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## Note

# Synthesis of 6- and 6′-deoxy derivatives of methyl 4-*O*-α-D-galactopyranosyl-β-D-galactopyranoside for studies of inhibition of pyelonephritogenic fimbriated *E. coli* adhesion to urinary epithelium-cell surfaces

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The structural unit 4-*O*-α-D-galactopyranosyl-β-D-galactopyranose is part of the receptor sites in urinary tract epithelium involved in adhesion by fimbriated *E. coli* bacteria[1,2]. Methyl, *p*-nitrophenyl, and 2-bromoethyl glycosides, and derivatives of the latter, as well as of 4-*O*-(4-*O*-α-D-galactopyranosyl-β-D-galactopyranosyl)-β-D-glucopyranose, have been synthesised[3–9].

A major contribution to binding between carbohydrates and proteins has been proposed to arise from hydrophobic interactions[10,11]. This hypothesis may be tested by removing hydroxyl groups from selected positions of an oligosaccharide hapten and comparing the binding properties of the deoxy compounds thus obtained with those of the parent compound[11]. Another impetus for the present work was the expectation that D-fucosyl analogues of the 4-*O*-α-D-galactopyranosyl-β-D-galactopyranose unit should be less rapidly degraded *in vivo* than the parent compound.

Partial benzoylation of methyl β-D-galactopyranoside gave the expected[5] 2,3,6-tribenzoate **1**, which was glycosylated with 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl chloride using silver trifluoromethanesulfonate as promoter[12], and the product was catalytically hydrogenolysed to give 80% of the disaccharide derivative **2**. Compound **2** was then converted into the key 4′,6′-*O*-benzylidene derivative **3** by treatment with benzaldehyde and formic acid[13]. Acetylation of **3**, followed by treatment of the product with *N*-bromosuccinimide[14], yielded the 6′-bromo derivative **4** which was deacylated and then catalytically hydrogenolysed to yield methyl 4-*O*-α-D-fucopyranosyl-β-D-galactopyranoside (**5**).

Partial debenzoylation of **3** with methanolic sodium methoxide gave 66% of the 2,3-dibenzoate **6** with HO-6 free. The primary hydroxyl group was converted into a deoxyiodo group in 94% yield by treatment with the triphenylphosphine–imidazole–iodine reagent[15]. The resulting 6-deoxy-6-iodo compound **7** was catalytically hydrogenated in the presence of triethylamine, and the product **8** was hydrolysed and deacylated to yield methyl 4-*O*-α-D-galactopyranosyl-β-D-fucopyranoside (**9**).

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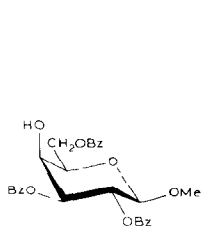

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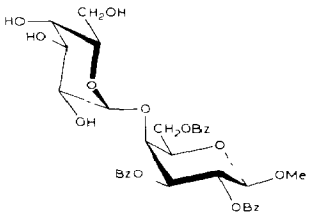

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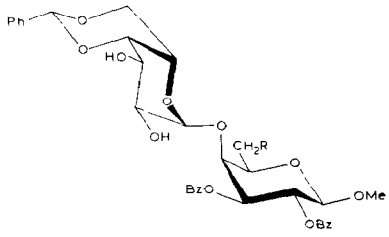
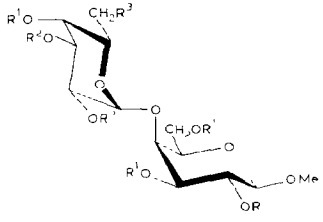
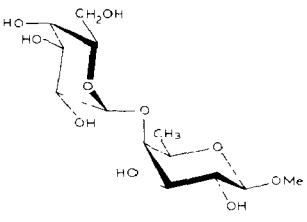


3 R = OBz
6 R = OH
7 R = I
8 R = H



4 R' = Bz R² = Ac, R³ = Br
5 R' = R² = R³ = H



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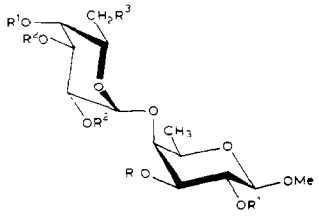

10 R' = Bz R² = Ac, R³ = Br
11 R = R² = R³ = H

Acetylation of **8** and treatment of the product with *N*-bromosuccinimide[14] gave the 6′-bromo-6′-deoxy compound **10**. Partial deacylation of **10** and catalytic hydrogenation in the presence of triethylamine, followed by deacylation, yielded methyl 4-*O*-α-D-fucopyranosyl-β-D-fucopyranoside (**11**).

Immunochemical and other work on the disaccharides **5, 9,** and **11** will be reported elsewhere.

## EXPERIMENTAL

*General methods.* — These were the same as those previously reported[16]. N.m.r. spectra ($^1$H at 100 MHz, $^{13}$C at 25 MHz), recorded for all new compounds, accorded with the postulated structures. Syrupy compounds were not subjected to elemental analysis.

*Methyl 2,3,6-tri-O-benzoyl-β-D-galactopyranoside* (**1**). — Benzoyl chloride (13 mL) in pyridine (20 mL) was added, dropwise with stirring, to a solution of methyl β-D-galactopyranoside (7.0 g) in pyridine (50 mL) at −40°. The solution was left at room temperature overnight. After the usual work-up followed by column chromatography (toluene–ethyl acetate, 9:1), **1** (10.0 g, 55%) was obtained; m.p. 142–143° (from ethanol), $[\alpha]_D$ +54° (*c* 1.1, chloroform). $^{13}$C-N.m.r. data ($CDCl_3$, internal $Me_4Si$); δ 56.7, 63.3, 67.4, 69.7, 72.6, 74.4, 102.2, 128.4, 129.1, 129.2, 129.7, 133.1, 133.2, 133.3, 165.6, 166.0, 166.5.

*Anal.* Calc. for $C_{28}H_{26}O_9$: C, 66.4; H, 5.17. Found: C, 66.3; H, 5.21.

*Methyl 2,3,6-tri-O-benzoyl-4-O-α-D-galactopyranosyl-β-D-galactopyranoside* (**2**). — A solution of 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl chloride (7.5 g) in dry toluene (20 mL) was added with stirring to a solution of **1** (5.0 g), silver trifluoromethanesulfonate (3.8 g), and 2,4,6-trimethylpyridine (1.9 g) in dry toluene (125 mL) at −40°. The mixture was stirred at room temperature for 2 h, filtered, and concentrated. Column chromatography (toluene–ethyl acetate, 19:1) of the residue gave a crude product (11.2 g) which was hydrogenated in acetic acid (20 mL) over 10% Pd/C (1 g). The mixture was filtered and concentrated. Column chromatography (ethyl acetate) of the residue gave **2** (5.3 g, 80%), $[\alpha]_D$ +73° (*c* 0.9 chloroform). $^{13}$C-N.m.r. data ($CDCl_3$): δ 57.3, 62.2, 62.6, 69.4, 70.0, 71.1, 72.9, 73.6, 74.2, 101.2, 102.4, 128.4, 128.6, 129.1, 129.4, 129.8, 133.3, 133.6, 165.7, 166.1, 166.3.

A portion of **2** was benzoylated to give methyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl)-β-D-galactopyranoside, m.p. 217–218°, $[\alpha]_D$ +99° (*c* 1.6, chloroform); lit.[5] m.p. 222–223°, $[\alpha]_D$ +96°. The $^{13}$C-n.m.r. spectra were superimposable.

*Methyl 2,3,6-tri-O-benzoyl-4-O-(4,6-O-benzylidene-α-D-galactopyranosyl)-β-D-galactopyranoside* (**3**). — Compound **2** (3.4 g) was dissolved in formic acid (5 mL) and benzaldehyde (5 mL) at room temperature. T.l.c. (toluene–ethyl acetate, 1:3) showed complete reaction after 2 min. The solution was poured into a stirred mixture of saturated aqueous sodium hydrogencarbonate (30 mL) and light petroleum (b.p. 40–60°, 30 mL). The organic phase was decanted off and the remainder was extracted with dichloromethane. The extract was washed twice with saturated aqueous sodium hydrogencarbonate, dried ($Na_2SO_4$), filtered, and concentrated. Column chromatography (toluene–ethyl acetate, 1:1) of the residue gave **3** (3.5 g, 91%), $[\alpha]_D$ +54° (*c* 1.5, chloroform). $^{13}$C-N.m.r. data ($CDCl_3$): δ 57.2, 61.3, 63.5, 68.6, 68.8, 69.6, 69.7, 72.4, 74.2, 74.8, 76.0, 100.7, 102.1, 102.3, 126.2, 126.6, 128.0, 128.2, 128.4, 128.5, 128.7, 128.9 129.0, 129.2, 129.5, 129.7, 133.3, 133.7, 137.6, 165.7, 165.8, 166.0.

*Methyl 2,3,6-tri-O-benzoyl-4-O-(2,3-di-O-acetyl-4-O-benzoyl-6-bromo-6-deoxy-α-D-galactopyranosyl)-β-D-galactopyranoside* (**4**). — Compound **3** (500 mg) was treated with acetic anhydride (2 mL) in pyridine (5 mL) at room temperature until t.l.c. (toluene–ethyl acetate, 2:1) showed complete reaction. The solution was concentrated and then co-concentrated several times with toluene. *N*-Bromosuccinimide (120 mg) and barium carbonate (400 mg) were added to a solution of the product (550 mg) in tetrachloromethane (35 mL). The mixture was boiled under reflux for 30 min, filtered, and concentrated. Column chromatography (toluene–ethyl acetate, 9:1) of the residue yielded **4** (480 mg, 79%), $[\alpha]_D$ +108° (*c* 1.26, chloroform). $^{13}$C-N.m.r. data ($CDCl_3$): δ 20.6, 20.7, 27.8, 56.6, 62.4, 67.8, 68.5, 68.9, 69.5, 72.4, 73.5, 75.3, 98.2, 102.1, 125.3, 128.3, 128.6, 128.8, 129.1, 129.3, 129.8, 133.2, 133.4, 133.6, 165.2, 165.4, 166.0, 166.2, 169.7, 170.5.

*Methyl 4-O-α-D-fucopyranosyl-β-D-galactopyranoside* (**5**). — Compound **4** (270 mg) was deacylated using a catalytic amount of sodium methoxide in methanol at room temperature overnight. The solution was neutralised with Dowex 50 ($H^+$) resin, filtered, and concentrated. A solution of the product in ethanol (2 mL) containing triethylamine (15 μL) was hydrogenated over 10% Pd/C (0.2 g) at 400 kPa for 3 days, filtered, and concentrated. Column chromatography (chloroform–methanol, 6:1) of the residue yielded **5** (70 mg, 70%), $[\alpha]_D$ +96° (*c* 0.7, water). $^{13}$C-N.m.r. data ($D_2O$, external $Me_4Si$): δ 16.5, 58.5, 61.4, 68.4, 69.7, 70.6, 72.2, 73.2, 73.5, 76.4, 78.3, 101.4, 105.2. In addition, methyl 4-*O*-(6-bromo-6-deoxy-α-D-galactopyranosyl)-β-D-galactopyranoside (30 mg, 25%) was obtained.

*Methyl 2,3-di-O-benzoyl-4-O-(4,6-O-benzylidene-α-D-galactopyranosyl)-β-D-galactopyranoside* (**6**). — Methanol (20 mL) containing sodium methoxide (from 20 mg of sodium) was added to a solution of **3** (2.8 g) in methanol (75 mL) at 0°. The reaction was monitored by t.l.c. (chloroform–methanol, 9:1). After 2 h, the mixture was neutralised with Dowex 50 ($H^+$) resin, filtered, and concentrated. Column chromatography (chloroform–methanol, 9:1, or, alternatively, toluene–ethyl acetate, 1:3, and then ethyl acetate) yielded, in various fractions, **3** (0.5 g, 18%), monobenzoates, deacylated material (0.2 g, ~13%), and **6** (1.6 g, 66%), m.p. 138–140° (from ethyl acetate–hexane), $[\alpha]_D$ +111°, (*c* 0.6, chloroform). $^{13}$C-N.m.r. data (25 MHz, $CD_3OD$): δ 56.9, 59.9, 64.4, 69.1, 69.3, 69.5, 70.3, 70.9, 75.2, 75.5, 77.3, 101.1, 102.6 (two signals), 127.1, 128.6, 129.3, 129.4, 130.2, 130.3, 130.4, 130.6, 134.0, 134.3, 139.6, 166.1, 166.2.

*Anal.* Calc. for $C_{34}H_{36}O_{13}$: C, 62.5; H, 5.56. Found: C, 62.2; H, 5.65.

*Methyl 2,3-di-O-benzoyl-4-O-(4,6-O-benzylidene-α-D-galactopyranosyl)-6-deoxy-6-iodo-β-D-galactopyranoside* (**7**). — A solution of **6** (1.35 g), triphenylphosphine (1.35 g), iodine (1.3 g), and imidazole in toluene–acetonitrile (2:1, 50 mL) was kept at 70° overnight. Concentration and column chromatography (toluene–ethyl acetate, 1:1) yielded **7** (1.49 g, 94%), $[\alpha]_D$ +80° (*c* 1, chloroform). $^{13}$C-N.m.r. data (25 MHz, $CDCl_3$): δ 1.7, 57.4, 63.5, 68.7, 69.3, 69.6, 74.3, 75.2, 75.6, 75.9, 76.1, 100.7, 102.1, 102.3, 126.2, 128.1, 128.4, 128.7, 129.0, 129.1, 129.4, 129.7, 133.3, 133.7, 137.7, 165.7, 165.8.

*Methyl 2,3-di-O-benzoyl-4-O-(4,6-O-benzylidene-α-D-galactopyranosyl)-β-D-fucopyranoside* (**8**). — A solution of **7** (1.2 g) in ethanol (10 mL) containing triethylamine (0.2 mL) was hydrogenated at atmospheric pressure over 10% Pd/C (0.1 g). The mixture was filtered and concentrated, and a solution of the residue in chloroform was washed with water and aqueous sodium thiosulfate, dried ($Na_2SO_4$), filtered, and concentrated to yield **8** (0.85 g, 85%), m.p. 136–137° (from ethyl acetate–hexane), $[\alpha]_D$ +117° (*c* 0.5, chloroform). $^{13}$C-N.m.r. data ($CDCl_3$): δ 16.3, 57.2, 63.4, 68.6, 68.9, 69.5, 69.7, 71.1, 74.7, 75.8, 76.0, 77.1, 100.7, 102.2 (two signals), 126.2, 128.0, 128.4, 128.8, 129.2, 129.7, 133.2, 133.6, 137.8, 165.7, 165.9.

*Anal.* Calc. for $C_{34}H_{36}O_{12}$: C, 64.1; H, 5.70. Found: C, 63.0; H, 5.74.

*Methyl 4-O-α-D-galactopyranosyl-β-D-fucopyranoside* (**9**). — Compound **8** (200 mg) was treated with aqueous 90% trifluoroacetic acid (2 mL) at 0° for 10 min.

The solution was diluted with toluene (20 mL) and concentrated, and toluene was codistilled twice from the residue which was then debenzoylated conventionally with sodium methoxide in methanol. The solution was neutralised with Dowex 50 ($H^+$) resin, filtered, and concentrated, and a solution of the residue in water was washed with ether and ethyl acetate, passed through a column (3 × 80 cm) of Biogel P-2, and lyophilised to yield **9** (90 mg, 84%), $[\alpha]_D$ +105° (*c* 1.0, water). $^{13}$C-N.m.r. data ($D_2O$): δ 16.7, 58.4, 61.7, 70.2, 70.4, 71.7, 72.0, 72.2, 73.8, 80.6, 101.7, 104.9.

*Methyl 2,3-di-O-benzoyl-4-O-(2,3-di-O-acetyl-4-O-benzoyl-6-bromo-6-deoxy-α-*D*-galactopyranosyl)-β-*D*-fucopyranoside* (**10**). — Compound **8** (400 mg) was treated with acetic anhydride (2 mL) in pyridine (5 mL) at room temperature, the product was worked-up and treated with *N*-bromosuccinimide and barium carbonate in tetrachloromethane as described above for the preparation of **4**, except that the reaction time in the bromination step was 1 h instead of 30 min. The mixture was filtered and concentrated, and column chromatography (toluene–ethyl acetate, 4:1) of the residue yielded **10** (420 mg, 83%), m.p. 179–180° (from ethyl acetate–hexane), $[\alpha]_D$ +164° (*c* 0.4, chloroform). $^{13}$C-N.m.r. data ($CDCl_3$): δ 16.9, 20.6, 20.7, 27.8, 56.3, 67.7, 68.7, 69.2, 69.4, 70.6, 73.9, 77.6, 97.8, 101.9, 128.2, 128.6, 128.7, 129.0, 129.2, 129.7, 129.8, 129.9, 133.0, 133.4, 133.5, 165.4, 166.1, 169.7, 170.5.

*Anal.* Calc. for $C_{38}H_{39}BrO_{14}$: C, 57.1; H, 4.92; Br, 10.00. Found: C, 56.9; H, 4.97; Br, 9.82.

*Methyl 4-O-α-*D*-fucopyranosyl-β-*D*-fucopyranoside* (**11**). — Compound **10** (75 mg) was partially deacylated with sodium methoxide (9.5 μmol) in methanol (2 mL) at room temperature for 1 h. The solution was concentrated, and a solution of the residue in ethanol (2 mL) containing triethylamine (10 μL) was hydrogenated over 10% Pd/C (50 mg) at 400 kPa for 3 days. The mixture was filtered and concentrated, and the residue was deacylated with methanolic sodium methoxide overnight. Column chromatography (chloroform–methanol, 8:1) of the product gave **11** (20 mg, 67%), $[\alpha]_D$ +79° (*c* 0.9, water). $^{13}$C-N.m.r. data ($D_2O$): δ 16.9, 17.3, 58.8, 68.7, 70.5, 71.3, 72.6, 72.7, 73.7, 74.4, 81.1, 102.2, 105.6.

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