The structure of a glycerol teichoic acid-like O-specific polysaccharide of *Hafnia alvei* 1205

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ABSTRACT

The O-specific polysaccharide of *Hafnia alvei* 1205 contained D-glucose, D-galactose, 2-acetamido-2-deoxy-D-glucose, 4-acetamido-4,6-dideoxy-D-glucose (Qui4NAc), glycerol, phosphate, and O-acetyl groups. On the basis of 1D and 2D shift correlated homonuclear and 13C-1H heteronuclear NMR spectroscopy, methylation analysis, Smith degradation, and dephosphorylation with hydrofluoric acid, it was concluded that the O-antigen was a partially O-acetylated teichoic acid-like polysaccharide having the following structure:

\[
\alpha-\text{D-Glc p} \\
\downarrow \\
\rightarrow 3)-\beta-\text{D-Gal p}(1 \rightarrow 3)-\alpha-\text{D-Glc pNAc}(1 \rightarrow 3)-\beta-\text{D-Qu i pNAc}(1 \rightarrow 1)-\text{Gro(3-P)} \\
\downarrow \\
\beta-\text{D-Glc pNAc} \\
\downarrow 3,6 \\
(\text{OAc})_2
\]

INTRODUCTION

The structures of the O-antigens of *Hafnia alvei* strains ATCC 13337, 2, 38, 39, 1187, and 1211 have been elucidated\(^1\) and contain repeating units that range from a disaccharide\(^5\) for that of strain 38 to a sialic acid-containing octasaccharide\(^3\) for that of strain 2.

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We now report the structure of the O-specific polysaccharide of *H. alvei* strain 1205.

**RESULTS AND DISCUSSION**

The lipopolysaccharide of *H. alvei* 1205 was isolated (2.8%) from dry bacterial cells by phenol–water extraction followed by gel filtration on Sepharose 2B. The O-specific polysaccharide (PS-I), obtained by hydrolysis of the lipopolysaccharide with aqueous 1% acetic acid (100°C, 1 h) followed by fractionation on Sephadex G-50, had [α]_D +53° (c 2, water).

The $^{13}$C-NMR spectrum (Fig. 1) indicated that PS-I lacked a strictly regular structure, most probably owing to non-stoichiometric O-acetylation (signals for COCH$_3$ at 21.5–21.7 ppm). However, the O-deacetylated polysaccharide (PS-II) had a regular structure with a pentasaccharide repeating unit. Thus, the $^1$H-NMR spectrum (Table I) contained, inter alia, signals at 4.4–5.1 (5 s, 5 H-1), 1.16 (d, $J_{5,6}$, 6.4 Hz, CHMe), and 1.95–2.07 ppm (3 s, 3 NaC). The $^{13}$C-NMR spectrum (Table II) contained, inter alia, signals at 98–104 (5 C-1), 52.5–57.4 (3 C-N), 17.4 (CMe), 23.0–23.5 (3 NOCH$_3$), and 174.2–174.9 ppm (3 NOCOCH$_3$).

The total number of $^{13}$C signals and the number of the signals for CH$_2$O groups (six as determined by using the attached proton test, including four at 61.0–62.0 ppm, and one each at 67.4 and 71.8 ppm) showed that PS-II contained five sugar residues and glycerol. The $^{31}$P-NMR spectrum of PS-II contained one signal at 0.54 ppm (s) belonging to a monophosphate ester and indicative tentatively of glycerol phosphate.

Using enzymic methods, PS-I was found to contain glucose, galactose, 2-amino-2-deoxyglucose, and O-acetyl groups in the ratios 1.0:0.44:1.7:0.6. The α configuration of each monosaccharide was established by reactions with α-glucose oxidase, α-galactose oxidase, and hexokinase, respectively. The 6-deoxy sugar was identified tentatively as 4-acetamido-4,6-dideoxyglucose (Qui$_4$NaC) by PC ($R_{Rha}$)
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<tr>
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<th>H-3</th>
<th>H-4</th>
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<td>β-β-Quip4NAc</td>
<td>4.47 (d)</td>
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<td>3.68 (t)</td>
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<td>$J_{2,3} 8.5$</td>
<td>$J_{3,4} \sim 9$</td>
<td>$J_{4,5} \sim 9$</td>
<td>$J_{5,6} 6.2$</td>
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<td>β-β-Quip4NAc</td>
<td>4.46 (d)</td>
<td>3.35 (t)</td>
<td>3.50 (dd)</td>
<td>3.54–3.61 (m)</td>
<td>1.20 (d)</td>
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<td>$J_{1,2}$ 8</td>
<td>$J_{2,3} 8.4$</td>
<td>$J_{3,4} 10.4$</td>
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<td>$J_{5,6} 5.7$</td>
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*a Additional signals: NAc at 1.95–2.07 ppm; CH$_2$O at 3.7–4.0 ppm. $^b J_{11}P \sim 10$ Hz.
0.92) after hydrolysis of PS-I (10 M HCl, 80°, 0.5 h). The content of phosphate (P) in PS-I was estimated by the method of Ames and Dubin10 to be 2.6%.

Hydrolysis of PS-II with 2 M trifluoroacetic acid (120°, 1 h) followed by GLC of the alditol acetates derived from the products revealed glycerol and the ratios of Glc, Gal, GlcN, and Qui4N to be 1.3:1:1.7:0.1. The reduced proportion of Qui4N was probably due to partial decomposition, and the structure was confirmed by GLC–MS of its derivative, which had a fragmentation identical to that10 of 4-acetamido-1,2,3,5-tetra-O-acetyl-4,6-dideoxyglucitol.

Therefore, PS-II had a repeating unit that contained 2 GlcNAc, Glc, Gal, Qui4NAc, glycerol, and phosphate.

Methylation analysis11 of PS-I gave 2,3,4,6-tetra-0-methylglucose, 3,6-di-O-methylgalactose, 4,6-dideoxy-2-O-methyl-4-methylaminoglucose, 2-deoxy-3,4,6-tri-O-methyl-2-methylaminoglucose, and 2-deoxy-4,6-di-O-methyl-2-methylaminoglucose in the ratios 1.0:0.3:0.7:1.3:1.2. The mass spectrum of the alditol acetate derived from Qui4N accorded with the fragmentation described10. These data indicated that PS-I was branched with 2,4-disubstituted Gal at the branch point, Glc and GlcNAc as branches, 3-substituted GlcNAc, and 3-substituted Qui4NAc. The presence of two branches and only one disubstituted residue (Gal) may be accounted for by the original presence of a phosphodiester linkage attached to Gal, which was partially split under the alkaline conditions of methylation. The reduced proportion of 3,6-di-O-methylgalactose was probably associated with incomplete hydrolysis of the galactose–phosphate linkage.

The 1H-NMR spectrum of PS-II was assigned by a sequential selective spin-decoupling procedure, 2D homonuclear shift-correlated spectroscopy (COSY, Fig. 2), and one-step relayed coherence transfer COSY (COSYRCT, Fig. 3). As a result, the chemical shifts and J values of the signals for H-1,2,3,4,5,6 of Qui4NAc, H-1,2,3,4,5 of Glc and α-GlcNAc, and H-1,2,3,4 of Gal and β-GlcNAc were determined (Table I). The position of the signals for H-5 of Gal and β-GlcNAc and H-2 of glycerol were clarified by using 2D 13C–1H heteronuclear shift-correlated spectroscopy (Fig. 4) that also allowed assignment of the 13C-NMR spectrum of PS-II (Table II).

In accord with the methylation analysis data, the 3JH1,H2 values (Table I) indicated the five sugar residues to be pyranosidic, and the Gal (J1,2 8.0 Hz) and Qui4NAc (J1,2 8.2 Hz) to be β. The two GlcNAc residues were distinguished by the relatively lowfield positions of the signals for H-2 at 4.04 and 3.73 ppm as compared to that of H-2 of Glc at 3.50 ppm and their correlation with the signals for C-2 in the region of carbon atoms bearing nitrogen (δ 52.5 and 56.5 ppm, respectively). The Glc was α (J1,2 3.5 Hz), one GlcNAc was α (J1,2 3.8 Hz), and one was β (J1,2 8.6 Hz).

The lowfield position of the signal for Gal H-3 and its additional splitting (J11,1p ~ 10 Hz) was indicative of phosphorylation at position 3. This conclusion was confirmed by the lowfield position (78.4 ppm) of the signal for Gal C-3 in the 13C-NMR spectrum of PS-II, as compared to that12 (74.1 ppm) when this position
Fig. 2. 2D Homonuclear shift-correlated spectrum (COSY) of the O-deacetylated polysaccharide PS-I (I, PS-II). The corresponding 1D $^1$H-NMR spectrum is displayed along the $F_2$ axis.

is unsubstituted, and splitting due to C,P coupling. Analysis of the effects of glycosylation$^{12}$ in this spectrum led to results consistent with the methylation analysis data and confirmed the modes of substitution of the sugar residues. The
Fig. 3. 2D Homonuclear shift-correlated spectrum with one-step relayed coherence transfer (COSYRCT) of PS-II (I). The corresponding 1D $^1$H-NMR spectrum is displayed along the $F_2$ axis.

glycerol was unsubstituted at position 2, since the chemical shift ($\delta$ 70.4 ppm) of the C-2 signal was not shifted downfield. The following significant inter-residue NOEs were observed for PS-II: from H-1
of Glc to H-4 of Gal, from H-1 of β-GlcNAc to H-2 of Gal, from H-1 of Gal to H-3 of α-GlcNAc, and from H-1 of α-GlcNAc to H-3 of Qui4NAc. These data accorded with the pattern of substitution established above and indicated that α-Glc and β-GlcNAc were attached to β-Gal at positions 4 and 2, respectively, and that β-Gal was linked to α-GlcNAc which, in turn, was linked to β-Qui4NAc.

No interpretable NOE results were obtained on pre-irradiation of H-1 of Qui4NAc. This residue was not phosphorylated at HO-1 (no coupling of H-1 to P). Moreover, none of the sugar residues in PS-II was 6-glycosylated (methylation analysis), but there was a signal (71.8 ppm) in the $^{13}$C-NMR spectrum for a glycosylated CH$_2$OH group, and it was concluded that Qui4NAc was linked to a CH$_2$OH group of glycerol. The second CH$_2$OH group was phosphorylated, as indicated by another downfield-shifted $^{13}$C signal for a CH$_2$OH group [67.4 ppm.

### TABLE II

$^{13}$C-NMR data $^a$ (δ in ppm)

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<th>Compound</th>
<th>C-1</th>
<th>C-2</th>
<th>C-3</th>
<th>C-4</th>
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<tr>
<td>β-β-GlcN-ac-(1 →)</td>
<td>100.7</td>
<td>56.5</td>
<td>76.6</td>
<td>71.3</td>
<td>77.2</td>
<td>61.4 $^b$</td>
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<tr>
<td>α-β-GlcN-ac-(1 →)</td>
<td>101.0</td>
<td>73.0</td>
<td>73.7</td>
<td>70.6</td>
<td>73.2</td>
<td>61.3 $^b$</td>
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<tr>
<td>→ 3)-β-β-Gal p-(1 →)</td>
<td>100.7</td>
<td>75.1 $^c$</td>
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<td>77.8</td>
<td>75.7</td>
<td>62.0 $^b$</td>
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<tr>
<td>→ 3)-α-β-GlcN-ac-(1 →)</td>
<td>98.0</td>
<td>52.5</td>
<td>80.8</td>
<td>68.8</td>
<td>72.0</td>
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<td>103.7</td>
<td>73.4</td>
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<td>→ 1)-Gro-(3 →)</td>
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<td>70.4 $^c$</td>
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<td>53.3</td>
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<td>70.5 $^c$</td>
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<td>→ 3)-β-β-Qui4N-ac-(1 →)</td>
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<td>78.9</td>
<td>57.6</td>
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<tr>
<td>→ 1)-Gro</td>
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<tr>
<td>Oligosaccharide 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-β-GlcN-ac-(1 →)</td>
<td>99.4</td>
<td>54.7</td>
<td>71.8</td>
<td>70.5</td>
<td>72.6</td>
<td>61.0 $^b$</td>
</tr>
<tr>
<td>→ 3)-β-β-Qui4N-ac-(1 →)</td>
<td>103.5</td>
<td>73.5</td>
<td>78.8</td>
<td>57.6</td>
<td>72.2</td>
<td>17.4</td>
</tr>
<tr>
<td>→ OCH$_2$CH$_2$OH</td>
<td>72.1</td>
<td>61.7 $^b$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Assignment of the spectrum of PS-II was made with the help of 2D heteronuclear $^1$H–$^{13}$C shift-correlated (XHCOORD) spectroscopy. Tentative assignments of the spectra of PS-III (2), 3, and 4 were based on comparison with the spectrum of PS-II (1) and published data.$^{10}$ Additional signals: 23.0–23.5 (NHCOCH$_3$) and 174.2–175.9 ppm (NHCOCH$_3$). $^b$ Assignments could be interchanged. $^c$ The signal was split due to coupling to phosphorus.
Fig. 4. 2D Heteronuclear $^{13}$C-$^1$H shift-correlated spectrum (XH CorrD) of PS-II (1). The corresponding 1D $^{13}$C- and $^1$H-NMR spectra are displayed along the $F_2$ and $F_1$ axes, respectively.

cf. 63.8 ppm for the unsubstituted CH$_2$OH (ref. 13) and 67.5 ppm in $\rightarrow 1$-Gro-(3-P $\rightarrow$ (ref. 14)], and the splitting of this signal and that for C-2 of glycerol was due to coupling to phosphorus.

Therefore, it was concluded that PS-II had structure I.

\[
\begin{align*}
\alpha-\text{d-Glc}p \\
\downarrow \\
(1 \rightarrow 3)-\beta-\text{d-Gal}p-(1 \rightarrow 3)-\alpha-\text{d-Glc}p\text{NAc}-(1 \rightarrow 3)-\beta-\text{d-Qui}p4\text{NAc}-(1 \rightarrow 1)-\text{Gro}(3-P \rightarrow 2) \\
\uparrow \\
\beta-\text{d-Glc}p\text{NAc}
\end{align*}
\]

I (PS-II)

The structure I was confirmed by selective cleavages of PS-II. Thus, Smith degradation resulted in a polymeric product (2, PS-III) with a trisaccharide repeating unit that contained Gal, GlcNAc, Qui4NAc, glycerol, and phosphate.
Dephosphorylation of PS-II with aqueous 48% hydrofluoric acid also split off \( \beta\)-GlcNAc and gave a tetraosyl-glycerol (3). Two successive Smith degradations of 3 gave a biosyl-(ethylene glycol) (4) and a glycosyl-(ethylene glycol) (5), respectively. The structures of 2–5 were established, as described above for PS-II, by 1D and 2D \(^1\)H-NMR spectroscopy (Table I), including NOE experiments, and confirmed by the \(^{13}\)C-NMR data (Table II).

\[
\rightarrow 3\)-\( \beta\)-d-Gal\( ^1\)p-(1 \( \rightarrow \) 3)-\( \alpha\)-d-Glc\( ^1\)pNAc-(1 \( \rightarrow \) 3)-\( \beta\)-d- Qui\( ^1\)p4NAc-(1 \( \rightarrow \) 1)-Gro-(3-P \( \rightarrow 
2 \)

(PS-III)

\[
\begin{align*}
\alpha\)-d-Glc\( ^1\)p \\
\downarrow
\beta\)-d-Gal\( ^1\)p-(1 \( \rightarrow \) 3)-\( \alpha\)-d-Glc\( ^1\)pNAc-(1 \( \rightarrow \) 3)-\( \beta\)-d- Qui\( ^1\)p4NAc-(1 \( \rightarrow \) 1)-Gro
\end{align*}
3

\[
\alpha\)-d-Glc\( ^1\)pNAc-(1 \( \rightarrow \) 3)-\( \beta\)-d- Qui\( ^1\)p4NAC-(1 \( \rightarrow \) OCH\(_2\)CH\(_2\)OH
4

\beta\)-d- Qui\( ^1\)p4NAc-(1 \( \rightarrow \) OCH\(_2\)CH\(_2\)OH
5

The formation of PS-III (2) and 3–5 and their structures were consistent with the structure (1) established above for PS-II. The relatively large \( ^3J_{HH} \) values (8–10 Hz), determined from the \(^1\)H-NMR spectrum of PS-III, confirmed that Qui4NAc had the \( \text{gluco} \) configuration. The absolute configuration of Qui4NAc was determined as follows. The signal (57.6 ppm) for C-4 of the 3-substituted Qui4NAc in the \(^{13}\)C-NMR spectrum of 4 was shifted due to the \( \beta \)-effect of glycosylation by only 0.35 ppm (cf. 57.95 ppm, which is characteristic\(^\text{10} \) of the unsubstituted Qui4NAc). This relatively small \( \beta \)-effect indicated that the (1 \( \rightarrow \) 3)-linked \( \alpha\)-d-GlcNAc and Qui4NAc in 4 had the same absolute configuration (a shift > 1 ppm would be expected\(^\text{12} \) if the absolute configurations were different); hence, Qui4NAc was a \( \text{d} \) sugar.

The positions of the O-acetyl groups in PS-I were determined from the \(^{13}\)C-NMR spectrum. The signals for COCH\(_3\) in the region 21.5–21.7 ppm (Fig. 1) showed that the repeating unit contained at least two OAc groups, one of which was located at position 6 of a sugar residue as indicated by the downfield position (64.7 ppm) of one C-6 signal as compared to that for PS-II. The signals for C-3,4,5 of \( \beta \)-GlcNAc were split due to the presence of O-acetylated and non-O-acetylated forms, whereas the signals for the other sugar residues had chemical shifts similar to those of the corresponding resonances for PS-II, except those for C-1 of \( \beta \)-Gal and C-3 of \( \alpha\)-GlcNAc which were close to the site of O-acetylation. Therefore, one OAc group was at position 6 of \( \beta\)-GlcNAc. The presence, as a result of the
The effect of O-acetylation\textsuperscript{15}, of a minor signal at 53.6 ppm and a corresponding decrease in the intensity of the signal at 55.1 ppm (C-2 of β-GlcNAc) indicated that the second OAc group was attached to position 3 of β-GlcNAc.

The location of the OAc groups at positions 3 and 6 of β-GlcNAc was confirmed by the presence of at least three signals for H-1 of the β-GlcNAc residue at 4.87, 4.89, and 4.93 ppm in the $^1$H-NMR spectrum of PS-I (not shown), which corresponded to the presence of non-acetylated and two O-acetylated forms of this residue (PS-II gave only one signal for H-1 of β-GlcNAc at 4.93 ppm. The chemical shifts for the H-1 resonances of the other residues were the same (Qui4NAc) or differed by ≤ 0.02 ppm. On the basis of the integrated intensities of the appropriate $^1$H and $^{13}$C signals for PS-I, the degrees of O-acetylation of β-GlcNAc at positions 3 and 6 were ~10 and ~40%, respectively.

Thus, it was concluded that the O-specific polysaccharide of \textit{H. alvei} 1205 had the structure 6.

\[
\begin{align*}
\alpha-\delta-Glc p \\
\quad 1 \\
\quad 4 \\
\rightarrow 3)-\beta-\delta-Gal p-(1 \rightarrow 3)-\alpha-\delta-Glc p NAc-(1 \rightarrow 3)-\beta-\delta-Qu i p 4 NAc-(1 \rightarrow 1)-Gro-(3-P \rightarrow \\
\quad 2 \\
\quad 1 \\
\beta-\delta-Glc p NAc \\
\quad 1 \quad 3, 6 \\
\quad \text{OAc}_2 \\
\end{align*}
\]

6 (PS-I of \textit{H. alvei} 1205)

\[
\begin{align*}
\rightarrow 4)-\beta-\delta-Gal p-(1 \rightarrow 3)-\alpha-\delta-Glc p NAc-(1 \rightarrow 3)-\beta-\delta-Qu i p 4 NAc-(1 \rightarrow 2)-\alpha-\delta-Man p-(1 \rightarrow \\
\quad 3 \\
\quad \uparrow \\
\quad 1 \\
\alpha-L-Rhap \\
\end{align*}
\]

7 (\textit{E. coli} O:7)

The teichoic acid-like structure 6 is uncommon for bacterial O-antigens. To the best of our knowledge, the only reported O-antigens of this type are those of several \textit{Yersinia kristensenii} serotypes, which have hexasaccharide repeating units connected via 2-substituted glycerol 1-phosphate\textsuperscript{16-18}.

Another chemical feature of PS-I (6) is the presence of the rare sugar 4-acetamido-4,6-dideoxy-\textit{d}-glucose. The first polysaccharide shown\textsuperscript{10} to contain this monosaccharide was the O-antigen of \textit{Escherichia coli} O:7, the structure 7 of which has the trisaccharide fragment β-\textit{d}-Galp-(1 → 3)-α-\textit{d}-Glc p NAc-(1 → 3)-β-\textit{d}-Qui p 4 NAc in common with PS-I (6). Various 2-amino-2,6-dideoxy-, 3-amino-3,6-dideoxy-, and 4-amino-4,6-dideoxy-hexoses have been identified\textsuperscript{14-19} in strains 23, 1204, 1211, 1216, and 1220 of \textit{H. alvei}, and that in strain 1211 has been identified\textsuperscript{4} as 3,6-dideoxy-3-\{(R)-3-hydroxybutyroamido\} \textit{d} galactose.
EXPERIMENTAL

*General methods.*—Optical rotations were measured with a Jasco DIP 360 polarimeter for solutions in water at 25°. PC was carried out using 6:4:3 1-butanol–pyridine–water. GLC was performed with a Hewlett-Packard 5890 instrument equipped with a flame-ionisation detector and a glass capillary Ultra 1 column (0.2 mm × 25 m). GLC–MS was performed with a Hewlett-Packard 5971 A system, using an HP-1 glass capillary column (0.2 mm × 12 m) and a temperature program of 150 → 270° at 8°/min. Gel-permeation chromatography was performed on a column (2 × 100 cm) of Sephadex G-50 in pyridine–acetic acid buffer (pH 5.75) or on a column (1.6 × 80 cm) of Fractogel TSK HW 40(S) in water, and eluates were monitored by the phenol–H$_2$SO$_4$ method$^{20}$ or with a Knauer differential refractometer.

*Hafnia alvei* strain 1205, from the collection of the Pasteur Institute (Paris), was grown in a liquid medium as described$^{21}$.

*NMR spectroscopy.*—The $^1$H-NMR and NOE spectra were recorded with a Bruker WM-250 instrument for solutions in D$_2$O at 30° (internal acetone, δ 2.23). Sequential, selective spin-decoupling experiments were performed as described$^{22}$. The 1D NOE spectra were obtained using the Bruker NOEMULT program in the difference mode where the on-resonance irradiated spectrum was subtracted from that in which the irradiation frequency was off resonance.

The 2D homonuclear shift-correlated spectrum (COSYHG) and one-step relayed coherence transfer shift-correlated spectrum (COSYRCTG) of PS-II were obtained with suppression of the peak for HDO under the following conditions: 90° pulse of 5.7 μs, the spectral width was 475 Hz, and the spectral size in the time domain was 512 (F$_2$) × 256 (F$_1$). For each $t_1$, 64 transients were accumulated, the relaxation delay D1 was 1 s, and D2 was 80 μs for COSYHG and 32 μs for COSYRCTG. The matrix was zero-filled in each dimension, multiplied by an unshifted sine-bell window function, and Fourier transformed in the magnitude mode.

The $^{13}$C-NMR spectra were recorded with a Bruker AM-300 instrument for solutions in D$_2$O at 60° (internal acetone, δ 31.45).

The 2D heteronuclear $^{13}$C–$^1$H shift correlated spectrum (XHCORRD) of PS-II was obtained under the following conditions: 90° pulses of 25 μs for $^1$H and 14 μs for $^{13}$C, the time domain in F$_2$ was 2K, 64 spectra were collected with 1000 scans, the spectral windows were 4500 Hz in the F$_2$ domain and 600 Hz in the F$_1$ domain (the region for the resonance of ring carbons and protons only), the relaxation delay D1 was 0.6 s, and D3 and D4 were 3.3 μs and 2.2 μs, respectively. The matrix was zero-filled in each dimension, multiplied by a phase-shifted (π/2) squared sine-bell window function, and Fourier transformed in the magnitude mode.

*O-Deacetylation of PS-I.*—Aqueous 12% ammonia was used at room temperature overnight and the O-deacetylated polysaccharide (1, PS-II, 90%) was isolated by gel-permeation chromatography on TSK HW 40.
Dephosphorylation of PS-II. — Aqueous 48% hydrofluoric acid was used at room temperature for 48 h, the solution was concentrated in vacuo at room temperature over solid NaOH, and the oligosaccharide 3 (20%) was isolated by gel-permeation chromatography on TSK HW 40.

Smith degradation. — Oligosaccharide 3 (8 mg) was treated with 0.1 M NaIO₄ (1 mL) at room temperature in the dark for 24 h, and the product was reduced with NaBH₄ (15 mg) for 2 h, neutralised with concd HClO₄, desalted by gel-permeation chromatography on TSK HW 40, and hydrolysed with aq 1% HClO₄ (1 mL) at 100° for 1 h to give oligosaccharide 4 (80%), isolated by gel-permeation chromatography on TSK HW 40. In a similar manner, 4 was converted into the glycoside 5 (90%), [a]₀ +4.2°, and PS-II (I) into PS-III (2, 70%).

REFERENCES

19 E. Katzenellenbogen and E. Romanowska, unpublished data.