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Structural comparison of the O6 specific polysaccharides from *E. coli* O6 : K2 : H1, *E. coli* O6 : K13 : H1, and *E. coli* O6 : K54 : H10

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Abstract

Two distinct forms of the O6 antigen (LPS) from *E. coli* were analysed using ^{1}H and ^{13}C NMR spectroscopoy. Their structures were found to be

$$\rightarrow 4)\text{-}\beta\text{-D-Man}p\text{-}(1 \rightarrow 3)\text{-}\alpha\text{-D-Glc}p\text{NAc-}(1 \rightarrow 4)\text{-}\alpha\text{-D-Gal}p\text{NAc-}(1 \rightarrow 3)\text{-}\underset{\underset{\displaystyle X}{\uparrow}}{\overset{}{\beta\text{-D-Man}p}}{}^{\!\!2}\text{-}(1 \rightarrow$$

In the O6-specific polysaccharide from *E. coli* O6 : K2 and O6 : K13, X is β-D-Glc*p*, as had previously been shown for the O6 polysaccharide from *E. coli* O6 : K15; in the O6 specific polysaccharide from *E. coli* O6 : K54, X is β-D-Glc*p*NAc.

Keywords: Escherichia coli; O6 Polysaccharides, structure; NMR spectroscopy

1. Introduction

The O antigens of *Escherichia coli* are lipopolysaccharides (LPS) which consist of a lipid moiety (lipid A), an oligosaccharide region (core), and a polysaccharide moiety. The latter expresses the serological O-specificity of the bacteria and is termed the O-specific polysaccharide [1,2]. Over 150 distinct *E. coli* O groups are

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known today which are defined by the epitope structure of the respective O antigens (LPS). It was found that some O groups of *E. coli* can be further divided into subgroups [3–6]. We have recently elucidated the structures of three O1 antigens, two O4 antigens, and four O18 antigens [7–10]. Together with *E. coli* O1, O4, and O18, strains with the O6 antigen belong to the most frequent extraintestinal *E. coli* strains [2]. In a comparative NMR study, we obtained evidence for the structural identity of the O6-specific polysaccharides from *E. coli* strains O6:K2:H1 and O6:K13:H1 with that from *E. coli* O6:K15:H16 (F8316-41), which has been published previously [11]. The NMR data for the O-specific polysaccharide from *E. coli* O6:K54 were, however, indicative of structural differences. Here we present the NMR analysis of the structures of the O6-specific polysaccharides from *E. coli* O6:K54:H10 and *E. coli* O6:K2:H1. We also show the structural identity of the O6-specific polysaccharides from *E. coli* strains O6:K2 and O6:K13 with that of *E. coli* O6:K15, which has been published before.

2. Results and discussion

Isolation and characterisation of the O-specific polysaccharides from E. coli *strains O6:K2, O6:K13, and O6:K54.*—The LPS were obtained by extraction of the bacteria with aqueous 45% phenol and subsequent ultracentrifugation of the material from the aqueous phases [12]. The O6 polysaccharides, obtained from the sedimented LPS by mild acid degradation, were purified by gel permeation chromatography on Sephadex G-50. They were eluted with water directly after the void volume (K_D 0.9–0.95).

The polysaccharides from *E. coli* O6:K2 and *E. coli* O6:K13 consisted of glucose, mannose, 2-acetamido-2-deoxyglucose (GlcNAc) and 2-acetamido-2-deoxygalactose in the molar ratios shown in Table 1. Table 1 also shows the effect of periodate oxidation on these polysaccharides. The polysaccharide from *E. coli* O6:K54 had no glucose and one additional GlcNAc residue.

NMR analysis.—The ^{13}C NMR spectra of the polysaccharides from *E. coli* O6:K2 and O6:K13 were identical (Fig. 1A) and superimposable on that of *E.*

Table 1
Composition of the polysaccharides from *E. coli* O6:K2 and O6:K54 before (PS) and after (PS_{ox}) periodate oxidation

Polysaccharide	Sugar composition (molar ratio)			
	Glc	Man	GlcNAc	GalNAc
O6:K2 PS [a]	1	2	1	1
O6:K2 PS_{ox}		1	1	1
O6:K54 PS		2	2	1
O6:K54 PS_{ox}		1	1	1

[a] The polysaccharide from *E. coli* O6:K13 has the same composition.

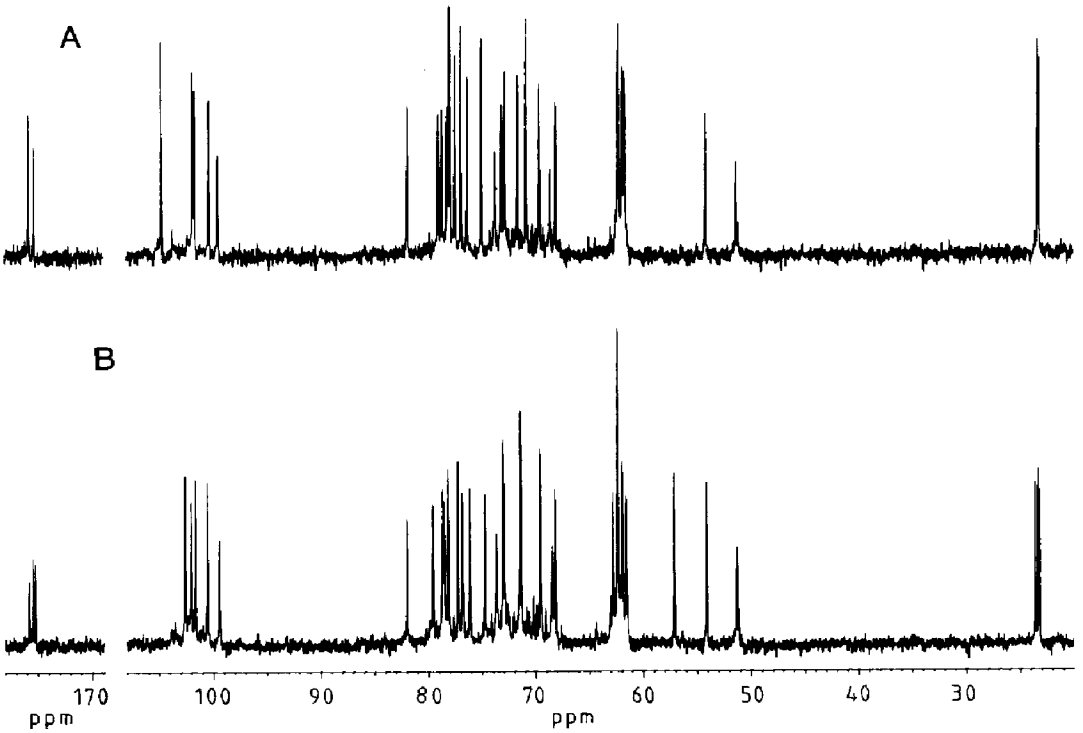

Fig. 1. 75-MHz ^{13}C NMR spectra (δ 22–108; 169–178) of the O6 polysaccharides from *E. coli* O6:K2 (A) and *E. coli* O6:K54 (B), recorded in D_2O (70°C); acetone (δ_C 31.45) as internal standard.

coli O6:K15. Their region of anomeric carbons contained five signals (δ 104.7, 101.8, 101.6, 100.3, and 99.4). The polysaccharide from *E. coli* O6:K54 exhibited a ^{13}C NMR spectrum (Fig. 1B) in which one of the anomeric signals was distinctly different (δ 102.0 versus 104.7) whereas all other signals in the region of anomeric carbons were almost identical (102.5, 101.6, 100.5, and 99.4). The spectra of *E. coli* O6:K2 and O6:K13 contained signals of two *N*-linked carbon atoms (δ 51.2 and 54.1), and that of *E. coli* O6:K54 had signals of three *N*-linked carbon atoms (δ 51.3, 54.0, and 57.1). These data are in accord with the chemical data and indicate that the O6 polysaccharides from strains O6:K2 and O6:K13 contain two amino sugars and that of strain O6:K54 contains three amino sugars, each in a pentasaccharide repeating unit.

The ^1H NMR spectra (Fig. 2) of the polysaccharides contained two signals for α-anomeric protons in the *gluco/galacto* configuration ($J_{1,2}$ 3.5–4 Hz), one signal for a β-anomeric proton in the *gluco/galacto* configuration ($J_{1,2}$ 7.5–8 Hz), and two signals for protons in the *manno* configuration ($J_{1,2} < 2$ Hz). Assignments of the signals (Tables 2 and 3) were obtained using 2D COSY, one-, two-, and three-step relayed coherence transfer (RCT) [13,14], and with the help of 1D homonuclear double resonance in the difference mode [15]. The latter method was also used for the determinations of visual multiplicities and coupling constants.

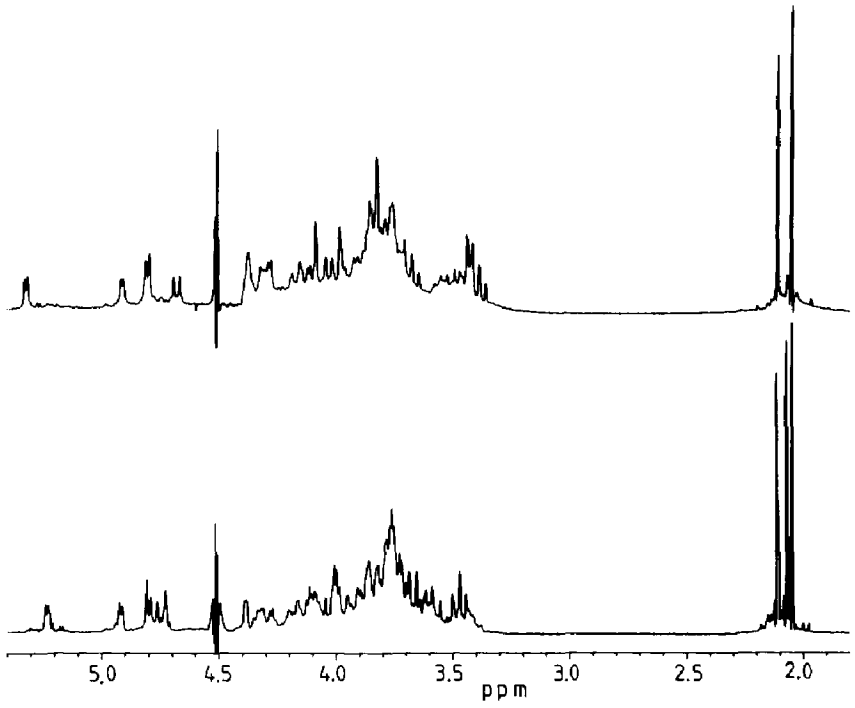

Fig. 2. 300-MHz ^{1}H NMR spectrum of the O6 polysaccharides from *E. coli* O6:K2 (top) and from *E. coli* O6:K54 (bottom), recorded in D_2O (55°C); acetone (δ_H 2.225) as internal standard.

NOE experiments with preirradiation of anomeric protons from each residue (Tables 4 and 5) showed that all O6 polysaccharides had the same sequence and linkage of sugars in the backbone, and differed only in the nature of the side-chain substituent.

The signals of the ^{13}C NMR spectrum were assigned (Tables 2 and 3) with a 2D heteronuclear COSY spectrum. The absolute configurations of the sugar residues were calculated from the glycosylation effects [16,17] with D-glucose as a basis, as derived from its reactivity with D-glucose oxidase. The results obtained allow the formulation of the O6 polysaccharides as:

$$\rightarrow 4)\text{-}\beta\text{-D-Man}p\text{-}(1 \rightarrow 3)\text{-}\underset{\mathbf{A}}{\alpha\text{-D-Glc}p\text{NAc}}\text{-}(1 \rightarrow 4)\text{-}\underset{\mathbf{B}}{\alpha\text{-D-Gal}p\text{NAc}}\text{-}(1 \rightarrow 3)\text{-}\underset{\mathbf{C}}{\beta\text{-D-Man}p}\text{-}(1 \rightarrow \underset{\mathbf{D}}{\overset{2}{\mathbf{E}}}$$

In the O6-specific polysaccharides from *E. coli* strains O6:K2 and O6:K13, **E** is β-D-Glc$p$, as had been demonstrated for the polysaccharide from *E. coli* O6:K15 (strain 8316/41) [11]. In the O6 polysaccharide from *E. coli* O6:K54, **E** is β-D-Glc$p$NAc. The differences in the side-chain substituents in the various O6 polysaccharides indicate that they are not essential for the serological definition of the O6-specificity.

The O6 antigen is another case in which an *E. coli* O antigen is represented by more than one LPS structure. Similar situations have been encountered with the

Table 2
Assignments of the signals in the ^{1}H and ^{13}C NMR spectra of the O6 polysaccharide from *E. coli* O6:K2

Residue	Proton	δ	Coupling		Carbon	δ	$J_{C\text{-}1,H\text{-}1}$ (Hz)	(Glycosylation effect)
			$J_{H,H}$	Hz				
→ 4)-β-D-Man*p*-(1 →	H-1	4.80	$J_{1,2}$	< 2	C-1	101.6	166	
(A)	H-2	3.98	$J_{2,3}$	3.5	C-2	71.5		
	H-3	3.81	$J_{3,4}$	10	C-3	72.8		(−1.3)
	H-4	3.84	$J_{4,5}$	10	C-4	78.1		(+10.2)
	H-5	3.55			C-5	76.2		
	H-6a	3.86			C-6	62.2		
	H-6b	3.74						
→ 3)-α-D-Glc*p*NAc-(1 →	H-1	4.91	$J_{1,2}$	3.5	C-1	99.4	171	
(B)	H-2	4.13	$J_{2,3}$	10	C-2	54.1		
	H-3	4.01	$J_{3,4}$	10	C-3	81.8		
	H-4	3.68	$J_{4,5}$	10	C-4	69.5		(−1.1)
	H-5	4.17			C-5	73.1		
	H-6a	3.84			C-6	61.8		
	H-6b	3.77						
→ 4)-α-D-Gal*p*NAc-(1 →	H-1	5.32	$J_{1,2}$	3.5	C-1	100.3	173	
(C)	H-2	4.30	$J_{2,3}$	10	C-2	51.2		
	H-3	4.06	$J_{3,4}$	3	C-3	68.5		
	H-4	4.09	$J_{4,5}$	< 2	C-4	78.6		
	H-5	4.29			C-5	73.7		
	H-6a	3.79			C-6	61.5		
	H-6b	3.73						
→ 3)-β-D-Man*p*-(1 →	H-1	4.81	$J_{1,2}$	< 2	C-1	101.8	159	
2 ↑	H-2	4.37	$J_{2,3}$	3.5	C-2	78.9		
(D)	H-3	3.85	$J_{3,4}$	10	C-3	77.9		
	H-4	3.84	$J_{4,5}$	10	C-4	68.0		(−0.1)
	H-5	3.46			C-5	77.9		
	H-6a	3.95			C-6	62.2		
	H-6b	3.80						
β-D-Glc*p*-(1 →	H-1	4.68	$J_{1,2}$	7.5	C-1	104.7	155	
(E)	H-2	3.38	$J_{2,3}$	9	C-2	74.9		
	H-3	3.49	$J_{3,4}$	9	C-3	76.8		
	H-4	3.44	$J_{4,5}$	9	C-4	70.8		
	H-5	3.42			C-5	77.4		
	H-6a	3.87			C-6	61.6		
	H-6b	3.74						

O1, O4, and O18 antigens [3–10]. Thus, the serological definition of *E. coli* O antigens may be more akin to those of *Salmonella*, in which O subgroups were established [18]. The predominant changes observed are changes in side-chain

Table 3
Assignments of the signals in the ^{1}H and ^{13}C NMR spectra of the O6 polysaccharide from *E. coli* O6:K54

Residue	Proton	δ	Coupling		Carbon	δ	$J_{C\text{-}1,H\text{-}1}$ (Hz)	(Glycosylation effect)
			$J_{H,H}$	Hz				
→4)-β-D-Man*p*-(1→	H-1	4.81	$J_{1,2}$	< 2	C-1	101.6	167	
(A)	H-2	4.01	$J_{2,3}$	3	C-2	71.3		
	H-3	3.84	$J_{3,4}$	10	C-3	72.9		
	H-4	3.75	$J_{4,5}$	10	C-4	78.6		
	H-5	3.57 [a]			C-5	76.1		
	H-6a	3.87	$J_{6a,6b}$	12	C-6	62.3		
	H-6b	3.73	$J_{5,6b}$	7				
→3)-α-D-Glc*p*NAc-(1→	H-1	4.92	$J_{1,2}$	4	C-1	99.4	174	
(B)	H-2	4.14	$J_{2,3}$	10	C-2	54.0		
	H-3	4.01	$J_{3,4}$	10	C-3	82.0		
	H-4	3.69	$J_{4,5}$	10	C-4	69.5		(−1.1)
	H-5	4.18			C-5	73.0		
	H-6a	3.84	$J_{6a,6b}$	11	C-6	61.9		
	H-6b	3.78						
→4)-α-D-Gal*p*NAc-(1→	H-1	5.24	$J_{1,2}$	3.5	C-1	100.5	177	
(C)	H-2	4.29	$J_{2,3}$	9	C-2	51.3		
	H-3	4.09	$J_{3,4}$	3	C-3	68.4		
	H-4	4.09	$J_{4,5}$	< 2	C-4	78.5		
	H-5	4.31 [a]			C-5	73.6		
	H-6a,6b	3.77			C-6	61.5		
→3)-β-D-Man*p*-(1→	H-1	4.73	$J_{1,2}$	< 2	C-1	102.5	160	
2	H-2	4.39	$J_{2,3}$	3	C-2	76.8		
↑	H-3	3.77	$J_{3,4}$	10	C-3	79.5		
(D)	H-4	3.67	$J_{4,5}$	10	C-4	68.1		
	H-5	3.46	$J_{5,6a}$	9	C-5	78.1		
	H-6a	3.97	$J_{6a,6b}$	12	C-6	62.8		
	H-6b	3.63	$J_{5,6b}$	3				
β-D-Glc*p*NAc-(1→	H-1	4.78	$J_{1,2}$	8	C-1	102.0	156	
(E)	H-2	3.73	$J_{2,3}$	9	C-2	57.1		
	H-3	3.59	$J_{3,4}$	9	C-3	74.7		
	H-4	3.47	$J_{4,5}$	9	C-4	71.4		
	H-5	3.39	$J_{5,6a}$	< 2	C-5	77.2		
	H-6a	3.89	$J_{6a,6b}$	11	C-6	62.3		
	H-6b	3.75	$J_{5,6b}$	3				

[a] From $^{13}C/^{1}H$-COSY.

substitution (from none to Glc*p*, or from Glc*p* to Glc*p*NAc), as well as minor changes in linkages within a main chain. These changes may have their genetic basis either within different fine structures of the *rfb* genes which direct the structure of the repeating O oligosaccharide [19], in the specificity of a polymerase,

Table 4
NOE data [a] for the O6 polysaccharide from *E. coli* O6:K2

NOE observed on			Pre-irradiated proton				
Residue		Proton	**A**, H-1	**B**, H-1	**C**, H-1	**D**, H-1	**E**, H-1
-4)-β-D-Man*p*-(1 →	**(A)**	H-2	+			+ [b]	
		H-3	+			+	
		H-5	+ [b]				
-3)-α-D-Glc*p*NAc-(1 →	**(B)**	H-2		+			
		H-3	+	+ [b]			
-4)-α-D-Gal*p*NAc-(1 →	**(C)**	H-2			+		
		H-4		+			
-3)-β-D-Man*p*(1 →	**(D)**	H-2				+	+
2 ↑		H-3			+	+	+ [b]
		H-5				+ [b]	
β-D-Glc*p*-(1 →	**(E)**	H-2					+
		H-3					+ [b]

[a] The experiment was performed using standard Bruker software NOEMULT.
[b] Small signal due to spin diffusion.

Table 5
NOE data [a] for the O6 polysaccharide from *E. coli* O6:K54

NOE observed on			Pre-irradiated proton				
Residue		Proton	**A**, H-1	**B**, H-1	**C**, H-1	**D**, H-1	**E**, H-1
-4)-β-D-Man*p*-(1 →	**(A)**	H-2	+				
		H-3	+ [b]				
		H-4				+	
-3)-α-D-Glc*p*NAc-(1 →	**(B)**	H-2		+			
		H-3	+	+ [b]			
-4)-α-D-Gal*p*NAc-(1 →	**(C)**	H-2			+		
		H-4		+			
-3)-β-D-Man*p*-(1 →	**(D)**	H-2			+ [b]	+	+
2 ↑		H-3			+	+	
		H-5				+	
β-D-Glc*p*NAc-(1 →	**(E)**	H-2					+
		H-3					+
		H-4					+ [b]
		H-5					+ [b]

[a] The experiment was performed using standard Bruker software NOEMULT.
[b] Small signal due to spin diffusion.

and/or in the specificity of a modifying enzyme [20,21] which attaches a sugar such as glucose or GlcNAc to the finished polysaccharide.

3. Experimental

Bacteria and cultivation.—*E. coli* strains Bi7458/41 (O6:K2:H1), Su4344/41 (O6:K13:H1), and A12b (O6:K54:H10) were used. The bacteria were grown to the stationary phase (ca. 5 h) in 14-L batch cultures at 37°C in a medium containing, per L, tryptone (7.5 g), yeast extract (10 g), D-glucose (10 g), NaCl (3 g), $Na_2HPO_4 \cdot 12H_2O$ (8 g), $MgSO_4 \cdot 7H_2O$ (0.2 g), and poly(ethylene glycol) (0.3 g). D-Glucose and $MgSO_4$ were sterilised separately. At the end of the cultivation, the bacteria were killed with phenol (1% final concentration) and harvested by centrifugation.

Isolation and characterisation of the lipopolysaccharides and preparation of the polysaccharides.—The LPS were isolated from the bacteria with aq 45% phenol at 65°C (5 min) and the material obtained from the aqueous phase was purified by repeated ultracentrifugation as described [12]. The polysaccharides were obtained from the LPS by hydrolysis in aq 1% AcOH (100°C, 90 min) and purified by chromatography on Sephadex G-50 [21].

Analytical procedures.—Glucose and mannose were determined as their alditol acetates by gas–liquid chromatography (GLC); glucosamine and galactosamine were determined [22] as alditol acetates by GLC on PolyA103 at 220°C. The absolute configuration of glucose was determined with D-glucose oxidase (Boehringer, Mannheim).

NMR spectroscopy.—^{1}H NMR and ^{13}C NMR spectra were recorded with a Bruker WM-300 spectrometer in D_2O, using acetone (δ_H 2.225; δ_C 31.45) as the internal standard, at 70°C (^{13}C spectra) and 55°C (^{1}H spectra). Homonuclear 2D COSY spectra, H-relayed H,H-COSY spectra (one-, two-, and three-step), and heteronuclear ^{13}C/^{1}H-COSY spectra were obtained by using standard Bruker software for ASPECT 2000 (COSYHG, COSYRCT, COSYRCT2, and XH-CORRD, respectively). NOE experiments were performed in the truncated driven (TOE) mode [23] with the Bruker NOEMULT program. The relaxation delay was 1 s, the irradiation time of every component of multiplets (D_2) was 0.1 s, and the total pre-irradiation time of whole multiplets was 1.0–1.2 s.

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References

[1] K. Jann and B. Jann, in E.T. Rietschel (Ed.), *Handbook of Endotoxin*, Vol. 1, *Chemistry of Endotoxin*, Elsevier, Amsterdam, 1984, pp 138–186.

[2] I. Ørskov, F. Ørskov, B. Jann, and K. Jann, *Bacteriol. Rev.*, 41 (1977) 667–710.
[3] M. Achtman, A. Mercer, B. Kusecek, A. Pohl, M. Heuzenroeder, W. Aaronson, A. Sutton, and R.P. Silver, *Infect. Immun.*, 39 (1983) 315–335.
[4] B. Kusecek, H. Wloch, A. Mercer, V. Vaisänen, G. Pluschke, T. Korhonen, and M. Achtman, *Infect. Immun.*, 43 (1984) 368–379.
[5] A. Moll, B. Kusecek, G. Pluschke, G. Morelli, M. Kamke, B. Jann, K. Jann, and M. Achtman, *Infect. Immun.*, 53 (1986) 257–263.
[6] D.S. Gupta, B. Jann, and K. Jann, *Infect. Immun.*, 45 (1984) 203–209.
[7] B. Jann, A.S. Shahskov, D.S. Gupta, and K. Jann, *Eur. J. Biochem.*, 210 (1992) 242–248.
[8] B. Jann, A.S. Shashkov, D.S. Gupta, S.M. Panasenko, and K. Jann, *Carbohydr. Polym.*, 18 (1992) 51–57.
[9] B. Jann, A.S. Shashkov, H. Kochanowski, and K. Jann, *Carbohydr. Res.*, 248 (1993) 241–250.
[10] D.S. Gupta, A.S. Shashkov, B. Jann, and K. Jann, *J. Bacteriol.*, 174 (1992) 7963–7970.
[11] P.-E. Jansson, B. Lindberg, J. Lönngren, and C. Ortega, *Carbohydr. Res.*, 134 (1984) 277–283.
[12] O. Westphal and K. Jann, *Methods Carbohydr. Chem.*, 5 (1965) 83–91.
[13] J. Jeener, B.H. Meier, P. Bachmann and E.E. Ernst, *J. Chem. Phys.*, 71 (1979) 4546–4553.
[14] J. Dabrowski, *Methods Stereochem. Anal.*, 9 (1987) 349–386.
[15] N.A. Kocharova, Y.A. Knirel, A.S. Shashkov, N.K. Kochetkov, and G.B. Pier, *J. Biol. Chem.*, 263 (1988) 11291–11295.
[16] A.S. Shashkov, G.M. Lipkind, Y.A. Knirel, and N.K. Kochetkov, *Magn. Reson. Chem.*, 26 (1988) 735–747.
[17] G.M. Lipkind, A.S. Shashkov, Y.A. Knirel, E.V. Vinogradov, and N.K. Kochetkov, *Carbohydr. Res.*, 175 (1988) 57–75.
[18] F. Kauffmann, *The Bacteriology of Enterobacteriaceae*, 1966, Munksgaard, Copenhagen, p 55.
[19] B.A.D. Stocker and P.H. Mäkelä, *Proc. R. Soc. London, Ser. B*, 202 (1978) 5–30.
[20] K. Nikaido and H. Nikaido, *J. Biol. Chem.*, 246 (1971) 3912–3919.
[21] H. Nikaido, K. Nikaido, T. Nakae, and P.H. Mäkelä, *J. Biol. Chem.*, 246 (1971) 3902–3911.
[22] W. Niedermayer and M. Thomana, *Anal. Biochem.*, 57 (1974) 363–368.
[23] G. Wagner and K. Wüthrich, *J. Magn. Reson.*, 33 (1979) 675–680.