Structural Determination of the Capsular Polysaccharide Antigen of Type II Group B Streptococcus*

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The native polysaccharide antigen isolated from type II group B Streptococcus contains D-galactose, D-glucose, 2-acetamido-2-deoxy-D-glucose, and sialic acid in the molar ratio of 3:2:1:1. The application of methylation and 13C nmr spectroscopic analyses to the native type II antigen and some of its specifically degraded products enabled the structure of the native type II antigen to be elucidated. The native type II antigen is composed of the following repeating unit

\[
\beta-D-Galp(1 \rightarrow 4) \beta-D-Glep(1 \rightarrow 3) \beta-D-Glep(1 \rightarrow 2) \beta-D-Galp(1 \rightarrow 3) \beta-D-GlepNAC(1 \rightarrow 3) \beta-D-GlepNAC(1 \rightarrow 2) \beta-D-Galp(1 \rightarrow 3) \beta-D-GlepNAC(1 \rightarrow 2) \beta-D-Galp(1 \rightarrow 3) \beta-D-GlepNAC(1 \rightarrow 2) \beta-D-Galp(1 \rightarrow 3) \beta-D-GlepNAC(1 \rightarrow 2) \beta-D-Galp(1 \rightarrow 3)
\]

Unlike the native types Ia, Ib, and III group B streptococcal polysaccharides, the native type II antigen contains terminal \(\beta-D-galactopyranosyl\) residues in addition to terminal sialic acid residues. The terminal sialic acid residues of the native type II antigen are also unique among those of the other group B streptococcal antigens in that they are not neuraminidase-sensitive and are attached directly to the backbone of the type II antigen.

Lancefield (1–3) characterized the type-specific antigens (types Ia, Ib, II, and III) of group B Streptococcus in early immunological studies. The type-specific antigens were polysaccharides and were originally isolated by hot hydrochloric acid extractions of the whole organisms which produced immunologically complete antigens. The incomplete antigens form a lower molecular weight core to the complete native antigens (4–12). This was first demonstrated for the type II polysaccharide antigen in 1966 when Lancefield and Friemer (4) found that the extraction of type II organisms using milder acidic conditions (trichloroacetic acid) yielded a more immunologically complete antigen. It was postulated (4, 11) that this was due to the presence of an acid-labile component in the original type II native polysaccharide. Since that time, the complete native types Ia, Ib, and II, and III polysaccharides have been isolated using neutral or buffered (pH 7.0) extractions of the whole organisms (7, 8, 11–13) and terminal sialic acid residues have been identified in them all (7–10). This paper describes the extraction of the complete native type II polysaccharide using these latter conditions and also describes some definitive degradation procedures which led to its structural elucidation. Unlike type Ia (10, 15), Ib (15), and III (9) native polysaccharides, the native type II antigen contains terminal \(\beta-D-galactopyranosyl\) residues in addition to terminal sialic acid residues.

**EXPERIMENTAL PROCEDURES**

Growth of the organism. Prototype strain 18RS21 type II group B Streptococcus was kindly supplied by the late Dr. Rebecca Lancefield, Rockefeller University. The lyophilized strain was

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1 "Experimental Procedures" are presented in miniprint as pre-
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conditions Z-amino-1-deoxy-D-glucose is converted to 1,2-oxido-D-mannose. The solution was dialyzed against 0.2 M sodium phosphate buffer, pH 7.0, against ethanol (90:10), and was lyophilized. The solution was analyzed by GC-MS using column I following the conversion of the glycosides to their trimethylsilyl derivatives. The solution was assigned to both the galactose and glucose residues by GC analysis. The analysis and analogous assignment of the core group B Streptococcus contained oxidase.

The methylated polysaccharides were recovered by chloroform extraction of an aqueous solution of the methylated mixture with subsequent concentration of the chloroform solution and after adjustment of the aqueous solution, and the methylated oligosaccharides were recovered by chloroform extraction. The methylated oligosaccharides were assigned to both the galactose and glucose residues by GC analysis using column I. The mass-to-charge ratio of the 2.8:2.0:1.0. The b-configuration was assigned to the galactose and glucose constituents by virtue of the characteristic retention times of their tetraacetylated (+)-2-octanol glycosides (19) and to the 2-acetamido-2-deoxyglucosyl constituent by virtue of the characteristic retention time of its trimethylsilylated derivative (17). From the low value of the optical rotation of the type II core antigen (c2, +2° c, 1.0 in water), it could be deduced that all of its glucose constituents were in the b-configuration. This was also consistent with the 1H nmr spectrum of the type II core antigen which exhibited three distinguishable anomeric signals representing five anomeric carbons at 104.5, 103.8, and 102.8 ppm. The value of these chemical shifts is consistent with their all having the b-configuration (31).

The type II core antigen was permethylated and on subsequent hydrolysis yielded a number of partially methylated sugars which were quantified by GC analysis and identified by GC-MS analysis (23, 24). The individual methylated sugars detected in this analysis and their respective molar ratios are shown in Table 1. The methylation analysis indicated the presence of terminal b-D-galactopyranosyl residues, two interchain b-D-galactopyranosyl residues, one linked at O-3 and one at O-4, and interchain b-D-galactopyranosyl and 2-acetamido-2-deoxyglucosyl residues linked at O-2 and O-6 respectively. The presence of one branched b-D-galactopyranosyl residue linked both at O-3 and O-6 was also indicated. The methylation analysis is consistent with the structure of the type II core antigen shown in Fig. 1 and confirmation of this structure was obtained from further degradation experiments on both the native and core type II polysaccharides. The linkage analysis was confirmed and some evidence of the sequence of sugar components in the type II core polysaccharide was obtained using a modification of the Smith degradation procedure (9, 10). In this degradation, the polycarboxylate obtained following periodate oxidation and sodium borohydride reduction of the polysaccharide was methylated and the permethylated polymer was partially hydrolyzed. The products of this partial hydrolysis were then further methylated using rideuomethyl iodide. By this procedure, labeled methyl groups were introduced at exposed hydroxyl groups, providing sites of linkage positions in the original type II core antigen. The major product of this procedure detected by GC analysis was the methylated oligosaccharide shown in Fig. 2. The oligosaccharide was subjected to GC-MS analysis and the mass spectrum showed the following major fragments: mass-to-charge ratio m/e 45, 71, 74, 92, 101, 104, 111, 115, 150, 210, 231, and 263. The sequence of the sugars in the methylated oligosaccharide (Fig. 2) was elucidated from the masses 263 and 150, the erythritol residue of the oligosaccharide being derived from the 4-O-b-D-galactopyranosyl residue in the original polysaccharide. This evidence indicated that the major type II core antigen contained a b-D-GlcNAcp-(1→3)-b-D-Galp-(1→4)-b-D-Glpk trisaccharide. The labeled erythritol derivative was detected following further hydrolysis of the labeled methylated products of the partial hydrolysis. The final hydrolysis yielded the individual methylated sugars which were analyzed as their alditol acetate derivatives by GC-MS using sodium boroduteride to label their reducing hemiacetal carbons. The individual methylated sugars detected are shown in Table II together with those obtained by the direct hydrolysis of the methylated polycarboxylate obtained from the Smith degradation of the type II core antigen. In addition to the anticipated labeled methylated sugars derived from oligosaccharide (Fig. 2), a further fully methylated glucopyranosyl residue labeled at O-3 was detected. This was

RESULTS

Structure of the Core Type II Antigen — The type II acid-extracted core antigen of group B Streptococcus contains galactose, glucose, and 2-acetamido-2-deoxyglucose in the molecular ratio of 2.8:2.0:1.0. The b-configuration was assigned to the galactose and glucose constituents by virtue of the characteristic retention times of their tetraacetylated (+)-2-octanol glycosides (19) and to the 2-acetamido-2-deoxyglucosyl constituent by virtue of the characteristic retention time of its trimethylsilylated derivative (17). From the low value of the optical rotation of the type II core antigen (c2, +2° c, 1.0 in water), it could be deduced that all of its glucose constituents were in the b-configuration. This was also consistent with the 1H nmr spectrum of the type II core antigen which exhibited three distinguishable anomeric signals (representing five anomeric carbons) at 104.5, 103.8, and 102.8 ppm. The value of these chemical shifts is consistent with their all having the b-configuration (31).

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**Type II Group B Streptococcus Antigen Structure**

<table>
<thead>
<tr>
<th>Methylation analysis of the native and modified native type II group B streptococcal polysaccharides</th>
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<tbody>
<tr>
<td>Methylated glucose derivatives</td>
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<tr>
<td>-------------------------------</td>
</tr>
<tr>
<td>2,3,4,6-Tetra-O-methyl-D-galactose</td>
</tr>
<tr>
<td>2,4,6-Tri-O-methyl-D-glucose</td>
</tr>
<tr>
<td>2,4,6-Tri-O-methyl-D-galactose</td>
</tr>
<tr>
<td>3,4,6-Tri-O-methyl-D-galactose</td>
</tr>
<tr>
<td>2,3,6-Tri-O-methyl-D-glucose</td>
</tr>
<tr>
<td>4,6-Di-O-methyl-D-galactose</td>
</tr>
<tr>
<td>2,4-Di-O-methyl-D-galactose</td>
</tr>
<tr>
<td>3,6-Di-O-methyl-N-methyl-N-acetyl-D-glucosamine</td>
</tr>
<tr>
<td>4,7,8,9-Tetra-O-methyl-N-methyl-N-acetyl-D-neuraminic acid</td>
</tr>
</tbody>
</table>

* Retention times of the alditol acetates relative to 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-D-glucitol.
* +, slight nonquantitative response; -, not detected.
* Identified as alditol acetates on column iv.
* Identified as the methyl ester methyl glycoside on column iv.

Fig. 1. Structure of the native (top), core (center), and backbone (bottom) type II polysaccharide antigens of group B Streptococcus.

![Structure of the native (top), core (center), and backbone (bottom) type II polysaccharide antigens of group B Streptococcus.](http://www.jbc.org/)

derived from the periodate-stable 3-O-β-D-linked glucopyranosyl residue of the original type II core antigen.

The above evidence is consistent with the structure of the type II core antigen being represented by the repeating unit shown in Fig. 1. However, this structure could not be verified definitively until further degradation procedures including the removal of the terminal β-D-galactopyranosyl residues from the type II core antigen had been carried out.

**Removal of the Terminal β-D-Galactopyranosyl Residues from the Type II Core Antigen**—An attempt was made to remove the terminal β-D-galactopyranosyl residues from the type II core antigen using an enzymatic procedure previously described for the type III core antigen (32). In this procedure, the primary hydroxyl groups of the terminal β-D-galactopyranosyl residues of the type III core were specifically oxidized to aldehyde groups using galactose oxidase. This rendered the terminal residues susceptible to basic elimination and in the eliminated form to their subsequent facile removal using mild acidic conditions. This method did not prove to be satisfactory for the type II core polysaccharide due in large part to the unusual extensive overoxidation of this polysaccharide by D-galactose oxidase. Two of the polysaccharide β-D-galactopyranosyl residues were fairly rapidly oxidized, and they were identified as the terminal and 2-O-β-D-galactopyranosyl residues in the following experiment. An aliquot of the reaction mixture was removed after only 15 min of oxidation and the polysaccharide containing the aldehyde groups was isolated and reduced with sodium borodeuteride in order to selectively label the oxidized galactose residues. The labeled polysaccharide was subjected to a methylation analysis and the methylated sugar components bearing deuterium labels at 0-6 were identified as 2,3,4,6-tetra-O-methyl-D-galactose and 3,4,6-tri-O-methyl-D-galactose in the molar ratio of 9:1, respectively.

The preferred method of selectively removing the terminal β-D-galactopyranosyl residues from the type II core antigen was by controlled periodate oxidation of the native type II polysaccharide. The core type II polysaccharide could not be used due to the susceptibility of the interchain 2-O-β-D-galactopyranosyl residue to periodate oxidation. In the oxidation of the native type II antigen, both the terminal β-D-galactopyranosyl and sialic acid residues were oxidized, and the remnants of both were removed following reduction and mild acid hydrolysis of the oxidized native type II polysaccharide.
The methylation analysis of the degraded type II antigen is shown in Table I and in comparison with that of the type II core antigen indicates the absence of 2,3,4,6-tetra-O-methyl-d-galactose and 3,4-di-O-methyl-d-galactose among its methylated components and the appearance of a new 2,4,6-tri-O-methyl-d-galactose component. This established that the degraded type II polysaccharide forms a linear backbone, the structure of which is shown in Fig. 1, to both the native and core type II antigens and that the terminal β-D-galactopyranosyl residues are linked directly to O-6 of the interchain 3-O-β-D-galactopyranosyl residues of the type II core antigen. Thus, the presence of the tetrasaccharide sequence shown in Fig. 3 can be established in the type II core antigen.

Structure and Sequence of the Native Type II Antigen—
The native type II antigen has \([\alpha]_D -2^{2}$ (c, 1.0 in water) and contains d-galactose, d-glucose, 2-acetamido-2-deoxy-d-glucose, and sialic acid in the molar ratio of 2.8:1.9:1.0:0.9. The sialic acid was estimated by the thiobarbituric method of Aminoff (21) following its removal from the type II native polysaccharide by mild acid hydrolysis. Using readily available neuraminidases, all attempts to remove the sialic acid residues were unsuccessful, even after prolonged incubation periods and the addition of further enzyme (33). The α-configuration was assigned to the sialic acid residues by virtue of the characteristic chemical shifts (174.8 ppm) of their carboxylate signals (34) in the $^1$C nmr spectrum of the type II native antigen.

To complete the structure of the type II native antigen, it was necessary to locate the position of linkage of the additional sialic acid residues to the type II core structure, and to elucidate the remaining undetermined sequence data. Methylation analysis of the native type II antigen established that all the sialic acid residues were present as single nonreducing residues because in the methanalysis of the permethylated native antigen only fully methylated derivatives of sialic acid (25) were detected (Table I). Hydrolysis of the permethylated native antigen also yielded a number of other partially methylated sugars (Table I). The difference between these partially methylated sugars and those yielded by the type II core antigen clearly demonstrated the position of linkage of the terminal sialic acid residues. The methylated native type II antigen contained 4,6-di-O-methyl-d-galactose residues which replaced the 3,4,6-tri-O-methyl-d-galactose residues of the core antigen (Table I). This is indicative of the sialic acid residue being linked to O-3 of the core interchain 2-O-β-D-galactopyranosyl residues. This evidence established a further structural feature of the native type II antigen which together with the tetrasaccharide sequence established previously accounted for six of the seven components in the repeating unit of the native antigen (Fig. 1). This left only the location of the 3-O-β-D-glucopyranosyl residue to be established. This was accomplished by subjecting the native type II antigen to the same modification of the Smith degradation procedure previously used on the core type II antigen. The polyalcohols obtained from the complete periodate oxidation of the native type II antigen was methylated and the methylated polymer was partially hydrolyzed. The products of the partial hydrolysis were then remethylated using trideuteriomethyl iodide. Hydrolysis of this methylation mixture yielded a number of methylated labeled and unlabeled sugars which are listed in Table II. The identification of 2,3,4,6-tetra-O-methyl-d-glucose labeled at O-3 is only consistent with the structure of the native type II antigen shown in Fig. 1. Because of the stability of periodate oxidation of the 2-O-β-D-galactopyranosyl residues of the type II native antigen, the alternate location of the 3-O-β-D-glucopyranosyl residues between the former residue and the 2-acetamido-2-deoxy-4-O-β-D-glucopyranosyl residues would have resulted in a different labeling pattern. In this case, 2,3,4,6-tetra-O-methyl-d-galactose doubly labeled at O-2 and O-3 would have been detected instead of the fully methylated glucose derivative labeled at O-3.

![Fig. 2. Permethylated reduced trisaccharide from the type II core antigen obtained after Smith degradation, methylation, mild acid hydrolysis, and remethylation with trideuteriomethyl iodide. Some characteristic fragments in its mass spectrum are indicated by broken lines.](image-url)

### Table II

<table>
<thead>
<tr>
<th>Methylated glucose derivatives</th>
<th>Relative retention times* using columns</th>
<th>Molar ratios†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(i)</td>
<td>(ii)</td>
</tr>
<tr>
<td>2,3*,4,6-Tetra-O-methyl-d-glucose</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>2,4,6-Tri-O-methyl-d-glucose</td>
<td>1.95</td>
<td>1.42</td>
</tr>
<tr>
<td>2,4,6-Tri-O-methyl-d-galactose</td>
<td>2.28</td>
<td>2.03</td>
</tr>
<tr>
<td>4,6-Di-O-methyl-d-galactose</td>
<td>3.00</td>
<td>3.20</td>
</tr>
<tr>
<td>2,4-Di-O-methyl-d-galactose</td>
<td>6.40</td>
<td>5.10</td>
</tr>
<tr>
<td>3,6-Di-O-methyl-N-methyl-N-acetyl-d-glucosamine</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3,4,6-Tri-O-methyl-N-methyl-N-acetyl-d-glucosamine</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>1,3*,4-Tri-O-methyl-d-erythritol</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

* Retention times of the alditol acetates relative to 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-d-glucitol.
† +, slight nonquantitative response; –, not detected.
‡, deuterium-labeled methyl group (–ODJ).
§, identified as alditol acetates on column iv.

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Type II Group B Streptococcus Antigen Structure

\[
\beta-D-GlcnAcp(1 \rightarrow 3) \beta-D-Galp(1 \rightarrow 4) \beta-D-Glcp \xrightarrow{6 \rightarrow 1} \beta-D-Galp
\]

Fig. 3. Structure of the part of the type II core antigen from which the permethylated reduced trisaccharide shown in Fig. 2 was obtained.

DISCUSSION

Types Ia (10, 15), Ib (7, 15), II, and III (9), acid-extracted incomplete core antigens of group B Streptococcus all contain D-galactose, D-glucose, and 2-acetamido-2-deoxy-D-glucose as their only sugar components. However, while types Ia, Ib, and III, contain these respective components in the ratio of 2:1:1, the type II core antigen contains them in the molar ratio of 3:2:1. The type II core antigen contains two structural features common to all the group B streptococcal incomplete core antigens, namely, terminal β-D-galactopyranosyl residues and a β-D-GlpNAc-(1 \rightarrow 3)-β-D-Galp-(1 \rightarrow 4)-β-D-GlcP trisaccharide unit. In the types II and III core antigens, this trisaccharide is linearly disposed in the backbone of the polysaccharide and it constitutes the repeating unit of the type III backbone (9). In the types Ia and Ib core antigens, this trisaccharide is not linearly disposed, the 2-acetamido-2-deoxy-D-glucopyranosyl residues forming the penultimate residues of disaccharide branches (15). Despite structural similarities, the type Ia, Ib, II, and III core antigens retain their serological specificity, being the antigens responsible for Lancefield’s typing system (1–9). However, cross-reactions involving types Ia and Ib have been reported which have been attributed to the lab determinants (2, 7, 15). Recently, using sensitive radioimmunoassay methods for antibody detection and quantification, more extensive cross-reactivity among all the acid-extracted core antigens (types Ia, Ib, II, and III) has been detected.

To obtain the sequence of sugars in the type II backbone, it was necessary to remove the terminal β-D-galactopyranosyl residues from the core antigen. This had been accomplished in the type III core antigen by the oxidation of its terminal β-D-galactopyranosyl residues using D-galactose oxidase followed by the basic elimination of the oxidized residues, and the facile cleavage of these eliminated residues by mild acid (32). For the types Ia and Ib core antigens, removal of the terminal β-D-galactopyranosyl residues was accomplished by an improved procedure involving a Smith degradation in which only their terminal β-D-galactopyranosyl residues were selectively oxidized with periodate (15). However, the selectivity of neither of the above oxidation procedures was maintained on the type II core antigen because of its backbone 2-O-β-D-galactopyranosyl residues. These residues proved to be almost if not equally as sensitive to both methods of oxidation as the terminal β-D-galactopyranosyl residues. The ability of D-galactose oxidase to oxidize the backbone 2-O-β-D-galactopyranosyl residues indicated that substitution at O-2 of an interchain β-D-galactopyranosyl residue does not significantly inhibit the action of D-galactose oxidase. This is probably due to the fact that the hydroxyl at C-2 of this residue is not essential for the enzymatic activity, which is consistent with the established sensitivity of a series of 2-substituted monomeric β-D-galactopyranosides to oxidation by D-galactose oxidase (27).

Because the 2-O-β-D-galactopyranosyl residues of the type II core antigen were as sensitive to periodate oxidation as its terminal β-D-galactopyranosyl residues, the selective removal of these latter residues could not be achieved by the modified Smith degradation procedure previously employed for the selective removal of terminal β-D-galactopyranosyl residues from the type Ia and Ib core antigens (15). However, in the type II native polysaccharide, these interchain 2-O-β-D-galactopyranosyl residues are rendered stable to periodate oxidation by having additional sialic acid residues attached to their O-3 positions. Therefore, the type II backbone antigen could be obtained by the standard Smith degradation procedure of the type II native antigen. It is interesting to note that while the type II backbone antigen contains the same linearly disposed β-D-GlcPNAc-(1 \rightarrow 3)-β-D-Galp-(1 \rightarrow 4)-β-D-GlcP trisaccharide which constitutes the repeating unit of the type III backbone antigen, it also contains an additional β-D-GlcP(1 \rightarrow 2)-β-D-Galp disaccharide unit. It is also interesting to note that by simply replacing the 3-O-β-D-glucopyranosyl residue of the pentasaccharide repeating unit of the type II backbone antigen with a 3-O-β-D-galactopyranosyl residue one obtains the backbone of the specific capsular polysaccharide of type 15F Streptococcus pneumoniae (35).

All the galp B streptococcal native polysaccharide antigens have terminal sialic acid residues. In types Ia and Ib, they form the terminal residues of trisaccharide branches (15), in type III, they form the terminal residues of disaccharide branches (9), and in type II, they are linked directly to the backbone. The type II native polysaccharide is also unique among the native group B streptococcal polysaccharides in that it has additional terminal β-D-galactopyranosyl residues. Lancefield and Friemer (4) demonstrated that the native type II polysaccharide produced antibodies with a specificity for both the core and native polysaccharides, and in inhibition experiments, Friemer (11) showed that the core specificity probably involved determinants having terminal galactose residues. Antibodies with specificities for the native types Ia (10) and III (9, 32) antigens are dependent on terminal sialic acid residues, although these residues are only immunodominant in the sense that they exert control over the determinants responsible for these specificities. For the native type III antigen, the terminal sialic acid residues exert conformational control over this determinant (32), and preliminary serological evidence would suggest that terminal sialic acid residues perform the same function in the type II native antigen.

While the terminal sialic acid residues of types Ia (10), Ib (7), and III (9) native polysaccharides are readily removed by treatment with bacterial neuraminidases, those of the type II native antigen are resistant to both viral and bacterial neuraminidases, this despite the fact that 13C nmr studies indicate that the sialic acid residues are in the α-D-configuration (31) and are free of O-acetyl substituents (36), characteristics that are favorable for the removal of terminal sialic acid residues by neuraminidase (33). The resistance associated with the type II native antigen could be attributed to steric hindrance to the approach of the enzyme due to the sialic acid residues being attached directly to its backbone. Another contributing factor, however, could be that the sialic acid is linked to O-3 of a backbone β-D-galactopyranosyl residue which also has a vicinal β-D-glucopyranosyl substituent at O-2. In consequence, the vicinal linkages of these two sugar constituents to the same β-D-galactopyranosyl residue place them in very close proximity to each other. The situation is similar to that of the neuraminidase-resistant GM1 ganglioside (II2 NeuAc-GgOseCer) (37) in which the terminal sialic acid is also linked directly to O-3 of an interchain β-D-galactopyranosyl residue which also has a vicinal β-D-galactopyranosyl residue at O-4 (38).
Acknowledgment—We wish to thank Fred Cooper for obtaining the mass spectra.

REFERENCES
1. Lancefield, R. C. (1933) J. Exp. Med. 57, 571-595
4. Lancefield, R. C., and Friemer, E. H. (1966) J. Hg. 64, 191-203
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