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Preliminary communication

Synthesis of a branched pentasaccharide fragment of the O-antigen of *Shigella flexneri* serotype 5b

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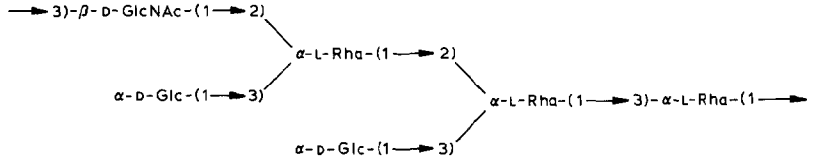
The structures of the O-antigens of all the serotypes of *Sh. flexneri* have been established[1] and the repeating unit of the polysaccharide of serotype 5b has the structure **1**.



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We now present data on the synthesis of the tetra- and penta-saccharide fragments of this repeating unit, aimed at elucidating more precisely the immunological determinants responsible for the O-factors Y and 7,8 (for preliminary immunological studies, see ref. 2).

The α-D-glucosyl-containing trisaccharide derivatives **2a**[3] and **3a**[4] were used as precursors for the stepwise synthesis of the pentasaccharide fragment. The route of synthesis involved selective acylation of diols in the rhamnose series and removal of *O*-acetyl groups in the presence of *O*-benzoyl groups by acid-catalysed methanolysis[5]. Another approach[6] to the synthesis of oligosaccharide fragments of the O-antigens of serotypes 5a, 5b, and variant X is based on the use of a combination of other protecting groups.

Zemplén deacetylation of **2a** gave the syrupy diol **2b** {97%, $[\alpha]_D$ +23° (*c* 1.35, chloroform)}, which was treated with 1.5 mol of acetyl chloride in dichloromethane–pyridine at 0° to yield the syrupy monoacetate **2c** {65%, $[\alpha]_D$ +1° (*c* 1.2, chloroform)} which was easily separated from the minor, isomeric monoacetate by column chromatography. The structure of **2c** followed from the low-field ^{1}H-n.m.r. signal (δ 5.33, dd, *J* 1.5 and 3.5 Hz) for H-2 of the non-glycosylated rhamnosyl group; ^{1}H- and ^{13}C-n.m.r. spectra were recorded for solutions in $CDCl_3$ (internal Me_4Si) unless otherwise stated. The position (98.1 p.p.m., *cf.* 101.2 p.p.m. for **2b**) of the C-1 signal of this rhamnosyl group was also in accord with the structure assigned.

Reaction of **2c** with 2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl bromide (~ 2 equiv.) in dichloromethane in the presence of $Hg(CN)_2$ (2 equiv.) and molecular sieves

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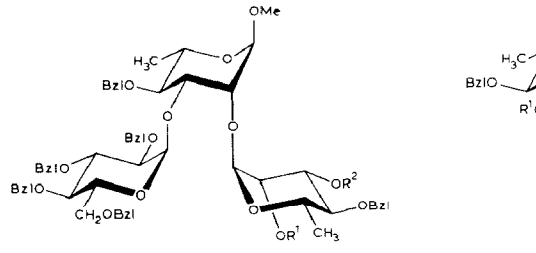
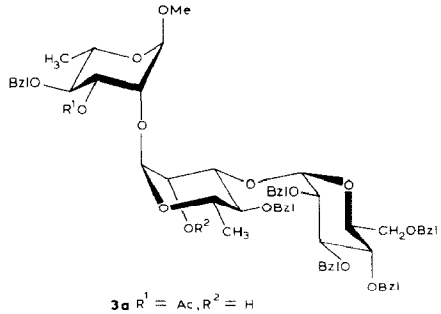
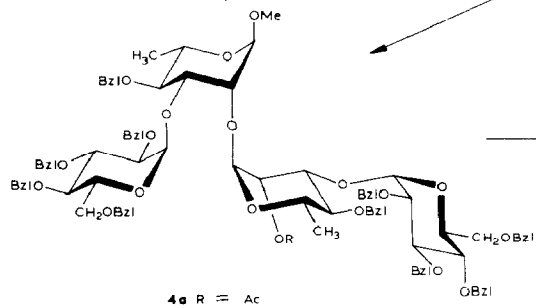
(3 Å; 20°, 1 h) gave a mixture of two tetrasaccharide derivatives differing in configuration of the newly formed glucosidic bond (^{13}C-n.m.r. data), from which 47% of ~90%-pure **4a** was isolated by column chromatography and had $[\alpha]_D$ +40° (*c* 1.3, chloroform). The ^{13}C-n.m.r. spectrum of **4a** contained signals for anomeric carbon atoms at 99.9, 98.1, 95.8, and 92.3 p.p.m. Deacetylation of **4a** afforded amorphous **4c** (80%), $[\alpha]_D$ +45° (*c* 1, chloroform), which gave n.m.r. signals for anomeric carbon atoms at 100.8, 100.1, 95.0, and 93.6 p.p.m., with $^1J_{C,H}$ values of 172, 170, 168, and 168 Hz, respectively, thus



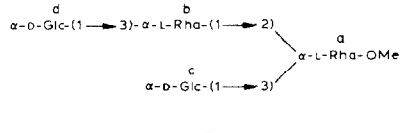
2a $R^1 = R^2 = Ac$
2b $R^1 = R^2 = H$
2c $R^1 = Ac, R^2 = H$



3a $R^1 = Ac, R^2 = H$
3b $R^1 = Ac, R^2 = Bz$
3c $R^1 = H, R^2 = Bz$



4a R = Ac
4b R = Bz
4c R = H



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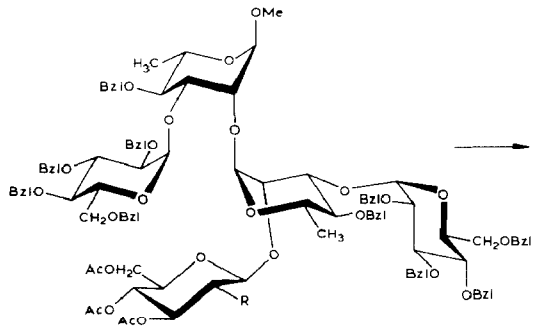
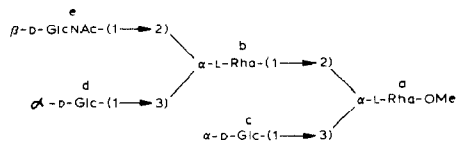


6a R = NPhth
6b R = NHAc



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proving that all the glycosidic bonds were α. The chemical shift values for the signals for C-1 of the α-D-glucopyranosyl groups (95.0 and 93.6 p.p.m.) are close to those for **2a–c** (95.9–94.9 p.p.m.) and **3a–c** (94.1–93.0 p.p.m.), respectively.

Alternatively, **4c** was prepared from **3a**, benzoylation of which gave **3b** (93%), $[\alpha]_D$ +41° (*c* 1, chloroform), and deacetylation[5] then afforded amorphous **3c** (82%), $[\alpha]_D$ +28° (*c* 1.1, chloroform). The ^{1}H-n.m.r. data indicated that the acyl substituent was at O-2′ in **3c** (H-2′, δ 5.75, t, *J* 2.5 Hz) and at O-3 in **3a** (H-3, δ 5.26, dd, *J* 3.2 and 9.5 Hz).

Glucosylation of **3c** under the conditions described above gave a ~3:1 αβ-mixture (^{13}C-n.m.r. data), column chromatography of which gave the di-α-D-glucosylated tetrasaccharide derivative **4b** (60%), $[\alpha]_D$ +25° (*c* 1.1, chloroform). ^{13}C-N.m.r. data: δ 99.95, 98.1, 96.3, and 92.7 (anomeric carbon atoms), $^1J_{C,H}$ 170, 174, 168, and 168 Hz. Debenzoylation of **4b** gave amorphous **4c** in almost quantitative yield, $[\alpha]_D$ +46° (*c* 1.4, chloroform).

Hydrogenolysis (10% Pd/C, methanol–ethyl acetate, 35°, 8 h) of the benzyl groups in **4c** gave the amorphous methyl glycoside **5** (90%), $[\alpha]_D$ +81° (*c* 0.8, methanol), the ^{13}C-n.m.r. spectrum of which contained, *inter alia*, the following, characteristic signals: 100.7 (C-1a), 102.4 (C-1b), 96.5 (C-1c,d) (all $^1J_{C,H}$ values were 170 Hz), 69.7, 70.25 (C-5a,b), and 72.85 p.p.m. (C-5c,d). Chemical shifts for the signals for all C-5 atoms and the $^1J_{C,H}$ values proved all the glycosidic linkages to be α. The high-field position of the signals for C-1c,d accords with the regularities found by Shashkov *et al.*[7] for (1→3)-linked disaccharides having HO-2 or HO-4 axial in the "aglycon" moiety.

The tetrasaccharide derivative **4c** was treated with 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-D-glucopyranosyl bromide (~5 equiv.) in acetonitrile with $Hg(CN)_2$ and $HgBr_2$ (5 equiv. each) under argon. Solvents and reagents were preconditioned using the vacuum technique[8]: solvents were distilled from CaH_2, the glycosyl bromide solution in benzene was lyophilised, and the reagents were dried at 4×10^{-3} mmHg. The pentasaccharide derivative **6a** was obtained (69%) as a syrup, $[\alpha]_D$ +59° (*c* 1.4, chloroform). ^{13}C-N.m.r. data: 99.95, 99.45, 98.1, 97.05, and 94.2 p.p.m. (anomeric carbon atoms).

Attempted silver triflate-assisted glycosylation of **4c** with 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-D-glucopyranosyl bromide (dichloromethane, 2,4,6-trimethylpyridine, −50–−70° → 20°) gave (t.l.c.) only traces of the pentasaccharide derivative **6a**, and 90% of **4c** was recovered together with 13% of 3,4,6-tri-*O*-acetyl-1,5-anhydro-2-deoxy-2-phthalimido-D-*arabino*-hex-1-enitol, $[\alpha]_D$ −2° (*c* 1.5, chloroform), which was identified on the basis of its ^{1}H-n.m.r. spectrum: δ 6.80 (s, H-1), 5.61 (bd, *J* 4 Hz, H-3), 5.35 (t, *J* 4.5 Hz, H-4), 4.37–4.60 (m, H-5,6,6′), 1.94, 2.13, 2.17 (3 s, 3 OAc). The reported[9] $[\alpha]_D$ value is −15°, and ^{1}H-n.m.r. data are δ 6.77 (s), 5.61 (d, *J* 4 Hz), and 5.32 (t, *J* 4 Hz) for H-1,3,4.

Hydrazinolysis of **6a** (99% hydrazine hydrate in boiling ethanol, 5 h) followed by treatment with acetic anhydride–pyridine gave 90% of the *N*-acetyl derivative **6b**, as a chromatographically homogeneous syrup, $[\alpha]_D$ +26° (*c* 1.1, chloroform). ^{13}C-N.m.r. data: 102.5, 99.7, 99.1, 94.2, and 93.9 p.p.m. (anomeric carbon atoms). Zemplén deacetylation of **6b** and hydrogenolysis of the product gave 85% of the target pentasaccharide-glycoside **7** as a white powder, $[\alpha]_D$ +68° (*c* 1, methanol), +71° (*c* 1.2,

water). The ^{13}C-n.m.r. spectrum of 7 (D_2O, internal MeOH) was interpreted with the aid of data for related oligosaccharides[3,4]. That the 2-amino-2-deoxy-D-glucopyranosidic linkage was β was indicated by the $^1J_{C,H}$ value (163 Hz) for the signal of C-1e (102.9 p.p.m.). Other linkages were α, as evidenced from $^1J_{C,H}$ values (174, 170, 170, and 170 Hz) for the C-1a,b,c,d signals (100.7, 102.2, 95.7, and 95.7 p.p.m., respectively). The chemical shifts for the signals of the other carbons were also in accord with the structure 7.

Immunochemical studies of **5** and 7 will be reported elsewhere.

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