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Note

Antigenic determinant of *Salmonella* serogroup B. Synthesis of a trisaccharide glycoside for use as an artificial antigen*

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Considerable effort has been devoted to the synthesis of antigenic determinants related to the O-antigens of *Salmonella* serogroups A, B, and D_1. Thus far, several reports[1–6] have appeared on the synthesis of disaccharides and higher-order, linear oligosaccharide haptens of the O-specific chain. In addition, we have recently reported[7] the synthesis of two-branched-trisaccharide haptens of *Salmonella* serogroups A and D_1, suitably derivatized to permit covalent attachment to proteins, cell surfaces, and immunoabsorbent supports[8]. It was anticipated[7] that this trisaccharide sequence, namely, α-D-Gal-(1→2)-[3,6-dideoxy-α-D-hexopyranosyl-(1→3)]-α-D-Man, would be related to the dominant role played by the 3,6-dideoxyhexoses in *Salmonella* serology, owing to the considerable conformational rigidity imposed by the pyranosyl substituents at vicinal oxygen atoms (O-2 and O-3)[9,10]. We now report the synthesis of the branched trisaccharide structure corresponding to the O-antigenic determinant of *Salmonella* serogroup B. The compounds synthesized in this program of research are of interest, as they can be used to infer a model of O-antigen conformation[9,10], to assist in the selection of hybrid-myeloma antibodies from somatic cell-fusion experiments[11,12], and to study the interaction of the *Salmonella* antigens with monoclonal antibodies.

The synthetic route employed herein utilizes the disaccharide intermediate from our previous work[7]. The crucial, synthetic step is then the addition of the 3,6-dideoxy-α-D-*xylo*-hexopyranose (abequose) residue.

Our synthesis of the appropriately protected abequose residue is analogous to that[13] of its enantiomer, namely, the 3,6-dideoxy-α-L-*xylo*-hexopyranose (colitose) residue. Thus, methyl α-D-fucopyranoside (**1**) was converted into a mixture of (*R*) and (*S*) isomers of the 3,4-*O*-benzylidene acetals **2a**, **2b** by use of α,α-di-

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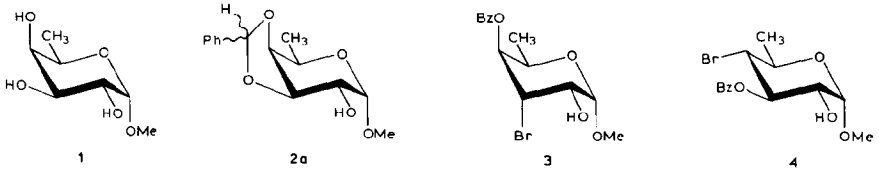

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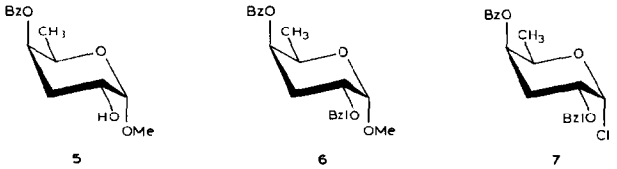

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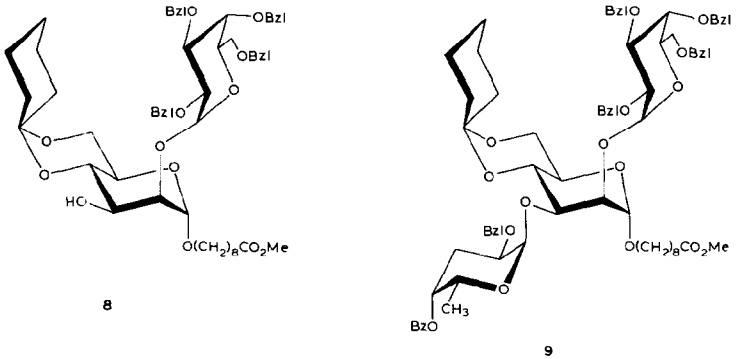

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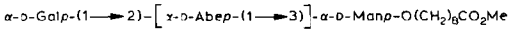

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methoxytoluene in acetonitrile. Treatment of a mixture of **2a** and **2b** with *N*-bromosuccinimide in refluxing carbon tetrachloride[14] yielded a mixture of methyl 4-*O*-benzoyl-3-bromo-3,6-dideoxy-α-D-gulopyranoside (**3**) and methyl 3-*O*-benzoyl-4-bromo-4,6-dideoxy-α-D-glucopyranoside (**4**) in the ratio of 5:1. Proof of structures was provided by comparison of the ^{13}C-n.m.r. parameters with those of the corresponding, L enantiomers[13]. The bromo sugar **3** was readily reduced to the 4-*O*-benzoyl dideoxyhexoside **5** by hydrogenation in the presence of palladium in ethanol solution. Owing to the base-lability of the benzoic ester, the 2-hydroxyl group in **5** was benzylated with benzyl trichloroacetimidate[15] under catalysis with triflic acid. Thus, methyl 4-*O*-benzoyl-2-*O*-benzyl-3,6-dideoxy-α-D-*xylo*-hexopyranoside (**6**) was obtained in a yield of 80%. Compound **6** was converted into the glycosyl chloride (**7**), using dichloromethyl methyl ether in the presence of zinc bromide[7,16,17].

Freshly prepared **7** reacted with the selectively protected disaccharide[7] **8**, using silver triflate and 1,1,3,3-tetramethylurea in dichloromethane solution[18], the yield of the α-linked trisaccharide **9** being 67% after chromatography. The protected trisaccharide **9** was trans-esterified, and the product hydrogenolyzed in the presence of palladium-on-charcoal in aqueous acetic acid solution. The crude trisaccharide thus obtained was purified by chromatography on silica gel. The product was analytically pure, and its ^{13}C-n.m.r. and ^{1}H-n.m.r. data[10] were consistent with the anticipated structure **10**.

N.m.r. measurements of trisaccharide **10** and other related oligosaccharides have been used to infer a model of the conformation[10] of *Salmonella* O-antigen. Compound **10** has also been converted into the corresponding glycoconjugate by a simplified, acyl azide methodology[8], and used in conjunction with the hybridoma technique to characterise serogroup B specific monoclonal antibodies.

EXPERIMENTAL

General. — The general methods and materials employed in this work were similar to those described in recent publications from this laboratory[19,20]. Chromatography on a column of silica gel, performed at a pressure of 300–500 kPa according to a published procedure[21], was used for separations. ^{13}C-N.m.r. spectra were recorded at 20 MHz. Chemical shifts are expressed relative to internal and external tetramethylsilane for solutions in chloroform-*d* or methanol-d_4, and deuterium oxide, respectively. Chemical-shift assignments were made by analogy with previous work[7,13,22,23], and were confirmed, when necessary, by means of gated-decoupling experiments.

*Methyl α-*D*-fucopyranoside* (**1**). — 6-Deoxy-6-iodo-1,2:3,4-di-*O*-isopropylidene-α-D-galactose[24] (54 g) in ethanol (180 mL) containing triethylamine (29 mL) was hydrogenated at 462 kPa with hydrogen in the presence of 10% palladium-on-charcoal (1.3 g) for 12 h. After filtration, and evaporation of the filtrate, the residue was dissolved in dichloromethane, succesively washed with water, sodium thiosulfate, and water, dried (anhydrous sodium sulfate), and evaporated, to afford a liquid (34.9 g) which was treated with boiling 60% acetic acid under reflux for 5 h. After evaporation, the residual syrup was dried under vacuum to yield a foam. Crystallization from ethanol afforded D-fucose as a white solid (21.1 g).

The solid was dissolved in anhydrous methanol (150 mL), and the solution mixed with a solution of trifluoromethanesulfonic acid (2.7 mL) in methanol (140 mL), boiled under reflux for 12 h, cooled, and the acid neutralized by addition of Rexyn 201 (OH^-) resin. The suspension was filtered, and the filtrate was evaporated, to afford a solid. Recrystallization from ethyl acetate gave methyl α-D-fucopyranoside (**1**; 13.1 g) as white crystals; m.p. 156–157°, lit.[25] m.p. 157–158°; ^{13}C-n.m.r. (CD_3OD): δ_C 101.5 (C-1), 73.5 (C-4), 71.6 (C-3), 69.9 (C-2), 67.3 (C-5), 55.6 (OCH_3), and 16.6 (C-6).

*Methyl (*S *and* R*)-3,4-*O*-benzylidene-α-*D*-fucopyranoside* (**2a** *and* **2b**). — To a solution of **1** (5 g, 28.8 mmol) in dry acetonitrile (50 mL) were added α,α-di-

methoxytoluene (7 g, 46 mmol) and *p*-toluenesulfonic acid (50 mg), and the mixture was stirred for 12 h at room temperature and then heated for 5 h at 50–60° under diminished pressure. Triethylamine (1 mL) was added, the solution was evaporated, the residue was dissolved in ethyl acetate, and the solution was washed successively with sodium hydrogencarbonate and water, dried (Na_2SO_4), and evaporated to a syrup Chromatography using 4:1 Skellysolve B–ethyl acetate as the eluant, followed by recrystallization from dichloromethane–Skellysolve B, afforded a crystalline mixture of diastereoisomers (5.04 g, 68%); ^{13}C-n.m.r. ($CDCl_3$): Isomer **2a**, δ_C 103.0 (Ph*C*H), 99.0 (C-1), 77.5 (C-4), 76.0 (C-3), 67.7 (C-2), 63.7 (C-5), 55.5 (OMe), and 16.4 (C-6); Isomer **2b**, 104.0 (Ph*C*H), 98.7 (C-1), 77.7 (C-4), 76.2 (C-3), 69.5 (C-2), 63.7 (C-5), 55.5 (OMe), and 16.3 (C-6). Assignments were facilitated by chromatographic separation of a small sample into its two components.

Anal. Calc. for $C_{14}H_{18}O_5$: C, 63.15; H, 6.81. Found: C, 63.01; H, 6.88.

*Methyl 4-O-benzoyl-3-bromo-3,6-dideoxy-α-*D*-gulopyranoside* (**3**) *and methyl 3-O-benzoyl-4-bromo-4,6-dideoxy-α-*D*-glucopyranoside* (**4**). — *N*-Bromosuccinimide (2.02 g, 11.5 mmol) was added to a suspension of a mixture of **2a** and **2b** (2.27 g, 8.53 mmol) and barium carbonate (1.01 g) in carbon tetrachloride (115 mL), and the mixture was boiled under reflux for 3 h. The solids were removed by filtration, and the filtrate was diluted with dichloromethane, washed with water, dried (Na_2SO_4), and evaporated, to afford a syrup (3.25 g). Chromatography using 4:1 Skellysolve B–ethyl acetate as the eluant yielded pure **3** (1.98 g, 59%) as a syrup; $[\alpha]_D^{20}$ +159.6° (*c* 0.8, dichloromethane); ^{13}C-n.m.r. ($CDCl_3$): δ_C 99.3 (C-1), 74.6 (C-4), 64.0 (C-2), 60.3 (C-5), 55.5 (OMe), 50.2 (C-3), and 16.2 (C-6).

Anal. Calc. for $C_{14}H_{17}BrO_5$: C, 48.71; H, 4.96; Br, 23.15. Found: C, 48.64; H, 4.81; Br, 22.97.

Compound **4** was obtained as a solid, and was recrystallized from dichloromethane–Skellysolve B (yield, 0.41 g, 12.2%); m.p. 143–144°, $[\alpha]_D^{20}$ +77.7° (*c* 0.5, dichloromethane); ^{13}C-n.m.r. ($CDCl_3$): δ_C 99.5 (C-1), 75.5 (C-3), 72.5 (C-2), 68.2 (C-5), 55.7 (OMe), 52.8 (C-4), and 19.2 (C-6).

Anal. Calc. for $C_{14}H_{17}BrO_5$: C, 48.71; H, 4.96; Br, 23.15. Found: C, 48.87; H, 4.85; Br, 23.29.

*Methyl 4-O-benzoyl-3,6-dideoxy-α-*D*-*xylo*-hexopyranoside* (**5**). — The 3-bromo compound **3** (1.98 g, 5.7 mmol) was hydrogenated at 462 kPa for 12 h at room temperature in the presence of 10% palladium-on-charcoal (0.5 g) in ethanol (100 mL) containing triethylamine (0.9 g). After filtration and evaporation, a solution of the resulting residue in dichloromethane was successively washed with sodium hydrogencarbonate and water, dried (Na_2SO_4), and evaporated, to yield a syrup. Crystallization from 3:1 Skellysolve B–ethyl acetate afforded **5** as white prisms (1.2 g, 80%); m.p. 118–119°, $[\alpha]_D^{20}$ +115.9° (*c* 0.7, dichloromethane); ^{13}C-n.m.r. ($CDCl_3$): δ_C 99.4 (C-1), 71.8 (C-4), 64.8 (C-5), 64.2 (C-2), 55.3 (OMe), 32.2 (C-3), and 16.3 (C-6).

Anal. Calc. for $C_{14}H_{18}O_5$: C, 63.15; H, 6.81. Found: C, 63.31; H, 6.92.

*Methyl 4-O-benzoyl-2-O-benzyl-3,6-dideoxy-α-*D*-*xylo*-hexopyranoside* (**6**). —

A suspension of the 2-hydroxy compound **5** (0.9 g, 3.38 mmol) and benzyl trichloroacetimidate[15] (1.71 g, 6.8 mmol) in cyclohexane (12 mL) and carbon tetrachloride (12 mL) was treated with trifluoromethanesulfonic acid (3 drops), and the mixture was stirred for 18 h at room temperature. The mixture was filtered, and the filtrate was diluted with dichloromethane, successively washed with sodium hydrogencarbonate and water, dried (Na_2SO_4), and evaporated, to give a syrup and solid. Chromatography using 6:1 Skellysolve B–ethyl acetate as the eluant afforded **6** as a colorless syrup (0.93 g, 78%); $[\alpha]_D^{20}$ +85.8° (*c* 1.1, dichloromethane); ^{13}C-n.m.r. ($CDCl_3$): δ_C 98.1 (C-1), 71.8 (C-4), 71.0 (CH_2Ph), 70.7 (C-2), 64.9 (C-5), 55.3 (OMe), 29.1 (C-3), and 16.4 (C-6).

Anal. Calc. for $C_{21}H_{24}O_5$: C, 70.79; H, 6.74. Found: C, 70.64; H, 6.82.

*4-O-Benzoyl-2-O-benzyl-3,6-dideoxy-α-*D-xylo-*hexopyranosyl chloride* (**7**). — A solution of **6** (0.47 g, 1.32 mmol) in dichloromethyl methyl ether (5 mL) was treated with zinc bromide (~50 mg), and the mixture was stirred under an atmosphere of nitrogen for 2 h, and filtered through glass wool; the latter was rinsed with anhydrous dichloromethane, and the filtrate and washings were combined and evaporated under high vacuum, to give light-orange, syrupy **7** that was dried for 1.5 h under high vacuum and then used in the glycosylation step; ^{13}C-n.m.r. ($CDCl_3$): δ_C 94.7 (C-1), 70.8, 70.7 (C-2, C-4, CH_2Ph), 68.1 (C-5), 29.2 (C-3), and 16.1 (C-6).

*8-Methoxycarbonyloctyl 3-O-(4-O-benzoyl-2-O-benzyl-3,6-dideoxy-α-*D-xylo-*hexopyranosyl)-4,6-O-cyclohexylidene-2-O-(tetra-O-benzyl-α-*D-*galactopyranosyl)-α-*D-*mannopyranoside* (**9**). — A mixture of 8-methoxycarbonyloctyl 4,6-*O*-cyclohexylidene-2-*O*-(tetra-*O*-benzyl-α-D-galactopyranosyl)-α-D-mannopyranoside[5] (**8**) (0.49 g, 0.51 mmol), silver trifluoromethanesulfonate (0.47 g, 1.81 mmol), 1,1,3,3-tetramethylurea (0.75 mL, 6.3 mmol), and 4A molecular sieves in anhydrous dichloromethane (6 mL) was stirred under an atmosphere of nitrogen for 4 h. The mixture was then cooled to −70°, a solution of the glycosyl halide **7** (1.3 mmol) in anhydrous dichloromethane (4 mL, previously stirred with 4A molecular sieves for 0.5 h under nitrogen) at −70° was added by means of a double-tipped needle under nitrogen, and the flask was rinsed with additional dichloromethane (5 mL). The final mixture was concentrated to 8 mL under high vacuum and then allowed to warm gradually to room temperature. The mixture was stirred for 48 h under nitrogen, the solids were filtered off, and the filtrate was evaporated to a syrup. Chromatography using 5:1 Skellysolve B–ethyl acetate as the eluant yielded pure **9** as a syrup (0.435 g, 67%); $[\alpha]_D^{20}$ +95.2° (*c* 0.3, dichloromethane); ^{13}C-n.m.r. ($CDCl_3$): δ_C 100.3 (acetal C), 99.8 (C-1), 97.3, and 96.9 (C-1′, C-1″).

Anal. Calc. for $C_{76}H_{92}O_{17}$: C, 71.47; H, 7.21. Found: C, 71.53; H, 7.15.

Some unreacted, starting disaccharide **8** (0.135 g, 27%) was recovered.

*8-Methoxycarbonyloctyl 3-O-(3,6-dideoxy-α-*D-xylo-*hexopyranosyl)-2-O-α-*D-*galactopyranosyl-α-*D-*mannopyranoside* (**10**). — A solution of the trisacharide **9** (0.36 g, 0.28 mmol) in dry methanol (40 mL) and dry dichloromethane (10 mL) at 0° was treated with 0.9M sodium methoxide in methanol (10 mL). The mixture was allowed to warm to room temperature, and was stirred for 12 h. Following neutrali-

zation of the base with Rexyn 101 (H^+) resin, the latter was removed by filtration, and the filtrate was evaporated to a syrup. This was dissolved in 80% aqueous acetic acid (25 mL) and hydrogenolyzed in the presence of 10% palladium-on-charcoal (0.2 g) at 482 kPa for 24 h. The mixture was filtered through Celite, which was then washed with ethanol, and the filtrate and washings were combined, and evaporated, to give a syrup. Chromatography using 7:2:1 ethyl acetate–methanol–water as the eluant afforded pure, amorphous **10** (0.122 g, 67%); $[\alpha]_D^{20}$ +106.8° (*c* 0.4, water); ^{13}C-n.m.r. (D_2O): δ_C 102.4 (C-1″), 101.6 (C-1′), 99.3 (C-1), 53.3 (OMe), and 16.7 (C-6″).

Anal. Calc. for $C_{28}H_{50}O_{16}$: C, 52.33; H, 7.84. Found: C, 52.09; H, 7.88.

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