Complete lipopolysaccharide of *Plesiomonas shigelloides* O74:H5 (strain CNCTC 144/92). Part 2. Lipid A, its structural variability, the linkage to the core oligosaccharide, and biological activity of the lipopolysaccharide

Jolanta Lukasiewicz, Monika Dzieciatkowska, Tomasz Niedziela, Wojciech Jachymek, Anna Augustyniuk, Lennart Kenne and Czeslaw Lugowski

L. Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, R. Weigla 12, PL-53114 Wroclaw, Poland, and Department of Chemistry, Swedish University of Agricultural Sciences, SE-750 07 Uppsala, Sweden

SUPPORTING INFORMATION

Figure S1. The fragment of TOCSY spectrum of the affinity-purified decasaccharide isolated from *P. shigelloides* O74 LPS (strain CNCTC 144/92) (**C**) and 1D traces from a 2D ^{1}H-^{31}P HMBC spectrum at δ_P 1.58 ppm (**A**) and δ_P 1.76 ppm (**B**). The capital letters refer to carbohydrate residues as shown on the structure at figure S2, and the numbers refer to protons and carbons in the respective residue. (*) - Unresolved signal, visible at the lower contour level. Additional signals, assigned with primed capital letters, revealed the microheterogeneity of the decasaccharide, previously observed for the core oligosaccharide [2].

Figure S2. The fragment of NOESY spectrum of the affinity-purified decasaccharide isolated from *P. shigelloides* O74 LPS (strain CNCTC 144/92). The capital letters refer to carbohydrate residues as shown on the structure, and the numbers refer to protons and carbons in the respective residue. Additional signals, assigned with primed capital letters, revealed the microheterogeneity of the decasaccharide, previously observed for the core oligosaccharide [2].

[2] Niedziela, T. *et al.*, Complete lipopolysaccharide of *Plesiomonas shigelloides* O74 (strain CNCTC 144/92). Part 1. Structural analysis of the highly hydrophobic lipopolysaccharide including the O-specific antigen, its biological repeating unit, the core oligosaccharide, and the linkage between them. Submitted as an accompanying paper.

Table S1. ^{1}H and ^{13}C NMR chemical shifts of the affinity purified decasaccharide isolated from de-N,O-acylated LPS of *P. shigelloides* O74 (strain CNCTC 144/92) [a]

		Chemical shift (ppm)							
		H-1	**H-2 (H-3*ax*)**	**H-3(H-3*eq*)**	**H-4**	**H-5**	**H-6a, 6b**	**H-7a, 7b**	**H-8a, 8b**
RESIDUE		**C-1**	**C-2**	**C-3**	**C-4**	**C-5**	**C-6**	**C-7**	**C-8**
A	→4,5)-α-Kdo-(2→		1.93	2.12	4.14	4.27	3.71	3.87	3.63, 3.90
		–[b]	100.0	34.8	70.8	70.2	72.6	71.0	64.1
B	→3,4)-L-α-D-Hep*p*-(1→	5.30	4.06	4.07	4.4	4.21	4.1	3.80, 3.97	
		99.3	71.2	76.5	72.3	72.8	69.4	64.4	
C	→6)-β-D-Glc*p*-(1→	4.54	3.28	3.5	3.43	3.62	3.84		
		101.9	74.3	76.0	70.4	75.3	67.3		
D	α-D-Gal*p*A-(1→	5.03	3.85	3.96	4.25	4.41			
		98.2	68.5	70.2	71.7	72.3	176.4		
E	→3,7)-L-α-D-Hep*p*-(1→	5.23	4.07	4.14	3.87	3.66	4.21	3.71, 3.74	
		102.4	70.5	76.0	66.7	72.6	68.6	71.3	

F	L-α-D-Hep*p*-(1→	4.91	3.99	3.88	3.77	3.66	4.02	3.74[c]	
		101.5	70.5	71.0	67.1	72.1	69.9	63.5	
G	4-deoxy-β-L-*threo*-hex-4-enopyranosyl	5.13	3.87	4.35	5.81				
		98.9	70.4	66.4	107.5	145.5	–[b]		
M	α-Kdo-(2→		1.77	2.17	4.08	4.04	3.64	4.02	3.71, 3.98
		175.3	102.2	35.1	66.6	67.0	72.7	69.6	63.6
N	→6)-β-D-Glc*p*N4P-(1→	4.85	3.11	3.89	3.82	3.76	3.46, 3.69		
		99.7	55.9	72.1	74.7	74.4	62.8		
O	→6)-α-D-Glc*p*N1P	5.71	3.45	3.93	3.64	4.14	3.78, 4.3		
		91.6	54.7	69.9	69.9	72.9	70.1		

[a] Spectra were recorded at 30 °C. Acetone (δ_H 2.225 ppm, δ_C 31.05) was used as internal reference. [b] Not determined signal. [c] The signals were not resolved.

Table S2. Selected inter-residue NOE and $^{3}J_{H,C}$-connectivities from the anomeric protons and carbons of the affinity purified decasaccharide isolated from the de-N,O-acylated LPS of *P. shigelloides* O74 (strain CNCTC 144/92).

RESIDUE		Atom (ppm)	Connectivities to (ppm)		Inter-residue atom/residue
		δ_H/δ_C	δ_C	δ_H	
		H-1/C-1			
A	→4,5)-α-Kdo-(2→	H3*ax* 1.93		3.63[a]	H-6 of M
		H3*eq* 2.12		3.63[a]	H-6 of M
		34.8			
B	→3,4)-L-α-D-Hep*p*-(1→	5.3	70.4	4.28	C-5, H-5 of A
		99.3			
C	→6)-β-D-Glc*p*-(1→	4.54	71.9	4.4[a]	C-4, H-4 of B
		101.9			
D	α-D-Gal*p*A-(1→	5.03	67.0	3.84[a]	C-6, H-6b of C
		98.2			
E	→3,7)-L-α-D-Hep*p*-(1→	5.23	76.2	4.07[a]	C-3, H-3 of B
		102.4			
F	L-α-D-Hep*p*-(1→	4.91	71.0	3.71	C-7, H-7a of E
		101.5		3.74	H-7b of E
G	4-deoxy-β-L-*threo*-hex-4-enopyranosyl-(1→	5.13		4.13[a]	H-3 of E
		98.9			

N	→6)-β-D-Glc*p*N4P-(1→	4.85	70.0	3.77[a]	C-6, H-6a of O
		99.7		4.30	H-6b of O

[a] Marked values represent NOE connectivities only.

Figure S1

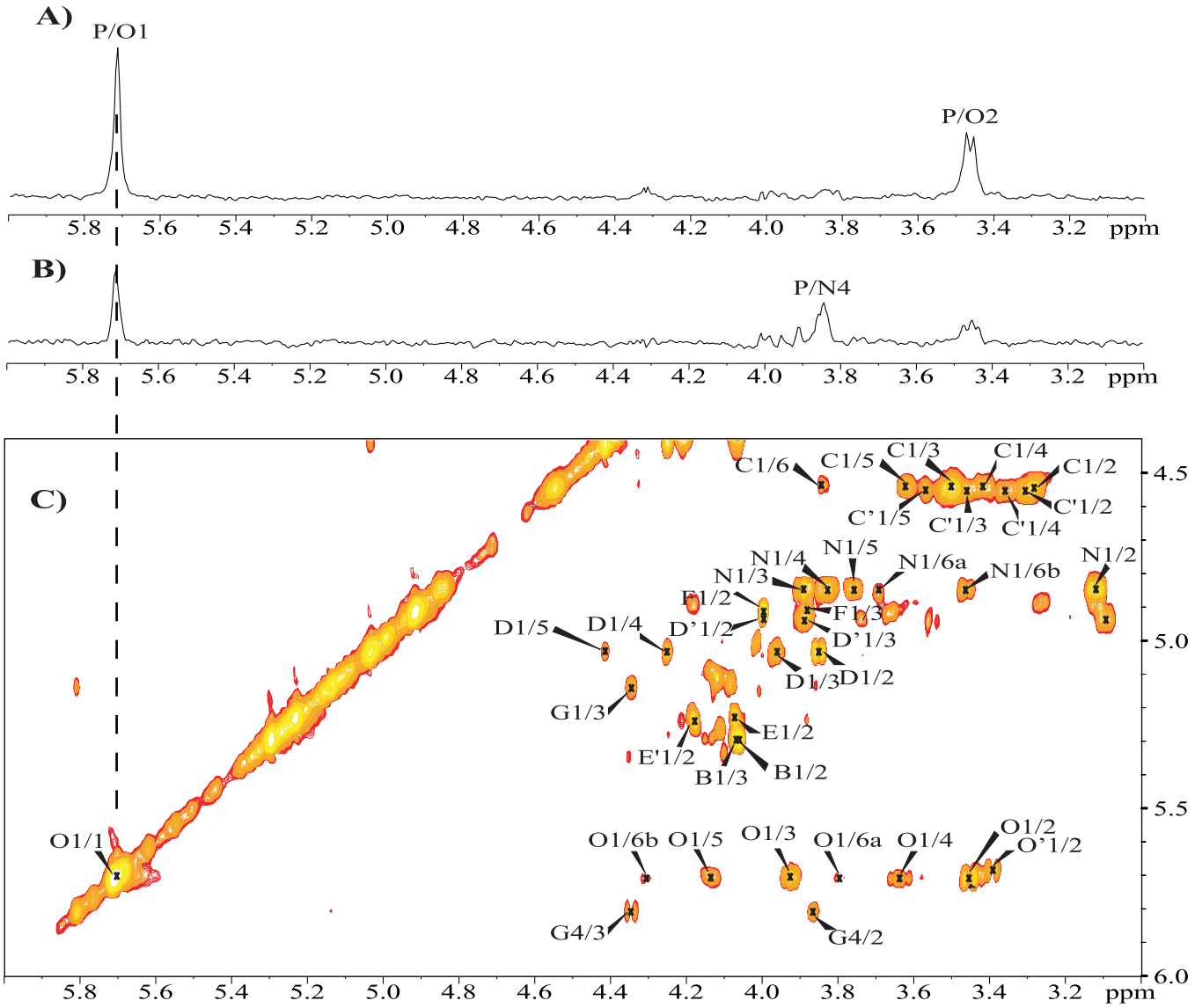
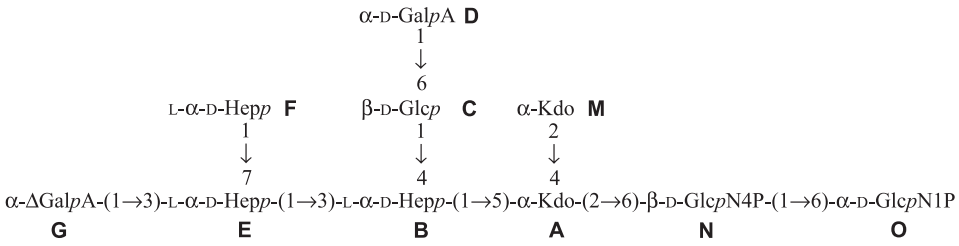

A)
P/O1
P/O2
5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 ppm
B)
P/N4
5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 ppm
C)
C1/6
C1/5
C1/3
C1/4
C1/2
C'1/2
C'1/4
C'1/3
C'1/5
N1/4
N1/5
N1/6a
N1/6b
N1/2
N1/3
E1/2
F1/3
D'1/3
D'1/2
D1/5
D1/4
D1/3
D1/2
G1/3
E1/2
E'1/2
B1/3
B1/2
O1/1
O1/6b
O1/5
O1/3
O1/6a
O1/4
O1/2
O'1/2
G4/3
G4/2
4.5
5.0
5.5
6.0
5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 ppm

Figure S2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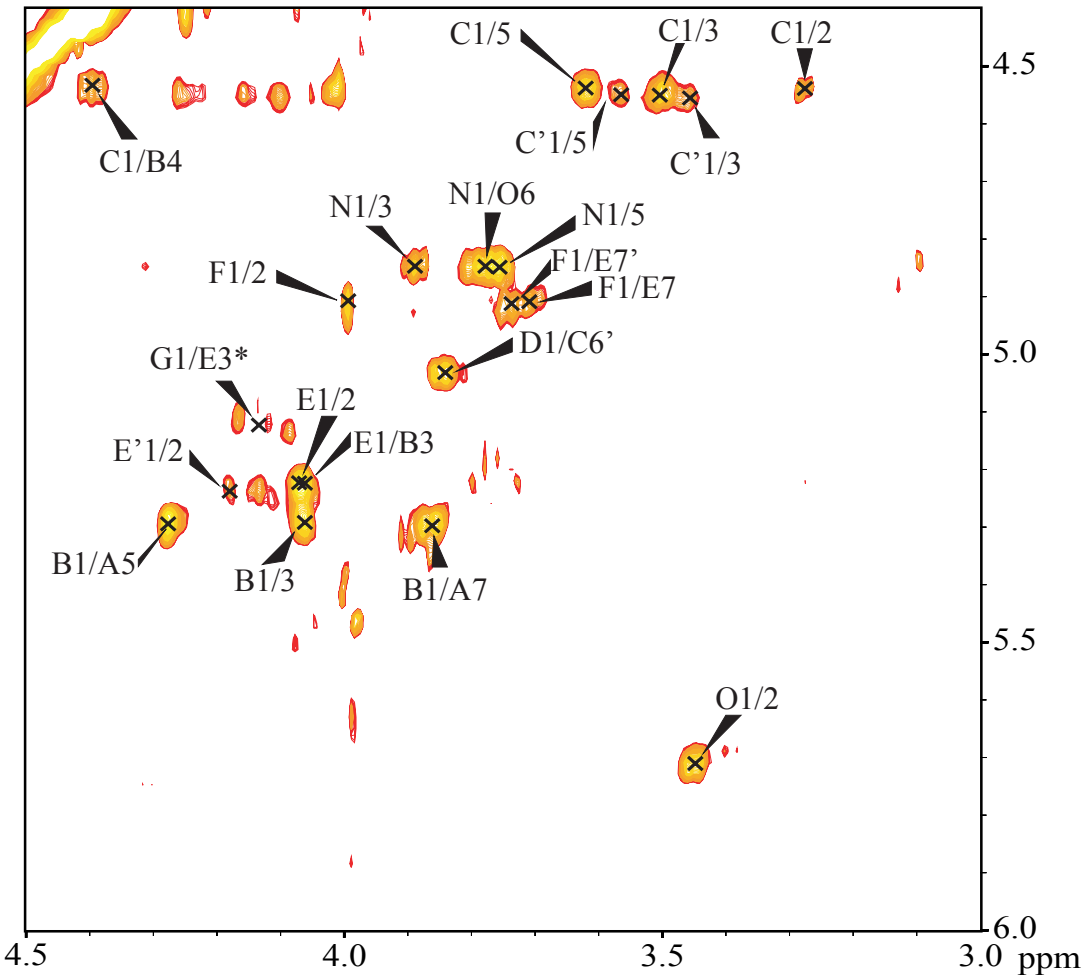
1
↓
6
L-α-D-Hepp F
1
↓
7
β-D-Glcp C
1
↓
4
α-Kdo M
2
↓
4
α-ΔGalpA-(1→3)-L-α-D-Hepp-(1→3)-L-α-D-Hepp-(1→5)-α-Kdo-(2→6)-β-D-GlcpN4P-(1→6)-α-D-GlcpN1P
G
E
B
A
N
O


C1/5
C1/3
C1/2
C1/B4
C'1/5
C'1/3
N1/3
N1/O6
N1/5
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F1/E7'
F1/E7
D1/C6'
G1/E3*
E1/2
E'1/2
E1/B3
B1/A5
B1/3
B1/A7
O1/2
4.5
5.0
5.5
6.0
4.5
4.0
3.5
3.0
ppm