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THE STRUCTURE OF AN R-TYPE OLIGOSACCHARIDE CORE OBTAINED FROM SOME LIPOPOLYSACCHARIDES OF *Neisseria meningitidis**

HAROLD J. JENNINGS, KENNETH G. JOHNSON,
Division of Biological Sciences, National Research Council of Canada, Ottawa, Ontario K1A OR6 (Canada)

AND LENNART KENNE
Department of Organic Chemistry, Arrhenius Laboratory, University of Stockholm, Stockholm S-106 91 (Sweden)

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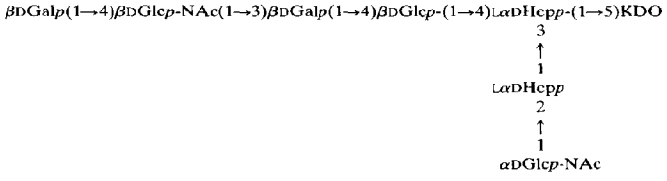
ABSTRACT

The main structure of a lipopolysaccharide R-type core oligosaccharide common to a number of different strains of *Neisseria meningitidis* has been elucidated. Methylation analysis, specific degradations, and nuclear magnetic resonance spectroscopic analysis of the dephosphorylated cores indicated that they all have the following structure.



The determinants responsible for the L3, L7, and L9 meningococcal lipopolysaccharide serotypes are situated in this oligosaccharide.

INTRODUCTION

Subcapsular antigens of *Neisseria meningitidis* have been implicated in the immune response to natural infections[1,2], and have been the focus of extensive studies[3–5]. They have assumed considerable importance in recent years due to their potential as vaccines, alternative to the poorly immunogenic group B polysac-

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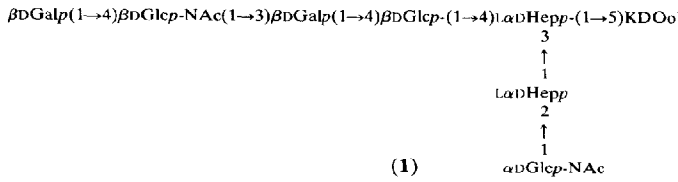
charide[6], against group B meningococcal infection. Like the well defined, serotype protein antigens[5], the meningococcal lipopolysaccharides (LPS) are situated in the outer membrane of the organism. Zollinger and Mandrell[3,4] have shown that the meningococcal LPS are serotype antigens, independent of protein serotype, and have identified eleven different, serotype determinants among the groups A, B, and C organisms. Some of these determinants are shared to a certain extent by all of the LPS, thus producing a complex, serological response[3,4].

Recently, it has been demonstrated[6,7] that these determinants are situated in the glycose moieties of the LPS that had previously been identified as low-molecular-weight, core oligosaccharides of the R-type[7,8]. Structural diversity in the meningococcal core oligosaccharides has been established[8] which is consistent with the type-specificity exhibited by their homologous LPS. To complete the antigenic profile, it was necessary to elucidate the structures of the cores related to the different LPS serotypes. We now describe the structural elucidation of a meningococcal LPS core oligosaccharide in which the L3, L7, and L9 determinants[3,4] predominate.

RESULTS AND DISCUSSION

The lipopolysaccharides from the different serogroups of *N. meningitidis* were isolated by a modified, phenol-extraction technique[9] in which the cells were disrupted by lysozyme prior to extraction, as previously described[8]. In this procedure, the LPS were recovered from the aqueous phase of the phenol extraction. However, it has now been established that further quantities of the LPS can be isolated from the phenol phase; this modification of the extraction procedure resulted in improved overall yields (sometimes as much as double) of the LPS. Presumably, the lack of a hydrophilic O-chain increases the solubility of these R-type lipopolysaccharides in the phenol phase.

The core oligosaccharides were prepared from the LPS by mild hydrolysis with acid, and purified by gel filtration[8]. Sugar analysis indicated that they all contained residues of D-galactose, D-glucose, 2-acetamido-2-deoxy-D-glucose, L-*glycero*-D-*manno*-heptose (LD-Hep), and 3-deoxy-D-*manno*-octulosonic acid (KDO) in the ratios of 2:1:2:2:1, as previously reported[8]. In addition, the presence of nonstoichiometric but equimolar amounts of 2-aminoethanol and phosphate have also been reported in these core oligosaccharides[8]. A signal at 41.3 p.p.m. ($^3J_{C-1,P}$ 8.0 Hz) in the ^{13}C-n.m.r. spectra of the oligosaccharides indicated that these components exist as 2-amino-*O*-phosphonoethanol substituents, and this was confirmed by the detection of a free amino group in these oligosaccharides by means of 1-fluoro-2,4-dinitrobenzene. As previously described[8], the oligosaccharides were modified by removal of the 2-amino-*O*-phosphonoethyl groups with 48% aqueous hydrogen fluoride, followed by reduction of the terminal KDO to 3-deoxyoctitols (KDOols) residues, to yield compound **1**.



(1)

The 300-MHz, ^{1}H-n.m.r. spectrum of **1** exhibited, *inter alia*, signals for anomeric protons at δ 5.39 [$^3J_{1,2}$ small (not resolved)], 4.97 ($^3J_{1,2}$ 3.0 Hz), 4.96 [$^3J_{1,2}$ small (not resolved)], 4.67 ($^3J_{1,2}$ 6.5 Hz), 4.48 ($^3J_{1,2}$ 7.5 Hz), 4.39 ($^3J_{1,2}$ 7.5 Hz), and 4.38 ($^3J_{1,2}$ 7.5 Hz). The chemical shifts and coupling constants of the first three, lowest-field signals indicated that both of the L-*glycero*-D-*manno*-heptosyl residues in **1** exist as L-α-D-heptopyranosyl residues, and that one α-D-hexopyranosyl unit is present in **1**; those of the remaining four (highest-field) signals indicated that **1** contains four β-D-hexopyranosyl units. Two singlets in the ^{1}H-n.m.r. spectrum, at δ 2.06 (3 H) and 2.04 (3 H), indicated that both of the 2-amino-2-deoxy-D-glucosyl units are *N*-acetylated.

The 25-MHz, ^{13}C-n.m.r. spectrum of **1** is complex, but it exhibits signals, at 176.0, 175.3, 23.5 and 23.2 p.p.m., associated with the two *N*-acetyl groups, at 56.4 and 55.5 p.p.m. for the carbon atoms linked to acetamido groups, at 36.7 p.p.m. for the CH_2 group of the 3-deoxyoctitol residue, and at 104.0 (2 C), 103.5, 102.9, and 100.8 (2 C) for a total of 6 anomeric carbon atoms.

The methylation analyses of compound **1** obtained from a number of strains of *N. meningitidis* [608 (B), 550 (29E), 665 (W-135), 247 (X) , 546 (Y), and 565 (Z)] have been reported[8], and the results are in agreement with the conclusion that all of the original octasaccharides have a terminal (nonreducing) D-galactopyranosyl and a 2-acetamido-2-deoxy-D-glucopyranosyl group, and a branching heptose residue linked through O-3 and O-4. The octasaccharides terminate with a KDO residue linked through O-5, a linkage common in the core oligosaccharides of other Enterobacteriaceae[10,11].

In order to obtain information on the sequences, a number of chemical degradations were performed on **1**. Oligosaccharide **1** was *N*-deacetylated[12], and part of the product was deaminated[13] with nitrite in aqueous acetic acid, and the product reduced with sodium borodeuteride. Two fragments containing 2,5-anhydro-D-mannitol-*1d* were isolated by gel filtration, and identified as 2,5-anhydro-D-mannitol-*1d* and *O*-β-D-galactopyranosyl-(1→4)-2,5-anhydro-D-mannitol-*1d*. The β-D-configuration of the disaccharide was confirmed from its ^{1}H-n.m.r. spectrum, which exhibited an anomeric doublet at δ 4.40 ($^3J_{1,2}$ 7.0 Hz).

Another portion of the *N*-deacetylated **1** was subjected to acid hydrolysis, in which all of the glycosidic linkages, except those of the 2-amino-2-deoxy-D-glucopyranosyl (GlcN) residues, were cleaved. Following reduction of the hydrolysis products with sodium borodeuteride, the GlcN-containing, reduced disaccharides

were separated from the neutral components (alditols) by ion-exchange chromatography. The neutral sugars liberated by the hydrolysis were D-galactose, D-glucose, L-*glycero*-D-*manno*-heptose, and reduced KDO in the ratios of 1:1:1:1, as identified by g.l.c. of their alditol acetates. After recovery from the ion-exchange column, the GlcN-containing, reduced disaccharides were *N*-reacetylated, and the ^{1}H-n.m.r. spectrum of the mixture exhibited two anomeric protons, at δ 5.12 (1 H, $^3J_{1,2}$ 3 Hz) and 4.65 (1 H, $^3J_{1,2}$ 8 Hz), and signals from two *N*-acetyl groups, at δ 2.10 (3 H) and 2.07 (3 H); these data are consistent with the presence of two disaccharides, one in the α-D, and the other in the β-D configuration. Equimolar amounts of D-galactitol-*1d* and L-*glycero*-D-*manno*-heptitol-*1d* were liberated from the two disaccharides by acid hydrolysis.

The mixture of GlcN-containing reduced disaccharides was acetylated, and the product subjected to chromic acid oxidation[14], using per-*O*-acetylated *myo*-inositol as internal standard. D-Galactitol-*1d* was released on treatment of the chromic acid degradation product with methoxide, whereas equimolar amounts of the heptitol-*1d* were released only by methanolysis of the reaction products. These results indicated that galactitol-*1d* and heptitol-*1d* were the "aglycons" of 2-acetamido-2-deoxy-β- and -α-D-glucose, respectively. Methylation, acid hydrolysis of the product, and acetylation of the resulting disaccharides gave 3-*O*-acetyl-1,2,4,5,6-penta-*O*-methyl-D-galactitol-*1d* and 2-*O*-acetyl-1,3,4,5,6,7-hexa-*O*-methyl-L-*glycero*-D-*manno*-heptitol-*1d*, identified by g.l.c.–m.s. All of these results indicated that the structures of the disaccharides obtained by *N*-deacetylation of **1** and acid hydrolysis were β-D-Glc*p*N-(1→3)-D-Gal and α-D-Glc*p*N-(1→2)-Hep. The degradation of *N*-deacetylated **1** gave the following partial structures: D-Glc*p*NAc-(1→, β-D-Glc*p*NAc-(1→3)-β-Gal*p*, β-D-Gal*p*-(1→4)-D-Glc*p*NAc, and α-β-Glc*p*NAc-(1→2)-Hep*p*.

Further information on the sequence of sugars in **1** was obtained when it was subjected to a Smith degradation[15]. This degradation should leave the 3-*O*-β-D-Gal*p* and 4-*O*-β-D-Glc*p*NAc residues intact, convert the 3,4-di-*O*-substituted α-Hep*p* residue into a 3,4-di-*O*-substituted α-D-Man*p* residue, and convert the 3-deoxyoctitol into a 2-deoxypentitol. In the ^{1}H-n.m.r. spectrum of the periodate-oxidized and reduced, intermediate Smith-degradation products, the signal for the α-D-acetamido sugar (δ 4.97, $^3J_{1,2}$ 3 Hz) had disappeared, thus proving that, of the two GlcN units, the one in the terminal position had the α-D configuration. In a sugar analysis, with deamination of the Smith-degradation products, the following components were identified by g.l.c.: glycerol, erythritol, a 2-deoxypentitol, 2,5-anhydromannose, galactose, and glucose, the last four being in the ratios of 0.5:1.2:1.0:0.7. This result is not entirely consistent with the proposed structure for **1**, because the 4-*O*-substituted β-D-Glc*p* residue should have been completely oxidized in this procedure. However, underoxidations of potentially oxidizable sugar residues of polysaccharides have been reported[16], and have been attributed to hemiacetal formation. That this was also the case in the oxidation of **1** was confirmed when no glucose could be detected in the products of a further periodate

oxidation of the first oxidized and reduced, intermediate Smith-degradation product of **1**.

The underoxidized intermediate from the first Smith degradation of **1** was reduced with sodium borodeuteride, and the product treated with acid under mild conditions, the hydrolyzate reduced with sodium borohydride, and the alditols subjected to methylation analysis. This analysis indicated the following methylated components: 2-deoxy-1,3,5-tri-*O*-methylpentitol-*1*,*5d*$_2$, 2,3,4,6-tetra-*O*-methylmannose-*6d*, 2,4,6-tri-*O*-methylgalactose, and 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-glucose. As a result of the underoxidation of the glucosyl residue in the Smith degradation of **1**, equimolar amounts of 2,3,6-tri-*O*-methylglucose and 2,3,6-tri-*O*-methylmannose-*6d* were also detected. This indicated that the partially oxidized β-D-glucopyranosyl residue was linked to O-4 of the di-*O*-substituted heptopyranosyl residue in **1**. The results of the foregoing Smith degradations are consistent with the presence in **1** of the following oligosaccharide unit: β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→4)-L-α-D-Hep*p*-(1→5)-KDOols.

The combined results of all of the different analyses made are consistent only with structure **1** for the dephosphorylated and reduced oligosaccharide obtained from the lipopolysaccharides of a number of strains of *N. meningitidis*. All of the core oligosaccharides obtained from *N. meningitidis* 608 (B), 550 (29E), 665 (W-135), 247 (X), 546 (Y), and 565 (Z) gave **1** on dephosphorylation, and reduction of their terminal KDO residues. This was indicated by the similar sugar and methylation analyses given by these modified oligosaccharides, and was confirmed by their superposable ^{13}C- and ^{1}H-n.m.r. spectra, respectively. The ^{13}C-n.m.r. spectra of the oligosaccharides prior to modification were, however, not superposable, thus indicating differences in their *O*-substitution with 2-amino-*O*-phosphonoethanol. LPS serotyping of the aforementioned organisms by the method of Zollinger and Mandrell[3,4] demonstrated the presence of multiple determinants, the L3, L7, and L9 determinants being predominant. However, these serotypes are closely related, and have more recently all been designated serotype 1, according to the system of Poolman *et al.*[17]. Preliminary serological studies[6a] indicated that these determinants are situated in the LPS R-type oligosaccharides; however, the role of the *O*-(2-amino-*O*-phosphonoethanol) substituents, if any, in the aforementioned serotyping systems has not yet been established.

EXPERIMENTAL

General methods. — Solutions were evaporated under diminished pressure below 40°. Gas–liquid chromatography (g.l.c.) was performed with a Hewlett–Packard 5830 A instrument, using columns and conditions as previously described[8] and an SP-1000 glass capillary-column (25 m × 0.25 mm). Combined gas–liquid chromatography–mass spectrometry (g.l.c.–m.s.) was conducted with a Finnegan 3100 D or a Varian MAT 311-SS100 instrument, using the same columns. N.m.r. spectra were recorded with a Varian CFT20, a JEOL FX-100, and a Bruker WM-

300 spectrometer in the pulsed, Fourier-transform mode, using, as reference standards, external tetramethylsilane for ^{13}C-n.m.r., and internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate for ^{1}H-n.m.r. spectroscopy.

Sugar and methylation analyses were conducted as previously described[5]. Following their deamination[13], and conversion into alditol acetates, the sugars were identified, and quantified, by g.l.c.[18]. Methylations were performed by the method of Hakomori[19], and the individual methylated components were identified, and quantified, by[20] g.l.c.–m.s. The absolute configurations of the sugars obtained by hydrolysis of oligosaccharide **I** were determined by using the method of Gerwig *et al.*[21,22]. The retention times of their (trimethylsilyl)ated (−)-2-butyl glycosides in an SE-30 W.C.O.T., glass capillary-column at 175° were compared to the retention times of the same derivatives of the corresponding reference sugars of known absolute configuration. Authentic L-*glycero*-D-*manno*-heptose was obtained by hydrolysis of the *Escherichia coli* J5 core oligosaccharide.

Growth of organisms. — The strains of the different groups (listed in parentheses) of *N. meningitidis* used in this study were 608 (B), serotype L3 (d), L9 (m); 550 (29-e), serotype L3,9 (d), L7 (m); 247 (X), serotype L4 (d), L6,7,9 (m); 546 (Y), serotype L3,9 (d), L7 (m); and 565 (Z), serotype L7,9 (d). The LPS serotyping was kindly performed by Dr. Wendell D. Zollinger of the Walter Reed Army Institute of Research, Washington, D.C. 20012, according to his procedures[3,4] where (d) is a dominant and (m) is a minor determinant. Inocula were prepared from lyophilized cultures which were grown overnight at 37° on sheep blood-agar plates (BAP) in candle jars. Cellular proceeds from 5–6 BAP were dispersed in 1 L of *Neisseria* Chemically Defined Medium(NCDM; General Biochemicals Inc., Chagrin Falls, OH), presterilized by passage through 0.22-μm filters (Millipore), and agitated in a 4-L. baffled flask for 7 h at 37°. The inoculum was then transferred to a 25-L. New Brunswick Microferm containing NCDM (20 L). Aeration at 25 L.min^{-1} and agitation at 200 r.p.m. were maintained. After growth for 18 h at 37°, cells were killed by the addition of 1% formalin, and harvested by centrifugation.

Isolation of the LPS. — The LPS from the different serogroups were isolated by a modified phenol-extraction technique[7] previously described[5]. In this technique, the LPS was obtained from the aqueous extract in yields of ~1% (depending on the serogroup), based on the dry weight of the organisms. Further quantities of the LPS, in some cases equal to the amount obtained from the aqueous extract, were recovered from the phenol layer. To recover the LPS, the phenol phase was dialyzed extensively against water, thus removing the phenol and producing another aqueous phase. Filtration, and lyophilization of the filtrate, yielded the crude LPS, which was purified, as described for the first aqueous phase, by dissolution in water and centrifugation of the solution for 12–16 h at 105,000*g*.

Isolation and modification of the LPS core oligosaccharides. — The core oligosaccharides were obtained by heating the LPS in 1% acetic acid for 2 h at 100°, and purified by gel filtration[5]. The core oligosaccharide used in all of the degrada-

tion studies was obtained from the LPS of the group-29E organisms, and gave an elemental analysis:C, 41.28; H, 6.02; N, 2.61; and ash, 6.0%. Free amino groups were detected in the cores by reacting with 1-fluoro-2,4-dinitrobenzene and measuring[23] the optical absorbance of the reaction mixture at 420 nm. Dephosphorylation of the cores, and reduction of their terminal KDO residues[8], yielded the modified oligosaccharide **1**. Elemental analysis of **1** obtained from the LPS of the group-29E organisms gave: C, 43.18; H, 6.48; N, 1.51; and ash, 0.0%. The oligosaccharide had $[\alpha]_D^{24}$ +51° (*c* 1.0, water).

Deamination of **1**. — Prior to deamination of the modified oligosaccharide, it was *N*-deacetylated by the method of Kenne and Lindberg[12]. The modified oligosaccharide (40 mg) was treated with sodium hydroxide (80 mg) in a mixture of benzenethiol (20 μL), water (0.4 mL), and dimethyl sulfoxide (3.0 mL) for 16 h at 80°, with stirring. The solution was cooled, made neutral with M HCl, and evaporated to dryness *in vacuo*. The product was purified by gel filtration on a column (50 × 1 cm) of Sephadex G-25, using 0.1% aqueous pyridine, and yielded 32 mg of *N*-deacetylated product. Complete *N*-deacetylation was confirmed by the failure to detect the characteristic *N*-acetyl methyl proton signals at δ ~2.

The deamination of the *N*-deacetylated core was achieved essentially by the method of Dmitriev *et al.*[13]. The *N*-deacetylated core (16 mg) in water (0.6 mL) was treated with acetic acid (1.0 mL) and 5% aqueous sodium nitrite (1.0 mL) for 40 min at room temperature, water (10 mL) was added, and the mixture was lyophilized. The product was redissolved in water (2 mL), and sodium borodeuteride (30 mg) was added. After 2 h, the pH was adjusted to 4 with acetic acid, and the solution was evaporated to dryness, and codistilled with methanol (3 × 2 mL). The product was treated with acetic anhydride (1.0 mL) in pyridine (1.0 mL) for 30 min at 100°, the solution evaporated to dryness, and the residue partitioned between chloroform and water. The chloroform phase was concentrated to ~1 mL, the concentrate applied to a column of Sephadex LH-20, and the column eluted with 1:1 acetone–chloroform. The fractions were examined by t.l.c., ^{1}H-n.m.r. spectroscopy, and g.l.c., and the fractions of lower molecular weight were *O*-deacetylated; the product was studied by ^{1}H-n.m.r. spectroscopy and methylation analysis.

Hydrolysis of N-*deacetylated* **1**. — *N*-Deacetylated **1** (16 mg) was hydrolyzed with 0.5M trifluoroacetic acid (3 mL) for 16 h at 100°, and the solution cooled, and evaporated to dryness. The residue was dissolved in water (2 mL), and the solution was treated with sodium borodeuteride (20 mg) for 4 h, added to a column (1 × 10 cm) of Dowex-50 (H^+) ion-exchange resin, and the column eluted first with water (to remove the neutral alditols), and then with 0.5M HCl (to remove hexosamine-containing disaccharides). The aqueous eluate was evaporated to dryness, codistilled with methanol (3 × 2 mL), the residue acetylated, and the acetates examined by g.l.c.–m.s. The acidic eluate was evaporated to dryness, and investigated by ^{1}H-n.m.r. spectroscopy; this showed two anomeric protons of approximately equal intensity, indicative of the presence of two different, hexosamine-

containing disaccharides. Portions of this mixture were subjected to sugar and methylation analyses, and to oxidation with chromic acid.

Chromic acid oxidation. — The foregoing mixture of disaccharides was acetylated, and the products were treated with chromic acid (20 mg) in acetic anhydride (0.2 mL) for 30 min at 50° as previously described[14], per-*O*-acetylated *myo*-inositol being used as the internal standard. The reaction mixture was partitioned between chloroform and water, and the chloroform layer was evaporated to dryness. The residue was treated with sodium methoxide in methanol, to release "aglyconic" alditols of oxidized sugars, the product acetylated, and the acetates investigated by g.l.c. The reacetylated mixture was then treated in a sealed tube with 3% methanolic hydrogen chloride for 16 h at 80°, to release "aglyconic" alditols of unoxidized sugars. The methanol solution was evaporated to dryness, the residue acetylated, and the contents of alditol acetates were determined by g.l.c.

Smith degradation[15]. — Oligosaccharide **1** (15 mg) was dissolved in water (5.5 mL). Sodium metaperiodate (47 mg) was added, and the solution was kept in the dark for 40 h at 4°. The excess of periodate was reduced with ethylene glycol (0.1 mL), and the product was reduced with sodium borodeuteride (20 mg) for 16 h. The pH was adjusted to 5 with 2M hydrochloric acid, the solution lyophilized, and boric acid removed by addition of methanol (3 × 3 mL) to, and evaporation from, the residue. Part of the material (1/15th) was used for sugar analysis, and another part (1/10th) was treated once more with sodium metaperiodate as already described, and the products of the oxidation were investigated by g.l.c.–m.s. The rest of the material was hydrolyzed with 0.5M trifluoroacetic acid (5 mL) for 18 h at 25°, diluted with water (15 mL), and lyophilized, and the products were subjected to methylation analysis.

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REFERENCES

1 I. GOLDSCHNEIDER, E. C. GOTSCHLICH AND M. S. ARTENSTEIN, *J. Exp. Med.*, 129 (1969) 1327–1348.
2 D. L. KASPER, J. L. WINKELHAKE, B. L. BRANDT AND M. S. ARTENSTEIN, *J. Infect. Dis.*, 127 (1973) 378–387.
3 W. D. ZOLLINGER AND R. E. MANDRELL, *Infect. Immun.*, 18 (1977) 424–433.
4 W. D. ZOLLINGER AND R. E. MANDRELL, *Infect. Immun.*, 28 (1980) 451–458.
5 C. E. FRASCH, in I. WEINSTEIN AND B. N. FIELDS (Eds.), *Seminars in Infectious Disease*, Vol. II, Stratton Intercontinental, Medical Book Corp., New York, 1979, pp. 303–337.
6 E. WYLE, M. S. ARTENSTEIN, B. L. BRANDT, E. C. TRAMONT, D. L. KASPER, P. L. ALTIERI, S. L. BERMAN AND J. P. LOWENTHAL, *J. Infect. Dis.*, 126 (1972) 514–522.
6a H. J. JENNINGS, unpublished results.
7 H. J. JENNINGS, G. B. HAWES, G. A. ADAMS AND C. P. KENNY, *Can. J. Biochem.*, 51 (1973) 1347–1354.

8 H. J. JENNINGS, A. K. BHATTACHARJEE, L. KENNE, C. P. KENNY, AND G. CALVER, *Can. J. Biochem.*, 58 (1980) 128–136.
9 K. G. JOHNSON AND B. S. PERRY, *Can. J. Microbiol*, 22 (1976) 29–34.
10 C. GALANOS, O. LUDERITZ, E. T. REITSCHEL, AND O WESTPHAL, in W. T GOODWIN (Ed.), *Biochemistry of Lipids*, University Park Press, Baltimore, 1977, pp 239–335.
11 P. PREHM, S. STIRM, B. JANN, AND K. JANN, *Eur. J. Biochem.*, 56 (1975) 41–45.
12 L. KENNE AND B. LINDBERG, *Methods Carbohydr. Chem.*, 8 (1980) 295–296.
13 B. A. DMITRIEV, L. V. BACKINOWSKY, V. L. LVOV, N. K. KOCHETKOV, AND I. L. HOFFMAN, *Eur. J. Biochem*, 50 (1975) 539–547.
14 J. HOFFMAN AND B. LINDBERG, *Methods Carbohydr. Chem.*, 8 (1980) 117–122.
15 I. J. GOLDSTEIN, G. W. HAY, B. A. LEWIS, AND F. SMITH, *Methods Carbohydr. Chem*, 5 (1965) 361–370.
16 M. F. JSUAK AND T. J. PAINTER, *Acta Chem. Scand.*, 27 (1973) 1268–1276.
17 J. T. POOLMAN, C. T. P. HOPMAN, AND H. C ZANEN, *FEMS Microbiol. Lett.*, 13 (1982) 339–348.
18 J. S. SAWARDEKER, J. H SLONEKER, AND A. R. JEANES, *Anal. Chem.*, 37 (1965) 1602–1604.
19 S. HAKOMORI, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
20 B. LINDBERG, *Methods Enzymol.*, 28 (1972) 178–195.
21 G. J. GERWIG, J. P. KAMERLING, AND J. F G. VLIEGENTHART, *Carbohydr Res.*, 62 (1978) 349–357.
22 G. J. GERWIG, J. P. KAMERLING, AND J. F. G. VLIEGENTHART, *Carbohydr. Res.*, 77 (1979) 1–7.
23 J.-M. GHUYSEN AND J. L. STROMINGER, *Biochemistry*, 2 (1963) 1110–1119.