Carbohydrate Research, 163 (1987) 285–288
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

Synthesis of *O*-(β-D-glucopyranosyluronic acid)-(1→4)-*O*-β-D-galactopyranosyl-(1→4)-D-glucopyranose

ARUN K. SARKAR AND NIRMOLENDU ROY
Department of Macromolecules, Indian Association for the Cultivation of Science, Calcutta 700 032 (India)

(Received October 15th, 1986; accepted for publication, January 15th, 1987)

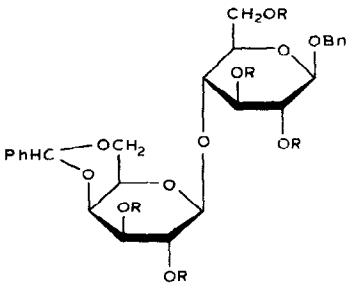
We have reported[1] the synthesis of *O*-(β-D-glucopyranosyluronic acid)-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-D-glucopyranose by condensation of benzyl 2,3,6,2′,6′-penta-*O*-benzyl-β-lactoside with methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate and removal of protecting groups. No substitution occurred at position 4′ of the lactose derivative. We now report the synthesis of the title trisaccharide (**7**), which is a constituent[2] of *Klebsiella* type 25 capsular polysaccharide.

Benzyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3-di-*O*-acetyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (**2**), prepared by reaction of benzyl-β-lactoside[3] (**1**) with α,α-dimethoxytoluene[4] followed by acetylation, was benzylated[5] to give the penta-*O*-benzyl derivative **3**. Removal of the benzylidene group from **3** yielded benzyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,3-di-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**4**). Selective benzylation of **4** by the phase-transfer method[6], using benzyl bromide and tetrabutylammonium hydrogensulfate, gave benzyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**5**). Condensation of **5** with methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate[7] in the presence of silver triflate and tetramethylurea in dichloromethane gave 41% of the trisaccharide derivative **6**. Debenzylation of **6** and deacetylation of the product gave **7**, the structure of which was confirmed by acid hydrolysis and methylation analysis.

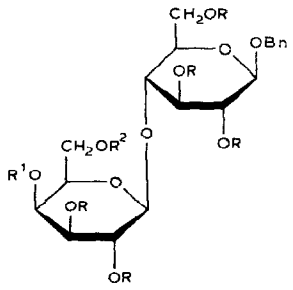
EXPERIMENTAL

General. — All reactions were monitored by t.l.c. on Silica Gel G (Merck). Column chromatography was performed on Silica Gel 60 (Merck). P.c. was performed on Whatman No. 1 paper with 9:2:2 ethyl acetate–acetic acid–water and 8:2:1 ethyl acetate–pyridine–water; detection was effected with alkaline silver

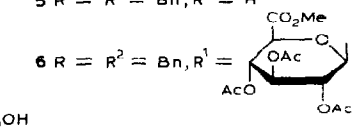
0008-6215/87/$ 03.50



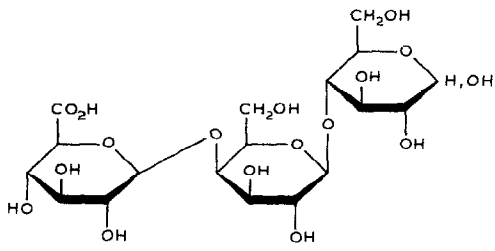
2 R = Ac
3 R = Bn



4 R = Bn, R^1 = R^2 = H
5 R = R^2 = Bn, R^1 = H



6 R = R^2 = Bn, R^1 =



7

nitrate. G.l.c. was performed on a Hewlett–Packard Model 5730A instrument fitted with a glass column (1.83 m × 6 mm) packed with 3% of ECNSS-M on Gas Chrom Q (100–120 mesh), at 180° for the alditol acetates of unsubstituted sugars and at 170° for methylated sugars. All solvents were distilled before use, and all evaporations were done at 50° under vacuum unless otherwise stated. Optical rotations were measured with a Perkin–Elmer Model 241 MC Spectropolarimeter. N.m.r. spectra were recorded with a Varian Model T-60A spectrometer for solutions in $CDCl_3$ (internal Me_4Si).

Benzyl 2,3,6-tri-O-acetyl-4-O-(2,3-di-O-acetyl-4,6-O-benzylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (**2**). — A solution of **1** (ref. 3) (2.5 g) in *N,N*-dimethylformamide (25 mL), α,α-dimethoxytoluene (1 mL), and toluene-*p*-sulfonic acid (20 mg) in a round-bottom flask attached to a Buchler evaporator was rotated and evacuated (water pump) at 65–70° for 1.5 h. The mixture was then concentrated under vacuum at 90°. To a solution of the syrupy residue in pyridine (20 mL) was added acetic anhydride (15 mL). The mixture was kept overnight at room temperature and then concentrated. A solution of the syrupy residue in chloroform (25 mL) was washed with water (3 × 20 mL), dried (Na_2SO_4), and concentrated. Crystallisation of the residue from ethanol gave **2** (2.75 g, 65%),

m.p. 220–222°, $[\alpha]_D^{25}$ +8° (c 1.8, chloroform). ^{1}H-N.m.r. data: δ 1.96, 2.00, 2.08 (3 s), 5.40 (s, 1 H, H-1), 7.20 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{36}H_{42}O_{16}$: C, 59.17; H, 5.79. Found: C, 58.99; H, 5.92.

*Benzyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-*D*-galactopyranosyl)-β-*D*-glucopyranoside* (**3**). — Compound **2** (2 g) was benzylated[1] and the product was crystallised from ethanol–ethyl acetate (10:1) to give **3** (1.8 g, 69%), m.p. 118–120°, $[\alpha]_D^{25}$ −9° (*c* 1.5, chloroform).

Anal. Calc. for $C_{61}H_{62}O_{11}$: C, 75.44; H, 6.43. Found: C, 75.61; H, 6.57.

*Benzyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,3-di-*O*-benzyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranoside* (**4**). — Compound **3** was treated[8] with aqueous 80% acetic acid at 85°. The product crystallised from ethanol to give **4** (76%), m.p. 143°, $[\alpha]_D^{25}$ +13.5° (*c* 1.5, chloroform).

Anal. Calc. for $C_{54}H_{58}O_{11}$: C, 73.44; H, 6.62. Found: C, 73.65; H, 6.74.

*Benzyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,3,6-tri-*O*-benzyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranoside* (**5**). — To a solution of **4** (1 g) in dichloromethane (25 mL) was added benzyl bromide (0.24 mL), tetrabutylammonium hydrogensulfate (0.08 g), and aqueous 5% sodium hydroxide (2.5 mL). The suspension was boiled under reflux for 3 days, cooled, washed with water (3 × 20 mL), dried (Na_2SO_4), and concentrated to dryness. Column chromatography (benzene–ether, 9:1) of the residue gave **5** (660 mg, 60%), m.p. 106–108°, $[\alpha]_D^{25}$ +4° (*c* 1.6, chloroform).

Anal. Calc. for $C_{61}H_{64}O_{11}$: C, 75.29; H, 6.63. Found: C, 75.50; H, 6.75.

*Benzyl 2,3,6,2′,3′,6′-hexa-*O*-benzyl-4′-*O*-[methyl (2,3,4-tri-*O*-acetyl-β-*D*-glucopyranosyl)uronate]-β-lactoside* (**6**). — To a stirred solution of **5** (600 mg) in dichloromethane (25 mL) was added methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate (600 mg) and tetramethylurea (1 mL). Silver triflate (0.5 g) was added in the dark and stirring was continued under nitrogen for 3 days at 20°. The suspension was then filtered through Celite, washed with saturated aqueous sodium hydrogencarbonate and water, dried (Na_2SO_4), and concentrated. T.l.c. (benzene–ether, 9:1) of the syrupy residue revealed one major and three minor spots. Column chromatography, using the same solvent mixture, gave **6** (320 mg, 41%), m.p. 180–182° (from ethanol), $[\alpha]_D^{25}$ −12° (*c* 1.8, chloroform). ^{1}H-N.m.r. data: δ 1.74, 1.93, and 1.96 (3 s, each 3 H, 3 OAc), 3.65 (s, 3 H, COOMe), 7.20 (m, 35 H, 7 Ph).

Anal. Calc. for $C_{74}H_{80}O_{20}$: C, 68.93; H, 6.25. Found: C, 69.11; H, 6.32.

O-(β-D-Glucopyranosyluronic acid)-(1→4)-O-β-D-galactopyranosyl-(1→4)-D-glucopyranose (**7**). — A solution of **6** (140 mg) in dry methanol (5 mL) was stirred under hydrogen for 36 h at room temperature in the presence of 10% Pd/C (500 mg), then filtered through Celite, and concentrated to dryness. To a solution of the residue in dry methanol (5 mL) was added sodium methoxide (27 mg) followed, after 3 h, by a few drops of water. The mixture was kept at room temperature for 1 h, neutralised with Dowex 50W-X8 (H^+) resin, filtered, and concentrated to dryness, to give **7** (50 mg), $[\alpha]_D^{25}$ −16° (*c* 1.2, water), $R_{Lactose}$ 0.51 (p.c.).

Anal. Calc. for $C_{18}H_{30}O_{17}$: C, 41.70; H, 5.83. Found: C, 41.35; H, 6.14.

The trisaccharide **7** (2 mg) was hydrolysed with 2M trifluoroacetic acid for 20 h at 100°. P.c. of the hydrolysate showed the presence of D-glucose, D-galactose, D-glucuronic acid, and an aldobiouronic acid. G.l.c. of the alditol acetates showed glucose and galactose to be in the ratio 1:0.7.

Compound **7** (5 mg) was methylated by the Kuhn method as described previously[9]. The methylated product was hydrolysed with 2M trifluoroacetic acid for 20 h and the products were converted into alditol acetates. G.l.c. then revealed derivatives of 2,3,6-tri-*O*-methylglucose and 2,3,6-tri-*O*-methylgalactose.

REFERENCES

1 L. BATAVYAL AND N. ROY, *Carbohydr. Res.*, 145 (1986) 328–331.
2 H. NIEMANN, B. KWIATKOWSKI, U. WESTPHAL, AND S. STIRM, *J. Bacteriol.*, 130 (1977) 366–374.
3 D. BEITH-HALAHMI, H. M. FLOWERS, AND D. SHAPIRO, *Carbohydr. Res.*, 5 (1967) 25–30.
4 P. J. GAREGG AND C. G. SWAHN, *Methods Carbohydr. Chem.*, 8 (1980) 317–319.
5 C. P. J. GLAUDEMANS AND H. G. FLETCHER, JR., *Methods Carbohydr. Chem.*, 6 (1972) 373–376.
6 P. J. GAREGG, T. IVERSEN, AND S. OSCARSON, *Carbohydr. Res.*, 50 (1976) c12–c14.
7 G. N. BOLLENBACK, J. W. LONG, D. G. BENJAMIN, AND J. A. LINDQUIST, *J. Am. Chem. Soc.*, 77 (1955) 3310–3312.
8 N. ROY, *Indian J. Chem.*, 16B (1978) 846–848.
9 A. SARKAR AND N. ROY, *Carbohydr. Res.*, 152 (1986) 205–215.