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Note

Synthesis of methyl 2-*O*-α-D-galactopyranosyl-3-*O*-β-D-glucopyranosyl-α-D-glucopyranoside, a trisaccharide of the R-1 core antigen of *Enterobacteriaceae*

Sumanta Basu* and Jitendra N. Pal
Department of Biological Chemistry, Indian Association for the cultivation of Science, Jadavpur, Calcutta 700 032 (India)

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Five different complete R-core structures have so far been reported from *Escherichia coli* serotypes[1,2]. They are disgnated as R-1–R-4 and K-12 core types. They all differ in the outer hexose region, whereas the inner region composed of L-*glycero*-D-*manno*-heptose and 3-deoxy-α-D-*manno*-octulopyranosonic acid (Kdo) seems to be the same, as revealed by ^{1}H-n.m.r. analysis[3]. Core types with R-1 and R-4 structures were found to be covalently linked to the enterobacterial common antigen (ECA), which is a cell-surface antigen shared by all members of the *Enterobacteriaceae*[4]. In this communication we report the synthesis of the title trisaccharide unit, a component of the R-1 core pentasaccharide structure which could be used for mapping the combining sites of a bacteriophage receptor and monoclonal antibodies which recognize this structure.

Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (**1**) (ref. 5) was prepared from methyl α-D-glucopyranoside by reacting with α,α-dimethoxytoluene in presence of *p*-toluenesulfonic acid. Partial allylation of **1** by the phase-transfer technique[6,7] gave methyl 2-*O*-allyl-4,6-*O*-benzylidene-α-D-glucopyranoside (**2**). Condensation of the product with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**3**) gave methyl 2-*O*-allyl-4,6-*O*-benzylidene-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-D-glucopyranoside (**4**). Removal of the acetyl and benzylidene groups from **4** gave methyl 2-*O*-allyl-3-*O*-(β-D-glucopyranosyl)-α-D-glucopyranoside (**5**). Benzylation[8] of **5**, followed by deallylation[9] of the product (**6**), gave methyl 4,6-di-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-α-D-glucopyranoside (**7**). Reaction of compound **7** with ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (**8**), promoted by methyl triflate[10,11,12], gave the trisaccharide derivative **9** in 46% yield. Removal of protecting groups from **9** with hydrogen in presence of palladium-on-charcoal gave the title trisaccharide **10**. Methylation analysis of **10** gave 2,3,4,6-tetra-*O*-methyl-D-glucopyra-

* Author for correspondence.

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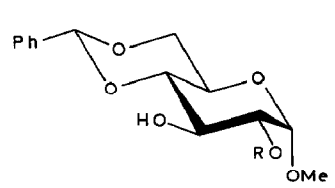
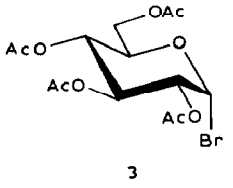


1 R = H
2 R = allyl



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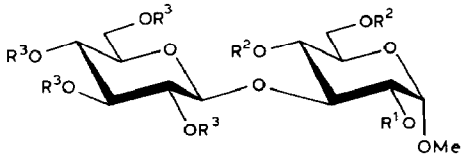
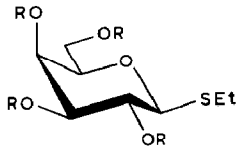
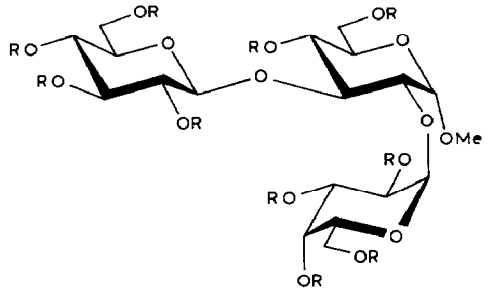


4 R^1 = allyl; R^2, R^2 = CHPh, R^3 = OAc
5 R^1 = allyl; R^2, R^3 = H
6 R^1 = allyl; R^2, R^3 = CH_2Ph
7 R^1 = H; R^2, R^3 = CH_2Ph



8 R = CH_2Ph



9 R = CH_2Ph
10 R = H

nose, 2,3,4,6-tetra-*O*-methyl-D-galactopyranose and 4,6-di-*O*-methyl-D-glucopyranose, which were identified by g.l.c. as their alditol acetates.

EXPERIMENTAL

General methods. — Optical rotations were measured with a Perkin–Elmer Model 241 MC spectropolarimeter. N.m.r. spectra were recorded with a JEOL FX-100 spectrometer for solutions in chloroform-*d* containing Me_4Si as an internal standard and for solution in methyl sulfoxide-d_6, using the solvent ^{13}C resonance (δ 39.5) as reference. All reactions were monitored by t.l.c. on Silica Gel G (Merck). Column chromatography was performed on Silica Gel 60 (Merck). All evaporations were carried out under reduced pressure at a temperature $<40°$. G.l.c. was performed at 190° for alditols acetates and 170° for partially methylated alditol acetates with a Hewlett-Packard Model 5730-A instrument fitted with a Model 3380-A electronic integrator and a glass

column (1.83 m × 6 mm) packed with 3% ECNSS-M on Gas Chrom Q (100–120 mesh).

Preparation of methyl 2-O-allyl-4,6-O-benzylidene-α-D-glucopyranoside (**2**). — A solution of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside[5] (**1**, 6 g, 21.3 mmol) in dichloromethane (230 mL), allyl bromide (2 mL, 24 mmol), tetraethylammonium bromide (1.8 g, 5.5 mmol) and 5% aq. sodium hydroxide (30 mL) in a round bottom flask was vigorously stirred for 3 days at 25°. The organic layer was washed with water (4 × 50 mL), dried (Na_2SO_4), and concentrated. T.l.c. (2:1, benzene–ether) of the syrupy residue revealed one major and three minor spots. Column chromatography, using the same solvent mixture, gave **2** (3.6 g, 52.5%) together with the 3-*O*-allyl derivative (20%) and **1**. Compound **2** had m.p. 110–112°; $[\alpha]_D^{30}$ +79.4° (*c* 1.8, chloroform); lit.[13,14] m.p. 115–116°, 116°, $[\alpha]_D$ +75.8°, +78.8°. ^{1}H-N.m.r. data: δ 7.20 (m, 5 H, 1 Ph), 5.88 (dddd, 1 H, allyl C*H*), 5.47 (s, 1 H, C*H*Ph), 4.81 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 3.40 (s, 3 H, OMe).

Methyl 2-O-allyl-4,6-O-benzylidene-3-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-α-D-glucopyranoside (**4**). — To a mixture of $Hg(CN)_2$ (4.5 g, 17.81 mmol), powdered 4A molecular sieves (4 g), Drierite (4 g) and compound **2** (2.2 g, 6.83 mmol) in 1:1 benzene–nitromethane (60 mL) was added a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide[15] (**3**, 4.7 g, 11.43 mmol) in 1:1 benzene–nitromethane (20 mL). The mixture was stirred for 72 h at 35° under nitrogen, diluted with dichloromethane (150 mL), and the solids were removed by filtration through a Celite bed. The filtrate was washed with water, M KI solution, saturated aq. $NaHCO_3$, and water in succession, and then evaporated to dryness. Examination of the crude reaction product by t.l.c. (4:1 benzene–ether) revealed the presence of a major product, slower migrating than **2**, along with some unchanged **2**. Column chromatography of the mixture gave **4** (2 g, 45%): m.p. 139–141° (ether–petroleum ether); $[\alpha]_D^{30}$ +10° (*c* 2.0, chloroform). ^{1}H-N.m.r. data: δ 7.40 (m, 5 H, 1 Ph), 5.84 (dddd, 1 H, allyl C*H*), 5.46 (s, 1 H, C*H*Ph), 4.72 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 5.30 (d, 1 H, $J_{1,2}$ 8 Hz, H-1'), 3.40 (s, 3 H, OMe), 2.04, 1.96 (12 H, 4 Ac).

Anal. Calc. for $C_{31}H_{40}O_{15}$: C, 57.05; H, 6.18. Found: C, 57.12; H, 6.15.

Methyl 4,6-di-O-benzyl-3-O-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)-α-D-glucopyranoside (**7**). — A solution of **4** (1.5 g, 2.3 mmol) in 0.01M NaOMe–MeOH (100 mL) was stirred for 2 h at 20°, decationized with Amberlite IR-120 [H^+] resin, filtered, and the solution was evaporated to dryness (1.1 g, 2.27 mmol). The product was heated with 80% aq. acetic acid (25 mL) for 3 h. Evaporation of the solvents under reduced pressure gave methyl 2-*O*-allyl-3-*O*-(β-D-glucopyranosyl)-α-D-glucopyranoside (**5**) as a foam (0.85 g, 2.15 mmol). To a cooled solution of **5** (0.85 g, 2.15 mmol) in dry DMF (15 mL), NaH (50% in oil, 1.9 g, 39.6 mmol) was carefully added. To this mixture, benzyl bromide (5 mL, 40 mmol) was added dropwise with stirring. After stirring for 2 h at 0°, the reaction mixture was allowed to stand at room temperature for an additional 3 h. The excess NaH was then decomposed by the addition of methanol. The mixture was diluted with dichloromethane, and the organic layer was washed with water, dried (Na_2SO_4), and the solvent was evaporated to give an oily residue. Column chromatography of this residue in 10:1 benzene–ether afforded compound **6** (1.8 g, 1.92 mmol) as a syrup.

Acetic acid (0.15 mL, 2.33 mmol) was added to a solution of this syrup in 1,4-

dioxane (20 mL) in the presence of selenium(IV) dioxide (229 mg, 2.31 mmol). The mixture was heated under reflux with stirring for 1 h, filtered through Celite, and concentrated. Column chromatography (4:1 benzene–ether) of the residue gave **7** (1.38 g, 1.54 mmol, 75%) as a syrup: $[\alpha]_D^{30}$ +65.9° (*c* 7.0, chloroform). ^{1}H-N.m.r. data: δ 7.55–7.5 (m, 30 H, 6 Ph), 4.82 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 4.35 (d, 1 H, $J_{1,2}$ 7 Hz, H-1′), 3.40 (s, 3 H, OMe).

Anal. Calc. for $C_{55}H_{60}O_{11}$: C, 73.64; H, 6.74. Found: C, 73.61; H, 6.64.

*Ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-*D*-galactopyranoside* (**8**). — Ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside[16,17] (3 g, 7.65 mmol) was dissolved in 0.01M NaOMe (70 mL) and stirred for 3 h at room temperature. The solution was neutralized with Amberlite IR-120 [H$^+$] resin and evaporated to give the thioglycoside as a solid mass (1.71 g). The thioglycoside (1.7 g) was added portionwise to a suspension of NaH (50% in oil, 1.68 g, 35 mmol) in DMF (20 mL). The mixture was stirred for 30 min at 25°, and $PhCH_2Br$ (5 mL, 40 mmol) was added dropwise at 0–5°. The mixture was then stirred for 10 h at 25°. After usual work-up, the product **8** (3.6 g, 81%) was isolated and crystallised from ethanol: m.p. 52°; $[\alpha]_D^{30}$ −15° (*c* 0.9, chloroform). ^{1}H-N.m.r. data: δ 7.5–7.2 (m, 20 H, 4 Ph), 4.35 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 2.64 (q, 2 H, CH_2), 1.24 (t, 3 H, CH_3).

Anal. Calc. for $C_{36}H_{40}O_5S$: C, 73.94; H, 6.89. Found: C, 74.00; H, 6.81.

*Methyl 4,6-di-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-benzyl-α-*D*-galactopyranosyl)-3-*O*-(2,3,4,6-tetra-*O*-benzyl-β-*D*-glucopyranosyl)-α-*D*-glucopyranoside* (**9**). — Methyl trifluoromethanesulfonate (0.7 mL, 6 mmol) was added to a stirred mixture of compounds **7** (1 g, 1.12 mmol) and **8** (900 mg, 1.54 mmol) in ether (10 mL) which contained powdered 4A molecular sieves (4 g). The mixture was stirred for 36 h at room temperature. Triethylamine (2 mL) was added, and stirring was continued for 10 min. The mixture was filtered through Celite, concentrated, and purified by column chromatography using 3:1 benzene–ether as eluant to yield **9** as a syrup (725 mg, 0.51 mmol, 46%): $[\alpha]_D^{30}$ +36° (*c* 4.0, chloroform). ^{1}H-N.m.r. data: δ 7.40–7.20 (m, 50 H, 10 Ph), 3.40 (s, 3 H, OMe).

Anal. Calc. for $C_{89}H_{94}O_{16}$: C, 75.30; H, 6.67. Found: C, 75.37; H, 6.77.

*Methyl 2-*O*-α-*D*-galactopyranosyl-3-*O*-β-*D*-glucopyranosyl-α-*D*-glucopyranoside* (**10**). — A solution of **9** (700 mg) in 1:1 methanol–ethyl acetate (20 mL) was stirred under hydrogen for 36 h at room temperature in the presence of 10% Pd–C (800 mg), then filtered through Celite, and concentrated to dryness. The product was purified on a column (2.5 × 80 cm) of Bio-Gel P-2 using water as eluant. After freeze drying, **10** was obtained as an amorphous powder (180 mg, 70%): $[\alpha]_D^{30}$ +56° (*c* 5.0, water). ^{1}H-N.m.r. data (Me_2SO-d_6): δ 4.86 (d, 1 H, $J_{1,2}$ 3 Hz, H-1), 4.82 (d, 1 H, $J_{1,2}$ 4 Hz, H-1′), 4.38 (d, 1 H, $J_{1,2}$ 8 Hz, H-1″), 3.39 (s, 3 H, OMe); ^{13}C-n.m.r. δ 102.62 (C-1″), 95.89 (C-1′), 95.60 (C-1), 60.7, 60.6, 60.3 (3 C-6), 54.2 (OMe).

Anal. Calc. for $C_{19}H_{34}O_{16}$: C, 44.02; H, 6.61. Found: C, 43.97; H, 6.61.

*Characterisation of methyl 2-*O*-allyl-4,6-benzylidene-α-*D*-glucopyranoside* (**2**). — Compound **2**, after methylation, was *O*-deallylated[9], then O-debenzylidenated, and converted into the alditol acetate[19]. G.l.c. analysis showed it to be the alditol acetate derivative of 3-*O*-methyl-D-glucose.

Acidic hydrolysis and methylation analysis of **10**. — Acidic hydrolysis and methylation analysis were carried out as has been described[19].

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