Synthesis and Characterization of Novel *lacZ* Gene Reporter Molecules: Detection of β-Galactosidase Activity by ^{19}F Nuclear Magnetic Resonance of Polyglycosylated Fluorinated Vitamin B_6

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Gene therapy has emerged as a promising strategy for treatment of various diseases. However, widespread implementation is hampered by difficulties in assessing the success of transfection, in particular, the spatial extent of expression in the target tissue and the longevity of expression. Thus, the development of noninvasive reporter techniques based on appropriate molecules and imaging modalities may help to assay gene expression. We have previously demonstrated the ability to detect β-galactosidase (β-gal) activity on the basis of ^{19}F NMR chemical shift associated with release of fluorophenyl aglycons from galactopyranoside conjugates. Use of fluoropyridoxol as the aglycon provides a potential less toxic alternative and we now report the design, synthesis, and structural analysis of a series of novel polyglycosylated fluorinated vitamin B_6 derivatives as ^{19}F NMR-sensitive aglycons for detection of *lacZ* gene expression. In particular, we report the activity of 3,α^4,α^5-tri-*O*-(β-D-galactopyranosyl)-6-fluoropyridoxol **4**, 3-*O*-(β-D-galactopyranosyl)-α^4,α^5-di-*O*-(β-D-glucopyranosyl)-6-fluoropyridoxol **12**, and 3-*O*-(β-D-galactopyranosyl)-α^4,α^5-di-*O*-(α-D-mannopyranosyl)-6-fluoropyridoxol **13**. Compounds **4**, **12**, and **13** all show promising characteristics including highly sensitive ^{19}F NMR response to β-gal activity ($\Delta\delta = 9.0 \sim 9.4$ ppm), minimal toxicity for substrate or aglycon, and good water solubility. However, the differential glycosylation of **12** and **13** appears more advantageous for assessing *lacZ* gene expression in vivo.

Introduction

Gene therapy holds great promise for the treatment of diverse diseases, but widespread implementation is hindered by difficulties in assessing the success of transfection. The development of noninvasive in vivo reporter techniques based on appropriate molecules and imaging modalities would be of considerable value for assessing the location, magnitude, and persistence of expression.

The *lacZ* gene encoding β-galactosidase (β-gal) is widely used in molecular biology as a reporter gene to assay clonal insertion, transcriptional activation, protein expression, and protein interaction. Many colorimetric reporter molecules have been described to detect β-gal activity and these form the basis of highly effective spectrophotometric assays in vitro.[1−3] However, optical methods are less practical for applications in animals in vivo or ultimately in man in the clinic due to extensive light scattering and absorption by tissues. Toward such applications new reporter molecules are being developed. Recently, Tung et al.[4] presented a near-infrared approach in vivo based on 9*H*-(1,3-dichloro-9,9-dimethylacridin-2-on-7-yl) β-D-galactopyranoside to detect β-gal activity in transfected tumors in live mice. Lee et al.[5] described use of a radiolabeled competitive inhibitor 2-(4-[^{125}I/^{123}I]iodophenyl)ethyl 1-thio-β-D-galactopyranoside to detect β-gal activity in mice. Louie et al.[6] introduced an NMR approach using 1-[2-(β-D-galactopyranosyloxy)propyl]-4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane)gadolinium(III) based on proton MRI contrast to detect β-gal activity in developing frog embryos following direct injection of substrate into eggs. We have been developing in vivo reporter molecules based on ^{19}F NMR with structures exploiting fluorophenol, trifluorophenol, and fluoropyridoxol aglycons.[7−11] To date our published investigations have focused on development of reporter molecules and we have demonstrated detection of β-gal activity in cultured tumor cells with preliminary examples of detectability in tumors in living mice.[12] In a continuing effort to develop enhanced approaches for in vivo detection of β-gal, we now report the synthesis and evaluation of polyglycosylated fluorinated vitamin B_6 reporter molecules, designed to enhance water solubility, cellular penetration, and enzyme response.

Design

The diversity of substrates and reporter molecules for β-gal activity is indicative of broad substrate specificity. Agents have been tailored for specific imaging modalities or with particular characteristics, such as thermal stability suitable for autoclaving. However, some substrates suffer from poor aqueous solubility and inability to reach targets in vivo and some aglycon products are toxic. Our initial investigations used fluorophenyl β-D-galactopyranosides.[7,9] This approach was particularly facile, being a simple analogy of the classic "yellow" agent *o*-nitrophenyl β-D-galactopyranoside (ONPG). However, the product aglycon appears somewhat toxic, being closely similar to the uncoupler dinitrophenol. We were able to reduce the requisite concentration of reporter molecule by introducing a trifluoromethyl reporter moiety in place of a single fluorine atom, but this is characterized by a much smaller chemical shift response.[11] Toxicity could be largely avoided by using 6-fluoropyridoxol (**1**, FPOL) as the aglycon and we recently demonstrated proof of principle. Introduction of a D-galactose at the 3-phenolic group of FPOL, 3-*O*-(β-D-galactopyranosyl)-6-fluoropyridoxol (GFPOL), yielded a ^{19}F NMR gene expression reporter exhibiting a large chemical shift response to β-gal cleavage but having only moderate kinetic sensitivity to β-gal.[8] GFPOL was also modestly water-soluble.

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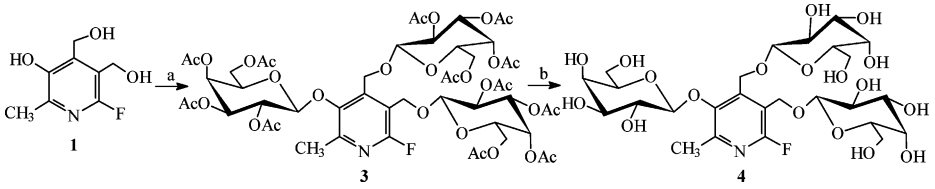

Figure 1. Reagents and conditions: (a) 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide **2**, $Hg(CN)_2$, 4 Å molecular sieve, CH_2Cl_2, RT, 12 h, 89%; (b) NH_3/MeOH, 0 °C → RT, 24 h, quantitative yields.

We considered that introduction of additional sugar moieties could enhance water solubility and potentially improve enzyme sensitivity. Pertinent to this approach were reports that modification the α^4- and α^5-position hydroxymethyl moieties of FPOL produces modification of its pK_a with relatively minor changes in chemical shift and chemical shift range.[13] Further, *Escherichia coli* (*lacZ*) β-gal catalyzes the hydrolysis of galactopyranosides by cleavage of the C−O bond between D-galactose and the aglycon with a double-displacement mechanism involving the formation (glycosylation step) and breakdown (deglycosylation step) of a glycosyl-enzyme intermediate via oxocarbonium ion-like transition states. It has been observed that hydrogen-bonding interaction between the enzyme and the glycosidic substrate is important in the formation of the enzyme−substrate complex and the hydrolysis rate.[14,15] The involvement of fluorine atoms in hydrogen bonding is well documented and exemplified by some of the strongest known hydrogen bonds.[16] Considerable evidence suggests that a C−F moiety can act as a weak proton acceptor and may form hydrogen bonds between the enzyme and the substrate.[17−21]

We have found evidence of intramolecular hydrogen bonding between α^5-OH and 6-F in ^{1}H NMR spectra of FPOL and its derivatives. For example, the signal of α^5-OH in α^4-OH and α^5-OH unprotected analogues, such as FPOL or 3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-6-fluoropyridoxol, always appears downfield and is coupled with 5-CH_2 as triplet due to the α^5-OH exchange limitation by the α^5-OH and 6-F hydrogen bonding. Meanwhile, α^4-OH occurs as an upfield singlet. Introduction of two additional carbohydrate residues at α^4- and α^5-hydroxymethyl positions of GFPOL would inhibit α^5-OH and 6-F hydrogen bonding and facilitate hydrogen bonding between the enzyme and the new substrates. Thus, substrate affinity should increase and both water solubility and enzyme sensitivity could be improved, while the virtues of GFPOL are retained.[8]

Results and Discussion

Syntheses. Our initial approach used a one-pot technique to introduce three D-galactose moieties at the 3 phenolic and α^4,α^5-hydroxymethylic sites, simultaneously. Reaction of **1** with 3.3 equiv of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide **2** in anhydrous dichloromethane catalyzed by $Hg(CN)_2$ afforded the fully galactopyranosylated 6-fluoropyridoxol (**3**) in 89% yield, which was deacetylated with NH_3/MeOH, giving the free galactopyranoside 3,α^4,α^5-tri-*O*-(β-D-galactopyranosyl)-6-fluoropyridoxol **4** in quantitative yield (Figure 1). The ESI-MS of **3** showed the expected molecular ion at *m*/*z* 1178 and quasi-molecular ion at *m*/*z* 1179 [M + H], corresponding to the fully adorned derivative with three fully acetylated galactosides. The identity of **3** was established by ^{1}H and ^{13}C NMR. The anomeric protons H-1′, H-1″, and H-1‴ of D-galactoses linked to 3, α^4-, and α^5-positions of FPOL at 5.24, 4.66, and 4.52 ppm, respectively, with three well-resolved doublets ($J_{1,2}$ = 8.0 Hz), as well as $J_{2,3} \sim 10$ Hz, confirming that all D-galactoses are in the β-configuration with the $^4C_1$ chair conformation, whereas in the ^{13}C NMR spectrum, the anomeric carbons C-1′, C-1″, and C-1‴ occurred at 103.34 and 100.22 ppm.

Compound **4** was stable in buffer and gave a single sharp ^{19}F NMR signal. Exposure of **4** to β-gal indicated that all three β-D-galactopyranosyl $C_{1(gal)}$−O linkages are sensitive resulting in multiple ^{19}F signals around 3 ppm and 12∼20 ppm (Figure 2). These results demonstrate the principle of polyglycosylation to enhance water solubility while retaining sensitivity to β-gal, but the complex spectra suggested a need for a more sophisticated approach. We therefore designed two further molecules, 3-*O*-(β-D-galactopyranosyl)-α^4,α^5-di-*O*-(β-D-glucopyranosyl)-6-fluoropyridoxol **12** and 3-*O*-(β-D-galactopyranosyl)-α^4,α^5-di-*O*-(α-D-mannopyranosyl)-6-fluoropyridoxol **13**, featuring differential glycosylation: galactosylation at the 3-phenolic group being sensitive to β-gal, and glucopyranosylation or mannopyranosylation at the α^4,α^5-hydroxymethyl groups to aid water solubility but resist β-gal activity. Retrosynthetic analysis suggested two approaches through differentially protected intermediates as key synthons.

6-Fluoro-α^4,α^5-isopropylidenepyridoxol **5** was previously prepared as part of the synthesis of 6-fluoro-3,α^4-isopropylidenepyridoxol.[8,13] Testing various acids as catalysts showed 2% H_2SO_4 acetone solution to provide the best yield of **5** (26%). The regioselectivity of the acetonation reaction was confirmed by comparing ^{1}H NMR of **5** and 6-fluoro-3,α^4-isopropylidenepyridoxol, in which the 5-CH_2 signal of **5** appeared at 5.03 ppm as a singlet, while in 6-fluoro-3,α^4-isopropylidenepyridoxol it appeared at 4.97 ppm as a doublet ($J_{H-5,HO-5}$ = 1.2 Hz) due to the coupling of 5-OH.[13] Treatment of **5** with **2** by the Koenigs−Knorr glycosylation gave 3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α^4,α^5-isopropylidene-6-fluoropyrodoxol **6** in 85% yield. The $\delta_{H-1'}$ at 4.64 ppm is a well-resolved doublet ($J_{1,2}$ = 8.0 Hz), and $\delta_{C-1'}$ at 100.03 ppm demonstrated that the D-galactose was in the β-configuration. The correlation between 2-CH_3 and H-1′ of sugar ring from the NOESY spectrum of **6** verified that 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl residue connected at the 3-phenolic site, providing further evidence that the acetonation had occurred regioselectively on 4,5-hydroxymethyl groups.

3-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-6-fluoropyridoxol **7** was obtained by cleavage of acetonide **6**, but the yields were quite low (≤15%), based on several hydrolysis conditions, such as 80% AcOH, 1% HCl, or 90% CF_3CO_2H in MeOH, CH_2Cl_2, or 1,4-dioxane at various temperatures (60∼100 °C). A moderate amount of **1** was recoverable, indicating that the β-D-galactopyranosyl $C_{1'}$(gal)−O_3 bond became weak and sensitive to acid hydrolysis, presumably due to the presence of the 6-fluorine atom. Condensation of **7** with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide **8** or 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide **9** in dry CH_2Cl_2 with $Hg(CN)_2$ as a promoter gave 3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α^4,α^5-di-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-6-fluoropyridoxol **10** or 3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α^4,α^5-di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-6-fluoropyridoxol **11** in yields of 80% or 78%, respectively.

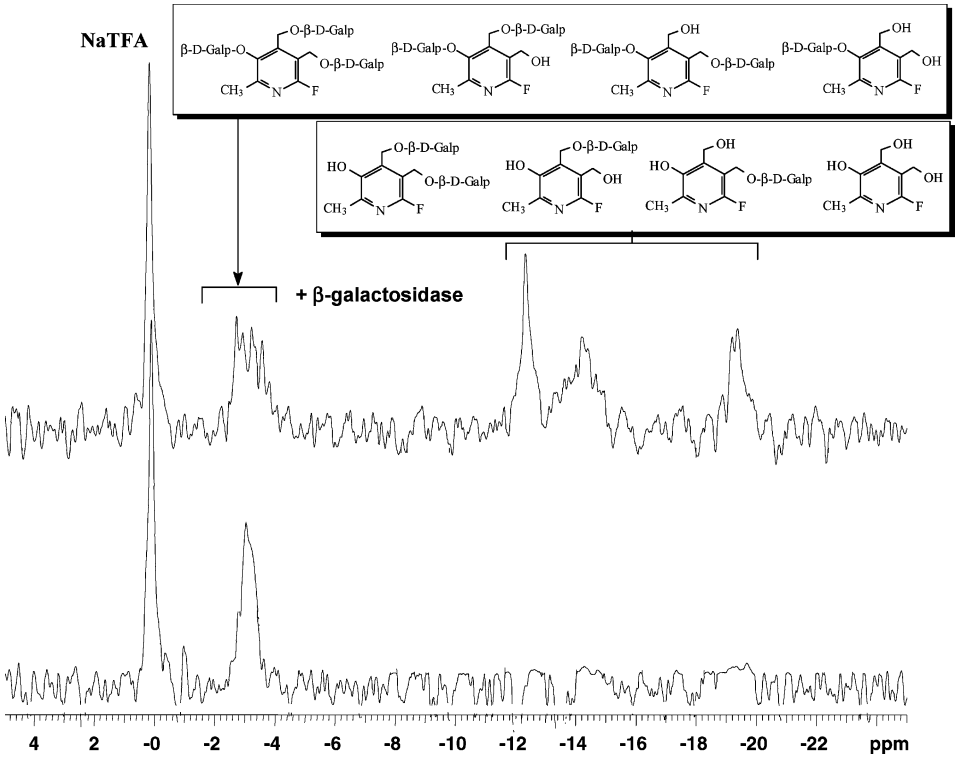

Figure 2. ^{19}F NMR spectra of 3,α^4,α^5-tri-*O*-(*β*-D-galactopyranosyl)-6-fluoropyridoxol **4** (10.1 mg, 15 mmol, lower trace) and its products resulting from addition of *β*-gal (E801A, 15 units) in PBS (pH = 7.4) at 37 °C (upper trace). Spectra were acquired in 51 s and enhanced with an exponential line broadening 40 Hz; *β*-D-Galp = *β*-D-galactopyranosyl.

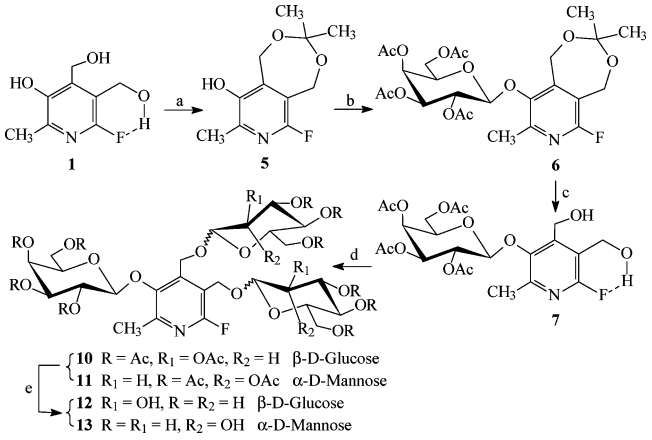

Figure 3. Reagents and conditions: (a) 2% H_2SO_4, acetone, RT 4∼5 h, 26%; (b) 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide **2**, $Hg(CN)_2$, 4 Å molecular sieve, CH_2Cl_2, RT, 12 h, 85%; (c) 80% AcOH, 80 °C, 4∼5 h, 15%; (d) 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide **8** or 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide **9**, $Hg(CN)_2$, 4 Å molecular sieve, CH_2Cl_2, RT, 12 h, 80% (→ **10**) or 78% (→ **11**), respectively; (e) NH_3/MeOH, 0 °C → RT, 24 h, quantitative yields.

Deacetylation of **10** or **11** in NH_3/MeOH from 0 °C to room temperature (RT) gave the target molecules **12** and **13** in quantitative yields (Figure 3). However, the overall yields for **12** and **13** through the five-step reactions were only 3%, with limiting steps in the α^4,α^5-isopropylidene group formation and hydrolysis procedures.

The acidic 3-phenolic group para to the 6-fluorine atom in FPOL should be easily converted into the monoanion under mild base conditions,[8,13,22] suggesting an alternate approach to selectively benzylate the 3-OH under carefully controlled conditions. Benzyl bromide (1.1 equiv) was added dropwise over a period of 4∼5 h to the well-stirred reaction mixture of **1** in a dichloromethane/aqueous biphasic system (pH 10∼11) with tetrabutylammonium bromide (TBAB) as the phase-transfer catalyst, yielding 3-*O*-benzyl-6-fluoropyridoxol **14** in 76% yield. The structure was established on the basis of the coupling characteristics of α^4,α^5-CH_2 as doublets ($J_{H-4,HO-4}$ = 6.0 Hz, $J_{H-5,HO-5}$ = 5.4 Hz) and α^4,α^5-OH as triplets in the ^1H NMR spectrum. Condensation of **14** with **8** or **9** gave 3-*O*-benzyl-α^4,α^5-di-*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-6-fluoropyridoxol **15** or 3-*O*-benzyl-α^4,α^5-di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-6-fluoropyridoxol **16** in satisfactory yields. Removal of the benzyl-protecting group afforded acceptors α^4,α^5-di-*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-6-fluoropyridoxol **17** or α^4,α^5-di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-6-fluoropyridoxol **18** in quantitative yields, which were subjected to a procedure similar to that described above for the preparation of galactosides, giving **10** or **11** in high yields (88% or 85%, respectively). After workup and deacetylation, the target compounds **12** and **13** were obtained in 57% and 52% overall yields over five-step reactions (Figure 4).

Recognizing the differential reactivity of the 3-phenolic group over the hydroxymethyl groups, most recently, we have successfully specifically galactopyranosylated **1** on the 3-phenolic group directly with **2** via the above phase-transfer catalysis technique, yielding **7**.[8] Figure 5 depicts a very efficient route to synthesize the target compounds **12** and **13** in just three steps with higher overall yields (67% and 65%, respectively).

Characteristics. Compounds **4**, **12**, and **13** each gave a single narrow ^{19}F NMR signal between *δ* −2.0 and −3.3 ppm, essentially invariant (Δ*δ* ≤ 0.06 ppm) with pH in the range 3−12 and temperatures from 25 to 37 °C in whole rabbit blood, 0.9% saline, or phosphate-buffered saline (PBS). Addition of *β*-gal (E801A) in PBS at 37 °C to **4**, **12** and **13** caused rapid hydrolysis, releasing the aglycons α^4,α^5-di-*O*-(*β*-D-galactopyranosyl)-6-fluoropyridoxol, α^4,α^5-di-*O*-(*β*-D-glucopyranosyl)-6-fluoropyridoxol, and α^4,α^5-di-*O*-(α-D-mannopyranosyl)-6-flu-

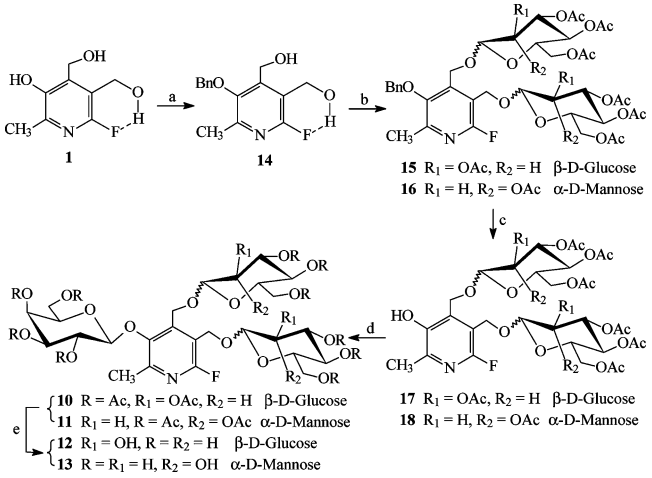

Figure 4. Reagents and conditions: (a) benzyl bromide (1.1 equiv), CH_2Cl_2−H_2O, pH 10∼11, 50 °C, TBAB, 4∼5 h, 76%; (b) 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide **8** or 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide **9**, $Hg(CN)_2$, 4 Å molecular sieve, CH_2Cl_2, RT, 12 h, 90% (→ **15**) or 85% (→ **16**), respectively; (c) 25 psi H_2, Pd/C, RT, 12 h, quantitative yields; (d) 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide **2**, $Hg(CN)_2$, 4 Å molecular sieve, CH_2Cl_2, RT, 12 h, 88% (→ **10**) or 85% (→ **11**), respectively; (e) NH_3/MeOH, 0 °C → RT, 24 h, 95% (→ **12**) or 94% (→ **13**), respectively.

oropyridoxol, which also appeared as single narrow ^{19}F signals between δ −11.20 and −12.40 ppm ($\Delta\delta$ = 9.0∼9.4 ppm) (Table 1). Action of β-gal on **4** was complicated by action on each of the galactose residues, apparently randomly, to generate multiple signals representing **1** together with partially galactosylated products (Figure 2). The β-gal hydrolysis of **4**, **12**, and **13** proceeded in a smooth manner, indicating that the liberated aglycons have no inhibitory effects on β-gal (Figure 6). The kinetic curves suggest straightforward first-order kinetics, which were much more rapid for all substrates than for GFPOL. Compounds **12** and **13** gave single products upon exposure to β-gal (Figure 7). Addition of **12** and **13** to stably transfected human breast MCF-7-*lacZ* tumor cells showed cleavage of **12** or **13** (Figure 8) and this proceeded in an initially smooth monotonic manner at rates of 18.6 or 19.6 μmol min^{-1} (million MCF7-*lacZ* cells)$^{-1}$, respectively. Compounds **12** and **13** have much higher aqueous solubility than GFPOL (GFPOL, 75 mM vs **12**, 196 mM, and **13**, 173 mM, all in PBS).

The products α^4,α^5-di-*O*-(β-D-galactopyranosyl)-6-fluoropyridoxol (DGFPOL), α^4,α^5-di-*O*-(β-D-glucopyranosyl)-6-fluoropyridoxol (DUFPOL), and α^4,α^5-di-*O*-(α-D-mannopyranosyl)-6-fluoropyridoxol (DMFPOL) of the action of β-gal on **4**, **12**, and **13** also exhibit large ^{19}F NMR chemical shift response to pH ($\Delta\delta$ = ∼ 11.0 ppm) in the range of pH 1∼12 (Figure 9, Table 2), but there is no spectral overlap with the substrates.

Conclusion

These results provide further evidence for the broad specificity of β-gal and the feasibility of modifying substrate structures to enhance enzyme sensitivity and water solubility. The additional sugar residues in **4**, **12**, and **13** compared with GFPOL all lead to faster cleavage kinetics with β-gal. Significantly, the differential glycosylation provides structures that respond to β-gal with generation of single products. The results with stably transfected breast cancer cells indicate the potential for future studies in vivo.

Experimental Section

General Methods. NMR spectra were recorded on a Varian Inova 400 spectrometer (400 MHz for ^1H, 100 MHz for ^{13}C, 376 MHz for ^{19}F) with $CDCl_3$ or DMSO-d_6 as solvent. ^1H and ^{13}C chemical shifts are referenced to tetramethylsilane (TMS) as internal standard, and ^{19}F to a dilute solution of sodium trifluoroacetate (NaTFA) in a capillary as external standard (37 °C). Compounds were characterized by acquisition of ^1H, ^{13}C, distortionless enhancement by polarization transfer (DEPT), $^1H-^1H$ correlation spectroscopy (COSY), or nuclear Overhauser enhancement spectroscopy (NOESY) experiments at 25 °C. Microanalyses were performed on a Perkin-Elmer 2400CHN microanalyzer. Mass spectra were obtained by positive and negative electrospray ionization mass spectrometry (ESI-MS) on a Micromass Q-TOF hybrid quadrupole/time-of-flight instrument (Micromass UK Ltd.). Reactions requiring anhydrous conditions were performed under nitrogen or argon. $Hg(CN)_2$ was dried before use at 50 °C for 1 h, CH_2Cl_2 was dried over Drierite, and acetonitrile was dried on CaH_2 and kept over molecular sieves under N_2. Solutions in organic solvents were dried with anhydrous sodium sulfate and concentrated in vacuo below 45 °C. 2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl bromide **2** and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide **8** were purchased from Sigma Chemical Co. 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl bromide **9** was prepared according to the literature method.[23] Column chromatography was performed on silica gel (200∼300 mesh) by elution with cyclohexane/EtOAc, and silica gel GF_{254} (Aldrich) was used for analytical thin-layer chromatography (TLC). Detection was effected by spraying the plates with 5% ethanolic H_2SO_4 (followed by heating at 110 °C for ∼10 min) or by direct UV illumination of the plate.

For enzyme kinetic experiments, **4**, **12**, and **13** (10.1 mg, 15 mmol) were dissolved in PBS (0.1 M, pH = 7.4, 600 μL), and a PBS solution of β-gal (0.1 M, pH = 7.4, 15 μL, 1 unit/μL, E801A, Promega, Madison, WI) was added and NMR data were acquired immediately at 37 °C.

MCF7-*lacZ* human breast cancer cells stably transfected to express β-gal were grown in culture under standard conditions and harvested. Compound **12** or **13** was added to suspension of cells (5×10^6) in PBS and observed by NMR for 1 h.

Syntheses: 3,α^4,α^5-Tri-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-6-fluoropyridoxol 3. A solution of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide **2** (1.35 g, 3.3 mmol, 1.1 equiv) in anhydrous CH_2Cl_2 (8 mL) was added dropwise to a solution of 6-fluoropyridoxol **1** (0.18 g, 1.0 mmol) and $Hg(CN)_2$ (1.01 g, 4.0 mmol) in dry MeCN (10 mL) containing powdered molecular sieves (4 Å, 2.0 g) with vigorous stirring at RT under argon in the dark for 12 h. The mixture was diluted with CH_2Cl_2 (30 mL), filtered through Celite, washed, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified on a silica gel column (1:3 cyclohexane/EtOAc) to yield **3** (1.05 g, 89%) as syrup, R_f 0.30 (1:3 cyclohexane/EtOAc). NMR ($CDCl_3$) δ_H 5.24 (1H, d, $J_{1',2'}$ = 8.0 Hz, H-1′), 5.04 (1H, dd, $J_{2',3'}$ = 9.8 Hz, H-2′), 4.73 (1H, dd, $J_{3',4'}$ = 3.4 Hz, H-3′), 3.98 (1H, dd, $J_{4',5'}$ = 2.4 Hz, H-4′), 4.02∼4.10 (3H, m, H-5′ and H-6′), 4.66 (1H, d, $J_{1'',2''}$ = 8.0 Hz, H-1″), 4.52 (1H, d, $J_{1''',2'''}$ = 8.0 Hz, H-1‴), 5.15 (2H, dd, $J_{2'',3''}$ = $J_{2''',3'''}$ = 10.0 Hz, H-2″ and H-2‴), 5.07 (2H, dd, $J_{3'',4''}$ = $J_{3''',4'''}$ = 3.6 Hz, H-3″ and H-3‴), 5.52 (2H, dd, $J_{4'',5''}$ = $J_{4''',5'''}$ = 3.2 Hz, H-4″ and H-4‴), 3.88 (2H, m, H-5″ and H-5‴), 4.18 (2H, dd, $J_{5'',6a''}$ = $J_{5''',6a'''}$ = 3.6 Hz, $J_{6a'',6b''}$ = $J_{6a''',6b'''}$ = 9.2 Hz, H-6a″ and H-6a‴), 4.11 (2H, dd, $J_{5'',6b''}$ = $J_{5''',6b'''}$ = 6.8 Hz, H-6b″ and H-6b‴), 4.48 (2H, d, $J_{CH2-4a,CH2-4b}$ = $J_{CH2-5a,CH2-5b}$ = 13.2 Hz, CH_2-4a and CH_2-5a), 4.12 (2H, d, $J_{CH2-4a,CH2-4b}$ = $J_{CH2-5a,CH2-5b}$ = 13.2 Hz, CH_2-4b and CH_2-5b), 2.43 (3H, s, CH_3-2), 2.18, 2.17, 2.16, 2.15, 2.12, 2.11, 2.10, 2.09, 2.08, 2.07, 2.06, 2.05 (36H, 12s, 12 CH_3CO). δ_C 170.84, 170.79, 170.77, 170.73, 170.68, 170.54, 170.53, 170.49, 170.45, 170.35, 170.31, 170.28 (12 CH_3**C**O), 146.47 (d, $^3J_{F-C}$ = 14.5 Hz, Py-C_2), 148.16 (d, $^4J_{F-C}$ = 3.8 Hz, Py-C_3), 133.10 (s, Py-C_4), 112.51 (d, $^2J_{F-C}$ = 31.3 Hz, Py-C_5), 155.04 (d, $^1J_{F-C}$ = 231.2 Hz, Py-C_6), 103.34 (s, C-1′), 100.22 (s, C-1″ and

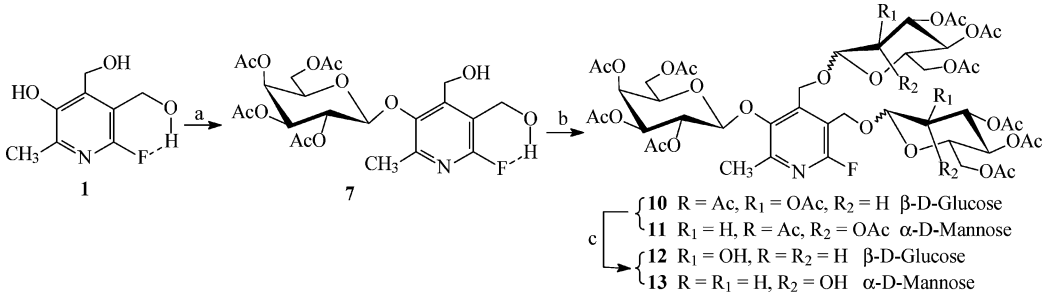

Figure 5. Reagents and conditions: (a) 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide **2**, $CH_2Cl_2-H_2O$, pH 10∼11, RT, TBAB, 4∼5 h, 88%; (b) 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide **8** or 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide **9**, $Hg(CN)_2$, 4 Å molecular sieve, CH_2Cl_2, RT, 12 h, 80% (→ **10**) or 78% (→ **11**), respectively; (c) NH_3/MeOH, 0 °C → RT, 24 h, 95% (→ **12**) or 94% (→ **13**), respectively.

Table 1. ^{19}F Chemical Shifts[a] and Hydrolytic Rates[b]

reporters	4	12	13	GFPOL
$\delta_{F(substrate)}$	−3.02	−2.85	−2.14	−3.22
$\delta_{F(product)}$	−12.37	−12.16	−11.22	−11.21
$\Delta\delta_F$	9.35	9.31	9.08	7.99
v (μmol min^{-1} unit^{-1})	34.0	35.0	38.0	4.3

[a] Chemical shifts are given in parts per million (ppm) with respect to sodium trifluoroacetate. [b] β-gal (E801A) was added at 37 °C in PBS (0.1 M, pH = 7.4).

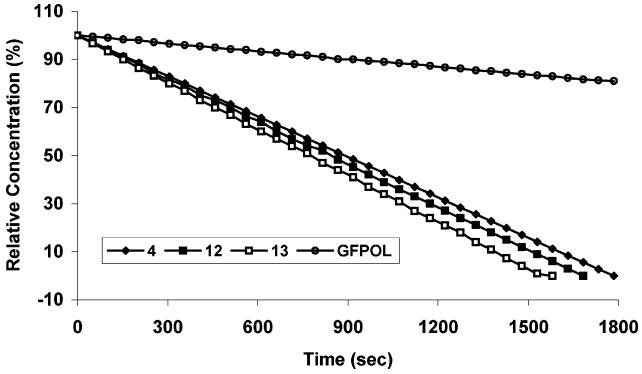

Figure 6. Kinetic hydrolysis time courses of **4** (◆), **12** (■), **13** (□) (15.0 mmol each), and GFPOL (○) (10.0 mmol) by β-gal (E801A, 15 units) hydrolysis in PBS (0.1 M, pH = 7.4, 600 μL) at 37 °C.

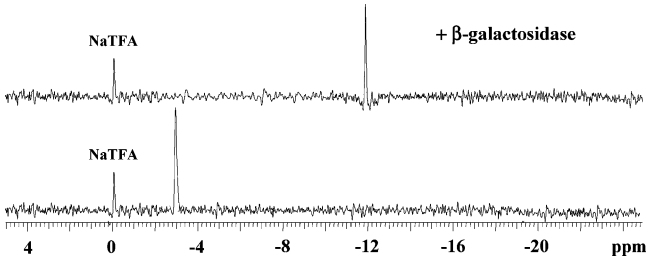

Figure 7. ^{19}F NMR spectra of 3-*O*-(β-D-galactopyranosyl)-α^4,α^5-di-*O*-(β-D-glucopyranosyl)-6-fluoropyridoxol **12** (10.1 mg, 15 mmol, lower trace) and its hydrolysis by β-gal (E801A, 15 units) in PBS (0.1 M, pH = 7.4, 600 μL) at 37 °C (upper trace). Spectra were acquired in 205 s and enhanced with exponential line broadening = 40 Hz.

C-1‴), 70.75 (s, C-2′), 71.14 (s, C-3′), 70.61 (s, C-4′), 71.56 (s, C-5′), 67.03 (s, C-6′), 67.45 (s, C-2″ and C-2‴), 68.39 (s, C-3″ and C-3‴), 66.31 (s, C-4″ and C-4‴), 68.55 (s, C-5″ and C-5‴), 61.99 (s, C-6″ and C-6‴), 61.54 (s, CH_2-4), 61.67 (s, CH_2-5), 21.03, 20.94, 20.90, 20.89, 20.87, 20.85, 20.83, 20.79, 20.77, 20.76, 20.74, 20.72 (12s, 12 CH_3CO), 18.77 (s, CH_3-3). ESI-MS *m*/*z* 1178 [M$^+$] (26%), 1179 [M + 1] (14%). Anal. Calcd for $C_{50}H_{64}NO_{30}F$: C, 50.96, H, 5.48, N, 1.19. Found: C, 50.93, H, 5.46, N, 1.15.

3,α^4,α^5-Tri-*O*-(β-D-galactopyranosyl)-6-fluoropyridoxol 4. A solution of **3** (0.9 g) in anhydrous MeOH (20 mL) containing 0.5 M NH_3 was vigorously stirred from 0 °C to RT overnight, until TLC showed complete reaction, and then evaporated to dryness in vacuo. Chromatography of the crude syrup on silica gel with EtOAc/MeOH (4:1) afforded **4** (0.52 g) as a syrup in quantitative yield, R_f 0.10 (1:4 MeOH/EtOAc). NMR (DMSO-d_6) δ_H 4.95 (1H, d, $J_{1',2'}$ = 8.2 Hz, H-1′), 4.76 (1H, dd, $J_{2',3'}$ = 10.0 Hz, H-2′), 4.91 (1H, dd, $J_{3',4'}$ = 2.8 Hz, H-3′), 5.11 (1H, dd, $J_{4',5'}$ = 2.3 Hz, H-4′), 3.77 (1H, m, H-5′), 3.90 (1H, dd, $J_{5',6a'}$ = 6.4 Hz, $J_{6a',6b'}$ = 12.4 Hz,

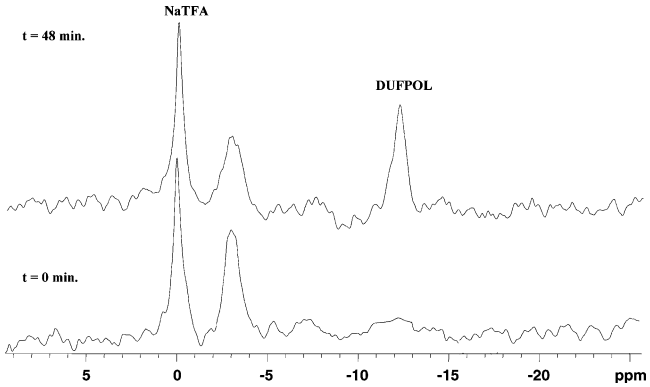

Figure 8. ^{19}F NMR spectra of 3-*O*-(β-D-galactopyranosyl)-α^4,α^5-di-*O*-(β-D-glucopyranosyl)-6-fluoropyridoxol **12** (5.1 mg, 7.5 mmol) with stably transfected MCF7-*lacZ* cells (5 × 10^6) in PBS (0.1 M, pH = 7.4, 600 μL) at 37 °C. Spectra were acquired in 51 s and enhanced with an exponential line broadening = 100 Hz. [DUFPOL = α^4,α^5-di-*O*-(β-D-glucopyranosyl)-6-fluoropyridoxol.]

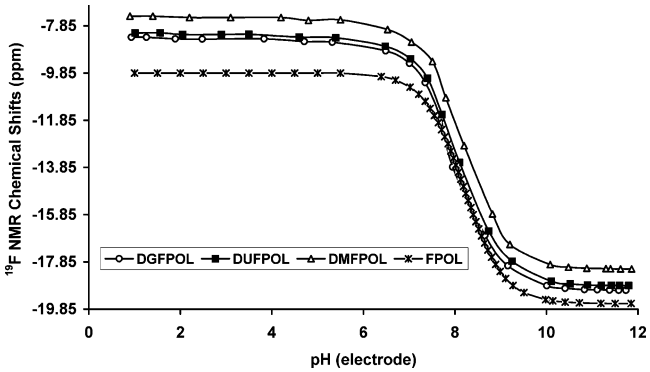

Figure 9. ^{19}F NMR chemical shift pH titration curve of DGFPOL, DUFPOL, and DMFPOL in 0.9% saline at 37 °C. [DGFPOL = α^4,α^5-di-*O*-(β-D-galactopyranosyl)-6-fluoropyridoxol; DUFPOL = α^4,α^5-di-*O*-(β-D-glucopyranosyl)-6-fluoropyridoxol; DMFPOL = α^4,α^5-di-*O*-(α-D-mannopyranosyl)-6-fluoropyridoxol.]

Table 2. Acidities and ^{19}F NMR/pH Properties of DGFPOL, DUFPOL, DMFPOL, and FPOL in Saline at 25 °C[a]

pH indicators	DGFPOL	DUFPOL	DMFPOL	FPOL[24]
pK_a	7.95	8.08	8.18	8.20
δ_{Facid}	−8.34	−8.15	−7.44	−9.85
δ_{Fbase}	−19.05	−18.85	−18.15	−19.61

[a] Chemical shifts are given in parts per million (ppm) with respect to sodium trifluoroacetate.

H-6a′), 3.68 (1H, dd, $J_{5',6b'} = 3.6$ Hz, H-6b′), 4.22 (2H, d, $J_{1'',2''} = J_{1''',2'''} = 8.0$ Hz, H-1″ and H-1‴), 3.29 (2H, dd, $J_{2'',3''} = J_{2''',3'''} = 10.6$ Hz, H-2″ and H-2‴), 3.51 (2H, dd, $J_{3'',4''} = J_{3''',4'''} = 3.2$ Hz, H-3″ and H-3‴), 3.62 (2H, dd, $J_{4'',5''} = J_{4''',5'''} = 2.4$ Hz, H-4″ and H-4‴), 3.46 (2H, m, H-5″ and H-5‴), 3.66 (2H, dd, $J_{5'',6a''} = J_{5''',6a'''} = 3.6$ Hz, $J_{6a'',6b''} = J_{6a''',6b'''} = 10.4$ Hz, H-6a″ and H-6a‴), 3.39 (2H, dd, $J_{5'',6b''} = J_{5''',6b'''} = 6.6$ Hz, H-6b″ and H-6b‴), 4.48 (2H, d, $J_{CH2-4a,CH2-4b} = J_{CH2-5a,CH2-5b} = 13.0$ Hz, CH_2-4a and CH_2-5a), 4.44 (2H, d, $J_{CH2-4a,CH2-4b} = J_{CH2-5a,CH2-5b} = 13.0$ Hz, CH_2-4b and CH_2-5b), 2.32 (3H, s, CH_3-2). δ_C 144.65 (d, $^3J_{F-C} = 14.5$ Hz, Py-C_2), 147.87 (d, $^4J_{F-C} = 3.9$ Hz, Py-C_3), 137.36 (d, $^4J_{F-C} = 3.8$ Hz, Py-C_4), 115.15 (d, $^2J_{F-C} = 32.3$ Hz, Py-C_5), 154.26 (d, $^1J_{F-C} = 226.6$ Hz, Py-C_6), 103.19 (s, C-1′), 101.67 (s, C-1″ and C-1‴), 70.36 (s, C-2′), 73.94 (s, C-3′), 69.44 (s, C-4′), 76.08 (s, C-5′), 62.88 (s, C-6′), 72.10 (s, C-2″ and C-2‴), 73.50 (s, C-3″ and C-3‴), 68.26 (s, C-4″ and C-4‴), 75.02 (s, C-5″ and C-5‴), 60.60 (s, C-6″ and C-6‴), 68.77 (s, CH_2-4), 68.92 (s, CH_2-5), 19.19 (s, CH_3-3). ESI-MS *m/z* 673 [M^+] (6%), 674 [M + 1] (10%). Anal. Calcd for $C_{26}H_{40}NO_{18}F$: C, 46.34, H, 5.99, N, 2.08. Found: C, 46.30, H, 5.96, N, 2.05.

α⁴,α⁵-*O*-Isopropylidene-6-fluoropyridoxol 5. A suspension of **1** (0.50 g, 2.67 mmol) in anhydrous acetone (40 mL) containing 2% concentrated H_2SO_4 was stirred for 4∼5 h, at the end of which time TLC (4:1 cyclohexane/EtOAc) indicated complete reaction, and then cold saturated Na_2CO_3 solution was added with vigorous stirring up to pH between 8 and 9. The precipitate was filtered off, and concentration of the reaction mixture under reduced pressure followed by purification on flash silica gel column (4:1 cyclohexane/EtOAc) gave **5** (0.64 g, 26%) as a syrup, R_f 0.34 (4:1 cyclohexane/EtOAc), NMR ($CDCl_3$) δ_H 7.45 (1H, s, HO-3), 5.03 (2H, s, CH_2-5), 4.57 (2H, s, CH_2-4), 2.33 (3H, s, CH_3-2), 1.55 (6H, s, 2 CH_3). δ_C 146.40 (d, $^3J_{F-C} = 14.5$ Hz, Py-C_2), 144.32 (d, $^4J_{F-C} = 3.8$ Hz, Py-C_3), 130.68 (s, Py-C_4), 111.21 (d, $^2J_{F-C} = 32.8$ Hz, Py-C_5), 152.20 (d, $^1J_{F-C} = 231.2$ Hz, Py-C_6), 100.15 (s, **C**Me_2), 58.70 (d, $^3J_{F-C} = 3.0$ Hz, CH_2-5), 54.51 (s, CH_2-4), 31.62 [s, C(**C**H_3)$_2$], 17.58 (s, CH_3-2). Anal. Calcd for $C_{11}H_{14}NO_3F$: C, 58.13, H, 6.21, N, 6.17. Found: C, 58.08, H, 6.16, N, 6.11.

3-*O*-(2,3,4,6-Tetra-*O*-acetyl-*β*-D-galactopyranosyl)-α⁴,α⁵-*O*-isopropylidene-6-fluoropyridoxol 6. To a solution of **5** (0.62 g, 2.72 mmol) and $Hg(CN)_2$ (0.88 g, 3.50 mmol) in dry CH_2Cl_2 (10 mL) containing freshly activated 4 Å molecular sieves (2.0 g) was added dropwise compound **2** (1.23 g, 3.0 mmol, 1.1 equiv). The mixture was stirred overnight in the dark at RT under N_2 until TLC indicated complete reaction. Workup as for **3** gave **6** (1.29 g, 85%), R_f 0.40 (2:3 cyclohexane/EtOAc). NMR ($CDCl_3$) δ_H 4.64 (1H, d, $J_{1',2'} = 8.0$ Hz, H-1′), 5.25 (1H, dd, $J_{2',3'} = 10.0$ Hz, H-2′), 5.02 (1H, dd, $J_{3',4'} = 3.6$ Hz, H-3′), 5.41 (1H, dd, $J_{4',5'} = 3.2$ Hz, H-4′), 3.97 (1H, m, H-5′), 4.21 (1H, dd, $J_{5',6a'} = 4.4$ Hz, $J_{6a',6b'} = 11.2$ Hz, H-6a′), 4.13 (1H, dd, $J_{5',6b'} = 7.2$ Hz, H-6b′), 5.10 (1H, d, $J_{CH2-4a,CH2-4b} = 8.0$ Hz, CH_2-4a), 4.67 (1H, d, $J_{CH2-4a,CH2-4b} = 8.0$ Hz, CH_2-4b), 5.14 (1H, d, $J_{CH2-5a,CH2-5b} = 9.6$ Hz, CH_2-5a), 5.12 (1H, d, $J_{CH2-5a,CH2-5b} = 9.6$ Hz, CH_2-5b), 2.42 (3H, s, CH_3-2), 2.17, 2.09, 2.08, 1.99 (12H, 4s, 4 CH_3CO), 1.61, 1.59 (6H, 2s, 2 CH_3). δ_C 170.78, 170.39, 170.26, 170.11 (4s, 4 CH_3**C**O), 145.48 (d, $^3J_{F-C} = 15.2$ Hz, Py-C_2), 133.16 (d, $^4J_{F-C} = 4.0$ Hz, Py-C_3), 126.26 (s, Py-C_4), 116.95 (d, $^2J_{F-C} = 32.1$Hz, Py-C_5), 154.30 (d, $^1J_{F-C} = 229.0$ Hz, Py-C_6), 101.41 (s, **C**Me_2), 100.03 (s, C-1′), 68.70 (s, C-2′), 70.82 (s, C-3′), 67.12 (s, C-4′), 71.53 (s, C-5′), 64.28 (s, C-6′), 55.38 (s, CH_2-4), 61.58 (s, CH_2-5), 31.88 (s, C(**C**H_3)$_2$), 20.90, 20.89, 20.82, 20.77 (4s, 4 CH_3CO), 18.77 (s, CH_3-2). Anal. Calcd for $C_{25}H_{32}NO_{12}F$: C, 53.84, H, 5.79, N, 2.51. Found: C, 53.79, H, 5.74, N, 2.49.

3-*O*-(2,3,4,6-Tetra-*O*-acetyl-*β*-D-galactopyranosyl)-6-fluoropyridoxol 7. A mixture of **6** (1.25 g, 2.50 mmol) in 80% AcOH (40 mL) was stirred at 80 °C for 4∼5 h, till TLC (1:3 cyclohexane/EtOAc) showed complete reaction. The cooled mixture was neutralized with cold saturated Na_2CO_3 solution, extracted with EtOAc (4 × 30 mL), and concentrated and purified by flash silica gel column with 1:4 cyclohexane/EtOAc, giving **7** (0.17 g, 15%) as a syrup, R_f 0.18 (1:4 cyclohexane/EtOAc). NMR ($CDCl_3$) δ_H 4.79 (1H, d, $J_{1',2'} = 8.0$ Hz, H-1′), 5.55 (1H, dd, $J_{2',3'} = 10.6$ Hz, H-2′), 5.10 (1H, dd, $J_{3',4'} = 3.6$ Hz, H-3′), 5.41 (1H, dd, $J_{4',5'} = 3.6$ Hz, H-4′), 3.88 (1H, m, H-5′), 4.24 (1H, dd, $J_{5',6a'} = 4.4$ Hz, $J_{6a',6b'} = 12.0$ Hz, H-6a′), 4.09 (1H, dd, $J_{5',6b'} = 6.0$ Hz, H-6b′), 5.01 (2H, d, $J_{CH2-4a,CH2-4b} = J_{CH2-5a,CH2-5b} = 12.4$ Hz, CH_2-4a and CH_2-5a), 4.62 (1H, d, $J_{CH2-4a,CH2-4b} = 12.4$ Hz, CH_2-4b), 4.66 (1H, d, $J_{CH2-5a,CH2-5b} = 12.4$ Hz, CH_2-5b), 3.50 (1H, m, α⁴-HO, exchangeable with D_2O), 3.56 (1H, m, α⁵-HO, exchangeable with D_2O), 2.47 (3H, s, CH_3-2), 2.23, 2.17, 2.02, 2.00 (12H, 4s, 4 CH_3CO). δ_C 170.32, 170.28, 170.18, 169.48 (4 CH_3**C**O), 150.33 (d, $^3J_{F-C} = 15.2$ Hz, Py-C_2), 147.62 (d, $^4J_{F-C} = 4.6$ Hz, Py-C_3), 146.32 (d, $^3J_{F-C} = 4.5$ Hz, Py-C_4), 120.17 (d, $^2J_{F-C} = 32.0$ Hz, Py-C_5), 157.60 (d, $^1J_{F-C} = 235.8$ Hz, Py-C_6), 102.39 (s, C-1′), 68.91 (s, C-2′), 70.74 (s, C-3′), 67.19 (s, C-4′), 71.93 (s, C-5′), 61.98 (s, C-6′), 55.91 (s, **C**H_2-4), 59.60 (s, **C**H_2-5), 20.99, 20.85, 20.70, 20.67 (4s, 4 CH_3CO), 19.46 (s, CH_3-2). Anal. Calcd for $C_{22}H_{28}NO_{12}F$: C, 51.05, H, 5.46, N, 2.71. Found: C, 51.00, H, 5.39, N, 2.68.

Alternately **7** was synthesized from **1** directly by phase transfer catalysis: to a well-stirred CH_2Cl_2 (10 mL)/H_2O (10 mL) biphasic mixture (pH 10∼11) of **1** (0.5 g, 2.67 mmol) and TBAB (0.1 g, 0.31 mmol), a solution of **2** (1.21 g, 2.94 mmol, 1.1 equiv) in CH_2Cl_2 (10 mL) was added dropwise over a period of 4∼5 h at RT, and the stirring continued for an additional hour. The products were extracted (EtOAc; 4 × 20 mL), washed free of alkali, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel (1:4 cyclohexane/EtOAc) to afford **7** (1.08 g, 88%) as syrup, which is identical in all respects to the product obtained above.

3-*O*-(2,3,4,6-Tetra-*O*-acetyl-*β*-D-galactopyranosyl)-α⁴,α⁵-di-*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-6-fluoropyridoxol 10 and 3-*O*-(2,3,4,6-Tetra-*O*-acetyl-*β*-D-galactopyranosyl)-α⁴,α⁵-di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-6-fluoropyridoxol 11. Condensation of **7** (0.5 g, 1.1 mmol) with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide **8** or 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide **9** (1.0 g, 2.40 mmol, 1.1 equiv) in dry CH_2Cl_2 (10 mL) with $Hg(CN)_2$ (0.63 g, 2.50 mmol) as a promoter, according to the procedures described for the preparation of **3** and **6**, furnished 3-*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl)-α⁴,α⁵-di-*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-6-fluoropyridoxol **10** and 3-*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl)-α⁴,α⁵-di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-6-fluoropyridoxol **11**, respectively.

3-*O*-(2,3,4,6-Tetra-*O*-acetyl-*β*-D-galactopyranosyl)-α⁴,α⁵-di-*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-6-fluoropyridoxol 10: 1.04 g, 80%, syrup, R_f 0.30 (1:3 cyclohexane/EtOAc). NMR ($CDCl_3$) δ_H 5.06 (1H, d, $J_{1',2'} = 7.8$ Hz, H-1′), 5.28 (1H, dd, $J_{2',3'} = 8.8$ Hz, H-2′), 4.98 (1H, dd, $J_{3',4'} = 4.8$ Hz, H-3′), 4.73 (1H, dd, $J_{4',5'} = 2.8$ Hz, H-4′), 3.95 (1H, m, H-5′), 4.19 (1H, dd, $J_{5',6a'} = 3.6$ Hz, $J_{6a',6b'} = 10.8$ Hz, H-6a′), 4.02 (1H, dd, $J_{5',6b'} = 5.2$ Hz, H-6b′), 5.36 (1H, d, $J_{1'',2''} = 8.0$ Hz, H-1″), 5.39 (1H, d, $J_{1''',2'''} = 8.0$ Hz, H-1‴), 5.12 (1H, dd, $J_{2'',3''} = 7.2$ Hz, H-2″), 5.15 (1H, dd, $J_{2''',3'''} = 6.8$ Hz, H-2‴), 5.04 (1H, dd, $J_{3'',4''} = 3.2$ Hz, H-3″), 5.07 (1H, dd, $J_{3''',4'''} = 3.6$ Hz, H-3‴), 4.76 (1H, dd, $J_{4'',5''} = 2.8$ Hz, H-4″), 4.78 (1H, dd, $J_{4''',5'''} = 2.8$ Hz, H-4‴), 3.91 (1H, m, H-5″), 3.93 (1H, m, H-5‴), 4.08 (1H, dd, $J_{5'',6a''} = 3.2$ Hz, $J_{6a'',6b''} = 9.4$ Hz, H-6a″), 4.10 (1H, dd, $J_{5''',6a'''} = 3.0$ Hz, $J_{6a''',6b'''} = 10.0$ Hz, H-6a‴), 4.04 (1H, dd, $J_{5'',6b''} = 7.6$ Hz, H-6b″), 4.07 (1H, dd, $J_{5''',6b'''} = 6.8$ Hz, H-6b‴), 4.55 (2H, d, $J_{CH2-4a,CH2-4b} = J_{CH2-5a,CH2-5b} = 11.2$ Hz, CH_2-4a and CH_2-5b), 4.49 (1H, d, $J_{CH2-4a,CH2-4b} = 11.2$ Hz, CH_2-4b), 4.91 (1H, d, $J_{CH2-5a,CH2-5b} = 11.2$ Hz, CH_2-5a), 2.34 (3H, s, CH_3-2), 2.05, 1.98, 1.97, 1.96, 1.95, 1.94, 1.93, 1.92, 1.91, 1.90, 1.89, 1.88 (36H, 12s, 12 CH_3CO). δ_C 170.83, 170.80, 170.76, 170.72, 170.70, 170.56, 170.28, 170.20, 170.17, 170.00, 169.82, 169.75 (12 CH_3CO), 152.14 (d, $^3J_{F-C} = 16.0$ Hz, Py-C_2), 149.81 (s, Py-C_3), 138.42 (d, $^3J_{F-C} = 11.4$ Hz, Py-C_4), 117.48 (d, $^2J_{F-C} = 32.0$ Hz, Py-C_5), 157.56 (d, $^1J_{F-C} = 233.5$ Hz, Py-C_6), 102.66 (s, C-1′), 98.00 (s, C-1″), 98.06 (s, C-1‴), 71.09 (s, C-2′), 68.65 (s, C-2″), 68.95 (s, C-2‴), 74.46 (s, C-3′), 70.84 (s, C-3″), 71.51 (s, C-3‴), 70.05 (s, C-4′), 68.13 (s, C-4″), 68.22 (s, C-4‴), 75.10 (s, C-5′), 72.20 (s, C-5″), 74.24 (s, C-5‴), 63.75 (s, C-6′), 61.91 (s, C-6″), 63.86 (s, C-6‴), 56.77 (s, CH_2-4), 57.16 (s, CH_2-5), 21.20, 20.95, 20.93, 20.91, 20.89, 20.87, 20.85, 20.75, 20.67, 20.62, 20.58,

20.54 (12s, 12 CH_3CO), 19.76 (s, CH_3-3). ESI-MS *m*/*z* 1178 [M^+] (28%), 1179 [M + 1] (12%). Anal. Calcd for $C_{50}H_{64}NO_{30}F$: C, 50.96, H, 5.48, N, 1.19. Found: C, 50.92, H, 5.44, N, 1.16.

3-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-α⁴,α⁵-di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-6-fluoropyridoxol 11: 1.01 g, 78%, syrup, R_f 0.35 (1:3 cyclohexane/EtOAc). NMR ($CDCl_3$) δ_H 4.80 (1H, d, $J_{1',2'}$ = 8.2 Hz, H-1′), 5.13 (1H, dd, $J_{2',3'}$ = 9.8 Hz, H-2′), 5.36 (1H, dd, $J_{3',4'}$ = 4.2 Hz, H-3′), 5.30 (1H, dd, $J_{4',5'}$ = 3.6 Hz, H-4′), 4.01 (1H, m, H-5′), 4.33 (1H, dd, $J_{5',6a'}$ = 3.2 Hz, $J_{6a',6b'}$ = 10.0 Hz, H-6a′), 4.11 (1H, dd, $J_{5',6b'}$ = 4.6 Hz, H-6b′), 4.71 (2H, d, $J_{1'',2''} = J_{1''',2'''}$ = 2.4 Hz, H-1″ and H-1‴), 4.74 (2H, dd, $J_{2'',3''} = J_{2''',3'''}$ = 6.2 Hz, H-2″ and H-2‴), 5.22 (2H, dd, $J_{3'',4''} = J_{3''',4'''}$ = 3.8 Hz, H-3″ and H-3‴), 3.95 (2H, dd, $J_{4'',5''} = J_{4''',5'''}$ = 2.0 Hz, H-4″ and H-4‴), 4.02 (2H, m, H-5″ and H-5‴), 4.11 (2H, dd, $J_{5'',6a''} = J_{5''',6a'''}$ = 2.0 Hz, $J_{6a'',6b''} = J_{6a''',6b'''}$ = 7.4 Hz, H-6a″ and H-6a‴), 4.07 (2H, dd, $J_{5'',6b''} = J_{5''',6b'''}$ = 5.6 Hz, H-6b″ and H-6b‴), 4.87 (2H, d, $J_{CH2-4a,CH2-4b} = J_{CH2-5a,CH2-5b}$ = 13.6 Hz, CH_2-4a and CH_2-5b), 4.67 (1H, d, $J_{CH2-4a,CH2-4b} = J_{CH2-5a,CH2-5b}$ = 13.6 Hz, CH_2-4b and CH_2-5a), 2.33 (3H, s, CH_3-2), 2.07, 2.04, 2.03, 2.00, 1.99, 1.98, 1.97, 1.96, 1.95, 1.94, 1.93, 1.92 (36H, 12s, 12 CH_3CO). δ_C 171.27, 171.23, 171.15, 171.06, 170.87, 170.83, 170.76, 170.63, 170.58, 170.44, 170.29, 170.25 (12 CH_3**C**O), 153.06 (d, $^3J_{F-C}$ = 16.0 Hz, Py-C_2), 149.41 (d, $^4J_{F-C}$ = 4.6 Hz, Py-C_3), 145.38 (d, $^3J_{F-C}$ = 4.6 Hz, Py-C_4), 117.21 (d, $^2J_{F-C}$ = 31.3 Hz, Py-C_5), 159.55 (d, $^1J_{F-C}$ = 235.0 Hz, Py-C_6), 103.62 (s, C-1′), 98.32 (s, C-1″), 98.61 (s, C-1‴), 70.75 (s, C-2′), 70.22 (s, C-2″), 70.26 (s, C-2‴), 71.83 (s, C-3′), 70.36 (s, C-3″), 70.39 (s, C-3‴), 69.38 (s, C-4′), 67.04 (s, C-4″), 68.60 (s, C-4‴), 72.54 (s, C-5′), 71.90 (s, C-5″), 72.45 (s, C-5‴), 61.47 (s, C-6′), 62.46 (s, C-6″), 63.53 (s, C-6‴), 56.44 (s, CH_2-4), 56.46 (s, CH_2-5), 21.29, 21.21, 21.19, 21.17, 21.13, 21.10, 21.08, 21.05, 21.00, 20.95, 20.90, 20.88 (12s, 12 CH_3CO), 20.35 (s, CH_3-3). ESI-MS *m*/*z* 1178 [M^+] (20%), 1179 [M + 1] (17%). Anal. Calcd for $C_{50}H_{64}NO_{30}F$: C, 50.96, H, 5.48, N, 1.19. Found: C, 50.94, H, 5.45, N, 1.16.

3-*O*-(β-D-Galactopyranosyl)-α⁴,α⁵-di-*O*-(β-D-glucopyranosyl)-6-fluoropyridoxol 12 and 3-*O*-(β-D-Galactopyranosyl)-α⁴,α⁵-di-*O*-(α-D-mannopyranosyl)-6-fluoropyridoxol 13. Compounds **10** and **11** (1.00 g, 0.85 mmol) were deacetylated as described above for **4** to yield **12** and **13** in quantitative yields.

3-*O*-(β-D-Galactopyranosyl)-α⁴,α⁵-di-*O*-(β-D-glucopyranosyl)-6-fluoropyridoxol 12: 0.57 g, foam solid, R_f 0.20 (1:4 MeOH/EtOAc). NMR (DMSO-d_6) δ_H 5.01 (1H, d, $J_{1',2'}$ = 8.2 Hz, H-1′), 5.22 (1H, dd, $J_{2',3'}$ = 9.0 Hz, H-2′), 4.92 (1H, dd, $J_{3',4'}$ = 4.6 Hz, H-3′), 4.70 (1H, dd, $J_{4',5'}$ = 2.6 Hz, H-4′), 3.91 (1H, m, H-5′), 4.12 (1H, dd, $J_{5',6a'}$ = 3.2 Hz, $J_{6a',6b'}$ = 10.2 Hz, H-6a′), 4.00 (1H, dd, $J_{5',6b'}$ = 5.6 Hz, H-6b′), 5.14 (2H, d, $J_{1'',2''}$ = 10.0 Hz, H-1″ and H-1‴), 4.82 (2H, dd, $J_{2'',3''} = J_{2''',3'''}$ = 8.2 Hz, H-2″ and H-2‴), 4.69 (2H, dd, $J_{3'',4''} = J_{3''',4'''}$ = 3.4 Hz, H-3″ and H-3‴), 4.93 (2H, dd, $J_{4'',5''} = J_{4''',5'''}$ = 3.2 Hz, H-4″ and H-4‴), 3.65 (2H, m, H-5″ and H-5‴), 3.55 (2H, dd, $J_{5'',6a''} = J_{5''',6a'''}$ = 4.8 Hz, $J_{6a'',6b''} = J_{6a''',6b'''}$ = 12.0 Hz, H-6a″ and H-6a‴), 3.31 (2H, dd, $J_{5'',6b''} = J_{5''',6b'''}$ = 5.6 Hz, H-6b″ and H-6b‴), 4.29 (1H, d, $J_{CH2-4a,CH2-4b}$ = 7.6 Hz, CH_2-4a), 4.36 (1H, d, $J_{CH2-4a,CH2-4b}$ = 7.6 Hz, CH_2-5a), 4.20 (2H, d, $J_{CH2-4a,CH2-4b} = J_{CH2-5a,CH2-5b}$ = 7.6 Hz, CH_2-4b and CH_2-5b), 4.18∼3.65 (12H, br, HO-2′, 3′, 4′, 6′, 2″, 3″, 4″, 6″, 2‴, 3‴, 4‴, and 6‴, exchangeable with D_2O), 2.42 (3H, s, CH_3-2). δ_C 144.43 (d, $^3J_{F-C}$ = 15.0 Hz, Py-C_2), 136.26 (d, $^4J_{F-C}$ = 3.8 Hz, Py-C_3), 124.40 (d, $^3J_{F-C}$ = 3.8 Hz, Py-C_4), 120.39 (d, $^2J_{F-C}$ = 32.8 Hz, Py-C_5), 148.98 (d, $^1J_{F-C}$ = 259.7 Hz, Py-C_6), 103.65 (s, C-1′), 101.76 (s, C-1″ and C-1‴), 72.34 (s, C-2′), 71.28 (s, C-2″ and C-2‴), 74.55 (s, C-3′), 73.88 (s, C-3″ and C-3‴), 69.82 (s, C-4′), 68.89 (s, C-4″ and C-4‴), 76.62 (s, C-5′), 77.29 (s, C-5″ and C-5‴), 61.36 (s, C-6′), 60.95 (s, C-6″ and C-6‴), 60.54 (s, CH_2-4), 60.78 (s, CH_2-5), 19.88 (s, CH_3-3). ESI-MS *m*/*z* 673 [M^+] (8%), 674 [M + 1] (14%). Anal. Calcd for $C_{26}H_{40}NO_{18}F$: C, 46.34, H, 5.99, N, 2.08. Found: C, 46.32, H, 5.97, N, 2.07.

3-*O*-(β-D-Galactopyranosyl)-α⁴,α⁵-di-*O*-(α-D-mannopyranosyl)-6-fluoropyridoxol 13: 0.57 g, foam solid, R_f 0.26 (1:4 MeOH/EtOAc). NMR (DMSO-d_6) δ_H 5.00 (1H, d, $J_{1',2'}$ = 8.0 Hz, H-1′), 5.23 (1H, dd, $J_{2',3'}$ = 10.0 Hz, H-2′), 5.16 (1H, dd, $J_{3',4'}$ = 3.8 Hz, H-3′), 5.08 (1H, dd, $J_{4',5'}$ = 3.2 Hz, H-4′), 4.21 (1H, m, H-5′), 4.51 (1H, dd, $J_{5',6a'}$ = 3.6 Hz, $J_{6a',6b'}$ = 10.2 Hz, H-6a′), 4.31 (1H, dd, $J_{5',6b'}$ = 4.8 Hz, H-6b′), 4.84 (2H, d, $J_{1'',2''} = J_{1''',2'''}$ = 2.6 Hz, H-1″ and H-1‴), 4.68 (2H, dd, $J_{2'',3''} = J_{2''',3'''}$ = 6.0 Hz, H-2″ and H-2‴), 5.02 (2H, dd, $J_{3'',4''} = J_{3''',4'''}$ = 3.6 Hz, H-3″ and H-3‴), 4.05 (2H, dd, $J_{4'',5''} = J_{4''',5'''}$ = 2.2 Hz, H-4″ and H-4‴), 3.94 (2H, m, H-5″ and H-5‴), 4.21 (2H, dd, $J_{5'',6a''} = J_{5''',6a'''}$ = 2.4 Hz, $J_{6a'',6b''} = J_{6a''',6b''}$ = 8.4 Hz, H-6a″ and H-6a‴), 4.17 (2H, dd, $J_{5'',6b''} = J_{5''',6b'''}$ = 6.5 Hz, H-6b″ and H-6b‴), 4.77 (2H, d, $J_{CH2-4a,CH2-4b} = J_{CH2-5a,CH2-5b}$ = 11.6 Hz, CH_2-4a and CH_2-5b), 4.57 (1H, d, $J_{CH2-4a,CH2-4b} = J_{CH2-5a,CH2-5b}$ = 11.6 Hz, CH_2-4b and CH_2-5a), 2.45 (3H, s, CH_3-2), 4.30∼3.70 (12H, br, HO-2′, 3′, 4′, 6′, 2″, 3″, 4″, 6″, 2‴, 3‴, 4‴, and 6‴, exchangeable with D_2O). δ_C 151.07 (d, $^3J_{F-C}$ = 15.3 Hz, Py-C_2), 148.61 (d, $^4J_{F-C}$ = 4.8 Hz, Py-C_3), 144.27 (d, $^3J_{F-C}$ = 3.6 Hz, Py-C_4), 116.29 (d, $^2J_{F-C}$ = 32.0 Hz, Py-C_5), 157.25 (d, $^1J_{F-C}$ = 233.5 Hz, Py-C_6), 103.68 (s, C-1′), 98.56 (s, C-1″), 98.68 (s, C-1‴), 71.76 (s, C-2′), 70.66 (s, C-2″), 70.86 (s, C-2‴), 72.38 (s, C-3′), 71.46 (s, C-3″), 71.32 (s, C-3‴), 70.48 (s, C-4′), 66.87 (s, C-4″), 67.90 (s, C-4‴), 73.64 (s, C-5′), 72.20 (s, C-5″), 72.65 (s, C-5‴), 62.77 (s, C-6′), 63.56 (s, C-6″), 64.83 (s, C-6‴), 57.54 (s, CH_2-4), 58.41 (s, CH_2-5), 20.12 (s, CH_3-3). ESI-MS *m*/*z* 673 [M^+] (5%), 674 [M + 1] (9%). Anal. Calcd for $C_{26}H_{40}NO_{18}F$: C, 46.34, H, 5.99, N, 2.08. Found: C, 46.31, H, 5.97, N, 2.05.

3-*O*-Benzyl-6-fluoropyridoxol 14. To a well-stirred CH_2Cl_2 (10 mL)/H_2O (10 mL) biphasic mixture (pH 10∼11) of **1** (0.50 g, 2.67 mmol) and TBAB (0.10 g, 0.31 mmol), a solution of benzyl bromide (0.51 g, 2.94 mmol, 1.1 equiv) in CH_2Cl_2 (10 mL) was added dropwise over a period of 4∼5 h, while the reaction temperature was maintained at 50 °C, and the stirring continued for an additional hour. Products were extracted (CH_2Cl_2, 4 × 20 mL), washed free of alkali, dried (Na_2SO_4), and concentrated, and the residue was purified by column chromatography on silica gel with 1:2 cyclohexane/EtOAc to afford major product **14** (0.56 g, 76%), white crystalline, R_f 0.38 (1:2 cyclohexane/EtOAc). NMR ($CDCl_3$) δ_H 7.39 (5H, m, Ar-H), 4.90 (2H, s, $PhCH_2$), 4.75 (2H, d, $J_{H-5,HO-5}$ = 5.4 Hz, CH_2-5), 4.72 (2H, d, $J_{H-4,HO-4}$ = 6.0 Hz, CH_2-4), 3.57 (1H, t, $J_{H-5,HO-5}$ = 5.4 Hz, α⁵-OH, exchangeable with D_2O), 3.49 (1H, t, $J_{H-4,HO-4}$ = 6.0 Hz, α⁴-OH, exchangeable with D_2O), 2.44 (3H, s, CH_3-2). δ_C 151.34 (d, $^3J_{F-C}$ = 9.6 Hz, Py-C_2), 146.97 (d, $^4J_{F-C}$ = 2.9 Hz, Py-C_3), 149.55 (d, $^3J_{F-C}$ = 3.1 Hz, Py-C_4), 119.09 (d, $^2J_{F-C}$ = 20.8 Hz, Py-C_5), 156.30 (d, $^1J_{F-C}$ = 216.2 Hz, Py-C_6), 136.33, 128.96, 128.88, 128.57 (Ph-C), 55.99 (s, $PhCH_2$, CH_2-4), 56.76 (s, CH_2-5), 19.31 (s, CH_3-2). Anal. Calcd for $C_{15}H_{16}NO_3F$: C, 64.96, H, 5.82, N, 5.05. Found: C, 64.95, H, 5.79, N, 5.04.

3-*O*-Benzyl-α⁴,α⁵-di-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-6-fluoropyridoxol 15 and 3-*O*-Benzyl-α⁴,α⁵-di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-6-fluoropyridoxol 16. Glycosylation of **14** (0.46 g, 2.0 mmol) with **8** or **9** (1.83 g, 4.45 mmol, 1.1 equiv) was carried out as for **3**, **10**, and **11** to give **15** and **16**, respectively.

3-*O*-Benzyl-α⁴,α⁵-di-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-6-fluoropyridoxol 15: 0.32 g, 95%) syrup, R_f 0.35 (3:2 cyclohexane/EtOAc). NMR ($CDCl_3$) δ_H 7.41 (5H, m, Ar-H), 5.36 (1H, d, $J_{1',2'}$ = 8.2 Hz, H-1′), 5.41 (1H, d, $J_{1'',2''}$ = 8.2 Hz, H-1″), 5.14 (2H, dd, $J_{2',3'} = J_{2'',3''}$ = 7.4 Hz, H-2′ and H-2″), 4.45 (2H, dd, $J_{3',4'} = J_{3'',4''}$ = 3.3 Hz, H-3′ and H-3″), 4.84 (2H, dd, $J_{4',5'} = J_{4'',5''}$ = 3.8 Hz, H-4′ and H-4″), 3.96 (2H, m, H-5′ and H-5″), 4.80 (2H, dd, $J_{5',6a'} = J_{5'',6a''}$ = 2.6 Hz, $J_{6a',6b'} = J_{6a'',6b''}$ = 10.1 Hz, H-6a′ and H-6a″), 4.10 (2H, dd, $J_{5',6b'} = J_{5'',6b''}$ = 3.0 Hz, H-6b′ and H-6b″), 4.94 (2H, s, $PhCH_2$), 4.55 (1H, d, $J_{CH2-4a,CH2-4b}$ = 10.4 Hz, CH_2-4a), 4.48 (1H, d, $J_{CH2-4a,CH2-4b}$ = 10.4 Hz, CH_2-4b), 4.60 (1H, d, $J_{CH2-5a,CH2-5b}$ = 11.0 Hz, CH_2-5a), 4.52 (1H, d, $J_{CH2-5a,CH2-5b}$ = 11.0 Hz, CH_2-5b), 2.37 (3H, s, CH_3-2), 2.00, 1.99, 1.98, 1.97, 1.96, 1.95, 1.94, 1.93 (24H, 8s, 8 CH_3CO). δ_C 170.84, 170.76, 170.31, 170.29, 170.26, 169.95, 169.92, 169.84 (8 CH_3**C**O), 152.18 (d, $^3J_{F-C}$ = 14.5 Hz, Py-C_2), 142.64 (d, $^4J_{F-C}$ = 4.6 Hz, Py-C_3), 150.12 (d, $^3J_{F-C}$ = 3.8 Hz, Py-C_4), 116.20 (d, $^2J_{F-C}$ = 32.0 Hz, Py-C_5), 157.40 (d, $^1J_{F-C}$ = 234.3 Hz, Py-C_6), 136.37, 129.00, 128.94, 128.87, 128.16, 127.77 (Ph-C), 100.23 (s, C-1′), 100.41 (s, C-1″), 71.41 (s, C-2′ and C-2″), 72.08 (s, C-3′), 72.19 (s, C-3″), 68.34 (s, C-4′), 68.51 (s, C-4″), 72.86 (s, C-5′), 72.93 (s, C-5″),

61.86 (s, C-6′), 61.98 (s, C-6″), 60.98 (s, CH_2-4), 61.28 (s, CH_2-5), 20.88, 20.85, 20.82, 20.75, 20.73, 20.60, 20.59, 20.58 (8s, 8 CH_3CO), 19.43 (s, CH_3-3). ESI-MS *m/z* 937 [M^+] (35%), 938 [M + 1] (25%). Anal. Calcd for $C_{43}H_{52}NO_{21}F$: C, 55.05, H, 5.59, N, 1.49. Found: C, 55.03, H, 5.57, N, 1.48.

3-*O*-Benzyl-α⁴,α⁵-di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-6-fluoropyridoxol 16: 0.30 g, 90%, syrup, R_f 0.40 (3:2 cyclohexane/EtOAc). NMR ($CDCl_3$) δ_H 7.38 (5H, m, Ar-H), 5.38 (1H, d, $J_{1',2'} = 2.6$ Hz, H-1′), 5.41 (1H, d, $J_{1'',2''} = 2.6$ Hz, H-1″), 5.36∼3.95 (18H, m, H-2′, 3′, 4′, 5′, 6′, 2″, 3″, 4″, 5″, 6″, $PhCH_2$, CH_2-4, and CH_2-5), 2.38 (3H, s, CH_3-2), 2.02, 2.00, 1.99, 1.98, 1.97, 1.96, 1.95, 1.94 (24H, 8s, 8 CH_3CO). δ_C 171.25, 171.18, 170.89, 170.85, 170.78, 170.66, 170.60, 170.48 (8 $CH_3\mathbf{C}O$), 153.28 (d, $^3J_{F-C} = 15.8$ Hz, Py-C_2), 145.48 (d, $^4J_{F-C} = 4.8$ Hz, Py-C_3), 150.16 (d, $^3J_{F-C} = 3.8$ Hz, Py-C_4), 116.30 (d, $^2J_{F-C} = 31.0$ Hz, Py-C_5), 157.77 (d, $^1J_{F-C} = 206.8$ Hz, Py-C_6), 98.42 (s, C-1′), 100.03 (s, C-1″), 72.60∼56.54 (13C, C-2′, 3′, 4′, 5′, 6′, 2″, 3″, 4″, 5″, 6″, $Ph\mathbf{C}H_2$, CH_2-4, and CH_2-5), 21.23, 20.94, 20.92, 20.90, 20.88, 20.86, 20.84, 20.80, 20.78 (8s, 8 CH_3CO), 18.37 (s, CH_3-3). ESI-MS *m/z* 937 [M^+] (32%), 938 [M + 1] (20%). Anal. Calcd for $C_{43}H_{52}NO_{21}F$: C, 55.05, H, 5.59, N, 1.49. Found: C, 55.01, H, 5.55, N, 1.45.

α⁴,α⁵-Di-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-6-fluoropyridoxol 17 and α⁴,α⁵-Di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-6-fluoropyridoxol 18. A mixture of **15** or **16** (0.29 g, 0.30 mmol) and Pd−C (5%, 50 mg) in MeOH (40 mL) was stirred for 24 h at RT under H_2 (25 psi). Evaporated filtrate gave **17** and **18** in quantitative yields.

α⁴,α⁵-Di-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-6-fluoropyridoxol 17: 0.26 g, syrup, R_f 0.28 (1:3 cyclohexane/EtOAc). NMR ($CDCl_3$) δ_H 7.33 (1H, s, HO-3, exchangeable with D_2O), 5.30 (1H, d, $J_{1',2'} = 8.4$ Hz, H-1′), 5.35 (1H, d, $J_{1'',2''} = 8.4$ Hz, H-1″), 5.09 (2H, dd, $J_{2',3'} = J_{2'',3''} = 7.6$ Hz, H-2′ and H-2″), 4.35 (2H, dd, $J_{3',4'} = J_{3'',4''} = 3.4$ Hz, H-3′ and H-3″), 4.80 (2H, dd, $J_{4',5'} = J_{4'',5''} = 3.6$ Hz, H-4′ and H-4″), 3.89 (2H, m, H-5′ and H-5″), 4.77 (2H, dd, $J_{5',6a'} = J_{5'',6a''} = 2.4$ Hz, $J_{6a',6b'} = J_{6a'',6b''} = 10.6$ Hz, H-6a′ and H-6a″), 4.05 (2H, dd, $J_{5',6b'} = J_{5'',6b''} = 3.2$ Hz, H-6b′ and H-6b″), 4.51 (1H, d, $J_{CH2-4a,CH2-4b} = 10.3$ Hz, CH_2-4a), 4.45 (1H, d, $J_{CH2-4a,CH2-4b} = 10.3$ Hz, CH_2-4b), 4.57 (1H, d, $J_{CH2-5a,CH2-5b} = 11.1$ Hz, CH_2-5a), 4.49 (1H, d, $J_{CH2-5a,CH2-5b} = 11.1$ Hz, CH_2-5b), 2.35 (3H, s, CH_3-2), 1.99, 1.98, 1.97, 1.96, 1.95, 1.94, 1.93, 1.91 (24H, 8s, 8 CH_3CO). δ_C 170.82, 170.78, 170.65, 170.58, 170.46, 169.85, 169.82, 169.80 (8 $CH_3\mathbf{C}O$), 152.28 (d, $^3J_{F-C} = 14.2$ Hz, Py-C_2), 148.28 (d, $^4J_{F-C} = 3.2$ Hz, Py-C_3), 142.69 (d, $^3J_{F-C} = 4.8$ Hz, Py-C_4), 116.26 (d, $^2J_{F-C} = 32.2$ Hz, Py-C_5), 157.56 (d, $^1J_{F-C} = 231.4$ Hz, Py-C_6), 100.35 (s, C-1′), 100.54 (s, C-1″), 71.37 (s, C-2′ and C-2″), 72.18 (s, C-3′), 72.29 (s, C-3″), 68.38 (s, C-4′), 68.56 (s, C-4″), 72.83 (s, C-5′), 72.88 (s, C-5″), 61.82 (s, C-6′), 61.89 (s, C-6″), 60.90 (s, CH_2-4), 61.19 (s, CH_2-5), 20.85, 20.83, 20.82, 20.80, 20.78, 20.76, 20.73, 20.65 (8s, 8 CH_3CO), 19.32 (s, CH_3-3). ESI-MS *m/z* 847 [M^+] (30%), 848 [M + 1] (21%). Anal. Calcd for $C_{36}H_{46}NO_{21}F$: C, 50.99, H, 5.47, N, 1.65. Found: C, 50.96, H, 5.45, N, 1.62.

α⁴,α⁵-Di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-6-fluoropyridoxol 18: 0.26 g, syrup, R_f 0.27 (1:3 cyclohexane/EtOAc). NMR ($CDCl_3$) δ_H 7.33 (1H, s, HO-3, exchangeable with D_2O), 5.33 (1H, d, $J_{1',2'} = 2.7$ Hz, H-1′), 5.37 (1H, d, $J_{1'',2''} = 2.7$ Hz, H-1″), 5.45∼4.07 (16H, m, H-2′, 3′, 4′, 5′, 6′, 2″, 3″, 4″, 5″, 6″, CH_2-4, and CH_2-5), 2.35 (3H, s, CH_3-2), 2.01, 2.00, 1.99, 1.98, 1.97, 1.96, 1.95, 1.94 (24H, 8s, 8 CH_3CO); δ_C: 171.33, 171.21, 170.85, 170.83, 170.76, 170.61, 170.56, 170.53 (8 $CH_3\mathbf{C}O$), 153.67 (d, $^3J_{F-C} = 15.8$ Hz, Py-C_2), 149.08 (d, $^4J_{F-C} = 3.0$ Hz, Py-C_3), 145.68 (d, $^3J_{F-C} = 4.6$ Hz, Py-C_4), 118.23 (d, $^2J_{F-C} = 31.2$ Hz, Py-C_5), 157.59 (d, $^1J_{F-C} = 223.1$ Hz, Py-C_6), 98.67 (s, C-1′), 100.33 (s, C-1″), 72.8∼56.56 (12C, C-2′, 3′, 4′, 5′, 6′, 2″, 3″, 4″, 5″, 6″, CH_2-4, and CH_2-5), 20.99, 20.97, 20.93, 20.90, 20.88, 20.86, 20.84, 20.80 (8s, 8 CH_3CO), 18.45 (s, CH_3-3). ESI-MS *m/z* 847 [M^+] (25%), 848 [M + 1] (18%). Anal. Calcd for $C_{36}H_{46}NO_{21}F$: C, 50.99, H, 5.47, N, 1.65. Found: C, 50.97, H, 5.44, N, 1.63.

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