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4-*O*-β-D-GALACTOPYRANOSYL-3-*O*-METHYL-D-GLUCOSE: A NEW SYNTHESIS AND APPLICATION TO THE EVALUATION OF INTESTINAL LACTASE*

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ABSTRACT

4-*O*-β-D-Galactopyranosyl-3-*O*-methyl-D-glucose (**1**, 3-*O*-methyl-lactose) has been prepared from benzyl 2,6-di-*O*-benzyl-4-*O*-(2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (**5**) and from benzyl 2,6-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**15**). Partial benzylation of benzyl 3′,4′-*O*-isopropylidene-β-lactoside (**4**) gave **5** and partial benzylation of either benzyl β-lactoside (**13**) or benzyl hepta-*O*-acetyl-β-lactoside (**24**) gave **15**. All other products from the partial benzylation of **4**, **13**, and **24** were also isolated and characterised. The hydrolysis of **1** *in vitro* by intestinal lactase was linear during 20 h; the V_{max} was 5% of that with lactose and the K_m was 120mM (*cf.* 30mM for lactose). Oral administration of **1** to suckling rats led to urinary excretion of 3-*O*-methyl-D-glucose.

INTRODUCTION

We have reported[1,2] a synthesis of 4-*O*-β-D-galactopyranosyl-3-*O*-methyl-D-glucose (**1**, 3-*O*-methyl-lactose) and the use of this substance in the evaluation *in vivo* of intestinal lactase[1]. Partial benzoylation[3] of lactose gave the heptabenzoate **2**, which was methylated to give **3**[3] and then debenzoylated to give **1**. Methylation of **2** was carried out with diazomethane[1,3] or methyl triflate[2], and neither reagent is convenient for large-scale operations. We now report new syntheses of **1** which avoid the use of these methylating agents.

*Dedicated to the memory of the late Professor Arne Dalqvist.
**On leave of absence from Universidad del Atlántico, Barranquilla, Colombia.

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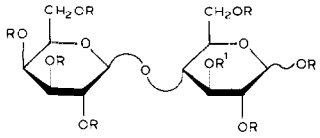
RESULTS AND DISCUSSION

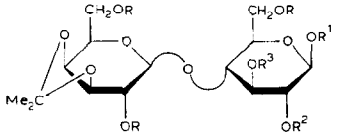
Partial benzylation of benzyl 3′,4′-*O*-isopropylidene-β-lactoside (**4**) with benzyl chloride–potassium hydroxide gives[4] a mixture of products from which benzyl 2,6-di-*O*-benzyl-4-*O*-(2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (**5**) can be isolated (30%) by column chromatography. Treatment of benzyl β-lactoside[5] (**13**) with acetone–toluene-*p*-sulfonic acid gave[5] the 3′,4′-*O*-isopropylidene derivative **4**. From the mother liquors, the 4′,6′-*O*-isopropylidene derivative **6** was isolated and transformed into **4** in the presence of silica gel. Compound **4** was the main product when **13** reacted with 2,2-dimethoxypropane. Acetylation of **6** gave the penta-acetate **7**. Treatment of **4** with benzyl bromide under phase-transfer conditions afforded, after column chromatography, benzyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (**8**, 18%), **5** (38%), and a mixture of **5** and benzyl 3,6-di-*O*-benzyl-4-*O*-(2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (**9**, 4%). Compound **5** could also be isolated by crystallisation from the benzylation mixture, although in lower yield. Acetylation of **5** gave the 3-acetate **10**. Methylation of **5** with methyl iodide afforded the 3-*O*-methyl derivative **11** in almost quantitative yield. Hydrogenolysis of **11** gave 3′,4′-*O*-isopropylidene-3-*O*-methyl-lactose (**12**, 92%) and acid hydrolysis of **12** afforded 3-*O*-methyl-lactose (**1**, 94%).

In an alternative synthesis, partial benzylation of benzyl β-lactoside (**13**) with benzyl chloride and potassium hydroxide gave, after column chromatography, benzyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**14**, 8%), benzyl 2,6-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**15**, 25%), a mixture (30%) of **16** and **17** [which, after acetylation and chromatography, gave benzyl 4-*O*-(3-*O*-acetyl-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**19**) and benzyl 4-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**20**)], and **21** (9%) acetylation of which afforded benzyl 3-*O*-acetyl-4-*O*-(3-*O*-acetyl-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-2,6-di-*O*-benzyl-β-D-glucopyranoside (**22**). The structures of these compounds were determined by ^{1}H- and ^{13}C-n.m.r. spectroscopy. Acetylation of **15** gave the 3-acetate **18**, and methylation of **15** with methyl iodide gave the 3-*O*-methyl derivative **23** (84%). Hydrogenolysis of **23** gave **1**.

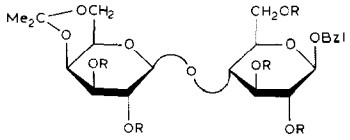
Finally, treatment of benzyl hepta-*O*-acetyl-β-lactoside (**24**) with benzyl chloride under phase-transfer conditions gave, after column chromatography, **14** (7%), **15** (26%), **16** and **17** [17%; which could be isolated as the corresponding acetates (**19** and **20**)], **21** (9%), and benzyl 2,3,6-tri-*O*-benzyl-(3,6-di-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**25**, 5%) which was characterised as diacetate **26**. The yield of **15** was almost the same as that from the partial benzylation of benzyl β-lactoside (**13**) described above. This procedure improves the synthesis of **1** since **24** is the precursor of **13**.



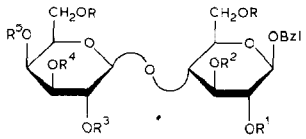
1 R = H, R^1 = Me
2 β R = Bz, R^1 = H
3 β R = Bz, R^1 = Me



4 R = R^2 = R^3 = H, R^1 = Bzl
5 R = R^1 = R^2 = Bzl, R^3 = H
8 R = R^1 = R^2 = R^3 = Bzl
9 R = R^1 = R^3 = Bzl, R^2 = H
10 R = R^1 = R^2 = Bzl, R^3 = Ac
11 R = R^1 = R^2 = Bzl, R^3 = Me
12 α,β R = R^1 = R^2 = H, R^3 = Me



6 R = H
7 R = Ac



13 R = R^1 = R^2 = R^3 = R^4 = R^5 = H
14 R = R^1 = R^2 = R^3 = R^4 = R^5 = Bzl
15 R = R^1 = R^3 = R^4 = R^5 = Bzl, R^2 = H
16 R = R^1 = R^2 = R^3 = R^5 = Bzl, R^4 = H
17 R = R^1 = R^2 = R^4 = R^5 = Bzl, R^3 = H
18 R = R^1 = R^3 = R^4 = R^5 = Bzl, R^2 = Ac
19 R = R^1 = R^2 = R^3 = R^5 = Bzl, R^4 = Ac
20 R = R^1 = R^2 = R^4 = R^5 = Bzl, R^3 = Ac
21 R = R^1 = R^3 = R^5 = Bzl, R^2 = R^4 = H
22 R = R^1 = R^3 = R^5 = Bzl, R^2 = R^4 = Ac
23 R = R^1 = R^3 = R^4 = R^5 = Bzl, R^2 = Me
24 R = R^1 = R^2 = R^3 = R^4 = R^5 = Ac
25 R = R^1 = R^2 = R^4 = Bzl, R^3 = R^5 = H
26 R = R^1 = R^2 = R^4 = Bzl, R^3 = R^5 = Ac

The hydrolysis of **1** by the intestinal lactase, partially purified from sheep, was essentially linear during 20 h (70% hydrolysis). In a parallel experiment, lactose was completely hydrolysed within 1 h. When an equimolecular mixture of lactose and 3-*O*-methyl-lactose was incubated with the enzyme, the hydrolysis of lactose was not appreciably hindered by **1**. Fig. 1 shows quantitatively the difference in kinetic parameters of the enzyme for the substrates lactose and **1**. The V_{max} for **1** was only 5% of that for lactose and the K_m was 120mM for **1** (*cf.* 30mM for lactose). These kinetic values are similar to those reported[6]. The initial report, that intestinal lactase could hydrolyse **1** almost as efficiently as lactose, is in error.

For the *in vivo* experiments, 15-day-old suckling rats and 4-month-old adult rats were used (Wistar strain and both sexes). Both suckling and adult rats were of a single litter. To one group, **1** (50 mg/0.5 mL of water) was administered orally by intragastric intubation after a 5-h fast during which time the urine was collected. To another group, **1** (25 mg) and D-galactose (25 mg) were administered similarly. Urine collection was carried out by transabdominal pressure several times during

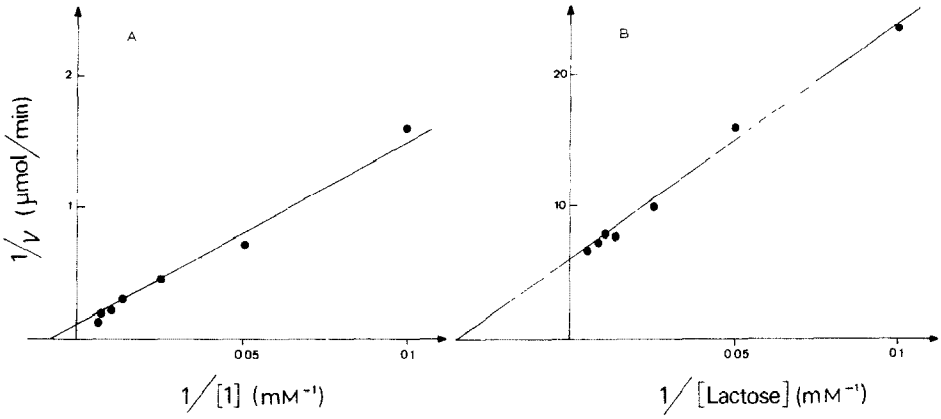


Fig. 1. Plots of the hydrolysis of **1** (A) and lactose (B) by intestinal lactase from sheep.

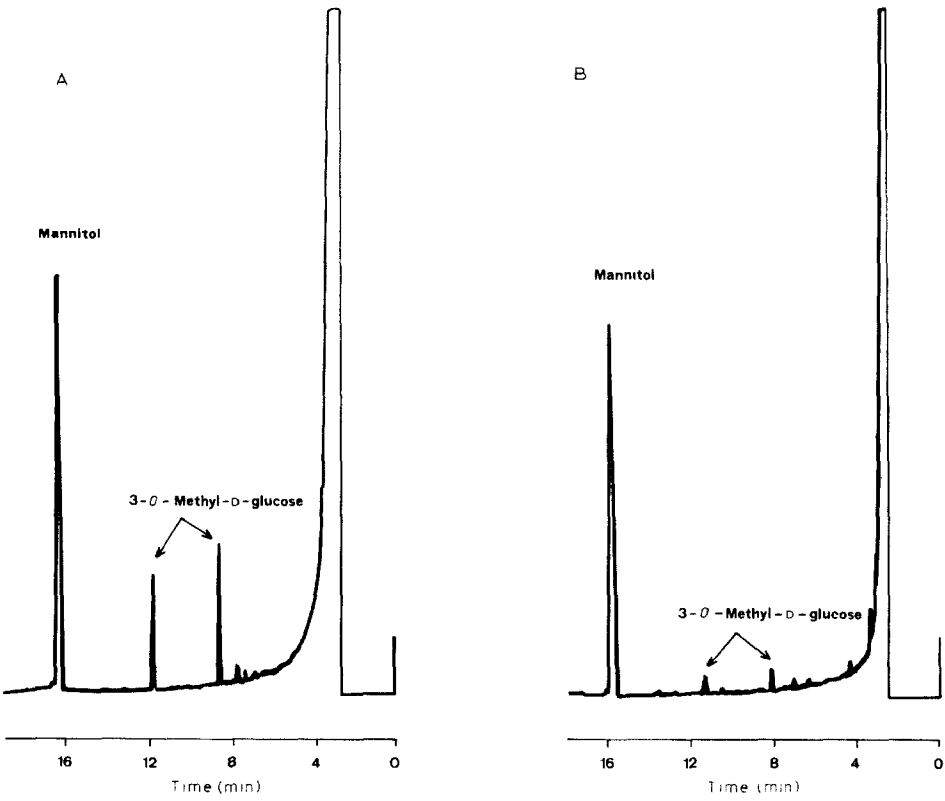


Fig. 2. Gas chromatogram of products obtained from the urine of a suckling rat (A) and an adult rat (B) after oral administration of 50 mg of **1**. A Perkin–Elmer 900 gas chromatograph was used and the carbohydrates were determined as trimethylsilyl derivatives with mannitol as the internal reference.

TABLE I

URINARY RECOVERY OF 3-*O*-METHYL-D-GLUCOSE

	Recovered 3-O-methyl-D-glucose (%)[a]		
	After administration of 3-O-methyl-D-glucose	*After administration of* **1**	*Specific intestinal lactase activity*
Adult rats (during 20 h)	97	<2	25
Suckling rats (during 8 h)	76	12	100

[a]Averages of three experiments. Amounts of 3-*O*-methyl-D-glucose and **1** administered are given in the text.

5 h for the suckling rats, and by accumulation of the urine eliminated during 18 h in metabolic cages devoid of food but provided with water. The urines collected before and after the administration of the indicated compounds were lyophilised, and the residues were trimethylsilylated. Fig. 2 shows typical gas chromatograms corresponding to suckling and adult rats that had received **1**; the greater elimination of 3-*O*-methyl-D-glucose by the suckling rats is evident. The chromatograms obtained for the products in basal urines had no detectable peaks at the position corresponding to 3-*O*-methylglucose. Table I shows the partial recovery of 3-*O*-methyl-D-glucose after administration of **1** to suckling rats in contrast to the negligible recovery from adult rats. Table I also shows that, after administration of **1** to adult rats, there was essentially complete recuperation from the urine during 20 h, and that the specific activity of lactase in the intestine of adult rats was only 25% of that of suckling rats.

EXPERIMENTAL

Material and methods. — Melting points were measured in capillary tubes and are uncorrected. T.l.c. was performed on silica gel GF_{254} (Merck) with detection by charring with sulfuric acid. Column chromatography was performed on Merck silica gel (70–230 mesh). ^{1}H-N.m.r. spectra (300 MHz) were recorded with a Varian XL-300 spectrometer, and ^{13}C-n.m.r. spectra (20 MHz) were recorded with a Bruker WP-80 spectrometer. Optical rotations were determined with a Perkin–Elmer 141 polarimeter.

Intestinal lactase was purified from young sheep essentially as described by Skovbjerg *et al.*[7]. Intestinal mucosa was scraped off and homogenised in 4 vol. of aqueous 1% Triton X-100. The supernatant solution obtained after centrifugation of the homogenate at 50,000*g* for 1 h was eluted from a column of DEAE cellulose, and the fractions containing >24 U of lactase/mL were combined and fractionated by gel filtration on Ultrogel AcA-34. Fractions containing more than 1 U of lactase/mL were combined and concentrated for use in the assays. The purification

factor was 20 unless otherwise stated. A standard reaction mixture contained, in a final volume of 200 μL, 80mM maleate (pH 6.0), 80 mL of lactose or **1**, and 20 μL of enzyme preparation. After incubation for 0.5 to 6 h at 37°, the reaction was stopped by addition of either 200mM Tris buffer (pH 8.6, 250 μL), for galactose determination, or 250mM Tris buffer (pH 7.0, 690 μL, containing 0.5 mg/mL of ABTS), if glucose was to be determined, and boiling for 2 min. D-Galactose and D-glucose were determined[8] with D-galactose dehydrogenase and D-glucose oxidase, respectively. One unit of enzyme activity is defined as the amount of enzyme that can produce 1 μmol of D-glucose or D-galactose per min at 37°. Enzyme specific activity is expressed as U/mg of protein. Protein was determined by the method of Lowry *et al.*[9]. Bovine serum albumin was used as standard. For the determination of 3-*O*-methylglucose in urine, a Perkin–Elmer 900 gas chromatograph was used. The urine was lyophilised after addition of mannitol (4 mg), and a solution of the dry residue in pyridine (1 mL) was heated to 70° for 3 min, filtered, and treated with chlorotrimethylsilane (0.5 mL) and hexamethyldisilazane (0.5 mL).

Benzyl 4-O-(4,6-O-isopropylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (**6**). — Benzyl β-lactoside[5] (2.5 g) was treated[5] with acetone (760 mL) in the presence of anhydrous toluene-*p*-sulfonic acid (0.5 g) to give, after concentration of the neutralised reaction mixture, a residue (3.0 g) which was stirred with warm acetone (30 mL) for 30 min. After cooling, the chromatographically pure (10:3 ethyl acetate–methanol) 3′,4′-*O*-isopropylidene derivative (**4**, 2 g) was isolated; m.p. 196°; lit.[5] m.p. 196–197°. Concentration of the mother liquors gave a syrupy residue (0.46 g) which solidified after the addition of hexane. Crystallisation from ethyl acetate–ether afforded **6**, m.p. 168–169°, $[\alpha]_D^{25}$ −42° (*c* 1.3, methanol). N.m.r. data (CD_3OD): ^1H, δ 7.20–7.40 (5 H, m, Ph), 1.47 and 1.40 (2 s, 6 H, Me); ^{13}C, 138.8 (C-ipso), 129.2, 129.0, 128.6 (aromatic), 104.5 (CMe_2), 103.1 (C-1′), 100.0 (C-1), 79.9, 76.4, 76.3, 74.7, 73.2, 71.8, 71.4, 69.7, 67.8, 63.5, 61.8, 29.5, and 18.7 p.p.m.

Anal. Calc. for $C_{22}H_{32}O_{11}$: C, 55.92; H, 6.83. Found: C, 55.80; H, 6.85.

Compound **6** was transformed into **4** (t.l.c., 10:3 ethyl acetate–methanol) on attempted chromatography on silica gel.

Acetonation of **13** (0.25 g) with 2,2-dimethoxypropane (0.18 mL) in *N,N*-dimethylformamide (2.5 mL) in the presence of toluene-*p*-sulfonic acid (35 mg) gave **6** as the main product (t.l.c.).

Benzyl 2,3,6-tri-O-acetyl-4-O-(2,3-di-O-acetyl-4,6-O-isopropylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (**7**). — Acetylation of **6** (0.12 g) with acetic anhydride in pyridine gave, after column chromatography (7:5 ethyl acetate–hexane), **7** (0.17 g), m.p. 167–169°, $[\alpha]_D^{25}$ −6° (*c* 1.15, chloroform). N.m.r. data ($CDCl_3$): ^1H, δ 7.20–7.40 (5 H, m, Ph), 5.16 (m, 2 H, H-2′,3), 4.97 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 9.7 Hz, H-2), 4.86 (d, 1 H, *J* 12.3 Hz, C*H*Ph), 4.77 (dd, 1 H, $J_{2',3'}$ 10.2, $J_{3',4'}$ 3.6 Hz, H-3′), 4.59 (d, 1 H, *J* 12.3 Hz, C*H*Ph), 4.53 (dd, 1 H, $J_{6a,6b}$ 11.5, $J_{5,6a}$ 2.1 Hz, H-6a), 4.50 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1′), 4.37 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 4.28

(dd, 1 H, $J_{3',4'}$ 3.6, $J_{4',5'}$ 0.8 Hz, H-4'), 4.10 (dd, 1 H, $J_{6b,6a}$ 11.5, $J_{6b,5}$ 5 Hz, H-6b), 4.01 (dd, 1 H, $J_{6a',6b'}$ 12.6, $J_{5',6'a}$ 1.8 Hz, H-6'a), 3.91 (dd, 1 H, $J_{6'a,6'b}$ 12.6, $J_{5',6'b}$ 1.5 Hz, H-6'b), 3.75 (t, 1 H, $J_{3,4} \simeq J_{4,5} \simeq 9.5$ Hz, H-4), 3.56 (m, 1 H, H-5), 3.28 (m, 1 H, H-5'), 2.13, 2.11, 2.06, 2.04, 2.00 (5 s, 15 H, 5 Ac), 1.42 and 1.36 (2 s, 6 H, Me); ^{13}C, 170.6, 170.4, 170.2, 169.6, 169.0 (CO), 136.9 (C-ipso), 128.5, 128.0, 127.8 (aromatic), 101.0 (CMe_2), 99.4 (C-1'), 29.1 and 18.6 (2 Me), and 20.7 p.p.m. (Ac).

Anal. Calc. for $C_{32}H_{42}O_{16}$: C, 56.30; H, 6.20. Found: C, 55.88; H, 6.61.

*Benzyl 3-*O*-acetyl-2,6-di-*O*-benzyl-4-*O*-(2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-β-*D*-galactopyranosyl)-*D*-glucopyranoside* (**10**). — A mixture of **4** (1 g, 2.1 mmol), benzene (40 mL), aqueous 20% sodium hydroxide (20 mL), and tetrabutylammonium hydrogensulfate (0.4 g) was vigorously stirred for 15 min. Benzyl bromide (1.67 mL, 14 mmol) was then added and stirring was continued for 24 h. The mixture was diluted with water, and the organic layer was washed with water, M sulfuric acid, and water, dried (Na_2SO_4), and concentrated. The syrupy residue (2.25 g) was eluted from a column of silica gel with 7:3 hexane–ethyl acetate to give, first, benzyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (**8**; 0.34 g, 18%); then a mixture (0.07 g, 4%) of benzyl 2,6-di-*O*-benzyl-4-*O*-(2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (**5**) and, probably, benzyl 3,6-di-*O*-benzyl-4-*O*-(2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (**9**); and finally **5** (0.67 g, 38%), m.p. 108–109° (from ethanol); lit.[4] m.p. 110°.

Acetylation of **5** (0.10 g), conventionally with acetic anhydride–pyridine, gave **10** (0.11 g) as a syrup, $[\alpha]_D^{20}$ +20° (*c* 1.2, chloroform). N.m.r. data ($CDCl_3$): ^{1}H, δ 7.20–7.40 (m, 25 H, 5 Ph), 5.14 (t, 1 H, $J_{2,3} \simeq J_{3,4} \simeq 9.5$ Hz, H-3), 4.19 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'), 4.04 (d, 1 H, $J_{3',4'}$ 5.5 Hz, H-4'), 3.95 (dd, 1 H, $J_{2',3'}$ 6.8, $J_{3',4'}$ 5.5 Hz, H-3'), 3.86 (t, 1 H, $J_{3,4} \simeq J_{4,5} \simeq 9.5$ Hz, H-4), 3.80 (dd, 1 H, $J_{5,6a}$ 3.7, $J_{6a,6b}$ 10.9 Hz, H-6a), 3.68 (s, 4 H, H-6b,5',6'a,6'b), 3.43 (dd, 1 H, $J_{1,2}$ 7.7, $J_{2,3}$ 9.5 Hz, H-2), 3.43 (m, 1 H, H-5), 3.22 (dd, 1 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 6.8 Hz, H-2'), 1.92 (s, 3 H, Ac), 1.34 and 1.30 (2 s, 6 H, CMe_2); ^{13}C, 170.3 (CO), 138.5, 138.3 (C-ipso), 128.4, 128.2, 127.9, 127.8, 127.5 (aromatic), 109.9 (CMe_2), 102.6 (C-1'), 102.1 (C-1), 80.4, 79.3, 74.9, 74.2, 73.9, 73.8, 73.6, 73.3, 71.9, 71.1, 69.4, 67.9, 27.8, 26.4, and 21.0 p.p.m.

Anal. Calc. for $C_{52}H_{58}O_{12}$: C, 71.37; H, 6.68. Found: C, 71.92; H, 6.93.

*3',4'-*O*-Isopropylidene-3-*O*-methyl-lactose* (**12**). — To a solution of **5** (2.14 g) in anhydrous tetrahydrofuran (17 mL) was added sodium hydride (0.45 g), and the stirred mixture was heated to 50°. Methyl iodide (9.2 mL) was then added, with more (4 mL) after 1.5 h, and the reaction was continued for 5 h. The mixture was cooled, methanol and then water were added, and the mixture was extracted with ether. The extract was dried ($MgSO_4$) and concentrated to give benzyl 2,6-di-*O*-benzyl-4-*O*-(2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-3-*O*-methyl-β-D-glucopyranoside (**11**, 2.20 g) which, after purification by column chromatography (20:20:5 benzene–hexane–ethyl acetate), had $[\alpha]_D^{20}$ −4° (*c* 1, chloroform); lit.[4] $[\alpha]_D^{20}$ −4.4°.

A solution of **11** (4 g) in ethanol (225 mL) and water (9 mL) was hydrogenated over 10% Pd/C (3.4 g) at room temperature for 4 h, filtered, and concentrated. Column chromatography (3:1 ethyl acetate–methanol) of the residue (1.75 g, 92%) gave **12**, m.p. 83–85°, $[\alpha]_D^{20}$ +63.5 → +76.5° (*c* 0.4, methanol). N.m.r. data (CD_3OD): ^1H, δ 5.07 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1α), 4.47 (d, 1 H, $J_{1,2}$ 7.4 Hz, H-1β), 1.45 and 1.31 (2 s, each 3 H, CMe_2); ^{13}C, 111.0 (CMe_2), 103.8 (C-1′), 93.8 (C-1β), 86.4 (C-1α), 83.7 (C-3β), 81.1 (C-3′), 77.9 (C-3′α), 77.5 (C-4α), 76.9 (C-4β), 28.5 and 26.6 p.p.m. (2 Me).

Anal. Calc. for $C_{16}H_{28}O_{11}$: C, 48.48; H, 7.12. Found: C, 48.29; H, 7.36.

Partial benzylation of benzyl β-lactoside (**13**). — A mixture of **13** (1 g, 2.3 mmol), benzyl chloride (6 mL, 52.4 mmol), and powdered potassium hydroxide (1.5 g) was stirred at 100° for 3.5 h and then cooled. Chloroform (60 mL) was added, and the mixture was washed with water, 0.5M sulfuric acid, and water, dried (Na_2SO_4), and concentrated. Column chromatography (7:2 hexane–ethyl acetate) of the residue afforded benzyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**14**; 0.2 g, 8%) as a syrup, $[\alpha]_D^{20}$ −2° (*c* 1, chloroform).

Anal. Calc. for $C_{68}H_{70}O_{11}$: C, 76.81; H, 6.64. Found: C, 76.95; H, 6.87.

Elution with 14:5 hexane–ethyl acetate then gave, first, benzyl 2,6-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**15**; 0.55 g, 25%), m.p. 97–99° (from ethanol), $[\alpha]_D^{20}$ −9° (*c* 0.56, chloroform).

Anal. Calc. for $C_{61}H_{64}O_{11}$: C, 75.29; H, 6.63. Found: C, 75.05; H, 6.62.

Treatment of **15** conventionally with acetic anhydride–pyridine gave benzyl 3-*O*-acetyl-2,6-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**18**), m.p. 96–97° (from ethanol), $[\alpha]_D^{20}$ +11° (*c* 0.7, chloroform). N.m.r. data ($CDCl_3$): ^1H, δ 7.1 (m, 35 H, 7 Ph), 5.12 (t, 1 H, $J_{3,4} = J_{3,2}$ = 9.5 Hz, H-3), 4.24 (d, 1 H, $J_{1',2'}$ 7.5 Hz, H-1′), 3.84 (d, 1 H, $J_{3',4'}$ 2.9, $J_{4',5'}$ ~0 Hz, H-4′), 3.83 (t, 1 H, $J_{3,4} \simeq J_{4,5} \simeq$ 9.5 Hz, H-4), 3.75 (dd, 1 H, $J_{6a,6b}$ 10.8, $J_{5,6a}$ 3.9 Hz, H-6a), 3.67 (m, 1 H, H-6b), 3.65 (dd, 1 H, $J_{2',3'}$ 9.8, $J_{1',2'}$ 7.5 Hz, H-2′), 3.54 (m, 2 H, H-6′a,6′b), 3.32–3.44 (m, 4 H, H-2,5,3′,5′), 1.80 (s, 3 H, Ac); ^{13}C, 170.2 (CO), 139.0, 138.6, 138.3, 138.0 and 137.4 (C-ipso, aromatic), 128.3, 128.2, 127.8, 127.7, and 127.4 (aromatic), 102.5 (C-1 and C-1′), 82.3, 79.8, 75.2, 75.0, 74.4, 74.3, 74.0, 73.9, 73.4, 73.2, 72.7, 72.1, 68.6, 68.1, and 20.9 p.p.m.

Anal. Calc. for $C_{63}H_{66}O_{12}$: C, 74.53; H, 6.55. Found: C, 74.54; H, 6.76.

Eluted second was a mixture (0.68 g, 30%) of benzyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**16**) and benzyl 2,3,6-tri-*O*-benzyl-4-*O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**17**). The mixture was acetylated under the usual conditions, and column chromatography (4:1 hexane–ethyl acetate) of the products afforded, first, benzyl 4-*O*-(3-*O*-acetyl-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**19**; 0.15 g) as a syrup, $[\alpha]_D^{20}$ +8° (*c* 0.8, chloroform). N.m.r. data ($CDCl_3$): ^1H, δ 7.1–7.2 (m, 35 H, 7 Ph), 4.80 (dd, 1 H, $J_{3',4'}$ 3.1, $J_{2',3'}$ 10.2 Hz, H-3′), 3.98 (t, 1 H, $J_{4,5} \simeq J_{3,4} \simeq$ 9.0 Hz, H-4), 3.92 (d, 1 H, $J_{3',4'}$ 3.1, $J_{4',5'}$ ~0 Hz,

H-4'), 3.79 (dd, 1 H, $J_{6a,6b}$ 11.0, $J_{5,6a}$ 3.9 Hz, H-6a), 3.73 (dd, 1 H, $J_{6a,6b}$ 11.0, $J_{5,6b}$ 2.2 Hz, H-6b), 3.72 (dd, 1 H, $J_{1',2'}$ 7.6, $J_{2',3'}$ 10.2 Hz, H-2'), 3.55 (t, 1 H, $J_{2,3} \simeq J_{3,4} \simeq 9.0$ Hz, H-3), 3.4–3.5 (m, 3 H, H-2,6'a,6'b), 3.35 (m, 2 H, H-5,5'), 1.92 (s, 3 H, Ac); ^{13}C, 170.2 (CO), 139.2, 138.8, 138.6, 138.3, 138.1, and 137.6 (C-ipso), 129.2, 129.0, 128.4, 128.3, 128.1, 128.0, 127.7, and 127.1 (aromatic), 102.7 (C-1 and C-1'), 83.0, 82.0, 77.9, 76.8, 75.5, 75.3, 75.0, 74.7, 73.3, 72.9, 70.95, 68.3, 67.8, and 20.85 p.p.m.

Anal. Calc. for $C_{63}H_{66}O_{12}$: C, 74.53; H, 6.55. Found: C, 74.63; H, 6.89.

Eluted third was benzyl 4-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**20**, 0.33 g) as a syrup, $[\alpha]_D^{20}$ +7° (*c* 0.6, chloroform). N.m.r. data ($CDCl_3$): ^{1}H, δ 7.1–7.2 (m, 35 H, 7 Ph), 5.30 (dd, 1 H, $J_{2',3'}$ 10.1, $J_{1',2'}$ 8.0 Hz, H-2'), 4.51 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'), 4.45 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1), 3.92 (d, 1 H, $J_{3',4'}$ 2.7 Hz, H-4'), 3.87 (t, 1 H, $J_{3,4} \simeq J_{4,5} \simeq 9.2$ Hz, H-4), 3.72 (d, 2 H, $J_{5,6}$ 2.8 Hz, H-6a,6b), 3.55 (t, 1 H, $J_{2,3} \simeq J_{3,4} \simeq 9.2$ Hz, H-3), 3.45 (dd, 1 H, $J_{2,3}$ 9.2, $J_{1,2}$ 7.7 Hz, H-2), 3.49 (m, 1 H, H-5'), 3.20–3.40 (m, 4 H, H-5,3',6'a,6'b), and 1.96 (s, 3 H, Ac); ^{13}C, 169.0 (CO), 139.3, 138.8, 138.3, 138.1, 137.6 (C-ipso), 128.4, 128.2, 127.9, 127.7, 127.3, and 127.0 (aromatic), 102.6 (C-1), 100.8 (C-1'), 82.9, 81.9, 80.6, 76.8, 75.2, 75.0, 74.9, 74.6, 73.4, 72.8, 72.2, 71.8, 71.1, 68.1, and 21.0 p.p.m.

Anal. Calc. for $C_{63}H_{66}O_{12}$: C, 74.53; H, 6.55. Found: C, 74.28; H, 6.81.

Deacetylation of **19** (0.15 g) with methanolic 0.2M sodium methoxide (0.44 mL) gave **16** (0.14 g, 97%) which, after purification by column chromatography (14:5 hexane–ethyl acetate), had $[\alpha]_D^{20}$ −6° (*c* 0.5, chloroform).

Anal. Calc. for $C_{61}H_{64}O_{11}$: C, 75.29; H, 6.63. Found: C, 75.62; H, 6.99.

Deacetylation of **20** (0.33 g) with methanolic 0.2M sodium methoxide (0.96 mL) gave **17** (0.29 g, 92%) as a syrup, $[\alpha]_D^{20}$ +2° (*c* 1, chloroform).

Anal. Calc. for $C_{61}H_{64}O_{11}$: C, 75.29; H, 6.63. Found: C, 75.22; H, 6.73.

The last fraction isolated from the benzylation mixture was a syrup (0.18 g, 9%) which was acetylated to give benzyl 3-*O*-acetyl-4-*O*-(3-*O*-acetyl-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-2,6-di-*O*-benzyl-β-D-glucopyranoside (**22**) as a syrup, $[\alpha]_D^{20}$ +19° (*c* 1.5, chloroform). N.m.r. data ($CDCl_3$): ^{1}H, δ 7.1–7.2 (30 H, 6 Ph), 5.12 (t, 1 H, $J_{2,3} \simeq J_{3,4} \simeq 9.6$ Hz, H-3), 4.75 (dd, 1 H, $J_{2',3'}$ 10.2, $J_{3',4'}$ 3.2 Hz, H-3'), 4.53 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1), 4.29 (d, 1 H, $J_{1',2'}$ 7.6 Hz, H-1'), 3.88 (d, 1 H, $J_{3',4'}$ 3.2 Hz, H-4'), 3.85 (t, 1 H, $J_{3,4} \simeq J_{4,5} \simeq 9.6$ Hz, H-4), 3.73 (dd, 1 H, $J_{6a,6b}$ 10.8, $J_{5,6a}$ 3.7 Hz, H-6a), 3.66 (dd, 1 H, $J_{5,6b}$ 1.7 Hz, H-6b), 3.60 (dd, 1 H, $J_{1',2'}$ 7.6, $J_{2',3'}$ 10.2 Hz, H-2'), 3.52 (s, 3 H, H-5',6'a,6'b), 3.42 (dd, 1 H, $J_{1,2}$ 7.7, $J_{2,3}$ 9.6 Hz, H-2), 3.42 (m, 1 H, H-5), 1.89 and 1.86 (2 s, 6 H, 2 Ac); ^{13}C, 170.3 (CO), 138.5, 138.2, 138.0, 137.5 (C-ipso), 128.4, 128.2, 127.8 and 127.7 (aromatic), 102.6 (C-1 and C-1'), 79.3, 77.4, 75.3, 75.0, 74.6, 74.1, 74.0, 73.3, 72.8, 71.4, 68.1, 20.9, and 20.8 p.p.m.

Anal. Calc. for $C_{58}H_{62}O_{13}$: C, 72.03; H, 6.46. Found: C, 72.03; H, 6.76.

*Treatment of benzyl hepta-*O*-acetyl-β-lactoside* (**24**) *with benzyl chloride under phase-transfer conditions.* — A solution of **24** (2 g) in benzene (40 mL) was vigorously stirred with aqueous 20% sodium hydroxide (20 mL), tetrabutyl-

ammonium hydrogensulfate (0.4 g), and benzyl chloride (6 mL) at 75° for 8 h. The organic layer was washed with water, 0.5M sulfuric acid, and water, dried (Na_2SO_4), and concentrated. Column chromatography (7:2 hexane–ethyl acetate) of the residue gave **14** (0.2 g, 7%), **15** (0.7 g, 26%), a mixture of **16** and **17** [0.45 g, 17%, which was acetylated and fractionated by column chromatography (4:1 hexane–ethyl acetate) to give **19** (0.20 g) and **20** (0.22 g)], **21** (0.22 g, 9%), and **25** (0.12 g, 5%) which, after acetylation, afforded benzyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,4-di-*O*-acetyl-3,6-di-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**26**) as a syrup, $[\alpha]_D^{20}$ +13° (*c* 1.4, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.1–7.2 (30 H, 6 Ph), 5.55 (d, 1 H, $J_{3',4'}$ 3.3 Hz, H-4'), 5.03 (dd, 1 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 10.0 Hz, H-2'), 3.90 (dd, 1 H, $J_{3,4}$ 9.2, $J_{4,5}$ 9.8 Hz, H-4), 3.70 (d, 2 H, $J_{5,6}$ 2.9 Hz, 2 H-6), 3.54 (t, 1 H, $J_{3,4} \simeq J_{2,3} \simeq$ 9.2 Hz, H-3), 3.46 (dd, 1 H, $J_{1,2}$ 7.7, $J_{2,3}$ 9.2 Hz, H-2), 3.42 (t, 1 H, $J_{5',6'}$ 6.5 Hz, H-5'), 3.34 (m, 1 H, $J_{4,5}$ 9.8, $J_{5,6}$ 2.9 Hz, H-5), 3.31 (dd, 1 H, $J_{3',4'}$ 3.3, $J_{2',3'}$ 10.0 Hz, H-3'), 3.27 (d, 2 H, $J_{5',6'}$ 6.5 Hz, 2 H-6'), 2.05 and 1.95 (2 s, 6 H, 2 Ac).

Anal. Calc. for $C_{58}H_{62}O_{13}$: C, 72.03; H, 6.46. Found: C, 72.26; H, 6.58.

Benzyl 2,6-di-O-benzyl-3-O-methyl-4-O-(2,3,4,6-tetra-O-benzyl-β-D-galacto-pyranosyl)-β-D-glucopyranoside (**23**). — A solution of **15** (0.4 g) in dry tetrahydrofuran (3.5 mL) was stirred with sodium hydride (0.085 g) and methyl iodide (1.8 mL) at 50° for 1 h and then cooled. Water was added, the mixture was extracted with ether (2 × 30 mL), and the combined extracts were dried (Na_2SO_4) and concentrated to give **23** (0.34 g, 84%) as a syrup which, after purification by column chromatography (7:2 hexane–ethyl acetate), had $[\alpha]_D^{20}$ −9° (*c* 0.95, chloroform).

Anal. Calc. for $C_{68}H_{66}O_{11}$: C, 75.43; H, 6.74. Found: C, 75.37; H, 7.01.

3-O-Methyl-lactose (**1**). — *(a) From* **12**. Compound **12** (1.8 g) was treated with aqueous 30% acetic acid (26 mL) at 100° for 1 h. The solution was then concentrated to give **1** (1.5 g, 94%) as a solid.

(b) From (**23**). A solution of **23** (0.34 g) in ethanol (25 mL) was hydrogenated over 10% Pd/C (0.2 g) for 2 h and then filtered. Column chromatography (1:1 chloroform–methanol) of the white solid (0.12 g, 94%) gave **1**, m.p. 77–80°, $[\alpha]_D^{20}$ +50 → +46° (*c* 0.5, methanol); lit.[2] m.p. 73–77°, $[\alpha]_D^{20}$ +47°. ^{13}C-N.m.r. data (D_2O): 103.7 (C-1'), 96.7 (C-1β), 92.7 (C-1α), 84.4 (C-3β), 81.8 (C-3α), 76.6 (C-4α), 76.3 (C-4β), 76.0, 74.1, 73.6 (C-3'), 72.2, 71.6, 69.5, 61.9, 60.9, 60.2 (Meα), and 59.8 p.p.m. (Meβ).

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