The Synthesis of 4-*O*-α-D-Glucopyranosyl-L-rhamnopyranose

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4-*O*-α-D-Glucopyranosyl-L-rhamnose has been synthesized in 40% overall yield by condensation of 2-*O*-benzyl-3,4,6-tri-*O*-*p*-nitrobenzoyl-β-D-glucopyranosyl bromide with methyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside in nitromethane using mercuric cyanide as acid acceptor. The methyl glycoside hexaacetate was obtained crystalline.

4-*O*-α-D-Glucopyrannosyl-L-rhamnose a été synthétisé avec un rendement de 40% par condensation du bromo-2-*O*-benzyle-3,4,6-tri-*O*-*p*-nitrobenzoyl-β-D-glucopyrannose avec le méthyl 2,3-*O*-isopropylidène-α-L-rhamnopyrannoside en nitrométhane et le cyanure de mercure. Le glycoside méthyl hexaacétate a été obtenu sous forme cristallin.

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The importance of *cis*-glycosides in nature has led to many attempts to synthesize them (1–7). We now report the synthesis in 40% yield of 4-*O*-α-D-glucopyranosyl-L-rhamnose using an intermediate with a nonparticipating 2-*O*-benzyl group as suggested by the work of Ishikawa and Fletcher (6). This disaccharide is required for structural studies on capsular polysaccharides from *Klebsiella* and was originally thought to be an antigenic determinant in *Shigella* (8).

2-*O*-Benzyl-D-glucose (**1**) was prepared as described in the literature (10, 11) except that a higher yield in shorter time was obtained by using only 0.2 *M* excess of benzyl bromide, adding Drierite to the reaction and, in addition, using freshly prepared silver oxide (12).

Condensation of the derived 2-*O*-benzyl-3,4,6-tri-*O*-*p*-nitrobenzoyl-β-D-glucopyranosyl bromide (**2**) with methyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside (**3**) with mercuric cyanide as an acid acceptor in nitromethane at 40° and subsequent purification by column chromatography, gave the fully blocked disaccharide (**4**) in 80% yield. Deacylation of **4** gave crystalline methyl 4-*O*-(2-*O*-benzyl-α-D-glucopyranosyl)-2,3-*O*-isopropylidene-α-L-rhamnopyranoside (**5**). In addition, a small amount of β-linked disaccharide was detected which was conveniently identified as the trimethylsilyl derivative by p.m.r. (13) after removal of the benzyl group. Hydrogenolysis of **5** gave the debenzylated compound (**6**) which when reacted with trifluoroacetic acid, yielded methyl 4-*O*-α-D-glucopyranosyl-α-L-rhamnopyranoside as a syrup (**8**).

Acetylation of **8** gave the crystalline hexaacetate (**10**), m.p. 136–137°. Acetolysis of **10** gave the corresponding heptaacetate (**11**). Deacetylation then afforded 4-*O*-α-D-glucopyranosyl-L-rhamnopyranose (**12**) as a syrup, $[\alpha]_D$ +10° (water).

The structure of the disaccharide follows from the method of synthesis and was confirmed by the products obtained by periodate oxidation (14) and methylation (15) of **8**.

The nature of the anomeric linkage is confirmed by the p.m.r. spectra of compounds **5**, **6**, **8**, **9**, **12**, and **13**, all of which gave signals in the region τ 4.76–5.03 with a splitting of about 3.8 Hz, characteristic of the α-D-configuration. In addition **12** was hydrolyzed by maltase but not by β-D-glucosidase. This disaccharide (**12**) represents the first α-D-glycosyl-L-rhamnose to be synthesized.

Experimental

General methods and the preparation of several of the intermediates have been described previously (9). In some cases where only small quantities of material were available p.m.r. spectra were obtained on a Varian XL-100 instrument equipped with Fourier transform.

2-O-Benzyl-D-glucose (1)

3,4,6-Tri-*O*-acetyl-1-deoxy-1-piperidino-β-D-glucopyranose (16) (35 g), freshly prepared (12) powdered dry silver oxide (40 g) and ground Drierite (30 g) were stirred in dry benzene (250 ml) for 30 min in the dark at room temperature with exclusion of moisture. The mixture was cooled to about 15° and benzyl bromide (13 ml, 0.2 *M* excess) was added. After stirring at room temperature for 3½ h t.l.c. (solvent A) indicated that the reaction was complete. The mixture was filtered, the salts were washed with benzene, and the combined solvents were removed after which the product crystallized. Recrystallization from hot methanol (3 ml/g) and drying gave pure 3,4,6-tri-*O*-acetyl-2-*O*-benzyl-1-deoxy-1-piperidino-β-D-glucopyranose 38.7 g (89%), m.p. 99–100°; $[\alpha]_D$ +42° (*c* 2.2,

methanol) (lit. (10) m.p. 100°; $[\alpha]_D$ +41.5° (*c* 0.9, methanol)). The above compound (35 g) was treated with 2% sodium methoxide in anhydrous methanol and t.l.c. (solvent A) showed that the reaction was complete within 30 min. Molar sulfuric acid was added until the p*H* of the solution was about 3 and the mixture was refluxed for 1 h (11). After neutralization (barium carbonate) and concentration, 2-*O*-benzyl-D-glucose (**1**) crystallized. Recrystallization from hot methanol (10 ml/g) gave pure **1** (16 g), m.p. 176–177°; $[\alpha]_D$ +47° (*c* 2.0, methanol) (lit. (10, 11) m.p. 176–177°; $[\alpha]_D$ +47° (*c* 1.0, methanol)).

2-O-Benzyl-3,4,6-tri-O-p-nitrobenzoyl-β-D-glucopyranosyl Bromide (2)

This compound was prepared by the method of Ishikawa and Fletcher (6) in 25% overall yield from **1**, m.p. 142–143°; $[\alpha]_D$ +3° (*c* 2.0, dichloromethane) (lit. (6) m.p. 143–144°; $[\alpha]_D$ +2.4° (*c* 2.1, dichloromethane)).

Methyl 4-O-(2-O-Benzyl-3,4,6-tri-O-p-nitrobenzoyl-α-D-glucopyranosyl)-2,3-O-isopropylidene-α-L-rhamnopyranoside (4)

To a solution of methyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside (17) (**3**, 0.78 g, 3.58 mmol) and mercuric cyanide (1.08 g, 4.3 mmol) in nitromethane (50 ml, distilled from calcium hydride) was added the bromide (**2**) (3.4 g, 4.3 mmol) and the mixture was stirred at 40° with exclusion of moisture. After 5 h t.l.c. (benzene–ether (9:1) (7)) showed a small amount of unreacted **3** (R_f 0.06, no decrease on the addition of more bromide and cyanide) and a large yellow-brown spot (R_f 0.33) corresponding to the disaccharide (**4**). In addition, a faster running component (R_f 0.56, a monosaccharide tri-*p*-nitrobenzoate as indicated by p.m.r.) was detected. The reaction solvent was removed and the remaining syrup was dissolved in benzene (100 ml) and washed successively with water, sodium bicarbonate and water, then dried ($CaSO_4$). The syrup obtained after removal of the solvent was purified on a 25 cm × 7 cm silica gel pressure column (18) (180 g silica) using benzene–ether (9:1) as solvent. Fractions 50–70 (each fraction 20 ml, collected at 90 s intervals) contained the disaccharide. The combined fractions gave a syrup (**4**) (2.65 g, 80%) which was homogeneous on t.l.c., $[\alpha]_D$ +65° (*c* 2.0, chloroform); p.m.r. (CCl_4) τ 1.6–2.1 (12H, *p*-nitrobenzoates), 2.8–3.0 (5H, phenyl), 6.65 (3H singlet, C-1, OCH_3), 8.43, 8.67 (3H, singlets, isopropylidene CH_3), 8.70 (3H, doublet, $J_{5,6}$ = 6 Hz, CH_3).

Methyl 4-O-(2-O-Benzyl-α-D-glucopyranosyl)-2,3-O-isopropylidene-α-L-rhamnopyranoside (5)

Fully blocked disaccharide (**4**) (1.5 g) was refluxed in a mixture of potassium hydroxide (6 g) in water (15 ml) and ethanol (60 ml). After about 90 min (t.l.c., solvent B) the spot corresponding to starting material (R_f 0.84) was replaced by a large product spot (R_f 0.74) corresponding to **5** and a faint spot running slightly faster (R_f 0.76); this was more conveniently identified after hydrogenolysis. The cooled solution was neutralized with Dry Ice and the solvent was removed by evaporation. Residual water was removed by azeotroping with dry benzene or ethanol. The disaccharide was then extracted from the insoluble salts by refluxing with diethyl ether (300 ml × 3). The syrup obtained crystallized after seeding with a sample obtained by preparative t.l.c., yield 565 mg. Alternatively **5** was obtained by dissolving the fully blocked disaccharide (**4**) (1 g) in chloroform (50 ml) and adding 0.2 *M* sodium methoxide in methanol (40 ml). After 1 h at room temperature the solution was neutralized with Dry Ice or, while cold, with Amberlite IR 120 H^+ resin. In the latter case the resin must be removed as soon as the mixture is neutral to avoid deacetalation. Purification by preparative t.l.c. gave crystalline **5** which was recrystallized from 2-propanol (12 ml/g), m.p. 158–159°; $[\alpha]_D$ +71.5° (*c* 1.8, methanol); p.m.r. ($CDCl_3$) τ 2.68 (5H, phenyl), 5.04 (1H doublet, $J_{1',2'}$ = 3.7 Hz, H′-1), 5.18 (1H singlet, H-1), 6.67 (3H singlet, C-1, OCH_3), 8.50, 8.68 (3H singlets, isopropylidene CH_3), 8.70 (3H doublet, $J_{5,6}$ = 6 Hz, CH_3).

Anal. Calcd. for $C_{23}H_{34}O_{16}$: C, 58.69; H, 7.29. Found: C, 58.36; H, 7.34.

Methyl 4-O-α-D-Glucopyranosyl-2,3-O-isopropylidene-α-L-rhamnopyranoside (6)

Methyl 4-*O*-(2-*O*-benzyl-α-D-glucopyranosyl)-2,3-*O*-isopropylidene-α-L-rhamnopyranoside (**5**) (400 mg, m.p. 158–159°) was hydrogenated with 5% palladium-on-carbon (1 g) in absolute ethanol (15 ml) at 50 p.s.i. at room temperature for 16 h. After filtration, washing of the carbon with ethanol and removal of the solvent, a chromatographically pure syrup (320 mg) was obtained, $[\alpha]_D$ + 73° (*c* 2.0, methanol); p.m.r. τ 6.64 (3H singlet, C-1, OCH_3), 8.50, 8.68 (3H singlets, isopropylidene CH_3). The p.m.r. spectrum of the trimethylsilyl derivative (13) of (**6**) showed (benzene, external TMS) τ 5.03 (1H doublet, $J_{1',2'}$ = 4, H′-1), 5.11 (1H singlet, H-1), 7.0 (3H singlet, C-1, OCH_3), 8.40, 8.76 (3H singlets, isopropylidene CH_3), 8.56 (3H doublet, $J_{1,2}$ = 6 Hz, CH_3), 9.65, 9.68, 9.78, 9.90 (9H singlets, 4 trimethylsilyl groups).

Similarly, a sample of **5** (30 mg) containing a small amount of a faster running compound was hydrogenolyzed giving **6** (R_f 0.34, solvent B) and a second component (**7**) (R_f 0.43), which was isolated by preparative t.l.c. (1 mg obtained). The p.m.r. (Fourier transform) of **7** was similar to that of **6** but was unclear. The p.m.r. (Fourier transform) of the trimethylsilyl derivative of **7** showed (benzene-d_6, external TMS) τ 4.86 (1H doublet, $J_{1',2'}$ = 7.3, H′-1), 5.06 (1H, singlet, H-1), 6.92 (3H singlet, C-1, OCH_3), 8.43 (3H doublet, $J_{5,6}$ = 6 Hz, CH_3), 8.46, 8.76 (3H singlets, isopropylidene CH_3), 9.70, 9.71, 9.75, 9.84 (9H singlets, 4 trimethylsilyl groups) and was identical to the p.m.r. of the trimethylsilyl derivative of an authentic sample of methyl 4-*O*-β-D-glucopyranosyl-2,3-*O*-isopropylidene-α-L-rhamnopyranoside obtained by deacetylation of the crystalline tetraacetate (9).

Methyl 4-O-α-D-Glucopyranosyl-α-L-rhamnopyranoside (8)

The isopropylidene compound (**6**) (250 mg) in chloroform (10 ml) was treated at room temperature for 2½ h with trifluoroacetic acid (TFA, 1 ml) containing 1–2% water. The mixture was concentrated and small amounts of TFA removed by treatment with Duolite A-4 (OH^-) resin, or by evaporation with toluene. After filtration and evaporation a syrup (**8**) (211 mg) was obtained, $[\alpha]_D$ +43° (*c* 1.2, methanol), $R_{glucose}$ (solvent C) 2.1; p.m.r. (D_2O, external TMS) τ 4.97 (1H doublet, $J_{1',2'}$ = 3.3 Hz), 5.35 (1H doublet, $J_{1,2}$ = 1.7 Hz), 8.64 (3H doublet, $J_{5,6}$ = 6 Hz, CH_3), 6.64 (3H singlet, OCH_3).

Periodate Oxidation

The disaccharide methyl glycoside (**8**) consumed 3.0 mol of sodium metaperiodate and reduction of the product

followed by methanolysis gave 1-deoxy-D-erythritol and glycerol as determined by paper chromatography in solvent D or as acetates on column *b* (9).

Methylation

The glycoside (**8**) was methylated (9, 15) to give the permethylated compound **9** which, after purification by t.l.c. (R_f 0.13, solvent A; 0.61, solvent B), had $[\alpha]_D$ +80° (*c* 0.9, methanol); p.m.r. ($CDCl_3$) τ 4.97 (1H doublet, $J_{1',2'}$ = 3.7 Hz, H'-1), 5.27 (1H doublet, $J_{1,2}$ = 1.8 Hz, H-1), 6.35–6.65 (21H, 7-OCH_3), 8.65 (3H doublet, $J_{5,6}$ = 6 Hz, CH_3).

The sugars obtained on methanolysis or hydrolysis of **9** were characterized as 2,3-di-*O*-methyl-L-rhamnose and 2,3,4,6-tetra-*O*-methyl-D-glucose by g.l.c. and mass spectra (9).

*Methyl 2,3-Di-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl)-α-L-rhamnopyranoside (**10**)*

The disaccharide methyl glycoside (**8**) (200 mg) was acetylated in pyridine (5 ml) and acetic anhydride (5 ml) at room temperature overnight to give **10** which was recrystallized from ethanol (6 ml/g), yield 290 mg, R_f 0.41 (solvent A); m.p. 136–137°; $[\alpha]_D$ +62.3° (*c* 2.6, chloroform); p.m.r. ($CDCl_3$) τ 6.62 (3H singlet, C-1, OCH_3), 7.90–8.02 (18H, OAc's), 8.60 (3H doublet, $J_{5,6}$ = 6 Hz, CH_3).

Anal. Calcd. for $C_{25}H_{36}O_{16}$: C, 50.65; H, 6.13; Found: C, 50.45; H, 6.11.

*4-O-(2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl)-1,2,3-tri-O-acetyl-L-rhamnopyranose (**11**)*

To the hexaacetate (**10**) (80 mg) in acetic anhydride (1 ml) was added a solution of 2% (v/v) sulfuric acid in acetic anhydride (2 ml). After 3 h at room temperature, cold saturated sodium bicarbonate (15 ml) was added to the mixture and stirred for 35 min. The product was extracted with chloroform (2 × 15 ml) which was washed with water (2 × 5 ml) and dried ($CaSO_4$). Thin-layer chromatography (solvent A) showed one major spot (R_f 0.41, indistinguishable from starting material) and a small amount of cleavage products. Preparative t.l.c. gave chromatographically pure heptaacetate (**11**) as a syrup, yield 78 mg, $[\alpha]_D$ +55° (*c* 2.0, chloroform); p.m.r. ($CDCl_3$) τ 4.00 (1H doublet, $J_{1,2}$ = 1.6 Hz, H-1), 7.82–8.02 (21H, OAc's), 8.62 (3H doublet, $J_{5,6}$ = 6 Hz, CH_3).

*4-O-α-D-Glucopyranosyl-L-rhamnopyranose (**12**)*

The heptaacetate (**11**) (70 mg) was deacetylated in 0.2 *M* sodium methoxide in methanol (5 ml, 1 h, 20°) and passage through Amberlite IR-120 (H^+) resin gave the free disaccharide (**12**) as a syrup, 35 mg. Paper chromatography gave one component with $R_{glucose}$ 0.68 (solvent C) and $[\alpha]_D$ +10° (*c* 1.0, water); p.m.r. (D_2O, external TMS) τ 4.88 (1H doublet, $J_{1,2}$ = 1.3 Hz, H-1 of α-L-form), 5.14 (1H doublet, $J_{1,2}$ = 0.8 Hz, H-1 of β-L-form), 4.94 (1H doublet, $J_{1',2'}$ = 3.8 Hz, H'-1), 8.64 (3H doublet, $J_{5,6}$ = 6 Hz, CH_3).

Gas–liquid chromatography of the per(trimethylsilyl) disaccharide (column *a*, 250°) gave one peak (77%) at 6.4 min and a second peak at 8.7 min, (per(trimethylsilyl)sucrose, 9.0 min). Enzymatic hydrolysis of the disaccharide (2 mg) with maltase (2 mg) at p*H* 6.5 at 37° gave over 90% cleavage in 30 min as shown by paper chromatography. Incubation with β-glucosidase (19) at p*H* 5.1 gave only trace cleavage in 16 h.

*4-O-α-D-Glucopyranosyl-L-rhamnitol (**13**)*

The disaccharide (**12**) (15 mg) was reduced with sodium borohydride (30 mg) in water (2 ml) at room temperature overnight. Passage through IR-120 (H^+) cation exchange resin, concentration, and distillation with methanol gave a syrup (**13**), 14 mg, $R_{glucose}$ 0.58 (solvent C); p.m.r. (D_2O, external TMS) τ 4.76 (1H doublet, $J_{1',2'}$ = 3.8 Hz, H'-1), 8.70 (CH_3 doublet, $J_{5,6}$ = 6 Hz). Gas-liquid chromatography of the per(trimethylsilyl)alditol on column *a* at 250° gave one peak at 9.6 min, (per(trimethylsilyl)sucrose, 9.0 min). Acetylation of the alditol (12 mg, pyridine-acetic anhydride) gave a syrup $[\alpha]_D$ +56° (*c* 1.25, chloroform); p.m.r. ($CDCl_3$) τ 7.90–8.02 (24H, OAc's), 8.66 (CH_3 doublet, $J_{5,6}$ = 6 Hz). Injection of the alditol acetate onto column *a* at 275° gave one peak at 5.8 min (sucrose octaacetate, 7.2 min).

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