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FLUORINATED CARBOHYDRATES AS POTENTIAL PLASMA MEMBRANE MODIFIERS AND INHIBITORS. SYNTHESIS OF 2-ACETAMIDO-2,6-DIDEOXY-6-FLUORO-D-GALACTOSE*

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ABSTRACT

Reaction of benzyl 2-acetamido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-mesyl-α-D-galactopyranoside with cesium floride gave benzyl 2-acetamido-3,6-anhydro-4-*O*-benzyl-2-deoxy-α-D-galactopyranoside instead of the desired 6-fluoro derivative. Acetonation of benzyl 2-acetamido-2-deoxy-6-*O*-mesyl-α-D-galactopyranoside gave the corresponding 3,4-*O*-isopropylidene derivative. The 6-*O*-mesyl group was displaced by fluorine with cesium fluoride in boiling 1,2-ethanediol, and hydrolysis and subsequent *N*-acetylation gave the target compound. In another procedure, treatment of 2-acetamido-1,3,4-tri-*O*-acetyl-2-deoxy-α-D-galactose with *N*-(diethylamino)sulfur trifluoride gave 2-acetamido-1,3,4-tri-*O*-acetyl-2,6-dideoxy-6-fluoro-D-galactose which, on acid hydrolysis followed by *N*-acetylation, gave 2-acetamido-2,6-dideoxy-6-fluoro-D-galactose.

INTRODUCTION

Since hydrogen bonding is often involved with enzyme–substrate interaction, and fluorine and hydroxyl groups may form hydrogen bonds in the same fashion, fluorinated substrates or metabolites, or both, may bind especially to allosteric enzymes and exert some interesting metabolic effects[2]. This binding can be readily studied by ^{19}F-n.m.r. spectroscopy[3]. A number of interesting studies on carbohydrate transport in intestinal membranes have also been carried out with fluorosugars[4]. They have also been used in enzyme-specificity studies in connection with the development of potential antitumor agents[5]. Several D-hexose derivatives were found to possess significant antitumor activity[6–9]. Therefore, fluorinated sugars appear to be of interest both for the study of enzyme–substrate interactions and as potential antitumor agents.

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In membrane glycoconjugates, *N*-acetyl-*D*-galactosamine is glycosidically linked to proteins through serine and threonine residues, as in the case of mucin, fetuin, and glycophorin. This type of linkage between carbohydrate and protein is found also in the antifreeze glycoproteins. The linkages of this sugar to the other carbohydrates in the oligosacharide component of glycoproteins are at the C-3 or C-6. By substituting C-6 with fluorine, the fluoro sugar may act as a carbohydrate chain terminator in glycoconjugates.

RESULTS AND DISCUSSION

Since the attempted synthesis of 2-amino-2,6-dideoxy-6-fluoro-D-galactose using the method[10] developed to prepare 2-acetamido-2,6-dideoxy-6-fluoro-D-glucopyranose did not give the analogous 2-amino-2,6-dideoxy-6-fluoro-D-galactose derivative, two alternative methods have been developed to obtain our target compound.

Sulfonic esters of carbohydrates have been found to be very useful intermediates in the synthesis of aminosugars[11], thiosugars[12], and deoxysugars[13] because of the ease with which they undergo replacement reactions with a variety of nucleophiles[14,15]. Generally, the displacement reaction at C-6 of hexoses can be readily carried out without much complication. However, difficulties have been encountered during the displacement reaction of 6-sulfonic esters of galactopyranoside derivatives that have not been found with their corresponding glucopyranosides[16]. This has been explained by a consideration of the polar-field effect operating from the lone pair of electrons on the axial O-4 and the ring oxygen atom[15-18], and the polar repulsive forces in the transition state[15].

Benzyl 2-acetamido-2-deoxy-α-D-galactopyranoside (**1**) was prepared from 2-acetamido-2-deoxy-D-galactose according to a method[10] analogous to that used previously for the 2-acetamido-2-deoxy-D-glucose derivative[19]. After two crystallizations from 2-propanol, the product was found to be pure from β-D-anomer. Mesylation of **1** with one equivalent of methanesulfonyl chloride in pyridine at −45−−40° gave the 6-*O*-mesyl derivative **2**, along with a dimesyl derivative, in 5–10% yield. The mesyl derivatives were seperated by column chromatography. The remaining hydroxyl groups of **2** were protected by benzylation to give benzyl 2-acetamido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-mesyl-α-D-galactopyranoside (**5**). Formation of an analogous dimesyl derivative as in the case of benzyl 2-acetamido-2-deoxy-α-D-glucopyranoside was not observed[10]. Although OH-4 is axially oriented in benzyl 2-acetamido-2-deoxy-α-D-galactopyranoside, the additional mesylation occured at O-3 instead of O-4, to give the 3,6-di-*O*-mesyl derivative **3**. The same dimesyl derivative was also obtained by further mesylation of **2**, and this dimesyl derivative was benzylated to give (presumably) **4**. Benzyl 2-acetamido-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside[20] (**9**) was converted into its 4,6-di-*O*-mesyl derivative which was in turn converted into **6** by treatment with lithium benzoate in boiling *N,N*-dimethylformamide. After hydrolysis of the benzoate groups to give **7** and subsequent mesylation in the usual

way, benzyl 2-acetamido-3-*O*-benzyl-2-deoxy-4,6-di-*O*-mesyl-α-D-galactopyranoside (**8**) was obtained. It was found to differ from the dimesyl derivative **4** obtained by mesylation of benzyl 2-acetamido-2-deoxy-α-D-galactopyranoside **1** on the basis of m.p., i.r. and n.m.r. data. Thus the structure of **3** was attributed to the dimesyl derivative obtained by mesylation of **1**.

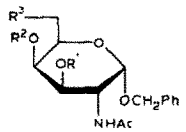
When the 6-*O*-mesyl derivative **5** was subjected to displacement reaction using cesium fluoride[21] in boiling 1,2-ethanediol, the anhydrosugar **11** was obtained as the only product instead of the desired fluoro derivative. Elemental analysis and ^{1}H-n.m.r. spectroscopy indicated the loss of a benzyloxy group. A similar type of anhydrosugar formation had been formed by solvolysis of methyl 2,3-di-*O*-dibenzyl-6-*O*-mesyl-β-D-galactopyranoside[22]. The benzyloxy group was shown to be a powerful anchimeric assistant in the ethanolysis of 4-*O*-benzyl-1-*p*-tolylsulfonylpentane-1,4-diol and other related compounds to give oxolane derivatives[23]. Neighboring-group participation by the 3-benzyloxy group of **5** is suggested to account for the formation of the anhydrosugar. Because of extensive overlap in the region δ 4.0–4.4 of the ^{1}H-n.m.r. spectrum of **11**, initial peak assignments were determined by adding europium (III) tris(1,1,1 2,2 3,3-heptafluoro-7,7-dimethyl-4,6-octadionate)[24] shift reagent to the (^{2}H) chloroform solution to a final molar ratio of (EuFOD) to **11** of 0.27. On the basis of spectral data, elemental analysis, and a previously reported analogous reaction, the structure of benzyl 2-acetamido-3,6-anhydro-2-deoxy-4-*O*-benzyl-α-D-galactopyranoside was assigned to **11**.

An alternative route to 2-amino-2,6-dideoxy-6-fluoro-D-galactose was the acetonation of **1** with acetone in presence of sulfuric acid to give, in a poor yield, 3,4-*O*-isopropylidene derivative **12**. Analogous attempts[25] to improve the yield of **12** failed. Acetonation of the 6-*O*-mesyl derivative **2** in the presence of sulfuric acid gave a very good yield of the 3,4-*O*-isopropylidene derivative **13**. When this compound was subjected to displacement reaction using cesium fluoride in boiling 1,2-ethanediol, benzyl 2-acetamido-2,6-dideoxy-6-fluoro-3,4,*O*-isopropylidene-α-D-galactopyranoside (**14**) was obtained in good yield. This was hydrolyzed to give **19**, isolated as the hydrochloride. Catalytic hydrogenolysis of **14** removed the glycosidic benzyl group as well as the 3,4-*O*-isopropylidene group to give **20** in very good yield, and subsequent acetylation yielded 2-acetamido-1,3,4-tri-*O*-acetyl-2,6-dideoxy-6-fluoro-D-galactopyranoside (**18**).

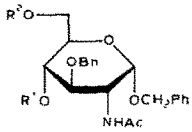
In another, more convenient approach, 2-acetamido-2-deoxy-α-D-galactopyranose was treated with chlorotriphenylmethane to give, in excellent yield, the 6-*O*-trityl derivative **15**, which was further acetylated into the corresponding tetraacetate **16**. Removal of the trityl group with hot aqueous acetic acid yielded 2-acetamido-1,3,4-tri-*O*-acetyl-2-deoxy-D-galactopyranose **17** in moderate yield. Alternatively, catalytic hydrogenolysis of **16** gave a very poor yield of **17**. Fluorination of **17** with (diethylamino)sulfur trifluoride[26] afforded **18** in 55% yield, and acid hydrolysis provided 2-amino-2,6-dideoxy-6-fluoro-D-galactose (**19**), as the hydrochloride, in excellent yield. Subsequently, **19** was *N*-acetylated to give **20**, the ^{13}C-n.m.r. spectrum of which showed that the introduction of fluorine deshielded

the C-6-signal from δ 62.2 (ref. 27) to 81.08 ($J_{C,F}$ 178,70 Hz).

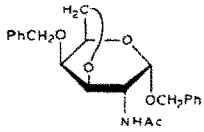
The compounds synthesized in this study were tested as inhibitors of growth of murine L1210 leukemia cells in culture. When the ID_{50} of a compound was greater than 10^{-3}M, the compound was designated as inactive. Fully acetylated **18** and the nonacetylated derivative **19** had ID_{50} values of 1×10^{-4} and 1×10^{-3}M, respectively, whereas the *N*-acetylated derivative **20** was found to be inactive. It was reported that *O*-acetylated sugars are more inhibitory than their *O*-deacetylated counterparts and that *O*-acetylation promotes uptake by passive diffusion[28]. The *N*-acetyl derivatives of aminosugars are only poorly taken-up by cells[9] and this factor most likely explains the inactivity of **20**. The non-fluorinated compound, 2-amino-2-deoxy-D-galactose, inhibited the growth of L1210 cells only by 26% at a 10^{-3}M concentration whereas its *N*-acetylated derivative was found inactive at this concentration.



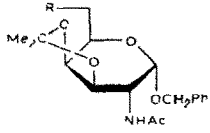
1 $R^1 = R^2 = H; R^3 = OH$
2 $R^1 = R^2 = H; R^3 = OMs$
3 $R^1 = Ms; R^2 = H; R^3 = OMs$
4 $R^1 = Ms; R^2 = Bn; R^3 = OMs$
5 $R^1 = R^2 = Bn; R^3 = OMs$
6 $R^1 = Bn; R^2 = Bzl; R^3 = OBzl$
7 $R^1 = Bn; R^2 = H; R^3 = OH$
8 $R^1 = Bn; R^2 = Ms; R^3 = OMs$



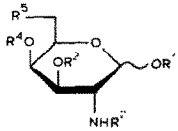
9 $R^1 = R^2 = H$
10 $R^1 = R^2 = Ms$



11



12 R = OH
13 R = OMs
14 R = F



15 $R^1 = R^3 = R^4 = H; R^2 = Ac; R^6 = OTr$
16 $R^1 = R^2 = R^3 = R^4 = Ac; R^6 = OTr$
17 $R^1 = R^2 = R^3 = R^4 = Ac; R^6 = OH$
18 $R^1 = R^2 = R^3 = R^4 = Ac; R^6 = F$
19 $R^1 = R^2 = R^3 = R^4 = H; R^6 = F$
20 $R^1 = R^3 = R^4 = H; R^2 = Ac; R^6 = F$

EXPERIMENTAL

General methods — Melting points are uncorrected. Optical rotations were measured for solutions in a 10-cm cell with a Perkin–Elmer 141 polarimeter. I.r. spectra were recorded with a Perkin–Elmer model 457 spectrometer and ^{1}H-n.m.r. spectra (δ values) with Varian 390 and XL100 instruments, the latter operating in the F.t. mode. ^{13}C-N.m.r. and ^{19}F-n.m.r. spectra were recorded with a Varian XL100 instrument. Evaporations were performed in a rotary evaporator *in vacuo* at bath temperatures $<40°$. Column chromatography was performed on silica gel (Bio-Rad Bio-Sil A-100–200 mesh), and t.l.c. on an Analtech uniplate Silica gel GF-250; the spots were detected with an H_2SO_4-in-mehanol spray at 100°.

Benzyl 2-acetamido-2-deoxy-α-D-galactopyranoside[29] (**1**) — 2-Acetamido-2-deoxy-D-galactose (5 g) was suspended in a solution of HCl (1 g) in benzyl alcohol

(50 mL) and stirred at 70-80° for 4 h. The solution was cooled and poured into anhydrous ether (400 mL) with vigorous stirring, and kept at 0-4° for 16 h, the precipitate was filtered off, washed with anhydrous ether, and crystallized from hot 2-propanol. Recrystallization from the same solvent gave **1** (3.85 g, yield 55%), m.p. 197-198°, lit.[29], m.p. 202-204°, $[\alpha]_D^{22}$ +233° (*c* 1, methanol); $[\alpha]_D$ +204° (*c* 0.98, water); ν_{max}^{KBr} 3560-3260 (OH and NH), 1640 and 1555 (C=O, amide), and 710 cm^{-1} (arom.); ^{1}H-n.m.r. [($^2H_3$)Me_2SO]: δ 1.90 (s, 3 H, NAc), 4.74 (d, 1 H, H $J_{1,2}$ 3.1 H-1) and 7.42 (s, 5 H, arom).

Anal. Calc. for $C_{15}H_{21}NO_6$: C, 57.87; H, 6.80; N, 4.50. Found: C, 57.66; H, 6.92; N. 4.29.

*Benzyl 2-acetamido-2-deoxy-6-*O*-mesyl-* (**2**) *and -3,6-di-*O*-mesyl-α-*D*-galacto-pyranoside* (**3**) — A solution of methanesulfonyl chloride (1.26 g) in dry pyridine (7 mL) was added dropwise to a stirred solution of **1** (3.1 g) in dry pyridine (25 mL) in 1 h at −45°. The solution was stirred at −45°--40° for 4 h and then overnight in the freezer (−5°). It was filtered and evaporated to give an oily residue which was chromatographed on a silica gel column and eluted with 1:9 methanol–chloroform. The compound eluted first was characterized as **3** (552 mg, 13%); it was crystallized from methanol–ether, m.p. 191-192°, $[\alpha]_D^{22}$ +170° (*c* 1, methanol); ν_{max}^{KBr} 3520 (OH), 3300 (NH), 1650 and 1545 (C=O, amide), 1355 and 1180 (SO_2), and 700 cm^{-1} (arom.); ^{1}H-n.m.r. ($CDCl_3$): δ 1.85 (s, 3 H, NAc), 3.82 (s, 6 H, 2 Ms), 4.81 (d, 1 H, $J_{1,2}$ 3.00 Hz H-1), and 7.42 (s, arom.).

Anal. Calc. for $C_{17}H_{25}NO_{10}S_2$, H_2O: C, 43.68; H, 5.35; S, 13.70. Found: C, 43.94; H, 5.60; S, 14.01.

The second compound, obtained by further elution with the same solvent, was the desired product **2** (2.7g 67%), m.p. 169-170° (dec.), $[\alpha]_D^{22}$ +198° (*c* 1, methanol); ν_{max}^{KBr} 3420 (OH), 3290 (NH), 1635 and 1545 (C=O, amide), 1350 and 1180 (SO_2), and 710 cm^{-1} (arom.); ^{1}H-n.m.r. (CD_3OD): δ 1.75 (s, 3 H, NAc), 2.92 (s, 3 H, mesyl), 4.74 (d, 1 H, $J_{1,2}$ 3.95 Hz, H-1), and 7.25 (s, 5 H, arom).

Anal. Calc for $C_{16}H_{23}NO_8S{\cdot}H_2O$: C, 47.18; H, 6.13; S, 7.86. Found: C, 47.23; H, 6.01; S, 8.03.

*Benzyl 2-acetamido-4-*O*-benzyl-2-deoxy-3,6-di-*O*-mesyl-α-*D*-galactopyrano-side* (**4**). — A solution of **3** (1.7 g) and benzyl bromide (2.5 g) in dry *N,N*-dimethylformamide (15 mL) was cooled to 0° and a mixture of BaO (4 g) and $Ba(OH)_2$ (1 g) was added. The mixture was stirred at room temperature for 20 h. It was diluted with chloroform and a solution of M formic acid was added until complete dissolution. After usual extraction with chloroform, the residue was chromatographed on a silica gel column. After removal of the oily impurities by eluting with chloroform, the product was eluted with 1:1 chloroform–ethyl acetate. The product crystallized from chloroform–ether (1.35 g, 67%), m.p. 148-149°, $[\alpha]_D^{22}$ +93.5° (*c* 1, chloroform); ν_{max}^{KBr} 3350 (NH), 1655 and 1545 (C=O, amide), 1350 and 1180 (SO_2), and 700 cm^{-1} (arom.); ^{1}H-n.m.r. ($CDCl_3$): δ 1.98 (s, 3 H, NAc), δ 2.88 (s, 3 H, Ms), 2.98 (s, 3 H, Ms), 4.95 (d, 1 H, $J_{1,2}$ 3.00 Hz, H-1), 5.75 (d, *J*, 9.00 Hz, NH), and 7.31 (m, 10 H, arom).

Anal. Calc. for $C_{24}H_{31}NO_{10}S$: C 51.69; H, 5.60; N, 2,50; S, 11.50. Found: C, 51.67; H, 5.68; N, 2.35; S, 11.38.

*Benzyl 2-acetamido-3-*O*-benzyl-2-deoxy-4,6-di-*O*-mesyl-α-*D*-glucopyranoside*[30] (**10**). — Benzyl 2-acetamido-2-deoxy-3-*O*-benzyl-α-D-glucopyranoside[20] (**9**; 550 mg) was treated with methanesulfonyl chloride (2 mL) in pyridine (15 mL) at 0°. After overnight reaction at 0–5°, it was poured into ice-water. After extraction with chloroform, the product crystallized from chloroform–ether (530 mg 70%), m.p. 180–181°, $[\alpha]_D^{22}$ +117° (*c* 1, chloroform), lit.[30] m.p. 173–174°, $[\alpha]_D^{20}$ +110° (*c* 1, chloroform)]; ν_{max}^{KBr} 3290 (NH), 1640 and 1545 (C=O), amide), 1350 and 1180 (SO_2) and 700 $^{-1}$ (arom.); ^{1}H-n.m.r. ($CDCl_3$): δ 1.85 (s, 3 H, NAc), 2.95 (s, 3 H, 4-*O*-Ms), 3.10 (s, 3 H, 6-*O*-Ms), 4.95 (d, 1 H, $J_{1,2}$ 3.20 Hz, H-1), 5.52 (d, 1 H, *J*, 9.61 Hz, NH), and 7.45 (m, 10 H, arom.).

Anal. Calc. for $C_{24}H_{31}NO_{10}S_2$: C, 51.71; H, 5.57; N, 2.51; S, 11.49. Found: C, 51.46; H, 5.39; N, 2.47; S, 11.23.

*Benzyl 2-acetamido-4,6-di-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-*D*-galactopyranoside* (**6**). — A solution of **10** (400 mg) in dry *N,N*-dimethylformamide (15 mL) was refluxed with lithium benzoate (4 g) for 20 h, when the reaction was complete. The solution was cooled, diluted with chloroform, washed with water, dried (Na_2SO_4), and evaporated to dryness. The residue was chromatographed on a silica gel column and the product was eluted with 1:4 ethyl acetate–chloroform. (It crystallized from chloroform–ether–petroleum ether (350 mg 80.2%), m.p. 135–136°, $[\alpha]_D^{22}$ +151° (*c* 1, chloroform); ν_{max}^{KBr} 3290 (NH), 1725 (C=O), 1645 and 1545 (C=O, amide), and 700 cm^{-1} (arom.); ^{1}H-n.m.r. ($CDCl_3$): δ 1.85 (s, 3 H, NAc), 3.70 (dd, 1 H, $J_{2,3}$ 9.5, $J_{1,2}$ 3.60 Hz, H-2), 5.10 (d, 1 H, $J_{1,2}$ 3.60 Hz, H-1), 5.75 (d, 1 H, *J* 8.46 Hz, NH), and 7.20–8.00 (m, 20 H, arom).

Anal. Calc. for $C_{36}H_{35}NO_8$; C, 70.92, 5.79; N, 2.30. Found: C, 70.97; H, 5.81; N, 2.20.

*Benzyl 2-acetamido-3-*O*-benzyl-2-deoxy-α-*D*-galactopyranoside* (**7**). — A solution of **6** (300 mg) in absolute methanol (30 mL) containing sodium methoxide (10 mg) was refluxed for 5 h. The solution was evaporated to dryness, the residue taken up in chloroform, and the solution washed with water, dried (Na_2SO_4), and evaporated. The residue was washed with petroleum ether to remove methyl benzoate and the product crystallized from methanol–ether–petroleum ether (180 mg 88%), m.p. 187–188°, $[\alpha]_D^{22}$ +165.5° (*c* 1, methanol); ν_{max}^{KBr} 3350–3600 (OH), 3300 (NH), 1650 and 1560 (C=O amide), and 700 cm^{-1} (arom.); ^{1}H-n.m.r. ($CDCl_3$): δ 1.85 (s, 3 H, NAc), 2.75 (2 H, OH), 3.55 (dd, 1 H, $J_{1,2}$ 3.65, $J_{2,3}$ 10.40 Hz, H-2), 4.95 (d, 1 H, $J_{1,2}$ 3.65 Hz, H-1), 5.30 (d, 1 H, *J* 9.66 Hz, NH), and 7.32 (s, 10 H, arom.).

Anal. Calc. for $C_{22}H_{27}NO_6$: C, 65.82; H, 6.78; N, 3.49. Found: C, 65.81; H, 6.60; N, 3.54.

*Benzyl 2-acetamido-3-*O*-benzyl-2-deoxy-4,6-di-*O*-mesyl-α-*D*-galactopyranoside* (**8**). — Compound **7** (150 mg) was treated with methanesulfonyl chloride (1 mL) in dry pyridine (5 mL) at 0°, and the solution kept at 0–5° for overnight. The red solution was poured into ice-water and extracted with chloroform. The residue was

chromatographed on a short column of silica gel and the product was eluted with ethyl acetate to give a crystalline solid which was recrystallized from methanol–ether (120 mg, 57%), m.p. 175–176°, $[\alpha]_D^{22}$ +126.5° (*c* 1, chloroform); ν_{max}^{KBr} 3300 (NH), 1660 and 1545 (C=O, amide), 1350 and 1180 (SO_2), and 700 cm^{-1} (arom.); ^{1}H-n.m.r. ($CDCl_3$): δ 1.85 (s, 3 H, NAc), 3.05 (s, 3 H, OMs), 3.15 (s, 3 H, OMs), 3.65 (dd, 1 H, $J_{1,2}$ 3.63, $J_{2,3}$ 1.50 Hz, H-2), 4.90 (d, 1 H, $J_{1,2}$ 3.65 Hz, H-1), and 7.28 (s, 10 H, arom).

Anal. Calc. for $C_{24}H_{31}NO_{10}S$: C, 51.69; H, 5.60; N, 2.50; S, 11.50. Found: C, 51.58; H, 5.63; N, 2.52; S, 11.27.

*Benzyl 2-acetamido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-mesyl-α-*D*-galactopyranoside* (**5**). — A solution of **2** (1.34 g) and benzyl bromide (3.6 g) in anhydrous *N,N*-dimethylformamide (15 mL) was cooled to 0° and a mixture of BaO (4 g) and $BA(OH)_2$ (1 g) was added. The mixture was stirred at 0° for 1 h and then at room temperature for 20 h. It was diluted with chloroform, filtered, and the chloroform solution washed with water, dried (Na_2SO_4), and evaporated to dryness. The residue crystallized from chloroform–ether (1.72 g, 64%), m.p. 186–187°, $[\alpha]_D^{22}$ +108° (*c* 1, chloroform); ν_{max}^{KBr} 3300 (NH), 1645 and 1550 (C=O, amide), 1355 and 1180 (SO_2), and 709 cm^{-1} (arom.); ^{1}H-n.m.r. ($CDCl_3$): δ 1.88 (s, 3 H, NAc), 2.93 (s, 3 H, OMs), 5.01 (d , 1 H, $J_{1,2}$ 3.50 Hz, H-1), and 7.36 (m, 15 H, arom).

Anal. Calc. for $C_{30}H_{35}NO_8S$: C, 63.25; H, 6.20; S, 5.60. Found: C, 63.41; H, 6.18; S, 5.71.

*Benzyl 2-acetamido-3,6-anhydro-4-*O*-benzyl-2-deoxy-α-*D*-galactopyranoside* (**11**). — The mesyl derivative **5** (200 mg) was added to a boiling solution of CsF (1.2 g) in freshly distilled 1,2-ethanediol (5 mL), and the solution was refluxed for 5 min, poured into ice–water, and extracted with chloroform. The chloroform solution yielded **5** as the major product which crystallized from acetone–ether (45 mg, 23%), m.p. 169–170°, $[\alpha]_D^{22}$ −6.9° (*c* 1, chloroform); ν_{max}^{KBr} 3340 (NH), 1660 and 1535 (C=O), amide), and 710 cm^{-1} (arom.); ^{1}H-n.m.r. ($CDCl_3$): δ 1.25 (s, 3 H, NAc), 4.04 (br. s, 2 H, H_2-6), 4.07 (d, 1 H, $J_{4,5}$ 2.1 Hz, H-5), 4.13 (m, 1 H, H-2), 4.37 (br., 1 H, $J_{3,4}$ 1.5 Hz not resolved, H-3), 5.05 (d, 1 H, $J_{1,2}$ 3.60 Hz, H-1), 5.84 (d, 1 H, *J*, 8.1 Hz, NH), and 7,36 (m, 10 H, arom).

Anal. Calc. for $C_{22}H_{25}NO_5$: C, 68.91; H, 6.57; N, 3.65. Found: C, 68.86; H, 6.59; N, 3.48.

*Benzyl 2-acetamido-2-deoxy-3,4-*O*-isopropylidene-α-*D*-galactopyranoside* (**12**). — A suspension of benzyl glycoside **1** (150 mg) in dry acetone (10 mL) containing H_2SO_4 (0.01 mL) was stirred at room temperature for 20 h. It was made neutral with $Ba(OH)_2$ and filtered. The filtrate was diluted with chloroform, washed with water, dried ($MgSO_4$), and evaporated to dryness. The residue crystallized from ether and was recrystallized from chloroform–ether (50 mg, 27%), m.p. 197–198°, $[\alpha]_D^{22}$ +175.5° (*c* 1, chloroform); ν_{max}^{KBr} 3515 (OH), 3310 (NH), 1640 and 1545 (C=O, amide), and 709 cm^{-1} (arom.); ^{1}H-n.m.r. [($^2H_3$) Me_2SO]: δ 1.32 (s, 3 H, CH_3) 1.41 (s, 3 H, CH_3), 1.85 (s, 3 H, NAc), 4.75 (d, 1 H, $J_{1,2}$ 3.15 Hz, H-1), and 7.31 (s, 5 H, arom).

Anal. Calc. for $C_{18}H_{25}NO_6$: C, 61.52; H, 7.17; N, 3.99. Found: C, 61.32; H, 6.98; N, 3.98.

Benzyl 2-acetamido-2-deoxy-3,4-O-isopropylidene-6-O-mesyl-α-D-galactopyranoside (**13**). — The mesyl derivative **2** (1 g) was shaken in dry acetone (15 mL) containing H_2SO_4 (0.01 mL) at room temperature for 24 h. The solution was made neutral with $Ba(OH)_2$, filtered, and evaporated. The residue was taken up in chloroform, and the solution washed with water, dried (Na_2SO_4), and evaporated to yield a colorless solid, which crystallized from methanol–ether (885 mg, 84%), m.p. 163–164°, $[\alpha]_D^{22}$ +162° (*c* 1, chloroform); ν_{max}^{KBr} 3325 (NH), 1650 and 1545 (C=O, amide), 1345 and 1170 (SO_2), and 710 cm^{-1} (arom.); ^{1}H-n.m.r. ($CDCl_3$): δ 1.33 (s, 3 H, CH_3), 1,57 (s, 3 H, CH_3), 1.98 (s, 3 H, NAc), 3.07 (s, 3 H, Ms), 4.93 (d, 1 H, $J_{1,2}$ 3.10 Hz, H-1), and 7.36 (m, 5 H, arom).

Anal. Calc. for $C_{19}H_{27}NO_8S \cdot H_2O$: C, 50.97; H, 6.52; S, 7.16. Found: C, 51.12; H, 6.41; S, 7.24.

Benzyl 2-acetamido-2,6-dideoxy-6-fluoro-3,4-O-isopropylidene-α-D-galactopyranoside (**14**). — Compound **13** (200 mg) was added to a solution of CsF (1.5 g) in boiling 1,2-ethanediol (10 mL) and refluxed for 15 min. It was then cooled, poured into water, and extracted with chloroform, and the extract dried (Na_2SO_4) and evaporated to a syrup. The residue was chromatographed on a column of silica gel and the product, eluted with ether, crystallized from chloroform–ether–petroleum ether (110 mg, 67%), m.p. 131–132°, $[\alpha]_D^{22}$ +199° (*c* 1, chloroform); ν_{max}^{KBr} 3295 (NH), 1655 and 1543 (C=O, amide), 710 (arom.), and 750 cm^{-1} (C-F); ^{1}H-n.m.r. ($CDCl_3$): δ 1.25 (s, 3 H, CH_3), 1.45 (s, 3 H, CH_3), 1.91 (s, 3 H, NAc), 4.85 (d, 1 H, $J_{1,2}$ 3.15 Hz, H-1), 5.75 (d, 1 H, NH, *J* 8.46 Hz), and 7.25 (s, 5 H, arom.); ^{19}F-n.m.r. ($CDCl_3$–$CFCl_3$): δ −224.00 (sext., $J_{F,H-6}$ 47.10 $J_{F,H-5}$ 15.8 Hz).

Anal. Calc. for $C_{18}H_{24}FNO_5$; C, 61.17; H, 6.84; F, 5.38. Found: C, 61.24; H, 7.03; F, 5.65.

2-Amino-2,6-dideoxy-6-fluoro-D-galactose hydrochloride (**19**). — The fluoro compound **14** (100 mg) was deblocked with 3M Hcl (5 mL) at reflux for 4 h. The solution was evaporated to dryness and the residue crystallized from methanol–ether, (45 mg, 77%), m.p. 188–189°, $[\alpha]_D^{22}$ +71.5° → +45°; (24 h, *c* 1, water); ^{19}F-n.m.r. (D_2O–$CFCl_3$ ext.). δ −237.00 (complex sext.).

Anal. Calc. for $C_6H_{13}ClFNO_4$; C, 33.03; H, 5.96; F, 8.73. Found: C, 33.20; H, 6.12; F, 8.84.

2-Acetamido-2,6-dideoxy-6-fluoro-D-galactose (**20**). — Compound **14** (400 mg) was hydrogenolyzed in the presence of Pd–C (350 mg, 10%) in acetic acid (20 mL). The uptake of H_2 was complete within 48 h. The mixture was filtered, the filtrate evaporated to dryness, and the residue crystallized from methanol–ether, (150 mg), m.p. 135–137°, $[\alpha]_D^{22}$ −15.2° (*c* 1, water); ν_{max}^{KBr} 3200–3500 (NH, OH), 1630 and 1560 (C=O, amide); ^{1}H-n.m.r. [($^2H_3$)Me_2SO] δ 1.85 (s, 3 H, NAc) 5.70 (m, 1 H, H-1), and 7.95 (d, 1 H, *J* 9.2 Hz, NH) ; ^{19}F-n.m.r. (D_2O–CF_3CO_2H ext.) δ −151.20 (sext., $J_{F,H-6}$ 47.00 $J_{F,H-5}$ 19.60 Hz).

Anal. Calc. for $C_8H_{14}FNO_5$; C, 43.05; H, 6.28; N, 6.25; F, 8.52. Found: C,

42.91; H, 6.37; N, 6.06; F, 8.44.

*2-Acetamido-1,3,4-tri-*O*-acetyl-2,6-dideoxy-6-fluoro-*D*-galactose* (**18**). — Compound **20** (190 mg) was acetylated with acetic anhydride (2 mL) in pyridine (7 mL) overnight. The solution was poured into ice-water and then evaporated to dryness. The residue was chromatographed on a silica gel column. After elution with chloroform to remove the impurities, the product was eluted with 9:1 chloroform–methanol, amorphous solid (154 mg, 78%), $[\alpha]_D^{22}$ +47.5° (*c* 1, chloroform); ν_{max}^{KBr} 3325 (NH), 1740 (C=O, OAc), 1650 and 1540 cm^{-1} (C=O, amide); ^{19}F-n.m.r. ($CDCl_3$–$CFCl_3$): δ –230.8 (sext., $J_{F,\ H\text{-}6}$ 47.05, $J_{F,\ H\text{-}5}$ 19.40 Hz).

Anal. Calc. for $C_{14}H_{20}FNO_8$; C, 48.12; H, 5.73; N, 4.00; F, 5.44. Found: C, 47.94; H, 5.72; N, 3.99; F, 5.32.

*2-Acetamido-2-deoxy-6-*O*-trityl-*D*-galactose* (**15**). — A solution of 2-acetamido-2-deoxy-D-galactopyranose (4.52 g) and chlorotriphenylmethane (5.6 g) in dry pyridine (25 mL) was stirred at room temperature for 5 days. The reaction mixture was heated at 80° for 1 h, cooled, and poured into ice-water. After 30 min, the semi-solid precipitate was filtered off and dissolved in chloroform. The filtrate was extracted once with chloroform and the combined chloroform solutions were washed with cold water, dried (Na_2SO_4), and evaporated. The residue was freed from pyridine by addition and evaporation of toluene, and chromatographed on a silica gel column. Chloroform eluted the fast moving triphenylmethanol and the product was eluted with 1:9 methanol–chloroform, as colorless amorphous solid (9 g, 95%), m.p. 130–135°, $[\alpha]_D^{22}$ +17° (*c* 1, chloroform); ν_{max}^{KBr} 3300–3500 (OH, NH), 1650 and 1545 (C=O, amide), and 700 cm^{-1} (arom.); ^{1}H-n.m.r. [$^2H_3$]Me_2SO]: δ 1.85 (s, 3 H, NAc) 4.90 (d, $J_{1,2}$ 3.05 Hz, H-1α), 4.50 (d, $J_{1,2}$ 4.50 Hz, H-1β), and 7.35 (m, 15 H, arom.).

Anal. Calc. for $C_{27}H_{29}NO_6$; C, 69.96; H, 6.31; N, 3.02. Found: C, 69.74; H, 6.28; N, 2.97.

*2-Acetamido-1,3,4-tri-*O*-acetyl-2-deoxy-6-*O*-trityl-*D*-galactose* (**16**). — The trityl compound **15** (5 g) was acetylated with acetic anhydride (20 mL) and dry pyridine (40 mL) overnight. It was poured into ice-water, and extracted with chloroform, and the solution washed with water, dried (Na_2SO_4) and evaporated. The residue was freed from pyridine by addition and evaporation of toluene and passed through a column of silica gel. The product was eluted with chloroform and crystallized from ether–petroleum ether (6 g, 94%), m.p. 120–125°, $[\alpha]_D^{22}$ +12.4° (*c* 1, chloroform); ν_{max}^{KBr} 3300 (NH), 1750 (C=O), 1660 and 1545 (C=O, amide), and 710 cm^{-1} (arom.)

Anal. Calc. for $C_{33}H_{35}NO_9$; C, 67.22; H, 5.98; N, 2.35. Found: C, 66.92; H, 5.92; N, 2.09.

*2-Acetamido-1,3,4-tri-*O*-acetyl-2-deoxy-*D*-galactose* (**17**). — To a hot solution of **16** (6 g) in acetic acid (90 mL), at 95°, was slowly added water (15 mL) with stirring. The clear solution was stirred at the same temperature for 2 h, cooled, and evaporated to dryness. The residue was freed from acetic acid and chromatographed on a silica gel column. After removal of the triphenylmethanol with chloroform, the product was eluted with (9:1) chloroform–methanol. The residue solidified in chlo-

roform–ether, (2.2 g, 68%), amorphous, m.p. 100–105°, $[\alpha]_D^{22}$ +87.5° (*c* 1, chloroform); ν_{max}^{KBr} 3200 (OH and NH), 1720 (C=O), 1665 and 1545 cm^{-1} (C=O, amide).

Anal. Calc. for $C_{14}H_{21}NO_9$; C, 48.41; H, 6.09; N, 4.03. Found: C, 48.63; H, 6.28; N, 3.90.

*2-Acetamido-1,3,4-tri-*O*-acetyl-2,6-dideoxy-6-fluoro-*D*-galactose* (**18**). — A solution of **16** (350 mg) in dry Diglyme (1 mL) was added to a stirred solution of (diethylamino)sulfur trifluoride (1 mL) in Diglyme (1 mL) at −10° under an N_2 atmosphere. After the addition, the mixture was stirred at room temperature for 4 h. The excess reagent was carefully decomposed with absolute ethanol at −20° and then with ice. After extraction with ethyl acetate and removal of solvents by evaporating *in vacuo*, the dry residue was chromatographed on a silica gel column. The product was eluted with ethyl acetate as a waxy solid, homogeneous on t.l.c. (250 mg, 65%), $[\alpha]_D^{22}$ +44° (*c* 1, chloroform); ν_{max}^{KBr} 3320 (NH), 1740 (C=O), 1650 and 1545 cm^{-1} (C=O, amide); ^{1}H-n.m.r. and ^{19}F-n.m.r. spectra were identical with those of the product obtained by acetylation of **20**.

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