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SYNTHESIS OF *O*-α-L-FUCOPYRANOSYL-(1→2)-*O*-β-D-GALACTOPYRANOSYL-(1→4)-D-GLUCOPYRANOSE (2′-*O*-α-L-FUCOPYRANOSYL-LACTOSE)*

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ABSTRACT

Selective benzoylation of 2,3:5,6:3′,4′-tri-*O*-isopropylidenelactose dimethyl acetal (**1**) with benzoyl chloride in dichloromethane afforded the 6′-*O*-benzoyl derivative (**2**). The constitution of **2** was inferred from its n.m.r. spectrum, and confirmed by methylation and subsequent hydrolysis to 2-*O*-methyl-D-galactose and D-glucose. Glycosidation of **2** (catalyzed by bromide ion) with 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide gave the 2′-*O*-linked, fully protected trisaccharide (**9**). *O*-Debenzoylation of **9**, followed by deacetalation, furnished the tri-*O*-benzyl trisaccharide (**11**). Subsequent hydrogenolysis of the benzyl groups of **11** afforded the title trisaccharide. The acetal **1** was converted into a monoacetate and a dibenzoate, and a simple synthesis of 2′-*O*-methyl-lactose, starting from **1**, is also described.

INTRODUCTION

Fucosyltransferases catalyze the transfer of L-fucose from GDP-L-fucose to appropriate, carbohydrate acceptors. Recently, the use of human fucosyltransferases as possible tumor-markers has been strongly suggested[2–4]. In our laboratory, we have initiated a program of purification and characterization of this class of enzyme. In one of the approaches, we are attempting the preparation of reference compounds having an α-L-fucosyl group (or groups) that can be effectively used for characterization of the product formed by the action of a fucosyltransferase and its respective acceptor. For example, the availability of aryl 2-*O*-α-L-fucopyranosyl-β-D-galactopyranosides [α-Fuc-(1→2)-β-Gal-OR, R = Ph, $C_6H_4NO_2$-*o* or -*p*] led us to the development of a facile, assay procedure for α-(1→2)-L-fucosyltransferase when the corresponding β-D-galactopyranosides (β-Gal-OR, R = Ph, $C_6H_4NO_2$-*o* or -*p*) were used as the acceptors[5]. To the best of our knowledge, at least three different, human fucosyltransferases are known to exist[6,7]. According to Watkins *et al.*[8], the readily accessible lactose (β-Gal-(1→4)-Glc) acts as an acceptor for the α-(1→2)- and

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α-(1→3)-L-fucosyltransferases present in human serum, to give α-Fuc-(1→2)-β-Gal-(1→4)-Glc and β-Gal-(1→4)-[α-Fuc-(1→3)]-Glc, respectively. Based upon this report, we have attempted the chemical synthesis of these compounds, and we now describe a facile synthesis of **12**. 2′-*O*-α-L-Fucopyranosyl-lactose (**12**) has been isolated from human milk[9], but, to the best of our knowledge, it had not hitherto been synthesized. We also describe a synthesis of 2′-*O*-methyl-lactose (**8**).

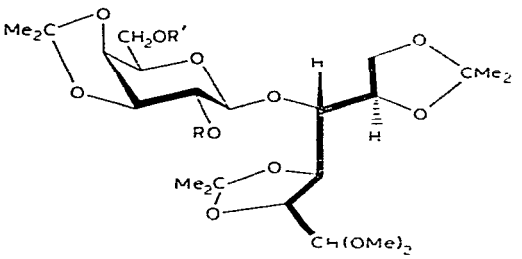
RESULTS AND DISCUSSION

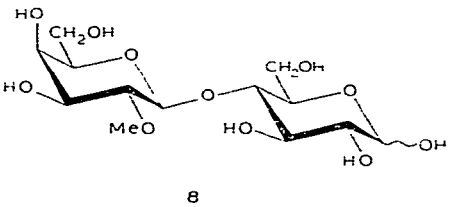
Isopropylidenation of lactose with 2,2-dimethoxypropane under various reaction conditions has been reported[10–12]. For example, in *N*,*N*-dimethylformamide at 25°, in the presence of *p*-toluenesulfonic acid, the kinetically favored 4′,6′-isopropylidene acetal is the major product[10], whereas, at a higher temperature (~85°), the ratio of the 3′,4′- to the 4′,6′-acetal is[11] 2:1. However, treatment of lactose with an excess of 2,2-dimethoxypropane at reflux in the presence of *p*-toluenesulfonic acid, but in the absence of *N*,*N*-dimethylformamide, gives mainly the tris-isopropylidene acetal **1**, which is suitably protected for modification of lactose at HO-2′ and HO-6′. For the present studies, we utilized **1** for the synthesis of the title trisaccharide (**12**).

Hough and co-workers[12] reported the conversion of compound **1** into di-*O*-acetyl and di-*O*-(methylsulfonyl) derivatives, but it appears that no attempt was made to monoacylate **1**. In our laboratory, **1** was treated with 1.1 molar equivalents of benzoyl chloride in dichloromethane containing ~13% (v/v) of triethylamine for 3.5 h at −10°. Thin-layer chromatography (t.l.c.) of the crude product isolated revealed the presence of one major product, identified as the 6′-monobenzoate (**2**). A trace of the 2′,6′-di-*O*-benzoyl derivative (**5**) and a negligible amount of a slower-moving product (faster than **1**) were also revealed by t.l.c. The last compound was, presumably, the isomeric 2′-*O*-benzoyl derivative of **1**, but it was neither isolated nor characterized. The n.m.r. spectrum of purified **2** was consistent with a monobenzoyl derivative of **1**. A five-proton complex (δ 7.4–8.2) indicated that only one benzoyl group had been introduced. The signals for the two (magnetically nonequivalent) methoxyl groups resonated as two singlets (δ 3.33 and 3.35), and the isopropylidene groups were accounted for by two singlets (δ 1.51 and 1.54), along with a cluster of singlets centered at δ 1.37.

The fact that the benzoyl group was not attached to O-2′ was evident from comparison of the n.m.r. spectrum of **2** with those of the dibenzoate **5** and the diacetate **7**. A proton attached to a carbon atom carrying the grouping R-C(=O)-O- would be expected to resonate at a lower field. Thus, in the spectrum of **5**, a low-field proton-triplet (δ 5.36, *J* 7.5 Hz) could only be assigned to H-2′. A similar signal in the spectrum of the diacetate **7** appeared at δ 5.05, with a spacing of ~8 Hz, which is in agreement with O-2′ carrying an acyl group and with H-2′ being in an axial–axial disposition with respect to both H-1′ and H-3′. In the spectrum of **2**, no signal

attributable to H-2′ was present in the region δ 5.0–5.4. Instead, a proton triplet (δ 3.63, J 7 Hz) could reasonably be assigned to H-2′. However, the methylene protons on C′-6 could not be assigned with certainty, but it is likely that they occurred at low field within the signals at δ 3.7–4.7 in the spectra of **2**, **5**, and **7**.





8

1 R = R′ = H
2 R = H, R′ = Bz
3 R = Me, R′ = Bz
4 R = Me, R′ = H
5 R = R′ = Bz
6 R = H, R′ = Ac
7 R = R′ = Ac

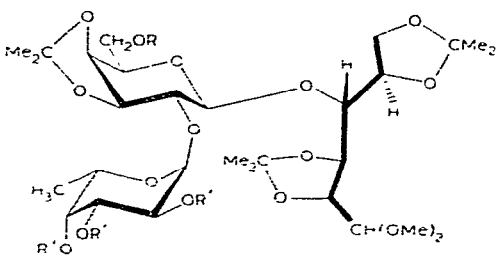
The structure of **2** was further confirmed by converting it into 2′-*O*-methyllactose (**8**). Methylation of **2** with methyl iodide–silver oxide in *N*,*N*-dimethylformamide for 16 h at room temperature, followed by purification by column chromatography, afforded monomethyl derivative **3** as a syrup. That **3** was a monomethyl derivative of **2** was evident from its n.m.r. spectrum, which revealed that a methoxyl group (δ 3.59) had been introduced; otherwise, all of the signals (except OH) in the spectrum of **2** were accounted for in that of **3**. *O*-Debenzoylation of **3** with methanolic sodium methoxide for 3 h at room temperature furnished **4** as a syrup, in 86% yield. Deacetalation of compound **4** in aqueous acetic acid for 4 h at 80° gave one major product and a small proportion of a faster-moving compound (t.l.c., solvent *C*). The latter compound was, presumably, incompletely deacetalated material. Column-chromatographic separation of the mixture afforded crystalline **8** in 78% yield. The relatively high, initial rotation, $[\alpha]_D$ +68 → +54.8° (24 h; H_2O), and the noticeable, downward mutarotation, are indicative of an anomeric mixture rich in the α-D anomer.

Acid hydrolysis of **8**, and examination of the hydrolyzate by paper chromatography, gave only glucose and 2-*O*-methylgalactose. The latter (R_{Gal} 2.28) was clearly distinguishable from 6-*O*-methylgalactose (R_{Gal} 1.96, solvent *E*). The isomeric 6-methyl ether would have arisen had the benzoyl group of **2** been situated on O-2′. Benzoylation of **1** with an excess of benzoyl chloride in pyridine afforded the dibenzoate **5**, which, in t.l.c., had the same chromatographic mobility as the faster-moving product in the aforementioned, selective benzoylation of **1**. Compound **5**

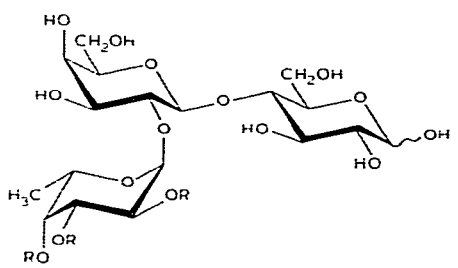
(a foam) was obtained in 86% yield, and its n.m.r. spectrum was consistent with its being a dibenzoate. Thus, a ten-proton complex (δ 7.25–8.25) clearly indicated that two benzoyl groups were present. Occurrence of one of the benzoyl groups at O-2′ was revealed by the presence of a one-proton triplet (δ 5.36, J 7.5 Hz). However, as already pointed out, the signals due to the methylene protons on C-6′ could not be assigned with certainty. A doublet (δ 5.1, J 7.5 Hz) was tentatively assigned to H-1′. Assuming that the D-galactopyranosyl group adopts the $^4C_1$ conformation, H-1′ would be the only proton having a *trans*-diaxial disposition with respect to another proton on an adjacent carbon atom.

Acetylation of **1** with approximately one molar equivalent of acetyl chloride in pyridine at −15° afforded the 6-*O*-acetyl derivative **6** and the 2,6-di-*O*-acetyl derivative **7** in 78.6 and 11.5% yield, respectively, after column-chromatographic separation. The n.m.r. spectrum of **6** showed signals in agreement with a monoacetyl derivative of **1**, and, by comparison with the spectrum of **2**, compound **6** could only be the 6-*O*-acetyl derivative. Thus, a one-proton triplet (δ 3.57, J 8 Hz) in the spectrum of **2** was assigned to H-2′; this clearly indicated that no acetate group was situated at C-2′. The acetal methoxyl groups, in contrast to those of **2**, resonated as a singlet at δ 3.46, and a three-proton singlet at δ 2.1 accounted for the presence of only one acetyl group. Although the diacetate **7** was not obtained crystalline, its n.m.r. spectrum was in accord with that expected, and its rotation ($[\alpha]_D$ +26.1°, chloroform) was in close agreement with that reported[12] ($[\alpha]_D$ +25.2°, chloroform).

Bromide ion-catalyzed glycosidation[13] of the monobenzoate **2** with 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide[13,14] afforded, after column chromatography, the protected trisaccharide **9** as a foam in 50% yield. However, **9** was slightly contaminated with a marginally faster-moving (t.l.c.) compound that could not be removed by column chromatography. Nevertheless, the n.m.r. spectrum of **9** showed signals in support of the proposed structure. After *O*-debenzoylation of **9** with methanolic sodium methoxide, the resulting trisaccharide derivative **10** was readily separated from the contaminant by column chromatography.



9 R = Bz, R′ = PhCH$_2$
10 R = H, R′ = PhCH$_2$



11 R = PhCH$_2$
12 R = H

Deacetalation of **10** in aqueous acetic acid at 80° afforded, after purification by preparative-layer chromatography, crystalline tri-*O*-benzyl trisaccharide **11** as the

dihydrate (the isomeric 3′-*O*-α-L-tri-*O*-benzyl trisaccharide is, also, a dihydrate, whereas the 6′-*O*-α-L-isomer is a hemihydrate[11]).

Catalytic hydrogenolysis of **11** in glacial acetic acid in the presence of a palladium catalyst afforded the free trisaccharide **12** in high yield as a white solid, homogeneous in t.l.c. (solvent *C*) and migrating more slowly than lactose. Its rotation ($[\alpha]_D$ −48.6 → −50.2°) agrees well with that reported[9]. Incubation of a sample of **12** for 1 h at 37° with partially purified (1→2)-α-L-fucosidase free from other α-L-fucosidases[15,16] released both fucose and lactose, and, as the enzyme preparation also contained some β-D-galactosidase, both galactose and glucose were also detected on the paper chromatogram.

EXPERIMENTAL

General methods. — Melting points were determined with a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured at room temperature with a Perkin–Elmer 241 polarimeter. T.l.c. was conducted on plates coated with a 0.25-mm layer of silica gel 60PF-254 (E. Merck, Darmstadt, Germany); the components were located either by exposure to u.v. light, or by spraying the plates with 5% sulfuric acid in ethanol and heating. The following solvent systems (v/v) were used for chromatography: *A*, 1:1 ethyl acetate–hexane; *B*, 1:2 ethyl acetate–hexane; *C*, 3:2:1 ethyl acetate–2-propanol–water; and *D*, 1:4 ethyl acetate–hexane. Descending paper-chromatography was performed on Whatman No. 1 paper, and the spots were detected with periodate, followed by silver nitrate[17]. Solvent *E* for development was 4:1:1 1-butanol–ethanol–water. Organic solutions were generally dried over anhydrous magnesium sulfate. Elemental analyses were performed by Robertson Laboratory, Florham Park, New Jersey, U.S.A. I.r. spectra were recorded with a Perkin–Elmer 297 spectrophotometer, and n.m.r. spectra with a Varian XL-100 instrument at 100 MHz, for solutions in chloroform-*d*, unless otherwise indicated, with Me_4Si as the internal standard.

2,3:5,6-Di-O-isopropylidene-4-O-(3,4-O-isopropylidene-β-D-galactopyranosyl)-D-glucose dimethyl acetal (**1**). — Prepared as described by Hough and co-workers[12], compound **1** had m.p. 129–131° (from ether–hexane); $[\alpha]_D$ +37.3° (*c* 1.3, chloroform); lit.[12] m.p. 133–134°, $[\alpha]_D$ +39.1° (chloroform).

4-O-(6-O-Benzoyl-3,4-O-isopropylidene-β-D-galactopyranosyl)-2,3:5,6-di-O-isopropylidene-D-glucose dimethyl acetal (**2**). — To a cold (−10°), stirred solution of **1** (3.55 g, 7 mmol) in dichloromethane (60 mL) containing triethylamine (8 mL) was added a solution of benzoyl chloride (1.83 g, 8 mmol) in dichloromethane (20 mL), dropwise, during 0.5 h. After being stirred for 3 h at −10°, the mixture was allowed to warm to room temperature (0.5 h). It was then successively washed with ice-cold water, ice-cold 10% hydrochloric acid, cold saturated sodium hydrogencarbonate solution, and water, dried, and evaporated under diminished pressure, to yield a syrup (~4.7 g) which, in t.l.c. (solvent *A*), showed one major (R_F 0.66) product, together with a trace of a faster-moving (R_F 0.88) product (identical in mobility to the

2,6-dibenzoate **5**; see later) and a small amount of unchanged **1**. After purification on a column of silica gel by elution with solvent *B*, compound **2** (3.6 g, 84%) was a foamy gum; $[\alpha]_D$ +33.1° (*c* 1.2, chloroform); n.m.r. data: δ 7.4–8.2 (complex, 5 H, aromatic), 3.63 (t, 1 H, *J* 7.5 Hz, H-2'), 3.33 and 3.35 (s, 2 × 3 H, 2 OMe), 1.51 and 1.54 (s, 2 × 3 H, CMe_2), 1.37 (center of cluster, 12 H, 2 CMe_2), and 3.8–4.7 (unresolved signals, 14 H).

Anal. Calc. for $C_{30}H_{44}O_{13}$: C, 58.81; H, 7.24. Found: C, 58.99; H, 7.31.

*4-O-(6-O-Benzoyl-3,4-O-isopropylidene-2-O-methyl-β-*D*-galactopyranosyl)-2,3:5,6-di-O-isopropylidene-*D*-glucose dimethyl acetal* (**3**). — A solution of the monobenzoate **2** (3.5 g) in *N*,*N*-dimethylformamide (40 mL) was stirred with methyl iodide (4 mL) and freshly prepared silver oxide (5 g) for 16 h at room temperature; t.l.c. (solvent *B*) then showed one faster-moving product and a little unchanged **2**. The mixture was filtered through Celite, and the residue washed with *N*,*N*-dimethylformamide. The filtrate and washings were combined, and evaporated under vacuum (~40°, bath). The residue was stirred with chloroform, the insoluble material removed by filtration, and the filtrate successively washed with water, aqueous sodium thiosulfate solution, and water, dried, and evaporated to a syrup which was applied to a column of silica gel (~100 g) and eluted with solvent *B*. On evaporation, fractions containing the methylated product gave **3** as a slightly yellowish syrup (2.8 g, 78.2%); $[\alpha]_D$ +1.0° (*c* 1.2, chloroform); ν_{max}^{film} [no absorption near 3500 (OH)] 1725 cm^{-1} (C = O); n.m.r. data: δ 7.35–8.20 (complex, 5 H, aromatic), 3.59 (s, 3 H, OMe), 3.39 and 3.35 [s, 2 × 3 H, $CH(OMe)_2$], and 1.30–1.54 (s, and a cluster of singlets, 18 H, CMe_2).

Anal. Calc. for $C_{31}H_{46}O_{13}$: C, 59.41; H, 7.40. Found: C, 59.52; H, 7.59.

*2,3:5,6-Di-O-isopropylidene-4-O-(3,4-O-isopropylidene-2-O-methyl-β-*D*-galactopyranosyl)-*D*-glucose dimethyl acetal* (**4**). — A solution of **3** (2.5 g) in methanol (120 mL) containing a catalytic amount of sodium methoxide was kept for 3 h at room temperature; t.l.c. (solvent *A*) then showed the disappearance of **3** and the formation of one product (R_F 0.48). The base was neutralized by dropwise addition of glacial acetic acid, and the solvent removed under vacuum. Small portions of toluene were added to, and evaporated from, the residue and the resulting syrup was purified on a short column of silica gel, using solvent *B* as the eluant. Compound **4** was obtained as a syrup (1.8 g, 85.7%); $[\alpha]_D$ +16.8° (*c* 1.0, chloroform); ν_{max}^{film} 3500 cm^{-1} (OH); n.m.r. data: δ 3.57 (s, 3 H, OMe), 3.48 [s, 2 × 3 H, $CH(OMe)_2$], and 1.20–1.50 (18 H, CMe_2).

Anal. Calc. for $C_{24}H_{42}O_{12}$: C, 55.16; H, 8.10. Found: C, 55.11; H, 8.26.

*4-O-(2-O-Methyl-β-*D*-galactopyranosyl)-*D*-glucopyranose* (**8**). — A solution of **4** (1.5 g) in 60% aqueous acetic acid was heated for 4 h at ~80°. T.l.c. (solvent *C*) then showed the disappearance of **4**, and the appearance of a major product (R_F 0.46); a small amount of a faster-moving contaminant (R_F 0.69) was also present. The solution was evaporated under diminished pressure (~35°, bath), and small portions of toluene were added to, and evaporated from, the residue; the white solid so obtained was applied to a column of silica gel and eluted with 65:15:2 chloroform–

methanol–water. Fractions containing the product were combined, and evaporated, to give a white solid (0.8 g, 78.4%) that was homogeneous in t.l.c. (2:1 chloroform–methanol, and solvent *C*). On recrystallization from alcohol, **7** had m.p. 156–158°, $[\alpha]_D$ +68 (initial) → +54.5° (24 h; *c* 0.6, H_2O).

Anal. Calc. for $C_{13}H_{24}O_{11} \cdot 0.5\ H_2O$: C, 42.73; H, 6.91. Found: C, 43.02; H, 7.05.

A small portion of **8** was hydrolyzed with 0.5M sulfuric acid for 5 h at 98°. The acid was neutralized with barium carbonate, the suspension filtered, the filtrate evaporated, and the residue dissolved in a small volume of water, and examined by paper chromatography. In addition to glucose, 2-*O*-methylgalactose was the only product of hydrolysis; these were identified by comparison with authentic samples[18]. 2-*O*-Methylgalactose (R_{Gal} 2.28) was clearly distinguishable from 6-*O*-methylgalactose* (R_{Gal} 1.96) in solvent *E* (70 h, descending).

*4-O-(2,6-Di-O-benzoyl-3,4-O-isopropylidene-β-*D*-galactopyranosyl)-2,3:5,6-di-O-isopropylidene-*D*-glucose dimethyl acetal* (**5**). — To a solution of **1** (0.51 g, 1 mmol) in pyridine (8 mL) was added benzoyl chloride (3 mL). After being kept for 16 h at room temperature, t.l.c. (solvent *A*) showed only one product (R_F 0.88, see before). After processing as described for the monobenzoate **2**, the product was freed of a faster-moving (t.l.c.), u.v.-visible contaminant (presumably benzoic acid or benzoic anhydride) by chromatography on a short column of silica gel, using 1:3 ethyl acetate–hexane as the eluant. Compound **5** was obtained as a foam (0.61 g, 85.9%); $[\alpha]_D$ +5.9° (*c* 1.05, chloroform); n.m.r. data: δ 7.25–8.25 (complex, 10 H, aromatic), 5.36 (t, 1 H, *J* 7.5 Hz, H-2′), 5.1 (d, 1 H, 7.5 Hz, H-1′), 3.34 and 3.35 [2 s, 6 H, $CH(OMe)_2$], 1.20–1.70 (s, and cluster of singlets, 18 H, CMe_2), and 3.76–4.70 (unresolved signals, 12 H).

Anal. Calc. for $C_{37}H_{48}O_{14}$: C, 62.00; H, 6.75. Found: C, 62.17; H, 6.80.

*4-O-(6-O-Acetyl-3,4-O-isopropylidene-β-*D*-galactopyranosyl)-2,3:5,6-di-O-isopropylidene-*D*-glucose dimethyl acetal* (**6**), *and the 2,6-di-O-acetyl derivative* (**7**). — To a cold (−15° bath), stirred solution of **1** (1.26 g, 2.5 mmol) in pyridine (30 mL) was added dropwise a solution of acetyl chloride (0.22 g, 2.8 mmol) in pyridine (5 mL). Stirring was continued for 2 h at −15°, and then the mixture was kept overnight at room temperature, poured onto ice–water, and extracted with chloroform. The extracts were combined, successively washed with ice-cold 10% hydrochloric acid, cold water, cold saturated sodium hydrogencarbonate, and water, dried, and evaporated. T.l.c. (solvent *A*) showed the presence of one major product; there were also a small amount of a faster-moving product and a trace of unchanged **1**. The mixture was applied to a column of silica gel and eluted with solvent *B*. The faster-moving diacetate **7** was eluted first. It was obtained as a foam (0.17 g, 11.5%), $[\alpha]_D$ +26.1° (*c* 1.3, chloroform); lit.[12] m.p. 113–115°, $[\alpha]_D$ +25.2° (chloroform); n.m.r. data: δ 5.05 (t, 1 H, *J* 8 Hz, H-2′), 4.8 (d, 1 H, *J* 8 Hz, H-1′), 3.45 [s, 6 H, $CH(OMe)_2$], 2.10 and 2.14 (s, 6 H, 2 OAc), 1.57, 1.48, 1.38, and 1.34 (s, 18 H, CMe_2), and 3.9–4.6 (unresolved signals, 12 H).

Continued elution of the column with solvent *B* gave the major product,

*Kindly provided by Dr. E. G. Gros, Buenos Aires, Argentina.

compound **6**, as a foam (1.1 g, 78.6%); $[\alpha]_D$ +41° (*c* 1.3, chloroform); n.m.r. data: δ 3.57 (t, 1 H, *J* 8 Hz, H-2'), 3.46 [s, 6 H, $CH(OMe)_2$], 2.10 (s, 3 H, OAc), 1.51, 1.40, and 1.35 (broad s and 2 s, 18 H, CMe_2), and 3.85–4.60 (unresolved signals, 13 H).

Anal. Calc. for $C_{25}H_{42}O_{13}$: C, 54.53; H, 7.69. Found: C, 54.18; H, 7.67.

O-(*2,3,4-Tri*-O-*benzyl*-α-L-*fucopyranosyl*)-(*1*→*2*)-O-(*6*-O-*benzoyl*-*3,4*-O-*isopropylidene*-β-D-*galactopyranosyl*)-(*1*→*4*)-*2,3*:*5,6*-*di*-O-*isopropylidene*-D-*glucose dimethyl acetal* (**9**). — A solution of 2,3,4-tri-O-benzyl-α-L-fucopyranosyl bromide[13,14] [2 g, 4 mmol; freshly prepared from the 1-(*p*-nitrobenzoate)[13]] and tetraethylammonium bromide (0.84 g, 4 mmol) in dichloromethane (20 mL) was stirred for 0.5 h with molecular sieves (type 4A) (3 g) under protection from light and moisture. Then a solution of **2** (1.2 g, 2 mmol) in dichloromethane (10 mL) was added, followed by ethyldiisopropylamine (0.6 g, 4.6 mmol). The mixture was stirred for 5 days at room temperature; t.l.c. (solvent *B*) then indicated the formation of one major product (R_F 0.63), accompanied by a trace of a marginally faster-moving component. 2,3,4-Tri-*O*-benzyl-L-fucose (R_F 0.42) and some unchanged **2** were also present, in addition to some faster-moving spots (possibly due to decomposition of the L-fucosyl bromide). A further amount of 2,3,4-tri-*O*-benzyl-L-fucosyl bromide [prepared from 0.8 g of the 1-(*p*-nitrobenzoate)] was added, and the mixture was stirred for 24 h. The mixture was filtered through Celite, the solids were thoroughly washed with dichloromethane, and the filtrate and washings were combined, washed with water, dried, and concentrated to a small volume. The concentrate was applied to a column of silica gel (~100 g) and eluted with solvent *D*. On evaporation, fractions containing the major product yielded **9** as a foam (0.76 g; 50.3%, based on reacted **2**). Re-examination by t.l.c. (solvent *B*) revealed a slight contamination with the marginally faster-moving component. A sample of **9**, purified by preparative-layer chromatography (p.l.c.; solvent *D*), had $[\alpha]_D$ −54.1° (*c* 1.1, chloroform); n.m.r. data: δ 7.2–8.2 (complex, 20 H, aromatic), 5.62 (d, 1 H, *J* 2.5 Hz, H-1''), 3.36 [s, 2 × 3 H, $CH(OMe)_2$], 1.20–1.65 (s, 18 H, CMe_2), 1.12 (d, 3 H, *J* 6 Hz, CMe). The rest of compound **9** was subjected to debenzoylation (see later).

Continued elution of the column with solvent *D* gave, first, 2,3,4-tri-*O*-benzyl-L-fucose (1 g), and then, unchanged **2** (0.3 g).

O-(*2,3,4-Tri*-O-*benzyl*-α-L-*fucopyranosyl*)-(*1*→*2*)-O-(*3,4*-O-*isopropylidene*)-β-D-*galactopyranosyl*)-(*1*→*4*)-*2,3*:*5,6*-*di*-O-*isopropylidene*-D-*glucose dimethyl acetal* (**10**). — Compound **9** (0.7 g; slightly contaminated with the marginally faster-moving component) was dissolved in methanol (70 mL) containing a catalytic amount of sodium methoxide. After being kept for 16 h at room temperature, t.l.c. (solvent *B*) showed the disappearance of **9** and the formation of a slower-moving (R_F 0.13) product; the spot corresponding to the faster-moving contaminant was not affected. The base was neutralized with a few drops of glacial acetic acid, the solution was evaporated under diminished pressure (~35°, bath), the residue was dissolved in chloroform, and the solution was washed three times with water, dried, and evaporated to a syrup. Elution from a short column of silica gel with solvent *B* afforded compound **10** as a foam (0.34 g); $[\alpha]_D$ −55.8° (*c* 0.5, chloroform); n.m.r. data: δ 7.25–7.55

(complex, 15 H, aromatic), 3.55 [s, 6 H, $CH(OMe)_2$], 1.20–1.50 (18 H, CMe_2), 1.10 (d, 3 H, *J* 6 Hz, CMe), and 3.60–5.10 (unresolved signals, 25 H).

Anal. Calc. for $C_{50}H_{68}O_{16}$: C, 64.92; H, 7.41. Found: C, 65.24; H, 7.44%.

O-(*2,3,4-Tri*-O-*benzyl*-α-L-*fucopyranosyl*)-(*1*→*2*)-O-β-D-*galactopyranosyl*-(*1*→*4*)-α,β-D-*glucopyranose* (**11**). — A solution of **10** (0.24 g) in 60% aqueous acetic acid (8 mL) was heated for 2 h at 75–80°; t.l.c. (5:1 chloroform–methanol) then indicated the presence of one major, slower-moving (R_F 0.41) product, accompanied by traces of faster-moving contaminants (possibly due to incomplete deacetalation). After purification by p.l.c. (same solvent), and recrystallization from methanol–ether, compound **11** had m.p. 122–125°, $[\alpha]_D$ −41° (*c* 0.9; 2:1 chloroform–methanol) n.m.r. data (CD_3OD): δ 7.15–7.55 (complex, 15 H, aromatic), 5.55 (d, 1 H, *J* 3 Hz, H-1″), and 1.12 (d, 3 H, *J* 6 Hz, CMe).

Anal. Calc. for $C_{39}H_{50}O_{15} \cdot 2\ H_2O$: C, 58.93; H, 6.85. Found: C, 59.53; H, 6.52.

O-α-L-*Fucopyranosyl*-(*1*→*2*)-O-β-D-*galactopyranosyl*-(*1*→*4*)-D-*glucopyranose* (**12**). — A solution of **11** (0.12 g) in glacial acetic acid (25 mL) was shaken under hydrogen at 50 lb.in.$^{-2}$ for 48 h at room temperature in the presence of 10% palladium-on-carbon (0.1 g). The suspension was filtered (a bed of Celite), and the filtrate evaporated under diminished pressure (~35°, bath). Addition and evaporation of methanol, and then toluene, gave a white solid; this was taken up in water, and the suspension filtered through Celite. The filtrate was evaporated, and addition and evaporation of absolute ethanol gave compound **12** as a white solid (70 mg, 91%): homogeneous in t.l.c. (solvent *C*); R_F 0.24, $R_{Lactose}$ 0.69; $[\alpha]_D$ −48.6 (initial) → −50.2° (72 h; *c* 0.5, H_2O), lit.[9] m.p. 230–231°, $[\alpha]_D$ −53.5° (initial) → −57.5° (72 h; *c* 2.0, H_2O).

Enzymic studies. — The assay mixture for α-(1→2)-fucosidase from *Aspergillus niger* contained 0.01M acetate buffer, pH 4.0, 5μM 2′-fucosyl-lactose, and the enzyme. Mixtures containing the enzyme, along with controls lacking the enzyme, or the substrate, were incubated in a total volume of 50 μL for 1 h at 37°. Reactions were terminated by chilling to 4°. Assay mixtures, and appropriate reference-compounds, were chromatographed on Whatman No. 1 paper, using solvent *E*, for 70 h. Compounds were detected with the silver nitrate reagent, following periodate oxidation. Incubation clearly showed the presence of fucose and lactose [and also galactose and glucose (resulting from cleavage of lactose)].

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