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FLUORINATED CARBOHYDRATES AS POTENTIAL PLASMA MEMBRANE MODIFIERS. SYNTHESIS OF 4- AND 6-FLUORO DERIVATIVES OF 2-ACETAMIDO-2-DEOXY-D-HEXOPYRANOSES*

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ABSTRACT

2-Amino-2,4-dideoxy-4-fluoro- and 2-amino-2,4,6-trideoxy-4,6-difluoro-D-galactose, and 2-amino-2,4-dideoxy-4-fluoro- and 2-amino-4-deoxy-4,4-difluoro-D-*xylo*-hexose were synthesized, as potential modifiers of tumor cell-surface glycoconjugate, from benzyl 2-acetamido-3-*O*-benzyl-2-deoxy-4,6-di-*O*-mesyl-α-D-glucopyranoside and benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-mesyl-α-D-glucopyranoside, which were converted into the corresponding 4,6-difluoro-2,4,6-trideoxy and 2,4-dideoxy-4-fluoro derivatives. Benzyl 2-acetamido-2-deoxy-4-*O*-mesyl-α-D-galactopyranoside and benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-*xylo*-hexo-4-ulopyranoside were treated with diethylaminosulfur trifluoride to give 2-amino-2,4-dideoxy-4-fluoro-D-glucose and 2-amino-2,4-dideoxy-4,4-difluoro-D-*xylo*-hexose derivatives, respectively, to give after deprotection the target compounds. Several of the peracetylated sugar derivatives inhibited L1210 tumor-cell growth *in vitro* at concentrations of 1–5 10^{-5}M. The peracetylated derivative of 2-amino-2,4-dideoxy-4-fluoro-D-galactose inhibited protein and glycoconjugate biosynthesis, and also exhibited antitumor activity in mice with L1210 leukemia.

INTRODUCTION

Membrane glycoconjugates are altered after oncogenic transformation by carcinogen or virus[2,3], and modification of cell surface glycoprotein(s) may eventually alter tumorgenicity, immunogenicity, or metastatic potential of cancer cells[4]. As part of our program for the development of antitumor, plasma-membrane modifiers and inhibitors, several analogs of cell surface carbohydrates[5] have been synthesized by substituting a fluorine atom for a hydroxyl group, as the C–F bond more closely resembles[6] C–OH than the C–H bond. These analogs were designed

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to inhibit the various enzymic processes of glycoconjugate metabolism[7,8], or to act as terminators of carbohydrate-chain elongation by being incorporated into the cell surface oligosaccharides. They also were expected to modify the nucleotide pool size and, hence, provide a potential use in combination chemotherapy with nucleoside analogs[7–9].

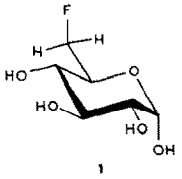
As modified hexosamines could serve as metabolic precursors for several sugars of both the peripheral and core region in the membrane glycoconjugates, several fluorinated analogs of amino sugars, such as 6-deoxy-6-fluoro-D-galactose, 2-acetamido-2,6-dideoxy-6-fluoro-D-glucose[10], 2-amino-2,6-dideoxy-6-fluoro-D-galactose[11], and *N*-acetyl-9-deoxy-9-fluoroneuraminic acid[12] have been synthesized and their effects on cell growth, cell surface macromolecular incorporation, and chemotherapeutic activity have been examined[13,14].

Since the hydroxyl groups are involved in the formation of oligosaccharide linkages in the membrane glycoconjugates, introduction of a fluorine atom at C-4 of the hexosamines, 2-acetamido-2-deoxy-D-glucopyranose and 2-acetamido-2-deoxy-D-galactopyranose, may result in biologically-active, membrane-sugar analogs.

RESULTS AND DISCUSSION

For the synthesis of 2-acetamido-4,6-dideoxy-4,6-difluoro-D-galactopyranose (**18**), the starting compound, benzyl 2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside[15], was prepared in an improved yield, and treatment with aqueous acetic acid gave **3** (ref. 15). Conversion of this compound into the difluoro compound **2** by treatment with *N,N*-diethylaminosulfur trifluoride was unsuccessful owing to the low reactivity of OH-4. Therefore, **3** was treated with methanesulfonyl chloride in pyridine to give **4** (78%), and then tetrabutylammonium fluoride to give **2**. Removal of the benzyl protecting groups by catalytic hydrogenolysis smoothly afforded 2-acetamido-2,4,6-trideoxy-4,6-difluoro-D-galactopyranose (**18**), which was subsequently acetylated to give **19**. A sample of **4** was hydrogenolyzed to give the dimesyl compound **5** for biological evaluation (Table I). Oxidation of **18** in aqueous solution in the presence of platinum-on-charcoal at pH 7.5–8 afforded the corresponding aldonolactone **26** which is a strong inhibitor of *N*-acetyl-β-D-glucosaminidase (K_i 41.4μM). Hydrolysis of **18** with aqueous hydrochloric acid gave 2-amino-2,4,6-trideoxy-4,6-difluoro-D-galactose hydrochloride (**16**).

The synthesis of 2-acetamido-2,4-dideoxy-4-fluoro-D-galactopyranose (**21**) *via* methyl 2-benzamido-2,4-dideoxy-4-fluoro-α-D-glycopyranoside[16] was not satisfactory as benzyl 2-acetamido-3-*O*-benzyl-2-deoxy-6-*O*-trityl-α-D-glucopyranoside[17] gave a poor yield of the 4-deoxy-4-fluoro derivative when treated with *N,N*-diethylaminosulfur trifluoride. Prolonged treatment of the corresponding 4-*O*-methylsulfonyl derivative **10** with an excess of tetrabutylammonium fluoride was also unsuccessful. A portion of **10** was deblocked by catalytic hydrogenolysis in acetic acid



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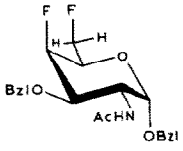

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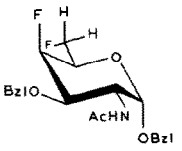

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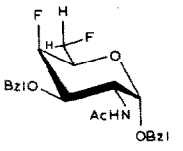

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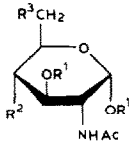
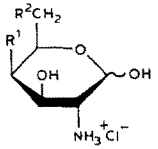
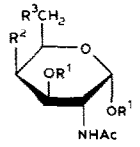


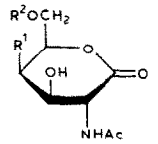
3 $R^1 = Bzl; R^2 = R^3 = OH$
4 $R^1 = Bzl; R^2 = R^3 = OMs$
5 $R^1 = H; R^2 = R^3 = OMs$
6 $R^1 = Ac; R^2 = R^3 = OMs$
7 $R^1 = Bzl; R^2 = OMs; R^3 = OBzl$
8 $R^1 = H; R^2 = OMs; R^3 = OH$
9 $R^1 = Ac; R^2 = OMs; R^3 = OAc$
10 $R^1 = Bzl; R^2 = OMs; R^3 = OTr$
11 $R^1 = Bzl; R^2 = OH; R^3 = OBzl$
12 $R^1 = Bzl; R^2 = OMs; R^3 = OH$
13 $R^1 = Bzl; R^2 = F; R^3 = OBzl$
14 $R^1 = H; R^2 = F; R^3 = OH$
15 $R^1 = Ac; R^2 = F; R^3 = OAc$



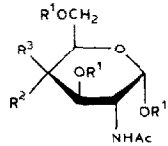
16 $R^1 = R^2 = F$
17 $R^1 = F; R^2 = OH$



18 $R^1 = H; R^2 = R^3 = F$
19 $R^1 = Ac; R^2 = R^3 = F$
20 $R^1 = Bzl; R^2 = F; R^3 = OBzl$
21 $R^1 = H; R^2 = F; R^3 = OH$
22 $R^1 = Ac; R^2 = F; R^3 = OAc$
23 $R^1 = Bzl; R^2 = Bz; R^3 = OBzl$
24 $R^1 = Bzl; R^2 = OH; R^3 = OBzl$
25 $R^1 = Bzl; R^2 = OMs; R^3 = OBzl$



26 $R^1 = R^2 = F$
27 $R^1 = F; R^2 = OH$



28 $R^1 = Bzl; R^2, R^3 = O$
29 $R^1 = Bzl; R^2 = R^3 = F$
30 $R^1 = H; R^2 = R^3 = F$
31 $R^1 = Ac; R^2 = R^3 = F$

in the presence of palladium-on-charcoal to afford the monomesyl compound **8**, which was subsequently acetylated to give **9** for evaluation of the antitumor activity (Table I). In order to decrease the probable steric interference of the trityl group in the displacement of OMs-4 with a fluoride nucleophile, **10** was detritylated to afford benzyl 2-acetamido-3-*O*-benzyl-4-*O*-mesyl-α-D-glucopyranoside (**12**), and then benzylated to give **7** in good yield (87%). Compound **7** was prepared more conveniently from benzyl 2-acetamido-4,6-*O*-benzylidene-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside[17] in two steps by reductive cleavage[18] with sodium cyanoborohydride to give **11**, which was then methanesulfonylated. Treatment of **7** with tetra-

TABLE I

EFFECTS OF SUGAR ANALOGS ON CELL GROWTH OF L1210 LEUKEMIA CELLS[a]

Compound	*Concentration for ID_{50} (mM)*
5	1
6	0.018
8	[b]
9	0.035
14	[b]
15	0.034
16	[b]
17	[b]
18	[b]
19	0.024
21	[b]
22	0.035
30	
31	0.02

[a]See Experimental section. [b]Not active at 1mM.

butylammonium fluoride in acetonitrile gave benzyl 2-acetamido-3,6-di-*O*-benzyl-2,4-dideoxy-4-fluoro-α-D-galactopyranoside (**20**, 90%), and hydrogenolysis led to 2-acetamido-2,4-dideoxy-4-fluoro-D-galactopyranose (**21**) which was acetylated to give the peracetylated derivative **22**. The aldonolactone **27** was obtained from **21** by oxidation in the presence of platinum-on-charcoal at pH 7.5–8.5. It was found to be a strong inhibitor of *N*-acetyl-β-D-hexosaminidase[19] (27.2μM). Hydrolysis of **21** with aqueous hydrochloric acid afforded 2-amino-2,4-dideoxy-4-fluoro-D-galactose hydrochloride (**17**) for biological evaluation (Table I).

2-Acetamido-1,3,6-tri-*O*-acetyl-2,4-dideoxy-4-fluoro-D-glucopyranose (**15**) was synthesized by starting from benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-mesyl-α-D-glucopyranoside (**12**), which was converted into **23** by treatment with lithium benzoate in boiling *N,N*-dimethylformamide. After debenzoylation to give **24**, and subsequent treatment with *N,N*-dimethylaminosulfur trifluoride, benzyl 2-acetamido-3,6-di-*O*-benzyl-2,4-dideoxy-4-fluoro-D-glucopyranoside (**13**) was obtained in a high yield (85%). Preparation of this compound from **25** by treatment with tetrabutylammonium fluoride in anhydrous acetonitrile was not successful. Compound **13** was hydrogenolyzed in the presence of palladium-on-charcoal and subsequently acetylated to give **15**.

The biological activity of the aforementioned fluorinated amino sugars suggested the preparation of a 2-amino-2,4-dideoxy-4,4-difluoro derivative. Benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**11**) was oxidized with chromium trioxide–pyridine complex[20] or with dimethylsulfoxide and acetic anhydride to afford benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-*xylo*-hexo-4-ulopyranoside (**28**) in 30 and 90% yield, respectively. Treatment of **28** with *N,N*-dimethylaminosulfur trifluoride gave, in excellent yield, benzyl 2-acetamido-3,6-di-

O-benzyl-2,4-dideoxy-4,4-difluoro-α-D-*xylo*-hexopyranoside (**29**). Catalytic hydrogenolysis of **29** in the presence of palladium-on-carbon smoothly afforded 2-acetamido-2,4-dideoxy-4,4-difluoro-α-D-*xylo*-hexopyranose (**30**), which was acetylated to afford **31**.

The ^{19}F-n.m.r. spectrum of **2** showed signals for the presence of an axially oriented[21] F-4 and the characteristic signals (sext., $J_{F\text{-}6,H\text{-}6}$ 46.26 Hz) for F-6. Characteristic differences for the axially and equatorially oriented fluorine atoms were observed in both the geminal and vicinal ^{19}F–H coupling constants. As described previously[22], the preferred conformation of the three possible rotamers of 6-deoxy-6-fluorohexopyranose is **1**, where the C-6–F and C-5–H bonds are antiplanar, this is consistent with the observed coupling constant[10,22,23], $J_{F,H\text{-}5}$ 27.5 Hz. In compound **2** where F-4 is axially oriented, the observed coupling constant ($J_{F\text{-}6,H\text{-}5}$ 12.30 Hz) is nearly equal to the value (13.50 Hz) calculated[22] for the rotamer **2c** where the C-6–F and C-5–H bonds are in *gauche* configuration and the C-6–F bond anti-periplanar to C-4–C-5, with F-6 away from F-4; the rotamers **2a** and **2b** contribute little to the average population of the rotamers.

In the evaluation of the biological effect of the aminosugars, the *N*-acetyl derivatives **14, 18, 21,** and **30** are only poorly taken up by the cells[7,24] as compared to the fully acetylated derivatives **15, 19, 22,** and **31**. Peracetylated sugars are more lipophilic and their uptake by passive diffusion[7], and, thereby, their effect on the inhibition of cell growth is increased. Generally, they inhibited 50% L1210 leukemia cell growth at concentrations of 1–5 10^{-5}M (Table I). Some of these sugar analogs may also mimic the parent sugar by competing for incorporation into macromolecular components[25]. D-Mannose (62% control) and 2-amino-2-deoxy-D-glucose (72% control) incorporation into L1210 leukemia cells appeared to be inhibited selectively by 3 10^{-4}M of **22** (Table II), although incorporation of leucine was also decreased (84%). Antitumor activity was measured by administration (i.p.) of **22** into mice with L1210 leukemia; a dosage of 50 mg kg^{-1} d^{-1} for five consecutive days resulted in a 68% increase in life (% ILS). A dosage of 200 mg kg^{-1} d^{-1} for

TABLE II

EFFECT OF 2-ACETAMIDO-1,3,6-TRI-*O*-ACETYL-2,4-DIDEOXY-4-FLUORO-D-GALACTOPYRANOSE (**22**) ON MACROMOLECULAR BIOSYNTHESIS[a]

Compound (mM)	*Macromolecular precursor*				
	Thymidine	*Uridine*	*Leucine*	*D-Mannose*	*2-Amino-2-deoxy-D-glucose*
0	100	100	100	100	100
0.1	112	108	94	88	112
0.3	116	100	84	62	72
1.0	70	74	74	57	48

[a]See Experimental section.

TABLE III

EFFECT OF 2-ACETAMIDO-1,3,6-TRI-*O*-ACETYL-2,4-DIDEOXY-4-FLUORO-D-GALACTOPYRANOSE (**22**) ON L1210 LEUKEMIA IN DBA/2HA MICE IN VIVO[a]

Dose (MKD × 5)[b]	*Survival (days)*	*ILS (%)*
Control	7	
1	7	0
10	7.4	6
50	11.8	68
100	11.0	57
200	8.2	17
500	4.0	Toxic
1000	3.6	Toxic

[a]See Experimental section. [b]Mg kg^{-1} day^{-1}.

five days and higher doses were toxic for the mice (Table III). Compound **22** also exhibited antiviral activity against HSV-1 and VSV virus at a concentration of 100μM, where a plaque-forming assay[26] was used.

EXPERIMENTAL

General methods. — Melting points (uncorrected) were determined by the capillary method. Optical rotations were measured on solutions in a 10-cm cell with a Perkin–Elmer 141 polarimeter. I.r. spectra were recorded with a Perkin–Elmer 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. Column chromatography was performed on Silica Gel Bio-Sil A (100–200 mesh; Bio-Rad) and t.l.c. on an Analtech Uniplate Silica Gel GF-250. The spots were detected with H_2SO_4 in methanol (10%) spray at 100°.

Benzyl 2-acetamido-3-O-benzyl-2-deoxy-α-D-glucopyranoside[15] (**3**). — To a mixture of BaO (3.2 g) and $Ba(OH)_2$ (800 mg) in *N,N*-dimethylformamide (20 mL) containing benzyl bromide (1.2 mL) was added benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside[15] at 0°. The mixture was stirred at room temperature for 18 h, diluted with water, and neutralized with aqueous formic acid (10%). The white precipitate was filtered, washed with water, and dried to give benzyl 2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside[15] (yield 1.6 g, 90%), m.p. 176–177° (lit.[15] m.p. 167°); ν_{max}^{KBr} 3245 (NH), 1645 and 1550 (C=O, amide), and 730 cm^{-1} (arom.); ^{1}H-n.m.r. [($^2H_3$)Me_2SO]: δ 1.85 (s, 3 H, NAc), 4.85 (d, 1 H, $J_{1,2}$ 4.10 Hz, H-1), 5.72 (s, 1 H, C_6H_5CH), 7.40 (m, 15 H, arom.), and 8.19 (d, 1 H, *J* 10 Hz, NH). The solid product (1.6 g) was dissolved in hot acetic acid (35 mL) at 90–100°, and water (10 mL) was added slowly with stirring. The solution was stirred at 90–100° for 1.5 h, and then it was cooled and concentrated to dryness.

The colorless residue was washed with petroleum ether to remove benzaldehyde and crystallized from methanol–ether to give **3** (yield 1.1 g, 84%), m.p. 178–179° (lit.[15] 178–179°); ν_{max}^{KBr} 3500–3200 (OH, NH), 1640 and 1545 (C=O, amide), and 730–700 cm^{-1} (arom.); ^{1}H-n.m.r. [$(^2H)_3Me_2SO$]: δ 1.81 (s, 3 H, NAc), 5.32 (d, 1 H, $J_{1,2}$ 4.50 Hz, H-1), 7.40 (m, 10 H, arom.), and 8.12 (d, 1 H, J 10.00 Hz, NH).

*Benzyl 2-acetamido-3-*O*-benzyl-2-deoxy-4,6-di-*O*-mesyl-α-*D*-glucopyranoside* (**4**). — Compound **3** (550 mg) was treated with methanesulfonyl chloride (2 mL) in pyridine (7 mL) at 0°. After an overnight reaction at 0–5°, ice–water was added, and the mixture was extracted with chloroform, washed with water, dried (Na_2SO_4), and concentrated. The residue crystallized from chloroform–ether to give **4** (530 mg, 78%) m.p. 180–181°, $[\alpha]_D^{22}$ +117° (*c* 1, chloroform); ν_{max}^{KBr} 3200 (NH), 1640 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), 2.95 (s, 3 H, CH_3SO_3-4), 3.10 (s, 3 H, CH_3SO_3-6), 4.95 (d, 1 H, $J_{1,2}$ 3.20 Hz, H-1), 5.52 (d,. 1 H, J 9.6 Hz, NH), and 7.42 (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.

*2-Acetamido-2-deoxy-4,6-di-*O*-mesyl-α,β-*D*-glucopyranose* (**5**). — Compound **4** (2 g) was hydrogenolyzed in acetic acid (35 mL) in the presence of Pd–C (1 g, 10%) for 48 h at room temperature. After the usual workup, the product crystallized from methanol–ethyl acetate (1.2 g, 89%) m.p. 178–179°, $[\alpha]_D^{22}$ +49° (*c* 1, methanol); ν_{max}^{KBr} 3400 (OH), 3210 (NH), 1640 and 1525 (C=O, amide), 1330 and 1160 cm^{-1} (SO_2); ^{1}H-n.m.r. (D_2O): δ 1.82 (s, 3 H, NAc), 3.15 (s, 3 H, CH_3SO_3-4), 3.25 (s, 3 H, CH_3SO_3-6), and 4.90 (d, 1 H, $J_{1,2}$ 1.80 Hz, H-1).

Anal. Calc. for $C_{10}H_{19}NO_{10}S_2$: C, 31.83; H, 5.04; N, 3.76; S, 16.98. Found: C, 31.64; H, 5.13; N, 3.81; S, 16.88.

*2-Acetamido-1,3-di-*O*-acetyl-2-deoxy-4,6-di-*O*-mesyl-α-*D*-glucopyranose* (**6**). — Compound **5** (1 g) was acetylated with acetic anhydride (4 mL) and pyridine (7 mL) overnight. Ice–water was added and the solution concentrated to dryness. The residue was freed from pyridine by distillation with toluene. The syrupy residue crystallized from chloroform–ether (750 mg, 85%), m.p. 90–92°, $[\alpha]_D^{22}$ +73° (*c* 1, chloroform); ν_{max}^{KBr} 3335 (NH), 1745 (C=O, Ac), 1655 and 1525 (C=O, amide), 1350 and 1175 cm^{-1} (SO_2); ^{1}H-n.m.r. ($CDCl_3$): δ 1.95 (s, 3 H, NAc), 2.17 (s, 3 H, OAc), 2.25 (s, 3 H, OAc), 3.10 (s, 3 H, CH_3SO_3-4), 3.17 (s, 3 H, CH_3SO_3-6), 5.80 (d, 1 H, NH), and 6.18 (d, 1 H, $J_{1,2}$ 3.60 Hz, H-1).

Anal. Calc. for $C_{14}H_{23}NO_{12}S_2$: C, 36.44; H, 4.98; N, 3.03; S, 13.88. Found: C, 36.18; H, 4.92; N, 2.86; S, 13.59.

*Benzyl 2-acetamido-3-*O*-benzyl-2,4,6-trideoxy-4,6-difluoro-α-*D*-galactopyranoside* (**2**). — A mixture of **4** (640 mg) and tetrabutylammonium fluoride (2.5 g) in anhydrous acetonitrile (15 mL) was refluxed for 112 h. After concentration in the rotary evaporator, the residue was taken up in chloroform, and the solution washed with water, dried (Na_2SO_4), and concentrated. The residue was chromatographed on a column of silica gel and the product was eluted with 1:1 chloroform–ethyl

acetate. The colorless solid crystallized from chloroform–ether (440 mg, 88%), m.p. 196–197°, $[\alpha]_D^{22}$ +154° (*c* 1, chloroform); ν_{max}^{KBr} 3290 (NH), 1635 and 1540 (C=O, amide), and 700 cm^{-1} (arom.); ^{1}H-n.m.r. ($CDCl_3$): δ 1.90 (s, 3 H, NAc), 5.20 (d, 1 H, $J_{1,2}$ 3.10 Hz, H-1), 5.35 (d, 1 H, *J* 9.52 Hz, NH), and 7.37 (10 H, arom.); ^{19}F-n.mr. ($CDCl_3$–$CFCl_3$): δ −219.22 (sext. $J_{F\text{-}4,H\text{-}4}$ 50.40, $J_{F\text{-}4,H\text{-}5} = J_{F\text{-}4,H\text{-}3}$ 28.13 Hz, F-4), and −231.68 (sext., $J_{F\text{-}6,H\text{-}6}$ 46.26, $J_{F\text{-}6,H\text{-}5}$ 12.30 Hz, F-6).

Anal. Calc. for $C_{22}H_{25}F_2NO_4$: C, 65.19; H, 6.17; N, 3.46. F, 9.38. Found: C, 64.93; H, 6.34; N, 3.43; F, 9.20.

*2-Acetamido-2,4,6-trideoxy-4,6-difluoro-*D*-galactopyranose* (**18**). — A solution of **2**, (3.05 g) in acetic acid (30 mL) was hydrogenolyzed in the presence of Pd–C (3 g, 10%) for 48 h. After the usual workup, the residue obtained after evaporation of solvent was a crystalline solid; it was recrystallized from methanol–ether (1.3 g, 77%), m.p. 205–206° (dec.), $[\alpha]_D^{22}$ +113° (*c* 1, methanol); ν_{max}^{KBr} 3500–3200 (NH, OH), 1625 and 1545 (C=O, amide); ^{13}C-n.m.r. (D_2O): δ 23.10 ($CH_3\alpha$), 23.35 ($CH_3\beta$), 83.4 (compl., $J_{C,F}$ 163.5, $J_{C,F}$ 166.4 Hz, C-4,6), 175.65 (C=Oα), and 175.9 (C=Oβ); ^{19}F-n.m.r. (D_2O; external $CFCl_3$): δ −230 and −231.5 (2 sext., $J_{F\text{-}6,H\text{-}6}$ 46.8, $J_{F\text{-}6,H\text{-}5}$ 12.25 Hz, F-6α,β), −220.20 (m, $J_{F\text{-}4,H\text{-}4}$ 50.28, $J_{F\text{-}4,H\text{-}3} = J_{F\text{-}4,H\text{-}5}$ 26.4 Hz, F-4β), and −218.20 (m, $J_{F\text{-}4,H\text{-}4}$ 50.25, $J_{F\text{-}4,H\text{-}3} = J_{F\text{-}4,H\text{-}5}$ 26.7 Hz, F-4α).

Anal. Calc. for $C_8H_{13}F_2NO_4$: C, 42.67; H, 5.78; N, 6.22; F, 16.89. Found: C, 42.39; H, 6.08; N, 6.04; F, 16.66.

*2-Acetamido-1,3-di-*O*-acetyl-2,4,6-trideoxy-4,6-difluoro-*D*-galactopyranose* (**19**). — Compound **18** (1.5 g) was treated with acetic anhydride (6 mL) and pyridine (10 mL) at room temperature overnight. After addition of ice–water, the solution was concentrated to dryness. The residue, freed from pyridine, crystallized from ethanol–ether (1.8 g, 90%), m.p. 215–216° (dec.), $[\alpha]_D^{22}$ +112° (*c* 1, chloroform); ν_{max}^{KBr} 3290 (NH), 1740 (C=O, Ac), 1650 and 1525 (C=O, amide); ^{1}H-n.m.r. ($CDCl_3$): δ 1.95 (s, 3 H, NAc), 2.15 (s, 3 H, OAc), 2.20 (s, 3 H, OAc), 5.55 (d, 1 H, *J* 9.54 Hz, NH), and 6.25 (d, 1 H, $J_{1,2}$ 3.90 Hz, H-1); ^{19}F-n.m.r. ($CDCl_3$–$CFCl_3$): δ −232.27 (sext., $J_{F\text{-}6,H\text{-}6}$ 46.4, $J_{F\text{-}6,H\text{-}5}$ 10.1 Hz, F-6) and 218.1 (quint., $J_{F\text{-}4,H\text{-}4}$ 50.0, $J_{F\text{-}4,H\text{-}5} = J_{F\text{-}4,H\text{-}3}$ 26.7 Hz, F-4).

Anal. Calc. for $C_{12}H_{17}F_2NO_6$: C, 46.60; H, 5.50; N, 4.53; F, 12.30. Found: C, 46.73; H, 5.77; N, 4.39; F, 12.07.

*2-Acetamido-2,4,6-trideoxy-4,6-difluoro-*D*-galactono-1,5-lactone* (**26**). — Oxygen gas was passed through a stirred solution of **18** (240 mg) in water (7 mL) at room temperature, in the presence of Pt–C (200 mg, 5%) at pH 7–8 maintained by addition of $NaHCO_3$. The reaction was completed within 4 h. The mixture was filtered, the catalyst washed with water, and the filtrate neutralized with Dowex-50W-X8 (H^+) cation-exchange resin. After filtration, the solution was concentrated to dryness and the residue crystallized from ethyl acetate–ether (190 mg, 94%), m.p. 95°, $[\alpha]_D^{22}$ −4.9° (*c* 1, ethyl acetate); ν_{max}^{KBr} 3500–3200 (NH, OH), 1720 (C=O, lactone), 1625 and 1530 cm^{-1} (C=O, amide); ^{19}F-n.m.r. (D_2O; external $CFCl_3$): δ −231.62 (compl. sext., $J_{F\text{-}6,H\text{-}6}$ 46.29, $J_{F\text{-}6,H\text{-}5}$ 16.30 Hz, F-6) and −208.0 (compl. oct., F-4).

Anal. Calc. for $C_8H_{11}F_2NO_4 \cdot H_2O$: C, 39.83; H, 5.39; N, 5.80; F, 15.76. Found: C, 40.01; H, 5.68; N, 5.59; F, 16.01.

*2-Amino-2,4,6-trideoxy-4,6-difluoro-*D*-galactose hydrochloride* (**16**). — A solution of **18** (500 mg) in 3M HCl (15 mL) was heated at 95–100° with stirring for 3 h. The mixture was diluted with water and concentrated to dryness in vacuum. The residue crystallized from ethanol–ether, m.p. 204–205° (dec.), $[\alpha]_D^{22}$ +42.5° (*c* 1, water); ν_{max}^{KBr} 3430 (OH), 3220 (NH), and 2900 cm^{-1}; ^{19}F-n.m.r. (D_2O; external $CFCl_3$): δ −217.78 (sext., $J_{F\text{-}4,H\text{-}4}$ 50.55, $J_{F\text{-}4,H\text{-}3} \approx J_{F\text{-}4,H\text{-}5}$ 29.71 Hz, F-4b), −220.26 (compl. sext., $J_{F\text{-}4,H\text{-}3} = J_{F\text{-}4,H\text{-}5}$ 29.62 Hz, F-4α), and −230.61 (two closed sext. F-6α,β).

Anal. Calc. for $C_6H_{12}ClF_2NO_3$: C, 32.80; H, 5.46; N, 6.38; F, 17.31. Found: C, 33.08; H, 5.73; N, 6.36; F, 17.28.

*Benzyl 2-acetamido-3-*O*-benzyl-2-deoxy-4-*O*-mesyl-6-*O*-trityl-α-*D*-glucopyranoside* (**10**). — To a solution of benzyl 2-acetamido-3-*O*-benzyl-3-deoxy-6-*O*-trityl-α-D-glucopyranoside[17] (6 g) in pyridine (50 mL) was added, at 0° with stirring, a solution of methanesulfonyl chloride (5 mL) in pyridine (7 mL). The solution was kept at 0-4° for 20 h and then poured into ice–water. The semisolid material was filtered off and taken up in chloroform, and the solution washed with water, dried (Na_2SO_4), and concentrated. The residue crystallized from dichloromethane–ether to give **10** (6 g, 90%), m.p. 215–216°, $[\alpha]_D^{22}$ +82° (*c* 1, chloroform); ν_{max}^{KBr} 3410 (NH), 1655 and 1535 (C=O, amide), 1350 and 1175 (SO_2), and 700 cm^{-1} (arom.); ^{1}H-n.m.r. ($CDCl_3$): δ 1.85 (s, 3 H, NAc), 2.61 (s, 3 H, CH_3SO_3), 5.40 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), and 7.45 (m, 25 H, arom.).

Anal. Calc. for $C_{42}H_{43}NO_8S \cdot 0.5\ H_2O$: C, 69.04; H, 6.02; N, 1.91; S, 4.38. Found: C, 68.89; H, 6.15; N, 1.82; S, 4.28.

*Benzyl 2-acetamido-3-*O*-benzyl-2-deoxy-4-*O*-mesyl-α-*D*-glucopyranoside* (**12**). — To a solution of **10** (5 g) in acetic acid (50 mL) at 90–100° (bath temp.) was slowly added water (12 mL) with stirring, and the heating was continued for 2 h. The solution was concentrated to dryness, and the residue washed with ether. It crystallized from hot methanol (2.8 g, 84%), m.p. 227–228°, $[\alpha]_D^{22}$ +113° (*c* 0.5, chloroform); ν_{max}^{KBr} 3450 (OH), 3290 (NH), 1645 and 1545 (C=O, amide), 1345 and 1145 (SO_2), and 725 cm^{-1} (arom.); ^{1}H-n.m.r. ($CDCl_3$): δ 1.90 (s, 3 H, NAc), 3.01 (s, 3 H, CH_3SO_3), 5.30 (d, 1 H, $J_{1,2}$ 3.25 Hz, H-1), 5.51 (d, 1 H, *J* 9.40 Hz, NH), and 7.40 (br. s, 10 H, arom.).

Anal. Calc. for $C_{23}H_{29}NO_8S$: C, 57.61; H, 6.10; N, 2.92; S, 6.61. Found: C, 57.76; H, 6.33; N, 3.06; S, 6.71.

*Benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-mesyl-α-*D*-glucopyranoside* (**7**). — (*a*) *From* **12**. To a mixture of BaO (1 g), $Ba(OH)_2$ (250 mg), and benzyl bromide (2.5 mL) in dry *N,N*-dimethylformamide (25 mL) was added, at 0° with stirring a solution of **12** (1 g) in *N,N*-dimethylformamide (5 mL). The mixture was stirred at room temperature overnight. A solution of 10% aqueous formic acid was added at 0° to neutralize the basic solution. This was diluted with water and extracted with chloroform, and the extract washed with water, dried (Na_2SO_4), and

concentrated. The residue was chromatographed on a column of silica gel. After elution with petroleum ether to remove impurities, the product was eluted with ethyl acetate, and it crystallized from chloroform–ether (1.2 g, 87%), m.p. 176–177°, $[\alpha]_D^{22}$ +110° (*c* 1, chloroform); ν_{max}^{KBr} 3290 (NH), 1645 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 (d, 3 H, NAc), 2.85 (s, 3 H, CH_3SO_3), 4.95 (d, 1 H, $J_{1,2}$ 3.58 Hz, H-1), 5.49 (d, 1 H, *J* 9.62 Hz, NH), and 7.40 (m, 15 H, arom.).

Anal. Calc. for $C_{30}H_{35}NO_8S$: C, 63.26; H, 6.19; N, 2.46; S, 5.62. Found: C, 63.33; H, 6.27; N, 2.34; S, 5.85.

(*b*) *From benzyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-α-*D*-glucopyranoside* (**11**). Compound **11**[17,18] (1 g) was treated with methanesulfonyl chloride (2.0 g) in pyridine (10 mL) overnight at 0–5°. The solution was poured into ice–water, and the precipitate filtered off and taken up in chloroform. The solution was washed with water, dried (Na_2SO_4), and concentrated. The residue crystallized from chloroform–ether to give **7** (980 mg, 84%), m.p. 175–176°, $[\alpha]_D^{22}$ +108.5° (*c* 1, chloroform).

*2-Acetamido-2-deoxy-4-*O*-mesyl-*D*-glucopyranose* (**8**). — A solution of **10** (1 g) in acetic acid (20 mL) was hydrogenolyzed in the presence of Pd–C (400 mg, 10%) for 48 h. After the usual workup, the product crystallized from methanol–ethyl acetate (380 mg, 94%), m.p. 117–120° (dec.), $[\alpha]_D^{22}$ +46° (*c* 1, methanol); ν_{max}^{KBr} 3550–3200 (OH, NH), 1645 and 1545 (C=O, amide), 1345 and 1170 cm^{-1} (SO_2).

Anal. Calc. for $C_9H_{17}NO_8S \cdot 0.5H_2O$: C, 35.04; H, 5.84; N, 4.54; S, 10.38. Found: C, 34.94; H, 6.06; N, 4.32; S, 10.61.

*2-Acetamido-1,3,6-tri-*O*-acetyl-2-deoxy-4-*O*-mesyl-α-*D*-glucopyranose* (**9**). — Compound **8** (250 mg) was acetylated with acetic anhydride (2 mL) and pyridine (5 mL) overnight at room temperature. Ice–water was added and the solution concentrated to dryness. The residue was freed from pyridine by distillation with toluene and it crystallized from ethanol–ether (280 mg, 70%), m.p. 195–196°, $[\alpha]_D^{22}$ +62° (*c* 1, chloroform); ν_{max}^{KBr} 3225 (NH), 1735 (C=O, Ac), 1650 and 1545 (C=O, amide), 1350 and 1170 cm^{-1} (SO_2); ^{1}H-n.m.r. ($CDCl_3$): δ 1.95 (s, 3 H, NAc), 2.15 (d, 6 H, 2 OAc), 2.25 (s, 3 H, OAc-1), 3.75 (s, 3 H, CH_3SO_3), 5.65 (d, 1 H, *J* 9.20 Hz, NH), and 6.20 (d, 1 H, $J_{1,2}$ 2.50 Hz, H-1).

Anal. Calc. for $C_{15}H_{23}NO_{11}S$: C, 42.35; H, 5.41; N, 3.29; S, 7.53. Found: C, 42.09; H, 5.61; N, 3.09; S, 7.64.

*Benzyl 2-acetamido-3,6-di-*O*-benzyl-2,4-dideoxy-4-fluoro-α-*D*-galactopyranoside* (**20**). — (*a*) *From* **11**. A solution of **11** (refs. 11, 18; 500 mg) in dichloromethane (5 mL) was added to a stirred solution of *N,N*-diethylaminosulfur trifluoride (1 mL) in dichloromethane (2 mL) at 0°. The mixture was stirred at 50° (bath temp.) for 2 h, cooled to −10°, and absolute ethanol (2 mL) was added, followed by ice–water. After extraction with chloroform, the organic phase was washed with water, dried (Na_2SO_4), and concentrated. The residue was chromatographed on a silica gel column and the product eluted with chloroform, it crystallized from chloro-

form–ether (105 mg, 25%), m.p. 185–186°, $[\alpha]_D^{22}$ +116° (*c* 1, chloroform); ν_{max}^{KBr} 3295 (NH), 1640 and 1535 (C=O, amide), and 700 cm^{-1} (arom.); ^{1}H-n.m.r. ($CDCl_3$): δ 1.90 (s, 3 H, NAc), 5.5 (d, 1 H, $J_{1,2}$ 3.2 Hz, H-1), 5.45 (d, 1 H, *J* 9.40 Hz, NH), and 7.35 (m, 15 H, arom.); ^{13}C-n.m.r. ($CDCl_3$): δ 23.3 (CH_3, NAc), 48.3 (d, C-2), 67.8 (m, C-5), 69.9, 70.7, 73.60 (d, C-3), 74.4, 85.3 (d, $J_{C,F}$ 185.40 Hz, C-4), 97.1 (C-1), 127.8 (m, arom.), 137.0 and 137.7 (quatern. arom. C), and 169.6 (C=O); ^{19}F-n.m.r. ($CDCl_3$–$CFCl_3$): δ −220.10 (sext., $J_{F\text{-}4,H\text{-}4}$ 50.40, $J_{F\text{-}4,H\text{-}5} = J_{F\text{-}4,H\text{-}3}$ 27.58 Hz, F-4).

Anal. Calc. for $C_{29}H_{32}FNO_5$: C, 70.59; H, 6.49; N, 2.84; F, 3.85. Found: C, 70.58; H, 6.55; N, 3.10; F, 4.02.

(*b*) *From* **7**. A solution of **7** (2.1 g) in anhydrous acetonitrile (60 mL) was refluxed with tetrabutylammonium fluoride (15 g) for 72 h. Most of the acetonitrile was evaporated, the residue taken up in chloroform, and the solution washed with water, dried (Na_2SO_4), and concentrated to dryness. The residue precipitated from chloroform–ether as an amorphous solid (1.65 g, 91%), m.p. 186–187°, $[\alpha]_D^{22}$ +117° (*c* 1, chloroform).

*2-Acetamido-2,4-dideoxy-4-fluoro-*D*-galactopyranose* (**21**). — A solution of **20** (1.8 g) in acetic acid (50 mL) was hydrogenolyzed in the presence of Pd–C (1.5 g, 10%) for 48 h. After the usual workup, the product crystallized from ethanol–ether (800 mg, 85%), m.p. 195–198°, $[\alpha]_D^{22}$ +85.5° (*c* 1, methanol); ν_{max}^{KBr} 3500–3200 (OH, NH), 1630 and 1550 cm^{-1} (C=O, amide); ^{1}H-n.m.r. (D_2O; external $SiMe_4$): δ 2.50 (s, 3 H, NAc), 5.57 (d, 1 H, $J_{1,2}$ 3.2 Hz, H-1); ^{13}C-n.m.r. (D_2O; external $SiMe_4$): δ 23.10 (CH_3, NAcα), 23.35 (CH_3, NAcβ), 82.97 (d, $J_{C,F}$ 165.10 Hz, C-4α), 83.68 (d, $J_{C,F}$ 171.71 Hz, C-4β), 92.11 (d, C-1α), 96.02 (d, C-1β), and 175.5 (C=O, NAc); ^{19}F-n.m.r. (D_2O; external $CFCl_3$): δ −218.75 (compl. oct., $J_{F\text{-}4,H\text{-}4}$ 50.10 Hz, F-4β) and −222.96 (compl. sext. $J_{F\text{-}4,H\text{-}4}$ 50.70 Hz, F-4α).

Anal. Calc. for $C_8H_{14}FNO_5$: C, 43.05; H, 6.33; N, 6.28; F, 8.52. Found: C, 42.92; H, 6.44; N, 6.16; F, 8.75.

*2-Acetamido-1,3,6-tri-*O*-acetyl-2,4-dideoxy-4-fluoro-α-*D*-galactopyranose* (**22**). — Compound **21** (1 g) was acetylated with acetic anhydride (5 mL) in pyridine (10 mL) overnight. Ice–water was added and the solution was concentrated to dryness. The residue was freed from pyridine by evaporation with toluene and the product crystallized from ether. It was recrystallized from ethanol–ether (1.5 g, 90%), m.p. 155–158°, $[\alpha]_D^{22}$ +144° (*c* 1, chloroform); ν_{max}^{KBr} 3225 (NH), 1740 (C=O, OAc), 1650 and 1545 cm^{-1} (C=O, amide): ^{1}H-n.m.r. ($CDCl_3$): δ 1.97 (s, 3 H, NAc), 2.1 (s, 3 H, OAc), 2.15 (s, 3 H, OAc), 2.21 (s, 3 H, OAc), 5.52 (d, 1 H, *J* 10.10 Hz, NH), and 6.25 (d, 1 H, $J_{1,2}$ 3.95 Hz, H-1); ^{13}C-n.m.r. ($CDCl_3$): δ 20.66, 20.74 and 20.84 (CH_3, OAc), 22.97 (CH_3, NAc), 46.85 (d, $J_{C\text{-}2,F\text{-}4}$ 2.4 Hz, C-2), 61.36 (d, $J_{C,F}$ 6.1 Hz, C-6), 68.06 (d, $J_{C,F}$ 18.6 Hz, C-5), 68.79 (d, $J_{C,F}$ 18.6 Hz, C-3), 85.81 (d, $J_{C,F}$ 186.6 Hz, C-4), 91.04 (C-1), 168.63, 170.10, 170.17, and 171.05 (C=O, OAc and NAc): ^{19}F-n.m.r. ($CDCl_3$–$CFCl_3$): δ −219.37 (sext., $J_{F\text{-}4\text{-}H\text{-}4}$ 50.30, $J_{F\text{-}4,H\text{-}3} = J_{F\text{-}4,H\text{-}5}$ 26.41 Hz, F-4).

Anal. Calc. for $C_{14}H_{20}FNO_8 \cdot H_2O$: C, 45.79; H, 5.99; N, 3.81; F, 5.18. Found: C, 46.09; H, 5.76; N, 3.66; F, 5.10.

*2-Amino-2,4-dideoxy-4-fluoro-*D*-galactose hydrochloride* (**17**). — A solution of **21** (500 mg) in 3M HCl (25 mL) was heated with stirring for 3 h at 95–100°. The solution was concentrated to dryness and the residue crystallized from methanol–ether to give **17** (240 mg, 78%), m.p. 187–188° (dec.), $[\alpha]_D^{22}$ +32.5° (*c* 1, water); ν_{max}^{KBr} 3500–3200 (OH, NH), 3100–2900 and 1600 cm^{-1} (salt); ^{19}F-n.m.r. (D_2O; external $CFCl_3$): δ −218.35 (compl. sext., $J_{F\text{-}4,H\text{-}4}$ 50.40, $J_{F\text{-}4,H\text{-}3} = J_{F\text{-}4,H\text{-}5}$ 29.10 Hz, F-4β) and −220.97 (sext., $J_{F\text{-}4,H\text{-}4}$ 50.60, $J_{F\text{-}4,H\text{-}3} = J_{F\text{-}4,H\text{-}4}$ 29.60 Hz, F-4α).

Anal. Calc. for $C_6H_{13}ClFNO_4 \cdot 0.5H_2O$: C, 31.70; H, 6.14; N, 6.18; F, 8.38. Found: C, 31.35; H, 6.14; N, 6.06; F, 8.21.

*2-Acetamido-2,4-dideoxy-4-fluoro-*D*-galactono-1,5-lactone* (**27**). — A solution of **21** (400 mg) in water (15 mL) was treated as described for the preparation of **26**. After 7 h, the reaction was complete, the mixture was filtered, and the filtrate was neutralized with Dowex 50W-X8 (H^+) cation-exchange resin. After filtration, the solution was concentrated and the residue solidified by addition of ethanol–ether to give **27** (320 mg, 75%), m.p. 62°, $[\alpha]_D^{22}$ +2.5° (*c* 1, methanol); ν_{max}^{KBr} 3400–3100 (OH, NH), 1720 (C=O, lactone), 1635 and 1540 cm^{-1} (C=O, amide); ^{19}F-n.m.r. (D_2O; external $CFCl_3$): δ −208.8 (q, $J_{F\text{-}4,H\text{-}4}$ 48.85, $J_{F\text{-}4,H\text{-}3} = J_{F\text{-}4,H\text{-}5}$ 28.50 Hz, F-4).

Anal. Calc. for $C_8H_{12}FNO_5 \cdot 0.5H_2O$: C, 41.79; H, 5.65; N, 6.08; F, 8.26. Found: C, 42.01; H, 5.90; N, 6.29; F, 7.89.

*Benzyl 2-acetamido-4-*O*-benzoyl-3,6-di-*O*-benzyl-2-deoxy-α-*D*-galactopyranoside* (**23**). — A solution of **7** (5 g) and lithium benzoate (30 g) in dry *N,N*-dimethylformamide (100 mL) was heated for 48 h at 160–165° (bath temp.) with stirring. The mixture was cooled and poured into ice–water, and the precipitate filtered off, washed with water, and taken up in chloroform. The solution was washed once with water, dried (Na_2SO_4) and concentrated. The residue crystallized from ether–petroleum ether to give **23** (5 g, 96%), m.p. 105–106°, $[\alpha]_D^{22}$ +176° (*c* 1, chloroform); ν_{max}^{KBr} 3300 (NH), 1730 (C=O, Bz), 1645 and 1550 (C=O, amide), and 720–700 cm^{-1} (arom.); ^{1}H-n.m.r. ($CDCl_3$): δ 1.90 (s, 3 H, NAc), 3.60 (m, 2 H, H_2-6), 3.75 (m, 1 H, H-5), 5.10 (d, 1 H, $J_{1,2}$ 3.50 Hz, H-1), 5.25 (d, 1 H, *J* 9.45 Hz, NH), 5.85 (d, 1 H, *J* 2.85 Hz, H-4), 7.50 and 8.10 (20 H, arom.).

Anal. Calc. for $C_{36}H_{37}NO_7$: C, 72.59; H, 6.26; N, 2.35. Found: C, 72.79; H, 6.46; N, 2.36.

*Benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-*D*-galactopyranoside* (**24**). — A solution of **23** (4.2 g) in absolute methanol (160 mL) containing sodium methoxide (0.85 mg) was refluxed for 7 h. The solution was concentrated, the residue taken up in chloroform, washed with water, dried (Na_2SO_4), and evaporated. The residue was chromatographed on a column of silica gel. After elution with ether to remove impurities (mostly methyl benzoate), the product was eluted with ethyl acetate. After evaporation, the residue crystallized from chloroform–ether (3.1 g, 92%), m.p. 148–149°, ν_{max}^{KBr} 3540 (OH), 3310 (NH), 1645 and 1545 (C=O, amide), and 700–720 cm^{-1} (arom.); ^{1}H-n.m.r. ($CDCl_3$): δ 1.85 (s, 3 H, NAc), 2.65 (br. s, 1 H, OH), 4.85 (d, 1 H, $J_{1,2}$ 3.62 Hz, H-1), 5.3 (d, 1 H, *J* 9.10 Hz, NH), and 7.25 (br. s, 15 H, arom.).

Anal. Calc. for $C_{29}H_{33}NO_6$: C, 70.86; H, 6.77; N, 2.85. Found: C, 70.77; H, 6.76; N, 3.01.

Benzyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-4-O-mesyl-α-D-galactopyranoside (**25**). — To a solution of **24** (5 g) in dry pyridine (40 mL) was added a solution of methanesulfonyl chloride (7 mL) in pyridine (10 mL) as described for the preparation of **7**. The residue was chromatographed on a silica gel column and the product was eluted with dichloromethane as a crystalline solid. It was recrystallized from chloroform–ether (5.5 g, 95%), m.p. 167–168°, $[\alpha]_D^{22}$ +127° (*c* 1, chloroform); ν_{max}^{KBr} 3320 (NH), 1650 and 1555 (C=O, amide), 1351 and 1180 (SO_2), and 700 cm^{-1} (arom.); ^{1}H-n.m.r. ($CDCl_3$): δ 1.85 (s, 3 H, NAc), 3.10 (s, 3 H, CH_3SO_3), 4.30 (d, 1 H, $J_{1,2}$ 3.60 Hz, H-1), and 7.25 (m, 15 H, arom.).

Anal. Calc. for $C_{30}H_{35}NO_8S$: C, 63.26; H, 6.19; N, 2.46; S, 5.62. Found: C, 63.18; H, 6.36; N, 2.59; S, 5.65.

Benzyl 2-acetamido-3,6-di-O-benzyl-2,4-dideoxy-4-fluoro-α-D-glucopyranoside (**13**). — To a solution of *N,N*-diethylaminosulfur trifluoride (2 mL) in dichloromethane (2 mL), cooled to −20° was added a solution of **24** (1 g) in dichloromethane (3 mL) with stirring. The solution was stirred at −20° for 30 min, and then at room temperature for 3 h after addition of anhydrous pyridine (1 mL). The mixture was worked up as described for the preparation of **20**. The colored residue was chromatographed on a column of silica gel. The product was eluted with 9:1 dichloromethane–ethyl acetate and the residue crystallized from chloroform–ether (700 mg, 82%), m.p. 169–170°, $[\alpha]_D^{22}$ +105.5° (*c* 1, chloroform); ν_{max}^{KBr} 3310 (NH), 1650 and 1545 (C=O, amide), and 690–730 cm^{-1} (arom.); ^{1}H-n.m.r. ($CDCl_3$): δ 1.80 (s, 3 H, NAc), 4.90 (d, 1 H, $J_{1,2}$ 3.11 Hz, H-1), 5.25 (d, 1 H, *J* 8.45 Hz, NH), and 7.28–7.31 (15 H, arom.); ^{13}C-n.m.r. ($CDCl_3$–$CFCl_3$): δ 23.20 (CH_3, NAc), 51.65 (d, C-2), 68.45 (d, C-5), 69.7, 73.66 (d, C-3), 75.6, 76.9, 90.5 (d, $J_{C,F}$ 182.10 Hz, C-4), 136.7, 137.8, and 138.05 (quaternary arom. C), and 169.4 (C=O); ^{19}F-n.m.r. ($CDCl_3$–$CFCl_3$): δ −194.70 (q, $J_{F\text{-}4,H\text{-}4}$ 50.5, $J_{F\text{-}4,H\text{-}3} = J_{F\text{-}4,H\text{-}5}$ 14.11 Hz, F-4).

Anal. Calc. for $C_{29}H_{32}FNO_5$: C, 70.59; H, 6.49; N, 2.84; F, 3.85. Found: C, 70.53; H, 6.60; N, 2.80; F, 3.59.

2-Acetamido-2,4-dideoxy-4-fluoro-D-glucopyranose (**14**). — Compound **13** (800 mg) in acetic acid (20 mL) was hydrogenolyzed in the presence of Pd–C (1 g, 10%) for 48 h. After the usual workup and evaporation of solvent, the residue crystallized from ethanol–ether (300 mg, 70%), m.p. 174–175°, $[\alpha]_D^{22}$ +61.5° (*c* 1, methanol); ν_{max}^{KBr} 3500–3200 (OH, NH), 1640 and 1555 cm^{-1} (C=O, amide); ^{13}C-n.m.r. (D_2O; external $SiMe_4$): δ 20.20 and 20.60 (CH_3, NAcα,β), 54.55 (d, C-2α), 57.1 (C-2β), 59.7 (d, C-6α,β), 68.6, 68.7, 70.3, 71.2, 72.1, 73.1, 88.3 (d, $J_{C,F}$ 180.7 Hz, C-4α,β), 89.4 (C-1α), 93.4 (C-1β), and 176.2 (C=O); ^{19}F-n.m.r. (D_2O; external $CFCl_3$): δ −197.04 (q, $J_{F\text{-}4,H\text{-}4}$ 50.00, $J_{F\text{-}4,H\text{-}3} = J_{F\text{-}4,H\text{-}5}$ 13.20 Hz, F-4β) and −199.3 (q, $J_{F\text{-}4,H\text{-}4}$ 49.90, $J_{F\text{-}4,H\text{-}3} = J_{F\text{-}4,H\text{-}5}$ 13.60 Hz, F-4α).

Anal. Calc. for $C_8H_{14}FNO_8$: C, 43.05; H, 6.33; N, 6.28; F, 8.52. Found: C, 43.11; H, 6.49; N, 6.54; F, 8.30.

A sample of **14** (500 mg) was oxidized with molecular O_2 as described for the

preparation of **27** to give 2-acetamido-2,4-dideoxy-4-fluoro-D-glucono-1,5-lactone, isolated as an amorphous solid from ethanol–ether, $[\alpha]_D^{22}$ +11.5° (*c* 1, methanol); ν_{max}^{KBr} 3500–3200 (OH, NH), 1730 (C=O, lactone), 1640 and 1545 cm^{-1} (C=O, amide). It was a good inhibitor of *N*-acetyl-β-D-hexosaminidase; K_i 1.1mM.

Anal. Calc. for $C_8H_{12}FNO_5 \cdot 0.5H_2O$: C, 41.70; H, 5.65; N, 6.08; F, 8.26. Found: C, 41.91; H, 5.76; N, 6.22; F, 7.92.

*2-Acetamido-1,3,6-tri-*O*-acetyl-2,4-dideoxy-4-fluoro-α-*D*-glucopyranose* (**15**). — Compound **14** (1.7 g) was acetylated with acetic anhydride (15 mL) in pyridine (25 mL) overnight. After addition of ice–water, the solution was concentrated to dryness and the residue chromatographed on a short column of silica gel. The product was eluted with 1:4 ethyl acetate–chloroform as an amorphous solid (2 g, 73%), $[\alpha]_D^{22}$ +45° (*c* 1, chloroform); ν_{max}^{KBr} 3300 (NH), 1735 (C=O, OAc), 1650 and 1550 cm^{-1} (C=O, amide); ^{1}H-n.m.r. ($CDCl_3$): δ 1.85 (s, 3 H, NAc); 2.10 (m, 9 H, 3 OAc), 5.95 (d, 1 H, *J* 9.20 Hz, NH), 6.15 (d, 1 H, $J_{1,2}$ 3.50 Hz, H-1); ^{13}C-n.m.r. ($CDCl_3$): δ 20.7 (CH_3, OAc), 22.80 (CH_3, NAc), 50.6 (d, $J_{F\text{-}4,C\text{-}2}$ 7.04 Hz, C-2), 61.5 (C-6), 68.6, 69.5, 70.2, 70.9, 86.1 (d, $J_{F\text{-}4,C\text{-}4}$ 186.90 Hz, C-4), 90.27 (C-1), 168.5, 170.2, 170.3, and 171.3 (C=O, NAc, OAc); ^{19}F-n.m.r. ($CDCl_3$–$CFCl_3$): −194.01 (q, $J_{F\text{-}4,H\text{-}4}$ 49.8, $J_{F\text{-}4,H\text{-}3} = J_{F\text{-}4,H\text{-}5}$ 13.7 Hz, F-4).

Anal. Calc. for $C_{14}H_{20}FNO_8 \cdot 0.5H_2O$: C, 46.89; H, 5.86; N, 3.90; F, 5.30. Found: C, 47.12; H, 6.09; N, 3.74; F, 5.05.

*Benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-*D*-*xylo*-hexo-4-ulopyranoside* (**28**). — (*a*) *Oxidation of* **11** *with chromium trioxide–pyridine complex.* To a solution of chromium trioxide–pyridine complex [prepared from Cr_2O_3 (200 mg) and dry pyridine (0.3 mL)] in dichloromethane (40 mL) was added a solution of **11** (250 mg) in dichloromethane (5 mL), followed by acetic anhydride (0.25 mL). After stirring for 30 min, 1:1 ethyl acetate–absolute ethanol (3 mL) was added, and the solution was poured onto a column of silica gel and eluted with ethyl acetate in one fraction. After evaporation of the solvent, the residue was taken up in chloroform, and the solution washed with water, dried (Na_2SO_4) and concentrated to dryness. The residue crystallized from ether–petroleum ether to give **28** (75 mg, 30%), m.p. 110–111°, $[\alpha]_D^{22}$ +163° (*c* 1, chloroform); ν_{max}^{KBr} 3300 (NH), 1740 (C=O), 1640 and 1550 (C=O, amide), 690 and 725 cm^{-1} (arom.); ^{1}H-n.m.r. ($CDCl_3$): δ 1.85 (s, 3 H, NAc), 5.25 (d, 1 H, $J_{1,2}$ 3.20 Hz, H-1), 5.45 (d, 1 H, *J* 9.45 Hz, NH), and 7.3 (br. s, 15 H, arom.).

Anal. Calc. for $C_{29}H_{31}NO_6$: C, 71.15; H, 6.38; N, 2.86. Found: C, 70.94; H, 6.17; N, 3.02.

(*b*) *Oxidation of* **11** *with dimethyl sulfoxide and acetic anhydride.* To a solution of **11** (1.6 g) in anhydrous dimethyl sulfoxide (8 mL) was added acetic anhydride (5 mL), and the mixture was stirred at room temperature for 140 h. Ice–water was added and the semisolid precipitate was filtered off, washed once with water, and taken up in chloroform. The solution was washed once with water, dried (Na_2SO_4), and concentrated. The residue was chromatographed on a column of silica gel and eluted with 1:4 dichloromethane–ethyl acetate. The product crys-

tallized from ether–petroleum ether (1.1 g, 82%), m.p. 109–111°, $[\alpha]_D^{22}$ +160.5° (*c* 1, chloroform).

*Benzyl 2-acetamido-3,6-di-*O*-benzyl-2,4-dideoxy-4,4'-difluoro-α-*D*-xylo-hexopyranoside* (**29**). — To a cold solution of *N,N*-diethylaminosulfur trifluoride (2 mL) in dry dichloromethane (2 mL) at −20°, was added a solution of **28** (1.0 g) in dichloromethane (5 mL) under N_2. The solution was stirred at room temperature for 6 h and 1 h at 40°, cooled to −20°, and absolute ethanol (2 mL) was added and stirred for 4 min, followed by addition of ice–water. After the usual workup, the semisolid, light-colored residue was chromatographed on a column of silica gel. The product was eluted with 9:1 dichloromethane–ethyl acetate and crystallized from ether (510 mg, 51%), m.p. 175–176°, $[\alpha]_D^{22}$ +80.5° (*c* 0.5, chloroform); ν_{max}^{KBr} 3310 (NH), 1650 and 1545 (C=O, amide), 710 and 690 cm^{-1} (arom.); ^{1}H-n.m.r. ($CDCl_3$): δ 1.80 (s, 3 H, NAc), 4.95 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 5.50 (d, 1 H, *J* 9.4 Hz, NH), and 7.25 (m, 15 H, arom.); ^{19}F-n.m.r. ($CDCl_3$): δ −115.41 (dd, $J_{F\text{-}4e,H\text{-}3} = J_{F\text{-}4e,H\text{-}5}$ 3.30 Hz, F-4*e*), and −134.20 (qq, $J_{F\text{-}4a,H\text{-}3} = J_{F\text{-}4a,H\text{-}5}$ 19.00 Hz, $J_{F\text{-}4a,F\text{-}4e}$ 249.30 Hz, F-4*a*).

Anal. Calc. for $C_{29}H_{31}F_2NO_5$: C, 68.09; H, 6.11; N, 2.74; F, 7.43. Found: C, 67.86; H, 6.11; N, 2.73; F, 7.44.

*2-Acetamido-2,4-dideoxy-4,4-difluoro-*D*-xylo-hexopyranose* (**30**). — A solution of **29** (350 mg) in acetic acid (20 mL) was hydrogenolyzed in the presence of Pd–C (300 mg, 10%) for 48 h. After the usual workup, the product crystallized from ethanol–ether (155 mg, 89%), m.p. 211–212°, $[\alpha]_D^{22}$ +61° (*c* 1, water); ν_{max}^{KBr} 3500–3200 (NH, OH), 1620 and 1540 cm^{-1} (C=O, amide); ^{19}F-n.m.r. (D_2O; external $CFCl_3$): δ −114 (d, $J_{F\text{-}4a,F\text{-}4e}$ 249.1 Hz, F-4*eβ*), −120.4 (d, $J_{F,F}$ 249.2 Hz, F-4*eα*), −136.3 (q, $J_{F,F}$ 249.2 Hz, F-4*aβ*), and −137.9 (sext., $J_{F,F}$ 249.3 Hz, F-4*aα*).

Anal. Calc. for $C_8H_{13}F_2NO_5$: C, 39.84; H, 5.43; N, 5.81; F, 15.75. Found: C, 40.01; H, 5.52; N, 5.64; F, 15.55.

*2-Acetamido-1,3,6-tri-*O*-acetyl-2,4-dideoxy-4,4-difluoro-*D*-xylo-hexopyranose* (**31**). — Compound **30** (300 mg) was acetylated with acetic anhydride (4 mL) and pyridine (7 mL) overnight. Ice–water was added and the solution concentrated to dryness. The residue was chromatographed on a silica gel column. The product was eluted with ethyl acetate and crystallized from chloroform–ether (350 mg, 90%), m.p. 128–129°, $[\alpha]_D^{22}$ +81° (*c* 1, chloroform); ν_{max}^{KBr} 3230 (NH), 1745 (C=O, OAc), 1645 and 1550 (C=O, amide); ^{1}H-n.m.r. ($CDCl_3$): δ 1.85 (s, 3 H, NAc), 2.05 (s, 3 H, OAc), 2.10 (s, 3 H, OAc), 2.15 (s, 3 H, OAc), 5.45 (d, 1 H, *J* 9.45 Hz, NH), and 6.10 (t, 1 H, H-1); ^{19}F-n.m.r. ($CDCl_3$–$CFCl_3$): −116.58 (dd, $J_{F\text{-}4e,H\text{-}5} = J_{F,H\text{-}3}$ 3.5 Hz, F-4*eβ*), −119.7 (dd, $J_{F\text{-}4e,H\text{-}3} = J_{F\text{-}4a,H\text{-}5}$ 5.4 Hz, f-4*eα*), 132.5 (qq, $J_{F\text{-}4a,H\text{-}5} = J_{F\text{-}4a,H\text{-}3}$ 19.4 Hz, F-4*aβ*), and −133.6 (qq, $J_{F\text{-}4a,H\text{-}5} = J_{F\text{-}4a,H\text{-}3}$ 19.6, $J_{F\text{-}4a,4\text{-}e}$ 250.7 Hz, F-4*aα*).

Anal. Calc. for $C_{14}H_{19}F_2NO_8$: C, 45.78; H, 5.21; N, 3.81; F, 10.37. Found: C, 45.89; H, 4.99; N, 3.79; F, 10.18.

Determination of effect on L1210 leukemia cell growth (Table I). — Cultures were inoculated with 5 10^4 cells mL^{-1} in RPMI 1640 medium containing 10% fetal bovine serum. Sugar analogs were added, and the cell number was measured and %

control (no sugar analog added) growth was calculated after 24 h. The ID_{50} (50% growth inhibitory concentration) was determined from the dose-response curve. All assays were performed in duplicate on at least two separate occasions.

Determination of effect of compound **22** *on macromolecular biosynthesis in L1210 leukemia cells (Table II).* — L1210 cells were incubated in microtiter wells (200 μL per well) containing various radiolabeled precursors for 5 h. Quadruplicate wells were assayed for each time and dose determination. After the incubation, cells were harvested on filter paper and washed in an automatic cell harvester apparatus. Discs were cut out, and cellular radioactivity quantitated by scintillation counting. results are expressed as percentage of control incorporation and represent the mean of three separate experiments.

Determination of effect of compound **22** *on L1210 leukemia cells in DBA/2HA mice* in vivo *(Table III).* — DBA/2J Mice (19–20 g) were inoculated i.p. with 10^6 L1210 leukemia cells on day zero. Starting on day one, mice were given various doses (5 mice per dosage level) of the test agent i.p., once per day through day 5. Life span was monitored daily. The % measure in life span (% ILS) was calculated as compared to the control group consisting of 8–10 animals.

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