAc-(1→ | 100.3 | 57.8 | 79.3 | 70.0 | 77.2 | 62.0 |
| →4)-α-D-GalpNAc-(1→ | 99.7 | 50.6 | 74.3 | 72.3 | 71.9 | 61.3 |
| 3 ↑ | | | | | | |
| →4)-α-D-Galp-1-*P*-(O→ | 96.7 | 69.3 | 69.8 | 77.2 | 73.7 | 61.3 |
| α-L-QuipNAc$^{II}$-(1→ | 99.2 [a] | 54.5 [b] | 71.9 | 76.4 | 70.2 | 17.6[c] |

Chemical shifts for the *N*-acetyl groups are $\delta_H$ 1.99–2.10, $\delta_C$ 23.1–23.8 ($CH_3$) and 174.8–175.4 (CO).
[a–c]Assignment could be interchanged.

is located at position 6 of most of the Glc*p*NAc$^{II}$ residues, and OPS of *P. vulgaris* OX2 has structure **3**.

### 3.3. Structural studies of the O-specific polysaccharide of P. vulgaris OX19

Delipidation of the *P. vulgaris* OX19 LPS by mild acid hydrolysis at pH 4.5 was accompanied by depolymerization of OPS. Therefore, LPS was O-deacylated under basic conditions to give an OPS attached to a core oligosaccharide and a modified lipid moiety containing N-linked fatty acids only. Comparison of the $^{13}$C-NMR spectra of the initial and O-deacylated LPSs showed that OPS contained no O-acyl groups that could be lost during alkaline treatment. Sugar analysis revealed the presence in OPS of GlcN, GalN and QuiN in the ratios 1:0.8:1.2, as well as galactose.

The $^{13}$C-NMR spectrum of the O-deacylated LPS (Table 1) demonstrated a pentasaccharide repeating unit in OPS (there were signals for five anomeric carbons at δ 96.7–100.3) containing four N-acetylamino sugars (signals for four carbons linked to nitrogen (C2) at δ 50.6–57.8 and four N-acetyl groups at δ 23.1–23.8 (CO) and 174.8–175.4 ($CH_3$)) and two 6-deoxy sugars (signals for two $CH_3$–C groups at δ 17.6 and 17.8). Accordingly, the $^1$H-NMR spectrum of the O-deacylated LPS (Table 1) contained, inter alia, signals for five anomeric protons at δ 4.87–5.63 ppm and four N-acetyl groups at δ 1.99–2.10. The coupling constant values ($J_{1,2}$ 8 Hz for GlcNAc and $J_{1,2} < 4$ Hz for the four other monosaccharide residues) showed that GlcNAc is β-linked residue, while Gal, GalNAc and QuiNAc are α-linked.

In the $^{13}$C-NMR spectrum, the signals for C3 of GlcNAc, C4 of Gal, C4 of one of the QuiNAc residues (QuiNAc$^I$), C3 and C4 of GalNAc were shifted downfield by 4–10 ppm as compared with their positions in the spectra of the corresponding free monosaccharides [23,24], while no such displacement was observed for any signal of the second QuiNAc residue (QuiNAc$^{II}$). These shifts were evidently due to the α-effects of glycosylation [23] and, thus, revealed the substitution pattern in OPS.

The $^{31}$P-NMR spectrum contained a signal for one phosphate group at −1.2 ppm. Doublet splitting in the $^{13}$C-NMR spectrum of the signals for C1 and C2 of Gal and C4 and C5 of QuiNAc$^I$ ($J_{C,P}$ 4–7 Hz) due to their coupling to phosphorus allowed identification of the 1-*P*-(O→4 phosphodiester linkage between these two residues. This conclusion was confirmed by a one-dimensional $^1$H,$^{31}$P HMQC experiment which revealed correlations P/Gal H1 at δ 1.2/5.63 and P/QuiNAc$^I$ H4 at δ 1.2/3.87.

A ROESY experiment revealed correlations between the following transglycosidic protons: QuiNAc$^I$ H1/GlcNAc H3, GlcNAc H1/GalNAc H4, GalNAc H1/Gal H4 and H6, and QuiNAc$^{II}$ H1/GalNAc H3; no interresidue correlations was observed for H1 of Gal. These data were consistent with the glycosylation and phosphorylation patterns of OPS and allowed unambiguous determination of the monosaccharide sequence in the repeating unit.

Therefore, the repeating unit of OPS of *P. vulgaris* OX19 has structure **4**.

α-L-Qui*p*NAc$^{II}$
1
↓
3
→4)-α-L-Qui*p*NAc$^I$-(1→3)-α-D-Glc*p*NAc-(1→4)-α-D-Gal*p*NAc-(1→4)-α-D-Gal*p*-1-*P*-(O→

**4**

Mild acid degradation of LPS resulted in a phosphorylated pentasaccharide which had a Gal residue at the reducing end and, hence, resulted from selective cleavage of the galactosyl phosphate linkage in OPS. The electrospray ionization mass spectrum of the pentasaccharide showed an intense peak of the quasimolecular ion [M–H]$^-$ at *m/z* 1039 which corresponded to the chemical repeating unit of OPS. In addition, the mass spectrum contained less prominent peaks at *m/z* 959 and 877 which were assigned to the [M–H]$^-$ ions of a dephosphorylated pentasaccharide and a phosphorylated galactose-lacking tetrasaccharide, respectively. Structure **5** was established for the phosphorylated pentasaccharide using NMR spectroscopy as described above for OPS.

α-L-Qui*p*NAc$^{II}$
1
↓
3
*P*-(O→4)-α-L-Qui*p*NAc$^I$-(1→3)-α-D-Glc*p*NAc-(1→4)-α-D-Gal*p*NAc-(1→4)-D-Gal

**5**

These data confirmed independently the structure **4** of OPS.

## 4. Discussion

From OPSs of the three *Proteus* group OX strains used in Weil-Felix test for diagnosis of rickettsiosis, that of *P. vulgaris* OX2 is neutral polysaccharide (**3**), while OPSs of two other strains are acidic, OPS of *P. mirabilis* OXK (**1**) containing D-glucuronic acid and an amide of D-galacturonic acid with L-lysine and OPS of *P. vulgaris* OX19 (**4**) a glycosyl phosphate group. While hexuronic acids and their amides with amino acids, including amides with lysine, are rather common in *Proteus* OPSs [15,25,26], none oligosaccharide phosphate repeating unit has been hitherto reported in any *Proteus* OPS, and structures of this sort are uncommon for other bacterial LPSs as well. The presence of the phosphodiester linkage in the main chain accounts for the known lability of *P. vulgaris* OX19 OPS towards acids.

In the three *Proteus* group OX OPSs, there is no structural fragment in common which could be sufficient for providing serological cross-reactivity. This clarifies the structural basis for serospecificity of *P. vulgaris* OX19, *P. vulgaris* OX2, and *P. mirabilis* OXK, which are classified into different O-serogroups in the Kauffmann-Perch classification scheme [5,6], namely, into serogroups O1, O2, and O3, respectively.

Previously, the same structure **1** has been established for OPS of *P. mirabilis* strain S1959 [19,20], which had not been serologically classified and, on the other hand, a different structure has been reported for OPS of *P. mirabilis* O3 (type strain 6/57) [28]. However, later serological investigations showed that the latter strain did not belong to serogroup O3 and should be reclassified [27].

Structure **2** of the O-deacetylated *P. vulgaris* OX2 OPS has been found earlier in another OPS reported as that of *P. vulgaris* OX19 (strain 5/43) [13]. However, while no cross-reactivity was observed between anti-*P. vulgaris* OX2 antibodies and LPS of *P. vulgaris* OX19 (strain PZH-24) studied in this work, LPS of strain 5/43 strongly cross-reacted in this system [22,27]. Therefore, it was concluded that the strain studied earlier [13] was erroneously described as *P. vulgaris* OX19 and, in fact, belonged to *P. vulgaris* OX2. These data suggested also that both structures **2** and **3** define the O2 specificity of *Proteus* and, thus, the O-acetyl group has a little

serological importance, as followed also from direct serological studies with the *P. vulgaris* OX2 OPS and O-deacetylated OPS [22].

Results of serological studies which will be published elsewhere, showed that human anti-rickettsial antibodies bind to OPSs of *Proteus* group OX strains. Polyclonal rabbit anti-*P. mirabilis* OXK antibodies inhibited the reaction of the homologous OPS with human anti-*Orientia tsutsugamushi* sera and, hence, antibodies against both bacteria utilize the same epitope or similar epitopes on the *Proteus* OPS. In contrast, rabbit anti-*P. vulgaris* OX2 and OX19 antibodies did not inhibit the reactions of the corresponding OPSs with sera from patients with Japanese spotted fever and typhus, respectively, thus indicating that human and rabbit antibodies recognize different epitopes on the *Proteus* OPSs.

A few is known about rickettsial surface antigens. Purified LPSs of two strains of the spotted fever group, strain Katayama (*Rickettsia japonica*) and Thai tick typhus strain TT-118 (an unclassified rickettsial sp.), were found to contain GlcN and QuiN as the major components [9], i.e. the same amino sugars which are present in the *P. vulgaris* OX2 OPS. However, no cross-reactivity was observed between rabbit anti-*P. vulgaris* OX2 antibodies and spotted fever group rickettsial cells [7,22] and, thus, no epitope which can be recognized by rabbit anti-*P. vulgaris* OX2 antibodies, is present on the rickettsial LPS or another surface antigen. The amino sugar composition of the rickettsial LPS and *P. vulgaris* OX2 OPS may be similar merely by chance, the more so that the absolute configuration of QuiN in the rickettsial LPS was not determined and may differ from the rather uncommon L configuration of QuiN present in the *P. vulgaris* OX2 OPS.

Similar results were obtained with rabbit anti-*P. vulgaris* OX19 and anti-*P. mirabilis* OXK antibodies which did not bind to the cell surface of *Rickettsia typhi* and *Orientia tsutsugamushi* [7]. One-way cross-reactivity is not uncommon with antigens having similar but not identical structures. One can also speculate that epitopes in common with the *Proteus* group OX OPSs are exposed on the rickettsial cell surface only after antigen processing and presentation [29]. Further chemical and immunochemical studies are necessary to elucidate structures of the rickettsial cell surface antigens and to reveal common epitopes responsible for Weil-Felix test.

*Acknowledgements:* This work was supported by Grants MRB000 from the International Science Foundation, 96-04-50460 from the Russian Foundation for Basic Research for Y.A. Knirel, and 4-PO5A 12208 from the State Committee for Scientific Research (KBN, Poland) for W. Kaca.

## References

[1] Vinson, J.W. (1976) in: Rose, R. and Friedman, F. (Eds.), Manual of Clinical Immunology, pp. 500–504, American Society for Microbiology, Washington.
[2] Ormsbee, O. (1980) in: Lenetti E.H. (Ed.), Manual of Clinical Microbiology, pp. 922–933, American Society for Microbiology, Washington.
[3] Dash, G.A. and Weiss, E. (1991) in: Balows, A., Trüper, H.G., Dworkin, M., Harder, W. and Schleifer, K.-H. (Eds.), The Procaryotes. A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications, pp. 2407–2470, Springer, Heidelberg.
[4] Drancourt, M. and Raoult, D. (1994) FEMS Microbiol. Rev. 13, 13–24.
[5] Kauffman, F. (1966) The Bacteriology of Enterobacteriaceae, pp. 333–360, Williams and Wilkins, Baltimore, MD.
[6] Larsson, P. (1984) Methods Microbiol. 14, 187–214.
[7] Amano, K.-I., Mizushiri, S., Fujii, S., Fukushi, K. and Suto, T. (1990) Microbiol. Immunol. 34, 135–145.
[8] Mizushiri, S., Amano, K.-I., Fujii, S., Fukushi, K. and Watanabe, S. (1990) Microbiol. Immunol. 34, 121–133.
[9] Amano, K.-I., Fujita, M. and Suto, T. (1993) Infect. Immun. 61, 4350–4355.
[10] Amano, K.-I., Suzuki, N., Fujita, M., Nakamura, Y. and Suto, T. (1993) Microbiol. Immunol. 37, 927–933.
[11] Amano, K.-I., Kyohno, K., Knirel, Y.A. and Kaca, W. (1994) Jpn J. Med. Sci. Biol. 47, 308–309.
[12] Amano, K.-I., Kyohno, K., Aoki, S. and Suto, T. (1995) Microbiol. Immunol. 39, 63–65.
[13] Vinogradov, E.V., Kaca, W., Rozalski, A., Shashkov, A.S., Cedzynski, M., Knirel, Y.A. and Kochetkov, N.K. (1991) Eur. J. Biochem. 200, 195–201.
[14] Westphal, O. and Jann, K. (1965) Methods Carbohydr. Chem. 5, 83–89.
[15] Shashkov, A.S., Toukach, F.V., Paramonov, N.A., Ziolkowski, A., Senchenkova, S.N., Kaca, W. and Knirel, Y.A. (1996) FEBS Lett. 386, 247–251.
[16] Leontein, K., Lindberg, B. and Lönngren, J. (1978) Carbohydr. Res. 62, 359–362.
[17] Gerwig, G.J., Kamerling, J.P. and Vliegenthart, J.F.G. (1979) Carbohydr. Res. 77, 1–7.
[18] Rance, M.R. (1985) J. Magn. Reson. 64, 533–535.
[19] Vinogradov, E.V., Shashkov, A.S., Knirel, Y.A., Kochetkov, N.K., Kholodkova, E.V. and Stanislavsky, E.S. (1987) Bioorg. Khim. 13, 660–669.
[20] Kaca, W., Knirel, Y.A., Vinogradov, E.V. and Kotelko, K. (1987) Arch. Immunol. Ther. Exp. 35, 431–437.
[21] Swierzko, A., Cedzynski, M., Knirel, Y.A., Senchenkova, S.N., Kocharova, N.A., Shashkov, A.S., Amano, K.-I., Kyohno, K. and Kaca, W. (1997) Biochemistry (Moscow) 62, 21–27.
[22] Cedzynski, M., Knirel, Y.A., Amano, K.-I., Swiersko, A., Paramonov, N.A., Senchenkova, S.N. and Kaca, W. (1997) Biochemistry (Moskow) 62, 15–20.
[23] Lipkind, G.M., Shashkov, A.S., Knirel, Y.A., Vinogradov, E.V. and Kochetkov, N.K. (1988) Carbohydr. Res. 175, 59–75.
[24] Knirel, Y.A., Vinogradov, E.V., Shashkov, A.S., Dmitriev, B.A., Kochetkov, N.K., Stanislavsky, E.S. and Mashilova, G.M. (1987) Eur. J. Biochem. 163, 627–637.
[25] Radziejewska-Lebrecht, J., Shashkov, A.S., Grosskurth, H., Bartodziejska, B., Knirel, Y.A., Vinogradov, E.V., Rozalski, A., Kaca, W., Kononov, L.O., Chernyak, A.Y., Mayer, H. and Kochetkov, N.K. (1995) Eur. J. Biochem. 230, 705–712.
[26] Knirel, Y.A., Vinogradov, E.V., Shashkov, A.S., Sidorczyk, Z., Rozalski, A., Radziejewska-Lebrecht, I. and Kaca, W. (1993) J. Carbohydr. Chem. 12, 379–414.
[27] Amano, K.-I., Cedzynski, M., Swierzko, A.S., Kyohno, K. and Kaca, W. (1996) Arch. Immunol. Ther. Exp. 44, 235–240.
[28] Vinogradov, E.V., Kaca, W., Shashkov, A.S., Krajewska-Pietrasik, D., Rozalski, A., Knirel, Y.A. and Kochetkov, N.K. (1990) Eur. J. Biochem. 188, 645–651.
[29] Kawamura, A., Takanaka, H. and Tamura, A. (1995) in: Tsutsugamushi Disease, pp. 275–307, University of Tokyo Press, Tokyo.