Carbohydrate Research, 152 (1986) 301–304
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

Synthesis of disaccharide analogues of methyl 4-*O*-α-D-galactopyranosyl-β-D-galactopyranoside ("methyl urobioside"), the minimum structure recognised by *p*-fimbriated *E. coli*

THOMAS NORBERG, STEFAN OSCARSON, AND MARIA SZÔNYI
Department of Organic Chemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm (Sweden)

(Received August 16th, 1985; accepted for publication, November 1st, 1985)

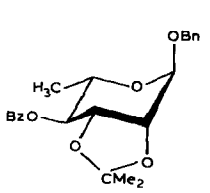
The ability of bacteria to adhere to mammalian cell membranes is of importance in the initiation of many bacterial infections. *E. coli* strains that cause pyelonephritis adhere to human uroepithelial cells and the capacity to adhere is correlated with virulence[1–3]. Adhesion is mediated by bacterial protein appendages (fimbriae). Of these, the "*p*-fimbriae" bind specifically to blood group P, P_1, and p^k carbohydrate structures on the mammalian cell surfaces. The simplest carbohydrate structure shown to bind to these fimbriae was the disaccharide methyl α-D-Gal*p*-(1→4)-β-D-Gal*p* ("methyl urobioside", **8**). We have synthesised[4] deoxy analogues of this structure and now report the synthesis of the disaccharide α-D-Gal*p*-(1→2)-L-Rha (**5**), which is structurally related to methyl urobioside. The disaccharide α-D-Gal*p*-(1→2)-D-Man (**7**) was also synthesised for comparison purposes. Investigations on the binding properties of **5** and **7** will be described elsewhere.

Benzyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside[5] was treated with benzoyl chloride in pyridine to give 81% of the 4-benzoate **1**. Acid hydrolysis of **1** gave the diol **2**, which was partially benzoylated with benzoyl chloride in pyridine–dichloromethane at −25° to give the 3,4-dibenzoate **3** (62% from **1**). Glycosidation of **3** with methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside[6], using methyl triflate[7] as promoter, gave 67% of the disaccharide derivative **4**. Treatment of **4** with methanolic sodium methoxide followed by catalytic hydrogenation gave **5** (81% from **4**). The disaccharide **7** was synthesised by silver triflate-promoted glycosidation of benzyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranoside[8] with 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl chloride[9] followed by catalytic hydrogenation of the product **6**. The yield in the glycosidation step was 80%.

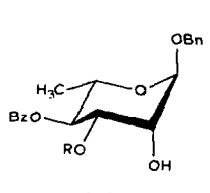
EXPERIMENTAL

General methods. — Melting points are corrected. Concentrations were performed at 1–2 kPa at <40° (bath). Optical rotations were recorded with a Perkin–

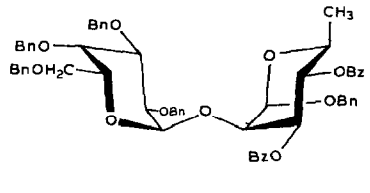
0008-6215/86/$ 03.50



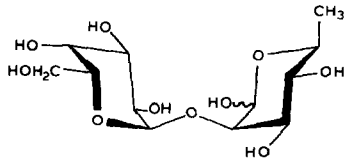
1



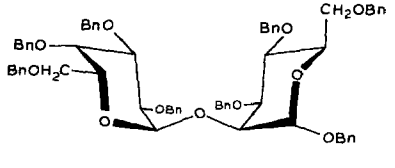
2 R = H
3 R = Bz



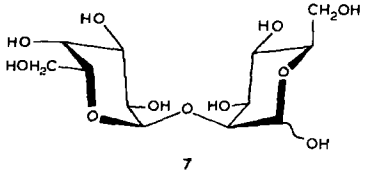
4



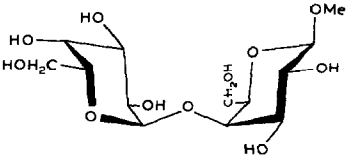
5



6



7



8

Elmer 241 polarimeter. N.m.r. spectra were recorded with a JEOL FX-100 or GX 400 instrument. The following reference signals were used: internal Me_4Si, δ 0.00 (^{13}C in $CDCl_3$); $CHCl_3$, δ 7.27 (^1H in $CDCl_3$); external Me_4Si, δ 0.00 (^{13}C and ^1H in D_2O). Only selected n.m.r. data are reported. T.l.c. was performed on silica gel F_{254} (Merck) with detection by u.v. light when applicable or by charring with sulfuric acid. Column chromatography was performed on silica gel 60 (0.04–0.063 mm, Merck) with loadings in the range 1/25–1/100. Molecular sieves (4 Å, Union Carbide) were desiccated in a vacuum at 300° overnight and ground immediately before use.

Benzyl 4-O-benzoyl-2,3-O-isopropylidene-α-L-rhamnopyranoside (**1**). — Benzoyl chloride (3.2 mL) was added to a stirred and cooled (0°) solution of benzyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside[5] (3.1 g) in pyridine (20 mL). After 1 h, water was added and stirring was continued for 15 min. The precipitate was collected and washed with water. Recrystallisation from ethanol gave **1** (3.4 g, 81%), m.p. 146°, $[\alpha]_D$ −199° (*c* 0.5, chloroform). ^{13}C-N.m.r. data ($CDCl_3$): δ 17.2 (C-6), 26.4, 27.8 (CH_3), 64.4, 69.4, 75.1, 75.9, 76.1 (C-2,3,4,5 and PhCH_2), 96.3 (C-1), 109.8 (acetal C), 165.7 (Ph*C*O).

Anal. Calc. for $C_{23}H_{26}O_6$: C, 69.3; H, 6.58. Found: C, 69.1; H, 6.62.

Benzyl 3,4-di-O-benzoyl-α-L-rhamnopyranoside (**3**). — A solution of **1** (1.1 g) in aqueous 70% acetic acid (50 mL) was heated (80°) for 2 h, then concentrated,

and co-concentrated with toluene. To a solution of the residue (1 g) in pyridine–dichloromethane (1:2, 10 mL) at −25° was added a solution of benzoyl chloride (0.35 mL) in pyridine–dichloromethane (1:2, 10 mL) dropwise with stirring. After 1 h at room temperature, water (0.2 mL) was added, and stirring was continued for 15 min at room temperature. The mixture was diluted with dichloromethane, washed with water, 2M sulfuric acid, and aqueous sodium hydrogencarbonate, dried (Na_2SO_4), and concentrated. The residue was subjected to column chromatography (toluene–ethyl acetate, 14:1). The main fraction was **3** (0.79 g, 62%), $[\alpha]_D$ +10° (*c* 0.5, chloroform); lit.[10] $[\alpha]_D$ +13° (*c* 0.5, chloroform). N.m.r. data ($CDCl_3$): ^{13}C, δ 17.5 (C-6), 66.9, 69.4, 69.5, 71.8, 72.9 (C-2,3,4,5 and Ph*C*H_2), 99.1 (C-1), 165.9, 166.0 (Ph*C*O); ^1H, δ 1.33 (d, $J_{5,6}$ 6.4 Hz, H-6), 4.16 (m, $J_{5,6}$ 6.3 Hz, H-5), 4.34 (dd, $J_{2,3}$ 2.9 Hz, H-2), 4.63 (d, *J* 12.0 Hz, PhC*H*), 4.82 (d, PhC*H*), 4.99 (d, $J_{1,2}$ 1.8 Hz, H-1), 5.61 (dd, $J_{4,5}$ 9.5 Hz, H-4), 5.66 (dd, $J_{3,4}$ 10 Hz, H-3).

*Benzyl 3,4-di-*O*-benzoyl-2-(2,3,4,6-tetra-*O*-benzyl-α-*D*-galactopyranosyl)-α-*L*-rhamnopyranoside* (**4**). — Methyl triflate (0.26 mL) was added at room temperature to a stirred solution of **3** (0.15 g) and methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside[6] (0.28 g) in dry ether (20 mL) containing molecular sieves. After 1 h, triethylamine (0.68 mL) was added, the mixture was filtered and concentrated, and the residue was subjected to column chromatography (toluene–ethyl acetate, 30:1, then iso-octane–acetone, 2:1). The main fraction was **4** (0.23 g, 67%), $[\alpha]_D$ +54° (*c* 0.5, chloroform). ^{13}C-N.m.r. data ($CDCl_3$): δ 17.7 (C-6), 67.1, 67.9, 69.4, 69.6, 71.8, 71.9, 72.8, 72.9, 74.8, 75.1, 76.4, 78.3 (C-2,3,4,5, C-2′,3′,4′,5′,6′, and Ph*C*H_2; some overlap), 96.7, 97.9 (C-1, C-1′), 165.7, 165.8 (Ph*C*O).

*2-*O*-α-D-Galactopyranosyl-L-rhamnopyranose* (**5**). — A solution of **4** (0.14 g) in methanolic 0.05M sodium methoxide was kept overnight at room temperature, then neutralised with Dowex 50 (H^+) resin, and concentrated. A solution of the residue in ethyl acetate–ethanol (1:1, 3 mL) was hydrogenated over Pd/C (10%, 150 mg) at 400 kPa for 16 h. The reaction mixture was applied directly to a column of silica gel packed in methanol–chloroform (3:1). Elution with the same solvent gave fractions containing **5**, which were concentrated, and a solution of the residue in water was applied to a column of Bio-gel P-2. Elution with water gave **5** (38 mg, 81%), $[\alpha]_D$ +94°. N.m.r. data (D_2O): ^{13}C, δ 17.9 (C-6α,6β), 92.5, 95.0, 99.0, 102.6 (C-1α,1β, C-1′α,1′β); ^1H, δ 1.26 (d, *J* 6.5 Hz, H-6α), 1.27 (d, *J* 6.5 Hz, H-6β), 4.89 (d, *J* <1 Hz, H-1β), 5.02 (d, *J* 3.7 Hz, H-1′α), 5.09 (d, *J* 3.7 Hz, H-1′β), 5.21 (d, *J* 1.6 Hz, H-1α).

Anal. Calc. for $C_{12}H_{22}O_{10}$: C, 44.2; H, 6.8. Found: C, 38.3; H, 6.4. The reason for the bad correspondence between calculated and found carbon content must be attributed to the presence of material not containing carbon, since ^{13}C- and ^1H-n.m.r. spectroscopy showed no signals other than those expected. The impurity is not water alone, however, as shown by calculations. Because of the bad elemental analysis, the optical rotation value must be considered of low accuracy.

*Benzyl 3,4,6-tri-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-benzyl-α-*D*-galactopyranosyl)-α-*D*-mannopyranoside* (**6**). — A solution of 2,3,4,6-tetra-*O*-benzyl-α-D-galacto-

pyranosyl chloride[9] (1.2 g) in toluene (20 mL) was added dropwise to a stirred and cooled (−30°) solution of benzyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranoside[8] (1.0 g) silver trifluoromethanesulfonate (0.65 g), and 2,4,6-trimethylpyridine (0.38 mL) in toluene (50 mL) containing molecular sieves. After 12 h, the mixture was filtered and concentrated. The residue was subjected to column chromatography (toluene–ethyl acetate, 25:1) to give **6** (1.6 g, 80%), $[\alpha]_D$ +26°. ^{13}C-N.m.r. data ($CDCl_3$): δ 69.0, 69.4, 69.8, 71.2, 72.4, 72.9, 73.2, 74.7, 74.9, 75.1, 75.3, 75.7, 76.4, 77.0, 78.3, 78.4, 80.4 (C-2,3,4,5,6, C-2′,3′,4′,5′,6′, and PhCH_2), 98.0, 98.5 (C-1, C-1′).

2-O-α-D-Galactopyranosyl-D-mannopyranose (**7**). — A solution of **6** (0.35 g) in ethanol–ethyl acetate (2:1) was hydrogenated over Pd/C (10%, 0.35 g) at 400 kPa for 12 h, then filtered, and concentrated. The residue was subjected to column chromatography (acetic acid–ethyl acetate–methanol–water, 12:3:3:1). The fractions containing **7** were combined, concentrated, and applied to a column of Bio-gel P-2. Elution with water gave **7** (96 mg, 83%), $[\alpha]_D$ +96° (*c* 0.5, water). N.m.r. data (D_2O): ^{13}C, δ 93.7, 102.3 (C-1α, C-1′α); ^{1}H, δ 4.87 (d, *J* 1.0 Hz, H-1β), 5.12 (d, *J* 3.7 Hz, H-1′α), 5.17 (d, *J* 3.7 Hz, H-1′β), 5.36 (d, *J* 1.5 Hz, H-1β).

Anal. Calc. for $C_{12}H_{22}O_{11} \cdot 2\ H_2O$: C, 38.1; H, 6.9. Found: C, 38.1; H, 6.9.

REFERENCES

1 G. KÄLLENIUS, R. MÖLLBY, S. B. SVENSON, J. WINBERG, A. LUNDBLAD, S. SVENSSON, AND B. CEDERGREN, *FEMS Microbiol. Lett.*, 7 (1980) 297–302.
2 G. KÄLLENIUS, S. B. SVENSON, R. MÖLLBY, B. CEDERGREN, H. HULTBERG, AND J. WINBERG, *Lancet*, (1981) 604–606.
3 G. KÄLLENIUS, S. B. SVENSON, R. MÖLLBY, T. KORHONEN, J. WINBERG, B. CEDERGREN, I. HELIN, AND H. HULTBERG, *Scand. J. Infect. Dis. Suppl.*, 33 (1982) 52–60.
4 P. J. GAREGG AND S. OSCARSON, *Carbohydr. Res.*, 137 (1985) 270–275.
5 J. S. BRIMACOMBE AND L. C. N. TUCKER, *Carbohydr. Res.*, 5 (1967) 36–44.
6 P. J. GAREGG AND S. OSCARSON, *Carbohydr. Res.*, 136 (1985) 207–213.
7 H. LÖNN, *Carbohydr. Res.*, 139 (1985) 105–113.
8 H. BAUMANN, H. LÖNN, AND J. LÖNNGREN, *Carbohydr. Res.*, 114 (1983) 319–321.
9 P. J. GAREGG, H. HULTBERG, AND C. LINDBERG, *Carbohydr. Res.*, 83 (1980) 157–162.
10 N. E. BYRAMOVA, M. V. OVCHINNIKOV, L. B. BACKINOWSKY, AND N. K. KOCHETKOV, *Carbohydr. Res.*, 124 (1983) c8–c11.