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Note

Synthesis of 2-acetamido-2,4-dideoxy-4-fluoro-3-*O*-β-D-galactopyranosyl-D-glucopyranose. A potential specific substrate for (1→2)-α-L-fucosyltransferase*

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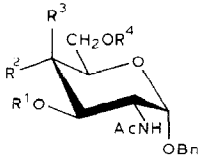
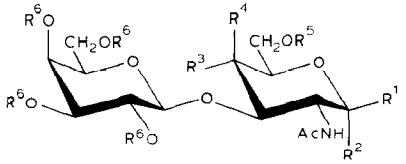
In a preceding communication in this series[1], we described the syntheses of some partially fluorinated, mucin-type oligosaccharide fragments. Such compounds were required as modified substrates for studies related to glucosyltransferases. We have also previously demonstrated that *N*-acetyl-2′-*O*-methyllactosamine could act as a specific acceptor for (1→3)-α-L-fucosyltransferase because it lacks a free OH-2 on its D-galactopyranosyl group[2]. By a similar token, 2-acetamido-2,4-dideoxy-4-fluoro-3-*O*-β-D-galactopyranosyl-D-glucopyranose (**8**) could be anticipated to act as a specific acceptor for (1→2)-α-L-fucosyltransferase. It is noteworthy that the parent disaccharide, β-D-Gal*p*-(1→3)-D-GlcNAc, can act as an acceptor for both (1→2)- and (1→4)-α-L-fucosyltransferases[3]. In continuation of these investigations, we now describe the synthesis of **8**.





1 R^1 = All, R^2 = H, R^3, R^4 = PhCH
2 R^1 = All, R^2 = H, R^3 = OH, R^4 = Bn
3 R^1 = All, R^2 = F, R^3 = H, R^4 = Bn
4 R^1 = R^3 = H, R^2 = F, R^4 = Bn

5 R^1 = R^3 = H, R^2 = OBn, R^4 = OH, R^5 = Bn, R^6 = Ac
6 R^1 = R^4 = H, R^2 = OBn, R^3 = F, R^5 = Bn, R^6 = Ac
7 R^1 = R^4 = R^6 = H, R^2 = OBn, R^3 = F, R^5 = Bn
8 R^1, R^2 = OH, H, R^3 = F, R^4 = R^5 = R^6 = H

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Two procedues were equally feasible. In the first, benzyl 2-acetamido-6-*O*-benzyl-2,4-dideoxy-4-fluoro-α-D-galactopyranoside (**4**) was required as a glycosyl acceptor. It was obtained in three steps from benzyl 2-acetamido-3-*O*-allyl-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside (**1**; ref. 4). Reductive ring-opening [5] of the benzylidene group of **1**, in acidic medium and in the presence of sodium cyanoborohydride, followed by column chromatographic purification on silica gel, afforded crystalline **2** in 48% yield. Treatment of **2** with diethylaminosulfur trifluoride under conditions similar to those previously described[6], and purification of the crude product mixture by column chromatography gave, in 49% yield, benzyl 2-acetamido-3-*O*-allyl-6-*O*-benzyl-2,4-dideoxy-4-fluoro-α-D-glucopyranoside (**3**). The ^{1}H-n.m.r. spectra of both **2** and **3** contained signals in support of their overall structures. Removal of the allyl group of **3** was readily accomplished with palladium chloride–sodium acetate in acetic acid–water[7] to furnish, in 71% yield, benzyl 2-acetamido-6-*O*-benzyl-2,4-dideoxy-4-fluoro-α-D-glucopyranoside (**4**).

Glycosylation of **4** with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide in benzene in the presence of mercuric cyanide gave, after column chromatographic purification, the disaccharide derivative **6** in 75% yield. Compound **6** was also obtained by reaction of DAST with benzyl 2-acetamido-6-*O*-benzyl-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-galactopyranoside (**5**). *O*-Deacetylation of **6** with Amberlyst A-26 (OH^-) anion-exchange resin in methanol[8] gave, in 78% yield, amorphous **7**. Hydrogenolysis of the benzyl groups of **7** in glacial acetic acid and in the presence of 10% palladium-on-charcoal, followed by column chromatography, furnished **8** in 82% yield.

EXPERIMENTAL

General methods. — Melting points were determined with a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured at 25–27° with a Perkin–Elmer 241 polarimeter. T.l.c. was conducted on aluminium sheets, precoated with 0.2 mm layers of Silica Gel 60F-254 (E. Merck Darmstadt, Germany); the components were located either by exposure to u.v. light, or by spraying the plates with 5% H_2SO_4 in ethanol and heating. Silica gel used for column chromatography was Baker Analyzed (60–200 mesh). The following solvent systems (v/v) were used for chromatography: (*A*) 1:1 chloroform–acetone, (*B*) 2:1 chloroform–acetone, and (*C*) 13:6:1 chloroform–methanol–water. N.m.r. spectra were recorded at ~25°; ^{1}H-n.m.r. spectra with a Varian EM-390, and ^{19}F-n.m.r. spectra with a Varian XL-100 instrument at 90 and 94 MHz, respectively; the positions of the peaks (δ or ϕ) are expressed from the Me_4Si or $CFCl_3$ signals, respectively. Organic solutions were generally dried with anhydrous Na_2SO_4. Elemental analyses were performed by Robertson Laboratory, Florham Park, New Yersey, U.S.A.

*Benzyl 2-acetamido-3-*O*-allyl-6-*O*-benzyl-2-deoxy-α-D-galactopyranoside* (**2**). — A mixture of **1** (ref. 4; 4.3 g, 9.8 mmol) and sodium cyanoborohydride (5.5 g, 88.1 mmol) in dry oxolane (135 mL) containing molecular sieves 4A (20 g) was cooled (0°,

bath) and stirred. A saturated solution of HCl in diethyl ether (~60 mL) was added dropwise with stirring until evolution of gas ceased, and the solution became acidic (pH paper). After 1.5 h at 0°, when t.l.c. (2:1 chloroform–acetone) indicated complete reaction, the mixture was filtered (a bed of glass wool) into ice–water (1 L), and extracted with chloroform (3 × 300 mL). The combined extracts were successively washed with water, saturated aqueous $NaHCO_3$, and water, dried, and evaporated to give a yellowish syrup, which was applied to a column of silica gel. Elution with 5:1 (v/v) chloroform–acetone and then with solvent *B*, followed by evaporation of the fractions corresponding to the product, gave a solid which crystallized from dichloromethane–ether–hexane to afford **2** (2.1 g, 48%), m.p. 119–121°, $[\alpha]_D^{27}$ +103° (*c* 1.0, 1:1 chloroform–methanol); ^{1}H-n.m.r. ($CDCl_3$): δ 7.35–7.20 (m, 10 H, arom.), 6.00–5.50 (m, 1 H, $CH=CH_2$), and 1.90 (s, 3 H, NAc).

Anal. Calc. for $C_{25}H_{31}NO_6$: C, 68.01; H, 7.08; N, 3.17. Found: C, 67.81; H, 7.16; N, 2.99.

Benzyl 2-acetamido-3-O-allyl-6-O-benzyl-2,4-dideoxy-4-fluoro-α-D-glucopyranoside (**3**). — A solution of **2** (1.6 g, 3.7 mmol) in dry 2-methoxyethyl ether (Diglyme, 30 mL) was slowly introduced into a cold (−10°, bath) and stirred solution of diethylaminosulfur trifluoride (2.1 g, 12.9 mmol) in Diglyme (30 mL). The mixture was allowed to gradually warm to room temperature, and stirring was continued for an additional 45 min at room temperature. T.l.c. (1:1 chloroform–ethyl acetate) revealed the disappearance of **2** and the presence of a major product, faster-migrating than **2**. The mixture was then cooled (−10°, bath) and methanol (40 mL) was cautiously added to destroy excess reagent. It was then evaporated to a yellowish syrup which was subjected to column chromatography with 3:1 (v/v) chloroform–ethyl acetate as the eluant to give a solid which crystallized from dichloromethane–ether–hexane to give **3** (0.8 g, 49%), m.p. 156–157°, $[\alpha]_D^{26.5}$ +109° (*c* 0.9, chloroform); ^{19}F-n.m.r. ($CDCl_3$): φ −190.5 (dd, *J* ~50 and 15 Hz); ^{1}H-n.m.r. ($CDCl_3$): δ 7.35–7.20 (m, 10 H, arom.), 6.00–5.50 (m, 1 H, $CH=CH_2$), and 1.90 (s, 3 H, NAc).

Anal. Calc. for $C_{25}H_{30}FNO_5$: C, 67.70; H, 6.82; N, 3.16. Found: C, 67.46; H, 6.61; H, 2.95.

Benzyl 2-acetamido-6-O-benzyl-2,4-dideoxy-4-fluoro-α-D-glucopyranoside (**2**). — A mixture of **3** (0.7 g, 1.58 mmol), sodium acetate (0.32 g, 3.78 mmol), and $PdCl_2$ (0.3 g, 1.65 mmol) in acetic acid (6 mL) and water (0.3 mL) was stirred overnight at room temperature. T.l.c. (solvent *A*) revealed the disappearance of **3** and the presence of a major product slower-migrating than **3**. The mixture was then evaporated *in vacuo* (<30°), and several portions of toluene were added to, and evaporated from the residue, which was taken up in chloroform (100 mL) and successively washed with water, saturated aqueous $NaHCO_3$, and water, dried, and evaporated. The residue was applied to a column of silica gel and eluted with solvent *B*. Evaporation of the fractions corresponding to the product, followed by crystallization of the residue from dichloromethane–ether, afforded **4** (0.45 g, 71%), m.p. 152–154°, $[\alpha]_D^{25}$ +88° (*c* 0.7, chloroform); ^{1}H-n.m.r. (1:1 $CDCl_3$–CD_3OD): δ 7.20–7.35 (m, 10 H, arom.) and 1.90 (s, 3 H, NAc).

Anal. Calc. for $C_{22}H_{26}FNO_5$: C, 65.49; H, 6.50; N, 3.47. Found: C, 65.43; H, 6.40; N, 3.26.

Benzyl 2-acetamido-6-O-benzyl-2,4-dideoxy-4-fluoro-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-glucopyranoside (**6**). *Method (a).* A stirred solution of **4** (0.4 g, 1 mmol) in dry benzene (80 mL) was boiled until 30 mL of the solvent had distilled off. It was then cooled to room temperature, $Hg(CN)_2$ (0.5 g, 2 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (0.82 g, 2 mmol) were added, stirring was continued for 5 h at room temperature, more portions of $Hg(CN)_2$ (0.25 g, 1 mmol) and galactosyl bromide (0.4 g, 1 mmol) were added, and stirring was continued for 16 h at room temperature. After processing in the usual manner, t.l.c. (solvent *A*) showed the disappearance of **4** and the presence of a major product, faster-migrating than **4**; some slower-migrating contaminants (presumably due to the decomposition of the galactosyl bromide) were also revealed in t.l.c. The crude mixture was applied to a column of silica gel and eluted with 1:1 (v/v) ethyl acetate–hexane. Evaporation of the fractions corresponding to the major product gave a solid residue which crystallized from ether–hexane to afford **6** (0.55 g, 75%), m.p. 78–81°, $[\alpha]_D^{26}$ + 51° (*c* 0.7, chloroform); ^{19}F-n.m.r. ($CDCl_3$): ϕ − 190.8 (dd, $J \sim 49$ and 15 Hz); ^{1}H-n.m.r. ($CDCl_3$): δ 7.35–7.20 (m, 10 H, arom.) and 2.15–1.95 (s, 15 H, H OAc and NAc).

Anal. Calc. for $C_{36}H_{44}FNO_{14}$: C, 58.93; H, 6.04; N, 1.91. Found: C, 58.88; H, 6.23; N, 1.83.

Method (b). Compound **5** (ref. 4; 0.74 g, 1 mmol) was treated with diethylaminosulfur trifluoride (0.49 g, 3.5 mmol) in a manner analogous to that described for **3** (to give **4**). After the described processing, t.l.c. (solvent *B*) showed the disappearance of **5** and the presence of a major product having identical chromatographic mobility with a sample of **6** obtained by (*a*). The crude product was purified by column chromatography on silica gel with 3:1 (v/v) chloroform–acetone as the eluent to give **6** (0.4 g, 54%), m.p. 78–80°, undepressed by admixture with a sample from (*a*), $[\alpha]_D^{25}$ + 54° (*c* 0.3, chloroform).

Benzyl 2-acetamido-6-O-benzyl-2,4-dideoxy-4-fluoro-3-O-galactopyranosyl-α-D-glucopyranoside (**7**). — Compound **6** (0.5 g) in methanol (20 mL) containing Amberlyst A-26 (OH^-) anion-exchange resin was stirred for 4 h at room temperature. T.l.c. (solvent *C*) then showed the disappearance of **6** and the presence of a major product, slower-migrating than **6**. The resin was filtered off and washed with methanol, and the filtrate and washings were combined and evaporated to give a solid, which was dissolved in methanol. Addition of ether caused the precipitation of **7** (0.3 g, 78%), amorphous, $[\alpha]_D^{27}$ + 87° (*c* 2.0, methanol); ^{1}H-n.m.r. (CD_3OD): δ 7.20–7.35 (m, 10 H, arom.) and 1.95 (s, 3 H, NAc).

Anal. Calc. for $C_{28}H_{36}FNO_{10} \cdot 1.5\ H_2O$: C, 57.72; H, 6.74; N, 2.40. Found: C, 57.61; H, 6.60; N, 2.40.

2-Acetamido-2,4-dideoxy-4-fluoro-3-O-β-D-galactopyranosyl-D-glucopyranose (**8**). — A mixture of **7** (0.25 g) and 10% Pd–C (0.2 g) in glacial acetic acid (10 mL) was shaken under H_2 at ~345 kPa for 16 h at room temperature. The suspension was

filtered through a bed of Celite, the solid thoroughly washed with glacial acetic acid, and the filtrate and washings were combined and evaporated under reduced pressure. The residue was applied to a column of silica gel. Elution with solvent *C* and evaporation of the fraction corresponding to the product gave a solid which was dissolved in water. The solution was filtered and lyophilized to furnish **8** (0.14 g, 82%), amorphous, $[\alpha]_D^{27}$ +35° (*c* 1.2, methanol); ^{19}F-n.m.r. [(^{2}H)Me_2SO]: ϕ −190.4 (dd, $J \sim 51$ and 15 Hz).

Anal. Calc. for $C_{14}H_{24}FNO_{11} \cdot 1.5\ H_2O$: C, 40.77; H, 6.61; N, 3.40. Found: C, 40.69; H, 6.49; N, 3.09.

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