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## Note

# Synthesis of 6-*O*-α-L-fucopyranosyl-D-galactose*

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Aryl α-L-fucopyranosides have been employed for isolation of various α-L-fucosidases from biological sources[1,2]. Certain fucosidases hydrolyze the non-reducing α-L-fucosidic linkage regardless of the nature of the aglycon. However, there is another class of α-L-fucosidases that does not act on simple α-L-fucopyranosides; they require both a specific intersugar linkage and a specific aglycon for their action[3–5]. For characterization and specificity studies of such α-L-fucosidases from human tissues, we have undertaken the synthesis of various α-L-fucosyldisaccharides, reporting here a simple synthesis of 6-*O*-α-L-fucopyranosyl-D-galactose. Schiffman, *et al.*[6] isolated this disaccharide in low yield by mild acid hydrolysis of human blood-group B substance from ovarian-cyst fluid, but it was not well characterized.

Interestingly, 2-*O*-α-L-fucopyranosyl-D-galactose[7], 2-*O*-α-L-fucopyranosyl-L-fucose[8], and 2-acetamido-2-deoxy-4-*O*-α-L-fucopyranosyl-D-glucose[9] have been synthesized by reaction of 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl bromide and appropriately protected sugar derivatives. However, attempts to obtain other fucosyl disaccharides in which L-fucose is attached at O-3 or O-6 of 2-acetamido-2-deoxy-D-glucose[10–12] or D-galactose[12] resulted in the formation of β-L-anomers. The reaction of 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl bromide and 1,3,4,6-tetra-*O*-acetyl-α-D-galactose under similar conditions, or in the presence of mercuric cyanide and mercuric bromide in acetonitrile[13], produced a mixture of α- and β-L- anomers[14]. It has been shown recently that 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide undergoes Koenigs–Knorr condensation with a low degree of selectivity[15]. However, Dejter-Juszynski and Flowers[16] have made use of the 2-*O*-benzyl-3,4-di-*O*-(*p*-nitrobenzoyl) bromide **1** for an unambiguous synthesis of 2-acetamido-2-deoxy-6-*O*-α L-fucopyranosyl-D-glucose.

It is well known that the 4-hydroxyl group of D-galactopyranosyl group is unreactive towards glycoside formation[16]. Flowers *et al.*[17,18] have shown that protected galactopyranose derivatives having the 3- and 4-hydroxyl groups free react preferentially at O-3 in glycosidation reactions. Recently, we have employed 1,2,3-tri-

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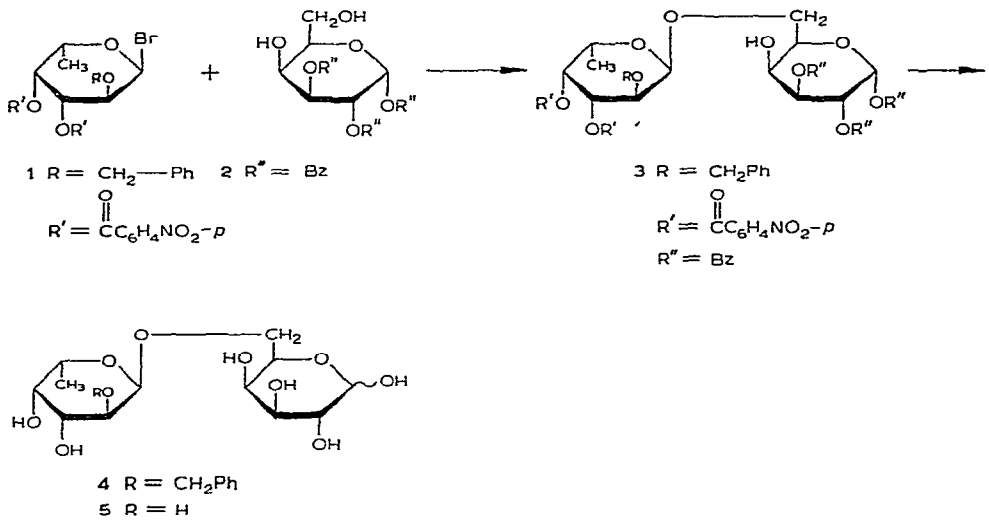
*O*-benzoyl-α-D-galactopyranose[19] (2) for the synthesis of 6-*O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-D-galactose[20].



In the present investigation, reaction of bromide **1** with compound **2** in nitromethane–benzene in the presence of mercuric cyanide produced compound **3**. Catalytic deacylation of **3** followed by chromatography on silica gel gave amorphous 6-*O*-(2-*O*-benzyl-α-L-fucopyranosyl)-D-galactose (**4**). Pure compound **4** was hydrogenolyzed catalytically to afford the desired disaccharide **5** in good yield.

The structure of the synthetic disaccharide was supported by periodate-oxidation data. A strongly negative optical rotation suggested the α-L-configuration for the compound. Neither paper chromatography nor t.l.c. served to distinguish compound **5** from 6-*O*-β-L-fucopyranosyl-D-galactose[12]. Similar observations have been made for some other L-fucosyl disaccharides[14,15]. However, the homogeneity of compound **5** was established by g.l.c. analysis of the per(trimethylsilyl) ether of the alditol. The latter was obtained by reduction of the disaccharide with sodium borohydride.

## EXPERIMENTAL

*General.* — Melting points were determined with a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer Model 141 Polarimeter with sodium light. N.m.r. spectra were recorded with a Varian Associates A-60 instrument. I.-r. spectra were taken for potassium bromide discs with a Perkin–Elmer Model 457 spectrophotometer. Ascending t.l.c. was performed on plates coated with a 0.25-mm layer of silica gel G (Merck, Darmstadt) and the spray reagent was potassium permanganate and sulfuric acid[21]. Column chromatography was perfor-

med on silica gel Bio-SIL A (100–200 mesh) (Bio-Rad Laboratories), which was used without pretreatment. Paper chromatography was effected on Whatman No. 3 paper and spots were detected with ammoniacal silver nitrate method[22].

G.l.c. was performed with a Bendix Gas chromatograph, model 2500, having a 6-foot column of chromosorb W.H.P. (100–120 mesh coated with 5% OV-1), temperature-programmed for a rise of 10° per min from 220 to 280°. $T_s$ refers to the retention time of a compound relative to that of per-*O*-(trimethylsilyl)sucrose.

*6-O-[2-O-Benzyl-3,4-di-O-(p-nitrobenzoyl)-α-L-fucopyranosyl]-1,2,3-tri-O-benzoyl-α-D-galactose* (**2**). — The starting material (**2**) was obtained from 1,2,3-tri-*O*-benzoyl-4,6-*O*-benzylidene-α-D-galactose [m.p. 171–172°, n.m.r. data: $\tau$ 3.05 (doublet, *J* 2.5 Hz, H-1α)] as described by Gros and Deulofeu[19]. Our material had m.p. 74–76°, $[\alpha]_D^{23}$ +237° (*c* 1, chloroform); n.m.r. doublet at $\tau$ 3.1 (*J* 2.5 Hz, H-1α)[23]; lit.[19] m.p. 75–78°, $[\alpha]_D^{25}$ +237.1° (*c* 1.4, chloroform).

A solution of **2** (0.7 g) in 1:1 nitromethane–benzene (80 ml) was boiled until approximately 25 ml of the solvent mixture had distilled off, to ensure complete removal of any moisture, and then cooled to room temperature. Mercuric cyanide (0.6 g) and bromide **1** (0.84 g in 8 ml of anhydrous dichloromethane) were added and the contents were stirred for 48 h. Additional amounts of mercuric cyanide (0.64 g) and bromide **1** (0.64 g) were added, and the reaction mixture was stirred for another 24 h. The mixture was diluted with benzene (200 ml) and washed with a cold, saturated solution of sodium hydrogen carbonate, and then water. It was dried over anhydrous sodium sulfate, filtered, and the filtrate evaporated to a syrup (1.8 g). The syrup (1.6 g) in benzene was chromatographed on a silica gel column (65 × 2.5 cm) that was eluted with benzene (150 ml) followed by 49:1 benzene–methanol (800 ml). Early fractions having $R_2$ 4.48 were combined and evaporated to give pure **3** (0.2 g) as syrup $[\alpha]_D^{23}$ −70.6° (*c* 1, chloroform); $\nu_{max}^{film}$ 3500 (OH), 1740 (ester), 1600, 1495, 710 (phenyl), 1530, and 1280 $cm^{-1}$ (nitro); n.m.r. data: $\tau$ 1.75–2.15 (multiplet, 8 H, *p*-nitrobenzoate) 2.3, 2.4, 2.52 (15 H, 3 Bz) 2.7 (5 H, Ph) 3.1 (doublet *J* 2.5 Hz, H-1α)[23] and 8.8 (doublet, *J* 6.5 Hz, 3 H, CH–$CH_3$).

*Anal.* Calc. for $C_{54}H_{46}N_2O_{19}$: C, 63.15; H, 4.15; N, 2.72. Found: C, 63.13; H, 4.42; N, 3.01.

Later fractions rich in compound **3** but contaminated with a slower-moving compound were pooled and evaporated to give a syrup (1.1 g) that was deacylated without further purification.

*6-O-(2-O-Benzyl-α-L-fucopyranosyl)-D-galactose* (**4**). — The foregoing partially purified syrup (1 g) was dissolved in chloroform (10 ml) and diluted with anhydrous methanol (60 ml). The solution was cooled and treated with catalytic amount of sodium methoxide. The contents were kept for 24 h at 4°, neutralized with dilute acetic acid, and evaporated. The residue obtained was shaken with water and ether. The aqueous layer was separated, extracted with ether, and then stirred with Dowex 50W-X8 ($H^+$) ion-exchange resin for 30 min and filtered. The filtrate was evaporated to give a syrup. T.l.c. indicated the presence of 2-*O*-benzylfucose (minor) and a

disaccharide. The mixture was taken up in 65:15:2 chloroform–methanol–water and chromatographed on a column (20 × 25 cm) of silica gel equilibrated with the same solvent. Early fractions (130 ml) gave 2-*O*-benzylfucose. The next fraction (60 ml) did not elute the desired material. The following fraction (200 ml) was rich in disaccharide and was evaporated to a syrup, tituration of which with ethanol–ether gave amorphous **4** (0.25 g, 45% from **2**); $[\alpha]_D^{25}$ −64.4° (*c*, 0.5, 50% ethanol). The purity of **4** was confirmed by t.l.c.: (*a*) $R_{Fuc}$ 1.75 in 65:15:2 chloroform–methanol–water; (*b*) $R_{Fuc}$ 1.47 in 13:6:1 chloroform–methanol–water; and (*c*) $R_{Fuc}$ 1.23 in 7:5:2 1-propanol–ethyl acetate–water; $\nu_{max}$ 3330–3410 (OH) and 745, 700 $cm^{-1}$ (phenyl).

*Anal.* Calc. for $C_{19}H_{28}O_{10}$: C, 54.80; H, 6.78. Found: C, 54.50; H, 7.05.

Pure compound **3** (100 mg), when deacylated as described, gave 6-*O*-(2-*O*-benzyl-α-L-fucopyranosyl)-D-galactose, isolated amorphous without chromatography on a column of silica gel.

*6-O-α-L-Fucopyranosyl-D-galactose* (**5**). — The foregoing compound **4** (120 mg) in 95% ethanol (120 ml) was shaken for 48 h at room temperature with 10% palladium-on-charcoal (50 mg) and hydrogen at pressure of 50 $lb.in^{-2}$. The catalyst was removed by filtration. The filtrate was evaporated to a syrup that was taken up in 13:6:1 chloroform–methanol–water and chromatographed on a column (10 × 2.2 cm) of silica gel to remove traces of compound **4**. The compound was eluted with the same solvent and fractions rich in disaccharide **5** were pooled. Evaporation gave a residue from which abs. ethanol was evaporated to give the desired, amorphous disaccharide **5** (80 mg, 80%), $[\alpha]_D^{25}$ −61.1° (*c* 1, water) (lit.[6] $[\alpha]_D$ −25°, 6-*O*-β-L-fucopyranosyl-D-galactose $[\alpha]_D^{25}$ +28.0°).

*Anal.* Calc. for $C_{12}H_{22}O_{10}$: C, 44.17; H, 6.79. Found: C, 44.07; H, 6.80.

The pure disaccharide **5** was dissolved in excess aqueous sodium metaperiodate and the periodate uptake was measured with a Gilford spectrophotometer at 222.5 $\mu m$[24]. After 24 h, 5.2 moles per mole of periodate had been consumed and no formaldehyde was detected[25]. Melibiose and methyl α-D-glucopyranoside used as reference compounds consumed 5.1 and 2 moles per mole of periodate, respectively, and no formaldehyde was liberated. Paper chromatography of the disaccharide showed a single spot having $R_{Fuc}$ 0.52 (10:4:3 ethyl acetate–pyridine–water), $R_{Fuc}$ 0.46 (6:4:3 1-butanol–pyridine–water); t.l.c. data: $R_{Fuc}$ 0.35 and $R_{Fuc}$ 0.56, respectively, in solvents (*b*) and (*c*) described in the preparation of **4**. These chromatographic techniques failed to differentiate the disaccharide from its β anomer[12]. The disaccharide (1 mg) in water (1 ml) was treated with sodium borohydride (5 mg). The solution was kept for 24 h at room temperature and then neutralized with dilute acetic acid (25%) and passed through a column of Dowex X-8 ($H^+$). The column was eluted with 7% aqueous ethanol (8 ml) and the eluate evaporated in *vacuo*. Methanol was evaporated several times from the residue in order to ensure complete removal of boric acid. The dry residue was converted into the per-*O*-(trimethylsilyl) derivative by the usual method[15]. G.l.c. of the derivative from pure **5** showed a single peak having $T_s$ 1.19, which was clearly separated from that of the derivative obtained from the β anomer ($T_s$ 1.28).

The foregoing compound **4** (obtained as a syrup without trituration with ethanol–ether), after hydrogenolysis followed by chromatography was reduced with sodium borohydride and converted into the per(trimethylsilyl) ether. G.l.c. showed two peaks having $T_s$ 1.19 (95%, $\alpha$ anomer) and $T_s$ 1.28 (5%, $\beta$ anomer).

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