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SYNTHESIS OF DI- AND TRI-SACCHARIDES CORRESPONDING TO RECEPTOR STRUCTURES RECOGNISED BY PYELONEPHRITOGENIC *E. coli* FIMBRIAE (PILI)

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ABSTRACT

Syntheses are described of di- and tri-saccharides required for studies of the inhibition of adhesion by means of fimbriae of pyelonephritogenic *E. coli* bacteria to epithelium cells in the urinary tract containing suitable receptor sites. The disaccharides are the *p*-nitrophenyl and methyl glycosides of 4-*O*-α-D-galactopyranosyl-β-D-galactopyranose, and the trisaccharides are the *p*-nitrophenyl and methyl glycosides of 4-*O*-(4-*O*-α-D-galactopyranosyl-β-D-galactopyranosyl)-β-D-glucopyranose. The key aglycons were the 1,2,3,6-tetrabenzoate of α-D-galactose and the 1,2,3,6,-2′,3′,6′-heptabenzoate of α-lactose. Glycosidations were performed with 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl chloride as glycosylating agent and silver triflate as promoter.

INTRODUCTION

Adhesion of *E. coli* to the urinary-tract epithelium is related to the specific binding to receptor structures present on the urinary-tract cells. These receptor structures are present not only on the urinary-tract epithelium, but also on human erythrocytes[1]. The result of haemagglutination studies suggested that the receptor structure relevant for pyelonephritogenic *E. coli* was the trihexosylceramide glycosphingolipid corresponding to the P^k antigen[2]. The structure of the oligosaccharide moiety of this glycosphingolipid is 4-*O*-(4-*O*-α-D-galactopyranosyl-β-D-galactopyranosyl)-β-D-glucopyranose. In order to study these interactions, the following di- and tri-saccharides were synthesised: *p*-nitrophenyl 4-*O*-α-D-galactopyranosyl-β-D-galactopyranoside (**6**), methyl 4-*O*-α-D-galactopyranosyl-β-D-galactopyranoside (**8**), *p*-nitrophenyl 4-*O*-(4-*O*-α-D-galactopyranosyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**15**), and methyl 4-*O*-(4-*O*-α-D-galactopyranosyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**17**). Of these, **17** has previously been synthesised[3] by a different route.*

*After the submission of the present paper, the synthesis of **8** by a different route was reported [M.-L. Milat, P. A. Zollo, and P. Sinaÿ, *Carbohydr. Res.*, 100 (1982) 263–271].

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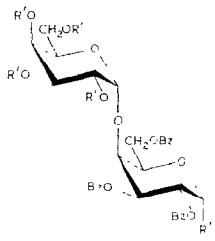

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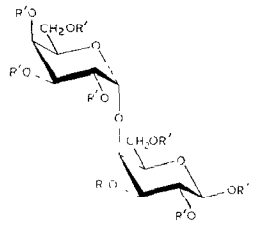
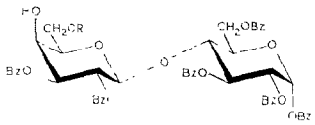
3 R′ = Bz, R″ = OBz
4 R′ = Bz, R″ = Br



5 R′ = Bz, R″ = p-$NO_2C_6H_4$
6 R′ = H, R″ = p-$NO_2C_6H_4$
7 R′ = Bz, R″ = Me
8 R′ = H, R″ = Me



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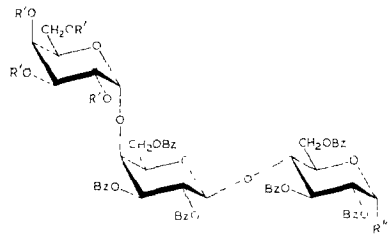
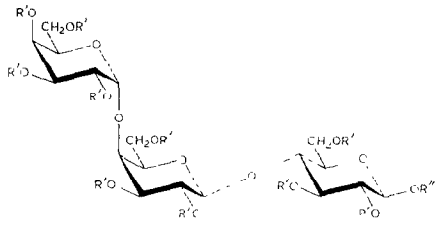


11 R′ = H, R″ = OBz
12 R′ = Bz, R″ = OBz
13 R′ = Bz, R″ = Br



14 R′ = Bz, R″ = p-$NO_2C_6H_4$
15 R′ = H, R″ = p-$NO_2C_6H_4$
16 R′ = Bz, R″ = Me
17 R′ = H, R″ = Me

RESULTS AND DISCUSSION

D-Galactose was benzoylated with 4 mol of benzoyl chloride in pyridine at 0°, and subsequent chromatography yielded 38% of the 1,2,4,6-tetrabenzoate **1**. The constitution of **1** was proved, *inter alia*, by methylation analysis, including methylation with methyl triflate[4,5]. Glycosidation of **1** with 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl chloride, using silver triflate as promoter[6], followed by catalytic hydrogenolysis of the product yielded 77% of the α-galactoside **2**. A high yield on glycosidation of a sterically hindered hydroxyl group using this galactosylation procedure was demonstrated earlier[7]. The disaccharide **2** was fully benzoylated to give the octabenzoate **3** (68% from **1**). Treatment of **3** with hydrogen bromide in acetic acid gave the α-bromide **4**, which was converted into the protected *p*-nitrophenyl disaccharide **5** by using silver imidazolate and zinc chloride as promoter[8] for the glycosidation reaction. Debenzoylation of **5** afforded the first target substance **6**. Silver triflate-promoted treatment of **4** with methanol gave the methyl β-D-glycoside **7**, debenzoylation of which afforded the second target substance **8**. The anomeric configurations of **6** and **8** were demonstrated by ^{1}H- and ^{13}C-n.m.r. spectroscopy, and the (1→4) linkages by methylation analysis[9].

A synthesis strategy analogous to that described above was used in order to obtain the remaining two target substances **15** and **16**. Partial benzoylation of **9**[10] with benzoyl chloride in pyridine at 0° afforded 75% of the lactose derivative **10**

having the axial HO-4′ free; **10** was then galactosylated with 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl chloride, and the product was debenzylated by catalytic hydrogenolysis to give the trisaccharide heptabenzoate **11**. Benzoylation of **11** yielded **12** (59% from **9**) which, with hydrogen bromide and glacial acetic acid in dichloromethane, afforded the α-bromide **13**. The reaction of **13** with *p*-nitrophenol gave the glycoside **14** and the reaction with methanol gave the glycoside **16**, as in the corresponding syntheses of glycosides **6** and **8** described above. Debenzoylation of **14** and **16** yielded the target compounds **15** and **17**, respectively. The structures of **15** and **17** were confirmed by ^{1}H- and ^{13}C-n.m.r. spectroscopy, and by methylation analysis[9].

The above routes for synthesis demonstrate facile access to galactose and lactose derivatives having free axial HO-4 and HO-4′, respectively. These are useful aglycons for the synthesis of α-D-galactosides using silver triflate as promoter. The synthesis route is flexible, in that various aglycons may be linked to C-1 of the disaccharides obtained.

Immunological studies using the above di- and tri-saccharides have been described elsewhere[11].

EXPERIMENTAL

General methods were the same as those previously reported[7]. Column chromatography on silica gel was performed in the flash mode[12]. Sugar[13,14] and methylation[9] analyses were in agreement with the postulated structures.

*1,2,3,6-Tetra-*O*-benzoyl-α-*D*-galactopyranose* (**1**). — A solution of benzoyl chloride (16 g) in pyridine (5 mL) was added dropwise to a stirred suspension of D-galactose (5.0 g) in pyridine (100 mL) at 0°. After 1 h, ice was added and the mixture was extracted with dichloromethane. The organic layer was washed with 2M sulfuric acid, saturated aqueous sodium hydrogencarbonate and water, dried (Na_2SO_4), filtered, and concentrated. Column chromatography (toluene–ethyl acetate, 4:1) then afforded amorphous **1** (6.0 g, 38%), $[\alpha]_D^{22}$ +147° (*c* 0.8, chloroform). N.m.r. data: ^{1}H (100 MHz, $CDCl_3$, internal Me_4Si), δ 6.80 (d, 1 H, $J_{1,2}$ 4 Hz, H-1); ^{13}C (25 MHz, $CDCl_3$, internal Me_4Si), δ 90.9 (C-1). Treatment of **1** in sequence with methyl triflate[4], sodium methoxide, sodium borohydride, and acetic anhydride–pyridine gave 1,2,3,5,6-penta-*O*-acetyl-4-*O*-methylgalactitol as the only product, as shown by g.l.c.–m.s.

*1,2,3,6-Tetra-*O*-benzoyl-4-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-*D*-galactopyranosyl)-α-*D*-galactopyranose* (**3**). — A solution of 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl chloride[7] (10.0 g) in dry toluene (20 mL) was added dropwise to a stirred mixture of **1** (4.0 g), silver trifluoromethanesulfonate (5.5 g), 2,4,6-trimethylpyridine (2.1 g), and dry toluene (20 mL) at −10°. The mixture was allowed to attain room temperature, and then stirred overnight, filtered, washed with dilute hydrochloric acid, water, saturated aqueous sodium hydrogencarbonate, and water, dried (Na_2SO_4), filtered, and concentrated. The syrupy residue was hydrogenolysed overnight in glacial acetic

acid (20 mL) over 10% Pd/C (0.2 g). The mixture was filtered and concentrated, and the residue was subjected to column chromatography (chloroform–methanol, 9:1), to yield syrupy **2** (4.9 g, 77%), $[\alpha]_D^{22}$ +166° (*c* 1.5, chloroform). N.m.r. data: ^{1}H, δ 6.80 (d, 1 H, $J_{1,2}$ 4 Hz, H-1); ^{13}C, δ 90.9 (C-1) and 101.1 (C-1′).

Benzoyl chloride (5.8 g) was added dropwise with stirring to a solution of **2** (4.0 g) in pyridine (100 mL) at 0°. After 2 h at room temperature and work-up as described above in the preparation of **1**, the product was purified by column chromatography (toluene–ethyl acetate, 12:1), to yield syrupy **3** (5.5 g, 89%), $[\alpha]_D^{22}$ +182° (*c* 1.3, chloroform). N.m.r. data: ^{1}H, δ 6.89 (d, 1 H, $J_{1,2}$ 4 Hz, H-1); ^{13}C, δ 90.8 (C-1) and 98.7 (C-1′).

p-*Nitrophenyl 2,3,6-tri*-O-*benzoyl-4*-O-(*2,3,4,6-tetra*-O-*benzoyl*-α-D-*galactopyranosyl*)-β-D-*galactopyranoside* (**5**). — Compound **3** was treated with acetic acid saturated with hydrogen bromide, and the resulting glycosyl bromide **4**, obtained after the usual work-up[15], was used without purification. A mixture of **4** (0.90 g), *p*-nitrophenol (0.16 g), silver imidazolate (0.10 g), and dry zinc chloride (0.60 g) in dichloromethane (25 mL) containing 4Å molecular sieves was stirred in the dark overnight at 40° and then filtered, washed with saturated aqueous sodium hydrogencarbonate and water, dried (Na_2SO_4), filtered, and concentrated. The product was purified by column chromatography [light petroleum (b.p. 40–60°)–chloroform–ethyl acetate, 3:1:1], to yield amorphous **5** (0.80 g, 87%), $[\alpha]_D^{22}$ +124° (*c* 1.1, chloroform). ^{13}C-N.m.r. data: δ 98.3 and 98.7 (C-1 and C-1′).

Anal. Calc. for $C_{67}H_{53}NO_{20}$: C, 67.5; H, 4.48; N, 1.17. Found: C, 67.7; H, 4.59; N, 1.19.

p-*Nitrophenyl 4*-O-α-D-*galactopyranosyl*-β-D-*galactopyranoside* (**6**). — Debenzoylation of **5** (0.12 g) with a catalytic amount of sodium methoxide in methanol followed by elution of the product from a column of Bio-gel P-2 afforded amorphous **6** (50 mg, 91%), $[\alpha]_D$ +53° (*c* 1, chloroform). N.m.r. data (D_2O): ^{1}H, δ 5.02 (d, 1 H, $J_{1',2'}$ 1 Hz, H-1′) and 5.23 (d, 1 H, $J_{1,2}$ 8 Hz, H-1); ^{13}C, δ 102.0 and 102.6 (C-1, C-1′).

Methyl 2,3,6-tri-O-*benzoyl-4*-O-(*2,3,4,6-tetra*-O-*benzoyl*-α-D-*galactopyranosyl*)-β-D-*galactopyranoside* (**7**). — A solution of **4** (0.60 g) in toluene (2 mL) was added with stirring to a mixture of silver trifluoromethanesulfonate (0.154 g), 2,4,6-trimethylpyridine (30 mg), methanol (60 mg), and toluene (5 mL) at −0°. The mixture was allowed to attain room temperature, and was then stirred overnight, washed successively with aqueous sodium thiosulfate, water, 2M sulfuric acid, and saturated aqueous sodium hydrogencarbonate, dried ($MgSO_4$), filtered, and concentrated. The product was purified by column chromatography (toluene–ethyl acetate, 2:1), to yield **7** (0.50 g, 90%), m.p. 222–223°, $[\alpha]_D$ +96° (*c* 1.3, chloroform). ^{13}C-N.m.r. data: δ 56.4 (OMe), 98.4 (C-1′), and 101.8 (C-1).

Anal. Calc. for $C_{62}H_{52}O_{18}$: C, 68.6; H, 4.83. Found: C, 68.6; H, 4.94.

Methyl 4-O-α-D-*galactopyranosyl*-β-D-*galactopyranoside* (**8**). — Debenzoylation of **7** (0.10 g) with a catalytic amount of sodium methoxide in methanol followed by elution of the product from a column of Bio-gel P-2 afforded amorphous **8**

(28 mg, 85%), $[\alpha]_D^{22}$ +88° (*c* 1.2, water). N.m.r. data (D_2O): ^{13}C, δ 58.3 (OMe), 101.3 (C-1′), and 104.8 (C-1); ^1H, δ 4.32 (d, 1 H, $J_{1,2}$ 7.1 Hz, H-1) and 4.96 (d, 1 H, $J_{1',2'}$ 2.2 Hz, H-1′).

1,2,3,6-Tetra-O-*benzoyl*-*4*-O-(*2,3,6-tri*-O-*benzoyl*-β-D-*galactopyranosyl*)-α-D-*glucopyranose* (**10**). — A solution of benzoyl chloride (10.7 g) in pyridine (5 mL) was added dropwise to a solution of 1,2,3,6,2′,3′-hexa-*O*-benzoyl-α-D-lactose[10] (**9**, 4.0 g) in pyridine (50 mL) at 0°. After 2 h at room temperature, the mixture was poured onto ice and stirred for 30 min. The crystals were collected, washed with cold water, and recrystallised from ethanol, to yield **10** (3.5 g, 75%). Conventional work-up and chromatography of the mother liquor yielded more (0.60 g, 15%) **10**, m.p. 196–197°, $[\alpha]_D^{22}$ +98° (*c* 1, water). ^{13}C-N.m.r. data: δ 89.8 (C-1) and 101.3 (C-1′).

Anal. Calc. for $C_{61}H_{50}O_{18}$: C, 68.4; H, 4.70. Found: C, 68.1; H, 4.69.

1,2,3,6-Tetra-O-*benzoyl*-*4*-O-[*2,3,6-tri*-O-*benzoyl*-*4*-O-(*2,3,4,6-tetra*-O-*benzoyl*-α-D-*galactopyranosyl*)-β-D-*galactopyranosyl*]-α-D-*glucopyranose* (**12**). — Compound **10** (2.50 g) was treated with 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl chloride[7] (2.5 g), silver trifluoromethanesulfonate (1.20 g), and 2,4,6-trimethylpyridine (0.50 g) in toluene (20 mL), the mixture was worked-up, and the product hydrogenolysed, as described above, to yield amorphous **11** (1.80 g, 64%), $[\alpha]_D^{22}$ +124° (*c* 1, chloroform). ^{13}C-N.m.r. data: δ 89.7 (C-1), 100.2 (C-1″), and 101.6 (C-1′).

Benzoylation of **11** (1.5 g), as described above, yielded amorphous **12** (1.84 g, 92%), $[\alpha]_D^{22}$ +72° (*c* 1, chloroform). ^{13}C-N.m.r. data: δ 89.8 (C-1), 98.9 (C-1″), and 101.8 (C-1′).

p-*Nitrophenyl 2,3,6-tri*-O-*benzoyl*-*4*-O-[*2,3,6-tri*-O-*benzoyl*-*4*-O-(*2,3,4,6-tetra*-O-*benzoyl*-α-D-*galactopyranosyl*)-β-D-*galactopyranosyl*]-β-D-*glucopyranoside* (**14**). — A solution of **12** in dry dichloromethane was treated with glacial acetic acid saturated with hydrogen bromide. After 1 h at room temperature, the solution was washed with water, saturated aqueous sodium hydrogencarbonate, and water, dried (Na_2SO_4), filtered, and concentrated, to give **13** which was used directly.

Compound **13** (0.95 g) was treated with *p*-nitrophenol (0.13 g), silver imidazolate (0.084 g), and zinc chloride (0.80 g) in dry dichloromethane (30 mL) containing powdered 4Å molecular sieves. Work-up, as described above, gave amorphous **14** (0.80 g, 80%), $[\alpha]_D^{22}$ +51° (*c* 1, chloroform). ^{13}C-N.m.r. data: δ 97.4 (C-1), 98.8 (C-1″), and 101.4 (C-1′).

p-*Nitrophenyl* 4-O-(*4*-O-α-D-*galactopyranosyl*-β-D-*galactopyranosyl*)-β-D-*glucopyranoside* (**15**). — Debenzoylation of **14** (0.50 g) and purification of the product, using the procedures described above, yielded **15** (0.17 g, 91%), $[\alpha]_D^{22}$ +12° (*c* 0.76, water). N.m.r. data (D_2O): ^1H, δ 4.51 (d, 1 H, $J_{1',2'}$ 7 Hz, H-1′), 4.96 (d, 1 H, $J_{1'',2''}$ 2 Hz, H-1″), and 5.21 (d, 1 H, $J_{1,2}$ 7.3 Hz, H-1); ^{13}C, δ 100.4 (C-1″), 101.5 (C-1), and 104.5 (C-1′).

Methyl 2,3,6-tri-O-*benzoyl*-*4*-O-[*2,3,6-tri*-O-*benzoyl*-*4*-O-(*2,3,4,6-tetra*-O-*benzoyl*-α-D-*galactopyranosyl*)-β-D-*galactopyranosyl*]-β-D-*glucopyranoside* (**16**). — Compound **13** (1.5 g) was treated with methanol (0.50 mL), silver trifluoromethanesulfo-

nate (0.20 g), and 2,4,6-trimethylpyridine (0.09 g) in dry toluene (30 mL) as described above. Column chromatography (toluene–ethyl acetate, 15 : 1) of the product afforded **16** (1.2 g, 76%), m.p. 223–224° (from ethanol), $[\alpha]_D$ +70° (*c* 1, chloroform). ^{13}C-n.m.r. data: δ 98.8 (C-1″), 101.3 (C-1), and 101.6 (C-1′).

Anal. Calc. for $C_{89}H_{74}O_{26}$: C, 68.5; H, 4.78. Found: C, 68.5; H, 4.85.

Methyl 4-O-(4-O-α-D-galactopyranosyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**17**). — Treatment of **16** (0.70 g) with a catalytic amount of sodium methoxide in methanol afforded, after chromatography on Bio-gel P-2, amorphous **17** (0.20 g, 86%), $[\alpha]_D$ +65° (*c* 1, water); lit.[3] $[\alpha]_D$ +63° (water). N.m.r. data (D_2O): ^{1}H, δ 4.94 (d, 1 H, $J_{1'',2''}$ ~1.7 Hz, H-1″), 4.47 (d, 1 H, $J_{1',2'}$ ~7.6 Hz, H-1′), and 4.34 (d, 1 H, $J_{1,2}$ ~7.8 Hz, H-1); ^{13}C, δ 58.4 (OMe), 61.3 (C-6), 61.6 (C-6′), 61.7 (C-6″), 101.5 (C-1″), 104.2 (C-1′), and 104.5 (C-1).

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