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SOMATIC ANTIGENS OF *Shigella*: STRUCTURE OF THE O-SPECIFIC POLYSACCHARIDE CHAIN OF THE *Shigella dysenteriae* TYPE 7 LIPOPOLYSACCHARIDE*

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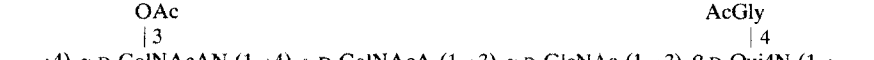
ABSTRACT

4-(*N*-Acetylglycyl)amino-4,6-dideoxy-D-glucose has been identified as a component of the *Shigella dysenteriae* type 7 O-specific polysaccharide, in addition to the previously reported 2-acetamido-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-galacturonic acid. On the basis of selective cleavage with anhydrous hydrogen fluoride and analysis by ^{1}H- and ^{13}C-n.m.r. spectroscopy and f.a.b.-mass spectrometry, it was concluded that the tetrasaccharide repeating-unit of the polysaccharide has the following structure:



where D-GalNAcAN is 2-acetamido-2-deoxy-D-galacturonamide and D-Qui4N is 4-amino-4,6-dideoxy-D-glucose.

INTRODUCTION

The structures of the O-specific polysaccharide chains of the *Shigella dysenteriae* types 1–5 and 8–10 lipopolysaccharides, as well as a partial structure of that of type 6, have been established[1]. 2-Acetamido-2-deoxy-D-glucose, 2-acetamido-2-deoxy-D-galacturonic acid, and glycine have been identified[2] as constituents of the type 7 polysaccharide (strain 408), but the structure of the repeating unit has

*Dedicated to Professor Bengt Lindberg.

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not been determined. The structure of the *S. dysenteriae* type 7 (strain NCTC 519/66) O-specific polysaccharide has been reported[3], but the sugar composition differs markedly from that of the polysaccharide of strain 408.

We now report the composition and structure of the *S. dysenteriae* type 7, strain 408, O-specific polysaccharide. The preliminary results have been reported[4].

RESULTS AND DISCUSSION

The ^{13}C-n.m.r. spectrum of the polysaccharide (Fig. 1) contained signals for the methyl carbon of a 6-deoxy sugar (17.7 p.p.m.), four *N*-acetyl groups (22.9–23.5 p.p.m.), one *O*-acetyl group (21.4 p.p.m.), four anomeric carbons (97.9–104.7 p.p.m.), four carbons carrying nitrogen (50.9–57.8 p.p.m.), one hydroxymethyl carbon (61.4 p.p.m.), twelve other carbons carrying oxygen (67.9–80.0 p.p.m.), eight carbonyl groups (172.5–175.8 p.p.m.), and C-2 of glycine (44.1 p.p.m.). These data showed that the polysaccharide has a regular structure built up of tetrasaccharide repeating-units involving four amino sugars, two of which were 2-amino-2-deoxyhexuronic acids, the third was a 2-amino-2-deoxyhexose, and the fourth was a 6-deoxyhexosamine. Each repeating unit also contained one *O*-acetyl and four *N*-acetyl groups, and a glycyl residue.

Hydrolysis of the polysaccharide with 2M hydrochloric acid gave three monosaccharides (**1–3**), which were isolated by preparative p.c. Prolonged hydrolysis of **2** and **3** under the same conditions yielded glycine and **1**, which were identified by

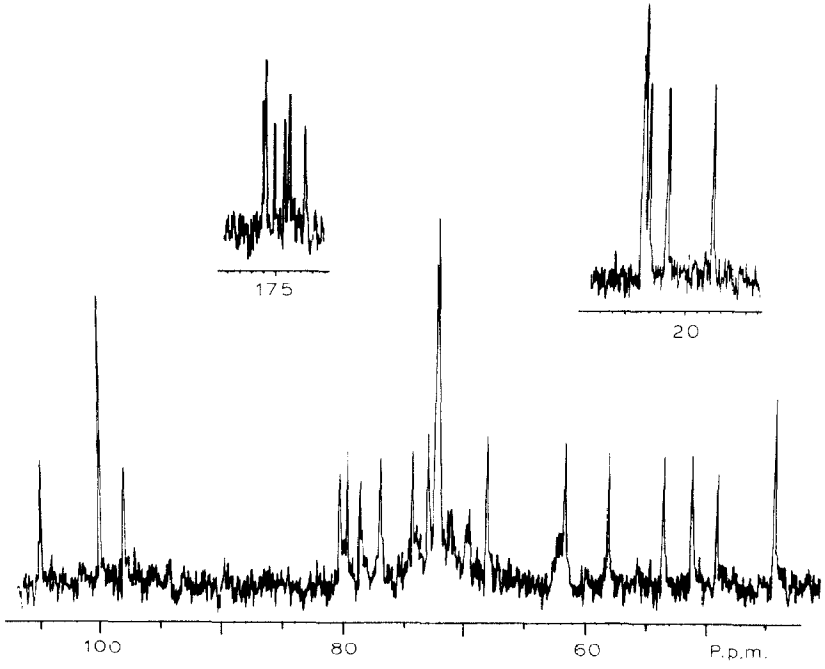
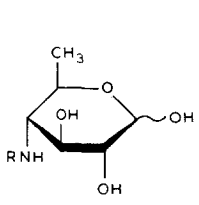


Fig. 1. ^{13}C-N.m.r. spectrum of *S. dysenteriae* O-specific polysaccharide.

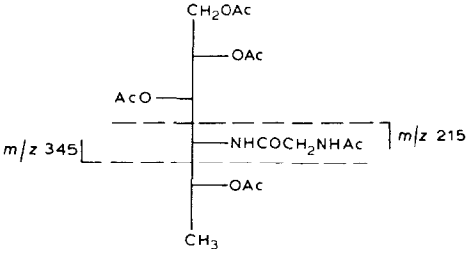
using an amino acid analyser, the retention time of **1** being identical to that of authentic 4-amino-4,6-dideoxyglucose (viosamine). Reduction of **2** with sodium borohydride, followed by acetylation, gave the alditol acetate **4**, the mass spectrum of which contained peaks at *m/z* 345 and 215 for the fragments C-1/4 and C-4/6, respectively, and also for ions arising from the primary fragments by the loss of ketene, acetic acid, and/or acetamidoketene (AcNHCH=CO). These data proved the presence of a deoxy unit at position 6 and an (*N*-acetylglycyl)amino group at position 4. Thus, **4** is derived from a 4-amino-4,6-dideoxyhexose *N*-acylated with glycine.

The ^{1}H- and ^{13}C-n.m.r. spectra of **2** and **3** contained signals for an amino-6-deoxy sugar and glycine, and differed from each other only by the presence in the spectra of **3** of extra signals for an *N*-acetyl group (the *N*-substituent of glycine). On the basis of the $J_{2,3}$, $J_{3,4}$, and $J_{4,5}$ values (9.5–10 Hz), determined from the ^{1}H-n.m.r. spectra of **2** and **3**, it was concluded that these monosaccharides had the *gluco* configuration[5]. The D configuration of the 4-amino sugar was established by analysis of the glycosidation effects in the ^{13}C-n.m.r. spectrum of the polysaccharide (see below).

Thus, the *S. dysenteriae* type 7 polysaccharide contains 4-(*N*-acetylglycyl)-amino-4,6-dideoxy-D-glucose (**3**), which has not been found in Nature previously; **1** and **2** are formed during the acid hydrolysis of **3** by successive removal of *N*-acetyl and *N*-glycyl groups.



1 R = H
2 R = Gly
3 R = AcGly



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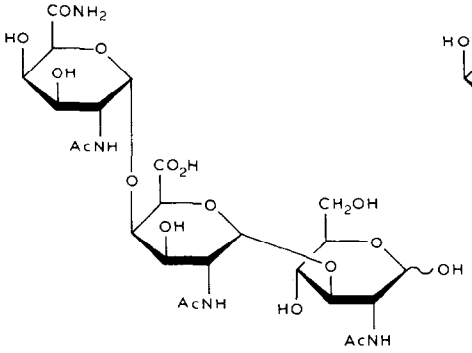

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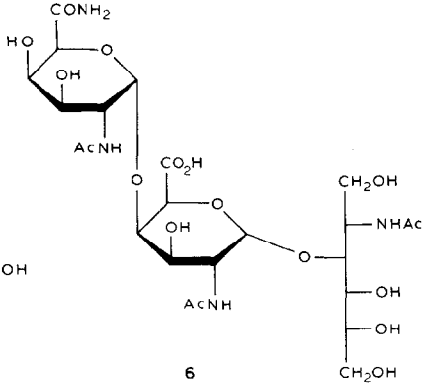


6

Solvolysis of the polysaccharide with anhydrous hydrogen fluoride afforded **3** and the oligosaccharide **5**. Treatment of **5** with methanolic hydrogen chloride, followed by re-*N*-acetylation and acid hydrolysis, gave 2-amino-2-deoxyglucose and 2-amino-2-deoxygalacturonic acid, which were identified by using an amino acid analyser. These monosaccharides were identified also after analogous treatment of the polysaccharide, and shown[2] to be D sugars.

The ^{13}C-n.m.r. spectrum of **5** (Table I) proved it to be a trisaccharide with a residue of 2-acetamido-2-deoxyglucose [δ 96.1 (C-1β), 92.1 (C-1α), 61.7, 61.6 (C-6α,6β), 56.5 (C-2β), 53.8 (C-2α)] at the reducing terminus. This conclusion was confirmed by the reduction of **5** with sodium borohydride to yield the oligosaccharide **6**, which contained, in place of 2-acetamido-2-deoxyglucose, 2-acetamido-2-deoxyglucitol [δ 64.0 (C-6), 61.8 (C-1), 55.0 (C-2)] and two glycosidically linked aminodeoxyhexuronic acid residues [δ 99.9, 99.8 (both C-1), 50.5, 50.5 (both C-2)]. The ^{1}H-n.m.r. spectrum of **6** was interpreted by sequential, selective spin-decoupling experiments (see Experimental). The coupling constants, $J_{1,2}$ 3.6–3.9, $J_{2,3}$ 11.5, $J_{3,4}$ 3, and $J_{4,5}$ 1–1.4 Hz, showed[5] that each of these aminodeoxyhexuronic acid derivatives had the *α-galacto* configuration. The ^{1}H- and ^{13}C-n.m.r. spectra of **6** contained signals for three *N*-acetyl groups [δ_H 2.13, 2.07, 2.04; δ_C 23.3, 23.2, 23.1 (CH_3CO)] and, hence, each of the three amino sugars was *N*-acetylated. From these data, it followed that the trisaccharide **5** was composed of two 2-acetamido-2-deoxy-D-galacturonic acid residues and one 2-acetamido-2-deoxy-D-glucose residue.

Irradiation of H-1 of one of the aminodeoxyhexuronic acid residues (unit A) at 5.13 p.p.m. in **6** caused a n.O.e. of the signal for H-4 of the other aminodeoxyhexuronic acid residue (unit B) at 4.49 p.p.m., whereas irradiation of H-1 of unit B at 5.27 p.p.m. caused a n.O.e. of the signal for one of the protons of the 2-acetamido-2-deoxyglucitol residue (unit C) at 4.19 p.p.m. Unit A, consequently, is a terminal sugar and 4-linked to unit B.

All the signals for the α and β anomers of the 2-acetamido-2-deoxyglucose residue (unit C) in the ^{13}C-n.m.r. spectrum of **5** were assigned by comparison with the spectrum of **6** (Table I) and taking into account their relatively low intensities. The noticeable downfield shift of the signal for C-3 [δ 80.7 (C-3β), 79.2 (C-3α)] and a somewhat upfield shift of the signals for C-2,4 [δ 72.3 (C-4α), 72.2 (C-4β), 56.5 (C-2β), 53.8 (C-2α)] of unit C in the spectrum of **5**, as compared with their position in the spectrum of 2-acetamido-2-deoxy-D-glucose[6], were caused by the α- and β-effects of glycosylation at O-3, respectively, and, hence, the 2-acetamido-2-deoxyglucose residue in **5** was substituted at position 3.

The molecular weight of **6**, as determined by f.a.b.-mass spectrometry, was 656 (intense peak for $[M + H]^+$ at *m/z* 657), thus indicating that one of the aminodeoxyhexuronic acid residues was in the form of a primary amide. In fact, the resonance for H-5 of only one of the uronic acid derivatives (unit B) in **6** was shifted (from 4.40 to 4.63 p.p.m.) on changing the pH from 5 to 1, which is typical of a uronic acid having a free carboxyl group[7]. In contrast, H-5 of the other uronic

TABLE I

^{13}C-N.M.R. CHEMICAL SHIFTS (P.P.M.)[a]

Unit	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
Monosaccharide **2**						
Qui4NGly α	93.1	73.2	71.7	58.2	67.3	18.0
β	96.8	75.9	74.5	58.1	71.7	18.0
Oligosaccharide **5**[b]						
α-GalNAcAN-(1→	100.0	50.5	68.7	70.0	72.7	
	99.9					
→4)-α-GalNAcA-(1→	99.2	50.5	68.0	79.6	72.5	
	99.8	50.6		79.8		
→3)-α-GlcNAc	92.1	53.8	79.2	72.3	72.7	61.6
→3)-β-GlcNAc	96.1	56.5	80.7	72.2	77.0	61.7
Oligosaccharide **6**						
α-GalNAcAN-(1→	99.9	50.5	68.2	69.9	72.7	
→4)-α-GalNAcA-(1→	99.8	50.5	67.2	78.6	71.7	
→3)-GlcNAcol	61.8	55.0	78.0	71.1	71.5	64.0
O-*Deacetylated polysaccharide*						
→4)-α-GalNAcAN-(1→	100.2	51.2	69.8	79.7	72.8	
→4)-α-GalNAcA-(1→	99.9	50.9	68.3	78.2	72.4	
→3)-α-GlcNAc-(1→	97.7	53.3	79.7	72.1	72.8	61.4
→3)-β-Qui4N(AcGly)-(1→	104.9	74.2	80.1	57.8	72.1	17.7
Original polysaccharide						
→4)-α-GalNAcAN-(1→	99.8	48.9	71.9	76.7	72.1	
→4)-α-GalNAcA-(1→	99.9	50.9	67.9	78.4	72.1	
→3)-α-GlcNAc-(1→	97.9	53.3	79.4	71.9	72.7	61.4
→3)-β-Qui4N(AcGly)-(1→	104.7	74.0	80.0	57.8	72.1	17.7
O-*Acetylated polysaccharide*[c]						
→4)-α-GalNAcAN-(1→	99.7	48.5				
→4)-α-GalNAcA-(1→	99.7	48.5				
→3)-α-GlcNAc-(1→	96.3	52.5				63.9
→3)-β-Qui4N(AcGly)-(1→	101.7			57.8		17.3

[a] CH_3COO, 21.3–21.4; CH_3CON, 22.8–23.6; CH_3CO, COOH, $CONH_2$, and C-1 of glycine, 172.3–176.1; C-2 of glycine, 41.8 (in the spectrum of **2**) or 44.1 p.p.m. (in the spectra of the polysaccharides). Assignments of signals having differences in chemical shift of less than 0.6 p.p.m. can be interchanged. [b] Some carbon atoms of the 2-amino-2-deoxygalacturonic acid derivatives gave two lines due to the presence of the 2-amino-2-deoxyglucose residue in the α and β forms. [c] The signals whose chemical shifts are omitted lie in the region 68–76 p.p.m.

acid derivative (unit A) resonated at 4.86 p.p.m. irrespective of the pH value, and, hence, unit A is a 2-acetamido-2-deoxygalacturonamide. The same derivative of 2-amino-2-deoxygalacturonic acid has been identified[7,8] as a constituent of the O-specific polysaccharide of *Pseudomona aeruginosa* O4 and related strains.

These data allowed the determination of the structures of **5** and **6**, which were confirmed by the interpretation of their ^{13}C-n.m.r. spectra (Table I). The

assignment was accomplished by comparison with the ^{13}C-n.m.r. data for oligosaccharide fragments of the *P. aeruginosa* O4 O-specific polysaccharides which also contain two derivatives of 2-amino-2-deoxygalacturonic acid[7,8]. Thus, solvolysis of the *S. dysenteriae* type 7 polysaccharide with anhydrous hydrogen fluoride cleaved selectively the glycosidic linkages of the 2-amino-2-deoxyglucose and 4-amino-4,6-dideoxyglucose derivatives and removed the *O*-acetyl groups, but did not affect the glycosidic linkages of the aminodeoxyhexuronic acid derivatives and the *N*-acyl substituents to give **3** and **5**.

The foregoing showed the tetrasaccharide repeating-unit of the *S. dysenteriae* type 7 O-specific polysaccharide to be composed of the monosaccharide **3** (unit D), three constituents of oligosaccharide **5** (units A–C), and an *O*-acetyl group. The last group was removed by treatment of the polysaccharide with aqueous triethylamine to give the *O*-deacetylated polysacharide, comparison of the ^{13}C-n.m.r. spectrum of which with those of **2, 5,** and **6** allowed assignment of the signals for C-1,2 of units A and B (100.2 and 99.9 p.p.m., 51.2 and 50.9 p.p.m., respectively) and C-4 of unit D (57.8 p.p.m.). Therefore, the fourth signal at 53.3 p.p.m. in the region for carbon atoms carrying nitrogen belonged to C-2 of unit C, and, judging[6] from the chemical shift, this unit, *i.e.*, the 2-amino-2-deoxyglucose residue, was α. Of the two unassigned signals at 97.7 and 104.9 p.p.m. in the region of anomeric carbons, only the former could belong to C-1 of the α-linked unit C; therefore, the latter belonged to C-1 of unit D, *i.e.*, the 4-amino-4,6-dideoxyglucose residue, which, thus, was β.

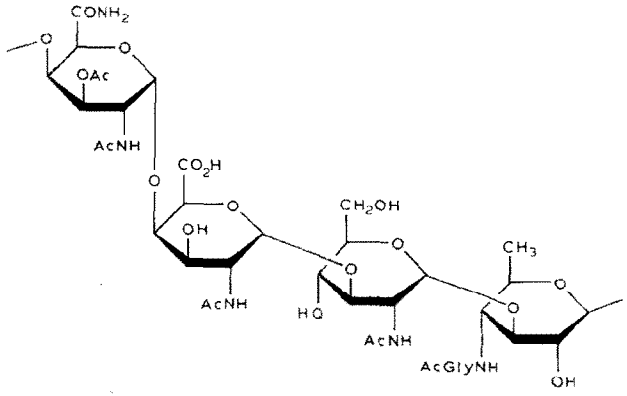
The configurations of the glycosidic linkages were confirmed by the $^{1}J_{C\text{-}1,H\text{-}1}$ values determined from the gated-decoupled ^{13}C-n.m.r. spectrum of the *O*-deacetylated polysaccharide. These values (174, 174, and 172 Hz for the signals at 100.2, 99.9, and 97.7 p.p.m., respectively) were relatively large, and, hence, the corresponding monosaccharides (units A–C) were α, whereas the coupling constant for the fourth signal at 104.9 p.p.m. was only 162 Hz, thus indicating[9] the remaining monosaccharide (unit D) to be β.

Attempts to methylate the polysaccharide under Hakomori conditions resulted in depolymerisation most probably due to β-elimination involving the 2-amino-2-deoxygalacturonamide residue. Attention was then turned to the fully acetylated polysaccharide which was water soluble and, hence, its ^{13}C-n.m.r. spectrum could be compared with that of the *O*-deacetylated polysaccharide. Acetylation shifted the signals (78–80 p.p.m.) for all the substituted carbons to higher field (<76 p.p.m.) due to the β-effects of neighbouring *O*-acetyl groups[11]. This displacement showed each of the monosaccharide residues to be monoglycosylated, and, hence, the polysaccharide to be linear. That the 2-amino-2-deoxyglucose was not glycosylated at position 6 followed from the position (61.4 p.p.m.) of the signal for C-6 in the ^{13}C-n.m.r. spectrum of the *O*-deacetylated polysaccharide. Acetylation at O-2 caused the displacement of the signal for C-1 of unit D from 104.9 to 101.7 p.p.m., but the position of the signal for C-4 of unit D near 58 p.p.m. was unaffected. Thus, the 4-amino-4,6-dideoxyglucose residue was

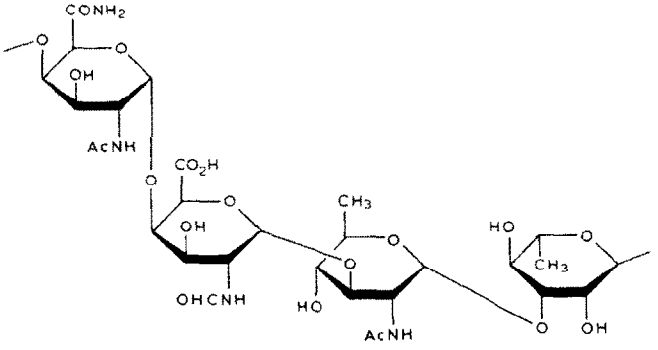
glycosylated at position 3. The signals for C-2 of units A and B were shifted upfield from 51.2 and 50.9 p.p.m. to 48.5 p.p.m., whereas the signal for C-2 of unit C was affected insignificantly. Therefore, each of the 2-amino-2-deoxygalacturonic acid derivatives was glycosylated at position 4, and the 2-amino-2-deoxyglucose residue was glycosylated at position 3, in accord with the structure of oligosaccharide **5**.

The data above established the structure of the *O*-deacetylated polysaccharide and allowed a tentative assignment of the other signals in its ^{13}C-n.m.r. spectrum, again making use of the spectra of **2** and **5** (Table I). It was possible also to determine the absolute configuration of the 4-amino-4,6-dideoxyglucose residue. Comparison of the ^{13}C-n.m.r. spectra of the *O*-deacetylated polysaccharide and **β-2** showed the displacement of the signal for C-4 of the 4-amino-4,6-dideoxy sugar, caused by the glycosylation at O-3 by the 2-acetamido-2-deoxyglucose residue, to be only −0.3 p.p.m. which indicated the α-(1→3)-linked residues to have the same absolute configuration (a shift of $\lesssim$ −1 p.p.m. would be expected[10] if the absolute configurations were different). Thus, the 4-(*N*-acetylglycyl)amino-4,6-dideoxyglucose moiety was a D sugar.

In order to locate the *O*-acetyl group, the ^{13}C-n.m.r. spectra of the original



7 (*Shigella dysenteriae* type 7)



8 (*Pseudomonas aeruginosa* O4a, 4c)

and *O*-deacetylated polysaccharides were compared. The shifts of the signals for C-2,3,4 of one of the 2-amino-2-deoxygalacturonic acid derivatives (unit A) from 51.2, 69.8, and 79.7 p.p.m. to 48.9, 71.9, and 76.7 p.p.m., respectively, were observed. These shifts are characteristic of the α- and β-effects of acetylation[11] at O-3, and, hence, the acetyl group in the original polysaccharide occurs at position 3 of unit A, and the polysaccharide has the structure **7**.

The structure **7** differs markedly from that proposed formerly[3] for the *S. dysenteriae* type 7 O-specific polysaccharide, namely, an octasaccharide repeating-unit involving residues of D-glucose, D-galactose, and 2-acetamido-2-deoxy-D-glucose in the ratios 2:1:1. Since no n.m.r. data were reported[3], further comparison with this work is not possible.

The *S. dysenteriae* type 7 O-specific polysaccharide has some structural features which are similar to those of the O-specific polysaccharides of *P. aeruginosa* O4 and related strains[7,8,12], which involve two derivatives of 2-amino-2-deoxygalacturonic acid. Formula **8** depicts the structure of one of the *P. aeruginosa* polysaccharides and the others differ by the presence of an *O*-acetyl group at O-3 of one of the 2-amino-2-deoxy-D-galacturonic acid derivatives, the mode of substitution of the rhamnose residue, the configuration of the quinovosamine residue, and the partial or full carboxyl-amidation of the 2-deoxy-2-formamido-D-galacturonic acid residue. This similarity of the O-antigens seems to determine the known[13] serological cross-reactivity between *S. dysenteriae* type 7 and *P. aeruginosa* O4.

EXPERIMENTAL

General. — ^{1}H-N.m.r. spectra were recorded with a Bruker WM-250 instrument for solutions in D_2O at 30°. N.O.e. data were obtained by the TOE method[14] and performed in the difference mode; the constants used were D1 = 4 s, relaxation delay; D2 = 0.5 s, build-up n.O.e. ^{13}C-N.m.r. spectra were run with an AM-300 instrument for solutions in D_2O at 60° for polysaccharides and 30° for oligo- and mono-saccharides (internal methanol, δ 50.15). Optical rotations were measured with a Perkin–Elmer Model 141 polarimeter for solutions in water at 20°. The mass spectrum was obtained with a Varian CH-6 instrument. The f.a.b.-mass spectrum was recorded with a VG 70/70 HS instrument, using an ION TECH argon source and glycerol (Serva) as the matrix. Solutions were freeze-dried or concentrated *in vacuo* at <40°.

Ascending p.c. was performed on FN-11 paper with 1-butanol–pyridine–water (6:4:3) and detection with alkaline silver nitrate or ninhydrin. Gel chromatography was performed on a column (70 × 3.5 cm) of Sephadex G-50 with a pyridine–acetate buffer (pH 4.5) and on a column (80 × 1.7 cm) of TSK HW 40 with aqueous 1% acetic acid. Elution profiles were recorded with a Technicon sugar analyser or a Knauer differential refractometer, respectively. Ion-exchange chromatography was carried out on a column (20 × 1 cm) of DEAE-Trisacryl M

with a linear gradient (0 to 0.5M) of sodium chloride in 0.05M sodium phosphate buffer (pH 6.5); the elution profile was recorded with a Technicon sugar analyser. Amino sugars were analysed with a BC-200 amino acid analyser, using a column (27 × 0.9 cm) of Chromex UA-8 resin and elution with a step-wise gradient of standard chloride/citrate buffers (0.2M, pH 3.25; 0.2M, pH 4.25; 0.35M, pH 5.28) at 65°.

The lipopolysaccharide was isolated[15] from dry bacterial cells of *S. dysenteriae* type 7, strain 408, and cleaved[2] with aqueous 1% acetic acid at 100°. Gel filtration on Sephadex G-50 yielded the O-specific polysaccharide, $[\alpha]_D$ +144° (*c* 0.15).

The polysaccharide was hydrolysed with 2M hydrochloric acid (2 h, 100°), the hydrolysate was concentrated, and water was repeatedly evaporated from the residue. The monosaccharides **1–3**, isolated by preparative p.c., had R_F 0.45, 0.80, and 0.85, respectively, and **2** had $[\alpha]_D$ +125° (*c* 0.4). ^{1}H-N.m.r. data for **3**: δ 5.26 (d, $J_{1,2}$ 3.6 Hz, H-1α), 4.66 (d, $J_{1,2}$ 8.0 Hz, H-1β), 4.05 (dq, $J_{5,6}$ 6.1 Hz, H-5α), 3.97 (2 H, s, H-2,2 of glycine), 3.82 (t, $J_{3,4}$ 9.7 Hz, H-3α), 3.66 (dd, $J_{2,3}$ 9.5 Hz, H-2α), 3.62–3.69 (2 H, m, H-4β,5β), 3.65 (t, $J_{4,5}$ 10 Hz, H-4α), 3.60 (t, $J_{3,4}$ 10 Hz, H-3β), 3.34 (t, $J_{2,3}$ 9 Hz, H-2β), 2.10 (3 H, s, AcN), 1.22 (3 H, d, $J_{5,6}$ 6.1 Hz, H-6,6,6β), 1.18 (3 H, d, H-6,6,6α). Reduction of **2** conventionally with sodium borohydride and acetylation gave **4**. Mass spectrum: *m/z* 345 (43%), 303 (8), 285 (25), 246(27), 226(7), 215(8), 187 (17), 186 (100), 171(7), 155 (11), 144 (8), 127 (16), 126 (15), 116 (25), 100 (22), 84 (56), 73 (38), 72 (28), 43 (78).

The polysaccharide was solvolysed with anhydrous hydrogen fluoride, freshly distilled over cobalt trifluoride[16], for 0.5 h at 20°. The hydrogen fluoride was removed *in vacuo* over solid sodium hydroxide and the solvolysate was diluted with aqueous 1% acetic acid. Gel chromatography on TSK HW 40 afforded **5**, which was subjected to ion-exchange chromatography on DEAE-Trisacryl M, and then heated with methanolic M hydrogen chloride (20 h, 100°, sealed tube). The methanolysate was concentrated, the residue was treated[17] with acetic anhydride in methanol and hydrolysed with 2M hydrochloric acid (2 h, 100°), the hydrolysate was concentrated, and the residue was analysed with an amino acid analyser. Reduction of **5** with an excess of sodium borohydride (24 h, 0°), followed by acidification with conc. acetic acid, and deionisation by gel filtration on TSK HW 40 gave **6**. ^{1}H-N.m.r. data (pH 5): δ 5.27 (d, 1 H, $J_{1,2}$ 3.9 Hz, H-1B), 5.13 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1A), 4.86 (d, 1 H, $J_{4,5}$ 1.4 Hz, H-5A), 4.49 (dd, 1 H, $J_{3,4}$ 3 Hz, H-4B), 4.40 (d, 1 H, $J_{4,5}$ 1 Hz, H-5B), 4.35 (dd, 1 H, $J_{3,4}$ 3.1 Hz, H-4A), 4.32 (dd, 1 H, $J_{2,3}$ 11.5 Hz, H-2B), 4.26 (dd, 1 H, $J_{2,3}$ 11.6 Hz, H-2A), 4.19 (m, 1 H, H-3C), 4.14 (dd, 1 H, H-3B), 4.09 (dd, 1 H, H-3A), 3.6–4.0 (the remaining protons of unit C), 2.13, 2.07, 2.04 (3 s, each 3 H, 3 AcN).

The polysaccharide was *O*-deacetylated with aqueous 1% triethylamine (2 h, 50°) and the product was isolated by gel filtration on TSK HW 40.

The polysaccharide was acetylated[18] with acetic anhydride in pyridine–formamide. The mixture was diluted with water, dialysed against distilled water, and freeze-dried, and the residue was re-acetylated under the same conditions to give the fully *O*-acetylated polysaccharide.

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REFERENCES

1 B. A. DMITRIEV AND N. K. KOCHETKOV, *Dokl. Akad. Nauk SSSR*, 245 (1979) 765–768.
2 B. A. DMITRIEV, V. M. DASHUNIN, Y. A. KNIREL, N. K. KOCHETKOV, AND I. L. HOFMAN, *Bioorg. Khim.*, 5 (1979) 77–82.
3 T. K. GHOSH, C. C. V. N. RAO, A. N. CHAKRABORTY, AND S. G. DASTIDAR, *Carbohydr. Res.*, 102 (1982) 241–252.
4 Y. A. KNIREL, V. M. DASHUNIN, A. S. SHASHKOV, B. A. DMITRIEV, N. K. KOCHETKOV, AND I. L. HOFMAN, *Bioorg. Khim.*, 13 (1987) 1002–1005.
5 C. ALTONA AND C. A. G. HAASNOOT, *Org. Magn. Reson.*, 13 (1980) 417–429.
6 K. BOCK AND C. PEDERSEN, *Adv. Carbohydr. Chem. Biochem.*, 41 (1983) 27–65.
7 E. V. VINOGRADOV, Y. A. KNIREL, A. S. SHASHKOV, AND N. K. KOCHETKOV, *Carbohydr. Res.*, 170 (1987) c1–c4.
8 Y. A. KNIREL, E. V. VINOGRADOV, A. S. SHASHKOV, B. A. DMITRIEV, N. K. KOCHETKOV, E. S. STANISLAVSKY, AND G. M. MASHILOVA, *Eur. J. Biochem.*, 150 (1985) 541–550.
9 K. BOCK AND C. PEDERSEN, *J. Chem. Soc., Perkin Trans. 2*, (1974) 293–297.
10 N. K. KOCHETKOV, O. S. CHIZHOV, AND A. S. SHASHKOV, *Carbohydr. Res.*, 133 (1984) 173–185.
11 D. GAGNAIRE, D. MANCIER, AND M. VINCENDON, *Org. Magn. Reson.*, 11 (1978) 344–349.
12 S. YOKOTA, S. KAYA, T. KAWAMURA, Y. ARAKI, AND E. ITO, *J. Biochem. (Tokyo)*, 99 (1986) 1551–1561.
13 B. LÁNYI, S. VÖRÖS, AND M. ÁDÁM, *Acta Microbiol. Acad. Sci. Hung.*, 20 (1973) 249–254.
14 G. WAGNER AND K. WÜTHRICH, *J. Magn. Reson.*, 33 (1979) 675–680.
15 O. WESTPHAL AND K. JANN, *Methods Carbohydr. Chem.*, 5 (1965) 83–91.
16 A. J. MORT AND D. T. A. LAMPORT, *Anal. Biochem.*, 82 (1977) 289–309.
17 K. NAGASAVA AND Y. INOUE, *Methods Carbohydr. Chem.*, 8 (1980) 291–294.
18 J. HOFMAN, B. LINDBERG, AND S. SVENSSON, *Carbohydr. Res.*, 26 (1972) 661–666.