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Note

Synthesis of *O*-α-L-fucopyranosyl-(1→6)-*O*-β-D-galactopyranosyl-(1→4)-D-glucopyranose (6′-*O*-α-L-fucopyranosyllactose)

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In a preceding paper[1], we have surveyed the literature on the occurrence, in Nature, of higher oligosaccharides that are structurally derived from lactose and contain one or more L-fucopyranosyl groups. The first member of this family to be isolated and elucidated[2], 2′-α-L-fucopyranosyllactose of human milk, was found[3] to have a modest ability to inhibit hemagglutination of human O(H) blood cells by eel serum. More recently, it was shown to be an effective inhibitor of the precipitation of human H substance by certain lectins, notably those from *Lotus tetragonolobus*[4] and *Ulex europaeus*[5], and has thus served as an important aid in the immunochemical study of blood-group specificity[6]. It seems reasonable to anticipate that various, isomeric fucosyllactoses, thus far unknown, might prove useful in future investigations into the specificity of combining sites on antibodies and on lectins. We have therefore initiated a project of synthesis in this direction. As already reported[1], kinetically controlled isopropylidenation of lactose with 2,2-dimethoxypropane gave a 4′,6′-*O*-isopropylidene derivative which, upon acetylation followed by deacetonation, provided crystalline 1,2,3,6,2′,3′-hexa-*O*-acetyl-β-lactose (**1**). When this hexaacetate was condensed[1] with tri-*O*-acetyl-α-L-fucopyranosyl bromide under Koenigs–Knorr conditions, concomitant 3′→6′ acetyl migration occurred, and the L-fucopyranosyl group became attached β-glycosidically in position 3′. The present Note describes the synthesis of the title trisaccharide.

Compound **1** was condensed with tri-*O*-benzyl-α-L-fucopyranosyl bromide (**2**) under catalysis by bromide ion according to Lemieux and co-workers[7], who have demonstrated, on related examples, that this procedure leads without complication to α-fucosylation. A crystalline 6′-*O*-(tri-*O*-benzyl-α-L-fucopyranosyl)-β-lactose hexaacetate (**3**) was obtained in over 80% yield. Although the substituent resonances in the ^1H-n.m.r. spectrum of **3** were in full accord with the structure depicted, the position of the fucose residue could not be determined spectroscopically; the 1″→6′ linkage was proved, at a later stage, by permethylation. That the primary hydroxyl group, rather than the secondary (and axial) OH-4′, would be the strongly favored (if not exclusive) site of reaction in **1** was, of course, to be expected. In contrast to

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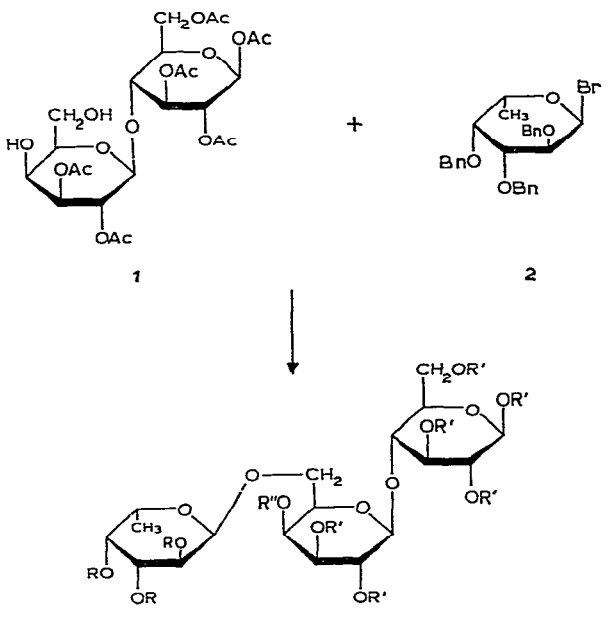
the aforedescribed[1] Koenigs–Knorr synthesis, acetyl-group migration to OH-6′ evidently did not intervene, even though a base (ethyldiisopropylamine) serving as an acid acceptor was used in the condensation reaction. There was no evidence, either, to indicate migration to OH-4′. The proton chemical-shifts of the acetoxyl resonances in **3**, in chloroform-*d* solution, were all in a range (δ 1.99–2.09) compatible with equatorial ester groups[8], suggesting that the axial OH-4′ group had remained unesterified. Complete acetylation of **3** afforded the crystalline heptaacetate **3a**. This derivative exhibited, in addition to six acetoxyl signals present in a similar range (δ 1.95–2.10), a seventh signal which, resonating at a marginally lower field (δ 2.13), may have been that of a newly introduced, axial OAc-4′ group. However, chemical-shift data reported[7a] for some analogous oligosaccharides suggest that a benzylated glycosyl moiety may cause changes in the shielding of acetoxyl protons present in neighboring saccharide residues, thus invalidating the conformational rule[8], and the position of the free hydroxyl group in **3** as formulated therefore remains unascertained. In any event, the problem was irrelevant to the operations that followed. These involved Zemplén deacetylation of **3** to give, quantitatively, the tri-*O*-benzyl trisaccharide **4**, and subsequent hydrogenolysis over a palladium catalyst to furnish the title compound **5**. The latter, an amorphous but chromatographically homogeneous, white powder, showed levorotation, as postulated[1] for an α-L-fucopyranosyl-lactose on the basis of Hudson's rule of isorotation: $[\alpha]_D$ −21° (equil., water). The



3 R = Bn, R′ = Ac, R″ = H
3a R = Bn, R′ = R″ = Ac
4 R = Bn, R′ = R″ = H
5 R = R′ = R″ = H (anomeric mixture)

isomeric 2′-α-L-fucopyranosyllactose[2] from human milk is also levorotatory ($[\alpha]_D$ −57.5°), whereas the synthetic 3′-β-L-fucopyranosyl isomer[1] is dextrorotatory ($[\alpha]_D$ +51°).

To determine the position of the fucosyl group, compound **5** was reduced with sodium borohydride and the resulting alditol subjected to permethylation followed by acid hydrolysis. Thin-layer chromatography of the hydrolyzate gave three well-separated spots which were attributed, in order of decreasing mobility, to 2,3,4-tri-*O*-methyl-L-fucose, 1,2,3,5,6-penta-*O*-methyl-D-glucitol, and a tri-*O*-methyl-D-galactose. The various, isomeric D-galactose trimethyl ethers may be distinguished in t.l.c. by use of solvent systems specially recommended for that purpose[9]; our product proved indistinguishable, in three different systems, from an authentic sample of 2,3,4-tri-*O*-methyl-D-galactose, whereas it clearly differed from a sample of the 2,4,6-tri-*O*-methyl isomer. A 1″→6′ linkage in **5** was thus indicated.

EXPERIMENTAL

General methods. — General and instrumental methods were the same as those employed previously[1]. Unless otherwise indicated, the solvents used for t.l.c. were (v/v): *A*, 4:1 chloroform–methanol; *B*, 10:1 chloroform–methanol; *C*, 3:2:1 ethyl acetate–2-propanol–water; *D*, 1:1 benzene–acetone; and *E*, 83:17 isopropyl ether–methanol. Petroleum ether refers to the fraction having b.p. 30–60°. Optical rotations were recorded at ~25°.

2,3,4-Tri-O-benzyl-α-L-fucopyranosyl bromide (**2**). — The preparation[10] of **2** from L-fucose was simplified, and rendered more economical, by using an anomeric mixture of methyl L-fucopyranosides[11] for benzylation; the pure α anomer crystallizes from such a mixture in yields of only ~45%. After the benzylation[12], the resulting syrup of tribenzyl ethers was freed from fast- and slow-moving impurities by column chromatography on silica gel using 8:1 toluene–ether as the irrigant. Hydrolysis[10] of the glycoside mixture then gave crystalline 2,3,4-tri-*O*-benzyl-α-L-fucopyranose (overall yield from L-fucose, 60%), m.p. 102–104°, $[\alpha]_D$ −25.4° (*c* 1.3, chloroform); lit.[10] m.p. 102–103°, $[\alpha]_D$ −26.5°. The sugar was converted[10] into its 1-*p*-nitrobenzoate which, in our hands, showed m.p. 134–136° and $[\alpha]_D$ +55.6° (*c* 1.1, chloroform). These data are at variance with those first recorded[10] but agree satisfactorily with later[7c] values (m.p. 135–136°, $[\alpha]_D$ +60.9°). The bromide **2** was generated[7b,7c,10] from the *p*-nitrobenzoate immediately prior to its intended use.

O-(*2,3,4-Tri*-O-*benzyl-α-L-fucopyranosyl*)-(*1→6*)-O-(*2,3-di*-O-*acetyl-β-D-galactopyranosyl*)-(*1→4*)-*1,2,3,6-tetra*-O-*acetyl-β-D-glucopyranose* (**3**). — A solution of the fucosyl bromide **2** (approximately 8.5 mmol, freshly prepared from 5.0 g of the *p*-nitrobenzoate just mentioned) and tetraethylammonium bromide (2.5 g) in dichloromethane (25 mL) was stirred with 5 g of molecular sieves (type 4A) for 0.5 h at room temperature, with exclusion of light. A solution of β-lactose 1,2,3,6,2′,3′-hexaacetate[1] (**1**; 2.4 g, 4 mmol) in dichloromethane (25 mL), and ethyldiisopropylamine (1.0 g) were then added successively, and stirring was continued for 48 h.

Inspection by t.l.c. (ether) indicated the formation of one major product, which moved faster than **1**, and a trace of a marginally less-mobile product. There were also three additional spots that moved faster than the main product; they appeared to originate from side-reactions of the glycosyl bromide. The mixture was filtered with the aid of Celite, and the filter residue was washed thoroughly with dichloromethane. The combined filtrate and washings were extracted with water, dried (magnesium sulfate), and evaporated to give a solid (~6.3 g). Dissolved in a small volume of dichloromethane, the product was applied to a column of silica gel (150 g). Elution with ether removed first the faster-moving contaminants and then gave fractions containing mainly the chief product, accompanied by a small proportion of the less-mobile by-product. Evaporation of solvent gave crystalline **3** (3.6 g) which, upon recrystallization from ethyl acetate–petroleum ether, furnished pure **3** (3.3 g, 82%), m.p. 195–197°, $[\alpha]_D$ −26.5° (*c* 0.5, chloroform); n.m.r. data (100 MHz, $CDCl_3$): δ 7.31 (m, 15 H, Ph), 5.67 (d, 1 H, *J* 8 Hz, H-1), 2.71 (d, 1 H, *J* 4.5 Hz, exchangeable by addition of D_2O to the solution, OH-4'), 2.09, 2.09, 2.07, 2.03, 2.01, and 1.99 (each s, total 18 H, six OAc), and 1.10 (d, 3 H, *J* 6 Hz, *C*-Me). Unresolved ring-proton signals in the region δ 4.0–5.2, and *Ph*–CH_2O signals centered at δ 3.75, integrated to a total of 24 protons.

Anal. Calc. for $C_{51}H_{62}O_{21}$ (1011.0): C, 60.58; H, 6.18. Found: C, 60.75; H, 6.25.

Continued elution of the column with ether produced a mixture (0.16 g) of **3** and unidentified, slow-moving material, according to t.l.c.

O-(*2,3,4-Tri*-O-*benzyl*-α-L-*fucopyranosyl*)-(*1*→*6*)-O-(*2,3,4-tri*-O-*acetyl*-β-D-*galactopyranosyl*)-(*1*→*4*)-*1,2,3,6-tetra*-O-*acetyl*-β-D-*glucopyranose* (**3a**). — A sample of the hexaacetate **3** (150 mg) was acetylated by treatment for 1 min with boiling acetic anhydride (3 mL) in the presence of anhydrous sodium acetate (0.2 g). The excess of anhydride was decomposed with ice–water and the product extracted into chloroform, which was washed with sodium hydrogencarbonate solution and water, dried, and evaporated to give a white solid [160 mg, homogeneous in t.l.c. (ether), migrating faster than **3**]. Recrystallized from ether–petroleum ether, the product (**3a**) had m.p. 110–113°, $[\alpha]_D$ −36.4° (*c* 1, chloroform); n.m.r. data (100 MHz, $CDCl_3$): δ 7.3 (m, 15 H, 3 Ph), 5.65 (d, 1 H, *J* 8 Hz, H-1), 5.41 (narrow dd, 1 H, H-4'), 5.3–3.4 (unresolved multiplets; sugar ring and benzylic methylene protons), and 1.04 (d, 3 H, *J* 6 Hz, *C*-Me). The OAc resonances, measured at 50-Hz sweep width, occurred at δ 2.126, 2.099, 2.096, 2.039, 2.025, 1.974, and 1.946.

Anal. Calc. for $C_{53}H_{64}O_{22}$ (1053.0): C, 60.45; H, 6.13. Found: C, 60.70; H, 6.21.

O-(*2,3,4-Tri*-O-*benzyl*-α-L-*fucopyranosyl*)-(*1*→*6*)-O-β-D-*galactopyranosyl*-(*1*→*4*)-D-*glucopyranose* (**4**). — A few drops of methanolic, M sodium methoxide were added to a solution of the hexaacetate **3** (0.40 g) in methanol (40 mL). After 4 h at room temperature, all of **3** had disappeared, and a slightly slower-moving product was present (t.l.c. in solvent *A*). The solution was deionized with Amberlite IR-120 (H^+) resin, the mixture filtered through a mixed bed of Celite, silica gel, and

some additional resin, and the filtrate evaporated to give crystalline **4** (0.30 g, quantitative). Recrystallized from methanol–ether, the product (0.27 g, 90%) showed m.p. 160–162°, $[\alpha]_D$ −25° (*c* 0.3, chloroform). Despite prolonged drying at 65° *in vacuo*, several samples consistently gave analytical data fitting for a hemihydrate.

Anal. Calc. for $C_{39}H_{50}O_{15} \cdot 0.5\ H_2O$ (767.8): C, 61.00; H, 6.70. Found: C, 60.97; H, 6.83.

Another sample of **3** (0.50 g) was deacetylated with 1:11:4 triethylamine–methanol–water (40 mL) during 3 h at room temperature. The solvent was removed by evaporation, and the residue was dissolved in methanol. Treatment of the solution with a small amount of Amberlite IR-120 (H^+) and subsequent evaporation gave crystalline **4** that weighed 0.35 g (93%) after recrystallization from methanol–ether.

O-α-L-*Fucopyranosyl*-(*1*→*6*)-O-β-D-*galactopyranosyl*-(*1*→*4*)-D-*glucopyranose* (**5**). — A solution of the tribenzyl derivative **4** (0.40 g) in a mixture of 95% ethanol (25 mL) and methanol (15 mL) was hydrogenated for 72 h at room temperature and 4 atm. pressure, in the presence of 10% palladium-on-charcoal (0.4 g). Filtration and evaporation of the filtrate gave **5** (0.25 g, 97%) as a white, hygroscopic solid. An inorganic contaminant (6%, by combustion analysis) was removed with a cation-exchange resin. The recovered trisaccharide, still slightly hygroscopic, was homogeneous in t.l.c. (solvent *C*), showing a mobility slightly lower than that of lactose; $[\alpha]_D$ −28.3 (*c* 0.24, methanol) and −23 (initial) →−21° (22 h, *c* 0.3, water). Dried in a high vacuum at 80°, the analytical sample appeared to retain a mol. equiv. of water.

Anal. Calc. for $C_{18}H_{32}O_{15} \cdot H_2O$ (506.5): C, 42.68; H, 6.77. Found: C, 42.50; H, 6.63.

Successive reduction, permethylation, and hydrolysis of **5**. — A solution of **5** (0.10 g) and sodium borohydride (0.15 g) in water (8 mL) was maintained for 0.5 h at room temperature, whereupon t.l.c. (solvent *C*) showed complete conversion of **5** into the marginally more-slowly moving alditol derivative. After cation exchange, evaporation of the solution, and several evaporations of added portions of methanol from the residue (to remove boric acid), the solid alditol showed $[\alpha]_D$ −55° (*c* 0.25, methanol). It was permethylated for 24 h by the Kuhn procedure[13], as described[1] for an isomeric trisaccharide. Processing[1] gave a yellowish syrup that was shown by t.l.c. (solvent *B*) to contain, besides the main product, a faster-moving contaminant. The latter was removed by chromatography of the material on a small column of silica gel by means of solvent *B*. The purified product (42 mg) was hydrolyzed in 3 mL of 0.5M sulfuric acid (4 h at 98°). The hydrolyzate was made neutral by use of Amberlite IR-45 (OH^-) resin and evaporated to dryness. The residue was extracted with acetone, and the extract concentrated to low volume. This solution contained three, widely separated components (2,3,4-tri-*O*-methyl-L-fucose, 1,2,3,5,6-penta-*O*-methyl-D-glucitol, and 2,3,4-tri-*O*-methyl-D-galactose) having high, medium, and low mobility, respectively (t.l.c. in solvent *B*). The galactose derivative moved at a rate identical with that of an authentic sample[1] in solvent *B* and in the solvents[9] *D* and *E* which permit differentiation of the 2,3,4-, 2,3,6-, 2,4,6-, and 3,4,6-trimethyl ethers[9].

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