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Structural studies of the saccharide part of the cell envelope lipooligosaccharide from *Haemophilus influenzae* strain galEgalK

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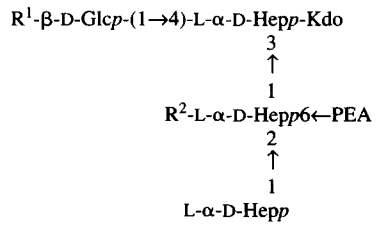
Abstract

The structure of the saccharide part of the lipooligosaccharide from *Haemophilus influenzae* strain galEgalK has been investigated. On treatment of the lipooligosaccharide with acid under mild conditions, followed by reduction with sodium borohydride and gel permeation chromatography, a main fraction was obtained which was studied by methylation analysis, NMR spectroscopy, and FABMS. The material was heterogeneous and contained two major compounds, A and B, and one minor, C.



A R^1 = β-D-Glc*p*-(1→4); R^2 = β-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→3)

B R^1 = H; R^2 = β-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→3)

C R^1 = β-D-Glc*p*-(1→4); R^2 = H

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In the structure, PEA is phosphoethanolamine, and L-D-Hep is L-*glycero*-D-*manno*-heptose. Kdo exists in reduced anhydro forms. The carbohydrate backbone is the same as that proposed for the saccharide part of the major component from *H. influenzae* type b strain A2 [N. J. Phillips, M. A. Apicella, J. M. Griffiss, and B. W. Gibson, *Biochemistry*, 32 (1993) 2003–2012]

Keywords: Structural analysis; *Haemophilus influenzae*; Lipooligosaccharide; NMR; FABMS

1. Introduction

The lipooligosaccharide (LOS) of *Haemophilus influenzae* resembles that of enterobacterial rough mutants (R-mutants). In the relatively few *H. influenzae* lipopolysaccharides (LPSs) studied so far lipid A is joined via a 3-deoxy-D-*manno*-octulosonic acid (Kdo) residue to a heterogeneous oligomer of neutral sugars, including L-*glycero*-D-*manno*-heptose, D-glucose, and D-galactose [1]. Phase variation is often observed for *H. influenzae* which means that the expression of LPS antigenic structures may vary from generation to generation thus making the elucidation of fine structural details difficult [2]. At least three chromosomal loci (*lic1, lic2, lic3*) have been identified as being involved in the biosynthesis of the saccharide part of the LOS [3]. The *gal* locus from *H. influenzae* has been cloned and sequenced, and the genes *galT, galK*, *galM*, and *galR* identified [4].

The double mutant galEgalK of *H. influenzae* type b strain RM 7004 has been generated by a deletion–insertion mutation constructed in *galK* and *galE*, which is located in *lic3*, moved into the *H. influenzae* chromosome [4]. This mutant is unable to convert UDP-glucose into UDP-galactose (via galE) and to synthesize UDP-galactose from exogeneous galactose (via galK). Thus galactose should be absent from its surface structures. The structure of the major saccharides of the LOS of the double mutant galEgalK of *H. influenzae* type b strain RM 7004 has now been investigated.

2. Results and discussion

The LOS was obtained by phenol extraction of the bacteria as described by Galanos et al. [5], followed by precipitation from the phenol phase with diethyl ether–acetone [6], and oligosaccharides were obtained by hydrolysis with acid under mild conditions and finally reduced with sodium borohydride. The resulting oligosaccharide-alditols were separated by gel permeation chromatography on Bio-Gel P4. The major fraction was rechromatographed to give a fraction called OS-1. On dephosphorylation of OS-1 with 48% hydrofluoric acid, OS-2 was obtained.

OS-1 contained inter alia Kdo and phosphoethanolamine (PEA) as evident by NMR spectroscopy (discussed below). A hydrolysate showed D-glucose and L-*glycero*-D-*manno*-heptose (Hep) in the relative proportions 3.6:1.0, analysed as the alditol acetates. The absolute configuration of glucose was determined by the method devised by Gerwig et al. [7]. The heptitol acetate co-chromatographed with the derivative from authentic L-*glycero*-D-*manno*-heptose. Methylation analysis of OS-1 showed terminal D-glucose,

4-substituted D-glucose, terminal L-*glycero*-D-*manno*-heptose, 3,4-substituted L-*glycero*-D-*manno*-heptose, and 2,3-substituted L-*glycero*-D-*manno*-heptose (Table 1). The stoichiometry in the analysis was not satisfactory but the presence of two branch-point residues indicates a doubly branched major structure.

The FAB mass spectrum of OS-1 obtained in the negative mode showed two major pseudomolecular ions at m/z 1568 and 1406, originating from what are later referred to as structures **6** and **7**, and a minor ion at m/z 1244. The molecular weight of an octasaccharide with the formula Hex_4Hep_3Kdo-olPEA is 1587. The ion at m/z 1568 is thus in accord with $(M - 18 - H)^-$. A difference of 18 amu between the expected pseudomolecular ion and that actually found has been observed for Kdo-containing oligosaccharides isolated from other *Haemophilus* LPS [8–12]. The probable explanation for this is the presence of an anhydro-Kdo moiety (Kdo-anh) formed during delipidation by β-elimination of a phosphate group from C-4 of Kdo [13]. The ions at m/z 1406 and 1244 are thus consistent with the heptasaccharide Hex_3Hep_3Kdo-anh-olPEA and the hexasaccharide Hex_2Hep_3Kdo-anh-olPEA, respectively.

The ^{1}H-NMR spectra of OS-1 reflected the sample heterogeneity observed by FABMS. In the low-field region (Fig. 1) signals were observed at δ 5.81, 5.79 (1 H together, *J*, small, not resolved), 5.27, 5.26 (1 H, *J* 3.9 Hz), 5.15, 5.13, 5.12, 5.10 (1 H, *J*, small, not resolved), 5.05, 5.04 (1 H, *J*, small, not resolved), and 4.56, 4.55, 4.51, 4.46 (4 H, *J* 8.0 Hz). It was evident from the COSY spectrum that additional protons resonated at δ 4.55 to 4.53, see below. Major signals for methylene protons of the Kdo derivatives are found at δ 2.25, 2.23, 2.20, 2.18, 1.94, and 1.87, indicating that the Kdo moiety occurs as different forms. It has been shown that up to eight different Kdo-derivatives can be formed upon hydrolysis with elimination [13]. Cross-peaks in a COSY spectrum, obtained at 600 MHz, from the signals at δ 4.55 and 4.53 to 3.90 and 3.73, respectively, showed complex coupling patterns due to an additional coupling (*J*, not resolved). This indicated these protons to be vicinal to a phosphate group, and not anomeric protons. A broad signal at δ 3.28 correlated to a signal at δ 4.15 is indicative of a methylene group next to nitrogen in PEA. PEA is a common substituent in *H. influenzae* LOSs and the chemical shifts recorded for the signals from this substituent were in agreement with previously observed values [8]. Its presence in *H. influenzae*

Table 1
Methylation analyses of oligosaccharide samples

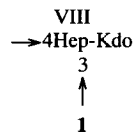
Sugar [a]	t_r [b]	Detector response (%)	
		OS-1	OS-2
2,3,4,6-Glc	1.00	33	28
2,3,6-Glc	1.32	32	30
2,3,4,6,7-Hep	1.55	17	14
3,4,6,7-Hep	1.94	+	+
2,6,7-Hep	2.02	11	11
4,6,7-Hep	2.18	7	17

[a] 2,3,4,6-Glc = 2,3,4,6-tetra-*O*-methyl-D-glucose, etc. [b] Retention time of the corresponding alditol acetate on a DB-5 capillary column using the temperature program 160° (1 min) → 250° (10 min) at 3°/min, relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol (1.00) and hexa-*O*-acetylgalactitol (2.21).

galEgalK was further confirmed by FABMS data on dephosphorylated OS-1 (discussed below). The signals at δ 5.27, 5.26 and 4.56, 4.55, 4.51, 4.46 were assigned to four β-linked and one α-linked hexose because of their $J_{1,2}$ values, and the residues were designated I–V, respectively (Table 2). The remaining signals between δ 5.04 and 5.81 were accordingly assigned to the three heptose residues, designated VI–VIII. The chemical shifts of most of the protons of the glycose residues could be assigned from the combined results from H,H-correlating 2D NMR experiments, namely DQF-COSY and HOHAHA. The ^{1}H NMR chemical shift data of residues I, II, and III are in agreement with those observed for terminal glucose residues, as no large chemical shift displacements were observed. Since OS-1 is a mixture, some of the anomeric protons in the ^{1}H NMR spectrum must be derived from both **6** and **7**, and some from either of them. As terminal heptose is also present, only two of the terminal glucose residues I–III can belong to **6** and the third to **7**. The ^{1}H NMR chemical shift data of residue IV are in agreement with chemical shifts observed for a 1,4-substituted β-D-glucose residue [14]. The ^{1}H NMR chemical shifts of the Glc residue V with significant displacements of signals for H-3 and H-4 clearly demonstrate this residue to be α-1,4-substituted. The corresponding values for the Hep residue VII imply that this residue is 2,3-substituted as the H-2 signal is shifted ca. 0.25 ppm relative to L-α-D-Hep.

The coupled ^{1}H,^{13}C-HMQC spectrum of OS-1 (Fig. 2) showed inter alia the correlation of the anomeric protons with the corresponding anomeric carbons and the $^1J_{C,H}$ values. Signals for anomeric carbons were observed at δ 103.7, 103.4 ($J_{C,H}$ 162 Hz, I, II, III, IV), 102.1 ($J_{C,H}$ 171 Hz), 101.3 ($J_{C,H}$ 174 Hz, V), 99.00 ($J_{C,H}$ 179 Hz, VII), and 97.29, 98.74, 98.73 ($J_{C,H}$ 174, 172, 176 Hz, respectively).

In earlier studies of *H. influenzae* and *H. ducreyi* LOS [8–10] it was observed that the anomeric ^{1}H and ^{13}C NMR resonances of the residue which is linked to Kdo are split because Kdo exists in several different anhydro forms. As a consequence, the intensities of the individual signals are lowered in both the ^{1}H and ^{13}C NMR spectra. We observed strong signals for anomeric protons of two heptosyl residues at δ 5.04 and 5.13, which both correlated with a strong ^{13}C signal at δ 102.1 (Fig. 2). The remaining signals of the anomeric protons from δ 5.04 to 5.15 all correlate with ^{13}C signals at δ 97.29, 98.74, and 98.73. These signals are weak, reflecting heterogeneity, and are assigned to the heptose adjacent to the Kdo residue. The chemical shift data from *H. influenzae* and *H. ducreyi* [8,9] for the corresponding residue linked to Kdo are similar, and we suggest that the anomeric ^{1}H NMR signals at δ 5.05, 5.12, and 5.15 with the corresponding ^{13}C NMR resonances at δ 97.29, 98.74, and 98.79 are assigned to residue VIII and the signals at δ 5.04 and 5.13 with the corresponding ^{13}C signal at δ 102.1 are assigned to residue VI. The following structural element **1** should thus be present in OS-1. In this and other structures Kdo denotes the reduced anhydro form.



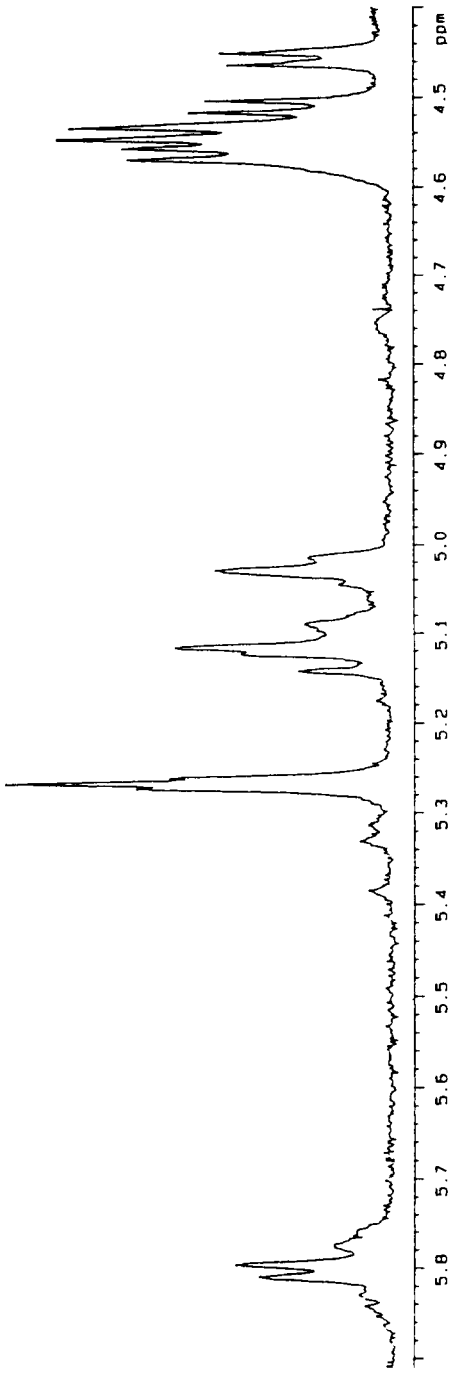


Fig. 1. The anomeric region of the 600 MHz ^{1}H NMR spectrum of OS-1 at 30°C.

Table 2
^1H and ^{13}C NMR data [a] for *H. influenzae* mutant strain galEgalK OS-1 obtained in D_2O at 30°C

Sugar residue		C-1	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b	H-7a	H-7b
β-D-Glc*p*-(1 →	I	103.7	4.46	3.34	3.53	3.47	3.53	3.77	3.90		
		(162)	(8.0)								
β-D-Glc*p*-(1 →	II	103.4	4.51	3.34	3.51	3.44	3.51	3.76	3.94		
		(162)	(8.0)								
β-D-Glc*p*-(1 →	III	103.4	4.55	3.36	3.47	3.53	3.47	3.76	3.96		
		(162)	(8.0)								
→ 4)-β-D-Glc*p*-(1 →	IV	103.4	4.56	3.40	3.64	3.67	3.68	3.86	4.07		
		(162)	(8.0)								
→ 4)-α-D-Glc*p*-(1 →	V	101.3	5.26	3.60	3.92	3.75		3.98			
		(171)	(3.9)								
		101.3	5.27	3.60	3.92	3.75		3.98			
		(171)	(3.9)								
Hep*p*-(1 →	VI	102.1	5.04	3.85							
		(171)	(n. r.)								
		102.1	5.13	3.85							
		(171)	(n. r.)								
→ 2,3)-L-α-D-Hep*p*-(1 →	VII	99.00	5.79	4.26			3.73	4.55		3.90	3.90
		(179)	(n. r.)								
		99.00	5.81	4.26	4.06		3.73	4.55		3.90	3.90
		(179)	(n. r.)								
→ 3,4)-L-α-D-Hep*p*-(1 →	VIII	97.9	5.05	3.98		4.30 [b]		4.09 [b]			
		(174)	(n. r.)								
		98.8	5.12	4.04		4.30 [b]		4.09 [b]			
		(176)	(n. r.)								
		98.8	5.15	4.04		4.30 [b]		4.09 [b]			
		(172)	(n. r.)								
Kdo [c]											

[a] $^1J_{C,H}$ and $^3J_{H,H}$ values are given in parentheses; (n.r.) small coupling, not resolved. [b] Tentative assignments from NOESY data. [c] Pairs of deoxy protons of reduced, anhydro Kdo are recognized in the DQF-COSY spectrum at δ 2.275/1.93, 2.20/1.90, 2.20/1.91, 2.18/1.75, 2.18/1.75, and 2.08/1.94.

The magnitude of the carbon–proton coupling constants observed in the HMQC spectrum confirmed the anomeric configurations of the residues. Thus, all heptose and one of the glucose residues are α-linked and the remaining four glucose residues are β-linked.

The results from FABMS indicated OS-1 to be composed of two major components. From NMR data and methylation analysis it is obvious that the structures are closely related and differ only in the number of hexose residues. Residue V, assigned to a 4-substituted α-Glc residue, shows only little heterogeneity in its chemical shifts, indicating that it occurs in both major structures. The largest saccharide, **6**, thus contains two terminal β-D-Glc groups, one terminal α-Hep group, one α- and one β-linked, and 4-substituted Glc residue, one 2,3- and one 3,4-substituted α-Hep residue, together with a Kdo residue and a PEA group. Saccharide **7** lacks one terminal β-Glc group relative to **6**.

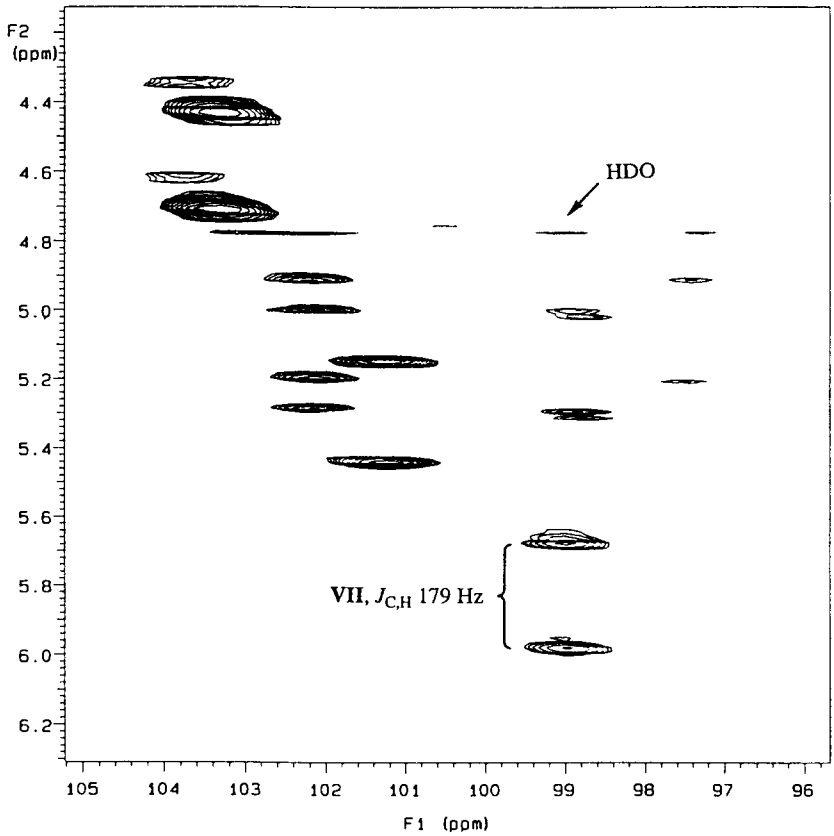


Fig. 2. The anomeric region of the $^{13}C,^{1}H$-coupled HMQC spectrum of OS-1 obtained at 30°C.

A NOESY experiment with a mixing time of 200 ms gave information on inter-residue NOEs, summarized in Table 3. H-1 of residue VI (δ 5.04, 5.13) showed inter-residue NOEs to δ 5.81, 5.79, and 4.26, which correspond to signals from H-1, H-1, and H-2, respectively, of residue VII and indicate the occurrence of the structural element **2** in OS-1.

VI VII
L-α-D-Hep*p*-(1→2)-L-α-D-Hep*p*
3
↑

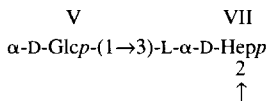
2

Table 3
Observed NOE contacts from anomeric protons of *H. influenzae* galEgalK

Anomeric protons	NOE contacts to
4.46 (t-Glc, I)	3.53 (Glc, I, H-3, H-5), 3.45 (n.r.) [a]
4.51 (t-Glc, II)	3.51 (t-Glc, II, H-3, H-5), 3.68 (→ 4-Glc, IV, H-4)
4.55 (t-Glc, III)	3.47 (Glc, III, H-3, H-5), 3.67 (n.r.), 3.71 (n.r.), 4.09 (→ 3,4-Hep, H-6, VIII), 4.30 (→ 3,4-Hep, H-4, VIII)
4.56 (→ 4-Glc, IV)	3.64 (→ 4-Glc, IV, H-3, H-5), 3.67 (n.r.), 3.71 (n.r.), 4.09 (→ 3,4-Hep, H-6, VIII), 4.30 (→ 3,4-Hep, H-4, VIII)
5.26 (→ 4-Glc, V)	3.60 (→ 4-Glc, V, H-2), 4.06 (→ 2,3-Hep, H-3, VII)
5.27	3.60 (→ 4-Glc, V, H-2), 4.06 (→ 2,3-Hep, H-3, VII)
5.04 (t-Hep, VI)	3.85 (t-Hep,VI, H-2), 4.26 (→ 2,3-Hep, VII, H-2), 5.81 (→ 2,3-Hep, VII, H-1), 3.58 (n.r.), 3.84 (n.r.)
5.13 (t-Hep, VI)	3.85 (t-Hep,VI, H-2), 4.26 (→ 2,3-Hep, VII, H-2), 5.79 (→ 2,3-Hep, VII, H-1),
5.79 (→ 2,3-Hep, VII)	4.26 (→ 2,3-Hep, VII, H-2), 4.04 (→ 3,4-Hep, H-3, VIII)
5.81	4.26 (→ 2,3-Hep, VII, H-2), 4.04 (→ 3,4-Hep, H-3, VIII)
5.05 (→ 3,4-Hep, VIII)	3.98 (→ 3,4-Hep, VIII, H-2), 4.16 (n.r.)
5.12	4.04 (→ 3,4-Hep, VIII, H-2), 4.18 (n.r.)
5.15	4.04 (→ 3,4-Hep, VIII, H-2), 4.18 (n.r.)

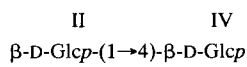
[a] n.r. = Not rationalized.

H-1 of residue V (δ 5.27, 5.26) showed NOE to δ 4.06, which is the chemical shift of H-3 of residue VII, giving evidence of structural element **3**.



3

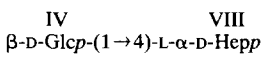
H-1 of residue II (δ 4.51) showed NOE to δ 3.68 which is the chemical shift of the H-4 signal of IV. This confirms structural element **4**.



4

H-1 of residue IV showed two inter-residue NOEs to δ 4.09 and 4.30. Neither of these should be the H-4 signal of glucose residue V, as it would be expected far below δ 4, but they are either signals for H-3 of VII or H-4 of VIII and another proton as these are the remaining linkage sites. None of these could be assigned. However, strong NOEs from one anomeric proton to two protons of the adjacent residue are expected for a β-D-Glc-(1 → 4)-D-Glc glycosidic bond. Conformational studies of some (1 → 4)-linked

disaccharides by Backmann et al. [14] showed that the anomeric proton is at approximately the same distance to both H-4 and H-6 of the neighbouring sugar in such a glycosidic linkage. This should also be true for other glycosidic linkages with the same stereochemistry. By analogy with observations described by us for *H. ducreyi* strain 4747 [9] it is thus reasonable to assign the signals at δ 4.09 and 4.30 to H-4 and H-6, or vice versa, of VIII, respectively, giving evidence for structure element **5**.

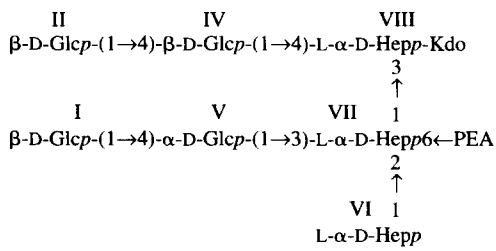


5

The glycosyl group I consequently must be linked to the 4-position of residue V. Residue III is assigned to structure **7**, see below.

OS-2 was analysed by FABMS and pseudomolecular ions at m/z 1445 and 1283 were observed. The shift of 123 amu between the molecular ions observed for OS-1 and OS-2 gives evidence for the presence of PEA. Methylation analysis of OS-2 showed significantly more 4,6,7-tri-*O*-methylheptose than was observed for OS-1 (Table 1) indicating that PEA is located on residue VIII. We propose that PEA is located at O-6 of this residue on the basis of chemical shift data as follows. It is resonable to assume that a proton on a phosphorylated site gives a signal substantially shifted downfield. The signal at δ 4.55 correlates with signals at δ 3.73 and 3.90, which do not correlate with each other. This signal, which also contains additional couplings, is assigned to H-6 in VIII and the other signals to H-5 and H-7a,b, or vice versa. This is a reasonable assignment as the terminal heptose in the disaccharide L-α-D-Hep*p*-(1 → 7)-L-α-D-Hep*p*-(1 → OMe has its signals for H-4, H-5, H-6, and H-7a,b in the heptosyl group at δ 3.88, 3.62, 4.04, and 3.72, respectively [15].

From the combined results presented, structure **6** is proposed for the structure of one of the major saccharides for the galEgalK mutant of *Haemophilus influenzae* type b strain RM 7004.



6

Structure **7** has one Glc residue less than structure **6.** Residue III is a terminal glucose not present in **6** but should be present in **7**. Either residue IV or V should be affected by the omission of a substituting glucosyl residue. These residues have their H-1 chemical

shifts at δ 4.56 (IV) and 5.27, 5.26 (V), respectively, and only at the former is a significant chemical shift heterogeneity observed. Furthermore III has the same NOEs as IV, assigned to heptosyl protons (Table 3). We therefore conclude that II is missing in **7**.

III
β-D-Glc*p*-(1→4)-L-α-D-Hep*p*-Kdo
3
↑
1
β-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→3)-L-α-D-Hep*p*6←PEA
2
↑
1
L-α-D-Hep*p*

7

From the fact that no terminal α-D-Glc*p* residue is detected in ^{1}H NMR spectra we conclude that **8**, lacking two hexosyl residues, has the following structure.

β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→4)-L-α-D-Hep*p*-Kdo
3
↑
1
L-α-D-Hep*p*6←PEA
2
↑
1
L-α-D-Hep*p*

8

The same carbohydrate backbone was observed for *H. influenzae* type b strain A2 LOS [12]. No anomeric configurations were determined in this investigation. It should be noted that the two b strains studied, i.e., galEgalK and A2, are from two entirely different sources and not related with each other.

The mutant strain galEgalK is constructed from the same parent strain (*H. influenzae* RM 7004) as *H. influenzae* AH1-3 [8] but should be without the galactose residues found in AH1-3. Where strain AH1-3 has a β-Gal*p*-(1 → 2)-α-Hep*p* unit, that of strain galEgalK has a terminal heptose. The heptose region α-Hep-(1 → 2)-α-Hep-(1 → 3)-α-Hep-Kdo as well as the cellobiose residue linked to the 3-position of Hep VII are the same in strains AH1-3 and galEgalK. In addition the galEgalK strain has a β-Glc-(1 → 4)-α-Glc-(1 → 3)-α-Hep unit.

3. Experimental

General methods.—Concentrations were performed under diminished pressure at 35°C (bath) or by flushing with nitrogen. For GLC, a Hewlett–Packard 5890 instrument

fitted with a flame-ionisation detector was used. Separation of alditol acetates and partially methylated alditol acetates was performed on a DB-5 fused-silica capillary column using a temperature gradient of 160°C(1 min) → 250°C(10 min) at 3°C/min. GLC/EI/MS was performed on a Hewlett–Packard 5970 mass spectrometer equipped with an HP 5890 gas-chromatograph, using the same conditions. FAB mass spectra were recorded on a NERMAG R10-10L quadrupole instrument. Ions were produced from a matrix of triethanolamine.

Methylation analysis was performed essentially as described [16]. Butyl-lithium was used as base and the methylated products were purified on Sep-Pak C_{18}-cartridges. Phosphorus was determined according to Chen et al. [17].

Bacteria and cultivation.—Bacteria were cultivated to late logarithmic phase in brain–heart infusion broth (Difco) supplemented with yeast extract (10 g/L, Difco), hemin (10 mg/L, Sigma) and nicotinamide adenine dinucleotide (10 mg/L, Sigma). Cultivation was done at constant pH 7.0 with aeration at 37°C in a 35-L fermentor (Belach Bioteknik AB, Stockholm, Sweden). The bacteria were harvested using a Sorvall RC-5B centrifuge at 4°C and 9000*g* for 30 min, then washed with deionized water and lyophilized.

Preparation of LOS.—LOS was extracted from lyophilized bacteria as described by Galanos et al. [5] with the modification of using 6 volumes of diethyl ether–acetone (1:5, v/v) instead of water to 1 volume of the phenol solution [6].

Separation of oligosaccharides.—Oligosaccharide material was obtained after mild hydrolysis of the LOS (102 mg, 1% acetic acid, 100°C, 2 h). Insoluble lipid A (69 mg) was separated from the hydrolysis mixture by centrifugation. The water-soluble part (19 mg) was freeze-dried, reduced overnight with $NaBH_4$ (10 mg/mL), and purified by gel permeation chromatography on a Bio-Gel P4 (2.5 × 90 cm) column using pyridinium acetate buffer (0.1 mM, pH 5) as eluant. Fractions were monitored using a differential refractometer (R403, Waters-Millipore Corporation, Bedford, MA, USA) and fractions were analysed by NMR. The main oligosaccharide fraction (4.9 mg) was further purified by chromatography on Bio-Gel P4.

HF-treatment of OS-1.—OS-1 (1 mg) in a 1.5-mL poly(propylene) tube containing aq 48% HF (0.2 mL) was kept for 18 h at 4°C when aq HF was evaporated under a stream of N_2. The residue was freeze-dried.

NMR spectroscopy.—NMR spectra of solutions in deuterium oxide were recorded at 30°C. Chemical shifts are reported in ppm, using internal sodium 3-trimethylsilylpropanoate-d_4 (δ 0.00,^{1}H) or external 1,4-dioxane (δ 67.4,^{13}C) as reference. The DQF-COSY, HOHAHA, NOESY, and HMQC experiments were performed on a Varian UNITY-600 spectrometer operating at 600 MHz with mixing times of 50 and 100 ms (HOHAHA) and 200 ms (NOESY).

NMR assignments.—Chemical shifts observed for the signals of H-1 to H-7a,7b of the sugar residues in OS-1 are given in Table 2. The signals at δ 4.46, 4.51, 4.55, 4.56 and 5.27, 5.26 were assigned to glucose residues because of their $^3J_{H\text{-}1,H\text{-}2}$ coupling constants. The remaining signals were accordingly assigned to the heptoses. The chemical shifts of most of the proton signals could be assigned from combined results from DQF-COSY and HOHAHA spectra.

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References

[1] S.E. Zamse and E.R. Moxon, *J. Gen Microbiol.*, 133 (1987) 1443–1451.
[2] J.N. Weiser, D.J. Maskell, P.D. Butler, A.A. Lindberg, and E.R. Moxon, *J. Bacteriol.*, 172 (1990) 3304–3309.
[3] A. Kimura and E.J. Hansen, *Infect. Immun.*, 51 (1986) 69–79.
[4] D.J. Maskell, M.J. Szabo, M.E. Deadman, and E.R. Moxon, *Mol. Microbiol.*, 6 (1992) 3051–3063.
[5] C. Galanos, O. Lüderitz, and O. Westphal, *Eur. J. Biochem.*, 9 (1969) 245–249.
[6] N. Qureshi, K. Takayama, and E. Ribi, *J. Biol. Chem.*, 19 (1982) 11808–11815.
[7] G.J. Gerwig, J.P. Kamerling, and J.F.G. Vliegenthart, *Carbohydr. Res.*, 77 (1979) 1–7.
[8] E.K.H. Schweda, O.E. Hegedus, S. Borelli, A.A Lindberg, J.N. Weiser, D.J. Maskell, and E.R. Moxon, *Carbohydr. Res.*, 246 (1993) 319–330.
[9] E.K.H. Schweda, A.Ch. Sundström, L.M. Eriksson, J.A. Jonasson, and A.A. Lindberg, *J. Biol. Chem.*, 269 (1994) 12040–12048.
[10] W. Melaugh, N.J. Phillips, A.A. Campagnari, R. Karalus, and B.W. Gibson, *J. Biol. Chem.*, 267 (1992) 13434–13439.
[11] N.J. Phillips, M.A. Apicella, J.M. Griffiss, and B.W. Gibson, *Biochemistry*, 31 (1992) 4515–4526.
[12] N.J. Phillips, M.A. Apicella, J.M. Griffiss, and B.W. Gibson, *Biochemistry*, 32 (1993) 2003–2012.
[13] F.-I. Auzanneau, D. Charon, and L. Szabó, *J. Chem. Soc., Perkin Trans. 1*, (1991) 509–517.
[14] I. Backman, B. Erbing, P.-E. Jansson, and L. Kenne, *J. Chem. Soc., Perkin Trans. 1*, (1988) 889–898.
[15] E.K.H. Schweda, unpublished results.
[16] S.B. Levery and S. Hakomori, *Methods Enzymol.*, 138 (1987) 13–25.
[17] P.S. Chen, T.Y. Toribara, and H. Warner, *Anal. Chem.*, 28 (1956) 1756–1761.