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6-Trifluoromethylpyridoxine: Novel ^{19}F NMR pH Indicator for in Vivo Detection

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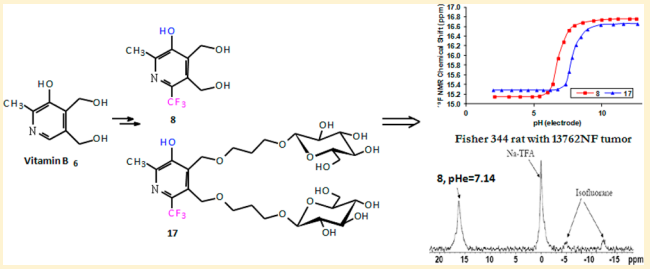
ABSTRACT: pH plays an important role in tumor proliferation, angiogenesis, metabolic control, and the efficacy of cytotoxic therapy, and accurate noninvasive assessment of tumor pH promises to provide insight into developmental processes and prognostic information. In this paper, we report the design, synthesis, and characterization of two novel pH indicators 6-trifluoromethylpyridoxine **8** and α^4,α^5-di-*O*-[3′-*O*-(β-D-glucopyranosyl)propyl]-6-trifluoromethylpyridoxine **17** and demonstrate **8** as an extracellular ^{19}F NMR pH probe to assess pH_e of various tumors in vivo.



INTRODUCTION

The pH gradient between the interstitial and intracellular compartments is involved in many cell regulatory processes and strongly influences drug uptake.[1] Tumor pH also influences cell thermosensitivity, radiation sensitivity, proliferation, and the efficacy of cancer therapy.[2,3] The accurate noninvasive assessment of tumor pH promises to provide insight into the developmental process and prognostic information regarding therapeutic outcome. Previously, we demonstrated that 6-fluoropyridoxine (FPOL, Figure 1) can be used to measure both intra- and extracellular pH simultaneously providing exceptional sensitivity to pH changes in whole blood and the perfused rat heart.[1a,b,4−6] However, its pK_a = 8.2 is not ideal for measurements under normal physiological conditions (pH 6.5−7.5). For the continuing work, we report herein another strategy through introduction of a trifluoromethyl (CF_3) group instead of a fluorine atom at the 6-position of vitamin B_6 with the aim of modifying the pK_a to physiological conditions and raising the ^{19}F signal-to-noise ratio.

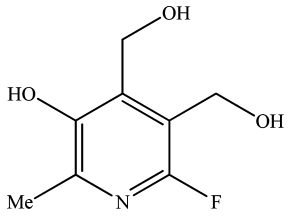


Figure 1. Structure of 6-fluoropyridoxine (FPOL).

RESULTS AND DISCUSSION

Design and Synthesis. The introduction of a trifluoromethyl (CF_3) group into an organic compound can bring about remarkable changes in its physical, chemical, and biological properties, making it suitable for diverse applications in pharmaceutics and agrochemistry.[7,8] We demonstrated that the introduction of a CF_3 group in place of fluorine atom in phenols can enhance the ^{19}F NMR signal and modify the pK_a value.[9,10]

A wide variety of methods have been developed for introducing a CF_3 group into organic compounds,[11] with (trifluoromethyl)trimethylsilane (Me_3SiCF_3) as a nucleophilic trifluoromethylating reagent becoming the method of choice.[12,13] In this study we demonstrate a strategy that utilizes the iodination derivative **3** of vitamin B_6 (**1**) to react with Me_3SiCF_3 for synthesis of target compound 6-trifluoromethylpyridoxine **8** (Figure 2).

A three-step procedure for halogenation of vitamin B_6 via 6-aminopyridoxine has been reported for the synthesis of the ^{19}F NMR pH indicator 6-fluoropyridoxine by the modified Schiemann reaction, resulting in ∼28% overall yield.[1,6,14,15] We have now developed a more effective and direct method for obtaining the key intermediate 6-iodopyridoxine (**2**) in high yield. Reaction of **1** with iodine in 10% aqueous K_2CO_3 solution and avoiding light afforded **2** in 73% yield. For convenient blocking and deprotection, we initially proposed the acetylation as protecting strategy. Acetylation of **2** as the usual workup gave 3,α^4,α^5-tri-*O*-acetyl-6-iodopyridoxine (**3**) in high yield (93%). However, trifluoromethylation of **3** with Me_3SiCF_3 gave the desired compound 3,α^4,α^5-tri-*O*-acetyl-6-trifluoromethylpyridoxine (**4**) in only 40% yield. Further purification separated 12% of α^4,α^5-di-*O*-acetyl-6-trifluoromethylpyridoxine (**5**). Presumably this resulted from the introduction of the highly electron-withdrawing 6-trifluoromethyl group in **4** that makes the para Ac−O_3 bond much polarized and activates its C═O group, which competed against the C_6−I bond in **3** to consume the trifluoromethylation reagent Me_3SiCF_3. The proposed mechanism is depicted in Scheme 1.

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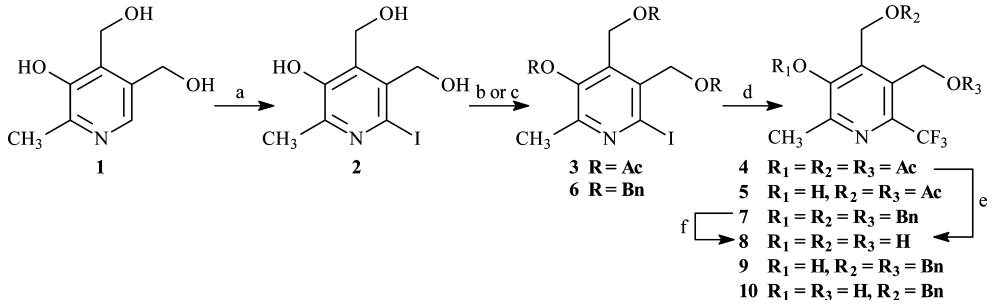
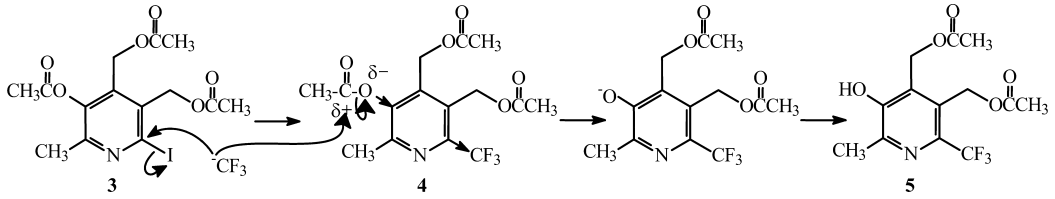


Figure 2. Reagents and conditions: (a) I_2, 10% K_2CO_3, rt 2−3 h, in the dark; Na_2SO_3; HCl, 73%; (b) Ac_2O−pyridine, 0 °C → rt, 24 h, 93% (→**3**); (c) NaH, benzyl bromide (3.3 equiv), DMF, rt, 5−7 h, 100% (→**6**); (d) Me_3SiCF_3 (1.2 equiv), CuI (1.0 equiv), KF (1.2 equiv), Ar, DMF−NMP (1:1 v/v′), 80 °C, 24 h, 40% (→**4**) or 96% (→**7**); (e) NH_3−MeOH, 0 °C → rt, 24 h, quantitative yield (→**8**); (f) H_2 (30 psi), AcOH/EtOH (1:10 v/v′), Pd/C (5% w/w′), rt, 2 days, quantitative yield (→**8**).

Scheme 1. Proposed Reaction Mechanism for the Formation of 5



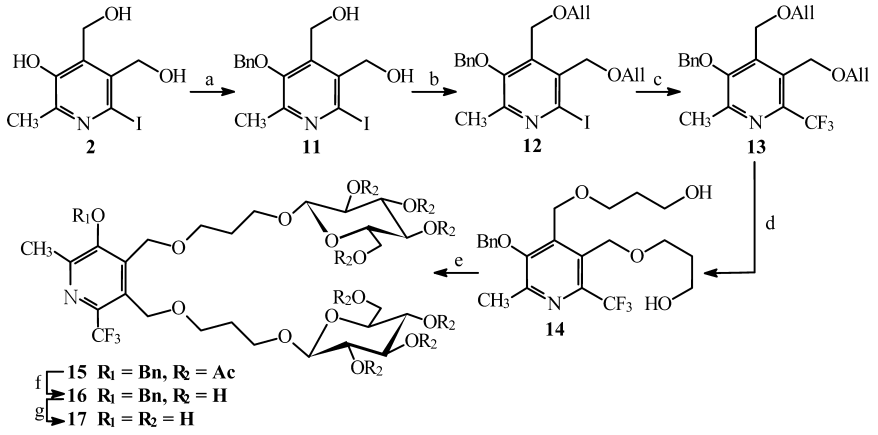


Figure 3. Reagents and conditions: (a) benzyl bromide (1.1 equiv), CH_2Cl_2−H_2O, pH 9−10, rt, TBAB, 4−5 h, 74%; (b) NaH, allyl bromide (1.5 equiv), DMF, rt, 4 h, 100%; (c) Me_3SiCF_3 (1.2 equiv), CuI (1.0 equiv), KF (1.2 equiv), Ar, DMF-NMP (1:1 v/v′), 80 °C, 24 h, 92%; (d) 9-BBN (2.0 equiv), Ar, dioxane, 0 °C, 24 h; NaOH, H_2O_2, rt, 48 h, 86%; (e) 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (1.2 equiv), $Hg(CN)_2$ (1.5 equiv), 4 Å molecular sieves, CH_2Cl_2, rt, 12 h, 88%; (f) NH_3−MeOH, 0 °C → rt, 48 h, quantitative yield; (g) 30 psi of H_2, EtOH, Pd/C (5% w/w′), rt, 12 h, quantitative yield.

To improve the process, we employed benzyl ether as an alternative protecting group for 3,α^4,α^5-hydroxyl. Benzylation of **2** with benzyl bromide afforded 3,α^4,α^5-tri-*O*-benzyl-6-iodopyridoxine (**6**) in quantitative yield, which reacted with Me_3SiCF_3 in a similar procedure for the preparation of **4**, giving 3,α^4,α^5-tri-*O*-benzyl-6-trifluoromethylpyridoxine (**7**) in excellent yield (96%). Overnight hydrogenation of **7** in ethanolic solution with the catalyst of 5% Pd/C provided the partial debenzylation product α^4,α^5-di-*O*-benzyl-6-trifluoromethylpyridoxine (**9**) in quantitative yield. However, the α^4,α^5-di-*O*-benzyl groups could not be removed even with extended reaction times up to 1 week. Testing various acids as cosolvents and catalysts showed that anhydrous AcOH−EtOH (1:10 v/v′) allowed stepwise cleavage of the 3,α^5-di-*O*-benzyl groups in **7**, yielding **10** in 1 day and then the α^4-*O*-benzyl group in another day, resulting in **8** with total yield of 100%. As expected, 6-trifluoromethylpyridoxine **8** yielded higher signal-to-noise than 6-fluoropyridoxine (FPOL) and its derivatives.[1a,b] Importantly, it has an ideal pK_a = 6.83 but with much less ^{19}F NMR sensitivity to pH ($\Delta\delta$ = 1.61 ppm) and poorer water solubility ((FPOL) 17.8 mg/mL and (**8**) 5.8 mg/mL in H_2O at room temperature).

We found that modification of 4- and/or 5-methylenehydroxyl moieties of 6-fluoropyridoxine resulted in changes of the pK_a and ^{19}F chemical shifts.[1a,b] Previously, we successfully enhanced the solubility of ^{19}F NMR and ^{1}H MRI β-galactosidase reporters by conjugating them with carbohydrates.[16] Prompted by these results, we designed another novel molecule **17** with two D-glucoses coupled to the 4- and 5-methylenehydroxyl moieties of 6-trifluoromethylpyridoxine **8** (Figure 3).

Starting with **2** as the initial molecule, the primary challenge was the regioselective protection among its three hydroxyl groups. However, pK_a calculations using the advanced chemistry development software (www.acdlabs.com) indicated

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that the 3-phenolic hydroxyl (pK_a = 9.27 ± 0.10) is much more acidic than 4- and 5-methylenehydroxyls (pK_a = 13.31 ± 0.10 and 13.81 ± 0.10, respectively), which suggests that phase-transfer-catalysis at pH 9−10 could provide regioselective protection of the 3-phenolic hydroxyl. To the well-stirred mixture of **2** in biphasic CH_2Cl_2−H_2O (pH 9−10) at room temperature, which was catalyzed by tetrabutylammonium bromide (TBAB), was added benzyl bromide (1.1 equiv) dropwise over a period of 4−5 h. 3-*O*-Benzyl-6-iodopyridoxine **11** was isolated as a major product in 74% yield. Its stereochemistry was confirmed by its ^{1}H NMR spectrum where α^4,α^5-CH_2 exhibits doublets with coupling constants of $J_{H\text{-}4,HO\text{-}4}$ = 5.6 Hz at 4.87 ppm and $J_{H\text{-}5,HO\text{-}5}$ = 6.4 Hz at 4.73 ppm. Treatment of **11** with an excess of allyl bromide gave 3-*O*-benzyl-α^4,α^5-di-*O*-allyl-6-iodopyridoxine **12** in quantitative yield. It was then subjected to the procedure described for the preparation of **4** and **7**, giving 3-*O*-benzyl-α^4,α^5-di-*O*-allyl-6-trifluoromethylpyridoxine **13** in excellent yield (92%). The diallylated derivative **13** underwent with regioselective hydroboration by using 9-borabicyclo-[3.3.1]nonane (9-BBN) and subsequent alkaline oxidation with H_2O_2, giving 3-*O*-benzyl-α^4,α^5-di-*O*-(3-hydroxypropyl)-6-trifluoromethylpyridoxine **14** in 86% yield.

Condensation of **14** with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl bromide gave 3-*O*-benzyl-α^4,α^5-di-*O*-[3′-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)propyl]-6-trifluoromethylpyridoxine **15** in 88% yield. The ESI-MS displayed the expected molecular ion at *m*/*z* 1103 and quasimolecular ion at *m*/*z* 1104 [M + H], corresponding to the fully adorned derivative with two 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl units. The identity of **15** was established using ^{1}H and ^{13}C NMR based on the anomeric protons H-1′ and H-1″ of D-glucoses at 4.48 and 4.41 ppm, respectively, with two well resolved doublets $J_{1',2'} = J_{1'',2''} \approx 8.0$ Hz and $J_{2',3'} = J_{2'',3''} \approx 10$ Hz, which confirmed both D-glucoses in the β-configuration with $^4C_1$ chair conformation, and the anomeric carbons C-1′ and C-1″ at 101.02 ppm are in accordance.

Compound **15** was deacetylated with NH_3/MeOH from 0 °C to room temperature, giving 3-*O*-benzyl-α^4,α^5-di-*O*-[3′-*O*-(β-D-glucopyranosyl)propyl]-6-trifluoromethylpyridoxine **16** in quantitative yield, followed by hydrogenation catalyzed with 5% Pd/C overnight, affording quantitative yield of target compound α^4,α^5-di-*O*-[3′-*O*-(β-D-glucopyranosyl)propyl]-6-trifluoromethylpyridoxine **17** with an overall yield of 52%.

Characterization as ^{19}F NMR pH Indicator. The ^{19}F chemical shifts upon pH changes (pH electrode) were measured with respect to sodium trifluoroacetate (NaTFA, δ_F = 0 ppm). Both **8** and **17** exhibited single narrow ^{19}F NMR signals in 0.9% saline or PBS essentially invariant ($\Delta\delta \leq 0.03$ ppm) with temperatures ranging from 25 to 37 °C. Figure 4 shows the titration curves of **8** and **17** in saline between pH 2 and pH 13 at 25 °C. From the titration curves their pK_a, $\delta_{(acid)}$, and $\delta_{(base)}$ were determined from the Henderson−Hasselbach equation[1a,b] (Table 1).

Given that both **8** and **17** show feasibility as ^{19}F NMR pH indicators, **8** has a more ideal pK_a for sampling biological system in vivo, making it favored for further evaluation.

^{19}F NMR pH Assessment in Perfused Heart and Whole Blood. The ^{19}F NMR spectrum (376 MHz) of **8** in Langendorff perfused rat heart exhibited only a single ^{19}F resonance at δ_F = 16.41 ppm corresponding to pH 7.39 (Figure 5a). The intra- and extracellular inorganic phosphates of Langendorff perfused rat heart was sampled by ^{31}P NMR,

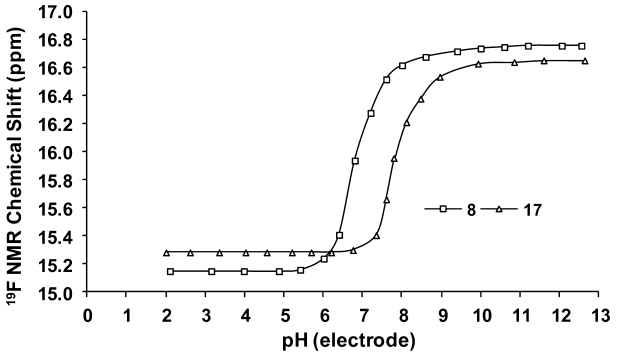

Figure 4. Titration curves of **8** and **17** in saline at 25 °C.

Table 1. Acidities and ^{19}F NMR/pH Properties of 8 and 17 at 25 °C

		^{19}F NMR				
compd	pK_a	$\delta_{(acid)}$, ppm	$\delta_{(base)}$, ppm	$\Delta\delta$, ppm	ppm/pH unit	pH range
8	6.83	15.15	16.76	1.61	0.40	5.40−9.40
17	7.84	15.28	16.66	1.38	0.43	6.75−9.93

which provided the intracellular pH_i 7.04 (δ_{Pi} = 4.88 ppm) and extracellular pH_e 7.42 (δ_{Pe} = 5.32 ppm).[1a,b] When the ^{19}F NMR of **8** is compared to these results, it becomes evident that **8** does not enter cells and reports only the extracellular pH_e. This was confirmed by the ^{19}F NMR spectrum of **8** in whole rabbit blood which showed a single ^{19}F peak at δ_F = 16.52 ppm (pH_e = 7.44) (Figure 5b).

In Vivo ^{19}F NMR pH Measurements in Tumors. Extracellular pH_e in human tumors has been shown to be associated with tumorigenic transformation, chromosomal rearrangements, induction of growth factors and proteases, extracellular matrix breakdown, and increased migration and invasion.[2c,e] To evaluate the efficacy of this novel ^{19}F NMR pH_e reporter molecule for sampling the tumor microenvironment, it was directly injected into rats bearing pedicle tumors. First, pH_e reporter **8** (320 mg/kg) and NaTFA (200 mg/kg) in DMSO/saline (1:3 v/v′) were injected ip into an anesthetized (with isoflurane) Copenhagen rat bearing a Dunning R3327-AT1 prostate tumor (tumor size, 2.4 cm × 3.1 cm × 1.8 cm). **8** was detected by ^{19}F NMR in the tumor 30 min after injection. The single broad ^{19}F signal centered at δ_F = 16.28 ppm indicated tumor heterogeneity with mean pH_e of 7.20.[1b]

Similarly, **8** was detected in a 13762NF rat mammary tumor (tumor size, 1.8 cm × 1.0 cm × 2.0 cm) after 8 min following an ip injection of the same doses into a Fisher 344 rat. A broad single ^{19}F resonance was obtained at δ_F = 16.23 ppm (pH_e 7.14) with biological clearance over 100 min (Figure 6A). This pH was commensurate with microelectrode measurements in three Fisher 344 rats bearing 13762NF breast tumors of 3.2, 7.5, and 9.2 cm^3, showing the most frequent pH_e of 7.03 (Figure 6B).

Encouraged by these in vivo measurements of tumor pH_e, we also investigated 6-trifluoromethylpyridoxine **8** in other animal models. After ip injection of **8** (12 mg, 54 μmol) in DMSO/H_2O (1:3 v/v′) into a nude mouse bearing a MatLu rat prostate tumor grown on the thigh (tumor size, 1.6 cm × 1.4 cm × 1.0 cm), a broad, single ^{19}F peak was observed at $\delta_{F(8)}$ = 15.96 ppm (pH_e 6.48) with an acquisition time of 7 min (Figure 7).

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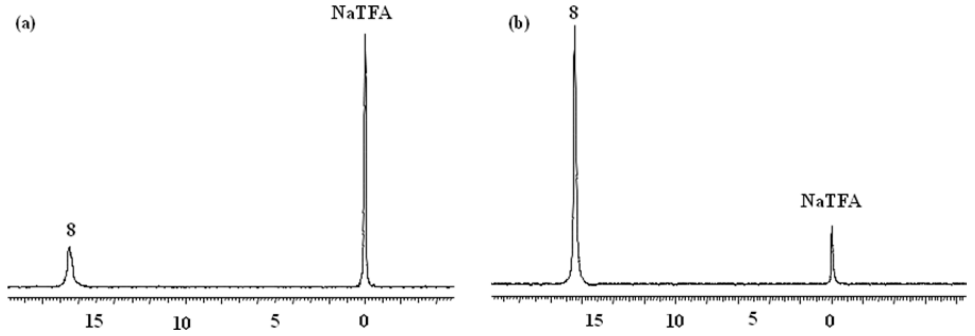

Figure 5. ^{19}F NMR spectra (376 MHz, 25 °C) of **8** in (a) Langendorff perfused rat heart, δ_F = 16.41 ppm (pH$_e$ 7.39) and (b) whole rabbit blood, δ_F = 16.52 ppm (pH$_e$ 7.44).

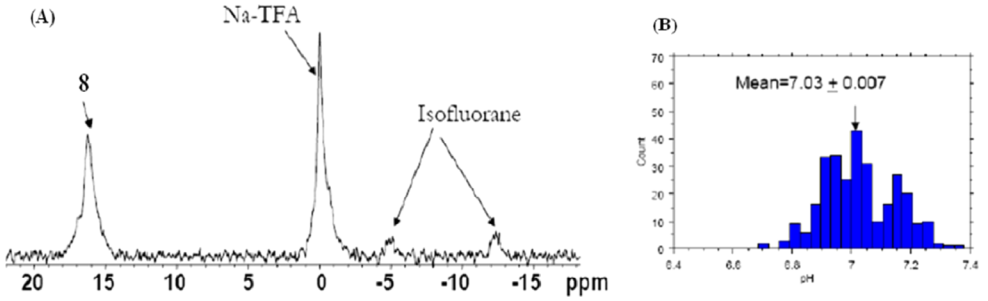

Figure 6. (A) In vivo ^{19}F NMR spectrum (188 MHz, 37 °C) of **8** (320 mg/kg) in Fisher 344 rat bearing pedicle 13762NF breast tumor: ip injection, tumor size of 1.8 cm × 1.0 cm × 2.0 cm, δ_F = 16.23 ppm corresponding to pH$_e$ 7.14, acquisition time of 8 min. (B) Distribution of pH$_e$ values measured in a group of three Fisher 344 rats bearing 13762NF breast tumors using needle electrode: mean pH of 7.03 (arrow).

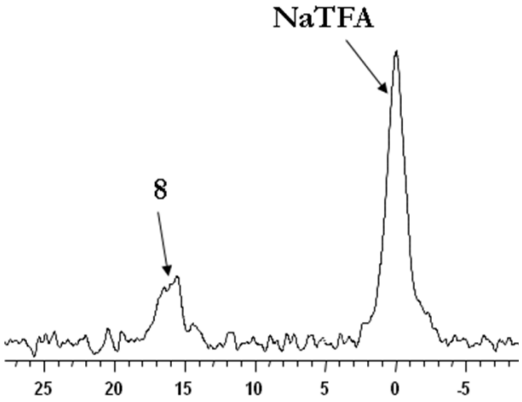

Figure 7. In vivo ^{19}F NMR spectrum (188 MHz, 37 °C) of **8** (12 mg) in nude mouse bearing MatLu rat prostate tumor on thigh: ip injection, tumor size of 1.6 cm × 1.4 cm × 1.0 cm, $\delta_{F(8)}$ = 15.96 ppm corresponding to pH$_e$ 6.48, acquisition time of 7 min.

CONCLUSION

Given the relevance of pH$_i$/pH$_e$ to tumor development and prognostic outcome, noninvasive techniques to sample cellular pH in vivo have great potential and are increasingly important in therapeutic oncology.[1−3] As the most electronegative element, fluorine has played a key role in medicinal chemistry; the incorporation of fluorine and/or fluorine-containing groups into an organic molecule often drastically perturbs the properties of the parent compound.[7,8] ^{19}F MRS has been widely utilized in in vivo studies on drug absorption, distribution, metabolism, and excretion because of its favorable MR properties, simplicity, and high sensitivity.[1a,b] In this study, we have successfully synthesized two novel ^{19}F NMR pH indicators **8** and **17** and identified the following useful characteristics that make them well-suited for the in vivo assessment of pH using ^{19}F MRS: (a) ideal pK_a (6.83−7.84); (b) sensitivity to pH (∼0.40 ppm/pH unit); (c) ^{19}F chemical shift response to pH ($\Delta\delta_F$ = 1.38−1.61 ppm); (d) ^{19}F signal enhancement, in which **8** was shown to be capable of in vivo sampling pH$_e$ in various tumor models. Noting these features of **8** as a ^{19}F MRS pH$_e$ molecular probe, we believe it has promising potential in ^{19}F MRI investigations of the tumor microenvironment for effective characterization of tumor heterogeneity with spatial and temporal resolution.

EXPERIMENTAL SECTION

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, 121 MHz for ^{31}P). ^{1}H and ^{13}C chemical shifts are referenced to TMS as internal standard with $CDCl_3$ or DMSO-d_6 as solvents. ^{19}F shifts are referenced to a dilute solution of NaTFA in a capillary as external standard. Chemical shifts are given in ppm. Mass spectra were obtained by positive and negative ESI-MS using a Micromass Q-TOF hybrid quadrupole/time-of-flight instrument (Micromass UK Ltd.). Microanalyses were performed on a Perkin-Elmer 2400 CHN microanalyser.

$Hg(CN)_2$ 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 nitrogen. 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-glucopyranosyl bromide was purchased from the Sigma Chemical Co. Column chromatography was performed on silica gel (200−300 mesh), and silica gel GF_{254} used for TLC was purchased from the Aldrich Chemical Co. 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. The purity of the final products was determined by HPLC with ≥95%.

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6-Iodopyridoxine 2. To a solution of pyridoxine **1** (3.4 g, 20.0 mmol) in 10% K_2CO_3 aqueous solution (60 mL) was added iodine (5.04 g, 20.0 mmol). The reaction mixture was vigorously stirred in the dark at room temperature for 2−3 h. After addition of Na_2SO_3 (320 mg), the reaction was quenched with concentrated HCl up to pH 3. Then the precipitate was filtered and dried in vacuo over NaOH to give **2** (4.28 g, 73%) as a yellow powder. ^{1}H NMR (DMSO-d_6), δ_H: 9.51 (1 H, s, HO-3), 5.82 (1 H, br, α^5-OH), 5.15 (1H, br, α^4-OH), 4.80 (2 H, d, J = 2.8 Hz, CH_2-5), 4.57 (2 H, d, J = 3.2 Hz, CH_2-4), 2.31 (3 H, s, CH_3-2) ppm. ^{13}C NMR (DMSO-d_6), δ_C: 150.43 (s, Py-C), 148.25 (s, Py-C), 136.21 (s, Py-C), 134.97 (s, Py-C), 112.11 (s, Py-C), 63.99 (s, CH_2-5), 57.05 (s, CH_2-4), 18.93 (s, CH_3-2) ppm. Anal. Calcd for $C_8H_{10}NO_3I$ (%): C, 32.56; H, 3.42; N, 4.75. Found: C, 32.51; H, 3.39; N, 4.71.

3,α^4,α^5-Tri-*O*-acetyl-6-iodopyridoxine 3. A solution of **2** (0.90 g, 3.0 mmol) in pyridine (20 mL) was treated with acetic anhydride (9 mL). After being stirred from 0 °C to room temperature overnight, the mixture was coevaporated with toluene under reduced pressure and the residue purified by flash silica gel column chromatography (eluent, 2:1 cyclohexane−EtOAc) to afford **3** (1.19 g, 93%) as white crystals. R_f = 0.50 (3:2 cyclohexane−EtOAc). ^{1}H NMR ($CDCl_3$), δ_H: 5.18 (2 H, s, CH_2-5), 5.15 (2 H, s, CH_2-4), 2.40 (3 H, s, CH_3-2), 2.05, 2.03, 2.01 (9 H, 3s, 3 × CH_3CO) ppm. ^{13}C NMR ($CDCl_3$), δ_C: 173.97, 170.73, 168.43 (3s, 3 × CH_3CO), 152.83 (s, Py-C), 150.33 (s, Py-C), 132.99 (s, Py-C), 129.83 (s, Py-C), 113.02 (s, Py-C), 66.33 (s, CH_2-5), 58.76 (s, CH_2-4), 20.94, 20.88, 19.63 (3s, 3 × **C**H_3CO), 19.61 (s, CH_3-2) ppm. Anal. Calcd for $C_{14}H_{16}NO_6I$ (%): C, 39.92; H, 3.83; N, 3.33. Found: C, 39.90; H, 3.80; N, 3.30.

3,α^4,α^5-Tri-*O*-acetyl-6-trifluoromethylpyridoxine 4. To a well stirred mixture of **3** (1.0 g, 2.4 mmol), CuI (456 mg, 2.4 mmol, 1.0 equiv), and KF (168 mg, 2.9 mmol, 1.2 equiv) in *N*,*N*-dimethylformamide (DMF, 5 mL) and *N*-methylpyrrolidine (NMP, 5 mL) was added Me_3SiCF_3 (537 μL, 2.9 mmol, 1.2 equiv) under an argon atmosphere in the dark. Then the reaction mixture was stirred at 80 °C in a sealed glass pressure tube (15 mL) for 24 h. The mixture was diluted with CH_2Cl_2 (120 mL), filtered through Celite, washed with water, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified on a silica gel column (2:1 cyclohexane−EtOAc) to yield **4** (0.35 g, 40%) as a syrup. R_f = 0.37 (2:1 cyclohexane−EtOAc). ^{1}H NMR ($CDCl_3$), δ_H: 5.34 (2 H, s, CH_2-5), 5.16 (2 H, s, CH_2-4), 2.39 (3 H, s, CH_3-2), 2.38, 2.10, 2.02 (9 H, 3s, 3 × CH_3CO) ppm. ^{13}C NMR ($CDCl_3$), δ_C: 170.54, 170.22, 168.41 (3s, 3 × CH_3**C**O), 145.04 (s, Py-$C_{2'}$), 137.81 (s, Py-$C_{3'}$), 154.80 (s, Py-$C_{4'}$), 133.90 (q, $^3J_{F-C}$ = 20.6 Hz, Py-$C_{5'}$), 140.27 (q, $^2J_{F-C}$ = 32.6 Hz, Py-$C_{6'}$), 121.53 (q, $^1J_{F-C}$ = 272.6 Hz, CF_3), 66.27 (s, CH_2-5), 57.39 (s, CH_2-4), 20.89, 20.72, 20.68 (3s, 3 × **C**H_3CO), 19.63 (s, CH_3-2) ppm. Anal. Calcd for $C_{15}H_{16}NO_6F_3$ (%): C, 49.59; H, 4.44; N, 3.86. Found: C, 49.55; H, 4.43; N, 3.84.

α^4,α^5-Di-*O*-acetyl-6-trifluoromethylpyridoxine 5. Yield: 88 mg, 12% as a syrup. R_f = 0.27 (2:1 cyclohexane−EtOAc). ^{1}H NMR ($CDCl_3$), δ_H: 5.24 (2 H, s, CH_2-5), 5.06 (2 H, s, CH_2-4), 2.33 (3 H, s, CH_3-2), 2.09, 2.02 (6 H, 2s, 2 × CH_3CO) ppm. ^{13}C NMR ($CDCl_3$), δ_C: 170.12, 168.38 (2s, 2 × CH_3**C**O), 144.84 (s, Py-$C_{2'}$), 137.41 (s, Py-$C_{3'}$), 154.77 (s, Py-$C_{4'}$), 133.30 (q, $^3J_{F-C}$ = 18.6 Hz, Py-$C_{5'}$), 141.67 (q, $^2J_{F-C}$ = 32.8 Hz, Py-$C_{6'}$), 121.13 (q, $^1J_{F-C}$ = 272.5 Hz, CF_3), 66.17 (s, CH_2-5), 57.29 (s, CH_2-4), 20.68, 20.57 (2s, 2 × **C**H_3CO), 19.56 (s, CH_3-2) ppm. Anal. Calcd for $C_{13}H_{14}NO_5F_3$ (%): C, 48.60; H, 4.39; N, 4.36. Found: C, 48.55; H, 4.36; N, 4.32.

3,α^4,α^5-Tri-*O*-benzyl-6-iodopyridoxine 6. A solution of benzyl bromide (1.43 g, 8.0 mmol) in dry DMF (15 mL) was added dropwise over a period of 1−2 h to a well stirred dry DMF (70 mL) solution of **2** (0.69 g, 2.4 mmol) and NaH (341 mg, 8.6 mmol, 60% dispersion in mineral oil), and the stirring continued for an additional 4−5 h. At the end of the time, TLC (4:1 cyclohexane−EtOAc) showed the reaction to be complete. Then MeOH (15 mL) was added slowly to react with the excess of the NaH. After most of the DMF was removed under reduced pressure at 55 °C, the residue was dissolved in CH_2Cl_2 (125 mL) and washed with water, dried (Na_2SO_4), filtered, and evaporated. The residue was purified by column chromatography on silica gel with 4:1 cyclohexane−EtOAc as the eluent to afford quantitatively **6** (1.32 g) as a syrup. R_f = 0.56 (4:1 cyclohexane−EtOAc). ^{1}H NMR ($CDCl_3$), δ_H: 7.38−7.27 (15 H, m, Ph-H), 4.84 (2 H, s, Ph**C**H_2-O_3), 4.64 (2 H, s, α^4-O**C**H_2Ph), 4.56 (2 H, s, CH_2-4), 4.54 (2 H, s, CH_2-5), 4.41 (2 H, s, α^5-O**C**H_2Ph), 2.48 (3 H, s, CH_3-2) ppm. ^{13}C NMR ($CDCl_3$), δ_C: 155.28 (s, Py-C), 152.76 (s, Py-C), 140.29 (s, Py-C), 136.24 (s, Py-C), 128.66−128.16 (m, Ph−C), 119.57 (s, Py-C), 76.97 (s, Ph**C**H_2-O_3), 73.68 (s, α^4-O**C**H_2Ph), 73.51 (s, α^5-O**C**H_2Ph), 71.87 (s, CH_2-4), 63.24 (s, CH_2-5), 19.74 (s, CH_3-2) ppm. Anal. Calcd for $C_{29}H_{28}NO_3I$ (%): C, 61.60; H, 4.99; N, 2.48. Found: C, 61.56; H, 4.96; N, 2.45.

3,α^4,α^5-Tri-*O*-benzyl-6-trifluoromethylpyridoxine 7. Trifluoromethylation of **6** (0.89 g, 1.6 mmol) with Me_3SiCF_3 (356 μL, 1.9 mmol, 1.2 equiv) in the presence of CuI (304 mg, 1.6 mmol, 1.0 equiv) and KF (110 mg, 1.9 mmol, 1.2 equiv) in DMF−NMP (10 mL, 1:1 v/v′) under an argon atmosphere in the dark, according to the procedures described for the preparation of **4**, furnished 3,α^4,α^5-tri-*O*-benzyl-6-trifluoromethylpyridoxine **7** (0.78 g, 96%) as a syrup. R_f = 0.64 (4:1 cyclohexane−EtOAc). ^{1}H NMR ($CDCl_3$), δ_H: 7.40−7.28 (15 H, m, Ph-H), 4.89 (2 H, s, Ph**C**H_2-O_3), 4.65 (2 H, s, α^4-O**C**H_2Ph), 4.59 (2 H, s, CH_2-4), 4.45 (2 H, s, CH_2-5), 4.40 (2 H, s, α^5-O**C**H_2Ph), 2.54 (3 H, s, CH_3-2) ppm. ^{13}C NMR ($CDCl_3$), δ_C: 141.44 (s, Py-$C_{2'}$), 136.50 (s, Py-$C_{3'}$), 153.44 (s, Py-$C_{4'}$), 137.60 (q, $^3J_{F-C}$ = 21.4 Hz, Py-$C_{5'}$), 142.07 (q, $^2J_{F-C}$ = 32.1 Hz, Py-$C_{6'}$), 122.36 (q, $^1J_{F-C}$ = 273.9 Hz, CF_3), 128.92−128.10 (m, Ph-C), 73.86 (s, Ph**C**H_2-O_3), 73.70 (s, α^4-O**C**H_2Ph), 64.08 (s, α^5-O**C**H_2Ph), 64.05 (s, CH_2-4), 62.63 (s, CH_2-5), 20.01 (s, CH_3-2) ppm. Anal. Calcd for $C_{30}H_{28}NO_3F_3$ (%): C, 70.99; H, 5.56; N, 2.76. Found: C, 70.96; H, 5.54; N, 2.73.

6-Trifluoromethylpyridoxine 8. Hydrogenation (H_2, 30 psi) of **7** (0.70 g, 1.4 mmol) in anhydrous AcOH−EtOH (70 mL, 1:10 v/v′) catalyzed by Pd/C (5%, 250 mg) for 2 days furnished the target molecule **8** (0.33 g, 100%) as crystals. R_f = 0.34 (1:1 cyclohexane−EtOAc). ^{1}H NMR (DMSO-d_6), δ_H: 4.88 (2 H, s, CH_2-5), 4.59 (2 H, s, CH_2-4), 4.50 (3 H, br, 3-OH, α^4-OH, α^5-OH), 2.40 (3 H, s, CH_3-2) ppm. ^{13}C NMR (DMSO-d_6), δ_C: 132.23 (s, Py-$C_{2'}$), 134.51 (s, Py-$C_{3'}$), 153.97 (s, Py-$C_{4'}$), 145.85 (s, Py-$C_{5'}$), 133.51 (q, $^2J_{F-C}$ = 31.3 Hz, Py-$C_{6'}$), 122.94 (q, $^1J_{F-C}$ = 272.4 Hz, CF_3), 56.55 (s, CH_2-4), 55.21 (s, CH_2-5), 19.27 (s, CH_3-2) ppm. Anal. Calcd for $C_9H_{10}NO_3F_3$ (%): C, 45.58; H, 4.25; N, 5.91. Found: C, 45.54; H, 4.23; N, 5.89.

α^4,α^5-Di-*O*-benzyl-6-trifluoromethylpyridoxine 9. Yield: 0.58 g, 100%, syrup. R_f = 0.52 (4:1 cyclohexane−EtOAc). ^{1}H NMR ($CDCl_3$), δ_H: 8.22 (1 H, s, 3-OH), 7.62−7.55 (10 H, m, Ph-H), 4.91 (2 H, s, α^4-O**C**H_2Ph), 4.79 (2 H, s, CH_2-4), 4.73 (2 H, s, CH_2-5), 4.72 (2 H, s, α^5-O**C**H_2Ph), 2.66 (3 H, s, CH_3-2) ppm. ^{13}C NMR ($CDCl_3$), δ_C: 138.12 (s, Py-$C_{2'}$), 138.17 (s, Py-$C_{3'}$), 149.47 (s, Py-$C_{4'}$), 147.26 (s, Py-$C_{5'}$), 140.02 (q, $^2J_{F-C}$ = 32.3 Hz, Py-$C_{6'}$), 121.54 (q, $^1J_{F-C}$ = 273.2 Hz, CF_3), 128.50−127.20 (m, Ph-C), 71.96 (s, α^4-O**C**H_2Ph), 71.64 (s, α^5-O**C**H_2Ph), 67.09 (s, CH_2-4), 62.58 (s, CH_2-5), 19.88 (s, CH_3-2) ppm. Anal. Calcd for $C_{23}H_{22}NO_3F_3$ (%): C, 66.18; H, 5.31; N, 3.36. Found: C, 66.13; H, 5.30; N, 3.34.

α^4-*O*-Benzyl-6-trifluoromethylpyridoxine 10. Yield: 0.46 g, 100%, syrup. R_f = 0.34 (4:1 cyclohexane−EtOAc). ^{1}H NMR (DMSO-d_6), δ_H: 7.38−7.34 (5 H, m, Ph-H), 5.05 (2 H, s, α^4-O**C**H_2Ph), 4.70 (2 H, br, 3-OH, α^5-OH), 4.63 (2 H, s, CH_2-4), 4.54 (2 H, d, $J_{H\text{-}5,HO\text{-}5}$ = 6.2 Hz, CH_2-5), 2.52 (3 H, s, CH_3-2) ppm. ^{13}C NMR (DMSO-d_6), δ_C: 132.03 (s, Py-$C_{2'}$), 137.30 (s, Py-$C_{3'}$), 153.76 (s, Py-$C_{4'}$), 147.93 (s, Py-$C_{5'}$), 136.58 (q, $^2J_{F-C}$ = 32.6 Hz, Py-$C_{6'}$), 122.54 (q, $^1J_{F-C}$ = 272.8 Hz, CF_3), 128.78−128.27 (m, Ph-C), 73.22 (s, α^4-O**C**H_2Ph), 64.40 (s, CH_2-4), 61.07 (s, CH_2-5), 18.99 (s, CH_3-2) ppm. Anal. Calcd for $C_{16}H_{16}NO_3F_3$ (%): C, 58.71; H, 4.93; N, 4.28. Found: C, 58.68; H, 4.90; N, 4.27.

3-*O*-Benzyl-6-iodopyridoxine 11. To a well stirred CH_2Cl_2−H_2O (20 mL, 1:1 v/v′) biphasic mixture (pH 9−10) of **2** (1.0 g, 3.4 mmol) and TBAB (0.10 g, 0.31 mmol) as the phase-transfer catalyst was added a solution of benzyl bromide (0.65 g, 3.73 mmol, 1.1 equiv) in CH_2Cl_2 (10 mL) dropwise over a period of 4−5 h at room temperature, and the stirring continued for an additional hour. The mixture was extracted with CH_2Cl_2 (4 × 20 mL), washed free of alkali, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel with 3:2 cyclohexane−EtOAc as the eluent to afford major product **11** (1.31 g, 74%) as a white

crystalline solid. R_f = 0.68 (1:2 cyclohexane−EtOAc). ^{1}H NMR ($CDCl_3$), δ_H: 7.41−7.37 (5 H, m, Ar−H), 4.92 (2 H, s, $PhCH_2$), 4.87 (2 H, d, $J_{H\text{-}4,HO\text{-}4}$ = 5.6 Hz, CH_2-4), 4.73 (2 H, d, $J_{H\text{-}5,HO\text{-}5}$ = 6.4 Hz, CH_2-5), 3.73 (2 H, br, α^4-OH, α^5-OH, exchangeable with D_2O), 2.50 (3 H, s, CH_3-2) ppm. ^{13}C NMR ($CDCl_3$), δ_C: 155.56 (s, Py-C), 152.03 (s, Py-C), 142.98 (s, Py-C), 136.22 (s, Py-C), 129.04−128.69 (m, Ph-C), 117.97 (s, Py-C), 76.93 (s, Ph**C**H_2-O_3), 67.03 (s, CH_2-4), 57.44 (s, CH_2-5), 19.83 (s, CH_3-2) ppm. Anal. Calcd for $C_{15}H_{16}NO_3I$ (%): C, 46.77; H, 4.19; N, 3.64. Found: C, 46.74; H, 4.17; N, 3.62.

3-*O*-Benzyl-α^4,α^5-di-*O*-allyl-6-iodopyridoxine 12. To a well stirred dry DMF (80 mL) solution of **11** (1.20 g, 3.1 mmol) and NaH (0.50 g, 12.5 mmol, 60% dispersion in mineral oil) was added allyl bromide (1.13 g, 9.33 mmol) in dry DMF (10 mL) dropwise over a period of 1−2 h, and the stirring continued for an additional 4−5 h. At the end of the time, TLC (4:1 cyclohexane−EtOAc) showed the reaction to be complete. Then MeOH (15 mL) was added slowly to react with the excess of the NaH. After most DMF was removed under reduced pressure at 55 °C, the residue was dissolved in CH_2Cl_2 (150 mL) and washed with water, dried (Na_2SO_4), filtered, and evaporated. The residue was purified by column chromatography on silica gel with 4:1 cyclohexane−EtOAc as the eluent to afford quantitatively **12** (1.45 g) as a syrup. R_f = 0.58 (4:1 cyclohexane−EtOAc). ^{1}H NMR ($CDCl_3$), δ_H: 7.43−7.36 (5 H, m, Ar-H), 5.98 (1 H, dq, $^3J_{1',2'}$ = 1.8 Hz, $^3J_{2',3a'}$ = 20.0 Hz, $^3J_{2',3b'}$ = 9.0 Hz, H-2′), 5.91 (1 H, dq, $^3J_{1'',2''}$ = 1.8 Hz, $^3J_{2'',3a''}$ = 22.4 Hz, $^3J_{2'',3b''}$ = 9.0 Hz, H-2″), 5.35 (1 H, dt, $^4J_{1',3'}$ = 1.0 Hz, $^2J_{3a',3b'}$ = 2.4 Hz, H-3′), 5.26 (1 H, dt, $^4J_{1',3'}$ = 1.0 Hz, $^2J_{3a'',3b''}$ = 2.4 Hz, H-3″), 4.89 (2 H, s, $PhCH_2$), 4.67 (2 H, s, CH_2-4), 4.60 (2 H, s, CH_2-5), 4.11 (2 H, dt, H-1′), 4.02 (2 H, dt, H-1″), 2.49 (3 H, s, CH_3-2) ppm. ^{13}C NMR ($CDCl_3$), δ_C: 155.27 (s, Py-C), 152.84 (s, Py-C), 140.40 (s, Py-C), 136.65 (s, Py-C), 134.63 (s, α^4-OCH_2**C**H=CH_2), 134.26 (s, α^5-OCH_2**C**H=CH_2), 128.89−128.14 (m, Ph-C), 119.45 (s, Py-C), 118.25 (s, α^4-OCH_2CH=**C**H_2), 118.03 (s, α^5-OCH_2CH=**C**H_2), 76.97 (s, Ph**C**H_2-O_3), 72.46 (s, α^4-O**C**H_2CH=CH_2), 72.29 (s, α^5-O**C**H_2CH=CH_2), 71.96 (s, CH_2-4), 63.29 (s, CH_2-5), 19.76 (s, CH_3-2) ppm. Anal. Calcd for $C_{21}H_{24}NO_3I$ (%): C, 54.20; H, 5.20; N, 3.01. Found: C, 54.16; H, 5.18; N, 3.00.

3-*O*-Benzyl-α^4,α^5-di-*O*-allyl-6-trifluoromethylpyridoxine 13. Trifluoromethylation of **12** (1.10 g, 2.4 mmol) with Me_3SiCF_3 (537 μL, 2.9 mmol, 1.2 equiv) in the presence of CuI (456 mg, 2.4 mmol, 1.0 equiv) and KF (168 mg, 2.9 mmol, 1.2 equiv) in DMF−NMP (10 mL, 1:1 v/v′) under an argon atmosphere in the dark, according to the procedures described for the preparation of **4** and **7**, yielded **13** (0.90 g, 92%) as a syrup. R_f = 0.71 (3:1 cyclohexane−EtOAc). ^{1}H NMR ($CDCl_3$), δ_H: 7.45−7.34 (5 H, m, Ar-H), 5.97 (1 H, dq, $^3J_{1',2'}$ = 4.8 Hz, $^3J_{2',3a'}$ = 20.8 Hz, $^3J_{2',3b'}$ = 9.2 Hz, H-2′), 5.91 (1 H, dq, $^3J_{1'',2''}$ = 1.8 Hz, $^3J_{2'',3a''}$ = 22.0 Hz, $^3J_{2'',3b''}$ = 9.0 Hz, H-2″), 5.32 (1 H, dt, $^4J_{1',3'}$ = 1.2 Hz, $^2J_{3a',3b'}$ = 2.6 Hz, H-3′), 5.23 (1 H, dt, $^4J_{1',3'}$ = 0.8 Hz, $^2J_{3a'',3b''}$ = 2.2 Hz, H-3″), 4.96 (2 H, s, $PhCH_2$), 4.71 (2 H, s, CH_2-4), 4.61 (2 H, s, CH_2-5), 4.10 (2 H, dt, H-1′), 4.05 (2 H, dt, H-1″), 2.58 (3 H, s, CH_3-2) ppm. ^{13}C NMR ($CDCl_3$), δ_C: 136.50 (s, Py-$C_{2'}$), 136.12 (s, Py-$C_{3'}$), 153.32 (s, Py-$C_{4'}$), 141.73 (q, $^3J_{F-C}$ = 6.8 Hz, Py-$C_{5'}$), 134.94 (q, $^2J_{F-C}$ = 32.4 Hz, Py-$C_{6'}$), 122.31 (q, $^1J_{F-C}$ = 273.9 Hz, CF_3), 133.38 (s, α^4-OCH_2**C**H=CH_2), 131.18 (s, α^5-OCH_2**C**H=CH_2), 129.64−127.45 (m, Ph-C), 118.24 (s, α^4-OCH_2CH=**C**H_2), 118.21 (s, α^5-OCH_2CH=**C**H_2), 78.41 (s, Ph**C**H_2-O_3), 72.69 (s, α^4-O**C**H_2CH=CH_2), 72.39 (s, α^5-O**C**H_2CH=CH_2), 71.21 (s, CH_2-4), 64.04 (s, CH_2-5), 20.59 (s, CH_3-2) ppm. Anal. Calcd for $C_{22}H_{24}NO_3F_3$ (%): C, 64.86; H, 5.94; N, 3.44. Found: C, 64.82; H, 5.91; N, 3.42.

3-*O*-Benzyl-α^4,α^5-di-*O*-(3-hydroxypropyl)-6-trifluoromethylpyridoxine 14. To a solution of **13** (0.41 g, 1.0 mmol) in dry dioxane (10 mL) was added 9-BBN (8 mL, 4 mmol, 0.5 M solution in THF) dropwise at 0 °C under argon. The reaction mixture was stirred at room temperature for 24 h and cooled to 0 °C. Aqueous NaOH (3 M, 8 mL) and 30% H_2O_2 (1.3 mL) were added. The reaction mixture was stirred at room temperature for 2 days. The aqueous phase was extracted with ethyl acetate (4 × 50 mL). The combined organic phases were washed with saturated NaCl solution and dried (Na_2SO_4). The solution was filtered and the filtrate concentrated in vacuo to give an almost colorless syrup, which was purified by column chromatography on silica gel with 1:3 cyclohexane−EtOAc as the eluent to afford **14** (0.38 g, 86%) as a syrup. R_f = 0.35 (1:4 cyclohexane−EtOAc). ^{1}H NMR ($CDCl_3$), δ_H: 7.42−7.36 (5 H, m, Ar-H), 4.93 (2 H, s, $PhCH_2$), 4.86 (2 H, s, CH_2-4), 4.62 (2 H, s, CH_2-5), 3.74−3.60 (8 H, m, α^4-OC**H**$_2$C**H**$_2$C**H**$_2$OH, α^5-OC**H**$_2$C**H**$_2$C**H**$_2$OH), 2.55 (3 H, s, CH_3-2), 2.92 (2H, br, α^4-O$(CH_2)_3$O**H**, α^5-O$(CH_2)_3$O**H**), 1.86−1.76 (4 H, m, α^4-OCH$_2$C**H**$_2$CH$_2$OH, α^5-OCH$_2$C**H**$_2$CH$_2$OH) ppm. ^{13}C NMR ($CDCl_3$), δ_C: 135.70 (s, Py-$C_{2'}$), 130.81 (s, Py-$C_{3'}$), 153.36 (s, Py-$C_{4'}$), 136.34 (q, $^3J_{F-C}$ = 8.3 Hz, Py-$C_{5'}$), 141.59 (q, $^2J_{F-C}$ = 32.0 Hz, Py-$C_{6'}$), 122.22 (q, $^1J_{F-C}$ = 273.9 Hz, CF_3), 129.00−127.84 (m, Ph-C), 76.85 (s, Ph**C**H_2-O_3), 72.66 (s, CH_2-4), 69.57, 69.56 (2s, α^4-O**C**H_2(CH_2)$_2$OH, α^5-O**C**H_2(CH_2)$_2$OH), 63.72 (s, CH_2-5), 60.79, 60.63 (2s, α^4-O-(CH_2)$_2$**C**H_2OH, α^5-O(CH_2)$_2$**C**H_2OH), 32.36, 32.27 (2s, α^4-OCH$_2$**C**H$_2$CH$_2$OH, α^5-OCH$_2$**C**H$_2$CH$_2$OH), 19.78 (s, CH_3-2) ppm. Anal. Calcd for $C_{22}H_{28}NO_5F_3$ (%): C, 59.59; H, 6.36; N, 3.16. Found: C, 59.56; H, 6.34; N, 3.14.

3-*O*-Benzyl-α^4,α^5-di-*O*-[3′-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)propyl]-6-trifluoromethylpyridoxine 15. A solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (0.75 g, 1.45 mmol, 1.2 equiv) in anhydrous CH_2Cl_2 (5 mL) was added dropwise into a solution of **14** (0.34 g, 0.75 mmol) and $Hg(CN)_2$ (0.52 g, 1.21 mmol) as a promoter in dry acetonitrile (10 mL) containing powdered molecular sieves (4 Å, 1.3 g) with vigorous stirring at room temperature under an argon atmosphere in the dark for 12 h. The mixture was diluted with CH_2Cl_2 (60 mL), filtered through Celite, washed with water, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified on a silica gel column (1:1 cyclohexane−EtOAc) to yield the title compound **15** (0.73 g, 88%) as a syrup. R_f = 0.50 (1:1 cyclohexane−EtOAc). ^{1}H NMR ($CDCl_3$), δ_H: 7.45−7.39 (5 H, m, Ar-H), 4.94 (2 H, s, $PhCH_2$), 4.68 (2 H, s, CH_2-4), 4.64 (2 H, s, CH_2-5), 4.15−4.09 (4 H, m, α^4-OC**H**$_2$(CH_2)$_2$O-, α^5-OC**H**$_2$(CH_2)$_2$O-), 3.62−3.54 (4 H, m, α^4-O(CH_2)$_2$C**H**$_2$O-, α^5-O(CH_2)$_2$C**H**$_2$O-), 2.57 (3 H, s, CH_3-2), 1.94−1.84 (4 H, m, α^4-OCH$_2$C**H**$_2$CH$_2$O-, α^5-OCH$_2$C**H**$_2$CH$_2$O-), 4.48 (1 H, d, $J_{1',2'}$ = 8.0 Hz, H-1′), 4.41 (1 H, d, $J_{1'',2''}$ = 7.6 Hz, H-1″), 5.00 (1 H, dd, $J_{2',3'}$ = 10.0 Hz, H-2′), 4.96 (1 H, dd, $J_{2'',3''}$ = 9.8 Hz, H-2″), 5.20 (1 H, dd, $J_{3',4'}$ = 3.2 Hz, H-3′), 5.17 (1 H, dd, $J_{3'',4''}$ = 3.6 Hz, H-3″), 5.10 (1 H, dd, $J_{4',5'}$ = 5.6 Hz, H-4′), 5.04 (1 H, dd, $J_{4'',5''}$ = 5.4 Hz, H-4″), 3.70 (1 H, m, H-5′), 3.68 (1 H, m, H-5″), 4.26 (1 H, dd, $J_{5',6a'}$ = 4.8 Hz, $J_{6a',6b'}$ = 13.6 Hz, H-6a′), 4.23 (1 H, dd, $J_{5'',6a''}$ = 4.4 Hz, $J_{6a'',6b''}$ = 12.4 Hz, H-6a″), 3.93 (1 H, dd, $J_{5',6b'}$ = 5.6 Hz, H-6b′), 3.90 (1 H, dd, $J_{5'',6b''}$ = 4.8 Hz, H-6b″), 2.06−1.99 (24 H, 8s, 8 × CH_3CO) ppm. ^{13}C NMR ($CDCl_3$), δ_C: 171.23−169.37 (8s, 8 × CH_3**C**O), 136.37 (s, Py-$C_{2'}$), 131.00 (s, Py-$C_{3'}$), 153.41 (s, Py-$C_{4'}$), 141.18 (q, $^3J_{F-C}$ = 11.5 Hz, Py-$C_{5'}$), 141.82 (q, $^2J_{F-C}$ = 32.8 Hz, Py-$C_{6'}$), 122.27 (q, $^1J_{F-C}$ = 274.0 Hz, CF_3), 129.00−127.88 (m, Ph-C), 76.88 (s, Ph**C**H_2-O_3), 71.49 (s, CH_2-4), 68.14 (s, α^4-OCH$_2$(CH_2)$_2$O-), 68.02 (s, α^5-OCH$_2$(CH_2)$_2$O-), 67.07 (s, α^4-O(CH_2)$_2$**C**H$_2$O-), 67.01 (s, α^5-O(CH_2)$_2$**C**H$_2$O-), 63.52 (s, CH_2-5), 30.00 (s, α^4-OCH$_2$**C**H$_2$CH$_2$O-), 29.13 (s, α^5-OCH$_2$**C**H$_2$CH$_2$O-), 20.10 (s, CH_3-2), 101.02 (s, C-1′, C-1″), 68.58 (s, C-2′), 68.51 (s, C-2″), 71.95 (s, C-3′), 71.87 (s, C-3″), 67.76 (s, C-4′), 67.66 (s, C-4″), 73.01 (s, C-5′), 72.92 (s, C-5″), 61.60 (s, C-6′), 61.50 (s, C-6″), 21.14−20.63 (8s, 8 × **C**H$_3$CO) ppm. ESIMS: m/z 1103 [M^+] (40%), 1104 [M + 1] (28%). Anal. Calcd for $C_{50}H_{64}NO_{23}F_3$ (%): C, 54.40; H, 5.84; N, 1.27. Found: C, 54.36; H, 5.83; N, 1.25.

3-*O*-Benzyl-α^4,α^5-di-*O*-[3′-*O*-(β-D-glucopyranosyl)propyl]-6-trifluoromethylpyridoxine 16. A solution of **15** (0.70 g) in anhydrous MeOH (20 mL) containing 0.5 M NH_3 was vigorously stirred from 0 °C to rt for 2 days until TLC showed the reaction to be complete. The mixture was then evaporated to dryness in vacuo. Chromatography of the crude syrup on silica gel with EtOAc−MeOH (4:1) afforded **16** (0.49 g) as a syrup in quantitative yield. R_f = 0.36 (1:4 MeOH−EtOAc). ^{1}H NMR ($CDCl_3$), δ_H: 7.52−7.39 (5 H, m, Ar−H), 5.00 (2 H, s, $PhCH_2$), 3.60 (2 H, s, CH_2-4), 3.49 (2 H, s, CH_2-5), 3.69−3.63 (4 H, m, α^4-OC**H**$_2$(CH_2)$_2$O-, α^5-OC**H**$_2$(CH_2)$_2$O-), 3.47−3.37 (4 H, m, α^4-O(CH_2)$_2$C**H**$_2$O-, α^5-O(CH_2)$_2$C**H**$_2$O-), 2.52 (3 H, s, CH_3-2), 1.83−1.78 (4 H, m, α^4-OCH$_2$C**H**$_2$CH$_2$O-, α^5-OCH$_2$C**H**$_2$CH$_2$O-), 4.29 (2 H, d, $J_{H\text{-}2,OH\text{-}2}$ = 7.6 Hz, HO-2′, 2″), 4.61 (1 H, d, $J_{H\text{-}3',OH\text{-}3'}$ = 5.2 Hz, HO-3′), 4.54 (1 H, d, $J_{H\text{-}3'',OH\text{-}3''}$ = 5.2 Hz,

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HO-3″), 4.95 (1 H, d, $J_{H\text{-}4',OH\text{-}4'}$ = 4.6 Hz, HO-4′), 4.91 (1 H, d, $J_{H\text{-}4'',OH\text{-}4''}$ = 4.4 Hz, HO-4″), 4.44 (2 H, br, HO-6′, 6″), 4.11 (1 H, d, $J_{1',2'}$ = 8.0 Hz, H-1′), 4.08 (1 H, d, $J_{1'',2''}$ = 7.6 Hz, H-1″), 2.96 (1 H, dd, $J_{2',3'}$ = 10.1 Hz, H-2′), 2.91 (1 H, dd, $J_{2'',3''}$ = 9.6 Hz, H-2″), 3.08 (1 H, dd, $J_{3',4'}$ = 3.0 Hz, H-3′), 3.05 (1 H, dd, $J_{3'',4''}$ = 3.4 Hz, H-3″), 3.84 (1 H, dd, $J_{4',5'}$ = 6.4 Hz, H-4′), 3.80 (1 H, dd, $J_{4'',5''}$ = 6.4 Hz, H-4″), 3.47 (1 H, m, H-5′), 3.43 (1 H, m, H-5″), 3.67 (1 H, dd, $J_{5',6a'}$ = 4.6 Hz, $J_{6a',6b'}$ = 11.2 Hz, H-6a′), 3.64 (1 H, dd, $J_{5'',6a''}$ = 4.8 Hz, $J_{6a'',6b''}$ = 9.6 Hz, H-6a″), 3.58 (1 H, dd, $J_{5',6b'}$ = 5.2 Hz, H-6b′), 3.54 (1 H, dd, $J_{5'',6b''}$ = 4.4 Hz, H-6b″) ppm. ^{13}C NMR ($CDCl_3$), δ_C: 136.50 (s, Py-$C_{2'}$), 131.27 (s, Py-$C_{3'}$), 153.16 (s, Py-$C_{4'}$), 141.36 (q, $^3J_{F-C}$ = 16.0 Hz, Py-$C_{5'}$), 140.25 (q, $^2J_{F-C}$ = 32.1 Hz, Py-$C_{6'}$), 122.32 (q, $^1J_{F-C}$ = 273.9 Hz, CF_3), 128.80–128.53 (m, Ph-C), 76.88 (s, Ph**C**H_2-O_3), 72.07 (s, CH_2-4), 68.04 (s, α^4-O**C**H_2(CH_2)$_2$O-), 67.82 (s, α^5-O**C**H_2(CH_2)$_2$O-), 66.00 (s, α^4-O(CH_2)$_2$**C**H_2O-), 65.89 (s, α^5-O(CH_2)$_2$**C**H_2O-), 63.98 (s, CH_2-5), 32.66 (s, α^4-OCH$_2$**C**H_2CH$_2$O-), 29.76 (s, α^5-OCH$_2$**C**H_2CH$_2$O-), 22.57 (s, CH_3-2), 103.11 (s, C-1′), 103.04 (s, C-1″), 68.18 (s, C-2′), 67.96 (s, C-2″), 70.38 (s, C-3′), 70.15 (s, C-3″), 65.98 (s, C-4′), 65.89 (s, C-4″), 73.52 (s, C-5′), 73.15 (s, C-5″), 61.30 (s, C-6′), 61.16 (s, C-6″) ppm. ESIMS: *m*/*z* 767 [M^+] (30%), 768 [M + 1] (18%). Anal. Calcd for $C_{34}H_{48}NO_{15}F_3$ (%): C, 53.19; H, 6.30; N, 1.82. Found: C, 53.14; H, 6.28; N, 1.80.

α^4,α^5-Di-*O*-[3′-*O*-(β-D-glucopyranosyl)propyl]-6-trifluoromethylpyridoxine 17. Hydrogenation (H_2, 30 psi) of **16** (0.45 g) in anhydrous EtOH (30 mL) catalyzed by Pd/C (5%, 125 mg) for 1 day furnished the target molecule **17** (0.40 g, 100%) as a syrup. R_f = 0.30 (1:2 MeOH–EtOAc). ^{1}H NMR (DMSO-d_6), δ_H: 4.78 (2 H, s, CH_2-4), 4.54 (2 H, s, CH_2-5), 4.94–4.85 (4 H, m, α^4-OC**H**$_2$(CH_2)$_2$O-, α^5-OC**H**$_2$(CH_2)$_2$O-), 3.40–3.15 (4 H, m, α^4-O(CH_2)$_2$C**H**$_2$O-, α^5-O(CH_2)$_2$C**H**$_2$O-), 2.38 (3 H, s, CH_3-2), 1.89–1.80 (4 H, m, α^4-OCH$_2$C**H**$_2$CH$_2$O-, α^5-OCH$_2$C**H**$_2$CH$_2$O-), 4.40 (1 H, d, $J_{1',2'}$ = 7.6 Hz, H-1′), 4.07 (1 H, d, $J_{1'',2''}$ = 8.8 Hz, H-1″), 3.06 (1 H, dd, $J_{2',3'}$ = 10.0 Hz, H-2′), 3.02 (1 H, dd, $J_{2'',3''}$ = 9.6 Hz, H-2″), 3.18 (1 H, dd, $J_{3',4'}$ = 2.8 Hz, H-3′), 3.10 (1 H, dd, $J_{3'',4''}$ = 3.4 Hz, H-3″), 3.85 (1 H, dd, $J_{4',5'}$ = 5.4 Hz, H-4′), 3.81 (1 H, dd, $J_{4'',5''}$ = 5.6 Hz, H-4″), 3.57 (1 H, m, H-5′), 3.54 (1 H, m, H-5″), 3.77 (1 H, dd, $J_{5',6a'}$ = 4.6 Hz, $J_{6a',6b'}$ = 11.4 Hz, H-6a′), 3.62 (1 H, dd, $J_{5'',6a''}$ = 4.4 Hz, $J_{6a'',6b''}$ = 9.8 Hz, H-6a″), 3.56 (1 H, dd, $J_{5',6b'}$ = 5.0 Hz, H-6b′), 3.52 (1 H, dd, $J_{5'',6b''}$ = 4.4 Hz, H-6b″) ppm. ^{13}C NMR (DMSO-d_6), δ_C: 148.05 (s, Py-$C_{2'}$), 132.81 (s, Py-$C_{3'}$), 154.84 (s, Py-$C_{4'}$), 130.25 (q, $^3J_{F-C}$ = 12.6 Hz, Py-$C_{5'}$), 135.45 (q, $^2J_{F-C}$ = 32.1 Hz, Py-$C_{6'}$), 123.71 (q, $^1J_{F-C}$ = 273.6 Hz, CF_3), 78.15 (s, CH_2-4), 74.37 (s, α^4-O**C**H_2(CH_2)$_2$O-), 74.14 (s, α^5-O**C**H_2(CH_2)$_2$O-), 71.13 (s, α^4-O(CH_2)$_2$**C**H_2O-), 71.08 (s, α^5-O(CH_2)$_2$**C**H_2O-), 77.27 (s, CH_2-5), 30.92 (s, α^4-OCH$_2$**C**H_2CH$_2$O-), 29.44 (s, α^5-OCH$_2$**C**H_2CH$_2$O-), 21.48 (s, CH_3-2), 103.73 (s, C-1′, C-1″), 68.97 (s, C-2′), 68.55 (s, C-2″), 70.98 (s, C-3′), 70.89 (s, C-3″), 67.23 (s, C-4′), 67.18 (s, C-4″), 73.09 (s, C-5′), 72.29 (s, C-5″), 62.17 (s, C-6′), 62.02 (s, C-6″) ppm. ESIMS: *m*/*z* 677 [M^+] (25%), 678 [M + 1] (12%). Anal. Calcd for $C_{27}H_{42}NO_{15}F_3$ (%): C, 47.86; H, 6.25; N, 2.07. Found: C, 47.81; H, 6.23; N, 2.04.

^{19}F NMR. The ^{19}F NMR data versus pH were measured in NMR tubes using a combination pH electrode (Wilmad, Buena, NJ) attached to a pH meter (Corning 220, Sudbury, U.K.), and for titration curves the pH was altered by addition of NaOH or HCl aqueous solutions. In vivo ^{19}F NMR data were acquired using a 4.7 T horizontal bore magnet with a Varian INOVA Unity system (Palo Alto, CA, U.S., 188 MHz ^{19}F).

Blood. Fresh whole blood was drawn from the lateral ear of New Zealand white rabbits and stored chilled in the presence of heparin prior to ^{19}F NMR studies.

Heart Perfusion. Langendorff retrograde perfusion was performed with recycled phosphate-free, modified Kres–Henseleit buffer oxygenated with carbogen at 37 °C under a pressure of 100 cmH_2O, as described in detail previously.[4−6]

Animal Studies. Animal studies were performed in accordance with protocols approved by the UT Southwestern Institutional Animal Care and Use Committee. Dunning prostate tumor R3327-AT1 and rat mammary tumor 13762NF were implanted in a skin pedicle on the fore-back of a Copenhagen–Fisher 344 rat, and Dunning prostate tumor Mat-Lu cells were implanted subcutaneously in thighs of nude mice. Anesthesia was induced in an induction chamber with isoflurane and maintained during the surgery with a nose cone at 1.3% isoflurane/air (1.0 dm^3/min). Once the tumor had grown to the requisite sizes, animals were anesthetized (isoflurane/air), injected ip with a solution of pH indicator **8** and NaTFA, then placed on a platform with a 2 cm diameter home-built volume coil around the tumor. The animal bed was inserted into the bore of the MR scanner, and ^{19}F NMR spectra were acquired immediately after tuning the coil to the ^{19}F resonance frequency. Animal temperature was maintained at 37 °C by a warm pad with circulating water during acquisition.

Tumor Microelectrode Studies. Measurement of pH_e was accomplished with the 20G combination needle electrode (model 818, Diamond General, Ann Arbor, MI) and digital pH meter (Model 820A, Orion Research). Spatial pH_e was mapped in three 13762NF breast tumor bearing female Fisher 344 rats. After anesthetizing the animal, the tumor was gently clamped into a fixed position. The pH electrode was clamped to a microcaliper insertion device and inserted along the central track of the central plane of the tumor until the needle reached the opposing side of the tumor wall. The pH needle was withdrawn in 0.5 mm steps and allowed to stabilize for 2 min prior to measurement. The pH response was recorded at each step, and the electrode was stepped backward until withdrawn. This process was repeated along two additional parallel tracks, 0.5 cm anterior and 0.5 cm posterior to the central track.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

FPOL, 6-fluoropyridoxine; CF_3, trifluoromethyl; Me_3SiCF_3, (trifluoromethyl)trimethylsilane; 9-BBN, 9-borabicyclo[3.3.1]-nonane; NaTFA, sodium trifluoroacetate; pH_e, extracellular pH value; pH_i, intracellular pH value; DMSO, dimethyl sulfoxide; MRS, magnetic resonance spectroscopy; NMR, nuclear magnetic resonance; MRI, magnetic resonance imaging; TLC, thin layer chromatography; DMF, *N*,*N*-dimethylformamide; NMP, *N*-methylpyrrolidine

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