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SYNTHESIS OF *O*-β-L-FUCOPYRANOSYL-(1→3)-*O*-β-D-GALACTOPYRANOSYL-(1→4)-D-GLUCOPYRANOSE (3′-*O*-β-L-FUCOPYRANOSYLLACTOSE)

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ABSTRACT

Isopropylidenation of lactose with 2,2-dimethoxypropane in *N*,*N*-dimethylformamide afforded 4′,6′-*O*-isopropylidenelactose as the main product. Its constitution was proved by permethylation and hydrolysis giving 2,3-di-*O*-methyl-D-galactose. By standard procedures, the β-hexaacetate and the α-hexabenzoate of the cyclic acetal were obtained, and removal of the isopropylidene group gave 1,2,3,6,2′,3′-hexa-*O*-acetyl-β-lactose (**4**) and 1,2,3,6,2′,3′-hexa-*O*-benzoyl-α-lactose, respectively. Koenigs–Knorr condensation of **4** with 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl bromide involved an acetyl migration from O-3′ and gave a crystalline nonaacetate **6** (that could be acetylated to a decaacetate) of *O*-β-L-fucopyranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-D-glucose (**8**). The free trisaccharide **8** was obtained from **6** by Zemplén deacetylation, and its constitution was established by permethylation, followed by hydrolysis which produced 2,4,6-tri-*O*-methyl-D-galactose. A simplified synthesis of the latter compound from methyl α-D-galactopyranoside is also described.

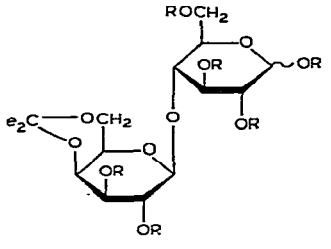
INTRODUCTION

Simultaneous with and subsequent to the isolation and elucidation of 2′-*O*-α-L-fucopyranosyllactose[1] and 2′,3-di-*O*-α-L-fucopyranosyllactose[2], some thirty structurally related oligosaccharides have been identified as ingredients of human milk[3–9]. Most of these contain a lactose moiety as the reducing end group and α-L-fucopyranosyl residues as terminal or branch units, and the majority are characterized, moreover, by the presence of amino sugar components. Many of the same or similar oligosaccharides have been found to occur in normal[10–14], pregnancy[15,16], and pathological[13,14,17] urines, where characteristic patterns of distribution are associated with the blood-group systems of individuals. At least five types of glycosidic linkages of the L-fucosyl group to D-galactose or D-glucose residues have been encountered in these compounds: α-(1→2), α-(1→3), and α-(1→6) to D-galactose, and α-(1→2) and α-(1→3) to D-glucose. L-Fucosyl group linkages to 2-acetamido-2-deoxy-D-glucose are of the α-(1→3), α-(1→4), or α-(1→6) type. Although Lemieux and Driguez[18] have synthesized the trisaccharides α-L-Fuc*p*-(1→2)-[α-D-Gal*p*-(1→3)]-D-Gal and α-L-Fuc*p*-(1→4)-[β-D-Gal*p*-(1→3)]-D-Glc*p*NAc, which are the determinant struc-

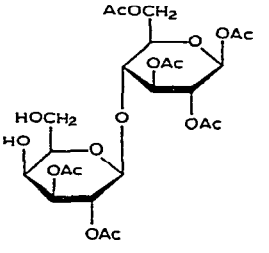
tures of human blood-group B and Lewis[a] antigens, respectively, no higher fucosido-oligosaccharides comprising the lactose unit seem to have been made by chemical synthesis as yet, and we have therefore embarked upon a program in this direction. Our initial concern was to elaborate economical approaches to partially blocked lactose derivatives which might be suitable for fucosylation by one or the other of the common condensation procedures. This first article reports the synthesis of lactose 1,2,3,6,2′,3′-hexaacetate and its Koenigs–Knorr condensation with 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl bromide, which was shown to involve a 3′→6′ acetyl migration and led to the title compound, β-L-Fuc*p*-(1→3)-β-D-Gal*p*-(1→4)-D-Glc*p*.

RESULTS AND DISCUSSION

Although benzyl 3′,4′-*O*-isopropylidene-β-lactoside was available and had served as a starting compound in a trisaccharide synthesis[19] similar to those now contemplated, we considered that the use of a partially blocked, but nonglycosidic, lactose derivative might reduce the number of necessary steps in the approach to fucosyllactoses. In our search of the literature for conveniently accessible derivatives of this type, we were unable to find any account of successful or attempted isopropylidenation of the free disaccharide*. We therefore first studied this possibility,



1 R = H (α,β anomers)
2 R = Ac (β anomer)
3 R = Bz (α anomer)



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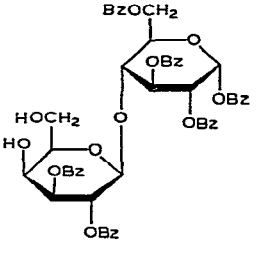

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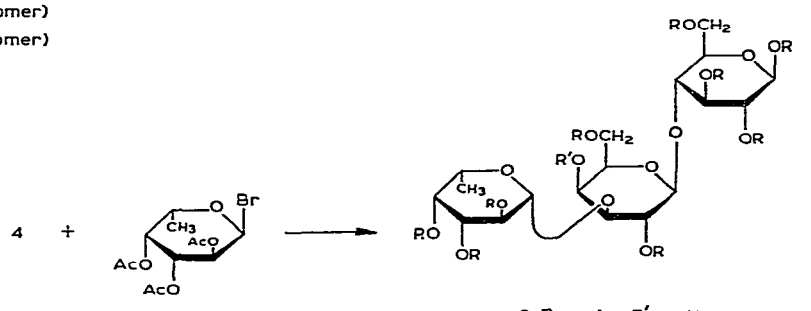

6 R = Ac, R′ = H
7 R = R′ = Ac
8 R = R′ = H

*A remark[19] citing older literature on a controversial isopropylidene acetal of lactose actually refers to a derivative of lactose dibenzyl dithioacetal.

and we applied to lactose the kinetic acetal formation[20] with 2,2-dimethoxypropane in *N*,*N*-dimethylformamide. When the anhydrous sugar was treated with this reagent at room temperature in the presence of a catalytic amount of *p*-toluenesulfonic acid, the formation and gradual increase of a single product was indicated by t.l.c. When, after 3 h, traces of additional products had begun to appear, the reaction was interrupted, notwithstanding the presence of remaining starting material. Processing then furnished a 76% yield of 4′,6′-*O*-isopropylidenelactose (**1**). Although contaminated by a small proportion of a by-product (which moved marginally faster in t.l.c.), the product was suitable for use in the next step. Further chromatographic purification gave pure **1** as an amorphous solid that showed correct microanalytical data, reduced hot Fehling solution, and underwent sodium borohydride reduction to give a monoisopropylidenelactitol. The location of the isopropylidene group was determined by permethylation of **1** according to Kuhn *et al.*[21], followed by acid hydrolysis, which produced 2,3-di-*O*-methyl-D-galactose. The dimethyl ether was characterized by its R_F value, mutarotation, and crystalline anilide, and its identity was corroborated by comparison with authentic samples of 2,3-di-*O*-methyl-D-galactose and its 2,6 isomer. The isomeric diether would have been formed if the isopropylidene group had been located at O-3′ and -4′. The two reference compounds were prepared from methyl α-D-galactopyranoside by following published procedures[22–26], with minor modifications (see Experimental). The anomeric configuration of **1** was not established; the product showed a slight, downward mutarotation ($[\alpha]_D$ +30.5→+25°, in water), but the low initial value suggested that it was probably a mixture rich in the β-D anomer.

Acetylation of **1** with acetic anhydride–sodium acetate readily afforded the crystalline β-hexaacetate **2**, and benzoyl chloride in pyridine gave the crystalline α-hexabenzoate **3**. The anomeric configurations were evidenced by the spacings of the H-1 doublets (*J* 8 and 3.5 Hz) and by the specific optical rotations ($[\alpha]_D$ +30.9° and +104.8°, in chloroform). Removal of the acetal groups from **2** and **3** with 90% trifluoroacetic acid gave crystalline 1,2,3,6,2′,3′-hexa-*O*-acetyl-β-lactose (**4**) and 1,2,3,6,2′,3′-hexa-*O*-benzoyl-α-lactose (**5**), respectively. In order to ascertain that no acetyl migration had taken place during the removal of the acetal group of **2**, a sample of **4** was treated with periodate in aqueous methanol; no glycol cleavage occurred as was evidenced by the production of glucose and galactose upon subsequent, total hydrolysis. This observation was consistent with structure **4** and militated against a possible acetyl migration from O-3′ to O-6′, although it did not exclude a migration to O-4′. More definitive proof was furnished by treatment of **4** with 2,2-dimethoxypropane which regenerated **2**.

Koenigs–Knorr condensation of the hexaacetate **4** with 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl bromide[27] in chloroform in the presence of silver carbonate led to a mixture of products from which a homogeneous, crystalline trisaccharide derivative was isolated by column chromatography in 27% yield. The n.m.r. spectrum of this product was consistent with the structure of a fucosyllactose nonaacetate, and complete acetylation yielded an amorphous, but microanalytically verified

decaacetate. In addition to the main product, a mixed fraction was obtained in 14% yield. According to t.l.c. and n.m.r. evidence, it appeared to consist largely of the same product, but contained a noticeable proportion of a second, slightly less-polar component. (A similar result was observed when silver oxide was used as the condensing agent.) The second component could not be isolated, but doubtless was also a trisaccharide, perhaps an anomeric or positionally isomeric, or a partially deacetylated product. Both fractions became indistinguishable in t.l.c. upon Zemplén deacetylation, and subsequent acid hydrolysis gave glucose, galactose, and fucose as indicated by paper chromatography.

Catalytic deacetylation of the crystalline nonaacetate furnished the unprotected trisaccharide as an amorphous solid showing $[\alpha]_D$ +54.2→+51°. The molecular rotation at equilibrium, $[M]_D$ +24 900°, indicated a β-L configuration of the L-fucopyranosyl group when it was compared with the values for α,β-lactose (+18900°) and those[28] of the anomeric methyl fucopyranosides (β-L, +2 500°; α-L, −35 000°), Thus, superposition gives +21 400° for a β-L, and −16 100° for an α-L-fucopyranosyl-α,β-lactose. Although such calculations cannot be expected to predict rotations accurately, because they do not take into account any influences due to linkage site, they are generally recognized as adequate guides to the assignment of interglycosidic configuration. (The molecular rotation of 2′-α-L-fucopyranosyllactose is[1] −28 000°). It is interesting that previous Koenigs–Knorr condensations using 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl bromide have variously generated β-linkages[27,29] and α-linkages[27,30,31], both when performed in the presence of silver oxide[27] or silver carbonate[31], or with mercuric cyanide[27,29–31] under Helferich's conditions; the outcome evidently depended upon the aglycon with which the glycosyl halide reacted. It is clear that over-all constitution[31] and configuration[32] of the acceptor are important factors in the stereochemical course of the condensation.

As concerns the site of condensation in the present synthesis, it could reasonably be expected that the primary OH-6′ of **4** would prevail over the secondary (and axial) OH-4′. However, it was found that the L-fucopyranosyl group had been linked to neither of these two positions, but to O-3′ (**6–8**). The borohydride-reduced trisaccharide was subjected to methylation followed by acid hydrolysis to give 2,4,6-tri-*O*-methyl-D-galactose, no 2,3,4-tri-*O*-methyl-D-galactose being found. Authentic samples of the two trimethyl ethers, which are clearly distinguishable[33] in t.l.c., were prepared for comparison essentially as described in the literature[24,34], although some procedural improvements were made (see Experimental). For the formation of **6** it is difficult to conceive of any explanation other than acetyl migration that must have taken place in **4** under the Koenigs–Knorr reaction conditions, prior to the condensation. However, we were unable to demonstrate such an occurrence with **4** in the absence of the glycosyl bromide under otherwise identical conditions.

EXPERIMENTAL

General methods. — Melting points were determined with an electrically-

heated, aluminum-block apparatus and are not corrected. Optical rotations were determined, for solutions in 1-dm tubes at ~25°, with a Perkin–Elmer Model 141 polarimeter. N.m.r. spectra were recorded with a Varian T-60 or a Varian HA-100 spectrometer for solutions in chloroform-*d* with tetramethylsilane as the internal standard, unless otherwise indicated. Column chromatography was performed on Silica gel Merck (70–325 mesh; E. Merck, Darmstadt, Germany). For qualitative t.l.c., precoated silica gel plates SIL G-25 UV_{254} (Macherey–Nagel & Co., Germany) were used, and spots were made visible by spraying the plates with 5% sulfuric acid in ethanol and heating them briefly on a hot plate. Unless otherwise indicated, the following solvent combinations (v/v) were used for chromatography: (*A*) 4:1 chloroform–methanol, (*B*) 7:1 chloroform–methanol, (*C*) 10:1 chloroform–methanol, (*D*) 8:1 toluene–ethyl acetate, (*E*) 8:1 toluene–methanol, (*F*) 10:1 toluene–methanol, (*G*) 18:1 ether–methanol, (*H*) 6:1 ether–ethyl acetate, (*I*) 2:1 ethyl acetate–acetone, and (*J*) 3:3:2 ethyl acetate–2-propanol–water.

4-O-(*4,6*-O-*Isopropylidene*-β-D-*galactopyranosyl*)-α,β-D-*glucopyranose* (**1**). — Anhydrous lactose (10.0 g) was suspended and partially dissolved in *N,N*-dimethylformamide (150 mL, reagent grade, without pretreatment) containing *p*-toluenesulfonic acid (150 mg). A solution of 2,2-dimethoxypropane (20 mL) in *N,N*-dimethylformamide was added dropwise at 25° over a period of 30–40 min, with magnetic stirring which was thereafter continued. Progress of the reaction was monitored by t.l.c. (*A*). A major product migrating slightly faster than reference lactose soon appeared and increased in intensity while the lactose suspended in the reaction mixture gradually dissolved. After some time, traces of faster-moving products became visible and, after a total of 3 h, the reaction mixture was processed, although some unchanged lactose was still present in solution. The mixture was stirred for 30 min with ~10 g of anion exchanger (Rexyn 203 [OH^-]), filtered, and evaporated *in vacuo* (bath temp., 50–60°) with the addition of several portions of toluene. The amorphous, yellowish residue was dried in an oil-pump vacuum (11 g). It was dissolved in warm ethanol, and insoluble parts (lactose, by t.l.c.) were filtered off. The filtrate was concentrated under reduced pressure and some additional lactose, which thereby separated in gelatinous form, was removed by filtration through a layer of Celite. Addition of ether to the filtrate caused precipitation of **1** as a white powder (7.0 g after drying) which contained only a trace of remnant lactose. (In other experiments, petroleum ether or acetone followed by ether was used for precipitating **1**, with similar results.) Crude **1** so obtained could be used directly for the preparation of **2** or **3** although it contained, in addition to traces of lactose, a small proportion of a marginally faster-migrating by-product detected in t.l.c. with solvent *A* or, better, solvent *J*.

Compound **1** was prepared in purer form and better yield when the reaction mixture obtained after evaporation of the *N,N*-dimethylformamide was chromatographed on a column of silica gel (200 g) with 8:1 (v/v) chloroform–methanol as eluent. This procedure gave **1** (8.5 g, 76%), which was free of lactose and the other impurities that had been present in the crude reaction mixture, except for the afore-

mentioned, small amount of marginally faster moving by-product; $[\alpha]_D^{25}$ +26 (5 min)→+25° (36 h; *c* 0.3, water). An analytical sample was further purified by chromatography on a small column with 3:2:1 (v/v) ethyl acetate–2-propanol–water as the eluent. This furnished pure **1** that gave a single spot in t.l.c. (solvents *A* and *J*) and showed $[\alpha]_D^{25}$ +30.5° (initial)→+25° (12 h, final; *c* 0.93, water). The substance was readily soluble in water, methanol, and ethanol; slightly soluble in acetone; and practically insoluble in ether and chloroform. It gradually softened and melted over a wide temperature range (120–160°).

Anal. Calc. for $C_{15}H_{26}O_{11} \cdot 0.33\ H_2O$: C, 46.38; H, 6.92. Found: C, 46.40; H, 6.87.

Compound **1** reduced hot Fehling solution. It was readily reduced by sodium borohydride in ethanol to give a product that moved slower in t.l.c. and still contained the isopropylidene group, according to the n.m.r. spectrum in dimethyl sulfoxide-*d* (2 singlets at δ 1.38 and 1.28, unchanged from the spectrum of **1**).

*1,2,3,6-Tetra-*O*-acetyl-4-*O*-(2,3-di-*O*-acetyl-4,6-*O*-isopropylidene-β-*D*-galacto-pyranosyl)-β-*D*-glucopyranose* (**2**). — Compound **1** (7.0 g, purified by column chromatography with 8:1 (v/v) chloroform–methanol) was introduced, in small portions, into a boiling mixture of acetic anhydride (60 ml) and anhydrous sodium acetate (6 g) with swirling and temporary removal from the heater during additions. At the end, the mixture was brought to brief boiling again, and then allowed to cool to room temperature, poured onto crushed ice, and processed in the usual manner by chloroform-extraction. The crude hexaacetate (10.0 g, 86%) showed one major spot in t.l.c. (solvent *E*), together with traces of slow-moving contaminants. Recrystallization from 95% ethanol gave 8.0 g (69%) of **2**, m.p. 172–174°, $[\alpha]_D^{25}$ +30.9° (*c* 0.6, chloroform); ν_{max}^{Nujol} no OH absorption; n.m.r. (60 MHz): δ 5.73 (d, 1 H, *J* 8 Hz, H-1), 2.07 (center of OAc signal cluster), 1.42 and 1.37 (s, 2 × 3 H, CMe_2).

Anal. Calc. for $C_{27}H_{38}O_{17}$: C, 51.10; H, 6.04. Found: C, 50.89; H, 5.88.

*1,2,3,6-Tetra-*O*-benzoyl-4-*O*-(2,3-di-*O*-benzoyl-4,6-*O*-isopropylidene-β-*D*-galac-topyranosyl)-α-*D*-glucopyranose* (**3**). — To a cooled (0°) solution of crude **1** (10 g, containing a small amount of lactose and dried *in vacuo* for several hours), in dry pyridine (100 mL), was added benzoyl chloride (30 mL) over a period of 20 min. The reaction mixture was then kept for 1 h at room temperature and for 4 h at 60°, after which water (3 mL) and dichloromethane (200 mL) were added. The solution was washed successively with water, 1.5M sulfuric acid, saturated aqueous sodium hydrogencarbonate solution, and again water. After being dried ($MgSO_4$) and filtered through a layer of charcoal and Celite, the solution was evaporated to give a syrup that was dissolved in hot methanol under addition of a little chloroform. Upon cooling, a solid was deposited, which was shown to consist largely of one product although it was accompanied by small amounts of slower-moving impurities (t.l.c. with solvent *D*). Three recrystallizations from acetone–methanol gave a nearly pure product (18 g, 68%). An analytical sample was recrystallized once more, m.p. 245–247°, $[\alpha]_D^{25}$ +104.8° (*c* 0.96, chloroform); n.m.r. (60 MHz): δ 8.3–7.2 (30 H, OBz), 6.83 (d, 1 H, *J* ~4 Hz, H-1), 1.53 and 1.28 (s, 2 × 3 H, CMe_2).

Anal. Calc. for $C_{57}H_{50}O_{17}$: C, 67.98; H, 5.01. Found: C, 68.16, H, 4.91.

1,2,3,6-Tetra-O-*acetyl-4*-O-(*2,3-di*-O-*acetyl*-β-D-*galactopyranosyl*)-β-D-*glucopyranose* (**4**). — The isopropylidene acetal **2** (4.0 g) was dissolved in trifluoroacetic acid (9 mL), and water (1 mL) was added. After 2–3 min, a 2:1 (v/v) mixture (~100 mL) of ether and petroleum ether (b.p. 30–60°) was added, whereby **4** was quantitatively precipitated in the form of fine, white crystals. These were washed thoroughly with ether and recrystallized from ethyl acetate–ether to give pure **4** (2.8 g, 75%), homogeneous in t.l.c. (solvents *C*, *G*, or EtOAc); m.p. 207–209°, $[\alpha]_D^{25}$ +5.4° (*c* 1, chloroform); n.m.r. (60 MHz): δ 5.70 (d, 1 H, *J* 8 Hz, H-1), 2.15–2.10 (cluster of singlets, OAc), no signals attributable to CMe_2; an OH signal, removable by D_2O exchange, was at δ 3.00.

Anal. Calc. for $C_{24}H_{34}O_{17}$: C, 48.48; H, 5.72. Found: C, 48.59; H, 5.66.

A sample of **4** (1.0 g) was isopropylidenated essentially as described for lactose, with a reaction time of 75 min. Processing gave crystalline **2** (from 95% ethanol), which was identified by t.l.c. (solvent *E*) and by an undepressed mixture m.p. of 172–174°.

A solution of **4** (30 mg) and sodium metaperiodate (13 mg) in methanol (3 mL) containing some water (~0.3 mL) was stored at room temperature in the dark. No change in t.l.c. was noticed after 16 and 48 h. After solvolysis, first with methanolic and then with aqueous hydrochloric acid, glucose and galactose were detected as spots of equal intensity in paper chromatography (10:3:3, v/v, 1-butanol–pyridine–water, 48 h, descending).

1,2,3,6-Tetra-O-*benzoyl-4*-O-(*2,3-di*-O-*benzoyl*-β-D-*galactopyranosyl*)-α-D-*glucopyranose* (**5**). — The isopropylidene acetal **3** (3.0 g) was dissolved in trifluoroacetic acid (18 mL), and water (2 mL) was immediately added. The mixture was kept at room temperature for 20 min and then concentrated *in vacuo* to give a syrup which turned into a white solid upon addition of methanol. The material was filtered off and washed with methanol, and t.l.c. (solvent *F*) then indicated complete absence of **3**, and the presence of one product (**5**), accompanied by only a trace of a slow-moving contaminant. The product was recrystallized from acetone with addition of methanol and a few drops of water, m.p. 225–228°, $[\alpha]_D^{25}$ +83.3° (*c* 0.25, chloroform); n.m.r. (60 MHz): δ 8.3–7.3 (2 m, OBz), 6.80 (d, 1 H, *J* 4 Hz, H-1), and 3.00 (broad s, 2 H, exchangeable with D_2O, OH).

Anal. Calc. for $C_{54}H_{46}O_{17}$: C, 67.07; H, 4.80. Found: C, 67.32; H, 5.00.

O-(*2,3,4-Tri*-O-*acetyl*-β-L-*fucopyranosyl*)-(1→3)-O-(*2,6-di*-O-*acetyl*-β-D-*galactopyranosyl*)-(*1*→*4*)-*1,2,3,6-tetra*-O-*acetyl*-β-D-*glucopyranose* (**6**). — A solution of the hexaacetate **4** (1.0 g) in ethanol-free, dry chloroform (30 mL) was stirred for 1 h with freshly prepared silver carbonate (1 g) and Drierite (5 g). A solution of syrupy 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl bromide[27] (1.0 g) in dry chloroform (10 mL) was then added dropwise, and the reaction was monitored by t.l.c. (*H*). The formation of a product of medium mobility (greater than that of **4**) and several spots that moved still faster and originated from decomposition of the fucosyl bromide were observed. Two additional 1-g portions of fucosyl bromide were introduced after 1 and 2 h,

respectively, and stirring was then continued for another 3 h. Thereafter, the reaction mixture was processed, even though a substantial proportion of **4** (or a compound migrating like **4** in t.l.c.) was still present. The inorganic material was filtered off with the aid of Celite and washed with chloroform. The filtrate was washed twice with water, dried ($MgSO_4$), concentrated to a small volume, and applied to a column of silica gel (150 g). Elution with 5:1 (v/v) ether–petroleum ether (b.p. 30–60°) removed the fast-moving contaminants that originated from decomposition of the fucosyl bromide. Subsequent elution with pure ether furnished **6** (0.40 g, 27%) as a crystalline solid showing a single spot in t.l.c. (solvent *H*, or ether alone), m.p. 222–224° (recrystallized from ethyl acetate–ether–petroleum ether), $[\alpha]_D^{25}$ +7.7° (*c* 0.7, chloroform). The mother liquor of recrystallization showed only **6** in t.l.c. Continued elution of the column with ether gave a fraction (0.20 g, 13.7%) consisting mainly of **6** but containing a noticeable proportion of a slightly less-mobile product. The latter could not be isolated or identified but most likely was a trisaccharide compound. The n.m.r. spectra of crystalline **6** and the mixed fraction were quite similar, and both fractions gave the same spot in t.l.c. (solvent *J*), somewhat slower than that of lactose, when they were deacetylated catalytically with sodium methoxide. Acid hydrolyzates (0.5M H_2SO_4) of **6** and the mixed fraction were shown by paper chromatography to contain fucose, galactose, and glucose. N.m.r. (100-MHz) of **6**: δ 5.68 (d, 1 H, *J* 8 Hz, H-1), 5.3–3.6 (3 groups of multiplets, 20 H), 2.17, 2.12, 2.09, 2.06, 2.02, 1.97 (s of 3, 6, 3, 9, 3, and 3 H intensity, 9 OAc), and 1.20 (d, 3 H, *J* 6 Hz, *C*Me).

Anal. Calc. for $C_{36}H_{50}O_{24}$: C, 49.88; H, 5.81. Found: C, 49.74; H, 5.77.

A similar condensation was performed with **4** (0.6 g) in chloroform (20 mL), but with silver oxide (0.6 g, freshly prepared) substituted for silver carbonate. A small crystal of iodine was added to the mixture, and 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl bromide (1.2 g) was added in three portions in the course of 3 h, after which stirring was continued for 2 h. Processing including chromatography on 60 g of silica gel was performed as aforedescribed. Nearly pure **6** (0.30 g) as well as a mixed fraction (0.1 g) similar to that obtained before were obtained, as well as a third fraction (0.1 g) in which the unidentified component preponderated.

A sample of **6** (100 mg) was acetylated with boiling acetic anhydride and sodium acetate. Upon usual processing, a colorless, amorphous solid was obtained; it could not be induced to crystallize and was not characterized except by microanalysis, which agreed with the structure of a fucosyllactose decaacetate (**7**).

Anal. Calc. for $C_{38}H_{52}O_{25}$: C, 50.22; H, 5.77. Found: C, 50.39; H, 5.78.

O-β-L-*Fucopyranosyl*-(*1*→*3*)-O-β-D-*galactopyranosyl*-(*1*→*4*)-D-*glucopyranose* (**8**). — A solution of crystalline **6** (220 mg) in methanol (35 ml) containing a few drops of M sodium methoxide solution was kept overnight at room temperature and then deionized with Amberlite IR 120 (H^+) cation-exchange resin, filtered through a mixed bed of Celite, silica gel, and Amberlite resin, and evaporated to give the free trisaccharide **8** as a white solid (120 mg, 97%), $[\alpha]_D^{25}$ +54.2° (initial)→+51° (4 h, constant; *c* 0.3, water).

Anal. Calc. for $C_{18}H_{32}O_{15}$: C, 44.26; H, 6.60. Found: C, 44.08; H, 6.64.

Permethylation and hydrolysis of **1**. — A solution of **1** (2.0 g) in *N*,*N*-dimethylformamide (40 mL) and methyl iodide (10 mL) was stirred overnight at room temperature with barium oxide (8 g) and barium hydroxide octahydrate (8 g). After the reaction mixture had been chilled in an ice bath, an equal volume of chloroform was added, and the insoluble material was separated and washed several times with cold chloroform. The combined organic phases were washed successively with water, sodium thiosulfate solution, and water, then dried ($MgSO_4$) and evaporated to give a syrup (2.5 g). T.l.c. (solvent *C*) indicated that the main product was accompanied by traces of several slower-moving (presumably incompletely methylated) products. These disappeared when the material was remethylated as just described, by use of one-third the amounts of reagents. The product (2 g) then was a faintly yellow syrup showing, in the n.m.r. spectrum, an α-anomeric proton doublet (1 H, J ~3.7 Hz) at δ 4.83, six sharp singlets in the region δ 3.66–3.40 (OMe) and, interestingly, a 6-proton singlet at δ 1.43 for CMe_2. It is noteworthy that the two isopropylidene methyl groups of methyl 4,6-*O*-isopropylidene-α-D-galactopyranoside and (see below) its 2,3-dimethyl ether show also identical chemical shifts[23] but those of methyl 3,4-*O*-isopropylidene-α-D-galactopyranoside and (see below) its 2,6-dimethyl ether show well-separated shifts.

A part of the permethylated disaccharide (1.0 g) was hydrolyzed with 0.5M sulfuric acid (30 mL) on a steam bath for 4 h. The cooled hydrolyzate was neutralized with Amberlite IR-45(OH^-) anion-exchange resin and evaporated to give a syrup which showed in t.l.c. (solvent *C*) a fast- and a slow-moving spot of comparable intensities, attributed to 2,3,6-tri-*O*-methyl-D-glucose and 2,3-di-*O*-methyl-D-galactose, respectively. The slow-moving compound was isolated as a syrup (480 mg) by silica gel column chromatography with 20:1 (v/v) dichloromethane–methanol as the eluent. The dimethyl ether exhibited a mutarotation, $[\alpha]_D^{25}$ +79.6° (10 min)→ +87.7° (30 min)→ +88.1° (20 h)→ +90.7° (48 h; *c* 0.4, water), in fair agreement with that observed by Bell and Greville[35]. A part of the product (200 mg) was heated under reflux for 4 h with aniline (94 mg) in ethanol (4 mL). The residue obtained upon solvent evaporation was crystallized from acetone–ether–petroleum ether to give 2,3-di-O-methyl-D-galactose anilide as fine, colorless needles, m.p. 153–155°, $[\alpha]_D^{25}$ −53.1° (10 min to 12 h)→ −45° (72 h)→ −37.9° (120 h; at this point, a small drop of glacial acetic acid was added to the 10-ml sample, whereupon the mutarotation continued more rapidly) → −13.6° (2 min)→ +8.5° (10 min)→ +11.8° (12 h, constant; *c* 0.9, ethanol); lit.[35] m.p. 154–155°, $[\alpha]_D$ −56.8° (20 min)→ +12.1° (120 h; ethanol).

The dimethyl galactose just described showed in t.l.c. with solvent *B*, a mobility identical with that of authentic 2,3-dimethyl ether, and it was clearly distinguishable from authentic, somewhat faster migrating 2,6-dimethyl ether.

*2,3-Di-*O*-methyl-*D*-galactose and 2,6-di-*O*-methyl-*D*-galactose from methyl* α-D-*galactopyranoside*. — Methyl α-D-galactopyranoside was condensed with acetone[22], and the resulting 3,4- and 4,6-acetals were isolated[23,25] as the major and minor products, respectively. The 3,4-acetal, moving faster in t.l.c. (*I*), was crystalline, m.p. 101–103°, $[\alpha]_D^{25}$ +147° (*c* 0.4, chloroform); lit.[22] m.p. 97–98°, $[\alpha]_D$ +135°

(chloroform); lit.[25] m.p. 103–104°, $[\alpha]_D$ +161° (chloroform). The 4,6-acetal was a syrup as was also observed earlier[23,25] when its structure was established by various chemical interconversions. It gave a single, 6-proton resonance at δ 1.49 for CMe_2 as reported[23], and showed $[\alpha]_D^{25}$ +137.6° (*c* 0.4, chloroform) and +150° (*c* 0.4, water); lit.[25] $[\alpha]_D$ +166° (ethanol).

Both acetals were methylated[21] with methyl iodide in *N,N*-dimethylformamide in the presence of barium oxide and barium hydroxide, essentially as described for **1** (reaction time, 3 h). The 3,4-acetal gave syrupy methyl 3,4-*O*-isopropylidene-2,6-di-*O*-methyl-α-D-galactopyranoside, $[\alpha]_D^{25}$ +152° (*c* 0.4, water); n.m.r. (60 MHz): δ 4.85 (d, 1 H, *J* 3.5 Hz, H-1), 3.54 and 3.43 (s, 3 + 6 H, OMe), 1.53 and 1.35 (s, 3 H each, CMe_2); lit.[24] $[\alpha]_D$ +155° (water). The 4,6-acetal gave crystalline methyl 4,6-*O*-isopropylidene-2,3-di-*O*-methyl-α-D-galactopyranoside, m.p. 135–137°, $[\alpha]_D^{25}$ +192° (*c* 0.12, chloroform); n.m.r. (60 MHz): δ 4.97 (d, 1 H, *J* 3 Hz, H-1), 3.53, 3.50, and 3.43 (s, 3 H each, OMe), 1.50 (s, 6 H, CMe_2); lit.[25] m.p. 130°, $[\alpha]_D$ +189° (chloroform).

Hydrolysis[24] of the methylated 3,4-acetal with 5% aqueous hydrochloric acid gave 2,6-di-*O*-methyl-β-D-galactose, m.p. 122–124° (from ethyl acetate), $[\alpha]_D^{25}$ +45.5° (initial)→+86.5° (22 h, constant; *c* 0.3, water); lit.[26] m.p. 119–120°, $[\alpha]_D$ +48→+84°. Similar hydrolysis of the methylated 4,6-acetal yielded syrupy 2,3-di-*O*-methyl-D-galactose, $[\alpha]_D^{25}$ +78.2° (20 min)→+86.8° (12 h, final; *c* 0.4, water); lit.[36] $[\alpha]_D$ +64.7→+80.9° (water).

Reduction, permethylation, and hydrolysis of **8**. — A solution of the trisaccharide **8** (90 mg) in water (3 mL) was treated with sodium borohydride (100 mg) for 30 min at 25°. After de-ionization of the solution with Amberlite IR-120 (H^+) cation-exchange resin and removal of the boric acid by multiple evaporations with added methanol, a solid alditol derivative was obtained, $[\alpha]_D^{25}$ +28° (*c* 0.2, water). The product differed from **8** by a slightly slower mobility in t.l.c. (solvent *J*). It was permethylated[21] by two successive treatments (5 and 16 h) with methyl iodide (2 mL), barium oxide (0.5 g), and barium hydroxide octahydrate (0.5 g) in *N,N*-dimethylformamide (5 mL). Processing as described for the methylation of **1** gave a yellowish syrup. A fast-moving contaminant revealed to be present by t.l.c. (*C*) was removed by passage of the material, dissolved in chloroform, through a small column of silica gel that was eluted with solvent *C*. The purified, syrupy product (60 mg) was hydrolyzed on a steam bath for 7 h with 0.5M sulfuric acid (5 mL). The hydrolyzate was neutralized with lead carbonate, the filtrate evaporated, and the organic residue dissolved in a small amount of acetone. T.l.c. of this solution (double irrigation with solvent *C*) showed 3 widely separated spots that were attributed to 2,3,4-tri-*O*-methyl-L-fucose (fast), 1,2,3,5,6-penta-*O*-methyl-D-glucitol (medium), and a tri-*O*-methyl-D-galactose. The last-mentioned compound moved, in solvent *C* as well as in two other, specially recommended[33] systems for distinguishing methylated sugars (1:1, v/v, benzene–acetone and 83:17, v/v, isopropyl ether–methanol), at a rate identical with that of authentic 2,4,6-tri-*O*-methyl-D-galactose and clearly different from that of slower-moving, authentic 2,3,4-tri-*O*-methyl-D-galactose.

2,3,4-Tri-O-methyl-D-galactose. — This compound was prepared according to Smith[34] from methyl α-D-galactopyranoside *via* its 6-trityl ether. However, the trityl ether was freed from residual triphenylcarbinol by column chromatography on silica gel by means of ethyl acetate as eluent, and was obtained in crystalline form, m.p. 126–129° (from ethyl acetate–hexane), $[\alpha]_D^{25}$ +54.9° (acetone) and +68.9° (chloroform). The 6-trityl ether was then methylated by the Kuhn procedure (see preceding sections) rather than the Haworth procedure[34]; the product had $[\alpha]_D^{25}$ +61° (chloroform) and showed substituent resonances in agreement with expectations [n.m.r. (60 MHz): δ 7.6–7.2 (m, Ph), 3.52, 3.50, 3.42, and 3.39 (s, OMe)]. Detritylation with hydrogen chloride in chloroform at 0° gave methyl 2,3,4-tri-*O*-methyl-α-D-galactopyranoside as a syrup, which was purified by column chromatography on silica gel, $[\alpha]_D^{25}$ +150° (*c* 1, methanol) and +190.5° (*c* 0.6, water); lit.[37] $[\alpha]_D$ +160.8° (methanol) and +198.4° (water). The n.m.r. spectrum indicated the absence of aromatic protons and showed OMe resonances at δ 3.59, 3.53 (2), and 3.43. Hydrolysis of the glycoside with 5% aqueous hydrochloric acid (7 h at 98°) afforded 2,3,4-tri-*O*-methyl-α-D-galactopyranose, m.p. 73–76° (from ether–petroleum ether), $[\alpha]_D^{25}$ +146.4°→+114.3° (*c* 0.14, water) in good agreement with recorded data[38].

Simplified synthesis of 2,4,6-tri-O-methyl-D-galactopyranose. — Two different syntheses of this compound, necessitating either 10 distinct steps from methyl β-D-galactopyranoside or 13 steps from D-galactose, have been described by Bell and Williamson[24]. We record an approach comprising 7 steps from methyl α-D-galactopyranoside, the first two being those leading to methyl 3,4-*O*-isopropylidene-2,6-di-*O*-methyl-α-D-galactopyranoside as outlined in a preceding section. The isopropylidene group of this derivative (0.50 g) was hydrolyzed with 90% trifluoroacetic acid (5 mL) at room temperature for 5 min, followed by evaporation and coevaporation of the residual solvent with added methanol. The syrupy product (470 mg) was free from starting acetal (t.l.c., solvent *I*); $[\alpha]_D^{25}$ +132° (*c* 0.4, chloroform); n.m.r. (60 MHz): δ 4.95 (d, 1 H, *J* 3.5 Hz, H-1), 3.50 and 3.42 (s, 3 + 6 H, OMe), 2.93 (broad s, 2 H, exchangeable with D_2O, OH). This glycoside dimethyl ether (420 mg) was monotosylated by treatment with *p*-toluenesulfonyl chloride (400 mg, 1.6 mol. equiv.) in pyridine (25 mL), initially at −20° but continuing at room temperature for 3 days as t.l.c. (solvent *I*) revealed a rather slow progress of the reaction. No attempt was made to crystallize the syrupy product (~0.5 g) obtained after customary processing; it was homogeneous in t.l.c., had $[\alpha]_D^{25}$ +128° (*c* 0.6, chloroform), and gave n.m.r. signals consistent with a methyl mono-*O*-tosylglycoside dimethyl ether: δ 7.63 (center of AB pattern, 4 H, arom.), 4.9 (m, 2 H, H-1 and -3), 3.45 and 3.21 (s, 6 + 3 H, OMe), 2.45 (s, 3 H, Me of Ts). Methylation of this product (0.40 g) by the Kuhn method as described in preceding examples furnished known[24] methyl 2,4,6-tri-*O*-methyl-3-*O*-*p*-tolylsulfonyl-α-D-galactopyranoside as a syrup (0.3 g) which crystallized on trituration with petroleum ether, m.p. 110–112°, $[\alpha]_D^{25}$ +143° (*c* 0.25, chloroform); n.m.r. (60 MHz): δ 7.47 (center of AB quartet, 4 H, arom.), 4.77 (d, 1 H, *J* 3.5 Hz, H-1), 4.75 (dd, 1 H, *J* 3.5 and 10 Hz, H-3), 3.59, 3.37, 3.33, and 3.17 (s, OMe), 2.42 (s, 3 H, Me of Ts); lit.[24] m.p. 112°, $[\alpha]_D$ +150°. Removal of the

sulfonyl group with sodium methoxide[24] gave methyl 2,4,6-tri-*O*-methyl-α-D-galactopyranoside as fine needles from ether–petroleum ether, m.p. 72–73° (lit.[24] m.p. 73–74°), and subsequent hydrolysis[24] of this glycoside with M hydrochloric acid yielded crystalline 2,4,6-tri-*O*-methyl-D-galactopyranose, m.p. 99–100° (from ether–petroleum ether), $[\alpha]_D^{25}$ +88.3° (equil.; *c* 0.2, water); lit.[24] m.p. 102–105°, $[\alpha]_D$ +90.4° (equil.).

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