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Synthesis of 4-deoxy-4-fluoro analogues of 2-acetamido-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-galactose and their effects on cellular glycosaminoglycan biosynthesis

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Abstract

4-Deoxy-4-fluoro analogues of 2-acetamido-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-galactose were synthesized and evaluated as inhibitors of hepatic glycosaminoglycan biosynthesis. 2-Acetamido-1,3,6-tri-*O*-acetyl-2,4-dideoxy-4-fluoro-D-glucopyranose (**16**) exhibited a reduction of [^{3}H]GlcN and [^{35}S]SO_4 incorporation into hepatocyte cellular glycosaminoglycans to 12 and 18%, respectively, of the control cells, at 1.0 mM. Similarly, 2-acetamido-1,3,6-tri-*O*-acetyl-2,4-dideoxy-4-fluoro-D-galactopyranose (**31**) exhibited a reduction of [^{3}H]GlcN and [^{35}S]SO_4 incorporation to 1 and 9%, respectively, of the control cells, at 1.0 mM. Unlike **16**, **31** exhibited a reduction of [^{14}C]Leu incorporation into cellular protein to 57% of control cells, at 1.0 mM.

Keywords: Glycosaminoglycans; 2-Acetamido-2,4-dideoxy-4-fluoro-D-glucopyranose derivatives; 2-Acetamido-2,4-dideoxy-4-fluoro-D-galactopyranose derivatives

1. Introduction

Cell-surface heparan sulfate (HS) proteoglycans play an important role in regulating several biological functions of a cell, including cell–cell migration, adhesion, growth and communication, and can serve as receptors for various proteins. HS has been identified as a receptor for herpes simplex virus [1] and is implicated in tumor invasion and metastasis [1,2], arthritis [3], adult-onset diabetes [4], and Alzheimer's disease [5].

The availability of synthetic HS analogues to perturb glycosaminoglycan (GAG) biosynthesis could greatly affect the above-mentioned disorders. The GAG chain of HS is composed of repeating disaccharides of (1→4)-linked D-glucosamine and uronic acid residues that are variably N- and O-sulfonated. This polysaccharide chain is attached by a tetrasaccharide linkage region consisting of -D-GlcA-β-(1→3)-D-Gal-β-(1→3)-D-Gal-β-(1→4)-D-Xyl-β- to L-serine, and the serine residue is attached to the core protein. We have shown [6] that 3-deoxy-D-*xylo*-hexose ('3-deoxy-D-galactose') and a 4-chloro-4-de-

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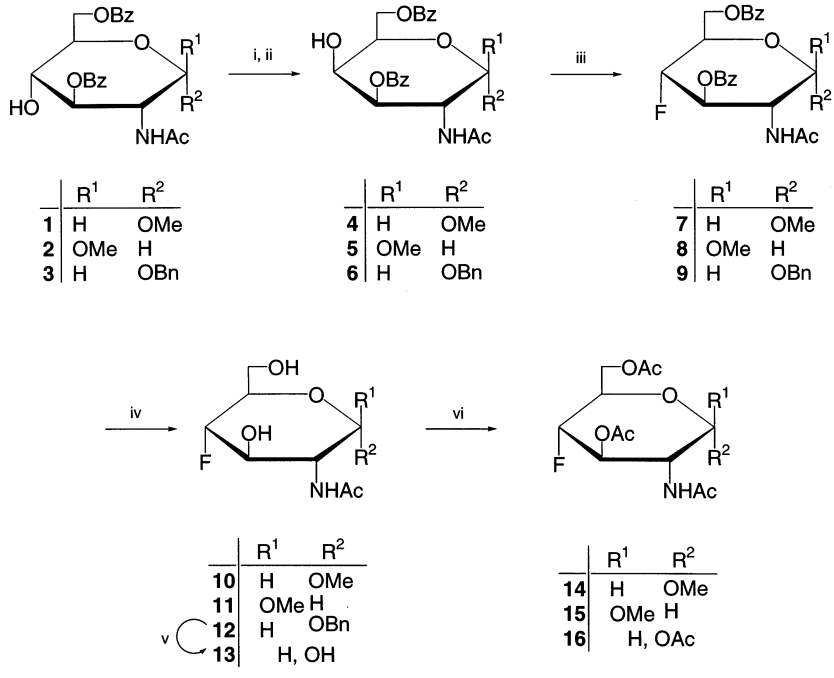
oxy analogue of a uronic acid, namely methyl (methyl 4-chloro-4-deoxy-β-D-galactopyranosid)uronate [6], significantly reduced D-[^{3}H]glucosamine ([^{3}H]GlcN) and [^{35}S]sulfate ([^{35}S]SO_4) incorporation into cellular GAGs of mouse hepatocytes in culture. The latter compound also exhibited a significant reduction of L-[^{14}C]leucine ([^{14}C]Leu) incorporation into total cellular protein synthesis in vitro. Recently, we also found [7] that some 4-deoxy analogues of 2-acetamido-2-deoxy-D-glucose were significant inhibitors of both protein and glycoconjugate biosynthesis.

Fluorinated carbohydrates have significant biological activities [8] and are of interest as biological probes, antitumor agents, and inhibitors of hexokinases. The altered hydrogen-bonding properties of fluorine and its atomic size relative to that of hydrogen make it of interest in the synthesis of other HS analogues. Sharma et al. [9] have evaluated a series of 4- and 6-fluoro derivatives of 2-acetamido-2-deoxy-D-hexopyranoses on L1210 leukemia cell growth in vitro and found significant inhibition of both total protein and glycoconjugate biosynthesis.

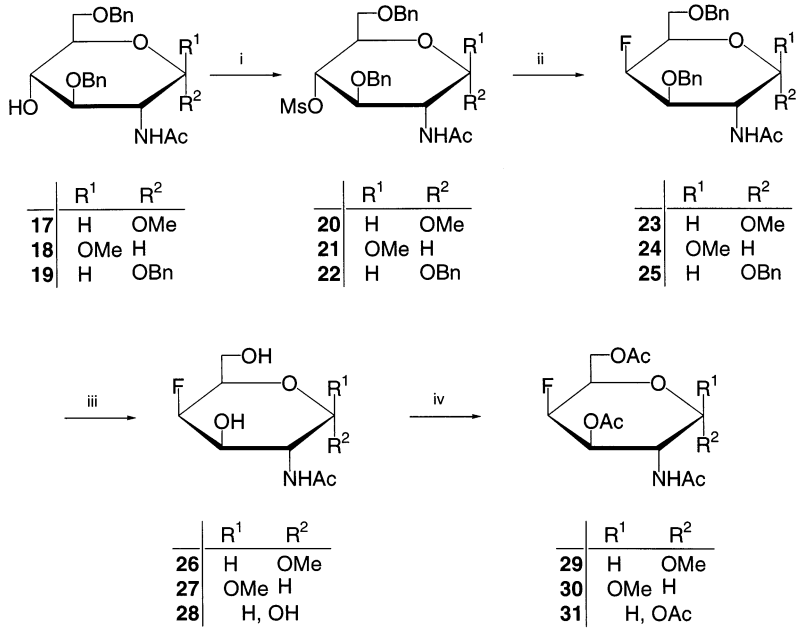
Herein we report the synthesis and biological evaluation of a series of 2-acetamido-2,4-dideoxy-4-fluoro-D-glucopyranoses and of 2-acetamido-2,4-dideoxy-4-fluoro-D-galactopyranoses, directed towards influencing cellular GAG and total protein biosynthesis.

2. Results and discussion

Chemical synthesis.—4-Fluoro analogues of 2-acetamido-2,4-dideoxy-D-glucose (Scheme 1) were synthesized by double inversion of the configuration at C-4 of the corresponding 3,6-di-*O*-benzoyl analogues. The 3,6-di-*O*-benzoyl analogues **1**–**3** were prepared from the corresponding methyl and benzyl glycosides in a one-step fashion following the procedure of Wang and Lee [10]. Briefly, methyl 2-acetamido-2-deoxy-α-D-glucopyranoside [11], methyl 2-acetamido-2-deoxy-β-D-glucopyranoside [11] and benzyl 2-acetamido-2-deoxy-α-D-glucopyranoside [12] were each treated with benzoyl chloride in pyridine at −45 °C to afford **1** (82.4%), **2** [10] (70.9%), and **3** [13] (76.9%), respectively, following flash chromatography. Compounds **1**, **2**, and **3** were each treated with trifluoromethanesulfonic anhydride in pyridine to afford the 4-*O*-triflyl derivatives, which were isolated and immediately treated with sodium nitrite in DMF to produce the inversion products **4** [10] (58.8%),



Scheme 1. Synthesis of 4-fluoro analogues of 2-acetamido-2,4-dideoxy-D-glucopyranose. Reagents: (i) Tf_2O, pyr; (ii) $NaNO_2$, DMF; (iii) DAST, CH_2Cl_2; (iv) KOH, MeOH; (v) H_2, Pd–C, AcOH; (vi) Ac_2O, pyr.



	R1	R2
17	H	OMe
18	OMe	H
19	H	OBn

	R1	R2
20	H	OMe
21	OMe	H
22	H	OBn

	R1	R2
23	H	OMe
24	OMe	H
25	H	OBn

	R1	R2
26	H	OMe
27	OMe	H
28	H, OH	

	R1	R2
29	H	OMe
30	OMe	H
31	H, OAc	

Scheme 2. Synthesis of 4-fluoro analogues of 2-acetamido-2,4-dideoxy-D-galactopyranose. Reagents: (i) MsCl, pyr; (ii) TBAF, MeCN; (iii) H_2, Pd–C, AcOH; (iv) Ac_2O, pyr.

5 [10] (50.8%), and **6** [14] (58.2%), respectively. ^{1}H NMR spectroscopy provided evidence for the inversion of configuration at C-4 for each of compounds **4**–**6** by the disappearance of a discernible H-4–H-5 coupling, an observation indicative of the *galacto* configuration, which was confirmed by NOE experiments.

Treatment of each of compounds **4**, **5**, and **6** with (diethylamino)sulfur trifluoride (DAST) afforded **7** (81.4%), **8** (40.2%), and **9** (85.2%), respectively. The low yield for the conversion of **5** into **8** is a result of the formation of two unknown side-products, one of which was a fluorinated compound. The inversion of configuration at C-4 for each of compounds **7**–**9** was determined by ^{1}H NMR spectroscopy and NOE experiments. Also, ^{19}F NMR spectroscopy of **7**–**9** revealed in each case a doublet of doublets at $\delta \sim 197$ ($J \sim 14$ and 51 Hz), a result indicative of an equatorially disposed fluorine atom at C-4 [15].

Compounds **7**, **8**, and **9** were each O-debenzoylated using KOH in MeOH to afford **10** (90.2%), **11** (81.4%), and **12** (92.5%), respectively. Removal of the benzyl protecting group of **12** by catalytic hydrogenolysis in the presence of 10% Pd–C and hydrogen afforded **13** [9a] (89.1%), which was acetylated with acetic anhydride and pyridine to afford 2-acetamido-1,3,6-tri-*O*-acetyl-2,4-dideoxy-4-fluoro-D-glucopyranose [9a] (**16**, 93.2%). ^{1}H NMR spectroscopy of **13** indicated a mixture of α and β anomers in a ratio of 5.7:1, respectively. Compounds **10** and **11** were each acetylated, as above, to afford methyl 2-acetamido-3,6-di-*O*-acetyl-2,4-dideoxy-4-fluoro-α-D-glucopyranoside (**14**, 88.2%) and methyl 2-acetamido-3,6-di-*O*-acetyl-2,4-dideoxy-4-fluoro-β-D-glucopyranoside (**15**, 86.8%), respectively.

The synthesis of 4-fluoro analogues of 2-acetamido-2,4-dideoxy-D-galactose (Scheme 2) was attempted from **1**–**3** using DAST, but was unsuccessful. An alternative route employing the 3,6-di-*O*-benzyl analogues, **17**–**19**, was investigated. Compounds **17** [16], **18** [17], and **19** [18] were each prepared according to literature procedures and were treated with methanesulfonyl chloride in pyridine to afford **20** (75.2%), **21** (73.6%), and **22** [9a,19] (77.5%), respectively. Compounds **20**, **21**, and **22** were each treated with anhydrous tetra-*n*-butylammonium fluoride (TBAF) [20] in MeCN to afford **23** (54.3%), **24** (64.0%), and **25** [9a,19] (62.0%), respectively. The difficulty of fluorine substitution at C-4 of **20**–**22** is evident by the low yields and long reaction times (MeCN at reflux temperature, 4–6 days). Notably, the TBAF must be completely dry before use. ^{1}H NMR spectroscopy and NOE experiments

confirmed the *galacto* configuration for each of **23**–**25**. Furthermore, the ^{19}F NMR spectrum for each of **23**–**25** exhibited a doublet of triplets at $\delta \sim 220$ ($J \sim 28$ and 50 Hz) indicative of an axially disposed fluorine atom at C-4 [9a,19].

Removal of the benzyl protecting groups of each of **23**–**25** by catalytic hydrogenolysis in the presence of 10% Pd–C and hydrogen afforded **26** (98.2%), **27** (86.5%), and **28** [9a] (87.6%), respectively, which were each acetylated to afford compounds **29** (84.1%), **30** (88.9%), and **31** [9a] (quant), respectively. ^1H NMR spectroscopy of **31** indicated a mixture of α and β anomers in a ratio of 3.2:1, respectively.

Biological evaluation.—Murine hepatocytes were isolated and placed in culture as described previously [6], and these were then treated with [^{3}H]GlcN and [^{35}S]SO_4 or with

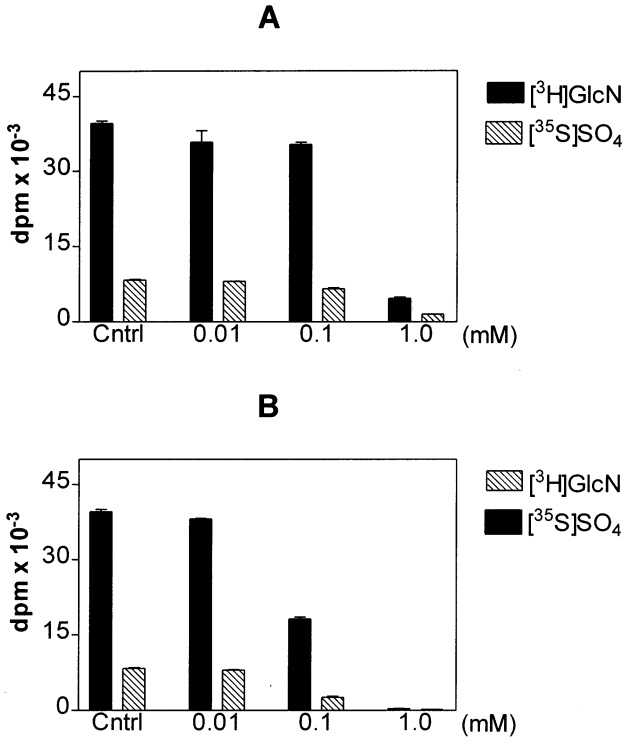


Fig. 1. Effects of increasing concentrations of compounds **16** and **31** on hepatocyte cellular GAG synthesis. Hepatocyte cultures were incubated with D-[^{3}H]glucosamine and [^{35}S]sulfate for 24 h in the absence (control) and presence of compounds **16** (panel A) and **31** (panel B) at 0.01, 0.1, and 1.0 mM. The values represent the mean ± S.D. of triplicate cultures. Statistical analyses using an unpaired *t*-test revealed that in panel A, control vs. 0.01 or 0.1 mM, not significant; control vs. 1.0 mM, $P < 0.01$. In panel B, control vs. 0.01 mM, not significant; control vs. 0.1 mM, $P < 0.01$; control vs. 1.0 mM, $P < 0.01$.

[^{14}C]Leu in the presence and absence of sugar analogues for 24 h. Peracetylated sugars are more lipophilic than their non-acetylated counterparts, a feature that allows for increased cellular uptake by passive diffusion [21]. Therefore, the fluorinated analogues were fully acetylated for in vitro testing.

No significant inhibitory effect was observed (results not shown) for the 4-fluoro analogues of methyl 2-acetamido-2,4-dideoxy-D-glucopyranoside (**14** and **15**), whereas the analogue **16** displayed a marked reduction of [^{3}H]GlcN and [^{35}S]SO_4 incorporation into hepatocyte cellular GAGs to 12 and 18%, respectively, of the control cells, at 1.0 mM (Fig. 1(A)). The 4-fluoro analogues of methyl 2-acetamido-2,4-dideoxy-D-galactopyranoside (**29** and **30**) exhibited no significant inhibitory effects on [^{3}H]GlcN and [^{35}S]SO_4 incorporation into isolated GAGs up to 1.0 mM (results not shown). The corresponding analogue **31** exhibited a large inhibition of cellular GAG synthesis by a reduction of [^{3}H]GlcN and [^{35}S]SO_4 incorporation to 1 and 9%, respectively, of the control cells, at 1.0 mM (Fig. 1(B)). It is possible that the methyl glycosides **14**, **15**, **29**, and **30** are poor substrates for lysosomal glycosidases as has been observed for 4-deoxy-D-glucopyranosides and 4-deoxy-4-fluoro-D-galactopyranosides [22,23].

The 4-fluoro analogues of *N*-acetyl-D-glucosamine and *N*-acetyl-D-galactosamine (**16** and **31**, respectively) were also evaluated for total protein inhibition by the measurement of [^{14}C]Leu incorporation into isolated glycoconjugates. Compound **16** exhibited no inhibition of cellular protein synthesis up to 1.0 mM (Fig. 2(A)); however, **31** exhibited a reduction in [^{14}C]Leu incorporation into cellular protein to 57% of the control cells, at 1.0 mM (Fig. 2(B)). The reduction in total protein synthesis observed for **31** may not be surprising since D-galactosamine is known to be rapidly converted into a UDP-analogue, a process which acts to trap uridine and deplete intracellular UDP pools, and which has been shown to inhibit protein biosynthesis [24,25].

Sharma et al. [9a] have shown that compounds **16** and **31** demonstrated ID_{50} values of 0.035 and 0.034 mM, respectively, for the inhibition of L1210 leukemia cell growth. Fur-

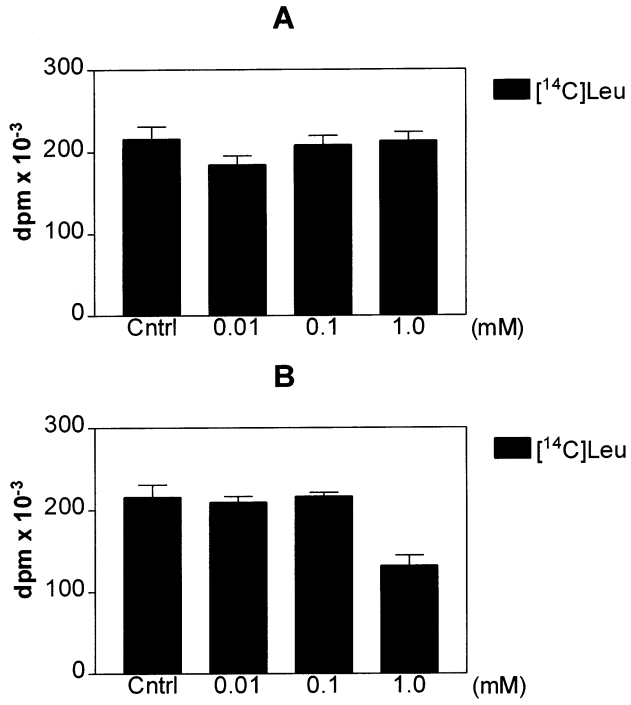


Fig. 2. Effects of increasing concentrations of compounds **16** and **31** on hepatocyte cellular total protein synthesis. Hepatocyte cultures were incubated with L-[^{14}C]leucine for 24 h in the presence of compounds **16** (panel A) and **31** (panel B) at 0.01, 0.1, and 1.0 mM. The values represent the mean $\pm$ S.D. of triplicate cultures. Statistical analyses using an unpaired t-test revealed that in panel A, control vs. 0.01, 0.1, or 1.0 mM, not significant. In panel B, control vs. 0.01 or 0.1 mM, not significant; control vs. 1.0 mM, $P < 0.01$.

thermore, in their study, compound **31** reduced the incorporation of [^{3}H]GlcN and [^{14}C]Leu into the total biosynthesized glycoconjugates of L1210 leukemia cells to 48 and 74%, respectively, of control cells at 1.0 mM [9a].

The mechanism of action of the monosaccharide derivatives described in the present work is under investigation.

3. Experimental

General methods and materials.—Melting points were determined on a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer 241 polarimeter for solutions in a 1 dm cell at room temperature (rt). ^{1}H, ^{13}C, and ^{19}F NMR spectra were recorded on a Bruker AM 400 spectrometer at 400.1, 100.6, and 376.5 MHz, respectively. The signals arising from residual protons in the deuterated solvents were used as internal standards. Chemical shifts (δ) are reported in ppm downfield from Me_4Si for ^{1}H and ^{13}C NMR spectra and downfield from $CFCl_3$ for ^{19}F NMR spectra. Thin-layer chromatography (TLC) was performed using glass plates precoated with EM Science Silica Gel 60 F_{254}. Flash chromatography was performed using EM Science Silica Gel 60 (230–400 mesh).

D-(1,6-$^{3}H_2$)Glucosamine·HCl (42.3 Ci/mmol), L-[^{14}C]leucine (320 mCi/mmol), and $Na_2^{35}SO_4$ (867 mCi/ml) were purchased from either Dupont or ICN Biomedicals. Laboratory chemicals of reagent grade were purchased from Sigma–Aldrich, Fisher Scientific, or BDH Chemicals. Williams' Medium E with L-glutamine, 10% fetal bovine serum, and antibiotic–antimycotic mixtures were supplied by Gibco. Fibronectin, collagenase, papain, and the GAG reference standards (chondroitin sulfate, hyaluronic acid, heparin) as sodium salts were purchased from Sigma–Aldrich.

*Preparation of methyl 2-acetamido-3,6-di-O-benzoyl-2-deoxy-α-*D*-galactopyranoside* (**4**).—To an ice-cold solution of **1** [7] (3.75 g, 8.46 mmol) in CH_2Cl_2 (50 mL) and pyr (7.0 mL) was added dropwise trifluoromethanesulfonic anhydride (4.76 g, 2 equiv). The solution was stirred for 20 min, diluted with CH_2Cl_2 (200 mL), and washed successively with ice-cold, aq solutions of $KHSO_4$ (10%) and $NaHCO_3$ (satd), and water, dried ($MgSO_4$), and concentrated under reduced pressure. The crude triflate was used without further purification. To a stirred solution in DMF (100 mL) was added $NaNO_2$ (1.75 g, 25.4 mmol), and after 40 h at rt, the mixture was concentrated and the residue was coevaporated with toluene under reduced pressure to afford an oil that was subjected to flash chromatography on silica gel (1:1 EtOAc–hexanes). Compound **4** was isolated as tiny, white needles (2.20 g, 58.8%): R_f 0.31 (3:1 EtOAc–hexanes); mp 202–205 °C, lit. 188–189 °C [10]; $[\alpha]_D$ +85.1° (c 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.86 (s, 3 H, NAc), 3.42 (s, 3 H, OMe), 4.22 (dd ~ t, 1 H, $J_{5,6} = J_{5,6'}$ 6.4 Hz, H-5), 4.26 (d, 1 H, H-4), 4.54 and 4.61 (dq, 2 H, J_{gem} 11.4 Hz, H-6, H-6′), 4.82 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 4.90

(ddd, 1 H, H-2), 5.32 (dd, 1 H, $J_{3,4}$ 3.0, $J_{3,2}$ 11.1 Hz, H-3), 5.79 (d, 1 H, $J_{NH,2}$ 9.8 Hz, NH), 7.38–7.55 (m, 6 H, Ph), 8.01–8.03 (m, 4 H, Ph); ^{13}C NMR ($CDCl_3$): δ 23.3 ($NCOCH_3$), 47.3 (C-2), 55.3 (OMe), 63.7 (C-6), 67.4 (C-4), 68.2 (C-5), 72.1 (C-3), 98.8 (C-1), 128.4–133.4 (Ph), 166.4 and 166.6 (2 Ph*C*=O), 170.2 (NC=O). Anal. Calcd for $C_{23}H_{25}NO_8$: C, 62.30; H, 5.68; N, 3.16. Found: C, 62.09; H, 5.84; N, 3.16.

*Preparation of methyl 2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-β-*D*-galactopyranoside* (**5**).—To a solution of trifluoromethanesulfonic anhydride (1.28 mL, 7.62 mmol) in CH_2Cl_2 (25 mL) at −15 °C was added dropwise a solution of pyr (1.24 mL, 15.3 mmol) in CH_2Cl_2 (4.0 mL); a solution of **2** [7,10] (2.11 g, 5.09 mmol) in CH_2Cl_2 (10 mL) was added, and the mixture was stirred at −15 °C for 1 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL), washed successively with cold 5% HCl, an aq solution of $NaHCO_3$ (satd), and water, dried ($MgSO_4$), and concentrated under reduced pressure. The crude triflate was used without further purification. To a stirred solution in DMF (10 mL) was added $NaNO_2$ (3.51 g, 50.9 mmol), and after 10 h at rt, the mixture was diluted with CH_2Cl_2 (150 mL), washed with brine, and concentrated, and the residue was coevaporated with toluene under reduced pressure to afford an oil that was subjected to flash chromatography on silica gel (2:1 EtOAc–hexanes). Compound **5** was isolated as tiny, white needles (1.07 g, 50.8%): R_f 0.30 (3:1 EtOAc–hexanes); mp 249–252 °C, lit. 237–242 °C [10]; $[\alpha]_D$ +13.6° {*c* 1, (1:1 MeOH–$CHCl_3$)}; ^{1}H NMR (1:1 CD_3OD–$CDCl_3$): δ 1.96 (s, 3 H, NAc), 3.61 (s, 3 H, OMe), 4.11 (dd, 1 H, $J_{5,6} = J_{5,6'}$ 6.3 Hz, H-5), 4.34 (d, 1 H, H-4), 4.53 (dd, 1 H, $J_{2,3}$ 11.0 Hz, H-2), 4.64 (d, 1 H, $J_{1,2}$ 8.6 Hz, H-1), 4.65 and 4.70 (dq, 2 H, *J* 11.2 Hz, H-6, H-6′), 5.22 (dd, 1 H, $J_{3,4}$ 3.2 Hz, H-3), 7.52–8.15 (m, 10 H, Ph); ^{13}C NMR (1:1 CD_3OD–$CDCl_3$): δ 22.9 ($NCOCH_3$), 50.8 (C-2), 56.9 (OMe), 64.2 (C-6), 66.8 (C-4), 73.3 (C-5), 75.2 (C-3), 102.9 (C-1), 129.1–134.0 (Ph), 167.2 and 167.4 (2 Ph*C*=O), 173.0 (NC=O). Anal. Calcd for $C_{23}H_{25}NO_8$: C, 62.30; H, 5.68; N, 3.16. Found: C, 62.18; H, 5.48; N, 3.05.

*Preparation of benzyl 2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-α-*D*-galactopyranoside* (**6**).—To an ice-cold solution of **3** [7,13] (4.39 g, 8.45 mmol) in CH_2Cl_2 (150 mL) and pyr (10 mL) was added dropwise trifluoromethanesulfonic anhydride (5.20 g, 2.2 equiv). The mixture was stirred for 20 min, diluted with CH_2Cl_2 (200 mL), and washed sequentially with ice-cold, aq solutions of $KHSO_4$ (10%) and $NaHCO_3$ (satd), and water, dried ($MgSO_4$), and concentrated to afford the 4-triflate, which was used without further purification. To a stirred solution in DMF (50 mL) was added $NaNO_2$ (1.75 g, 25.4 mmol), and after 16 h at rt, the mixture was concentrated, and the residue was coevaporated with toluene under reduced pressure to afford an oil that was subjected to flash chromatography on silica gel (1:2 EtOAc–hexanes). Compound **6** was isolated and recrystallized (EtOAc–hexanes) to afford white needles (2.56 g, 58.2%): R_f 0.48 (1:1 EtOAc–hexanes); mp 187–189 °C, lit. 180 °C [14]; $[\alpha]_D$ +100.2° (*c* 1.25, $CHCl_3$), lit. +86.4° (*c* 1, $CHCl_3$) [14]; ^{1}H NMR ($CDCl_3$): δ 1.82 (s, 3 H, NAc), 2.65 (br s, 1 H, OH), 4.27 (d, 1 H, $J_{4,3}$ 2.5 Hz, H-4), 4.32 (dd ~ t, 1 H, $J_{5,6} = J_{5,6'}$ 6.4 Hz, H-5), 4.52 and 4.76 (2 d, 2 H, *J* 11.7 Hz, PhCH_2), 4.53 and 4.63 (dq, 2 H, J_{gem} 11.4 Hz, H-6, H-6′), 4.91 (m, 1 H, H-2), 5.02 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 5.36 (dd, 1 H, H-3), 5.72 (d, 1 H, $J_{NH,2}$ 9.8 Hz, NH), 7.29–8.05 (m, 15 H, Ph); ^{13}C NMR ($CDCl_3$): δ 23.2 ($NCOCH_3$), 47.3 (C-2), 63.5 (C-6), 67.5 (C-4), 68.5 (C-5), 69.8 (PhCH_2), 72.0 (C-3), 97.0 (C-1), 128.1–136.7 (Ph), 166.3 and 166.5 (2 Ph*C*=O), 170.0 (NC=O). Anal. Calcd for $C_{29}H_{29}NO_8$: C, 67.04; H, 5.63; N, 2.70. Found: C, 67.10; H, 5.71; N, 2.61.

*Methyl 2-acetamido-3,6-di-*O*-benzoyl-2,4-dideoxy-4-fluoro-α-*D*-glucopyranoside* (**7**).—To a solution of DAST (5.70 g, 35.3 mmol) in CH_2Cl_2 (20 mL) in a Teflon Erlenmeyer flask was added dropwise a solution of **4** (1.84 g, 4.16 mmol) in CH_2Cl_2 (40 mL) at −5 °C. The reaction mixture was allowed to warm to rt overnight and then cooled to −5 °C. Methanol (20 mL) was added dropwise to destroy the excess of DAST, and the solution was concentrated under reduced pressure to afford a yellowish oil that was subjected to flash chromatography on silica gel (3:2

EtOAc–hexanes). Compound **7** was isolated and recrystallized from EtOAc–hexanes to afford tiny, white needles (1.51 g, 81.4%): R_f 0.45 (3:1 EtOAc–hexanes); mp 149–152 °C; $[\alpha]_D$ +87.2° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.86 (s, 3 H, NAc), 3.45 (s, 3 H, OMe), 4.18 (m, 1 H, H-5), 4.48 (ddd, 1 H, $J_{2,1}$ 3.6, $J_{2,3}$ 10.3, $J_{2,NH}$ 9.9 Hz, H-2), 4.56 (ddd, 1 H, $J_{6,5}$ 4.7, $J_{6,F}$ 1.3, J_{gem} 12.1 Hz, H-6), 4.67 (m, 1.5 H, H-4, H-6′), 4.79 (m, 1.5 H, H-1, H-4), 5.63 (dddd, 1 H, $J_{3,4}$ 9.0, $J_{3,F}$ 13.7 Hz, H-3), 5.90 (br d, 1 H, NH), 7.43–7.61 (m, 6 H, Ph), 8.02–8.07 (m, 4 H, Ph); ^{13}C NMR ($CDCl_3$): δ 23.0 ($NCOCH_3$), 51.7 (*J* 6.8 Hz, C-2), 55.5 (OMe), 62.5 (C-6), 67.5 (*J* 23.2 Hz, C-5), 71.7 (*J* 18.4 Hz, C-3), 87.0 (*J* 187.7 Hz, C-4), 98.3 (C-1), 128.4–133.4 (Ph), 166.1 and 166.8 (2 Ph*C*=O), 170.0 (NC=O); ^{19}F NMR ($CDCl_3$): δ 197.0 (dd, $J_{F,H3}$ 13.6, $J_{F,H4}$ 51.2 Hz). Anal. Calcd for $C_{23}H_{24}FNO_7$: C, 62.02; H, 5.43; F, 4.26; N, 3.14. Found: C, 61.82; H, 5.57; F, 4.07; N, 3.12.

*Methyl 2-acetamido-3,6-di-*O*-benzoyl-2,4-dideoxy-4-fluoro-β-*D*-glucopyranoside* (**8**).—To a mixture of **5** (0.875 g, 2.11 mmol) in CH_2Cl_2 (20 mL) in a Teflon Erlenmeyer flask was added dropwise DAST (2.89 g, 17.9 mmol) at −40 °C. The reaction mixture was allowed to warm to rt overnight and then cooled to −5 °C. Methanol (5 mL) was added dropwise to destroy the excess of DAST, and the solution was concentrated under reduced pressure to afford a yellowish oil that was subjected to flash chromatography on silica gel (1:1 EtOAc–hexanes). Compound **8** was isolated and recrystallized from EtOAc–hexanes to afford an amorphous, white solid (0.353 g, 40.2%): R_f 0.62 (EtOAc); mp 173–175 °C; $[\alpha]_D$ −7.5° (*c* 2, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.86 (s, 3 H, NAc), 3.45 (s, 3 H, OMe), 4.18 (m, 1 H, H-5), 4.48 (ddd, 1 H, $J_{2,1}$ 3.6, $J_{2,3}$ 10.3, $J_{2,NH}$ 9.9 Hz, H-2), 4.56 (ddd, 1 H, $J_{6,5}$ 4.7, $J_{6,F}$ 1.3, J_{gem} 12.1 Hz, H-6), 4.67 (m, 1.5 H, H-4, H-6′), 4.79 (m, 1.5 H, H-1, H-4), 5.63 (dddd, 1 H, $J_{3,4}$ 9.0, $J_{3,F}$ 13.7 Hz, H-3), 5.90 (br d, 1 H, NH), 7.43–7.61 (m, 6 H, Ph), 8.02–8.07 (m, 4 H, Ph); ^{13}C NMR ($CDCl_3$): δ 23.0 ($NCOCH_3$), 51.7 (*J* 6.8 Hz, C-2), 55.5 (OMe), 62.5 (C-6), 67.5 (*J* 23.2 Hz, C-5), 71.7 (*J* 18.4 Hz, C-3), 87.0 (*J* 187.7 Hz, C-4), 98.3 (C-1), 128.4–133.4 (Ph), 166.1 and 166.8 (2 Ph*C*=O), 170.0 (NC=O); ^{19}F NMR ($CDCl_3$): δ 197.0 (dd, $J_{F,H3}$ 13.6, $J_{F,H4}$ 51.2 Hz). Anal. Calcd for $C_{23}H_{24}FNO_7$: C, 62.02; H, 5.43; F, 4.26; N, 3.14. Found: C, 62.12; H, 5.42; F, 4.43; N, 3.18.

*Benzyl 2-acetamido-3,6-di-*O*-benzoyl-2,4-dideoxy-4-fluoro-α-*D*-glucopyranoside* (**9**).—To a solution of DAST (1.39 g, 8.63 mmol) in CH_2Cl_2 (5 mL) in a Teflon Erlenmeyer flask was added dropwise a solution of **6** (0.500 g, 1.02 mmol) in CH_2Cl_2 (10 mL) at −5 °C. The reaction mixture was allowed to warm to rt overnight and then cooled to −5 °C. Methanol (5 mL) was added dropwise to destroy the excess of DAST, and the solution was concentrated under reduced pressure to afford an oil which was subjected to flash chromatography on silica gel (1:2 EtOAc–hexanes). Compound **9** was isolated as a clear, colorless oil (0.426 g, 85.2%): R_f 0.30 (1:2 EtOAc–hexanes); $[\alpha]_D$ +101.2° (*c* 1.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.80 (s, 3 H, NAc), 4.24 (m, 1 H, H-5), 4.47–4.63 (m, 3 H, H-2, H-6, H-6′), 4.56 and 4.77 (2 d, 2 H, *J* 11.8 Hz, PhCH_2), 4.76 (ddd ~ dt, 1 H, $J_{4,5}$ 9.4, $J_{4,F}$ 51.0 Hz, H-4), 4.98 (dd ~ t, 1 H, $J_{1,2} = J_{1,F}$ 3.3 Hz, H-1), 5.68 (dddd, 1 H, $J_{3,4}$ 9.0, $J_{3,2}$ 10.9, $J_{3,F}$ 13.6 Hz, H-3), 5.93 (d, 1 H, $J_{NH,2}$ 9.6 Hz, NH), 7.32–7.59 (m, 9 H, Ph), 8.02–8.09 (m, 6 H, Ph); ^{13}C NMR ($CDCl_3$): δ 22.9 ($NCOCH_3$), 51.7 (*J* 6.8 Hz, C-2), 62.4 (C-6), 67.8 (*J* 22.7 Hz, C-5), 70.1 (PhCH_2), 71.6 (*J* 18.7 Hz, C-3), 87.0 (*J* 187.7 Hz, C-4), 96.5 (C-1), 128.1–136.3 (Ph), 166.0 and 166.7 (2 Ph*C*=O), 170.0 (NC=O); ^{19}F NMR ($CDCl_3$): δ 197.0 (dd, $J_{F,H3}$ 13.6, $J_{F,H4}$ 51.2 Hz); ESIMS (positive-ion): Expected for $C_{29}H_{28}FNO_7$ $[M+H]^+$: 522.2. Found: 522.3. Anal. Calcd for $C_{29}H_{28}FNO_7 \cdot H_2O$: C, 64.55; H, 5.60; F, 3.52; N, 2.60. Found: C, 64.79; H, 5.59; F, 3.26; N, 2.51.

*Methyl 2-acetamido-2,4-dideoxy-4-fluoro-α-*D*-glucopyranoside* (**10**).—To a solution of compound **7** (0.35 g, 0.78 mmol) in MeOH (20 mL) was added KOH (0.5 g), and the mixture was stirred for 30 min at rt. The mixture was neutralized with Amberlite IR-120 (H^+) resin and filtered, and the filtrate was concentrated under reduced pressure to give a crude product that was recrystallized from absolute EtOH to afford **10** (0.18 g, 90.2%) as white

needles: R_f 0.65 (1:3 MeOH–$CHCl_3$); mp 189–190 °C; $[\alpha]_D$ +139.0° (*c* 1, MeOH); ^{1}H NMR (CD_3OD): δ 1.92 (s, 3 H, NAc), 3.32 (s, 3 H, OMe), 3.64 (m, 2 H, H-5, H-6), 3.73 (m, 1 H, $J_{6',F}$ 2.1, J_{gem} 10.0, H-6′), 3.78–3.91 (m, 2 H, $J_{2,3}$ 10.8 Hz, H-2, H-3), 4.23 (ddd ~ dt, 1 H, $J_{4,3} = J_{4,5}$ 8.1, $J_{4,F}$ 51.1 Hz, H-4), 4.60 (t, 1 H, $J_{1,2} = J_{1,F}$ 3.2 Hz, H-1); ^{13}C NMR (CD_3OD): δ 22.5 (NCO*C*H$_3$), 54.9 (*J* 8.2 Hz, C-2), 55.7 (OMe), 61.7 (C-6), 70.9 (*J* 18.9 Hz, C-3), 71.1 (*J* 24.6 Hz, C-5), 91.3 (*J* 180.1 Hz, C-4), 99.6 (C-1), 173.7 (NC=O); ^{19}F NMR (CD_3OD): δ 197.1 (dd, $J_{F,H3}$ 14.5, $J_{F,H4}$ 51.8 Hz). Anal. Calcd for $C_9H_{16}FNO_5$: C, 45.57; H, 6.37; F, 8.01; N, 5.90. Found: C, 45.72; H, 6.50; F, 8.27; N, 5.89.

*Methyl 2-acetamido-2,4-dideoxy-4-fluoro-β-*D*-glucopyranoside* (**11**).—To a solution of **8** (0.196 g, 0.440 mmol) in MeOH (15 mL) was added KOH (0.2 g), and the mixture was stirred for 30 min at rt. The mixture was neutralized with Amberlite IR-120 (H^+) resin and filtered, and the filtrate was concentrated under reduced pressure to afford a crude product that was recrystallized from absolute EtOH to afford **11** (0.085 g, 81.4%) as an amorphous solid: R_f 0.46 (1:3 MeOH–$CHCl_3$); mp 192–194 °C; $[\alpha]_D$ −50.0° (*c* 0.61, MeOH); ^{1}H NMR (CD_3OD): δ 1.89 (s, 3 H, NAc), 3.35–3.42 (m, 1 H, H-5), 3.38 (s, 3 H, OMe), 3.57 (m, 1 H, H-2), 3.65 (m, 1 H, H-6), 3.69 (m, 1 H, H-3), 3.75 (m, 1 H, J_{gem} 12.3 Hz, H-6′), 4.18 (ddd, 1 H, $J_{4,3} = J_{4,5}$ 8.7, $J_{4,F}$ 50.8 Hz, H-4), 4.29 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1); ^{13}C NMR (CD_3OD): δ 22.9 (NCO*C*H$_3$), 57.0 (*J* 8.3 Hz, C-2), 57.1 (OMe), 61.7 (C-6), 73.7 (*J* 18.6 Hz, C-3), 75.3 (*J* 24.6 Hz, C-5), 91.0 (*J* 181.0 Hz, C-4), 103.4 (C-1), 173.8 (NC=O); ^{19}F NMR (CD_3OD): δ 197.0 (dd, $J_{F,H3}$ 16.1, $J_{F,H4}$ 50.6 Hz); FABMS (positive-ion): Expected for $C_9H_{17}FNO_5$: 238.1091. Found: 238.1080.

*Benzyl 2-acetamido-2,4-dideoxy-4-fluoro-α-*D*-glucopyranoside* (**12**).—To a solution of compound **9** (1.45 g, 2.94 mmol) in MeOH (40 mL) was added KOH (1.0 g), and the mixture was stirred for 30 min at rt. The mixture was neutralized with 1N HCl and concentrated under reduced pressure to give a solid that was recrystallized from absolute EtOH to afford **12** (0.81 g, 92.5%) as tiny, white crystals: R_f 0.38 (1:9 MeOH–CH_2Cl_2); mp 197–198 °C; $[\alpha]_D$ +206.3° (*c* 1, MeOH); ^{1}H NMR (CD_3OD): δ 1.85 (s, 3 H, NAc), 3.61 (ddd, 1 H, $J_{6,5}$ 4.9, J_{gem} 12.3, $J_{6,F}$ 1.5 Hz, H-6), 3.67–3.73 (m, 2 H, H-5, H-6′), 3.81–3.91 (m, 2 H, H-2, H-3), 4.22 (ddd, 1 H, $J_{4,3}$ 8.1, $J_{4,5}$ 9.8, $J_{4,F}$ 50.9 Hz, H-4), 4.43 and 4.65 (2 d, 2 H, *J* 12.1 Hz, PhC*H*$_2$), 4.76 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 7.23 (m, 5 H, Ph); ^{13}C NMR (CD_3OD): δ 22.5 (NCO*C*H$_3$), 54.9 (*J* 8.4 Hz, C-2), 61.7 (C-6), 70.4 (Ph*C*H$_2$), 70.6 (*J* 18.8 Hz, C-5), 71.5 (*J* 24.7 Hz, C-3), 91.4 (*J* 180.0 Hz, C-4), 97.3 (C-1), 128.9–138.7 (Ph), 173.6 (NC=O); ^{19}F NMR (CD_3OD): δ 169.6 (dd, $J_{F,H3}$ 14.4, $J_{F,H4}$ 51.6 Hz). Anal. Calcd for $C_{15}H_{20}FNO_5$: C, 57.50; H, 6.43; F, 6.06; N, 4.47. Found: C, 57.42; H, 6.50; F, 5.93; N, 4.37.

*Preparation of 2-acetamido-2,4-dideoxy-4-fluoro-*D*-glucopyranose* (**13**).—A mixture of **12** (0.58 g, 1.85 mmol), acetic acid (15 mL), and 10% Pd–C (0.5 g) was subjected to a hydrogen pressure (55 psig) for 3 days. The mixture was filtered through Celite 521 (Aldrich) and the residue washed with MeOH. The filtrate and washings were combined and concentrated under reduced pressure to afford an oil that was purified by flash chromatography on silica gel (1:9 to 1:5 MeOH–CH_2Cl_2) to yield **13** (0.37 g, 89.1%) as a mixture of α and β anomers in a ratio of 5.7: 1 as determined by ^{1}H NMR spectroscopy. R_f 0.25 and 0.31 (1:3 MeOH–$CHCl_3$); mp 176–180 °C (dec), lit. 174–175 °C [9a]; $[\alpha]_D$ +59.3° (equil) (*c* 1, MeOH), lit. +61.5° (*c* 1, MeOH) [9a]. α Anomer: ^{1}H NMR (CD_3OD): δ 1.91 (s, 3 H, NAc), 3.52–3.75 (m, 2 H, H-6, H-6′), 3.78–3.94 (m, 3 H, H-2, H-3, H-5), 4.24 (m, $J_{4,F}$ 46.3 Hz, H-4), 5.03 (m, 1 H, $J_{1,2}$ 3.2 Hz, H-1); ^{13}C NMR (CD_3OD): δ 22.6 (NCO*C*H$_3$), 55.5 (*J* 8.0 Hz, C-2), 61.8 (C-6), 70.5 and 70.7 (C-3, C-5), 91.5 (*J* 182.8 Hz, C-4), 92.4 (C-1), 173.7 (NC=O); ^{19}F NMR (CD_3OD): δ 199.6 (dd, $J_{F,H3}$ 14.3, $J_{F,H4}$ 50.6 Hz). Anal. Calcd for $C_8H_{14}FNO_5$: C, 43.05; H, 6.32; F, 8.51; N, 6.28. Found: C, 42.86; H, 6.37; F, 8.61; N, 6.22.

*Methyl 2-acetamido-3,6-di-*O*-acetyl-2,4-dideoxy-4-fluoro-α-*D*-glucopyranoside* (**14**).—Compound **10** (0.10 g, 0.42 mmol) was treated with Ac_2O (1.0 mL) in pyr (2.0 mL) at 0 °C, and the mixture was stirred overnight at rt.

The solution was concentrated, and the residue was coevaporated with toluene under reduced pressure to afford a clear oil which was crystallized from EtOAc–hexanes to give **14** (0.13 g, 88.2%) as white crystals: R_f 0.64 (EtOAc); mp 112–113 °C; $[\alpha]_D$ +58.9° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.95 (s, 3 H, NAc), 2.08 and 2.10 (2 s, 6 H, 2 OAc), 3.40 (s, 3 H, OMe), 3.95 (m, 1 H, H-5), 4.23–4.29 (m, 2 H, H-2, H-6), 4.39 (m, 1 H, H-6′), 4.49 (ddd ~ dt, 1 H, $J_{4,3} = J_{4,5}$ 9.5, $J_{4,F}$ 51.0 Hz, H-4), 4.68 (t, 1 H, $J_{1,2} = J_{1,F}$ 3.3 Hz, H-1), 5.31 (ddd, 1 H, $J_{3,2}$ 9.0, $J_{3,4}$ 10.8, $J_{3,F}$ 14.2 Hz, H-3), 5.72 (d, 1 H, $J_{NH,2}$ 9.5 Hz, NH); ^{13}C NMR ($CDCl_3$): δ 20.8 ($NCOCH_3$), 51.7 (*J* 6.8 Hz, C-2), 55.5 (OMe), 62.0 (C-6), 67.2 (*J* 23.6 Hz, C-5), 71.2 (*J* 18.6 Hz, C-3), 86.6 (*J* 186.5 Hz, C-4), 98.2 (C-1), 170.0, 170.6, and 171.3 (2 $OCOCH_3$, NC=O); ^{19}F NMR ($CDCl_3$): δ 227.3 (dd, $J_{F,H3}$ 13.8, $J_{F,H4}$ 51.2 Hz). Anal. Calcd for $C_{13}H_{20}FNO_7$: C, 48.60; H, 6.27; F, 5.91; N, 4.36. Found: C, 48.18; H, 6.35; F, 5.69; N, 4.42.

Methyl 2-acetamido-3,6-di-O-*acetyl-2,4-dideoxy-4-fluoro-β*-D-*glucopyranoside* (**15**).—Compound **11** (0.085 g, 0.357 mmol) was treated with Ac_2O (1.5 mL) in pyr (2.5 mL) at 0 °C, and the mixture was stirred overnight at rt. The solution was concentrated, and the residue was coevaporated with toluene under reduced pressure to afford a crude solid that was recrystallized from EtOAc–Et_2O to afford **15** (0.100 g, 86.8%) as clear, colorless rods: R_f 0.30 (EtOAc); mp 174–176 °C; $[\alpha]_D$ −61.4° (*c* 1, MeOH); ^1H NMR ($CDCl_3$): δ 1.80 (s, 3 H, NAc), 1.96 and 1.99 (2 s, 6 H, 2 OAc), 3.40 (s, 3 H, OMe), 3.95 (m, 1 H, H-5), 4.23–4.29 (m, 2 H, H-2, H-6), 4.39 (m, 1 H, H-6′), 4.49 (ddd ~ dt, 1 H, $J_{4,3} = J_{4,5}$ 9.5, $J_{4,F}$ 51.0 Hz, H-4), 4.68 (t, 1 H, $J_{1,2} = J_{1,F}$ 3.3 Hz, H-1), 5.31 (ddd, 1 H, $J_{3,2}$ 9.0, $J_{3,4}$ 10.8, $J_{3,F}$ 14.2 Hz, H-3), 5.72 (d, 1 H, $J_{NH,2}$ 9.5 Hz, NH); ^{13}C NMR ($CDCl_3$): δ 20.6 and 22.7 ($NCOCH_3$, 2 $OCOCH_3$), 55.2 (*J* 5.9 Hz, C-2), 57.3 (OMe), 63.4 (C-6), 72.4 (*J* 23.6 Hz, C-5), 74.1 (*J* 18.8 Hz, C-3), 88.7 (*J* 184.3 Hz, C-4), 102.9 (C-1), 171.8, 172.4, and 173.5 (2 $OCOCH_3$, NC=O); ^{19}F NMR ($CDCl_3$): δ 227.2 (dd, $J_{F,H3}$ 14.9, $J_{F,H4}$ 49.4 Hz). Anal. Calcd for $C_{13}H_{20}FNO_7$: C, 48.60; H, 6.27; F, 5.91; N, 4.36. Found: C, 48.74; H, 6.42; F, 6.16; N, 4.43.

2-Acetamido-1,3,6-tri-O-*acetyl-2,4-dideoxy-4-fluoro*-D-*glucopyranose* (**16**).—Compound **13** (0.10 g, 0.45 mmol) was treated with acetic anhydride (1.0 mL) in pyr (2.0 mL) at 0 °C, and the mixture was stirred overnight at rt. The solution was concentrated, and the residue was coevaporated with toluene under reduced pressure to produce an opaque oil that was crystallized from EtOAc–hexanes to give the β anomer of **16** (0.032 g) as colorless rods. The remainder of **16** crystallized as a mixture of α and β anomers (0.125 g, 93.2%). β Anomer: R_f 0.38 (EtOAc); mp 188–189 °C; $[\alpha]_D$ −29.2° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.92 (s, 3 H, NAc), 2.09 and 2.11 (3 s, 9 H, 3 OAc), 3.83 (m, 1 H, H-5), 4.21–4.31 (m, 2 H, H-2, H-6), 4.40 (d, 1 H, J_{gem} 12.3 Hz, H-6′), 4.53 (dt, 1 H, $J_{4,3} = J_{4,5}$ 9.3, $J_{4,F}$ 50.4 Hz, H-4), 5.23 (ddd, 1 H, $J_{3,2}$ 8.9, $J_{3,4}$ 10.7, $J_{3,F}$ 14.2 Hz, H-3), 5.67 (d, 1 H, $J_{1,2}$ 8.8 Hz, H-1), 5.77 (d, 1 H, $J_{NH,2}$ 9.5 Hz, NH); ^{13}C NMR ($CDCl_3$): δ 20.8 and 20.9 (3 $OCOCH_3$), 23.2 ($NCOCH_3$), 52.6 (*J* 7.4 Hz, C-2), 61.8 (C-6), 72.1 (*J* 24.1 Hz, C-5), 86.1 (*J* 187.0 Hz, C-4), 92.5 (C-1), 169.5, 170.3, 170.6, and 171.2 (3 $OCOCH_3$, NC=O); ^{19}F NMR ($CDCl_3$): δ 200.0 (dd, $J_{F,H3}$ 13.5, $J_{F,H4}$ 50.1 Hz). Anal. Calcd for $C_{14}H_{20}FNO_8$: C, 48.14; H, 5.77; F, 5.44; N, 4.01. Found: C, 47.90; H, 5.52; F, 5.63; N, 3.99.

Methyl 2-acetamido-3,6-di-O-*benzyl-2-deoxy-4*-O-*mesyl-α*-D-*glucopyranoside* (**20**).—To a solution of methyl 2-acetamido-3,6-di-*O*-benzyl-α-D-glucopyranoside [16] (**17**, 4.75 g, 11.4 mmol) in pyr (60 mL) was added a solution of methanesulfonyl chloride (5 mL) in pyr (7 mL) with stirring at 0 °C. The solution was kept at 0 °C for 11 h and then poured into ice–water. The resulting solid was collected by filtration and dissolved in $CHCl_3$; the solution was washed with H_2O, dried ($MgSO_4$), and concentrated to a solid that was recrystallized from EtOAc–hexanes to give **20** (4.24 g, 75.2%) as short, glassy needles: R_f 0.39 (3:1 EtOAc–hexanes); mp 194–195 °C (dec); $[\alpha]_D$ +90.2° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.87 (s, 3 H, NAc), 2.84 (s, 3 H, SMe), 3.37 (s, 3 H, OMe), 3.70 (dd, 1 H, $J_{6,5}$ 5.2, J_{gem} 11.0 Hz, H-6), 3.79–3.84 (m, 2 H, H-3, H-6′), 3.88 (m, 1 H, H-5), 4.41 (ddd ~ dt, 1 H, $J_{2,1}$ 3.6, $J_{2,3} = J_{2,NH}$ 10.0 Hz,

H-2), 4.55 and 4.62 (2 d, 2 H, *J* 11.8 Hz, PhC*H*$_2$), 4.67–4.72 (m, 4 H, H-1, H-4, PhC*H*$_2$), 5.56 (d, 1 H, NH), 7.25–7.36 (m, 10 H, Ph); ^{13}C NMR ($CDCl_3$): δ 23.3 (NCO*C*H$_3$), 38.5 (SMe), 52.1 (C-2), 55.3 (OMe), 68.4 (C-6), 69.2 (C-5), 73.5 and 73.9 (2 Ph*C*H$_2$), 78.0 (C-3, C-4), 98.3 (C-1), 127.5–128.6, 137.1, and 137.8 (Ph), 169.7 (NC=O). Anal. Calcd for $C_{24}H_{31}NO_8S$: C, 58.40; H, 6.33; N, 2.84; S, 6.50. Found: C, 58.44; H, 6.57; N, 2.82; S, 6.61.

Methyl 2-acetamido-3,6-di-O-*benzyl-2-deoxy-4*-O-*mesyl-β*-D-*glucopyranoside* (**21**).—To a solution of methyl 2-acetamido-3,6-di-*O*-benzyl-β-D-glucopyranoside [17] (**18**, 2.00 g, 4.81 mmol) in pyr (30 mL) was added a solution of methanesulfonyl chloride (3 mL) in pyr (5 mL) with stirring at 0 °C. The solution was kept at 0 °C for 24 h and processed as described for the preparation of **20** to afford **21** (1.75 g, 73.6%) as white rods: R_f 0.31 (2:1 EtOAc–hexanes); mp 170–171 °C (dec); $[\alpha]_D$ +24.0° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.89 (s, 3 H, NAc), 2.87 (s, 3 H, SMe), 3.40 (m, 1 H, H-2), 3.49 (s, 3 H, OMe), 3.72 (m, 2 H, H-5, H-6), 3.86 (dd, 1 H, $J_{6',5}$ 3.9, J_{gem} 12.8 Hz, H-6′), 4.33 (dd, 1 H, $J_{3,2}$ 9.4 Hz, H-3), 4.61 (d, 2 H, *J* 1.6 Hz, PhC*H*$_2$), 4.64 (m, 1 H, H-4), 4.67 and 4.78 (2 d, 2 H, *J* 11.1 Hz, PhC*H*$_2$), 4.83 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 5.84 (d, 1 H, $J_{NH,2}$ 7.6 Hz, NH), 7.27–7.38 (m, 10 H, Ph); ^{13}C NMR ($CDCl_3$): δ 23.5 (NCO*C*H$_3$), 38.6 (SMe), 56.9 (OMe), 57.6 (C-2), 68.8 (C-6), 73.3 (C-5), 73.6 (Ph*C*H$_2$), 74.0 (Ph*C*H$_2$), 78.2 (C-3), 78.5 (C-4), 100.3 (C-1), 127.7–128.6, 137.6, and 137.9 (Ph), 171.5 (NC=O). Anal. Calcd for $C_{24}H_{31}NO_8S$: C, 58.40; H, 6.33; N, 2.84; S, 6.50. Found: C, 58.32; H, 6.51; N, 2.87; S, 6.73.

Preparation of benzyl 2-acetamido-3,6-di-O-*benzyl-2-deoxy-4*-O-*mesyl-α*-D-*glucopyranoside* (**22**).—To a solution of benzyl 2-acetamido-3,6-di-*O*-benzyl-α-D-glucopyranoside [18] (**19**, 0.390 g, 0.793 mmol) in pyr (5 mL) was added a solution of methanesulfonyl chloride (0.5 mL) in pyr (0.7 mL) with stirring at 0 °C. The solution was kept at 0 °C for 24 h and processed as described for the preparation of **20** to afford **22** (0.45 g, 77.5%) as tiny, white needles: R_f 0.62 (2:1 EtOAc–hexanes); mp 172–173 °C, lit. 168–170 °C [19]; $[\alpha]_D$ +103.2° (*c* 1, $CHCl_3$), lit. +98° (*c* 0.8, $CHCl_3$) [19]; ^{1}H NMR ($CDCl_3$): δ 1.84 (s, 3 H, NAc), 2.86 (s, 3 H, SMe), 3.73 (dd, 1 H, $J_{6,5}$ 5.1, J_{gem} 11.0 Hz, H-6), 3.81 (dd, 1 H, $J_{6',5}$ 2.4 Hz, H-6′), 3.89 (dd ~ t, 1 H, $J_{3,4}$ 9.1 Hz, H-3), 4.01 (m, 1 H, H-5), 4.45 (ddd ~ dt, 1 H, $J_{2,3} = J_{2,NH}$ 9.7 Hz, H-2), 4.49 and 4.74 (2 d, 2 H, *J* 11.6 Hz, PhC*H*$_2$), 4.58 and 4.65 (2 d, 2 H, *J* 11.8 Hz, PhC*H*$_2$), 4.69 (d, 2 H, *J* 2.1 Hz, PhC*H*$_2$), 4.74 (m, 1 H, H-4), 4.92 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1), 5.52 (d, 1 H, NH), 7.28–7.41 (m, 15 H, Ph); ^{13}C NMR ($CDCl_3$): δ 23.3 (NCO*C*H$_3$), 38.5 (SMe), 52.2 (C-2), 68.3 (C-6), 69.7 (C-5), 69.8 (Ph*C*H$_2$), 73.6 and 73.9 (2 Ph*C*H$_2$), 78.0 (C-4), 78.1 (C-3), 96.6 (C-1), 127.5–128.6, 136.6, 137.1, and 137.9 (Ph), 169.6 (NC=O).

Methyl 2-acetamido-3,6-di-O-*benzyl-2,4-dideoxy-4-fluoro-α*-D-*galactopyranoside* (**23**).—To a solution of **20** (2.1 g, 4.25 mmol) in anhyd MeCN (30 mL) was added a solution of anhyd tetra-*n*-butylammonium fluoride [20] (10.9 g, 9.8 equiv) in anhyd MeCN (15 mL), and the solution was heated at reflux temperature for 6 days. The solvent was removed under reduced pressure, and the residue was dissolved in CH_2Cl_2; the solution was washed with H_2O and concentrated to a brownish solid. The aqueous layer was extracted with CH_2Cl_2, and the extracts were dried ($MgSO_4$) and concentrated to a brownish solid. The combined samples of crude brownish solids were recrystallized from EtOAc–hexanes to afford **23** (0.96 g, 54.3%) as tiny, white needles: R_f 0.47 (2:1 EtOAc–hexanes); mp 203–204 °C; $[\alpha]_D$ +131.7° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.93 (s, 3 H, NAc), 3.32 (s, 3 H, OMe), 3.55 (dddd, 1 H, $J_{3,4}$ 2.3, $J_{3,2}$ 11.0, $J_{3,F}$ 27.8 Hz, H-3), 3.64 (dddd, 2 H, $J_{6,F}$ 1.4, J_{gem} 9.4 Hz, H-6, H-6′), 3.72 (m, 1 H, H-2), 3.85 (ddd ~ dt, 1 H, $J_{5,6} = J_{5,6'}$ 6.6, $J_{5,F}$ 28.8 Hz, H-5), 4.47 and 4.76 (2 d, 2 H, *J* 12.3 Hz, PhC*H*$_2$), 4.57 (d, 2 H, *J* 3.3 Hz, PhC*H*$_2$), 4.77 (overlapping d, 1 H, H-1), 4.92 (dd, 1 H, $J_{4,F}$ 50.2 Hz, H-4), 5.34 (d, 1 H, $J_{NH,2}$ 8.8 Hz, NH), 7.29–7.35 (m, 10 H, Ph); ^{13}C NMR ($CDCl_3$): δ 23.4 (NCO*C*H$_3$), 48.4 (C-2), 55.4 (OMe), 68.0 (*J* 6.5 Hz, C-6), 68.2 (*J* 17.9 Hz, C-5), 70.7 and 73.7 (2 Ph*C*H$_2$), 74.0 (*J* 18.6 Hz, C-3), 85.3 (*J* 184.9 Hz, C-4), 98.7 (C-1), 127.7–128.5, 137.1, and 137.8 (Ph), 169.8 (NC=O); ^{19}F NMR

($CDCl_3$): δ 219.9 (dt, $J_{F,H3}$=$J_{F,H5}$ 28.2, $J_{F,H4}$ 50.3 Hz). Anal. Calcd for $C_{23}H_{28}FNO_5$: C, 66.17; H, 6.76; F, 4.55; N, 3.36. Found: C, 65.95; H, 6.57; F, 4.39; N, 3.26.

Methyl 2-acetamido-3,6-di-O-*benzyl-2,4-dideoxy-4-fluoro-β*-D-*galactopyranoside* (**24**).—To a solution of **21** (0.80 g, 1.62 mmol) in anhyd MeCN (25 mL) was added a solution of anhyd tetra-*n*-butylammonium fluoride [20] (6.36 g, 15 equiv) in anhyd MeCN (7.5 mL), and the mixture was heated at reflux temperature for 4 days. The reaction mixture was processed as described for the preparation of **23** to afford a brownish solid that was subjected to flash chromatography on silica gel (3:1 EtOAc–hexanes). The resulting solid was recrystallized from EtOAc–hexanes to afford **24** (0.43 g, 64.0%) as an amorphous, white solid: R_f 0.38 (2:1 EtOAc–hexanes); mp 208–210 °C; $[\alpha]_D$ +29.9° (*c* 1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.93 (s, 3 H, NAc), 3.31 (m, 1 H, H-2), 3.49 (s, 3 H, OMe), 3.66–3.76 (m, 3 H, H-5, H-6, H-6′), 4.38 (dddd, 1 H, $J_{3,4}$ 2.4, $J_{3,2}$ 10.9, $J_{3,F}$ 28.1 Hz, H-3), 4.52 and 4.70 (2 d, 2 H, *J* 11.6 Hz, PhCH_2), 4.57 (s, 2 H, PhCH_2), 4.88 (dd, 1 H, $J_{4,F}$ 50.2 Hz, H-4), 4.99 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1), 5.75 (br s, 1 H, NH), 7.26–7.37 (m, 10 H, Ph); ^{13}C NMR ($CDCl_3$): δ 23.7 (NCOCH_3), 55.2 (C-2), 56.9 (OMe), 67.7 (C-6), 71.8 (PhCH_2), 72.0 (*J* 18.5 Hz, C-5), 73.6 (PhCH_2), 74.5 (*J* 18.9 Hz, C-3), 85.1 (*J* 184.7 Hz, C-4), 100.2 (C-1), 127.3–128.5, and 137.6 (Ph), 171.0 (NC=O); ^{19}F NMR ($CDCl_3$): δ 220.1 (dt, $J_{F,H3} = J_{F,H5}$ 28.2, $J_{F,H4}$ 50.5 Hz). Anal. Calcd for $C_{23}H_{28}FNO_5$: C, 66.17; H, 6.76; F, 4.55; N, 3.36. Found: C, 66.32; H, 7.00; F, 4.34; N, 3.41.

Benzyl 2-acetamido-3,6-di-O-*benzyl-2,4-dideoxy-4-fluoro-α*-D-*galactopyranoside* (**25**).—To a solution of **22** (1.78 g, 3.10 mmol) in anhyd MeCN (60 mL) was added a solution of anhyd tetra-*n*-butylammonium fluoride [20] (9.41 g, 11.6 equiv) in anhyd MeCN (40 mL), and the mixture was heated at reflux temperature for 4 days. The reaction mixture was processed as described for the preparation of **23** to afford a brownish solid that was subjected to flash chromatography on silica gel (1:1 EtOAc–hexanes). The resulting solid was recrystallized from EtOAc–hexanes to afford **25** (0.96 g, 62.0%) as an amorphous, white solid: R_f 0.22 (1:1 EtOAc–hexanes); mp 180–182 °C, lit. 186–187 °C [9a]; $[\alpha]_D$ +137.8° (*c* 1, $CHCl_3$), lit. +117° (*c* 1, $CHCl_3$) [9a]; ^{1}H NMR ($CDCl_3$): δ 2.00 (s, 3 H, NAc), 3.65 (m, 2 H, H-3, H-6), 3.72 (m, 1 H, H-6), 3.94 (ddd ~ dt, 1 H, $J_{5,6} = J_{5,6'}$ 6.5, $J_{5,F}$ 29.2 Hz, H-5), 4.45 and 4.67 (2 d, 2 H, *J* 11.8 Hz, PhCH_2), 4.48 and 4.75 (2 d, 2 H, *J* 12.2 Hz, PhCH_2), 4.56 (s, 2 H, PhCH_2), 4.57 (m, 1 H, H-2), 4.95 (dd, 1 H, $J_{4,3}$ 2.0, $J_{4,F}$ 50.1 Hz, H-4), 5.00 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 5.33 (d, 1 H, $J_{NH,2}$ 8.9 Hz, NH), 7.24–7.38 (m, 15 H, Ph); ^{13}C NMR ($CDCl_3$): δ 23.3 (NCOCH_3), 48.4 (C-2), 67.9 (*J* 5.1 Hz, C-6), 68.6 (*J* 18.6 Hz, C-5), 69.9 (PhCH_2), 70.8 and 73.7 (2 PhCH_2), 74.2 (*J* 18.5 Hz, C-3), 85.3 (*J* 185.2 Hz, C-4), 97.2 (C-1), 127.7–128.6, 137.1, and 137.8 (Ph), 169.8 (NC=O); ^{19}F NMR ($CDCl_3$): δ 220.3 (dt, $J_{F,H3} = J_{F,H5}$ 28.6, $J_{F,H4}$ 50.9 Hz). Anal. Calcd for $C_{29}H_{32}FNO_5$: C, 70.57; H, 6.53; F, 3.85; N, 2.84. Found: C, 70.44; H, 6.49; F, 4.04; N, 2.86.

Methyl 2-acetamido-2,4-dideoxy-4-fluoro-α-D-*galactopyranoside* (**26**).—A mixture of **23** (0.83 g, 1.5 mmol), acetic acid (20 mL), and 10% Pd–C (1.0 g) was subjected to a hydrogen pressure (55 psig) for 4 days. The mixture was diluted with MeOH, neutralized with Amberlite IRA-400 (OH^-) resin, filtered through Celite 521 (Aldrich), and the filtrate was concentrated under reduced pressure to a white solid that was recrystallized (EtOH–acetone) to afford **26** (0.30 g, 80.6%) as an amorphous, white solid: R_f 0.50 (1:3 MeOH–$CHCl_3$); mp 227–228 °C; $[\alpha]_D$ +173.4° (*c* 1, MeOH); ^{1}H NMR (CD_3OD): δ 1.98 (s, 3 H, NAc), 3.32 (s, 3 H, OMe), 3.64 (m, 1 H, H-6), 3.69 (m, 1 H, H-6′), 3.75–3.83 (m, 2 H, $J_{3,4}$ 2.6 Hz, H-3, H-5), 4.18 (dd, 1 H, $J_{2,3}$ 11.2 Hz, H-2), 4.64 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 4.70 (dd, 1 H, $J_{4,3}$ 2.6, $J_{4,F}$ 47.4 Hz, H-4); ^{13}C NMR (CD_3OD): δ 22.6 (NCOCH_3), 51.7 (C-2), 55.8 (OMe), 61.4 (*J* 5.2 Hz, C-6), 68.2 (*J* 19.4 Hz, C-3), 71.3 (*J* 18.5 Hz, C-5), 90.4 (*J* 180.7 Hz, C-4), 100.0 (C-1), 173.8 (NC=O); ^{19}F NMR (CD_3OD): δ 221.2 (dt, $J_{F,H3} = J_{F,H5}$ 29.0, $J_{F,H4}$ 50.6 Hz). Anal. Calcd for $C_9H_{16}FNO_5$: C, 45.57; H, 6.80; F, 8.01; N, 5.90. Found: C, 45.39; H, 6.88; F, 8.24; N, 5.72.

Methyl 2-acetamido-2,4-dideoxy-4-fluoro-β-D-galactopyranoside (**27**).—A mixture of **24** (0.448 g, 0.833 mmol), acetic acid (20 mL), and 10% Pd–C (0.6 g) was subjected to a hydrogen pressure (55 psig) for 4 days. The mixture was processed as described for **26** to afford a solid that was recrystallized from EtOH–hexanes to give **27** (0.17 g, 86.5%) as tiny, colorless crystals: R_f 0.35 (1:3 MeOH–$CHCl_3$); mp 209–211 °C; $[\alpha]_D$ −24.8° (*c* 1, MeOH); ^{1}H NMR (CD_3OD): δ 1.97 (s, 3 H, NAc), 3.45 (s, 3 H, OMe), 3.58 (ddd ~ dt, 1 H, $J_{5,6} = J_{5,6'}$ 6.6, $J_{5,F}$ 27.6 Hz, H-5), 3.72 (dddd, 1 H, $J_{3,2}$ 10.9, $J_{3,4}$ 2.5 Hz, H-3), 3.73 (d, 2 H, H-6, H-6′), 3.86 (dd, 1 H, H-2), 4.36 (dd, 1 H, $J_{1,2}$ 8.3, $J_{1,F}$ 0.8 Hz, H-1), 4.72 (dd, 1 H, H-4); ^{13}C NMR (CD_3OD): δ 23.0 ($NCOCH_3$), 54.4 (C-2), 57.0 (OMe), 61.1 (*J* 5.1 Hz, C-6), 71.7 (*J* 19.3 Hz, C-3), 75.2 (*J* 18.1 Hz, C-5), 89.6 (*J* 181.0 Hz, C-4), 103.6 (C-1), 174.0 (NC=O); ^{19}F NMR (CD_3OD): δ 217.1 (dt, $J_{F,H3} = J_{F,H5}$ 28.3, $J_{F,H4}$ 50.6 Hz). Anal. Calcd for $C_9H_{16}FNO_5$: C, 45.57; H, 6.80; F, 8.01; N, 5.90. Found: C, 45.70; H, 6.94; F, 8.19; N, 5.86.

2-Acetamido-2,4-dideoxy-4-fluoro-D-galactopyranose (**28**).—A mixture of **25** (0.600 g, 1.22 mmol), acetic acid (15 mL), and 10% Pd–C (1.0 g) was subjected to a hydrogen pressure (55 psig) for 4 days. The mixture was processed as described for **26** to afford a solid that was recrystallized from EtOH–Et_2O to obtain **28** (0.27 g, 86.7%) as tiny, white needles: R_f 0.17 (1:3 MeOH–$CHCl_3$); mp 205–209 °C, lit. 195 °C [9a]; $[\alpha]_D$ +87.9° (equil) (*c* 1, MeOH), lit. +85.5° (*c* 1, MeOH) [9a]. α Anomer: ^{1}H NMR (CD_3OD): δ 1.93 (s, 3 H, NAc), 3.58 (dddd, 1 H, $J_{6,5}$ 6.7, $J_{6,F}$ 1.0, J_{gem} 11.0 Hz, H-6), 3.65 (dd, 1 H, H-6′), 3.84 (ddd, 1 H, $J_{3,2}$ 11.1, $J_{3,4}$ 2.5, $J_{3,F}$ 28.8 Hz, H-3), 4.00 (ddd ~ dt, 1 H, $J_{5,6'}$ 6.8, $J_{5,F}$ 30.3 Hz, H-5), 4.13 (dd, 1 H, H-2), 4.71 (dd, 1 H, H-4), 5.07 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1); ^{13}C NMR (CD_3OD): δ 22.6 ($NCOCH_3$), 52.2 (C-2), 61.3 (C-6), 68.0 (*J* 18.9 Hz, C-3), 70.6 (*J* 17.5 Hz, C-5), 90.5 (*J* 179.6 Hz, C-4), 92.8 (C-1), 173.9 (NC=O); ^{19}F NMR (CD_3OD): δ 221.1 (dt, $J_{F,\ H3} = J_{F,H5}$ 29.5, $J_{F,H4}$ 50.8 Hz).

Methyl 2-acetamido-3,6-di-O-acetyl-2,4-dideoxy-4-fluoro-α-D-galactopyranoside (**29**).—Compound **26** (0.200 g, 0.843 mmol) was treated with acetic anhydride (1.5 mL) in pyr (2.5 mL) at 0 °C, and the mixture was stirred overnight at rt. The solution was concentrated, and the residue was coevaporated with toluene under reduced pressure to obtain a crude product that was recrystallized from EtOAc–hexanes to afford **29** (0.23 g, 84.1%) as white needles: R_f 0.18 (3:1 EtOAc–hexanes); mp 166–168 °C; $[\alpha]_D$ +101.6° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.95 (s, 3 H, NAc), 2.06 and 2.07 (2 s, 6 H, 2 OAc), 3.38 (s, 3 H, OMe), 3.98 (ddd ~ dt, 1 H, $J_{5,6}$ 6.5, $J_{5,F}$ 28.4 Hz, H-5), 4.22 (dd, 1 H, J_{gem} 11.3 Hz, H-6), 4.29 (ddd, 1 H, $J_{6',5}$ 6.8, $J_{6',F}$ 0.7 Hz, H-6′), 4.61 (m, 1 H, H-2), 4.75 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.77 (dd, 1 H, $J_{4,3}$ 2.3, $J_{4,F}$ 50.7 Hz, H-4), 5.09 (dddd, 1 H, $J_{3,2}$ 11.3, $J_{3,F}$ 27.8 Hz, H-3), 5.63 (d, 1 H, $J_{NH,2}$ 9.6 Hz, NH); ^{13}C NMR ($CDCl_3$): δ 20.7 and 20.8 (2 $OCOCH_3$), 23.3 ($NCOCH_3$), 47.7 (C-2), 55.5 (OMe), 61.9 (*J* 5.3 Hz, C-6), 67.0 (*J* 18.0 Hz, C-5), 68.8 (*J* 17.4 Hz, C-3), 86.4 (*J* 186.0 Hz, C-4), 98.6 (C-1), 169.9, 170.4, and 171.2 (2 $OCOCH_3$, NC=O); ^{19}F NMR ($CDCl_3$): δ 219.2 (dt, $J_{F,H3} = J_{F,H5}$ 28.1, $J_{F,H4}$ 50.7 Hz). Anal. Calcd for $C_{13}H_{20}FNO_7$: C, 48.60; H, 6.27; F, 5.91; N, 4.40. Found: C, 48.69; H, 6.05; F, 6.12; N, 4.43.

Methyl 2-acetamido-3,6-di-O-acetyl-2,4-dideoxy-4-fluoro-β-D-galactopyranoside (**30**).—Compound **27** (0.10 g, 0.42 mmol) was treated with Ac_2O (1.0 mL) in pyr (2.0 mL) at 0 °C, and the mixture was stirred overnight at rt. The solution was processed as described for **29** to afford a solid that was recrystallized from EtOAc to obtain **30** (0.12 g, 88.9%) as tiny, white needles: R_f 0.20 (EtOAc); mp 235–236 °C; $[\alpha]_D$ −19.4° {*c* 1, (1:1 MeOH–$CHCl_3$)}; ^{1}H NMR (1:1 CD_3OD–$CDCl_3$): δ 1.97 (s, 3 H, NAc), 2.13 and 2.12 (2 s, 6 H, 2 OAc), 3.52 (s, 3 H, OMe), 3.91 (ddd ~ dt, 1 H, $J_{5,6}$ 6.5, $J_{5,F}$ 26.4 Hz, H-5), 4.09 (dd, 1 H, $J_{2,3}$ 11.1 Hz, H-2), 4.26 (dd, 1 H, J_{gem} 11.3 Hz, H-6), 4.37 (ddd, 1 H, $J_{6',5}$ 6.7, $J_{6',F}$ 0.8 Hz, H-6′), 4.59 (dd, 1 H, $J_{1,2}$ 8.4, $J_{1,F}$ 1.0 Hz, H-1), 4.84 (dd, 1 H, $J_{4,3}$ 2.5, $J_{4,F}$ 50.6 Hz, H-4), 5.13 (dddd, 1 H, $J_{3,F}$ 27.7 Hz, H-3); ^{13}C NMR (1:1 CD_3OD–$CDCl_3$): δ 20.0 and 20.1 (2 $OCOCH_3$), 22.2 ($NCOCH_3$), 50.2 (C-2), 56.2 (OMe), 61.5 (*J* 5.3 Hz, C-6), 70.5 (C-3, C-5), 85.3 (*J* 185.3 Hz, C-4), 101.1 (C-1), 170.5,

170.7, and 171.7 (2 O*C*OCH$_3$, NC=O); ^{19}F NMR (1:1 CD$_3$OD–CDCl$_3$): δ 219.2 (dt, $J_{F,H3} = J_{F,H5}$ 28.1, $J_{F,H4}$ 50.7 Hz). Anal. Calcd for $C_{13}H_{20}FNO_7$: C, 48.60; H, 6.27; F, 5.91; N, 4.40. Found: C, 48.65; H, 5.91; F, 5.77; N, 4.36.

*Preparation of 2-acetamido-1,3,6-tri-*O*-acetyl-2,4-dideoxy-4-fluoro-*D*-galactopyranose* (**31**).—Compound **28** (0.10 g, 0.42 mmol) was treated with Ac_2O (1.0 mL) in pyr (2.0 mL) at 0 °C, and the mixture was stirred overnight at rt. The solution was processed as described for **29** to afford a solid that was recrystallized from EtOAc–hexanes to obtain **31** (0.16 g, quant) as clear, colorless rods: R_f 0.13 and 0.19 (3:1 EtOAc–hexanes); mp 140–142 °C, lit. 155 – 158 °C [9a]; $[\alpha]_D$ + 114.0° (*c* 1, $CHCl_3$), lit. + 144° (*c* 1, $CHCl_3$) [9a]. α Anomer: ^{1}H NMR ($CDCl_3$): δ 1.93 (s, 3 H, NAc), 2.12 and 2.15 (2 s, 9 H, 3 OAc), 4.09 (ddd ~ dt, 1 H, $J_{5,6}$ 6.6, $J_{5,F}$ 27.8 Hz, H-5), 4.19 (dd, 1 H, J_{gem} 11.2 Hz, H-6), 4.37 (ddd, 1 H, $J_{6',5}$ 6.7, $J_{6',F}$ 0.8 Hz, H-6′), 4.75 (m, 1 H, H-2), 4.83 (dd, 1 H, $J_{4,3}$ 2.2, $J_{4,F}$ 50.0 Hz, H-4), 5.16 (dddd, 1 H, $J_{3,2}$ 11.6, $J_{3,F}$ 26.9 Hz, H-3); 5.57 (d, 1 H, H-2), 6.20 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1); ^{13}C NMR ($CDCl_3$): δ 20.7, 20.8, and 20.9 (3 OCO*C*H$_3$), 23.1 (NCO*C*H$_3$), 46.8 (C-2), 61.4 (*J* 6.2 Hz, C-6), 68.0 (*J* 18.4 Hz, C-5), 68.9 (*J* 18.2 Hz, C-3), 85.8 (*J* 186.7 Hz, C-4), 91.2 (C-1), 168.7, 170.0, 170.4, and 171.4 (3 O*C*OCH$_3$, NC=O); ^{19}F NMR ($CDCl_3$): δ 219.0 (dt, $J_{F,H3} = J_{F,H5}$ 27.4, $J_{F,H4}$ 50.2 Hz).

Biological evaluation.—Hepatocytes were obtained from 6–8-week-old, female Swiss white mice (Charles River Canada, St. Constant, Quebec) by the procedure described previously [26,27]. Briefly, the liver was perfused with 50 mL of 0.01 M Hepes buffer (pH 7.4) containing 0.5 mM EGTA, followed by 50 mL of a collagenase type-IV solution (0.5 mg/mL) in 0.1 M Hepes (pH 7.6). The liver was removed, and the hepatocytes separated from the capsule by gentle teasing. The pooled cells were centrifuged at 200 rpm at 5 °C for 5 min and washed once with fresh medium. After resuspension in 20 mL of Williams' Medium E and filtration through a Nitex 110 nylon membrane, the cells were exposed to Trypan Blue and counted on a hemocytometer to determine the viability and cell number. The viability was usually greater than 85%. The cells were plated in triplicate on fibronectin-coated tissue culture dishes (Falcon 35 × 10 mm) at a density of 2×10^6 cells per plate. They were incubated in 2 mL of Williams' Medium E containing 10% Fetal bovine serum and 1% antibiotic–antimycotic mixtures. After 2 h, the non-adherent cells were removed, and the attached cells were fed with fresh plating medium for a 24-h period. The cells were then provided with fresh medium containing the necessary isotopes and/or monosaccharide derivative. For GAG labeling, [^{3}H]GlcN (2 μCi/mL) and [^{35}S]SO_4 (4 μCi/mL) were included in the medium. For experiments monitoring protein synthesis, the cells were incubated with [^{14}C]Leu (0.5 μCi/mL). The labeled cellular GAGs, or total proteins, were harvested 24 h later.

GAG isolation.—After the 24-h labeling period, the medium was separated from the cells, and the cells were solubilized in 4 M guanidine·HCl, 2% Triton X-100 in 0.05 M acetate buffer (pH 6.0). A GAG carrier (1 mg/mL each of chondroitin sulfate, hyaluronan, and heparin) was added to all of the samples. The isolation of radioactive GAGs was based on the cetylpyridinium chloride (CPC) precipitation technique described by Hronowski and Anastassiades [28]. Briefly, media and cell fractions were each subjected to papain digestion, and the GAGs were precipitated as sodium salts by CPC. After lyophilization, the samples were dissolved in a known volume of water for analysis. Media GAGs were analyzed only during preliminary experiments. Cellular GAGs served as the assay material for the effects of the sugar analogs.

Total protein isolation.—Total cell protein synthesis was determined by measuring [^{14}C]Leu incorporation. After the cells had been incubated in the labeling medium for the specified period of time, the culture medium was removed, and the cell fractions were solubilized by treatment with 1% sodium dodecyl sulfate and then combined with the medium. The proteins were precipitated by the addition of 3 mL of a 10% aq solution of trichloroacetic acid (TCA) containing 10 mg/mL of DL-leucine. The samples were heated at

90 °C for 30 min to destroy leucine tRNA and then cooled at 4 °C for 30 min. Using a Millipore 1225 sampling manifold, each sample was passed through a glass microfiber filter (Whatman 934-AH), and the filter was washed three times with 5 mL of a cold, 5% aq solution of TCA containing 10 mg/mL of DL-leucine and once with 3 mL of EtOH. The filters were air-dried and immersed in 5 mL of scintillation solution, and the radioactivity was measured. Nonspecific binding of [^{3}H]Leu was not more than 5% of the total incorporated radioactivity.

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