Supplementary Materials:

Targeting the chondroitin sulfate proteoglycans: Evaluating fluorinated glucosamines and xylosides in screens pertinent to multiple sclerosis

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Materials and Methods

Mixed glial cultures and enrichment for oligodendrocyte precursor cells and astrocytes

All murine *in vitro* experiments were in accordance with ethical animal care guidelines by the Animal Care Committee at the University of Calgary and were performed with CD-1 mice as previously described[1,2] and also illustrated in Fig. **3**. In brief, cortices from postnatal days 1-3 mouse brains were dissociated with papain digestion and then plated as a mixed culture for 7 days in a 37°C incubator at 8.5% CO_2 whereupon OPCs and microglia tended to be loosely attached on top of a monolayer of astrocytes; the OPCs and microglia were then shaken off when placed on an orbital shaker at 220 r.p.m. at 37°C and 5% CO_2 overnight. The media containing microglia and OPCs were collected and added to a 100 mm tissue culture dish and left at 37°C and 5% CO_2 for 30 min, to allow preferential adhesion of microglia. The media were collected again, now containing OPCs, and used as the OPC-enriched isolates for experiments. For the remaining adhered cells, largely astrocytes, these were removed with 0.25% trypsin and after centrifugation and washing, these astrocytes were plated at a density of 1.0 x 10^5 cells in flat bottom 96-well plates coated with 10µg/ml poly-L-lysine. Cells were grown for 7 days at 37°C and 5% CO_2 in MGM and daily media changes. Astrocytes were carefully removed with EDTA treatment (0.2g EDTA (Na_4) per liter PBS, 30 minutes at 37°C and 5% CO_2) and mechanical dislodgement with a micropipetter. The remaining ECM left behind was covered with PBS at 4°C until OPCs were seeded. Enriched OPCs from mixed glial cultures were seeded at a density of 5–10 x 10^4 cells per well in OPC media and grown at 37 °C and 8.5% CO_2 for 18–24 h and then were fixed with 4% ice-cold paraformaldehyde at 4°C for 10 min, rinsed with PBS, and stored at 4°C until immunocytochemistry.

Immunocytochemistry to sulfatide O4 on oligodendrocyte lineage cells

OPCs were blocked with Licor Odyssey blocking buffer for 60 minutes. The primary antibody to sulfatide O4 on oligodendrocyte lineage cells (EMD Millipore, MAB345) was diluted in Licor blocking buffer (1:250) and incubated overnight at 4°C. A secondary antibody and nuclear yellow for detecting cell nuclei were then added for 60 minutes. Cells were imaged with ImageXpress® Micro Cellular Imaging and Analysis System (Molecular Devices, Sunnyvale, CA) and analyzed by MetaExpress multiwavelength cell scoring program. Data from 12 images per well were averaged to a single data point per well, with four well replicates per treatment.

ImageXpress acquisition and MetaXpress analysis

96-well plates were imaged with ImageXpress® Micro Cellular Imaging and Analysis System (Molecular Devices, Sunnyvale, CA). Twelve images per well were collected at x10 magnification. Images were processed with MetaXpress analysis software. OPC outgrowth was calculated by the MetaExpress® "neurite outgrowth" software module which uses fluorescence and user-modified parameters to document the overall extent of processes emanating from the cell soma, regardless of size of the protrusions or the number of branches. This was previously detailed elsewhere[3]. Live-dead cell assay was calculated with MetaXpress analysis software with the "multiwavelength cell scoring", which uses fluorescent intensity to measure co-localization of calcein AM, propidium iodide, and nuclear yellow. Data from the 12 images were averaged to a single data point per well, wherein the OPC outgrowth data calculated from the MetaExpress® "neurite outgrowth" readout was divided by data from the same 12 fields of cell number that was acquired through the multiwavelength cell scoring. This provided mean outgrowth per cell per well, with four well replicates per treatment. Experiments were repeated at least twice.

Western blot of astrocyte conditioned medium

Astrocytes were plated in uncoated 6-well plates (3 replicate wells per treatment) at $1x10^6$ cells/well in mixed glial media, and incubated at 5% CO_2 and 37 °C. Astrocytes were treated with compounds at 50μM in AIM-V media for 48 hours. Media was pooled from 3 replicate wells in 15ml 100K cutoff centricon tubes (EMD Millipore, #UFC905008), which were spun on an ultracentrifuge at 5000 x g for 2 cycles of 10 minutes each. When harvested, the cell lysate was kept on ice for 5 minutes with 250μL of RIPA buffer (ThermoFisher Scientific #89900) containing proteinase inhibitor, and then collected and centrifuged at 14000g for 5 minutes. Total protein was quantified using a Bradford assay. Conditioned media that were to be probed for chondroitin 4-sulfate GAGs (Millipore, #MAB2030) were first digested with 0.2 U/ml chondroitinase ABC for overnight at 37 °C to remove chondroitin sulfate sidechains. Blots that were to be probed with CSA (2H6, Cosmo) or CS56 (Abcam #ab11570) were not exposed to chondroitinase ABC. Samples were heat denatured with NuPAGE® LDS Sample buffer at 70 °C for 10 minutes, loaded into 3–8% tris-acetate pre-cast gels, electrophoresed and then transferred to a 0.2 mm polyvinylidene fluoride membrane. Following a blocking step, primary antibodies were added to membranes in 3% skim milk and incubated overnight at 4 °C, followed by HRP-conjugated secondary antibodies, then developed using an ECL chemiluminescence kit. Bands were normalized from the same gel to a lane with untreated astrocytes on the same gel to compare relative densities across different gels.

Splenocytes cell cultures and thymidine proliferation assays

Spleens were isolated from 8-10-week-old female C57BL/6 mice and homogenized. The cells were separated by Ficoll gradient (30 min at 1,800 rpm) and were cultured in RPMI containing 10% fetal bovine serum. Cells were plated at $2.5x10^5$ cells per well in a round-bottom 96-well plate and exposed to 1 μg/ml plate-bound anti-CD3 and 1 μg/ml anti-CD28 suspended in media to preferentially activate T lymphocytes. Compounds were added at final concentrations of 25 μM. The cells were kept at 37 °C and 5% CO_2 for 30 h, after which 10 μl/well (1 μCi per well) of 3H-thymidine was added for 18h. The cells containing thymidine were harvested onto filter mats using a cell harvester and mats were allowed to dry for 24h. The results were read by liquid scintillation counts.

Flow cytometry for propidium iodide staining for DNA cell cycle analysis and Annexin V/propidium iodide staining

Cells were collected, resuspended in 350 μl of PI staining buffer (50μg/ml propidium iodide, 0.1% TritonX100, 0.2mg Dnase free Rnase A in PBS) and incubated for 30-45 minutes. Annexin V was detected using the Annexin V FITC apoptosis detection kit (BD Biosciences, #556547), which was performed according to manufacturer's instructions.

Bone marrow-derived macrophage (BMDM) cultures

Femurs from female C57Bl/6 mice were flushed for marrow into a culture plate. Cells were spun at 1100 rpm for 10 minutes, resuspended in fresh growth medium and plated at 10 x 10^6 cells/ml in a 10 cm culture dish. Cells were grown in DMEM with supplements and L929 supernatant for 5 days, then half the medium was replaced with fresh growth medium. On day 7 growth medium

was replaced with DMEM with 10% FBS and supplements. Cells were used on day 8, and experiments were conducted in DMEM with 1% FBS and supplements unless otherwise specified.

TNFα ELISA

BMDM were plated at 25,000 cells in 96-well plates in DMEM+L929 media. After 24 hours media was changed to DMEM + 1% FBS. One hour later, cells were treated with 25 μM compounds or PBS. After 1 hour LPS (final concentration 100ng/ml) was added. Following 24 hours, conditioned media was harvested for TNFα ELISA (Thermo Scientific), which was performed according to manufacturer's instructions.

Human neuron cell cultures and the ATP assay

Human fetal neurons were plated at $10x10^4$ cells/well in 96 well plates as previously described[4]. After 24 hours neurons were treated with 100 μM compounds and incubated for 24 hours. The ATP assay (CellTiter-Glo Luminescent Cell Viability Assay; Promega, Madison, WI, USA) was then performed to test for toxicity according to manufacturer's instructions.

Propidium iodide/calcein AM immunocytochemistry

A mixture was created containing propidium iodide (10 μg/ml) to stain dead cells, 10μM Calcein AM (ThermoFisher, #C3100MP) to identify live cells, and 2 drops per ml of media of NucBlue™ Live ReadyProbes™ Reagent (ThermoFisher, #R37605) to identify nuclei. The mixture was added to plate media and incubated for 20-30 minutes at 37°C and 5% CO_2 before imaging. Excitation/emission of calcein-AM (λ_{ex} 490 nm, λ_{em} 515 nm), propidium iodide

(λ_{ex} 535 nm, λ_{em} 617 nm), and nuclear blue (λ_{ex} 350 nm, λ_{em} 461 nm) was performed using ImageXpress® Micro Cellular Imaging and Analysis System (Molecular Devices, Sunnyvale, CA) and analyzed by MetaExpress multiwavelength cell scoring program.

Experimental autoimmune encephalomyelitis (EAE)

All procedures were in accordance with guidelines of the Canadian Council of Animal Care and have received approval by local ethics committee. EAE experiments used seven to ten-week-old female C57Bl/6 female mice. Mice were injected with 50µl (200 µg) of MOG_{35-55} (peptide 35-55, University of Calgary), emulsified in complete Freud's adjuvant (CFA) containing 10 mg/ml of heat inactivated *Mycobacterium tuberculosis* H37RA injected subcutaneously into each hind flank. At time of MOG_{35-55} immunization and again 2 days later, each animal received 300 ng of pertussis toxin. Mice were evaluated daily for weight loss, and scored daily for clinical signs of EAE with a 15-point scale [1]. All treatment and EAE clinical scoring and analysis were done blinded to treatment groups.

For treatment regimen 1, mice were randomized into two groups of eight mice on day of MOG_{35-55} immunization. Intraperitoneal treatment with either Ac-4,4-diF-GlcNAc (25mg/kg, dissolved in saline) or vehicle (saline) was done blinded, and began on day 7 and continued once a day until sacrifice on day 15. Following lethal anaesthesia with intraperitoneal ketamine/xylosine (10mg/kg), blood was taken for FACs analysis and mice were PBS-perfused. Lumbar/thoracic spinal cord were used for FACs analysis. Cervical spinal cord and the cerebellum were taken for immunohistochemistry.

For treatment regimen 2, mice were randomized into two groups of nine on day of MOG_{35-55} immunization. One mouse from the Ac-4,4-diF-GlcNAc group was removed due to an

unrelated skin lesion. Intraperitoneal treatment with either Ac-4,4-diF-GlcNAc (25mg/kg, dissolved in saline) or vehicle (saline) was done blinded, and began on day 15 and continued once a day until sacrifice on day 24. Mice and tissue were processed in the same manner as regimen 1.

Treatment with 50mg/kg of Ac-4-F-GlcNAc, 50mg/kg Ac-4,4-diF-GlcNAc, or saline was intraperitoneal and conducted as treatment regimen 1. Thirty mice were immunized for EAE and then randomized into three groups of ten. Treatments were administered daily from pre-onset (day 7) until peak (day 15). Mice and tissue were processed in the same manner as regimen 1.

Flow cytometry of spinal cord and circulating leukocytes

To assess the inflammatory profile of circulating leukocytes, we performed flow cytometry using a modified protocol published previously[5]. Briefly, mice were anaesthetized using ketamine/xylazine, after which blood was drawn via cardiac puncture. Heparin-coated syringes were used to draw approximately 130 μL of blood, which was then subsequently diluted with 70 μL of Hanks' Balanced Salt Solution (HBSS; Gibco) without calcium and magnesium. Fc receptors were then blocked by addition of Mouse BD Fc Block (1:100; BD Pharmingen) for 30 minutes at 4°C. Primary antibodies (1:50) were then added and incubated for 45 minutes at 4°C. The primary antibodies used were CD45-PerCP (BD Pharmingen; Clone 30-F11), CD11b-FITC (BD Pharmingen; Clone M1/70), Ly6G-APC-Cy7 (BD Pharmingen; Clone 1A8), Ly6C-V450 (BD Horizon; Clone AL-21), and CD3-PE (BD Pharmingen; Clone 17A2). Red blood cells were then lysed by rocking samples at room temperature for 12 minutes with 1 mL of BD FACS Lysing Solution (BD Biosciences). Samples were then washed at 1200 rpm for 10 minutes at 4°C, followed by two more washes at 2000 rpm for 3 minutes at 4°C. Prior to acquisition on a

flow cytometer (BD LSRII), cells were then fixed in 1% formalin for 10 minutes and then resuspended in HBSS without calcium and magnesium.

For flow cytometry of the spinal cord, spinal cords were dissected following a PBS perfusion. The thoracic and lumber sacral part of isolated spinal cords were separated into neural and leucocyte populations by density gradient centrifugation using isotonic Percoll (GE Healthcare). The leukocytes samples were prepared at 4°C in fluorescence-activated cell sorting (FACS) buffer solution (BD Biosciences) and Fc receptors were then blocked by addition of Mouse BD Fc Block (1:100; BD Pharmingen) for 30 minutes at 4°C. The cells were stained with antibodies against CD45-PerCP (BD Pharmingen; Clone 30-F11), CD11b-FITC (BD Pharmingen; Clone M1/70) and CD3-APC-Cy7 (BD Pharmingen; Clone 17A2) for 45 minutes and then washed three times with FACS buffer. The cells were fixed in 1% buffered formalin for 10 min and resuspended in the 200µl of the FACS buffer.

Data acquisition was performed on a flow cytometer (BD FACSAria; BD Biosciences) and analysed with FlowJo software (version 8.6, TreeStar). To ensure proper compensation and gating, unstained samples, appropriate isotype controls, and single-stain controls were included. All data was analyzed using FlowJo software.

Quantification of number of perivascular cuffs per spinal cord and Imaris quantification of CD45+ cells

Perivascular cuffs were identified with pan-laminin staining of two basement membranes with CD45+ cells clustered within the perivascular space. To obtain the average number of perivascular cuffs per spinal cord, perivascular cuffs were counted on 4 cervical spinal cord sections (each >200 µm apart), and then averaged to obtain the average perivascular cuffs per

spinal cord per mouse. The Imaris software (Bitplane, Switzerland) was used to quantify the number and distance of CD45+ cells around perivascular cuffs. CD45+ cells were registered as spots and the laminin-positive membranes were registered as surfaces. The Xtension component in Imaris 'distance transformation' calculated the distance of every CD45+ cell from the perivascular cuff.

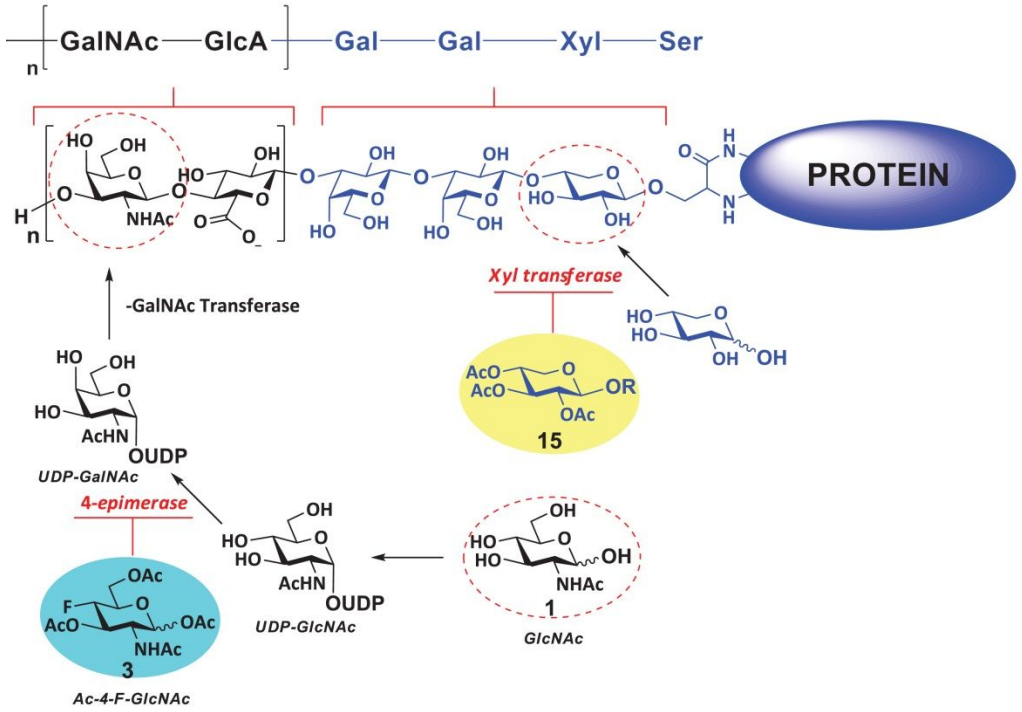
Statistical analysis

Where multiple groups were compared, a one-way ANOVA with Tukey-Kramer's *post hoc* test for multiple comparisons was used. If the multiple comparisons were against a control group, a Dunnett's *post hoc* test was used. For comparisons between two groups, unpaired two-tailed Student's *t*-tests were applied. EAE disease scores were analyzed with two-way repeated-measures ANOVA with Sidak's *post-hoc* test. $P<0.05$ was considered statistically significant. All graphs presented are mean with standard deviation, unless otherwise specified. A linear regression analysis was also used for Suppl. Fig. **6**. All the statistical analyses were performed with Prism 6.0 software (GraphPad).

Safety Statement

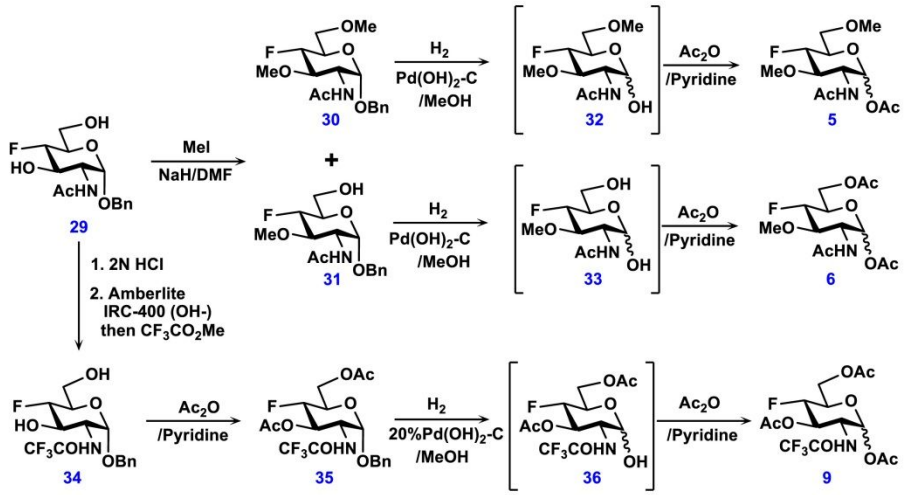
No unexpected or unusually high safety hazards were encountered.

Supplementary Figures

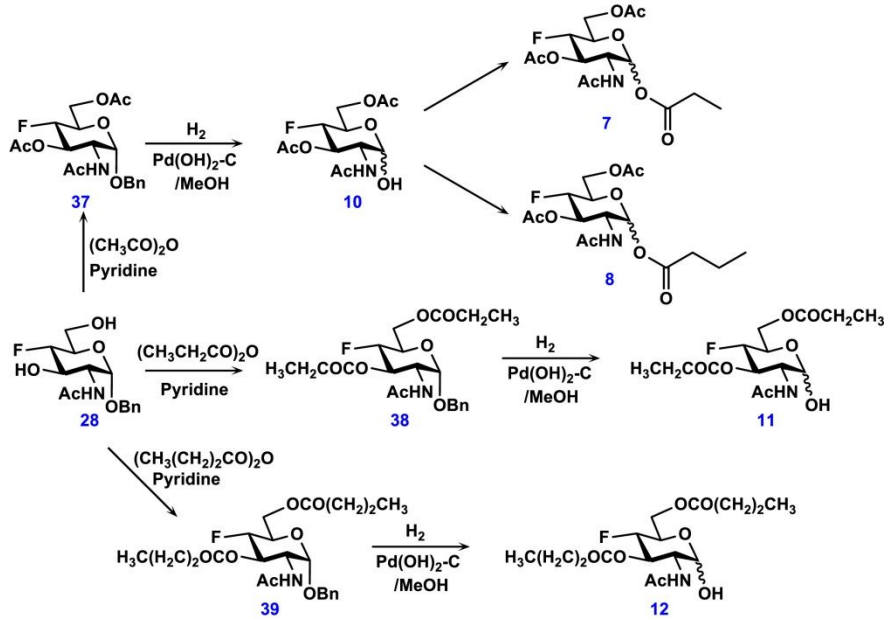


Supplemental Figure 1. Synthesis of CSPGs. Following formation of trisaccharide linker (xylose, galactose, galactose), chondroitin sulfate GAG chains are elongated with the disaccharides glucuronic acid (GlcA) and N-acetyl-galactosamine (GalNAc). UDP-GalNAc is created from UDP-N-acetyl-glucosamine (UDP-GlcNAc) by the enzyme 4-epimerase through an oxidation and reduction process. Fluorinated analogs (3, blue) perturb chondroitin sulfate GAG synthesis, potentially by acting as inhibitors of 4-epimerase. Xyloside analogs (15, yellow) perturb synthesis by competing for binding with xyloside.

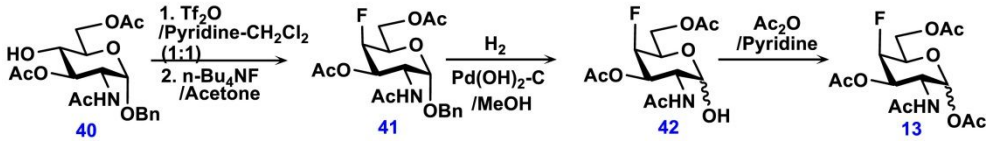
a). Synthesis routes to compounds 5, 6 and 9.



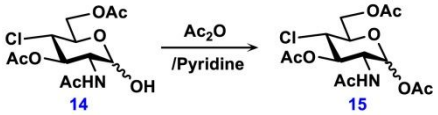
b). Synthesis routes to compounds 7, 8, 11 and 12.



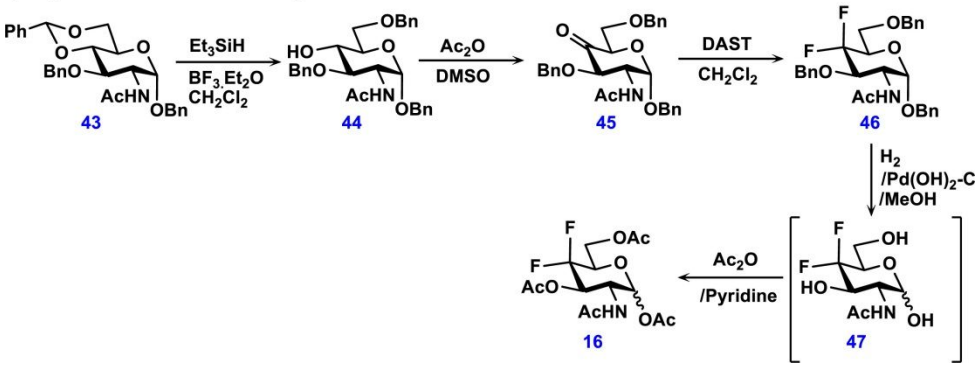
c). Synthesis routes to compounds 13.



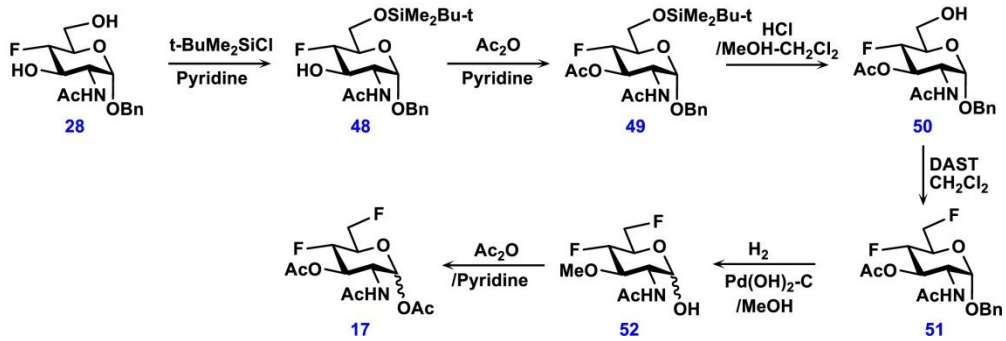
d). Synthesis routes to compounds 15.



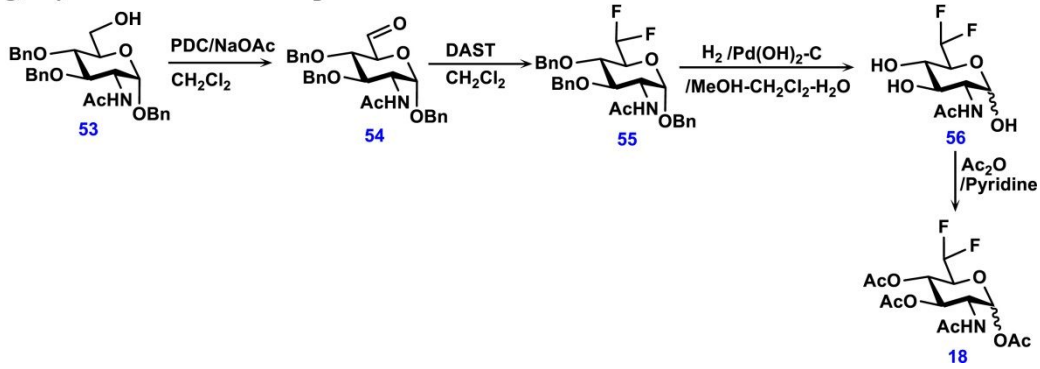
e). Synthesis routes to compounds 16.



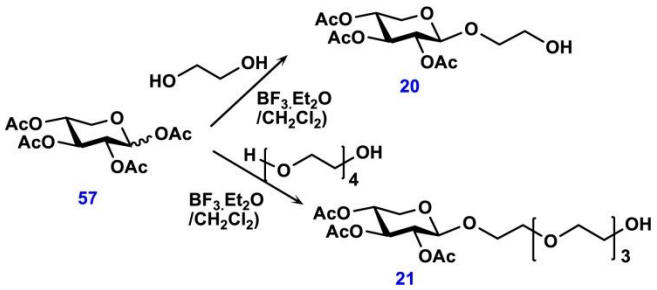
f). Synthesis routes to compounds 17.



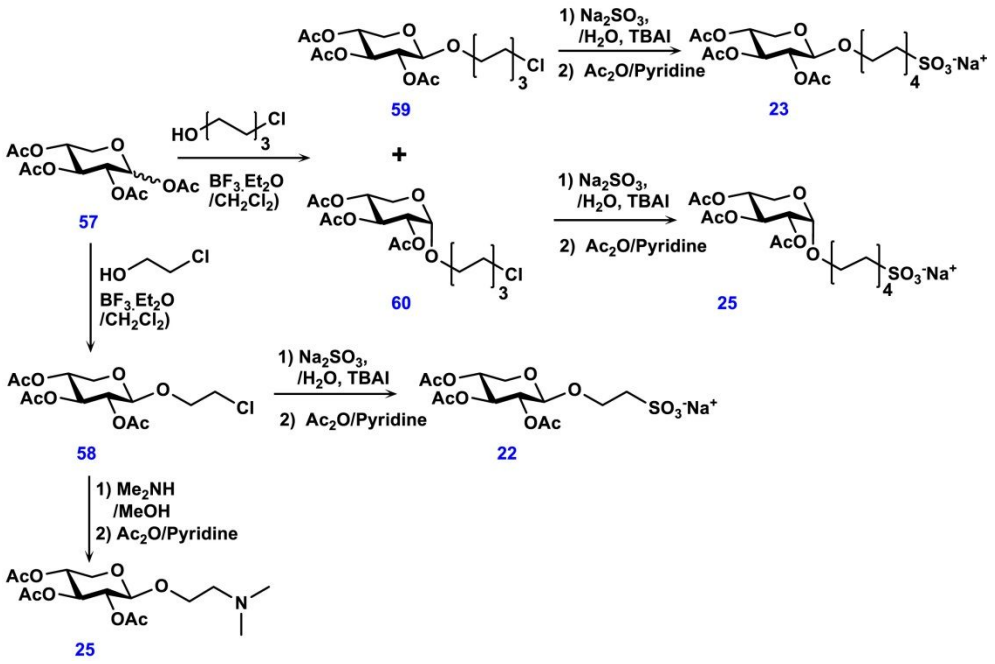
g). Synthesis routes to compounds 18.



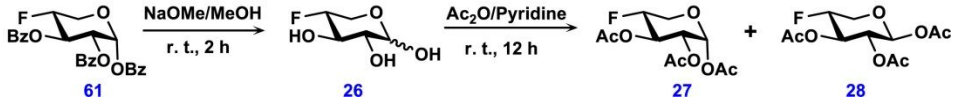
h). Synthesis routes to xylosides 20 and 21.



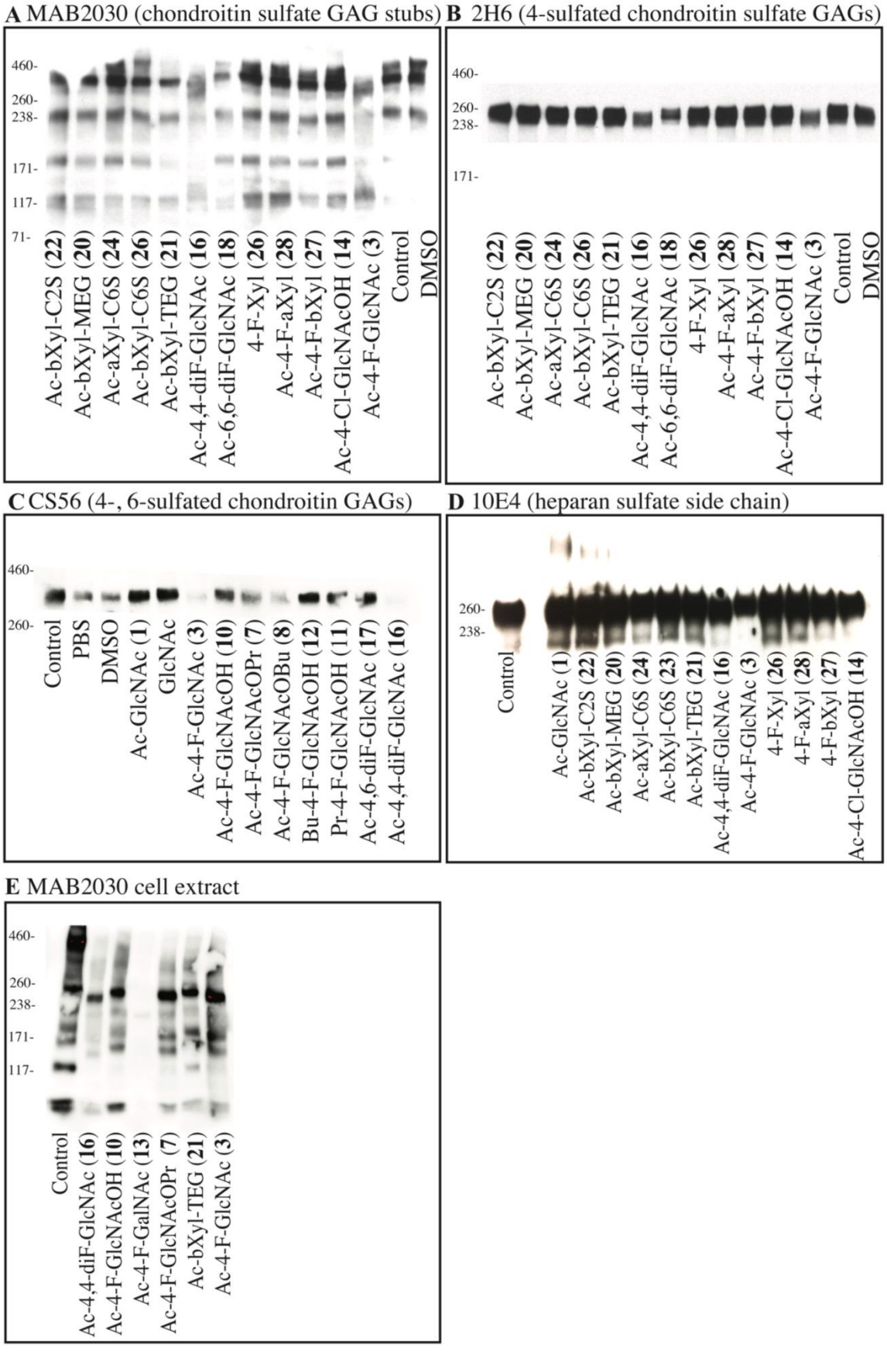
i). Synthesis routes to xylosides 22, 23, 24 and 25.



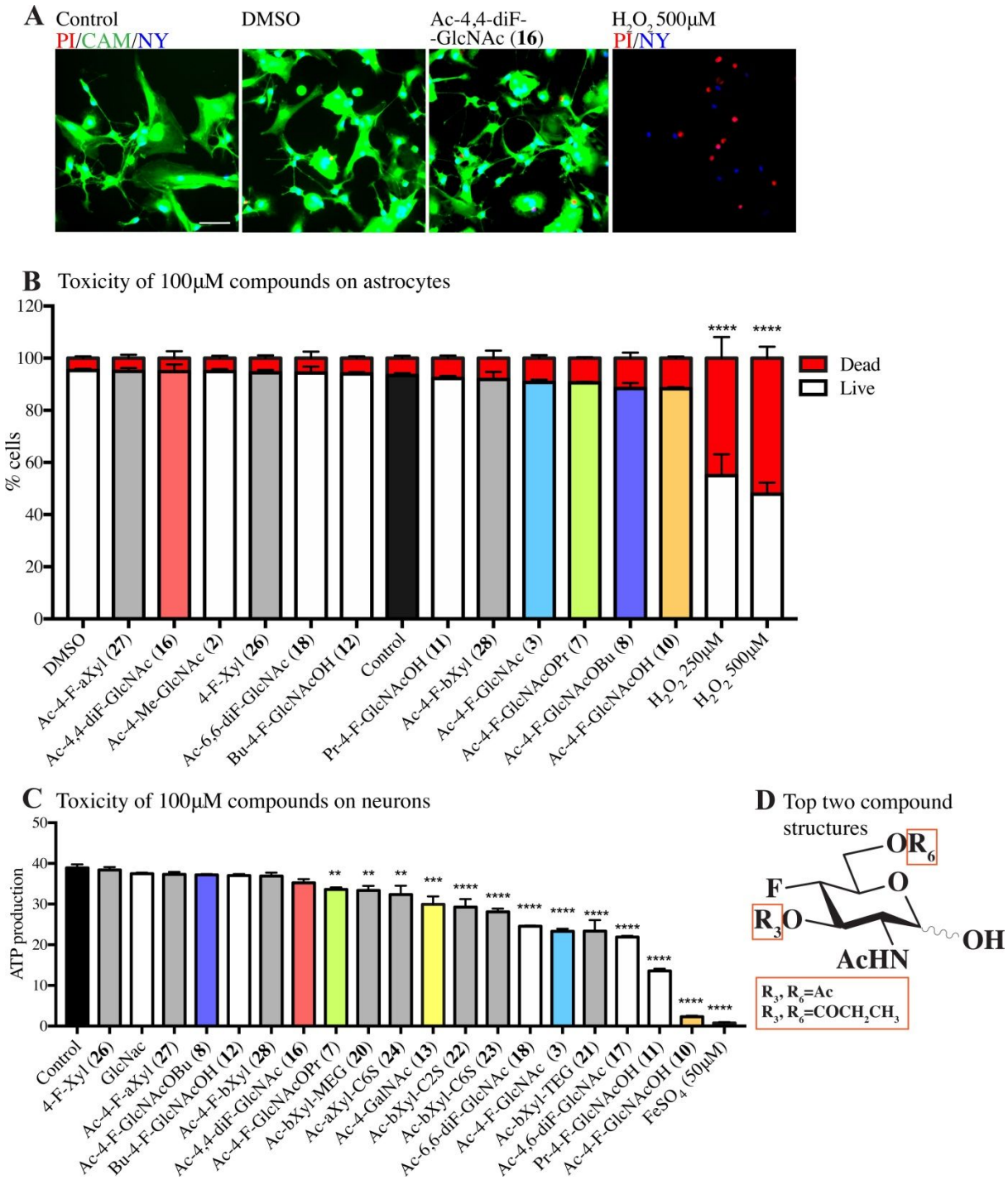
j). Synthesis routes to 4-fluorinated xylosides 26, 27 and 28.



Supplemental Figure 2. Synthesis of fluorinated glucosamine derivatives **5**-**9**, **11**-**13**, **15**-**18**, and xylosides **20**-**28**. Synthetic routes to substituted *N*-acetyl-D-glucosamine derivatives: **a)** compounds **5**,**6** and **9**, **b) 7**, **8**, **11** and **12**, **c) 13**, **d) 15**, **e) 16**, **f) 17**, **g) 18**, **h) 20** and **21**; substituted D-xylopyranose derivatives: **i)** compounds **22**-**25** and **j) 26**-**28.**

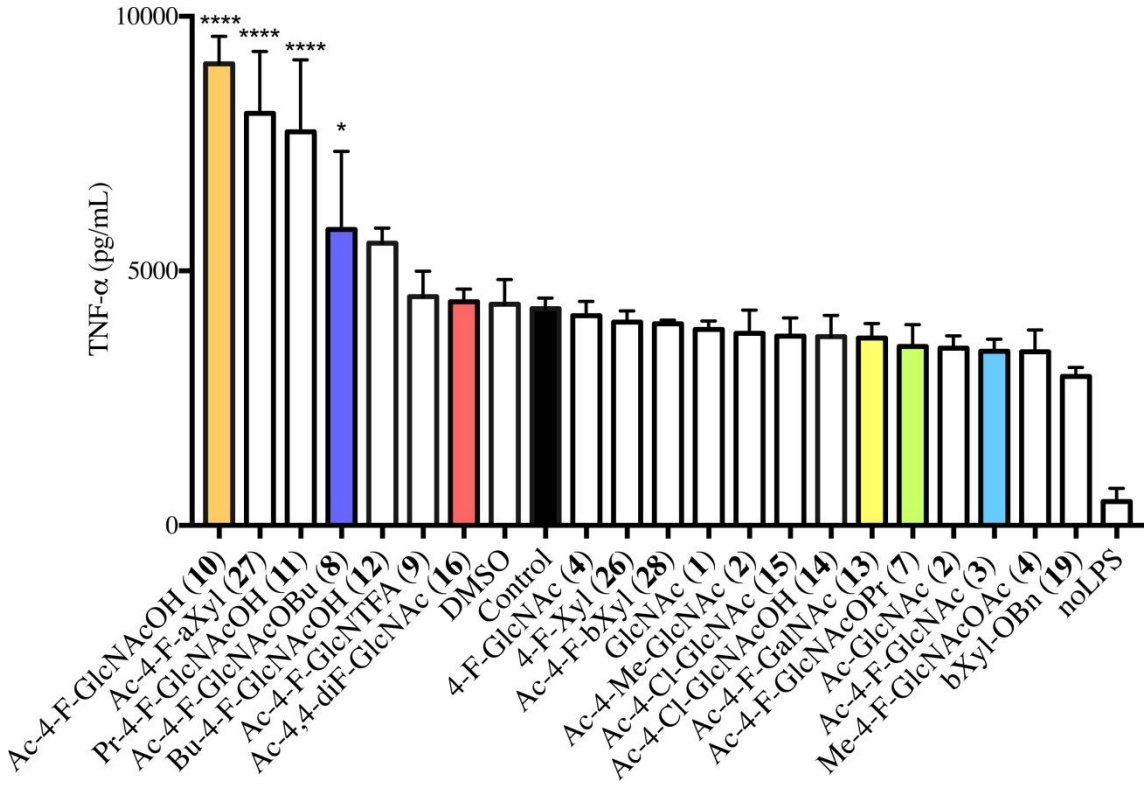

A MAB2030 (chondroitin sulfate GAG stubs)
460-
260-
238-
171-
117-
71-
Ac-bXyl-C2S (22)
Ac-bXyl-MEG (20)
Ac-aXyl-C6S (24)
Ac-bXyl-C6S (26)
Ac-bXyl-TEG (21)
Ac-4,4-diF-GlcNAc (16)
Ac-6,6-diF-GlcNAc (18)
4-F-Xyl (26)
Ac-4-F-aXyl (28)
Ac-4-F-bXyl (27)
Ac-4-Cl-GlcNAcOH (14)
Ac-4-F-GlcNAc (3)
Control
DMSO
B 2H6 (4-sulfated chondroitin sulfate GAGs)
460-
260-
238-
171-
Ac-bXyl-C2S (22)
Ac-bXyl-MEG (20)
Ac-aXyl-C6S (24)
Ac-bXyl-C6S (26)
Ac-bXyl-TEG (21)
Ac-4,4-diF-GlcNAc (16)
Ac-6,6-diF-GlcNAc (18)
4-F-Xyl (26)
Ac-4-F-aXyl (28)
Ac-4-F-bXyl (27)
Ac-4-Cl-GlcNAcOH (14)
Ac-4-F-GlcNAc (3)
Control
DMSO
C CS56 (4-, 6-sulfated chondroitin GAGs)
460-
260-
Control
PBS
DMSO
Ac-GlcNAc (1)
GlcNAc
Ac-4-F-GlcNAc (3)
Ac-4-F-GlcNAcOH (10)
Ac-4-F-GlcNAcOPr (7)
Ac-4-F-GlcNAcOBu (8)
Bu-4-F-GlcNAcOH (12)
Pr-4-F-GlcNAcOH (11)
Ac-4,6-diF-GlcNAc (17)
Ac-4,4-diF-GlcNAc (16)
D 10E4 (heparan sulfate side chain)
260-
238-
Control
Ac-GlcNAc (1)
Ac-bXyl-C2S (22)
Ac-bXyl-MEG (20)
Ac-aXyl-C6S (24)
Ac-bXyl-C6S (23)
Ac-bXyl-TEG (21)
Ac-4,4-diF-GlcNAc (16)
Ac-4-F-GlcNAc (3)
4-F-Xyl (26)
4-F-aXyl (28)
4-F-bXyl (27)
Ac-4-Cl-GlcNAcOH (14)
E MAB2030 cell extract
460-
260-
238-
171-
117-
Control
Ac-4,4-diF-GlcNAc (16)
Ac-4-F-GlcNAcOH (10)
Ac-4-F-GalNAc (13)
Ac-4-F-GlcNAcOPr (7)
Ac-bXyl-TEG (21)
Ac-4-F-GlcNAc (3)

Supplemental Figure 3. Fluorinated glucosamines and xylosides reduce the synthesis of CSPGs and HSPGs by astrocytes. **(A)** Representative western blot of MAB2030 (chondroitin sulfate GAG stubs) and **(B)** 4-sulfated GAGs produced by astrocytes treated with a variety of xylosides and glucosamines. **(C)** Western blot with CS56 antibody against 4- and 6-sulfated GAG chains. (**D**) Probing for heparan sulfate GAGs (10E4) shows a reduction by Ac-4-F-GlcNAc **3** and Ac-4,4-diF-GlcNAc **16.** (**E**) MAB2030 western blot of astrocyte cell lysates showing less immunoreactive bands in cultures treated with compounds previously found to reduce CSPG levels in the conditioned media.

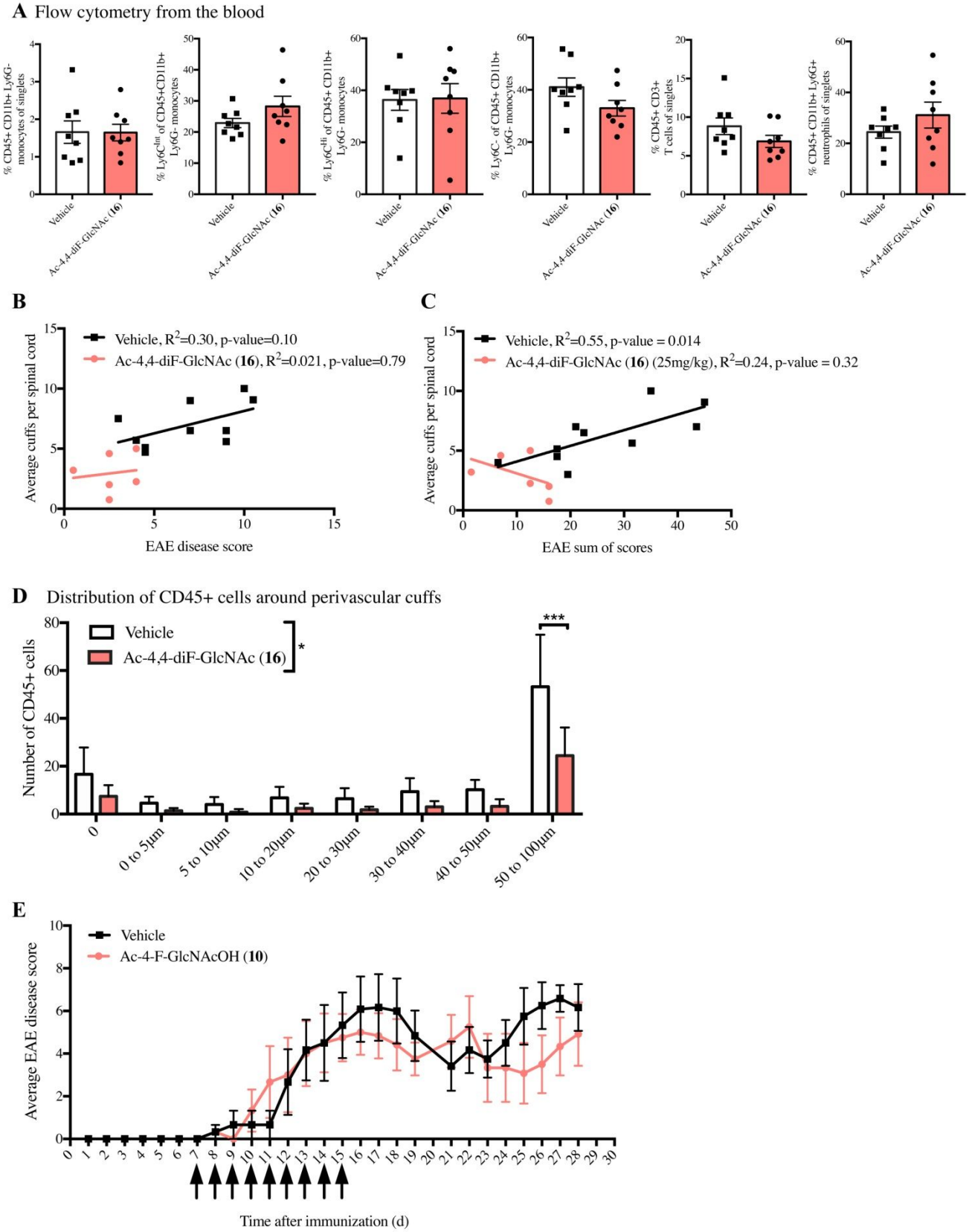


Supplemental Figure 4. Toxicity of compounds. **(A)** Representative images of mouse astrocytes stained for propidium iodide (PI), calcein AM (CAM), and nuclear yellow (NY), following treatment with control (PBS), DMSO and 100 µM of Ac-4,4-diF-GlcNAc **16**. Only the positive

control H_2O_2 caused a significant increase in toxicity, as shown by propdidium iodide (PI)-positive cells (scale bar=50μm). **(B)** Quantified propidium iodide and calcein AM staining of mouse astrocyte cultures showing the percentage of cells (identified by nuclear yellow) that were propidium iodide positive (dead) and calcein AM positive (live). Astrocytes were treated with 100 μM of compounds for 48 hours and only the positive control H_2O_2 caused a significant increase in toxicity. **(C)** ATP assay of neurons treated with 100 μM of select N-acetyl-D-glucosamine and xylose derivatives. **(D)** Structure of the two compounds identified in neuronal cell ATP assay that reduced ATP production by greater than 50% (Ac-4-F-GlcNAcOH **3** and Pr-4-F-GlcNAcOH **11**). *P<0.05, **P<0.01, ****P<0.0001 one-way analysis of variance (ANOVA) with Dunnett's *post hoc test* (**B**,**C**) comparing treatments against control.

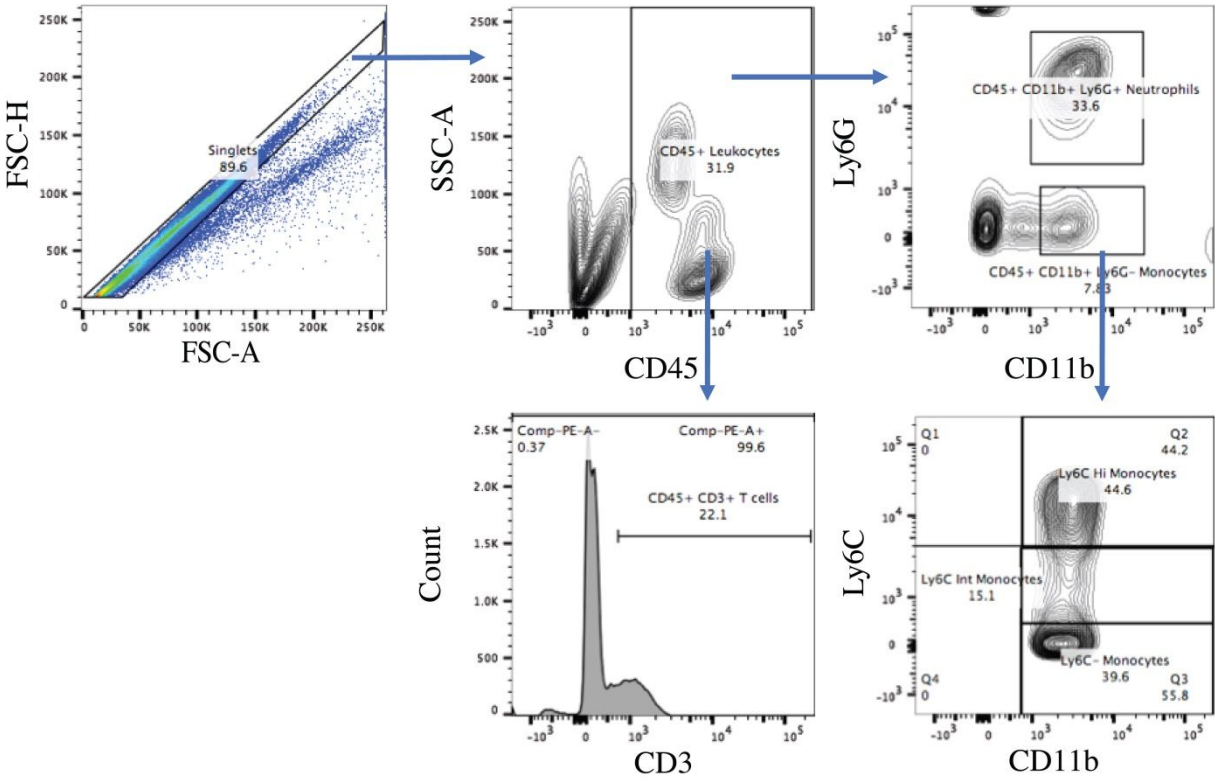


Supplemental Figure 5. TNFα production in bone marrow-derived macrophages (BMDMs) treated with sugar analogs. BMDMs were stimulated with LPS and treated with 50 μM of sugar analogs for 24 hours ***P<0.001, ****P<0.0001 compared with astrocyte ECM control; one-way analysis of variance with Dunnett's *post hoc* test. Error bars are mean±s.d.

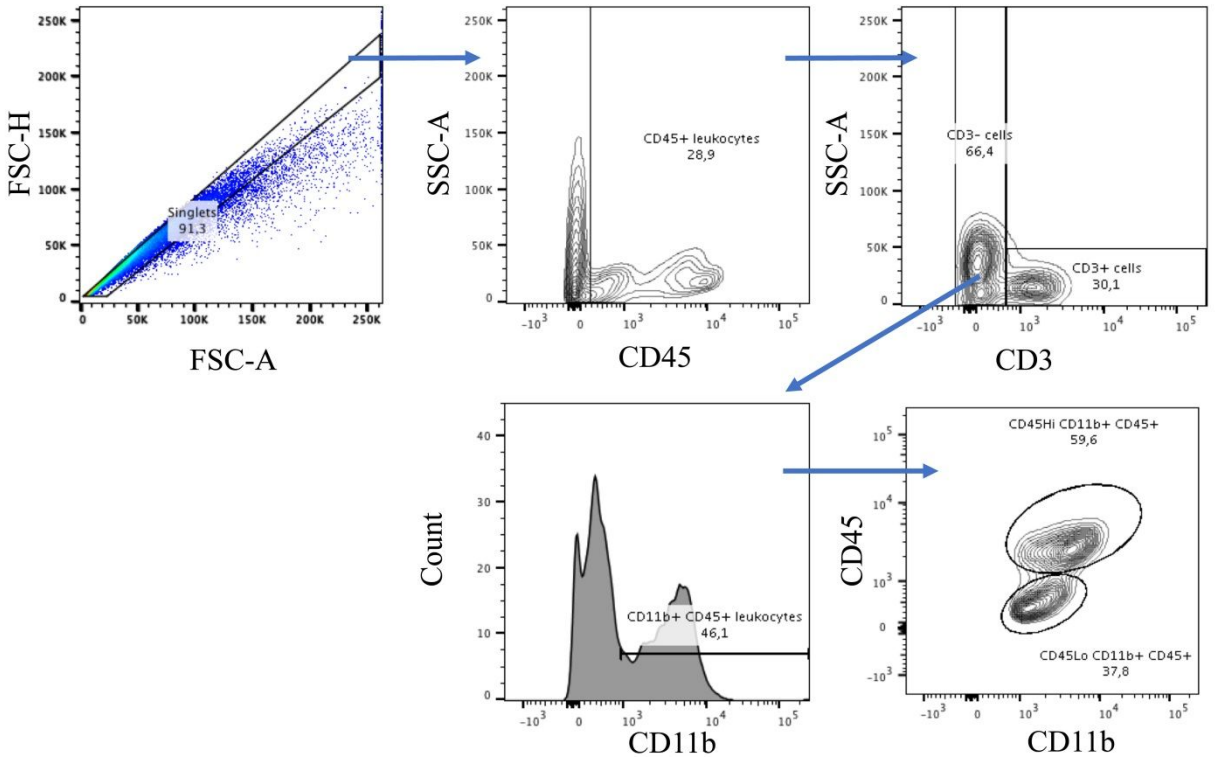

A Flow cytometry from the blood
% CD45+ CD11b+ Ly6G- monocytes of singlets
% Ly6C^int of CD45+CD11b+ Ly6G- monocytes
% Ly6C^hi of CD45+ CD11b+ Ly6G- monocytes
% Ly6C- of CD45+ CD11b+ Ly6G- monocytes
% CD45+ CD3+ T cells of singlets
% CD45+ CD11b+ Ly6G+ neutrophils of singlets
Vehicle
Ac-4,4-diF-GlcNAc (16)
B
Vehicle, R2=0.30, p-value=0.10
Ac-4,4-diF-GlcNAc (16), R2=0.021, p-value=0.79
Average cuffs per spinal cord
EAE disease score
C
Vehicle, R2=0.55, p-value = 0.014
Ac-4,4-diF-GlcNAc (16) (25mg/kg), R2=0.24, p-value = 0.32
Average cuffs per spinal cord
EAE sum of scores
D Distribution of CD45+ cells around perivascular cuffs
Vehicle
Ac-4,4-diF-GlcNAc (16)
*
***
Number of CD45+ cells
0
0 to 5µm
5 to 10µm
10 to 20µm
20 to 30µm
30 to 40µm
40 to 50µm
50 to 100µm
E
Vehicle
Ac-4-F-GlcNAcOH (10)
Average EAE disease score
Time after immunization (d)

Supplemental Figure 6. Further analysis of mice treated daily with 25mg/kg Ac-4,4-diF-GlcNAc **16** or saline vehicle (N=8, day 7 to 14). **(A)** Flow cytometry from blood at peak EAE with no significant difference between Ac-4,4-diF-GlcNAc **16** or vehicle of %Ly6C^{Hi}, Ly6C^{Int}, Ly6C^{Lo}, or total Ly6C+ monocytes, circulating neutrophils (Ly6G+ CD11b+ CD45+) or T cells (CD45+ CD3+). **(B)(C)** Correlation between the average cuffs per spinal cord per mouse and EAE disease score (**B**), and EAE sum of scores (**C**). Linear regression analysis with a non-zero slope for the relationship between average cuffs and EAE disease score and sum of scores for vehicle and Ac-4,4-diF-GlcNAc **16**-treated mice. There was no significant difference between slopes of treated and vehicle-treated mice for either graph. Each point represents the average cuffs per spinal cord per mouse (red circle=Ac-4,4-diF-GlcNAc **16,** black square=vehicle). **(D)** Imaris-quantified CD45+ cells and their distance from perivascular cuffs (0μM, 0-5 μm, 5-10μm, 10-20μm, 20-30μm, 30-40μm, 40-50μm, and 50-100μm). A two-way repeated measures ANOVA with Sidak's multiple comparison test found an overall significant difference between the vehicle and Ac-4,4-diF-GlcNAc **16** distances (*, $p<0.05$), and a significant difference between the number of CD45+ cells vehicle and Ac-4,4-diF-GlcNAc **16** at 50-100 μm (***, $p<0.001$). (E) EAE experiment of Ac-4-F-GlcNAcOH (**10**) (n=6 mice each group) daily 50mg/kg intraperitoneal treatment from day 7 to day 15.

A Gating strategy for blood



B Gating strategy for spinal cord



Supplemental Figure 7. Flow cytometry gating strategy used for the EAE experiment where EAE mice were treated with vehicle or 25mgkg^{-1} Ac-4,4-diF-GlcNAc **16** from day 7 to day 15. **(A)** Murine blood was isolated and stained for CD45, CD11b, CD3, Ly6C, and Ly6G and **(B)** spinal cord was isolated and stained for CD45, CD11b, and CD3. For both methods, the forward scatter height and area was used to isolate singlets, after which CD45+ cells were separated. T cells were identified by CD45+ and CD3+ staining. From the CD45 gate, CD11b+ cells were isolated. For the blood, Ly6G gated neutrophils (CD45+ CD11b+ Ly6G+) were separated from other myeloid cells (CD45+ CD11b+ Ly6G-). Ly6C was used as a marker to differentiate subtypes of proinflammatory monocytes (Ly6C^{Hi}), anti-inflammatory monocytes (Ly6C^{Lo}) and other monocytes (Ly6C^{Int}). For the spinal cord, CD11b+CD45+ cells were further separated into CD45^{Hi} cells (which may represent monocyte-derived macrophages) and CD45^{Lo} cells (which may represent microglia).

Chemical synthesis of compounds

Benzyl 2-acetamido-2,4-dideoxy-4-fluoro-3,6-di-O-methyl-α-D-glucopyranoside (**30**) and *benzyl 2-acetamido-2,4-dideoxy-4-fluoro-3-O-methyl-α-D-glucopyranoside* (**31**). Compound **29** (100 mg, 0.319 mmol) was dissolved in anhydrous DMF (2.0 ml) to 0 °C; sodium hydride (60% in mineral oil, 20.4 mg, 0.51 mmol) was then added followed by methyl iodine (32 µl, 0.51 mmol). After stirring the mixture for 1 h at room temperature, MeOH (100 µl) was added to quench the reaction. The mixture was diluted with EtOAc (~30 ml), and washed with 10% brine (~30 ml) and water (30 ml). The organic solution was dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel using 15% acetone– hexanes as an eluent to afford compound **30** (59.4 mg, 55% yield). Further increasing the polarity of the eluent to 20% acetone – hexanes afforded the compound **31** (28.2 mg, 27% yield). **Data for 30**: R_f =0.40 (MeOH/CH_2Cl_2, 3 : 97). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.46 – 7.29 (m, 5H, Bn), 5.66 (d, J = 9.2 Hz, 1H, NH), 4.92 (dd, J = 3.4, 3.4 Hz, 1H, H-1), 4.74 (d, J = 11.8 Hz, 1H, Bn), 4.52 (ddd, J = 9.9, 8.6 Hz, J_{H-F} = 50.6 Hz, 1H, H-4), 4.48 (d, J = 11.8 Hz, 1H, Bn), 4.21 (m, 1H, H-2), 3.91 (m, 1H, H-5), 3.67 – 3.48 (m, 6H, H-3 + H-6a + H-6b + OMe), 3.43 (s, 3H, OMe), 1.98 (s, 3H, Ac). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 169.82 (CO), 136.88, 128.61, 128.24, 128.16 (Ar), 96.86 (C-1), 89.87 (d, J_{C-F} = 182.8 Hz, C-4), 79.34 (d, J_{C-F} = 17.1 Hz, C-3), 70.62 (C-6), 69.93 (CH$_2$Ph), 69.00 (d, J_{C-F} = 23.8 Hz, C-5), 59.54 (OMe-6), 59.45 (d, J_{C-F} = 2.3 Hz, OMe-3), 51.49 (d, J_{C-F} = 9.3 Hz, C-2), 23.28 (Ac). HRMS (ESI, positive) *m/z* calc'd for $C_{17}H_{25}O_5FN$ $[M+H]^+$: 342.1711; found: 342.1714. **Data for 31**: R_f =0.13 (3% MeOH/CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.44 – 7.30 (m, 5H, Bn), 5.64 (d, J = 8.9 Hz, 1H, NH), 4.94 (dd, J = 3.4, 3.4 Hz, 1H, H-1), 4.71 (d, J = 11.3 Hz, 1H, Bn), 4.56 (ddd, J = 9.0, 9.0 Hz, J_{H-F} = 50.8 Hz, 1H, H-4), 4.49 (d, J = 11.3 Hz, 1H, Bn), 4.18 (m, 1H, H-2), 3.93 – 3.72 (m, 3H, H-6a + H-6b

+ H-5), 3.60 (ddd, J = 8.6, 10.7 Hz, $J_{H\text{-}F}$ = 14.3 Hz, 1H, H-3), 3.52 (d, $J_{H\text{-}F}$ = 1.2 Hz, 3H, OMe), 1.98 (s, 3H, Ac). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 169.98 (CO), 136.76, 128.66, 128.66, 128.34, 128.16 (Ar), 96.83 (C-1), 89.69 (d, $J_{C\text{-}F}$ = 181.5 Hz, C-4), 79.06 (d, $J_{C\text{-}F}$ = 17.6 Hz, C-3), 70.13 (CH$_2$Ph), 69.82 (d, $J_{C\text{-}F}$ = 25.6 Hz, C-5), 61.10 (C-6), 59.54 (d, $J_{C\text{-}F}$ = 2.5 Hz, OMe), 51.64 (d, $J_{C\text{-}F}$ = 9.0 Hz, C-2), 23.29 (Ac). HRMS (ESI, positive) *m/z* calc'd for $C_{16}H_{23}O_5FN$ $[M+H]^+$: 328.1555; found: 328.1556.

2-Acetamido-1-O-acetyl-2,4-dideoxy-4-fluoro-3,6-di-O-methyl-α/β-D-glucopyranose (**5**). Compound **30** (50 mg, 146 μmol) was dissolved in a mixture of MeOH (5.0 ml) and CH_2Cl_2 (2.0 ml). To the solution, was added 20% $Pd(OH)_2$ on charcoal (~30 mg) and AcOH (2 drops), and the mixture was purged with hydrogen gas and stirred under the hydrogen atmosphere for 24 h. The insoluble solid was filtered off with a 0.22 μM membrane syringe filter, and the solution was evaporated under reduced pressure to affor crude compound **32** (35 mg, 95% yield). The residue was dissolved in pyridine (1.0 ml) and Ac_2O (0.5 ml) was added, and the reaction was stirred at room temperature for 2 h. The solution was evaporated to dryness and the residue was purified by column chromatography on silica gel using 4% methanol – CH_2Cl_2 as an eluent to afford compound **5** (α/β: 95.7/4.3) (38 mg, 94% yield). R_f =0.15 (MeOH/CH_2Cl_2, 5 : 95). ^{1}H NMR (400 MHz, $CDCl_3$) for α-anomer: δ_H 6.15 (dd, J = 3.3, 3.3 Hz, 1H, H-1), 5.98 (d, J = 8.3 Hz, 1H, NH), 4.58 (ddd, J = 9.8, 8.7 Hz, $J_{H\text{-}F}$ = 50.4 Hz, 1H, H-4), 4.26 (m, 1H, H-2), 3.88 (m, 1H, H-5), 3.67 – 3.50 (m, 6H, H-3 + H-6a + H-6b + OMe), 3.38 (s, 3H, OMe), 2.14 (s, 3H, Ac), 1.99 (s, 3H, Ac). ^{13}C NMR (101 MHz, $CDCl_3$) for α-anomer: δ_C 170.34 (CO), 168.87 (CO), 90.81 (C-1), 89.47 (d, $J_{C\text{-}F}$ = 182.6 Hz, C-4), 78.40 (d, $J_{C\text{-}F}$ = 17.6 Hz, C-3), 71.03 (d, $J_{C\text{-}F}$ = 24.4 Hz, C-5), 70.22 (C-6), 59.54 (OMe), 59.37 (d, $J_{C\text{-}F}$ = 1.7 Hz, OMe), 50.78 (d, $J_{C\text{-}F}$ = 9.5 Hz, C-2), 23.06

(Ac), 20.86 (Ac). Selected ^{1}H NMR (400 MHz, $CDCl_3$) for β-anomer: δ_H 5.83 (d, J = 8.4 Hz, 1H, H-1), 5.61 (d, J = 10.7 Hz, 1H, NH), 4.50 (ddd, J = 9.7, 8.2 Hz, J_{H-F} = 50.4 Hz, 1H, H-4), 3.38 (s, 3H, OMe), 2.06 (s, 3H, Ac), 1.97 (s, 3H, Ac). Selected ^{13}C NMR (101 MHz, $CDCl_3$) for β-anomer: δ_C 92.08 (C-1), 89.47 (d, J_{C-F} = 182.6 Hz, C-4), 80.40 (d, J_{C-F} = 17.0 Hz, C-3), 73.48 (d, J_{C-F} = 25.6 Hz, C-5), 69.65 (C-6), 54.2 (d, J_{C-F} = 9.4 Hz, C-2). HRMS (ESI, positive) *m/z* calc'd for $C_{12}H_{20}O_6FNNa$ $[M+Na]^+$: 316.1167; found: 316.1159.

2-Acetamido-1,6-di-O-acetyl-2,4-dideoxy-4-fluoro-3-O-methyl-α/β-D-glucopyranose (**6**). Compound **31** (30 mg, 91.6 μmol) was hydrogenated in a mixture of MeOH (8.0 mL), CH_2Cl_2 (2.0 mL) and AcOH (2 drops) in the presence of 20% $Pd(OH)_2$ on charcoal (~20 mg) for 24 h. The reaction mixture was filtered off with a 0.22 μM membrane syringe filter, and the filtrate was evaporated to dryness. The residue was acetylated in a mixture of pyridine (1.0 mL) and Ac_2O (0.5 mL) for 2 h at room temperature. The solution was evaporated to dry mixture and the residue was purified by column chromatography on silica gel using 4% methanol – CH_2Cl_2 as an eluent to afford compound **6** (α/β: 84.7/15.3, 26 mg, 88% yield). R_f =0.18 (MeOH/CH_2Cl_2, 5 : 95). ^{1}H NMR (400 MHz, $CDCl_3$) for α-anomer: δ_H 6.15 (dd, J = 3.3, 3.3 Hz, 1H, H-1), 5.62 (d, J = 8.5 Hz, 1H, NH), 4.56 (ddd, J = 9.8, 8.5 Hz, J_{H-F} = 50.1 Hz, 1H, H-4), 4.38 – 4.21 (m, 3H, H-2 + H-6a + H-6b), 3.99 (m, 1H, H-5), 3.61 (ddd, J = 10.9, 8.5 Hz, J_{H-F} = 13.6 Hz, 1H, H-3), 3.58 (d, J = 1.4 Hz, 3H, OMe), 3.55 (t, J = 2.0 Hz, 1H), 2.18 (s, 3H, Ac), 2.09 (s, 3H, Ac), 2.03 (s, 3H, Ac). ^{13}C NMR (101 MHz, $CDCl_3$) for α-anomer: δ_C 170.62 (CO), 170.19 (CO), 168.63 (CO), 90.70 (d, J_{C-F} = 1.3 Hz, C-1), 89.7 (d, J_{C-F} = 183.5 Hz, C-4), 78.48 (d, J_{C-F} = 17.33 Hz, C-3), 69.25 (d, J_{C-F} = 24.6 Hz, C-5), 61.84 (C-6), 59.76 (d, J_{C-F} = 1.3 Hz, OMe), 50.70 (d, J_{C-F} = 9.0

Hz, C-2), 23.16 (Ac), 20.86 (Ac), 20.68 (Ac). ^{1}H NMR (400 MHz, $CDCl_3$) for β-anomer: δ_H 5.99 (d, J = 7.3 Hz, NH), 5.89 (high order d, J = 8.2 Hz, 1H, H-1), 4.45 (overlapped, 1H, H-4), 3.92 – 3.73 (m, 3H, H-2 + H-3 + H-5), 3.56 (d, J = ~1 Hz, 3H, OMe). Selected ^{13}C NMR (101 MHz, $CDCl_3$) for β-anomer: δ_C 91.86 (C-1), 71.99 (d, $J_{C\text{-}F}$ = 24.6 Hz, C-5), 62.15 (C-6), 53.96 (d, $J_{C\text{-}F}$ = 8.6 Hz, C-2). HRMS (ESI, positive) *m/z* calc'd for $C_{13}H_{21}FNO_7$ $[M+H]^+$: 322.1297; found: 322.1289.

Benzyl 3,6-di-O-acetyl-2,4-dideoxy-4-fluoro-2-trifluroacetamido-α-D-glucopyranoside (**35**). Compound **29** (50 mg, 159.6 μmol) was dissolved in 2N HCl (3.0 ml), and the mixture was refluxed for 4 h, the mixture was evaporated to dryness. The residue was redissolved in MeOH, and neutralized with Amberlite IRA-400 (CO_3^{2-}) resin. After stirring for 1 h, methyl trifluoroacetate (0.5 ml) was added, and reaction was stirred for 30 min. The resin was filtrated off and concentrated under reduced pressure. The residue containing crude **34** was acetylated in a mixture of pyridine (1.5 ml) and Ac_2O (1.0 ml) for 2 h, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using 15% EtOAc –hexanes as the eluent to afford compound **35** (41 mg, 57% yield). R_f =0.55 (EtOAc/hexanes, 30 : 70). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.46 – 7.30 (m, 5H, Bn), 6.62 (d, J = 9.1 Hz, 1H, NH), 5.45 (ddd, J = 10.7, 8.9 Hz, $J_{H\text{-}F}$ = 13.9 Hz, 1H, H-3), 4.96 (dd, J = 3.3, 3.3 Hz, 1H, H-1), 4.77 (d, J = 11.9 Hz, 1H, Bn), 4.58 (d, J = 11.9 Hz, 1H, Bn), 4.57 (ddd, J = 9.5, 9.5 Hz, $J_{H\text{-}F}$ = 50.6 Hz, 1H, H-4), 4.38 (ddd, J = 12.3, 1.9 Hz, $J_{H\text{-}F}$ = 1.9 Hz, 1H, H-6a), 4.33 – 4.20 (m, 2H, H-6b + H-2), 4.08 (m, 1H, H-5), 2.16 (s, 3H, Ac), 2.11 (s, 3H, Ac). ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 171.13 (CO), 170.45 (CO), 128.86, 128.76, 128.38 (Ar), 95.23 (C-1), 86.22 (d, $J_{C\text{-}F}$ = 187.4 Hz, C-4), 70.69 (d, $J_{C\text{-}F}$ = 19.8 Hz, C-3), 70.40 (Ph*C*H_2), 67.63 (d, $J_{C\text{-}F}$ = 23.39 Hz, C-5), 61.72 (C-6), 52.37 (d, $J_{C\text{-}F}$

= 7.2 Hz, C-2), 20.71 (Ac), 20.50 (Ac). HRMS (ESI, positive) *m/z* calc'd for $C_{19}H_{21}O_7F_4NNa$ $[M+Na]^+$: 474.1146; found: 474.1167.

1,3,6-Tri-O-acetyl-2,4-dideoxy-4-fluoro-2-trifluroacetamido-α/β-D-glucopyranose (**9**). Compound **35** (30 mg, 66.5 μmol) was dissolved in a mixture of MeOH (5.0 ml), CH_2Cl_2 (1.0 ml) and AcOH (1 drop), and 20% $Pd(OH)_2$ on charcoal (~30 mg) was added to the solution. The reaction flask was purged with hydrogen gas and stirred under an atmosphere of hydrogen for 24 h. The reaction mixture was filtered off through a 0.22 μM membrane syringe filter, and the solution was concentrated under reduced pressure. The residue containing crude compound **36** was acetylated in a mixture of pyridine (1.5 ml) and Ac_2O (1.0 ml). After stirring at ambient temperature for 2 h, the mixture was concentrated under reduced pressure. The obtained residue was purified by column chromatography on silica gel using 20% EtOAc –hexanes as the eluent to afford **9** (α/β: 78.7/21.3) (24.6 mg, 92% yield). R_f =0.16 (EtOAc/hexanes, 20 : 80). ^{1}H NMR (400 MHz, $CDCl_3$) for α-anomer: δ_H 5.98 (d, J = 9.0 Hz, 1H, NH), 6.23 (dd, J = 3.2, 3.2 Hz, 1H, H-1), 4.48 (ddd, J = 9.0, 11 Hz, J_{H-F} = 13.7 Hz, 1H, H-3), 4.64 (ddd, J = 9.4, 9.9 Hz, J_{H-F} = 50.3 Hz, 1H, H-4), 4.46 – 4.26 (m, 3H, H-2 + H-6a + H-6b), 3.87 (m, 1H, H-5), 2.19 (s, 3H, Ac), 2.16 (s, 3H, Ac), 2.13 (s, 3H, Ac). ^{13}C NMR (101 MHz, $CDCl_3$) for α-anomer: δ_C 171.89 (CO), 170.44 (CO), 168.32 (CO), 89.42 (C-1), 86.78 (d, J_{C-F} = 187.2 Hz, C-4), 70.20 (d, J_{C-F} = 19.4 Hz, C-3), 69.18 (d, J_{C-F} = 23.4 Hz, C-5), 61.46 (C-6), 51.60 (d, J_{C-F} = 7.3 Hz, C-4), 20.63 (Ac), 20.60 (Ac), 20.50 (Ac). Selected ^{1}H NMR (400 MHz, $CDCl_3$) for β-anomer: δ_H 7.20 (d, J = 9.5 Hz, 1H, NH), 5.77 (d, J = 8.7 Hz, 1H, H-1), 5.44 (ddd, J = 9.1, 10.9 Hz, J_{H-F} = 19.9 Hz, 1H, H-3), 4.59 (ddd, J = 9.4, 9.4 Hz, J_{H-F} = 50.3 Hz, 1H, H-4), 4.67 – 4.07 (m, 3H, H-2 + H-6a + H-6b), 3.86 (m, 1H, H-5), 2.14 (s, 3H, Ac), 2.13 (s, 3H, Ac), 2.06 (s, 3H, Ac). Selected ^{13}C NMR (101 MHz,

$CDCl_3$) for β-anomer: δ_C 171.89 (CO), 170.44 (CO), 169.10 (CO), 91.86 (C-1), 86.1 (d, $J_{C\text{-}F}$ = 188.1 Hz, C-4), 72.39 (d, $J_{C\text{-}F}$ = 24.5 Hz, C-5), 71.85 (d, $J_{C\text{-}F}$ = 19.7 Hz, C-3), 61.61 (C-6), 53.07 (d, $J_{C\text{-}F}$ = 7.4 Hz, C-2), 20.66 (Ac), 20.56 (Ac), 20.41 (Ac). HRMS (ESI, positive) *m/z* calc'd for $C_{14}H_{17}O_8F_4NNa$ $[M+Na]^+$: 426.0783; found: 426.0776.

2-Acetamido-3,6-di-O-acetyl-2,4-dideoxy-4-fluoro-1-O-propanoyl-α/β-D-glucopyranoside (**7**). Compound **10** (70 mg, 0.23 mmol) was dissolved in a mixture of anhydrous pyridine (1.0 ml) and CH_2Cl_2 (1.5 ml); propionic anhydride (58 μL, 0.46 mmol) was added followed by a catalytic amount of 4-N,N-dimethylaminopyridine, and the mixture was stirret at room temeprarure overnight. A few drops of MeOH were added to quench the reaction, and the mixture was evaporated under reduce pressure. The residue was purified by column chromatography on silica gel using a mixture of 50% EtOAc – hexanes as the eluent to yield compound **7** (α/β 95.5/4.5, 73 mg, 88% yield). R_f =0.12 (EtOAc/hexanes, 50 : 50). ^{1}H NMR (400 MHz, Acetone-d_6) for α-anomer: δ_H 7.20 (d, J = 9.1 Hz, 1H, NH), 6.10 (dd, J = 3.3, 3.3 Hz, 1H, H-1), 5.38 (ddd, J = 8.9, 11 Hz, $J_{H\text{-}F}$ = 13.8 Hz, 1H, H-3), 4.67 (ddd, J = 9.3, 9.3 Hz, $J_{H\text{-}F}$ = 50.7 Hz, 1H, H-4), 4.39 (dddd, J = 3.7, 9.3, 11.2 Hz, $J_{H\text{-}F}$ = 1.1 Hz, 1H, H-2), 4.34 (ddd, J = 1.7, 11.9, Hz, $J_{H\text{-}F}$ = 1.1 Hz, 1H, H-6a), 4.27 – 4.16 (m, 2H, H-6b + H-5), 2.51 (q, J = 7.5 Hz, 2H, CH_3CH_2CO), 2.05 (s, 6H, 2 × Ac), 1.85 (s, 3H, Ac), 1.12 (t, J = 7.5 Hz, 3H, CH_3CH_2CO). ^{13}C NMR (101 MHz, Acetone-d_6) for α-anomer: δ_C 172.20 (CO), 169.88 (× 2, CO), 169.76 (CO), 89.99 (d, $J_{C\text{-}F}$ = 1.5 Hz, C-1), 87.06 (d, $J_{C\text{-}F}$ = 184.4 Hz, C-4), 70.25 (d, $J_{C\text{-}F}$ = 18.1 Hz, C-3), 69.22 (d, $J_{C\text{-}F}$ = 23.3 Hz, C-5), 61.48 (C-6), 50.36 (d, $J_{C\text{-}F}$ = 7.5 Hz, C-2), 26.77 (CH_3CH_2CO), 21.70 (Ac), 19.78 (Ac), 19.68 (Ac), 8.15 (CH_3CH_2CO). Selected ^{1}H NMR (400 MHz, Acetone-d_6) for β-anomer: δ_H 7.17 (overlapped, 1H, NH), 5.87 (d, J = 8.8 Hz, 1H, H-1), 5.43(ddd, partially overlapped, J = 8.8,

10.7 Hz, $J_{H\text{-}F}$ = ~13 Hz, 1H, H-3), 4.69 (ddd, J = 9.0, 9.7 Hz, $J_{H\text{-}F}$ = 50.6 Hz, 1H, H-4), 4.11 (m, 1H, H-2), 4.02 (m, 1H, H-5), 2.35 (q, J = 7.5 Hz, 2H, CH_3CH_2CO), 2.04 (s, 6H, 2 × Ac), 1.84 (s, 3H, Ac), 1.07 (t, J = 7.5 Hz, 3H, CH_3CH_2CO). HRMS (ESI, positive) *m/z* calc'd for $C_{15}H_{22}O_8F_4NNa$ $[M+Na]^+$: 386.1222; found: 386.1218.

2-Acetamido-3,6-di-O-acetyl-1-O-butanoyl-2,4-dideoxy-4-fluoro-α/β-D-glucopyranoside (**8**). Compound **10** (31 mg, 0.10 mmol) was dissolved in a mixture of anhydrous pyridine (1.0 ml); butyric anhydride (48 μL, 0.30 mmol) was added followed by a catalytic amount of 4-N,N-dimethylaminopyridine, and the mixture was stirret at room temeprarure overnight. A few drops of MeOH were added to quench the reaction, and the mixture was evaporated under reduce pressure. The residue was purified by column chromatography on silica gel using a mixture of 50% EtOAc – hexanes as the eluent to yield compound **8** (α/β 94/6, 30 mg, 78% yield). R_f =0.15 (EtOAc/hexanes, 50 : 50). ^{1}H NMR (400 MHz, Acetone-d_6) for α-anomer: δ_H 7.19 (d, J = 8.9 Hz, 1H, NH), 6.11 (dd, J = 3.3, 3.3 Hz, 1H, H-1), 5.38 (ddd, J = 9.0, 11.1 Hz, $J_{H\text{-}F}$ = 13.8 Hz, 1H, H-3), 4.66 (ddd, J = 9.0, 9.6 Hz, $J_{H\text{-}F}$ = 50.6 Hz, 1H, H-4), 4.39 (dddd, , J = 3.8, 9.1, 11.4 Hz, $J_{H\text{-}F}$ = 1.1 Hz, 1H, H-2), 4.34 (m, 1H, H-6a), 4.26 – 4.15 (m, 2H, H-6b + H-5), 2.54 – 2.39 (m, 2H, $COCH_2CH_2CH_3$), 2.05 (s, 3H, Ac), 2.05 (s, 3H, Ac), 1.85 (s, 3H, Ac), 1.73 – 1.62 (m, 2H, $COCH_2CH_2CH_3$), 0.95 (t, J = 7.4 Hz, 3H, $COCH_2CH_2CH_3$). ^{13}C NMR (101 MHz, Acetone-d_6) for α-anomer: δ_C 171.26 (CO), 169.83 (CO), 169.82 (CO), 169.67 (CO), 89.82 (d, $J_{C\text{-}F}$ = 1.3 Hz, C-1), 87.12 (d, $J_{C\text{-}F}$ = 184.5 Hz, C-4), 70.18 (d, $J_{C\text{-}F}$ = 18.4 Hz, C-3), 69.25 (d, $J_{C\text{-}F}$ = 23.4 Hz, C-5), 61.51 (C-6), 50.31 (d, $J_{C\text{-}F}$ = 7.5 Hz, C-2), 35.28 ($COCH_2CH_2CH_3$), 21.65 (Ac), 19.74 (Ac), 19.64 (Ac), 17.87 ($COCH_2CH_2CH_3$), 12.81 ($COCH_2CH_2CH_3$). Selected ^{1}H NMR (400 MHz, Acetone-d_6) for β-anomer: δ_H 7.19 (overlapped, 1H, NH), 5.87 (d, J = 8.8 Hz, 1H, H-1),

5.43(ddd, partially overlapped, J = 8.9, 10.7 Hz, $J_{H\text{-}F}$ = ~13 Hz, 1H, H-3), 4.58 (ddd, J = 8.9, 9.8 Hz, $J_{H\text{-}F}$ = 50.7 Hz, 1H, H-4), 4.11 (m, 1H, H-2), 4.01 (m, 1H, H-5), 2.32 (t, J = 7.4, 1H, $COCH_aH_bCH_2CH_3$), 2.31 (t, J = 7.4, 1H, $COCH_aH_bCH_2CH_3$), 1.65 – 1.55 (m, 2H, $COCH_aH_bCH_2CH_3$), 0.91 (t, J = 7.4 Hz, 3H, $COCH_2CH_2CH_3$). HRMS (ESI, positive) *m/z* calc'd for $C_{16}H_{24}O_8F_4NNa$ $[M+Na]^+$: 400.1378; found: 400.1370.

Benzyl 2-acetamido-2,4-dideoxy-4-fluoro-3,6-di-O-propanoyl-α-D-glucopyranoside (**38**). Compound **29** (50 mg, 159.58 μmol) was dissolved in a mixture of CH_2Cl_2(1.0 ml) and pyridine (1.0 ml) at 0 °C, and propionic anhydride (122 μL, 957 μmol) was added. The reaction was stirred at room temperature for 2 h. The solution was evaporated to dryness and the residue was purified by column chromatography on silica gel using 20% EtOAc – hexanes as an eluent to afford compound **38** (37.9 mg, 56% yield). R_f =0.13 (EtOAc/Toluene, 30 : 70). $[\alpha]^{25}_D$ +58° (*c* 9.2 mg/ml, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.44 – 7.30 (m, 5H, Bn), 5.75 (d, J = 9.5 Hz, 1H, NH), 5.39 (ddd, J = 10.9, 9.0 Hz, $J_{H\text{-}F}$ = 14.1 Hz, 1H, H-3), 4.90 (dd, J = 3.4, 3.4 Hz, 1H, H-1), 4.74 (d, J = 11.8 Hz, 1H, Bn), 4.60 (ddd, 1H, J = 9.4, 9.4 Hz, $J_{H\text{-}F}$ = 50.9 Hz, 1H, H-4), 4.53 (d, J = 11.8 Hz, 1H, Bn), 4.50 – 4.43 (m, 1H), 4.38 (ddd, J = 12.2, 2.0 Hz, $J_{H\text{-}F}$ = 2.0 Hz, 1H, H-6a), 4.34 – 4.22 (m, 2H, H-6b + H-2), 4.04 (m, 1H, H-5), 2.47 – 2.33 (m, 4H, 2 × CH_3CH_2CO), 1.90 (s, 3H, Ac), 1.18 (t, J = 7.4 Hz, 3H, CH_3CH_2CO), 1.14 (t, J = 7.6 Hz, 3H, CH_3CH_2CO). ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 174.76 (CO), 173.99 (CO), 169.95 (CO), 136.42, 128.71, 128.43, 128.20 (Ar), 96.50 (C-1), 86.71 (d, $J_{C\text{-}F}$ = 186.4 Hz, C-4) 71.02 (d, $J_{C\text{-}F}$ = 18.7 Hz, C-3), 70.21 (CH_2Ph), 67.64 (d, $J_{C\text{-}F}$ = 23.2 Hz, C-5), 61.82 (C-2), 51.67 (d, $J_{C\text{-}F}$ = 7.2 Hz, C-2), 27.54 (CH_3CH_2CO), 27.37 (CH_3CH_2CO), 23.04 (Ac), 9.11 (CH_3CH_2CO), 9.04 (CH_3CH_2CO). HRMS (ESI, positive) *m/z* calc'd for $C_{21}H_{29}O_7FN$ $[M+H]^+$: 426.1923; found: 426.1932.

2-Acetamido-2,4-dideoxy-4-fluoro-3,6-di-O-propanoyl-α/β-D-glucopyranose (**11**). Compound **38** (30 mg, 70.5 μmol) was hydrogenated in a mixture of MeOH (5.0 ml) and CH_2Cl_2 (1.0 ml) in the presence of 20% $Pd(OH)_2$ on charcoal (~30 mg) and AcOH (1 drop) for 24 h. The mixture was filtered off with a 0.22 μM membrane syringe filter, and the solution was evaporated to dryness. The residue was purified by column chromatography on silica gel using 70% EtOAc – hexanes as an eluent to afford compound **11** (α/β: 93.4/6.5) (19.6 mg, 83% yield). R_f =0.22 (EtOAc/hexanes, 80 : 20). ^{1}H NMR (600 MHz, $CDCl_3$) for α-anomer: δ_H 6.11 (d, J = 9.4 Hz, 1H, NH), 5.45 (ddd, J = 10.9, 9.0 Hz, $J_{H\text{-}F}$ = 14.0 Hz 1H, H-3), 5.21 (ddd, J = ~3.6, 3.6, 3.2 Hz, 1H, H-1), 4.52 (ddd, J = 9.5, 9.5 Hz, $J_{H\text{-}F}$ = 51.1 Hz, 1H, H-4), 4.45 (m, 1H, H-6a), 4.29 – 4.19 (m, 3H, H-6b + H-5 +H-2), 2.42 – 2.35 (m, 4H, 2 × CH_3CH_2CO), 1.97 (s, 3H, Ac), 1.90 (br, 1H, OH), 1.16 (t, J = 7.6, 3H, CH_3CH_2CO), 1.14 (t, J = 7.6, 3H, CH_3CH_2CO). ^{13}C NMR (151 MHz, $CDCl_3$): δ_C 174.97 (CO), 174.37 (CO), 170.62 (CO), 91.51 (C-1), 86.72 (d, $J_{C\text{-}F}$ = 186.6 Hz, C-4), 70.64 (d, $J_{C\text{-}F}$ = 187.2 Hz, C-4), 70.64 (d, $J_{C\text{-}F}$ = 18.5 Hz, C-3), 67.13 (d, $J_{C\text{-}F}$ = 23.4 Hz, C-5), 61.88 (C-6), 52.07 (d, $J_{C\text{-}F}$ = 6.7 Hz, C-2), 27.55 (CH_3CH_2CO), 27.34 (CH_3CH_2CO), 23.04 (Ac), 9.10 (CH_3CH_2CO), 9.00 (CH_3CH_2CO). Selected ^{1}H NMR (600 MHz, $CDCl_3$) for β-anomer: δ_H 6.45 (d, J = 7.3 Hz, 1H, NH), 5.26 (m, 1H, H-1), 5.15 5.45 (ddd, J = 10.9, 8.7 Hz, $J_{H\text{-}F}$ = 14.3 Hz 1H, H-3), 3.74 (m, 1H, H-5), 2.01 (s, 3H, Ac). HRMS (ESI, positive) *m/z* calc'd for $C_{14}H_{23}O_7NF$ $[M+H]^+$: 336.1453; found: 336.1450.

Benzyl 2-acetamido-3,6-di-O-butanoyl-2,4-dideoxy-4-fluoro-α-D-glucopyranoside (**39**). To a solution of compound **29** (50 mg, 159.58 μmol) in anhydrous CH_2Cl_2(1.0 ml) and pyridine(1.0 ml) at 0 °C, was added butyric anhydride (157 μL, 957 μmol)dropwise, and the reaction was

stirred at room temperature for 2 h. The mixture was evaporated to dryness under reduced pressure. The obtained residue was purified by column chromatography on silica gel using 25% EtOAc – hexanes as the eluent to afford compound **39** (66.1 mg, 91.3% yield). R_f = 0.25 (EtOAc/Toluene, 30 : 70). $[\alpha]^{25}_D$ +81.7° (*c* 0.92, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.43 – 7.30 (m, 5H, Bn), 5.81 (d, *J* = 9.5 Hz, 1H, NH), 5.39 (ddd, *J* = 10.9, 9.0 Hz, $J_{H\text{-}F}$ = 14.1 Hz, 1H, H-3), 4.90 (dd, *J* = 3.3, 3.3 Hz, 1H, H-1), 4.73 (d, *J* = 11.9 Hz, 1H, Bn), 4.52 (d, *J* = 11.9 Hz, 1H, Bn), 4.51 (ddd, , *J* = 9.4, 9.4 Hz, $J_{H\text{-}F}$ = 51.0 Hz, 1H, H-4), 4.37 (ddd, , *J* = 12.2, 1.9 Hz, $J_{H\text{-}F}$ = 1.9 Hz, 1H, H-6a), 4.34 – 4.21 (m, 2H, H-6b + H-2), 4.04 (m, 1H, H-5), 2.42 – 2.29 (m, 4H, 2 × $CH_3CH_2CH_2CO$), 1.89 (s, 3H, Ac), 1.76 – 1.54 (m, 4H, 2 × $CH_3CH_2CH_2CO$), 0.98 (t, *J* = 7.5 Hz, 3H, $CH_3CH_2CH_2CO$), 0.94 (t, *J* = 7.5 Hz, 3H, $CH_3CH_2CH_2CO$). ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 173.87 (CO), 173.16 (CO), 169.93 (CO), 136.44, 128.68, 128.40, 128.18 (Ar), 96.47 (C-1), 86.76 (d, $J_{C\text{-}F}$ = 186.8 Hz, C-4), 70.81 (d, $J_{C\text{-}F}$ = 18.6 Hz, C-3), 70.16 (CH_2Ph), 67.63 (d, $J_{C\text{-}F}$ = 23.0 Hz, C-5), 61.73 (C-6), 51.64 (d, $J_{C\text{-}F}$ = 6.9 Hz, C-2), 36.04 ($CH_3CH_2CH_2CO$), 35.95 ($CH_3CH_2CH_2CO$), 23.03(Ac), 18.40 ($CH_3CH_2CH_2CO$), 18.37 ($CH_3CH_2CH_2CO$), 13.63($CH_3CH_2CH_2CO$), 13.45 ($CH_3CH_2CH_2CO$). HRMS (ESI, positive) *m/z* calc'd for $C_{23}H_{33}O_7FN$ $[M+H]^+$: 454.2236; found: 454.2245.

2-Acetamido-3,6-di-O-butanoyl-2,4-dideoxy-4-fluoro-α/β-D-glucopyranose (**12**). Compound **39** (50 mg, 110 μmol) was dissolved in a mixture of MeOH (5.0 ml) and CH_2Cl_2 (1.0 ml); to this solution was added 20% $Pd(OH)_2$ on charcoal (~30 mg) and AcOH (1 drop). The reaction flask was purged with hydrogen gas, and the reaction was stirred in the presence of hydrogen atmosphere for 24 h. The mixture was filtered off through a 0.22 μM membrane syringe filter and the solution was evaporated under reduced pressure. The residue was purified by column

chromatography on silica gel using 70% EtOAc – hexanes as the eluent to provide compound **12** (α/β: 96.2/3.8) (35.9 mg, 89.8% yield). R_f =0.31 (EtOAc/hexanes, 80 : 20). ^{1}H NMR (600 MHz, $CDCl_3$) for α-anomer: δ_H 6.23 (d, J = 9.4 Hz, 1H, NH), 5.46 (ddd, J =10.9, 9.0 Hz, $J_{H\text{-}F}$ = 13.9 Hz, 1H, H-3), 5.19 (ddd, J = ~3.4, ~3.4, ~3.4 Hz, 1H, H-1), 4.91 (dd, J = 4.0, 1.1 Hz, 1H, OH), 4.51 (ddd, J = 9.3, 9.3 Hz, $J_{H\text{-}F}$ = 51.0 Hz, 1H, H-4), 4.43 (dd, J =11.9, 1.8 Hz, $J_{H\text{-}F}$ = 1.8 Hz, 1H, H-6a), 4.27 – 4.17 (m, 3H, H-2 + H-5 + H-6b), 2.36 – 2.30 (m, 4H, 2 × $CH_3CH_2CH_2CO$), 1.95 (s, 3H, Ac), 1.70 – 1.59 (m, 4H, 2 × $CH_3CH_2CH_2CO$), 0.95 (t, J = 7.4 Hz, 3H, $CH_3CH_2CH_2CO$), 0.93 (t, J = 7.4 Hz, 3H, $CH_3CH_2CH_2CO$). ^{13}C NMR (151 MHz, $CDCl_3$): δ_C 174.10 (CO), 173.62 (CO), 170.77 (CO), 91.43 (C-1), 86.78 (d, $J_{C\text{-}F}$ = 186.1 Hz, C-4), 70.50 (d, $J_{C\text{-}F}$ = 18.2 Hz, C-3), 67.00 (d, $J_{C\text{-}F}$ = 23.1 Hz, C-5), 61.79 (C-6), 52.10 (d, $J_{C\text{-}F}$ = 7.0 Hz, C-2), 36.04 ($CH_3CH_2CH_2CO$), 35.92 ($CH_3CH_2CH_2CO$), 22.98 (Ac), 18.37 ($CH_3CH_2CH_2CO$), 18.33 ($CH_3CH_2CH_2CO$), 13.57 ($CH_3CH_2CH_2CO$), 13.43 ($CH_3CH_2CH_2CO$). Selected ^{1}H NMR (600 MHz, $CDCl_3$) data for β-anomer: δ_H 6.51 (d, J = 7.3 Hz, 1H, NH), 5.24 (m, 1H, H-1), 3.73 (m, 1H, H-5), 1.99 (s, 3H, Ac). HRMS (ESI, positive) *m/z* calc'd for $C_{16}H_{27}O_7NF$ $[M+H]^+$: 364.1766; found: 364.1751.

Benzyl 2-acetamido-3,6-di-O-acetyl-2,4-dideoxy-4-fluoro-α-D-galactopyranoside (**41**). A solution of compound **40** (600 mg, 1.51 mmol) in a mixture of anhydrous CH_2Cl_2 (2.5 ml) and anhydrous pyridine (2.5 ml) was cooled to -10 °C; Tf_2O (789 μL, 4.53 mmol) was added. After 1 h at -10 °C, MeOH (250 μl) was added to quench the reaction. The mixture was diluted with EtOAc (~30 ml), and the solution was washed with 2N HCl (~30 ml), 10% $NaHCO_3$ (~30 ml) and 10% NaCl (~30 ml), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to afford crude 4-triflate, which was redissolved in a solution of *n*-Bu_4NF in acetone solution

(0.2 g/ml, 5.0 ml). After stirring at room temperature overnight, the mixture was concentrated to dryness. The obtained residue was purified by column chromatography on silica gel using 30% EtOAc –toluene as the eluent to afford compound **41** (320 mg, 53% yield). R_f =0.17 (EtOAc/hexanes, 50 : 50). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.42 – 7.26 (m, 5H, Bn), 5.69 (d, J = 9.6 Hz, 1H, NH), 5.13 (ddd, J = 11.4, 2.3 Hz, $J_{H\text{-}F}$ = 27.6 Hz, 1H, H-3), 4.98 (d, J = 3.5 Hz, 1H, H-1), 4.81 (ddd, J = <1, 2.5 Hz, $J_{H\text{-}F}$ = 50.6 Hz, 1H), 4.71 (d, J = 11.7 Hz, 1H, CH_aH$_b$Ph), 4.63 (ddd, J = 3.7, 9.8 11.4 Hz, 1H, H-2), 4.51 (d, J = 11.7 Hz, 1H, CH$_a$$H_b$Ph), 4.28 (ddd, J = 11.3, 6.8 Hz, $J_{H\text{-}F}$ = 0.7 Hz, 1H, H-6a), 4.22 (dd, J = 11.3, 6.3 Hz, 1H, H-6b), 4.08 (dddd, J = ~6.5, ~6.5, <1 Hz, $J_{H\text{-}F}$ = 28.3 Hz, 1H, H-5), 2.09 (s, 3H, Ac), 2.08 (s, 3H, Ac), 1.90 (s, 3H, Ac). ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 171.12 (CO), 170.40 (CO), 169.89 (CO), 136.53, 128.67, 128.36, 128.21 (Ar), 96.90 (C-1), 86.36 (d, $J_{C\text{-}F}$ = 186.0 Hz, C-4), 70.14 (*C*H$_2$Ph), 68.77 (d, $J_{C\text{-}F}$ = 7.8 Hz, C-3), 67.28 (d, $J_{C\text{-}F}$ = 18.0 Hz, C-5), 61.90 (d, $J_{C\text{-}F}$ = 5.9 Hz, C-6), 47.58 (d, $J_{C\text{-}F}$ = 2.3 Hz, C-2), 23.16 (Ac), 20.79 (Ac), 20.72 (Ac). HRMS (ESI, positive) *m/z* calc'd for $C_{19}H_{25}O_7NF$ $[M+H]^+$: 398.1610; found: 398.1619.

2-Acetamido-1,3,6-tri-O-acetyl-2,4-dideoxy-4-fluoro-α/β-D-galactopyranose (**13**). Compound compound **41** (165 mg, 415 μmol) was dissolved in a mixture of MeOH (10.0 mL), CH_2Cl_2 (3.0 mL) and H_2O (4 drops); 20% $Pd(OH)_2$ on charcoal (50 mg) was added, and the flask was purged with hydrogen gas and the reaction was continued under an atmosphere of hydrogen gas for 24 h. The reaction mixture was filtered off through a 0.22 μm membrane syringe filter, and the solution was concentrated under reduced pressure. The obtained residue containing crude hemiacetal **42** was acetylated in a mixture of pyridine (3.0 mL) and Ac_2O (2.0 mL). After stirring at room temperature for 1 h, the mixture was concentrated. The mixture was purified by column

chromatography on silica gel using 40% EtOAc –toluene as the eluent to afford compound **13** (α/β: 88/12) (135 mg, 93% yield). R_f =0.05 (50% EtOAc/hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) data for α-anomer: δ 6.21 (d, *J* = 3.6 Hz, 1H, H-1), 5.70 (d, *J* = 9.1 Hz, 1H, NH), 5.17 (ddd, *J* = 11.7, 2.3 Hz, J_{H-F} = 26.7 Hz, 1H, H-3), 4.86 (ddd, *J* = <1, 2.3 Hz, J_{H-F} = 50.4 Hz, 1H, H-4), 4.75 (ddd, *J* = 3.7, 9.4, 11.4 Hz, 1H, H-2), 4.27 (ddd, *J* = 6.5, 11.2 Hz, J_{H-F} = 1.1 Hz, 1H, H-6a), 4.20 (dd, *J* = 11.1, 6.4 Hz, 1H, H-6b), 4.11 (ddd, *J* = 6.6, 6.6 Hz, J_{H-F} = 27.8 Hz, 1H, H-5), 2.16 (s, 3H, Ac), 2.13 (s, 3H, Ac), 2.06 (s, 3H, Ac), 1.94 (s, 3H, Ac). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.38 (Ac), 170.42 (Ac), 170.08 (Ac), 168.79 (Ac), 91.13 (C-1), 85.85 (d, J_{C-F} = 187.4 Hz, C-4), 68.85 (d, J_{C-F} = 18.4 Hz, C-3), 68.03 (d, J_{C-F} = 17.9 Hz, C-5), 61.37 (d, J_{C-F} = 6.4 Hz, C-6), 46.84 (d, J_{C-F} = 3.0 Hz, C-2), 23.05 (Ac), 20.89 (Ac), 20.77 (Ac), 20.67 (Ac). Selected ^{1}H NMR (400 MHz, $CDCl_3$) data for β-anomer: δ 5.94 (d, *J* = 9.3 Hz, 1H, NH), 6.78 (dd, *J* = 8.8 Hz, J_{H-F} = 0.8 Hz, 1H, H-1), 3.94 (ddd, *J* = 6.4, 6.4 Hz, J_{H-F} = 26.3 Hz, 1H, H-5), 2.12 (s, 3H, Ac), 2.11 (s, 3H, Ac), 2.07 (s, 3H, Ac), 1.93 (s, 3H, Ac). HRMS (ESI, positive) *m/z* calculated for $C_{14}H_{20}O_8NFNa$ $[M+Na]^+$: 372.1065; found: 372.1071.

2-Acetamido-1,3,6-tri-O-acetyl-2,4-dideoxy-4-chloro-α/β-D-galactopyranoside (**15**). Compound **14** (30 mg, 92.7 μmol) was acetylated in a mixture of pyridine (3.0 ml) and Ac_2O (2.0 ml). After stirring at room temperature for 1 h, the mixture was concentrated. The mixture was purified by column chromatography on silica gel using a 1→5% gradient of methanol – dichloromethane as the eluent to afford the α-anomer of compound **15** (15 mg, 44% yield). $[\alpha]^{25}_D$ +96.4° (*c* 0.12, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 6.20 (d, *J* = 3.6 Hz, 1H, H-1), 5.61 (d, *J* = 9.1 Hz, 1H, NH), 5.27 (dd, *J* = 10.8, 9.5 Hz, 1H, H-3), 4.45 (ddd, *J* = 10.8, 9.2, 3.6 Hz, 1H, H-2), 4.43 – 4.36 (m, 2H, H-6a + H-6b), 4.08 (ddd, *J* = 2.3, 2.3, 10.5 Hz, 1H, H-5), 4.03 (dd, *J* = 9.6, 10.6 Hz, 1H,

H-4), 2.23 (s, 3H, Ac), 2.16 (s, 3H, Ac), 2.13 (s, 3H, Ac), 1.96 (s, 3H, Ac). ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 171.48 (CO), 170.42 (CO), 169.98 (CO), 168.57 (CO), 90.75 (C-1), 72.35 (C-3), 71.94 (C-5), 62.33 (C-6), 54.63 (C-4), 51.79 (C-2), 23.04 (Ac), 20.93 (Ac), 20.69 (Ac), 20.64 (Ac). HRMS (ESI, positive) *m/z* calc'd for $C_{14}H_{20}{}^{35}ClNO_8Na$ $[M+Na]^+$: 388.0770; found: 388.0768; calculated for $C_{14}H_{20}{}^{37}ClNO_8Na$ $[M+Na]^+$: 390.0740; found: 390.0750.

Benzyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranoside (**44**). To a solution of compound **43** (9.0 g, 18.4 mmol) in anhydrous CH_2Cl_2 (100 ml) at 0 °C, Et_3SiH (14.7 ml, 92 mmol) was added, followed by $BF_3.Et_2O$ (3.5 mL, 27.6 mmol), and the mixture was stirred at 0 °C for 2 h. After neutralizing the reaction mixture with NEt_3, the mixture was evaporated under reduced pressure and the crude mixture was purified by column chromatography on silica gel using a mixture of 60% ethyl acetate - hexanes as the eluent to afford the desired compound **44** (6.51 g, 72% yield). R_f =0.44 (AcOEt/hexanes, 60 : 40). $[\alpha]^{25}_D$ +92.8° (*c* 0.80, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H 7.42-7.26 (m, 15H, Ph), 5.53 (d, *J* = 5.2 Hz, 1H, NH), 4.91 (d, *J* = 3.6 Hz, 1H, H-1), 4.83-4.22 (m, 6H, CH_2), 4.29 (td, *J* = 9.6, 3.6 Hz, 1H, H-2), 3.90-3.61 (m, 5H, H-3, H-4, H-5, H-6), 3.13 (d, *J* = 2.8 Hz, 1H, OH), 1.85 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C 169.86 (CO), 138.63 (C), 137.96, 137.24, 128.54, 128.49, 128.42, 128.05, 128.01, 127.97, 127.75, 127.72, 127.64 (Ar), 97.17 (C-1), 79.92 (C-3), 73.80 (CH_2), 73.64 (CH_2), 71.97 (C-5), 70.73 (C-4), 70.15 (C-6), 69.58 (CH_2), 51.95 (C-2), 23.31 (Ac). HRMS (ESI, positive) *m/z* calc'd for for $C_{29}H_{34}NO_6$ $[M + H]^+$: 492.2381, found 492.2386.

Benzyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-α-D-xylo-hexopyranosid-4-ulose (**45**). Acetic anhydrous (25.0 ml) was added to anhydrous DMSO (50.0 ml) at 0 °C, and the mixture was

stirred for 10 minutes. Compound **44** (5.3 g, 10.78 mmol) was then added, and the mixture was stirred at room temperature overnight. The reaction was diluted with EtOAc (~300 ml) and the solution was extracted with 10% brine (2 × 400 ml), and the organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The solid was purified by column chromatography on silica gel using a mixture of 20% EtOAc - toluene as the eluent to afford the desired compound **45** (3.48 g, 66% yield). R_f =0.40 (EtOAc/hexanes, 50 : 50). $[\alpha]^{25}_D$ +154° (*c* 0.76, $CHCl_3$). ^{1}H NMR (CD_3COCD_3, 400 MHz): δ_H 7.42-7.27 (m, 15H, Ph), 5.56-5.46 (m, 1H, NH), 5.15 (d, *J* = 3.6 Hz, 1H, H-1), 4.93 (d, *J* = 12.0 Hz, 1H, CH_2-a), 4.79 (d, *J* = 12.0 Hz, 1H, CH_2-b), 4.70-4.43 (m, 5H, H-2, CH_2), 4.39 (dd, *J* = 6.0, 3.6 Hz, 1H, H-5), 4.14 (t, *J* = 11.2 Hz, 1H, H-3), 3.96 (dd, *J* = 10.8, 3.6 Hz, 1H, H-6a), 3.96 (dd, *J* = 10.8, 6.0 Hz, 1H, H-6b), 1.90 (s, 3H, CH_3). ^{13}C NMR (CD_3COCD_3, 100 MHz): δ_C 201.59 (C-4), 169.57 (CO), 137.98, 137.67, 136.62, 128.74, 128.50, 128.48, 128.44, 128.23, 128.12, 128.06, 127.79, 127.79 (Ar), 96.72 (C-1), 79.54 (C-3), 73.87 (C-5), 73.78 (CH_2), 72.65 (CH_2), 70.44 (CH_2), 67.73 (C-6), 54.25 (C-2), 23.30 (Ac). HRMS (ESI, positive) *m/z* calc'd for for $C_{29}H_{32}NO_6$ $[M + H]^+$: 490.2224, found 490.2236.

Benzyl 2-acetamido-3,6-di-O-benzyl-2,4-dideoxy-4,4-difluoro-α-D-xylo-hexopyranoside (**46**). To a cold solution of compound **45** (2.5 g, 5.1 mmol) in anhydrous dichloromethane (20.0 ml) at 0 °C, was added DAST (2.0 ml, 15.3 mmol), and the mixture was stirred at 0 °C for 10 minute. The reaction was then stirred at ambient temperature overnight. MeOH (1.0 ml) was added to quench the reaction, and the mixture was diluted with EtOAc (~150 ml), washed with 10% brine (~100 ml) and water (~100 ml), and the organic solution was dried over anhydrous Na_2SO_4, and evaporated. The crude mixture was purified by column chromatography on silica gel using 20%

EtOAc – hexanes as the eluent to afford compound **46** (1.38 g, 53% yield). R_f =0.27 (EtOAc/toluene, 30 : 70). $[\alpha]^{25}_D$ +108.6° (*c* 0.7, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ_H 7.48 – 7.25 (m, 15H, Bn), 5.41 (d, *J* = 9.1 Hz, 1H, NH), 4.97 (m, 2H, H-1 + PhC*H*aHb), 4.79 (d, *J* = 11.2 Hz, 1H, PhC*H*aHb), 4.69 (d, *J* = 12.0 Hz, 1H, PhC*H*aHb), 4.65 – 4.56 (m, 2H, 2 x PhCHa*H*b), 4.55 – 4.41 (m, 2H, H-2 + PhCHa*H*b), 4.19 (ddd, *J* = 24.7, 7.4, 2.5 Hz, 1H, H-3), 4.04 – 3.94 (m, 1H, H-6a), 3.90 – 3.71 (m, 2H, H-3 + H-6b), 1.86 (s, 3H, Ac). ^{13}C NMR ($CDCl_3$, 150 MHz): δ_C 169.71 (CO), 137.91, 137.56, 136.57, 128.65, 128.50, 128.46, 128.31, 128.16, 128.09, 127.77, 127.58 (Ar), 118.62 (dd, J_{C-F} = 250.9, 255.2 Hz, C-4), 96.29 (C-1), 75.33 (dd, J_{C-F} = 19.5, 19.5 Hz, C-3), 74.69 (Ph*C*H_2), 73.64 (Ph*C*H_2), 70.05 (dd, J_{C-F} = 22.8, 28.3 Hz, C-5), 69.85 (Ph*C*H_2), 66.49 (d, J_{C-F} = 4.6 Hz, C-6), 51. 07 (J_{C-F} = 7.9 Hz, C-2), 23.21 (Ac). HRMS (ESI, positive) *m/z* calc'd for for $C_{29}H_{32}F_2NO_5$ $[M + H]^+$: 512.2243, found 512.2228.

2-Acetamido-1,3,6-tri-O-acetyl-2,4-dideoxy-4,4-difluoro-α/β-D-xylo-hexopyranose (**16**). To a solution of compound **46** (1.5 g, 2.93 mmol) in a mixture of MeOH (10.0 ml) and CH_2Cl_2 (5.0 ml) was added 20% $Pd(OH)_2$ on charcoal (~150 mg) and AcOH (2 drops), and the mixture was purged with hydrogen gas and stirred under a hydrogen atmosphere for two days. The solution was filtered off with a 0.22 μM membrane syringe filter, and the solution was concentrated under reduced pressure to afford the intermediate **47** (721 mg, 90% yield). R_f =0.19 (10% MeOH/CH_2Cl_2). Compound **47** (900 mg, 3.7 mmol) was dissolved in pyridine (10.0 ml) and acetic anhydride (8.0 ml) was added. After stirring the reaction for 2 hours at room temperature, the solution was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using a mixture of 45% EtOAc - hexanes as the eluent to provide desired compound **16** (α/β, 87 : 13) (1.32 g, 96% yield). R_f: =0.20 (EtOAc/toluene, 60 : 40). ^1H

NMR (400 MHz, $CDCl_3$) for α-anomer: δ_H 6.21 (dd, J = 2.3, 3.3 Hz, 1H, H-1), 5.57 (d, J = 9.1 Hz, 1H, NH), 5.36 (ddd, J = 5.1, 11.5 Hz, $J_{H\text{-}F}$ = 19.3 Hz, 1H, H-3), 4.68 (m, 1H, H-2), 4.47 (dd, J = 3.2, 11.8 Hz, 1H, H-6a), 4.28 (dd, J = 7.2, 12.0 Hz, H-6b), 4.20 (m, 1H, H-5), 2.20 (s, 3H, Ac), 2.19 (s, 3H, Ac), 2.08 (s, 3H, Ac), 1.96 (s, 3H, Ac). ^{13}C NMR ($CDCl_3$, 101 MHz) for α-anomer: δ_C 171.27 (CO), 170.54 (CO), 169.87 (CO), 168.31 (CO), 115.51 (dd, $J_{C\text{-}F}$ = 253.4, 255 Hz, C-4), 90.25 (C-1), 69.63 (dd, $J_{C\text{-}F}$ = 23.2, 27.9 Hz, C-5), 68.21 (dd, $J_{C\text{-}F}$ = 20.0, 20.0 Hz, C-3), 59.80, (d, $J_{C\text{-}F}$ = 5.7 Hz, C-6), 49.94 (d, $J_{C\text{-}F}$ = 6.4 Hz, C-2), 23.00 (Ac), 20.83 (Ac), 20.64 (Ac), 20.54 (Ac). Selected ^{1}H NMR (400 MHz, $CDCl_3$) for β-anomer: δ_H 5.85 (d, J = 8.7 Hz, 1H, H-1), 5.66 (d, J = 9.3 Hz, 1H, NH), 5.36 (overlapped, 1H, H-3), 4.50 (overlapped, 1H, H-6a), 4.36 (m, 1H, H-2), 4.03 (ddd, J = 3.2, 4.5 Hz, $J_{H\text{-}F}$ = 21.9 Hz, C-5), 2.17 (s, 3H, Ac), 2.14 (s, 3H, Ac), 2.09 (s, 3H, Ac), 1.94 (s, 3H, Ac). Selected ^{13}C NMR ($CDCl_3$, 101 MHz) for β-anomer: δ_C 170.45 (CO), 170.10 (CO), 169.22 (CO), 167.03 (CO), 91.98 (C-1), 59.86 (d, $J_{C\text{-}F}$ = 6.4 Hz, C-6), 52.26 (d, $J_{C\text{-}F}$ = 6.9 Hz, C-2), 23.13 (Ac), 20.78 (Ac), 20.66 (Ac), 20.43 (Ac). HRMS (ESI, positive) *m/z* calc'd for $C_{14}H_{20}F_2NO_8$ ($M+H^+$): 368.1151; found: 368.1147.

Benzyl 2-acetamido-3-O-acetyl-6-O-t-butyldimethylsilyl-2,4-dideoxy-4-fluoro-α-D-glucopyranoside (**49**). To a solution of compound **29** (103 mg, 328.7 μmol) in anhydrous pyridine (3.0 mL), was added *t*-butyldimethylsilyl chloride (54.5 mg, 361.6 μmol), and the mixture was stirred at ambient temperature for 3 h. MeOH (100 μl) was added to quench the reaction, and the mixture was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel using 20% acetone –toluene as the eluent to obtain compound **48** (117.3 mg, 83.5% yield). R_f =0.62 (Acetone/toluene: 60 : 40). A portion of compound **48** (111 mg, 259.6 μmol) was acetylated in a mixture of pyridine (2.0 mL) and Ac_2O

(1.0 mL) for 2 h at ambient temperature. The mixture was concentrated under reduced pressure and co-evaporated with toluene (2 × 20 mL) obtain compound **49** (117.3 mg) without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.47 – 7.30 (m, 5H, Ph), 5.74 (d, J = 9.5 Hz, 1H, NH), 5.37 (ddd, J = 10.8, 9.0 Hz, $J_{H\text{-}F}$ = 14.3 Hz, 1H, H-3), 4.91 (dd, J = 3.4, 3.4 Hz, 1H, H-1), 4.74 (d, J = 11.8 Hz, 1H, Bn), 4.56 (high order ddd, J = 9.2, 9.2 Hz, $J_{H\text{-}F}$ = 51 Hz, 1H, H-4), 4.51 (d, J = 11.8 Hz, 2H), 4.26 (m, 1H, H-2), 3.91-3.79 (m, 3H, H-5 + H-6a + H-6b), 2.10 (s, 3H, Ac), 1.91 (s, 3H, Ac), 0.93 (s, 9H, t-butyl), 0.11 (s, 3H, MeSi), 0.10 (s, 3H, MeSi). ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 171.32 (Ac), 169.93 (Ac), 136.69, 128.62, 128.27, 128.18, 96.37 (d, $J_{C\text{-}F}$ = 1.2 Hz, C-1), 86.3 (d, $J_{C\text{-}F}$ = 185.0 Hz, C-4), 71.70 (d, $J_{C\text{-}F}$ = 18.9 Hz, C-3), 70.23 (d, $J_{C\text{-}F}$ = 23.3 Hz, C-5), 69.79 (Ph*C*H_2), 61.40 (C-6), 51.83 (d, $J_{C\text{-}F}$ = 7.1 Hz, C-1), 25.91 (C(*C*H_3)$_3$), 23.11 (Ac), 20.85 (Ac), 18.42 (*C*$(CH_3)_3$), -5.31 (SiMe), -5.41 (SiMe). HRMS (ESI, positive) *m/z* calc'd for $C_{23}H_{37}FNO_6Si$ ($M+H^+$): 470.2369; found: 470.2378.

Benzyl 2-acetamido-3-O-acetyl-2,4-dideoxy-4-fluoro-α-D-glucopyranoside (**50**). Compound **49** (110 mg, 234 μmol) was dissolved in a mixture of CH_2Cl_2 (2.0 mL) and MeOH (1.0 mL); a solution of HCl (1.0 N) was added to adjust pH to 1. After stirring for 2 h, the reaction mixture was evaporated under reduced pressure. The obtained residue was purified by column chromatography on silica gel using 25% acetone –toluene as the eluent to afford the alcohol **50** (70 mg, 84% yield). R_f =0.43 (Acetone/toluene, 30 : 70). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.42 – 7.28 (m, 5H, Ph), 5.83 (d, J = 9.4 Hz, 1H, NH), 5.36 (ddd, J = 10.9, 9.0 Hz, $J_{H\text{-}F}$ = 14.3 Hz, 1H, H-3), 4.90 (dd, J = 3.4, 3.4 Hz, 1H, H-1), 4.73 (d, J = 11.9 Hz, 1H, Bn), 4.59 (ddd, J = 9.4, 9.4 Hz, $J_{H\text{-}F}$ = 50.5 Hz, 1H, H-4), 4.51 (d, J = 11.8 Hz, Bn), 4.25 (m, 1H, H-2), 3.92 – 3.73 (m, H-5 + H-6a + H-6b), 2.35 (dd, J = 7.4, 5.7 Hz, 1H, OH-6), 2.08 (s, 3H, Ac), 1.89 (s, 3H, Ac). ^{13}C NMR

(101 MHz, $CDCl_3$): δ_C 171.36 (Ac), 170.14 (Ac), 136.47, 128.63, 128.34, 128.15, 128.14, 128.13, 128.11, 128.11, 128.10, 96.41 (d, $J_{H\text{-}F}$ = 1.5 Hz, C-1), 86.21 (d, $J_{C\text{-}F}$ = 185.0 Hz, C-1), 71.27 (d, $J_{C\text{-}F}$ = 18.9 Hz, C-3), 70.08 (Ph*C*H_2), 69.78 (d, $J_{C\text{-}F}$ = 24.4 Hz, C-1), 60.74 (C-6), 51.81 (d, $J_{H\text{-}F}$ = 7.2 Hz, C-2), 23.01 (Ac), 20.78 (Ac). HRMS (ESI, positive) *m/z* calc'd for $C_{17}H_{22}FNO_6$ ($M+H^+$): 356.1504; found: 356.1510.

Benzyl 2-acetamido-3-O-acetyl-2,4,6-trideoxy-4,6-difluoro-α-D-glucopyranoside (**51**). To a solution of compound **50** (80 mg, 225 μmol) in CH_2Cl_2 (2.0 mL) at 0 °C, was added DAST (59 μL, 450 μmol), and the reaction was allowed to warm up to ambient temperature and stirred overnight. MeOH (50 μL) was added to quench the reaction. The mixture was evaporated under reduced pressure and the residue was purified by column chromatography on silica gel using 10% acetone –toluene as the eluent to afford **51** (42.8 mg, 53% yield). R_f =0.31 (Acetone/toluene, 20 : 80). $[\alpha]^{25}_D$ +122° (*c* 0.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.46 – 7.31 (m, 5H, Ph), 5.72 (d, *J* = 9.3 Hz, 1H, NH), 5.39 (ddd, *J* = 10.8, 9.1 Hz, $J_{H\text{-}F}$ = 14.3 Hz, 1H, H-3), 4.95 (dd, *J* = 3.3, 3.3 Hz, 1H, H-1), 4.74 (d, *J* = 11.9 Hz, 1H, Bn), 4.72 – 4.47 (m, 4H, H-4 + H-6a + H-6b + Bn), 4.35 – 4.24 (m, 1H, H-2), 3.99 (m, 1H, H-5), 2.11 (s, 3H, Ac), 1.91 (s, 3H, Ac). ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 171.27 (Ac), 169.93 (Ac), 136.42, 128.70, 128.45, 128.26, 96.61 (d, $J_{C\text{-}F}$ = 1.3 Hz, C-1), 85.56 (dd, $J_{C\text{-}F}$ = 186.0, 7.5 Hz, C-4), 80.75 (d, $J_{C\text{-}F}$ = 175.0 Hz, C-6), 71.20 (d, $J_{C\text{-}F}$ = 18.8 Hz, C-3), 70.43 (Ph*C*H_2), 68.65 ((dd, $J_{C\text{-}F}$ = 18.8, 23.4 Hz, C-5), 51.67 (d, $J_{C\text{-}F}$ = 7.1 Hz, C-2), 23.07 (Ac), 20.79 (Ac). HRMS (ESI, positive) *m/z* calc'd for $C_{17}H_{22}F_2NO_5$ ($M+H^+$): 358.1461; found: 358.1456.

2-Acetamido-1,3-di-O-acetyl-2,4,6-trideoxy-4,6-difluoro-α/β-D-glucopyranose (**17**). The 4,6-difluroride **51** (30 mg, 84 μmol) was dissolved in a mixture of MeOH (5.0 mL), CH_2Cl_2 (1.0 mL) and H_2O (2 drops). To this solution, was added 20% $Pd(OH)_2$ on charcoal (30 mg), and the flask was purged with hydrogen gas; the mixture was then stirred under a hydrogen atmosphere for 24 h. The reaction mixture was filtered off through a 0.22 μM membrane syringe filter, and the solution was concentrated under reduced pressure to afford the crude compound **52**, which was acetylated in a mixture of pyridine (1.0 mL) and Ac_2O (0.5 mL) at room temperature for 2 h. The reaction mixture was evaporated to dryness and the residue was purified by column chromatography on silica gel using 20% acetone – toluene as the eluent to afford compound **17** (24 mg, 92% yield). R_f =0.09 (Acetone/toluene, 20 : 80). ^{1}H NMR (400 MHz, $CDCl_3$) for α-anomer: δ_H 6.18 (dd, J = 3.3, 3.3 Hz, 1H, H-1), 5.63 (d, J = 8.9 Hz, 1H, NH), 5.38 (ddd, J = 8.9, 11.2 Hz, $J_{H\text{-}F}$ = 14.1 Hz, 1H, H-3), 4.68 (ddd, J = 9.2, 10.0 Hz, $J_{H\text{-}F}$ = 50.5 Hz, 1H, H-4), 4.71 – 4.57 (m, 2H, H-6a + H-6b), 4.42 (dddd, J = 3.7, 8.9, 11.4 Hz, $J_{H\text{-}F}$ = 1.1 Hz, 1H, H-2), 3.99 (m, 1H, H-5), 2.21 (s, 3H, Ac), 2.15 (s, 3H, Ac), 1.95 (s, 3H, Ac). ^{13}C NMR (101 MHz, $CDCl_3$) for α-anomer: δ_C 171.67 (Ac), 170.10 (Ac), 168.63 (Ac), 90.47 (d, $J_{C\text{-}F}$ = 1.1 Hz, C-1), 85.03 (dd, $J_{F\text{-}C}$ = 7.8, 186.2 Hz, C-4), 80.22 (d, $J_{C\text{-}F}$ = 176.3 Hz, C-6), 70.61 (d, $J_{C\text{-}F}$ = 19.3 Hz, C-3), 70.32 (dd, $J_{F\text{-}C}$ = 18.6, 23.9 Hz, C-5), 50.81 (d, $J_{C\text{-}F}$ = 7.2 Hz, C-2), 22.93 (Ac), 20.82 (Ac), 20.78 (Ac). Selected ^{1}H NMR (400 MHz, $CDCl_3$) for the β-anomer: δ_H 5.71 (d, J = 8.7 Hz, 1H, H-1), 5.59 (d, J = 8.9 Hz, 1H, NH), 5.31 (ddd, J = 9.0, 10.6 Hz, $J_{H\text{-}F}$ = 14.3 Hz, 1H, H-3), 4.73 – 4.53 (m, 3H, H-4 + H-6a + H-6b), 4.27 (m, 1H, H-2), 3.82 (m, 1H, H-5), 2.14 (s, 2H), 2.12 (s, 2H). Selected ^{13}C NMR (101 MHz, $CDCl_3$) for β-anomer: δ_C 170.97 (Ac), 170.24 (Ac), 169.43 (Ac), 92.42 (d, $J_{C\text{-}F}$ = 1.1 Hz, C-1), 85.22 (dd, $J_{C\text{-}F}$ = 186.5, 7.2 Hz, C-4), 73..06 (dd, $J_{C\text{-}F}$ = 19.1, 24.6 Hz, C-5), 72.43 (d, $J_{C\text{-}F}$ = 19.3 Hz, C-3), 52.58 (d, $J_{C\text{-}F}$ = 7.2 Hz, C-2), 23.08 (Ac), 21.41 (Ac),

20.69 (Ac). HRMS (ESI, positive) *m/z* calc'd for $C_{12}H_{17}F_2NO_6Na$ ($M+Na^+$): 332.0916; found: 332.0910.

Benzyl 2-acetamido-3,4-di-O-benzyl-2-deoxy-α-D-gluco-hexodialdo-1,5-pyranoside (**54**). A suspension of pyridinium chlorochromate (65 mg, 300 μmol), sodium acetate (50 mg, 600 μmol) and 4 Å molecular sieves (200 mg) in dichloromethane (20.0 mL) was stirred for 1 h. To this mixture was added drop-wise a solution of compound **53** (49.2 mg, 100 μmol) (*45*) in dry dichloromethane (10.0 mL). The reaction mixture was stirred for 2 h before a 1:1 mixture of hexanes and ether (25 mL) was added. The solution was filtered through a bed of silica and the filtrate was concentrated to give the crude aldehyde. The residue was purified by column chromatography on silica gel using 60% EtOAc – hexanes as eluent to afford the desired aldehyde **54** (15.7 mg, 32%) as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H 9.63 (d, J = 1.0 Hz, 1H, CHO), 7.40-7.26 (m, 15H, Ph), 5.35 (d, J = 9.6 Hz, 1H, NH), 4.99 (d, J = 3.6 Hz, 1H, H-1), 4.86-4.46 (m, 6H, CH_2), 4.26 (ddd, J = 9.7, 9.7, 3.6 Hz, 1H, H-2), 4.19 (dd, J = ~1.0, 9.6 Hz, 1H, H-5), 3.83 (dd, J = 9.6, 8.4 Hz, 1H, H-3), 3.74 (dd, J = 9.6, 8.4 Hz, 1H, H-4), 1.79 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C 197.26 (CHO), 169.85 (CO), 138.06, 137.27, 136.82, 128.73, 128.68, 128.66, 128.43, 128.37, 128.32, 128.25, 128.12, 128.09, 97.19 (C-1), 79.56 (C-3), 78.01 (C-4), 75.52 (C-5), 75.13 (Ph*C*H_2), 75.00 (Ph*C*H_2), 70.46 (Ph*C*H_2), 51.89 (C-2), 23.38 (Ac). HRMS (ESI, positive) *m/z* calc'd for for $C_{29}H_{32}NO_6$ $[M + H]^+$: 490.2224, found 490.2242.

Benzyl 2-acetamido-3,4-di-O-benzyl-2,6-dideoxy-6,6-difluoro-α-D-glucopyranoside (**55**). To a solution of compound **54** (15.7 mg, 32 μmol) in dry dichloromethane (10 mL), cooled to 0° C, was added DAST (42.3 μL, 320 μmol) by small portions, and the mixture was stirred at room temperature overnight. The reaction was then quenched with MeOH (5.0 mL), diluted with

dichloromethane (100 mL), and washed with H_2O (2 × 30 mL). The organic solution was dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by column chromatography on silica gel using 30% EtOAc – hexanes as eluent to afford the 6,6-difluoride **55** (15.9 mg, 95% yield) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H 7.42-7.26 (m, 15H, Ph), 5.92 (td, J = 54.2, 1.1 Hz, 1H, H-6), 5.29 (d, J = 9.6 Hz, 1H, NH), 4.94 (d, J = 4.0 Hz, 1H, H-1), 4.89-4.44 (m, 6H, CH_2), 4.29 (ddd, J = 9.8, 9.8, 3.7 Hz, 1H, H-2), 4.00-3.86 (m, 1H, H-5), 3.82-3.70 (m, 2H, H-3, H-4), 1.80 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C 169.81 (CO), 138.18 (C), 137.47 (C), 136.75 (C), 128.75 (CH), 128.70 (CH), 128.65 (CH), 128.40 (CH), 128.29 (CH), 128.26 (CH), 128.26 (CH), 128.23 (CH), 128.06 (CH), 113.93 (t, $J_{C\text{-}F}$ = 243.6 Hz, C-6), 97.14 (C-1), 80.12 (C-3), 77.66 (C-4), 75.37 (Ph*C*H_2), 75.20 (Ph*C*H_2), 70.07 (Ph*C*H_2), 69.93 (t, J = 20.8 Hz, C-5), 52.29 (C-2), 23.41 (Ac). HRMS (ESI, positive) *m/z* calc'd for for $C_{29}H_{32}F_2NO_5$ $[M + H]^+$: 512.2243, found 512.2239.

2-acetamido-1,3,4-tri-O-acetyl-2,6-dideoxy-6,6-difluoro-α/β-D-glucopyranose (**18**). Compound **55** (15.9 mg, 31.1 μmol) was dissolved in a mixture of methanol : dichloromethane (v/v 9 : 1, 10 mL) and a catalytic amount of $Pd(OH)_2$ (20% on charcoal) was added. The reaction mixture was stirred under hydrogen for 48 h at room temperature. The catalyst was filtered off and the filtrate was concentrated to afford the crude compound **56** (α/β = 8/1) without further purification. ^{1}H NMR (CD_3OD, 400 MHz) for the α-anomer: δ_H 6.04 (dt, J = 1.1 Hz, $J_{H\text{-}F}$ = 54.1 Hz, H-6), 5.14 (d, J = 3.4 Hz, 1H, H-1), 3.98 (m, 1H, H-5), 3.85 (dd, J = 3.5, 10.5 Hz, 1H, H-2), 3.71 (dd, J =7.5, 10.5 Hz, 1H, H-3), 3.46 (m, 1H, H-4), 1.99 (s, 3H, Ac). ^{13}C NMR (CD_3OD, 100 MHz) for the α-anomer: δ_C 115.8 (d, $J_{C\text{-}F}$ = 242.1, C-6), 92.75 (C-1), 72.44 (C-3), 71.67 (dd, $J_{C\text{-}F}$ = 5.4, ~1 Hz, C-4), 70.08 (t, $J_{C\text{-}F}$ = 19.4 Hz, C-5), 55.52 (C-2), 22.59 (Ac). Selected ^{1}H NMR (CD_3OD, 400 MHz) for the β-anomer: δ_H 6.06 (dt, J = 1.1 Hz, $J_{H\text{-}F}$ = 53.9 Hz, H-6), 4.65 (d, J = 8.2 Hz, H-

1). ^{13}C NMR (CD_3OD, 100 MHz) for the β-anomer: δ_C 97.33 (C-1), 58.58 (C-2). To a solution of crude **56** in pyridine (2 mL) was added acetic anhydride (1 mL) and the reaction mixture was stirred at room temperature overnight. The reaction was evaporated and the residue was purified by column chromatography on silica gel using to afford the desired target **18** (6.6 mg, 58% yield in two steps) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H 5.77 (dt, J = 3.6 Hz, $J_{H\text{-}F}$ = 54.5 Hz, H-6), 5.59 (d, J = 9.6 Hz, NH), 5.24 – 5.19 (m, 2H, H-3 + H-4), 4.87(d, J = 3.6 Hz, 1H, H-1), 4.31 (m, 1H, H-2), 3.92 (m, 1H, H-5), 2.04 (s, 3H, Ac), 2.03 (s, 3H, CH_3), 1.95 (s, 3H, CH_3). HRMS (ESI, positive) *m/z* calc'd for $C_{14}H_{20}F_2NO_8$ ($M+H^+$): 368.1151; found: 368.1146.

2-Hydroxyethyl 2,3,4-tri-O-acetyl-β-D-xylopyranoside (**20**). Compound **57** (1.0 g, 3.14 mmol) and ethylene glycol (0.26 ml, 4.71 mmol) were dissolved in anhydrous CH_2Cl_2 (10.0 ml), and $BF_3.Et_2O$ (0.78 ml, 6.28 mmol) was added. After stirring at ambient temperature for 4 h. Et_3N (3.0 ml) was added to quench the reaction. The mixture was evaporated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel using a 40 → 60% gradient of EtOAc – hexanes to afford the desired compound **20** (473 mg, 47%). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 5.18 (dd, J = 8.9, 8.9 Hz, 1H, H-3), 5.00-4.91 (m, 2H, H-2 + H-4), 4.52 (d, J = 7.1 Hz, 1H, H-1), 4.14 (dd, J = 11.8, 5.2 Hz, 1H, H-5a), 3.88 – 3.67 (m, 4H, O$CH_aH_bCH_cH_d$O), 3.36 (td, J = 11.7, 5.7 Hz, 1H, H-5b), 2.31 (t, J = 6.1 Hz, 1H, OH), 2.06 (s, 3H, Ac), 2.04 (s, 3H, Ac), 2.04 (s, 3H, Ac). ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 170.05 (Ac), 169.81 (Ac), 169.57 (Ac), 101.23 (C-1), 71.81 (OCH_aH_b), 71.50 (C-3), 70.99 (C-2), 68.79 (C-4), 62.24 (C-5), 61.72 ($OCH_aH_bCH_cH_dOH$), 20.69 (× 3, 3 × Ac). HRMS (ESI, positive) *m/z* calc'd for $C_{13}H_{24}NO_9$ ($M+NH_4^+$): 338.1446; found: 338.1440.

2-(2-(2-(2-Hydroxyethoxy)ethoxy)ethoxy)ethyl 2,3,4-tri-O-acetyl-β-D-xylopyranoside (**21**). Compound **57** (0.5 g, 1.57 mmol) and tetraethylene glycol (0.41 ml, 2.26 mmol) were dissolved in anhydrous CH_2Cl_2 (5.0 ml), and $BF_3.Et_2O$ (0.38 ml, 3.14 mmol) was added. After stirring at ambient temperature for 4 h. Et_3N (1.0 ml) was added to quench the reaction. The mixture was evaporated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel using a 0 → 5% gradient of MeOH – CH_2Cl_2 as the eluent to afford the desired compound **21** (404 mg, 57%). $[\alpha]^{20}_D$ -36.6° (*c* 0.58, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 5.17 (dd, *J* = 8.5, 8.5 Hz, 1H, H-3), 4.99-4.90 (m, 2H, H-2 + H-4), 4.58 (d, *J* = 6.8 Hz, 1H, H-1), 4.14 (dd, *J* = 11.8, 5.1 Hz, 1H, H-5a), 3.91 (ddd, *J* = 9.1, 5.2, 4.2 Hz, 1H, OC*H*aHb), 3.78 – 3.59 (m, 15H, OCHa*H*bC$H_c$$H_d$(OC$H_2CH_2$)$_3$), 3.38 (dd, *J* = 11.8, 8.8 Hz, 1H, H-5b), 2.57 (s, 1H, OH), 2.07 (s, 2H, Ac), 2.06 (s, 2H, Ac), 2.04 (s, 2H, Ac). ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 170.04 (Ac), 169.81 (Ac), 169.43 (Ac), 100.69 (C-1), 72.49 (OC$H_a$$H_b$), 71.44 (C-3), 70.78 (C-2), 70.65, 70.57, 70.56, 70.32, 70.25, 68.91 (C-4), 68.65, 61.96 (C-5), 61.65 (CH_2OH), 20.68 (Ac), 20.66 (× 2, 2 × Ac). HRMS (ESI, positive) *m/z* calc'd for $C_{19}H_{32}O_{12}Na$ ($M+Na^+$): 475.1786; found: 475.1801.

2-Bromoethyl 2,3,4-tri-O-acetyl-β -D-xylopyranoside (**58**). Compound **57** (1.0 g, 3.14 mmol), 2-bromoethanol (0.45 mL, 6.28 mmol) and $BF_3.Et_2O$ (0.78 mL, 6.28 mmol) were reacted in anhydrous CH_2Cl_2 (10.0 ml) according literature procedure (*46*). The desired compound **58** (634 mg,) was obtained by column chromatography on silica gel using a 20% → 35% gradient of EtOAc – hexanes as an eluent.

2-Sulfoethyl 2,3,4-tri-O-acetyl-β -D-xylopyranoside, sodium salt (**22**). Compound **58** (100 mg, 0.261 mmol) was dissolved in a 1:1 mixture of ethanol – water (5 ml); the solution was then heated to 70 °C, and a solution of Na_2SO_3 (100 mg, 0.794 mmol) in water (0.7 ml) was added dropwise, and the mixture was heated to reflux for 24 h. The mixture was cooled to room temperature and evaporated under reduced pressure. The residue was acetylated using a mixture of 1:1 acetic anhydride – pyridine (2 ml) at 50 °C for 4 h, and the solution was evaporated under reduced pressure, and co-evaporated with toluene several times. The residue was purified by column chromatography on reverse phase C18 silica gel using a 0% → 30% gradient of water - methanol as the eluent to afford the desired compound **22** (81 mg, 76% yield). $[\alpha]^{20}_D$ -44.5° (*c* 0.37, H_2O). ^{1}H NMR (400 MHz, D_2O): δ_H 5.19 (dd, J = 7.9, 7.9 Hz, 1H, H-3), 4.98 (ddd, J = 8.1, 8.1, 4.8 Hz, 1H, H-4), 4.88 (dd, J = 6.2, 8.0 Hz, 1H, H-2), 4.80 (d, J = 6.3 Hz, 1H, H-1), 4.21 – 4.07 (m, 2H, H-5a + OC*H*aHb), 3.94 (ddd, J = 6.4, 6.4, 11.2 Hz, 1H, OCH_aH_b), 3.58 (dd, J = 12.2, 8.3 Hz, 1H, H-5b), 3.17 (t, J = 6.5 Hz, 2H, $CH_2SO_3^-Na^+$), 2.09 (s, 3H, Ac), 2.06 (s, 6H, 2 × Ac). ^{13}C NMR (101 MHz, D_2O): δ_C 173.10 (Ac), 172.94 (Ac), 172.74 (Ac), 99.81 (C-1), 71.18 (C-3), 70.39 (C-2), 68.61 (C-4), 64.81 (OCH_aH_b), 61.09 (C-5), 50.61 ($CH_2SO_3^-Na^+$), 20.21 (× 3, 3 × Ac). HRMS (ESI, negative) *m/z* calc'd for $C_{13}H_{19}NaO_{11}S$ $C_{17}H_{27}O_{11}S$ (M^-): 383.0648; found: 383.0650.

6-Chlorohexyl 2,3,4-tri-O-acetyl-β-D-xylopyranoside (**59**) and *6-chlorohexyl 2,3,4-tri-O-acetyl-α-D-xylopyranoside* (**60**). Compound **57** (1.0 g, 3.14 mmol) and 6-chlorohexanol (0.35 ml, 6.28 mmol) were dissolved in anhydrous CH_2Cl_2 (10.0 ml); the mixture was then cooled to 0 °C, and $BF_3.Et_2O$ (0.78 ml, 6.28 mmol) was added. After stirring at 0 °C for 4 h. Et_3N (3.0 ml) was added to quench the reaction. The mixture was diluted with EtOAc (~50 ml) and the organic

solution was worked up as above. The residue was purified by column chromatography on silica gel using a 5 → 20% gradient of EtOAc – toluene to afford the desired compound **60** (351 mg, 28.3%) and compound **59** (588 mg, 47% yield) in pure forms. **Data for 59**: $[\alpha]^{20}_{D}$ + 8.7° (*c* 0.39, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 5.17 (dd, *J* = 8.6, 8.6 Hz, 1H, H-3), 4.96 (ddd, *J* = 5.1, 8.8, 8.8 Hz, 1H, H-4), 4.92 (dd, *J* = 6.8, 8.8 Hz, 1H, H-2), 4.48 (d, *J* = 6.8 Hz, 1H, H-1), 4.13 (dd, *J* = 11.8, 5.1 Hz, 1H, H-5a), 3.82 (ddd, *J* = 9.6, 6.4, 6.4 Hz, 1H, OC*H*$_a$H$_b$), 3.54 (t, *J* = 6.7 Hz, 2H, CH_2Cl), 3.48 (ddd, *J* = 9.6, 6.4, 6.4 Hz, 1H, OCH$_a$*H*$_b$), 3.37 (dd, *J* = 11.8, 8.9 Hz, 1H, H-5b), 2.07 (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.05 (s, 3H, Ac), 1.82 – 1.74 (m, 2H, OCH$_a$H$_b$C*H*$_2$), 1.64 – 1.56 (m, 2H, C*H*$_2$CH$_2$Cl), 1.50 – 1.33 (m, 4H, CH_2CH_2). ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 170.08 (Ac), 169.82 (Ac), 169.35 (Ac), 100.70 (C-1), 71.54 (C-3), 70.90 (C-2), 69.37 (C-4), 68.95 (OCH$_a$H$_b$), 62.05 (C-5), 44.93 (CH_2Cl), 32.49 (OCH$_a$H$_b$*C*H$_2$), 29.29 (*C*H$_2$CH$_2$Cl), 26.52 (CH_2), 25.21 (CH_2), 20.69 (× 3, 3 × Ac). HRMS (ESI, positive) *m/z* calc'd for $C_{17}H_{27}ClO_8Na$ ($M+Na^+$): 417.1287; found: 485.1280. **Data for 60**: $[\alpha]^{20}_{D}$ + 113.6° (*c* 0.28, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 5.51 – 5.43 (dd, *J* = 9.8, 9.8 Hz, 1H, H-3), 4.98 (d, *J* = 3.5 Hz, 1H, H-1), 4.95 (ddd, *J* = 6.0, 9.5, 10.8 Hz, 1H, H-4), 4.79 (dd, *J* = 3.6, 10.1 Hz, 1H, H-2), 3.77 (dd, *J* = 6.0, 10.8 Hz, 1H, H-5a), 3.69 (ddd, *J* = 9.8, 6.5, 6.5 Hz, 1H, OC*H*$_a$H$_b$), 3.61 (dd, *J* = 10.8, 10.8 Hz, 1H, H-5b), 3.54 (t, *J* = 6.7 Hz, 2H, CH_2Cl), 3.39 (ddd, *J* = 9.8, 6.5, 6.5 Hz, 1H, OCH$_a$*H*$_b$), 2.05 (s, 3H, Ac), 2.02 (s, 6H, 2 × Ac), 1.83 – 1.75 (m, 2H, OCH$_a$H$_b$C*H*$_2$), 1.67 – 1.57 (m, 2H, C*H*$_2$CH$_2$Cl), 1.53 – 1.34 (m, 4H, CH_2CH_2). ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 170.18 (Ac), 170.01 (Ac), 169.92 (Ac), 95.69 (C-1), 71.16 (C-2), 69.68 (C-3), 69.46 (C-4), 68.26 (O*C*HaHb), 58.27 (C-5), 44.91 (CH_2Cl), 32.49 (OCH$_a$H$_b$*C*H$_2$), 29.11 (*C*H$_2$CH$_2$Cl), 26.55 (CH_2), 25.37 (CH_2), 20.74 (Ac), 20.68 (×2, 2 ×Ac).). HRMS (ESI, positive) *m/z* calc'd for $C_{17}H_{27}ClO_8Na$ ($M+Na^+$): 417.1287; found: 417.1283.

6-Sulfohexyl 2,3,4-tri-O-acetyl-β-D-xylopyranoside, sodium salt (**23**). Compound **59** (70 mg, 0.13 mmol) was dissolved in a 1:1 mixture of ethanol – water (10 ml), and tetra-*n*-butylammonium iodide (34 mg, 0.09 mmol) was added. The solution was then heated to 70 °C, and a solution of Na_2SO_3 (46 mg, 0.37 mmol) in water (0.5 ml) was added dropwise. After refluxing for 40 h, the mixture was cooled to room temperature and evaporated under reduced pressure. The residue was acetylated using a mixture of 1:1 acetic anhydride – pyridine (3 ml) at 50 °C for 4 h, and the solution was evaporated under reduced pressure, and co-evaporated with toluene several times. The residue was purified by column chromatography on reverse phase C18 silica gel using a 0 → 30% gradient of water - methanol as the eluent to afford the desired compound **23** (50 mg, 61% yield). $[\alpha]^{20}_D$ -34.5° (*c* 0.22, H_2O). ^{1}H NMR (400 MHz, D_2O): δ_H 5.17 (dd, *J* = 8.4, 8.4 Hz, 1H, H-3), 4.96 (ddd, *J* = 5.2, 8.7, 8.7 Hz, 1H, H-4), 4.81 (dd, *J* = 7.0, 8.4 Hz, 1H, H-2), 4.70 (overlapped, 1H, H-1), 4.07 (dd, *J* = 12.2, 5.0 Hz, 1H, H-5a), 3.79 (ddd, *J* = 6.3, 6.3, 10.0 Hz, 1H, OCH_aH_b), 3.57 (ddd, *J* = 10.2, 6.5, 6.5 Hz, 1H, OCH_aH_b)), 3.51 (dd, 1H, *J* = ~12.1, 8.9, 1H, H-5b), 2.82 (high order t, *J* = 7.7 Hz, 2H, $CH_2SO_3^-Na^+$), 2.05 (s, 3H, Ac), 2.01 (s, 3H, Ac), 2.00 (s, 3H, Ac), 1.65 (m, 2H, $OCH_aH_bCH_2$), 52 (m, 2H, $CH_2CH_2SO_3^-Na^+$), 1.41 – 1.22 (m, 4H, CH_2CH_2). ^{13}C NMR (101 MHz, D_2O): δ_C 173.03 (Ac), 172.85 (Ac), 172.61 (Ac), 99.86 (C-1), 71.44 (C-3), 70.75 (C-2), 70.19 (OCH_aH_b), 68.65 (C-4), 61.16 (C-5), 50.90 ($CH_2SO_3^-Na^+$), 28.24 ($OCH_aH_bCH_2$), 27.23 ($CH_2CH_2SO_3^-Na^+$), 24.56 (CH_2), 23.88 (CH_2), 20.12 (× 3, 3 × Ac). HRMS (ESI, positive) *m/z* calc'd for $C_{17}H_{27}Na_2O_{11}S$ ($M+Na^+$): 485.1064; found: 485.1077.

6-Sulfohexyl 2,3,4-tri-O-acetyl-α-D-xylopyranoside, sodium salt (**24**). Compound **60** (70 mg, 0.18 mmol) was dissolved in a 1:1 mixture of ethanol – water (10 ml), and tetra-n-butylammonium iodide (33 mg, 0.09 mmol) was added. The solution was then heated to 70 °C, and a solution of Na_2SO_3 (89 mg, 0.71 mmol) in water (0.5 ml) was added dropwise. After refluxing for 40 h, the mixture was cooled to room temperature and evaporated under reduced pressure. The residue was acetylated using a mixture of 1:1 acetic anhydride – pyridine (3 ml) at 50 °C for 4 h, and the solution was evaporated under reduced pressure, and co-evaporated with toluene several times. The residue was purified by column chromatography on reverse phase C18 silica gel using a 0% → 30% gradient of water - methanol as the eluent to afford the desired compound **24** (30 mg, 34% yield). $[\alpha]^{20}_D$: + 80.9° (*c* 43, H_2O). ^{1}H NMR (400 MHz, D_2O): δ_H 5.37 (dd, *J* = 9.3, 9.3 Hz, 1H, H-3), 5.11 (d, *J* = 3.6 Hz, 1H, H-1), 5.04 (ddd, *J* = 5.6, 9.2, 10.2 Hz, 1H, H-4), 4.99 (dd, *J* = 3.7, 9.8 Hz, 1H, H-2), 3.88 (dd, *J* = 11.4, 5.7 Hz, 1H, H-5a), 3.75 (ddd, *J* = 6.7, 6.7, 10.2 Hz, 1H, OC*H*$_a$H$_b$), 3.72 (dd, 1H, *J* = ~11.2, 11.2 Hz, 1H, H-5b), 3.56 (ddd, *J* = 10.2, 6.3, 6.3 Hz, 1H, OCH$_a$*H*$_b$)), 2.92 – 2.86 (high order t, *J* = 7.9 Hz, 2H, $CH_2SO_3^-Na^+$), 2.10 (s, 3H, Ac), 2.07 (s, 3H, Ac), 2.06 (s, 3H, Ac), 1.73 (m, 2H, OCH$_a$H$_b$C*H*$_2$), 1.69 – 1.58 (m, 2H, C*H*$_2$CH$_2$SO$_3^-$Na$^+$), 1.50 – 1.34 (m, 4H, CH_2CH_2). ^{13}C NMR (101 MHz, D_2O): δ_C 173.24 (Ac), 172.98 (Ac), 172.80 (Ac), 95.30 (C-1), 70.53 (C-3), 70.34 (C-2), 69.06 (C-4), 68.49 (OCH$_a$H$_b$), 58.23 (C-5), 51.04 ($CH_2SO_3^-Na^+$), 28.11 (OCH$_a$H$_b$*C*H$_2$), 27.48 (*C*H$_2$CH$_2$SO$_3^-$Na$^+$), 24.92 (CH_2), 24.02 (CH_2), 20.21 (× 2, 2 × Ac), 20.13 (Ac). HRMS (ESI, positive) *m/z* calc'd for $C_{17}H_{27}Na_2O_{11}S$ (M+Na$^+$): 485.1064; found: 485.1071.

2-N,N-Dimethylaminoethyl 2,3,4-tri-O-acetyl-β -D-xylopyranoside (**25**). Compound **58** (100 mg, 0.261 mmol) was dissolved in methanol (5 ml); a solution of dimethylamine in methanol (2.0 M,

1.0 mL) was added, and the solution was stirred at room temperature for 48 h. The mixture was concentrated under reduced pressure. The residue was acetylated using a mixture of 1:1 acetic anhydride – pyridine (2 ml) at 50 °C for 4 h, and the solution was evaporated under reduced pressure, and co-evaporated with toluene several times. The residue was dissolved in AcOEt (~20 mL), the organic solution was washed with 10% $NaHCO_3$ (20 mL), 10% brine, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography on reverse phase C18 silica gel using a 0 → 30% gradient of water - methanol as the eluent to afford the desired compound **25** (74 mg, 82% yield). $[\alpha]^{20}_D$ -40° (*c* 0.29, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 5.18 (dd, *J* = 9.1, 9.1 Hz, 1H, H-3), 4.99 – 4.87 (m, 2H, H-2 + H-4), 4.52 (d, *J* = 7.3 Hz, 1H, H-1), 4.25 (ddd, *J* = 3.5, 5.2, 12.0 Hz, 1H, OC*H*aHb), 4.17 (ddd, *J* = 3.7, 6.1, 12.0 Hz, 1H, OCHa*H*b), 4.11 (dd, *J* = 11.8, 5.4 Hz, 1H, H-5a), 3.36 (dd, *J* = 11.8, 9.7 Hz, 1H, H-5b), 3.33 – 3.27 (m, 2H, Me_2NC*H*c*H*d), 2.83 (s, 6H, Me_2N), 2.06 (s, 3H, Ac), 2.04 (s, 3H, Ac), 2.02 (s, 3H, Ac). ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 169.86 (x 2, Ac), 169.58 (Ac), 100.65 (C-1), 71.47 (C-3), 70.85 (C-2), 68.72 (C-4), 63.85 (OCHaHb), 62.53 (C-5), 57.06 (Me_2N*C*HcHd), 43.77 (Me_2N), 20.78 (Ac), 20.67 (Ac), 20.63 (Ac). HRMS (ESI, positive) *m/z* calc'd for $C_{15}H_{25}NO_8$ ($M+H^+$): 348.1653; found: 348.1667.

4-Deoxy-4-fluoro-D-xylopyranose (**26**). To a solution of 1,2,3-tri-*O*-benzoyl-4-deoxy-4-fluoro-α-D-xylopyranose **61** (227 mg, 0.49 mmol) (*47*) in dry methanol (10 mL) was added 2 drops of a freshly prepared 0.1 M sodium methoxide solution. The mixture was stirred and monitored by TLC at room temperature for 0.5 h. The reaction was quenched by adding Amberlite IR120 (H^+) resin. Once the pH reached 6-7, the mixture was filtered and the filtrate was concentrated to dryness. The crude product was purified by HPLC chromatography (C18) using a gradient of MeOH - water (5 : 95 → 10 : 90) to afford compound **26** (34 mg, 48 %). R_f =

0.57 (CH_2Cl_2 / MeOH, 8 : 2). ^{1}H NMR (D_2O, 400 MHz) for α-anomer: δ_H 5.23 (dd, J = 3.6 Hz, $J_{H\text{-}F}$ = 3.6 Hz, 1H, H-1), 4.66-4.43 (dm, $J_{H\text{-}F}$ = 50.3 Hz, 1H, H-4), 3.99 (ddd, J = 9.2, 7.4 Hz, $J_{H\text{-}F}$ = 15.1 Hz, 1H, H-3), 3.93 (m, 2H, H-5), 3.61 (ddd, J = 3.6, 9.2 Hz, $J_{H\text{-}F}$ = 1 Hz, 1H, H-2). ^{13}C NMR (D_2O, 100 MHz) for α-anomer: δ_C 91.9 (d, $J_{C\text{-}F}$ = 1.4 Hz, C-1), 89.1 (d, $J_{C\text{-}F}$ = 177.9 Hz, C-4), 71.1 (d, $J_{C\text{-}F}$ = 18.2 Hz, C-3), 70.8 (d, $J_{2\text{-}F}$ = 7.7 Hz, C-2), 58.6 (d, $J_{5\text{-}F}$ = 27.8 Hz, C-5). ^{1}H NMR (D_2O, 400 MHz) for β-anomer: δ_H 4.67 (d, J = 7.8 Hz, 1H, H-1), 4.66-4.43 (dm, $J_{H\text{-}F}$ = 50.3 Hz, 1H, H-4), 4.16 (ddd, J = 5.6, 11.6 Hz, $J_{H\text{-}F}$ = 1.3 Hz, 1H, H-5a), 3.79 (ddd, J = 9.3, 9.3 Hz, $J_{H\text{-}F}$ = 15.7 Hz, 1H, H-3), 3.56 (ddd, J = 3.9, 11.6 Hz, $J_{H\text{-}F}$ = 10.2 Hz, 1H, H-5b), 3.33 (ddd, J = 7.8, 9.3 Hz, $J_{H\text{-}F}$ = 1 Hz, 1H, H-2). ^{13}C NMR (D_2O, 100 MHz) for β-anomer: δ_C 96.5 (d, $J_{C\text{-}F}$ = 1.2 Hz, C-1), 89.2 (d, $J_{C\text{-}F}$ = 177.9 Hz, C-4), 74.0 (d, $J_{C\text{-}F}$ = 18 Hz, C-3), 73.3 (d, $J_{C\text{-}F}$ = 9.1 Hz, C-2), 62.3 (d, $J_{C\text{-}F}$ = 28.7 Hz, C-5). HRMS (ESI, positive) *m/z* calc'd for for $C_5H_9O_4FNa$ $[M + Na]^+$: 175.0377, found 175.0370.

1,2,3-Tri-O-acetyl-4-deoxy-4-fluoro-α-D-xylopyranose (**27**) and *1,2,3-tri-O-acetyl-4-deoxy-4-fluoro-β-D-xylopyranose* (**28**) To a solution of **26** (21 mg, 0.14 mmol) in dry pyridine (5 mL) was added acetic anhydride (0.18 mL, 1.38 mmol, 10 eq) at 0 °C under inert atmosphere. The mixture was stirred for 6 h at room temperature, cooled to 0 °C and quenched with methanol. The mixture was concentrated to dryness and the residue was dissolved in EtOAc. The organic layer was successfully washed with an aqueous solution of 1 M HCl, saturated aqueous $NaHCO_3$ and brine before been dried over Na_2SO_4, filtered, and concentrated to dryness. The crude product was purified by chromatography (hexane / EtOAc, 9 : 1) to afford compound **27** (18.5 mg, 49 %) and **28** (18.7 mg, 49 %). **Data for 27**: R_f = 0.41 (EtOAc/hexanes, 3 : 7). $[\alpha]^{20}_D$ + 46° (*c* 0.5, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H 6.22 (dd, J = 3.6 Hz, $J_{H\text{-}F}$ =

3.5 Hz, 1H, H-1), 5.52 (ddd, J = 10.1, 8.9 Hz, $J_{H\text{-}F}$ = 13.3 Hz, 1H, H-3), 4.97 (ddd, J = 3.6, 10.1 Hz, $J_{H\text{-}F}$ = 0.9 Hz, 1H, H-2), 4.61 (dddd, J = 8.9, 6, 10.9 Hz, $J_{H\text{-}F}$ = 49.9 Hz, 1H, H-4), 4.01 (dd, J = 6.0, 11.3 Hz, 1H, H-5a), 3.85 (ddd, J = 10.9, 11.3 Hz, $J_{H\text{-}F}$ = 4.8 Hz, 1H, H-5b), 2.18 (s, 3H, Ac), 2.10 (s, 3H, Ac), 2.02 (s, 3H, Ac). ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C 170.0 (Ac), 169.9 (Ac), 169.0 (Ac), 89.1 (d, $J_{C\text{-}F}$ = 1 Hz, C-1), 86.5 (d, $J_{C\text{-}F}$ = 185.8 Hz, C-4), 70.2 (d, $J_{C\text{-}F}$ = 20 Hz, C-3), 69.1 (d, $J_{C\text{-}F}$ = 8.1 Hz, C-2), 61.0 (d, $J_{C\text{-}F}$ = 27.7 Hz, C-5), 21.0 (Ac), 20.9 (Ac), 20.6 (Ac). HRMS (ESI, positive) *m/z* calc'd for $C_{11}H_{15}O_7FNa$ $[M + Na]^+$: 301.0694, found, 301.0698. **Data for 28:** R_f = 0.38 (EtOAc/hexanes, 3 : 7). $[\alpha]^{20}_D$ -56° (*c* 0.86, $CHCl_3$)**.** ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H 5.76 (d, J = 6.3 Hz, 1H, H-1), 5.25 (ddd, J = 7.8, 7.4 Hz, $J_{H\text{-}F}$ = 13.4 Hz, 1H, H-3), 4.97 (ddd, J = 6.3, 7.8 Hz, $J_{H\text{-}F}$ = 0.5 Hz, 1H, H-2), 4.6 (dddd, J = 7.4, 4.6, 7.8 Hz, $J_{H\text{-}F}$ = 48.5 Hz, H-4), 4.18 (ddd, J = 4.6, 12.4 Hz, $J_{H\text{-}F}$ = 12.9 Hz, 1H, H-5a), 3.73 (ddd, J = 7.8, 12.4 Hz, $J_{H\text{-}F}$ = 7.8 Hz, 1H, H-5b), 2.1 (s, 3H, Ac), 2.09 (s, 3H, Ac), 2.06 (s, 3H, Ac). ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C 169.5 (Ac), 169.4 (Ac), 168.9 (Ac), 91.8 (C-1), 85.7 (d, $J_{C\text{-}F}$ = 185 Hz, C-4), 70.8 (d, $J_{C\text{-}F}$ = 23.3 Hz, C-3), 68.9 (d, $J_{C\text{-}F}$ = 6.1 Hz, C-2), 62.6 (d, $J_{C\text{-}F}$ = 26.2 Hz, C-5), 20.7 (Ac), 20.6 (Ac), 20.5 (Ac). HRMS (ESI, positive) *m/z* calc'd for $C_{11}H_{15}O_7FNa$ $[M + Na]^+$: 301.0694, found, 301.0698.

Supplemental References

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