Synthesis of UDP-4-deoxy-4-fluoroglucose and UDP-4-deoxy-4-fluorogalactose and Their Interactions with Enzymes of Nucleotide Sugar Metabolism

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Fluorinated carbohydrates can be used as probes of enzymatic active sites. We report the synthesis of 4-deoxy-4-fluoro-α-D-galactose-1-phosphate and the substrate analogues of UDP-galactose, UDP-4-deoxy-4-fluoro-α-D-galactose (UDP-FGal), and of UDP-glucose, UDP-4-deoxy-4-fluoro-α-D-glucose (UDP-FGlc), which may be useful in analyzing the binding properties of enzymes that utilize nucleotide sugars as substrates. As a first step in this study, we determine the kinetic and inhibition parameters for UDP-FGal and UDP-FGlc interacting with UDP-glucose dehydrogenase and UDP-galactose 4-epimerase. UDP-FGlc is a substrate for bovine liver UDP-glucose dehydrogenase: $K_m = 30.2 \pm 4.5$ μM slightly higher than the value 9.6 ± 0.7 μM for UDP-glucose, and $V_{mUDP\text{-}FGlc} = 0.46V_{mUDP\text{-}Glc}$. UDP-FGal is not a substrate for UDP-glucose dehydrogenase but is a competitive inhibitor with respect to UDP-glucose ($K_i = 19.9 \pm 6.6$ μM). These analogs also bind to UDP-galactose 4-epimerase from *E. coli* with dissociation constants K_d of 1.4 and 1.1 mM for UDP-FGlc and UDP-FGal, respectively.

Considerable efforts have been expended in the synthesis of deoxyfluorosugars,[1] primarily for use as analogues in probing enzymatic active sites.[2] Fluorinated analogues are thought to provide insight into the interactions between enzymatic binding sites and hydroxyl groups replaced by fluorine. This stems from similarities between the F-atom and the OH group, with particular reference to polarity and hydrogen bonding. Studies with fluorinated analogues can provide useful information about the specificity of carbohydrate binding to a variety of proteins, including enzymes, transport proteins, and antibody–antigen complexes; they are also potentially useful in studies of metabolism.[3] In this paper, we report the chemical synthesis of the new products UDP-4-deoxy-4-fluoro-α-D-galactose (UDP-FGal) and UDP-4-deoxy-4-fluoro-α-D-glucose (UDP-FGlc) as well as of 4-deoxy-4-fluoro-α-D-galactose 1-phosphate. We also describe preliminary studies of the interactions of these nucleotide fluorosugars at the active sites of UDP-glucose dehydrogenase and UDP-galactose 4-epimerase.

The UDP-4-deoxy-4-fluorosugars should not be substrates for UDP-galactose 4-epimerase because of the substitution of F for OH on carbon-4, which undergoes transient oxidation in the epimerization mechanism.[4,5] However, these molecules should bind to the active site in a manner similar to the binding of UDP-galactose (UDP-Gal) and UDP-glucose (UDP-Glc). We are interested in UDP-FGal and UDP-FGlc as probes for the active sites of enzymes such as UDP-galactose 4-epimerase and UDP-glucose dehydrogenase that utilize uridine nucleotide sugars as substrates.

Results and Discussion

The synthetic steps to UDP-FGal and UDP-FGlc, **1a** and **1b**, are shown in Figure 1. The syntheses of peracetylated fluorogalactose, 1,2,3,6-tetra-*O*-acetyl-4-deoxy-4-fluoro-β-D-galactopyranoside (**10a**), and 4-deoxy-4-fluoro-α-D-glucopyranose 1-phosphate [bis(cyclohexylammonium) salt] (**11b**) are described in the literature. We briefly survey the overall synthesis of compounds **1a** and **1b** and describe in detail the steps to compounds **11a**, **1a**, and **1b** that have not been reported in the literature.

4-Deoxy-4-fluoro-α-D-galactopyranose 1-Phosphate (11a, Figure 1). Standard protection of methyl α-D-glucopyranoside (**2**) with trityl chloride[6,7] afforded methyl 6-*O*-trityl-α-D-glucopyranoside (**3**) (68%), which was regiospecifically fluorinated by the DAST reagent to give methyl 4-deoxy-4-fluoro-6-*O*-trityl-α-D-galactopyranoside (**4**) in 30% yield.[8,9] Detritylation[8] of **4** by aqueous acetic acid (methyl 4-deoxy-4-fluoro-α-D-galactopyranoside (**8a**); 78%), followed by deprotection of the anomeric center by use of Dowex 50W-X8 (H^+) ion exchange resin,[10] afforded free 4-deoxy-4-fluoro-D-galactopyranoside (**9a**, quant). With acetic anhydride–sodium acetate,[11] the fluoro sugar **9a** gave 1,2,3,6-tetra-*O*-acetyl-4-deoxy-4-fluoro-β-D-galactopyranoside (**10a**, 44.3%). Phosphorylation, by the

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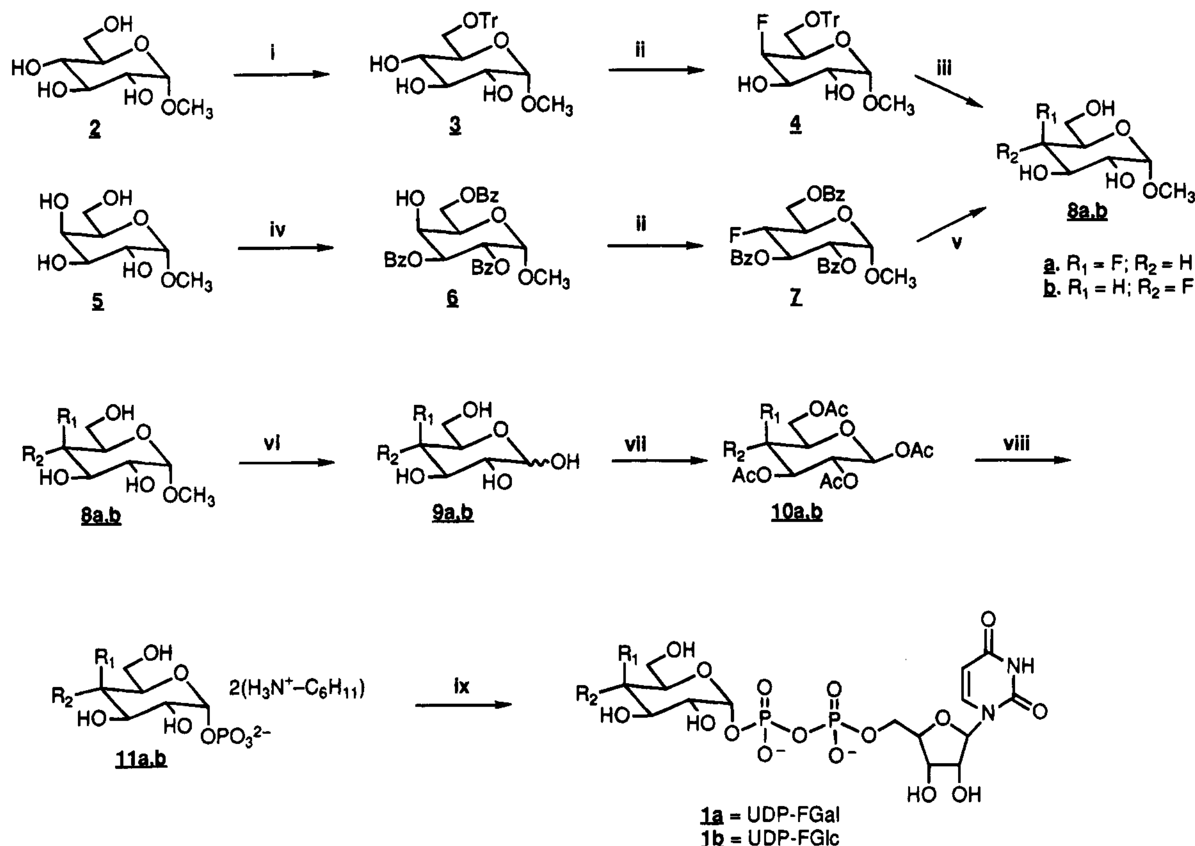

Figure 1. Synthesis of UDP-4-deoxy-4-fluoro-α-D-glycosides. Key: (i) TrCl, DMAP, DMF, TEA; (ii) DAST, CH_2Cl_2; (iii) AcOH, H_2O, Δ; (iv) BzCl, Pyr; (v) NaOMe, MeOH; (vi) AG50W-X8 resin, Δ or HCl, H_2O, Δ; (vii) NaOAc, Ac_2O, Δ or Ac_2O/Pyr, HBr-AcOH/Ac_2O, AcOH/HgOAc; (viii) H_3PO_4, LiOH, cyclohexylamine; (ix) UMP-morpholidate, n-octylamine, Pyr.

MacDonald procedure,[12] was carried out by fusion of the β-peracetate **10a** with anhydrous phosphoric acid affording **11a**, which was crystallized as its bis (cyclohexylammonium) salt (74%).

UDP-4-deoxy-4-fluoro-α-D-galactose (1a, Figure 1). Condensation[13] of **11a** with the 4-morpholine *N,N'*-dicyclohexylcarboxamidine salt of uridine 5'-monophosphomorpholidate followed by ion-exchange chromatography with gradient elution by 0.01−0.1 N lithium chloride (elution of the desired product at 0.06 N) and precipitation with ether/acetone yielded the desired UDP-4-deoxy-4-fluoro-α-D-galactose **1a** containing traces of UMP and its dimer P^1,P^2-bis-5'-uridine diphosphate (UPPU), although these had been separated from the desired product by the ion-exchange chromatography. Modifications of the chromatographic conditions, including adjustment of the pH to 4 and use of the alternative anion exchanger DEAE-Sephadex A-25,[14] gave better separations but did not prevent the partial hydrolysis of **1a** during the precipitation step. Extensive repurification under almost neutral conditions allowed the recovery of pure **1a** in 17% yield: reversed phase HPLC with 6 mM phosphate buffer at pH 6 as the mobile phase (UDP-FGal is eluted first, then UMP, and finally UPPU), followed by desalting on a Biogel P2 column with elution by water.

UDP-4-deoxy-4-fluoro-α-D-glucose (1b, Figure 1). Methyl α-D-galactopyranoside (**5**) reacted with benzoyl chloride to give, in one step, methyl 2,3,6-tri-*O*-benzoyl-α-D-galactopyranoside (**6**, 61%).[15] Treatment with DAST afforded the methyl 2,3,6-tri-*O*-benzoyl-4-deoxy-4-fluoro-α-D-glucopyranoside (**7**, 50.5%).[16] The deoxyfluorosugar was deprotected (**8b**: 65.4%, **9b**: 94.5%), and the pure 1,2,3,6-tetra-*O*-acetyl-4-deoxy-4-fluoro-β-D-glucopyranoside was obtained by way of the bromo-α-D-glucopyranoside as described by Withers et al. (**10b**: 45%).[10] Pure β **10b** in identical yield (44.5%) was obtained more quickly by use of acetic anhydride−sodium acetate.[11] Phosphorylation was carried out as previously described for the galactose derivative, and 4-deoxy-4-fluoro-α-D-glucopyranosyl[bis(cyclohexylammonium) phosphate] (**11b**) was recovered in 51% yield. Under these conditions, contamination of **11b** by its β-analogue was observed (ratio $\alpha/\beta = 12$). The mixture was used for the next reaction without further purification. UDP-FGlc was obtained under the conditions previously described for UDP-FGal, in 10% yield. A shortcut was used for subsequently purifying UDP-FGlc: after the ion-exchange chromatography with elution by lithium chloride, the fractions corresponding to UDP-FGlc were pooled, neutralized, loaded onto a column of Biogel P2, and eluted with water. UDP-FGlc emerged first, closely followed by UPPU. No UMP was observed in the final product when using this modified purification method.

Enzyme Studies. Kinetic characterizations of the interactions of UDP-FGlc (**1b**) and UDP-FGal (**1a**) with UDP-glucose dehydrogenase have been conducted. The enzyme catalyzes the NAD^+-linked dehydrogenation of

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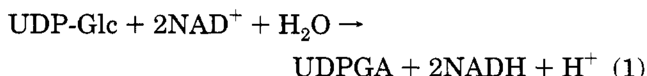
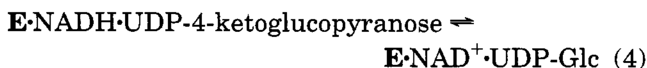
UDP-Glc to UDP-glucuronic acid (UDPGA) according to eq 1.



UDP-glucose dehydrogenase recognizes UDP-FGlc as a substrate and UDP-FGal as an inhibitor just as it recognizes UDP-Glc as a substrate and UDP-Gal as an inhibitor. The kinetic parameters for UDP-FGlc as a substrate are $K_m = 30.2 \pm 4.5$ μM and $V_m = 0.55 \pm 0.05$ μmol/min/unit at pH 8.5 in 0.125 M K–bicinate buffer and 25 °C. Under identical conditions, the kinetic parameters for the reaction of UDP-Glc are $K_m = 9.6 \pm 0.7$ μM and $V_m = 1.19 \pm 0.03$ μmol/min/unit. The ratio of 2.2 for the maximal velocities qualifies UDP-FGlc as an excellent substrate for UDP-glucose dehydrogenase under substrate-saturating conditions. Recognition of substrates in dilute solutions is best assessed by the relative values of the parameter V_m/K_m; the ratio in this case is 6.8 in favor of UDP-Glc, which qualifies UDP-FGlc as a good substrate. In contrast, UDP-FGal is not accepted by UDP-glucose dehydrogenase, even with 20 times the usual amount of enzyme. It does, however, inhibit the enzyme competitively ($K_i = 19.9 \pm 6.6$ μM) with respect to UDP-Glc.

The recognition by UDP-glucose dehydrogenase of UDP-FGlc as a substrate but not UDP-FGal is interesting given the differences between fluorine and hydroxyl substituents. Like oxygen, fluorine is electronegative and may accept hydrogen bonds; however, fluorine is significantly smaller than the hydroxyl group and cannot donate a hydrogen bond. The fact that UDP-FGlc is a good substrate for UDP-glucose dehydrogenase indicates that its interactions with the active site must be very similar to those of UDP-Glc. To the extent that the glycosyl-C-4 hydroxyl group of UDP-Glc interacts with the active site of the enzyme, the glycosyl-C-4 fluorine of UDP-FGlc interacts in a similar way. If the productive binding of UDP-Glc required hydrogen bond donation from the 4-OH group to an enzymatic group in the active site, UDP-FGlc could not participate in this interaction and should not be a substrate. The substrate reactivity of UDP-FGlc indicates that hydrogen bond donation by the 4-OH group is not required. The 4-epimer, UDP-FGal, like UDP-Gal cannot make the correct contacts with the active site and is not a substrate, although it is bound very well as a competitive inhibitor.

UDP-galactose 4-epimerase catalyzes the interconversion of UDP-Gal and UDP-Glc by a mechanism that requires the reversible oxidation of the substrates at glycosyl-C-4. The enzyme contains tightly bound NAD^+ that functions as the coenzyme for epimerization by reversibly oxidizing the substrates according to eqs 2–5.[4]



UDP-FGlc and UDP-FGal are not substrates for this enzyme because of the substitution of fluorine for the hydroxyl group on glycosyl-C-4. However, both compounds bind at the active site of UDP-galactose 4-epimerase as detected by a fluorescence binding assay, in which the displacement of the competitive inhibitor 8-anilinonaphthalene-1-sulfonate from the active site by uridine nucleotide inhibitors such as UMP, UDP, and others is measured fluorimetrically.[17] The dissociation constants (K_d at 27 °C and pH 8.5 in 0.125 M K–bicinate buffer) for UDP-FGlc and UDP-FGal are 1.4 and 1.1 mM, respectively. These values of K_d are similar to that for UMP dissociating from the active site under the same conditions.[17]

In addition to binding reversibly to UDP-galactose 4-epimerase as competitive inhibitors, UDP-FGal and UDP-FGlc irreversibly inactivate the enzyme at different rates on a slow time scale. They appear to act as suicide inactivators of the enzyme. This process is under investigation and will be described in a forthcoming paper.

In conclusion, UDP-FGlc and UDP-FGal have been synthesized and their interactions with UDP-glucose dehydrogenase and UDP-galactose 4-epimerase examined. One of the extended objectives in using these molecules for enzyme studies is to probe the details of their interactions with the active site of UDP-galactose 4-epimerase, the structure of which is under investigation.[5] The interactions of these inhibitors with crystals of UDP-galactose 4-epimerase may reveal the differences between the binding of UDP-Glc and UDP-Gal to the active site.

Experimental Section

Materials and Methods. Methyl α-D-galactopyranoside was purchased from Sigma Chemical Co. (St. Louis, MO). (Diethylamino)sulfur trifluoride (DAST), phosphoric acid (crystals) were obtained from Aldrich Chemical Co. (Milwaukee, WI). Methyl α-D-glucopyranoside was purchased from Fluka Co. (Ronkonkoma, NY). Melting points are uncorrected. Column chromatography was performed on Fisher chromatographic silica gel (100–200 mesh). Thin-layer chromatography was performed on precoated plates of silica gel (Analtech), with detection by quenching of fluorescence, or by charring after spraying with 7% molybdic acid in ethanol. Nuclear magnetic resonances were recorded at either 125, 200, or 500 MHz. ^{1}H-Chemical shifts are given relative D_2O, $CDCl_3$, or acetone-d_6, ^{13}C-NMR relative to external standard 60/40 benzene-d_6 (128 ppm):dioxane-d_6 (67 ppm) or internal standard $CDCl_3$ (77 ppm) or acetone-d_6 (29.8 ppm) when used, ^{31}P-NMR relative to pure 85% H_3PO_4 (0 ppm) as external standard, and ^{19}F-NMR relative to TFA (−76.5 ppm) as external standard. The matrix for FAB mass spectra was 3-nitrobenzyl alcohol. Elemental analyses were performed by Galbraith Laboratories (Knoxville, TN).

Synthesis. Methyl 6-*O*-trityl-α-D-glucopyranoside (**3**), was prepared according to the procedure of Chaudhary and Hernandez[6] as modified by Bernet and Vasella[7] in 68% yield. ^{13}C-NMR ($CDCl_3$): 143.8 (C1 Ph), 128.7 (C2 Ph), 127.8 (C3 Ph), 127.0 (C4 Ph), 99.1 (C1), 86.6 (CPh_3), 74.5 (C3), 72.0 (C2), 71.4 (C5), 70.3 (C4), 63.9 (C6), 54.3 (CH_3).

Methyl 4-deoxy-4-fluoro-6-*O*-trityl-α-D-galactopyranoside (**4**) was prepared according to the procedure of Card and Reddy[8] in 30% yield.

Methyl 4-deoxy-4-fluoro-α-D-galactopyranoside (**8a**) was prepared from **4** by the procedure of Card and Reddy in 78% yield.

4-Deoxy-4-fluoro-D-galactopyranoside (**9a**). A solution of **8a** (0.3 g, 1.53 mmol) in water (20 mL) containing Dowex 50W-X8 (H^+) ion-exchange resin (20 mL) was heated at reflux with stirring for 25 h. The reaction was monitored by TLC (EtOAc/MeOH, 5:1). After cooling, filtering, and evaporating, chro-

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matography of the residue (EtOAc/MeOH, 8:1) gave a white powder (278.mg, 1.53 mmol, quant), mp 147.5–149 °C. ^{19}F-NMR (^{1}H-coupled; D_2O): −221.1 ppm (dt, $J_{F,3}$ = 30.1, $J_{F,5}$ = 30.5, $J_{F,4}$ = 49.6 Hz, α anomer), −218.6 ppm (dt, $J_{F,3}$ = 29.7 Hz, $J_{F,5}$ = 30.5 Hz, $J_{F,4}$ = 50.1 Hz, β anomer).

1,2,3,6-Tetra-*O*-acetyl-4-deoxy-4-fluoro-β-D-galactopyranoside (**10a**). 4-Deoxy-4-fluoro-α-D-galactopyranoside (**9a**) (300 mg, 1.53 mmol) was treated with a boiling solution of sodium acetate (170 mg, 2.04 mmol) in acetic anhydride (8.5 mL, 90 mmol) for 50 min followed by stirring for 2 h at room temperature. Evaporated of the mixture to dryness, followed by column chromatography (CH_2Cl_2:MeOH, 19/1), gave an αβ tetraacetate from which the β-anomer **10a** (237 mg, 0.7 mmol, 44.3% yield) was crystallized from ether, mp 118–119 °C. ^{19}F-NMR ($CDCl_3$): −218.2 ppm (dt, $J_{F,3}$ = 26.7 Hz, $J_{F,5}$ = 26.0 Hz, $J_{F,4}$ = 49.7 Hz, ^{1}H-coupled).

4-Deoxy-4-fluoro-α-D-galactopyranosyl [Bis(cyclohexylammonium) phosphate] (**11a**). Anhydrous phosphoric acid (385 mg, 3.85 mmol), dried overnight in vacuum with P_2O_5, was heated at 55 °C under vacuum until molten, and 1,2,3,6-tetra-*O*-acetyl-4-deoxy-4-fluoro-β-D-galactose (**10a**) (189 mg, 0.54 mmol) was then added. The melt was heated under vacuum for 2.5 h at 55 °C in an oil bath, after which the reaction mixture was placed in an ice bath and aqueous lithium hydroxide (2 M, 8 mL) added. The mixture was left overnight at rt. Celite was used to filter off the lithium phosphate. In the cold, the filtrate was loaded on a Dowex 50W-X8 (H^+) ion-exchange resin (1 × 5 cm) and eluted into an excess of cyclohexylamine (0.4 mL) in deionized water. The solution was evaporated *in vacuo* at 30 °C and the residue crystallized by addition of acetone. Compound **11a** was obtained in 74% yield (190 mg, 0.4 mmol). ^{1}H-NMR (D_2O): 5.53 (dd, 1, H_1, $J_{1,2}$ = 2.8 Hz, $J_{1,P}$ = 7.1 Hz), 4.33 (d, 1, H_4, $J_{4,F}$ = 51.1 Hz), 4.27 (dt, 1, H_5, $J_{5,4}$ = 6 Hz, $J_{5,F}$ = 31.8 Hz), 4.06 (dd, 1, H_3, $J_{3,2}$ = 10.3 Hz, $J_{3,4}$ = 10.3 Hz, $J_{3,F}$ = 30.4 Hz), 3.84–3.76 (m, 3, H_6, $H_{6'}$, H_2), 3.16, 2.02–1.12 (m, 22, cyclohexyl). ^{13}C-NMR: 96.5 (C1), 93.4 (d, C4, J = 177 Hz), 72.6 (d, C5, J = 17 Hz), 71.8 (C2), 71.1 (d, C3, J = 18 Hz), 62.8 (C6), 53.1, 33.1, 27.0, 26.6 (cyclohexyl). ^{19}F-NMR: −220.2 ppm (s; ^{1}H-decoupled). ^{31}P-NMR: 3.36 ppm (s, ^{1}H-coupled). Anal. Calcd: C, 46.95; N, 6.08. Found: C, 47.01; N, 6.24

UDP-4-deoxy-4-fluoro-α-D-galactose (**1a**). An aqueous solution of 4-deoxy-4-fluoro-α-D-galactopyranosyl [bis (cyclohexylammonium) phosphate] (**11a**) (74.5 mg, 0.158 mmol) is passed through a column of Dowex 50 (chloride form, 1 × 5 cm), dropped in excess of pyridine. The eluate was evaporated to a few mL *in vacuo* to which tri-*n*-octylamine (63 μL, 0.158 mmol) was added. After evaporation to dryness, the mixture was rendered anhydrous by three evaporations with dry pyridine. Separately, the 4-morpholine *N,N'*-dicyclohexyl carboxamidine salt of uridine 5'-phosphoromorpholidate (162 mg, 0.236 mmol) was dissolved in anhydrous pyridine (4–10 mL) and evaporated to dryness *in vacuo* three times. The residue was dissolved in pyridine and added to **11a**. The mixture was evaporated twice more, dissolved in dry pyridine (4–10 mL), and stored under nitrogen at rt for 2 days. The solvent was then evaporated, and the oily residue was resuspended in water and stirred with lithium acetate (24 mg, 0.236 mmol). Trioctylamine was then extracted with ether. The aqueous phase was passed through a DEAE-Sephadex A-25 column (1.5 × 20 cm).[14] The column was washed with water, and the products were eluted with a linear gradient of 3 L of lithium chloride pH 4.0 (0.01–0.1 M salt). Unreacted morpholidate and uridine 5'-monophosphate were eluted first. The fractions with UDP-4-deoxy-4-fluoro-galactose emerged at roughly 0.06 M salt and were pooled and evaporated to dryness *in vacuo*. The UDP derivative was then precipitated as described by Moffat.[13] After precipitation, UMP along with its dimer UPPU was identified present with UDP-FGal. HPLC on reversed phase C_{18} with 6 mM potassium phosphate buffer pH 6 (elution order: UDP-FGal, UMP, and UPPU), followed by desalting on a Biogel P2 column (5 × 110 cm) with water as solvent, gave pure **1a** in 17% yield. H-NMR (D_2O): 7.79 (d, 1, $H_{6''}$, J = 8.3 Hz), 5.82 (m, 2, $H_{5''}$, $H_{1'}$), 5.51 (dd, 1, H_1, $J_{1,2}$ = 3.6 Hz, $J_{1,P}$ = 7 Hz), 4.79 (d, 1, H_4, $J_{4,F}$ = 50.6 Hz), 4.38–4.17 (m, 6, $H_{2'}$, $H_{3'}$, $H_{5R'}$, $H_{5S'}$, H_5), 4.03 (dd, 2, H_3, $J_{2,3}$ = 10.2 Hz, $J_{3,F}$ = 28.6 Hz), 3.87–3.78 (m, 3, $H_{6'}$, $H_{6''}$, H_2). ^{13}C-NMR: 166.8 (C4''), 152.4 (C2''), 142.2 (C6''), 103.2 (C5''), 96.2 (C1), 89.5 (d, C4, J = 176.5 Hz), 89.1 (C1'), 83.8 (C4'), 74.4 (C3'), 71.1 (d, C5, J = 16 Hz), 70.3 (C2), 68.8 (d, C3, J = 20 Hz), 68.7 (C2'), 65.6 (C5'), 60.5 (C6). ^{31}P-NMR: −10.5 (d, uridine P, J = 20.1 Hz), −12.2 (dd, hexose P, J = 5.7, 20.2 Hz). ^{19}F-NMR: −220.6 ppm (s, ^{1}H-decoupled). FAB mass spectroscopy on desalted samples[18] gave M + 1= 569 (free acid $C_{15}H_{23}N_2O_{16}FP_2$ = 568.16) and M + 1= 670 ($C_{15}H_{23}N_2O_{16}FP_2{\cdot}(C_2H_5)_3N$ = 568.16 + 101.19).

Methyl 2,3,6-tri-*O*-benzoyl-α-D-galactopyranoside (**6**) was prepared by the procedure of Reist et al.[15] in 61% yield. ^{1}H-NMR ($CDCl_3$): 8.04–7.95 (m, 6, benzoyl ortho), 7.57–7.33 (m, 9, benzoyl meta and para), 5.74 (dd, 1, H_3, $J_{3,4}$ = 2.8 Hz, $J_{3,2}$ = 10.4 Hz), 5.67 (dd, 1, H_2, $J_{2,1}$ = 3.8 Hz, $J_{2,3}$ = 11.0 Hz), 5.20 (d, 1, H_1, $J_{1,2}$ = 3.65 Hz), 4.70–4.32 (m, 4, H_4, H_5, H_6, $H_{6'}$), 3.44 (s, 3, CH_3O−), 2.55 (−OH). ^{13}C-NMR: 166.5, 166.1, 165.7 (benzoyl *C*=O), 133.4, 133.3 (quartenary C, benzoyl), 129.8, 129.8, 129.7, 129.4, 129.3, 128.5, 128.4 (benzoyl ortho, meta, para C), 97.6 (C1), 70.8 (C5), 68.9 (C4), 68.2 (C2), 67.7 (C3), 63.3 (C6), 55.5 (CH_3).

Methyl 2,3,6-tri-*O*-benzoyl-4-deoxy-4-fluoro-α-D-glucopyranoside (**7**) was prepared from **6** by the procedure of Card[16] in 50.5% yield. ^{13}C-NMR ($CDCl_3$): 166.1, 165.8, 165.5 (benzoyl *C*=O), 133.5, 133.3 (quartenary C, benzoyl), 129.9, 129.7, 129.2, 128.8, 128.4, 128.3 (C ortho , meta, para benzoyl), 96.8 (C1), 87.3 (C4, d, J = 189.9 Hz), 71.3 (C2, d, J = 6.4 Hz), 70.4 (C3, d, J = 19.6 Hz), 67.0 (C5, d, J = 23.1 Hz), 62.5 (C6), 55.6 (CH_3).

Methyl 4-deoxy-4-fluoro-α-D-glucopyranoside (**8b**) was prepared by the procedure of Withers et al.[10] in 65.9% yield.

4-Deoxy-4-fluoro-D-glucopyranoside (9b) was prepared by the procedure of Withers et al.[10] in 94.5% yield.

1,2,3,6-Tetra-*O*-acetyl-4-deoxy-4-fluoro-β-D-glucopyranoside (**10b**) was prepared by the procedure of Withers et al.[10] in 45% yield or by the procedure of Barford et al.[11] in 44.4% yield. ^{13}C-NMR ($CDCl_3$): 170.6, 169.6, 169.2, 168.6 (acetate C=O), 92.0 (C1), 87.5 (C4), 72.8 (C3), 72.4 (C2), 70.4 (C5), 62.0 (C6), 20.9, 20.8, 20.7, 20.6 (acetate CH_3). ^{19}F-NMR (^{1}H-coupled): −196.8 ppm (dd, $J_{F,3}$ = 14.6 Hz, $J_{F,4}$ = 50.2 Hz).

4-Deoxy-4-fluoro-α-D-glucopyranosyl [Bis(cyclohexylammonium) phosphate] (**11b**). Reaction of **10b** (137 mg, 0.39 mmol) with anhydrous phosphoric acid (300 mg, 2.99 mmol) was performed as for **11a**, yielding **11b** in 51.3% yield (93 mg, 0.2 mmol). ^{1}H-NMR (D_2O): 5.44 (dd, 1, H_1, $J_{1,2}$ = 3.3 Hz, $J_{1,P}$ = 7.3 Hz), 4.33 (dt, 1, H_4, $J_{4,3}$ = 9.6 Hz, $J_{4,5}$ = 9.6 Hz, $J_{4,F}$ = 51.0 Hz), 4.09 (m, 1, H_2), 4.04 (dt, 1, H_3, $J_{3,2}$ = 9 Hz, $J_{3,4}$ = 9 Hz, $J_{3,F}$ = 15.9 Hz), 3.86–3.74 (m, 2, H_6, $H_{6'}$), 3.51 (m, 1, H_5), 3.14, 2.05–1.10 (m, 22, cyclohexyl). ^{13}C-NMR: 94.0 (C1), 89.8 (C4), 72.4 (C3), 72.1 (C2), 70.0 (C5), 60.6 (C6), 51.0, 31.0, 24.9, 24.4 (cyclohexyl). ^{19}F-NMR: −200.3 ppm (s; ^{1}H-decoupled). ^{31}P-NMR: 3.38 ppm (s; ^{1}H-coupled). Anal. Calcd for $C_{18}H_{38}FN_2O_8P$: C, 46.95, N, 6.08. Found: C, 47.02, N, 6.09.

UDP-4-deoxy-4-fluoro-α-D-glucose (**1b**). Synthesis of **1b** (15.4 mg, 0.02 mmol, 10%) from **11b** (122 mg, 0.26 mmol) was performed as for **1a**. ^{1}H-NMR (D_2O): 7.95 (d, 1, $H_{6''}$, J = 8.2 Hz), 5.98 (m, 2, $H_{5''}$, $H_{1'}$), 5.60 (dd, 1, H_1 $J_{1,2}$ = 3 Hz, $J_{1,P}$ = 6 Hz), 4.40 (dt, 1, H_4, $J_{4,3}$ = 9.3 Hz, $J_{4,5}$ = 9.3 Hz, $J_{4,F}$ = 50.6 Hz), 4.40–4.16 (m, 5, $H_{2'}$, $H_{3'}$, $H_{4'}$, $H_{5R'}$, $H_{5S'}$), 4.08 (m, 1, H_2), 4.05 (m, 1, H_3), 3.91–3.79 (m, 2, $H_{6'}$, $H_{6''}$). ^{1}H-NMR of β anomer: 5.04 (dd, H_1), 4.62 (dt, 1, H_4, $J_{4,3}$ = 9.5 Hz, $J_{4,5}$ = 9.8 Hz, $J_{4,F}$ = 51.7 Hz). ^{13}C-NMR: 166.8 (C4''), 152.4 (C2''), 142.2 (C6''), 103.2 (C5''), 95.8 (C1), 89.1 (d, C4, J = 187 Hz), 89.0 (C1'), 83.7 (C4'), 74.4 (C3'), 71.7 (C3), 71.6 (C2), 70.8 (d, C5, J = 24.4 Hz), 70.1 (C2'), 65.5 (C5'), 60.2 (C6). ^{31}P-NMR: −11.5 (d, uridine P, J = 20.0 Hz), −13.3 (dd, hexose, J = 5, 19.9 Hz). ^{19}F-NMR: −199.2 ppm (s, ^{1}H-decoupled). FAB mass spectroscopy on desalted samples[18] gave M + 1= 670 ($C_{15}H_{23}N_2O_{16}FP_2{\cdot}(C_2H_5)_3N$= 568.16 + 101.19), addition of HCl gave M + 1 = 613 ($C_{15}H_{21}N_2O_{16}FP_2KLi$ = 612.32).

Enzymatic Binding and Kinetics. K_m: The initial velocities of the enzymatic reactions were determined by measuring the rate of UDP-4-deoxy-4-fluoro glucuronic acid or UDP-glucuronic acid formation at 25 °C using a modification

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of the method of Strominger et al.[19] All reactions were carried out in quartz cuvettes containing 125 mM potassium bicinate buffer (pH 8.5), 1.25 μmol of NAD^+, and 0.002 unit of UDP-glucose dehydrogenase, in a total volume of 1 mL. Eight concentrations of **1b** (varying from 5 to 200 μM) or UDP-Glc (varying from 5 to 250 μM) were used in these assays. The formation of NADH concomittent to UDP-4-deoxy-4-fluoro glucuronic acid or UDP-glucuronic acid is monitored spectrophotometrically at 340 nm, and the reaction rates were estimated by use of the extinction coefficient 6.22 mM^{-1} cm^{-1} for NADH. Similar conditions were used to determine the K_i value for **1a**. The initial velocity of the enzymatic reactions were determined by measuring the rate of UDP-glucuronic acid formation at 25 °C. The cuvettes contained 125 mM potassium bicinate buffer (pH 8.5), 5−250 μM of UDP-Glc, 1.25 μmol of NAD^+, and 0.002 of unit UDP-glucose dehydrogenase, in a total volume of 1 mL. (One unit of UDP-glucose dehydrogenase oxidizes 1.0 μmol of UDP-Glc per min at 25 °C and pH 8.7 under standard assay conditions). Three concentrations of **1a** were used in this assay varying from 35 to 100 μM. The K_d values for **1a** and **1b** were determined by the fluorescence titration method of Wong and Frey.[17] The solutions contained 1.8 μM epimerase·NAD^+ and 25 μM 8-anilinonaphthalene-1-sulfonate in 125 mM potassium bicinate buffer (pH 8.5) at 25 °C. The most intense emission spectrum was obtained on this solution upon excitation at 375 nm and the quenched spectra upon addition of **1a** (0.1 to 1 mM) or **1b** (0.1 to 1.5 mM).

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