Synthesis and Characterization of Novel *lacZ* Gene Reporter Molecules: Detection of β-Galactosidase Activity Using ^{19}F NMR of Polyglycosylated Fluorinated Vitamin B$_6$

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Abstract

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 non-invasive reporter techniques based on appropriate molecules and imaging modalities may help to assay gene expression. We have previously demonstrated the ability to detect β-gal activity based on ^{19}F NMR chemical shift associated with release of fluorophenyl aglycones from galactopyranoside conjugates. Use of fluoropyridoxol as the aglycone 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 aglycones 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. 4**, **12,** and **13** all show promising characteristics including highly sensitive ^{19}F NMR response to β-gal activity (Δδ = 9.0 ~ 9.4 ppm), minimal toxicity for substrate or aglycone, and good water solubility. However, the differential glycosylation of **12** and **13** appears more advantageous for assessing *lacZ* gene expression *in vivo*.

Keywords

β-galactosidase; ^{19}F NMR; *lacZ* gene reporter; 6-fluoropyridoxol; pH

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 non-invasive *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 for the basis of highly effective spectrophotometric assays *in vitro*.[1–3]

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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. Towards 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-one-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 aglycones.[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 aglycone 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 aglycone 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 aglycone 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.

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* (*lac*Z) β-gal catalyses the hydrolysis of galactopyranosides by cleavage of the C-O bond between *D*-galactose and the aglycone 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 to 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 retaining the virtues of **GFPOL**. [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 equivalents 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 quasimolecular ion at *m/z* 1179 [M+H], corresponding to the fully adorned derivative with three fully acetylated galactosides. The identity of **3** was established using ^{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 $J_{2,3}$ ~ 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.

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. Retro-synthetic 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$_2$SO$_4$ 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 ($J_{\text{H-5,HO-5}}$ = 1.2 Hz) due to the coupling of 5-OH. [13] Treatment of **5** with **2** using 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 $\delta_{\text{H-1'}}$ at 4.64 ppm is a well-resolved doublet ($J_{1,2}$ = 8.0 Hz) and $\delta_{\text{C-1'}}$ 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 NOSEY spectrum of **6** verified that 2, 3, 4, 6-tetra-*O*-acetyl-β-*D*-galactopyranosyl residue connected

at 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$_3$CO$_2$H in MeOH, CH$_2$Cl$_2$ 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$_2$Cl$_2$ 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. Deacetylation of **10** or **11** in NH$_3$/MeOH from 0°C to room temperature 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 of 3% with limiting steps in the α^4, α^5-isopropylidene group formation and hydrolysis procedures.

The acidic 3 phenolic group *para* to 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) using 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 ^{1}H-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 work up 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** using the above phase-transfer catalysis technique yielding **7**.[8] Figure 5 depicts a very efficient route to synthesize the target compounds **12** and **13** using just three-steps with higher overall yields (67% and 65%, respectively).

Characteristics

4, **12** and **13** each gave a single narrow ^{19}F NMR signal between δ −2.0 ~ −3.3 ppm essentially invariant (Δδ ≤ 0.06 ppm) with pH in the range 3 to 12 and temperatures from 25 to 37 °C in whole rabbit blood, 0.9% saline or PBS. Addition of β-gal (E801A) in PBS at 37 °C to **4**, **12** and **13** caused rapid hydrolysis releasing the aglycones 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-fluoropyridoxol, which also appeared as single narrow ^{19}F signals between δ −11.20 ~ −12.40 ppm (Δδ = 9.0 ~ 9.4 ppm) (Table 1). Action of β-gal on **4** was complicated by action on each of the galactose residue 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 aglycones 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. 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 per million MCF7-*lacZ* cells, respectively. **12** and **13** have much higher aqueous solubility than **GFPOL** (75 mM, vs. **12**, 196 mM and **13**, 173 mM 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

General methods

NMR spectra were recorded on a Varian Inova 400 spectrometer (400 MHz for ^{1}H, 100 MHz for ^{13}C, 376 MHz for ^{19}F) with CDCl$_3$, or DMSO-d_6 as solvents. ^{1}H and ^{13}C chemical shifts are referenced to TMS as internal standard, and ^{19}F to a dilute solution of NaTFA in a capillary as external standard (37 °C). Compounds were characterized by acquisition of ^{1}H, ^{13}C, DEPT, ^{1}H-^{1}H COSY or NOESY experiments at 25 °C. Microanalyses were performed on a Perkin-Elmer 2400CHN microanalyser. Mass spectra were obtained by positive and negative ESI-MS using 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 1h, CH$_2$Cl$_2$ 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 the Sigma Chemical Company. 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) used for analytical TLC. Detection was effected by spraying the plates with 5% ethanolic H$_2$SO$_4$ (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, USA) 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. **12** or **13** were 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-fluoro-pyridoxol 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 the 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 r.t. under argon in the dark for 12h. 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 (1 H, d, $J_{1',2'} = 8.0$ Hz, H-1′), 5.04 (1 H, dd, $J_{2',3'} = 9.8$ Hz, H-2′), 4.73 (1 H, dd, $J_{3',4'} = 3.4$ Hz, H-3′), 3.98 (1 H, dd, $J_{4',5'} = 2.4$ Hz, H-4′), 4.02~4.10 (3 H, m, H-5′, H-6′), 4.66 (1 H, d, $J_{1'',2''} = 8.0$ Hz, H-1″), 4.52 (1 H, d, $J_{1''',2'''} = 8.0$ Hz, H-1‴), 5.15 (2 H, dd, $J_{2'',3''} = J_{2''',3'''} = 10.0$ Hz, H-2″, H-2‴), 5.07 (2 H, dd, $J_{3'',4''} = J_{3''',4'''} = 3.6$ Hz, H-3″, H-3‴), 5.52 (2 H, dd, $J_{4'',5''} = J_{4''',5'''} = 3.2$ Hz, H-4″, H-4‴), 3.88 (2 H, m, H-5″, H-5‴), 4.18 (2 H, dd, $J_{5'',6a''} = J_{5''',6a'''} = 3.6$ Hz, $J_{6a'',6b''} = J_{6a''',6b'''} = 9.2$ Hz, H-6a″, H-6a‴), 4.11 (2 H, dd, $J_{5'',6b''} = J_{5''',6b'''} = 6.8$ Hz, H-6b″, H-6b‴), 4.48 (2 H, d, $J_{CH2-4a,CH2-4b} = J_{CH2-5a,CH2-5b} = 13.2$ Hz, CH_2-4a, CH_2-5a), 4.12 (2 H, d, $J_{CH2-4a,CH2-4b} = J_{CH2-5a,CH2-5b} = 13.2$ Hz, CH_2-4b, CH_2-5b), 2.43 (3 H, 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 (36 H, 12s, $12\times CH_3CO$) ppm; 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 ($12\times CH_3CO$), 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″, 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″, C-2‴), 68.39 (s, C-3″, C-3‴), 66.31 (s, C-4″, C-4‴), 68.55 (s, C-5″, C-5‴), 61.99 (s, C-6″, 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, $12\times CH_3CO$), 18.77 (s, CH_3-3) ppm. ESIMS: *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 r.t. overnight until TLC showed complete reaction and 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 (1 H, d, $J_{1',2'} = 8.2$ Hz, H-1′), 4.76 (1 H, dd, $J_{2',3'} = 10.0$ Hz, H-2′), 4.91 (1 H, dd, $J_{3',4'} = 2.8$ Hz, H-3′), 5.11 (1 H, dd, $J_{4',5'} = 2.3$ Hz, H-4′), 3.77 (1 H, m, H-5′), 3.90 (1 H, dd, $J_{5',6a'} = 6.4$ Hz, $J_{6a',6b'} = 12.4$ Hz, H-6a′), 3.68 (1 H, dd, $J_{5',6b'} = 3.6$ Hz, H-6b′), 4.22 (2 H, d, $J_{1'',2''} = J_{1''',2'''} = 8.0$ Hz, H-1″, H-1‴), 3.29 (2 H, dd, $J_{2'',3''} = J_{2''',3'''} = 10.6$ Hz, H-2″, H-2‴), 3.51 (2 H, dd, $J_{3'',4''} = J_{3''',4'''} = 3.2$ Hz, H-3″, H-3‴), 3.62 (2 H, dd, $J_{4'',5''} = J_{4''',5'''} = 2.4$ Hz, H-4″, H-4‴), 3.46 (2 H, m, H-5″, H-5‴), 3.66 (2 H, dd, $J_{5'',6a''} = J_{5''',6a'''} = 3.6$ Hz, $J_{6a'',6b''} = J_{6a''',6b'''} = 10.4$ Hz, H-6a″, H-6a‴), 3.39 (2 H, dd, $J_{5'',6b''} = J_{5''',6b'''} = 6.6$ Hz, H-6b″, H-6b‴), 4.48 (2 H, d, $J_{CH2-4a,CH2-4b} = J_{CH2-5a,CH2-5b} = 13.0$ Hz, CH_2-4a, CH_2-5a), 4.44 (2 H, d, $J_{CH2-4a,CH2-4b} = J_{CH2-5a,CH2-5b} = 13.0$ Hz, CH_2-4b, CH_2-5b), 2.32 (3H, s, CH_3-2) ppm; 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″, 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″, C-2‴), 73.50 (s, C-3″, C-3‴), 68.26 (s, C-4″, C-4‴), 75.02 (s, C-5″, C-5‴), 60.60 (s, C-6″, C-6‴), 68.77 (s, CH_2-4), 68.92 (s, CH_2-5), 19.19 (s, CH_3-3) ppm. ESIMS: *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.

α^4, α^5-*O*-isopropylidene-6-fluoropyridoxol 5—A suspension of **1** (0.50 g, 2.67 mmol) in anhydrous acetone (40 mL) containing 2% c. H_2SO_4 was stirred for 4 ~ 5h, at the end of which time TLC (4:1 cyclohexane-EtOAc) indicated complete reaction, then cold saturated Na_2CO_3 solution was added with vigorous stirring up to pH between 8 ~ 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 (1 H, s, HO-3), 5.03 (2 H, s, CH$_2$-5), 4.57 (2 H, s, CH$_2$-4), 2.33 (3 H, s, CH$_3$-2), 1.55 (6 H, s, 2×CH$_3$) ppm; 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) ppm. 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)-α^4, α^5-*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$_2$Cl$_2$ (10 mL) containing freshly activated 4Å molecular sieves (2.0 g) was added dropwise **2** (1.23 g, 3.0 mmol, 1.1 equiv.). The mixture was stirred overnight in the dark at r.t. under N$_2$ until TLC indicated complete reaction. Work up as for **3** gave **6** (1.29 g, 85%), R_f 0.40 (2:3 cyclohexane-EtOAc), NMR (CDCl$_3$), δ_H: 4.64 (1 H, d, $J_{1',2'}$ = 8.0 Hz, H-1′), 5.25 (1 H, dd, $J_{2',3'}$ = 10.0 Hz, H-2′), 5.02 (1 H, dd, $J_{3',4'}$ = 3.6 Hz, H-3′), 5.41 (1 H, dd, $J_{4',5'}$ = 3.2 Hz, H-4′), 3.97 (1 H, m, H-5′), 4.21 (1 H, dd, $J_{5',6a'}$ = 4.4 Hz, $J_{6a',6b'}$ = 11.2 Hz, H-6a′), 4.13 (1 H, dd, $J_{5',6b'}$ = 7.2 Hz, H-6b′), 5.10 (1 H, d, $J_{CH2-4a,CH2-4b}$ = 8.0 Hz, CH$_2$-4a), 4.67 (1 H, d, $J_{CH2-4a,CH2-4b}$ = 8.0 Hz, CH$_2$-4b), 5.14 (1 H, d, $J_{CH2-5a,CH2-5b}$ = 9.6 Hz, CH$_2$-5a), 5.12 (1 H, d, $J_{CH2-5a,CH2-5b}$ = 9.6 Hz, CH$_2$-5b), 2.42 (3 H, s, CH$_3$-2), 2.17, 2.09, 2.08, 1.99 (12 H, 4s, 4×CH$_3$CO), 1.61, 1.59 (6 H, 2s, 2×CH$_3$) ppm; 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×**C**H$_3$CO), 18.77 (s, CH$_3$-2) ppm. 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 reaction complete. The cooled mixture was neutralized with cold saturated Na$_2$CO$_3$ solution, extracted with EtOAc (4×30 mL), 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 (1 H, d, $J_{1',2'}$ = 8.0 Hz, H-1′), 5.55 (1 H, dd, $J_{2',3'}$ = 10.6 Hz, H-2′), 5.10 (1 H, dd, $J_{3',4'}$ = 3.6 Hz, H-3′), 5.41 (1 H, dd, $J_{4',5'}$ = 3.6 Hz, H-4′), 3.88 (1 H, m, H-5′), 4.24 (1 H, dd, $J_{5',6a'}$ = 4.4 Hz, $J_{6a',6b'}$ = 12.0 Hz, H-6a′), 4.09 (1 H, dd, $J_{5',6b'}$ = 6.0 Hz, H-6b′), 5.01 (2 H, d, $J_{CH2-4a,CH2-4b}$ = $J_{CH2-5a,CH2-5b}$ = 12.4 Hz, CH$_2$-4a, CH$_2$-5a), 4.62 (1 H, d, $J_{CH2-4a,CH2-4b}$ = 12.4 Hz, CH$_2$-4b), 4.66 (1 H, d, $J_{CH2-5a,CH2-5b}$ = 12.4 Hz, CH$_2$-5b), 3.50 (1 H, m, α^4-HO, exchangeable with D$_2$O), 3.56 (1 H, m, α^5-HO, exchangeable with D$_2$O), 2.47 (3 H, s, CH$_3$-2), 2.23, 2.17, 2.02, 2.00 (12 H, 4s, 4×CH$_3$CO)ppm; 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,

CH$_2$-5), 20.99, 20.85, 20.70, 20.67 (4s, 4×**CH$_3$**CO), 19.46 (s, CH$_3$-2) ppm. 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$_2$Cl$_2$ (10 mL)-H$_2$O (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$_2$Cl$_2$ (10 mL) was added dropwise over a period of 4 ~ 5h at r.t., and the stirring continued for an additional hour. The products were extracted (EtOAc; 4×20 mL), washed free of alkali, dried (Na$_2$SO$_4$), 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)-α^4, α^5-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)-α^4, α^5-di-*O*-(2, 3, 4, 6-tetra-*O*-acetyl-α-*D*-manno-pyranosyl)-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$_2$Cl$_2$ (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)-α^4, α^5-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)-α^4, α^5-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)-α^4, α^5-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 (1 H, d, $J_{1',2'}$ = 7.8 Hz, H-1'), 5.28 (1 H, dd, $J_{2',3'}$ = 8.8 Hz, H-2'), 4.98 (1 H, dd, $J_{3',4'}$ = 4.8 Hz, H-3'), 4.73 (1 H, dd, $J_{4',5'}$ = 2.8 Hz, H-4'), 3.95 (1 H, m, H-5'), 4.19 (1 H, dd, $J_{5',6a'}$ = 3.6 Hz, $J_{6a',6b'}$ = 10.8 Hz, H-6a'), 4.02 (1 H, dd, $J_{5',6b'}$ = 5.2 Hz, H-6b'), 5.36 (1 H, d, $J_{1'',2''}$ = 8.0 Hz, H-1''), 5.39 (1 H, d, $J_{1''',2'''}$ = 8.0 Hz, H-1'''), 5.12 (1 H, dd, $J_{2'',3''}$ = 7.2 Hz, H-2''), 5.15 (1 H, dd, $J_{2''',3'''}$ = 6.8 Hz, H-2'''), 5.04 (1 H, dd, $J_{3'',4''}$ = 3.2 Hz, H-3''), 5.07 (1 H, dd, $J_{3''',4'''}$ = 3.6 Hz, H-3'''), 4.76 (1 H, dd, $J_{4'',5''}$ = 2.8 Hz, H-4''), 4.78 (1 H, dd, $J_{4''',5'''}$ = 2.8 Hz, H-4'''), 3.91 (1 H, m, H-5''), 3.93 (1 H, m, H-5'''), 4.08 (1 H, dd, $J_{5'',6a''}$ = 3.2 Hz, $J_{6a'',6b''}$ = 9.4 Hz, H-6a''), 4.10 (1 H, dd, $J_{5''',6a'''}$ = 3.0 Hz, $J_{6a''',6b'''}$ = 10.0 Hz, H-6a'''), 4.04 (1 H, dd, $J_{5'',6b''}$ = 7.6 Hz, H-6b''), 4.07 (1 H, dd, $J_{5''',6b'''}$ = 6.8 Hz, H-6b'''), 4.55 (2 H, d, $J_{CH2-4a,CH2-4b}$ = $J_{CH2-5a,CH2-5b}$ = 11.2 Hz, CH$_2$-4a, CH$_2$-5b), 4.49 (1 H, d, $J_{CH2-4a,CH2-4b}$ = 11.2 Hz, CH$_2$-4b), 4.91 (1 H, d, $J_{CH2-5a,CH2-5b}$ = 11.2 Hz, CH$_2$-5a), 2.34 (3 H, 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 (36 H, 12s, 12×CH$_3$CO) ppm; 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$_3$**CO**), 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$_3$CO), 19.76 (s, CH$_3$-3) ppm. ESIMS: *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)-α^4, α^5-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 (1 H, d, $J_{1',2'}$ = 8.2 Hz, H-1'), 5.13 (1 H, dd, $J_{2',3'}$ = 9.8 Hz, H-2'), 5.36 (1 H, dd, $J_{3',4'}$ = 4.2 Hz, H-3'), 5.30 (1 H, dd, $J_{3'',4''}$ = 3.6 Hz, H-4'), 4.01 (1 H, m,

H-5′), 4.33 (1 H, dd, $J_{5',6a'}$ = 3.2 Hz, $J_{6a',6b'}$ = 10.0 Hz, H-6a′), 4.11 (1 H, dd, $J_{5',6b'}$ = 4.6 Hz, H-6b′), 4.71 (2 H, d, $J_{1'',2''}$ = $J_{1''',2'''}$ = 2.4 Hz, H-1″, H-1‴), 4.74 (2 H, dd, $J_{2'',3''}$ = $J_{2''',3'''}$ = 6.2 Hz, H-2″, H-2‴), 5.22 (2 H, dd, $J_{3'',4''}$ = $J_{3''',4'''}$ = 3.8 Hz, H-3″, H-3‴), 3.95 (2 H, dd, $J_{4'',5''}$ = $J_{4''',5'''}$ = 2.0 Hz, H-4″, H-4‴), 4.02 (2 H, m, H-5″, H-5‴), 4.11 (2 H, dd, $J_{5'',6a''}$ = $J_{5''',6a'''}$ = 2.0 Hz, $J_{6a'',6b''}$ = $J_{6a''',6b''}$ = 7.4 Hz, H-6a″, H-6a‴), 4.07 (2 H, dd, $J_{5'',6b''}$ = $J_{5''',6b'''}$ = 5.6 Hz, H-6b″, H-6b‴), 4.87 (2 H, d, $J_{CH2-4a,CH2-4b}$ = $J_{CH2-5a,CH2-5b}$ = 13.6 Hz, CH$_2$-4a, CH$_2$-5b), 4.67 (1 H, d, $J_{CH2-4a,CH2-4b}$ = $J_{CH2-5a,CH2-5b}$ = 13.6 Hz, CH$_2$-4b, CH$_2$-5a), 2.33 (3 H, 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 (36 H, 12s, 12×CH$_3$CO); 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×**C**H$_3$CO), 20.35 (s, CH$_3$-3) ppm. ESIMS: 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-fluoro-pyridoxol 12 and 3-O-(β-D-galactopyranosyl)-α⁴, α⁵-di-O-(α-D-mannopyranosyl)-6-fluoropyridoxol 13

Compounds **10**, **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 (1 H, d, $J_{1',2'}$ = 8.2 Hz, H-1′), 5.22 (1 H, dd, $J_{2',3'}$ = 9.0 Hz, H-2′), 4.92 (1 H, dd, $J_{3',4'}$ = 4.6 Hz, H-3′), 4.70 (1 H, dd, $J_{4',5'}$ = 2.6 Hz, H-4′), 3.91 (1 H, m, H-5′), 4.12 (1 H, dd, $J_{5',6a'}$ = 3.2 Hz, $J_{6a',6b'}$ = 10.2 Hz, H-6a′), 4.00 (1 H, dd, $J_{5',6b'}$ = 5.6 Hz, H-6b′), 5.14 (2 H, d, $J_{1'',2''}$ = 10.0 Hz, H-1″, H-1‴), 4.82 (2 H, dd, $J_{2'',3''}$ = $J_{2''',3'''}$ = 8.2 Hz, H-2″, H-2‴), 4.69 (2 H, dd, $J_{3'',4''}$ = $J_{3''',4'''}$ = 3.4 Hz, H-3″, H-3‴), 4.93 (2 H, dd, $J_{4'',5''}$ = $J_{4''',5'''}$ = 3.2 Hz, H-4″, H-4‴), 3.65 (2 H, m, H-5″, H-5‴), 3.55 (2 H, dd, $J_{5'',6a''}$ = $J_{5''',6a'''}$ = 4.8 Hz, $J_{6a'',6b''}$ = $J_{6a''',6b'''}$ = 12.0 Hz, H-6a″, H-6a‴), 3.31 (2 H, dd, $J_{5'',6b''}$ = $J_{5''',6b'''}$ = 5.6 Hz, H-6b″, H-6b‴), 4.29 (1 H, d, $J_{CH2-4a,CH2-4b}$ = 7.6 Hz, CH$_2$-4a), 4.36 (1 H, d, $J_{CH2-4a,CH2-4b}$ = 7.6 Hz, CH$_2$-5a), 4.20 (2 H, d, $J_{CH2-4a,CH2-4b}$ = $J_{CH2-5a,CH2-5b}$ = 7.6 Hz, CH$_2$-4b, CH$_2$-5b), 4.18 ~ 3.65 (12 H, br, HO-2′, 3′, 4′, 6′, 2″, 3″, 4″, 6″, 2‴, 3‴, 4‴, 6‴, exchangeable with D$_2$O), 2.42 (3 H, 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″, C-1‴), 72.34 (s, C-2′), 71.28 (s, C-2″, C-2‴), 74.55 (s, C-3′), 73.88 (s, C-3″, C-3‴), 69.82 (s, C-4′), 68.89 (s, C-4″, C-4‴), 76.62 (s, C-5′), 77.29 (s, C-5″, C-5‴), 61.36 (s, C-6′), 60.95 (s, C-6″, C-6‴), 60.54 (s, CH$_2$-4), 60.78 (s, CH$_2$-5), 19.88 (s, CH$_3$-3) ppm. ESIMS: 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 (1 H, d, $J_{1',2'}$ = 8.0 Hz, H-1′), 5.23 (1 H, dd, $J_{2',3'}$ = 10.0 Hz, H-2′), 5.16 (1 H, dd, $J_{3',4'}$ = 3.8 Hz, H-3′), 5.08 (1 H, dd, $J_{3'',4''}$ = 3.2 Hz, H-4′), 4.21 (1 H, m, H-5′), 4.51 (1 H, dd, $J_{5',6a'}$ = 3.6 Hz, $J_{6a',6b'}$ = 10.2 Hz, H-6a′), 4.31 (1 H, dd, $J_{5',6b'}$ = 4.8 Hz, H-6b′), 4.84 (2 H, d, $J_{1'',2''}$ = $J_{1''',2'''}$ = 2.6 Hz, H-1″, H-1‴), 4.68 (2 H, dd, $J_{2'',3''}$ = $J_{2''',3'''}$ = 6.0 Hz, H-2″, H-2‴), 5.02 (2 H, dd, $J_{3'',4''}$ = $J_{3''',4'''}$ = 3.6 Hz, H-3″, H-3‴), 4.05 (2 H, dd, $J_{4'',5''}$ = $J_{4''',5'''}$ = 2.2 Hz, H-4″, H-4‴), 3.94 (2 H, m, H-5″, H-5‴), 4.21 (2 H, dd, $J_{5'',6a''}$ = $J_{5''',6a'''}$ = 2.4 Hz, $J_{6a'',6b''}$ = $J_{6a''',6b''}$ = 8.4 Hz, H-6a″, H-6a‴), 4.17 (2 H, dd, $J_{5'',6b''}$ = $J_{5''',6b'''}$ = 6.5 Hz, H-6b″, H-6b‴), 4.77 (2 H, d,

$J_{\text{CH2-4a,CH2-4b}} = J_{\text{CH2-5a,CH2-5b}} = 11.6$ Hz, CH$_2$-4a, CH$_2$-5b), 4.57 (1 H, d, $J_{\text{CH2-4a,CH2-4b}} = J_{\text{CH2-5a,CH2-5b}} = 11.6$ Hz, CH$_2$-4b, CH$_2$-5a), 2.45 (3 H, s, CH$_3$-2), 4.30 ~ 3.70 (12 H, br, HO-2′, 3′, 4′, 6′, 2″, 3″, 4″, 6″, 2‴, 3‴, 4‴, 6‴, exchangeable with D$_2$O); δ_C: 151.07 (d, $3J_{\text{F-C}} = 15.3$ Hz, Py-C$_2$), 148.61 (d, $4J_{\text{F-C}} = 4.8$ Hz, Py-C$_3$), 144.27 (d, $3J_{\text{F-C}} = 3.6$ Hz, Py-C$_4$), 116.29 (d, $2J_{\text{F-C}} = 32.0$ Hz, Py-C$_5$), 157.25 (d, $1J_{\text{F-C}} = 233.5$ 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) ppm. ESIMS: 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$_2$Cl$_2$ (10 mL)-H$_2$O (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$_2$Cl$_2$ (10 mL) was added dropwise over a period of 4 ~ 5h, while the reaction temperature was maintained at 50 °C, and the stirring continued for an additional hour. Products were extracted (CH$_2$Cl$_2$, 4×20 mL), washed free of alkali, dried (Na$_2$SO$_4$), and concentrated, 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 (5 H, m, Ar-H), 4.90 (2 H, s, PhCH$_2$), 4.75 (2 H, d, $J_{\text{H-5,HO-5}} = 5.4$ Hz, CH$_2$-5), 4.72 (2 H, d, $J_{\text{H-4,HO-4}} = 6.0$ Hz, CH$_2$-4), 3.57 (1 H, t, $J_{\text{H-5,HO-5}} = 5.4$ Hz, α^5-OH, exchangeable with D$_2$O), 3.49 (1 H, t, $J_{\text{H-4,HO-4}} = 6.0$ Hz, α^4-OH, exchangeable with D$_2$O), 2.44 (3 H, s, CH$_3$-2); δ_C: 151.34 (d, $3J_{\text{F-C}} = 9.6$ Hz, Py-C$_2$), 146.97 (d, $4J_{\text{F-C}} = 2.9$ Hz, Py-C$_3$), 149.55 (d, $3J_{\text{F-C}} = 3.1$ Hz, Py-C$_4$), 119.09 (d, $2J_{\text{F-C}} = 20.8$ Hz, Py-C$_5$), 156.30 (d, $1J_{\text{F-C}} = 216.2$ 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) ppm. Anal. Calcd. for C$_{15}$H$_{16}$NO$_3$F(%): C, 64.96, H, 5.82, N, 5.05; Found: C, 64.95, H, 5.79, N, 5.04.

3-*O*-Benzyl-α^4, α^5-di-*O*-(2, 3, 4, 6-tetra-*O*-acetyl-β-*D*-glucopyranosyl)-6-fluoro-pyridoxol 15 and 3-*O*-Benzyl-α^4, α^5-di-*O*-(2, 3, 4, 6-tetra-*O*-acetyl-α-*D*-manno-pyranosyl)-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-α^4, α^5-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 (5 H, m, Ar-H), 5.36 (1 H, d, $J_{1',2'} = 8.2$ Hz, H-1′), 5.41 (1 H, d, $J_{1'',2''} = 8.2$ Hz, H-1″), 5.14 (2 H, dd, $J_{2',3'} = J_{2'',3''} = 7.4$ Hz, H-2′, H-2″), 4.45 (2 H, dd, $J_{3',4'} = J_{3'',4''} = 3.3$ Hz, H-3′, H-3″), 4.84 (2 H, dd, $J_{4',5'} = J_{4'',5''} = 3.8$ Hz, H-4′, H-4″), 3.96 (2 H, m, H-5′, H-5″), 4.80 (2 H, dd, $J_{5',6a'} = J_{5'',6a''} = 2.6$ Hz, $J_{6a',6b'} = J_{6a'',6b''} = 10.1$ Hz, H-6a′, H-6a″), 4.10 (2 H, dd, $J_{5',6b'} = J_{5'',6b''} = 3.0$ Hz, H-6b′, H-6b″), 4.94 (2 H, s, PhCH$_2$), 4.55 (1 H, d, $J_{\text{CH2-4a,CH2-4b}} = 10.4$ Hz, CH$_2$-4a), 4.48 (1 H, d, $J_{\text{CH2-4a,CH2-4b}} = 10.4$ Hz, CH$_2$-4b), 4.60 (1 H, d, $J_{\text{CH2-5a,CH2-5b}} = 11.0$ Hz, CH$_2$-5a), 4.52 (1 H, d, $J_{\text{CH2-5a,CH2-5b}} = 11.0$ Hz, CH$_2$-5b), 2.37 (3 H, s, CH$_3$-2), 2.00, 1.99, 1.98, 1.97, 1.96, 1.95, 1.94, 1.93 (24 H, 8s, 8×CH$_3$CO); 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, $3J_{\text{F-C}} = 14.5$ Hz, Py-C$_2$), 142.64 (d, $4J_{\text{F-C}} = 4.6$ Hz, Py-C$_3$), 150.12 (d, $3J_{\text{F-C}} = 3.8$ Hz, Py-C$_4$), 116.20 (d, $2J_{\text{F-C}} = 32.0$ Hz, Py-C$_5$), 157.40 (d, $1J_{\text{F-C}} = 234.3$ 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′, 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×**C**H$_3$CO), 19.43 (s, CH$_3$-3) ppm. ESIMS: 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 (CDCl₃), δ_H: 7.38 (5 H, m, Ar-H), 5.38 (1 H, d, $J_{1',2'}$ = 2.6 Hz, H-1'), 5.41 (1 H, d, $J_{1'',2''}$ = 2.6 Hz, H-1''), 5.36 ~ 3.95 (18 H, m, H-2', 3', 4', 5', 6', 2'', 3'', 4'', 5'', 6'', PhCH₂, CH₂-4, CH₂-5), 2.38 (3 H, s, CH₃-2), 2.02, 2.00, 1.99, 1.98, 1.97, 1.96, 1.95, 1.94 (24 H, 8s, 8×CH₃CO); δ_C: 171.25, 171.18, 170.89, 170.85, 170.78, 170.66, 170.60, 170.48 (8×CH₃**C**O), 153.28 (d, 3J_{F-C} = 15.8 Hz, Py-C₂), 145.48 (d, 4J_{F-C} = 4.8 Hz, Py-C₃), 150.16 (d, 3J_{F-C} = 3.8 Hz, Py-C₄), 116.30 (d, 2J_{F-C} = 31.0 Hz, Py-C₅), 157.77 (d, 1J_{F-C} = 206.8 Hz, Py-C₆), 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'', PhCH₂, CH₂-4, CH₂-5), 21.23, 20.94, 20.92, 20.90, 20.88, 20.86, 20.84, 20.80, 20.78 (8s, 8×CH₃CO), 18.37 (s, CH₃-3) ppm. ESIMS: *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 24h at r.t. under H₂ (25 psi). Evaporated filtrate gave **17**, **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 (CDCl₃), δ_H: 7.33 (1 H, s, HO-3, exchangeable with D₂O), 5.30 (1 H, d, $J_{1',2'}$ = 8.4 Hz, H-1'), 5.35 (1 H, d, $J_{1'',2''}$ = 8.4 Hz, H-1''), 5.09 (2 H, dd, $J_{2',3'}$ = $J_{2'',3''}$ = 7.6 Hz, H-2', H-2''), 4.35 (2 H, dd, $J_{3',4'}$ = $J_{3'',4''}$ = 3.4 Hz, H-3', H-3''), 4.80 (2 H, dd, $J_{4',5'}$ = $J_{4'',5''}$ = 3.6 Hz, H-4', H-4''), 3.89 (2 H, m, H-5', H-5''), 4.77 (2 H, dd, $J_{5',6a'}$ = $J_{5'',6a''}$ = 2.4 Hz, $J_{6a',6b'}$ = $J_{6a'',6b''}$ = 10.6 Hz, H-6a', H-6a''), 4.05 (2 H, dd, $J_{5',6b'}$ = $J_{5'',6b''}$ = 3.2 Hz, H-6b', H-6b''), 4.51 (1 H, d, $J_{CH2-4a,CH2-4b}$ = 10.3 Hz, CH₂-4a), 4.45 (1 H, d, $J_{CH2-4a,CH2-4b}$ = 10.3 Hz, CH₂-4b), 4.57 (1 H, d, $J_{CH2-5a,CH2-5b}$ = 11.1 Hz, CH₂-5a), 4.49 (1 H, d, $J_{CH2-5a,CH2-5b}$ = 11.1 Hz, CH₂-5b), 2.35 (3 H, s, CH₃-2), 1.99, 1.98, 1.97, 1.96, 1.95, 1.94, 1.93, 1.91 (24 H, 8s, 8×CH₃CO); δ_C: 170.82, 170.78, 170.65, 170.58, 170.46, 169.85, 169.82, 169.80 (8×CH₃**C**O), 152.28 (d, 3J_{F-C} = 14.2 Hz, Py-C₂), 148.28 (d, 4J_{F-C} = 3.2 Hz, Py-C₃), 142.69 (d, 3J_{F-C} = 4.8 Hz, Py-C₄), 116.26 (d, 2J_{F-C} = 32.2 Hz, Py-C₅), 157.56 (d, 1J_{F-C} = 231.4 Hz, Py-C₆), 100.35 (s, C-1'), 100.54 (s, C-1''), 71.37 (s, C-2', 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, CH₂-4), 61.19 (s, CH₂-5), 20.85, 20.83, 20.82, 20.80, 20.78, 20.76, 20.73, 20.65 (8s, 8×CH₃CO), 19.32 (s, CH₃-3) ppm. ESIMS: *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 (CDCl₃), δ_H: 7.33 (1 H, s, HO-3, exchangeable with D₂O), 5.33 (1 H, d, $J_{1',2'}$ = 2.7 Hz, H-1'), 5.37 (1 H, d, $J_{1'',2''}$ = 2.7 Hz, H-1''), 5.45 ~ 4.07 (16 H, m, H-2', 3', 4', 5', 6', 2'', 3'', 4'', 5'', 6'', CH₂-4, CH₂-5), 2.35 (3 H, s, CH₃-2), 2.01, 2.00, 1.99, 1.98, 1.97, 1.96, 1.95, 1.94 (24 H, 8s, 8×CH₃CO); δ_C: 171.33, 171.21, 170.85, 170.83, 170.76, 170.61, 170.56, 170.53 (8×CH₃**C**O), 153.67 (d, 3J_{F-C} = 15.8 Hz, Py-C₂), 149.08 (d, 4J_{F-C} = 3.0 Hz, Py-C₃), 145.68 (d, 3J_{F-C} = 4.6 Hz, Py-C₄), 118.23 (d, 2J_{F-C} = 31.2 Hz, Py-C₅), 157.59 (d, 1J_{F-C} = 223.1 Hz, Py-C₆), 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'', CH₂-4, CH₂-5), 20.99, 20.97, 20.93, 20.90, 20.88, 20.86, 20.84, 20.80 (8s, 8×CH₃CO), 18.45 (s, CH₃-3) ppm. ESIMS: *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.

Acknowledgements

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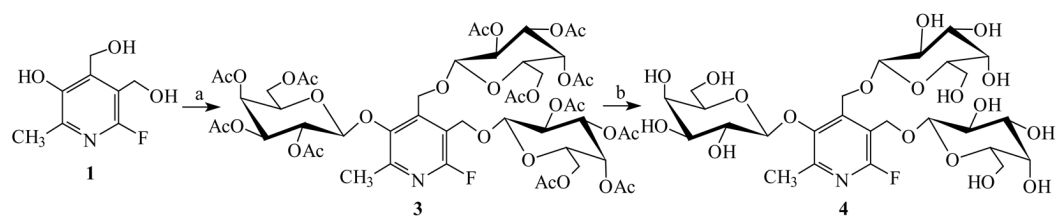
Figure 1. Reagents and conditions
(a) 2, 3, 4, 6-tetra-*O*-acetyl-α-*D*-galactopyranosyl bromide **2**, Hg(CN)$_2$, 4Å M.S., CH$_2$Cl$_2$, r.t., 12 h, 89%; (b) NH$_3$-MeOH, 0 °C→r.t., 24 h, quantitative yields.
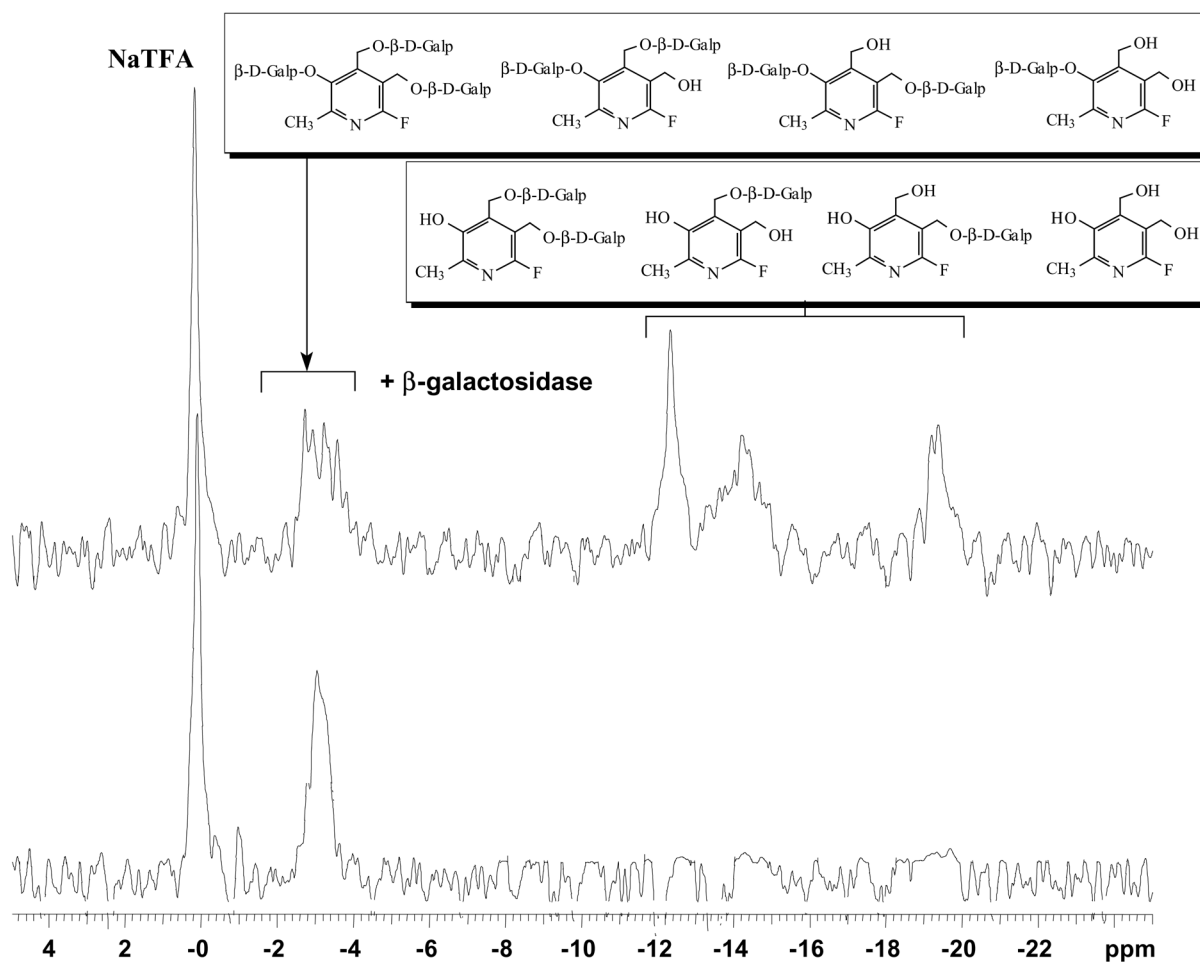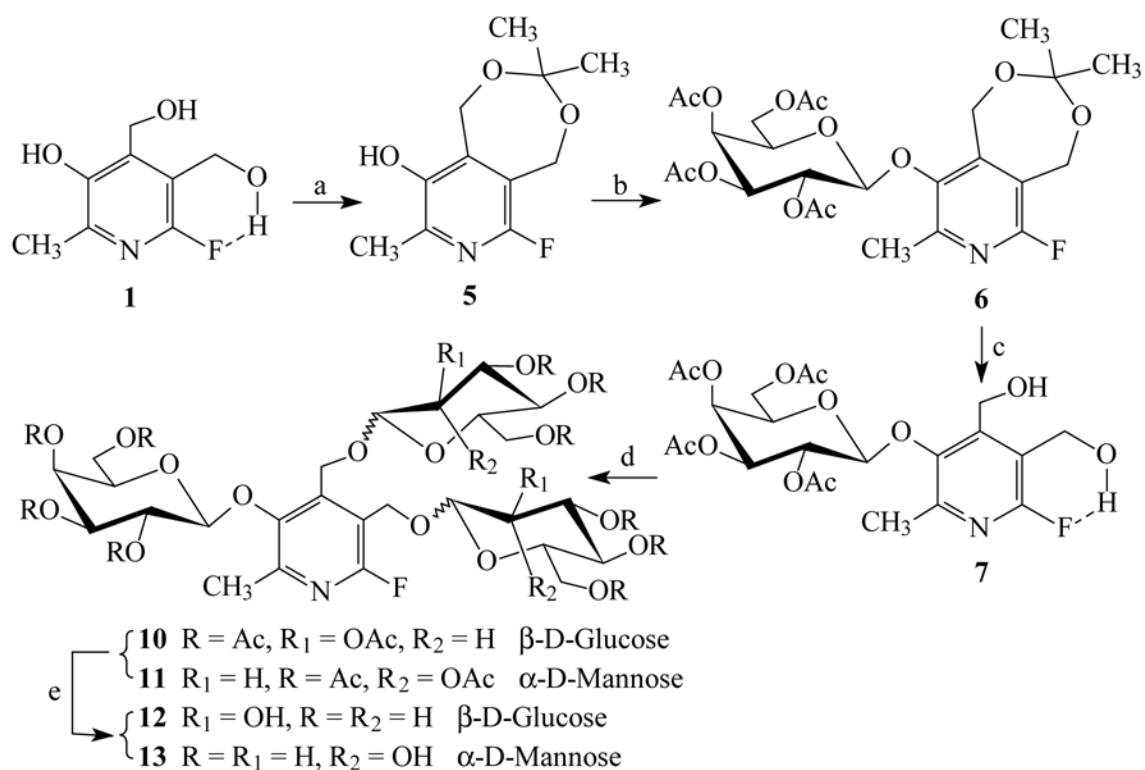
Figure 2.
^{19}F-NMR spectra of 3, α^4, α^5-tri-*O*-(β-*D*-galactopyranosyl)-6-fluoropyridoxol **4** (10.1 mg, 15 mmol, **lower**) and its products resulting from addition of β-gal (E801A, 15 units) in PBS (pH=7.4) at 37 °C (**upper**). Spectra acquired in 51 s and enhanced with an exponential line broadening 40 Hz; β-***D*-Galp** = β-***D*-galactopyranosyl.**

Figure 3. Reagents and conditions
(a) 2% H_2SO_4, acetone, r.t. 4~5 h, 26%; (b) 2, 3, 4, 6-tetra-*O*-acetyl-α-*D*-galactopyranosyl bromide **2**, $Hg(CN)_2$, 4Å M.S., CH_2Cl_2, r.t., 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**, $Hg(CN)_2$, 4Å M.S., CH_2Cl_2, r.t., 12 h, 80%(→**10**) or 78% (→**11**), respectively; (e) NH_3-MeOH, 0°C→r.t., 24 h, quantitative yields.
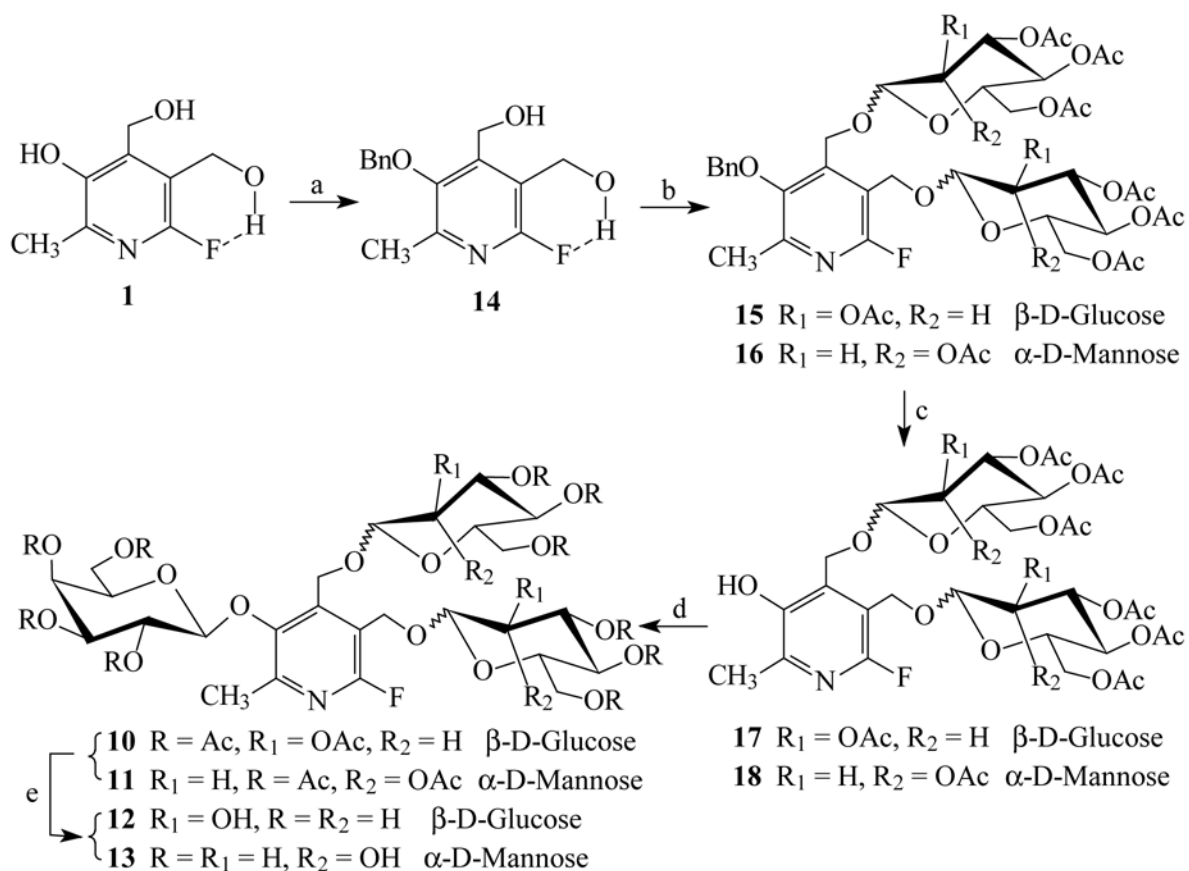
Figure 4. Reagents and conditions
(a) benzyl bromide (1.1 equiv.), CH_2Cl_2-H_2O, pH 10~11, 50°C, TBAB, 4~5 h, 76%; (b) 2, 3, 4, 6-tetra-*O*-acetyl-α-*D*-glucopyranosyl bromide **8** or 2, 3, 4, 6-tetra-*O*-acetyl-α-*D*-mannopyranosyl bromide **9**, Hg(CN)$_2$, 4Å M.S., CH_2Cl_2, r.t., 12 h, 90%(→**15**) or 85% (→**16**), respectively; (c) 25psi H$_2$, Pd/C, r.t., 12 h, quantitative yields; (d) 2, 3, 4, 6-tetra-*O*-acetyl-α-*D*-galactopyranosyl bromide **2**, Hg(CN)$_2$, 4Å M.S., CH_2Cl_2, r.t., 12 h, 88%(→**10**) or 85%(→**11**), respectively; (e) NH$_3$-MeOH, 0°C→r.t., 24 h, 95%(→**12**) or 94%(→**13**), respectively.
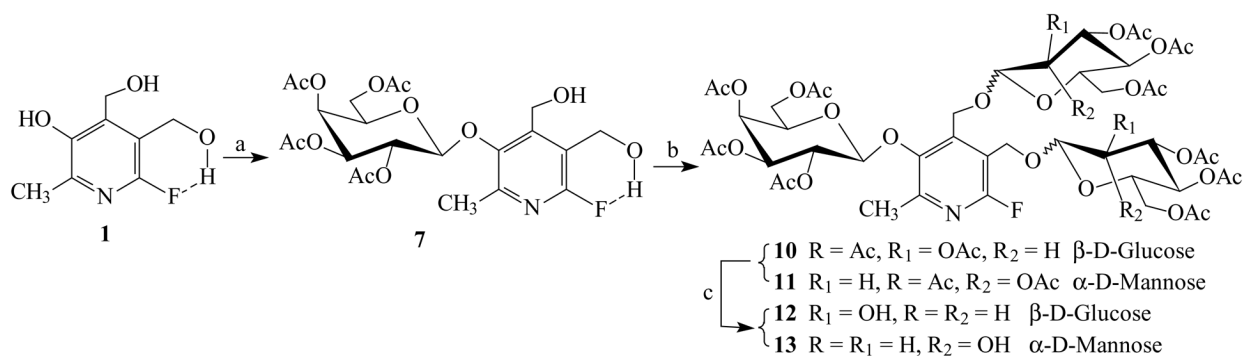
Figure 5. Reagents and conditions

(a) 2, 3, 4, 6-tetra-O-acetyl-α-*D*-galactopyranosyl bromide **2**, CH_2Cl_2-H_2O, pH 10~11, r.t., 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**, $Hg(CN)_2$, 4Å M.S., CH_2Cl_2, r.t., 12 h, 80% (→**10**) or 78%(→**11**), respectively; (c) NH_3-MeOH, 0°C→r.t., 24 h, 95%(→**12**) or 94% (→**13**), respectively.
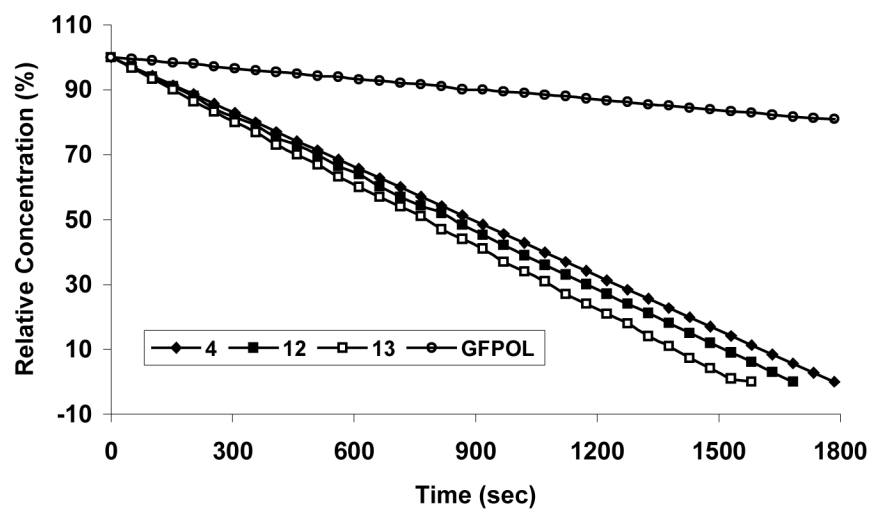
Figure 6.
The kinetic hydrolysis time courses of **4** (◆), **12** ( ), **13** (□) (15.0 mmol each) and **GFPOL** (○) (10.0 mmol) by β-gal (E801A, 15 units) hydrolysis in PBS (0.1 M, pH=7.4, 600 μL) at 37 °C.
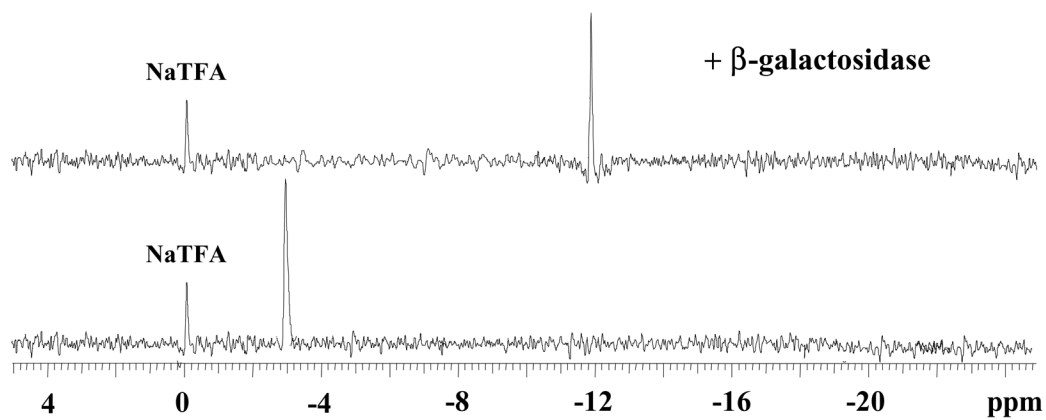
Figure 7.
^{19}F-NMR spectra of 3-*O*-(β-*D*-galactopyranosyl)-α^4, α^5-di-*O*-(β-*D*glucopyranosyl)-6-fluoropyridoxol **12** (10.1 mg, 15 mmol, lower) and its hydrolysis by β-gal (E801A, 15 units) in PBS (0.1 M, pH=7.4, 600 μL) at 37 °C (upper). Spectra acquired in 205 s and enhanced with exponential line broadening = 40 Hz.
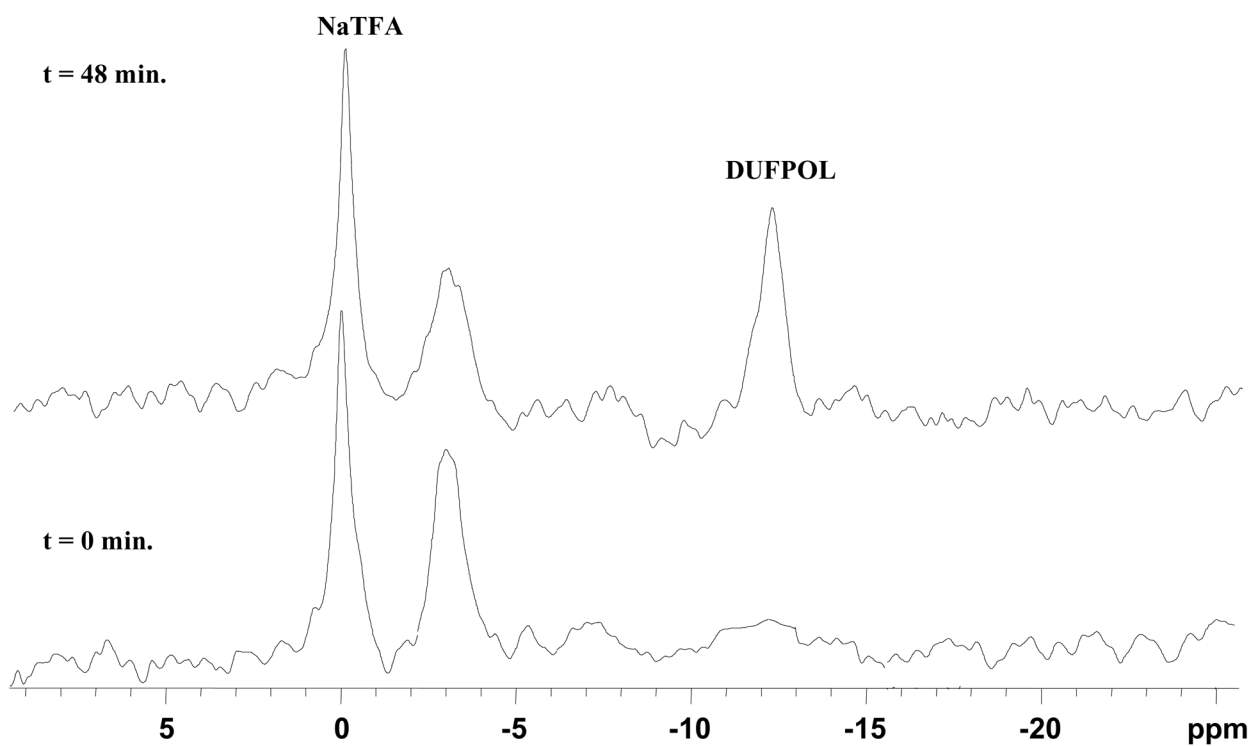
Figure 8.
^{19}F-NMR spectra of 3-*O*-(β-*D*-galactopyranosyl)-α^4, α^5-di-*O*-(β-*D*-glucopyranosyl)-6-fluoropyridoxol **12** (5.1 mg, 7.5 mmol) with stably transfected MCF7-*lacZ* cells (5×10^6) in PBS (0.1M, pH=7.4, 600 μL) at 37 °C. Spectra acquired in 51 s and enhanced with an exponential line broadening = 100 Hz. (**DUFPOL:** α^4, α^5-di-*O*-(β-*D*-glucopyranosyl)-6-fluoropyridoxol).
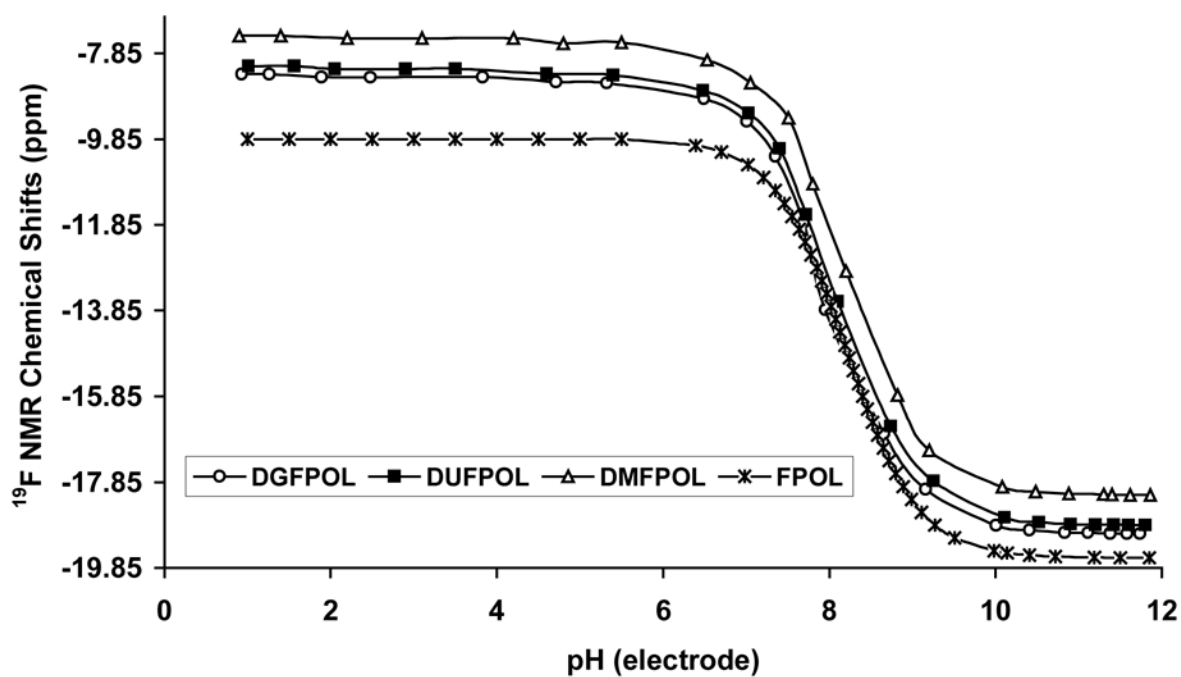
Figure 9.
^{19}F NMR chemical shifts pH titration curve of **DGFPOL**, **DUFPOL** and **DMFPOL** in 0.9% saline at 37°C. (**DGFPOL:** α^4, α^5-di-*O*-(β-*D*-galactopyranosyl)-6-fluoropyridoxol; **DUFPOL:** α^4, α^5-di-*O*-(β-*D*-glucopyranosyl)-6-fluoropyridoxol; **DMFPOL:** α^4, α^5-di-*O*-(α-*D*-mannopyranosyl)-6-fluoropyridoxol).

Table 1

^{19}F chemical shifts [+] and hydrolytic rates [*]

Reporters	4	12	13	GFPOL
δ_F (substrate)	−3.02	−2.85	−2.14	−3.22
δ_F (product)	−12.37	−12.16	−11.22	−11.21
$\Delta\delta_F$	9.35	9.31	9.08	7.99
v (μmol/min/unit)	34.0	35.0	38.0	4.3

[+] ppm with respect to sodium trifluoroacetate.

[*] β-gal (E801A) added at 37°C in PBS (0.1 M, pH=7.4).

Table 2

Acidities and ^{19}F NMR/pH properties of **DGFPOL**, **DUFPOL**, **DMFPOL** and **FPOL** in saline at 25 °C

pH Indicators	DGFPOL	DUFPOL	DMFPOL	FPOL[24]
pKa	7.95	8.08	8.18	8.20
$\delta_{F\ acid}$	−8.34	−8.15	−7.44	−9.85
$\delta_{F\ base}$	−19.05	−18.85	−18.15	−19.61

[+] ppm with respect to sodium trifluoroacetate.