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Note

Synthesis of 4-*O*-β-D-galactopyranosyl-3-*O*-methyl-D-glucose

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Several methods have been developed for the diagnosis of intestinal lactase deficiency[1]. Most of them are invasive, *i.e.*, they require either intestinal mucosal biopsy or blood sampling. A non-invasive method has been developed which utilises the fact that unabsorbed lactose, which reaches the large intestine, is fermented with the formation of hydrogen that can be detected in the expired air[2–4]. However, this method is indirect. Some pitfalls have been observed with individuals having an abnormal colonic flora[5] or after oral intake of antibiotics[6].

Martinez-Pardo *et al.*[7] have described a non-invasive method, which is more direct and is based on 4-*O*-β-D-galactopyranosyl-3-*O*-methyl-D-glucose (3-*O*-methyl-lactose), a substrate that is hydrolysed by the intestinal lactase at about the same rate and with the same K_m as for lactose. The 3-*O*-methyl-D-glucose formed on hydrolysis is actively absorbed, but not metabolised, and is quantitatively excreted in the urine, where it can be selectively detected, *e.g.*, using g.l.c. or h.p.l.c. However, the routine use of 3-*O*-methyl-lactose in lactase-deficiency determination is critically dependent on the availibility of gram quantities of 3-*O*-methyl-lactose. We have therefore investigated two different synthetic routes to this compound, starting from lactose or from D-glucose and D-galactose.

Benzyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (**1**, prepared[8] from D-glucose in 72% yield) was deacetylated with methanolic sodium methoxide, and the product was converted into the 4,6-*O*-benzylidene derivative **2**. Partial benzylation of **2** under phase-transfer conditions[9] gave benzyl 2-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (**3**, 50% from **1**). Methylation of **3** then gave 95% of **4**. Reductive opening[10] of the benzylidene acetal in **4**, using sodium cyanoborohydride under acidic conditions, gave 80% of the 2,6-dibenzyl ether **5** and 12% of the 2,4-di-

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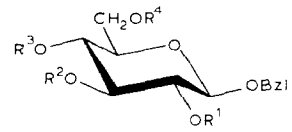
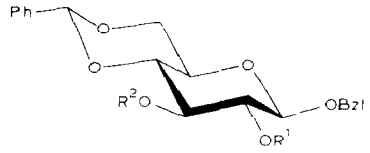
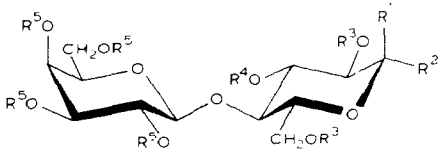


1 $R^1 = R^2 = R^3 = R^4 = Ac$
5 $R^1 = R^4 = Bzl, R^2 = Me, R^3 = H$
6 $R^1 = R^3 = Bzl, R^2 = Me, R^4 = H$



2 $R^1 = R^2 = H$
3 $R^1 = Bzl, R^2 = H$
4 $R^1 = Bzl, R^2 = Me$

benzyl ether **6**. Glycosidation of **5** with 2,3,4,6-tetra-*O*-benzoyl-α-D-galacto-pyranosyl bromide[11], using silver triflate as promoter[12] gave 84% of the di-saccharide derivative **7** which, on debenzoylation and subsequent catalytic hydrogenation, gave 3-*O*-methyl-lactose (**8**, 88%). The overall yield of **8** from D-glucose, using this ten-step pathway, was 24%.



7 $R^1 = H, R^2 = OBzl, R^3 = Bzl, R^4 = Me, R^5 = Bz$
8 $R^1 = R^2 = H$ or $OH, R^3 = R^5 = H, R^4 = Me$
9 $R^1 = OBz, R^2 = H, R^3 = R^5 = Bz, R^4 = H$
10 $R^1 = OBz, R^2 = H, R^3 = R^5 = Bz, R^4 = Me$

An overall yield of 18% was obtained starting from lactose, using a modification of the 3-step pathway reported by Martinez-Pardo *et al.*[7]. Partial benzoylation of lactose gave 1,2,6,2′,3′,4′,6′-hepta-*O*-benzoyl-α-lactose[13] (**9**, 24%). Methylation of **9** with methyl triflate[14] gave the 3-methyl ether (**10**, 81%). Deblocking with methanolic sodium methoxide then gave **8** (96%).

The latter procedure is by far the most convenient for the preparation of up to 1-gram quantities of 3-*O*-methyl-lactose (**8**). However, the toxicity of methyl triflate and the cost of the necessary 2,6-di-*tert*-butyl-4-methylpyridine make larger scale preparations inconvenient. A cheaper and less toxic procedure for the methylation of **9** is being sought.

EXPERIMENTAL

General methods. — Melting points are corrected. Concentrations were performed at 1–2 kPa at <40° (bath). Optical rotations were recorded for 0.5–1.0% solutions in chloroform, unless otherwise stated, using a Perkin–Elmer 241 polarimeter. N.m.r. spectra were recorded in the F.t. mode for solutions in $CDCl_3$ (internal Me_4Si) unless otherwise stated, using a JEOL JNM FX-100 instrument.

N.m.r. spectra, recorded for all new compounds, were in agreement with the postulated structures, and only selected 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 loading in the range 1/25–1/100 and elution with toluene–ethyl acetate mixtures unless otherwise stated. Organic solutions were dried over $MgSO_4$. Molecular sieves (4 Å, Union Carbide) were desiccated in a vacuum at 300° overnight and ground immediately before use.

Benzyl 2-O-benzyl-4,6-O-benzylidene-β-D-glucopyranoside (**3**). — Benzyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside[8] (**1**; 2.0 g, 4.6 mmol) was suspended in methanolic sodium methoxide (50 mL, 0.01M). When t.l.c. indicated complete reaction, the mixture was neutralised with Dowex 50 (H^+) resin, filtered, and concentrated. The residue was suspended in benzaldehyde (10 mL), and toluene-*p*-sulfonic acid monohydrate (75 mg) was added. After being stirred for 4 h at room temperature, the mixture was poured into a stirred 1:1 mixture of light petroleum and aqueous sodium hydrogencarbonate (200 mL). The precipitate was collected, dried, and dissolved in dichloromethane (90 mL); benzyl bromide (0.86 mL), tetrabutylammonium hydrogensulfate (0.36 g), and aqueous sodium hydroxide (5%, 7.5 mL) were added, and the mixture was boiled under reflux overnight. Methanol was added and boiling was continued for 1 h. The organic layer was washed with water, dried, and concentrated. Column chromatography of the residue gave **3** (0.93 g, 50%) as the main fraction. Crystallisation from toluene gave material with m.p. 164–165°, $[\alpha]_D$ −45°.

Anal. Calc. for $C_{27}H_{28}O_6$: C, 72.3; H, 6.29. Found: C, 71.9; H, 6.30.

Benzyl 2-O-benzyl-4,6-O-benzylidene-3-O-methyl-β-D-glucopyranoside (**4**). — Sodium hydride (40 mg) was added to a stirred solution of **3** (0.25 g) in *N,N*-dimethylformamide (3 mL) followed by methyl iodide (0.18 mL). After 5 min, methanol (1 mL) was added, stirring was continued for 5 min, and the mixture was then partitioned between water and 1:1 dichloromethane–ether. The organic layer was dried and concentrated. Column chromatography of the residue gave **4** (0.25 g, 95%). Recrystallisation from chloroform–methanol gave material with m.p. 160–163°, $[\alpha]_D$ −56°.

Anal. Calc. for $C_{28}H_{30}O_6$: C, 72.7; H, 6.54. Found: C, 72.0; H, 6.60.

Benzyl 2,6-di-O-benzyl- (**5**) *and 2,4-di-O-benzyl-3-O-methyl-β-D-glucopyranoside* (**6**). — A solution of **4** (0.46 g) in tetrahydrofuran (15 mL) containing sodium cyanoborohydride (0.57 g) was treated with ethereal hydrogen chloride until the solution was acidic to pH-paper. When t.l.c. indicated complete reaction, the mixture was poured into ice–water and extracted with dichloromethane, and the extract was washed with aqueous sodium hydrogencarbonate, dried, and concentrated. Column chromatography of the residue gave, first, **6** (56 mg, 12%), m.p. 142° (from ethyl acetate–hexane), $[\alpha]_D$ −4°. ^{13}C-N.m.r. data: δ 61.3, 62.2 (OCH_3, C-6), 102.8 (C-1).

Anal. Calc. for $C_{28}H_{32}O_6$: C, 72.4; H, 6.94. Found: C, 70.4; H, 6.80.

Further elution gave syrupy **5** (0.37 g, 80%), $[\alpha]_D$ −49°. ^{13}C-N.m.r. data: δ 61.1 (OCH_3), 70.2, 71.1, 71.3, 73.7, 74.3, 74.5, 81.7, 86.0 (C-2/6, OCH_2Ph), 102.6 (C-1).

Benzyl 2,6-di-O-benzyl-3-O-methyl-4-O-(2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**7**). — A solution of tetra-*O*-benzoyl-α-D-galactopyranosyl bromide[11] (0.40 g, 0.60 mmol) and **5** (0.28 g, 0.60 mmol) in dry dichloromethane (3.0 mL) containing molecular sieves was cooled to −30°, and silver triflate (0.19 g) in toluene (2 mL) was added. After 15 min, the mixture was filtered, washed with aqueous sodium thiosulfate, water, M sulfuric acid, and aqueous sodium hydrogencarbonate, dried, and concentrated. The syrupy residue was subjected to column chromatography to give **7** as the main product (0.53 g, 84%), $[\alpha]_D$ +36°. ^{13}C-N.m.r. data: δ 61.1 (OCH_3), 62.0, 68.3, 68.5, 70.7, 71.1, 71.3, 72.0, 73.4, 74.3, 74.8, 77.9, 82.0, 85.0 (C-2/6, C-2′/C-6′, OCH_2Ph), 101.1, 102.4 (C-1, C-1′).

1,2,6-Tri-O-benzoyl-3-O-methyl-(2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl)-α-D-glucopyranose (**10**). — A solution of **9**[13] (2.14 g), methyl triflate (0.56 mL), and 2,6-di-*tert*-butyl-4-methylpyridine (2.04 g) in 1,2-dichloroethane (5 mL) was stirred and heated to 85° in an ampoule for 48 h. Solid material was removed, the filtrate and washings were concentrated, and the residue was subjected to column chromatography to give **11** (1.76 g, 81%). Recrystallisation from chloroform–methanol gave material with m.p. 210–211°, $[\alpha]_D$ +56°; lit.[13] m.p. 203–204°, $[\alpha]_D$ +52.9°.

4-O-β-D-Galactopyranosyl-3-O-methyl-D-glucose (**8**, *3-O-methyl-lactose*). — *(a) From* **7**. To a solution of **7** (0.38 g) in 1:1 chloroform–methanol (25 mL) was added methanolic sodium methoxide (0.5 mL, 0.5M). When t.l.c. indicated complete reaction, the mixture was neutralised with Dowex 50 (H^+) resin, filtered, and concentrated, and the residue was eluted from a short column of silica gel with chloroform–methanol (20:3). The appropriate fractions were concentrated and the residue was hydrogenated over Pd/C (10%, 150 mg) in ethanol solution at 400 kPa. Filtration and concentration of the reaction mixture gave a syrup which was chromatographically homogeneous **8** (0.11 g, 88%), $[\alpha]_D$ +18° (*c* 0.8, equil., water). ^{13}C-N.m.r. data (D_2O, external Me_4Si): δ 93.1, 97.1, 104.1 (C-1, C-1′).

(b) From **10**. Compound **10** (0.20 g) was debenzoylated as described above, and the product was purified by column chromatography (ethyl acetate–methanol–acetic acid–water, 5:3:3:2) to give **8** (0.066 g, 96%).

Acid hydrolysis of **8** followed by borohydride reduction and acetylation gave hexa-*O*-acetylgalactitol and penta-*O*-acetyl-3-*O*-methylglucitol, identified[15] by g.l.c.–m.s.

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