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STUDIES ON THE KOENIGS–KNORR REACTION

PART V. SYNTHESIS OF 2-ACETAMIDO-2-DEOXY-3-*O*-α-L-FUCOPYRANOSYL-α-D-GLUCOSE

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ABSTRACT

Optically pure 2-acetamido-2-deoxy-3-*O*-α-L-fucopyranosyl-α-D-glucose was synthesized by the Koenigs–Knorr reaction of 2-*O*-benzyl-3,4-di-*O*-*p*-nitrobenzoyl-α-L-fucopyranosyl bromide with benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside. Reaction of 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl bromide gave the β-L-fucopyranosyl anomer. In contrast to the stereospecificity shown in this reaction by these two bromides, 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide afforded a mixture of α-L and β-L anomers in almost equimolar proportions. The disaccharides synthesized were crystallized and characterized, and their optical purity demonstrated by g.l.c. of the per(trimethylsilyl) ethers of the corresponding alditols.

INTRODUCTION

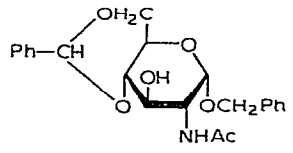
The synthesis of α-(D or L)-linked disaccharides from 2-*O*-benzyl substituted bromides and the effect of substituent groups in the bromides on the stereochemical course of the Koenigs–Knorr reaction have been discussed in previous papers of this series[1–4]. It was considered of interest to extend our investigations to include reactions of L-fucopyranosyl bromides with secondary hydroxyl groups of hexopyranosides.

The disaccharide 2-acetamido-2-deoxy-3-*O*-α-L-fucopyranosyl-D-glucose has been shown to be a part of the oligosaccharide chains of A, B, H, and Le blood-group substances[5,6], and an enzyme that catalyzes the transfer of L-fucose from GDP-L-fucose to the *O*-3 of a 2-acetamido-2-deoxy-D-glucose residue has been isolated from human blood[7]. The β-L anomer has been synthesized[8], but no determination was made of the optical purity of the initial product of condensation prior to purification by crystallization. Thus, no conclusive information is available on the stereospecificity of the reaction between 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl bromide and the secondary hydroxyl group at C-3 of the protected 2-acetamido-2-deoxy-D-glucose derivative.

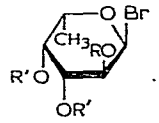
RESULTS AND DISCUSSION

Reaction of benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside[9] (**1**) with 2-*O*-benzyl-3,4-di-*O*-*p*-nitrobenzoyl-α-L-fucopyranosyl bromide[3] (**2**)

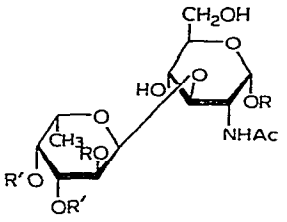
under Koenigs–Knorr conditions was completely stereospecific. The product obtained was not further purified, but treated directly with dilute acid and purified by column chromatography to give a disaccharide fraction isolated in 45% overall yield from **1** as a syrup (**5**). Catalytic deacylation afforded a solid in quantitative yield, a portion of which crystallized from ethanol. The crude product was hydrogenolized, and the free disaccharide obtained converted directly into the per(trimethylsilyl) ether of the corresponding glycitol, as described previously[1], care being taken during the sodium borohydride reduction to avoid alkaline conditions, under which 3-*O*-substituted disaccharides are hydrolyzed[10]. G.l.c. of the ether showed that it was homogeneous, a single peak ($T_{sucrose}$ 1.68) being detected. A portion of the free disaccharide was crystallized, and the analytically pure product (**7**) showed a high, negative optical rotation.



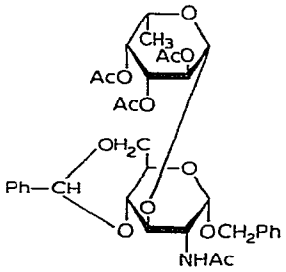
1



2 R=CH_2Ph; R′=$OCC_6H_4NO_2$-*p*
3 R=R′=Ac
4 R=R′=CH_2Ph



5 R=CH_2Ph; R′=$OCC_6H_4NO_2$-*p*
6 R=CH_2Ph; R′=H
7 R=R′=H
8 R=R′=CH_2Ph



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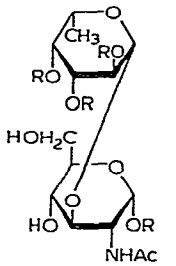

10 R=H
11 R=CH_2Ph

Reaction of **1** with 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl bromide[11] (**3**) afforded a disaccharide, a portion of which was crystallized. The protective substituents of the chromatographically homogeneous material, which had not been crystallized and contained all disaccharides obtained in the reaction, were split off and the product obtained was converted into the per(trimethylsilyl) ether of the glycitol. G.l.c. showed a single peak ($T_{sucrose}$ 1.90), clearly different from that shown by the derivative of **7**. Crystalline **10** had the same m.p. as that reported previously for the β-L linked disaccharide[8], and exhibited a low, positive optical rotation. The disaccharides **7** and

10 are anomers, the one with the negative rotation (**7**) having the α-L-fucopyranosyl configuration.

Koenigs–Knorr reaction of **1** with 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide[2] (**4**), followed by debenzylidenation, afforded a disaccharide in approximately 40% yield. In previous syntheses of similar derivatives[2–4], we have been unable to separate anomers on silica gel columns. In this particular case, however, the debenzylidenated condensation product was separated by silica gel chromatography into two disaccharide fractions, which were in almost equimolar amounts and were separately crystallized and characterized. Their optical purity and anomeric configuration were established by removal of the substituents, reduction, and g.l.c. of the per(trimethylsilyl) ethers of the resulting glycitols. One of the trimethylsilyl ethers obtained was identical with that prepared from **7** and the other with that from **10**.

It would appear that the stereochemistry of the Koenigs–Knorr reaction is critically influenced by the nature of the protecting groups of the reacting bromide. Both **2** and **3** gave complete stereospecificity of reaction, the former producing an α-L-linked and the latter a β-L-linked disaccharide. On the other hand, **4** afforded a mixture of anomers with little stereoselectivity being shown. The results are in general agreement with those that we have obtained for other synthetic fucopyranosyl disaccharides[2–4]. It is not clear whether **3** and 2-*O*-acetyl-3,4-di-*O*-benzyl-α-L-fucopyranosyl bromide (which gave 25% of 2-acetamido-2-deoxy-6-*O*-α-L-fucopyranosyl-D-glucose) react fundamentally by different mechanisms or whether the variation in anomeric composition is due to the different hydroxyl groups involved. A change in nucleophile can, indeed, affect the configuration of the product, as illustrated[11–13] by the preparation of α-L-fucopyranosyl-(1→2)- and -(1→4)-linked compounds from **3**. Further work is required before definite predictions of the configuration of the interglycosidic linkage are possible. Results so far do, however, point to **2** as the most useful glycosyl bromide hitherto described for the synthesis of α-L-fucopyranosides.

EXPERIMENTAL

For General Methods, see Ref. 1.

*Benzyl 2-acetamido-3-*O-(*2-*O*-benzyl-3,4-di-*O-p*-nitrobenzoyl-α-L-fucopyranosyl)-2-deoxy-α-D-glucopyranoside* (**5**). — A solution of benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside[9] (**1**, 2.0 g, 5.0 mmoles) in 1:1 (v/v) nitromethane–benzene (100 ml) was concentrated until approximately 20 ml of the solvent mixture had distilled and then cooled to 40°. Mercuric cyanide (1.3 g, 5.0 mmoles) and 2-*O*-benzyl-3,4-di-*O*-*p*-nitrobenzoyl-α-L-fucopyranosyl bromide[3] (**2**, 3.2 g, 5.0 mmoles) were added, and the reaction mixture was stirred for 48 h at 40°, a further addition of **2** (1.6 g, 2.5 mmoles) being made after 24 h. The mixture was diluted with benzene, washed successively with sodium hydrogen carbonate solution and water, dried (calcium chloride), and evaporated *in vacuo*. The residue was suspended in *p*-dioxane (100 ml), and 0.5M sulfuric acid (40 ml) was added. The mixture was stirred for 1 h at

100° and, after being cooled, diluted with chloroform. The organic layer was washed with sodium hydrogen carbonate solution and water, dried, and evaporated *in vacuo*. The residue was dissolved in benzene and chromatographed on a column of silica gel. A homogeneous fraction (t.l.c.) was eluted with 1:2 (v/v) benzene–ethyl acetate, and evaporation *in vacuo* afforded a syrup (1.9 g, 45% from **1**); $[\alpha]_D^{23}$ −101° (*c* 1.09, chloroform); n.m.r. data: τ 1.85–2.12 (m, 8 H, 2 *p*-nitrobenzoate groups), 2.75 and 2.84 (10 H, 2 Ph), 8.4 (3 H, NAc), and 8.78 (d, *J* 6.5 Hz, 3 H, CH-Me). No other disaccharide material was obtained.

Anal. Calc. for $C_{42}H_{43}N_3O_{16}$: C, 59.64; H, 5.12. Found: C, 59.71; H, 5.39.

Benzyl 2-acetamido-3-O-(2-O-benzyl-α-L-fucopyranosyl)-2-deoxy-α-D-glucopyranoside (**6**). — Compound **5** (1.5 g) was dissolved in methanol (50 ml) containing a catalytic amount of sodium methoxide. The solution was kept overnight at room temperature, neutralized with acetic acid, and evaporated *in vacuo*. The material was purified by column chromatography on silica gel. 2-Propanol–water (7:3, v/v) eluted fractions that were homogeneous on t.l.c. Evaporation of the solvent *in vacuo* afforded a solid (0.92 g, 95%), a portion of which was crystallized from absolute ethanol to give **6**, m.p. 224–226°; $[\alpha]_D^{23}$ +37° (*c* 1.16, methanol); n.m.r. data [dimethyl sulfoxide-d_6]: τ 2.72 (10 H, 2 Ph), 8.28 (3 H, NAc), and 8.92 (d, *J* 6.5 Hz, 3 H, CH-Me).

Anal. Calc. for $C_{28}H_{37}NO_{10}$: C, 61.41; H, 6.81. Found: C, 61.18; H, 6.66.

2-Acetamido-2-deoxy-3-O-α-L-fucopyranosyl-α-D-glucose (**7**). — Crude **6** (0.50 g) was dissolved in 90% ethanol (100 ml, containing a drop of acetic acid), and 10% palladium-on-charcoal (50 mg) was added. The mixture was shaken with hydrogen at 3.5 atm for 48 h at room temperature, the catalyst removed by filtration, and the solvent evaporated *in vacuo*. The residue was dissolved in 3:3:1 (v/v) ethyl acetate–2-propanol–water and the solution chromatographed on silica gel. Earlier fractions contained apparently incompletely hydrogenolyzed material and were not investigated further. Later, combined fractions eluted from the column gave 0.27 g (81%) of material, which was homogeneous on t.l.c. in 3:3:2 (v/v) ethyl acetate–2-propanol–water, 13:6:1 (v/v) chloroform–methanol–water, and 9:1 (v/v) acetone–methanol. The solid product obtained was crystallized from ethanol–methanol–water to give **7**, m.p. 218–220° (dec.); $[\alpha]_D^{23}$ −60→−74° (*c* 0.83, water).

Anal. Calc. for $C_{14}H_{25}NO_{10}\cdot H_2O$: C, 43.63; H, 7.06; N, 3.63. Found: C, 43.52; H, 6.88; N, 3.80.

A portion of crude **7** (10 mg), dissolved in water (5 ml), was reduced with sodium borohydride (50 mg added portionwise over 3 h) in the presence of boric acid (100 mg). The solution was kept overnight at room temperature, neutralized with glacial acetic acid, treated with Amberlite IR-120 (H^+) ion-exchange resin, and evaporated *in vacuo*. Methanol was added and evaporated several times to remove the borate ions. The disaccharide glycitol was dried and converted into the per(trimethylsilyl) ether; g.l.c. showed one sharp peak having $T_{sucrose}$ 1.68.

Benzyl 2-acetamido-4,6-O-benzylidene-2-deoxy-3-O-(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-α-D-glucopyranoside (**9**). — A solution of **1** (1.5 g, 3.8 mmoles) in 1:1 (v/v)

benzene–nitromethane (150 ml) was concentrated until 30 ml of the solvent mixture had distilled and then cooled to room temperature. Mercuric cyanide (0.9 g, 3.5 mmoles) and a solution of 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl bromide[11] (**3**, 2.4 g, 3.8 mmoles) in dichloromethane (20 ml) were added, and the reaction mixture was stirred for 24 h, a further addition of **3** (1.2 g) being made after 12 h. The solution was diluted with dichloromethane, washed successively with cold sodium hydrogen carbonate solution and water, dried (calcium chloride), and evaporated *in vacuo*. The residue was dissolved in benzene and chromatographed on silica gel. Fractions that were eluted with 1:1 (v/v) benzene–ether and were identical and homogeneous on t.l.c. were combined to give a solid (**9**, 1.1 g, 43%). A portion was crystallized from acetone–petroleum ether, m.p. 202–204°; $[\alpha]_D^{25}$ +68° (*c*, 1.02, chloroform); n.m.r. data: τ 2.88 and 2.92 (10 H, 2 Ph), 7.84 (3 H, *ax.* OAc), 8.04 (6 H, 2 *eq.* OAc), 8.24 (3 H, NAc), and 8.78 (d, *J* 6.5 Hz, 3 H, CH-Me). No other disaccharide material was obtained.

Anal. Calc. for $C_{34}H_{41}NO_{13}$: C, 60.79; H, 6.15. Found: C, 60.59; H, 6.05.

2-Acetamido-2-deoxy-3-O-β-L-fucopyranosyl-α-D-glucose (**10**). — A portion of **9** (0.50 g) was dissolved in acetone (10 ml), and 1% sulfuric acid (w/v, 5 ml) was added. The clear solution was kept for 6 h at 60°, the reaction being monitored by t.l.c. The reaction mixture was diluted with chloroform and washed with a saturated solution of sodium hydrogen carbonate and water, dried with calcium chloride, and evaporated *in vacuo*. The residue was dissolved in methanol (20 ml) containing a catalytic amount of sodium methoxide. The solution was kept overnight at room temperature, treated batchwise with Amberlite IR 120 (H^+) ion-exchange resin, filtered, and the filtrate evaporated *in vacuo*. The residue was hydrogenolyzed as described previously and, after hydrogenolysis, purified by column chromatography on silica gel. The disaccharide (0.18 g, 67%) was indistinguishable from **7** on t.l.c. in the solvent systems previously specified. Crystallization from ethanol–ether gave needles, m.p. 148–150°; $[\alpha]_D^{23}$ +8 → +1° (*c* 1,1, water); lit.[8]: m.p. 148–150°; $[\alpha]_D^{20}$ +25 → +11° (*c* 0.90, 60% methanol). The per(trimethylsilyl) ether of the glycitol showed on g.l.c., a single peak having $T_{sucrose}$ 1.90.

Benzyl 2-acetamido-2-deoxy-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-α-D-glucopyranoside (**8**) *and benzyl 2-acetamido-2-deoxy-3-O-(2,3,4-tri-O-benzyl-β-L-fucopyranosyl)-α-D-glucopyranoside* (**11**). — Reaction of compound **1** (2.0 g, 5.0 mmoles) with 2,3,4 tri-*O*-benzyl-α-L-fucopyranosyl bromide[2] (**4**, 3.8 g, 7.5 mmoles) in the presence of mercuric cyanide (1.3 g, 5.0 mmoles) as described for the preparation of **5**, followed by the same processing and procedure of partial hydrolysis, afforded a syrup which was dissolved in 1:1 (v/v) dichloromethane–ether and chromatographed on silica gel. Two homogeneous disaccharide components were eluted from the column. The slower-migrating one (0.72 g, 20% from **1**) crystallized from acetone–ether to give **8**, m.p. 178–180°; $[\alpha]_D^{25}$ +26.4° (*c* 1.0, chloroform); n.m.r. data: τ 2.65 and 2.68 (20 H, 4 Ph), 8.62 (3 H, NAc), and 8.86 (d, *J* 6.5 Hz, 3 H, CH-Me).

Anal. Calc. for $C_{42}H_{49}NO_{10}$: C, 69.31; H, 6.79. Found: C, 69.03; H, 6.34.

Hydrogenolysis of a portion of **8** in the usual fashion afforded a material

indistinguishable from **7** (t.l.c., m.p., and optical rotation). The per(trimethylsilyl) ether of the glycitol showed, on g.l.c., one sharp peak having $T_{sucrose}$ 1.68.

The second disaccharide obtained (0.66 g, 18% from **1**) was crystallized from acetone–methanol to give **11**, m.p. 200–202°; $[\alpha]_D^{25}$ +66° (*c* 1.08, chloroform); n.m.r. data: τ 2.68 (20 H, 4 Ph), 8.14 (3 H, NAc), and 8.80 (d, *J* 6.5 Hz, 3 H, CH-Me).

Anal. Calc. for $C_{42}H_{49}NO_{10}$: C, 69.31; H, 6.79. Found: C, 68.91; H, 6.65.

A portion of **11** was hydrogenolyzed, as described previously, and the disaccharide obtained after purification by column chromatography was identical with **10** (t.l.c., optical rotation). The per(trimethylsilyl) ether of the glycitol showed, on g.l.c., one sharp peak having $T_{sucrose}$ 1.90.

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